Discover Environment

Research

Interactive effects of carbendazim and cold shock on haematology and behaviour of juvenile *Labeo rohita*

Muskan Kosriya[1] · Pravesh Kumar[1] · Sujit Kumar Nayak[1] · Ankur Jamwal[1,2]

Received: 25 November 2024 / Accepted: 7 April 2025
Published online: 19 April 2025
© The Author(s) 2025 OPEN

Abstract
Fungicides, including carbendazim, are prevalent agricultural pesticides that contaminate surface waters, posing a threat to aquatic organisms like fish. The study addresses a critical knowledge gap regarding the mechanistic effects of carbendazim toxicity in fish, especially its influence on cold shock response, a major concern in aquaculture, particularly in the Indian subcontinent where winter fish mortality is common. This research investigates the impact of carbendazim exposure on juvenile *Labeo rohita* (rohu), an economically important carp species, focusing on health, behaviour, and cold shock response. The 96-h LC_{50} of carbendazim was determined, and haematological and behavioural responses were measured in fish exposed to carbendazim, both independently and in conjunction with cold shock. The 96-h LC_{50} of carbendazim for rohu juveniles was found to be 2 mg L^{-1}. Sub-lethal carbendazim exposure induced behavioural and physical abnormalities. Sub-lethal exposure to carbendazim also significantly impacted the ability of rohu to withstand cold shock. The research has important implications for aquaculture management and pesticide regulation, particularly in regions where carbendazim use is high and cold stress is a recurring issue.

Keywords Fungicide · Winter stress · Aquaculture · Pesticide · Water pollution

1 Introduction

Fungicides are the second most used category of agricultural pesticides and frequently contaminate surface waters causing behavioural changes, neurotoxicity, oxidative stress, and mortality in fish [1–6]. Thermal stress can further exacerbate pesticide insult by overwhelming the stress response mechanisms in fish [7–10].

Carbendazim (methyl-1-H-benzimidazol-2-yl-carbamate), though banned in many countries, is one of the most used fungicides and frequently detected in surface waters [11–13]. The use of carbendazim is particularly high in Asian countries. For example, Thailand, India and Malaysia consumed 1437.89, 726.47, and 139.38 metric tons (M.T.) of carbendazim (Tech. Grade), respectively in various forms and combinations in the year 2020–21 [14, 15]. Carbendazim, with its resistance to photolysis and persistent nature, can persist in non-sterile soil and water for up to 61 and 320 days, respectively [16]. Furthermore, it can partition into sediments and have a half-life of 743 days under anaerobic conditions [16]. Such conditions are often found in deeper lakes, ponds, and water bodies with thermal stratification. Due to its exceptionally

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s44274-025-00244-4.

✉ Ankur Jamwal, ankur.jamwal@usask.ca | [1]Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar 848125, India. [2]Present Address: School of Climate Change and Sustainability, Azim Premji University, Bengaluru, Karnataka 562125, India.

long half-life, carbendazim can accumulate to concentrations exceeding 1 µg L^{-1} of water in agricultural drainage canals [17–19]; 291.3 µg of carbendazim L^{-1} of water was reported in Ergene River in Turkey [20].

Pesticides are biocides by design and because living organisms share a large number of conserved biochemical and physiological mechanisms, pesticides can also elicit toxicity in non-targeted organisms [21, 22]. Therefore, carbendazim, though used as a fungicide, is known to cause developmental and behavioral abnormalities, metabolic aberrations, and oxidative stress in fish [23–26].

Cold shock and winter fish mortality syndrome is a major problem in many parts of the world [27, 28]. Winter fish mortality is also a common occurrence in the Indian subcontinent where the night temperatures during December and early January fall drastically below 15 °C. However, the extant literature primarily focuses on the effects of elevated thermal stress in fish, and low-temperature stress is often overlooked [29, 30]. While cold shock in fish typically triggers a surge in blood glucose to fuel the acute stress response [29], prolonged environmental stress, including that from xenobiotic exposure, can lead to metabolic depression and suppressed feeding, potentially hindering this initial energy mobilization [31].

The existent literature presents a significant knowledge gap regarding the mechanistic effects of carbendazim toxicity in fish, particularly concerning its influence on cold shock response in ectotherms. Furthermore, there is a dearth of threshold toxicity values of carbendazim in freshwater Asian fishes, especially carps. Threshold toxicity values are often translated into policy guidelines concerning the use of a pesticide. However, due to lack of toxicity values of carbendazim for aquatic organisms of the Indian subcontinent, the policymakers must depend on the research conducted on non-native fish that may respond differently to various carbendazim concentrations in comparison to the native organisms. This study addresses this critical gap by investigating the impact of carbendazim exposure on juvenile *Labeo rohita* (rohu), specifically focusing on health, behaviour, and cold shock response. Haematological and behavioural responses were measured to provide a comprehensive assessment of carbendazim toxicity, both independently and in conjunction with cold shock exposure. Currently there is no published data on how temperature modulates carbendazim toxicity. Recognizing the scarcity of lethal concentration data for carbendazim and other pesticides in Asian carps, we also determined the 96-h LC_{50} for carbendazim in rohu juveniles.

Rohu, is an ecologically and economically important carp species native to the Ganga–Brahmaputra-Indus River system. It is a major contributor to Indian subcontinent's inland aquaculture and capture fisheries and contributed 4.3% to global inland finfish production [32].

In subcontinent, rohu is frequently cultured in earthen ponds, often in polyculture or integrated with other farming systems. These aquaculture systems are frequently interspersed with agricultural lands, making them susceptible to runoff containing complex mixtures of pesticides and nutrients. Winter conditions exacerbate stress in these systems, with increased carp morbidity and mortality often observed due to cold shock from fluctuating diurnal temperatures, especially in ponds receiving agricultural runoff. Therefore, we hypothesized that prior exposure to carbendazim compromises the ability of rohu juveniles to withstand cold shock.

2 Materials and methods

2.1 Test fish and its husbandry

Rohu fingerlings, weighing 15 ± 4 g, were procured from a local carp hatchery and stocked in 1000 L FRP circular tanks in the fish rearing facility of the institutional research center. Groundwater from the borewell was used for the husbandry and 24 h aeration was provided in the fish tanks. Fish were fed with commercially available sinking pelleted feed at the rate of 6% of the body weight, divided into two equal portions. Fish feces and uneaten food were siphoned, and 50% of the water in tanks was exchanged daily. The fish were allowed to habituate to the laboratory conditions for 10 days before their use in the experiments. The physicochemical parameters of water used in husbandry were tested on every alternate day and found within the acceptable range (hardness 80 ppm, alkalinity 150 mg L^{-1}, $CaCO_3$, pH 8.1, temperature 25 ± 2 °C, dissolved oxygen 7.3 ± 0.2 mg L^{-1}, ammonia nitrogen < 0.15 mg L^{-1}, nitrite nitrogen < 0.002 mg L^{-1}, nitrate nitrogen < 0.03 mg L^{-1}). Photoperiod was not artificially manipulated. The fish used in the experiment, or their parents, had no known history of exposure to environmental contaminants.

2.2 Test chemical

Commercially available form of carbendazim (Brand name- Tagstin, Tropical Agrosystem (INDIA) Pvt. Ltd, 51% w/w) was used because of its high-water solubility and it is also most used by the farmers in India. For use in the experiment, a stock solution of carbendazim was prepared daily by accounting for the composition of the material. The stock solution was prepared fresh each time and used as per the requirement of the experiment.

2.3 Experimental design

2.3.1 Acute toxicity test

A semi-static procedure was performed as per the OECD guidelines number 203 for testing of acute toxicity of chemicals and median lethal concentration (LC_{50}) was calculated. Fish were stocked in 150 L capacity food-grade plastic tanks containing 60 L of stagnant water, half of which was replaced every day. Aeration was provided in all the tanks. Feeding of fish was stopped 24 h before the start of the experiment and starvation was continued during the test. A total of nine concentrations of carbendazim were tested; 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 10 mg of carbendazim L^{-1} of water. Each concentration was evaluated in duplicate, using 5 fish in each replicate tank (a total of 10 fish per concentration). Fish were assigned randomly to the tanks. Fish mortality was assessed at 12, 24, 36, 48, 60, 72, 84, and 96 h. The dead fish were removed immediately. The experiment was conducted under ambient temperature (22–25 °C).

General fish behavior like erratic swimming, loss of equilibrium, darting, schooling, and body and gill color was also recorded after 24 h of exposure to carbendazim. Darting, gulping and hyperactivity were quantified through direct observation as number of events in 5 min. Darting behavior in fish, for the sake of current study, was defined as a short burst of swimming activity, often resulting in changing direction and hitting the sides of the fish tank. Gulping referred to a surfacing of fish accompanied by rapid opening and closing of their mouth to intake surface layer of water. Hyperactivity was differentiated from darting and referred to erratic swimming patterns involving spiraling or slightly longer durations of swimming at higher speeds. The loss of equilibrium was quantified as the number of fish that showed equilibrium loss during the 96 h interval. The rest of the observations were recorded as number of fish with abnormal physical appearance when they demonstrated morbidity or after 96 h of exposure, if the fish survived.

