Sources, Distribution, and Conveyance of Opportunistic Pathogens in Estuaries and the Oceans

D. JAY GRIMES
College of Marine Sciences
The University of Southern Mississippi
P.O. Box 7000
Ocean Springs, Mississippi 39566-7000 USA

ABSTRACT
In 1981, water samples collected from 14 stations distributed along a track-line from Barbados to Bermuda revealed a preponderance of bacteria belonging to the genus Vibrio (Grimes et al. 1984a). Ten years earlier, using the same experimental design along the same approximate track-line, Sieburth (1971) had reported that nearly 100% of the culturable bacterial isolates belonged to the genus Pseudomonas. Explanations offered for this apparent shift in the dominance of culturable bacteria included chronic pollution of the ocean with anthropogenic hydrocarbons, resulting in the selection of hydrocarbon-degrading vibrios (Grimes et al. 1984a). It is well known that some species Vibrio cause a variety of diseases in marine fishes, marine invertebrates, and humans. Vibrio species are also autochthonous inhabitants of estuaries and the oceans, and they possess an array of degradative enzymes that allow them to metabolize compounds ranging from chitin and squalene to short chain fatty acids and polyaromatic hydrocarbons. Clearly, the vibrios possess an impressive set of habitat-adaptive enzymes, and this general adaptation was recently confirmed for one species by the completion of the genomic sequence of V. cholerae (Heidelberg et al. 2000). In 1997 and 1998, the largest outbreaks of human disease ever caused by V. parahaemolyticus in North America occurred from the consumption of contaminated oysters. Interestingly, these years were also years of increased sea surface temperature associated with the El Niño Southern Oscillation, and V. parahaemolyticus grows best under mesophilic conditions. The focus of this paper will be the niches that are filled by members of the bacterial genus Vibrio that are capable of degrading anthropogenic compounds, responding to global climate change, and infecting and causing disease in vertebrate and invertebrate hosts.

KEY WORDS: Autochthonous, allochthonous, anthropogenic chemicals, nutrients, pathogens, opportunistic, Vibrio, ENSO, currents, degradation

INTRODUCTION
Recent literature abounds with reports of marine diseases affecting marine organisms and humans (Grimes 1991, Harvell et al. 1999, DePaola et al. 2000). After a thorough review of the literature on this matter, Harvell et al. (1999) concluded that reports of diseases in the oceans are on the rise. They noted that there have been many epidemics affecting economically and ecologically important species, and that unknown species may be disappearing without notice. They went
on to note that most “new” diseases occur by host shifts and not by the emergence of “new” microorganisms. Clearly, pathogens have always existed in estuaries and the oceans and they continue to plague humans and the living resources available to humans for sustainable use.

SOURCES AND DISTRIBUTION

Opportunistic pathogens are organisms that do not normally cause disease in a host unless that host has been in some way compromised. There are many such pathogens living in oceans (Grimes et al. 1986) and estuaries (Grimes 1991), some of which normally reside in those habitats and some of which are transients. The indigenous pathogens are referred to as autochthonous by ecologists and their physiology is ideally suited to survival and life in estuaries and the oceans. Unfortunately, there are also mechanisms of attachment and growth that allow these microorganisms to infect susceptible hosts and therein cause disease. Some of the better known examples of autochthonous opportunistic pathogens are listed in Table 1. The non-indigenous pathogens are transients that gain entrance to oceans and estuaries through some point or non-point source of pollution, and they do not usually survive well in these saline aquatic environments. Ecologists refer to non-indigenous microorganisms as allochthonous, and the better known examples of allochthonous opportunists are listed in Table 2.

Table 1. Genera containing opportunistic pathogens autochthonous to estuaries and oceans

<table>
<thead>
<tr>
<th>Known autochthonous bacteria</th>
<th>Possibly allochthonous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vibrio*</td>
<td>Staphylococcus</td>
</tr>
<tr>
<td>Aeromonas*</td>
<td>Clostridium</td>
</tr>
<tr>
<td>Renibacterium</td>
<td>Pseudomonas*</td>
</tr>
<tr>
<td>Mycobacterium*</td>
<td>Flavobacterium</td>
</tr>
<tr>
<td>Plesiomonas</td>
<td>Klebsiella*</td>
</tr>
<tr>
<td></td>
<td>Acinetobacter</td>
</tr>
<tr>
<td></td>
<td>Legionella*</td>
</tr>
</tbody>
</table>

*Some members of the genus have demonstrated ability to become nonculturable in response to stress

An important survival mechanism that allows both autochthonous and allochthonous microorganisms to exist in spite of environmental stress was first described by Xu et al. (1982) for *Vibrio cholerae* and *Escherichia coli* - good examples of an autochthonous opportunist and an allochthonous opportunist, respectively. Since that initial description, many laboratories throughout the world have observed the viable but nonculturable phenomenon for numerous pathogens and non-pathogens alike. The essence of this mechanism is illustrated in the survival
curves shown in Figure 1. Briefly, when stressed by a physical or chemical factor (e.g., loss of nutrients, adverse temperature, chlorine), microbes examined thus far respond to the stress by undergoing a series of structural and physiological changes that result in a dormant or "nonculturable" stage of growth. They tend to become smaller, less permeable, refractory to cultivation on culture media normally supportive of their vegetative growth, and some lose their flagella. Little is known about the mechanism or mechanisms that return normal vegetative growth, but it is clear that a susceptible host will suffice for many of the opportunistic pathogens when they are in a nonculturable state. Recently, Colwell and Grimes (2000) collected and published several reviews of this survival strategy that appears to play an important role in the conveyance of opportunistic pathogens in estuaries and oceans. The microorganisms listed in Tables 1 and 2 that have been shown to be capable of this survival strategy are so denoted.

**Table 2. Genera containing opportunistic pathogens allochthonous to estuaries and oceans**

<table>
<thead>
<tr>
<th>Known allochthonous bacteria</th>
<th>Possibly autochthonous</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em></td>
<td><em>Staphylococcus</em></td>
</tr>
<tr>
<td><em>Shigella</em></td>
<td><em>Clostridium</em></td>
</tr>
<tr>
<td><em>Leptospira</em></td>
<td><em>Pseudomonas</em></td>
</tr>
<tr>
<td><em>Escherichia</em></td>
<td><em>Flavobacterium</em></td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
<td><em>Klebsiella</em></td>
</tr>
<tr>
<td><em>Enterococcus</em></td>
<td><em>Acinetobacter</em></td>
</tr>
<tr>
<td><em>Morganella</em></td>
<td><em>Legionella</em></td>
</tr>
<tr>
<td><em>Enterobacter</em></td>
<td></td>
</tr>
<tr>
<td><em>Listeria</em></td>
<td></td>
</tr>
</tbody>
</table>

*Some members of the genus have demonstrated ability to become nonculturable in response to stress*

Of all the known opportunistic pathogens in estuaries and oceans throughout the world, the *Vibrio* species generally predominate the culturable microbial community (Grimes et al. 1984a). This was an unexpected finding by Grimes et al. (1984a), because since the mid-1900s it was almost axiomatic that *Pseudomonas* species dominated the culturable bacterial communities of the world oceans (ZoBell and Upham 1944, Sieburth 1971). In 1981, water samples collected along a track-line from Barbados east into the Caribbean Sea and north through the Mona Passage to Bermuda demonstrated that vibrios had replaced pseudomonads (Grimes et al. 1984a). *Vibrio* species comprised up to 100% of the culturable community, followed by acinetobacters (Figure 2, Grimes et al. 1984a). Now, it is known that *Vibrio* species are ubiquitous in oceans and estuaries—throughout the water column, in bottom sediments, attached on marine plants and animals, and within marine animals. Their distribution and density are, ultimately, dependant upon known environmental limiting factors, including salinity (vibrios preferentially generate
energy by means of a Na-dependant ATP pump), temperature (vibrios are mesophiles), organic and inorganic nutrients (all vibrios are chemoheterotrophs, some can fix N, and most can use nitrate in anaerobic respiration), currents (bacteria are colloids that are easily conveyed by tides, currents, upwellings, and rings), and possibly light.

Figure 1. Survival curves and morphological changes typical of vibrios as they transition from vegetative growth into the viable but nonculturable stage of growth.
Figure 2. Distribution of culturable, heterotrophic bacteria in water samples collected at 14 stations along a track-line from Barbados, into the Caribbean Sea, and through Mona Passage into the Atlantic Ocean to Bermuda.
Consistent with their ubiquitous distribution in oceans and estuaries, vibrios and acinetobacters are capable of metabolizing a wide variety of hydrocarbons. Short chain volatile fatty acids, alcohols, toluene, benzene, dimethyianiline, and polyaromatic hydrocarbons (PAHs) are all subject to biodegradation by vibrios and acinetobacters (Okpokwasili et al. 1986), and these hydrocarbons were characteristic of wastes being disposed of at the Puerto Rico dumpsite and of petroleum being transported from South America (Grimes et al. 1984a). Clearly, the vibrios are capable of metabolizing a wide variety of anthropogenic chemicals.

Vibrios are also capable of metabolizing a wide variety of biopolymers. Most can hydrolyze and utilize monomers from proteins, complex carbohydrates, and lipids. This ability not only confers metabolic versatility on the vibrios, but it also allows them to colonize and grow in a variety of animal hosts. They can hydrolyze the building blocks of connective and soft tissues; in other words, they can easily exploit the habitat provided by chance hosts and act as opportunistic pathogens. Table 3 illustrates the metabolic versatility of the vibrios, by listing some of the compounds that can be used by the shark pathogen V. carchariae (Grimes et al. 1984c, Grimes et al. 1989).

