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Immunosuppression Cascade in the Florida Manatee (*Trichechus manatus latirostris*)

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Abstract

The Florida manatee (*Trichechus manatus latirostris*) is a federally endangered marine mammal that resides on both coasts of the Florida peninsula. The species faces many anthropogenic and natural threats, including boat strikes, exposure to harmful algal blooms, and cold water temperatures. We have developed a conceptual model that depicts how changes in the environment, such as reduced water temperature, can trigger an immunosuppressive cascade of interrelated diseases and pathological conditions ultimately leading to the death of the animal. Although the Florida manatee has a relatively robust immune system, rendering it resistant to several diseases, the onset of unfavorable environmental conditions has been shown to compromise the immune system, often leading to infections that make an animal more susceptible to opportunistic pathogens. In this paper, we review several common diseases of the Florida manatee and compare and contrast their symptoms. We then interrelate these diseases and generate a conceptual model of a cascade of immunosuppressive conditions originally triggered by adverse environmental conditions or one of the diseases. The end result of the cascade is the death of the animal.

Key Words: West Indian manatee, *Trichechus manatus latirostris*, immunology, disease, immunosuppression, cold stress syndrome, harmful algal blooms

Introduction

Florida manatees are marine mammals in the order Sirenia and the family Trichechidae. The West Indian manatee (*Trichechus manatus*) is divided into two subspecies: (1) the Florida manatee (*Trichechus manatus latirostris*) of the Florida peninsula and (2) the Antillean manatee (*Trichechus manatus manatus*) of the Caribbean and Central and South America (Garcia-Rodriguez et al., 1998; U.S. Fish and Wildlife Service, 2001).

While manatees are susceptible to some natural hazards in their habitat, most threats to their population are from anthropogenic sources. A major focus of wildlife conservation in the State of Florida over the past 25 years has been the protection and conservation of the Florida manatee. In 2007, watercraft-related mortality was the second leading cause of death of the Florida manatee, accounting for approximately 23% of all known manatee deaths (Fish and Wildlife Research Institute, 2008). The *Preliminary Biological Status Review of the Florida Manatee* (Haubold et al., 2006) considered a variety of factors, including past and future population trends, the geographic range of the species, critical habitats, the number of mature individuals, and the probability of extinction. The review concluded that there is a 12.1% chance of a 50.0% decline in the manatee population over the next three generations, a 46.5% chance of a 30.0% decline, a 55.5% chance of a 20.0% decline, and 77.1% chance of a 10.0% decline in the same time frame. Although manatee numbers appear to have increased in the past few decades, many anthropogenic threats to the Florida manatee population continue to increase and may affect the survival of the species. Natural catastrophes, although infrequent, have the capacity to cause significant declines in the population and are expected to continue to occur in the future.

Natural threats to the Florida manatee include cold water temperatures, hurricanes, harmful algal blooms, and the spread of diseases or pathogens (Table 1). In addition, compared to other marine organisms, manatees have long life spans, high ages of sexual maturity, low reproductive rates, and high parental investment, making their survival even more problematic (Haubold et al., 2006). Such threats and natural history traits have made it nearly impossible for manatees to recover from past population declines or to maintain viable population levels.

Table 1. Causes and clinical features of manatee brevetoxicosis, cold stress syndrome, papillomavirus, and morbillivirus (Sundberg et al., 1985, 1996, 2000; Lipscomb et al., 1994; Duignan et al., 1995; Bossart et al., 1996, 1998, 2003; Purkerson et al., 1999; Flewelling et al., 2005; Woodruff et al., 2005; Berta et al., 2006)

Causes and Clinical Features
• Brevetoxicosis
• Caused by large blooms of brevetoxin-producing *Karenia brevis*
• Brevetoxins depolarize and open voltage-gated sodium-channels in nerve cells
• Clinical features include congestion, edema, and hemorrhage
• May lead to fatal toxic shock syndrome due to discharge of inflammatory mediators and/or initiation of apoptosis
• Cold stress syndrome
• Caused by continuous exposure to cold water temperatures
• Cold temperatures elicit a series of physiological and pathological events
• Clinical features include lesions with signs of enterocolitis, lyphoid reduction, emaciation, fat store exhaustion and serous fat atrophy, epidermal hyperplasia, pustular dermatitis, and myocardial deterioration
• May lead to death if cold temperatures persist or the animal is left untreated
• Papillomavirus
• Caused by contact with infected animals or environmental surfaces
• Virus is an infectious pathogenic epitheliotrophic non-enveloped DNA virus
• Clinical features include verrucous warts and keratotic flat skin lesions
• Papillomas are benign but can threaten the animal if they grow large enough to interfere with feeding, sight, or breathing
• Morbillivirus
• Caused by a hazardous pathogen
• Transmittal may be via infected cetaceans, crowding, environmental contamination in food, harmful algal blooms, or high water temperatures
• Clinical features include lung, nervous, and lymphoid lesions; interstitial pneumonia; interstitial fibroplasias; and leukocyte infiltration
• Although manatees have antibodies to it, morbillivirus has not been isolated from them

