



# Comparative Study of Season-based Haematology of *Mugil cephalus* and *Sillago sihama* from Ennore Creek, Southeast Coast, India

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## ABSTRACT

**Background:** Haematological parameters are valuable indicators of fish health status. This study aims to provide baseline data on the blood profile of *Mugil cephalus* and *Sillago sihama* from Ennore Creek, southeast coast, India and UPRS, Arambakkam from September 2022 to August 2023, at bimonthly intervals.

**Methods:** Blood samples of the said two fish species were collected from Ennore Creek and UPRS Arambakkam during all four seasons to analyse haematological parameters like WBC, Lymphocytes, RBC, HGB, HCT, MCV, MCH and MCHC.

**Results:** The values of haematological parameters were found less in these two fish of Ennore than Arambakkam. Between the two stations (Ennore and Arambakkam), the differences in the values of haematological parameters were found to be highly significant ( $p < 0.01$ ) for both fish. Reduction in the values of haematological parameters of these two fish in Ennore Creek showed that the fishes exposed to pollution-induced stress make them weak, anaemic, and vulnerable to diseases.

**Key words:** Ennore Creek, Haematology, Lymphocytes, RBC, WBC

## INTRODUCTION

Flathead grey mullet and *Sillago sihama* has a good market in many countries of the world namely Egypt, Taiwan, India, Israel, etc. Mullet species marketed fresh, frozen, salted and dried and its eggs are considered a delicacy. In India the species has a good demand in the domestic market fetching up to Rs. 300/kg. Since *Mugil cephalus* is a high valued fish in India, there is good scope for scaling up of seed production technology in the country. This also requires a thorough understanding of the species / stock status of *M. cephalus* in Indian waters. *Sillago sihama* is a high value fishes in India the species have a good demand in the domestic market fetching up to Rs 600/kg. Haematological parameters are commonly used as vital indicators to assess fish health status (Gabriel *et al.*, 2004). Variations in blood parameters depend upon the fish species, aquatic biotope, health and nutritional status, age and sexual maturity (Blaxhall 1972; Chaudhuri *et al.*, 1986; Wilhem *et al.*, 1992; Hrubec *et al.*, 2001; Fazio *et al.*, 2016). Moreover, the blood parameters of fish are sensitive to environmental changes. Quality of water, oxygen, temperature and salinity reflect blood parameters (Lea Master *et al.*, 1990; Luskovav, 1997; Sheikh and Ahmed 2016), as well as ecological factors such as feeding regime and stocking density (Což-Rakovac *et al.*, 2005; Ferri *et al.*, 2011). A correct interpretation of fish haematology depends on the availability of reference values, help in understanding the relationship of blood characteristics to the phylogeny, activity, habitat and adaptability of fish to the environment (Blaxhall, 1972; Wilhem *et al.*, 1992). This study aimed to provide baseline

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data of the haematological profile of two teleost fish living in different aquatic environments, namely the Flathead Grey Mullet *Mugil cephalus* and Silver sillago *Sillago sihama*. Haematological and biochemical parameters are indicators to measure health status and toxicological symptoms of fish. While providing information about the health status of organisms, these parameters may also indicate abnormal environmental conditions. Health status and disease in fishes are assessed by determining values of various haematological and biochemical parameters. Anthropogenic activities like industrial activities, thermal power plants, petrochemicals, automobiles, harbour and suburban residential areas of fishing hamlets are causing pollution in the estuary (Karthikeyan *et al.*, 2020). Ennore estuary is highly dynamic with geographic changes in the bar mouth and characterized by the influence of discharge from various industries and wastewater from municipal sewage. The

haematological profiles of the Flathead Grey Mullet *Mugil cephalus* and the Silver sillago, *Sillago sihama* were determined by an automated system. The present study aims to provide baseline data on the blood profile of *Mugil cephalus* and *Sillago sihama* from Ennore Creek, southeast coast, India and UPRS, Arambakkam.

## MATERIALS AND METHODS

### Fish collection and acclimatization

Flathead Grey Mullet, *Mugil cephalus* and Silver sillago, *Sillago sihama* were caught from Ennore Creek and UPRS, Arambakkam. The Ennore Creek (13°13' 54.48" N, 80°19' 26.60" E) is located approximately 24 km in the northeastern part of Chennai City, Tamil Nadu, India at the coast of the Bay of Bengal (Fig 1). The Creek is connected with Pulicat Lake in the north through Buckingham Canal and Kosasthalaiyar River in the northwest. It runs parallel to the sea coast and extends over a distance of 36 km. This Creek was chosen as the test site as it receives untreated sewage from the Royapuram sewage outfall, and untreated/treated industrial effluents from the Manali Industrial Belt, which houses many chemical industries like fertilizer, oil refineries, sugar, chemicals, etc. In addition, fishing and navigational activities take place in the area. A fishing village, Arambakkam (13°33' 16.9" N 80° 04' 40.3" E) is located along the coast of Pulicat Lake is a relatively Unpolluted

Reference Site for the collection of test animals, water and sediment samples compared to Ennore Creek. There are no industries in the vicinity of this fishing village. Further, this location is a part of the Pulicat Lake Bird Sanctuary.

