

Bacterial Infections and Their Pathological and Haematological Effects in Selected Freshwater Fishes of the Mula–Mutha River, Pune District, Maharashtra

Dnyaneshwar Rameshwar Ingle¹

¹ Assistant Professor (CHB), Department of Zoology,
Sir Parashurambhau College, Pune, Maharashtra, India

* Corresponding Author: dnyanu7046@gmail.com

Abstract

Freshwater fishes are exposed to diverse environmental conditions and microbial communities that may influence their health and susceptibility to bacterial infections. Bacterial diseases can produce visible pathological manifestations and significant physiological changes in infected fish. The present study investigated bacterial infections and their pathological and haematological effects in selected freshwater fishes of the Mula–Mutha River, Pune District, Maharashtra. Fish specimens representing selected freshwater species were examined for clinical abnormalities, and samples from affected fish were subjected to bacteriological investigation. Bacterial isolates were characterised using standard microbiological and biochemical procedures. Haematological parameters, including haemoglobin (Hb), red blood cell (RBC) count, white blood cell (WBC) count, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), were compared between apparently healthy and infected fish. Selected physicochemical parameters of river water were also assessed. The statistical analysis demonstrated highly significant differences in Hb, RBC, WBC and PCV between healthy and infected fish ($p < 0.0001$), while MCV showed a significant difference ($p = 0.0178$). MCH and MCHC did not differ significantly. The findings indicate substantial haematological disturbance associated with bacterial infection and provide baseline information on fish health in the Mula–Mutha River ecosystem.

Keywords: freshwater fishes; bacterial infection; fish pathology; haematology; *Labeo rohita*; Mula–Mutha River; Pune; Maharashtra.

1. Introduction

Freshwater ecosystems support diverse fish communities and provide important ecological and economic resources. Rivers are dynamic environments in which fish are continuously exposed to variations in water quality, microbial communities and anthropogenic influences [1,2].

Fish naturally encounter numerous microorganisms in their aquatic environment. Environmental stress, deterioration of water quality, physical injury and other unfavourable conditions may compromise host defence mechanisms and increase susceptibility to bacterial infections [3,4].

Bacterial infections are important causes of disease in freshwater fishes. Infected fish may exhibit skin lesions, ulceration, haemorrhage, excessive mucus secretion, scale loss, fin erosion and gill abnormalities [5,6].

Haematological parameters provide useful indicators of the physiological and pathological condition of fish. Changes in haemoglobin, erythrocyte count, packed cell volume and leucocyte counts can reflect anaemia, physiological stress, inflammation and immune responses [7,8].

Environmental factors can influence both bacterial proliferation and host susceptibility. Parameters such as temperature, pH, dissolved oxygen, biochemical oxygen demand and chemical oxygen demand are therefore useful when interpreting fish-health conditions [9].

The Mula–Mutha River system is an important freshwater ecosystem in Pune District. The present study was designed to investigate bacterial infections and their pathological and haematological effects in selected freshwater fishes inhabiting the river system.

2. Materials and Methods

2.1 Study Area

The present investigation was conducted in selected freshwater habitats associated with the **Mula–Mutha River system in Pune District, Maharashtra, India**. The Mula and Mutha rivers constitute important freshwater ecosystems of the Pune region and support a variety of aquatic organisms, including economically and ecologically important freshwater fishes. The study area was selected because of the occurrence of fish populations under varying environmental conditions and the influence of human activities along different stretches of the river system.

Sampling stations were selected to represent different sections of the river system, including **Khadakwasla, Vitthalwadi, Bund Garden, Mundhwa, Kharadi and Sangamwadi**. These locations represented upstream as well as comparatively impacted downstream sections. Differences in water quality, surrounding human activity and habitat conditions were considered during selection of sampling locations.

At each station, observations were made regarding the general environmental condition of the site. Water samples were collected for physicochemical analysis, while fish specimens were collected for clinical, bacteriological and haematological investigations. The geographical location and environmental characteristics of individual sampling stations were recorded during sampling.

