Plesiomonas shigelloides *(Lone Gram)*

a) The disease and some epidemiological aspects

The genus *Plesiomonas* belongs to the Vibrionaceae family and consists of a single species, *P. shigelloides*. The species can be sub-typed and contain many serovars (Kirov, 1997). *P. shigelloides* can cause wound infections and septicemia but has also been suspected as cause of gastroenteritis. Thus the same serotype was found in tap water and in patients suffering diarrhoea (Tsukamoto *et al.,* 1978) and the organism has been isolated from patients with watery (mild) diarrhoea. In the USA, *P. shigelloides* has mainly been linked to consumption of raw oysters. There appears to be seasonal variation, with the peak occurring during the warm summer months. The role of *P. shigelloides* in the described cases may be doubtful since volunteers participating in feeding studies have failed to develop diarrhoea after ingestion of 10^9 organisms. Mild diarrhoea has, however, been induced in piglets (Kirov, 1997).

b) Prevalence in fish and fishery products

P. shigelloides is an environmental organism but is mostly associated with aquatic environments, both freshwater and marine waters (Farmer *et al.,* 1997). The organism is mesophilic and typically growth does not occur below 8°C, however, it has been isolated from freshwater environments in cold climates (Krovacek *et al*., 2000). The bacterium can be isolated from different food products but is typically found in fish and seafood.

c) Growth and survival in fish and fishery products

As mentioned, *P. shigelloides* is a mesophilic bacteria and does not grow at chill temperatures. The organism does survive freezing. The organism is sensitive to low pH and growth is slowed at moderate salt-concentrations (> 3.5% WPS).

d) Prevention and control

The evidence of *P. shigelloides* as a human food-borne pathogen is not completely convincing. As an organism indigenous to the aquatic environment, it must be expected to be present on fish and shellfish. Bearing in mind the volunteer studies, it must be anticipated that some growth to high concentrations is needed for any (potential) disease to occur. Thus its control in foods is straight forward since chill storage or moderate salting/acidifying conditions will prevent growth of the organism.

Aeromonas *(Lone Gram)*

a) The disease and some epidemiological aspects

The genus *Aeromonas* is a member of the Aeromonodaceae family which was created in 1986. Formerly the genus belonged to the Vibrionaceae family. It contains species pathogenic to animals (fish) and man. In humans, *Aeromonas* species may cause several adverse conditions including skin or soft tissue infection through invasion via burn injury. Such infections are most commonly associated with immunosuppression (Monteil and Harf-Monteil, 1997). The taxonomy of *Aeromonas* is rather confusing, but the species *A. hydrophila, A. sobria* and *A. caviae* which are motile and often (but not always) mesophilic have been linked to human gastroenteritis. They are, even more commonly than *P. shigelloides*, isolated from patients with mild diarrhoea, often as the sole potential human pathogen. Several classical virulence factors (extracellular enzymes, exotoxins (including enterotoxins), siderophores) have been identified in these *Aeromonas* species. There is no evidence that toxins preformed in the food play any role (Ahmed, 1991). As for *P. shigelloides*, feeding high levels to human volunteers have failed to cause disease (Morgan *et al.,* 1985) and the association between eating fish and shellfish and *Aeromonas*-gastroenteritis is at best circumstantial.

b) Prevalence in fish and fishery products

The *Aeromonas* species is a common, natural member of freshwater environments and between 33 and 100% of water samples contain the bacterium (Palumbo *et al.,* 2000). These organisms can also be isolated from marine and estuarine environments (Knøchel, 1989). The *A. hydrophila* group is very commonly found in fish and fish products at levels between 10^2 and 10^6 cfu/g but is also readily isolated from meat, milk, poultry and vegetable products (Palumbo *et al.,* 2000). Several studies have implicated *Aeromonas* species as spoilage organisms of raw meat (Dainty *et al.,* 1983), raw packed salmon (Gibson, 1992), fish from warm tropical waters (Gram *et al.,* 1990) and milk (Eneroth *et al.,* 1998). In such products the organisms may grow to 10^7-10^9 cfu/g.

c) Growth and survival in fish and fishery products

Although the motile aeromonads as a group are mesophilic, several studies have demonstrated that many environmental (food derived) strains grow well at chill temperatures (Knøchel, 1990; Eneroth *et al.,* 1998). Growth is inhibited by approximately 5% NaCl (Gram, 1991) and at pH 5. The organisms are able to grow in both vacuum and modified atmosphere packed products (Palumbo *et al.,* 2000).

d) Prevention and control

Aeromonas species are readily isolated from water, fish and shellfish and must be expected to be present. Limitation of growth requires a combination of chilling, salting and/or acidification. Growth of aeromonads will not be a problem in foods with pH below 6.5 and NaCl > 3% WPS.

5.1.1.2 Bacteria indigenous to the human/animal reservoir (Lone Gram)

Bacteria from the human/animal reservoir may, as presented in the tables in section 4.1 on statistics, cause seafood-borne diseases. Thus cases of staphylococcal enterotoxin gastroenteritis have been reported from cooked crustaceans, and oysters and other ready-to-eat products have caused salmonellosis or shigellosis. Several of these diseases are zoonotic since the major source of human illness is infected animals. The tolerance of these organisms to food-relevant preservation parameters is presented in Table 5.16.

Table 5.16 Growth limiting factors of pathogenic bacteria indigenous to the human and animal reservoir (adapted from Huss, 1994; ICMSF, 1996)

Pathogenic bacteria	Temperature, °C		pH	a_w	NaCl (%)
	minimum	optimum	minimum	minimum	maximum
Salmonella	5[1]	35-43	3.8	0.94	6
Shigella	6	35-40	4.9	0.96	5
Escherichia coli	7	35-40	4.4	0.95	8
Yersinia enterocolitica	-1.3	25-37	4.2	0.96	7
Campylobacter	30	42	4.9	0.99	1.5
Staphylococcus aureus	7	37	4	0.83	20-25[2]
toxin production	10	40-45	4.5	0.87	10-15[2]

1. *Some authors report growth at temperatures as low as 2°C (D'Aoust 2000)*
2. *Different maximum limits are reported in the literature*

Salmonella species or Serovars

The genus *Salmonella* is a member of the Enterobacteriaceae family. Salmonellosis is a leading cause of bacterial enteric disease in both humans and animals (Brenner *et al.,* 2000). The nomenclature is complex and causes confusion. At the Centre for Disease Control (CDC) in USA, the following scheme is used: only two species of *Salmonella* are recognised; the *S. enterica* and

the *S. bongori*. The former occurs in 6 sub-species. Within each of these, several serotypes exists. Thus *S. enterica* subsp. *enterica* as the largest group covers approximately 1500 serotypes. Examples of such serotypes are Enteritidis, Typhimurium or Typhi (Brenner *et al.,* 2000). The serotypes of the other sub-species are not named but identified by antigenic formula (D'Aoust, 2000).

a) The disease (adverse health effect) and some epidemiological aspects

Salmonellosis manifests itself clinically either as the enteric fever syndrome caused by typhoid or paratyphoid strains or as the nontyphoid dependent gastroenteritis. The latter may progress to a more severe systemic infection. Symptoms of the non-typhoid salmonellosis include nausea, abdominal cramps, diarrhoea with watery and possibly mucoid stools, fever and vomiting appearing 8-72 hours after exposure to the pathogen (D'Aoust, 2000). Systemic spread may occur leading to cardiac and circulatory problems. Poultry, pork and beef products are important sources of salmonellosis and eggs have, especially due to transovarian infection of the egg with *S.* Enteritidis, been involved in many outbreaks. Recently, also a variety of ready to eat vegetables, including bean-sprouts, have caused salmonellosis. Seafoods are relatively uncommon as causes of salmonellosis, however, the number of cases seems to be increasing. The infectious dose of salmonellae is, in general, high – typically around 10^6 cells, however, much lower infectious doses (10-100 cells) are reported if the organism is protected against stomach acidity e.g. by fat and if the product is eaten by more susceptible groups such as children.

b) Prevalence in fish and fishery products

Salmonellae are typically mesophilic bacteria with a global distribution. However, their main reservoir is the gastrointestinal tract of man and animals, including birds. Also, environments, such as water reservoirs, contaminated with human or animal excreta may harbour *Salmonella*. In particular shellfish growing in contaminated waters may accumulate *Salmonella* and raw oysters have been the cause of salmonellosis outbreaks (Ahmed, 1991).

Open marine waters are free from *Salmonella* but estuaries and contaminated coastal waters may harbour the pathogen. Also, poor personal hygiene may transmit the organism. *Salmonella* is rarely detected in fish from temperate waters but may occur in tropical waters and on fish and shellfish from such waters. Up to 10-15% of fish samples from India and Mexico were positive of *Salmonella* which has also been detected in several crustacean and molluscan products from India and Malaysia (D'Aoust, 2000). There is evidence that specific serotypes of *Salmonella* are common in fish farms and become part of the indigenous micro flora (Feldhusen, 2000).

The integrated fish farming in some areas of South-East Asia and Asia where poultry manure and/or so-called "night soils" are sometimes used as fertilisers for the ponds may add to the *Salmonella* contamination. However, the use of chicken manure does not *per se* lead to *Salmonella* detection as demonstrated by Dalsgaard *et al.* (1995) who did not find a single positive sample out of 158 samples from shrimp production in Thailand. To further complicate the situation, *Salmonella* may also, in these warm climates, originate from the environment itself (Reilly *et al.,* 1992; Bhaskar *et al.,* 1995) and does not necessarily indicate poor hygiene. In a Japanese study, *Salmonella* was detected in approximately one fifth of eel culture ponds (Saheki *et al.,* 1989).

Cooked shrimp may be post-process contaminated from the raw crustaceans or by employees and since no competing micro flora is present, it may constitute a high risk product if the bacterium is allowed to grow e.g. following temperature abuse.

c) Growth and survival in fish and fishery products

The vast majority of salmonellae are mesophilic bacteria growing from just above 5°C to approximately 45°C with an optimum at 37°C. Vegetative, unstressed cells are heat-sensitive and are easily destroyed at pasteurisation (hot-smoking) temperatures. D-values at 60°C are typically 1-3 minutes. Although *Salmonella* does not grow well at low water activity, it has been found to

survive well in dry environments and (re)-contaminate products such as fish meal. *Salmonella* does not grow below pH 4.5.

Shigella

Four species of *Shigella* are known all of which are human pathogenic. The genus *Shigella* is very closely related to another Enterobacteriaceae genus, *Escherichia*.

a) The disease and some epidemiological aspects

Sh. dysenteriae causes the most severe condition of bacilliary dysentery whereas *Sh. sonnei* causes the mildest of the diseases. The infectious dose is low, approximately 10-100 cells and from 7 hours to 7 days may lapse before symptoms present themselves. These include abdominal pain, vomiting, fever and diarrhoea which may contain bloody stools. The disease is an infectious disease. *Sh. dysenteriae* occurs on the Indian subcontinent, in Africa and Asia whereas the mildest of the species, *Sh. sonnei* is the most common in the western countries (Lampel *et al.,* 2000). In children, particularly in developing countries, the disease may be severe and *Shigella* diarrhoea accounts for hundreds of thousands deaths every year.

The primary route of infection is the faecal-oral route with person-to-person being the most common route of transmission. Shigellosis outbreaks follow a seasonal pattern with the largest number of outbreaks in the warm (summer) months.

b) Prevalence in fish and fishery products

Unlike *Salmonella*, Shigella is not associated with particular food raw materials but its presence is exclusively a question of poor hygienic handling and humans are its natural reservoir. Outbreaks have been caused by a multitude of food products, including shrimp and clams (Lampel *et al.,* 2000). *Shigella* are not naturally present in water but may survive for up to 6 months in water (Wachsmuth and Morris, 1989) and may survive for long time in clams and oysters (Feldhusen, 2000). Outbreaks have typically involved contamination of raw or previously cooked foods during preparation by an infected, asymptomatic carrier with poor personal hygiene.

In the US, FDA in 1994 and 1995 reported 7 cases of shigellosis caused by seafood and estimate that the annual total number of seafood-related shigellosis cases is approximately 200 cases (Feldhusen, 2000).

c) Growth and survival in fish and fishery products

Shigella species are truly mesophilic and do not grow below 6-7°C. They are sensitive to salting and heating. As mentioned, they may survive for long periods of time in bivalves.

Escherichia coli

The genus *Escherichia* is a member of the Enterobacteriaceae family and *E. coli* is the most common aerobic organism in the intestinal tract of man and warm-blooded animals. Most of the *E. coli* strains are harmless commensals that colonise the intestinal tract and probably play important roles in maintaining intestinal physiology. However, some strains of *E. coli* are pathogenic and can cause diarrhoeal disease. *E. coli* strains are differentiated based on a serotyping scheme involving O (somatic), H (flagellar) and K (capsular) antigens. Pathogenic *E. coli* are divided into specific groups depending on virulence, clinical symptoms and distinct O:H antigens. The important groups are (Doyle *et al.,* 1997):

- enteropathogenic *E. coli* (EPEC)
- enterotoxigenic *E. coli* (ETEC)
- enteroinvasive *E. coli* (EIEC)

- ∉ diffuse-adhering *E. coli* (DAEC)
- ∉ enteroaggregative *E. coli* (EAggEC) and
- ∉ enterohemorrhagic *E. coli* (EHEC) or verotoxic *E. coli* (VTEC).

a) The disease and some epidemiological aspects.

EPEC causes a watery type of diarrhoea accompanied by vomiting and fever. It typically occurs in infants and young children. The EIEC produced a diarrhoeal disease similar to *Shigella* whereas ETEC causes diarrhoea resembling *V. cholerae* diarrhoea. ETEC are a major cause of diarrhoea in children in developing countries and also a cause of so-called travellers' diarrhoea in adults. ETEC strains produce two types of toxin of which one resembles the cholera toxin. Also, the DAEC and EAggEC cause various variants of diarrhoea.

Due to recent outbreaks of EHEC in developed countries, much research has been directed against these organisms. By the early 1980ies it was realised that some *E. coli* strains caused hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). Following diarrhoea and a sub-set of symptoms, EHEC may result in renal failure; the HUS condition. Whilst the disease may affect all age groups, in particular children are susceptible (Willshaw *et al.,* 2000). *E. coli* O157:H7 is the most common EHEC serotype. The attachment capability appear to be important virulence factors for EHEC strains which produce two Shiga-like toxins capable of killing Vero (African Green Monkey Kidney) cells. The infectious dose is low and levels of 2 – 2 000 cells have been recorded as ingested concentrations in outbreaks.

b) Prevalence in fish and fishery products

The main source of *E. coli* infections have been (faecally) contaminated water and contaminated food handlers. Outbreaks by EHEC have mostly involved undercooked ground beef and raw milk. Also vegetables, such as alfalfa sprouts, washed or cultured in contaminated water have caused outbreaks. A number of famous outbreaks have been related to unpasteurised apple juice. Due to the relatively low pH, these juices were considered safe, however EHEC strains have an unusual acid tolerance and thus survived in the product.

Neither of the *E. coli* strains are typical of water or of aquatic products. However, poor hygiene, cross contamination by food handlers or dirty water may transfer the organism. Also, such strains may accumulate in filter feeding bivalves cultured in contaminated waters.

Whilst *E. coli* is not indigenous to the aquatic environment, it may survive and even multiply in warm tropical waters (Rhodes and Kator, 1988; Jiménez *et al.,* 1989) and thus also be isolated from presumed unpolluted waters. There are no reports of isolation of O157:H7 strains from seafood products.

c) Growth and survival in fish and fishery products

All *E. coli* strains are mesophilic organisms with optimum growth at 37°C. They do not grow at chill temperatures and are readily destroyed by mild heating. Most isolation procedures rely on incubation at 44°C, however, EHEC strains do not grow on selective media at 44°C. In general, the organisms are sensitive to salting and acidifying. A notable exception is the acid tolerance seen in EHEC strains.

Prevention and control of mesophilic Enterobacteriaceae

Although both *Salmonella* and *E. coli* can be isolated from non-contaminated tropical waters, the main source of these organisms and *Shigella* are human and animal (faecal) contamination. Therefore adherence to Good Hygienic Practices with emphasis on clean water and personnel hygiene will control the organisms. As all are sensitive to heating, the GHP-programme must be particularly strict when ready-to-eat foods are processed.

Proper treatment (e.g. chlorination) of water and sanitary disposal of sewage are essential parts in a control programme.

The infectious dose of *Shigella* and *E. coli* is low and thus it is their mere presence that must be avoided. In contrast, most Salmonellae have a higher infectious dose if they are not consumed in very fatty (protective) products. Therefore their growth in the product must be avoided. Growth will be inhibited at chill temperatures and by salting.

Current levels of *Salmonella* in various foods and its importance in human food-borne infections underline that bacteriological testing and stringent bacteriological standards (e.g. absence) of most foods are insufficient measures in the control of salmonellosis. Even the microbiological quality of harvest water (for live bivalves) appears not to be a good predictor for *Salmonella* contamination, because oysters removed from closed and open beds had the same level of contamination (4%) and no correlation was observed between the presence of *E. coli* and *Salmonella* (D'Aoust *et al.,* 1980).

