Hematological and cytochemical characteristics of peripheral blood cells in the argus snakehead (*Ophiocephalus argus* Cantor)

Xue Wang [1] , **Jie Zheng Wu** [1] , **Mei Sheng Wu** [1] , **Xian Xian Chen** [1] , **Hanif Misbah** [2] , **Zhou Sheng Zhang** [Corresp. 1]

[1] College of Life Sciences, Anhui Normal University, Wuhu, China

[2] College of Life Sciences, Anhui Normal University, Faisalabad, Pakistan

Corresponding Author: Zhou Sheng Zhang
Email address: szzhang@mail.ahnu.edu.cn

Background. The argus snakehead (*Ophiocephalus argus* Cantor) is a highly nutritious, freshwater, cultured bony fish with a high economic value. The health of the fish is closely related to its blood cells, which are critical for oxygen transport, natural defense, and immunity. We investigated the morphometry, microstructure, and cytochemical characteristics of the peripheral blood cells of *O. argus*. Our results may provide the basic reference values needed to monitor the health of this fish for large-scale cultivation.

Methods. The number of blood cells in *O. argus* were counted on a hemocytometer and their size was measured using a micrometer under light microscope. The morphology and classification of the blood cells were studied using Wright's staining and the cytochemical characteristics were studied using seven chemical stains including peroxidase (POX), Sudan black B (SBB), periodic acid-Schiff (PAS), acid phosphatase (ACP), alkaline phosphatase (ALP), chloroacetic acid AS-D naphthol esterase (AS-D), and α-naphthol acetate esterase (α-NAE).

Results. The peripheral blood cells in *O. argus* can be classified as erythrocytes, leukocytes, and thrombocytes; of which, females had 2.9597 million/mm^3, 88,400/mm^3, and 43,600/mm^3, respectively, and males had 3.0105 million/mm^3, 105,500/mm^3, and 34,000/mm^3, respectively. Leukocytes consisted of neutrophils, monocytes, large lymphocytes, and small lymphocytes. Eosinophils and basophils were not found. Monocytes were the most numerous leukocytes identified, followed by neutrophils and small lymphocytes, while large lymphocytes were the least frequently identified. Cytochemical staining showed that erythrocytes were only positive for PAS staining. Neutrophils were strongly positive for POX, SBB, and ACP, and positive for all the other cytochemical stains. Monocytes were positive for PAS and α-NAE and were weakly positive for ACP and AS-D staining. Large lymphocytes were positive for PAS and were weakly positive for ALP, AS-D, and α-NAE staining. Small lymphocytes were positive for PAS and weakly positive for AS-D and α-NAE staining. Thrombocytes were positive for PAS and were weakly positive for ACP and AS-D, but negative for the remaining cytochemical stains. The morphology of peripheral blood cells in *O. argus* was generally similar to that of other fish species, while the cytochemical staining patterns showed clear species specificity.

1 Hematological and cytochemical characteristics of peripheral blood cells in the argus snakehead

2 (*Ophiocephalus argus* Cantor)

3 Xue Wang, Zhengjie Wu, Shengmei Wu, Xianxian Chen, Misbah Hanif, Shengzhou Zhang

4 Key Laboratory for Conservation and Use of Important Biological Resources of Anhui Province, College

5 of Life Sciences, Anhui Normal University, Wuhu, Anhui, China

6 Corresponding author:

7 Shengzhou Zhang

8 College of Life Sciences, Anhui Normal University, 1 Beijing East Road, Wuhu, Anhui Province 241000,

9 People's Republic of China

10 Email: szzhang@mail.ahnu.edu.cn

20 **Abstract**

21 **Background**. The argus snakehead (*Ophiocephalus argus* Cantor) is a highly nutritious,

22 freshwater, cultured bony fish with a high economic value. The health of the fish is closely

23 related to its blood cells, which are critical for oxygen transport, natural defense, and immunity.

24 We investigated the morphometry, microstructure, and cytochemical characteristics of the

25 peripheral blood cells of *O. argus*. Our results may provide the basic reference values needed to

26 monitor the health of this fish for large-scale cultivation.

27 **Methods**. The number of blood cells in *O. argus* were counted on a hemocytometer and their

28 size was measured using a micrometer under light microscope. The morphology and

29 classification of the blood cells were studied using Wright's staining and the cytochemical

30 characteristics were studied using seven chemical stains including peroxidase (POX), Sudan

31 black B (SBB), periodic acid-Schiff (PAS), acid phosphatase (ACP), alkaline phosphatase (ALP),

32 chloroacetic acid AS-D naphthol esterase (AS-D), and α-naphthol acetate esterase (α-NAE).

33 **Results**. The peripheral blood cells in *O. argus* can be classified as erythrocytes, leukocytes, and

34 thrombocytes; of which, females had 2.9597 million/mm^3, 88,400/mm^3, and 43,600/mm^3,

35 respectively, and males had 3.0105 million/mm^3, 105,500/mm^3, and 34,000/mm^3, respectively.

36 Leukocytes consisted of neutrophils, monocytes, large lymphocytes, and small lymphocytes.

37 Eosinophils and basophils were not found. Monocytes were the most numerous leukocytes

38 identified, followed by neutrophils and small lymphocytes, while large lymphocytes were the

39 least frequently identified. Cytochemical staining showed that erythrocytes were only positive

40   for PAS staining. Neutrophils were strongly positive for POX, SBB, and ACP, and positive for

41   all the other cytochemical stains. Monocytes were positive for PAS and α-NAE and were weakly

42   positive for ACP and AS-D staining. Large lymphocytes were positive for PAS and were weakly

43   positive for ALP, AS-D, and α-NAE staining. Small lymphocytes were positive for PAS and

44   weakly positive for AS-D and α-NAE staining. Thrombocytes were positive for PAS and were

45   weakly positive for ACP and AS-D, but negative for the remaining cytochemical stains. The

46   morphology of peripheral blood cells in *O. argus* was generally similar to that of other fish

47   species, while the cytochemical staining patterns showed clear species specificity.

48

49   **Introduction**

50   Vertebrate blood consists of plasma and blood cells. Fish blood cells can be classified as

51   erythrocytes, leukocytes, and thrombocytes, and are vital to the body for gas transportation,

52   immune defense, and coagulation, respectively (Chen et al., 2019b; Palmer et al., 2015). Blood

53   cells are very sensitive to changes in internal physiological conditions and external stimuli

54   (Palmer et al., 2015). Variations in blood cell counts, morphology, and various intracellular

55   functional components can be used as direct markers to determine the health status of fish (Fang

56   et al., 2014; Ishikawa, Ranzani-Paiva & Lombardi, 2008).

57   Traditional Wright's staining can be used to study the microstructure and classification of

58   fish blood cells (Zhang et al., 2019). Cytochemical staining is commonly used to detect

59   biological macromolecules and the activity of enzymes, and can improve our understanding of

60   the function and physiological state of blood cells and cell lineages (Massar et al., 2012; Shigdar,

61   Harford & Ward, 2009). A number of studies have been conducted on the classification,

62   microstructure, and cytochemical characteristics of peripheral blood cells in fish, especially

63   commercially cultured fish. Tripathi, Latimer & Burnley (2004) determined the hematological

64   reference intervals for koi (*Cyprinus carpio*), including blood cell morphology, cytochemistry,

65   and ultrastructure. Tavares-Dias & Moraes (2006) described the morphology, cytochemistry, and

66   ultrastructure of thrombocytes and leukocytes in neotropical fish (*Brycon orbignyanus*). Tavares-

67   Dias (2006) studied the morphology and cytochemistry of erythrocytes, thrombocytes, and

68   leukocytes in four freshwater teleosts: big head carp (*Aristichthys nobilis*), oscar (*Astrootus*

69   *ocellatus*), traíra (*Hoplias malabarus*), and lambari (*Astyanax bimaculatus*). Fang et al. (2014)

70   observed the morphology and cytochemistry of peripheral blood cells in *Schizothorax prenanti*

71   by light and electron microscopy. Bianchi et al. (2014) described the cell morphology and

72   cytochemical characteristics of a native South America catfish (*Sorubim lima*). Zheng et al.

73   (2016) investigated the ultrastructure and cytochemical properties of the peripheral blood cells of

74   the piebald naked carp (*Gymnocypris eckloni*) by transmission electron microscopy. Zhang et al.

75   (2019) compared the microstructure and cytochemical characteristics of peripheral blood cells in

76   the crucian carp (*Carassius auratus*) and grass carp (*Ctenpharyngodon idellus*). These studies

77   indicated that the major groups and micromorphology of peripheral blood cells in different fish

78   were generally similar. However, there were obvious species-specific differences in the

79   leukocytes, the proportion of various leukocyte types, and the cytochemical characteristics of

80   blood cells.