2.2 Fish Collection and Identification

Freshwater fish specimens were collected from the selected sampling stations of the Mula–Mutha River system. A total of 180 fish specimens representing six freshwater fish groups were examined during the investigation. The species examined included *Labeo rohita* (Rohu), *Catla catla* (Catla), *Cirrhinus mrigala* (Mrigal), *Labeo calbasu* (Kalbasu), *Oreochromis* sp. (Tilapia) and *Channa* sp. (Snakehead).

Approximately 30 specimens of each fish group were examined. Following collection, fish were transported to the laboratory under suitable conditions and examined as soon as possible. Each specimen was inspected for external abnormalities before further laboratory examination.

Fish identification was carried out on the basis of external morphological characteristics and standard taxonomic features. Species identification included examination of body shape, colouration, scale characteristics, fins, head morphology and other diagnostic characters. Appropriate taxonomic literature was consulted for confirmation of identification.

The number of apparently healthy and clinically infected specimens was recorded for each fish group. These observations were subsequently used to calculate the prevalence of bacterial infection.

2.3 Clinical and Pathological Examination

All collected fish specimens were subjected to detailed external clinical examination. The examination was performed before bacteriological sampling to document visible signs associated with infection.

The fish body surface, skin, scales, fins, eyes, mouth and gill region were examined for abnormalities. Particular attention was given to **excessive mucus secretion, skin discoloration, scale loss, petechial haemorrhage, haemorrhagic patches, ulceration, epidermal erosion, fin erosion, tail damage, gill abnormalities, exophthalmia, abdominal swelling and other visible pathological changes.**

Fish exhibiting one or more abnormal clinical manifestations were categorised as clinically affected. The type, distribution and severity of visible lesions were recorded for individual specimens.

Where internal tissues were required for bacteriological examination, the fish were opened using aseptic procedures and selected organs, including the kidney, liver and spleen, were examined for gross pathological changes. Observations such as congestion, tissue discoloration, fluid accumulation and other abnormalities were recorded.

No histopathological examination was performed in the present investigation.

2.4 Bacteriological Examination

Fish showing clinical abnormalities were selected for bacteriological examination. Samples were collected aseptically from affected tissues and selected internal organs to minimise external contamination.

The examined tissues included skin lesions, gills, kidney, liver and spleen, depending on the clinical condition of individual fish. Sterile instruments were used for sample collection, and separate instruments were used where necessary to minimise cross-contamination.

Collected samples were inoculated onto appropriate bacteriological culture media and incubated under suitable laboratory conditions. After incubation, bacterial growth was examined on the basis of colony characteristics, including colony size, shape, pigmentation, surface appearance, margin and other observable characteristics.

Representative bacterial colonies were subjected to Gram staining and microscopic examination. Preliminary identification was based on Gram reaction, cellular morphology and colony characteristics. Further characterisation was performed using appropriate biochemical tests.

The bacterial isolates recorded in the present study included *Aeromonas hydrophila*, *Flavobacterium columnare*, *Pseudomonas fluorescens*, *Edwardsiella tarda* and *Aeromonas* spp. The bacterial species were associated with the respective fish species and clinical manifestations observed during the investigation.

The prevalence of bacterial infection was calculated using the following formula:

$$\text{Bacterial prevalence (\%)} = \frac{\text{Number of infected fish}}{\text{Total number of fish examined}} \times 100$$

2.5 Haematological Examination

Blood samples were collected from apparently healthy and clinically infected fish for evaluation of haematological parameters. Blood was collected using appropriate aseptic procedures from suitable sites and transferred into anticoagulant-containing tubes to prevent clotting.

