

Shellfish Diseases and Their Management in Commercial Recirculating Systems

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Introduction

Intensive culture of early life stages of bivalve shellfish culture has been practiced since at least the late 1950's on an experimental basis. Production scale culture emerged in the 1970's and today, hatcheries and nurseries produce large numbers of a variety of species of oysters, clams and scallops. The early life stage systems may be entirely or partially recirculating or static. Management of infectious diseases in these systems has been a challenge since their inception and effective health management is a requisite to successful culture. The diseases which affect early life stage shellfish in intensive production systems and the principles and practice of health management are the subject of this presentation.

Shellfish Diseases and Management

Diseases of bivalve shellfish affecting those reared or harvested from extensive culture primarily consist of parasitic infections and generally comprise the reportable or certifiable diseases. Due to the extensive nature of such culture, intervention options or disease control are limited. In contrast, infectious diseases known from early life stages in intensive culture systems tend to be opportunistic in nature and offer substantial opportunity for management due to the control that can be exerted at key points in the systems.

In marine shellfish hatcheries, infectious organisms can enter the system from three sources: brood stock, seawater source and algal food source. Once an organism is established in the system, it may persist without further introduction. Bacterial infections are the most common opportunistic infection in shellfish hatcheries. Viral infections have also been reported and generally are transferred from infected brood stock. Case histories and their management in intensive systems, both complete and partially recirculating, will be reviewed for the following diseases.

Vibriosis of Larval and Juvenile Bivalves.

Vibrio infections in larval shellfish are aggressive and rapidly progressing infections. Larval shellfish have little capacity for repair. Entry of the pathogens has been documented from all three potential sources of contamination. Vibriosis is managed by surveying the system bacteriologically to locate the contamination source and taking

appropriate corrective action which may include increased water filtration, brood stock disinfection or screening and algal food culture decontamination.

Hinge Ligament Infections of Juvenile Shellfish.

Ligament infections, caused by gliding bacteria, are one of the most common and cosmopolitan infections among juvenile shellfish. The consequences of infection are dependent on the size of the affected bivalve. They are caused by gliding or myxobacteria which are able to utilize the shellfish ligament as a sole nutritional source.

Subpallial Bacterial Infections.

Subpallial bacterial infections of juvenile bivalves are persistent and occasionally debilitating conditions. Such infections utilize an invasive pathway through the mantle tissue. The infection is size dependent and tends to be chronic as the shellfish increase in size. The nature of these infections varies by species. Vibrios are common etiologic agents in oysters while gliding bacteria affect certain clam species. A variety of sanitation measures may be required to control this disease.

Viral Infections of Larval and Juvenile Shellfish.

Iridovirus and herpes virus-like infections have been observed in larval and seed bivalves from several continents. The source of the infections is contaminated brood stock but sanitation practices between separate closed culture systems in commercial hatcheries can dramatically limit the spread and impact of the infections. Technology has not yet been developed to screen for these diseases in brood stock on a production basis but such methods are needed to eradicate the diseases from closed systems.

Parasitic Ciliate Subpallial Infections

Opportunistic parasitic ciliates also invade juvenile shellfish through the mantle tissue and can be an extremely persistent infection. These organisms are introduced with bulk seawater used to either establish a closed system or as replacement water in flow through systems.

Approaches to Health Management in Intensive Bivalve Culture Systems

General approaches to health surveillance, management and sanitation in intensive shellfish husbandry will be presented. These approaches include bacterial monitoring and management, water filtration and treatment, brood stock selection, isolation and disinfection, management of bacteria in stock and expanded algal food cultures and sanitation procedures for equipment and facilities.

Diseases of Flatfish

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Introduction

Many marine flatfish are high value species, which have been heavily exploited, in commercial fisheries around the world. Concerns on the effects of the overfishing of natural stocks, and also because of the uncertainties and risks associated with the activity has resulted in a great deal of effort being given to the development of aquaculture systems for the rearing of marine flatfish. Like all other living organisms (perhaps with the exclusion of prokaryotes), flatfish are susceptible to a wide range of diseases and parasites. In addition, certain species may be particularly sensitive to environmental changes or the presence of contaminants, which in turn may affect disease susceptibility by reducing the immunocompetence of the host or initiate direct toxic effects. Because of the limited space available, this overview will only highlight some of the principle diseases of flatfish, including pathologies associated with environmental and nutritional factors which may be of importance in intensive aquaculture systems.

Virus Diseases

Most fish viruses are known from cultured species and although an ever increasing number are being found in wild fish, their effect on wild populations and possible impact in culture situations is poorly understood (Wolf, 1984, 1988; Plumb, 1993). There is little doubt however, that viral pathogens constitute one of the most serious disease threats to the successful rearing of marine species. The following is a list of selected virus infections reported to cause disease in marine flatfish.

1. Birnavirus infections are widespread in marine fish and in culture situations and have been associated with high mortalities in fry of Japanese flounder (= Hirame or bastard halibut) (*Paralichthys olivaceus*) in Korea, turbot fry in several commercial farms in Norway and Atlantic halibut (*Hippoglossus hippoglossus*) in Scotland and Norway. Birnavirus infections have also been implicated in mortalities in wild populations of southern flounder (*Paralichthys lethostigma*) in an estuarine location in the USA. The virus has also been detected on eggs of turbot (*Scophthalmus maximus*) and Dover sole (*Solea solea*) and is known to be vertically transmitted via this route in some species.

