1. Stentiford, G. D., Feist, S. W., Stone, D. M., Bateman, K. S. and Dunn, A. M., Microsporidia: diverse, dynamic, and emergent pathogens inaquatic systems. *Trends Parasitol.,* 2013, **29**, 567–578.
2. Vavra, J. and Lukes, J., Microsporidia and 'the art of living together'. *Adv. Parasitol.,* 2013*,* **82**, 253–319.
3. Tourtip, S. *et al.*, *Enterocytozoon hepatopenaei* sp. nov. (Microsporida: enterocytozoonidae), a parasite of the black tiger shrimp *Penaeus monodon* (Decapoda: Penaeidae): fine structure and phylogenetic relationships. *J. Invertebr. Pathol.,* 2009*,* **102**, 21–29.
4. Tangprasittipap, A. *et al.*, The microsporidian *Enterocytozoon hepatopenaei* is not the cause of white feces syndrome in whiteleg shrimp *Penaeus* (*Litopenaeus*) *vannamei. BMC Vet. Res*., 2013, **9**, 139–148.
5. NACA, Hepatopancreatic microsporidiosis caused by *Enterocytozoon hepatopenaei*: disease card, Network of Aquaculture Centres in Asia-Pacific (NACA), Bangkok, Thailand, 2015; www.enaca.org
6. Thitamadee, S. *et al.*, Review of current disease threats for cultivated penaeid shrimp in Asia. *Aquaculture*, 2016, **452**, 69–87.
7. CIBA, Annual Report 2014–15. Central Institute of Brackishwater Aquaculture, Chennai, 2015, p. 96; www.ciba.res.in
8. Rajendran, K. V. *et al.*, Emergence of *Enterocytozoon hepatopenaei* (EHP) in farmed *Penaeus* (*Litopenaeus*) *vannamei* in India. *Aquaculture*, 2016, **454**, 272–280.
9. Didier, E. S., Vossbrinck, C. R., Stovall, M. E., Green, L. C., Bowers, L., Fredenburg, A. and Didier, P. J., Diagnosis and epidemiology of microsporidia infections in humans. *Southeast Asian J. Trop. Med. Public Health* (*Suppl.*), 2004, **35**, 65–81.
10. Bukhari, Z. and Smith, H. V., Effect of three concentration techniques on viability of *Cryptosporidium parvum* oocysts recovered from bovine faeces. *J. Clin. Microbiol*., 1995, **33**, 2592–2595.
11. Dryden, M. W., Payne, P. A., Ridley, R. and Smith, V., Comparison of common fecal flotation techniques for the recovery of parasite eggs and oocysts. *Vet. Ther.*, 2005, **6**, 15–28.
12. Ryan, N. J. *et al.*, A new trichrome blue stain for detection of microsporidial species in urine, stool and nasopharyngeal specimens. *J. Clin. Microbiol.*, 1993, **31**, 3264–3269.
13. Lom, J., On a new taxonomic character in myxosporidia, as demonstrated in description of two new species of *Myxobolus*. *Folia Parasitol.*, 1969, **16**, 97–103.
14. Bell, T. A. and Lightner, D. V., *A Handbook of Normal Penaeid Shrimp Histology*, World Aquaculture Society, Baton Rouge, LA, USA, 1988, p. 114.
15. Tang, K. F. J., Pantoja, C. R., Redman, R. M., Han, J. E., Tran, L. H. and Lightner, D. V., Development of *in situ* hybridization and PCR assays for the detection of *Enterocytozoon hepatopenaei* (EHP), a microsporidian parasite infecting penaeid shrimp. *J. Invertebr. Pathol*., 2015, **130**, 37–41.
16. Weber, R., Bryan, R. T., Owen, R. L., Wilcox, C. M., Gorelkin, L. and Visvesvara, G. S., Improved light-microscopical detection of microsporidia spores in stool and duodenal aspirates. *N. Engl. J. Med*., 1992, **326**, 161–166.

ACKNOWLEDGEMENTS. This study was carried out under the National Surveillance Programme for Aquatic Animal Diseases, coordinated by the National Bureau of Fish Genetic Resources, Lucknow. We thank the National Fisheries Development Board, Hyderabad for financial support and the Director, ICAR-Central Institute of Brackishwater Aquaculture, Chennai for providing the necessary facilities to carry out this work.

