J. Aqua. **23** (2015)

HAEMATOLOGICAL AND INNATE IMMUNOLOGICAL REFERENCE INTERVALS FOR FARMED YELLOW CATFISH *HORABAGRUS BRACHYSOMA* AND THEIR SEASONAL VARIATIONS

P. K. Sahoo, S. K. Sahoo, B. R. Mohanty, A. Das, S. S. Giri and M. Paramanik

ICAR-Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar-751 002, Odisha, India
**Corresponding author: pksahoo1@hotmail.com*

The reference intervals for haematological and innate immune response variables of healthy adult yellow catfish *Horabagrus brachysoma* raised in captivity were determined. Reference ranges were established for all parameters and significant ($P < 0.05$) seasonal variations in most of the haematological and innate immune parameters were observed. Total erythrocyte count, packed cell volume, serum lysozyme and myeloperoxidase activities were found to be higher during winter season whereas most of the red blood cell indices, superoxide production by phagocytes, serum ceruloplasmin and anti-protease activities were marked to be significantly higher during rainy season of the year. Except high total leucocyte count and mean corpuscular haemoglobin, other parameters were found to be significantly lower during summer, possibly indicating a higher disease risk in summer months for this species. However, no significant variations in these variables were obtained between male and female catfish during breeding season. The information generated would be indirectly helpful for determining health status of this endangered species.

INTRODUCTION

Hematological evaluation is gradually becoming a routine practice for determining health status, disease or stress conditions of intensively cultured farmed fish (Bowden *et al*., 2004; De Pedro *et al*., 2005; Sahoo *et al*., 2005; Swain *et al*., 2007; Tavares-Dias and Moraes, 2007a, b). Haematological and immunological variables vary substantially between species (Hine, 1992; Sahoo *et al*., 2005; Swain *et al*., 2007) and even within a major group of fish species cultured in the similar environment (Sahoo *et al*., 2005). The major biotic and abiotic factors such as temperature, season, sex, species, age, strain, photoperiod, nutritional status and environmental factors influence blood parameters in fish (De Pedro *et al*., 2005; Tavares-Dias and Moraes, 2007a, b). Thus, the establishment of species-specific reliable reference values under standard environmental conditions is a prerequisite before haematology or immunological parameters being used for determining biomarkers of health status of fish (Handy and Depledge, 1999) or exposed to pollutants, stress or infections (Sahoo *et al*., 2005).

Red blood cell indices are used to diagnose anaemia and to indicate systemic responses to external stimulus (Tavares-Dias and Moraes, 2007b). Total leucocyte count may reveal leucopenia or leucocytosis, suggesting possible immune function alterations (Huffman *et al*., 1997). The total leucocyte count (TLC) level increases in infected fish (Harikrishanan *et al*.,

2003). Previous studies have indicated a reduction in total erythrocyte count (TEC), haematocrit and haemoglobin (Hb) in infected fish (Rehulka, 2002; Harikrishanan *et al*., 2003) or fish exposed to toxic chemicals (Svobodova *et al.,* 2003). Reduction in blood glucose and total protein has been recorded in fish exposed to *Aeromonas* spp. (Rehulka, 2002; Harikrishanan *et al*., 2003).

Immunity is an important physiological defence mechanism to protect against infection and maintain internal homeostasis (Ingram, 1980). Many cells (leucocytes, nonspecific cytotoxic cells, eosinophilic granular cells, macrophages and other cells) and their products [myeloperoxidases (MPO), superoxides, acute-phase proteins, lysozyme, interferon, complement, properdin, lysins and agglutinins] contribute to the general immunological defence mechanism. Seasonal influence dominates the life cycle of fish and is also believed to co-ordinate their immune response (Bromage *et al*., 2001). Fish appear to exhibit seasonal fluctuations in their susceptibility to different infectious diseases (Lillehaug *et al*., 2003; Kumari *et al*., 2006). For example, Karvonen *et al*. (2010) showed higher disease incidences during summer with prolong high water temperature in farms. However, the pattern was opposite or there was no pattern. Reference ranges for each parameter are needed to be known in order to access the health status of fish. Reference intervals for each variable are defined by upper and lower limits that cover the majority of the values obtained for healthy individuals in the reference population (i.e., a set of individuals meeting certain criteria, particularly absence of any disease) (Rehulka *et al*., 2004; Tavares-Dias and Moraes, 2007a). *Horabagrus brachysoma* or Asian sun catfish or yellow catfish is an endangered species endemic to few southern states of India (Bhat, 2001; Kurup *et al*., 2004), and also found in few Asian countries. The positive attributes in this species viz., adaptability in varied environment conditions, maturity in captivity, and acceptance to wide range of food and good growth within short time span in culture conditions enable it as a perspective species for aquaculture. However, there is lack of information on haematological and immunological indices in *H. brachysoma.*

