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Biochemical Responses of Ornamental Fish to Oxidative Stress

SRI SAIPRAJWAL AND BELA ZUTSHI*
Department of Zoology, Fish Biology Section, Bangalore University, Bengaluru, India
E-mail: srisaiprajwal1919@gmail.com, bela_zutshi@yahoo.co.in
***Corresponding author**
ORCID: 0000-0003-1879-3398 (BZ)

ABSTRACT
During short-term or long-term transportation, ornamental fish have stress-related effects due to their exposure to degrading water quality levels, e.g., pH (acidic or alkaline), oxygen, ammonia, temperature levels, etc., and captivity in the container. The present study estimated the biochemical parameters, such as lipid peroxidation (LPO) and antioxidant enzymatic activities (SOD-superoxide dismutase, CAT-catalase, GST-glutathione-s-transferase) during transportation and exposure to pH shift response in liver and muscle of three families of ornamental fish such as black wagtail platy, rosy barb and lemon-yellow cichlid during and exposure to pH 5 and 10. 100% survivability was noted among three fish species, and oxidative stress was marked by increased LPO levels in all fish transported and exposed to pH 5 and pH 10. Exposure of Rosy Barb to pH 10 and platy and cichlid to pH 5 induced a significant increase in LPO in liver tissue compared to all transported fish, whereas the muscle tissue of platy and cichlid showed increased activities of LPO during transportation compared to exposed ones, and a control group of fish. Significantly elevated levels of SOD activity in both tissues of all experimental fishes, whereas CAT activity was more in the liver tissue of transported fishes to counteract stress response and detoxify products of lipid peroxidation. Therefore, understanding the variation in stress levels of ornamental fishes during transportation and exposure to pH levels, which are tissue- and species-specific, becomes critically important for their welfare in aquaculture practices, as observed in this study.

Key words: Ornamental fish, Oxidative stress, Lipid peroxidation, Superoxide dismutase, Catalase, Glutathione-s-transferase

INTRODUCTION

Transportation of fish, although a global necessity, is known to cause acute and/or chronic stress due to duration and distance covered between the source of initial travel and their final destination, causing changes in the water quality of the container consisting of different types of fish species crowded together in different types of containers. Transportation of ornamental fishes is an essential practice in aquaculture, but for a successful method of transportation, the aqua culturist has to understand many technical issues. Fish welfare during transportation is rapidly gaining importance, and many scientists have identified conditions to be fulfilled regarding the welfare criteria of fish to reduce stress, suffering, and pain during transportation. The data available in the literature regarding the transport of live fish included scientific information about 8 hr or less (short transport) and more than 8 hr duration (long transport) (Stieglitz et al. 2012). According to Davis (2006), the effects of duration of transport stress can be categorized into acute (short-term) or chronic (long-term). Reviews by Harmon (2009) focused on stress associated with alteration in water quality during transportation.

In water chemistry, pH is an intensity factor, whereas acidity or alkalinity of water is a capacity factor, which is defined based on the carbonate system (Stumm and Morgan 1996). CO_2 causes acidity, while bicarbonate and carbonate cause alkalinity of water (Boyd 2000). The reaction of water molecules with ammonia released by fish resulted in the formation of ammonium (NH_4^+) and hydroxyl ions (OH^-) ions into the water, which further reacted with CO_2 to produce HCO_3^- (bicarbonate) that in turn increased water alkalinity (Boyd 1990). Water with a pH less than 4.5 does not have alkalinity, while a pH greater than 8.5 does not have acidity. The transported fish are exposed to fluctuations in water quality when repacked from different sources by the stakeholders from the time in the wild or aquaculture farm to their final destination of aquarist vendors or domestic aquarium. Lim et al. (2003) reported that ornamental fish are transported for long distances, mainly in plastic sealed bags in high

densities causing damage to fish health or mortality (Braun and Nuner 2014). Sampaio and Freire (2016) observed that important factors of water, viz., pH, dissolved oxygen, temperature and ammonia should be monitored while studying the simulated commercially transported fish to understand their effect on physiology of the fish in question (Paterson et al. 2003, Manuel et al. 2014).

