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Shell Disease in *Callinectes rathbunae* Contreras, 1930, Parasitized by *Loxothylacus texanus* Boschma, 1933

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ABSTRACT

Two-hundred forty two *Callinectes rathbunae* crabs parasitized with *Loxothylacus texanus* were caught; of them, 233 showed some type of lesion related to shell disease. The organisms with virgin externa showed grooming activity and the organisms with mature externa did not show that behavior; they only groomed merely the externa, like egg-bearing females do. Sediment accumulation was observed on the shells of these organisms. Another aspect observed was that the organisms with virgin and immature externa had the ability to swim and the crabs with mature externa lost swimming mobility; some of these organisms lost even mobility of their walking legs. The most common lesions were ruptures or cuts (104), followed by brown spots and ulcerations (95), brown spots (20) and ulcerations (7). This is the first report where serious injuries in the abdomen of crustaceans affected with shell disease are documented, showing even loss of exoskeleton and cuts, since this type of injuries were not found in the consulted literature. Summarizing, shell disease is characterized by: (1) the appearance of brown spots, (2) the appearance of brown/black spots with brown/black halos, (3) the appearance of ulcerations with brown/black halos, (4) perforations and (5) loss of large amounts of exoskeleton. We can conclude that this is the first record on shell disease in *C. rathbunae* parasitized by *L. texanus* and the presence of *L. texanus* on *C. rathbunae* is probably a stress cause that facilities the establishment of bacteria on parasitized dark crabs.

Key words: Disease crabs, parasitism, rhizocephala, brown spots, injuries, cuts, Alvarado lagoon, Mexico

INTRODUCTION

Shell disease syndrome can be described as a progressive degradation of the crustacean cuticle and is characterized externally by the appearance of black spot lesions in the exoskeletal surface (Getchell, 1989; Vogan and Rowley, 2001). The shell disease syndrome has been reported from many freshwater and marine crustaceans of economic importance (Sindermann and Lightner, 1988; Sindermann, 1989).

The decapod crustaceans affected by shell disease exhibit brown/black colored lesions, as a consequence of melanization, a defense response triggered by cuticular damage (Nyhlen and Unestam, 1980), exoskeletal erosion is largely attributed to the chitinolytic activities of microorganisms (Getchell, 1989; Mancuso *et al*., 2010). The early stage of shell disease manifests

itself as numerous punctiform brown marks with reddish-brown depressed centers (Rosen, 1970). As the disease progresses, these lesions deepen and eventually coalesce (Johnson, 1983). The lesions are round, oblong, or irregularly shaped with brown to black foci. They are characterized by loss of epicuticle and exocuticle and either loss or severe fragmentation and necrosis of calcified endocuticle (Rosen, 1967; Young and Pearce, 1975; Comely and Ansell, 1989; Getchell, 1989; Sindermann, 1989; Andersen *et al*., 2000; Noga *et al*., 2000; Vogan *et al*., 2001, 2002, 2008; Vogan and Rowley, 2001; Smolowitz *et al*., 2005; Augusto-Gregati and Negreiros-Fransozo, 2009; Jithendran *et al*., 2010; Mancuso *et al*., 2010).

This shell disease has been observed in blue crab *Callinectes sapidus* Rathbun, 1896 (Rosen, 1967; McKenna *et al*., 1990; Noga *et al*., 1994, 2000), grey crab *C. ornatus* Ordway, 1863 (Mantellatto *et al*., 2000), freshwater crayfish *Astacus astacus* Linnaeus, 1758 (Nyhlen and Unestam ,1980), American lobster *Homarus americanus* H. Milne Edwards, 1837 (Smolowitz *et al*., 2005), edible crab *Cancer pagurus* Linnaeus 1758 (Vogan *et al*., 2001; Vogan and Rowley, 2001; Vogan *et al*., 2002), green crab *Carcinus maenas* Linnaeus, 1758 and brown shrimp *Crangon crangon* Linnaeus, 1758 (Stentiford and Feist, 2005), mud crab *Scylla* spp. (Jithendran *et al*., 2010), *S. serrata* Forskal, 1775 (Andersen *et al*., 2000), crab *Neohelice granulata* Dana, 1851 (Augusto-Gregati and Negreiros-Fransozo, 2009) and spiny lobsters *Palinurus elephas* Fabricius, 1787 (Mancuso *et al*., 2010). In Mexico, there are no studies on the presence of this disease in crustaceans. The objective of the present work was to describe the different types of lesions that occur in *Loxothylacus texanus*-parasitized *C. rathbunae*.