MATERIALS AND METHODS

Fish and experimental design

H. brachysoma juveniles were collected from their natural habitat and transported to the Institute farm. They were reared in cement tanks of 20 m^2 size for a period of two years under brood stock raising programme. The cement tank covered with linen shed was provided with 2-3 cm soil base and water depth of 45 cm was maintained. The tanks were provided with plastic pipes for hiding of fish to simulate natural conditions. Water exchange was carried out periodically to maintain optimum water quality. Fish were provided with pellet feed (30% crude protein) once daily at 2% of their body weight. The broods raised in cement tanks were collected during the month of July for induced breeding. The larvae thus obtained were reared for a period of two years till they mature. The mature fish were collected and equal size (50-55 g) fish were segregated before releasing those to experimental tanks for further study. Three cement tanks of

16 m^2 size were prepared as described previously and each tank was stocked 48 fish (three fish/m^2) for a period of one year under continuous supply of freshwater under natural photoperiod and temperature. The aerated ground water was stored in overhead tank before supply to experimental tanks. Other managemental protocols were similar as mentioned earlier for brood stock raising. The blood samplings were undertaken in last week of December, April and July representing winter, summer and rainy seasons, respectively. The size ranges of fish used were 72-85 g, 75-95 g and 85-105 g during December, April and July, respectively. The water temperature ranges recorded during three major seasons varied from 31.0-34.0 ºC (average 33.0 ºC) in summer (March-June), 29-31 ºC (average 30.5 ºC) in rainy (July-September) and 18.7-20.2 ºC (average 19.4 ºC) in winter (November-January) seasons. The mean of water quality parameters measured during entire period of study were dissolved oxygen 5.65 ± 0.70 ppm, pH 7.2 ± 0.6, total ammonia 0.109 ± 0.024 ppm, nitrites 0.015 ± 0.009 ppm and hardness 92.0 ± 8.2 ppm. During the study period, fish were observed at quarterly interval for any clinical signs of diseases and found to be free from any gross signs of disease.

Blood was collected in the morning (10:00-11:00 hours) after 24 h fasting from caudal vein of rapidly caught anaesthetized (0.1 ml/l 2-phenoxy ethanol) fish through plastic syringe. Heparinized syringe was used to collect blood from 15-20 fish (randomly collected from three tanks) to measure haematological indices, blood (plasma) glucose level and nitroblue tetrazolium assay. Similarly, sera were obtained from blood collected with non-heparinized syringe from 17 to 32 fish (representing equal numbers approx. from each tank) to measure immune parameters during each season. The sample size details vary in each season and are given in results section against each test. The blood collected during July (when morphological sex differentiation was prominent) was processed separately sex-wise. Two of the innate immune parameters such as natural haemolysin titre and bacterial agglutination titre were not recorded during rainy season and hence, not incorporated in seasonal or sex impact analysis study. All the assays were carried out in triplicate.

Haematology and immunology

TEC and TLC were carried out manually after dilution (200 × and 50 ×, respectively) using modified Dacie's fluid (Blaxhall and Daisley, 1973). Values were expressed as number of cells/mm^3 of blood. The haematocrit was measured by microhaematocrit method and Hb concentration using the cyanomethaemoglobin method with Drabkin's reagent (Blaxhall and Daisley 1973). Secondary Wintrobe indices, such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were derived from the primary indices. Plasma glucose content was quantified by enzymatic colorimetric method with GLUCOSE FL kit (Chema Diagnostica, Italy).

The oxygen radical production by blood phagocytes during respiratory burst activity was measured through nitroblue tetrazolium (NBT) assay as described previously by Anderson and Siwicki (1995). The total myeloperoxidase content present in serum was measured according to Quade and Roth (1997) and a partial modified technique (Sahoo *et al.*, 2005). A turbidometric assay utilizing lyophilized *Micrococcus lysodeikticus* cells (Sigma) was used to determine lysozyme activity in serum following Kumari *et al*. (2006). Lyophilized hen egg white lysozyme, HEWL (Sigma) was used to develop a standard curve. Serum lysozyme values were expressed as μg/ml equivalent of hen egg white lysozyme activity. Alternative complement activity was assayed following a previously described technique (Matsuyama *et al*., 1988; Yano, 1992) with partial modifications (Kumari and Sahoo, 2005) by using rabbit red blood cells (RaRBC). The results are expressed as ACH_{50} (U/ml), the reciprocal serum dilution giving 50% haemolysis. The total protein content in serum was measured following Bradford (1976) method, using bovine serum albumin as a standard protein. The natural haemolysin titre was performed as per Sahoo *et al*. (2005) using rabbit RBC. The titre was defined as the last dilution of serum showing complete lysis of RBC. Values are expressed as reciprocal of haemolysin titre. Natural agglutinin levels in the serum of individual fish were determined by plate agglutination technique using formalin-killed *Aeromonas hydrophila* (Sahoo *et al*., 2005). The bacterial agglutination titre was defined as the last dilution of serum showing minimal positive agglutinin. Values were expressed as reciprocal of the agglutination titre. Total serum antiprotease in fish serum was determined according to Zuo and Woo (1997) with partial modification. Serum (10 μl) was mixed with 100 μl of trypsin (bovine pancreas type I, Sigma; 200 μg ml of PBS) and incubated at 25° C for 30 min. It was further incubated with 1 ml of casein dissolved in PBS (2.5 mg/ml) for 15 min at 25° C. The reaction was terminated with the addition of 500 μl of 10% trichloroacetic acid (TCA). The sample was centrifuged at 10,000 × g for 5 min to remove protein precipitates. The OD of the supernatant was measured at 280 nm and the percentage trypsin inhibition was calculated. Ceruloplasmin activity in serum sample was measured as p-phenylene diamine (PPD) oxidase activity (Sigma) according to methods of Pelgrom *et al.* (1995) with slight modification (Sahoo *et al*., 2008).

