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PATHOPHYSIOLOGICAL CHANGES IN RESPONSE TO PATHOGENS ADMINISTERED TO HEALTHY *ETROPLUS MACULATUS* (TELEOSTEI: CICHLIDAE) AND *PUNTIUS TICTO* (TELEOSTEI: CYPRINIDAE)

The pathogens in fish generate alterations in physiology of fish with consequences in life function decreasing and death. ***Objective.*** *To analyzed the response of ornamental fish Etroplus maculatus (Teleostei: Cichlidae) and Puntius ticto (Teleostei: Cyprinidae) infected by Pseudomonas aeruginosa (PSA1) and Enterobacter cloacae (EMS1).* ***Methods.*** *Strains of bacteria Pseudomonas aeruginosa (PSA1) and Enterobacter cloacae (EMS1) were obtained from moribund individuals of Etroplus maculatus (Teleostei: Cichlidae) and Puntius ticto (Teleostei: Cyprinidae), also hemaglobin, hematocrit, erythrocyte, and hemoglobin in an erythrocyte, oxygen consumption and ammonium excretion, and histological studies were measured.* ***Results.*** *Hematological parameters (hemaglobin, hematocrit, erythrocyte, hemoglobin in an erythrocyte) denoted an inverse relation with bacterial infection for both species, which was supported by high oxygen consumption, high ammonium excretion, and cell damage showed using histological analysis.* ***Conclusions.*** *These results are similar to observations for inland water and marine fish under culture conditions. The artificial infection of two fish species with two pathogen bacteria generates effects in pathophysiology and can be used for the development of treatment approaches.*

Keywords: *Etroplus maculatus, Puntius ticto, Pseudomonas aeruginosa, Enterobacter cloacae, hematological parameters.*

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The pathogenicity of a specific pathogen can be studied by artificially administering it to the host, so as to produce the same symptoms and changes in the physiological condition of fish living in natural environment that are known to harbor pathogenic bacteria (Chalamassetti et al., 2022). Invasion of fish muscles due to the breakage of immunological barriers of fish with pathogens is likely to occur when the fish is reared in aquaculture ponds contaminated with fecal coliforms, *Escherichia coli,* and *Salmonella,* greater than 10^3, 10^4, and 10^5 per 100 mL of pond water, respectively (Saharan, 2020) [1]. After entering the host, the pathogens spread to the various organs and tissue chiefly through blood. The rate with which the various organs and tissues of non-immune hosts are colonized by the various pathogens depends on a number of factors, including the infectious dose actually entering the fish and the rate at which the pathogen can multiply in the host tissue (Chalamassetti et al., 2022).

Though there are several studies on fish bacteria identification, experimental infection, or disease resistance, only a few of them relate the hematological parameters to bacterial experimental infection. The hematological parameters are an important tool of diagnosis that reveals the state of fish health [2]. The degree of changes occurring in the external environment and their impact on the infection and pathogenesis can often be measured by determining various blood parameters as suggested by Amenyogbe (2022) [3].

Erythrocytes, leucocytes, and thrombocytes are essential cellular components of fish blood, and their concentration is maintained within well-defined limits in different fish species unless the balance between production and elimination is disturbed by the pathological process (Pandey et al., 2022). Generally, erythrocytes not only pump out sodium and pump in potassium against the electrochemical gradient but also reduce methemoglobin to hemoglobin (Hb) to transport oxygen to the body tissues [3]. The importance of the packed cell volume (PCV), or hematocrit (Hct), as an index of anemia and the erythrocyte sedimentation rate (ESR) revealing almost all types of infections is well known in clinical medicine. White blood corpuscles (WBC) play a major role in the defense mechanism and mainly comprise granulocytes, monocytes, and lymphocytes [3]; the former two function as phagocytes to salvage debris from injured tissue, and the latter produces antibodies (Pandey et al., 2022). There is no valid information on the disease associated with the hematological changes in *Etroplus maculatus* and *Puntius ticto*, particularly during disease condition caused by bacterial pathogens such as *Pseudomonas aeruginosa* and *Enterobacter cloacae* respectively.

On the basis of importance of *Etroplus maculatus* and *Puntius ticto* in the ornamental fish aquaculture industry and the scarce data about physiological response to the bacterial infection of both fish species, it was proposed as an objective to study the physiological response (by hematological, physiological, and histological techniques) of ornamental fish *Etroplus maculatus* (Teleostei: Cichlidae) and *Puntius ticto* (Teleostei: Cyprinidae) artificially intraperitoneally infected by *Pseudomonas aeruginosa* (PSA1) and *Enterobacter cloacae* (EMS1).

Materials and methods. ***Bacterial isolates:*** Pathogenic isolates, namely *Pseudomonas aeruginosa* (PSA1) and *Enterobacter cloacae* (EMS1) from diseased and moribund *Etroplus maculatus,* were used for the investigation. From the pure cultures of *P. aeruginosa* and *E. cloacae*, three or four colonies were transferred separately with the help of a sterile inoculum needle to 25 mL of nutrient broth and incubated at 37 °C for 24 h until the broth became turbid. 10 mL of nutrient broth with the bacterial cells were centrifuged in a refrigerated centrifuge (Remi, India) at a speed of 10,000 rpm for 10 min. The supernatant was discarded, and 10 mL of 0.8% sterile saline was added to the sediment. The bacterial cells were dispersed in the saline by thorough mixing. The broth containing the bacterial population was

taken for streaking on nutrient agar plates (Hi Media) to isolate the causative bacteria using the standard streak plate method. The plates were incubated overnight and examined for size and shape of the colonies [4, 5]. Two colonies were dominant, and those were re-isolated in nutrient agar plates, incubated, and pure cultures were obtained by streaking on nutrient agar slants and stored in the refrigerator. Samples of both colonies of cultures were streaked in agar slant tubes once in a month, and these were inoculated through the same species of fish for retaining the virulence. Each sample was serially diluted, and the microbial load of 1 mL of the diluted sample was enumerated. The colonies were counted and expressed in CFU/mL. Different densities of *P. aeruginosa* (1.3×10^5, 1.3×10^6, 1.3×10^7, 1.3×10^8 CFU/mL) and *E. cloacae* (1.5×10^5, 1.5×10^6, 1.5×10^7, 1.5×10^8 CFU/mL) thus prepared were used for intraperitoneal administration among the apparently healthy experimental groups of fish.

The *Etroplus maculatus* (4.7 — 9.5 cm, 7.2 ± 1.0 cm of total length; 1.9 — 12.6 g; 7.0 ± 2.5 g of biomass) and *Puntius ticto* (9.1 — 10.8 cm, 9.8 ± 0.6 cm of total length; 14.0 — 24.0 g, 17.83 ± 3.39 g of biomass) of the same weight class were taken from the aquaria and divided into 8 independent groups in 60 L tanks, (pH = 8.0 + 1.0; temperature 24.0 + 1.0 °C). Each group was intraperitoneally administered with the following log concentrations of *P. aeruginosa:* 1.3×10^5, 1.3×10^6, 1.3×10^7, and 1.3×10^8 CFU/mL and *E. cloacae:* 1.5×10^5, 1.5×10^6, 1.5×10^7, and 1.5×10^8 CFU/mL and kept under observation after fixing the time of administration in separate aquaria. Controls were maintained by injecting 0.85% saline.

Fish blood sampling for hematological examination was carried out at 3, 9, 12, 24, and 48 h of the pathogen exposure. Blood was collected from the ventral aorta by severing the caudal peduncle. The first few drops of blood were discarded to prevent contamination and the rest was collected in a clean dry watch glass containing oxalate mixture as an anticoagulant. The anticoagulated blood was used for estimation of all peripheral blood variables (Dogra, 2020).

Total counts of erythrocytes (TEC) and Leukocytes (TLC): The total counts of RBC (TEC) and WBC (TLC) were simultaneously estimated by employing the conventional hemocytometer method. A haemocytometer with improved Neubaeur ruling (Weber and Sons, England) was used. Blood was diluted 200 times with Hendrick's diluting fluid (Hendricks, 1952) stained with methylene blue and counted at 10×45 magnification. Erythrocyte and Leucocyte counts were expressed as the number of cells per mm^3.

Hemoglobin (Hb) determination: Hemoglobin was estimated employing Sahli's acid hematin method as described by Ahmad (2022) [6].

Hematocrit /Packed Cell Volume (PCV) measurement (Ht%): Hematocrit, or packed cell volume, was measured by the microhematocrit method using non-heparinized capillaries. The tube was charged with blood and sealed at one end with sealing wax and centrifuged in a tabletop centrifuge at 3000 rpm for 30 min. Ht was expressed in percents.

Erythrocyte constants: Erythrocyte constants such as the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC) were calculated from RBC, Hb, and Ht values as follows:

MCV mean volume of erythrocyte:

$$\frac{\text{Ht}}{\text{RBC} \in \text{millions}/\text{mm}^3}\times 10\ \text{pg}$$

MCH mean weight of hemoglobin in an erythrocyte:

$$\frac{\text{Hb} \in \text{g}\%}{\text{RBC} \in \text{millions}/\text{mm}^3}\times 10\ \text{pg}$$

MCHC mean concentration of hemoglobin in the unit volume of blood:

$$\frac{\text{Hb} \in \text{g}\%}{\text{Ht}}\times 100\%$$

Oxygen consumption: The rate of oxygen consumption in *Etroplus maculatus* and *Puntius ticto* of the same weight class administered with different densities of *P. aeruginosa* (1.3×10^5, 1.3×10^6, 1.3×10^7, and 1.3×10^8 CFU/mL) and *E. cloacae* (1.5×10^5, 1.5×10^6, 1.5×10^7, and 1.5×10^8 CFU/mL) for unit time at 3, 6, 9, 24, and 48 h after administration were determined through modified Winkler's method (Winkler, 1987) with a sealed vessel respirometer (Brett,1964). Replicates were maintained throughout the experiment, and the results were recorded.

Ammonia excretion: The amount of ammonia excreted by the experimental animals of the same weight class administered with *P. aeruginosa* (1.3×10^5, 1.3×10^6, 1.3×10^7, and 1.3×10^8 CFU/mL) and *E. cloacae* (1.5×10^5, 1.5×10^6, 1.5×10^7, and 1.5×10^8 cells/mL) during the experiment gaps viz., 3, 6, 9, 24, and 48 h, were determined through the Indo-phenol method of Solorzano (1969), and the rate of ammonia excretion was calculated as follows:

$$\text{Ammonia \quotient} = \frac{\text{Rate \of \oxygen \consumed}}{\text{Rate \of \ammonia \excreted}}$$

The correlations were analyzed using a parametric Spearman correlation analysis [7] between the time in hours and the last mentioned parameters, because in previous verification of normal distribution and variance homogeneity both conditions were absent [7] with a 95% confiability, using the software R [8].

