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RESEARCH

Differential blood cells associated with parasitism in the wild puffer fish *Lagocephalus laevigatus* (Tetraodontiformes) of the Campeche Coast, southern Mexico

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Abstract

In aquaculture conditions, severe parasitic infections cause negative impacts on fish health and economic losses. The parasite load has been associated with anemia, which reduces the number of erythrocytes in fish. Therefore, the evaluation of hematological parameters as a feasible tool for diagnosing and monitoring fish health allows us to determine the indirect effect of parasites on the health status of fish. Our aim was to evaluate changes in the blood cell parameters of *Lagocephalus laevigatus* associated with parasitism. A total of 99 puffer fish were collected from the coast of Seybaplaya, Campeche. Each fish had 20 µl of peripheral blood drawn, and blood smears were performed in triplicate. The smears were stained with Giemsa stain, and a quantitative analysis of blood cells (erythrocytes, leukocytes, and monocytes) was obtained with an optical microscope at 100 ×. The parasites recovered from each fish were fixed and identified, and the infection parameters were calculated. Through generalized additive model analysis (GAMLSS), we observed that the infection intensity of puffer fish influenced changes in hematological parameters, principally in erythrocytes, neutrophils, thrombocytes, the total fish length, and the condition factor of the fish. In conclusion, this is the first study that provides baseline data on the hematological parameter variations in uninfected and infected *L. laevigatus*, the tropical wild puffer fish, as well as the possible effects on fish health. It is necessary to establish reference hematological patterns in wild populations for diagnosis and timely management with emphasis on aquaculture fish.

Keywords Hematological parameters · Parasitic infections · Puffer fish · *Lagocephalus laevigatus* · Aquaculture

Introduction

Parasitic infections are thought to be a serious underrecognized health problem in wild aquatic organisms because infection levels can lead to disease and sometimes mortality, resulting in a serious impact on ecological interactions in ecosystems. For economically important fish, these factors can affect food safety with economic and public health consequences (Giari et al. 2022). Likewise, parasitic infections can alter the health conditions of wild and farmed fish (Guidelli et al. 2011). Physiological alterations, such as anemia, in host fish are characterized by a reduced hemoglobin concentration, hematocrit level, and erythrocyte number (Witeska 2015), and this is reflected in the condition factor of fish (Ryberg et al. 2020). Hematological parameters change in infected fish due to factors such as the consumption of blood by a parasite as it feeds (Clauss et al. 2008) and activation of host defense cells (Alvarez-Pellitero 2008). For example, hematocrit reduction observed in *Hemigymnus melapterus* (Labridae) Bloch, 1791 is related to the presence of ectoparasites such as isopods (*Gnathia* sp.), which take periodic large blood meals before leaving the host to mate (Jones and Grutter 2005). Additionally, the increase in eosinophil counts in the zebrafish *Danio rerio* (Cyprinidae) Hamilton, 1822

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infected with the nematode *Pseudocapillaria tomentosa* suggests an inflammatory response to the parasitic infection (Balla et al. 2010). Changes in hematological parameters have also been observed in *Cyprinus carpio* infected with *Philometra pellucida* (Kumar 2023).

Hematological disorders in fish occur under natural and aquaculture conditions, and they can be diagnosed in a timely manner by the measurement of blood cell parameters. One suitable, noninvasive, and feasible diagnostic method used to help define alterations and test health status in fish is hematologic analysis (Clauss et al. 2008; Fazio 2019; Witeska et al. 2022). Although some authors consider this method to be controversial because the counts in some species seem to have a wide range of variation (Blaxhall 1972; Blaxhall and Daisley 1973), it is commonly used to assess the welfare of some economically important aquaculture species and in veterinary practice and scientific research (Clauss et al. 2008; Fazio 2019; Witeska et al. 2022). However, while the total amount of available information on fish diversity is high, references addressing fish health and their hematological disorders are limited. Therefore, it is often difficult to identify and determine possible alterations in the hematological parameters of wild fish with aquaculture potential, especially those affected by parasitic infections during management.

The morphology and defense system of the puffer fish (Tetraodontiformes, Tetraodontidae) include inflation and the presence of tetrodotoxin (neurotoxin), which is highly toxic to humans (Isbister et al. 2002). Despite the toxic risk to humans, puffer fish are biologically and economically important in many regions, especially in Asia, where the meat is considered a delicacy (NMFS 1989, Stump et al. 2018).