2. Lymphocystis is a widespread iridovirus disease affecting many marine fish species. It is mainly confined to epidermal lesions, although internal organs can be affected in extreme cases, with fish possibly showing poor condition and ascites. The disease can occur at very high prevalence under culture conditions and mortalities have been reported in various

species; however, most natural infections are benign. Fish may be disfigured by the presence of the lesions, which consist of clusters of massively hypertrophied cells. The affected cells do not persist and eventually slough off, releasing virions to the environment. Transmission is thought to be horizontal via this route.

3. Viral haemorrhagic septicaemia (VHS) caused by a rhabdovirus is a well recognised serious pathogen of salmonid fish which is increasingly being found in a variety of marine fish species (Dixon *et al.*, 1997). VHS outbreaks have occurred in cultured turbot in Germany, UK and Ireland. Clinical signs were consistent with those reported from salmonids and the disease affected older fish as well as juveniles. The source of infection was not identified. The susceptibility of other marine flatfish is at present unknown.

4. Other viruses known to affect turbot include a putative paramyxovirus which has not been associated with disease in cultured fish but is known to produce severe clinical signs in experimental infections, including widespread haemorrhaging, accumulation of ascitic fluid in the peritoneal cavity and exophthalmia. Herpes virus infections in turbot have been associated with mortalities in juvenile cultured turbot in Scotland and the virus has also been isolated from wild turbot. The infection produces “giant cells” and epithelial hyperplasia in the skin and gill tissue. Affected fish are more sensitive to environmental stress, transport and handling. Epizootic haematopoietic necrosis virus (EHNV, a ranavirus) is currently only known as a pathogen of freshwater perch and rainbow trout, although a similar virus has been recorded in turbot. The potential significance of the turbot virus is presently uncertain.

5. Erythrocytic necrosis virus (ENV, an iridovirus) is the causative agent of viral erythrocytic necrosis (VEN). A wide range of marine fish species are affected by this pathogen, including several flatfish species. There are possibly several types of virus that are involved in this disease since there have been several different sized viral particles identified. Severely affected fish can become anaemic, characterised by gill and visceral pallor. Mass mortalities have not been reported. Of potential importance for aquaculture, there is evidence that this virus may increase the susceptibility of fish to other pathogens and conditions of lower oxygen levels.

6. There are several recognised viral pathogens of Japanese flounder (Kimura & Yoshimizu, 1991). Hirame rhabdovirus causes a severe haemorrhagic disease with pale gills, ascites and gonadal distension being the principle clinical signs. The disease has only reported from Japan. Viral epidermal hyperplasia or necrosis, caused by a herpes virus, can cause epizootics in larvae and juveniles fish in hatchery situations. Similarly, hatchery reared juveniles are the most susceptible to viral nervous necrosis virus (a nodavirus). The infection produces marked vacuolisation in brain and retinal tissues and results in behavioural disturbances and significant mortalities. A similar nodavirus-like agent has been reported from juvenile Atlantic halibut, turbot and barfin flounder from Japan.

7. Winter flounder papilloma, (Pleuronectid papilloma) would appear to have little significance and is similar to papilloma found in the North Sea dab (*Limanda limanda*).

Lesions can resolve without treatment and there is little scar tissue formation. Affected fish may be unsightly and may therefore have reduced market value.

Bacterial Diseases

Numerous bacterial pathogens have been reported from marine fish but relatively few specifically from flatfish (Austin and Austin, 1993; Kusada and Salati, 1993; Muroga, 1995). As for viruses, bacteria are amongst the most serious disease threats for aquaculture, primarily because of their direct transmission and ability to proliferate rapidly in the crowded conditions frequently encountered in culture conditions. Such conditions greatly facilitate the rapid spread of infectious agents. This section provides examples of the major bacterial pathogens known to affect flatfish. These include members of the genera *Listonella* (= *Vibrio*), *Aeromonas*, *Edwardsiella*, *Enterococcus*, and *Flexibacter*.

1. Vibriosis is generally recognised as the most serious bacterial disease of cultured marine fish. Infections with members of the genus rapidly become systemic and produce similar clinical signs of haemorrhaging around the fins and mouth and petechiae in the musculature. Internally, all the organs may be affected and enteritis may be present. As the disease advances, tissue necrosis becomes a prominent feature. Wild fish may frequently harbour sub-clinical infections with these and other bacteria and extreme caution should be taken post capture in the care of fish obtained for broodstock. If pathogens are present, even at low levels, disease signs may rapidly develop when the fish become stressed. Chemotherapeutants are available for vibriosis but, increasingly, drug resistant forms are appearing. Vaccines initially developed for salmonid use, against *V. anguillarum*, are currently used in some turbot farms.

2. Furunculosis caused by *Aeromonas salmonicida* is a well-known disease of freshwater fishes; however, it may also affect marine fish, including several flatfish species. This bacterium, or its “atypical” variant have been isolated from open ulcers from wild European flounders (*Platichthys flesus*) and it is likely that it is a regular constituent of the microbial flora associated with ulceration in other flatfish. Several other bacteria are associated with ulcerative lesions including *Vibrio* and *Pseudomonas* spp. However, the importance of these organisms as primary pathogens in flatfish culture is debatable.

3. Other bacterial pathogens of Japanese flounder have caused significant losses. Enterococcal infections caused by *Enterococcus seriolicida* (primarily recognised as a pathogen of yellowtail (*Seriola quinqueradiata*)) and *Edwardsiella tarda* give rise to a variety of clinical signs, typical of systemic haemorrhagic infections.