### Experimental animal

Grey mullets with a length of (15.7±0.32cm) and weight of (717±22.93g) and Indian whiting of length of (13.2±0.51cm) and weight of (445±23.33g) were collected once in two months from Ennore Estuary and UPRS, Arambakkam (Fig 2) using the gill nets and brought to the laboratory on the same day. The temperature, pH, salinity, dissolved oxygen (DO) and ammonia (NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup>) levels, were checked using a multi-parametric probe C 203 (Hanna-Instruments, United Kingdom). No mortality was recorded during the acclimation period. The research period is from September 2022 to August 2023 at fortnightly intervals. The work is carried out in Tamil Nadu Dr. J. Jayalathaa Fisheries University, Dr M.G.R. Fisheries College and Research Institute, Ponneri.

### Fish blood collection

Blood samples from *Mugil cephalus* and *Sillago sihama* were collected through a sterile syringe. The blood sample was drawn from the caudal vein by introducing a disposable sterile syringe (2.5 ml) and transferred in a Miniplast 0.5 ml tube containing EDTA (1.26 mg/ 0.6 ml) as an anticoagulant for haematology studies. The samples were transferred immediately into heparinized sterile tubes 1-1.5 ml for the haematological analysis. Through centrifugation for 15 min at 3000 rpm serum were obtained from blood samples and subjected to haematological analysis (Priyatharshni *et al.*, 2024; Jackqulin *et al.*, 2024).

### Automatic haematological analysis

To study the haematological parameters, a fully automatic 3 part, Haematology analyser cell tech 380, Radiant Biomed Pvt. Ltd, New Delhi. This apparatus uses an impedance analysis system that was already used and validated by comparative manual tests in the veterinary field to investigate haematological profiles in various fish species (Faggio *et al.*, 2013; Fazio *et al.*, 2013, 2016). Evaluation of the haemogram involved the determination of the red blood cell count (RBC), haematocrit (Hct), haemoglobin concentration (Hbc), white blood cell count (WBC), mean corpuscular volume (MCV),



Fig 1: Sampling stations.



Fig 2: Experimental animal.

mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

### Statistical analysis

All the data were presented as the mean  $\pm$  standard deviation of three replicates. One-way ANOVA, followed by Tukey's test for multiple comparisons at the significance level of 0.05 was used to compare the differences between the dietary groups. The data were statistically analyzed by SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA).

## RESULTS AND DISCUSSION

The results of haematological parameters of *M. cephalus* (UPRS) at Arambakkam were given in Table 1. The total red blood cell (cells/mm<sup>3</sup>) count in the mullet, *M. cephalus* of the reference (Arambakkam) site was estimated to be the highest value ( $3.51 \times 10^6 \pm 0.56^b$ ) was registered in pre-monsoon and the lowest ( $3.11 \times 10^6 \pm 0.52^{ab}$ ) in monsoon similarly, the total red blood cell (cells/mm<sup>3</sup>) count in the mullet, *M. cephalus* of the Ennore was estimated to be the highest value ( $2.71 \times 10^6 \pm 0.35^c$ ) was registered in pre-monsoon and the lowest ( $2.12 \times 10^6 \pm 0.58^c$ ) in post-monsoon. The total red blood cell (cells/mm<sup>3</sup>) count in *Sillago sihama* of the reference (Arambakkam) site was estimated to be the highest value ( $2.41 \times 10^6 \pm 0.22^a$ ) was registered in pre-monsoon and the lowest ( $2.18 \times 10^6 \pm 0.45^{bc}$ ) in summer similarly, the total red blood cell (cells/mm<sup>3</sup>) count in *Sillago sihama* of Ennore was estimated to be the highest value ( $1.33 \times 10^6 \pm 0.79^a$ ) in summer and the lowest ( $1.08 \times 10^6 \pm 0.72^{ab}$ ) in monsoon. Between the stations, (Arambakkam and Ennore) RBC (cells/mm<sup>3</sup>) count were found to be highly significant at 99% level of significance for both the fish species. In a healthy fish, the total RBC count acts as an index of the well-being of the fish, the concentration of haemoglobin reflects the oxygen supply and the Hct% reflects the blood cell volume. Variations may be due to the divergent physiological activeness of the examined fish species. As previously reported by (Svobodova *et al.*, 2008), active species have higher values of haematological parameters compared to less active forms. High RBC values are usually associated with fast movement and high activity with streamlined bodies, as documented in various studies

