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Epizootiology of the parasitic dinoflagellate *Hematodinium* sp. in the American blue crab *Callinectes sapidus*⋆

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ABSTRACT: *Hematodinium* sp. is a parasitic dinoflagellate that infects and kills blue crabs *Callinectes sapidus*. Periodic outbreaks of dinoflagellate infections with subsequent high host mortalities prompted a study of the epizootiology and distribution of the crab pathogen. Hemolymph samples from over 13 000 crabs were assessed for infections over 8 yr. Moderate to high prevalences were found at several locations along the Atlantic and Gulf coasts of the United States. In the coastal bays of Maryland and Virginia, prevalence followed a seasonal pattern, with a sharp peak in late autumn. Infections were significantly more prevalent in crabs measuring less than 30 mm carapace width; host sex did not influence prevalence. Prevalences were highest in crabs collected from salinities of 26 to 30‰; no infected crabs were found in salinities below 11‰. Intensity of infection did not vary among crab sizes, molt stages, or sexes. Naturally and experimentally infected crabs died over 35 and 55 d in captivity, with a mean time to death of approximately 13 and 42 d, respectively. Several other crustaceans, including gammaridean amphipods, xanthid (mud) crabs, and the green crab *Carcinus maenus*, were found with *Hematodinium*-like infections. Considering its widespread distribution and high pathogenicity, we suggest that *Hematodinium* sp. represents a significant threat to blue crab populations in high salinity estuaries along the Atlantic and Gulf coasts of the USA.

KEY WORDS: *Hematodinium* sp. · *Callinectes sapidus* · Seasonality · Size · Salinity · Disease · Dinoflagellate

INTRODUCTION

The blue crab *Callinectes sapidus* Rathbun supports valuable commercial fisheries along the Atlantic and Gulf coasts of the United States. Annual landings of blue crabs exceeded 97 metric tons from 1989 to 1993, with US dockside values in 1994 estimated at $137 million (Johnson et al. 1998). Blue crabs sustain the largest extant fishery in Chesapeake Bay. Outbreaks of disease caused by the parasitic dinoflagellate *Hematodinium* sp. in blue crabs have been reported in several coastal states (Newman & Johnson 1975, Couch & Martin 1982, Messick 1994). In the laboratory, experimentally infected blue crabs suffer high mortality rates (>86%) to the resultant disease, a level 7 to 8 times higher than uninfected controls (Shields & Squyars 2000). Current models project crab abundance based on constant low levels of natural mortality (Lipcius & Van Engel 1990, Abbe & Stagg 1996, Rugolo et al. 1998). They do not consider the potential epizootics and resulting mortalities caused by *Hematodinium* sp. or other diseases.

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Members of the genus *Hematodinium* are parasitic syndinid dinoflagellates that invade the hemolymph and other tissues of their crustacean hosts (Shields 1994). Several commercially important crustaceans have been reported infected with *Hematodinium* spp. Epizootics have damaged fisheries for the Tanner crab *Chionoecetes bairdi* in Alaska (Meyers et al. 1987, 1990), the snow crab *C. opilio* in Alaska and Newfoundland (Meyers et al. 1990, 1996, Taylor & Khan 1995), the Norway lobster *Nephrops norvegicus* in Scotland (Field et al. 1992), the velvet swimming crab *Necora puber* in France (Wilhelm & Mialhe 1996), and the blue crab *Callinectes sapidus* in the USA (Newman & Johnson 1975, Messick 1994). Other commercial species are also hosts to *Hematodinium* sp. infections, including rock crabs *Cancer irroratus* and *C. borealis* (MacLean & Ruddell 1978), the Australian blue crab *Portunus pelagicus*, and the mangrove crab *Scylla serrata* (Shields 1992, Hudson & Shields 1994). Infections also occur in lady crabs *Ovalipes ocellatus* (MacLean & Ruddell 1978), and obligate coral-dwelling crabs *Trapezia* spp. (Hudson et al. 1993). *Hematodinium*-like dinoflagellates also occur in amphipods (Johnson 1986), and dinoflagellate-like infections occur in spot shrimp *Pandalus platyceros* (Meyers et al. 1994).

Hematodinium perezi was first reported as a rare parasite in green crabs *Carcinus maenus* from Europe (Chatton & Poisson 1931). Newman & Johnson (1975) identified the dinoflagellate in blue crabs as *H. perezi*, based on similarities in morphological characteristics reported by Chatton & Poisson (1931). The dinoflagellate in blue crabs we previously identified as *H. perezi* (Messick 1994, Shields & Squyars 2000) is not designated a species in this report. Additional molecular and morphological comparisons must be made between the type species, *H. perezi* in the green crab from the type locality (France) and the blue crab parasite, before assigning a species identification.

The goal of this study was to clarify the potential threat of *Hematodinium* sp. infections to blue crab populations. We report the distribution and prevalence of the parasite along the Atlantic and Gulf coasts of the United States, document seasonality of infections, potential relationships between infections and several host factors, relate the distribution and epizootics of the parasite to hydrographic features where infections progress and spread, and identify several potential reservoir hosts for the parasite.

MATERIALS AND METHODS

Sampling concentrated on crabs from waters surrounding the Delmarva Peninsula to obtain seasonal and long-term data. In Maryland (MD), blue crabs were collected semimonthly, from 1991 through 1997, from 21 stations within the coastal bays, in conjunction with the MD Department of Natural Resources Coastal Bay Fisheries Project. Crabs were collected with a 4.9 m (16 ft) semiballoon shrimp trawl and tickler chain towed for 6 min at 3 knots. The trawl had a 3.8 cm (1.5 inch) mesh in the body, a 3.2 cm (1.25 inch) mesh in the cod end, and a 1.3 cm (0.5 inch) liner. Some crabs within coastal bays were collected in a 30.5 m (100 ft) × 1.8 m (6 ft) seine net with a 0.6 cm (0.25 inch) ace mesh central bag. Additionally, 5 sites in Virginia were sampled via trawl in 1994 and 1995: portions of Chincoteague Bay, a site near Kiptopeke National Wildlife Refuge, a site near Wachapreague, a site near Occahannock, and off Cape Charles within Chesapeake Bay.

In Virginia (VA), crabs were collected by several methods. In October 1993, 1994, 1996, and throughout 1997, crabs were taken with commercial crab pots from 2 reference locations adjacent to the Delmarva Peninsula: Red Bank and Hungars Creeks. In October 1993, additional samples were obtained from crab pots set in Chincoteague Bay, Mattawoman Creek, Nassawadox Creek, Wachapreague Creek, and Wachapreague Inlet. Crab pot samples were biased towards mature crabs. Broad-scale sampling within lower Chesapeake Bay was done in conjunction with the Virginia Institute of Marine Science (VIMS) Trawl Survey (April through December 1996 and 1997), and the VIMS Blue Crab Dredge Survey (part of the Chesapeake Bay Stock Assessment Program, November through March 1996 and 1997). Trawls consisted of 5 min tows using a 9.144 m (30 ft) semiballoon otter trawl (Marinivich Gulf shrimp trawl) with a 38.1 mm (1.5 inch) stretch mesh body, a 9.05 mm (0.75 inch) cod end, and a 6.35 mm (0.25 inch) mesh cod-end liner with attached tickler chain (0.375 inch link). Dredge tows consisted of a 1.83 m wide Virginia crab dredge fitted with 1.25 cm (0.5 inch) Vexar mesh dragged on the bottom for 1 min at 3 knots. For the 1997 collection, up to 60 crabs from each trawl or dredge sample were examined for dinoflagellates. All crabs over 28 to 30 mm carapace width (CW) were sampled from the high salinity sites in the VIMS Dredge Survey. After 1994, low salinity locations (e.g., York River near West Point) were not sampled for the disease, but several crabs (n = 60 to 75) were later sampled from the mouths of the York and James Rivers. Temperature and salinity were recorded for each station in the Trawl and Dredge Surveys. Due to variations in sampling gear and effort, pot and trap collections from the lower Chesapeake Bay were biased toward mature crabs, whereas trawl collections from coastal bays of Maryland were biased toward immature crabs.