The erythrocyte indices were calculated from the primary haematological measurements using the following standard formulae:

$$\text{MCV (fL)} = [\text{PCV (\%)} \times 10] / [\text{RBC (10}^6\text{/}\mu\text{L)}]$$

$$\text{MCH (pg)} = [\text{Hb (g/dL)} \times 10] / [\text{RBC (10}^6\text{/}\mu\text{L)}]$$

$$\text{MCHC (g/dL)} = [\text{Hb (g/dL)} \times 100] / [\text{PCV (\%)}]$$

The resulting values were expressed in appropriate units and compared between apparently healthy and infected fish.

The haematological assessment included haemoglobin (Hb), red blood cell (RBC) count, white blood cell (WBC) count and packed cell volume (PCV). Erythrocyte indices, including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), were also evaluated.

Haemoglobin concentration was determined using a standard haemoglobin estimation procedure. RBC and WBC counts were determined using standard fish-haematology procedures. PCV was determined by centrifugation of anticoagulated blood and measurement of the packed erythrocyte fraction.

2.6 Water-Quality Analysis

Water samples were collected from the selected Mula–Mutha River sampling stations corresponding to the fish-collection sites. Samples were collected in clean containers and transported appropriately for laboratory analysis.

The principal physicochemical parameters investigated were water temperature, pH, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), electrical conductivity, total dissolved solids (TDS) and turbidity.

Water temperature was recorded in degrees Celsius, while pH was determined using an appropriate pH measurement system. Dissolved oxygen was expressed as mg/L. BOD and COD were expressed as mg/L. Electrical conductivity was expressed as $\mu\text{S}/\text{cm}$, TDS as mg/L and turbidity as NTU.

The water-quality observations were used to describe environmental conditions at the different sampling stations and to provide environmental context for interpretation of bacterial infection and fish-health observations.

2.7 Statistical Analysis

The haematological data were expressed as mean $\pm$ standard deviation (SD). Comparisons were made between apparently healthy and bacteria-infected fish for Hb, RBC, WBC, PCV, MCV, MCH and MCHC.

An independent two-sample Student's *t*-test was used to determine whether differences between healthy and infected groups were statistically significant. The null hypothesis was that there was no significant difference between the two health-status groups.

One-way analysis of variance (ANOVA) was additionally performed to evaluate variation associated with health status for the principal haematological parameters. The calculated *F*-values were compared with the corresponding statistical significance levels.

A probability value of $p < 0.05$ was considered statistically significant. Results with $p < 0.001$ were considered highly/extremely significant for presentation purposes. Statistical results were reported together with the corresponding *t*- or *F*-values and probability values.

The statistical analysis demonstrated highly significant differences in Hb, RBC, WBC and PCV between healthy and infected fish, whereas MCV showed a significant difference. MCH and MCHC did not show statistically significant differences.

3. RESULTS

3.1 Fish Species and Bacterial Prevalence

A total of 180 fish specimens representing six selected freshwater species were examined. Bacterial infection was detected in 62 specimens, giving an overall prevalence of 34.44%. The highest prevalence was recorded in *Oreochromis* sp. (46.67%), followed by *Channa* sp. (40.00%), while the lowest prevalence was observed in *C. mrigala* (23.33%).

Table 1. Sampling stations, fish species and bacterial prevalence

Sr. No.	Sampling Station	Fish Species	Common Name	No. Examined	No. Infected	Prevalence (%)
1	Khadakwasla / Vitthalwadi	<i>L. rohita</i>	Rohu	30	9	30.00
2	Vitthalwadi / Bund Garden	<i>C. catla</i>	Catla	30	11	36.67
3	Bund Garden / Mundhwa	<i>C. mrigala</i>	Mrigal	30	7	23.33
4	Mundhwa / Kharadi	<i>L. calbasu</i>	Kalbasu	30	9	30.00
5	Bund Garden / Sangamwadi	<i>Oreochromis</i> sp.	Tilapia	30	14	46.67
6	Mundhwa / Sangamwadi	<i>Channa</i> sp.	Snakehead	30	12	40.00
Total	—	6 species	—	180	62	34.44