Statistical analysis

The data expressed as Mean ± SE and had a non-Gaussian distribution (except for TEC, Hb, haematocrit, lysozyme and total protein). Thus, reference intervals (25^{th} and 75^{th} percentiles) were established using non-parametric methods. The normality of the data was assessed using Kolmogorov-Smirnov test. Difference between means were assessed by Student's T-test (for difference between male and female, and also for ACH_{50} activity in seasonal study) or by one-way ANOVA (for seasonal study) followed by Duncan's multiple range tests using SPSS 13.0 package (SPSS Inc., Chicago, USA). A probability level of $P < 0.05$ was considered statistically significant. Data from males and females of rainy season were pooled when statistical differences between sexes were absent.

RESULTS

The mean values, lower (25^{th}) and upper (75^{th}) percentiles and range of each parameter obtained from samples collected during all the seasons over one year duration are presented in Table 1. No difference in haematological and immunological indices was found between male and female catfish sampled during rainy season (Table 2). However, a marked influence of season on most of the parameters studied was observed (Table 3).

TEC, Hb and haematocrit were found to be significantly ($P<0.05$) lowest during summer season compared to other seasons whereas TLC showed a higher value during summer followed by rainy and winter seasons. The MCH level was significantly higher during summer season when compared with winter and rainy seasons. On the other hand, MCV and MCHC showed no significant fluctuation over the year. Similarly, the blood glucose level was consistent over different seasons. Superoxide production, serum antiprotease, total protein and ceruloplasmin were significantly higher during rainy season; whereas lysozyme and myeloperoxidase activities in the serum of catfish were shown to be higher during winter season. This was not the case for ACH_{50} level, as no clear trend with regard to seasonal variations was observed (Table 3).

Table 1 : Haematological and innate immune parameters reference intervals for farmed yellow catfish obtained from observations made over one year.

Parameter	N	Mean ± SE	25^{th}-75^{th} percentile	Range
TEC (x 10^6/ mm^3 of blood)	52	1.88 ± 0.07	1.53-2.18	0.68-3.28
TLC (x 10^3/ mm^3 of blood)	53	16.78 ± 1.16	8.73-22.5	4.43-34.45
Hb (g/ dL^{-1})	54	7.21 ± 0.25	6.0-8.2	3.6-11.4
Haematocrit (%)	59	27.44 ± 0.91	22.5-33.0	12.0-40.0
MCV (fL)	52	157.36 ± 10.82	117.97-171.99	59.07-492.75
MCH (pg)	52	40.12 ± 1.62	31.92-46.51	21.71-68.24
MCHC (%)	54	27.71 ± 1.24	23.13-30.99	12.67-51.82
Glucose (mg/ dL)	56	75.21 ± 3.29	57.06-89.69	37.02-133.95
NBT activity (OD at 540 nm)	58	0.34 ± 0.01	0.29-0.40	0.21-0.53
ACH_{50} activity (units/ mL)	47	40.27 ± 2.23	30.70-48.25	12.47-68.18
Lysozyme activity (µg/ mL)	53	7.39 ± 0.39	4.67-9.88	2.22-12.33
Myeloperoxidase activity (OD at 450 nm)	86	0.71 ±0.04	0.46-0.90	0.14-1.78
Ceruloplasmin (units 25 µL of serum)	82	0.21 ± 0.01	0.12-0.26	0.08-0.63
Anti-protease (% inhibition)	89	52.89 ± 1.63	41.39-62.19	24.84-85.07

Parameter	N	Mean ± SE	25^{th}-75^{th} percentile	Range
Total protein (g/ dL)	75	7.14 ± 0.34	4.80-8.61	2.26-13.92
Haemolysin titre	27	1.26 ± 0.13	1.0-2.0	1.0-4.0
Bacterial agglutination titre	24	4.25 ± 0.61	2.0-4.0	2.0-16.0

TEC, total erythrocyte count; TLC, total leucocyte count; Hb, haemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; NBT, nitroblue tetrazolium activity; N, number of fish sampled

Table 2 : Effect of sex on various haematological and innate immune parameters measured in farmed yellow catfish during rainy season. Data are presented as Mean ± SE with number of fish sampled in the parenthesis.

