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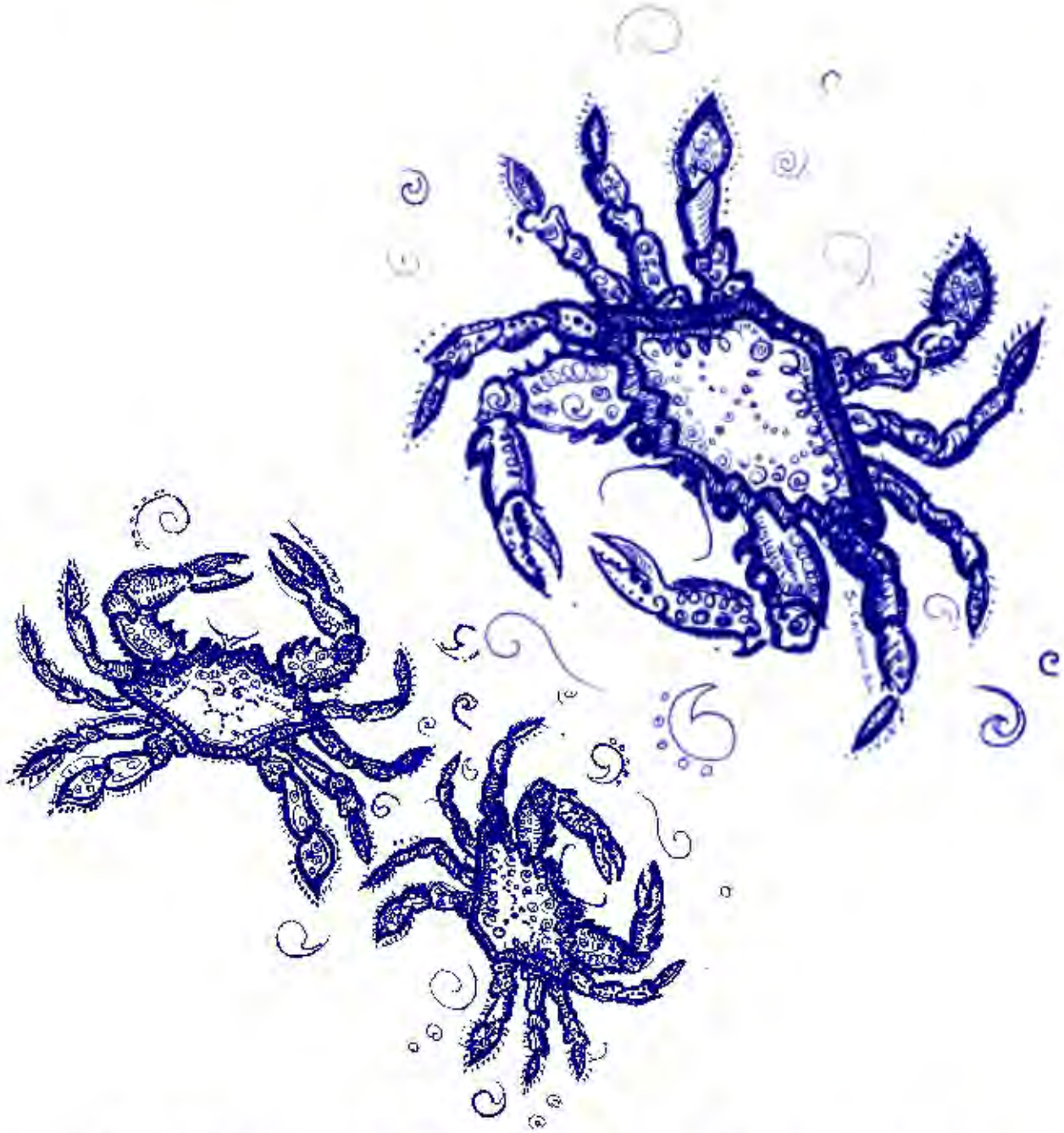
Infection and mortality studies with Hematodinium perezi in blue crabs

Jeffrey D. Schields
Virginia Institute of Marine Science

Proceedings:

Blue Crab Mortality Symposium



Gulf States Marine Fisheries Commission

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PROCEEDINGS: BLUE CRAB MORTALITY SYMPOSIUM

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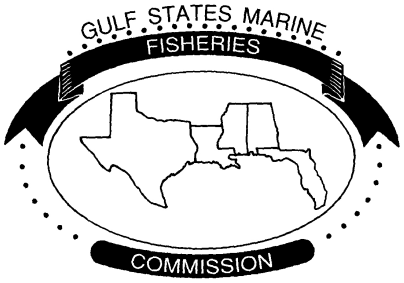

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Preface

A symposium on sources and measurement of blue crab (*Callinectes sapidus* Rathbun) mortality was held in conjunction with the Annual Meeting of the Crustacean Society in Lafayette, Louisiana, on May 28 and 29, 1999. The symposium was sponsored by the Crab Subcommittee of the Gulf States Marine Fisheries Commission (GSMFC). The symposium addressed sources of juvenile and adult blue crab mortality in the form of both review papers and original research findings. The information provided by this Proceedings should stimulate additional questions, investigations, and insights into the sources of mortality on the blue crab population and its implications for the blue crab fishery.

Appreciation is extended to Ms. Cindy Yocom and Ms. Lucia Hourihan for their assistance in the final editing of the proceedings. Thanks also goes to numerous reviewers who provided valuable comment on the papers submitted for this publication.

