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Research Article

Baseline studies on selected hematological parameters of Indian major carps, exotic carps and catfishes

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ABSTRACT

The aim of the current investigation was to obtain a basic knowledge of the hematology of the commercially important select fishes. Blood hematological parameters are often used to assess the health status and as stress indicators in fishes. The present study was undertaken to understand and compare the hematological parameters of Indian major carps, exotic carps and select catfishes to establish the baseline values and normal ranges of blood parameters which would help to assess the health the fish as well as reference point for future comparative surveys. For all the fishes selected, five sexually immature and disease-free fish were examined and indices measured, include: Total Erythrocyte Counts (TEC), Total Leucocytes Counts (TLC), Packed Cell Volume (PCV), Hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Erythrocyte Sedimentation Rate (ESR), leucocyte counts and cell morphology. The mean values were determined for each hematological parameter evaluated and the significance was set at 0.05. The study revealed a maximum number of erythrocytes, Hb and PCV for catfishes and minimum in common carp. However, significantly higher MCV, MCH, MCHC was in mrigal, common carp and catla compared to other fishes. Further, highest platelet counts were observed in rohu and lowest in silver carp and maximum ESR in catla and minimum in rohu among the fish species studied. The results imply that the variation in hematological parameters depends on various intrinsic and extrinsic parameters and these could be used for biomarker and biomonitoring studies.

INTRODUCTION

Studies on hematology have not widely researched in fishes and examination on these could be used as a suitable diagnostic tool to understand the physiological and pathological changes. Various factors such as seasons, physicochemical parameters of the water, stressors, age and sexual maturity stage, species and health status which affect the hematological parameters. However, one of the bottlenecks in understanding health status of fish is the lack of reliable and reference conditions. In this view, studies have aimed to understand the hematology, since these parameters are providing a valuable diagnostic tool in evaluating human health. Determination of these parameters can provide substantial information; upon standardized reference values are established. Moreover, recent attention has also been given to the biochemical characterization of fish blood as an internal index. Currently, the base line levels of blood parameters in several fishes are established by earlier investigators for different teleosts (Darvish *et al.*, 2009; Satheeshkumar *et al.*, 2012).

In addition, knowledge on the hematological parameters is an important tool that can be used as an effective index to monitor the physiological and pathological changes (Rambhaskar and Rao 1986; Xiaoyun *et al.*, 2009). These indices have proven to be a valuable approach for analyzing the health of the fish (De Pedro *et al.*, 2005) or any other animals it could be and also provided reliable information on metabolic disorders, deficiencies and chronic stress status (Bahmani *et al.*, 2001; Cnaani *et al.*, 2004). For example, significant fluctuations detected in the concentrations of cortisol, glucose, cholesterol and other basic components in response to handling and hypoxic stress (Skjervold *et al.*, 2001). The levels of cortisol and glucose are considered to be specific indicators of sympathetic activation during stress conditions (Lermen *et al.*, 2004). Basic ecological factors, such as feeding regime and stocking density also have a direct influence on certain biochemical parameters (Coz-Rakovac *et al.*, 2005). To date, data reported in the literature refer mainly to acute stress status in different fish species and there have been an insufficient number of studies on the comparative hematological differences between different feeding behaviors of fish that share the same ecological zone.

The baseline hematological studies on Indian major carps, exotic carps and select catfishes have not been explored completely, hence the present study focused on Indian major carps; catla (*Catla catla),* rohu *(Labeo rohita)* and mrigal *(Cirrhinus mrigala),* exotic carps; common carp *(Cyprinus carpio),* grass carp *(Ctenopharyngodon idella),* and silver carp *(Hypophthalmichthys molitrix)* and catfishes; pangas *(Pangasius sp)* and singhi *(Heteropneustes fossilis)*. These fishes were selected for the current study, because of their commercial importance and information on hematological investigations of these fishes is extremely inadequate, hence the study would help understanding the health condition of the fishes, which undergo biotic and or abiotic stressors

MATERIAL AND METHODS

Fish samples and blood collection

Fish belonging to different groups (Indian major carps, exotic carps and selected catfishes) were collected for baseline hematological studies. Before collection of blood, fish were anaesthetized using MS-222 (tricaine methanesulfonate) at the rate of 30-50 mg/L of water. The dosage of MS-222 varied between the species due to their size variations. The needle size for collection varied between the species according to their size. Generally it varied from 20-26 gauge. For each fish species, 5 fish samples (n=5) were used for assays. From each fish, blood sample was collected at the caudal vein near the anal fin, then samples were placed in anti-coagulant vial (10% EDTA; AcCuvet-Plus; K_3EDTA) and stored at 4 ^oC for short time and the samples were immediately processed.

