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ECOLOGICAL ASPECTS OF THE INVADING TREMATODE *CENTROCESTUS FORMOSANUS* (NISHIGORI, 1924) (TREMATODA: HETEROPHYIDAE) IN THE NILE TILAPIA *OREOCHROMIS NILOTICUS* (LINNAEUS, 1758) (PERCIFORMES, CICHLIDAE), IN THE WETLAND LOS PANTANOS DE VILLA, LIMA, PERU

ASPECTOS ECOLÓGICOS DEL TREMÁTODO INVASOR *CENTROCESTUS FORMOSANUS* (NISHIGORI, 1924) (TREMATODA: HETEROPHYIDAE) EN LA TILAPIA DEL NILO *OREOCHROMIS NILOTICUS* (LINNAEUS, 1758) (PERCIFORMES: CICHLIDAE), EN EL HUMEDAL LOS PANTANOS DE VILLA, LIMA, PERÚ

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ABSTRACT

Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) is a freshwater fish native to Africa. The present work's objective was to evaluate some ecological aspects of the invading metacercaria of *Centrocestus formosanus* (Nishigori, 1924) (Trematoda: Heterophyidae) in *O. niloticus* in the wetlands of Pantanos de Villa, Lima, Peru. Twenty specimens of *O. niloticus* collected in the Genesis, and Marvilla lagoons in Pantanos de Villa's wetlands were inspected parasitologically during February and October of 2012. The fish had an average weight and length of 221.3 g ± 111.4 and 19.6 cm ± 4.24 cm, respectively. The invasive ectoparasite metacercariae were cataloged and evaluated using standard parasitological protocols. During the entire sampling, a total of 130 *C. formosanus* trematodes was collected, with an average abundance of sixty percent of the Nile tilapias parasitized by *C. formosanus*. The relative condition factor (k_n), an indicator of host fish health, was not influenced by the presence of *C. formosanus*. *Centrocestus*

Keywords: *Centrocestus* – fish parasites – *Oreochromis niloticus* – parasite ecology – Peru

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formosanus has been recorded in birds and mammals, including humans producing heterophils, a worldwide emerging disease in humans transmitted by raw fish consumption. *Centrocestus formosanus* in Peru has a potential zoonotic impact on public health. In addition, the presence of the dynozoan ectoparasite *Amyloodinium ocellatum* Brown & Hovasse, 1946, can cause pathological alterations in marine fish and saline environments found in gills in a single host.

Keywords: *Centrocestus* – fish parasites – *Oreochromis niloticus* – parasite ecology – Peru

RESUMEN

La tilapia del Nilo *Oreochromis niloticus* (Linnaeus, 1758) es un pez de agua dulce endémico originario de África. El objetivo del presente trabajo fue evaluar algunos aspectos ecológicos del tremátodo invasor *Centrocestus formosanus* (Nishigori, 1924) (Trematoda: Heterophyidae) en *O. niloticus* en los humedales de Pantanos de Villa, Lima, Perú. Durante febrero y octubre de 2012 se inspeccionaron parasitológicamente 20 ejemplares de *O. niloticus* recolectados en las lagunas Génesis y Marvilla en los humedales de Pantanos de Villa, Lima, Perú. Los peces tuvieron un peso y longitud promedio de 221.3 g ± 111.4 y 19.6 cm ± 4.24 cm, respectivamente. Las metacercarias de ectoparásitos invasores fueron catalogadas y evaluadas utilizando protocolos parasitológicos estándares. Durante todo el muestreo, se recolectaron un total de 130 trematodos de *C. formosanus*, con una prevalencia promedio del 60% de las tilapias del Nilo parasitadas por *C. formosanus*. El factor de condición relativo (k_n), un indicador de la salud de los peces huéspedes, no fue influenciado por la presencia de *C. formosanus*. *Centrocestus formosanus* se ha registrado en aves y mamíferos, incluidos los humanos, que producen heterofiosis, una enfermedad emergente mundial en humanos transmitida por el consumo de pescado crudo. *Centrocestus formosanus* en Perú representa un potencial impacto zoonótico en la salud pública. En adición, se registró en branquias en un solo huésped, la presencia del ectoparásito dinozoario *Amyloodinium ocellatum* Brown & Hovasse, 1946, que puede causar alteraciones patológicas en peces marinos y ambientes salinos.