Histological studies: Both control and infected (administered with *E. cloacae*) fish were carefully netted out and immobilized using ice cubes. Tissues like intestine, liver, and gill were dissected out, cleaned in saline, and fixed in 10% neutral buffered formalin for 24 h. After fixation, the tissues were graded in an ascending alcohol series and cleared in xylene. Then they were decalcified in 30% nitric acid before alcohol grading and embedded in paraffin wax. After paraffin filtration, sections were cut to a 5 μm thickness using a microtome (Sipcon SP 1120-Model) and stained with hematoxylin and eosin. The sections were examined under low and high resolutions of the light compound microscope (Olympus Make) and photographed.

Results. *Hematological changes in experimental fish exposed to live cells of P. aeruginosa (PSA1) and E. cloacae (EMS1)*: The hematological indices for both control fish (injected with 0.85% saline) are presented in Table 1. The changes in the hematological parameters in response to administration of live *P. aeruginosa* (PSA1) and *E. cloacae* (EMS1) cells at 10^5, 10^6, 10^7, and 10^8 CFU/ mL to healthy *Etroplus maculatus* and *Puntius ticto* are given in Tables 2 and 3.

Hematological changes in Etroplus maculatus exposed to live cells of P. aeruginosa (PSA1): The total erythrocyte count (TEC) of *Etroplus maculatus* administered with both pathogens decreased with increasing concentration with respect to increasing exposure time. The fish administered with 10^5 cells of *P. aeruginosa* showed a gradual decrease in TEC. At 3 h after administration, the TEC was 2.92×10^6 cells/mm^3 while

Table 1. **Hematological parameters in healthy *Etroplus maculatus* and *Puntius ticto* — Control (injected with 0.85% saline)**

Fish	RBC (10^6 cells/mm^3)	Hb%	Ht%	MCV	MCH	MCHC	WBC (10^4 cells/mm^3)	RBC:WBC
E.maculatus	3.11	12.32	30.16	96.97	39.61	40.84	3.09	100
P. ticto	2.35	8.10	31.02	132	34.46	26.11	2.29	102.6

Table 2. **Hematological changes in *Etroplus maculatus* injected with different concentrations of *P. aeruginosa* (PSY1) and *Enterobacter cloacae* (EMS1). (RBC in 10^6 cells/ mm^3; WBC in 10^4 cells/ mm^3)**

Time, h	RBC	Hb%	Ht%	MCV	MCH	MCHC	WBC	RBC:WBC	RBC	Hb%	Ht%	MCV	MCH	MCHC	WBC	RBC:WBC
	10^5 cells/fish of *Pseudomonas aeruginosa* (PSY1)								10^5 cells/fish of *Enterobacter cloacae* (EMS1)							
3	2.92	12.21	29.92	102.40	41.81	40.80	3.75	77.86	2.84	12.16	29.86	105.14	42.81	40.72	4.11	69.09
9	2.89	12.19	29.89	103.42	42.17	40.78	3.86	74.87	2.80	12.11	29.80	106.42	43.25	40.63	4.19	66.82
12	2.86	11.12	29.83	104.30	38.88	37.27	4.12	69.41	2.75	11.98	29.75	108.18	43.56	40.26	4.26	64.55
24	2.82	11.10	29.80	105.67	39.36	37.24	4.25	66.35	2.68	11.90	29.69	110.78	44.40	40.08	4.28	62.61
48	2.76	11.00	29.75	107.78	39.85	36.97	4.38	63.01	2.59	11.85	29.78	114.98	45.75	39.79	4.39	58.99
	10^6 cells/fish of *Pseudomonas aeruginosa* (PSY1)								10^6 cells/fish of *Enterobacter cloacae* (EMS1)							
3	2.83	12.10	29.83	105.40	42.75	40.56	3.82	74.08	2.70	2.11	29.82	110.4	44.85	40.61	4.19	64.43
9	2.76	12.04	29.77	107.86	43.62	40.44	3.94	70.05	2.68	12.02	29.79	111.15	44.85	40.34	4.23	63.35
12	2.70	11.99	29.70	110.0	44.40	40.37	4.23	63.82	2.59	11.98	29.71	114.71	46.25	40.32	4.29	60.37
24	2.65	11.90	29.66	111.9	44.90	40.12	4.34	61.05	2.43	11.92	29.66	122.05	49.05	40.18	4.35	55.86
48	2.59	11.86	29.60	114.28	45.79	40.06	4.56	56.79	2.39	11.86	29.61	123.89	49.62	40.05	4.39	54.44
	10^7 cells/fish of *Pseudomonas aeruginosa* (PSY1)								10^7 cells/fish of *Enterobacter cloacae* (EMS1)							
3	2.71	12.01	29.79	109.92	44.31	40.31	3.96	68.43	2.65	12.03	29.79	112.41	45.39	40.38	4.21	62.94
9	2.65	11.96	29.70	112.70	45.13	40.26	4.15	63.85	2.59	11.96	29.74	114.82	46.17	40.21	4.26	60.79
12	2.53	11.83	29.65	117.19	46.75	39.89	4.39	57.63	2.46	11.89	29.67	120.60	48.33	40.07	4.30	57.20
24	2.43	11.78	29.60	121.81	48.47	39.79	4.41	55.10	2.41	11.86	29.65	123.02	49.21	40.00	4.39	54.89
48	2.32	11.70	29.57	127.45	50.43	38.89	4.60	50.43	2.65	12.03	29.79	112.41	45.39	40.38	4.21	62.94
	10^8 cells/fish of *Pseudomonas aeruginosa* (PSY1)								10^8 cells/fish of *Enterobacter cloacae* (EMS1)							
3	2.63	11.96	29.71	112.96	45.47	40.25	4.12	63.83	2.51	11.98	29.76	118.5	47.72	40.25	4.25	59.05
9	2.56	11.89	29.68	115.93	46.44	40.06	4.21	60.80	2.50	11.95	29.71	118.84	47.80	40.23	4.29	58.27
12	2.49	11.79	29.60	118.87	47.34	39.83	4.43	50.76	2.45	11.87	29.65	121.02	48.44	40.03	5.01	48.90
24	2.36	11.68	29.58	125.33	49.49	39.48	4.56	51.74	2.39	11.81	29.62	123.93	49.41	39.87	5.09	46.95
48	2.25	11.59	29.50	131.11	51.51	39.28	4.64	48.49	2.51	11.98	29.76	118.5	47.72	40.25	4.25	59.05

Table 3. **Hematological changes in *Puntius ticto* injected with different concentrations of *P. aeruginosa* (PSY1) and *Enterobacter cloacae* (EMS1). (RBC in 10^6 cells/ mm^3; WBC in 10^4 cells/ mm^3)**

Time, h	RBC	Hb%	Ht%	MCV	MCH	MCHC	WBC	RBC:WBC	RBC	Hb%	Ht%	MCV	MCH	MCHC	WBC	RBC:WBC
	10^5 cells/fish of *Pseudomonas aeruginosa* (PSY1)								10^5 cells/fish of *Enterobacter cloacae* (EMS1)							
3	2.31	7.98	31	134.19	4.54	25.74	2.65	87.16	2.19	7.86	30.92	141.1	35.89	25.42	2.81	77.93
9	2.28	7.90	30.95	135.74	4.64	25.52	2.69	84.75	2.15	7.84	30.89	143.6	36.46	25.38	2.85	75.43
12	2.25	7.86	30.93	137.46	4.93	25.41	2.74	82.11	2.11	7.79	30.86	146.25	36.91	25.24	2.89	71.76
24	2.21	7.80	30.87	139.6	5.29	25.26	2.76	80.07	2.08	7.74	30.80	148.07	37.21	25.12	2.94	70.74
48	2.19	7.76	30.82	140.73	5.43	25.17	2.80	78.21	2.04	7.70	30.75	150.73	37.74	25.04	2.99	68.22
	10^6 cells/fish of *Pseudomonas aeruginosa* (PSY1)								10^6 cells/fish of *Enterobacter cloacae* (EMS1)							
3	2.27	7.95	30.96	136.38	35.02	25.67	2.72	83.45	2.14	7.85	30.90	144.39	36.68	25.40	2.84	75.00
9	2.24	7.89	30.92	138.03	35.22	25.51	2.79	80.28	2.13	7.81	30.87	144.92	36.66	25.29	2.87	74.21
12	2.23	7.85	30.91	138.60	35.20	25.39	2.83	78.79	2.09	7.78	30.84	147.55	37.22	25.22	2.91	71.82
24	2.19	7.79	30.88	141.04	35.57	25.22	2.86	76.57	2.06	7.72	30.81	149.56	37.47	25.05	2.96	70.00
48	2.14	7.75	30.80	143.92	36.21	25.16	2.90	73.79	2.01	7.69	30.74	152.93	38.25	25.01	3.01	67.00
	10^7 cells/fish of *Pseudomonas aeruginosa* (PSY1)								10^7 cells/fish of *Enterobacter cloacae* (EMS1)							
3	2.24	7.92	30.95	138.16	35.35	25.58	2.75	81.45	2.11	7.84	30.88	150.00	37.15	25.38	2.87	73.51
9	2.20	7.88	30.91	140.50	35.81	25.49	2.81	78.29	2.08	7.80	30.84	148.20	37.50	25.29	2.91	71.47
12	2.18	7.83	30.88	141.65	35.91	25.35	2.85	76.49	2.04	7.79	30.80	150.98	38.18	25.29	2.95	69.15
24	2.13	7.77	30.85	144.83	36.47	25.18	2.90	73.44	1.99	7.71	30.79	154.72	38.74	25.04	2.99	66.55
48	2.09	7.74	30.79	147.32	37.03	20.90	2.93	71.33	1.94	7.68	30.73	158.40	39.58	24.99	3.04	63.81
	10^8 cells/fish of *Pseudomonas aeruginosa* (PSY1)								10^8 cells/fish of *Enterobacter cloacae* (EMS1).							
3	2.22	7.88	30.94	139.3	35.49	25.46	2.78	79.85	2.09	7.81	30.84	147.55	37.36	25.32	3.01	69.43
9	2.18	7.83	30.89	141.69	35.81	25.34	2.82	77.30	2.06	7.78	30.81	149.56	37.76	25.22	3.04	67.76
12	2.14	7.79	30.85	144.15	36.40	25.25	2.86	74.82	2.01	7.75	30.78	153.13	38.55	25.17	3.07	65.47
24	2.10	7.76	30.80	146.66	36.95	25.19	2.95	71.18	1.95	7.70	30.75	157.69	39.48	25.04	4.01	48.62
48	2.05	7.72	30.76	150.04	37.65	25.09	2.98	68.79	1.90	7.65	30.70	161.57	40.26	24.91	4.03	47.14

after 48 h it was 2.76×10^6 cells/mm^3. The TEC of fish administered with 10^6, 10^7, and 10^8 cells/ fish followed the same pattern. The Hb content in fish administered with 10^5, $10^{6,}$ 10^7, and 10^8 cells/fish of *P. aeruginosa* decreased with increasing exposure time. The Hb content of control fish was 12.32%. In the experimental fish administered with 10^5 cells, at 3 h, the Hb content reduced to 12.21% from 12.32% of control fish. Hb of fish administered with 10^8 cells decreased from 11.96% (3 h) to 11.59% (48 h).