MATERIALS AND METHODS

Dark crabs of either sex, parasitized with *L. texanus*, were captured from the lagoon-estuarine subsystem of Alvarado, Veracruz, Mexico, during the spring of 2002; crabs were captured using a handmade fishing net, locally called "aro jaibero" (crab hoop). Dark crabs were transported to the laboratory of the National Collection of Crustaceans in the National Autonomous University of Mexico (UNAM), in Mexico City. In the laboratory, all specimens were measured with a 0.1 mm precision Vernier caliper and classified following Wardle and Tirpak (1991) criteria on externa type. The crabs were placed individually in 20-liter aquaria, the salinity was maintained at 15±2 PSU (practical salinity units) and the temperature at 25±2°C. The external morphology of crabs was observed and all abnormal features were characterized as lesions and classified as follows: normal appearance, with brown spots, with ulcerations, with brown spots and ulcerations, with ruptures and/or cuts. The description of the lesions was made according to Williams (1984), Andersen *et al*. (2000) and Noga *et al*. (2000) criteria. All lesions were photographed. Crabs included hosts with virgin externa (externa that have not been fecundated and presented a milky white color) and fecundated externa (immature externa presenting a yellow color and about to expel larvae and mature externa which are dark brown color and are the ones that expel larvae periodically) (Wardle and Tirpa, 1991; Vazquez-Lopez *et al*., 2006). Data were analyzed with Chi-square (to observe independence between the type of externa and the kind of lesions observed) ($\alpha_{0.05,4\ fg}$) and Kruskal-Wallis multiple-comparison test ($z<1.96$).

RESULTS

Crabs with carapace width interval of 5.8 to 9.6 cm, mean of 8.5 cm, were captured. Of them, 139 were females (57%) with virgin externa, 12 females (5%) with fecundated externa, 77 males (32%) with virgin externa and 14 males (6%) with fecundated externa. The chi-square test showed no relationship between the type of externa and the kind of lesions observed; the Kruskal-Wallis test showed no significant differences between the size of the organisms and the type of lesion.

DISCUSSION

The Chi-square test ($\chi^2 = 1.99$, $\chi^2_{\alpha} = 0.05$, 4fa1 = 9.49) suggested that shell disease may affect equally the organisms with virgin and fertilized externa (immature and mature externae), whereas the Kruskal-Wallis test showed no significant differences between the size of the organisms and the type of lesion, suggesting that shell disease affects all crabs, independently of their size. These lesions have also been observed in healthy, collected and cultured *Callinectes sapidus* organisms of either sex (Noga *et al*., 2000). However, Andersen *et al*. (2000) observed 121 crabs *Scylla serrata* with shell lesions. These crabs were predominantly female (78.8%). Same researchers observed that lesions were greater in females that in males. Comely and Ansell (1989) found no relationship between the capture depth of *Carcinus maenas* Linnaeus, 1758 and the presence of lesions.

