Bitter Crab Syndrome: A Major Player in the Global Theater of Marine Crustacean Disease

by
Frank Morado

Bitter Crab Syndrome (BCS) is a fatal disease of crustaceans that is caused by a parasitic dinoflagellate of the genus *Hematodinium*. To date nearly thirty species of crustaceans are known to be infected by *Hematodinium* spp. world-wide, and the large majority of parasitized hosts are located in the North Pacific and Atlantic Oceans (Fig. 1). Affected species include several commercially important decapod species such as the snow crab (*Chionoecetes opilio*) from the Gulf of Alaska, the Bering and Chukchi Seas, eastern Canada and Greenland; Tanner crab (*C. bairdi*) from Southeast Alaska, the Gulf of Alaska, and Bering Sea; the grooved Tanner crab (*C. tanneri*) from western Vancouver Island; the blue crab (*Callinectes sapidus*) from the eastern U.S. seaboard and Gulf of Mexico; the Norway lobster (*Nephrops norvegicus*) from the North and Baltic Seas and North Atlantic Ocean; and the velvet (*Necora puber*) and edible (*Cancer pagurus*) crabs from the North Atlantic Ocean. The disease in the edible crab is generally known as Pink Crab Disease, but by whatever common name *Hematodinium*-associated infections are known, the presented infection pattern is similar. The data indicate that while infections may be common in commercial size animals, the disease is more frequent in pre-recruits. Yet the impact of *Hematodinium*-associated diseases on commercial crustacean populations has not been adequately determined. The remainder of affected crustacean species, though not necessarily economically important, play important ecological roles in benthic and intertidal environments.

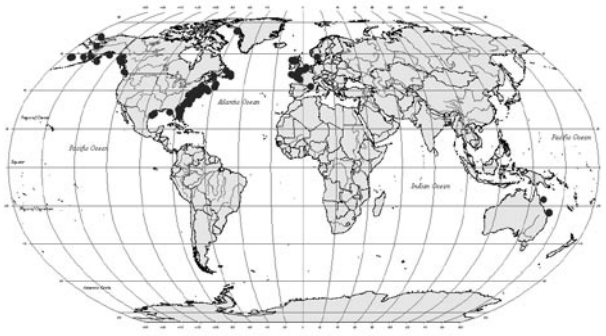

Figure 1. Worldwide occurrence of *Hematodinium* spp. Black dots denote reported cases.

The type species of the parasitic dinoflagellate, *Hematodinium perezi*, was described from France in *Leocarcinus depurator* and *Carcinus maenus* in 1931. In 1975, a similar parasitic dinoflagellate was encountered in the blue crab from several North Carolina, Georgia, and Florida estuaries. *Hematodinium* spp. was subsequently reported in Southeast Alaska Tanner crabs in 1987. This later report was significant because it was the first instance where prevelances approaching 100% were encountered, indicating that a disease could significantly affect abundance and distribution patterns of a crustacean population. In addition, the impact on product quality was first recognized and became the basis for the common name of the disease. During this encounter, it was estimated that 175,000 pounds of live Tanner crabs purchased by local Southeast Alaska processors were unfit for human consumption because of a markedly bitter aftertaste.

The life history of *Hematodinium* is not entirely known. Within an infected crab, the most common life history stages encountered are trophonts and pre-spores. A trophont is a vegetative stage that reproduces slowly by simple division and which possess a foamy cytoplasm. It is in this parasite stage that the typical dinokaryon nucleus, which is diagnostic of a dinoflagellate, is evident. A pre-spore is a smaller more compact stage that undergoes more rapid division and does not possess a foamy cytoplasm. Depending upon the host species, macro- and micro-dinospores may also develop which eventually escape from the infected host into the surrounding water. Both dinospore types possess two flagella in the typical dinoflagellate fashion: the longitudinal flagellum extends towards the posterior, while the transverse flagellum forms a

lateral circle at mid-body. What occurs from this point on until other hosts become infected is unknown. As a result, the number and morphology of free-living stages of *Hematodinium* is unknown, the method of infection has not been determined, and whether tissue tropism exists with respect to early infection is unanswered. This uncertainty exists for all *Hematodinium*-like infections.

