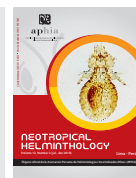




Neotropical Helminthology



ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

IMPLICATIONS OF THE SANITARY QUALITY OF BEACHES IN THE PARASITIC ECOLOGY OF *EMERITA ANALOGA* (STIMPSON, 1857) (DECAPODA: HIPPIDAE)

IMPLICANCIAS DE LA CALIDAD SANITARIA DE LAS PLAYAS EN LA ECOLOGÍA PARASITARIA DE *EMERITA ANALOGA* (STIMPSON, 1857) (DECAPODA: HIPPIDAE)

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ABSTRACT

The power of contamination of domestic effluents lies in the amount of organic matter and microorganisms they contain. The objective of the present study was to determine the implications of effluent impact on the parasitic ecology of the mole sand crab *Emerita analoga* in the central coast of Peru. A total of 5287 specimens of *E. analoga* were collected from 13 beaches off Lima, Peru between July 2001 and June 2016. The influence of the sanitary quality established by the quantity of thermotolerant coliforms (NMP·100mL⁻¹) in relation to parasitic indexes, the length of the cephalothorax and sex of *E. analoga*, according to date and place of collection, and distance from the tide line. The parasites found were: *Profilocollis altmani* (Perry, 1942) Van Cleave, 1947 (Acanthocephala) and *Proleptus carvajali* (Fernandez & Villalba, 1985) (Nematoda). The prevalence of *P. altmani* and *P. carvajali* was 57.8% and 45.3%, reaching an average abundance of 4.27 and 1.10, respectively. The shortest length of the cephalothorax of *E. analoga* was found on the beach "Conchán" characterized as "Unhealthy", according to the level of thermotolerant coliforms, in September 2013. No differences were observed in the prevalence ($p = 0.72$) and average abundance ($p = 0.37$) of *P. altmani* with respect to sanitary quality. The prevalence ($p = 0.02$) and mean abundance ($p = 0.03$) of *P. carvajali* was significantly different in relation to sanitary quality. The 9 collection dates showed uniformity in the prevalence and average abundance of *P. altmani* ($p = 0.11$, $p = 0.16$) and *P. carvajali* ($p = 0.59$, $p = 0.18$). The 13 beaches showed a significant discontinuity around the prevalence and average abundance of *P. altmani* ($p = 0.01$, $p = 0.05$) and *P. carvajali* ($p = 0.02$, $p = 0.01$). "Pucusana", qualified as "Healthy", presented the highest prevalence and average abundance of *P. altmani*. "San Pedro" qualified as "Healthy" presented the highest prevalence and average abundance of *P. carvajali*. There is a relationship between the season of the year and the parasite

mean abundance ($p = 0.006$), but not in relation to prevalence ($p = 0.31$). The degree of environmental disturbance derived from the discharge of wastewater, urban and industrial, leads to the proposal of the use of the helminths present in *E. analoga* as indicators of sanitary quality.

Keywords: sanitary quality – crustacean – *Emerita analoga* - helminth – parasite – Lima beaches

RESUMEN

El poderío de contaminación de los efluentes domésticos radica en la cantidad de materia orgánica y microorganismos que contienen. El objetivo del presente estudio fue determinar las implicancias de la calidad sanitaria de las playas en la ecología parasitaria del “Muy Muy” *Emerita analoga* en la costa central del Perú. Se recolectaron 5287 especímenes de *E. analoga* de 13 playas del departamento de Lima, Perú entre julio del 2001 a junio del 2016. Se analizó la influencia de la calidad sanitaria establecida por la cantidad de coliformes termotolerantes ($\text{NMP} \cdot 100\text{mL}^{-1}$) en relación a los índices parasitarios, la longitud del cefalotórax y sexo de *E. analoga*, según fecha y lugar de colecta, y distancia desde la línea de marea. Los parásitos encontrados fueron: *Profilicollis altmani* (Perry, 1942) Van Cleave, 1947 (Acanthocephala) y *Proleptus carvajali* (Fernandez & Villalba, 1985) (Nematoda). La prevalencia de *P. altmani* y *P. carvajali* fue de 57,8 % y 45,3 %, alcanzando una abundancia media de 4,27 y 1,10, respectivamente. La menor longitud del cefalotórax de *E. analoga* se encontró en la playa “Conchán” calificada como “No Saludable”, según el nivel de coliformes termotolerantes, en septiembre del 2013. No se observó diferencias en la prevalencia ($p=0,72$) y abundancia media ($p=0,37$) de *P. altmani* con respecto a la calidad sanitaria. La prevalencia ($p=0,02$) y abundancia media ($p=0,03$) de *P. carvajali* fue significativamente distinta en relación a la calidad sanitaria. Las 9 fechas de colecta exhibieron uniformidad en la prevalencia y abundancia media de *P. altmani* ($p=0,11$; $p=0,16$) y *P. carvajali* ($p=0,58$; $p=0,18$). Las 13 playas demostraron una discontinuidad significativa en torno a la prevalencia y abundancia media de *P. altmani* ($p=0,01$; $p=0,05$) y *P. carvajali* ($p=0,02$; $p=0,01$). “Pucusana”, calificada como “Saludable”, presentó la mayor prevalencia y abundancia media de *P. altmani*. “San Pedro” calificado como “Saludable” presentó la mayor prevalencia y abundancia media de *P. carvajali*. Existe una dependencia entre la estación del año y el parásito, expresada en la abundancia media ($p=0,006$), pero no en relación a la prevalencia ($p=0,31$). El grado de disturbio ambiental derivado del vertido de aguas residuales, urbanas e industriales conlleva a la propuesta del uso de los helmintos presentes en *E. analoga* como indicadores de la calidad sanitaria.

