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META-ANALYSIS OF HAEMATOLOGICAL PARAMETERS OF NON-PARASITIZED AND PARASITIZED FISH *LIZA PARSIA* WITH THE ISOPOD PARASITE *NORILECA INDICA*

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ABSTRACT
Isopods belong to the subphylum of crustaceans and are a great threat to both marine and freshwater fisheries. By piercing the attached area, these isopods began to feed on the host's blood. The severity of the infestation causes morbidity and mortality in host fishes. The aim of the study was to compare the *Norileca indica* isopod infested fishes of *Liza parsia* with healthy *Liza parsia* fishes with reference to important haematological parameters. Infested fish had shown significant reduction of haematological parameters. The parasitized *L. parsia* haemoglobin content was reduced by 2.7 g/dL from the 35.4±0.36 g/dL of non-parasitized fish total Hgb. The red cell indices such as Haematocrit (Hct (%), Mean Corpuscular Volume (MCVfl), and Mean Corpuscular Haemoglobin (MCHpg) were reduced to 2.02%, 3.21, and 16 from 28.50 (P<0.05), 12.35 (P<0.05), and 154 (P<0.05) of non-parasitized fish haematological parameters, respectively. The total RBC was reduced from 2.7 million//L (P<0.05) of non-parasitized fish to 2.53 million//L (P<0.05). The WBC total cell count shows a significant difference between the two groups of fishes. The current study may give enlightenment on the host's health and the cause of mortality due to parasitic infestations.

Keywords: *Liza parsia, Isopods, Haematology, Morbidity, Mortality*

INTRODUCTION
Isopods parasitize numerous commercially important marine fish families such as Mugilidae; Jarocki, 1822, Atherinidae; Risso, 1827, Serranidae; Swainson, 1839, Carangidae; Rafinesque, 1815, Sciaenidae; Cuvier, 1829, Embiotocidae; Agassiz, 1853, Bothidae; Smitt, 1892, Clupeidae; Cuvier, 1817, Pleuronectidae; Cuvier, 1816, Scombridae; Rafinesque, 1815; and Haemulidae; Gill, 1885. These isopods attach to the host in various places, such as the body surface, gills, and buccal cavity. (Castro and Filho, 1946). These obligate parasites pierce the skin or organs and damage the dermis of subcutaneous regions; later they feed on the blood and mucous of the host. Infestations result in serious physical and physiological damage such as reduced growth, impaired reproduction, behavioural alterations and, in the most extreme cases it leads to death (Smit *et al*., 2014; Ravichandran *et al*., 2019). It causes morbidity and mortality in host animals, as well as significant financial losses in the fishing industry (Aneesh *et al*., 2017). Sahadevan *et al*., 2019 cited the cymothoid genus *Norileca* (Edwards, 1840); so far this genus reported with other three species such as: *N. borealis* (Javed and Yasmeen, 1999), *N. triangulata* (Rameshkumar and Ravichandran, 2015) (Richardson 1910), and *N. indica* (Edwards 1840), all of which infect the branchial cavity of pelagic marine fishes worldwide (Remeshkumar and Ravichandran. 2015; Serita *et al*., 2017). Recently, the species *Norileca indica* parasitizing *Rastrelliger kanagurta* host also

reported on the west coast of India (Rameshkumar *et al*., 2015). In the present contribution, the species *N. indica* infestation on *Liza parsia* also reported from the east coast of India. Edwards collected the *N. indica* in the branchial cavity of the big eye scad Selar crumenophthalmus in Pilobah, Great Nicobar Islands in 1840. The apparently damaged morphology of fishes caused by parasitic infestation, such as damage to fins, skin, and gills, would reduce their marketability and cause economic loss to fishermen and the fishing industry.

MATERIALS AND METHODS

Vellar Estuary

Healthy and parasitized marine teleost *Liza parsia* fishes were collected from the Vellar Estuary (Lat.11°29 N and Long 79°46 E), Parangipettai, of the southeast coast of Tamil Nadu, India.

Parasitological Examination

A parasitological examination was carried out for the identification of external parasites on the buccal cavity, skin, and gills of the hosts. Blood samples were collected from host fishes immediately for the haematological process, and subsequently, all isopod parasites were removed from hosts. Isolated parasites were stored in 70% ethanol and brought to the laboratory for identification based on literature data (Trilles, 1969; 1994, Trilles *et al*., 2011, 2013, Ravichandran *et al*., 2019) The sample size of the fish was twenty, and the weight of the fish was in the range of 151-200 g, with 10–12 cm, and 200–250 g, with 11–15 cm.

