

Shellfish Diseases

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ABSTRACT—An overview of commercial bivalve shellfish aquaculture is presented. The advantages and disadvantages of shellfish production as compared with other forms of food animal production is discussed. The common shellfish diseases are listed and the known specific etiologic agents are indicated. The latter include viral, bacterial, fungal, protozoan, and metazoan parasitic and infectious agents. In addition, predators, toxic agents, and fouling organisms produce serious economic losses.

The specialized problems of shellfish hatcheries are discussed. The importance of monitoring the qualitative physical, chemical, and bacteriological changes in shellfish larval cultural media and its ingredients for optimum production is indicated.

A description of a laboratory model for evaluating the pathogenicity of pure bacterial cultures for larval shellfish is presented. The experimental optimal and lethal concentrations of bacteria for shellfish larvae are defined. An interrelationship between bacteria and protozoa in the pathogenesis of shellfish larval diseases is reported. The shellfish industry has encouraged and supported the reported research to increase the efficiency of shellfish production by reducing economic losses due to shellfish diseases.

Less is known about the subject of shellfish diseases, and, accordingly, there is a wider latitude in discussing it. There are many unique problems, some of which overlap with fish diseases.

One problem is that the molluscan bivalves are filter feeders. Their ability to concentrate harmful chemicals and infectious agents pose serious problems in controlling both shellfish and human diseases. Since shellfish are estuarine dwellers, they are subjected to environmental variations such as changes in salinity and temperature, seasonal tidal variations, and varying degrees of

exposure to urban and industrial pollutants discharged into estuarine waters.

In spite of these hazards, shellfish hold one of the greatest potentials for the economic production of food protein. Shellfish hold great promise for the efficient recycling of organic waste materials, such as agricultural wastes, and the capture of energy for food production from thermal effluents, such as that discharged from atomic power plants. In addition, there are more species of shellfish than any other group of animals, with the exception of arthropods. Genetic selection for greater food yields from these abundant varieties should be rewarding. Also, in terms of reproductive potential, there are no other food animals that even approach their fecundity. For example, a single pair of oysters can produce as

many as 120 million offspring from a single mating.

There is another unique aspect of shellfish production that exceeds the economic efficiency of other forms of animal protein production and that is free food. Unlike the rising food costs of other animal feeds, shellfish foods are naturally generated planktonic foods. Since shellfish are an important source of food, we should learn more of their diseases as a part of the technical development necessary to increase production.

The following discussion of shellfish diseases is an overview and a short consideration of one specific bacterial disease problem in larval shellfish production being currently studied.

DISEASES OF SHELLFISH

A list of organisms that cause common diseases in oysters is shown in Table 1.

Viral Diseases

Of the known reported virus infections of oysters, "Ovacystis" infection is the most common, but it is probably of little economic importance. It can be detected histologically as hypertrophy of the ovarian follicles. The affect of this virus upon reproductive performance has not been evaluated.

A herpes virus infection has been described by Austin Farley (1972) in oysters. Apparently, expression of the disease was temperature dependent and was found in oysters cultivated in the heated effluent of a power plant. When

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Table 1.—Causes of common diseases in oysters.

Group	Species
Viral	Ovacystis virus
	Herpes virus
	Others
Bacterial	<i>Achromobacter</i> sp.
	<i>Aeromonas</i> sp.
	<i>Vibrio</i> sp.
	<i>Mytilomus ostreareum</i>
	"Maladie Du Pied"
	<i>Nocardia</i> sp.
Fungal	<i>Cladothrix dichotoma</i> (Actinomycetes)
	Others
	<i>Dermocystidium (Labyrinthomyxa) marinum</i>
	<i>Sirolopidium</i> sp.
Parasite protozoan	Others
	1. Sarcodina (Amoeba)
	<i>Flabellula</i> sp.
	2. Mastigophora (Flagellate)
	<i>Hexamita nelsoni</i>
Helminthic	3. Sporozoan
	a. Gregarine
	b. Haplosporidia
	4. Ciliates—many
Arthropods	1. Trematoda (larval)
	<i>Bucephalus</i> sp.
	Others
	2. Cestoda (larval)
Other Organisms	<i>Tylocephalum</i> sp.
	Others
	1. Copepods
	<i>Mytilicola intestinalis</i>
	Others
	2. Decapods
	Pinnotherid crabs
	3. Annelids ("Mud blisters")
	<i>Polydora websteri</i>
	Others
4. Sponges ("Boring sponges")	
<i>Clinona celata</i>	
Others	

the environmental temperature dropped, the disease was not apparent. While there are undoubtedly other shellfish viral diseases present, they have not been defined.

The two previous viral diseases mentioned were demonstrated upon the basis of diagnostic inclusion bodies and electron microscopic demonstration of viral particles in affected cells. Virus-free molluscan tissue culture systems are needed to isolate and identify molluscan viruses and human viral pathogens that may be carried by shellfish.

