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Modulation of the innate immune responses in the striped snakehead murrel, *Channa striata* upon experimental infection with live and heat killed *Aeromonas hydrophila*

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Abstract
It is well-known that the innate immune mechanisms in fish serve as the first line of defence against wide variety of pathogens. In most of the situations, innate responses get induced and enhanced after the pathogen invasion. It would be interesting to look into the inducibility of various innate immune mechanisms and the level of enhancement after infection with the pathogen. Hence, in the present investigation, modulation of the innate immune responses in the striped snakehead murrel, *Channa striata* on experimental challenge with either live virulent or heat killed *Aeromonas hydrophila* at a dose of $1x10^7$ CFU (suspended in 0.2 mL PBS) were measured. Most of the non-specific (both humoral and cellular) immune responses tested were substantially induced or enhanced in both the experimental groups in comparison with the unchallenged control group. Significant increase in the lysozyme, total peroxidase, antiprotease and respiratory burst activities were observed after the pathogen challenge. Thus, most of the innate non-specific immune responses are inducible though they are constitutive of fish immune system exhibiting a basal level of activity even in the absence of pathogen challenge.
Keywords: *Aeromonas hydrophila*, Experimental challenge, Innate immune response, Striped snakehead murrel.

Introduction

Vertebrates have developed a complex immune system to recognize non-self and mount a neutralizing response towards foreign invaders like the microbes. The microbial invasion is initially countered by innate defences that begin to act within minutes of encounter with the infectious agent (Riera Romo *et al*., 2016). Hence, normally, the innate immunity is sufficient to prevent the body from being routinely overwhelmed by the vast number of microorganisms that live on and in it (Sorci *et al*., 2013).

As in other vertebrates, in fish, the innate immunity is the primary defence mechanism which is fairly active and diverse (Buchmann, 2014). The innate immune response includes cellular and molecular components that are present and ready for action even before an antigen challenge is encountered. Nevertheless, several humoral substances and cell secretions are thought to lead to the natural resistance of fish to pathogenic and infectious factors. It has been considered for a long time that these innate components are less specific than those of the adaptive immune system and they are non-inducible in nature after pathogen invasion (Akira *et al*., 2006).

Recently, it has been shown that innate immunity can sometimes be mobilized to mount a more specific response to individual pathogens than was once thought possible (Subramani *et al*., 2016a; Martin-Gayo and Yu, 2017). Thus, the innate immune response is not only constitutive but also inducible by external molecules involved in the non-specific defence mechanisms of organisms (Begam and Sengupta, 2015; Kumaresan *et al*., 2016). Further, the innate immune system appears to be important in the induction of adaptive immunity when a pathogen is present but fails to prime such responses in the absence of infection (Zoccola *et al*., 2017). The innate immune responses could be modulated by various external factors like water temperature, fish population density and the presence of pathogen (Magnadottir, 2010) and among them, the pathogen exert relentless modulation of the innate immune responses.

An understanding of the immune system of the fish species being cultured is important for improved husbandry and health management of the species. The striped snakehead murrel, *Channa striata* is one of the economically important freshwater fish (US$ 8-10 per kg) that contributes 13% of the marketable airbreathing fish of India (Jawahar *et al*., 2016). It is considered as a valuable food fish not only because of its deliciousness but also of its medicinal value especially, the wound healing properties after surgical operations and reduction of the post-operative pain (Mohd and Abdul Manan, 2012; Haniffa *et al*., 2014).

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However, its production has been dwindling mainly because of outbreaks of diseases caused by pathogens including *Aphanomyces invadans* and *Aeromonas hydrophila* (Jawahar *et al*., 2016).

Despite the fact that there are reports available regarding medicinal value and many other related details about *C. striata*, details regarding the nature of immune defences of this species is scarce. In one of the earlier studies, antibody response against formalin killed *A. hydrophila* was studied in *C. striata* (Rauta *et al*., 2013) but there was no mention of innate immune responses.

The objective of the present study was to study the basal level of the innate immune responses of *C. striata* and modulation of them if they are inducible, on exposure to experimental infection with *A. hydrophila*, one of the associated bacterial pathogens.

Materials and Methods

Fish maintenance

The striped snakehead murrel, *C. striata* (n=240, average weight, 100±25 g) were collected from the local farm in Madurai (9.9252° N, 78.1198° E), India. The fish were acclimated for 2 weeks in fibre-reinforced plastic (FRP) tanks of 500 L capacity with the stocking density of $5gL^{-1}$, under continuous aeration and recirculation of water by external biofilters (Eheim, Germany).

Fish were fed with standard pellet feed prepared in this laboratory according to Vincent (1987) at 2% of body weight per day. Briefly, the feed contained 35% crude protein and 6.5% dietary lipid. About one-fourth of the water was siphoned daily to remove the faeces and unused food particles with subsequent water level adjustment.