Received 19 August 2016; revised accepted 18 May 2018

doi: 10.18520/cs/v115/i4/758-763

Haematobiochemical parametrs of fishes of preferential nutritional habits

Gayatri Acharya*, Prafulla K. Mohanty, Sujata Puspamitra, Aryadhara Das and Sarbeswar Nayak

Postgraduate Department of Zoology, Utkal University, Vani Vihar, Bhubaneswar 751 004, India

This study compares the haematobiochemical parameters of fishes of different food habits, viz. *Labeo rohita* (herbivore), *Channa striatus* (carnivore), *Clarias batrachus* (omnivore) and *Cirrhinus reba* (detrivore). Statistical analyses reveal the significant differences in haematobiochemical parameters like haemoglobin, red blood cell, packed cell volume, mean corpuscular haemoglobin and while blood cells with respect to nutritional habits. The monocytes have been observed to vary significantly at $P < 0.001$ level, mean corpuscular volume deviates at $P < 0.05$, whereas both eosinophils and albumin show significant variation at $P < 0.01$ level. Haematobiochemical parameters have been regarded as a valuable tool for monitoring the health of fish.

Keywords: Freshwater fish, haematobiochemical parameters, nutritional habit, statistical analysis.

FISHES are the first successful aquatic vertebrates which are characterized by the presence of paired fins with fin rays and breathe through gills. These are a rich source of simple animal protein and omega three fatty acids which are highly digestible and absorbable by humans. The omega three fatty acids help in protecting the heart. Therefore, healthy and disease-free fish need to be consumed for human beings for better health. One of the difficulties in assessing the health status of natural fish population is the lack of reliable references in normal condition. Therefore, ichthyologists have developed interest in haematological studies. Moreover, these haematological parameters are influenced by many factors such as age, sex, dietary state and stress[1]. The variation in haematological parameters in relation to sex is observed in common carp *Cyprinus carpio*[2] and *Heterobranchus longifilis*[3]. Significant seasonal variation is recorded in *Mastacembelus armatus*[4]. Concentration of salt is reported to show significant increase in blood parameters in common carp[5]. Since the aquaculture industry is expanding, standardized methods are essential for monitoring the health status of fishes. The application of haematological indices in fish farming may enhance disease-free production by identifying early detection of stress and diseases[6]. This, in turn will contribute to more specific, timely and effective disease treatments in the future. Haematological

*For correspondence. (e-mail: gayatri.acharya65@gmail.com)

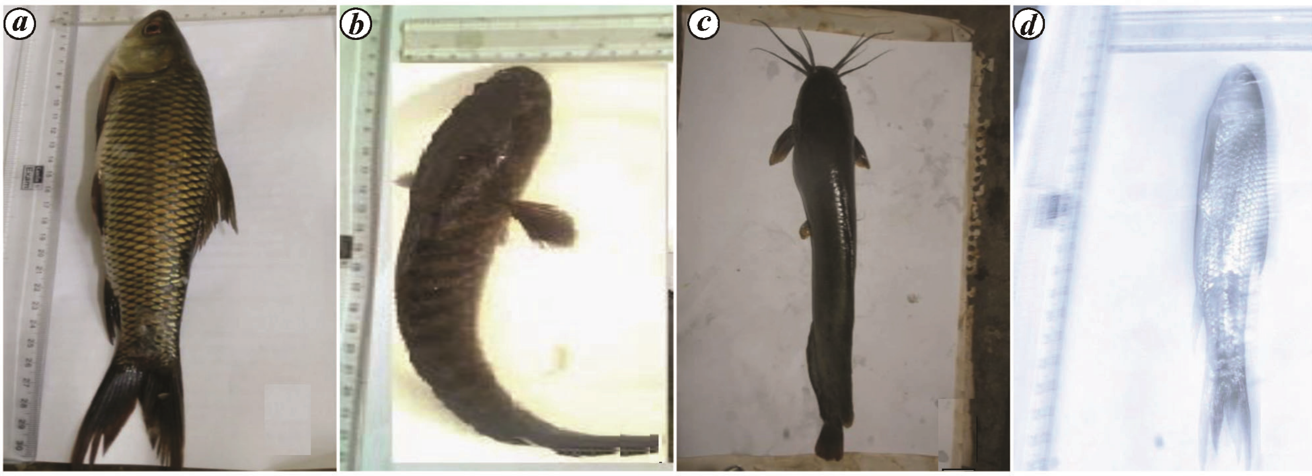

Figure 1. Freshwater fishes of different food habits. ***a***, *Labeo rohita* (herbivore); ***b***, *Channa striatus* (carnivore); ***c***, *Clarias batrachus* (omnivore); ***d***, *Cirrhinus reba* (detrivore).

