Distribution of *Octalasmis lowei* and *Carcinonementes carcinophia* in the branchial chamber of *Callinectes danae* and *Callinectes ornatus*.

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Abstract

This paper reports the distribution of *Octolasmis lowei* and *Carcinonemertes carcinophila imminuta* in the branchial chambers of the crabs *Callinectes danae* and *Callinectes ornatus*. Gills were checked for the presence and position of the symbionts along the axis. The hypobranchial or epibranchial gill surface of the gills, the walls and floor of the branchial chamber were recorded as attachment sites only for the barnacles. The observed number of symbionts were tested (Chi-square test) against expected numbers calculated with the proportion of total gill area (for *O. lowei*) and total gill volume (for *C. c. imminuta*) corresponding to each gill. Higher concentration of both symbionts were observed in the proximal and in the medial thirds of the gills and in gills more centrally located. The majority of the cirripeds was found on the hypobranchial surface. The distribution of the symbionts was related to the distribution of water flow and oxygen in the branchial chamber. The results suggest that higher intensity of infestation by *O. lowei* affected the establishment of small sized *C. c. imminuta.*

Key words: symbionts, cirriped, nemertean, crab, distribution

Introduction

The branchial or gill chambers of many decapod crustaceans serve as an attachment site for symbiotic organisms. Some of these symbionts are not invasive and are found on the exoskeleton-lined surface of the gills and walls of the branchial chamber. These epibionts find shelter and receive food and oxygen that are continuously brought in with the water current produced by the gill bailer, or scaphognathite (Walker, 1974). This water flow is also important for removing the metabolic wastes produced by the symbionts.

Species of the symbiotic egg predator *Carcinonemertes* (Hoplonemertea) and of the pedunculate barnacle *Octolasmis* commonly occur within the gill chambers of brachyuran crabs (Humes, 1942; Newman, 1967). Juveniles of the nemertean *Carcinonemertes carcinophila imminuta* Humes, 1942 are found encysted between the gill lamellae of portunids (Humes, 1942). In female hosts that become ovigerous, these nemertean worms migrate to the egg mass, feed on the eggs, attain sexual maturity, and reproduce (Kuris, 1993). After the host larvae hatch, the worms migrate back to the branchial chamber (Humes, 1942; Hopkins, 1947).

The pedunculate cirriped *Octolasmis lowei* (Darwin, 1852) attaches permanently on the gill surface or, less frequently, on the walls of the gill chamber of its host. Nauplii of *Octolasmis* are planctotrophic, feeding mainly on algae (Moyse, 1987; Jeffries *et al.*, 1995). As in other species of symbiont cirripedes, association to host begins when the animal reaches the cypris stage and starts living as a sessile organism on a living substrate.

There are many benefits to epizoans living on mobile benthic host. Movement of the host may improve the dispersal and expand the biogeographic distribution of the epizoans by increasing the range of larval dispersal (Key *et al.*, 1996). Currents generated by the movement, breathing, and/or feeding of the host may improve the food supply to suspension feeding epizoans as well as improve the removal of wastes produced by the epizoans (Wahl, 1989; Gili *et al.*, 1993; Key *et al.*, 1996).

Epizoans can negatively affect the host blue crabs (Key *et al.*, 1999). According to Walker (1974), *Octolasmis* infestation may contribute to the accumulation of debris on the gills of crabs. However, the grooming action of the epipodites of the maxillipeds and the reversal of direction of the water flow within the gill chamber may help alleviate the build up of debris and detritus on the gills and gill chamber apertures (Taylor, 1982). This grooming action may also affect barnacle survival, especially during and immediately after settlement of the cypris larva (Gannon, 1990). However, heavy epibiont infestations may overwhelm the cleaning capacity of grooming appendages and make respiration difficult (Overstreet, 1983; Messick and Sinderman, 1992). McDermott (1967) reported that a high infestation by nemerteans on the branchial chambers of *Zaops ostreum* (Say, 1817) makes gas exchange difficult for the host. *Callinectes sapidus* Rathbun, 1896 infested by *Octolasmis mülleri* (Coker, 1902) hyperventilate and exhibit tachycardia and minor changes in hemolymph parameters probably because of obstruction of the host ventilatory stream (Gannon and Wheatly, 1992, 1995).

