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NOTE

Hematology and condition factor of tui chub and fathead minnow parasitized by nematode from Upper Klamath Lake, Oregon, USA

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ABSTRACT: This study evaluated the hematological profile and condition factor (Kn) of tui chub *Siphateles bicolor* and fathead minnow *Pimephales promelas* and their associations with larvae of *Contracaecum* sp. infection of the heart. A total of 30 tui chub and 17 fathead minnow were collected from Upper Klamath Lake, Oregon, USA, measured, and weighed and blood was drawn for hematological analysis. Nematode larvae parasitized tui chub with a prevalence of 50% and mean intensity of 1.40, while 11.8% of fathead minnow were parasitized at a mean intensity of 1.0. Non-parasitized tui chub were significantly larger than the parasitized fish, indicating that small fish could be easily predated by the definitive host, a piscivorous bird. Although the relatively large worm occupied a large portion of the atrium, the presence of the larvae did not affect tui chub Kn, possibly associated with low parasite intensity and a harmonic co-evolution. Only parasitized fathead minnow showed significant differences in red blood cell measurements (greater cell width and larger nuclei) compared to non-parasitized fish. Lymphocytes were the most common white blood cells found in tui chub, followed by neutrophils, monocytes, and periodic acid-Schiff positive granular leukocytes; in fathead minnow lymphocytes were followed by heterophils, monocytes, neutrophils and eosinophils. This study is the first report of Kn and description of blood cells and hematological parameters in these fish species.

KEY WORDS: Fish · Cyprinid · Condition factor · Blood · Nematode

INTRODUCTION

We investigated parasites and their impacts on fishes in Upper Klamath Lake, Oregon, USA. In addition to endangered sucker species, we investigated tui chub *Siphateles bicolor* (Girard, 1856) and fathead minnow *Pimephales promelas* (Rafinesque, 1920) in the lake. The normal geographic range of fathead minnows includes freshwaters in the central USA, extending into central Canada (Page & Burr 1991). They were introduced to Upper Klamath Lake, Oregon, about 50 yr ago and now represent the most abundant fish species in the lake. Tui chub are native to Klamath Lake and are also very abundant in the lake (Simon & Markle 1997).

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Parasites are very common in wild fishes, and they are often associated with significant mortality (Lester 1987, Molnár et al. 1991). They often cause reduction in fish growth, health, and reproduction and can cause physiological alterations and negatively affect swimming performance (Santos et al. 2013). Predation is one of the main causes of mortality in wild fishes, and there are numerous examples of parasites causing increased predation (Lafferty & Morris 1996, Johnson et al. 2010). This is particularly the case when fish serve as intermediate hosts of trophically transmitted parasites. The condition factor (Kn) is an indicator of the health status of a fish population with respect to growth and nutritional condition (Le Cren 1951) and to parasite load (Corrêa et al. 2013). Hematological parameters are also useful indicators of fish health in relation to parasitic diseases, providing a complementary tool for describing the clinical impacts of parasites in fish (Martins et al. 2004, Ranzani-Paiva et al. 2013). Studies correlating parasite infections with hematological endpoints include alterations in red blood cell (RBC) parameters, thrombocytes and leukocytes number (Martins et al. 2004, Corrêa et al. 2013, Ranzani-Paiva et al. 2013)

Several surveys have been made of parasites in fathead minnow (Sutherland & Holloway 1979, McDowell 1992, Kuperman et al. 2002) and tui chub (Kuperman et al. 2002), but none have been conducted on these host species in the Upper Klamath Lake. The most profound parasite infection that we have discovered to date in these species is that with *Contracaecum* sp., which infects the heart of fathead minnow (Dick 1987). Sutherland & Holloway (1979) reported *Contracaecum* species in the stomach of fathead minnows from North Dakotan rivers. Madi & Silva (2009) found *Contracaecum* sp. in *Hoplias malabaricus*, *Rhamdia quelen* and *Salminus hilarii* from a reservoir in the state of São Paulo, Brazil, and noted that Kn was not affected by the parasitism. In contrast, Olivero-Verbel et al. (2006) found low and negative correlations between the Kn and parasite intensity in wolf-fish *H. malabaricus* from the Amazon River, Colombia, suggesting that the parasites have an impact on fish health. Regarding hematological parameters, Corrêa et al. (2015) observed a positive correlation of hematocrit with the mean intensity of infection by *Contracaecum* sp. L_3 larvae and a negative correlation with the mean corpuscular volume of the trahira *H. malabaricus*. For that species, Corrêa et al. (2013) noted a significant difference in the hematocrit, RBCs and a decreased number of thrombocytes in *H. malabaricus* infected by L_3 larvae of *Contracaecum* sp. However, *Contracaecum* spp. have not been reported from tui chub.

