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Notes on the behaviour of the portunid crab *Charybdis longicollis* Leene parasitized by the rhizocephalan *Heterosaccus dollfusi* Boschma

G. INNOCENTI†, M. VANNINI† and B. S. GALIL‡

†*Museo Zoologico 'La Specola', Università di Firenze, via Romana 17, 50125 Firenze, Italy*
‡*Israel Oceanographic and Limnological Research, P.O. Box 8030, Haifa 31080, Israel*

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A significant portion of the population of *Charybdis longicollis* (Decapoda: Portunidae) along the Mediterranean coast of Israel is parasitized by the rhizocephalan *Heterosaccus dollfusi*. The behaviour of the parasitized crabs, in different stages of infection, is examined and compared with the behaviour of non-parasitized crabs. Locomotion, rest, body-, egg- and parasite-grooming, digging and burying, courtship and mating are described qualitatively and analysed quantitatively under standardized conditions. Behaviour patterns of parasitized crabs lacking *externae*, either immature or scarred, were similar to those of non-parasitized crabs. The presence of *externae* was observed to modify digging and to inhibit burying behaviour. The presence of *externae* induces an imitation of egg-grooming behaviour pattern in both males and females. *Externae*-bearing crabs engaged in parasite-grooming more intensely than ovigerous crabs in egg-grooming.

KEYWORDS: *Charybdis*, *Heterosaccus*, Rhizocephala, parasitism, behaviour.

Introduction

The lessepsian portunid crab *Charybdis longicollis* Leene is one of the most common decapods along the Mediterranean coast of Israel, where it was first recorded about 40 years ago (Lewinsohn and Holthuis, 1964). Since 1993, it has been parasitized by the rhizocephalan *Heterosaccus dollfusi* Boschma, a lessepsian migrant too (Galil and Lützen, 1995).

H. dollfusi penetrates through the gills of young *C. longicollis* and, for a certain period, develops inside the crab ('*interna*'). Over several moults, the abdominal shape of the parasitized male is modified, becoming more similar to that of the female, while the pleopods disappear in both sexes. Later, the reproductive part of the parasite protrudes out of the crab's abdomen, assuming a sacciform shape ('*externa*'), occupying the position normally taken by the eggs in berried females. At the end of the reproductive period, the *externa* drops off and leaves a scar (Galil and Lützen, 1995).

Studies on parasitized crabs have focused on the morphological and histological effects rhizocephalans have on their hosts (see Lawler and Shepard, 1978 and Høeg,

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1995 for a review), only a few studies describe the behaviour of the parasitized crabs. Rasmussen (1959) noted that abdomen grooming behaviour in *externae*-bearing *Carcinus maenas* (Linnaeus) of both sexes resembled that of berried females, and suggested that the stimulus for that behaviour was due to the *interna* and was not merely tactile. Bishop and Cannon (1979) observed similar behaviour in *Portunus pelagicus* (Linnaeus) parasitized by *Sacculina granifera* Boschma, as did Ritchie and Høeg (1981) in rhizocephalized porcellanid crabs. Overstreet (1983) and Wardle and Tirpak (1991) noted the behaviour of the blue crab, *Callinectes sapidus* Rathbun infested by *Loxothylacus texanus Boschma*, and Gherardi and Cassidy (1995) of the hermit crab *Discorsopagurus schmitti* (Stevens) parasitized by *Peltogaster boschmae* Reinhard.

However, these behavioural accounts furnished us mainly with qualitative descriptions, few studies have employed controlled ethological experiments and the ones which did so were all conducted on *externae*-bearing crabs.

Materials and methods

Charybdis longicollis specimens were caught by trawl on sandy bottoms at depths of 15–20 m, in Haifa Bay (Israel), in November 1995. Specimens measuring more than 30 mm across the carapace were taken to Israel Oceanographic and Limnological Research Institute in Tel Shikmona, Haifa.

Parasitized crabs were separated from non-parasitized. The presence of parasites in specimens lacking *externae* was verified by gently prying loose the abdomen and looking for pleopods. The crabs were reared in separate compartments in tanks and fed on fish and molluscs. For observation they were transferred to a small aquarium (20 × 20 × 10 cm), the bottom of which was covered in 4 cm of fine shell grit. Observations were made in dim light and crab movements were recorded every 15 s for 30 min. The overall time distribution, for all crab categories, was then analysed and usually was found to follow a plurimodal distribution. The four main peaks were used to split each into four time classes.