Yersinia enterocolitica

As *Salmonella, Shigella* and *E. coli*, *Yersinia enterocolitica* is a member of the Enterobacteriaceae. It is discussed separately from the above as it is psychrotrophic and thus capable of growth at chill temperatures. The species is divided into sero- and phage types and only certain sub-types are pathogenic.

a) The disease and some epidemiological aspects

Y. enterocolitica causes a gastrointestinal disease characterized by abdominal pain, diarrhoea and mild fever. Whilst the diarrhoeal disease is self-limiting, sequela may occur resulting in arthritis and red skin lesions. These post-infection conditions may last several months. *Y. enterocolitica* produces an enterotoxin but its exact role in disease is not known.
b) Prevalence in fish and fishery products

Y. enterocolitica is associated with pigs which are chronic carriers of the serotypes involved in human infection. Food products washed in contaminated water or contaminated milk also causes yersiniosis. The bacterium has only sporadically been detected in seafoods.

c) Growth and survival in fish and fishery products

Although seldomly occurring in seafoods, *Y. enterocolitica* should be considered when evaluating the risks of seafood products with long, chilled shelf lives. In particular ready-to-eat products may become hazardous if contaminated with the organisms. Jeppesen and Huss (1993) demonstrated that *Y. enterocolitica* serotype O3 may grow well in brined (salted) shrimp stored at 5°C.

d) Prevention and control

Proper hygienic conditions may prevent cross-contamination from agricultural sources. Due to its psychrotrophic nature, chill storage may not be sufficient to prevent growth of the bacterium in products where the competing Gram-negative spoilage flora has been eliminated. Heating (cooking) will destroy the organism as will the salting and acidifying procedures used in semi-preserved seafood products.

Campylobacter

The genus *Campylobacter* is the most prominent species of the family Campylobacteriaceae. The genus contains several species of which especially one, *C. jejuni* causes gastrointestinal disease in humans. The disease is zoonotic with several animals serving as reservoirs. Although very sensitive to a range of environmental conditions, *Campylobacter* can commonly be isolated from waters close to agricultural run-off and waste waters.

a) The disease and some epidemiological aspects

Campylobacter jejuni and in some cases *C. coli,* cause diarrhoeal disease. Symptoms develop between one and 11 days after ingestion and abdominal pain, fever and diarrhoea are the main ones. The disease is self-limiting, but in a few instances, *Campylobacter* has been the cause of the neurological disease, Guillain-Barrè syndrome. Although the bacteria are sensitive to acid (and thus low stomach pH), the infective dose appears to be low (< 1 000 cfu) (Nachamkin, 1997).

b) Prevalence in fish and fishery products

Although poultry appears to be the main source of *Campylobacter* it may be isolated from several other food products. Milk has been linked to outbreaks. Up to 14% of oyster flesh samples have been found to contain campylobacters and an outbreak in the US has been ascribed to raw clams (Adams and Moss, 2000). Campylobacters are frequently isolated from water and water supplies (Nachamkin, 1997). Whilst the bacteria die quickly in open marine waters, they may accumulate in shellfish where they appear to be protected. One study has reported that up to 42% of (Irish) shellfish were positive for mesophilic *Campylobacter* (cf Feldhusen, 2000).

c) Growth and survival in fish and fishery products

Campylobacter species have a narrow growth spectrum and do not grow at temperatures below 28-30°C and are sensitive to oxygen. Thus they will not grow in chill stored products but may survive under chill temperatures. The organism is sensitive to heating (D_{55} of approximately 1 minute).

d) Prevention and control

Due to its sensitivity to food-relevant parameters, the control of *Campylobacter* in seafood appears simple. Avoidance of seafood from contaminated waters will control the hazard. This applies in particular to live bivalves.

Staphylococcus aureus

The *Staphylococcus* genus comprises several species of which especially *S. aureus* is associated with food-borne disease. The staphylococci are Gram-positive cocci with their primary habitat in the skin, glands and mucous membranes of warm-blooded animals including humans. Infected sores and scratches are often harbourage sites for *S. aureus.* The bacteria survive well in the environment and may also be isolated from a range of sources that come into contact with man and animals.

a) The disease and some epidemiological aspects

The disease caused by *S. aureus* is intoxication. The bacteria produce enterotoxin that upon ingestion causes nausea, vomiting, stomach cramps and, sometimes, diarrhoea. The enterotoxins are preformed in the food – thus growth of the organisms is a prerequisite for disease – and the incubation period is short, typically 2-4 hours. Seven antigenically different proteins cause the disease. All the enterotoxins have molecular weight of approximately 27 kD. The primary effect of the toxins is really a neurological (and not an enterotoxic) effect stimulating the vomiting centre in the brain. The disease is self-limiting and typically lasts only 24-48 hours, however, it may be extremely unpleasant. Due to the relatively short-lived nature of the disease, it is believed that only a small fraction (1-5%) of cases are reported. A higher frequency is seen during the warmer months and in November and December. The latter peak is probably correlated to left over holiday foods and buffets (Jablonski and Bohach, 1997).

b) Prevalence in fish and fishery products

Staphylococci may be isolated from newly caught fish, especially in warm waters (Gram and Huss, 2000). However, enterotoxigenic strains are typically transferred from food handlers with hand infections or with a cold or a sore throat. *S. aureus* has been isolated at levels of 2-10% in fish and bivalves but much more commonly in cooked, handled crustaceans where as much as 24-52% of samples may be positive (Jablonski and Bohach, 1997).

c) Growth and survival in fish and fishery products

Growth (to levels above 10^6 cfu/gram) is required for toxin formation and since *S. aureus* is a mesophilic organism some degree of temperature abuse typically precedes intoxication. Staphylococci are poor competitors and do not grow well in the presence of other microorganisms. Although they may be detected on raw fish (and meat), they will not be able to grow to toxigenic levels. The bacterium is tolerant to high levels of salt and toxin may be produced in up to 10-15% NaCl. Growth and toxin production may occur in products such as cooked crustaceans where the heat processed meat is virtually sterile and where the hand peeling operations provides ample opportunity for contamination with staphylococci.

d) Prevention and control

Growth and toxin formation may easily be prevented by proper chilling of products. Avoidance of cross contamination of heat treated (cooked) products is also important. The toxins are compact molecules and are not degraded by gut proteases. Also, they are resistant to heat and will resist boiling for some time. Toxins have not been detected in canned foods.

EU has set a microbiological criteria for *S. aureus* in cooked crustaceans where none of five samples may exceed 1000 cfu/g and only two samples may exceed 100 cfu/g (EC 2001a).

5.1.2 Production of biogenic amines *(Lahsen Ababouch/Lone Gram)*

a) the disease and some epidemiological aspects

Histamine poisoning is a food-borne chemical intoxication occurring few minutes to several hours following the ingestion of foods that contain unusually high levels of histamine (Taylor 1983, 1986).

It is usually a mild disorder with a variety of symptoms. The primary symptoms are cutaneous (rash, urticaria, oedema, localized inflammation), gastrointestinal (nausea, vomiting, diarrhoea), haemodynamic (hypotension) and neurological (headache, tingling, oral burning and blistering sensation, flushing and perspiration, itching). More serious complications such as cardiac palpitations are rare. The toxicity of histamine is probably potentiated by other biogenic amines (Taylor, 1986; Lehane and Olley, 2000).

Histamine poisoning occurs throughout the world and is perhaps the most common form of toxicity caused by the ingestion of fish (see Tables 4.3 and 4.5). However, good statistics about its incidence do not exist because the poisoning incidents are often unreported due to the mild nature of the illness, to lack of adequate system for reporting food-borne diseases or ignorance by medical personnel who misdiagnose histamine poisoning as a food allergy (Taylor, 1986; Lehane and Olley, 2000). Japan, the USA and the UK are the countries with the highest number of reported incidents, although this possibly implies better reporting on their part. Less frequent incidents have been reported elsewhere in Europe, Asia, Africa, Canada, New Zealand and Australia (Ababouch, 1991; Lehane and Olley, 2000).

Despite its toxicity, histamine is not a substance foreign to the human body. It is stored in specialized cells where its release is regulated. In small physiological doses, histamine is a necessary and desirable substance involved in the regulation of such critical functions as the release of stomach acid. But in large doses, histamine becomes toxic and can precipitate poisoning symptoms.

Although compelling evidence exists for the involvement of histamine as the causative agent of histamine food poisoning, it has been virtually impossible to reproduce the illness in oral challenge studies with human volunteers. The paradox between the lack of toxicity of pure histamine and the apparent toxicity of even smaller doses of histamine in spoiled fish has been attributed to the possible occurrence of histamine toxicity potentiators in the spoiled fish. Other biogenic amines (agmatine, putrescine, cadaverine, anserine, spermine and spermidine) trimethylamine or trimethylamine oxide have been suggested as potentiators (Taylor, 1986). Three theories have been advanced to explain the mechanism of histamine toxicity potentiation.

- the potentiators inhibit histamine-metabolizing enzymes (diamine oxidase (DAO) or histaminase and histamine N-methyltransferase (HMT)) which are present in the intestinal tract
- the potentiators interfere with the possible protective action of intestinal mucin which prevents histamine absorption by binding it (Lehane and Olley, 2000). Taylor (1986) estimates, however, that inhibition of histamine binding to mucin in the gastrointestinal tract would play only a secondary role in the potentiation of histamine toxicity
- the potentiators cause release of endogenous (mast cell) histamine (Clifford *et al.,* 1991; Ijomah *et al.,* 1991,1992; Bartholomew *et al.,* 1987; Gessner *et al.,* 1996). Clifford *et al.* (1993) even suggested that saxitoxins, which were found at low levels in the mackerel used during the feeding experiment may be responsible for the release of endogenous histamine. However, this theory and the role of endogenous histamine release, if any, remain uncertain and unproven (Lehane and Olley, 2000).

There is uncertainty regarding the threshold toxic concentration because potentiators of toxicity may be present in fish and lower the effective dosage compared with pure histamine. Different fish could contain different potentiators, and the levels of potentiators could also vary considerably from one fish to another.

Simidu and Hibiki (1955*)* estimated the threshold toxic dose for histamine in fish at approximately 60 mg/100g (600 ppm). Shalaby (1996) reviewed the oral toxicity to humans of histamine and other biogenic amines in foods. He considered that histamine-induced poisoning is, in general, slight at 8–40 mg/100g, moderate at > 40 mg/100g and severe at >100 mg/100g. Based on an analysis of recent poisoning episodes, Shalaby (1996) suggested the following guideline levels for histamine content of fish:

- < 5 mg/100 g (safe for consumption)
- 5–20 mg/100 g (possibly toxic)
- 20–100 mg/100 g (probably toxic), and
- >100 mg/100 g (toxic and unsafe for human consumption).

b) prevalence in fish and fishery products

Biogenic amines are produced in foods by decarboxylation of the corresponding free amino acid (Table 5.17). This decarboxylation reaction is catalyzed by bacterial amino acid decarboxylases. Figure 5.5 represents the decarboxylation of histidine into histamine.

Table 5.17
Amino acid precursors and biogenic amines formed in food products.

Amino acid precursor	Biogenic amine
Histidine	Histamine
Ornithine	Putrescine
Putrescine[1]	Spermidine
Lysine	Cadaverine
Tyrosine	Tyramine
Arginine	Agmatine

1. Not an amino acid

Histamine poisoning is often referred to as scombrotoxin poisoning because of the frequent association of the illness with the consumption of spoiled scombroid fish such as tuna (*Thunnus* spp.), skipjack (*Katsuwonus pelamis*), saury (*Kololabis saira*), bonito *(Sarda* spp.) and mackerel (*Scomber* spp.). However, non-scombroid fish such as sardines (*Sardinella* spp.), herring (*Clupea* spp.), pilchards (*Sardina pilchardus*), anchovies (*Engraulis* spp.), marlin (*Makaira* spp.), bluefish (*Pomatomus* spp.) and mahi-mahi (*Coryphaena* spp.) have also been implicated in outbreaks of this illness (Taylor, 1986; Lehane and Olley, 2000). More recent reports indicate the implication of salmon (*Arripis truttaceus*, *Oncorrhynchus nerka*) as well (Lehane and Olley, 2000).

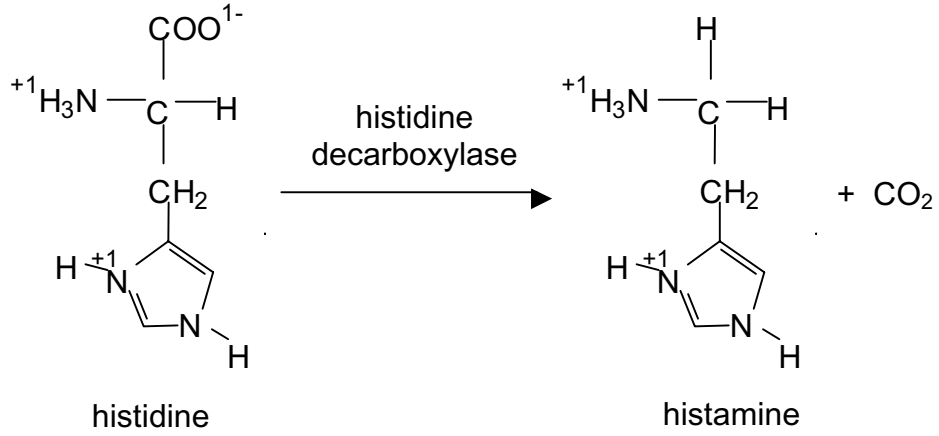


Figure 5.5 Formation of histamine.

Many of these fish species have significant amounts of histidine in their muscle tissues that serves as a substrate for bacterial histidine decarboxylase. Free histidine is generally found in large amounts in the muscle of fatty, red-meat active and migratory species as compared to its amount in the white meat of slower species. The level of other amino acids, precursors of biogenic amines (Table 5.17) has not been sufficiently studied.

c) growth of biogenic amine forming bacteria and stability of toxin in fish products

Most studies have investigated histidine decarboxylation into histamine, whereas fewer reports exists on production of other biogenic amines (Flick *et al.,* 2001).

In some studies (Taylor, 1986; Middlebrooks *et al,.* 1988), the potential for histamine and biogenic amines formation was evaluated by measuring the decarboxylase activity. This is not always appropriate as it ignores the role of histaminase for example, which has been found in some bacterial species (Taylor, 1986). Therefore, measurement of the actual amines must be done.

In general, the amino acid decarboxylase enzymes, especially histidine decarboxylase, can be found in species of Enterobacteriaceae, *Clostridium, Lactobacillus, Vibrio, Pseudomonas and Photobacterium* (Ababouch, 1991; Taylor, 1986; Lehane and Olley, 2000; Flick *et al.,* 2001). *Vibrio, Pseudomonas* and *Photobacterium* species are indigenous bacteria found naturally in the marine environment and on fish whereas the mesophilic Enterobacteriaceae and *C. perfringens* typically occur as a result of post-harvest contamination. The enteric bacteria (especially *Morganella morganii*) tend to prevail during the summer season, whereas the indigenous bacteria may predominate during the winter (Okuzumi *et al.,*1984). The group of psychrophilic and halophilic bacteria named “N-group bacteria” were later identified as *Photobacterium phosphoreum* by Fujii *et al*. (1997).

Enterobacteriaceae species are the most important biogenic amines forming bacteria in fish. These include *Morganella morganii, K. pneumoniae, Proteus vulgaris* and *Hafnia alvei* (Frank, 1985). Since the most prolific histamine forming bacteria are mesophilic enteric bacteria, the formation of histamine, and probably of other biogenic amines, takes place at high rates at high temperatures (> 15 - 20°C) (Ababouch, 1991; Lehane and Olley, 2000; Flick *et al.,* 2001). However, several other studies have also demonstrated that histamine and other biogenic amines can accumulate in

fish to reach toxic levels even at low temperatures (Ababouch *et al.,* 1991; Flick *et al.,* 2001). Thus Jørgensen *et al.* (2000, 2000a) demonstrated that several biogenic amines were formed in vacuum-packed cold-smoked salmon stored at 5°C. Psychrotrophic lactic acid bacteria, Enterobacteriaceae and, especially, *P. phosphoreum* were the producing organisms. Whilst biogenic amines clearly may be formed in some fish at low temperatures, this is not common (Lehane and Olley, 2000; Flick *et al.,* 2001) indicating that several factors, other than time and temperature play a major role.

Klausen and Huss (1987) reported that large amounts of histamine were formed by *M. morganii* at low temperatures (0 – 5°C) following storage at higher temperatures (10-25°C) even though bacterial growth did not take place at 5°C or below. It was argued that the enzyme histidine decarboxylase generated during storage at high temperature was responsible for subsequent histamine production at 5°C or below. Similar findings were reported by van Spreekens (1986). Biogenic amines are very heat stable and once formed, they will not be destroyed even by dramatic heat treatment such as autoclaving (Figure 5.6).

d) prevention and control

Histamine poisoning following the consumption of fish requires that:

- ∉ The fish muscle contains the amino acid(s) precursor of histamine and other biogenic amines
- ∉ the fish contains and/or gets contaminated with bacteria capable of decarboxylating the amino acid(s)
- ∉ the handling and storage conditions (especially hygiene and time –temperature conditions) are conducive to the growth of these bacteria
- ∉ consumers eat fish with high levels of histamine and other biogenic amines.

