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First Record on Larval Development of the Cirripedian Parasite *Loxothylacus texanus* (Cirripedia-Rhizocephala) Under Laboratory Conditions in México

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Abstract: Crabs with mature parasites were put on individual aquariums of 20 L of capacity and with an initial salinity of 10‰. Salinity was gradually increased 1‰ until it reached a point in which parasites expeled their eggs or larvae. Larvae at different developmental stages obtained from each sample were preserved in 70 and ethanol to be studied afterwards. From 20 crabs employed, only 14 of them produced eggs and other 6 died after a few days at the aquariums. The mature externas expelled from 8000 to 160000 clustered eggs. After 24 h from the beginning of cellular division, larvae hatch. This larvae are called nauplius 1. This stage lasted 24 h, after which all larvae entered an excitation period, where they rapidly moved, to then pass a new molt process resulting in a new larval stage, nauplius 2. Elevating salinity a unity, externas started to expel eggs, nauplis larvae 1 hatched from them, but they only survived less than 24 h. As salinity increased, nauplius larvae lived longer, even to realized a molt. At a 15‰ salinity, larvae reached cypris stage with no problem, but with a salinity higher than 25‰, larvae died after a few hours of hatching. We can conclude that: the rhizocephalan *Loxothylacus texanus* has two nauplius stages, the nauplius and cypris larvae presented similar morphological characteristics to related species of rhizocephalans and a salinity interval from 15 to 20‰ report the same result.

Key words: Parasitism, Rhizocephala, *Loxothylacus*, naupli, barnacle, *Callinectes*

Introduction

Crabs from the genus *Callinectes* are an important group of eatable crustaceans and in Mexico they are known as jaibas. They are distributed all over the tropical coasts of America, Western Africa, South Pacific islands and Western Atlantic ocean (Durá, 1985; Willuams, 1984).

One of the most important biotic factors that negatively affect to blue (*C. sapidus*) and black crab (*C. rathbunae)* populations in the Gulf of México, is the parasitism caused by rhizocephalans barnacle, due to its periodic apparition, high frequency and effects on its host (Wardle and Tirpak, 1991; Lorán *et al*., 1993; Alvarez and Calderón, 1996). Rhizocephalans in larval planctonic stage are able to parasite shrimps and crabs (Alvarez and Calderón, 1996). The most important effect caused by this parasites, is the so called parasitic castration, in which the host´s gonads remain immature (O'Brien and Wyk, 1984).

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There exist almost 200 species of rhizocephalans, all of them very specialized parasites of other crustaceans. Their principal hostages are decapods, but they can also parasite isopods, cumaceans, stomatopods, or balanomorf barnacles. It seems that rhizocephalans exist in the same marine environment of their hostages, including deep sea (Lützen, 1985). Some rhizocephalan species parasite semiterrestrial crabs, while others parasite freshwater crabs (Andersen *et al*., 1990; Høeg, 1992).

Life cycle of rhizocephalans is highly modified if compared with that of non parasitic barnacles. However, rhizocephalans posses the both typical dioic larval stages of all barnacles: nauplius and cypris (Høeg, 1991). The typical life cycle of a rhizocephalan is as follows: nauplius sexually differentiated larvae hatch from eggs, being males the biggest individuals and females the smallest ones. After four molts, nauplius larvae turn into cypris larvae, then, female cypris larvae infect susceptible hosts and turn into endoparasites (Høeg, 1992; Høeg and Lützen, 1995). After a sexual maturation period, a reproductive body emerges from the host´s abdomen (externa). This externa can be fertilized by a male cypris larvae. Afterwards it begins to mature (Høeg and Lützen, 1995). The externa contains the reproductive organs of the female as well as the mantle cavities (used as an incubator), which open to the exterior through a simple orifice in the mantle. Adult rhizocephalans are different from other cirripedians because they lack of segmentation, appendages and digestive tract. They also lack of a calcarean shell (Høeg, 1992; Høeg and Lützen, 1995).

The rhizocephalan *Loxothylacus texanus* Boschma, is parasite of the blue crab. Its principal area of distribution is the Gulf of Mexico and is said to be responsible of great economic loses (Wardle and Tirpak, 1991; Daugherty, 1952; Christmas, 1969; Park, 1969; Adkins, 1972; Ragan and Matherne, 1974; Hochberg *et al*., 1992). Eventhough local fishermen knew of the existence of this parasite in mexican waters since a long time ago, studies of the affected populations are very recent (Alvarez and Calderón, 1996; Lázaro-Chávez *et al*., 1996; Alvarez *et al*., 1999). In the particular case of Mexico, literature relative to *L. texanus* is scarce and it lacks of information about basic biological aspects.

