Pakistan J. Zool., vol. 56(4), pp 1917-1922, 2024 DOI: https://dx.doi.org/10.17582/journal.pjz/20220926220926

Modifying the Design of Pond Production Systems can Improve the Health and Welfare of Farmed Nile Tilapia, *Oreochromis niloticus*

Wasseem Emam[1], Mohamed E. Bakr[2]*, Marwa F. Abdel-Kader[3], Mohamed M. Abdel-Rahim[4], Ashraf I.G. Elhetawy[4] and Radi A. Mohamed[1]

[1]Institute of Aquaculture, University of Stirling, Stirling, FK9 4LA, United Kingdom
[2]Department of Aquaculture, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt
[3]Department of Fish Diseases and Management, Sakha Aquaculture Research Unit, Central Lab for Aquaculture Research, A.R.C., Kafr El-Sheikh, Egypt
[4]Aquaculture Division, National Institute of Oceanography and Fisheries, Cairo, Egypt

Article Information
Received 26 September 2022
Revised 05 November 2022
Accepted 28 November 2022
Available online 09 June 2023 (early access)
Published 14 June 2024

Authors' Contribution
WE, MEB, MFA, MMA, RAM and AIGE presented the concept and performed acquisition. MMA, WE, MEB and RAM interpreted data. WE, MMA, AIGE, MEB, MFA and RAM wrote the manuscript. WE, RAM and MMA revised the manuscript. MEB, and MFA did samples analysis. AIGE performed statistical analysis.

Key words
Pond modification, Greenhouse, Behavioural fever, Growth performance, Immune response

ABSTRACT

Infected fish have been known to recover from mild illness when they are able to locate to warmer water. This study aimed to replicate this 'behavioural fever' effect in an aquaculture setting by artificially heating a section of a fish pond (thereby introducing a thermal gradient) and effectively modifying pond design. This was achieved through the construction of a 'greenhouse' type structure above a section of the pond. Over the length of the production cycle at three typical Nile tilapia (*Oreochromis niloticus*) farms, the study collected data on water quality and fish growth and at the end of the cycle, blood samples were taken and total production was recorded. At each farm, fish were divided into two identical ponds, one with a greenhouse covering 3% of the pond surface area and one without. Results showed that greenhouse was effective in warming the surface of the water immediately below it. Oxygen levels were also higher under the greenhouse than outside of it and higher than in the control pond. Fish reared in the greenhouse ponds tended to be larger than the control ponds and had improved physiological and immune status (*i.e.*, better liver and kidney function, higher antioxidant activity and lysozyme count; $p < 0.05$). The results of this study suggest that low-cost interventions that introduce thermal gradients in aquaculture systems may hold promise for improving health and welfare status of farmed fish in developing countries.

INTRODUCTION

Ectothermic species, unlike endotherms, do not experience fever as one of their key immune responses to infection. In the absence of this innate fever immune response, ectothermic animals such as fish have been shown to seek out warmer waters when struck with disease, a phenomenon that was first discovered in the bluegill (*Lepomis macrochirus*) by Reynolds *et al*. (1976) and subsequently termed behavioural fever. Behavioural fever is defined as an abrupt shift in an organism's temperature preferences brought about by the detection of a pathogen. Previous studies have reported higher survival in infected fish exposed to higher temperatures than their normal optimum temperature (Covert and Reynolds, 1977; Cerqueira *et al*., 2016). This implies that infected fish can potentially recover from mild illness when they are able to locate to warmer water.