This study presents data on the Kn and hematological parameters and describes the red and white blood cells of wild fathead minnow and tui chub captured from the Upper Klamath Lake. The relationship between parasitism with *Contracaecum* sp. (Nematoda: Anisakidae) larvae in the heart chamber and hematology is also presented.

MATERIALS AND METHODS

A total of 30 tui chub (7.95 ± 1.97 cm total length and 6.80 ± 6.51 g weight, mean ± SD) and 17 fathead minnows (5.51 ± 0.45 cm total length and 1.43 ± 0.40 g weight) were collected from Upper Klamath Lake, Oregon, USA, in September 2015. On the collection day the water temperature was 11°C, and transparency was 1.20–1.40 m. At the collection site, the fish were anesthetized by immersion in 100 mg l^{-1} tricaine methanesulfonate solution (MS-222, Western Chemical), and blood was withdrawn with a micropipette for blood smears posteriorly stained with May Grunwald/Giemsa /Wright. An aliquot was transferred to microtubes and diluted 1:200 with Dacie solution to determine the RBC count using a Neubauer chamber (Ranzani-Paiva et al. 2013). The total number of thrombocytes and leukocytes (WBC) were calculated using an indirect method (Ranzani-Paiva et al. 2013). The mean length and width of the erythrocytes and their nucleus were measured in 150 cells from different blood smears using a microscope equipped with a Spot 2.2.0 (Diagnostic Instruments). Photomicrographs were made using a Leica microscope (DMLB100S). Immediately after blood sampling, the fish were euthanized by cerebral concussion and individually identified. They were kept in ice until transport to the laboratory, where the presence of nematodes in the heart chamber could be verified. Prevalence, mean intensity and mean abundance were calculated according to Bush et al. (1997), and the nematode was identified based on criteria provided by specialized literature. Total length (L) and weight (W) data were used to determine the relative Kn. Body weight (g) and total length (cm) were also used to calculate the relative Kn of fish using the W–L relationship ($W = aL^b$) after logarithmic transformation of length and weight and subsequent adjustment of 2 straight lines, obtaining $\ln y = \ln a + b \ln x$ (Le Cren 1951). Fish collection was approved by the Institutional Animal Care and Use Committee, Oregon State University.

For statistical analysis, Kolmogorov-Smirnov and Bartlett tests were performed to verify the normality and homoscedasticity of the data, respectively. Posteriorly, the data were subjected to analysis of variance (ANOVA), and means were compared using the Tukey test. All analyzes were performed using the STATISTICA software (StatSoft). Data were transformed, and differences were considered significant when $p < 0.05$. To verify the difference between the means of hematological parameters of parasitized and non-parasitized fathead minnow, the *Z*-test was also applied at $p < 0.05$.

RESULTS

Contracaecum sp. prevalence and intensity

From 30 examined tui chubs, 15 (50%) were parasitized with *Contracaecum* sp. larvae in the heart chamber with a mean (±SD) intensity of 1.40 ± 0.51 and a mean abundance of 0.70 ± 0.79 parasites per host. From 17 examined fathead minnows, 2 (11.8% prevalence) were parasitized with a mean intensity of 1.00 ± 0 and a mean abundance of 1.00 ± 0 parasites.

Body parameters

For tui chub, the equation for the *W–L* relationship was $W = 0.0068L^{3.2213}$ ($r^2 = 0.983$), with isometry, indicating increases in body weight and length at the same proportion. There was no significant difference in Kn associated with the infection (Table 1). For fathead minnow, the equation for the *W–L* relationship was $W = 0.8037L^{2.9981}$ ($r^2 = 0.804$), negative allometric, indicating a larger increase in body weight than length. Fathead minnow showed no difference in size and Kn (1.10 ± 1.77) between parasitized and non-parasitized hosts ($t = 0.253$, $p = 0.804$).