The physicochemical parameters such as temperature, pH, dissolved oxygen, hardness and salinity were measured using standard methods (Albertson, 2007) and the fish were maintained at an ambient photoperiod (about 12L:12D), temperature of 26 ± 2°C, pH 7.2–7.5, DO 3.2–3.5 mg L^{-1}, hardness 760 mg L^{-1} $CaCo_3$ and salinity 3ppt-5ppt at the optimal levels throughout the study period. The physicochemical parameters such as temperature, dissolved oxygen and hardness were measured using standard methods (Albertson, 2007) and maintained at the optimal levels throughout the study period. After acclimation for 2 weeks, the fish were transferred to 160 L FRP tanks and again acclimated for a week prior to the challenge experiments.

Culturing of pathogen

The virulent strain of *A. hydrophila* was kindly provided by Dr. P.K. Sahoo (Central Institute of Freshwater Aquaculture, Bhubaneswar, India). The pure *A. hydrophila* colony was isolated with Aeromonas isolation medium (HiMedia, India) and then enriched in tryptone soya broth (HiMedia, India) for experimental purpose. Single cell colony of *A. hydrophila* from tryptone soya agar plate was inoculated in 30 mL of tryptone soya broth. After overnight incubation at 37°C, the culture was centrifuged at 800g for 15 min. The packed cells were washed thrice with PBS; concentrations were adjusted spectrophotometrically; confirmed by viable counts after serial dilution and plating on tryptone soya agar and the required dose was prepared by adjusting with phosphate buffered saline (Karunasagar *et al*., 1996). Prior to the experiment, LD_{50} dose of $1x10^7$ CFU (suspended in 0.2 mL phosphate buffered saline [PBS]) was determined for virulent *A. hydrophila* and the same dose was also used for the injection of heat killed *A. hydrophila* bacterin. Heat killed bacterin was prepared by heating *A. hydrophila* overnight culture to 60°C for 1h in water bath.

Experimental design

To a group of 20 fish maintained in a triplicate (20x3=60fish), the live virulent *A. hydrophila* was injected intraperitoneally with an LD_{50} dose of 10^7 CFU (suspended in 0.2 mL PBS) per fish and mortality pattern was recorded for 4 days (Fig. 1). Our earlier studies did not show significant mortality beyond day 4. The surviving fishes were (10±1 fish per tank) were subsequently used for investigation.

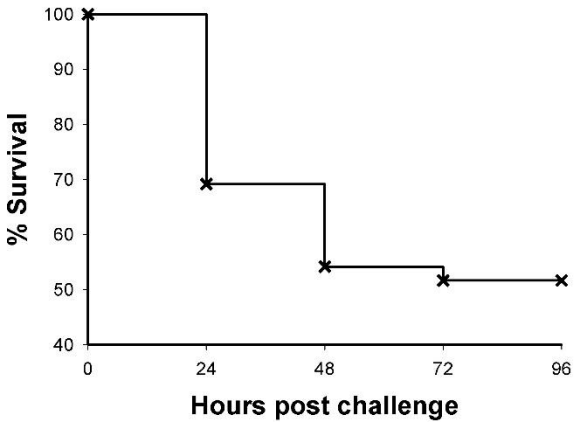

Fig. 1. Mortality pattern of *Channa striata* injected with LD_{50} dose of *Aeromonas hydrophila*. Fishes were injected with 0.2 ml suspension of 10^7 CFU of *A. hydrophila* and the mortality was recorded for 96 hours. Each point represent mean obtained from triplicates (n=10 fish per tank, in triplicates).

Another experimental group (in triplicate, n=10 each; 10x3=30 fish) were injected with heat killed *A. hydrophila* bacterin at 10^7 CFU (suspended in 0.2 mL PBS) per fish. A third group, the untreated control fish group (in triplicate, n=10 each; 10x3=30 fish) were injected with just 0.2 mL PBS.

These fishes were used for assaying serum lysozyme, peroxidase and antiprotease activities and the same experimental design was used with different fishes to assay cellular parameters.

Blood collection and serum separation

Fish from each group were sampled just before antigen challenge (day 0) and 5, 10, 15 and 20 days post challenge. In the live *A. hydrophila* injected group, fishes that survived the challenge during the sampling time were only bled. Before blood collection or an injection, the fish were anaesthetized using 100 ppm (100 mg L^{-1}) MS-222 (Sigma, USA). Blood was drawn from the anterior cardinal vein (Michael and Priyadarshini, 2012) with a 26-gauge needle attached to a 1 mL sterile glass tuberculin syringe. The blood samples were allowed to clot for overnight at 4ºC in serological tubes (70x10mm) and the serum was separated by centrifugation at 400g for 10 min and stored at -20º C until used for an assay.