Parameter	Male	Female
TEC (x 10^6/mm^3 of blood)	2.07 ± 0.05 (13)	2.07 ± 0.18 (11)
TLC (x 10^3/mm^3 of blood)	17.60 ± 0.89 (13)	19.82 ± 1.44 (10)
Hb (g/dL)	8.48 ± 0.43 (13)	8.29 ± 0.36 (11)
Haematocrit (%)	31.21 ± 1.33 (14)	30.73 ± 1.71 (11)
MCV (fL)	153.26 ± 6.26 (13)	154.66 ± 10.10 (11)
MCH (pg)	41.19 ± 2.30 (13)	41.94 ± 2.40 (11)
MCHC (%)	27.44 ± 2.23 (13)	27.40 ± 1.09 (11)
Glucose (mg/dL)	85.22 ± 7.98 (11)	85.92 ± 7.48 (14)
NBT activity (OD at 540 nm)	0.41 ± 0.02 (14)	0.38 ± 0.02 (11)
ACH_{50} activity (units/mL)	42.37 ± 4.42 (16)	35.65 ± 3.16 (18)
Lysozyme activity (µg/mL)	5.26 ± 0.74 (11)	5.44 ± 0.45 (14)
Myeloperoxidase activity (OD at 450 nm)	0.49 ± 0.05 (15)	0.61 ± 0.05 (13)
Ceruloplasmin (units/25 µL of serum)	0.25 ± 0.03 (22)	0.25 ± 0.03 (19)
Anti-protease (% inhibition)	61.75 ± 3.65 (23)	59.93 ± 3.47 (21)
Total protein (g/dL)	9.36 ± 0.61 (20)	9.89 ± 0.74 (11)

TEC, total erythrocyte count; TLC, total leucocyte count; Hb, haemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; NBT, nitroblue tetrazolium activity

Table 3 : Effect of season on various haematological and innate immune parameters measured in farmed yellow catfish. Data are presented as Mean ± SE with number of fish sampled in the parenthesis. Means bearing common superscript in a row are not significantly ($P < 0.05$) different.

Parameter	Winter season	Summer season	Rainy season
TEC (x 10^6/mm^3 of blood)	2.10 ± 0.11^b (15)	1.47 ± 0.11^a (18)	2.09 ± 0.11^b (19)
TLC (x 10^3/mm^3 of blood)	12.84 ± 0.38^a (17)	23.98 ± 1.57^c (18)	18.96 ± 0.88^b (18)
Hb (g/dL)	7.24 ± 0.20^{ab} (16)	6.21 ± 0.55^a (19)	8.18 ± 0.31^b (19)
Haematocrit (%)	28.60 ± 0.96^b (20)	22.89 ± 1.91^a (19)	30.60 ± 1.28^b (20)
MCV (fL)	136.36 ± 9.22^a (15)	161.05 ± 9.07^a (18)	147.13 ± 6.51^a (18)
MCH (pg)	36.42 ± 2.18^a (15)	43.32 ± 2.41^b (18)	40.37 ± 2.07^{ab} (19)
MCHC (%)	26.60 ± 1.03^a (16)	27.14 ± 0.59^a (19)	27.23 ± 1.61^a (19)
Glucose (mg/dL)	71.27 ± 4.82^a (17)	71.82 ± 5.81^a (19)	81.77 ± 6.08^a (20)
NBT activity (OD at 540 nm)	0.31 ± 0.01^a (19)	0.35 ± 0.02^{ab} (19)	0.37 ± 0.02^b (20)
ACH_{50} activity (units/mL)	ND	37.05 ± 3.12^a (26)	44.26 ± 3.03^a (21)
Lysozyme activity (µg/L)	9.43 ± 0.35^c (27)	3.86 ± 0.49^a (19)	6.02 ± 0.46^b (17)
Myeloperoxidase activity (OD at 450 nm)	0.93 ± 0.07^b (26)	0.69 ± 0.06^a (32)	0.55 ± 0.04^a (28)
Ceruloplasmin (units/25 µL of serum)	0.16 ± 0.01^a (25)	0.19 ± 0.02^a (27)	0.27 ± 0.02^b (30)
Anti-protease (% inhibition)	45.62 ± 1.56^a (27)	43.24 ± 1.68^a (31)	68.88 ± 2.12^b (31)
Total protein (g/L)	6.18 ± 0.30^a (26)	5.55 ± 0.35^a (32)	11.60 ± 0.23^b (17)

TEC, total erythrocyte count; TLC, total leucocyte count; Hb, haemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; NBT, nitroblue tetrazolium activity; ND, not done

DISCUSSION

The haematological and immunological assessment of intensively farmed fish can be considered as an integral part of the evaluation of their health status. Any deviation in the proportion of the parameters leads to diagnostic significance. Hence, the establishment of

reference intervals for these variables are important and the same has been defined for many species viz., hybrid striped bass *Morone saxatilis* (Walbaum) × *Morone chrysops* (Rafinesque) (Hrubec *et al*., 2001), channel catfish *Ictalurus punctatus* (Tavares-Dias and Moraes, 2007b), Indian major carps *Labeo rohita*, *Catla catla*, *Cirrhinus mrigala* (Sahoo and Mukherjee, 1999; Sahoo *et al*., 2005), common carp *Cyprinus carpio* (Tripathi *et al*., 2003), hybrid tilapia *Oreochromis niloticus* (L.) × *Oreochromis mossambicus* (Peters) (Hrubec *et al*., 2000) and southern bluefin tuna *Thunnus maccoyii* (Rough *et al*., 2005).

