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Ectoparasitic copepod infestation on a wild population of Neotropical catfish *Sciades herzbergii* Bloch, 1794: Histological evidences of lesions on host

Deborah Fogel[a], José Luis Fuentes[b], Luz Marina Soto[c], Wilson Lafuente[c], Rodrigo Moncayo-Estrada[d], Carlos López[c,*]

[a] Programa de Maestría en Ciencias Biológicas, mención Ecología Acuática, División de Estudios para Graduados, Facultad Experimental de Ciencias, Universidad del Zulia, Maracaibo, Venezuela
[b] Centro Regional de Investigaciones Ambientales, Universidad de Oriente, Venezuela
[c] Escuela Superior Politécnica del Litoral, ESPOL, Carrera de Biología, Facultad de Ciencias de la Vida, Campo Gustavo Galindo, Km. 30,5 Vía Perimetral, Guayaquil, Ecuador
[d] Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, COFAA, La Paz, Baja California Sur, Mexico

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ABSTRACT

We analyzed the infestation of the attached copepod species *Lepeophtheirus* sp on a wild population of *Sciades herzbergii*. The infestation and occurrence of attached copepods were related to body size, maturity and sex of host and the presence of lesions on fish skin were described. In 61 fish specimens (37 males and 24 females), total of 218 ectoparasitic copepods, including 204 mature and 14 immature stages were found. Copepods were attached to different regions of fish body without any regular pattern. The prevalence of infestation was 80.3% and intensity between 1 and 15 copepods/fish. No significant differences were found between sex or maturity and the presence of attached *Lepeophtheirus* sp. However, a contingency table including both sex and maturity status, and the total number of attached copepod per combined category showed a significant association. A positive correlation was found between body length of fish and intensity of infestation. Similarly, when comparing the infested fish according to group size, we found more copepods on larger individual. Attached copepods were associated with the presence of lesions visible to the naked eye. Histological analyses showed changes in cell architecture when sections of copepod-free tissues and attached copepods were compared.

1. Introduction

Parasitic copepods infect virtually all aquatic animal groups and show a staggering diversity in body form and life cycle strategies (Boxshall and Halsey, 2004). Particularly within the order Siphonostomatoida, that contains 40 families of exclusively parasitic forms, Caligidae is the richest in number of species (Boxshall, 2014). Caligid copepods are commonly known as Sea Lice and comprise 31 genera, of which, *Caligus* Müller, 1785 and *Lepeophtheirus* Von Nordmann, 1832 are the most diverse (Dojiri and Ho, 2013; Boxshall, 2014).

Sea lice are dominant ectoparasitic of farmed and wild fish populations around the world (Costello, 2006). They feed on the epithelial tissue of their hosts and may promote diseases in fish populations (Johnson et al., 2004; Venmathi et al., 2016). Sea lice also are considered as a economically important parasitic species around the world (Johnson et al., 2004; Rosenberg, 2008). Particularly, infestations of the species of *Lepeophtheirus* has been associated with decreased of body growth, changes in swimming behaviour, and increased of fish host morbidity by bacterial diseases in skin lesions and damages produced by attached copepods (Bui et al., 2016; Godwins et al. 2017; Llewellyn et al., 2017).

Historically, most of the studies on infestation from Caligid copepods are related to farmed fish populations in temperate zones, due to the economic costs they represent and their higher incidence at high population densities such as those occurring in aquaculture (Shepard and Gargano 2017). In contrast, fewer researches have examined copepod infestations on wild fish populations or assemblages (Todd et al., 2006; Jensen et al., 2016; Godwins et al. 2017). Similarly, in the Neotropical Regions, most of the detailed studies related to the ecological interaction between Caligid copepods and fish host refer essentially to the salmonids of the Chilean Pacific Ocean (Johnson et al., 2004; Costello, 2006). Although, the occurrence of *Lepeophtheirus* species have been widely recorded on different fish species in these regions (Luque et al., 2016; Morales-Serna et al., 2012, 2016) and particularly, they have been reported as the predominant metazoan parasites on host catfish (Luque and Tavares, 2007), the characteristics and effects of

* Corresponding author.
E-mail address: cllopez@espol.edu.ec (C. López).

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infestation are poorly known.

In this study, the infestation of an attached copepod (*Lepeophtheirus* sp) on a wild population of *Sciades herzbergii* Bloch, 1794, a Neotropical catfish species in a coastal lagoon in Northeastern Venezuela was analyzed. This fish species is endemic of South America and is located in Caribbean regions of Colombia and Venezuela and Atlantic coasts of Brazil (Cervigón, 1991; Marceniuk, 2015). *S. herzbergii* was selected because it is a common and one of the dominant species in the study area, as well as in estuarine waters in other locations (Cervigón, 1991; Giarrizzo and Krumme, 2007; Froese and Pauly, 2017). Also, it is part of the fishery resources in some areas like northeastern of Venezuela (Betancur et al., 2004) and Brazil (Barletta and Costa, 2009). The present study was carried out in order to describe infestation of parasitic copepod and analyze its relationships with total body length, sex, maturity and lesions on skin of the host fish.

2. Material and methods

2.1. Fish sampling and examinations

Specimens of *S. herzbergii* for the study were collected in July and December of 2008 in "Las Marites" lagoon, located at Southeastern coast of Margarita Island in Northeastern of Venezuela (10º53′50″-10º55′N 63º53′54″- 63º57′20″ W). More information at this lagoon is given in Palazón et al. (1996).

