ZOOLOGIA 30 (3): 307–311, June, 2013
http://dx.doi.org/10.1590/S1984-46702013000300007

Epibiosis in decapod crustaceans by stalked barnacle *Octolasmis lowei* (Cirripedia: Poecilasmatidae)

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ABSTRACT. Stalked barnacles *Octolasmis lowei* Darwin, 1851 are frequently found attached to decapod crustaceans. Their epibiotic association depends on many factors, which are mainly related to characteristics of the host's biology. This study evaluated the infestation and distribution of stalked barnacles in the branchial chambers of crabs, and analyzed the data with respect to the host's sex, maturity stage, molt cycle and size. The crab species *Arenaeus cribrarius* Lamarck, 1818, *Callinectes danae* Smith, 1869, *Callinectes ornatus* Ordway, 1863, *Hepatus pudibundus* Herbst, 1785, *Libinia ferreirae* Brito Capello, 1871, and *Persephona punctata* Linnaeus, 1758 were sampled and found to be infested by *O. lowei*. No juvenile crabs were infested. The prevalence of infestation by *O. lowei* was significantly different among *C. danae*, *C. ornatus*, and *H. pudibundus* males and females. All infested hosts were in the intermolt period. The mean size of infested crabs was larger than that observed for non-infested individuals. Internally, stalked barnacles were concentrated on the central gills or walls and floor of branchial chambers, suggesting that these gills provide more favorable conditions for the settlement and development of these epibionts. These results highlight the relationship between epibiont infestation and host biology, as well as the role of decapod crustaceans as a suitable substrate for the development of stalked barnacle *O. lowei*.

KEY WORDS. Brachyurans; epibiont; infestation; host biology; symbiosis.

In soft substrate environments, decapod crustaceans are among the few solid surfaces available for colonization by benthic invertebrates (Abelló *et al.* 1990). In their association, known as epibiosis, the settler (benthic invertebrate) benefits without harming the host (decapod crustacean). Epibiosis has been extensively documented in marine environments (Wahl 1989, Wahl & Mark 1999, Alvarez *et al.* 2003, Blomsterberg *et al.* 2004, Cordeiro & Costa 2010). The biology of the host can often influence epibiont settlement and development. During molting, crustaceans reduce the density of symbionts on them (Walker 1974). Crustacean behavior, age, and maturity can also affect their colonization by benthic invertebrates (Abelló *et al.* 1990, Jeffries *et al.* 1992, Becker 1996).

Stalked barnacles of the genus *Octolasmis* Gray, 1825 (Poecilasmatidae) are sessile invertebrates frequently found attached to decapods, mainly lobsters, and in the branchial chambers of crabs (Jeffries & Voris 1996). This association can offer the epibiont some advantages, such as protection against predators. Moreover, the movements of the host can optimize epibiont dispersion, gene flow, and the host's movement or breathing generates water currents that improve access to food and remove metabolic residues produced by the epibionts (Wahl 1989, Key *et al.* 1997).

Although there is an extensive literature on Brazilian brachyuran species, there are few studies addressing the role of these crabs as hosts for benthic invertebrates (Negreiros-Fransozo *et al.* 1995, Santos *et al.* 2000, Santos & Bueno 2002, Mantelatto *et al.* 2003, Cordeiro & Costa 2010, Costa *et al.* 2010). Due to the symbiotic relationship of *Octolasmis* spp. with their hosts, the study of infestation patterns associated with host biology can provide significant information about this interaction, which can also be of great value for, and enrich, biodiversity research (Abelló *et al.* 1990, Gili *et al.* 1993, Becker 1996). Thus, the aim of this study was to evaluate the infestation and distribution of the stalked barnacle *Octolmasmis lowei* Darwin, 1851 in the branchial chambers of decapod crustaceans, and analyze this data with respect to the host's sex, maturity, molt cycle and size.

MATERIAL AND METHODS

Samples were collected monthly between October 2005 and September 2006 at the Guarujá (23°55'51"S, 46°10'20"W) and Praia Grande (24°01'33"S, 46°24'32"W) Bays, along the southern coast of the state of São Paulo, Brazil. Crabs were captured using an otter-trawl towed by a commercial shrimp fishing boat, at depths from 5 to 20 m. The sampled individuals were frozen for laboratory analysis.

Brachyurans were identified according to Melo (1996). Each crab was sexed based on the shape of the abdomen and number of pleopods. Molt stages were determined based on the consistency of the carapace, and were classified as intermolt or molt activity (Skinner 1985). The maximum carapace width (CW in mm) was estimated for all species using a caliper.

