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Original

Community structure of metazoan parasites in the Splittail bass *Hemanthias peruanus* (Serranidae) from northern Peru

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ABSTRACT

Objective. To assess the community structure of helminths and parasitic crustaceans in the splittail bass *Hemanthias peruanus* (Steindachner, 1875) from northern Peru. **Materials and methods.** 75 specimens (34 males and 41 females) of *H. peruanus* were captured from Puerto Cabo Blanco, Piura, Peru. Total length and sex data of the fish were recorded. For the analysis of the parasitic community, the ecological parasitological indices, aggregation indices, alpha diversity indices and association between the biometric parameters of the fish and the parasitological indices were calculated. **Results**. The percentage of total prevalence in splittail bass infected with at least one parasitic metazoan species was 65.33%, that is, 49 parasitized hosts. The community component of the parasitic eumetazoan fauna in the evaluated fish was dominated by the presence of ectoparasites (three species of monogeneans and one species of isopod). Endoparasite abundance (two species of trematodes, one species of tapeworms and one species of acanthocephalan) was comparatively low. Biotic factors such as fish length and sex were not found to be related to parasitological indices of any parasitic species. The lack of association is probably due to the influence of other biotic or abiotic factors. The most prevalent parasites presented an aggregate type distribution. The Chao-1 estimator indicates that the expected richness was eight. **Conclusions**. We registered *H. peruanus* as a new host for *Ceratothoa gaudichaudii*, *Corynosoma australe*, *Parancylodiscoides signifer*, *Pronotogrammella scholzi* and *Scolex pleuronectis*.

Keywords: Animal parasitology; animal ecology; helminths; *Hemanthias peruanus*; metazoan; parasite *(Source: ICYT).*

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RESUMEN

Objetivo. Evaluar la estructura comunitaria de helmintos y crustáceos parásitos en la doncella *Hemanthias peruanus* (Steindachner, 1875) procedente del norte de Perú. **Materiales y métodos.** Se capturaron 75 especímenes (34 machos y 41 hembras) de *H. peruanus* procedentes del Puerto Cabo Blanco, Piura, Perú. Se registraron los datos de longitud total y el sexo de los peces. Para el análisis de la comunidad parasitaria, fueron calculados los índices ecológicos parasitológicos, índices de agregación, índices de diversidad alfa y asociación entre los parámetros biométricos de los peces y los índices parasitológicos. **Resultados.** El porcentaje de prevalencia total en las doncellas infectadas con al menos una especie metazoaria parásita fue del 65.33%, es decir 49 hospederos parasitados. El componente comunitario de la fauna de eumetazoos parasitaria en los peces evaluados estuvo dominada por la presencia de ectoparásitos (tres especies de monogeneos y una especie de isópodo) frente a la poca abundancia de especies endoparásitos (dos especies de tremátodos, una especie de céstodo y una especie de acantocéfalo). Los factores bióticos como la longitud y el sexo de los peces no se encontraron relacionados con los índices parasitológicos de ninguna especie parásita. Probablemente la falta de asociación se deba a la influencia de otros factores bióticos o abióticos. Los parásitos más prevalentes presentaron una distribución del tipo agregada. El estimador de Chao-1 mide que la riqueza esperada fue de ocho. **Conclusiones.** Registramos a *H. peruanus* como nuevo hospedero para *Ceratothoa gaudichaudii*, *Corynosoma australe*, *Parancylodiscoides* signifer, *Pronotogrammella scholzi* y *Scolex pleuronectis*.

Palabras clave: Ecología animal; Helmintos; *Hemanthias peruanus*; Metazoos; parasitología animal; parásito *(Fuente: ICYT).*

INTRODUCTION

The Humboldt Current system has three areas of high richness for marine species, the maritime north of Peru being one of them (1). However, the evaluation or assessment of parasitic species has not been considered despite being an important fraction of the planet's biodiversity (2,3). Despite this, more and more investigations are being carried out on parasites in marine fish (4), thus increasing the documentation of reports in recent years of new species, new geographic records and new host-parasite interactions (2,5,6,7).

In Peru there are registered, to date, more than 1,630 species of fish (marine and freshwater), of which only 207 species have been reported as hosts of metazoan parasites in records published up to May 2016 (6,7). According to this information, 1,423 species of fish in Peru still do not have any study of parasites.

"Splittail bass" is the common name of *Hemanthias peruanus* (Steindachner, 1875), a marine, pelagic-neritic species, with a depth range of 20–120m and of importance in the artisanal fishery (8). This species belongs to the Serranidae family and is distributed in the eastern Pacific, from Baja California, Mexico to northern Chile, including the Galapagos Islands (9,10). It is a tropical species that is part of the diet of cartilaginous marine fish (11).

