ISSN: 2278-5213

RESEARCH MANUSCRIPT

Effect of transportation stress on the humoral immunity of catla fry and fingerlings

Iqlas Ahmed[1] and K.B. Shenoy[2]
[1]Dept. of Aquaculture, College of Fisheries, Mangalore-575002, India
[2]Dept. of Applied Zoology, Mangalore University, Mangalagangotri-574199, Mangalore, India
iqlas.ahmed@gmail.com; +91 824 2249256; +91 9739769190; Fax: +91 824 2248366

Abstract

Routine aquaculture procedures such as, handling, netting, crowding, confinement and live transport (in approximate order of duration) evoke stress response in fish. This study was carried out to quantify the effect of simulated transport on the production of IgM in catla fry and fingerlings. Fry (0.052-0.078 g) and fingerlings (1.18–1.34 g) of catla were packed in 18 L capacity polyethylene bags and two batches bags were kept in an orbital shaker for 10 and 20 h to simulate transport of fish seed. Immunoglobulin levels were measured after 2, 4, 8 and 20 d intervals post stress. Reduction in the IgM concentration as a consequence of transportation of stress was observed. While, the production of immunoglobulin increased steadily over time in control unstressed group, there was an initial decline in the levels of immunoglobulin both in the fry and fingerlings as a result of 10 h as well as 20 h of transport which increased later. But the increase in the levels of immunoglobulin post initial depression was less prominent in stressed fish than in the control group. The decline in IgM levels post transportation stress was sharper and for a longer period in the fry than in the fingerlings (up to 8 d in fry compared to 4 d in fingerlings). Hence, smaller catla seed appear to be more sensitive to stress than the larger ones. This study has clearly demonstrated that transportation stress causes reduction in immunity of catla seed.

Keywords: Stress response, catla, immunoglobulin, live transport, fry and fingerlings, immunity.

Introduction

Routine aquaculture procedures such as, handling, netting, crowding, confinement and live transport (in approximate order of duration) evoke stress response in fish (McDonald and Milligan, 1997). Quantifying stress is necessary to understand the effect of handling on fish seed performance. Stressors in intensive systems are unavoidable (Iwama *et al.,* 1997). While severe stress can be fatal, sub-lethal stress can compromise the performance of fish in terms of survival, growth and immuno-competance leading to sub-optimal production (Iwama *et al*., 1997) and reduced welfare of fish (Ashley, 2007). Hence it is prudent to understand effect of stress on fish, in order to alleviate stress in fish culture systems and increase production and welfare of farmed fish. Catla is one of the most widely cultivated species in the Asian sub-continent. The production of catla in 2008 through aquaculture was 2.4 million tons valued at 3.5 billion USD (FAO, 2010). The seed of catla are routinely transported from hatcheries and rearing centres to grow-out farms, in sealed plastic bags filled with water and pure oxygen. Live fish transport, particularly of fish seed is an important part of fish husbandry. There is a need to reduce stress during these procedures. The stress response is first primary, then secondary and finally tertiary. Lowered immune-competence is one of the most important tertiary responses to stress. The tertiary response has a direct bearing on the survival of the fish, particularly of the early life history stages such as seed of catla. Stress increases susceptibility of fish to diseases, resulting many times in mortality (FSBI, 2002; Ashley, 2007). Quantifying the tertiary response would help to devise ways to mitigate stress in catla seed during transport. Thus, the objective of the study was to quantify the effect of transportation stress on the humoral immunity of catla fry and fingerlings.

Materials and methods

Fry (0.052-0.078 g) and fingerlings (1.18–1.34 g) of catla were packed in 18 L capacity polyethylene bags. A total of six bags were used for fry and another six for fingerlings. Three bags containing fry and another three bags containing fingerlings were kept in an orbital shaker for 10 h to simulate transport of fish seed. The procedure was repeated with another similar set of bags for 20 h. At the end of the experimental period, the bags were opened and the fish were maintained in plastic pools separately for each bag for further sampling. Pooled samples (n=8) from each of the pool were collected at 2, 4, 8 and 20 d intervals for preparation of whole body extracts for estimation of immunoglobulin levels. Samples collected before packing were considered as 0 d samples (pre-stress levels). Whole body extracts of samples of catla fry and fingerlings were prepared according to Breuil *et al*. (1997) with slight modifications in the speed of centrifugation. The samples were homogenized with three volumes of phosphate buffered saline (PBS), pH 7.4.