Innate/Non-specific immune parameters

Serum lysozyme activity

The serum lysozyme activity was determined by the turbidimetric assay with the microplate adaptation of (Hutchinson and Manning, 1996).

Serum total peroxidase activity

The total peroxidase content of serum was determined colorimetrically as described in our earlier protocol (Rajendran *et al*., 2016).

Serum antiprotease activity

Serum antiprotease assay was performed colorimetrically according to the protocol described by Bowden *et al*. (1997).

Reactive oxygen species (ROS) and Reactive nitrogen species (RNS) production

Peripheral blood leukocytes (PBL) collection and measurement of ROS and RNS produced by PBL was done according to the earlier protocols described by Green *et al*. (1982) and Stolen (1990).

Statistical analysis

The data were expressed as arithmetic mean ± standard error obtained from 30 fishes. Statistical analysis involved one-way analysis of variance (ANOVA) followed by Tukey's multiple pair wise comparison test. SigmaPlot (Systat Software, San Jose, CA) was used for the analyses.

Results

Modulation of the innate immune parameters of *C. striata* on experimental challenge with live virulent or heat-killed *A. hydrophila* is presented below.

Serum lysozyme activity

Serum lysozyme activity was significantly (P<0·05) enhanced in both the groups challenged with live or heat-killed *A. hydrophila* in comparison with that of the control group on day 5 and there is no difference (P>0.05) between the experimental groups in the degree of enhancement (Fig. 2). However, the enhancement of lysozyme activity persisted and was more pronounced on day 10 in both the experimental group. On the rest of the days tested (day 15 and 20) there is no difference in the lysozyme activity among control and the experimental groups.

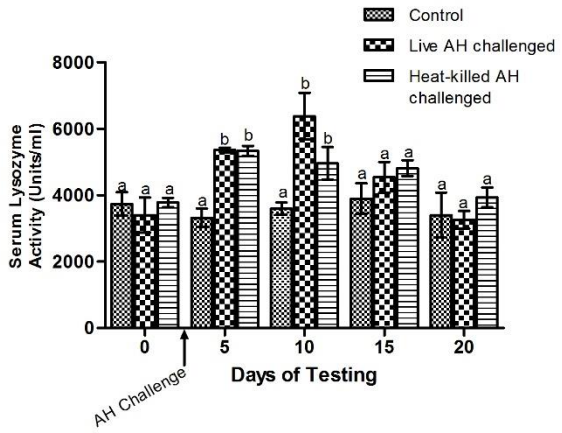

Fig. 2. Serum lysozyme activity. Fishes were injected with PBS or live virulent or heat-killed *Aeromonas hydrophila.* Each column and bar represents mean ± SE obtained from 30 fishes respectively. Different alphabets represent a statistical difference between means (P<0.05).

Serum peroxidase activity

Serum peroxidase activity was significantly (P<0·05) enhanced in both the groups challenged with live or heat-killed *A. hydrophila* in comparison with that of the control group on day 5 and 10 post challenge and there was no difference (P>0.05) between the experimental groups in the degree of enhancement (Fig. 3). The enhancement persisted till day 15 in the group challenged with live *A. hydrophila*, whereas the enzyme activity in group challenged with killed *A. hydrophila* was not modulated and it was comparable to that of the control. On day 20, there was no difference in the peroxidase activity among the control and the experimental groups.

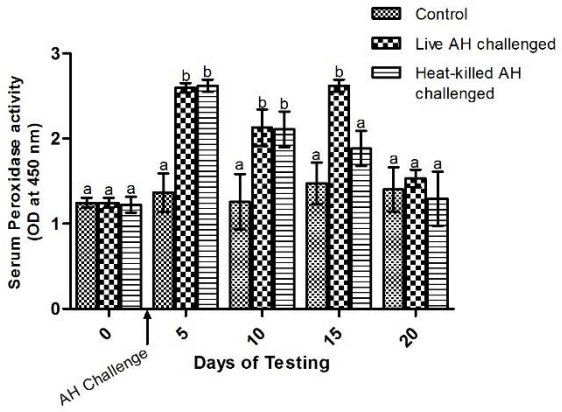

Fig. 3. Serum myeloperoxidase activity. Fishes were injected with PBS or live virulent or heat-killed *Aeromonas hydrophila*. Each column and bar represents mean ± SE obtained from 30 fishes respectively. Different alphabets represent a statistical difference between means (P<0.05).

Serum antiprotease activity

Significant enhancement (P<0·05) of antiprotease activity of serum in terms of percent trypsin inhibition after challenge with either live or heat killed *A. hydrophila* was depicted when compared to the control group on all the days tested (Fig. 4). There was no significant difference in the antiprotease activity between the experimental groups till day 15 post challenge.