The reference intervals obtained for the parameters studied in yellow catfish were more or less similar to those defined for other species. A minimal difference that was observed in all the variables might be due to species difference, age and environment.

No variation was observed in any of the immune parameters studied between male and female fish that were bled during rainy or breeding season. Similar observations were also made for few other species viz., *L. rohita* (Swain *et al*., 2007) for innate immune parameters, and wild yellowfish (*Barbus holubi*), *C. carpio*, and two mudfish species (*Labeo umbratus* and *L. capensis*) (Van Vuren and Hattingh, 1978) for haematological parameters suggesting that both the sexes equally immuno-competent.

The present results indicate the existence of seasonal variations in haematological and innate immunological parameters in the blood of the yellow catfish. This is the first time a normal range for all these variables being established for this endangered species, which has both food and ornamental value. Thus, season must be considered as a key factor when blood parameters are used as biomarkers for prediction of pollution, stress, disease problems or environmental alterations.

Erythrocyte profiles (red blood cells count, haemoglobin content and haematocrit value) exhibited almost similar levels in rainy and winter seasons whereas significantly lower levels during summer. The rise in water temperature for a prolonged period during summer season might be playing detrimental role on the physiology of fish leading to unnoticed reduced feed intake, thereby reducing red blood cells and its related parameters. The effect of temperature on number of physiological and immune parameters of fish has already been described (Hernandez and Tort, 2003). TLC is an important defence activity of fish (De Pedro *et al*., 2005). The variation in TLC of yellow catfish clearly depicts the variation in immune response with respect to season. Similar to our study, a higher TLC was marked in tench (Tinca tinca) during summer and autumn compared to winter and spring suggesting shortened day length as possible cause inducing changes in immune system (Collazos *et al*., 1998). Secondary Wintrobe indices viz., MCV, MCH and MCHC are indicative of types of anaemia. There was no influence of season on MCV and MCHC values whereas MCH value was higher during summer.

The blood glucose level is indicative of stress and in our study, it was observed that the plasma glucose level remained stable in different seasons. As the fish were deprived of food for 24 h before sampling, the effect of change in feed intake due to seasonal differences in temperature is minimized. Further, these results indicate absence of any external stress during the period of study. Hence, blood glucose can actively be considered as a valuable test for evaluating the general physiological state of fish.

Many researchers have studied the changes in non-specific immune parameters of fish with relation to infection, toxicity, diet, stressors, temperature fluctuations or pollution (Ingram, 1980; Studnicka *et al*., 1986; Anderson *et al*., 1992; Dalmo *et al*., 1997; Ellis, 2001; Kumari and Sahoo, 2006). Nevertheless, just a few of these studies are related to catfish species i.e. in *Clarias batrachus* (Kumari *et al*., 2006; Kumari and Sahoo, 2006) and *Ictalurus punctatus* (Plumb and Areechon, 1990). Thus, the present work was undertaken to find the normal physiological presence and ranges for some of the important non-specific immune parameters of this endangered yellow catfish species.

Natural haemolysins are considered to be important in innate immunity in vertebrates. Potent haemolytic activity was also observed in the sera of yellow catfish. Other investigators have also reported natural haemolytic activity in normal serum of different fish species against a diverse array of cellular antigens (Ingram, 1980; Sakai, 1983a, b; Sahoo *et al*., 2005; Swain *et al*., 2007). Natural factors found in normal, healthy fish such as lysins, agglutinins and precipitins may help to overcome various diseases much earlier than that required to produce specific immunity. These natural agglutinins are structurally different from known immunoglobulins. These natural agglutinins also react with a wide variety of bacteria causing agglutination. The observed bacterial agglutination titre against a common contaminant/pathogen is clearly indicative of the presence of agglutinins in yellow catfish sera.

Phagocytes produce large quantities of superoxide anion during phagocytosis or upon stimulation. The NBT reduction product obtained after reaction with superoxides is a very good indicator of the health status or the immunization effectiveness in fish (Anderson et al., 1992). A higher NBT activity was noticed in yellow catfish in rainy season. However, earlier studies have indicated higher superoxide production at low water temperature (Le Morvan *et al.*, 1998) and during winter in *C. batrachus* (Kumari *et al*., 2006).

MPO is an important enzyme having antimicrobial activity. It utilizes hydrogen peroxide during respiratory burst to produce hypochlorous acid (Dalmo *et al*., 1997). Reduced activity may indicate the presence of contaminants or stress (Anderson and Siwicki, 1995). The highest MPO activity in yellow catfish was noticed in winter as compared to the lowest activity in *C. batrachus*

during the same time (Kumari *et al*., 2006). The trend obtained for MPO and NBT activities in yellow catfish thus reverse as compared to Asian catfish *C. batrachus*.