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Infection and Mortality Studies with *Hematodinium perezi* in Blue Crabs

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Abstract. — Blue crabs, *Callinectes sapidus*, from the Delmarva Peninsula experience recurring epizootics of a pathogenic dinoflagellate. The parasite, *Hematodinium perezi*, fulminates in late summer and autumn causing significant mortalities in high salinity embayments and estuaries. Mortality rates during epizootics are difficult to estimate because dead crabs quickly deteriorate. Host mortality was investigated in naturally- and experimentally-infected crabs. Detection of the parasite, its proliferation in the hemolymph, and distribution in lower Chesapeake Bay were also examined. The dinoflagellate was highly pathogenic, killing 100% of naturally infected crabs, and 86% of inoculated crabs over 35 and 40 days, respectively. Inoculated hosts began dying 14 days after infection, with a median time to death of 30.3 $\pm$ 1.5 d (se). Proliferation of the parasite was rapid at 20°C, with infections progressing to high levels over one to two weeks. Detection of the parasite was, however, variable. In infection experiments some hosts presented infections after two weeks while others were not detected until four weeks post inoculation. The dinoflagellate was prevalent in the seaside bays of the Delmarva Peninsula. In fall 1996, the prevalence of the disease along the Virginia portion of the Delmarva Peninsula varied from 20-50% in legal crabs. Prevalences of 1-30% were noted for crabs caught between Cape Henry and Cape Charles, i.e., the mouth of the bay. The mortality studies indicate that *H. perezi* represents a significant threat to the blue crab fisheries in high salinity estuaries and may preferentially infect mature females that move to higher salinities to breed.

KEYWORDS: *Callinectes sapidus, Hematodinium perezi*, epizootics, crab fisheries, parasitic dinoflagellate

Hematodinium perezi is a parasitic dinoflagellate found in the hemolymph of blue crabs, *Callinectes sapidus,* and other decapods. The disease occurs in high salinity waters (>11‰) from Delaware to Florida, and into the Gulf of Mexico (Newman and Johnson, 1975; Messick and Sinderman, 1992; Messick and Shields, in press). In Virginia and Maryland, *H. perezi* has caused serious losses to the crab fishery of the Delmarva Peninsula (Messick, 1994; S. Rux and M. Oesterling, VIMS, personal communication). In 1991 and 1992, an epizootic of the parasite affected the blue crab fishery in seaside bays of the Delmarva Peninsula. Watermen reported reduced catches and lethargic, moribund and dead crabs in pots and shedding facilities. The Delmarva Peninsula has several characters that may facilitate epizootics of *H. perezi*, including relatively closed crab populations (i.e., those with little immigration and emigration of juveniles and adults), relatively little water exchange between the open ocean and backwaters, and stressful conditions such as heat stress, seasonal hypoxia, seasonal fishing and predation pressure (Shields, 1994). Similar conditions exist in many small estuaries along the mid-Atlantic and southeastern USA.

In 1997, blue crabs supported the largest commercial fishery within Chesapeake Bay and the second largest fishery in Virginia (Kirkeley, 1997). The crab industry harvests from 80-120 million

pounds from Chesapeake Bay annually; of that, approximately 10-14 million pounds are soft-shell crabs (Kirkeley, 1997; Rugolo et al., 1998a). Declines in crab catches in 1998 and projected declines in 1999 indicate that mortality processes in blue crabs are not well understood. Current models project estimates of natural mortalities, but they do not account for the potential epizootics and mortalities caused by *Hematodinium perezi.* While fishing pressure may be high (Rugolo et al., 1998b), disease and environmental processes have not been well examined; yet, both processes contribute to declines and population cycles in other crustacean fisheries (e.g., Hobbs et al., 1992; Kuris and Lafferty, 1992). Given the importance of the fishery to the region's economy, and the relative effect of the disease in blue crabs, it is imperative that we clarify the epizootiology and pathology of the disease.

Hematodinium spp. threaten other important crustacean fisheries. Recent outbreaks of *Hematodinium*-like parasites have been reported from Alaskan stocks of Tanner crab, *Chionoecetes bairdi* (Meyers et al., 1987, 1990, 1996); Eastern Canadian stocks of snow crab, *C. opilio* (Taylor and Khan, 1995); the Scottish fishery for Norway lobster, *Nephrops norvegicus* (Field et al., 1992; Field and Appleton, 1995); and the French fisheries for the velvet crab, *Necora puber* (Wilhelm and Miahle, 1996) and rock crab, *Cancer pagurus* (Latrouite et al., 1988). In Australia, *H. australis* occurs at low levels in stocks of sand crabs, *Portunus pelagicus,* and mangrove crabs, *Scylla serrata* (Shields, 1992; Hudson and Shields, 1994). An unusual dinoflagellate has also been reported from spot and pink shrimp, *Pandalus borealis* and *P. platyceros* (Bower et al., 1993; Meyers et al., 1994).

Unfortunately, background mortalities due to *Hematodinium* are often difficult to assess because dead crabs quickly become undiagnosable. The timing of host mortalities was investigated in natural and experimental infections as well as the detection and proliferation of the parasite in infected crabs. In addition, the prevalence of the parasite was reported for Chesapeake Bay.

