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Effects of common salt, vitamin C and taurine on transportation-induced HSP70 levels in African catfish, *Clarias gariepinus*

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Abstract

The transportation of fish from farm to farm, farms to markets, and farms to homes are necessary stressful practices in aquaculture, hence the need to look for how to mitigate its impact. The present study investigated the effects of common salt, vitamin C and taurine administered through fish water on transportation-induced stress using shock protein 70 (heat shock protein 70 or HSP70) as a biomarker of stress. Group I contained the control and not transported fish and Group II contained the transported fish without common salt, vitamin C or taurine in fish water while Groups III, IV, and V contained the transported fish with common salt (6 gL^{-1}), vitamin C (200 mgL^{-1}), and taurine (200 mgL^{-1}), respectively. There was significant ($p<0.05$) decline in HSP70 levels in the transported fish. However, significantly ($p<0.05$) elevated HSP70 levels occurred in fish transported with common salt and vitamin C but no significant ($p>0.05$) changes occurred in HSP70 levels of fish transported with taurine. There should be cautious use of common salt and vitamin C administration via the fish water for live fish transportation.

Keywords: *Clarias gariepinus*, Common salt, HSP70, Stress, Taurine, Vitamin C

Introduction

Live fish transportation is a normal aquaculture practice on fish farms involving the movement of fish of a particular age from one fish pond to another on the same farm or between farms, as well as from farms to markets for the edible species or from farms to homes and museums for the ornamental species (NAERLS, 2001; Crosby *et al.*, 2023). Some of the transportation might be over short or long duration depending upon the distance involved in each case. Live fish transportation is known to cause stress in fish (Refaey & Li, 2018). Although stress and stressors associated with live fish transportation are well documented with a focus on maintaining water quality during transport to reduce the stress, far less attention has been given to practices that would enhance fish health and welfare during transport such as the use of dietary or water-borne additives before and during the transportation (Vanderzwalmen *et al*., 2018) hence the present study.

In addition to stress-induced primary, secondary, and tertiary physiological responses (Barton, 2002), stress has been known to increase the levels of heat shock proteins (HSPs) synthesis at the cellular level (Rafaey & Li, 2018). Animals express HSPs in response to stressful conditions (Hofmann, 1999) because HSPs play vital protective roles in cellular homeostasis (Ghosh *et al*. 2023) by preventing protein aggregation in addition to helping to refold/unfold misfolded proteins (Ranford *et al*., 2000). That might be why HSP70, which is also known as stress protein, is now recognized not only as chaperones for all proteins but also, as chaperones for lipid metabolic cellular activities (Roberts *et al*., 2010). Therefore, HSP70 expression may serve as a bioindicator of stress in fish (Winarti *et al*., 2022). More so elevated HSP70 levels is often a marker of cellular stress in fish (Teles *et al*., 2019) or a marker of proteotoxic effects (Dieterich *et al*., 2013). Although there are reports of the transportation effects of HSP70 on fish, such reports are presently limited (Refaey & Li, 2018) to necessitate the present study. The role of HSPs as part of the secondary physiological stress response has necessitated its use in the evaluation of fish welfare conditions (Poltronieri *et al*., 2007), as well as a marker of cellular stress in fish (Teles *et al*., 2019). More so HSPs evaluation in fish is still in the early stages compared to what is obtainable in bacteria, yeast, and mammals. Besides, few HSPs investigations in whole fish subjects currently exist with most of them involving HSPs as the stressor (Iwama et al., 1998) rather than fish transportation as the stressor, as in the present study.

