Biological Relationship of *Callinectes sapidus* (Rathbun) with Carcinonemerteans, Bryozoan, and Barnacles[1] (34460)

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The blue crab *Callinectes sapidus* (Rathbun) is known to host: the nemertean worm *Carcinonemertes carcinophila* (Kölliker) (1, 2); the bryozoan *Acanthodesia tenuis* (3); the barnacles, *i.e., Chelonibia patula* (4), *Balanus eburneus* (Gould) (4, 5), and *Octolasmis lowei* (2, 5); and the sea anemone *Diadumene leucolena*. This biological association appears to be influenced by the sex and age of the crabs (2, 6). These phenomena as well as the provenience, water salinity, and the nature of the biological association were the object of the present study.

Materials and Methods. The blue crabs were collected in Sarah's Creek or in the mouth of the York River, Virginia; Hampton Roads, Virginia; Chesapeake Bay, Maryland; and in Cape Hatteras, North Carolina. Water salinities of these various localities were determined by titration with silver nitrate. Juvenile and adult crabs of both sexes were measured for their maximum body width and examined for the presence of various associated organisms. A dissecting microscope was used for the detection of young nemertean worms in capsules occurring on the crab's gills. The gills were also examined for the presence of *Octolasmis lowei* and the shells for *Balanus eburneus, Chelonibia patula, Acanthodesia tenuis* and *Diadumene leucolena.*

The nature of biological relationship between the crabs and their associated nemertean worms was studied by injection of various dyes, with a hypodermic needle, into the hearts of a number of mature female crabs. The dyes used (dissolved in saline and adjusted to pH 7.0–7.5) were neutral red, vegetable green food coloring (both in concentration of 1.5%), methylene blue (20%), and Evans blue (0.4%). Some of the crabs were injected twice, with the same dye on the same or the next day. The worms in the capsules found on the gills of these crabs were examined then under a dissecting microscope 1, 2, or several hours, and also a day or two, after the injection of the dye. Uninjected crabs, with encapsulated worms served as controls.

In order to determine whether *Carcinonemertes* is parasitic on the gills of *Callinectes,* tests were performed to find if the nemertean worms can live isolated from the crabs. For this purpose, and also in order to investigate the effect of water salinity on the survival of isolated worms, some nemertean worms were placed into Syracuse watch glasses containing sea water of various salinities ranging from 26 ppt to 3.5 ppt using distilled water to obtain the appropriate dilutions. One group of nemertean worms was placed in the watch glass containing tap water. Crabs' eggs were added to some bowls to serve as food for the worms. The waters in various containers were changed daily.

Results and Discussion. The incidence of associated organisms according to age of male and female crabs, provenience, and the water salinities is summarized in Tables I and II. The results of these studies show that many of the adult female crabs had red (adult) nemertean worms on their gills. Most of them were also infested with *Chelonibia patula, Balanus eburneus, Acanthodesia tenuis,* and *Octolasmis lowei* (Table II). On the contrary, the juvenile female crabs had no nemerteans or other associated organisms

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TABLE I. Associated Organisms, Size of Male Crabs, and Water Salinity.

Specimens	Maximum body width (mm)	Total no. Nemerteans	Total no. Associated organisms
Low salinity (16.2–28.2 ppt)			
4[a]	65–100	None	None
3[a]	107–145	None	None
High salinity (35.3 ppt)			
1[b]	105	1	1 Chelonibia
7[b]	122–165	None	8 Chelonibia 2 Octolasmis 1 Acanthodesia

[a] Sarah's Creek or mouth of York River, Va.
[b] Cape Hatteras, N.C.

while the males had no nemerteans and only a few of the other types of associated organisms (Tables I and II). It is known that mature female crabs have more barnacles and nemerteans since, in order to spawn, they migrate more than males, which tend to stay in shallow water and so usually do not come in contact with the larvae of nemertean worms (2). The ciliated larva of *Carcinonemertes* escapes from the egg membrane by secreting an enzyme which softens the membrane until the larva can push its way out mechanically (7). Parasitic on the crabs' eggs (8), the nemertean larvae migrate then to female crabs' gills where they metamorphose and become encapsulated (Fig. 1) in mucous sheaths between the gill lamellae (9). The present results show no correlation between the frequency of the nemerteans and the low and high salinities of the water ranging from 16 ppt to 35.3 ppt (Tables I and II). Many of the barnacles of *Octolasmis lowei* and *Chelonibia patula* were found on adult female crabs from Cape Hatteras, North Carolina where the salinity is higher (35.3 ppt) than in localities of other crabs examined (16 up to 26 ppt). It appears that these associated organisms prefer waters of high salinity (Table II). On the other hand, there may be an association between *Chelonibia patula* and *Octolasmis lowei* since both are very often seen on the same female crab.