Palabras clave: *Centrocestus* – ecología de parásitos – *Oreochromis niloticus* – parásitos de peces – Perú

INTRODUCTION

The gray tilapia *Oreochromis niloticus* (Linnaeus, 1758) (Perciformes: Cichlidae) is an endemic freshwater fish native to Africa and the Near East; it is a species widely distributed worldwide for cultivation due to its relative ease of adaptation and easy management in production in aquatic crops (FAO, 2016; Abd El-Naby *et al*., 2019). In Peru, starting in the 70s, this species' introduction and cultivation began for research and commercial cultivation purposes (Ramos & Gálvez, 2000; Baltazar, 2007). The introduction of this species began from Brazil in 1978 until it became an invasive species, displacing species such as native fish from the lower area of the Rio Grande, on the coast of Ica, Peru (Ortega *et al*., 2007). It is currently considered a naturalized species in Amazonas and Arequipa environments, to name a few (Ortega *et al*., 2007; Cossíos, 2010).

In Peru, the nile tilapia, O. niloticus, have been registered few jobs in relation to their parasitofauna and other pathogens. (Amin *et al*., 2010; Gonzales-Fernández, 2012; Chiclla-Salazar & Tantas-García, 2015; Luque *et al*., 2016; Ortega *et al*., 2017).

Among the known adverse effects of introducing exotic or alien species is introducing diseases through pathogens and parasites (Davis, 2009). One of the species mentioned as introduced alien parasites is the trematode *Centrocestus formosanus* (Nishigori, 1924) (Trematoda: Heterophyidae), which has among its exotic paratenic hosts *O. niloticus* and among its strange intermediate hosts *Melanoides tuberculata* (OF

Müller, 1774) (Ximenes *et al*., 2017). It is found in the form of metacercaria in the gills of freshwater fish. It is of aquaculture sanitary importance due to the damage it can cause to the gill filaments, which can end up being destroyed, thus reducing the surface area of the respiratory epithelial tissue (Sumuduni *et al*., 2018; Leibowitz *et al*., 2019; Pace *et al*., 2020). This trematode has caused the death of high populations of fish in hatcheries, causing severe economic losses and the death of wild fish and becoming a threat to those in danger of extinction (Mitchell *et al*., 2005).

Other risk factors that make *C. formosanus* a vital species is its zoonotic capacity because it can cause heterophiosis, an emerging global disease in humans transmitted by the consumption of raw fish, which in some cases can cause significant heart disease, brain and spinal cord, being often fatal in these organs (Lima dos Santos & Howgate, 2011). Despite infecting humans, it is only considered an accidental host of *C. formosanus* since birds and wild mammals associated with freshwater bodies are those that play the role of definitive hosts (Pinto & Melo, 2012; Leibowitz *et al*., 2019).

One of the ideal settings for the establishment and development of the biological cycle of *C. formosanus* is the Pantanos de Villa wildlife refuge in Lima, Peru, since part of the fauna of this wetland harbors *M. tuberculata* (Torres-Zevallos *et al*., 2020) and *O. niloticus*, both documented hosts of the immature forms (redia, cercaria and metacercaria), and species of wild piscivorous birds, both migratory and resident (Iannacone *et al*., 2010) available as definitive hosts for *C. formosanus*.

Due to the above, this work aims to evaluate the ecological aspects of the invasive trematode *C. formosanus* in the Nile tilapia *O. niloticus*, in the wetlands of Pantanos de Villa, Lima, Peru.