Puffer fish are also considered an economically important resource in the southern Gulf of México and Pacific regions, and those with the potential for aquaculture (Chávez-Sánchez et al. 2008) include *S. annulatus* from the Pacific and *L. laevigatus* and *Sphoeroides* spp. from the Gulf of México, particularly from the Campeche Coast. Notwithstanding this potential, knowledge of the hematological parameters of puffer fish is scarce, including a lack of information about the blood cell parameter imbalance associated with parasitic infections.

Fajer-Ávila et al. (2011) suggested that the infection levels with the copepod *Pseudochondrcanthus diceraus* can be associated with changes in the hematological parameters of the puffer fish *Sphoeroides annulatus* from the Mexican Pacific. Usually, fish harbor more than one species of parasite (Bush et al. 1997). Therefore, it is important to consider and evaluate these interactions together with the variability in hematological parameters to establish an information base. This allows for precise diagnoses, treatment or preventive measures in wild and cultured fishes.

Consequently, the aim of this study was to assess the parasite levels in infected *Lagocephalus laevigatus*, a wild tropical puffer fish of the coastal zone of Campeche, Mexico, and the associated changes in blood parameters.

Materials and methods

A total of 99 *L. laevigatus* were collected from Seybaplaya, Campeche (19°42.580'N, 90°44.155'W) during the months of November 2020 (27 fish), January 2021 (30 fish), April 2021 (12 fish) and September 2021 (30 fish) using a line and hook. After capturing a fish, 20 µl of blood was collected from the caudal vein using a hypodermic syringe without anticoagulant and applied to glass slides to perform blood smears in triplicate for at least 3,000 erythrocytes per fish; at the same time, leukocyte and thrombocyte numbers were determined in the fields (Murray 1983; Schultz et al. 1993, Ek-Huchim et al. 2022). The smears were air dried and fixed in absolute methanol for 10 min (Al-Sabti and Metcalfe 1995). Subsequently, for blood collection, the fish were first euthanized through a brain puncture and kept on ice for a maximum of 8 h (Vidal- Martínez et al. 1998; Ek-Huchim et al. 2022). The smears and fish were transported to the Laboratory of Aquatic Parasitology of EPOMEX (Instituto de Ecología, Pesquerías y Oceanografía del Golfo de México), Universidad Autónoma de Campeche (UAC). In the laboratory, the total length (TL) in cm and weight (W) in g of each fish were measured. Next, the fish were examined under a compound stereomicroscope (Leica EZ4) to determine the presence of any parasites on the body surface. Then, all organs were removed (gills, intestines, liver, bladder, and kidney), placed in bowls with 4% formaldehyde solution, and examined under a stereomicroscope.

The methods used for fixing, staining, and clearing the parasite specimens for identification were as follows: Trematoda were stained with Gomori's trichrome stain and Nematoda, Copepoda and Crustacea were cleared with glycerin at different concentrations (1:10, 1:5, 1:2) (Vidal-Martínez et al. 2002; May-Tec et al. 2022). The parasites were identified according to the keys of Anderson (2004), Palm (2004), Jones and Bray (2005), Ho and Lin (2004) and Lin and Ho (2006) using an Olympus microscope (DM 2500). The prevalence, mean abundance, infection intensity and mean infection intensity were determined based on methods proposed by Bush et al. (1997).

To assess the condition factor of wild *L. laevigatus*, the length-weight relationship (LWR) of the puffer fish was estimated using the Equation $W = \alpha SL^{\beta}$ (Ricker 1975), where W = total weight (g), SL = standard length (cm), α = ordinate to the origin, and β = the intercept, also known as the allometry coefficient. Based on the estimation of the LWR, the condition factor of the puffer fish was calculated according to the formula proposed by Bagenal and Tesch (1978) ($K = 100 * W/SL\beta$).