81   The argus snakehead (*Ophiocephalus argus* Cantor) belongs to the family Channidae,

82   perciformes, and is commonly found in the Yangtze River basin and the lower Yellow River in

83   China, as well as in various river systems in Korea, Japan, and Russia (Courtenay & Williams,

84   2004). *O. argus* is a very popular, nutritious, economically-farmed freshwater fish (Xiao et al.,

85   2017). In China, the annual production of *O. argus* is about 510,000 tons (worth approximately

86   1.6 billion US dollars) (Sagada et al., 2017). The incidence of diseases in *O. argus* has increased

87   as cultivation has increased (Xu et al., 2017). Fish hematology is one diagnostic tool that can

88   provide useful information in guiding treatment options (Grant, 2015). However, the hematology

89   and cytochemistry of peripheral blood cells in *O. argus* has not been well-studied. We

90   investigated the number, microstructure, and cytochemical characteristics of peripheral blood

91   cells in *O. argus* using cell counts, Wright's staining, and cytochemical methods under a light

92   microscope. Our results may improve the understanding of fish hematology, provide a reference

93   for monitoring the health of artificially-bred *O. argus*, and provide basic information for further

94   study of the physiology and immunology of this species.

95

96   **Materials & Methods**

97   **Animals and blood smear preparation**

98   Thirty healthy adult *O. argus* specimens were selected (15 males and 15 females), with an

99   average body length of 30.56-42.78 cm and weight of 960.20-1850.32 g. All fish were obtained

100   from a local Wuhu aquaculture farm between May and September, 2019. Blood samples were

101   collected by caudal vein puncture, and about 2 ml of blood was taken from each fish using a

102   sterile 5 ml syringe and 22 G needle. K_2-EDTA was used as an anticoagulant to avoid blood

103   coagulation and blood smears were prepared immediately after blood collection. This work was

104   approved by the ethics committee of Anhui Normal University (permit no. 20190312). Fish

105   handling and sampling techniques were carried out in accordance with standard vertebrate

106   procedures and veterinary practices and in accordance with national and provincial guidelines.

107   **Wright's staining**

108   The prepared blood smears were air-dried at room temperature and treated with Wright's

109   reagent according to the guidelines by Hefei Tianda Diagnostic Reagent Co., Ltd. (Hefei, China).

110   The blood smears were placed in a box containing a parallel support frame and little water,

111   stained with Wright's A solution for 1 min at room temperature, and treated with Wright's B

112   buffer for 10 min. The samples were rinsed with distilled water several times and were air-dried

113   again at room temperature. Stained blood smears were examined under a light microscope with

114   oil-immersion at 1,000× magnification (BM2000, Jiangnan Yongxin Co., Ltd. Nanjing, China).

115   **Cytochemical staining**

116   Cytochemical staining was carried out according to the methods described by Xu (2003),

117   with minor modifications. The prepared blood smears were fixed with formaldehyde vapor for

118   Sudan black B (SBB) and acid phosphatase (ACP) staining, 10% methanol-formaldehyde

119   solution for alkaline phosphatase (ALP) and chloroacetic acid AS-D naphthol esterase (AS-D)

120   staining, and 95% ethanol solution for periodic acid-schiff's (PAS) and α-naphthol esterase (α-

121   NAE) staining. The specific staining procedures are briefly described as follows:

122   POX staining: 2 ml 0.1% tetramethylbenzidine ethanol solution (0.1 g tetramethylbenzidine

123   (Sangon, 54827-17-7) dissolved in 100 ml 88% ethanol solution) was mixed with 20 μl sodium

124   nitroferricyanide (Sangon, 13755-38-9) saturated solution and dropped on the smears. Then 0.7

125   ml dilute H_2O_2 solution (50 μl 1% H_2O_2 solution mixed with 10 ml distilled water) was added

126   after the smears had been standing for 1 min, and was air blown until distributed evenly and

127   oxidized for 6 min.

128   SBB staining: dried blood smears were placed in Sudan Black B (Sangon, 4197-25-5)

129   staining solution for 60 min at 37℃, then rinsed in 70% ethanol solution and distilled water for

130   1-2 min.

131   PAS staining: blood smears were oxidized with 10 mg/ml periodic acid for 18-20 min, and

132   rinsed in distilled water for 2 min. Samples were placed in Schiff's solution for 60-90 min at

133   37℃. After rinsing in a sulfuric acid solution (0.6 g sodium bisulfite (Sangon, 7681-57-4)

134   dissolved in 5 ml 1mol/l hydrochloric acid and 100 ml distilled water) three to four times, the

135   smears were washed with distilled water for 2-3 min.

136   ACP staining: blood smears were stained with the reaction solution (0.1 g lead nitrate

137   (Xilong, 10099-74-8) and 0.128 g β-sodium glycerophosphate (Sangon, 819-83-0) dissolved in

138   74 ml distilled water and 12 ml pH 4.7 acetic acid buffer) for 4-4.5 hours at 37°C. Samples were

139   washed with distilled water for 5 min and were immersed in 2% ammonium sulfide solution

140   (Aladdin, 12135-76-1) for 30 min.

141   ALP staining: the smears were immersed in a substrate incubation solution (10 mg naphthol

142   AS-BI phosphate (Sangon, 1919-91-1), dissolved in 10 ml 0.05 mol/l propanediol buffer, mixed

143   with 10 mg fast blue B salt (Yuanye, 14263-94-6), and then filtered) for 45-60 min at 37℃, and

144   rinsing in distilled water for 2 min.

145   AS-D staining: blood smears were stained with the incubation solution (10 mg chloroacetic

146   acid AS-D naphthol (Sangon, 528-66-5), dissolved in 0.5 ml acetone solution, and then mixed in

147   5 ml distilled water, 5 ml pH 7.5 Veronal acetic acid buffer, and 10 mg fast blue B salt (Yuanye,

148   14263-94-6) were added). Samples were then incubated for 60-80 min at 37℃, and washed with

149   distilled water.

150   α-NAE staining: the smears were placed in the reaction solution (100 ml phosphate buffer

151   mixed with 1 ml 4mg/ml α-naphthol acetate (Sangon, 90-15-3), then 100 mg fast blue B salt was

152   added (Yuanye, 14263-94-6), and filtered by oscillation) and samples were incubated for 45-60

153   min at 37℃ and washed with distilled water three to four times.

154   After cytochemical staining, the smears were counterstained with Wright's reagent for POX

155   and SBB, 20 mg/ml methyl green (Sangon, 7114-03-6) for PAS, ACP, and α-NAE, and 1 mg/ml

156   hematoxylin (Sangon, 517-28-2) for ALP and AS-D.

157   **Evaluation of cytochemical staining results**

158   The results of cytochemical staining were expressed in terms of the intensity of

159   cytochemical reactions (negative reaction (-), weak positive reaction (+), positive reaction (+ +)

160   and strong positive reaction (+ + +)), according to the evaluation method described by Bianchi et

161   al. (2014).

162   **Blood cell counts and measurements**

163   The total number of blood cells was calculated using a hemocytometer under an Olympus

164   BX61 microscope (Tokyo, Japan). The number of erythrocytes (RBC), leukocytes (WBC), and

165   thrombocytes (TC) were calculated according to the proportions of these cells counted on the

166   Wright's blood smears (total number × the percentage of cells). The percentages of different

167   leukocyte types were calculated after counting 3,000 randomly-selected leukocytes from males

168   and females. The cell sizes (the length and width of various cells and nuclei) were obtained using

169   an ocular micrometer scale by measuring 20 randomly-selected cells for each cell type from male

170   and female specimens. The hemoglobin (Hb), hematoceit (HCT), and erythrocyte sedimentation

171   rate (ESR) were determined according to the methods described previously (Peng, et al., 2018).

172   The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean

173   corpuscular hemoglobin concentrations (MCHC) were calculated from RBC, HCT, and Hb

174   according to the formulae below (Gao et al., 2007a):

175   MCV (fl) = HCT/RBC

176   MCH (pg) = Hb /RBC

177   MCHC (g/dl) = Hb/HCT

178 **Statistical analysis**

179   The experimental data were represented by mean ± SD. The significant differences in

180 morphometric values among different cell types or between sexes were compared by one-way

181 ANOVA analysis using SPSS 21.0 software (SPSS Inc, Chicago, USA). A P-value less than 0.05

182 was used to indicate a significant difference, and a P-value less than 0.01 indicated an extremely

183 significant difference.

184 # Results

185 **Classification and counting of peripheral blood cells**

186   The blood smears treated with Wright's staining were observed under a light microscope

187 with oil-immersion at 1,000× magnification. According to the morphology and size of cells and

188 nuclei, nucleo-cytoplasmic ratio, the presence or absence of particles, and tinctorial feature in the

189 cytoplasm, the peripheral blood cells of *O. argus* could be divided into erythrocytes, leukocytes,

190 and thrombocytes, and the leukocytes could be further subdivided into neutrophils, monocytes,

191 large lymphocytes, and small lymphocytes.

192   The number of erythrocytes, leukocytes, thrombocytes, and various hematological

193 parameters in *O. argus* were calculated and shown in Table 1. There was no significant

194 difference in the number of erythrocytes and total leukocytes between sexes (P > 0.05), while the

195 number of thrombocytes in females was significantly higher than that in males (P <0.05). The

196 number of different leukocytes were also shown in Table 1. Monocytes were the most abundant

197 leukocytes in *O. argus*, followed by neutrophils and small lymphocytes, and   large lymphocytes

198 were the least numerous (one-way ANOVA: $F_{4,11}=354.476. P < 0.01$). The number of large

199 lymphocytes and small lymphocytes in females was significantly lower than that in males (P

200 <0.05). No statistically significant differences in Hb, HCT, ESR, MCV, MCH, and MCHC were

201 found between females and males (P > 0.05).