pH exposure can be the forerunner to oxidative stress in fish species was confirmed by a few scientists, e.g., Gilmour and Perry (1994) checked the pH shift and acid-base equilibrium from the physiological regulation perspective, Halliwell and Gutteridge (1999) studied the oxidative damage during pH shift in liver and kidney tissue and Fenner (2001) considered pH above or below 1.5points to have a negative effect beyond a time. Das et al. (2006) studied the influence of alteration in environmental (water) conditions concerning pH levels during transportation, which influences their welfare since they cannot maintain acid-base and ion regulation. Hence, altered pH levels were considered a common stressor among the potential stressors during fish transportation due to its denaturing effect on cellular membranes (EIFAC 1971). Previous studies by Sies (1985) on suboptimal pH or salinity exposed fishes showed enhancement in free radical production, which resulted in oxidative damage. Winston and Giulio (1991) observed the presence of low and high molecular weight antioxidant defenses such as reduced glutathione (GSH) and superoxide dismutase (SOD), catalase CAT, glutathione-s-transferase (GST) to scavenge free radical elements in stressed fish. Such conditions were exhibited by evaluating LPO levels through MDA values. Later Hermes-Lima (2004), Husak et al. (2014), and Moniruzzaman et al. (2017) proposed that an anti-oxidant system in fish either prevented or counterbalanced the elevated free radical (ROS) by triggering the release of antioxidant enzymes such as SOD, CAT, and enzymes related with GPx since they acted in a synchronised manner for protection against oxidative stress. Bagnyukova et al. (2006) reported that different stressors induced external stress in goldfish, stimulating variable patterns of antioxidant enzyme activities in the liver and kidney. The liver, a metabolically active organ, regulates homeostasis by breaking down metabolites and toxic elements to maintain natural body physiology. Thus, it is the preferred organ to assess oxidative stress status in aquatic organisms.

The energy demand of transported fish is also compromised with variable metabolic responses, causing changes in plasma glucose levels while combating oxidative stress (van der Boon et al. 1991). The antioxidant defence systems play a role in the maintenance of the physiology of cells and tissues (Mourente et al. 2002) but, in turn, get affected due to pH stress during transportation that disrupts the removal of ROS, resulting in tissue dysfunction (Mukherjee et al. 2017a, b). Suggestions of Bagnyukova et al. (2006) regarding obscure knowledge about the effect of pH shift processes on free radical mechanism led to assumptions that a rise in lipid oxidation resulted due to a shift of pH from 8.25 (control) to 8.67 (limestone water) and antioxidant enzymes response. The information regarding the protocol for transporting live ornamental fish is incipient. There are lacunae in the literature about the ornamental fish response to physiological stress due to alterations in water quality and the interaction of these factors. Thus, it becomes highly relevant for studying environmental stress on transported ornamental fishes. Eyckmans et al. (2011) reported that variation in antioxidant stimulation between fish species depends on their flexibility to combat stress causing oxidative damage. Counterbalancing the response of enzymatic antioxidants to oxidative stress might vary among fish species with different tolerance limits to water quality and pH alteration. Interpretation of such variation may support the identification of essential mechanisms involved in the sensitivity of fishes to different pH values. Due to lacuna in the field, the present study was focused on explicating oxidative stress and its complex effects on the antioxidant status of vital organs, viz., liver and muscle of three commercially important ornamental fish species, black wagtail platy (live bearer) and rosy barb and lemon-yellow cichlid (egg layers) that differed in their sensitivity towards pH. These fishes are mildly tolerant and generally survive a small range of stressful conditions. For example, cichlid can survive prolonged exposure (48 hr) to clove oil (Kaiser et al. 2006), and platy survived temperature alterations from 22 to 28°C (Singh and Zutshi 2020).

These attractive ornamental fish models were used to analyze oxidative stress when exposed to pH 5 and pH 10 points in lab conditions and those of transported fish species for 6 hrs from the source to their destination in containers with altered water quality. Biochemical parameters, viz., LPO and antioxidant enzymes (SOD, CAT and GST) were assessed as an endpoint to measure oxidative stress due to pH shift.

MATERIAL AND METHODS

Fish sampling and maintenance

A total of 75 ornamental fish, black wagtail platy (*Xiphophorus maculatus*), rosy barb (*Pethia conchonius*), and lemon-yellow cichlid (*Labidochromis caeruleus*) belonging to family Poecillid, Cyprinid and Cichlid, respectively, were collected from Ornamental Fish farm, Hessarghatta, Bangalore District. Live and healthy fishes (15 individuals per bag) were brought to the laboratory in 5 polythene airtight bags, half-filled with oxygenated water, and quarantined in 0.1% potassium permanganate solution. The experimental fish group was acclimatized for a week in a pre-washed, dried, and disinfected fiberglass aquarium filled with well-aerated tap water and fed with standard commercial ornamental fish food ("Taiyo Staple" by Taiyo Feed Mill Pvt. Ltd.) for platy fish, ("Hikari Micro pellets" by Kyorin Food Ind. Ltd., Japan) for rosy barb and ("Optimum Cichlid" by Perfect Companion Group Co. Ltd.,) for cichlid fish. Fishes were not fed two days before experimentation to allow the gut to be emptied and stabilize nitrogenous waste excretion. 10-15% of the water was siphoned off along with fecal matter and replaced with fresh dechlorinated tap water every alternate day. The transported fish were collected immediately from the vendors in Bangalore and transported from the primary source in Chennai for 6-8 hrs by road.

