Short- and long-term strategies for managing and mitigating the effects of *Hematodinium* on host species

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Abstract

Bitter crab disease has had severe implications on the population of several decapod crustacean species worldwide, with many fisheries facing extreme loses as a direct result of the pathogen. The disease is caused by parasitic dinoflagellates from the genus *Hematodinium*, which interferes with the host's immune system, alters carapace pigmentation, gives the meat a bitter and unpalatable taste, and may ultimately lead to death. Despite these negative effects, little is known the biodiversity of this parasite and how to control it. Here, I have synthesized the current knowledge on the life history of *Hematodinium*, factors influencing host susceptibility, and the diagnostic tools used to identify infected individuals. Subsequently, I have also identified and proposed a few long-term studies to fill in the knowledge gaps need to inform management strategies. However, commercial fisheries are being affected by *Hematondinium* now, so I have also suggested some short-term strategies to help mitigate the effects of the parasite through modified fishing practices.

Introduction

Parasitic dinoflagellates of the genus *Hematodinium* cause potentially lethal infections in crustaceans (Meyers et al., 1987). They have been found in crabs from multiple regions of the world, including tanner and snow crabs (*Chionoecetes bairdi* and *C. opilo*, respectively) from the Gulf of Alaska, blue crab (*Callinectes sapidus*) from the eastern U.S., brown crab (*Cancer pagurus*) from the English Channel, and flower crab (*Portunus pelagicus*) from Australia (Stentiford & Shields, 2005). *Hematodinium* also infects many other crab species, lobsters, and amphipods worldwide. The pathological effect of the dinoflagellates are consistent across hosts and include, but are not limited to altering host pigmentation and causing an unpalatable flavor (Stentiford & Shields, 2005). The latter is a common problem with severe consequences on commercially valuable species, and is popularly known as "bitter crab disease" (Taylor & Khan, 1995; Meyers et al., 1996). The effects of the *Hematodinium* outbreaks are highly variable, but may lead to increased mortalities and the loss of marketable products. The disease infects a third of commercial *C. bairdi* populations in Southeast Alaska, and nearly decimated the velvet crab (*Necora puber*) fishery in France (Meyers et al., 1990; Wilhelm & Mialhe, 1996). The economic losses attributed to *Hematodinium* infections also vary and often exceed $500,000 per year for the blue crab fishery in Virginia during non-epidemic years, whereas in Scotland, the Norwegian lobster fishery loses £2-4 million annually to the parasite (Field et al., 1992; Messick & Shields, 2000). The negative effects of *Hematodinium* are clear, and this globally widespread disease warrants control and preemptive action to prevent future outbreaks.

Hematodinium *life cycle*

The majority of knowledge on the *Hematodinium* life cycle is derived from an *in vitro* study of *Hematodinium* found in Norwegian lobsters, *Nephrops norvegicus* (Appleton & Vickerman, 1998). The parasite has micro- and macro-dinospore stages, which germinate and become filamentous trophonts. These trophonts merge to form large Gorgonlock trophont colonies, which either become compact clump colonies that produce more filamentous trophonts, or transition into web-like arachnoid trophonts (Appleton & Vickerman, 1998). The latter may grow outward or fuse with other arachnoid trophonts, but soon develop into arachnoid sporonts that produce masses of sporoblasts. The sporoblasts can either become secondary arachnoid sporonts, similar to the initial sporonts, or in the presence of sufficient nutrients the sporoblast will undergo sporogony to generate new flagellated micro- or macro-dinospores (Appleton & Vickerman, 1998). The full cycle may ensue over the course of five weeks and occurs within the hemolymph of the host (Appleton & Vickerman, 1998).

Only partial life cycles of *Hematodinium* species found in crab hosts are known, but they strong provide evidence for interspecific variation in the life cycles of *Hematodinium*. For example, the Gorgonlock trophont stage has not been observed in *Hematodinium* parasites from the blue crab, *C. sapidus*, but rather, the filamentous trophonts undergo budding or fission to produce additional amoeboid trophonts. At some point (probably at high cell density), the amoeboid trophonts produce rounded trophonts that undergo sporogony and generate dinospores (Shields & Squyars, 2000). In contrast, other studies have found slightly more similarities in the life history stages of parasites from *C. bairdi* and *C. opilio* with ones from *N. norvegicus* (Meyers et al., 1987; Eaton et al., 1991; Appleton & Vickerman, 1998). The differences in parasite life history, particularly in *C. sapidus*, corroborates the idea of multiple undescribed

species, especially given that the *Hematodinium* from several hosts have not been thoroughly studied (Stentiford & Shields, 2005).