2.3.2 Interaction study of cold shock with sub-lethal concentration of carbendazim

A 15 days experiment was conducted with 60 rohu juveniles allocated randomly to four treatment groups. The first group (control) was not exposed to carbendazim or cold shock. The fish in the second treatment group (CBZ) were exposed to 0.5 mg carbendazim L^{-1} of water (0.25×96 h LC_{50}) with no exposure to cold shock. The fish in the third group (CS) were given cold shock from the 12th day onward (last 4 days) and no exposure to carbendazim. Fish in the last treatment group (CBZ + CS) were exposed to 0.5 mg carbendazim L^{-1} of water for the entire duration of the experiment and cold-shock in the last 4 days. Thus, a complete factorial design was followed to evaluate the individual and interactive effects of cold shock and carbendazim. All treatment groups were in triplicate with 5 fish in each replicate tank (Fig. 1).

For the cold shock, fish from the respective treatment groups were taken and dipped for 1 h in water chilled to 15 °C. The internal monitoring data from the University's fish farm indicated that the water temperature in earthen ponds (at 0.5 m depth) drops below 15 °C in the months of November and December for about 1 h at about 5–6 A.M, after which the temperature may again rise with the sunrise when the skies are clear. Therefore, the cold shock was given at 6 A.M. in the last 4 days of the experiment. Fish that did not receive cold shock were also exposed to handling stress similar to the cold-shocked fish but water at ambient temperature was used for the dip. The ambient water temperature ranged between 22 and 25 °C.

All fish were fed during the entire duration of the experiment, except 24 h before euthanization. Uneaten feed and feces were removed promptly. No mortality was reported in any treatment group during the experiment. Water quality parameters were tested on alternate days and found within acceptable limits (data not shown). At the end of the exposure (16th day since the start of the experiment), fish were euthanized with an overdose of buffered MS-222 (Ethyl 3-aminobenzoate methanesulfonate). Blood of fish was drawn through a caudal vein puncture and used for hematological tests on the same day.

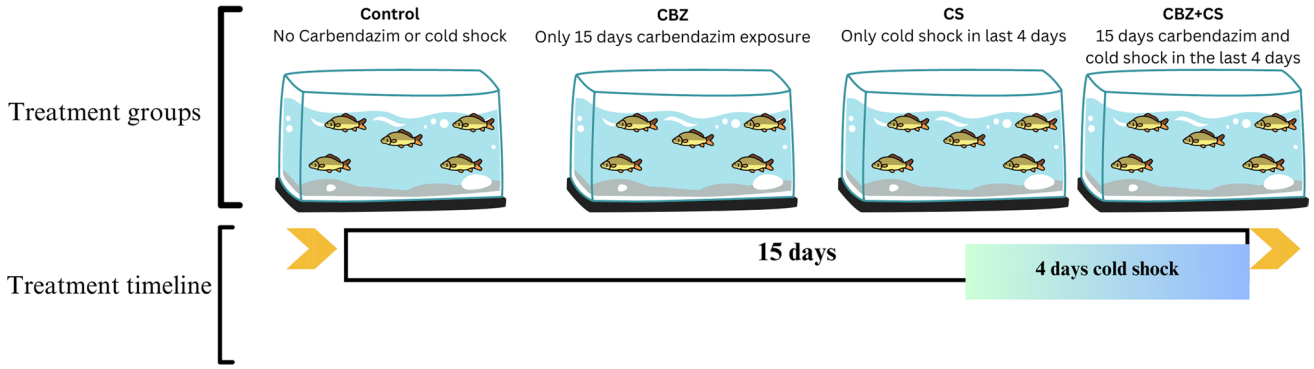

Fig. 1 Representation of experimental design followed to evaluate the interactive effects of cold shock on sub-lethal toxicity of carbendazim. The experiment was conducted for a total of 15 days and had four treatment groups. Cold shock was given in the last 4 days to the respective treatment groups

2.4 Hematological parameters

Because of the small fish size and low volume of blood in each fish, blood from three fish per treatment replicate was pooled to evaluate hematological parameters. The final value of each hematological parameter was calculated as the mean of three treatment replicates (n = 3). Hemoglobin (Hb) was estimated as g dL^{-1} by cyanmethemoglobin method [33]. Hematocrit (Hct) or the packed cell volume (PCV) was evaluated by the Wintrobe's method [34]. The whole blood was diluted in Hayem's (1:200) or Turk's (1:20) solution to count the red blood cells (RBC) and white blood cells (WBC), respectively using a hemocytometer. The mean corpuscular volume (MCV), which represents an average volume of an RBC, was calculated using Eq. 1.

$$\text{MCV}\left(\mu m^{-3}\right) = \frac{\text{hematocrit (\%)} \times 10}{\text{RBC (in million)}} \tag{1}$$

Mean corpuscular hemoglobin (MCH) is the average amount of Hb in an RBC expressed as pg $cell^{-1}$ (Eq. 2).

$$MCH\left(pgcell^{-1}\right) = \frac{Hb\left(gdL^{-1}\right)}{RBC(inmillion)} \times 10 \tag{2}$$

Mean corpuscular hemoglobin concentration (MCHC) is a measure of the concentration of hemoglobin in each volume of packed RBC and is expressed as percentage (Eq. 3).

$$MCHC = \frac{Hb\left(gdL^{-1}\right)}{Hct(\%)} \tag{3}$$

Total serum protein and albumin were determined by biuret and bromo-cresol green methods, respectively. Globulin content of the blood was calculated by subtracting albumin content from the total protein concentration. Blood glucose was estimated by the standard glucose oxidase method on 2nd, 10th, and 15th day to evaluate the effect of immediate exposure to carbendazim, 10-day exposure to carbendazim, and effect of cold shock alone or in combination with carbendazim on the 15th day, respectively.

2.5 Statistical analysis

The data were analyzed using R version 4.2.2. [35]. Median lethal concentrations were calculated using Finney's statistical method with the use of ecotox package for R [36, 37]. The data were assessed for normality and homoscedasticity using Shapiro's and Levene's tests, respectively. The data met the assumptions of normality and equality of variance, and no transformation was necessary. The mains and interactive effects of carbendazim exposure and thermal shock on fish were evaluated with ANOVA followed by Tukey's-HSD test. All the data are represented as mean ± SEM unless stated otherwise.

3 Results

3.1 Acute toxicity of carbendazim

3.1.1 Median lethal concentration of carbendazim

The 96-h median lethal concentration (96-h LC_{50}) of carbendazim for the juveniles of rohu was 2 mg L^{-1} with lower and upper confidence levels of 1.3 mg L^{-1} and 2.9 mg L^{-1}, respectively (Fig. 2).

There was no mortality in the control. Fish mortality was first observed after 36 h in treatments exceeding 0.5 mg L^{-1} concentration of carbendazim. No fish survived at concentrations ≥ 4 mg L^{-1}. Complete mortality was observed with carbendazim ≥ 4 mg L^{-1} (see Supplementary Information, Table S1).

3.1.2 Fish behavioural response and physical trauma

Fish in the control group exhibited no signs of distress. In contrast, the fish exposed to carbendazim demonstrated an array of behavioural and physical abnormalities (see Table 1). Darting, hyperirritability to sound and light, and aggregation at the darker corners of the tank was apparent in some fish exposed to carbendazim ≥ 1.0 mg L^{-1} and in all the fish exposed to 8 or 10 mg L^{-1} of carbendazim. Fish also avoided schooling when carbendazim exceeded 2 mg L^{-1} and lost equilibrium at concentrations ≥ 3.0 mg L^{-1}.

Respiratory distress, in form of air gulping, was demonstrated in fish exposed to carbendazim > 3 mg L^{-1}. Physical damage to gills was apparent as black and oedematous gill lamellae in fish exposed to carbendazim ≥ 4 mg L^{-1}. Fish exposed to 10 mg L^{-1} of carbendazim showed focal haemorrhage in addition to oedema of gill lamellae (see Fig. 3a–c). Tail rot was observed in fish exposed to carbendazim ≥ 6 mg L^{-1}, which was also accompanied by body discolouration and white patches (Fig. 3d). Yellow discolouration around the pectoral region in fish exposed to carbendazim > 4 mg L^{-1} suggested hepatocellular dysfunction (Fig. 3e).