Hematology

There were no statistical differences in any of the hematological parameters of parasitized and non-parasitized tui chub fish

Table 1. Biometric data (mean ± SD) of tui chub and fathead minnow from Upper Klamath Lake, Oregon, USA, parasitized by *Contracaecum* sp. larvae. T(/S)L: total(/standard) length; Kn: condition factor. Different superscript letters indicate significant differences by Tukey test ($p < 0.05$); low sample numbers for fathead minnow precluded statistical comparison between parasitized and non-parasitized fish

Health status	n	TL (cm)	SL (cm)	Weight (g)	Kn
Tui chub					
Parasitized	15	6.81±0.62[b]	5.71±0.49[b]	3.42±0.97[b]	1.00±0.05
Non-parasitized	15	9.09±2.22[a]	7.61±2.00[a]	10.19±7.90[a]	1.00±0.08
Fathead minnow					
Parasitized	2	5.65±1.06	4.95±0.49	1.67±1.01	0.55±0.09
Non-parasitized	15	5.49±0.39	4.57±0.29	1.40±0.32	1.10±1.71

Table 2. Total number of red blood cells (RBCs), thrombocytes, white blood cells (WBC) and differential counting of leukocytes in tui chub and fathead minnow from Upper Klamath Lake, Oregon, USA, parasitized by *Contracaecum* sp. larvae. PAS-GL: periodic acid Schiff stain-positive granular leukocytes. Different superscript letters indicate significant differences by Tukey test ($p < 0.05$). Low sample numbers for fathead minnow precluded statistical comparison between parasitized and non-parasitized fish; p-values for parasitized and non-parasitized fathead minnow analyzed by *Z*-test

Health status	n	RBC ($\times 10^6$ µl^{-1})	Thrombocytes ($\times 10^3$ µl^{-1})	WBC ($\times 10^3$ µl^{-1})						Heterophils ($\times 10^3$ µl^{-1})
				Total	Lymphocytes	Monocytes	Neutrophils	Eosinophils	PAS-GL	
Tui chub										
Parasitized	15	1.18±0.22[a]	22.43±14.02[a]	26.00±15.69[a]	19.67±19.21[a]	3.24±1.98[a]	4.78±3.64[a]	0	0.69±0.87[a]	0
Non-parasitized	15	1.04±0.30[a]	29.86±23.00[a]	27.38±14.44[a]	15.98±10.86[a]	5.29±2.02[a]	5.57±1.16[a]	0	0.80±0.79[a]	0
Fathead minnow										
Parasitized	2	0.64±0.20	22.33±7.31	31.26±20.87	14.19±4.67	6.22±5.53	1.42±0.60	0	0	9.44±10.08
Non-parasitized	15	0.69±0.22	16.04±11.79	24.74±10.90	11.79±6.69	3.03±1.69	2.35±2.13	0.30±0.44	0.02±0.06	7.26±3.91
p-value		0.7501	0.281	0.6632	0.5098	0.4203	0.1507	–	–	0.7615

(Table 2). The leukocytes observed are presented in Fig. 1. Blood cells of the tui chub and fathead minnow observed included monocytes, neutrophils, lymphocytes, periodic acid-Schiff positive granular leukocytes (PAS-GL), heterophils and eosinophils. In both fish species, lymphocytes varied in size, were spherical, with basophilic cytoplasm and apparently without granulations. Monocytes exhibited basophilic cytoplasm and frequently eccentric, occasionally horseshoe-shaped, nuclei. Neutrophils were rod-shaped and occasionally segmented with eccentric nuclei and cytoplasm with typical neutrophilic granules. PAS-GL showed abundant cytoplasm rich in granules resembling heterophils, but without staining of the granules. Eosinophils were mainly spherical, with generally eccentric nuclei and compact chromatin, and the cytoplasm presented eosinophilic granules. Heterophils were round cells, containing eosinophilic and basophilic granules randomly distributed in the cytoplasm, and the number of granules depending on the degree of cell maturation (Fig. 1).

