

## Chapter 8

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# Diseases, Parasites, and Other Symbionts

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*And this great structural diversity [of marine life] is paralleled by habits and ways of life which are often bizarre to the point of fantasy. Against such a background, it is not surprising that the whole spectrum of animal associations, from the obviously casual to the intimately complex, can be seen.*

R.V. Gotto (1969)

### INTRODUCTION

We present a critical review of the microbial diseases, parasites, and other symbionts of the blue crab. Previous reviews have provided brief synopses of the diseases of the blue crab (Messick and Sinderman 1992; Noga et al. 1998), overviews and synthesis of crustacean diseases in general (Couch 1983; Johnson 1983; Overstreet 1983; Brock and Lightner 1990; Meyers 1990), or aspects of specific parasitic taxa (Couch and Martin 1982; Overstreet 1982; Bradbury 1994). Infectious diseases of blue crabs have received far less attention than those of the intensively cultured eastern oyster or the penaeid shrimps, primarily because of differences in resource management as well as the dramatic detrimental influences of protozoal and viral diseases in the latter hosts, respectively. Nonetheless, given the appropriate environmental conditions, several pathogenic agents (e.g., viruses, *Vibrio* spp., *Hematodinium perezii*, *Paramoeba perniciosus*, *Loxothylacus texanus*) have the capacity to severely affect blue crab fisheries. Several bacteria and a few parasites (e.g., *Vibrio* spp., microphallid trematodes) represent minor human health concerns, and a few bacteria (*Listeria monocytogenes*, *Clostridium botulinum*) represent safety hazards to the seafood industry. In addition, several symbionts (e.g.,

*Carcinonemertes carcinophila*, *Octolasmis muelleri*) may serve as markers of host biology by indicating migration or molting patterns, and one syndrome, shell disease, may even serve as a useful indicator of poor water quality associated with pollution.

Our synthesis is meant to show the gaps in our understanding of the primary diseases of the blue crab and guide future work on their ecological influences and their pathological processes in the host. Aspects of the immune system of the blue crab are discussed in relation to selected diseases with the caveat that immune functions are poorly understood in the Crustacea in general.

Throughout the chapter the term "symbiont" is used broadly as an organism in some form of close or intimate association with its host (Overstreet 1978). "Symbiosis" means "living together," and we view it as spanning the gamut of disease, parasitic, commensalistic, mutualistic, and phoretic, but not predatory, relationships. A "disease" imparts abnormal function within the host. "Pathogens" cause disease by damaging physiological functions within the host. "Parasites" may or may not be pathogens that cause disease, but they have the potential to produce a negative effect on the host, especially in heavy infections. "Hyperparasitism," a second order parasitism, signifies the condition when one parasite

infects another. A “facultative symbiont” is not physiologically dependent on a host but can establish a relationship with it when the opportunity occurs. It contrasts with an “obligate symbiont,” which has a physiological dependency on its host. The terms “infection” and “infestation” refer to internal and external invasion of the host by endo- and ectosymbionts, respectively. “Commensalism” and “phoresy” are relationships where the symbiont derives benefit, but the host is not affected by the association. Commensalism results when the symbiont shares nutritional resources or a living space with the host. Phoresy results when the symbiont uses the host for transportation. An “epibiont” is an organism that lives on the external surface of the host. In “mutualism,” both the symbiont and the host benefit from the association. These definitions represent a continuum that exists among symbiotic associations.

Standard parasitological terms were defined by Margolis et al. (1982) and updated by Bush et al. (1997). Briefly, “prevalence” is the number of infected hosts divided by the total number of hosts examined, usually expressed as a percentage. “Intensity” is the number of parasites infecting a host, with “mean intensity” representing the mean number of parasites per infected host. “Density” refers to the number of parasites per unit of host or habitat measured in area, volume, or weight (e.g., parasites per ml hemolymph), and is often used with bacterial and protozoal agents. An “epizootic,” or “epidemic” if related to people, is an outbreak of a disease, usually expressed as a large increase in prevalence or intensity of infection in the host population. When occurring over a wide geographic area, such an epizootic is referred to as a “panzootic.” An “enzootic,” or native, disease is one caused by factors consistently present in the affected host population, environment, or region.

The first reported symbionts of the blue crab were the rhizocephalan barnacle *Loxothylacus texanus* by Boschma (1933), the fungus *Lagenidium callinectes* by Couch (1942), and the nemertean worm *Carcinonemertes carcinophila* by Humes (1942). The

late 1960s and early 1970s saw the advent of scientific interest in diseases of the blue crab, especially as crab fisheries became more fully exploited. With the expansion of the softshell industry has come an increased awareness of the role of diseases in the short-term culture of the blue crab and their negative effects on the fisheries. Blue crabs are now known to be infected by a large, disparate fauna comprised of viruses, bacteria, fungi, protozoa, helminths, and other crustaceans. Most of the parasites and diseases are relatively benign and cause little pathological alteration in the crab host. Several, however, cause considerable alteration and occasionally fulminate into epizootics, or outbreaks, resulting in crab mortalities. Unlike dead fish that float, dead crabs generally sink, hence large mortalities often go unnoticed or underreported. The true influence of several diseases, therefore, may be difficult to assess without intensive sampling in enzootic locations.

## VIRAL INFECTIONS

Other than those from some penaeid shrimps, viral infections in the blue crab are some of the better known from a marine invertebrate host. These blue crab infections are known primarily from descriptive ultrastructural studies by Johnson (e.g., 1986a). There are seven or eight reported viruses infective to *C. sapidus* along the Atlantic Coast, with at least two occurring in the Gulf of Mexico. Three species are lethal and two are found concurrently with other viruses, with strong experimental evidence indicating a synergistic pathogenic effect. The identification and characterization of the viruses infecting crabs are less well established than those infecting shrimps, perhaps because there is less economic incentive to culture crabs than to rear penaeid shrimps. However, when one of the shrimp viruses is introduced to the blue crab, it can produce an infection and induce mortality. In addition to hosting viruses that are apparently specific to the blue crab and its relatives, the blue crab can also accumulate human enteric viruses.



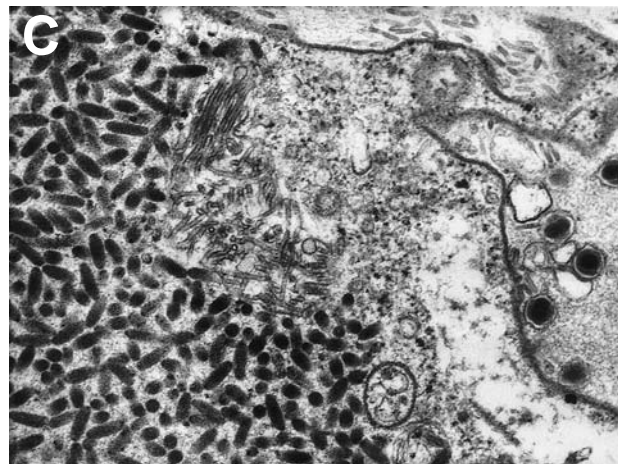
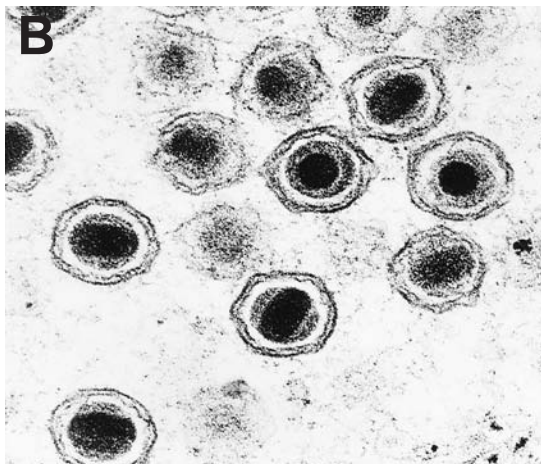
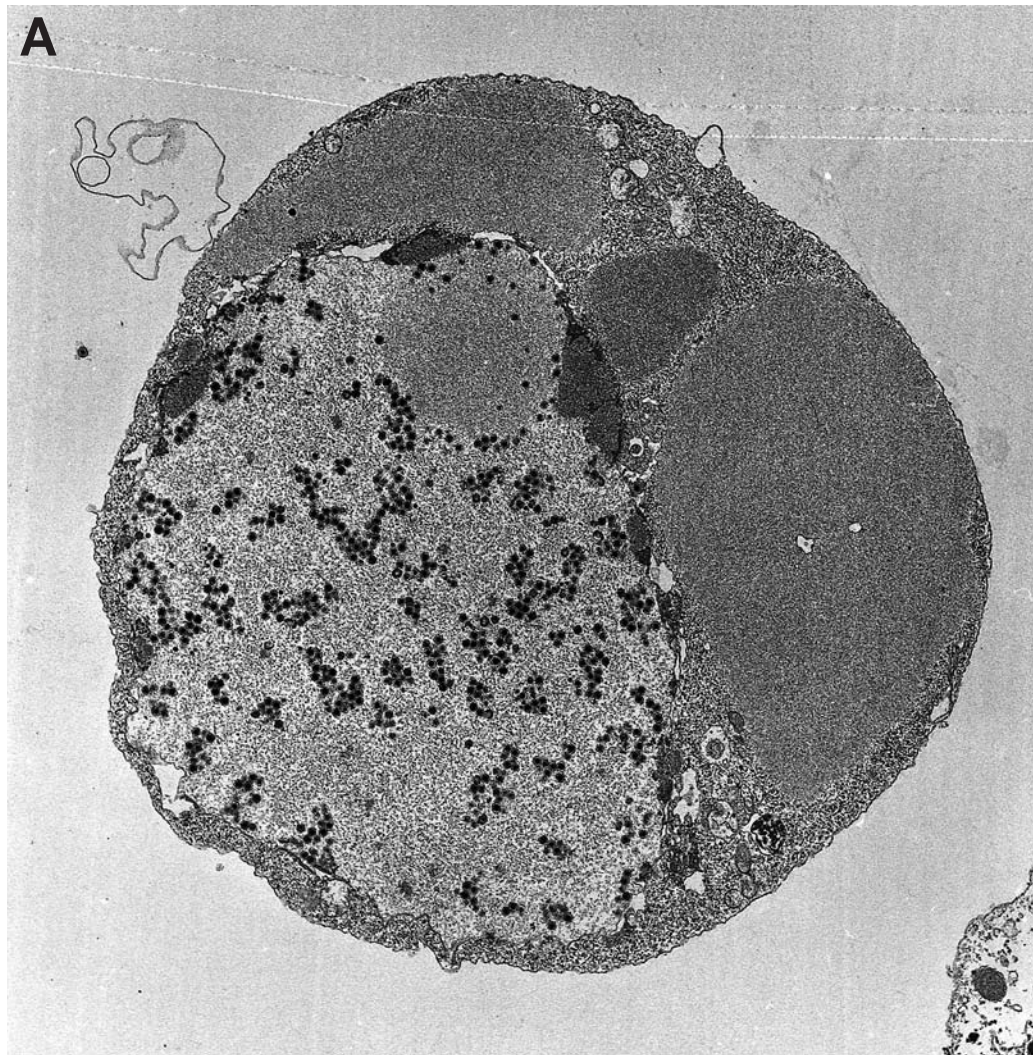


Figure 1. Bi-facies virus (BFV). (A) Infected hemocyte with large viral induced inclusions in both cytoplasm and nucleus. From Johnson (1988). (B) Type-A mature particle showing electron-dense core bound by electron-dense sphere, which in turn is surrounded by an inner and outer membrane. From Overstreet (1978). (C) Type-B mature particle showing single envelope. Note mass of rods (perhaps core of undeveloped BFV) in cytoplasmic material associated with fixed phagocyte. Note small rhabdo-like virus in same cell. From Johnson (1988).

## DNA Viruses

### Bi-facies Virus (BFV)

#### *Biology*

Bi-facies virus, formerly referred to as herpes-like virus (HLV), is a dsDNA, enveloped virus that is extracellular or in the nucleus or cytoplasm of hemocytes and hemopoietic cells (Fig. 1A); it was originally described by Johnson (1976c). Initially considering the virus to be a herpes-like virus, Johnson (1988) altered her view because herpes viruses become enveloped after — rather than before — leaving the nucleus, as in BFV. Bonami and Lightner (1991) still considered the virus to be HLV and related it to Herpesviridae. The virus may also infect connective tissue and epithelial cells of the gill, but it has not been observed in the skeletal muscle, heart, gut epithelium, gonad, or nervous tissues. Infected cells have hypertrophied nuclei with Feulgen-positive granules or homogeneously stained nuclei. Such infected cells may also contain Feulgen-negative inclusions in both nuclei and cytoplasm. When the hemocytes lyse, free refractile virus and lysed cellular debris can fill the hemolymph, producing a diagnostic chalky white hemolymph that does not gel when exposed to air.

The complete development of Bi-facies virus occurs in the nucleus, where this hexagonal virus has two types of development leading to two final forms. The enveloped Type A particles (Fig. 1B) with two envelopes (face to face) measure 197 to 233 nm in diameter and the Type B particles (Fig. 1C) with just one envelope measure 174 to 191 nm (Johnson 1988).

#### *Animal Health and Fisheries Implications*

Other than from captive stocks, Bi-facies virus is known only from Assawoman, Delaware, and Chincoteague bays on the east coast of the USA. In those locations, the prevalence of infection in a natural population of juveniles has been as high as 13% (Johnson 1983).

Once the nucleus of an infected cell hypertrophies and lyses, the hemocyte becomes dysfunctional and necrotic. The advanced condition proba-

bly causes the death of infected crabs, but the condition is not necessarily a stress-related disease. When hemolymph from a moribund crab was injected into healthy crabs or infected tissue was fed to such a crab, death resulted 30 to 40 d later (Johnson 1978), much sooner than that for naturally infected individuals. Naturally infected crabs may survive for at least 60 d (Johnson 1983). Healthy juveniles maintained in separate containers supplied with water from a common source containing infected individuals developed disease that often resulted in mortality. Infected crabs appeared healthy until right before death when they became inactive and stopped feeding.

A similar “herpes-like” virus from the bladder and antennal gland of the Alaskan blue king crab *Paralithodes platypus* has been implicated in a decline of the host population and perhaps the populations of two related crabs in the Bering Sea (Sparks and Morado 1986).

#### *Future Research*

Because of the pathogenic nature of Bi-facies virus, there is a need to establish its geographic range, hosts, and effects on host population. There is also a need to characterize better the virus using biochemical and biophysical features.

### Baculovirus A (Baculo-A)

#### *Biology*

Baculovirus-A is actually a bacilliform virus or nudibaculovirid. It is a nonoccluded, rod-shaped, enveloped virus that infects juvenile and adult blue crabs along the Atlantic coast and perhaps throughout the range of the crab. It infects the nuclei of hepatopancreatic epithelium (Fig. 2A, B), causing hypertrophy, with the nucleus usually reaching twice its normal size and weakly staining Feulgen-positive. The trilaminar enveloped dsDNA virion measures about 260 to 300 by 60 to 70 nm; with the nucleocapsid, it measures 240 to 254 by 43 nm. Virions occur in ordered paracrystalline arrays along the nuclear membrane (Johnson 1976a).



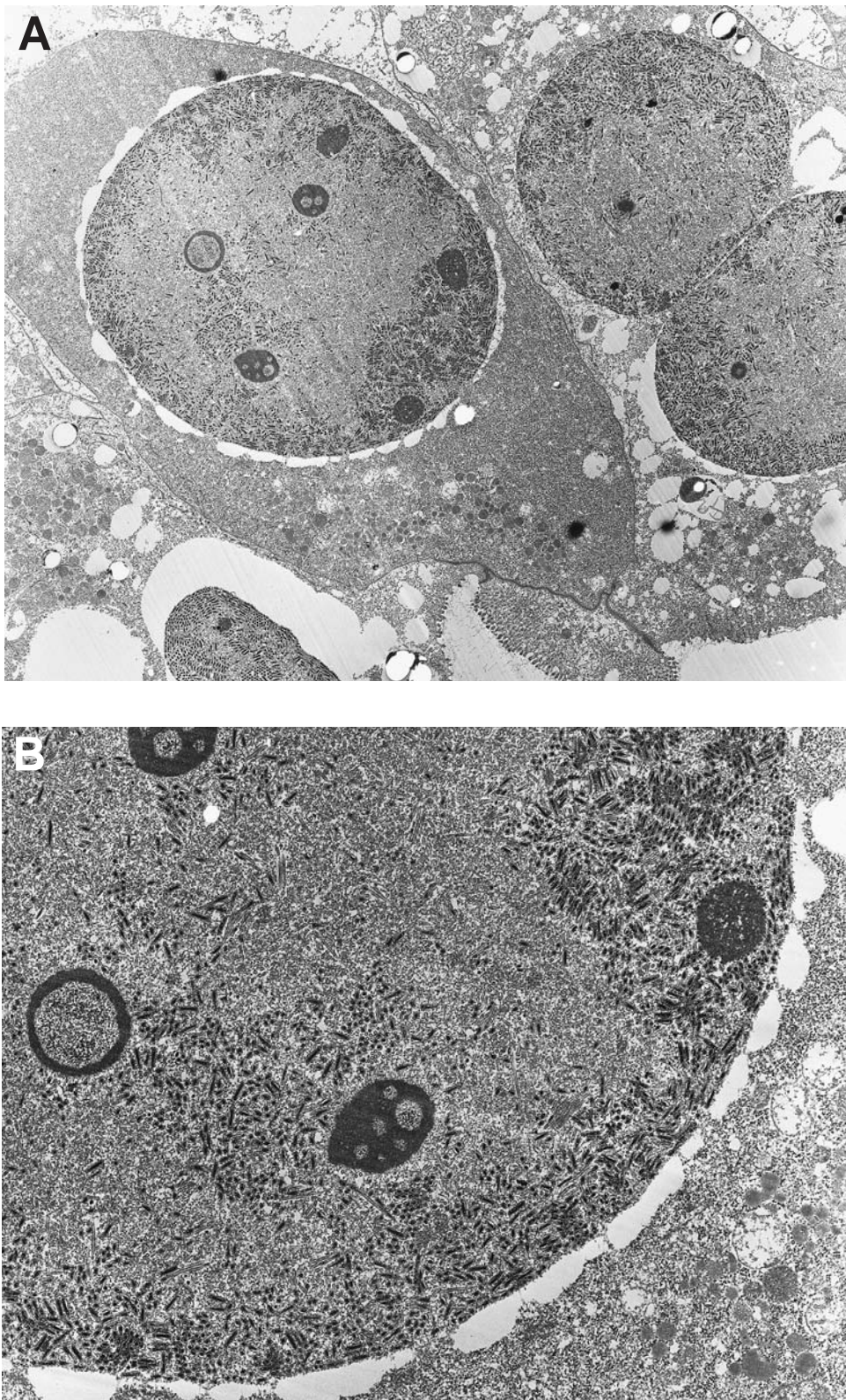


Figure 2. Baculovirus A (Baculo-A) in nuclei of hepatopancreatic epithelium. (A) One of two cells is binucleate and all nuclei exhibit remains of nucleoli in addition to the mature virions. (B) Close-up of (A). From same material as reported by Johnson (1983).

### ***Animal Health and Fisheries Implications***

Johnson (1983) thought that Baculo-A might be the most ubiquitous of all the blue crab viruses. Its prevalence typically ranged from 4 to 20% in all stages of the molt cycle of the blue crabs between Long Island Sound, Connecticut, and Chesapeake Bay, though Johnson (1983) reported 52% prevalence in one collection from Chincoteague Bay, Virginia. Johnson (1976a) found the agent in all collections from crab populations in low to high salinity locations. There, however, was no indication that any infected crab was harmfully affected. Nevertheless, microscopical signs of focal infection were observed; hypertrophied nuclei occurred most commonly in absorptive cells (reserve cells, or R-cells) and less often in secretory (B-cells) and fibrillar (F-cells) cells of infected crabs.

### ***Future Research***

Research might show that larval crabs are affected or even killed by Baculo-A. For example, an occluded baculovirus typically kills larval and post-larval penaeids but seldom older individuals (Overstreet 1994). The apparently nonoccluded "tau" virus of the green crab *Carcinus maenas*, which may be related to Baculo-A, kills its crab host (Bazin et al. 1974). Feeding or injection can experimentally transmit both the occluded and nonoccluded agents. Because Johnson (1983) thought Baculo-A might be the most ubiquitous of all the crab viruses, blue crabs from the Gulf of Mexico surely should be surveyed for this as well as other viruses.

## **Baculovirus B (Baculo-B)**

### ***Biology***

The nonoccluded Baculo-B virus exhibits similarities to Baculo-A, but it infects hemocytes and hematopoietic cells, often producing diagnostic hyperchromatic areas in the center of the nucleus (Johnson 1983, 1986a). Nuclear and cellular hypertrophy is not as marked as in cells infected with BFV or Baculo-A, but the cells stain more strongly with Feulgen. The infected hemopoietic cells and hemocytes exhibit pale, hypertrophied nuclei that can be either homogeneous or rimmed with chromatin,

occasionally with hyperchromatic areas in the center. Virions mature after the nucleus becomes hypertrophied. The enveloped virions appear ovoid, measuring about 100 by 335 nm with tapered and rounded ends; developing virions are associated with intranuclear vesicles as has been observed in the hemocytes and some connective tissue cells of *Carcinus maenas* (see Bazin et al. 1974), rather than long tubule-like structures as in Baculo-A. Virions occur in ordered arrays in the nucleoplasm. The cytoplasm becomes a narrow rim around the nucleus with few or no granules. Mature granulocytes are apparently not infected. Once a nucleus is infected, it ruptures and the virions invade the cytoplasm and then disperse into the extracellular space upon lysis of the cell.

### ***Animal Health and Fisheries Implications***

Infections occur at least in Chesapeake Bay, Maryland, and its tributaries and in Chincoteague Bay, Virginia. Experimentally infected crabs became sick, but at least one was infected with other viruses (RhVA and EHV) (Johnson 1983). Infections are not known to harm naturally-infected crabs.

### ***Future Research***

The pathogenic effect of the virus on the blue crab, especially on larvae and young juveniles, needs to be determined. As with Baculo-A, biochemical and biophysical data are needed. The lack of infection in granulocytes is intriguing. The specificity of Baculo-B for certain hemocyte-types should be further examined.

## **RNA Viruses**

### **Reo-like Virus (RLV)**

### ***Biology***

Reo-like virus, a nonoccluded member of the Type I Reoviridae, infects primarily hemocytes, hemopoietic tissues, and glial cells (Fig. 3A, B). Various other ectodermally and mesodermally derived tissues such as epidermis, gill, bladder, blood vessel endothelium, Y-organ, and connective tissue cells, including fixed phagocytes, can also be infected



(Johnson 1983). Originally placed incorrectly in Picornaviridae, this reovirus has icosahedral virions. The virion is a nonenveloped dsRNA that measures 55 to 60 nm in cross-section and occurs in the cytoplasm, producing Feulgen-negative inclusions and increased cytoplasmic volume. These inclusions, basophilic and angulate to rounded in shape, constitute paracrystalline arrays of virus particles as well as sinuous proteinaceous filaments 20 to 30 nm in diameter (Fig. 3C, D). Infected hemocytes invade the glia of the brain and thoracic ganglia, which become necrotic. Because of this tissue destruction, the crab becomes sluggish and exhibits tremor and ultimately paralysis (Johnson 1983).

Based on what is known about the virus, it may be the same as that found in the harbor crab *Liocarcinus depurator* (as *Macropipus depurator*) from the Mediterranean Sea (Hukuhara and Bonami 1991).

### **Animal Health and Fisheries Implications**

Reo-like virus has been found commonly in juvenile and adult crabs from Chincoteague and Chesapeake bays, where it was associated with mortalities (Johnson and Bodammer 1975; Johnson 1983, 1984). Infected crabs occurred in high and low salinity habitats.

Also present in RLV-infected crabs was Rhabdo-like virus A (RhVA), which seemed to produce a synergistic response (Fig. 4) in the resulting glial necrosis and paralysis (Johnson 1983, 1984). Other viruses can also be present in RLV-infected crabs, such as another rhabdo-like virus, Baculo-A, and Baculo-B. The association between RLV and each of those viruses requires investigation. When hemolymph infected with RLV plus RhVA was injected into healthy naïve crabs, those crabs died in as little as 3 to 4 d for pre- or postmolt individuals and 11 d for intermolt individuals. When crabs were administered infected tissues orally, it took 12 to 32 d for the intermolt crabs to die (Johnson 1978, 1983, 1986a). The virus can probably also enter by other routes, and the resulting infection represents a potential threat to crabs in shedding-tank systems. Diagnosis usually consists of examination of hemocytes or hemopoietic tissues of sluggish crabs possessing hemolymph that either will not clot or that

exhibits a reduced clotting rate. In many cases, the exoskeleton becomes discolored, and the gills of the crab turn a reddish to brownish color.

### **Future Research**

Reo-like virus infects juveniles and adults in culture, but its prevalence in nature has not been established. The virus needs to be better characterized.

## **Rhabdo-like Virus A (RhVA)**

### **Biology**

Rhabdo-like virus A probably occurs as a ubiquitous virus in the blue crab along both the Atlantic and Gulf of Mexico coasts. This small virus measures 20 to 30 nm by 110 to 170 nm in bacilliform stages with rounded ends or 20 to 30 nm by up to 600 nm long in a filamentous flexuous stage (Jahromi 1977). It buds into the endoplasmic reticulum, infecting glial cells of ganglia and large nerves as well as hemocytes, hemopoietic tissue, connective tissues, and epithelium other than that of the alimentary tract and antennal gland. Rhabdo-like virus A infects similar sites as Reo-like virus. It does not infect axons or striated muscle (Johnson 1978, 1983), but it does infect the mandibular organ. Initially, the virus was incorrectly reported from the ecdysal gland, and consequently the virus was originally called EGV2 (Yudin and Clark 1978, 1979).

### **Animal Health and Fisheries Implications**

Rhabdo-like virus A may exemplify a virus associated with host stress. Infected crabs (Figs. 1C, 3A) usually exhibited disease when they had been maintained under stressful laboratory conditions or were infected with other viruses (RLV, EHV, CBV, BaculoB, or HLV) (Johnson 1983). Those not infected with other viruses or not under stress apparently do not exhibit disease. Infection by RhVA produced no pathological signs visible with the light microscope. As indicated above, sick crabs infected with RLV had a mixed infection with RhVA (Figs. 3A, 4). Experimentally, an injected inoculum of both of those viruses can kill a crab in as little as 3 d. The taxonomic relationship with a

similar virus in *Carcinus mediterraneus* from the Mediterranean coast of France is uncertain (Mari 1987).

### Rhabdo-like Virus B (RhVB)

#### Biology

Rhabdo-like virus B, originally called EGV1 for "ecdysal gland virus 1," has been reported once (Yudin and Clark 1978). The reported size was 50 to 70nm by 100 to 170 nm, or much wider than RhVA. The enveloping membrane exhibited surface projections. The virus occurred extracellularly under

the basal lamina of the mandibular organ in crabs from the Gulf of Mexico.

#### Animal Health and Fisheries Implications

Rhabdo-like virus B occurred in 3% of 60 mandibular organs of crabs from the Gulf of Mexico examined by transmission electron microscopy (TEM). None of the infected crabs appeared sick. In one case, RhVA co-occurred with RhVB and the viral particles developed in the interlamellar space of the nuclear envelope, forming various kinds of clusters in the cytoplasm (Yudin and Clark 1978).

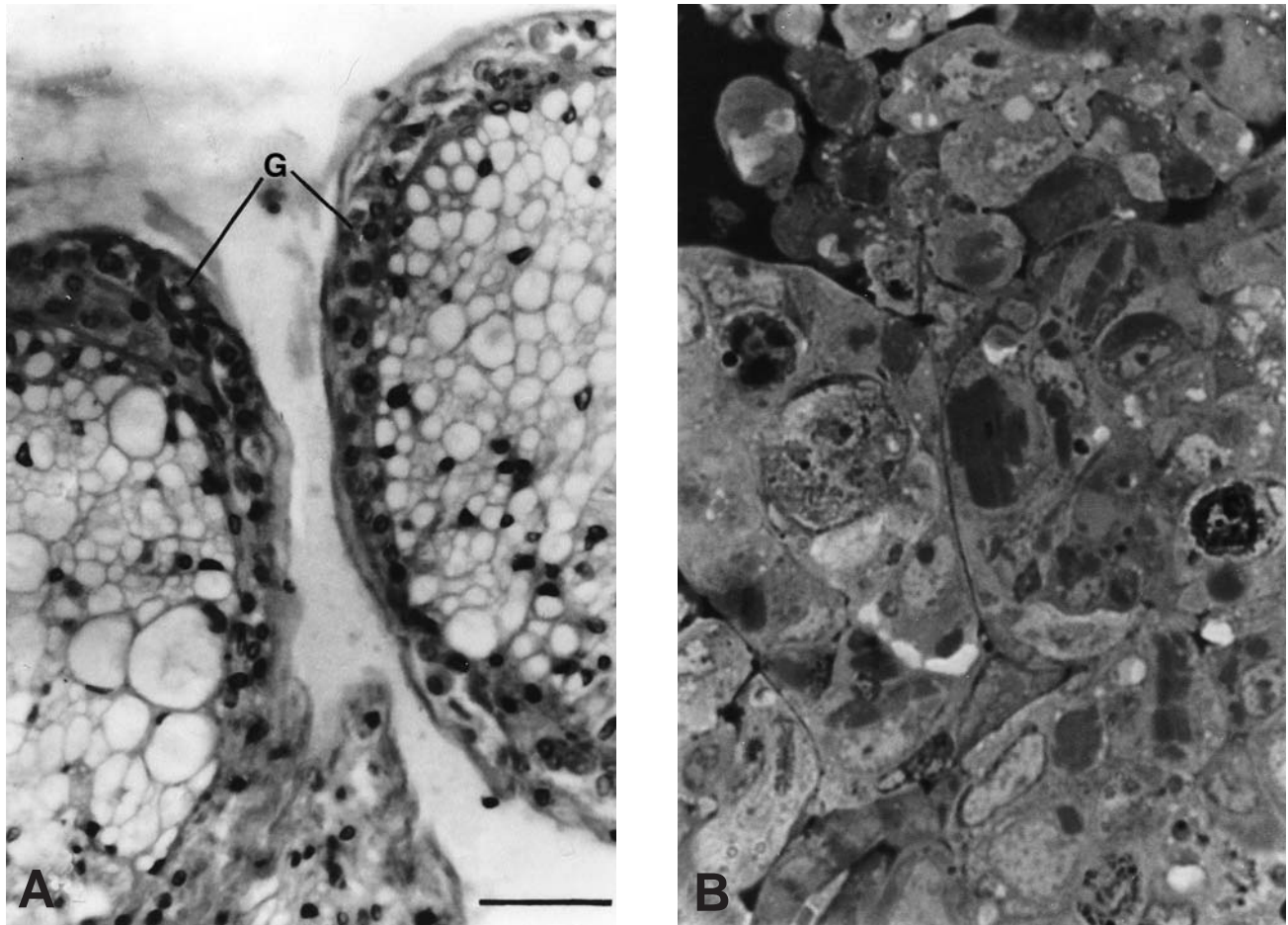
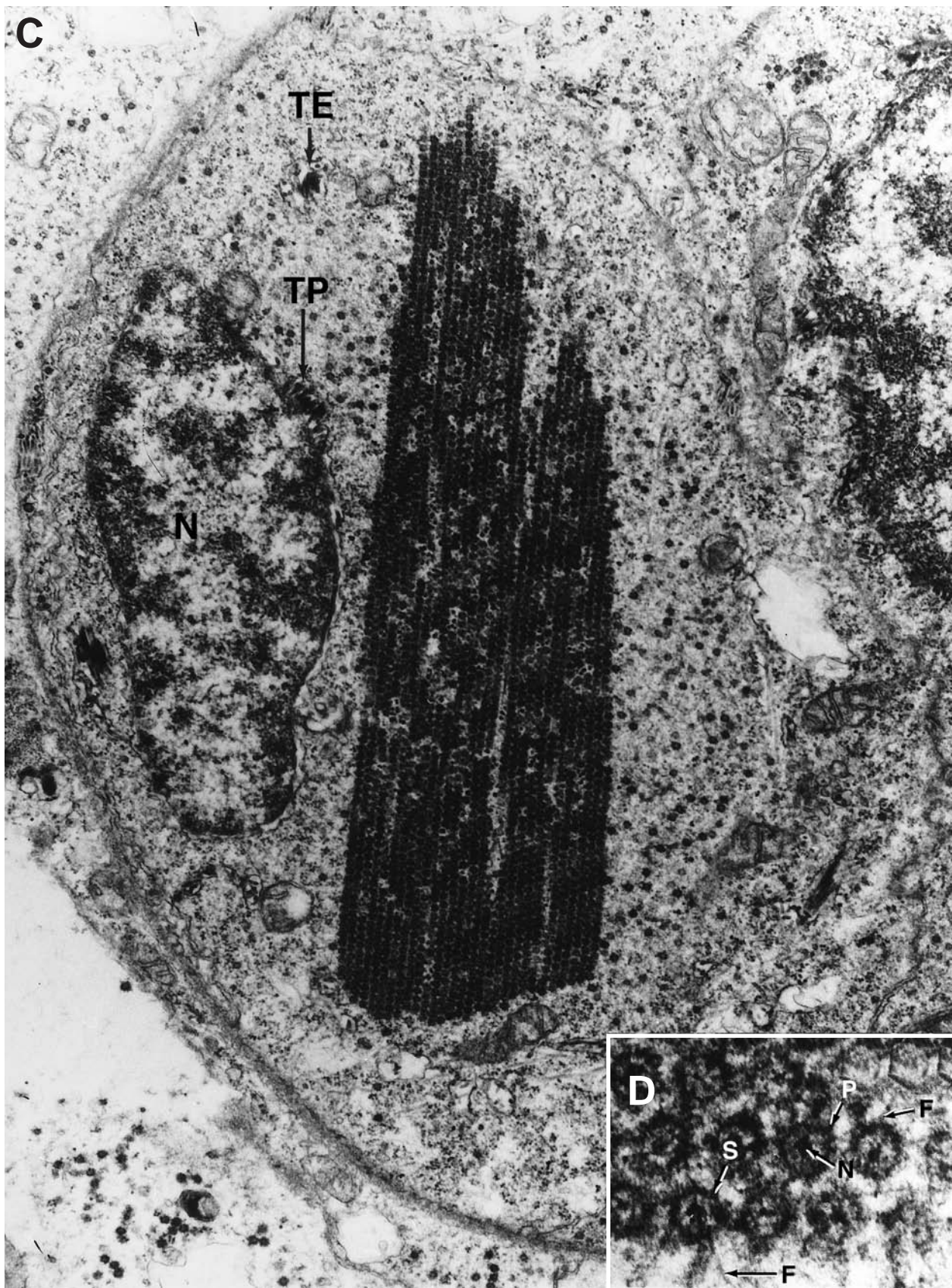


Figure 3. Reo-like virus (RLV). Above: (A) Cross-section of large nerve with hemocytes present in necrotic glial area [G]; tissue also infected with RhVA. From Johnson (1984); (B) epoxy-embedded hemopoietic tissue, toluidine blue stain, exhibiting darkly staining crystalline inclusion. Opposite page: (C) Ultrastructure of cytoplasmic inclusion of viral particles, associated with atypical tubules in perinuclear cistern [TP] of nucleus [N] and in endoplasmic reticulum cistern [TE]. (D) Insert shows close-up of virus with cytoplasmic fibrils [F], nucleoid-like density [N], projections [P], and outer wall subunits [S]. (B) - (D) from Johnson and Bodammer (1975). Bar in (A) = 2-  $\mu$ m.







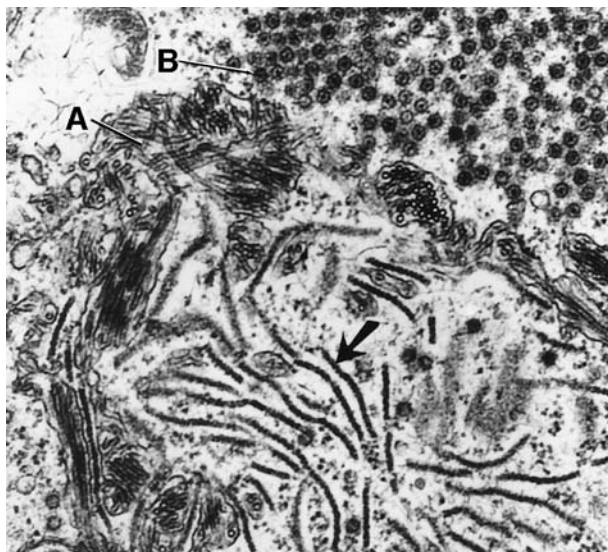


Figure 4. Rhabdo-like virus A (RhVA). (A) In endoplasmic reticulum of cell additionally infected with reo-like virus (RLV). (B) The sinuous strands (arrow) are associated with the development of RLV. From Johnson (1984).

### **Future Research**

The status of RhVB requires investigation. It is important to know what this virus is before an effort is spent on determining its host range, prevalence of infection, host specificity, and means of infection. With improved molecular techniques, RhVB may be more easily studied and classified.

## **Enveloped Helical Virus (EHV)**

### **Biology**

Enveloped helical virus is another wide ssRNA virus. It is an extracellular virus usually associated with the basal lamina or lying between the basal lamina and plasma membrane of hemocytes, or cells of hemopoietic tissue, or certain other connective tissue cells (Fig. 5). Johnson and Farley (1980) tentatively associated it with Paramyxoviridae and Orthomyxoviridae, but Johnson (1986a) later considered it to be a rhabdo-like virus. The virus is either ovoid (approximately 105 by 194 nm) or

bacilliform (105 by up to 300 nm long). Like a similarly appearing virus from the Y-organ of *Carcinus maenas*, it buds virions through the plasma membrane and has flexuous nucleocapsids and granular areas of development in the cytoplasm of cells (Johnson 1983). Projections from EHV occur on the outer surface of the envelope. Mature virions bud through the plasma membrane where they occur extracellularly (Fig. 5B, C).

### **Animal Health and Fisheries Implications**

Johnson and Farley (1980) found this virus in crabs from Chincoteague and Chesapeake bays and the east coast of Florida. They found it only with TEM and only concurrent with other viruses. No evidence presently exists linking an infection with ill health. Multiple infections, however, are common (Fig. 5A).

### **Future Research**

There is a need to characterize this virus as well as determine host range, prevalence of infection, host specificity, association with other viruses, and means of infection. Also, in spite of the purported difference in size between EHV and RhVB in the blue crab, perhaps EHV and RhVB are the same virus.

## **Chesapeake Bay Virus (CBV)**

### **Biology**

Chesapeake Bay virus is a ssRNA, nonenveloped, icosahedral, picorna-like virus about 30 nm in diameter that occurs in the cytoplasm of ectodermally derived cells (Johnson 1978, 1983). It occurs in neurosecretory cells (Fig. 6A), but not glial cells, as well as in the epidermis and in the epithelium of the gill (Fig. 6B), bladder, foregut, and hindgut of crabs from Chesapeake Bay. It has also been observed in hemocytes and hemopoietic tissue. Hypertrophied cells contain a Feulgen-negative homogenous material consisting mostly of virus, often focally arranged in a paracrystalline array (Fig. 6C) that makes detection possible with a light microscope.



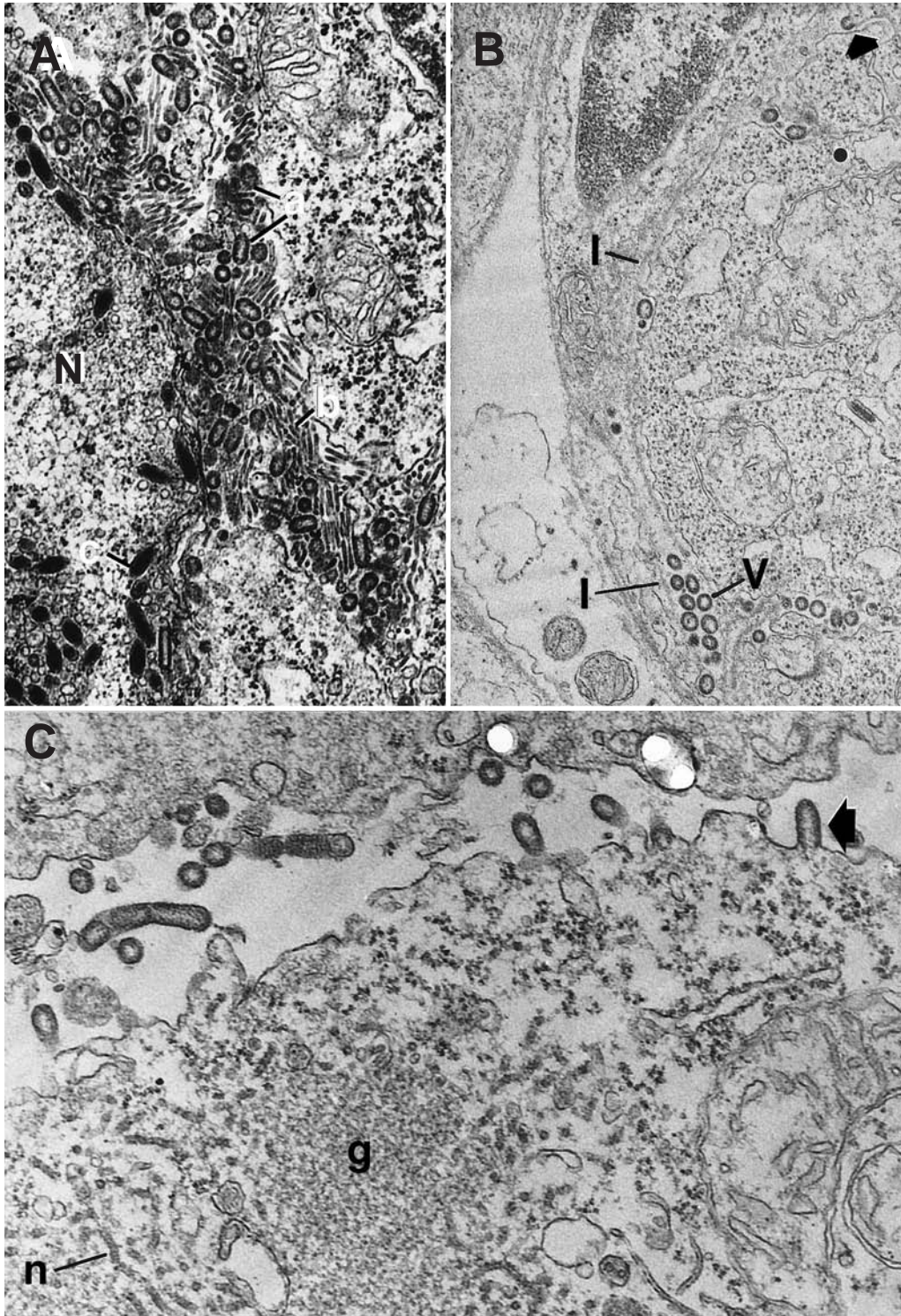


Figure 5. Enveloped helical virus (EHV). (A) Infection with the enveloped EHV [a] as well as the rhabdo-like virus RhVA [b] occurring in the extracellular space, while nucleus [N] contains the baculovirus Baculo-B [c]. (B) Infected hemopoietic tissue showing EHV virions [v] free between cells and under basal lamina [l]. Note budding form (broad arrow). (C) Close-up showing EHV virions budding (arrow) through plasma membrane of a hemocyte, with nucleocapsids [n] and granular area [g] in cytoplasm. From Johnson and Farley (1980).

### ***Animal Health and Fisheries Implications***

Infections with CBV usually have a limited focal nature, sometimes resulting in blindness when the retina becomes infected. However, it can cause extensive destruction of central nervous system, gill epithelium, bladder epithelium, and neurosecretory cells. Infections can result in death, but mortality often takes a month or two because of the focal distribution. Abnormal behavior (including difficulties in gas exchange and osmotic control, erratic swimming, and blindness) presumably allows predators to readily feed on infected crabs.

The virus infects captive juveniles and probably infects juveniles and adults in wild populations. Diseased crabs with focal lesions suggestive of CBV have been observed in Chesapeake and Assawoman bays. Experimental infections indicate that the virus is pathogenic. Most crabs experimentally infected with CBV died, but RLV, RhVA, or EHV co-occurred in at least some of those individuals (Johnson 1983).

### ***Future Research***

There is a need to confirm that the virus is a picornavirus, to assess prevalence in natural populations, and to determine if the deleterious response can result from a sole infection by CBV.

### ***Non-Callinectes Viruses***

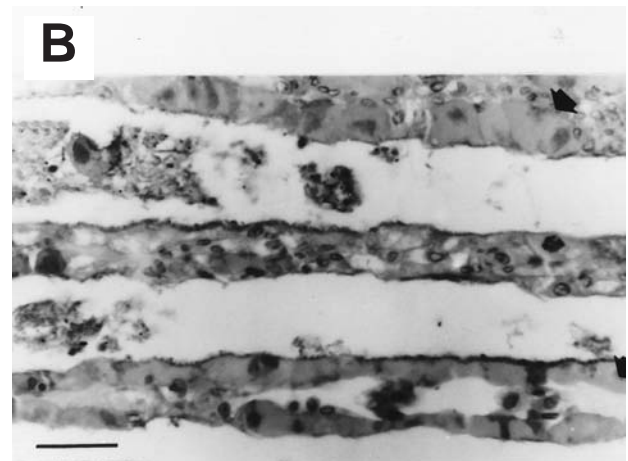
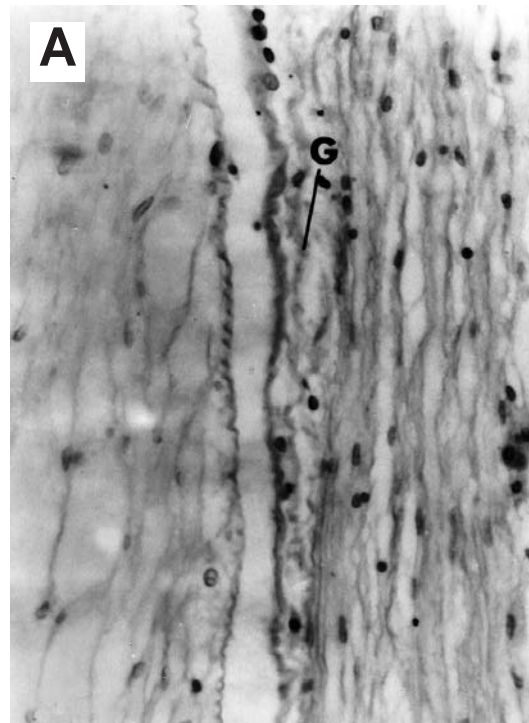
One could argue without evidence that some of the viruses reported above are not primarily agents of the blue crab. Perhaps the primary host for some of those could be other crustaceans or invertebrates, but, without additional evidence, there is little point in such speculation. More important, there are probably many viruses that have a wide host range but that have never been associated with natural infections in the blue crab. One of these is what was previously known as a baculovirus or bacilliform virus but which is now being proposed as a species of *Whispovirus* (Nimaviridae) and is commonly known as white spot virus (WSV). Because it is a double stranded DNA virus, it was not included in the listing above. Nevertheless, even though it is not con-

sidered a blue crab virus, it is a serious threat to wild and cultured blue crab stocks, and as such is an example of a threat from an introduced species.

