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NOTE

Hematodinium perezi infections in adult and juvenile blue crabs *Callinectes sapidus* from coastal bays of Maryland and Virginia, USA

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ABSTRACT: In coastal bays of Maryland and Virginia, USA, adult and juvenile blue crabs *Callinectes sapidus* were severely infected with the parasitic dinoflagellate *Hematodinium perezi*. Dinoflagellates were observed in the hemocoel of all infected crabs; associated histopathological changes were evident in some tissues. Dinoflagellates could be observed with an inverted microscope through the 5th pleopod of heavily infected juvenile crabs (5 to 29 mm) without invasion. This note documents a high prevalence of *H. perezi* infections in juvenile blue crabs from coastal bays in Maryland and Virginia. The seasonal infection prevalence cycle reported by previous authors is consistent with observations made during this study.

KEY WORDS: *Hematodinium perezi* · *Callinectes sapidus*

Hematodinium perezi was originally reported as a rare hemolymph-infecting parasite in the European crabs *Carcinus maenas* and *Liocarcinus depurator* (Chatton & Poisson 1931). Rare infections in the cancer crabs *Cancer irroratus* and *Cancer borealis* and in the portunid crab *Ovalipes ocellatus* from the New York Bight area of the northeastern United States have also been reported (MacLean & Ruddell 1978). In 1975, the parasite was reported in up to 30 % of adult blue crabs *Callinectes sapidus* sampled from coastal areas of North Carolina, Georgia, and Florida (USA) (Newman & Johnson 1975), and in Gulf of Mexico blue crabs (Couch & Martin 1979). In recent years, *Hematodinium*-like organisms have been reported in additional crab species and other crustaceans. A 21 % prevalence was found in the cancer crab *Cancer pagurus* from the west coast of France (Latrouite et al. 1988). A 100 % prevalence has been reported in Tanner crabs *Chionoecetes bairdi* from southeast Alaskan waters, and *Chionoecetes opilio* from the Bering Sea (Meyers et al. 1987, 1990, Eaton et al. 1991). Additionally, *Hematodinium*-like parasites have been observed in 87 % of the crab *Necora* (*Liocarcinus*) *puber* from France (Wilhelm & Boulo 1988), and in up to 70 % of the Norway lobster *Nephrops novegicus* from the west coast of Scotland (Field et al. 1992). On the east coast of Australia a parasitic dinoflagellate infects the sand crab *Portunus pelagicus*, the mud crab *Scylla serrata* (Hudson & Shields 1994) and the coral crab *Trapezia aerolata* (Hudson et al. 1993). A syndinid dinoflagellate similar to *H. perezi* has been reported in 13 species of benthic amphipods, with prevalences as high as 67 % (Johnson 1986). Up to 18 % of spot prawns *Pandalus platyceros* from British Columbia have been reported with a *Hematodinium*-like protozoan (Bower et al. 1993).

A blue crab disease survey was initiated in late summer 1992, following reports of reduced catches and mortality of trapped crabs from coastal bays near Ocean City, Maryland, and Franklin City, Virginia. This note extends the reported size range for blue crabs infected by *Hematodinium perezi*, provides information on increased seasonal prevalences in blue crab populations, and extends the geographic range of the parasite to include coastal bays in Maryland and Virginia.

Materials and methods. Monthly samples of live and dead adult [90 to 180 mm carapace width (CW)], juvenile (30 to 89 mm CW), and early-juvenile (5 to 29 mm CW) blue crabs were collected using both commercial traps and an otter trawl in coastal bays near Ocean City, Maryland, and Franklin City, Virginia. Carapace width was measured from point to point, and sex was recorded; pre-molt and post-molt crabs were noted when molt stage was apparent. Crabs were bled from

the hemal sinus at the joint between the thorax and the 5th pleopod (swimmer fin) using a 1 cc insulin syringe equipped with a 28-gauge needle. Expressed cells were allowed to adhere to an acid-cleaned, 0.1 % w/v poly-L-lysine-coated microscope slide. Cells were observed live using an inverted microscope with Hoffman modulation or phase contrast optics, before fixation in Bouin's fluid and staining with Mayer's hematoxylin and eosin (H&E) (Luna 1968). Selected crabs were fixed whole, or after dissection, in Bouin's fluid, and processed for histologic examination by standard methods (Johnson 1980, Howard & Smith 1983). The parasitic dinoflagellate encountered in this survey was identified as *Hematodinium perezi,* based on morphologic similarities with *H. perezi* in stained preparations deposited at this facility by Newman & Johnson (1975). Characteristic similarities to *H. perezi* noted by Newman & Johnson (1975) to the dinoflagellate described by Chatton & Poisson (1931) which were identified in this study include plasmodial forms, dinokaryon-type nuclei arranged in V-shapes, absence of a nuclear membrane, and apparent continuous mitotic activity in the nucleus.

