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Infestation of *Probopyrus pacificensis* (Isopoda: Bopyridae) in *Macrobrachium tenellum* (Caridea: Palaemonidae) in the Ameca River, Jalisco, Mexico: prevalence and effects on growth

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Abstract. The present paper examined the prevalence and size relationship of *Probopyrus pacificensis* and *Macrobrachium tenellum* at Ameca River. Also, the effect of this parasite on the growth and survival of *M. tenellum* were reported. Four sampling sites were selected in the Ameca River México: two at upstream and two near to the opening. This study confirms the occurrence of *P. pacificensis* in prawns of this region. The parasite infested mainly female prawns. Our results suggested that *P. pacificensis* has no negative effect on the growth of its host, neither affects survival, at least in the studied.

Key words: Isopod, epibranchial parasite, prawns, Pacific coast

Resumen. **Infestación de *Macrobrachium tenellum* (Caridea: Palaemonidae) por *Probopyrus pacificensis* (Isopoda: Bopyridae) en el río Ameca, México: prevalencia y efecto en el crecimiento.** El presente estudio examinó la prevalencia y la relación de talla de *Probopyrus pacificensis*, y *Macrobrachium tenellum* en el río Ameca. También, se reporta el efecto de este parásito en el crecimiento y supervivencia de *M. tenellum.* Cuatro estaciones de muestreo se seleccionaron en el río Ameca México: dos estaciones río arriba y dos cerca de la desembocadura. Este estudio confirma la presencia de *P. pacificensis* en langostinos de esta región. El parásito mostró preferencia por langostinos hembra. Nuestros resultados sugieren que *P. pacificensis* no produce un efecto negativo sobre el crecimiento y tampoco parece afectar su supervivencia al menos en este estudio.

Palabras claves: Isópodo, parasito epibranquial, langostinos, costa del Pacifico

Introduction

Most parasitic isopods are ecto parasites of fish but some also are crustacean specific (Lester 2005). A common example of that is the Family Bopyridae, which consists of approximately 605 species- all parasitic isopods (Boyko & Williams 2009). Those Isopods may reduce gametogenesis in their host, and can also modify its secondary sexual features (Beck 1980). In particular, genus *Probopyrus* is constituted by epibranchial parasites affecting freshwater prawns of genus *Macrobrachium, Palaemon* and *Palaemonetes* (Markham 1985). On the Mexican Pacific coast four species of this genus have been reported: *P. pandalicola*; *P. bithynis*; *P. pacificensis* and *P. markhami* (Román-Contreras 2004). *P. pacificensis* has a distribution recorded from southern Nayarit, Jalisco, Michoacan and Guerrero (Ocaña-Luna *et al.* 2009). Guzmán & Román-Contreras (1983), mentioned that the greater abundance of the parasite occurs in areas with high human disturbance problems (water pollution by domestic and industrial effluents).

The river prawn *Macrobrachium tenellum* is host of *P. pacificensis* (Román-Contreras 1993, 2004, Ocaña-Luna *et al*. 2009). This prawn is distributed from Mulege in Baja California, in Mexico, to the Chira river in northern of Peru (Holthuis 1952) and a species of economic importance because it is subject of artisanal fishing in coastal regions of Mexico, El Salvador and Guatemala (Cabrera 1983), either for self-consumption or sale (Espino-Barr *et al*. 2006, Pérez-Velázquez *et al.* 2011). The magnitude of the damage made by this parasite in the populations and life cycle of this particular species is unknown since this phenomenon has received little attention; however, it has been demostrated the parasite affects the host physiology (Neves *et al*. 2000, Choong *et al*. 2011). The parasite attaches to the branchiosteguite wall within the gill chamber and feeds on the host hemolymph by perforating the integument with its mandibles (Lester 2005). Issues such as the prevalence or the effect of this parasite in its host have not been executed for the study area. Hence this paper examines the parasitic relation of *P. pacificensis* on *M. tenellum* in the Ameca river and its effect on the growth and survival of the host. An assessment of the relationship between the size of the parasite and the host is included.

Materials and Methods

Prevalence of Probopyrus pacificensis on Macrobrachium tenellum at Ameca River. Study area is located within the hydrological region "AMECA" (RH-14). The main surface currents that drain the basin are the Ameca and Mascota rivers, the latter is a tributary of the first. Ameca River begins in “La Primavera” forest, on Jalisco state, just 23 km west of the Guadalajara city; forms the boundary between Jalisco and Nayarit states, and flows into the Pacific Ocean in Banderas Bay near to Puerto Vallarta, Jalisco (INEGI 2004).