Also, considerable changes were observed in the hematocrit value. The PCV or Ht offish reduced from 3h to 48 h of the exposure period for all different densities viz., 10^5, $10^{6,}$ 10^7, and 10^8 cells/fish. The Ht value was 29.6% after 48 h in the fish administered with 10^6 cells/fish. The percent of reduction was 29.5% after 48 h in the fish administered with 10^8 cells/fish. The MCV of control fish was 96.97 μ whereas the fish inoculated with 10^5 cells showed an increase to 107.78 μ after 48 h. Similar trend to increasing MCV was observed in increasing concentrations of pathogens viz., 10^6, 10^7 and 10^8 cells/fish. A slight fluctuation was noted in the MCH of fish administered with 10^5 cells. The MCH at 3 h was 41.81 pg and gradually increased to 42.17 pg after 9 h, then decreased to 38.88 pg after 12 h, and again slightly increased to 39.36 pg at 24 h and to 39.85 pg after 48 h. In the case of fish administered with 10^6,10^7 and 10^8 cells/ fish, the MCH increased with increasing concentration and exposure duration**.**

After 3 h of exposure, the MCHC of fish administered with 10^5 cells was 40.8%, then it gradually decreased to 36.97% at 48 h. Similarly, in the fish administered with 10^6 cells, the MCHC was 40.56% at 3 h and reduced to 40.06% at 48 h. Reduction in MCHC was noted in fish administered with 10^7 and 10^8 cells/fish. Data on TLC count in the infected fish showed that it increased with increasing exposure time and densities of *P. aeruginosa*. At 48 h, TLC count was 4.38×10^4 cells/m^3 observed in fish administered with 10^5 cells while it was 3.09×10^4 cells/m^3 in control fish injected with 0.85% saline. TLC count was 4.56×10^4 cells/m^3, 4.60×10^4 cells/m^3, and 4.64×10^4 cells/m^3 in the fish inoculated with 10^6,10^7, and 10^8 cells/fish, respectively, after 48 h of exposure. The WBC count showed an inverse to the RBC relationship for 10^5, 10^6, 10^7 and 10^8 cells/fish.

Hematological changes in Etroplus maculatus exposed to live cells of E. cloacae (EMS1). The TEC of fish administered with 10^5 cells of *E. cloacae* at 3 h after administration was 2.84×10^6 cells/mm^3 and at 12 h it reduced to 2.75×10^6 cells/mm^3, then to 2.59×10^6 cells/mm^3 at 48 h after administration. The same trend was noted for the rest of the pathogen densities with respect to the exposure time. The Hb content of fish administered with 10^5 cells gradually decreased from 12.32 g % (control) to 11.85% after 48 h of exposure. In the fish administered with 10^6, 10^7 and 10^8 cells, the same pattern emerged through the reduction from 12.11 g % to 11.86 g %, 12.03 g % to 11.80 g % and 11.98 g % to 11.79 g %, respectively.

The hematocrit (PCV or Ht) of fish administered with 10^5 cells gradually reduced from 29.71% (3 h) to 29.50% (48 h). The same trend was repeated for 10^6, $10^7$and 10^8 cells. After 3 h, the MCV of fish administered with 10^5 cells was 105.14 μ and gradually increased to 114.98 μ after 48 h. In the case of fish administered with 10^6, 10^7 and 10^8, the MCV after 3 h was 110.4 μ, 112.41 μ, and 118.5 μ and showed an increase to 123.89 μ, 126.53 μ, and 129.17 μ, respectively, at 48 h.

The MCH also showed significant changes during 48 h experiment. It exhibited an increasing trend for all the densities with increasing exposure time. The data on MCHC showed a declining pattern with respect to increasing concentration and increasing exposure time for all different densities of pathogen cells. The TLC count increased as exposure period progressed. In the fish inoculated with 10^5 cells, TLC was 4.11 after 3 h, and then gradually increased to 4.39×10^4 cells/m^3 at 48 h. In the case of fish ad-

ministered with 10^6 cells, the TLC count was 4.19×10^4 cells/m^3 at 3 h, and gradually increased to 4.39×10^4 cells/m^3 at 48 h after the administration of cells. A similar pattern of increase in TLC count was observed with 10^7 and 10^8 cells. The WBC count showed an inverse relationship to that of the RBC values for the concentrations of 10^5, 10^6, 10^7 and 10^8 cells/fish.

Hematological changes in Puntius ticto exposed to live cells of P. aeruginosa (PSA1): The TEC of fish administered with 10^5 cells of *P. aeruginosa* showed a gradual decrease. At 3 h after the administration, the TEC was 2.31×10^6 cells/mm^3, whereas at 48 h it was 2.19×10^6 cells/mm^3. The TEC for cell concentrations of 10^6, 10^7 and 10^8 cells/fish followed the same pattern of decrease as exposure time increased. The Hb content of fish administered with 10^5, 10^6, 10^7 and 10^8 cells decreased with increasing exposure time. The Hb content of control fish was 8.10%. In the experimental fish administered with 10^5 cells, the Hb content at 3 h reduced to 7.98% from 8.10% of control fish, and in the fish administered with 10^8 cells decreased from 7.88% (3 h) to 7.72% (48 h).

Considerable changes were also observed in the hematocrit value. The PCV, or Ht, of fish reduced from 3 h to 48 h of exposure period for all the densities studied viz., 10^5, 10^6 10^7, and 10^8 cells/fish. In the fish administered with 10^8 cells, a reduction down to 30.76% was noted after 48 h from 30.94% at 3 h. The MCV of fish administered with 10^5 cells was 134.19 μ after 3 h and then gradually increased to 140.73 μ after 48 h. In the case of fishadministered with 10^6, 10^7 and 10^8 cells/fish, the MCV after 3 h was 136.38, 138.16, and 139.3 μ, and then showed an increase to 143.92, 147.32, and 150.04 μ, respectively, after 48 h.

The MCH exhibited an increasing trend in all the pathogen densities with increasing exposure time. In the fish administered with 10^5 cells, MCH was 34.54 pg after 3 h and increased to 35.43 pg after 48h. Similarly, MCH for increasing densities of 10^6, 10^7, and 10^8 were 36.21, 37.03, and 37.65 pg after 48 h. After 3 h, the MCHC offish administered with 10^5 cells was 25.74%, which gradually decreased to 25.17% after 48 h. Similarly, in the fish administered with 10^6 cells, the MCHC was 25.67% at 3 h and reduced to 25.16% at 48 h. Data on TLC count in the infected fish showed that their TLC count increased with increasing time and increasing densities of *P. aeruginosa*. At 48 h, TLC count was 2.65×10^4 cells/m^3 observed in fish administered with 10^5 cells while it was 2.29×10^4 cells/m^3 in control fish injected with 0.85% saline. TLC counts were 2.90×10^4, 2.93×10^4, and 2.98×10^4 cells/m^3 in the fish inoculated with 10^6, 10^7 and 10^8 cells/fish, respectively, after 48 h of exposure. The WBC count showed an inverse relationship with that with the RBC values, for 10^5, 10^6, $10^7$and 10^8 cells/fish.

Hematological changes in Puntius ticto exposed to live cells of E. cloacae (EMS1). The TEC of fish administered with 10^5 cells of *E. cloacae* at 3 h after administration was 2.19×10^6 cells/mm^3 and after 24 h the value reduced to 2.08×10^6 cells/mm^3, then to 2.04×10^6 cells/mm^3 after 48 h. The same trend was noted in the rest of the pathogen densities with respect to the exposure time. The Hb content of fish administered with 10^5, 10^6, 10^7 and 10^8 cells decreased with increasing exposure time. The Hb content of control fish was 8.10%. In the experimental fish administered with 10^5 cells, the Hb content reduced to 7.86% at 3 h (from 8.10% for the control fish). In the case of fish administered with 10^7 cells, Hb content reduced to 7.68% at 48 h. In the fish with 10^8 cells, Hb decreased from 7.84% (at 3 h) to 7.68% (at 48 h).

The hematocrit (PCV or Ht) of fish administered with 10^5 cells gradually reduced from 30.92% (3 h) to 30.75% (at 48 h). In the case of fish administered with 10^6 cells, the Ht was reduced to 30.74% at 48 h. The MCV of control group's fish was 132 μ whereas the fish inoculated with 10^5 cells showed an increase to 150.73 μ after 48 h. Similar trend of increase in MCV was observed for increasing concentrations of pathogens viz., 10^6, 10^7 and 10^8 cells/fish with increasing exposure time.

The MCH exhibited an increasing trend for all the densities with increasing exposure time. In the fish administered with 10^5 cells of MCH was 35.89 pg after 3 h and increased to 37.74 pg after 48 h. Similarly, MCH for different increasing densities such as 10^6, 10^7, and 10^8 were 38.25, 39.58, and 40.26 pg, respectively, after 48 h. The data on MCHC showed a declining pattern with respect to increasing concentration and increasing exposure time for all the considered densities of cells.