Morphological and morphometric analysis of blood cells

The blood smears were stained with Giemsa 10% for 12 min. After being air dried, they were observed under a microscope (Olympus BX51) at 100 × magnification using immersion oil (Baršiene et al. 2004). Descriptive data were obtained by observing the shape of the cell and its nucleus, the lobulation of the nucleus, and the color of the cytoplasm, as well as the color and density of the cytoplasmic granules. Quantitative cell analysis included measurements of erythrocytes, leukocytes (lymphocytes, neutrophils, eosinophils, basophils, and monocytes), and thrombocytes, also called platelets. The parameters measured involved the long axis (LA) of each blood cell. At least 3,000 erythrocytes were analyzed per fish and were used for differential (percent) determination of leukocytes. The morphological identification of blood cells was based on the work of Conroy and Conroy (2007) and Fajer-Ávila et al. (2011).

Statistical analysis

Kruskal–Wallis (K) analysis was applied to the data to determine the existence of any meaningful difference in the mean intensity of the parasite species and size of the fish.

The statistical relationship between the infection intensity (dependent variable) and hematological parameters (independent variables) in *L. laevigatus* was determined using generalized additive models of location, scale, and shape (GAMLSS). These models considered the statistical distribution of each variable (e.g., exponential, Gaussian, linear, distribution, etc.) (Rigby and Stasinopoulos 2005). The multicollinearity of the independent variables was evaluated through a variance inflation factor (VIF < 4) index and Spearman's correlation (rs > 0.70) (R Core Team 2018). The selection of the best statistical distribution was made using the Akaike information criterion (AIC) (Burnham and Anderson 2002), where the best-fitted distribution had the lowest AIC value. The best statistical model was selected using the stepGAIC function in the GAMLSS package (Rigby and Stasinopoulos 2005) in R programming language (R Core Team 2018). In addition, the power of the fit of each model was evaluated through the explained deviance (ED), expressed as a percentage (Rigby and Stasinopoulos 2005).

Results

Fish status and parasitism

We collected 99 specimens of *L. laevigatus* with TLs from 18.9−48.5 cm (29.18 ± 6.53) and Ws from 87−1562 g (449.50 ± 305.73). The highest frequency of size was observed in the class of 27−36.9 cm (Fig. 1a). The LWR values showed a negative allometric growth of *L. laevigatus* (β = 2.97), and the condition factor values of *L. laevigatus* oscillated between 0.65 and 2.09 (1.02 ± 0.23).

A total of 554 individual parasite specimens were found on *L. laevigatus*, which included 19 parasite species, of which 16 spp. were endoparasites and 3 spp. were ectoparasites, with a range of infection intensities of 0−53 individual parasites per fish. The cestode (Tetraphyllidea) had the highest prevalence at 41.17%, followed by the copepod *Caligus haemulonis* (Caligidae), with a prevalence of 38.23% (Table 1). The number of parasites infecting *L. laevigatus* were categorized into four levels: uninfected (0 parasites), 1 (1−5 parasites), 2 (6−14 parasites), and 3 (15−53 parasites).

The highest infection intensity was 53 parasites per fish, and the puffer fish were 38.9 ± 7.31 cm long, and weighed 861.54 ± 400.23 g. The length of uninfected fishes was 28.58 ± 5.84 cm, and the weight was 380.75 ± 215.82 g. The infection intensity values were significantly different among different sizes of *L. laevigatus* (K-W $_{(2,99)}$ = 17.31, P< 0.05) (Fig. 1b).

