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# Oxidative Stress and Antioxidant Defense to Acute Changes of Water Ph in Liver and Muscle of Three Ornamental Fish

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# Abstract

Ornamental fish during short-term or long-term transportation have stress-related effects due to their exposure to degrading levels of water quality e.g., pH (acidic or alkaline), oxygen, ammonia, temperature levels, etc., and captivity in container. Thus, estimation of biochemical parameters, as lipid peroxidation (LPO) and antioxidant enzymatic activities (SOD-superoxide dismutase, CAT-catalase, GST-glutathione-s-transferase) during pH shift response in liver and muscle of three ornamental fish such as black wagtail platy, rosy barb and lemon-yellow cichlid was reported here. Although 100% survivability was noted among all fish species but oxidative stress was marked with an increase in LPO levels in all fishes transported in different containers /plastic bags for a travel of 6hours by road and those exposed to short-term for 6hours/day for 96 hours to pH5 and pH10 maintained under lab conditions. Exposure of Rosy Barb to pH10 and platy and cichlid to pH5 induced significant increase in LPO in liver tissue compared to all transported fish whereas, muscle tissue of platy and cichlid showed increased activities of LPO during transportation compared to exposed ones and control group of fish. Significantly elevated levels of SOD activity in both tissues of all experimental fishes whereas CAT activity was more in liver tissue of transported fishes to counteract stress response and detoxify products of lipid peroxidation. Therefore, understanding variation in stress levels of ornamental fishes during transportation and exposure to pH levels which is tissue as well as species-specific becomes critically important for their welfare in aquaculture practices as observed in this study.


# Introduction

Transportation of fish, although a global necessity, are known to cause acute and/or chronic stress due to duration and distance covered between source of initial travel and their final destination causing changes in 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 important practice in aquaculture but for successful method of transportation the aquaculturist has to understand many technical issues. Fish welfare during transportation is rapidly gaining importance and many scientists have identified conditions to be fulfilled regarding welfare criteria of fish so as to reduce stress, suffering and pain during transportation. The data available in literature regarding transport of live fish, included scientific information about 8 h or less (short transport) and more than 8 h duration (long transport) (Stieglitz et al., 2012). According to Noga (2000) and Davis (2006) effects of duration of transport stress can be categorized into acute (short-term) or chronic (long-term). Reviews by Berka (1986) and Harmon (2009) was mainly 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 capacity factor which is defined based on carbonate system (Stumm and Morgan, 1996). $CO_2$ causes acidity while bicarbonate and carbonate cause alkalinity of water (Boyd, 2000). Reaction of water molecules with ammonia released by fish resulted in formation of ammonium ($NH_4^+$) and hydroxyl ions ($OH^-$) ions into water which further reacted with $CO_2$ to produce $HCO_3$ (bicarbonate)- that in turn increased water alkalinity (Boyd, 1990). Water with pH less than 4.5 do not have alkalinity while pH greater than 8.5 don’t

have acidity. The transported fish are exposed to fluctuation of water quality when repacked from different sources by the stakeholders from the time in wild or aquaculture farm to their final destination of aquarist vendors or domestic aquarium. Lim et al. (2003) and Pramod et al. (2010) accepted that ornamental fish are transported for long distances and mainly in plastic sealed bags in high densities causing damage to fish health or mortality (Braun and Nuner 2014). Bower and Turner, (1982), Silva et al., (2015) and 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 so as to understand their effect on physiology of the fish in question (Paterson et al. 2003, Abreu et al. 2008 and Manuel et al. 2014).

pH exposure can be the forerunner to oxidative stress in fish species was confirmed by 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 negative effect beyond a period of time. Das et al., (2006) studied the influence of alteration in environmental (water) conditions with respect to pH levels during transportation which influences their welfare since they are unable to maintain acid-base and ion regulation. Hence, altered pH levels were considered common stressor among the potential stressors during transportation of fish due to its denaturing effect on cellular membrane (EIFAC, 1971). Previous studies by Sies (1985) on suboptimal pH or salinity exposed fishes showed enhancement in the free radical production which resulted in oxidative damage. Winston and Giulio, (1991) observed the presence of low and high molecular weight anti-oxidants defences 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 due to evaluation of LPO levels through MDA values. Later Droge (2002), Hermes-Lima, (2004), Husak et al., (2014) and Moniruzzaman et al., (2017) proposed that anti-oxidant system in fish either prevented or counter balanced 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 type of stressors induced external stress in goldfish which stimulated variable patterns of antioxidant enzyme activities in liver and kidney. Liver, a metabolically active organ regulates homeostasis process by breakdown of metabolites and toxic elements to maintain natural body physiology and thus, it is preferred organ to assess the status of oxidative stress 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 defense systems plays a role in maintenance of physiology of cells and tissues (Mourente et al. 2002) but in turn gets 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 effect of pH shift processes on free radical mechanism led to assumptions that 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 protocol for

transportation of live ornamental fish is incipient and there are lacunae in literature about the ornamental fish response to physiological stress due to alteration in water quality and interaction of these factors. Thus it becomes extremely relevant for studying environmental stress on transported ornamental fishes. Eyckmans et al., (2011) reported that variation in antioxidants stimulation between fish species is dependent 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 alteration in water quality in general and pH in particular. Interpretation of such variation may support identification of essential mechanisms involved in sensitivity of fishes to different pH values. Due to lacuna in the field, the present study was focused to explicate 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 can survive a small range of stressful conditions in general for example, cichlid can survive prolonged exposure (48 hour) to clove oil (Kaiser et al., 2006) and platy survived temperature alterations from 22 to 28°C (Singh and Zutshi, 2020).