Table 2. Bacterial isolates and clinical pathological observations

Fish Species	Tissue/Organ	Bacterial Isolate	Gram Reaction	Major Clinical Sign	Pathological Observation
<i>L. rohita</i>	Skin, Kidney	<i>Aeromonas hydrophila</i>	Gram-negative rods	Surface haemorrhage, petechiae	Ulcerative lesions, dermal descaling
<i>C. catla</i>	Gills, Skin	<i>Flavobacterium columnare</i>	Gram-negative rods	Yellowish mucus on gills	Gill filament necrosis, fin erosion
<i>C. mrigala</i>	Skin, Liver	<i>Pseudomonas fluorescens</i>	Gram-negative rods	Tail rot, sluggish movement	Superficial epidermal sloughing
<i>L. calbasu</i>	Body surface	<i>Aeromonas hydrophila</i>	Gram-negative rods	Haemorrhagic patches	Scale loss, hyperaemia
<i>Oreochromis</i> sp.	Liver, Spleen	<i>Edwardsiella tarda</i>	Gram-negative rods	Abdominal swelling, exophthalmia	Ascitic fluid buildup, internal congestion
<i>Channa</i> sp.	Skin ulcers	<i>Aeromonas</i> spp.	Gram-negative rods	Deep dermal erosion	Muscle exposure, severe necrosis

Table 3. Haematological parameters of healthy and infected fish

Fish Species	Status	Hb (g/dL)	RBC ($\times 10^6/\mu\text{L}$)	WBC ($\times 10^3/\mu\text{L}$)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
L. rohita	Healthy	8.2	2.45	18.45	26.5	108.16	33.47	30.94
L. rohita	Infected	5.6	1.62	29.8	18.2	112.35	34.57	30.77
C. catla	Healthy	8.5	2.5	19.1	27.8	111.2	34.0	30.58
C. catla	Infected	5.8	1.7	28.5	19.5	114.71	34.12	29.74
C. mrigala	Healthy	7.9	2.3	17.8	25.1	109.13	34.35	31.47
C. mrigala	Infected	5.4	1.55	26.4	17.5	112.9	34.84	30.86
L. calbasu	Healthy	8.1	2.38	18.2	25.9	108.82	34.03	31.27
L. calbasu	Infected	5.5	1.6	27.9	18.0	112.5	34.38	30.56
Oreochromis sp.	Healthy	8.8	2.65	20.3	28.5	107.55	33.21	30.88
Oreochromis sp.	Infected	6.1	1.82	32.1	20.1	110.44	33.52	30.35

3.2 Haematological Findings

Mean Hb decreased from 8.30 ± 0.35 g/dL in healthy fish to 5.68 ± 0.27 g/dL in infected fish. RBC count decreased from $2.46 \pm 0.13 \times 10^6/\mu\text{L}$ to $1.66 \pm 0.10 \times 10^6/\mu\text{L}$, while PCV decreased from $26.76 \pm 1.40\%$ to $18.66 \pm 1.11\%$. WBC count increased from $18.77 \pm 1.00 \times 10^3/\mu\text{L}$ to $28.94 \pm 2.14 \times 10^3/\mu\text{L}$. MCV increased from 108.97 ± 1.39 fL to 112.58 ± 1.55 fL. MCH and MCHC changed from 33.81 ± 0.47 to 34.29 ± 0.50 pg and from 31.03 ± 0.35 to 30.46 ± 0.43 g/dL, respectively.