conducted on wild and farmed species, including grey mullets (Fazio *et al.*, 2012a, 2012b, 2013, 2016). Factors like water salinity directly affect various blood parameters such as RBC and Hct through their effect on the haemoglobin oxygen binding properties and oxygen transport (Witeska, 2013). The increased number of erythrocytes and concomitant reduction in their volume recorded herein in mullets is due to an adaptive process to the salinity of seawater habitat. Oxygen transportation in salt water moves faster than in freshwater, and this implies a degeneration of part of the red cells resulting in an increased erythropoiesis. This leads to an augmented production of new erythrocytes with a decreased volume unit. Similar data were provided by Ilergina *et al.* (2007), after investigating the influence of water salinity on the physiological status of juvenile chum salmon. However, continuous exposure of fish for 96hrs to acute toxic conditions resulting in the low levels of RBC, Hb and HC an indicative of the physiological damage under severe stressful conditions. The increase in HC% may be attributed to the swelling of the RBC under stressful conditions (Murad and Mustafa, 1989). Low RBC counts may be due to the inhibition of erythropoiesis or an increase in erythrocyte destruction in severe haemolytic anaemia. Reduced Hb concentration may be due to the impaired gill function resulting in hypoxia, but the shrinkage in the blood cell surface may have resulted in low haematocrit percentages. A similar reduction of Hc% was reported in the case of rabbit fish *Siganus rivulatus* exposed to Senai crude oil and Iranian crude oil (Eisler and Kissil, 1975). The prolonged reduction of haemoglobin and haematocrit values in fish blood could be associated with haemolytic crisis caused by toxic metals, thus signifying fish anaemia (Gaafar *et al.*, 2010). Haemoglobin, an oxygen-carrying molecule, thereby acts as an indicator of fish anaemic conditions (Parekh and Tank, 2015).

The total haemoglobin (g/dl) in the mullet, *M. cephalus* of the reference (Arambakkam) site was estimated to be the highest value ( $3.36 \pm 0.13^c$ ) was registered in post-monsoon and the lowest ( $3.12 \pm 0.85^b$ ) in summer. The total haemoglobin (g/dl) in the mullet, *M. cephalus* of Ennore site was estimated to be the highest value ( $2.92 \pm 0.21^a$ ) was registered in pre-monsoon and the lowest ( $2.16 \pm 0.84^a$ ) in

**Table 1:** Haematological parameters of *M. cephalus* (UPRS) at Arambakkam.

Haematological parameters	Pre mon'22	monsoon'22	Post mon'23	Summer'23
WBC (gm/dl)	$35.69 \times 10^5 \pm 0.68^{ab}$	$33.29 \times 10^5 \pm 0.21^b$	$32.75 \times 10^5 \pm 0.15^b$	$34.86 \times 10^5 \pm 0.36^b$
LYM (gm/dl)	$30.25 \times 10^5 \pm 0.55^a$	$27.59 \times 10^5 \pm 0.34^a$	$25.20 \times 10^5 \pm 0.42^{ab}$	$21.65 \times 10^5 \pm 0.75^{ab}$
RBC (cells/mm <sup>3</sup> )	$3.51 \times 10^6 \pm 0.56^b$	$3.11 \times 10^6 \pm 0.52^{ab}$	$3.29 \times 10^6 \pm 0.81^b$	$3.19 \times 10^6 \pm 0.58^a$
HGB (g/dl)	$3.28 \pm 0.19^a$	$3.6 \pm 0.31^c$	$3.36 \pm 0.13^c$	$3.12 \pm 0.85^b$
HCT (%)	$4.84 \pm 0.21^c$	$4.39 \pm 0.28^a$	$4.54 \pm 0.74^a$	$4.16 \pm 0.98^a$
MCV (fL)	$172.21 \pm 0.88^a$	$163.14 \pm 0.71^c$	$160.15 \pm 0.75^{ab}$	$162.89 \pm 0.50^b$
MCH (pg)	$84.06 \pm 0.41^b$	$81.51 \pm 0.42^b$	$88.4 \pm 0.94^a$	$77.76 \pm 0.28^c$
MCHC (gm %)	$92.54 \pm 0.96^{ab}$	$92.14 \pm 0.51^b$	$97.49 \pm 0.59^c$	$95.29 \pm 0.15^b$

Values in the same column with different superscripts differ significantly ( $P < 0.05$ ) for each parameter post hoc analysis. One-way ANOVA was used following Tukey's test.