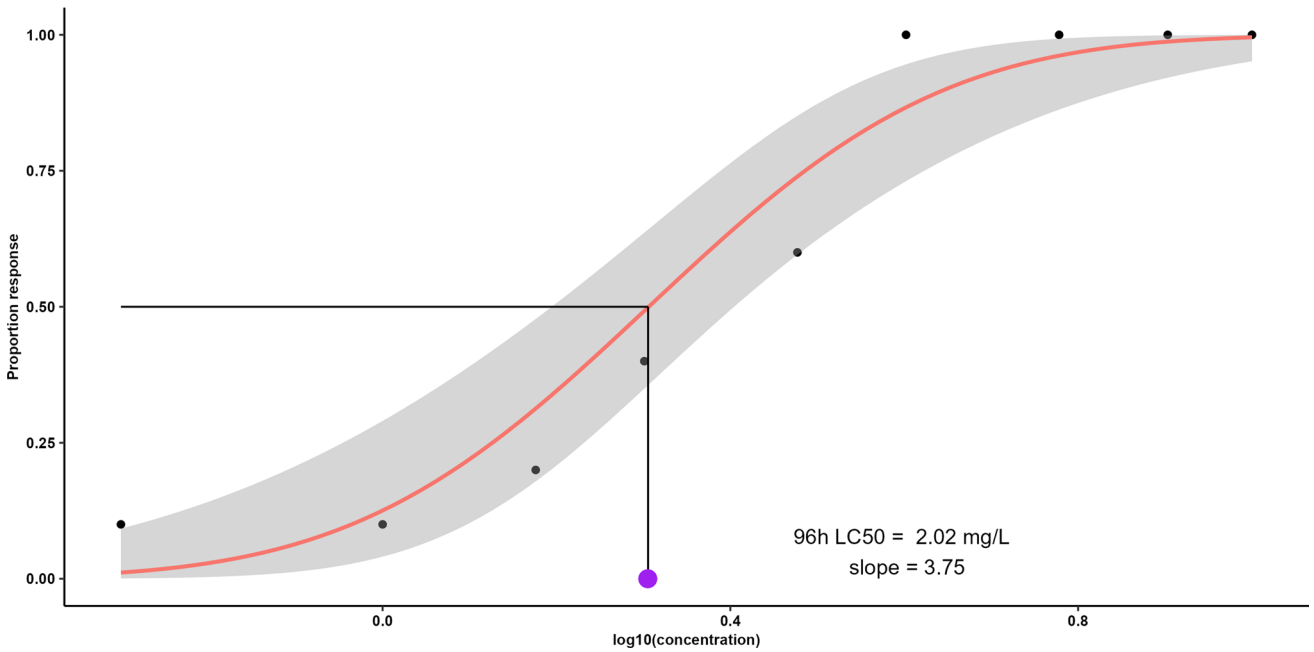

Fig. 2 Dose–response curve for 96-h LC_{50}. The value of LC_{50} is the antilogarithm (log_{10}(concentration)) when the proportion response is 0.5

Table 1 Aberrant behavioural and physical characteristics observed in juvenile rohu from 96 h acute exposure to varying concentrations of carbendazim

Abnormalities	Carbendazim (mg L^{-1})									
	0.0	0.5	1.0	1.5	2.0	3.0	4.0	6.0	8.0	10.0
Darting movement	–	–	+	+	+	+	++	++	+++	+++
Scototaxis	–	–	+	+	+	+	++	+++	+++	+++
Schooling behaviour [present (+) or absent (–)]	–	–	–	–	–	+	+	+	+	+
Gulping	–	–	–	–	–	–	+	+	++	++
Branchial haemorrhage	–	–	–	–	–	–	–	–	–	+
Blackening of gills	–	–	–	–	–	–	+	++	++	++
Loss of equilibrium	–	–	–	–	–	–	+	++	+++	+++
Body discoloration	–	–	–	–	–	–	–	++	+++	+++
Hepatic cellular dysfunction	–	–	–	–	–	–	–	+	++	+++
Tail rot	–	–	–	–	–	–	–	+	++	+++

'–'=absent, '+'=mild, '++'=moderate, '+++'=high, 'refer to supplementary data for more information on quantification of behavioural effects'

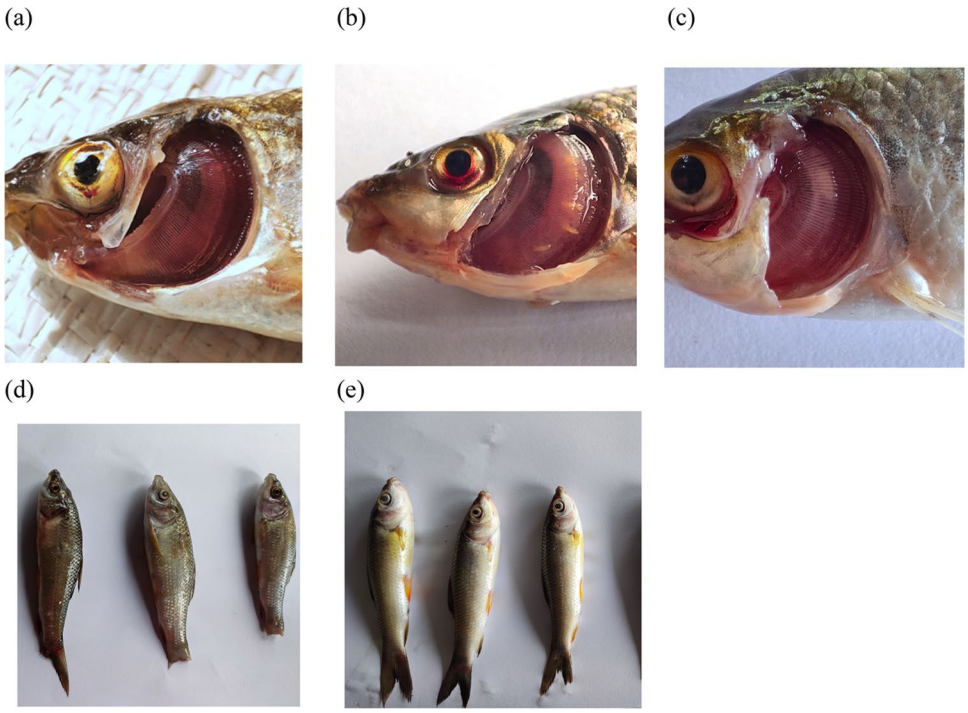

Fig. 3 Exposure to waterborne carbendazim induced branchial trauma in fish as evident from blackening of gills (**a**), fused gill lamellae (**b**), and focal hemorrhage (**c**). Tail rot and body discolouration (**d**), hepatocellular damage (**e**) was also observed in fish exposed to carbendazim

3.2 Interaction of cold shock with sub-lethal concentration of carbendazim

3.2.1 Hematological response to carbendazim and cold shock

Cold shock did not interact with carbendazim to influence the RBC count of fish ($F_{1,8}=1.08$, $p=0.33$). However, a simple main effects analysis revealed significant effects of both carbendazim exposure ($F_{1,9}=17.22$, $p=0.003$) and cold stress ($F_{1,9}=57.37$, $p=0.004$). A treatment-wise analysis showed that exposure to carbendazim reduced the mean RBC count in fish by 16%, in comparison to the control, though the difference was not statistically significant ($p=0.20$). Conversely, a cold shock treatment resulted in statistically significant 28% increase in the RBC count in comparison to the control ($p=0.03$). However, a cold shock failed to elicit a similar increase in the RBC count in the carbendazim-exposed fish Fig. 4a.

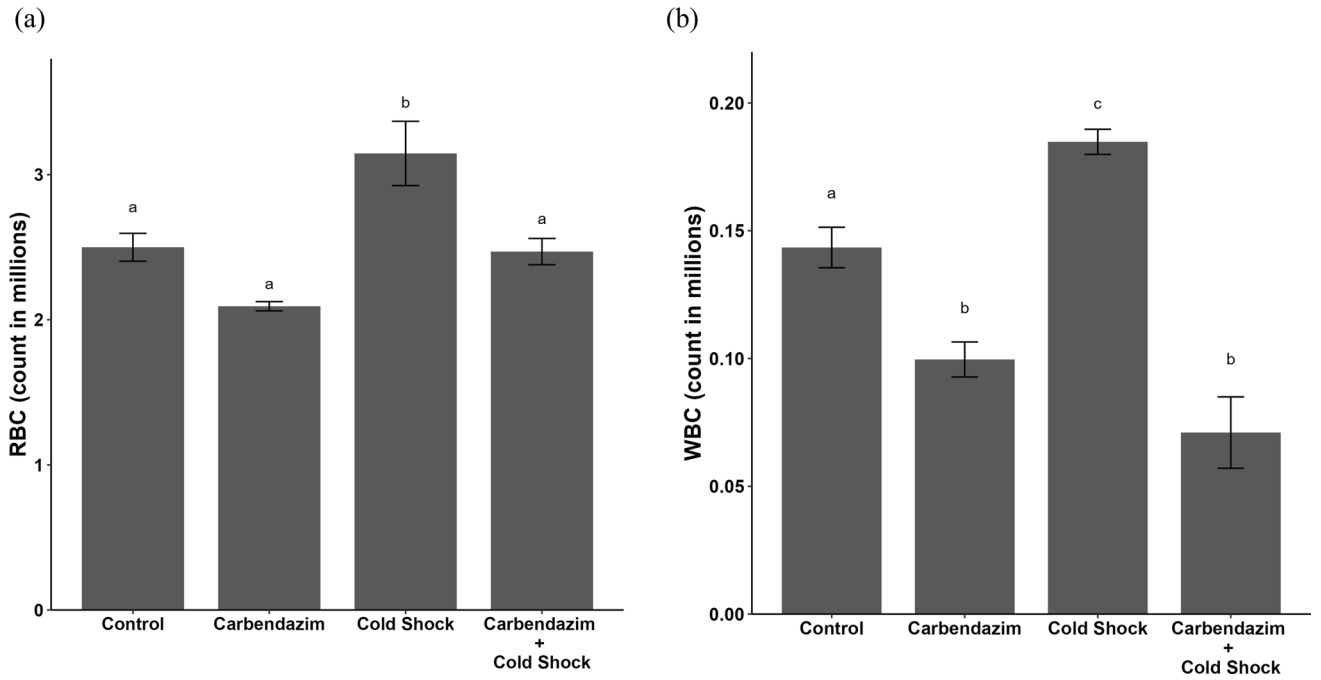

Fig. 4 The RBC (**a**) and WBC (**b**) count of fish exposed to carbendazim, alone or in combination with cold shock. The data are presented as mean ± S.E.M. (n = 3), where n represents the number of true independent measurements. Bars with different alphabetical notations are significantly different from each other (p > 0.05), as determined by ANOVA and post hoc analysis

Carbendazim exposure and cold shock demonstrated a statistically significant interaction on the WBC count of the fish ($F_{1,8} = 14.9$, $p = 0.005$). A pair-wise comparison among the treatments showed that carbendazim, alone or with cold shock, reduced the blood WBC by 28.6 ($p = 0.04$) and 50% ($p = 0.002$), respectively, relative to the control. While cold shock alone significantly increased the WBC count by 35.7% ($p = 0.048$), it was unable to induce a significant change in the WBC count in the fish that were previously exposed to carbendazim ($p = 0.19$) Fig. 4b.