The homogenate was centrifuged in a refrigerated centrifuge maintained at 4°C, at 12,000 rpm for 15 min. The supernatant was re-centrifuged for 10 min at the same rpm, which was then stored at -40°C till they were analyzed for immunoglobulin levels. A protease inhibitor cocktail (Sigma, USA) was added @ 100 µL per 1 mL of the supernatant to prevent degradation of the immunoglobulin. The extracts so obtained were stored at -40°C till the analysis.

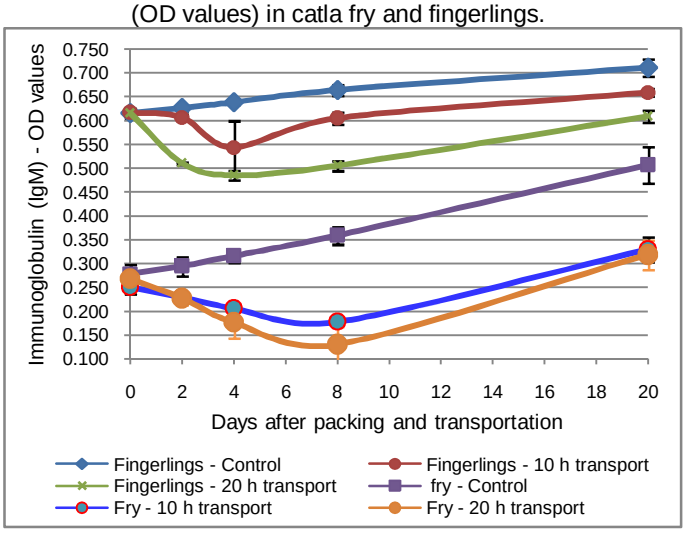
Protein estimation: Protein concentration of the whole body extracts was determined using the method of Lowry *et al*. (1951) by employing a protein estimation kit (Genei India Pvt. Ltd., Bangalore). The extract was suitably diluted to get the desired concentration of 10 µg/mL since this concentration was found to be the optimum level for getting good results (Lokesh, 2009).

Enzyme-linked immunosorbent assay: ELISA was carried out according to Furuta *et al*. (1995) with some modifications. Microtitre plates were coated with 100 µL of the whole body extracts having a protein concentration of 10 µg/mL and incubated at 4°C, overnight. Unbound proteins were poured off and the plates were washed with PBS-T20. After three minutes they were again washed with PBS. 300 µL of PBS-5% skimmed milk was then added to each of the wells and incubated for 2 h at the ambient temperature (25°C) to block the residual protein binding sites. The blocking solution was poured off and the plate was washed once with PBS-T20 and again with PBS. 100 µL solution of monoclonal antibodies (MAb) against catla IgM was poured into each well except the antibody blank, and incubated for 3 h at the ambient temperature. The excess solution of MAb was poured off and the plates were washed once with PBS-T20 and twice with PBS. Rabbit anti-mouse IgG peroxidase conjugate (Genei India Pvt. Ltd., Bangalore) was then added to the wells, which were incubated for 45 min at the room temperature. The antibody solution was poured off and the wells were washed three times with PBS-T20 and once with PBS. 100 µL of substrate solution containing tetramethylbenzidine and hydrogen peroxide, diluted in distilled water in a ratio of 1:20 was added to the wells and incubated for 10 min at the ambient temperature in the dark. The reaction was stopped after 10 min by adding 50 µL of 2N H_2SO_4. The optical density (OD) values of the colour developed were read using a microplate reader (Bio-tek Instruments Inc. USA) at 450 nm.

Results

Figure 1 depicts the trend of effect of transportation stress on the immunoglobulin levels of catla fry and fingerlings after 10 and 20 h of simulated transport. The immunoglobulin levels in control group of both fry and fingerling continuously increased during the experimental period of 20 d.

Fig. 1. Effect of transportation stress on immunoglobulin levels (OD values) in catla fry and fingerlings.