Control of histamine poisoning can be achieved by eliminating one or more of these steps. It is worthy to recall that histamine is thermostable and once it is in the fish, there is no treatment capable of removing it. The most used methods for the control of histamine and biogenic amines formation in the fish industry are (FDA, 2001a):

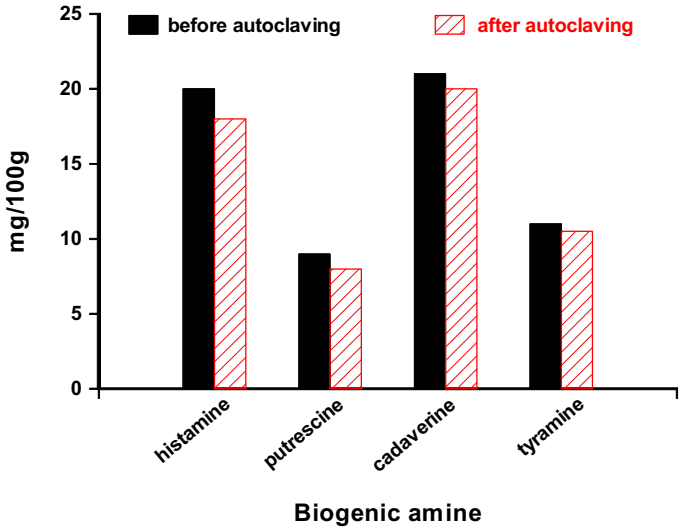


Figure 5.6
Effect of heating on mackerel spiked with biogenic amines before autoclaving (Luten *et al.,* 1992).

1) Rapid chilling of fish immediately after death. This is particularly important for fish that are exposed to warmer waters or air, and for large tuna that generate heat in the tissues of the fish following death. It is recommended that:

- ∉ Generally, fish should be placed in ice or in refrigerated seawater, in chilled sea water or brine at 4.5°C or less within 12 hours of death, or placed in refrigerated seawater, chilled sea water or brine at 10°C or less within 9 hours of death
- ∉ Fish exposed to air or water temperatures above 28°C, or large tuna (i.e. above 20 lbs.) that are eviscerated before on-board chilling, should be placed in ice (including packing the belly cavity of large tuna with ice) or in refrigerated seawater or brine at 4.5°C or less within 6 hours of death
- ∉ Large tuna (i.e., above 20 lbs.) that are not eviscerated before on-board chilling should be chilled to an internal temperature of 10°C or less within 6 hours of death.

This will prevent the rapid formation of the decarboxylase enzymes. Once histidine decarboxylase is formed, control of the hazard is unlikely. Further chilling towards the freezing point is also desirable to safe-guard against longer-term, low temperature development of histamine. Additionally, the shelf-life of the fish is significantly compromised when product temperature is not rapidly dropped to near freezing.

2) Good hygienic practices on-board, at landing and during processing to avoid contamination or recontamination of the fish by bacteria capable of amino acid decarboxylation.

Freezing of the fish can significantly reduce the bacterial load, but will not limit the activity of decarboxylase enzymes that may have been produced prior to freezing. Therefore, it is important to know the temperature history of the frozen fish since outbreaks of histamine poisoning can be caused by the ingestion of thawed- frozen fish containing biogenic amines if the fish was previously temperature-abused (Flick *et al.,* 2001).

Conflicting results have been reported on the effect of salting on biogenic accumulation (Flick *et al.,* 2001). This reflects the diversity of the bacteria that are involved and their adaptation to different levels of salts. Likewise, bacteria producing biogenic amines are not equally affected by smoking and vacuum packaging and these procedures cannot therefore be relied upon to control biogenic amine accumulation. They have to be combined with refrigeration and limits on storage time to be efficient.

Because of the recurrence of histamine poisoning in many parts of the world and the importance of international trade of the concerned fish species, many countries have enacted maximal limits or guidelines on histamine levels in traded fish. Thus, the US Food and Drug Administration guidelines has established for tuna, mahi-mahi and related fish specify 50 mg/100 g (500 ppm) as the toxicity level, and 5 mg/100g (50 ppm) as the defect action level because histamine is not uniformly distributed in a decomposed fish. Therefore, if 5 mg/100g found in one section, there is a possibility that other units may exceed 50 mg/100g (FDA, 2001a). FDA requires the use of the AOAC fluorometric method (Rogers and Staruszkiewicz, 1997).

The European Union (EC 1991a, 1995) requires that nine samples must be taken from each batch of fish species of the following families: Scombridae, Clupeidae, Engraulidae and Coryphaenidae. These samples must fulfil the following requirements

- ∉ the mean value must not exceed 10 mg/100g (100 ppm)
- ∉ two samples may have a value of more than 10 mg/100g (100 ppm) but less than 20 mg/100g (200 ppm)
- ∉ no sample may have a value exceeding 20 mg/100g (200 ppm).

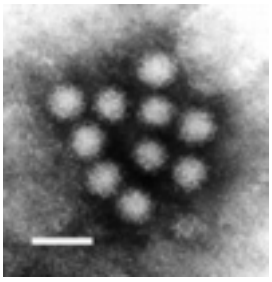
However, fish belonging to these families which have undergone enzyme ripening treatment in brine may have higher histamine levels but not more than twice the above values, i.e. in preserved anchovies, it can be as high 200 and 400 ppm instead of 100 and 200 ppm. Examinations must be carried out in accordance with reliable, scientifically recognized methods, such as high-performance liquid chromatography (HPLC) (EC 1991a, 1995).

In Australia and New Zealand, the level of histamine in a composite sample of fish or fish products, other than crustaceans and molluscs must not exceed 10 mg/100g (100 ppm). A 'composite sample' is a sample, taken from each lot, consisting of five portions of equal size taken from five representative samples. This clause, which came into force in October 1994, is currently under review, with a proposal to increase the maximum allowable level of histamine in fish and fish products to 20 mg/100g (200 ppm) (Lehane and Olley, 2000).

5.1.3 Viruses *(Lone Gram)*

Viruses are very small microorganisms (typically 25-70 nm) which consist of genetic material (RNA or DNA) and a protein cover. Viruses are obligatory intracellular pathogens and cannot, as bacteria, yeasts and filamentous fungi, multiply outside host cells. Thus virus particles *per se* are totally inert. The marine environment is full of viruses which represent the most abundant life form in the sea, typically numbering ten billion per litre, however, none of these are pathogenic to man (Lees, 2000). Viruses being implicated in seafood-borne diseases all have their niche in the human gastro-intestinal (GI) tract and their presence in water and seafood is a consequence of poor hygiene; either water being contaminated with sewage or products being contaminated by food handlers.

Figure 5.7
Norwalk virus as observed by Transmission Electron Microscopy; Bar represents 100 σm. By F.P. Williams, US Environmental Protection Agency.



The diseases caused by human enteric viruses fall into two major categories: viral gastroenteritis and viral hepatitis (Caul, 2000). As is evident from data in section 4.2, viruses are responsible for the largest number of cases of seafood-borne diseases and is in particular associated with raw (under-cooked) molluscan shellfish. Notably, the largest outbreak of food-borne disease ever to be recorded was an outbreak of Hepatitis A in Shanghai, China, in 1988 where more than 290 000 people were infected by eating clams harvested in a sewage polluted area (Lees, 2000; Halliday *et al.,* 1991; Tang *et al.,* 1991).

Viruses may cause a range of diseases in humans and are the cause of mild diseases as flu and cold as well as more serious diseases as AIDS. Viruses traced to seafood-borne diseases are primarily so-called Norwalk-like virus and Hepatitis A virus.

Viruses are divided into groups depending on the organization and transcription of the genetic material (Table 5.18). The genetic material is DNA or RNA and they carry a single or a double stranded strand of genetic information. Many viral orders and families exist. Norwalk virus belongs to the viral family, Caliciviridae, which also includes three other genera, including the Sapporo-like virus. Norwalk-like virus are sometimes also called small-round-structured virus (SRSV). Hepatitis A belongs to the Picornaviridae family. Taxonomy of virus was for a long time dependent on electron microscopically classification (Caul, 2000) but has been greatly facilitated by molecular techniques allowing sequencing and molecular phylogenetic studies.

Studies of viral (seafood-borne) diseases have been and are greatly hampered by lack of methods for culturing and enumeration. Several viruses, including the Norwalk-like virus cannot be cultured on cell lines and enumeration relies on molecular (e.g. PCR-based) detection. Some laboratory adapted strains of hepatitis A can be cultured but most wild type strains escape culturing.

Table 5.18 Groups of viruses causing gastrointestinal diseases from seafood. Based on Lees (2000) and Caul (2000).

Virus	Type	Family	Associated with seafood-borne disease	Comment
Norwalk-like	SS[1] RNA	Caliciviridae	Frequently	
Hepatitis A	SS RNA	Picornaviridae	Frequently	
Hepatitis E	SS RNA	Caliciviridae ?	not documented	cause of enteric non-A and non-B hepatitis. Outbreaks associated with drinking water
Astrovirus	SS RNA	Astroviridae	astrovirus from oysters were suspected in one outbreak	few food-borne cases
Rotavirus	DS[2] RNA	Reoviridae	not documented	isolated from sewage
Adenovirus	DS DNA	Adenoviridae	not documented	isolated from sewage and seafood

1. SS = Single Stranded
2. DS = Double Stranded

a) The disease and some epidemiological aspects

Norwalk-like virus (NLV) forms a distinct group of viruses which includes the "classical" Norwalk virus as well as Snow mountain virus, the Hawaii agent and the Montgomery agent. Disease is caused by ingesting viruses and symptoms appear after approximately 24 hours. These are sudden in onset and typically include nausea, vomiting, low-grade fever and diarrhoea. In general, NLV infections are mild and self-limiting and cease after 1-4 days. Due to the short duration and the self limiting disease, the number of NLV cases (from all sources) is probably underreported (EC, 2002). The infective dose of NLV – and most other viruses – is not known but several studies with human volunteers ingesting enteric virus point to low MIDs; probably less than 50 plaque forming units (PFU) (Gerba and Haas, 1988).

NLV is highly transmissible and the attack rate, i.e. the number of people becoming ill following ingestion, is high, typically between 50 and 90%. NLV is transmitted by perosn-to-person contact, by contaminated environments and by water and food (EC, 2002). Food-borne NLV gastroenteritis is especially caused by consuming contaminated molluscan shellfish. The link between molluscan shellfish and NLV was made in the UK where electron microscopy of faecal material from "winter vomiting disease" patients revealed virus particles.

Hepatitis A virus (HAV) causes a food- and water-borne infectious viral disease which lasts for several weeks. The liver is typically infected and jaundice, anorexia, vomiting and profound malaise are characteristic symptoms. The incubation period range from 15 to 50 days. The patient develops immunity but relapses and sequela may appear. Vaccines are available in both Europe and the United States and it has been suggested that food handlers should be immunized (Cliver, 1997).

b) The niche and prevalence in fish and fishery products

Like all viral diseases transmitted by seafood, NLV and HAV are associated with the gastrointestinal tract of humans and are shed in large quantities on faeces of infected persons. NLV are shedded from infected people, and food handlers must not work with foods for at least two days after symptoms have disappeared. In contrast, HAV is often shed in faeces from infected people 10-14 days before onset of disease and continues 1-2 weeks after onset.

The most common cause of viral gastroenteritis is live molluscan shellfish in which viral particles from the surrounding (contaminated) water are filtered and accumulated in the animals. However, a range of other foods have been implicated in viral diseases. Hepatitis A has been caused by orange juice, salads, bakery goods and lettuce. NLV has cause outbreaks involving butter cream, cool drinks and fresh cut fruits.

c) Growth and survival in fish and fishery products

Viruses do not multiply outside the host, and thus their numbers will not increase after the initial contamination event. Subsequent processing will affect the survival of the viruses, although little is known about the effect of food processing parameters on NLV and HAV. In general, viruses are more resistant to preservation parameters and processing steps than vegetative bacteria. Virus particles are stable at refrigeration temperatures when they are not de-stabilised by other factors, and frozen storage will only cause a slight increase in rate of inactivation (ICMSF, 1996). Heat inactivates viruses and D-values are typically measured in seconds at temperatures > 60°C but in minutes at temperatures in the range of 50 to 60°C. This means that household cooking / steaming often is not sufficient to inactivate viruses.

HAV is more resistant to heat and drying than other enterovirus but heating to 85-90°C caused a 4 log reduction in PFUs (Millard *et al.,* 1987). HAV is resistant to short exposures to acid (pH 2). Due to the lack of culture methods for NLV, studies on the influence of food relevant parameters on virus survival are almost impossible to conduct. Based on studies of food-borne outbreaks it can be concluded that infectivity persists for 3 h at pH 2.7 at room temperature and for 60 min at neutral pH at 60°C.

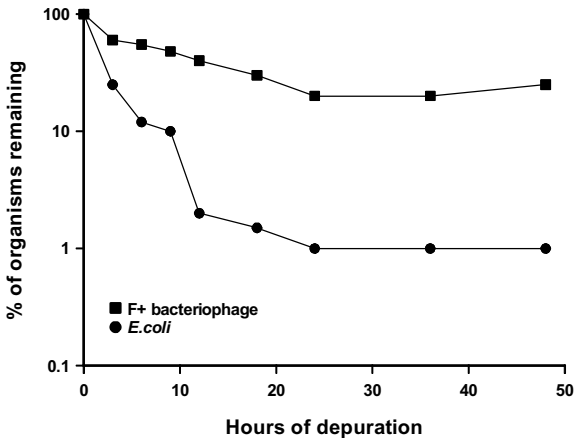
Temperature has a major impact on survival of virus in seawater. At 4°C, it took 671 days to reduce HAV with 90% whereas the same reduction was obtained in 25 days at 25°C (Gantzer *et al.,* 1998). UV-light inactivates virus and HAV was reduced with 90% in 2.6 minutes at 42 mW s /cm (Gantzer *et al.,* 1998).

d) Prevention and control

Control of seafood-borne viral disease is, in principle, simple since the source of disease is indirect or direct faecal contamination. Thus, measures that prevent this contamination control the disease. Bivalve shellfish are suited for human consumption if harvested from waters free from sewage and pollution. Alternatively, the processing can include a virucidal treatment such as heat treatment at high temperatures (e.g. canning), or the viruses can be removed from the shellfish before consumption.

Depuration. Molluscan shellfish are filter feeding animals and the viruses – and other pathogenic agents accumulated – may be removed by depuration. This involves the transfer of the animals to clean water enabling them to shed the virus and other agents. The process is very difficult to control and there is no simple test to indicate that a shellfish has been depurated effectively. Several studies have shown that viruses are retained in the animals longer than bacteria. Epidemiological evidence indicate strongly that depuration may fail to eliminate enteric viruses from contaminated shellfish and that compliance with bacterial standards do not guarantee absence of viruses (Lees, 2000) (Figure 5.8). Several studies have suggested the use of a viral indicator such as the F+ RNA bacteriophage. This enteric virus is culturable and numbers correlates with the presence of NLV and the outbreaks of diseases (Doré *et al.,* 2000).

Figure 5.8
Depuration of *Escherichia coli* and F+ bacteriophage from oysters following the exposure to crude sewage discharge (redrawn from Lees (1995).



However, since such viral indicators are not widely accepted and most viruses like NLV cannot be cultured, waters in which bivalves are harvested are monitored using bacterial counts. The EU and the US both have several guidelines and standards relating to bacteriological quality of live bivalves or shellfish growing waters. Due to the lack of correlation between water quality and presence of pathogens in the animals, EU has set standards for the animals (EC, 1991) while US standards refer to the quality of the water in harvesting areas (see section 11.2).

Hygienic practices. Contamination by food handlers can be prevented by good personal hygiene and education. As mentioned, food handlers must not handle foods for 2 days following an outbreak of NLV. Disposable gloves may be worn since viruses are difficult to remove by hand washing. Viruses are relatively resistant to disinfectants (e.g. phenolics, quaternary ammonium compounds, ethanol) while halogens (chlorine, iodine) inactivate viruses in water and on clean surfaces. The sensitivity to halogens is, however, lower than that of vegetative bacterial cells. Levels of > 10 mg chlorine / litre for 30 min are sufficient to inactivate the viruses.

5.1.4 Parasites (Hans Henrik Huss/Peter Karim Ben Embarek)

The presence of parasites in fish is very common, but most of them are of little concern with regard to economics or public health. Reviews have been published by Higashi (1985), Olson (1987) and Cross (2001) and recently a scientific status summary was prepared by Orlandi *et al.,* (2002).

