Marine Bivalve Mollusks as Reservoirs of Viral Finfish Pathogens: Significance to Marine and Anadromous Finfish Aquaculture

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ABSTRACT—Filter-feeding bivalve mollusks can bioaccumulate various disease agents significant in the management of public health. Recent evidence has indicated that marine bivalves can harbor viruses that are virulent for salmonid species. Examples of finfish viruses occurring in shellfish tissues and those that have potential of occurring are presented.

Participation of shellfish in the epizootiology of endemic and/or exotic finfish diseases is possible and should be considered for future management of fish health. To reduce potential introductions of finfish disease agents which may be incidental in shellfish tissues, certification of bivalve stocks is advised before movement into new waters.

Introduction

Epidemiologists have long known that bivalve mollusks are capable of harboring disease agents significant to human health. The mollusks accomplish this phenomenon by filter feeding, whereby seawater is pumped in through the gills which entrap larger planktonic food organisms within the cilia and mucus of the respiratory epithelium (Purchon, 1977). As a result, these mollusks also take in smaller incidental particles including bacteria and viruses suspended in ambient seawater or adsorbed onto the surfaces of organic and inorganic solids.

Consequently, human pathogens including coliform and *Vibrio* species of bacteria as well as polio and hepatitis viruses have been detected in bivalve species from clean and organically polluted waters (Ross, 1956; Mitchell et al., 1966; Hamblet et al., 1967; Liu et al., 1967; Lovelace et al., 1968; Kampelmacher et al., 1972; Ayres, 1975; Thompson and Vanderzant, 1976). Disease outbreaks caused by some of these agents have occurred in the human populace following ingestion of poorly cooked or raw shellfish from contaminated areas (Mason and McLean, 1962; Liu et al., 1967; Sakazaki, 1969.

Recent evidence has shown that marine shellfish are potential reservoirs for certain finfish pathogens through the same bioaccumulation process of filter feeding. This information has some significance when considering finfish disease epizootiology in both marine and freshwater finfish rearing systems having bivalve mollusks directly in the water supply or immediately downstream. Any epizootic occurring within a finfish population can result in the release of high concentrations of the pathogen into ambient water from dead or dying fish. As an example, infectious hematopoietic necrosis virus (IHNV) in hatchery effluents during acute disease outbreaks has been reported at levels as high as 400 p.f.u./ml (Leong and Turner, 1979).

Recorded epizootics in feral finfish populations, though uncommon when fish are normally dispersed, become more evident when fish are concentrated in smaller areas as occurs during periods of spawning or smolt outmigration. As much as 1,600 p.f.u./ml of IHNV has been reported in fresh water near spawning sockeye salmon, *Oncorhynchus nerka,* (Mulcahy et al., 1983). Nearby shellfish beds filtering water contaminated by diseased finfish, would be capable of entrapping the causative disease agent. Filter feeding shellfish can accumulate viruses and bacteria within their tissues at much higher concentrations than present in surrounding seatwater (Mitchell et al., 1966).

The actual concentration in the tissues is dependent upon: The rate at which bivalves pump water through the gills; the concentration and physical characteristics of the microbe in the ambient seawater; and seawater temperature, salinity, and turbidity (Galtsoff, 1964; Hamblet et al., 1967; Liu et al., 1967). Eventually, incidental contaminants are depurated or expelled from the tissues back into the seawater. This virus or contaminant loss occurs over a period of time and is called the depuration rate. This rate is enhanced by exposure of the shellfish to waters free of the contaminant and is proportional to the degree of tissue contamination.

Other factors influencing the depuration rate are the same as those described for bioaccumulation (Liu et al., 1967). Consequently, depuration

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and bioaccumulation rates for a given contaminant vary among species of bivalve and within a species depending upon animal size and environmental parameters influencing animal physiology. Hard clams, *Mercenaria mercenaria* can depurate human polio virus type 1 within 96 hours if placed in clean seawater at 13-15°C (Liu et al., 1967). However, after 40-60 days the European flat oyster, *Ostrea edulis*, still cannot completely depurate certain strains of molluscan IPN viruses, some of which are pathogenic to finfish (Hill and Alderman, 1977; Hill et al., 1982).