Four sampling sites were selected in the Ameca river section located on the coastal plain between Jalisco and Nayarit two upstream, El Colomo town (ELC) (20 ° 53'22.79 "N 105 ° 8'2.27" O), and San Juan de Abajo town (SJ), 23.4 km (20 ° 48'34.49 "N 105 ° 10'31.20" W), and two stations near the opening, Las Juntas town (PTE), at 3.3 km from the river mouth (20 ° 41'31.84 "N 105 ° 15'40.50" O) and Boca (BCA) just 1 km from the river mouth (20 ° 40'59.78 "N 105 ° 16'29.64" W). The first two stations are in relatively rural areas compared to the other two, which are located in areas of large residential and hotels areas (Fig. 1).

Specimens of *M. tenellum* were collected during February 2015 (one sample per site) with electrofishing equipment (Samus 725g) adjusted to 150-200 w (approximately 400 volts). At each site sampling effort of 30 minutes was carried out. The specimens were captured and transported alive in a container with water to the laboratory, and stored at -20°C until use. Sampled organisms were identified using the taxonomic keys of Holthuis (1952) and Hendrickx (1995). Sexual differentiation was based on the presence (in males) or absence (in females) of the appendix masculina, located in the second pair of pleopods (Ismael & New 2000). The prawns with large parasites were detected by visual inspection because of the obvious bulge in the exoskeleton in the gill chamber. The presence of parasites of small size or immature females was recognized by the pattern of spots on the branchiostegite (Conner & Bauer 2010). Prevalence of parasitized prawn at each collecting site was determined according to Muñoz (1997), defined as the number of hosts infected with one or more individuals of a particular parasite species (or taxonomic group) divided by the number of hosts examined for the parasite species (Bush *et al.* 1997).

The host´s carapace length (CL) was measured from the postorbital margin to the posterior end of the cephalotorax. The parasites were identified as *P. pacificensis* based on the description of Román-Contreras (1993). The total length (TL) of

the female parasite was recorded from the anterior

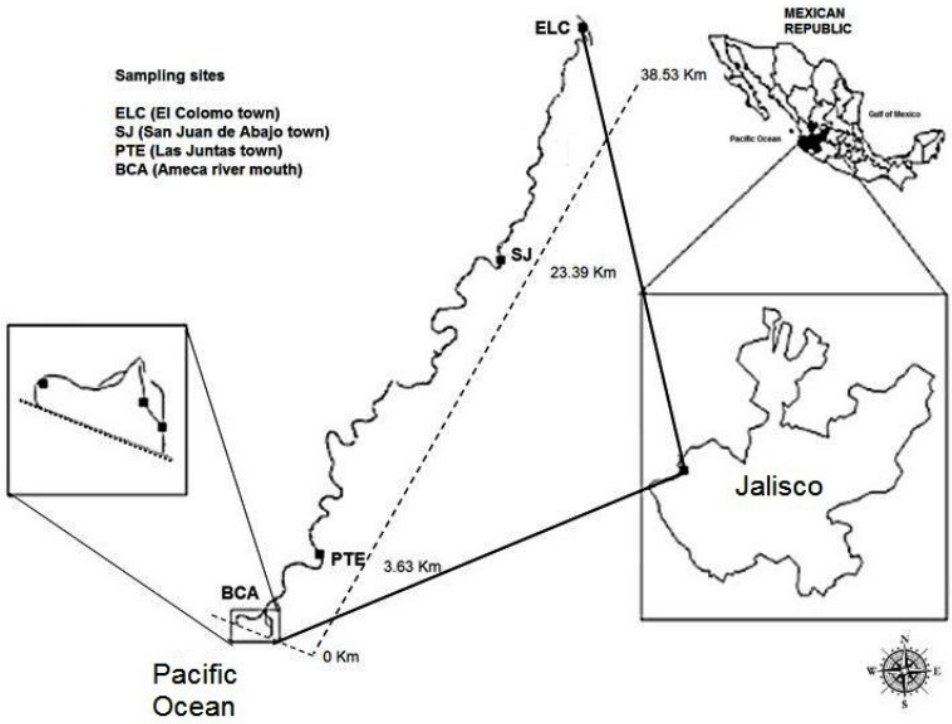

Figure 1. Sampling sites of infested prawns along the coastal region of Ameca river.

edge of cephalon to the tip of telson. In each sampling site, the salinity (conductimeter YSI 30 ®), temperature, pH (potentiometer YSI EcoSense ® 10A) and the concentration of dissolved oxygen (oximeter YSI 550A ®) were recorded, and variation between the size of the parasite and the host was analyzed using linear regression.