The process by which infections are transmitted is still not well known, but it is thought that the dinospores may be the primary infective stage, as in other parasitic dinoflagellates (Eaton et al., 1991; Stentiford & Shields, 2005; Frischer et al., 2006). In several hosts, sporulation is correlated with the release of dinospores through the gills of the infected host and into the water column (Love et al., 1993; Appleton & Vickerman, 1998; Shields & Squyars, 2000). Frischer et al. (2006) found evidence for waterborne transmission of *Hematodinium* for *C. sapidus*, probably through passive intake of free-living dinospores. It has not yet been determined how long free-living dinospores can survive before requiring a host, which may have implications related to the spread of the parasite. Additionally, other life stages, such as the trophonts, may be infectious and are often passed through trophic transmission (Meyers et al., 1987; Hudson & Shields, 1994; Shields & Squyars, 2000). Cannibalism is a common behavior in *C. sapidus* and *C. opilo* and may serve as an efficient mode of transmission (Sheppard et al., 2003). Alternatively, it has been speculated that infected amphipods, which are also common prey items for *Chionoecetes* spp. and *N. norvegicus*, may serve as paratenic or reservoir hosts, but further work is required to determine their role (Hudson & Shields, 1994).

Factors influencing host susceptibility

The susceptibility of a given host depends greatly on a variety of individual factors such as species-specific molt stage, size, and sex (Eaton et al., 1991; Field et al., 1992; Messick, 1994; Messick & Shields, 2000; Stentiford et al., 2001). Generally, newly molted hosts have a greater abundance of *Hematodinium* than do “old” shelled crab that have not molted within the past year

(Meyers et al., 1990; Eaton et al., 1991). In many of parasite–host systems, *Hematodinium* sporulation is intricately tied with the molting seasons of their host, with sporulation occurring right before or soon after, depending on the species, and causing a peak in infection prevalence (Field et al., 1992; Stentiford et al., 2001). However, in *C. sapidus* the peak infection time is entirely disjunct from the parasite sporulation, suggesting that other factors are at play (Messick, 1994). Infections are typically more abundant in juveniles or smaller individuals, probably because they undergo ecdysis and risk exposure to infection more frequently (Messick & Shields, 2000; Mullowney et al., 2011). This suggests that the parasite invade the juveniles through penetration of the cuticle when it is soft and vulnerable. Alternatively, the juveniles of several host species feed heavily on potentially infected amphipods and may obtain the infection through trophic transmission (Stentiford & Shields, 2005). Furthermore, the degree to which prevalence varies between sexes is highly dependent on the species, but those with more sexual dimorphic differences in size often show greater prevalence differences (Field et al., 1992; Stentiford et al., 2001).

The prevalence of *Hematodinium* infections exhibits strong seasonal fluctuations, but the patterns vary greatly from host to host. Temperature likely plays a large role as boreal species (*C. bairdi* and *C. opilio*) experience peaks in the summer or fall, respectively; while outbreaks in temperate species may occur in the fall (*C. sapidus*) or late winter and spring (*N. norvegicus*) (Meyers et al., 1990; Eaton et al., 1991; Love et al., 1993; Sheppard et al., 2003). Because warm temperature are also a determining factor for ecdysis, it is possible that sporulation and peak infection rates are directly tied to molting periods (Cadman & Weinstein, 1988). For example, peak infection rates in *N. norvegicus* are correlated with high proportions of recently molted and intermolt individuals, supporting the hypothesis that the parasite enters the host through

integument pathways (Stentiford et al., 2001). These seasonality differences provide additional evidence for the likelihood of undescribed taxonomic diversity among *Hematodinium* species. Despite the seasonal differences in prevalence, peak seasons are often followed by periods of extremely low prevalence or even undetectable levels of infection (Stentiford & Shields, 2005). While this is partially due to increased mortality of infected individuals, it raises questions about the where the parasite goes when it is not found in the host. Thus, it seems necessary to investigate the possibility of amphipods as a potential reservoir host, or even the possibility of a dormant cyst stage to better understand the life history of this parasite.