Although, with variable results, common salt addition to fish water reduces stress during handling and transportation (Tacchi *et al*., 2015), thereby necessitating its continuous evaluation in that regard. This is because of common salt's ability to reduce or eliminate fish salt loss by equilibrating or minimizing differences in electrolyte gradient between the environmental water and fish blood (Wurts, 1995). Vitamin C is a known anti-stress in fish (Varghese *et al*., 2021), which is often administered through feed (Aboseif *et al*., 2022; Mazini *et al*., 2022). However, vitamin C has also been administered via the water to dechlorinate fish water (Land, 2005) among other beneficial effects (AquaNature, 2024; Brightwell Aquatics, 2024). Although, Yang *et al*. (2023) have reported the use of vitamin C in fish water during fish transportation, available information on the use of water-borne vitamin C to counter stress in fish during transportation is currently limited, hence the present study. Taurine is an anti-stress in fish by modulating the stress axis (Liu *et al*., 2022) in addition to its vital cellular osmoregulatory role (Ito *et al*., 2018). However, there is a dearth of information concerning the use of taurine to ameliorate transportation stress in fish either as a feed or water-borne additive. *Clarias gariepinus* (*C. gariepinus*) is a hardy fish that withstands unfavourable harsh conditions (Ng, 2021) and prolonged drought (Nwizugbo *et al*., 2023), which makes it an ideal fish for the present study. More so, during damp conditions, the fish can transport itself or move between water bodies (Ng, 2021). Therefore, the present work aimed to evaluate the effects of common salt, vitamin C and taurine on the HSP70 status of transported African catfish, *C. gariepinus.*

Materials and Methods

Preparation of chemicals

The stock solutions used in the present study were made from analytical grades of common salt (Merck, Denmark), Vitamin C (Sigma Aldrich, Germany) and taurine (Sigma Aldrich, Japan).

Fish collection and preparation

The experiment involved the acclimatization of *C. gariepinus* juveniles purchased from a commercial catfish farm in Gwagwalada, Abuja, Nigeria, for 14 days under natural photoperiod. During the period, there was a complete fish water change once on alternate days while fish were fed twice daily to satiation with 3 mm commercial catfish feed (BLUECROWN(R) by Crown Flour Mill Ltd., Ilorin, Nigeria.

Experimental design

Clarias gariepinus juveniles of 318.10 ± 4.50 g mean weight and 35.05 ± 0.29 cm mean length were divided into five groups of 10 fish each and subjected to transportation alone and along with either common salt, vitamin C or taurine separately over a 5-hour (5-h) period as follows:

Group I: Control fish not subjected to any form of transportation

Group II: Fish subjected to a 5-h uninterrupted transportation stress alone

Group III: Fish subjected to a 5-h uninterrupted transportation stress along with common salt (6 gL^{-1}) in fish water.

Group IV: Fish subjected to a 5-h uninterrupted transportation stress along with vitamin C (200 mgL^{-1}) in fish water.

Group V: Fish subjected to a 5-h uninterrupted transportation stress along with taurine (200 mgL^{-1}) in fish water.

Common salt, vitamin C and taurine concentrations used in the present study were as or adapted from previous reports (Urbinati & Carneiro, 2006; Peng *et al*., 2013). Each group had 10 fish in 40 L of water in a 50 L black plastic container (jerry can) with a semi-cut-out upper end. The physicochemical characteristics of the fish water were as follows: 6.63 ± 0.33 mgL^{-1} for dissolved oxygen, 6.99 ± 0.17 for pH, 30.07 ± 0.03°C for temperature, 0.09 ± 0.00 $mScm^{-1}$ for electrical conductivity, and 0.04 ± 0.00 ppt for total dissolved solids, respectively. Although experimental fish were starved some 24 hours before the transportation, according to Wedemeyer (1996), fish were neither sedated nor was the fish water aerated during the 5-h transportation period.

Heat-shock protein (HSP70) evaluation

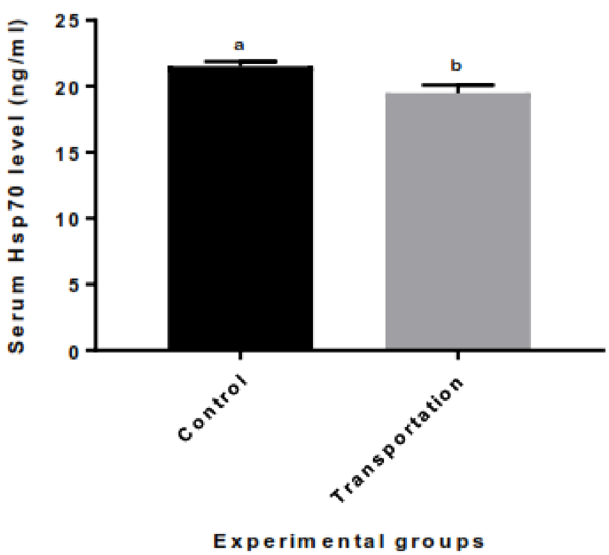

Figure 1: Serum heat shock protein (HSP70) levels in *Clarias gariepinus* following a 5-hour transportation period
Bars with different superscripts denoted significant ($p<0.05$) differences between the groups based on Student's *t*-test analysis