Bacterial Diseases

Little is known of the bacterial diseases of shellfish, and the list in Table 1 is limited to those that have been described. From the standpoint of human health, outbreaks of cholera have been related to the consumption of shellfish in Africa and Italy.

Fungal Diseases

Dermocystidium (Labyrinthomyxa) marinum is a very important shellfish

pathogen that produces serious economic losses in adult shellfish in warm climates. *Sirolopidium* sp. is a common infection of hatchery-reared larval shellfish.

Helminthic Diseases

Among the helminth parasites of shellfish, trematode, cestode, and nematode parasites may be found. Larval forms of trematodes (especially *Bucephalus* sp.) and cestodes (especially *Tylocephalum* sp.) are of economic importance as shellfish pathogens that often produce sterility in affected shellfish. Most of the larval forms mature in fish which serve as definitive hosts. Some are of public health significance.

Arthropods and Other Organisms

In addition to helminth parasites, copepod crustacean and polychaete annelids, during some stage of their life cycles, may parasitize shellfish with resultant serious economic losses.

A great variety of marine organisms are found in shellfish beds in apparent symbiotic or commensal relationships to shellfish. Some, as pinnotherid crabs, enter and leave the pallial cavity of shellfish freely. Crabs may serve as the intermediate host for the primitive gregarine sporozoans (*Nemotopsis* sp.) whose spores infect shellfish with little resultant tissue damage. Macroalgae and sponges grow on the surface of shellfish. The boring sponges (*Clinona* sp.) may damage the external shell and the shell may then become porous and crumble.

Diseases of Unknown Etiology

In addition to the known diseases, many unexplained die-offs have been reported that have decimated shellfish populations. Often these populations do not recover, and new stock, introduced to repopulate, are quickly affected and die. Such diseases are often named for the locality in which they occurred, such as "Malpeque Bay" and "Denman Island" disease. Often serious losses are attributed to climatic conditions, water quality changes, and

pollution without adequate evidence that disease was not responsible.

Parasitic Diseases

Protozoans

Shellfish protozoan infections are very common. Whether these organisms are primary infectious agents is often questionable. This is especially true of the ciliates that are common inhabitants of shellfish tissues. They become especially active when other pathogens such as bacterial agents are present. Of the flagellated protozoa, *Hexamita* sp. and the amoeboid protozoa are pathogenic. When shellfish are maintained under adverse conditions, such as extreme temperatures, protozoa may actively invade shellfish tissues and produce deterioration or spoilage. These conditions may also be found in "winter-kills" of shellfish where high mortality associated with protozoan infections may be found in sustained low temperature exposures.

Protozoans can be primary shellfish pathogens. The most important single shellfish pathogen that has produced the greatest economic losses to the shellfish industry is a haplosporidian, *Minchinia nelsoni*. This organism has destroyed the great oyster industry of the Delaware and Chesapeake Bays. Haplosporidians are very poorly understood, poorly classified sporozoans, distinct from myxosporidia, or coccidial organisms. Their exact taxonomic position and life cycles are unknown. In addition to the areas mentioned, *M. nelsoni*, commonly called MSX, is present in other geographic locations of the northeastern U.S. coastline. This organism is apparently salinity-dependent. It is seasonal in its incidence. There are many other haplosporidians, of varying pathogenicity found as parasites in a variety of aquatic animals. They are found as hyperparasites in trematodes. These organisms tend to sterilize the trematode host.

SHELLFISH HATCHERY OPERATION STUDIES

When I began working with the Long Island shellfish industry, the problems were overwhelming and it was difficult

to select a single starting point. Perhaps the most important economic problems were based in shellfish hatchery production. If hatchery production could be increased, and livability of larvae and juveniles were improved, restocking and harvesting from shellfish beds would yield greater production and efficiency. The techniques of hatchery operation are well known, but consistent production of healthy larvae is difficult. Shellfish larval disease losses are serious hatchery problems, often of epizootic proportions.

Although specific pathogens were occasionally responsible for such losses, it became apparent that there were many unexplained phenomena associated with the more common losses. In an attempt to resolve these problems, studies of hatchery media were undertaken. These included physical, chemical and microbiological examination of hatchery water supply, stock algal cultures, pooled algal food cultures, and spawn obtained from hatchery breeding stock. Each hatchery operation was distinctive. Some operated all year, others limited their operation to warm weather only. Hatchery water supply was either raw bay water, or from deep saltwater wells. Some operations pumped water into the plant on demand; others held water in large storage tanks that was later gravity fed into the operation on demand. Various methods of screening, filtration, and centrifugation are employed for water clarification. In addition some plants utilize ultraviolet treatment of incoming water, or recycled water for disinfection.

Physical and chemical examination of shellfish larval culture media included measurement of pH, salinity, chemical oxygen demand, suspended and total solids. Other tests including nitrogen determinations are currently being explored. Quantitative counts and identification of dominant bacterial populations of the larval culture media ingredients are also being made.