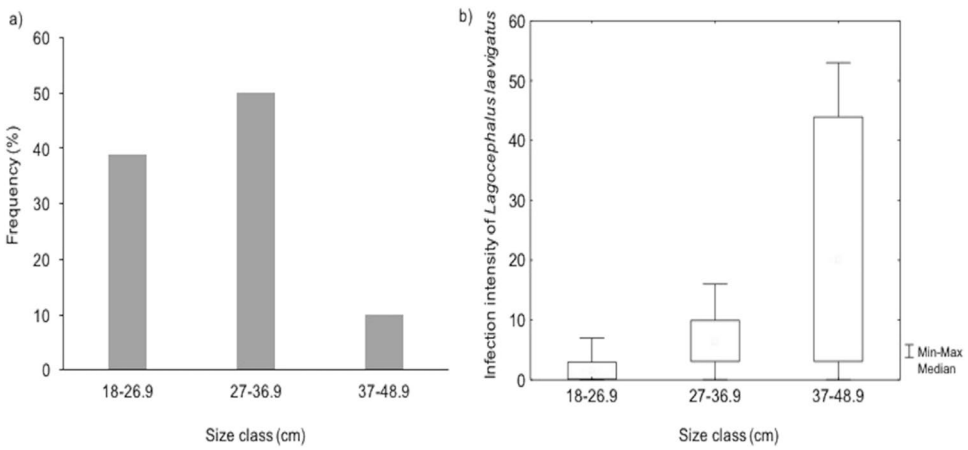

Fig. 1 The size of the puffer fish *Lagocephalus laevigatus* from Seybaplaya, Campeche **a** Frequency of size class of *L. laevigatus*, **b** Infection intensity and size class of *L. laevigatus*

Table 1 Infection parameters of parasites on *Lagocephalus laevigatus* from Seybaplaya, Campeche, Mexico

Parasite species	P (%)	MA ± SD	MI ± SD
Trematoda			
Helicometrina nimia[A] (Linton, 1910)	17.64	0.26 ± 0.67	1.5 ± 0.67
Opecoelidae gen. sp.[A]	1.47	0.01 ± 0.0	1 ± 0.0
*Clinostomum*sp.[A]	1.47	0.01 ± 0.0	1 ± 0.0
Diplostomidae gen. sp.[A]	5.88	0.10 ± 1.5	0.17 ± 1.5
Haplosplanchidae gen. sp.[A]	1.47	0.01 ± 0.0	1 ± 0.0
Stephanostomum sp1[L]	2.94	0.04 ± 0.70	1.15 ± 0.70
Didymozoidae gen sp.[L]	1.47	0.02 ± 0.0	2 ± 0.0
Cestoda			
Tetraphyllidea gen sp.[L]	41.17	1.39± 6.14	3.39 ± 6.14
Trypanorhyncha gen sp.[L]	1.47	0.01 ± 0.0	1 ± 0.0
Nematoda			
Terranova sp.[L]	11.76	0.25 ± 1.24	2.12 ± 1.24
Cucullanus sp.[A]	2.94	0.04 ± 0.70	1.15 ± 0.70
Porrocaecum sp.[L]	1.47	0.01 ± 0.0	1 ± 0.0
*Contracecum*sp. type 2.[L]	1.47	0.01 ± 0.0	1 ± 0.0
Hysterothylacium reliquens[L] (Norris & Overstreet, 1975) Deardorff & Overstreet, 1981	2.94	0.05 ± 1.41	2 ± 1.41
Nematoda gen. sp[L]	10.29	2.22 ± 21.53	2.57 ± 21.53
Acantocephala			
Echinorhynchida gen. sp.[A]	5.88	0.13 ± 1.89	2.25 ± 1.89
Crustacea			
Argulus sp.[L]	7.35	0.10 + 0.89	1.4 + 0.89
Copepoda			
Caligus haemulonis[A](Krøyer, 1863)	38.23	2.97+ 7.17	7.76 + 7.17
Taeniacanthus lagocephali[A] (Pearse, 1952)	29.41	0.44 + 1	1.5 + 1

P: Prevalence (%), MA±SD: Mean abundance ± standard deviation, MI ± SD: Mean intensity ± standard deviation. [A]=Adult parasite, [L]=Larval parasite

Blood cell morphology

The blood cell morphologies of the puffer fish (*L. laevigatus*) are presented in Figure 2. Erythrocytes with a blue cytoplasm were round with compact and oval nuclei and centrally positioned, and their LA was 7.97 ± 0.75 µm in diameter (Fig. 2a). The differential count of erythrocytes was 97.46% ± 2.38% (90.50%–99.96%).

Five leukocyte types were identified in the peripheral blood of puffer fish. The lymphocytes were smaller in size