Haematological analysis

Blood samples were collected by caudal vein or direct heart puncture using a sterile plastic syringe (2.5 mL) and transferred into 2 different tubes, one (Miniplast 0.5 ml, LP Italiana Spa, Milano) containing EDTA (1.26 mg/ 0.6 mL) as an anticoagulant agent and the other without EDTA. The blood samples were collected in EDTA tubes were used for the determination of haematological profile. Heparin sodium (1%) was used as an anticoagulant (Svoboda *et al*., 2012). The collected blood samples were immediately subjected to hematological analysis. Evaluation of the haemogram involves the determination of the Red Blood Count (RBC), Haematocrit (Hct), Hemoglobin concentration (Hgb), White Blood Cell Count (WBC), Red cell indices include Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC).

The blood was diluted with appropriate diluting fluids, and RBC and WBC counts were determined using an improved Neubauer haemocytometer and calculated (Blaxhall and Daisley, 1973; Shah and Altindag, 2005). Repeated counts were made for each blood sample to minimise the error. Hematocrit was determined by micro-hematocrit centrifugation. Microcapillary tubes were filled, plugged with clay, and centrifuged at 19.000 g for 5 minutes. Measure the length of the columns containing packed red cells, and packed red cells plus supernatant. The calculation of hematocrit is as follows: (packed red cells/packed red cells plus supernatant)/100%. Haemoglobin concentration was measured with Hb test kit (Roach GmbH Mannheim, Germany) using the cyan methemoglobin method (Larsen and Snieszko 1961) and Sahili Haemoglobinometer. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) were calculated indirectly by the above direct parameter values using standard formulas.

Statistical Analysis

Differences in haematological parameters between the parasitized and non-parasitized fishes were statistically analyzed by the Unpaired T-test. The mean and standard error (SEM) were calculated for each parameter, and differences were considered to be significant at $p < 0.05$. All statistical analyses were carried out with the help of the statistical software Prism v. 4.00 (Graphpad Software Ldt., USA, 2003).

RESULTS

The parasitized and non-parasitized categories of the fish *Liza parsia's* haemotological parameters show a significant decrease. The parasitized *L. parsia* Hemoglobin was reduced 2.7 g/dL from the 35.4±0.36 g/Dl of non-parasitized fish total Hgb. Haematocrit (Hct (%)), Mean Corpuscular Volume (MCVfl), and Mean