Observations were carried out on 85 crabs, subdivided into the following categories: M ($n = 9$), unparasitized males, M_{int} ($n = 5$), males with internal infection, M_{ext} ($n = 21$), males bearing *externae*, M_{scar} ($n = 5$), males bearing scars, F_{eggs} ($n = 12$), ovigerous females, F ($n = 5$), unparasitized females without eggs, F_{int} ($n = 8$), females with internal infection, F_{ext} ($n = 13$), females bearing *externae*, F_{scar} ($n = 7$), females bearing scars. We observed the six following patterns: (i) walking or swimming, (ii) digging, (iii) staying buried in the sand, (iv) grooming of the eggs or of the *externae*, (v) grooming of the limbs or carapace, (vi) stance position. Movements of the antennulae, antennae and maxillipeds were not considered.

After observation, the crabs were all transferred to communal tanks (80 × 50 × 50 cm), still keeping uninfected crabs separated from infected crabs. This allowed observation of courtship and mating in a total of six couples of uninfected crabs, no sexual behaviour was observed among the infected crabs. Spawning of *externa* was not observed over the study period. Prior to the experimental observations, some nocturnal observations were carried out. Since no relevant differences were found between nocturnal and diurnal behaviour, we carried out the observations during the day, between 10 a.m. and 5 p.m.

Data frequencies were compared by applying the G-test (with Williams correction for $n < 50$; Sokal and Rohlf, 1981).

Results

Eggs and parasite grooming

The cleaning of *externae* and eggs is similar: the crabs use the 2nd, 3rd and 4th pereopods to rub, scratch, pinch and smooth the eggs or the *externae* whilst contracting the abdomen, so the mass (eggs or *externae*) is lowered and adducted to it. Infested crabs use the dactyls of the 2nd–4th pereopods to clean inside the mantle cavity of the *externae*. The effect of this behaviour on the parasite has never been investigated in detail. It could be related to cleaning proper as well as oxygenating, helped by contracting and expanding the parasite's mantle cavity. Ovigerous females shake their pleopods, lacking in parasitized crabs. In one instance, the moult of an *externa* was removed by the crab using the dactyls of the 2nd–3rd pereopods. Similar behaviour was observed by Ritchie and Høeg (1981).

The *externae* grooming behaviour was not observed in M_{int} and F_{int}, while, a few M_{scar} and F_{scar} flap their abdomen occasionally—without the *externa*—by bending the 2nd to 4th left and right pereopods inside. Crabs bearing *externae* modify their behaviour and spend most of their time looking after the parasite, cleaning and fanning like ovigerous females (F_{ext} vs. F_{eggs}, $G_{[3]} = 7.35$, ns). In fact, this behaviour was performed significantly more frequently, both in parasitized females (lacking eggs) and in males (table 1a). Marked differences appear in the duration of the eggs or *externa* grooming pattern as the infection progresses (uninfected animals, those with *interna* and scarred vs. those with *externae*, $G_{[3]} = 48.63$ $P < 0.001$), while there was no difference between the sexes (M_{ext} vs. F_{ext}: $G_{[3]} = 1.57$, ns). Overall distribution of the pattern was multimodal.

To determine whether the physical presence of an *externa* stimulated grooming behaviour, nine M, one F, two M_{int}, four F_{int}, three M_{scar} and one F_{scar} were fitted with a rounded piece of foam rubber (15 × 15 mm), simulating the size and consistency of an *externa*, stuck inside the abdomen with a drop of epoxide glue. The crabs were observed continuously for 30 min. Their activity and behaviour were similar to non parasitized crabs, i.e. they did not perform grooming behaviour, except for the raised posture of the body as seen in *externae*-bearing crabs and non-coordinated digging of the sand. Within a few minutes, five M were observed trying to pull out the artificial *externa* with their chelipeds, while two M_{scar} were observed contracting their abdomen and cleaning the false *externa*.

Thus in uninfected crabs, grooming behaviour is apparently not stimulated by the physical presence of *externa per se*; however, a mechanical device probably could elicit grooming behaviour in crabs recently freed from the parasite, although some postural modification does occur when the artificial *externa* is applied, for instance, the marked raised position of the body.