Given the increasing number of disease outbreaks reported in aquaculture globally and in developing countries particularly, there is a need to find low-cost solutions to improving fish health (Emam *et al*., 2022). As such, the aim of this study was to explore the potential of modifying the systems in which fish are farmed in order to replicate the aforementioned behavioural prophylaxis effect. The easiest context in which to do this is in inland freshwater farming which is typically based on rearing fish in earthen ponds. One way of potentially creating a temperature gradient in such an earthen fish pond is through the construction of a greenhouse covering part of

* Corresponding author: OWO_Health@kfs.edu.eg
0030-9923/2024/0004-1917 $ 9.00/0

it (Kassem *et al*., 2016). A typical greenhouse consists of a concave-shaped structure of galvanised iron covered by a film of transparent ultraviolet low-density polyethylene (LDPE) (Kassem *et al*., 2016). This structure helps to retain the solar heat and can raise water temperature by 3–4°C inside and underneath the greenhouse compared to the external temperature, thereby theoretically allowing fish to express their natural thermoregulatory behaviour in the face of disease (Zhu *et al*., 1998). This study explores the effect of fish pond design modification in the form of installing a greenhouse on the growth, survival and health status of fish in inland pond aquaculture.

Egypt was chosen as the candidate country to test out this idea given its prominent status as a global aquaculture producer and the fact that fish diseases and mortalities are on the rise in this country (Emam *et al*., 2022). Egypt is also a developing country where fish farming provides an important source of protein and makes a key contribution to food security. The most commonly farmed species in Egypt is the Nile tilapia (*Oreochromis niloticus*) with a total production volume of 1.6 million tonnes in 2020 (Kaleem and Sabi, 2021). As such, this study centred on tilapia farms in particular. Within Egypt, the study sites were intentionally selected in the region most representative of national tilapia pond production, the Nile Delta governorate of Kafr El-Sheikh, where more than 50% of Egyptian tilapia are produced (GAFRD, 2014).

MATERIALS AND METHODS

Ethical approval

The protocol and conduct of this trial was approved by the Institutional Aquatic Animal Care and Use Committee, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Egypt (approval number: IAACUC-KSU-19-2018).

Production cycle and stocking parameters

This study followed the typical production cycle of three representative tilapia farms in Kafr El-Sheikh where two identical ponds in each farm were set up with the only difference being the addition of a greenhouse in one of the two ponds (the other pond was treated as a control). In April 2018, the six ponds across the three farms were stocked with mono-sex Nile tilapia fry (average body weight of 0.5 g) sourced from a local hatchery (Abbassa strain) at a density of 3.5 fish per m^2.

Greenhouse design

The greenhouses consisted of a galvanised iron frame covered by a translucent LDPE film of 250-micron thickness that has been treated to stabilise ultraviolet rays. The structures were designed to occupy 3% of the pond's total surface area and were attached to the pond's edge by pillars.

Fish growth performance

On the last day of the production cycle, at each study pond, a random sample of six adult Nile tilapia was taken from three random crates of different size grades. Sampled fish were then anesthetised in a solution of 25 mg/L buffered tricaine methane sulfonate (MS-222; Agent Laboratories, Redmond WA, USA) before being weighed and measured. Growth assessment variables were calculated as follows (where W_0 is the initial weight in grams, W_1 is the final weight in grams, and t is the experimental period in days):

Body weight gain (BWG) = final body weight (W_1) − initial body weight (W_0);

Specific growth rate (SGR % /day) = 100 × (ln(W_1) – ln(W_0)) / t;

Feed conversion ratio (FCR) = feed intake in grams / BWG in grams.

Haematological sampling

On-farm blood samples were collected from a total of 18 fish per study pond. Samples were extracted from the fish caudal vein using a 3 ml syringe and placed in Eppendorf tubes on ice. Upon return to the laboratory, blood samples were instantly centrifuged at 3000 rpm for 15 min at 4 °C for serum separation before being stored in the deep freezer at -20°C for future analysis.

Levels of serum albumin and total protein in the blood samples were estimated using the colorimetric methods by Doumas *et al*. (1971, 1981). Liver enzyme levels, serum creatinine and urea were similarly measured using standard colorimetric methods (Heinegård and Tiderström, 1973; Coulombe and Favreau, 1963) and total cholesterol and serum triglycerides were measured using the GPO-PAP commercial clinical kit method and the CHOD-PAP method (Fynn-Aikins *et al*., 1992).