Hematological analysis

Various hematological parameters were determined as per standard protocols. Briefly, total erythrocyte counts (TEC), total leucocytes counts (TLC) and packed cell volume (PCV) or hematocrit (Ht) value was calculated according to Haney *et al.*,(1992) and hemoglobin (Hb) was estimated by acid haematin method (Sahli, 1962). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) was calculated using the above parameters. For other parameters viz., platelet counts, red cell distribution width, erythrocyte sedimentation rate (ESR), neutrophils and lymphocytes, 100 uL of sample was used for assay in Sysmex Kx-2 instrument. To understand the blood cell morphology, Leishman's stain was used according to the manufacturer's instructions and the cells were observed using Leica DM 3000 microscope and photographed.

Statistical analysis

Statistical analysis of the data was performed with a Statistical Package for the Social Sciences (SPSS v16.0). One-way ANOVA was applied to understand the difference in hematological parameters between and among the fish groups and the significance was set at 0.05

RESULTS AND DISCUSSION

Hematological parameters are used as an index for health status and to detect the physiological changes in different conditions (Satheeshkumar *et al.,* 2012). In the present study, baseline levels of different hematological parameters were determined in Indian major carps, exotic carps and select catfishes. The parameters analyzed include: TEC (RBC), TLC (WBC) count, hemoglobin, hematocrit value, MCV, MCH, MCHC, red cell distribution width, erythrocyte sedimentation rate, platelets, neutrophils, lymphocytes and cell morphology. Studies on these parameters had proven to be a valuable approach for analyzing the health of fish and help understanding the relationship of blood characteristics to the habitat and adaptability of the species to the environment. The ranges of baseline values of the key hematological parameters are still undefined for some of the cultured fish species. Understanding the levels of these parameters would help to know the homeostasis of fish and if continuously affected by the changes or variation in levels of biotic and abiotic factors.

Total Erythrocyte Counts (TEC) and Total Leucocyte Counts (TLC)

The study did not reveal any significant difference in TEC within the groups. However, mrigal and common carp showed significantly less TEC in their respective groups (Fig. 1A). Further, the maximum number of erythrocytes was observed in pangas and singhi and minimum in common carp, and these values were significant to other fishes ($p<0.05$). Among the groups, highest TLC was observed for catfishes followed by Indian major carps and exotic carps. However, in all the fishes studied minimum TLC was found in mrigal and common carp (Fig. 1B). The TLC variations observed between the groups and within the groups were significant ($P<0.05$). Both TEC and TLC showed similar levels in all the groups. The erythrocyte levels in fish determine the capacity to transport the oxygen, whereas TLC is part of the immune system and fight against pathogens (Nikinmaa 1997). The difference in erythrocyte counts observed in the study could be either due to the type of species or habitat they live in or feeding habit. In the present study, the differences in TEC and TLC among the three fish groups also could be due to different size of fish and attributed to other biotic factors viz., age, season and maturity, and abiotic factors (Jawad *et al.,* 2004; Xiaoyun *et al.,* 2009). Further variations between the hematological parameters among fish species could also depend on the sampling technique, method of analysis, age of the fish, habit and habitat (Harikrishnan et al 2003; Celik, 2004). However in the present study, sampling and analysis for all the fish was performed simultaneously, hence intra assay error is excluded.

Hemoglobin (Hb)

Hemoglobin is an oxygen transport molecule from gills to different parts of the body in the form of oxyhaemoglobin and it collects carbon dioxide from different parts of the body to the gills in the form of carboxyhaemoglobin (Southamani *et al.,* 2015; Jensen 2017). The present study observed maximum hemoglobin content for pangas and singhi followed by catla, rohu, silver carp, grass carp and mrigal and lowest in common carp (Fig. 1C, P<0.05). The variation in Hb observed could be due to variation in erythrocytes, and it is deduced that the level of Hb is directly proportional to the erythrocyte numbers. The Hb value in all the three groups of fishes was found varied significantly which could be attributed to the fact that the oxygen carrying capacity of the fish was affected by different feeding habits, their habitat and metabolism (Southamani et al 2015). Among the fishes studied, it is found that catfishes mainly require protein rich feeds than other fishes such as herbivorous and omnivores, hence the variation in Hb is observed.