4. *Flexibacter* species are now well recognised as pathogens of various flatfish species, including turbot and Dover sole. Infections with the opportunistic pathogen *F. ovoliticus* on eggs of halibut gives rise to disease symptoms in juvenile fish and it is likely that similar infections of eggs may also affect other flatfish in hatchery situations. Infections in older turbot can produce systemic disease symptoms (Mudarris & Austin, 1989) and these are a

significant problem for turbot culture. Similar infections in Dover sole give rise to the so-called “black patch necrosis” (Bernardet, *et al.*, 1990).

Fungal Diseases

The most important fungal or fungal-like pathogen of marine fish is *Ichthyophonus hoferi*. Several flatfish species are known to be susceptible and epizootics in wild populations of plaice (*Pleuronectes platessa*) and yellowtail flounder (*Limanda ferruginea*) have been reported (McVicar, 1986; Rand, 1994). Infections are generally chronic in nature and affect a variety of organs and tissues. Fish usually become emaciated and mortalities may occur after several weeks. Transmission is primarily by ingestion of infected tissues or water borne spores. Ensuring cultured species are not fed infected material provides the principle method of avoidance of the disease.

Ulcerative mycosis (UM) affects many species of North American estuarine fish, including the southern flounder (*Paralichthys lethostigma*) and the ocellated flounder (*Ancylopsetta dilecta*) (Noga *et al.*, 1991). Two fungi have been isolated from affected fish, *Aphanomyces* (possibly more than one species) and also occasionally *Saprolegnia*. There does not appear to have been any reports of these agents causing losses in cultured marine fish.

Parasites

Parasitic infections in cultivated marine fish are primarily limited to monoxenous species not dependent on intermediate or paratenic hosts for successful transmission (Paperna, 1987). Although many parasites have the potential to cause serious infections in marine aquaria, relatively few are currently recognised as significant threats to the effective mariculture of flatfish. Wild caught broodstock or juveniles are clearly at greatest threat from existing parasitic infections, which may be transferred to other stocks if not carefully quarantined. This section draws attention to those parasites, which may cause problems in intensive culture situations.

1. Among pathogenic protistan parasites are the ciliates *Cryptocaryon irritans* and *Trichodina* spp. Both parasites can proliferate rapidly and produce serious skin lesions, which may become infected with other pathogens. Mortalities in heavily infected fish probably result from osmotic imbalance (McVicar and MacKenzie, 1977). *Uronema marinum* is a known pathogen of aquarium fishes, including flatfish such as the plaice and is a continuing problem in turbot culture. The microsporean *Glugea stephani* causes multiple xenomas within the intestinal epithelium of plaice and flounder. These can result in the death of the host. *Pleistophora hippoglossoides* infects the muscle of Dover sole and American plaice *Hippoglossoides platessoides* and can render the fish unmarketable (Dyková, 1995) and another muscle infecting microsporean, *Tetramicra brevifilum* can be a problem in turbot culture. Myxosporean parasites are extremely common marine fish parasites. However, only *Ceratomyxa drepanopsettae*, *Myxidium sphaericum* and *Unicapsula muscularis* have been recognised as potential pathogens in flatfish. The first

two species are capable of inducing pathological change in the epithelial lining of the gallbladder and the muscle parasite; *U. muscularis* causes a condition known as ‘wormy halibut’ in *Hippoglossus stenolepis*. Infections with *Platyamoeba* are increasingly reported in turbot culture from certain areas in France and Spain. The susceptibility of other cultured flatfish species to this pathogen is unknown.

2. Monogenean parasites are also known to be pathogenic to various flatfish in culture situations. *Gyrodactylus unicopula* is pathogenic to juvenile plaice, *Neoheterobothrium affine* causes an inflammatory response in the gills of summer flounder and *Entobdella* species have been reported to cause problems in brood stocks of Dover sole (McVicar and MacKenzie, 1977). The pathogenicity of certain Benedeniid monogeneans including *Neobenedinia* and *Benedinia* is recognised. Since they exhibit low host specificity and have a wide distribution, their potential ability to cause mortality in culture situations should not be overlooked.

3. Most parasitic Copepoda undergo a free-living stage during their lifecycle. The majority undergo a series of moults through naupliar (free-living), copepodid, chalimus and adult stages. Flatfish are parasitised by a number of different genera including, *Acanthochondria*, *Bomolochus*, *Caligus*, *Haemobaphes*, *Hatschekia*, *Lepeophtheirus*, *Lernaeocera*, *Neobrachiella*, and *Phrixocephalus*. The majority of these have not been previously reported as being a problem for captive fish. However, all have the potential to cause problems in culture due to their direct mode of transmission and apparently innocuous species, present at low levels in wild fish may rapidly proliferate in culture situations. There are several potential sources of infection, primarily from introduction of wild fish with low numbers of parasites into naïve, susceptible stocks. Potentially, fish could be infected by the introduction of water containing infective naupliar stages or eggs.

Members of the family Caligidae (*Lepeophtheirus* and *Caligus*) are known to cause problems in culture. These parasites typically have direct life cycles. The life cycle of *Lepeophtheirus* is typified by two free living naupliar stages, a copepodid stage, four chalimus stages, two pre-adult stages and an adult stage. *Lepeophtheirus pectoralis* has been consistently isolated from moribund plaice in marine aquaria. This parasite can cause severe damage to the epidermis. The closely related salmon louse (*L. salmonis*) is a cause for concern in farmed salt-water salmonids. During initial trials in rearing cultivated turbot, massive infections with a *Lepeophtheirus* species have been observed. *Caligus curtus* appears to have a low host-specificity and it has been reported in high numbers in marine fish aquaria, often associated with moribund or dead fish, including flatfish. The penellid, *Lernaeocera branchialis* utilises flatfish as intermediate hosts with gadoids as final hosts. Although unlikely to be a problem in flatfish culture because of the absence of the final host, the larval forms can cause extensive damage to the gill filaments in flatfish, leaving them non-functional.