### **White Spot Virus (WSV)**

#### ***Biology***

The penaeid shrimp aquaculture community has been well aware of WSV under that name or any of several others since 1993 because it has killed large numbers of cultured shrimp. This is a non-





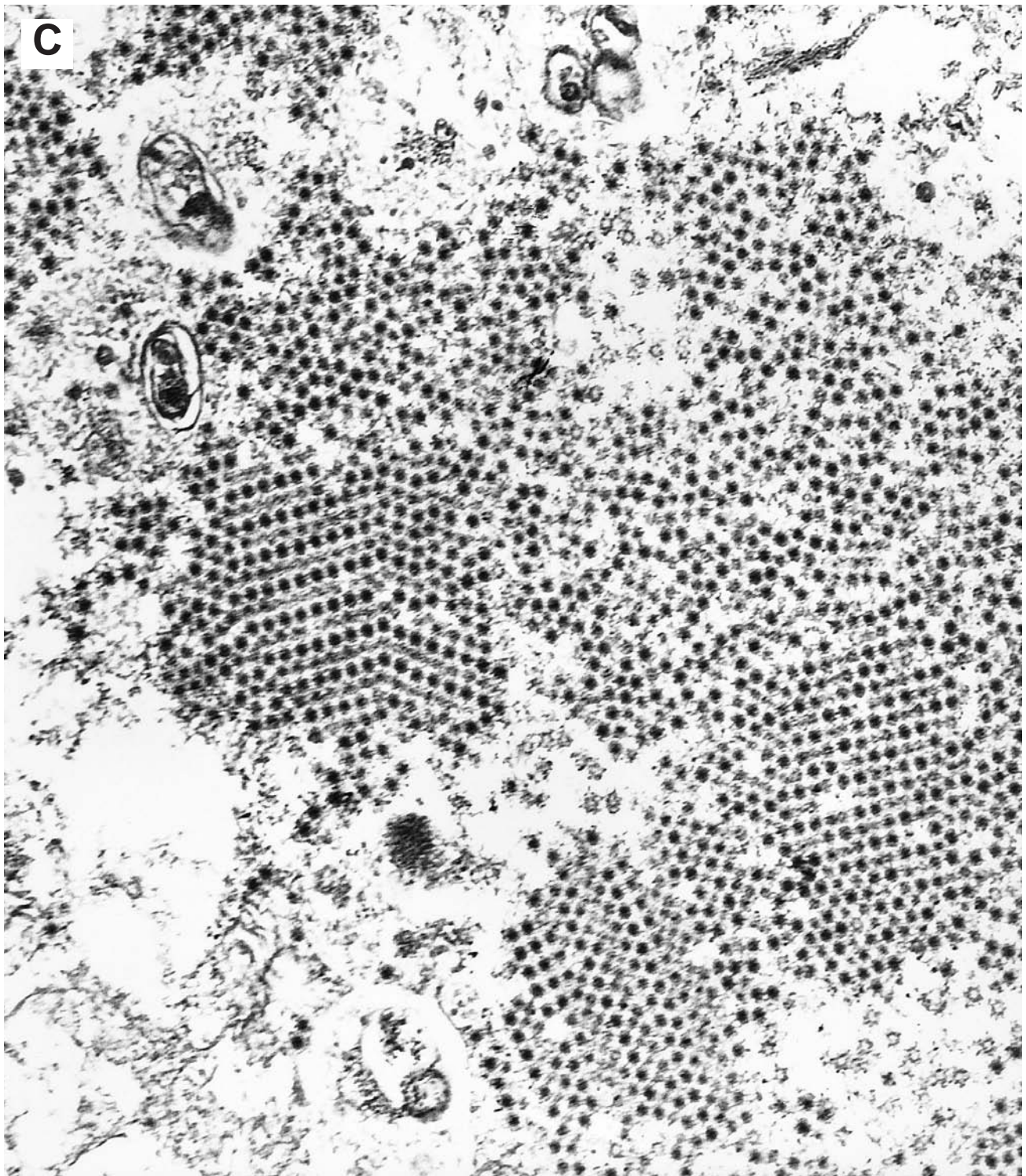


Figure 6. Chesapeake Bay virus (CBV), alcian blue-nuclear fast red stained tissues. Opposite page: (A) Longitudinal section through large nerve of infected crab. The glia [G] are normal. (B) Gill lamellae with epithelial cells in upper and lower aspects are hypertrophied and heavily infected with Feulgen negative material consisting almost entirely of virus (arrows), while middle lamella is normal. Above: (C) Paracrystalline arrays of picorna-like virus in cytoplasm of degenerating cell. Spikes project from the surface of virions. (A) and (B) from Johnson (1984). Bar in (B) = 20  $\mu\text{m}$ .

occluded rod-shaped particle with an apical envelope extension. The nucleocapsid is cylindrical with asymmetric ends and a superficially segmented appearance (Durand et al. 1997). Virions measure 70 to 150 nm by 275 to 380 nm. Infected cells can be diagnosed histologically by prominent eosinophilic to pale basophilic (with H&E staining), Feulgen-positive intranuclear inclusion bodies in hypertrophied nuclei of cuticular epithelial (Fig. 7) and connective tissue cells. It can also be detected in the antennal gland epithelium, lymphoid organ sheath cells, hematopoietic tissues, and fixed phagocytes of the heart (Lightner 1996). Because of the interest in this disease in aquaculture, gene probes and PCR detection methods have been developed to quickly detect an infection. Confirmation of bioassays is usually made with light microscopy (LM), TEM, PCR, and *in situ* DNA hybridization. In addition to infecting several different penaeid shrimps, the virus can infect a variety of other crustaceans, including the blue crab (Flowers et al. 2000; Krol 2002).

### ***Animal Health and Fisheries Implications***

White spot virus has been prevalent in penaeid aquaculture facilities in Asia and the Indo-Pacific where it has caused enormous losses to commercial shrimp farms. After Hurricane Georges in 1998, the virus was introduced into South, Central, and North America, where it caused major losses to penaeids. To assess for potential reservoir and carrier hosts, experimental work has been conducted on several marine and estuarine organisms. In Asia, the portunid sand crab *Portunus pelagicus* and mud or mangrove crab *Scylla serrata* as well as *Acetes* sp. were exposed experimentally to WSV (Supamattaya et al. 1998). All exposed specimens of *Acetes* sp. died in 3 or 5 d following injection or immersion, respectively. All of the injected sand crabs died by day 8 but only 20% of the mud crabs had died by day 9. Neither species of crab died when fed the virus, but both demonstrated an infection histologically and certainly can serve as reservoir hosts. On the other hand, all naïve specimens of the injected blue crab from Mississippi and 66% of those fed infected tissue died, and the infection in the bioassay animals was confirmed with PCR, TEM and LM histology

(Flowers et al. 2000; Krol 2002). White spot virus demonstrates the potential for a virus common in shrimp aquaculture to have a serious influence as a pathogen on wild or cultured stocks of the blue crab.

### ***Future Research***

As indicated, WSV is an example of the ability of the blue crab to serve as a host to a virus that is extremely pathogenic to members of another crustacean group. Regarding WSV, we need to know what environmental, host, and viral conditions it would take to transform an infected individual or stock into a panzootic with heavy mortalities. The same approach can be directed to other viruses that could be introduced into a habitat in which the blue crab occurs.

### **Public Health Implications of Viruses**

None of the crab viruses discussed above is harmful to humans or pose any public health threat. However, although the blue crab is not a filter feeder like the eastern oyster and other bivalves that concentrate large numbers of human pathogenic viruses, it can readily accumulate human enteric viruses. Hejkal and Gerba (1981) experimentally determined that poliovirus and other viruses in high concentrations in water surrounding the crab were rapidly (within 2 h) acquired throughout the tissues of the crab. Highest levels occurred in the digestive tract and hemolymph. Virus uptake was not affected much by salinity, but the levels were clearly influenced by temperature. Some virus survived 6 d in the hemolymph at 15°C, but at 25°C the rates of both uptake and removal were significantly increased, with none detected after 20 h. Consequently, especially in cool water, the blue crab can accumulate (but not concentrate) harmful viruses from the surrounding water or from contaminated food in a polluted location, and then migrate to an uncontaminated location. Moreover, all or nearly all the virus accumulated in crabs originating from areas contaminated with municipal sludge and other dumped wastes. Hejkal and Gerba (1981) also



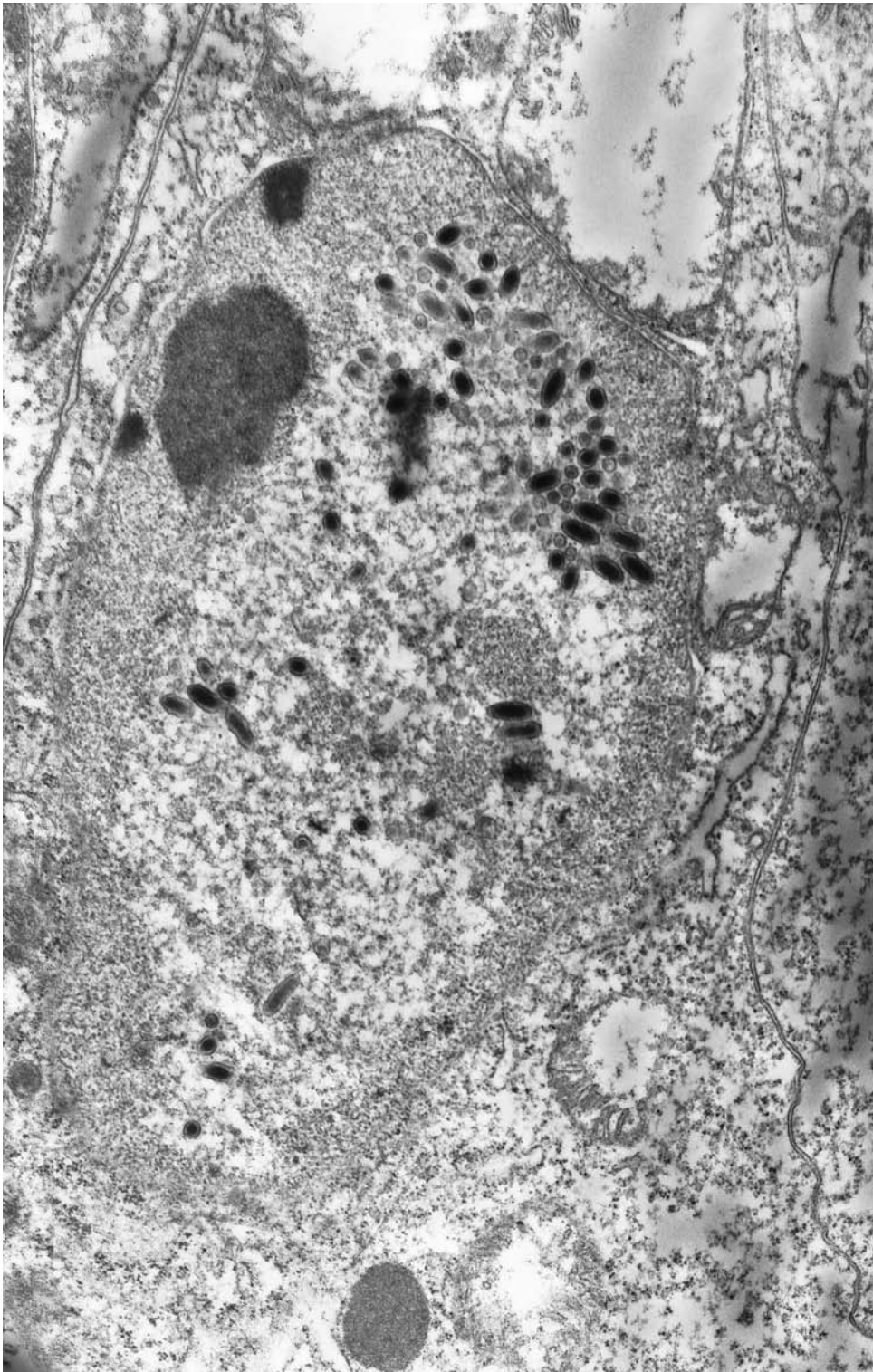


Figure 7. White spot virus (WSV) in nucleus of gill epithelial cell of an experimentally infected blue crab in Mississippi.

showed that although boiling a crab for 8 min (internal T of 70°C) inactivated 99.9% of the virus, in rare cases some active virus was still detected in swimming muscle after 16 min (internal T of 94°C).

Rotavirus and enteroviruses can be detected simply by separating the virus from tissue homogenates at pH 9.5, concentrating by absorption to protein precipitates at pH 3.5, followed by elution from the precipitates at pH 9.2 (Seidel et al. 1983). Recovery effectiveness averaged 52% with poliovirus and others when using the polyelectrolyte cat-floc precipitation to remove toxic factors from cell cultures without loss in virus recovery. Without such removal, the final elute had a toxic effect on the cells used for the assay.

### General Future of Viral Research

Research possibilities dealing with blue crab viruses are begging for attention. As an example, The Crustacean Society meeting in May 1999 (Lafayette, Louisiana) included a 16-paper symposium entitled "Blue Crab Mortality Symposium" that did not include a single paper that mentioned viral infections even though at least RLV, BFV, and CBV can be fatal and potentially serious pathogens to crab stocks. Increased experience with penaeid shrimp viruses has demonstrated how devastating a few agents can be to wild and cultured stocks. Some of the matters that require future attention have been indicated above under the separate viruses. There are, however, general approaches that should be addressed. A basic need exists to fully characterize each known virus and to determine its host range, prevalence of infection, host specificity, and means of transmission. We speculate that many viruses, under the appropriate conditions, can have a devastating effect on cultured and wild crabs. Considering the assumed potential for catastrophes, we think there exists a plethora of avenues to investigate. Most information about viruses in the blue crab comes from descriptive studies by Phyllis Johnson and colleagues. One must assume that the crab, an apparently good host for viruses, could or does harbor numerous others. Any of these could be either highly specific to the blue crab or infective to a wide range of crustacean hosts. Both the geographic range

and the specificity (host range) of the known viruses require additional documentation. Prevalence of infection from a few locations should be documented even though values for a given virus are dependent on environmental conditions and will differ yearly, seasonally, and geographically. Assuming that the viruses all act differently, similar to what one finds in penaeid viruses (e.g., Overstreet 1994; Lightner 1996), we think the conditions necessary for infection and inactivation for these should be established. The primary question is what conditions or interactions are necessary to shift a harmless infection in equilibrium into a disease state and mass mortality?

Based on the lack of critical examination for viruses along the range of the crab, at least some of the agents other than RhVA and RhVB can be predicted to occur in the Gulf of Mexico and some probably also in the Caribbean Sea. Considering the importation of crabs from the Gulf of Mexico to the Chesapeake Bay area, we think perhaps some Chesapeake cases could have originated from the Gulf. Perhaps the agent for some of these diseases has established an equilibrium with its host in the wild, including in the Gulf of Mexico. In any event, given the high population densities of blue crabs and short-term culture of soft-shell crabs, we suggest that high mortalities in shedding facilities may foment spread of viral pathogens. Given the fishing practices involved in soft-shell culture, the movement of diseased crabs and introduction of pathogens to new areas is highly likely, especially in Chesapeake Bay where molting crabs are shed in different watersheds from whence they came. For these reasons, we caution against importation of soft-shell and hard crabs into the Chesapeake area or the Gulf of Mexico.

As already indicated, the wealth of information on viral infections in the blue crab has resulted from ultrastructural studies by Johnson, including experimental infections, stressing animals in confined systems, and surveys. This work should be followed with molecular and other techniques that are continually being updated for viruses in penaeid shrimps (e.g., Lightner 1996). With additional research, infections could be detected and distinguished by genetic probes, PCR, *in situ* hybridiza-



tion, dot blot hybridization, antibody tests (e.g., ELISA and fluorescent), direct fresh microscopical evaluation, and histopathological criteria as well as ultrastructure of the viruses. Unfortunately, there is no well-established, continuous cell line developed from any crustacean. Hence, viral culture and plaque tests cannot be conducted as they are for viruses from most other animals.

Multiple infections often infect the same individual, and the pathogenic relationship among the different species needs to be assessed. In some cases, an infection can become patent or an outbreak of disease can occur when crabs are in culture, crowded, or in a stressed environment (Johnson 1978, 1984, 1986; Yudin and Clark 1979; Messick and Kennedy 1990). Consequently, in addition to producing mortalities in cultured products, viral infections may provide a good indication of environmental health in the natural environment. Knowledge of these agents suggests the need for increased funding opportunities, if the crab is to be cultured on an economically successful basis.

### BACTERIAL DISEASES

Bacteria are ubiquitous in the marine environment, and, not surprisingly, they are ubiquitous in the blue crab. Although most bacteria are relatively benign, several species of *Vibrio* have been implicated in several crab mortalities. Other bacteria such as *Listeria monocytogenes* and *Clostridium botulinum* represent significant concerns to food safety (Williams-Walls 1968; Rawles et al. 1995; Peterson et al. 1997). Bacterial diseases of blue crabs sparked some debate in the 1970s. At that time, the hemolymph of blue crabs was thought to be sterile like that of vertebrates and many other invertebrates (Bang 1970). Colwell et al. (1975) and Tubiash et al. (1975) found high prevalences of bacteria in the hemolymph of healthy blue crabs. Johnson (1976d), however, posited that bacterial infections were simply acquired through wounds received by the rough handling of crabs en route to markets. In 1982, Davis and Sizemore definitively showed that species of *Vibrio* were present at low to moderate levels in the hemolymph and various other tissues of freshly caught, unstressed crabs. Mean intensities were

found to range from  $10^3$  to  $10^5$  bacteria per ml, a level too low in some cases to detect with microscopy (Davis and Sizemore 1982).

Stress plays a major role in the etiology and prognosis of bacterial infections in crustaceans (see Brock and Lightner 1990). Blue crabs suffer considerable stress as a result of capture, handling, crowding, transport, temperature, wounding, and poor water quality, especially in poorly managed recirculating systems (Johnson 1976d). Given the background of normal bacterial loads in healthy crabs, some stressed hosts will develop rampant, lethal infections of species of *Vibrio* and other bacteria. Mortalities of crabs affecting the fishery are periodically reported in the spring and summer, seasons when water temperatures and handling stress can trigger outbreaks of bacterial disease.

Blue crabs have a diverse fauna of opportunistic bacterial invaders. Using an elegant numerical approach, Sizemore et al. (1975) and Colwell et al. (1975) identified several genera of bacteria from the hemolymph of market-bought, and freshly caught, "hardshell" blue crabs. The following species were cultured from *C. sapidus* in Maryland: *Aeromonas* spp., *Pseudomonas* spp., *Vibrio* spp., *Bacillus* spp., *Acinetobacter* spp., *Flavobacterium* spp., and coliform bacteria similar to *Escherichia coli*; several isolates could not be identified. Babinchack et al. (1982) isolated *Escherichia coli*, *Enterobacter aerogenes* and *Vibrio* spp. on the gills of blue crabs from South Carolina. Marshall et al. (1996) reported the presence of streptomycin-resistant *Plesiomonas shigelloides* from blue crabs from Mississippi. They implied that antibiotic resistance was due to contamination of estuarine areas by wastewaters. There are other reports, including some in the grey literature. For example, Overstreet and Rebarchik (unpublished) found 49 different bacterial isolates from blue crabs collected near Pensacola, Florida (Table 1). Forty-one of these were isolated from the hemolymph. Sterile hemolymph was noted in only 24.3% of the 111 crabs examined.

Bacterial surveys have also been undertaken in other species of blue crabs. Rivera et al. (1999) cultured 23 different bacterial isolates from the hemolymph of six specimens of *C. boucourti* from the eutrophic Mandry Channel, Puerto Rico. They

Table 1. Summary of bacterial isolates identified from hemolymph and shell samples from 111 specimens of *Callinectes sapidus* collected from Pensacola Bay, Bayou Chico, Bayou Grande, and Bayou Texar, Florida, on 15-18 August 1994 (Overstreet and Rebarchik, unpublished data). Isolates are from H (hemolymph), S (shell), or both and identified with BioLog MicroLog software version 3.2. A plus symbol (+) next to the isolate denotes the bacterium as chitinoclastic, producing chitinase.

Isolate identification	Isolate source	Isolate identification	Isolate source
<i>Aeromonas cavaie</i>	H	<i>Aeromonas hydrophila</i> +	H&S
<i>Aeromonas sobria</i> +	S	<i>Acinetobacter baumannii</i>	H&S
<i>Acinetobacter calcoaceticus</i>	H&S	<i>Acinetobacter johnsonii</i>	H&S
<i>Acinetobacter Iwoffii</i>	H	<i>Alcaligenes latus</i>	H
<i>Alcaligenes xylosoxydans</i>	S	<i>Citrobacter freundii</i>	H
<i>Clavibacter michagenese</i>	H	<i>Enterobacter aerogenes</i>	H
<i>Enterobacter agglomerans</i>	H	<i>Enterobacter cloacae</i>	H
<i>Enterobacter intermedium</i>	H	<i>Escherichia vulneris</i>	H
<i>Haemophilus parainfluenzae</i>	H	<i>Haemophilus parasuis</i>	H
<i>Haemophilus somnus</i>	H	<i>Kingella kingae</i> +	H&S
<i>Klebsiella oxytoca</i>	H	<i>Klebsiella phenmonaie</i>	H&S
<i>Klebsiella terrigena</i>	H	<i>Moraxella</i> sp.	H
<i>Pasturella</i> sp.	H	<i>Proteus mirabilis</i>	H
<i>Proteus penneri</i>	H	<i>Providencia ruttgeri</i>	S
<i>Pseudomonas</i> sp. +	H&S	<i>Psychrobacter immobilis</i>	S
<i>Salmonella</i> sp.	H	<i>Serratia marscescens</i> +	H
<i>Serratia rubidea</i>	H	<i>Shigella</i> sp.	H
<i>Shewanella putrifaciens</i>	H	<i>Vibrio anguillarum</i> +	H&S
<i>Vibrio alginolyticus</i>	H&S	<i>Vibrio cholerae</i> +	S
<i>Vibrio fluvialis</i> +	H&S	<i>Vibrio harveyii</i> +	H
<i>Vibrio mediterraneii</i> +	S	<i>Vibrio mimicus</i> +	H&S
<i>Vibrio parahaemolyticus</i> +	H&S	<i>Vibrio splendidus</i> +	H
<i>Vibrio vulnificus</i> +	H&S	<i>Xanthomonas albilineans</i>	S
<i>Xanthomonas campestris</i>	S	<i>Yersinia</i> sp.	H
Gram + <i>Bacillus</i> sp.	H&S		



found several human pathogens including *Aeromonas hydrophila*, *Pasteurella multocida*, *Pseudomonas mallei*, *P. cepacia*, *P. putrefaciens*, *Salmonella* sp., *Shigella flexeri*, *V. cholerae* and *Yersinia pseudotuberculosis*, but, surprisingly, not *V. parahaemolyticus*.

### ***Vibrio* and Related Bacterial Infections**

*Vibrio* spp. are aerobic, heterotrophic, straight- or comma-shaped, Gram-negative rods. Strains of *V. parahaemolyticus* exhibit lipase and lecithinase activity, liquefy gelatin, and hydrolyze casein (Krantz et al. 1969). Such biochemical features may aid in their invasiveness (Krantz et al. 1969). At least three other pathogens, *V. vulnificus*, *V. cholerae*, and *V. alginolyticus*, are also found in blue crabs (Colwell et al. 1975; Tubiash et al. 1975; Davis and Sizemore 1982). *Vibrio* spp. make up the largest portion of bacterial species present in blue crabs. Indeed, virtually pure cultures of *Vibrio* spp. were isolated directly from two heavily infected crabs (Davis and Sizemore 1982).

### **Biology**

*Vibrio parahaemolyticus*, *V. vulnificus*, and *V. cholerae* have been isolated from the carapace, hemolymph, and digestive tract of the blue crab (see also Table 1). *Vibrio parahaemolyticus* is the most common species of bacteria isolated from crab hemolymph (Sizemore et al. 1975; Davis and Sizemore 1982). Davis and Sizemore (1982) identified *V. parahaemolyticus*, *V. vulnificus*, and *V. cholerae* from the hemolymph of 23, 7, and 2% and externally on 8, 2 and 1.5% of 140 crabs, respectively. *Vibrio cholerae* was isolated from five crabs; none of the isolates consisted of the human pathogen O1 serovar, but non-O1 serovars can also be pathogenic (Aldova et al. 1968). *Vibrio cholerae* may represent a significant public health threat as infectious doses ( $10^4$  to  $10^8$  organisms) are possible from eating infected crabs (Davis and Sizemore 1982).

Isolations of vibrios are typically made on thio-sulfate citrate bile salts (TCBS) agar followed by culturing on selective media to confirm physiological and biochemical characteristics. The TCBS agar is highly selective for vibrios, but species in a few other

bacterial genera such as *Photobacterium* and others will grow on it. Formulations for TCBS are inexpensive and simple to prepare. The difficulty in identifying vibrios lies in the multitude of species and the large number of strains within each species. Growth characteristics on selective media, immunoprobes with various antigens, primers for polymerase chain reactions, and DNA probes have all been used to identify the multitude of species. At present, the leading method for identification uses variations in gene sequences from the small-subunit 16S ribosomal region analyzed with the maximum-likelihood and maximum-parsimony methods (e.g., Lambert et al. 1998; Farto et al. 1999). Strain variation is analyzed by ribotyping using restriction enzymes and restriction fragment length polymorphisms (Farto et al. 1999). Specific serovars of *Vibrio cholerae* are associated with disease, so ribotyping to identify the pathogenic forms is extremely important for proper diagnosis.

### **Animal Health and Fisheries Implications**

The portal of bacterial entry into the crab may be through wounding, limb autotomy, or rough handling at time of capture (Tubiash et al. 1975; Johnson 1976d). The prevalence and community characteristics of the bacterial flora on the external surfaces, however, is quite different from that reported internally (Davis and Sizemore 1982). Invasion through the stomach appeared to provide the primary avenue of entrance because the flora was more representative of that found in the hemolymph. Later, Sizemore and Davis (1985) concluded that the source of infections was from the carapace, and crabs were likely to become infected from injury or molting. Babinchak et al. (1982) equated the dark brown coloration of the gills with increased densities of *Vibrio* spp. and fecal coliform bacteria that were assumed to be acquired from the sediments. They did not examine internal infections in the crab.

Injured crabs generally demonstrate heavier infections than intact crabs. Tubiash et al. (1975) found that crabs injured during fishing had heavy infections ( $> 6,600$  MPN [most probable number],  $2.71 \times 10^3$  bacteria  $\text{ml}^{-1}$ ), while intact crabs had light

infections (4.6 - 240 MPN,  $1.67 \times 10^3$  bacteria ml<sup>-1</sup>). The difference in prevalences between injured and intact crabs was not discussed, but it was also significant (86.6% vs. 77%, Chi-square,  $P < 0.05$ ). In contrast, Davis and Sizemore (1982) reported no difference in the intensity of infection between injured and intact crabs. Welsh and Sizemore (1985), however, found no difference in prevalence but did observe a significant difference in intensity of bacterial infections between injured and intact crabs from lightly stressed and highly stressed groups. Sample size in the unstressed population was too small to assess any relationship with injury. They concluded that although injured or stressed crabs were more likely to suffer bacterial infections, bacteria were also present in the healthy population.

Crabs trapped in cages during periods of rapid salinity or other environmental changes can die from rapidly developing bacterial infections. For example, Overstreet (unpublished data) noted high levels of bacteria (predominantly *V. parahaemolyticus*) in a large number of crabs not exhibiting conspicuously high levels of shell lesions but dying in traps in an area of Mississippi Sound where salinity had recently decreased. Similarly caged crabs were not dying in nearby areas that did not experience decreased salinity.

There are few outward signs that crabs possess bacterial infections. Heavily infected crabs become abnormally weak (Krantz et al. 1969), sluggish, and moribund (Welsh and Sizemore 1985). These signs, however, are also common to infections with other disease agents (i.e., viruses, *Hematodinium perezii*, *Ameoson michaelis*, *Paramoeba perniciosus*, *Mesanothryx chesapeakeensis*). Upon dissection, moribund crabs show characteristic and extensive "anterior" acellular clots in the anterodorsal and frontal blood sinuses and incomplete clotting of the hemolymph (Johnson 1976d). "Cloudy" aggregations of hemocytes are frequently visible in the translucent regions of the gill lamellae and the 5<sup>th</sup> walking leg (Johnson 1976d). The gross signs of infection may change in the fall, presumably because of decreasing water temperatures. At that time, few crabs exhibit cellular aggregations and "anterior" clotting.

Histological observations showed a general

decline in hyalinocytes and granulocytes in infected animals (Johnson 1976d). Bacteria were observed in the hemocyte aggregations and within phagosomes of individual hyalinocytes. Declines in hemocyte density were presumably the result of cellular aggregation and infiltration. Aggregations of hemocytes occurred within 24 h in the heart, gill lamellae, antennal gland, and Y-organ (Fig. 8). Infiltration with

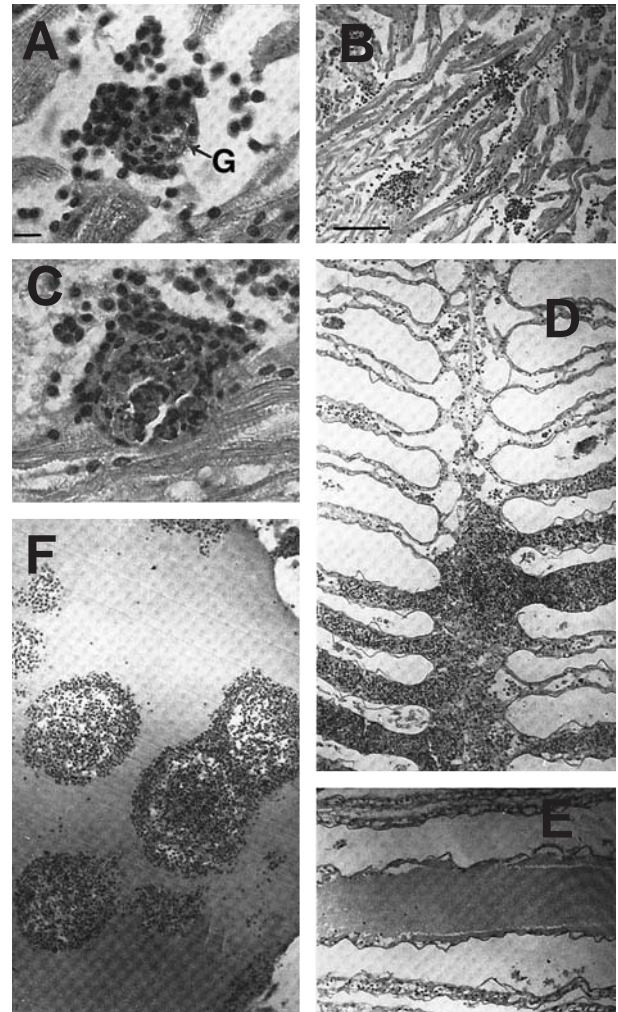


Figure 8. Systemic bacterial infection. (A-C) Granuloma-like aggregation of degenerating hemocytes in the heart of a blue crab with a bacterial infection (granulocyte, [G]). (D) Gill showing distension from hemocyte aggregations. (E) Gill lamella distended with hemolymph. (F) Large aggregates of hemocytes in clotted blood. From Johnson (1976d). Bar in (A) = 10  $\mu$ m (Figs. C, D to same scale); bar in (B) = 100  $\mu$ m (Figs. E, F to same scale).



marked encapsulation was not apparent. Thromboemboli formed as the aggregates were sloughed from the gill lamellae. Such emboli apparently caused ischemia through hemolymph stasis and further clot formation (Johnson 1976d). As the infection progressed, nodules occurred less frequently in the heart and blood sinuses, but more frequently in the other organs. Large aggregations of hemocytes embolized and led to extensive focal necrosis and degeneration in heavy infections. The hepatopancreas showed significant involvement in infected crabs. The acinar epithelium exhibited massive sloughing and few mitotic figures, while karyolysis occurred in affected cells (Johnson 1976d). Fixed hemocytes demonstrated karyorrhexis, pycnosis, and karyolysis, possibly as a result of their phagocytosis of the virulent bacteria (Johnson 1976d).

Pathological effects occur quickly in bacterial infections with mortalities occurring in as few as 2 to 3 d (Johnson 1976d). Bacterial populations are typically controlled by cellular and humoral defenses (i.e., phagocytosis, lectins, and callinectins; see Defensive Responses below), but proliferation in the hemolymph may occur quickly in relation to water temperature, handling, or other stressors (Davis and Sizemore 1982).

As expected, seasonality contributes significantly to the prevalence and intensity of bacteria in crabs (Fig. 9). In Chesapeake Bay, bacterial prevalence was 84.1% in the summer versus 77.2% in the winter, but the intensity of infection was significantly higher in spring, summer, and fall versus winter ( $2.52 \times 10^3$  vs.  $1.02 \times 10^3$  MPNs, respectively) (Tubiash et al. 1975). In Galveston Bay, the intensity of infection peaked in summer (mean intensity of  $10^6$  *Vibrio* spp. ml<sup>-1</sup>) and declined with cooler water temperatures (mean intensity of  $10^5$  *Vibrio* ml<sup>-1</sup>), but the trends were not significant (Davis and Sizemore 1982). Near Wilmington, North Carolina, bacterial infections showed strong positive correlations between mean intensity and mean water temperature in unstressed and lightly stressed crabs (Welsh and Sizemore 1985). Mean intensity and prevalence of *Vibrio* infections in highly stressed crabs were higher than in unstressed crabs and remained high throughout the different seasons. Handling stresses obscured any

seasonal trends in the stressed crabs. In unstressed conditions, *Vibrio* spp. represented 52% (winter) to 70% (summer) of the bacterial community in crabs from Galveston Bay (Davis and Sizemore 1982).

Host sex may be an important factor in bacterial infections, but the data are not conclusive. Tubiash et al. (1975) reported that infected male crabs had a mean intensity of  $2.76 \times 10^3$  MPN and females had  $1.30 \times 10^3$  MPN. Unfortunately, their analysis was flawed because male crabs sustained more injuries and were collected in the summer, while larger numbers of females were sampled in the winter when *Vibrio* infections were declining. Davis and Sizemore (1982) showed significant differences between male and female crabs, and injuries did not explain those difference. Welsh and Sizemore (1985) found no difference between sexes.

Field prevalences of *Vibrio* are high. Tubiash et al. (1975) found that 82% of 290 crabs had bacteria in the hemolymph. Heavy infections ( $>6,600$  MPN) were found in 31% of the crabs. Davis and Sizemore (1982) found a prevalence of *Vibrio* spp. of 78% in crabs that were trapped and trawled and held for less than 1 h. Colwell et al. (1975) reported mean intensities of  $10^5$  to  $10^6$  MPN ml<sup>-1</sup> in hemolymph, which is quite high compared to a mean intensity of

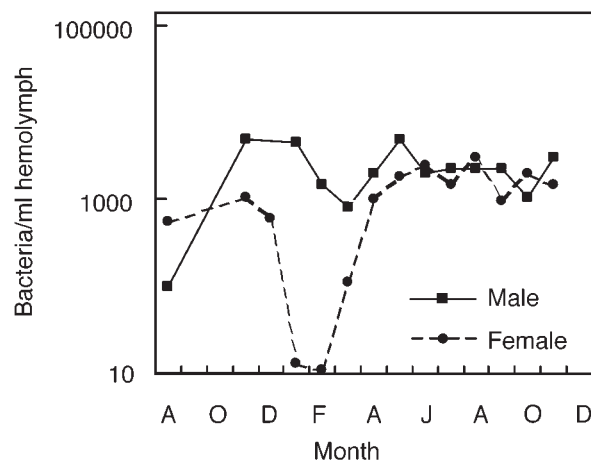


Figure 9. Intensity (log scale) of bacterial infections in the hemolymph of naturally infected blue crabs. Redrawn from Tubiash et al. (1975). Combined prevalence of bacteria in the hemolymph in winter was 69.8% versus 80.6% in summer.

$2.4 \times 10^4$  colony forming units (CFU)  $\text{ml}^{-1}$  (Davis and Sizemore 1982) and  $1.8 \times 10^3$  CFU  $\text{ml}^{-1}$  (Welsh and Sizemore 1985). Welsh and Sizemore (1985) speculated that the absence of crabs with infections greater than  $10^4$  CFU  $\text{ml}^{-1}$  might have been due to mortality or to moribund crabs not entering traps. Rivera et al. (1999), however, reported densities of  $2.9 \times 10^7$  CFU  $\text{ml}^{-1}$  in *C. boucourti*, with direct counts of  $3.53 \times 10^9$  to  $4.64 \times 10^{11}$  bacteria  $\text{ml}^{-1}$ .

Johnson (1976d) suggested that crabs acquired bacterial infections from the stress of capture and handling. Her results supported Bang's (1970) view that the hemolymph of blue crabs was sterile and that infections resulted from stress and trauma from handling. She did not, however, attempt to isolate and culture bacteria from crabs nor did she quantify infections that were observable with the light microscope (i.e., infections  $\geq 10^6$  bacteria  $\text{ml}^{-1}$ ) (Johnson et al. 1981). Microscopic and histological analyses were conducted for crabs that were roughly handled (commercially trapped, transported, and held out of water from 4 to 8 h) and for those that were trawled by research personnel (held out of water for  $< 6$  h). The commercially caught crabs suffered 80% mortality over 12 d compared to 23% for the trawled crabs. Bacterial infections were diagnosed histologically in 85% of the mortalities from commercially fished crabs versus 45% in carefully handled crabs. Mortalities declined after 9 d, but the observations may have been confounded by a significant decline in water temperature. Mortality to bacterial infections was further reduced in animals that were collected and handled gently ( $< 2$  h exposure time).

In a rigorously controlled assessment of the stress issue, Welsh and Sizemore (1985) showed that the prevalence of bacteria in the spring and summer was high (75%) in unstressed, freshly caught (pyramid traps) crabs. Lightly stressed crabs from pots (research collections using pots, with crabs held *in situ* for up to 24 h), and highly stressed crabs (purchased live from the market) showed somewhat higher prevalences (81 and 91%, respectively). The intensity of infection was significantly lower in the unstressed crabs than in both of the stressed groups, and the lightly stressed crabs had significantly lower intensities than the highly stressed group (mean

intensities of 14 vs. 19 vs. 46 CFU  $\text{ml}^{-1}$ , respectively). *Vibrio* spp. comprised mean percentages of 27, 26, and 44% of the bacterial community, respectively, with the heaviest infections represented almost solely by *Vibrio* spp. From the stressed groups, one can infer that heavy infections develop quickly from lightly infected crabs.

Bacterial mortalities are common in shedding facilities. Messick and Kennedy (1990) used a split-plot design to examine host mortality and prevalence of bacterial and viral infections in relation to the type of holding system (flow-through vs. recirculating) and crab density. Moribund crabs were examined histologically but not with isolation and culture techniques. Although there was no difference in the mean number of mortalities between systems, the total number of mortalities was higher in the recirculating systems (separately by month and in total). Most of the mortalities could be attributed to bacterial and viral infections. Mortality rates were highest in the recirculating system in June and July and declined in August. Interestingly, bacterial infections were common in crabs from the flow-through system. Messick and Kennedy's (1990) findings suggest that flow-through systems may place less stress on crabs because mortalities were lower, even though bacteria were prevalent in the systems. The study confirmed the importance of careful handling of crabs to reduce mortalities in shedding systems.

### **Public Health Implications**

Infections of *Vibrio* spp. in crabs warrant some public health concern. Raw or poorly prepared crabmeat may be contaminated with pathogenic forms. Fortunately, neither the Southeast Asian delicacy of drunken crab (live crab marinated in wine before eating) nor the habit of flavoring dishes with raw crab juices is popular in the USA. Thus, there are few cases in the USA of bacterial poisoning from eating crabs. Nonetheless, blue crabs should be cooked thoroughly and eaten immediately or stored properly before eating (Overstreet 1978). In addition, *V. cholerae* shows a predilection for chitin (Huq et al. 1983; see Pruzzo et al. 1996), and attaches to the chitin in the hindgut of blue crabs (Huq et al. 1986). Because the bacterium attaches to chitin, it



may potentially be transmitted by contaminated copepods (Huq et al. 1983, 1984; Chowdhury et al. 1997; Montanari et al. 1999). Food handlers should be aware of the potential for exposure to cholera, but at present, the possibility appears negligible in the USA (Blake et al. 1980). Other species such as *V. parahaemolyticus* are often transmitted to cooled, cooked crabs from contact with live crabs or from the juices of uncooked crabs. In any case, live or raw crabs should not be in contact with, be stored above, or otherwise contaminate cooked foods (Overstreet 1978).

### **Future Research**

Periodically the soft-shell industry experiences crab mortalities from species of *Vibrio*. Blue crab mortalities are often localized regionally and can significantly reduce the short-term production of soft-shell crabs. In most cases, poor water quality, high stocking density, and other factors, such as temperature, influence the level of stress in the host. Bacteria are ubiquitous, and stress results in an increase in bacterial intensity. Nevertheless, given the importance of crab mortalities in the soft-shell industry, it is remarkable that no one has published conclusive experiments to fulfill Koch's postulates as has been done with infections in lobster (Bowser et al. 1981). Because bacteria can be inoculated into crabs and recovered (Shields, pers. obs.) and because bacterial clearance occurs quickly in some species (White and Ratcliffe 1982; Martin et al. 1993), Koch's postulates should be relatively easy to fulfill. Control groups of uninfected crabs must be established with prior sampling. Injection studies, well-controlled mortality studies, and research to show better the associations among foci of infection, water quality, and other stressors should be considered priorities.

There is at present no therapeutic treatment for symptomatic crabs. As with many bacterial problems in aquaculture, good culture practices and handling techniques are the best prophylaxis against bacterially induced mortalities in shedding facilities. Lastly, crabs and other shellfish should be further assessed as indicators of pathogenic strains of *Vibrio* including *V. parahaemolyticus*, *V. vulnificus*, and *V. cholerae* in monitoring programs for public health. Bacterial contam-

ination of crabs and other shellfish should not be ignored.

### **Shell Disease (Chitinoclastic Bacteria)**

Shell disease is typically a non-fatal external bacterial infection of the blue crab and other crustaceans that have been subjected to stress. Injuries sustained from high stocking densities, long-term confinement, molting, and environmental pollutants have been implicated as stressors inducing shell disease in many decapods (Rosen 1967; Iversen and Beardsley 1976; Overstreet 1978; Johnson 1983; Getchell 1989; Sindermann 1989; Smolowitz et al. 1992). Chitinoclastic bacteria are a part of the normal fauna found on crustaceans. Although bacteria are clearly involved in the etiology of the disease, pollutants (i.e., sewage sludge, dredge spoils, heavy metals, organic debris) and other symbionts can play a significant role in the syndrome (Young and Pearce 1975; Couch 1983; Morado et al. 1988; Gemperline et al. 1992; Weinstein et al. 1992; Ziskowski et al. 1996; Andersen et al. 2000).

### **Biology**

Shell disease was first described from the American lobster *Homarus americanus* (see Hess 1937). A similar disease was observed in freshwater crayfish in the 1880s, but it was later determined to be a fungus (krebsspest, or burn-spot disease caused by *Aphanomyces astaci*). While fungal infections of crustaceans also cause shell lesions, few fungi have been isolated from the characteristic lesions of shell disease (Rosen 1967). Chitinoclastic bacteria are isolated by streaking infected shell onto difco-marine agar with precipitated chitin (Skerman medium) (Cook and Lofton 1973) or by swabbing the lesion with a sterile loop and inoculating into enrichment broth with chitin (Malloy 1978).

Shell disease in blue crabs is typically caused by small, chitinoclastic, gram-negative rods (Rosen 1967). The genera of bacteria have been tentatively identified as *Vibrio*, *Beneckea* (now *Vibrio*), and *Pseudomonas* (Cook and Lofton 1973). As noted in Table 1, 14 of the 49 bacteria collected from near Pensacola, Florida, produced chitinase, an enzyme

allowing the bacteria to break down the crab's chitinous exoskeleton (Overstreet and Rebarchik, unpublished). The bacteria belonged in the genera *Vibrio*, *Aeromonas*, *Pseudomonas*, *Kingella*, and *Serratia*. As in other studies, *V. parahaemolyticus* was the predominant species. *Kingella kingae*, also common in the crab and also found in the eastern oyster and shrimps in the Gulf of Mexico, is a non-motile gram-negative, straight rod that has not been regarded before now as a chitinoclastic bacterium. It is known from the human respiratory system and does not grow in media with NaCl concentrations of 4% and higher; perhaps it was introduced into the ecosystem following a heavy rainfall and is not a typical part of the normal microbial flora of the blue crab. Other bacteria (*Photobacterium* sp., *V. anguillarum*, and *Vibrio* spp.) have been isolated from lesions on *Chionoecetes bairdi* (Baross et al. 1978).

The crustacean exoskeleton is comprised of three layers: the epicuticle, the exocuticle, and the membranous layer overlying the living epidermis (Skinner 1962, 1985; Green and Neff 1972; O'Brien et al. 1991). Polyphenolic substances contained in the epicuticle provide resistance to microbial degradation (Dennell 1960), but a wide range of bacteria colonizes the surface of the epicuticle, and slow degradation of the cuticle over the molt cycle of the host may allow penetration of chitinoclastic bacteria (Baross et al. 1978). Damage to the exoskeleton provides a portal of entry for chitinoclastic bacteria (Cook and Lofton 1973; Malloy 1978), and the developing lesions represent a portal of entry and media for other infectious agents. Trauma, fungi, and other events may also effect portals of entry (Getchell 1989). In shrimp, lipolytic enzymes may initiate the lesion, with chitinase, lipase, and proteases important to lesion development (Cipriani et al. 1980). Lipases may be important in initiating invasion through the waxy epicuticle, with chitinases and proteases facilitating expansion into the chitin-rich exocuticle and membranous layer.

### ***Animal Health and Fisheries Implications***

Early stages of shell disease initiate as numerous, small, brown puncture- or crater-like marks on the

ventral carapace (sternum) or legs (Rosen 1967; Johnson 1983). As the epicuticle of the carapace is disrupted, the exposed chitin is infected by chitinoclastic bacteria (Figs. 10-12). The condition coalesces in the later stages to form broad, irregular lesions with deep necrotic centers (Rosen 1967) that may or may not penetrate through the shell (Overstreet 1978; Johnson 1983; Noga et al. 2000). Lesions tend to spread along the integument rather than through it. Affected areas are friable and turn black

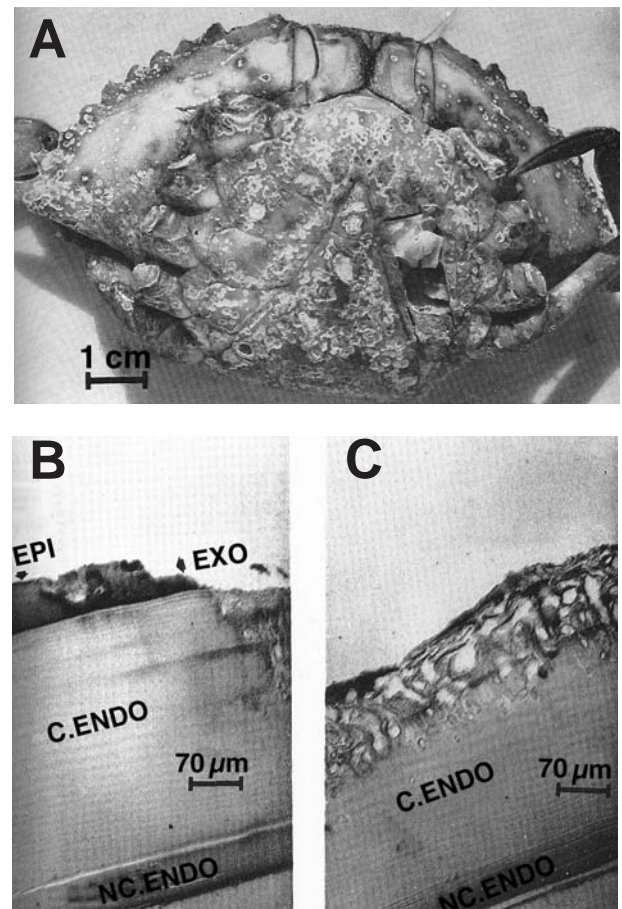


Figure 10. Bacterial shell disease. (A) Advanced case. (B) Section through cuticle of a blue crab showing mild erosion of the epicuticle (epi) and exocuticle (exo). (C) Section through a late stage of shell disease showing necrosis of the epicuticle and exocuticle and lack of penetration into the endocuticle (endo). [C. ENDO] = calcified endocuticle, [NC. ENDO] = noncalcified endocuticle. From Rosen (1967).