Concentrations of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and malonaldehyde (MDA) were quantified using a commercially available ELISA kit (Inova Biotechnology, China). Similarly, serum lysozyme activity and immunoglobulin M (IgM) were quantified using commercially available ELISA kits (Cusabio, China).

Water quality parameters

Water temperature and dissolved oxygen concentrations were measured weekly using a handheld oxygen-temperature meter (Oxy-guard, Handy Polaris) at fixed points in both the modified and control ponds at each of the three farms. At each point, two readings were taken, one near the surface layer of the water column (30 cm from the water surface) and one near the bottom (30 cm from the pond bottom). Salinity, pH and total ammonia nitrogen (TAN) were measured at one point in each pond using a portable salinity refractometer (ATC, Generic, USA), a pH meter (Hanna Instruments, model AD11) and an ammonia medium-range photometer (Milwaukee Instruments, model Mi 405), respectively.

Total production data collection

Given the speed at which the harvest process is conducted and the fact that a large quantity of harvested fish are sold alive, total production for each pond at harvest had to be estimated using a method of representative sampling. This was done by weighing 30 fish from each size grade and counting the number of fish per crate in 3 crates of each size grade. The total number of crates was also recorded. The average weight, number of fish per crate of each size and total number of crates were used to estimate total production.

Statistical analysis

Data were examined for linearity, homogeneity of variance and distribution normality before analysis in SPSS (v16.0) and graphing in GraphPad Prism 6 (San Diego, CA, USA). Tests of significance were conducted using unpaired Student's *t*-test at a standard significance level of $p < 0.05$.

RESULTS

Introduction of a thermal gradient and environmental parameters

Surface water temperature immediately below the greenhouse was significantly higher than anywhere else in the pond or in the control pond. Dissolved oxygen concentrations were also significantly higher in the ponds with a greenhouse than in the control ponds (Table I). The same trend was observed across all three farms. However, there was no significant difference between greenhouse and control ponds in terms of bottom temperature, pH or total ammonia nitrogen.

Total production, growth performance and feed utilisation

Total yield or production was similar between both greenhouse and control ponds across all farms except that the proportion of larger-size fish ('super-size') tended to be greater in the ponds with a greenhouse (Table II). Fish reared in the greenhouse pond had a significantly larger final body weight than the fish reared in the control ponds without the greenhouse cover ($p < 0.05$; Table III).

Table I. Mean water quality parameters of the earthen ponds sampled in this study. The six ponds in the three farms were grouped together for this analysis. Surface and bottom refer to where in the water column the measurements were taken.

Environmental parameter	Control pond	Greenhouse pond
Dissolved oxygen (mg/L) - Surface	11.38±1.36[a]	13.02±2.12[b]
Bottom	8.39±2.80[a]	11.45±3.16[b]
Temperature (°C) - Surface	26.12±2.91[a]	28.06±2.34[b]
Bottom	26.42±2.31	26.82±1.95
Salinity (ppt)	4.33±1.30	4.321±1.25
pH	8.87±1.59	8.77±1.21
Total ammonia nitrogen (mg/L)	0.55±0.02	0.51±0.01

Means within the same row with different superscripts are significantly different ($P<0.05$).

Table II. Mean total production/yield (kg) of the 6 stocked ponds at the end of the production cycle. Totals are calculated by size grades. The six ponds in the three farms were grouped together for this analysis.

Size grade	Control pond	Greenhouse pond
Super size	765.3 ± 97.44[a]	1400 ± 630.1[b]
Grade 1	625.3 ± 186.7	849.3 ± 236.7
Grade 2	87.33 ± 82.38	23 ± 16.92
Trash fish	214.7 ± 103.9[b]	21.33 ± 4.06[a]
Total	1478 ± 217.4	2272 ± 844.9

Means within the same row with different superscripts are significantly different ($P<0.05$).