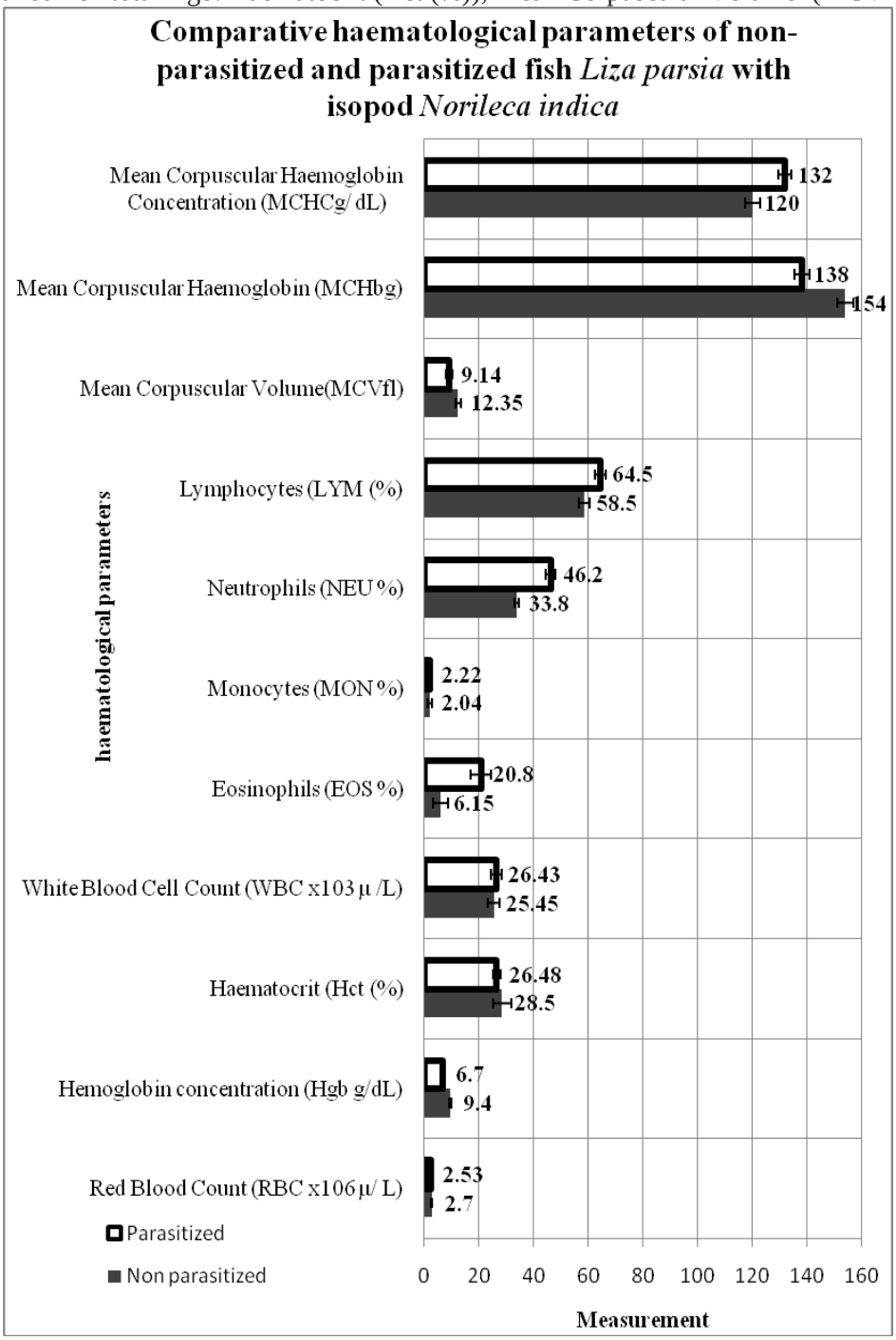

Figure 1: Comparison of hematological parameters of non-parasitized and parasitized fish *Liza parsia* with isopod *Norileca indica* (Values are mean ± SE (n=20), P< Value=0.05

Corpuscular Haemoglobin (MCHpg) were reduced to 2.02%, 3.21, and 16 from 28.50 (P<0.05), 12.35 (P<0.05), and 154 (P<0.05) of non-parasitized fish haematological parameters, respectively. The total RBC was reduced from 2.7 million//L (P<0.05) of non-parasitized fish to 2.53 million//L (P<0.05). The WBC total cell count showing significant difference between the two groups of fishes. The total WBC count was significantly increased (P<0.05) however the granulocyte eosinophil and agranulocyte lymphocyte were increased notably. The Total WBC decreased to 25.45±0.21 (P<0.05) from 26.43±0.20 (P<0.05). The Granulocytes of WBCs such as Eosinophils (EOS %) and Neutrophils (NEU %) were significantly increased to 20.8 ±2.25, 46.20±1.20 from 6.15 ±5.93 (P<0.05), 33.80±1.27 (P<0.05) respectively. In spite of this, the agranulocytes cells viz; Monocytes (MON %) and Lymphocytes (LYM %) increased moderately when compared with granulocytes. The Monocyte and Lymphocytes were increased to 2.22±1.50 (P<0.05) from 2.04±1.00 (P<0.05) and 64.50 ±2.00 (P<0.05) from 58.50 ±5.02 (P<0.05) respectively.