Figure 11. Shell disease in exoskeleton of claw of crab in Mississippi. From Overstreet (1978).

or blue from melanin deposition (Johnson 1983). Rosen (1970) viewed the necrotic pits as miniature communities of bacterial colonizers, including chitinoclastic and non-chitinoclastic forms.

In advanced cases, the lesion penetrates into the noncalcified membranous layer, and limbs and spines may become necrotic and are lost (Rosen 1967). The gills can also be attacked (Johnson 1983). Infected American lobsters show varying stages of host response ranging from cellular infiltration, epicuticle deposition, and melanization to pseudomembrane formation (Smolowitz et al. 1992). Lightly and moderately infected individuals can overcome the disease by molting (Rosen 1967), but the area of the lesions may not reflect the severity of the disease (Noga et al. 2000). Newly molted crabs are usually free of shell disease, but in advanced cases, the new instar dies from an inability to cast off the old molt (Sandifer and Eldridge 1974; Fisher et al. 1976; Overstreet 1978; Johnson 1983). Older blue crabs, which molt less frequently, are most affected by the disease (Sandifer and Eldridge 1974). Heavily infected crabs are lethargic, weak, and die when stressed.

Cook and Lofton (1973) inoculated cultures of bacteria directly onto sterile, rasped, or scraped surfaces of crab exoskeletons. After a few weeks, shell necrosis was observed on all of the rasped surfaces. Inoculated but undamaged areas did not obtain dis-

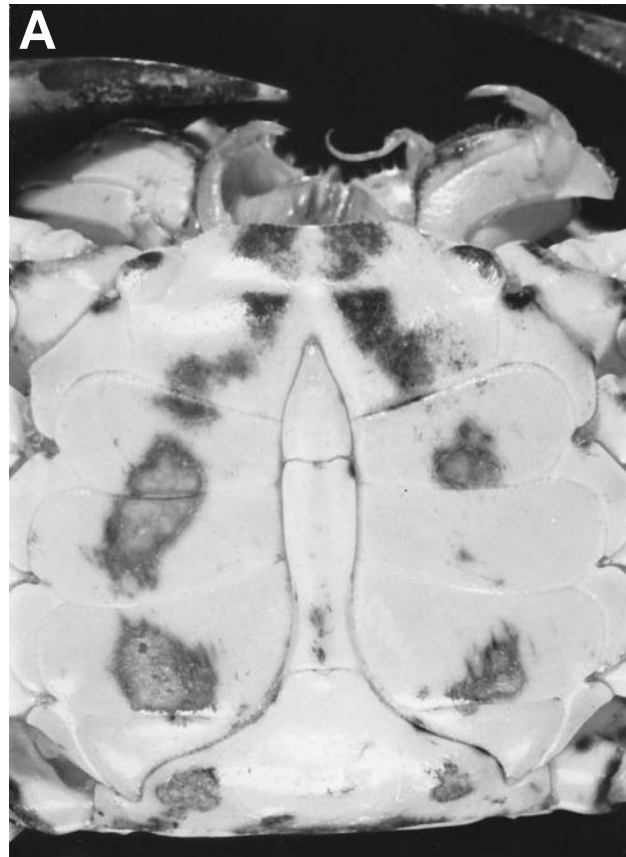


Figure 12. Shell disease on underside of male crabs in Mississippi, not indicative of wounds. (A) early lesions. (B) More advanced lesions. From Messick and Sindermann (1992).

ease. McKenna et al. (1990) undertook sentinel studies with "rasped" versus control crabs in "high" and "low" risk areas (with risk based on prevalence of shell disease). Blue crabs in the high-risk areas took approximately 4 d to develop early lesions with all of the rasped crabs (n=20) exhibiting lesions after 10 d. Those from low risk areas took approximately 8 d to develop lesions with 80% (n=20) exhibiting lesions after 21 d. Interestingly, only one crab developed severe shell disease and only after 30 d. None of the unrased control animals developed lesions.

Shell disease typically indicates a significant problem with water quality. Several studies on blue crabs have focused on the high prevalence of shell disease in the Pamlico River, North Carolina. The river has experienced significant deterioration in water quality with freshwater runoff, erosion, sedimentation, nutrients (primarily phosphate), heavy metals, salinity, and low dissolved oxygen (Rader et al. 1987). McKenna et al. (1990) suggested that cadmium and fluorine may interfere with calcium deposition and thus interfere with shell synthesis. Gemperline et al. (1992) and Weinstein et al. (1992) found a relationship between the presence of several heavy metals and shell disease in crabs from the Pamlico River. Compared with levels in crabs from outside the area, levels of aluminum, arsenic, cadmium, manganese, tin, and vanadium were higher in the gill tissues while aluminum, manganese, and vanadium were higher in hepatopancreas and muscle. Since calcium deposition is driven by cationic shifts in pH (Cameron 1985b), heavy metals likely could interfere with the deposition process and, thus, render the carapace susceptible to invasion.

Alternatively, Noga et al. (1994, 2000) found that shell disease was significantly correlated with a decline in the antibacterial activity of serum in crabs from polluted sites. Crabs from polluted sites had less activity than crabs from relatively pristine sites. Additionally, at polluted sites, crabs with shell disease had lower serum activity than those without lesions. They speculated that shell disease was correlated with declines in immune function. The antibacterial activity was recently identified as being due to a polypeptide, callinectin (Khoo et al. 1996), with specific activity against species of *Vibrio* and other Gram

negative bacteria isolated from the blue crab (Noga et al. 1996). In contrast, Engel et al. (1993) found that hemocyanin, the primary serum protein in blue crabs, was significantly depressed at several sites on the Neuse and Pamlico rivers, North Carolina. The depressed hemocyanin levels indicate that afflicted crabs may be compromised in several ways. Engel et al. (1993) suggested that hemocyanin level may be a useful marker in identifying or monitoring polluted or otherwise impacted sites. Burkholder et al. (1995) suggested that toxins produced by the dinoflagellate *Pfiesteria piscicida* caused the shell lesions observed on blue crabs from these rivers. No experimental evidence, however, was presented on the possible link in etiologies nor was the association with pollution and shell disease presented.

Overstreet and Rebarchik (unpublished) investigated the presence and degree of shell disease in Pensacola Bay, Florida, and three variously contaminated nearby bayous. The presence of lesions appeared to serve as an indication of environmental health. In contrast, their incomplete data showed no correlation between chitinoclastic bacteria in the hemolymph and degree of exoskeletal lesions. Considering crabs with lesions, they identified chitinoclastic bacteria from 46, but 41 others had no identified chitinoclastic bacteria. Shell disease was common in crabs from all four localities, with the highest prevalence and the greatest number of lesions exhibiting moderate to heavy intensities at Bayou Chico. Several crabs from Pensacola Bay exhibited a heavy degree of lesions, but these consisted mostly of females in anecdyosis, some of which had probably migrated recently from Bayou Chico, in relatively close proximity to Pensacola Bay. The presence of lesions in adults was higher in females that had ceased molting than in males that continued to molt. No specific chitinoclastic bacterium was linked to environmental condition, and none was linked to fecal coliform pollution. All corresponding water samples had enterocci, *E. coli*, and fecal coliforms within the Environmental Protection Agency's acceptable limits, except for one from Bayou Chico and all from Bayou Texar. Total numbers of *Vibrio* spp. were highest in Bayou Grande and lowest in Bayou Texar.



The benthic life style of the blue crab contributes to the transmission of shell disease. Sediments foster high densities of chitinoclastic bacteria (Seki 1965; Cook and Lofton 1973; Hood and Meyers 1977); hence, burying activity places crabs in direct contact with the highest densities of chitinolytic forms. Vogan et al. (1999) found a higher prevalence of lesions on the posterodorsal carapace, and the ventral surfaces of the legs of *Cancer pagurus*. They suggested that lesions develop from sand abrasion with subsequent infection resulting from the burying activity of the crab. Young and Pearce (1975) showed that lesions developed in lobsters held in aquaria containing sludge from a dumpsite. McKenna et al. (1990) found that lesions on blue crabs were more frequent on the anterodorsal carapace and on the posterodorsal carapace than elsewhere on the carapace or limbs, but they did not record lesions on the sternum, one of the more common areas for initial infections (Rosen 1967; Iversen and Beardsley 1976; Overstreet 1978; Johnson 1983; Getchell 1989). Dredged crabs from Chesapeake Bay exhibit the characteristic pinpoint lesions on the sterna and limbs in late winter presumably from their residence in the sediments (Shields, pers. obs.). Although Hood and Meyers (1974) found peak populations of chitinoclastic bacteria in the spring and summer, shell disease in blue crabs was highest in fall and winter (Sandifer and Eldridge 1974). Thus, shell disease on blue crabs arises from abrasions acquired from their burying activities, from other wounds to the epicuticle, and from stress due to poor water quality.

Shell disease is contagious, especially in long-term, crowded conditions such as those found at American lobster holding facilities (Rosen 1970; Sandifer and Eldridge 1974). Mortality can be high in lobster facilities, but less so with blue crabs where culture conditions are generally of short duration (e.g., soft-shell production). Brock and Lightner (1990) note that shell disease is often associated with stress and that the underlying causes of stress must be determined in a differential diagnosis. Wound avoidance, proper attention to hygiene, and proper husbandry lessen the prevalence of shell disease in lobster culture systems (Stewart 1980).

Heavily diseased animals can be difficult to treat. Dips of malachite green have been used for lobsters (Fisher et al. 1978), and antibiotic baths (penicillin-streptomycin, furanace, erythromycin, oxolinic acid) and malachite green and formalin have been used for shrimp (Tareen 1982; Brock 1983; El-Gamal et al. 1986). Disinfection of aquaria can be achieved with bleach solutions. Advanced cases should be destroyed to prevent further spread and to avoid excessive trauma.

In general, shell disease is not a significant factor in mortality of wild stocks of crustaceans. High prevalences of it, however, may indicate significant issues involving water quality or stress in resident crustacean populations. Shell disease does have a small economic impact in that afflicted animals are not aesthetically pleasing to eat; thus there may be a lower grading of meat value (Rosen 1970; Getchell 1989). Because blue crabs are not held for long periods, shell disease in culture systems is not a significant issue. Rosen (1967) recorded 3% prevalence in three shedding houses in Maryland. Sandifer and Eldridge (1974) reported monthly prevalences in field and commercial samples of 15,000 crabs collected from four locations in South Carolina. Prevalences ranged from 0.0 to 53.1%, with field samples having a higher overall prevalence (9.2%) compared with that in commercial samples (3.4%). Males were slightly more susceptible than females, but the data were not consistent. McKenna et al. (1990) did an extensive survey of shell disease in the Pamlico River (trawl, pot, and sentinel studies). In July 1987, 5% of 1459 trawled crabs had shell disease. Males had a prevalence of 5.1%, females, 16.2%, and immature females, 2.5%. Although more crabs were found in shallower waters, there was no association between shell disease and depth (5.1 vs. 4.4% prevalence, 0-1.82 m vs. 1.83-3.65 m depth, respectively, Chi-square).

### **Future Research**

Shell disease is relatively innocuous in blue crabs. However, significant research questions can be addressed through study of the disease. Stress is a significant issue in the onset of numerous diseases and

conditions in invertebrates. Further investigation of the association between different stressors and the onset of shell disease will provide a useful model to study the effects of stress on crustaceans and other invertebrates. Indeed, the decrease in immune function and decline in hemocyanin in diseased crabs from polluted waters (e.g., Engel et al. 1993; Noga et al. 1994, 1996) highlights the need for just such studies. In addition, the prevalence of shell disease may be an excellent indicator of water quality (e.g., pollution, nutrient enrichment). Thus, monitoring for shell disease may provide an inexpensive early warning tool for pollutants or other stressors.

### Other Bacteria

Other bacterial infections have been reported from the blue crab. A rare, Gram-negative bacterium has been observed in the midgut and hepatopancreas (Johnson 1983). It was associated with focal necrosis of the hepatic tubules. An unusual filamentous, non-septate Gram-negative bacterium ("strand-like" organism - Johnson 1983; Messick 1998) was also observed attached to the tubules of the hepatopancreas. It was reported from 2% of crabs from the Atlantic and Gulf coasts (Johnson 1983). The prevalence of the bacterium was lower in flow-through (12%) versus recirculating seawater (31%) systems in the summer (Messick and Kennedy 1990). The bacterium exhibited a peak in prevalence in the summer (up to 16% in Maryland), and significantly higher prevalences in juveniles than adults (Messick 1998). The localized effect of the bacterium included subtle changes in the epithelial cells with the formation of syncytia. The strand-like organisms are of doubtful pathogenicity to their crab host as the epithelial cells of the hepatopancreas are being replaced continuously (Johnson 1983; Messick 1998).

Rickettsiales-like organisms (RLOs) are typically considered obligate intracellular bacterial parasites. They are rarely reported from crustaceans. Mass mortalities of shrimp, however, have been associated with RLOs (Krol et al. 1991; Lightner et al. 1992; Loy et al. 1996). In the blue crab, the prevalence of

RLOs from field samples is low, probably because of the few histological analyses available. Rickettsiales-like organisms had a prevalence of 2.3% in a Maryland shedding facility, but heavy infections were not fatal (Messick and Kennedy 1990). In a later study, a single infected crab exhibited a focal infection of the hepatopancreas, with moderate increases in hemocyte numbers in the adjacent hemal spaces (Messick 1998). Given the emerging importance and abundance of RLOs in invertebrates, especially molluscs, it is surprising that so few have been described in Crustacea.

*Leucothrix mucor* is an ubiquitous filamentous bacterium found on the external surfaces, gills, and eggs of crabs, algae, and various other surfaces (Johnson et al. 1971; Bland and Brock 1973). It is common in the egg masses and occasionally on the gills of the blue crab (Bland and Amerson 1974; Shields, pers. obs.). Bacterial fouling of the egg masses occurs with several crustaceans. *Leucothrix mucor* was suspected of contributing to egg mortalities in *Cancer* spp. (Fisher et al. 1976), but its contribution to mortalities was negligible; rather, nemertean worms were shown to cause significantly more egg mortality through predation (Shields and Kuris 1988a). The presence of many strains of *L. mucor* and related species typically develop on hosts in the presence of excess nutrients, such as in natural areas contaminated with domestic sewage or in overfed aquaculture facilities. Solangi et al. (1979) tested a variety of treatments on infestations on the brine shrimp. Bacterial mats and associated debris sloughed as a single unit, most effectively with 100 ppm Terramycin® (an oxytetracycline formulation). In the field, some crustaceans preen themselves of infestations with their third maxillipeds and, thus, control their infestations (Bauer 1979).

Lastly, bdellovibrios occur on the external surfaces of the carapace and gills of the blue crab (Kelley and Williams 1992). Bdellovibrios are predatory bacteria that are ubiquitous in the marine environment. They are not pathogenic and apparently feed on other bacteria. They should be considered fouling organisms.



## FUNGI

Historically, the oomycetes (water molds) were described as fungi. With the advent of TEM and advanced molecular techniques, they have been removed from the fungi and placed in their own phylum. For the sake of simplicity, we refer to the oomycetes and other “lower fungi” as fungi. Fungal infections in crustaceans range from the benign to the severe. Epizootic outbreaks have affected several crustaceans including copepods, crayfishes, tanner crab, penaeid shrimps, and American lobster. One of the most notorious and disastrous is krebsspest, *Aphanomyces astaci*. Introduced into Europe with the American signal crayfish *Pacifastacus leniusculus* in the 1880s, it has since wiped out most stocks of the European crayfish *Astacus astacus* (Unestam 1973). Fungal diseases in crustaceans have been reviewed by Unestam (1973), Alderman (1976), Lightner (1981), and Johnson (1983). In aquaculture, infections of *Lagenidium callinectes*, *Haliphthoros milfordensis*, and *Fusarium solani* are relatively common in penaeid shrimp and American lobster embryos, imposing significant threats to the culture of these crustaceans (Lightner 1981). The embryos of the blue crab can be experimentally infected with *H. milfordensis*, but natural infections have not been reported in that host (Tharp and Bland 1977).

### *Lagenidium callinectes*

Fortunately, there are few serious fungal infections reported from the blue crab. A significant pathogen, *Lagenidium callinectes* was first isolated from the embryos of the blue crab (Couch 1942). It has since been reported from the embryos and larvae of several decapod crustaceans and algae.

### Biology

*Lagenidium callinectes* is a holocarpic oomycete fungus-like protist that attacks and kills crab embryos. The fungal thallus consists of coenocytic, intramatrical hyphae within a crab embryo, and extramatrical hyphae that function in sporogenesis and spore discharge (Fig. 13). The life cycle of *L. callinectes* was elucidated by Bland and Amerson (1973)

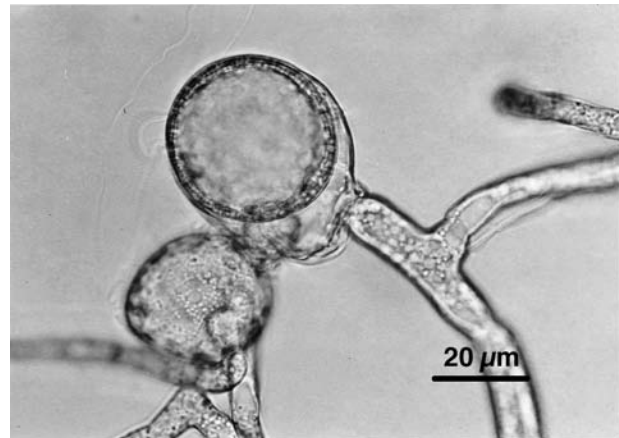


Figure 13. *Lagenidium callinectes*. Sporangial development in a modified Vishniac medium.

with additional work on an algal isolate by Gotelli (1974a, b). Sporangia start discharging spores after 12 to 15 h in sterile sea water, with continued release extending over 48 h. The pyriform zoospores, 10 by 13  $\mu\text{m}$ , have two flagella arising from a groove that spans the length of the spore. Polycomplexes indicative of meiosis have been observed in the encysting spores (Amerson and Bland 1973). Cysts, 9 to 11  $\mu\text{m}$  in diameter, germinate a single germ tube that penetrates the egg and grows rapidly into vegetative hyphae that in turn ramify throughout the embryo. Young hyphae have few septa. Septa usually delimit sporangia or separate older sections of the hyphae (Gotelli 1974b).

Upon death of the embryo, several holocarpic sporangia form at the end of discharge tubes (Fig. 14) and release monoplanetic zoospores. Sporogenesis begins at the tips of the extramatrical hyphae with the formation of septa to differentiate the sporangium (Gotelli 1974a). After the discharge tube develops, cytoplasm is discharged (5-30 min) into a gelatinous vesicle. Flagellar formation precedes cleavage and the flagella can be observed actively beating inside the sporangium (Gotelli 1974a). Cleavage is rapid and spore release occurs within 10 min of sporogenesis. From 20 to 200 zoospores are produced by a single sporangium. Zoospores swim to, encyst on, and infect new embryos, but extramatrical hyphae also grow into and infect new embryos.

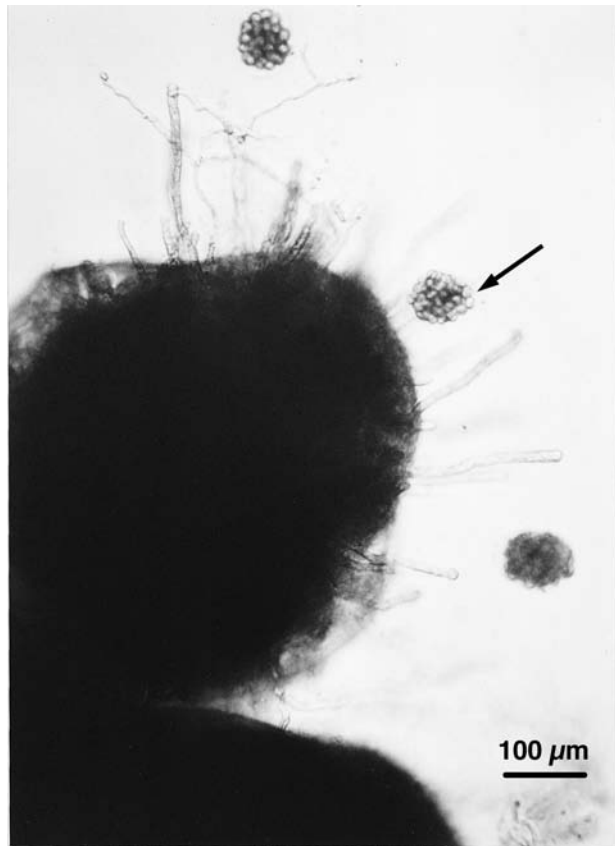


Figure 14. *Lagenidium callinectes*. Dead crab egg showing extramatrical hyphae with sporangia containing developing zoospores (arrow). Note the opacity of the egg.

Several strains of *L. callinectes* have been isolated from decapod embryos and algae. Couch (1942) described one with short extramatrical hyphae, and Bland and Amerson (1973) described another with elongate extramatrical hyphae. Bahnweg and Bland (1980) indicated the need for a review of the taxonomy of the group based on biochemical attributes between different isolates. In a statistical analysis using morphological and physiological parameters, Crisp et al. (1989) showed that strain differences are part of intraspecific variation. Molecular taxonomy would no doubt improve our understanding of this taxon.

Nutrient requirements vary significantly among strains of *Lagenidium callinectes*. Fungal growth is rapid, with hyphal tips recognizable after 12 h in seawater PYG-agar (Bland and Amerson 1973). An

algal strain was grown on a defined medium containing glutamate, glucose, vitamin B constituents, and trace metal mix (Gotelli 1974a,b). Vitamin B<sub>1</sub> was required for the isolate from the blue crab (L-1), and cultures grew better on simple sugars (e.g., fructose, glucose) than on complex carbohydrates and polysaccharides (Bahnweg and Bland 1980). Most strains are obligate marine forms, but isolates from the American lobster and the Dungeness crab *Cancer magister* do not require NaCl (Bahnweg and Gotelli 1980). Isolate L-1 is strongly proteolytic, and chitin is not used for growth (Bahnweg and Bland 1980). Chitin is not present in hyphal walls, but beta-glucans and both 1-3 and 1-6 glucosamines are abundant (Bertke and Aronson 1992). The nitrogen sources and simple energy requirements of *L. callinectes* appear representative of a marine saprophyte, not a "fastidious" parasite (Bahnweg and Bland 1980); this is not surprising when one considers the rich "medium" of an undifferentiated crab embryo.

### ***Animal Health and Fisheries Implications***

In the blue crab, *Lagenidium callinectes* naturally infects embryos. It has not been observed in zoeae, albeit experimentally infected larvae quickly lose the ability to swim; hence, larvae sink and their mortality may be difficult to document in the field (Rogers-Talbert 1948). The presence of the fungus can be observed as brown or gray patches in the clutch. Dead eggs are opaque and smaller than healthy ones (Couch 1942; Rogers-Talbert 1948). The fungus is usually restricted to the periphery of the clutch, penetrating rarely more than 3 mm into it (Fig. 15). Diseased eggs die before hatching. In heavy infections, the fungus may kill approximately 25% of the clutch. Older clutches are attacked more heavily than recently laid ones (Fig. 16).

Transmission to new hosts is rapid. Zoeae of *Cancer magister* acquire infections within 48 h of molting, and uninfected females can develop infections within 2 to 3 d of being housed with infected females or exposed to water that contains infected clutches (Armstrong et al. 1976). Infections establish in the visceral organs of the larvae and then ramify throughout the body and tissues (Armstrong et al.



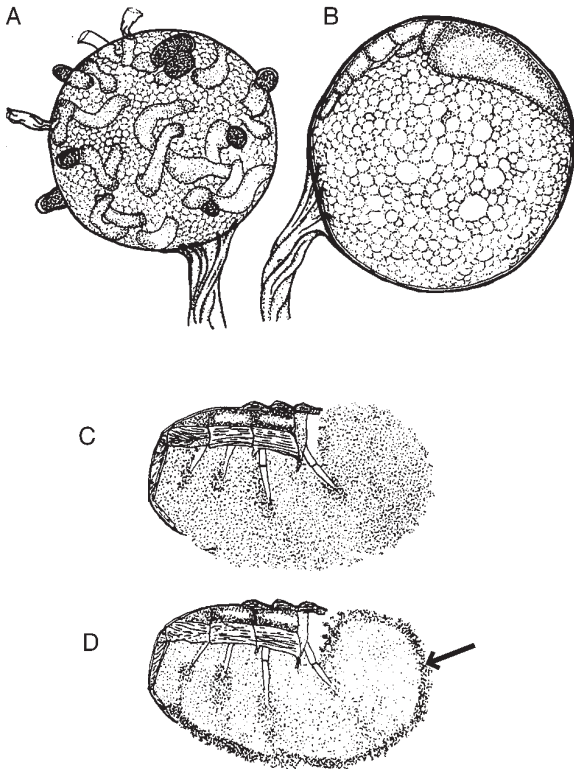


Figure 15. (A) Dead blue crab egg with fungal hyphae. (B) Live crab egg for comparison. (C) Representative cross-section through a healthy clutch of a blue crab. (D) Cross-section through a clutch infected with *Lagenidium callinectes* showing the peripheral location (arrow) of most infected eggs. From Rogers-Talbert (1948).

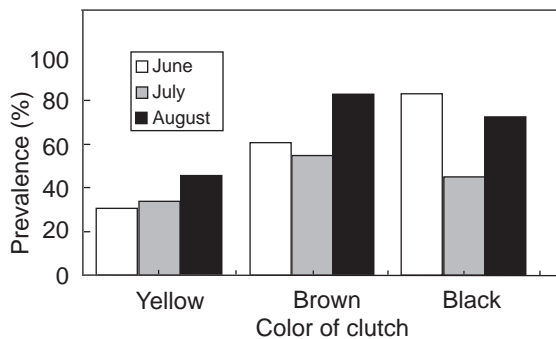


Figure 16. Prevalence of *Lagenidium callinectes* in clutches of blue crabs during summer months. Yellow (early), brown (mid) and black (late) refer to the relative age of the clutch. Data from Rogers-Talbert (1948).

1976). Zoal stages may become infected during the molt (Armstrong et al. 1976), but because the germination tube can penetrate the chorion of the egg, the tube can probably penetrate through the thin cuticle of the larva. In larval specimens of *C. magister*, the fungus was highly pathogenic, with 40% mortality over 7 d. Transmission can be difficult to control and may involve other crustaceans such as brine shrimp that are often used as food (Ho and Lightner, personal communication in Armstrong et al. 1976).

Ovigerous blue crabs experience a high prevalence of the fungus throughout the summer in Chesapeake Bay (Fig. 16) (Rogers-Talbert 1948). There is an apparent lag period in May when crabs are not infected. In North Carolina, the fungus has a high prevalence from May through June (95%) with prevalence in July (after the main ovigerous period) dropping to 30%, and to zero thereafter (Bland and Amerson 1973, 1974). Anecdotally, the prevalence of the fungus increased with the density of female crabs (Bland 1974).

Although the fungus has a wide salinity tolerance, in Chesapeake Bay it is primarily restricted to the lower regions of the Bay where salinity is high (Fig. 17; Rogers-Talbert 1948; Bahnweg and Bland 1980). In the 1940s, the fungus occurred at high prevalences (40-62%) in the Virginia Marine Resources Commission's Crab Sanctuary (Rogers-Talbert 1948) and was moderate to high in York River, Virginia, in the 1960s (Scott 1962).

Natural infections have been reported from the embryos of the blue crab (Sandoz et al. 1944; Rogers-Talbert 1948), the barnacle *Chelonibia patula* (Johnson and Bonner 1960), the zoeae of *Cancer magister* (Armstrong et al. 1976), and algae (Fuller et al. 1964; Gotelli 1974a,b). The embryos of the crabs *Dyspanopeus texana*, *Panopeus herbstii*, and *Pinnothereos ostreum* but not those of *Libinia emarginata*, *Menippe mercenaria*, or *Sesarma cinereum* are susceptible to infection (Rogers-Talbert 1948; Bland and Amerson 1974). The reason for such differences in susceptibility has not been resolved, though the depth of the chorion or its resiliency may be factors (but see below).

In an elegant study of bacterial and fungal pathogenesis, Fisher (1983) showed that *L. callinectes*

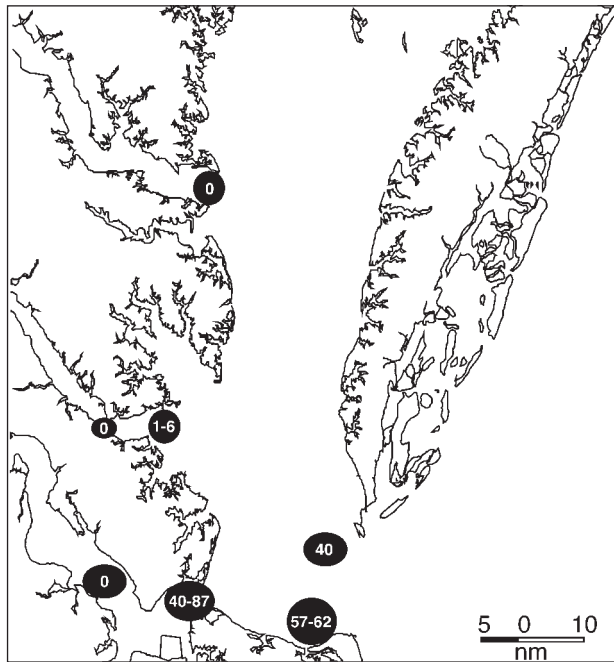


Figure 17. Prevalence (% , numbers in circles) of *Lagenidium callinectes* in portions of lower Chesapeake Bay during 1946. From Rogers-Talbert (1948). The fungus is still quite prevalent in the clutches of blue crabs from the region (Shields, pers. obs.). nm = nautical miles.

grew only on the detached eggs of the oriental shrimp *Palaemon macrodactylus* except when the first pleopods had been excised. Caridean shrimp, including *P. macrodactylus*, are excellent groomers; they may limit the spread of egg diseases by periodic preening of the clutch (Bauer 1979, 1981, 1998, 1999). Nonetheless, the fungus presents a significant problem to crustacean aquaculture (Lightner and Fontaine 1973; Nilson et al. 1975).

Curiously, the embryos of the oriental shrimp and the American lobster exhibit some resistance to infection by *L. callinectes* (Gil-Turnes et al. 1989; Gil-Turnes and Fenical 1992). Gram-negative bacteria have been isolated from these embryos and the presence of penicillin-sensitive bacteria is correlated with resistance. On *P. macrodactylus*, the bacterium *Alteromonas* sp. attaches to the outer chorionic coat of the embryo and releases 2,3 indolinedione, or isatin, and on lobster, a similar Gram-negative bacterium releases 4-hydroxyphenethyl alcohol, or tyrosol; both are known antifungal compounds (Gil-

Turnes et al. 1989; Gil-Turnes and Fenical 1992). The presence of the 2,3 indolinedione alone was sufficient to inhibit mortality in embryos exposed to the fungus. We speculate that poor water conditions may limit bacterial protection of the embryos in aquaculture systems.

Presumably several different fungi infect the blue crab and its eggs, but few, if any, recent studies have explored the possibility. *Haliphthoros millfordensis*, a phycomycete that infects the eggs of several decapods and a gastropod and that appears grossly similar to *L. callinectes*, can infect blue crab eggs experimentally; however, it has not been found in naturally occurring infections (Fisher et al. 1976, 1978; Tharp and Bland 1977).

### Future Research

*Lagenidium callinectes* may very well represent the greatest fungal threat to the successful culture of several marine decapods. Hence, various fungicides have been examined for use in shrimp aquaculture. Early studies that reported on the efficacy of malachite green in shrimp culture used reduced zoopore motility as the primary measure (Bland et al. 1976). Trifluralin and captan showed efficacy (as reductions in mortalities) over 96-h exposures with minimal larval mortalities (Armstrong et al. 1976). Benomyl was toxic to larvae in 96-h exposures but showed some efficacy over 48 h. Furanace showed excellent efficacy against *H. millfordensis* in shrimp aquaculture (Lio-Po et al. 1985). None of these compounds is currently available for use in USA aquaculture.

*Lagenidium callinectes* is often found in association with a filamentous bacterium and the nemertean worm *Carcinonemertes carcinophila* (see Rogers-Talbert 1948; Bland and Amerson 1973). The bacterium was thought to be *Chlamydobacterium*, but it is more likely the common filamentous *Leucothrix mucor* (see Bland and Amerson 1974; Shields, pers. obs.). *Leucothrix mucor* is a common constituent on the surface of crab eggs (Johnson et al. 1971). It was at one time implicated in egg mortalities in *Cancer magister* (see Fisher 1976; Fisher and Wickham 1976). Although the bacterium may be a significant fouling organism, at moderate densities it was not a causal factor in egg mortality involving the yellow



rock crab *Cancer anthonyi* (see Shields and Kuris 1988a). The cumulative effect of bacterial and fungal agents and predatory worms may cause significant mortality to the clutches of crabs (Wickham 1986; Shields and Kuris 1988a; Kuris et al. 1991). One can speculate on the adaptive significance of egg predation by the worm inside the clutch versus fungal mortality (and abrasion) on the periphery.

### Other Fungi

*Leptolegnia marina* is a saprolegnid oomycete that was first reported in ova, embryos, and body organs of the pea crab *Pinnotheres pisum* by Atkins (1954). While seemingly rare, it has been reported from a number of invertebrate eggs, including those of other crabs *Pinnotheres pisum*, *P. pinnotheres*, and *Callinectes sapidus* and the bivalves *Barnea candida* and *Cardium echinatum* (Atkins 1954; Johnson and Pinschmidt 1963). It or a related species also occurs as a facultative parasite on cultured salmonid eggs (Kitancharoen et al. 1997). The fungus has profusely branched, intramatrical and extramatrical hyphae, but often the latter appear as the "apical portions of the sporangia" (Johnson and Pinschmidt 1963). Sporangia are similar to hyphae in appearance with an apical or lateral discharge pore. Spores form in two to three rows within a sporangium. The diplanetic, biflagellate spores encyst and excyst as reniform biflagellate spores 8 to 14  $\mu\text{m}$  long by 3 to 4  $\mu\text{m}$  wide. Oogonia are 6 to 40  $\mu\text{m}$  in breadth, generally with hypogynous antheridia or lacking antheridia, with potential oospores developing in the form of a large oogonial mass. The saprolegnids on salmonid eggs have been successfully treated with ozone (Benoit and Matlin 1966).

Another fungus-like protist, a thraustochytrid, also occurs on the egg masses of *C. sapidus* (Rogers-Talbert 1948). Most thraustochytrids are saprophytic, but some are serious pathogens to molluscs (QPX) (Maas et al. 1997) and sea grasses (wasting disease) (Short et al. 1987). In the milieu of the egg mass, several fungus-like protists, including thraustochytrids and oomycetes, are attracted to dying and dead eggs, which make an excellent medium for benthic saprobes (Shields 1990).

Yeast infections are rare in crustaceans. None has been reported from *C. sapidus*. However, yeast infections have been observed in the hemolymph of blue crabs from Virginia and Mississippi (Overstreet and Shields, pers. obs.), but their prevalence and role in disease in nature have not been investigated.

## PROTOZOANS

### Microspora

The blue crab hosts a variety of microsporidians that are in many cases destructive to the crab. Microspora is a phylum containing strictly intracellular parasitic species that produce small (usually < 6  $\mu\text{m}$ ) unicellular spores with an imperforate wall. The spores lack mitochondria, but contain a sporoplasm and a hatching apparatus, including an extrusible hollow polar tube that injects the sporoplasm into the host cell. In species of many genera, including "*Pleistophora*" and *Thelohania* parasitic in crabs, spores develop inside a membranous structure termed the sporophorous vesicle (SPV, previously termed pansporoblast). In members of other genera, such as *Ameeson*, the spores do not develop in an SPV. The phylogenetic position of the phylum is uncertain (Müller 1997). It has been considered by some as one of the earliest diverging eukaryotes, related to blue-green algae (e.g., Sogin et al. 1989). In contrast, other evidence involving the loss of mitochondria (Germot et al. 1997; Hirt et al. 1997) and the presence of cytokeratin filaments and desmosomal analogues suggests the group may be more recently derived, at least in the sense of acquiring the latter features from a vertebrate host (Weidner et al. 1990). Several recent studies also have suggested a close relationship between Microspora and fungi (e.g., Keeling and McFadden 1998). All microsporidians are parasitic, with some infecting vertebrates and others infecting invertebrates; some have a direct life cycle not requiring an intermediate or additional host, but others need another host or life stage. The life cycle for most species is not known. Whereas morphological features seen under the light microscope are still useful to identify and classify species, other characteristics involving developmental, ultrastructural,

biochemical, and molecular features are now being used in the systematics of the phylum and are continually being updated (e.g., Larsson 1986, 1999; Sprague et al. 1992; Pomport-Castillon et al. 1997).

### Biology

Most well-known and probably most common of the species infecting the blue crab is *Ameson michaelis* (Fig. 18), previously known as *Nosema michaelis* (see Sprague 1965, 1970). It will serve as a representative for this group of parasites (Fig. 19), but other known species will be discussed. The life cycle of *A. michaelis* was first described by Weidner (1970), who demonstrated a direct cycle not requiring another host. The fresh spore is relatively small and ovoid; its size varies. For example, in crabs from Maryland, spores measured 2.2  $\mu\text{m}$  long by 1.7  $\mu\text{m}$  wide, with a polar tube about 40  $\mu\text{m}$  long (Sprague 1977). In crabs from Louisiana, spores were 1.2 to 3.5  $\mu\text{m}$  long by 0.9 to 2.0  $\mu\text{m}$  wide, averaging 1.3 by 1.0  $\mu\text{m}$  (Weidner 1970). In Louisiana and Mississippi, spores averaged 1.9 by 1.5  $\mu\text{m}$  (Overstreet 1988). When infected crab tissues or spores are ingested by an uninfected crab, the spore everts its coiled polar tube by means of its lamellar polaroplast and rapidly injects the sporoplasm into an epithelial

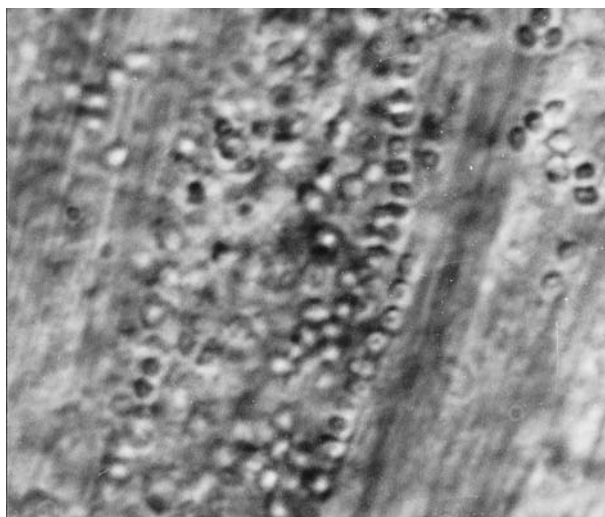


Figure 18. Oil emersion micrograph of fresh skeletal muscle tissue exhibiting the microsporidian *Ameson michaelis* in Louisiana. From Overstreet (1978).

cell lining the lumen of the mid-gut. The vegetative cell then invades, develops, and multiplies in the hemocytes in the adjacent submucosal connective tissue of the midgut. When the hemocytes reach skeletal muscles, the parasite in these cells undergoes further development in the myofibrils, first forming chains of eight meronts (merogony) that separate into pairs of cells that finally result in isolated mature spores (sporogony) (Weidner 1970; Weidner and Overstreet, unpublished data).

Aspects of the nutrition, invasion into the host cell, and other features of *A. michaelis* are considered representative of the phylum in general (e.g., Weidner 1972, 1976). One should appreciate the fact that if the sporoplasm is injected into a general culture media such as medium 199 or blue crab gut extract, rather than being injected into a host cell, it will disintegrate within minutes (Weidner 1972). When adenosine triphosphate, but not various other compounds, is added to the culture medium, the sporoplasm maintains its structural integrity for at least 6 h (Weidner and Trager 1973).

At least five species in addition to *A. michaelis* infect the blue crab. One is a species of *Thelohania* that is presently being described (Weidner et al. 1990; Overstreet and Weidner, in preparation). *Thelohania* sp. has eight spores developing in an SPV. This vesicle has a highly persistent membrane. The species, which infects skeletal muscles as do the other species known to infect blue crabs, has cytoskeletal features, as indicated above, that suggest microsporidians are highly evolved and that *Thelohania* sp. is especially complex (Weidner et al. 1990). *Nosema sapidi* (referred to as *Ameson sapidus* by Couch and Martin [1982], Noga et al. [1998], and others) has single spores massed in the skeletal muscle that measure 3.6  $\mu\text{m}$  long by 2.1  $\mu\text{m}$  wide. It has been reported in the blue crab from North Carolina only (DeTurk 1940a). What was originally described as *Nosema sapidi* in an unpublished thesis (DeTurk 1940a) actually consisted of both *A. michaelis* and *N. sapidi* (see Sprague 1977). *Pleistophora cargo* has an SPV containing 32 to >100 ellipsoidal, mononucleate mature spores measuring approximately 5.1  $\mu\text{m}$  long by 3.3  $\mu\text{m}$  wide (4-6 by 3-4  $\mu\text{m}$ ) when live (Sprague 1977). The extruded polar tube is usually



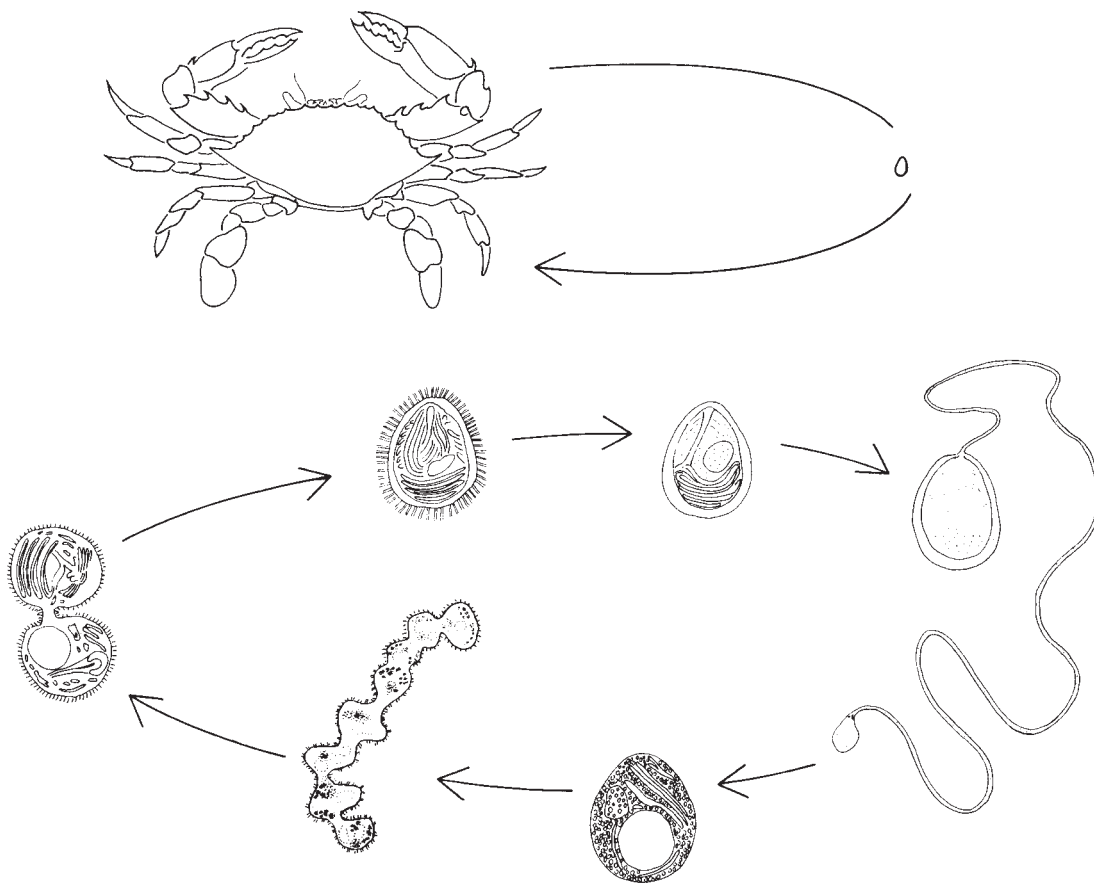


Figure 19. Life cycle of *Ameson michaelis*. This direct life cycle proceeds without an intermediate host. The mature spore is acquired when an uninfected crab feeds on infected crab tissue or on spores ultimately released from such tissue. The polar tube everts from the ingested spore and the sporoplasm passes through the tube and apparently infects a crab hemocyte. Once reaching muscle tissue, the intracellular parasite undergoes development first in a string of eight cells and then of two cells before producing single spores, which occur in large numbers within the muscle. The muscle becomes chalky and weakened from this vegetative multiplication. From Overstreet (1978).

unevenly thick along most of its 80- $\mu$ m length except where it narrows abruptly near the distal end. When additional material is examined, *P. cargoi* will most likely be transferred to another genus because species of *Pleistophora sensu stricto* do not infect crustaceans. A cooked crab from Mississippi contained a species of "*Pleistophora*," but it was too altered to identify (Overstreet, unpublished data). A related species, *Pleistophora* sp. of Johnson (1972) from North Carolina is reported to have smaller spores than *P. cargoi* (see Sprague 1977). Finally, a hyperparasite identified as *Nosema* sp. infects an unidentified micropallid metacercaria in *C. sapidus*, rather than

infecting crab tissue directly (Sprague and Couch 1971).

### **Animal Health and Fisheries Implications**

Ranges for the different species are not known, but at a minimum they extend from at least Delaware and Chesapeake bays to Louisiana and probably farther south for *A. michaelis* (see Overstreet and Whatley 1976; Overstreet 1988). In the Lake Pontchartrain area, commercial crabbers estimated prevalence to be less than 1% year-around, with more than 1% in relatively warm areas, lagoons, and close to shore (Overstreet and Whatley 1976),

where infections occurred in crabs measuring 2 to 13 cm in carapace width. Prevalences of up to 10% occur in restricted locations from Chesapeake Bay to Georgia (Messick 2000).

Of the other species infecting the blue crab in the Gulf of Mexico, the species of *Thelohania* presently being described by Overstreet and Weidner (in preparation; Weidner et al. 1990) occurs in crabs from at least Mississippi and Central Florida. What may be the same species occurs in Chesapeake Bay (Shields, unpublished data). In the Gulf Coast locations, infections are associated with morbidity and mortalities (Steele and Overstreet, unpublished data). At least one other species of *Thelohania* exists in the Gulf, but nothing is known about it. In regard to the other species reported from the blue crab along the Atlantic coast, *Nosema sapidi* occurs in Beaufort, North Carolina, as does *A. michaelis* (see Sprague 1977). Only 3 of 120 crabs examined by DeTurk (1940a) exhibited microsporidian infections. *Pleistophora cargo* was originally described from one crab in the Patuxent River, Maryland (Sprague 1966), and we are unaware of any other reports. *Pleistophora* sp. from North Carolina with smaller spores than *P. cargo* infected 2% of a population in coastal waters of salinity less than 17<sup>1</sup>, but not in higher salinity water (Johnson 1972). The hyperparasite identified as *Nosema* sp. occurred in Pry Cove on the Eastern Shore of Chesapeake Bay, Maryland. What may be the same microsporidian occurred also in North Carolina, South Carolina, and Georgia (Sprague and Couch 1971).