202 **The microstructure of peripheral blood cells**

203 **Erythrocytes**

204   Mature erythrocytes (Fig. 1A) were oval in shape, with a smooth surface, and contained an

205 ovoid or rod-shaped purple nucleus in the center of the cell, with a light brown or yellowish

206 cytoplasm. The size of the mature erythrocytes and their nuclei are shown in Table 2. The cell

207 length, and nuclear length and width of mature erythrocytes in females were significantly larger

208 than those in males (P < 0.01). A small number of immature erythrocytes (Fig. 1A) were also

209 observed on the blood smears treated with Wright's staining, and were round and smaller than

210 mature erythrocytes, with round or elliptic, dark purplish-stained nuclei.

211 **Neutrophils**

212   Neutrophils (Fig. 1B, C) were spherical or round in shape, with purplish stained nuclei. The

213 nuclei had a variety of shapes, including bilobate, trilobed, kidney-shaped (or non-bilobed), and

214   bilobed, which were the most frequently observed. The cytoplasm was rich and stained light blue,

215   containing numerous fine mauve and reddish particles.

216   **Monocytes**

217   Monocytes (Fig. 1D) were the largest leukocytes in *O. argus* (Table 2). Most of them were

218   round and oval and a few were irregular. The nuclei were oval, pear-shaped, or horseshoe-shaped,

219   and generally stained purple. The most obvious morphological feature of monocytes was that the

220   cytoplasm contained a large number of vacuoles of different sizes with pseudopodia

221   protuberances at the cell edges.

222   **Large lymphocytes**

223   Lymphocytes could be classified as large and small lymphocytes. Large lymphocytes (Fig.

224   1E) were generally or irregularly round, with large, oval nuclei on one side of the cells; the

225   purplish nucleus occupied almost the entire cytoplasm. Some large lymphocytes had smooth

226   surfaces and some had small finger-like protuberances on the surface of the cells.

227   **Small lymphocytes**

228   Small lymphocytes (Fig. 1F) were oval in shape with an eccentric, purple, rounded, gapped

229   nucleus that occupied almost the entire cell, and contained a thin rim of pale blue cytoplasm.

230   Small lymphocytes were characterized by numerous microvilli protuberances from the

231   cytoplasmic margins.

232   **Thrombocytes**

233   Thrombocytes showed different shapes in the smears, including round, oval, long ovoid,

234   and spindle. Spindle-shaped thrombocytes (Fig. 1G) were usually isolated and their nuclei were

235   consistent with the shape of the cells, most of which were centered and purplish, and the

236   cytoplasm was nearly colorless. Round thrombocytes (Fig. 1H) usually appeared in clusters with

237   multiple cells, with round, dark purple-stained nuclei, and less cytoplasm was flocculent around

238   the nucleus.

239   **The cytochemical staining characteristics of peripheral blood cells**

240   **POX staining**

241   Neutrophils (Fig. 2AB) were strongly positive with blue-black, coarse, and rod-shaped

242   granules in the cytoplasm. The cytoplasms of erythrocytes (Fig. 2AA), monocytes (Fig. 2AC),

243   large lymphocytes (Fig. 2AD), small lymphocytes (Fig. 2AE), and thrombocytes (Fig. 2AF)

244   were light blue without granules; all of these cells were negative.

245   **SBB staining**

246   Neutrophils (Fig. 2BC) were covered with a large number of diffusely-distributed dark

247   black granules in the cytoplasm, which were strongly positive. The cytoplasms of erythrocytes

248   (Fig. 2BB), monocytes (Fig. 2BD), large lymphocytes (Fig. 2BE), small lymphocytes (Fig. 2BF),

249   and thrombocytes (Fig. 2BG) were pale purple without granules; all of these cells were negative.

250   **PAS staining**

251   The cytoplasms of erythrocytes, neutrophils, monocytes, large lymphocytes, small

252   lymphocytes, and thrombocytes (Fig. 2CC-CH) were purple or dark purple with diffusely

253   granular matter; all of these cells were positive.

254   **ACP staining**

255   Neutrophils (Fig. 2DE) contained a large number of brown-black granules or tablets in the

256   cytoplasm and were strongly positive. Monocytes (Fig. 2DF) and thrombocytes (Fig. 2DI) were

257   weakly positive, with a small number of brown granules in the cytoplasm. The cytoplasms of

258   erythrocytes (Fig. 2DD), large lymphocytes (Fig. 2DG), and small lymphocytes (Fig. 2DH) were

259   pale purple without granules; all of these cells were negative.

260   **ALP staining**

261   Both neutrophils (Fig. 2EF) and large lymphocytes (Fig. 2EH) were weakly positive, with

262   many fine, uniformly-distributed purple granules in the cytoplasm. The cytoplasms of

263   erythrocytes (Fig. 2EE), monocytes (Fig. 2EG), small lymphocytes (Fig. 2EI), and thrombocytes

264   (Fig. 2EJ) were pale yellow without stained granules; all of these cells were negative.

265   **AS-D staining**

266   Neutrophils (Fig. 2FG) were positive, uniformly-distributed red granules. The cytoplasms

267   of monocytes (Fig. 2FH), large lymphocytes (Fig. 2FI), small lymphocytes (Fig. 2FJ), and

268   thrombocytes (Fig. 2FK) were pale red with fine granules; all of these cells were weakly positive.

269   Erythrocytes (Fig. 2FF) were negative with a pale pink cytoplasm.

270   **α-NAE staining**

271   The cytoplasm of monocytes (Fig. 2GI) was filled with gray-black diffused or granular

272   deposits, which were positive. The cytoplasms of neutrophils (Fig. 2GH), large lymphocytes (Fig.

273   2GJ), and small lymphocytes (Fig. 2GK) were purple with dark brown or purple granules; all of

274   these cells were weakly positive. Erythrocytes (Fig. 2GG) and thrombocytes (Fig. 2GL) were

275   negative; their cytoplasm were purplish without granules.

276   **The cytochemical staining patterns of peripheral blood cells**

277   The cytochemical staining patterns of peripheral blood cells in *O. argus* are summarized in

278   Table 3. Erythrocytes were positive for PAS and negative for POX, SBB, ACP, ALP, AS-D, and

279   α-NAE staining. Neutrophils exhibited a strongly positive reaction for POX, SBB, and ACP;

280   positive for PAS, and AS-D; and weakly positive for ALP and α-NAE staining. Monocytes were

281   positive for PAS and α-NAE; weakly positive for ACP and AS-D; and negative for POX, SBB,

282   and ALP staining. Large lymphocytes were positive for PAS and weakly positive for ALP, AS-D,

283   and α-NAE, while negative for POX, SBB and ACP staining. Small lymphocytes were positive

284   for PAS, and weakly positive for AS-D and α-NAE, while negative for POX, SBB, ACP, and

285   ALP staining. Thrombocytes showed a positive reaction for PAS and a weakly positive reaction

286   for ACP and AS-D, but were negative for all the other cytochemical staining.

287

288   **Discussion**

289   **The number and morphology of erythrocytes in *O. argus***

290   Transporting oxygen and carbon dioxide through intracellular hemoglobin is the primary

291   function of the erythrocyte (Minasyan, 2014). Erythrocytes are the predominant blood cell type

292   in the vast majority of fish (Satheeshknmar et al., 2011; Satheeshknmar et al., 2012). The

293   erythrocyte counts were significantly different among various fish (Table 4). The number of

294   erythrocytes in *O. argus* was comparable to that of spotted rose snapper (*Lutjanus guttatus*) and

295   cichlid fish (*Cichlasoma dimerus*), lower than that of the Persian sturgeon (*Acipenser persicus*)

296   and shovelnose catfish (*Sorubim cuspicaudus*), and higher than that of the Siamese fighting fish

297   (*Betta splendens*), Asian sun catfish (*Horabagrus brachysoma*), and piebald naked carp. Hb and

298   HCT values in *O. argus* were comparable to those of the spotted rose snapper, and higher than

299   those of cichlid fish, the Persian sturgeon, Siamese fighting fish, and piebald naked carp. The

300   value of MCV in *O. argus* was comparable to that of the spotted rose snapper, cichlid fish, Asian

301   sun catfish, and piebald naked carp, and lower than that of the Persian sturgeon and Siamese

302   fighting fish. RBC and Hb values are related to the ability of the blood to carry dissolved oxygen

303   (Fazio et al., 2012; Tavares-dias & Moraes, 2004). RBC, Hb, HCT, and MCV values in fish are

304   related to various factors, including diet, fish body length, age, sex, water temperature, salinity,

305   and living environment (Jawad, Al-Mukhtar & Ahmed, 2004; Kori-Siakpere, Ake & Idoge, 2005;

306   Martins et al., 2011; Milad et al., 2016). Fish with higher values of RBC, Hb, HCT, and MCV

307   were mostly carnivorous, with a wide range of motion and high activity. These results were