Since a phase of the development of *Carcinonemertes* is dependent on the eggs of the crab, young nemerteans appear to be unable to attain a sexual maturity if attached to male crabs (9). Also, immature female crabs molt more frequently than adult ones. Thus, any organisms living on them are shed with

TABLE II. Associated Organisms, Size of Female Crabs, and Water Salinity.

Specimens	Maximum body width (mm)	Total no. Nemerteans	Total no. Associated organisms
Low salinity (16–30 ppt)			
8	50– 97	None	None
4	100–127	None	None
6	143–152[a]	Very many; some large (red)	12 Chelonibia 3 Acanthodesia 1 Octolasmis
5[b]	159–165	Very many large (red); some in external egg mass	9 Chelonibia 2 Acanthodesia 1 Balanus
High salinity (35.3 ppt)			
7[c]	152–172	Present (very many) or absent[d]	51 Chelonibia Many Octolasmis 0 Acanthodesia

[a] Adult crabs. Those smaller than 127 mm are juvenile crabs.
[b] Hampton Roads, Va. The other crabs came mainly from Chesapeake Bay, Md.
[c] Cape Hatteras, N.C.
[d] One third of the crabs had no nemerteans.

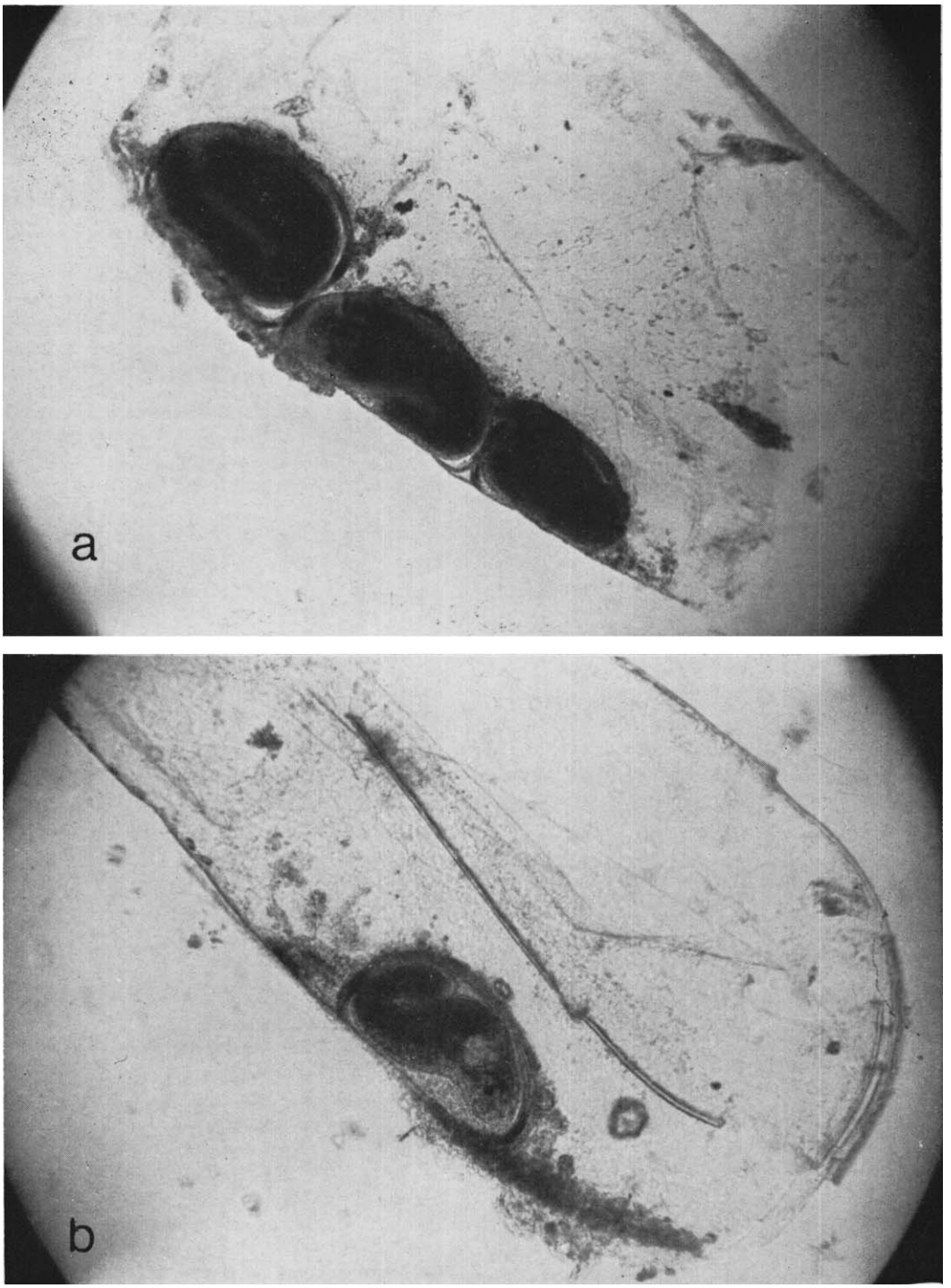

FIG. 1. Encapsulated nemertean worms occurring among the lamellae of the gills of the crab *Callinectes sapidus* (Rathbun).

each molt. Only a slight nemertean infestation with young (white) worms is observed when the seminal receptacles are swollen and the ovaries are not enlarged (6). The nemertean infestation increases with the development of crab and enlargement of the crab's ovaries, and if the crabs spawned at least once, adult (red) nemertean worms on their gills become very frequent (6).

According to the present results, there appears to be no correlation between the size of adult female crabs and the number of the nemerteans on their gills (Table II, crabs between 152 and 172 mm of the body width). These observations confirm Humes' findings reported in 1942 (2).

