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Potential pathological effects of blood flukes (Digenea: Sanguinicolidae) on pen-reared marine fishes

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ABSTRACT

Sanguinicolids, 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, sanguinicolids, 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 sanguinicoliasis. Dwindling wild stocks of marine fishes and increased demand for fish products has catalyzed aquaculture production, but sanguinicoliasis 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

Sanguinicolids 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 211 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

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host death. This, along with the transparent appearance of many sanguinicolids 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 sanguinicolids 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 (Køie, 1982). The blood fluke *Aporocotyle simplex* develops in the terebellid polychaete intermediate host (*Artacama proboscidea*) 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 spirorchiids, 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 sanguinicolids 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 *Paracardicoloides yamagutii* 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 cagecultured 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

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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

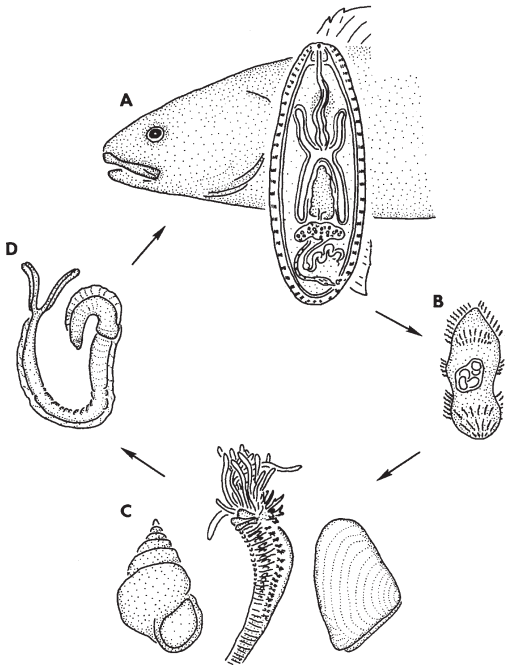

Figure 1. Generalized sanguinicolid life cycle. **A.** The adult fluke in the vascular system of the definitive host, a fish, releases eggs into the blood. **B.** The first freeswimming stage of the fluke, the miracidium, hatches from the egg in the gill, emigrates through the gill epithelium, and seeks 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 freeswimming 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.

contents. In its flatfish hosts, the adult of *A. simplex* infects the heart, ventral aorta, and gill arteries (Køie, 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-yearold 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 or[illegible] 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, *Sanguinicola fontinalis* probably is restricted to the cyprinid *Rhinicthys 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 differentaged 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.

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 *Sanguinicola 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

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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 nonspecific 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 serranids contained an abundance of macrophage aggregates (MA's) in the ventriculus, and they and a few other fishes contained free macrophages among ventricular trabeculae. 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 serranid 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, mesentery, 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 serranids 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 campechanus*) from the northern Gulf of Mexico (Figs. 26), 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 (Figs. 2-4). Recently deposited eggs in early stages of degeneration were encapsulated by a thin epitheliod or fibroid layer (Fig. 2). More degenerated eggs exhibited some yellowish ceroid pigment (Fig. 3). Some eggs in a later phase of degeneration exhibited more conspicuous light to dark brown ceroid pigment (Fig. 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. (Fig. 5). Sequestered eggs in the gill mostly contained living miracidia and were readily apparent by their refractive excretory products in fresh wet-mounted preparations (Fig. 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 (Fig. 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 acanthus* 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 (Fig. 8). Large clusters of eggs with the associated host inflammatory response occluded both large and small vessels (Figs. 9-13). A given fish typically demonstrated both recently deposited eggs containing miracidia and a slight granulomatous response (Fig. 10) and more advanced cases with eggs in various phases of degeneration (Figs. 11-13). The more advanced cases, especially when

