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## DISEASES, PARASITES, AND SYMBIONTS OF BLUE CRABS (*CALLINECTES SAPIDUS*) DREDGED FROM CHESAPEAKE BAY

Gretchen A. Messick

### ABSTRACT

A 3-year histological study of disease prevalence in 657 blue crabs, *Callinectes sapidus*, dredged from 31 sites within Maryland portions of Chesapeake Bay during autumn and winter revealed the presence of many diverse parasites and symbionts. A large number of crabs exhibited hemocytic infiltration and encapsulation. Parasites and symbionts identified included viruses (Baculo-B and RLV-RhVA), a rickettsia-like microorganism (RLM), an unusual strandlike organism, unidentified microsporidians and gregarines, both parasitic (*Mesanoophrys chesapeakeensis*) and symbiotic (*Lagenophrys callinectes* and *Epistylis* sp.) ciliates, the nemertean *Carcinonemertes carcinophila*, and trematode metacercariae, some hyperparasitized by the haplosporidian *Urosporidium crescens*. Significant differences in disease and parasite frequencies were observed among survey periods. The prevalence of some tissue responses and parasites exhibited seasonal patterns of infection and infestation.

Blue crabs, *Callinectes sapidus* Rathbun, are one of the most valuable commercial fisheries in Chesapeake Bay. The 1996 Maryland landings were 38 million pounds, valued at \$31 million (Maryland Department of Natural Resources, 1996). Blue crab landings fluctuate yearly (Holliday and O'Bannon, 1990) due to factors such as winds and currents, which influence larval transport and recruitment from the Atlantic Ocean back into Chesapeake Bay (Boicourt, 1982; Sulkin and Epifanio, 1986). Recent indications of decreased crab landings and increased fishing pressure in Chesapeake Bay have prompted legislation of conservation measures for the fishery since 1994 (Abbe and Stagg, 1996). Other factors, such as predation, food availability (Van Heukelem, 1991), and disease (Sprague, 1965; Johnson, 1983) may also affect fluctuations in crab abundance. Reduced catches or mortalities associated with diseases or parasites have been documented in several commercial crustacean fishery populations including Chesapeake Bay (Sprague and Beckett, 1966), other blue crab fisheries (Overstreet, 1978), king crabs (*Paralithodes camtschatica* (Tilesius)) (Kuris *et al.*, 1991), Tanner crabs (*Chionoecetes bairdi* Rathbun) (Meyers *et al.*, 1987), and the Norway lobster *Nephrops norvegicus* (Linnaeus) (see Field *et al.*, 1992), and the velvet swimming crab *Necora puber* (Linnaeus) (see Wilhem and Mialhe, 1996). This study examines the prevalences of diseases and parasites in blue crabs

within Chesapeake Bay in order to better understand the impact that diseases may have on blue crab populations.

Numerous publications have described the diseases, parasites, and symbionts of blue crabs (Sprague, 1970; Johnson, 1978, 1983; Overstreet, 1978; Couch, 1983; Millikin and Williams, 1984; Messick and Sindermann, 1992), but most reports lack information on the prevalence of infections. I report prevalences of several diseases, parasites, and symbionts in blue crabs and suggest relationships with temperature and salinity.

### MATERIALS AND METHODS

Crabs were sampled from the Maryland portion of Chesapeake Bay (Fig. 1). Crabs sampled in the winter were obtained in cooperation with the Maryland Department of Natural Resources' (MDNR) annual winter dredge survey (WDS) of overwintering blue crab population densities. Crabs sampled in the autumn (ADS) were obtained as bycatch from MDNR's oyster-bar surveys.

Stations sampled during the winter were chosen randomly using longitude and latitude and water depth of 1.8 m (6 ft) as required for the research vessel. Crabs were caught by dragging a 1.8-m (6-ft) dredge, lined with 13-mm (0.5-in) hardware cloth, for 1 min. Crabs sampled during autumn months were a bycatch of oyster dredge tows with a 91-cm (3-ft) wide dredge (Smith and Jordan, 1993). Crabs were transported to the Cooperative Oxford Laboratory, Maryland, and placed in tanks with flowing estuarine water from the Tred Avon River for up to 24 h before dissection. MDNR's Resource Assessment Service provided mean salinity and temperature data taken for 3 water quality monitoring stations from the main stem of the bay (Fig. 1).

Carapace width (CW, greatest width between the epi-

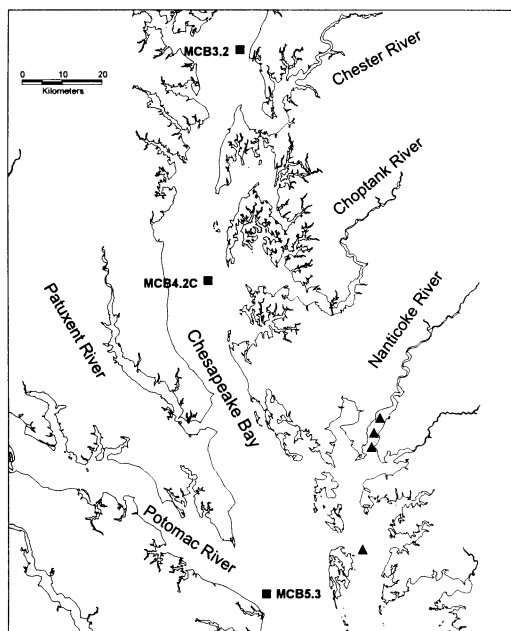


Fig. 1. Maryland portion of Chesapeake Bay showing various sites where blue crabs were sampled during the autumn and winter from 1990–1992. ▲ = sites where crabs infected with *Mesanothrips chesapeakeensis* were found. MCB3.2, MCB4.2C, and MCB5.3 are reference salinity and temperature stations.

branchial spines), sex, and maturity were assayed by morphology of the abdomen and telson (male crabs under 90-mm CW were considered immature) (Millikin and Williams, 1984). Crabs from each river system were dissected, with gill, gut, hepatopancreas, epidermis, heart, hemopoietic tissue, brain, antennal gland, thoracic ganglion, and muscle tissues removed for histological processing (Messick, 1995). Tissues were placed either in Helly's or Bouin's fixative for 18 h, embedded in paraffin, cut at 5  $\mu$ m, and stained with Mayer's hematoxylin and eosin (Luna, 1968) or alcian blue (Howard and Smith, 1983). Gram (Brown and Hops, 1973), periodic acid-Schiff, PAS (Howard and Smith, 1983), and acid-fast bac-

teria, AFB (Howard and Smith, 1983) staining techniques were also utilized. Since histological preparations included only a small portion of the whole animal, false negatives may have been recorded during this survey.

Slides were examined for pathology, presence of parasites, or any abnormal histological characteristics (Johnson, 1980; Messick, 1995). Diagnoses of diseases and identification of parasites were based on light microscopy, electron microscopy (EM), and literature descriptions. Although only a presumptive diagnosis of viral infections can be made without EM analysis, light microscopy demonstration of previously published histological characteristics of viral infections which were assayed using EM (Johnson, 1984) were used to assign the type of viral infection. No biochemical analyses were performed on crab tissues from this survey.