The TLC count increased as exposure time progressed. In the fish inoculated with 10^5 cells, TLC was 2.81×10^4 cells/m^3 after 3 h and gradually increased to 2.99×10^4 cells/m^3 after 48 h. In the case of fish administered with 10^6 cells, the TLC count was 2.84×10^4 cells/m^3 after 3 h and gradually increased to 3.01×10^4 cells/m^3 after 48 h. The TLC of the fish administered with 10^7 cells was 3.04×10^4 cells/m^3 after 48 h and that of fish injected with 10^8 cells was 4.03×10^4 cells/m^3 after 48 h. The WBC count showed an inverse relationship as compared to the RBC values for 10^5, 10^6, 10^7, and 10^8 cells/fish.

Relationship between different densities of P. aeruginosa (PSA1) and hematological indices in Etroplus maculatus: The relationship between various densities of *P. aeruginosa* (PSA) and hematological indices in *Etroplus maculatus* are presented in Table 4. The relationship between specific time of administration and RBC in fish

Table 4. **Relationship between various densities of *P. aeruginosa* (PSA1), *Enterobacter cloacae* (EMS1) and hematological indices in *Etroplus maculatus* and *Puntius ticto***

Pathogen densities (CFU/ml)	r-values, h						
	Vs RBc	Vs Hb	Vs Ht	Vs MCV	Vs MCH	Vs MCHC	Vs WBC
Pseudomonas. aeruginosa (PSA1) in *Etroplus maculatus*							
1.3×10^5	–0.97699	–0.72812	–0.9273	0.9823	–0.4798	–0.7167	0.8924
1.3×10^6	–0.9196	–0.9201	–0.9142	0.9299	0.9262	–0.9163	0.91826
1.3×10^7	–0.9422	–0.9028	–0.8579	0.9601	0.9608	–0.9790	0.8758
1.3×10^8	–0.9629	–0.9373	–0.9357	0.9726	0.9769	–0.9338	0.8780
Enterobacter cloacae (EMS1) in *Etroplus maculatus*							
1.5×10^5	–0.9758	–0.8836	–0.3839	0.9887	0.9963	–0.9316	0.9369
1.5×10^6	–0.9113	–0.9191	–0.9157	0.9159	0.9059	–0.8952	0.9184
1.5×10^7	–0.8885	–0.8983	–0.8701	0.9024	0.8979	–0.9092	0.9179
1.5×10^8	–0.9866	–0.8700	–0.8918	0.9908	0.9940	–0.8509	0.7306
Pseudomonas aeruginosa (PSA1) in *Puntius ticto*							
1.3×10^5	–0.9118	–0.9000	–0.9576	0.9131	0.9129	–0.8831	0.9028
1.3×10^6	–0.9854	–0.9196	–0.9901	0.9862	0.9971	–0.8861	0.8852
1.3×10^7	–0.9543	–0.9111	–0.9655	0.9581	0.9736	–0.9322	0.8961
1.3×10^8	–0.9468	–0.9124	–0.9254	0.9544	0.9583	–0.9133	0.9230
Enterobacter cloacae (EMS1) in *Puntius ticto*							
1.5×10^5	–0.9253	–0.9379	–0.9654	0.9265	0.9218	–0.9184	0.9493
1.5×10^6	–0.9665	–0.9294	–0.9827	0.9685	0.9688	–0.9002	0.9568
1.5×10^7	–0.9568	–0.9407	–0.9386	0.9470	0.9607	–0.9215	0.9468
1.5×10^8	–0.9473	–0.9669	–0.9654	0.9531	0.9487	–0.9694	0.8729

administered with 1.3×10^5 of *P. aeruginosa* cells was found insignificant (p = 0.0743) and negatively correlated (r = –0.9769). The relationship between the incubation time and Hb of fish administered with 1.3×10^5 cells was insignificant (p > 0.05: p = 0.3640) and negatively correlated (r^2 = –0.7281). Similarly, the relationship between specific time of administration and Ht was insignificant (p = 0.2187) and negatively correlated (r^2 = –0.9273). A significant (p =5.28×10^{-6}) and positive correlation (r^2 = 0.9823) existed between incubation time and MCV. A significant (p = 0.0291) and positive correlation (r^2 = 0.4798) was noted between incubation time and MCH. In the case of MCHC, a significant (p = 0.0418) and negative correlation (r^2 = –0.7167) was observed. An insignificant (p = 0.0943) and positive correlation (r^2 = 0.8924) was noted between the incubation time and WBC of fish.

The relationship between the incubation time and RBC in fish administered with 1.3×10^6 of *P. aeruginosa* cells was insignificant (p = 0.0723) and negatively correlated (r^2 = –0.9196). The relationship between the incubation time and Hb was also insignificant (p = 0.3914) and negatively correlated (r^2 = –0.9201). Similarly, the relationship between the specific time of administration and Ht was insignificant (p = 0.2237) and negatively correlated (r^2 = –0.9142). A significant (p = 3.7×10^{-6}) and positive correlation (r^2 = 0.9299) was noted between the incubation time and MCV. Also, a significant (p = 0.0137) and positive correlation (r^2 = 0.9262) was observed between incubation time and MCH. In the case of MCHC, a significant (p = 0.0293) and negative correlation (r^2 = –0.9163) was observed unlike that for WBC (p = 0.0962, r^2 = 0.9198).

An insignificant (p = 0.0698) and negative correlation (r^2 = –0.9422) was noted between the exposure time and RBC of fish with 1.3×10^7 cells. Similarly, the relationship between the specific time of administration and Ht was insignificant (p = 0.2257) and negatively correlated (r^2 = –0.8579). A significant and positive correlation characterized the relationships of the incubation time —MCV (p = 2.95×10^{-6}, r^2 = 0.9601) and the incubation time — MCH (p = 0.0086, r^2 = 0.9608). In the case of MCHC, an insignificant (p = 0.1191) and negative (r^2 = –0.9790) correlation was observed. The relationship between the incubation time and WBC of fish was found insignificant (p = 0.0986) and positively correlated (r^2 = 0.8780).

The relationship between incubation time and RBC in fish administered with 1.3×10^8 *P. aeruginosa* cells was insignificant (p = 0.0689) and negatively correlated (r^2 = –0.9629). The relationship between specific time of administration and Ht was also insignificant (p = 0.2277) and negatively correlated (r^2 = –0.9357). An insignificant relationship (p = 2.45×10^{-6}) and positive correlation (r^2 = 0.9726) were noted between the incubation time and MCV, and significant (p = 0.0071) and positive (r^2 = 0.9769) correlation — between incubation time and MCH. The incubation time and WBC of fish were related insignificantly (p = 0.1003) and correlated positively (r^2 = 0.8780).

Relationship between various densities of E. cloacae (EMS1) and hematological indices in Etroplus maculatus: The relationship between various densities of *E. cloacae* (EMS1) and hematological indices in *Etroplus maculatus* are listed in Table 4. The relationship between the specific time of administration and RBC of fish exposed to 1.5×10^5 *E. cloacae* cells was found insignificant (p = 0.0727) and negatively correlated (r^2 = –0.9758) like that between the incubation time and Hb (p = 0.3927, r^2 = –0.8836) and between the specific time and Ht (p = 0.2212, r^2 = –0.3839). A significant (p = $4.1.10^{-6}$) and positive (r^2 = 0.9887) correlation was observed between incubation time and MCV. In the case of MCHC, a significant (p = 0.0294) and negative correlation (r^2 = –0.9316) was noted. The incubation time vs. WBC of fish was insignificant (p = 0.0975) and positively correlated (r^2 = 0.9369).

The relationship between the incubation time and RBC in fish administered with 1.5×10^6 cells was insignificant (p = 0.0703) and negatively correlated (r^2 = –0.9113) like that for the incubation time and Hb (p = 0.3914, r^2 = –0.9191). Similarly, the specific time of administration and Ht were related insignificantly (p = 0.2235) and negatively correlated (r^2 = –0.9157). A significant ($p = 2.95 \times 10^{-6}$) and positive (r^2 = 0.9159) correlation was observed between incubation time and MCV. Also, a significant (p = 0.0086) and positive (r^2 = 0.9059) correlation was noted between the incubation time and MCH. A significant (p = 0.0293) and negative correlation (r^2 = –0.8952) was revealed in the case of MCHC. The relationship between the incubation time and WBC of fish was insignificant (p = 0.0983) and positively correlated (r^2 = 0.9184).

The exposure time vs. RBC of fish administered with 1.5×10^7 cells was insignificant (p = = 0.2160) and negatively correlated (r^2 = –0.8885). A similar relationship was revealed for the specific time of administration and Ht (p = 0.2245, r^2 = –0.8701). On the contrary, the incubation time — MCV relationship was significant ($p = 2.20 \times 10^{-6}$) and positively correlated (r^2 = 0.9024). Significant (p = 0.0072) and positive (r^2 = 0.8979) correlation was found between the incubation time and MCH. In the case of MCHC, an insignificant (p = 0.0305) and negative correlation (r^2 = –0.9092) was observed. The incubation time vs. WBC of fish was insignificant (p = 0.0988) and positively correlated (r^2 = 0.9179).

The incubation time vs. RBC relationship in 1.5×10^8-cells-administered fish was insignificant (p = 0.0685) and negatively correlated (r^2 = –0.9866). Similar relationships were fixed between the incubation time and Hb (p = 0.3851 r^2 = –0.8700) as well as between specific time of administration and Ht (p = 0.2256 r^2 = = –0.8918). A significant ($p = 1.52 \times 10^{-6}$) and positive correlation (r^2 = 0.9908) was characteristic for incubation time vs. MCV unlike an insignificant (p = 0.1150) and negative correlation (r^2 = –0.8509) in the case of MCHC. The relationship between the incubation time and WBC of fish was insignificant (p = 0.1075) and positively correlated (r^2 = 0.7306).

Relationship between various densities of P. aeruginosa (PSA1) and hematological indices in Puntius ticto: The relationships between various densities of *P. aeruginosa* (PSA1) and hematological indices in *Puntius ticto* are shown in Table 4. The relationship between specific time of administration and RBC in fish administered with 1.3×10^5 cells of *P. aeruginosa* was found insignificant (p = = 0.0661) and negatively correlated (r^2 = –0.9118). Similarly, the specific time of administration and Ht was found insignificant (p =0.1798) and negatively correlated (r^2 = –0.9576). A significant ($p = 4.55 \times 10^{-6}$) and positive correlation (r^2 = 0.9131) was noted between incubation time and MCV. An insignificant (p = 0.0833) and positive correlation (r^2 = 0.9129) was between the incubation time and MCH. In the case of MCHC, an insignificant (p = 0.4577) and negative correlation (r^2 = –0.8831) was observed. The relationship between the incubation time and WBC of fish was insignificant (p = 0.0726) and positively correlated (r^2 = 0.9028).