Table 1. Physico-chemical parameters of the study site

Physico-chemical parameters of water	Mean ± SE
Temperature (°C)	22.42 ± 2.15
Alkalinity (mg/dl)	26.75 ± 0.35
Dissolved oxygen (mg/l)	6.34 ± 0.30
pH	7.09 ± 0.41

findings are among the most desirable and useful laboratory diagnostic aid[7].

Fishes are found in different regimes like surface, middle and bottom portions of aquatic body where food of their preference is available. According to their feeding habit, fishes are named as herbivores, carnivores, omnivores and detrivores. Since haematobiochemical data with respect to different feeding habits are inadequate and inconsistent, the aim of this study is to examine the influence of different feeding habits on haematobiochemical parameters of four commercially important freshwater fishes.

For the present study, water samples and healthy freshwater fishes, namely *Labeo rohita (*Figure 1 *a*), *Channa striatus* (Figure 1 *b*), *Clarias batrachus* (Figure 1 *c*) and *Cirrhinus reba* ((Figure 1 *d*) were collected from Daya river, Odisha, India. Fifteen live fishes from each species weighing 120–150 g and with body length 25–30 cm were selected for the study. Blood samples were taken from the caudal vein using 22 gauge needle[8]. Blood smears (three slides per fish) were prepared immediately after collection of blood to avoid morphological changes. The smeared slides were air-dried, fixed with methanol, stained with Giemsa's stain and maintained for differential leucocyte count. The remaining blood samples were used immediately for haematobiochemical analyses. Total red blood cell (RBC) and total white blood cell (WBC) counts were made using a Neubauer's haemocytometer. The haemoglobin (Hb) concentration was analysed by Sahli's haemometer[9]. Packed cell volume (PCV) was determined using the microhaematocrit method[10]. Erythrocyte indices like mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated[11]. Biochemical parameters like serum protein, albumin, globulin, glucose and cholesterol were analysed with the help of commercial kits (Crest Biosystem, India). Differences in the haematobiochemical parameters among the four categories of fishes of different food habits were statistically analysed by one-way ANOVA followed by Tukey's pairwise comparison.

The physico-chemical parameters of water of Daya river such as temperature (22.42 ± 2.15°C), alkalinity (26.75 ± 0.35 mg/l), dissolved oxygen (6.34 ± 0.30 mg/l), and pH (7.09 ± 0.41) were taken into account (Table 1). Haematological and biochemical parameters of the four freshwater fishes of different feeding habits reflected significant variation (Table 2). Total RBC was significantly variable among the fishes at $P < 0.001$. Hb was within the range 6.56 to 9.5 gm/dl. The maximum level of Hb was found in *C. batrachus* (9.5 ± 0.31), whereas the least was in *C. reba* (6.56 ± 0.32). PCV was within the range 22.26–30.2%. MCH and MCHC significantly deviated at $P < 0.001$ level, whereas the level of deviation for MCV was at $P < 0.05$. Higher WBC count was recorded in *C. batrachus*, followed by *C. striatus*, *C. reba*, and *L. rohita*. The percentage of lymphocyte in *L. rohita* (Figure 2 1.*a*), *C. striatus* (Figure 2 1.*b*), *C. batrachus* (Figure 2 1.*c*), and *C. reba* (Figure 2 1.*d*) was found to be 69.73 ± 0.88, 69.46 ± 0.56, 67.2 ± 0.97 and 69.06 ± 0.94 respectively. Monocytes were found to be 6.8 ± 0.41 in *L. rohita* (Figure 2 2.*a*), 5.73 ± 0.28 in *C. striatus* (Figure 2 2.*b*), 10.46 ± 0.82 in *C. batrachus* (Figure 2 2.*c*) and 5.26 ± 0.39 in *C. reba* (Figure 2 2.*d*) which deviated significantly at $P < 0.001$. The percentage of eosinophils