The dorsal carapace of all specimens was removed to inspect epibionts in the branchial chambers, and to observe gonads (classified as active and inactive, as described by Benetti *et al*. 2007). The sites epibionts were attached to were recorded according to their location on the gill (counting from anterior to posterior end) and considering the gill pairs (left and right) (Voris *et al*. 1994, Santos *et al*. 2000). The branchiostegite and the branchial chamber floor were also inspected as additional attachment sites, and epibionts were recorded. For further analysis, branchial chambers were divided into four regions: anterior (gills 1, 2, and 3), central (gills 4, 5, and 6), posterior (gills 7 and 8), and walls and floor of branchial chambers. Brachyurans with immature gonads were considered juveniles (Costa & Negreiros-Fransozo 1998). Since infested juveniles were not found, they were not considered in the data analysis.

Infestation prevalence (percentage of infested individuals), infestation intensity (number of epibionts on each infested host) and distribution of epibionts in the branchial chambers (number of epibionts by region) were evaluated for each of the host species (Bush *et al*. 1997). Differences in the infestation prevalence between sexes were evaluated using the Chi-square test, as well as the proportion of adult hosts with active and inactive gonads.

Before performing tests to evaluate size and infestation intensity according to sex or branchial chamber regions, data variance homogeneity was verified using the Levene's test. When homogeneity was not confirmed after data transformation (fourth roots or logarithms), an equivalent non-parametric test was applied. To compare infestation intensity between sexes and size between infested and non-infested crabs, the parametric Student's t-test or the non-parametric Mann-Whitney test was applied.

The infestation intensity according to branchial chamber regions was evaluated using ANOVA on randomized data, considering branchial chamber regions as treatments (fixed factor), and the Tukey' test was applied to verify differences among regions. Even if variance homogeneity was not met after data transformation, the test was performed with original data, since ANOVA is considered robust, mainly where data are balanced (Underwood 1997). Significance level of 5% was considered for all analyses.

Voucher specimens of crabs and epibiont are deposited in the collection of the Museu de Zoologia, Universidade de São Paulo (MZUSP28380 to MZUSP28386), São Paulo, SP, Brazil.

RESULTS

All six crab species sampled were infested by *O. lowei*: *Arenaeus cribrarius* Lamarck, 1818 (Portunidae), *Callinectes danae* Smith, 1869 (Portunidae), *C. ornatus* Ordway, 1863 (Portunidae), *Hepatus pudibundus* Herbst, 1785 (Aethridae), *Libinia ferreirae* Brito Capello, 1871 (Epialtidae), and *Persephona punctata* Linnaeus, 1758 (Leucosiidae) (Table I) (classification according to Ng *et al*. 2008). Infestation intensity analysis according to the sex of the host, size and distribution on the gills were not carried out for three species, *viz*. *A. cribrarius*, *L. ferreirae* and *P. punctata*, due to the few numbers of infested samples.

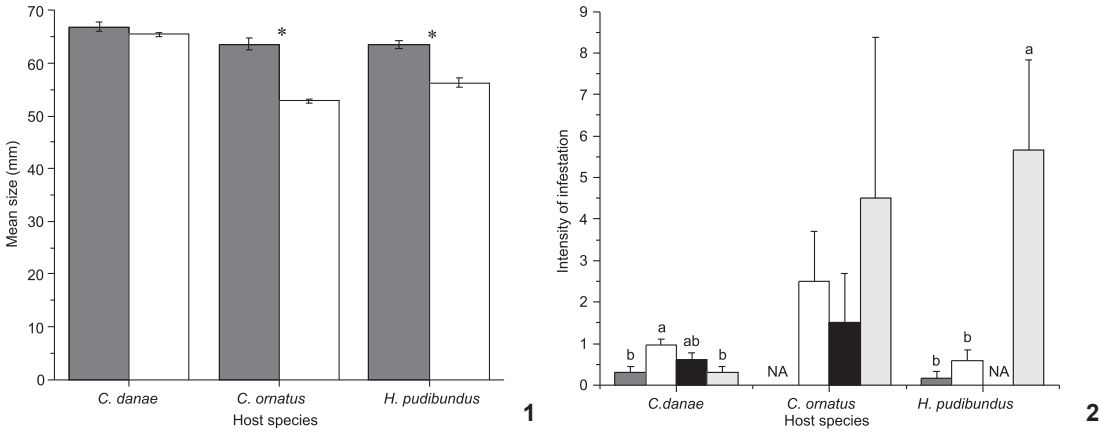
The proportion of adult hosts with active and inactive gonads differed among species (and among sexes), and for *A. cribrarius* and *L. ferreirae*, the proportion of individuals with inactive gonads (i.e., rudimentary stage) was greater than that of crabs with active gonads ($p < 0.001$), while for the other species, crabs with active gonads predominated ($p < 0.05$) (Table I). Adult males of *C. ornatus* and *H. pudibundus* had higher infestation prevalence when compared to females (*C. ornatus*: $\chi^2 = 5.93$, d.f. = 1, $p = 0.015$; *H. pudibundus*: $\chi^2 = 6.42$, d.f. = 1, $p = 0.011$). Among *C. danae*, females had higher prevalence compared to males ($\chi^2 = 6.63$, d.f. = 1, $p = 0.01$). The infestation intensity of all species was similar between the sexes (*C. danae*: $t = 0.86$, d.f. = 63, $p = 0.391$; *C. ornatus*: $t = 1.49$, d.f. = 15, $p = 0.158$; *H. pudibundus*: $t = 0.26$, d.f. = 53, $p = 0.794$) (Table I).