More than 50 species of helminth parasites from fish and shellfish are known to cause diseases in man. Most are rare and involve only slight to moderate injury but some pose serious potential health risks. The most important are listed in Table 5.19.

Table 5.19 Pathogenic parasites transmitted by seafood.

Parasite	Geographical distribution
Nematodes or round worms	
Anisakis spp.	Worldwide
Gnathostoma spp.	Worldwide
Capillaria philippensis	The Philippines
Angiostrongylus spp.	Worldwide
Cestodes or tape worms	
Diphyllobothrium spp.	Worldwide
Trematodes or flukes	
Clonorchis spp.	South East Asia
Opisthorchis spp.	South East Asia, Eastern Europe
Heterophyes spp.	Worldwide
Paragonimus spp.	Worldwide
Metagonimus yokagawai	Asia, Egypt

All the parasitic helminths have complicated life cycles. They do not spread directly from fish to fish but must pass through a number of intermediate hosts in their development. Very often sea-snails or crustaceans are involved as first intermediate host and marine fish as second intermediate host, while the sexually mature parasite is found in mammals as the final host. In between these hosts, one or more free living stages may occur. Infection of humans may be part of this life cycle or it may be a side track causing disruption of the life cycle as shown in Figure 5.9. In most cases, infections of man are acquired by eating intermediate hosts that are raw or incompletely cooked, partially pickled or smoked or poorly preserved. The infections are preventable if the food is prepared sufficiently to destroy the infective stages of the parasite. However, it is extremely difficult to change cultural and eating habits, and therefore these parasites will continue to prevail.

Anisakis species

a) The disease and epidemiological aspects

Anisakiasis is a gastrointestinal parasitosis caused by the larval stages of anisakid nematodes. Humans acquire the disease by eating raw or improperly cooked or preserved seafood. Surviving worms will then penetrate the gut wall and enter the peritoneal cavity. Symptoms are often non-specific with abdominal pain, nausea and vomiting. Vague abdominal pain and possibly fewer may persist for weeks. Anisakiasis is common in Europe (the Netherlands), Japan and the US. The complete life cycle of *Anisakis* species is shown in Figure 5.9. Humans become infected by eating fish containing life third stage larvae. However, humans are accidental hosts, since transfer of parasites to humans cannot result in a complete life cycle for the parasite.

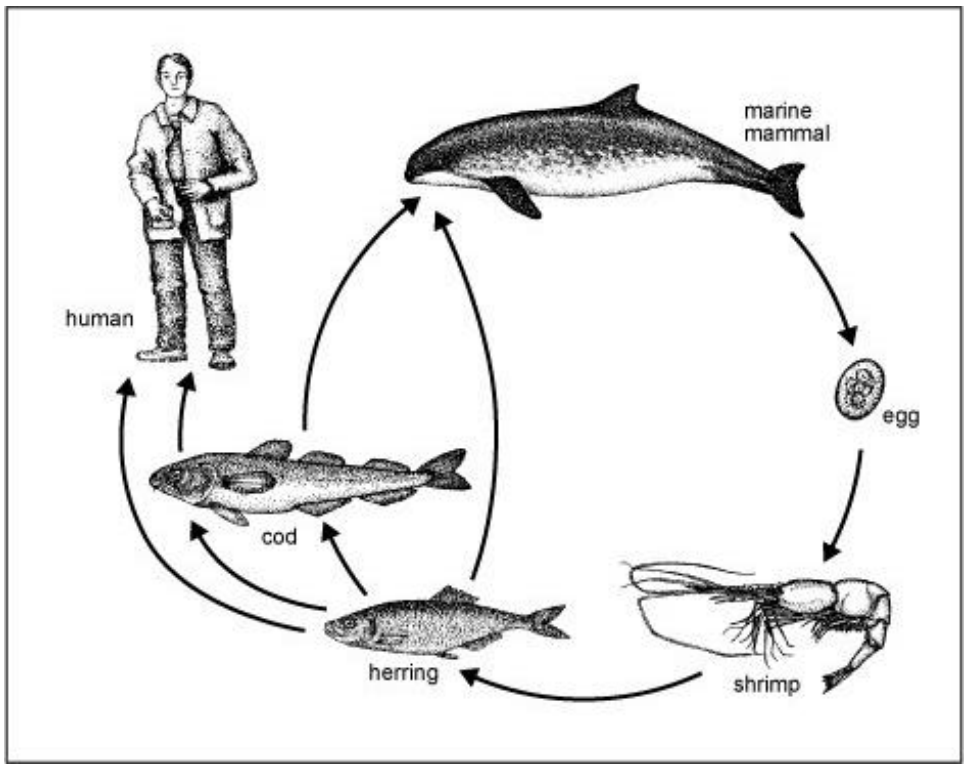


Figure 5.9. Life-cycle of *Anisakis* species.

The species of anisakidae most often associated with disease are *Anisakis simplex* (the herring worm) and *Pseudoterranova dicipiens* (the cod worm). The infective larval stage of the parasites can be found in the viscera and in the musculature of a variety of fish (see Figure 5.10). It is easy to distinguish between the two species as seen in Table 5.20.

Table 5.20 Characteristics of *Anisakis* species.

Species	Size	Colour	Common		
			name	feature	host
Anisakis simplex	18-36 mm long 0.3-0.7 mm wide	White	Herring worm	Curled up in a spiral	Herring
Pseudoterranova dicipiens	25-60 mm long 0.3-1.2 mm wide	Yellowish, brownish or reddish	Cod worm	Straight or in S-shape	Cod

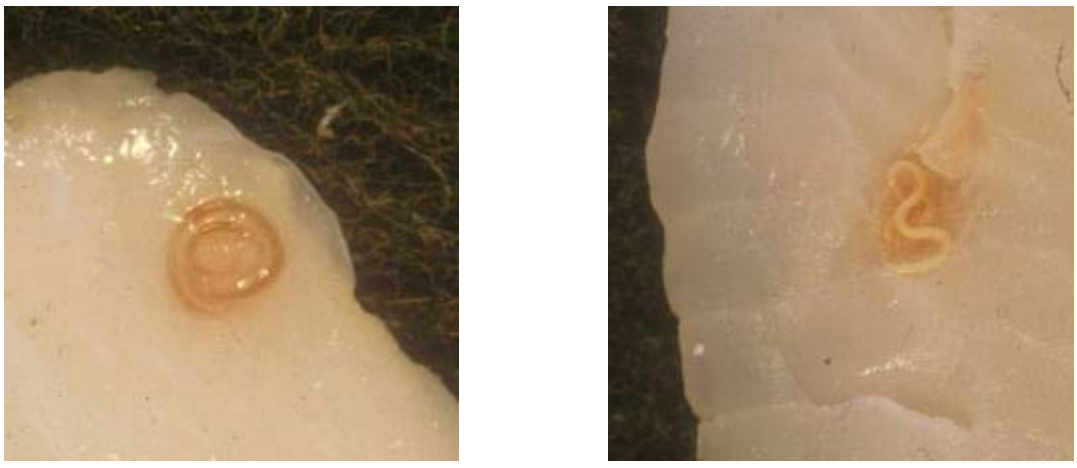


Figure 5.10 *Anisakis simplex* (left) and *Pseudoterranova dicipiens* (right) both in cod (photos courtesy of Dr. Stig Mellergaard).

b) Prevalence in fish and fishery products

Anisakis spp. are widely distributed geographically as well as within numerous fish hosts (cod, herring, squid, salmon a.o.). Thus prevalence reached more than 75% in fresh US commercial salmon (Deardoff and Overstreet, 1991) and nearly 100% in herring from the North Sea (Roepstorff *et al.,* 1993). In areas with no presence of sea-mammals, the prevalence of *Anisakis* will naturally also be very low. It should also be noted, that the parasite has never been detected in a large number of aquaculture salmon examined as shown in Table 5.21 (Angot and Brasseur, 1993; Deardoff and Kent, 1989; Bristow and Berland, 1991).

Table 5.21. Prevalence of *Anisakis simplex* in reared and wild caught marine fish species (after ICMSF,2003).

Fish	Origin	Number of samples	% positive
Farmed salmon,	Washington	50	0
Farmed salmon	Norway	2 832	0
Farmed salmon	Scotland	867	0
Farmed coho salmon	Japan	249	0
Farmed rainbow trout	Japan	40	0
Wild salmon	Washington	237	100
Wild salmon	North Atlantic	62	65
Wild salmon	West Atlantic	334	80-100
Wild salmon	East Atlantic	34	82
Wild coho salmon	Japan	40	100
Sardines	Mediterranean	7	14
Herring	Mediterranean	4 948	86
Herring	Pacific Ocean	127	88
Cod	Pacific Ocean	509	84

c) Survival in fishery products

Low or high temperatures or high salt concentrations may be used to kill or inactivate nematodes in fish (Table 5.22). In contrast, acid conditions are not affecting the nematodes.

Table 5.22 Conditions to kill or inactive nematodes in fish

Condition	Value	Time to inactivation	Reference
Temperature	- 20°C	24 h	Howgate 1998
	55°C	1 min	Huss unpublished data
NaCl, % WPS	4-5	>17 weeks	Karl *et al.* 1995
	6-7	10-12 weeks	
	8-9	5-6 weeks	

d) Prevention and control

In the EU, conditions concerning control of parasites are laid down in Council Directive no. 91/493/EEC (EC, 1991a). All fish and fish products must be subject to a visual inspection during processing for the purpose of detecting and removing any visible parasite. Further, all fish that are to be consumed raw or almost raw must be subjected to a freezing process (-20∀for at least 24 h in all parts of the fish). This also applies to fish products that are heated (e.g. hot smoked) to a temperature of less than 60∀C. As far as salted fish is concerned, the process must be sufficient to destroy the larvae of nematodes. The US regulations stipulate that the freezing process to destroy parasites should be -20∀C for 7 days or -35∀C for 15 h (FDA, 2001a).

Thus, the best prevention and control of anisakiasis is eating well-cooked or well-frozen fish only. A number of well-known fish products can be unsafe. This applies to all lightly preserved fish products (< 5% NaCl in water phase) such as cold smoked fish, gravad fish, matjes herring, lightly salted caviar, ceviche and several other local traditional products. A short period of freezing – either of the raw material or the final product – must be included in the processing as a mean to control parasites.

Gnathostoma

This nematode has carnivores (dogs, cats, wild animals) as the natural definitive hosts. *Gnathostoma* is acquired by eating raw or under-cooked freshwater fish or by drinking contaminated water. Clinical manifestation of gnathomiasis is caused by migrating larval. The larval can reach 10 mm. Acute pain is experienced as the larval penetrate and migrate through abdominal and thoracic organs and eventually makes its way to the subcutaneous tissues causing swellings ("creeping eruption"). In serious cases the larval may reach the eye or central nervous system.

***Capillaria* species**

These nematodes are of great public health importance although with restricted focus. Infection with *C. phililppensis* causes serious illness and usually leads to death if not treated in time.

The adult parasite inhabits the intestinal tract, causing severe diarrhoea and death attributed to fluid loss. Eggs of the parasite are passed with faeces into soil or water, and the larval are found in intestines of freshwater fish having ingested embryonated eggs. The adult worm is most likely a parasite of piscivorous of birds with humans being accidental host.

***Angiostrongylus* species**

Angiostrongylus is a 25-30 mm long nematode having rats as the final host (Figure 5.11).

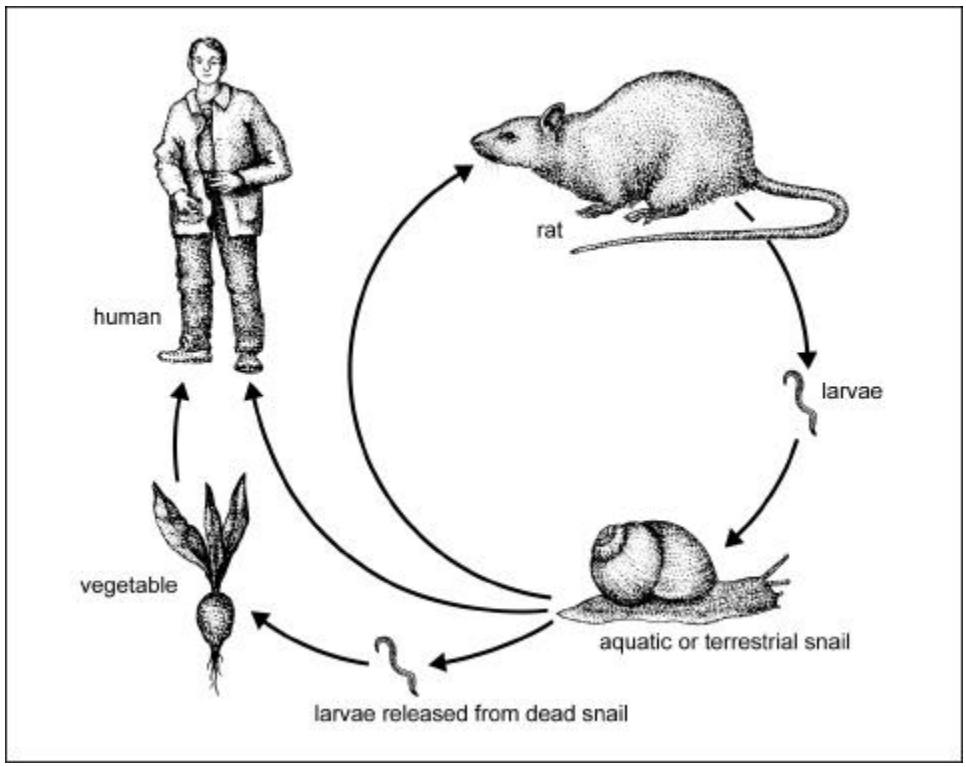


Figure 5.11 Life cycle of *Angiostrongylus* sp.

Humans become infected by eating infected snails or molluscs. The worms migrate to the brain causing life-threatening meningo-encephalitis. The parasite was originally seen in certain parts of Asia, but parasitosis caused by this worm continues to be reported from new areas of the world. This could possibly be attributed to stow away rats.

***Diphyllobothrium* species**

Diphyllobothrium are cestodes or tape worms. *D. latum* is the largest human tape worm and can reach more than 10 m in length. It resides in the small intestines of fish.

Diphyllobothriasis is a long lasting infection (decades). Most infections are asymptomatic but manifestations may include abdominal discomfort, diarrhoea, vomiting and weight loss. The distribution of the tape worm is widespread in the temperate and sub-Arctic regions of the Northern Hemisphere where freshwater fish are eaten (Figure 5.12).

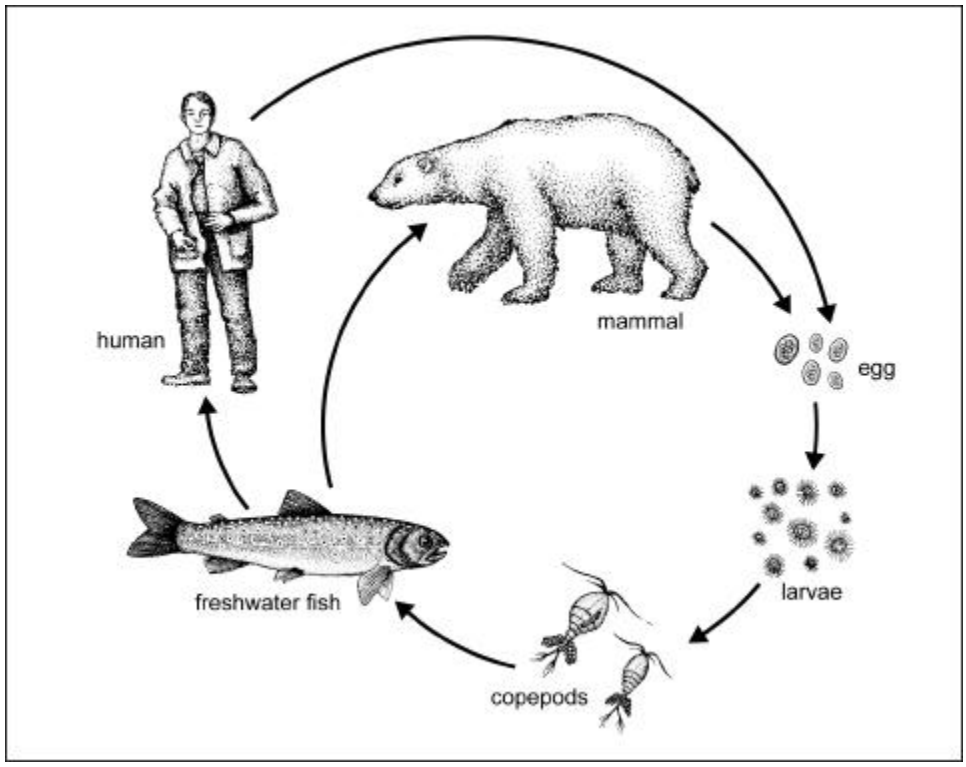


Figure 5.12 Life cycle of the broad fish tapeworm, *Diphyllobothrium* sp.