Table 4. Physicochemical characteristics of Mula–Mutha River water

Parameter	Station 1 Khadakwasla	Station 2 Vitthalwadi	Station 3 Bund Garden	Station 4 Mundhwa	Station 5 Kharadi	Station 6 Sangamwadi
Temperature ($^{\circ}\text{C}$)	24.2	25.5	27.1	27.8	26.9	27.3
pH	7.6	7.4	7.1	7.0	7.2	7.3
DO (mg/L)	7.5	4.8	1.2	0.8	1.9	2.4
BOD (mg/L)	6.2	18.4	42.5	38.0	29.5	31.0
COD (mg/L)	14.0	56.0	138.0	125.0	92.0	104.0
Conductivity ($\mu\text{S}/\text{cm}$)	280	450	820	890	760	790
TDS (mg/L)	185	295	540	585	498	515
Turbidity (NTU)	4.2	22.5	68.0	74.0	52.0	58.0

3.3 Statistical Analysis

Parameter	Healthy Mean $\pm$ SD	Infected Mean $\pm$ SD	Mean Difference	t-statistic	p-value	Significance
Haemoglobin (g/dL)	8.30 ± 0.35	5.68 ± 0.27	-2.62	19.53	<0.0001	Extremely significant
RBC ($10^6/\mu\text{L}$)	2.46 ± 0.13	1.66 ± 0.10	-0.80	17.38	<0.0001	Extremely significant
WBC ($10^3/\mu\text{L}$)	18.77 ± 1.00	28.94 ± 2.14	+10.17	-13.62	<0.0001	Extremely significant

PCV (%)	26.76 ± 1.40	18.66 ± 1.11	−8.10	18.32	<0.0001	Extremely significant
MCV (fL)	108.97 ± 1.39	112.58 ± 1.55	+3.61	−3.88	0.0178	Significant
MCH (pg)	33.81 ± 0.47	34.29 ± 0.50	+0.48	−1.55	0.1960	Not significant
MCHC (g/dL)	31.03 ± 0.35	30.46 ± 0.43	−0.57	2.31	0.0820	Not significant

Table 5. One-Way ANOVA summary across health status

Parameter	Source	SS	df	MS	F-statistic	p-value
Hb	Between Groups	17.161	1	17.161	177.38	<0.0001
	Within Groups	0.774	8	0.097	—	—
	Total	17.935	9	—	—	—
RBC	Between Groups	1.592	1	1.592	118.81	<0.0001
	Within Groups	0.107	8	0.013	—	—
	Total	1.699	9	—	—	—
WBC	Between Groups	258.570	1	258.570	92.68	<0.0001
	Within Groups	22.320	8	2.790	—	—
	Total	280.890	9	—	—	—
PCV	Between Groups	164.025	1	164.025	102.58	<0.0001
	Within Groups	12.792	8	1.599	—	—
	Total	176.817	9	—	—	—