308   consistent with previous reports that the carnivorous fish with high activity needed to consume

309   more oxygen and had correspondingly higher values of RBC, Hb, and HCT (Engel & Davis,

310   1964; Molnár & Tamássy, 1970; Rambhaskar & Rao, 1987). This study showed that there was

311   no significant difference in the values of RBC, Hb, and HCT between males and females, which

312   was consistent with most fish, such as the Persian sturgeon (Milad et al., 2016), Siamese fighting

313   fish (Motlagh et al., 2012), shovelnose catfish (Negrete et al., 2010), and cichlid fish (Vázquez &

314   Guerrero, 2007).

315   The morphological characteristics of mature erythrocytes of *O. argus* were similar to those

316   of other fish (Ahmed & Sheikh., 2020; Chen et al., 2019a; Negrete et al., 2010; Vázquez &

317   Guerrero, 2007), which were usually oval in shape with an oval or long-oval nucleus. The size of

318   erythrocytes in *O. argus* was smaller than in the Chinese sturgeon (*Acipenser sinensis*) and

319   sisorid catfish (*Glyptosternum maculatum*), larger than in the Siamese fighting fish and cichlid

320   fish, and similar to the piebald naked carp (Table 4). The erythrocyte size reflects the oxygen

321   transport capacity, and small erythrocytes are better able to transport oxygen (Fang et al., 2014).

322   We found a small number of immature erythrocytes in the peripheral blood of *O. argus*,

323   which was consistent with reports in other fish. However, the morphology of immature

324   erythrocytes in *O. argus* were round with a smaller and mostly-round nucleus, which was

325   somewhat different from that of other fish, such as *S. prenanti* (Fang et al., 2014), the spotted

326   rose snapper (Rio-Zaragoza et al., 2011), piebald naked carp (Tang et al., 2015), crucian carp,

327   and grass carp (Zhang et al., 2019), whose immature erythrocytes were mostly ovoid or oval in

328   shape, with a larger, elliptic nucleus.

The proportion and morphology of leukocytes in *O. argus*

329

330   Four types of leukocytes, neutrophils, monocytes, large lymphocytes, and small

331 lymphocytes, were found in the peripheral blood of *O. argus*. There were three types of

332 granulocytes in vertebrates: neutrophils, eosinophils, and basophils (Fang et al., 2014). Almost

333 all   bony fish contain neutrophils (or heterophils), but eosinophils and/or basophils exist only in

334 certain species (Zhou et al., 2006). Few fish other than tilapia (*Oreochromis niloticus*) (Ueda et

335 al., 2001) have both eosinophils and basophils. Most fish have only eosinophils without

336 basophils (Gao et al., 2007b; Milad et al., 2016; Motlagh et al., 2012; Rio-Zaragoza et al., 2011;

337 Vázquez & Guerrero, 2007; Zheng et al., 2016). Few fish have only basophils without

338 eosinophils (Shigdar, Harford & Ward, 2009; Zhang et al., 2019), and some fish have neither

339 eosinophils nor basophils (Chen et al., 2019a; Fang et al., 2014; Tavares-dias & Moraes, 2006;

340 Da Silva et al., 2011; Zhang et al., 2011). We did not find eosinophils and basophils in the

341 peripheral blood of *O. argus*.

342   The percentages of leukocytes were different among various fish species. Lymphocytes

343 were the most abundant leukocytes in most of the fish, such as the South American catfish

344 (Bianchi et al., 2014), turbot (*Psetta maxima*) (Burrows, Fletcher & Manning, 2001), Siamese

345 fighting fish (Motlagh et al., 2012), shovelnose catfish (Negrete et al., 2010), spotted rose

346 snapper (Rio-Zaragoza et al., 2011), piebald naked carp (Tang et al., 2015), cichlid fish (Vázquez

347 & Guerrero, 2007), and sisorid catfish (Zhang et al., 2011). Neutrophils were most common in

348 some fish, such as the Persian sturgeon (Milad et al., 2016) and *S. prenanti* (Fang et al., 2014).

349 Monocytes were found to be the most abundant leukocytes in *O. argus*, similar to the Chinese

350 sturgeon (Gao et al., 2007b).

351   Fish monocytes are mostly round and oval (though a few are irregular in shape), have

352 phagocytic functions, and are extremely sensitive to environmental variations (Zheng et al.,

353 2016). Mononcytes were the largest leukocytes in *O. argus*, and were significantly larger than

354 those of *S. prenanti* (Fang et al., 2014), the spotted rose snapper (Rio-Zaragoza et al., 2011),

355 piebald naked carp (Tang et al., 2015), and neotropical fish (Tavares-Dias & Moraes, 2006). The

356 most distinct morphological characteristic of monocytes in *O. argus* was that their cytoplasm

357 contained vacuoles of different sizes and pseudopodia protuberances on the cell edge, which was

358 consistent with the monocytes reported in other fish (Tavares-Dias, 2006; Tripathi, Latimer &

359 Burnley, 2004; Zheng et al., 2016) and may be related to their phagocytic function (Zheng et al.,

360 2016).

361   Lymphocytes belong to agranulocytes and play an important role in both innate and

362 acquired immunity (Shigdar, Harford & Ward, 2009). Compared with the lymphocytes in some

363 other fish (Tavares-dias, 2006; Zheng et al., 2016), the lymphocytes in *O. argus* were variable in

364 size and were classified as large lymphocytes and small lymphocytes. Most of the large

365 lymphocytes had small finger-like protuberances on the cell surface. Many microvilli

366 protuberances can also be found in the cytoplasmic edges of small lymphocytes. These features

367 have also been reported in other fish (Burrows, Fletcher & Manning, 2001; Rio-Zaragoza et al.,

368 2011; Vázquez & Guerrero, 2007; Da Silva et al., 2011), and the protuberances on the surface of

369   lymphocytes may be related to the immune function of antigen binding receptor molecules

370   (Scapigliati, 2013).

371   Neutrophils in *O. argus* were usually round or spherical with a bilobate nucleus, their

372   cytoplasm contained numerous fine mauve and reddish granules; these morphological

373   characteristics were somewhat different from the reports in some other fish. For instance, the

374   neutrophils of *S. prenanti* (Fang et al., 2014) were round or irregular-shaped, their nuclei were

375   usually kidney-shaped or trilobed, and their cytoplasm contained a large number of light blue or

376   pink granules. The neutrophils of the shovel-nose catfish (Negrete et al., 2010) had eccentric and

377   round nuclei with light blue granules in the cytoplasm; neutrophils of neotropical fish (Tavares-

378   Dias & Moraes., 2006) were round with an oval-shaped, eccentric nucleus; their cytoplasm

379   contained many purple granules of different sizes. The heterophils of the sisorid catfish (Zhang

380   et al., 2011) were round and regular in shape with kidney-shaped or round nuclei, and the

381   cytoplasm contained pale blue granules.

382   **The morphology and number of thrombocytes in *O. argus***

383   Different thrombocyte shapes, including round, oval, oblong, and spindle-shaped, were

384   observed in *O. argus*, which was consistent with the reports in other fish (Fang et al., 2014; Gao

385   et al., 2007b; Micha et al., 2019; Rio-zaragoza et al., 2011; Zhang et al., 2011; Zheng et al.,

386   2016). The spindle-shaped thrombocytes often existed alone with some vacuoles in the

387   cytoplasm and were related to cell phagocytosis (Nagasawa, Somamoto & Nakao, 2015; Stosik

388   et al., 2002). The round thrombocytes usually appeared in clusters with two to eight cells, which

389   may be related to their hemostatic function (Chen et al., 2019b; Peng et al., 2018).

390   The number of thrombocytes in *O. argus* was lower than that of the Siamese fighting fish

391   (Motlagh et al., 2012), shovel-nose catfish (Negrete et al., 2010), and spotted rose snapper (Rio-

392   Zaragoza et al., 2011); higher than that of the Chinese sturgeon (Gao et al., 2007b) and piebald

393   naked carp (Tang et al., 2015); and similar to grass carp, blunt snout bream (*Megalobrama*

394   *amblycephala*), yellow catfish (*Pelteobagrus fulvidraco*) (Chen et al., 2019), and cichlid fish

395   (Vázquez & Guerrero, 2007). The differences in the number of thrombocytes in different species

396   of fish may be related to biotic and abiotic factors and their adaptability to the environment

397   (Pavlidis et al., 2007; Prasad & Charles, 2010).

398   **The cytochemical staining patterns of blood cells in *O. argus***

399   We studied the cytochemical characteristics of peripheral blood cells of *O. argus* using

400   seven staining methods, including POX, SBB, PAS, ACP, ALP, AS-D, and α-NAE for the first

401   time. POX is enzyme-specific to neutrophils in mammals and participates in the defense

402   mechanism of bacterial infection (Tavares-Dias, 2006). SBB and PAS staining were used to

403   detect intracellular glycogen and lipids, which may provide energy for phagocytosis (Ueda et al.,

404   2001). ACP and ALP are lysosomal enzymes involved in phagocytosis and degradation (Shigdar,

405   Harford & Ward, 2009; Da Silva et al, 2011). AS-D is a specific esterase, which is associated

406   with cellular defense and phagocytic material processing (Casaletti-Rosa & Lunardi, 1997;

407 Tavares-Dias et al., 2007). As a non-specific esterase, α-NAE plays an important role in

408 phagocytosis and antigen presentation (Fang et al., 2014).