A technique modified from Field et al. (1992) was used to detect *Hematodinium perezi* infections in several live, early-juvenile blue crabs. Crabs were positioned on the stage of an inverted microscope to allow the flattened portion of the 5th pleopod to lie flat on a microscope slide or petri dish. Light transmitted through the exoskeleton illuminated internal tissues, allowing visualization of hemocytes and parasites. The reliability of this modified Field et al. (1992) technique (MFT) was verified by examining fixed, stained hemolymph and leg tissue from the same crabs for the presence of dinoflagellate parasites. The MFT for diagnosing *H. perezi* infections in early-juvenile crabs avoided invasion of infected individuals and allowed continuous monitoring of the parasite *in vivo.*

Results. *Hematodinium perezi* infection prevalence in blue crabs followed the seasonal trend reported by

Table 1. *Hematodinium perezi* infections in adult (90 to 180 mm), juvenile (30 to 89 mm), and early-juvenile (5 to 29 mm) blue crabs *Callinectes sapidus* from coastal bays in Maryland (MD) and Virginia (VA) from August 1992 to November 1993. CW: carapace width. Combined sample prevalence is prevalence in all size crabs during each survey sample date. na: not available

Sample date	Sample site	Size range (mm CW)	No. of crabs examined	Infection prevalence (%)	Combined sample prevalence	Temp. (°C)	Salinity (ppt)
August 1992	MD	130–145	9	44		23	22
October 1992	VA	110–155	13	77			
	VA	7–27	17	100	90	16	32
November 1992	MD	8–26	32	59		10	24
December 1992	MD	7–17	13	38			
	MD	36–53	2	100			
	MD	90–160	8	37	43	7	24
January 1993	MD	5–18	37	16			
	MD	130–165	13	15	16	4	22
March 1993	MD	12–86	84	0		5	24
April 1993	MD	8–45	100	0		na	19
July 1993	MD	45–89	25	4			
		90–180	13	15	8	26	26
29 July 1993	MD	30–89	48	10			
		90–180	44	9	10	26	29
August 1993	MD	5–29	4	74			
		30–89	86	43			
		90–180	81	32	39	26	27
September 1993	MD	5–29	56	83			
		30–89	72	49			
		90–180	39	44	57	23	30
October 1993	VA	5–29	20	95		17	30
October 1993	MD	5–29	76	82			
		30–89	41	49			
		90–180	46	37	60	17	30
November 1993	MD	5–29	1	100			
		30–89	53	75			
		90–180	40	60	69	15	24

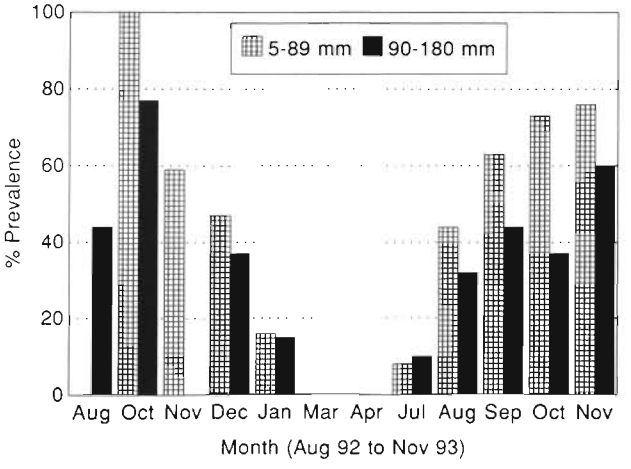

Fig. 1. *Hematodinium perezi* infecting *Callinectes sapidus*. Comparison of percent prevalence in blue crabs with carapace width 5 to 89 mm and 90 to 180 mm from coastal bays of Maryland and Virginia, August 1992 to November 1993. Months omitted were not surveyed

