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Hematological changes in *Eleginops maclovinus* during an experimental *Caligus rogercresseyi* infestation

Alterações hematológicas em *Eleginops maclovinus* durante uma infestação experimental com *Caligus rogercresseyi*

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Abstract

Eleginops maclovinus has been an important fishery resource in Chile since 1957. *Caligus rogercresseyi* is one of the most prevalent ectoparasite species found on *E. maclovinus*. Hematocrit, hemoglobin level, red blood cell count (RBC), white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and differential white blood cell count were determined before and after an experimental infestation with *C. rogercresseyi*. We found significant differences in the hemoglobin level, WBC, MCV, MCH, MCHC, hematocrit level and RBC between infested and uninfested fish. Furthermore correlations between number of *C. rogercresseyi* with hematocrit, MCHC, neutrophil, eosinophil and lymphocyte counts were found. Hematological reference ranges of *E. maclovinus* in captivity conditions were also established.

Keywords: Blood parameters, erythrocytes, leukocytes.

Resumo

Eleginops maclovinus tem sido um importante recurso pesqueiro no Chile desde 1957. *Caligus rogercresseyi* é uma das espécies ectoparasitas mais comumente encontradas em *E. maclovinus*. Hematócrito, nível de hemoglobina, contagem de eritrócitos, contagem de leucócitos, volume corpuscular médio (VCM), hemoglobina corpuscular média (HCM), concentração de hemoglobina corpuscular média (CHCM) e contagem diferencial de leucócitos foram determinadas antes e após uma infestação experimental com *C. rogercresseyi*. Foram encontradas diferenças significativas no nível de hemoglobina, leucócitos, VCM, HCM, CHCM, hematócrito e eritrócitos entre peixes infestados e não infestados. Além disso, foram encontradas correlações entre o número de *C. rogercresseyi* com hematócrito, CHCM, neutrófilos, eosinófilos e linfócitos. Foram estabelecidos intervalos de referência para *E. maclovinus* em condições de cativeiro.

Palavras-chave: Parâmetros sanguíneos, eritrócitos, leucócitos.

Introduction

The Chilean rock cod, also known as robalo (*Eleginops maclovinus*), is a common fish in littoral zones, estuaries and rivers (under tidal effects) of southern South America, including the Falkland islands (PEQUEÑO et al., 2010). Although this species is traditionally considered an opportunistic benthic omnivore, recent research indicates that juveniles and adults are opportunistic carnivores capable of feeding in both marine and freshwater environments (EASTMAN; LANNOO, 2008). *E. maclovinus* is a coastal species generally associated with estuarine systems, has been exploited as a fishery resource in Chile since 1957 (GACITÚA et al., 2008) and also has a great potential to be farmed (VALENZUELA et al., 1999).

Caligus species is found in continental waters on native fish (*E. maclovinus*) as well as on a cultured species, *Oncorhynchus mykiss,* from the south of Chile (CARVAJAL et al., 1998). *C. rogercresseyi* (Copepoda: Caligidae) is one of the most prevalent ectoparasite species found on *E. maclovinus* (CARVAJAL et al., 2001; HENRIQUEZ et al., 2011). Parasites negatively influence host fitness, and in response, hosts develop anti-parasitic defenses, for example, a functional immune system, to reduce the fitness cost induced by parasitism (SHELDON; VERHULST, 1996). Sea louse cause a local inflammatory response and blood loss at the site of attachment but do not appear to suppress host defense mechanisms during the early stages of infestation (TAVARES-DIAS et al., 2007;

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WAGNER et al., 2008). Parasitic infestations can influence blood parameters, such as anemia and white cells count unbalances in other host fishes (HORTON; OKAMURA, 2003; MARTINS et al., 2004; SILVA-SOUZA et al., 2000; TAVARES-DIAS et al., 1999, 2002, 2007; WAGNER et al., 2008).

The aim of this work is to study the hematological changes in *E. maclovinus* caused by *C. rogercresseyi*-induced experimental infestation.

Materials and Methods

The experimental hatchery of Fundación Chile in Quillaipe (Región de los Lagos, Chile) has *E. maclovinus* stocks that were grown since hatching. In this study 160 *E. maclovinus* (weight = 488 ± 138 g, length = 32.9 ± 2.89 cm) belonging to this group were used. They have always been fed with an Ewos transfer commercial diet. Prior to the experiment fish were acclimatized to the experimental tank for two weeks. During acclimatization and experimental periods, fish were maintained in a 3 m^3 tank with a density of 26.03 kg m^{-3}. The maximum density for *E. maclovinus* farming is 30 kg m^{-3} (Fundación Chile unpublished results). The water temperature during the experiment was 12.7 ± 0.8 °C. Fish were infested with copepodid stage *C. rogercresseyi* parasites (150 copepodid/fish), and infestation was maintained up to 4 weeks post-infestation (wpi).