There was no statistically significant interaction between carbendazim and cold shock exposure on the blood hemoglobin (Hb) content of fish ($F_{1,8} = 1.74$, $p = 0.22$). A simple mains effects of both carbendazim and cold shock was significant ($F_{1,9} = 87.48$, $p < 0.001$; $F_{1,9} = 8.60$, $p = 0.02$). When compared to the control, exposure to carbendazim caused a 27.7% reduction in fish Hb ($p < 0.001$). In contrast, cold shock demonstrated no apparent influence on blood Hb, relative to control. Similarly, cold shock treatment to carbendazim exposed fish also failed to elicit any significant change in Hb relative to the fish exposed to only carbendazim Fig. 5a.

Carbendazim and cold shock showed no statistically significant interaction with regards to the PCV ($F_{1,8} = 0.50$, $p = 0.5$). However, a simple mains effect of carbendazim was apparent from the statistical analysis ($F_{1,9} = 46.78$, $p < 0.001$). A pair-wise comparison among the treatments revealed that cold shock did not influence the PCV of the blood. In contrast, carbendazim reduced PCV by 28% ($p = 0.01$). The PCV of fish given a combined exposure to carbendazim and cold shock was 33.3% lower than the control ($p = 0.005$), but statistically similar to the fish exposed to carbendazim alone Fig. 5b.

The treatments had no significant effect on MCV, MCH and MCHC whose mean value ranged between 70 and 96.6 μm^3, 22.6–38.7 pg $cell^{-1}$, and 32.5–46.0%, respectively.

3.2.2 Effects of treatments on the fish blood protein content

3.2.2.1 Total serum protein Carbendazim and cold shock had no significant interactive effect on the total serum protein of fish, which was significantly affected only by the exposure to carbendazim ($F_{1,9} = 93.21$, $p < 0.001$). When compared treatment-wise, the exposure to carbendazim, alone or in combination with cold shock, significantly reduced the serum protein by 40–50%, respectively (Fig. 6a). Similarly, only carbendazim had a significant main effect on the serum albumin content ($F_{1,9} = 6.68$, $p = 0.03$), with no interaction between the two main variables. Carbendazim exposure, without cold shock, caused a 44% reduction in serum albumin content, in comparison to the control.

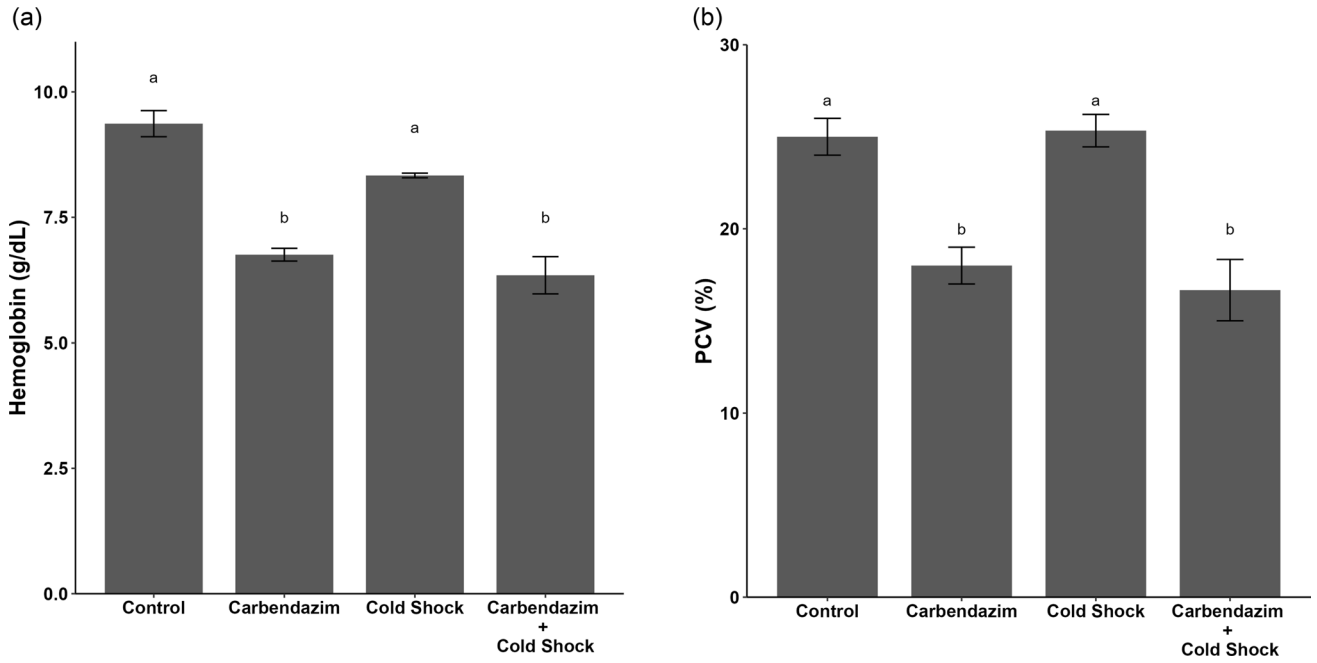

Fig. 5 The hemoglobin (g dL^{-1}) (**a**) and packed cell volume (PCV %) (**b**) in blood of the fish exposed to carbendazim, alone or in combination with cold shock. The data are presented as mean ± S.E.M. (n = 3), where n represents the number of true independent measurements. Bars with different alphabetical notations are significantly different from each other (p > 0.05), as determined by ANOVA and post hoc analysis

The mean albumin in fish given carbendazim and cold shock exposure was also 21% lower in comparison to the control; however, the difference was not statistically significant due to high amount of variability in the data (Fig. 6b). Carbendazim and cold shock had no interactive effects on the serum globulin content ($F_{1,8} = 2.14$, $p = 0.18$), but there was significant mains effect of carbendazim ($F_{1,9} = 298.99$, $p < 0.001$) and cold shock ($F_{1,9} = 7.09$, $p = 0.03$). Treatment-wise comparison (Fig. 6c) showed a 52.9 and 48.4% reduction in serum globulin level in fish exposed to carbendazim alone or in combination with cold-shock, respectively. No change in serum globulin, with respect to the control, was observed in fish exposed to only cold shock. The globulin content in all fish exposed to carbendazim, irrespective of cold shock exposure, was statistically similar.

A two-way ANOVA showed no interactive effect of carbendazim and cold shock on albumin:globulin ratio (A:G) [$F_{1,8} = 1.86$, $p = 0.21$]. Only carbendazim had a significant mains effect on the A:G ($F_{1,9} = 9.27$, $p = 0.014$). Among the different treatment groups, the A:G was reduced only in the fish given cold-shock; however, the difference was significantly lower only in comparison to the fish given carbendazim and cold shock (Fig. 6d, p = 0.048).

The effects of treatments on blood glucose level were evaluated on 2nd, 10th, and 15th day, and compared for statistical significance. A 24 h carbendazim exposure caused 50% increase in the blood glucose concentration ($t_4 = 3.3$, $p = 0.03$, Fig. 7). The difference in blood glucose level between the control and carbendazim-exposed fish was similar on the 10th and 15th day. In contrast, the blood glucose was significantly higher in all the cold-shocked fish (15th day) in comparison to the fish given no stress or only carbendazim exposure ($p < 0.001$ and $p = 0.001$, respectively).