The crabs main protection is ecdysis since epibionts may be shed along with the exuviae (Walker, 1974; Gannon, 1990). However, Wickham *et al.* (1984), who studied *Carcinonemertes errans* Whickham, 1978 infestation in the crab *Cancer magister* Dana, 1852, demonstrated that these nemerteans are able to transfer from old to new exoskeleton. The transfer of these worms is facilitated because they are found under the abdomen and between the limb axillae rather than between the gill lamellae as in *C. carcinopihla* (Santos and Bueno, 2001). However, *Carcinonemertes mitsukurii* Takakura, 1910, a species also found between the gill lamellae of *Portunus pelagicus* (Linnaeus, 1766), was observed on the external surfaces of crabs in late premolt and early postmolt stages (Shields, 1992).

Reports on nemertean and cirriped infestation in brachyuran crabs in Brazil are very limited. Humes (1942) provided the first record of a nemertean-crustacean association from Brazil, reporting the occurrence of *C. c. imminuta* in the portunid crab, *Callinectes danae* Smith, 1869 from Rio de Janeiro. More recently, Santos and Bueno (2001) investigated the pattern of infestation by juvenile *C. c. imminuta* with regard to sex, maturity, condition of adult female (ovigerous or non ovigerous), molt stage and size of *C. danae* and *Callinectes ornatus* Ordway, 1863 from São Sebastião.

Young (1990) reported the presence of *O. lowei* in the branchial chamber of the brachyuran crabs *Libinia spinosa* Milne Edwards, 1834, *Portunus spinimanus* (Latreille, 1819), *Portunus spinicarpus* (Stimpson, 1871), *Hepatus pudibundus* (Herbst, 1785) and an unidentified species of *Callinectes*. According to Jeffries and Voris (1996), references that provide details on the distribution and abundance of *Octolasmis* are few in number. Walker (1974), Jeffries and Voris (1983), and Gannon (1990) provided data on the distribution of *O. mülleri* in the branchial chamber of *C. sapidus*. More recently, Santos and Bueno (2002) studied the prevalence and intensity of infestation by *O. lowei* with respect to sex, maturity, condition of adult female (ovigerous or non ovigerous), molt stage and size of *C. danae* and *C. ornatus*.

The purpose of this study was to analyze the distribution pattern of the hoplonemertean *C. c. imminuta* and the cirriped *O. lowei* in the branchial chamber of the blue crabs *C. danae* and *C. ornatus*, from Brazil.

Materials and Methods

Blue crabs were collected monthly by a trawler at the region of the "Enseada" beach, São Sebastião, Brazil (23°43'18"S, 45°23'54"W) between September 1995 and June 1996. The identification of *Callinectes* species followed Williams (1974) and Melo (1996) and was confirmed by Dr. Gustavo Augusto S. de Melo from Museu de Zoologia da Universidade de São Paulo. Identification of nemerteans followed Humes (1942) and Shields and Kuris (1990). Identification of barnacles followed Young (1990) and was confirmed by Dr. Paulo S. Young from Museu Nacional do Rio de Janeiro.

To access the gills in both branchial chambers, the crab carapace was cut along its lateral margins and removed. Gills (8 pairs) were removed with the aid of a forceps and transferred to Petri dishes filled with saltwater. Gills were arranged in sequence so as to reflect the relative position they occupied in each branchial chamber.

Attachment sites were recorded with respect to gill number (from 1 to 8, anterior to posterior) and distance along the gill (each gill was divided into thirds of equal lengths - proximal, medial and distal). The inside (hypobranchial) and the outside (epibranchial) surfaces of gills, and the walls and floor of the branchial chambers were also considered as attachment sites for the pedunculate barnacles (Walker, 1974; Jeffries *et al.* 1982, 1992; Gannon, 1990; Voris *et al.* 1994).