A series of crab samples was collected during spring and summer months from 1994 to 1997 in Delaware

(DE) in conjunction with the DE Division of Fish and Wildlife Blue Crab Survey. Sampled sites included Indian River, Rehoboth Bay, and Delaware Bay. Samples were collected with a 4.9 m (16 ft) semiballoon otter trawl using a 3.6 cm (1.4 inch) stretch mesh body with a 1.3 cm (0.5 inch) knotless stretch mesh liner in the cod end.

Samples of blue crabs were also obtained from various coastal bays or tributaries of New Jersey, North Carolina, South Carolina, Georgia, Mississippi, Louisiana, Texas, and the Atlantic Ocean off MD. These crabs were collected with a dredge, trawl, traps, or pots.

Water salinity and temperature were recorded, when provided, for sites where crabs were collected in coastal bays of MD. For data analyses, salinity was divided into 5 groups: 11–15, 16–20, 21–25, 26–30, and 31–35‰; temperature was also divided into 5 groups: 3–9, 10–16, 17–23, 24–30, and 31–37°C. CW was measured as the longest distance between epibranchial spines. Crabs were divided into 6 size groups: 3–30, 31–60, 61–90, 91–120, 121–150, and >150 mm CW for data analyses. Sex and maturity were recorded; male crabs under 90 mm CW were considered immature (Millikin & Williams 1984). The molt stage (postmolt, intermolt, premolt) of some crabs was noted from visual examination of the propodus of the 5th leg (swimmer), and the flexibility of the carapace.

Hemolymph was assayed using the methods described by Messick (1995). In some cases, crabs were shipped live to Oxford, MD; in other cases, hemolymph samples were removed and fixed on site and sent to Oxford for processing. Crabs were bled from arthrodial membranes using a 1 cc insulin syringe with a 1.0 cm, 27 or 28 ga needle. Hemolymph was allowed to adhere to acid-cleaned, 0.1% w/v poly-L-lysine-coated microscope slides for 20 s to 5 min; longer times reflect observing cells live, under an inverted microscope with Hoffman modulation or phase contrast optics. Hemolymph samples were fixed in Bouin's fluid and stained with Mayer's hematoxylin and eosin (H&E) (Luna 1968). Mean intensity and prevalence are defined in Margolis et al. (1982). Briefly, intensity is the number of individuals of a particular parasite species in each infected host. We estimated intensity by counting at least 300 cells per hemolymph preparation, divided the number of individual parasites by the total number of cells (parasites + hemocytes) counted, and multiplied by 100. The mean intensity of infection for a sample was calculated using infected hosts only. Prevalence was determined as the number of infected crabs divided by the total number of crabs sampled and expressed as a percentage. For some analyses, infections were further divided into light (0.3 to 3.0 parasites per 100 host cells), moderate (3.1 to 10 parasites per 100 host cells), or high (>10 parasites per 100 host cells) intensity categories. Intensity is not density, which is the number of individuals of a parasite species per unit volume; cell densities were obtained for selected samples using a hemacytometer (Shields & Squyars 2000).

Mortality rates in infected crabs were assayed in naturally infected and inoculated crabs held at 20°C in 24‰ seawater. Naturally infected crabs (n = 22) were held individually for observation. Previously assayed naive crabs (n = 15) had 100 µl of infected hemolymph containing 10^2 plasmodia ml^{-1} injected directly into the axillae of the 5th leg. Both male and female crabs were inoculated (n = 12, 3, respectively). The onset and course of infections were monitored via weekly hemolymph assays as described above. Uninfected control crabs were not included, as the initial purpose of this ancillary study was to examine infection dynamics; controlled mortality experiments have been reported elsewhere (Messick et al. 1999, Shields & Squyars 2000).

Additional crustacean species were assayed for *Hematodinium* spp. infections. These included the following: 25 mud crabs *Hexapanopeus angustifrons*, *Neopanope sayi*, and *Panopeus herbstii* from the coastal bays of MD, and Core Sound, North Carolina; 13 green crabs *Carcinus maenus* from the coastal bays of MD and Long Island Sound, New York; 9 rock crabs *Cancer irroratus* from the coastal bays of MD, and the Atlantic Ocean off MD; 29 lady crabs *Ovalipes ocellatus* from the coastal bays of MD; over 140 *Callinectes similis* from coastal bays of MD, VA, North Carolina, and Georgia; 7 grass shrimp *Paleomonetes* spp. from Core Sound, North Carolina; and 77 unidentified gammaridean amphipods from the coastal bays of MD. In November 1994 and January 1995, 13 *C. irroratus* and 5 *Portunus gibbesii* from lower Chesapeake Bay were examined. In June 1996, 13 *C. irroratus*, 9 *O. ocellatus*, and 1 *Libinia emarginata* from Wachapreague Inlet, VA, were examined. Crab species were collected and assayed for *Hematodinium* spp. in hemolymph, as described above. Due to their small size, amphipods and grass shrimp were fixed in Bouin's fluid and processed for histological examination by routine methods (Johnson 1980, Howard & Smith 1983).

Data from crabs collected from the coastal bays of MD were analyzed using factorial analysis of variance (ANOVA) techniques (SAS 1999). The analyses tested the effects of host characteristics (size, sex, molt stage) and physical characteristics (water salinity and temperature) on the prevalence and mean intensity of *Hematodinium* sp. infections; percent intensity was square root transformed (as appropriate for percentages). The large number of stations and repeated sampling allowed reasonable sample sizes for estimates of mean prevalence for ANOVA. Observations with missing data were omitted from the analyses. Effects were considered significant at $p \leq 0.05$.

RESULTS

Distribution

Hemolymph samples from over 13 000 blue crabs were examined for *Hematodinium* sp. infections. Collections were comprised of 4830 crabs from coastal bays of MD; 1542 from coastal bays of VA; 5076 from lower Chesapeake Bay; 695 from coastal bays of DE; and 1138 from other coastal areas from New Jersey to Texas.

Hematodinium sp. infections were widespread along the mid-Atlantic seaboard. The parasite was found in blue crabs from New Jersey to Florida, and along the Gulf coast in Texas (Fig. 1, Table 1). The widespread sampling effort indicated the parasite was present in many locations. A consistent pattern in prevalence was not observed among sampling locations and sampling times. Due to difficulty obtaining multiple samples, some areas were not sampled repeatedly; thus, areas such as Louisiana and Mississippi may be underrepresented.