An important factor to understand dispersion and distribution of these parasites within lagoonal systems, is the knowledege and characterization of larval development.

The objective of present research was characterize the larval development of *L. texanus* in laboratory conditions.

Materials and Methods

The crabs *Callinectes rathbunae* were captured in the Alvarado lagoon in Alvarado, Veracruz, México in January 2000. Organisms were transported to the Coleccion Nacional de Crustáceos Laboratory in Mexico City. Once at the laboratory, crabs were individually put on recirculation water systems, with an initial salinity of 5‰, which then was gradually increased until it reached 10‰. Acclimation period lasted one week. After this period, mature parasites were selected from the crabs and put on individual aquariums of 20 L of capacity and with an initial salinity of 10‰. Salinity was gradually increased 1‰ until it reached a point in which parasites expeled their eggs or larvae.

There are no references that indicate wether *L. texanus* expels living larvae or eggs.

Crabs were fed with little fishes (Poecilidae), fresh chopped fish meat, or live cambarids.

Temperature at the laboratory was always at 27°C and represented and average of the temperatures registrated at field, being the minimum 25°C and the maximum 30°C. Salinity and temperature were daily measured. As it is unknown where, the mature externa of *L. texanus* expels eggs or living larvae, daily filtrations of water were performed at least once a day, with a net aperture of 60 μ.

When eggs were observed in the aquariums, the host crab was taken away and the water filtrated to a volume of 1 L, obtaining samples of 1 mL that were then passed into a 1 mL capacity counting camera, to be observed at a stereoscopic microscope (OLYMPUS model S2H10). Measurements of the eggs were performed considering their tips. Afterwards they were returned to the aquaria so their development could continue. When larvae were observed, the same procedure was performed, with the exception that in this case, some drops of 70% ethanol were added to the camera to make easier the account of individuals.

For both cases, 30 samples of 1 mL each, were taken (following Alvarez criteria, pers. com.). An average of the number of eggs and larvae was obtained and then extrapolated to 1 L.
Obtained larvae were separated by sex, being males those bigger and larger individuals and females those smaller and rounded ones (Høeg and Lützen, 1995).

Sex ratio for each spawning was obtained and measurements of larvae were done too using an ocular micrometer. Larvae at different developmental stages obtained from each sample were preserved in 70% ethanol to be studied afterwards. A video equipment was adapted to stereoscopic microscope, so larval development could be followed. Finally, external morphology of some organisms was detailed by photography.

Results

From 20 collected crabs, only 14 of them produced eggs and other 6 died after a few days at the aquariums. The mature externas expelled from 8000 to 160000 clustered eggs (Table 1), which sank to the bottom in all cases. The eggs presented a greenish coloration when expelled what disappeared after 2 h.

Eggs were only of two sizes: 142 and 174 μ and presented variable developmental stages. In the eggs less developed, significant changes ocurred within 24 h, with the beginning of the celular division and then the appearance of a black spot that could be the ocular primordium. The appearance of this spot occurs within an interval of 6 h. In more developed eggs, larval naupli hatched within 24 h. The larvae was sizes: 222 and 320 μ of length.

Sex ratio varied in each of the 14 reported spawn and ranged from 8:1 to 1:6 males: females. The mature externas that could be measured had a volume ranged from 2.4 to 3.6 cm^3.

Table 1: Eggs produced by the *C. rathbunae* parasite, *L. texanus* in laboratory conditions

Spawn No.	Eggs produced	Host´s shell width (cm)	Host´s sex
1	51000	9.67	Female
2	8000	8.72	Female
3	160000	8.76	Female
4	110000	7.96	Female
5	96000	8.19	Male
6	29800	8.81	Female
7	12800	6.94	Male
8	156000	7.34	Male
9	12960	9.18	Female
10	57860	5.24	Male
11	8667	8.76	Female
12	140000	6.94	Male
13	12000	8.76	Female
14	76800	5.80	Female

After 24 h from the beginning of cellular division, larvae hatch. This larvae are called nauplius 1 (Høeg and Lützen, 1995; Walker, 1985; Walker, 1988 and Walker and Lester, 1998) and only presented two sizes: 222 and 320 μ of length. Hatching process (from the rupture of membrane until larvae freely swims) lasted in most of larvae, 1/2 h, but it was observed that it could be as long as 3 h for some larvae.