Once a crustacean decapod becomes infected, trophonts (Fig. 2) reproduce to spread throughout the body of the host. Why decapod hemocytes (which have a similar function as white blood cells do in humans) are unable to recognize the invader is unknown, but as the disease progresses, host hemocytes are removed from circulation. The method of hemocyte removal is unknown, but it is unlikely that parasite phagocytosis plays a role. Rather, host hemocytes are most likely passively removed from circulation by the release of uncharacterized parasite metabolites that cause hemocyte lysis. The parasite continues to proliferate throughout the body of the host, eventually filling all spaces formerly occupied by host hemocytes. Proliferation of the parasite is such that parasite densities will significantly exceed hemocyte densities of a normal uninfected decapod. This results in another characteristic feature of BCS which is the presence of milky and opaque hemolymph in late stages of the disease. It is during this period that the external features of the disease such as a chalky abdomen or cooked appearance of the crustacean become apparent. With further disease progression, pre-spores arise from trophonts and will eventually develop into either micro- or macrodinospores, but only one type of dinospore develops within an infected host. Curiously, dinospores have not been observed within infected snow and Tanner crabs from the eastern Bering Sea, but both dinospore types have been observed in Southeast Alaska Tanner crabs. Observations and limited experiments do not support the original notion that dinospores are gametes, although research is currently under way to determine whether they are diploid or haploid. Release of dinospores into the surrounding water is believed to occur when marked changes in hemolymph chemistry cause general lysis or rupture of host tissues.

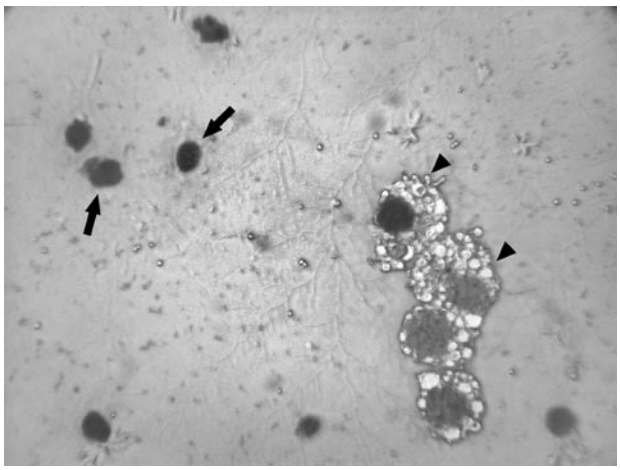

Figure 2. Trophonts (arrowheads) and host hemocytes (arrows) in a bloodsmear.

From a pathology perspective, trophonts and pre-spores may be observed throughout the body of an infected decapod. In early or moderately advanced stages of the disease, little change may occur in host tissues. However, in late stages of the disease, trophonts and pre-spores may occlude vascular pathways and are easily visible in the connective tissues throughout the body of the infected decapod. Within the gills of late-stage infected crabs (Fig. 3), the epithelium becomes attenuated, and pillar cells, which are critical for structural integrity, are destroyed, leading to increased dilation of the gill lamellae. This pattern of tissue change is consistent throughout an infected host.

Despite the fact that neither trophonts or prespores appear to be capable of perforating basement membranes and epithelia, general epithelial tissue changes occur and include increased vacuolization and necrosis. In essence, the parasite outcompetes the host for its own nutrients, leading to the general decrease in energy reserves and increased lethargy. These observations indicate that because of reduced fitness, molting mortality could be significant in BCS-infected decapods, especially in pre-recruits.

The Alaska Fisheries Science Center's Fisheries Resources Pathobiology program has been monitoring BCS in eastern Bering Sea snow and Tanner crabs since 1988. Disease monitoring was initially dependent upon microscopic analysis of bloodsmears, but the program recently developed a conventional PCR- (polymerase chain reaction) based protocol that is more rapid and sensitive. PCR is a laboratory technique to greatly amplify a specific region of DNA that can be used, for example, in diagnostic assays and DNA sequencing. Since the program's monitoring began, annual prevalences of infection have varied from less than 1% to more than 20% (Fig. 4), with overall prevalences at about 3.5%. Closer examination of prevalences indicates that infections are more common in small crab less