These interesting ornamental fish models were used to analyse oxidative stress when exposed to pH5 and pH10 points in lab conditions and those of transported fish species for a period of 6 hours from the source to its 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.

# Materials and Methods

### 2. *1 Collection of fish*

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 (15no.per bag) were brought to the laboratory in 5 polythene airtight bags, half-filled with oxygenated water and quarantined in 0.1% potassium-per-manganate solution. The experimental fish group was acclimatized for a week in 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 2 days prior to experimentation, so as to provide time for the gut to be emptied and to stabilize nitrogenous waste excretion. 10–15% of the water was siphoned off along with faecal matter and replaced with fresh dechlorinated tap water every alternate day. The transported fishes were collected immediately from the vendors in Bangalore transported from the main source in Chennai for 6-8hours by road.

### 2.2 *Experimental protocol*

The experimental set up consisted of exposing each of the three species of fish to two different pH standardized by conducting LC50 for all fish species and fixed at pH5 (pH4.5-5.5) and pH10 (9.5–10.5) for a period of 6 hrs intermittently for 04 days. The experimental fish were exposed in 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 ± 0.2g black wagtail platy (*Xiphophorus maculatus)*, 1.8 ± 0.3g rosy barb (*Pethia conchonius*) and 2.5 ± 0.5g 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 ± 1◦C and a natural light–dark cycle of about 12:12h 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 as given in Table 1. The transported experimental fishes were collected from vendors and anesthetized immediately on site for further procedures.

**2.** *3 Preparation and procedure for analyses of anti-oxidant enzyme activity*

Fish were removed from aquaria (n = 5) with the help of scoop net, anesthetized using neutralized MS222/few drops of clove oil (pH 8.0, ethyl 3-aminobenzoate methane-sulfonic acid, 1 g/L, Acros Organics, Geel, Belgium). Fish was dissected on ice to excise liver and muscle tissue of control and experimental group of fish species exposed to pH 5.0 and 10.0 after 96hours of intermittent exposure for 6 hours but those of transported fish was immediately excised and an assay on oxidant and anti-oxidant enzymes was conducted by following the standard procedures. The liver and muscle tissue weighing 100mg 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 minutes. 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 as Thiobarbituric Acid Reactive Substances (TBARS) method of Nehius and Samuelson (1986). 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 were read at 560nm.

Statistical analysis: The results were expressed as mean value ± S.E. Within species, no significant differences were observed among the control values. Thus, controls value was pooled for each experimental group. To compare the means among the variables Tukey–Bonferroni multiple comparison test was used. All data were subjected to two-way analysis of variance (ANOVA). The data were analyzed by Statistical Package for the 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.

Table 1
Details of physico-chemical parameters of control, transported and experimental tanks

| Parameters | BIS: 2012 | Control tank | Transported container | Experimental tanks pH5 | Experimental tanks pH10 |
|---|---|---|---|---|---|
| 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 |

# Results

In the present study water parameters assessed from control and experimental tanks (pH5 & pH10) and before and after transportation containers carrying fishes are represented in Table 1. Water parameters recorded in control tanks were within BIS limits whereas in experimental tanks there was a slight increase in temperature, ammonia and alkalinity due to the pH variation from normal. The containers before transportation showed almost similar values of water quality as control tanks but those after transportation revealed changes in water quality parameters such as changes from pH7 to pH4.5 and 10.5, temperature ranging from 26°C to 28°C in control condition with minimum decrease of 16°C to maximum of 34°C during transportation, dissolved oxygen (4–6 $mg^{-1}l$ as control) depletion to 2 $mg^{-1}l$, increase in alkalinity from 128 mg $CaCO3^{-1}l$ to 145 mg $CaCO3^{-1}l$ and ammonia from 0.37 to 1.4 with alteration of 1.38 to 1.45 when compared to control tank water was observed. There were slight changes in water parameters of the tank water with pH 5 and pH 10 exposed fish when compared to control tanks (Table 1).

## 3.1 Behaviour observations at different pH level

All fish species exposed to both the extremes of pH, >pH4.5 and < pH10.5 (acidic and alkaline conditions) showed mortality within 01 hour of exposure due to intolerance of acute and lethal stress levels of pH. The fishes showed erratic jerking swimming movement assuming diagonal position with the head upwards towards the water surface so as to engulf atmospheric oxygen. The body was covered with abundant quantity of mucous; the gill epithelium showed mucous covering and discolouration with its subsequent destruction. Thus the pH level for their sustainability and survivability was standardized

to pH 5.0 and pH 10.0 as oxidative stress markers for the conduct of experiments for an intermittent period of 6 hrs for 92hrs.