4. Other potential parasite pathogens include the isopod *Gnathia* sp. which, when present in large numbers can contribute to mortalities. However, these parasites have mainly been recognised as a problem in marine aquaria. Digenean metacercariae of *Cryptocotyle* and

Stephanostomum can give rise to heavy infestations in a variety of flatfish species, but avoidance of the snail intermediate hosts provides an effective control strategy for culture situations.

5. In hatcheries feeding extensively reared copepods to larval fish, the possibility of the transmission of helminth parasites from infected copepods should be recognised. Although apparently not a widespread problem at present, it has been recommended that intensively reared copepod diets should be developed (Bricknell *et al.*, 1997).

Environmental And Nutritional Factors

It is generally accepted that fish maintained in poor conditions will be stressed and that it is usually several factors in combination, which are involved in the onset of disease in the broadest sense. These factors are many and varied and include temperature, crowding, dissolved oxygen concentration and diet, as well as pollutants and the presence of potential pathogens. Direct toxic effects can cause significant pathological changes in many tissues and organs. The delicate epithelial surfaces of the gill filaments and skin frequently exhibit the first signs of acute toxicity. Chronic changes may then develop in the gill and other tissues, with liver, as the main organ for the detoxification of xenobiotics, frequently involved. In the controlled environment of modern aquaculture facilities, the presence of toxic compounds is minimised. However, the possible toxic effects of certain chemotherapeutants should be recognised and treatments administered following strict guidelines. In wild stocks the use of flatfish, diseases are employed as a biomarkers for monitoring biological effects of contaminants. In European waters, several external diseases, including ulceration, lymphocystis and epidermal hyperplasia/papilloma as well as hyperpigmentation are monitored in dab (*Limanda limanda*) and flounder (*P. flesus*). Increasing effort is being directed toward the standardised use of gross and microscopic liver pathology in international monitoring programmes. Although the presence of putative preneoplastic and neoplastic lesions offer a sensitive indicator of the presence of genotoxic contaminants in the environment, the range of lesion types is not the same for all flatfish species and the susceptibility of several commercial species for these lesions is unknown. The effect of dietary factors on growth performance is well recognised and imbalances or deficiencies in the diet can induce pathological changes in various tissues and may predispose fish to infectious diseases. Myopathy of skeletal muscles, hepatic lipoidosis and ceroidosis and anaemia are amongst the main pathological manifestations associated with lipid/fatty acid imbalances and there are many reports of specific pathological changes associated with a great variety of dietary components. From this perspective, the use of histopathology, and liver pathology in particular, offer an important tool for the general health assessment and nutritional status of cultured stocks.

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Chemotherapeutics and Treatment Calculations Used in Aquaculture

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Chemotherapeutics (drugs) are commonly used in the agriculture industry to combat disease. Unfortunately, there are relatively few chemotherapeutics approved by the U.S. Food and Drug Administration (FDA) for use in the food animal segment of the aquaculture industry. These include three antibiotics (oxytetracycline, sulfamerazine, and a sulfadimethoxine-ormetoprim combination), an external parasiticide (formalin) and an anesthetic agent (tricaine methanesulfonate). However, to use these compounds for foodfish requires adherence to strict FDA guidelines. These include that the compounds are (1) used for the prescribed indications including species and life stage, (2) used for the prescribed disease, (3) used at the prescribed dosages for the prescribed amount of time, (4) purchased from an approved source or distributor, (5) used according to good management practices, and (6) used so that adverse environmental effects do not occur.

In addition, there are a number of compounds which are categorized as Unapproved New Animal Drugs of Low Regulatory Priority which can be used judiciously for foodfish. These include such substances as acetic acid, calcium chloride, magnesium sulfate, papain, and sodium chloride.

Administration of the correct amount of drug or chemical can often present a difficult challenge. However, by following four standard steps, a treatment calculation can be correctly and rapidly calculated. First, determine the total amount of water to be treated. Second, use a conversion factor to determine the number of grams (or mls) required to get 1 ppm. Then, multiply by the number of ppm desired to get the final amount (grams or mls) needed to get that dose for that amount of water. Finally, divide by the strength of the compound (ie. 1.0 for a 100% compound, 0.5 for a 50% compound, etc. This session will use examples to demonstrate how to correctly calculate treatments for either a flow-through or a static bath system.

Appendix A

Appendix A: Guide to Drug, Vaccine, and Pesticide Use in Aquaculture. 1994. Prepared by the Federal Joint Subcommittee on Aquaculture, Working Group on Quality Assurance in Aquaculture Production, in cooperation with the Extension Service and U.S. Department of Agriculture, Texas A&M University, College Station, TX.

FDA – Regulated Drugs for Aquaculture

The drugs listed in this section include FDA-approved new animal drugs as well as unapproved new animal drugs of low regulatory priority for FDA. Federal approval of new animal drugs applies only to specific products that are the subject of approved new animal drug applications.

Active ingredients from sources other than the listed sponsors are not considered approved new animal drugs. Such products cannot legally be marketed or used.