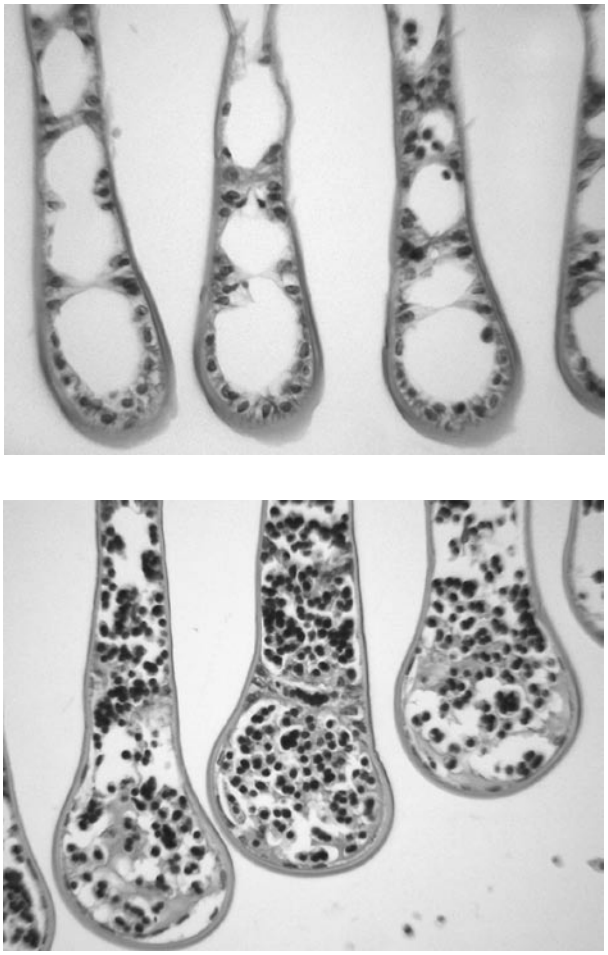

Figure 3. Normal (top) and infected (bottom) gills of snow crabs. Note the robust epithelium underlying the chitin covering of the gill, pillar cells, and a few hemocytes in the normal gill. Infected gills are occluded by the proliferating parasite, the epithelium is attenuated, and the lamellae are slightly dilated.

than 50-mm carapace width, and no significant differences exist between males and females (Fig. 5). As a result, the data suggest that BCS could affect recruitment of both snow and Tanner crabs. Similar disease/size relationships have been observed in other commercially important decapods such as the Norway lobster and the blue crab.

In Southeast Alaska, several embayments are known to harbor prevalences of BCS in Tanner crabs that exceed 40% and may approach 100%. In general, these areas are well known by commercial fishers and avoided. Unfortunately, because assessment surveys have not been routinely conducted in these areas, the impact of BCS on affected populations cannot be determined.

In the Bering Sea, BCS does not appear to be a concern to the industry as prevalences in legal snow and Tanner crabs are low. However, BCS is present in small crabs throughout much of the distribution range of snow crab (Fig. 6). The overall distribution of BCS is such that snow crab prevalences increase with increase in latitudes, attaining remarkable prevelances (>30%) in Norton Sound and the Chukchi Sea. In association with the general increase in prevalence with increase in latitude, a greater proportion of sampled stations are BCS-positive in higher latitudes. This is in sharp contrast to the distribution of BCS in Tanner crabs. In the mid- to early 1990s a few Tanner crab BCS-positive stations were encountered in Bristol Bay, but since that time, Tanner crab BCS-positive stations have been along the shelf edge. This apparent segregation of BCS in snow and Tanner crabs is unexplained considering that only one parasite species is believed to infect both crab species while the distribution ranges of both host species overlap considerably.

Currently, only two species of *Hematodinium* (*H. perezi* and *H. australis* from Austalia) have been morphologically described, although more are suspected. DNA sequencing of two loci indicates that at least two parasite species or clades exist in the North Pacific and Atlantic Oceans—the species type *H. perezi* and an undescribed species. The relatedness of *H. australis* is uncertain because its DNA has not been sequenced. Apparent differences in life history, virulence, and ecology of *Hematodinium* appear to exist, suggesting that a complex of similar but different parasite species or strains may be impacting susceptible decapods. Further supporting this hypothesis is the observation that host-pathogen relationships appear to vary between regions and infected hosts.