Manatees cannot physiologically withstand waters below 20° C for more than 2 or 3 d (Bossart et al., 2003). Such prolonged exposure can lead to cold stress syndrome, weakening their immune system and predisposing them to pathogens and disease (Bossart et al., 2003; Walsh et al., 2005). Due to their inability to tolerate cold water, the geographic distribution of manatees is severely limited. During winter months, many populations reside in natural and industrial warm-water resources, including springs and power plant outflows (Ackerman et al., 1995). When water temperatures rise, manatees are typically found no farther north than Rhode Island and no farther west than Texas (Deutsch et al., 2003).

Although the immune systems of manatees are relatively strong, they can be afflicted with several diseases and infections (Bossart, 1999; Bossart et al., 2003). These diseases and infections are often the result of immunosuppression as the consequence of an initial infection, leading to several other syndromes whose signs and symptoms are similar, implying a causal relationship between them (Bossart et al., 1998, 2003).

Diseases and Pathogens of Manatees

Harmful Algal Blooms

Harmful algal blooms (HABs), or red tides, occur when the marine dinoflagellate *Karenia brevis* releases brevetoxins into the ambient water. These brevetoxins initially enter the manatee via inhalation or ingestion, leading to brevetoxicosis (Flewelling et al., 2005). Upon inhalation, brevetoxins undergo phagocytization by macrophages and internalization by lymphocytes. The brevetoxins later act as competitive inhibitors of degradative enzymes in the brevetoxin-containing cells, programming them for apoptosis. The initiation of apoptosis and/or the release of inflammatory mediators by brevetoxins is speculated to possibly cause lethal toxic shock syndrome (Bossart et al., 1998).

The effects of brevetoxins have been examined in other mammals. For example, in 1999 and 2000, 120 bottlenose dolphins (*Tursiops truncatus*) were found stranded along the coast of the Florida panhandle. The strandings occurred during a HAB of *K. brevis*. The highest level of brevetoxins in the dolphins was found in their stomachs along with

the remains of consumed fish that introduced the toxins into their gastrointestinal systems (Mase et al., 2000). Humans can also be affected by HABs by consuming poisoned shellfish leading to neurotoxic shellfish poisoning. Additionally, humans can inhale the toxins, causing respiratory irritation (particularly in asthmatic individuals) (Kimm-Brinson & Ramsdell, 2001). Rats who inhale brevetoxins have reduced antibody production by splenic lymphocytes (Benson et al., 2005).

Symptoms of brevetoxicosis in manatees include gross histological lesions, hemorrhage, and edema, possibly leading to death of the animal in cases of continuous toxin inhalation or ingestion (Bossart et al., 1998). Continuous exposure to HABs may also impair the cellular immune response of individual animals by reducing lymphocyte proliferation, perhaps making them more susceptible to toxic shock syndrome and other diseases or infections (Walsh et al., 2005; Baskin, 2006). Additionally, manatees that come into contact with toxins from red tides have elevated levels of nitric oxide (a reactive nitrogen intermediate that is generated during natural host defense), which could lead to decreased lymphocyte proliferation (Lancaster & Stueher, 1996; Walsh et al., 2007). Brevetoxins can also competitively inhibit cathepsin enzymes in lymphocytes, reducing cellular immune function and the competence of the manatee immune system (Sudarsanam et al., 1992; Bossart et al., 1998; Trainer & Baden, 1999).

Cold Stress Syndrome

Low metabolic rates, the inability to create adequate body heat, and intolerance to prolonged exposure to cold water render manatees susceptible to cold stress syndrome (CSS). Continuous exposure to cold water (less than 20° C) triggers a succession of physiological and pathological events leading to CSS. Manatees exposed to cold water have reduced lymphocyte proliferation as compared to healthy animals (Walsh et al., 2005). Lymphoid depletion occurs in the humoral and cell-mediated immune systems (Bossart et al., 2003). Reduced lymphocytes may possibly contribute to excessive and prolonged stress-related glucocorticoid release and nutritional deficiencies, and such reductions may suppress the manatee immune system, leaving such individuals susceptible to other diseases in addition to CSS. If water temperatures remain low or the disease is left untreated, CSS often results in the death of an affected manatee (Bossart et al., 2003). Morbidity and mortality related to CSS have been shown to decrease with animal size (O'Shea, 1998).