Experimental protocol

The experimental setup consisted of exposing each of the three species of fish to two different pH standardized by conducting LC_{50} for all fish species and fixed at pH 5 (pH 4.5-5.5) and pH 10 (9.5-10.5) for a period of 6 hrs intermittently for four days. The experimental and control treatment fish were reared in a 6 L glass aquaria (water volume set to 4 L). Control groups of fish were kept in similar aquaria parallel to the period of experimental groups. The uniformly sized experimental fish (n = 5) viz., with an average mass (mean ± standard deviation) 1.2±0.2 g black wagtail platy (*Xiphophorus maculatus)*, 1.8±0.3 g rosy barb (*Pethia conchonius*) and 2.5±0.5 g lemon-yellow cichlid (*Labidochromis caeruleus*) in triplicate were placed in an individual glass aquarium in dechlorinated and aerated water with the temperature maintained at 26 ± 1æ%C and a natural light–dark cycle of about 12:12 hrs and with pH 7.0-7.5 in lab conditions. Dissolved oxygen, temperature, ammonia, total alkalinity, and total hardness were measured in transported water and experimental tanks (Table 1). The transported experimental fishes were collected from vendors and anesthetized immediately on-site for further procedures.

Procedure for analyses of enzymatic activity

Fish were removed from aquaria (n=5) with the help of a scoop net, anesthetized using neutralized MS222/few drops of clove oil (pH 8.0, ethyl 3-aminobenzoate methane-sulfonic acid, 1 g/L, Across Organics, Geel, Belgium). Fish was dissected on ice to excise liver and muscle tissue of the control and experimental group of fish species exposed to pH 5.0 and 10.0 for 96 hrs with intermittent exposure for 6 hrs. However, those transported fish were immediately excised, and an assay on oxidant and anti-oxidant enzymes was conducted by following the standard procedures. The liver and muscle tissue weighing 100 mg each, excised from both control and experimental fish species, were homogenised in potassium phosphate buffer at pH 7.0 and later centrifuged at 5000 rpm for 15 mins. The supernatant was collected for assay of LPO (lipid peroxidation/ malondialdehyde), superoxide dismutase (SOD), catalase (CAT), and glutathione-S-transferase (GST). Lipid peroxides (LPO) were determined using the Thiobarbituric Acid Reactive Substances (TBARS) method by Niehaus and Samuelson (1968). TBARS concentrations were determined from a standard curve established with TBA-malondialdehyde (MDA, 1,1,3,3-tetramethoxypropane) adducts. GST activity was determined spectrophotometrically by Habig et al. (1974) method, superoxide dismutase

(SOD) by Beauchamp and Fridovich (1971), and catalyse activity by Beers and Sizer (1952). The optical density of all the reaction mixtures was read at 560 nm.

Statistical analysis

The results were expressed as mean value ± S.E. Within species, no significant differences were observed among the control values. Thus, the control value was pooled for each experimental group. The Tukey–Bonferroni multiple comparison test was used to compare the means among the variables. All data were subjected to a two-way analysis of variance (ANOVA). The data were analyzed by Statistical Package for Graph Prism 5 to calculate probability level $p<0.001$, $p<0.01$, and $p<0.05$ was used for rejection of the null hypothesis.

RESULTS AND DISCUSSION

An organism undergoing stress responds by activating a corresponding protective mechanism to either maintain the previous status or attain a new stable state. Analysis of water parameters assessed from control tanks showed all were within BIS limits, whereas in experimental tanks (pH 5 and pH 10), there was a slight increase in temperature, ammonia, and alkalinity due to the pH variation from normal (Table 1). Many scientists have reported that aquatic organisms undergo oxidative stress due to changes in water parameters, such as pH, from a given normal range during transportation and other climatic conditions (Doudoroff and Katz 1950, Droge 2002). In the present study, it was observed that containers with ornamental fishes before transportation showed no changes as compared to control tanks. However, those after transportation revealed changes in pH from pH 7 to pH 4.5 and 10.5, temperatures ranging from 26 to 28°C in control conditions altered to a minimum of 16°C and maximum of 34°C during transportation. Dissolved oxygen (4-6 mg L^{-1} as control) recorded depletion to 2 mg L^{-1}, alkalinity increased from 128 to 145 mg $CaCO_3$ L^{-1}, and ammonia from 0.37 to 1.4, 1.45 (pH 5) and 1.38 (pH 10) (Table 1). Thus, transportation resulted in oxidative stress among all fish groups, and similarly, stress was noted in the experimental fish species exposed to pH 5 and pH 10. Sampaio and Freire (2016) mentioned that during transportation, increases in CO_2 levels were observed, which led to acidification and acidosis of water with progression in hypoxic conditions, including a rise in ammonia levels along with other secondary factors that contributed to alteration in parameters of water quality. The scientists have also noted that pH, DO, and ammonia are important during short and long transport of ornamental fish to evaluate physiological changes in the fish (Lim et al. 2003). Along similar lines, Treasurer (2012) reported that long transport caused a rise in pH and ammonia levels due to dissolved CO_2 produced by transported fish in aerobic and ammonia during anaerobic conditions, resulting in water acidification (Marshall and Grosell 2006). Doudoroff and Katz (1950) measured the tolerance limit of fish regarding pH. They reported that fishes can identify and avoid carbon dioxide when pH is 7.4 to about 5.5. However, their indifference to pH ranging between 5.5 and 10.5 can be considered the tolerance limit exhibited by most freshwater fishes. Similarly, the tolerance limit in the present experimental fish species (cichlid, rosy barb, and platy) when exposed to pH ranging from