In dark crabs with normal appearance (16) no external injuries (Table 1, Fig. 1c, d) were observed as in healthy organisms (Fig. 1a, b). In 20 crabs (8%), brown spots of irregular shape were observed, mainly in the ventral part of the carapace and pereiopods. In seven hosts (3%),ulcerations were observed in the dorsal and ventral cephalothorax, including the abdomen and on both sides of the pereiopods; in 95 crabs (39%) brown spots and ulcerations were observed in diverse regions of the body, 91 organisms (38%) had ruptures in the

Table 1: Lesions observed in the crabs

	Host category			
	Females		Males	
Lesion category	With virgin externa	With fecundated externa	With virgin externa	With fecundated externa
Normal appearance	8	1	4	3
Brown spots	9	1	9	1
Ulcerations	5	0	1	1
Brown spots and ulcerations	56	5	30	4
Ruptures and/or cuts	61	5	33	5

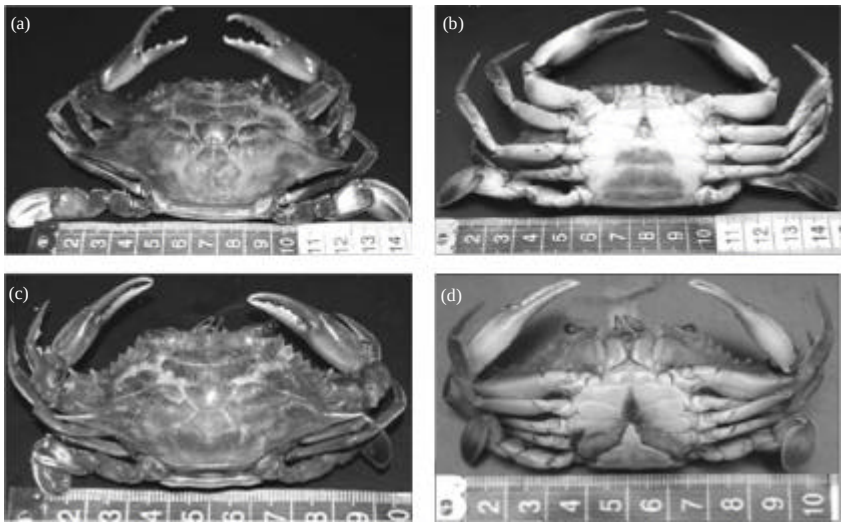

Fig. 1(a-d): Female with normal appearance; (a) Dorsal view and (b) Ventral view and Male with normal appearance; (c) dorsal view and (d) Ventral view

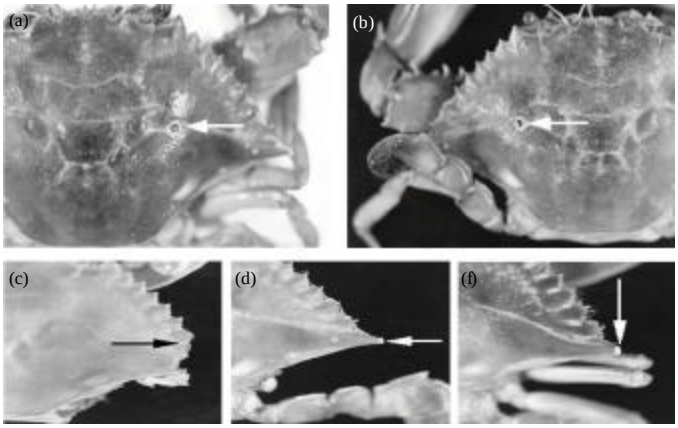

Fig. 2(a-e): (a) Circular ulceration in the mesogastric region (female) (b) Circular ulceration in the mesobranchial region (male), (c) Cut in the anterolateral region (female), (d) Rupture on the anterolateral spine in female crab and (e) Rupture on the anterolateral spine in male crab, Arrows indicate point of abnormality