409 The erythrocytes observed in this study were only positive for PAS, which was different

410 from results in other fish, such as *S. prenanti* (Fang et al., 2014), tilapia (Ueda et al., 2001),

411 crucian carp, grass carp (Zhang et al., 2019), and piebald naked carp (Zheng et al., 2016), whose

412 erythrocytes were negative for PAS. The erythrocytes in *O. argus* were negative for POX, SBB,

413 ACP, ALP, AS-D, and α-NAE staining, which was consistent with the fish listed above. PAS

414 positivity and SBB negativity indicated that glycogen is the main energy source of erythrocytes

415 in *O. argus*.

416 The neutrophils of *O. argus* exhibited a strongly positive reaction for POX, SBB, and ACP,

417 and were positive for PAS and AS-D, and weakly positive for ALP and α-NAE staining. These

418 results were generally similar to the staining results of the Murray cod (*Maccullochella peelii*

419 *peelii*) (Shigdar, Harford & Ward, 2009), but different from reports in other fish. For instance,

420 neutrophils of the fat snook (*Centropomus parallelus*) (Da Silva et al., 2011) were positive for

421 PAS, SBB, ACP, and NAE, but negative for ALP staining. The American paddlefish (*Polyodon*

422 *spathula*) (Petrie-Hanson & Peterman, 2005) and piebald naked carp (*Gymnocypris eckloni*)

423 (Zheng et al., 2016) were positive for ACP, but negative for SBB staining. The Asian sun catfish

424 (*Horabagrus brachysoma*) (Prasad & Charles., 2010) was negative for ALP, NAE, and ASD

425 staining. Neutrophils in human and mammals are mainly involved in the phagocytosis and

426 degradation of invading microorganisms (Azevedo and Lunardi, 2003; Rieger and Barreda, 2011;

427   Wang et al., 2019). The strongly positive reactions for POX, SBB, and ACP and the positive

428   reactions for PAS, ACP, AS-D, and α-NAE indicated that the neutrophils of *O. argus* are similar

429   to those of mammals, which have strong phagocytic and bactericidal abilities.

430   Monocytes of *O. argus* were positive for PAS and α-NAE, and weakly positive for ACP

431   and AS-D, but negative for SBB, POX and ALP staining. These results were fairly consistent

432   with those of other fish, such as the *S. prenanti* (Fang et al., 2014), American paddlefish (Petrie-

433   hanson & Peterman, 2005), Murray cod (Shigdar, Harford & Ward, 2009), tilapia (Ueda et al.,

434   2001), fat snook (Da Silva et al., 2011), and piebald naked carp (Zheng et al., 2016), indicating

435   that fish monocytes have phagocytotic and antigen-presenting functions. Glycogen is the main

436   energy source of phagocytosis and the lack of POX and ALP suggested that fish monocytes had

437   weaker bactericidal abilities.

438   Lymphocytes of *O. argus* were positive for PAS, which was different from those of the

439   South American catfish (Bianchi et al., 2014), four freshwater teleosts (Tavares-Dias, 2006),

440   channel catfish (*Ictalurus punctactus*) (Tavares-Dias & Moraes, 2007), and fat snook (Da Silva

441   et al., 2011), whose lymphocyte were negative for PAS. However, the results were consistent

442   with *S. prenanti* (Fang et al., 2014), tilapia (Ueda et al., 2001) and piebald naked carp (Zheng et

443   al., 2016), suggesting that there were certain glycogen in the lymphocytes of *O. argus*.

444   Thrombocytes were positive for PAS, and weakly positive for ACP and AS-D, while

445   negative for all other cytochemical staining. These results were similar to those of the Murray

446   cod (Shigdar, Harford & Ward, 2009), tilapia (Ueda et al., 2001), and piebald naked carp (Zheng

447   et al., 2016). The thrombocytes of lower vertebrates are functionally similar to platelets in

448   mammals (Micha et al., 2019), playing an important role in the process of hemostasis and

449   coagulation (Chen et al., 2018; Peng et al., 2018). Studies have investigated whether

450   thrombocytes in some fish have phagocytotic abilities (Michał et al., 2019; Shigdar et al., 2007;

451   Shigdar, Harford & Ward, 2009; Da Silva et al, 2011; Zhang et al., 2019). In this study, the

452   thrombocytes of *O. argus* were positive for PAS, ACP, and AS-D staining, indicating that they

453   may have some phagocytic and material-processing functions.

454   We conducted a comprehensive study on the morphological metrology, microstructure, and

455   cytochemical characteristics of peripheral blood cells in *O. argus* for the first time. The results

456   showed that the number of erythrocytes and leukocytes in *O. argus* was consistent with that of

457   carnivorous fish. The morphology and microstructure of peripheral blood cells in *O. argus* was

458   similar to those of other fish, while the cytochemical staining patterns have clear species

459   specificity. For example, all of the blood cell types of *O. argus* were positive for PAS;

460   neutrophils were strongly positive or positive for all the seven kinds of cytochemical staining;

461   monocytes, large lymphocytes, and small lymphocytes were negative for POX and SBB; and

462   thrombocytes were weakly positive for AS-D. Our results may enrich the understanding of the

463   morphology and function of peripheral blood cells of fish, and provide basic data for health

464   assessments in   *O. argus* aquaculture.

465

466   **References**

467   **Ahmed I, Sheikh AZ. 2020.** Comparative study of hematological parameters of snow trout

468   *Schizopyge plagiostomus* and *Schizopyge niger* inhabiting two different habitats. *The*

469   *European Zoological Journal* **87(1)**:12–19.

470   **Azevedo A, Lunardi LO. 2003.** Cytochemical characterization of eosinophilic leukocytes

471   circulating in the blood of the turtle (*Chrysemys dorbignih*). *Acta Histochemica* **105(1)**:99–

472   105.

473   **Bianchi MB, Jerônimo GT, Pádua SB, Satake F, Ishikawa MM, Tavares-Dias M, Martins**

474   **ML. 2014.** The hematological profile of farmed *Sorubim lima*: reference intervals, cell

475   morphology and cytochemistry. *Veterinarski Arhiv* **84(6)**:677–690.

476   **Burrows AS, Fletcher TC, Manning MJ. 2001.** Haematology of the turbot, *Psetta maxima* (L.):

477   ultrastructural, cytochemical and morphological properties of peripheral blood leucocytes.

478   *Journal of Applied Ichthyology* **17(2)**:77–84.

479   **Casaletti-Rosa L, Lunardi LO. 1997.** Comparative study of the localization of nonspecific

480   esterase activity (Naphthyl butyrate) in leukocytes from reptiles, birds and fish. *Brazilian*

481   *Journal of Morphology Science* **14**,72.

482   **Chen HJ, Yuan GL, Su JG, Liu XL. 2019a.** Hematological analysis of *Ctenopharyngodon*

483   *idella*, *Megalobrama amblycephala* and *Pelteobagrus fulvidraco*: Morphology,

484   ultrastructure, cytochemistry and quantification of peripheral blood cells. *Fish & Shellfish*

485   *Immunology* **90**:376–384.

486 **Chen XX, Wang J, Wei QQ, Misbah H, Li E, Zhang SZ. 2019b.** Morphology and

487   cytochemical patterns of peripheral blood cells in domestic pigeon (*Columba livia*). *Tissue*

488   *and Cell* **59**:10–17.

489 **Chen XX, Wei QQ, Wang J, Peng F, Li E, Zhou YK, Zhang SZ. 2018.** Cytochemical patterns

490   of the peripheral blood cells in Chinese alligator (*Alligator sinensis*). *Tissue and Cell* **55**:71–

491   76.

492 **Courtenay JrWR, Williams JD. 2004.** Snakeheads (Pisces, Channidae)-A Biological Synopsis

493   and Risk Assessment. *U.S. Geological Survey Circular* **1251**:1–143.

494 **Da Silva WF, Egami MI, Santos AA, Antoniazzi MM, Silva M, Gutierre RC,   Paiva MJR.**

495   **2011.** Cytochemical, immunocytochemical and ultrastructural observations on leukocytes

496   and thrombocytes of fat snook (*Centropomus parallelus*). *Fish and Shellfish Immunology*

497   **31(4)**:571–577.

498 **Engel DM, Davis EM. 1964.** Relationship Between Activity and Blood Composition in Certain

499   Marine Teleosts. *Copeia* **3(4)**:586–587.

500 **Fang J, Chen K, Cui HM, Peng X, Li T, Zuo ZC. 2014.** Morphological and Cytochemical

501   Studies of Peripheral Blood Cells of *Schizothorax prenanti*. *Anatomia Histologia*

502   *Embryologia* **43(5)**:386–394.