Newman & Johnson (1975), with the highest prevalence observed during the period of August through November (Fig. 1, Table 1). Prevalences were higher in small crabs (5 to 89 mm), than in larger crabs (90 to 180 mm) (Fig. 1) collected at salinities from 19 to 32 ppt and temperatures from 4 to 26 °C (Table 1). Limited numbers of obvious pre-molt and post-molt crabs were captured, and smaller crabs were difficult to stage. These limited data showed 1 of 5 (20 %) pre-molt and 3 of 8 (37.5 %) post-molt crabs were infected with *H. perezi*. There was no difference in prevalence of *H. perezi* infection between male and female crabs.

Occasional gross signs of *Hematodinium perezi* infection included sluggishness, opaque muscles seen through the ventral carapace, or a pink carapace. Blood removed from some severely infected crabs appeared opaque, while gills and other tissues were occasionally pink. However, most infected crabs appeared normal.

Several morphological forms of the parasite were observed. Uninucleate cells with dinokaryon-type nuclear morphology (Chatton & Poisson 1931) were most common; occasional dividing cells and multinucleate cells having prominent chromatin and no apparent nuclear membrane were also observed (Fig. 2A). Some multinucleate cells were elongate (Fig. 2B) with amoeboid motility when observed live. Other parasites had hyperchromatic, dense nuclei rather than typical dinokaryon-type nuclear morphology (Fig. 2B). The average diameter of fixed and stained uninucleate parasites in blood smears was 10.5 µm. No flagellated dinospores were observed during this study. One crab was infected with *Paramoeba perniciosa*, 2 others by a ciliate in the hemolymph, and several others by a microsporidan parasite in addition to *H. perezi*.

Severely infected crabs had reduced hemocyte numbers, with apparent replacement by *Hematodinium perezi*. Host response to the parasitic dinoflagellate included formation of hemocytic nodules in hemal spaces (Fig. 3). Pathology associated with infection included vacuolization and lysis of hepatopancreatic tubule epithelia, and degeneration of muscle cells. Using the MFT, severely infected animals showed virtually no flow of hemocytes, and hemal sinuses appeared occluded by the multitude of parasites (Fig. 4).

Discussion. This report is the first documentation of *Hematodinium perezi* in early-juvenile blue crabs. Newman & Johnson (1975) reported *H. perezi* in crabs from 70 to 170 mm CW. The highest prevalence reported by them was 30 % in one sample from Florida. This report shows a prevalence as high as 100 % in one sample of early-juvenile crabs (Table 1).

Although additional data are necessary to validate the observation, preliminary results suggest a higher prevalence of *Hematodinium perezi* infections in post-molt crabs than in pre-molt crabs. Other investigators have noted that recently-molted Tanner crabs are more likely to harbor dinoflagellate infections than pre-molt crabs (Meyers et al. 1990, Eaton et al. 1991). Molting was hypothesized to predispose crabs to invasion by the infectious stage of the parasite, by allowing entry through interruptions in the integument (Meyers et al. 1990). A similar portal of entry was proposed by Couch & Martin (1979) for the blue-crab pathogenic amoeba *Paramoeba perniciosa*. These authors suggested host defense mechanisms may be compromised by ecdysis, rendering new-shell crabs more susceptible to invading organisms.

The seasonal increase in prevalence observed in blue crabs from August through November during this study is consistent with seasonal prevalences reported by Newman & Johnson (1975) and is similar to the seasonal increase in prevalence reported in *Chionoecetes bairdi* from southeast Alaskan waters during summer months (Eaton et al. 1991). Eaton et al. (1991) suggested that seasonality was due either to environmental factors, such as salinity, temperature, or photoperiods, which may control the life cycle of the parasite, or to the possibility that summer mortality of infected crabs was so high that by fall and winter only uninfected and lightly infected crabs remained to be sampled, thus reducing prevalences. The seasonal variation in prevalence, and the inverse relationship between parasite prevalence and crab size observed during this survey, may reflect processes suggested by other authors. The higher infection prevalences for frequently molting juvenile crabs and the seasonal prevalence cycle noted in this study are consistent with etio-