A recent mass occurrence of human infection with *Diplogonoporus grandis,* which is a cestode belonging to Diphyllobothridae, has been recorded in Japan (Kino *et al.,* 2002). It was suggested that the transmission was due to consumption of raw, juvenile Japanese anchovies. The tapeworms recovered from patients had a mean length of 230 cm and a mean with of 9 mm. The life cycle of *D. grandis* has not been established.

Trematodes

While more than 750 million people around the world are at risk for food-borne trematode infections (FBT), an estimated 40 million people are infected with one or more of these parasites (WHO, 1995). The majority of these infections (around 38 million) are fish-borne infections and are mainly occurring in some 20 countries where the parasites are endemic. Although seldom fatal, trematode diseases can cause morbidity and complications leading to death. The cause of infection is the ingestion of viable trematode metacercariae, which can be present in the flesh of, raw, inadequately cooked or minimally processed freshwater fish, molluscans and crabs. Infections are prevalent in several countries and among communities where eating raw, fermented or inadequately cooked fish is a cultural habit.

To control a disease, it is important to know where it is endemic. In trematode disease it is also necessary to have a complete understanding of the biology of the parasite, the life cycle and each stage of the life cycle must be known from the egg via the miracidium to the cercaria to the metacercaria to the adult parasite. All hosts must be determined: the snail (first intermediate), the animal host or vegetation (second intermediate) upon which metacercaria may encyst.

The adult worms are small, flat, slender and measures from a few up to 20 mm in length and 3-5 mm at the widest area. The general life cycle of trematodes, having fish as the second intermediate host is shown in Figure 5.13.

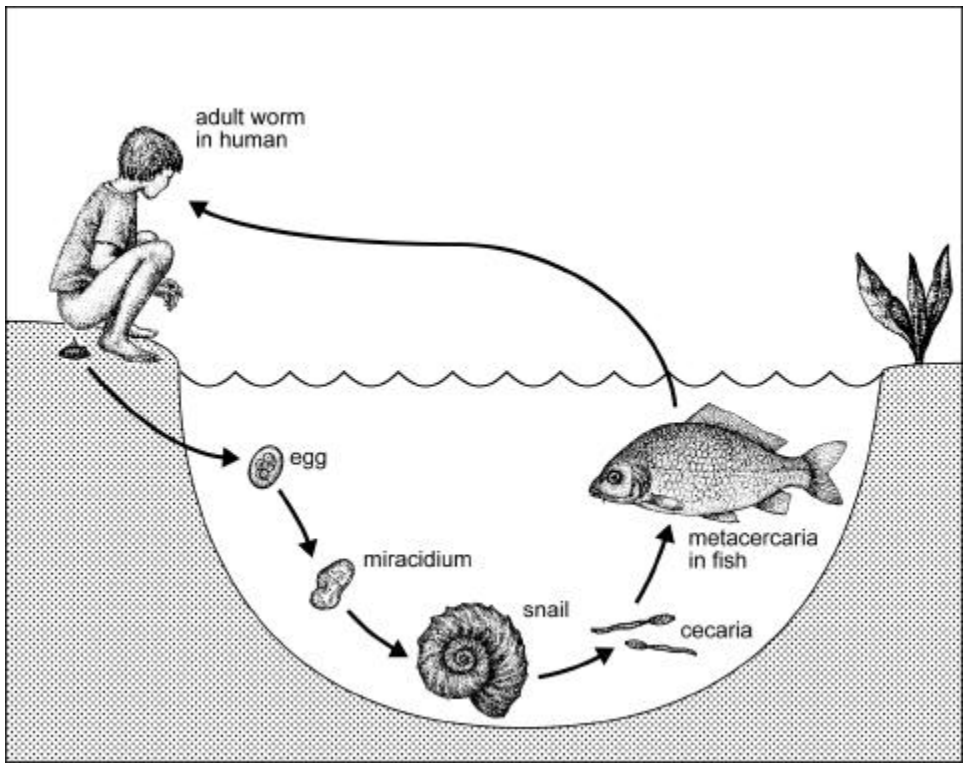


Figure 5.13 Life cycles of trematodes having fish as an intermediate host (redrawn from Strauss 1996).

There are three main groups of fish-borne trematodes infecting man (Table 5.23):

- ∉ the liver flukes
- ∉ the lung flukes
- ∉ the intestinal flukes.

Table 5.23 Trematode parasites transmitted by fish

Parasite	Second intermediate host	Geographical area[1]	Estimated no. of infections (millions)
Liver flukes:			
Opisthorchis viverini	> 35 species of freshwater fish	SEA	8
Opisthorchis felineus	> 35 species of freshwater fish	EE, R, V	2
Clonorchis sinensis	> 100 species of freshwater fish, mainly carp	C, RK, J, R, V	7-13
Lung flukes:			
Paragonimus spp.	crustaceans – freshwater crab	SEA, P, E, W+CA	21
Intestinal flukes:			
Metagonimus yokagawai	freshwater fish	A, (EU)	1.3
Heterophyes heterophies	brackish water fish, bivalves, molluscs	EU, ME, A	

1. Geographical area: SEA: South East Asia, EE: East Europe, R: Russia, U: Ukraine, C: China, RK: Republic of Korea, J: Japan, P: Peru, E: Ecuador, W+CA: West- and Central Africa, EU: Europe, ME: Middle East

Liver flukes
These parasites are named as liver flukes due to their preference for migrating to the bile ducts and the liver. Small amounts of adult trematodes can be present without causing any disease or symptoms of disease. Large numbers of trematodes (up to 20 000 have been counted at autopsy of a patient) causes obstruction of bile ducts and possibly secondary bacterial infections followed by hepatitis. Also, the incidence of liver carcinoma is high in patients with liver flukes. The clinical symptoms are fever, epigastric pain, anorexia, diarrhoea, jaundice and abdominal pain.

Lung flukes
The adult lung fluke is located in the lungs of man and a number of domestic and wild animals (dogs, cats, pigs, tigers, leopards). The eggs from the parasite are passed into the bronchioles and expectorated from the body or swallowed and passed with the faeces.

The first intermediate host is a snail and the second intermediate host is crustaceans or freshwater crab. Humans become infected by eating raw or undercooked crustaceans or crab. The immature worms will penetrate the intestinal tissue, enter the body cavity and penetrate the diaphragm into the pulmonary cavity and the lungs.

The pathology associated with lung flukes depends on the number of worms ingested. A few worms are harmless, but large numbers cause chronic pulmonary disease. A complicating factor is the tendency of lung worms to enter the central nervous system (CNS). Invasion of the brain may result in mental disorder and meningitis.

The clinical symptoms may be diarrhoea and abdominal pain. Once the worms are established in the lungs, there might be general malaise and cough. In severe cases, and when other organs are involved, the outcome of infection may be fatal.

Intestinal flukes
In recent years various species of fish-borne intestinal trematodes have gained epidemiological significance. An estimated 1.3 million people suffer from metagonimiasis, heterophyasis and echinostomiasis caused by approximately 70 species of intestinal flukes of which the *Heterophyidae* and *Echinostomatidae* are the main families. The two most important species are *Metagonimus yokagawai* and *Heterophyes heterophies*. They are very small flukes (1-2 mm) living in the intestines of the final host, causing inflammation, symptoms of diarrhoea and abdominal pain. The primary intermediate hosts are snails. Freshwater fish act as the second intermediate

host for the metacercarial stage of *Metagonimus* sp. and brackish water fish for the *Heterophyes* sp.; while brackish water bivalves, molluscs and oysters serves as secondary intermediate hosts to a range of other species of intestinal flukes (Chai and Lee, 2002). Raw or improperly cooked freshwater-, brackish water fish and bivalves including oysters are the major sources of infections. Clinical symptoms differ depending on the parasites involved and include acute abdominal pain, diarrhoea, lethargy, weight loss, fever and malabsorption. In some cases, eggs of the parasite mature deep in the intestinal tissues and may enter the circulatory system and cause cardiac damage. Light infections are asymptomatic.

Control of diseases caused by trematodes

Fish-borne trematode infections are a major public health problem that has largely gone unrecognised by the health sector and the fish inspection services in recent years. All parasites of concern are transmitted to man by eating raw or uncooked fish products. Transmission of fish-borne trematodes is associated with behavioural patterns determined by socio-economic and cultural conditions in endemic areas. Consumption of trematode-borne fish and shellfish occurs most often around lakes, streams and ponds. Korean men will eat raw fish while drinking sake at social gatherings acquiring clonorchiasis. In South China people like to eat congee (rice gruel) with slices of raw fish. In Hong Kong freshwater fish is imported from the mainland and therefore expensive resulting in the more affluent groups acquiring clonorchiasis and possibility cholangiocarcinoma. Paragonimiasis is acquired by eating wine soaked "drunken" crabs in parts of China and in Thailand and the Philippines crab juice is used for medicinal purposes as well as using it in food preparations. Opisthorchiasis is acquired in Thailand by eating raw fish salads or low-salt fermented fish. Echinostome infections are from eating snails and raw fish in Northern Luzon in the Philippines and in Korea.

Eating habits are deeply rooted in a culture and are resistant to changes. In some cultures raw animals and plants are eaten for medicinal as well as nutritional purposes. Raw crayfish is used to treat measles and transmits paragonimiasis. In the Cameroon, raw crab is thought to increase fertility and in Ecuador macerated crab supernatant is given to sick children. Raw foods are often eaten out of necessity because of the lack of cooking fuel. The use of human and animal faeces (night soil) for fertiliser and indiscriminate defecation contaminates the environment and water bodies. In some areas toilets are built over fishponds, thereby perpetuating the infectious cycle in rural aquaculture. The relative contributions of farmed fish and wild caught fish to the burden of these diseases are yet unclear. In countries such as China and Vietnam, aquaculture fish in small traditional ponds are heavily infected with *C. sisensis* and play an important role in the spread of the disease.

Although there are effective drugs for treatment of most fish-borne trematode disease, it is more important to prevent infections. Control of trematode infections is difficult and the measures that have been employed have not been successful.

The parasites involved in FBT infections have complex life cycles involving one or two intermediate hosts. Effective control strategies are therefore difficult to implement.

The WHO Technical Report on trematode infections (WHO, 1995) details the basis of strategies for the control of fish-borne trematode infections. Many sectors are important and collaboration between all of them is necessary: i.e. public health, agriculture, aquaculture, food industry, food control and education. Methods for controlling food-borne trematodes in freshwater fish have shown promising results in countries like Korea and Thailand. These involve case detection and treatment, health education, improved sanitation, legislation of food safety measures and management of human faeces. Application of preventive approaches based on HACCP (Hazard Analysis and Critical Control Points) could also contribute to provide a high degree of food safety. Control of snail populations could also be envisaged together with the promotion of infestation resistant fish species for aquaculture purpose in endemic areas.

So far very little have been done to control the infections in the food, i.e. the fish products. Most preservative parameters (temperature, pH, salt) used in processing fish, shellfish and aquatic animals have been only the subject of limited studies for their potential to control trematodes (Table 5.24).

Heat inactivation of parasites is an effective method for eliminating the risk of parasitic infections. (Adams *et al.,* 1997). The only data available for trematodes would seem to indicate a higher heat resistance of trematodes compared to nematodes. For nematodes, a recommended min of 63°C for 15 sec should be enough to inactivate the parasites (FDA, 2001a). Obviously, more work should be undertaken to gain a better knowledge of the necessary heat treatment needed to inactivate trematodes in the fish. Freezing provides an effective mean of inactivating parasites in raw fish. Again, for nematodes, 15 h at –35°C or 7 days at –20°C will be effective (FDA, 2001a) while data on trematodes (Fan 1998) indicates that 7 days at –20°C had no inhibitory effect on the viability of metacercariae of *C. sinensis* in naturally infected fish. Based on the work of Fattakhov (1989), the Ministry of Health of the USSR recommended in 1990, holding fish at –28°C for 32 h or at –40°C for 7h to inactivate the trematode *O. felinus* in fish (Table 5.24). Storage at refrigeration temperatures does not seem to affect trematodes. *O. vevirrini* was virtually unaffected when stored in saline solution at 4°C for 5 weeks (Sithithaworn *et al.,* 1991). The large differences observed in the experiments reported in Table 5.24 reflect the differences in the methodology applied and in the way the viability of metacercariae are determined (visually or by artificial infection in laboratory animals).

Table 5.24 Preservative parameters necessary to inactivate trematodes. Adapted from WHO (1995).

Preservative parameter	Parasite	Process variable	Time	Reference
Salting	*Opisthorchis* meta-cercariae in fermented fish	13.6%	24 h	Kruatrachue *et al.* 1982
	C. sinensis in naturally infected fish.	30% (wt based)	8 days	Fan 1998
	O. viverrini metacercariae in fermented fish	20% (wt based)	5 h[1]	Tesana *et al.* 1986
Freezing	*C. sinensis* in naturally infected fish	-12°C	20 days[2]	Fan 1998
	C. sinensis in naturally infected fish	-20°C	3-4 days[3]	Fan 1998
	O. felinus in fish	-28°C	32 h	Recommendation, Ministry of Health, USSR, 1990.
	O. felinus in fish	-40°C	7 h	-"-
	O. felinus in fish	-28°C	20 h	Fattakhov 1989
		-35°C	8 h	-"-
		-40°C	2 h	-"-

1. *Viability was markedly reduced but not completely inhibited*
2. *10 days had no inactivating effect and 18 days had only marginal inactivating effect*
3. *7 days at –20°C had no inhibitory effect on 10 rats infected but 3 days storage at –20C, followed by thawing and re-freezing for 4 days had 100% inhibitory effect on 10 infected rats.*

Protozoan

A large number (nearly 40) of parasitic protozoans are known to be infectious to humans. The most important of those being transmitted primarily via water are shown in Table 5.25.

Table 5.25
Protozoans transmitted via water.

Name	Reservoir host
Cryptosporidium sp.	>130 species of mammals
Entamoeba histolytica	Human
Giardia sp.	Human and animals
Cyclospora sp.	Human

All of these protozoan parasites are excreted in the faeces of the host. The protozoan can enter water and be transmitted directly by drinking the water or indirectly via contaminations of food, utensils, hands of food handlers or flies and other pests. Direct person to person contact is also possible as no intermediate host is required for protozoan parasites.

Infections can range from asymptomatic or mild bowel discomfort to diarrhoea or dysentery with or without blood in the stools and can last several months (amoebiasis). Cryptosporiasis often begins with an influenza-like illness and with possible development of diarrhoea, abdominal pain, nausea, vomiting and fever. Infections with *Giardia* can range from asymptomatic to fatal following months with severe symptoms of discomfort in the upper intestines.

The important steps in preventing protozoan infections are good personal hygiene, proper sanitation of toilet seats, avoid eating raw fruit and vegetables and treatment of drinking water. Slow sand filtration combined with chemical flocculation has been recommended as the best method (Fayer 2001). A review of water purification methods has been given by Ives (1990).

5.1.5 Aquatic biotoxins *(Hans Henrik Huss)*

The possible presence of natural toxins in fish and shellfish has been known for a long time. Most of these toxins are produced by species of naturally occurring marine algae (phytoplankton). There are over 4,000 species of marine algae, but only 70-80 species (~2%) are known to produce toxins (Scoging, 1998).

A proportion of the toxic phytoplankton has a red-brown pigmentation, giving rise to the naming of algal blooms as “red tides”. However, it should be emphasised that not all coloured algae are toxic, and incidence of poisoning have occurred in the absence of red tides. Visible red tides may contain from 20 000 to > 50 000 algal cells per ml. Concentrations as low as 200 cells/ml may produce toxic shellfish. During a bloom, bivalves can accumulate sufficient toxin to cause human illness after filter feeding for only 24h (Scoging, 1998) (see Figure 5.14).

Molluscan shellfish are filter feeders and continually pump water through their gills where particulate matters is removed and ingested. Mussels ingest food particles of any type of 2 to 90 σm in size with a rate of ingestion dependent on water temperature and environment. Optimally, they can filter 2.5 l/h extracting 98% of the available algae. Consequently, any toxin associated with the phytoplankton ingested can rapidly accumulate and hence become concentrated in the bivalve mollusc. The consumption of these toxic shellfish by humans causes illness with symptoms ranging from mild diarrhoea and vomiting to memory loss, paralysis and death.

Toxins associated with phytoplankton are known as phycotoxins. These toxins have been responsible for incidents of wide-scale death of sea-life and are increasingly responsible for human intoxication. There are a number of different seafood poisoning syndromes associated with toxic marine algae and these include paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP), diarrhetic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP) and azaspiracid shellfish poisoning (AZP). There are also different types of food poisoning associated with finfish and these include ciguatera poisoning and puffer fish poisoning. Consumption of raw molluscan shellfish poses well-known risks of food poisoning, however, intoxication from finfish is not so well known. Most of the algal toxins associated with seafood poisoning are heat stable and are not inactivated by cooking. It is also not possible to visually distinguish toxic from non-toxic fish and shellfish. Many countries rely on biotoxin monitoring programmes to protect public health and close

harvesting areas when toxic algal blooms or toxic shellfish are detected. In non-industrialized countries, particularly in rural areas, monitoring for harmful algal blooms does not routinely occur and death due to “red tide toxins” commonly occurs.