As with brevetoxicosis, manatees afflicted with CSS exhibit skin lesions and reduced lymphocyte proliferation as well as other related symptoms, including broncho-pneumonia, dermatitis, secondary enterocolitis, epidermal hyperplasia, myocardial degeneration, emaciation, exhaustion of fat stores, and serous fat atrophy (Bossart et al., 1998, 2003; Bossart, 2001). Due to the reduction in lymphocyte proliferation, it is possible that CSS allows opportunistic neurotoxins, such as brevetoxins, to further compromise the manatee immune system should the animal encounter a HAB (Bossart et al., 2003).

Papillomavirus

Papillomaviruses (non-enveloped DNA viruses) are infectious epitheliotrophic pathogens that induce benign tumors in several species of mammals and birds (Sundberg et al., 1985, 1996, 2000). These viruses infect manatees suffering from immune system suppression (Bossart et al., 2002; Woodruff et al., 2005). Immunosuppression can be caused by cold water temperatures (see above). The small size of the manatee population also contributes by reducing the diversity of immune defense genes as a consequence of population "bottlenecks" (Rector et al., 2004). Thus, papillomavirus infection may occur secondary to CSS or other afflictions because of the already weakened immune system of the manatees. Papillomavirus has also been found in killer whales (*Orcinus orca*), Burmeister's porpoise (*Phocoena spinipinnis*), harbor porpoises (*Phocoena phocoena*), dusky dolphins (*Lagenorhynchus obscurus*), and several species of sea turtles (Bossart et al., 1996; Van Bressem et al., 1999).

Papillomavirus was first detected in seven captive manatees held at Homosassa Springs State Wildlife Park, Florida, in 1997. The infected manatees initially exhibited cutaneous, pedunculated papillomas on their skin. Four previously infected manatees had sessile, solid papillomas that differed topographically and clinically from their first papillomas three years prior (Bossart et al., 2002). Since its discovery, however, papillomavirus has also been found in free-ranging, wild manatees (Woodruff et al., 2005).

Virus-caused papillomas are benign but can threaten the animal if they grow large enough to interfere with feeding, sight, or breathing. Similar tumors caused by papillomavirus in other mammals have also been shown to spontaneously develop into malignant tumors and cancer. Due to their development on common contact areas of the manatee's body, it is believed that the virus can be spread by contact between infected and non-infected animals (Bossart et al., 2002). The cutaneous tumors of the Florida manatee are of a unique manatee papillomavirus known as *Trichechus manatus latirostris* Papillomavirus Type 1 (Rector et al., 2004).

The most common symptoms of papillomavirus infection are epidermodysplasia verruciformis (an inherited immunological defect that causes increased susceptibility to papillomavirus infections), verrucous warts, and keratotic flat skin lesions (Berkhout et al., 1995; Woodruff et al., 2005). As mentioned previously, similar lesions are seen in brevetoxicosis and CSS, although these are not virus-derived. In addition to warts, manatees infected by papillomavirus also exhibit signs of lowered lymphocyte proliferation and lymphocyte T-cell stimulation as found in brevetoxicosis and CSS (see above) (Bossart et al., 1998; Rector et al., 2004). However, a recent study of papillomas on Atlantic bottlenose dolphins revealed higher incidences of B-lymphocyte proliferation in infected animals, implying an upregulated immune humoral reaction to the virus (Bossart et al., 2008). Decreased T-cell production was not observed in the dolphins, the opposite of the effects seen in manatees (Bossart et al., 1996, 2008). Such contradictory results indicate that responses to the papillomavirus may vary from species to species. Therefore, the results of the studies of papillomavirus in other marine mammals may not be representative of what happens in manatees.

Morbillivirus

Morbillivirus infection has been demonstrated in manatees based on serological findings (Duignan et al., 1995). The virus is caused by a dangerous pathogen that is responsible for distemper in dogs and measles in humans as well as illnesses in cetaceans and phocids. Epizootics of the virus occur rapidly in large populations that lack previous immunity (Lipscomb et al., 1994).

Several hypotheses have been developed regarding the origin and spread of morbillivirus in manatees. The illness could originate from dolphins afflicted with morbillivirus because they share the same coastal ranges and cohabitate with manatees during winter months in warm-water areas. Infected, stranded pelagic odontocetes might spread morbillivirus. Other means of transmission may be related to crowding, environmental contamination in food, HABs, or warm water temperatures (Berta et al., 2006). Manatee physiology might allow for sufficient viral replication to elicit a neutralizing antibody reaction that prevents disease transmission to other animals, rendering the animal a dead-end host (Duignan et al., 1995).