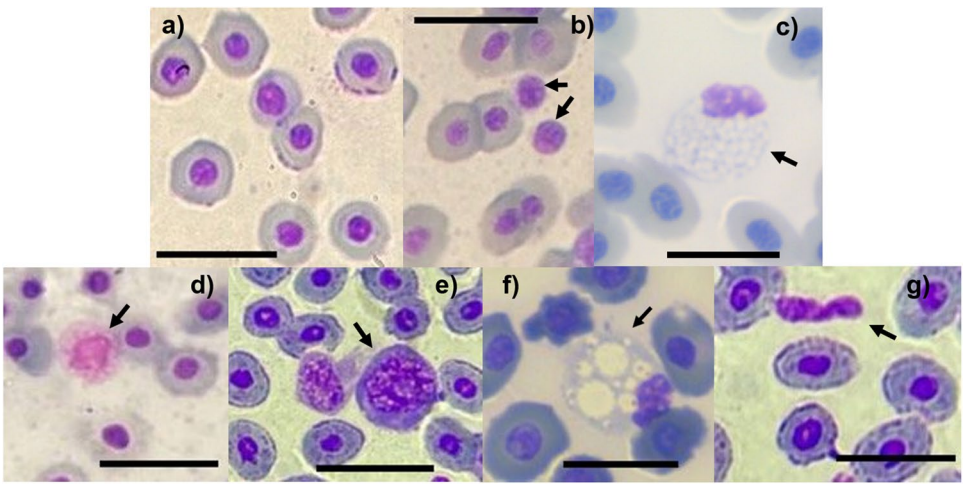

Fig. 2 The blood cell morphology of the pufferfish *Lagocephalus laevigatus* from Seybaplaya, Campeche. The arrow points to the indicated cell type **a** Erythrocytes, **b** Lymphocytes, **c** Neutrophils, **d** Eosinophils, **e** Basophils, **f** Monocytes, **g** Thrombocytes, (bar = 10 µm)

and round with a 5 ± 4.57 µm diameter, and a round, purple nucleus with dense chromatin that occupied almost all of the cytoplasm was observed (Fig. 2b). The differential count was 83% ± 16.74% (7.89%–100%).

Neutrophils were the most common granulocytes found in the peripheral blood of puffer fish. Unlike the other leukocytes, the neutrophils were ovoid to rounded with a diameter of 10.5 ± 1.50 µm; the nucleus was stained purple; and the cytoplasm had a pale blue color (Fig. 2c). The differential count of neutrophils was 6.68% ± 9.16% (0%–42.85%). Eosinophils were ovoid to rounded with a diameter of 8.59 ± 0.74 µm, and unsegmented peripheral purple nuclei, and a cytoplasm with numerous reddish-orange granules were observed (Fig. 2d). The differential count of eosinophils was 3.97% ± 12.37% (0–92.10). The last type of granulocyte was basophils; these cells were ovoid with a diameter of 9.04 ± 0.92 µm, and the cytoplasm was filled with deep blue–purple stained granules with an eccentric nucleus (Fig. 2e). The differential basophil percentage was 4.24% ± 7.05% (0%− 36%).

Monocytes were recognized by their ovoid to rounded shape with a diameter of 11.05 ± 0.82 µm. Their cytoplasm was stained clear blue with vacuoles and a bluish-purple eccentric nucleus (Fig. 2f). The differential count of monocytes was 1.95% ± 4.33% (0% − 20%).

Thrombocytes were observed as purple fragments in the blood (Fig. 2g) and commonly formed small clusters. The differential thrombocyte counts was 0.96% ± 0.71% (0%−3.15%).

Association of blood cell parameters and infection intensity of parasites on *Lagocephalus laevigatus*

Infection intensities from 0 to 53 parasites per fish resulted in a significant reduction in the percentage of erythrocytes and an increase in leukocytes. There was a significant difference between the infection intensity and erythrocyte count ($K\text{-}W_{3,68} = 30.26$; $P < 0.05$), leucocyte count ($K\text{-}W_{3,82} = 34.26$; $P < 0.05$), lymphocyte count ($K\text{-}W_{3,68} = 27.04$; $P < 0.05$), neutrophil count ($K\text{-}W_{3,68} = 14.83$; $P < 0.05$), eosinophil count ($K\text{-}W_{3,68} = 17.73$; $P < 0.05$), monocyte count ($K\text{-}W_{3,68} = 9.4$; $P < 0.05$), and thrombocyte count ($K\text{-}W_{3,68} = 23.30$; $P < 0.05$); however, no significant difference was observed for the basophil count.