503   **Fazio F, Faggio C, Marafioti S, Torre A, Piccione G. 2012.** Comparative study of

504   haematological profile on *Gobius niger* in two different habitat sites: Faro Lake and

505   Tyrrhenian Sea. *Cahiers De Biologie Marine* **53(2)**:213–219.

506   **Ferdous F, Scott TR. 2015.** A comparative examination of thrombocyte/platelet immunity.

507   *Immunology Letters* **163(1)**:32–39.

508   **Gao ZX, Wang WM, Khalid A, Zhou XY, Yang Y, James SD, Wang HP, Wang HL, Yang**

509   **L, Sun YH. 2007a.** Haematological characterization of loach *Misgurnus anguillicaudatus*:

510   Comparison among diploid, triploid and tetraploid specimens**.** *Comparative Biochemistry*

511   *and Physiology - Part A Molecular & Integrative Physiology* **147(4)**:1001-1008.

512   **Gao ZX, Wang WM, Yang Y, Khalid A, Li DP, Zou GW, James SD. 2007b.** Morphological

513   Studies of Peripheral Blood Cells of the Chinese sturgeon, *Acipenser sinensis*. *Fish*

514   *Physiology and Biochemistry* **33(3)**:213–222.

515   **Grant KR. 2015.** Fish Hematology and Associated Disorders, *Veterinary Clinics of North*

516   *America Exotic Animal Practice* **18(1)**:83–103.

517   **Ishikawa NM, Ranzani-Paiva MJT, Lombardi JV. 2008.** Total leukocyte counts methods in

518   fish, *Oreochromis niloticus*. *Archives of Veterinary Science* **13(1)**:54–63.

519   **Jawad LA, Al-Mukhtar MA, Ahmed HK. 2004.** The relationship between haematocrit and

520   some biological parameters of the Indian shad, *Tenualosa ilisha* (Family Clupeidae).

521   *Animal Biodiversity and Conservation* **27(2)**:47–52.

522 **Kori-Siakpere O, Ake JEG, Idoge E. 2005.** Haematological characteristics of the African

523 snakehead, *Parachanna obscura. African Journal of Biotechnology* **4(6)**:527–530.

524 **Martins ML, Xu DH, Shoemaker CA, Klesius PH. 2011.** Temperature effects on immune

525 response and hematological parameters of channel catfish *Ictalurus punctatus* vaccinated

526 with live theronts of *Ichthyophthirius multifiliis. Fish and shellfish immunology* **31(6)**:774–

527 780.

528 Massar B, Dey S, Barua R, Dutta K. 2012. Microscopy and Microanalysis of Hematological

529 Parameters in Common Carp, *Cyprinus carpio*, Inhabiting a Polluted Lake in North East

530 India. *Micros, Microscopy & Microanalysis* **18(5)**:1077–1087.

531 **Michał S, Beata T, Wiesław D. 2019.** Characterisation of Thrombocytes in Osteichthyes.

532 *Journal of veterinary research* **63**:123–131.

533 **Milad A, Kumar PS, Shafigh S, Hasan F, Jalil ZM. 2016.** Comparative study of

534 haematological, serum electrolyte and nonelectrolyte parameters of male and female Persian

535 sturgeon (*Acipenser persicus*) brood stocks. *Acta Oceanologica Sinica* **35(8)**:39–43.

536 **Minasyan HA. 2014.** Erythrocyte and Leukocyte: Two Partners in Bacteria Killing.

537 *International Reviews of Immunology* **33**:490–497.

538 **Molnár G, Tamássy E. 1970.** Study of the haemoglobin content of a single erythrocyte (M

539 index) in various cultured fish species. *Journal of Fish Biology* **2**:171–267.

540 **Motlagh SP, Zarejabad AM, Nasrabadi RG, Ahmadifar E, Molaee M. 2012.** Haematology,

541   morphology and blood cells characteristics of male and female Siamese fighting fish (*Betta*

542   *splendens*). *Comparative Clinical Pathology* **21(1)**:15–21.

543   **Nagasawa T, Somamoto T, Nakao M. 2015.** Carp thrombocyte phagocytosis requires

544   activation factors secreted from other leukocytes. *Developmental and Comparative*

545   *Immunology* **52**:107–111.

546   **Negrete JCC, Correa AAG, Guevara MJP, García VJA, Carrasco SCP. 2010.**

547   Characterization of blood cells and hematological parameters in trans-andean shovelnose

548   catfish *Sorubim cuspicaudus*. *Zootecnia Tropical* **27(4)**:393–405.

549   **Palmer L, Briggs C, Mcfadden S, Zini G, Burthem J, Rozenberg G, Proytcheva M, Machin**

550   **SJ. 2015.** ICSH recommendations for the standardization of nomenclature and grading of

551   peripheral blood cell morphological features. *International Journal of Laboratory*

552   *Hematology* **37(3)**:287–303.

553   **Pavlidis M, Futter WC, Katharios P, Divanach P. 2007.** Blood cell profile of six

554   Mediterranean mariculture fish species. *Journal of Applied Ichthyology* **23(1)**:70–73.

555   **Peng F, Chen XX, Meng T, Li E, Zhou YK, Zhang SZ. 2018.** Hematology and serum

556   biochemistry parameters of captive Chinese alligators (*Alligator sinensis*) during the active

557   and hibernating periods. *Tissue and Cell* **51**: 8–13.

558 **Petrie-Hanson L, Peterman AE. 2005.** American paddlefish leukocytes demonstrate

559   mammalian-like cytochemical staining characteristics in lymphoid tissues. *Journal of Fish*

560   *Biology* **66(4)**:1101–1115.

561 **Prasad G, Charles S. 2010.** Haematology and leucocyte enzyme cytochemistry of a threatened

562   yellow catfish *Horabagrus brachysoma* (Gunther 1864). *Fish Physiology and Biochemistry*

563   **36(3)**:435–443.

564 **Rambhaskar B, Rao SK. 1987.** Comparative haematology of ten species of marine fish from

565   Visakhapatnam Coast. *Journal of Fish Biology* **30(1)**:59–66.

566 **Rieger AM, Barreda DR. 2011.** Antimicrobial mechanisms of fish leukocytes. *Developmental*

567   *and Comparative Immunology* **35(12)**:1238–1245.

568 **Rio-Zaragoza OBD, Fajer-ávila EJ, Almazán-Rueda P, Abdo de la Parra MI. 2011.**

569   Hematological characteristics of the spotted rose snapper *Lutjanus guttatus* (Steindachner,

570   1869) healthy and naturally infected by dactylogyrid monogeneans. *Tissue and Cell*

571   **43**:137–142.

572 **Sagada G, Chen JM, Shen BQ, Huang AX, Sun LH, Jiang JH, Jin CH. 2017.** Optimizing

573   protein and lipid levels in practical diet for juvenile northern snakehead fish(*Channa argus*).

574   *Animal Nutrition* **3(2)**:156–163.

575 **Satheeshkumar P, Ananthan G, Senthilkumar D, Khan AB, Jeevanantham K. 2012.**

576   Comparative investigation on haematological and biochemical studies on wild marine

577   teleost fishes from Vellar estuary, southeast coast of India. *Comparative Clinical Pathology*

578   **21(3)**:275–281.

579   **Satheeshkumar P, Senthilkumar D, Ananthan G, Soundarapandian P, Khan AB. 2011.**

580   Measurement of hematological and biochemical studies on wild marine carnivorous fishes

581   from Vellar estuary, southeast coast of India. *Comparative Clinical Pathology* **20(2)**:127–

582   134.

583   **Scapigliati G. 2013.** Functional aspects of fish lymphocytes. *Developmental and Comparative*

584   *Immunology* **41(2)**:200–208.

585   **Shigdar S, Cook D, Jones P, Harford A, Ward AC. 2007.** Blood cells of Murray cod

586   *Maccullochella peelii peelii* (Mitchell). *Journal of Fish Biology* **70(3)**:973–980.

587   **Shigdar S, Harford A, Ward AC. 2009.** Cytochemical characterisation of the leucocytes and

588   thrombocytes from Murray cod (*Maccullochella peelii peelii*, Mitchell). *Fish and Shellfish*

589   *Immunology* **26(5)**:731–736.

590   **Stosik M, Deptula W, Trávniček M, Baldy-Chudzik K. 2002.** Phagocytic and bacterial

591   activity of blood thrombocytes in carps (*Cyprinus carpio*). *Veterinarni Medicina*, **47(1)**:21–

592   25.

593   **Tang Y, Peng X, Fang J, Cui HM, Zuo ZC, Deng JL. 2015.** Characterization of hematological

594   parameters and blood cells of cultured *Gymnocypris eckloni* Herzenstein, 1891. *Journal of*

595   *Applied Ichthyology* **31(5)**:931–936.

596   **Tavares-Dias M. 2006.** A morphological and cytochemical study of erythrocytes, thrombocytes

597   and leukocytes in four freshwater teleosts. *Journal of Fish Biology* **68(6)**:1822–1833.

598   **Tavares-Dias M, Moraes F. 2004.** Hematologia de peixes teleósteos. *Ribeirão Preto, São Paulo:*

599   *Villimpress* 144p.