Table 1. Physico-chemical parameters of water from control, experimental tanks and transported media

Parameters	BIS: 2012	Control tank	Transported media	Experimental tanks with	
				pH 5	pH 10
Temperature (°C)	24 -26	26	32	32	28
D.O (mg/l)	4.5-6.5	4.5	2.6	2.5	3.0
NH_3 (mg/l)	≥0.5	0.37	1.4	1.45	1.38
Total alkalinity (mg $CaCO_3L^{-1}$)	≥200	136	145	147	147
Total hardness (mg $CaCO_3L^{-1}$)	≥200	207	212	207	207
pH	6.5-8.5	7.2	5.5 - 9.5	5	10

4.5 to 7 (acidic) and pH 7 to 10.5 (alkaline) was pH 5 and 10.

In the present study, the behaviour of fish when transported was unavailable due to non-accessibility. However, Vanderzwalmen et al. (2021) reported that neon tetra, small-sized fish, showed gasping and crowding during transportation while the fishes were still in the bag, as observed in the recordings of transported fishes. These fishes showing gasping indicated low- -water quality (depletion in DO) (Kramer 1987), and fishes, when grouped (crowding), increased with stress due to the perception of threat among fishes swimming close together. All fish species exposed to both the extremes of pH, below pH4.5 and above Ph 10.5 (acidic and alkaline conditions), showed mortality within 1 hr of exposure due to intolerance of acute and lethal stress levels of pH. These fishes showed erratic jerking swimming movement, assuming a diagonal position with the head upwards towards the water surface to engulf atmospheric oxygen, indicating increased stress or distress conditions. The body was found to be covered with an abundant quantity of mucous; the gill epithelium showed mucous covering and discoloration with its subsequent destruction. Above pH 4.5 and below 10.5, the fishes showed normal behaviour. However, swimming was slightly rapid with the gill operculum's fast movement and a copious amount of mucus in initial exposure. Subsequently, the fishes adapted to pH 5 and pH 10, showing slow swimming and a regular beat of the operculum. Thus, the pH level for their sustainability and survivability was standardized to pH 5 and pH 10 as oxidative stress markers for the experiments conducted with an intermittent 6 hrs for 92 hrs.

Alteration in the fish environment during transportation was determined by using stress markers that challenged fish homeostasis. The physiological responses of fish were grouped into primary, secondary, and tertiary due to different stressors (Barton 2002, Iwama et al. 2006). Hermes-Lima (2004) suggested that occurrence of oxidative stress can be assessed by changes in levels of oxidative damage markers as products of protein and lipid peroxidation. Alterations in water quality sampling from fish transportation containers and those of pH-exposed fishes encouraged us to assess and identify the physiological changes in the liver and muscle tissue of fish transported (short and long transport) and exposed ones in question.

The outcome (mean ± SD) of physiological stress responses such as lipid peroxidation levels (LPO) that caused reactive oxygen species (ROS) production and activities of antioxidant enzymes due to pH shift in liver and muscle tissues of three fish species during transportation and those exposed to pH 5 and 10 are represented in Figures 1 to 4. The results revealed significantly high LPO levels, the biomarkers of oxidative stress ($P<0.001$) in liver and muscle tissue of all fish species compared to those of control ones. Increased levels of LPO (259 ±1.35) ($P<0.001$) noted in the liver tissue of rosy barb exposed to pH10 indicated it was under alkaline stress when compared to platy and cichlid fish (238.2±1.85; 237±1.45) as well as to those of transported and control ones (Fig. 1). Cichlid fish showed intolerance to acid stress since LPO levels (297.8±1.31) of liver tissue were significantly ($P<0.001$) high when the fish was exposed to pH5. Our results agree with Bagnyukova et al. (2006), who reported increased levels of lipid peroxidation products (TBARS and LOOH) in goldfish liver exposed to pH shift due to the addition of limestone water in rearing tanks.

Interestingly, during short-term transportation (6 hrs), LPO activity in rosy barb fish liver was significantly less (206±1.04) than platy and cichlid (211.2±1.75 and 228.5±1.63), respectively, which might be due to hypoxic condition as was also reported by Lushchak and Bagnyukova (2006) in common carp liver with reduction in LPO levels but an increase in TBARS, the end product of LPO under hypoxic condition. Similarly, Lushchak et al. (2005a) observed a decrease in LPO levels in goldfish during hypoxia with an effective detoxifying system to maintain cell integrity. The low levels of LPO in rosy barb liver during transportation could also be attributed to a decrease in water pH from 7.5 in the presence of intermediate ammonia levels during anaerobic metabolism, i.e., consumption of oxygen resulting in the production of CO_2 causing hypoxic condition as observed by Sampio and Freire (2016).