post-monsoon. The total haemoglobin (g/dl) in *Sillago sihama* of the reference (Arambakkam) site was estimated to be the highest value ( $2.72 \pm 0.52^a$ ) was registered in post-monsoon and the lowest ( $2.16 \pm 0.58^a$ ) in summer. The total haemoglobin (g/dl) in *Sillago sihama* of Ennore site was estimated to be the highest value ( $2.35 \pm 0.71^b$ ) was registered in pre-monsoon and the lowest at ( $2.05 \pm 0.27^{bc}$ ) in post-monsoon. Between the stations, (Arambakkam and Ennore) haemoglobin (g/dl) were found to be highly significant at 99% level of significance for both the fish species. The decrease in erythrocyte number and haemoglobin content observed in this study may be due to the disruptive action of the pesticides on the erythropoietic tissue as a result of which the viability of the cells might be affected (Köprücü *et al.*, 2006). The decrease in RBC was because heavy metals destroy RBC, so less oxygen binds to hemoglobin due to the inhibition of erythropoiesis (Akbar *et al.*, 2017). The reduction in RBC count and Hb are often accompanied by a decrease in Hct and demonstrates the physiological dysfunction of the hemopoietic system. Reductions in Hb and Hc% probably have resulted from the disturbance in hematopoietic trends due to heavy metal toxicity (Lavanya *et al.*, 2011). A significant decrease in haematocrit values in the metal-administered groups could be due to aggregates of red blood cells in the gills of stressed fish. These aggregates lessen the circulation of red blood cells and impair osmoregulation, thus resulting in anemia and hemodilution (Parekh and Tank, 2015). The difference in the RBC count in *M. cephalus* collected from polluted Ennore creek and UPRS was 1.01 cells/mm<sup>3</sup> and the RBC count of Ennore was 30.23% lower than UPRS. Similarly, the difference in the RBC count in *S. sihama* collected from polluted Ennore creek and UPRS was recorded as 1.1 cells/mm<sup>3</sup> and the RBC count of Ennore was 48.24% lower than UPRS. It was found that there was a difference of 1.02 gm/dl in the HGB count of Mullet, *M. cephalus*, between the specimen collected from polluted Ennore creek and the difference in the HGB count in *M. cephalus* collected from polluted Ennore creek and UPRS was 0.92 gm/dl and the HGB count of Ennore was 27.54% lower than UPRS. Similarly, the difference in HGB count of Silver sillago, *S. sihama*, between the specimens collected from polluted

Ennore Creek and UPRS was 11.76% lower than UPRS. The difference in the HGB count in *S. sihama* collected from polluted Ennore Creek and UPRS was recorded as 0.35 gm/dl and the HGB count of Ennore was 14% lower than UPRS.

The results of haematological parameters of *M. cephalus* at Ennore (2022-2023) were given in Table 2. The total HCT (%) in the mullet, *M. cephalus* of the reference (Arambakkam) site was estimated to be the highest value ( $4.84 \pm 0.21^c$ ) was registered in pre-monsoon and the lowest ( $4.16 \pm 0.98^a$ ) in summer. The total HCT (%) in the mullet, *M. cephalus* of Ennore site was estimated to be the highest value ( $2.55 \pm 0.36^a$ ) was registered in monsoon and the lowest ( $2.19 \pm 0.61^a$ ) in post-monsoon. The total HCT (%) in *Sillago sihama* of the reference (Arambakkam) site was estimated to be the highest value ( $3.72 \pm 0.85^b$ ) was registered in post-monsoon and the lowest ( $3.12 \pm 0.16^a$ ) in summer. The total HCT (%) in *Sillago sihama* of the Ennore site was estimated to be the highest value ( $1.69 \pm 0.51^a$ ) was registered in post-monsoon and the lowest ( $1.56 \pm 0.69^c$ ) in pre-monsoon. Between the stations, (Arambakkam and Ennore) HCT (%) were found to be highly significant at 99% level of significance for both the fish species. The difference in HCT (%) of Mullet, *M. cephalus*, collected from polluted Ennore Creek and UPRS during the year 2022-23, the difference in the HCT (%) in *M. cephalus* collected from polluted Ennore Creek and UPRS was recorded as 2.07% and HCT (%) of S2 was 46% lower than UPRS. It was found that there was a difference of 1.87% in the HCT (%) of Silver sillago, *S. sihama*, between the specimens collected from polluted Ennore Creek. The difference in the HCT (%) in *S. sihama* collected from polluted Ennore Creek and UPRS was recorded as 1.75% and the HCT (%) of Ennore was 51.47% lower than UPRS.

The total WBC count in the mullet, *Mugil cephalus* of the reference (Arambakkam) site was estimated to be the highest value ( $35.69 \times 10^5 \pm 0.68^{ab}$ ) was registered in pre-monsoon and the lowest ( $32.75 \times 10^5 \pm 0.15^b$ ) in post-monsoon. The results of haematological parameters of *S. sihama* (UPRS) at Arambakkam (2022-2023) were given in Table 3. The total WBC count in the Mullet, *M. cephalus* of Ennore site was estimated to be the highest value ( $22.86 \times 10^5 \pm 0.72^b$ ) was