The first documented report of parasites in *H. peruanus* is the copepod *Blias prionoti* Krøyer, 1863, off the coast of Piura, Peru (12). Later, Luna et al (13) made the first records of the digeneans *Lecithochirium magnaporum* Manter, 1940 (Hemiuridae), *Gonocerca crassa* Manter, 1934 (Derogenidae) and *Tentacularia coryphaenae* Bosc, 1797 (Tentaculariidae) in *H. peruanus* from Tumbes, Peru. Finally, Cruces et al (14) describe a new species of monogenean of the Dactylogyridae family: *Parancylodiscoides peruensis* Cruces, Chero, Sáez & Luque, 2017, obtained from the same locality.

Due to the scarce information about parasitological studies in *H. peruanus*, the objective is to determine the community structure of parasitic helminths and crustaceans in *H. peruanus* from Northern Peru, as well as the degree of association between biometric parameters of the fish and their parasites.

MATERIALS AND METHODS

Collection of the material. 75 specimens of the splittail bass fish *H. peruanus* were acquired at the fishing terminal in the district of Villa María del Triunfo, Lima, Peru. The fish were caught by artisanal fishermen and brought from Puerto Cabo Blanco, Talara province, Piura department, Peru. For the identification of *H. peruanus*, specialized taxonomic keys were used (15). Before the necropsy of the fish, the total length (LT) and sex (S) data were taken. To collect the parasites, the oral cavity, gills, coelomic cavity, stomach, small intestine, large intestine, pyloric cecum, gonads, heart, swim bladder, kidneys, liver and spleen of the fish were checked. The parasites were collected and preserved in 70% ethyl alcohol (16).

Processing of samples. For the taxonomic study, the helminths were stained in carmine acetic acid and alternatively in Gomori trichrome, dehydrated in concentrations of 50%, 70%, 90% and 100% of ethyl alcohol, diaphanized in eugenol and mounted in Canada balsam (16,17). The specimens were deposited in the Helminths Parasites and Related Invertebrates collection - HPIA of the zoological collection of the Natural History Museum of Universidad Nacional Federico Villarreal - MUFV, the codes are shown in table 1.

Analysis of the samples. For the analysis of the parasitic community, the parasitological ecological indices of prevalence (P), abundance (AM) and mean intensity (MI) of infection were calculated following the indications by Bautista-Hernández et al. (18). The type of strategy (TE) of each parasitic species was evaluated according to the prevalence percentage, for which the species were classified as "core" species for species with prevalence's greater than 45%, "secondary" species for prevalence's between 45-10% and "satellite" species for prevalence's less than 10% (7,18). The specific importance index (IE) calculated as the importance of each parasitic species in the ecological assembly was used, in order to obtain an integrated infection index of both ecological descriptors (19): IE=P+(AM x 100). Where: IE = index of specific importance, P = Prevalence, AM = Mean abundance of infection.

For the case of parasitic species with prevalence's greater than 10%, the dispersion indices (ID) were used to determine the type of spatial distribution of the parasitic populations, Poulin's discrepancy (DP) and K of the negative binomial equation with its respective value of Chi square (X^2) to determine the degree of aggregation (20). The calculations were performed using the statistical package Quantitative Parasitology 3.0 (21).

The TL of the fish was separated into ranges using Sturges rule as a criterion to determine the number of intervals, from which their own P values were calculated. To evaluate the association between this parameter and the TL, the Spearman correlation coefficient was calculated (7). Similarly for AM and MI, Pearson's correlation coefficient was used to determine the relationship of fish TL with the AM and MI of each parasite species.

2x2 contingency tables were used to calculate the degree of association between the sex of the host and P% of each parasite using X^2 and the Likelihood Ratio test. Student's t test was used to compare the AM of each parasite and the sex of the host. The analysis of the parasites in relation to the size and sex of the host was carried out only for species with prevalence greater than 10% (7). In all the previous cases, the normality of the data was verified using the Kolmogorov-Smirnov test with the modification of Lillierfors and the homocesticity of variances based on the Levene test.

The level of significance was evaluated at a level of alpha = 0.05. For the determination of the descriptive and inferential statistics, the statistical package IBM SPSS Statistics 24 was used.

The parasitic community component was the next level evaluated, for which the richness (S), the Menhinick richness index (DMn), the parasitic diversity with the Shannon-Wiener index (H) were determined, the equity was determined by the Pielou uniformity index (J), the Simpson dominance (D) and the estimate of the number of expected species was calculated with the Chao-1 index (19).