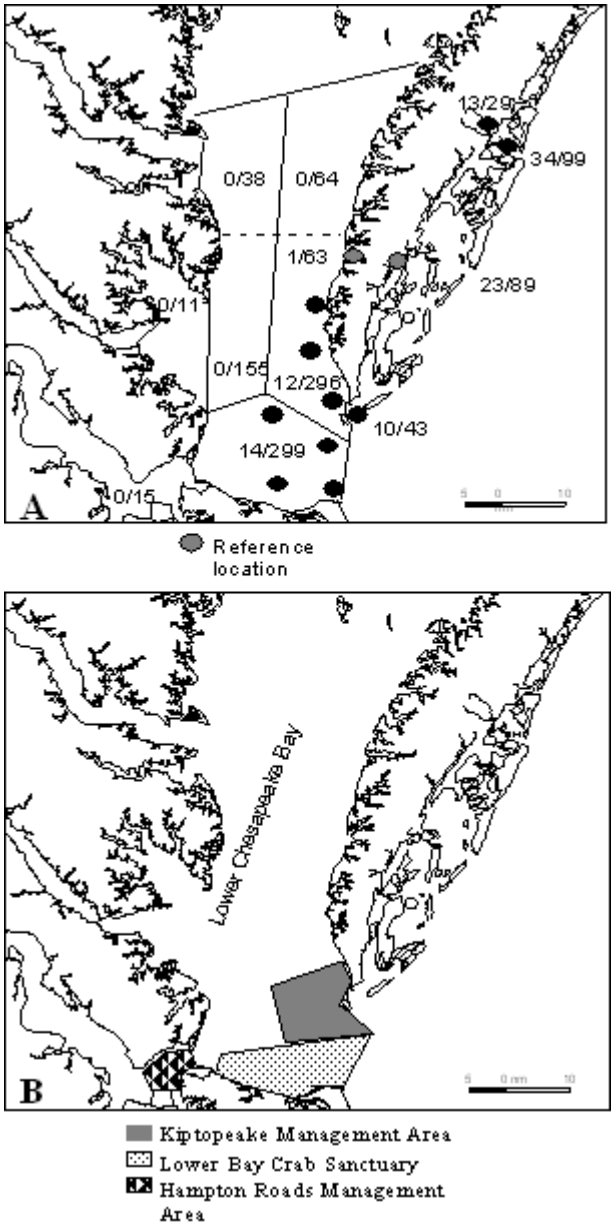

FIGURE 1. — (A) Distribution and prevalence of *H. perezi* in blue crabs from Chesapeake Bay, fall, 1996. Black circles represent locations where the disease was found. Gray dots represent reference locations (Hungars and Red Bank creeks). Numbers indicate infected crabs versus total number sampled. Schematic of aggregate locations used in the analysis (cf. strata used by VIMS Trawl Survey). (B) Crab management areas designated by the Virginia Marine Resources Commission. The Kiptopeake and Hampton Roads areas are closed to dredging in winter. The Lower Bay is closed to pot fishing but open to dredging.

Methods

Crab Collections

Blue crabs were collected from the coastal bays and creeks on the "seaside" (e.g., Red Bank Creek, Wachapreague) and "bayside" (e.g., Nassawadox Creek, Hungars Creek, Cape Charles) of the Delmarva Peninsula and from many locations within the mainstem of the lower Chesapeake Bay (Figure 1). Additional collections were made in the York, James, and Rappahannock rivers. Regular monthly sampling was done via pot and trap fishing at two reference locations: bayside at Hungars Creek and seaside at Red Bank Creek. The broad-scale sampling was done in conjunction with the Virginia Institute of Marine Sciences (VIMS) trawl (April through December, December through March, respectively) and dredge surveys. Additional samples were collected via trawls and crab pots. In most cases, crabs were chilled on ice for transportation to the laboratory. Up to 60 crabs from each trawl or dredge were examined for *Hematodinium perezi*. Low salinity locations (<11‰) were not sampled for the disease, but subsamples (n=60-75) of several hundred crabs were sampled from the York River as a baseline.

Laboratory Analyses

Crab sex; carapace width (with and without spines); condition (maturity, natural vs. trawl damage); molt stage; and collection location were recorded. Crab hemolymph was taken from the axillae of the 5th walking leg with a 27 ga. needle and tuberculin syringe. Hemolymph was examined as a wet smear, with an additional smear being processed and stained as described in Messick (1994). Briefly, acid-cleaned, poly-l-lysine-coated microslides were smeared with 2-3 drops of fresh blood, allowed to sit for 2-3 min, then fixed in Bouin's fixative. The smears were then processed through a routine hematoxylin and eosin procedure. Wet smears were read at 400x using phase contrast; matching prepared smears were read with oil immersion at 1000x. The density of infection was estimated via direct cell counts using a hemocytometer (Neubauer, Bright-line). Intensity was the proportion of parasites (trophonts and plasmodia; Figure 2) to the total number of host cells multiplied by 100, giving parasites per 100 host cells.

For infection studies, *Hematodinium perezi* was maintained in the laboratory by serial passage of infected hemolymph. Raw hemolymph or buffer-washed (see below) parasites from naturally infected crabs was injected directly into naive crabs. Naive crabs were obtained from areas outside the enzootic region, and their hemolymph was examined prior to challenge. Uninfected crabs were acclimated for 3-7days prior to treatment to ensure absence of overt bacterial or protozoal diseases. During experiments, crabs were fed fish and squid semiweekly and held individually in 5gal. (19 l) aquaria (static, box filters with activated charcoal) at 20°±2°C and 24‰ salinity.

Proliferation and Mortality Studies

Observations on the proliferation of the disease were undertaken in two Proliferation Studies. In Proliferation I, naturally infected crabs were held individually for observation and hemolymph sampled regularly as described above. In Proliferation II, unexposed, naive crabs were inoculated with infected hemolymph containing plasmodia of *H. perezi*. Hemolymph was taken from an infected crab, the density of the parasites was counted with a hemocytometer (10^3 plasmodia ml^{-1}), and 100 µl of hemolymph was injected directly into the axillae of the fifth walking leg of uninfected, naive crabs. Both male and female crabs were inoculated (n = 12 and 3, respectively). The onset and course of the infections were monitored via weekly hemolymph smears.