**Table 2:** Haematological parameters of *M. cephalus* at Ennore (2022-2023).

Haematological parameters	Pre mon'22	monsoon'22	Post mon'23	Summer '23
WBC (gm/dl)	$22.86 \times 10^5 \pm 0.72^b$	$19.71 \times 10^5 \pm 0.16^a$	$19.17 \times 10^5 \pm 0.18^{bc}$	$21.03 \times 10^5 \pm 0.11^b$
LYM (gm/dl)	$17.12 \times 10^5 \pm 0.18^b$	$16.21 \times 10^5 \pm 0.18^b$	$16.90 \times 10^5 \pm 0.11^c$	$17.02 \times 10^5 \pm 0.16^{ab}$
RBC (cells/mm <sup>3</sup> )	$2.71 \times 10^6 \pm 0.35^c$	$2.18 \times 10^6 \pm 0.31^{bc}$	$2.12 \times 10^6 \pm 0.58^a$	$2.33 \times 10^6 \pm 0.36^a$
HGB (g/dl)	$2.92 \pm 0.21^a$	$2.21 \pm 0.4^a$	$2.16 \pm 0.84^a$	$2.36 \pm 0.18^a$
HCT (%)	$2.52 \pm 0.12^c$	$2.55 \pm 0.36^a$	$2.19 \pm 0.61^a$	$2.49 \pm 0.53^a$
MCV (fL)	$136.2 \pm 0.25^b$	$138.41 \pm 0.62^b$	$139.05 \pm 0.51^a$	$143.61 \pm 0.29^b$
MCH (pg)	$42.12 \pm 0.26^{ab}$	$44.92 \pm 0.21^{bc}$	$49.51 \pm 0.54^b$	$43.61 \pm 0.18^c$
MCHC (gm %)	$77.35 \pm 0.59^c$	$70.01 \pm 0.68^b$	$73.01 \pm 0.18^{bc}$	$69.55 \pm 0.77^{ab}$

Values in the same column with different superscripts differ significantly ( $P < 0.05$ ) for each parameter post hoc analysis. One-way ANOVA was used following Tukey's test.



registered in pre-monsoon and the lowest ( $19.17 \times 10^5 \pm 0.18^{bc}$ ) in post-monsoon. The total WBC count in *Sillagosihama* of the reference (Arambakkam) site was estimated to be the highest value ( $20.05 \times 10^5 \pm 0.75^{bc}$ ) was registered in pre-monsoon and the lowest ( $18.55 \times 10^5 \pm 0.38^b$ ) in monsoon. The total WBC count in *Sillago sihama* of Ennore site was estimated to be the highest value ( $15.42 \times 10^5 \pm 0.19^{bc}$ ) was registered in summer and the lowest ( $14.69 \times 10^5 \pm 0.19^a$ ) in pre-monsoon. Between the stations, (Arambakkam and Ennore) WBC (gm/dl) count were found to be highly significant at 99% level of significance for both the fish species. It is known that WBC plays a vital role in the immune defense system of fish (Magnadottir, 2006). The number of WBCs may change due to various environmental parameters or stimuli such as infection, as well as multiple other factors, from the age of fish to species characteristics or nutritional differences (Romano *et al.*, 2017), which may explain the variations in WBC values observed herein in mullets and silver sillago of Ennore creek compared to UPRS. The reduction in the WBC count of the treatment groups may be due to the release of epinephrine during stress is capable of causing the contraction of the spleen and a decrease in leucocyte count, which can result in the weakening of the immune system (Svoboda, 2001; Witesta, 2003; Ruby *et al.*, 2022). The decreased number of white blood cells (leucopenia) may result in bioconcentration of the metal in the kidney and liver. Other authors have associated the cause with hindering granulopoiesis or lymphopoiesis, induced by primary or secondary changes in haematopoietic organs (Tomaszewski, 1997). There was a difference in the WBC count of Mullet, *M. cephalus* between the specimen collected from polluted Ennore Creek and UPRS. The difference in the WBC count in *M. cephalus* collected from polluted Ennore Creek and UPRS was recorded as 13.49 gm/dl and the WBC count of Ennore was 39.46% lower than UPRS. Similarly, the difference in the WBC count of Silver sillago, *S. sihama*, the difference in the WBC count in *S. sihama* collected from polluted Ennore Creek and UPRS was recorded as 4.46 gm/dl and the WBC count of Ennore was 22.98% lower than UPRS.

The total MCV (fL) in the mullet, *M. cephalus* of the reference (Arambakkam) site was estimated to be the highest value ( $172.21 \pm 0.88^a$ ) was registered in pre-monsoon and the lowest

( $160.15 \pm 0.75^{ab}$ ) in post-monsoon. The total MCV (fL) in the mullet, *M. cephalus* of Ennore site was estimated to be the highest value ( $143.61 \pm 0.29^b$ ) was registered in summer and the lowest ( $136.2 \pm 0.25^b$ ) in pre-monsoon. The total MCV (fL) in *Sillago sihama* of the reference (Arambakkam) site was estimated to be the highest value ( $126.54 \pm 0.57^b$ ) was registered in summer and the lowest ( $122.5 \pm 0.59^a$ ) in post-monsoon. The total MCV (fL) in *Sillago sihama* of Ennore site was estimated to be the highest value ( $121.16 \pm 0.42^b$ ) was registered in monsoon and the lowest ( $101.6 \pm 0.24^a$ ) in post-monsoon. Between the stations, (Arambakkam and Ennore) MCV (fL) were found to be highly significant at 99% level of significance for both the fish species. There was a difference in the MCV (fL) of Mullet, *M. cephalus*, between the specimen collected from polluted Ennore Creek and UPRS, the difference in the MCV (fL) in *M. cephalus* collected from polluted Ennore Creek and UPRS was recorded as 25.18 fL and the MCV (fL) of Ennore was 15.30% lower than UPRS. The difference in the MCV (fL) in *S. sihama* collected from polluted Ennore Creek and UPRS was recorded as 13.19 fL and the MCV (fL) of Ennore was 10.66% lower than UPRS. The results of haematological parameters of *Sillago sihama* at Ennore (2022-2023) were given in Table 4.