3.2 *Oxidative stress marker during transportation, exposed to pH (acidic and basic levels) in three fish species*

The outcome (mean ± SD) of physiological stress responses due to pH shift in liver and muscle tissues of three fish species, during transportation and those exposed to pH 5.0 and 10.0 compared to control ones are represented in Fig. 1–4. The tissues of transported and exposed fish species tested for LPO activity revealed significantly high LPO levels ($P < 0.001$) when compared to those of control ones. The liver of rosy barb exposed to pH10 and cichlid and platy fish exposed to pH 5.0 and pH10 showed maximum LPO activity (significance $P < 0.001$) when compared to those of transported and control ones (Fig. 1). However, muscle tissue of platy and cichlid fish group transported and those exposed to pH5 and pH10 showed significant LPO activity ($P < 0.001$) that was followed by rosy barb compared to control ones. LPO activity was insignificant in muscle of transported and pH5 exposed barb when compared to those exposed to pH10 whereas, cichlid exposed to pH10 showed moderate activity ($P < 0.01$).

SOD activity was significantly higher ($P < 0.001$) in liver tissue of all the three fish species exposed to pH5 and pH10 compared to transported fish 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 pH5 (Fig. 2). However, muscle tissue of all transported and pH5 and pH10 exposed fishes showed a significant increase ($P < 0.001$) in SOD activity compared to control ones and liver tissue as well. Fish species exposed to pH10 showed minimum SOD activity to compensate the enhanced levels of LPO.

A significantly high CAT ($P < 0.001$) activity was observed in liver tissue of all transported fishes and those exposed to pH10 when compared to pH5 exposed and control ones with rosy barb showing a minimum ($P < 0.01$) CAT activity (Fig. 3). Muscle tissue of all transported fishes and those exposed to pH5 and 10 fish showed higher CAT activity when compared to control but it was insignificant when compared to that in liver tissue. Interestingly in transported barb and cichlid, ph5 exposed platy and pH10 exposed cichlid the CAT levels showed a further decrease ($P < 0.01$) when compared to fishes under other treatments.

A good correlation was observed in SOD and CAT activity in liver and muscle tissue of all fish species to counteract oxidative stress damage and ROS production in all treatments. Reduced levels of SOD in the liver of transported lemon-yellow cichlid, platy and barb fish and GST was compensated by enhanced activity of CAT whereas an increase in SOD activity was noted in those exposed to pH5 and pH10. Hence, elevated catalase activity showed an improvement of defense and detoxification of LPO products in transported fish species whereas enhanced SOD levels in acidic and alkaline exposed fish counteracted the reduced CAT and GST levels (Fig. 4). Rosy Barb showed acute stress levels when exposed to pH10 whereas platy and cichlid were stressed when exposed to pH5 compared to transported fish for 04 days.

Conclusion: Elevated levels of CAT in transported fish compensated the reduced activities of SOD and GST whereas deficiency of CAT and GST activity in fish exposed to pH5 & pH10 due to its inhibition was counteracted by increased SOD levels which helped to detoxify aldehydic products of lipid peroxidation. GST activities were inversely related with LPO and SOD levels which proved the involvement of liver GST in 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.

# Discussion

An organism undergoing stress responds by activating corresponding protective mechanism to either maintain the previous status or may go to new stable state. Many scientists have reported about the aquatic organism undergoing oxidative stress due to change in water parameters such as pH, from given normal range during transportation and other climatic conditions. (Doudoroff & Katz, 1950; Dorge, 2002). In the present study, changes in water quality parameters e.g., pH, temperature, dissolved oxygen, alkalinity and free ammonia after transportation had resulted in oxidative stress among all fish groups and similar changes were noted in the experimental fish species exposed to pH5 and pH10. Sampaio and Freire (2016) mentioned that during transportation increase in $CO_2$ leads to acidification and acidosis of water with progression in hypoxic condition including rise in ammonia levels along with other secondary factors contributed to alteration in parameters of water quality. Lim et al. (2003) confirmed that reduction in oxygen-carrying capacity of the blood (Root effect) was due to presence of low plasma pH and high plasma $CO_2$ inspite of high levels of DO in water. The scientist 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. Treasurer (2012) found that long transport causes rise in pH and ammonia levels due to dissolved $CO_2$ produced by transported fish in aerobic and ammonia during anaerobic condition resulting in acidification of water (Marshall and Grosell 2006).

Doudoroff & Katz (1950) reported that fishes within the pH range of 7.4 to about 5.5 can identify and avoid carbondioxide and their indifference to pH ranging between 5.5 to 10.5 can be considered as the tolerance limit exhibited by most freshwater fishes. Similar tolerance limit was observed in the experimental fish species (cichlid, rosy barb and platy) when exposed to pH4.5 to 5 and pH 9.5 to10 in the present study. So, the present aim to assess the physiological changes identified in the fish possibly could be associated with transportation of the fish in question, (short and long transport) as discussed due to changes in the water quality. Many scientists proved that stress markers were used to evaluate the cause of changes in fish environment during transportation (means of transport) challenging the extra- and intracellular homeostasis of fish. Barton (2002) and Iwama et al. (2006) grouped physiological responses of fish to different stresses into primary, secondary and tertiary responses. An increase in plasma glucose and a decreased hepatic glycogen are some of the primary metabolic consequences for an increased demand on energy, so as to deal with stressful conditions (Zeppenfeld et al. 2014).