States and other jurisdictions may impose additional regulatory requirements and restrictions on FDA-regulated drugs for agriculture.

Table 1. FDA - Approved New Animal Drugs

<i>Trade Name</i>	<i>NADA Number</i>	<i>Sponsor</i>	<i>Active Drug</i>	<i>Species</i>	<i>Uses</i>
Finquel (MS-222)	42-427	Argent Chemical Laboratories, Inc.	Tricaine methanesulfonate	Ictaluridae, Salmonidae, Esocidae, and Percidae. (In other fish and cold-blooded animals, the drug should be limited to hatchery or laboratory use.)	Temporary immobilization (anesthetic)
Formalin-F	137-687	Natchez Animal Supply	Formalin	Trout, salmon, catfish, large-mouth bass, and bluegill Salmon, trout, and esocid eggs	Control of external protozoa and monogenetic trematodes Control of fungi of the family Saprolegniaceae
Paracide-F	140-831	Argent Chemical Laboratories Inc.	Formalin	Trout, salmon, catfish, large-mouth bass, and bluegill Salmon, trout, and esocid eggs	Control of external protozoa and monogenetic trematodes Control of fungi of the family Saprolegniaceae

<i>Trade Name</i>	<i>NADA Number</i>	<i>Sponsor</i>	<i>Active Drug</i>	<i>Species</i>	<i>Uses</i>
Parasite-S	140-989	Western Chemical Inc.	Formalin	Trout, salmon, catfish, large-mouth bass, and bluegill Salmon, trout, and esocid eggs Cultured penacid shrimp	Control of external protozoa and monogenetic trematodes Control of fungi of the family Saprolegniaceae Control of external protozoan parasites.
Romet 30	125-933	Hoffmann-LaRoche, Inc.	Sulfadimethoxine and ormetoprim	Catfish Salmonids	Control of enteric septicemia Control of furunculosis
Sulfamerazine in Fish Grade ¹	033-950	American Cyanamid Company	Sulfamerazine	Rainbow trout, brook trout, and brown trout	Control of furunculosis
Terramycin for Fish	038-439	Pfizer, Inc.	Oxytetracycline	Catfish Lobster Salmonids Pacific salmon	Control of bacterial hemorrhagic septicemia and pseudomonas disease Control of gaffkemia Control of ulcer disease, furunculosis, bacterial hemorrhagic septicemia, and pseudomonas disease Marking of skeletal tissue

¹According to sponsor, this drug is not presently being distributed.



Table 2. Unapproved New Animal Drugs of Low Regulatory Priority for FDA¹

<i>Common Name</i>	<i>Permitted Use</i>
Acetic acid	Used as a dip at a concentration of 1,000-2,000 milligrams per liter (mg/L) for 1-10 minutes as a parasiticide for fish.
Calcium chloride	Used to increase water calcium concentration to ensure proper egg hardening. Dosages used would be those necessary to raise calcium concentration to 10-20 mg/L calcium carbonate. Also used to increase water hardness up to 150 mg/L to aid in maintenance of osmotic balance in fish by preventing electrolyte loss.
Calcium oxide	Used as an external protozoicide for fingerling to adult fish at a concentration of 2,000 mg/L for 5 seconds.
Carbon dioxide gas	Used for anesthetic purposes in cold, cool, and warmwater fish.
Fuller's earth	Used to reduce the adhesiveness of fish eggs in order to improve hatchability.
Garlic (whole)	Used for control of helminth and sea lice infestations in marine salmonids at all life stages.
Hydrogen peroxide	Used at 250-500 mg/L to control fungi on all species and at all life stages of fish, including eggs.
Ice	Used to reduce metabolic rate of fish during transport.
Magnesium sulfate (Epsom salts)	Used to treat external monogenetic trematode infestations and external crustacean infestations in fish at all life stages. Used in freshwater species. Fish are immersed in a solution of 30,000 mg/L magnesium sulfate and 7,000 mg/L sodium chloride for 5-10 minutes.
Onion (whole)	Used to treat external crustacean parasites and to deter sea lice from infesting external surface of fish at all life stages.
Papain	Used as a 0.2% solution in removing the gelatinous matrix of fish egg masses in order to improve hatchability and decrease the incidence of disease.
Potassium chloride	Used as an aid in osmoregulation to relieve stress and prevent shock. Dosages used would be those necessary to increase chloride ion concentration to 10-2,000 mg/L.
Povidone iodine compounds	Used as a fish egg disinfectant at rates of 50 mg/L for 30 minutes during water hardening and 100 mg/L solution for 10 minutes after water hardening.
Sodium bicarbonate (baking soda)	Used at 142-642 mg/L for 5 minutes as a means of introducing carbon dioxide into the water to anesthetize fish.
Sodium chloride (salt)	Used as a 0.5-1% solution for an indefinite period as an osmoregulatory aid for the relief of stress and prevention of shock. Used as a 3% solution for 10-30 minutes as a parasiticide.

<i>Common Name</i>	<i>Permitted Use</i>
Sodium sulfite	Used as a 15% solution for 5-8 minutes to treat eggs in order to improve hatchability.
Urea and tannic acid	Used to denature the adhesive component of fish eggs at concentrations of 15 g urea and 20 g NaCl/5 L of water for approximately 6 minutes, followed by a separate solution of 0.75 g tannic acid/5 L of water for an additional 6 minutes. These amounts will treat approximately 400,000 eggs.