MATERIALS AND METHODS

Collection of material and processing of samples

Twenty specimens of *O. niloticus* were necropsied between February and October 2012 at the Los Pantanos de Villa Wildlife Refuge, Lima, Peru (12 ° 12′49 ″ S; 76 ° 59′20 ″ W) to study its community of metazoan parasites (Eiras *et al*., 2006). Ten specimens were obtained from Génesis lagoon in February-2012 and ten from Marvilla lagoon in October-2012. The fish were collected using gillnets with 25 mm internode meshes. The nets were placed randomly and crosswise in each lagoon for ten hours, and were checked every 60 min and then relocated to the site (UNMSM - MINAM, 2014). The average physical-chemical characteristics of both lagoons of the wetlands of Villa, Lima, Peru evaluated *in situ* were: temperature of 25 ± 5.2 ° C, pH of 8.42 ± 0.03, Electrical conductivity (EC) of 5.67 ± 0.61 $mS \cdot cm^{-1}$, surface dissolved oxygen (DO) of 3.19 ± 1.58 $mg \cdot L^{-1}$, background dissolved oxygen (DO) of 1.88 ± 1.66 $mg \cdot L^{-1}$, using a multiparametric HANNA@ (HI98130, Solitec, Lima, Peru) and likewise, the transparency of the water was 37.91 ± 17.37 cm, which was evaluated using the Secchi disk. Fish were anesthetised in a benzocaine solution (50 $mg \cdot L^{-1}$) and a two mL blood sample was taken from the caudal vein using a syringe containing a drop of 10% EDTA solution (Jerônimo *et al*., 2011). Two hematological parameters were evaluated: hematocrit (Ht) and total red cell count (RBC). Ht (%) determinations were made by the microhematocrit method and the RBC (x $10^6 \cdot uL^{-1}$) was carried out in a Neubauer chamber (Blaxhall & Daisley, 1973). We measured total weight (0.1 g sensitivity) and full length (TL) (0.1 cm sensitivity). All fish underwent a systematic external and internal examination of tissues, including skin, fins, gills, eyes (lens and vitreous humor), body cavity, mesentery, and visceral organs (stomach, intestine, liver, swim bladder, heart, and gonads). The recovered parasites were fixed and preserved using commonly applied methods (Özer *et al*., 2016).

Sample analysis

The ecological approach of the metazoan parasite community was made at the component and intracommunity levels (Esch *et al*., 1990). For the case of parasitic species with prevalences higher than 10% (Esch *et al*., 1990), the dispersion indices (DI) were used to determine the type of spatial distribution of the parasitic populations, the Poulin discrepancy (PDI), and the K of the negative binomial equation with its respective Chi-square value (X^2) to determine the degree of aggregation (Bego & Von-Zuben, 2010). The calculations were performed using the statistical package Quantitative Parasitology 3.0 (Rózsa *et al*., 2000; Reiczigel *et al*., 2019).

Relative condition factor (K_n)

The theoretically expected weight for a given length was calculated using the estimated weight (W) -total length (LT) ratio curve. Then, the relative condition factor was obtained by the relationship between the observed weight (W) and the expected weight (We) as shown below: K_n = W/We. In this way, K_n's mean values for fish infected by a particular parasite alone were calculated (Özer *et al*., 2016). A student's t-test was performed to compare k_n deals between parasitized and non-parasitized and between the two lagoons evaluated, after analysis of the Levene test for the homogeneity of the variances Shapiro-Wilk test for normality (Zar, 2014).

Pearson's correlation coefficient (r_p) was used to indicate the relationship between the host's total length and the abundance of parasites. The Spearman rank correlation coefficient (r_s) was calculated to determine a possible correlation between the host's full size and the parasite's prevalence, with the anterior arcsine transformation of the prevalence data (Zar, 2014; Bautista-Hernández *et al*., 2013). The ecological terminology used follows Bush *et al*. (1997). The level of statistical significance was evaluated at $p \leq 0.05$. The SPSS version 25.0 statistical package was used for all descriptive and inferential statistics calculations.

Ethic aspects: The authors indicate that all the ethical requirements of the country and international were met.

RESULTS

The fish had an average length of 19.6 cm ± 4.24 (13 cm - 30.6 cm) and a weight of 221.3 g ± 111.4 (99 g - 486.5 g). Two parasites were found: *C. formosanus* (Trematoda) and *Amyloodinium ocellatum* (Alveolata). It was observed that 60% of the total population of *O. niloticus* were parasitized by the metacercariae of *C. formosanus* in the branchial area. Likewise, this parasite records an aggregate type distribution according to the dispersion indices according to the variance/mean

Table 1. Ecological descriptors of *Centrocestus formosanus* parasites of the Nile tilapia *Oreochromis niloticus* collected in Pantanos de Villa, Lima, Peru.