**Table 3. Compounds metabolized by Vibrio carchariae, an opportunistic pathogen of sharks (Grimes et al. 1984c and 1989)**

| Lysine, ornithine, tryptophan, glycine | Starch, algin |
| Chitin, chondroitin, hyaluronic acid | Xanthine, salicin |
| Casein, gelatin, collagen | y-aminobutyrate |
| Lecithin, squalene | a-ketoglutarate |
| Tweens 20, 40, 60, 80 | Glucose, sucrose, mannose |
| Urea | Trehalose, arabinose, mannitol |
| Nitrate | Ethanol, 1-propanol |

Clearly, the vibrios are elegantly equipped to quickly exploit a wide variety of potential nutrient sources - inputs and fluxes - in estuaries and oceans. The full extent of this capability was recently demonstrated for the type species of the genus, *V. cholerae*, by Heidelberg et al. (2000). The vibrios closely follow primary producer as well as primary consumer population increases, as they grow in response to fluxes in dissolved organic material and on living and dead zooplankton (Harvell et al. 1999, Kaneko and Colwell 1973). They respond to anthropogenic inputs (Grimes et al. 1984a) and to climate change (Lobitz et al. 2000). In every sense, they are "r-strategists" or copiotrophs, living off the "fat of the land" (Grimes 1991). Vibrios grow quickly in response to new sources of nutrients in natural aquatic habitats, they rapidly disseminate in compromised hosts, and they become dormant or nonculturable (Colwell and Grimes 2000) during famine or otherwise adverse environmental conditions.
CONVEYANCE

There is ample evidence that vibrios, like most marine bacteria, are conveyed throughout estuaries and oceans of the world by both natural and anthropogenic influences. Currents, tides, waves, gyres, and other natural water movements convey bacteria as they would any comparable sized particle. In 1984, it was hypothesized that pharmaceutical wastes released at a dumpsite north of Puerto Rico meandered throughout the Atlantic Ocean and, eventually, into the Caribbean Sea (Grimes et al. 1984a). In turn, it was further hypothesized that these wastes stimulated the growth of a community of vibrio bacteria in the affected waters. In a related study, it was shown that pharmaceutical wastes introduced into the ocean by means of a long sewage outfall also had an influence that was related to prevailing currents (Grimes et al. 1984b).

In 1997, consumption of raw oysters from the Pacific Northwest resulted in a major disease outbreak of *V. parahaemolyticus* that caused 210 cases, including one death from septicemia (CDCP 1998). The *V. parahaemolyticus* strains involved (O1, O4, and O5) were serogroups commonly found in the U.S., and there was reason to believe that their increased prevalence in oysters harvested from Pacific NW waters may have been associated with increased water temperatures caused by the El Niño Southern Oscillation. The following year, *V. parahaemolyticus* appeared again, this time predominantly in oysters harvested from Galveston Bay, Texas, and it exclusively involved an Asian serogroup (O3:K6) not reported from the U.S. since 1972 (DePaola et al. 2000). The 1998 “VP” outbreak was the largest ever recorded in the U.S., involving over 500 individuals but no deaths. This time, there was reason to believe that the exotic “VP” may have been introduced through ship ballast water.

CONCLUSIONS

It has been documented that a shift in the culturable community of heterotrophic bacteria in the Atlantic Ocean occurred sometime in the late 1970s. A variety of influences may have caused this shift, including anthropogenic nutrients – both organic and inorganic. The resulting “climax” bacterial community is characterized by a predominance of *Vibrio* species, including autochthonous vibrios pathogenic for humans and fishes. Global climate change may have also influenced, either directly or indirectly, this bacterial community-shift. It is hypothesized that the chemicals which cause bacterial community-shift also stress potential animal hosts (e.g., fishes), thereby allowing the increased densities of opportunistic pathogens to easily invade, colonize, and cause disease in their hosts. With regard to human hosts, demographics (e.g., most people live close to the coastal ocean) and food preferences (e.g., seafood is becoming increasingly popular) are also contributing to risk. Overall, it would appear that anthropogenic influences – inorganic nutrients, toxic chemicals, organic chemicals, greenhouse gases, global transportation – are influencing the sources, distribution, and conveyance of opportunistic pathogens in estuaries and the oceans.
LITERATURE CITED


Potential Pathological Effects of Blood Flukes (Digenea: Sanguinicolidae) on Pen-reared Marine Fishes

STEPHEN A. BULLARD and ROBIN M. OVERSTREET
Gulf Coast Research Laboratory
Department of Coastal Sciences
The University of Southern Mississippi
Ocean Springs, Mississippi 39564 USA

ABSTRACT

Sanguinicolidids, or fish blood flukes, infect the vascular system of both marine and freshwater fishes, and some act as serious pathogens of hosts in aquaculture. Blood flukes typically possess a relatively benign relationship with wild fishes; however, cultured hosts near appropriate intermediate hosts (i.e., snail, bivalve, or polychaete) may accumulate heavy infections of the worms and their eggs. The resulting disease, sanguinicoliasis, has caused mass mortalities of fish reared in ponds and cages in North America, Europe, and Asia. In the life cycle, the cercaria emerges from the intermediate invertebrate host and penetrates into and matures in the definitive fish host, and the resulting adult releases eggs into the fish’s vascular system. These eggs may be sequestered in gill, heart, kidney, liver, spleen, pancreas, or other organs, where they cause inflammation and decrease the physiological and mechanical efficiency of these organs. In some cases, they kill the host. Treatment of debilitated fishes is difficult, and the combination of stock destruction and facility disinfection is a realistic option for managing cases in freshwater systems. However, because this is usually not possible in marine systems, early detection and identification of the parasite, careful site selection and construction of culture facilities, and elimination of infected hosts (definitive or intermediate) are important. Much information needs to be acquired about each parasite’s biology and geographic range, and imported fishes and fish products should be quarantined or examined fresh for infections prior to potential contamination of culture systems.

KEY WORDS: Aquaculture, disease, parasite

INTRODUCTION

Blood flukes of fishes, sanguinicolidids, represent an unusual group of digeneans because their life cycles lack a second intermediate host and an encysted or encapsulated metacercaria. In addition, unlike all other families of flukes, members of which have a molluscan first intermediate host, some blood flukes utilize a polychaete rather than a snail or bivalve (e.g., Køie 1982, Smith 1997). Not having to eat a second intermediate host means that to complete a life cycle depends on the proximity of definitive and intermediate hosts to the free-swimming infective stages of the parasite. Manipulating the location of the fish host to its fluke’s intermediate host, such as occurs in some aquaculture activities, can easily dictate infections, which in turn produces sickness or death of the fish host. Understanding the life cycles, seasonality of the cycles, specificity of the flukes, immunological responses of the fishes, and environmental interactions with the infections allows a biologist and manager to assess better the potential for infections and the resulting associated
disease sanguinicoliosis. Dwindling wild stocks of marine fishes and increased demand for fish products has catalyzed aquaculture production, but sanguinicoliosis could threaten the economic growth and sustainability of the industry. In the present paper, we summarize the biology and host-parasite associations, discuss some physical and biological factors that affect blood fluke infections in confined fishes, and describe some pathological alterations to host tissue caused by blood flukes in both wild and captive fishes.

**Fish Blood Flukes**

Sanguinicoloids comprise a morphologically and ecologically diverse family of flukes (Platyhelminthes: Digenea) that infects both marine and freshwater fishes and is usually reported from large blood vessels such as the heart, branchial vessels, or mesenteric vessels. They are roughly 2 - 11 mm in total length, thin-bodied, dorsoventrally flat, narrow and elongate or oval in shape, and opaque or nearly transparent in life. Adults of some species are concave and capable of using the ventral surface of the body as a suction cup that allows them to adhere to smooth surfaces such as the walls of arteries, veins, or even a petri dish. Adults of many species possess tread-like rows of lateral tegumental spines that facilitates both attachment and rapid motion. Adults of other species possess minute spines and partially embed themselves in the wall of the heart. Some blood flukes are extremely active, and, if removed from heart and placed in a dish with physiologic saline solution, they rapidly crawl or swim about and make quick, repeated, flapping body movements. Live specimens are therefore relatively easy to notice and collect because the movements signal their location in a dissection tray or petri dish. They differ from mammalian blood flukes because they are usually wider and are hermaphroditic rather than having separate males and females. The tegument of many fish blood flukes is delicate and deteriorates rapidly after host death. This, along with the transparent appearance of many sanguinicoloids and the atypical sites they infect, results in many being overlooked during parasitological examinations.

In the Gulf of Mexico, just as in most regions around the world, there presently exists little information on blood fluke diversity, host ranges, life cycles, intermediate hosts, geographic ranges, and pathogenicity. Only four adult sanguinicoloids belonging in three genera are known from six Gulf of Mexico fishes, an adult blood fluke has not been described from a Gulf fish in 46 years, none has been described from the northern Gulf west of Mobile Bay, a study on the distribution or seasonality of any blood fluke in a Gulf fish population has not been conducted, and few details of wild host-parasite relationships have been described (e.g., Overstreet and Thulin 1989).