On day 20, in the live *A. hydrophila* challenged group, the enhancement of the antiprotease activity was higher than (P<0·05) that of the heat killed *A. hydrophila* challenged group.

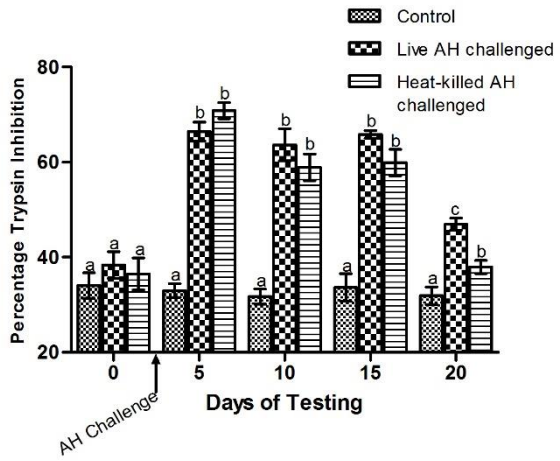

Fig. 4. Serum anti-protease activity. Fishes were injected with PBS or live virulent or heat-killed *Aeromonas hydrophila.* Each column and bar represents mean ± SE obtained from 30 fishes respectively. Different alphabets represent a statistical difference between means (P<0.05).

Cellular ROS production

The ROS productions in *C. striata* after challenge with live or heat killed *A. hydrophila* was estimated. ROS production by the peripheral blood leukocytes has significantly (P<0·05) increased in both the experimental groups compared to that of the control group, on day 5 post challenge and the response persisted till day 10 in both the experimentally challenged group. However, there was no difference among the control and experimental groups on other testing days (Fig. 5).

Cellular RNS production

Reactive nitrogen species (RNS) production by peripheral blood leukocytes measured in terms of nitrite was significantly (P<0.05) enhanced in both the groups challenged with live or heat killed *A. hydrophila* only on day 5 post challenge compared to that of the unchallenged control group.

On rest of the days tested, there was no significant difference in RNS production among the control and experimental groups (Fig. 6).

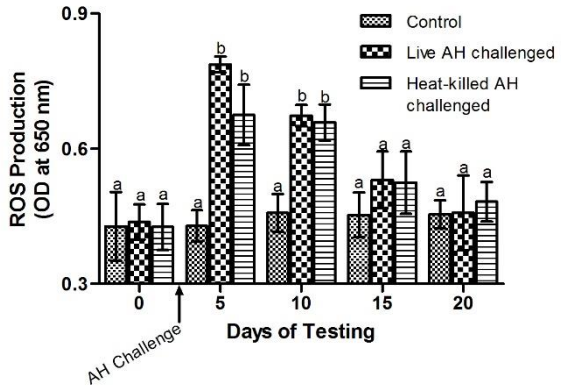

Fig. 5. Reactive oxygen species (ROS) production by peripheral blood leukocytes (PBL). Fishes were injected with PBS or live virulent or heat-killed *Aeromonas hydrophila.* Each column and bar represents mean ± SE obtained from 30 fishes respectively. Different alphabets represent a statistical difference between means (P<0.05).

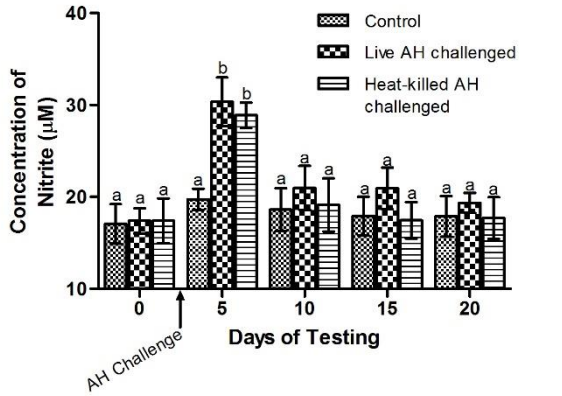

Fig. 6. Reactive nitrogen intermediate (RNI) production by peripheral blood leukocytes (PBL). Fishes were injected with PBS or live virulent or heat-killed *Aeromonas hydrophila.* Each column and bar represents mean ± SE obtained from 30 fishes respectively. Different alphabets represent a statistical difference between means (P<0.05).

Discussion

Innate immunity plays a foremost role in fish in defending against the infection by pathogenic bacteria. This study envisages the modulation of the innate immune parameters of the striped snakehead murrel, *C. striata* on an experimental challenge with either live or heat killed *A. hydrophila* and would throw more light on the inducibility of piscine innate immune responses in general and of this species in particular.

