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Short Communication

Blood parameters and parasitic load in *Sardinops sagax* (Jenyns, 1842) from Todos Santos Bay, Baja California, Mexico

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ABSTRACT. Some studies have characterized and evaluated the parasitic load of the Pacific sardine. However, there are few studies related to its physiology and parasites load. This study aimed to evaluate the blood parameters and the abundance of helminth parasites of sardines obtained from two samples from Todos Santos Bay during autumn 2013. The digenea *Myosaccium ecaude* parasite was the only species registered. Sardines had a prevalence of 28%, an average abundance of 25 and an intensity of 7 parasites. There were no statistically significant differences ($P > 0.05$) in most of the analyzed blood parameters (red blood cell count, hematocrit, leukocyte cellular differentiation, mean corpuscular volume, total protein, glucose, alanine aminotransferase, osmotic pressure, Na, K, and pH) of fish with and without parasites, except in white blood cell count and in Ca concentration that showed a marked decrease. The relationship between parasite abundance and blood parameters, based on the results of this study, it is not clear due to the low parasitic load.

Keywords: blood parameters, *Sardinops sagax, Myosaccium ecaude*, helminth parasites, Mexican Pacific coast.

The geographic distribution of the Pacific sardine, *Sardinops sagax*, extends from Alaska to northwestern Mexico (Félix-Uraga *et al*., 2005). Sardines have a significant role in marine ecosystems as a primary consumer and as prey in the food chain, which allows the transmission of parasites (Baldwin *et al.*, 2012). Previous studies have described the parasite load of the Pacific sardine, *S. sagax* as: Digenea (*Myosaccium ecaude, Parahemiurus merus, P. noblei, Lecithaster gibbosus* and *Bucephalus* sp.), cestodes (Tetraphyllidea) and nematodes larvae (*Anisakis* spp., *Hysterothylacium* sp., *Contracecum* spp. and *Pseudoterranova* sp.) (Love & Moser, 1983; Baldwin *et al.*, 2011; Sánchez-Serrano & Cáceres-Martínez, 2017). Some studies show that the prevalence and intensity of helminths larvae infections may vary in the same area and the same host species over time (Baldwin *et al*., 2011; George-Nascimento & Moscoso, 2013).

Alterations of environmental factors and the presence of parasites can result in changes in physiological and behavioral responses of the fish due to the stress (Martins *et al*., 2004; Del Río-Zaragoza *et al*., 2008, 2011). In sardine there are few works related to these topics, Martínez-Porchas *et al*. (2011) addressed the physiological status of the *S. sagax* by evaluating the effect of temperature changes on the physiological condition of the sardine through blood parameters analysis. The authors highlighted that the sardines showed susceptibility to high temperatures (≥23°C), specifically during the summer cycle (symmetric and asymmetric) which caused an increase in the concentration of glucose, cortisol, alanine and aspartate aminotransferase.

Sardine is a species of high commercial value for human consumption and bluefin tuna farming in Baja California (Del Moral-Simanek *et al*., 2010). The

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Fishing Secretary of the State of Baja California reported that more than 38,000 t of sardine were captured (more than 3.89 million dollars) in 2013, while in 2017 the catch tripled, representing more than 9 million dollars (SEPESCABC, 2013, 2017). The presence of parasites in fish is a topic of great ecological interest and for aquaculture. This study aimed to evaluate the relationship between blood parameters and the abundance of parasites of the Pacific sardine from Todos Santos Bay, Baja California, Mexico, to relate its health condition with its parasite load.

Sardines (n = 110, survival of 82%, after 24 h of arrival at the laboratory) were obtained during autumn 2013 (two samples) from a live bait sport fishing business that captures them in Todos Santos Bay (31°40'-31°56'N, 116°36'-116°50'W). Sardines were transported to the laboratory in plastic bags (12-15 sardines per bag) with 50 L of seawater at 20.2 ± 1.4°C, and oxygen supply >7 mg L^{-1}. Fish were released in 7 m^3 tanks connected to a recirculation system and held for ten days to enable its recovery from the stress to transport. The next day after arriving at the laboratory, a commercial trout diet (protein 42%, 1 mm granule size, Silvercup ®) was offered *ad libitum*. Every day, leftover food and faeces were removed from the tanks. The average water temperature in the tank was 18 ± 2.25°C; dissolved oxygen 7.66 ± 0.69 mg L^{-1} and salinity 33.08 ± 0.29.