A chi-square contingency table with  $\alpha = 0.05$  and 56 degrees of freedom was used to determine if frequencies of the various diseases and parasites varied statistically from one survey period to the other (Steele and Torrie, 1980, p. 498). Data were further evaluated using confidence limits for percentages (Sokal and Rohlf, 1973) to determine the degree to which overall prevalence of a disease or parasite varied between survey periods. Confidence limits for zero prevalence indicated the probability that the disease or parasite may have been present in the population, but was missed due to sampling error, such as low sample numbers in relation to a large population or the small portion of tissue assayed.

## RESULTS

A total of 657 crabs were sampled from 31 sites within the Maryland portion of Chesapeake Bay (Fig. 1). The random sampling method prevented repetitive sampling, although certain river systems were sampled several times. Numbers of crabs collected, mean salinity, and temperature for three reference stations varied for each sampling period (Table 1). Generally, northern stations had lower salinity and temperature than southern stations. In all samples combined, 60% of the crabs were male and 40% were female (Table 2); 40% of male crabs were im-

Table 1. Number of blue crabs dredged from Maryland portions of Chesapeake Bay and mean salinity and temperature for three reference stations during the autumn and winter from 1990–1992. WDS = winter dredge survey, ADS = autumn dredge survey.

Station	WDS 90	WDS 91	ADS 91	WDS 92	ADS 92
Number sampled	113	224	60	188	72
MCB3.2					
Salinity	15.8	14.7	17.2	17.9	17.8
Temperature	1.7	6.3	17.9	4.7	16.0
MCB4.2C					
Salinity	21.5	17.9	23.2	21.2	17.5
Temperature	1.6	6.6	20.3	5.1	22.1
MCB5.3					
Salinity	22.8	22.2	24.9	24.8	16.4
Temperature	2.8	7.2	19.5	5.6	22.8

Table 2. Number of *Callinectes sapidus* collected and percentage with diseases and parasites by sex and maturity. Crabs dredged from Maryland portions of Chesapeake Bay during the winter and autumn from 1990–1992.

Condition	Reference number	Female	Male	Immature	Mature
Number collected		257	393	381	269
Infiltration		31	31	27	38
Nodules		13	11	11.5	12
Gill necrosis		0	1.5	0	2
Baculo-B virus	USNM 47929	3	2	3	2
RLV–RhVA	USNM 47925	1	0.2	1	0
RLM	USNM 47920	0	0.2	0	0.4
Strandlike	USNM 47923	4	4	6	1.5
Microsporidians	USNM 47914,				
	USNM 47915	4	4	5	3
<i>Mesanophrys chesapeakensis</i>	USNM 47816				
	ATCC 50563	0	0	0.8	0.4
<i>Lagenophrys callinectes</i>	USNM 47919	3	5	3	4
<i>Epistylis</i> sp.	USNM 47919	4	7	4	8.5
Unidentified gregarine	USNM 47916	17	19	17	21
Metacercariae	USNM 47918	3	7	4.5	7
<i>Urosporidium crescens</i>	USNPC 87468,				
	USNPC 87469	3	1.5	1	4
<i>Carcinonemertes carcinophila</i>	USNM 177942	12	9	8	14.5

Reference number = Accession number for type slides deposited at either USNM = National Museum of Natural History, Smithsonian Institution, Washington, D.C., U.S.A.; USNPC = U.S. Department of Agriculture, National Animal Parasite Collection, Beltsville, Maryland, U.S.A.; ATCC = American Type Culture Collection, Rockville, Maryland, U.S.A.

mature, and 72% of female crabs were immature. Insufficient numbers of infected crabs from maturity and sex categories, and individual collection sites prevented statistical analysis to determine whether significant variations occurred between disease conditions and these groups.

Three tissue responses, two presumptive viruses, two bacterial agents, six protozoans, unidentified metacercariae of digenetic trematodes, and one nemertean were identified (Tables 2, 3). Significant differences in disease and parasite frequencies were observed be-

tween survey periods as determined by a chi-square contingency table test ( $\chi^2_{.05[56]} = 203.529, P < 0.05$ ). Confidence limits for percentages showed that hemocytic infiltration, encapsulation, the strandlike organism, microsporidians, and the nemertean *Carcinonemertes carcinophila* (Kölliker) exhibited significant differences in prevalence between at least two survey periods (Figs. 2–4). Focal gill lesions, RLV–RhVA, Baculovirus, RLMs, *Mesanophrys chesapeakensis* Messick and Small, *Epistylis* sp., *Lagenophrys callinectes* Couch, unidentified gregarine and trematode

Table 3. Percentage of blue crabs with diseases dredged from Maryland portions of Chesapeake Bay during the autumn and winter 1990–1992. WDS = winter dredge survey, ADS = autumn dredge survey.

Condition	WDS1990	WDS1991	ADS1991	WDS1992	ADS1992	Average
Infiltration	29	30	13	36	7	28
Encapsulation	4	18	3	13	6	11
Gill necrosis	0	0	0	3	0	1
Baculo-B	0	3	0	3	0	2
RLV–RhVA	0	1	0	0	0	0.3
RLM	1	0	0	0	0	0.2
Strandlike	3	0	15	1	16	4
Microsporidian	0	10	2	0.5	3	4
<i>Mesanophrys chesapeakensis</i>	3	0.4	0	0	0	0.8
<i>Lagenophrys callinectes</i>	5	1	8	3	10	4
<i>Epistylis</i> sp.	1	1	8	6	10	4
Unidentified gregarines	8	19	22	20	14	17
Metacercariae	11	8	7	3	0	7
<i>Urosporidium crescens</i>	3	3	0	3	2	3
<i>Carcinonemertes carcinophila</i>	7	11	10	14	0	10

RLV–RhVA = Reolike–Rhabdolike virus, RLM = rickettsia-like microorganisms.