The results of this investigation clearly indicate that most of the innate immune responses in the groups challenged with live or killed *A. hydrophila* were enhanced compared to those of the unchallenged control group and also significant difference was not observed between the live and killed *A. hydrophila* challenged groups on most of the days tested. Thus, these results demonstrate the concept of the inducible

nature of the innate immune responses in *C. striata* that are discussed below in detail. An important defence enzyme of the innate immune system is lysozyme which mediates protection against microbial invasion (Kumaresan *et al*., 2015a). In fish, lysozyme is mainly distributed in the head kidney which is rich in leukocytes, and at sites such as the gill, skin, gastrointestinal tract and eggs, where the risk of bacterial invasion is very high (Scapigliati, 2013; Brinchmann, 2016). Experimental infection of Atlantic salmon (*Salmo salar*) with *A. hydrophila* resulted in increased lysozyme activity two days post infection (Du *et al*., 2015). A similar increase in plasma lysozyme level was observed in *Silurus glanis* experimentally infected with *Edwardsiella tarda* (Caruso *et al*., 2002). In greater amberjack, *Seriola dumerili* an increase in lysozyme was detected on an experimental challenge with the bacterial pathogen *Listonella (Vibrio) anguillarum* (Caruso *et al*., 2011).

The lysozyme producing immune related genes such as lysozyme G, lysozyme C genes were found to be significantly up-regulated (induced) in the kidney of *Puntius sarana* (Olive Barb) following an intraperitoneal challenge with *A. hydrophila* (Das *et al*., 2011). The inducible nature of lysozyme activity reported earlier is again confirmed in the present investigation where the serum lysozyme response was found to be induced to a significantly ($P<0{\cdot}05$) higher level in the group challenged with the live virulent or heat killed *A. hydrophila.*

Peroxidases (PO), yet another immune component of the innate immune responses that are released from the cytoplasmic granules of phagocytes, can participate in the oxidative responses against pathogens (Castro *et al*., 2008). In the present study, the serum peroxidase level was significantly ($P<0{\cdot}05$) enhanced in the group challenged with live or killed *A. hydrophila* till day 5 and 10 respectively after challenge. In another study, a significant increase in serum myeloperoxidase was observed as an early infection period response in *P. sarana* following an intraperitoneal challenge with *A. hydrophila* (Das *et al*., 2011). Similarly, serum PO levels increased in gilt-head seabream *(Sparus aurata)* exposed to *Enteromyxum leei* (Cuesta *et al*., 2006) or heat inactivated probiotic bacteria (Diaz-Rosales *et al*., 2006). Thus, the higher peroxidase activity observed in challenged fish, not only documents the stimulatory effect of bacterial factors on neutrophilic granulocytes i.e. the inducible nature of peroxidases, but also indicates the role of the peroxidase – hydrogen peroxide system in elimination of pathogenic factors.

Antiprotease also actively participate in the innate immune responses of fish (Li *et al*., 2017). Several bacterial pathogens invade the host and obtain their nutrients by using their extracellular proteolytic enzymes. To neutralize the pathogen proteases, the host uses various protease inhibitors like α1-antiproteinase and α2-macroglobulin (α2M) present in the serum and other body fluids which have anti-enzyme activity (Kumaresan *et al*., 2015b).

These protease inhibitors could selectively arrest the replication of bacterial pathogens without untoward toxicity to the host. Inhibition of trypsin activity is the most convenient way of measuring anti-protease activity of the serum. Our study indicates that the antiprotease level was significantly elevated in both the live and killed *A. hydrophila*- challenged groups of *C. striata* on all the post challenge days tested. The results indicate that antiprotease neutralizes the bacterial proteases and thus gradually getting rid of infection. This finding is in agreement with the observation in *C. striata* experimentally infected with *A. hydrophila*, the expression of Kazal type antiprotease was found to be higher than in uninfected (control) fish (Kumaresan *et al*., 2015b). Macrophages directly recognize pathogens and thereafter induce a protective immune response. As potent effector cells, they are able to produce small effector molecules such as reactive oxygen (ROS) and nitrogen (RNIs) intermediates which are able to kill bacteria, fungi and protozoan pathogens or control their growth. ROS and RNIs are microbicidal by inducing oxidative damage to the pathogen and inactivation of essential enzymes respectively (Secombes and Fletcher, 1992).

We have earlier showed that culture supernatant of *A. hydrophila* increases the production of ROS by PBL of Nile tilapia, *Oreochromis niloticus* (Subramani *et al*., 2016b). Vaccinating Mozambique tilapia, *O. mossambicus* with heat-killed *A. hydrophila* resulted in a strain dependent modulation of ROS production by PBL (Subramani *et al*., 2016a). The increased production of O_2^- by rainbow trout macrophages infected with *Pasteurella piscicida* involved in the killing of pathogen by macrophages, since the highest level of O_2^- production (after 5 h incubation) coincided with the highest bactericidal activity (Skarmeta *et al*., 1995).