Muscle tissue showed significant LPO activity in response to pH stress in all fish species but was comparatively less than in liver tissue. Since liver

and muscle tissue are metabolically active in acid-base regulation for maintaining body physiology, they were expected to respond prominently to pH shifts in the present study. Both tissues of fish in control conditions projected comparatively negligible LPO levels. However, significant LPO activity ($P<0.001$) was noted in the muscle tissue of the transported platy and cichlid fish group (134.5±1.50 and 132.3±1.52). Those exposed to pH 5 (118.6±2.35 and 116.1±1.12) and pH 10 (127.1±1.45 and 119.6±0.90), respectively, followed by rosy barb transported (108.3±1.13) and exposed to pH 10 (124.7±1.35; $P<0.001$) compared to control ones. Elevated levels of LPO in muscle raised the fish metabolites or accelerated reactive oxygen species (ROS) production due to oxidative stress (Halliwell and Gutteridge 1999), assumed to be caused by crowding or erratic swimming activity in containers with a high fish density. Production of free radicals during pH shift by changing the superoxide state into superoxide anion (O_2^-) and hydroperoxyl radical (HO_2) that caused oxidative damage to cellular components due to differences in crossing the biological membrane was also reported. Urbinati et al. (2004) and Braun and Nuner (2014) also confirmed that the density of fish during transportation is essential because a more significant number of fish in one bag/container corresponds to cost economy for fish farmers. However, in turn, the crowding of fish caused stress or even mortality, thus compromising fish health that was not economically viable. Subsequently, on exposure of the three fish species (barb, platy, and cichlid) to acidic (pH 5) and alkaline water conditions (pH 10), a significant increase in LPO activity of their muscles was noted more so in pH 10 (124.7±1.35; 127.1±1.45 and 119.6±0.90) (Fig. 1**)** resulting in oxidative stress that caused disturbance in their behaviour of swimming activity and osmoregulation capacity. Our results on behaviour are in agreement with those of Zahangir et al. (2015), who reported an increase in erratic swimming movement, restlessness, fish lying at the tank bottom, mouth and gill opercula wide open with erect gill filaments in zebrafish when exposed to pH4 and below and pH11and above. This behaviour varied in intensity with the magnitude of the stimulus of pH. Kane et al. (2005) suggested that alteration in behaviour is an important tool to identify and determine the influence of exposure to environmental stress in ornamental fishes.

The anti-oxidants such as SOD, catalase and glutathione S transferase levels assessed along with LPO levels showed positive results in compensating stress levels. Although, the defensive approach of antioxidant mechanism in controlling ROS production in the liver of cichlid dealing with pH 5 and barb liver with pH 10 was less effective since the LPO levels remained high, despite activities of SOD (64.36±1.69 and 56.03±1.18) and CAT (34.84±1.01 and 41.49±0.84) followed by those of platy liver questions the efficiency of antioxidant enzymes. Thus, a significant increase in SOD activity

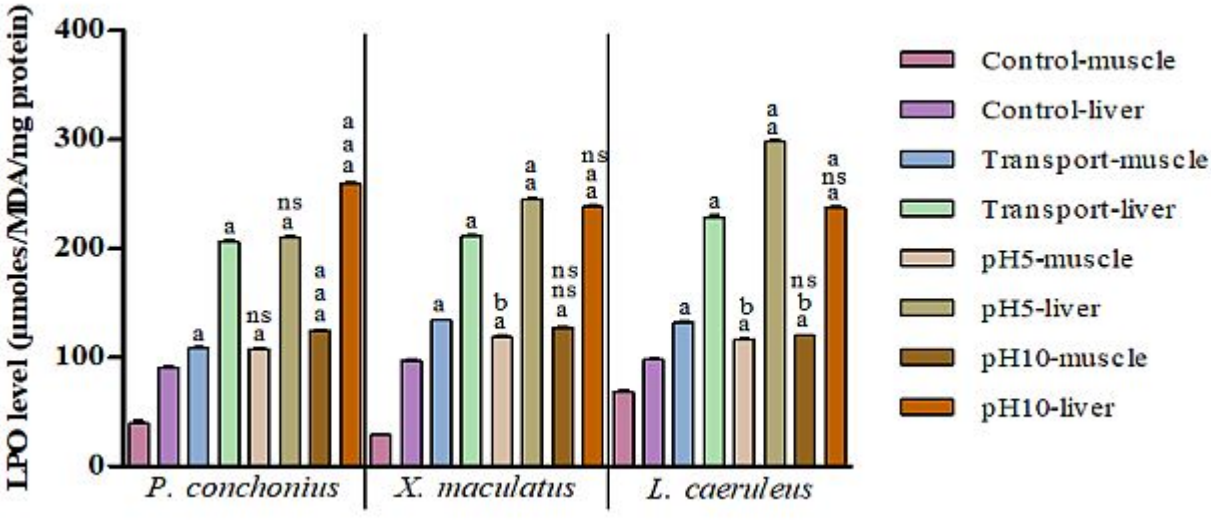

Figure 1. Lipid peroxidation level (µmoles/MDA/mg protein) in muscle and liver tissue of *P. conchonius, X. maculatus,* and *L. caeruleus* during transportation and exposure to pH 5 and pH 10