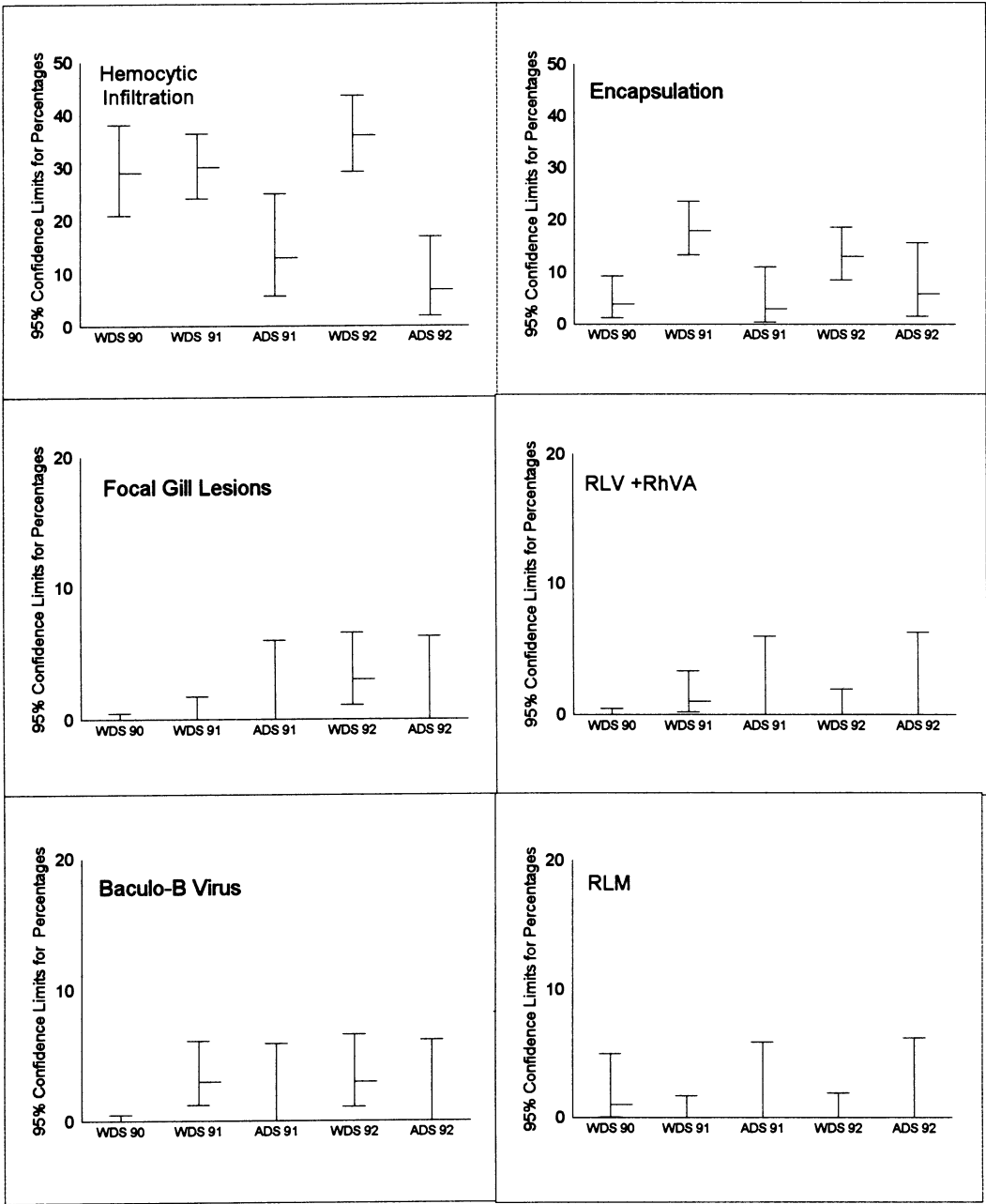


Fig. 2. Confidence limits for percentages and observed prevalence of hemocytic infiltration, encapsulation, focal gill lesions, RLV–RhVA virus, Baculo-B virus, and RLM in crabs (*Callinectes sapidus*) dredged during the autumn and winter from 1990–1992. Confidence limits for zero prevalence indicate the probability that the disease or parasite may have been present in the population, but was missed due to sampling error. (Observed prevalence = central mark.) RLV–RhVA = Reolike–Rhabdolike virus, RLM = rickettsia-like microorganisms.

metacercariae, and metacercariae infested with *Urosporidium crescens* De Turk, showed no significant differences in prevalence among survey periods (Figs. 2–4).

Generalized Tissue Responses

Hemocytic infiltration occurred in 28% of the crabs sampled (Table 2). Crabs sampled

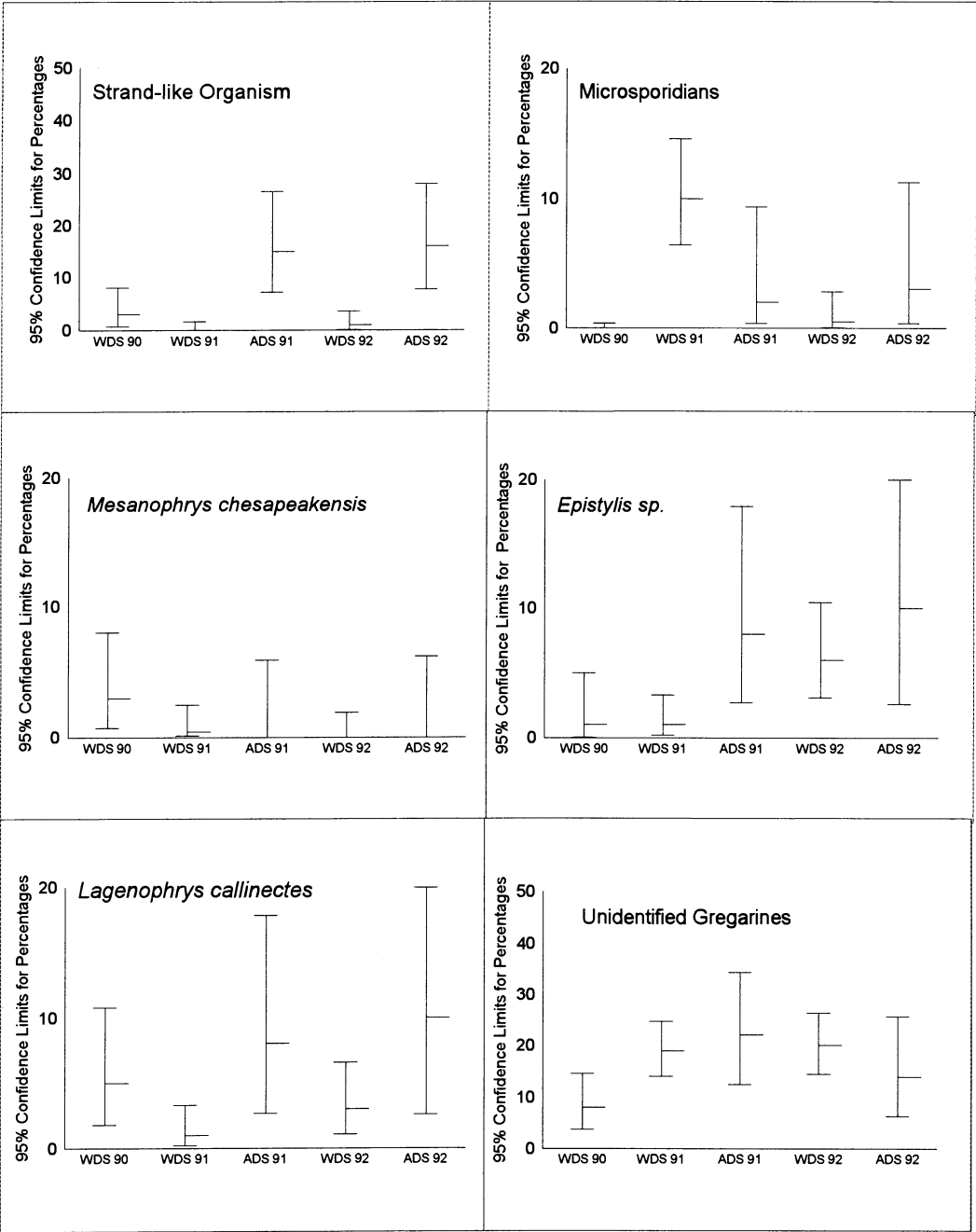


Fig. 3. Confidence limits for percentages and observed prevalence of strandlike organisms, microsporidians, *Mesanoophrys chesapeakeensis*, *Epistylis* sp., *Lagenophrys callinectes*, and unknown gregarines in crabs (*Callinectes sapidus*) dredged during the autumn and winter from 1990–1992. Confidence limits for zero prevalence indicate the probability that the disease or parasite may have been present in the population but was missed due to sampling error. (Observed prevalence = central mark.)