600   **Tavares-Dias M, Moraes F. 2006.** Morphological, cytochemical, and ultrastructural study of

601   thrombocytes and leukocytes in neotropical fish, *Brycon orbignyanus* Valenciennes, 1850

602   (Characidae, Bryconinae). *Journal of Submicroscopic Cytology and Pathology* **38(2–**

603   **3)**:209–215.

604   **Tavares-Dias M, Moraes F. 2007.** Leukocyte and thrombocyte reference values for channel

605   catfish (*Ictalurus punctatus* Raf), with an assessment of morphologic, cytochemical, and

606   ultrastructural features. *Veterinary Clinical Pathology* **36(1)**:49–54.

607   **Tavares-Dias M, Ono EA, Pilarski F, Moraes F. 2007.** Can thrombocytes participate in the

608   removal of cellular debris in the blood circulation of teleost fish? A cytochemical study and

609   ultrastructural analysis. *Journal of Applied Ichthyology* **23(6)**:709–712.

610   **Tripathi NK, Latimer KS, Burnley VV. 2004.** Hematologic reference intervals for koi

611   (*Cyprinus carpio*), including blood cell morphology, cytochemistry, and ultrastructure.

612   *Veterinary Clinical Pathology* **33(2)**:74–83.

613   **Vázquez GR, Guerrero GA, 2007.** Characterization of blood cells and hematological

614   parameters in *Cichlasoma dimerus* (Teleostei, Perciformes). *Tissue and Cell* **39**:151–160.

615 **Ueda IK, Egami MI, Sasso WDS, Matushima ER. 2001.** Cytochemical aspects of the

616 peripheral blood cells of *Oreochromis (Tilapia) niloticus*. (Linnaeus, 1758) (Cichlidae,

617 Teleostei): part II. *Brazilian Journal of Veterinary Research and Animal Science* **38(6)**:

618 273–277.

619 **Wang Z, Lin LY, Chen WJ, Zheng X, Zhang YX, Liu Q, Yang DH. 2019.** Neutrophil plays

620 critical role during *Edwardsiella piscicida* immersion infection in zebrafish larvae. *Fish and*

621 *Shellfish Immunology* **87**:565–572.

622 **Xiao MS, Hu QS, Zhao Y, Bao FY, Cui F, Zheng RQ. 2017.** Development of 36 SNP markers

623 in *Ophiocephalus argus* Cantor based on high-throughput sequencing. *Conservation*

624 *Genetics Resources* **10(11)**:35–38.

625 **Xu J , Bian C, Chen KC, Liu GM, Jiang YL, Luo Q, You XX, Peng WZ, Li J, Huang Y, Yi**

626 **YH, Dong CJ, Deng H, Zhang SH, Zhang HY, Shi Q, Xu P. 2017.** Draft genome of the

627 Northern snakehead, *Channa argus*. *Gigaence*, **6(4)**:1–5.

628 **Xu WR. 2003.** *Clinical Hematology and blood testing experimental guidance.* Beijing: People's

629 Medical Publishing House (in Chinese).

630 **Zhang F M, Feng RR, Fang W, Shi YH, An LG, Yang GW. 2019.** Cytochemical

631 characterization of peripheral blood cell populations of two Cyprinidae, *Carassius auratus*

632 and *Ctenopharyngodon idellus*. *Anatomia Histologia Embryologia* **48**:22–32.

633   **Zhang HJ, Xie CX, Li DP, Liu HP, Yang XF. 2011.** Blood cells of a sisorid catfish

634   *Glyptosternum maculatum* (Siluriformes: Sisoridae), in Tibetan Plateau. *Fish Physiology*

635   *and Biochemistry* **37(1)**:169–176.

636   **Zheng ZX, Tang Y, Fang J, Peng X, Fan JD, Cui HM, Yang LZ. 2016.** Ultrastructural and

637   cytochemical properties of peripheral blood cells of piebald naked carp (*Gymnocypris*

638   *eckloni*). *Anatomia Histologia Embryologia* **46(1)**:17–24.

639   **Zhou Y, Pan FG, Li YS, Yan GM. 2006.** Morphological study on peripheral blood cells of

640   kalugaa, *Huso dauricus*. *Journal of Fishery Sciences of China* **13**:480–484 (in Chinese).

Haematological parameters of female and male argus snakehead.

Each data point represents the mean of three replicates ± SD and the range. *indicates significant differences in blood cell counts and haematological parameters between males and females (P < 0.05); different letters (a, b, c, d) in the same column indicate significant differences between leukoyte types (P < 0.05). Coefficient of Variation (CV) (%) = (Standard Deviation / Mean)×100%. TBC: total blood cell counts.

1 **Table 1: Haematological parameters of female and male argus snakehead.**

parameters	Female (N = 15)		Male (N = 15)	
	Mean ± SD	Range	Mean ± SD	Range
TBC ($\times 10^6$/mm³)	3.09 ± 0.21	2.90–3.30	3.15 ± 0.24	2.85–3.45
CV of TBC (%)	6.95		7.70	
RBC ($\times 10^6$/mm³)	2.96± 0.16	2.78−3.16	3.01 ± 0.24	2.72−3.30
CV of RBC (%)	5.41		7.97	
Hb (g/dl)	10.53± 0.37	10.17−11.03	10.60 ± 0.29	10.30−11.0
HCT (%)	42.41 ± 2.38	39.45−45.27	43.21 ± 1.77	41.52−45.66
ESR (mm/h)	1.52 ± 0.22	1.23−1.76	1.49 ± 0.10	1.42−1.63
MCV (fl)	143.51 ± 13.54	124.84−162.85	144.86 ± 14.15	125.83−167.87
MCH (pg)	35.72 ± 2.57	32.17−39.69	35.46 ± 3.03	31.21−40.44
MCHC (g/dl)	24.56 ± 2.59	21.95−29.44	24.73 ± 2.03	22.50−27.56
WBC ($\times 10^4$/mm³)	8.84 ± 0.47	8.29−9.44	10.55 ± 0.82	9.55−11.56
CV of WBC (%)	5.33		7.78	
Neutrophil($\times 10^4$/mm³)	1.82 ± 0.10[c]	1.71−1.94	2.03 ± 0.16[b]	1.83−2.22
Monnocyte ($\times 10^4$/mm³)	5.11 ± 0.27[d]	4.79−5.46	6.09 ± 0.47[c]	5.51−6.67
Large lymphocyte ($\times 10^4$/mm³)	0.60 ± 0.03[a]*	0.56−0.64	0.77 ± 0.06[a]*	0.70−0.84
Small lymphocyte ($\times 10^4$/mm³)	1.31 ± 0.07[b]*	1.23−1.40	1.67 ± 0.13[b]*	1.51−1.83
TC ($\times 10^4$/mm³)	4.36 ± 0.23*	4.09−4.65	3.40 ± 0.27*	3.08−3.73
CV of TC (%)	5.28		7.94	

2 Notes: Each data point represents the mean of three replicates ± SD and the range. *indicates

3 significant differences in blood cell counts and haematological parameters between males and

4 females (P < 0.05); different letters (a, b, c, d) in the same column indicate significant differences

5 between leukoyte types (P < 0.05). Coefficient of Variation (CV) (%) = (Standard Deviation /

6 Mean)×100%. TBC: total blood cell counts.

The size of the peripheral blood cells in argus snakehead (Mean ± SD, μm, N = 20).

** indicates that extremely significant differences between males and females (P < 0.01); different letters (a, b, c, d) in the same column indicate significant differences among different cells (P < 0.05).

1 **Table 2: The size of the peripheral blood cells in argus snakehead (Mean ± SD, μm, N = 20).**

Cell types	Females		Males	
	Cell length	Cell width	Cell length	Cell width
Erythrocytes	14.33 ± 1.16 **	10.43 ± 1.05	13.11 ± 0.88	10.47 ± 0.98
(nuclei)	(8.71 ± 0.88**)	(4.60 ± 0.70**)	(7.21 ± 0.90)	(3.55 ± 0.79)
Neutrophils	16.64 ± 2.16^c	14.83 ± 2.45^c	15.21 ± 2.21^c	13.55 ± 2.16^c
Monocytes	16.60 ± 1.77^c	14.61 ± 1.43^c	15.51 ± 1.34^c	14.29 ± 1.10^c
Large lymphocytes	11.38 ± 2.67^b	9.92 ± 2.50^b	11.94 ± 2.17^b	10.31 ± 2.09^b
Small lymphocytes	6.75 ± 1.36^a	6.00 ± 1.30^a	6.77 ± 1.13^a	6.17 ± 1.06^a
Thrombocytes	19.18 ± 3.19^d	6.99 ± 0.74^a	14.70 ± 2.50^c	6.87 ± 1.03^a

2 Notes: ** indicates that extremely significant differences between males and females (P < 0.01);

3 different letters (a, b, c, d) in the same column indicate significant differences among different

4 cells (P < 0.05).

Cytochemical staining patterns of peripheral blood cells in argus snakehead.

"+ + +" strongly positive; "+ +" positive;"+" weakly positive;"-" negative.