Descriptors	Total	Génesis lagoon	Marvilla lagoon
Parasitized fish	12	4	8
Prevalence (P) %	60	40	80
Medium abundance (MA)	6.5 ± 1.74	4.5 ± 2.81	8.5 ± 1.99
Medium intensity (MI)	10.83 ± 2.24	11.25 ± 4.44	10.63 ± 2.23
type of strategy	Core	Secondary	Core
Variance / mean ratio (DI)	9.27	17.49	4.66
Poulin's discrepancy Index (PDI)	0.58	0.72	0.35
Negative binomial exponent k	NA	0.15	NA
Total length (TL) (cm)	19.9 ± 4.24	22.9 ± 5.18	18.6 ± 5.18
Weight (W) (g)	247.8 ± 111.39	264 ± 133.56	226.7 ± 133.56
K_n	1.12 ± 1	1.23 ± 1	1 ± 1
Student's t between k_n between lagoons		t = 2.12, p=0.06	
K_n for parasitized and non - parasitized		K_n parasitized 0.86 ± 0.36	K_n not parasitized 1.07 ± 0.26
Student's t between parasitized k_n and non-parasitized k_n		t = 1.48, p=0.15	

K_n = Relative condition factor. NA = cannot calculate maximum likelihood estimate of k.

relationship (DI = 9.27) and Poulin's discrepancy Index (PDI = 0.58). Compared to the two lagoons, it is observed that in the Marvilla lagoon, the P and AM are higher than in Genesis lagoon, except for the MI, where the opposite occurs. In the type of distribution of *C. formosanus*, for the two environments, they are classified as aggregate or contagious (Table 1).

According to the Relative Condition Factor (K_n), the fish parasitized by *C. formosanus* did not show the loss or low levels of weight about the total population of fish (infected and not infected); on the contrary, the factor was above 1 ($K_n = 1.12 \pm 1$) indicating a satisfactory health condition concerning weight (Table 1). No significant differences were observed in kn between lagoons and between parasitized and non-parasitized (Table 1).

The degree of association between the morphological parameters of *O. niloticus* (TL and W) was sought about the ecological descriptors of *C. formosanus* (prevalence, mean abundance and mean intensity of infection), were an only significant association of TL with relation to the mean abundance (Table 2).

Table 2. Correlation between the total length (TL) and weight (W) of *Oreochromis niloticus* and the prevalence, abundance, and mean intensity of infection of *Centrocestus formosanus* in the Pantanos de Villa wildlife refuge, Lima, Peru.

	r	p
TL *vs* P%	*0.11	1.00
TL *vs* MA	**0.96	0.04
TL *vs* MI	**0.81	0.19
W *vs* P%	*0.60	0.23
W *vs* MA	**0.82	0.09
W *vs* MI	**0.15	0.81

TL = Total length, P% = Prevalence, MA = mean abundance, MI = mean intensity, W = Weight. r = Correlation coefficient, * Spearman, ** Pearson p = significance.

About *A. ocellatum*, this dinoflagellate was found only in one individual parasitizing a single host of Nile tilapia in the gills (5% prevalence) from the Genesis Lagoon. This parasite was found in the trophont stage of development, and an MA and MI of 0.05 and 1, respectively, were observed. Due to the low prevalence, this species was considered rare.

Table 3 shows the physical-chemical parameters (temperature, pH, dissolved oxygen, and water transparency) of the two lagoons (Génesis and Marvilla) that make up the Pantanos de Villa wildlife refuge Lima, Peru.

Table 3. The Génesis and Marvilla lagoons' physical-chemical parameters make up the Pantanos de Villa wildlife refuge, Lima, Peru.

Physico-chemical parameters	Génesis lagoon	Marvilla lagoon
Temperature (°C)	29	21.6
pH	8.4	8.4
Electric Conductivity ($\mu S \cdot cm^{-1}$)	6.1	5.2
DO ($mg \cdot L^{-1}$) (surface)	4.31	2.1
DO ($mg \cdot L^{-1}$) (bottom)	3.05	0.7
BOD ($mg \cdot L^{-1}$) (surface)	1.26	9.6
Transparency (Secchi) (cm)	50.40	25.4

Two hematological parameters in Nile tilapia, hematocrit (Ht) and total red cell count (RBC) presented mean values of 30.04 ± 5.41 % and 3.83 ± 0.47 ($x\ 10^6 \cdot uL^{-1}$), respectively.