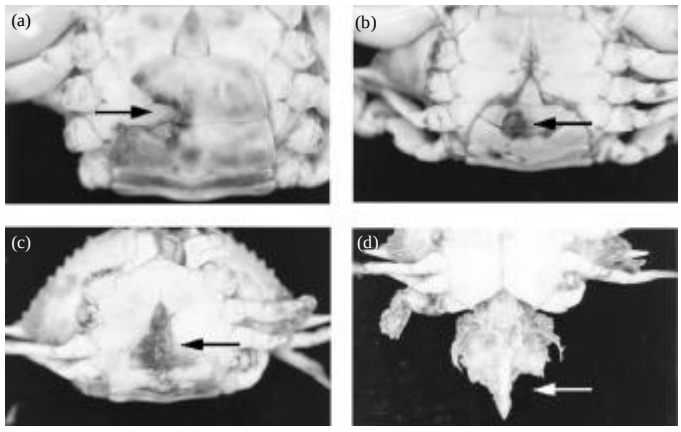

Fig. 3(a-d): (a) Rupture in the right side of abdomen, around it a brown spot is (indicate with arrow) observed (female), (b) Ulceration in the right side of abdomen, in the periphery a brown spot is observed (male), (c) Rupture in the left side of abdomen, in the right side, a big brown spot is observed (female) and (d) Same rupture from (c) seen inside the abdomen, arrows indicate point of abnormality

propodus, the dactyl or the anterolateral spines and 13 crabs (5%) had cuts on some of the walking legs, swimming legs and/or abdomen (Fig. 2-5).

Rosen (1967) stated that the signs of the disease are best seen on the ventral side of *Callinectes sapidus* where commensal organisms rarely attach themselves; even in the early stages of shell disease, the brown depressed necrotic spots are clearly visible against the cream-colored background, necrotic lesions also occur on the dorsal area of the carapace, observing also irregularly shaped areas with a deep necrotic center and necrosis of the lateral spines and the dactylopodites. In one advanced case, the distal section of the lateral spine was practically detached from its base; the same was observed in this study (Fig. 2c).

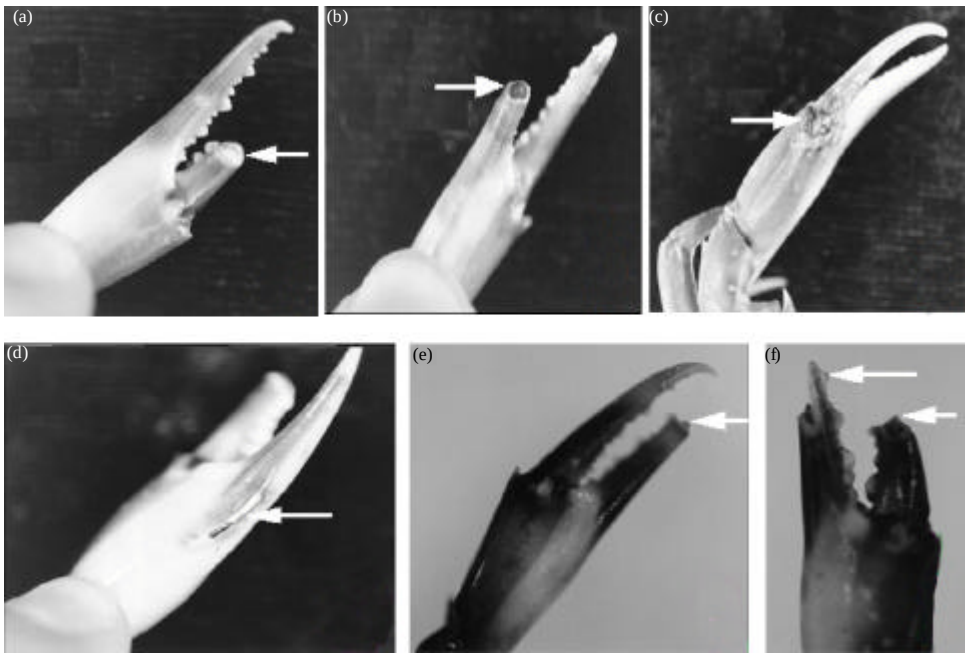

Fig. 4(a-f): (a) Rupture on dactyl in the right cheliped, a brown hoop is (indicated with arrow) observed around the lesion (female), (b) Rupture in the propodus of the right cheliped (male), (c) Irregular ulceration in the left cheliped, an irregular brown spot is observed in the dactyl base (male), (d) Elongated ulceration under the propodus in the left chela (male), (e) Rupture at the tip of the cheliped in left propodus, an irregular shape of the brown area in the propodus and dactyl, exposure of muscle tissue are observed (male) and (f) Right cheliped cut in the propodus and dactyl, despite the severity of the injury, there is no exposure of muscle tissue; in both, an irregular shaped brown area is observed (male), Arrow indicate point of abnormality