during the winter surveys had a significantly higher prevalence of infiltration than crabs sampled during the autumn of 1992 (Fig. 2). The mean winter prevalence of infiltration

was 32%, whereas the mean autumn prevalence was 10%. The prevalence of encapsulation, generally characterized as the sequestering of tissue debris or an injurious agent

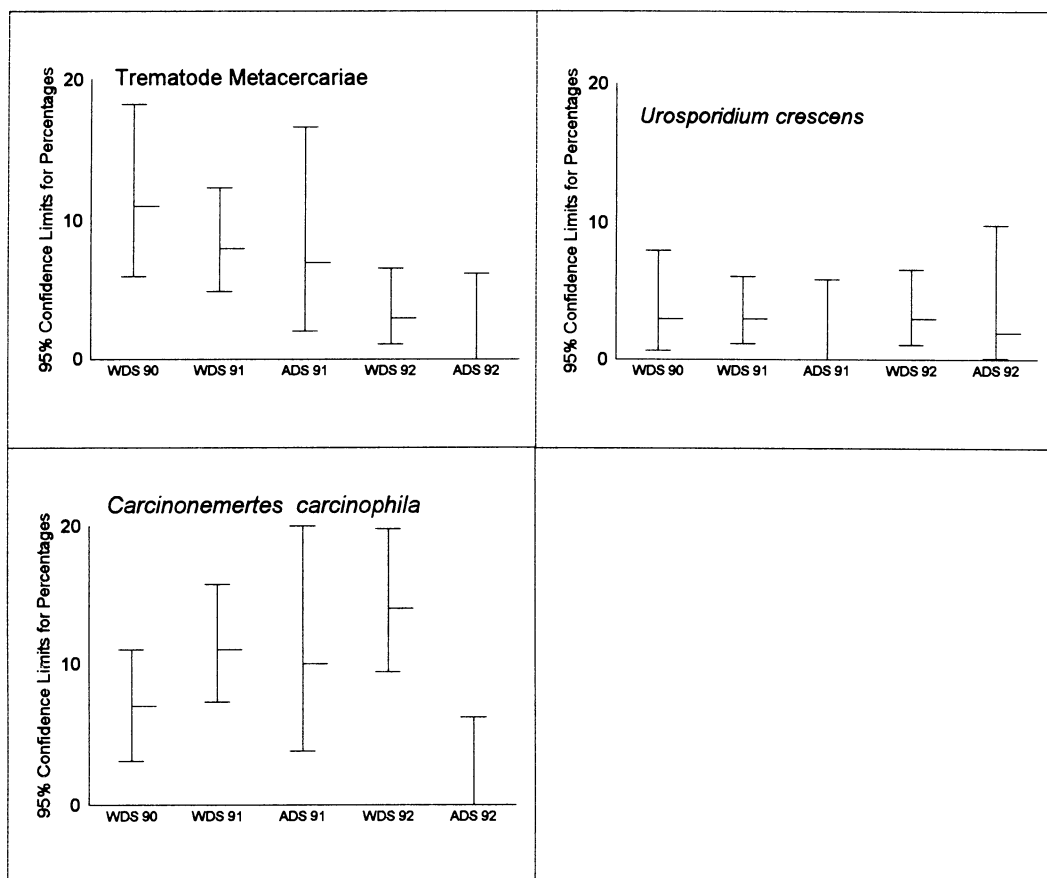


Fig. 4. Confidence limits for percentages and observed prevalence of metacercariae, *Urosporidium crescens*, and the nemertean *Carcinonemertes carcinophila* in crabs (*Callinectes sapidus*) dredged during the autumn and winter from 1990–1992. Confidence limits for zero prevalence indicate the probability that the disease or parasite may have been present in the population but was missed due to sampling error. (Observed prevalence = central mark.)

within a sheath of differentiated hemocytes, was statistically higher during the winter of 1991 than either the winter of 1990 or the autumn of 1991 (Fig. 2). The prevalence of encapsulation in crabs fluctuated from 3% during the autumn of 1991 to 18% during the winter of 1991 (Table 3, Fig. 2). Encapsulation was equally prevalent in male, female, mature, and immature dredged crabs (Table 2). Hemocytic infiltration and encapsulation were most often found in hemal sinuses and connective tissue.

Gill lesions were focal accumulations of what appeared to be degranulated hemocytes and vacuolate cytoplasmic material between layers of cuticle on gill lamellae. Lesions had a walled-off appearance with eosinophilic layers of cuticle lining the base of lesions. Gill epithelia associated with lesions had de-

creased cellular integrity and appeared to be separating from the cuticle (Fig. 5). Some hemal channels had increased numbers of granulocytes. One or several lesions could be found on a gill section. Gill lesions were noted only in mature males (110–160-mm CW) during the winter 1992 survey with a prevalence of 3% (Fig. 2).

#### Viral and Microorganism Infections

Histological indications of viral infections were found in 2.3% of crabs examined. Infections occurred at 11 stations between the upper Chesapeake Bay and the Annesmessex River. Cytological characteristics of tissues indicated the presence of Baculo-B virus (2%) and RLV-RhVA (0.3%). Baculo-B infections were found during the winters of 1991 and 1992; only two immature crabs



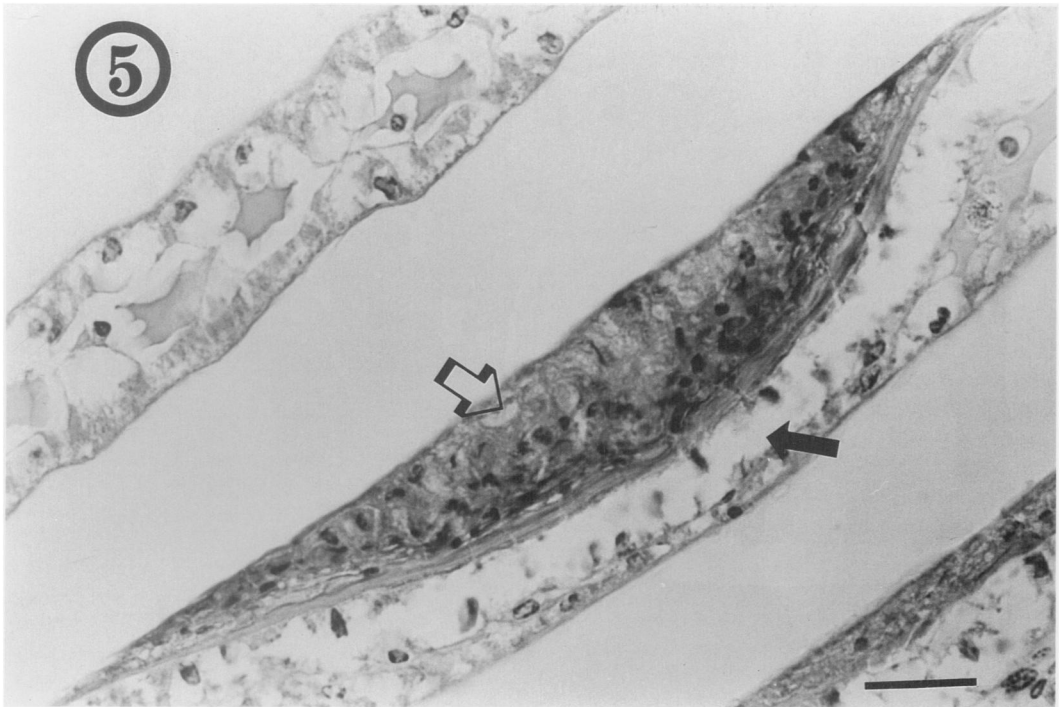


Fig. 5. Gill lesion in *Callinectes sapidus* showing focal accumulation of degranulated hemocytes and vacuolate cytoplasmic material (open arrow). Note the walled-off appearance of the lesion. Gill epithelia associated with lesions had decreased cellular integrity and appeared to be separating from the cuticle (black arrow). Line = 50  $\mu$ m.