The incubation time and RBC in fish administered with 1.3×10^6 *P. aeruginosa* cells was insignificant (p = 0.0656) and negatively correlated (r^2 = –0.9854). The relationship between the incubation time and Hb was found insignificant (p = = 0.1921) and negatively correlated (r^2 = –0.9196). Similarly, the specific time of administration and Ht was found to be related insignificantly (p = = 0.1805) and negatively correlated (r^2 = –0.9901). However, a significant ($p = 4.05 \times 10^{-7}$) and positive correlation (r^2 = 0.9862) was noted between the incubation time and MCV. An insignificant (p = 0.0760) and positive correlation (r^2 = 0.9971) existed between incubation time and MCH. The incubation time and WBC of fish were related insignificantly (p = 0.0739) and correlated positively (r^2= 0.8852).

The exposure time vs. RBC in fish administered with 1.3×10^7 cells of *P. aeruginosa* was insignificant (p = 0.0651) and negatively correlated (r^2 = –0.9543). The incubation time and Hb were found to be related insignificantly (p = 0.1915) and correlated negatively (r^2 = –0.9111). Similar was the relationship between specific time of administration and Ht (p =0.1811, r^2 = –0.9655). A significant (p = 3.55 10^{-7}) and positive (r^2 = = 0.9581) correlation was observed between incubation time and MCV. An insignificant (p = 0.0667) and positive (r^2 = 0.9736) correlation was between incubation time and MCH. The incubation time — WBC relationship was insignificant (p = 0.0743) and positively correlated (r^2 = 0.8961).

The incubation time vs. RBC in fish administered with 1.3×10^8 of *P. aeruginosa* cells was insignificant (p = 0.0647) and negatively correlated (r^2 = –0.9468). The same trend was characteristic for the relationships between the incubation time and Hb (p = 0.1904, r^2 = –0.9124), as well as between the specific time of administration and Ht (p = 0.1820, r^2 = –0.9254). An insignificant (p = 3.33 10^{-7}) and positive correlation (r^2 = 0.9544) was noted between incubation time and MCV, whereas insignificant (p = 0.4685) and negative correlation — in the case of MCHC. The incubation time vs. WBC of fish was insignificant (p = 0.0747) and positively correlated (r^2 = 0.9230).

Relationship between various densities of Enterobacter cloacae (EMS1) and hematological indices in Puntius ticto: The relationships between various densities of *Enterobacter cloacae* (EMS1) and hematological indices in *Puntius ticto* are shown in Table 4. The incubation time and RBC in fish administered with 1.5×10^5 cells were related insignificantly (p = 0.0644) and correlated negatively (r^2 = –0.9253). Such kind of relationships was found between the incubation time and Hb (p = 0.1900, r^2 = –0.9379) and between the specific time of administration and Ht (p = 0.1822, r^2 = –0.9654). There was significant (p = 2.90×10^{-7}) and positive correlation (r^2 = 0.9265) between incubation time and MCV. An insignificant (p = 0.0579) and positive (r^2 = 0.9218) correlation was noted between incubation time and MCH, whereas an insignificant (p = 0.0750) and negative (r^2 = –0.9184) correlation — in the case of MCHC. The incubation time—WBC relationship was significant (p = 0.0001) and positively correlated (r^2 = 0.9493).

The exposure time vs. RBC in fish administered with 1.5×10^6 cells was insignificant (p = 0.0640) and negatively correlated (r^2 = -0.9665). The same tendency was found for relationships between the incubation time and Hb (p = 0.1895, r^2 = –0.9294) as well as between the specific time of administration and Ht (p = 0.1826,r^2 = –0.9827). A significant (p = 2.53×10^{-7}) and positive (r^2 = 0.9685) correlation was characteristic for incubation time and MCV, and an insignificant (p = 0.4736) and negative (r^2 = –0.9002) correlation - in the case of MCHC. The incubation time vs. WBC of fish was insignificantly (p = 0.0753) and positively (r^2 = 0.9568) correlated.

The incubation time vs. RBC in fish administered with 1.5×10^7 cells was insignificant (p = = 0.0634) and negatively correlated (r^2 = –0.9568). The same tendency was found for relationships between the incubation time and Hb (p = 0.4487, r^2 = –0.9407) as well as between the specific time of administration and Ht (p = 0.1834, r^2 = –0.9386). A significant (p = 2.02×10^{-7}) and positive (r^2 = 0.9470) correlation was observed between incubation time and MCV whereas an insignificant (p = 0.4733) and negative (r^2 = –0.9215) correlation — in the case of MCHC. The incubation time vs. WBC of fish was insignificant (p = 0.0758) and positively correlated (r^2 = 0.9694).

The specific time of administration vs. RBC in fish administered with 1.5×10^8 of *E. cloacae* cells was insignificant (p = 0.0630) and negatively (r^2 = –0.9475) correlated. A negative (r^2 = –0.9669) and insignificant (p = 0.188 4) correlation was observed between the incuba-

tion time and Hb as well as between the specific time of administration and Ht ($p = 0.1845$, $r^2 = -0.9654$). On the contrary, a significant ($p = 2.25 \times 10^{-7}$) and positive ($r^2 = 0.9531$) correlation was noted between incubation time and MCV as well as between incubation time and MCH ($p = 0.0406$, $r^2 = 0.9487$). An insignificant ($p = 0.4780$) and negative ($r = -0.9694$) correlation was revealed between exposure time and MCHC. The incubation time—WBC relationship was insignificant ($p = 0.0834$) and positively correlated ($r^2 = 0.8729$).

Rate of oxygen consumption and ammonia excretion for control fish, Etroplus maculatus: The *Etroplus maculatus* fish consumed oxygen with a rate of 362 mg/kg/h and excreted ammonia with a rate of 0.091 mg/kg/h at 3 h after administration of 0.85% saline. Ammonia quotient was 0.00025. At 6 h, the fish consumed oxygen with a rate of 358 mg/kg/h and excreted ammonia with a rate of 0.091 mg/kg/h at 0.00025 A.Q. The fish consumed oxygen with a rate of 336 mg/kg/h and excreted ammonia with a rate of 0.090 mg/kg/h at 9 h after administration of 0.85% saline with 0.00026 A.Q. At 24 h, the fish consumed oxygen with a rate of 318 mg/kg/h and excreted ammonia with a rate of 0.089 mg/kg/h at 0.00027 A.Q. At 48 h of administration, the fish consumed oxygen with a rate of 298 mg/kg/h and excreted ammonia with a rate of 0.088 mg/kg/h at 0.00029 A.Q. (Table 5).

Rate of oxygen consumption and ammonia excretion in Etroplus maculatus administered with various densities of P. aeruginosa (PSA1): The fish administered with 1.3×10^5 *P. aeruginosa* cells consumed oxygen with a rate of 355 mg/kg/h at 3 h at A.Q. of 0.00028. A gradual reduction in the rate of oxygen consumption was observed at 6, 9, 24, and 48 h of incubation. The rate of ammonia excretion was 0.100 mg/kg/h at 3 h and gradually decreased to 0.070 mg/kg/h at 48 h (Table 5). *Etroplus maculatus* administered with 1.3×10^6 cells consumed oxygen with a rate of 336 mg/kg/h at 3h, which gradually decreased to 254 mg/kg/h after 48 h. The rate of ammonia excretion was 0.119 mg/kg/h at 3 h and gradually decreased to 0.059 mg/kg/h after 48 h. The ammonia quotient was 0.00035 at 3 h and at 48 h was reduced to 0.00023 (Table 5).

The fish administered with 1.3×10^7 of *P. aeruginosa* cells consumed oxygen with a rate of 315 mg/kg/h at 3 h. A gradual rate reduction was observed at 6, 9, 24, and 48 h of incubation. The rate of ammonia excretion was 0.125 mg/kg/h at 3 h and gradually decreased to 0.080 mg/kg/h at 48 h. At 3 h after administration, ammonia quotient was 0.00039 and then reduced to 0.00034 at 48 h (Table 5).

On administration of 1.3×10^8 cells, fish consumed oxygen with a rate of 285 mg/kg/h at 3 h, which gradually reduced at 6, 9, 24 and 48 h of incubation. The rate of ammonia excretion was 0.145 mg/kg/h at 3 h and gradually decreased to 0.092 mg/kg/h at 48 h. The ammonia quotient was 0.00050 at 3 h and after 48 h it fell down to 0.00043 (Table 5).

Rate of oxygen consumption and ammonia excretion in Etroplus maculatus administered with varying densities of E. cloacae (EMS1): The fish administered with 1.5×10^5 of *E. cloacae* cells consumed oxygen with a rate of 342 mg/kg/h at 3 h, which gradually reduced to 250 mg/kg/h at 48 h. The rate of ammonia excretion was 0.110 mg/kg/h at 3 h and gradually decreased to 0.051 mg/kg/h at 48 h. At 3 h after administration, the ammonia quotient was 0.00048 and reduced to 0.00020 at 48 h (Table 5).

On administration of fish with 1.5×10^6 cells, it consumed oxygen with a rate of 318 mg/kg/h at 3 h. A gradual reduction in the rate of oxygen consumption was observed at 6, 9, 24 and 48 h of incubation. The rate of ammonia excretion was 0.132 mg/kg/h at 3 h and gradually decreased to 0.071 mg/kg/h at 48 h. The ammonia quotient was 0.00041 at 3 h and at 48 h it fell down to 0.00039 (Table 5).

Etroplus maculatus administered with 1.5×10^7 pathogen cells consumed oxygen with a rate of

284 mg/kg/h at 3 h, which gradually decreased to 176 mg/kg/h at 48 h. The rate of ammonia excretion was 0.0151 mg/kg/h at 3 h and gradually decreased to 0.088 mg/kg/h at 48 h. At 3 h, the administration ammonia quotient was 0.00053 and decreased to 0.00050 at 48 h (Table 5).

The fish administered with 1.5×10^8 cells consumed oxygen at a rate of 271 mg/kg/h at 3 h, which gradually declined to 135 mg/kg/h at 48 h. The rate of ammonia excretion was 0.191 mg/kg/h at 3 h and gradually declined to 0.111 mg/kg/h at 48 h. A slight fluctuation was seen in ammonia quotient. At 3 h it was 0.00070, then it reduced to 0.00068 at 6 h and further increased to 0.00082 at 48 h (Table 5).