Table 2. Haematobiochemical parameters of fishes of different food habits

	Labeo rohita	*Channa striatus*	*Clarias batrachus*	*Cirrhinus reba*	
Haematological parameters	Herbivore	Carnivore	Omnivore	Detrivore	*P*-value
RBC (10^6 mm^3)	1.95 ± 0.07^a	2.11 ± 0.07^b	2.97 ± 0.07^{a,b,c}	1.94 ± 0.06^c	4.825E-15***
Hb (g/dl)	7.92 ± 0.20^{a,b}	8.4 ± 0.26^{a,c,d}	9.5 ± 0.31^{b,c,e}	6.56 ± 0.32^{d,e}	7.576E-11***
PCV (%)	24.4 ± 0.64^a	26.06 ± 0.75^{b,c}	30.2 ± 1.31^{a,b,d}	22.26 ± 1.06^{c,d}	4.522E-06***
MCV (fl)	128.33 ± 6.48^a	124.36 ± 6.20	109.64 ± 4.53^a	115.91 ± 5.87	0.05*
MCH (pg)	41.67 ± 2.12^{a,b}	40.05 ± 1.93^{c,d}	33.25 ± 0.74^{a,c}	35.57 ± 1.99^{b,d}	0.001***
MCHC (%)	32.48 ± 0.25^{a,b}	32.27 ± 0.43	31.95 ± 1.15^b	30.61 ± 0.56^b	0.0004***
WBC (10^3 mm^3)	3.75 ± 0.23^{a,b}	7.40 ± 0.46^{a,c}	7.63 ± 0.35^{b,d}	3.90 ± 0.25^{c,d}	8.334E-08***
Lymphocyte (%)	69.73 ± 0.88	69.46 ± 0.56	67.2 ± 0.97	69.06 ± 0.94	0.11NS
Monocyte (%)	6.8 ± 0.41^a	5.73 ± 0.28^b	10.46 ± 0.82^{a,b,c}	5.26 ± 0.39^c	2.654E-08***
Eosinophil (%)	2.86 ± 0.36^a	4.33 ± 0.27^a	3.93 ± 0.38	2.6 ± 0.48	0.01**
Neutrophil (%)	20.6 ± 0.77	20.46 ± 0.78	19 ± 1.15	23.06 ± 0.80	0.11NS
Protein (g/dl)	5.33 ± 0.39	5.27 ± 0.36	6.74 ± 0.43	6.18 ± 0.68	0.11NS
Albumin (g/dl)	3.19 ± 0.18^a	4.06 ± 0.21^{a,b}	3.60 ± 0.22	3.08 ± 026^b	0.01**
Globulin (g/dl)	2.14 ± 0.38	1.21 ± 0.46	3.14 ± 0.57	3.09 ± 0.64	0.31NS
Glucose (mg/dl)	95.18 ± 5.42	94.81 ± 5.56	98.86 ± 3.96	101.73 ± 3.42	0.68NS
Cholesterol (mg/l)	206.20 ± 11.14	229.56 ± 16.08	204.41 ± 19.10	208.95 ± 11.41	0.87NS

Rows with similar letters show significant difference.
*Significant at $P < 0.05$, **Significant at $P < 0.01$, ***Significant at $P < 0.001$. NS, Not significant.

