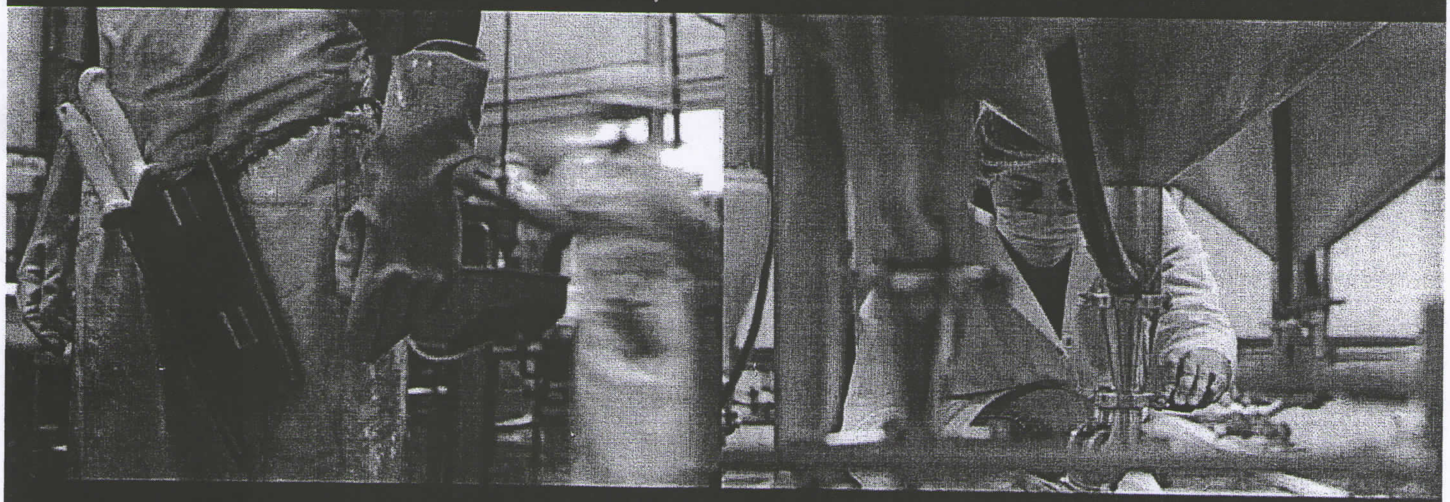
Food Safety Management

A Practical Guide for the Food Industry



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FOOD SAFETY MANAGEMENT

A PRACTICAL GUIDE FOR THE FOOD INDUSTRY

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14 15 16 17   10 9 8 7 6 5 4 3 2 1

20 Chilling and Freezing

STEPHEN J. JAMES, CHRISTIAN JAMES

21 Detection of Physical Hazards

GILLES DEMAUREX, LAURENT SALLÉ

III
FOOD SAFETY ASSURANCE SYSTEMS

22 Principles and Systems for Quality and Food Safety Management

PETER OVERBOSCH, SARAH BLANCHARD

23 Hygiene in Primary Production

GISELA KOPPER, SLAVKO MIRECKI, IGOR S. KLJUJEV, VERA B. RAICEVIC, BLAZO T. LALEVIC, JELENA PETROVIC-JOVICIC, STOJMIR STOJANOVSKI, DIJANA BLAZEKOVIC-DIMOVSKA

24 Hygiene in Food Processing and Manufacturing

JOHN HOLAH

CHAPTER

23

Hygiene in Primary Production

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BOX 23.13

INFORMATION THAT SHOULD BE RECORDED ON FARM

- Information on farm management, i.e. personnel information, training, health status.
- Information on pesticides and fertilizations applied to each production lot.
- Results of water analysis.
- Cleaning and sanitizing procedures.
- Information on production management, i.e. history of each animal, feed and water; farm management; animal health; production records and quality of animal products such as raw milk quality.

tasks of agricultural production is to preserve the environment and biodiversity, with simultaneous, economically justified, agricultural production.

Record Keeping

Farms should have a well-planned and established system of documentation. Important information should be archived for at least 3 years for the purposes of traceability.

PART 3: FISH HYGIENE

BACKGROUND

Statistics published by the FAO (Food and Agriculture Organization of the United Nations) show that inland capture fisheries production follows the general trend of most of the world's sea fishing areas, which have apparently reached their maximum potential, with the majority of stocks being fully exploited. In contrast, growth in aquaculture production has shown the opposite trend. While capture fisheries production has increased only very slightly, output from aquaculture (farmed fish, shellfish and algae) increased significantly from just over 13 million tonnes in 1990 to 33 million tonnes in 1999.

Despite its healthy growth, the aquaculture industry still faces problems with diseases which can affect its sustainability. Infectious diseases caused by viruses, bacteria and parasites are continuing threats to consistent industry growth. With increasing intensification, the incidence of diseases is also expected to increase proportionately.

DISEASE PREVENTION

Most diseases can be prevented through good husbandry practices and proper screening of incoming animals to the facility. When possible, bring in only eggs from a reputable supplier that can provide disease-free animals. Commercial blood test kits are available to screen fish for antibodies of several important fish pathogens. These kits normally do not determine active infections but can provide evidence of previous exposure by vaccination or live-disease-causing agents. More expensive laboratory DNA tests of fish tissue using polymerase chain reaction (PCR) can provide an even higher degree of sensitivity than antibody-based tests to identify subclinical infections or previous use of vaccinations.

Isolation, rapid removal and necropsy of dead animals will reduce the spread of disease and help to provide early diagnosis and treatment of the problem. Prophylactic external (e.g. chloramine T, etc.) treatments during and after handling procedures will prevent the start of many infections. Separate nets for each tank and iodophore disinfection baths for equipment will reduce cross-contamination problems.

Vaccines are commercially available to protect against vibriosis, furunculosis, enteric red mouth and enteric septicemia bacteria. New vaccines are in development for several commercially important viral and rickettsial fish pathogens. Specialty orders of "autogenous" vaccines can also be manufactured to protect against unique or emerging bacterial pathogens. All vaccines require that the fish be held for a period of disease-free conditions (usually 3–5 weeks) after vaccination to build up immunity before any significant exposure to infectious diseases. Vaccinations against vibriosis, enteric red mouth and enteric septicemia bacteria can be delivered to the fish by immersion. For other diseases, intraperitoneal injection is the preferred route for maximum protection and duration of immunity.

Selective breeding using quantitative genetics can be used to produce strains of fish with enhanced resistance to specific diseases. The traits for selection must be based on genes with sufficient heritability for this process to be successful. Furunculosis-resistant strains of brook trout and brown trout are examples of the great potential of these efforts.

DISEASE TREATMENT

Bacterial, parasitic and fungal diseases can all be controlled with chemo-therapeutants. Viral diseases are best prevented or eliminated by isolation and quarantine procedures. The key to successful treatment is the proper identification of the primary cause of the observed losses. For example, the observation of a single external parasite is not a reason for immediately beginning treatment without further investigation of other underlying infections or water quality problems. Consultation with a fish health pathologist or experienced veterinarian is strongly recommended before starting any treatment.

All compounds can have side effects and it is essential that caution be used in handling and use of any chemical.

Fungal infections of eggs can be treated with methylene blue or formalin. However, frequent removal of dead eggs is critical to the success of hatching survival and may limit the need for such treatments. Formalin is also useful for the treatment of external parasitic infections in juvenile or adult fish.

Damaged gills or skin can often be treated with sodium chloride to improve the osmotic balance between the water and the fish tissues. Dissolved salt treatments improve the effect of other surface compounds by removing debris and mucus from the gills and skin.

Judiciously applied, potassium permanganate can remove surface parasites and bacteria from fish in freshwater systems.

If a pathogenic bacterium is isolated, tests for antibiotic resistance should be done to select the best drug and treatment regime. Gram-negative organisms are commonly treated with oxytetracycline or sulfamethoxazole plus trimethoprim. Gram-positive bacteria are more responsive to erythromycin or doxycycline. Caution should be exercised if the biological filter media will be exposed to these compounds because water quality may quickly deteriorate if the nitrifying bacteria are lost.