4 Discussion

4.1 Acute carbendazim toxicity

The 96-h LC_{50} of carbendazim for rohu juveniles was 2.02 mg L^{-1} (Fig. 2). On comparison with other fish species, rohu appears to be more sensitive to carbendazim. For example, LC_{50} for temperate Amazonian fish species and crustaceans exceeded 4.1 mg L^{-1} [38], and that for Zebrafish (*Danio rerio*), a tropical freshwater cypriniformes and native to the Indian subcontinent, is 8.53 mg L^{-1} [39]. Rohu is also more sensitive than the juvenile African sharptooth catfish (*Clarias gariepinus*) for which the LC_{50} was 4.33 mg L^{-1} [40]. In contrast, temperate rainbow trout (*Oncorhynchus mykiss*) and channel

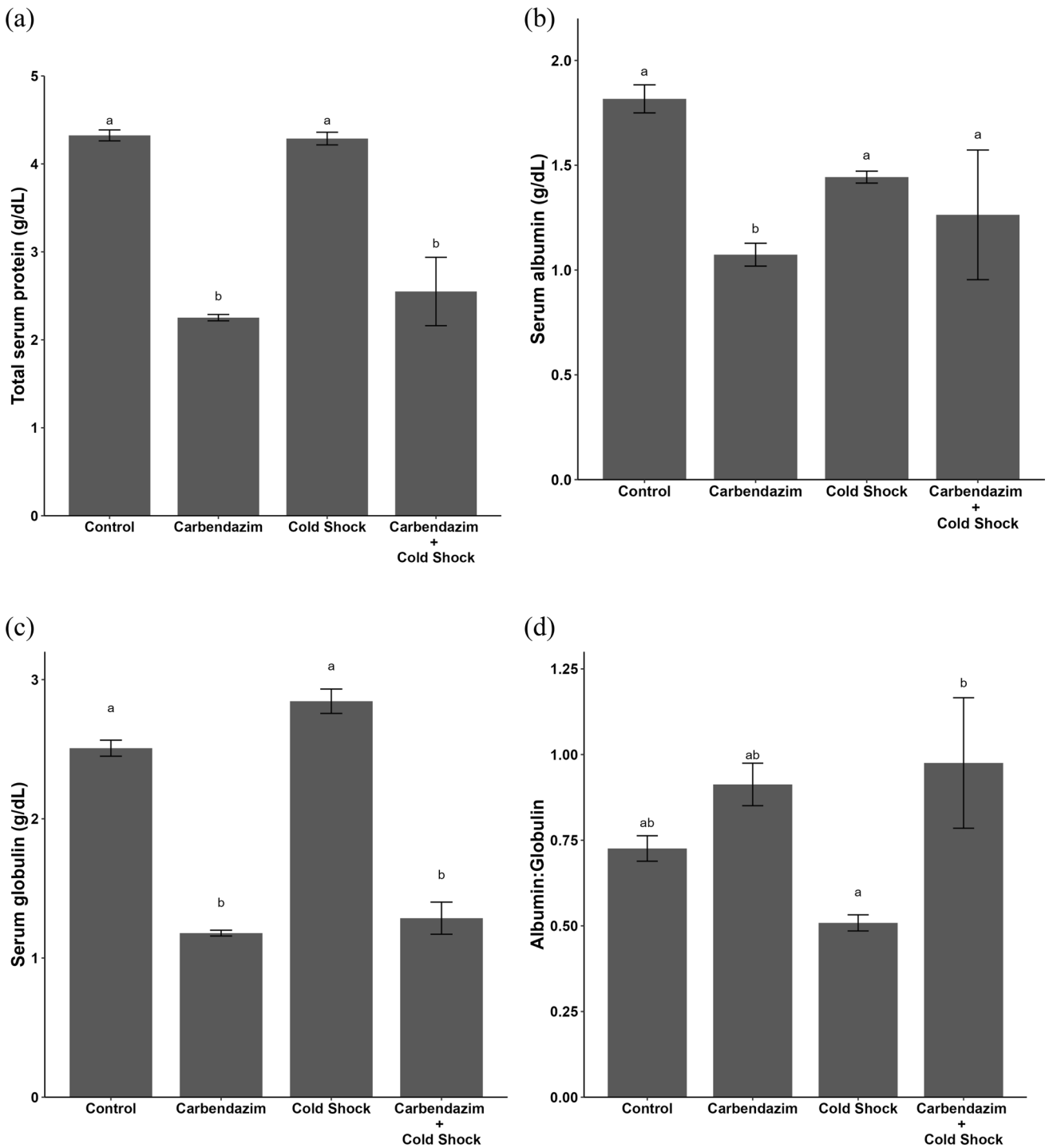

Fig. 6 The total serum protein (g L^{-1}) (**a**), albumin (**b**), globulin (**c**), and albumin:globulin (**d**) in e fish exposed to carbendazim, alone or in combination with cold shock. The data are presented as mean ± S.E.M. (n = 3), where n represents the number of true independent measurements. Bars with different alphabetical notations are significantly different from each other ($p > 0.05$), as determined by ANOVA and post hoc analysis

catfish (*Ictalurus punctatus*) juveniles are more sensitive than rohu with LC_{50} values of 0.87 and 0.019 mg L^{-1}, respectively [41]. Currently, milkfish (*Chanos chanos)* is the only commercial food fish species, native to the Indian subcontinent, for which the LC_{50} values are reported in the literature [42]. The reported 96-h LC_{50} value for milkfish is 11.5 µg L^{-1}, indicating a higher sensitivity compared to rohu. However, it is important to note that direct comparisons between these two

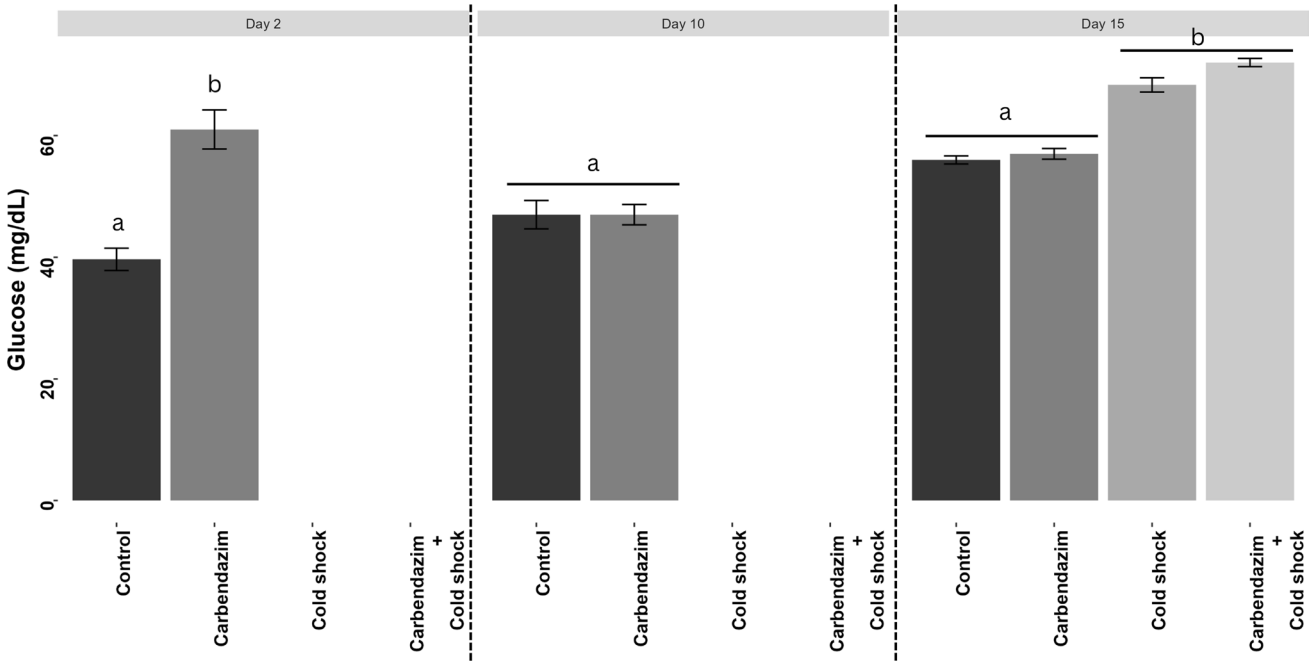

Fig. 7 Blood glucose (mg L^{-1}) in response to various treatments on day 2, day 10, and day 15. Cold shock was given only the 15th day. The data are presented as mean ± S.E.M. (n = 3), where n represents the number of true independent measurements. Bars with different alphabetical notations are significantly different from each other ($p > 0.05$), as determined by ANOVA and post hoc analysis

species may be misleading, as milkfish inhabits brackish water environments that are fundamentally different from the freshwater habitat occupied by rohu.

The lethal concentration values for tropical carps are not readily available in the published scientific literature, despite their significance in Asian economy and food security. The current lack of species-specific data necessitates reliance on studies of non-native fish, a practice that introduces uncertainty and can potentially lead to erroneous ecological risk assessments with long-term environmental ramifications. To the best of our knowledge, the LC_{50} value reported herein represents the first such determination for a freshwater food fish species of the Ganga–Brahmaputra-Indus riverine system within the family Cyprinidae.

Though mortality provides a clear endpoint in toxicity assessments, relying solely on lethal concentration data overlooks ecologically significant sublethal impacts. Environmental contaminants can trigger behavioral alterations that, while not immediately fatal, compromise critical activities such as foraging, predator avoidance, and reproduction [43–45]. This, while reduces individual fitness, also impacts population dynamics. Hence, an understanding of ecological risks requires examining non-lethal behavioral patterns, including shoaling, courtship, and foraging [44].

In the current study, exposure to carbendazim elicited scototaxis (tendency to remain in the dark regions of the tank), thigmotaxis (tendency to remain close to structures or the walls of a tank), and startling response, indicating anxiety (Table 1). The scototaxis was dose dependent. Given that self-preservation and adaptive behaviour in animals, including fish, are mediated by highly regulated neuroendocrine pathways, our results indicate that carbendazim affects these mechanisms. The existing literature demonstrates that pesticides can interfere with cholinergic and GABAergic systems, leading to anxiety-like symptoms, including scototaxis, and thigmotaxis [46–50]. Oxidative stress is also recognized as an important factor in nervous system dysfunction in fish leading to abnormal behaviour [51, 52].