Injection of pathogenic *A. hydrophila* into *C. striata* resulted in the upregulation of tumour necrosis factor-α (TNF-α) within 24 hours which subsequently led to ROS production by phagocytic cells (Palanisamy *et al*., 2015). These findings are in agreement with or confirmed by the present study where the peripheral blood leukocytes of *C. striata* challenged with live or heat killed *A. hydrophila*-challenged groups showed significantly enhanced ROS production. Thus, the activated respiratory burst activity that was not further enhanced after 15-day post challenge treatment ensures higher resistance against both live and heat killed *A. hydrophila* challenged groups. In the same way, fish are able to generate reactive nitrogen species such as nitric oxide (NO) implicated in the destruction of

certain pathogens.In the present study, the RNS production in *C. striata,* got induced after challenge with live or killed *A. hydrophila* on day 5 post challenge. In a similar experiment, elsewhere, an increase in inducible nitric oxide synthase (iNOS) activity has been detected in channel catfish head kidney leukocytes following intraperitoneal injection with live *Edwardsiella ictulari* (Secombes *et al*., 1988). The gene for iNOS has been partially sequenced in both trout (Laing *et al*., 1999) and goldfish (Laing *et al*., 1996) and it is shown to be inducible after challenge *in vivo* with bacterial pathogen or *in vitro* with LPS. iNOS was also upregulated in channel catfish, *Ictalurus punctatus* after immunization with live theronts of *Ichthyophthirius multifiliis* (Moreira *et al*., 2017). However, NO production is not selectively targeted to microorganisms and has the potential to be toxic to host tissues in high concentrations. Because of this, the production of NO must be tightly regulated. This argument is favourable to the NO response observed in this study, where its production increased only during day 5 post challenge and then returned to normal level. The reason for its tight regulation might be due to the synthesis of increased cyclic AMP (cAMP) levels leading to reduction of NO production (Joerink *et al*., 2006; Pietsch *et al*., 2008), pointing to an important role of this second messenger in modulating fish antimicrobial responses.

Since the molecular basis underlying the pathogen recognition in *C. striata* is not yet known, future studies should delineate the underlying activation pathways further and demonstrate the resulting effector molecules eventually eliminating invading pathogens.

In conclusion, the present study, the non-specific immune response parameters in general, are shown to be sensitive and inducible. Serum and cellular immune response parameters studied herein can be used to assess the health status of *C. striata* and *A. hydrophila* infection can be reported. Future studies should focus the cellular and molecular events taking place during *A. hydrophila* infection in *C. striata* at transcriptomic and proteomic levels.

Acknowledgements

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Conflict of interest

The authors declare that there is no conflict of interests.

References

Akira, S., Uematsu, S. and Takeuchi, O. 2006. Pathogen recognition and innate immunity. Cell 124(4), 783-801.

Albertson, O.E. 2007. Changes in the biochemical oxygen demand procedure in the 21st edition of Standard Methods for the Examination of Water and Wastewater, James C. Young, Lenore S. Clesceri, sabry M. Kamhawy, 77, 404-410 (2005). Water Environ Res. 79(4), 453-456.

Begam, M. and Sengupta, M. 2015. Immunomodulation of intestinal macrophages by mercury involves oxidative damage and rise of pro-inflammatory cytokine release in the fresh water fish Channa punctatus Bloch. Fish Shellfish Immunol. 45(2), 378-385.

Bowden, T.J., Butler, R., Bricknell, I.R. and Ellis, A.E. 1997. Serum trypsin-inhibitory activity in five species of farmed fish. Fish Shellfish Immunol. 7(6), 377-385.

Brinchmann, M.F. 2016. Immune relevant molecules identified in the skin mucus of fish using -omics technologies. Mol. Biosyst. 12(7), 2056-2063.

Buchmann, K. 2014. Evolution of Innate Immunity: Clues from Invertebrates via Fish to Mammals. Front. Immunol. 5, 459. https://doi.org/10.3389/fimmu.2014.00459.

Caruso, D., Schlumberger, O., Dahm, C. and Proteau, J.P. 2002. Plasma lysozyme levels in sheatfish Silurus glanis (L.) subjected to stress and experimental infection with Edwardsiella tarda. Aquacul. Res. 33(12), 999-1008.

Caruso, G., Caruso, R., Denaro, M.G. and Genovese, L. 2011. Non-Specific immune parameters in some new candidate species for Mediterranean aquaculture: Results of first Studies. Open Marine Biol. J.. 5(1), 3-11.