Palabras clave: calidad sanitaria – crustáceo – helminto – parásito – playa

INTRODUCCIÓN

The concept of ecosystem health has played a role in environmental management, the public domain and even in our current scientific and legislative lexicon (Marcogliese, 2005), the justification for incorporating the concept lies in the search for the stability of ecological systems (Merrill et al., 2018).

The Coastal-Marine Zone (ZCM), is the geomorphological space inside and outside the

mediolitoral zone, in which the interaction between the marine and terrestrial systems takes place (CE, 2008ab). This space is under constant anthropogenic pressure, due to a high human population. Beaches are important because they give us a chance to reconnect with nature nearby. The depopulation of the Andean area in favor of the coast, established as littoralization (Meneses, 1998), is evidenced by the fact that 58% of the population inhabits the coasts of Peru (INEI, 2018).

Marine pollution is a problem that has been

growing exponentially, exceeding the limits of the self-purifying power of the sea (Savichtcheva & Okabe, 2006), according to DIGESA (Direction of environmental health of Peru) (1997) in Metropolitan Lima, about $15 \text{ m}^3 \cdot \text{s}^{-1}$ of domestic wastewater are discharged into the sea by means of six collectors, one of them through the Rímac River, the main source of water for the capital of Peru.

Faced with this situation, different protocols for the detection and monitoring of marine pollution have been proposed (Williams & Mackenzie, 2003; Borja *et al.*, 2008; Crain *et al.*, 2008), from the use of faecal contamination indicator organisms (Sumampouw & Riskani, 2014); Benthic invertebrates as accumulators of toxic substances (Birk *et al.*, 2012); tools for assessing nested environmental status (NEAT), this multi-proxy approach being one of the most integrative methods (Andersen *et al.*, 2014; Nemati *et al.*, 2017); to a perspective of the beaches as socioecological systems (Botero & Hurtado, 2009).

The use of parasites as potential indicators of marine pollution has been analyzed in different studies (Möller, 1987; Khan & Thulin, 1991; Mackenzie *et al.*, 1995; Lafferty, 1997; Mackenzie, 1999; Lewis *et al.*, 2003; Pietrock & Marcogliese, 2003; Williams & Mackenzie, 2003; Marcogliese, 2005; Monserrat *et al.*, 2007; Blonar *et al.*, 2009; Gilbert & Avenant-Oldewage, 2017).

Parasites are omnipresent (Marcogliese, 2005), parasitism being the most common interspecific relationship on Earth (Price, 1980). Each species of parasite reflects the participation and trophic interaction of different links in an ecosystem (Latham & Poulin, 2002; Marcogliese, 2005), some stages of transmission of free life (heteroxene cycle), are highly sensitive to environmental conditions (Mackenzie, 1999), finally, the eggs of certain parasites may be in contact with the marine sediment, expressing the health of the ecosystem (Williams & Mackenzie, 2003).

Emerita analoga (Stimpson, 1857) (Anomura: Hippidae) is a crustacean hermaphrodite protandrid (Subramonian & Gunamalai, 2003). This marine organism lives in a sedentary state as an adult on sandy beaches in the middle and upper

infralittoral zones (Contreras *et al.*, 1999; Bhaduri *et al.*, 2018), in a bathymetric range between 0 and 3 m (Retamal, 2000). Its geographic distribution extends from Alaska, USA (58° N) to Aysén, Chile (55° S), although it is absent in tropical zones (Tam *et al.*, 1996).

Emerita analoga occupies a central position, constituting an important component in the food chain of the coastal marine sandy ecosystem (Alvitres *et al.*, 1999b). This crustacean acts as an intermediate host for some parasites (Kolluru *et al.*, 2011). In Peru, seven metazoan parasites have been recorded and described for *E. analoga*, among which is thorny-headed worms *Profilicollis altmani* (Perry, 1942) Van Cleave, 1947 and the nematode *Proleptus carvajali* Fernández & Villalba, 1985 (Fernández & Villalba, 1985; Oliva *et al.*, 1992; Alvitres *et al.*, 1999b; Nickol *et al.*, 1999; Tantaleán *et al.*, 2005; Iannacone *et al.*, 2007; Iannacone & Alvariano, 2009; Oliva *et al.*, 2008; Chero *et al.*, 2014).

One of the causes of alteration of the sanitary quality of the beaches is due to an increase in the quantity of thermotolerant coliforms, generating eutrophication processes that hinder the development of the life cycle of numerous organisms from benthic organisms to the parasites that they can harbor. In this sense, the present investigation determines the implications of the sanitary quality of the beaches on the parasitic ecology of *E. analoga*, in the central coast of Peru.