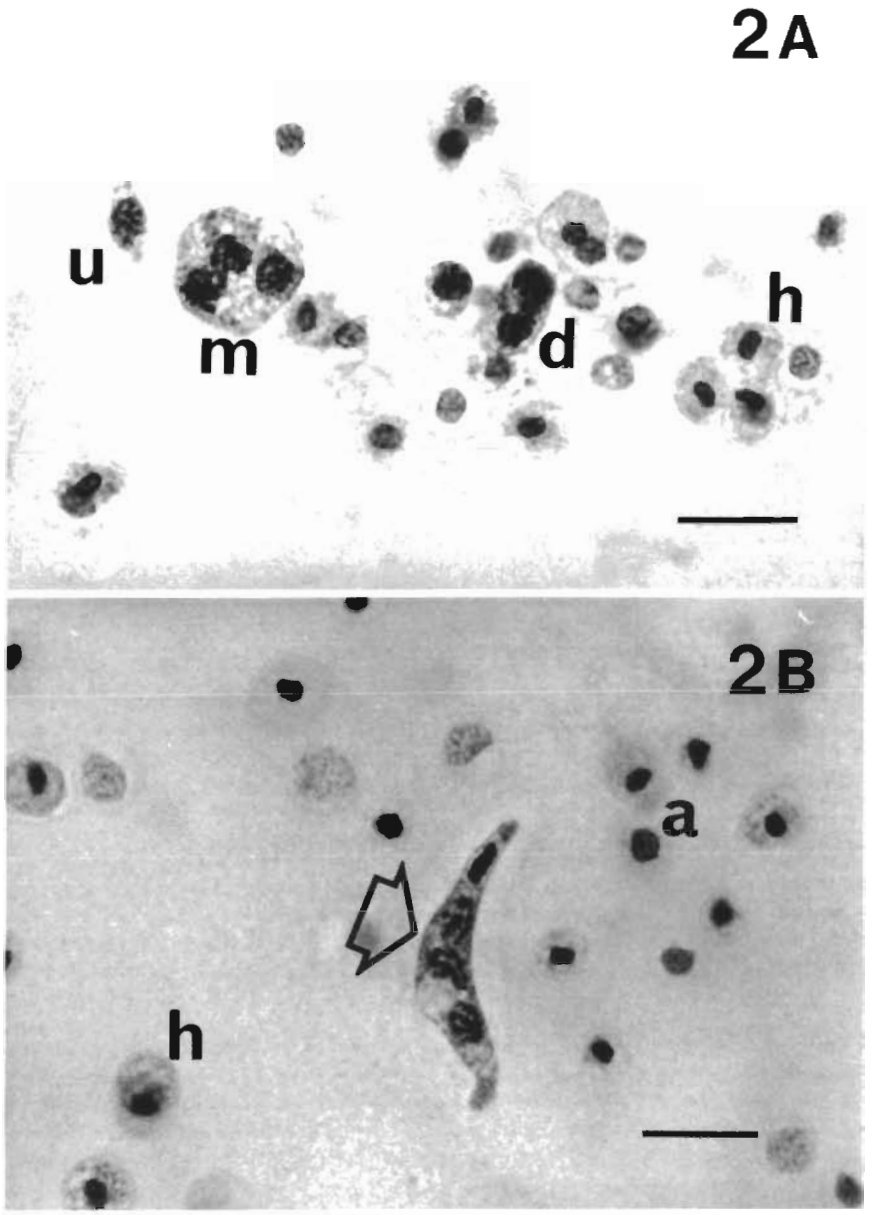

Fig. 2. *Hematodinium perezi* infecting *Callinectes sapidus* as seen in hemolymph smear preparations stained with H&E. (A) Uninucleate (u), multinucleate (m), and dividing (d) parasites associated with crab hemocytes (h). Scale bar = 20 µm. (B) Multinucleate elongate parasite (arrow), dense, hyperchromatic parasite (a), and crab hemocyte (h). Scale bar = 20 µm.

logical hypotheses suggested by other authors which couple infection acquisition and seasonal prevalence to molting and environmental control of the parasite life cycle.

Hematodinium perezi is hypothesized to cause mortality in blue crabs due to systemic proliferation and replacement of hemocytes (Couch & Martin 1979). It is uncertain what parasite characteristics or numbers are required to compromise effective crab defense response, or what cellular and molecular alterations occur in host tissues to cause mortality. Studies are in progress to investigate the virulence, distribution, and impact of *H. perezi* on crab populations from coastal bays of Maryland and Virginia.