Castro, R., Piazzon, M.C., Noya, M., Leiro, J.M. and Lamas, J. 2008. Isolation and molecular cloning of a fish myeloperoxidase. Mol. Immunol. 45(2), 428-437.

Cuesta, A., Munoz, P., Rodriguez, A., Salinas, I., Sitja-Bobadilla, A., Alvarez-Pellitero, P., Esteban, M.A. and Meseguer, J. 2006. Gilthead seabream (Sparus aurata L.) innate defence against the parasite Enteromyxum leei (Myxozoa). Parasitology 132(Pt 1), 95-104.

Das, A., Sahoo, P.K., Mohanty, B.R. and Jena, J.K. 2011. Pathophysiology of experimental Aeromonas hydrophila infection in Puntius sarana: early changes in blood and aspects of the innate immune-related gene expression in survivors. Vet. Immunol. Immunopathol. 142(3-4), 207-218.

Diaz-Rosales, P., Salinas, I., Rodriguez, A., Cuesta, A., Chabrillon, M., Balebona, M.C., Morinigo, M.A., Esteban, M.A. and Meseguer, J. 2006. Gilthead seabream (Sparus aurata L.) innate immune response after dietary administration of heat-inactivated potential probiotics. Fish Shellfish Immunol. 20(4), 482-492.

Du, Y., Yi, M., Xiao, P., Meng, L., Li, X., Sun, G. and Liu, Y. 2015. The impact of Aeromonas

salmonicida infection on innate immune parameters of Atlantic salmon (Salmo salar L). Fish Shellfish Immunol. 44(1), 307-315.

Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S. and Tannenbaum, S.R. 1982. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal. Biochem. 126(1), 131-138.

Haniffa, M.A., Sheela, P.A., Kavitha, K. and Jais, A.M. 2014. Salutary value of haruan, the striped snakehead Channa striatus - a review. Asian Pac. J. Trop. Biomed. 4(Suppl. 1), S8-S15.

Hutchinson, T.H. and Manning, M.J. 1996. Seasonal trends in serum lysozyme activity and total protein concentration in dab (Limanda limandaL.) sampled from Lyme Bay, U.K. Fish Shellfish Immunol. 6(7), 473-482.

Jawahar, S., Nafar, A., Vasanth, K., Musthafa, M.S., Arockiaraj, J., Balasundaram, C. and Harikrishnan, R. 2016. Dietary supplementation of Zeolite on growth performance, immunological role, and disease resistance in Channa striatus against Aphanomyces invadans. Fish Shellfish Immunol. 51, 161-169.

Joerink, M., Forlenza, M., Ribeiro, C.M., de Vries, B.J., Savelkoul, H.F. and Wiegertjes, G.F. 2006. Differential macrophage polarisation during parasitic infections in common carp (Cyprinus carpio L.). Fish Shellfish Immunol. 21(5), 561-571.

Karunasagar, I., Ali, A. and Otta, S. 1996. Immunization with bacterial antigens: infections with motile aeromonads. Develop. Biol. Stand. 90, 135-141.

Kumaresan, V., Bhatt, P., Ganesh, M.R., Harikrishnan, R., Arasu, M., Al-Dhabi, N.A., Pasupuleti, M., Marimuthu, K. and Arockiaraj, J. 2015a. A novel antimicrobial peptide derived from fish goose type lysozyme disrupts the membrane of Salmonella enterica. Mol. Immunol. 68(2 Pt B), 421-433.

Kumaresan, V., Harikrishnan, R. and Arockiaraj, J. 2015b. A potential Kazal-type serine protease inhibitor involves in kinetics of protease inhibition and bacteriostatic activity. Fish Shellfish Immunol. 42(2), 430-438.

Kumaresan, V., Ravichandran, G., Nizam, F., Dhayanithi, N.B., Arasu, M.V., Al-Dhabi, N.A., Harikrishnan, R. and Arockiaraj, J. 2016. Multifunctional murrel caspase 1, 2, 3, 8 and 9: Conservation, uniqueness and their pathogen-induced expression pattern. Fish Shellfish Immunol. 49, 493-504.

Laing, K.J., Grabowski, P.S., Belosevic, M. and Secombes, C.J. 1996. A partial sequence for nitric oxide synthase from a goldfish (Carassius auratus) macrophage cell line. Immunol. Cell Biol. 74(4), 374-379.

Laing, K.J., Hardie, L.J., Aartsen, W., Grabowski, P.S. and Secombes, C.J. 1999. Expression of an inducible nitric oxide synthase gene in rainbow trout Oncorhynchus mykiss. Dev. Comp. Immunol. 23(1), 71-85.