All species of microsporidians from the blue crab are relatively rare. Infected crabs become inactive and the occasional "outbreaks" may be artifacts caused by the departure of uninfected crabs from shallow water habitats during seasonal migrations. Findley et al. (1981) demonstrated lactate concentrations six to seven times higher in the muscle tissue and hemolymph of infected crabs compared with those in uninfected ones. Commercial fishers com-

monly collect crabs infected by *A. michaelis* near or among the vegetation along the shorelines of Louisiana lakes. Perhaps infected crabs have a buildup of lactic acid that inhibits their innate migration and spawning behaviors (Overstreet and Whatley 1976; Overstreet 1988). Because of the parasite's direct life cycle, dead or weakened crabs serve as a source of the disease for their cannibalistic cohorts, resulting in deleterious effects on stocks both in culture or in confined habitats in the wild. Infected crabs, including dead individuals and spoiled tissues, should not be returned to the water because the infection could spread (Overstreet 1978, 1988).

Crabs infected by *A. michaelis* in Chesapeake Bay and Louisiana are considered to have "sick crab disease," or "cotton crab disease." Experimentally infected crabs are clearly weakened by the agent, with at least some dying (Weidner 1970; Overstreet and Whatley 1976); but in the natural environment, infected individuals may seem to be healthy. The parasite lyses infected muscle and adjacent tissues (Fig. 20). The actin and myosin filaments of the host disassemble in the presence of the sporoblasts (Weidner 1970; Overstreet and Weidner 1974), and a cell-free extract of infected tissue can produce lysis of normal blue crab muscle tissue (Vernick and Sprague 1970). Grossly, the infected muscles appear chalky white through joints of the appendages, and the abdomen may appear greyish (Overstreet 1988). This species does not have an SPV, also called a "pansporoblast wall," like species in some other genera such as *Inodosporus spraguei* in the grass shrimp *Palaemonetes pugio* and *P. kadiakensis*. The SPV in *I. spraguei* was suggested by Overstreet and Weidner (1974) to function as a sink for toxic byproducts of parasitic metabolism. Such a sink could provide protection for the host shrimp, and would be unavailable in infections of *A. michaelis* in the blue crab.

*Ameson michaelis* also promotes an internal biochemical imbalance in the host. Findley et al. (1981) found significant changes in hemolymph protein and ion concentrations in infected blue crabs. This imbalance is in marked contrast to the condition in infections of *Thelohania maenadis* in *Carinus maenas* (see Vivarès and Cuq 1981). The difference was con-

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<sup>1</sup> Salinity is presented as a pure ratio with no dimensions or units, according to the Practical Salinity Scale (UNESCO 1985).



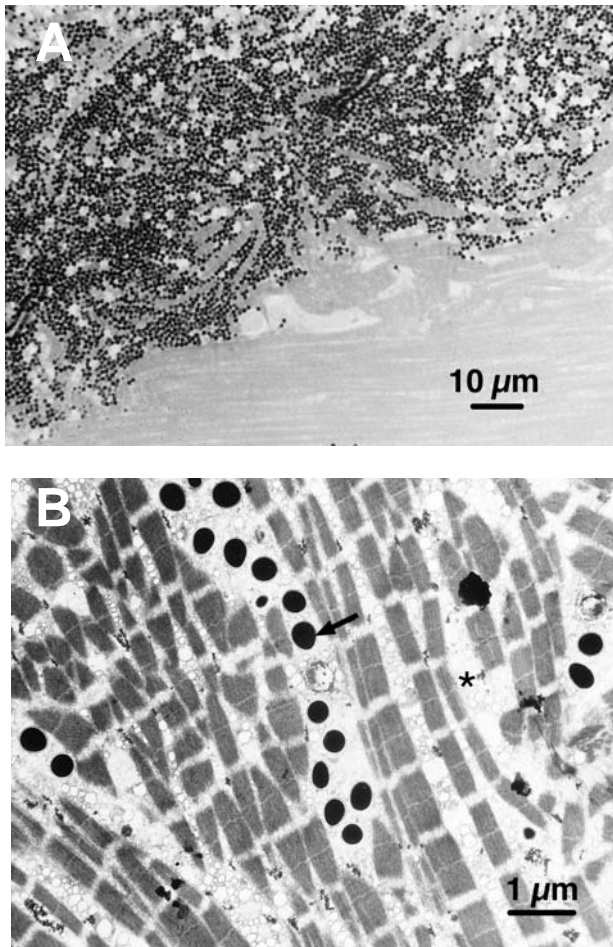


Figure 20. (A) *Ameson* sp. infection in the muscle of *Portunus pelagicus*. Note the presence of uninfected muscle tissue and the density of the microspores in lysed muscle tissue (thick section, toluidine blue). Virtually identical pathologies occur in *Callinectes sapidus*. (B) Electron micrograph of an infection of *Ameson* sp. from *P. pelagicus*. Note how the parasites (arrow) grow along and lyse (star) a muscle syncytium with no lysis of nearby muscle.

sidered to be related to the lower salinities and higher temperatures experienced by the experimentally infected blue crab (Findley et al. 1981). The hemolymph of the blue crab infected with *A. michaelis* exhibited lower levels of  $\text{Cl}^-$  and  $\text{Na}^+$  and higher levels of free amino acids. In addition, infected individuals showed increased levels of lactate and decreased levels of blood glucose in the hemolymph, thoracic muscle, and hepatopancreas.

Increased levels of amino acids in the hemolymph may have resulted from proteolysis of muscle or leakage of amino acids from cell membranes. Increased lactic acid levels may result from the use of muscle tissue as an energy source by the parasite (Findley et al. 1981) or from parasite-induced hypoxia in muscle tissues that may arise in heavy protozoal infections (Taylor et al. 1996).

The simple life cycle of *A. michaelis* allowed Overstreet and Whatley (1976) and Overstreet (1975, 1988) to conduct experiments screening drugs and assessing control methods for this agent. In culture facilities, infections can be prevented using monensin or buquinolate concurrent with or before administration of the agent. Neither drug is available for use in aquaculture in the USA. Disinfection was achieved with commercial bleach or an iodine-containing solution such as Wescodyne®. Treatment of crabs containing developed spores was not successful when attempted with a variety of compounds. Some spores tolerated freezing for at least 67 d at  $-22^\circ\text{C}$  (Overstreet and Whatley 1976).

### Future Research

A need exists to determine the taxonomy, biology, and distribution of the various microsporidian species and to develop complete diagnostic features of each species that includes molecular analyses. Presently, molecular data on many microsporidians are being accumulated in several laboratories worldwide. The life cycles of species other than *A. michaelis* also need to be determined. Not all life cycles are direct like that of *A. michaelis*. For example, efforts to transmit *Thelohania* sp. from crab to crab by direct feeding have been unsuccessful (Overstreet, unpublished data).

Some species are infective, develop, multiply, and detrimentally affect their hosts only within a specific environmental range. For example, low oxygen levels produced more rapid mortality of crangonid shrimp infected with *Pleistophora crangoni* than of uninfected shrimp. Large shrimp were also more heavily infected than smaller, young ones (Breed and Olson 1977). Prevalence of that microsporidian reached 30 and 41% in two of the four infected host species during winter. Infections appeared to shift

the sex ratio in favor of females as well as effectively castrating those females. The relationship between environmental factors and infection by *A. michaelis* also needs critical investigation. Apparently, *A. michaelis* infects the blue crab most readily in areas of low salinity, high temperature, and reduced oxygen (Findley et al. 1981). Alternatively, these conditions may represent those where infected crabs survive longer (Overstreet and Whatley 1976).

The effect of infections on the crab population may be more pronounced in juveniles than in mature crabs (Overstreet and Whatley 1976). Experimental infections of *A. michaelis* clearly demonstrate that infected crabs are not as active or strong as non-infected ones, but no critical evaluation has been conducted. In the case of *Thelohania* sp., infections may weaken the blue crab host sufficiently for it to be eaten by the next host in the life cycle of the parasite. Vivarès (1975) indicated that *Carinus maenas* infected with *Thelohania maenadis* when compared with uninfected ones exhibited increased exploratory activity and decreased aggressive reactions, agonistic reaction time, strength, and locomotive speed when out of the water. Most likely, blue crabs infected with any microsporidian are more vulnerable prey than their uninfected counterparts.

Host resistance may explain the relatively low prevalence of infection considering the potential for high rates of infection. Overstreet and Whatley (1976) collected *A. michaelis*-infected crabs in Louisiana (Lake Pontchartrain and Lake Borgne) and administered the spores *per os* to wild crabs collected from inshore waters nearby in Mississippi where infections had not been observed. Exposures to several groups of crabs usually produced infections in 40 to 65% of the individuals over a several-year period. Then, abruptly and continually for several attempts with additional groups of crabs, spores that had proven to be infective as well as additional fresh spores from Louisiana crabs no longer produced infections. At that time, two crabs infected with *A. michaelis* were collected in Back Bay of Biloxi, Mississippi. Perhaps, weather conditions and current flow had allowed crabs or the parasite from Louisiana to enter the Mississippi estuary and with it promote some type of resistance. Regardless, much

remains to be learned about factors that influence the temporal and spatial dynamics of microsporidian infections in the blue crab.

### Haplosporidia

The Haplosporidia is a small phylum of spore-forming protozoans that have a multinucleated naked plasmodial stage in their life cycle. Members contain uninucleated spores without extrudible polar tubules, but they contain mitochondria, characteristic haplosporosomes, and an anterior orifice or operculum. Most of the approximately 40 known species occur in molluscs, but a few infect crustaceans and other invertebrates. Little is known about them, and various unclassified spore-forming protists have at one time or another been placed within the group (e.g., Sprague 1979). There is considerable attention directed to the group because of the severe panzootics caused by *Haplosporidium nelsoni* in the eastern oyster *Crassostrea virginica* along the Middle Atlantic USA and to a lesser extent other species in wild oysters and in hatchery-produced spat. (See recent references by Burreson et al. [2000], who discussed increased virulence in introduced *H. nelsoni*, and by Hine and Thorne [1998, 2000], who discussed recent mortalities in Australia.) Problems occur in identifying and classifying members of the Haplosporidia *sensu stricto*. The group is considered to be a phylum by some (Perkins 1990; Flores et al. 1996) and a major component of the phylum Asctosporida by others (e.g., Sprague 1979; Corliss 1994).

Presently, all or nearly all species are placed into three genera, but authors disagree as to what features are diagnostic for each. Members of the genus *Urosporidium*, perhaps the basal genus of the extant genera (Siddall et al. 1995; Flores et al. 1996), have an oval or spherical spore with an anterior orifice covered by a tongue of wall material tucked inside the aperture. The several species in the genus are primarily hyperparasites of digeneans or nematodes and have characteristic extensions of the epispore cytoplasm. Members of the genera *Haplosporidium* and *Minchinia* have an oval spore with an operculum covering the orifice. Differentiating species into these two genera is contentious, especially because



what are considered species of *Haplosporidium* do not form a monophyletic assemblage (e.g., McGovern and Bureson 1990; Flores et al. 1996; Perkins 1996); nevertheless, many taxonomists temporarily consider those of *Haplosporidium* to have a spore with filaments wrapped around it that do not form a prominent extension visible with the light microscope. Using that criterion, the genus includes several freshwater and marine species occurring in decapods and a variety of invertebrates, whereas *Minchinia* contains species parasitizing marine polychaetes and molluscs only. Members of genus *Minchinia* have spores possessing prominent extensions, or "tails," visible with the light microscope (Perkins 2000). No life cycle of a species of haplosporidian has been established, but an intermediate host in the cycle is suspected (e.g., Perkins 1996, 2000).

### Biology

Most obvious of the haplosporidians in the blue crab is *Urosporidium crescens* because it causes "pepper crabs," crabs exhibiting "pepper-spot," or "buck-shot," in the skeletal muscles, visceral organs, and gills (Fig. 21, see also Fig. 38). The haplosporidian does not infect the actual crab tissue, but rather it hyperparasitizes the encysted metacercaria of the digenean *Microphallus basodactylophallus* that infects the crab. When this parasite infects the fluke and undergoes extensive multiplication, its brownish colored spores in the greatly enlarged worm create a black spot readily visible to the naked eye. The ultrastructure and development of *U. crescens* was described by Perkins (1971). A hyperparasitized worm can be up to three or more times larger (up to 1 mm) than the uninfected 450- $\mu$ m long worm within its 200- $\mu$ m diameter cyst. The infected worm has no functional reproductive organs, can hardly move when excysted, and serves only as a culture medium for *U. crescens*. Couch (1974) described details of the histological changes in the same species misidentified as *Megalophallus* sp. (see Heard and Overstreet 1983).

At least one other haplosporidian infects crab tissue directly. Newman et al. (1976) reported a *Haplosporidium*-like agent in a few moribund crabs in North Carolina and Virginia. Affected crabs exhib-

ited opaque hemolymph with uninucleated cells containing perinuclear haplosporosomes and mitochondria; interstitially, multinuclear plasmodial stages of this parasite occupied much of the vascular spaces. As with members of the Haplosporidia, the nuclei of the plasmodial stage contained bundles of microtubules and the cytoplasm of these cells contained no haplosporosome, but they did have empty membrane-bound vesicles the same size as the haplosporosomes (Newman et al. 1976). No spore was observed in those cases.

A focal haplosporidian infection with developed spores in the hepatopancreas of the blue crab was

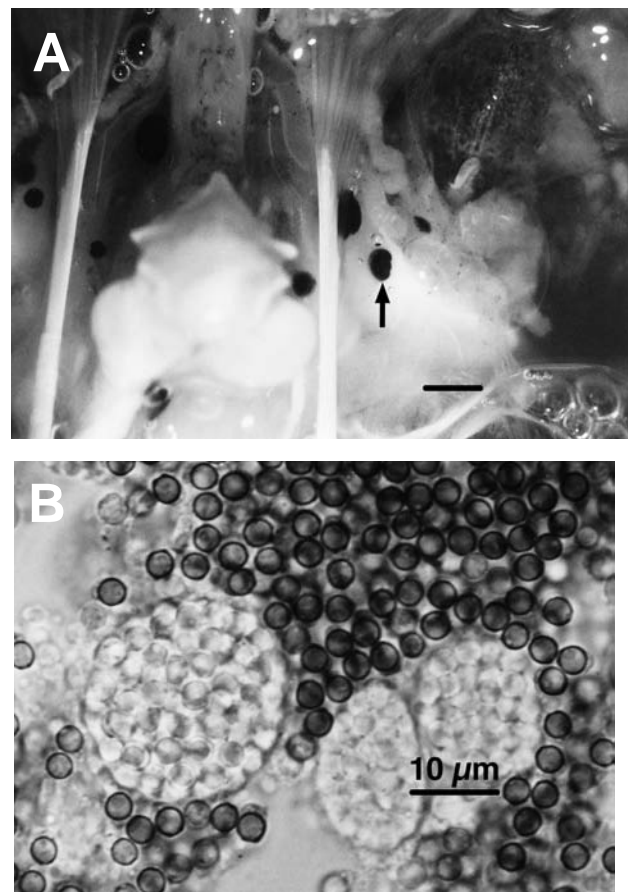


Figure 21. (A) Pepper-spot disease (arrow) in the connective tissues surrounding the foregut of a blue crab. (B) Spores of *Urosporidium crescens* in the tissues of a trematode metacercaria infecting the blue crab (wet smear). Note the dark coloration of the mature spores. Bar in (A) = 5 mm.

found in Mississippi (Overstreet, pers. obs.); this species may or may not be the same as that found along the Middle Atlantic coast. The ultrastructure of the agent is presently being examined to determine its identity and to find out whether it is conspecific with that from the blue crab in the Atlantic, conspecific with *U. crescens* from a concurrent digenean infection, conspecific with one from other crabs or hosts in the area, or a unique species (Krol and Overstreet, pers. obs.).

Few species of Haplosporidia have been reported from crustaceans, although *Haplosporidium louisiana* infects xanthid mud crabs from both the Atlantic seaboard and the Gulf of Mexico. The paucity of known species probably reflects the lack of attention given to investigation of this group because we have seen species in majids, penaeids, and amphipods as well as molluscs (Heard, Overstreet, and Shields, unpublished data).

### **Animal Health and Fisheries Implications**

Reports, several of them unsubstantiated, of *U. crescens* exist from microphallids found north of Chesapeake Bay to as far south as at least Texas. This haplosporidian was originally described from *Spelotrema nicolli* (probably a misidentification of *Microphallus basodactylophallus*) from North Carolina (DeTurk 1940b). Because the "pepper-spots" produce an unappetizing appearance, the infected crabs are either unmarketable as a seafood product or have a lesser value and therefore negatively influence the crab fishery (Perkins 1971; Couch and Martin 1982; Overstreet 1983; Noga et al. 1998). From a human health perspective, *U. crescens* will not infect humans, but the hyperparasitized, hypertrophied, darkly-colored, infected, encysted metacercaria may keep the seafood consumer from eating the nearly invisible, opaque, co-occurring uninfected metacercaria of *M. basodactylophallus*, which may be infective and pathogenic to humans who eat the crab host uncooked (Heard and Overstreet 1983).

Sprague (1982) indicated that although *U. crescens* and other species of *Urosporidium* in trematodes were not known to be of any practical significance as pathogens, the metacercaria infected with

*U. crescens* became hypertrophied and eventually transformed into a large bag of haplosporidian spores. Nevertheless he continued, "it remains apparently healthy and active." We note that an infected metacercaria can hardly move and does not possess the reproductive organs typical of the healthy specimen; consequently, infected worms are neither healthy nor active, and the haplosporidian, whereas not pathogenic to the crab, is highly pathogenic to the worm.

The unidentified *Haplosporidium*-like agent reported by Newman et al. (1976) was suspected to cause morbidity in the crabs from Chincoteague Bay, Virginia, and coastal North Carolina. As indicated above, no spore stage was present. From Virginia, more than 475 crabs examined throughout the year by Phyllis Johnson included only three infected with the haplosporidian; those cases were in May and July (Newman et al. 1976). Unlike the apparent rare pathogenic infection along the Atlantic coast, the one in Mississippi did not seem to severely harm the host; it infected the alimentary tract epithelium and formed brownish spores.

### **Future Research**

One of the most important problems concerning the Haplosporidia is to determine a representative life cycle of a member of the group and ascertain if any additional host exists. The one or more haplosporidian species within tissues of the blue crab should be identified and the distributions determined. More important, the question of whether the agent(s) causes disease and mortality in the host needs to be confirmed. If it does cause disease, what is the relationship among salinity, temperature, size of crab, and parasite? A better understanding of the biology of *U. crescens* may allow for a better understanding of the biology of other crab- and oyster-infecting species.

### **Dinoflagellata/Dinophyceae**

#### ***Hematodinium perezii***

*Hematodinium perezii* is a parasitic dinoflagellate that proliferates internally in the hemolymph and tissues of crustaceans. It infects the blue crab, where

it is highly pathogenic, and in most cases kills its host. Two species in the genus, *H. perezii* and *H. australis*, have been described, but several forms warrant species status. *Hematodinium perezii*, the type species, was originally described from the green crab *Carcinus maenas* from France (Chatton and Poisson 1931). The parasite in the blue crab is morphologically identical to *H. perezii*, and until there is convincing molecular or ultrastructural data to the contrary, we refer to *H. perezii sensu lato* Chatton and Poisson 1931 as the infectious form in the American blue crab (see Newman and Johnson 1975; MacLean and Ruddell 1978).

Species of *Hematodinium* or *Hematodinium*-like dinoflagellates have been identified from a wide range of host species from many geographic regions. On the eastern seaboard of the USA, *H. perezii* infects the blue crab (Newman and Johnson 1975; Couch 1983), the rock crabs *Cancer irroratus* and *C. borealis*, and the lady crab *Ovalipes ocellatus* (see MacLean and Ruddell 1978). A possibly related parasite has been reported from several genera of amphipods (Johnson 1986b). Well-studied but undescribed species of *Hematodinium*-like dinoflagellates have caused significant damage to fisheries for the Tanner crab *Chionoecetes bairdi* and snow crab *C. opilio* (see Meyers et al. 1987, 1990, 1996; Taylor and Khan 1995), the edible crab *Cancer pagurus* (see Latrouite et al. 1988), the velvet crab *Necora puber* (see Wilhelm and Miahle 1996), the harbor crab *Lio-carcinus depurator* (see Wilhelm and Boulo 1988), and the Norway lobster *Nephrops norvegicus* (see Field et al. 1992; Field and Appleton 1995). In Tanner and snow crabs, the parasite causes "bitter crab disease," resulting in unpalatable, unmarketable crabs. Infected blue crabs do not taste bitter; perhaps they die before acquiring the vinegary, bitter flavor.

### Biology

Infections with *H. perezii* can be difficult to diagnose. The vermiform plasmodium is the most readily identifiable stage in the hemolymph (Fig. 22), but the trophont or vegetative stage is that most frequently observed; to the layperson, it is often confused with a hemocyte. Previously, infections were

described as a neoplastic granulocytopenia from the blue crab (Newman 1970). Neutral red is an excellent vital stain for the parasite in fresh hemolymph, because the dye is taken up in the Golgi apparatus of the actively synthesizing parasites; hemocytes do not generally acquire the stain. Electron micrographs reveal the distinctive dinokaryon, alveolate pellicle, and trichocysts that firmly establish affinities of the parasite to the dinoflagellates (Fig. 23).

At least four different stages can be observed in the hemolymph. The vermiform, multinucleate plasmodia are motile and range in length from approximately 15 to 100  $\mu\text{m}$ . They are found in early infections and probably arise from an infectious dinospore. The ameboid trophont resembles an immature or atypical hemocyte ranging in size from 9 to 22  $\mu\text{m}$ . Trophonts occur in at least two morphologically different stages: an ameboid form with few, small refractile granules and a large rounded form with many, large refractile granules. The latter may represent a sporont because it is generally observed in later stages of infection. Dinospores are rarely observed in the hemolymph. Distinct macrospores and microspores (cf. Meyers et al. 1987, 1990; Appleton and Vickerman 1998) occur in the blue crab, but they have not been characterized (Shields, pers. obs.). In massive infections, few host hemocytes remain, and rounded forms of the parasite (prespore or effete stages) can be observed in the hemolymph. Effete stages of the parasite have many large vacuoles and are typically associated with large quantities of cellular debris in the hemolymph.

Partial progression of the life cycle of *H. perezii* has been observed in primary cultures of the parasite (Shields, unpublished data). The multinucleate plasmodium initially reproduces by budding and further develops by merogony to produce the vegetative trophont (Fig. 24). Ameboid trophonts undergo successive rounds of binary fission to produce prespores (rounded trophonts) or sporonts that undergo rapid division to produce dinospores. Sporulation is rapid and occurs over 2 to 4 d in blue crabs (Shields and Squyars 2000); the ephemeral sporont may develop over a very brief period; it is rarely observed in prepared smears. Dinospore density in the hemolymph can be extraordinarily high ( $1.6 \times 10^8$  dinospores



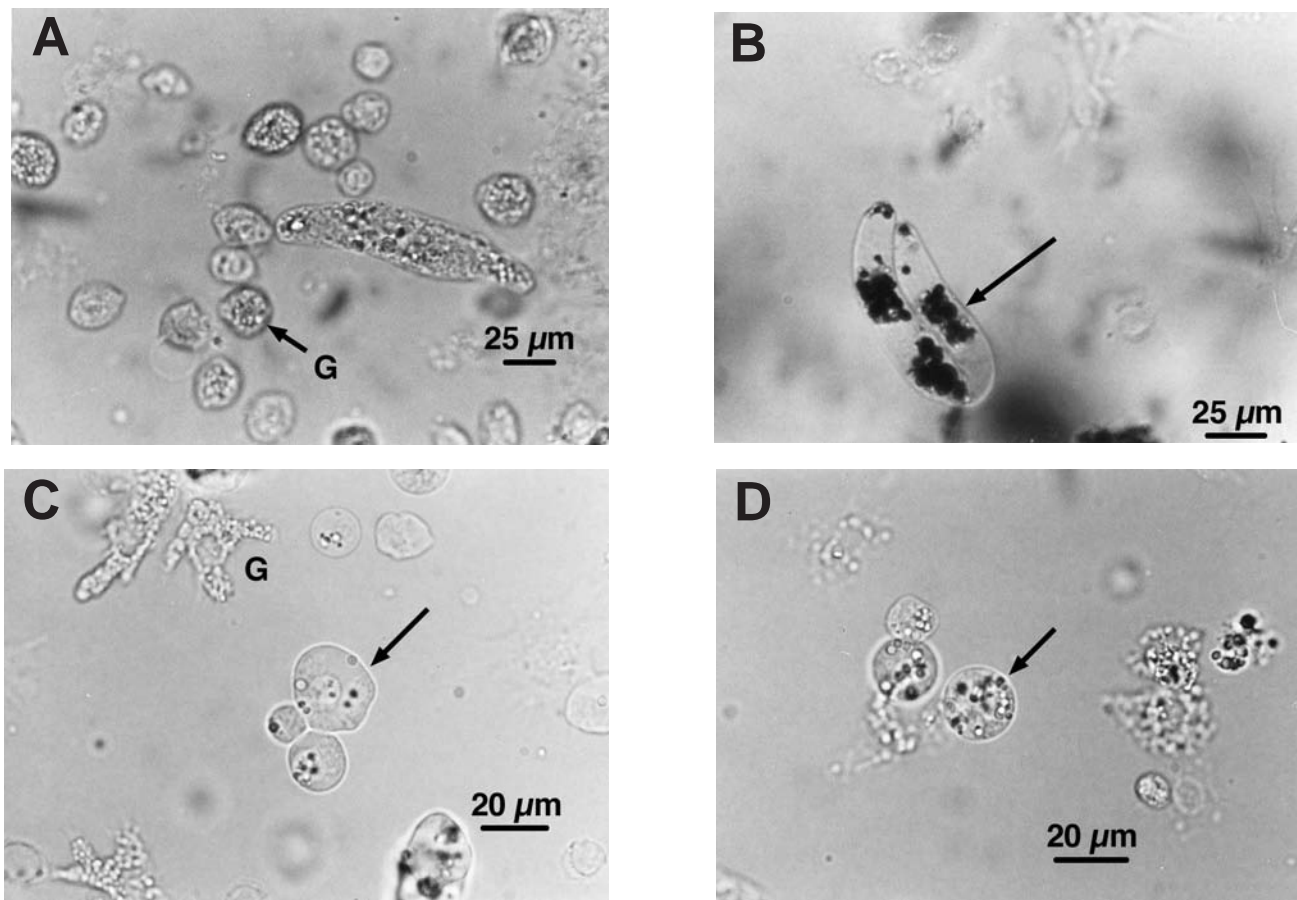


Figure 22. *Hematodinium perezi* from the blue crab, *Callinectes sapidus*. (A) Vermiform plasmodia in hemolymph (granulocytes [G]). (B) Plasmodium stained with neutral red (arrow). (C) Amoeboid trophonts (arrow) with few refractile granules (granulocytes, [G]). (D) Round trophonts (arrow) with many refractile granules. From Shields and Squarys (2000).

ml<sup>-1</sup>) during sporulation. Sporulation can be prolific enough to turn large (38-200 L) aquaria milky or cloudy with discharged dinospores.

Culture attempts have succeeded with a related "species" of *Hematodinium*. Appleton and Vickerman (1998) achieved the continuous culture of *Hematodinium* sp. from the Norway lobster at 6 to 10°C. The life cycle of *Hematodinium* sp. from the Norway lobster is different from that reported for other parasitic dinoflagellates and includes filamentous trophonts (cf. the plasmodium of Chatton and Poisson 1931) developing into unusual colonies of plasmodia termed "gorgonlocks," followed by arachnoid trophonts (filamentous forms), clump colonies, arachnoid sporonts, sporoblasts, and finally

dinospores (Appleton and Vickerman 1998). Continuous cultures of *Hematodinium* sp. from the Norway lobster are not infectious (Appleton and Vickerman 1998), suggesting a loss of infectivity or viability in cultured parasites. The arachnoid sporonts that occur in in vitro cultures of *Hematodinium* sp. from the Norway lobster (Field and Appleton 1995; Appleton and Vickerman 1998) have not been observed in natural infections or in laboratory inoculations of *H. perezi* in blue crabs, but they do occur in culture (Shields, pers. obs.).

Culture of *H. perezi* was not successful using a modification of the medium of Appleton and Vickerman (1998) (Shields and Small, unpublished data). Infections, however, have been maintained in the

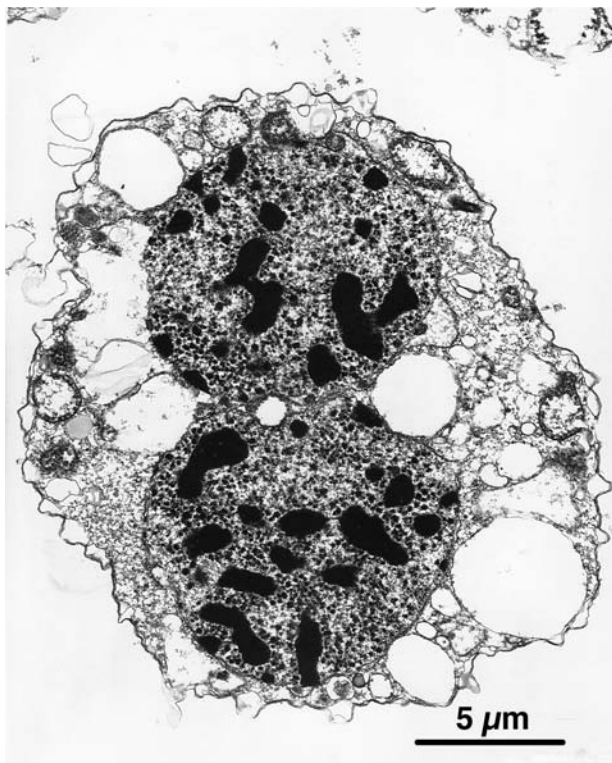


Figure 23. Electron micrograph of a plasmodium of *Hematodinium perezii* showing characteristic condensed chromatin bands in the nuclei, alveolate pellicle, and large vacuoles. Trichocysts not shown.

laboratory for over 7 months using serial passage in crabs (Shields and Squyars 2000). Three other species have also been transmitted by inoculation into their host crabs: Tanner crab (Meyers et al. 1987), snow crab (Shields and Taylor, unpublished data), and Australian sand crab *Portunus pelagicus* (see Hudson and Shields 1994). Trophonts from primary cell cultures were successful in establishing infections in the Tanner and blue crabs (Meyers et al. 1987; Shields, unpublished data). Natural transmission with dinospores has yet to be achieved.

### **Animal Health and Fisheries Implications**

The main sign of infection by *H. perezii* in heavily infected blue crabs is lethargy. Lightly and moderately infected crabs exhibit no overt sign of infection. Anecdotal observations suggest that most infected crabs cease feeding approximately 14 d after infection (at 20°C, Shields, pers. obs.). Acutely

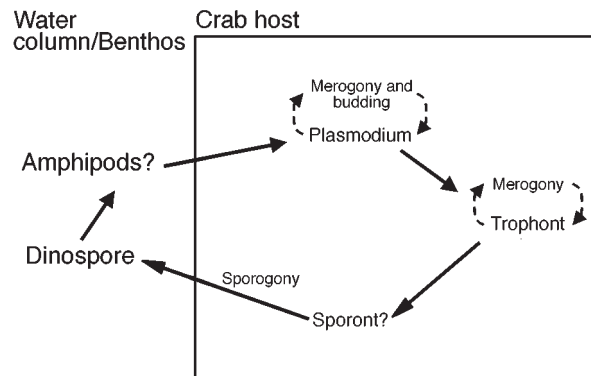


Figure 24. Presumptive life cycle of *Hematodinium perezii* in *Callinectes sapidus*.

infected crabs tend to die from stress before their hemolymph exhibits gross discoloration (Shields and Squyars 2000; Shields 2001a). Heavily infected crabs exhibit radical changes in the chemistry of the hemolymph. Gross changes include chalky or yellow discoloration of the hemolymph, lack of clotting ability, and the above-noted decline in total hemocyte density. Loss of clotting is a common endpoint for the hemolymph in many different decapod infections. The changes leading to the loss are not readily apparent. Declines in hyalinocytes, hemolymph proteins, and various enzyme systems suggest that an overall decrease in proteins associated with clotting may result from infection (Shields et al. 2003). Alternatively, the clotting mechanisms may be specifically suppressed or reduced. Pauley et al. (1975) suggested that the lack of clotting in *Paramoeba* infections in the blue crab was due to alterations of the hemolymph resulting from parasite-modulated proteolytic activity or from the loss of serum fibrinogen as a component of the total serum protein.

The route of infection by *H. perezii* is unknown. However, in the Norway lobster the route of infection appears to be through the midgut (Field et al. 1992; Field and Appleton 1995). The disease then progresses with the degeneration of the hepatopancreas and muscles, followed by general congestion of gill filaments and hemal sinuses with trophonts and plasmodia (Meyers et al. 1987; Latrouite et al. 1988;

Field et al. 1992; Hudson and Shields 1994; Messick 1994). Respiratory dysfunction is indicated by the low oxygen-carrying capacity, decreased hemocyanin levels, and reduced copper concentrations of the hemolymph of infected lobster (Field et al. 1992; Shields et al. 2003). Reduced hemocyanin levels and respiratory dysfunction may explain the induced lethargy, resulting in tissue hypoxia, necrosis, and eventual death of the host (Field et al. 1992; Taylor et al. 1996; Stentiford et al. 2000; Shields et al. 2003).

In experimental infections, hemocyte densities declined rapidly, approaching an 80% decrease within the first week of infection (Fig. 25) (Shields and Squyars 2000). The decline in circulating hemocytes is evident within 3 d, possibly sooner (Persson et al. 1987), although the parasites are not detectable in hemolymph. Experimentally infected crabs also exhibit marked shifts in hemocyte populations with proportionally more granulocytes than hyalinocytes. The large decline in hemocyte density also occurs with other hosts infected with *Hematodinium* spp. (Meyers et al. 1987; Hudson and Shields 1994; Field and Appleton 1995). Disruption of the hemocytes may result from the sheer number of trophonts in

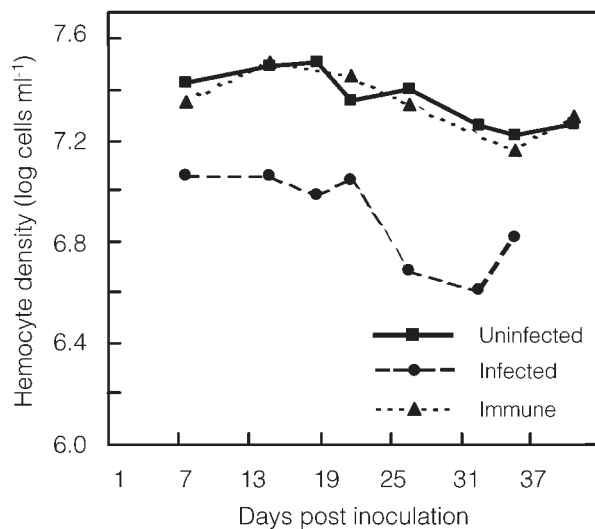


Figure 25.  $\text{Log}_{10}$ -transformed total hemocyte densities in uninfected, infected, and “immune” crabs. “Immune” crabs were inoculated with  $10^5$  parasites but never developed infections. From Shields and Squyars (2000).

the blood and their contact with the host cells. The shift in hemocyte subpopulations may result from the mobilization of tissue-dwelling reserves, differential cell death (Mix and Sparks 1980), increased mitotic stimuli of hemopoetic tissue (Hose et al. 1984), sequestration, or loss of specific hemocyte types during infection.

*Hematodinium perezii* was highly pathogenic in experimentally infected blue crabs, with a mortality rate of 87% over 40 d (Fig. 26) (Shields and Squyars 2000; Shields 2001a). Survival analysis indicated that inoculated crabs were seven to eight times more likely to die than uninfected crabs. Mortality rates of 50 to 100% over several months have been reported for laboratory-held Tanner crabs and Norway lobster (Meyers et al. 1987; Field et al. 1992). Interestingly, during challenge studies, a small number of blue crabs was refractory to infection (Shields and Squyars 2000). These “immune” crabs exhibited significant relative and absolute increases in granulocytes, and they did not develop hemocytopenia, loss of clotting ability, or changes in morbidity. On several occasions other “immune” crabs were serially challenged with infectious doses of *H. perezii* and did not develop infections (Shields, unpublished data).

Few physiological markers have been investigated in *Hematodinium*-infected blue crabs. Serum proteins, hemocyanin, and tissue glycogen levels show gradual changes with infection, and indicate a decline in the metabolic resources of the host

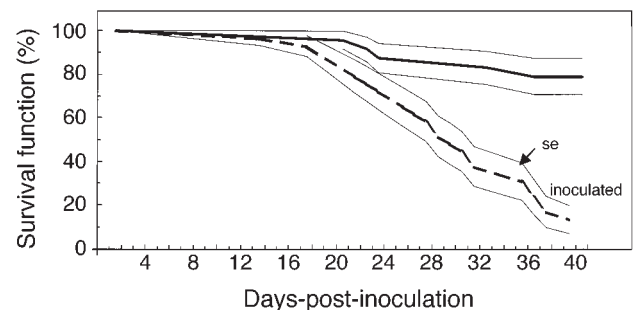


Figure 26. Survival function (Kaplan-Meier method) for uninfected blue crabs and blue crabs experimentally inoculated with *Hematodinium perezii*. Upper and lower standard errors (se) are shown. From Shields and Squyars (2000).



(Shields et al.2003). Total serum protein level has been used as a marker for physiological condition in American lobster (Stewart et al. 1967; Stewart et al. 1972) and snow crab (Courmier et al. 1999). In blue crabs, heavily infected males had significantly lower serum proteins and hemocyanin levels than uninfected males (Fig. 27A,B; Shields et al.2003). Infected females did not show different levels of serum proteins and hemocyanin levels compared with uninfected females. Acid phosphatase activity, however, increased with intensity of infection (Fig. 27C; Table 2; Shields et al., in press). Acid phosphatase activity was localized intracellularly in the parasite (as opposed to lysozymes that occur extracellularly in oysters); hence, acid phosphatase activity may be a useful marker for early or latent infections.

Physiological alterations have been investigated in Norway lobster infected with a *Hematodinium*-like dinoflagellate. Free amino acids in the plasma showed significant changes with high intensity

infections; serine concentrations decreased with intensity of infection but glutamate concentrations increased 10-fold, and taurine concentrations increased 13-fold with high intensity infections (Stentiford et al. 1999). Taurine and taurine-serine ratios were good indicators of intensity of infection and status of infection. Plasma lactate concentrations were significantly higher in infected Norway lobsters and, when coupled with high parasite loads, may cause hypoxic stress resulting in muscle necrosis (Taylor et al. 1996).

Glycogen levels showed significant decreases with infection, and males showed proportionally greater declines in glycogen than females (Fig. 27D). Glycogen is the main storage substrate in many invertebrates, providing energy for several physiological processes including chitin synthesis (Gabbot 1976; Stevenson 1985). Glycogen apparently is used for energy metabolism during summer (with lipid storage) followed by glycogen storage over winter

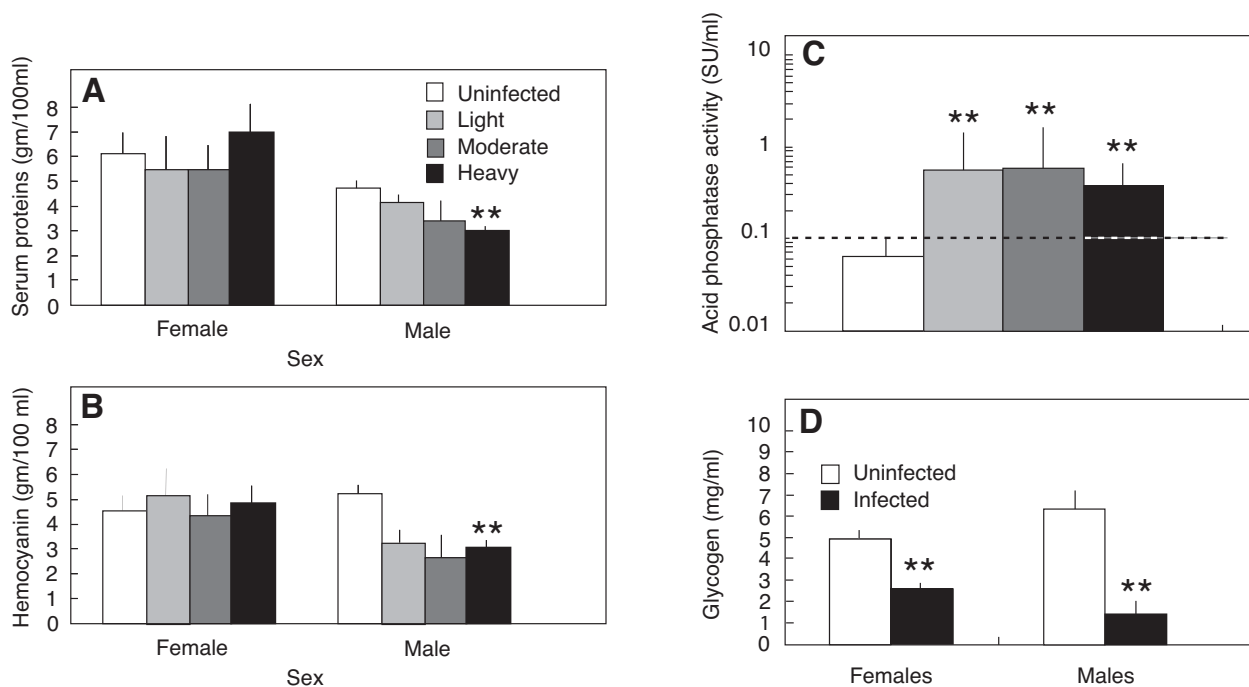


Figure 27. Pathophysiological indices in *Callinectes sapidus* naturally infected with *Hematodinium perezii*. Intensity levels were lightly (0.3-3.2 parasites per 100 host cells), moderately (3.3-10), and heavily (10+) infected crabs. (A) Total serum proteins (Biuret method); (B) serum hemocyanin levels; (C) acid phosphatase activity (log scale) in whole hemolymph; dashed line, detection limit for activity level. (D) glycogen levels in the hepatopancreas of uninfected and heavily infected male and female crabs. Vertical lines indicate standard errors; \*\*,  $P < 0.01$ . From Shields et al.

(with lipid metabolism) (Heath and Barnes 1970; Nery and Santos 1993). Large quantities of glycogen can be found in the epidermis and underlying connective tissues before ecdysis (Travis 1955; Johnson 1980). The juvenile green crab *C. carinus* stores glycogen in the hepatopancreas before molting whereas large, adult crabs (anecdysial) do not store large quantities (Heath and Barnes 1970). The cessation of feeding in infected blue crabs may hasten the depletion of glycogen, but lightly infected blue crabs have successfully molted in the laboratory (Shields, unpublished data). Because there is no difference in the prevalence of *H. perezii* in postmolt, intermolt, and premolt crabs (Messick and Shields 2000), the reduction in glycogen may not occur before molting in lightly infected blue crabs.

*Hematodinium perezii* is endemic to the high salinity waters of the mid-Atlantic states (Messick and Shields 2000). The parasite occurs in blue crabs in high salinity (>11) waters from Delaware to Florida and in the Gulf of Mexico (Newman and Johnson 1975; Messick and Sinderman 1992; Messick and Shields 2000). It has occurred along the Delmarva Peninsula of Delaware, Maryland, and Virginia at moderate prevalences for several years (Messick and Shields 2000). The parasite occurs in small foci along the Peninsula throughout much of the year, with small to moderate outbreaks occurring in the mouth and southeastern portions of the mainstem of Chesapeake Bay during fall (Fig. 28). In

Table 2. Number of crabs naturally infected with *Hematodinium perezii* with detectable levels of acid phosphatase in their hemolymph. From Shields et al. 2003. SU = standard units.

Infection level (parasites per 100 host cells)	Below detection (0.1 SU/ml)	Above detection
Uninfected (0)	16	3
Light (0.3-3.2)	4	4
Moderate (3.3-10)	5	11
Heavy (10 <sup>+</sup> )	2	13

1975, 30% prevalence was reported in blue crabs from Florida, where it was thought to have a large effect on the crab population (Newman and Johnson 1975). In 1991 and 1992, an epizootic of the parasite affected 70 to 100% of the juvenile blue crabs in the seaside bays of Maryland and Virginia (Messick 1994). Commercial watermen reported reduced catches, as well as lethargic, moribund, and dead crabs in pots and shedding facilities. The epizootic virtually shut down the blue crab fishery in seaside bays of the Delmarva Peninsula. In 1996 and 1997, prevalence ranged from 10 to 40% on the eastern embayments of the Delmarva Peninsula and 1 to 10% in the eastern portions of lower Chesapeake Bay (Messick and Shields 2000).

*Hematodinium perezii* exhibits a strong peak in prevalence in fall and a rapid decline in winter (Fig. 28B) (Messick and Shields 2000). Temperature experiments with infected crabs suggest that some crabs either lose their infections or overwinter with latent infections (Messick et al. 1999). Messick (1994) found that juvenile crabs had a higher prevalence of infection than older, mature crabs (as high as 100 vs. 70%, respectively; prevalence in juveniles was generally 20% higher than in adults). The fall peak in prevalence in mature females and the predilection of the disease for juvenile crabs indicates that during epizootics the disease may threaten reproduction in the fishing sanctuaries and possibly have a negative effect on survivorship of the next season's crabs. Peak periods of mortality may kill a large portion of the population, leaving only uninfected and lightly infected crabs. Sudden mortalities may explain the decline in mean intensity in November and prevalence in December, and the low to moderate prevalences occurring through spring. The possible effect of the disease on recruitment to the stock has not been modeled as has been done for *Cancer magister* and the egg-predator nemertean worm *Carcinonemertes errans* (see Hobbs and Botsford 1989).