Despite the many uncertainties surrounding *Hematodinium*-associated diseases, the potential impact of this disease complex on crustacean populations cannot be ignored. Animal diseases are generally present at some low or background level of prevalence (i.e., endemic or enzootic). In contrast, epizootics and epidemics are disease outbreaks that exceed expected or measured background levels. Epizootics and epidemics are generally short-lived, lasting a few months or a few years. Examples of such diseases include the Spanish flu epidemic in 1918, phocine distemper virus disease of harbor seals in 1988-1989 and then again in 1998, 2000, and 2002, and ciliate disease in Dungeness crabs from 1990 to 1992. Epizootics and epidemics that occur over many years or decades are uncommon, so the continued presence of BCS or *Hematodinium*-

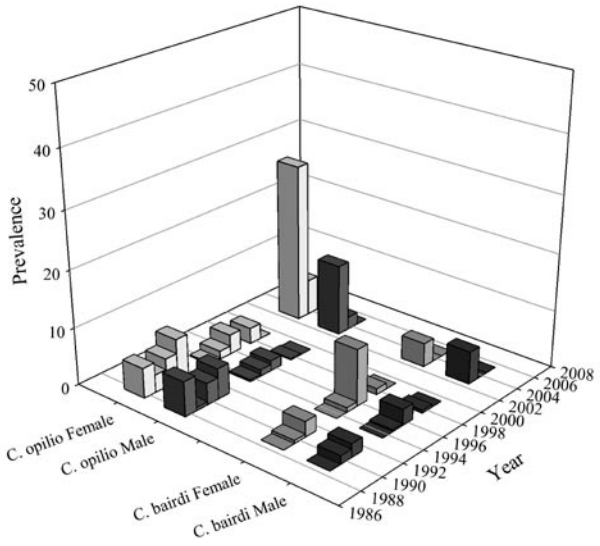

Figure 4. Annual prevalences of BCS in eastern Bering Sea snow and Tanner crabs. Note the high BCS prevalences in snow crabs in 2003.

associated diseases in some crustacean populations (over 20 years in Southeast Alaska Tanner crabs) is remarkable. In addition, anecdotal observations suggest that the disease was present in Southeast Alaska Tanner crabs as early as the mid-1970s, prior to exploitation of the resource.

In recent years, concern has surfaced as to whether or how climate change may be playing a role in emerging infectious disease. There is evidence to suggest that warmer temperatures are affecting coral health worldwide and facilitating the spread of amphibian diseases. *Hematodinium*-associated diseases have become more prevalent in the North Pacific over the past 22 years which corresponds to a recognized regime shift. Off Newfoundland, BCS has increased in prevalence and distribution since 1995 which also corresponds with a noticeable warming trend. The possible relationship between climate change and *Hematodinium*-associated diseases warrants further investigation.

In light of the fact that numerous unanswered questions surround BCS and other *Hematodinium*-associated diseases, it was apparent that researchers, managers, and fishers needed to meet and exchange ideas, data, and observations about this potentially important disease complex. With this need as a backdrop, an international workshop, "Hematodinum Associated Diseases: Research Status and Future Directions" was held 20-22 September 2007 in Charlottetown, Prince Edward Island, Canada (www.lobsterscience.ca/bcdworkshop/). Experts in the disciplines of invertebrate pathology, dinoflagellate ecology, host biology, disease modeling, genetics, and crustacean management were invited to identify common ground as well as knowledge gaps of this potentially important disease complex. Represented countries included Norway, Sweden, Ireland, Scotland, England, Greenland, Denmark, Canada, Australia, and the United States, including the States of Alaska, Arizona, Maryland, Virginia, Georgia, Mississippi and Washington. Rick Cawthorn of the Lobster Science Center, Atlantic Veterinary College, University of Prince Edward Island and Frank Morado of the AFSC Fisheries Resources Pathobiology program convened the workshop.