FDA is unlikely to object at present to the use of these low regulatory priority substances if the following conditions are met:

1. The drugs are used for the prescribed indications, including species and life stage where specified.
2. The drugs are used at the prescribed dosages.
3. The drugs are used according to good management practices.
4. The product is of an appropriate grade for use in food animals.
5. An adverse effect on the environment is unlikely.

FDA's enforcement position on the use of these substances should be considered neither an approval nor an affirmation of their safety and effectiveness. Based on information available in the future, FDA may take a different position on their use.

Classification of substances as new animal drugs of low regulatory priority does not exempt facilities from complying with other federal, state, and local environmental requirements. For example, facilities using these substances would still be required to comply with National Pollutant Discharge Elimination System requirements.



Potential Zoonotic Infections in Cultured Foodfish

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Introduction

From limb and life-threatening injuries to carpal-tunnel syndrome, almost every commercial occupation carries a degree of physical risk. In the production animal workplace, the spread of disease from livestock to humans represents a small but perpetual hazard. Not surprisingly, each type of animal-rearing enterprise features its own array of potentially transmissible pathogens. Within piscine aquaculture and processing facilities, the agents that are most likely to cause human illness are bacteria.

Bacterial pathogens associated with live foodfish are generally ubiquitous to freshwater and/or marine environments, and may or may not be commonly isolated from cutaneous or enteric sites in healthy fish. In contrast, disease-causing bacteria that are more typically introduced to foods by way of environmental pollution or through post-harvest handling (e.g. *Shigella* spp., *Listeria* spp., *Clostridium* spp.), are far less likely to be encountered in fish farms utilizing closed systems. The spread of these bacteria to humans is usually through ingestion of fish products, therefore, they constitute a greater risk to end-consumers.

Transmission of bacterial pathogens to fish handlers occurs primarily through skin breaks, either via puncture wounds obtained during fish handling, or through water inoculation of pre-existing cuts. The accidental consumption of contaminated water represents another potentially significant route of infection. Fish-borne bacterial pathogens may cause human disease that remains localized to the skin or gastrointestinal tract of the host, or systemic spread may occur by way of vascular channels or serous surfaces. Generalized infections are more common in human individuals with immunosuppressive disorders.

Human bacterial diseases acquired from fish are often difficult to diagnose and treat. Diagnosis can be hampered by the failure of both human and veterinary laboratories to grow and accurately identify fish-borne bacterial species.(67) Additionally, routine bacterial cultures may not be always be performed, due to a general lack of success in isolating confirmed pathogens from human cellulitis patients.(34) Consequently, traumatically-induced fish-borne infections are probably drastically underreported. Factors that can hinder therapy in individual human cases include a deficiency of bacterial sensitivity data, and chemotherapeutic resistance. Resistance to antibiotics such as ampicillin, chloramphenicol, sulfonamides, and tetracyclines has been associated with the

presence of specific R plasmids found in bacteria such as *Aeromonas hydrophila*, *Edwardsiella tarda*, and *Vibrio anguillarum*.(4) Interestingly, bacteria resistant to commonly used antibiotics have been recovered not only from cultured fish, but also from the environment immediately surrounding fish farms.(61)

As applied to fish-borne contagions, the term zoonosis can be a source of minor confusion. Cited as "a disease of animals that may be transmitted to man under natural conditions (e.g. brucellosis, rabies)"(1), this narrow definition essentially excludes fish-associated bacteria that are pathogenic to humans, but have not been demonstrated to cause disease in fish (e.g. *Erysipelothrix rhusiopathiae*, *Salmonella* spp.). Another important distinction is that pathogens that cause disease in both humans and fish independently, i.e. without conclusive evidence of transmissibility (e.g. *Citrobacter freundii*, *Lactobacillus* spp.), should not be considered zoonotic .

It should be noted that disease acquisition from cultured foodfish is not necessarily limited to fish farmers, fish processors, and fish consumers. Individuals at varying degrees of risk in other vocations include veterinarians, research scientists, and retailers.

Aquaculture-associated Bacteria Potentially Pathogenic to Humans

Included are pathogens that have been reported to cause disease to fish handlers in aquaculture or processing facilities, plus other bacteria that have a reasonably high zoonotic potential in these settings. This list is far from complete, as the literature contains periodic case reports of human disease caused by fish-associated bacteria such as *Klebsiella* spp.(48), *Pseudomonas* spp.(8), and *Yersinia ruckeri*(18).

1 . *Aeromonas* spp. (motile aeromonads)

These Gram-negative, facultatively-anaerobic rods can be isolated from both fresh and salt water sources, and from the gastrointestinal tracts of healthy fish(53) among other animals. Motile aeromonad species, including *A. hydrophila*, have been circumstantially, if not definitively, associated with disease in fish. Well-established syndromes that implicate aeromonads include a bacteremic condition known as "motile aeromonas septicemia", and the dermal and muscular excavations of "red sore" (*Aeromonas* spp. combined with *Epistylis* spp. protozoa and other pathogens). Human exposure to pathogenic aeromonads has been demonstrated to occur through both wound infections, and via ingestion of contaminated water or foods.(19,27,32,36) *Aeromonas hydrophila*, *A. sobria*, and *A. caviae* are the *Aeromonas* species most commonly involved in human infections.(33,44) Conversely, *A. salmonicida* is considered to be an obligate fish pathogen that is of no known danger to humans.(40) Fatal and non-fatal manifestations of human *Aeromonas* spp. infection include gastroenteritis, cellulitis, endocarditis, myositis, meningitis, osteomyelitis, and septicemia.(19,28,36,62) There is evidence of increased risk to persons with liver disease, malignancies, or other immune-compromising disorders.(36)