Injuries can occur during capture and transport of fish. Sardines with some injury were not considered for blood sampling, because, the blood parameters could be altered. Fish were not fed for 24 h before blood samples were taken. Sardines (n = 40) were carefully handled to minimize stress. They were anesthetized with MS-222 (150 mg L^{-1}), and after 3 min, blood samples were collected from the caudal vein using 1 mL non-anticoagulant syringes. The blood sample was immediately placed into two tubes: The first one had K_2EDTA (BD Microtainer, Franklin Lakes, NJ, USA) to prevent coagulation. This tube was used for total red blood cell count (RBC) and total white blood cell count (WBC). The second tube without anticoagulant was used for the rest of the analysis, *i.e.*, blood smears, and hematocrit (Hct). The leftover blood of the last tube was centrifuged for 10 min, and serum was stored in a -80°C ultra freezer for further analysis of blood chemistry.

Natt & Herrick (1952) method was used for total RBC and WBC-plus-thrombocyte count. Leukocyte cellular differentiation was observed through blood smears treated with a Hemacolor stain (Harleco, Mexico) in an optical microscope (Zeiss, PrimoStar). From each blood smear, 100 cells were counted and reported as percentages. The presence of thrombocyte was also recorded. The cells were measured with a ruler added in the microscope. Hct was measured with the blood sample in a heparinized 2/3 filled capillary tube (Corning). The tube was sealed and placed for 10 min in a micro-hematocrit centrifuge (Clay Adams®) and using a hematocrit reader (Clay Adams®) the packed cell was measured and denoted as a percentage. The mean corpuscular volume (MCV) was calculated with the standard formula using Hct and RBC data.

Serum samples were centrifuged for 10 min at 500 g and then analyzed for blood chemistry determined by colorimetric methods adapted to the microplate device (Beckman Coulter, AD200, ADLD software ver. 1.6). Total protein was analyzed with the Bradford method (Amresco, USA) using bovine serum albumin as standard. Glucose concentration was analyzed with glucose-oxidase method (Kit Randox ® Gluc-Pap). Alanine aminotransferase (ALT) was determined using a kit (Randox ®). Readings of the absorbance were performed in a microplate reader at 595 nm for total protein and 546 nm for glucose and ALT. The osmotic pressure was measured with an osmometer (Wescor vapor 5520) using 10 mL of serum. Electrolyte and pH were measured in an automated Na/K/Ca/pH analyzer (Medica Corporation's EasyLyte® Calcium).

After blood sampling, weight and fork length for each specimen were measured, and Fulton´s condition factor (K) was calculated as $K = 100 \times W\ L^{-3}$. All the fish were examined under the stereomicroscope (Zeiss, Stemi 2000-C) to locate parasites. The extraction and processing (fixation, staining, and assembly) of helminths was carried out with the technique described by Pérez-Ponce de León *et al*. (1999). The parasites found were identified taxonomically to the lowest possible level (trematodes; Montgomery, 1957). The amount of each parasite was described by prevalence, mean abundance and mean intensity of infection according to Bush *et al.* (1997). Blood parameter data were performed by triplicate for each fish. Results are expressed as means ± standard deviation (SD). Normality tests and t-tests ($\alpha = 0.05$) were applied to all data, using Sigma Stat 4.0 software (Systat Software, Inc., San Jose, CA, USA). All percentage data were arcsine transformed before statistical comparisons. Sardines had an average weight of 91.46 ± 10.76 g and a standard length of 22 ± 1.19 cm. The condition factor of sardines with parasites (n = 10) and without parasites (n = 30) is not significantly different ($P > 0.05$), with an average value of 0.85 ± 0.06. The 247 parasite load of the sardines were represented only by *Myosaccium ecaude* (Trematoda: Digenea) and located in the esophagus region of the fish. The quantitative indicators of the parasite population were: prevalence of 28%, average abundance of 25 and an intensity of 7 parasites per fish.

Table 1. Blood parameters of Pacific sardine (*S. sagax*) with and without parasites Digenea (*M. ecaude)*. Mean ± SD. Blood parameters ranges are displayed in parentheses. Values with asterisks (*) within a row indicate significant differences at $P < 0.05$, using the t-tests. RBC: red blood cell, MCV: mean corpuscular volume, WBC: white blood cell.