Two mortality experiments were undertaken and are briefly described here (for details, see Shields and Squyars, 2000). In Mortality I, raw, infected hemolymph was the inoculant. In Mortality II, buffer-washed parasites were adjusted to the density of the inoculant used in Mortality I. Mortality II was a close replicate of Mortality I except for the use of centrifugation with buffer washes and the use of mostly plasmodial versus uninucleate stages of the parasite (see below). Controls consisted of injecting uninfected hemolymph (or buffer in Mortality II) into uninfected crabs and handling uninfected crabs similarly. The controls served as comparisons for the onset and severity of the infection in the

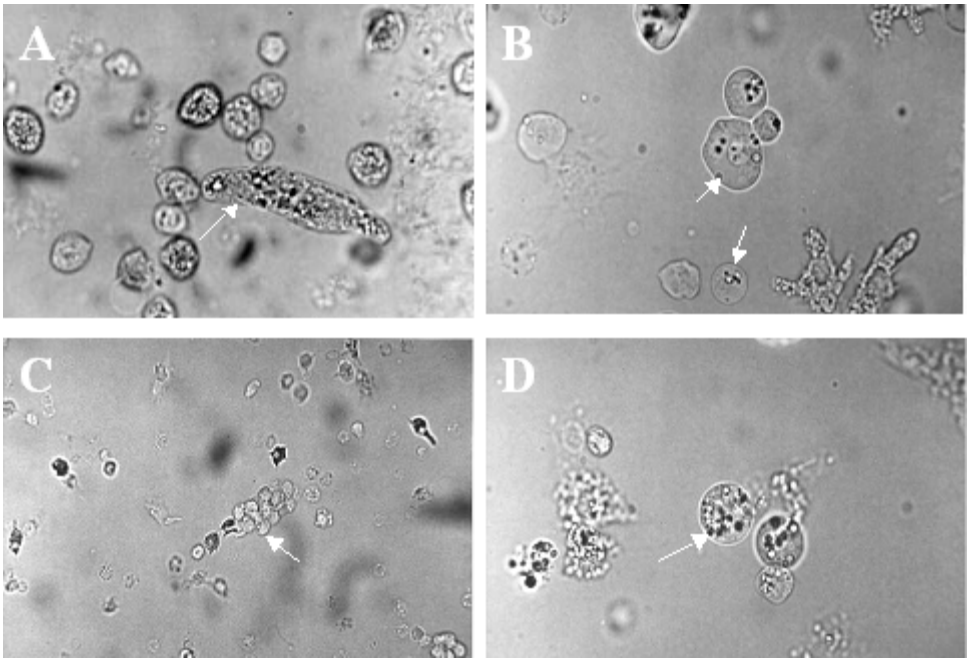

FIGURE 2. — (A) Small plasmodium in the hemolymph (approx. 20 um long) (arrow). (B) Amoeboid trophont (12-14 um) in the hemolymph (arrow). (C) Schizogony of a plasmodial stage (approx. 80 um) (arrow). (D) Rounded trophont (12-14 um, arrow) in the hemolymph.

inoculated crabs, as well as for monitoring survivorship. Crabs were monitored daily for mortalities and bled weekly to assess infection status.

Mortality I consisted of an experimental group (n = 20 crabs) inoculated individually with 100 µl of infected hemolymph containing 1.3 x 10^5 parasites (100% trophonts). Controls (n = 22 crabs) were inoculated with 100 µl of uninfected hemolymph. Mortality II consisted of two experimental groups (n = 10, 10 crabs respectively) inoculated with 100 µl of buffer containing either 1.0 x 10^5 parasites per crab (high dose; 97% plasmodia, 3% trophonts) or 1.0 x 10^3 parasites per crab (low dose, same ratio). Controls (*n* = 8 crabs) were inoculated with 100 µl physiological buffer. In Mortality II, infected hemolymph was diluted 1:1 with filter-sterilized buffer (modified from Appleton and Vickerman, 1998; NaCl 19.31 g/l, KCl 0.65 g/l, $CaCl_2 \bullet 2H_2O$ 1.38 g/l, $MgSO_4 \bullet 7H_2O$ 1.73 g/l, Na_2SO_4 0.38 g/l, HEPES 0.82 g/l, adjusted to pH 7.8, with added glucose 1.0 mg/ml), centrifuged at 4000 rpm for ten minutes, washed twice more as above, and the parasites resuspended in buffer to obtain 1.0 x 10^6 parasites ml^{-1} and 1.0 x 10^4 parasites ml^{-1}.

The proportional hazards model was used to analyze survival data (Cox and Oakes, 1984). The Tarone-Ware log-rank test was used to examine differences between survivorship curves (Wilkinson, 1997). SYSTAT (Wilkinson, 1997) and SAS were used to perform the statistics. A significance level of $p < 0.05$ was accepted as significant.

Results

Proliferation Studies

In aquaria, naturally infected crabs with light infections (0.33 to 3.00 plasmodia/100 host cells) developed heavy infections (>100 trophonts/100 host cells) over two to three weeks (Figure 3). Proliferation of the parasite was faster in moderate