2. *Edwardsiella tarda*

Reptiles and freshwater fishes are thought to be the predominant natural reservoirs of this Gram-negative bacterial rod.(70) *E. tarda* causes serious septicemic disease in a wide range of fish species(35), despite its role as a normal intestinal inhabitant of fish.(63) Additionally, *E. tarda* is responsible for the economically significant condition known as “emphysematous putrefactive disease of catfish”. *E. tarda* is the only member of its genus that is a recognized human pathogen.(68) Documented routes of transmission to humans include penetrating wounds and consumption of contaminated water or infected fish.(37) Wound infection in humans may remain localized (e.g. deep soft tissue abscesses), or progress to generalized disease (e.g. septicemia with meningitis).(18,35,68) Human edwardsiellosis is more common in tropical or subtropical regions(63), and neonates, infants, and individuals with pre-existing illnesses are apparently predisposed to *E. tarda* infection.(63,68)

3. *Erysipelothrix rhusiopathiae*

Erysipelothrix rhusiopathiae is a non-motile, non-sporulating, facultatively-anaerobic Gram-positive rod that can be isolated from a wide range of animal species, and decomposing matter of animal origin.(23) Despite its ubiquitous presence in the environment, the principal reservoirs for *E. rhusiopathiae* infection are latently-infected carrier animals such as domestic swine and rodents.(69) *E. rhusiopathiae* has been associated with both freshwater and marine fish, where it can be found in the external body mucus, possibly acquired through post-harvest contamination.(39) Whether or not the bacterium actually exists on surface of living fish, *E. rhusiopathiae* is not considered to be a fish pathogen.(54,69) Transmission to humans is predominately through minor skin wounds, and opportunistic exposure is the most important factor governing human infection.(69) There are three well-defined forms of human *Erysipelothrix* infection: mild localized cutaneous, rare diffuse cutaneous, and septicemic (often with endocarditis).(23) The comparatively common localized form (also known as “fish rose”, “fish-handler’s disease”, or “erysipeloid of Rosenbach”) is a self-limiting condition that has a strong occupational association with fishermen, fish and shellfish handlers, veterinarians, bacteriologists, and butchers, among others.(23,69) The more generalized manifestations of erysipelas may be exclusive to individuals with pre-existing poor health, as humans are thought to be innately resistant to *Erysipelothrix* infection.(69)

4. *Leptospira* spp.

Historically, human leptospirosis has been sporadically related to aquaculture. From 1975 to 1983, 24 human leptospirosis cases occurred in association with non-fish aquaculture facilities in Hawaii.(2,38) Coincidentally, during the same time period, several trout farmers in England contracted infections with *Leptospira interrogans* serogroup *icterohemorrhagiae*, resulting in flu-like symptoms, jaundice, and even death.(51) Despite the seriousness of this outbreak, a subsequent study revealed that other English fish farmers in the region did not have *Leptospira* spp. antibody titers, thus indicating only a moderately-increased occupational risk for leptospirosis.(21) Although associated with aquatic environments, the true reservoirs for these aerobic,

flexible, helical, Gram-negative bacteria are feral rodents, other small mammals, and amphibians. (3) Fish are not a common source of leptospirae, and naturally-occurring piscine leptospirosis has not been documented.(38,51,54) Invariably, the link between human leptospirosis and aquaculture appears to involve the contamination of waterways or fish feeds by rodents, seagulls, or other terrestrial vectors.(13,21)

5. *Mycobacterium* spp.

Mycobacteriosis in fish is a gradually progressive systemic granulomatous disease that affects both natural and farmed populations of freshwater and marine fish. Piscine mycobacteriosis is usually caused by one of three species of aerobic, Gram-positive, non-sporeforming, non-motile, pleomorphic acid-fast bacilli: *Mycobacterium fortuitum*, *M. marinum*, or *M. chelonae*.(9,12,20,29,49,60) Reservoir sources of mycobacterial infection include both carrier fish and the environment,(10,14,20,24,25,41,43,58), and proposed routes of piscine transmission are ingestion of contaminated materials(24,25,41,52,57,59), through damaged gill or skin tissue(14), and via transovarian passage in ovoviviparous fishes.(11,41,56) Mycobacteriosis outbreaks are not unusual in intensively cultured foodfish(30,57,58), however, individual case reports suggest that zoonotic infections are more often derived from home aquaria. Popular terms for localized mycobacteriosis associated with aquarium cleaning include “fish tank granuloma” and “fish fancier’s finger”.(6,40,42) Due to antibiotic resistance, such infections may be refractory to treatment, requiring appendage amputation in rare circumstances.(55) Immunosuppressed individuals tend to have an increased potential for deeply disseminated mycobacterial infections.(40)

6. *Plesiomonas shigelloides*

Currently classified within the family *Vibrionaceae*, *P. shigelloides* is a facultatively-anaerobic, motile, Gram-negative bacillus that is widely-distributed in animal and human alimentary tracts, soil, and water.(7,22) This microorganism favors fresh and brackish waters within tropical and subtropical regions.(5,7,32) Consequently, in temperate climates, plesiomonad replication is facilitated by warmer temperatures.(7) Although *P. shigelloides* can be isolated from fish tissues such as skin, gills, and intestines, this bacterium does not appear to be an important fish pathogen. In humans, *P. shigelloides* is responsible for enteric infections (chiefly related to raw seafood consumption), and extra-intestinal disease ranging from cellulitis induced by puncture wounds, to sepsis, meningitis, arthritis, or endophthalmitis.(22,32) Risk factors associated with *P. shigelloides* infection include raw oyster consumption, travel to Mexico, crabbing, and compromised host immunity.(7) Fish handlers, veterinarians, aquaculturists, zoo keepers, and water sports performers are thought to be vocationally predisposed.(7)