Young and Pearce (1975) observed in crabs and lobsters collected in the vicinity of sewage sludge and dredge spoil disposal areas, most frequently skeletal erosions on the tips of the dactylopodites of the walking legs, on the ventral sides of the chelipeds, around areas of articulation where contaminated sediments could accumulate and on parts of the exoskeleton that formed prolongations or spines, the same researchers stated that the lesions were certainly not limited to these areas. Regard, in the present study were observed rounded ulceration on the ventral side of the palm and irregular shaped ulceration on the ventral side of the carpus (Fig. 5a), rounded ulceration on the lateroventral side of propodus and ulceration rounded on the ventral side in the carpus (Fig. 5b), rounded ulceration on the ventral side of the cheliped and in the propodus from the walking appendages (Fig. 5d) and irregular shaped ulceration in the palm, carpus and merus (Fig. 5f). Getchell (1989) mentioned finding *Homarus americanus* with shell disease and abdominal membrane lesion; *Callinectes sapidus,* shell disease; *Cancer magister* (Dana, 1852), exoskeleton lesions; *Penaeus* spp., brown spot disease; and the giant prawn *Macrobrachium rosenbergii* (De Man, 1879), with brown spot disease, bacterial necrosis and burn spot disease, this last is similar to that observed in a male *C. rathbunae* (Fig. 3b). Noga *et al.* (1994) observed shell

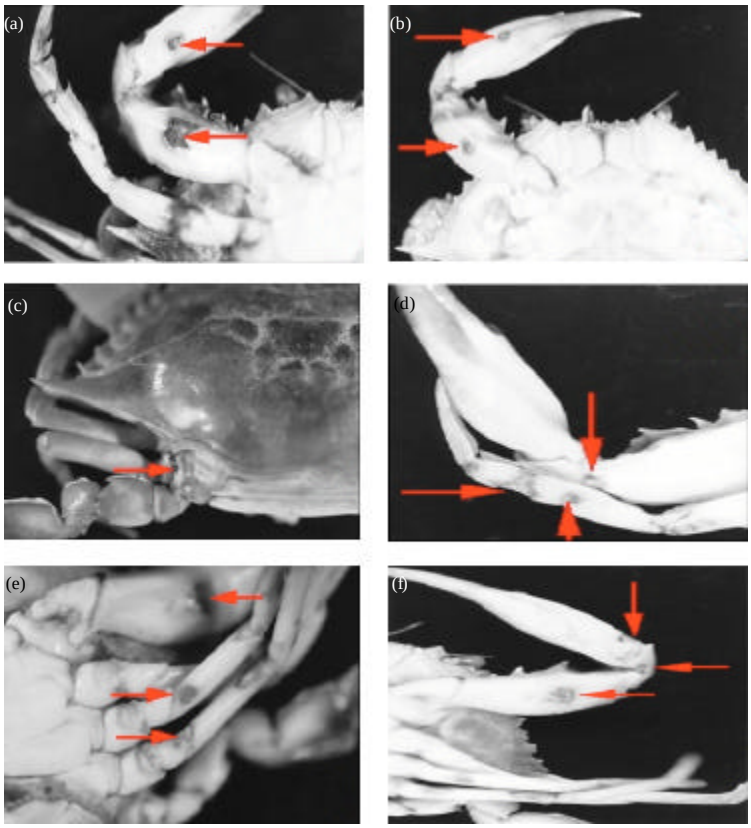

Fig. 5(a-f): (a) Rounded ulceration on the ventral side of the palm, irregular shaped ulceration on the ventral side of the carpus, a brown hoop (indicate with arrow) is observed in both lesions (female), (b) Rounded ulceration on the lateroventral side of propodus, ulceration rounded on the ventral side in the carpus, between the carpus and palm area an irregular shaped brown area is observed (female), (c) Rupture in the coxa (male), (d) Rounded ulceration on the ventral side of the cheliped and in the propodus from the first walking appendage, in the same walking leg an irregular ulceration-rupture shape and a brown hoop are observed (male), (e) Oval ulceration in the ventral side of the carpus, rounded ulcerations in the merus of the second an third walking leg (female) and (f) Irregular shaped ulceration in the palm, carpus, and merus (male), Arrows indicate point of abnormality

