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Natural transmission of *Hematodinium perezi* in juvenile blue crabs (*Callinectes sapidus*) in the laboratory

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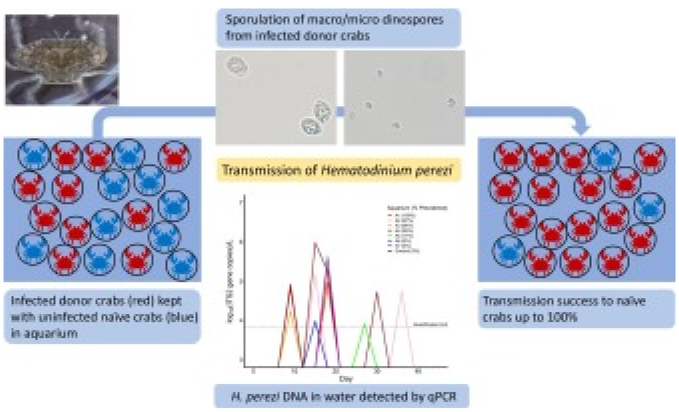
Highlights

- Natural water-borne transmission of *Hematodinium perezi* was achieved in the laboratory.
- Transmission success in juvenile blue crabs was highly variable.
- More than half of infected naïve crabs had heavy infections by day 30.
- Mortality of infected donor crabs was associated release of *H. perezi* into the water.

Abstract

Hematodinium perezi is a dinoflagellate endoparasitic in marine crustaceans, primarily decapods. It occurs in juvenile blue crabs, *Callinectes sapidus*, at high prevalence levels and has severe pathogenic effects in this host. The life history outside the host has not been experimentally investigated and, until now, transmission using dinospores has not been successful. We investigated the natural transmission dynamics of *H. perezi* in the laboratory using small juvenile crabs, which are highly susceptible to infection in the field, and elevated temperatures, which are known to stimulate dinospore production. Natural water-borne transmission to naïve crabs varied between 7 and 100% and was not correlated with dinospore densities measured from their aquaria water. Infections appeared to develop quickly in naïve hosts at 25 °C, suggesting that elevated temperatures as seen in the late summer and early autumn have a strong influence on the transmission of *H. perezi* in natural systems.

Graphical abstract



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Introduction

Hematodinium species are parasitic dinoflagellates that infect more than 40 species of crustaceans, all of which are found in the hemolymph of the host (Stentiford and Shields, 2005, Small, 2012). The type species, *H. perezi* was first described from *Carcinus maenas* and *Liocarcinus depurator* in France (Chatton and Poisson, 1931). It was first reported in blue crabs *Callinectes sapidus* from North Carolina, Georgia, and Florida as *Hematodinium* sp. by Newman and Johnson (1975) and then reported in other states, ranging from Delaware to Texas (Messick, 1994, Messick and Shields, 2000). Molecular analysis of the ITS1 region of *H. perezi* shows that three genotypes of this species infect disparate crustacean hosts from different geographic regions. Genotype I is found in decapods from northern European waters, genotype II from portunids from China, and genotype III from blue crabs and other crustaceans from the eastern United States of America (Small, 2012).

Hematodinium perezi is an obligate parasite and obtains nutrition from the host through osmotrophy (Shields, 1994, Shields et al., 2003). It is highly pathogenic in adult blue crabs, with 100% mortality in naturally infected crabs held for 35 days (Messick and Shields, 2000) and 86% mortality in experimentally infected crabs held for 40 days (Shields and Squyars, 2000). The mortality is associated with glycogen depletion (Shields et al., 2003), organ malfunction, and respiratory dysfunction (Field et al., 1992; Shields and Squyars, 2000) resulting from proliferation of the parasite and its resulting burden on the host's metabolic processes. In juvenile blue crabs, progression of the parasite is strongly temperature dependent, leading to rapid and high mortality of infected crabs at 25 °C (Huchin-Mian et al., 2018).