Whether a depurated disease agent remains infectious for its natural host depends upon its stability within the environment outside normal host tissues. It is highly probable that beds of filter feeding shellfish contaminated with a stable, viable finfish pathogen could disseminate waterborne infectious particles to other finfish hosts during depuration.

The following discussion presents examples of finfish pathogens actually isolated from bivalve mollusks and others which have potential for bioaccumulation in shellfish tissues. These agents are all viruses which can infect salmonid fishes and at least one centrarchid species. However, other finfish species may also be susceptible to some of these viral agents. There are bacterial agents (*Vibrio* sp., etc.) common to bivalve tissues which are pathogenic for both finfish and other shellfish, but these will not be included herein (Tubiash et al., 1965, 1970; Lovelace et al., 1968; DiSalvo et al., 1978; Leibovitz, 1978; Garland et al., 1983).

Viral Finfish Pathogens Isolated From Bivalves

$13p_2$ Reovirus

A new serotype of reovirus was isolated from juvenile American oysters, *Crassostrea virginica*, held in rearing facilities of two different Long Island, N.Y., shellfish hatcheries (Meyers, 1979; Meyers and Hirai, 1980). The virus, designated as $13p_2$, is very stable, remaining viable in both seawater or oyster tissues for longer than 60 days (Meyers, 1980). It does not replicate in oyster tissues but is infectious for various fish cell lines and for at least two finfish species.

The virus produces a 44 percent mortality in bluegill, *Lepomis macrochirus,* fingerlings causing severe necrotic hepatitis (Meyers, 1980). It also produces chronic self-limiting granulomatous hepatitis and occasional pancreatitis in adult and fingerling rainbow trout, *Salmo gairdneri* (Meyers, 1983). Thus, for rainbow trout, $13p_2$ is weakly virulent for the age classes tested, but its effect on sac fry or on other salmonid species has not been examined. In salmonid aquaculture, this virus may not be significant as a primary pathogen but would contribute to undesirable stress and lowered host resistance to other biological or environmental pressures affecting fish survival.

JOV-1

A second reovirus-like agent, labeled JOV-1, has been isolated from Japanese oysters, *C. gigas*, reared in a Japanese hatchery (Nagabayashi and Mori[1]). Little information is yet available, but apparently the virus is infectious for 10-week-old rainbow trout, producing a 40 percent mortality in infected fish. Fifteen-week-old trout are reported refractory to clinical disease.

IPN Molluscabirnaviruses

Infectious pancreatic necrosis virus (IPNV) was first islated from brook trout, *Salvelinus fontinalis* (Snieszko et al., 1957), and has traditionally caused disease in juvenile salmonids. However, other nonsalmonid strains of IPNV have recently been isolated from many other freshwater and marine finfish species as reviewed by Hill (1982). Many of these new isolates are not responsible for clinical disease in their respective hosts and are variable in their infectivity and virulence for rainbow trout (Hill, 1982). Included among these nonsalmonid IPN viruses are strains which have been isolated from several species of marine bivalves, two species of gastropod, and one decapod crustacean (Hill, 1976a, b; Hill, 1982). Such isolates are nearly all biochemically, biophysically, and serologically (three exceptions represent different serotypes) indistinguishable from reference strains of IPNV in fish. At least eight of these shellfish isolates are pathogenic in rainbow trout fry producing typical clinical signs of IPN disease and fry mortality (Hill, 1982). Definitive evidence is still lacking to substantiate whether such molluscan IPN viruses can replicate in shellfish tissues or are merely bioaccumualted contaminants. Regardless of this final determination, the confirmed pathogenicity of these viruses for rainbow trout and their prolonged stability in shellfish tissues (≥ 50 days; Hill and Alderman, 1977) make them significant disease agents to be considered in the management of anadromous salmonid health.

Viral Finfish Pathogens Having Potential To Occur in Bivalves

There are other viral finfish pathogens which have prolonged viability within the natural environment and could potentially accumulate in nearby mollusk tissues if released from clinically diseased or carrier fish.

CSV

The chum salmon virus (CSV) is another recently discovered reovirus similar in many respects to the $13p_2$ agent. However, CSV is a different virus (Winton[2]) which was isolated

[1]Nagabayashi T., and S. Mori. 1983. XVI Annual Meeting of the Society for Invertebrate Pathology, Cornell Univ., Ithaca, N.Y. Unpubl. abstr.