Serum HSP70 level determination in five randomly selected fish from each of the experimental group at the end of the 5-h transportation duration were via the method of Zhang *et al*. (2021) with commercial HSP70 kit (StressXpress HSP70 ELISA, StressGen Biotechnologies), according to the manufacturer's directions. Blood collection was as described by Abalaka *et al*. (2021) before subjection to centrifugation at 3000 xg for 15 minutes to obtain the serum (Mohapatra *et al*., 2016) after clotting at room temperature.

Data analysis

Data expression was as mean (± SEM) before subjecting them to student's t-test and a One-way Analysis of Variance (ANOVA) followed by post hoc Dunnett's multiple comparison test for statistical significance ($p<0.05$) using the GraphPad statistical program (GraphPad Prism, version 7, www.graphpad.com).

Results

Fish transportation significantly ($p<0.05$) reduced the HSP70 level compared to the control as shown in Figure 1. Although increased HSP70 levels also occurred in *C. gariepinus* transported with either common salt, vitamin C, or taurine, significant ($p<0.05$) elevation occurred only in fish transported with common salt and vitamin C (Figure 2).

Discussion

Although transportation has induced HSP70 expression in affected *Dicentrarchus labrax* (Poltronieri *et al*., 2007), the significant ($p<0.05$) reduction in HSP70 level following *C. gariepinus* transportation might be due to the collapse of the HSP70 protective system in the stressed fish (Scheil *et al*., 2011). This is because HSP70 levels often rises with increasing proteotoxic stress in a distinct reaction curve (Tomanek, 2002) to gradually peak with no further induction with increasing stress (Arts *et al*., 2004; Köhler *et al*., 2009). Beyond such levels, further increasing stress reportedly leads to a rapid decrease in the HSP70 level resulting in either serious tissue damage or the death of the organism (Scheil *et al*., 2011).

Schiel *et al*. (2011) also observed elevated HSP70 levels in Mediterranean land snails up to 2-h of stress exposure followed by a decline until 16-h when it peaked again to decline further to baseline level by the 24th hour. The decline was attributed to a non-compensatory destruction phase that resulted in the impairment or breakdown of the stress-protein apparatus, as earlier reported by Eckwert *et al*. (1997) in *Oniscus asellus* (woodlouse). In the present study, HSP70 levels were assayed only once immediately following the 5-h transportation stress whose time frame corresponded to the decline phase reported by Schiel *et al*. (2011). The recorded decreased HSP70 levels in the transported fish could probably be due to "overwhelming" physiological stress, as earlier reported by Tomanek (2002). Similarly, there was a gradual increase in HSP70 levels in rat myocardium followed by a sharp decrease at the 100 minutes of the stress exposure in an *in-vivo* experiment (Chen *et al*., 2015). Although Ren *et al*. (2022) reported significantly increased HSP70 expression in the gills and muscles of *Oncorhynchus mykiss* during transportation, they also reported significantly decreased liver HSP70 levels to highlight the possible role of fish tissue in HSP70 expression during live fish transportation. Likewise, although increased HSP70 expression was recorded in the rumen, omasum, and abomasum of the caprine stomach following transportation, decreased reticulum HSP70 expression was reported after 6-h of transportation (Hu *et al*., 2020). However, Rafaey & Li (2018) reported a significantly ($p<0.05$) higher hepatic HSP70 mRNA expression only at 0-h, 6-h, and 24-h following the transportation of *Ictalurus punctatus* over a 168-h period to also highlight the possible role of the time of assay. Similarly, Nasrullah *et al*. (2022) reported increased hepatic HSP70 expression only at 0-h, 6-h, and 12-h following the transportation of *C. gariepinus*. Therefore, animal species, animal tissue and time of HSP70 assay, and the type, duration, and degree of the stress could be responsible for the varying levels of HSP70 expressions in transported animals.