Antibiotics added to the feed may not be appropriate if the affected population of fish is refusing to eat. Fish oil additives to enhance palatability or direct injection of antibiotics are alternatives to consider before applying oral drug treatments. Particular attention must be paid to local and federal regulations regarding restrictions on the use and withdrawal periods of antibiotics in food fish.

An indication of the magnitude of economic losses is illustrated by farm surveys conducted in 16 Asian countries, which show that annual losses due to disease in the region total more than USD 3 billion. Probably the most striking example of disease spread through international trade and consequential major economic loss in aquaculture is white spot disease in farmed shrimp. The disease first emerged in 1991 in a shrimp farm in an OIE member country and apparently has since spread to most other shrimp-farming countries of Asia and the Americas. This has been attributed by some experts to the uncontrolled international trade in live shrimp for aquaculture purposes and in dead shrimp for processing. Some countries with shrimp-farming activities continue to be free of the disease, almost certainly due to strict controls on imports of live shrimp and uncooked dead shrimp, in particular for use as fish bait.

The adverse social, economic and environmental consequences of uncontrolled movement of live aquatic animals and their products have increased global awareness of the need for improved health management standards. The serious impact of unrestricted international movement of aquatic animals has led to the development of health certification and risk reduction methodologies. The International Aquatic Animal Health Code and the Diagnostic Manual of Aquatic Animal Diseases are published by the OIE and provide recommendations and standards for reducing the spread of specific aquatic animal diseases considered to be of significance for international trade. They are recognized by the World Trade Organization as the international standards for trade.

The importance of containing the threat of diseases in fish production is a matter of global concern especially with increased trade and increased transboundary movements of goods which include live fish and other aquatic organisms. Due to this concern, the minimum EU measures for the control of the fish diseases are referred to in list I and II of Annex A to Council Directive 91/67/EEC (EC, concerning the animal health conditions governing the placing on the market of aquaculture animals and products, 1991). The diseases are categorized in three lists (Table 23.8).

An outbreak of a fish disease can quickly take on epizootic proportions, causing mortality and disturbances on a scale liable to reduce severely the profitability of aquaculture. Therefore it is important that control measures are taken when the presence of such a disease is suspected so that immediate and effective actions can be implemented as soon as its presence is confirmed. Such measures are aimed at preventing the spread of the disease, in particular by carefully controlling movements of fish and products liable to spread the infection.

TABLE 23.8 Listed Diseases/Pathogens of Fish, Mollusks and Crustacea (Annex A of Directive 91/67/EC)

Disease/Pathogen	Susceptible Species
LIST I	
Fish	
Infectious salmon anemia (ISA)	Atlantic salmon (*Salmo salar*)
LIST II	
Fish	
Viral hemorrhagic septicemia (VHS)	Salmonid species Grayling (*Thymallus thymallus*) Whitefish (*Coregonus* spp.) Pike (*Esox lucius*) Turbot (*Scophthalmus maximus*)
Infectious hematopoietic necrosis (IHN)	Salmonid species Pike fry (*Esox lucius*)
Mollusks	
Bonamia ostreae	Flat oyster (*Ostrea edulis*)
Marteilia refringens	Flat oyster (*Ostrea edulis*)
LIST III	
Fish	
Infectious pancreatic necrosis (IPN) Spring viremia of carp (SVC) Bacterial kidney disease (BKD) (*Renibacterium salmonidarum*) Furunculosis (*Aeromonas salmonicida*) Enteric red mouth disease (ERM) (*Yersinia ruckeri*) *Gyrodactylus salaries* Crustaceans Crayfish plague (*Aphanomyces astaci*)	To be specified in the program referred to in Articles 12 and 13 of Directive 91/67/EC

When fish on a farm are suspected of being infected with a list I disease, infectious salmon anemia (ISA), the official services in the member states must initiate official investigations to confirm or rule out the presence of the disease. No movement of fish, whether dead or alive, eggs and gametes are allowed without the authorization of the official service. When the presence of the disease is confirmed, fish infected with the disease are killed and destroyed as soon as possible to prevent the spread of the disease. Member states must have contingency plans for list I diseases.

List II diseases are important endemic diseases that should be contained and eradicated in the long term. Where fish are suspected of being infected with a list II disease, i.e. viral hemorrhagic septicemia (VHS) and infectious hematopoietic necrosis (IHN), an official investigation must be initiated to confirm or rule out the presence of the disease. Approved farms and zones will lose their status as free from the disease until it is proven that the disease is eradicated.

All farms rearing or keeping fish susceptible to list I or list II disease must be registered by the official service and keep records of mortality and the movement into and out of the farm.

Council Directive 2006/88/EC (EC 1991) on animal health requirements for aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals, establishes:

- animal health requirements for the placing on the market, importation and transit of aquaculture animals (fish, mollusks and crustaceans) and their products;
- minimum measures to prevent diseases in aquaculture animals;
- minimum measures to be taken in response to suspected or established cases of certain diseases in aquatic animals.

MAJOR FISH DISEASES

Fish Viral Diseases

Prevention and control of viral diseases in fish are rather limited. Efficient chemotherapeuticals for viral diseases do not exist. Also, efficient vaccines are obtained only for a certain number of viruses. Unique practical measures for the control of viral diseases are: quarantine, control of trading of fish and their products, as well as strict disinfection and harmless removal of diseased fish.

In this respect, all reproductive centers for fish young have to be free of viral infections. Water-supplying systems in aquaculture have to be protected from the ingress of wild fish, as well as other water organisms, that might be carriers of viruses. If viruses enter an aquarium and infect the fish, it is very difficult to remove fish infected with that virus.

Viral diseases that affect the health of fish are: infectious pancreatic necrosis of salmonid fry (IPN), spring viremia of carp (SVC), pox disease of carp, viral hemorrhagic septicemia (VHS), infectious hematopoietic necrosis (IHN), infectious salmon anemia (ISA), and lymphocystis disease.

Infectious pancreatic necrosis (IPN) is an infectious, contagious disease, which attacks salmonid fry. It passes in acute stadium, characterized by a sudden explosive outbreak with high mortality. Affected fish become dark and rotate their bodies while swimming. They usually have exophthalmia and distended abdomens with the presence of a gelatinous material in the stomach and anterior intestine.

Spring viremia of carp (SVC) is an infectious, very contagious disease, which appears in acute form, and is manifested by symptoms of hemorrhagic diathesis, enteritis and peritonitis.

Pox disease of carp (*Epithelioma papillosum*) is an infectious, contagious disease, which attacks cyprinid fish species. It appears in chronic stadium, with hyperplasia of epidermis of skin and fins, such as hard gelatinous milky-white tumoroid proliferates, and in advanced cases with metabolic disorders of mineral matters. This disease has a benign character.

Viral hemorrhagic septicemia (VHS) is an infectious, contagious disease, which attacks mainly rainbow trout. It is the most serious viral disease of farmed rainbow trout, and is manifested by variable clinical symptoms: hemorrhagic syndrome, hydropsy characteristics and anemia, up to nervous disorders.

Infectious hematopoietic necrosis (IHN) of salmonid fry is manifested with hemorrhagiae and edema, accompanied by necrotic alterations of the wall of blood vessels. The hematopoietic tissues of the kidney and spleen of young fish are the most severely affected and are the first tissues to show extensive necrosis. This disease is very similar to VHS.

Lymphocystis disease is an infectious disease, with chronic progression and benign character. It is manifested with the appearance of pebble or wart-like nodules most commonly seen on the fins, skin or gills, although other tissues may be affected.

Infectious salmon anemia (ISA) is an infectious disease. It is associated with high mortalities and is of great economic significance for the Norwegian fish farming industry. Infected fish are lethargic and severely anemic. Other typical signs are ascites, petechiae in internal organs and hemorrhagic liver necrosis.

More information on viral diseases can be found in FDA (2011), Hristovski and Stojanovski (2005), OIE (2012) and Woo and Bruno (1999).

Fish Bacterial Diseases

Bacteria exist in different environments in nature. Bacteria have a major role in the circulation of matter in natural ecosystems, but certain bacteria can cause serious diseases in fish.