In control fry group not subjected to transportation stress, the OD values of immunoglobulin on 0 d were 0.279 ± 0.018, which increased to 0.507 ± 0.039 after 20 d. In fingerlings control group, the OD value on 0 d was 0.616 ± 0.010 which increased to 0.711 ± 0.019 on day 20. The rate of increase in OD values of immunoglobulin was faster in fry as compared to fingerlings not subjected to transportation stress. The OD values of immunoglobulin declined sharply in both fry and fingerlings subjected to 10 and 20 h of transportation stress. In fry, it declined up to 8 d post stress before it started to increase up to the day 20 of experimental period, but the magnitude of increase was less than that of control group. Unlike in fry, the OD values of immunoglobulin in fingerlings declined only up to 4 d post stress after which they started to increase, but not to the level of control group. The decline in OD values was more drastic in fry and fingerlings subjected to 20 h transportation stress as compared to 10 h transportation stress. The magnitude of decline in OD values of immunoglobulin was more severe in fingerlings as compared to fry but the time of recovery post-stress was earlier in fingerlings.The OD values in fry subjected to 10 h post stress decreased from pre-stress levels of 0.251 ± 0.015 to 0.229 ± 0.011 after 2 d, and to 0.207 ± 0.006 after 4 d and 0.178 ± 0.007 after 8 d. Thereafter, the value increased to 0.330 ± 0.026 after 20 d. In fry subjected to 20 h of transportation stress, the OD values decreased to 0.227 ± 0.013 from pre-stress levels of 0.238 ± 0.028 after 2 d post stress. It further decreased to 0.177 ± 0.034 and 0.130 ± 0.034 after 4 and 8 d post stress respectively. Thereafter, the values increased to 0.318 ± 0.031 after 20 d. In fingerlings transported for 10 h the OD value of immunoglobulin decreased to 0.607 ± 0.007 after 2 d from pre-stress levels of 0.617 ± 0.006. After 4 d it further decreased to 0.544 ± 0.056, while after 8 d post stress it increased to 0.605 ± 0.013. After 20 d post-stress the value of OD increased to 0.660 ± 0.008.

The OD values of immunoglobulin in catla fingerlings post 20 h transportation stress decreased sharply to 0.511 ± 0.002 after 2 d from the initial pre-stress values of 0.616 ± 0.002 and continued to decrease to 0.486 ± 0.010. It increased to a value of 0.505 ± 0.010 on day 8 and finally to 0.609 ± 0.013 on day 20, the last day of the experimental period.

Discussion

It is well known that stress results in elevation of cortisol. Immuno-suppressive effects of stress, as a result of cortisol elevation have been well documented (Pickering and Pottinger, 1985; Maule *et al*., 1989; Barton and Iwama, 1991). *In vitro* administration of cortisol resulted in reduced number of IgM secreting cells as well as the production of IgM by these cells in the carp *Cyprinus carpio* (Saha *et al*., 2004). Handling resulted in reduced plasma proteins and immunoglobulin levels in the carp (Raune *et al.,* 1999) and red sea bream (Biswas *et al*., 2006).

Reduction in the IgM concentration as a consequence of transportation of stress was also observed in this study. While, the production of immunoglobulin increased steadily over time in control unstressed group, there was an initial decline in the levels of immunoglobulin both in the fry and fingerlings as a result of 10 h as well as 20 h of transport which increased later. But the increase in the levels of immunoglobulin post initial depression was less prominent in stressed fish than in the control group. Lokesh (2009) has recorded a steady increase in the levels of IgM with the age in catla and rohu fry and fingerlings from day 21 post hatch to 91 d post hatch. Prakruthi (2011) working on the effect of stress on the ontogeny of humoral immunity in catla reported that although stress did not affect the ontogeny of immune response, it was weaker in catla fry and fingerlings subjected to crowding and handling stress. She too has recorded a steady increase in the levels of immunoglobulin in both unstressed and stressed catla seed from 21 d post hatch onwards. Since the levels of immunoglobulin differ significantly between fish of different size but of same age (Johnson *et al*., 1982a, b; Lokesh, 2009; Prakruthi, 2011), the increase observed in the levels of IgM in the studies of Lokesh (2009) and Prakruthi (2011) in catla and rohu seed is a result of increase in weight with the age rather than an increase in the age. The decline in the IgM levels post transportation stress was sharper and for a longer period in the fry than in the fingerlings (up to 8 d in fry compared to 4 d in fingerlings). Hence smaller catla seed appear to be more sensitive to stress than the larger ones.

Conclusion

It can be concluded from the study that transportation stress affects production of immunoglobulin in catla fry and fingerlings and that fry are more affected by transportation stress than fingerlings.