In the present investigation exposure to pH5 or pH10 caused significant ($P < 0.001$) LPO activity in liver and muscle of platy and it was accompanied by a differential oxidative stress response whereas in transported platy the LPO levels in liver were not as significant as in the exposed fish. Bagnyukova et al., (2006) reports also showed increased levels of lipid peroxidation products (TBARS and LOOH) in goldfish liver exposed to pH shift due to addition of limestone water in rearing tanks. Similar increases in lipid peroxidation were recorded in response to ammonia exposure in Nile tilapia and silver carp by Sun et al. (2011) and Hegazi (2011) respectively. Occurrence of oxidative stress can be assessed by changes in levels of oxidative damage markers as products of protein and lipid peroxidation (Storey, 1996; Hermes-Lima, 2004). ROS generation was noted by Baraboy and Sutkovoy, (1997) and Halliwell and Gutteridge, (1999) as a response to increased LPO levels due to a various type of stress. Thus, anti-oxidants such as catalase, SOD and glutathione S transferase levels were assessed along with LPO levels as stress biomarker.

The LPO levels in transported rosy barb, platy and cichlid liver were not as significant as in the pH5 and pH10 exposed ones revealed the antioxidants are more active during transportation whereas, elevated LPO levels in liver of rosy barb exposed to pH10 and cichlid and platy to pH5 revealed that antioxidants were differentially effective in different fish tissues and species under experimental condition during direct exposure to changes in pH. It is very likely that during pH stress SOD was more active as anti-oxidant defense in liver and muscle tissue of rosy barb but during transportation catalase took the lead as effective antioxidant in liver of all fish groups. Muscle tissue showed significant LPO activity in response to pH stress but it was comparatively less than liver tissue. Since liver and muscle tissue are metabolically active in acid-base regulation for the maintenance of body physiology, they were expected to respond prominently to pH shift in the present study. Both tissues of fish in control conditions projected comparatively negligible LPO levels.

Halliwell and Gutteridge, (1999) had reported production of free radical during pH shift by changing the superoxide state into superoxide anion ($O_2^-$) and hydroperoxyl radical ($HO_2$) causing oxidative damage to cellular components due to their difference in crossing the biological membrane. Consequently, oxidative stress level and antioxidant system is regulated by the rate of ROS production in liver in aquatic organisms. Sinha et al., (2014) reported ammonia stressed carp and goldfish with high accumulation of $H_2O_2$ and MDA (end product of LPO) in liver unlike those in trout. The defensive approach related with antioxidant mechanism is less effective in controlling ROS production in liver of cichlid dealing with pH5 and barb liver with pH10 showing high LPO levels, despite high SOD and CAT activity followed by those platy liver. Carneiro et al., (2021) reported increase in SOD activity in sea horse exposed to acidic environment when in brackish water but CAT activity remained unaffected when exposed to different pH. Halliwell and Gutteridge, (2015) also mentioned that insufficient removal of ROS might be due to imbalance of SOD and CAT activities leading to accumulation of LPO causing oxidative stress. Further, barb exposed to pH5 and cichlid and platy to pH10 could effectively remove ROS and limit LPO levels due to significant ($P < 0.001$) activity of SOD with compensatory levels of CAT. The above results indicated disparity in anti-oxidative compensatory responses toward pH exposure by these fish species.

The present findings perhaps clarify in part that barb has high resistance towards acid waters with pH5 and above whereas cichlid and platy can tolerate alkaline water with pH10 and below.

Interestingly, during short-term transportation (6 hours), LPO activity in cichlid fish **liver** was lower than platy and barb, 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, decrease in LPO levels was observed by Lushchak et al., (2005a) in goldfish during hypoxia with an effective detoxifying system to maintain cell integrity. The low levels of LPO in cichlid during transportation could also be attributed to a decrease in water pH from 7.5 in presence of intermediate ammonia levels during anaerobic metabolism i.e., consumption of oxygen resulting in production of CO2 causing hypoxic condition as observed by Sampio and Freire, (2016). Unlike in platy and barb low levels of SOD in transported cichlid liver undergoing oxidative stress during transportation suggested that SOD, a first antioxidant defense to counter excessive ROS production, is not the only effective mechanism to regulate LPO process. Although SOD being the key enzyme that catalyses $H_2O_2$ synthesis to reduce LPO levels, cichlid depended on catalase activity. $H_2O_2$ is a secondary by-product of spontaneous or enzymatic dismutation of O2. CAT an antioxidant enzyme is involved in the destruction / elimination of $H_2O_2$ which 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 as anti-oxidative sentinels with minimum of SOD and GST 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 with 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 presence of high LPO levels for a period of 6 days of exposure despite the activities of SOD and CAT questions the efficiency of anti-oxidant enzymes.

The muscle of platy and cichlid fish groups when transported for short-term period of 6hours revealed significantly ($P < 0.001$) high levels of LPO compared to control which was due to oxidative stress assumed to be caused by crowding or erratic swimming activity in containers with high density of fishes. Elevated levels of LPO that increased fish metabolites or accelerated reactive oxygen species (ROS) production, resulted in oxidative stress. Urbinati et al. (2004) and Braun and Nuner (2014) confirmed that density of fish during transportation is an important feature because large number of fish in a bag corresponded to cost economy but in turn the crowding of fish caused stress or even mortality thus compromising with fish health proving not economically viable. Subsequently, on exposure of these fish to acidic (pH5) and alkaline water condition (pH10), a significant increase in LPO activity of their muscles was noted caused by disturbance in their behaviour of swimming activity. Similar results of increase in pectoral fin movement and erratic swimming were reported by Hoglund & Hardig (1969) among parr of Atlantic salmon (*Salmo salar*) when exposed to acidic waters and this behaviour varied in