**Life Cycle**

Life cycles for most blood flukes are cryptic, making eradication or control of the parasites in aquaculture difficult. The life cycles may be challenging to study because a definitive host's diet does not necessarily indicate the probable intermediate host; however, comparisons of molecular sequences from various cercariae and adult flukes could elucidate the cycles. Of the roughly 60 known marine species, the life cycle of only one has been demonstrated experimentally (Koie 1982). The blood fluke *Aporocotyle simplex* develops in the terebellid
polychaete intermediate host (*Artacmaproboascidea*) before infecting the flatfishes *Limanda limanda*, *Pleuronectes platessa*, or *Platichthyes flesus*. Life cycles of several freshwater blood flukes are known, and each of these also lacks a second intermediate host (Schell 1974, Hoffman et al. 1985, Kirk and Lewis, 1992, 1993). The following serves as a generalized life cycle based on those studies (Fig. 1). Adult flukes deposit thin-shelled, pliable eggs in the vascular system of the definitive host. Some eggs travel to and lodge in the fish's gill, where the embryo develops in and hatches from the egg. After hatching, the first free-swimming form of the fluke, the miracidium, emigrates through the gill epithelium and searches for an appropriate snail, bivalve, or polychaete intermediate host. After penetration of the intermediate host, the parasite undergoes asexual reproduction. A sporocyst or redial stage may produce the second free-swimming infective stage, the cercaria, which is shed from the intermediate host and either infects an appropriate fish host or dies. The cercaria penetrates the fish host through its gill, skin, eye, fin, or alimentary tract and develops through the juvenile stage as a schistosomule before ending up in a specific site in the circulatory system of the host. Often in the heart or branchial vessels, the fluke ultimately matures, copulates, and releases eggs. We suspect that these sites are advantageous for egg deposition because eggs released there immediately are carried to the gill; however, adults of other fish blood flukes occur in vessels involving the mesentery, intestine, kidney, and brain (e.g., Smith, 1972, 1997), and perhaps eggs of these flukes exit the fish host from different sites. Further investigations are needed to determine other pathways for eggs. For example, they may pass through the intestine, as shown by schistosomes and sporocysts, blood flukes that infect mammalian and reptilian hosts, respectively. On the other hand, *Cruoricola lates* infects the vessels of the mesentery and other visceral organs but miracidia seem to develop in the gills and heart only.

**Dynamics of Life Histories**

Some blood fluke life cycles exhibit seasonal development, and monthly data on the prevalence and intensity of these infections could elucidate details about the life history of sanguinicolid and dictate schedules for both stocking and harvest of cultured fishes. Some blood flukes migrate to branchial vessels and lay eggs during a specific season, creating peak periods of intense infections and subsequent fish kills (e.g., Ogawa et al. 1989). On the other hand, Ewens et al. (1994) showed that the adult of *Sanguinicola inermis* overwintered in wild common carp and released eggs in the spring. Hine (1978) readily observed adult specimens of *Paracardiocoleides yamaguttii* in the gills of eels (*Anguilla australis* and *Anguilla dieffenbachii*) only when the flukes migrated there to lay eggs in spring and autumn. Maillard and Ktari (1978) found that the uterus of adult specimens of *Hyperandrotrema cetorhini* collected from a basking shark (*Cetorhinus maximus*) either lacked eggs or possessed only a few deformed eggs, suggesting that viable eggs were produced during a specific period other than when collected. Of course, that host could have been an abnormal host. Concurrent infections of the blood flukes *Paradeontacylix grandispinus* and *Paradeontacylix kampachi* caused mass mortalities of cage-cultured greater amberjack from December 1983 to 1984 (Ogawa et al. 1989). Later study of that relationship showed that the number of blood fluke eggs in the gill entered the gills in November and increased until March, when it decreased
toward July (Ogawa et al. 1993). An interacting set of abiotic factors probably drives this apparent seasonality, but knowledge on how each of those factors affects the miracidium, schistosomule, and adult for any species is scant, if known at all. Abiotic factors such as water temperature and salinity probably significantly influence the behavior and physiology of blood fluke miracidia and cercariae as well as the complex of hosts (e.g., Overstreet 1982). Because these factors are site- or season-dependent, information on how they affect the free swimming infective stages of blood flukes could be invaluable in selecting culture sites and in predicting seasons in which epizootics are likely to occur. Ewens et al. (1994) reported that under laboratory conditions the life cycle of S. inermis was dependent on temperature, and Smith (1997) stated that discrepancies in the times for penetration and migration of S. inermis as reported by several workers were possibly a result of different temperatures for host maintenance. Temperature also appears to dictate developmental time of the schistosomule. It and other features such as site in the host probably affect how this stage can influence health of the host. Examples expressing different sites for this preadult stage developing between the cercaria and adult, taking the place of the metacercaria of other digeneans, include the dermis for S. inermis in the carp. The schistosomule remains in the dermis much longer at low compared to higher temperatures. Adults occur in branchial arteries, bulbus arteriosus, and aortas (Kirk and Lewis 1993). The juvenile of S. klamathensis occurs throughout the circulatory system of O. clarki but is restricted to the efferent renal vein as an adult (Meade 1967), and that of A. simplex occurs in the lymphatic system, under the skin, and between muscles containing light yellow gut contents whereas adults in blood has yellow-brown contents. In its flatfish hosts, the adult of A. simplex infects the heart, ventral aorta, and gill arteries (Kaele 1982). Seasonal assessments of the effect of salinity on different blood flukes infecting cultured hosts are necessary. For example, at the time of this manuscript's preparation, salinities in the northern Gulf of Mexico (e.g., Gulf-proper, Mississippi Sound, Back Bay) were higher than they had been for many years, and seemingly there was a greater prevalence of adult blood flukes in the heart of 1- to 3-year-old red drum and black drum from those waters as well as a few infections in other hosts not seen previously.

Host specificity

Knowing which host or hosts a blood fluke can infect helps identify aquaculture candidates that might be vulnerable to developing sanguinicoliasis and those that are not. Some parasites infect a wide range of fish hosts, while others apparently infect only a single fish species. Parasites that infect one or relatively few hosts are more “host specific” than those that infect several hosts. A list compiled by Smith (1997) shows that of the roughly 76 described sanguinicolids (20 freshwater, 56 marine), 53 (70%) have been reported from a single host, 11 (14%) from two hosts, 5 (7%) from three hosts, and 7 (9%) from four or more hosts. Those flukes from more than one host infect hosts in the same genus or family. However, exceptions, including experimental infections, occur. For example, Sanguincola fontinalis probably is restricted to the cyprinid Rhinichthys cataractae (longnose dace) in nature, but it can infect the brook trout, Salvelinus fontinalis. Because it killed so many brook trout in culture and was refractive to a variety of other salmonids, the contaminated
Pennsylvania fish hatchery discontinued producing brook trout. This example illustrates the implications of "host specificity" for aquaculture where infective cercariae could infect an abnormal host. Further collections of blood flukes from different-aged fishes and during different seasons are needed to accurately assess identifications and host range; susceptible hosts may not be infected throughout the year. The true host range of a blood fluke could be underestimated if hosts were sampled only once or during a season of low prevalence of infection.

Figure 1. Generalized sanguinicolid life cycle. A. The adult fluke in the vascular system of the definitive host, a fish, releases eggs into the blood. B. The first free-swimming stage of the fluke, the miracidium, hatches from the egg in the gill, emigrates through the gill epithelium, and seeks and penetrates the appropriate intermediate host. C. In the intermediate host (snail, polychaete, or bivalve), asexual reproduction (mother and daughter sporocyst or redial stage) ultimately produces the second free-swimming stage, the cercaria. D. The tailed cercaria is shed from the intermediate host and penetrates a definitive host. In the fish, it loses its tail, develops to an adult as a schistosomule, and finally migrates to the specific site in the circulatory system of the host where it copulates and produces eggs.
Immunity

At least some fish have a documented immune response to sanguinicolids. Presumably, all hosts have significant immune responses, but the responses probably differ according to a number of factors. A series of papers by Richards et al. (e.g., 1994, 1996a) has provided considerable information on the response of the carp to *Sanguincola inermis*. The fish clearly elicits a cellular response against the adult, egg, and cercaria, involving eosinophils, neutrophils, and macrophages (Richards et al. 1994, 1996a). Lymphocyte production was experimentally enhanced in response to both cercarial and adult extracts at 20°C. At 10°C, the adult extract produced a greater response. Perhaps the reduced ability of the lymphocytes to proliferate in carp at lower temperatures in response to the fluke extracts suggests an impairment of the immune system. This can serve the parasite in a number of ways. It would allow the adult worm to overwinter, since at the higher temperature the adult may survive a couple of months only (Kirk and Lewis 1993). Periods of cool weather also correspond to periods of cercarial shedding, enhancing cercarial penetration into host and survival of the young worm. The proliferation of lymphocytes in carp is even more complicated because at 10°C, it occurs in the pronephric (kidney) and splenic lymphocytes. But at 20°C, most proliferation occurs in the pronephric cells. Perhaps during warmer months, specific humoral immune responses mediated by kidney lymphocytes influence infections, but during cool months, more non-specific responses, probably mediated by T-cells in the spleen, predominate (Richards et al. 1996b). The ability of leucocytes to adhere to cercariae and encapsulate and degrade eggs appears to be inhibited by live adult worms (Richards et al. 1996a). Other immune responses also play a role in the relationship between carp and blood fluke as well as those of other fish and their parasites. As a marine example, Ogawa et al. (1989) presented data on prevalence and intensity of sanguinicolids infecting young and old samples of greater amberjack in a net pen. Individuals less than 1-year-old had a relatively high number of eggs in cardiac muscle and gill, suggesting that the older fish acquired some level of immunity to infections after receiving an initial exposure to the flukes a year earlier. Further studies are needed; however, individual hosts may respond to infections differently, and infection intensity and abiotic factors probably have an interacting effect on the host's immune response. In summary, little is known about specifics of the immune responses directed at sanguinicolids, but the responses differ among species, differ between other digeneans, and presumably play an important role in infections in both wild and cultured fishes.