While manatees have been previously demonstrated to have antibodies to morbillivirus, it is not known if they are afflicted with this disease. Therefore, the specifics of morbillivirus infection in manatees have yet to be described. However, symptoms of morbillivirus infection have been described in bottlenose dolphins (Lipscomb et al., 1994). These symptoms are similar to those seen in canine distemper, including lung, nervous, and lymphoid lesions (resembling those seen in brevetoxicosis, CSS, and papillomavirus infections in manatees); interstitial pneumonia; interstitial fibroplasias; and leukocyte infiltration (Lipscomb et al., 1994; Bossart et al., 1998; Kennedy, 1998). Canine distemper is also thought to reduce immunocompetence by depleting the lymphoids and potentially causing secondary infections (Moro et al., 2003).

Other Infections

Most manatee deaths are caused by boat collisions, debris entanglement, brevetoxicosis, and CSS, often resulting from secondary bacterial or fungal infections (Bossart, 2001). Several secondary infections due to immunosuppression can afflict Florida manatees. These infections often exhibit symptoms similar to other common manatee diseases, implying previously unrecognized connections between them. In 1978 and 1981, two Florida manatees residing in a marine aquarium in Japan died from pyogranulomatous pneumonia caused by a mycobacterial infection (Sato et al., 2003). Pyogranulomatous pneumonia is a rare skin disorder often seen in canines, usually resulting in a chronic relapsing disorder. *Mycobacterium marinum*, *M. fortuitum*, and *M. kansasii* were cultured from infected tissues in the lungs and lymph nodes of the manatees. The infected manatees also had signs of edema and tissue lesions that are seen in other diseases (Bossart et al., 1998; Sato et al., 2003).

A link has been suggested between papillomavirus skin lesions and biofouling communities that have been shown to concurrently establish on the dorsal epidermis (Bledsoe et al., 2006). Organisms found on the dorsum included the freshwater filamentous and toxin-producing cyanobacteria, *Lyngbya*, which forms large mats at the water surface that could be encountered by manatees (Dubose et al., 1992). Pappilomavirus skin lesions that lower the manatee immune system response could facilitate the establishment of toxin-producing biofouling organisms (Harr et al., 2008). The toxins produced by *Lyngbya* are cytotoxic to mammalian cell lines (Carmichael et al., 1997; Teneva et al., 2003) and thus could potentially contribute to the spread of the virus or permit other diseases and infections to occur.

The Immunosuppression Cascade

Florida manatees have strong immune systems that easily safeguard them from injuries or the environment. Although disease in Florida manatees is relatively uncommon, epizootics and other

infections have occurred. It has been suggested that it is a weakening of their normally robust immune system that ultimately makes them susceptible to diseases and pathogens (Bossart, 1999; Bossart et al., 2002). Such immune system deterioration is often related to changes in their environment. Environmental fluctuations may act to inhibit manatee immune defense mechanisms (Walsh et al., 2007). Events such as HAB outbreaks, anthropogenic insults, or cold water temperatures can cause the onset of one or more diseases or afflictions in manatees.

We have developed a conceptual model that depicts how environmental changes can trigger the development of several successive diseases through a cascade of immunosuppression events in the Florida manatee (see Figure 1). The model demonstrates how a single environmental change can initiate a succession of infections and diseases due to the suppression of the manatee's immune system. The model contains several feedback loops that depict how one infection or disease can facilitate the progression of others based on the similarities between their causes and symptoms.

For example, during the winter, if ambient water temperatures drop to less than 20° C, a manatee may develop CSS. CSS will then reduce the robustness of the immune system, rendering the individual susceptible to other diseases or infections.

Florida HABs occur primarily in the late summer and autumn months when water temperatures are beginning to decline. The manatee immune system could be stressed because of the low water temperatures as described above. The coincidence of a HAB and brevetoxicosis could compromise the immune system even further. Alternatively, the manatee may already have a weakened immune system because of a HAB exposure during the months prior to the reduction in water temperature. Thus, the animal may become more susceptible to cold stress once water temperatures fall below 20° C.