[2]Winton, J.R. 1984. Department of Microbiology, Oregon State University Marine Science Center, Newport, Oreg. Unpubl. data.

from spawning chum salmon, *Oncorhynchus keta*, caught in Japan. This virus can infect chum, chinook, *O. tshawytscha*; and kokanee, *O. nerka*, salmon fingerlings, producing a self-limiting necrotic hepatitis (Winton et al., 1981). As with the $13p_2$ agent, CSV remains viable for months outside fish host cells. Thus, it could potentially accumulate in filter-feeding bivalve reservoirs near diseased fish hosts. This virus could be significant in salmonid husbandry if it caused similar host debilitation as discussed for the $13p_2$ agent.

IHNV

Infectious hematopoietic necrosis virus (IHNV) is an extremely virulent pathogen of juvenile salmonids in western North America. Consequently, this rhabdovirus is particularly important to Pacific salmon, *Oncorhynchus* spp., and steelhead, *S. gairdneri,* husbandry in the Pacific Northwest and Alaska (Grischkowsky, 1981; Groberg et al., 1982, Groberg, 1983; Rohovec, 1983). Whether IHNV could accumulate in filter feeding mollusks is speculative, but physically and biologically possible.

It is clear that IHN virus can remain infectious for several months in freshwater rivers (Wedemeyer et al., 1978; Mulcahy et al., 1983), and recent laboratory studies indicate detectable levels of virus after 27 and 22 days in estuarine and saltwaters, respectively (Toranzo and Hetrick 1982). It becomes conceivable that high concentrations of IHNV shed by infected spawning salmon or outmigrating clinically diseased smolts could accumulate in marine bivalve mollusks located directly below short stream systems. Shellfish could then participate in waterborne transmission of the virus for a period of time during the depuration process. Although considerable dilution of virus concentrations would be involved, evidence suggests that low levels of infectious particles may be sufficient for transmission of the disease (Mulcahy et al., 1983).

Salmonid IPN Piscibirnaviruses

Although infectious for salmonids, IPN molluscan birnaviruses are less virulent than the true salmonid strains. However, these true salmonid strains are also capable of surviving for long periods of time in fresh, estuarine, and saltwaters (Ahne, 1982; Wedemeyer et al., 1978; Toranzo and Hetrick, 1982). Consequently, they are stable enough in the environment to make possible their bioaccumulation and later dissemination by filter feeding mollusks.

Conclusions

Marine bivalve mollusks are unavoidable in estuarine and salt waters which may be used in the rearing of anadromous fishes or which may be directly downstream of freshwater egg and fry facilities operating on short watersheds. No less abundant are the many freshwater species of filter feeding clams and mussels which populate certain stream and lake bottoms around the world. The established occurrence of certain viral finfish pathogens in common species of marine bivalve mollusks indicates that shellfish could serve two roles in the epizootiology of these disease agents, and possibly others: 1) Shellfish may be seasonal reservoirs for some endemic finfish pathogens originating from finfish epizootics and/or from subclinically diseased "carrier fish"; 2) shellfish could introduce exotic finfish diseases when transported to other waters for commercial or experimental purposes from areas where such agents may be endemic.

In terms of practical fishery management, there is little that could be done about involvement of marine bivalve mollusks in endemic finfish disease epizootiology. However, realization of their potential as disease reservoirs would add a new dimension to the future investigation of certain finfish disease outbreaks.

Conversely, there are obvious prophylactic measures for reducing the possible dissemination of exotic finfish diseases by shellfish introductions into "new" waters. Specific invertebrate diseases, particularly those affecting commercially important marine bivalve mollusks, are becoming increasingly important regarding their geographic containment and control. This concept is emphasized by a growing recognition for the importance of health certification of imported and exported shellfish species (Rosenfield and Kern, 1978; Elston, 1981; Elston[3]). Consequently, if certification procedures are exercised for shellfish pathogens, some additional effort made in screening mollusk tissues for incidental viral finfish pathogens would provide significant protection against the introduction of these agents by movement of shellfish stocks.

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