To determine what proportion of the total gill area and the total gill volume were made up by each gill, the length and the basal diameter of gills of the crabs subsamples comprising all carapace width class categories collected were measured with a caliper. The results were averaged (Gannon, 1990) and the area of each gill was calculated using the formula for the area of a right cone ($a = \pi rh$) (Jeffries and Voris, 1983); the gill volumes were calculated using the formula for the volume of a cone ($v = 1/3\pi r^2h$). The resulting relative areas and volumes were used for calculation of the expected number of *O. lowei* and of *C. c. imminuta* for each gill for both crab species. The observed number of symbionts was compared with the expected number of symbionts with Chi-square analysis (Zar, 1996).

The dependence of symbionts distribution to gill segment, to gill surfaces (hypobranchial and epibranchial) and to host species was tested with contingency table analysis (Zar, 1996). Values of $p < 0.05$ were accepted as significant.

Maintenance of categories with expected frequencies lower than 5 in some Chi-square analyses followed the mean relative frequencies criteria presented by Zar (1996).

Results

Octolasmis lowei

The study of the distribution of *O. lowei* on the gills of crabs was based on 49 specimens of *C. danae* and 40 specimens of *C. ornatus*.

Barnacles were most frequently cemented to the gills but they also occurred on the walls of the branchial chambers (16 in *C. danae* and 28 in *C. ornatus*) and attached to the floor of the branchial chamber (10 in *C. danae* and 2 in *C. ornatus*). The analyses described bellow were done with barnacles found exclusively on the gills.

In both crab species, barnacles were primarily located in the proximal segment of gills 3-7 (figures 1 and 2). The medial segment of the hypobranchial surface was also favored by the barnacles. Significantly more barnacles were found on the hypobranchial side of the gills of both *C. danae* (82.98%, 78 of 94) and *C. ornatus* (91.78%, 134 of 146) than on the epibranchial side.

The distribution of *O. lowei* over gills 1 through 8 differed significantly between the hypobranchial and epibranchail surfaces for both *C. danae* ($\chi^2 = 23.82$; d. f. = 6; $p = 0.0006$) and *C. ornatus* ($\chi^2 = 23.86$; d. f. = 6; $p = 0.0006$). The number of barnacles along the gill segments (proximal, medial and distal) was independent of the side of the gills for *C. danae* ($\chi^2 = 0.73$; d. f. = 2; $p = 0.70$) but not for *C. ornatus* ($\chi^2 = 9.92$; d. f. = 2; $p = 0.007$). The proximal segment of the gills of *C. ornatus* presented a higher number of barnacles on the epibranchial side than expected.

The observed distribution of *O. lowei* on the hypobranchial side of gills 1 through 8 was not significantly different from the expected distribution (according to the relative area of each gill) for *C. danae* ($\chi^2 = 13.21$; d. f. = 7; $p = 0.07$) (figure 3). In the gill chambers of *C. ornatus*, the observed distribution differed significantly from the expected distribution ($\chi^2 = 42.43$; d. f. = 6; $p = 0.0001$) (figure 4). In this latter crab species, gills 3 and 6 presented higher numbers of barnacles than expected and gill 8 presented a lower number of barnacles than expected.

The distribution of *O. lowei* on the hypobranchial side of gills 1 through 8 did not differ between *C. danae* and *C. ornatus* ($\chi^2 = 12.28$; d. f. = 6; $p = 0.056$). The distribution of barnacles along the segments of gills (proximal, medial and distal) on the hypobranchial side was also independent of the two host crab species ($\chi^2 = 3.76$; d. f. = 2; $p = 0.15$)