Nauplius larvae 1 presented anterolateral horns of two sizes, 27.57 and 16.9 μ. They also had a furcal spine at the posterior tip of their bodies, being of two sizes: 23.63 and 15.2 μ. A nauplius eye, a pair of antennules and fat granules were located at the anterior tip of the body.

This stage lasted 24 h, after which all larvae entered an excitation period, where they rapidly moved, to then pass a new molt process. The molt process lasted 15 min in most of the cases, resulting in a new larval stage, nauplius 2, but some individuals remained 2.1/2 h in the process. Once it was over, the only observable difference between this new stage and the last one, is the diminish of the lenght of the furcal spine, there even were individuals that did not had this spine. Nauplius 2 larvae individuals were also of two sizes. This stage lasted 12 h, after which a new molt process began. Within the old shells, completely different organisms are observable, they are called cypris larvae (Høeg and Lützen, 1995; Walker, 1985; Walker, 1988; Walker and Lester, 1998). These larvae were also of two sizes: 228.5 and 325.4 μ and did not possess anterolateral horns and furcal spine, but did have fat granules, thoracic appendages and musculature accompaning the appendages. United to musculature, there was a caudal appendage, located at the end or behind the thoracic appendages. Both larval sizes had a nauplius eye, a pair of antennules, which was bigger in males and an aestetasc (sensorial structure). None of the larval stages described above fed and show the same morphological characters of related species. Larval development was completed in about 60 h, from hatching to cypris larvae stage and 84 h from spawning to cypris larvae stage.

It was observed that at a 10‰ salinity, externas show no activity, except for some rythmic contractions. Elevating salinity a unity, externas started to expel eggs, nauplis larvae 1 hatched from them, but they only survived less than 24 h. As salinity increased, nauplius larvae lived longer, even to realized a molt. At a 15‰ salinity, larvae reached cypris stage with no problem, but with a salinity higher than 25‰, larvae died after a few hours of hatching. From this we could conclude that a salinity interval from 15 to 20‰ reports the same result.

Discussion

In all cases, surviving externas expeled clustered eggs, being only of two sizes, 142 and 174 μ. Yanagimachi (Yanagimachi, 1961) and Høeg and Lützen (1995) mention only two sizes of eggs for *Pellogasterella gracilis* and *Lemaeodiscus porcellanae,* respectively.

Høeg and Lützen (1995) established an interval of 50-400 μ for rizocephalan´s eggs, but 90% of the studied species show an interval of 125-175 μ, so the measurements presented in this study fit on this last interval.

Høeg and Lützen (1995) mention that the number of eggs expeled by the rizocephalans in each spawning varies from a few hundreds as in the thompsonids, to millions as in *Briarosaccus callosus*, a parasite litodid crabs.

In this study, from 8000 to 160,000 eggs were quantified (Table 1); this values are within the expected interval for a rizocephalan of the size of *L. texanus* in laboratory conditions.

A significative relation between the externa´s size and the number of eggs produced was not found. The studied externas had volumes from 2.4 to 3.6 cm^3; the size of the spawn varied a lot more

than the values of the externa´s volume. There were also observed some externas that presented more than one spawning. The difference between the number of eggs expeled in each one was of 10,000 eggs/larvae. Høeg and Lützen (1995) establish that in very few species, a relation between the externa´s size and the number of eggs has been found. An example of this is *Lemaeodiscus porcellanae*, where a little externa (3-4 mm) expel 1000 eggs, while a big externa (9-10 mm) expel 17,000. However, this observations have been carried out for Northern Sea and Scandinavian species.

Reisser and Forward (1991) Walker and Clare (1994) and Alvarez (1993) found that *Loxothylacus panopaei´*s externas expel living nauplius larvae but did not register expelling of eggs. Alvarez (1993) found weak evidence that relates the size of the externa of *Loxothylacus panopaei* with the number of eggs per spawning, when it parasites to *Panopeus lacustris, Eurypanopeus depresus* and *Rhithropanopaeus harrisii*. Walker and Lester (Walker and Lester, 1998) mentioned that mature externas of *Heterosaccus lunatus* expel living larvae and that when parasites died, externas were extirpated and eggs put into small containers where they hatched after a few minutes. In this last experiments, salinity was changed until favorable results were obtained.