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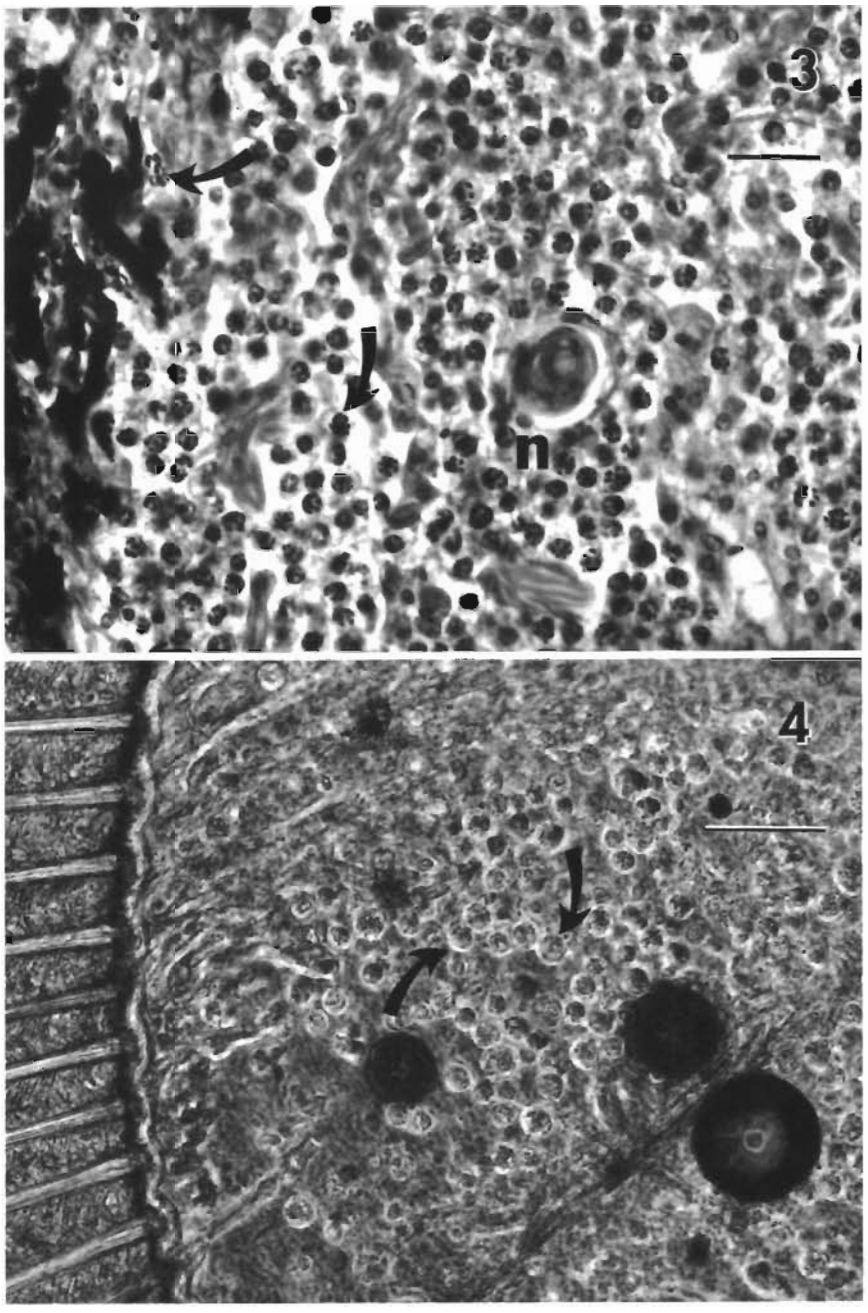

Figs. 3 & 4. *Hematodinium perezi* infecting *Callinectes sapidus*. Fig. 3. H&E-stained histological section showing many parasites in epidermal epithelium and subdermal spongy connective tissues (arrows). n: hemocytic nodule formation. Scale bar = 50 µm. Fig. 4. Phase contrast photomicrograph of flattened portion of 5th pleopod of early-juvenile crab using modified Field et al. (1992) technique showing aggregated parasites (arrows) in hemal sinuses. Scale bar = 50 µm

suggestions to this manuscript. Reference to trade names does not imply endorsement by the National Marine Fisheries Service.

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