Digging and burying movements

The crabs usually drag the sand under the abdomen with the 2nd, 3rd and 4th pereopods of both sides, then push it forward or laterally. Crabs bearing *externa* dig in an uncoordinated manner, gathering the sand less efficiently. In many cases, digging movements anticipate the burying of the crab in the sand: the animal sinks very quickly, moving the posterior part of the body backwards and forwards, while the 5th pereopods throw sand over the carapace until the animal is completely concealed in the sediment. The digging pattern seemed to be performed more in F_{eggs} (F_{eggs} vs. all the remaining categories, $G_{[6]} = 13.603$, $P < 0.05$), while no

Table 1. Number of crabs observed in (a) grooming and (b) staying buried for each category. The four time classes (in s) correspond to the four peaks shown by the overall time span multimodal distribution (30 min).

a. Grooming

	Time classes	0	1–150	151–300	> 300	*n*
Crab categories	M	9	0	0	0	9
	M_{int}	5	0	0	0	5
	M_{ext}	1	5	11	4	21
	M_{scar}	1	4	0	0	5
	F_{eggs}	3	4	5	0	12
	F	3	2	0	0	5
	F_{int}	7	0	1	0	8
	F_{ext}	0	5	5	3	13
	F_{scar}	3	2	2	0	7
	$M + M_{int} + M_{scar}$	15	4	0	0	19
	M_{ext}	1	5	11	4	21
	$G_{[3]} = 32.81$, $P < 0.001$					
	$F + F_{int} + F_{scar}$	13	4	3	0	20
	F_{ext}	0	5	5	3	13
	F_{eggs}	3	4	5	0	12
	$G_{[6]} = 21.95$, $P < 0.01$					

b. Staying buried

	Time classes	0	1–180	181–360	> 360	*n*
Crab categories	M	4	0	3	2	9
	M_{int}	2	1	2	0	5
	M_{ext}	17	4	0	0	21
	M_{scar}	5	0	0	0	5
	F_{eggs}	7	5	0	0	12
	F	3	2	0	0	5
	F_{int}	6	0	0	2	8
	F_{ext}	12	1	0	0	13
	F_{scar}	7	0	0	0	7
	$M + M_{int} + M_{scar}$	11	1	5	2	19
	M_{ext}	17	4	0	0	21
	$G_{[3]} = 11.13$, $P < 0.02$					
	$F + F_{int} + F_{scar}$	16	2	0	2	20
	F_{ext}	12	1	0	0	13
	F_{eggs}	7	5	0	0	12
	$G_{[4]} = 7.17$, NS					

M, normal males; M_{int}, males with internal infection; M_{ext}, males with externa; M_{scar}, males with scars; F_{eggs}, ovigerous females; F, normal females without eggs; F_{int}, females with internal infection; F_{ext}, females with externa; F_{scar}, females with scars.

difference was revealed between crabs of both sexes with and without *externae* ($G_{[3]} = 2.715$, ns) (see also table 2a).

Burying behaviour is very common in non-parasitized crabs and in ovigerous females but not in parasitized crabs. The *externae* bearing crabs can barely bury themselves because their digging behaviour is affected, while digging frequency remains constant. Differences in the pattern were observed as the infection progressed (uninfected animals, those with *interna* and scarred vs. those with *externae* vs. F_{eggs}, $G_{[6]} = 17.813$, $P < 0.01$), the crabs lacking *externae* were more prone to staying buried (table 1b).

General body grooming

Cleaning movements were frequently observed in both uninfected and infected crabs, and are identical throughout (uninfected animals, those with *interna* and scarred vs. those with *externae* vs. F_{eggs}, $G_{[6]} = 10.406$, ns; table 2b). The crabs clean their carapace by moving the dactyls of the pereopods and with their chelipeds, which are used to brush the carapace, sternite and limbs. Once the cleaning is completed, the dactyls are brushed against each other. They are then carefully cleaned by the maxillipeds. Faeces were expelled with the help of the 3rd–4th pereopods of both sides, afterwards the dactyls of the pereopods were always used to groom each other.

Locomotion

When the uninfected crabs move, they keep a low position, whereas crabs bearing *externae* or eggs walk with the body and pereopods markedly raised. At times, normal locomotion on the bottom was interrupted by short bouts of swimming, both in uninfected and infected crabs. When the crabs were submitted to an unexpected standardized object dropped on their body from above, both infected and uninfected crabs walked and swam. No differences in the duration of locomotive patterns were noted between uninfected and infected crabs (uninfected animals, those with *interna* and scarred vs. those with *externae* vs. F_{eggs}, $G_{[6]} = 6.485$, ns; table 3a).