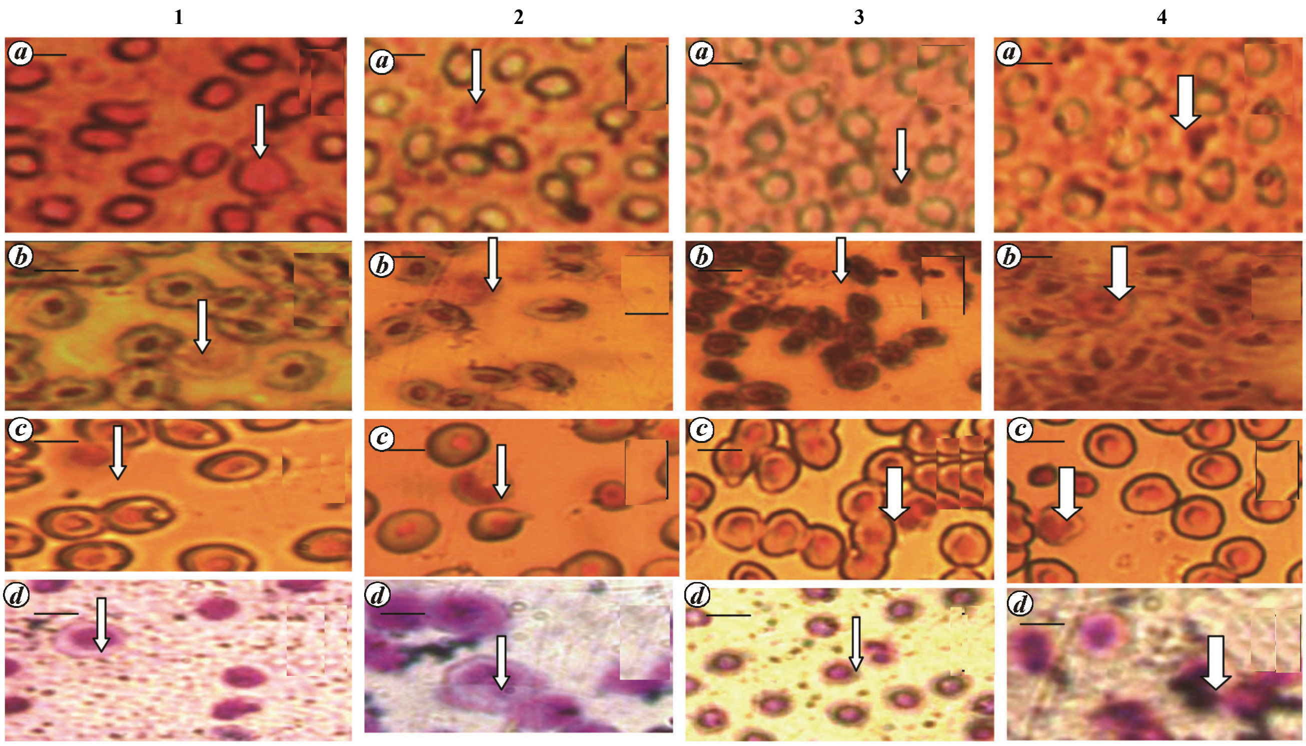

Figure 2. (**1**) Lymphocytes of fishes of different food habits. (**2**) Monocytes of fishes of different food habits. (**3**) Eosinophils of fishes of different food habits. (**4**) Neutrophils of fishes of different food habits. ***a***, *Labeo rohita* (herbivore); ***b***, *Channa striatus* (carnivore); ***c***, *Clarias batrachus* (omnivore); ***d***, *Cirrhinus reba* (detrivore). (Bar = 10 mm.)

was found to be 2.86 ± 0.36, 4.33 ± 0.27, 3.93 ± 0.38 and 2.6 ± 0.48 in *L. rohita* (Figure 2 3.*a*), *C. striatus* (Figure 2 3.*b*), *C. batrachus* (Figure 2 3.*c*), and *C. reba* (Figure 2 3.*d*) respectively, and showed significant deviation at $P < 0.01$. The neutrophils did not vary significantly among fishes with respect to feeding habit and were noted to be 20.6 ± 0.77, 20.46 ± 0.78, 19 ± 1.15 and 23.06 ± 0.80 in *L. rohita* (Figure 2 4.*a*), *C. striatus* (Figure 2 4.*b*), *C. batrachus* (Figure 2 4.*c*) and *C. reba* (Figure 2 4.*d*) respectively. Haematobiochemical parameters with significant variation ($P < 0.01$) were observed only for albumin concentration.

A high level of RBC was recorded in *C. batrachus*, followed by *C. striatus*, *L. rohita* and *C. reba*. The increased number of erythrocyte is associated with oxygen demand, which is essential to meet if requirement at higher metabolic rate[12]. In fact, lesser haemoglobin content is associated with the hypo activity of fishes[13]. The anaemic condition of fishes may be determined by PCV[14]. The status of health of fishes is related to the presence of leucocytes and in many cases, these are also helpful in the evaluation of the immune system. Therefore, variation in the proportion of these defence cells in the blood is usually expected.

Monocytes (macrophages) and lymphocytes are also involved in the immune response. Neutrophils are effector key cells in non-specific immunity as they migrate into the site of infection to recognize, ingest and destroy the pathogens. The range of serum biochemical parameters are species-specific[15]. An increased blood glucose and protein level was noted in *C. reba* and *C. batrachus* respectively. This might be because of an increased depletion of liver glycogen[16]. An increased concentration of plasma protein was found as a result of alteration in liver structure that reduced aminotransferase activity[17]. Albumin helps in transportation of lipid in fishes and also in their general metabolism[18]. Cholesterol concentration of *C. striatus* was higher due to high proportion of fat in the chemical composition of its food[19].

Haematological studies of fishes are of significance due to the increasing emphasis on pisciculture and greater awareness of pollution in the aquatic ecosystem. This study provides a comparative account of haematobiochemical parameters of fishes having different food habits. Based on the analysis using one-way ANOVA, it is confirmed that omnivores and carnivores are more active fishes, followed by herbivores and detrivores. This might be due to the variation of their feeding habits and modifications of their physiological system which leads to variation in haematobiochemical parameters.