Pathology and diagnosis of Hematodinium infections

The pathological effects of *Hematodinium* infections include altered pigmentation, reduced hemocyte count, muscle degradation, and loss of digestive functions. A common feature of infection is the hyperpigmentation of the carapace accompanied by a "chalky" appearance that serves as the simplest diagnostic method (Stentiford & Shields, 2005). Hemocytes in a healthy host serve several functions including nutrient transport, wound repair, and defense against foreign agents. However, infected hosts exhibit a rapid decline in hemocytes and suffer from reduced functions (Shields & Squyars, 2000). The presence of *Hematodinium* itself has been found within the muscles of its hosts and can change the biochemical structure as well as the degrade the muscle fibers (Meyers et al., 1987; Messick, 1994). This has severe commercial implications because, once cooked, the altered muscle acquires a chalky texture and bitter taste – symptoms for the condition known as bitter crab disease. The filamentous trophont and sporont stages of the parasite are also found in close association with the hepatopancreas and compromise its integrity and ability to produce digestive enzymes (Appleton & Vickerman,

1998). A combination of these pathologies coupled with the flooding of the gills with dinospores post-sporulation, often cause the host to die through starvation and suffocation. To make matters worse, the immune system of several host species are unable to detect the presence of *Hematodinium* and fail to elicit any form of cell-mediated response (Rowley et al., 2015).

Currently there are several diagnostic tools for identifying *Hematodinium* infections, but none are universal with a 100% success rate. Visual diagnosis based on carapace discoloration is a widely used diagnostic tool in the field; however, color changes are less pronounced in low intensity infections and diagnostics effectively miss 48% of infections in *C. opilo* (Pestal et al., 2003). For *N. norvegicus*, removing a pleopod and examining it for the presence of parasites under low-light microscopy has been a successful tool for field studies (Field et al., 1992). Its success rate for identifying infections is 4% to 50% better than only using carapace discoloration, but is not a widely applicable tool due to anatomical differences between hosts. Alternatively, examining prepared hemolymph smears under a microscope is the most reliable and cost-efficient means of diagnosis (Meyers et al., 1987; Love et al., 1993; Hudson & Shields, 1994; Messick, 1994). However, to the uninitiated, it can be difficult to distinguish the parasite from hemocytes and contrast stains are not effective for all hosts (Stentiford & Shields, 2005). In recent years, PCR and sequencing techniques have been used to detect infections; while it is currently too costly to be used on a large scale, it prove to be useful when delineating *Hematodinium* taxonomy (Hudson & Adlard, 1996; Stentiford & Shields, 2005).

Due to the taxonomic ambiguity and wide geographic range of the parasite, we do not currently have the information needed to execute an eradication plan. Instead, I will propose a series of baseline research studies that will close important knowledge gaps and provide sufficient information to control *Hematodinium* outbreaks. These initiatives include: 1) a

comprehensive taxonomic survey of *Hematodinium*, 2) an investigation of the variation in *Hematodinium* life cycles both *in vitro* and *in situ*, 3) an assessment of amphipods as potential reservoir hosts, and 4) discovering a new diagnostic method to identify low-level infections earlier and more efficiently. However, these studies will produce benefits only in the long-term; in order to provide options for immediate relief of *Hematodinium* impact on fisheries, I also recommend several fishery practices that can be implemented now. These strategies include: 1) altering the timing of fishing seasons, 2) targeting specific sexes or age classes, and 3) preemptively removing infected crabs from the system to avoid large outbreaks.

Long-term initiatives

Our inability to prevent outbreaks is partially inhibited by the taxonomic uncertainty associated with the parasite and the lack of species-specific information. Different parasite species may have distinct interactions with their hosts that may affect the timing of peak infection rates or transmission pathways (Eaton et al., 1991; Messick, 1994; Stentiford et al., 2001). Implementing efficient management strategies that target these interactions requires a comprehensive understanding of the hosts and an equal understanding of the parasites. For example, if the effects of a single parasite species are temporally similar across host species then we might be able to develop interventions that target specific parasite species rather than each case by case parasite—host system.

Therefore, the first necessary step towards controlling *Hematodinium* is to conduct a global genomic survey of the parasite. It is unknown whether the genus *Hematodinium* contains a few, widely distributed generalist species with low host specificity, or whether it contains several species with high host specificity (Stentiford & Shields, 2005). For example, the

Hematodinium species for which we have the most information on, is found in *N. norvegicus*, but we have no idea about what its other host species are (Appleton & Vickerman, 1998; Stentiford et al., 2001). To address this, I suggest that *Hematodinium* from every known host species should be sampled and compared. Currently, there are only two recognized species of *Hematodinium* (*H. perezi* and *H. australis*), but neither are the ones found in *N. norvegicus* and several more have also been proposed based on life history differences (Stentiford & Shields, 2005). It would be in our interest to describe new species in order to delineate which parasites are found in which hosts. Because dinoflagellates are morphologically cryptic and possess multiple life stages that vary in appearance, genetic data will be the primary tool used to distinguish species (Hamish J. Small et al., 2012).