Table III. Growth performance and feed utilization efficiency of the Nile tilapia (*Oreochromis niloticus*) sampled during this study. The six ponds in the three farms were grouped together for this analysis.

Morphological parameter	Control pond	Greenhouse pond
Initial body weight (g)	0.5 ±0.23	0.5 ±0.12
Final body weight (g)	157.7 ±33.51[a]	189.3 ±15.39[b]
Weight gain (g)	157.2 ±33.51[a]	188.8 ±15.39[b]
Feed conversion ratio	2.34 ±0.36	2.133 ±0.26
Specific growth rate (%/day)	3.26 ±0.55	3.375 ±0.47
Daily weight gain (g/day)	0.88 ±0.23	0.9933 ±0.2
Final length (cm)	20.46 ±0.64	20.98 ±0.72

Means within the same row with different superscripts are significantly different ($p<0.05$).

Table IV. Blood serum biochemical profile of the Nile tilapia (*Oreochromis niloticus*) sampled in this study. The six ponds in the three farms were grouped together for this analysis.

Blood parameter	Control pond	Greenhouse pond
Alanine aminotransferase (U/L)	30.17±1.26	27.93±0.54
Aspartate aminotransferase (U/L)	26.57±0.84[a]	21.76±0.57[b]
Total protein (g/dL)	3.36±0.05[b]	3.89±0.03[a]
Albumin (g/dL)	1.36±0.03[b]	1.59±0.03[a]
Globulin (g/dL)	1.99±0.05[b]	2.31±0.02[a]
Urea (mg/dL)	3.261±0.04[a]	3.07±0.02[b]
Creatinine (mg/dL)	0.37±0.004[a]	0.318±0.006[b]
Triglycerides (mg/dL)	107.7±2.35[a]	99.76±1.64[b]
Cholesterol (mg/dL)	111.7±1.73[a]	98.41±1.72[b]

Means within the same row with different superscripts are significantly different (P<0.05).

Blood analysis

The levels of kidney and liver function biomarkers (AST, ALT, albumin, total protein, urea, and creatinine) recorded in the greenhouse pond were significantly higher than the control group and closer to the reference range thereby indicating better performance (Table IV). However, the fish reared in the greenhouse ponds had higher concentrations of blood lipids (triglycerides and cholesterol). Similarly, serum anti-oxidants level were significantly higher (increased SOD, CAT and GPX, and decreased MDA) in fish reared in the greenhouse ponds than in the control one. Immunological parameters (IgM and lysozyme activity) were also significantly improved in the fish reared in the greenhouse ponds than in the control pond.

DISCUSSION

The fact that the greenhouse in this study heated the surface of the water directly below it suggests that this is an effective method of introducing a thermal gradient in an inland fish earthen pond, which is line with other studies on this topic (Kassem *et al*., 2016; Nzula *et al*., 2020). Given the established link between higher temperatures and higher photosynthetic activity, the reason dissolved oxygen concentrations (DO) were higher under the greenhouse than outside could be a result of higher photosynthetic activity associated with the higher temperatures under the greenhouse. Similarly, it is not clear whether the improvement in physiological and haematological parameters observed in the greenhouse-reared fish is a result of allowing fish to exhibit behavioural prophylaxis or just as a simple consequence of improving DO (which was unintentional and unexpected) as suggested by Mjoun *et al*. (2010).

Water temperature is undoubtedly a key driver of physiological and metabolic responses in all aquatic animals (Mjoun *et al*., 2010). For example, fish metabolic rates, appetite and food consumption are all known to increase in line with water temperature (Zvavahera *et al*., 2018; Nzula *et al*., 2020). Consequently, fish reared in the pond with a slightly warmer section could have a greater feed intake and therefore grow to a larger size. Previous studies have indeed documented a similar phenomenon (Likongwe *et al*., 1996; Tribeni *et al*., 2010; Musal *et al*., 2012; Hamed *et al*., 2021). This theory has been confirmed based on the larger fish harvested from the greenhouse ponds.