Hematocrit (Ht)

The maximum Ht was observed in both catfishes followed by exotic and Indian major carps (Fig.1D). However, within the Indian major carps, Ht for catla was lower compared to other two species and in exotic carps; Ht values for common carp was significantly lower than other two species. Whereas both catfishes showed similar levels of Ht, which is significantly higher than all other group of fishes studied. In the present study, high Ht values were observed in catfishes and low levels of Ht found in common carp and catla. The high correlation between Ht and Hb levels observed in the study could be due to the erythrocyte numbers (Sandstrom 1989).

Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC)

The maximum MCV was observed for mrigal, common carp and silver carp, followed by catfishes, rohu and grass carp and lowest MCV was observed in catla (Fig. 2A). However, MCH was significantly higher in common carp compared to other fishes, followed by mrigal, silver carp, catla, rohu, grass carp and catfishes. Among the Indian major carps and exotic carps, mrigal and common carps showed maximum MCH levels respectively (Fig. 2B). In all the species studied, common carp showed maximum MCH levels and minimum in catfishes. Whereas, the maximum MCHC was observed in catla, followed by rohu, common carp, grass carp, mrigal, silver carp and pangas and lowest in singhi (P<0.05) (Fig. 2C). Surprisingly, among all the species studied, either within the groups or between the groups, MCHC was significantly different. MCV value reflects the size of erythrocytes by expressing the volume occupied by a single cell and frequently used in assessments of osmoregulatory status. The present study showed significantly higher value of MCV in mrigal, common carp and silver carp which might reflect the lower Ht. However, there is no precise correlation noticed between Ht and MCV levels, hence it needs further studies. Among the parameters studied, MCHC is simply a hemoglobin content in a known volume of packed red cells (Schreck and Moyle, 1990) and significant difference found in this study could be due to the species variation.

Platelets

The maximum platelet counts were observed in rohu followed by catla, common carp, grass carp, pangas, singhi and mrigal and the lowest was observed in silver carp (Fig. 2D). Interestingly, within groups (Indian major carps and exotic carp) showed significant differences. However, in catfishes variations were discernible but there was no significant differences (P<0.05). The varied levels of platelets observed in the study could depend on the autoimmune system of the fish species. These factors are the clotting factors, which are transported in the plasma and help in clotting process called coagulation to seal a wound and prevent any loss of blood.

Erythrocyte Sedimentation Rate (ESR)

Maximum ESR was observed for catla among all the fish species studied, followed by silver carp, mrigal and catfishes. The minimum was found in rohu, common carp and grass carp (Fig. 3A). There was no appropriate trend observed for ESR between the groups (P<0.05). The present study revealed a significant variation in ESR and also earlier reports revealed that ESR is affected by stress and could be used as a biomarker. The rate of erythrocyte sedimentation could be due to the fragility of erythrocytes and disruptions caused by stressors (Akinortimi, 2006).

Red cell distribution and width (RDW)

In the current study, the maximum RDW was found in catla followed by rohu, common carp, mrigal, grass carp, pangas and singhi and least was found in silver carp. The difference in RDW among the species studied could be due to the variation in red blood cell size and or red blood cell volume and these changes might depend on the composition as well as morphology of cells with change in shape, size and density of cells (Schreck and Moyle 1990).

Neutrophils

The maximum number of neutrophils was found in silver carp followed by catla, pangas, rohu, singhi and grass carp. The lowest number of neutrophils was found in mrigal and common carp (P<0.05, Fig. 3C). The variation in neutrophils was observed among and within the groups and these variations could be due to their feeding habit and habitat. According to the earlier report (Ainsworth *et al.*, 1991), increase in neutrophils was observed in circulating blood due to various stress factors and the present study was performed only to understand the baseline levels of neutrophils and this could be used as a stress responding parameter in future studies for any teleost.

Lymphocytes

Variation in lymphocyte counts was observed between and among the groups. However, there were no significant changes found (P<0.05; Fig. 3D). These function as immunocompetent cells, and their precise mechanism of action is not explored yet (Ellis 1977), and the present study was only focused to establish base line levels of lymphocytes in selected fishes.

The present and earlier research on haematological parameters suggest that these parameters could change either with fish species (Langston *et al.*, 2002), age (Svetina *et al.*, 2002) or sexual maturity (Vazquez and Guerrero, 2007) and other factors viz., temperature (Langston *et al.*, 2002; Magill and Sayer, 2004), stress (Cnaani *et al.*, 2004), photoperiod (Leonardi and Klempau, 2003), feeding habits and nutritional status (Svetina *et al.,* 2002; Lim and Klesius, 2003). With the assistance of the blood parameters, the perturbation in the metabolism of the fishes could be assessed (Jamalzadeh *et al.,* 2009).