Resting position (stance)

The crabs remain immobile with the 2nd–4th pereopods and chelipeds flexed close to the body, and stay above the water-sediment interface. The resting position of crabs bearing *externae* differed in the higher position of the body on account of the encumbrance of the parasite and in the grooming posture; in fact no differences were found between the uninfected and infected crabs (uninfected animals, those with *interna* and scarred vs. those with *externae* vs. F_{eggs}, $G_{[6]} = 3.676$, ns; table 3b).

Moult

Moulting in *C. longicollis* was often observed in non-parasitized crabs, but only in three infected crabs, presumably in the last stage of internal infection. The moulted infected crabs were isolated, and seven to eight days after the moult a virgin *externa* was observed.

Behaviour of the externae

The *externae* swell and contract while the crabs move and when the abdomen is kept open. This is probably the reason why sand was noted inside their cavity,

Table 2. Number of crabs observed in (a) digging and (b) general body grooming for each category. The four time classes (in s) correspond to the four peaks shown by the overall time span multimodal distribution (30 min).

a.

	Digging					
	Time classes	0	1–90	91–180	> 180	*n*
Crab categories	M	3	5	1	0	9
	M_{int}	0	4	1	0	5
	M_{ext}	9	10	2	0	21
	M_{scar}	1	3	1	0	5
	F_{eggs}	0	7	5	0	12
	F	3	2	0	0	5
	F_{int}	1	4	2	1	8
	F_{ext}	7	4	1	1	13
	F_{scar}	3	3	1	0	7
	$M + M_{int} + M_{scar}$	4	12	3	0	19
	M_{ext}	9	10	2	0	21
	$G_{[2]} = 2.100$, NS					
	$F + F_{int} + F_{scar}$	7	9	3	1	20
	F_{ext}	7	4	1	1	13
	F_{eggs}	0	7	5	0	12
	$G_{[6]} = 13.242$, $P < 0.05$					

b.

	Body grooming					
	Time classes	0	1–90	91–180	> 180	*n*
Crab categories	M	4	3	1	1	9
	M_{int}	3	0	2	0	5
	M_{ext}	6	14	1	0	21
	M_{scar}	2	2	0	1	5
	F_{eggs}	4	7	1	0	12
	F	2	1	1	1	5
	F_{int}	2	5	1	0	8
	F_{ext}	3	8	2	0	13
	F_{scar}	0	4	1	2	7
	$M + M_{int} + M_{scar}$	9	5	3	2	19
	M_{ext}	6	14	1	0	21
	$G_{[3]} = 7.678$, NS					
	$F + F_{int} + F_{scar}$	4	10	3	3	20
	F_{ext}	3	8	2	0	13
	F_{eggs}	4	7	1	0	12
	$G_{[4]} = 5.203$, NS					

M, normal males; M_{int}, males with internal infection; M_{ext}, males with externa; M_{scar}, males with scars; F_{eggs}, ovigerous females; F, normal females without eggs; F_{int}, females with internal infection; F_{ext}, females with externa; F_{scar}, females with scars.

Table 3. Number of crabs observed in (a) locomotion and (b) resting position for each category. The four time classes (in s) correspond to the four peaks shown by the overall time span multimodal distribution (30 min).

a.

	Locomotion					
	Time classes	0	1–90	91–180	> 180	*n*
Crab categories	M	3	4	1	1	9
	M_{int}	2	3	0	0	5
	M_{ext}	5	15	1	0	21
	M_{scar}	0	4	1	0	5
	F_{eggs}	5	7	0	0	12
	F	3	2	0	0	5
	F_{int}	2	4	1	0	7
	F_{ext}	4	9	0	0	13
	F_{scar}	1	3	3	0	7
	$M + M_{int} + M_{scar}$	5	11	2	1	19
	M_{ext}	5	15	1	0	21
	$G_{[3]} = 1.807$, NS					
	$F + F_{int} + F_{scar}$	6	9	4	0	19
	F_{ext}	4	9	0	0	13
	F_{eggs}	5	7	0	0	12
	$G_{[4]} = 6.841$, NS					

b.