Carbendazim exposure ≥ 3 mg L^{-1} in the current study also resulted in cessation of schooling behaviour, suggestive of disruption in social interaction due to anxiety. Though adult rohu seldom exists in schools in rivers, their juveniles demonstrate schooling, perhaps to avoid predation. Therefore, deviation from schooling behaviour in nature would make juveniles susceptible to predation and affect fish population structure [44]. The current results also warrant an investigation into the effects of carbendazim on reproductive behaviour in adults, which can be affected by pesticide-induced oxidative stress [53].

The fish exposed to carbendazim ≥ 4 mg L^{-1} showed behaviour characteristic of branchial distress or hypoxia. The fish aggregated near the source of aeration, gulped air, and appeared sluggish (Table 1). Inspection of gills of the stressed

fish revealed lesions, melanisation and oedema of gill filaments (Fig. 3a, b). Petechial hemorrhage of gill filaments was also observed with the highest concentration of carbendazim (10 mg L^{-1}) (Fig. 3c). Gill lamellar oedema is a common response to waterborne xenobiotic insult in fish, mostly as a response to oxidative stress [54–56]. Similarly, hypermelanization is also considered an antioxidative stress response because of the ability of melanin and related pigments to sequester xenobiotics and reactive oxygen species [57, 58]. Thus, damage to the fish branchial ultrastructure through carbendazim-induced oxidative stress can be hypothesized, as already demonstrated in the existing literature [59, 60]. The behaviour in rohu, which is peculiar to hypoxia, could be the result of damage to branchial ultrastructure and inefficient gaseous exchange thereby.

Exposure to higher concentrations of carbendazim, for more than 36 h could have exhausted the fish, thus causing secondary infections observed as tail rot and body discolouration (Fig. 3d) [61, 62]. The yellowish colouration, an indication of hepatocellular dysfunction, could be due to secondary infection or the result of carbendazim toxicity itself and more investigations will be required to ascertain the cause behind it (Fig. 3e). Hepatotoxicity due to carbendazim-induced oxidative stress and metabolic impairment is also documented in zebrafish (concentration less than 100 µg L^{-1}) and similar mechanistic underpinnings could be hypothesized for rohu [24, 63, 64].

4.2 Interaction of cold shock with waterborne sub-lethal carbendazim

A suite of hematological parameters, including the change in blood protein and glucose concentrations, were used to understand the effects of cold shock on fish that were previously exposed to a sublethal concentrations of carbendazim (0.5 mg L^{-1}; 0.25 × 96 h LC_{50}) for 12 days. Although hematology is a nonspecific indicator of stress in vertebrates, it can be a highly sensitive and reliable technique to ascertain stress under controlled conditions, such as the ones provided in the current experiment [52].

The decrease in Hb and Hct levels induced by carbendazim may have compromised the oxygen-carrying capacity in rohu, as evidenced by the fish's hypoxic behaviour (Table 1). Carbendazim disrupts microtubule metabolism, which is crucial for RBC cytoskeleton integrity, thereby affecting erythrocyte membrane stability and Hb synthesis [65]. Additionally, carbendazim metabolism generates ROS [59], which increase RBC fragility, leading to erythrolysis and reduced Hct levels [66]. Oxidative stress can further diminish Hb and Hct by denaturing enzymes and proteins essential for erythropoiesis [40]. Furthermore, carbendazim is known to adversely impact Hb and Hct levels by inducing pathological changes in the kidneys, the primary organ responsible for erythropoiesis [67].

The observed reduction in MCV and PCV may be associated with microcytosis, characterized by a diminished size of mature RBCs. This phenomenon could potentially arise from impaired Hb synthesis or premature erythrocyte destruction because of oxidative stress. Although such findings have not been documented in carps, analogous results have been reported in African sharptooth catfish subjected to clotrimazole exposure [68].

Exposure to cold shock triggers release of acute corticosteroid stress hormones, epinephrine and cortisol, through hypothalamus-pituitary-interrenal (HPI) axis [29]. The immediate effect of acute stress hormones is to stimulate innate defence mechanism, including leukopoeisis, to counter potential pathogens and inflammation [69]. The observed increase in WBC count in the acute cold shock group aligns with this response, indicating an attempt by the fish to mobilize immune defenses against the acute stressor. However, chronic exposure to stressors often results in reallocation of energy and metabolic depression, causing immunosuppression [70]. This compromised immune response might explain the absence of significant leukocytosis in the groups exposed to both carbendazim and cold shock. The pre-existing stress from carbendazim exposure might have exhausted the immune system's resources, rendering it incapable of mounting a further response to the additional stress of cold shock. This aligns with stress-induced immune-suppression observed in other fish at lower temperatures [71].

Corticosteroid mediated acute stress repose is energy intensive and thereby induces glycogenolysis and gluconeogenesis, resulting in hyperglycemia [7, 72]. However, the hyperglycemic response does not last for long, and the blood glucose returns to normal levels after a day or two as the acute stress response subsides and the cells switch to energy conservation. The fish exposed to carbendazim and cold shock also demonstrated a predictable blood glucose pattern. Blood glucose levels were high on the second day of exposure to carbendazim, indicating an acute stress response to the xenobiotic. The blood glucose levels were normal on the 10th day, indicating that the initial stress response had subsided. However, on day 15th, exposure to cold shock again elevated blood glucose in fish, irrespective of their prior carbendazim exposure. It is apparent that cold shock could have triggered another round of gluconeogenesis and hyperglycemia in fish. While research, specifically on carbendazim and fish blood sugar is limited, fungicides like

tebuconazole are known invoke hyperglycemic response by catabolizing proteins and channeling energy away from non-critical anabolic pathways [8, 70].

During stress, fish prioritize energy production to cope with the challenge, which often involves mobilizing proteins, breaking them down to generate glucose for energy. Maintenance of energy levels to meet demands of stress response is also met by reducing the allocation of energy to de novo protein synthesis [73]. This explains the lower total serum protein levels, both albumin and globulin, observed in all treatment groups except for the acute cold shock group [74]. Thus, long-term exposure to carbendazim induces a systemic effect on energy metabolism, which impairs cold shock response in fish.

5 Conclusion

This study significantly advances the understanding of carbendazim toxicity in rohu juveniles, addressing critical knowledge gaps regarding its mechanistic effects, particularly in relation to cold shock response—a topic that has been largely overlooked in existing literature. The establishment of specific threshold toxicity values and the determination of the 96-h LC_{50} for this native species provide essential data that can inform local environmental policies and guidelines, underscoring the importance of region-specific research. The findings reveal that rohu exhibits heightened sensitivity to carbendazim compared to many other fish species, with acute exposure leading to anxiety-like behaviour and disrupted social interactions, thereby posing risks at the population level. Furthermore, chronic exposure alongside cold shock resulted in hyperglycaemia, indicative of metabolic depression or exhaustion, which compromises functions necessary for individual and population fitness in both natural habitats and under captivity. The novel documentation of anxiogenic and behaviour-disrupting effects of carbendazim, along with the haematological impacts observed in this study, contributes valuable insights into the potential environmental risks associated with pesticide pollution in aquatic ecosystems. By exploring the combined effects of cold shock and carbendazim exposure on fish physiology and behaviour, this research highlights a previously unaddressed aspect of pesticide toxicity in the context of changing environmental conditions, paving the way for future studies aimed at mitigating the impacts of agricultural chemicals on aquatic life.

Though there are evidence of carbendazim reaching 0.2–0.5 mg L^{-1} concentrations in agricultural runoff, the authors are aware that such high concentrations are rare. This experimental study demonstrates that the fungicides, like carbendazim, can induce non-target responses in fish at both lethal and sub-lethal concentrations. The anxiogenic and branchial effects of carbendazim observed herein necessitate further investigation into their mechanistic underpinnings. The effects of environmentally relevant concentrations on vital reproductive behaviour, such as courtship and nesting should also be studied to understand the ecological impact of fungicides, that may otherwise be considered safe for fish and other aquatic organisms.

Acknowledgements All the experiments were approved by the IRB of RPCAU, Pusa and conducted in accordance with guidelines prescribed by the institution and the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA), Government of India. Authors declare no conflict of interest. The research was funded by the institutional grant of RPCAU, Pusa.

Author contributions M.K.: Investigation, Formal Analysis, Data Curation, Writing—Original Draft, Writing—Review and Editing. A.J.: Conceptualization, Methodology, Software, Formal Analysis, Resources, Writing—Original Draft, Writing—Review and Editing, Visualization, Supervision. Manuscript revision. S.K.N.: Methodology, Supervision. P.K.: Supervision, Writing—Review and Editing. All authors reviewed the manuscript.