Høeg and Lützen (1995) mentioned that only very few rizocephalan species expel nauplius larvae instead of eggs, but they did not specify the experiments made to get to this conclusions.

In the present study was expected that expeled eggs from mature externas were not viable, however they did present cellular division and produced larvae in most of the cases.

No relation between this and physical or chemical factors was found, due to the fact that the incubation temperature was always the same (27°C) and that salinity was always between 10 and 25‰. These results suggest that larvae´s sex is genetically determined, unlike reptiles, which sex can be determined by temperarure variations. In a study carried out by Yanagimachi (Yanagimachi, 1961) with *Pellgasterella gracilis*, he found that spawnings were composed by females or by males exclusively, eventhough, he also observed spawn composed by both sexes, concluding that sex is determined genetically. Ritchie and Høeg (1981) studied the spawn of *Lernaeodiscus porcellanae*, founding that they expel females and males in an alternate way and that between each spawn there occurs one when both females and males are expeled. Lützen (1984) mentioned that in some temperate zone species, the appearance of new externas occurs at precise moments during the year and that the occurrence of males was higher when this new externas appeared.

Walker (Walker, 1985; 1988) found that more than 60% of the spawn of *Sacculina carcini* were composed by males and females. It was found by Høeg (1987) that larvae of both sexes were expeled by *Lernaeodiscus porcellanae*. Finally, Walker (1988) mentioned that populations of *Sacculina carcini* in the English Channel, were predominantly composed by male larvae at the beginning of spring and summer, when a high percentage of the externas are new.

The first larval stage found, presented the typical structures previously described for nauplius stage, for both sexes. Høeg and Lützen (1995) who realized the first deep study about Order Rhizocephala, established that nauplius larvae of *Sacculina carcini* and *Peltogasterella sulcata*, possess a nauplius eye, anterolateral horns, furcal spine and antennular appendages. The most notable difference between these and *L. texanus* is that they reported a longer furcal spine, not specifying if it belonged to a male or a female. The first species is 331.25 μ in length and its furcal spine is 18.2 μ. The second species is 348 μ in length, its furcal spine is 42.4 μ and its anterolateral horns were 34.3 μ. Walker and Lester (1998) described the same structures for both sexes of *Heterosaccus lunatus*. Male nauplius larvae 2 was 253 μ, its furcal spine was 27.7 μ and anterolateral horns were 18.3 μ; the size of female structures was 202.4, 25.4 and 13 μ. Høeg (1992) worked with *Peltogasterella sulcata* and only mentioned the presence of a tripartite nauplius eye and lateral horns associated with glandules.

Furcal spines of the first stage of *L. texanus* were 23.63 μ for the bigger individuals and 15.2 μ for the smaller ones. These lengths are similar to those of *Heterosaccus lunatus*. In the case of anterolateral horns, these were 27.57 μ for the bigger individuals and 16.9 μ for the smaller ones. These measurements are comparable to those of *S. carcini* and *H. lunatus*. Some authors like Walker and Clare (1994) and Høeg and Lützen (1995) found no morphological differences between sexes in nauplius 1 stage, except for the total size, being males 320 μ and females 222 μ.

The only reference of nauplius 2 stage is that of Walker and Lester (1998). They worked with *Hetyerosaccus lunatus*, but the described structures coincide with those of a nuaplius 1 from present study.

Walker *et al*. (1992) described a nauplius 4 of *Loxothylacus panopaei* and it coincides with the description of a nauplius 2 from our work and also mentioned a furcal spine as short as the one described here. Høeg and Lützen (1995) found no morphological differences between sexes of nauplius larvae of different species (*Briarosaccus tenellus, B. callosus, Peltogasterella gracilis* and *Lernaeodiscus porcellanae*) except for the total size, being females the rounded and smaller individuals and males the larger ones.

Taking as guide previous works, it was determined that the next larval stage observed was cypris (Høeg and Lützen, 1995; Walker, 1985; Walker and Lester, 1998; Reisser and forward, 1991; Walker and Clare, 1994; Walker *et al*., 1992). Cypris larvae differs completely form a nauplius one, it posses a laterally compressed shell, which is wider at the front. Nauplius stages have a pair of antennules and a pair of antennas as the only locomotive appendages, on the other side, cypris larvae have locomotive appendages in parts of the thorax and a pair of modified antennas which serve as adhesion structure and for the injection of the infective primordium for females and as adhesion and as fecundation structure for males (Høeg and Lützen, 1995).