Blood samples were taken from the caudal vessel (WEISS; WARDROP, 2010) one week pre (basal) and 2, 3 and 4 weeks post infection (wpi) (n=40 per each sample time). The fish were sedated using benzocaine (50 mg L^{-1}) prior to bleeding. Once the fishes were sampled (n=40) they were placed in a different tank to avoid fish reuse; afterwards water level corrections were performed to keep a constant density during the experiment.

Blood samples were stored in heparinized Eppendorf tubes that were transported at 4 °C and analyzed within 12 h of collection at the Catholic University of Temuco. The hematocrit, hemoglobin level, red blood cell count (RBC), white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and differential white cell count were determined using standard methods (BAIN et al., 2011; TODD et al., 1979).

Statistical analysis was performed using a one-way ANOVA and the Bonferroni post-test to make comparisons between the groups infested and uninfested. Associations of parasite count with neutrophil, eosinophil, and lymphocyte counts; hematocrit; and MCHC levels were tested using the Spearman correlation (*rs*) and linear regression. Statistical significance between the groups was indicated by $p<0.05$. All statistical analyses were performed using GraphPad Prism v5.0 (GraphPad Software Inc, CA, USA).

Results

The *C. rogercresseyi* infestation site was the skin of *E. maclovinus*. The mean parasite number ± standard deviation per fish at 2nd wpi was 6.73 ± 5.71, at 3rd wpi was 4.14 ± 3.58 and at the end of experiment was 1.11 ± 1.06.

The mean values and ranges of the blood parameters and white blood cells counts for uninfested and *C. rogercresseyi*-infested *E. maclovinus* are shown in Table 1. Table 2 describes the hematological reference ranges in *E. maclovinus* under cultivation.

Our results showed significant differences between infested and uninfested fish in hemoglobin levels (p=0.0104; df=154; F=3.883), WBC counts (p=<0.0001; df=154; F=37.12), MCV (p=<0.0001; df=154; F=8.3), MCH (p=<0.0001; df=154; F=21), MCHC (p=<0.0001; df=154; F=25), hematocrit (p=<0.0001; df=154; F=9.935) and RBC counts (p=<0.0001; df=154; F=14.47). No significant differences were observed in lymphocyte, neutrophil, eosinophil, basophil and monocyte counts.

Infested fish show an increase in WBC counts up to the 3rd wpi and an increase in eosinophils and basophils in the second week, with a subsequent decline to the 4th wpi, a decrease in neutrophils over all 4 wpi, an increase in lymphocytes at 4 wpi, and a decrease in monocytes up to the 3rd wpi followed by a slight increase in the last wpi. Red blood cell parameters (hemoglobin, MCV, MCH and MCHC) increased during infestation; however, RBC count and hematocrit decreases during infestation. The most common leukocytes in uninfested group (basal) were lymphocytes, followed by neutrophils, monocyte, basophils and a small perceptual of eosinophils. However, in the infested group, the most abundant cell types were lymphocytes, neutrophils and eosinophils (Table 1).

Table 1. Hematological parameters of uninfested and *Caligus rogercresseyi*-infested *Eleginops maclovinus*. Mean ± standard deviation (SD). Different lowercase letters indicate significant differences.

Parameters	Basal	2nd wpi	3rd wpi	4th wpi
	(n=40)	(n=40)	(n=40)	(n=40)
RBC (x10^6/μL)	1.15 ± 0.25 a	0.90 ± 0.15 b	0.92 ± 0.17 b	0.89 ± 0.22 b
Hematocrit (%)	51.2 ± 7.71 a	49.5 ± 2.86 ac	47.8 ± 3.17 bc	45.3 ± 4.22 b
Hemoglobin (g dL^{-1})	7.97 ± 1.16 a	8.51 ± 0.83 a	8.3 ± 0.99 a	8.71 ± 0.92 b
MCV (fL)	458 ± 76 a	564 ± 95.4 b	531 ± 78.2 b	540 ± 143 b
MCH (pg)	72.1 ± 16.4 a	96 ± 12.5 bc	92.1 ± 14.7 b	104 ± 26.6 c
MCHC (g dL^{-1})	15.8 ± 2.42 a	17.2 ± 1.62 b	17.4 ± 1.6 b	19.3 ± 1.06 c
WBC (x10^3/μL)	1.47 ± 0.24 a	1.85 ± 0.28 b	2.25 ± 0.53 c	2.19 ± 0.34 cd
Lymphocytes (%)	75.3 ± 7.84 a	82.6 ± 5.86 b	86.8 ± 6.53 b	87.3 ± 4.84 b
Neutrophils (%)	12.5 ± 4.93 a	11 ± 4.76 a	9.63 ± 4.35 a	9.03 ± 5.55 a
Eosinophils (%)	1.0 ± 0 a	7.31 ± 5.48 a	4.23 ± 3.44 a	4.45 ± 3.32 a
Monocytes (%)	10.9 ± 5.72 a	1.88 ± 1.43 b	1.0 ± 0 b	2.87 ± 2.64 b
Basophils (%)	2.63 ± 2.64 a	3 ± 1.41 a	1.0 ± 0 a	1.0 ± 0 a

weeks post infection (wpi).