Growth of Macrobrachium tenellum infested by Probopyrus pacificensis : To determine the possible effect of the parasite on the growth on its host, parasitized organisms were collected with baited traps at Ameca river during May 2013 and classified base on two factors, prawn weight (A=0.62 ± 0.10g, B=0.93 ± 0.11g and C=1.20 ± 0.10 g) and healthy conditions, in respect to isopod parasite (infested and non-infested) (Table II). Prawns were remained at least two weeks in laboratory conditions for acclimatization, until the experiment began in June 2013. The experimental design was multifactorial. All organisms were placed individually in experimental units (EU), which consisted of glass aquaria (45x30x30cm; 35 L), with clear waters and under controlled conditions of oxygen (5.95 ± 0.41 ‰), temperature (29.89 ± 0.72 ºC), salinity (0.1 PSU), pH (8.44 ± 0.15) and photoperiod (13:11 light: dark). During the 45 days of the experiment, the organisms were fed (10% of their biomass) with a commercial balanced food for marine shrimp with 40 % of protein (Camaronina ® Purina ®). The feed was offered once daily (15:00 h) and remain food and feces were removed from the EU after three hours.

The total length (TL, vernier) of all prawns were measured considering the distance between the tip of the rostrum to the tip of the telson and weighed with a Scout Pro digital scales OHAUS® at the beginning and end of the study. A simple linear regression analysis was applied to obtain the coefficient of determination between weight groups for each of the categories ($p < 0.05$). To determine the survival, the number of dead organisms was recorded daily. Normality and homogeneity of variances tests and one-way ANOVAs were applied to all data, using SigmaStat V3.1 (2004). Post hoc analyses between treatments were determined by Tukey multiple comparisons ($p < 0.05$). All percentage data were arcsine of the square root transformed before statistical comparisons. The prevalence was analyzed between sampling sites to determine whether there were statistical differences

through chi square (X^2) (α = 0.05). Excel 2013 statistical software was used.

Results

The values of temperature, salinity, dissolved oxygen and pH by location are shown in Table I. In the collection sites, a total 826 organisms were caught out of which 73 (8.83%) were found parasitized by *P. pacificensis* (except those farthest from the coast, ELC). Bopyrid prevalence was significantly different in the four sampling sites (X^2 = 8.61; df= 2; P= 0.01) (Table I). There are significant differences (X^2 = 65.8; df= 1; P< 0.05) between the number of females (72) and male (1) parasitized. No parasitic preference for left or right gill chamber (right 35, left 38) (X^2=0.12; df= 1; P>0.05) was found, the ratio was close to 1: 1. No bilateral infestation was recorded. The TL of the female isopod correlated with CL of the host (Fig. 2).

Survival data, initial and final weight, initial and final size of juvenile *M. tenellum*, healthy and infested by *P. pacificensis*, after 45 days of testing are presented in Table II. Regarding the effect of the parasite on the growth and survival of prawns under controlled conditions in the laboratory, mortality differences between groups were observed only in the group B in which infested prawns had a survival rate of 80%.

With respect to the final size (A, B, and C) significant differences (F= 7.21; d f= 5; P < 0.05) were observed between the three weight groups but such differences were not observed between healthy and infested organisms from each group (Initial weight (F=37.2; d f= 5; P>0.05) initial size (F=15.53; d f= 5; P>0.05) final weight (F= 10.62; d f= 5; P>0.05) final size (F= 7.21; d f= 5; P> 0.05). As interesting finding of present study, was the change in color observed in the ventral region of the parasites occupying the gill chambers of some females. Those parasites were extracted with dissecting forceps and observed under a microscope. It was observed that at different stages of embryonic development of the parasite the ovigerous mass has different colors ranging from white, to start developing embryos, then beige to intermediate stages of development and gray and dark gray for late embryonic stages.