Only two sizes of cypris larvae were observed: 326 μ for males and 228 μ for females. This sexual dimorfism has also been observed by Høeg (1984) who pointed out that in the case of *Sacculina carcini* there is a seasoual variation in size, founding bigger individuals (278 and 296 μ) in August 1982 and May-June 1983 and smaller individuals were 244 and 260 μ. Høeg (1992) mentioned that sexual dimorfism exists within rhizocephalans and that is more marked in some species. Walker and Lester (1998) found that *Heterosaccus lunatus´* cypris larvae are of two sizes (205 and 328 μ) being females the smaller individuals and males the bigger ones. In this study, two sizes of cypris larvae were found, being 228 μ for females and 326 μ for males. Aesthetascs were observed in both sexes, eventhough they are more developed in males; their lengths were similar to those of *Loxothylacus panopaei* (Høeg, 1984) and shorter than those of *Heterosaccus lunatus* (Walker and Lester, 1998). The only references for rhizocephalans antennules are those of Høeg and Lützen (1995), Høeg (1987) and Høeg and Rybacov (1995-96). Høeg and Rybakov (1995-96) pointed out that compared with the rise of literature about the adhesion mode of cypris, very few works have used electronic microscope to examine antennules of cypris and most of this works are only referred to one or two species of balanomorfs. They also established that these studies focus their attention to the adhesion organ localized in the third segment of the antennule, because of its importance in the selection of substrate. Such organ is visible in both sexes.

Nauplius eye was present in all larval stages, as in most of rhizocephalans. *Pellogaster paguri* is a species that does not show nauplius eye in any larval stage and is said that it has been change for an halo that functions as a floating structure, to compensate the phototrofism (Høeg and Lützen, 1995). Thoracic appendages of *L. texanus* are as long as in *Sacculina carcini* (Høeg and Lützen, 1995) and *Arcturossacus kussakini* (Høeg and Rybakov, 1995-96). In all studied larvae it was observed that all

thoracic appendages are united to musculature. This characteristic is clearly visible in both sexes and is proportional to body size. Both sexes of cypris larvae of *Loxothylacus texanus* present a caudal appendage similar to that in *Arcturosaccus kussakini* (Høeg and Rybakov, 1995-96), but different in size and form to that in *Duplorbis* sp. and *Cryptogaster cumacei* (Høeg and Rybakov, 1995-96). In *L. texanus*, such appendage is formed by two spines and is orientated backwards, while in *Duplorbis* sp. It is orientated forwards and in *C. cumacei* it is formed by four spines and is orientated downwards.

None of the examined stages possessed feeding structures, this fact confirms the believe that *L. texanus* has a lecitotrophic development. This observations agree with the work performed by Høeg and Lützen (1995) who said that all rhizocephalan species show a complete lecitotrophic development, because the great amount of vitelum gives the necessary nutrientes to complete development from hatching to invasion (in the females case) or to implantation within an externa (in the males case). The same authors established the existence of cells full of lipids within the body of cypris larvae, this observation was also performed in nauplius stage.

Different authors have established the existence in the Order Rhizocephala, of various nauplius stages, before reaching the cypris one. Walker (1988) and Høeg and Lützen (1995) mentioned that there exist four nauplius stages; Reisser and Forward (1991) did not mention the exact number of those stages and Walker and Lester (1998) mentioned hat there are four nauplius stages in *L. panopaei*. In all the experiments carried out in this study, it was observed that mature externas always expel eggs capable of have cellular division and hatch. Individuals product of this present two nauplius stages before reaching cypris stage.

We can conclude that the rhizocephalan *Loxothylacus texanus* has two nauplius stages. All larval stages presented similar morphological characteristics to related species of rhizocephalans. The fulcral spine diminish from nauplius 1 to nauplius 2. *L. texanus* is a gonocoristic specie in which sexual dimorfism is the size shown in nauplius stages and in aesthetasc in cypris stage. Nauplius 1 stage lasted 24 h and nauplius 2 lasted 12 h. Larval development is completed in 60 h from hatch to cypris stage and 84 h from spawning. Finally, larval development is completed within a salinity interval of 12-24‰.

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