Lysozyme is an important enzyme in blood that actively lyses bacteria; an increased level has been considered to be a natural protective mechanism in fish (Ingram, 1980). Neutrophils are thought to be the source of lysozyme, and the enzyme appears to be much more bactericidal in fish than that of higher vertebrates (Ellis, 2001). The lysozyme level varies widely among the fish species (Anderson and Siwicki, 1995), as was also observed in our study. Lysozyme activity was found to be higher in winter in yellow catfish, which is well correlated with high MPO in this species at the same period, thus indicating the release of these enzymes by similar cell population like neutrophils. Similar to our previous study in *C. batrachus* (Kumari *et al*., 2006), a lower lysozyme activity was noticed during summer in yellow catfish. On the other hand, a higher lysozyme activity was noticed in broods of *L. rohita* during summer (Swain *et al.*, 2007) and in dab (*Limanda limanda*) (Hutchinson and Manning, 1996).

The ACH_{50} activity is very active in fish serum when compared with mammals (Yano, 1996), suggesting that this pathway is very important in the defence mechanisms of fish (Ellis, 2001; Holland and Lambris, 2002). ACH_{50} values of fish serum were extremely high when compared with those of mammalian sera. The ACH_{50} values of *C. carpio*, yellow tail and eel displayed 68, 142, and 134 units/ml of serum, respectively (Matsuyama et al., 1988). Similarly, we also recorded high ACH_{50} value (40.27 units/ml) in yellow catfish. Saha *et al.* (1993) marked ACH_{50} values of 26 and 16 units/ml of serum in *C. batrachus* and *Heteropneustes fossilis*, respectively, which are comparatively lower than that of yellow catfish. In this study, the measured ACH_{50} in summer and rainy seasons did not vary significantly. Similarly, any change in alternative complement activity in snapper (*Pagrus auratus*) with relation to variable temperatures (12 or 24 °C) was not observed in an earlier study (Cook *et al.*, 2003). However, variable effects have been noticed in different fish species with relation to temperature variations or seasons in ACH_{50} activity (Collazos *et al*., 1994; Le Morvan *et al*., 1998; Kumari *et al*., 2006).

A higher activity of two important innate defence molecules viz., serum ceruloplasmin and anti-protease activities was observed in yellow catfish during rainy season. Thus, the higher values obtained in most of the haematological and innate immune parameters in catfish evident during rainy season i.e., breeding season of the year possibly indicates a natural physiological phenomenon for making a brood fish healthy from defence point of view to avoid post-breeding immune suppression and/or for maternal transfer of immunity to eggs.

CONCLUSION

In the present study on yellow catfish the normal baseline values for several haematological and innate immunological parameters have been established, even for different

sex. This study highlights the relevance of seasonal variations, when monitoring health or immune status of fish. Although a clear seasonal variation was marked in haematological and innate immune parameters of this species, the fluctuations are not consistent to any season, except summer season showing less value in most of the parameters. The probable compensatory mechanism among the various defence factors might be playing role to protect from diseases during different seasons. The data generated will help for subsequent studies with relation to immune-modulation or stress response in this species.

REFERENCES

Anderson, D. P. and A. K. Siwicki, 1995. Basic haematology and serology for fish health programs. *In:* Proceedings of the Diseases in Asian aquaculture, Asian Fisheries Society, 185 pp.

Anderson, D. P., T. Moritomo and R. de Grooth, 1992. Neutrophil, glass adherent, nitroblue tetrazolium assay gives early indication of immunization effectiveness in rainbow trout. *Vet. Immunol. Immunopathol.*, **30**: 419-429.

Bhat, A., 2001. New report of the species, *Horabagrus brachysoma* in the Uttarakhanda district of Karnataka. *J. Bombay Nat. Hist. Soc.*, **98**: 294-296.

Blaxhall P. C. and K. W. Daisley, 1973. Routine haematological methods for use with fish blood. *J. Fish Biol.*, **5**: 771-781.

Bowden, T. J., R. Butler and I. R. Bricknell, 2004. Seasonal variation of serum lysozyme level in Atlantic halibut (*Hipoglossus hipoglossus* L.). *Fish Shellfish Immunol.*, **17**: 129-135.

Bradford, M. M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein. *Anal. Biochem.*, **72**: 248-254.

Bromage, N., M. Porter and C. Randall, 2001. The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. *Aquaculture*, **197**: 1-4.

Collazos, M. E., C. Barriga and E. Ortega, 1994. Optimum conditions for the activation of the alternative complement pathway of a cyprinid fish (*Tinca tinca*, L.): Seasonal variations in the titres. *Fish Shellfish Immunol.*, **4**: 499-506.

Collazos, M. E., E. Ortega, C. Barriga and A. B. Rodriguez, 1998. Seasonal variation in haematological parameters in male and female *Tinca tinca*. *Mol. Cell. Biochem.*, **183**: 165-168.