The erythrocytes of both fishes were elliptical, but in the fathead minnow they were slightly rounded when compared to those of tui chub (Fig. 1). The measurements of the erythrocytes and their nuclei are presented as means followed by standard deviation (Table 3). The erythrocytes and cell nuclei of parasitized tui chub were larger ($p < 0.05$) than those observed in non-parasitized fish. In fathead minnow, parasitized fish had a higher mean blood cell width ($p < 0.05$) than non-parasitized ones. Erythrocytes in tui chub were longer and narrower ($p < 0.05$) compared to fathead minnow.

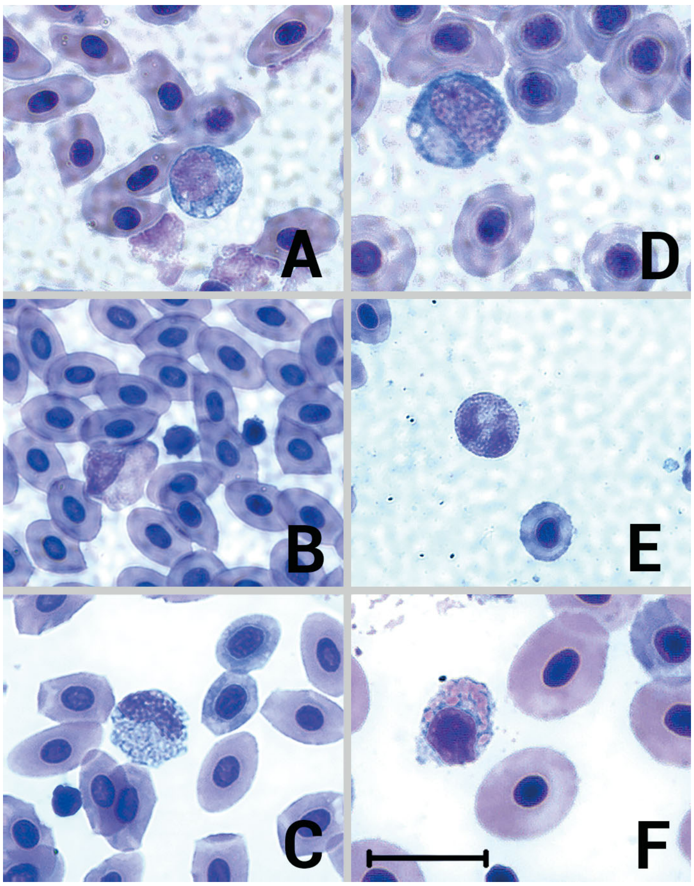

Fig. 1. Blood cells of tui chub and fathead minnow from Upper Klamath Lake, Oregon, USA. Tui chub: (A) monocyte, (B) neutrophil and lymphocytes, (C) periodic acid Schiff (PAS) positive granular leukocyte. Fathead minnow: (D) monocyte, (E) neutrophil, (F) heterophil. Scale bar = 5 µm

DISCUSSION

Several studies on *Contracaecum* larval infections in fishes have been conducted, but most of them have been based on parasite species biology (Madi & Silva 2009), life cycle (Huizinga 1967, Køie & Fagerholm 1995), prevalence (Sutherland & Holloway 1979) and zoonotic importance (Haenen et al. 2010). *Contracaecum* larval infections present severe pathogenicity in fish hosts (Dick 1987, Barros et al. 2004, Dezfuli et al. 2008). Most pertinent to the present study, Dick (1987) described the pathological effects of *Contracaecum* sp. in the hearts of fathead minnows and nine-spined stickleback (*Culaea inconstans* and *Pungitius pungitius*). Here the most profound effect was a severe enlargement of the atrium.

Larvae of *Contracaecum* did not have an effect on the Kn of tui chub, and these data corroborate the findings of Corrêa et al. (2013) and Madi & Silva (2009), who found no influence of *Contracaecum* on the Kn of *Hoplias malabaricus*. Likewise, Haenen et al. (2010) did not observe changes in the Kn of European eels *Anguilla anguilla* parasitized by *Anguillicola crassus* Kuwahara, Niimi & Itagaki, 1974 captured from the Rhine River in Western Europe. In contrast, Olivero-Verbel et al. (2006) observed a significant negative correlation between Kn and the parasite intensity of *Contracaecum* in *H. malabaricus*. Similarly, 2 of the parasitized fathead minnows in our study had a Kn half that of the uninfected fish. In addition, there was no change in all of the hematological parameters evaluated in tui chub and fathhead