Discussion

Bopyrids are very common parasites of prawns (Román-Contreras 1993). In this work, the parasite presence was significantly higher in females than males (72 females and 1 male) and no one of the females parasitized were ovigerous (sex ratio of uninfested host was 0.95:1). This is comparable to that mentioned by Beck (1979), Guzmán & Román-Contreras (1983) and Román-Contreras & Rodriguez-Romero (2005). A possible cause of this is because *P. pacificensis*, like other bopyrids isopods, are castrating parasites (by inhibiting gonadal development) as previously observed by Beck (1979, 1980), O´Brien & Van Wyk (1985) and Conner & Bauer (2010). This parasite does not cause gonadal destruction, but causes a temporary inhibition of its reproductive physiology (Pike 1960). The parasite damage the gills by compression (Bursey 1978), interfering with gas exchange (Neves *et al*. 2000). The parasite also interferes with feeding abilities of the host. The energy and nutrients that the host usually directs toward reproduction and growth is apparently deviated for growth and reproduction of the parasite (Conner & Bauer 2010). The most common effect of castration is the suppression of vitelogenesis in females and alteration of sexual characters in males (Odinetz-Collart 1990). The parasite can live as long as its host (Beck 1980) and may inhibit its reproduction permanently. For example, *P. pandalicola* on *Palaemonetes* sp. takes 10 % of host's energy intake and lowers egg production to a half (Anderson 1977).

Table I. Physicochemical parameters in the collecting sites, collected and parasitized prawns and prevalence rate.

Site	Salinity (PSU)	Dissolved oxygen (mg/l)	pH	Temperature (°C)	No. collected prawns (LC mm)	No. parasitized prawns (LT)	Prevalence %
El Colomo (ELC)	0.25	11.9	8.7	23.75	114 (8.3)	0	0
San Juan (SJ)	0.2	11.5	8.6	25.5	291 (8.0)	17 (4.9)	5.8
Las Juntas (PTE)	0.25	9.05	8.1	27.7	342 (8.2)	46 (5.1)	13.4
Boca (BCA)	3.65	6.85	8.35	25.1	79 (3.9)	10 (2.1)	12.66
Total					826	73	8.84

The effect on males and females hosts, are different but, there are reports that *P. pandalicola* and *P. floridensis* may not have a preference for one sex (Campos & Campos 1989, Masunari *et al.* 2000, Chaplin-Ebanks & Curran 2007), which, differs about our observations in this study.

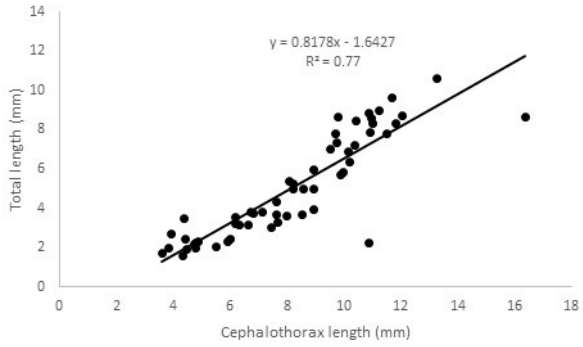

Figure 2. Relationship between the female total length of *Probopyrus pecificensis* Román-Conteras, 1993 and *Macrobrachium tenellum* CL, in the Ameca river (Smith, 1871).

Prevalence of *P. pacificensis* in the studied population was 8.83 %, which is low compared with that reported by Román-Contreras (1991), who mentioned that from 13,757 specimens of *M. tenellum* (Coyuca lagoon, Guerrero, Mexico), 17 % had the bopyrid and very low compared with those reported by Signoret & Brailovsky (2002), which mentioned a prevalence of 58.17 % in the same host (Coyuca lagoon, Guerrero, Mexico). In this study, the location with the highest prevalence were those found near the shore. According to Anderson (1990), the infective stage of *P. pandalicola* is located in the estuaries, place that is also the habitat of larvae and post larvae of *M. tenellum* (unpublished data). In agreement with the above, it was observed that in the BCA station the highest number of infested hosts were poslarvae and juveniles (3.96 mm ±1.3 LC). The same author mentioned that the host is liable to be infested during zoea or poslarvae stages, because ecdysis process is more frequent in these stages, increasing the possibility for the parasite to attach on to the host.