Haematological parameters of ectotherms are also influenced by temperature (Gillooly *et al*., 2001; Somero, 2002; Clarke and Fraser, 2004). The lower levels of liver and kidney biomarkers observed in the fish in the greenhouse ponds would generally imply improved liver function; however, levels observed across all study ponds were still reasonably close to reference range and therefore no signs of severe liver or kidney disease or damage were observed. Similarly, and despite significant differences in blood lipid concentrations, all observed levels were not excessively high. Such differences could be explained by the thermal effect given that fish are known to be able to rapidly modify their pattern of fatty acid production in response to temperature changes (Leslie and Buckley, 1976). However, given that the temperature ranges observed in this study are all within the Nile tilapia's optimum range of 12-30°C, it is not likely that these changes are driven by water temperature directly but perhaps by less severity and incidence of disease implying that the behavioural prophylaxis design has indeed been effective.

The observed increase in immunological parameters can also be attributed to the increased temperature. For example, Ndong *et al*. (2007) reported a significant increase in finfish lysozyme activity in animals exposed to higher temperatures within their temperature scope. Harrahy *et al*. (2001) suggest that in most teleost fishes, lysozyme levels are directly correlated with water temperature and that low water temperatures result in reduced lysozyme activity. This phenomenon could at least partially contribute to an improved immune status in fish cultured under a greenhouse.

Previous studies by Parihar *et al*. (1997) and Verlecar *et al*. (2007) reported improved antioxidant levels in aquatic animals when exposed to higher temperatures within their thermal tolerance range. Antioxidants can be an important biomarker of oxidative stress level in aquatic organisms (Davey *et al*., 2005). As such, it is conceivable that modifying inland culture ponds by the addition of a greenhouse could contribute to favourably reducing oxidative stress in the fish farmed in that pond. However, that effect would probably be limited to the fish directly exposed to the warmer surface waters under the greenhouse given that we did not record warmer water anywhere else in the pond.

CONCLUSIONS

The results of this study demonstrated that constructing a greenhouse over part of an inland fish pond in a subtropical region can increase the surface water temperature underneath it and thereby introduce a thermal gradient within the pond. As such, it is possible to apply the theory of behavioural fever to an aquaculture setting. The improvements in physiological and immune status observed in the greenhouse ponds suggest that fish health and welfare were favourable in these ponds. However, this did not lead to major overall differences in terms of survival rates or production. Different variations of this greenhouse design (e.g., covering a greater surface area or closer to water surface) may lead to more notable differences. We therefore conclude that it is worthwhile to further explore modifications to inland fish pond design that could introduce thermal gradients. Such interventions should ideally be simple to implement and affordable for small-scale farmers in developing countries.

ACKNOWLEDGEMENTS

The authors would like to thank the Department of Aquaculture, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Egypt, for providing facilities to carry out this experiment.

Funding

This study utilised existing resources put in place as part of a British Council-Newton Mosharafa Institutional Links grant (entitled 'BOLTI EGYPT') provided to the University of Stirling, Kafr El-Sheikh University and World Fish Egypt in 2016-18.

IRB approval and ethical statement

The Institutional Aquatic Animal Care and Use in Research Committee, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Egypt, approved the protocol and conduct of the present experiment (approval number: IAACUC-KSU-19-2018).