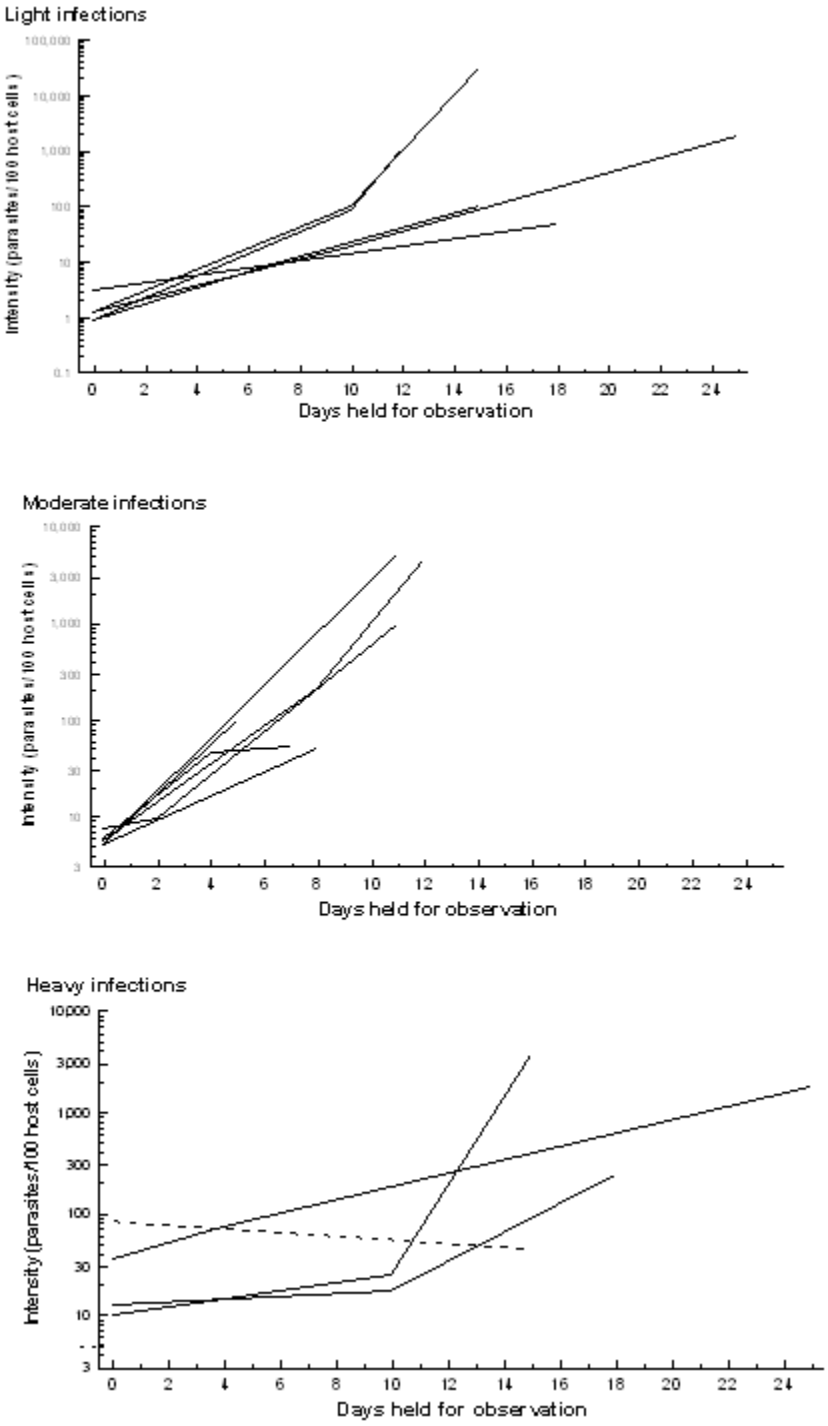

FIGURE 3. — Proliferation of *H. perezi* in naturally infected blue crabs with light infections (0.33-3 parasites/100 host cells), moderate infections (3.1-10 parasites/100 host cells), heavy infections (>10 parasites/100 host cells). Females (dashed lines) and males (solid lines). Endpoints represent crab death.

infections with log growth rapidly pushing intensities to enormous levels (>3000 parasites/100 host cells). Proliferation in heavy infections was not as rapid as the moderate infections, but log growth was apparent in two crabs after ten days in the laboratory.

Survival of the host was not consistent between light, moderate and heavily infected crabs. (Figure 3). Crabs with moderate infections appeared to die more quickly than those with light and moderate infections, and mortalities may have been associated with high parasite intensities. In some cases, heavily infected crabs survived as long as lightly infected crabs (Figure 3). A few crabs survived for up to 35 days before dying from the infection (Figure 4), with one female showing a decline in the intensity of the infection (Figure 3).

Inoculation of 10^2 plasmodia (Proliferation II) successfully transmitted the parasite to naive hosts. In general, no infections were observed before six days post inoculation. Light infections, characterized by plasmodia (1-3 plasmodia/100 host cells), were observed after approximately 13 days in 14 of 15 hosts (Table 1). Moderate infections (3–10 parasites/100 host cells) with plasmodia and trophonts occurred after 16 days, and heavy infections (>10 parasites/100 host cells) occurred after 30 days. Host mortalities to the infection occurred after 17 days, peaked between 45 to 52 days, with the last infected crab dying 55 days after the injection (Figure 4).

An important aspect regarding occult infections was revealed. Densities of less than 0.33 parasites/100 host cells (approximately 1.0 x 10^4 parasites/ml) cannot be effectively diagnosed using hemolymph smears. This was confirmed by reexamining crabs from Hungars and Red Bank Creek (reference locations) after they had been held separately for five to ten days. Several crabs that had been diagnosed as uninfected from field collections had converted to light infections. Hence, there was a minimum prepatent period of at least eight to ten days before infections could be observed.