MATERIAL AND METHODS

Periodically, from July 2001 to June 2016, specimens of *E. analoga* from 13 sandy beaches of the were collected, one was located in the province of Huaura and the other 12 in the province of Lima, from north to south, the beaches were the following: “Los Viños” ($10^\circ 89' \text{ S}$; $77^\circ 69' \text{ W}$), “Hermosa” ($11^\circ 77' \text{ S}$; $77^\circ 18' \text{ W}$), “Playa Chica” ($11^\circ 80' \text{ S}$; $77^\circ 17' \text{ W}$), “Costa Azul” ($11^\circ 87' \text{ S}$; $77^\circ 15' \text{ W}$), “Los Delfines” ($12^\circ 11' \text{ S}$; $77^\circ 05' \text{ W}$), “Los Yuyos” ($12^\circ 15' \text{ S}$; $77^\circ 02' \text{ W}$), “Agua Dulce” ($12^\circ 16' \text{ S}$; $77^\circ 02' \text{ W}$), “Venecia” ($12^\circ 23' \text{ S}$; $76^\circ 97' \text{ W}$), “Conchán” ($12^\circ 25' \text{ S}$; $76^\circ 93' \text{ W}$), “San Pedro” ($12^\circ 29' \text{ S}$; $76^\circ 87' \text{ W}$), “El Silencio”

(12°31'S; 76°83'W), "Santa María del Mar" (12°40'S; 76°77'W) and "Pucusana" (12°48'S; 76°79'W).

The collection methodology for *E. analoga* was carried out through transects, 4 transects were located on each beach and separated from each other by 100 m, each transect started on the tide line (lower level of the supralittoral) and finished offshore (Veas *et al.*, 2013). The collection was made every 5 m (Sánchez & Alamo, 1974; Lépéz *et al.*, 2001), starting from the tide line and ending at the "station - 40 m", therefore, there were 9 sampling stations. To obtain the sample, a PVC cylinder 1 m long with a diameter of 15 cm (0.003 m³) was introduced into the sand for up to 20 cm and then sifted in 2 mm sieve (Penchaszadeh, 1971).

In the laboratory the sex of the specimen was determined following Sánchez & Alamo (1974), for the male the genital apparatus ends in a genital papilla located at the base of the fifth pereopods, the abdomen lacks pleopods; On the other hand, in females, the genital pore is found in the coxa of the third pair of pereopods, in addition they present three pairs of pleopods whose function is to carry the eggs during incubation. In parallel, the length of the cephalothorax (LCT) was measured, which was considered as the size (Oliva *et al.*, 1992; Alvitres *et al.*, 1999a; Iannacone *et al.*, 2007). Subsequently, the search for parasites was carried out on the same day of collection, to avoid the effect of environmental stress (Iannacone *et al.*, 2010), searching exhaustively on the external body surface, in the hemocele, intestine and hepatopancreas (Iannacone *et al.*, 2007; Oliva *et al.*, 2008).

Information was obtained on the sanitary quality of the 13 sampling beaches provided by the Direction of environmental health (DIGESA), Lima, Peru, according to the date of collection or a date close to this date (week 33-2001 "Santa María del Mar; week 40-2006 "Hermosa, "El Silencio" and "Pucusana"; week 42-2011 "Los Viños"; week 12-2012 "Venecia"; week 36-2012 "San Pedro"; week 12-2013 "San Pedro"; week 32-2013 "Los Yuyos"; week 34-2013 "San Pedro", "Conchán", "El Silencio"; week 36-2013 "Agua Dulce", "Los Delfines"; week 38-2013 "Hermosa", "Costa Azul", "Santa Rosa o Chica"; week 42-2013

"Venecia"; week 36-2014 "San Pedro"; week 7-2016 "San Pedro"). The sanitary quality of beaches is defined according to the quantity of thermotolerant or fecal coliforms (NMP·100mL⁻¹), the different ranges of values and their qualification are: 0 - 200, healthy or good; 201 - 1000, regularly healthy; > 1000, unhealthy or bad (DS, 1983; DS, 2011; APHA – AWWA – WEF, 2017).

The possible bias of capture of specimens of *E. analoga* was evaluated through reciprocity between the number of individuals from the different collection sites, the prevalence and average parasitic abundance, for this, a linear regression was made (natural logarithms) and Rho non-parametric Spearman correlation (Beltrán-Saavedra, 2015).

Prevalence (PRE) and mean abundance (AM) were determined by taxon, according to Bush *et al.* (1997) and Bautista-Hernández *et al.* (2013), in relation to the sex of *E. analoga* (Alvitres *et al.*, 1998; Alvitres *et al.*, 1999a), the distance from the tide line, the season of the year and the sanitary quality of the beach; in the case of sex, specimens that were not determined for statistical analysis were discarded.

The sexual dimorphism of *E. analoga* was confirmed by the Mann-Whitney U test. A contingency table was made for the X² test, with the purpose of examining the possible dependence of the length of the cephalothorax of *E. analoga* and the sanitary quality of the beaches. The heterogeneity of the length of the cephalothorax of *E. analoga* per season of the year was evaluated with the Kruskal-Wallis test. The seasonal alternation of the year, probably associated with the life cycle of the parasites of *E. analoga*, was explained using a univariate general linear model.

The effect of the length of the cephalothorax of *E. analoga* on its parasitic load and total prevalence was evaluated through Spearman's nonparametric Rho test (Poulin, 1999; Iannacone *et al.*, 2010; Iannacone *et al.*, 2011; Leiva *et al.*, 2015; Violante-González *et al.*, 2016).