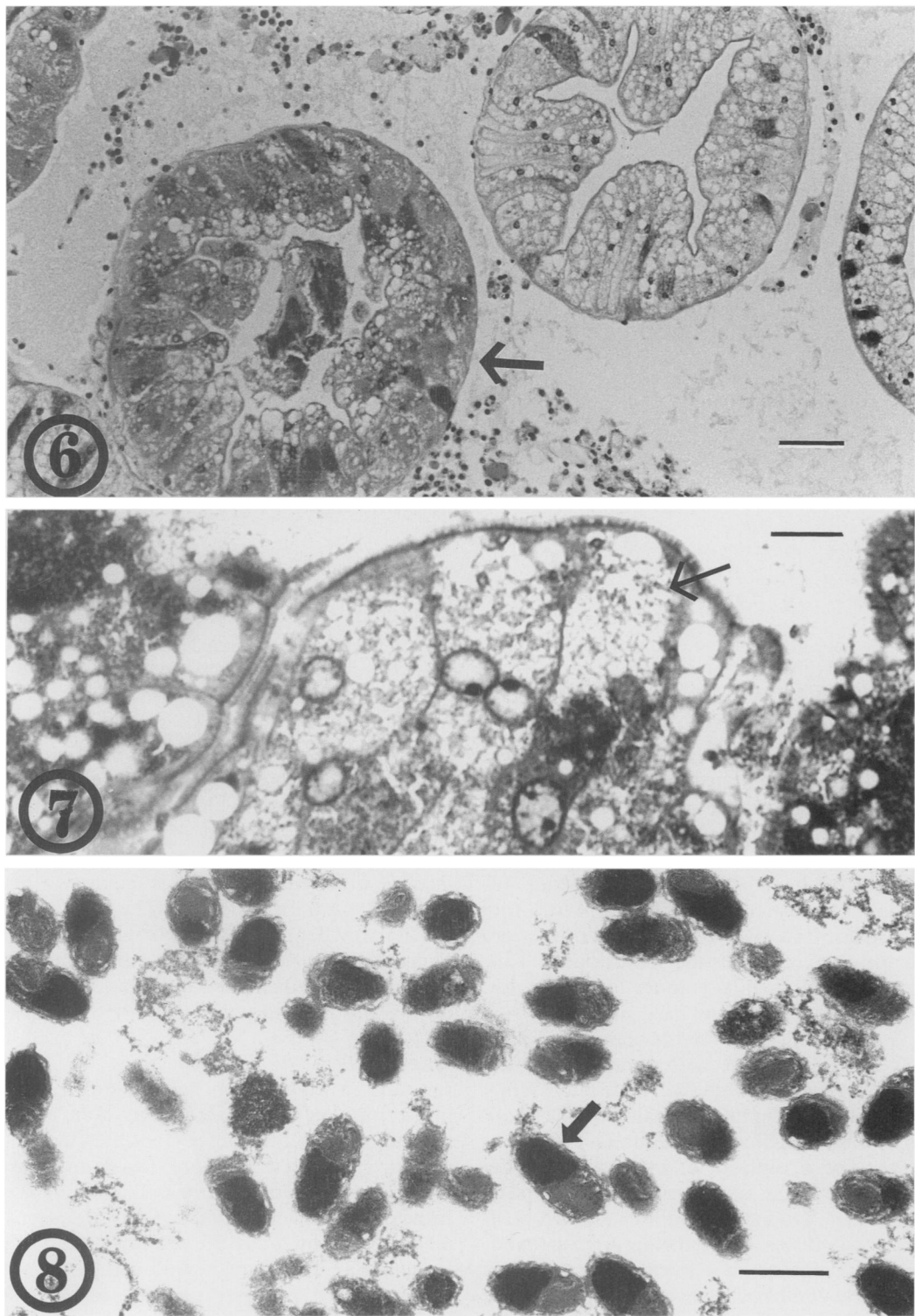
sampled during the winter of 1991 showed RLV–RhVA infections (Table 2, Fig. 2). Baculo-B-infected hemocytes and hemopoietic cells had hypertrophied nuclei which usually appeared as a dark rim around the circumference of the otherwise homogeneous nucleus; cytoplasm was condensed into a thin outer rim. RLV–RhVA viral infections were identified by cytoplasmic inclusions and increased cytoplasmic volume in hemocytes, hemopoietic tissue, and glial nerves.

A rickettsia-like microorganism (RLM) was observed in one crab during the winter of 1990 (Fig. 2, Table 3). Light microscopy preparations of epithelial cells of hepatopancreas tubules indicated that the infection was focal. The cytoplasm of infected epithelial cells was filled with granular, basophilic, Gram-negative material, which stained positive with nuclear fast red (Fig. 6). Surrounding hemal spaces occasionally had increased hemocyte numbers, indicating a host response to the RLMs. Electron micrographs of deparaffinized, postfixed tissues elucidated numerous short rods within the cytoplasm of infected epithelial cells (Figs. 7, 8). Most rods

occurred singly, although apparent budding pairs were occasionally seen. Rods were more electron dense on one end, and had a multilaminar plasma membrane. A granular material resembling ribosomes was the only apparent internal structure. RLM bodies averaged 0.25  $\mu$ m wide  $\times$  0.6  $\mu$ m long ( $N = 27$ ) (Fig. 7).

The prevalence of an unusual strandlike microorganism found in the lumen and attached to hepatopancreas tubules fluctuated from 0% during the winter of 1991 to 16% during the autumn of 1992, with an overall prevalence of 4% (Fig. 3, Table 3). Crabs sampled in the autumn had a significantly higher prevalence of infections than crabs sampled during the winter (Fig. 3). This unusual organism was found in 6% of immature crabs and 1.5% of mature crabs (Table 2). Infected crabs were found in 9 of 22 stations dispersed throughout the survey area from Eastern Bay to Tangier Sound and the lower western shore. In tissue sections, the organism appeared as a filamentous, nonseptate mass attached by a basal holdfast to hepatopancreas epithelial cells. Infections were focal; only a few tubules exhibited lesions





Figs. 6–8. Hepatopancreas of *Callinectes sapidus*. Fig. 6. Hepatopancreas tubule infected with rickettsia-like microorganisms (RLM) (arrow) adjacent to uninfected tubule. Line = 50  $\mu\text{m}$ . Fig. 7. Epithelial cells of hepatopancreas tubule infected with rickettsia-like microorganism (RLM) (arrow). Note the granular appearance of the cytoplasm. Line = 10  $\mu\text{m}$ . Fig. 8. Electron photomicrograph of rickettsia-like microorganism (RLM) in cytoplasm of hepatopancreas epithelial cell. Note the more electron-dense end (arrow). Line = 0.5  $\mu\text{m}$ .