Infested *C. ornatus* and *H. pudibundus* individuals were larger than non-infested individuals (*C. ornatus*: $U = 1221.5$, $N = 555$, $p < 0.001$; *H. pudibundus*: $U = 2714.0$, $N = 219$, $p < 0.001$). Among *C. danae*, no differences were found between infested and non-infested individuals ($t = 1.51$, d.f. = 672, $p = 0.13$) (Fig. 1). In all species, infested hosts were in the intermolt period, except for *H. pudibundus*, for which one specimen in the postmolt period was found.

The distribution of epibionts between branchial chamber regions was not homogeneous (Table II). For *C. danae*, the *O. lowei* amount was higher on the central gills compared to the anterior region and the walls and floor of the branchial chambers, but there was no difference when compared to the posterior region. No difference was found for *C. ornatus*. *Hepatus pudibundus* had a greater number of epibionts on the walls and floor of branchial chambers (Fig. 2).

DISCUSSION

Our results showed that infestation by *O. lowei* is associated with aspects of the biology of the host, for instance sex,



Figures 1-2. (1) Mean size (± standard error) of (■) infested and (□) non-infested crabs for each of the host species. (*) Groups differ significantly regarding size (Mann-Whitney test, $p < 0.05$). (2) Mean infestation intensity (± standard error) by branchial chamber region and host species. Same letters indicate that the means do not differ significantly between columns for each of the host species (Tukey's test, $p > 0.05$). (NA) Not available, (■) anterior, (□) central, (■) Posterior, (■) walls and floor, .

Table I. Brachyuran species infested by *Octolasmis lowei*. Total number of adults (N), proportion of actively reproducing and inactive male and female infested crabs, prevalence (%) and median infestation intensity (± SD = standard deviation) according to host species and sex for the three most abundant hosts. Same small letters in the same column indicate lack of significance between sexes for each of the host species ($p > 0,05$).

Host	N	Reproductively		Infested crabs	Infestation	
		Active % (N)	Inactive % (N)		Prevalence	Intensity
Arenaeus cribarius						
Male	59	20.3 (12)^{B}	79.7 (47)^{A***}			
Female	32	21.9 (7)^{B}	78.1 (25)^{A**}			
Total	91	20.9 (19)^{B}	79.1 (72)^{A***}	1	1.10	1.00
Callinectes danae						
Male	121	56.1 (68)^{NS}	43.9 (53)^{NS}		0.59^{b}	3.00 ± 0.82^{a}
Female	560	97.9 (548)^{A}	2.1 (12)^{B***}		8.96^{a}	8.77 ± 13.26^{a}
Total	681	90.5 (616)^{A}	9.5 (65)^{B***}	65	9.55	8.42 ± 12.92
Callinectes ornatus						
Male	335	64.5 (216)^{A}	35.5 (119)^{B***}		2.67^{a}	5.40 ± 10.83^{a}
Female	226	81.9 (185)^{A}	18.1 (41)^{B***}		0.36^{b}	19.00 ± 24.04^{a}
Total	561	71.5 (401)^{A}	28.5 (160)^{B***}	17	3.03	7.00 ± 12.64
Hepatus pudibundus						
Male	85	70.6 (60)^{A}	29.4 (25)^{B**}		13.24^{a}	4.31 ± 7^{a}
Female	134	64.2 (86)^{A}	35.8 (48)^{B**}		11.87^{b}	3.88 ± 4,62^{a}
Total	219	66.7 (146)^{A}	33.3 (73)^{B***}	55	25.11	4.11 ± 5.94
Libinia ferreirae						
Male	56	0.0^{B}	100 (56)^{A***}			
Female	45	31.1 (14)^{B}	68.9 (31)^{A*}			
Total	101	13.9 (14)^{B}	86.1 (87)^{A***}	1	0.99	4.00
Persephona punctate						
Male	22	81.8 (18)^{A}	18.2 (4)^{B**}			
Female	35	97.1 (34)^{A}	2.9 (1)^{B***}			
Total	57	91.2 (52)^{A}	8.8 (5)^{B***}	1	1.75	1.00

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Capital letters in the same row do not differ statistically for the same sex (or total) for each host species; ^{NS} not significant.