The workshop was organized into five sessions (Table 1), each with a list of questions that the speakers were asked to address during their presentations. A sixth session was added in which industry representatives were invited to speak on personal experience or discuss anecdotal evidence of the potential impacts of disease on the industry. The workshop could not adequately respond to all of the questions

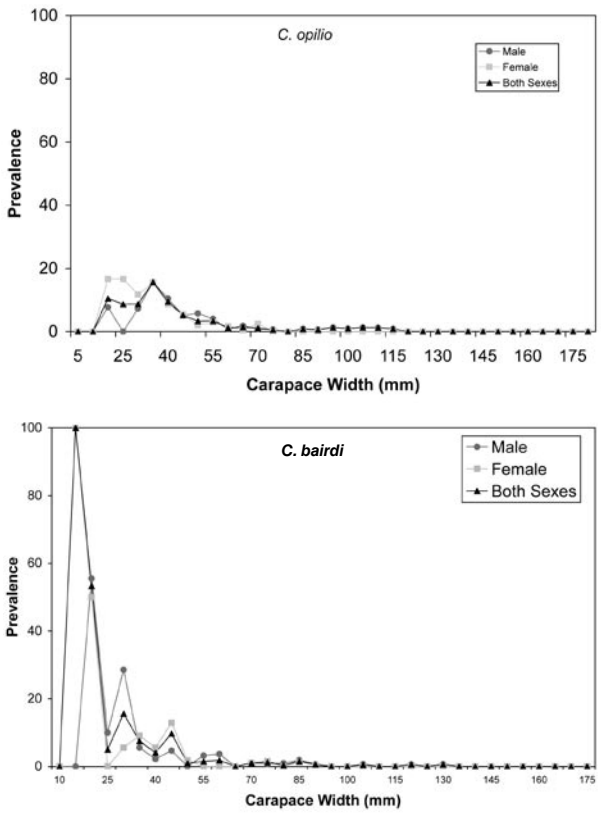

Figure 5. Cummulative prevalences of BCS by size in snow (top) and Tanner (bottom) crabs from 1988 to 2005.

Table 1. Bitter Crab Syndrome workshop modules for *Hematodinium*-associated disease and related questions.	
Module	Research Issues
Fisheries	1. What is the impact of disease on affected populations? 2. Is it possible to model this effect? 3. Can monitoring efforts be standardized? 4. Is a mechanism in place for reporting new affected species or dispersal of disease (e.g., OIE)?
Host Biology	1. Does a common factor exist between susceptible hosts? 2. Are all infections fatal in all species of susceptible hosts? 3. Do environmental factors exist that limit infections in susceptible hosts?
Parasite Genetics	1. How many species/clades/variants exist world-wide? 2. Are the identified species/clades native to areas where they are found? 3. Do they possess similar/different virulent factors?
Parasite Ecology	1. Are certain physical/environment factors associated with spread of the disease (e.g., global warming, sediment type)? 2. Do reservoirs exist? 3. What is the ecology of the differing species/clades/variants? 4. What is the life history of the parasite both within and outside of the host? 5. Does a common feature exist for *Hematodinium* associated diseases or what are the differences of disease progression with respect to host species, environment, geographic location? 6. Is climate change a contributing factor to epizootics?
Pathobiology	1. What pathophysiological changes occur over the course of disease? 2. What factors offer resistance to infection? 3. What are the factors that pre-dispose potential hosts to infection? 4. What is the mean time to death? 5. What is the method of infection and nature of the infective stage? 6. What pathophysiological changes are responsible for the bitter flavor? 7. How and at what rate are hemocytes removed from circulation? 8. Do *Hematodinium* associated diseases affect molting?

because sufficient data are not available, but the questions did stimulate considerable discussion among the attendees. For example, a consensus could not be reached on a pathogen-host-environment model. It became clear that while similarities do occur, sufficient differences exist that make it impossible to identify a model disease system. The chief problem may be that despite DNA analysis, several species/clades/variants may exist that confound research. Another obstacle is that individual diseases develop slowly over several months and may require up to 18 months from onset of infection to death of the host. In the end, a number of collaborations were proposed to address key research topics. As a result, the workshop attained the desired results, and attendees expressed the desire to meet again in the future. A synoptic paper that captures the essence of the workshop is planned for publication.