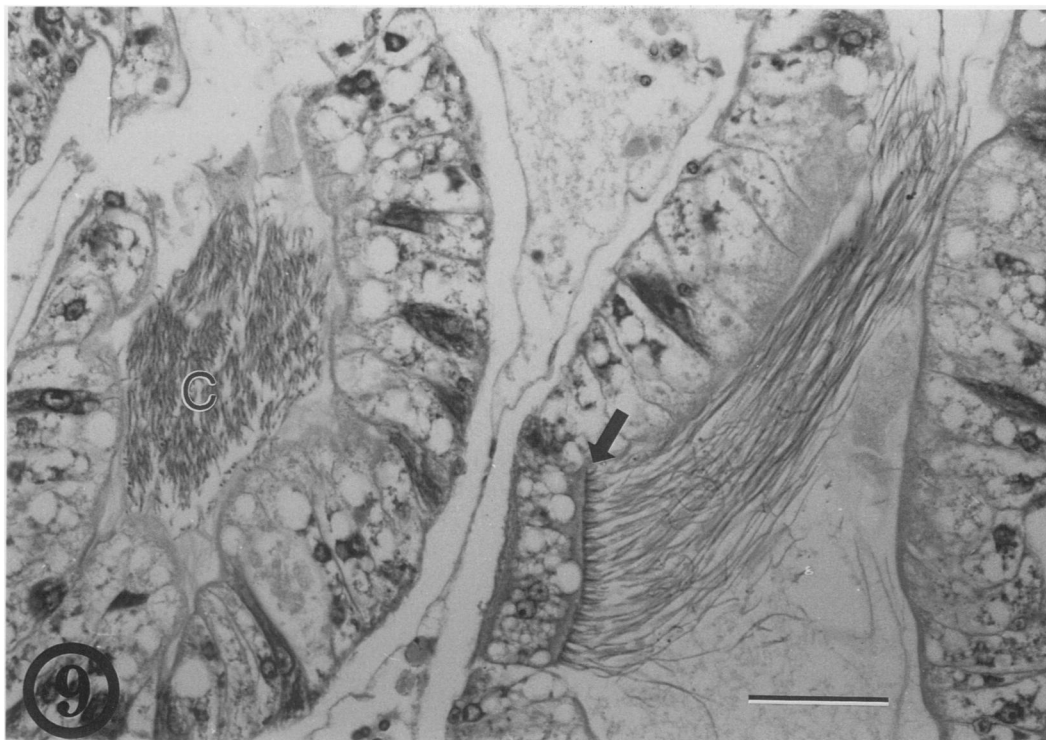


Fig. 9. Strandlike organism attached to the surface of the lumen of hepatopancreas tubule epithelial cells (arrow) in *Callinectes sapidus*. C = cross section of infected tubule. Line = 50  $\mu$ m.

(Fig. 8). The filamentous mass stained Gram negative, basophilic with hematoxylin, and was negative with PAS for fungus (Howard and Smith, 1983).

Microsporidians identified in muscle tissues of crabs from this study were either grouped within a capsule, such as *Pleistophora* sp. (Sprague, 1966, 1970; Couch and Martin, 1982) or *Thelohania* sp. (Weidner *et al.*, 1990), or ungrouped in direct contact with tissues such as *Ameson* sp. (Sprague, 1965, 1966). Muscle lysis was apparent in heavy infections. Prevalence fluctuated from 0–10%, averaging 4%, and was significantly higher in the winter of 1991 than either the winters of 1990 or 1992 (Fig. 3, Table 3). Infected crabs were found in 12 of 22 stations dispersed throughout the survey area during the winter of 1991. Spores stained basophilic with hematoxylin; the polar filament and anterior end of spores stained red with PAS and pink to red with AFB (Howard and Smith, 1983).

The histophagous ciliate *Mesanophrys chesapeakeensis* was identified in the hemolymph of four crabs in the winter of 1990 and in one crab in the winter of 1991 (Fig. 3).

*Mesanophrys chesapeakeensis* was seen in 0.8% of blue crabs sampled during this study. Four of the histophagous ciliate infections were clustered at two sites in close proximity to each other in the Nanticoke River ( $N = 3$ ) and Tangier Sound ( $N = 1$ ) (Fig. 1). Infected crabs ranged in size from 6–150-mm CW. One infected crab was lethargic. The ciliate measured 31.1  $\mu$ m long (range 15.2–51.8  $\mu$ m) by 13.8  $\mu$ m wide (range 9.1–19.8  $\mu$ m) ( $N = 22$ ) in histological preparations. Connective tissues and hemal sinuses were most often infected; ciliates also invaded heart, muscle, thoracic ganglion, and hemopoietic tissue. Hemocytic aggregation, infiltration, and encapsulation or nodule formation were typical tissue responses to infections with *M. chesapeakeensis*.

Two peritrichous ciliates, *L. callinectes* and *Epistylis* sp., were observed on the gills of crabs during this study. Each peritrich had a 4% prevalence (Table 3); some infected crabs harbored both species. Both *L. callinectes* and *Epistylis* sp. followed the same infection pattern; generally, more crabs were infected in the autumn than in the winter (Fig. 3).

Unidentified gregarines were often seen grossly during dissection as white oval cysts embedded among anterior midgut cecae. Overall, 17% of surveyed crabs had gregarine cysts (Table 2); prevalence did not vary significantly between sampling periods (Fig. 3). Gregarine cysts were found in 17% of female and 19% of male crabs, and in 21% of mature and 17% of immature crabs. Some infected crabs had hemocytic infiltration in surrounding connective tissues. Infected crabs were found throughout the sampling area.

The prevalence of unidentified trematode metacercariae varied from 11% during the winter of 1990 to 0% during the autumn of 1992; the overall prevalence was 7% (Fig. 4, Table 3). The prevalence of metacercariae did not vary significantly among the five sampling periods (Fig. 4) and infested crabs were found from Eastern Bay to Tangier Sound. Trematode metacercariae hyperparasitized by the haplosporidian *Urosporidium crescens* could be seen grossly during dissections as round black "pepper spots" in connective tissue; this hyperparasite had a 3% prevalence overall (Table 3) and did not vary significantly among the five sampling periods (Fig. 4). Crabs with hyperparasitized metacercariae were found from the Tred Avon River to Tangier Sound. Hyperparasitized metacercariae were found in 4% of mature and 1% of immature crabs (Table 2). Both unparasitized and hyperparasitized metacercariae were found in connective tissues of brain, thoracic ganglion, epidermis, and gut. Metacercariae were encysted within nerve tissues of the thoracic ganglion in some crabs.

The nemertean *Carcinonemertes carcinophila* was encysted between gill lamellae of 10% of all crabs sampled (Table 3). The prevalence of this nemertean was significantly lower during the autumn of 1992 than either the winters of 1991 or 1992 (Fig. 4). *Carcinonemertes carcinophila* was found in 9% of males and in 12% of females; 15% of mature crabs were infested, whereas only 8% of immature crabs were infested (Table 2). Infested crabs were found throughout the sampling area.