Availability of the data and materials

All relevant data are available from the authors upon request.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Cerqueira, M., Rey, S., Silva, T., Featherstone, Z., Crumlish, M., and MacKenzie, S., 2016. Thermal preference predicts animal personality in Nile tilapia *Oreochromis niloticus*. *J. Anim. Ecol.*, **85**: 1389-1400. https://doi.org/10.1111/1365-2656.12555

Clarke, A., and Fraser, K., 2004. Why does metabolism scale with temperature? *Funct. Ecol.*, **18**: 243-251. https://doi.org/10.1111/j.0269-8463.2004.00841.x

Coulombe, J., and Favreau, L., 1963. A new simple semimicro method for colorimetric determination of urea. *Clin. Chem.*, **9**: 102-108. https://doi.org/10.1093/clinchem/9.1.102

Covert, J.B., and Reynolds, W.W., 1977. Survival value of fever in fish. *Nature,* **267**: 43-45. https://doi.org/10.1038/267043a0

Davey, M., Stals, E., Panis, B., Keulemans, J., and Swennen, R., 2005. High-throughput determination of malondialdehyde in plant tissues. *Anal. Biochem.*, **347**: 201-207. https://doi.org/10.1016/j.ab.2005.09.041

Doumas, B.T., Bayse, D.D., Carter, R.J., Peters, Jr., T., and Schaffer, R., 1981. A candidate reference method for determination of total protein in serum I. Development and validation. *Clin. Chem.*, **27**: 1642-1650. https://doi.org/10.1093/clinchem/27.10.1642

Doumas, B.T., Watson, W.A., and Biggs, H.G., 1971. Albumin standards and the measurement of serum albumin with bromcresol green. *Clin. Chim. Acta,* **31**: 87-96. https://doi.org/10.1016/0009-8981(71)90365-2

Emam, W., El-Rewiny, M.N., Abou Zaid, A.A., El-Tras, W.F., and Mohamed, R.A., 2022. Trends in the use of feed and water additives in Egyptian tilapia culture. *Aquacult. Res.,* **53**: 3331–3336. https://doi.org/10.1111/are.15840

Fynn-Aikins, K., Hung, S.S., Liu, W., and Li, H., 1992. Growth, lipogenesis and liver composition of juvenile white sturgeon fed different levels of D-glucose. *Aquaculture,* **105**: 61-72. https://doi.org/10.1016/0044-8486(92)90162-E

GAFRD, 2014. General authority for fish resources development. In: *Fish statistics yearbook*. Ministry of Agriculture and Land Reclamation, Cairo, Egypt.

Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M., and Charnov, E.L., 2001. Effects of size and temperature on metabolic rate. *Science,* **293**: 2248-2251. https://doi.org/10.1126/science.1061967

Hamed, S.A., Abou-Elnaga, A., Salah, A.S., Abdel-Hay, A.H.M., Zayed, M.M., Soliman, T., Mohamed, R.A., 2021. Effect of water temperature, feeding frequency, and protein percent in the diet on water quality, growth and behavior of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758). *J. appl. Ichthyol.,* **37**: 462-473. https://doi.org/10.1111/jai.14193

Harrahy, L.N., Schreck, C.B., and Maule, A.G., 2001. Antibody-producing cells correlated to body weight in juvenile chinook salmon (*Oncorhynchus tshawytscha*) acclimated to optimal and elevated temperatures. *Fish Shellfish Immunol.,* **11**: 653-659. https://doi.org/10.1006/fsim.2001.0342

Heinegård, D., and Tiderström, G., 1973. Determination of serum creatinine by a direct colorimetric method. *Clin. Chim. Acta,* **43**: 305-310. https://doi.org/10.1016/0009-8981(73)90466-X

Kaleem, O., and Sabi, A.F.B.S., 2021. Overview of aquaculture systems in Egypt and Nigeria, prospects, potentials, and constraints. *Aquacult. Fish.,* **6**: 535-547. https://doi.org/10.1016/j.aaf.2020.07.017

Kassem, M.M., Elsbaay, A.M., AbouZaher, S.E., and Abdelmotaleb, I.A., 2016. Energetic performance assessment of a thermo-solar greenhouse fish (Nile tilapia) hatchery. *Misr J. agric. Eng.*, **33**: 1649-1674. https://doi.org/10.21608/mjae.2016.97625