Genomic techniques have already been implemented to detect the presence of *Hematodinium* and to identify distinct clades (Jensen et al., 2010; Hamish J. Small et al., 2012). The 18S ribosomal DNA and adjacent ITS1 region have proved to be useful DNA regions to target within host hemocoel to detect *Hematodinium* (Jensen et al., 2010). Many gene sequences from previous studies have already been published through peer-reviewed journals or online databases that should be compiled and used to produce preliminary phylogenetic hypotheses (Clark et al., 2016). However, our own genetic data needs to be collected to determine whether hosts populations can be infected with multiple *Hematodinium* species, with commercially relevant host species holding the highest priority. To minimize costs, I propose that already infected fishery species should be retained for sampling rather than be discarded. Obtaining non-commercially relevant specimens may be more difficult and costly, but remain necessary if we are to full understand the extent of *Hematodinium* diversity.

Once we have a better understanding about the diversity of parasite species, species-specific data needs to be collected on the life cycles of each species using lab studies. This phase of the initiative would primarily focus on commercially relevant parasite—host systems to identify the infectious life stages of each parasite and how they are acquired by the hosts. Although it would be reasonable to expect the life cycles of various *Hematodinium* species to be similar, there has been life stages found in some species, but not in others (Shields & Squyars, 2000). This is significant because it may affect how the parasites are transmitted between hosts (i.e. integuments pathways, passive consumption, or trophic transmission). I propose a series of studies similar to that of Appleton and Vickerman (1996) and Shields and Squyars (2000), in which the parasites were extracted from live hosts, cultured, and used to infect new hosts in the lab. Transmission studies will help determine whether intervention can help prevent the parasites from entering the host or whether efforts are best spent mitigating the effects. For example, if the parasite is consumed accidently from the water column, it may be nearly impossible prevent infection, but if trophic transmission plays a key role then that interaction may be disrupted.

Additional studies should also be conducted to continue investigating the modes of *Hematodinium* transmission, particularly in relation to potential reservoir hosts. This parasite is capable of infecting amphipods, but the parasite's life cycle within them has yet to be determined. Infections can be obtained through trophic transmission, and considering how many of host species feed on amphipods in large quantities, it is a worthwhile endeavor to investigate (Stentiford & Shields, 2005; H. J. Small et al., 2006; Li et al., 2011). Additionally, minimal *Hematodinium* infections have been found in decapod crustacean hosts during cold seasons, that suggests it is being harbored elsewhere (Stentiford & Shields, 2005). If the results show that amphipods do indeed play an important role in fostering the parasite in the winter and later

facilitating the transmission of the parasite to decapods, then reducing the local amphipod populations through a biocontrol may be a viable management strategy for controlling the parasite. This would be done by introducing a non-crustacean biocontrol agent to prey on local amphipods during the winter so that fewer crabs and lobsters are infected come spring time. However, we need to first be certain that this trophic link exists, then conduct an extensive review to identify a potential agent that will have minimal unintended impacts on the ecosystem.

To mitigate the effects of *Hematodinium* infections through modified fishing practices, we must first assess seasonal variation and host demographics. Field studies serve as an essential tool for identifying differences in prevalence, abundance, and the intensity of infections. Because fishery catch data is primarily skewed towards adult males, data on female and juvenile infections are lacking and many parasite-related deaths go undetected (Dawe et al., 2001). Additionally, the fishing seasons often coincide with the parasite sporulation and peak infection rates, and contributes to the skewed sampling (Stentiford & Shields, 2005). For some host species, the seasonal infection trends have already been mapped out, while in others, the proportion of infected individuals for each sex and age class have been determined (Eaton et al., 1991; Meyers et al., 1996; Stentiford et al., 2001). However, we lack both types of data for most host species, but this can be rectified through field sampling. This would require extensive and consistent periodic sampling of host species, but assuming we have both types of data we could alter fishing practices. For example, if we find that males are more likely to be infected during peak infections periods, we might be able to preemptively fish the host species during the window of time after their spawning events, but before parasite infections become more prevalent.