The statistical associations between the hematological parameters changed, and the infective parasite intensity of *L. laevigatus* was assessed through three generalized additive models for location scale and shape (GAMLSS) (Table 2). The first GAMLSS model showed that the number of individual parasites per fish was correlated with the number of erythrocytes, neutrophils, and thrombocytes, TL, and the condition factor, and this correlation had a 60% overall contribution to the explained deviance (ED). The second GAMLSS model showed that the number of individual ectoparasites per fish was correlated with the number of erythrocytes and thrombocytes, the total length, and the condition factor, which had a 57% contribution to the ED (Table 2). The last model showed that the number of individual endoparasites per fish was correlated with the neutrophil count and the total length, which had a 46% contribution to the ED (Table 2).

Discussion

The host-parasite interaction triggers the activation of different blood cells as lines of defense (Holzer et al. 2021). The variability in fish blood cell parameters (i.e., variation in the cell type composition) is an efficient and sensitive tool for assessing the health of wild and cultured fishes, providing key insights regarding their physiological status reflected by the condition factor as well the damage by parasitic infections (Omar et al. 2021). Our findings indicate that variation in the blood cell parameters of the wild puffer fish *L. laevigatus* of the Campeche Coast was associated with infective parasite levels, i.e., blood cell parameters of uninfected and infected puffer fish were significantly different. However, authors such as Marcogliese (2002), Lafferty et al. (2006), and Dunne et al. (2013) have mentioned that parasitized wild fish represent a healthy ecosystem in which a proper host-parasite interaction of the food web occurs. High levels of infection can cause important diseases in both wild (Barzegar and Jalali 2009) and farmed fish (Jithila and Prasadan 2019; Buchmann 2022; Nguyen et al. 2021). Our

Table 2 The general additive models (GAMLSS) for the number of parasites and hematological parameters of *Lagocephalus laevigatus* from Seybaplaya, Campeche, Mexico

Model	df	GD	ED (%)	AIC	FD
Np ~ cs (Neutrophils) + cs (Thrombocytes) + cs (Erythrocytes) + cs (K) + cs (TL) *	21	431	60	473	PO
Ect.Np ~ cs (Erythrocytes) + cs (Thrombocytes) + cs (K) + cs (TL) *	17	272	57	306	PO
End.Np ~ cs (Neutrophils) + cs (TL) *	10	431	46	451	ZIP

AIC= Akaike information criterion, cs= cubic spline, df= degrees of freedom, ED= explained deviance in percentage, Ect.Np= number of ectoparasites, End. Np= number of endoparasites, FD= family distribution, GD= global deviance, K= condition factor of *L. laevigatus*, Np= number of parasites, PO= Poisson distribution, TL= total lenght of *L. laevigatus*, ZIP= zero-inflated Poisson *$p < 0.001$

work is the first study on the hematological values of *L. laevigatus* and their parasites, and this study revealed that there is considerable variation in blood cell parameters associated with infective parasite levels in a natural environment.

The larval cestode Tetraphyllidea and the copepod *Caligus haemulonis* were the dominant species infecting *L. laevigatus*. These endoparasites (cestode) and ectoparasites (copepod) might have biological and morphological traits relevant to the variability in blood parameters of the puffer fish. The cestode Tetraphyllidea is an absorber parasite, and the copepod *C. haemulonis* is characterized as a depredator (Vidal-Martínez et al. 2022). Both groups of parasites damage the tissues of their hosts, and they are associated with hematological changes (Sharp et al. 1992; Jones and Grutter 2005). For example, the anemia and inflammatory response of fish have been observed in hosts infected with copepods such as *Caligus rogercresseyi* and cestodes *Cyathocephalus truncates* and *Bothriocephalus acheilognathi*, respectively (Sopinska 1985; Martins et al. 2004; Alvarez-Pellitero et al. 2008; Peña-Rehbein et al. 2013). Therefore, the present study suggests that the high infection intensity of cestodes and copepods could most likely affect the health of *L. laevigatus*.