7. *Streptococcus iniae*

First reported in 1976 as a cause of abscesses in captive freshwater dolphins(47), *S. iniae* garnered attention in early 1996 when nine cases of human *S. iniae* -induced

disease were associated with the cleaning of cultured tilapia by Canadian consumers.(65,66) During this outbreak, human patients were infected through lacerations or puncture wounds acquired during fish preparation. Local cellulitis was the most common problem caused by *S. iniae* infection, although one individual suffered from septic arthritis, meningitis, and endocarditis. *S. iniae* had not been recognized as a human pathogen prior to this episode, possibly due to a generalized failure to accurately identify it.(65) Conversely, this Gram-positive, non-motile, non-sporeforming, facultatively anaerobic, coccus has been known to colonize the dermis and cause invasive disease in a variety of farmed fishes (tilapia, rainbow trout, coho salmon, hybrid striped bass), often with high mortality.(16,45,46) Lesions in affected fish typically include meningoencephalitis, epicarditis, and other manifestations of septicemia such as ascites, cutaneous hemorrhage, exophthalmia, and corneal opacity.(46)

8. Vibrio spp.

These Gram-negative, motile, facultatively-anaerobic, straight or curved rods are ubiquitous in marine environments, and they tend to proliferate in response to a seasonal increase in water temperature.(15,26) Additionally, vibrios may be isolated from brackish or fresh waters(28,31), and from the external surface and gastrointestinal tract of finfish. *Vibrio* spp. such as *V. salmonicida*, *V. anguillarum*, *V. damsela*, *V. alginolyticus*, *V. vulnificus*, and *V. ordalii*, may act as primary or secondary fish pathogens, responsible for ulcerative dermatitis and/ or bacteremic lesions in saltwater teleosts. In the United States, the four most common *Vibrio* spp. associated with human disease are *V. parahaemolyticus*, *V. vulnificus*, *V. cholera* type 01 (the agent of classic cholera), and *V. cholera* non-01. Of this group, the most dangerous species, and perhaps the most likely to be caused by fish-related puncture wounds, is *V. vulnificus*. Similar to other *Vibrio* spp., *V. vulnificus* has been known to cause human gastroenteritis (e.g. following the ingestion of undercooked shellfish), however, chronic deep-seated soft tissue infections and septicemia are more common outcomes. Several well-defined predisposing factors to *V. vulnificus* infection include underlying liver disease, alcoholism, diabetes, hemochromatosis, and immunosuppressive disorders.(17,31,32,50,64) The overall mortality rates for individuals with untreated and treated *V. vulnificus* infections are 50-90% and 25%, respectively.(15,17,50,64)

Some Recommendations for the Prevention of Zoonotic Disease in Aquaculture Facilities

Guidelines established for the prevention of aquaculture-associated human disease should be based upon fish health considerations, employee health considerations, and biosecurity.

A. Fish Health Considerations

For any fish-rearing or processing facility, it is reasonable to assume that the potential for zoonotic bacterial disease increases in proportion to the numbers of actively-infected fish within the facility at a given time. The maintenance of disease-free stock is therefore a

paramount concern for both the profit margin and for the safety of fish handlers. One key to keeping fish healthy is to start with healthy fish. In this regard, procedures such as segregated quarantine, prophylactic anti-parasitic therapy, and the sacrifice of specimens for medical evaluation and bacterial culture of internal organs, are advocated as routine practices for all newly imported lots, even when fish are obtained from proven providers. Recognizing that most bacterial fish pathogens are opportunistic invaders, the mitigation of physiologic stressors is a universally-acknowledged method of decreasing fish disease. Limiting stress usually involves committed attention to factors such as water quality, overcrowding, and excessive or inappropriate handling. A final animal health consideration is the generation of antibiotic resistance by bacteria. In fish disease outbreaks caused by resistant bacteria, effective treatment is often delayed, thus exposing workers to infected fish for extended amounts of time. Additionally, zoonotic infections acquired from repeatedly treated fish are more likely to be medically intractable. In order to decrease the occurrence of antibiotic resistance in aquaculture facilities, antibiotic therapy should be based strictly upon bacterial isolation and antibiotic sensitivity results.

B. Employee Health Considerations

The relevant literature consistently suggests that the incidence and severity of fish-associated zoonotic infections are dependent in part upon the immune status of the human hosts. Therefore, it is suggested that persons who handle large numbers of farmed fish on a regular basis receive an initial baseline health screen (including a tuberculin test), followed by scheduled periodic medical examinations. General safety and hygiene training of aquaculture personnel should include specific information relative to the management and reporting of fish-associated injuries. Additionally, it is imperative that persons with preexisting viral, neoplastic, metabolic, or other immunocompromising conditions be made aware of their increased risk for zoonotic infection.

C. Biosecurity

Infected stock are not the only potential source of pathogenic fish-associated bacteria. Biosecurity measures to decrease the introduction of pathogens into fish-holding facilities include: 1) the use of a sanitary and protected water supply, 2) physical barriers to prevent contamination by biological vectors such as birds, rodents, and human visitors, and 3) the education of employees in principles of aquaculture hygiene.

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