The total MCHC (gm%) in the mullet, *M. cephalus* of the reference (Arambakkam) site was estimated to be the highest value ( $97.49 \pm 0.59^c$ ) was registered in post-monsoon and the lowest ( $92.14 \pm 0.51^b$ ) in monsoon. The total MCHC (gm%) in the mullet, *M. cephalus* of Ennore site was estimated to be the highest value ( $77.35 \pm 0.59^c$ ) was registered in pre-monsoon and the lowest ( $69.55 \pm 0.77^{ab}$ ) in summer. The total MCHC (gm%) in *Sillago sihama* of the reference (Arambakkam) site was estimated to be the highest value ( $48.15 \pm 0.26^{ab}$ ) was registered in summer and the lowest ( $43.26 \pm 0.32^a$ ) in monsoon. The total MCHC (gm%) in *Sillagosihama* of Ennore site was estimated to be the highest value ( $36.15 \pm 0.37^c$ ) was registered in monsoon and the lowest ( $33.19 \pm 0.22^c$ ) in post-monsoon. Between the stations, (Arambakkam and Ennore) MCHC (gm%) were found to be highly significant at 99% level of significance for both the fish species. There was a difference in the MCHC (gm %) of Mullet, *M. cephalus*, between the specimens collected from polluted Ennore Creek and UPRS. The

**Table 3:** Haematological parameters of *S. sihama* (UPRS) at Arambakkam (2022-2023).

Haematological parameters	Pre mon'22	monsoon'22	Post mon'23	Summer '23
WBC (gm/dl)	$20.05 \times 10^5 \pm 0.75^{bc}$	$18.55 \times 10^5 \pm 0.38^b$	$19.54 \times 10^5 \pm 0.95^{ab}$	$19.46 \times 10^5 \pm 0.54^b$
LYM (gm/dl)	$17.84 \times 10^5 \pm 0.52^b$	$17.15 \times 10^5 \pm 0.25^{ac}$	$17.99 \times 10^5 \pm 0.48^b$	$17.8 \times 10^5 \pm 0.3^{ab}$
RBC (cells/mm <sup>3</sup> )	$2.41 \times 10^6 \pm 0.22^a$	$2.19 \times 10^6 \pm 0.12^{ab}$	$2.36 \times 10^6 \pm 0.25^{ac}$	$2.18 \times 10^6 \pm 0.45^{bc}$
HGB (g/dl)	$2.63 \pm 0.42^a$	$2.5 \pm 0.75^a$	$2.72 \pm 0.52^{ac}$	$2.16 \pm 0.58^a$
HCT (%)	$3.40 \pm 0.64^a$	$3.38 \pm 0.51^b$	$3.72 \pm 0.85^{ba}$	$3.12 \pm 0.16^a$
MCV (fL)	$122.8 \pm 0.71^b$	$123.07 \pm 0.38^c$	$122.5 \pm 0.59^a$	$126.54 \pm 0.57^b$
MCH (pg)	$29.94 \pm 0.52^{bc}$	$28.19 \pm 0.23^c$	$28.56 \pm 0.15^b$	$26.53 \pm 0.33^b$
MCHC (gm %)	$46.71 \pm 0.26^c$	$43.26 \pm 0.32^a$	$47.71 \pm 0.75^b$	$48.15 \pm 0.26^{ab}$

Values in the same column with different superscripts differ significantly ( $P < 0.05$ ) for each parameter post hoc analysis. One-way ANOVA was used following Tukey's test.

**Table 4:** Haematological parameters of *Sillago sihama* at Ennore (2022-2023).

Haematological parameters	Pre mon' 22	monsoon' 22	Post mon' 23	Summer' 23
WBC (gm/dl)	14.69 $\times 10^5 \pm 0.19^a$	14.75 $\times 10^5 \pm 0.11^{bc}$	14.92 $\times 10^5 \pm 0.14^{ab}$	15.42 $\times 10^5 \pm 0.19^{bc}$
LYM (gm/dl)	11.71 $\times 10^5 \pm 0.18^{bc}$	10.88 $\times 10^5 \pm 0.16^a$	10.91 $\times 10^5 \pm 0.11^c$	9.8 $\times 10^5 \pm 0.42^a$
RBC (cells/mm <sup>3</sup> )	1.18 $\times 10^6 \pm 0.12^b$	1.08 $\times 10^6 \pm 0.72^{ab}$	1.16 $\times 10^6 \pm 0.28^{bc}$	1.33 $\times 10^6 \pm 0.79^a$
HGB (g/dl)	2.35 $\pm 0.71^b$	2.1 $\pm 0.29^c$	2.05 $\pm 0.27^{bc}$	2.11 $\pm 0.21^c$
HCT (%)	1.56 $\pm 0.69^c$	1.68 $\pm 0.56^b$	1.69 $\pm 0.51^a$	1.65 $\pm 0.61^b$
MCV (fL)	111.72 $\pm 0.21^{bc}$	121.16 $\pm 0.42^b$	101.6 $\pm 0.24^a$	107.66 $\pm 0.23^{ac}$
MCH (pg)	26.51 $\pm 0.16^a$	21.83 $\pm 0.2^a$	21.89 $\pm 0.16^a$	23.49 $\pm 0.16^a$
MCHC (gm %)	36.09 $\pm 0.52^a$	36.15 $\pm 0.37^c$	33.19 $\pm 0.22^c$	34.62 $\pm 0.83^b$