Some fish, which show no signs of having a certain disease, may be carriers of infective agents. But if those fish are exposed to stress factors, the disease may manifest itself and begin to excrete pathogenic microorganisms into the water, leading to repeated outbreaks of disease.

Exact identification of organisms that lead to the appearance of infective disease is particularly important, as well as determination of antimicrobial substances that successfully act against them. Different species of fish need different treatments. Usage of inappropriate antimicrobial components might create resistant lineages of bacteria.

Bacterial diseases harmful to fish health are: erythrodermatitis of carp, furunculosis of salmonids, motile aeromonas septicemia (MAS), vibriosis, yersiniosis, Edwardsiellosis, Edwardsiellosis enteritic septicemia of catfish, ulcer disease of salmonids, bacterial kidney disease, columnaris disease, bacterial cold water disease, mycobacteriosis and nokardiosis.

Erythrodermatitis of carp is an infective bacterial disease, which appears in subacute or chronic form, and is manifested with characteristic alterations of skin as erosions with progressive character and possible generalized form with hard clinical picture, where general hydropsy dominate.

Furunculosis of salmonids is an infective, very contagious bacterial disease of salmonid fish species, which appears in peracute, acute, subacute and chronic form, and is manifested with local alterations on the skin, but in certain clinical cases in the form of septicemia.

Motile aeromonas septicemia (MAS) is caused by ubiquitous *Aeromonas hydrophila* complex, and is manifested by hemorrhagic septicemia.

Vibriosis (*Erysipelosis anguillarum*) is an infective, very contagious bacterial disease of salmonid fish species, which appears in peracute, acute and chronic form, and is manifested with septicemia in acute form, and formation of abscesses and ulcers in chronic form. The losses produced by this disease are so disastrous that vibriosis caused by *V. anguillarum* has been recognized as a major obstacle for salmonid marine culture.

Yersiniosis (enteric redmouth disease) is a bacterial, subacute or acute disease of salmonids, which is manifested by hyperemia and hemorrhage on the head. It is present in a carrier state in many species of fish and remains undetected until stress.

Edwardsiellosis (*Edwardsiella septicemia*) is a serious systemic bacterial, subacute or chronic disease of warm water, rarely cold water fish species, commonly known as fish gangrene, emphysematous putrefactive disease of catfish or red disease of eels. The disease is manifested by formation of erosions, abscesses and ulcers on the skin. Infective agents can cause disease at other animals (reptiles, birds, mammals). It can cause gastroenteritis, abscesses and meningitis in humans. It sometimes produces a subclinical infection in fish intended for human consumption, where it may create problems during the cleaning process, which requires processing interruption, cleaning of equipment and disposing of infected fish.

Edwardsiellosis enteritic septicemia of catfish is a bacterial, subacute or chronic disease of cultured warm water fish species from the family Ictuliridae, which is manifested by formation of petechial hemorrhage, erosions, abscesses and ulcers on the skin. A characteristic clinical symptom is a longitudinal ulcerative lesion in the area between the eyes, which can even reach skull bones. There is no indication that *E. ictaluri* poses a health threat to aquatic animals and humans, probably due to temperature limitations under which bacteria grow.

Ulcer disease of salmonids is an infective disease, which might appear as local infection of the skin or as acute septicemia.

Bacterial kidney disease is an infective, very contagious bacterial disease of cultured and wild salmonid fish species, which appears in chronic form, and is manifested with necrotic alterations of kidneys, development of anemia and high rate of mortality.

Columnaris disease is one of the most frequent infective bacterial diseases, which appears in different fish species, and is manifested with alterations of skin and gills.

Bacterial cold water disease is a serious septicemic disease of the young of salmonid fish species bred in hatcheries. It appears at $t < 12°C$. At the start the disease has a local character, with alterations of fins, musculature, gills; later even internal organs, particularly kidneys, become affected.

Nokardiosis is a chronic, granulomatous disease of fresh- and saltwater fish, which is very similar to tuberculosis, according to clinical symptoms.

Mycobacteriosis is a bacterial, contagious, subacute to chronic, systemic, progressive disease, which appears in all fish species, and is manifested by nodules and ulcerations on the skin and tuberculous nodules in internal organs. It is not of major importance in intensive fish breeding, but is particularly harmful for breeding of aquarium fish, because they are often kept for long periods of time compared with fish raised for commercial purposes. Piscine tuberculosis is caused by three species of bacteria belonging to the genus *Mycobacterium*, which is also the causative agent of tuberculosis in humans. While the bacteria that causes this disease in fish prefers cooler temperatures than most bacteria that infect humans it is still possible for the illness to be passed on to humans. Such an infection in humans usually shows in the form of an infected nodule in the skin, although there is a chance of a more serious internal infection.

Fish pathogenic bacteria are harmful to humans and others: they are pathogenic for warm-blooded animals and humans; however, fish usually are not diseased by these pathogens, but they can be germ carriers (in internal organs, skin or gills) for some time (weeks or months). Fish pathogenic bacteria are:

1. *Salmonella*;
2. *Listeria* – salmonids might be diseased;

3. *Leptospira;*
4. *Erysipelotrix rusiopathiae* – erysipeloid diseases are found in humans who work in the fish processing industry or in fish trading. The condition known as "crayfish handler's disease" is well known in the fishing industry. It can be caused by various bacteria, but particularly *Erysipelothrix rhusiopathiae* and various species of the *Vibrio* genus. The bacteria enter the skin through abrasions, lacerations or fissures and cause a painful itching or burning sensation;
5. *Vibrio parahaemolyticus* – can cause mild disease in fish. But diseases in humans who eat fresh fish, crayfish or shellfish are frequent;
6. *Clostridium botulinum* – as with other *Clostridium* species, it is ubiquitous as well. Therefore, many kinds of food might be contaminated. Type E toxin is the most poisonous of all the toxins (A–E). It is present in sediments of open waters, near coastlines.

More information on bacterial diseases can be found in FDA (2011), Hristovski and Stojanovski (2005), OIE (2012) and Woo and Bruno (1999).

Fish Fungal Disease

Fish mycoses are considered difficult to prevent and treat, particularly in intensive freshwater systems, and are reported to be second only to bacterial disease in economic importance to aquaculture.

Ichthyophoniasis, due to infection with *Ichthyophonus hoferi,* has been known in fish since the end of the 19th century. The disease is recognized to be of economic significance, in both fish cultivation and wild fisheries, and to have a wide host and geographical distribution. Included as hosts have been various marine and freshwater crustaceans, fish (35 marine fish species and 48 freshwater species), amphibians, reptiles and piscivorous birds. *Ichthyophonus* has been recorded from many temperate and some tropical waters throughout the world. Manifested external signs include skin roughening ("sandpaper effect") and occasional ulceration. Inside the body are gross white or cream-colored nodular lesions 1–5 mm in size throughout most tissues.

Fungal infections of fish by oomycetes, commonly known as water molds, are widespread in fresh water and represent the most important fungal group affecting wild and cultured fish.

Four orders are recognized in this class and the most important are the Saprolegniales. Although eight genera have been reported in infections, namely *Saprolegnia, Achlya, Aphanomyces, Calyptratheca, Thraustotheca, Leptolegnia, Pythiopsis* and *Leptomitus,* only *Saprolegnia, Achlya* and *Aphanomyces* are significant in aquaculture.

Some species are consistently isolated from fish and generally these are assigned to a single major cluster, which form a coherent, separate taxon, *Saprolegnia parasitica* (synonym *Saprolegnia diclina* Humphrey type 1).

The Saprolegniaceae, in particular members of the genus *Saprolegnia,* are responsible for significant infections, involving both living and dead fish and eggs, particularly in aquaculture facilities. Oomycetes are classical saprophytic opportunists, multiplying on fish that are physically injured, stressed or infected. Fungal outbreaks among farmed fish stocks are frequently associated with poor water quality, injuries associated with handling and grading, temperature shock, infestation by parasites and spawning. However, there is evidence that some Saprolegniaceae act as primary pathogens.

The oomycetes are an economically important group of mycotic agents that affect salmonids and other teleosts. They are reported extensively in both wild and farmed fish and are considered ubiquitous in freshwater ecosystems. Oomycete infections have also been recorded in the marine fish species. In the marine environment, oomycetes are significant pathogens of lobsters and crayfish.