Ethical aspects. The procedures to collect the diversity of the parasitic fauna in *H. peruanus* minimized the number of organisms used, repetitions and using the three R's "Replacement, Reduction and Refinement" and Resolution 2558-2018-CU-UNFV that includes the code of Ethics for Research at the Universidad Nacional Federico Villarreal (UNFV), Peru. For the management of parasitic fauna, the guidelines of the Peruvian animal protection and welfare law (Law No. 30407: Article 19) were followed.

RESULTS

The population structure of the *H. peruanus* splittail bass was composed of 75 specimens, distributed between 34 males (45%) and 41 females (55%). The total length of the splittail bass was in the range of 22.4cm - 40cm (Mean ± SD = 28.67 ± 4.08cm). Splittail bass fish males ranged from 22.4cm - 39.5cm (29.8 ± 4.54 cm) and females 23cm - 40cm (27.74 ± 3.42cm).
The total prevalence percentage in the splittail bass infected with at least one parasitic metazoan species was 65.33%, that is, 49 parasitized hosts. The species with the highest prevalence and numerical abundance were the monogeneans *P. peruensis* with 33 hosts infected with this species, *Parancylodiscoides signifer* Cruces, Chero, Sáez & Luque, 2017 with 25 hosts and the isopod *Ceratothoa gaudichaudii* (H. Milne Edwards, 1840) with eight hosts. No core species were found in this fish, only species with prevalence's below 45% (Table 1).

Table 1. Ecological descriptors of parasites associated with *Hemanthias peruanus* from northern Peru.

Parásites	**SI**	**P %**	**MA ± SD**	**MI ± SD**	**SIM**	**TS**	**MUFV**
MONOGENEA							
Parancylodiscoides peruensis	G	44	4.97 ± 1.19	11.3 ± 0.31	541.33	secundary	HPIA:40
Parancylodiscoides signifer	G	33.33	2.15 ± 0.53	6.44 ± 0.19	248	secundary	HPIA:41
Pronotogrammella scholzi	G	8	0.31 ± 0.13	3.83 ± 0.19	38.67	satelite	HPIA:42
TREMATODA							
Lecithochirium magnaporum	S	2.67	0.04 ± 0.03	1.5 ± 0.13	6.67	satelite	HPIA:43
Digenea g. sp.	SI	8	0.08 ± 0.03	1 ± 0.05	16	satelite	HPIA:44
CESTODA							
Scolex pleuronectis	M	1.33	0.04 ± 0.04	3 ± 0.35	5.33	satelite	HPIA:45
ACANTHOCEPHALA							
Corynosoma australe	M	2.67	0.11 ± 0.08	4 ± 0.33	13.33	satelite	HPIA:46
CRUSTACEA							
Ceratothoa gaudichaudii	BC	10.67	0.19 ± 0.07	1.75 ± 0.07	29.33	secundary	HPIA:47

SI = Site of infection. P% = Percentage of prevalence. MA = mean abundance of infection. MI = mean intensity of infection. SIM = specific importance. TS = Type of strategy. HPIA = collection of Helminth Parasites and Allied Invertebrates - HPIA. MUFV = Zoological collection of the Natural History Museum of the Universidad Nacional Federico Villarreal. G = Gills. S = Stomach. BC = branchial cavity. SI = Small intestine, M = Mesenteries.

The aggregation indices were calculated for the species *P. peruensis*, *P. signifer* and *C. gaudichaudii* for presenting prevalences above 10%. The DI index shows that the three species are not homogeneously or randomly distributed in the splittail bass population, on the contrary, they presented an aggregate or contagious type distribution (ID> 1), which is expected in parasitic species in general. while the DP index indicates that *P. peruensis* presented a lower degree of aggregation than the other species, which would indicate that this species had a lower concentration in many hosts and that most of the hosts were infected by this species (Table 2).

Table 2. Aggregation indices to evaluate the dispersion of the most prevalent parasites in *Hemanthias peruanus*.

Indices	*PP*	*PS*	*CG*
Dispersion Index (DI)	21.43	9.98	1.84
p/ interpretation	99.04 / A	38.65 / A	1.91 / A
Poulin discrepancy (PD)	0.73	0.8	0.9
Interpretation	A	A	A
Negative binomial exponent (K)	0.19	0.16	0.13
p/ Interpretation	0 / A	0.05 / A	**

PP: *Parancylodiscoides peruensis; PS: Parancylodiscoides signifer; CG: Ceratothoa gaudichaudii;* A= aggregated. ** Sample too small to check the fit of the negative binomial distribution.