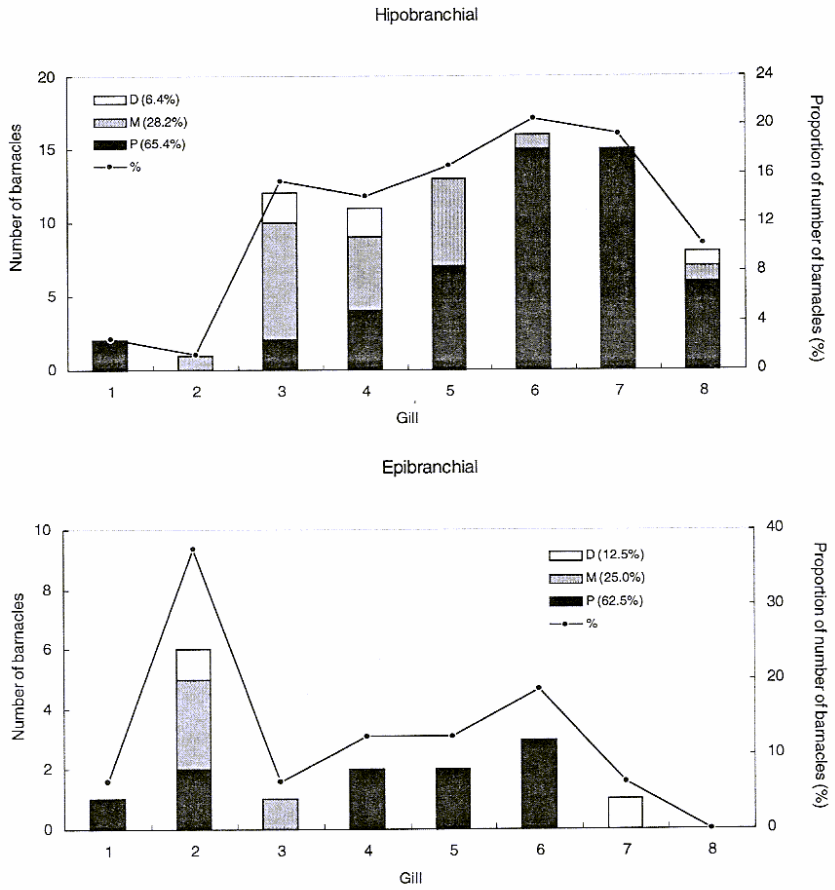

Figure 1: *O. lowei* distribution over the hypobranchial and epibranchial surface and along gill segments (P, proximal; M, medial; D, distal) of *C. danae*.