Saprolegniasis is frequently observed as a superficial and chronic infection. It may occur anywhere on the body of fish, but normally appears as a conspicuous, circular or crescent-shaped, white, cotton-like mycelium, on the integument and gills of host fish or eggs, particularly around the head and the caudal and anal fin, which may spread over the entire body surface. Most fish die due to osmotic or respiratory problems if the area of skin or gills is large.

Branchiomycosis (gill rot) is caused by two species *Branchiomyces sanguinis* and *B. demigrans*. It is primarily a problem in carp and eels. The disease occurs most commonly in ponds with abundant organic matter and high ammonia levels. Usually higher temperatures (20–25°C) bring about the disease. Affected fish usually show respiratory distress. There is prominent gill necrosis caused by thrombosis of blood vessels in the gills. Histologically the identification of nonseptated branching hyphae with an intrahyphal eosinophilic round body (apleospores) in and around blood vessels of the gill is diagnostic.

More information on fungal diseases can be found in FDA (2011), Hristovski and Stojanovski (2005), OIE (2012) and Woo and Bruno (1999).

Fish Parasitic Diseases

Causative agents of parasitic diseases in fish are different species of protozoa, helminths, leeches and crustaceans.

Protozoa: Mastigophora (flagellates), Rhizopoda (amoebae), Apicomplexa (sporozoa), Microsporidia, Myxozoa (myxosporidia), Ciliophora (ciliates).
Helminths: Trematoda, Cestoda, Nematoda and Acantocephala.

Fish parasites can be found on the skin, fins or gills – ectoparasites, or in their internal organs – endoparasites. Fish parasites appear either as direct causative agents of certain diseases or as factors leading to disorder or decrease of fish resistance, and therefore fish become sensitive to many infective diseases. Besides health problems, parasites are an important economic problem in intensive fish breeding, because their presence can cause excessive economic damages, e.g. impeding growth.

Development of parasites can occur in one or several hosts – mainly small water organisms. Higher numbers of helminth parasitize in fish as their final hosts in sexually matured (adult) form. But some of them parasitize only in their larval forms in fish, as their transitional hosts. In intensive fish culture there are outstanding conditions for the spread of substantial parasitic invasions. Usually parasites appear with simple life cycles. On the other hand, fish from open waters are found with a great number of different parasites with complex life cycles.

The following are some products that have been implicated in human parasite infection: ceviche (fish and spices marinated in lime juice); lomi lomi (salmon marinated in lemon juice, onion and tomato); poisson cru (fish marinated in citrus juice, onion, tomato and coconut milk); herring roe; sashimi (slices of raw fish); sushi (pieces of raw fish with rice and other ingredients); green herring (lightly brined herring); drunken crabs (crabs marinated in wine and pepper); cold-smoked fish; and undercooked grilled fish. Seafood-borne parasitic infections occur with sufficient frequency to recommend preventive controls during the processing of parasite-containing species of fish that are intended for raw consumption.

The process of heating raw fish sufficiently to kill bacterial pathogens is also sufficient to kill parasites.

The effectiveness of freezing to kill parasites depends on several factors, including the temperature of the freezing process, the length of time needed to freeze the fish tissue, the length of time the fish are frozen, the species and source of the fish, and the type of parasite present. For example, tapeworms are more susceptible to freezing than are roundworms. Flukes appear to be more resistant to freezing than roundworms.

Freezing and storing at an ambient temperature of −20°C or below for 7 days (total time), freezing at an ambient temperature of −35°C or below until solid and storing at an ambient temperature of −35°C or below for 15 hours, or freezing at an ambient temperature of −35°C or below until solid and storing at an ambient temperature of −20°C or below for 24 hours are sufficient to kill parasites. Note that these conditions may not be suitable for freezing particularly large fish.

Brining and pickling may reduce the parasite hazard in fish, but they do not eliminate it, nor do they minimize it to an acceptable level. Nematode larvae have been shown to survive 28 days in 21% salt by weight.

Trimming away the belly flaps of fish or candling and physically removing parasites are effective methods for reducing the numbers of parasites. However, they do not completely eliminate the hazard, nor do they minimize it to an acceptable level.

More information on parasitic diseases can be found in FDA (2011), Hristovski and Stojanovski (2005), OIE (2012) and Woo (2006).

Fish Helminth Zoonoses

Fish can also appear as carriers or act as transitional hosts of certain parasite species which attack humans. Numerous marine and freshwater fish serve as sources of medically important parasitic zoonoses. The majority of these zoonoses are found in coastal regions of the seas, big lakes and rivers, where fish and their products are consumed further. But with the increasing consumption of fish, as well as the new trend of so-called "natural cooking," the number of the registered zoonoses continuously increases. The potential danger of human infestation with certain helminths still exists, because in Europe, several helminth zoonoses have been recorded: metacercariae of trematodes *Opisthorchis felineus, Pseudamphistomum truncatum, Clinostomum complanatum, Metagonimus yokogawai, Heterophyes heterophyes, Cryptocotyle lingua, Echinochasmus perfoliatus*, plerocercoids of cestods of the genus *Diphyllobothrium* and larvae of nematodes: *Dioctophyme renale, Anisakis simplex* and *Gnathostoma hispidum*, etc. Zoonotic transmission of some bacterial diseases, such as streptococcosis or mycobacteriosis, is also possible.

In most cases fish zoonotic parasites do not lead to major health problems in fish.

Fish parasites usually cause small or moderate damages in the human body. But some of them are more frequent and are a serious threat for human health. Some show abdominal pains, diarrhea or constipation, nausea, vomiting, loss of weight, or anorexia. Hepatomegaly, eosinophilia, tetanic cramps, tremors and toxemia may also occur.

Generally, fish can be either an intermediate host of parasites involving a human as the definitive host or a carrier of larvae of animal parasites that only invade human tissues for a limited period without undergoing further development. The latter are considered incidental infections. The natural definitive hosts for parasites are usually marine mammals or birds. However, larval stages of a few fishborne parasites can mature in both animals and humans.

Fishborne trematodiasis is especially important in Southeast Asia, the Far East and regions where people are dependent on freshwater fish as the major source of protein. Infections by both large and small digenetic trematodes are common. Although the diseases are seldom fatal, they can cause morbidity and serious complications. The route of infection is by ingesting metacercariae located in muscles and subcutaneous and other tissues of fish.

There are relatively few cases of fishborne cestode infections in humans. The cestodes that mature in the small intestine of humans are not very pathogenic and the diseases are never fatal. Diphyllobothriasis is the major cestodiasis transmitted by freshwater, marine and anadromous fish.

Fishborne nematodiases are generally caused by the incidental infection of humans with nematodes whose natural definitive hosts are marine mammals, birds, pigs or other animals. Freshwater, brackish or marine fish are the second intermediate host. In most infections, the worms can only survive for a limited period after the initial invasion of the gastrointestinal tract. The method of infection is by ingesting the infective-stage larvae, which can be located in the muscles, intestine or viscera of fish. Unlike cestodiasis, some nematode infections can be fatal. In the Netherlands, since the passage of legislation against eating raw herring and requiring fish to be frozen prior to sale, anisakiasis has almost disappeared. Freezing fish for 24 hours or heating processed fish to 65°C can kill the larvae. Also, the gutting of fish soon after they are caught prevents the migration of larvae to muscles.

Theoretically, fishborne parasitic zoonoses can easily be prevented by refraining from eating raw seafood. However, in many parts of the world, such an eating habit represents an established way of life or part of the inherent culture. It cannot be easily changed, even by the implementation of a strong education program or the passage of legislation. Therefore, these diseases will remain as public health problems and there is a need to undertake regular epidemiological studies. These studies, however, cannot be carried out effectively without the development of more cost-effective, sensitive and specific diagnostic methods that can be used in large-scale screening of fish. The use of molecular biological techniques can also help to clarify species of dubious validity and to trace the source of infection. Stronger support for this neglected area of research is required.

More information on parasitic diseases can be found in FDA (2011), Hristovski and Stojanovski (2005), OIE (2012) and Woo (2006).