Table 3. Length and width (in µm) of red blood cells and their nuclei (n = number of measured cells) in tui chub and fathead minnow from Upper Klamath Lake, Oregon, USA, parasitized by *Contracaecum* sp. larvae. Different superscript letters indicate significant differences within each species by Tukey test ($p < 0.05$)

Health status	n	Cell		Nucleus	
		Length	Width	Length	Width
Tui chub					
Parasitized	150	13.45 ± 1.00[a]	7.46 ± 0.99[a]	4.78 ± 0.46[a]	3.12 ± 0.35[a]
Non-parasitized	150	13.34 ± 0.91[a]	7.16 ± 0.80[a]	4.73 ± 0.43[a]	2.98 ± 0.38[a]
Fathead minnow					
Parasitized	35	11.61 ± 0.98[a]	9.30 ± 0.78[a]	4.80 ± 0.55[a]	3.61 ± 0.51[a]
Non-parasitized	132	11.68 ± 0.89[a]	7.80 ± 0.85[b]	4.42 ± 0.48[b]	3.06 ± 0.43[b]

minnows, which may indicate 2 possible things: the existence of a well-established host–parasite relationship or a low parasitic infection in the studied fish. The later is not likely because, although only 1 or rarely 2 worms per fish were observed, the location in the heart and the size of the worm suggest that they would severely affect the host. In contrast, Corrêa et al. (2013) found normochromic–hypochromic anemia and thrombocytopenia, but no alteration in differential counting of leukocytes of *H. malabaricus* naturally infected by *Contracaecum* sp. The present study presents for the first time an assessment of the Kn, the hematological parameters of tui chub and fathead minnow, and their relation to *Contracaecum* infections in the heart of the fish.

There are very few other reports on the hematological changes in fish infected with other nematodes. These changes can indicate a possible effect of stress caused by these parasites (Haenen et al. 2010) as reported in *Leporinus macrocephalus* Garavello & Britski, 1988 infected with *Goezia leporini* Martins & Yoshitoshi, 2003 (Nematoda: Anisakidae). In the study above this fish showed a decrease in the hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration and lymphocytes percentages, along with an increased neutrophil and eosinophil percentage and anemia associated with the infection (Martins et al. 2004). In a subsequent study, Corrêa et al. (2015) found that the mean intensity of infection by *Contracaecum* sp. L_3 larvae in the visceral organs correlated positively with hematocrit and correlated negatively with the mean corpuscular volume of *H. malabaricus*. The erythrocytes were larger in infected than in non-parasitized fathead minnow, which could be explained by a need for the infected fish to increase the oxygen absorption area, because in these fish no alterations were found in the other RBC parameters.

Leukocytes characteristics of tui chub and fathead minnow were similar to other fish species (Ranzani-Paiva et al. 2013). In addition, Lefebvre et al. (2004) reported that the hematophagous behavior of *A. crassus* in European eels altered the haematological function of the spleen. Nevertheless, it is noteworthy that in our study, although *Contracaecum* sp. caused remarkable infections in the heart, there were few changes in the hematological parameters.

In conclusion, we observed little, if any, effect of the heart nematodes on the Kn and hematological endpoints in tui chub. The definitive host for most *Contracaecum* species are fish-eating birds. Pelicans, gulls and cormorants are abundant at Klamath Lake as this is a wild life refuge. There are countless examples of parasites predisposing their fish host to predation, particularly with trophically transmitted parasites (see reviews by Barber et al. 2000). We certainly have not excluded the possibility that the worms cause parasite-associated mortality through increased predation due to other health or fitness impacts that we have not elucidated. For example, parasites can cause reduced swimming speed (Ferguson et al. 2012) or aberrant swimming behavior (Lafferty & Morris 1996). There are multiple year classes of tui chub in the lake, and most of the smaller fish probably represented underyearlings. The larger fish, which were for the most part uninfected, were ≥1 yr old. This suggests parasite-associated mortality as defined by Method 3 of Lester (1987) in the section 'Observing a decrease in the prevalence of long-lived parasites with host age'. It is difficult to draw conclusions regarding *Contracaecum* sp. infection in fathead minnow as only 2 fish were infected in this study, but these preliminary results do suggest that the parasite has more effect on this fish host than on tui chub.

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