Fish were transported alive to laboratory, where they were anesthetized with Benzocaine. In each fish, body surface (integument) was revised in detail for presence of attached ectoparasitic copepods.

Copepods were removed from fish by performing skin scrapings using tweezers. All the samples (scrapings) were separately placed in Petri dishes with filtered seawater and observed under a stereoscopic microscopy.

The attached copepods were divided by sex and stage of development in phase I (sexually mature) and phase II (immature). For taxonomic identification, we used the keys proposed by Boxshall and Montú (1997) and Cressey (1991).

Every fish was measured to total length with a vernier of 1 mm of precision and individuals were identified using the taxonomic keys proposed by Cervigón (1991). Sex was determined by direct observation of the primary and secondary characters of the gonads in dissected specimens (Eslava, 2004). For both sexes, the stage of maturity was discriminated in two phases (immature and mature). We grouped the individuals into two length categories to determine the intensity of infestation of ectoparasitic copepod according to size, because it is expected to higher values in larger individuals, related mainly with more surface area. We chose two sizes to have an approximate number of individuals in each one (37 small fish and 24 big fish).

2.2. Histology of the tegument of fish host

Immediately after the death of the fish, skin samples were dissected from sites with and without attached copepods. Some skin samples were fixed for 24–48 h in 10% formalin, buffered with 0.1 M phosphate buffer at pH 7.2 and others in Bouin's liquid for 24 h. The formalin-fixed samples were then washed in running water for four (4) hours to remove excess fixative, while the liquid-fixed samples were washed repeatedly with 70% ethanol. Subsequently, all the skin samples were processed until their inclusion in paraffin, in order to obtain cuts of 8 μm thick, which were stained with hematoxylin–eosin. Samples were examined in the optical microscope to compare the architecture of the skin in infested and non-infested areas and to detect and describe lesions due to the infestation.

2.3. Data analysis

Prevalence, intensity and mean intensity of infestation were calculated according to Jensen et al. (2016).

Relationships of host length and the infestation intensity of *Lepeophtheirus* sp. were determined using the Pearson correlation coefficient (r). While the Wilcoxon rank X^2 test was used to determine the possible association between sex, sexual maturity or size length of the host with the infestation. In addition, a contingency table including both sexual aspects were created, and a X^2 test with Yates' correction for continuity to prevent overestimation of statistical significance for small dataset was implemented. In all analyses a significance level of $\alpha = 0.05$ was used. Statistics were computed using the R statistical language (R Foundation for Statistical Computing).

3. Results

3.1. Infestation of copepods on host fish

All sea louses found and observed microscopically were identified as *Lepeophtheirus* sp based on morphological features. These copepods were attached on skin at different regions of the body of juveniles and adults of host fish, showing not a regular pattern of preference. A total of 204 copepods were sexually mature and 14 were immatures.

Prevalence of infestation was 80.3% and intensity between 1 and 15 copepods/fish with a mean value of 3.5 copepods/fish.

3.2. Host fish conditions

A total of 61 specimens *S. herzbergii* were analyzed with total body length between 200.00 and 389.00 mm (mean ± SD = 292.86 ± 42.06 mm). Of the total number of individuals, 37 were females with body length between 222.50 and 389.00 mm (mean ± SD = 259.41 ± 110.44 mm) and 24 males with body length between 200.00 and 372.50 mm of total length (mean ± SD = 280.60 ± 34.56 mm). Examination of the external characters of juvenile and immature adults showed no visible differences between males and females.

The analysis of the relationship between total body length of host and intensity of the infestation, showed a weak positive correlation ($r = 0.328$; $p = 0.009$).

In *S. herzbergii,* 52.46% of total females were infested in contrast to 27.87% of infested males. However, the Wilcoxon rank test allowed to establish that the presence of the attached *Lepeophtheirus* sp is independent of the sex of *S. herzbergii* ($W = 458.5$; $p = 0.834$) or the

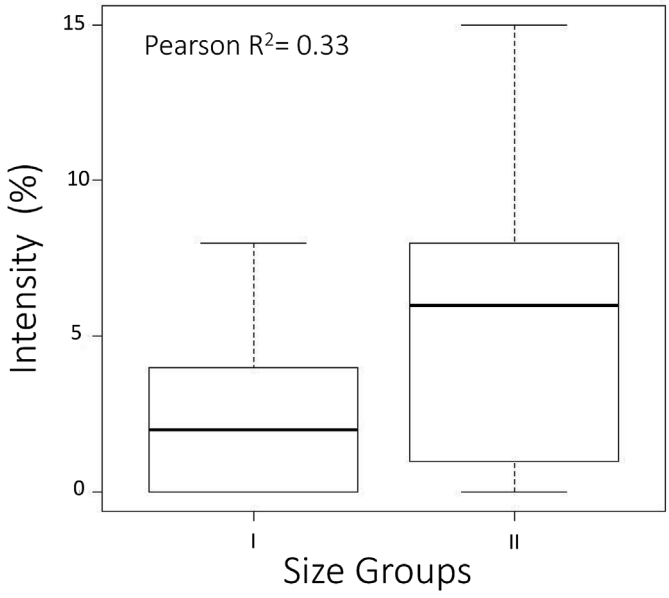

Fig. 1. Prevalence of *Lepeophtheirus* sp in two size groups of *Sciades herzbergii.*