intensity with the magnitude of the stimulus of pH. Schreck et al., (1997) suggested that alteration in swimming patterns is a behavioural marker for chronic stress in ornamental fishes. The occurrence of significantly high LPO levels in muscle of all fish species transported and pH exposed ones with rosy barb as exception, wasstabilized 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. Acute exposure to acidic stress in an aquatic environment reduced fertility of flounder fish that resulted in a decreased growth of the species 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 and liver of barb revealed insignificant rise in LPO levels during transportation and pH5 exposure but it was significantly more than control. Whereas, when exposed to pH10, barb showed high LPO activity in both tissue in response to oxidative stress by alkaline water which was well counteracted by significantly high SOD activity compared to CAT. Winston, (1991) reports suggested the presence of a well-developed recovery system or an antioxidant defense mechanism in fish to facilitate overcome stress, and to generate and degrade free radicals. Such observations regarding stimulation or production of intracellular ROS and antioxidant reactions of SOD and GST activities to compensate the pH oxidative stress have been proved in previous studies on pH shift (acidic or alkaline) by Maqsood and Benjakul, (2011). Thus, an increase in SOD activity in liver 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). In contrast to present result, Kim et al., (2021) found a significant reduction in SOD activity in liver of *P. olivaceus* when exposed to an 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).

A significant increase in SOD activity, the first-line of defense against free radicals compared to low levels of CAT observed in barb, platy and cichlid fish group' muscle during transportation, exposure to pH5 & pH10 including the liver of all fish exposed to pH5& 10 with slight variation suggested that all fish relied mainly on superoxide dismutase dependent defensive mechanism against increase in LPO activity. Previous studies have shown that decreased catalase activity might be due to its inactivation by overproduction of ROS (Pigeolet et al., 1990). Chitra and Maiby (2014) reported significant decrease in catalase activity in the liver of fresh water fish *Oreochromis mossambicus* on exposure to the sublethal concentration of bisphenol-A which is in accordance with the present study on exposure of fish to pH5 and pH10. Jin et al., (2010) had also suggested alteration in catalase activity following oxidative stress. In transported fish, it was likely due to stress from long crowding conditions, whereas in experimental ones the pH exposure 5 and pH10 leading to excessive ROS production. 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 of hybrid snapper and channel catfish, *Ictalurus punctatus* caused significant increase in SOD activity and MDA contents in fish respectively due to transport stress are in lines with the present study on transported fish (6 hours) inducing high SOD and

LPO levels. In the present experimentation high SOD and CAT activity and minimum GST levels in liver and muscle tissue of all test fishes possibly indicates major role of SOD and CAT in scavenging of $H_2O_2$ and superoxide anion (Parihar et al., 1997). Scientific research for the protocol development of live fish transport is still budding and has incomplete knowledge.

To conclude it can be anticipated that rosy barb followed by platy liver and muscle utilized the protective system moderately and showed more effective anti-oxidative compensatory responses throughout the experimental exposure and transportation period, while cichlid liver helped to stabilize in the transportation stress more effectively including its muscle tissue on pH exposure. The present work also indicated that all three species responded well in acidic environment by compensating a relatively mild oxidative stress in the **muscles.** This probably explains that oxidative stress, as well as the antioxidant potential to overcome the effect of stress that resulted in damaging of cells was different between the three species and their tissues.

## Declarations

# Competing interest:

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# Author Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Srisaiprajwal. The first draft of the manuscript was written by Srisaiprajwal and conceptualization, visualization, review and editing done by Dr.Bela Zutshi; all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript

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# Data Availability

The datasets generated during and/or analysed during the current study are not publicly available since it is not published yet but are available from the corresponding author on reasonable request.