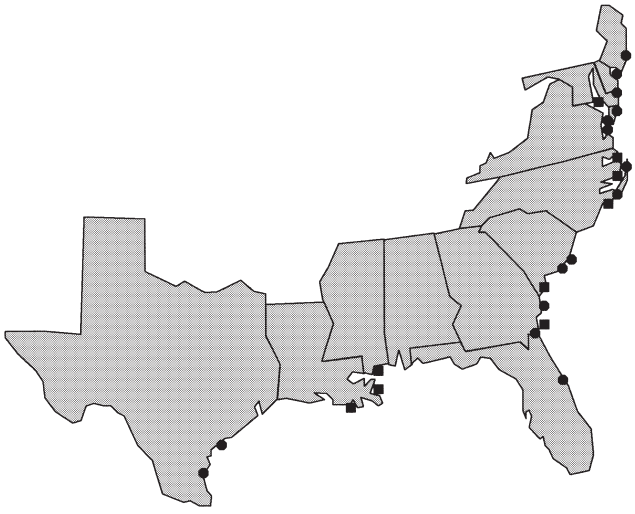

Fig. 1. Distribution of *Hematodinium* sp. infections in blue crabs sampled along the Atlantic and Gulf coasts of USA. (●) General area where sampled crabs had infections, (■) general area where sampled crabs had no infections

Hematodinium sp. was not found in crabs from the upper and northeastern reaches of Chesapeake Bay where salinities were below 18‰, but was more prevalent in the higher salinity waters in the lower portions of Chesapeake Bay (Table 2). Prevalences varied in these locations over sampling periods. Seasonal variation in prevalence was observed in lower portions of Chesapeake Bay, but *Hematodinium* sp. was rarely found in crabs from western portions of the lower bay (Fig. 2).

Coastal bays on the Delmarva Peninsula had high prevalences of *Hematodinium* sp. infections, especially during autumn months. In coastal bays of DE, prevalences ranged from 0 to 69% with a mean of 36.3% (± 23.6 SD, n = 11 samples) during autumn. Many samples had high intensity infections (Table 3). Prevalences for crabs collected from coastal bays of VA varied with location and season, with higher prevalences during autumn. From September through December 1992 to 1998, prevalence ranged from 0 to 95% with a mean of 32 (±30 SD, n = 22 samples) (Table 4).

Table 1. Location, prevalence, and mean intensity of *Hematodinium* sp. infections in blue crabs sampled along the Atlantic and Gulf coasts of USA with water salinity, temperature, sample number, and collection date. Prev. = prevalence, nr = not recorded

Site	Date (mo/d/yr)	n	Prev. (%)	Intensity (%)	Salinity (‰)	Temp. (°C)
Stone Harbor, NJ	09/15/98	34	9	23	32	28
Atlantic Ocean, MD	09/12/96	9	22	72	34	nr
Atlantic Ocean, MD	10/22/96	16	0		33	nr
Core Sound, NC	06/20/95	27	0		30	25
Jones Bay, NC	09/12/95	63	0		14	24
Roanoke Sound, NC	07/16/96	49	0		28	25
Newport River, NC	07/18/96	47	0		25	31
Core Sound, NC	07/19/96	76	5	28	33	27
Ashley River, SC	07/23/96	20	5	64	22	29
Wando River, SC	07/23/96	20	10	49	22	29
Charleston Harbor, SC	07/23/96	20	30	58	27	28
North Edisto System, SC	07/31/96	25	0		22	31
Lower Ashley River, SC	08/05/97	27	15	2	18	28
Port Royal Sound, SC	03/18/98	22	0		25	13
Wando River, SC	07/07/98	14	0		24	30
Ashley & Wando Rivers, SC	09/10/98	16	12	80	25	28
St. Simons Island, GA	11/09/96	23	4	2	27	19
St. Simons, GA	05/24/99	85	6	57	28	26
Wassaw, Ossabaw, St. Simons, Cumberland, St. Andrew, GA	06/02/99	105	22	24	24–34	27
Wassaw, Ossabaw, Sapelo, St. Simons, St. Andrew, GA	07/13/99	217	5	25	24–34	29
Ossabaw, Sapelo, St. Simons, GA	08/10/99	36	0		22–34	31
Ft. Pierce, FL	07/08/99	45	40	13	27	35
Davis Bayou, MS	09/15/97	32	0		18	27
Grand Isle, LA	09/15/97	32	0		23	32
Caillou Lake, LA	11/09/96	30	0		25	23
Corpus Christi Bay, TX	11/20/96	23	9	48	30	23
Corpus Christi Bay, TX	07/23/97	8	0		33	30
Aransas Bay, TX	10/30/96	17	6	15	30	nr

Table 2. Location, prevalence, and mean intensity of *Hematodinium* sp. infections in blue crabs sampled within Chesapeake Bay with water salinity, temperature, sample number, and collection date. Samples for 1996 VA sites are shown in Fig. 2. Prev. = prevalence, nr = not recorded

Site	Date (mo/d/yr)	n	Prev. (%)	Intensity (%)	Salinity (‰)	Temp. (°C)
Nanticoke River, MD	03/22/95	116	0		14	12
Tangier Sound, MD	11/04/93	68	0		12	16
Pocomoke Sound, MD	10/27/93	75	0		10	11
Smiths Beach, VA	09/29/94	19	0			
Occahannock, VA	07/15/94	26	0		15	30
Occahannock, VA	08/15/94	20	0			
Occahannock, VA	09/15/94	34	0		17	25
Occahannock, VA	10/18/94	13	0		16	17
Occahannock, VA	11/14/94	19	0		18	14
Occahannock, VA	07/13/95	57	0		19	29
Occahannock, VA	08/23/95	60	0		21	29
Occahannock, VA	09/19/95	31	0		21	24
Occahannock, VA	10/23/95	55	0		21	19
Nassawadox Creek, VA	08/23/93	24	0		20	
Nassawadox Creek, VA	08/23/93	21	14	nr		
Nassawadox Creek, VA	08/25/93	30	3	nr	20	
Hungars Creek, VA	08/24/93	21	5	nr	20	
Hungars Creek, VA	09/28/94	44	2	9		
Hungars Creek, VA	09/30/94	19	0			
Hungars Creek, VA	10/27/94	24	8	32		
Hungars Creek, VA	11/14/94	24	0			
Hungars Creek, VA	12/07/94	18	0			
Hungars Creek, VA	04/25/96	22	0			
Hungars Creek, VA	05/17/96	40	0			
Hungars Creek, VA	06/11/96	40	0			
Hungars Creek, VA	07/29/96	57	0			
Hungars Creek, VA	08/21/96	43	0			
Hungars Creek, VA	09/25/96	37	0			
Hungars Creek, VA	10/24/96	43	2	nr		
Hungars Creek, VA	11/20/96	21	0			
Hungars Creek, VA	05/02/97	40	0			
Hungars Creek, VA	09/25/97	40	18	42		
Hungars Creek, VA	10/30/97	47	0			
Hungars Creek, VA	05/12/98	20	5	nr		
Hungars Creek, VA	06/17/98	22	0			
Hungars Creek, VA	07/20/98	27	0			
Hungars Creek, VA	09/17/98	32	0			
Hungars Creek, VA	10/06/98	28	7	nr		
Mattawoman Creek, VA	08/24/93	21	0			
York River, VA	01/03/95	24	0			
York River, VA	12/14/94	94	0			
York River, VA	12/19/95	60	0			
Cape Charles, VA	07/15/94	44	0		20	30
Cape Charles, VA	08/15/94	34	0			
Cape Charles, VA	09/15/94	30	3	22	21	23
Cape Charles, VA	10/18/94	47	4	13	22	16
Cape Charles, VA	11/14/94	14	0		23	15
Cape Charles, VA	07/13/95	62	11	43	23	29
Cape Charles, VA	08/23/95	31	10	37	23	27
Cape Charles, VA	09/19/95	21	29	29	24	22
Cape Charles, VA	10/23/95	60	18	38	25	18
Lower bay, VA	11/29/94	62	0			
Lower bay, VA	12/21/94	34	0			
Lower bay, VA	03/06/95	19	0			
Lower bay, VA	12/18/95	98	2	14		
Tue Point, York River, VA	10/06/94	35	0			
James River, VA	12/30/99	67	0			