1. McCarthy, D. H., Stevenson, J. P. and Roberts, M. S., Some blood parameters of the rainbow trout (*Salmo gairdneri richardson*). *J. Fish Biol.*, 1973, **5**, 1–8.
2. Baghizadeh, E. and Khara, H., Variability in hematology and plasma indices of common carp *Cyprinus carpio*, associated with age, sex and hormonal treatment. *Iran. J. Fish Sci.*, 2014, **14**(1), 99–111.
3. Suleiman, B. and Abdullah, S. A., Haematological and serum biochemical parameters as biomarkers of growth performance in artificially spawned *Heterobranchus longifilis*. *J. Biosci. Biotechnol. Disc.*, 2016, **1**, 22–27.
4. Kandari, M. and Rauthan, J. V. S., Hematological changes in fish *Mastacembelus armatus* (Lacepede) of Song River. *Scholers Acad. J. Biosci.*, 2015, **3**(1A), 34–37.
5. Hasan, A., Hilali, A. I., Mohammed, S. and Khshali, A. I., Effect of water salinity on some blood parameters of common carp (*Cyprinus carpio*). *Int. J. Appl. Agric. Sci.*, 2016, **2**(1), 17–20.
6. Wells, B. B., *Clinical Pathology*, W.B. Saunders and Company, Philadelphia, USA, 1956, 5th edn, pp. 266–272.
7. Strickland, J. D. H. and Parsons, T. R., *A Practical Hand Book of Seawater Analysis*, Fish Research Board, Canada, 1972, p. 310.
8. Campbell, T. W., Clinical pathology of reptiles. In *Reptile Medicine and Surgery* (ed. Mader, D. R.), W.B. Saunders and Company, Philadelphia, USA, 2006, 2nd edn, pp. 453–470.
9. Sahli, H., *Lehrbuch d.klin. Untersuchungen Methode*, Williams and Williams & Co, 1909, 5th edn, p. 846.
10. Mcinroy, R. A., A micro-haematocrit for determining the packed cell volume and haemoglobin concentration on capillary blood. *J. Clin. Pathol.*, 1953, **7**, 32–36.
11. Dacie, J. V. and Lewis, S. M., *Practical Haematology*, Churchill Livingston, Edinburgh, UK, 1984, 6th edn, pp. 27–32.
12. Engel, D. M. and Davis, E. M., Relationship between activity and blood composition in certain marine teleosts. *Copeia*, 1964, **3**, 586–587.
13. Rambhaskar, B. and Srinivasa Rao, K., Comparative haematology of ten species of marine fish from Visakhapatnam Coast. *J. Fish Biol.*, 1986, **30**, 59–66.
14. Blaxhall, P. C. and Daisley, K. W., Routine haematological methods for use with fish blood. *J. Fish Biol.*, 1973, **5**, 771–781.
15. Jawad, L. A., Al-Mukhtar, M. A. and Ahmed, H. K., The relationship between haematocrit and some biological parameters of the Indian shad, *Tenualosa ilisha* (family Clupidae). *Anim. Biodivers. Conserv.*, 2004, **27**, 478–483.
16. Ojolick, E. J., Cusack, R., Benfey, T. J. and Kerr, S. R., Survival and growth of all female diploid and triploid *Clarias macrocephalus*. *Fish Genet. Biotrop. Spec. Publ.*, 1995, **52**, 79–86.
17. Kavadias, S., Castritsi-Catharios, J. and Dessypris, A., Annual cycles of growth rate, feeding rate, food conversion, plasma glucose and plasma lipids in the population of European sea bass (*Dicentrarchus labrax*) farmed in floating marine cages. *J. Appl. Ichthyol.*, 2004, **19**, 29–34.
18. Nguyen, H. T., *Transport Proteins. The Clinical Chemistry of Laboratory Animals*, Taylor and Francis, Philadelphia, USA, 1999, 2nd edn, pp. 309–335.
19. Hill, S., A literature review of the blood chemistry of rainbow trout, *Salmo gairdneri*. *J. Fish Biol.*, 1982, **20**, 535–569.

ACKNOWLEDGEMENTS. We thank the Postgraduate Department of Zoology, Utkal University, Bhubaneswar for providing the necessary facilities to carry out this work. G.A. thanks the DST-INSPIRE programme for funds. Authors declare no conflict of interest.

Received 28 February 2017; revised accepted 23 May 2018

doi: 10.18520/cs/v115/i4/763-766