However, a major caveat of increased sampling efforts is our ability to identify and diagnose early stage and juvenile infections (Stentiford & Shields, 2005). The ideal diagnostic tool would be cheap, easy to use, widely applicable, and with 100% success rate; however, no such method has been discovered. I suggest investigating the potential for using metabarcoding to detect the presence of *Hematodinium* out in the field and in commercial processing plants. In recent years, metabarcoding combined with next-generation sequencing have enhanced our ability to rapidly detect the presence of a given species within an entire community (Taberlet et al., 2012). If we strategically collect hemocoel samples during field surveys, we might able to detect not only the presence of *Hematodinium*, but also determine the relative proportion of parasite DNA to host DNA as a proxy for measuring infection prevalence or abundance. Metabarcoding may also help to identify infected batches of commercially caught crustaceans. This would be extremely beneficial for reducing the amount of spoiled catch as it would allow for infected batches to be scrutinized more intensely before processing. However, metabarcoding does not come without its own flaws, as it is also subjected to missing or losing genetic information (Taberlet et al., 2012). Additionally, while metabarcoding may prove to be useful for field studies where timing is not absolutely critical, it is not applicable for diagnosing individuals on fishing boats, as it still requires DNA processing to be outsourced. Although, with the rising interests in technology that allows for portable, real-time sequencing, it may not be long before on-board detection of *Hematodinium* becomes possible.

Proposed fishery practices

While the proposed long-term initiatives are meant to fill knowledge gaps, annual loses due to *Hematodinium* create the need for immediate, short term management plans. Based on the

current knowledge of *Hematodinium*, we can begin to propose changes in fishing practices that will help mitigate the effects of the parasite. The first proposed change is to fish earlier in the season, prior to peak infection periods. For example, the parasites within *Chionoecetes bairdi* and *Chionoecetes opilio* sporulate during the summer and fall, respectively, so fishing in the spring and summer, respectively, might help reduce the number of infected individuals in the population and thereby reduce transmission (Love et al., 1993; Meyers et al., 1996). It might be argued that doing so may result in smaller catches and lower profits, but marketable catch may increase overall and outweigh the accumulated losses attributed to *Hematodinium*. As we improve our understanding of the seasonal fluctuations between parasite—host systems, we can refine each optimal fishing window on a per system basis. Additionally, information on the sexual differences in infection rates, as well as age differences, might alter which proportion of the population should be targeted. Currently, retaining female crabs is often prohibited to ensure the population's survival; however, in populations where females are more likely to be infected with *Hematodinium* and castrated or die, it might be more beneficial to fish females (Kuris & Lafferty, 1992). This prompts the second proposed change of implementing additional management policies that take into account the fact that some parasites prefer one sex over the other and removal of that sex has the potential to control the parasite population (Kuris & Lafferty, 1992). While *Hematodinium* does not directly castrate females, it does limit hemocyte and digestive enzyme functions, which may lead to reduced overall fecundity (Stentiford & Shields, 2005).

The third proposed strategy focuses on culling and removing infected individuals from the system. Today, caught infected individuals and potentially infected bycatch, including non-targeted sexes and age classes, are discarded back into the water (Dawe et al., 2001). Doing so

indirectly promotes the spread of the parasite as it provides more opportunities for it to sporulate and infect other individuals. Instead of releasing infected individuals back in to the system, they should be removed altogether. Some studies have proposed using infected individuals as terrestrial fertilizer to ensure that they cannot infect others, but are also returned to the ecosystem (Stentiford & Shields, 2005). The question becomes whether populations should be removed through active searching and removal of infected individuals or only on a per catch basis. It is highly dependent on whether infected individuals are able to reproduce before infection related mortalities occur as well as the population's resilience against an increase in juvenile mortalities. Maintaining a sufficient number of fertile females is also of concern, so active removal of females may not be advantageous; however, retaining infected females have been shown to lower infection rates (Kuris & Lafferty, 1992). Moreover, our ability to assess the situation requires additional species-specific knowledge and improved diagnostic capabilities. It is apparent that as we learn more about *Hematodinium*, we will be able to better inform our management practices and mitigate its negative impact on commercially important species.

Conclusions

Hematodinium is a parasite that negatively impacts crustacean fisheries and warrants control. While there is a fair amount of published literature on the parasite, there are still several areas that require further investigation to help inform intervention strategies. I proposed several potential studies including a broad taxonomic survey of *Hematodinium* to help delineate parasite species and provide context for additional species-specific studies. Subsequently, each species of *Hematodinium* should be investigated *in vitro* and *in situ* to determine the modes of transmission, host specificity, and seasonal prevalences. We are also currently limited by our ability to identify

infected hosts, but with the advancements in metabarcoding, it may prove to be a useful diagnostic tool. Furthermore, I have also proposed a few short-term management strategies that involve altering fishing practices and could be refined later, once we have accumulated additional data. There is still so much more to learn about *Hematodinium*, and a long way to go before we can implement a robust management strategy.

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