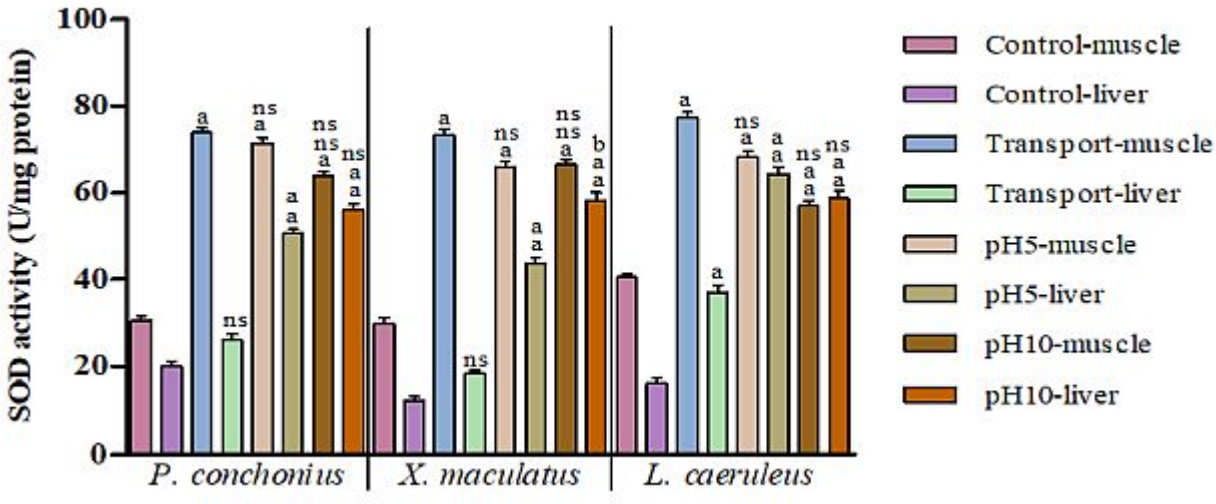

Figure 2. Superoxide dismutase content (U/mg protein) in muscle and liver tissue of *P. conchonius, X. maculatus,* and *L. caeruleus* during transportation and exposure to pH 5 and pH 10

($P<0.001$) in liver tissue of all the three fish species exposed to pH 5 and pH 10 compared to transported fish can be related to the augmented ROS generation since free radicals and ROS can damage fish livers through lipid peroxidation (LPO) (Lin et al. 2019) and to counteract an increase in their LPO activity. Surprisingly, SOD levels were insignificant in transported barb and platy compared to control ones, but a significant SOD activity was observed in cichlid fish, with the highest in those exposed to pH 5 **(**Fig. 2**)**.

Carneiro et al. (2021) reported an increase in SOD activity in sea horses exposed to acidic environments when in brackish water, but CAT activity remained unaffected when exposed to different pH. A significant decrease in catalase activity in the liver of freshwater fish *Oreochromis mossambicus* on exposure to the sublethal concentration of bisphenol-A was reported by Chitra and Maiby (2014), which is following the present study on exposure of fish species to pH5 and pH10 (Fig. 3). Previous studies have shown that decreased catalase activity might be due to its inactivation by overproduction of ROS (Pigeolet et al. 1990). Halliwell and Gutteridge (2015) also mentioned that insufficient removal of ROS might be due to an imbalance of SOD and CAT activities leading to the accumulation of LPO, causing oxidative stress. In contrast to the present result, Kim et al. (2021) found a significant reduction in SOD activity in the liver of *P. olivaceus* when exposed to acidic (pH 5 and 6) and alkaline (pH 9 and 10) water caused by excessive ROS generation as was also previously reported by Yu et al. (2020).

Low levels of SOD in the liver of transported cichlid (37.17±1.65) followed by barb (26.11±1.47) and platy (18.30±0.81) undergoing oxidative stress suggested that SOD, a first antioxidant defense to counter excessive ROS production, is not the only effective mechanism to regulate LPO process. Although SOD is the key enzyme that catalyzes H_2O_2 synthesis to reduce LPO levels, all fishes depend on catalase activity. H_2O_2 is a secondary by-product of spontaneous or enzymatic dismutation of O_2. CAT, an antioxidant enzyme, is involved in the destruction/ elimination of H_2O_2which is a by-product of the SOD activity (Sinha et al. 2014). Thus, we can say that cichlid liver used an up-regulation of CAT (63.41±1.47) as anti-oxidative sentinels with reduced SOD and GST levels to effectively remove ROS, limiting the accumulation of LPO (MDA) as was reported by Bagnyukova et al. (2005a) in goldfish liver showing positive correlation of CAT with LOOH levels. Lipid peroxidation level is the marker of oxidative damage to lipids and involves in inducing the release of antioxidant enzymes (Lushchak and Bagnyukova 2006c) to further supress LPO activity. Our results are in agreement with those of Sinha et al. (2014) in trout, carp, and goldfish in response to high environment ammonia and Chanu et al. (2014) in liver, muscle, and gill of *L. calbasu*

in response to acid stress possibly indicating the role of SOD and CAT in scavenging of superoxide anion.