The total length of the *H. peruanus* splittail bass examined in this study was not found to be related to the parasitological indices for any parasitic species. Likewise, no degree of association was observed between sex and the three most prevalent parasite species (Table 3).

Table 3. Inferential statistics of the parameters of total length and sex of the fish with the parasitological indices.

Parameter	*PP*	*PS*	*CG*
TL *vs* P	*r*=0.48 *p*=0.33	*r*=0.70 *p*=0.12	*r*=-0.80 *p*=0.05
TL *vs* MI	*r**=0.18 *p*=0.30	*r**=0.27 *p*=0.19	*r**=0.04 *p*=0.93
TL *vs* MA	*r**=0.15 *p*=0.19	*r**=0.17 *p*=0.14	*r**=0.15 *p*=0.19
Sex *vs* P	X^2=0.24 *p*=0.63	X^2=1.32 *p*=0.25	X^2 =1.06 *p*=0.30
Sex *vs* MI	*t*=1.59 *p*=0.13	*t*=0.99 *p*=0.35	*t* =1.37 *p*=0.22
Sex vs MA	t=1.63 *p*=0.11	t=0.15 *p*=0.88	t=1.38 *p*=0.18

PP: *Parancylodiscoides peruensis; PS: Parancylodiscoides signifer; CG: Ceratothoa gaudichaudii;* TL=Total length. MI=Medium intensity. MA=Medium abundance. P=prevalence. F=Levene's test. *p*=level of significance. r=Spearman's correlation. r* =Pearson's correlation. t=Student's t test. X^2=Chi square test.

Regarding the diversity indices, the species richness (S), the Shannon diversity (H), Menhinick (DMn), Equity (J) and the non-parametric estimator of Chao-1 show higher values in the female population. Only the number of parasite individuals and the Simpson dominance index (D) were slightly higher in males. The Chao-1 estimator estimates that the expected richness in this study is the same as that found, that is, the level of effort was optimal (Table 4).

Table 4. Alpha diversity for parasites according to sex and population of the fish evaluated.

Alpha Diversity	*Hemanthias peruanus*		
	Total	**Males**	**Females**
Richness S	8	6	8
Individuals	591	340	251
Menhinick DMn	0.33	0.33	0.51
Shannon H	1.02	0.84	1.19
Equity J	0.49	0.47	0.57
Dominance of Simpson D	0.48	0.55	0.40
Chao-1	8	6	8

DISCUSSION

The community component of the parasitic eumetazoan fauna in the evaluated fish was dominated by the presence of monogenean ectoparasites compared to the low abundance of endoparasitic species in *H. peruanus*. The high or low association of ectoparasites with their hosts is often influenced by factors such as habitat, behavior, and host density, as well as environmental characteristics (eg, depth and temperature) (22). The high dominance of ectoparasites in *H. peruanus* would also show a better adaptation of this group against endoparasites; however, in a hypothetical scenario where host extinction occurs, ectoparasites would be the most susceptible, producing the co-extinction phenomenon (23,24).

The endoparasites in this study, although they were low in abundance, have been present both in immature forms (tapeworms and acantocephalan) and in their adult state (trematodes). Fish endoparasite communities can vary and be determined by the feeding habits of the hosts (e.g., specialized versus generalist predators), their ontogenetic and geographic changes (25), as well as by the availability of different prey species (intermediate hosts) in a given environment. The presence of adult parasites and larvae in *H. peruanus* can be considered as a sign of an intermediate level in the food web, since it is part of the diet of predatory cartilaginous fish such as *Sphyrna zygaena* (Linnaeus, 1758) in the marine ecosystem (eleven).

In the species *C. gaudichaudi*, *P. peruensis* and *P. signifer*, the aggregation indices showed aggregate distribution, which is influenced by intrinsic and extrinsic factors such as: (a) spatial heterogeneity of the fish habitat that produces differences in susceptibility (b) influence on the evolutionary history of the parasite by food, spatial and reproductive competition; (c) improvement in the opportunity to infect the fish, and (d) prevention of the collapse of the host population due to the effects of parasitism (19). Likewise, aggregation was the expected model in species with prevalence's above 10% since this pattern is predominant in many natural host-parasite systems (7,20,26).

The lack of association between length and sex of *H. peruanus* in relation to parasitological parameters (P%, AM, IM) suggests that other local ecological factors may be more influential in regulating the *H. peruanus* parasite community, such as the geographical area, the decrease in intermediate hosts, the seasonality of the fish (27,28,29).