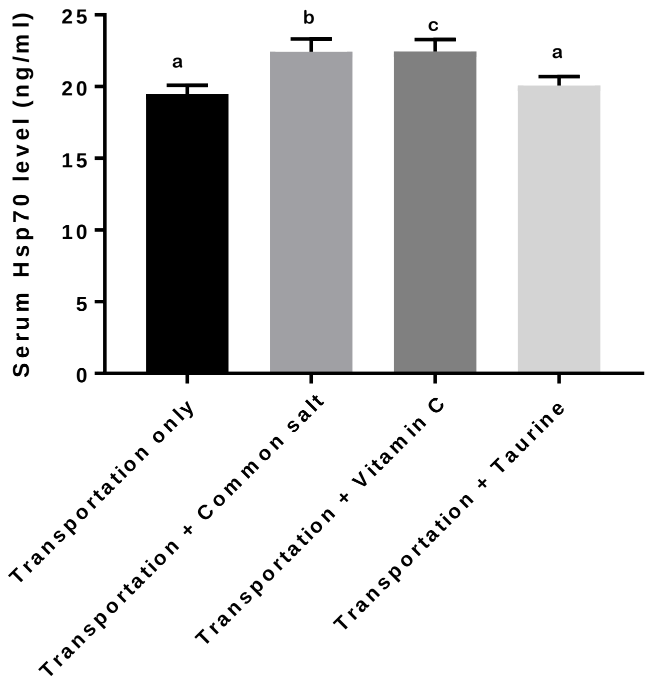

Figure 2: Effects of common salt, vitamin C, and taurine on Serum heat shock protein (HSP70) levels in *Clarias gariepinus* following a 5-hour transportation period

Bars with different superscripts denoted significant ($p<0.05$) differences between the transported and treated groups and the transported only group following post hoc Dunnett's multiple comparison test

The addition of common salt to fish water during the live fish transportation further induced stress in the exposed fish evidenced by the significantly ($p<0.05$) increased HSP70 levels. This might be due to hyperosmotic stress, according to Tine *et al*. (2010). However, the increased HSP70 levels might also be due to the important roles of HSPs in protein homeostasis (Kumar *et al*., 2022), cellular protection and stress response mechanisms (Ghosh *et al*. 2023). Similarly increased HSP70 levels was reported in *Sparus sarba* (Deane & Woo, 2004) and *Salmo trutta* (Larsen *et al*., 2008) following hyperosmotic stress. The increased HSP70 level in fish transported with vitamin C might probably be due to the pro-oxidative propensity of vitamin C under certain conditions (Podomore *et al*., 1998; Bergström *et al*., 2012; Otanwa *et al*., 2023), which probably turned it into a stressor in the present study. The addition of taurine into fish water during live fish transportation had no effects on the HSP70 profile of the affected fish.

In conclusion, although the transportation of fish is a known stressor, a significant ($p<0.05$) decline in HSP70 level occurred in the transported fish probably due to the time of fish sampling, type of fish species, and the fish tissue used for the assay in addition to the type, duration and degree of the stress. The significantly ($p<0.05$) elevated HSP70 levels in common salt and vitamin C transported fish suggested increased stress as HSP70 expressions are indicative of cellular stress (Guo *et al*., 2007). Therefore, the addition of common salt and vitamin C to the fish water during live fish transportation, as used in the present study, rather induced more stress in the transported fish. Nevertheless, there is a need to sample fish undergoing transportation stress periodically throughout the duration of the transportation to help determine the progressive pattern of fish stress HSP70 response. Similarly, the possible role of fish species and fish tissue used in HSP70 assay during live fish transportation needs further elucidation.

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Conflict of Interest
The authors declare that there is no conflict of interest.

References

Abalaka SE, Oyelowo FO, Akande MG, Tenuche OZ, Sani NA, Adeyemo BT, Idoko SI, Ogbe AO & Ejeh SA (2021). Effects of *Moringa oleifera* leaves extract, vitamin C, and taurine co-exposures on calcium and metallothionein levels, oxidative stress, and gill histopathological changes in *Clarias gariepinus* exposed to sub-lethal cadmium. *Environmental Science and Pollution Research*, **28**(37): 52258-52271.