The occurrence of significantly high LPO levels in the muscle of all fish species transported and pH exposed ones with rosy barb showing minimum LPO levels (in transported and pH 5 exposed), was stabilized by significant SOD activity that played an important role as antioxidants in removal or elimination of ROS production but with a minimum efficiency of CAT activity (Fig. 3). Acute exposure to acidic stress in an aquatic environment reduced fertility of flounder fish that resulted in decreased growth was reported by Fromm (1980). In contrast with the present result, limited activation of SOD was noted for rainbow trout in comparison to common carp and gibel carp when encountered water-borne copper (Eyckmans et al. 2011). Relatively to platy and cichlid, the muscle of rosy barb revealed an insignificant rise in LPO levels during transportation (108.3±1.13) and pH 5 (107.7±1.23) exposure, but it was significantly more than control (39.16 ± 2.49). Whereas, when exposed to pH 10, barb showed an increase in LPO activity of both tissue in response to oxidative stress by alkaline water which was well counteracted by significantly high SOD activity (65.25±1.30) compared to that of CAT (20.90±1.50). During transportation, it was likely due to stress from long crowding conditions. In contrast, in experimental ones, it was due to direct exposure to pH 5 and pH 10 that led to excessive ROS production. This rise in SOD levels against free radicals compared to low levels of CAT in barb, platy, and cichlid fish group' muscle during transportation and pH exposed ones and in the liver of all pH exposed fish indicated that all fish relied mainly on superoxide dismutase dependent defensive mechanism. The muscle tissue of all transported fishes and those exposed to pH 5 and pH 10 showed higher CAT activity when compared to the control, but it was insignificant when compared to that in liver tissue (Fig. 3). Such observations had been proved in previous studies on pH shift (acidic or alkaline) by Maqsood and Benjakul (2011) and alteration in catalase activity by Jin et al. (2010). Thus, a well-developed recovery system or an antioxidant defence mechanism in fish helped overcome stress and generate and degrade free radicals (Winston 1991). Tristan et al. (2021) suggested that hyperoxia/hypoxic environment during transportation could be the triggering cause for alteration in SOD activity or generation or removal of ROS due to oxidative stress. The findings of Qiang et al. (2017) and Refaey and Li (2018) on 6hrs of transportation stress in hybrid snapper and channel catfish, *Ictalurus punctatus* caused a significant increase in their SOD activity and MDA contents, respectively, are in line with the present study on transportation stress (6 hrs) inducing high LPO and SOD levels in fish. It is very likely that during pH stress, SOD was more active as an

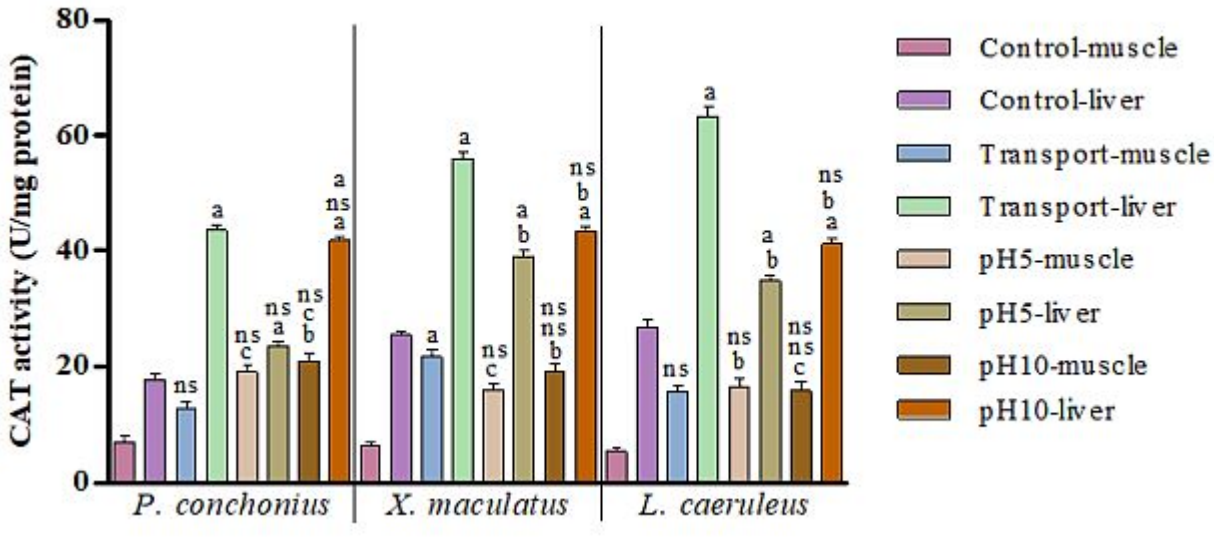

Figure 3. Catalase content (U/mg protein) in muscle and liver tissue of *P. conchonius, X. maculatus,* and *L. caeruleus* during transportation and exposure to pH 5 and pH 10