The *Parancylodiscoides* species Caballero & Bravo Hollis, 1961, were considered exclusive to the fish of the Ephippidae family (30), until four years ago when Kritsky and Bakenhaster (31) observed the *Haliotrema macrobaculum* species on *Parancylodiscoides, P. macrobaculum* representing the first record of this genus in a serranid host fish (Serranidae). Later Cruces et al. (14); describe two new species: *Parancylodiscoides peruanus* and *P. signiferi* in the fishes *H. peruanus* and *H. signifer*, respectively, supporting the observation that these monogeneans are not exclusive to a single family of fish. These same authors register values of P = 25% (3/12), P = 25% (4/16) and MI = 17, MI = 11, respectively. This is similar to what was observed in this study where the P% of both species *P. peruanus* and P. signiferi were between 33% - 44%, slightly higher than that recorded by Cruces et al. (14) however MI was lower in this study.

Another monogenean species found in this study was *Pronotogrammella scholzi* Cruces, Chero, Sáez & Luque, 2020, which was found in low prevalence (P% = 8). This species was originally described from *Pronotogrammus multifasciatus* Gill, 1863 collected in Puerto Pizarro, Tumbes, Peru (32). In our study we registered H. peruanus as a new host for *P. scholzi*, as well as information about its parasitological indices.

The isopod *C. gaudichaudii* has previously been reported infecting nine fish in Peru (6). The low host specificity of *C. gaudichaudii* was observed by Brusca (33), so it would behave as a generalist species. Likewise, it has been considered a satellite or rare species due to its low prevalence of infection in marine fish off the Peruvian coast (27,34). On the contrary, in this evaluation we report the behavioral strategy of the secondary type (P> 10%) of *C. gaudichaudii* in *H. peruanus*. Martin et al. (35) consider *C. gaudichaudii* as an species inquirenda, that is, uncertain, because they observed that these specimens were indistinguishable from the Australian specimens of *Ceratothoa imbricata* (Fabricius, 1775) and therefore should be considered as a minor synonym of *C. imbricata*. In our study we have followed Brusca (33) to determine this species as *C. gaudichaudii*, but we consider that a review should be made of the species of the genus *Ceratothoa* recorded in Neotropical Pacific fish.

Tetraphyllid metacestode larvae have been found parasitizing *H. peruanus*, as a rare species. Because they are difficult to identify due to the lack of features in the morphology of their scolex and the diagnostic characteristics of adult tapeworms, they were cataloged as *Scolex pleuronectis* Mueller, 1788 to encompass metacestodes that share characteristics common to this order of tapeworms (27). The numerous articles published in the last 30 years in South America, mainly ecological ones like this study, focused on marine teleost hosts as models, include a high number of records of unidentified larvae and most of them correspond to the Tetraphyllidea named as *Scolex* spp. (36). Of these studies carried out in South America, 12 hosts were reported for Peru (6), but not *H. peruanus*, making this study the first to report this host-parasite relationship.

Like the tetraphyllid larvae, the thorny-headed worms *C. australe* was also found in *H. peruanus*, with prevalence below 10%. *Corynosoma australe* was found in the form of a cysticanth in this host, which according to Hernández-Orts et al. (37) would act as a paratenic host and could have become infected by feeding on parasitized amphipods and thus use the trophic gap between intermediate hosts to reach their final hosts, which are usually marine mammals and birds. With the samples obtained in this study, *H. peruanus* is added as a new host for the genus *C. australe*.

Finally, the presence of *Lecithochirium magnaporum* was observed in *H. peruanus*, which was recorded in *H. peruanus* and *H. signifer* from Puerto Pizarro, Tumbes - Peru, by Luna et al. (13), being the first record in Peru for this Hemiuridae trematode. It is estimated that the P% of this trematode in the work of Luna et al. (13) was 12.1% and 28.8% in *H. peruanus* and *H. signifer*, respectively. On the contrary, in our study the infection rates were lower, coming to consider it a satellite species in this work.

In conclusion, the community of parasitic eumetazoa diversified between endoparasite and ectoparasite species, the latter being the one that dominated the *H. peruanus* parasitic community. Likewise, *H. peruanus* is considered as a new host for *C. gaudichaudii*, *C. australe*, *P. signifer*, *P. scholzi* and *Scolex pleuronectis*. This is the first study of an ecological aspect in the parasitic fauna of H. peruanus. Finally, the authors encourage to continue contributing with ecological studies of the parasitic fauna of *H. peruanus* to determine if the parasite populations tend to a sequential succession over time or a random succession (38).

Conflict of interests

The authors declare that there was no conflict of interest of any kind during the preparation and preparation of this work.

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