Wild host-parasite relationships

Relatively few digeneans reportedly cause significant disease of fishes in the wild, and blood flukes are no different. Based on what is known on sanguinicolid life cycles, the strategy of debilitating or killing the definitive host should not increase transmission within a host population. For most species, a few pliable eggs are released on a seasonal basis. Most of the eggs become entrapped in gill tissue. For those species investigated, the miracidium develops in that location. For *S. inermis*, the miracidium develops and is released, leaving the fish within 7 days (Kirk and Lewis 1993). Eggs that do not accumulate in the gills typically become lodged in viscera or connective tissue where the host's inflammatory granulomatous
response encapsulates them and degrades the miracidium. Overstreet and Thulin (1989) investigated the heart and tissues of a large number of marine teleosts and found that most sarranids contained an abundance of macrophage aggregates (MA's) in the ventriculus, and they and a few other fishes contained free macrophages among ventricular trabeculæ. This unusual abundance of macrophages in these locations appeared to have developed as a means to contend with blood flukes. One sanguinicolid, *Pearsonellum corventum*, in its sarranid host *Plectropomus leopardus* causes minimal harm to the wild host (Overstreet and Thulin 1989). In the heart, the macrophages along with neutrophils and lymphocytes surrounded the eggs as well as the adult worm, but no fibrotic encapsulation was evident. Eggs that ended up in the ventricular epicardium, pericardium, aortic serosa, mesentry, liver, spleen, and kidney were mostly encapsulated and being degraded in small granulomas, some associated with ceroid or MA’s. Moreover, the substantial cellular response of most sarranids and a few other fishes also serves to sequester a variety of parasites, prey spines, entire eels, and other foreign bodies within heavily pigmented fibro-encapsulations (Overstreet and Thulin 1989).

We observed sanguinicolid eggs in cardiac muscle, gill epithelium, and liver of several wild-caught fish, including the red snapper (*Lutjanus campechensis*) from the northern Gulf of Mexico (Figures 2-6), and they were like most cases in the literature. Eggs in cardiac muscle, which contained no MA’s, and liver were not abundant and typically present in various stages of degeneration (Figures 2-4). Recently deposited eggs in early stages of degeneration were encapsulated by a thin epithelial or fibroid layer (Figure 2). More degenerated eggs exhibited some yellowish ceroid pigment (Figure 3). Some eggs in a later phase of degeneration exhibited more conspicuous light to dark brown ceroid pigment (Figure 4). Cardiac muscle surrounding the degenerated eggs appeared normal except for invading leucocytes. Based on these observations, we doubt these eggs caused any significant decrease in the mechanical efficiency of the heart. Eggs in the liver primarily occurred in pancreatic nodules and were surrounded by a granulomatous response. (Figure 5). Sequestered eggs in the gill mostly contained living miracidia and were readily apparent by their refractive excretory products in fresh wet-mounted preparations (Figure 6). We estimated that fewer than ten of these thin-shelled, elongated eggs in the afferent vessels infected each filament of the wild-caught red snapper. For comparison, eggs in wild-caught red drum (Figure 7) differed by being more spherical, but neither elicited a severe host response.

Not all mature blood flukes occur in the heart and vessels leading to the gills. *Plethorchis aca nutus* from the intestine and mesenteric pancreas of wild-caught mullet (*Mugil cephalus*) from Queensland, Australia, provide a good example. In some geographic areas, most of the mullet exhibited infections with a grossly apparent host response. Histologically, the adult expanded the vessels and accommodated large numbers of surrounding leucocytes (Figure 8). Large clusters of eggs with the associated host inflammatory response occluded both large and small vessels (Figures 9-13). A given fish typically demonstrated both recently deposited eggs containing miracidia and a slight granulomatous response (Figure 10) and more advanced cases with eggs in various phases of degeneration (Figures 11-13). The more advanced cases, especially when heavily infected, typically exhibited
an extensive inflammatory response involving ceroid pigmentation. Perhaps some eggs with infective miracidia of this species are voided through the intestine.

The host response to fish blood flukes varies according to host species, site in the host, intensity of infection, season, and environmental conditions. Little information exists regarding the effect of these flukes on wild host populations, but some flukes may weaken their hosts, making them vulnerable prey and reducing their populations, especially during certain seasons. However, probably few wild host-parasite associations significantly influence population-structure.

Cultured host-parasite relationships

Confined fishes are either more or less prone to sanguinicoliasis than wild fishes. The infection, whether the parasite is present, and the disease, whether the parasite is present in high enough density to harm the host, depend on the closeness of the intermediate host to the fish host. As indicated above, wild fish get infected, but disease rarely occurs. Freshwater pond conditions that promote the presence of an intermediate host to be present therefore promote infections and disease. Marine net pens can be positioned such that the two hosts remain distant, preventing infections. On the other hand, marine fishes in ponds could easily get infected, if the intermediate host was one that could live in the pond habitat.

When confined fish come in contact with the cercariae, they probably will come in contact with large numbers. The more cercariae that penetrate a fish, the more likely it is to be detrimentally affected or die. Death can result from acute or chronic conditions. Acute cases can involve large numbers of either cercariae or miracidia. As an example, when 2,500 cercariae of S. inermis are exposed to carp, they produce in the fish severe edema, epidermal hemorrhage, and death within a few hours (Kirk and Lewis 1992). When lower numbers are exposed, the effects are reduced, and fish do not die. When large numbers of miracidia exit a host simultaneously, there can be severe hemorrhaging of the secondary lamella or elsewhere in the gills, with resulting mortalities. In contrast to the acute conditions, chronic conditions kill or debilitate hosts over a longer period. This disease usually results from the effects of a large number of eggs and associated host response occluding blood vessels. Consequently, various vital organs and tissues lose their blood supply, and the fish can die from a variety of specific causes.

As with the lodging of fluke eggs in wild fish, those in confined fish may be more numerous, accumulate faster, trigger a stronger immune response, form a more effective blockage, and result in greater infarctions and more reduced blood flow and hypoxia. Moreover, in some cases, the host can have severe reactions to the adults or juveniles, which can then cause blockage. We also see a greater need to investigate the effect of temperature on the health of the fish. One should consider the possibility of various scenarios. Assume that large number of eggs are lodged in vessels during a period of low temperatures. The fish might be sluggish from the temperature, but adequate blood flow would exist for most purposes. Now when the temperature increases, the host's immune response becomes active, and its cellular response produces masses of leucocytes, fibroblasts, and other components that quickly seal the vessels. The fish then dies as a compounded result of the increased temperature.
Figures 2-7. Sanguinicolid eggs in wild fish from Mississippi. Figs. 2-6. In red snapper. 2. Egg surrounded by few inflammatory cells in cardiac muscle. Scale bar = 30 μm. 3. Early granuloma involving two degenerating eggs in cardiac muscle. Scale bar = 30 μm. 4. More developed response with ceroid pigment surrounding egg in cardiac muscle. Scale bar = 30 μm. 5. Degenerating eggs in pancreatic nodule in liver, showing slight ceroid response surrounding eggs (arrows). Scale bar = 75 μm. 6. Elongated egg (arrow) in fresh preparation of gill tissue with live well-formed miracidium. Scale bar = 75 μm. 7. Spheroidal fresh egg in gill of different species in red drum. Note well-developed miracidium (arrow). Scale bar = 30 μm.
Figures 8-13. *Plethorchis acanthus* and its eggs in mesentery of wild striped mullet from Queensland, Australia. Sections are stained with hematoxylin and eosin except Fig. 9, which is stained in PAS. 8. Adult worm (w) and clusters of eggs surrounded by inflammatory cells in dilated mesenteric vein. Scale bar = 115 µm. 9. Eggs occluding wide and narrow mesenteric veins (arrowheads). Scale bar = 345 µm. 10. Early granuloma involving large cluster of eggs. Scale bar = 75 µm. 11. Granulomas containing clusters of eggs and surrounded by intensive leucocytic response. Scale bar = 115 µm. 12. Ceroid involvement in epithelioid granuloma. Scale bar = 115 µm. 13. Late fibroid granulomas surrounded by packed leucocytes and earlier granulomas (arrowheads) still containing egg fragments and ceroid response in completely occluded vein abutting intestine (i). Scale bar = 345 µm.
Pen and cage culture offer an excellent potential for development of sanguinicoliosis. If the intermediate host can develop within its cercaria's range of the confined fish, and this might be enhanced by the feed and waste products from the system, then fish can get infected. The intermediate host could obtain the initial infection from a wild fish. There is also the possibility that a blood fluke not normally infective to the reared organism could infect it under specific conditions.

Sanguinicoliosis was reported in a cage-cultured marine fish (greater amberjack, *Seriola dumerilli*) by Ogawa and Egusa (1986). Mass mortalities of this amberjack maintained in shallow-water floating net cages off the coast of Shikoku Island, Japan, occurred between December and March. Gill filaments of heavily-infected dead fish contained more than 1,000 eggs of *Paradeontoclytix grandispinus* and/or *P. kampachi* per filament, causing gill hyperplasia and extensive papillae formed from proliferation of endothelium in the afferent branchial arteries (Ogawa et al., 1989). Nodules of encapsulated eggs in the gill obstructed blood flow through afferent arteries, and those in the ventricle wall were surrounded by atrophied muscle. Heavily-infected fish gasped at the water surface and died soon after being fed, suggesting that the increased oxygen demand during feeding could not be met. A subsequent mass mortality occurred in May 10 years later off Kyushu, Japan, with fish exhibiting similar signs (Ogawa and Fukudome 1994). Crespo et al. (1992) reported mass mortalities of 0- and 1-year-old cultured greater amberjack from the Spanish Mediterranean Sea off Majorca, Catalonia, and Murcia. They attributed the primary cause of death in the 0-year-class fish to epitheliocystis, but one of the same or a related blood fluke was present, and some of the older infected fish without epitheliocystis also died. Other blood flukes infect marine and estuarine fishes in culture. For example, *Crucorica lates* in the centropomid *Lates calcarifer* in Malaysia (Herbert et al. 1995) occurs commonly in vessels of the mesentery, kidney, pericardium.