Juvenile blue crabs are highly susceptible to *H. perezi* in high salinity areas (26–34 psu) and become infected shortly after settlement and recruitment (Messick and Shields, 2000, Small et al., 2019). The parasite has a bimodal prevalence in blue crabs, with a small peak in summer and a much higher peak in autumn (Messick, 1994, Messick and Shields, 2000, Sheppard et al., 2003). In sentinel studies, naïve crabs held in off-bottom cages deployed in endemic areas become infected within 3 days of deployment and heavy infections develop within 7 to 14 days (Huchin-Mian et al., 2017, Shields et al., 2017).

Few studies have focused on natural transmission of *H. perezi*, but there is strong circumstantial evidence that the dinospores are the transmissive stage (Frischer et al., 2006, Huchin-Mian et al., 2017, Shields et al., 2017). *In vitro* culture has shown that species of *Hematodinium* produce two types of dinospores (Li et al., 2011, Appleton and Vickerman, 1998), the micro-dinospore and the macro-dinospore, but their function remains unknown. In blue crabs, these dinospores are released from their adult hosts into the water in large quantities (up to 10^8 dinospores/ml hemolymph) (Shields and Squyars, 2000, Huchin-Mian et al., 2018). Sporulation leads to damage of the gill lamellae as the spores exit through the less sclerotized tissues, resulting in perforation of the gills, which is fatal in many host species (Meyers et al., 1987, Wheeler et al., 2007, Appleton and Vickerman, 1998). After sporulation, motile dinospores can be observed in aquaria for 3–5 days with a microscope and are detectable with qPCR for up to 7 days (Li et al., 2010). The DNA of free-living stages of *H. perezi* in the environment was first detected in water samples using real-time PCR by Frischer et al. (2006). This observation has since been verified in both water and sediment samples at densities ranging from 1 to 100+ cells/l using additional molecular primers with higher sensitivity (Li et al., 2010, Hanif et al., 2013, Lycett and Pitula, 2017). A recent cohabitation trial using infected mudflat crabs, *Helice tientsinensis* and healthy swimming crabs, *Portunus trituberculatus*, showed that naïve crabs can become infected with *H. perezi* (genotype II) when exposed to infected individuals, further supporting water-borne transmission (Huang et al., 2021).

Our goal is to understand the transmission dynamics of *H. perezi* to blue crabs. We undertook three objectives as part of this goal: 1) to determine if natural water-borne transmission can occur in a laboratory setting; 2) to assess spore densities in relation to infections using natural transmission from donor crabs; 3) to examine patterns in spore densities in water samples in relation to mortality of donor crabs.

Section snippets

Crab collection

Juvenile blue crabs<30mm carapace width (CW) were collected using dipnets from two locations in October 2020 and September 2021. Unexposed naïve crabs were collected from a non-endemic area, Mobjack Bay (37°18′N, 76°24′W), VA, a sub-estuary of Chesapeake Bay. Mobjack Bay has average salinities around 20 psu and has been used as a site for collecting naïve crabs in sentinel studies (Huchin-Mian et al., 2017, Shields et al., 2017). Infected donor crabs were collected from a hyper-endemic area, …

Results

Prevalence levels in the pre-sample of crabs from Cobb Bay were 80% (n=10) and 90% (n=10) in 2020 and 2021, respectively; hence, crabs from this area were used as infected “donor” crabs. Pre-samples of crabs (n=10 in each year) from Mobjack Bay were uninfected as expected and served as naïve crabs.…

Discussion

This study is the first report of natural transmission of *Hematodinium perezi* to blue crabs in the laboratory. Our results are in general agreement with the cohabitation trial done by Huang et al. (2021), but we further support our findings with a quantitative assessment of *H. perezi* DNA demonstrating direct, waterborne transmission. Although transmission was natural in our system, the resulting prevalence in naïve crabs varied widely between aquaria. Prevalence levels from natural transmission ...

Conclusions

Our findings on transmission dynamics of *H. perezi* in juvenile blue crabs confirm the notion that the parasite is transmitted via water-borne transmission of dinospores without an intermediate host. The high mortality of donor crabs and subsequent peaks of *H. perezi* DNA in the water help to explain the natural seasonality of prevalence observed in the Delmarva Peninsula. With alterations in the experimental design, natural transmission in the laboratory may provide an opportunity to further...

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper....

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