Salinity

Within the coastal bays of MD, the distribution of *Hematodinium* sp. was significantly associated with high salinities ($p < 0.0001$) (Fig. 3). Prevalence was highest in the 26 to 30‰ salinity range with 38% infected (n = 2130). No crabs collected from salinities <11‰ (n = 45) were found with infections. Mean intensity did not vary significantly among infected crabs collected from the various salinity groups ($p = 0.36$) in coastal bays of MD. In Chesapeake Bay and its western tributaries, prevalence was zero in salinities below 18‰ (n = 833) (Table 2). No infected crabs were collected at salinities below 18‰ from other areas sampled along the Atlantic and Gulf coasts (Table 1).

Hydrography

In the coastal bays of MD, prevalence of *Hematodinium* sp. varied significantly among 20 trawl stations ($p < 0.0001$). Stns T01 to T07, located north of Ocean City Inlet, were among the stations with the lowest prevalence. Additionally, Stns T12 and T17 in the upper reaches of Newport Bay and Green Run Bay had relatively lower prevalences of infections than nearby stations (Fig. 4). Average salinities at Stns T05 and T12 were comparatively lower than nearby stations (Fig. 4).

Seasonality

Monthly or periodic sampling from 1992 to 1998 in coastal bays of MD showed that prevalence and intensity of *Hematodinium* sp. infections followed a seasonal trend, with a distinct peak in late autumn ($p < 0.001$, Fig. 5). Although infection prevalences gradually increased throughout the summer, they peaked in late autumn, with a sharp increase in average prevalence from 49 to 81% from October to November (Figs. 5 & 6). Prevalences precipitously declined in January, and were virtually undetectable from March through May. In June, infections became apparent, and mean prevalence rose to 20% (Figs. 5 & 6). Prevalences within

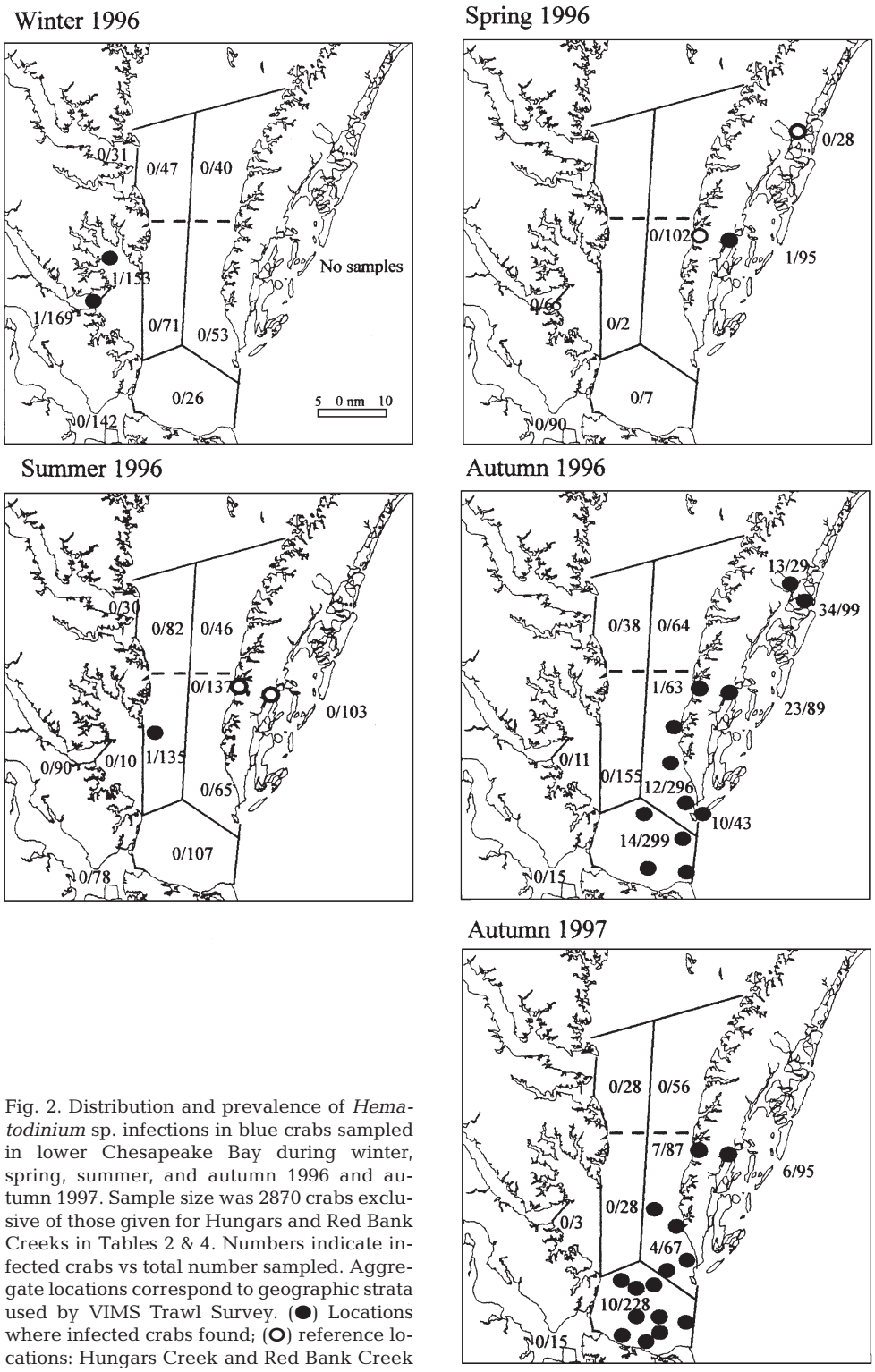

Fig. 2. Distribution and prevalence of *Hematodinium* sp. infections in blue crabs sampled in lower Chesapeake Bay during winter, spring, summer, and autumn 1996 and autumn 1997. Sample size was 2870 crabs exclusive of those given for Hungars and Red Bank Creeks in Tables 2 & 4. Numbers indicate infected crabs vs total number sampled. Aggregate locations correspond to geographic strata used by VIMS Trawl Survey. (●) Locations where infected crabs found; (○) reference locations: Hungars Creek and Red Bank Creek