Arts MJ, Schill RO, Knigge T, Eckwert H, Kammenga JE & Köhler HR (2004). Stress proteins (hsp70, hsp60) induced in isopods and nematodes by field exposure to metals in a gradient near Avonmouth, UK. *Ecotoxicology*, **13**(8): 739-755.

Aboseif AM, Ramadan EA & El-Hammady AKI (2022). Effectiveness of dietary vitamin C on the performance of common carp (*Cyprinus carpio*). *Egyptian Journal of Aquatic Biology and Fisheries*, **26**(5): 257 – 287.

AquaNature (2024). AquaNature Vitamin-C Superior Vitamin C Complex for Freshwater & Marine Fishes. https://www.aquanatureonline.com/product/aquanature-vitamin-c-superior-vitamin-c-complex-for-freshwater-marine-fishes/, retrieved 01-03-2024.

Barton BA (2002). Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and Comparative Biology*, **42**(3), 517–525.

Bergström T, Ersson C, Bergman J & Möller L (2012). Vitamins at physiological levels cause oxidation to the DNA nucleoside deoxyguanosine and to DNA – Alone or in synergism with metals. *Mutagenesis*, **27**(4): 511–517.

Brightwell Aquatics (2024). Vitamin C: Concentrated vitamin-C supplement for all marine fish & reef aquaria. Marine Aquaculture. https://www.brightwellaquatics.com/products/vitamarinct.php, retrieved 01-03-2024

Chen H, Adam A, Cheng Y, Tang S, Hartung J & Bao E (2015). Localization and expression of heat shock protein 70 with rat myocardial cell damage induced by heat stress in vitro and in vivo. *Molecular Medicine Reports*, **11**(3): 2276–2284.

Crosby TC, Hill JE, Martinez CV, Watson CA, Pouder DB & Yanong RPE (2023). On-farm transport of ornamental fish. IFAS Extension. University of Florida. https://edis.ifas.ufl.edu/publication/FA119, retrieved 28-02-2024.

Deane EE & Woo NYS (2004). Differential gene expression associated with euryhalinity in sea bream (*Sparus sarba*). *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 287(5): R1054-R1063.

Dieterich A, Fischbach U, Ludwig M, Di Lellis MA, Troschinski S, Gärtner U, Triebskorn R & Köhler H-R. (2013). Daily and seasonal changes in heat exposure and the Hsp70 level of individuals from a field population of *Xeropicta derbentina* (Krynicki 1836) (Pulmonata, Hygromiidae) in Southern France. *Cell Stress and Chaperones*, **18**(4): 405–414.

Eckwert H, Alberti G & Köhler H-R. (1997). The induction of stress proteins (hsp) in *Oniscus asellus* (Isopoda) as a molecular marker of multiple heavy metal exposure: I. Principles and toxicological assessment. *Ecotoxicology*, **6**(5): 249–262.

Ghosh S, Vashisth K, Ghosh S, Han SS, Bhaskar R & Sinha JK (2023). From sleep to cancer to neurodegenerative disease: the crucial role of HSP70 in maintaining cellular homeostasis and potential therapeutic implications. *Journal of Biomolecular Structure and Dynamics*, doi.10.1080/07391102.2023.2252509.

Guo S, Wharton W, Moseley P & Shi H (2007). Heat shock protein 70 regulates cellular redox status by modulating glutathione-related enzyme activities. *Cell Stress and Chaperones*, **12**(3): 245–254.

Hofmann GE (1999). Ecologically relevant variation in induction and function of heat shock proteins in marine organisms. *American Zoologist*, **39**(6): 889–900.

Hu W, Ye T, Yang Y, Liu B & Zheng W (2020). Effects of transport stress on pathological injury and expression of main heat shock proteins in the caprine stomach. *BMC Veterinary Research*, doi.10.1186/s12917-020-02569-z.

Ito T, Murakami S & Schaffer S (2018). Pathway analysis of a transcriptome and metabolite profile to elucidate a compensatory mechanism for taurine deficiency in the heart of taurine transporter knockout mice. *Multidisciplinary Scientific Journal*, **1**(1): 57-70.

Iwama GK, Thomas PT, Forsyth RB & Vijayan MM (1998). Heat shock protein expression in fish. *Reviews in Fish Biology and Fisheries*, doi.10.1023/A:1008812500650.