Cases of sanguinicoliosis in freshwater ponds from North America, Europe, and Asia also provide good examples because 1) they show what could happen in saltwater ponds where confined fish have a continual exposure of the larvae from an extraordinarily high infection of the abundant intermediate host and 2) they demonstrate the variation in disease signs in different fishes and from different flukes.

For example, Hoffman et al. (1985) reported a mass mortality of many of the 400,000 infected brook trout (*Salvelinus fontinalis*) caused by *Sanguinicolis fontinalis*. Severely infected fishes exhibited affected gills, but also damaged the kidneys. The latter demonstrated generalized nephrosis and moderate edema, with accumulation of basophilic material in both Bowman's capsule and tubule lumina. For lack of an effective treatment, all heavily infected fish had to be destroyed. That blood fluke was just one of the five known to infect North American salmonids: *Sanguinicolis davisi*, *Sanguinicolis klamathensis*, *Sanguinicolis alseae*, and *Sanguinicolis idahoensis*. Davis et al. (1961) reported mortalities of fingerlings of *Oncohrhynchus clarki* and *Oncohrhynchus mykiss* infected by *S. davisi*; they were anemic, and their gills were damaged by fluke eggs and exiting miracidia. Evans (1974a, 1974b) showed that eggs of *S. klamathensis* in *O. clarki*, when deposited over a period of time, produced progressive alterations in gills and kidney, resulting
in poor weight gain and mortality. Schell (1974) reported that the eye of *O. mykiss*
heavily infected with *S. idahoensis* exhibited a bulging retina, thickened iris, and
disrupted vascular stroma in addition to affected gills.

Commercially valuable fishes such as the red drum (*Sciaenops ocellatus*), red
snapper (*Lutjanus campechanus*), cobia (*Rachycentron canadum*), Florida pompano
(*Trachinotus carolinus*), and greater amberjack are all likely candidates for net pen
culture in the Gulf of Mexico. These plus other fishes host a blood fluke
(unpublished data), and awareness and understanding of these and the corresponding
diseases are needed so that these potential fish resources can be managed in an
economically and environmentally sound manner.

**Control and Management**

Control of infections of blood flukes in culture systems can be easier than
controlling most parasites with direct life cycles, those without intermediate hosts.
Nevertheless, a good management strategy involves keeping intermediate and
definitive hosts separate. Early detection and identification of a blood fluke could
be essential for elimination of susceptible intermediate hosts from the vicinity of
fishes in pens, ponds, or raceways. We advocate regular monitoring of several fish
from each group to assess all parasites and diseases, including blood fluke infections.
Infected fishes such as brood stock or fingerlings should not be transported to other
facilities, and inspection for blood fluke infections in each facility could help reduce
or halt the spread of disease from facility to facility by movements of fish.

Elimination of susceptible intermediate hosts could protect enclosed fishes
against sanguinicoliosis, and molluscicide application has been suggested for ponds
(Smith 1972, 1997). However, such application would probably be impractical and
cost-prohibitive for marine pens or cages. Moreover, biodiversity of endemic fauna
inhabiting sites below and near containments should be preserved and protected.

As demonstrated in other culture systems, biosecurity is needed in aquaculture
with regard to blood flukes, and quarantine protocols should be defined,
implemented, and enforced to protect cultured and wild fishes. Introduced parasites
can become pathogens of local, endemic fishes (Overstreet, 1990). Because of this
threat, imported fingerlings, brood stock, and fish products including frozen fish
should be quarantined until a subsample can be examined carefully for the presence
of either blood fluke eggs or adults or other parasites. For example, Ogawa and
Fukudome (1994) suggested that infected greater amberjack imported from China
eventually caused mass mortalities of that fish in Japanese culture systems.
Anderson and Shabaram-Harrison (1986) believed that *Sanguinicola armata* was
brought into Malaysia by importation of fingerling bighead carp (*Aristichthys
nobilis*) and grass carp (*Ctenopharyngodon idella*). Kirk and Lewis (1994) reported
that *S. inermis* was introduced to Britain when infected fishes were imported from
continental Europe in the 1950s and 1960s, and that the fluke has since spread to
various British rivers by movements of fishes that were caught for display or
recreation and subsequently released. Infections of *S. inermis* have been sustained
in British rivers and hatcheries because the intermediate hosts occur abundantly
there. Inadequate pre-transfer protocols were blamed for subsequent mass
mortalities in hatcheries. Studies on sanguinicoliosis among net-penned fishes in the
Gulf of Mexico are currently not possible because there are few, if any, pens or
cages. Nevertheless, now is the time to develop adequate protocols for preventing the spread of sanguinicoliasis based on studies of local wild parasite-host interactions combined with those investigating sanguinicoliasis in hatcheries and culture systems elsewhere.

Capture Fisheries and Aquaculture

Marine fishes traditionally have been overexploited. Some assessments are controversial, but 30-year trends show that stocks of most capture fisheries are eventually overfished. Presently, 44% of the major marine fish stocks are fully exploited and producing catches at their maximum limit; 16% are overfished with no room for increased production; 6% are depleted with declining production; and 3% are recovering slowly (FAO 1999). Capture fisheries reached their maximum production levels 10 - 20 years ago, and catches of some of those same stocks have now declined (FAO 1999). The need for alternate sources of fish products is becoming increasingly more evident.

The marine aquaculture industry expects to supplement the demand for fish products and relieve some pressure from wild stocks, as already demonstrated by the freshwater aquaculture industry. This would allow some wild stocks to begin recovery and increase above threshold levels. Humans consume predominantly wild-caught marine fishes; however, this may soon change. The aquaculture industry now includes greater than 220 species of finfish and shellfish, global fish and shellfish production more than doubled to 28 million metric tons between 1987 and 1997, with 40% from marine sources, (FAO 1999, Naylor et al. 2000), and aquaculture provided 22-29% of all fish consumed by humans in 1996 (New, 1997; FAO 1999). Growth of the industry will continue; however, with expansion comes disease, and sanguinicoliasis is an important one.

CONCLUSIONS

Blood flukes may debilitate or kill cultured fishes, and studies on sanguinicolid diversity, geographic range, host specificity, life histories, and host-parasite relationships all should be incorporated in cost-effective, environmentally-sound management practices for the emerging aquaculture industry in the northern Gulf of Mexico. The strategy for control of sanguinicoliasis in the Gulf or any marine habitat should include rigorous biosecurity measures consisting of quarantine and subsequent necropsies of subsamples of imported fishes and fish products, monitoring of local cultured stocks for the presence of blood flukes, specific identification of blood flukes from these stocks, and implementation and enforcement of quarantine protocols.

ACKNOWLEDGMENTS

We thank Ronnie Palmer for laboratory assistance; Kim Lamey and Marie Wright for sectioning and staining tissues; Kim Overstreet for reading a draft and translation services; and Dale Little, Catherine Schloss, Joyce Shaw, and Marjorie Williams for library assistance. This study was supported in part by a fellowship awarded to SAB by the Mississippi-Alabama Sea Grant Consortium and National
Oceanographic and Atmospheric Association, National Marine Fisheries Service, award No. NA96FL0358.

LITERATURE CITED


Koeie, M. 1982. The redia, cercaria and early stages of Aporocotyle simplex Odhner, 1900 (Sanguinicolidae)- a digenetic trematode which has a polychaete annelid as the only intermediate host. Ophelia 21:115-145.


Richards, D.T., D. Hoole, J.W. Lewis, E. EWens, and C. Arme. 1996b. Stimulation of carp Cyprinus carpio lymphocytes in vitro by the blood fluke Sanguinicola...


Factors Contributing to the 1999 Mass Mortality of Reef-Associated Fish in Barbados

STEPHAN WILLOUGHBY¹, C. PARKER¹, W. HUNTE², V.S. ST. JOHN³, C.J. ROACH², and H. FERGUSON⁴

¹Fisheries Division
Ministry of Agriculture and Rural Development
Princess Alice Highway
Bridgetown, Barbados
²School for Graduate Studies and Research
University of the West Indies
Cave Hill, St. Michael, Barbados
³Veterinary Diagnostic Laboratory
Ministry of Agriculture and Rural Development
Pine Plantation, St. Michael, Barbados
⁴Institute of Aquaculture
University of Sterling, Scotland

ABSTRACT

During the period August to November 1999, mass mortalities of several species of reef-associated fish were reported at a number of islands in the southern Caribbean, including Tobago, Grenada, Barbados, St. Vincent and the Grenadines. Histopathological and bacteriological studies conducted in Barbados revealed that the fish died from severe disseminated bacteraemic disease caused by the pathogen Streptococcus iniae which had not previously been reported in the marine environment in the southern Caribbean. Analysis of satellite imagery indicated that a plume of water originating in the region of the Orinoco River, and characterised by high chlorophyll concentrations and low nocturnal oxygen levels, impacted on Barbados and the neighbouring islands at the time of the fish kill. It is suggested here that several atypical environmental conditions, including elevated sea water temperature, high phytoplankton concentration and reduced oxygen levels worked in concert to stress the fish and increase their susceptibility to S. iniae.

KEY WORDS: Pathogen, Oxygen Depletion, Streptococcus iniae

INTRODUCTION

Barbados is the most easterly of the Caribbean islands. It is located at approximately 13°N and 59°W (Figure 1). During the period mid-September to mid-October 1999, large numbers of dead and moribund fish were deposited on the beaches in Barbados. In the early stages of the mass mortality, fish were deposited on the eastern and southeastern coast beaches, while towards the end of the event, dead fish also washed ashore along the northeast coast (Figure 2).
Figure 1. Map of the eastern Caribbean showing the location of the islands affected by the 1999 fish kill.