Chesapeake Bay also varied by season, with peaks during autumn (Fig. 2). Mean intensities within MD coastal bays followed a seasonal trend, but peaked earlier in September (Fig. 6). The prevalence of *Hematodinium* sp. varied significantly among crabs collected at different temperatures ($p < 0.0001$) (Fig. 7). The prevalence of infections was significantly higher in crabs from 3 to 9°C waters ($p < 0.0001$) than any other temperature range. Mean intensity varied significantly among crabs collected at different temperature ranges

Table 3. Prevalence and mean intensity of *Hematodinium* sp. infections in blue crabs sampled from Delaware (DE) embayments (Indian River, Rehoboth Bay, and Delaware Bay) with sample number and collection date. Prev = prevalence, Int = mean intensity

Date	Indian River			Rehoboth Bay			Delaware Bay		
(mo/d/yr)	Prev. (%)	n	Int. (%)	Prev. (%)	n	Int. (%)	Prev. (%)	n	Int. (%)
07/14/94	8	77	16						
09/01/94	32	37	23	22	58	32			
10/01/94				13	37	30	65	62	33
05/01/95				0	5	0			
06/01/95	24	25	35	0	31	0			
07/01/95	26	23	49	6	34	23			
09/01/95	0	23	0	33	6	78			
10/01/95	51	51	40						
07/01/96	27	30	42	33	36	67			
07/30/96									
09/01/96	56	16	76	50	8	42	8	49	91
07/01/97	28	29	75	31	29	48			
09/01/97							69	29	73

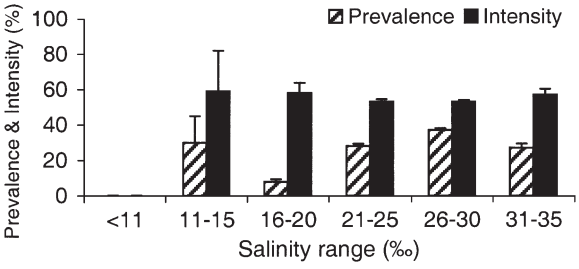

Fig. 3. Comparison of mean prevalence and mean intensity of *Hematodinium* sp. infections among blue crabs collected from different water salinity ranges within coastal bays of Maryland from 1992 to 1998. Error bar = SE

(p = 0.004); but in contrast to prevalence, mean intensity was significantly lower in crabs from 3 to 9°C waters than crabs from 10 to 16°C (p = 0.02), 17 to 23°C (p = 0.02), and 24 to 30°C (p = 0.02) waters.

Host factors

The prevalence of *Hematodinium* sp. infections in coastal bays of MD varied significantly among the 6 host size classes throughout the year (p = <0.0001) (Fig. 8). Prevalence was significantly higher in crabs measuring 3–30 mm CW, than in those measuring 31–60, 61–90, 91–120, 121–150, or >150 mm CW (p < 0.005). Additional analysis of data collected in autumn months, when disease prevalence peaked, indicated that mean prevalences in smaller crabs measuring ≤60 mm CW were significantly higher than prevalences in larger crabs measuring >60 mm CW (p < 0.0007). Mean intensity did not vary among size classes during autumn months (p < 0.20), nor throughout the year (p < 0.13).

In coastal bays of MD, the prevalence of *Hematodinium* sp. infections varied significantly between mature (26%) and immature (35%) crabs (p < 0.0005) throughout the year. Also, during autumn months when prevalence peaked, prevalence was significantly higher (p < 0.0001) in immature crabs (65%) than in mature crabs (38%).

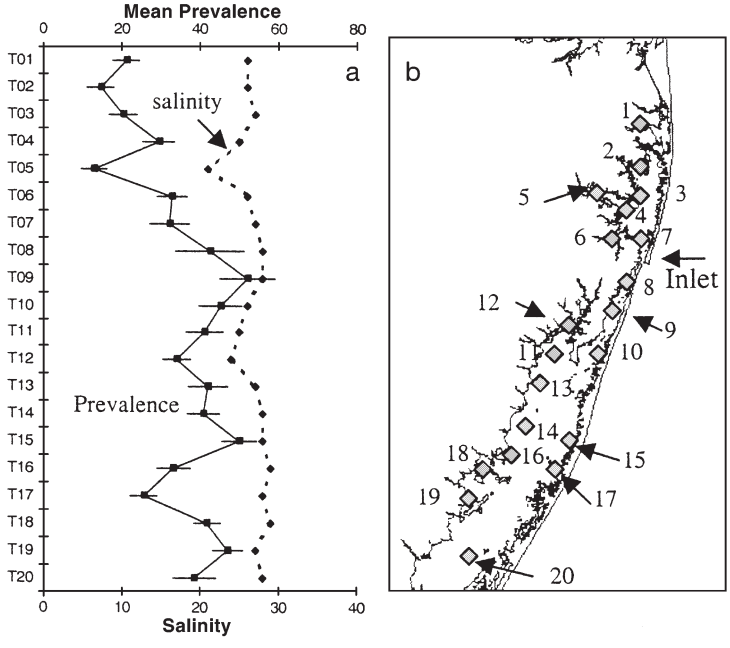

Fig. 4. (a) Mean prevalence of *Hematodinium* sp. infections in blue crabs and mean water salinity over time from sites within coastal bays of Maryland sampled from 1992 to 1998. Error bar = SE. (b) Location of various sites within coastal bays of Maryland sampled semi-monthly from 1992 to 1998

Table 4. Location, prevalence, and mean intensity of *Hematodinium* sp. infections in blue crabs sampled from Virginia (VA) coastal bays with water salinity, temperature, sample number, and collection date. Prev. = prevalence, nr = not recorded

Site	Date (mo/d/yr)	n	Prev. (%)	Intensity (%)	Salinity (‰)	Temp. (°C)
Chincoteague	10/08/92	40	93	39		
Chincoteague	08/25/93	24	13	nr		
Chincoteague	08/25/93	27	7	nr		
Chincoteague	10/26/93	20	95	24		
Chincoteague	06/15/95	81	44	35	23	23
Chincoteague	07/20/95	34	59	43	29	29
Chincoteague	08/23/95	28	43	29	33	26
Chincoteague	09/19/95	27	67	48	29	20
Chincoteague	10/23/95	31	61	37	23	18
Wachapreague	10/27/94	15	27	nr		
Wachapreague	08/30/95	20	15	9	32	26
Wachapreague	10/26/95	20	80	26	32	18
Wachapreague	10/21/96	99	34	21		
Finney Creek	10/21/96	29	48	14		
Red Bank Creek	08/24/93	37	16	nr	30	
Red Bank Creek	09/28/94	47	6	36		
Red Bank Creek	09/30/94	14	7	0.3		
Red Bank Creek	10/27/94	108	7	40		
Red Bank Creek	11/14/94	46	13	32		
Red Bank Creek	12/07/94	65	18	19		
Red Bank Creek	04/25/96	32	0			
Red Bank Creek	05/17/96	36	3	5		
Red Bank Creek	06/13/96	27	0			
Red Bank Creek	07/29/96	32	0			
Red Bank Creek	08/21/96	34	0			
Red Bank Creek	09/25/96	35	0			
Red Bank Creek	10/24/96	38	16	53		
Red Bank Creek	11/20/96	26	35	5		
Red Bank Creek	12/16/96	25	32	20		
Red Bank Creek	05/02/97	45	13	23		
Red Bank Creek	09/25/97	22	14	20		
Red Bank Creek	10/30/97	73	4	nr		
Red Bank Creek	05/12/98	40	7.5	7		
Red Bank Creek	06/17/98	49	0			
Red Bank Creek	07/20/98	37	22	26		
Red Bank Creek	09/17/98	37	5.4	51		
Red Bank Creek	10/06/98	52	13	39		
Kiptopeke	08/30/95	19	58	23	31	25
Fishermans Island	10/23/96	43	23	22		

Overall, the prevalence of *Hematodinium* sp. infections did not vary significantly between male (34%) and female (33%) crabs in coastal bays of MD ($p = 0.40$). However, during autumn 1996, in the coastal bays of VA, mature males (25.7%) had significantly higher prevalence than mature females (4.3%) (chi-square, $p < 0.001$). In addition, southeastern sites within Chesapeake Bay such as Cape Charles and Fishermans Island had significantly higher prevalences in males (18 to 31%) than in females (0 to 3%). Mean intensities did not vary significantly among mature and immature infected male and female crabs from the coastal bays of MD ($p = 0.18$).