Köhler HR, Lazzara R, Dittbrenner N, Capowiez Y, Mazzia C & Triebskorn R (2009). Snail phenotypic variation and stress proteins: do different heat response strategies contribute to Waddington's widget in field populations? *Journal of Experimental Zoology. Part B, Molecular and Developmental Evolution*, **312**(2): 136-147.

Kumar V, Roy S, Behera BK & Das BK (2022). Heat shock proteins (HSPs) in cellular homeostasis: A promising tool for health management in crustacean aquaculture. *Life (Basel)*, **12**(11): 1777.

Land B (2005). Using vitamin C to neutralize chlorine in water systems. United States Department of Agriculture. Forest Service Technology & Development Program. 0523 1301—SDTDC. https://www.fs.usda.gov/t-d/pubs/pdf/hi_res/05231301hi.pdf, retrieved 25-02-2024.

Larsen PF, Nielsen EE, Koed A, Thomsen DS, Olsvik PA & Loeschcke V (2008). Interpopulation differences in expression of candidate genes for salinity tolerance in winter migrating anadromous brown trout (*Salmo trutta* L.). *BMC Genetics,* doi.10.1186/1471-2156-9-12.

Liu C, Zhang W, Jiang P, Yv L & Lu W (2022). The anti-stress effect of taurine in fish: Assessments based on repeat acute stress and animal individuality. *Aquaculture*, doi.10.1016/j.aquaculture.2022.738685.

Mazini BSM, Martins GP, Menezes LLC & Guimarães IG (2022). Nutritional feed additives reduce the adverse effects of transport stress in the immune system of Tambaqui (*Colossoma macropomum*). *Fish and Shellfish Immunology Reports*, doi.10.1016/j.fsirep.2022.100051.

Mohapatra S, Chakraborty T, Kacem RH, Shimizu S, Matsubara T & Ohta K (2016) Starvation: an alternate measure to improve immunity and physiology of red sea bream during *Edwardsiella Tarda* infection. *Journal of Aquaculture Research and Development*, doi.10.4172/2155-9546.S2-007.

NAERLS. (2001). Transporting fish for culture. Extension Bulletin No 151. Fisheries Series No 6. National Agricultural Extension and Research Liaison Services, Ahmadu Bello University, Zaria. Pp 1-21. https://naerls.gov.ng/wp-content/uploads/2022/11/Transporting-Fish-for-Culture.pdf, retrieved 25-05-2024.

Ng WK (2021). *Clarias gariepinus*. CABI Compendium. doi.10.1079/cabicompendium.88683.

Nasrullah H, Layinatuzzain L, Soelistiyowati DT, Widanarni W & Alimuddin A (2022). Heat-shock protein 70: Sequence and expression analysis in African catfish *Clarias gariepinus* after bacterial infection and stress exposures. *Genetics of Aquatic Organisms*, doi.10.4194/GA509.

Nwizugbo KC, Ogwu MC, Eriyamremu GE & Ahana CM (2023). Alterations in energy metabolism, total protein, uric and nucleic acids in African sharptooth catfish (*Clarias gariepinus* Burchell) exposed to crude oil and fractions. *Chemosphere*, doi.10.1016/j.chemosphere.2023.137778.

Otanwa OO, Ndidi US, Ibrahim AB, Balogun EO & Anigo KM (2023). Prooxidant effects of high dose ascorbic acid administration on biochemical, haematological and histological changes in *Cavia porcellus* (Guinea pigs): A Guinea pig experimental model. *Pan African Medical Journal*, doi,10.11604/pamj.2023.46.18.36098.

Peng S, Shi Z, Fei Y, Gao QX, Sun P & Wang JG (2013). Effect of high-dose vitamin C supplementation on growth, tissue ascorbic acid concentrations and physiological response to transportation stress in juvenile silver pomfret, *Pampus argenteus*. *Journal of Applied Ichthyology*, **29**(6): 1337–1341.

Poltronieri C, Maccatrozzo L, Simontacchi C, Bertotto D, Funkenstein B, Patruno M & Radaelli G (2007). Quantitative RT-PCR analysis and immunohistochemical localization of HSP70 in sea bass *Dicentrarchus labrax* exposed to transport stress. *European Journal of Histochemistry*, **51**(2): 125–135.