Data availability The data that supports the findings of this study are not openly available due to institutional policies. However, the data can be made available by the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds

the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

References

1. FAO. Statistical Yearbook World Food and Agriculture 2023 [Internet]. 2023. Available from: https://openknowledge.fao.org/server/api/core/bitstreams/28cfd24e-81a9-4ebc-b2b5-4095fe5b1dab/content/cc8166en.html#chapter-2_3
2. Banaee M, Sagvand S, Sureda A, Amini M, Haghi BN, Sopjani M, Faggio C. Evaluation of single and combined effects of mancozeb and metalaxyl on the transcriptional and biochemical response of zebrafish (*Danio rerio*). Comp Biochem Physiol Part C Toxicol Pharmacol. 2023;268: 109597.
3. Gan W, Zhang R, Cao Z, Liu H, Fan W, Sun A, Song S, Zhang Z, Shi X. Unveiling the hidden risks: pesticide residues in aquaculture systems. Sci Total Environ. 2024;929: 172388.
4. Storck TR, do Amaral AMB, da Cruz TKT, Schneider SI, Cerezer FO, de Oliveira JA, Zanella R, Prestes OD, Giacomini SJ, Loro VL, Golombieski JI, Clasen B. Anthropogenic actions alter the aquatic environment quality: biomonitoring study of a river in Southern Brazil. Aquat Ecol. 2024;59;185–202. https://doi.org/10.1007/s10452-024-10156-9
5. Wei Y, Meng Y, Jia K, Lu W, Huang Y, Lu H. Dimethomorph induces heart and vascular developmental defects by disrupting thyroid hormone in zebrafish embryos. Ecotoxicol Environ Saf. 2025;289: 117413.
6. Shen C, Ding X, Rao W, Hu J, Lin T, Zhou XZ, Zheng Y, Dong F, Fan G. Prediction of potential risk for ten azole and benzimidazole fungicides with the aryl hydrocarbon receptor agonistic activity to aquatic ecosystems. J Agric Food Chem. 2025;73(2):1167–81.
7. Kumar S, Jamwal A. Climate Change and Stress Response in Teleost. In: Sinha A, Kumar S, Kumari K, editors. Outlook of Climate Change and Fish Nutrition. Singapore: Springer Nature; 2022; p. 171–8. https://doi.org/10.1007/978-981-19-5500-6_13
8. Toni M, Arena C, Cioni C, Tedeschi G. Temperature- and chemical-induced neurotoxicity in zebrafish. Front Physiol. 2023;14:1276941.
9. Verheyen J, Stoks R. Thermal performance curves in a polluted world: too cold and too hot temperatures synergistically increase pesticide toxicity. Environ Sci Technol. 2023;57(8):3270–9.
10. de Souza SS, Bruce KHR, da Costa JC, Pereira D, da Silva GS, Val AL. Effects of climate change and mixtures of pesticides on the Amazonian fish *Colossoma macropomum*. Sci Total Environ. 2024;922: 171379.
11. Carazo-Rojas E, Pérez-Rojas G, Pérez-Villanueva M, Chinchilla-Soto C, Chin-Pampillo JS, Aguilar-Mora P, Alpízar-Marín M, Masís-Mora M, Rodríguez-Rodríguez CE, Vryzas Z. Pesticide monitoring and ecotoxicological risk assessment in surface water bodies and sediments of a tropical agro-ecosystem. Environ Pollut. 2018;241:800–9.
12. de Souza RM, Seibert D, Quesada HB, de Jesus BF, Fagundes-Klen MR, Bergamasco R. Occurrence, impacts and general aspects of pesticides in surface water: a review. Process Saf Environ Prot. 2020;135:22–37.
13. Zhou T, Guo T, Wang Y, Wang A, Zhang M. Carbendazim: ecological risks, toxicities, degradation pathways and potential risks to human health. Chemosphere. 2023;314: 137723.
14. FAOSTAT. Pesticide Use [Internet]. 2023. Available from: https://www.fao.org/faostat/en/#data/RP
15. DPPQS. Pesticide wise consumption of Indigenous Pesticides During 2017–18 to 2021–22. Statistical Database. 2023. Available from: https://ppqs.gov.in/statistical-database
16. WHO. Environmental Health Criteria 149. Carbendazim. World Health Organization; 1993; Report No.: 149. Available from: https://apps.who.int/iris/bitstream/handle/10665/37443/9241571497-eng.pdf?sequence=1
17. Readman JW, Albanis TA, Barcelo D, Galassi S, Tronczynski J, Gabrielides GP. Fungicide contamination of Mediterranean estuarine waters: results from a MED POL pilot survey. Mar Pollut Bull. 1997;34(4):259–63.
18. Liu WR, Yang YY, Liu YS, Zhao JL, Zhang QQ, Yao L, Zhang M, Jiang YX, Wei XD, Ying GG. Biocides in the river system of a highly urbanized region: a systematic investigation involving runoff input. Sci Total Environ. 2018;624:1023–30.
19. Acayaba RD, de Albuquerque AF, Ribessi RL, de Umbuzeiro GA, Montagner CC. Occurrence of pesticides in waters from the largest sugar cane plantation region in the world. Environ Sci Pollut Res. 2021;28(8):9824–35.
20. Tokatlı C, Köse E, Çiçek A, Emiroğlu Ö. Pesticide accumulation in Turkey's Meriç river Basinwater and sediment. Pol J Environ Stud. 2020;29(1):1003–8.
21. Rice PJ, Rice PJ, Arthur EL, Barefoot AC. Advances in pesticide environmental fate and exposure assessments. J Agric Food Chem. 2007;55(14):5367–76.
22. Mauro AA, Ghalambor CK. Trade-offs, pleiotropy, and shared molecular pathways: a unified view of constraints on adaptation. Integr Comp Biol. 2020;60(2):332–47.
23. Jiang J, Wu S, Wu C, An X, Cai L, Zhao X. Embryonic exposure to carbendazim induces the transcription of genes related to apoptosis, immunotoxicity and endocrine disruption in zebrafish (*Danio rerio*). Fish Shellfish Immunol. 2014;41(2):493–500.
24. Bao Z, Zhao Y, Wu A, Lou Z, Lu H, Yu Q, Fu Z, Jin Y. Sub-chronic carbendazim exposure induces hepatic glycolipid metabolism disorder accompanied by gut microbiota dysbiosis in adult zebrafish (*Daino rerio*). Sci Total Environ. 2020;739: 140081.
25. Fan R, Zhang W, Li L, Jia L, Zhao J, Zhao Z, Peng S, Yuan X, Chen Y. Individual and synergistic toxic effects of carbendazim and chlorpyrifos on zebrafish embryonic development. Chemosphere. 2021;280: 130769.
26. Zhang W, Fan R, Luo S, Ying L, Jin Y, Li Y, Xiong M, Yuan X, Jia L, Chen Y. Antagonistic effects and mechanisms of carbendazim and chlorpyrifos on the neurobehavior of larval zebrafish. Chemosphere. 2022;293: 133522.
27. Hurst TP. Causes and consequences of winter mortality in fishes. J Fish Biol. 2007;71(2):315–45.
28. Gao X, Wang X, Wang X, Li H, Xu L, Fang Y, Cao S, Huang B, Chen H, Xing R, Liu B. Effect of winter feeding frequency on growth performance, biochemical blood parameters, oxidative stress, and appetite-related genes in *Takifugu rubripes*. Fish Physiol Biochem. 2022;48(5):1167–81.
29. Reid CH, Patrick PH, Rytwinski T, Taylor JJ, Willmore WG, Reesor B, Cooke SJ. An updated review of cold shock and cold stress in fish. J Fish Biol. 2022;100(5):1102–37.