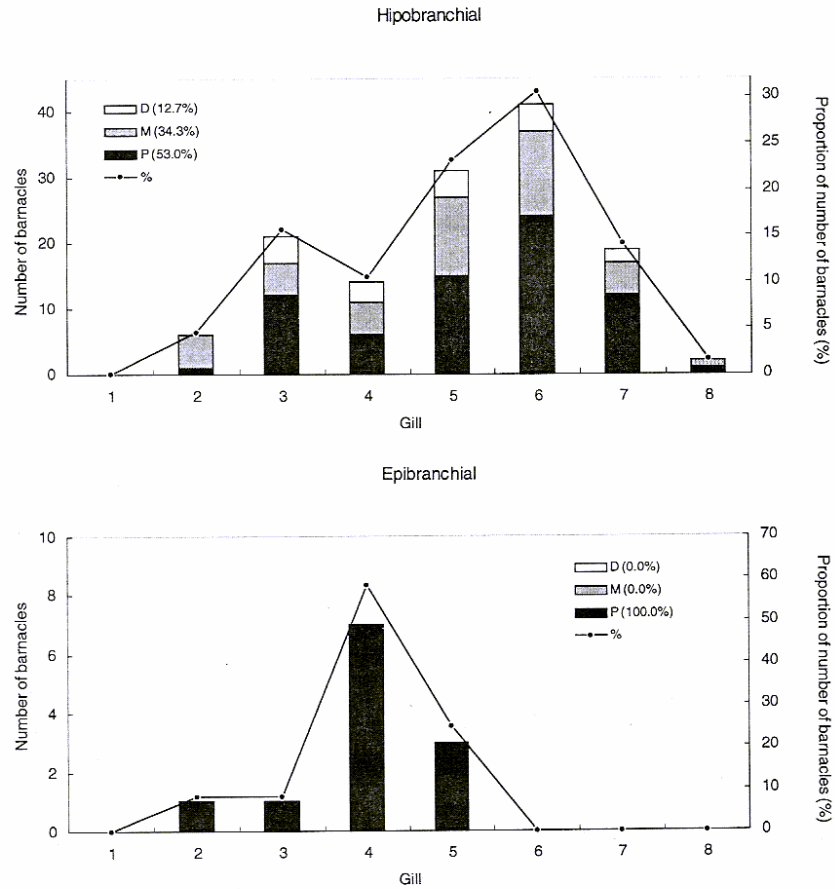

Figure 2: *O. lowei* distribution over the hypobranchial and epibranchial surface and along gill segments (P, proximal; M, medial; D, distal) of C. *ornatus*

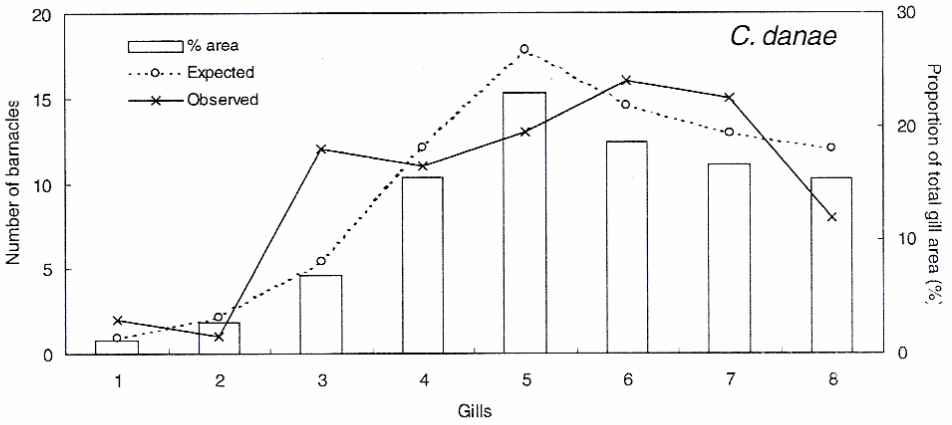

Figure 3: Observed and expected distribution of *O. lowei* on the hypobranchial surface of *C. danae* gills. The proportion of the total gill area represented by each gill (bars) was applied to the total number of barnacles for estimation of expected frequencies.

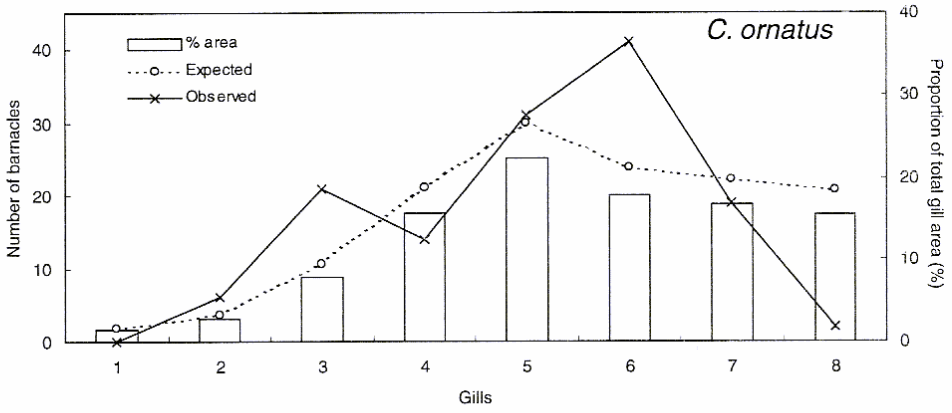

Figure 4: Observed and expected distribution of *O. lowei* on the hypobranchial surface of *C. ornatus* gills. The proportion of the total gill area represented by each gill (bars) was applied to the total number of barnacles for estimation of expected frequencies.

Carcinonemertes carcinophila imminuta

The study of the distribution of *C. c. imminuta* between the gill lamellae of crabs was based on 89 specimens of *C. danae* and 29 specimens of *C. ornatus*.