DISEASES OF MOLLUSCA AND CRUSTACEA

Some of the earliest records of mass mortalities of shellfish were caused by microbial disease agents, e.g. the phycomycete fungus *Ostracoblabe implexa*, responsible for "foot disease" in the European oyster (*Ostrea edulis*) and the iridoviral agent of "gill diseases" in Portuguese oysters (*Crassostrea angulata*). Increasing development of shellfish aquaculture, and recent advances in diagnostic techniques, along with diversification of cultured species, continue to provide a seemingly inexhaustible reserve of new or emerging microbial disease problems. They have also significantly broadened the scope of microbial pathogen research and are proving useful for differentiating between primary pathogens and the ubiquitous microbial fauna that surrounds shellfish in their natural environment. Note is also made of apparently non-significant pathogens, since, given the right conditions, even the most benign infectious organism may transform into a serious disease agent. Knowledge on how to distinguish between primary and opportunistic pathogens is also important for optimizing their control or treatment.

More information on diseases of Mollusca and Crustacea can be found in Hristovski and Stojanovski (2005) and Woo and Bruno (1999).

FISH TOXICITY

The discipline of toxicology involves studying the nature and mechanisms of toxic lesions, and evaluating in a quantitative manner the spectrum of biological changes produced by exposure to chemicals. It is important to realize that every chemical can be toxic to fish under certain exposure conditions. For every chemical there should be an exposure condition (i.e. dose or concentration) that is "safe" and an exposure condition that is "toxic" to fish. The range of concentrations or doses that are toxic to fish may span several orders of magnitude. It is also important to determine toxic "thresholds," that is, concentrations or doses above which toxicity occurs and below which it does not.

Until relatively recently, toxicological studies with fish focused almost exclusively on very toxic substances which produce "acutely lethal" responses, that is, mortalities in fish exposed to chemicals for only short periods. Recently, we have become concerned with substances that may produce "sublethal" responses in fish after "chronic" exposure.

There are several chemical, physical and biological factors that influence the toxicity of chemicals to fish, including the properties of the chemical in water, the water quality conditions, the route of exposure, and the species and life stage of the fish.

Chemical toxicity to fish is often affected by external factors, such as photoperiod, temperature, salinity, reproductive status, disease and exposure to other external stressors.

Factors Affecting Toxicity

Water Quality Conditions

Since fish live in water, the extent to which fish are exposed to a chemical is dependent on aqueous solubility.

The solubility of ionic chemicals, which include most salts of toxic metals and some ionic organic compounds, is usually much higher than that of non-ionic compounds.

Ions may be dissolved in water in non-toxic forms. For instance, ions may form complexes with inorganic and organic "ligands." Inorganic ligands for cations in fresh water include carbonate (CO_3^{2-}), sulfate (SO_4^{2-}), and fluoride (F^-) ions, and Cl^- is an important ligand in saline water. Complexes between cations and inorganic ligands tend to be fairly "labile" or reversible, depending on the concentration of the ion and the ligand, and the pH. However, complexes with organic ligands, such as humic acids, tend to be relatively non-labile. "Alkalinity," which is primarily the concentration of carbonate ions in solution, is an important measure of the cation-binding capacity of fresh water. Alkalinity and pH are important variables influencing the toxicity of metal ions to fish. Transformations of chemicals dissolved in water can occur by hydrolysis, photolysis and oxidation.

If we accept that the toxicity of ionic chemicals is usually dependent upon the concentrations of the free ion in solution, then various factors that affect speciation of ions will affect toxicity, including pH, alkalinity, hardness and concentrations of organic ligands.

The solubility of non-ionic chemicals, such as organic compounds and elemental forms of toxic metals (e.g. Hg), is influenced by the polarity of the compound.

The toxicity of non-ionizable chemicals, such as organic compounds, is affected to a lesser extent by water quality conditions such as pH, alkalinity and hardness. However, dissolved and particulate organic material in water can alter the toxicity of organic compounds by acting as ligands for hydrophobic substances.

For additional information on water quality conditions, see Di Giulio and Hinton (2008), Hristovski and Stojanovski (2005) and Ostrander (2000).

Biological Interactions

A chemical can be toxic to a fish in two possible ways. It may affect tissues on the surface of the organism (e.g. gill epithelium) or the chemical may enter the organism and cause toxicity. A toxic chemical must pass through cell membrane barriers to reach "target" organs or tissues. The epithelial and endothelial integument of fish is usually thickened and relatively impermeable to chemicals, except in the gill tissues, which are specialized for gas exchange, and in the gastrointestinal tract. Thus, branchial or gastrointestinal uptake routes are the most efficient mechanisms for uptake of toxic chemicals into fish.

The most prevalent route of exposure of fish for chemical agents is via gills. Fish gills have an enormous surface area, approximately 50% of the entire surface area of the fish. Gill secondary lamellae, flattened ridges protruding perpendicularly from the primary lamellae, provide an effective and extensive surface for gas exchange. Although designed to facilitate diffusion of respiratory gases, fish gills also provide routes for other molecules to be accumulated by fish. Small hydrophilic molecules (e.g. NH_3, CO_2 and urea) can pass through small aqueous pores or gaps between cells in the gill epithelium. Larger neutral hydrophobic molecules, including many drugs and toxic organic chemicals, readily diffuse across the gill epithelium into the vascular space. Diffusion or uptake efficiency of these chemicals by the gills depends primarily on their hydrophobicity and molecular size. In addition, free metal ions can bind to negatively charged sites on fish gills. Once bound to the gill epithelium many metals use existing ion transfer mechanisms, such as calcium channels or protein-mediated endocytosis for entry into the gill.

The next route of exposure of fish for chemical agents is with food. Systemic absorption of the ingested chemical is relatively rapid. Significant accumulation of radiolabeled methyltestosterone was detected in fish tissues 2 hours after feeding sprayed chow. Tissue levels of testosterone appeared to reach equilibrium concentrations 24 hours after feeding testosterone sprayed on food to coho salmon (*Oncorhynchus kisutch*). In a similar experiment with carp (*Cyprinus carpio*), 4 days' feeding was required for testosterone to reach equilibrium concentrations in fish tissues. Chemical absorption from food depends on the rate of chemical dissolution from the food, its absorption efficiency in the stomach and intestine and influences of other factors, such as chemical and microbial degradation in the gut and binding to tissues. Also, physiological or metabolic differences between the two fish species may have caused differences in absorption, distribution and metabolism of the testosterone. Thus absorption efficiencies and time needed to attain equilibrium concentrations in fish will vary, depending on chemical properties and the fish species.

The most efficient method of administering a drug to fish is by injection. Ideally the chemical agent is dissolved directly in physiological saline. Chemical agents may be injected directly into veins or arteries (intravascular), into the peritoneal cavity (intraperitoneal) or into the muscle (intramuscular) of adult fish. Fish eggs or embryos may be injected into the perivitelline space or yolk sac with a micro syringe. In general, injection techniques provide a high internal dose with rapid distribution to the tissues.

Uptake of chemicals by fish can be influenced by both the lipophilicity and molecular size of the chemical.

For some chemicals, the rate of uptake is strongly influenced by the physiology of the fish. Fish species differ widely in their sensitivity to the toxic effects of chemicals.

Gill ventilation rates and dietary intake are governed by the metabolic rates of fish. There is considerable variation in the metabolism of fish; from fast-swimming pelagic predators to slow-swimming benthivores, so toxicity thresholds may vary considerably, depending on the fish species tested. In poikilothermic organisms such as fish, metabolism changes with the water temperature, so temperature may be an important factor influencing toxicity. Similarly, dissolved oxygen concentrations may influence gill ventilatory rates. Early life stages of fish tend to have higher rates of metabolism than later life stages. Therefore, the most sensitive period for chemically induced toxicity in fish is the embryolarval or early juvenile stages.

"Bioaccumulation" of chemicals represents the uptake and retention of chemical from the environment into fish via any pathway (e.g. food, water), whereas "bio-concentration" represents uptake and retention of a chemical directly from water into fish.

Fish possess metabolic pathways capable of transforming chemicals, such as oxidation and binding of chemicals to proteins or other large biomolecules (i.e. conjugation).