30. Schleger IC, Pereira DMC, Resende AC, Romão S, Herrerias T, Neundorf AKA, Sloty AM, Guimarães IM, de Souza MRDP, Carster GP, Donatti L. Cold and warm waters: energy metabolism and antioxidant defenses of the freshwater fish *Astyanax lacustris* (Characiformes: Characidae) under thermal stress. J Comp Physiol B. 2022;192(1):77–94.
31. Yousefi M, Adineh H, Reverter M, Khademi Hamidi M, Vatnikov YA, Kulikov EV, Hoseinifar SH, Van Doan H. Protective effects of black seed (*Nigella sativa*) diet supplementation in common carp (*Cyprinus carpio*) against immune depression, oxidative stress and metabolism dysfunction induced by glyphosate. Fish Shellfish Immunol. 2021;109:12–9.
32. FAO. The State of World Fisheries and Aquaculture 2024–Blue Transformation in action. Rome, Italy: Food and Agricultural Organization; 2024. Available from: https://doi.org/10.4060/cc0461en
33. Whitehead RD Jr, Mei Z, Mapango C, Jefferds MED. Methods and analyzers for hemoglobin measurement in clinical laboratories and field settings. Ann NY Acad Sci. 2019;1450(1):147–71.
34. McGovern JJ, Jones AR, Steinberg AG. The hematocrit of capillary blood. N Engl J Med. 1955;253(8):308–12.
35. R Core Team. R: A Language and Environment for Statistical Computing [Internet]. R Foundation for Statistical Computing, Vienna, Austria; 2023.
36. Finney DJ. Probit analysis; a statistical treatment of the sigmoid response curve. Camb Univ Press. 1947.
37. Gama J. Ecotoxicology: methods for ecotoxicology. 2015. Available from: https://cran.r-project.org/web/packages/ecotoxicology/index.html
38. Rico A, Waichman AV, Geber-Corrêa R, van den Brink PJ. Effects of malathion and carbendazim on Amazonian freshwater organisms: comparison of tropical and temperate species sensitivity distributions. Ecotoxicology. 2011;20(4):625–34.
39. Hu G, Wang H, Wan Y, Zhou L, Wang Q, Wang M. Combined toxicities of cadmium and five agrochemicals to the larval zebrafish (*Danio rerio*). Sci Rep. 2022;12(1):16045.
40. Nwani CD, Omah MC, Ivoke N, Nwamba HO, Ani C, Ogbonna SU. Biochemical, haematological and morphological variations in juvenile *Clarias gariepinus* exposed to Carbendazim® fungicide. Afr J Aquat Sci. 2015;40(1):63–71.
41. Palawski DU, Knowles CO. Toxicological studies of benomyl and carbendazim in rainbow trout, channel catfish and bluegills. Environ Toxicol Chem. 1986;5(12):1039–46.
42. Palanikumar L, Kumaraguru AK, Ramakritinan CM, Anand M. Toxicity, biochemical and clastogenic response of chlorpyrifos and carbendazim in milkfish *Chanos chanos*. Int J Environ Sci Technol. 2014;11(3):765–74.
43. Weber D, Spieler R. Behavioral Mechanisms of Metal Toxicity in Fishes. In: Aquatic Toxicology: Molecular, Biochemical and Cellular Perspectives. CRC Press; 1994. p. 48.
44. Scott GR, Sloman KA. The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. Aquat Toxicol. 2004;68(4):369–92.
45. Salahinejad A, Meuthen D, Attaran A, Niyogi S, Chivers DP, Ferrari MCO. Maternal exposure to bisphenol S reduces anxiety and impairs collective antipredator behavior of male zebrafish (*Danio rerio*) offspring through dysregulation of their serotonergic system. Aquat Toxicol. 2024;267: 106800.
46. Qiu X, Nomichi S, Chen K, Honda M, Kang IJ, Shimasaki Y, Oshima Y. Short-term and persistent impacts on behaviors related to locomotion, anxiety, and startle responses of Japanese medaka (*Oryzias latipes*) induced by acute, sublethal exposure to chlorpyrifos. Aquat Toxicol. 2017;192:148–54.
47. Liu S, Yu M, Xie X, Ru Y, Ru S. Carbofuran induces increased anxiety-like behaviors in female zebrafish (*Danio rerio*) through disturbing dopaminergic/norepinephrinergic system. Chemosphere. 2020;253: 126635.
48. Thoré ESJ, Van Hooreweghe F, Philippe C, Brendonck L, Pinceel T. Generation-specific and interactive effects of pesticide and antidepressant exposure in a fish model call for multi-stressor and multigenerational testing. Aquat Toxicol. 2021;232: 105743.
49. de Oliveira MRB, Profeta IV, Saraiva Raimondi Lopes JV, Costa RM, Matos e Chaib VR, Domingues AG, Beirão MV, Santos Rubio KT, Martucci MEP, Eskinazi-Sant'Anna EM, de Azevedo CS. Effects of the fungicide carbendazim on the behaviour of the zebrafish *Danio rerio* (Cypriniformes, Cyprinidae). Acta Ethologica. 2024;27(2):101–11.
50. Bouly L, Jacquin L, Chapeau F, Bonmatin JM, Cousseau M, Hagimont A, Laffaille P, Lalot B, Lemarié A, Pasquet C, Huc L, Jean S. Fluopyram SDHI pesticide alters fish physiology and behaviour despite low *in vitro* effects on mitochondria. Ecotoxicol Environ Saf. 2024;288: 117400.
51. Dar OI, Sharma S, Singh K, Sharma A, Bhardwaj R, Kaur A. Biomarkers for the toxicity of sublethal concentrations of triclosan to the early life stages of carps. Sci Rep. 2020;10(1):17322.
52. Banaee M, Zeidi A, Haghi BN, Beitsayah A. The toxicity effects of imidacloprid and chlorpyrifos on oxidative stress and blood biochemistry in *Cyprinus carpio*. Comp Biochem Physiol Part C Toxicol Pharmacol. 2024;284: 109979.
53. Olivares-Rubio HF, Arce E. Effects of chemical pollution on the behaviour of cichlid fish. Environ Biol Fishes. 2023;106(5):1149–76.
54. Jiao W, Han Q, Xu Y, Jiang H, Xing H, Teng X. Impaired immune function and structural integrity in the gills of common carp (*Cyprinus carpio* L.) caused by chlorpyrifos exposure: through oxidative stress and apoptosis. Fish Shellfish Immunol. 2019;86:239–45.
55. Ogbeide O, Uhunamure G, Uwagboe L, Osakpamwan T, Glory M, Chukwuka A. Comparative gill and liver pathology of *Tilapia zilli*, *Clarias gariepinus* and *Neochanna diversus* in owan river (Nigeria): relative ecological risks of species in a pesticide-impacted river. Chemosphere. 2019;234:1–13.
56. Roberts RJ, Rodgers HD. The Pathophysiology and Systematic Pathology of Teleosts. In: Fish Pathology. 4th ed. 2023.
57. Cho K, Ryu CS, Jeong S, Kim Y. Potential adverse effect of tyrosinase inhibitors on teleosts: a review. Comp Biochem Physiol Part C Toxicol Pharmacol. 2020;228: 108655.
58. Fu W, Wu Z, Zheng R, Yin N, Han F, Zhao Z, Dai M, Han D, Wang W, Niu L. Inhibition mechanism of melanin formation based on antioxidant scavenging of reactive oxygen species. Analyst. 2022;147(12):2703–11.
59. Jiang J, Wu S, Wang Y, An X, Cai L, Zhao X, Wu C. Carbendazim has the potential to induce oxidative stress, apoptosis, immunotoxicity and endocrine disruption during zebrafish larvae development. Toxicol In Vitro. 2015;29(7):1473–81.
60. Ezeoyili IC, Mgbenka BO, Atama CI, Ngwu GI, Madu JC, Nwani CD. Changes in brain acetylcholinesterase and oxidative stress biomarkers in African catfish exposed to carbendazim. J Aquat Anim Health. 2019;31(4):371–9.
61. Austin B. The effects of pollution on fish health. J Appl Microbiol. 1998;85(S1):234S-242S.

62. Ibrahim RE, Elbealy MA, Salem GA, Abdelwarith AA, Younis EM, Wagih E, Elkady AA, Davies SJ, Rahman ANA. Acute mancozeb-fungicide exposure induces neuro-ethology disruption, health disorders, and immune-oxidative dysfunction in Nile tilapia (*Oreochromis niloticus*). Aquat Toxicol. 2023;261: 106630.
63. Shi J, Zhao M, Li K, Zhao Y, Li W, Peng Y, Zheng J. Metabolic activation and cytotoxicity of fungicide carbendazim mediated by CYP1A2. J Agric Food Chem. 2022;70(13):4092–101.
64. Costa RM, Matos e Chaib VR, Domingues AG, Rubio KTS, Martucci MEP. Untargeted metabolomics reveals lipid impairment in the liver of adult Zebrafish (*Danio rerio*) exposed to carbendazim. Environ Toxicol Chem. 2023;42(2):437–48.
65. Wei Y, Wang L, Liu J. The diabetogenic effects of pesticides: evidence based on epidemiological and toxicological studies. Environ Pollut. 2023;331: 121927.
66. Li HT, Feng L, Jiang WD, Liu Y, Jiang J, Li SH, Zhou XQ. Oxidative stress parameters and anti-apoptotic response to hydroxyl radicals in fish erythrocytes: protective effects of glutamine, alanine, citrulline and proline. Aquat Toxicol. 2013;126:169–79.
67. Junaid M, Abbas Z, Siddiqui JA, Liu S, Tabraiz S, Yue Q, Wang J. Ecotoxicological impacts associated with the interplay between micro(nano) plastics and pesticides in aquatic and terrestrial environments. TrAC Trends Anal Chem. 2023;165: 117133.
68. Melefa TD, Mgbenka BO, Aguzie IO, Andong FA, Nwakor U, Nwani CD. Morphological, haematological and biochemical changes in African catfish *Clarias gariepinus* (Burchell 1822) juveniles exposed to clotrimazole. Comp Biochem Physiol Part C Toxicol Pharmacol. 2020;236: 108815.
69. Guo H, Dixon B. Understanding acute stress-mediated immunity in teleost fish. Fish Shellfish Immunol Rep. 2021;2: 100010.
70. Bonga SEW. The stress response in fish. Physiol Rev. 1997. https://doi.org/10.1152/physrev.1997.77.3.591.
71. Dellagostin EN, Martins AWS, Blödorn EB, R. Silveira TL, Komninou ER, Varela Junior AS, Corcini CD, Nunes LS, Remião MH, Collares GL, Domingues WB, Giongo JL, Vaucher RA, Campos VF. Chronic cold exposure modulates genes related to feeding and immune system in Nile tilapia (*Oreochromis niloticus*). Fish Shellfish Immunol. 2022;128:269–78.
72. Barton BA. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids1. Integr Comp Biol. 2002;42(3):517–25.
73. Zhou Q, Hao B, Cao X, Gao L, Yu Z, Zhao Y, Zhu M, Zhong G, Chi F, Dai X, Mao J, Zhu Y, Rong P, Chen L, Bai X, Ye C, Chen S, Liang T, Li L, Feng XH, Tan M, Zhao B. Energy sensor AMPK gamma regulates translation via phosphatase PPP6C independent of AMPK alpha. Mol Cell. 2022;82(24):4700-4711.e12.
74. Atli G, Ariyurek SY, Kanak EG, Canli M. Alterations in the serum biomarkers belonging to different metabolic systems of fish (*Oreochromis niloticus*) after Cd and Pb exposures. Environ Toxicol Pharmacol. 2015;40(2):508–15.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.