1 **Table 3: Cytochemical staining patterns of peripheral blood cells in argus snakehead.**

Cell types	POX	SBB	PAS	ACP	ALP	AS-D	α-NAE
Erythrocytes	−	−	+ +	−	−	−	−
Neutrophils	+ + +	+ + +	+ +	+ + +	+	+ +	+
Monocytes	−	−	+ +	+	−	+	+ +
Large lymphocytes	−	−	+ +	−	+	+	+
Small lymphocytes	−	−	+ +	−	−	+	+
Thrombocytes	−	−	+ +	+	−	+	−

2 Notes: "+ + +" strongly positive; "+ +" positive;"+" weakly positive;"-" negative.

The values of RBC, Erythrocyte sizes, Hb, HCT and MCV in argus snakehead and some other fish species.

The values of RBC, Erythrocyte sizes, Hb, HCT and MCV in argus snakehead were compared with those of some other fish.

1   **Table 4: The values of RBC, Erythrocyte sizes, Hb, HCT and MCV in argus snakehead and some other fish species.**

Species	RBC (10^6 /mm^3)	Erythrocyte sizes (µm)	Hb (g/dl)	HCT(%)	MCV (fl)	References
Ophiocephalus argus	2.72–3.30	14.33 ± 1.16 × 10.43 ± 1.05	10.30−11.0	41.52−45.66	125.83−167.87	This study
Lutjanus guttatus	0.75–3.71	11.04 ± 0.85 (10–13)	7.29−17.03	33.53−71.14	135.66−369.80	Rio-Zaragoza et al. (2011)
Cichlasoma dimerus	1.68–4.27	9.4–10 × 6.2–7.3	5.23−8.33	22.5−39.12	70.14–198	Vázquez and Guerrero (2007)
Acipenser persicus	4.8–7.9		8.60−9.87	29.58−31.72	412.20−621.70	Milad et al. (2016)
Sorubim cuspicaudus	3.5–14.0	10.5×8.8	10.5 ± 2.3	25.5 ± 5.6		Negrete et al. (2010)
Betta splendens	1.70–2.21	10.12–15.26 × 7.37–12.59	7.1−9.4	31-39	187.28 ± 7.05	Motlagh et al. (2012)
Horabagrus brachysoma	1.66–2.43		7.2−9.9	21.40−55.61	88.07−335.0	Prasad and Charles (2010)
Gymnocypris eckloni	1.49–1.78	14.88 ± 0.76 × 10.02 ± 0.42	5.21−7.93	22.42−36.92	150.98−207.42	Tang et al. (2015)
Acipenser sinensis	0.85 ± 0.10	17.98 ± 0.96 × 12.65 ± 0.87				Gao et al. (2007)
Ctenopharyngodon idella	1.76 ± 0.23	12.31 ± 0.78 × 8.27 ± 0.72			4.08 ± 0.12	
Megalobrama amblycephala	1.53 ± 0.12	13.61 ± 0.85 × 7.47 ± 0.55			3.06 ± 0.10	Chen et al. (2019a)
Pelteobagrus fulvidraco	1.41 ± 0.10	12.22 ± 0.92 × 9.98 ± 0.83			3.02 ± 1.14	
Glyptosternum maculatum		19.39 ± 2.48 × 15.15 ± 1.91				Zhang et al. (2011)

2   Notes: RBC: red blood cell counts; Hb: hemoglobin; HCT: hematoceit; MCV: mean corpuscular volume.

Figure 1

Microstructure of peripheral blood cells in argus snakehead (Wright's staining).

(A) mature erythrocyte (arrow) : oval with a long oval nucleus; immature erythrocyte (arrowhead) : round, with a round nucleus. (B) neutrophil: globular with a bilobate nucleus, pale blue cytoplasm contained many tiny mauve and reddish particles. (C) neutrophil: round, with an eccentric and oval shape nucleus, cytoplasm contained a large number of fine light purple particles and vacuoles of different sizes. (D) monocyte: oval with a horseshoe-shaped nucleus, cytoplasm contained many small vacuoles. (E) large lymphocyte: round or irregularly round, with many projections on the surface. (F) small lymphocyte: elliptic, with minimal cytoplasm, and some microvilli protuberances at the margin. (G) thrombocyte: spindle-shaped with an oval and mostly centered nucleus. (H) thrombocyte: round with flocculent cytoplasm, often appeared in clusters with multiple cells. Scale bars = 10 μm. The magnification is 1000X.
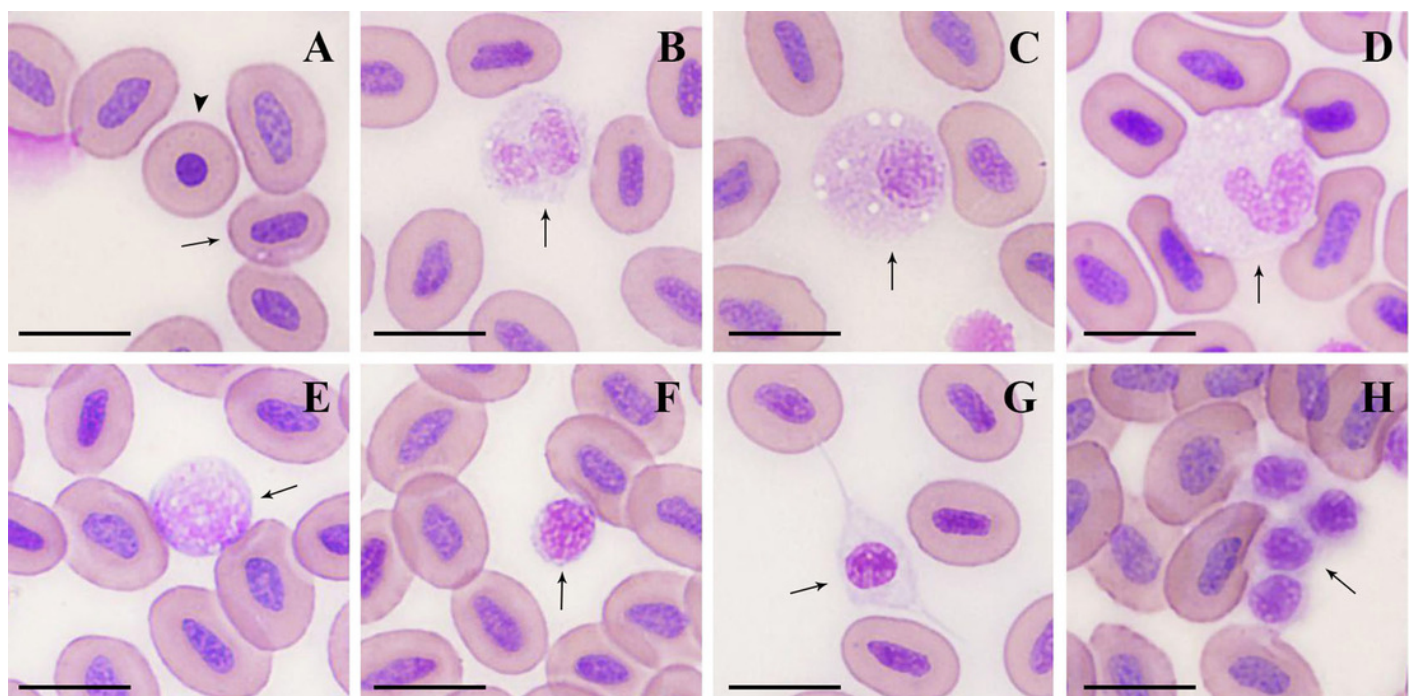
Figure 2

Cytochemical staining of peripheral blood cells in argus snakehead.

Erythrocytes were positive for PAS (CC), and negative for POX, SBB, ACP, ALP, AS-D and α-NAE staining (AA, BB, DD, EE, FF, GG); neutrophils exhibited strongly positive rection for POX, SBB and ACP (AB, BC, DE), positive for PAS and AS-D (CD, FG), and weakly positive for ALP and α-NAE staining (EF, GH); monocytes showed positive for PAS and α-NAE (CE, GI), and weakly positive for ACP and AS-D (DF, FH), while negative for POX, SBB and ALP staining (AC, BD, EG); large lymphocytes exhibited positive for PAS (CF), and weakly positive for ALP, AS-D and α-NAE (EH, FI, GJ), while negative for POX, SBB and ACP staining (AD, BE, DG); small lymphocytes were positive for PAS (CG), and weakly positive for AS-D and α-NAE (FJ, GK), while negative for POX, SBB, ACP and ALP staining (AE, BF, DH, EI); thrombocytes showed positive rection for PAS (CH), and weakly positive for ACP and AS-D (DI, FK), while negative for POX, SBB, ALP and α-NAE staining (AF, BG, EJ, GL). Scale bars=10 μm. The magnification is 1000×.

	Erythrocyte	Neutrophil	Monocyte	Large lymphocyte	Small lymphocyte	Thrombocyte
POX	AA	AB	AC	AD	AE	AF
SBB	BB	BC	BD	BE	BF	BG
PAS	CC	CD	CE	CF	CG	CH
ACP	DD	DE	DF	DG	DH	DI
ALP	EE	EF	EG	EH	EI	EJ
AS-D	FF	FG	FH	FI	FJ	FK
α-NAE	GG	GH	GI	GJ	GK	GL