disease lesions in *C. sapidus* collected in the Pamlico River, they observed complete erosive loss of the anterolateral spine; the same lesion was observed in the anterolateral region of a female (Fig. 2c), the same authors observed a deep erosive lesions on the chelipeds; in this study were observed a rounded ulceration on the lateroventral side of propodus and an ulceration rounded on the ventral side in the carpus in a female (Fig. 5b). Likewise, Andersen *et al.* (2000) found shell lesions in 146 *S. serrata* crabs, 121 (82.9%) out of a total of 673 had rust spot lesions of the carapace, these crabs were predominantly females (78.8%) and most crabs (>69%) had more than one lesion; of the rust spot lesions, 54.8% were bilaterally symmetrical but only 10.6% were

perforated. The same authors found that the prevalence of rust spot lesions for male crabs ranged from 67.5 to 0%, while that for female crabs ranged from 37.0 to 0.9%. In the present study, no symmetrical bilaterality was observed regarding lesions distribution, only two cases was observed, an ulceration/perforation on each side close to the meso-metagastric region (named as area 7 for Andersen *et al*., 2000); in the Fig. 2a, a circular ulceration in the meso-mesogastric region in right side is observed, in the Fig. 2b, is observed the same lesion but in left side in the (Fig. 2a), a circular ulceration in the meso-mesogastric region in right side is observed, in the Fig. 2b.

Noga *et al*. (2000) observed diffuse brown melanization on the ventral carapace linear black foci (arrow) on a cheliped, moderate ulceration on a cheliped and severe ulceration on a walking leg. In the present study were observed the next injuries: Diffuse brown spots in the abdomens of females and males (Fig. 3a-c), rounded ulceration on the lateroventral side of propodus and ulceration rounded on the ventral side in the carpus (Fig. 5b), rupture in the right side of abdomen (Fig. 3a), rounded ulceration on the ventral side of the palm and irregular shaped ulceration on the ventral side of the carpus too (Fig. 5a), brown hoops in both lesions (rounded ulceration and irregular shaped ulceration) and brown hoop around the lesions was observed on dactyls of some males (Fig. 4a). Smolowitz *et al*. (2005) examined *Homarus. americanus* lobsters and found shallow erosions in the epicuticle and exocuticle; the authors stated that the ventral and lateral edges of the erosions were characterized by a brown/gold color indicating activation of the melanistic inflammatory response. The same authors observed moderate lesions consisting of erosions of the calcified endocuticle, severe deep erosions of the uncalcified endocuticle/membranous layer and ulcerations of the carapace characterized by total loss of all cuticular material and the cuticular epithelium, thus exposing the connective tissues of the body to the environment. It is worth noting that the latter is similar to what is observed in Fig. 2a and 4a, b, d-f; Jithendran *et al*. (2010) mentioned that shell disease is not of infectious nature and that it is characterized by circular shaped lesions, commonly called "rust spot shell disease".

It has been observed that chitinolytic bacteria, such as genera *Aeromonas*, *Pseudomonads*, *Spirillum*, *Flavobacterium* and *Vibrio,* are responsible for the injuries in all cases (Rosen, 1967; Young and Pearce, 1975; Comely and Ansell, 1989; Getchell, 1989; Sindermann, 1989; Andersen *et al*., 2000; Noga *et al*., 2000; Smolowitz *et al*., 2005; Augusto-Gregati and Negreiros-Fransozo, 2009; Jithendran *et al*., 2010; Mancuso *et al*., 2010). Adhesion to the chitinous substrate may occur as a result of various proteins on the bacterial surface that are specific for chitin or non-specific adhesion processes. These interactions ultimately lead to the formation of biofilms containing mixed microbial assemblages (Vogan *et al*., 2008).