The Kruskal-Wallis test was used to determine if the sanitary quality of the beaches conditioned the load and parasitic frequency (AM and PRE) of *E.*

analoga. This test was also used to visualize differences in the parasitic indexes between the nine sampling periods (July - September 2001, September - October 2006, October 2011, May 2012, September 2012, April 2013, September 2013, September 2014 and June 2016) and the thirteen collection beaches (Hogue & Swig, 2007; Beltrán-Saavedra, 2015; Faulkes, 2017).

Finally, an assumed parasitic spatial segregation was verified, by means of the average abundance and prevalence of *E. analoga* (Oliva *et al.*, 1992), in association with the distance from the tide line using the nonparametric Kruskal-Wallis and Rho test of Spearman, this last test was done by converting the variable DIST (distance from the tide line) from factor to numerical vector.

All the statistical tests were evaluated with a level of significance (α) of 0.05. The R version 3.5.1 software was used with the "ggplot2" package for the graphics (Wickham, 2016).

Ethical aspects

The procedures for collecting the diversity of parasitic fauna in the mole sand crab followed the guidelines of the "Institutional Animal Care and Use Committee" (IACUC) (APA, 2012), minimizing the number of organisms used, repetitions and using the three Rs "Rs-replacement, reduction, and refinement, and resolution 2558-2018-CU-UNFV that includes the code of ethics for research at the National University Federico Villarreal (UNFV). For the management of the parasitic fauna, the guidelines of the protection and animal welfare law of Peru

were followed (Law No. 30407: Article 19). For the field collection of mole sand crab, the impact on the abundance of species was minimized so that it is minimal (Costello *et al.*, 2016).

Conflicts of interest

The authors declare that they do not present any conflicts of interest.

RESULTS

A total of 5288 individuals of *E. analoga* were captured, of which 2487 were males, 1478 females and the rest were undetermined individuals (1323). The percentage discarded of the latter was males 62.72% and females 37.28%. The males had a length of the cephalothorax of 1.42 ± 0.5 cm and the females 1.80 ± 0.71 cm, the size difference being a determining attribute in the sexual dimorphism ($U = 252, z = 2, 85; p = 0.004$).

The lowest LCT was found at the beach "Conchán" qualified as "Not Healthy" in September 2013, the X^2 test showed a dependence between the length of the cephalothorax and the sanitary quality of the beaches ($X^2 = 114.48$; $df = 70$; $p = 0.001$; Table 1). A remarkable heterogeneity was observed per season of the year in relation to the LCT ($X^2 = 136.69$, $df = 70$, $p = 0.001$; Table 2), and a causal dependence was found between the season and the parasitic taxon expressed in the MA ($F = 5.45$, $df = 2$, $p = 0.006$; Table 2), this relationship was not noted in the PRE ($F = 1.16$, $df = 2$, $p = 0.31$; Table 2).

Table 1. Parasite load (mean parasitic abundance and prevalence) and length of the cephalothorax of *E. analoga* according to the sanitary quality of the 13 beaches of the central coast of Peru between July 2001 to June 2016.

Sanitary quality	n	<i>P. altmani</i>		<i>P. carvajali</i>		LCT
		MA	PRE	MA	PRE	
Healthy	2935	6.48	74.08	1.04	42.06	1.99
Regularly healthy	784	1.43	63.40	1.29	62.33	2.09
Unhealthy	254	1.97	67.86	0	0	1.82

n = number of individuals. MA = mean parasitic abundance. PRE = prevalence. LCT = length of the cephalothorax of *E. analoga*.

Table 2. Parasite load (mean parasitic abundance and prevalence) and length of the cephalothorax of *E. analoga* according to the seasonal variation between July 2001 to June 2016, in the central coast of Peru.

Season	n	<i>P. altmani</i>		<i>P. carvajali</i>		LCT
		MA	PRE	MA	PRE	
Autumn	1190	1,37	55,28	1,18	50,18	1,8
Winter	1345	2,04	74,8	1,14	44,91	2,17
Spring	1438	19,28	76,36	0,55	31,76	1,62

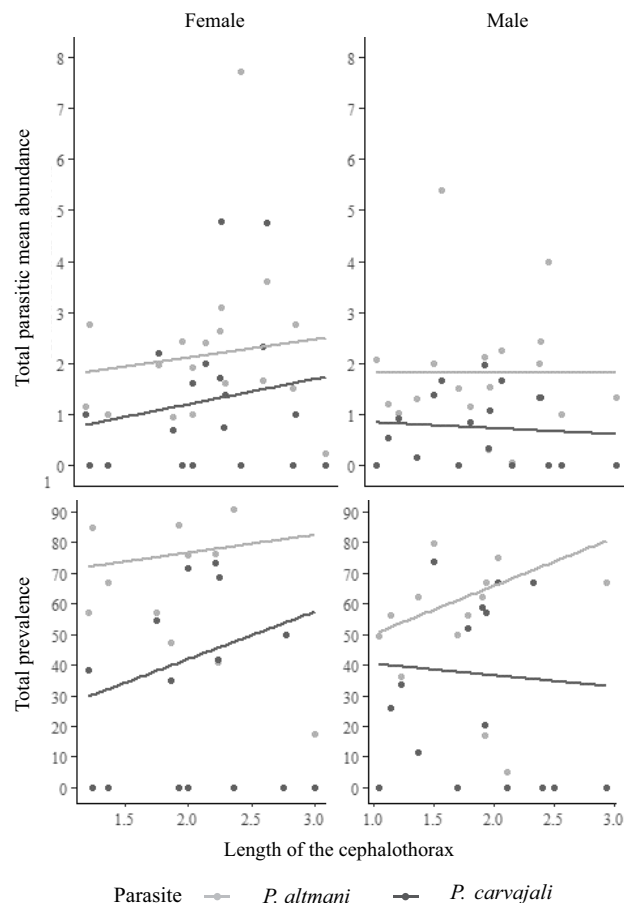
n = number of individuals. MA = mean parasitic abundance. PRE = prevalence. LCT = length of the cephalothorax of *E. analoga*.