DISCUSSION

The parasite community in the Nile tilapia was limited to one metazoan parasite species, the metacercariae of the trematode *C. formosanus* as the dominant species. Similar studies have also addressed the registration of ecological descriptors, as in Aguilar–Aguilar *et al*. (2009), where they found prevalence values of 100% and a mean abundance of 182.2, in *Cyprinella lutrensis* (Baird & Girard, 1853). On the contrary, Pinto *et al.* (2014) mentions low values of P = 31.9% (19/61), AM = 1.03 and IM = 3.42 in *O. niloticus*.

Fish age, behavior, and resistance to parasitism are factors that can influence the prevalence and intensity of trematode infection in Nile tilapia (Pinto *et al*., 2014). On the other hand, different authors from other countries (Kalantan *et al*., 1999; Ramadan *et al*., 2002; Chi *et al*., 2008) also recorded low intensities of infection by *C. formosanus* in *O. niloticus*. Pinto *et al*. (2014) suggest that the Nile tilapia has a relative resistance to the parasitosis of *C. formosanus*. They even offer that resistance to parasites is one reason why tilapia is chosen as a fish for culture; however, this study shows the high susceptibility to this trematode. These differences can be influenced by environmental aspects (temperature, humidity, luminosity) that each locality presents (Lo & Lee, 1996).

The type of distribution was classified as aggregated in the three indices, which is expected in populations of parasites with prevalence above 10% because aggregation is the predominant pattern in all-natural systems of parasites- host (Bego & Von-Zuben, 2010). In the case of the negative K index, it has not been possible to estimate the degree and type of aggregation for the total populations and the population of Laguna Marvilla; probably the reason could be (among others) that there is very little data or that are not aggregated at all (Reiczigel *et al*., 2019).

The association of the morphological parameters of *O. niloticus* and the parasitological indices did not show any degree of association except in the TL and the mean abundance of *C. formosanus,* which showed a significant positive association. Poulin (2011) suggests that larger body size hosts can deliver a greater supply of nutrients to parasites and, consequently, be the most susceptible to increased parasite infection.

Negative effects caused by pathogenic parasites can be expected on their hosts; however, it is difficult to define or quantify whether these effects directly caused any alteration in the health of the fish, this measure has been applied to individuals and, although in limited quantities, to varieties of parasites co-infecting their hosts (Özer *et al*., 2016). Health status based on the relative condition factor of *O. niloticus* was not affected by the individual presence of *C. formosanus*. Ht and RBC were within the expected range for Nile tilapia (Hah-Von-Hessberg *et al*., 2011; Jerônimo *et al.,* 2011).

Previously, *Centrocestus* sp. in the Green Terror *Andinoacara rivulatus* (Günther, 1860), and in the Swordtail *Xiphophorus hellerii* (Heckel, 1848), both reported in the department of La Libertad, Perú (Luque *et al*., 2016). These are the only records that exist of the genus *Centrocestus,* so that this study would be the first report of the species *C. formosanus* for the Pantanos de Villa life refuge for Lima's department and Peru.

It should also be considered that this trematode can not only cause pathologies in fish or their definitive hosts, birds and aquatic mammals (Pinto & Melo, 2012; Leibowitz *et al*., 2019), but is also responsible for zoonotic diseases, being able to produce in the human heterophiosis, which is emergent and transmitted to man due to the consumption of raw fish. This disease can be fatal in cases where it significantly affects the heart, spinal cord or brain (Lima dos Santos & Howgate, 2011).

In aquaculture, *A. ocellatum* has also been considered an important pathogen of marine and freshwater fish because it causes (amyloodyniosis) can be devastating and deadly in crowded and closed systems (Francis-Floyd & Floyd, 2011), as is the free-living refuge Pantanos de Villa. It is also known that the host range for this ectoparasitic dinoflagellate is quite wide, even reaching a group of teleost and elasmobranch fish (Alvarez-Pellitero, 2008). This generalist ability of the parasite can endanger the other fish species that accompany *O. niloticus* in Pantanos de Villa, such as the White Liza *Mugil curema* Valenciennes, 1836, which is also present in the same environment.

The extremely low prevalence of *A. oncellatum* could be because the parasite is still beginning to adapt to this environment and subsequently colonize fish populations. Therefore, it is essential to keep monitoring and reviewing possible intermediate hosts shortly. To ensure the health of the fish and its ecosystem of the swamps, since the loss of water resources such as fish could lead to imbalances in this environment's trophic chain and the organism's dependent on them.

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