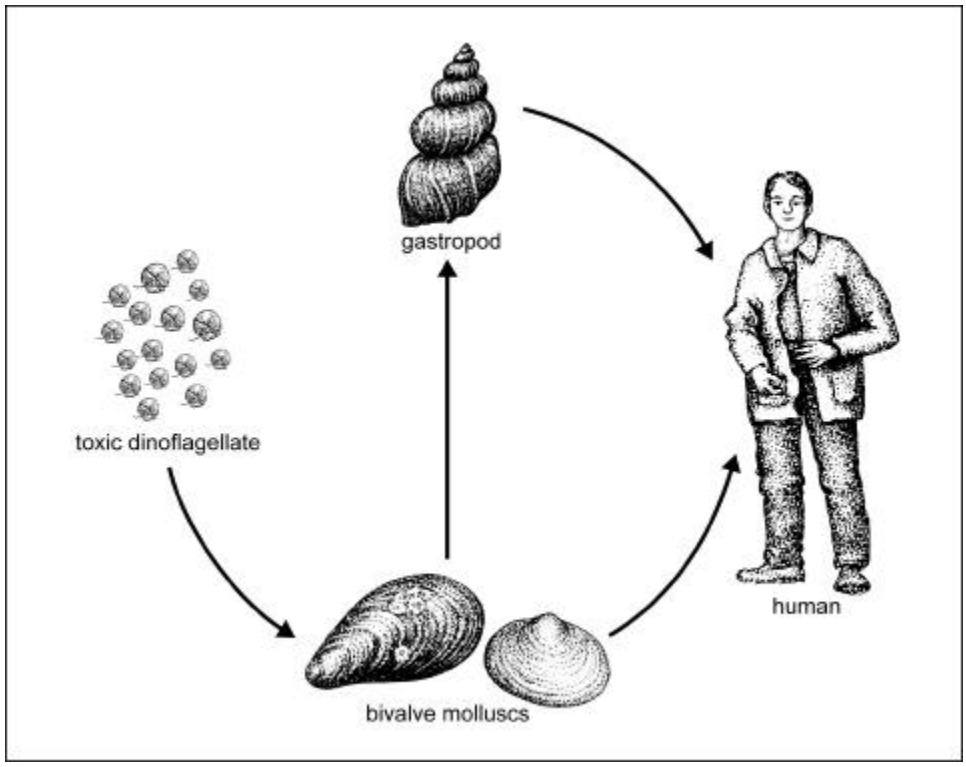


Figure 5.14 Generalized pathways of human intoxication with molluscan shellfish toxins via filter feeding bivalves and carnivorous and scavenging gastropods. (from Anderson *et al.*, 2001).

The toxins are accumulated in the digestive gland of the shellfish (hepatopancreas) and do not affect the shellfish themselves. The shellfish may reduce toxicity in clean water, but depuration times vary greatly according to the bivalve specie involved, the pumping activity of the bivalve and the hygrographic conditions.

Fish may also consume toxic algae and cause disease in humans (ciguatera). Also, there are toxins in some fish species that do not involve marine algae (puffer fish poisoning), see Table 5.26.

Paralytic shellfish poisoning (PSP)

a) The disease and epidemiological aspects

Intoxication after consumption of shellfish is a syndrome that has been known for centuries, the most common being PSP. It is caused by a group of toxins (saxitoxins and derivates) produced by dinoflagellates of the genera *Alexandrium*, *Gymnodium* and *Pyrodinium*.

Table 5.26 Marine biotoxins and the associated poisonings.

The disease	Toxins	Occurrence
PSP-Paralytic shellfish poisoning	Saxitoxin	Worldwide
DSP-Diarrheic shellfish poisoning	Okadaic acid dinophysis toxin	Worldwide
NSP-Neurotoxic shellfish poisoning	Brevetoxins	USA, Caribbean, New Zealand
ASP-Amnesic shellfish poisoning	Domoic acid	North America
Ciguatera fish poisoning	Ciguatoxin (CTX)	Tropical, subtropical
Puffer fish (tetrodotoxin) poisoning	Tetrodotoxin (TTX)	Japan, South Pacific

Symptoms of PSP initially involve numbness and a burning or tingling sensation of the lips and tongue that spread to the face and fingertips. This leads to a general lack of muscle coordination in the arms, legs and neck. Severe cases of PSP have resulted in respiratory paralysis and death. There are an estimated 1 600 annual cases of PSP world-wide, approximately 300 of these will be fatal (Scoging, 1998). Mortality rates in outbreaks of PSP have reached 40%. There is an extreme variation in sensitivity to the toxin, but intoxication has followed oral intake of 144 µg to 1,660 µg per person with fatalities occurring at levels of 300 µg to 12 400 µg PSP per person (van Egmond *et al.,* 1993).

b) Prevalence in fish and fishery products

PSP is the most widespread shellfish poisoning and outbreaks are occurring worldwide as shown in Figure 5.15.

Blooms of toxic algae – and outbreaks of PSP – occur regularly throughout Europe, and the EU-monitoring programmes regularly detect high toxin levels (van Egmond *et al.,* 1993). The dinoflagellates bloom as a function of water temperature, light, salinity, presence of nutrients and other environmental conditions. Blooms of toxic algae have recently become more prevalent, and many experts believe coastal pollution and shipping practices have contributed to this expansion (Anderson 1994). Water temperature must be ∅ 5-8∀C for blooms to occur. If temperature decreases to below +4∀C, the dinoflagellates will survive as cysts buried in the upper layer of the sediments.

Shellfish that have fed on toxic dinoflagellates retain the toxin for varying periods of time depending on the shellfish. Some clear the toxin very quickly and are only toxic during the actual bloom. Others retain the toxin for a long time, even years (Schantz, 1984).

c) Stability of toxin

The toxic compounds are water-soluble and heat stable. A 5-minute cook will reduce toxicity by only 30% and increasing this to 20 min. will only effect a 40% denaturation (Scoging, 1998).

Figure 5.15 World distribution of outbreaks of paralytic shellfish poisoning (black spots) and ciguatera (shaded area). See Huss (1994) for references.

Diarrheic shellfish poisoning (DSP)

Thousands of cases of gastrointestinal disorders caused by DSP have been reported in Europe, Japan, South East Asia, North- and South-America (Sechet *et al.,* 1990). The causative dinoflagellates, which produce the toxins are within the genera *Dinophysis* and *Prorocentrum*. These dinoflagellates are widespread, which means that this illness could also occur in any other parts of the world. A great number of toxins has been identified including okadaic acid (OA) and associated toxins (DTX 1-4). Levels producing diarrhoea in adults are estimated at $\geq$ 40 µg for OA and $\geq$ 35 µg for DTX 1 (Scoging, 1998).

Onset of disease is within half an hour to a few hours following consumption of shellfish, which have been feeding on toxic algae. Symptoms are gastrointestinal disorder (diarrhoea, vomiting, abdominal pain) and victims recover within 3-4 days with or without treatment. No fatalities have ever been observed.

The toxins are heat stable and survive normal cooking.

Neurotoxic shellfish poisoning (NSP)

The occurrence of NSP has historically been limited to the west coast of Florida, where blooms of the dinoflagellate *Gymnodinium breve* occurs regularly offshore and is carried inshore by wind and current conditions. However, also shellfish harvested on the southern Atlantic coast may be toxic and there have been reports of outbreaks of NSP in New Zealand.

The responsible toxins are a family of brevetoxins. The toxins are extraordinarily stable (survive heat up to 300∀C) and the oral LD_{50} value in rats being in the order of 520-6 600 µg/kg (Llewellyn 2001). Pathogenic dose for human is in the order of 42-72 mouse units (MU).

Typical symptoms of NSP are tingling in the face, throat and digits, dizziness, fever, chills, muscle pains, abdominal pains, nausea, vomiting, headache and reduced heart rate. There have been no recorded human deaths from NSP, but the toxin is fatal to fish and can cause massive fish kill.

Amnesic shellfish poisoning (ASP)

ASP is the only shellfish poison produced by a diatom. Disease was first identified in Canada in 1987, where more than 100 people became ill often consuming contaminated shellfish (Todd, 1993). The disease was named after one of the more curious symptoms, which was loss of short-term memory. Other symptoms include nausea, vomiting, diarrhoea, headache and neurological effects including dizziness, disorientation and confusion. In severe cases seizures followed by coma and death may occur. The short-term memory loss seems to be permanent in surviving victims.

Outbreaks have so far been confined to Canada and the USA, although the responsible algae has been found in many other areas.

The causative agent is domoic acid. In the Canadian 1987 outbreak, human toxicity occurred at 1-5 mg/kg (Todd, 1993).

Ciguatera fish poisoning (CFP)

CFP is one of the most common food-borne illnesses related to finfish consumption. Its true incidence is not known, but it has been estimated that 10 000-50 000 people a year suffer from this disease. It is caused by consumption of fish that have become toxic by feeding on toxic dinoflagellates or toxic herbivore fish. The principal source is the benthic dinoflagellates *Gambierdicus toxicus*, which is found primarily in the tropics where it lives in association with macro algae, usually attached to dead corals. More than 400 species of fish are known to be vectors of ciguatoxins. Toxins can be detected in the gut, liver and muscle tissue by means of mouse assay. Some fish may be able to clear the toxins from their systems (Taylor, 1988). The toxic fish may be found in tropical and subtropical Pacific and Indian Ocean regions and in the tropical Caribbean as shown in Figure 5.15.

Ciguatoxins arise from bio-transformation in the fish of precursor toxins produced in the dinoflagellates and it causes disease when present in $\geq$ 1 ppb (0.1 µg/kg) in the flesh of the fish (Lehane and Lewis, 2000).

Clinical symptoms vary widely but are characterized by gastrointestinal, neurological and cardiovascular disturbances often within 10 min but also up to 24 h after ingestion of toxic fish. The initial gastrointestinal symptoms are similar to any other food poisoning (abdominal pain, nausea, vomiting, diarrhea). The neurological symptoms most often encountered are tingling and numbness in the mouth, hand and feet, muscle cramping and weakness, temperature reversal, superficial hyperesthasia with a sensation of burning. Headache, vertigo, stiffness, convulsions, hallucinations, transient blindness, salivation, perspiration are symptoms that may occur. A slow, irregular pulse and law arterial pressure may follow. Cardiovascular disorders usually disappear within 48-72 h while neurological effects may persist for weeks, even years in severe cases. Death from CFP is rare (<1% worldwide).

Puffer fish (Tetrodotoxic) poisoning (PFP)

Tetrodotoxin (TTX) is one of the most potent non-proteinacous toxins known and responsible for numerous fish poisonings. The toxin is named after the order Tetraodontidae (common names: puffer fish, balloon fish, globe fish, fugu, toad fish, blow fish), since many of these fish often carry the toxin. Apart from Tetraodontidae toxin has been found in goby, blue-ringed octopus, various gastropods, newts and houseshoe crab.

PFP has frequently occurred in Japan, where these fish are a traditional food. Nearly 300 cases (nearly 500 patients) were recorded in the 10-year period 1987-1996 with an average mortality rate of 6.6% (Yoshikawa-Ebesu *et al.,* 2001). Sporadic cases of PFP are seen in other Asian and Pacific countries incl. USA. Symptoms of PFP occur within minutes and rarely more than 6 h after ingestion of toxic fish. Nausea and vomiting may or may not occur, but the most common

symptoms are tingling or pricking sensation and dizziness. Disease may progress to muscle and respiratory paralysis. Where death occurs it is usually within 6 h and sometimes as rapidly as 20 min following toxin ingestion. Persons who have not died within 24 h generally recover completely.

The distribution of the toxin in the fish is mainly in the ovaries (eggs), liver and skin. The muscle tissue is normally free of toxin. The origin of the toxin has historically been much debated (Figure 5.16). The question has been whether it is endogenous or exogenous. It is now assumed that TTX in fish comes directly from its feed. The toxin is produced by bacteria, absorbed on or precipitated with plankton, transmitted to TTX-bearing animals such as small gastropods, starfish, flatworks etc. and from here transmitted to fish and large gastropods. Fish, except those processing tetrodotoxin such as puffers and tropical goby, do not accumulate tetrodotoxin even where toxin-containing diets are fed to them at sub-lethal doses (Yoshikawa-Ebesu *et al.,* 2001).

TTX is a potent toxin with a LD_{50} of 2 mg for man. The minimum dose necessary to cause symptoms has been estimated to 0.2 mg (Yoshikawa-Ebesu *et al.,* 2001).

Control and prevention of natural toxins

Natural toxins are very heat stable. Normal household cooking (e.g. boiling, steaming, frying) has no or very little effect on toxin levels. Also a heat treatment of 70∀C in 20 min was insufficient to reduce the toxin level significantly and even after retorting (120∀C for 60 min) some toxicity remained (Nagashima *et al.,* 1991). The normal industrial canning process may significantly decrease the toxin levels present in shellfish, but it is only sufficient when the initial toxin level is relatively low. Thus in the European Union it is acceptable to utilise bivalve molluscs when the initial level of

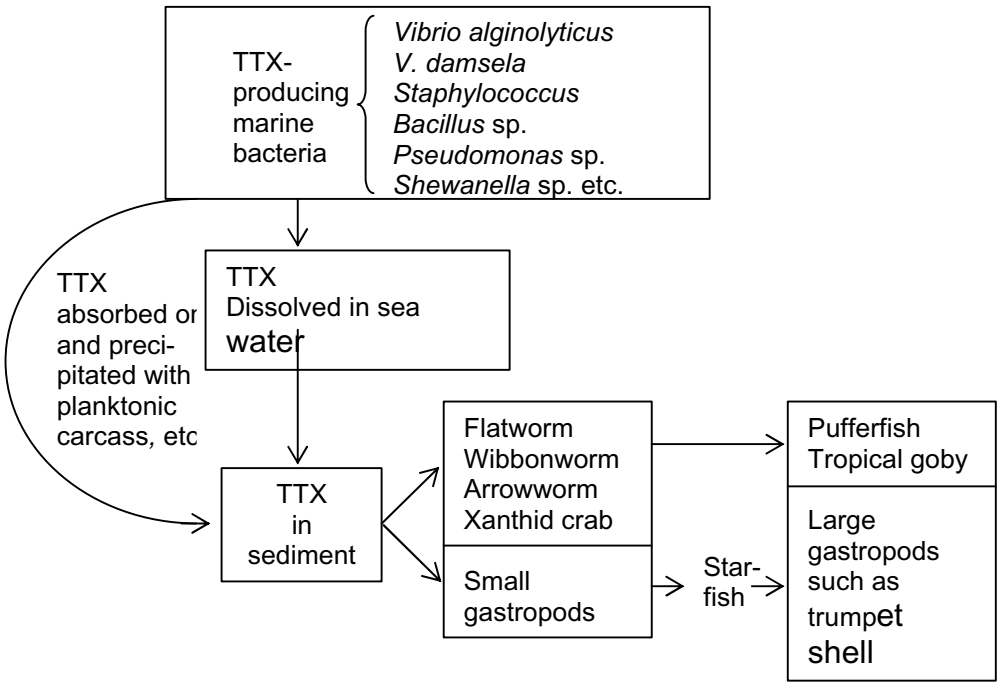


Figure 5.16 Assumed mechanism of toxification of tetrodotoxin-bearing animals (after Yoshikawa-Ebesu *et al.,* 2001).

contamination with PSP exceeds the limit of 80 µg/100 g laid down in Council Directive 91/492/EEC but is below 300 µg/100 g (EC, 1996). However, the molluscs have to undergo the following operations sequentially:

1. Preliminary cleaning in fresh water for a minimum of two minutes at a temperature of 20∀C, plus or minus 2∀C

2. Pre-cooking in fresh water for a minimum of three minutes at a temperature of 95∀C, plus or minus 5∀C
3. The separation of flesh and shells
4. Second cleaning in running fresh water for a minimum of 30 seconds at a temperature of 20∀C, plus or minus 2∀C
5. Cooking in fresh water for a minimum of nine minutes at a temperature of 98∀C, plus or minus 3∀C
6. Cooling in running cold fresh water for approximately 90 seconds
7. The separation of the edible parts (foot) from the non-edible parts (gills, viscera and mantle) mechanically with water pressure
8. Conditioning in containers closed hermetically in a non-acidified liquid medium
9. Sterilisation in autoclave at a minimum temperature of 116∀C for a time calculated according to the dimension of the containers used but which cannot be lower than 15 minutes.

A new chemical method for decontamination of PSP toxins in shellfish was recently developed by Lagos *et al.* (2001). The method involves one or two alkaline treatment (pH – 9) followed by boiling and washing. The method was reported to yield 99% decontamination.

Detection of natural toxins is mainly based on mouse-bioassays, while analytical methods may be used for confirmatory analysis of toxic compounds. Only in one case (analysis for domoic acid) is an analytical method – high-performance liquid chromatography (HPLC) – approved as a certified method.