Li, W., Wang, Q., Li, S., Jiang, A. and Sun, W. 2017. Molecular cloning, genomic structure, polymorphism analysis and recombinant expression of a alpha1-antitrypsin like gene from swamp eel, Monopterus albus. Fish Shellfish Immunol. 62, 124-138.

Magnadottir, B. 2010. Immunological control of fish diseases. Mar. Biotechnol. (NY). 12(4), 361-379.

Martin-Gayo, E. and Yu, X.G. 2017. Dendritic Cell Immune Responses in HIV-1 Controllers. Curr. HIV/AIDS Rep.

Michael, R.D. and Priyadarshini, S.K. 2012. A reliable method for repetitive bleeding in striped murrel, Channa striata (Bloch). Aquacul. Res. 43(11), 1738.

Mohd, S.M. and Abdul Manan, M.J. 2012. Therapeutic potential of the haruan (Channa striatus): from food to medicinal uses. Malays. J. Nutr. 18(1), 125-136.

Moreira, G.S., Shoemaker, C.A., Zhang, D. and Xu, D.H. 2017. Expression of immune genes in skin of channel catfish immunized with live theronts of Ichthyophthirius multifiliis. Parasite Immunol. 39(1). e12397. doi:10.1111/pim.12397.

Palanisamy, R., Kumaresan, V., Harikrishnan, R., Arasu, M.V., Al-Dhabi, N.A. and Arockiaraj, J. 2015. Functional roles and gene regulation of tumor necrosis factor receptor 1 in freshwater striped murrel. Mol. Immunol. 66(2), 240-252.

Pietsch, C., Vogt, R., Neumann, N. and Kloas, W. 2008. Production of nitric oxide by carp (Cyprinus carpio L.) kidney leukocytes is regulated by cyclic 3',5'-adenosine monophosphate. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 150(1), 58-65.

Rajendran, P., Subramani, P.A. and Michael, D. 2016. Polysaccharides from marine macroalga, Padina gymnospora improve the nonspecific and specific immune responses of Cyprinus carpio and protect it from different pathogens. Fish Shellfish Immunol. 58, 220-228.

Rauta, P.R., Mohanty, J., Garnayak, S.K. and Sahoo, P.K. 2013. Characterization of serum immunoglobulin in channa striata (BLOCH) and kinetics of its response to Aeromonas hydrophila antigen. J. Immunoassay Immunochem. 34, 283-293.

Riera Romo, M., Perez-Martinez, D. and Castillo Ferrer, C. 2016. Innate immunity in vertebrates: an overview. Immunology 148(2), 125-139.

Scapigliati, G. 2013. Functional aspects of fish lymphocytes. Dev. Comp. Immunol. 41(2), 200-208.

Secombes, C.J., Chung, S. and Jeffries, A.H. 1988. Superoxide anion production by rainbow trout macrophages detected by the reduction of ferricytochrome C. Dev. Comp. Immunol. 12(1), 201-206.

Secombes, C.J. and Fletcher, T.C. 1992. The role of phagocytes in the protective mechanisms of fish. Annual Rev. Fish Dis. 2, 53-71.

Skarmeta, A.M., Bandín, I., Santos, Y. and Toranzo, A.E. 1995. In vitro killing of Pasteurella piscicida by fish macrophages. Dis. Aquat. Organ. 23, 51-57.

Sorci, G., Cornet, S. and Faivre, B. 2013. Immune evasion, immunopathology and the regulation of the immune system. Pathogens 2(1), 71-91.

Stolen, J. 1990. Techniques in fish immunology. Fish immunology technical communications (USA).

Subramani, P.A., Daniels Gnanamuthu, A.A. and Michael, R.D. 2016a. Neutrophil activity affects Oreochromis mossambicus (Peters, 1852) antibody production against heat-killed Aeromonas hydrophila vaccine. J. Appl. Ichthyol. 32(6), 1113-1117.

Subramani, P.A., Narasimha, R.V., Balasubramanian, R., Narala, V.R., Ganesh, M.R. and Michael, R.D. 2016b. Cytotoxic effects of Aeromonas hydrophila culture supernatant on peripheral blood leukocytes of Nile tilapia (Oreochromis niloticus): Possible presence of a secreted cytotoxic lectin. Fish Shellfish Immunol. 58, 604-611.

Vincent, S. 1987. Effect of selected steroid on food utilization in the fresh water fish *Channa punctatus*. Ph. D., Madurai Kamaraj University.

Zoccola, E., Kellie, S. and Barnes, A.C. 2017. Immune transcriptome reveals the mincle C-type lectin receptor acts as a partial replacement for TLR4 in lipopolysaccharide-mediated inflammatory response in barramundi (Lates calcarifer). Mol. Immunol. 83, 33-45.