The metazoan parasitic fauna was formed by *P. altmani* (Acantocephala: Polymorphidae) in its larval form and *P. carvajali* (Nematoda: Physalopteridae) in its larval form. The MA of *P. altmani* and *P. carvajali* was 4.27 and 1.10, reaching a PRE of 57.8% and 45.3%, respectively, for both sexes of *E. analoga*.

There was no capture bias of *E. analoga* expressed

in the MA and PRE of *P. altmani* (MA: $p = 0.16$, PRE: $p = 0.06$) and *P. carvajali* (MA: $p = 0.11$; PRE: $p = 0.20$).

The LCT of *E. analoga* did not present a correlation in favor of the total MA ($r_s = 0.09$, $p = 0.41$, Fig. 1), but presented a positive weak correlation towards the total PRE ($r_s = 0.23$; $p = 0.04$, Fig. 1).

**Figure 1.** Scatter plot between mean parasitic abundance and prevalence of *P. altmani* and *P. carvajali* in relation to the length of the cephalothorax according to the sex of *E. analoga* collected in 13 beaches of the central coast of Peru, between July 2001 to June 2016.

The sanitary quality of the beaches had the highest MA (6.48) and PRE (74.08%) of *P. altmani* on the beaches classified as "Healthy", the nematode *P. carvajali* reached the highest MA (1.29) and PRE (62.33%) on the beaches classified as "Regularly Healthy" (Table 1). However, according to the non-parametric Kruskal-Wallis test, no differences

were observed in MA ($H = 1.98$, $df = 2$, $p = 0.37$) and PRE ($H = 0.62$, $df = 2$, $p = 0.72$) of *P. altmani* with respect to sanitary quality, on the contrary, MA ($H = 6.20$, $df = 2$; $p = 0.03$) and PRE ($H = 6.74$, $df = 2$; $p = 0.02$) of *P. carvajali* was significantly different (Fig. 2).

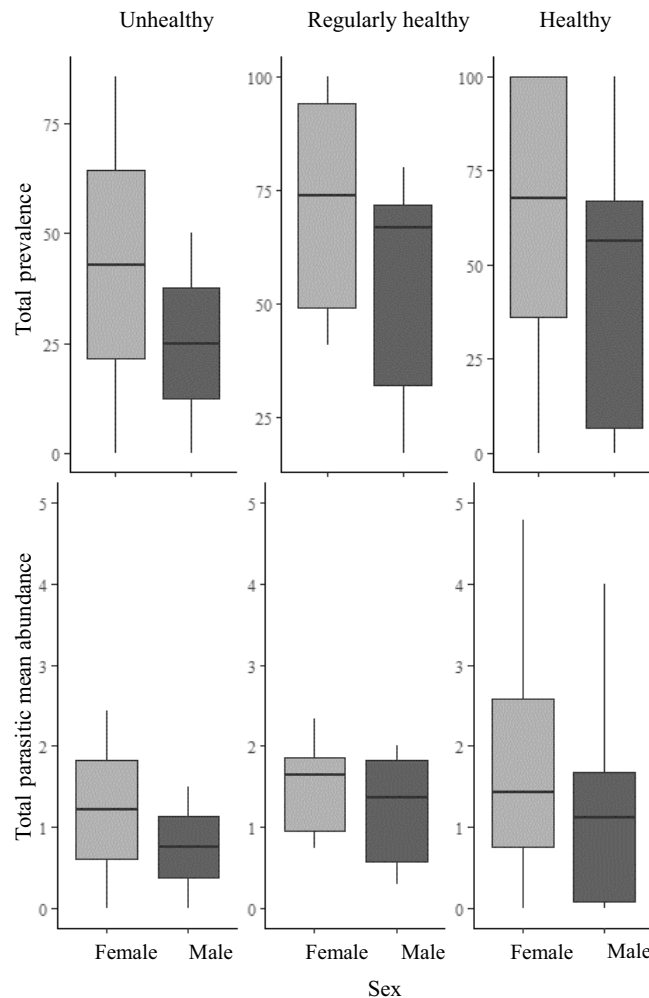


Figura 2. Box plot between total mean parasitic abundance and total prevalence in relation to the sanitary quality of the beaches according to the sex of *E. analoga* collected in 13 beaches of the central coast of Peru, between July 2001 to June 2016.

The highest MA (26.45) of *P. altmani* by dates occurred in September - October 2006, the largest PRE corresponded to September 2014 with 78.13%, in contrast, the date July - September 2001 was recorded with the lowest MA (0.08) and PRE (6.72%). As for *P. carvajali*, the highest MA (2.48) and PRE (72.66) occurred in September 2012 and September 2014, respectively. Found individuals

of *P. carvajali* on the date July-September 2001, having been collected only on the beach "Santa María del Mar" (Table 3). However, the 9 collection dates showed uniformity in the MA and PRE of *P. altmani* ($H = 11.74$, $df = 8$, $p = 0.16$ and $H = 12$; $df = 8$; $p = 0.11$) and *P. carvajali* ($H = 10.85$, $df = 8$, $p = 0.18$ and $H = 6.13$, $df = 8$, $p = 0.59$).