The molt condition of blue crabs from coastal bays of MD was not significantly associated with prevalence or intensity of *Hematodinium* sp. infections ($p = 0.52$, 0.41, respectively). Prevalence was 15% in intermolt, 19% in postmolt, and 17% in premolt crabs. Mean intensity was 47% (n = 273) in intermolt, 38% (n = 150) in postmolt, and 33% (n = 47) in premolt crabs. Some infected crabs molted in the laboratory (n = 3), but changes in intensity were not examined for molting crabs.

Mortality experiments

Naturally infected crabs held in captivity suffered 100% mortality over 35 d. The mean time to death was approximately 13 d, and infections ranged from light to heavy intensities. Mortalities in experimentally inoculated crabs began after 17 d, with a mean time to death of

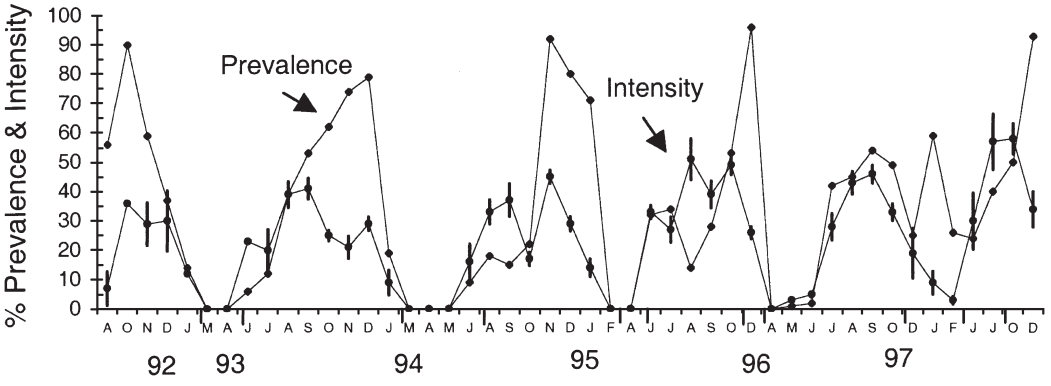

Fig. 5. Seasonal variation in mean prevalence and mean intensity of *Hematodinium* sp. infections in blue crabs collected semimonthly from coastal bays of Maryland from 1992 to 1997. Error bar = SE

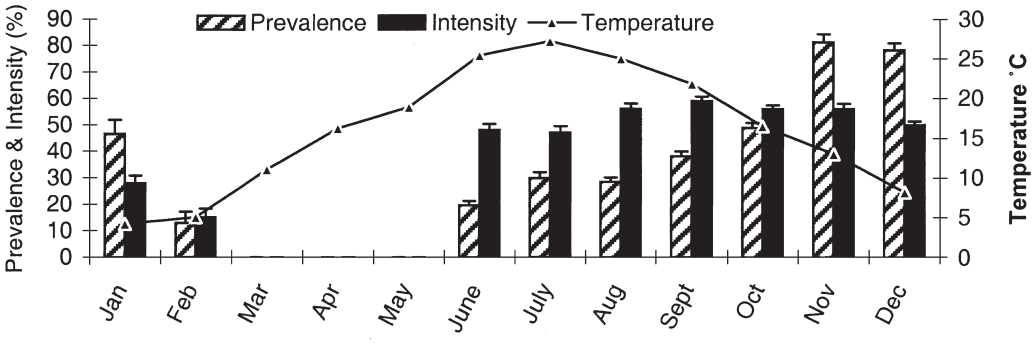

Fig. 6. Seasonal variation in mean prevalence and mean intensity of *Hematodinium* sp. infections in blue crabs from coastal bays of Maryland from 1992 to 1997 with mean monthly water temperature

approximately 42 d and 100% mortality after 55 d (Fig. 9). Naturally infected crabs with light infections developed heavy infections over 2 to 3 wk (Fig. 10). The proliferation of the parasite was not consistent between lightly, moderately and heavily infected crabs, and in some cases, heavily infected crabs survived as long as lightly infected crabs (Fig. 10). Crabs with high intensity infections on Day 1 likely had end-stage infections and lagged in parasite proliferation perhaps due to depleted host resources or due to chronic infections. Inocula of 10^2 plasmodia successfully transmitted the parasite to naive hosts. In general, no infections were observed prior to 6 d post-inoculation. Light infections were observed after 13 d in 14 of 15 hosts. Moderate infections occurred after 16 d, and heavy infections occurred after 30 d.

Additional parasites

Numerous other parasites were detected in hemolymph samples of crabs collected from the coastal bays of MD. Hemocytes with cytoplasmic inclusions similar to those described as virus infections (Johnson 1985) were found in 19 crabs; a histophagous ciliate, *Mesanophrys chesapeakensis* (Messick & Small 1996), was found in 4; unidentified microsporidians (Sprague 1977) were found in 28; *Paramoeba perniciosa* (Sprague & Beckett 1966, Johnson 1977) was found in 35; and a haplosporidian-like organism (Newman et al. 1976) was found in 2 sampled crabs. Some crabs which had *Hematodinium* sp. infections were co-infected with other parasites, including *P. perniciosa* (n = 6), an unidentified virus (n = 1), unidentified microsporidians (n = 3), and *M. chesapeakensis* (n = 3). Although many parasites can be found in hemolymph, parasite infections were likely higher than reported since parasites may have been present in tissues other than hemolymph.

Alternate hosts

Hematodinium sp. infections were detected in 8% of mud crabs, 8% of *Carcinus maenus* collected from the coastal bays of MD, and 0.7% *C. similis* collected from Georgia. A sample of gammarid amphipods collected on 22 April 1994 from MD coastal bays was not infected, but 8.5% of a sample collected on 21 August 1996 were infected with a *Hematodinium*-like dinoflagellate. Infections were not observed in *Paleomonetes* spp., *Cancer irroratus*, *Ovalipes ocellatus*, *Portunus gibbesii*, nor *Libinia emarginata*. In some cases, collections were not made in autumn when parasite prevalence peaks in blue crabs.