Rate of oxygen consumption and ammonia excretion in Puntius ticto administered with various densities of P. aeruginosa (PSA1): Puntius ticto administered with 1.3×10^5 cells of *P. aeruginosa* showed a drastic reduction in the

Table 5. **Relationship between densities of *Pseudomonas aeruginosa* (PSA1), *E. cloacae* (EMS1) and the rate of oxygen consumption and ammonia excretion in *Etroplus maculatus* and *Puntius ticto***

Pathogen densities (CFU/ml)	r-values		
	Hours Vs Rate of O_2 consumption	Hours Vs ammonia excretion	Rate of O_2 consumption Vs ammonia excretion
Pseudomonas aeruginosa (PSA1) in *Etroplus maculatus*			
Control	-0.9462	-0.9579	0.9971
1.3×10^5	-0.9215	-0.8221	0.9799
1.3×10^6	-0.8600	-0.9112	0.9719
1.3×10^7	-0.8970	-0.8919	0.9924
1.3×10^8	-0.9230	-0.8970	0.9911
Enterobacter cloacae (EMS1) in *Etroplus maculatus*			
Control	-0.9462	-0.9579	0.9971
1.3×10^5	-0.9296	-0.9286	0.9985
1.3×10^6	-0.9174	-0.9197	0.9944
1.3×10^7	-0.9311	-0.9233	0.9779
1.3×10^8	-0.9034	-0.9399	0.9844
Pseudomonas aeruginosa (PSA1) in *Etroplus maculatus* in *Puntius ticto*			
Control	-0.9089	-0.8961	0.7197
1.5×10^5	-0.9149	-0.8889	0.9925
1.5×10^6	-0.8800	-0.9291	0.9744
1.5×10^7	-0.9470	-0.9037	0.9920
1.5×10^8	-0.9136	-0.9094	0.9918
Enterobacter cloacae (EMS1) in *Puntius ticto*			
Control	-0.9089	-0.8961	0.9089
1.5×10^5	-0.8667	-0.9185	0.9878
1.5×10^6	-0.9162	-0.9069	0.9970
1.5×10^7	-0.9097	-0.9546	0.9769
1.5×10^8	-0.8990	-0.9346	0.9933

rate of oxygen consumption with increasing exposure time. The rate of oxygen consumption was 330 mg/kg/h at 3 h and reduced to 291 mg/kg/h at 48 h. Similarly, the rate of ammonia excretion was 0.085 mg/kg /h at 3 h and reduced to 0.025 after 48 h (Fig 8). Ammonia quotient was 0.00025 at 3 h and reduced to 0.000085 at 48 h (Table 5).

The fish administered with 1.3×10^6 cells consumed oxygen with a rate of 323 mg/kg/h at 3 h, which gradually declined to 283 mg/kg/h at 48 h. The rate of ammonia excretion was 0.098 mg/kg/h at 3 h and gradually declined to 0.041 mg/kg/h at 48 h, herein ammonia quotient was 0.0003and declined to 0.0001 at 48 h (Table 5).

On administration of 1.3×10^7 cells, the fish consumed oxygen at a rate of 312 mg/kg/h at 3 h. A gradual reduction in the rate of oxygen consumption was observed at 6, 9, 24, and 48 h of incubation. The rate of ammonia excretion was 0.120 mg/kg/h at 3 h and gradually decreased to 0.072 mg/kg/h after 48 h. The ammonia quotient was 0.00038 at 3 h and at 48 h it fell to 0.00027 (Table 5).

Similarly, the fish administered with 1.3×10^8 cells of *P. aeruginosa* showed a drastic reduction in the rate of oxygen consumption with increasing exposure. The rate of oxygen consumption was 301 mg/kg/h at 3 h and reduced to 252 mg/kg/h after 48 h. Similarly, the rate of ammonia excretion was 0.136 mg/kg/h at 3 h and reduced to 0.72 mg/kg/h after 48 h. Ammonia quotient was 0.00045 at 3 h and 0.00028 at 48 h (Table 5).

Rate of oxygen consumption and ammonia excretion in Puntius ticto administered with varying densities of E. cloacae (EMS1): The fish administered with 1.5×10^5 of *E.cloacae* cells consumed oxygen with a rate of 325 mg/kg/h at 3 h, which gradually reduced to 279 mg/kg/h at 48 h. The rate of ammonia excretion was 0.096 mg/kg/h at 3 h and gradually decreased to 0.048 mg/kg/h after 48 h. At 3 h after administration, ammonia quotient was 0.00029, then decreased to 0.00017 at 48 h. On administration of 1.5×10^6 cells, the fish consumed oxygen with a rate of 313 mg/kg/h at 3 h with further rate decreasing at 6, 9, 24 and 48 h of incubation. The rate of ammonia excretion was 0.112mg/kg/h at 3 h and gradually decreased to 0.051 mg/kg/h at 48 h. The ammonia quotient was 0.00035 at 3 h, and at 48 h it fell to 0.00019 (Table 5).

Similarly, the fish administered with 1.5×10^7 cells of *E. cloacae* showed a drastic reduction in the rate of oxygen consumption with increasing exposure time. The rate of oxygen consumption was 302 mg/kg/h at 3 h and reduced to 268 mg/kg/h at 48 h. Similarly, the rate of ammonia excretion was 0.136 mg/kg/h at 3 h and reduced to 0.071 mg/kg/h at 48 h. Ammonia quotient was 0.00045 at 3 h and reduced to 0.00026 at 48 h. The fish administered with 1.5×10^8 *E. cloacae* cells consumed oxygen with a rate of 294 mg/kg/h at 3 h, which gradually reduced to 269 mg/kg/h at 48 h. The rate of ammonia excretion was 0.158 mg/kg/h at 3 h and gradually decreased to 0.58 mg/kg/h at 48 h. At 3 h after administration, the ammonia quotient was 0.00053 and reduced to 0.00021 at 48 h (Table 5).

Relationship between various densities of Pseudomonas aeruginosa (PSA1) and the rate of oxygen consumption and ammonia excretion in Etroplus maculatus: The results of relationship between various densities of *Pseudomonas aeruginosa* (PSA1) and rates of oxygen consumption and ammonia excretion in *Etroplus maculatus* are presented in Table 6. The relationship between the incubation time and the rate of oxygen consumption for 1.3×10^5 cells of pathogen was found to be highly significant ($p = 1.27 \times 10^{-7}$) and negatively correlated ($r^2 = -0.9215$). The similar features were found for the rate of ammonia excretion ($p = 0.0001$ $r^2 = -0.8821$). A positive correlation was observed between the rates of oxygen consumption and ammonia excretion ($r^2 = 0.9799$). In the fish administered with 1.3×10^6 cells, the incubation time and the rate of oxygen consumption were related high-

ly significantly ($p = 3.15 \times 10^{-7}$) and negatively correlated ($r^2 = -0.8600$) as well as the incubation time and the rate of ammonia excretion ($p = 0.0007$, $r^2 = -0.9112$). A positive correlation was noted between rate of oxygen consumption and ammonia excretion ($r^2 = 0.9719$) (Table 5).

On administration of 1.3×10^7 cells to the fish, a highly significant ($p = 5.02 \times 10^{-7}$) and negative ($r^2 = -0.8970$) correlation was noted between the exposure time and the rate of oxygen consumption. Similarly, the relationship between the incubation time and the rate of ammonia excretion was highly significant ($p = 0.9.95 \times 10^{-7}$) and negatively correlated ($r^2 = -0.8919$). The rate of oxygen consumption and ammonia excretion were positively correlated ($r^2 = 0.9924$). The relationship between the incubation time and the rate of oxygen consumption for 1.3×10^8 cells of pathogen was highly significant ($p = 5.28 \times 10^{-7}$) and negatively correlated ($r^2 = -0.9230$). As for ammonia excretion, the relationship is also highly significant ($p = 0.0013$) and negatively correlated ($r^2 = -0.8970$). The rates of oxygen consumption and ammonia excretion were positively correlated ($r^2 = 0.9911$) (Table 5).

Relationship between the densities of E. cloacae (EMS1) and the rates of oxygen consumption and ammonia excretion in Etroplus maculatus: The results for the relationship between various densities of *E. cloacae* (EMS1) and the rate of oxygen consumption and ammonia excretion in *Etroplus maculatus* are presented in Table 5. On administration of 1.5×10^5 cells to fish, a highly significant ($p = 3.24 \times 10^{-7}$) and negative ($r^2 = -0.9296$) correlation was noted between the exposure time and the rate of oxygen consumption. Similarly, the incubation time and the rate of ammonia excretion was highly significant ($p = 0.0013$) and negatively correlated ($r^2 = -0.9286$). The rates of oxygen consumption and ammonia excretion were positively correlated ($r^2 = 0.9985$). In the fish administered with 1.5×10^6 cells, the incubation time vs. the rate of oxygen consumption was highly significant ($p = 1.51 \times 10^{-5}$) and negatively correlated ($r^2 = -0.9174$). Also, the relationship between the incubation time and the rate of ammonia excretion was highly significant ($p = 0.0002$) and negatively correlated ($r^2 = -0.9197$). A positive correlation was noted between rates of oxygen consumption and ammonia excretion ($r^2 = 0.9944$) (Table 5).

In the fishadministered with 1.5×10^7 cells, the relationship between the exposure time and oxygen consumption rate was highly significant ($p = 6.42 \times 10^{-6}$) and negatively correlated ($r^2 = -0.9311$). The incubation time vs. the rate of ammonia excretion was highly significant ($p = 3.93 \times 10^{-5}$) and negatively correlated ($r^2 = -0.9233$). Similarly, the exposure time vs. the oxygen consumption rate was highly significant ($p = 0.0001$) and negatively correlated ($r^2 = -0.9034$) in the fishadministered with 1.5×10^8 cells. As for the incubation time and the rate of ammonia excretion, this relationship was highly significant ($p = 3.16 \times 10^{-5}$) and negatively correlated ($r^2 = -0.9399$). The rates of oxygen consumption and ammonia excretion were positively correlated ($r^2 = 0.9844$) (Table 5).