Cook, M. T., P. J. Hayball, W. Hutchinson, B. F. Nowak and J. D. Hayball, 2003. Administration of a commercial immunostimulant preparation, EcoActiva as a feed supplement enhances macrophage respiratory burst and the growth rate of snapper (*Pagrus auratus*, Sparidae (Bloch and Schneider)) in winter. *Fish Shellfish Immunol.*, **14**: 333-345.

Dalmo, R. A., K. Ingebrightsen and J. Bogwald, 1997. Non-specific defense mechanisms in fish, with particular reference to the reticuloendothelial system (RES). *J. Fish Dis.*, **20**: 241-273.

De Pedro, N., A. I. Guijarro, M. A. Lopez-Patino, R. Martinez-Alvarez, M.J Delgado, 2005. Daily and seasonal variations in haematological and blood biochemical parameters in the tench, *Tinca tinca* Linnaeus, 1758. *Aquacult. Res.*, **36**: 1185-1196.

Ellis, A. E., 2001. Innate host defense mechanisms of fish against viruses and bacteria. *Dev. Comp. Immunol.*, **25**: 827-839.

Handy, R. D. and M. H. Depledge, 1999. Physiological responses: their measurement and use as environmental biomarkers in ecotoxicology. *Ecotoxicol.*, **8**: 329-349.

Harikrishnan, R., M. N. Rani and C. Balasundaram, 2003. Hematological and biochemical parameters in common carp, *Cyprinus carpio*, following herbal treatment for *Aeromonas hydrophila* infection. *Aquaculture*, **221**: 41-50.

Hernandez, A. and L. Tort, 2003. Annual variation of complement, lysozyme and haemagglutinin levels in serum of the gilthead seabream *Sparus aurata*. *Fish Shellfish Immunol.*, **15**: 479-481.

Hine, P. M., 1992. The granulocytes of fish: a review. *Fish Shellfish Immunol.*, **2**: 79-98.

Holland, M. C. H. and J. D. Lambris, 2002. The complement system in teleosts. *Fish Shellfish Immunol.*, **12**: 399-420.

Hrubec, T. C., J. L. Cardinale and S. A. Smith, 2000. Haematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis* hybrid). *Vet. Clin. Pathol.*, **29**: 7-12.

Hrubec, T. C., S. A. Smith and J. L. Robertson, 2001. Age-related changes in hematology and plasma chemistry values of hybrid striped bass (*Morone chrysops x Morone saxatilis*). *Vet. Clin. Pathol.*, **30**: 8-15.

Huffman, P. A., M. R. Arkoosh and E. Casillas, 1997. Characteristics of peripheral blood cells from rainbow trout evaluated by particle counter, image analysis and hemocytometric techniques. *J. Aquat. Anim. Health*, **9**: 239-248.

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

Ingram, G. A., 1980. Substances involved in the natural resistance of fish to infection-A review. *J. Fish Biol.*, **16:** 23-60.

Karvonen, A., P. Rintamaki, J. Jokela and E. Tellervo Valtonen, 2010. Increasing water temperature and disease risks in aquatic systems: Climate change increases the risk of some, but not all, diseases. *Int. J. Parasitol.*, **40:** 1483-1488.

Kumari, J. and P. K. Sahoo, 2005. Effects of cyclophosphamide on the immune system and disease resistance of Asian catfish *Clarias batrachus*. *Fish Shellfish Immunol.*, **19**: 307-316.

Kumari, J. and P. K. Sahoo, 2006. Non-specific immune response of healthy and immunocompromised Asian catfish (*Clarias batrachus*) to several immunostimulants. *Aquaculture*, **255**: 133-141.

Kumari, J., P. K. Sahoo, T. Swain, S. K. Sahoo, A. K. Sahu and B. R. Mohanty, 2006. Seasonal variation in the innate immune parameters of the Asian catfish *Clarias batrachus*. *Aquaculture*, **252**: 121-127.

Kurup, B. M., K. V. Radhakrishnan and T. G. Manoj Kumar, 2004. Biodiversity status of fish inhabiting rivers of Kerala (South India) with special reference to endemism, threats and conservation measures. In: Proceedings of the Second International Symposium on the Management of Large Rivers for Fisheries, Phnom Penh, Cambodia, 163-182.

Le Morvan, C., D. Troutand and P. Deschaux, 1998. Differential effects of temperature on specific and non-specific immune defences in fish. *J. Exp. Biol.*, **201**: 165-168.

Lillehaug, A., B. T. Lunestad and K. Grave, 2003. Epidemiology of bacterial diseases in Norwegian aquaculture- a description based on antibiotic prescription data for the ten-year period 1991-2000. *Dis. Aquat. Org.*, **53**: 115-125.

Matsuyama, H., K. Tanaka, M. Nakao and T. Yano, 1988. Characterization of the alternative complement pathway of carp. *Dev. Comp. Immunol.*, **12**: 403-408.

Pelgrom, S. M. G. J., R. A. C. Lock, P. H. M. Balm and S. E. W. Bonga, 1995. Integrated physiological response of tilapia, *Oreochromis mossambicus*, to sublethal copper exposure. *Aquat. Toxicol.*, **32**: 303-320.