In this puffer fish, the erythrocyte characteristics (oval nuclei and centrally positioned) were consistent with teleost fish (Palíková et al. 1999; Rough et al. 2005; Pavlidis et al. 2007; Fajer-Ávila et al. 2011; Grant 2015). However, the erythrocyte size of *L. laevigatus* (6–10 µm) differed from that suggested by Fänge (1992) for teleost fish with a tendency to live in brackish waters (8–15 µm) and as reported by Fajer-Ávila et al. (2011) for the puffer fish *Sphoeroides annulatus* (7.61 ± 0.66 µm) from the Pacific Coast. Larsson et al. (1976) and Glazova (1977) noted that erythrocytes are slightly smaller in active species than in nonactive species due to oxygen demands. The dissimilarity in the erythrocyte size of *S. annulatus* and *L. laevigatus* is likely due to their habitat. *S. annulatus* prefers shallow waters (<1–60 m) and represents the category of puffer fish that are slow swimmers, while *L. laevigatus* is an active species in marine waters (10–180 m) (Nader et al. 2012).

Although variation in leukocyte classification between fish species was observed (Barber et al. 1981; Tierney et al. 2004), the white cells of *L. laevigatus* were consistent with the general classification suggested by Fänge (1992) for teleost fishes. In *L. laevigatus*, lymphocytes were the most abundant, accounting for 83% of leukocytes. These results support early studies by Fänge (1992) and Fajer-Ávila et al. (2011) concerning teleost fish (50%–80%) and *S. annulatus* (84%–86%), respectively. The lymphocytes of *L. laevigatus* (6–10 µm) are apparently larger than those of *S. annulatus* (5.06 ± 0.52 µm) and teleost fish (4.5–8.2 µm). The lymphocytes constituted the basis of the immune response of fish, and some authors mentioned a size variation (small, medium, and large lymphocytes) between species of fish (Rough et al. 2005). Granulocytes were the second most frequently observed (14%) leukocyte of *L. laevigatus*, measuring between 7 and 14 µm. These values differ from the low values for *S. annulatus* (12.34%), with cell measurements of 8.38 ± 0.80 µm, while high values (18%) and cell measurements of 9–12 µm have been reported for teleost fish. This inconsistency can be attributed to the fact that granulocytes are active cells with properties such as phagocytosis, so they can vary in size between different groups of fish (Buchmann 2022). In granulocytic cells, neutrophils were the most abundant (6.6%), followed by basophils (4.2%) and eosinophils (3.9%). In contrast, neutrophils account for less than 5% of leukocytes, while basophils and eosinophils are scarce cells in teleost fish (Fänge 1992). Neutrophils are involved in host protection against pathogens and the inflammatory process (Havixbeck and Barreda 2015; Buchmann 2022). Therefore, the presence and abundance of neutrophils in *L. laevigatus* suggest an active host immune response most likely attributed to the presence of parasitic infections.

In this research, the additive model analysis showed that the infective parasite intensity (ectoparasites and endoparasites) of *L. laevigatus* was associated with concurrent changes in erythrocyte, neutrophil, and thrombocyte values, such as with the length and condition factor (k) of this puffer fish. Several studies have demonstrated that parasitism causes impaired condition factors in fish (Guidelli et al. 2011; Özer et al. 2016; Ryberg et al. 2020). This result suggested that the infection levels of wild puffer fish most likely affected their health status and metabolism due to energy consumption to maintain fish homeostasis (Sadauskas-Henrique et al. 2011; Ryberg et al. 2020). Several studies have recorded alterations in erythrocyte values in a wide variety of fish species infected with parasites (Martins et al. 2004; Montero et al. 2004; Belo et al. 2013; Restiannasab et al. 2014; Souza-Rocha et al. 2018). These alterations are related to anemia and attributed to the consumption of blood as a form of parasite feeding; in some cases, those with anemia cannot recover fully, leading to a fatal outcome (Witeska 2015). However, with the variation in erythrocytes found in *L. laevigatus*, we cannot indicate the presence of anemia because of the lack of parameters of normal erythrocytes established in this puffer fish. Then, the tendency of infected fish to have decreased erythrocytes is a determining factor in the physiology of the organism.

Likewise, the variation in neutrophil values with respect to infective parasite intensity can be attributed to the fish immune response and inflammation processes, as suggested by Buchmann (2022). It is known that parasites are host stressors and that neutrophil numbers are normally lower in uninfected fish than in infected fish (Havixbeck and Barreda 2015; Souza-Rocha et al. 2018). The variation in thrombocyte values is associated with the activation of the

coagulation system of fish, and this can be influenced by infective parasite damage. Studies have suggested that this damage occurs during ectoparasite infection, as monogeneans and copepods cause mechanical damage, resulting in hemorrhages (Ferguson 2006; Tavares-Dias and Oliveira 2009; Belo et al. 2013; Becker and Baldisserotto 2014; Fernandes and Moron 2014). Therefore, the condition factor, together with hematological parameters, is an important variable for monitoring the health status and infective parasite intensity of *L. laevigatus*.