Several other islands in the southeastern Caribbean also verbally reported the beaching of thousands of dead and dying fish along their eastern and southeastern Atlantic coasts, from as early as August and continuing into November 1999. The islands reportedly affected were Grenada, St. Vincent, the Grenadine islands and Tobago, (Figure 2).

Local fish kills on the individual islands are common in the southeast Caribbean. Reports of these fish kills are not usually published, but the Fisheries Officials in Grenada, St. Vincent, Tobago and Barbados have confirmed the occurrence of such
events in their respective countries (pers. com. and Heileman Leo, and A. Siung-Chang 1990), while the 1994 mass mortality of reef-associated fish in Barbados was investigated and documented (Alleyne 1998).

\[\text{Figure 2. Areas of Barbados where fish were beached during the 1999 fish kill.}\]

The 1999 fish kill in the southeastern Caribbean was unusual in that it was regional affecting several islands almost simultaneously. The only previous report of a regional mass mortality of a marine organism in the Caribbean was that of the black sea urchin \textit{Diadema antillarum} in 1983 - 1984 (Lessios et al. 1984, Hunte et al. 1986). The mortality of \textit{D. antillarum} started in Panama and was distributed throughout the Caribbean by water currents. It is believed to be caused by a species-specific water-borne pathogen.
Based on the results of the bacteriological and histo-pathological examinations of freshly dead and moribund fish collected in Barbados during the 1999 fish kill, Ferguson et al. (in press) concluded that the fish died from severe disseminated bacteraemic disease caused by the pathogenic bacterium, *Streptococcus iniae*. *S. iniae* was isolated in pure culture from inflamed tissues of the gills, epicardium, myocardium, and liver. However, Herman (1990) emphasised that outbreaks of bacterial diseases are seldom the result of a single factor. Consequently, although the bacterium *S. iniae* was the pathogen that ultimately caused the death of the fish, predisposing factors were probably also involved in the epizootic occurrence.

This paper reports the observations made of environmental conditions existing during the 1999 fish kill in Barbados and assesses their possible roles as predisposing factors of the mass mortality.

**METHODS**

Information about the 1999 fish kill in the southern Caribbean was obtained from several sources, including reports of local and international governmental and non-governmental agencies, observations of personnel of the Barbados Fisheries Division and interviews with local fishermen, divers and members of the public. The Internet was used extensively to access relevant information from individuals, institutions and international agencies, and to obtain information on regional water quality characteristics during the period of the fish kill. The species of fish deposited on the beaches in the affected areas were identified, counted, and necropsies performed on sick, freshly dead and moribund fish samples. A detailed report of the post-mortem investigations of the fish is presented by Ferguson et al. (in press).

**OBSERVATIONS**

**Beaching of Dead Fish**

Available records kept by the Ministry of Health of the Government of Barbados, indicated that an estimated 40 - 45 tons of fish were deposited on the beaches and removed by the Ministry of Health, fishermen and residents, during the clean-up exercise. The fish deposited on the beaches are believed to be less than half of the total fish killed, since divers and fishermen reported that fish deposited on the reefs, and those being carried offshore by the currents and tides, were together more than those deposited on the beaches. Many more fish were observed on the beaches in the early mornings, before the beaches were cleaned, than were deposited during the rest of the day.

**Size and Species Composition**

Among the affected shallow water reef species, mortality was highest among larger individuals. Forty fish species from 20 families were among the fish beached during the fish kill. The majority of dead fish were surgeon fish (46%), followed by Bermuda chubs (22%), parrot fishes (7%), sea basses (5%) and triggerfishes (4%).
All other species together accounted for only 16% of the fish beached, with individual species in this group accounting for less than 3% of the fish beached. Deep reef fish, coastal and offshore pelagics, and shellfish were not among the organisms deposited on the beaches. Divers also confirmed that these groups were not among the dead fish seen on the seabed.

**Gross Anatomical Observations**

Examination of the freshly dead or dying fish from the affected area revealed:

i) There were few external lesions such as haemorrhages, ulcerations and/or lesions.

ii) Gills, gut and livers were pale in colour.

iii) The gastro-intestinal tract of most species sampled, except the Bermuda chub, were empty.

iv) The stomachs of the Bermuda chubs examined were filled with unidentified green algae.

v) In some fish, the gall-bladder was enlarged or ruptured; in others, there was an excess of bile in the stomach.

vi) The spleens were enlarged and congested.

**Presence of Discoloured Water**

Fishermen reported the presence of murky green coloured water prior to and during early stages of the fish kill in the areas along the southeast coast of Barbados first affected by the fish kill. According to fishermen, murky green water is seen on occasions along the southeast coast during late summer, but it usually disappears within a day or two. However, in September 1999, the murky green water lingered for an extended period. Pilots flying aircraft in the southeastern Caribbean during the fish kill episode reported seeing large masses of green water around the islands. The neighbouring islands of Grenada and St. Vincent, reported discoloured coastal waters during the fish kill episode (CARICOM 1999). Satellite imagery of the southern Caribbean taken during the fish kill - specifically September 14 - 21, 1999 (Figure 3), showed that Barbados and other islands affected by the fish kill were engulfed by a mass of water with chlorophyll concentrations an order of magnitude (10 X) higher than normal (seawifs.gsfs.nasa.gov). This water mass appeared to have originated in the area of the Orinoco river, Venezuela. Similar satellite imagery for the period immediately prior to the fish kill (August 29 to September 5, 1999; Figure 4) shows the water mass heading north towards Barbados from the Orinoco area, but Barbados still surrounded by typical oceanic water of low chlorophyll concentration. Satellite imagery for the period following the fish kill (October 8 -15, 1999; Figure 5), shows Barbados again surrounded by oceanic water, that is, no longer engulfed by the high chlorophyll water mass. In a typical September, the water mass originating from the Orinoco region tends to move south and west of Barbados and the Lesser Antilles chain, in to the Caribbean Sea (Figure 6).
Figure 3. Satellite pictures for the period 14-21 September 1999, showing Barbados engulfed by water of chlorophyll concentration (shown as white) as much as 30 times normal seawater concentration. Arrow shows location of Barbados. Source: seawifs.nasa.gov.

Plant Material in the Discoloured Water

During the early stages of the fish kill, when fishermen were reporting the presence of the murky green water, the beaches were being littered with organic and plant material, including large numbers of seedlings from an unidentified legume. The seedlings were not from a local legume (Carrington, S pers.com.). This suggested that the plant and organic materials were brought in from outside of Barbados. Grenada also reported floating plant materials in the discoloured water observed along their coasts during the fish kill episode (CARICOM 1999).

No harmful algae were found in water samples taken from affected coastal areas in Barbados during the latter stages of the fish kill. Water samples were not taken in Barbados during the early and peak stages of the kill. However, water samples taken off Tobago and St. Vincent during the fish kill, and analysed by agencies within those territories, showed no evidence of harmful phytoplankton known to be associated with fish kills (CARICOM 1999).
Figure 4. Satellite pictures for the period 29 August - 8 September 1999, showing a plume of water of high chlorophyll concentration (shown as green) to the southeast of Barbados. Arrow shows location of Barbados. Source: seawifs.nasa.gov.

Sea Surface Temperatures

Fishermen and swimmers in the near shore waters of Barbados reported that the seawater was noticeably warmer than usual. The Barbados Meteorological Office reported that unusually high sea surface temperatures (29° - 33° C) were recorded during September 1999, between longitudes 7° and 10° N and latitudes 55° and 60° W. These sea surface temperatures were reported to the Meteorological Office by ships in the area. Seawater temperatures near Barbados in August and September for 1993 - 95 varied between 28° and 29°C (CARICOMP).

Oxygen Concentration

Oxygen readings taken in Grenada and to a lesser extent in Barbados, during the fish kill detected values significantly lower than normal, particularly in the early morning (Hunte, W. University of the West Indies; pers. com.)
Unusual Currents

Just prior to the fish kill, tropical storm Gert passed through the southern Caribbean. In Barbados, it provided very high sea-swells and rough seas that pounded coastal areas, especially the east and southeast coasts. Prior to the first report of beaching of dead fish, divers and fishermen observed a change in current direction from the normal northwesterly pattern to a northeasterly flow. The observed change is believed to have been caused by the passage of tropical storm Gert and may have been responsible for the movement of the water mass from the Orinoco region north towards Barbados and the Lesser Antilles.

Discussion

The pathogenic bacterium Streptococcus iniae ultimately caused the death of the fish in Barbados during the 1999 mass mortality of reef-associated fish, the fish dying from severe disseminated Streptococcus sepsicaemia, (Ferguson et al. in press). Pathogen-caused fish kills in natural systems usually involve hosts that are
susceptible to the contagion by the specific pathogen and exposure to predisposing environmental conditions that increase susceptibility of the hosts to the pathogen (Hermen 1990). The observations presented in this report indicate that the reef-associated fish affected by the 1999 fish kill were exposed to several predisposing environmental factors that may have increased the fishes’ susceptibility to *S. iniae*.

**Figure 6.** Satellite imagery for the period September 1998, showing the plume of water of high chlorophyll concentration (shown as green) to the south and west of Barbados and extending into the Caribbean. Arrow shows location of Barbados. 
*Source: seawifs.nasa.gov.*

In most fish kill investigations, information on the environmental conditions that contributed to the mortality are not accurately assessed, as the conditions often cease to exist before the mortality is noted and the investigation has begun. Snieszko (1964) listed temperature stress, decreased immunological response, pollution, unfavourable water chemistry, inadequate food supply and storms as some of the predisposing environmental conditions associated with fish kills. Herman and Meyer (1990) identified oxygen depletion, toxic algal blooms and sudden or excessive temperature changes as some of the agents that cause fish kills. Herman and Meyer (1990) also listed several observations that are evidence of a fish kill caused by oxygen depletion. Among these are that fish mortality occurs mainly at night and
subsides during the day, that mortality rate is highest among larger individuals and that there is discoloration of water. These conditions were observed during the 1999 mortality event, indicating that low oxygen levels were a contributing factor to fish death, either directly or through increasing susceptibility to the pathogen. Oxygen depletion occurs when the total demand for oxygen by aquatic organisms exceeds the dissolved oxygen available in the surrounding water, and it is typically associated with heavy algal growth or high concentrations of organic matter or plant material (Herman and Meyer 1990).