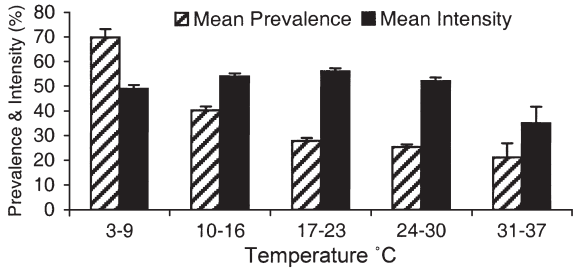

Fig. 7. Comparison of mean prevalence and mean intensity of *Hematodinium* sp. infections in blue crabs collected among different water temperature ranges within coastal bays of Maryland from 1992 to 1998. Error bar = SE

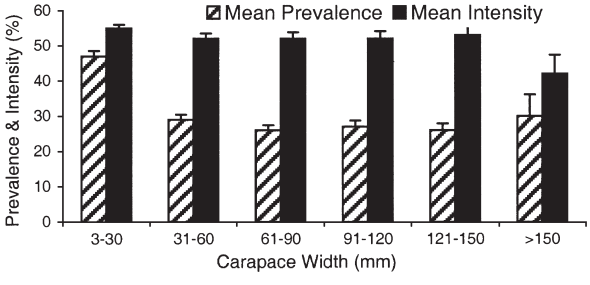

Fig. 8. Comparison of mean prevalence and mean intensity of *Hematodinium* sp. infections among blue crabs of different size ranges collected within coastal bays of Maryland from 1992 to 1998. Error bar = SE

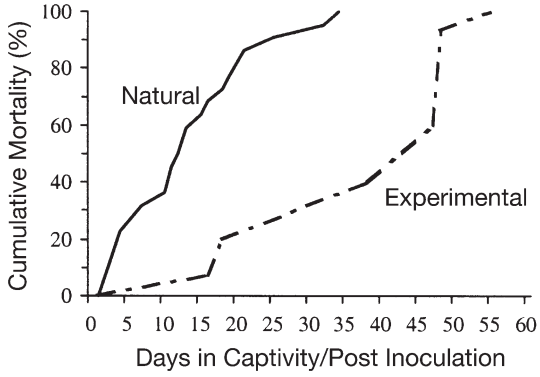

Fig. 9. Mortality over time in blue crabs held at 20°C in 24‰ seawater for up to 60 d; naturally infected crabs (n = 22) and crabs experimentally inoculated with *Hematodinium* sp. plasmodia (n = 15)

DISCUSSION

Hematodinium sp. infections in blue crabs are widespread along the Atlantic and Gulf coasts of the United States. Retrospective analysis of landings data shows a marked decline in the fishery which corresponds to initial reports of heavy mortalities and high prevalences of infected crabs in coastal bays of MD and VA (Messick 1994) (Fig. 11). *Hematodinium* sp. infections are highly pathogenic in blue crabs; in the present study, naturally infected crabs suffered 100% mortality over 35 d at 20 to 24°C. In a separate study, mortality rates were greater than 86% in experimentally infected crabs compared to 20% in uninfected controls (Shields & Squyars 2000). Natural infections appear to become latent at lower temperatures, but when crabs are held at higher temperatures, infections become active (Messick et al. 1999). The wide distribution, high prevalence, and high mortality rate of infected crabs indicate that *Hematodinium* sp. is a significant potential source of mortality in juvenile and adult blue crabs and may severely impact coastal fisheries during seasonal epizootics.

MD, VA, North Carolina, Louisiana, and other coastal states sustain major blue crab fisheries. Some areas of the USA have reported a downward trend in crab fisheries (Evans 1998, Guillory et al. 1998, Steele & Bert 1998). It is difficult to estimate mortality due to disease, and to separate disease effects from the numerous other sources of natural mortality; therefore, mortality is typically assigned a constant value in models designed to estimate crab stocks. Considering the widespread distribution and virulence of *Hematodinium* sp. in the blue crab, the development of differential models for exploitation should consider regional (coastal vs riverine) and environmental factors (reports of epizootics, salinity increases due to drought, warm winters that may enhance parasite survival) that may affect blue crab stocks.

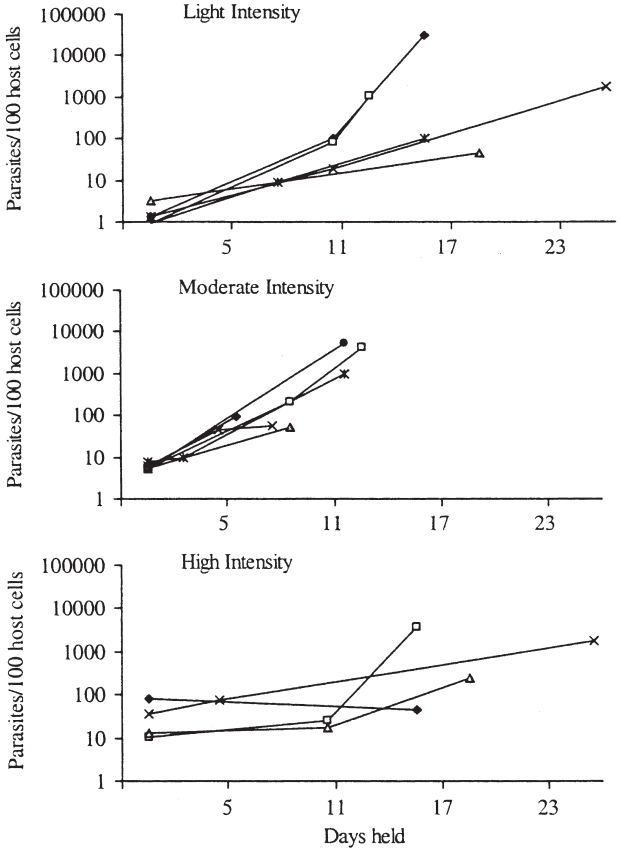

Fig. 10. Proliferation of *Hematodinium* sp. in naturally infected blue crabs with light (0.3 to 3.0 parasites/100 host cells), moderate (3.1 to 10 parasites/100 host cells) or heavy (>10 parasites/100 host cells) infections. Each line represents an individual crab. Note log scale for parasite proliferation

Hematodinium sp. occurs in crabs from waters above 11‰ salinity (Newman & Johnson 1975), including areas within Chesapeake and Delaware Bays; therefore, the disease is not strictly a high-salinity coastal bay phenomenon. Most of the crabs collected from the lower Chesapeake Bay were in, or adjacent to, sanctuaries designed to protect pre-ovigerous and spawning crabs. The presence of infections in adult female crabs (1 to 13%) from Chesapeake Bay, coupled with greater prevalences in juveniles and crabs from higher salinities (Messick 1994), may allow the disease to threaten reproduction in sanctuaries during major epizootics and to impact survival of juvenile crabs protected as recruits to the fishery. It is unknown why juvenile crabs are more prone to *Hematodinium* sp. infections than larger crabs. High population densities during the annual transient profusion in the juvenile population may predispose them to the parasite, or mature crabs may appear less susceptible due to their removal by the fishery or by premature removal by infections.