Relationship between various densities of Pseudomonas aeruginosa (PSA1) and the rates of oxygen consumption and ammonia excretion in Puntius ticto: The results of these relationships are shown in Table 5. The incubation time and the rate of oxygen consumption for 1.3×10^5 cells of pathogen were highly significantly related ($p = 3.8 \times 10^{-9}$) and negatively correlated ($r^2 = -0.9149$) similar to the case of ammonia excretion ($p = 0.0345$, $r^2 = -0.8889$). A positive correlation was observed between the rates of oxygen consumption and ammonia excretion ($r^2 = 0.9925$). In the fish administered with 1.3×10^6 cells, the relation of the incubation time to the rate of oxygen consumption was highly significant ($p = 6.94 \times 10^{-7}$) and negatively correlated ($r^2 = -0.8800$). The incubation time and the rate of ammonia excretion were highly significantly related ($p = 0.0015$) and negatively correlated ($r^2 = -0.9291$), whereas a positive

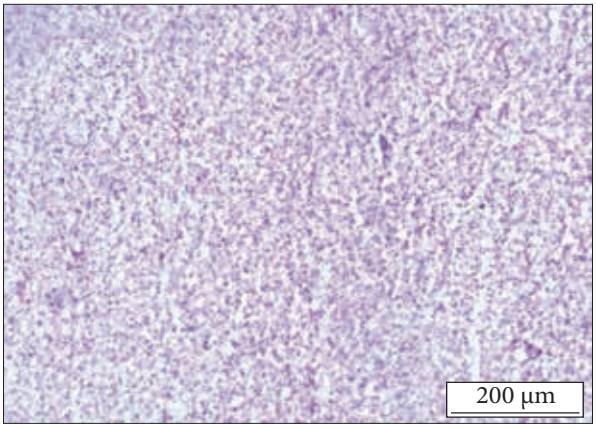

Fig. 1. Plate showing hepatocytes of *Etroplus maculatus* (control)

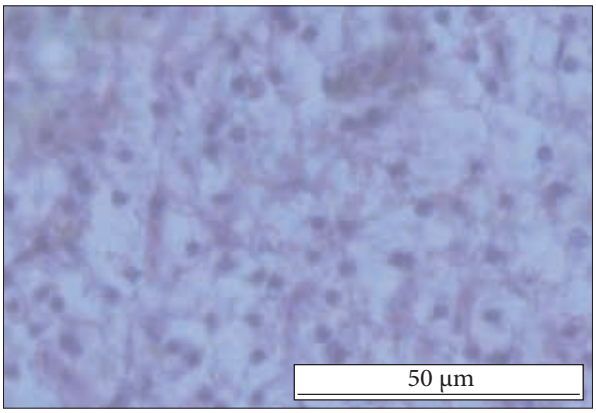

Fig. 3. Plate showing hepatocytes of *Puntius ticto* (control)

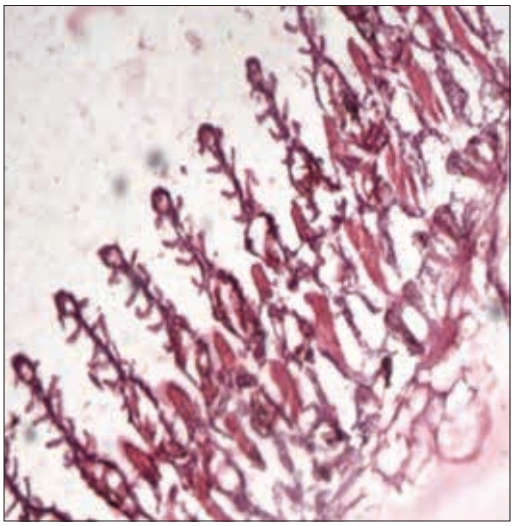

Fig. 5. Gill lamellae of *Etroplus maculatus* (control)

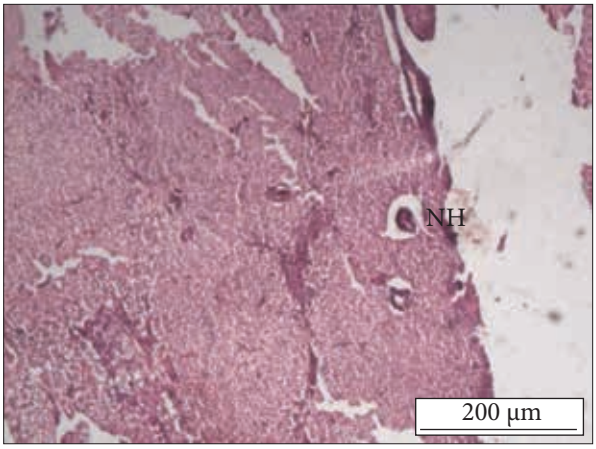

Fig. 2. Plate showing lipoid vacuole and necrosed hepatocytes of *Enterobacter cloacae* challenged *Etroplus maculatus*

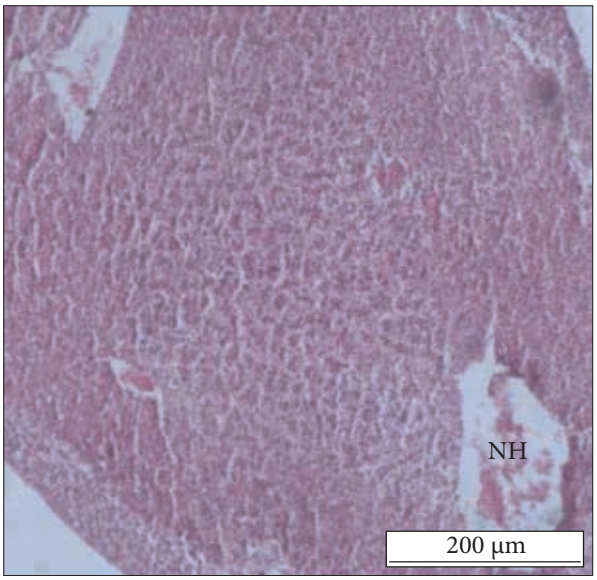

Fig. 4. Plate showing lipoid vacuole and necrosed hepatocytes of *Enterobacter cloacae* challenged *Puntius ticto*

correlation was observed between the rates of oxygen consumption and ammonia excretion ($r^2 = 0.9744$) (Table 5).

On administration of 1.3×10^7 cells into the fish, a highly significant ($p = 1.55.10^{-8}$) and negative correlation ($r^2 = -0.9470$) was noted between the exposure time and the rate of oxygen consumption. Similar relationship was revealed between the incubation time and the rate of am-

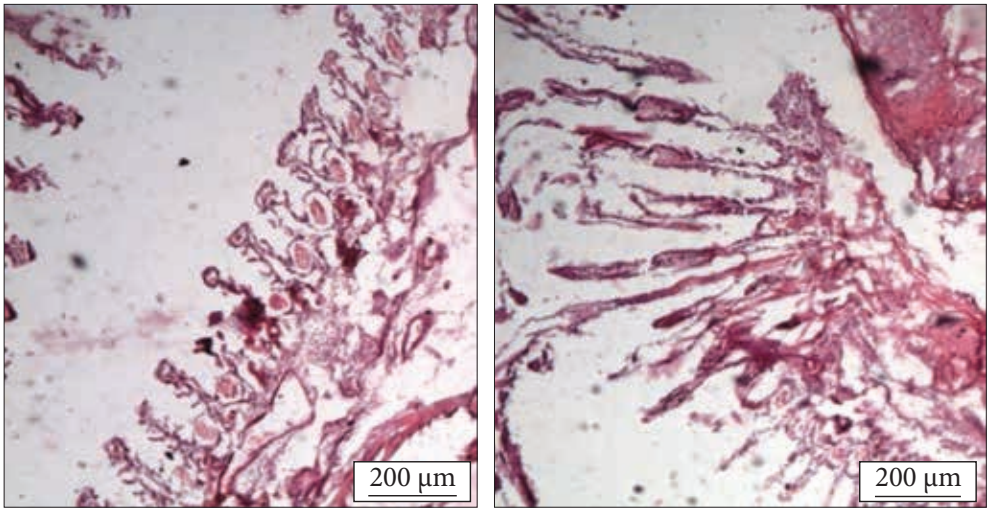

Fig. 6. Gill lamellae of *Etroplus maculatus* challenged with *Enterobacter cloacae*: *a* — in less infected condition; *b* — in highly infected condition

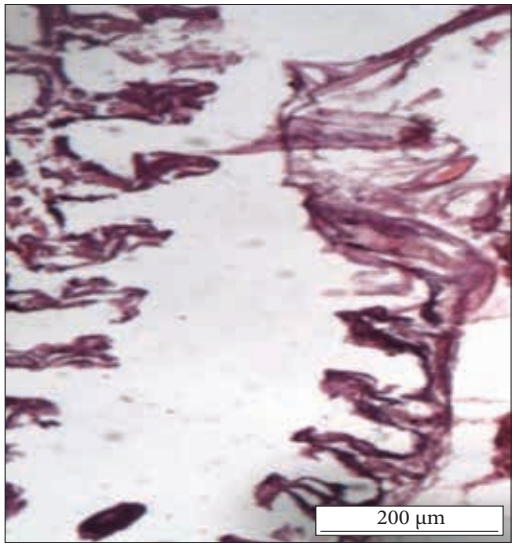

Fig. 7. Gill lamellae of *Puntius ticto* (control)

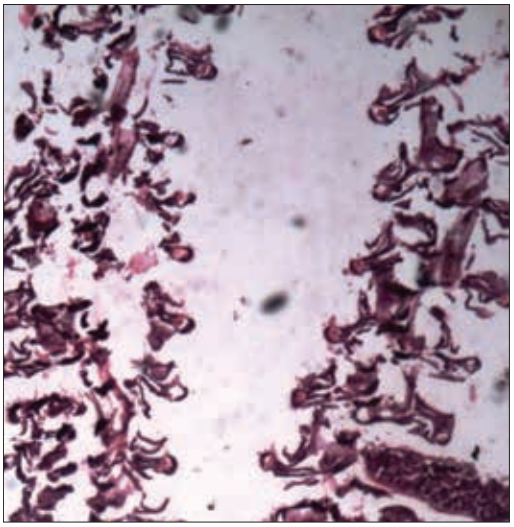

Fig. 8. Gill lamellae of *Enterobacter cloacae* challenged *Puntius ticto*

monia excretion ($p = 0.0015$, $r^2 = -0.9037$). A positive correlation existed between the rate of oxygen consumption and ammonia excretion ($r^2 = 0.9920$). The relationship between the incubation time and the rate of oxygen consumption for 1.3×10^8 cells of pathogen was highly significant ($p = 2.80 \times 10^{-8}$) and negatively correlated ($r^2 = -0.9136$). For the rate of ammonia excretion, a highly significant ($p = 0.0002$) and negative correlation ($r^2 = -0.9094$) was obtained. A positive correlation was observed between the rates of oxygen consumption and ammonia excretion ($r^2 = 0.9918$) (Table 5).