Satellite imagery for the period of the fish kill (September 1999) shows Barbados engulfed by a plume of water that is of higher chlorophyll concentration than that of normal seawater (<seawifs.nasa.gov> 1999). The high chlorophyll content indicates the presence of large quantities of photosynthetic (phytoplankton, floating plant) material in the water. The murky green water that lingered around Barbados during the early stages of the fish kill was directly observed to be carrying plant and organic material, including seedlings that were not from local plants. The respiratory needs of the dense assemblage of phytoplankton, and the oxidative decay of the dead plant material, would have sharply reduced the supply of dissolved oxygen in the water column, making less oxygen available for other marine organisms. During the daytime, oxygen levels would have been at least partially replenished through photosynthesis. However, at night in the absence of photosynthesis, oxygen levels would not have been maintained. It is therefore expected that oxygen levels would have been lowest during the nighttime.

Excessive temperature changes are one of the environmental factors often associated with fish kills (Herman and Meyer 1990). Above normal sea surface temperatures were reported around Barbados, and throughout the southern Caribbean, during the period of the fish kill. The solubility of oxygen in water is inversely related to temperature. The above normal sea-water temperatures observed would therefore, have resulted in even lower concentrations of dissolved oxygen in the seawater, that is the creation of hypoxic conditions in the water column. Such environmental changes can stress fish to the extent that a weakening of the immune response may predispose the fish to infectious diseases (Herman and Meyer 1990). Larger fish have higher oxygen demands than smaller individuals and may therefore be expected to be more acutely affected by lowered dissolved oxygen levels. Once infected with S. iniae, hypoxia is especially critical in the toxemic fish. Consequently, sick fish, especially the larger ones, would be more likely to die during the night when hypoxic conditions were most severe. This is consistent with the observation that many more fish were found on the beach in the early hours of the morning than later in the day.

The death of the fish in the 1999 fish kill was apparently not immediate, since the stomachs and guts of the freshly dead and dying fish were often empty. This suggests that the fish exposed to stressful environment conditions and to S. iniae were probably sick and not eating for several days before eventually succumbing to the effects of the pathogen.
The mass mortality of a wide variety of reef fish species along the east and southeast coasts, the complete absence of *S. iniae* in fish outside of the affected areas, suggests that the affected reef fish do not normally carry *S. iniae*, and were immunologically naïve. Once introduced, *S. iniae* would spread rapidly through the naïve population, already stressed by the deteriorated environmental conditions. These observations suggest that the virulent *S. iniae* may have been entrained in the same invading water mass as the predisposing environment conditions, and would therefore be available to infect fish that had not previously been exposed to the pathogen. However, it is uncertain how *S. iniae* was actually transported. It is unlikely that it was transported in the water outside the body of a host, since *S. iniae* does not survive very long in seawater. This suggests the involvement of a host, presently unknown, in carrying the pathogen in the water mass to Barbados. Once the pathogen was introduced to the area, it may have entered the fish either via the gills from the seawater or by the fish eating contaminated carriers.

In concluding, the satellite imagery and other observational data presented in this paper support the suggestion that there was a significant deterioration of water quality at the time of the 1999 Caribbean fish kill. High sea surface temperatures and the high oxygen demand resulting from the influx of large amounts of photosynthetic material into the area, depleted oxygen levels in surrounding near-shore waters. Although the oxygen levels may not have been low enough to kill healthy fish, they were low enough to critically stress fish, increase their susceptibility to *S. iniae* infection, and increase the morbidity of the disease.

LITERATURE CITED


CARICOM. Draft Summary Proceeding of the CARICOM Regional Workshop on the Fish Kill Events, held on 16th October, 1999 in Barbados. In prep.


Susceptibility of *Litopenaeus vannamei*, *Farfantepenaeus duorarum*, to White Spot Syndrome Virus (WSSV) and Infection of *Menippe adina* with WSSV

M. ANDRES SOTO, VIRGINIA R. SHERVETTE, and JEFFREY M. LOTZ
Department of Coastal Sciences
University of Southern Mississippi, Institute of Marine Science
Gulf Coast Research Laboratory
P.O. Box 7000
Ocean Springs, Mississippi 39566-7000 USA

ABSTRACT

White spot syndrome virus (WSSV) can cause 100 % cumulative mortality to farmed shrimp, and there is increasing concern over the possible introduction of this virus into wild shrimp and crab populations in the Gulf of Mexico. In this contribution, we compare the mortality rate of WSSV infected *Farfantepenaeus duorarum* to *Litopenaeus vannamei*. In addition, we demonstrate that the stone crab (*Menippe adina*) is susceptible to WSSV infection.

We used an experimental procedure that is based on a mathematical epidemiology model to compare the survival of *F. duorarum* to *L. vannamei* from exposures to WSSV. The experimental procedure involved exposing 12 uninfected susceptible shrimp to a single infected shrimp cadaver for a specified period of time and then isolating the exposed shrimp individually to determine the number of deaths. *Menippe adina* were challenged by injection of a homogenate containing WSSV and exposed *per os* to WSSV infected tissue. The *L. vannamei* used in the experiment were obtained from the United States Marine Shrimp Farming Program, and *F. duorarum* and *M. adina* were obtained from the wild.

The mean mortality rate from a WSSV exposure was 0.81 for *L. vannamei*, and 0.75 for *F. duorarum*. A statistical difference was not detected in final mean mortality rates between *L. vannamei* and *F. duorarum*. From the *M. adina* challenge, two of the four crabs injected with WSSV died, and both of those were found to be histologically positive for WSSV associated lesions. In addition to the WSSV inclusions, basophilic, intranuclear inclusions were found in hypertrophied nuclei of hepatopancreatic cells which may be caused by another pathogen. Our results suggest *F. duorarum* is as susceptible to mortality from WSSV as *L. vannamei*, and that *M. adina* is susceptible to infections by WSSV.

KEY WORDS: Crustaceans, Gulf of Mexico, viruses

INTRODUCTION

White spot syndrome virus (WSSV) is a recently described shrimp pathogen that is devastating the shrimp farming industry. WSSV can cause 100 % cumulative mortality to farmed shrimp. Because WSSV is known to have a broad host range, there is concern over the possible introduction of this virus into economically
valuable shrimp and crab populations in the Gulf of Mexico.

Two of the known shrimp hosts of WSSV are *Litopenaeus vannamei*, white-legged shrimp and *Farfantepenaeus duorarum*, pink shrimp (Lightner 1998). *Litopenaeus vannamei* is the most commonly cultured species in the Americas and is one of the most commercially important species comprising the wild shrimp fishery along the Pacific coast of the Americas. *Farfantepenaeus duorarum* is one of three species of shrimp comprising the wild shrimp fishery in the Gulf of Mexico. *Litopenaeus vannamei* is known to be highly susceptible to WSSV, however there is some confusion as to the relative susceptibility of *F. duorarum* to WSSV. Results from two studies have demonstrated that larval *F. duorarum* are as susceptible to WSSV as larval *L. vannamei* and that juvenile *F. duorarum* are less susceptible to WSSV infections than juvenile *L. vannamei* (Lightner et al. 1998 and Wang et al. 1999). However, in the study by Wang et al. (1999) they mention that in a preliminary experiment, juvenile *F. duorarum* were found to be as susceptible to WSSV as juvenile *L. vannamei* (Wang et al. 1999). In addition, preliminary studies performed in our lab have found little difference in mortality rates between juvenile *F. duorarum* and *L. vannamei*.

*Menippe adina* is a commercially valuable species of stone crab in the Gulf of Mexico (Stuck and Perry 1992). Various crab species have been found to be susceptible to WSSV, but it is not known if *M. adina* is susceptible to WSSV.

In this contribution, we compare the mortality rate of *F. duorarum* to *L. vannamei*, and demonstrate that *M. adina* is susceptible to WSSV infection. In addition, we describe an inclusion in hepatopancreatic cells of *M. adina* that may be caused by a different naturally occurring virus.

**MATERIALS AND METHODS**

**Test Animals and Viral Stock**

The *L. vannamei* used in these experiments were obtained from the Oceanic Institute, Hawaii. These shrimp are from the original unselected population of shrimp (Kona stock) that have been maintained by the United States Marine Shrimp Farming Program (Lotz 1997). *Farfantepenaeus duorarum* and *M. adina* used for the experiments were captured in Mississippi Sound, Mississippi, USA. All shrimp used weighed between 2 and 3 grams, and *M. adina* weighed between 0.5 to 25.0 g. The isolate of WSSV used was obtained from mainland China in 1996 and has been maintained in *L. vannamei*.

**Experiment 1: Estimating survival rates for *L. vannamei* vs. *F. duorarum***

We used an experimental procedure that is based on a mathematical epidemiology model to compare the survival rates of *F. duorarum* to *L. vannamei* from exposures to WSSV (Soto and Lotz 2000).
In epidemiology models with the Reed-Frost approach to pathogen transmission, transmission from infected host to susceptible individuals is represented by the following equation:

\[ S_t = S_0 - S_0 (1 - \beta)^t, \]

where \( S_0 \) and \( I_0 \) are the number of susceptible and infected hosts, respectively at the beginning of some time period, \( S_t \) is the number of susceptible hosts after that time period, and \( \beta \) is the transmission coefficient which is the probability that a contact between a susceptible (\( S_0 \)) and an infected (\( I_0 \)) host will result in a transmission. By solving for \( \beta \), an equation for estimating the transmission rate is obtained:

\[
\beta = 1 - \exp\left(\ln\left(\frac{S_t}{S_0}\right)/\ln\left(\frac{I_0}{I_0}\right)\right).
\]

The transmission rate can be estimated from knowledge of the initial numbers of susceptible (\( S_0 \)) and infected (\( I_0 \)) shrimp and the number of susceptible shrimp (\( S_t \)) at the end of the time period of interest.