Table II. Analysis of Variance (ANOVA) comparing infestation intensity between branchial chamber regions. (M.S.) Mean square, (D.F.) degrees of freedom.

Source of variation		M.S.	D.F.	F	p
C. danae	Region	1.518	3	4.24	<0.009
	Residue	0.358	60		
C. ornatus	Region	14.250	3	0.80	<0.516
	Residue	17.750	12		
H. pudibundus	Region	88.743	3	6.21	0.001
	Residue	14.301	44		

maturity, molt cycle and size. In spite of the clear relationship between epibionts and hosts, this interaction does not seem to be species-specific, since the stalked barnacle was found on six crab species. According to Wahl & Mark (1999), species-specific interactions between hosts and epibionts, such as *O. lowei*, are rare. In addition, the high standard deviation from the mean infestation intensity of some host species suggests an elevated inter-individual variability in infestation.

In contrast to our results, previous studies have reported intense infestations by *O. lowei* in *A. cribrarius* and *L. ferreirae* or their congeners (Mantelatto *et al*. 2003, Costa *et al*. 2010, Cordeiro & Costa 2010). In the present study, adults of *A. cribrarius* and *L. ferreirae* had predominantly inactive gonads (rudimentary stage), which is an evidence that they have just recently molted. Since epibionts are eliminated during ecdysis, this result was expected (Negreiros-Fransozo *et al*. 1995).

Few authors have reported the occurrence of *Octolasmis* spp in crabs from the family Leucosiidae (Humes 1941, Walker 1974, Jeffries *et al*. 1982), and in Brazil there is no record of infestation due to *Octolasmis* spp in other species from the same family (Mantelatto *et al*. 2003). The infestation by *Octolasmis* spp in Leucosiidae species do not seem to be very common, what corroborates our results that showed low infestation for *P. punctata* (only one crab infested).

Regarding the sex of the hosts, the differences observed in the infestation prevalence may be due to behavioral differences between sexes, such as efficiency in cleaning the gills, burying and hiding (Becker 1996, Cordeiro & Costa 2010). Nevertheless, further studies need to be conducted in order to verify whether any behavioral differences occur and how they affect infestation patterns. It must also be noted that the lack of differences regarding infestation intensity suggests that individuals of *O. lowei* display no preferences for either sex of the host species studied, as suggested by Cordeiro & Costa (2010) for *Libinia spinosa* H. Milne-Edwards, 1834.

The fact that all individuals were infected at the intermolt stage (except for one *H. pudibundus* specimen) reveals the relationship of the epibiont with the duration of this stage. The molting process affects the infestation of symbionts growing on the exoskeleton. As a result, the epibiont life cycle may be associated with the length of the intermolt stage of its host (Walker 1974, Gili *et al*. 1993). Furthermore, the rapid settlement and development of the cyprid larvae of *O. lowei* in hosts may explain the infestation of an individual in the postmolt period. The larger size of infested crabs in *C. ornatus* and *H. pudibundus,* in addition to the lack of infested juveniles in all host species, highlight the importance of the length of the intermolt period for the settlement and development of epibionts, since adults molt less frequently than juveniles (Abelló *et al*. 1990, Jeffries *et al*. 1992).

The spatial distribution of *O. lowei* in the branchial chambers does not seem to be random, since there is a greater number of epibionts in the central region, at least for *C. danae*. For *H. pudibundus*, we found that infestation intensity was higher on the walls and floor of the branchial chambers. The main factors that determine the settlement and the spatial distribution of stalked barnacles are water flow, which affects food availability, ventilation and removal of metabolites (Voris *et al*. 1994, Santos *et al*. 2000), and the presence of conspecifics, which may increase the reproductive success of these epibionts (Dinamani 1964, Crisp 1974). Since the central gills have larger area than others, this region may support more epibionts and provide more favorable conditions for the settlement and development of *O. lowei*. In addition, the presence of stalked barnacles on the walls and floor of the branchial chambers is relevant and reported in previous studies (Kumaravel *et al*. 2009), which suggests that the region may be a viable alternative space of settlement.

The occurrence of stalked barnacle *O. low*ei in several host species suggests that the decapod crustacean community plays an important role in ensuring the maintenance of this epibiont species. However, infestation may be limited by aspects of the host's biology such as maturity, length of the intermolt period and size.

ACKNOWLEDGMENTS

We thank Isabela C.A. da C. Bordon and Ana P. Garcia, respectively for providing material for study and help with sorting.

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Submitted: 16.VII.2012; Accepted: 21.II.2013.
Editorial responsibility: Marcus V. Domingues