Plumb, J. A. and N. Areechon, 1990. Effect of malathion on humoral immune response of channel catfish. *Dev. Comp. Immunol.*, **14**: 355-358.

Quade, M. J. and J. A. Roth, 1997. A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. *Vet. Immunol. Immunopathol.*, **58**: 239-248.

Rehulka, J., 2002. *Aeromonas* causes severe skin lesions in rainbow trout (*Oncorhynchus mykiss*): clinical pathology, haematology and biochemistry. *Acta Vet. Brno.*, **71**: 351-360.

Rehulka, J., B. Minarik and E. Rehulkova, 2004. Red blood cell indices of rainbow trout *Oncorhynchus mykiss* (Walbaum) in aquaculture. *Aquacult. Res.*, **35**: 529-546.

Rough, K. M., B. F. Nowak and R. E. Reuter, 2005. Haematology and leucocyte morphology of wild caught *Thunnus maccoyii*. *J. Fish Biol.*, **66:** 1649-1659.

Saha, K., K. Dash and A. Sahu, 1993. Antibody dependent haemolysin, complement and opsonin in sera of a major carp, *Cirrhina mrigala* and catfish, *Clarias batrachus* and *Heteropneustes fossilis*. *Comp. Immunol. Microbiol. Inf. Dis.*, **16**: 323-330.

Sahoo, P. K. and S. C. Mukherjee, 1999. Normal ranges for diagnostically important haematological parameters of laboratory-reared rohu (*Labeo rohita*) fingerlings. *Geobios*, **26**: 31-36.

Sahoo, P. K., J. Kumari and B. K. Mishra, 2005. Non-specific immune responses in juveniles of Indian major carp. *J. Appl. Ichthyol.*, **21**: 151-155.

Sahoo, P. K., K. Das Mahapatra, J. N. Saha, A. Barat, M. Sahoo, B. R. Mohanty, B. Gjerde, J. Ødegård, M. Rye and R. Salte, 2008. Family association between immune parameters and resistance to *Aeromonas hydrophila* infection in the Indian major carp, *Labeo rohita*. *Fish Shellfish Immunol.*, **25**: 163-169.

Sakai, D. K., 1983a. The assessment of the health condition of the salmonids by non-specific haemolytic activity of serum. *Bull. Jpn. Soc. Sci. Fish.*, **49**: 1487-1491.

Sakai, D. K., 1983b. Lytic and bactericidal properties of salmonid sera. *J. Fish Biol.*, **23**: 457-466.

Studnicka, M., A. Siwicki and B. Ryka, 1986. Lysozyme level in carp (*Cyprinus carpio* L.). *Israel J. Aqua.*, **38**: 22-25.

Svobodova, Z., V. Luskova, J. Drastichova, M. Svoboda and V. Zlabek, 2003. Effect of deltamethrin on haematological indices of common carp (*Cyprinus carpio* L.). *Acta Vet. Brno.*, **72**: 79-85.

Swain, P., S. Dash, P. K. Sahoo, P. Routray, S. K. Sahoo, S. D. Gupta, P. K. Meher and N. Sarangi, 2007. Non-specific immune parameters of brood Indian major carp *Labeo rohita* and their seasonal variations. *Fish Shellfish Immunol.*, **22**: 38-43.

Tavares-Dias, M. and F. R. Moraes, 2007a. Leukocyte and thrombocyte reference values for channel catfish (*Ictalurus punctatus* Raf.) with an assessment of morphological, cytochemical, and ultrastructural features. *Vet. Clin. Pathol.*, **36**: 49-54.

Tavares-Dias, M. and F. R. Moraes, 2007b. Haematological and biochemical reference intervals for farmed channel catfish. *J. Fish Biol.*, **71**: 383-388.

Tripathi, N. K., K. S. Latimer and V. V. Burnley, 2003. Biochemical reference intervals for koi (*Cyprinus carpio*). *Comp. Clin. Pathol.*, **12**: 160-165.

Van Vuren, J. H. J. and J. Hattingh, 1978. A seasonal study of the haematology of wild freshwater fish. *J. Fish Biol.*, **13**: 305-313.

Yano, T., 1992. Assays of haemolytic complement activity. *In*: Techniques in Fish Immunology, Vol. 2. (Eds. J.S. Stolen, T.C. Fletcher, D.P. Anderson, S.L. Kaatari, A.F. Rowley), SOS, USA, pp. 131-141.

Yano, T., 1996. The non-specific immune system. *In*: The Fish Immune System: organism, pathogen and environment (Eds. G. Iwama and T. Nakanishi), San Diego, USA, Academic Press, pp. 105-157.

Zuo, X. and P. T. K. Woo, 1997. Natural anti-proteases in rainbow trout, *Oncorhynchus mykiss* and brook charr, *Salvelinus fontinalis* and the *in vitro* neutralization of fish α 2-macroglobulin by the metalloprotease from the pathogenic haemoflagellate, *Cryptobia salmositica*. *Parasitology*, **114**: 375-381.