Although chemical contamination of our environment is often associated with human activities, plants and animals have evolved in an environment that has included continuous exposure to toxic materials. Basic mechanisms for resisting toxicity probably evolved with early life and are likely to be highly conserved in nature. Because of the large number and wide distribution of novel anthropogenic compounds introduced into the modern world, these mechanisms have become increasingly essential for survival. Organisms surviving environments heavily contaminated with anthropogenic chemicals demonstrate a diversity of mechanisms to tolerate or resist toxic effects.

Resistance or tolerance can be defined as the relative ability to function or survive during toxicant exposures that are harmful or lethal to susceptible individuals and populations. Fish and other organisms appear to develop tolerance through a variety of short-term and long-term processes. Physiological acclimation and genetic adaptation are general terms for short-term, transitory responses and long-term, heritable responses, respectively. Physiological acclimations occur in direct response to toxic exposures and likely involve temporary alterations in the levels of expression of proteins and enzymes involved in chemical defense. Following chemical exposure, protein expression returns to normal and the state of physiological acclimation declines. Genetic adaptation or evolved tolerance occurs when the genetic basis for advantageous responses is passed on to progeny. In genetic adaptation, tolerance is retained through successive generations, even when progeny are not exposed to chemicals.

The terms physiological acclimation and genetic adaptation have been used frequently for categorizing mechanisms of chemical tolerance in fish; however, other processes and conditions may contribute to tolerance as well. For example, abundant evidence indicates that some forms of chemically induced cancer represent adaptations to harsh chemical environments, providing survival value to individuals especially during the early stages of cancer. Cancer resulting from chemically induced mutations in somatic cells and concomitant alterations in protein expression would be considered to be a genetic but nonheritable adaptation. In addition to cancer, epigenetic alterations, such as hypermethylation of promoter regions of DNA, may affect responsiveness to drug and chemical exposures. Although gene silencing due to hypermethylation has been widely studied in mammalian cancer research, it has only recently being investigated as a tolerance mechanism in fish. Finally, nongenetic but heritable factors involving maternal transfer of toxicant from an exposed parent to offspring could contribute to tolerance in offspring. In cases involving maternal transfer, tolerance may appear to have a genetic basis (i.e. tolerant field-collected parents and their progeny) but is in fact physiologically based and related to direct exposure of offspring to toxicant. Because of the possibility of maternal transfer, genetic adaptation is established only when tolerance is maintained for two or more generations.

In populations inhabiting severely contaminated sites, multiple processes likely contribute to resistance during the lives of individuals; for example, physiological acclimations could provide individuals with the ability to survive and reproduce in moderately contaminated sites. As chemical contamination at a particular site increases with time, selection of progeny that carry genetic traits with adaptive significance could become a more dominant factor. Genetically adapted individuals may rely to some extent on epigenetic mechanisms or may respond to periodic pulses of contaminants through physiological responses. Tumor-bearing individuals may also exhibit features characteristic of genetic adaptation, physiological acclimation or epigenetic alteration.

Chemical resistance in fish has been observed in response to diverse environmental contaminants, including pesticides, dioxin-like compounds, polycyclic aromatic hydrocarbons (and other compounds associated with creosote) and metals (Table 23.9).

For more information on biological interactions, see Di Giulio and Hinton (2008), FDA (2011), Hristovski and Stojanovski (2005), Ostrander (2000) and Treves-Brown (2000).

Natural Toxins

Contamination of fish with natural toxins from the harvest area can cause consumer illness. Most of these toxins are produced by species of naturally occurring marine algae (phytoplankton). They accumulate in fish when they feed on the algae or on other fish that have fed on the algae. There are also a few natural toxins that are normal constituents of certain species of fish.

TABLE 23.9 Classes and Sources of Environmental Toxicants Addressed in Toxicity Resistance Studies

Toxicant	Source
Organochlorine pesticides	Includes DDT, the first modern highly toxic pesticide, followed by toxaphene, chlordane, aldrin, dieldrin, heptachlor, mirex, and kepone. Very persistent compounds that accumulate in fatty tissues and sediments, they are toxic to fish, wildlife and humans and are banned in the United States. Some of the earliest records of toxicity resistance involve DDT.
Dioxin-like compounds (DLCs)	Includes polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs) and other persistent polyhalogenated aromatic hydrocarbons (PHAHs). PCBs were valuable industrial materials used in capacitors, transformers and other products. PCDDs are produced inadvertently during a variety of processes (e.g. pesticide manufacture, chlorine bleaching of pulp). The most notorious DLC is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), often referred to simply as dioxin.
Polycyclic aromatic hydrocarbons (PAHs)	Complex mixtures of compounds produced during combustion of organic materials, especially fossil fuels; also, natural components of petroleum. PAHs are widely studied because of their abundance in the environment and because of the mutagenic and carcinogenic properties of some members (e.g. benzo(*a*)pyrene).
Creosote	Abundant pesticide mixture used to protect wood pilings, telephone poles, etc., from microbial decay. Creosote is composed primarily of PAHs; nitrogen-, sulfur- and oxygen-heterocyclic compounds; and phenols.
Metals	Naturally occurring elements (e.g. mercury, lead, cadmium, chromium), including some biologically essential elements (copper, iron, zinc). Human activities alter the environmental loading, availability and toxicity of metals through a variety of activities such as strip mining, fossil fuel combustion, smelting, and industrial processes.

For fish products in the United States there are six recognized fish poisoning syndromes that can occur from the consumption of fish or fishery products contaminated with natural toxins: paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), diarrheic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), ciguatera fish poisoning (CFP) and azaspiracid shellfish poisoning (AZP).

1. Paralytic shellfish poisoning (saxitoxin) is generally associated with the consumption of molluscan shellfish from environments ranging from tropical to temperate waters. Certain gastropods (e.g. conch, snails and whelk) are also known to accumulate PSP toxins.
   The effects of PSP are primarily neurological with respiratory paralysis. PSP toxin is an extremely potent toxin with a high mortality rate.
2. Neurotoxic shellfish poisoning (from brevetoxin) is generally associated with the consumption of molluscan shellfish from the Atlantic coast of the USA, New Zealand, and there are some suggestions of occurrence elsewhere. NSP is characterized by gastrointestinal and neurological symptoms. There are few, if any, after-effects and there have been no reported fatalities.
3. Diarrheic shellfish poisoning (from okadaic acid and dinophysis toxins) is generally associated with the consumption of molluscan shellfish. Outbreaks have been

documented in Japan, Southeast Asia, Scandinavia, Western Europe, Chile, New Zealand, the USA and eastern Canada. DSP is characterized by gastrointestinal symptoms, including: nausea, vomiting, diarrhea, abdominal pain, headache and fever. DSP is generally not considered life threatening but complications could occur as a result of severe dehydration in some patients.

4. Amnesic shellfish poisoning (from domoic acid) is generally associated with the consumption of molluscan shellfish from the northeast and northwest coasts of North America. In these regions, domoic acid has been identified in the viscera of Dungeness, tanner and red rock crab. Domoic acid has also been identified in several fish species including anchovies, Pacific sanddab, chub mackerel, albacore tuna, jack smelt and market squid. ASP is characterized by gastrointestinal symptoms.
5. Ciguatera fish poisoning (from ciguatoxin (CTX)) is associated with consumption of toxin-contaminated subtropical and tropical predatory reef fish. The toxin is introduced to the marine food chain by microscopic algae and moves up the food chain as small plant-eating reef fish eat the toxic algae and are then eaten by larger reef fish. CFP is characterized by gastrointestinal symptoms, followed by neurological and cardiovascular symptoms.
6. Azaspiracid shellfish poisoning (AZP) is caused by the consumption of molluscan shellfish contaminated with azaspiracids (AZA). AZP was first recognized following a 1995 outbreak in the Netherlands, linked to consumption of mussels harvested in Ireland. Since then, several outbreaks of AZP have been reported in various regions in Europe. AZP is characterized by severe gastrointestinal disorders. There have been no reported fatalities.

More information on natural toxins can be found in Di Giulio and Hinton (2008), FDA (2011) and Hristovski and Stojanovski (2005).