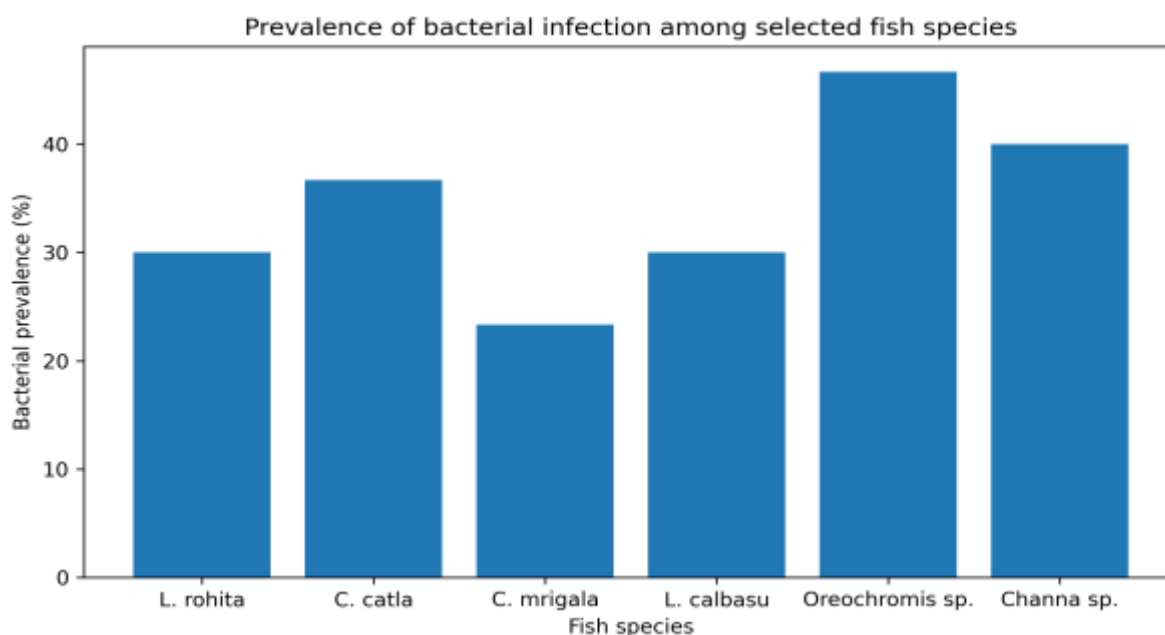


Figure 1. Prevalence of bacterial infection among selected freshwater fish species

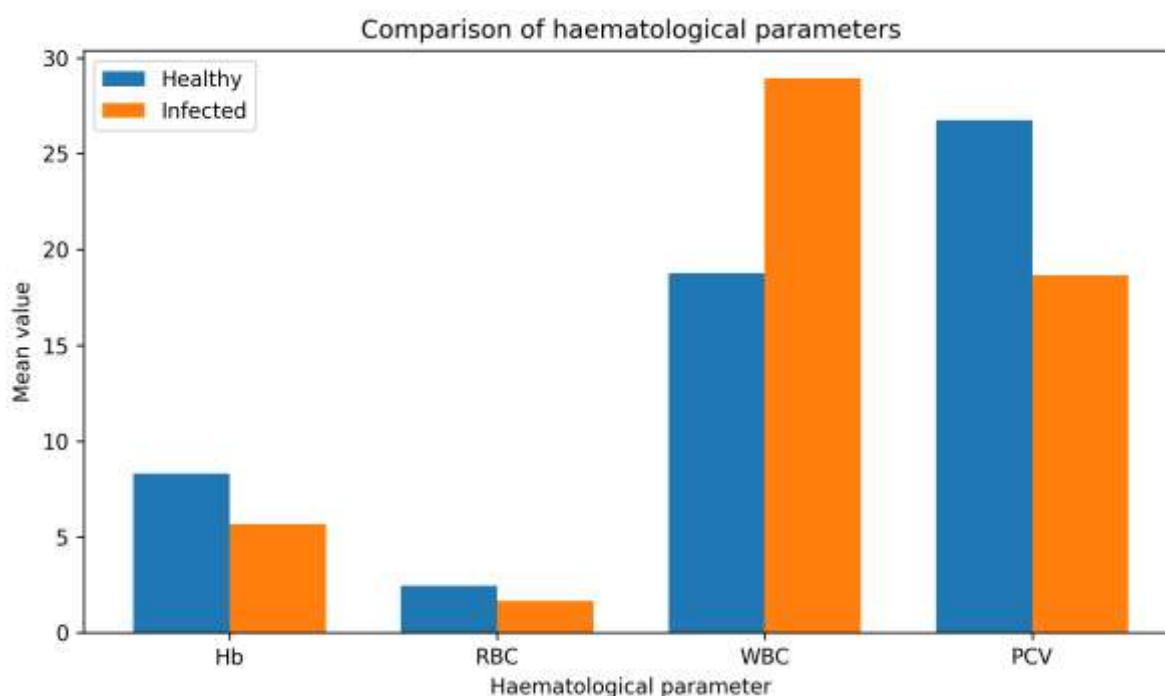


Figure 2. Comparison of major haematological parameters between healthy and infected fish

4. DISCUSSION

The present study demonstrates substantial differences in haematological parameters between apparently healthy and bacteria-infected freshwater fishes. The reduction in haemoglobin, RBC count and PCV indicates an infection-associated anaemic response. Similar haematological disturbances have been reported as useful indicators of physiological stress and bacterial disease in cultured and wild fishes [7,8].