Mortality Studies

Hematodinium perezi was highly pathogenic (Figures 4 and 5). Inoculated crabs that became infected began dying two weeks after inoculation. The mortality rate of the infected crabs was 86%, while only 20% of the controls died. Uninfected crabs (controls) experienced significantly fewer

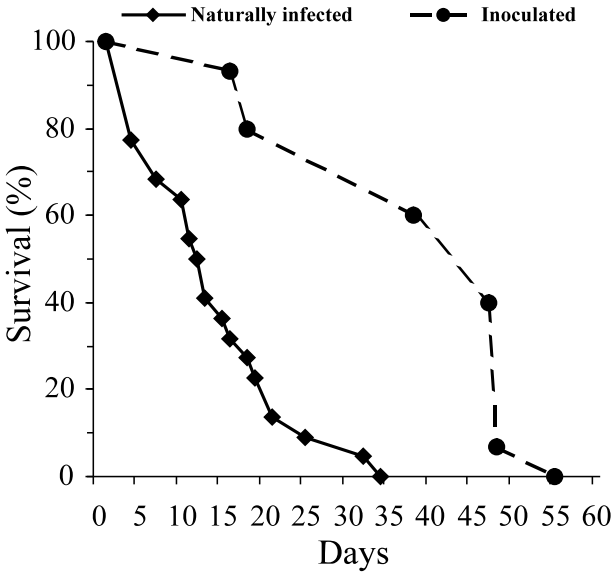

FIGURE 4. — Survival of of naturally infected crabs (Proliferation I; solid line, diamonds) and experimentally inoculated crabs (Proliferation II, dashed line, circles, 10^2 plasmodia per crab) held in the laboratory.

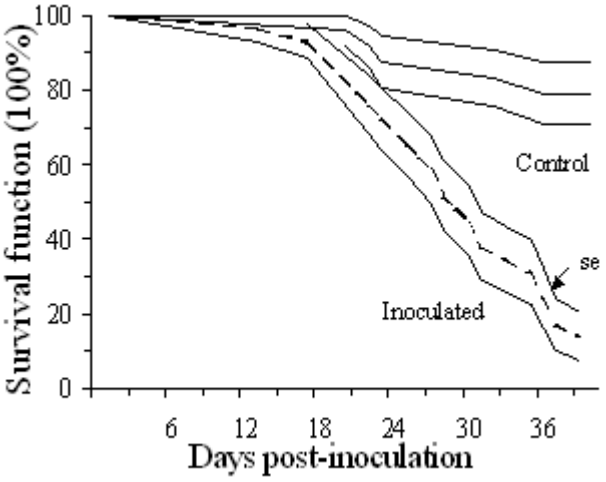

FIGURE 5. — Survival analysis (Kaplan-Meier, Weibull distribution) of experimentally inoculated crabs (Mortality I and II, 10^3 and 10^5 parasites per crab) held at 20° C, 24 ‰. N = 20 uninfected controls, 30 inoculated crabs. The proportional hazards model indicated a significant difference between uninfected and infected crabs (with a 14 day lag) at $P<0.001$.

TABLE 1. — Detection of *H. perezi* in hemolymph of experimentally inoculated blue crabs over the course of infection. Crabs in Proliferation I were given 10^2 plasmodia (n = 15 crabs). Crabs in Mortality experiments (combined results) were given 10^3 or 10^5 plasmodia or trophonts. Mean intensity is given for the Mortality experiments only. Numbers in parenthesis are sample sizes. Only those crabs that developed infections are included.

Days post inoculation	Proliferation II Prevalence (%)	Mortality I and II Prevalence (%)	Mean Intensity (parasites/100 host cells) (± se)
0	0.0	0.0 (21)	0.0 ± 0.0
7	0.0	8.7 (21)	0.037 ± 0.04
14	93.3	36.3 (11)	2.876 ± 1.66
18	100	30.0 (10)	0.256 ± 0.14
21		60.0 (10)	202.27 ± 197.55
26		87.5 (16)	48.27 ± 23.82
32		75.0 (4)	46.6 ± 24.28
35		100.0 (4)	46.74 ± 4.49

mortalities than infected hosts (Chi-square = 19.267, df = 1, $P<0.001$). There were no differences in mortalities between infected crabs in Mortality I and II (Chi-square = 1.212, df = 1, $P= 0.271$). There were also no differences in mortalities between uninfected crabs in the two experiments (Chi-square = 0.652, df = 1, $P = 0.419$); hence the data were grouped for further analysis. The median time to death (MTTD) for infected crabs was 30.3 ± 1.5 (se) days. The MTTD could not be calculated for the controls since they exhibited too few mortalities. Infected crabs had a significantly higher mortality rate that was 7 to 8 times greater than that of the uninfected controls (proportional hazards, Chi-square = 13.503, $P < 0.001$, relative risk = $e^{1.055/0.5174}$).

From Proliferation II and the mortality studies, parasites were detected in the hemolymph approximately two weeks after injection (Table 1). In Proliferation II, 93% of the inoculated crabs had detectable infections after two weeks, but in the mortality experiments, detection was low (30-35%) after 14 to 18 days, reaching 80 to 85% after 26 to 32 days and 100% after 35 days. (Detection was based solely on crabs that developed infections. Four crabs from Mortality II did not become infected and were excluded from the analysis of detection.) Proliferation of the parasite and related hematology of infected crabs in the mortality studies are reported elsewhere (Shields and Squyars, 2000).

Additional Observations

During the course of routine maintenance of the parasite, four crabs presented long-term chronic infections. One crab (#3977) was monitored weekly over the time course of infection (Figure 6). Observations on the other crabs were intermittent. Synchronous sporulation of the parasite occurred at least twice in Crab #3977, with each event lasting less than four days. Parasite density was extraordinarily high (1.6×10^8 dinospores ml^{-1}) during sporulation and dropped to moderate levels (3.3×10^6 trophonts ml^{-1}) thereafter. Dinospores were observed five times over the course of 26 days, beginning 43 days after injection. [Dinospores were only enumerated twice.] Surprisingly, the crab did not die during the mass sporulation events.