Parameters	Sardines without parasites (n = 30)		Sardines with parasites (n = 10)	
Hematocrit (%)	59.54 ± 10.76	(42.5-72)	56.60 ± 7.13	(44-70)
RBC×10^6 mm^3	5.45 ± 0.64	(4.15-6.82)	5.24 ± 0.73	(4.10-6.87)
MCV (fL)	114.79 ± 17.95	(77.41-159.40)	109.40 ± 18.31	(82.70-148.30)
WBC×10^3 mm^3	8.63 ± 4.61	(3.70-25.10)	5.93 ± 2.43*	(3.90-11.50)
Thrombocyte (%)	61.65 ± 8.13	(39.76-78.81)	66.32 ± 11.89	(49.49-83.76)
Leukocyte (%)	38.34 ± 8.13	(21.18-60.24)	33.67 ± 11.90	(16.23-50.50)
Lymphocyte (%)	81.52 ± 11.76	(52-98)	85.80 ± 10.68	(64-96)
Monocyte (%)	3.23 ± 1.73	(2-6)	2.67 ± 1.03	(2-4)
Eosinophil (%)	5.80 ± 5.53	(2-20)	3.33 ± 2.30	(2-6)
Neutrophil (%)	14.48 ± 9.09	(2-34)	11.6 ± 9.55	(4-30)
Total protein (mg mL^{-1})	15.07 ± 1.76	(9.06-17.18)	15.86 ± 0.93	(14.31-17.46)
Glucose (mg dL^{-1})	112.96 ± 19.01	(67.35-145.45)	110.32 ± 18.82	(80.95-134.03)
ALT (U L^{-1})	8.47 ± 4.58	(1.71-19.26)	6.11 ± 2.67	(0.20-9.92)
Osmotic pressure (mmol kg^{-1})	369.10 ± 31.44	(293-454.5)	364.65 ± 21.16	(343.5-414.5)
Na (mmol L^{-1})	186.69 ± 15.56	(169.7-216.2)	178.48 ± 9.08	(155.5-187.8)
K (mmol L^{-1})	4.25 ± 1.81	(1.55-10.49)	3.83 ± 2.18	(1.79-6.99)
Ca (mmol L^{-1})	1.66 ± 0.43	(1.13-2.3)	1.32 ± 0.21*	(1.08-1.61)
pH	7.62 ± 0.19	(7.29-7.92)	7.76 ± 0.13	(7.53-7.99)

Blood parameters were compared between sardines without parasites and those with an average intensity of 25 parasites (Table 1). There was no statistically significant difference ($P > 0.05$) in the blood parameters except in total leukocyte count and calcium (Ca) concentration.

In sardines the blood cells were identified and characterized by light microscopy as follows: Erythrocytes stained purple with a light grey cytoplasm and small dark granules dispersed in the nucleus. Immature erythrocytes had a blue-grey cytoplasm, purple nucleus and dense chromatin pattern compared to mature erythrocytes (Fig. 1a). Thrombocytes appeared separately or in clusters. The thrombocytes were round, ovoid or elongated cells with a dark purple nucleus and showed a clear basophilic cytoplasm visible and moderate chromatin (Fig. 1a).

Lymphocytes were round or ovoid cells with a big, round, and centric nucleus that occupied most of the cell.

The cytoplasm was limited to a small area around the nucleus. Frequently, lymphocytes had cytoplasmic pseudopods (Fig. 1a). Small lymphocytes had no visible cytoplasm and less compact chromatin. The nucleus stained dark-purple to violet compared to the larger lymphocytes that had some dispersed chromatin and a violet nucleus. Neutrophilic granulocytes cells were round with one to four-lobed nuclei with variable forms and fine granulation surrounded by a cytoplasm

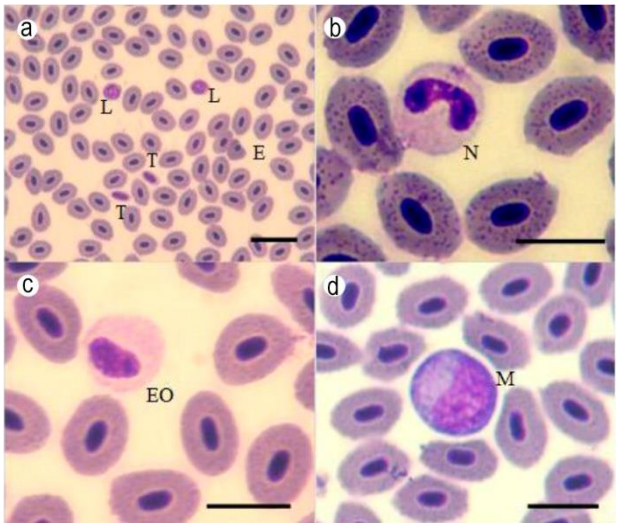

Figure 1. Light micrographs of hemacolor stain blood film of the blood cells of Pacific sardine (*Sardinops sagax*). a) Erythrocytes (E), thrombocytes (T) and lymphocytes (L) (scale bar = 20 µm), b) neutrophil (N), c) eosinophil (EO), d) monocyte (M). (Scale bar = 10 µm).