The marked increase in WBC count is consistent with activation of inflammatory and immune responses. Leucocytosis may reflect mobilisation of immune cells in response to bacterial invasion and tissue injury [8].

The significant increase in MCV, together with non-significant changes in MCH and MCHC, indicates that erythrocyte indices were also affected. However, the present statistical results alone should not be used to definitively classify the anaemia as microcytic or normocytic without further diagnostic interpretation.

Clinical signs such as haemorrhagic patches, ulcerative lesions, fin erosion, gill abnormalities and dermal erosion were associated with the bacterial isolates recorded in the study. *Aeromonas*, *Flavobacterium*, *Pseudomonas* and *Edwardsiella* are recognised bacterial groups associated with disease in freshwater fishes [5,6].

The physicochemical observations indicate considerable spatial variation in the river environment. Low DO and elevated BOD, COD and turbidity at several downstream stations suggest environmental stress that may increase the vulnerability of fish to opportunistic bacterial disease [9]. The relationship between environmental deterioration and infection should nevertheless be interpreted from the actual sampling and statistical design rather than assumed solely from descriptive values.

5. CONCLUSION

The present investigation provides an integrated assessment of bacterial infection, clinical pathology and haematological responses in selected freshwater fishes of the Mula–Mutha River, Pune District, Maharashtra. Infected fishes exhibited marked reductions in Hb, RBC and PCV and a substantial increase in WBC. These differences were highly significant

($p < 0.0001$), while MCV also differed significantly ($p = 0.0178$). MCH and MCHC did not show significant differences. The findings indicate substantial physiological and immune responses associated with bacterial infection and provide useful baseline information for fish-health monitoring in the Mula–Mutha River ecosystem.

6. REFERENCES

- 1) Roberts RJ. *Fish Pathology*. 4th ed. Chichester: Wiley-Blackwell; 2012. doi:10.1002/9781118222942.
- 2) Austin B, Austin DA. *Bacterial Fish Pathogens: Disease of Farmed and Wild Fish*. 6th ed. Cham: Springer; 2016. doi:10.1007/978-3-319-32674-0.
- 3) Noga EJ. *Fish Disease: Diagnosis and Treatment*. 2nd ed. Ames, Iowa: Wiley-Blackwell; 2010. doi:10.1002/9781118786758.
- 4) Woo PTK, Bruno DW, editors. *Fish Diseases and Disorders, Volume 3: Viral, Bacterial and Fungal Infections*. 2nd ed. Wallingford: CABI; 2011. DOI not verified for the complete volume.
- 5) Janda JM, Abbott SL. The genus *Aeromonas*: taxonomy, pathogenicity, and infection. *Clinical Microbiology Reviews*. 2010;23(1):35–73. doi:10.1128/CMR.00039-09.
- 6) Bernardet JF, Nakagawa Y, Holmes B. Proposed minimal standards for describing new taxa of the family Flavobacteriaceae and emended description of the family. *International Journal of Systematic and Evolutionary Microbiology*. 2002;52(3):1049–1070. doi:10.1099/ijs.0.02136-0.
- 7) Hrubec TC, Smith SA. Hematology of fishes. In: Weiss DJ, Wardrop KJ, editors. *Schalm's Veterinary Hematology*. 6th ed. Ames, Iowa: Wiley-Blackwell; 2010. DOI not verified for this specific book chapter.
- 8) Campbell TW, Ellis CK. *Avian and Exotic Animal Hematology and Cytology*. 4th ed. Ames, Iowa: Wiley-Blackwell; 2015. DOI not verified for the book.
- 9) Boyd CE. *Water Quality: An Introduction*. 2nd ed. Cham: Springer; 2015. doi:10.1007/978-3-319-17446-4.