The smaller coastal fisheries appear at most risk to *H. perezii* because the infection occurs in high salinity waters. Epizootics, however, tend to occur during fall, at or near the end of the primary migra-

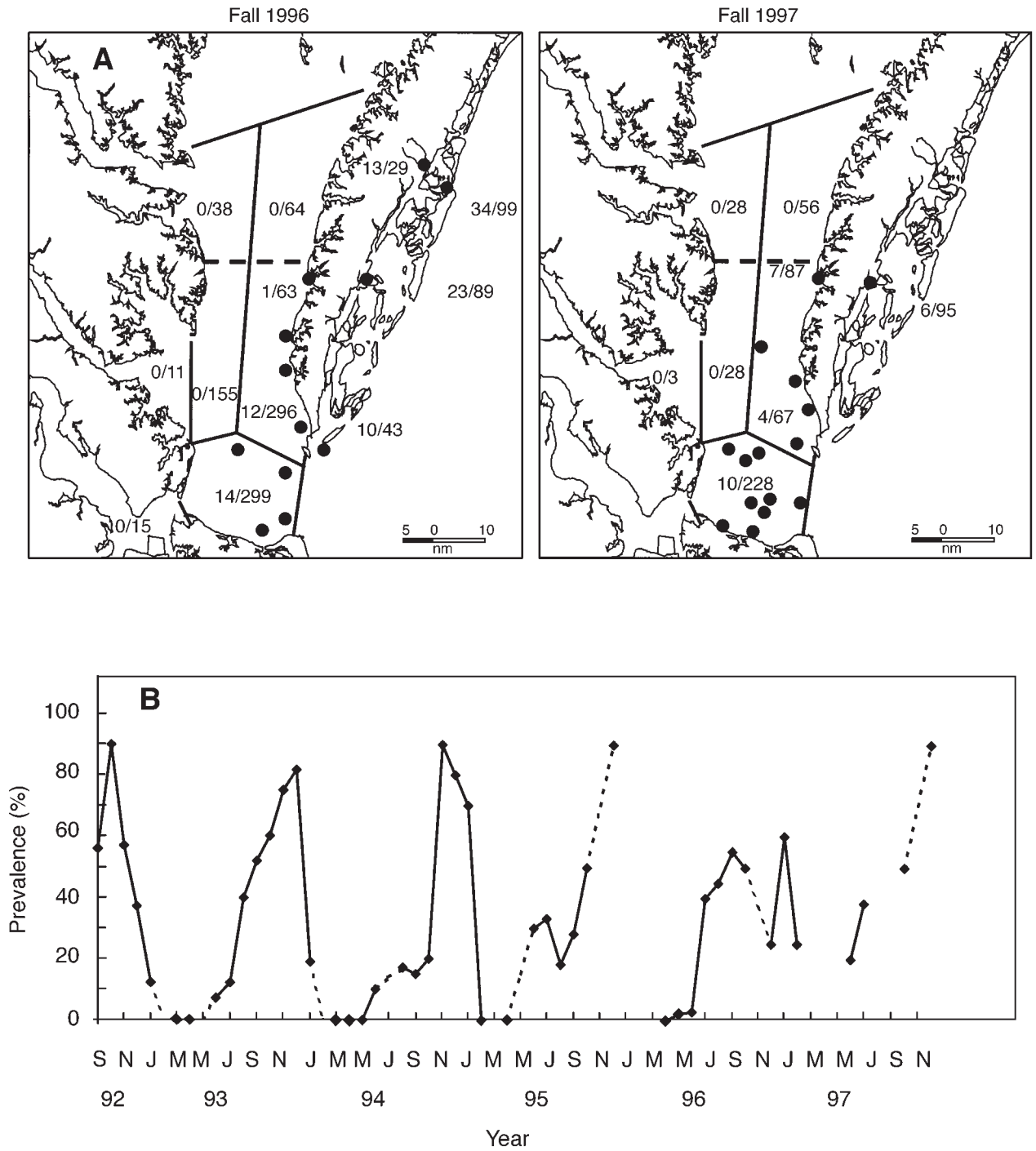


Figure 28. (A) Prevalence of *Hematodinium perezii* in *Callinectes sapidus* from lower Chesapeake Bay in Fall 1996 and 1997. Numbers are parasitized crabs/total examined within each delineation. Closed circles represent stations where infected crabs were found. Salinity differences within the lower Bay are divided roughly between upper and lower portions (broken line). nm = nautical miles. (B) Temporal patterns in prevalence of *H. perezii* in *C. sapidus* from coastal bays of Maryland. Dashed line indicates projected prevalence during unsampled months. From Messick and Shields (2001).



tion and reproductive periods. In 1996, the parasite was present at low prevalences during fall in the main spawning grounds of the blue crab in Chesapeake Bay. The peak season for crab reproduction is late spring and summer (Van Engel 1958, 1987; Hill et al. 1989). Therefore, although crab reproduction occurred during fall, the parasite was not present during the peak periods of reproduction. However, 1996 exhibited unusually high rainfall/runoff and lower than average temperatures. The parasite does not occur in salinities below 11; therefore, it could feasibly infect and cause significant mortalities to juvenile and adult crabs in much of the mainstem of the Bay.

The Delmarva Peninsula may be an ideal region for the growth and spread of parasitic diseases in blue crabs. Epizootics of both *H. perezii* and *Paramoeba perniciosus* recur there. The region possesses several characters that may facilitate such epizootics, including relatively closed crab populations (in this case, little immigration and emigration of juveniles and adults); relatively high salinity with the potential for entrainment of water within the backwaters and lagoonal systems; and stressful conditions such as high temperatures, seasonal hypoxia, seasonal fishing, and predation pressure (Shields 1994). Similar conditions exist in many small estuaries along the mid-Atlantic and southeastern USA.

Overfishing, density-dependent juvenile mortality, and predation are considered overarching causes for declining stocks. In stock assessment models, disease is considered as natural mortality, and it is typically given a low, stable, discrete value (e.g., Lipcius and Van Engel 1990; Abbe and Stagg 1996; Rugolo et al. 1998). Stock assessment models do not consider the potential for epizootics and resulting mortalities caused by *H. perezii*. Differential models of exploitation by region may be warranted, especially during or after epizootics (Shields and Squyers 2000). Given that all of the blue crabs in Chesapeake Bay migrate through high salinity waters, there is potential for the parasite to have a large effect on the fishery.

### **Future Research**

Clearly, the genus *Hematodinium* needs better taxonomic definition. Given the importance of the disease to several commercial fisheries, high priority should be given to the taxonomy of the genus. The type species requires ultrastructural study to compare it with other forms. Unfortunately, the type species may be rare in the green crab, in that Chatton and Poisson (1931) found three infected individuals out of 3500 crabs examined. Molecular and electron microscopic studies using parasites from the type host should be undertaken to resolve taxonomic questions to adequately diagnose species within the taxon.

Molecular probes should be developed to determine whether other crabs (*Cancer* spp., *Ovalipes* spp.) are alternate hosts for *H. perezii*, whether amphipods are involved in the life cycle or transmission of the parasite, and whether low level infections persist in crabs through the winter and spring. For example, because *Hematodinium*-like infections occur in amphipods (Johnson 1986b) that are a significant component of the diet of juvenile blue crabs, a DNA probe may be the best means of determining if amphipods are reservoir hosts for the crab parasite. The internal transcribed spacer region of the ribosomal RNA gene cluster from *H. perezii* has been described (Hudson and Adlard 1994, 1996). Although their study did not include the type species (*H. perezii* from *Carcinus maenas*), Hudson and Adlard (1996) found substantial sequence variation among the different species of *Hematodinium* from the Norway lobster, the Tanner crab, and the blue crab. Thus, species-specific PCR primers or custom-made DNA probes should provide exquisitely sensitive tools to investigate transmission pathways in the life cycle of the parasite.

Additional studies are needed to obtain *H. perezii* in continuous culture. Success with modified physiological saline will improve our efforts to study the parasite. Successful *in vitro* culture of the organism will facilitate study of many comparative aspects of its transmission and life history. For example, sporu-

lation is relatively rapid and difficult to observe in *H. perezii* from blue crabs. The development and production of dinospores for transmission studies and the determination if resting cysts occur in the life cycle are two immediate goals for culture experiments.

Mortality and life cycle/transmission studies should be done with juveniles and adults to refine models for better estimates of natural, disease-induced mortality, and to identify important factors in the host-parasite association. For example, experimental evidence indicates an innate resistance in some crab hosts. Such resistance needs to be documented and more fully characterized.

## RHIZOPODA

### *Paramoeba pernicioso*

“Gray crab” disease is a relatively common affliction recognized by watermen along the Delmarva Peninsula (Delaware, Maryland, and Virginia). It is caused by a systemic infection of the pathogenic amoeba *Paramoeba pernicioso*. The amoeba causes mortalities in high salinity waters, and the disease is named for the darkly discolored sternum and ventral surfaces of heavily infected and dead crabs (Sprague and Beckett 1966; Sprague et al. 1969). The disease was first thought to be viral in origin because the causative agent resembled a semigranulocyte with an inclusion-like “Nebenkörper,” a secondary nucleus (Sprague and Beckett 1966; Sprague et al. 1969). As with *Hematodinium perezii*, there is anecdotal evidence that the amoeba is limited to salinities over 25 (Sawyer et al. 1970).

### Biology

*Paramoeba pernicioso* is a lobose amoeba with few linguiform lobopods and a distinct, intensely basophilic nucleosome, or secondary nucleus (Fig. 29) (Sprague et al. 1969). The Nebenkörper is Feulgen positive, is siderophilic, and possesses two basophilic polar caps (Sprague et al. 1969). The organelle is comprised of an eukaryotic nucleus situated as a polar cap with finger-like extensions into a

prokaryotic-like nucleus, the whole enclosed in discrete cytoplasm (Fig. 30) (Perkins and Castagna 1971). Phagocytosis occurs in the cytoplasm of the Nebenkörper; the organelle is apparently a symbiont that has established a mutualistic relationship with the host amoeba (Grell 1968; Perkins and Castagna 1971). The formation of such a relationship has been well documented with a bacterial symbiont in *Amoeba proteus* and can occur over relatively short periods (see Jeon 1983).

The parasite has two forms that can both be found in the same individual (Fig. 30). The small form is spherical and ranges from 3 to 7  $\mu\text{m}$  long,

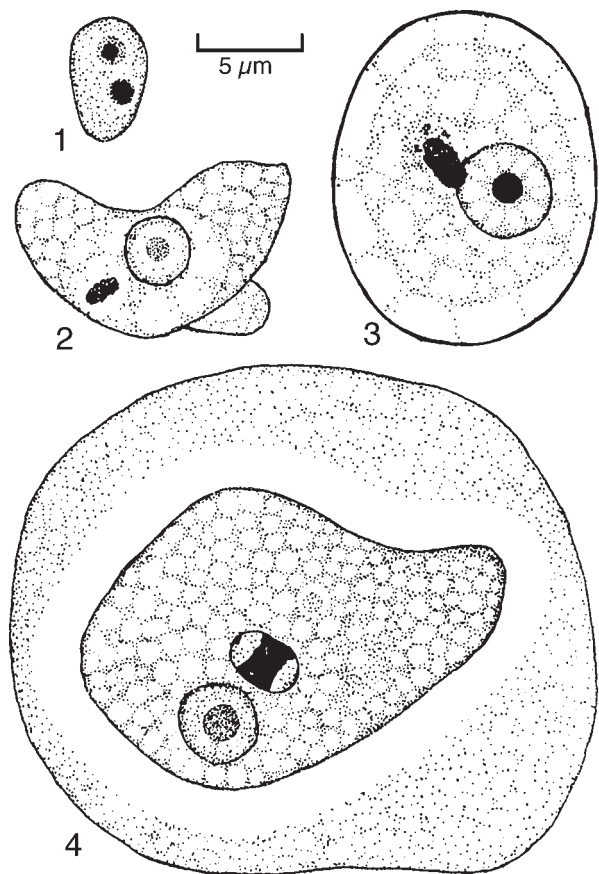


Figure 29. Representative forms of *Paramoeba pernicioso* from *Callinectes sapidus*. From Sprague and Beckett (1966).

but the large form is lobose and measures from 10 to 25  $\mu\text{m}$  (Sawyer 1969; Sprague et al. 1969; Johnson 1977b; Couch 1983). The small form (Fig. 30) is more commonly seen and can be observed in the hemolymph in the late stages of the disease (Johnson 1977b). The large form generally occurs in the connective tissues of the antennal gland, the endothelia of the blood vessels, and within the nervous system; it rarely occurs in the blood except in late-stage, terminal infections. Mitotic activity in the ameba was observed in 30 to 40% of the heavy infections and

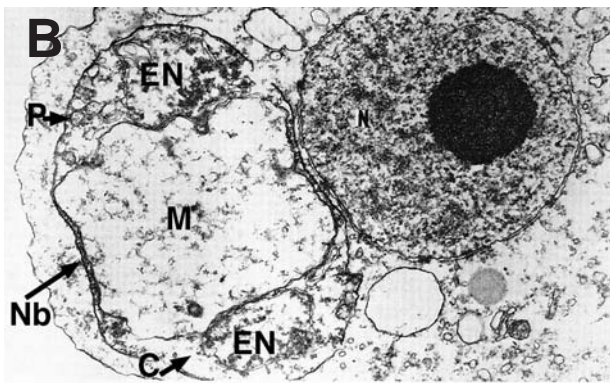
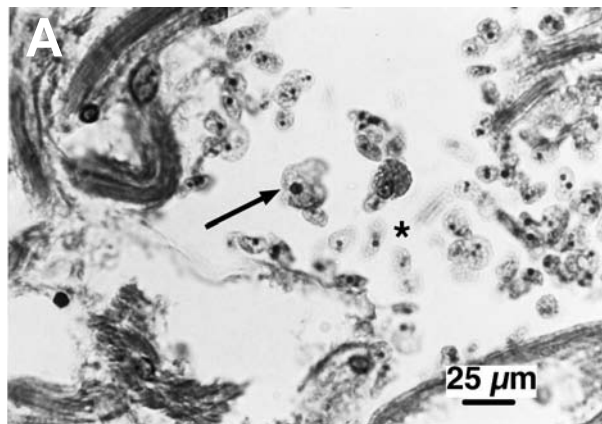


Figure 30. *Paramoeba perniciosae* in *Callinectes sapidus*. (A) Small (star) and large (arrow) forms of amebae in the heart. From Shields (pers. obs.). (B) Electron micrograph of the Nebenkörper or secondary nucleus of *P. perniciosae*: cytoplasm of Nebenkörper [C]; polar caps of Nebenkörper [EN]; prokaryotic-like nucleoid of Nebenkörper [M]; host nucleus [N], Nebenkörper [Nb]; phagosomes [P] within cytoplasm of Nebenkörper. From Perkins and Castagna (1971).

generally in large organisms. The presence of abundant small forms, the paucity of mitotic figures, and the distribution of the two forms indicate that mitotic activity may be synchronous and of short duration (Johnson 1977b). The ameba does not form cysts and does not form extensive pseudopodial extensions (Couch 1983).

*Paramoeba perniciosae* has not been grown in continuous culture. Primary cultures in 10% calf serum-agar overlaid with sterile sea water showed the best survival at 2 weeks (Sprague et al. 1969). Other media were also tested, including sterile seawater supplemented with bacteria, yeast, algae, or diatoms; 10% calf or crab serum in sterile sea water; and biphasic media of serum-agar overlaid with serum or sea water. Neither cysts nor trophonts of the ameba were found in cultured sediments obtained from aquaria containing infected crabs (Sprague et al. 1969). Couch (1983) considered *P. perniciosae* an opportunistic invader because other members of the genus are free-living. Because the parasite was not obtained in culture in standard media for nonparasitic forms, Sprague et al. (1969) considered the ameba an obligate rather than a facultative parasite.

### **Animal Health and Fisheries Implications**

Crabs with light and moderate infections exhibit no overt sign of disease. Heavily infected crabs, however, are sluggish and often die shortly after capture (Johnson 1977b). Infected "peelers" die shortly after molting. Mortality in laboratory-held animals can be sporadic (Johnson 1977b). During an epizootic, Newman and Ward (1973) examined crab mortality in relation to the disease. In an unusual and uncontrolled mortality study, they estimated that *P. perniciosae* caused a 30% loss to the population of crabs in Chincoteague Bay during June 1971.

In light infections, the ameba occurs in the antennal gland, Y-organ, and endothelial lining of the blood vessels. Connective tissues of the midgut are frequently involved, containing numerous amebae and infiltrating host hemocytes (Johnson 1977b). The hemal spaces of the gills and heart rarely have amebae in light infections, but those in the hepatopancreas are occasionally invaded. As the



infection progresses, the hemal spaces, connective tissues, muscles, and nerve tissues are infiltrated by the amoebae. In heavy infections, necrotic lesions occur in the hemopoietic tissues but never in the pericardial connective tissue (Johnson 1977b).

Hemolymph smears often reveal a large amount of lysed muscle (Sprague et al. 1969). Necrosis of the heart muscle and focal necrosis and lysis of skeletal muscle occasionally occur in heavily infected crabs (Johnson 1977b). Lysis of the connective tissues, hemocytes, hemopoietic tissues, and Y-organ can also occur in heavy infections. Because hypoxia can cause focal necrosis in the muscles of the blue crab (Johnson 1976b, d), and because hemocyanin is depleted in heavily infected crabs (Pauley et al. 1975), Johnson (1977b) speculated that necrosis of the muscles may result from hypoxia rather than directly from effects of the amoebae. A similar pathological condition that includes the additional burden of the parasite's respiration and intensity of infection has been posited for Norway lobster infected with *Hematodinium* sp. (Taylor et al. 1996).

In terminal infections, *P. perniciosus* is abundant in the hemolymph. The sheer number of organisms may cause extensive disruption of the connective tissues, hemopoietic tissues, and Y-organ (Johnson 1977b). The epidermis is also involved in heavy infections, with a displacement or lysis of epithelial cells. The effect on the metabolism of the host is significant. In approximately half of the heavily infected crabs, the reserve cells (R cells) of the hepatopancreas appeared to be depleted, and little fat was stored (Johnson 1977b). Hosts were clearly depleted of metabolic reserves.

Hemolymph from heavily infected crabs is cloudy and does not clot (Sprague et al. 1969; Sawyer et al. 1970; Johnson 1977b). The lack of clotting may result from alterations of the hemolymph by the proteolytic activity modulated by the parasite, from the loss of serum fibrinogen as a component of the total serum protein (Pauley et al. 1975), or from the loss of hyalinocytes which carry clotting factors (see Defensive Responses). There is some evidence for the destruction of the alpha subunits of hemocyanin (Pauley et al. 1975). The loss of hemocyanin indicates that death is due to a combination of

hypoxia and nutrient depletion (Pauley et al. 1975).

Amoebae do not generally occur in the hemolymph until late in the course of the infection. In heavy infections, amoebae can reach densities as high as 238,500 cells per mm<sup>3</sup> (Sawyer et al. 1970). In such infections, the parasite virtually replaces the hemocytes and may thus contribute to the loss of vital functions of the hemolymph, hemocytes, and muscle (Couch 1983). Hemocyte densities were variable in light and moderate infections, but in heavy infections, hemocytopenia was evident (Sawyer et al. 1970). The cause of the decline in hemocyte numbers is unknown, but lysis of phagocytic cells, aggregations of hemocytes, and endocrine disruption of the hemopoietic tissues have been postulated (Johnson 1977b). Johnson (1977b) suggested that a study of the mitotic index of the hemopoietic tissue during the molt cycle in relation to pathogenesis of the infection may help determine the fate of the hemocytes.

Dead or degraded amoebae occur in the lumen of the heart, even in light infections (Johnson 1977b). Free and fixed hemocytes are capable of phagocytizing amoebae; however, the hemocytes are often destroyed during the process (Johnson 1977b). Phagocytic hemocytes form aggregates in the hemal spaces of the antennal gland, not in the hepatopancreas or gills. The phagocytic hemocytes are rarely necrotic (Johnson 1977b). In contrast, in bacterial infections, bacteria-laden hemocytes are deposited in the antennal gland, Y-organ, hepatopancreas, and gills, and such hemocytes frequently form large aggregates of necrotic cells in the hemal spaces (Johnson 1976d). Granulocytes and hyalinocytes phagocytize bacteria, but apparently only hyalinocytes phagocytize amoebae (Johnson 1977b).

Encapsulation and nodule formation are less frequently observed in amoebic infections than in bacterial infections (Johnson 1977b). Hemocytes infiltrate areas with amoebae, especially the regions around the midgut and the antennal gland, but nodule formation is uncommon. The fixed phagocytes of the hepatopancreas do not appear to play a role in combatting the disease (Johnson 1977b). Whereas there is clearly a cellular defensive response, there has been no study on the potential role of specific humoral

factors in ameba-infected crabs. The loss of clotting and the drain on metabolic resources (see below) suggest that humoral factors may be compromised relatively early in the infection.

Few physiological markers have been evaluated in *Paramoeba*-infected blue crabs. Heavily infected crabs have significantly less total protein and glucose in the hemolymph than uninfected crabs (Pauley et al. 1975). Progressive loss of total protein was noted among uninfected, lightly, moderately, and heavily infected hosts. Infected males showed a 79% decrease in total serum proteins compared with uninfected males ( $9.4 \pm 4.6$  [standard deviation] vs.  $45.4 \pm 15.1$  mg ml<sup>-1</sup>). Total serum protein in infected females declined by over 49% ( $7.4 \pm 3.9$  vs.  $14.6 \pm 5.6$  mg ml<sup>-1</sup>). Serum copper levels, a measure of hemocyanin concentration, were reported, but the declines were not quantified. Serum glucose levels declined significantly. Glucose in infected males declined by 59% ( $14.1 \pm 15.8$  vs.  $34.7 \pm 18.4$  mg 100 ml<sup>-1</sup>) and that in infected females by 61% ( $9.4 \pm 10.4$  vs.  $24.4 \pm 18.6$  mg 100 ml<sup>-1</sup>). Serum glucose may not be a good indicator of pathophysiology because it varies considerably with season and physiological state of the organism (Lynch and Webb 1973). Nonetheless, for several heavily infected crabs, Pauley et al. (1975) observed values of zero glucose, indicating that the amebae were capable of outcompeting their host for short term energy resources.

The blue crab is the primary host for *P. perniciosus*. The green crab (Campbell 1984), Jonah crab, and American lobster have also been reported as hosts (Sawyer 1976 as cited by Sawyer and MacLean 1978), but the paucity of reports suggest these decapods are not important reservoirs for the ameba. Interestingly, a *Paramoeba*-like organism has recently been reported in *H. americanus* from western Long Island Sound. It apparently infects the nerves of the lobster and has been implicated in an epizootic that resulted in serious losses to the lobster industry there (Russell et al. 2000). In addition, *Paramoeba invadens* occurs in epizootics in green sea urchins *Strongylocentrotus droebachiensis* (see Scheibling and Hennigar 1997), and *P. pemaquidensis* causes disease in cultured salmonids (Kent et al. 1988; Roubal et al. 1989).

The mode of transmission of *P. perniciosus* remains unknown. Cannibalism may spread the disease (Johnson 1977b) because lethargic and moribund crabs are eaten by conspecifics. Infections, however, have not been experimentally established by feeding infected tissues to naïve hosts (Newman and Ward 1973; Couch 1983). The fact that infected crabs are found in high salinity waters, coupled with the fact that blue crabs are osmoregulators, suggest that cannibalism plays little role in transmission; otherwise, infections should be sustained at moderate salinities. Because mortalities peak in late spring, Newman and Ward (1973) and Couch (1983) speculated that transmission may occur during ecdysis or in post-molt when the carapace is soft. In general, mortalities may result from the stress of handling the pre-molt crab, especially during warm periods.

As in early *Hematodinium* sp. infections, amebae are typically present in the connective tissues surrounding the midgut (Johnson 1977b). This location suggests feeding as a route of entry. The antennal gland also shows early involvement with amebae, hemocyte aggregations, and granulomas. Amebae, however, occurred in the lumen of the antennal gland in advanced stages of the disease (Johnson 1977b). The antennal gland is probably not a portal of entry, but it may represent an exit for the ameba.

Inoculation experiments with the ameba have been inconclusive. Two of seven crabs inoculated with infected hemolymph developed infections and died after 34 and 39 d, but the density of the inocula was not assessed (Johnson 1977b). Progression from light (few individuals in the hemolymph, 1% amebae relative to host cells) to terminal infections (96% amebae relative to host cells) occurred over 1 to 2 weeks (Newman and Ward 1973). This finding is in contrast to Johnson's (1977b) histological study where amebae were found in the hemolymph only in late stages of the infection, and was probably an artifact of Newman and Ward's (1973) focusing on hemolymph instead of connective tissues. Couch (1983) was unable to transmit the disease by inoculation, but no sample size or condition was given. In reports by Johnson (1977b) and Couch (1983), crabs were held at lower salinities than those occurring where infections were normally present. Although

crabs are osmoregulators, hemolymph osmolality decreases significantly at lower salinities (Lynch et al. 1973); this decrease supports the hypothesis that high salinity plays a role in determining the distribution of the parasite.

In rare instances, crabs may be capable of clearing infections, but the evidence is anecdotal. Newman and Ward (1973) found 100% mortality of infected crabs, but they speculated that some crabs may survive the infection. Johnson (1977b) reported that six infected crabs possessed large numbers of dead or dying amoebae. Although two of these crabs died, the remaining four later had light, moderate, or heavy infections.

*Paramoeba perniciosus* infects blue crabs from Long Island Sound south to the Atlantic coast of Florida (Newman and Ward 1973; Johnson 1977b). It has not been found in the Gulf of Mexico (Overstreet 1978). Prevalence ranged from 3 to 30% from South Carolina to Florida (Newman and Ward 1973). Background losses occur at low levels, but epizootics fulminate to cause noticeable mortalities. Around Chesapeake Bay, mortalities occur in shedding houses from May to June and in the dredge fishery from October to February (Couch 1983). During epizootics, prevalences ranged from 17 to 35% in the hemolymph of peeler crabs at shedding facilities on Chincoteague Bay (Sawyer 1969; Sprague et al. 1969; Newman and Ward 1973). Newman and Ward (1973) assessed mortality at 30% per month from Chincoteague Bay. After peak mortality events, prevalence dropped to 8% in trawled crabs (Sawyer 1969). Such declines in prevalence probably reflect host mortality and not a seasonal reduction of disease or an increase in host resistance.

Newman and Ward (1973) found peak prevalences of *P. perniciosus* in June and July, but Johnson (1977b) found a peak of 57% in July during an epizootic (Fig. 31; Newman and Ward 1973; Johnson 1977b). Couch (1983) reported peaks of 20% prevalence in May and June and from October through February. Amoebae apparently overwinter in crabs (Johnson 1977b), but more histological study of overwintering crabs from high salinity areas is needed. Mortalities of the blue crab during winter are often thought to be caused by low water tem-

peratures. Although such mortalities may occur in low salinity regions, the amoeba cannot be ruled out as the cause of death in high salinity regions. Prevalences of over 20% have been reported from winter dredge samples from Chincoteague Bay (Couch 1983). Winter prevalence in lower Chesapeake Bay near the York Spit Light ranged from 3 to 13%, and was 3% in July 1969. Couch (1983) speculated that the lower Bay was not optimal habitat for *P. perniciosus*. The higher winter prevalence suggests that either crabs acquire the infection in late fall, or that infected crabs may be moving into the lower bay to overwinter.

### Future Research

*Paramoeba perniciosus* should not be introduced to the Gulf of Mexico. To avoid this, transportation of live crabs should be eliminated or minimized between Atlantic and Gulf states. Survival analyses and mortality estimates from amoeba-infected crabs are mostly anecdotal; they require better documentation. Infection and transmission studies with larger sample sizes should be pursued to investigate the issues of overwintering, the role of host defenses, and the prospect of alternate hosts in the life cycle of the amoeba. Because the parasite primarily occurs in the connective tissues, molecular techniques should be developed for detecting the parasite without killing the host. One possible technique may be to sample an autotomized leg or develop a relatively benign

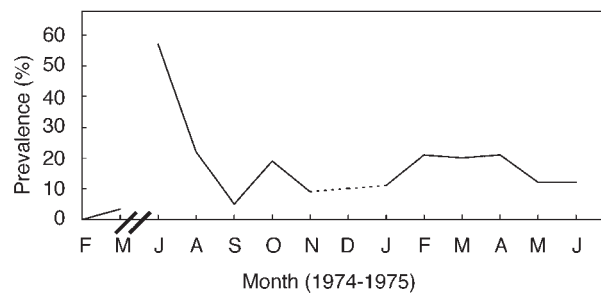


Figure 31. Prevalence of *Paramoeba perniciosus* over time in Chincoteague Bay from 1974 to 1975. Redrawn from Johnson (1977b). Dotted line indicates projected increase in prevalence during unsampled months. Hash marks indicate additional unsampled months.



needle biopsy for later testing with molecular primers or probes.

### Ciliophora

The blue crab hosts a variety of ciliates, both in terms of species, taxonomic groups, and potential for affecting health. Most of these occur externally on the gills or appendages. Because of this external habitat, infestations are generally strongly influenced by environmental conditions. On the other hand, internal ciliates are also affected, albeit indirectly, by environmental conditions.

#### *Mesano-phrys chesapeakensis*

Most ciliates associated with crustaceans are epibionts or ectocommensals. Records of internal ciliate infections in crustaceans have a long but sparse history (see Morado and Small 1994, 1995). With the exception of *Synophrya parasitica*, which has not been reported from *C. sapidus*, internal infections in the blue crab are considered to be cases of facultative parasitism generally caused by *Mesano-phrys chesapeakensis* (as *Anophrys* and *Paranophrys*). Infections occur more frequently in captive or injured hosts than in healthy, unstressed hosts.

#### Biology

*Mesano-phrys chesapeakensis* is an opportunistic, facultative scuticiliate parasitic in blue crabs (Messick and Small 1996). The ciliate has a fusiform body, 28 to 48  $\mu\text{m}$  long, with 10 ciliary bands (kineties) and three sets of oral polykinetids. Conjugating forms have been observed in the hemolymph. Culture studies have used a modified marine axenic medium and sterile artificial sea water. Both media are capable of supporting growth; albeit, the latter could not maintain the ciliate for extended periods. Cryopreservation with reconstitution was successful at  $-40^{\circ}\text{C}$  from aliquots stored in culture media containing 15% dimethyl sulfoxide, but the results were not consistent. The ciliate grows slowly at  $4^{\circ}\text{C}$ , a temperature encountered during mid Atlantic winter (Messick and Small 1996).

Blue crabs infected with *M. chesapeakensis* show lethargy and poor clotting of the hemolymph. The ciliate invades the connective tissues, the hemal

sinuses, heart, muscle, thoracic ganglion, and hemopoietic tissues (Fig. 32). It is most often observed in the connective tissues and the hemolymph. Infiltration and nodule formation by hemocytes may result from tissue damage caused by the ciliates (Messick and Small 1996).

#### Animal Health and Fisheries Implications

The ciliate occurs in blue crabs from Chesapeake Bay, Delaware Bay, and Assawoman Bay, Maryland. It has an extremely low prevalence of 0.3% (8 of 2500 crabs were infected) (Messick and Small 1996). A ciliate in the hemolymph, possibly *M. chesapeakensis*, in Mississippi estuaries infects both wild and confined crabs (Overstreet and Whatley 1975; Overstreet 1978). High intensity infections have been associated with mortalities from mid-salinity regions in Mississippi.

Transmission of *M. chesapeakensis* remains unknown. As in other protozoal infections, the ciliate may be an opportunist that enters the host either during ecdysis or through a "compromised" exoskeleton (Messick and Small 1996). A ciliate resembling *M. chesapeakensis* has been observed feeding in and around damaged gill cleaners and injured gill branchiae (Shields, pers. obs.). Although the parasite has been cultured *in vitro*, no transmission studies or experimental infections have been attempted. However, inoculation studies with another species from *Cancer magister* indicate a rapid growth rate and high host mortality with major declines in host hemocytes, presumably from phagocytosis by the parasite (Cain and Morado 2001).

#### Peritrich and Suctorian Epibionts

Several peritrich and suctorian ciliates occur as epibionts on the gills, carapace, eggs, and other external surfaces of the blue crab. Most are considered commensals, but at high intensities, some may be implicated in disease. *Lagenophrys callinectes*, *Lagenophrys epistylus*, *Epistylis* sp., and the suctorians *Acineta* sp. and *Ephelota* sp. are commonly found on blue crabs (Couch 1966, 1967; Sawyer et al. 1976; Overstreet 1978; Couch and Martin 1982). There have been few studies on their relationships with the host.

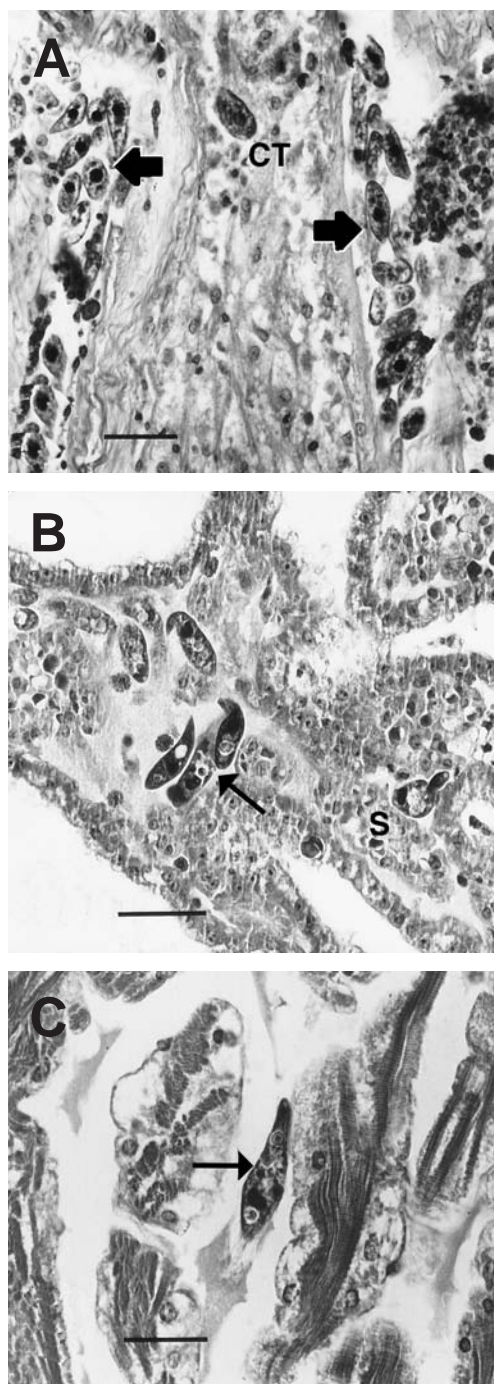


Figure 32. *Mesanophrys chesapeakensis* in *Callinectes sapidus*. (A) Ciliates (arrows) within the thoracic ganglion and in hemal spaces; connective tissue [CT]. (B) Ciliates (arrow) within antennal gland; a ciliate with an amorphous shape is noted [S]. (C) Ciliate (arrow) insinuated between fibers of thoracic muscle. From Messick and Small (1996). Bar in (A) and (B) = 35 $\mu$ m; bar in (C) = 20  $\mu$ m.

### Biology

*Lagenophrys callinectes* is an ectocommensal, loricate peritrich ciliate that shows distinct host specificity much like most species in the genus (Corliss and Brough 1965; Morado and Small 1995). It lives in a yellowish lorica, a transparent encasement, cemented to the flat surfaces of the gill lamellae of at least three species of *Callinectes* (Fig. 33). The distinctive protective lorica is 48 to 59  $\mu$ m long by 45 to 57  $\mu$ m wide and often remains on the crab or its molt even after the death of the ciliate (Couch and Martin 1982). The body of the ciliate has one medio-dorsal ciliary band (kinety), one set of polykinetids, and one set of haplokinetids. The lips of the buccal aperture are split into four elements and are useful in diagnosing the species. Asexual reproduction is through binary fission with the formation of a telotroch larva. Sexual reproduction involves the fusion of microconjugants with macroconjugants, but little else is known (Couch 1967).

*Epistylis* sp. is a stalked peritrich ciliate from the gills of the blue crab (Fig. 34). Members of the genus are typically host generalists (Nenninger 1948), but the species on the blue crab probably is restricted to

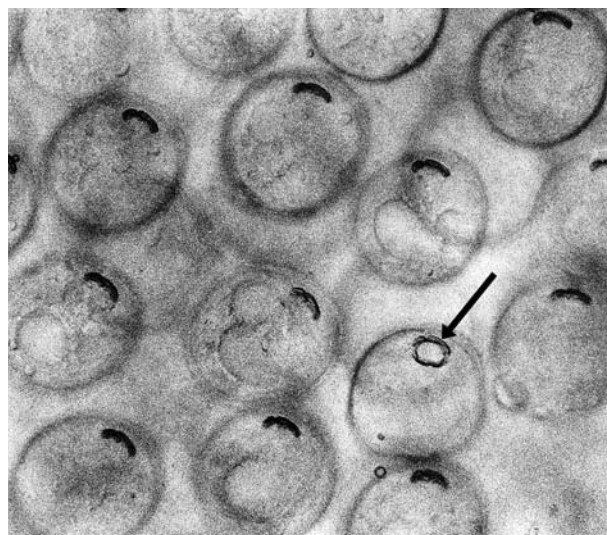


Figure 33. Heavy infestation on the flat portion of gill lamellae of the peritrich ciliate *Lagenophrys callinectes* from the northern Gulf of Mexico. Note ciliate within the transparent loricate with conspicuous aperture (arrow).



relatively few crab hosts. On the blue crab, the ciliate occurs in the margins and stems of the gill lamellae (Overstreet 1978) and infrequently on the eggs or pleopods of the females.

*Acineta* sp. is a suctorian ciliate that lives interspersed with *L. callinectes* on the flat surfaces of the gill lamellae (Overstreet 1978). Signs of a prior infestation include the remains of their small, disk-shaped holdfasts on the surface of the gill lamellae (Overstreet and Shields, pers. obs.). On *Portunus*

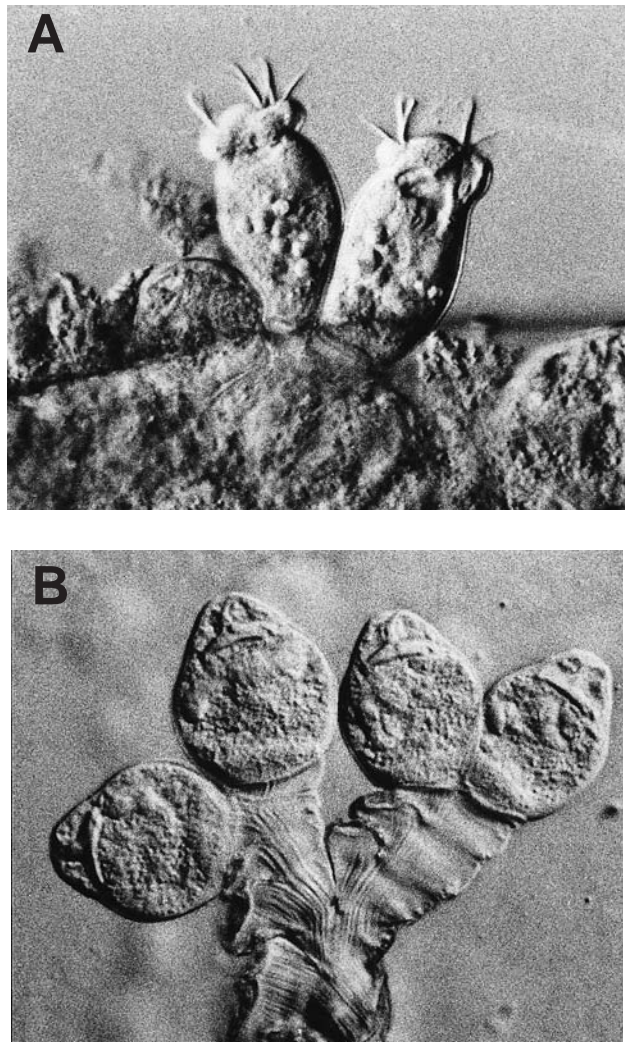


Figure 34. Infestation of *Epistylis* sp. on margin of gill lamella of blue crab from northern Gulf of Mexico. (A) Feeding specimens with cilia in motion and spherical food vacuoles at mid level. (B) Constricted specimens with cilia withdrawn. Note stalk with a few individual zooids missing.

*pelagicus*, a related species of *Acineta* showed a distinct preference for mature female crabs (Fig. 35) (Shields 1992). Little else is known about infestations of *Acineta* sp., but it does not seem to harm its host.

### Animal Health and Fisheries Implications

Peritrich ciliates do not penetrate the gill tissues of their crustacean hosts (e.g., Foster et al. 1978). In heavy infestations, they may occlude portions of the lamellae and interfere with respiration or excretion of the gill tissues (Couch 1967; Couch and Martin 1982), but this has not been examined in detail. Couch (1966) associated the high prevalence and intensity of *L. callinectes* on gills of crabs in floats, shedding tanks, and traps with high mortality. In observations lacking appropriate controls, he reported that several dying crabs maintained in running seawater for 3 months had extremely heavy infestations when compared to those on crabs fresh from Chesapeake Bay. Mortality could also involve a combination of other disease-causing agents, crowding, and lowered oxygen tension. Any of the sessile ciliates and other symbionts can compete with their host for available oxygen and can cover much of the cuticle, not allowing sufficient gas exchange between gill and water or excretion to maintain a healthy condition. For example, penaeid shrimp have suffered mortalities caused by heavy infestations

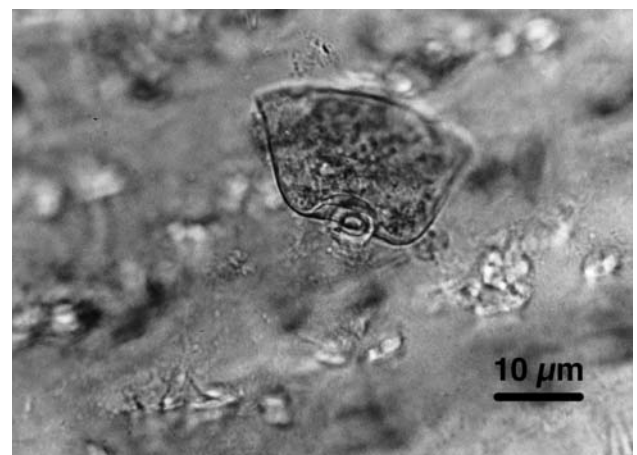


Figure 35. The suctorian *Acineta* sp. on a gill lamella of *Portunus pelagicus*. *Acineta* spp. are relatively common on the gills of portunid crabs.



of peritrichs in stressful culture conditions (Overstreet 1973). Heavy loads of peritrichs probably induce or add to the stress level of an infested crab. Experimental work with another gill symbiont, the gooseneck barnacle *Octolasmis muelleri*, indicates that heavily infested crabs are in a functional state of heavy exercise (see External Barnacles below; Gannon and Wheatley 1992, 1995). Heavy infestations of ciliates may cause a similar condition.

*Lagenophrys callinectes* is abundant along both the Atlantic and Gulf coasts. It ranges along the Atlantic coasts of North and South America and the Gulf of Mexico (Couch 1967; Overstreet 1978; Clamp 1989). The ciliate is probably the most common symbiont of the blue crab. Couch and Martin (1982) reported a low prevalence of infestation from December through April in Chincoteague Bay when crabs were mostly in winter dormancy and largely buried in the mud or sand (Fig. 36). As temperatures increased from April to August, the prevalence increased as did the mean intensity of infestation. In Mississippi, prevalence and intensity are less correlated with temperature. The water temperature is relatively high most of the year, promoting higher intensities of infestation. The frequent molting of the host results in individuals periodically harboring few, if any, organisms. When the crab molts, the infestations are lost, but the ciliates appear to respond to the upcoming molt, perhaps cued by ecdysal fluids,

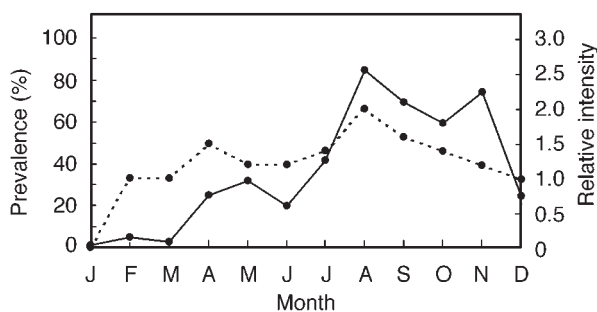


Figure 36. Prevalence (solid line) and relative intensity (dashed line) of *Lagenophrys callinectes* infestations on *Callinectes sapidus* from Chincoteague Bay, 1969. Redrawn from Couch (1983). Relative intensity is a semi-quantitative scale from 0, not infected, to 3, heavily infected.

allowing them to produce reproductive stages capable of re-infesting the fresh cuticle.

*Epistylis* sp. is generally less abundant than *L. callinectes* but also occurs along both Atlantic and Gulf coasts. In Mississippi, *Epistylis* sp. and *Acineta* sp. are typically found on old or heavily fouled crabs (Overstreet 1978). Molting rids the crab of the epibionts (Couch 1967); reinfestation of the related portunid *P. pelagicus* occurs quickly by similar external ciliates (Shields 1992).

### Apostome Ciliates

Apostome ciliates include both external and internal symbionts of invertebrates. On crustaceans they are generally nonpathogenic and feed on the exuvial fluids that remain within the molt. Small tomites settle and encyst as phoronts on the exoskeleton or gills of their host. Just before ecdysis, the phoronts develop into large tomonts and undergo rapid division to produce trophonts, which feed on exuvial fluids from host ecdysis. Two non-pathogenic species, *Gymnodinoides inkystans* and *Hyalophysa chattoni*, occur on the gills and carapace of the blue crab (Bradbury 1994). Surprisingly, the moderately pathogenic, histotrophic apostome *Synophrya hypertrophica* has not been observed in *C. sapidus* (Johnson and Bradbury 1976). Offshore specimens of *C. sapidus*, however, have not been examined. In other brachyuran decapods, including several portunids and especially those offshore in high salinity (>32) waters, the trophont of *S. hypertrophica* burrows into gill lamellae and causes localized damage to the site of infection. There it feeds on hemolymph and surrounding host tissues and eventually encysts as a tomont. The host response includes hemocytic encapsulation of the affected area, with subsequent melanization to encapsulate the intruder. The tomont normally divides preceding host ecdysis, producing trophonts that feed on the exuvial fluids (Johnson and Bradbury 1976; Bradbury 1994).

### Future Research

Blue crabs from high salinity waters should be assessed for ciliate infections, especially *S. hypertrophica*.

*trophica*, which infects a multitude of decapod species including several other portunid crabs (Johnson and Bradbury 1976; Haefner and Spacher 1985). The possible role of high intensity infections of *L. callinectes* in the health of the blue crab should be critically examined.

### Other Protozoans

*Isonema*-like euglenoid flagellates are extremely rare in the hemolymph of blue crabs (Fig. 37). They have been observed in the hemolymph on three separate occasions during field and culture studies with *H. perezii*. These flagellates are relatively easily cultured in blue-crab saline augmented with 10% fetal bovine serum, and, anecdotally, they do not appear to be pathogenic when inoculated into blue crabs (Shields, unpublished data). *Isonema*-like flagellates occur as pathogens in the larval geoduck *Panope abrupta* from Washington and at least as commensals in the hemolymph or mantle cavity of the eastern oyster from Maryland (Kent et al. 1987; Nerad et al. 1989). Some euglenoid flagellates, however, are relatively common pathogens of copepods (see Bradbury 1994).

Gregarine and coccidian apicomplexans are notably absent from *C. sapidus*. These protozoan groups are well represented in other crustacean hosts, including several common portunids (e.g., species of *Nematopsis* and *Aggregata* in species of

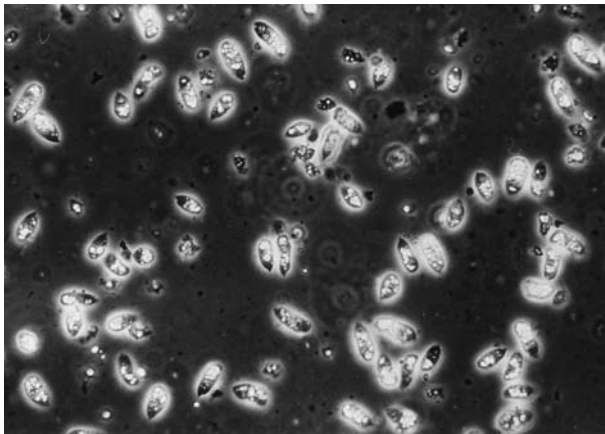


Figure 37. *Isonema*-like ameboflagellate cultured from the hemolymph of a blue crab from Chesapeake Bay (phase contrast).

*Carcinus*, *Necora*, and *Portumnus*) (Vivarès 1974). These and other gregarines and coccidians use molluscs as additional hosts in their life cycles. The lack of these parasites in blue crabs is even more surprising, because blue crabs eat a large number of molluscs.

## HELMINTHS

### Digeneans

Digeneans, flatworms that are also known as trematodes or flukes, are common parasites of the blue crab. Although some species from other hosts are quite large, those in the blue crab are all encysted metacercariae, and most are so small that one may not realize they are present. Digeneans are generally hermaphroditic platyhelminths that typically have a selectively absorptive tegument, a blindly ending alimentary tract, and two suckers. Each species has a complicated life cycle that includes a molluscan first intermediate host, usually a second intermediate host, occasionally a secondary or additional intermediate host, and a definitive host, which is usually a vertebrate such as a bird, fish, or mammal. Unlike the cestodes, which nearly always occur as adults in the alimentary tract of vertebrates, many adult flukes occur in a variety of sites in addition to the intestine. Nevertheless, those that use the blue crab as their intermediate host mature in the intestine of their definitive host.