Sures (2008) pointed out that the effects of pollution can be positive or negative in the levels of parasitism, the same author considered that infestations by ectoparasites (such as *P. pacificensis*) tend to increase in direct relation to the pollution; while endoparasites tend to fall when the pollution increases. The findings in this study suggested that environmental conditions promote a development of the parasite and its intermediate hosts, as prevalence were highest in PTE and BCA, which are urban areas that discharge pollutants into the river. This hypothesis should be supported with studies designed for this purpose. It has been already reported that the Ameca river is highly polluted due to municipal, agricultural and industrial discharges (López-López & Paulo-Maya 2001). Moreover, Cortés-Lara (2003) mentioned that the level of fecal coliforms in seawater is higher in sample areas close to BCA, indicating that this may be due to domestic effluents, farms, pig production and restaurants, discharged sewage directly into the river. This coincides with what was set by Guzmán & Román-Contreras (1983), who mentioned a greater abundance of the parasite in areas with higher pollution. The smallest parasites (presumably larvae) were associated with the smaller prawns, while the larger parasites were in the largest prawns. This is explained by the correlation between bopyrid TL and prawns CL, which allows establishing that the hosts are infested in early stages (Cash & Bauer 1993, Romero-Rodríguez & Román-Contreras 2013).

Table II. Survival, weight and Total Length of juvenile *M. tenellum* by health condition

Parameters	Group A (0.47 a 0.78 g)		Group B (0.79 a 1.08 g)		Group C (1.09 a 1.39 g)	
	Infested (n=7)	Healthy (n=7)	Infested (n=5)	Healthy (n=5)	Infested (n=6)	Healthy (n=4)
Initial weight (g)	0.61±0.04^{a}	0.63±0.13^{a}	0.90±0.10^{b}	0.98±0.12^{b}	1.18±0.05^{c}	1.23±0.17^{c}
Final weight (g)	0.76±0.14^{c}	0.76±0.20^{c}	1.07±0.34^{bc}	1.26±0.28^{ab}	1.21±0.10^{ab}	1.63±0.33^{a}
Initial size (mm)	37.99±1.84^{c}	38.54±2.75^{c}	43.29±2.24^{ab}	42.59±2.87^{b}	46.99±1.35^{a}	44.53±1.90^{ab}
Final size (mm)	41.06±3.72^{b}	42.19±3.16^{b}	46.13±4.53^{ab}	47.43±2.89^{ab}	48.85±2.10^{a}	51.11±4.17^{a}
Survival (%)	100^{a}	100^{a}	80^{b}	100^{a}	100^{a}	100^{a}

This suggested a synchronicity growth between host and parasite (Muñoz 1997, Romero-Rodríguez & Román-Contreras 2014). However in this study, a large-size prawn was found carrying a tiny parasite, a finding that has been reported by other authors such as Beck (1980) for *P. pandalicola-P. paludosus*, Campos & Campos (1989), for *P. pandalicola-P. ritteri* and Conner & Bauer (2010), for *P. panpdalicola-Macrobrachium ohione*. These authors mentioned that adult can be infested, despite low rates of molting (causing difficulty in the adhesion of the parasite). This could be due to the seasonal reproductive migration downstream to estuaries or near them, places in where the parasite has the higher infesting capacity and could be an additional explanation of the common presence of the parasite in female prawns. However, the cryptoniscus larva of the bopyrids can migrate upstream to infest its host (Beck 1979).

According the followed protocol in growth bioassays, the results suggesting that parasitism does not reduces the ability of organisms to gain the nutrients needed for optimal development, in contrast of that mentioned by Lester (2005), which affirms that the parasitic organism may cause a decrease in host growth . Dumbauld *et al*. (2011) demonstrated that the presence of isopod *Orthione griffenis* caused the shrimp *Upogebia pugettensis* not to reach their optimum size and weight and caused a decrease of the population in the wild. Our results did not show a lower weight gain in infected prawns but this could be because the experimental time was not sufficient to demonstrate this phenomenon. Furthermore, the ability of parasitized prawns to get food without move (a highly nutritious food) and with minimal energy expenditure could minimize the harmful effect of the parasite on the host growth. However, there is no evidence that infestation affect shrimp survival over a 108-day period in the laboratory (Dumbauld *et al*. 2011), and this seems to be confirmed by our results.

An association between the color of the ovigerous mass of female bopyrids and embryo development is suggested. Such observations must be supported by further studies conducted for this purpose. Other impacts of the parasites over its host must be studied. For example, parasites can also lower the rate at which hosts capture food, reduces its osmoregulation and migration capabilities (Lester 2005). A specific study on the life cycle of this particular parasite make give clues of its parasitic effect on this particular species.

Conclusions

This study confirms the occurrence of *P. pacificensis* in the prawns *M. tenellum* in Ameca river, Mexico. It showed that the parasite has a preference for female prawns, although this may vary in other seasons. Following the protocols in this study, it is suggested that the parasite has no negative effect on the growth of its host, neither affects survival, at least in the studied stages.

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