Although the gills of the crabs injected with various dyes were found to be intensely colored (this was particularly conspicuous in the case of Evans blue dye) no dye was detected in the body of the nemertean worms of these crabs, when as such or dissected in pieces they were examined under the microscope. This seems to indicate no parasitic relationship between the nemertean worms and their hosts. It has to be mentioned also that no injuries were observed on the gills of infested crabs when examined under the dissecting microscope.

The examination of the isolated worms maintained in waters of various salinities revealed that the worms kept in sea water of salinity higher than 10 ppt survived for at least 3 weeks, whereas those kept in tap water and in water of salinity as low as 3.5 ppt died within 2 days (Table II). This evidence further proves that the adult nemertean worms are not true parasites, but commensal on the gills of the crabs. Also, it appears that these worms are not solely dependent on crabs' eggs, since they cannot survive in water of low salinity in which the eggs are still present (Table III).

The relationship of other associated organisms borne on crab shells appears to be of a phoretic nature. *Octolasmis lowei* is found in the branchial chambers of large decapods with which it lives in a commensal relationship (10). Neither *Octolasmis* nor *Carcinonemertes* seems to impair the respiration of *Callinectes* at least as observed in the present study.

TABLE III. Survival of Isolated Nemerteans Maintained in Sea Water of Various Salinities.

Salinity of water[a] (ppt)	No. of worms	Crabs' eggs[b]	Survival[c]
Tap water	10	—	2 days
Tap water	5	+	2 days
3.5	5	+	2 days
10.0	5	+	3 weeks
15.0	5	+	3 weeks
18.0	10	—	3 weeks
18.0	10	+	3 weeks
20.0	10	+	3 weeks
26.0	5	—	3 weeks
26.0	10	+	3 weeks
26.0 (boiled)	5	+	3 weeks

[a] Distilled water was used to obtain appropriate dilutions from the sea water.

[b] Plus indicates that crabs' eggs were added.

[c] Experiment was terminated after 3 weeks.

Summary. A number of organisms occurring on blue crabs do not seem to affect their normal growth and survival.

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