Table 3. Parasite load (mean parasitic abundance and prevalence) of the parasites of *E. analoga* according to the date of collection, in the central coast of Peru, between July 2001 to June 2016.

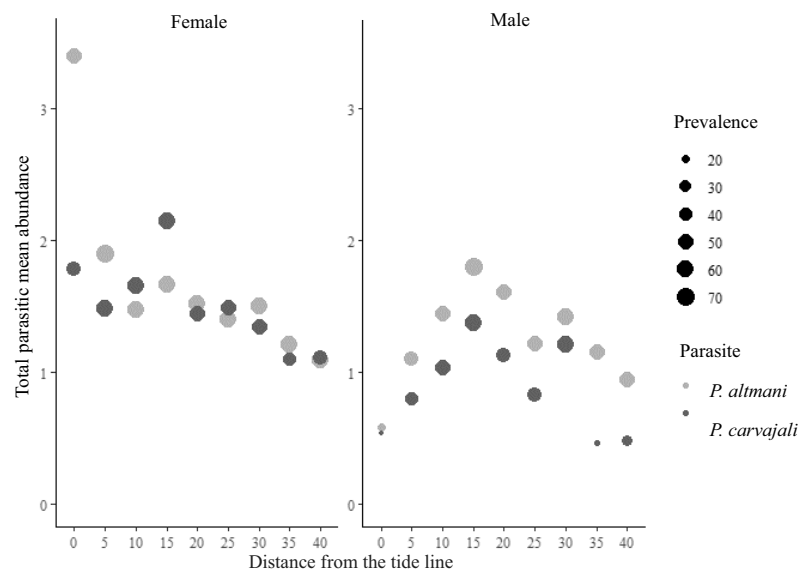
Collection date	n	<i>P. altmani</i>		<i>P. carvajali</i>	
		MA	PRE	MA	PRE
jul-sep 2001	1453	0.08	6.72	0	0
sep-oct 2006	339	26.45	71.89	0.38	24.60
oct-2011	854	1.17	56.54	0.77	32.41
may-2012	469	1.09	52.94	0.78	46.20
sep-2012	340	2.30	64.72	2.48	61.54
abr-2013	434	1.22	40.43	1.18	38.12
sep-2013	325	1.20	48.94	0.75	40.08
sep- 2014	785	1.95	78.13	1.48	72.66
abr - 2016	287	1.54	67.22	1.16	60.30

n = number of individuals. MA = mean parasitic abundance. PRE = prevalence. LCT = length of the cephalothorax of *E. analoga*.

A significant discontinuity was found, by means of the non-parametric Kruskal-Wallis test, in the 13 beaches for the MA and PRE of *P. altmani* ($H = 35.46$, $df = 19$, $p = 0.01$ and $H = 29.31$; $df = 19$; $p = 0.05$) and *P. carvajali* ($H = 31.59$, $df = 19$, $p = 0.02$ and $H = 32.75$, $df = 19$, $p = 0.02$). It should be noted that the highest MA (72.06) and PRE (97.46%) of *P. altmani* was found on the "Pucusana" beach in the period September-October 2006, being qualified for that date as a "Healthy beach". On the other hand, *P. carvajali* presented the highest MA (2.48) on the beach "San Pedro" for September

2012, date on which it was qualified as "Healthy"; September 2014 with its only beach "San Pedro" showed the highest PRE (72.65%), on that date it was classified as "Regularly Healthy" (Table 4).

The non-parametric Rho correlation of Spearman showed a negative association between the distance from the tide line and the MA of *P. altmani* ($r_s = -0.50$, $p = 0.15$), the PRE presented a slight correlation ($r_s = 0.33$, $p = 0.34$), likewise, in the MA of *P. carvajali* ($r_s = -0.32$, $p = 0.37$), while the PRE did not show any association ($r_s = 0.02$; $p = 0.96$);

**Figure 3.** Scatter plot of the mean parasitic abundance and prevalence per parasite in relation to the distance from the tide line according to the sex of *E. analoga* collected in 13 beaches of the central coast of Peru, between July 2001 to June 2016.

the probable parasitic spatial segregation was evidenced through the Kruskal-Wallis test, indicating a significant inequality in the MA of *P. altmani* ($H = 16.11$, $df = 8$, $p = 0.04$) and PRE of *P. carvajali* ($H = 18.57$, $df = 8$, $p = 0.01$) compared to

the different distances from the tide line, in contrast, uniformity was observed in MA of *P. carvajali* ($H = 11.99$, $df = 8$; $p = 0.15$) and PRE of *P. altmani* ($H = 14.46$, $df = 8$, $p = 0.07$) (Fig. 3).