with regular borders (Fig. 1b). Eosinophilic granulocytes were round cells and had round, dark-purple, often eccentric nucleus and compact chromatin with one round or sometimes lobulated nucleus. Cytoplasm had numerous eosinophilic granules ranging from small to few large ones (Fig. 1c). Monocytes were round to irregular cells characterized by a round or irregular,

Table 2. Size of blood cell type from Pacific sardine *Sardinops sagax*. Values are given as means (n = 30). Blood cell ranges are displayed in parentheses.

Cell type	Dimension (μm)	
Erythrocyte	10.85 ± 0.70	(8.8-12.1)
Oval thrombocyte	6.34 ± 0.30	(6-6.6)
Elongated thrombocyte	8.69 ± 0.72	(7.7-9.9)
Small lymphocyte	5.52 ± 0.22	(5-6.6)
Large lymphocyte	7.52 ± 0.66	(6.6-8.8)
Monocyte	11.72 ± 0.98	(11-14.3)
Eosinophil	9.57 ± 0.77	(8.8-11)
Neutrophil	10.09 ± 0.96	(8.8-12.1)

eccentric nucleus with an open chromatin pattern. These cells exhibited a blue-purple nucleus and a gray-blue cytoplasm, and frequently vacuoles were present (Fig. 1d). The results of the cell dimension analysis were tabulated (Table 2).

In this study, we found that most of the blood parameters were not affected by the parasitic load. On the other hand, a decrease in Ca and WBC values were observed in the infected Pacific sardine. However, the implication of these findings needs more detailed research and space-time distributed information, because in the present study is not clear the relationship between parasite abundance and blood parameters due to the low parasitic load represented only by a digenea *Myosaccium ecaude*. This digenea show an host specificity towards fish of the family Clupeidae distributed in temperate and tropical seas of America, and it is not reported to cause damage in fish (Love & Moser, 1983; León-Regagnon *et al*., 1997; Baldwin *et al*., 2011). Sánchez-Serrano & Cáceres-Martínez (2017) reported five species of helminths different to our study. Nevertheless, their fish were obtained during summer, fall (2004) and winter (2005) from landings of the sardine fisheries of Ensenada that operates on the western coast of the Baja California Peninsula and the Cedros Island. The differences of parasites found in both studies can be attributed to the prevailing environmental conditions.

Taking into account the information of the International Research Institute for Climate and Society (IRI), the sampling dates of Sánchez-Serrano & Cáceres-Martínez (2017) coincide with El Niño Southern Oscillation (ENSO) from 2004 to early 2005 when the conditions were warmer. Our study was carried out when neutral ENSO conditions were present throughout the last three months of 2013 and into early 2014, so these conditions could influence the parasitic load of the sardine. However, digenea mean intensity (*M. ecaude*) was similar in both works. George-Nascimento & Moscoso (2013) mentioned that the infracommunity of fish parasites have distinct quantitative and qualitative characteristics over space and time, which could explain the lack of parasites species found in our study compared with the results reported in sardine by Love & Moser (1983), Baldwin *et al*. (2011) and Sánchez-Serrano & Cáceres-Martínez (2017).

The variation exhibited in the nucleus and cytoplasm shape of the Pacific sardines in our study was consistent with the typical characteristics of blood cells in marine fish (Palíková *et al*., 1999; Rough *et al*., 2005; Pavlidis *et al*., 2007; Del Río-Zaragoza *et al*., 2011). However, we observed size differences of some blood cells of the Pacific sardine, reinforcing the need to characterize each species separately (Shigdar *et al*., 2007). Specifically, there was variability in mean values of erythrocytes and leukocytes sizes of the Pacific sardine in relation with other marine fish (Palíková *et al*., 1999; Pavlidis *et al*., 2007; Fajer-Ávila *et al*., 2011). The differences in leukocytes size are species-specific, and it is thought that they reflect their functional state, shape, and maturity of the cell at organism level and the physiology status of the fish (Del Río-Zaragoza *et al*., 2011).

Based on the results of this study, the relationship between parasite abundance and blood parameters is not clear due to the low parasitic load. Therefore, it is crucial to establish the space-time effect of environmental changes on the parasite-host interaction to know the behavior of the parasitic load of the sardine and its relationship with the health status evaluated through blood parameters.

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