Ranford JC, Coates ARM & Henderson B (2000). Chaperonins are cell-signalling proteins: The unfolding biology of molecular chaperones. *Expert Review in Molecular Medicine*, doi.10.1017/S1462399400002015.

Refaey MM & Li D (2018). Transport stress changes blood biochemistry, antioxidant defense system, and hepatic HSPs mRNA expressions of Channel catfish *Ictalurus punctatus*. *Frontiers of Physiology*, doi.10.3389/fphys.2018.01628.

Ren Y, Men X, Yu Y, Li B, Zhou Y & Zhao C (2022). Effects of transportation stress on antioxidation, immunity capacity and hypoxia tolerance of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Reports*, doi.10.1016/j.aqrep.2021.100940.

Roberts RJ, Agius C, Saliba C, Bossier P & Sung YY (2010). Heat shock proteins (chaperones) in fish and shellfish and their potential role in relation to fish health: A review. *Journal of Fish Diseases*, **33**(10): 789-801.

Scheil AE, Köhler H-R & Triebskorn R (2011). Heat tolerance and recovery in Mediterranean land snails after pre-exposure in the field. *Journal of Molluscan Studies*, **77**(2): 165–174.

Tacchi L, Lowrey L, Musharrafieh R, Crossey K, Larragoite ET & Salinas I (2015). Effects of transportation stress and addition of salt to transport water on the skin mucosal homeostasis of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, https://doi.org/10.1016/j.aquaculture.2014.09.027

Teles M, Oliveira M, Jerez-Cepa I, Franco-Martinez L, Tvarijonaviciute A, Tort L & Mancera JM (2019). Transport and recovery of gilthead Sea Bream (*Sparus aurata* L.) sedated with clove oil and MS222: Effects on oxidative stress status. *Frontiers of Physiology*, doi.10.3389/fphys.2019.00523.

Tine M, Bonhomme F, McKenzie DJ & Durand JD (2010). Differential expression of the heat shock protein HSP70 in natural populations of the tilapia, *Sarotherodon melanotheron*, acclimatised to a range of environmental salinities. *BMC Ecology*, doi.10.1186/1472-6785-10-11

Tomanek L (2002). The heat-shock response: Its variation, regulation and ecological importance in intertidal gastropods (genus Tegula). *Integrative and Comparative Biology*, **42**(4): 797-807.

Urbinati EC & Carneiro PCF (2006). Sodium chloride added to transport water and physiological responses of Matrinxã *Brycon amazonicus* (Teleost: Characidae). *Acta Amazonica*, **36**(4): 569-572.

Varghese T, Ebeneeza S Dar SA & Pal AK (2021). Mitigation of stress in fish through nutraceuticals. *World Journal of Aquaculture Research & Development*, **3**(1): 5-16.

Vanderzwalmen M, Eaton L, Henriquez F, Carey P, Snellgrove D & Sloman K (2018). The use of feed and water additives for live fish transport. *Reviews in Aquaculture*, **11**(1): 263-278.

Wedemeyer GA (1996). Interactions with water quality conditions. In: *Physiology of Fish in Intensive Culture Systems*. (GA Wedemeyer, editor). Chapman and Hall, New York. Pp 60-110.

Winarti M, Sulmartiwi L & Lutfiyah L (2022). The effect of salinity on the expression of heat shock protein 70 (hsp70) in the gills and kidneys of srikandi tilapia (*Oreochromis niloticus*). *IOP Conference Series: Earth and Environmental Science*, doi.10.1088/1755-1315/1036/1/012092.

Wurts, WA. (1995). Using salt to transport live fish. *World Aquaculture*, **26**(3): 80-81.

Yang W-L, Jiang S, Yang Q-B, Jiang S-G, Huang J-H, Yang L-S, Chen X, Li Y-D & Zhou F-L (2023). Effects of vitamin C on transport of *Penaeus monodon*. *Israeli Journal of Aquaculture – Bamidgeh*, **75**(1): 1-10.

Zhang Y, Shang Z, Wnag G, You K & Mi D (2021). High concentrations of environmental ammonia induced changes in large-scale loach (*Paramisgurnus dabryanus*) immunity. *Ecology and Evolution*, **11**(13): 8614-8622.