CONCLUSION

The results suggest that hematological parameters could be conveniently employed as health-monitoring tools in fish culture practices. These parameters are also indicative of the habits of fishes, and could be used for confirming the maturity and any changes in the quality of waters. The assessment of hematological parameters in Indian major carps, exotic carps and select catfish might provide fundamental information for researchers and used as biomarkers associated with stressors or as an available tool to diagnose and monitor diseases in these fish species. However, for further advanced studies of hematological parameters including combination of morphological and quantitative methods is warranted. In summary, the results of our investigation provide a contribution to the knowledge of the hematological parameters of the commercially important fishes under the normal conditions. This research could also help to monitor the health status of the fishes and response to either feeding trials or any contaminant exposures in fishes.

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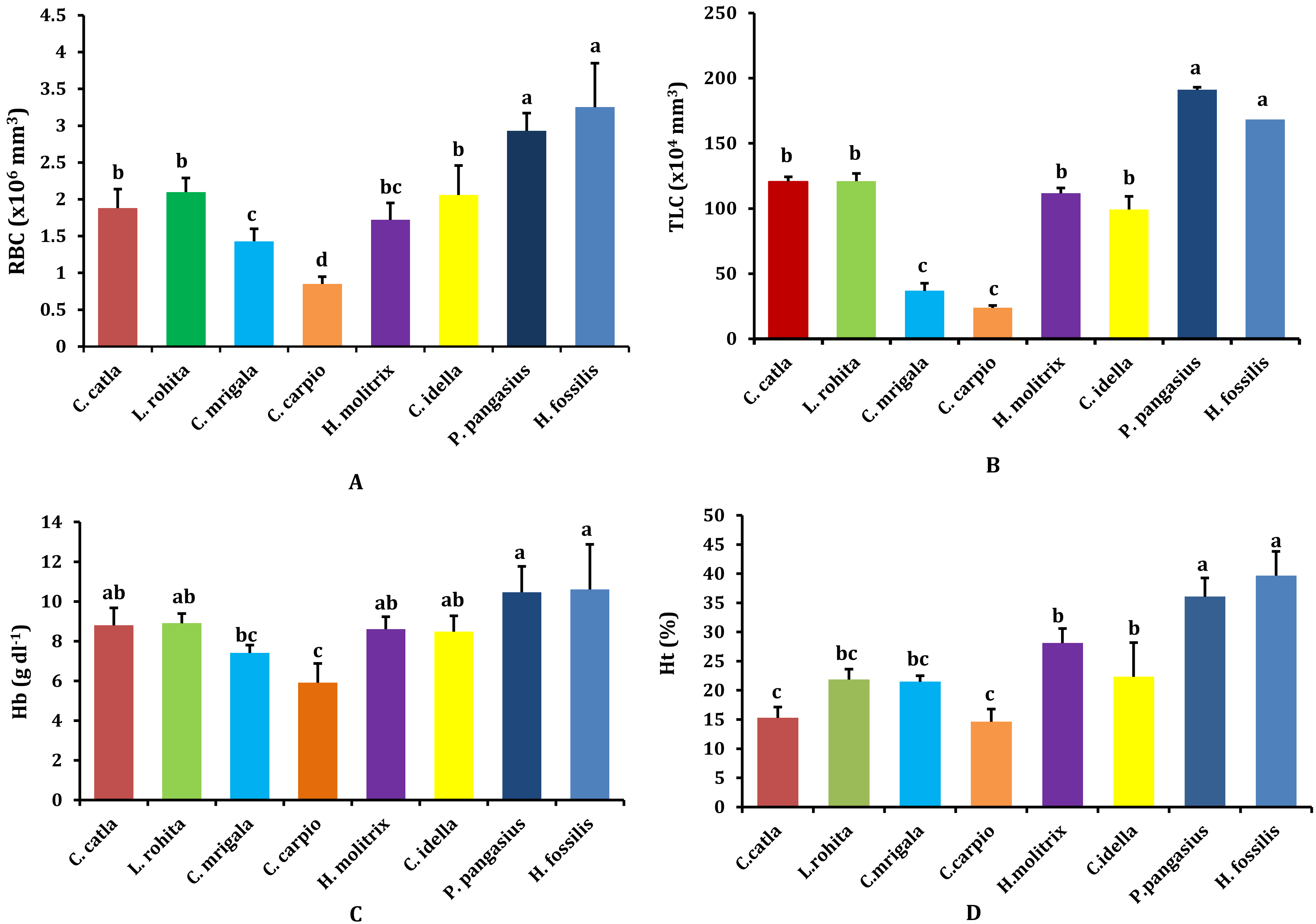