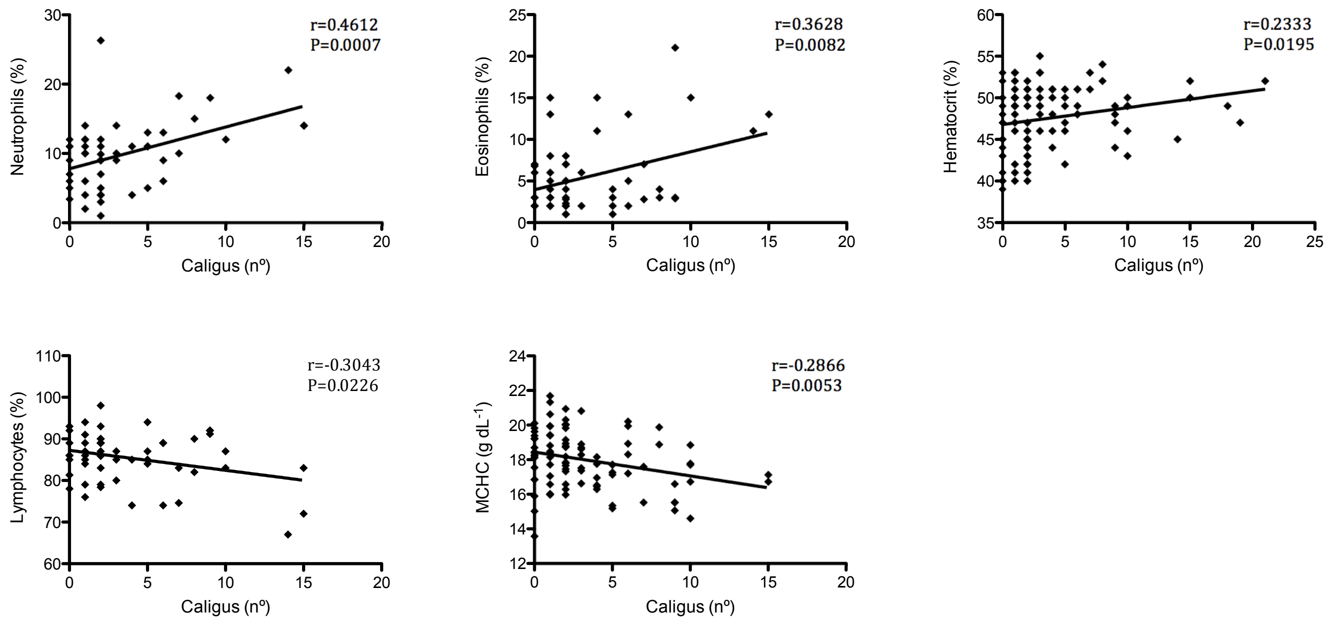

Figure 1. Correlations between *Caligus rogercresseyi* number and hematological parameters of *Eleginops maclovinus*.

Table 2. Reference ranges of hematological parameters in *Eleginops maclovinus* (n=40) under farm conditions. Lower (25^{th}) and upper percentiles (75^{th}).

Parameters	Uninfested Range (25^{th}-75^{th})
RBC (x10^6/µL)	0.90-1.30
Hematocrit (%)	38.0-57.8
Hemoglobin (g dL^{-1})	7.25-8.84
MCV (fL)	414-491
MCH (pg)	58.7-80.4
MCHC (g dL^{-1})	14.2-17.0
WBC (x10^3/µL)	1.26-1.65
Lymphocytes (%)	68.0-82.9
Neutrophils (%)	9.0-15.0
Eosinophils (%)	1.0-1.0
Monocytes (%)	6.85-14.0
Basophils (%)	1.0-3.0

Our results demonstrate that the number of *C. rogercresseyi* in the body was correlated with the neutrophil counts (r=0.4612; p=0.0007), eosinophil counts (r=0.3628; p=0.0082), hematocrit levels (r=0.2333; p=0.0195), MCHC (r=–0.2866; p=0.053) and lymphocyte counts (r=–0.3043; p=0.0226). No correlation was observed between the *C. rogercresseyi* number and red blood cell counts, hemoglobin levels, MVC, MCH, and white blood cell, monocyte and basophil counts (Figure 1).