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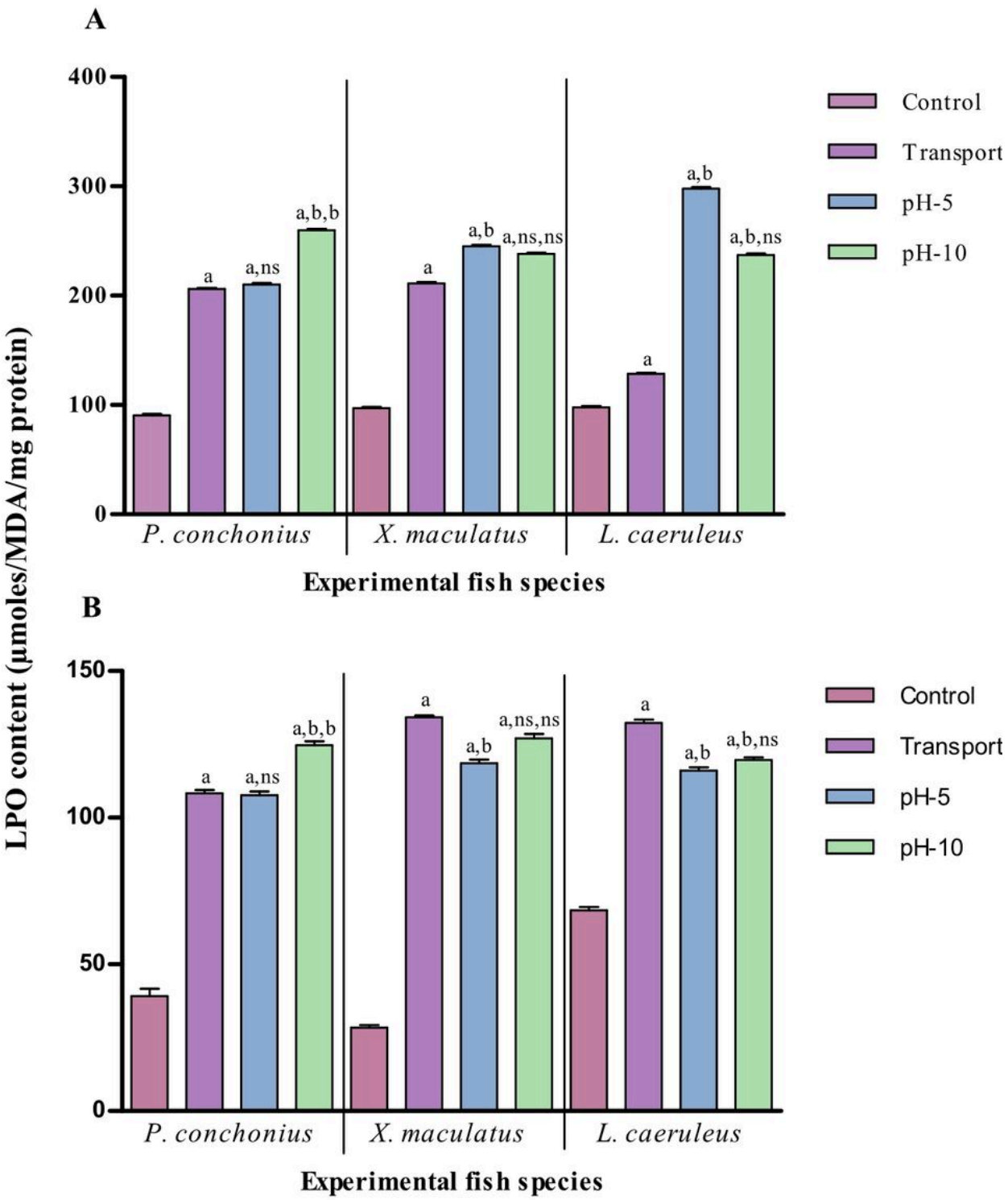
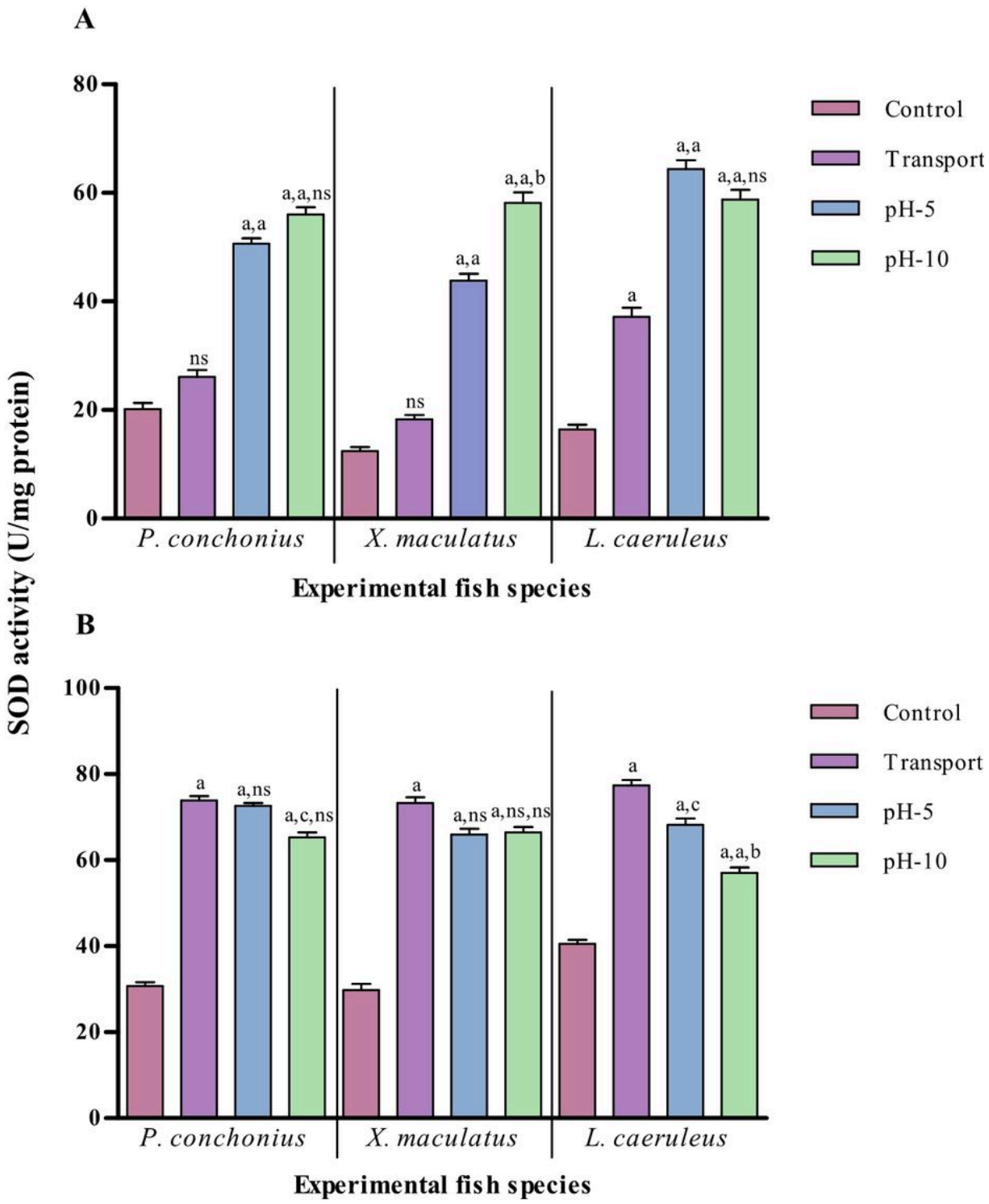
# Figures