Fig. 1. Baseline levels of A) Total Erythrocyte Count, B) Total Leucocyte Count, C) Hemoglobin and D) Hematocrit of selected fish species. Values described by the same letter on the bars are not significantly different from each other.

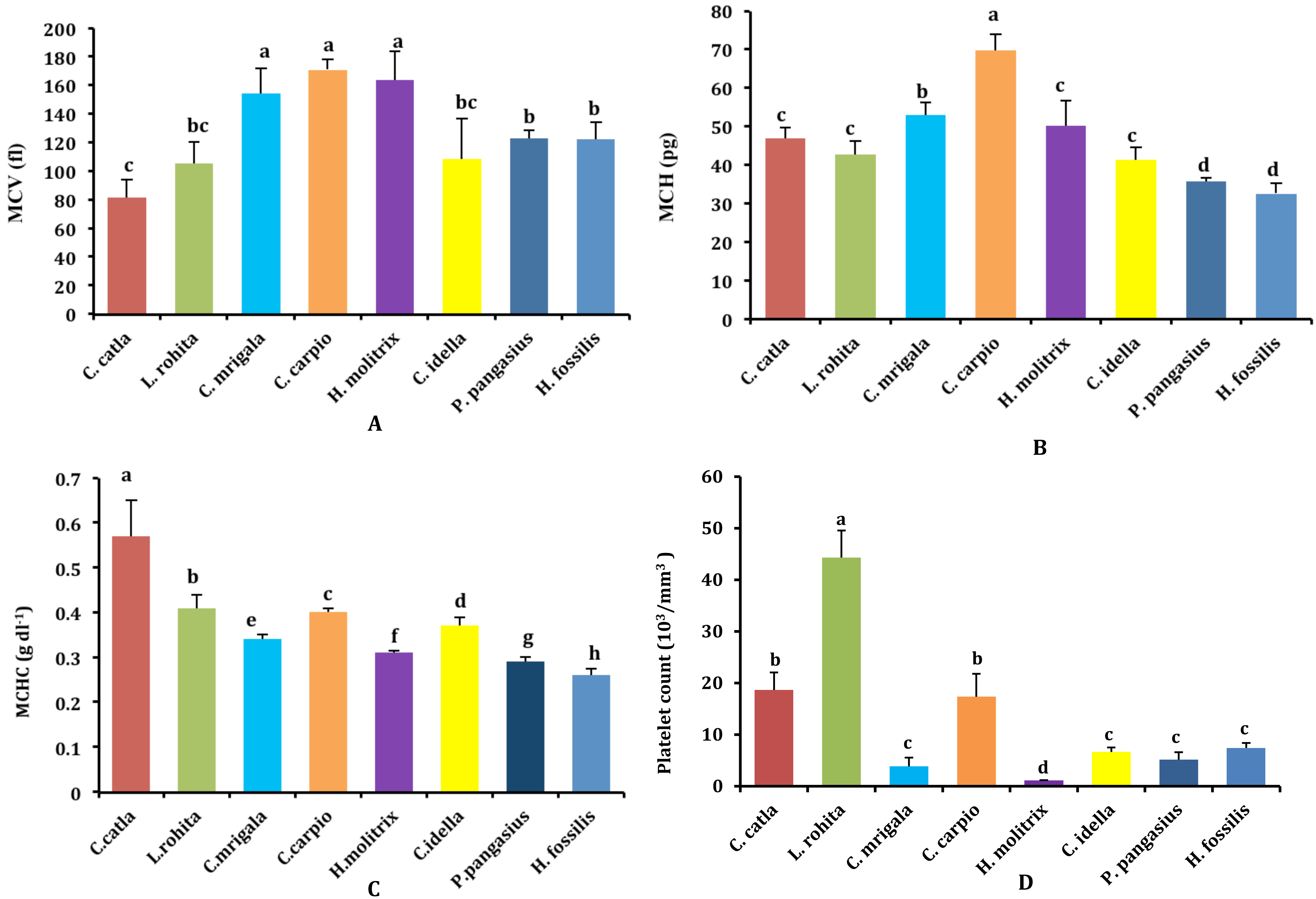

Fig. 2. Baseline levels of A) MCV, B) MCH, C) MCHC and D) Platelet counts of selected fish species. Values described by the same letter on the bars are not significantly different from each other.