Tabla 4. Parasitic load (mean parasitic abundance and prevalence) of the parasites of *E. analoga* according to the beach of origin, in the central coast of Peru, between July 2001 to June 2016.

beaches	n	<i>P. altmani</i>		<i>P. carvajali</i>	
		MA	PRE	MA	PRE
Los Viños	854	1.17	56.54	0.77	32.41
Hermosa	195	2.77	70.06	0	0
Chica	57	1	66.67	0	0
Venecia	826	0.81	41.24	0.66	38.76
Conchán	32	2.22	77.78	0	0
San Pedro	1631	1.91	70.10	1.57	66.52
El Silencio	111	1.56	73.40	0.99	47.48
S. María del Mar	1453	0.08	6.72	0	0
Pucusana	118	72.06	97.46	1.45	89.66

n = number of individuals. MA = mean parasitic abundance. PRE = prevalence.
LCT = length of the cephalothorax of *E. analoga*.

DISCUSSION

A small number of examined hosts will lead to an error in the search towards hosts with greater parasitic richness (Poulin, 2013), in turn generating an apparent narrow amplitude of niche (Poulin, 1992) or giving rise to the capture only of "active animals" (Beldomenico, 2008). The different sample sizes of *E. analoga*, collected between July 2001 and June 2016 along the central coast of Peru, did not show any capture bias expressed in the AM or PRE, as a result of the effort of sampling.

The general average of the length of the cephalothorax of *E. analoga* was 1.28 cm, the males had 1.42 ± 0.5 cm and the females 1.8 ± 0.71 cm, tending towards a marked sexual dimorphism in the difference of sizes confirming what was indicated by Lpez *et al.* (2001), Iannacone *et al.* (2007) and Jerez & George-Nascimento (2010). Sánchez & Alamo (1974) mention that external sex differences are observed from 3 mm and 4 mm, in males and females respectively, therefore it was not possible to determine some individuals (0.5 ± 0.25

cm), the size of these, reveals its stage in megalopa *sensu* Lpez *et al.* (2001) and Núñez *et al.* (1974). The proportion of sexes (62.3% males and 37.7% females) found in the present study shows us a clear protandric hermaphroditism (Subramonian & Gunamalai, 2003), confirming the findings of Penchaszadeh (1971).

Surface-volume relationships increase the rates of incorporation of toxic substances, especially in small species of crustaceans (Jiang *et al.*, 2012). The greater length of the cephalothorax corresponded to the category of "Regularly Healthy" beaches, and the shortest length was observed in the "Unhealthy" beaches. A dependence between the length of the cephalothorax and the sanitary quality of the beaches was demonstrated (Table 1). Zhang *et al.* (2010) point out that crustaceans are the most sensitive organisms, after echinoderms, to reduced levels of oxygen, a condition that is generated in eutrophic ecosystems, due to a constant accumulation of organic waste that depletes oxygen during decomposition (Zouiten, 2012; Veas *et al.*, 2013).

Bretz *et al.* (2002) point out that high concentration of the toxic PSPT in *E. analoga* limit its growth. Barca-Bravo *et al.* (2008) indicate that the asymmetry of certain morphological parameters in amphipods may reflect environmental stress on sandy beaches. The "Unhealthy" beaches are the busiest places for the population of Metropolitan Lima; the anthropogenic pressure, and in turn, the greater release of toxins confers a limitation to the body growth of *E. analoga*, likewise, the density and benthic richness can be affected in front of hypoxia / anoxia levels (Brazeiro, 2005; Aparicio, 2013). Guíñez *et al.* (2015) point out that *E. analoga* is a potential bioindicator of environmental health due to contamination by heavy metals, and could be used to alert the impact of this disturbance on the human population.

This study revealed an MA of *P. altmani* and *P. carvajali* of 4.27 and 1.10, reaching a PRE of 57.8% and 45.3%, respectively, for both sexes of *E. analoga*. The gradient of parasitic occurrence of *E. analoga* as reported in Peru is: Oliva *et al.* (1992) 44% of global PRE (*P. altmani* and *P. carvajali*) in 577 individuals from 11 beaches of the department of Lima; Tantaleán *et al.* (2002) PRE of 48% for *P. altmani* from the beach "Bujama", in Mala, Lima. Iannacone *et al.* (2007) 55.3% of PRE for *P. altmani*, while *P. carvajali*, reached 12.1% in 860 specimens acquired from the Fishing Terminal of Chorrillos, Lima, Peru; Alvitres *et al.* (1999ab) 75% of global PRE (*Eutetrarhynchus* sp. Pintner, 1913, *Nybelinia* sp. Poche, 1926, *Proleptus* sp. Dujardin, 1845, Spiruroidea, *P. altmani*, *Maritrema* sp. Nicoll, 1907 and Digenea) before "El Niño", and 53.2% during "El Niño" in 1331 individuals for Lambayeque; Rojas-Meza & Sebastián-Cabrera (2010), found 77.1% of PRE for *P. altmani* in 2300 individuals reviewed from 23 beaches south of Lima. These results indicate that the PRE for *P. altmani* found in the present study coincides with Iannacone *et al.* (2007) meanwhile, the PRE of *P. carvajali* agrees with that stipulated by Oliva *et al.* (1992). The niche amplitude observed in Alvitres *et al.* (1999ab).

A causal relationship has been found between the season of the year and the parasitic taxon expressed in the MA of *P. altmani* and *P. carvajali*, this association was not observed in the PRE. The highest MA and PRE of *P. altmani* occurs in spring with a value of 19.28 and 76.36 respectively, *P.*

carvajali presents the highest MA (1.18) and PRE (50.18) in autumn. The present work reveals an association between parasitic load and frequency in favor of warmer times; however, we cannot generalize due to the absence of evaluation in summer. Apparently, this relationship is due to the arrival of migratory birds that nest in the northern hemisphere and migrate to the southern hemisphere in search of food and shelter (García-Olaechea *et al.*, 2018), speculation would be approved according to Torres *et al.* (2006), who find the greatest abundance and diversity of birds in September and January in the Wildlife Refuge "Pantanos de Villa" and the adjacent beach. However, Leiva *et al.* (2015) point out that there is no relationship between the abundance of definitive hosts and the parasitic load of intermediary hosts for Chile.