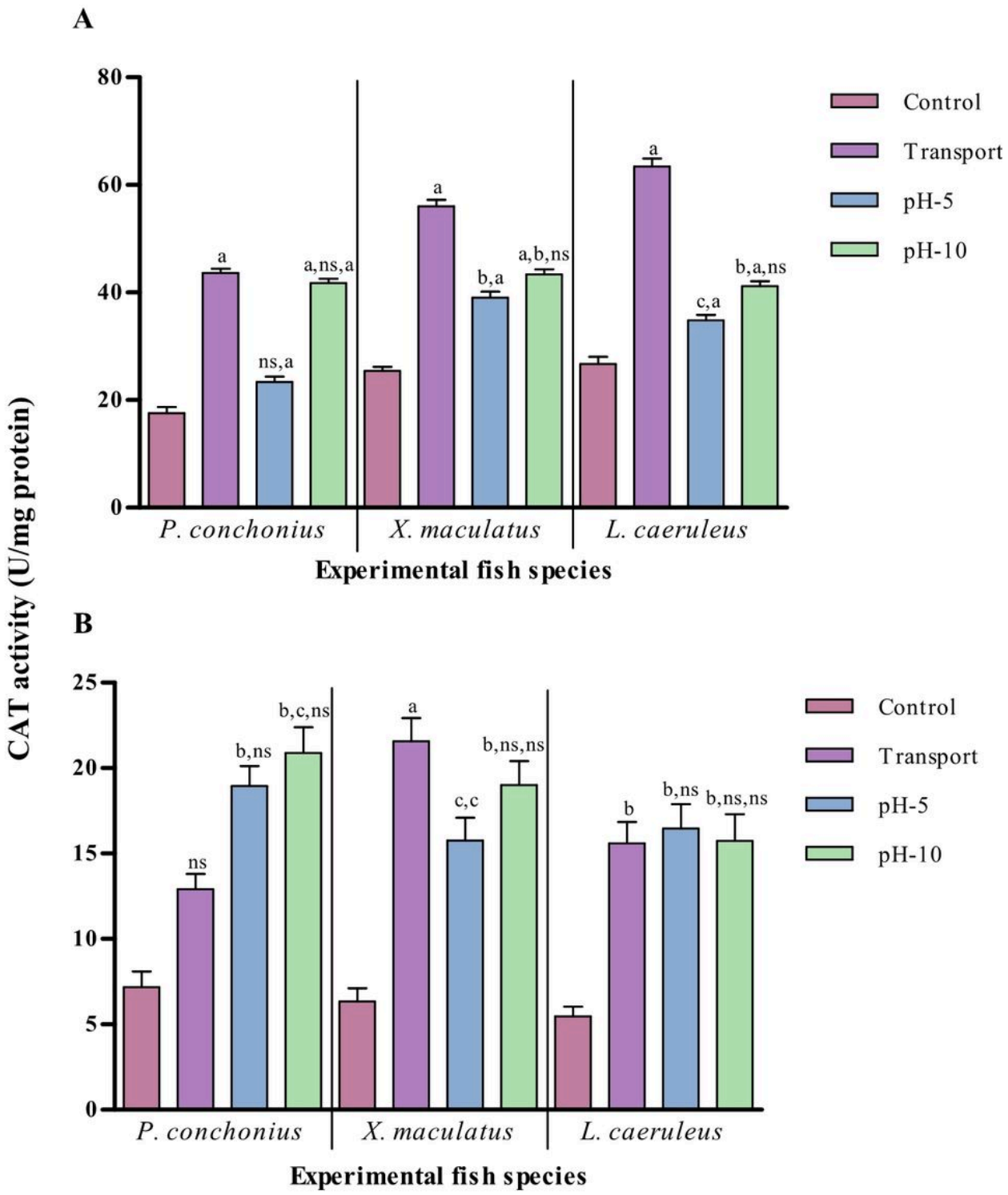

## Figure 1

Lipid peroxidation content (µmoles/MDA/mg protein) in (A) liver and (B) muscle of P. conchonius, X. maculatus, and L. caeruleus during transportation and exposure to pH (5&10). Data are shown as mean ± SE. Significance by two-way ANOVA and post-hoc test – Bonferroni (P<0.01) using GraphPad Prism 5.1. Superscripts a, b and ns indicate statistical mean differences between transport and exposure to pH (5&10) which are P<0.001, P<0.01 and P<0.05.