Discussion

Studies performed by Valenzuela et al. (1999) and Valenzuela et al. (2003) have characterized blood cells from wild fish native to Chile. To date there are no studies of the differences in the hematologic parameters between uninfested and infested captive robalo *E. maclovinus* with *C. rogercresseyi*.

The infestation site by *C. rogercresseyi* was the skin of *E. maclovinus*, this is consistent with Nolan et al. (2000), Costello (2006), Hamilton-West et al. (2012) and Venmathi Maran et al. (2012).

When compared our infested fish with data from Valenzuela et al. (1999), no differences was found in lymphocyte, eosinophil and monocyte counts, this suggests that the study by Valenzuela et al. (1999) was made with wild infested *E. maclovinus*. On the other hand, we found statistically significant differences in lymphocyte (p<0.0001, df=185, F=23.1), eosinophil (p<0.0001, df=185, F=15.1) and monocyte counts (p<0.0001, df=185, F=78.9) when compared Valenzuela et al. (1999) results with our uninfested (basal) fish group. The values of uninfested group are proposed as reference ranges for uninfested *E. maclovinus* maintained in captivity (Table 2). Blood parameters, although not regularly used in fish medicine, can provide substantial diagnostic information once reference intervals are established.

In both the infested and basal (uninfested) groups, we found that lymphocytes are the most abundant leukocytes in the peripheral blood; however, eosinophils were scarce in the uninfested fishes but abundant in the infested ones, which correlates with other studies in fishes (ALVAREZ-PELLITERO, 2008; EZE EVELYN et al., 2012; PIERRARD et al., 2012; RUANE et al., 2000; SILVA-SOUZA et al., 2000). Neutrophils decrease steadily during the 4 weeks of infestation, which is consistent with a study by Tavares-Dias et al. (2008). This decrease indicates that neutrophils are often the first leukocytes to migrate to the site of parasite infestation (TAVARES-DIAS et al., 2008; WITESKA et al., 2010). WBC counts increase significantly in infested *E. maclovinus* at 2, 3 and 4 wpi, correlating with the studies by Jori and Mohamad (2008) and Harikrishnan et al. (2012). The RBC counts decrease during the *C. rogercresseyi* infestation, which is similar to studies of other parasites (HORTON; OKAMURA, 2003; MARTINS et al., 2004; TAVARES-DIAS et al., 2002). Hematocrit and electrolyte levels are secondary indicators of chronic stress, indicating the osmoregulatory status of the fish (TAVARES-DIAS et al., 2007). The hematocrit reduction in infested fish can be explained by osmoregulatory failure as a result of exposed lesions (GRIMNES; JAKOBSEN, 1996) or blood ingestion (JONES; GRUTTER, 2005).

Higher values of MCV, MCH and MCHC was found in infested fish when compared to uninfested fish, this is probably due to the continued development in RBC which increase in size and hemoglobin content with time (WEISS; WARDROP, 2010).

Positive correlations found between *C. rogercresseyi* number and neutrophil count, could be explained by the hyperplastic and inflammatory responses involving rich neutrophil infiltration as response to *Lepeophtheirus salmonis* observed in salmonids (WOO; BUCHMANN, 2011). The positive correlation with eosinophils counts is due to that eosinophils are frequently involved in parasite disease responses (ALVAREZ-PELLITERO, 2008; THRALL et al., 2012). So, hematocrit positive correlation with parasite number is consistent with osmoregulatory failure as a result of exposed lesions or blood ingestion (GRIMNES; JAKOBSEN, 1996; JONES; GRUTTER, 2005). Negative correlation between number of *C. rogercresseyi* and MCHC is probably an indication of RBC swelling and/or a decrease in hemoglobin synthesis (KUMAR; RAMULU, 2013). The negative correlation of lymphocytes count with *C. rogercresseyi* is explained by the migration of lymphocytes into peripheral tissues such as skin and gills which has been reported in Atlantic salmon infected with adult *L. salmonis* and other salmonids (RUANE et al., 2000).

In conclusion, this study has demonstrated differences between *Caligus rogercresseyi*-infected and uninfected *E. maclovinus* in the hematological parameters RBC and WBC counts, hemoglobin, MCV, MCH, MCHC and hematocrit. The WBC count increased during infection and were mainly composed by lymphocytes, neutrophils, and eosinophils. Neutrophils, eosinophils and hematocrit increased with parasites number, confirming the importance of these leukocytes in the response against *C. rogercresseyi*. The Chilean rock cod, also known as robalo

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