Relationship between various densities of E. cloacae (EMS1) and rates of oxygen consumption and ammonia excretion in Puntius ticto: The results for relationship between various densities of *E. cloacae* (EMS1) and rates of oxy-

gen consumption and ammonia excretion in *Puntius ticto* are listed in Table 7. As seen, on administration of 1.5×10^5 cells to the fish, a highly significant ($p = 9.46 \times 10^{-9}$) and negative ($r^2 = -0.8667$) correlation was observed between the specific incubation time and the rate of oxygen consumption. Similarly, the incubation time *vs.* the rate of ammonia excretion was highly significant ($p = 0.0018$) and negatively correlated ($r^2 = -0.9185$). The rate of oxygen consumption vs. the rate of ammonia excretion was positively correlated ($r^2 = 0.9878$). The incubation time and the rate of oxygen consumption for 1.3×10^6 cells of pathogen were related highly significantly ($p = 1.41 \times 10^{-8}$) and negatively correlated ($r^2 = -0.9162$) as well as in the case of ammonia excretion ($p = 0.0015$ and $r^2 = -0.9185$). A positive correlation was observed between the rates of oxygen consumption and ammonia excretion ($r^2 = 0.9878$) (Table 5).

In the fish administered with 1.3×10^7 cells, the relationship between the incubation time and the rate of oxygen consumption was significant ($p = 4.5 \times 10^{-9}$) and negatively correlated ($r^2 = -0.9097$). The incubation time and the rate of ammonia excretion were related highly significantly ($p = 0.0002$) and negatively correlated ($r^2 = -0.9546$). A positive correlation was observed between the rates of oxygen consumption and ammonia excretion ($r^2 = 0.9769$). The specific time of incubation and the rate of oxygen consumption for 1.3×10^8 cells of pathogen were related highly significantly ($p = 2.82 \times 10^{-9}$) and negatively correlated ($r^2 = -0.8990$). However, in the case of ammonia excretion, the relationship was highly significant ($p = 0.0015$) and negatively correlated ($r^2 = -0.9346$). A positive correlation was observed between the rates of oxygen consumption and ammonia excretion ($r^2 = 0.9933$) (Table 5).

Histological studies of Etroplus maculatus and Puntius ticto. Section through liver of E. maculatus: In the contol *E. maculatus* fish, the liver section showed a normal hepatocyte with homogenously located spherical nuclei (Fig. 1). The hepatocytes of the liver tissue administered with *E. cloacae* showed a shrunken morphology with necrosis and degenerated parenchyma cells (Fig. 1).

Section through liver of P. ticto: The cross section of the infected fish liver showed some dilations in the vascular structures with lipoid vacuoles (Fig. 2). In control fish, the hemopoietic cells were found localized under normal healthy conditions (Fig. 3).

Section through gill lamellae of E. maculatus: The gill morphology of the untreated fish (control) of *E. maculatus* showed an ordinary structure in which lamellae are lined by squamous epithelium composed of non differentiated cells (Fig. 4). The *E. cloacae* challenged *E. maculatus* gill showed clubbing of the ends of the secondary lamellae and fusion of adjacent secondary gill lamellae (Fig. 5).

Section through gill lamellae of P. ticto: Vascular congestion, inflammatory mono-nuclear cell infiltration, and degeneration in gill cartilage were seen in experimentally infected fish (Fig. 6). No such pathologic features were observed in cross-sections of healthy fish (Figs. 7, 8).

Discussion. The physiological condition of fish is one of the key factors underlying the attainment of the required performance levels. Monitoring of the physiological status has become an integral part of the routine examination of the health of fish. To date, little has been known about the hematology of *Etroplus maculatus* and *Puntius ticto*, most important species in ornamental fish sector worldwide [9]. The results presented in this chapter have revealed an interesting pattern of response to the hematological variables in stressed fish [10].

The results presented in this study have revealed an interesting pattern showing that the number of total erythrocyte count (TEC), the level of Hb, values of PCV (Ht), and MCHC significantly decrease in fish injected with different densities of *P. aeruginosa* and *E. cloacae*

with appropriate increase in the exposure time in both the experimental fish species, *Etroplus maculatus* and *Puntius ticto,* compared to the control group of fish, leading to anaemia. Anaemia can be attributed to increased destruction or loss of erythrocytes and/or suppression of erythropoiesis [10, 11]. It may be due to hemodilution caused by impaired osmoregulation. Also, it was reported that a reason for the anemic condition may be hemodilution which seems to be brought by loss of body fluids from advanced hemorrhagic necrotic lesions in severely affected fish (Seibel et al., 2021). A decline in the values of RBCs, Ht, and Hb, associated with symptoms of severe anemia, was recorded by Boaventura et al. (2021) [11], such as was observed for salmonids [12].

For *Aeromonas* infection in Atlantic salmon, Sun et al. (2022) [13] described a decrease in Hb. Irrespective of the infecting microbial agent, there is reduction in the vital functioning of blood vascular system. From the present findings, it was established that a higher number of cells in the infectivity studies comparatively reduced the TEC, Hb, and Ht values. This could be correlated to the virulence capability of the bacterial isolates PSA1 and EMS1 as well.

Hafsan et al. (2022) [14] recorded a reduction in RBC, Ht, and Hb in IHN virus-infected rainbow trout. A decline in RBC, Ht, and Hb, combined with signs of anemia, was also described in cases of proliferative kidney disease (PKD). Sun et al. (2022) [13] reported low levels of RBC, Hb, Ht, and MCHC in salmonids in response to *A. hydrophilia* and EUS infection. Hb content was related to the growth and volume of RBCs. Decreased RBC counts along with hematocrit and hemoglobin concentrations indicated that RBCs were destroyed by the leukocytosis activity in an erythrocytic anemia with subsequent erythroblastosis (Das et al., 2021).

Hemodilution resulted from the impaired osmoregulation across the gill epithelium also might have contributed to such reduction in Hb content and TECs [15]. The decreased Hb trend may be a result of the RBC swelling as well as poor Hb mobilization from the spleen to other hemopoietic organs (Scott et al., 1981). These data support the present findings that the significant decrease in RBC and Hb content is possibly due to hypochromic microcytic anemia caused by *P. aeruginosa* (PSA1) and *E. cloacae* (EMS1). Bjorgen et al. (2021) [16] reported that the erythrocytic count and hematocrit level of *Arcobacter cryaerophilus*-infected rainbow trout were significantly lower compared to the healthy fish, which corroborates with the present findings. Although in the present work it was difficult to determine whether the pathogen entrance or the stress situation existed initially, fish showed altered physiological parameters due to the pathogen incidence resulting in a chronic stress status. Nakayasu et al. (2002) [17] described microcytic hypochromic anemia in *Paralichthys olivaceus* due to the decrease in the mean corpuscular volume, hypoglobulia, and the structural abnormality of erythrocytes.

The experimental fish showed increased MCH, MCV and WBC compared to the control fish. The WBC count determines the total number of white cells (leukocytes) in a blood sample. Fewer in number than the red cells, WBC is the body's primary means to fightthe infection. The study of Harikrishnan et al. (2003) [18] in carp (*Cyprinus carpio*) infected with A. *hydrophila* showed an increase in the leukocyte number, supports the present observations. However, the opposite was found in pacu *Piaractus mesopotamicus* experimentally infected with *A. hydrophila* [19]. Bailone et al. (2010) [20] have reported that *Enterococcus*-injected tilapia showed not only a high number of thrombocytes but also an increase in white blood cells and lymphocytes compared to non-injected fish.

Alterations in histological sections have been described for other teleost fish suffering from motile aeromonad septicemia [21]. In this sce-

nario, the direct association between anemia effects and bacterial virulence was similar to descriptions for fish [22—24]. Our findings indicate that physiological, hematological, and histopathological responses in *Etroplus maculatus* and *Puntius ticto* after artificial intraperitoneal infection with *P. aeruginosa* (PSA1) and *E. cloacae* (EMS1) reproduce those observed in fish from inland water and marine fish under culture conditions. Therefore, artificial infection of ornamental fishwith *P. aeruginosa* (PSA1) and *E. cloacae* (EMS1) using different bacterial load could be used for the deep insight into pathophysiology of these infectious diseases and for the development of efficient treatment approaches.

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ПАТОЛОГІЧНІ ЗМІНИ У ВІДПОВІДЬ НА ЗБУДНИКИ, ВВЕДЕНІ ЗДОРОВИМ *ETROPLUS MACULATUS* (TELEOSTEI: CICHLIDAE) ТА *PUNTIUS TICTO* (TELEOSTEI: CYPRINIDAE)

Патогени риб викликають зміни у фізіології риб, що призводить до зниження їхньої життєдіяльності та смерті. **Мета.** Проаналізувати реакцію декоративних риб *Etroplus maculatus* (Teleostei: Cichlidae) і *Puntius ticto* (Teleostei: Cyprinidae), інфікованих штамами бактерій *Pseudomonas aeruginosa* (PSA1) і *Enterobacter cloacae* (EMS1). **Методи.** Штами бактерій *Pseudomonas aeruginosa* (PSA1) і *Enterobacter cloacae* (EMS1) були отримані від мертвих особин *Etroplus maculatus* (Teleostei: Cichlidae) і *Puntius ticto* (Teleostei: Cyprinidae). Визначали гемоглобін, гематокрит, еритроцити, гемоглобін в еритроцитах, споживання кисню та виділення амонію, а також проводили гістологічні дослідження. **Результати.** Досліджені гематологічні показники (гемоглобін, гематокрит, еритроцит, гемоглобін в еритроциті) вказують на зворотний зв'язок їх із бактеріальною інфекцією для обох видів риб, що підтверджується високим споживанням кисню, високим виділенням амонію та пошкодженнями клітин, виявленими за допомогою гістологічного аналізу. **Висновки.** Ці результати подібні до спостережень за внутрішніми водоймами та морськими рибами в умовах культивування. Проведене штучне зараження двох видів риб двома патогенними бактеріями важливе для патофізіології та може бути використано для розробки нових підходів до лікування.

Ключові слова: *Etroplus maculatus, Puntius ticto, Pseudomonas aeruginosa, Enterobacter cloacae, гематологічні показники.*