Scombrotoxin (Histamine) Formation

Certain bacteria produce the enzyme histidine decarboxylase during growth. This enzyme reacts with histidine, a naturally occurring amino acid that is present in larger quantities in some fish than in others. The result is the formation of scombrotoxin (histamine). Scombrotoxin (histamine) formation is a result of time and temperature abuse of certain species of fish, and can cause consumer illness. Histamine is more commonly the result of high-temperature spoilage than of long-term, relatively low-temperature spoilage. Freezing may inactivate some of the enzyme-forming bacteria. Both the enzyme and the bacteria can be inactivated by cooking. However, once histamine is produced, it cannot be eliminated by heat (including retorting) or freezing. Rapid chilling of scombrotoxin-forming fish immediately after death is the most important element in any strategy for preventing the formation of scombrotoxin (histamine).

The illness is closely linked to the development of histamine in these fish. In most cases, histamine levels in illness-causing fish have been above 200 ppm, often above 500 ppm. A guidance level has been set at 50 ppm histamine in the edible portion of fish. If 50 ppm is found in one section of a fish or lot, there is the possibility that other sections may exceed 500 ppm.

However, there is some evidence that other chemicals (e.g. biogenic amines such as putrescine and cadaverine) may also play a role in the illness.

Symptoms of scombrotoxin poisoning include tingling or burning in or around the mouth or throat; rash or hives on the upper body; drop in blood pressure; headache; dizziness; itching of the skin; nausea; vomiting; diarrhea; asthmatic-like constriction of the air passage; heart palpitation; and respiratory distress. Symptoms usually occur within a few minutes to a few hours of consumption and last from 12 hours to a few days.

For more information, see Di Giulio and Hinton (2008) and FDA (2011).

Environmental Chemical Contaminants and Pesticides

Fish can be harvested from waters that are contaminated by varying amounts of industrial chemicals, including heavy metals and pesticides. These contaminants may accumulate in fish at levels that can cause human health problems (e.g. carcinogenic and mutagenic effects). The hazard is most commonly associated with exposure over a prolonged period of time (chronic exposure). Illnesses related to a single exposure (one meal) are very rare. Concern for these contaminants primarily focuses on fish harvested from aquaculture ponds, freshwater bodies, estuaries and near-shore coastal waters (e.g. areas subject to shoreside contaminant discharges), rather than from the open ocean. Environmental chemicals and pesticides may also accumulate in aquacultured fish through contaminated feed ingredients. Certain pesticides are applied directly to the water in aquaculture ponds to control weeds and algae and to eliminate fish and invertebrates.

Although some pesticides have not been produced or used for many years (e.g. dichlorodiphenyl-trichloroethane (DDT) and polychlorinated biphenyls (PCBs)), many are very persistent and tend to accumulate in soil and sediments. Once pesticides are introduced into the environment, they may travel beyond their point of application or discharge.

Many contaminants accumulate in the edible fatty tissues of fish. Concentrations of these contaminants can vary considerably in individual fish of the same species from the same location, depending on factors such as their fat content, size, age and gender.

In the case of components or extracts of whole fish (e.g. dietary supplements, dietary ingredients and flavors), the component or extract may contain higher or lower concentrations of environmental chemical contaminants and pesticides than the whole fish from which it was derived. For example, organochlorine contaminants, such as PCBs, are oil soluble. When producing fish oil and fish meal, any PCBs present will become more concentrated in the oil fraction and less concentrated in the water fraction, as compared with the levels in the whole fish.

Maximum residue levels (MRLs) are: (1) the maximum concentration of residue accepted by the European Union (EU) in a food product obtained from an animal that has received a veterinary medicine; (2) the upper legal levels of a concentration for pesticide residues in or on food or feed based on good agricultural practices and to ensure the lowest possible consumer exposure. The assessment for the safety of residues is carried out by the Committee for Medicinal Products for Veterinary Use (CVMP). In the United States, MRLs are established by the Environment Protection Agency (EPA) and the Food and Drug Administration (FDA).

Methylmercury

Mercury occurs naturally in the environment and can also be released into the air through industrial pollution. Mercury falls from the air and can accumulate in streams and oceans and is turned by bacteria into methylmercury in the water. Fish absorb the methylmercury as they feed in these waters and so it builds up in them. Nearly all fish and shellfish contain traces of methylmercury. However, larger fish (swordfish, shark, king mackerel, tuna and tilefish) that have lived longer have the highest levels of methylmercury because they have had more time to accumulate it. It is the type of mercury that can be harmful to young people. The FDA action level of methylmercury is 1.0 ppm in the edible portion of fish.

Aquaculture Drugs

A range of veterinary drugs including antimicrobial, antiparasitical and growth promoters (hormones) may be used in aquaculture to control bacterial, fungal and parasitic diseases and to control reproduction of fish. Farmers may also use a range of vitamins, immunostimulants, disinfectants and other chemotherapeutants and employ chemicals for pond soil and water treatment.

Abuse of veterinary drugs, non-respect of the withdrawal period or application of illegal drugs constitutes a potential food safety problem. The health consequences of excessive use of antimicrobial drug residues include allergies, toxic effects, changes in colonization patterns of human-gut flora and acquisition of drug resistance in pathogens. The establishment of appropriate withdrawal periods ensures that no harmful residues remain in edible tissues after use of a drug. Since fish are poikilotherms, their metabolic rate is determined by environmental temperatures. As a result, withdrawal periods are based on time and temperature, i.e. degree-days: for example, 10 days at 5°C equals 150 degree-days. Where the legislation or implementation of regulation is poor, the risk of non-compliance is greater.

Additionally, the impacts of many of these chemicals on the environment are unknown and their release into the environment is likely to have a negative effect. They can also affect microbial biodiversity and contribute to the development of antimicrobial drug resistance. Application of vaccines has been instrumental to reduce use of drugs in the farmed salmon industry.

Compliance with MRLs for products from aquaculture is beginning to be enforced. For instance, the European Union is in the course of implementing a monitoring program in which fish muscle tissue will be routinely sampled for the presence of a range of veterinary drug residues. Such monitoring programs help provide assurance that no unacceptable human health risk is posed by veterinary drug residues in products from aquaculture. Unfortunately, some countries implement monitoring programs for export products but do not offer the same assurance for domestic markets.

Reasons for the use of drugs in aquaculture include the need to (1) treat and prevent disease, (2) control parasites, (3) affect reproduction and growth, and (4) provide tranquilization (e.g. for weighing). Relatively few drugs have been approved for aquaculture. Use of unapproved drugs or misuse of approved drugs in aquacultured fish poses a potential human health hazard. These substances may be toxic, allergenic or carcinogenic, and/or may cause antibiotic resistance in pathogens that affect humans.

More details on environmental chemical contaminants and pesticides can be found in Di Giulio and Hinton (2008), FDA (2011), Hristovski and Stojanovski (2005) and Treves-Brown (2000).

PATHOGENIC BACTERIAL GROWTH AND TOXIN FORMATION

Time and Temperature Abuse

Pathogenic bacteria growth and toxin formation as a result of time and temperature abuse of fish and fishery products can cause consumer illness. This hazard is limited to bacterial pathogens since viral pathogens (viruses) are not able to grow in food. Of particular concern in seafood are the pathogenic forms of *Listeria monocytogenes, Vibrio vulnificus, Vibrio parahaemolyticus, Vibrio cholera, Escherichia coli, Salmonella* spp., *Shigella* spp., *Staphylococcus aureus, Clostridium perfringens, Bacillus cereus, Campylobacter jejuni* and *Yersinia enterocolitica*.

Pathogenic bacteria can enter the process on raw materials. They can also be introduced into foods during processing from the air, unclean hands, insanitary utensils and equipment, contaminated water or sewage and through cross-contamination between raw and cooked product. The primary method for control is to reduce levels through cooking or other treatments when feasible, and minimize the potential for recontamination and to maintain products at temperatures that do not support growth of pathogenic bacteria.

Growth rates of pathogens are highly temperature dependent. Ordinarily, pathogenic bacteria growth is relatively slow at temperatures below 20°C.