Papillomavirus has been shown to be secondary to CSS and more prevalent during cold water

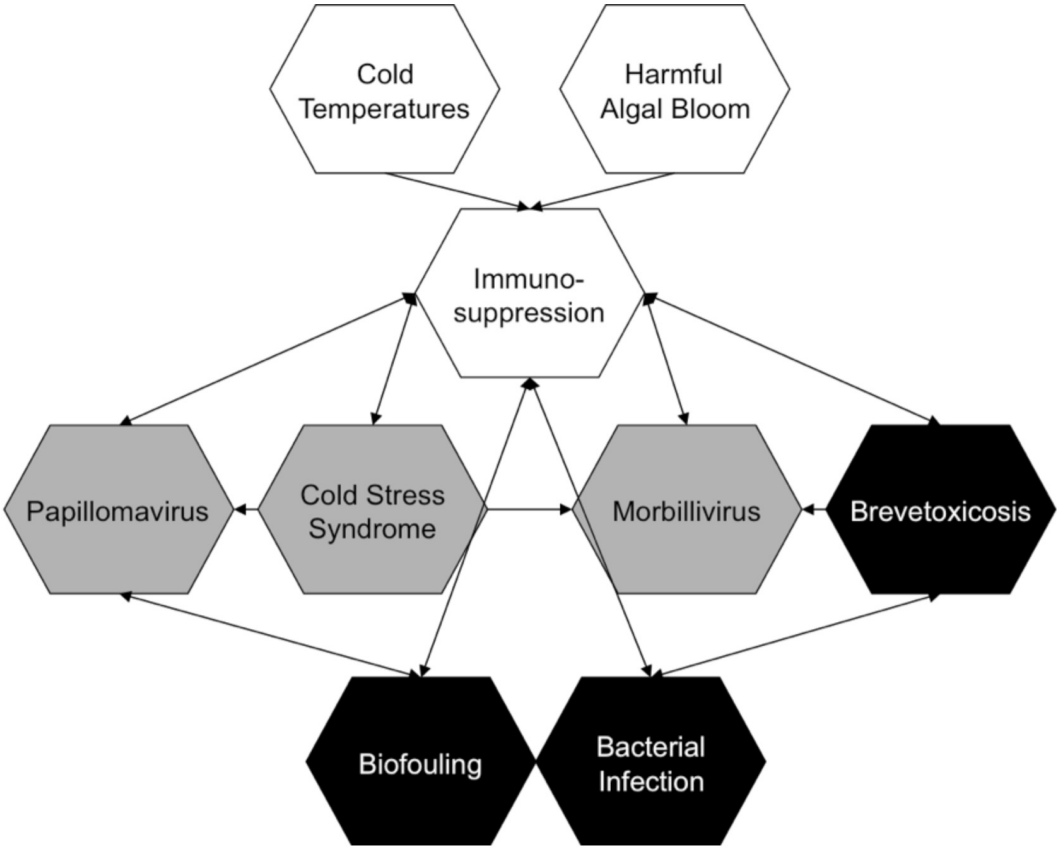

Figure 1. A conceptual model depicting a succession of events and feedback loops due to a change in the environment of the manatee (*Trichechus manatus latirostris*); an environmental change can lead to immunosuppression, potentially causing a cascade of disease(s) possibly leading to death. Diseases are depicted in gray, while infections are depicted in black.

temperatures because of the weakened state of the immune systems of the animals. Thus, papillomavirus infection could result from the cold stress. In addition, the possible communicative mechanism for the virus is through direct skin contact between infected and non-infected animals or through indirect contact with infected environmental surfaces (Bossart et al., 2002). Manatees often crowd into warm-water refuges such as springs and power plant effluents during cold temperature episodes. This close proximity could provide increased contact opportunities and thus increased dispersal of the virus.

Morbillivirus is thought to be transmitted due to crowding, HABs, or high water temperatures. As with papillomavirus, crowding could possibly allow for the infection and spread of morbillivirus. In addition, a concurrent HAB could provide another mechanism of infection and spread of morbillivirus. As stated above, HABs are common events in the summer during warm water temperatures when morbillivirus may be more prevalent. Such conditions may facilitate proliferation of morbillivirus since it is more virulent in warm water. The co-infection of manatees with brevetoxicosis, CSS, morbillivirus, or papillomavirus could permit secondary opportunistic bacterial or fungal infections as a consequence of the impaired status of the immune system.

Clearly, more research is needed to examine the impact of environmental changes on the physiology and immune systems of manatees to prevent the spread of disease and increase the survival probability of these populations. In addition, several manatee diseases, such as papillomavirus, plague humans. Thus, Florida manatees could be used as animal models for these diseases. Manatees may also have a role as sentinels of ecosystem health because they reflect changes in the environment and threats to human health (Bonde et al., 2004; Bossart, 2006). New insights concerning diseases in manatees could be applied to existing diagnosis and treatment methods to offer greater chances of survival for both humans and manatees.

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