Acknowledgements

The first author is grateful to Dr. K.M. Shankar, the Dean, College of Fisheries, Mangalore, for providing the laboratory facilities for carrying out this study.

References

1. Ashley, P.J. 2007. Fish welfare: Current issues in aquaculture. *App. Anim. Behav. Sci.* 104: 199-235.
2. Barton, B.A., and Iwama, G.K., 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Ann. Rev. Fish Dis*. 1: 3-26.
3. Biswas, A.K., Seoka, M., Tanaka, Y., Takii, K. and Kumai, H. 2006. Effect of photoperiod manipulation on the growth performance and stress response of Juvenile sea bream (*Pagrusmajor*). *Aquaculture.* 258: 350-356.
4. Breuil, G., Vassiloglou, B., Pepin, J.F. and Romestand, B. 1997.Ontogeny of IgM-bearing cells and changes in the immunoglobulin M-like protein level (IgM) during larval stages in sea bass (*Dicentrarchuslabrax*). *Fish Shellfish Immunol.*7: 29-43.
5. FAO. 2010. FAO Year Book, Fishery and Aquaculture Statistics 2008. Rome. p.72.
6. FSBI. 2002. Fish welfare briefing paper 2. Fisheries Society of the British Isles, Grant Information Systems, Swanston, Cambridge.
7. Furuta, T., Anichnam, T., Zakaguchi, J., Akaba, Y. and Ashi, H. 1995. Indirect Enzyme Linked Immunosorbant Assay (ELISA) for the detection of antibody in serum of Japanese flounder. *Fish Sci.*61: 663-667.
8. Iwama, G.K., Pickering A.D., Sumpter, J.P. and Schreck, C.B. 1997. Fish stress and health in aquaculture, Cambridge University Press, Cambridge.
9. Johnson, K.A., Flynn, J.K. and Amend, D.F. 1982a. Onset of immunity in salmonid fry vaccinated by direct immersion in *Vibrio anguillarum* and *Yersinia ruckeri*bactrins. *J. Fish Dis.*5: 197-205.
10. Johnson, K.A., Flynn, J.K. and Amend, D.F. 1982b. Duration of immunity in salmonids vaccinated by direct immersion with *Yersinia ruckeri and Vibrio anuillarum* bacterins. *J. Fish Dis.* 5: 207-213.
11. Lokesh, J. 2009. Ontogeny of humoral immunity in Indian major carps – studies using monoclonal antibody based ELISA. M.F.Sc., thesis, submitted to the Karnataka Veterinary Animal and Fisheries Sciences University, Bidar, India.
12. Lowry, O.H., Rosenbrough, N.H., Farr, A.L. and Randall, R.J. 1951. Protein measurements with folin phenol reagent. *J. Biol. Chem.* 183: 265-275.
13. Maule, A.G., Tripp, R.A, Kaattari, S.L. and Schreck, C.B. 1989. Stress alters immune function and disease resistance in Chinook salmon *Oncorhynchus tshawytscha. J. Endocrinol*. 120: 135-142.
14. McDonald, G. and Milligan, L. 1997. Ionic, osmotic and acid-base regulation in stress. In: Iwama, G.K., Pickering, A.D., Sumpter, J.P. and Schreck, C.B., (Eds.), Fish stress and health in aquaculture, Cambridge University Press, Cambridge, pp.119-144.
15. Pickering, A.D. and Pottinger, T.G. 1985. Cortisol can increase the susceptibility of brown trout, *Salmo trutta* to disease without reducing the white blood cell count. *J. Fish Biol*. 27: 611-617.
16. Prakruthi, G.S. 2011. Effect of stress on ontogeny of humoral immunity in catla. M.F.Sc., thesis submitted to the Karnataka Veterinary Animal and Fisheries Sciences University, Bidar, India.
17. Ruane, N.M., WendelaarBonga, S.E. and Balm, P.H.M. 1999. Differences between rainbow trout and brown trout in the regulation of the pituitary-interrenal axis and physiological performance during confinement. *Gen. Comp. Endocrinol*. 113: 210-219.
18. Saha, N.R., Usami, T. and Suzuki, Y. 2004. *In vitro* effects of steroid hormones on IgM-secreting cells and IgM secretion in common carp (*Cyprinus carpio*). *Fish Shellfish Immunol*. 17: 149-158.