Time and temperature abuse occurs when a product is allowed to remain at temperatures favorable to pathogenic bacteria growth for sufficient time to result in unsafe levels of pathogenic bacteria or their toxins in the raw fish and fishery products (e.g. raw molluscan shellfish). Certain pathogenic bacteria grow well, and others do not. Those that grow well in time and temperature-abused raw fish include: *V. vulnificus, V. parahaemolyticus, V. cholerae* and *L. monocytogenes*. Others may grow if the natural condition of the raw fish is changed, such as through salting or reduced oxygen packaging. Those that ordinarily do not grow well, because they compete poorly with the normal spoilage bacteria, include: *C. jejuni*, pathogenic strains of *E. coli, Salmonella* spp., *Shigella* spp., *S. aureus, C. perfringens, B. cereus* and *Y. enterocolitica*.

Most pathogenic bacteria will grow well in temperature-abused cooked fish if their growth is not controlled by means such as drying, salting or acidification, because competing bacteria are destroyed by the cooking process.

Certain pathogenic bacteria are associated with specific food sources, and it may not be necessary to assume that they will be present in other foods unless introduced from a contaminated source. For example, *V. vulnificus, V. parahaemolyticus* and *V. cholerae* non-O1 and non-O139 are generally associated with marine and estuarine species of fish and not with freshwater species or non-fishery ingredients.

The infective dose or toxic dose is the total number of a pathogen, or the total amount of a toxin, that is necessary to produce human illness. The dose often varies considerably for a single pathogen based on the health of the consumer and the virulence (infective capacity) of the particular strain of the pathogen.

In humans, usually, gastrointestinal symptoms are included: nausea, vomiting, abdominal pain, abdominal cramps, diarrhea, dehydration, electrolyte imbalance, high body fluid acidity, fever, headache, muscle pain, malaise and general discomfort. Septicemia rarely appears. Symptoms usually start from 1 or few hours – 1 or few days after consumption and usually last from 1–10 days. Everyone is susceptible to pathogenic bacteria food poisoning, but it is more common in infants, the young, the elderly, the infirm, those with underlying chronic disease, with reduced stomach acidity or malnutrition, and the immunocompromised.

Strategies for Control of Pathogenic Bacteria

Management of time and temperature of product exposure is important to produce a safe product. There are a number of strategies for the control of pathogenic bacteria in fish and fishery products. They include:

- Managing the amount of time that food is exposed to temperatures that are favorable for pathogen growth and toxin production.
- Killing pathogenic bacteria by cooking, pasteurization or by retorting.
- Killing pathogenic bacteria by processes that retain the raw product characteristics.
- Controlling the amount of moisture that is available for pathogen growth (water activity) in the product by drying or formulation.
- Controlling the amount of salt or preservatives, such as sodium nitrite, in the product.
- Controlling the level of acidity (pH) in the product.
- Controlling the introduction of pathogenic bacteria after the pasteurization process.
- Controlling the source of molluscan shellfish and the time from exposure to air (e.g. by harvest or receding tide) to refrigeration to control pathogens from the harvest area.

Inadequate Drying

Dried products are usually considered shelf stable and are, therefore, often stored and distributed unrefrigerated. Examples of shelf-stable dried fish products are salmon jerky, octopus chips, dried shrimp, stock fish and shark cartilage. The characteristic of dried foods that makes them shelf stable is their low water activity (a_w). Water activity is the measure of the amount of water in a food that is available for the growth of microorganisms, including pathogenic bacteria. Pathogenic bacteria growth and toxin formation in the finished product as a result of inadequate drying of fishery products can cause consumer illness. A water activity of 0.85 or below will prevent the growth and toxin production of all pathogenic bacteria, including primary pathogens *S. aureus* and *C. botulinum,* and is critical for the safety of a shelf-stable dried product. *S. aureus* grows at a lower water activity than other pathogenic bacteria, and should, therefore, be considered the target pathogen for drying for shelf-stable products.

Cooking or Pasteurization

The survival of pathogenic bacteria through cooking or pasteurization can cause consumer illness. The primary pathogens of concern are *Clostridium botulinum, Listeria monocytogenes, Campylobacter jejuni,* pathogenic strains of *Escherichia coli, Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica, Staphylococcus aureus, Vibrio cholera, V. vulnificus* and *V. parahaemolyticus.*

In addition to eliminating bacterial pathogens, cooking and pasteurization also greatly reduce the number of spoilage bacteria present in the fishery product. These bacteria normally restrict the growth of pathogens through competition. Elimination of spoilage bacteria allows rapid growth of newly introduced pathogenic bacteria. Pathogenic bacteria that may be introduced after cooking or pasteurization are, therefore, a concern. This is especially true for pasteurization, because that process can significantly extend the shelf-life of the fishery product, providing more time for pathogenic bacteria growth and toxin formation.

Retorting is a heat treatment that eliminates all foodborne pathogens and produces a product that is shelf stable.

There is a potential that *C. botulinum* type E or non-proteolytic types B and F will survive the pasteurization process and grow under normal storage conditions or moderate abuse conditions.

If the product is not reduced oxygen packaged, or contains a barrier that is sufficient to prevent the growth and toxin formation by *C. botulinum* type E or non-proteolytic types B and F, or is equipped with a time and temperature integrator, or is distributed frozen, then selection of another target pathogen may be appropriate. *L. monocytogenes* may be selected as the target pathogen for pasteurization of this type of product because it is the most resistant bacterial pathogen of public health concern that is reasonably likely to be present. Generally, *L. monocytogenes* is regarded as the most heat-tolerant, foodborne bacterial pathogen that does not form spores.

It is not practical to target viral pathogens in cooking or pasteurization processes because of their extreme heat resistance. Viral pathogens should be controlled through a rigorous sanitation regime as part of a prerequisite program or as part of hazard analysis critical control point (HACCP) itself.

Processes Designed to Retain Raw Product Characteristics

Some processes are designed to reduce specific pathogens to acceptable levels while retaining the sensory qualities (appearance, taste and texture) of the raw product. These processes are particularly useful in addressing the hazard associated with the target pathogen in raw products such as raw molluscan shellfish (i.e. oysters, clams, mussels and whole and roe-on scallops) that are intended for the raw ready-to-eat market. Because these processes do not eliminate all pathogens of public health concern, they are not considered cooking or pasteurization processes.

Examples of processes designed to retain raw product characteristics include:

- High hydrostatic pressure processing (HPP);
- Individual quick freezing (IQF) with extended frozen storage;
- Mild heat processing;
- Irradiation (ionizing radiation).

The survival of pathogenic bacteria through processes designed to retain raw product characteristics can cause consumer illness. The primary pathogens of concern are *Vibrio vulnificus* and *Vibrio parahaemolyticus*. *V. vulnificus* and *V. parahaemolyticus* are naturally occurring pathogens (i.e. not associated with human or animal sources) that may be present in fish and fishery products, and in particular raw molluscan shellfish.

Cross-Contamination of Fish and Fish Products

With fishery products, pasteurization is usually performed after the product is placed in the hermetically sealed finished product container. It is applied to fishery products that are distributed either refrigerated or frozen. Examples of pasteurized fishery products are: pasteurized crabmeat, pasteurized surimi-based analog products and pasteurized lobster meat. Because these products are cooked before they are packaged, they are at risk for recontamination between cooking and packaging. The risk of this recontamination may be minimized by filling directly from the cook kettle using a sanitary, automated, continuous-filling system (designed to minimize the risk of recontamination) while the product is still hot (i.e. hot filling). This control strategy may not be suitable for products such as crabmeat, lobster meat or crayfish meat.

There are three primary causes of recontamination after pasteurization and cooking performed immediately before reduced oxygen packaging:

- Defective container closures;
- Contaminated container cooling water;
- Recontamination between cooking and reduced oxygen packaging.

The introduction of pathogenic bacteria after pasteurization and certain specialized cooking processes can cause consumer illness. The primary pathogens of concern are *Clostridium botulinum, Listeria monocytogenes, Campylobacter jejuni*, pathogenic strains of *Escherichia coli, Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica, Staphylococcus aureus, Vibrio cholerae, V. vulnificus* and *V. parahaemolyticus*.

For more on the growth of and toxin production by pathogenic microbes see FDA (2011) and Hristovski and Stojanovski (2005).

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