Values in the same column with different superscripts differ significantly ( $P < 0.05$ ) for each parameter post hoc analysis. One-way ANOVA was used following Tukey's test.

difference in the MCHC (gm%) in *M. cephalus* collected from polluted Ennore creek and UPRS was recorded as 22.21 gm % and the MCHC (gm%) of Ennore was 11.22% lower than UPRS. The MCHC (gm %) of Ennore was 25.32% lower than UPRS. The difference in the MCHC (gm %) in *S. sihama* collected from polluted Ennore creek and UPRS was recorded as 11.12 gm % and the MCHC (gm %) of Ennore was 24.20% lower than UPRS. There was a difference in the MCHC (gm%) of Mullet, *M. cephalus*, between the specimens collected from polluted Ennore Creek and UPRS. During the year 2022-23, the difference in the MCHC (gm %) in *M. cephalus* collected from polluted Ennore Creek and UPRS was recorded as 22.21 gm % and the MCHC (gm %) of Ennore was 11.22% lower than UPRS. The MCHC (gm %) of Ennore was 25.32% lower than UPRS. The difference in the MCHC (gm %) in *S. sihama* collected from polluted Ennore creek and UPRS was recorded as 11.12 gm % and the MCHC (gm %) of Ennore was 24.20% lower than UPRS.

The total lymphocyte count in the mullet, *M. cephalus* of the reference (Arambakkam) site was estimated to be the highest value ( $30.25 \times 10^5 \pm 0.55^a$ ) was registered in pre-monsoon and the lowest ( $21.65 \times 10^5 \pm 0.75^{ab}$ ) in summer. The total lymphocyte count in the mullet, *M. cephalus* of Ennore site was estimated to be the value ( $17.12 \times 10^5 \pm 0.18^{ab}$ ) was registered in pre-monsoon and the lowest ( $16.21 \times 10^5 \pm 0.18^b$ ) in monsoon. ANOVA Results of various Haematology parameters of *S. sihama* (2022-23) were given in Table 5. The total lymphocyte count in *Sillago sihama* of the reference (Arambakkam) site was estimated to be the highest value ( $17.99 \times 10^5 \pm 0.48^{ab}$ ) was registered in post-monsoon and the lowest ( $17.15 \times 10^5 \pm 0.25^{ac}$ ) in monsoon. The total lymphocyte count in *Sillago sihama* of Ennore site was estimated to be the highest value ( $11.71 \times 10^5 \pm 0.18^{bc}$ ) was registered in pre-monsoon and the lowest ( $9.8 \times 10^5 \pm 0.42^a$ ) in summer. Between the stations, (Arambakkam and Ennore) lymphocytes (gm/dl) were found to be highly significant at 99% level of significance for both the fish species. The results of differences in the values of haematological parameters between Ennore and UPRS were given in Table 6. There was a difference in the lymphocyte count of Mullet, *M. cephalus*, between the specimens collected from polluted

Ennore Creek and UPRS. The difference in the lymphocyte count in *M. cephalus* collected from polluted Ennore Creek and UPRS was recorded as 9.33 gm/dl and the lymphocyte count of Ennore was 35.65% lower than UPRS. Similarly, difference in the lymphocyte count of Silver sillago, *S. sihama*, between the specimens collected from polluted Ennore Creek and UPRS. the difference in the lymphocyte count in *S. sihama* collected from polluted Ennore Creek and UPRS was recorded as 6.89 gm/dl and the lymphocyte count of Ennore was 38.90% lower than UPRS. To overcome hypoxic conditions in stressful media, fish usually respond by increasing the MCV and MCH of erythrocytes (Rauf and Arain, 2013). However, in the present study, MCV and MCH were also found to be less in polluted sites (Ennore creek). The significant change in the MCH of the experimental fish when compared with the control may be due to the reduction in cellular blood iron, resulting in reduced oxygen-carrying capacity of blood and eventually stimulating erythropoiesis (Hodson *et al.*, 1978).