#### DISCUSSION

Hemocytic infiltration and encapsulation were common in crabs dredged during winter and autumn months during this study. In-

filtration is a cellular mechanism in which crab hemocytes migrate from the hemocoel to injured, stressed, or parasitized tissues. Encapsulation and hemocytic nodule formation are natural defense mechanisms which develop in response to foreign material (Hose *et al.*, 1990), including bacteria (Johnson, 1976a; Newman and Feng, 1982), fungi (Lightner and Fontaine, 1975), paramoebae (Johnson, 1977), a parasitic dinoflagellate, *Hematodinium* sp. (Messick, 1994), and ascorbic acid deficiency (Magarelli *et al.*, 1979). Tissue alterations also develop in response to mechanical disruption of host tissue which can be caused by injury from various factors (Fontaine and Lightner, 1975; Morado and Small, 1994; Messick and Small, 1996).

Gill lesions were morphologically similar to localized areas of "focal necrosis" on gill filaments reported in *Cancer irroratus* Say from the New York Bight (see fig. 5.4 (d) in Sawyer *et al.*, 1985), in that both appeared to have focal accumulations of hemocytes, a walled-off appearance, and epithelial cells with decreased integrity which were separated from the cuticle. The focal necrosis of gill filaments of *C. irroratus* may have been associated with accumulations of diatoms or fine silt, or may have been the result of defense mechanisms destroying foreign bodies, making them no longer recognizable (Sawyer *et al.*, 1985). The layered and walled-off appearance of gill lesions from this present study was similar to lesions described for infections of *Synophrya hypertrophica* Chatton and Lwoff in gill lamellae of *Ovalipes stephensoni* Williams (see Haefner and Spacher, 1985). Lesions of *Synophrya* were never found in decapods sampled from estuaries, and decapods captured close to shore were only rarely infected (2%); salinities in sampled waters ranged from 15–35 ppt (Johnson and Bradbury, 1976). Although it is unlikely that the idiopathic gill lesions in blue crabs sampled from the estuarine waters of Chesapeake Bay were infections with *Synophrya*, they may be the manifestation of the crab's basic defense response: clotting of hemolymph due to endotoxin. A break or injury to the cuticle may have allowed ubiquitous endotoxin to infiltrate and trigger hemocytes to form a clot and eventually wall off the injury. Ecdysis likely alleviates lesions by replacing old gill cuticle with new cuticle.

Both Baculo-B virus and RLV-RhVA com-



binations are considered lethal to blue crabs, since they infect hemopoietic tissue and hemocytes which are vital for survival (Johnson, 1985; Messick and Sindermann, 1992). Johnson (1983) reported that of 12 crabs infected with Baculo-B virus, 3 (25%) were naturally acquired infections from the Tred Avon River, but the overall prevalence of infection in the population sampled was not reported. Viral infections often become patent when crabs are stressed under artificial conditions, such as holding in crowded aquaculture facilities (Johnson, 1978; Messick and Kennedy, 1990).

Rickettsia-like microorganisms have been reported in several crustacean species, but were rare among the blue crabs sampled in autumn and winter (Table 3). A thorough discussion of crustacean species infected with RLMs may be found in Bower *et al.* (1996). RLMs were previously reported in 2.3% of blue crabs confined in shedding tanks (Messick and Kennedy, 1990). Heavy RLM infections in hepatopancreas tubules of blue crabs are likely not fatal, since epithelial cells of the hepatopancreas are continually sloughed and regenerated (Johnson, 1980). Wild populations of blue crabs may naturally have low prevalences of virus and RLMs, or infections may not become apparent until stressed by factors such as extreme temperatures or crowded conditions in holding facilities.

Bacterial infections were not enumerated during this study. Antibacterial activity (Fries, 1984; Noga *et al.*, 1994), phagocytosis, and other natural defense responses remove and denature bacteria that cause tissue responses, such as hemocytic infiltration, encapsulation, and nodule formation (Bang, 1970; Johnson, 1976a, 1983). In the horseshoe crab *Limulus polyphemus* (Linnaeus) and probably other invertebrates, endotoxin from bacteria causes clumping of hemocytes, cell disruption, explosion of granules, and subsequent clotting or gel formation which immobilize the endotoxin-containing bacteria (Levin, 1967). In addition, crabs stressed by capture and handling develop transient bacterial infections (Johnson, 1976a), making it difficult to determine whether infections were naturally acquired or may have resulted from sampling artifact.

Further investigation is needed to identify the strandlike organism found in the lumen of hepatopancreas tubules of crabs sampled. It

morphologically resembles the gram-negative *Leucothrix*-like bacterium, but the filaments are not septate. No endotoxin-induced clotting response is apparent. Prevalence of the strandlike organism varied considerably during this study. Johnson (1976b) found a similar organism in hepatopancreas tubules from 2% of juvenile and mature male and female blue crabs from the Atlantic coast of North America and the Florida coast of the Gulf of Mexico, but did not report the time of year when the organism was found. A similar strandlike organism was also found in crabs held in flow-through (12%) and recirculating (31%) shedding systems from June through August (Messick and Kennedy, 1990); many of these crabs harbored other parasites (Roe, 1988). Based on data from this study and the shedding system study, infections may increase with water temperatures or confinement; and infected crabs may be prone to concurrent infections (Roe, 1988). This strandlike organism probably does not cause mortalities, since hepatopancreas epithelial cells are continuously regenerated and replaced.

Microsporidians infect blue crabs from Chesapeake Bay (Sprague, 1970) to the Gulf coast (Sprague, 1977; Weidner *et al.*, 1990). A prevalence of less than 1% was reported in Louisiana (Overstreet, 1978). Sprague (1970) reported that microsporidian infections were common and widely distributed in Chesapeake Bay, but no data on prevalence were presented. The unusually high prevalence of microsporidians in crabs collected throughout the sampled areas in the winter of 1992 indicates that the parasites are likely tolerant of low temperatures and a range of salinities.

Since 1888, ciliates have been reported in the hemolymph of wild and captive crustaceans from various locations [e.g., see Morado and Small (1994) for a review of ciliate parasites in Crustacea]. Most reports of ciliate infections in crustaceans have been from captive animals (Cattaneo, 1888; Bang *et al.*, 1972; Aiken *et al.*, 1973; Grolière and Leglise, 1977; Armstrong *et al.*, 1981; Sparks *et al.*, 1982). There have been few reports of ciliate infections in wild crustaceans. Poisson (1930) found only seven of 3,000 (0.2%) animals infected, and Aiken *et al.* (1973) found only three infected wild lobsters over 14 years. Crabs infected with *M. chesapeakensis* were found only during winter months in this survey, but water temperatures where in-

fects of crabs have been found ranged from 4–17°C (Messick and Small, 1996). Although most infections with *M. chesapeakensis* have been reported from two sites within Chesapeake Bay, infections have also been found in other regions, including Delaware Bay and Assawoman Bay, a Maryland coastal bay (Messick and Small, 1996). Sex, molt stage, season, and physical parameters characteristic of shallow bays may influence the prevalence of infections in blue crabs (Messick and Small, 1996).

*Lagenophrys callinectes*, *Epistylis* sp., and other gill epibionts are limited by ecdysis, which removes external fauna with the old cuticle; gills are then gradually repopulated with epibionts in intermolt crabs (Shields, 1992). Older crabs with delayed ecdysis have no mechanism to free themselves of ever-increasing numbers of branchial epibionts (Couch, 1967), although scaphognathites are reasonably effective at brushing organisms off the gill surface. The relatively low prevalence of epibionts on gills of intermolt crabs found in this study supports previous reports that crustaceans have efficient antifouling mechanisms to keep their body surfaces clean and that burying in the sediment is detrimental to colonization by epibionts (Shields, 1992; Becker, 1996). It is doubtful that crabs with epibionts sustain any direct injury, although large numbers of these may interfere with gas diffusion across gill membranes.