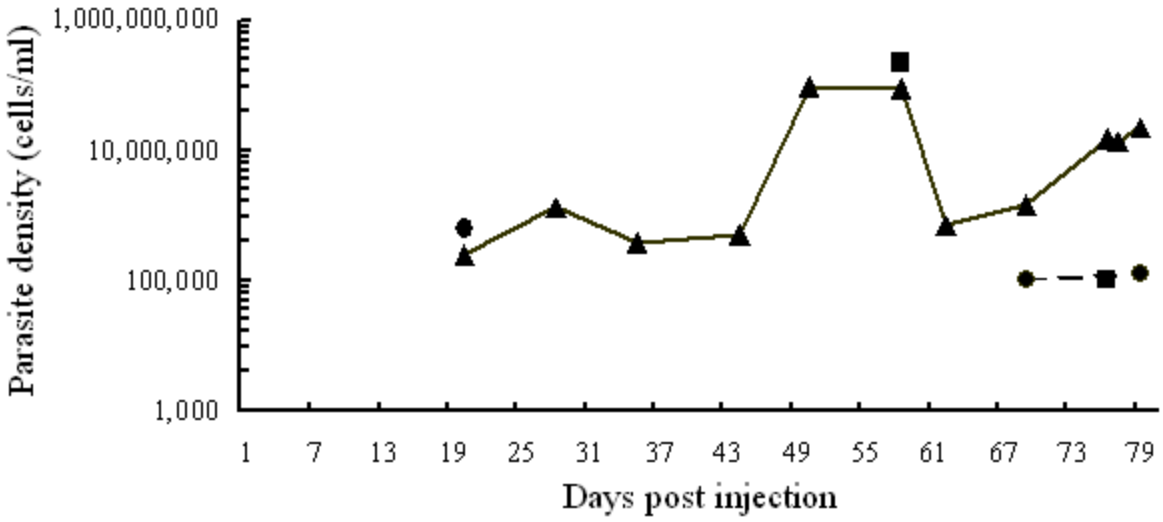

FIGURE 6. — Crab #3977 presenting a chronic, experimental infection of *H. perezi* (plasmodia, circles; trophonts, triangles; dinospores, boxes). The crab died after 80 days.

Prevalence in Lower Chesapeake Bay

In 1996, a total of 3259 crabs were sampled. Of these, 733 were sampled in the winter, 446 in the spring, 871 in the summer, and 1209 in the fall. The collection gear was biased toward mature crabs. Since most of the sampling was done in the mainstem of the bay, more female crabs were collected than males (1940 versus 1239, respectively). A total of 111 crabs were infected, with virtually all of the infections occurring in the fall (Figure 1).

In the fall 1996, a limited outbreak was documented along the Eastern Shore, at the mouth of Chesapeake Bay, and at several stations along the southeastern portion of the bay (Figure 1). The prevalence of the disease along the Virginia portion of the Delmarva Peninsula varied from 20-50% in legal size crabs. Lower prevalences (1-10%) were noted for crabs caught between Cape Henry and Cape Charles, i.e., at the mouth of the bay. In November, the prevalence of the disease was notably higher in crabs caught between Cape Henry and Cape Charles (10-30%).

In 1997, a total of 870 crabs were sampled. Of these, six were sampled in the winter, 148 in the spring, 358 in the summer, and 358 in the fall. At least 47 crabs were infected, with the infected crabs evenly spread through the three seasons. In 1997, *H. perezi* festered at moderate prevalences (1-10%) at both the seaside and bayside reference stations. In the summer and fall 1997, the prevalence and distribution of the disease were higher than that observed in 1996. The disease was present at both reference locations during spring and summer 1997. Hungars Creek had a surprisingly high prevalence of 20% during September 1997. This creek is located over 30 miles from the bay mouth on the bayside of Delmarva Peninsula.

Discussion

Experimental infections of *Hematodinium perezi* were highly pathogenic and proliferated rapidly in the hemolymph. The mortality rate of 86% at 20°C over 40 days is equivalent to that reported for bubonic plague in human populations. Lower mortality rates with longer survival duration have been observed at lower temperatures (12° and 16°C) in naturally infected crabs (Messick et al., 1999). Parasites are lost or become dormant below 10°C. High mortality rates have been noted in *Hematodinium*-like infections in Tanner crab, *Chionoecetes bairdi*, and Norway lobster, *Nephrops norvegicus*. Naturally-infected Tanner crabs experienced 67% mortality (n=11) over 158 days (Meyers et al., 1987). Mortalities occurred in assocation with secondary bacterial infections.

Uninfected controls exhibited no mortalities during the time course (Meyers et al., 1987). Naturally-infected Norway lobsters suffered mortality rates of 86% to 100% over 27 days and 75 days, respectively (Field et al., 1992). Mortality rates were two to four times higher than uninfected lobsters (Field et al., 1992). Infections in blue crabs kill the host very quickly; hence, infected crabs do not acquire the bitter flavor found in Tanner crabs with their lengthy *Hematodinium* infections (Meyers et al., 1987).

Hematodinium infections in blue crabs have four apparent stages: (1) the occult stage occurs when parasites are not detectable in the hemolymph (but detectable in the heart, Shields and Squyars, 2000); (2) the acute stage is when parasites are rarely observed in the hemolymph, and mortalities occur quickly (14 to 40 days); (3) the chronic stage occurs over lengthy periods (up to 90 days) with one to several mass sporulation events (10^8 dinospores ml^{-1} – a density far higher than most algal blooms) or sequelae; and (4) the refractory or immune state, where exposed crabs survive and do not develop infections.