The reduction in MCH, MCHC, and MCV levels may have been due to a compensation for reduced oxygen up-take which resulted from damage to gills caused by pollutants or was possibly due to immature RBCs being released into the blood circulation (Priyatharshni *et al.*, 2024; Kavitha *et al.*, 2010). The present study is in agreement with the study of Sing and Tando (2009) who reported that the fish, *Wallago attu* from the Suheli River showed significantly lesser values for MCV, MCHC and MCH as compared to fish from the Gomti River. Similarly, the lymphocyte count of fish of Ennore Creek was less than the UPRS. A decrease in the number of leucocytes and significant changes in their differential count are typical effects caused by several pollutants (e.g. phenols, metals, pesticides, *etc.*); a characteristic decrease in the percentage of lymphocytes and an associated increase in granulocytes can occur (Svobodová *et al.*, 1993). A reduction in haematological values indicates anemia in the pesticide-exposed fish, which may be due to erythropoietic, haemosynthetic and osmoregulatory dysfunction or due to an increase in the rate of erythrocyte destruction in haematopoietic organs (Joshna *et al.*, 2023; Jenkins *et al.*, 2003; Seth and Saxena, 2003). The optimum water quality range was observed in both sites of

**Table 5:** ANOVA results of various haematology parameters of *S.sihama* ( 2022-23).

Haematology parameters	Rows				Columns			
	df	f	P	SL	df	f	P	SL
WBC	3	1.732	0.2298	NS	3	130.7	9.73E-08**	S
LYM	3	1.889	0.2019	NS	3	135.9	8.19E-08**	S
RBC	3	3.812	0.05159	NS	3	184.6	2.13E-08**	S
HGB	3	2.596	0.1169	NS	3	4.999	0.02608*	S
HCT	3	4.976	0.0264*	S	3	209.8	1.21E-08**	S
MCV	3	1.637	0.2489	NS	3	10.8	0.00244**	S
MCH	3	8.586	0.00525**	S	3	35.61	2.54E-05**	S
MCHC	3	0.5427	0.6651	NS	3	40.84	1.44E-05**	S

\*Significant at 95% level of significance ( $p < 0.05$ ); \*\*Significant at 99% level of significance ( $p < 0.01$ ); df-Degrees of Freedom; F- F test statistic for regression analysis; P- P value; S- Significant; NS- Not significant; Rows- Between Bi-Monthly samples; Column- Between the sampling sites.

**Table 6:** Differences in the values of haematological parameters between Ennore and UPRS.

Haematology parameters	<i>M. cephalus</i>		<i>S. sihama</i>	
	Difference	Percentage %	Difference	Percentage %
WBC (gm/dl)	13.49	39.46%	4.46	22.98%
LYM (gm/dl)	9.33	35.65%	6.89	38.90%
RBC (cells/mm <sup>3</sup> )	1.01	30.23%	1.1	48.24%
HGB (g/dl)	0.92	27.54%	0.35	14%
HCT (%)	2.07	46%	1.75	51.47%
MCV (fL)	25.18	15.30%	13.19	10.66%
MCH (pg)	37.64	45.52%	5.53	18.47%
MCHC (gm %)	22.21	11.22%	11.12	24.20%

**Table 7:** Water quality parameters of Ennore and Arambakkam.

Parameters	ENNORE	ARAMBAKKAM
Temperature	29±0.5	30±1.2
pH	7.59±0.04	7.5±0.2
Ammonia mg/l	1.69±0.3	0.68±0.07
DO mg/l	5.3±0.6	4.5±0.2
BOD mg/l	31±2.1	6.0±0.4
COD mg/l	240±9.0	75±2.5
TDS	1510±	225±42.10
TSS	24±0.2	10±3.65
Salinity	27.56±0.78	27±0.9
Nitrite mg/l	0.61±0.08	0.12±0.01
Nitrate mg/l	0.42±0.02	0.31±0.01

Arambakkam and Ennore. The results of water quality parameters of Ennore and Arambakkam site were given in Table 7. In conclusion, the results acquired from the current study showed that the fish species, *Mugil cephalus* and *Sillago sihama* exposed to pollution-induced stress at Ennore Creek caused a significant reduction in all haematological parameter values. Ennore Creek has a strong potential to induce stress making the fish anaemic, weak and vulnerable to diseases.

## CONCLUSION

Haematological parameters are widely used as an early signal of changes in fish health status and have proven to

be a valuable approach for monitoring the effects of habitat changes on fish biology. Haematological parameters such as WBC (gm/dl), LYM (gm/dl), RBC (cells/mm<sup>3</sup>), Hb (g/dl), Hc (%), PLT (cells/cu.mm<sup>-1</sup>) and PCV (%) of Mullet, *M. cephalus* and Silver sillago, *S. sihama* collected from polluted Ennore creek was less than UPRS, indicating the polluted nature of the Ennore Creek. It is concluded that Ennore Creek was found to be less diverse as the pollution load is the highest. Analysis of water samples revealed that parameters like BOD, COD, TDS, TSS, Ammonia, Nitrite and Nitrate were higher at Ennore Creek than at the reference site. The dissolved oxygen content was less at Ennore Creek than at the reference site. The concentration of heavy metals was higher at Ennore Creek than the reference site indicating that Ennore Creek is polluted due to several anthropogenic activities. Finally, the results of histology and haematology acquired from the current study showed that the fish species were exposed to pollution-induced stress at Ennore Creek.

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## Conflict of interest

All procedures followed were in accordance with the ethical standards of the responsible, the authors declare that they have no conflict of interest.

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