In *C. danae, C. c. imminuta* were most abundant within the proximal and medial portions of gills 4-8 while in *C. ornatus* 40% of the total number of worms occurred in gill 4 (figure 5). In this latter crab species, the majority of *C. c. imminuta* occupied the proximal gill segment.

The observed distribution of worms on gills 1 through 8 was significantly different from the expected (according to the relative volume of each gill) for both *C. danae* (χ^2 = 66.64; d. f. = 7; $p < 0.0001$) (figure 6) and *C. ornatus* (χ^2 = 244.52; d. f. = 6; $p < 0.0001$) (figure 7). In *C. danae,* gill 4 presented a number of worms that was markedly higher than expected while gills 5 and 8 presented a lower number of worms than expected (figure 6). In *C. ornatus*, all gills, with the exception of gill 3, presented numbers of worms that departed markedly from the corresponding expected frequencies (figure 7).

The distribution of *C. c. imminuta* over gills 1 through 8 differed between *C. danae* and *C. ornatus* (χ^2 = 107.74; d. f. = 7; $p < 0.0001$). The distribution of worms along gill segments (proximal, medial and distal) also differed significantly between the host crab species (χ^2 = 65.15; d. f. = 2; $p < 0.0001$).

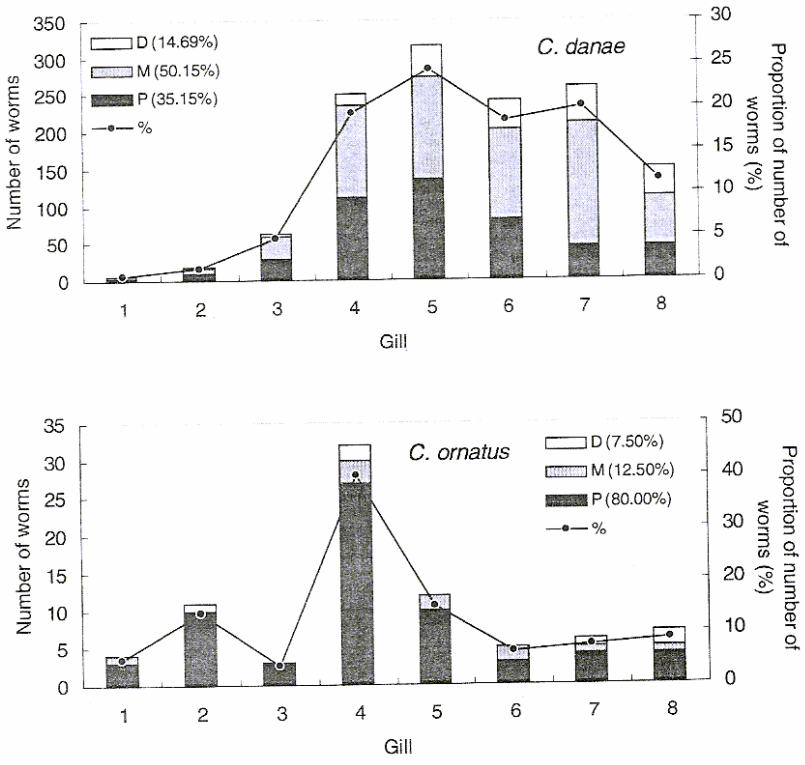

Figure 5: *C. c. imminuta* distribution along gill segments (P, proximal; M, medial; D, distal) in *C. danae* and *C. ornatus*.