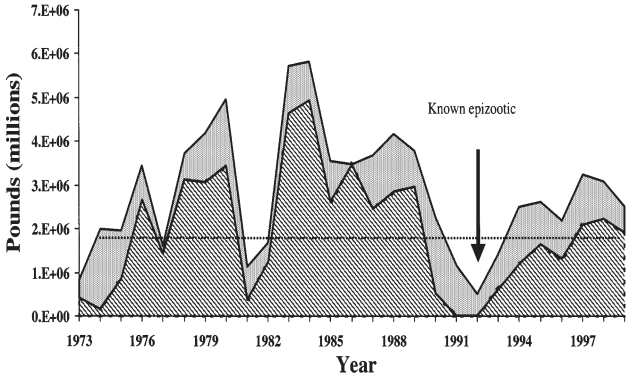

Fig. 11. Annual landings of blue crabs from seaside coastal bays of Maryland ( ) and Virginia ( ) shown with average yearly landings for Virginia (·····). Landings data from Virginia Marine Resources Commission, Stock Assessment Program

Hydrographic features may contribute to epizootics of *Hematodinium* sp. in blue crabs. In the coastal bays of MD, the prevalence of *Hematodinium* sp. varied by location in relation to salinity, and to general drainage or flushing patterns. Stations with some of the lowest prevalences were located north of the ocean inlet, and in tributaries (Fig. 4). The greatest drainage of this system is into the northern portions (Sieling 1960), i.e., increased flushing via this drainage pattern may partially explain the lower prevalence of infections in the northern coastal bays of MD. Hydrographic conditions may contribute to high prevalences of infections in crabs from Chincoteague Bay, Wachapreague, and Red Bank Creek. Limited flow of water through these shallow, high salinity lagoons may focus or amplify the infectious stages of the parasites. Hydrographic conditions are important features in epizootics of egg predatory nemerteans, rhizocephalan castrators, and other dinoflagellate infections of crabs and lobsters (Sloan 1984, Meyers et al. 1987, 1990, Kuris et al. 1991, Field et al. 1992). In Alaskan waters, hydrographic conditions such as fjords with shallow sills coupled with seasonal increases in water temperature contribute to epizootics of several parasites (Sloan 1984, Meyers et al. 1990, Kuris et al. 1991). Coastal bays along the Delmarva Peninsula appear ideal for the proliferation of epizootics of *Hematodinium* sp. and *Paramoeba perniciosa* (Shields 1994). The region includes relatively closed crab populations, based on low immigration and emigration rates of juveniles and adults (Kuris & Lafferty 1992), relatively high salinity with little water exchange between the open ocean and backwaters, and stressful conditions such as high temperatures and seasonal hypoxia. Similar conditions exist in many small estuaries along the mid-Atlantic and southeastern USA.

Hematodinium sp. infections showed marked autumnal seasonality in blue crabs. Distinct seasonal peaks in *Hematodinium* spp. infections are apparent in other crustacean fisheries. The seasonal prevalence of *Hematodinium* sp. in Alaskan Tanner crabs *Chionoecetes bairdi* peaks in summer, declines in winter, and increases again in the spring (Eaton et al. 1991, Love et al. 1993). *Hematodinium* sp. in *Necora puber* from France causes mortalities in winter, with prevalence peaks in May and June (Wilhelm & Boulo 1988, Wilhelm & Miahle 1996). *Hematodinium* sp. in *Cancer pagurus* is found throughout the year (Latrouite et al. 1988). Infections in the Norway lobster *Nephrops norvegicus* peak from April to May (Field et al. 1998) with latent infections from July to December (Appleton & Vickerman 1998). Infected Jonah crabs *C. borealis* were found throughout the year (MacLean & Ruddell 1978), but sample frequency was too low to determine seasonal patterns. The prevalence of a dinoflagellate-like protozoan reported in Alaskan shrimps *Pandalus platyceros* and *P. borealis* peaks in winter and the onset of infections are associated with colder temperatures (Meyers et al. 1994).

Several days to months are required for dinoflagellate infections in some crustaceans to progress from early subpatent infections to detectable infections (Meyers et al. 1987, Field & Appleton 1996, Appleton & Vickerman 1998, Field et al. 1998). The ability to detect infections may be delayed in naturally infected crabs due to low initial parasite numbers. Sensitivity (percentage of infected crabs exhibiting detectable parasites in the hemolymph) was relatively low (30 to 35%) in crabs inoculated with 10^3 or 10^5 parasites after 2 wk but increased to 80 to 85% after 26 to 32 d (Shields & Squyars 2000). In this study (Figs. 5 & 6), no patent infections were found during the early spring; low temperatures during winter may cause the parasite to become subpatent and there may be a delay in patency despite the warmer spring water temperatures. Although water temperatures are highest during summer, parasite prevalences were rather steady. Parasite proliferation may be hindered by high water temperatures during summer. Data collected from estuaries in Georgia during June, July, and August 1999 indicate the prevalence of infections decreased at higher summer temperatures (Table 1). The prevalence of infections in coastal bays of MD was highest during autumn when water temperatures were relatively cooler; this season may provide the optimal temperatures for parasite proliferation. Prevalence declined precipitously during winter months when water temperatures dropped. A water temperature of 9°C reduced parasite intensity in naturally infected crabs held for 73 d (Messick et al. 1999). Water temperature and parasite proliferation rates at these temperatures likely induce the

seasonality of *Hematodinium* sp. infections in blue crabs. There was no indication that infection seasonality was due to seasonal fluctuations in salinity.

Crab size is a determining factor in the prevalence and distribution of *Hematodinium* sp. in blue crabs. Smaller crabs are more prone to infection than mature crabs and were more prevalent than larger crabs in autumn samples; crab size may also influence the seasonality of infections. Juvenile crabs molt more frequently than larger crabs. Host defense mechanisms may be stressed by ecdysis, rendering juvenile crabs more susceptible to invading organisms. Newly molted Tanner crabs are more likely to harbor detectable dinoflagellate infections than pre-molt crabs (Meyers et al. 1990, Eaton et al. 1991), and infections in the Norway lobster *Nephrops norvegicus* peak from March to May, the period of highest molting activity (Field et al. 1992). Molting may predispose crabs to invasion by certain parasites by allowing entry through breaks in the cuticle (Couch & Martin 1982, Meyers et al. 1990, Morado & Small 1994, Hoeg 1995). Although no significant variation in prevalence was found in crabs at different molt stages in this study, sample numbers were relatively low. Additionally, although prevalence of disease was 0% in coastal bays of MD during May, when molting peaked, prevalence increased to 30% just 1 mo later in June.

Infections of *Hematodinium*-like dinoflagellates in other crustaceans suggest the presence of alternate or reservoir hosts. Hudson & Shields (1994) speculated that amphipods may serve as intermediate or reservoir hosts for infections. Since blue crabs eat amphipods and other crabs, predation or cannibalism may perhaps spread infections. We have not, however, been able to transmit *Hematodinium* spp. infections via feeding (Hudson & Shields 1994) or co-habitation (Messick et al. 1999). Parasites found in mud crabs, green crabs, *Callinectes similis*, or amphipods may be the same species as that found in the blue crab. Morphologically, the parasites show similarities in size, form, and nuclear staining characteristics. At present, molecular studies (Hudson & Adlard 1996) and ultrastructural investigations have not examined the type species from the locality of the type host. Clearly, additional assessments of green crabs for *Hematodinium* sp. would facilitate our understanding of the taxonomy of this important group of pathogens.

In summary, *Hematodinium* sp. infections in blue crabs from the USA are widely distributed, seasonal, and influenced by location, salinity, and host size. Numerous questions remain about how environmental and host factors modulate parasite prevalence, proliferation, and virulence. These variables are likely influenced by physiological characteristics of the host, the parasite, or they may synergistically influence infections.

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