### Biology

All digeneans reported from the blue crab belong in the family Microphallidae *sensu lato*. Consequently, they all have a similar general pattern in their life cycle, and this pattern will be represented by that of *Microphallus basodactylophallus*, the most prevalent species in *C. sapidus* (Fig. 38; Heard and Overstreet 1983; Overstreet 1983). The 450- $\mu$ m long adult occurs naturally, sometimes in very large numbers, in the intestine of the raccoon *Procyon lotor* or the marsh rice rat *Oryzomys palustris* and experimentally in mice or rats. Eggs of the flukes are dispersed in tidal marshes with the definitive host's feces and are subsequently eaten by one of at least

six different hydrobiid snails from four genera. Once the egg is ingested by the proper snail species, a miracidium hatches. Its germinal cells infect the snail and produce a mother sporocyst that reproduces asexually to form daughter sporocysts, which in turn produce a continual release of many free-swimming, tailed cercariae. In other words, the products of one egg (and millions can be deposited daily from a moderately infected raccoon or rat) can result in

hundreds to thousands of these 90- $\mu\text{m}$  long larvae with their 90- $\mu\text{m}$  long tails. The cercaria swims erratically, stopping when disturbed so that the respiratory currents of the crab sweep it into the branchial chamber where it subsequently penetrates a gill. The cercaria produces around itself a thin penetration cyst that permits leverage for that cercaria, along with assistance of its movable stylet, to penetrate the gill and enter the circulatory system of the

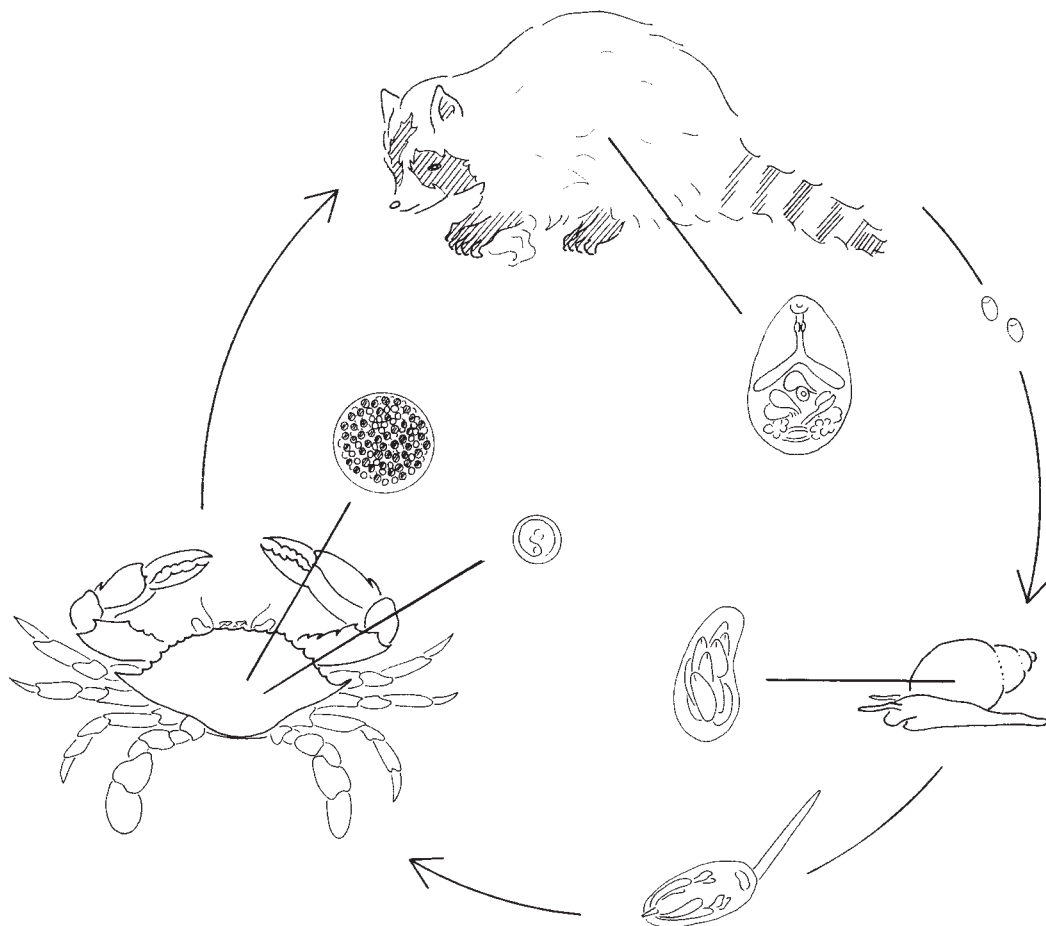


Figure 38. The life cycle of the digenean *Microphallus basodactylophallus*. The adult worm in the intestine of the raccoon, rice rat, Norway rat, and at least certain other mammals matures rapidly and deposits eggs in the host's feces. The feces and associated eggs are eaten by any of several species of hydrobiid snails. The miracidium in the egg produces a sporocyst that reproduces itself asexually and ultimately produces large numbers of swimming cercariae. The cercaria penetrates a crab and develops into an encysted metacercaria. After a period of development, the stage is infective to the raccoon and other hosts, and the cycle continues. The larger darker spherical cysts in the crab are commonly called "buckshot." This is a hyperparasitized cyst of *M. basodactylophallus* containing the haplosporidian *Urosporidium crescens*. This protozoan debilitates the worm without harming the crab. From Overstreet (1978).



crab. The now tailless larva ends up as a metacercaria in the skeletal muscle, hepatopancreas, connective tissue, or nervous tissue where it and the crab produce a thin, layered, spherical, encircling cyst about 230  $\mu\text{m}$  in diameter (Fig. 39). After about 25 d, the encysted metacercaria looks quite similar to the adult stage and becomes infective to any definitive host that feeds on the crab (Heard and Overstreet 1983). Bridgman (1969) reported larger cysts, up to 355  $\mu\text{m}$  in diameter, that did not develop the thick cyst wall after 40 d. Heard and Overstreet (1983) found at least three species of fiddler crabs that can also serve as the secondary intermediate host. The worm in the definitive host starts producing eggs within 48 h of infection and starts the cycle over again. One can appreciate the large number of eggs, larvae, intermediate hosts, and definitive hosts necessary to assure completion of the life cycle. The other microphallid species indicated below have fewer hosts and are not as common.

As indicated above, the metacercarial cyst of *M. basodactylophallus* is relatively small, about 300  $\mu\text{m}$  in diameter, relatively clear, and very difficult to see without a microscope. However, when the metacercaria is hyperparasitized by the haplosporidian *Urosporidium aescens* (Fig. 21), it enlarges several times its normal volume to greater than 650  $\mu\text{m}$  diameter and takes on the brownish black coloration of the haplosporidian spores infecting the worm. The hyperparasite was described in the previous section on Haplosporidia; it makes the metacercaria readily visible to a seafood consumer or biologist.

At least five other species of Microphallidae infect the blue crab. Two of these are *Microphallus nicolli* (reported by a few authors as *Spelotrema nicolli*), which occurs in cysts up to 0.5 mm in diameter in the connective fibers extending from the viscera to the bases of the walking legs, and *Megalophallus didontis*, which occurs primarily along the base of the gill filaments where it can impede the flow of blood. *Microphallus basodactylophallus* has occasionally been misidentified as one of those two species (Heard and Overstreet 1983). Additionally, Hutton (1964) reported *Microphallus pygmaeum* in the blue crab from the area of Tampa Bay, Florida, but did not indicate the site in the host or reference to any

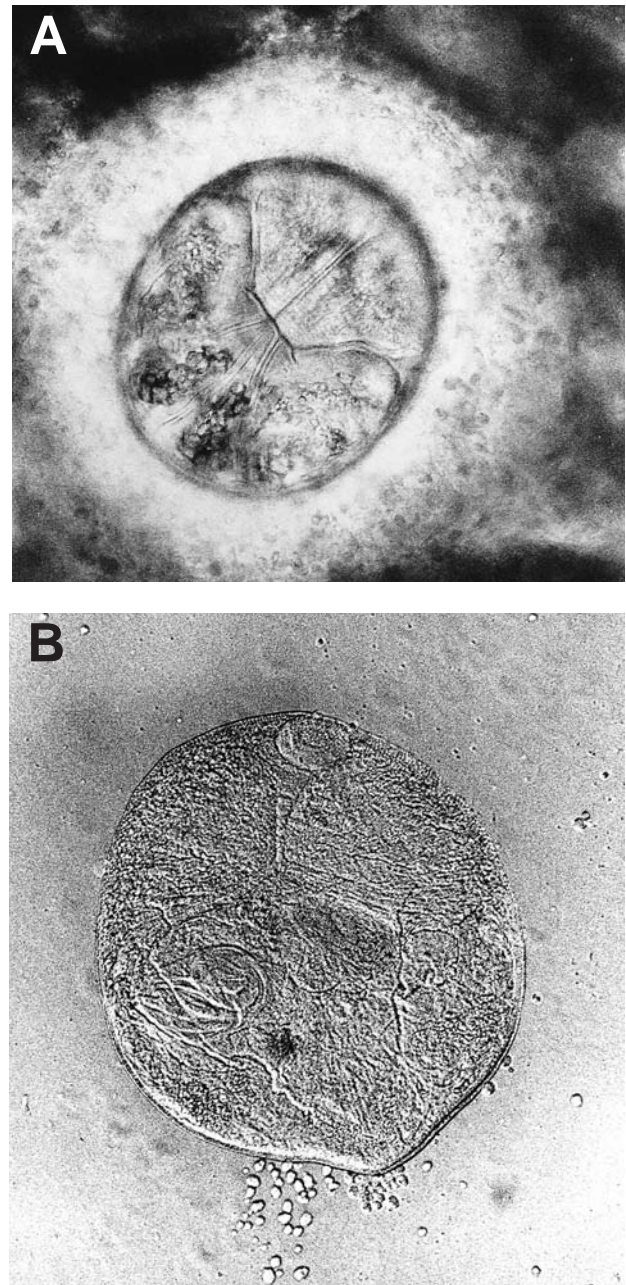


Figure 39. Live metacercariae of *Microphallus basodactylophallus* from hepatopancreas; specimens more commonly occurring in skeletal muscle tissue. (A) Encysted worm with thick cyst wall. Note rolled up specimen. (B) Specimen removed from cyst under coverslip pressure.

morphological data. The record is probably also a misidentification of *M. basodactylophallus*. Possibly the same species has been tentatively and incorrectly suggested as being *Microphallus simile*.

Additional described and at least two undescribed species of microphallids in the blue crab are presently under study by Heard and Overstreet. One of these, *Levinseniella capitanea*, differs considerably from *M. basodactylophallus*. It reaches nearly 4-mm long in the fixed state after being excysted from its spherical yellowish cyst and becomes even longer in the live state. In fact, this species is the largest member of this rather abundant digenean family. Also unusual but not unique to the family, the species has little or no gut and no well-developed pharynx and thereby receives its nutrients almost entirely through the tegument (Overstreet and Perry 1972). It also appears to be restricted to the raccoon and a specific fish definitive host rather than any of a variety of vertebrates. Heard, Semmes, and Overstreet (unpublished data) did not see it in birds and mammals that presumably had fed recently on infected crabs, and they could not establish it in rats, mice, or chicks, experimental hosts for a variety of other microphallids (Overstreet 1983). The cyst, appearing like tapioca among the gonads and hepatopancreatic tubules, measures up to 1.2 mm in diameter and can be seen easily with the unaided eye.

### **Animal Health and Fisheries Implications**

*Microphallus basodactylophallus* is probably the most wide ranging of the digeneans in the blue crab. Its range extends from at least Chesapeake Bay to Texas and probably to Costa Rica and further south, if it is indeed a junior synonym of *Microphallus skrjabini* as questioned by Heard and Overstreet (1983). Prevalence of infection varies considerably, depending on location and environmental conditions. For example, values ranged from 85% at Pass a Loutre to 0% at Bonne Carre Spillway, Louisiana (Bridgman 1969). The cercaria from one snail, *Litterodinops palustris*, appears to encyst in the thoracic ganglion only (Heard and Overstreet 1983). Its presence could influence the behavior of the crab host and put it in more jeopardy of being eaten by a predator

than a cohort not infected in the nervous tissue. Whereas this particular host-parasite relationship may facilitate the completion of the life cycle and serve the worm's population, it may be more detrimental to the individual or stock of infected crabs. Regardless, sufficiently high numbers of this metacercaria or any of the other species of microphallids in any tissue can weaken or kill a crab, especially if the infection occurs in a critical organ (Heard and Overstreet 1983).

Infection with *M. basodactylophallus* hyperparasitized by *U. crescens* also affects the aesthetic appearance of the infected crab. Fishermen, biologists, and seafood consumers readily see the cysts in both fresh and cooked crabs, especially in the mass of muscle at the bases of the swimming legs. They refer to these cysts as "buckshot" or "pepper spot" and to infected crabs as "pepper crabs." These black cysts, occurring from the Chesapeake Bay to Texas, can decrease the marketability of the infected product (Perkins 1971; Couch and Martin 1982; Overstreet 1983; Noga et al. 1998).

*Microphallus nicolli* has been reported as a common metacercaria in the blue crab from the Woods Hole Region, Massachusetts, where the first intermediate host is the cerith *Bittium alternatum*, and an experimental definitive host is the young herring gull *Larus argentatus*. Eggs of the 540- $\mu$ m long worm began passing with the feces 12 h after the bird acquired the infection (Cable and Hunninen 1940). There is no other verified report for this species; however, the 400- to 500- $\mu$ m diameter cysts reported from the blue crab in Rhode Island (Melzian and Johnson 1988) are probably conspecific with *M. nicolli*. The 1-mm long adult of *Megalophallus diodontis* occurs in specific fishes and mammals in Puerto Rico (Siddiqi and Cable 1960) and Florida (Overstreet and Heard 1995). *Levinseniella capitanea* has been reported from Louisiana and Mississippi (Overstreet and Perry 1972), but it extends farther eastwards (Heard and Overstreet, unpublished data).

All microphallids from the crab may impair the normal behavior of the host or may cause death if the intensity of infection is high (Heard and Overstreet, unpublished data). Even though mortalities are probably rare in the natural environment, the

agent should be considered a potential risk in aquaculture ponds. Stunkard (1956) exposed the green crab to large numbers of cercariae of *Microphallus similis*. Infected crabs died after 10 to 20 d. In the natural environment when juvenile blue crabs become heavily infected, they likely become more available as prey, but no data exist to support our assumption. Melzian and Johnson (1988) found a metacercaria encysted in the nerve tissue of 22 of 114 crabs in Rhode Island that were being used to determine the effects of No. 2 fuel oil on the crab. The unidentified worm, possibly *M. nicolli*, selectively infected the nerves in the hepatopancreas and muscle tissues. The authors detected localized compression and distortion of some nerves as well as peripheral-nerve necrosis and hemocytic aggregations in the vicinity of many cysts. Nevertheless, they, unlike Sparks and Hibbits (1981) who studied a metacercaria in the nerves of *Cancer magister*, detected no indication of ataxia.

### Public Health Implications

Some human cultures promote the use of raw crabs to enhance the flavor of a dish. The flavor of the blue crab would certainly enhance the recipe, but we definitely do not promote the practice of eating the blue crab raw. In addition to normal communities of *Vibrio parahaemolyticus* and other bacteria in and on the crab, which if not heated could cause gastric distress, *M. basodactylophallus* is a potential human pathogen. Whereas some of the other microphallids in crabs might also be able to infect humans, *M. basodactylophallus* is a stronger candidate. In the Philippines, the closely related *Microphallus brevicaeca* from a prawn has been implicated in adverse and fatal involvement of the heart, spinal cord, and other organs of people who eat the product raw (Africa et al. 1935, 1936, 1937). The irony is that metacercariae hyperparasitized by the haplosporidian probably keep American consumers from eating raw crabs, although it is not those individual metacercariae but the uninfected indistinct cohorts that are potentially harmful to humans (Heard and Overstreet 1983). Cooking blue crabs properly eliminates all risk of acquiring any parasite.

### Future Research

Digeneans offer a range of research problems. For example, do cercariae from specific snail hosts locate in specific sites within the crab (Heard and Overstreet 1983)? Do infections in the nervous tissue make the crab more vulnerable to predation? Melzian and Johnson (1988) found no indication of ataxic behavior in infected crabs. However, we stress the need to compare the effect of infections in a range of different sizes of juveniles and adults.

Taxonomic and life history studies of digeneans are presently underway. Other studies to help assess crab-fluke relationships include determination of geographic ranges, optimal and threshold salinity and temperature values, intensity necessary to produce morbidity by size of crab, ability to infect humans or human models, and ability to serve as biological tags, or indicators of host migration and position in food webs.

### Cestodes

Cestodes are tapeworms, and like the digeneans, they are all parasitic and mostly hermaphroditic. With few exceptions, adult tapeworms are restricted to vertebrate hosts. In addition to the blue crab, a variety of crustaceans serve as intermediate hosts for many marine cestodes (Overstreet 1983). Both the metacestode and the adult of most cestode groups have a diagnostically shaped scolex, or holdfast organ, that the adult uses to maintain itself in contact with the host tissue, which usually is the intestine.

Although rare, the metacestode (juvenile or incorrectly "larval") stage of at least a few species of cestodes infect the blue crab. The metacestodes that occur in the crab are relatively small, requiring microscopic examination of the viscera and skeletal muscles of the host. They occur free, encysted, or encapsulated, depending on the group of cestode. Regardless of which group, those metacestodes known from the blue crab all mature in an elasmobranch definitive host.

### Biology

In Mississippi, at least the trypanorhynch plerocercoid metacestode of *Prochristianella* sp. infects the



hepatopancreas of the crab (Fig. 40). This is the first report for a member of this genus in the blue crab. However, DeTurk (1940a) reported an infection by the plerocercoid of the trypanorhynch *Rhynchobothrium* sp. in North Carolina. It was encysted in the tissue surrounding the body cavity. This species, 5.6 to 11.3 mm long by 1.1 mm wide with two bothridia (as "suckers"), may well be conspecific with *Prochristianella* sp. from Mississippi, although the illustration by DeTurk (1940a: his Fig 31) is not diagnostic. Another member of the genus *Prochristianella* has a life cycle that has been partially determined by Tom Mattis (see Overstreet 1983). A dasyatid stingray is the definitive host, as suspected for the species from the blue crab, and the filamented eggs from the adult

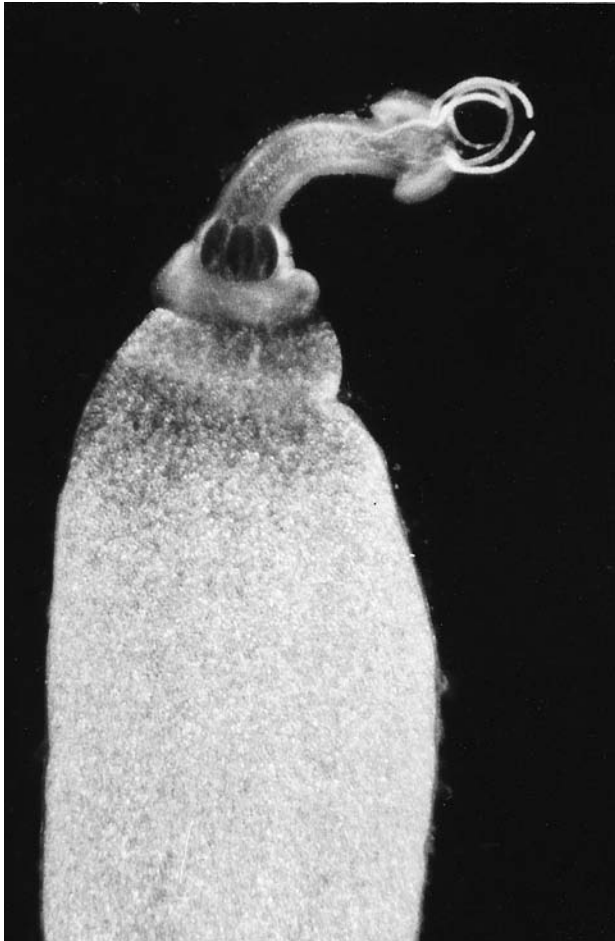


Figure 40. Removed metacystode stage of trypanorhynchean *Prochristianella* sp. from the hepatopancreas of a Mississippi blue crab.

worm are released in the feces of the ray. A harpacticoid copepod and presumably other specific copepods become infected by eating the egg, which contains the larval cestode. Whether the crab also can become infected from an egg or whether the crab would have to feed on an infected copepod to become infected is uncertain. Regardless, the ray probably becomes infected by feeding on the crab.

Other cestode plerocercoids also occur in *C. sapidus*, but none of these has been identified, described (e.g., Overstreet 1978), or seen in recent years. Hutton (1964) reported the lecanicephalan *Polypocephalus* sp. from the blue crab in Tampa Bay, Florida. However, he also reported and illustrated the same or similar species tentatively as a lecanicephalan from penaeids and other decapods earlier (Hutton et al. 1959). The species occurring in the intestine of penaeids was not identifiable, but it was definitely not *Polypocephalus* sp. (Overstreet 1973). Infection by the cestode in the blue crab probably occurs in high salinity water. A lecanicephalan, *Polypocephalus moretonensis*, occurs in large numbers in the thoracic ganglion of *Portunus pelagicus* from Australia, but it does not appear to harm the host (Shields 1992).

Another unidentified small tapeworm metacystode, about 200  $\mu\text{m}$  long by 25  $\mu\text{m}$  wide and possibly a tetraphyllidean (Fig. 41), occurs in high numbers free in the muscle tissue of at least the lesser blue crab *Callinectes similis* in Mississippi (Overstreet 1978); what appears to be the same species occurs in the same site in the blotched swimming crab *Portunus spinimanus* and in the shelligs *Callinectes ornatus* in North Carolina (DeTurk 1940a). As indicated above, this species or a complex of species probably incorporates elasmobranch definitive hosts in its life cycle.

### **Animal Health and Fisheries Implications**

*Rhynchobothrium* sp. infected just 2 of 83 specimens of the blue crab in the vicinity of Beaufort, North Carolina (DeTurk 1940a), and *Prochristianella* sp. (which may be conspecific, see above) was also uncommon in crabs in Mississippi. The geographic range of none of the trypanorhynch metacystodes in

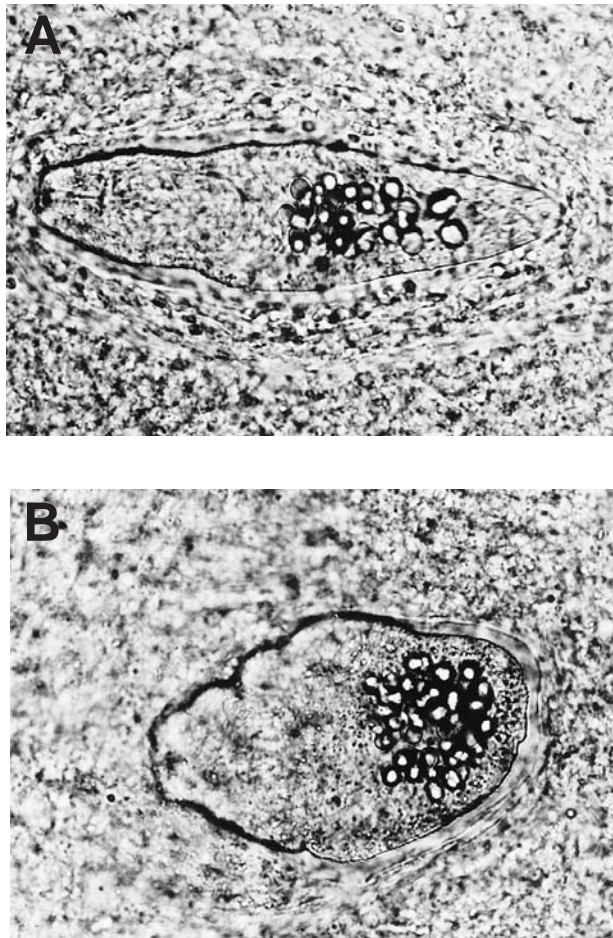


Figure 41. Metacestode of non-encapsulated unidentified tetraphyllidean moving within skeletal muscle of *Callinectes similis*. (A) Specimen in extended position. (B) Same specimen in constricted position moving by "inchworm" fashion through tissue. From Overstreet (1978).

the blue crab is certain. Because of the low prevalence of infection in North Carolina, DeTurk (1940a) suggested that the crab was not the normal host. Richard Heard (The University of Southern Mississippi, personal communication) has observed what may be the same cestode in *Callinectes marginatus* from the Florida Keys. Because of the relatively high host specificity in related trypanorhynch, we suggest that the blue crab is the normal host, but that infections probably occur more often in individuals in high salinity water than has been recognized previously. The unidentified plerocercoid in *Portunus spinimanus* and *Callinectes ornatus* in North

Carolina infected 31 and 8% of those hosts, respectively (DeTurk 1940a).

Once the life cycles of the cestodes in the blue crab are determined, the cestodes could serve as useful biological indicators of host range and migration patterns. Based on the taxonomic position of cestodes encountered to date, we doubt that any cestode influences the health of its hosts or represents any potential human health risk.

### **Future Research**

Critical examination of blue crabs from high salinity areas may reveal metacestodes of additional species that use elasmobranchs as definitive hosts. Such records need to be established. Other cestodes that mature in birds may use the crab in inshore areas as an intermediate host, but none has yet been reported. The identity and life cycles of species reported from the blue crab should be determined or resolved. These will be especially useful for assessing ecological aspects of the host involving migrations, predation, and food webs.

### **Nematodes**

Various species of crabs serve as hosts for nematodes, or roundworms, but the reported species and presumably several others that use the blue crab as an intermediate host do not seem to be specific to the blue crab or to portunids. Nevertheless, a juvenile ascaridoid nematode and an adult "free-living-like" species infect *C. sapidus*.

### **Biology**

The blue crab in the northern Gulf of Mexico is one of many hosts of the juvenile stage of the ascaridoid *Hysterothylacium reliquens* (also referred to as *Hysterothylacium* type MA) (Overstreet 1982). Deardorff and Overstreet (1981a) described this 4 to 9 mm-long third stage juvenile from the hemocoel among hepatopancreatic tubules (Fig. 42). It is often encapsulated, and it has a boring tooth, which allows the worm to penetrate into and migrate through a host such as the blue crab. The crab is a paratenic host, or a host in which no development occurs and that is used as an ecological "bridge," which is not

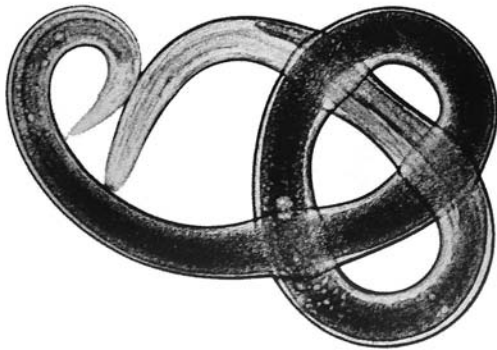


Figure 42. Live partially coiled ascaridoid specimen of *Hysterothylacium reliquens* removed from a host capsule located in the hemocoel of a blue crab from Mississippi. This nematode is a juvenile stage infective to a fish definitive host.

biologically necessary, to infect the definitive host. No significant development of the worm occurs in the crab. The blue crab can acquire its infection from either a copepod or another paratenic host. This nematode, as a third stage juvenile, can, in turn, infect either a wide range of other paratenic invertebrate and fish hosts (Deardorff and Overstreet 1981a) or an appropriate fish definitive host (Deardorff and Overstreet 1981b) in which it will mature and mate with another individual of the opposite sex.

What may be one or more monhysterid nematode species, or at least nematodes that are usually referred to as belonging to “free-living” groups, occur on or in the blue crab in the northern Gulf of Mexico (Mississippi and Louisiana). In this region, they probably infest crabs as symbionts because a few cases were observed where all stages of the life cycle of the unidentified nematodes were present on the gill, with individuals of one of the same species occurring among the hepatopancreas. The monhysterids *Diplolaimella ocellata* and *Theristus* cf. *bipunctatus* occurred in the gill chamber of an unidentified species of *Callinectes* in the Caribbean area (Riemann 1970).

Nematodes that superficially are thought of as free-living and that occur on blue crabs are not unique. Several species have been reported from crustaceans, and some appear restricted to crustacean hosts. For the Monhysteridae, a family considered to be a free-living group of marine, brackish, limnic, and terrestrial species with conspicuous cephalic setae and a large pair of round amphids (sensory organs), all members of the genus *Gammarinema*, occur on crustaceans, and some members of related genera also occur on crustaceans (Lorenzen 1978, 1986). For example, *Tripylium carcinicum*, *Monhysterium* aff. *transitans*, and *Monhysterium wilsoni* infested the gill chambers of the land crabs *Gecarcinus lateralis* and *Gecarcinus ruricola* in the Caribbean area (e.g., Baylis 1915b; Riemann 1970). In some cases, no male worm was observed, but hermaphrodites were reported (Baylis 1915b). Numerous free-living nematodes occur in the gill chambers of crayfish. For example, Schneider (1932) reported 14 species in 8 genera (e.g., *Actinolaimus*, *Prochromadorella*, *Chromadorita*, *Dorylaimus*, *Monhysteria*, *Rhabditis*, and *Trilobus*) in the crayfishes *Potamobius* sp. and *Cambarus* sp. from Germany, but only *Prochromadorella astacicola* was found associated consistently with the crayfish. Edgerton et al. (2002) provide more examples of crayfish infestations. Because of the range and habits of the blue crab, it probably is also a good host candidate for a variety of similar nematodes.

### **Animal Health and Fisheries Implications**

*Hysterothylacium reliquens* matures in various fishes, including the red drum *Sciaenops ocellatus*, which feeds heavily on the blue crab in Mississippi, Georgia (Overstreet and Heard 1979a), and elsewhere. It also occurs in other predators of the blue crab (Overstreet and Heard 1979b, 1982) but primarily infects batrachoids (Deardorff and Overstreet 1981b). During some years in Mississippi, it has occurred in such large numbers in the sheepshead *Archosargus probatocephalus* that it was named “*reliquens*” after the evacuation of individuals from the mouth, branchial chamber, and anus of the fish when lying in a boat or on the fisherman’s “cleaning table” (Norris and Overstreet 1975). This is an unusual nematode because it has such diverse groups



of both intermediate or paratenic hosts and fish definitive hosts. Infected hosts occur over a wide range in the Atlantic and Pacific oceans and the Gulf of Mexico (Deardorff and Overstreet 1981b). Because the worm ranges along the Atlantic coastline, the blue crab probably hosts the species there as well. DeTurk (1940a) reported an unidentified and non-illustrated juvenile that may have been *H. reliquens* from among the viscera of two specimens of *C. sapidus* in North Carolina.

Adult and juvenile monhysterids and probably other "free-living" nematodes infest the blue crab in Mississippi and Louisiana and probably elsewhere throughout its range. Even though the crab probably hosts a variety of juvenile nematode species that have wide host specificity, those symbionts may not play an important role in the health of the crab. Also, the crab may not be important in maintaining the life cycle of the nematodes. If crabs are reared in systems excessively rich in detritus and other organic matter, we would expect high levels of "free-living" nematodes, assuming the temperature and other environmental conditions were appropriate. Caughey (1991) exposed the freshwater monhysterid *Gammarinema* sp. from crayfish in Australia to five doses of toltrazuril (Baycox<sup>®</sup>, in doses of 1-50  $\mu\text{g ml}^{-1}$ ) and all doses were effective. Doses of 5  $\mu\text{g ml}^{-1}$  killed all the worms when exposed for 24 h and over 50% when exposed for 2 h.

The hemolymph of some crabs infested by a "monhysterid" nematode and dying in a Louisiana commercial soft-shell crab facility revealed a pinkish color. Bacteria were ruled out as a cause of death, but a virus, perhaps associated with the nematode, was not ruled out, even though there was no obvious light microscopic histopathological evidence (Overstreet, unpublished data).

In summary, at present there is no evidence that any juvenile or adult nematode plays an important role in the health of the blue crab. However, in certain circumstances such as when a young crab experiencing poor water quality becomes infested by numerous monhysterids or when a young crab receives a heavy ascaridoid infection, the crab could be negatively affected.

### **Public Health Implications**

The juvenile of *Hysterothylacium reliquens* possibly could be an irritant to the human alimentary tract, if an infected crab is eaten raw or inadequately prepared for human consumption. Nevertheless, such infections would seldom be intense, and the worm would probably have to be in an early stage of development (Overstreet and Meyer 1981). Digenans are a more common and more serious health risk, if one chooses to eat raw crab.

### **Future Research**

Additional crabs from different localities need to be surveyed, and all of their nematodes identified. For example, some of the different "free-living-like" species from the northern Gulf of Mexico that spend their entire life on the gill of crustaceans need to be identified and their life histories determined. We assume there exists for one species a complicated life history that depends upon the blue crab because all of its stages can be encountered internally and on the gills of the host. Understanding the life history of all the species and their relationship with the environment should provide an important indicator of host biology.

## **Nemertean**

### ***Carcinonemertes carcinophila***

Nemertean worms belong in a small phylum distinguished from flatworms by the presence of a rhynchocoelom, a body cavity housing the proboscis. Nemerteans are important but often overlooked worms of sand and mud benthos. Several species have developed symbiotic relationships that range from commensalistic (e.g., *Malacobdella grossa* in bivalves) to semi-parasitic (e.g., species of *Carcinonemertes* on crabs and lobsters). In general, species of *Carcinonemertes* are obligate, semi-parasitic egg predators that feed primarily on yolk. Epizootics of *C. regicides* and *C. errans* have been implicated in declining stocks of the red king crab *P. camtschaticus* and the Dungeness crab *C. magister*, respectively (Wickham 1979; Hobbs and Botsford 1989; Kuris et

al. 1991). Hence, the presence of nemertean worms should be considered when examining underlying causes of poor recruitment or lost fecundity in crabs or lobsters.

### Biology

*Carcinonemertes carcinophila* is a nemertean worm commonly found externally on the gills and egg masses of female blue crabs (Figs. 43, 44). One species of worm occurs on several species of blue crabs, with two subspecies, *C. c. carcinophila* and *C. c. imminuta*, occurring in the north and south of the ranges of the hosts, respectively (Humes 1942). Juvenile worms and nonfeeding adults live encapsulated in mucous sleeves cemented between the gill lamellae of host crabs. After the host oviposits, the worm moves into the clutch and begins feeding on crab embryos (Humes 1942). While in the egg mass of its host, the adult worm lives in a mucoid, parchment-like tube or sheath, where it lays several hundred (up to 1200) eggs in a gelatinous strand. The worm matures only after it has fed on host embryos. Unlike other species of *Carcinonemertes*, *C. carcinophila* does not reside in the limb axillae between host clutches; rather, worms migrate back into the branchial chamber and encapsulate between the gill lamellae. Although Humes (1942) and Hopkins

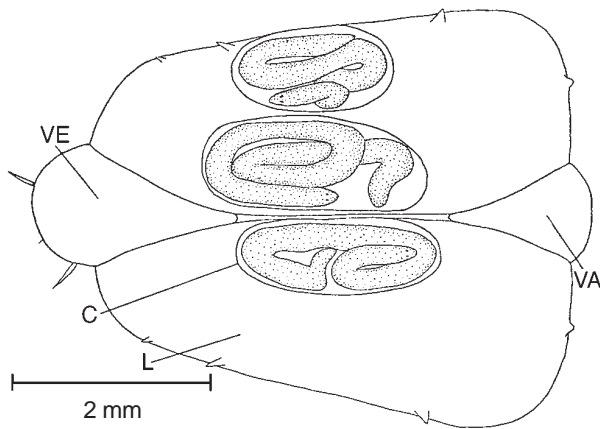


Figure 43. Several specimens of *Carcinonemertes carcinophila* ensheathed on a gill lamella of *Callinectes sapidus*. Afferent vessel [VA]; efferent vessel [VE]; "cyst" or sheath [C]; lamella [L]. From Humes (1942).

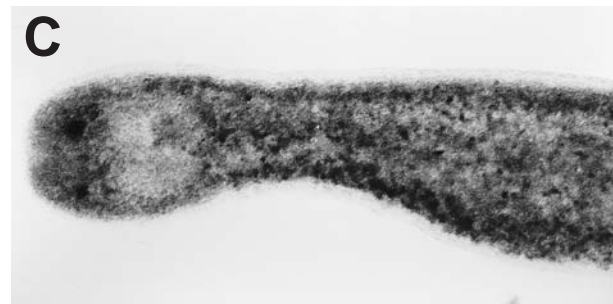
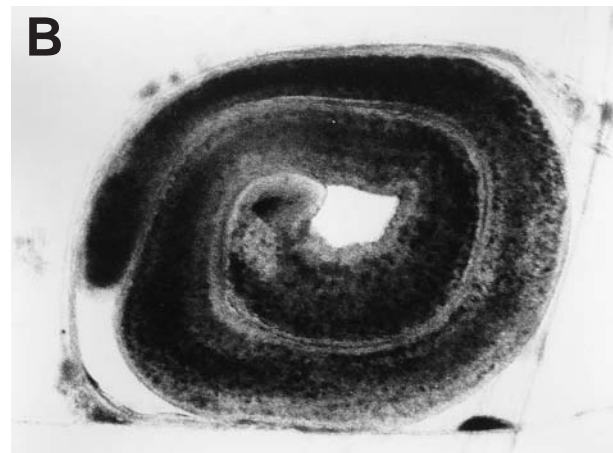


Figure 44. The ribbonworm *Carcinonemertes carcinophila* from between gill lamellae in a high salinity area of Mississippi. (A) Long specimen removed from between two lamellae, leaving additional specimens between adjacent lamellae. From Overstreet (1978). The worms are orangish, demonstrating that they have already fed on host eggs, an indication that the crab has spawned at least once. (B) Close-up of encapsulated specimen. (C) Anterior end of specimen.

(1947) noted that the worms were lost with host ecdysis, other species of *Carcinonemertes* move to the new instar (*C. errans*, *C. epialti*; Wickham et al. 1984) or show no reduction in prevalence between molts (*C. mitsukurii* on *Portunus pelagicus*; Shields 1992; Shields and Wood 1993). This retention of individuals may also occur with *C. carcinophila*.

*Carcinonemertes carcinophila* is a long, filiform, monostyliferous hoplonemertean with a greatly reduced, slightly extrusible proboscis. The species has separate sexes, with the males growing to 20 mm long and females to 25 mm long (*C. c. imminuta*) or 40 to 70 mm long (*C. c. carcinophila*). The male worm has a distinct seminal vesicle known as Takakura's duct (after Takakura 1910, from Humes 1941a). Fertilization of the eggs is internal but may also occur during oviposition (Humes 1942). Embryos develop over 11 to 12 d and hatch as planktonic, highly modified larvae (125  $\mu$ m long), with apical sensory tufts. With several species of *Carcinonemertes*, the larval worms hatch in synchrony with their host crab's zoea (Humes 1942; Roe 1988; Shields and Kuris 1990). Hatching of the worms may be stimulated by the vigorous pumping activity that the female host uses to stimulate the zoeae to hatch (Roe 1988). The newly hatched nemertean larva exhibits positive phototaxis and can swim for several days (Humes 1942; Davis 1965).

### **Animal Health and Fisheries Implications**

The presence of *C. carcinophila* can sometimes be detected by the large number of dead or empty crab eggs in clutches that have not reached eclosion. Egg mortality due to nemertean infections can be high at the periphery of the clutch or at the base of the pleopod, depending upon the degree of phototaxis and the host-parasite relationship of each species (Wickham and Kuris 1985; Shields and Kuris 1988a; Shields and Kuris 1990; Shields et al. 1990a; Kuris et al. 1991). Because adults of *C. carcinophila* are negatively phototactic (Humes 1942), egg mortality due to the worm should be greatest at the bases of the pleopods as is the case for other carcinonemertids (Shields et al. 1990a).

*Carcinonemertes carcinophila* has a broad host

range; it has been reported from 28 species of crabs, mostly portunids (reviewed in Wickham and Kuris 1985). The blue crab, however, is a very common host for *C. carcinophila* (see Humes 1942; Overstreet 1978, 1983). Worms on several of these hosts are likely not *C. carcinophila*, especially those on xanthids. Care should be taken to document histological features and characteristics of the symbiont in each host relationship.

Aspects of the biology of other species of *Carcinonemertes* are relevant to those of *C. carcinophila*. Low salinity appears to limit the distributions of *C. errans* and *C. mitsukurii* (see McCabe et al. 1987; Shields and Wood 1993). Neither Humes (1942) nor Hopkins (1947) examined infestations in relation to salinity. Salinities below 10 may limit distribution of *C. carcinophila*, but controlled studies are needed to define the lower limits and survival times of each species on their hosts (e.g., Scrocco and Fabianek 1970). Infestations of *C. errans* on several species of *Cancer* have been successfully treated with freshwater baths and low doses of malachite green (Wickham 1988; Shields, pers. obs.).

Juveniles of *C. errans* are found between the limb axillae and the sterna of infested Dungeness crabs. There they actively absorb amino acids (Roe et al. 1981). *Cancer magister* and presumably other crabs leak amino acids from the arthodial membranes. These nutrients are sufficient to meet the metabolic needs for the maintenance of juveniles and regressing adults but not reproductive adults of *C. errans* (see Crowe et al. 1982). Interestingly, the gills of blue crabs actively eliminate certain foreign proteins (Clem et al. 1984), and consequently they may "leak" nutrients used for the maintenance of *C. carcinophila* in the gills.

Although carcinonemertids have separate sexes, parthenogenesis occurs in *C. errans* on *Cancer magister* (see Roe 1986) and simultaneous sexual hermaphroditism occurs in *Ovicides julieae* on the xanthid crab *Chlorodiella nigra* (see Shields 2001b). Such asexual features are adaptations for low mate-finding potentials due to the rarity of adults, low transmission rates, or relatively sparse distributions of small populations of hosts and symbionts. Roe (1986)



found that both haploid and diploid larvae were produced and later speculated on the ecological significance of parthenogenesis (Roe 1988). Hermaphroditism and parthenogenesis are clear examples of the semi-parasitic adaptations present in the family. Curiously, several species of *Carcinonemertes* exhibit overdispersed, or contagious, distributions (Shields 1993); hence, their ability to find conspecifics should be high.

Juvenile carcinonemertids occur on both sexes of the crab host, albeit they are much more common on the female. In several carcinonemertid-host associations, the worms are found only on females (Shields and Kuris 1990). On the blue crab, *C. carcinophila* occurs at low prevalence on mature males (1.5%) and immature females (4.2%) when compared with mature (37.2%) and ovigerous females (55.6%) (Humes 1942), and this may result from the salinity preferences between the different host sexes. On male blue crabs, worms may still mature and contribute to the population as they may migrate from the male to the female crab during host copulation (Wickham et al. 1984; Shields, pers. obs.). Unlike some shrimps that preen, or remove, dead eggs from the clutch (Bauer 1979, 1981, 1998, 1999), the blue crab cannot respond to infestation by *C. carcinophila*.

The nemertean can reach high intensities of infestation on the blue crab. Humes (1942) reported an intensity of "at least 1000 worms" on one crab, and over 800 worms have been observed in infestations of the related *C. mitsukurii* on *P. pelagicus* (Shields 1992; Shields and Wood 1993). Hopkins (1947) reported that mature, pre-ovigerous overwintering female crabs had higher prevalence and intensity than those female hosts in less advanced reproductive states. Thus, larval settlement appears timed to occur when the female crab moves to high salinity water to reproduce and then overwinters in the sediment. Interestingly, worms decreased in intensity on post-ovigerous crabs during winter, a decline indicative of senescence or mortality from starvation. Prevalence was not correlated with size of mature crabs (Humes 1942), though intensity-size correlations were not examined. On *P. pelagicus*, *C.*

*mitsukurii* showed significant increase in prevalence with the progression of the ovarian cycle, although there was no increase in intensity (Shields and Wood 1993). This increase suggests that settlement is at least partially dependent upon cues related to the reproductive status and salinity exposure of the host.

In Chesapeake Bay, the prevalence of infestation generally increases through the summer spawning period of the crab and into autumn (Hopkins 1947). Prevalences (reported up to 85%) peak in August and September, or near the end of the spawning period. In Louisiana, sexually mature worms occurred from May through August (Humes 1942), with a prevalence of 27% in Barataria Bay and 13% in the Gulf of Mexico.

*Carcinonemertes carcinophila* can be used to indicate the spawning status of the host. Hopkins (1947) and Overstreet (1983) noted that worms from post-ovigerous females were large and bright reddish in color, but those on pre-ovigerous females were smaller and pale white to pinkish in color. Apparently, egg predation changed the color of the worm. Because the blue crab usually spawns two or more times per season, the presence of brightly colored worms can indicate at least one successful spawning event.

The effect of high intensity infestations of *C. carcinophila* on *C. sapidus* has not been documented. In other carcinonemertid infestations, high intensities can result in the complete loss of the egg clutch, a condition resembling parasitic castration (e.g., Shields and Kuris 1988b; Kuris et al. 1991). Outbreaks of nemerteans on other species of crabs have contributed to declines in certain fishery stocks (see Wickham 1986; Shields and Kuris 1988b; Kuris et al. 1991). Models indicate that the impact of *C. errans* on population density of *C. magister* is similar in effect to density-dependent recruitment mechanisms (Hobbs and Botsford 1989). Taken together, worm-derived egg mortality and density-dependent juvenile mortality can account for significant fluctuations in the Dungeness crab. Changes in salinity and temperature appear to limit infestations of *C. mitsukurii* on the related portunid *Portunus pelagicus* (see Shields and Wood 1993); such factors probably

limit infestations of *C. carcinophila*. Lastly, carcinonemertid worms represent no human health risk.

### Future Research

Nemertean worms can be ecologically important predators and are often overlooked in habitats where they are abundant. The crab or lobster host is one such habitat. Epizootics in commercially important hosts suggest that *C. sapidus* may be susceptible to outbreaks of *C. carcinophila*. Given that crabs with their high fecundity are expected to experience high larval and juvenile mortality, egg predation reaching over 50% of the clutch clearly represents a significant mortality factor to the host population (Shields and Kuris 1988b). In certain circumstances, egg predation by species of *Carcinonemertes* on hosts other than *C. sapidus* approaches 100% of the clutch and can occur at high prevalences in large sectors of the host population (Wickham 1986; Shields and Kuris 1988b; Kuris et al. 1991). Intensities of infestation, egg predation rates, host settlement patterns, transference at ecdysis, host preferences, migration cues, and other biological aspects are unknown for *C. carcinophila*. Comparative studies would greatly facilitate our understanding of these unusual worms.

The taxonomy of carcinonemertid worms requires observations on live specimens and qualitative histology using serial sections. With the plethora of host species thought to harbor *C. carcinophila*, and the recent description of *C. pinnotheridophila* from the pea crabs *Pinnixa chaetoptera* and *Zaops ostreum* (McDermott and Gibson 1993; McDermott 1998), it is apparent that several species remain to be described from the Atlantic and Gulf coasts.

### Annelids (Clitellata)

Annelids are not usually considered common symbionts of crabs, but a few species are, and these can be prevalent on blue crabs from low salinity water. These are readily seen, are commonly noted by recreational and commercial fishermen, and include leeches and a branchiobdellid worm (e.g., Overstreet and Cook 1972; Perry 1975; Overstreet 1983).