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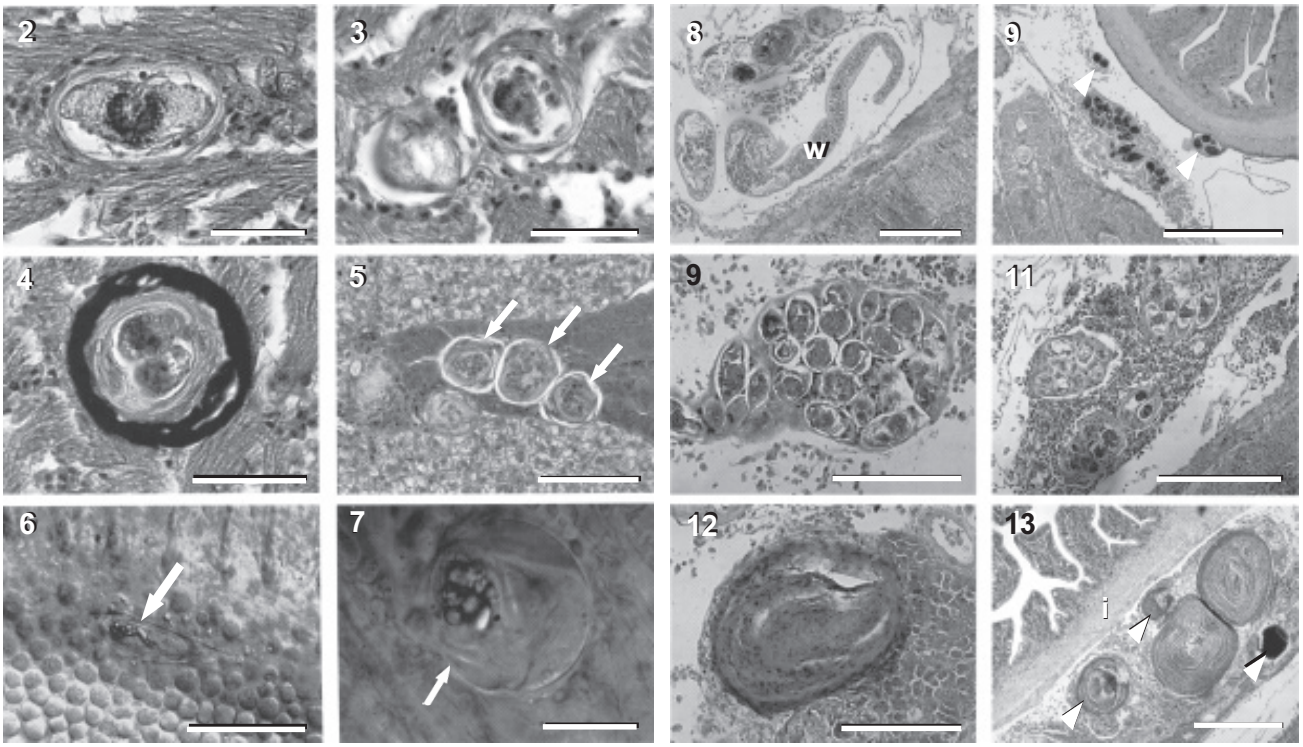
heavily infected, typically exhibited an extensive inflammatory response involving ceroid pigmentation. Perhaps some eggs with infective miracidia of this species are voided through the intestine.

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 cercaria, 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.



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 wellformed miracidium. Scale bar = 75 µm. **7.** Spheroidal fresh egg in gill of different species in red drum. Note welldeveloped miracidium (arrow). Scale bar = 30 µm. **Figs. 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

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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.

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.

Pen and cage culture offer an excellent potential for development of sanguinicoliasis. 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.

Sanguinicoliasis was reported in a cagecultured marine fish (greater amberjack, *Seriola dumerili*) by Ogawa and Egusa (1986). Mass mortalities of this amberjack maintained in shallowwater floating net cages off the coast of Shikoku Island, Japan, occurred between December and March. Gill filaments of heavilyinfected dead fish contained more than 1,000 eggs of *Paradeontacylix 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. Heavilyinfected 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, *Cruoricola lates* in the centropomid *Lates calcarifer* in Malaysia (Herbert *et al.*, 1995) occurs commonly in vessels of the mesentery, kidney, pericardium.

Cases of sanguinicoliasis 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 *Sanguinicola 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: *Sanguinicola davisi*, *Sanguinicola klamathensis*, *Sanguinicola alseae*, and *Sanguinicola 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.

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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 sanguinicoliasis, 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, bio-security 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 Shaharom-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 1950's and 1960's 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 sanguinicoliasis 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 30year 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 1020 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 2229% 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 bio-security 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.

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