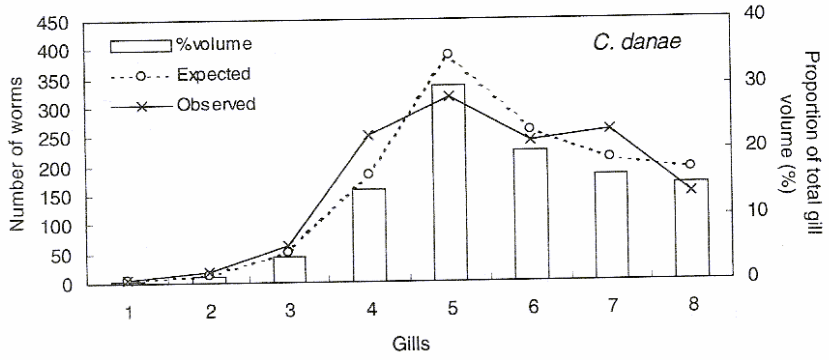

Figure 6: Observed and expected distribution of *C. c. imminuta* in *C. danae* gills. The proportion of the total gill volume represented by each gill (bars) was applied to the total number of worms for estimation of expected frequencies.

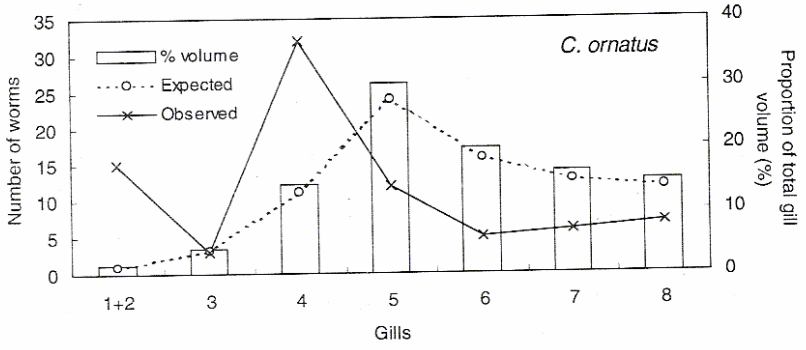

Figure 7: Observed and expected distribution of *C. c. imminuta* in *C. ornatus* gills. The proportion of the total gill volume represented by each gill (bars) was applied to the total number of worms for estimation of expected frequencies.

Discussion

The majority of barnacles and nemerteans occurred in the proximal and medial segments of gills and on the larger gills which are more centrally located. Interlamellar spaces are wider in larger gills and are also larger proximally than distally, thereby providing less resistance to water flow (Jeffries and Voris, 1983). This enhanced flow condition might favor deposition of symbionts, ventilation, food availability, and debris removal.

The number of both *O. lowei* and *C. c. imminuta* on gill 8 was lower than expected by the relative gill dimensions (similar to those of gill 4) for both host crab species. This might be resultant of reduced ventilatory current intensity on the hindmost gill, as described for *C. maenas* by Hughes *et al.* (1969).

There is also a gradual decrease in oxygen tension from the base to tip along the length of any given gill (Hughes *et al.*, 1969). Thus, both symbionts would have more access to oxygen in the proximal and medial segments of gills than in the distal segment.

Distribution of *C. c. imminuta* along the gill was clearly affected by space restriction due to intensity of infestation by the worms. According to Santos and Bueno (2001), *C. ornatus* presented a low intensity of infestation (mean ± s.e.: 2.7 ± 0.4; range: 1 to 9) and the majority of these worms (80% of total number) were established in the proximal segment of gills. On the other hand, *C. danae* presented a higher intensity of infestation by the same worm (mean ± s.e.: 12 ± 2.7; range: 1 to 268) (Santos and Bueno, 2001e) and the proportion of worms in the proximal segment was comparatively smaller (around 35%) while approximately half the total number of worms occurred in the medial segment. In this latter situation, proportion of worms in the distal segment was also larger than that verified for *C. ornatus*.

The occurrence of the majority of *O. lowei* on the hypobranchial side of the gills of *C. danae* and *C. ornatus* is in agreement with the results obtained by Walker (1974), Jeffries and Voris (1983), Gannon (1990) and Voris *et al.* (1994). In addition, the distribution of *O. lowei* on the proximal and medial segments of the hypobranchial and epibranchial surfaces of the gills of *C. ornatus* were distinct. These findings are consistent with those reported for *Octolasmis angulata* (Aurivillius, 1894) and *Octolasmis cor* (Aurivillius, 1892) in *Scylla serrata* (Forskal, 1755) (Voris *et al.*, 1994).