	Stance					
	Time classes	0	1–135	136–270	> 270	*n*
Crab categories	M	3	4	2	0	9
	M_{int}	0	3	1	1	5
	M_{ext}	1	15	4	1	21
	M_{scar}	0	2	1	2	5
	F_{eggs}	1	7	3	1	12
	F	0	1	2	2	5
	F_{int}	2	4	2	0	8
	F_{ext}	1	7	3	2	13
	F_{scar}	1	3	3	0	7
	$M + M_{int} + M_{scar}$	3	9	4	3	19
	M_{ext}	1	15	4	1	21
	$G_{[3]} = 3.169$, NS					
	$F + F_{int} + F_{scar}$	3	8	7	2	20
	F_{ext}	1	7	3	2	13
	F_{eggs}	1	7	3	1	12
	$G_{[6]} = 1.682$, NS					

M, normal males; M_{int}, males with internal infection; M_{ext}, males with externa; M_{scar}, males with scars; F_{eggs}, ovigerous females; F, normal females without eggs; F_{int}, females with internal infection; F_{ext}, females with externa; F_{scar}, females with scars.

probably aspirated by the above mechanism, also reported by Hartnoll (1967) in *Sacculina hartnolli* Boschma parasitizing *Geograpsus lividus* (Milne Edwards).

Courtship and mating

A similar behaviour pattern was observed in all cases of courtship and mating: male and female made contact with a pereopod, the male grasped the pereopods (2nd–3rd of both sides) of the female with one or both chelae. The male placed the female in front of him and started to swing her from side to side, the pair getting closer. Using a cheliped, the male patted the female's carapace and continued rocking her. The female began spreading her abdomen. In a sudden movement the male turned and pushed the female under him, while the female had her pereopods twisted round the male. Courtship lasted on average 20 min. During mating, the male tried to dig and the female grasped the males' pereopods with hers. Cleaning of the mouthparts with the maxillipeds was observed in both sexes. Crabs approaching the couple during mating were threatened by the male. Females' movements were minimal during mating. The male kept his abdomen curved, with the tips of the pleopods held out at the margins. At the end of mating, both clean inside the abdomen, the female shaking her pleopods; in two cases the male suddenly buried himself in the sand. Duration of mating ranged from 2 to 30 min.

Recognition is presumably by tactile and chemical stimuli on contact (Hartnoll, 1969). Fielder and Eales (1972) describe similar courtship in *Portunus pelagicus,* mentioning the 'motionless' female. Mating *Charybdis* females were hard shelled, as in *Thalamita crenata* H. Milne Edwards (S. Cannicci, personal communication), but unlike other portunid crabs (Hartnoll, 1969).

No traces of the above behavioural patterns—such as approaching, grasping and rocking—were observed in parasitized (both internally and externally infected) crabs.

Discussion

The presence of the *externae* of the rhizocephalan *Heterosaccus dollfusi* occupying the position of the egg mass of its host, *Charybdis longicollis,* induces the crabs to simulate egg-grooming behaviour. *Externae*-bearing *C. longicollis* spend most of their time cleaning and fanning the *externae.* This behaviour pattern was observed in higher frequency in parasitized crabs than in normal ovigerous crabs. It is of particular interest that the parasite succeeds in inducing that behaviour in male crabs, in which grooming and fanning of the abdomen normally do not occur.

Grooming behaviour serves to clean and ventilate the parasite, enhancing its survival. Indeed, Ritchie and Høeg (1981) reported the gradual death of *externae* in the porcellanid crab, *Petrolisthes cabrilloi* Glasell, parasitized by *Lernaeodiscus porcellanae* (Müller), when the hosts were prevented from grooming. Grooming behaviour appears to be controlled by the parasite, probably through hormones (Høeg, 1995), as it is stimulated by the emergence of the *externae* and declines after their degeneration. Bishop and Cannon (1979) surgically removed the *externae* in three crabs of unknown sex and observed that grooming behaviour declined after 2 weeks. Our preliminary findings using an artificial *externa*, suggest that the *externae* itself may play a role, at least in scarred crabs.

The presence of the parasite modifies digging and inhibits burying in the sand, a common activity in non-parasitized crabs. Similar modification was recorded by Wardle and Tirpak (1991) in *Callinectes sapidus* parasitized by *Loxothylacus texanus.*

Further studies are needed to elucidate the hormonal mechanisms by which the parasite presumably influences its host, effecting these behaviour patterns (Hartnoll, 1967; Høeg, 1995).

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