Regarding ectoparasite infection, the second model suggested that their variation influenced the number of erythrocytes and thrombocytes in puffer fish (Table 2). The copepod *Caligus haemulonis* was the second dominant species of *L. laevigatus*. It is known that copepods damage the tissues of their hosts; thus the increase in thrombocytes can be attributed to avoiding blood loss. These results are observed in some species of fish infected with copepods (Paperna and Zwerner 1982; Roubal 1989; Ferguson 2006). However, Fajer-Ávila et al. (2011) did not find an association between the blood parameters and the infection intensity of the copepod *Pseudochondracanthus diceraus* on *S. annulatus*. This difference can most likely be attributed to infection levels (i.e., $n = 17$ parasites on *S. annulatus* vs. $n = 53$ parasites in *L. laevigatus*). High levels of infective parasites promote a stress reaction in the host, with the presence of physiological responses (Urbinati et al. 2015; Holzer et al. 2021). Our results indicate that the health conditions of wild puffer fish can be especially affected by ectoparasites. This is in accordance with Guidelli (2011), who found a negative effect of the abundance of ectoparasites on the condition factor of wild fish. Therefore, our results suggest that erythrocyte variation is due to the loss of blood, and thrombocyte variability is a function of blood clot formation that occurs when the vasculature is damaged by these parasites.

Our third model suggested that the number of endoparasites is related to neutrophil variability. The aggregation of parasites can promote neutrophil cell variation as an immune response. We observed an aggregation of the larval cestode Tetraphyllidea and nematode larvae in the intestine of *L. laevigatus*. Buchmann (2022) indicated that this cell line of defense is stimulated by parasites such as monogeneans, cestodes, trematodes, and ciliates. Several studies mention that helminthic infection causes inflammation, impaired nutrient absorption, damage to the intestinal tract, and, in several cases, host mortality (Ogawa et al. 2007; Scholz et al. 2012; Wise et al. 2013; Jithila and Prasadan 2019; Nguyen et al. 2021). We consider that neutrophils are an immunological response of puffer fish to this parasitic aggregation in the intestine of *L. laevigatus*.

The size of *L. laevigatus* was an important factor for parasite infection in the three models due to larger puffer fish having the highest infection intensities. The influence of host size on infection intensity variability is a pattern documented in marine and freshwater fish (Poulin 2000; Luque and Alves 2001; Oniye et al. 2004; Barzegar and Jalali 2009). Some authors have mentioned that larger fish provide more internal and external areas for parasite establishment, and the highest infection intensity occurs because they eat more parasitized small hosts and have a longer contact time for the transmission of parasites (Rolbiecki 2006; Poulin 2000; May-Tec et al. 2020).

In conclusion, our results indicate that parasitism is reflected in the variation in the blood cells of *L. laevigatus*, which is related to the health status of the fish (condition factor). In addition, the present results contribute to the baseline knowledge of blood cell parameters in this puffer fish and could be useful for further fish hematology research, such as establishing diagnostic applications in the wild and in aquaculture. To establish a range of blood cell parameters in this species, it is essential to carry out bioassays comparing healthy fish and infected fish under controlled conditions. This interaction promotes understanding an identifying the variability in natural infections, such as the possible effects of some ecological disturbances (contaminants) on fish health in natural populations.

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Author contributions All authors conceived the study. Ana Luisa May-Tec: conceptualization, methodology, formal analyses, visualization, writing (reviewing and editing). Juan Pablo Ek-Huchim: methodology and review. Abril Rodríguez-González: review, and editing. Edgar Fernando Mendoza-Franco: visualization, resources, review, and editing. The authors read and approved the final manuscript.

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Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethical approval All applicable institutional, national, and international guidelines for the care of animals were followed.

Consent to participate, consent for publication All the authors read and approved the final version of the manuscript. All persons involved in the study gave their oral consent for publication.

Competing interests The authors declare no competing interests.

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