DISCUSSION

In this present investigation the blood parameters like total RBC count, Hgb, Hct, WBC, EOS, MON, NEU, LYM, MCVfl, and red *cell indices* such as MCH, MCV and MCHC were compared in parasitized and non-parasitized fishes. Haematological parameters and their diagnosis is the best tool for the assessment of the health status of fishes (Javed *et al*., 2016). It is the best indicator of the host-parasite relationship impact. It reveals the reduced level of blood parameters mentioned above except for EOS, LYM and *red cell indices* are the useful to study the etiology of anemia (Sarma, 1990). These parameters were highly correlated with the parasite, population, and attachment place (Jerônimo *et al*., 2014; Soberon and Mathews, 2014). *Ceratothoa oestroides* isopod infested bogue fishes have been showed the hemorrhage and significant reduction of RBC, Ht, MCH, MCHC, and Hb along with serum biochemical characters which agreed with our results (Özdemir *et al*., 2016). The *Nandus nandus* (Ham.) parasitized by metacercariae of digenetic trematode *Clinostomum complanatum* also reveals the reduced Hgb, RBC and increased granulocytes namely EOS and LYM. (Shah *et al*., 2005, Kaur *et al*., 2012); The RBC was not significantly reduced however the RBC and Hb are highly associated with hypochromic microcytic anemia (Sachar and Raina, 2014). *Red cell indices* (HT, MCV and MCHC) showed reduced trend in *Leporinus macrocephalus* which naturally infected by *Goezia leporini* (Nematoda) and certain parasites of *Mystus gulio* which dwelling in the south-east coast of India, (Natarajan and Felix, 1987). Most prevalence and intensity of digenea and crustacean parasites infestation with *Eustrongylides exises* fish showed reduced level of red cell indices and other WBC sub sets counts which correlated with our results (Fallah *et al*., 2015). The parasitized bogue fish shows a significant decrease in the number of RBC, Hb, Hct, MCH and MCHC values in comparison with the not parasitized fish (Özdemir *et al*., 2016). In our study no erythrocyte swelling reported however swelling has been reported, but *Tilapia zillii* infested with the isopod *Nerocila bivittata* showed contrary increase in MCV and MCHC (Elgendy *et al*., 2018). The haematocrit and total blood volume have reduced in the captive *Hemigymnus melapterus* infected with the cultured juvenile parasitic isopods; this may cause by the Gnathia isopod feeding of blood on fish blood (Jones and Grutter, 2005). The reduction of MCH, MCHC is directly correlated to reduced synthesis of Hgb in parasitized fish (Soivio and Nikinmaa, 1981). The Complete Blood Cell (CBC) is the best analytical tool and data for the indicator of fish health status with reference to the water quality, parasitic infection condition, nutrition quantity and quality assessment, and drug efficacy. Physiological parameters like lymph and blood are the important liquid tissue systems in circulatory system animals which give a genuine prognosis to interpret the health status of the host when it is affected by parasites. Hemoglobin and hematocrit levels are highly related to healthy red blood cell parameters; the 3% decrease RBC could be due to *N. indica* infection over *L. parsia*. Parasites primarily rely on body fluids and tissues for survival. They obtain it from the host's blood, lymph, and other bio-substances through sucking. The dog fish *Scyliorhinus canicula* showed significant reduction in

hematological parameters such as leukocytes in parasitized fish with high parasitic load (Francesca *et al*., 2022).This same result were observed in different fish families such as Anostomidae, Pimelodidae, Erythrinidae, Ictaluride, Characidae, Cichli-dae, Mugilidae and Cyprinidae (Tavares-Dias *et al*., 2007). Rodlet cells are exclusive to fishes and represent the eosinophilic granulocytes that are aggregated on epithelia of gills or the intestinal tract and mesothelial and endothelial tissues. These rodlet cells matured proliferate more to parasitic stimuli and cause inflammation (Ola and Oystein, 2006).

CONCLUSION

This present study is the first meta-analysis of hematological parameters of fish *L. parsia* and infested relationship of *N. indica* in the natural marine pelagic habitat. Finally, the infested fish do not show healthy muscle and weight due to high level of blood loss and body fluid due to parasitic infestation.

Compliance with Ethical Standards

In this study, no live animals were used. According to the IUCN, *Liza parsia* is the least concerning fish in India, there is no such permission required for this type of commercial fish used to conduct estimations or assays. All the parasitized and non-parasitized fish were procured a couple of hours after the fishing boat and initiated the above estimations and assays in very fresh condition without rigour mortis.

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Conflict of Interest

No conflict of Interest with anybody else

Authors Contribution statement

The concept of the study was obtained from the first author's doctoral degree. The Author collected the sample directly from the Vellar fishing area, and biochemical analysis of the sample has been done in CAS in Parangipettai, Annamalai University research Lab. Chart and table have been done in MS office package

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