In studies on the physiological effects of the gill parasite *Octolasmis muelleri* Coker, heavily infested blue crabs maintained the same oxygen uptake as uninfested crabs by compensating with increased ventilation volume and cardiac output (Gannon and Wheatly, 1992). Heavy infections of ciliates on the gills could potentially cause similar results. The higher prevalence of ciliates on gills during the autumn as opposed to the winter (Fig. 2) reiterates the seasonal prevalence of *L. callinectes* reported by Couch and Martin (1982), in which prevalence was lowest from December through April when water temperatures, molting frequency, and respiratory function are reduced. Actual prevalence may be considerably higher than reported here due to the nature of histological processing; thin (5 µm) rather than thicker (10 µm) sections inevitably missed some infections (Sawyer *et al.*, 1985).

There is generally a high degree of tolerance

between a gregarine and its host, although a few individual host cells may be destroyed (Sprague, 1970). In a study of captive intermolt crabs, held in either flow-through or recirculating shedding tanks from June through August, it was found that moribund crabs had a much higher prevalence of gregarine infections (29–62%) than cohort crabs which were not moribund (11–18%) (Messick and Kennedy, 1990). Dredged crabs from the present study revealed a relatively high infection rate (17%) and none appeared moribund. It may be that crabs from the shedding study were stressed by captivity, crowding, or high water temperatures, and became moribund from synergistic effects of stress, concurrent bacterial or viral infections, and gregarine infections.

Infestations with digenetic trematode metacercariae are common in crustaceans (Overstreet, 1978; Shields, 1992). Trematode metacercariae were found encysted within the nervous system of several crabs from this study, and have been reported in nervous system tissues of the Dungeness crab *Cancer magister* Dana (see Sparks and Hibbits, 1981) and the shrimp *Crangon alaskensis* Lockington (see Morado and Sparks, 1983). Infestations may affect nerve impulse transmission, cause lethargy (Sparks and Hibbits, 1981), and allow heavily parasitized animals to be quickly consumed by predators at the first sign of weakness (Sawyer *et al.*, 1984).

The ribbon worm *Carcinonemertes carcinophila* is a common parasite of the blue crab in Chesapeake Bay (Hopkins, 1947). The higher prevalence of this parasite in female and mature crabs found in this study supports earlier reports in which prevalence of female crabs infested in Chesapeake Bay is highest following the peak autumn spawning period (Millikin and Williams, 1984). Although this study showed no significant seasonality of infestation on gills, the abundance of *Carcinonemertes mitsukurii* Takakura on the eggs of *Portunus pelagicus* (Linnaeus) was correlated with salinity and temperature (Shields and Wood, 1993). The effects of *C. carcinophila* on blue crab gills appear to be negligible, since no host tissue response was observed. The higher prevalence in female and mature crabs may be due to the life cycle of the nemertean (Humes, 1942). As in other heavy gill parasite infestations, there may be some respiratory dysfunction which is likely

to be compensated for with increased ventilation volume and cardiac output (Gannon and Wheatly, 1992).

Seasonal fluctuations in the prevalences of diseases and parasites can be attributed to numerous interactions among the parasite, host, and environment. Crustaceans lack specific immune responses, such as antibodies, but rely on broad spectrum defenses, such as clotting, hemocyte infiltration, phagocytosis, encapsulation, nodule formation, and antibacterial activity. Natural immunity of the host can influence seasonal fluctuations in disease and parasite prevalence (Haefner and Spacher, 1985). A decreasing gradient of antibacterial activity in blue crabs from oceanic to riverine areas appears to be controlled by salinity (Noga *et al.*, 1994). Low water temperatures influence hemocyanin levels in blue crabs. Hemocyanin, a metalloprotein necessary for growth and survival, was found at reduced levels in the hemolymph of crabs overwintering in cold water (6°C) (Engel and Brouwer, 1987). The distribution of crabs within Chesapeake Bay during autumn and winter is controlled by environmental parameters; mature male and juvenile crabs overwinter in tributaries, where salinity and temperature are reduced, while adult females tend to overwinter in higher salinity, warmer waters near the mouth. Van Engel (1982) suggested that crabs overwintering in less saline upper portions of Chesapeake Bay are stressed and killed from cold temperatures more so than crabs overwintering in higher salinity waters at the mouth of the bay. The higher prevalence of hemocytic infiltration and encapsulation in crabs surveyed during the winter in upper, less saline portions of Chesapeake Bay during this study may be associated with colder temperatures and altered salinity, which may shift basic physiological parameters causing cellular defense mechanisms to manifest in tissues as infiltration and encapsulation.

Seasonal variations in parasite prevalences have been noted in numerous crustaceans. The parasitic dinoflagellate *Hematodinium* sp. has been reported to have seasonal prevalences in various crustaceans (Eaton *et al.*, 1991; Field *et al.*, 1992; Love *et al.*, 1993; Hudson and Shields, 1994; Messick, 1994). High salinities were associated with increased numbers of *Synophrya* sp. in decapods from coastal waters of the southeastern United States (Johnson and Bradbury, 1976). Seasonal

changes in temperature and salinity affected infestation and abundance of nemertean that prey upon eggs, *Carcinonemertes epialti* Coe on *Hemigrapsus oregonensis* Dana and *C. mitsukurii* on *Portunus pelagicus* (see Shields, 1993). Salinity also affects the survival of *Carcinonemertes errans* Wickham on *Cancer magister* (see Shields and Wood, 1993). Seasonal water temperatures affected prevalence of disease during this study. The higher prevalence of gill epibionts and the strandlike organism in autumn rather than winter was likely influenced by the warmer temperatures of autumn; the higher prevalence of hemocytic infiltration and encapsulation during winter rather than autumn was likely influenced by the colder temperatures of winter. Alternatively, cooler temperatures in autumn and winter likely influenced the reduced prevalences of RLM and the strandlike organism in dredged crabs in this study than those observed in crabs during the summer (Messick and Kennedy, 1990).

Temperature and salinity are two factors among many which may interact to cause fluctuations in prevalences of diseases. Seasonal changes in water quality factors, such as turbidity, dissolved oxygen, fresh-water runoff, and tidal flow into an estuary during the year may interact to influence disease prevalence. In addition, host-parasite interactions, such as parasites whose life cycles are associated with the molt cycle of their crustacean host (Haefner and Spacher, 1985), resistance of hosts to parasitism related to nutritional status (Yan and Larsson, 1988), host-parasite population dynamics and interplay (Yan and Larsson, 1988), and dispersion patterns of parasites (Shields, 1993), all interact and influence variations in disease prevalence. The movement of blue crabs within an estuary to spawn, mate, feed, or overwinter exposes them to a variety of potential pathogens. Larval crabs spawned in high salinity regions become burdened with salinity-tolerant parasites, while juvenile and adult crabs moving into less saline areas of an estuary become hosts for parasites found only at lower salinities. Life history, environmental conditions, and season of the year contribute to the prevalence of disease and parasitism among blue crabs from Chesapeake Bay. This three-year survey establishes baseline data for the prevalence of various disease conditions among overwintering blue crabs.



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