The experimental procedure involved exposing 12 uninfected susceptible shrimp to a single infected shrimp cadaver for a specified period of time and then isolating the exposed shrimp individually to determine the number of successful transmissions. The procedure for obtaining the transmission rate estimates is divided into three phases: preparation of \( I_0 \) (initially infected shrimp), exposure, and isolation.

**I₀ Preparation**

To prepare \( I_0 \) (the initial infected shrimp), we exposed two groups of 20 *L. vannamei* to WSSV. The shrimp were exposed in 115 L rectangular aquaria. Each aquarium was filled to a depth of 10 cm of chlorine disinfected seawater. Shrimp were exposed *per os* and received approximately 15 % body weight of frozen, minced cephalothoraces of shrimp known to have died of WSSV.

**Exposure**

In the exposure phase, susceptible shrimp (\( S_0 \)) are exposed to infected shrimp (\( I_0 \)). Twelve susceptible shrimp and one infected shrimp were placed in a cylindrical tank (1 m² bottom surface area by 0.6 m height). Each tank was filled to a depth of 10 cm of chlorine treated seawater. The susceptible shrimp were exposed to the infected shrimp for 16 hours. At the end of the exposure, the proportion of the dead infected shrimp consumed by the susceptible shrimp was noted. Water temperature in the exposure tanks was maintained at 27 ± 3 °C.
Isolation

To ensure no secondary transmission from newly infected dying shrimp, the exposed susceptible shrimp were isolated after the initial exposure period. After the 16-h exposure, all shrimp were placed in 1L jars. All jars were placed in a water bath, and each jar was supplied with air. Water temperature in the isolation jars was maintained at 26 ± 2 °C. The time of death of isolated shrimp was recorded. Shrimp were kept in these isolation jars for five days at which time the number of surviving specimens was recorded.

When a single infected shrimp is used, the transmission rate (β) is the proportion of susceptible shrimp getting infected. In a previous study using *L. vannamei*, we found that 100% of shrimp dying during the isolation phase, were WSSV positive, and 98.1% all shrimp that lived through the isolation phase were histologically negative for WSSV (Soto and Lotz 2000). Therefore, the mortality rate of WSSV is a good measure of transmission. We will report mortality rate, the proportion of susceptible shrimp dying, in this study.

Experiment 2: *Menippe adena* Challenge of WSSV

Each *M. adena* was exposed individually in 1 L jars. Each jar was placed in a water bath and supplied with air. Water temperature was maintained at 26 ± 1 °C. Four crabs were challenged by injection with a cell-free shrimp homogenate containing WSSV. The homogenate consisted of a 1:10 dilution of tissue of shrimp known to have died of WSSV in water. Each crab was injected with 0.02 ml per gram of crab into the infrabrachial sinus of the fifth pereiopod. Three crabs were injected with a virus free homogenate. For the per os exposures, 15 crabs received a piece of WSSV infected tissue weighing approximately 5% body weight, and four crabs received a piece of virus-free shrimp tissue.

Crabs dying were fixed in Davidson's solution following procedures outlined by Lightner (1996). Crabs were kept in these jars for five days at which time all surviving specimens were fixed in Davidson's solution. Each crab was examined histologically after staining with Hematoxylin and Eosin stains for the presence of WSSV intranuclear inclusions. For each crab, two non-serial sagittal sections were analyzed by routine histology. In addition, *in situ* hybridization was performed on a corresponding parallel section. *In situ* hybridization was performed with kits available from DiagXotics, Inc. *L. vannamei* were used as positive and negative controls.

RESULTS

Experiment 1: *L. vannamei* vs. *F. duorarum*

Susceptible *L. vannamei* and *F. duorarum* (S₀) in each tank completely consumed the infected material (I₀). The mean mortality rate from a WSSV exposure was 0.81 (95% CL 0.34 - 0.91) for *L. vannamei*, and 0.75 (95% CL 0.41-0.87) for *F. duorarum*. A statistical difference was not detected in final mean
mortality rates between *L. vannamei* and *F. duorarum* (chi-square test, $P = 0.45$). Most animals died between 24 and 60 hours post-exposure for both *L. vannamei* and *F. duorarum* (Figure 1). No animals from either negative control group died during the experiment.

![Graph showing mean cumulative survival](image)

**Figure 1.** Mean cumulative survival in four-hour increments of *Litopenaeus vannamei* and *Farfantepenaeus duorarum* exposed to WSSV. No difference in final mean survival was detected (chi-square test, $P = 0.45$).

**Experiment 2: Menippe adina Challenge of WSSV**

From the *M. adina* challenge, two of the four crabs injected with WSSV died, and both of those were found to be histologically positive for WSSV associated lesions (with H&E stains). The crabs died at 60 and 71 hours post-exposure. One of the 15 crabs exposed *per os* was found to be histologically positive for WSSV. No WSSV associated lesions were observed in crabs used as negative control animals. Tissue tropism was similar to WSSV infections of penaeid shrimps (Lightner 1996). In infected crabs WSSV intranuclear inclusions were observed in cuticular epithelium, connective tissue, antennal gland, hematopoietic tissue, heart, and gill. With *in situ* hybridization, light reactions were observed in WSSV infected cells from crabs despite getting dark reactions in WSSV infected cells from the positive control shrimp.

In addition to the inclusions typically caused by WSSV, intranuclear inclusions were found in hypertrophied nuclei in cells of the hepatopancreas. Affected nuclei
were usually large but varied in size, contained a single basophilic to lightly eosinophilic inclusion, and displayed margined chromatin. No occlusion bodies were observed in the inclusions. The inclusions were found in crabs exposed to WSSV, and in crabs used as negative control animals. Further, the inclusions were present in one of the two crabs that died during the experiment. Ten of the 24 crabs used in the experiment were positive for this hepatopancreas inclusion.

DISCUSSION

The results suggest that juvenile *F. duorarum* are as susceptible to mortality from WSSV as juvenile *L. vannamei*. In contrast, Lightner et al. (1998) and Wang et al. (1999) exposed juvenile *F. duorarum* and *L. vannamei* per os to WSSV and found juvenile *F. duorarum* to be more resistant to WSSV than juvenile *L. vannamei*. However in the study by Wang et al. (1999), they mention a preliminary experiment (unpublished) in which juvenile *L. vannamei* and *F. duorarum* were challenged per os to WSSV. In that study no difference in mortality rates was detected which corroborates our findings.

In a previous study the procedure for estimating transmission rate (β) used in this study was used to compare the transmission rates of WSSV by ingestion of an infected cadaver of *L. vannamei* to the transmission by ingestion of an infected cadaver of *L. setiferus* (Soto and Lotz 2000). In that study most shrimp were examined histologically to determine infection status. They determined that mortality rate was a good measure of transmission rate. In this study, since no histological examination has been performed on *F. duorarum*, it is possible that the transmission rate is even greater than the mortality rate.

*Menippe adina* were found to be susceptible to WSSV. *Callinectes sapidus* has also been found to be susceptible to WSSV (Flowers et al. 2000). Moreover, all three species of commercially important penaeid shrimps found in the Gulf of Mexico have been found to be susceptible to WSSV (Lightner et al. 1998, Wang et al. 1999, Soto and Lotz 2000). If successfully introduced, WSSV may pose a threat to the most economically important shrimp and crab fisheries in the Gulf of Mexico.

Tissue tropism of WSSV to *M. adina* was similar to WSSV infections of penaeid shrimps (Lightner 1996). WSSV typically infects cuticular epithelial cells, connective tissue cells, antennal gland epithelium, lymphoid organ sheath cells, hematopoietic tissues, and fixed phagocytes of the heart (Lightner 1996, Chang et al. 1996). WSSV has not been found to infect hepatopancreatic cells. Some researchers have found the hepatopancreas to be PCR positive for WSSV (Lo et al. 1997), but the hepatopancreas is composed of other types of cells including cuticular epithelial and connective tissue cells. The PCR positive results were probably due to the test reacting to infections in these cells.

It is possible that the inclusions observed in hepatopancreatic cells of *M. adina* were caused by another virus. The inclusions are similar to inclusions caused by Baculo-A virus described from *Callinectes sapidus* (Johnson 1980, 1983) and to Baculoviral Midgut Gland Necrosis (BMN), a virus that infects some penaeid
shrimps from south and southeast Asia, primarily Penaeus japonicus and P. monodon (Lightner 1996).

It is important to note that the M. adina used in the experiments were kept in holding tanks for up to one year prior to the start of this experiment. In these tanks, M. adina were cohabitating with other crabs, specifically C. sapidus, Panopeus simpsoni, and Eurypanopeus depressus. Therefore, little can be said of the prevalence (41.6%) of the inclusion of M. adina in wild populations.

ACKNOWLEDGEMENTS

We would like to thank Rena Krol for histological expertise, and Verlee Breland, Lesber Salazar, Anne Marie Moore, Charles Flowers, Jason Lemus, and Mike Turner for technical assistance. This research was supported by USDA/CSREES through grant # 98-38808-6019.

LITERATURE CITED


Lotz, J.M. and M.A. Soto. [2000]. A model of white spot syndrome virus (WSSV)