The adult form of the acanthocephalan *P. altmani* has been found in *Larus belcheri* (Vigors, 1829), *L. dominicanus* (Lichtenstein, 1823), *Leucophaeus modestus* (Tschudi, 1843), *Leucophaeus pipixcan* (Wagler, 1831), *Chroicocephalus serranus* (Tschudi, 1844), *Podiceps occipitalis* (Garnot, 1826), *Numenius phaeopus* (Linnaeus, 1758) and *Calidris* sp. (Oliva *et al.*, 1992; Tantaleán *et al.*, 2005; Riquelme *et al.* 2006; Rodríguez *et al.*, 2016). Although it has also been reported to *Haematopus palliatus* Temminck, 1820 and *Pluvialis squatarola* (Linnaeus, 1758) feeding on *E. analoga* (Castro & Myers, 1987). On the other hand, *P. carvajali* has registered in *Mustelus mento* (Cope, 1877); *Rhinobatos planiceps* (Garman, 1880), *Triakis maculata* (Kner & Steindachner, 1867), *Schroederichthys chilensis* (Guichenot, 1848), *Raja chilensis* (Guichenot, 1848) and *Discopyge tschudii* (Heckel, 1841) (George-Nascimento *et al.*, 1994; Iannacone *et al.*, 2011), arvae were occasionally found in *Labrisomus philippii* (Steindachner, 1866) and *Scartichthys gigas* (Steindachner, 1876), and in a variety of marine bony fishes (García-Varela *et al.*, 2013; Luque *et al.*, 2016).

Iannacone *et al.* (2007) found no correlation between the length of the cephalothorax of *E. analoga* with the PRE and MA of *P. altmani* and *Proleptus* sp. On the contrary, it was observed in the present study, since a positive correlation was found between the MA and total PRE coinciding with that indicated by Alvitres *et al.* (1999ab). The

finding of positive and significant coefficients allows us to infer a gradual accumulation of parasites throughout host ontogeny (Zambrano & George-Nascimento, 2010; Violante-Gonzalez *et al.*, 2015; Bhaduri *et al.*, 2018). Violante-Gonzalez *et al.* (2012) conjecture that the survival of the host is reduced by the pathological consequences attributable to the parasites, leading in some cases to an increase in host sensitivity to contaminants (Iannacone & Alvarino, 2003).

The change in sanitary quality is due to the increase in organic matter (OM), which is related to an increase in the amount of coliforms (Wynes & Wissing, 1981) and/or an increase, in rainfall (Espinal, 2008). The constant discharge of wastewater, coupled with the massive use of fertilizers, accelerate eutrophication processes, causing oxygen levels and turbidity that make the natural development of aquatic ecosystems impossible, and an effect on benthic macroinvertebrates as *E. analoga* (Elías *et al.*, 2003; Zouiten, 2012).

The sanitary quality of the beaches has been a determining factor in the parasitic load and frequency of *P. altmani* and *P. carvajali*. The lower MA and PRE of *P. altmani* occurs in the "Regularly Healthy" beaches, while *P. carvajali* is absent in "Unhealthy" beaches, the nematode presents more contrasting results compared to the 3 categories of sanitary quality of the Beaches. Lafferty (1997) points out that nematodes are good indicators because they are sensitive to eutrophication, while acanthocephali are more susceptible to heavy metals, even accumulating a xenobiotic agent to a greater degree than their host (Sures, 2003; Nachev & Sures, 2016; Gilbert & Avenant-Oldewage, 2017). Espinal (2008) finds that *Uvulifer ambloplitis* (Hughes, 1927) and *Clinostomum complanatum* (Rudolphi, 1814) Braun, 1899 are favored by the alteration in water quality (eutrophication), while acanthocephalan *Polymorphus* Lühe, 1911 does not present any association with water quality and was found only in the month of lowest salinity. Therefore, a greater susceptibility of *P. altmani* to the osmotic stress generated as a side effect of the wastewater discharge can be presumed.

The use of *P. altmani* as a bioindicator of fecal contamination is based on its spatial segregation,

the highest MA was observed in females and males between the intertidal until its peak in 15 m, from where it begins to decay (Fig. 3), the intertidal zone is exposed to reservoirs of faecal bacteria protected in biofilms (Hartz *et al.*, 2007), at the same time, grains of sand provide an environment potentially favorable for their survival and development (USEPA, 1999). *P. altmani* and *P. carvajali* are exposed to high organic matter load in the intertidal, postulating them as sentinel bioindicators in an early warning system that contemplates, from the morphological analysis of the host to its load and parasitic frequency.

The unhealthiness of the beaches limits the growth of *E. analoga*. The spring season favors the parasitic load of *P. altmani*, while autumn is favorable for *P. carvajali*. There is a gradual accumulation of parasites, dependent on the length of the cephalothorax of *E. analoga*. An early warning system is proposed around the size, load and parasitic frequency of *E. analoga*.

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