### Leeches (Hirudinea)

#### Biology

The most common leech on the blue crab is *Myzobdella lugubris* (Fig. 45). This piscicolid has a broad distribution that follows that of the blue crab from at least Massachusetts south through the Gulf of Mexico. The blue crab serves as a substratum for the deposition of egg cases and as a means of dispersal for the leech. In shallow, low salinity (<15) habitats rich in vegetation, young leeches attach to fishes and acquire multiple blood meals. Several different species of fishes serve as hosts, sometimes for the same individual leech. The most common fish hosts include *Paralichthys lethostigma*, *Mugil cephalus*, *Fundulus grandis*, *F. majalis*, and *Ictalurus catus*. Large numbers of the leech can infest the skin, fins, gills, mouth, and nostrils of an individual fish. Usually about late

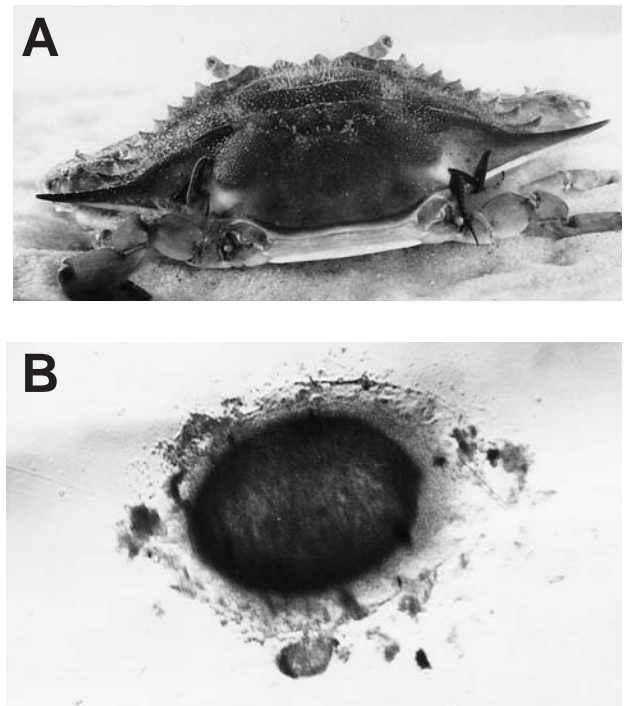


Figure 45. The leech *Myzobdella lugubris* from relatively low salinity in Mississippi. (A) A few engorged specimens near the posterior margin of the carapace. (B) Egg case which is deposited along the carapace's posterior margin. A single leech develops in and hatches from each cocoon to ultimately settle on fish to obtain blood meals. From Overstreet (1978).

autumn, the engorged leech drops off and is associated with vegetation and oyster shells until it can attach to a blue crab. The leech can also attach to a grass shrimp or a penaeid shrimp, but it has a predilection for the blue crab (Sawyer et al. 1975). Once one is on a host, additional specimens are attracted to the same host (Sawyer et al. 1975). The blue crab host is usually an adult male, at least in Mississippi, because most adult females migrate to relatively high salinity water where the leech cannot survive.

On the crab, the leech transforms from a thin, approximately 1 cm-long individual, which appears reddish because the gut is engorged with fish blood and its byproducts, to a larger, more robust, mature individual with a greenish-tan color. The mature leech can extend as long as 4 cm, is hermaphroditic, and presumably mates on the carapace of the crab. These two morphological forms, one from a fish and one from the crab, were once considered to represent separate species. The species can be characterized by having a distinct trachelosome and urosome region on its relatively smooth body that lacks tubercles or papillae. A pair of eyes are found on the small oral sucker which is only slightly wider than the neck and about 3/5 of the width of the caudal sucker. The caudal sucker is continuous with the posterior end of the body and conspicuously narrower than the maximal body width.

A group of mature specimens of *M. lugubris* usually occupies the posterior margin of the carapace of the infested blue crab (Fig. 45). There they deposit large numbers of egg cases (averaging 43 per individual under experimental conditions) and are out of reach of the claws of the crab; crab or shrimp hosts will eat the leech, if able. Egg cases usually are not deposited on the grass shrimp or other crustaceans other than the blue crab. One egg is laid per egg case, and developing egg cases are dark brown versus light tan in non-developing ones. The embryo develops over about 35 d, and the young swimming juvenile, about 1.5 mm long and appearing much like a miniature adult, hatches through a terminal pore. At 23 to 26°C, neither the juvenile nor the adult can survive long at salinities >15; at lower temperatures they can tolerate somewhat

higher salinities. After the young leech starts feeding on the blood of fishes, the cycle continues.

In the Eastern Pacific off Colombia, other species of *Callinectes*, primarily *Callinectes toxotes* and *Callinectes arcuatus*, host a leech identified as *Myzobdella* sp. (perhaps *M. lugubris*). That leech also attaches its egg cases to the posterior margin of the carapace (Norse and Estevez 1977), just as seen on *C. sapidus* in Mississippi and South Carolina (Sawyer et al. 1975; Overstreet 1978).

In addition to hosting species of *Myzobdella*, the blue crab in Mississippi can also harbor *Calliobdella vivida* (see Overstreet 1983). Unlike *M. lugubris*, this leech does not depend on the blue crab to deposit egg cases and to maintain its life cycle. Morphologically, it differs from *M. lugubris* by having lateral pulsatile vesicles and a caudal sucker distinct from the posterior end and roughly equal to the maximal body width.

## Branchiobdellida

### Biology

The branchiobdellids comprise an odd group of relatively primitive annelids that have a close symbiotic relationship with their freshwater crustacean hosts. They require a specific live crustacean to survive or at least as a site to deposit egg cases (e.g., Overstreet 1983). The taxonomic group is usually considered related to oligochaetes, with its suckered species having 15 body segments, no seta, and unpaired gonopores. It is presently considered an order (Martin 2001). What has been reported as *Cambarincola vitreus* occurs on the gills and carapace of the blue crab. The worm is often called a "mullet bug" in Mississippi, possibly because of its resemblance to one of the true leeches that are common on mullet. This leech-like branchiobdellid normally infests crayfish over a wide geographic range in freshwater, but it also occurs on the blue crab from Florida to Louisiana in oligohaline water (Overstreet 1983).

The identification of branchiobdellids from blue crabs presently remains confused. Specimens provided by Gretchen Messick (National Marine Fisheries Service, Oxford, Maryland) from *C. sapidus* in



Chesapeake Bay exhibited similarities to both *C. vitreus* and *Cambarincola osceola* (Overstreet, unpublished data). Gelder et al. (2001) identified *Cambarincola mesochoreus sensu lato* from an unidentified species of *Callinectes* that he received from an uncertain locality in the Gulf of Maine. Additional well-fixed specimens from Maine to Louisiana should permit an understanding of how many species infest blue crabs and how they differ biologically. The fact that specimens occur in large numbers on the blue crab in water with even a minimal concentration of salt is remarkable for the group. The branchiobdellid can be grossly differentiated from *M. lugubris* by its pinkish color and smaller length (approximately 3 mm). Most branchiobdellids feed on detritus, small protozoans, algae, and other microorganisms (e.g., Jennings and Gelder 1979), but a few have been reported to feed on their hosts. There is no evidence indicating that *C. vitreus* feeds on the blue crab host.

### **Animal Health and Fisheries Implications**

The leech *M. lugubris* has a wide geographic distribution on its blue crab host. Further examination may reveal the range of the leech to be even greater, probably comprising the entire range of the blue crab. The association typically occurs in low salinity when water temperature is relatively high (Daniels and Sawyer 1975; Sawyer et al. 1975). In contrast, *Myzobdella uruguayensis*, which apparently differs from *M. lugubris* by having two pairs of eyespots on the oral sucker rather than one, has never been observed on the blue crab in Uruguay (reported as *Callinectes sapidum acutidens*), which is at the southern limit of the crab's distribution (Mañé-Garzón and Montero 1977).

The distribution of *C. vivida* on the blue crab has been reported from Mississippi and Louisiana (Overstreet 1982) and probably includes an occasional infestation throughout the range of the crab. The leech should be most evident on the crab in the Gulf of Mexico during winter and spring when water temperature is relatively low. *Calliobdella vivida* harbors trypanoplasma and hemogregarine protozoans that infect fishes and, under specific conditions, the protozoans can cause mortality of the fish hosts (Burreson and Zwerner 1982). These blood

protozoans do not infect crustaceans. Consequently, the leech may influence the ecosystem by hosting pathogenic protozoans that reduce the fishery stock rather than directly by affecting the crab.

Although a few leeches other than *M. lugubris* or *C. vivida* consume crustacean tissues, *M. lugubris* clearly benefits from its association with *C. sapidus*. Hutton and Sogandares-Bernal (1959) suggested that the leech might be implicated in causing fatal lesions. However, Overstreet (1978, 1979, pers. obs.) questioned any severe action harming the crab host, based on observations of thousands of specimens of *M. lugubris* on crabs that had lesions on the carapace but with no leech nearby. However, there is no reason that leeches would not enter lesions already present, especially on crabs caught in traps or placed in containers. Most crabs with leeches attached have no lesions.

### **Future Research**

Because *M. lugubris* and *C. vitreus* occur externally on blue crabs, often in large numbers and occasionally on dying individuals or on those with lesions, fishermen occasionally think that the organisms inhibit host molting or cause mortality (Overstreet 1983). Consequently, experimental studies should be conducted to determine if the symbionts feed on the host or can cause any pathological effect. Both the leech and branchiobdellid and their egg cases can be indicators that the host crab has spent considerable time in fresh or oligohaline waters. Mortality of *C. vitreus* in fresh water can also be used as an indicator of specific toxicants and water quality. When used in combination with other indicator species that infect the crab, considerable information could be developed on the movements and health of the crab, and on environmental health.

More careful examination of blue crabs might also reveal symbiotic oligochaetes because at least two species of Enchytraeidae infest the gill chambers of gecarcinid land crabs (Baylis 1915a).

## **CIRRIPEDS (BARNACLES)**

The barnacle symbionts on the blue crab comprise an illustrative group because they include

species that are clearly fouling organisms, a symbiont that occurs on a number of different decapods, another that occurs on few crabs other than the blue crab, and another that is a true parasite that lives internally in a few different blue crab species. All of the barnacles influence the blue crab host, but they affect it at different life stages and under different ecological conditions. The true parasite, *Loxothylacus texanus*, may have a major influence on blue crab populations. Overstreet (1982) suggested that *L. texanus* probably influenced blue crab stocks in the Gulf of Mexico more than any other metazoan symbiont. That barnacle will be treated separately below.

### External Barnacles

#### Biology

The fouling balanid barnacles such as *Balanus venustus* and *Balanus eburneus* commonly attach to the carapace of the blue crab (e.g., Scrocco and Fabianek 1970; Overstreet and Cook 1972; Overstreet 1978, 1982). *Balanus venustus niveus* as reported from the northern Gulf of Mexico (Overstreet 1978) has been considered a color variant of *Balanus venustus* (see Henry and McLaughlin 1975), and it often encrusts on appendages and the carapace (Overstreet 1982). These balanomorphs are also mentioned under "Fouling Organisms" (below) because the crab offers nothing more than a hard substratum on which to attach.

The acorn barnacle *Chelonibia patula* has wide geographic and host distributions and is often found on a variety of decapods and the horseshoe crab *Limulus polyphemus*. In the southeastern United States, it has a preference for the carapace of *C. sapidus* (Fig. 46) and *L. polyphemus*, but in the northern Gulf of Mexico it also commonly infests spider crabs (e.g., Pearse 1952); it even has been found on gastropod shells (e.g., *Busycon* spp.) (Gittings et al. 1986). Just like other fouling organisms and *Octolasmis muelleri* (see below), *C. patula* feeds heavily on phytoplankton. Lang (1976a) demonstrated that the larvae completed development on three of eight experimental algal diets, and required 8 to 11 d to develop to a cypris at 24 to 27°C. Coker (1902)

reared the larvae to cypris on a culture of unidentified diatoms from the sediment. In Delaware Bay, crabs can spawn over a period of 2 years, and based on evaluations of ovarian stage and fouling on the carapace, Williams and Porter (1964) considered a large crab, estimated at 25 to 26 months old, to host the single largest reported specimen of *C. patula* from the blue crab. That specimen lacked competitor barnacles, and it was estimated to be at least 1 year old. The specimen was described as 36 mm across, but if the crab's stated width and the magnification value of the photograph were presented correctly, the measurement of the barnacle was actually 47 mm long by 47 mm wide.

More specific to the blue crab than those barnacles mentioned above is the gooseneck barnacle *Octolasmis muelleri*, a species originally named by Coker (1902) as *Dichelaspis muelleri* (spelled as *D. mül-*



Figure 46. The external acorn barnacle symbiont *Chelonibia patula* on a blue crab from high salinity water in Mississippi. From Overstreet (1978).

leri). It cements itself to the branchial chamber of various crabs, usually on the gill filaments and often fusing several lamellae together. For example, in Brazil it infested *Callinectes* spp., *Libinia spinosa*, *Portunus spinicarpus*, *Portunus spinimanus*, *Hepatus pudibundus*, and an unidentified species of Majidae (Young 1990). Young found that *L. spinosa*, *Calappa flammea*, and *Scylarides* sp. also were infested with *Octolasmis hoeki*. In Louisiana and South Carolina, *O. muelleri* was reported from *C. sapidus* and *C. danae* as well as species of *Libinia*, *Portunus*, and *Calappa* (see Causey 1961). In the northern Gulf of Mexico, *C. sapidus* is a principal host, but *Calappa sulcata* and *Menippe* spp. are also commonly infested. *Octolasmis hoeki*, a more highly calcified species, can infest the same individual host as *O. muelleri*, but it occurs outside the gill chamber (Gittings et al. 1986; Overstreet, unpublished observations from Mississippi). Humes (1941b) lists other hosts for *O. muelleri*. Most reports list the barnacle on the gills as *Octolasmis lowei*, a name considered a senior synonym of *O. muelleri* by many researchers (e.g., Gittings et al. 1986). Young (1990) described how to differentiate the two, but *O. lowei* from the type locality requires redescription. Presently, we refer to this important species on *C. sapidus* as *O. muelleri*.

Infestations of *O. muelleri* are well documented (e.g., Coker 1902; Humes 1941b; Walker 1974; Jeffries and Voris 1983; Gannon 1990; Key et al. 1997; Voris and Jeffries 2001). The inhalant aperture of the branchial chamber allows access for the cyprid to attach to the inner sides of the gills, where up to 90% of the individuals can reside (Fig. 47A, B). The barnacle ranges in capitular length (height) from 0.14 to 5.58 mm, with the smallest reproductively active individual 1.14 mm long (Jeffries and Voris 1983). The peduncle is usually 1.5 to 3.0 times longer than the capitulum (Fig. 47C), and it is translucent unless pinkish when colored by the ova.

All gills can contain infestations, but infestations primarily involve gills 5 and 6 of 8, counting from the anterior. Most individuals attach to the proximal segment, with nearly as many on the medial portion and a few on the distal portion. The relatively large optimal site on the basal and medial portions of the hypobranchial sides of gills 3 to 6 constituted 29% of

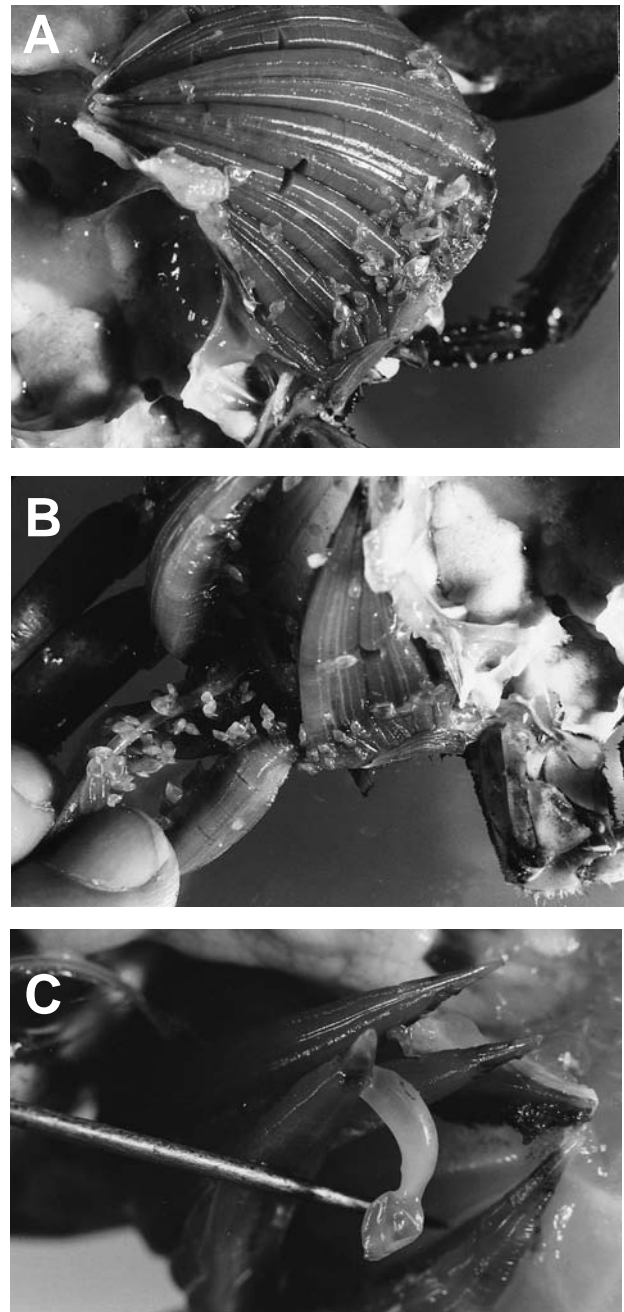


Figure 47. The gooseneck barnacle *Octolasmis muelleri* from a moderate infestation in high salinity water off Mississippi. The carapace of the host has been removed to readily view the gills. (A) A few individuals on the upper surface near the base of the gills. (B) The same crab as in A, showing the underside of a few gills (note few large and several medium-sized individuals). (C) Close-up of single relatively large individual. From Overstreet (1978).



the available gill surface and 6% of the available areas used for attachment, but it contained 61% of the infestation (Gannon 1990). Barnacle density, the number of barnacles per gill, did not correlate to gill size, although abundance, the number of barnacles per host, was greater in larger crabs. At least in Seahorse Key, Florida, there was no obvious seasonality for prevalence or intensity of infestation (Gannon 1990), although in areas like Mississippi where seasonal temperatures differ more, female crabs occur only off the mainland in the warmer months when they spawn and acquire barnacles. Brood size for mature individual barnacles ranges from 21 to 4459, with synchronous development. Crabs rarely contain more than a few hundred attached individuals. However, Coker (1902) and Overstreet (1978, 1983) have counted over 1000 per crab, although some individuals were relatively small and many also occurred on the exposed outer portion of the lamellae and elsewhere in the gill chamber. Overstreet (1978) noted that over 700 could coat the underside of the gills while hardly apparent on their dorsal surface.

The life history of *O. muelleri* includes six free-living nauplii and one cyprid (Lang 1976b). The larvae require at least 15°C to feed, and they develop when fed on a diet of either of two of eight tested algae. Development to cypris takes 2 to 3 weeks at 24 to 29°C. Gooseneck barnacle larvae are less tolerant to temperature than are larvae of *C. patula*, and they require a live crab on which to settle. Barnacles grow quickly on the blue crab. The growth rate of the capitulum of juvenile and adult *O. muelleri* was estimated at 0.016 mm per day over 68 d, while that of smaller juveniles was estimated at 0.023 mm per day (Jeffries and Voris 1998).

Species of *Octolasmis* can reinfest crabs quickly after ecdysis. Shields (1992) found individuals of *Octolasmis* spp. on postmolt *P. pelagicus* at the same prevalence of infestation as concurrent intermolt crabs, but with lower intensities of infection. A pulsed mode of colonization has been described for *O. cor* on newly molted specimens of *Scylla serrata* (see Jeffries et al. 1989). Molting of the host and the life cycle of the barnacle appear synchronized. However, infestations on the blue crab occur primarily

on the adult and slowly increase as a "trickle mode" of colonization (Voris and Jeffries 2001).

### ***Animal Health and Fisheries Implications***

*Balanus venustus* and *Balanus eburneus* are widely distributed, occurring in the Indian Ocean, Mediterranean Sea, and the Atlantic Ocean. *Balanus eburneus* is endemic to the Western Atlantic and has been introduced into other areas including the Pacific Ocean (Newman and Ross 1976). It is a euryhaline species but rarely encountered in normal marine salinities, perhaps because of competition with *Semibalanus balanoides* (see Henry and McLaughlin 1975). These and other balanoid barnacles occur on most species of blue crab and a few other crustaceans, including *Litopenaeus setiferus* (e.g., Dawson 1957).

*Chelonibia patula* occurs worldwide in warmer seas (Zullo 1979) and occurs on the blue crab as far north as Delaware Bay on the North Atlantic coast (Williams and Porter 1964). On the blue crab, it typically does not cause harm. However, the weight of a heavy infestation may burden a crab; encrusted appendages can hamper its movement and the extra weight can increase vulnerability to predation (e.g., Overstreet 1983). Infestations on crabs in the northern Gulf of Mexico occasionally become so great that the barnacles weigh as much the crab (Overstreet 1982). Eldridge and Waltz (1977) investigated *C. patula* on male and female crabs monthly for over 2 years from commercial pot catches in four areas in South Carolina. The total prevalence in those areas ranged from 1.1 to 3.2%, with most infestations restricted to a specific sex of crab in a specific locality at a specific time. For example, 5 to 57% monthly prevalences occurred on females in May to August of one year in St. Helena Sound relative to 0 to 25% on the male counterparts. In North Carolina, Pearce (1947a) found more infestations in relatively deep water compared with shallow nearshore water, with a maximum number of 485 specimens on a crab at 12 m depth. Nevertheless, the relationship is more likely to represent salinity than depth.

Salinity and temperature affect the presence of barnacles on the blue crab. For example, Crisp and Costlow (1963) determined that development of

nauplii of *C. patula* was normal between salinities of 25 and 40 at typically encountered high ambient temperatures; 50% of the eggs hatched, with 75 to 80% developing normally, but hatching success dropped sharply outside those ranges. Of those individuals that could tolerate 15 to 25 salinity, few hatched, development was delayed, and the resulting larvae were sluggish. Later stage larvae could tolerate salinities from 15 to 50. The tolerance to salinity was greater for *B. eburneus* and even greater for *Balanus amphitrite*. As an example of the effects of temperature, 90% of the embryos of *B. eburneus* developed at 15°C when in 29 to 43 salinity, but, with an increase to 30°C, they survived a range of 22 to 47 salinity. It took about 80 h at 30°C to reach the final stage compared with about 240 h at 15°C. The tolerance values were much more defined for *B. amphitrite* and *C. patula*. Lang (1976a, 1976b) investigated the biology of the nauplii and cyprid stages of *C. patula* and *O. muelleri* at high salinity. He determined that the former developed to a cypris in 8 to 11 d at 24 to 27°C and the latter took 14 to 18 d, with reduced or no development at lower temperatures.

The early study by Coker (1902) reported more female than male crabs (89 vs. 56%) infested with *O. muelleri* in North Carolina, and these infestations occurred in late rather than early summer. He found 80% of crabs infested with *O. muelleri* also had "*Balanus*" (presumably *C. patula*), and he found only the rare female crab with *C. patula* that did not also have *O. muelleri*. He speculated that when females were berried, they would be burdened by the eggs and were therefore less vigorous in their movements, with a correspondingly slower respiratory current, affording a better opportunity for the cyprids to attach. Humes (1941b) and More (1969) also found mostly older crabs and mostly females being infested in Louisiana and Texas. In Mississippi, it is the same, though we discount Coker's (1902) speculation because females in Mississippi and adjacent waters move offshore, usually without their male counterparts, to the high salinity waters around barrier islands and passes where they can spawn. Because the mature females do not molt, those that spawn and do not move inshore to lower salinities before spawning a second or third time accumulate more individuals of *O. muelleri* and other barnacles, which

also require high salinity water to survive. Also, males continue to molt, so although individual males that migrate to higher salinities can acquire epibionts, they lose them when they shed the encrusted exuvia.

*Octolasmis lowei* is widely distributed from 41°N to 43°S on decapod hosts. It is a shallow water species and does not occur deeper than 38 m in the Gulf of Mexico, and it apparently is not present in the eastern Pacific (Gittings et al. 1986). Jeffries et al. (1982) provided information on ten related species, including *O. lowei*, on a wide range of crustaceans near Singapore. If *O. muelleri* is a separate species rather than conspecific with *O. lowei*, it probably is restricted to the Western Atlantic.

*Callinectes sapidus* in warm marine waters often carries heavy burdens of barnacles and other associates. Both *C. patula* and *O. muelleri* are most prevalent in high salinity water (e.g., Overstreet 1982). The relationship of the various stages of the different barnacles with temperature, salinity, host sex, and geographic locality can be used as indicators of age, migration, and origin of the crab. For example, many female crabs in Florida and the Gulf of Mexico spawn two or even three times in high salinity water during one season. Also, mated but unspawned crabs in the autumn can overwinter, become ovigerous in March and April, and then be ready to spawn in the spring (Perry 1975). Spent females typically return from offshore or Gulf waters to inland areas to develop their subsequent egg clutches. Each case can result in a different pattern or appearance of infestation. The repeat spawners can be distinguished from the others with their clean, bright-colored shells by having dull-colored shells encrusted with *C. patula* and fouling organisms and by having ragged abdominal appendages and remnants of egg shells (Tagatz 1968). The reddish or orangish rather than opaque creamy coloration of typically concurrent specimens of *Carcinonemertes carcinophila* on the gills indicates that the colored worms had fed on a crab's egg clutch and consequently that crabs with those colored worms had previously spawned (Hopkins 1947).

Finding different sizes (ages) of different barnacle species either independently or concurrently allows one to estimate the age of the crab host, peri-

ods when crabs occurred in relatively high salinity water, whether an inshore crab had previously been offshore, and other features. For example, preliminary results by Overstreet and Rebarchik (unpublished data) tentatively suggested that shell lesions indicated contamination in tributaries of Pensacola Bay, Florida, and that the presence of attached organisms aided that evaluation. The presence of *O. muelleri* and *C. carcinophila* in conjunction with the presence or absence of other symbionts allows one to recognize crabs that have recently migrated inshore or that have migrated inshore a long time earlier, thereby representing different subpopulations.

Along the Eastern Pacific waters of Colombia, Norse and Estavez (1977) noted no barnacle on species of *Callinectes* located up rivers, but they reported *Balanus* sp. on some crabs near the mouths of rivers. They also found higher infestations on crabs from the continental shelf. In the latter sites, the different species of *Callinectes* also hosted *C. patula*, *O. lowei*, encrusting and arborescent bryozoans, and small sabellid polychaetes. Those crabs with heavy infestations of *O. lowei* also had abnormal purplish-black gills with substantial amounts of trapped sediments among their gill lamellae, similar to that encountered on *C. sapidus* along the eastern U.S. Coast (DeTurk 1940a; Walker 1974) and Gulf of Mexico Coast (Overstreet, pers. obs.s). Norse and Estevez (1977) found no associated symbionts on the gills and carapace of *Portunus asper* and few symbionts on *Euphyllax robustus*, presumably because these species were more effective cleaners.

Symbionts on the gills of crustaceans apparently can compete with their hosts for oxygen (e.g., Couch 1966, 1967; Overstreet 1973). Walker (1974) and Scrocco and Fabianek (1970) thought that neither *O. muelleri* nor *C. carcinophila* impaired respiration of the crab. However, Overstreet (1978) assumed that the combination of heavy infestations and debris on the gills of the blue crab could impede respiration. Moreover, once established on the gill, *O. muelleri* seems to impair gill cleaning, and with growth of any individual barnacle, cleaning of the filament surrounding it is further hampered until more barnacles attach, and heavy infestations soon occupy progressively more of the gills and gill

chamber.

Clearly, *O. muelleri* can be lethal to a crab. This effect becomes evident by the poor survival of crabs under stress such as handling and aerial exposure when there were over 50 individuals per crab (e.g., Gannon and Wheatly 1992). Gannon and Wheatly (1992, 1995) critically examined the physiological responses to the barnacle at different intensities. Oxygen uptake, lactate levels, pH, and other blood parameters were not different in infested (not heavily) and non-infested crabs. Still, infested crabs had an elevated heart rate and ventilation rate, apparently to compensate for the infestation, because the differences disappeared during exercise and recovery. At rest, the individuals without heavy infestations paused ventilating more frequently, often more than once every 2 min, but heavily infested ones paused only once every 20 min. Neither group paused during exercise. Crabs with extremely heavy infestations have to compensate more and probably do not survive long in nature. Overstreet (1978) noted a sluggish behavior most of the time in heavily infested crabs in captivity and assumed they attracted predators in nature. On occasion and especially during late summer, there have been many dead, spent, female crabs lining the barrier island beaches of Mississippi. Most of these crabs are heavily fouled with barnacles on their carapace and gills (e.g., Perry 1975; Overstreet, pers. obs.). Spent females during these periods are expected to die, but the fouling organisms presumably hastened their demise.

### Future Research

Use of external barnacles as indicators of host migration and previous locations of inhabitation provides an opportunity to assess polluted areas and various aspects of the biology of the crab and other conditions. When one can age the instar of a crab based on the age classes of barnacles, the infestations become an even more valuable tool. There is a need to determine the effect of infestations on crabs that spawn two or three times as well as the life histories of barnacles in estuaries compared with those that occur offshore.

No one has examined gill cleaning in *C. sapidus* in detail, but Bauer (e.g., 1981, 1998, 1999) and oth-



ers have critically investigated mechanisms of cleaning in other decapods. A comparison between mechanisms of *C. sapidus*, which can allow establishment of masses of barnacle symbionts and various fouling agents, and one or more species of *Portunus*, which rarely accumulates such organisms, would shed considerable insight into the biology of a female blue crab both before and following her first spawn.

### Rhizocephala (Internal Barnacles):

#### *Loxothylacus texanus*

Rhizocephalan barnacles provide some of the most unusual examples of parasitism and adaptations of a host-parasite relationship. Virtually all rhizocephalans castrate their hosts and many cause feminization of male crabs. The barnacles have complex life cycles and at several stages they are difficult for the novice to identify correctly.

#### Biology

An infection in the blue crab with the rhizocephalan barnacle *Loxothylacus texanus* can be recognized by an external brood sac, the externa, superficially appearing like crab eggs under the host's abdomen and by the host's modified secondary sexual characteristics. It is an obvious and potentially serious parasite to its host but not an obvious barnacle. Primarily by looking at the larval stages, one can realize its true taxonomic affiliation. The internal structure, or interna, of the barnacle exhibits rather strict host specificity; it occurs abundantly in some areas and is most widespread in *Callinectes sapidus*, although reported from a few other species of *Callinectes*.

The life cycle of *L. texanus* and other rhizocephalans involves initial separate female and male naupliar stages. These larvae, adapted for dispersal, develop into relatively small female or larger male cypris larvae, which separately produce stages that infect the crab or fertilize the female parasite, respectively. The female cypris of *L. texanus* attaches to the blue crab, metamorphosing into a kentrogen that penetrates the young juvenile host's exoskeleton (Glenner et al. 2000). This penetration typically



Figure 48. Detail of the rootlet (arrows) of *Sacculina granifera* infecting the ventral thoracic ganglion [N] of *Portunus pelagicus* (wet smear). The rootlet has penetrated the nerve tissue and has begun to ramify through it into the surrounding tissue.

occurs on the arthrodistal membranes of the joints, apparently in postmolt crabs less than about 18 mm wide (O'Brien et al. 1993b; Overstreet and O'Brien 1999). After about 3 d, a worm-like vermigon is released from the dart-like female kentrogen (Glenner et al. 2000). The vermigon migrates to the connective tissues surrounding the midgut where it eventually produces the interna (Fig. 48), or the internal complex web of root-like branches, that obtains its nutrition from the host. After appropriate growth, under appropriate environmental conditions, and following the host's final molt, the parasite extrudes the pouch-like structure externally as a bud, or virgin externa, under the host's abdomen. The male cypris must then encounter such an externa for the testis and associated structures to develop and subsequently for fertilization to occur. Fertilized eggs incubate to nauplii in a mantle cavity surrounding the visceral mass. Larval development promotes the externa, or mantle, to expand into the relatively large, characteristic reniform sac protruding from under the abdomen of the crab (Fig. 49).

Most infected blue crabs contain one externa, initially light yellowish-tan or creamy in appearance because of its eggs. The occasional host will have two or up to eight externae (Ragan and Matherne

1974), but these multiple sacs are correspondingly smaller than a single one (Fig. 49B). As the nauplii within the externa develop, the sac appears a brownish to purplish color until the larvae are shed. The externa in some senescent individuals appears dark brown. Nauplii are released from the parasite's mantle opening in a dozen or so pulses of many thousands every 2 to 7 d, with the number of broods per month increasing with water temperature. Based on experimental infections, O'Brien and co-workers (University of South Alabama personal communication) found that considerable variability occurred in the period between when a kentrogon infected a young juvenile and an externa appeared. It took from 3 to 7 months with up to nine molts of the host crab. The potential for considerable variability in growth during that period can mislead an observer about when an externa-bearing crab actually became infected.

#### **Animal Health and Fisheries Implications**

*Loxothylacus texanus* infects primarily *C. sapidus* but also infects *Callinectes ornatus* in the shallow warm inshore areas of Biscayne Bay (Miami), Florida Keys, and the west coast of Florida (Overstreet 1978, 1983); *Callinectes larvatus* (listed as the subjective junior synonym *Callinectes marginatus* by Boschma 1955 and others) in Panama (Rathbun 1930; Boschma 1933, 1955); and *Callinectes rathbunae* in southwestern Gulf of Mexico coasts in Mexico (Alvarez and Calderón 1996; Alvarez et al. 1999). Actually, Rathbun (1895) initially reported it as probably *Peltogaster* from *C. sapidus* in Texas (see also Boschma 1955). Several other species of *Callinectes* co-occur with the infected species indicated above, but they do not exhibit infections.

In *C. sapidus*, *L. texanus* has occasionally infected over half of the crabs collected in specific areas of the northern Gulf of Mexico (e.g., O'Brien and Overstreet 1991). The infection occurs, usually at low prevalence, up the Atlantic coast, at least along South Carolina in late summer and in overwintering females (Eldridge and Waltz 1977; James E. Jenkins, Marine Resources Division, South Carolina Department of Natural Resources, Charleston, personal

communication) and south into southern Mexico (Alvarez and Calderón 1996) and Colombia (Young and Campos 1988; Alvarez and Blain 1993). The rare occurrence of what may be the same barnacle, presumably by introduction of *C. sapidus*, has been reported from Greece in the Mediterranean Sea (Boschma 1972).

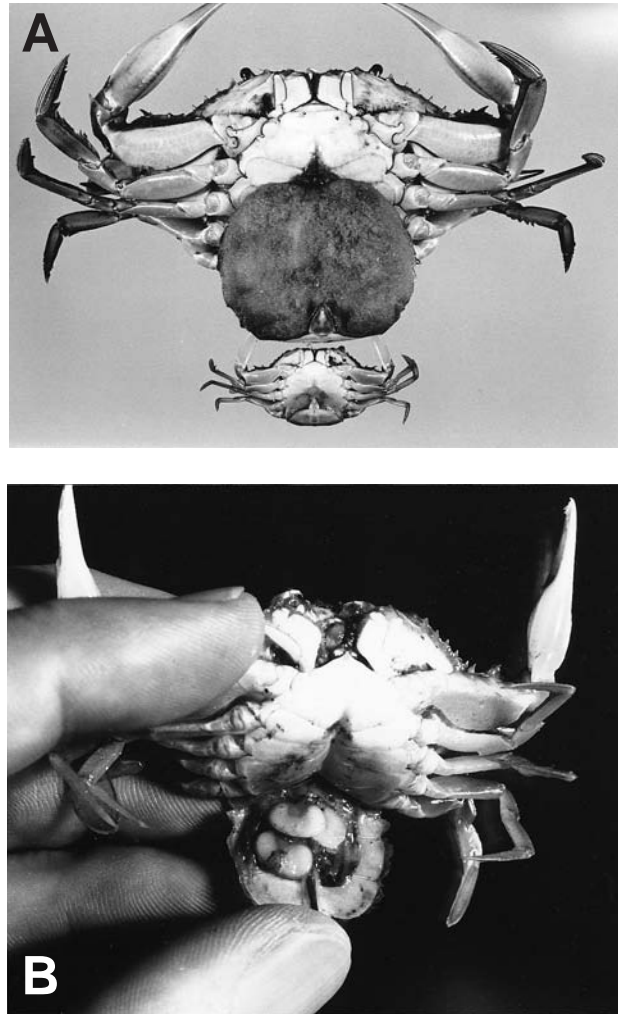


Figure 49. Ventral views of the internal rhizocephalan barnacle *Loxothylacus texanus* from crabs in Mississippi Sound. (A) An infected stunted individual showing externa below a normal sized berried female, both caught together and presumably of the same age. From Overstreet (1982). (B) Infected individual exhibiting three externae. From Overstreet (1978). Typically, only a single externa is present, though several can also be present.

In the case of *L. texanus* in the blue crab, but not necessarily all rhizocephalans in other families, the infected crab stops molting after the externa is produced. Molting, and therefore subsequent growth, is inhibited. An equal number of male and female crabs are usually infected, and they typically measure 3 to 6 cm wide (e.g., Overstreet et al. 1983), with the occasional one reaching 10 cm along the northern Gulf of Mexico (Overstreet 1983). Infected crabs, however, along Florida's Gulf of Mexico coast are much larger (Hochberg et al. 1992). Females in Mississippi having undergone their final ecdysis between the prepubertal and first mature instars were significantly larger than infected individuals; mature females reached a peak at 16 cm wide compared with 4 cm wide for individuals when infected (Overstreet et al. 1983). Moreover, those blue crabs infected with *L. texanus* in Mississippi occasionally accumulated large numbers of diverse fouling organisms (Fig. 50).

Infected male blue crabs show distinct changes in the morphology of their abdominal segments and pleopods (Reinhard 1950a,b; Alvarez and Calderón 1996). Infected males become feminized, and the physiological features of both sexes are altered. The normal, narrow, T-shaped abdomen of the uninfected male widens and becomes rounded in infected individuals, like that of an uninfected, mature female. Uninfected immature females have a



Figure 50. A hydroid, probably *Obelia bidentata* or a related species, attached to a stunted blue crab infected with *Loxothylacus texanus* in Mississippi.

triangularly shaped abdomen from the fourth segment to the extremity. The abdomen rounds off when the crab matures. Both sexes are castrated. In the sand crab *Portunus pelagicus* infected with *Sacculina granifera*, partial clutches have been observed on infected females (Shields and Wood 1993). Behavioral modifications also occur. In the case of *P. pelagicus*, the crab digs a hole and grooms and cares for the externa of *S. granifera*, a surrogate egg mass, just as the female would do for her developing egg mass (Bishop and Cannon 1979). O'Brien and Overstreet (unpublished observations) have not seen the digging behavior in crabs infected with *L. texanus*, but Wardle and Tirpak (1991) noted that externa-bearing crabs were generally less aggressive than non-externa-bearing crabs when presented with food, and the crabs with externa were unable to burrow. However, O'Brien and co-workers (University of South Alabama, personal communication) noted that crabs exposed to infective larvae appeared more active than their unexposed counterparts. Regardless of the sex of infected individuals, the gonad does not develop fully and the crab neither mates nor spawns, resulting in parasitic castration.

Infected crabs are altered in several ways, few of which have been analyzed in much detail. Manwell and Baker (1963) found, on the basis of four male crabs, that the infected male had higher serum levels of electrophoretically "fast" hemocyanin components and higher levels of "dianisidine oxidase" than the uninfected one. They found no change in the blood of blue crabs infested with *Octolasmis muelleri* or *Carcinonemertes carcinophila* on the gills. They did not find respiratory pigment in *L. texanus*. Uglow (1969), using more sophisticated methods also found no difference in the blood of *Carcinus maenas* infected with *Sacculina carcini* when compared with non-infected individuals.

Rhizocephalan infections typically cause castration and feminization of male hosts, which apparently results from destruction of the androgenic glands soon after infection (Veillet and Graf 1958). When Rubiliani (1985) injected an extract (multiple injections over 2 to 3 weeks) of interna of *L. panopaei* into the xanthid crab *Panopeus herbstii*, he observed pycnotic spermatogonia, hypertrophy of



residual primary gonad, and signs of degeneration of the androgenic glands. The related xanthid *Rhithropanopeus harrisi* was affected less severely by similar injections. Therefore, at least for *L. panopaei* in *P. herbstii*, feminization and castration appears to be effected through the biochemical destruction of the reproductive organs, not the androgenic gland. Degeneration of the glands was not apparent in the blue crab. Unlike the blue crab with *L. texanus*, *P. pelagicus* was susceptible to infection by *S. granifera* at any size (Shields and Wood 1993). Some species secrete pheromones that stimulate various reproductive behaviors (DeVries et al. 1989). O'Brien and Van Wyk (1985) discussed aspects of why different hosts are affected differently and why different parasites exhibit different effects.

Many of the features that affect the health of an individual crab also influence populations. *Loxothylacus texanus* has the potential to severely affect a crab stock. When large numbers of young crabs and infective cyprid larvae occur simultaneously in conjunction with optimal water conditions, a high percentage of crabs will be infected. Infected crabs in Mississippi exhibiting externae typically occur most frequently from May to August, with few such crabs occurring in February and March (O'Brien and Overstreet 1991). Infected crabs, however, can occur year-around as documented in Louisiana (Ragan and Matherne 1974). Infections have reached 50% or more of some samples in Mississippi (Christmas 1969; Overstreet 1978; O'Brien and Overstreet 1991), Galveston Bay, Texas (Wardle and Tirpak 1991), and Tamiahua Lagoon, Mexico (Lázaro-Chávez et al. 1996). Prevalence is no doubt underreported as most studies only note the prevalence of the external stage of the parasite.

Nauplii are attracted to light and to high salinity water, but cyprids, perhaps only males, have a weaker phototactic response (Cej et al. 1997). Larvae were not viable at salinities below 12 (O'Brien et al. 1993a, 1993b). Consequently, the nauplii in sea water occur near the surface, and the cyprids, especially males, occur near the benthos. Virgin externae appear to be fertilized near the bottom of the water column, where male cyprids are more likely to be encountered (Cej et al. 1997). A pheromone from

the immature female may attract the male cyprid (O'Brien et al. 1993a). The combination of environmental conditions and the presence of young vulnerable crabs determines the prevalence of infection and may ultimately influence individual crab mortality and abundance of the crab stocks.

In southwest Florida, infections of *L. texanus* in *C. sapidus* were not common (23 of 16,282 crabs), but the 0.2 to 5.1% local annual prevalence of infection along the west coast of Florida from Cape Sable to Apalachee Bay included much larger infected individuals than in the northern Gulf of Mexico (Hochberg et al. 1992). At least 51% of these infected crabs from West Florida had carapaces measuring 100 mm or more wide, with a few individuals reaching 17 cm across. There was a relationship between crabs with externae and salinity, but most infections were noted when the temperature was 21 to 25°C. Hochberg et al. (1992) hypothesized, based on an assumed 4- to 6-week period between maturation of externa to infective female cyprid stage, that because most crabs hatched during spring, infected crabs would be approximately 10- and 40-mm wide during autumn and early winter, respectively. Because the relative abundance of infections in Florida was greatest during August and September, it would allow infective female cyprids, which would have been abundant in October through December, to synchronize their presence with the abundance of juvenile crabs. This may account for the observed midwinter to early spring increase in crabs with a mature externa. This method of analysis, however, seems somewhat misleading as indicated earlier because of the variability in length of time and number of molts between infection and protrusion of externa. Perhaps blue crabs in South Florida grow faster between ecdyses due to relatively higher ambient temperature, producing larger infected crabs than those in the northern Gulf of Mexico where the temperature and salinity are typically lower. Such crabs could have become infected in spring through summer, resulting in the larger infected hosts. In warm southern coastal lagoons of Mexico, infected *C. sapidus* ranged from 7 to 13 cm wide and infected *C. rathbunae* ranged from 5 to 14 cm (Alvarez and Calderón 1996), although the mean

width was still less than 10 cm. Unlike in Florida where infections were not common, in Tamiahua Lagoon, Mexico, one seasonal October sample of *C. sapidus* had a 51.1% prevalence of infection, although overall prevalence was 13.3% (Lázaro-Chávez et al. 1996).

Unpublished studies by O'Brien, Overstreet, and colleagues show that the relationship between salinity and infection by *L. texanus* plays an important role in larval transmission and host health. Larvae of the barnacle cannot survive low salinity. Mortalities reach 85% at a salinity of 10 and 10% at a salinity of 15. Even infected crabs with externae cannot tolerate lower salinities; infected individuals survive best at salinities from 25 to 30. In a few cases when a crab was placed in low salinity water, it did not die, but the externa was shed and the crab survived.

Different rhizocephalans affect their hosts differently. Eight species of king crabs are infected by *Briarosaccus callosus*; those in Alaska, especially the blue king crab (*Paralithodes platypus*), have been assessed by Hawkes et al. (1985) and others. The golden king crab *Lithodes aequispina* and red king crab *P. camtschaticus* had a pronounced hemolymph response to the barnacle, but the blue king crab did not (Shirley et al. 1986). Unable to respond as well as its relatives, the blue king crab exhibited greater inhibition of growth, lethargy, and castration, with the prevalence of infection greater in smaller crabs and in females, reaching 90% of the female population versus 65% of males in southeastern Alaska. Because the infected blue king crab was not retained in the commercial harvest, Hawkes et al. (1985) recommended a potential management strategy for control of parasitism consisting of allowing the commercial harvest of infected crabs regardless of sex or size. Lester (1978) noted that a possible strategy for controlling *Sacculina granifera* in the *P. pelagicus* fishery would be to fish heavily and destroy infected crabs, but fishery regulations would have to be modified to allow the capture of seemingly small animals for removal. Kuris and Lafferty (1992) have developed models to simulate the effects of different harvesting strategies for the control of diseases in crustacean fisheries.

The portunid population of *Charybdis longicollis*

that resulted after migration of the species through the Suez Canal from the Red Sea to the Mediterranean Sea has been heavily infected by the rhizocephalan *Heterosaccus dollfusi*. Galil and Innocenti (1999) reported a rapidly established prevalence of 77%, with 58% of the crabs bearing more than one externa. They expect the Mediterranean crab population will suffer drastic perturbations as a result of the infection because of a dense host population and reproduction of the parasite throughout the year.

In summary, *L. texanus* can kill young blue crabs or weaken them so that they readily become prey. It can also affect larger crabs, probably making infected individuals more available prey than their non-infected counterparts, especially after they have left the marshes and migrated into open water. More important, they may compete with non-infected individuals for reproductive partners and space, thereby reducing the potential for a harvestable crab or for reproductive effort (see Shields and Wood 1993). To reiterate, infected crabs do not molt and are too small to enter the commercial and recreational harvest. These small crabs are not to be confused with the occasional dwarf specimens such as the berried specimen 20.0 mm long by 46.7 mm wide figured by Overstreet et al. (1983).

### **Future Research**

O'Brien, Sherman, and colleagues (University of South Alabama, personal communication) are developing a DNA probe for *L. texanus*, which will allow determination of infected juveniles that do not exhibit externae or altered morphological features (Woodard et al. 1989). This probe should allow for the assessment of infections in specific stocks and subsequent commercial crab production. In addition, the dwarf crabs in the fishery present problems involving management decisions, especially because dwarf crabs seldom exhibit the modified secondary sexual characteristics diagnostic for infected individuals. These stunted and parasitized crabs may accumulate in the population due to fishing of their uninfected counterparts (Meyers 1990), and thus may persist as reservoirs for further transmission of the parasite. Clarification of infection should help one evaluate the biological influence of infections

on populations. In addition, based on gonad size and host mortality, Obrebski (1975) modeled evolutionary strategies that may lead to parasitic castration. Rhizocephalan barnacles may be ideal candidates to explore such models further.

Research is also underway on elucidating the parasite's life cycle, which will clarify other aspects of infections and host management. Heavy mortalities of megalopae, especially in the Gulf of Mexico, have been attributed to predation (Heck and Coen 1995), which could include cannibalism (Dittel et al. 1995; Hines and Ruiz 1995). Much of this mortality could periodically result from infection of young crabs and possibly even megalopae by *L. texanus*, and this possibility deserves attention. In the case of *Rhithropanopeus harrisi* experimentally infected with *L. panopaei* while in a megalopal stage (<1 mm wide carapace), only 6% survived to juvenile 9 stage (C-9, about 9.5 mm wide) compared with 50% of the controls (Alvarez et al. 1995).