The shell disease has been associated with stressful environments, such as intensive aquaculture, polluted natural environments, exposure to pesticides, heavy metals, high nutrient loads and low dissolved oxygen (Young and Pearce, 1975; Engel and Noga, 1989; Sindermann, 1989; Khoo *et al*., 1999; Andersen *et al*., 2000; Noga *et al*., 2000). Robles *et al*. (2002) mention that *Callinectes rathbunae* crabs with mature parasites of *Loxothylacus texanus* had significantly higher oxygen consumption rates than control crabs, these authors suggest that mature externae of parasite are energetically costly to host crabs. Alvarez *et al*. (2002) mentioned that *L. texanus* affects the osmoregulation in *C. rathbunae* when exposed to changing salinity conditions, hence, it is understandable that parasitism caused by Rhizocephala induces stress in their hosts, easing the establishment of bacteria and other pathogen agents. Khoo *et al*. (1999) stated that callinectin's predominance as an antibacterial factor in blue crab (*C. sapidus*) hemocytes suggests that it plays a major role in blue crab immunity, these authors stated that the antibacterial activity of blue crab

hemolymph is severely depressed in polluted waters. Noga *et al.* (1994) found that increased prevalence of shell disease in blue crabs *C. sapidus* coincides with low serum antibacterial activity. It has been observed that inadequate nutrition causes shell disease, a reduction in hemocyte numbers in cultured *Homarus americanus* lobsters (Noga *et al.*, 1994). Vafopoulou (2009) mentioned that wound healing and repair in healthy crayfish involves the action of two physiological systems, the immune system and the neuroendocrine system, regulating synthesis of the steroid molting hormones, ecdysteroids; injury promotes a swift rise in hemolymph ecdysteroids to a low, sustained plateau, followed by a premolt peak and molting. The plateau is essential for wound healing since its principal targets are the circulating cells of the immune system, the hemocytes and healthy epidermal cells and fibrocytes. Massive migration of these cells occurs under the wound and their concerted efforts under ecdysteroid control are paramount to wound healing and repair. These cells are likely engaged in physiological and biochemical activities that promote cell communication and cell to cell adhesion, removal of dead and harmful material and production of molecules essential to tissue regeneration (Vafopoulou, 2009). Dillaman and Roer (1980) observed that injury in the calcified layers of the carapace from healthy crab *Carcinus maenas* stimulates the tissue under and adjacent to the injury to deposit a unique calcified cuticular material below the intact membranous layer.

It should be noted that only Comely and Ansell (1989) mentioned that the organisms studied were parasitized with rhizocephalan (*C. maenas* parasitized by *Sacculina carcini* Thompson, 1836). Comely and Ansell (1989) observed black spot to be initially more or less circular, with increasing size, the lesions may perforate the shell, becoming evident externally first as a general discoloration and finally as a black area; the same authors mention that, in general, it appears that the disease is as evident or even more evident on the external surface of the chelae than on the internal surface but it is very difficult to make accurate observations, as any such attempt involves considerable destruction of the limb and the part of the external skeleton in which the disease was most manifest varies between the different crab species. To the best of our knowledge, this is the first report where serious injuries in the abdomen of crustaceans affected with shell disease are documented, showing even loss of exoskeleton (Fig. 4f) and cuts (Fig. 3a, c, d), we did not find this type of lesions described in the consulted literature. Summarizing, shell disease is characterized by: (1) the appearance of brown spots, (2) the appearance of brown/black spots with brown/black halos, (3) the appearance of ulcerations with brown/black halos, (4) perforations and (5) loss of large amounts of exoskeleton. We can conclude that this paper is the first record on shell disease in *C. rathbunae* parasitized by *L. texanus*. The presence of *L. texanus* on *C. rathbunae* is probably a stress cause that facilitates the establishment of bacteria on parasitized dark crabs.

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