Many factors may influence the choice of attachment site by the cyprids: exposure to water currents, texture and contour of surface, light, pressure, and chemical factors (Crisp, 1974). According to Voris *et al.* (1994), current flow through the gill chamber is the most important factor influencing site selection by octolasmid cyprids. The direction, path, and intensity of current flow determine the distribution and disposal of food and oxygen to the symbionts, as well as the elimination of metabolic waste products.

Cyprids enter the branchial chamber with the inhalant respiratory current and pass into the hypobranchial side of the gill chamber. Being closely opposed to one another, the gills act as a passive filter that prevents the cyprids from passing through to the epibranchial side and therefore making them settle on the hypobranchial surface (Walker, 1974). Settlement on the hypobranchial side is more profitable to the barnacles since the water in the hypobranchial space has a higher oxygen pressure than that in the epibranchial space (Hughes *et al.*, 1969). The cyprids found on the epibranchial side of the gills had presumably been taken there during the reversal of respiratory current by the crab as previously observed in *C. sapidus* infested by *O. mülleri* (Walker, 1974).

Voris *et al.* (2000) found that *O. cor* and *O. angulata* tended to occur only on the inside gill surfaces when the total number of barnacles within the branchial chamber was less than 20. However, in the present study, crabs infested with less than 20 *O. lowei* presented barnacles on the epibranchial surface of gills.

Barnacles were more abundant on gills 3-7 of both crab species. Although ventilatory current flow in the brachial chamber is the primary factor that influences settlement of cyprids (Voris *et al.*, 1994), some evidences suggest that biological factors also contribute to the distribution of barnacles on the gills. Larval settlement is stimulated by the presence of conspecifics (Crisp, 1974; 1984) and

this leads to a aggregation of adults. For sessile species, such as *O. lowei*, this restriction in microhabitat certainly represents enhanced mating chances (Rohde, 1984) and consequently reproductive success. In the present study, higher proportions of ovigerous barnacles occurred on gills 3 and 6 of *C. ornatus* (Santos, unpublished data), which were the gills that presented number of *O. lowei* higher than expected, that is, with higher barnacles densities.

Although *C. c. imminuta* occurred in higher numbers than expected in some gills (4, 5, and 8) of *C. danae*, the overall distribution pattern is similar to the one presented by *O. lowei* on the hypobranchial surface of gills, that is, number of epizoans tend to be higher on larger gills. Although the present data could not demonstrate any statistically significant negative relation between the epizoan species, the distribution patterns of worms and barnacles in *C. ornatus* present clearly opposite trends. Two factors might potentially contribute for this patterns: the intensity of infestation by *O. lowei* was higher in *C. ornatus* (mean ± s. e.: 4.0 ± 1.3; range: 1 to 49) than in *C. danae* (mean ± s. e.: 2.2 ± 0.2; range: 1 to 8) (Santos and Bueno, *in press* b); the mean body size of *C. c. imminuta* was smaller in *C. ornatus* than in *C. danae* (Santos, unpublished data). The results suggest that either numerous barnacles on the surface of gills or small body size of worms, or both make establishment of worms in gills 5 to 7 difficult. The marked concentration of *C. c. imminuta* on gill 4, which is a large and centrally positioned gill and the one with the lowest number of *O. lowei*, support this hypothesis. The smaller body size of worms in *C. ornatus* is presumably related to food restriction since no ovigeorus crab occurred in the analyzed sample. *C. c. imminuta* individuals that feed on the eggs of the host crab grow larger than those that live in the branchial chambers only (Humes, 1942). Thus, we believe that larger worms found in *C. danae* returned to the branchial chamber, after migrating to the egg mass of crabs, feeding on the eggs and breeding as described by Humes (1942) and Hopkins (1947). Additional studies on possible interactions between epibiont species as well as studies on growth of *C. c. imminuta* under different food availability conditions would be of great interest for a enhanced understanding of variations in distribution pattern and population size structure of this worm species.

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