Leslie, J., and Buckley, J., 1976. Phospholipid composition of goldfish (*Carassius auratus* L.) liver and brain and temperature-dependence of phosphatidyl choline synthesis. *Comp. Biochem. Physiol. B Comp. Biochem.,* **53**: 335-337. https://doi.org/10.1016/0305-0491(76)90337-0

Likongwe, J.S., Stecko, T.D., Stauffer, Jr, J.R., and Carline, R.F., 1996. Combined effects of water temperature and salinity on growth and feed utilization of juvenile Nile tilapia *Oreochromis niloticus* (Linneaus). *Aquaculture,* **146**: 37-46. https://doi.org/10.1016/S0044-8486(96)01360-9

Mjoun, K., Rosentrater, K., and Brown, M.L., 2010. Tilapia: Environmental biology and nutritional requirements. *Fact sheets*. Paper 164., http://openprairie.sdstate.edu/extension_fact/164.

Musal, S., Orina, P.S., Aura, C.M., Kundu, R., Ogello, E.O., and Munguti, J.M., 2012. The effects of dietary levels of protein and greenhouse on growth, behaviour and fecundity of Nile tilapia (*Oreochromis niloticus* L.) broodstock. *Int. J. Sci. Res.*, **10**: 2271-2278.

Ndong, D., Chen, Y.Y., Lin, Y.H., Vaseeharan, B., and Chen, J.C., 2007. The immune response of tilapia *Oreochromis mossambicus* and its susceptibility to *Streptococcus iniae* under stress in low and high temperatures. *Fish Shellfish Immunol.,* **22**: 686-694. https://doi.org/10.1016/j.fsi.2006.08.015

Nzula, K., Yasindi, A., and Akidiva, A., 2020. Influence of greenhouse technology on selected pond water quality parameters and growth performance of Nile tilapia in high altitude areas. *Int. J. Fish. aquat. Stud.*, **8**: 142-147.

Parihar, M., Javeri, T., Hemnani, T., Dubey, A., and Prakash. P., 1997. Responses of superoxide dismutase, glutathione peroxidase and reduced glutathione antioxidant defenses in gills of the freshwater catfish (*Heteropneustes fossilis*) to short-term elevated temperature. *J. Therm. Biol.,* **22**: 151-156. https://doi.org/10.1016/S0306-4565(97)00006-5

Reynolds, W.W., Casterlin, M.E., and Covert, J.B., 1976. Behavioural fever in teleost fishes. *Nature,* **259**: 41-42. https://doi.org/10.1038/259041a0

Somero, G.N., 2002. Thermal physiology and vertical zonation of intertidal animals: Optima, limits, and costs of living. *Integr. Comp. Biol.,* **42**: 780-789. https://doi.org/10.1093/icb/42.4.780

Tribeni, D., Tiwari, G., and Bikash, S., 2010. Thermal performance of a greenhouse fish pond integrated with flat plate collector. *Int. J. agric. Res.,* **5**: 851-864. https://doi.org/10.3923/ijar.2010.851.864

Verlecar, X., Jena, K., and Chainy, G., 2007. Biochemical markers of oxidative stress in *Perna viridis* exposed to mercury and temperature. *Chem. Biol. Interact.,* **167**: 219-226. https://doi.org/10.1016/j.cbi.2007.01.018

Zhu, S., Deltour, J., and Wang, S., 1998. Modeling the thermal characteristics of greenhouse pond systems. *Aquacult. Eng.,* **18**: 201-217. https://doi.org/10.1016/S0144-8609(98)00031-4

Zvavahera, C.C., Hamandishe, V.R., Saidi, P., Saidi, P., Imbayarwo, V., and Nhiwatiwa, T., 2018. Modification of the *Oreochromis* spp. aquaculture production environment using greenhouses. *J. Aquacult. Eng. Fish. Res.,* **4**: 64-72.