The goal of the AFSC Fisheries Resources Pathobiology program is to understand the role of disease on distribution patterns and population abundance of fish and shellfish by examining individual responses. Effort is directed at documenting disease-related mortalities, but other side-effects of

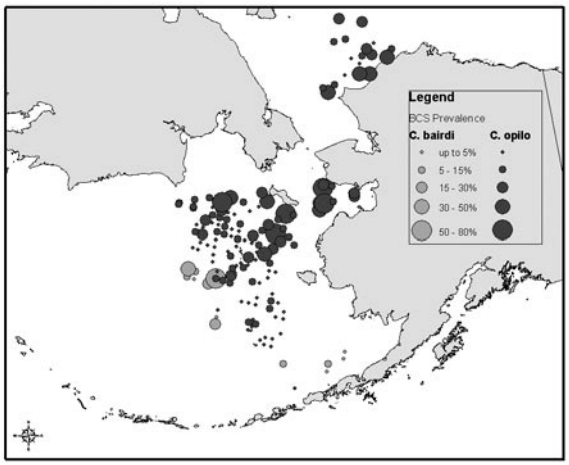

Figure 6. Cumulative distribution of BCS in Bering Sea and Chukchi Sea crabs. Note the apparent segregation of infections.

disease occur (i.e., reduced fitness as reflected by reduced growth, fecundity, physiological state, immunity, etc.) and are being investigated. In addition to its study of BCS, program research has indicated that infections by another protist, a microsporidan of the genus *Pleistophora,* affects juvenile walleye pollock (*Theragra chalcogramma*) fitness. The parasite infects the skeletal muscle causing severe disruption and dissolution, presenting the appearance of rice kernels in the muscle. As a result, swimming performance in infected juvenile walleye pollock is likely affected. Mortalities do not appear to directly result from microsporidan infections. Rather, high prevalences that occur in small fish and decrease with increase in fish size suggest that infected juvenile fish may find it difficult to evade predators or capture prey. Adult walleye pollock are also infected by the same parasite, but with a different outcome. In adults as in juveniles, the proliferating parasite causes disruption and dissolution of skeletal muscle. However, the impact of the observed rice kernels is a product quality issue. Severely affected muscle can only be used for surimi and not fillets which command a higher price. Product quality is also affected by other pathogens that include a primitive fungus, *Ichthyophonus* sp., and several species of myxozoa.

The Fisheries Resources Pathobiology program works to understand host-parasite interactions. These studies are important for understanding why one host may be susceptible to disease and another is not. This is particularly true for BCS. United Kingdom colleagues have observed that within an estuary, several species of brachyuran and anomuran crustaceans may be infected, however, not all crustacean species within that estuary harbor infections. To this end, our program is developing molecular tools for identifying pathogens, with a long-term plan to develop other molecular tools that measure host adaptive responses and identify markers that determine pathogen virulence.

The Fisheries Resources Pathobiology program has conducted integrated and comprehensive disease studies on eastern Bering Sea fish and shellfish species for over 25 years and collaborates with AFSC resource assessment programs to understand population biology. It is through these long-term efforts that BCS research in the North Pacific is now at the stage where modeling of a marine invertebrate disease, even though rare, is now possible.

FURTHER READING

Chatton, É. and Poisson, R. 1931. Sur l'existence dans le sang des crabes, de péridiniens parasites: *Hematodinium perezi* n.g., n.sp. (Syndinidae). Comptes Rendus des Séances de la Societé de Biologie et de ses Filiales, 105:553-557.

Gulland, F.M.D. 1998. The impact of infectious diseases on wild animal populations – a review. In: Ecology of Infectious Diseases in Natural Populations (Grenfell, B.T. & Dobson, A.P., eds.), Cambridge University Press, Cambridge.

Harvell, D.C., Kim, K., Burkholder, J., Colwell, R.R., Epstein, P., Grimes, J., Hofmann, E., Lipp. E., Osterhaus, A., Overstreet, R.R., Porter, J., Smith, G. and Vasta, G. 1999. Emerging marine diseases : climate links and anthropogenic factors. Science, 285:1505-1510.

Kim, K., Dobson, A.P., Gulland, F.M.D. and Harvell, C.D. 2005. Diseases and the conservation of marine biodiversity. In: Marine Conservation Biology (Norse, E.A. & Crowder, L.B., eds.), Island Press, Washington.

Meyers, T.R., Koeneman, T., Botelho, C. and Short, S. 1987. Bitter crab disease: a fatal dinoflagellate infection and marketing problem for Alaskan Tanner crabs, *Chionoecetes bairdi*. Diseases of Aquatic Organisms, 3:195-216.

Newman, M.W. and Johnson, C.A. 1975. A disease of blue crabs (*Callinectes sapidus*) caused by the parasitic dinoflagellate, *Hematodinium* sp. Journal of Parasitology, 63:554-557.