TESTING BACTERIAL PATHOGENICITY IN LARVAL SHELLFISH PRODUCTION

It became apparent that a pathogenicity model was needed to test the pure

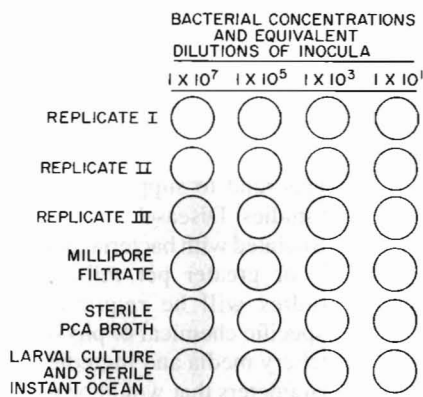


Figure 1.—Pathogenicity model system to test pure bacterial isolates obtained from larval cultures.

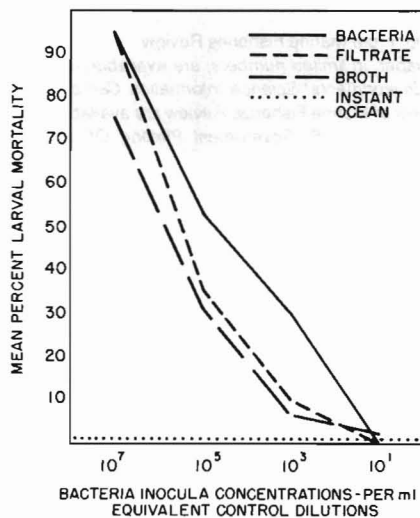


Figure 2.—Graphic representation of mean percent mortality values of equivalent concentrations of inocula of all tested isolates (35) (each replicated in triplicate), their cultural filtrate, uninoculated broth medium, and "Instant Ocean" controls.

bacterial isolates obtained from the larval cultures. The same model could be utilized to test environmental factors, drug efficacy, and other factors for their influence in such disease models. This model system was assembled in plastic "dispo" trays and consisted of 6 rows, of 4 wells per row, containing precalculated approximate numbers of shellfish larvae from 3 to 14 days of age (Fig. 1). To each row was added a

known dilution of the test substance. In testing for bacterial pathogenicity, pure 24-hour broth cultural bacterial isolates were added to each of the first 3 rows of the plate; from left to right, each well of each of the first three rows containing approximately 10^7 , 10^5 , 10^3 , and 10^1 bacteria per milliliter of well larval suspension. The fourth row was given the equivalent dilution of bacteria-free filtrate (Millipore filtrate) of the broth culture to correspond to the dilutions of the bacterial suspension wells above this row. The fifth row received again, equivalent dilutions of sterile culture broth (plate count agar broth - PCA). The last (sixth row) received equivalent dilutions of synthetic sea salts (Instant Ocean)¹ to the shellfish larval suspensions. The results of the above test were read at the end of a 24-hour incubation period. The number of alive and dead larvae in each well was counted and the percentage mortality for each well was determined. In this manner the effects of dilution and comparison of the affects of added ingredients could be measured to determine their relative influence on pathogenicity.

RESULTS

The results of the above pathogenicity tests (Fig. 2) suggest that almost all bacterial isolates at high concentrations ($>10^5$ /ml) are pathogenic for shellfish larvae; however, only "true" pathogens kill at very high dilutions ($<10^3$ /ml). The latter suggests that these true pathogens require larvae for growth. Note that the presence of higher concentrations of even sterile nutritive broth produces a lethal effect. Accordingly, this may suggest that food concentrations, dead or decaying algal foods, or larvae may aggravate the pathogenic effect of both extrinsic and intrinsic microbial concentration (within the larvae). Future studies are needed. At high concentrations of bacteria and/or equivalent culture media (10^7 or greater), lethal effects are rapid

¹Reference to trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

and are not associated with protozoan activity. However, at levels corresponding approximately to 10^5 /ml, lethal effects are indicated by more gradual losses and are associated with intense protozoan proliferation and activity. The latter probably originate from the normal intrinsic microbial flora of the shellfish larvae. In fact, if one were to observe such cultures without knowing of the presence of the experimental bacterial inoculum, the aggressive behavior of the protozoan attack on the shellfish larvae would suggest that they are the primary pathogen. The mechanism responsible for this phenomenon requires further study. Direct observation of the af-

ected larvae in this bacterial study support common diagnostic signs and lesions evident in diseased larvae and is a separate discussion in other studies.

The results of field studies of bacterial populations of hatchery media and its ingredients tend to support the experimental studies. Diseased larval cultures are associated with bacterial populations 10^7 or greater per milliliter. Further studies will be required to define the specific chemical or physical tests of hatchery media and ingredients and their parameters that would be useful in disease detection and diagnosis that could be related to specific pathogenic agents.

As a result of these studies, the need

to monitor and define hatcheries for optimum performance becomes more apparent. Since individual hatcheries are different, each hatchery must be evaluated for its operational methods and equipment.

ACKNOWLEDGMENTS

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