Mouse-bioassays are cheap to carry out, but it is to their disadvantage that they involve live animals, a practice, which has become increasingly unpopular, and that they require experienced personnel and careful standardisation of assay conditions. Also bioassays are less sensitive and less precise than analytical methods. For an overview of present and emerging technologies in detecting natural toxins see Kitts (2001), Price and Tom (1999) or Anderson *et al.* (2001).

The regulatory tolerances established for natural toxins by FDA (1998) and others are listed in Table 5.27.

Table 5.27 Monitoring of biotoxins

Toxin	Toxicity	Regulatory tolerance	Method of analysis
PSP	PD[1]: 0.1-2 mg; LD[2]: ∅0.3-12 mg	80 µg/100 g tissue	Mouse assay
DSP	35-40 µg	0-60 µg/100 g	Mouse assay
NSP	PD: 42-72 MU	0.8 ppm (20 MU/100g)	Mouse assay
ASP	PD: 1-5 mg/kg	20 ppm domoic acid	HPLC
CFP	PD: 23-230 µg	must not be detected	Mouse assay
PFP	LD_{50}: 2 mg; PD: 0.2 mg		HPLC

1. PD = Pathogenic dose for humans
2. LD = Lethal dose for humans.

The primary preventive tool for intoxications with natural toxins is the monitoring of toxin levels in algae in the harvesting areas (see Chapter 9). Based on the presence of toxins, waters can be classified and harvesting of shellfish forbidden if levels of toxin are too high. Other elements of a control programme will include (FDA, 1998):

- a requirement that containers of in-shell molluscan shellfish bear a tag that identifies the type and quality of shellfish, harvester, harvest location and date of harvest
- a requirement that molluscan shellfish harvesters be licensed

- a requirement that processes that chuck molluscan shellfish or ship, repack the chucked product be certified
- a requirement that containers of chucked shellfish bear a label with the processor's name, address and certification number.

Depuration and ozonation are not effective and are not used in reducing toxins in shellfish (Anderson *et al.*, 2001).

5.2 Chemical hazards

There are no toxic chemicals, but there are toxic concentrations of all chemicals. Very few chemicals are present in high enough concentrations to pose a threat to human health. Mass toxication has occurred in connection with accidental exposure to high concentrations but in reality the risk of acute chemical intoxication is very low. However, long-term low level exposure to some chemical contaminants may be associated with serious diseases such as neurological damage, birth defects and cancer.

The chemical contaminants with some potential for toxicity are (Ahmed, 1991):

- Inorganic chemicals: arsenic, cadmium, lead, mercury, selenium, sulphites (used in shrimp processing)
- Organic compounds: polychlorinated biphenyls, dioxins, insecticides (chlorinated hydrocarbons). This is a very diverse group with a wide range of industrial users. Unfortunately the chemical stability allow them to accumulate and persist in the environment
- Processing related compounds: nitrosamines and contaminants related to aquaculture (antibiotics, hormones).

5.2.1 Industrial and environmental contaminants *(Hans Henrik Huss)*

A modest concentration of contaminants is ubiquitous in the clean aquatic environment. A few metals such as copper, selenium, iron and zinc are essential nutrients for fish and shellfish. Contamination occurs when there is a statistically significant increase in the mean levels in the comparable organisms.

Problems related to chemical contamination of the environment are nearly all man-made. The ocean dumping of hundreds of millions tons of material from industrial processing, sludge from sewage treatment plants, draining into the sea of chemicals used in agriculture and raw untreated sewage from large urban populations all participate in contaminating the coastal marine environments or freshwater environments. From here the chemicals find their way into fish and other aquatic organisms. Increasing amounts of chemicals may be found in predatory species as a result of biomagnification, which is the concentration of the chemicals in the higher levels of the food chain. Or they may be there as a result of bioaccumulation, when increasing concentrations of chemicals in the body tissues accumulated over the life span of the individual. In this case, a large (i.e. an older) fish will have a higher content of the chemical concerned than a small (younger) fish of the same species. The presence of chemical contaminants in seafood is therefore highly dependent on geographic location, species and fish size, feeding patterns, solubility of chemicals and their persistence in the environment.

In a review on chemical residue concerns in seafood, Price (1992) concluded that risk from chemical contaminants in commercially harvested fish and shellfish is low and not a problem. Risk from chemical residues (mercury, selenium, dioxins, PCPs, kepone, chlordane, dieldrine and DDT) are primarily a concern with recreational fish and shellfish, caught in coastal waters and (possibly) in highly polluted waters.

In a more recent review, Smith and Gangolli (2002) similarly concluded that organochlorine levels in fish intended for human consumption are low and probably below levels likely to adversely affect human health. However, they are of potential concern for two groups: populations for whom seafoods form a major part of the diet and infants and young children who consume substantial quantities of oily fish.

A large section of a committee report concerned with Seafood Safety in U.S. (Ahmed, 1991) has been devoted to occurrence of chemical contamination and related health risks. Some of the general conclusions and recommendations from this report are cited below:

- From both natural and human sources, a small proportion of seafood is contaminated with appreciable concentrations of potentially hazardous organic and inorganic chemicals. Some of the risks that may be significant include reproductive effects from PCBs and methylmercury, and carcinogenesis from selected PCB congeners, dioxins, and some chlorinated hydrocarbon pesticides
- Consumption of some types of contaminated seafood poses enough risk that efforts toward evaluation, education and control of that risk must be improved
- Present quantitative risk assessment procedures used by government agencies can and should be improved and extended to non-cancer effects
- Current monitoring and surveillance programs provide an inadequate representation of the presence of contaminants in edible portions of domestic and imported seafood, resulting in serious difficulties in assessing both risks and specific opportunities for control
- Because of the unevenness of contamination among species and geographic areas, it is feasible to narrowly target control efforts and still achieve meaningful reductions in exposures
- The data base for evaluating the safety of certain chemicals that find their way into seafood via aquaculture and processing is too weak to support a conclusion that these products are being effectively controlled.

The principal recommendations of the committee are as follows:

- Existing regulations to minimize chemical and biological contamination of the aquatic environment should be strengthened and enforced
- Existing FDA and state regulations should be strengthened and enforced to reduce the human consumption of aquatic organisms with relatively high contaminant levels (e.g. certain species from the Great Lakes with high levels of PCBs, swordfish and other species with high methylmercury levels)
- Federal agencies should actively support further research to determine the actual risks from the consumption of contaminants associated with seafood and to develop specific approaches for decreasing these risks
- Increased environmental monitoring should be initiated at the state level, as part of an overall federal exposure management system
- States should continue to be responsible for site closures, and for issuing health and contamination advisories tailored to be specific consumption habits, reproductive or other special risks, and information sources of specific groups of consumers
- There should be an expanded program of public education on specific chemical contaminant hazards via governmental agencies and the health professions.

The conclusions from the Committee report (Ahmed, 1991) are still valid (2002), although some of the recommendations have been put into practice. Environmental monitoring at state level is done by many countries, and government agencies are responsible for closures of harvesting areas and management of risks related to chemical contaminants. Most countries have laws and regulations defining the conditions for use of agrochemicals. Usually, a holding period is required between the use of such chemicals and harvest or slaughter (aquaculture). Maximum levels have been

established for a number of compounds. Examples are shown in Table 5.28. Results from a large number of surveys have shown, that residues of chemical contamination normally are lower than the limits shown in Table 5.28, and do not give rise to any concerns regarding health of the consumer.

A class of compounds made up of the polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF), collectively known as dioxins has recently received widespread attention.

Table 5.28 Environmental chemical contaminants. Tolerances and critical limits in fish and fish products (EC, 2001a; FDA, 1998).

	Maximum levels		
Substance	**US (ppm)**	**EU (mg/kg wet weight)**	**Food commodity**
Arsenic	76-86		molluscs, crustaceans
Cadmium	3-4	0.05-1.0	fish, molluscs
Lead	1.5-1.7	0.2-1.0	fish, molluscs
Methyl mercury	1.0	1.0	all fish
PCB	2.0		all fish
DDT, TDE	5.0		all fish
Diedrin	0.0		all fish
Dioxin		0.000004	

Dioxins are commonly formed when organic substances smoulder or burn in the presence of chlorine. This may happen in industrial operations within metallurgy, paper mills, chemical industries (the Seveso case) and others. Due to the high persistence of dioxins these compounds are relatively stable once released into the environment. Due to the chemical nature of dioxins, the compounds will accumulate in the fat deposits of fish and animals and amounts will increase in higher levels of the food chain. The WHO has recently re-evaluated the toxicity of dioxins and is recommending a Tolerable Daily Intake (TDI) of max. 1-4 picogram TEQ (toxic equivalent)/kg body weight. Examples of dioxin amounts in food are shown in Table 5.29.

Table 5.29 Dioxin amounts in common foods (Compilation of EU dioxin exposure and health data, October 1999)

Food commodity	**Picogram TEQ/g fat**	
	Min	**Max**
Milk products	0.5	3.8
Meat and meat products	0.1	16.7
Poultry	0.7	2.2
Fish	2.4	214.3
Eggs	1.2	4.6
Fat and oils	0.2	2.6
Bread and cereals	0.1	2.4

5.2.2 Veterinary drugs *(Allan Reilly)*

As aquaculture has developed, a range of fish and shellfish diseases have been encountered that have led to major economic losses and the failure of the industry in some parts of the world. This has led to the increased use of veterinary drugs and vaccines in intensive production systems to combat diseases in farmed fish. Antibiotics are commonly used in aquaculture worldwide to treat infections caused by a variety of bacterial pathogens of fish including *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Edwardsiella tarda*, *Pasteurella piscidida*, *Vibrio anguillarum*, *Vibrio salmonicida* and *Yersinia ruckeri*. They are commonly used as in-feed medications or surface coated onto feed pellets and dispersed in water. There is a wide range of antimicrobial agents

used in aquaculture (Alderman and Hastings, 1998; GESAMP, 1997; ACMSF, 1999). Where antibiotics are approved for use, specific doses and withdrawal periods are specified by manufactures. Since fish are poikilotherms, their metabolic rate is determined by environmental temperatures. Withdrawal periods are specified as degree days, for example, 10 days at 5°C equals 50 degree-days. Some of the antibiotics in common use are shown in Table 5.30.

The use of antibiotics in fish farming is associated with new hazards in fishery products that are not encountered in wild captured species. The main hazards are antibiotic residues and the development of antimicrobial resistance in bacteria that may be transferred to consumers of farmed fish.

5.2.2.1 Antibiotic residues

With the increased use of veterinary drugs in food production, there is global concern about the consumption of low levels of antimicrobial residues in aquatic foods and the effects of these residues on human health. This concern is not limited to only aquaculture products but to all foods of animal origin where the use of antibiotics has become an integral part of intensive animal husbandry.

The potential hazards associated with the presence of antimicrobial drug residues in edible tissues of products from aquaculture include allergies, toxic effects, changes in the colonisation patterns of human-gut flora and acquisition of drug resistance in pathogens in the human body (WHO, 1999).

Table 5.30 Examples of antibiotics used in aquaculture.

Group	Compound	Comments
Sulphonamides	Sulphamerazine Sulphaimidine Sulfadimethoxine[1]	Bacteriostatic agents with broad-spectrum activity against furunculosis in salmonids (trout and salmon).
Potentiated Sulphonamide	Co-trimazine/Sulfatrim[1,2,3] (combination of trimetho-prim and sulfadiazine)	Used for treating diseases in salmon and trout (furunculosis, vibriosis and enteric red mouth).
Tetracyclines	Chlortetracycline Oxytetracycline[1,2,3,4]	Wide use in aquaculture. Effective against several fish pathogens and is relatively cheap. Used in salmon, trout, turbot and shrimp farming. Approved for prevention of "red tail" in lobsters in Canada.
Penicillins (Beta-lactams)	Ampicillin[4] Amoxycillin[2,4] Benzyl penicillin[3]	Used to treat furunculosis in salmon and rainbow trout fry syndrome (RTFS) in Europe. Used for yellowtail and sea bream in Japan
Quinolones	Ciprofloxacin Enrofloxacin Norfloxacin Oxolinic acid[2,3,4] Perfloxacin Flumequine[3,4] Sarafloxacin[2]	Used in shrimp farms in Asia Used in shrimp farms in Asia Used in shrimp farms in Asia EU MRL 150ug/kg fish muscle
Nitrofurans	Furazolidone	Broad-spectrum antimicrobial agent. Used in shrimp farms in Asia. Use discouraged as it is a potential carcinogen.
Macrolides	Erthromycin[4] Spiramycin	
Aminoglycosides	Gentamycin	
Other antibiotics	Chloramphenicol Florfenicol[1,3,4] Thiamephenicol[4] Tiamulin Nalidixic acid Milozacin	Residues in foods may cause aplastic anaemia in man[5]. Use banned in the European Union. Used to treat RTFS and furunculosis in salmon.

1. *Use permitted in Canada (http://www.syndel.com/msds/canada_approved.htm)*
2. *Licensed for use in the UK (Alderman and Hastings 1998)*
3. *Use permitted in Norway (Alderman and Hastings 1998)*
4. *Use permitted in Japan (Okamoto 1992)*
5. *Tan (1999).*

Antibiotics are used in aquaculture as prophylactics, as growth promoters and in the treatment of diseases. Prophylactic use of antibiotics is defined as the administration of antibiotics in advance of disease occurrence and this is a common practice in shrimp hatcheries in Asia to reduce the incidence of diseases (GESAMP, 1997). A recent review (Graslund and Bengtsson, 2001) report the widespread prophylactic use of antibiotics in both shrimp hatcheries and in shrimp ponds in Southeast Asia. Antibiotics are usually administered in aquatic feeds and most commercial shrimp feeds contain antibiotics (Flaherty *et al.*, 2000). In contrast, antibiotics are not used either as

prophylactic agents or as growth promoters in temperate water aquaculture production in Europe and North America (Alderman and Hastings, 1998). In recent years the use of antibiotics has fallen dramatically in the farmed salmon industry in Norway form about 50 tonnes to less than one tonne annually (Figure 5.17). This is largely as a result of the successful development and use of vaccines against the principle fish pathogens (Alderman and Hastings, 1998).

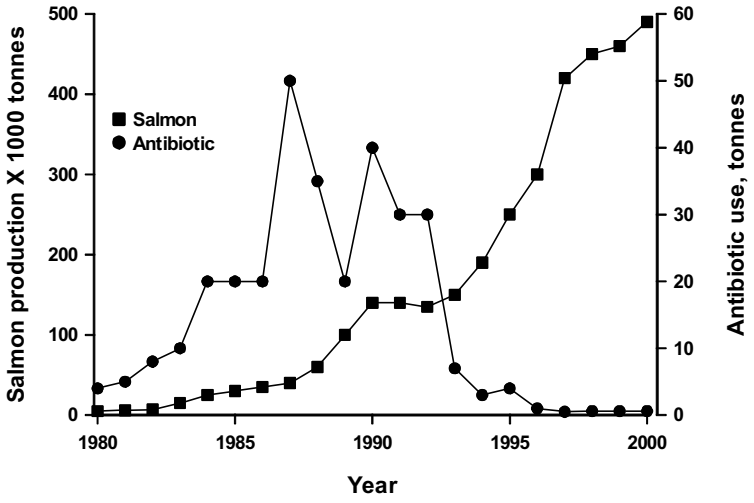


Figure 5.17
Increase in production of farmed salmon and decrease in use of antibiotics in Norway from 1984 to 2000 (modified from Buchmann and Larsen (2001)).

While vaccines have been developed for finfish, the same success story has not be true for farmed shrimp. Vaccines are of little use in shrimp culture because of the nature of the shrimp host defence system is such that no long term specific immune memory has been demonstrated to exist. Control over the sale and use of antibiotics in some shrimp producing countries is limited which has led to problems in overseas markets. The occurrence of antibiotic residues in cultured shrimp from Asia has led to the rejection of products in export markets (Saitanu *et al.,* 1994) and more recently, the European Union has introduced new legislation requiring the testing of all shipments of farmed shrimp from China, Vietnam and Indonesia for residues of chloramphenicol (EC, 2001b,c).

5.2.2.2 Antimicrobial resistance

There are a number of ways in which bacteria become resistant to antibiotics. When a population of sensitive bacteria is exposed to an effective antibiotic, the majority will be killed or their growth will be inhibited. However, within a population there may be a few relatively resistant organisms that are capable of survival and growth. These have a selective advantage over the sensitive organisms and are able to survive and grow. Bacteria develop resistance through random mutations in bacterial genes or they can acquire resistance from another bacterium. There are three ways in which genes can be transferred between cells:

- by transformation where naked DNA released by a bacterium is taken up and incorporated into the chromosome of the host cell
- by transduction, which involves a bacteriophage transferring DNA into the hosts' chromosome
- by conjugation, which involves direct transfer between cells and where genes are carried on plasmids or conjugative transposons.