Estimates of host mortality to disease are often difficult to obtain. They are important in predicting the scale or impact of parasites and diseases on a fishery. Blue crab catches fluctuate yearly in Chesapeake Bay but causes for these fluctuations are not well understood. Current models for blue crab populations in Chesapeake Bay are based on population assessments from various surveys (Lipcius and Van Engel, 1990; Abbe and Stagg, 1996; Rugolo et al., 1998a, 1998b). These models project crab abundance for the fishery as a whole but do not separate the larger, low salinity "bayside" fishery from the smaller, high salinity "seaside" fishery in the region. Natural mortality is often assigned a constant term in models, it does not vary with potential epizootics and resulting mortalities caused by *H. perezi.* Differential models of exploitation by region may be warranted

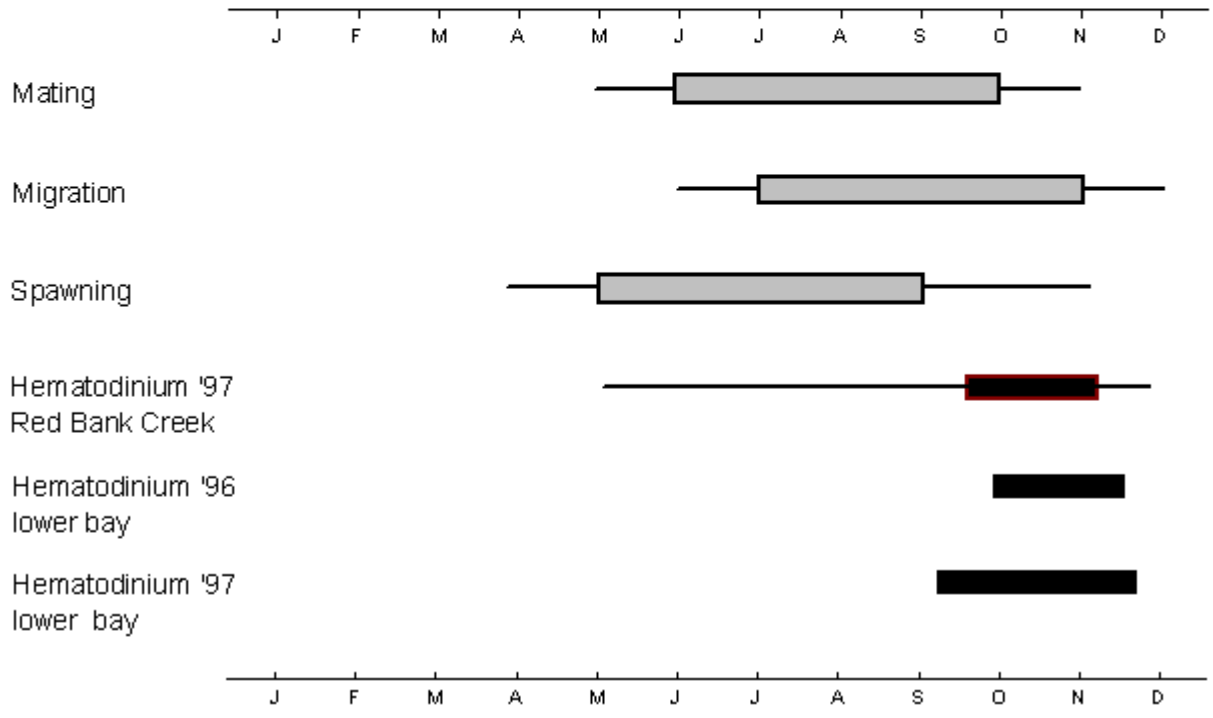

FIGURE 7. — Temporal patterns in reproductive patterns of female blue crabs shown with underlying prevalences of *H. perezi*. Bars represent peak periods of activity; lines, ranges of activity. Red Bank Creek is the "seaside" reference site; lower bay is the mouth, and adjacent strata.

especially during or immediately following epizootics.

Hematodinium perezi was present at low to moderate prevalences in the main spawning grounds of the blue crab near the mouth of Chesapeake Bay. Most of the crabs collected from the lower bay were in or adjacent to the Crab Management Areas/Sanctuaries designated by the Virginia Marine Resources Commission (Figure 1b). These sanctuaries are designed to protect preovigerous and spawning crabs during sensitive portions of their life cycles. The parasite occurred in the spawning grounds during the fall. The peak season for crab reproduction is late spring and summer (Van Engel 1958, 1987; Hill et al., 1989). In 1996, the prevalence of the parasite was low during the prebreeding and ovigerous season (Figure 7), but moderate to high later in the ovigerous season. The moderate prevalence and heavy infections in adult female crabs, coupled with the predilection of the disease for juvenile crabs (Messick, 1994), indicates that during epizootics the disease may threaten reproduction in the sanctuaries and may impact survivorship of the next season's harvest.

Hematodinium perezi occurs in high salinity waters (Newman and Jonson, 1975). However, the occurrence of the disease on both sides of the Delmarva Peninsula indicates that the problem is not strictly a "seaside" phenomenon. Apparently, the parasite fulminates in small foci along the Eastern Shore throughout much of the year with small to moderate outbreaks occurring in the mouth and southeastern portions of the mainstem of Chesapeake Bay during the fall (Messick and Shields, in press). The risk of infection to larval and migrating juvenile crabs has not been well assessed. Larval crabs must pass through high salinity waters, hence, they may risk exposure to infectious stages (dinospores?) of *H. perezi*. The possible impact of the disease on juvenile recruitment to the stock should be incorporated into further analyses of blue crabs in Chesapeake Bay.

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