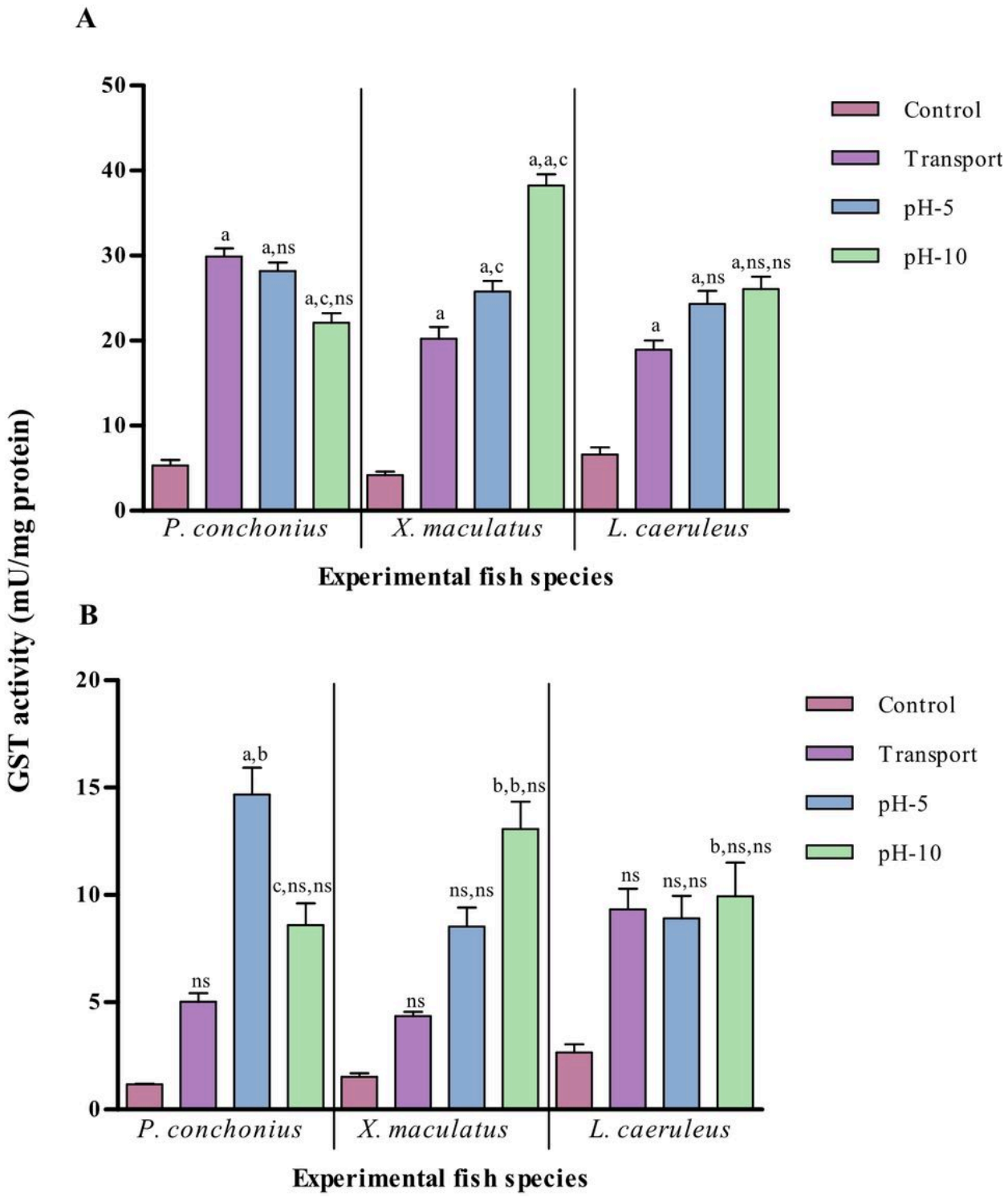

## Figure 2

Superoxide dismutase activity (U/mg protein) in (A) liver and (B) muscle of P. conchonius, X. maculatus, and L. caeruleus during transportation and exposure to pH (5&10). Data are shown as mean ± SE. Significance by two-way ANOVA and post-hoc test – Bonferroni (P<0.01) using GraphPad Prism 5.1. Superscripts a, b, c and ns indicate statistical mean differences between transport and pH (5&10) which are P<0.001, P<0.01 and P<0.05.




## Figure 3

Catalase activity (U/mg protein) in (A) liver and (B) muscle of *P. conchonius, X. maculatus,* and *L. caeruleus* during transportation and exposure to pH (5&10). Data are shown as mean ± SE. Significance by two-way ANOVA and post-hoc test – Bonferroni ($P<0.01$) using GraphPad Prism 5.1. Superscripts a, b, c and ns indicate statistical mean differences between transport and pH (5&10) which are $P<0.001$, $P<0.01$ and $P<0.05$.




## Figure 4

Glutathione-s-transferase (mU/mg protein) in (A) liver and (B) muscle of *P. conchonius, X. maculatus,* and *L. caeruleus* during transportation and exposure to pH (5&10). Data are shown as mean ± SE. Significance by two-way ANOVA and post-hoc test – Bonferroni (P<0.01) using GraphPad Prism 5.1. Superscripts a, b, c and ns indicate statistical mean differences between transport and pH (5&10) which are P<0.001, P<0.01 and P<0.05