Conjugation is thought to be the principal way in which transfer of antibiotic resistant genes occurs between bacteria. Large plasmids that encode resistance to several different antibiotics have been found in human pathogens such as *Salmonella* Typhimurium DT 104.

The emergence of antimicrobial resistance following the use of antimicrobial agents in aquaculture has been identified in fish pathogens. (WHO, 1999; Midvedt and Lingass, 1992). For instance,

plasmid-mediated resistance to antimicrobials have been identified in a number of bacterial fish pathogens including *Aeromonas salmonicida*, *A. hydrophila*, *Vibrio anguillarum*, *Pseudomonas fluorescens*, *Pasteurella piscicida*, *Edwardsiella tarda* (Aoki, 1988) and *Yersinia ruckeri* (DeGrandis and Stevenson, 1985). Transferable R-plasmids have been found in *A. salmonicida* encoding resistance to chloramphenicol, sulphonamide and streptomycin in Japan, and to combinations of sulphonamide, streptomycin, spectinomycin, trimethoprim and/or tetracycline in Ireland (Aoki, 1997). In Scotland, transferable R-plasmids were found in 11 out of 40 oxytetracycline-resistant *A. salmonicida* isolates (Inglis *et al*., 1993). Transferable resistance was detected to combinations of oxytetracycline, streptomycin, sulphamethoxine and/or trimethoprim. These are all examples of the emergence of antimicrobial resistance in fish pathogens following the use of antimicrobial agents in fish farming.

Use of antimicrobial agents in aquaculture also selects for resistance in bacteria in fish in the local environment and in sediments close to fish farms. Medicated feeds that are not eaten by fish fall to the bottom of ponds or through the bottom of cages. Additionally, some of the antimicrobials in medicated feed that is consumed by fish will be excreted in faeces into the local environment. Uneaten feeds may be consumed by other fish in the vicinity of a fish farm. Samuelson *et al.* (1992) reported residues of oxolinic acid in wild fish, crabs and mussels in the vicinity of a Norwegian fish farm up to 13 days post-treatment. A number of authors have reported oxytetracycline in sediments in the vicinity of salmon farms (Coyne *et al*., 1994; Bjorklund *et al*., 1991; Samuelson *et al*.,1992a).

Potential risks to consumer health exist in that antimicrobial resistance arising from the use of antibiotics in aquaculture can be transferred to human pathogens, such as *Vibrio parahaemolyticus* (Hayashi *et al*., 1982) and *Vibrio cholerae* (Nakjima *et al*., 1983). The strain of *Vibrio cholerae* O1 that caused the epidemic of cholera in South America in 1991 was multidrug-resistant and the epidemic in Ecuador began among persons working on shrimp farms (Weber *et al*., 1994). Similar multi-drug resistance was found in non-cholera *Vibrio* that were pathogenic to shrimp which may have been transferred to the *V. cholera* O1 (Weber *et al*., 1994). Bacteria on farmed fish and shrimp can be transmitted to humans when these are eaten or when such bacteria are transferred to food that are subsequently eaten. *Vibrio parahaemolyticus* is a common cause of food-borne illness in Japan and *Salmonella* species have been isolated from farmed fish and shrimp (Reilly and Twiddy, 1992). Other bacteria that are human pathogens, such as *Streptococcus iniae* and *Vibrio vulnificus,* have been associated with wound infections in fish handlers and can cause serious illness (Weinstein *et al*., 1997; Bisharat and Raz, 1996). Another route for the transfer of antimicrobial resistant bacteria to man is by ornamental fish. Multi-drug resistant strains of *Mycobacterium marinum* have been isolated from ornamental fish and are the cause of “fish tank granuloma” in man. There is also a potential for resistance development in bacteria in integrated fish/poultry/animal production systems in parts of Asia where the waste from animals is used to fertilize fish ponds. Antibiotics administered to poultry and animals are inadvertently dose the fish ponds in faeces and urine with subsequent selective pressure for resistance development.

5.2.2.3 Control Strategies

There can be little doubt that the use of antibiotics in aquaculture selects for antimicrobial resistance among bacteria in farmed fish and in the environment surrounding fish farms. It is well established that antibiotics given to animals have resulted in the emergence of some resistant germs that can infect humans via the food chain. Additionally, illegal residues have been reported in aquaculture products in export markets.

Antibiotics should never be used as an easy alternative to good fish farming practices. National governments need to put in place control programmes for residues of antimicrobials in aquaculture production. Such control programmes should control the approval or licensing of antimicrobials and should control their sale and use in fish farming. What is required at national level is up-to-date legislation and standards that are based on sound science, a monitoring programme and adequate resources for enforcement of the legislation.

Consumers can protect themselves against antibiotic resistant bacteria as these are just as susceptible to heat and hygiene as their non-resistant counterparts. Thorough cooking, frequent hand washing, prevention of cross-contamination by separating raw seafoods from other foods and proper chilled storage will minimise the incidence of seafood poisoning.

5.3 Physical Hazards (Hans Henrik Huss)

Physical hazards include any potentially harmful extraneous matter not normally found in food. The extraneous matter found in fish products can be divided or classified as:

- non-food safety hazards (e.g. filth)
- food safety hazards (e.g. glass, metal, wood, bones, stones, hard plastic).

The adverse health effect of physical hazards may be choking, injury incl. laceration and perforation of tissues in the mouth, throat, stomach or intestines. Broken teeth and damage to gums may also be the result. The FDA Health Hazard Board has found that foreign objects that are less than 7 mm maximum dimensions rarely cause trauma or serious injury except in special risk groups such as infants, elderly or surgery patients (FDA, 1998)

Although physical hazards rarely cause serious injury, they are among the most commonly reported consumer complaints, because the injury occurs immediately or soon after eating, and the source of the hazard is often easy to identify.

Control measures for physical hazards can include:

- for metal inclusions:
  periodically checking all equipment for damage or missing parts
  passing the product through metal detection or separation equipment
- for non-metallic objects:
  passing the product through an X-ray detector.

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Part II: Risk Management Tools

6 INTERNATIONAL REGULATORY FRAMEWORK FOR FISH SAFETY AND QUALITY (Lahsen Ababouch)

The increasing demand for fish and fishery products and the development in international fish trade have raised major concerns about the overexploitation of aquatic resources and the quality and safety of the products internationally traded. Globalisation of the economy and the development of regional economic groupings have highlighted the need for harmonizing fish safety and quality assurance approaches, with the view to ensure fish safety and fair trade practices.

Following is a description of the international regulatory framework for fish safety and quality assurance.

6.1 The World Trade Organization (WTO) agreement

The Final Act of the Uruguay Round of multilateral trade negotiations, which began in Punta del Este, Uruguay in September 1986 and concluded in Marrakesh, Morocco in April 1994, established the World Trade Organization (WTO) to succeed the General Agreement on Tariffs and Trade (GATT). The Uruguay Round negotiations were the first to deal with the liberalization of trade in agricultural products, an area excluded from previous rounds of negotiations.

Significant implications for food safety and quality arise from the Final Act of the Uruguay Round, especially from two binding agreements: the Agreement on the Application of Sanitary and Phytosanitary (SPS) Measures and the Agreement on Technical Barriers to Trade (TBT Agreement).

6.1.1 The agreement on the Application of Sanitary and Phytosanitary Measures (SPS)

The SPS agreement confirms the right of WTO member countries to apply measures necessary to protect human, animal and plant life and health. This right was included in the original 1947 GATT as a general exclusion from the provisions of the agreement provided that “such measures are not applied in a manner which would constitute a means of arbitrary or unjustifiable discrimination between countries where the same conditions prevail, or a disguised restriction on international trade”. Despite this general condition for the application of national measures to protect human, animal and plant life and health, it had become, whether by design or accident, effective trade barriers.

The purpose of the SPS Agreement is to ensure that measures established by governments to protect human, animal and plant life and health, in the agricultural sector, including fisheries, are consistent with obligations prohibiting arbitrary or unjustifiable discrimination on trade between countries where the same conditions prevail and are not disguised restrictions on international trade. It requires that, with regard to food safety measures, WTO members base their national measures on international standards, guidelines and other recommendations adopted by the *Codex Alimentarius* Commission (CAC) where they exist. This does not prevent a member country from adopting stricter measures if there is a scientific justification for doing so, or if the level of protection afforded by the *Codex* standards is inconsistent with the level of protection generally applied and deemed appropriate by the country concerned.

The SPS Agreement states that any measures taken that conform to international Codex standards, guidelines or recommendations are deemed to be appropriate, necessary and not discriminatory. Furthermore, the SPS Agreement calls for a programme of harmonization based on international standards. This work is guided by the WTO Committee on SPS measures, to which representatives of the CAC, the International Office of Epizootics (OIE) which deals with animal (including fish) health, and the International Plant Protection Convention (IPPC) which deals with plant protection are invited.

Finally, the SPS Agreement requires that SPS measures are to be based on an assessment of the risks to humans, animal and plant life and health using internationally accepted risk assessment techniques. Risk assessment should take into account the available scientific evidence, the relevant processes and production methods, the inspection/sampling/testing methods, the prevalence of specific illnesses and other matters of relevance.

6.1.2 The agreement on Technical Barriers to Trade (TBT)

The Agreement on TBT is a revision of the agreement of the same name first developed under the Tokyo round of negotiations (1973 – 1979). The objective of the TBT Agreement is to prevent the use of national or regional technical requirements, or standards in general, as unjustified technical barriers to trade. The agreement covers standards relating to all types of products including industrial products and quality requirements for foods (except requirements related to SPS measures). It includes numerous measures designed to protect the consumer against deception and economic fraud.

The TBT Agreement basically provides that all technical standards and regulations must have a legitimate purpose and that the impact or cost of implementing the standard must be proportional to the purpose of the standard. It also states that if there are two or more ways of achieving the same objective, the least trade restrictive alternative should be followed. The agreement also places emphasis on international standards, WTO members being obliged to use international standards or parts of them except where the international standard would be ineffective or inappropriate in the national situation.

Both the SPS and TBT Agreements call on Member countries to facilitate the provision of technical assistance, especially to developing countries, either bilaterally or through the appropriate international organizations. Also, special and differential treatment provisions call for the consideration of the needs of developing countries and especially the least developed countries when preparing and implementing SPS and quality measures. Such consideration include providing longer time frames for compliance on products of interest to developing countries

The aspects of food standards that TBT requirements cover specifically are quality provisions, nutritional requirements, labelling, packaging and product content regulations, and methods of analysis. Unlike the SPS Agreement, the TBT Agreement does not specifically name international standard setting bodies, whose standards are to be used as benchmarks for judging compliance with the provisions of the Agreement.

6.2 The Food and Agriculture Organization of the United Nations (FAO)

6.2.1 Codex Alimentarius

Since 1962, the *Codex Alimentarius* Commission (CAC) has been responsible for implementing the Joint FAO/WHO Food Standards Programme. The Commission's primary objectives are the protection of the health of consumers, the assurance of fair practices in food trade and the coordination of the work on food standards.

The CAC is an intergovernmental body with a membership of 165 Member governments. In addition, observers from international scientific organizations, food industry, food trade and consumer associations may attend sessions of the Commission and of its subsidiary bodies. An Executive Committee, six Regional Coordinating Committees and a Secretariat assist the Commission in administering its work programme and other activities.

The work of the *Codex Alimentarius* is divided between two basic types of committees:

- nine general subject matter(s) Committees that deal with general principles, hygiene, veterinary drugs, pesticides, food additives, labelling, methods of analysis, nutrition and import/export inspection and certification systems and

- 12 Commodity Committees which deal with a specific type of food class or group, such as dairy and dairy products, fats and oils, or fish and fish products.

The work of the Committees on hygiene, fish and fishery products, veterinary drugs and import/export inspection and certification systems are of paramount interest to the safety and quality of internationally traded fish and fishery products.

In the environment of the Uruguay round agreements, the work of the CAC has taken on unprecedented importance with respect to consumer protection and international food trade. The specific Codex food safety provisions, which are recognized by the SPS Agreement, include the maximum residue limits for pesticides and veterinary drugs, the maximum level of use of food additives, the maximum levels of contaminants, and food hygiene requirements of Codex standards.

In the specific area of Food Hygiene, the CAC has revised its main document on food hygiene (CAC 2001) to incorporate risk assessment principles and to include specific references to the Hazard Analysis and Critical Control Point (HACCP) System.

The *Codex* standards are meant to be voluntary and adopted by consensus. But under the new SPS/TBT agreements, the *Codex* standards can not be called voluntary, nor are they fully mandatory, falling in an area in between which looks like voluntarism under duress. This is changing the *Codex* deliberations into a highly charged political exercise, because countries know that the standards they are debating might subsequently be the subject of WTO dispute settlement, and act therefore accordingly.

Another major issue increasingly faced by the *Codex* is the critical problem of scientific uncertainty. It can only operate on the hypothesis that best fits the facts available at any given time. To deal with the uncertainty, some countries advocate the precautionary principle: "*Where there are threats, lack of full scientific certainty should not be used as a reason for postponing cost-effective measures to prevent the damage*". However, any precautionary measure taken should be accompanied by a search for greater scientific certainty, and periodic evaluation of the measures in light of new evidence.

6.2.2 The FAO Code of conduct for responsible fisheries

During the recent decades, world fisheries have become a market-driven, dynamically developing sector of the food industry and coastal States have striven to take advantage of their new opportunities by investing in modern fishing fleets and processing factories in response to growing international demand for fish and fishery products. By the late 1980s it became clear, however, that fisheries resources could no longer sustain such rapid and often uncontrolled exploitation and development, and new approaches to fisheries management embracing conservation and environmental considerations were urgently needed.

The FAO Committee on Fisheries (COFI) at its Nineteenth Session in March 1991 called for the development of new concepts which would lead to responsible, sustained fisheries. Subsequently, the International Conference on Responsible Fishing, held in 1992 in Cancûn (Mexico) further requested FAO to prepare an international Code of Conduct to address these concerns. The outcome of this Conference, particularly the Declaration of Cancûn, was an important contribution to the 1992 United Nations Conference on Environment and Development (UNCED), in particular its Agenda 21.

Noting these and other important developments in world fisheries, the FAO Governing Bodies recommended the formulation of a global Code of Conduct for Responsible Fisheries which would be consistent with these instruments and, in a non-mandatory manner, establish principles and standards applicable to the conservation, management and development of all fisheries. The Code, which was unanimously adopted on 31 October 1995 by the 28th Session of the FAO Conference, provides a necessary framework for national and international efforts to ensure

sustainable exploitation of aquatic living resources in harmony with the environment (FAO, 1995). Article 6 (General principles, provisions 6.7 and 6.140) and article 11 (Post-harvest practices and trade) are of particular relevance to fish trade, safety and quality. Provisions 11.1.2, 11.1.3 and 11.1.4 encourage States to establish and maintain effective national safety and quality assurance systems, to promote the implementation of the CAC standards and codes of practice and cooperate to achieve harmonization or mutual recognition, or both, of national sanitary measures and certification programmes.

The same 28th FAO Conference requested the elaboration of technical guidelines in support of the implementation of the Code of Conduct in collaboration with member states and relevant organizations. Volume No 7 provides technical guidelines for responsible fish utilization (FAO, 1998).

6.3 Conclusion

The globalization and further liberalization of world fish trade, while offering several benefits and opportunities, also presents new safety and quality challenges. Fish safety and quality assurance in the new millennium will require enhanced levels of international co-operation in setting up standards and regulations. The SPS/TBT agreements of the WTO and the benchmarking role of the Codex provide an international platform in this respect. Consequently, the major fish producing, exporting or importing countries have launched in the early 90's an overhaul of fish inspection regulations to set up the foundations for the implementation of the HACCP-based quality and safety systems, in conformity with the guidelines of the CAC. Regulations enacted by the EU (EC, 1991, 1994, 2000) and the USA (FDA, 1997) have set up the pace and the trend for many other countries, especially the major commercial partners of the EU or the USA, highlighting the need for better harmonization and recognition schemes. More recently, several countries have initiated national works on microbiological risk assessments. But several gaps and differences subsist. These differences entail questions such as:

- Should HACCP address fish safety (USA) only or safety and spoilage (European Union)?
- Where does the clear demarcation between GHP/GMP and HACCP lie?
- Should the control authority assist the industry in developing HACCP programs or should it confine its role to assessment and verification?
- Is HACCP always needed regardless of the product and process?
- In international trade, who should be responsible for verification of HACCP implementation?. Is it the importer, the exporter, the control authority of the importing country, of the exporting authority, a third party?
- How do we reconcile between the precautionary principle and science-based risk assessments
- How can we achieve common understanding of equivalency and of recognition/equivalency schemes?
- Why is the progressive implementation of HACCP not leading to a gradual decrease in end-product sampling and inspection?
- Is it realistic to expect a global harmonization of microbiological standards for fish and fishery products? Even at the European Union level, only one microbiological standard has been developed in 1993 for cooked crustaceans and shellfish. For all the other fish products, different national microbiological standards are applied.

On many of these issues, developing countries are at a disadvantage because of insufficient/inadequate national capacities and resources.

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