The threat of introduction of *L. texanus* along the middle Atlantic coastline should be investigated. Perhaps the salinity and cooler weather would or has already prevented such an introduction or spread further north than, perhaps, Cape Fear, North Carolina. A related species, *Loxothylacus panopaei* in mud crabs, was introduced from the Gulf of Mexico into Chesapeake Bay in 1963 (Van Engel et al. 1966; Overstreet 1983). Hines et al. (1997) have evaluated the long-term effects of that and other introductions of *L. panopaei* and determined that a lag of nearly 30 years occurred for the barnacle to disperse 200 km up Chesapeake Bay. Also, the spread of the barnacle south into mud crabs of the North Carolina sounds may have occurred by the late 1970s or early 1980s. Apparently, because of the limited dispersal capabilities of the crab larva in conjunction with the salinity tolerance of the parasite, spatial and temporal factors control the parasite's persistent nature and its ability for rapid build-up of infections in local crab populations. *Loxothylacus panopaei* seems to have acquired additional hosts, and the different availability of vulnerable host stages of different crab species promotes long-term stability in some species and sporadic and epizootic infections in others.

## FOULING ORGANISMS

The blue crab hosts a diverse fouling community, especially the female in her terminal molt in high salinity water. For example, Pearse (1947b) reported several fouling organisms from North Carolina and Overstreet (1982, 1983) reported several from Mississippi. Most fouling organisms (e.g., oysters, mussels, corals) show facultative phoresy, but especially one, the bryozoan *Triticella elongata*, exhibits an obligate phoretic relationship on crustaceans. The fouling community has been used to examine basic biological and ecological questions that include aspects of host molting, longevity, community succession, migration, and differences in the spatial patterns between host sexes. Important issues such as the presence or absence of a terminal molt for *Callinectes ornatus* and *C. danae* have been addressed through the study of the fouling community (Negreiros-Fransozo et al. 1995). Abelló et al. (1990), Becker (1996) and Key et al. (1996, 1997, 1999) have reviewed aspects of the fouling communities on crab hosts.

Crabs and other large-bodied crustaceans in general represent a hard substratum for settlement, and competition for space on such substrata is often intense (Connell 1961; Paine 1974), even when such space is molted frequently (Shields 1992; Key et al. 1996, 1997, 1999). Additionally, the crab carapace is a biologically active surface comprised of chitin, calcium, and bacterial films, all on a mobile platform. Thus, the carapace is an ideal habitat for several sessile, short-lived organisms. This may be particularly true over mud and sand substrata, habitats encompassing much of the range of the blue crab. Probably the best example of fouling communities on crabs are the epibionts found on various majid decorator crabs. The decorator crabs actively select small rocks and specific species of symbionts to facilitate camouflaging their carapaces (e.g., Wicksten 1980). The blue crab, however, has a characteristic and diverse fauna of its own.

The blue crab's fouling community represents a disparate fauna from several different phyla. Protozoans are common on the external surfaces and egg masses of blue crabs, but other than a few ciliates



(see Ciliates above), they have been largely ignored. Bacteria, diatoms, other algae, foraminiferans, amebae, thraustochytrids, and ciliates represent the fauna of the underlying benthos, and crabs acquire such organisms primarily from their association with the benthos. Such is particularly the case for ovigerous crabs, as they bury into the sediments during oviposition. Amphipods, in particular, can be found on the egg masses of ovigerous crabs, and they may represent opportunistic egg predators as has been observed on cancrid and lithodid crabs and lobsters (Kuris et al. 1991; Shields, pers. obs.). Pearse (1947b) noted the presence of amphipods, and Overstreet (1979) noted caprellid amphipods on the carapaces of blue crabs. Other fouling organisms include the stony coral *Astrangia danae*, soft coral *Leptogorgia virgulata*, zoanthid *Epizoanthus americanus*, tunicate *Molgula manhattensis* (Pearse 1947b), hydroids *Bougainvillia* sp. and *Obelia bidentata* (Fig. 50) (Overstreet 1983), and others (Williams and Porter 1964). Oysters, sponges, algae, and bryozoans occur on the abdomen of blue crabs but only on hosts in high salinity waters and typically on senescent hosts or hosts in terminal molts (Fig. 51), such as those infected by *Loxothylacus texanus* (Overstreet 1979). The slipper shell *Crepidula plana*, serpulid worms, and colonial ascidians occasionally foul the carapace (Pearse 1947b; Key et al. 1999).

In small numbers, fouling agents have little, if any, effect on their host. Heavily fouled crabs, how-



Figure 51. A relatively large specimen of the eastern oyster *Crassostrea virginica* attached to the ventral surface under the abdomen of a blue crab in Mississippi.

ever, may suffer from increased drag, impaired swimming ability, burdensome weight (especially with barnacle infestations), and thus, greater energy demands (e.g., Gannon and Wheatley 1992, 1995). From riverine systems, mature female crabs migrate to high salinity areas to reproduce. Thus, females generally have a higher prevalence and intensity of infestations than males in the freshwater reaches (Key et al. 1999). The egg clutches of females independently attract a community of fouling agents and saprophytic organisms. In addition, the female blue crab generally molts to a terminal instar at maturity, but the male may continue to molt and grow; thus, females, particularly older, senescent ones, appear most at risk to heavy fouling and such infestations can be considered harmful (Overstreet 1983; Williams 1984; Becker 1996). Heavily fouled dead crabs occasionally wash up on beaches, and the general public often assumes that the fouling agents killed the crabs. The underlying causes of such mortalities are rarely examined, but physiological stress, senescence, or microbial infection should be ruled out before considering fouling organisms as the sole pathogens (Overstreet 1982).

Although not strictly fouling organisms, adult sarcophagid flies have been observed emerging from baskets of blue crabs containing live and dead individuals, but the deposition of fly eggs on living crabs is uncertain (Overstreet, pers. obs.). Similarly, chironomid fly larvae are occasionally seen on dead peeler crabs, especially from freshwater floats (Shields, pers. obs.). Clearly, flies and other scavengers are attracted to dead flesh as well as the microorganisms feeding off the bacterial community on the dead crabs.

Other than external barnacles (see above), bryozoans are probably the best studied of the actual fouling organisms occurring on blue crabs (see review by Key et al. 1999). Several species have been recorded, including *Alcyonidium albescens* (reported as *A. polyoum* and *A. mytili*), *Alcyonidium verrilli*, *Conopeum tenuissimum*, *Membranipora arborescens*, *Membranipora crustulenta*, *Membranipora tenuis* (probably *M. arborescens*), and *Triticella elongata* (Van Engel, Virginia Institute of Marine Science, as personal communication in Overstreet 1982; Key et al. 1999). All but *T.*

*elongata* have facultative phoretic relationships. Triticellids are apparently obligate symbionts on crustaceans. Infestations originate in the branchial chamber and spread to the external surfaces (DeTurk 1940a; Watts 1957 in Key et al. 1999). The other bryozoans typically infest the ventral surfaces of the carapace or limbs, but dorsal infestations are not uncommon, especially for *A. albescens* (see Key et al. 1999).

Bryozoans are often rarer than barnacles on host crabs. Salinity, temperature, seasonality, location, and migration patterns of the host may influence their settlement. Bryozoan infestations are typically restricted to salinities above 8 with most occurring above 18 (Winston 1977). A prevalence of 16% was reported for bryozoans from the Newport River, near Wilmington, North Carolina (Key et al. 1999). Barnacles from the same crabs had a prevalence of 67% (Key et al. 1997). Negreiros-Fransozo et al. (1995) noted that barnacles were more common than bryozoans on *C. ornatus* and *C. danae* from Ubatuba Bay, Brazil. Watts (1957, cited in Key et al. 1999) reported a prevalence of 97% for bryozoans on blue crabs from Delaware Bay, a high number possibly indicative of older, mature female hosts.

Missing from the fouling community of blue crabs are obligate relationships with polychaetes and isopods. Eunicid polychaetes live in the branchial chambers of decapods (reviewed by Paris 1955). Portunid, majid, goneplacid, and cancrid crabs are infested with eunicid polychaetes in the genus *Iphitime* (Abello et al. 1988; Comely and Ansell 1989; Paiva and Nonato 1991). Notably, *I. cuenoti* was not found on host crabs from mud and sand benthos (Comely and Ansell 1989), a finding that may explain the lack of eunicids on blue crabs. Leeches and branchiobdellid annelids are treated elsewhere. Van Engel (1987) listed an unidentified isopod in the branchial chamber of blue crabs from the York River, and suspected it of having a close relationship with the blue crab. Bopyrid isopods are highly host-specific parasites of crustaceans that infest the branchial chamber or external surface of the host. Although bopyrid isopods are common parasites of crabs and shrimp, none has been reported from the blue crab. Other portunids, how-

ever, are commonly parasitized by bopyrids and other epicaridean isopods (e.g., Markham 1985, 1989; Shields and Early 1993).

The blue crab can also be highly susceptible to fouling when infected by an agent that impedes or completely inhibits molting. In the Gulf of Mexico, the rhizocephalan *Loxothylacus texanus* serves as a prime example in the blue crab, especially in late summer or autumn. The rhizocephalan can be easily detected without intrusive dissection and crabs infected by them can accumulate seemingly massive infestations of fouling organisms. The role of the rhizocephalan in the dynamics of the fouling community has not been published, but the association may be useful in exploring succession in fouling communities (Shields 1992), or in assessing positive and negative species associations (Shields, pers. obs.).

## OTHER SYNDROMES

Two other syndromes, winter mortality and gas-bubble disease, will be briefly addressed. Winter mortality was first reported by Van Engel (1982). Although the data are anecdotal, low temperatures (<5°C) may kill female crabs in the mainstem of Chesapeake Bay. Mortalities in dredge catches are highest in lower salinity waters (<15). Blue crabs are less able to osmoregulate at low temperatures (Johnson 1980, p. 99) and failure to osmoregulate, indicated grossly by swelling of the paddles and arthro-dial membranes, could be a major cause of death in winter crabs held at low salinities (Johnson, unpublished data, cited in Johnson 1976c). At higher salinities, winter mortalities may be related to protozoan (see *Paramoeba perniciosus* and *Hematodinium perezii*) infections that overwinter in the crab. In addition, hemopoietic tissues show little activity in winter (Johnson 1980); thus, crabs may be more susceptible to secondary infections, albeit most bacteria and presumably viruses grow optimally at relatively high temperatures.

Gas bubble disease occurs in invertebrates and fishes exposed to supersaturated air or other gases. Johnson (1976b) inadvertently caused a supersaturation event in the blue crab and followed the course of the syndrome and recovery of individuals. Gas

emboli were observed regularly in the crab gills over 13 d. The emboli became less prevalent and were not observed after 40 d. Emboli were present in the hemal sinuses after 20 d. Ischemia, recognized as focal necrosis, was evident from 4 to 13 d after the event. The gills were mechanically disrupted by the gas emboli, with lamellae being broken off or otherwise damaged by hemal stasis. The heart and antennal gland were damaged by ischemia; in some cases, the antennal gland was severely altered. On occasion, muscle and nervous tissues exhibited focal necrosis. Interestingly, there was no cellular response except for the infiltration of hemocytes into the gill lamellae, and that presumably was due to the physical damage from the emboli. The lack of cellular response is intriguing, especially because crab hemolymph clots quickly when exposed to air. Gut tissues and hepatopancreas were not overtly affected. The pathology appears most consistent with disruption of tissues as opposed to focal necrosis from ischemia. Most of the crabs showed signs of recovery

over 20 to 40 d after the event. Johnson (1976b) diagnosed *Paramoeba pernicioso* from several of the afflicted crabs. She speculated that the syndrome probably killed the most heavily infected individuals first.

## DEFENSIVE RESPONSES

Crustacean immunology presents a rich, if not contentious, area for new research. More studies are needed to clarify hemocyte classifications among taxa, the origin and development of hemocytes, the location(s) for synthesis of clotting factors, the putative roles of probable defensins, and aspects of inducible non-specific responses. The development of a conceptual model for the crustacean defense system should integrate the above with the role of the prophenoloxidase (proPO) system, proteases, and humoral factors such as lectins, callinectin, and other defensins (Fig. 52). Numerous morphological studies on hemocytes of crayfishes, lobsters, and shrimps

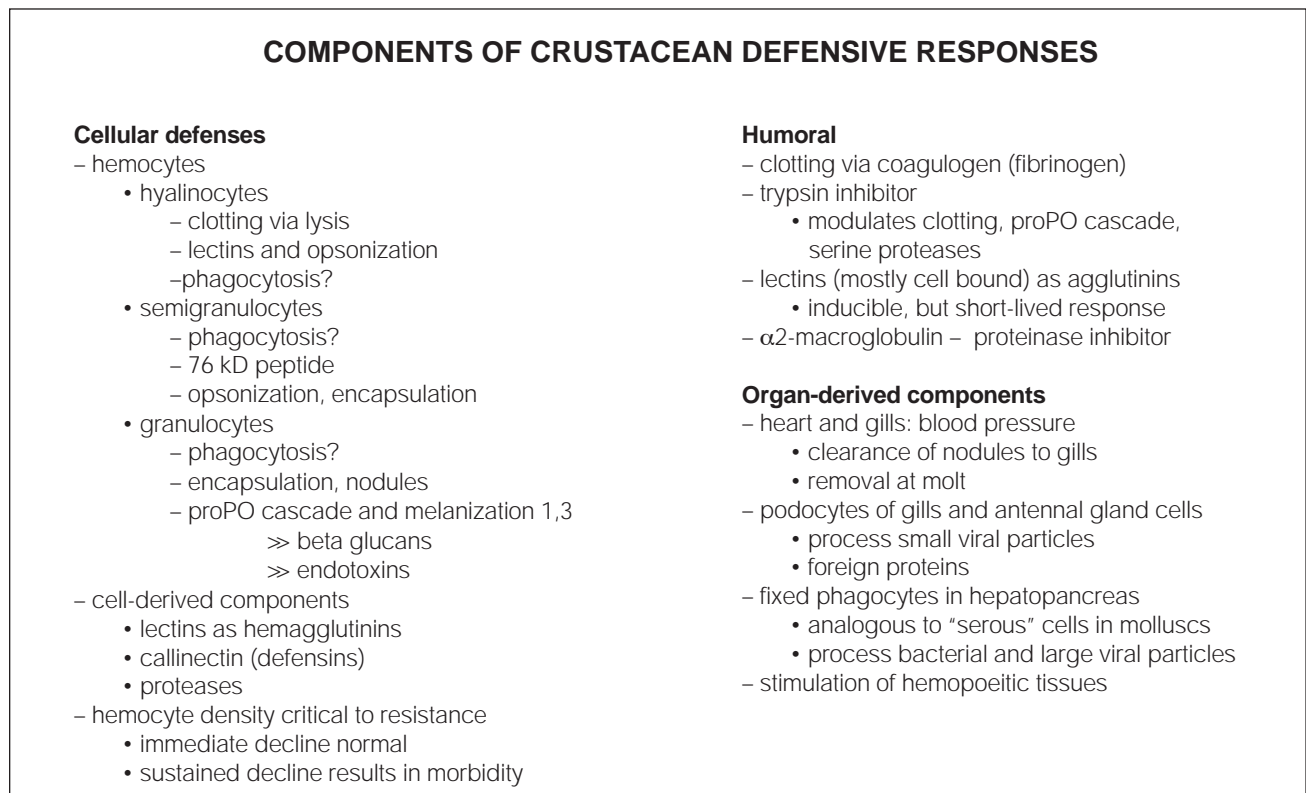


Figure 52. Components of the crustacean defensive response to infectious diseases. proPO = prophenoloxidase. Excerpted from Hose et al. (1990) and Söderhäll and Cerenius (1992).



have been reported, probably because of the ease of culturing these crustaceans as well as because of their commercial importance. A critical review of crustacean defensive responses is beyond the scope of this chapter. For reviews of hemocyte morphology and function, see Johnson (1980), Bauchau (1981) and Hose et al. (1990). For general reviews on crustacean immunity, see Sindermann (1971, 1990) for pertinent observations on the older literature, and Smith and Chisholm (1992), Söderhäll and Cerenius (1992), Bachère et al. (1995), Holmblad and Söderhäll (1999) for newer syntheses.

### Hemocytes and Cellular Defenses

The number and types of hemocytes differ among crustaceans and they can differ depending on how the cells are assessed. Typically, hemocytes are divided into large and small cells, whether with or without granules, or inclusion bodies. For example, Owens and O'Neill (1997) made light-microscopic counts on the four cell types they observed from the giant tiger shrimp *Penaeus monodon*. When those authors used flow cytometry, they detected five cell types, and the counts differed significantly between the two methods.

There are currently two conflicting views on the development of hemocytes in crustaceans. Cuénot (1893) suggested that hemocytes originate from a single stem cell lineage and that the different cell morphologies and categories represent a continuum. Several authors have supported his findings (e.g., Bodammer 1978; Johnson 1980; Bauchau 1981). However, there is some evidence that different portions of the hemopoietic tissues give rise to the disparate hemocytes (Clare and Lumb 1994). Accordingly, the hyalinocytes may develop along a separate line as they serve a very different role than the granulocytes (Hose et al. 1990; Clare and Lumb 1994; Martin et al. 1993).

Hemocytes of crustaceans have several known defensive functions, including phagocytosis, wound repair, encapsulation, and nodule formation. They also function in tanning of the cuticle, transport of nutrients, and coagulation or clotting to prevent blood loss. Hemocytes may also function in glucose regulation, hemocyanin synthesis, and possibly

osmotic regulation (see Bauchau 1981). The hemocytes of blue crabs are variously classified into three cell types: hyalinocytes, semigranulocytes or intermediate cells, and granulocytes. At the light microscopic level, as indicated above, the presence of granules is one of the defining characters for the cell types. All hemocytes, however, have granules at the sub-micron level (Bodammer 1978; Clare and Lumb 1994), and certain biochemical and defensive characters may serve as useful markers to differentiate cell types (Hose et al. 1990). Visualization of the granules with the light microscope, particularly with phase contrast, however, does serve to distinguish among cells (Johnson 1980).

Hyalinocytes of the blue crab are cells 6 to 13  $\mu\text{m}$  in diameter with a high nucleus-to-cytoplasm ratio and few sub-micron granules. The morphology of the hyalinocyte varies considerably with the technique and handling in each study. Unfortunately, this has led to some contention in identifying the role of hyalinocytes in the cellular defenses of the crab. In raw clotted hemolymph, hyalinocytes will spread out to become star-shaped with long filopodia or become flattened and difficult to see (Johnson 1980; Shields, pers. obs.). In hemolymph mixed with seawater, the hyalinocytes will lyse and cause clotting (see below, Clare and Lumb 1994); hence, their nuclear details, pseudopodia, and ability to phagocytize foreign particles are not apparent. In histological preparations, the hyalinocytes do not lyse, nor do they exhibit pseudopods.

The primary hemocytes involved in clotting have been classified as either hyalinocytes (Hose et al. 1990) or semigranulocytes (Stang-Voss 1971; Johnson 1980). Both cell types may actually be involved in clotting (Bauchau and DeBrouwer 1974). Hyalinocytes dehisce (lyse) upon contact with seawater, foreign bodies, or air. The products of their granules catalyze coagulogen, or fibrinogen (Fig. 53), the main clotting protein in the plasma of many crustaceans (Ghidalia et al. 1981; Martin et al. 1991), to effect coagulation and form the clot (Bauchau and DeBrouwer 1974; Durliat 1989; Hose et al. 1990; Martin et al. 1991). The type of clotting may be dependent on the titer of coagulogen, or fibrinogen, in the plasma (Bauchau 1981) or on the

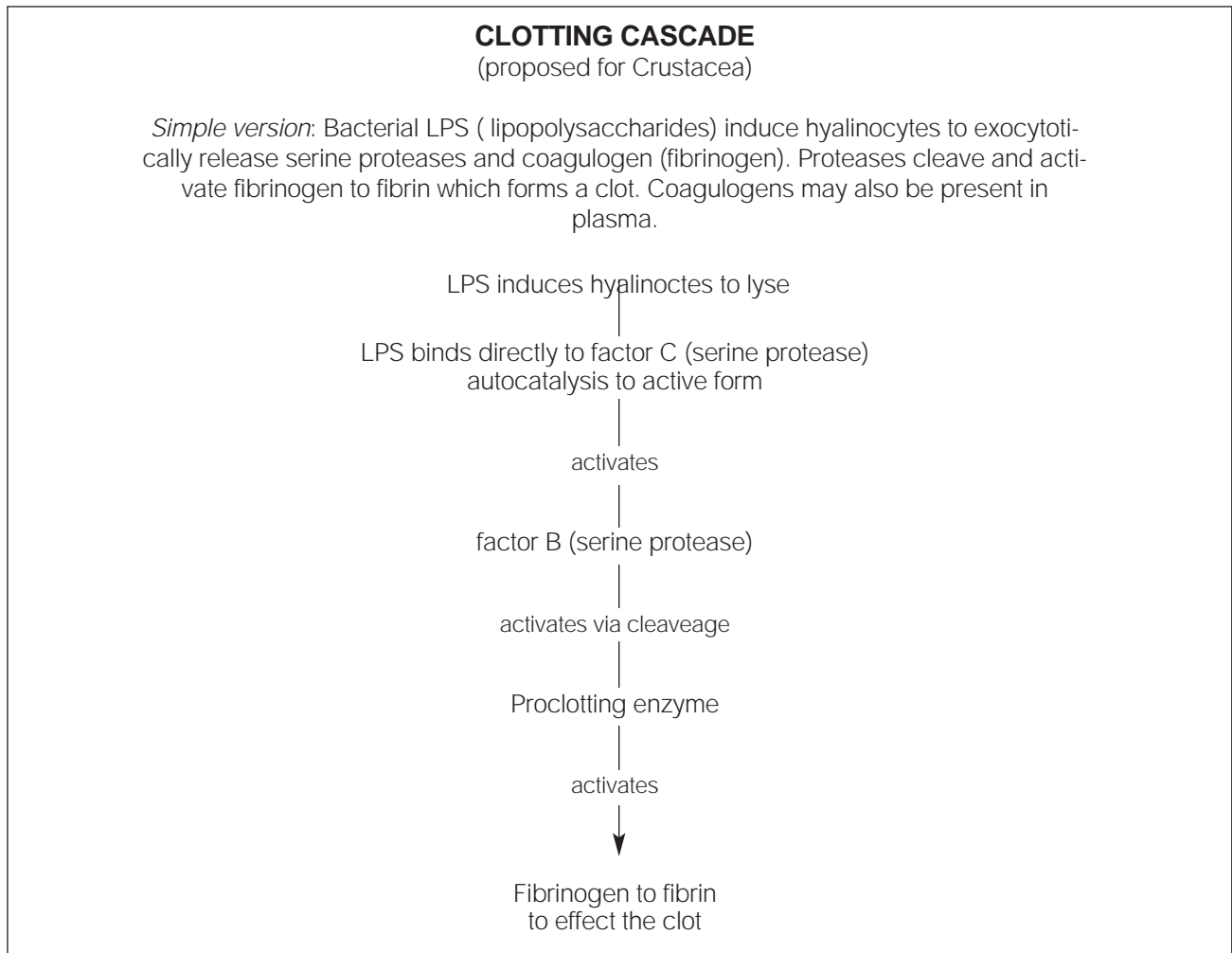


Figure 53. Generalized clotting cascade proposed for Crustacea. From Martin et al. (1991) and Söderhäll and Cerenius (1992).

number of hyalinocytes present in the hemolymph (Hose et al. 1990). The blue crab has Type C clotting, or explosive cytolysis (Clare and Lumb 1994). Transmission electron microscopy shows that the granules in the hyalinocytes dehisce by means of exocytosis and release their products into the extracellular matrix (Hose et al. 1990). Hemocytes form pseudopods rapidly and in association with clotting/coagulation (Bauchau and DeBrouwer 1974). Clotting in blue crabs is also temperature dependent, but few if any studies have investigated the role of temperature in activating and regulating the response.

In the blue crab, semigranulocytes or intermediate cells are 13 to 20  $\mu\text{m}$  long by 6 to 10  $\mu\text{m}$  wide,

have a low nucleus-to-cytoplasm ratio, and contain several sub-micron and micron-sized granules. They share similar morphological features with hyalinocytes and granulocytes, namely similar types of granules, highly organized organelles, and size (Bodammer 1978). Semigranulocytes are distinguished from granulocytes by the central or eccentric location of the nucleus, an intermediate number of granules, a mixture of granule sizes as opposed to a relatively constant size, and the presence of non-refractile granules (Bodammer 1978; Hose et al. 1990). Semigranulocytes are identical to the small granule hemocytes of Clare and Lumb (1994) and are consistent with the small granule hemocytes described by Hose et al. (1990).

Granulocytes are variable in size from 12 to 25  $\mu\text{m}$  in diameter, with a low nucleus-to-cytoplasm ratio compared to hyalinocytes. They can be distinguished from the semigranulocytes by the presence of numerous, large ( $>1 \mu\text{m}$ ) granules. Although not reported for the hemocytes of blue crabs, the granulocytes are often packed with either small ( $<1 \mu\text{m}$ ) or large ( $>1 \mu\text{m}$ ) granules (cf. Cornick and Stewart 1978). The latter are occasionally basophilic when stained with hematoxylin and eosin (Shields, pers. obs.). Granulocytes initiate encapsulation and nodule formation and show more intense staining for pro-PO than hyalinocytes (Hose et al. 1990). Granulocytes do not lyse during clotting (Bodammer 1978; Hose et al. 1990), but some may dehisce to release their granules when exposed to bacterial invaders (Söderhäll and Cerenius 1992).

Chisholm and Smith (1992) determined that antibacterial activity against Gram-positive and Gram-negative bacteria resides exclusively in the granulocyte, at least in *Carcinus maenas*. The factor or factors for this activity, effective within an hour, was heat stable, independent of divalent cations, and non-lytic for 8 of the 12 bacteria tested. Chisholm and Smith (1994) also determined that the activity of the hemocyte lysate supernatant responded to temperature. The factor in the supernatant, collected during all months except those with the highest and lowest temperatures, responded well, indicating the importance of temperature for immunity in the host and use of such biomarkers for assessing environmental health.

Phagocytosis of invaders, cell debris, and waste products is an important function of the hemocytes. All hemocyte types are capable of phagocytosis, but hyalinocytes and semigranulocytes are more actively phagocytic than granulocytes (Bauchau 1981). Johnson (1976d, 1977a) stated that the hyalinocytes and semigranulocytes are phagocytically active against Gram-negative bacteria while hyalinocytes are active against amebae. Smith and Ratcliffe (1980) used heat-killed Gram-negative bacteria and TEM to demonstrate phagocytosis in the hyalinocytes of *Carcinus maenas*. Söderhäll et al. (1986) indicated that hyalinocytes are the phagocytic cells in *C. maenas* and that hyalinocytes and semigranulocytes are

phagocytic in crayfish. However, Hose et al. (1990) elegantly demonstrated that granulocytes are the primary phagocytic cells in lobsters (*Panulirus interruptus* and *Homarus americanus*) and the majid crab *Loxorhynchus grandis*. The granulocyte is involved in encapsulation for all of these species, but the hyalinocytes and semigranulocytes are both involved in encapsulation in blue crabs (Johnson 1980). Some hyalinocytes lyse in the presence of bacteria and other protozoa (Johnson 1980; Hose et al. 1990), presumably as part of an activation mechanism for further defensive responses such as encapsulation.

The fixed phagocyte is a tissue-bound defensive cell capable of phagocytosis. In blue crabs and other brachyurans, the fixed phagocytes are found only in nodules or rosettes surrounding to the hepatic arterioles (Johnson 1980, 1987). They resemble semigranulocytes in size and appearance, but the clusters are always coated with a dense "interrupted layer" similar to a basal lamina. Circulating hemocytes may give rise to the fixed phagocytes (Johnson 1987). Phagocytosis of bacteria and viruses causes distinct changes to the pericellular space underlying the interrupted layer. The intracellular granules dehisce, and a finely granular material containing bacteria or large virions can be observed in the perinuclear space. Bacteria, bi-facies virus, and a baculovirus can be ingested by fixed phagocytes, while the smaller reovirus and picornavirus are not phagocytized (Johnson 1980, p. 325). Fixed phagocytes may die, becoming necrotic and frequently destroyed by the bacteria and viral pathogens they ingest.

### Cellular Responses and Melanization

A review of melanization and the related prophenoloxidase (proPO) cascade is beyond the scope of this chapter. For reviews, see Söderhäll (1982), Johansson and Söderhäll (1989), and Söderhäll and Cerenius (1992). Briefly, melanization is a chemical process initiated by the cellular defenses of invertebrates. It is typically an encapsulation response to isolate large foreign invaders or repair large wounds. Phenoloxidase is a key enzyme that triggers melanization. The inactive proenzyme proPO is



found in the semigranulocytes and granulocytes of crustaceans (Johansson and Söderhäll 1989; Hose et al. 1990). Activation of the proenzyme involves a complex cascade of peptides and enzymes (Söderhäll and Cerenius 1992). Bacterial cell wall components, primarily lipopolysaccharides and peptidoglycans, and presumably some metazoan invaders directly trigger degranulation of semigranulocytes (Fig. 54). The degranulated semigranulocytes release a 76 kD peptide into the hemolymph that causes further degranulation of the granulocytes, which then release proPO. Fungal cell wall components, primarily beta-1,3 glucans, and some bacterial components bind with beta-glucan binding protein in the

hemolymph which then stimulates degranulation of the granulocytes. A serine protease, ppA, or prophe-noloxidase activating enzyme, catalyzes proPO to phenoloxidase, which acts on phenols in the hemolymph to produce quinones that polymerize to form melanin. Several enzymes and other proteins are involved in regulating the level of encapsulation, melanization, cell adhesion, and degranulation (Johansson and Söderhäll 1989).

### Humoral Defenses

Various humoral factors contribute to extracellular and intracellular destruction of parasites in crustaceans (see Smith and Chisholm 1992; Söder-

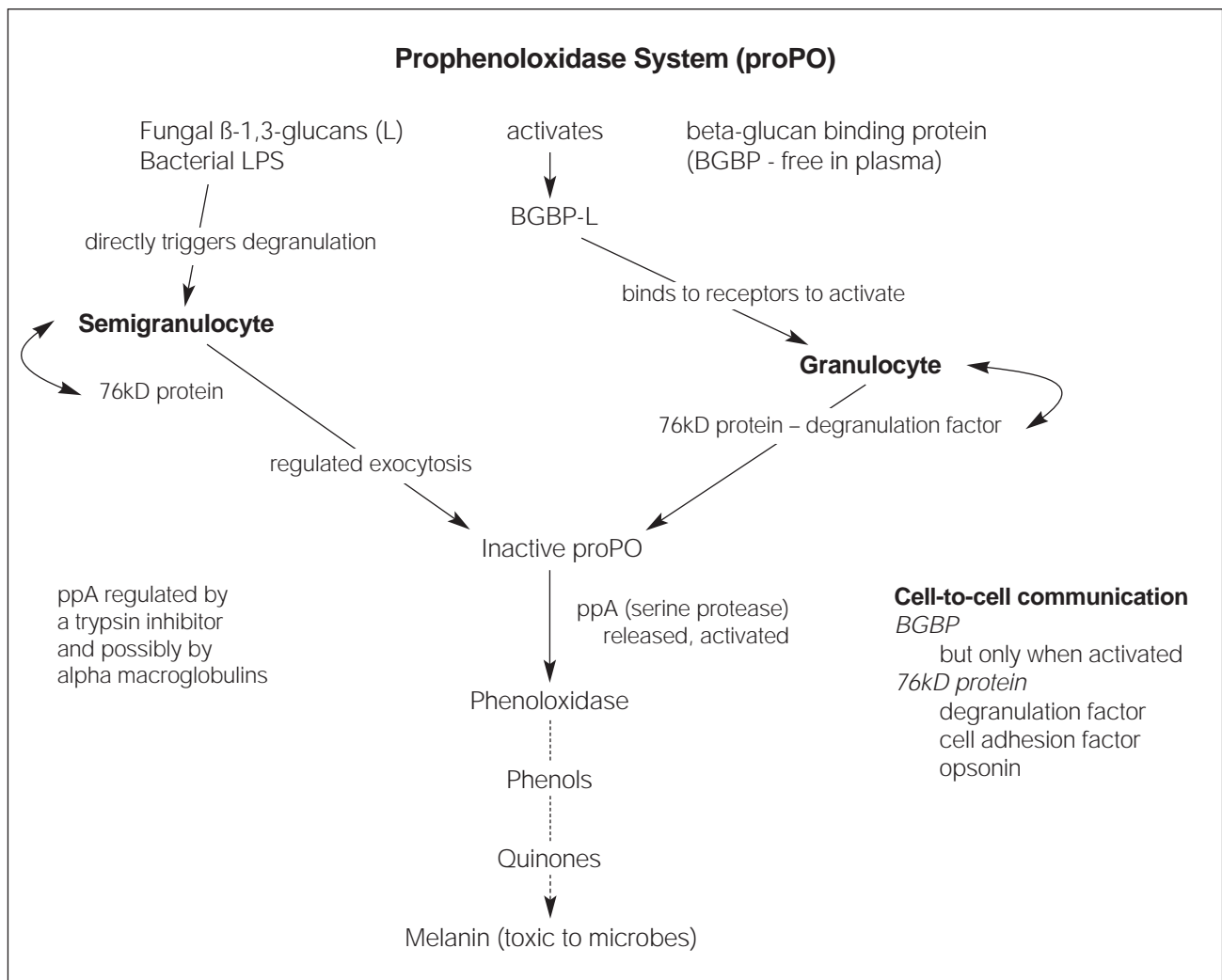


Figure 54. Aspects of the prophenoloxidase (proPO) cascade against microbial invaders. From Söderhäll and Cerenius (1992). LPS = lipopolysaccharides.

häll and Cerenius 1992). Lectins are part of the self/non-self recognition system in crustaceans and most invertebrates. They are polyvalent proteins or glycoproteins with binding affinities for specific carbohydrates, primarily polysaccharides and lipopolysaccharides. The suggested defensive roles for humoral factors include agglutination of bacteria by lectins and agglutinins leading to inactivation, lysis of inactivated bacteria by extracellular lysosomal enzymes, activation of hemocytes for encapsulation or phagocytosis of agglutinated particles, and opsonization for recognition of non-self particles by hemocytes (Hardy et al. 1977; Vasta and Marchalonis 1983; Ey 1991). Opsonin-like activation by agglutinins probably occurs in the blue crab when hemocytes form nodules in response to bacterial and protozoal infections (Johnson 1976d, 1977b; Messick 1994). For a comparative review of lectins in invertebrates and vertebrates, see Arason (1996).

In crustaceans, agglutinins and other lectins have been found that bind vertebrate erythrocytes (Pauley 1973), bacteria (Huang et al. 1981), invertebrate sperm (Smith and Goldstein 1971), protozoans (Bang 1962), and other cells (Tyler and Metz 1945). In most crustaceans, agglutinins occur naturally in the plasma or serum or bound to the cells (Cassels et al. 1986, 1993; Smith and Chisholm 1992; Chisholm and Smith 1995). Agglutinins generally have low titers in crustaceans when compared to other invertebrates (Smith and Chisholm 1992). Several lectins have been discovered in the blue crab (Pauley 1974; Cassels et al. 1986, 1993). These lectins can be bound to the hemocytes or free in the serum and show specificity to N-acylaminosugars that are common constituents of bacterial cell walls. Such sugars are found in a number of serotypes of *Vibrio parahaemolyticus* and may thus function in the innate resistance to this bacterium by the blue crab (see discussion in Cassels et al. 1986).

There is surprisingly little correlative evidence on the role of agglutinins or other humoral factors in the disease resistance of crustaceans. Foreign bodies such as vertebrate red blood cells (RBCs) induced increased agglutination titers in the blue crab (Pauley 1973). Rabbit and chicken RBCs induced a short but weak rise in the titers of agglu-

tinins over 2 d. Lobster sera, however, did not agglutinate *Aerococcus viridans*, but sera from three species of crabs, *Cancer irroratus*, *Chionoecetes opilio*, and *Geryon quinqueedens*, produced varying levels of agglutination (Cornick and Stewart 1968, 1975). Agglutination was not correlated with infectivity of the pathogen. Pooled sera of *Marsupenaeus japonicus* agglutinated horse, sheep, chicken, and human RBCs (Muramoto et al. 1995). In blue crabs, hemagglutination by individuals did not correlate with infection by *Hematodinium perezii* (Shields et al. 2003). Several infected crabs, however, did show relatively high titers of activity (>1:64). Interestingly, the serum of crabs that were refractory to infections of *H. perezii* showed marked precipitation when frozen at -80°C, but hemagglutination was not determined for these animals.

More recently, callinectin, a bacteriolytic killing factor, has been found in the sera and on the hemocytes of blue crabs (Noga et al. 1994, 1996; Khoo et al. 1996). Callinectin is a small peptide that exhibits specific activity against several marine bacteria, including *Vibrio* spp. Decreased levels of callinectin are associated with shell disease in the Pamlico River, North Carolina (Noga et al. 1994). Interestingly, blue crabs in similar areas in the Pamlico and Neuse Rivers were also reported to have decreased levels of hemocyanin (Engel et al. 1993), which normally comprises over 90% of the serum proteins. Correlations between hemocyanin concentrations and callinectin activity should be further investigated as biomarkers for stress in these crabs.

Lysozymes, key lytic enzymes in the hemolymph of vertebrates and other invertebrates including insects and molluscs, have not been reported extracellularly in crustaceans (Smith and Chisholm 1992). They are probably present in the lysosomes, but their absence in the hemolymph is notable.

Blue crabs also have humoral receptors that neutralize bacteriophages. Clearance is probably through passive circulation followed by adherence to cell-bound receptors in the gills and hepatopancreas (McCumber and Clem 1977; Clem et al. 1984). The plasma is apparently more effective than serum in neutralizing the bacteriophages (McCumber et al. 1979). The neutralizing factor is a polymer of non-

covalently linked subunits, each with a MW of 80 kDa.

Bacterial and protozoal diseases commonly lead to reduced hemocyte densities in crustaceans. Because many of the defensive reactions are cell-bound or cell-mediated, it is no surprise that morbidity and mortality are associated with declining hemocyte density. With *H. perezii*, hyalinocyte densities decline with infection, and hemocyte densities are correlated with host mortality (Shields and Squyars 2000). Loss of clotting is probably dependent on the decline in hyalinocytes in infections and may be associated with mortality. Because blue crabs have Type C clotting, known as explosive cytolysis (Tait 1911 cited in Clare and Lumb 1994), a rapid decline in hyalinocyte densities, coupled with changes in serum proteins, probably leads to loss of clotting ability.

### Organ-derived Components

The gills, antennal glands, and hemopoietic tissues are passively or actively involved in the cellular defenses of the blue crab. The gills act as passive filters trapping hemocyte-formed nodules that contain bacteria. The flow of hemolymph from the heart to the gills is under positive pressure (Maynard 1960). That positive pressure, coupled with the highly vascularized nature of the gills, makes them ideal "sinks" for foreign particles and reacting hemocytes (Smith and Ratcliffe 1980). Localization of nodules in the gills is a hallmark of bacterial and fungal infections in crustaceans (Cornick and Stewart 1968; Solangi and Lightner 1976; Smith and Ratcliffe 1978, 1980; White and Ratcliffe 1982; White et al. 1985; Martin et al. 1998). Gross observations of white nodules in the gills are often indicative of *Vibrio* spp. infections in blue crabs (Johnson 1976d; Overstreet and Shields, pers. obs.). Cell-bound lectins apparently recognize and agglutinate some bacteria, assist phagocytosis, initiate encapsulation and melanization of agglutinated bacteria, and facilitate deposition of nodules in the narrow spaces of gill lamellae (Smith and Ratcliffe 1980; Martin et al. 1999, 2000). The permeability of gill lamellae may also allow removal of waste products generated by host defenses or may permit sloughing of melanized capsules with ecdysis.

The gills and antennal glands actively clear foreign proteins and small particles such as virions. McCumber and Clem (1977) and Clem et al. (1984) injected blue crabs with radiolabeled bovine serum and examined the clearance of the protein from the hemolymph and various organs. The gills and antennal glands showed high levels of radioactivity over 1 to 4 h, while the hemolymph showed declines in activity after 30 minutes. Johnson (1980) found that large viruses may be selectively removed by the podocytes at the bases of the gill branchiae and lamellae. Podocytes possess membrane diaphragms that apparently act to filter the hemolymph. She speculated that foreign proteins and smaller viruses are removed by the podocytes while large viruses are taken up by the fixed phagocytes and hemocytes. Clem et al. (1984) found that the relatively large poliovirus was deposited in the gills, whereas the smaller bacteriophages were removed less actively through receptors in the hepatopancreas (McCumber and Clem 1977; McCumber et al. 1979).

Lastly, the hemopoietic tissues of the host may be stimulated by certain types of infection (Johnson 1980, p. 283). Hyperplasia of tissues with increased mitotic activity has been noted for bacterial infections but not for protozoal infections. The process for this stimulation has not been well studied, but it can occur quickly, within several hours for dormant crabs subjected to warmer laboratory conditions. Hemopoiesis also changes with the molt cycle (Johnson 1980), and that may obscure a response directed to infectious agents. Nonetheless, activation of the hemopoietic tissue and factors controlling increases in changes in the sequestration of hemocytes are ripe areas for investigating the nature of inducible cellular responses in crustaceans.

### Future Research

Several studies have highlighted aspects of cellular and humoral defenses in shrimps, crabs, crayfishes, and lobsters, but most of these have focused on single elements in the defensive system of the host. With the exception of *Aeromonas viridans* in the American lobster (for review see Stewart 1980), studies have not focused on specific pathogens and host responses *per se*. For example, whereas lectins are important molecules in recognizing self versus



non-self, their functional response to different pathogens is relatively unknown. Are they induced by pathogenic invaders? Does lectin activity decline with the loss of condition of the host? Similar arguments can be made for most elements of the defensive system, especially for hemocytes. Hemocytes may decline with pathogenic infections, but do all hemocyte types decline or are subpopulations affected differentially by specific pathogens? Blue crabs infected with *Hematodinium perezii* exhibit differential changes in cell types against the background of absolute declines in hemocyte densities (Shields and Squyars 2000). The relative decline in hyalinocytes in bacterial, amebic, and *Hematodinium* spp. infections (Johnson 1976d, 1977b; Johnson et al. 1981; Messick 1994; Field and Appleton 1995) probably represents their activation in encapsulation and nodule formation, but their loss may also be associated with loss of clotting ability and resulting host mortality.

Lastly, some blue crabs appear refractory to infection by *H. perezii* and presumably to other pathogens. What elements of the defense system are responsible for this resistance and do they confer immunity to other invaders? Clearly, our understanding of the defensive responses of the blue crab would advance through studies on lectins, defensins, inducible non-specific responses, proteases, and cell-derived components such as prophenyloxidase, phagocytosis, and hemopoietic responses, in relation to pathogens.

## CONCLUSIONS

Pathogenic diseases have obvious negative impacts on individuals, but extrapolation to host populations and their dynamics can be difficult. The importance of diseases in the population dynamics of the host is the subject of considerable debate, even in human populations where plagues have had major impacts on populations and the course of modern history. Parasitic diseases can negatively affect, and possibly even regulate, crustacean populations (Blower and Roughgarden 1989a, b). As we have indicated, pathogenic diseases such as *Hematodinium perezii*, *Paramoeba perniciosus*, and *Loxothylacus texanus* may play a key role in the population

dynamics of the blue crab. Before dying, diseased crabs are weakened by their infections, and they often succumb to stressors such as temperature (high or low), hypoxia, cannibalism, or increased predation. Measuring the effects of the diseased state on predation rates may be possible using tethering experiments as has been done for juvenile blue crabs in the field (Heck and Coen 1995).

Diseases can have important negative consequences to crab populations. As with most fisheries, the question arises as to why be concerned about diseases when nothing can be done to limit their effects on the fished population. First, natural mortality is often assumed to be 0.2 in pre-recruits in many fishery models. Unfortunately, background levels can be much higher, especially during outbreaks of pathogens such as *Hematodinium perezii* or *Paramoeba perniciosus* where mortalities to the pre-recruit and adult populations can approach 100% in endemic locations. Stock assessments and fishery models must incorporate losses to diseases if they are to be used in managing the resource. Second, several parasitic diseases cause marketability issues through stunting of the host or by causing unsightly lesions in the crabmeat. Market losses can influence public opinion about quality of product. Third, certain fishing practices such as transporting crabs between watersheds may help to spread diseases. By understanding transmission and pathogenicity of a disease, one can curtail or minimize such practices. Fourth, with the advent of shipping live crabs and lobsters, there is an increased potential for the inadvertent introduction of pathogenic agents to new regions. This is not a trivial issue as introduced diseases have wreaked havoc on the shrimp industry worldwide and have marginalized the aquaculture of abalone in California.

Diseases of the blue crab affect fecundity, recruitment, and mortality, yet there are few practical responses to control or mitigate effects of diseases in crustacean fisheries. Simple measures such as "culling" infected individuals on station or within a watershed, culling or removing dead animals to onshore fertilizer processing plants, limiting transportation of live animals, and changing "baiting practices" may limit the spread of pathogens to new locations. Changes in fishing policies may also be

warranted. Regulations on minimum size may enhance populations of parasites that stunt their hosts, and the accumulation of stunted crabs may further affect the fishery. By using outreach or other education programs, fishermen could practice destroying stunted and parasitically castrated crabs, but many fishermen are loathe to keep or kill small crabs for fear of penalties from enforcement agencies. Lastly, many state or regional agencies have monitoring programs for stock assessments. Rhizocephalan barnacles, which cause alterations and appear similar to egg masses, could easily be included in monitoring protocols. The wealth of information from such monitoring programs would enhance our understanding of disease prevalence and association with host and environmental variables and help to document effects on the individual hosts and the fished populations.

The role of stressors in crab mortalities cannot be overstated. Seasonal hypoxia and temperature extremes are often associated with crab mortalities, but neither stressor has received much attention when associated with infectious diseases. The sudden mortalities in *Hematodinium perezii*-infected crabs could be related to hypoxic events, especially given the oxygen demands of the parasite and the moribund host. Low temperatures are often cited as causes of winter mortalities reported for blue crabs, yet *Paramoeba perniciosus* is known to overwinter in blue crabs, and *Hematodinium perezii* may persist in crabs during winter (Messick et al. 1999). These parasites may contribute as underlying causes of winter mortalities, especially in mid to high salinity areas. Physico-chemical influences should be further examined in laboratory and mesocosm studies as they no doubt contribute to the mortality of diseased crabs.

Host factors such as size, sex, maturity status, ovigerous state, and molt stage often affect the nature of the crustacean host-symbiont relationships. Indeed, the blue crab and other crustaceans may be vulnerable to infection especially during ecdysis, oviposition, and dormancy. Throughout the range of symbioses, we see remarkable adaptations to host

molting. Such adaptations range from symbionts that simply migrate onto the new instar (e.g., *Carcinonemertes carcinophila* and possibly *Myzobdella lugubris*), to those that have exquisitely timed reproduction such that new propagules can find their mobile substratum (peritrich and apostome ciliates), to a parasite that interferes with ecdysis to enable its own reproductive efforts (*Loxothylacus texanus*). It is the very nature of these relationships and their association with host factors that provide insights into the biology and ecology of the crab host.

As we have shown, several symbionts can serve as indicators of the biology of the blue crab. Shell disease shows clear affinities with poor water quality and pollution; the nemertean *C. carcinophila* can be used to indicate spawning status; barnacles show relationships with host molting, longevity, and migration patterns; and leeches and branchiobdellid annelids can indicate host origin and water quality conditions. Fouling agents can indicate the timing of migration, the anecdysial molt stage, water quality, and more. The presence and abundance of these indicators are not difficult to assess, and their indications should be further developed to aid in assessments of impacts of migrations and water quality on the host.

We have focused attention on much needed research priorities for each symbiont. Although our comments are primarily directed to host-parasite relationships and effects on the fisheries, other avenues are open for exploration. For example, many of the pathogens associated with blue crabs show narrow salinity tolerances. We speculate that the extensive catadromous migrations of the host may have resulted from selection pressures induced by the myriad fouling organisms and pathogenic parasitic diseases. Regardless, blue crabs found in freshwater reaches generally have fewer species of parasites and diseases than those found in high salinity regimes. Why these patterns have evolved is intriguing. Addressing such evolutionary questions on host-symbiont relationships will enhance our understanding of how such intimate associations develop in invertebrate hosts.

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