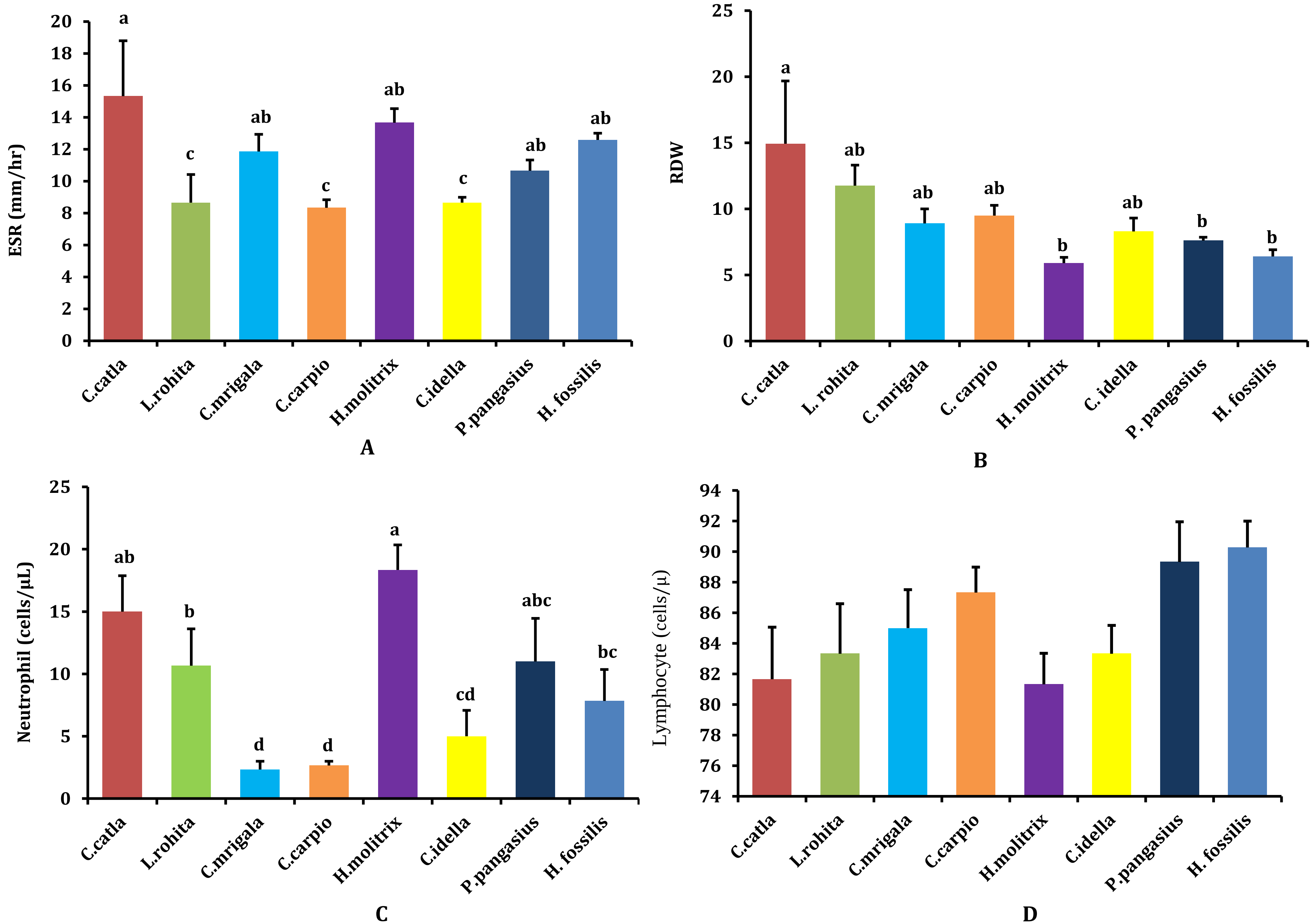

Fig. 3. Baseline levels of A) ESR, B) RDW, C) Neutrophil and D) Lymphocyte counts of selected fish species. Values described by the same letter on the bars are not significantly different from each other.