antioxidant defence in both liver and muscle tissue. However, catalase took the lead during transportation as an effective antioxidant in the liver of all fish groups. Therefore, the prominent role of SOD and CAT in scavenging H_2O_2 and superoxide anion with minimum GST levels in the liver and muscle tissue of all test fishes (Fig. 4) was also considered in the studies by Parihar et al. (1997). SOD and CAT activity exhibited a good correlation in the liver and muscle tissue of all fish species to counteract oxidative stress damage, i.e., reduced levels of SOD and GST in the liver of transported fish were compensated by enhanced activity of CAT. Hence, elevated catalase activity improved the defence and detoxification of LPO products in transported fish species. In contrast, enhanced SOD levels in acidic and alkaline exposed fish counteracted the reduced CAT and GST levels (Fig. 4). Scientific research for the protocol development of live fish transport is still budding and has incomplete knowledge.

The above results indicated a disparity in these fish species' anti-oxidative compensatory responses toward pH exposure. It can be anticipated that rosy barb followed by platy liver and muscle utilized the protective system moderately and showed more effective antioxidative compensatory responses throughout the experimental exposure and transportation period, while cichlid liver helped to stabilize the transportation stress more effectively than its muscle tissue for pH exposure. The present findings clarify that rosy barb had high resistance towards acid waters with pH 5 and above, whereas cichlid and platy could tolerate alkaline water with pH 10 and below. The results also revealed that the effect of antioxidants was dissimilar in different fish tissues and was species-specific during direct exposure to varied pH.

CONCLUSIONS

Elevated levels of CAT in transported fish compensated for the reduced activities of SOD and GST, whereas deficiency of CAT and GST activity in fish exposed to pH 5 and pH 10 due to its inhibition was counteracted by increased SOD levels which helped to detoxify aldehydic products of lipid peroxidation. GST activities were inversely related to LPO and SOD levels which proved the involvement of liver GST in the detoxification of aldehydic products of lipid peroxidation. Therefore, it can be concluded that variation in stress levels when exposed to elevated or lowered pH levels (acidic or alkaline condition) compared to control is fish species and tissue-dependent.

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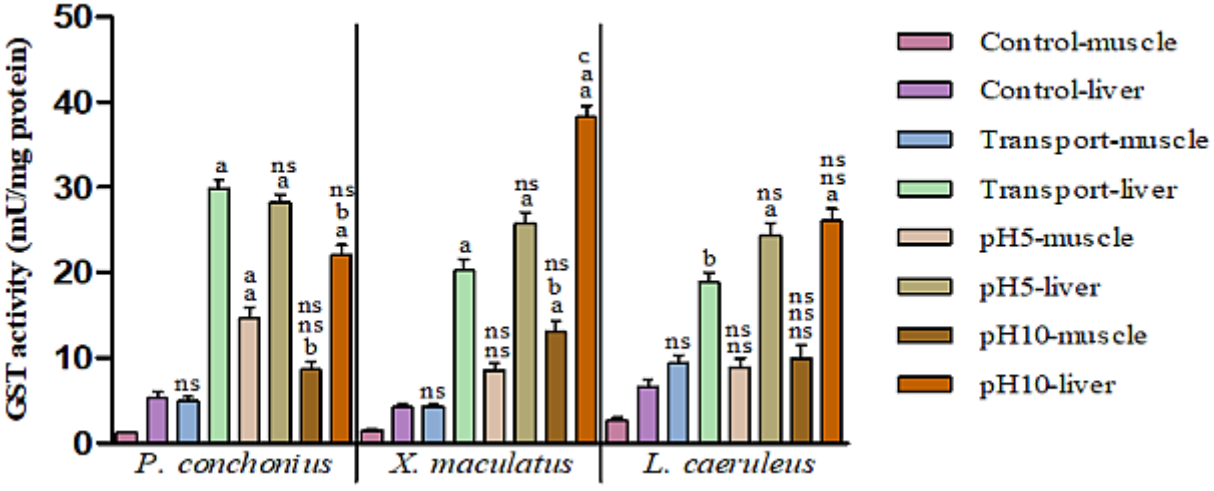

Figure 4. Glutathione-s-transferase (mU/mg protein) content in muscle and liver tissue of *P. conchonius, X. maculatus,* and *L. caeruleus* during transportation and exposure to pH 5 and pH 10

research work.

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