2. BIOLOGICAL HAZARDS IN SEAFOOD

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For the purpose of HACCP, hazards only refers to the conditions or contaminations in food that can cause illness or injury to the consumers. Hazards in seafood can be classified into three groups.

I. Biological hazards
II. Chemical hazards
III. Physical hazards

Biological hazards

Biological hazards include pathogenic bacteria, fungi, viruses and parasites when present in food can result in infection or toxicity. These hazards can come from raw materials or from food processing steps.

A. Bacterial hazards

Pathogenic bacteria can cause illness in human, either by infection or intoxication. Food borne infections are caused by swallowing live pathogens that grow within the body, usually in the intestinal tract. Intoxication is a condition caused by swallowing preformed toxins i.e. toxins produced by microorganisms in the food before it is eaten. Most of the pathogenic bacteria are not present in fish caught from off-shore waters, but contamination occurs during handling of the material. If the time and temperature conditions are favourable, these organisms get an opportunity to grow and multiply at a faster rate. Consumption of such fish is dangerous and it will lead to food poisoning.

Bacteria of public health significance in fish and fish products

1. *Escherichia coli (E. coli)*
2. Faecal *Streptococci*
3. *Staphylococcus aureus*
4. *Salmonella* spp.
5. *Shigella* spp.
6. *Vibrio cholerae*
7. *V. parahaemolyticus*
8. *V. vulnificus*

9. *Listeria monocytogenes*
10. *Campylobacter jejuni*
11. *Bacillus cereus*
12. *Clostridium botulinum*
13. *Clostridium perfringens*
14. *Aeromonas* spp.
15. *Plesiomonas shigelloides*

1. *Escherichia coli (E. coli)*

E. coli and the coliforms are Gram-negative, rod-shaped, non-spore forming facultatively anaerobic bacteria.

Primary habitat and source of contamination

The primary habitat of the organism is intestinal tract of man and animals. One gram of faeces contains 10^7 to 10^9 *E. coli.* Hence its presence in food is generally considered as an indication of faecal contamination. Natural water get contaminated with *E. coli* either by direct contact with faeces or by mixing up with sewage. This water, when used for seafood processing, contaminates the product. Similar possibilities arise when the ice used for preservation or the utensils used for processing are contaminated with *E. coli.*

Off-shore water is generally free from *E. coli*, but its incidence is noted in coastal waters. Fishing in these waters or washing the boat deck and fish containers using coastal water or harbour waters are known to be the major source of contamination with *E. coli.* Inadequately cleaned boat deck and containers used onboard trawlers also act as source of contamination. If the temperature is favourable, the contaminating organism multiplies rapidly and further aggravates the situation.

Generally this organism is harmless: Pathogenic strains of *E.coli* are considered to be harmful.

Enterotoxigenic *E.coli* (ETEC)	-	Gastroenteritis
Enteropathogenic *E.coli* (EPEC)	-	Infant diarrhoea
Enteroinvasive *E.coli* (EIEC)	-	Bacillary dysentery
Enterohemorrhagic *E.coli* (EHEC)	-	Hemorrhagic colitis (*E.coli* 0157:H7)
Enteroadherent *E.coli* (EAEC)	-	Newly added category

Effect of freezing and frozen storage

E.coli is very sensitive to freezing and frozen storage. By freezing 95% of the organism can be eliminated. Complete destruction is possible on frozen storage (-18°C) for about 3 months.

Preventive measures

a. Maintenance of proper hygiene and sanitation
b. Chlorination of process water
c. Adoption of scientific system or cleaning and disinfection
d. Avoid fishing from near-shore waters
e. Avoid washing the catch with coastal/harbour waters
f. Avoid delay in processing
g. Reduce the temperature of the material at every stage ie. <4°C
h. Strict personal hygiene

Allowable limit - 0 - 20/g

2. Faecal *Streptococci*

Faecal streptococci are Gram-positive, facultative anaerobic, non-spore forming non-motile and catalase negative cocci.

Primary habitat and source of contamination are same as in the case of *E.coli*. One gram of faeces contains 10^5 to 10^8 *faecal streptococci,* therefore their presence in food product is generally regarded as an indication of faecal contamination. Just like *E.coli* faecal streptococci are absent in off-shore water but are present in considerable numbers in coastal waters. Unclean boat deck, utensils, water and ice are the major source of contamination.

Effect of freezing and frozen storage

Faecal streptococci are comparatively resistant to many adverse conditions. About 30% reduction of faecal streptococci takes place during freezing at -40°C, during subsequent storage at -18°C not much of reduction in count takes place even after 2 years of storage. This organism is considered as a better indicator or sanitation than *E.coli,* because of their resistance to sub-zero temperature.

Symptoms: causes gastrointestinal and respiratory diseases.

Preventive measures: similar to *E.coli*

Allowable limit - 100/g.

3. Coagulase – positive *Staphylococci (Staphylococcus aureus)*

Since 1930, it is known that contamination of food with coagulase – positive staphylococci could cause food poisoning, as the organism growing in food materials in considerable numbers, secretes exotoxin.

Habitat and source of contamination

The primary habitat of *Staphylococcus aureus* is man. This organism is present in sweat, ear gum, tears, throat, ulcers, boils and nasal cavities. It is known that 50% of human beings are carriers of this organism. Investigations carried out by CIFT have shown that 45% of seafood handlers in the processing units at Cochin are carriers of *Staphylococcus aureus.*

Fish caught from the open sea doesn't contain *Staphylococcus aureus.* When the material is taken onboard and handled by workers, contamination takes place. So its presence in seafood indicates lapse in maintaining personal hygiene.

Toxin production

Few Staphylococci per gram of food material may be harmless, but food poisoning outbreak may occur if the product is handled carelessly during processing, so as to allow multiplication of the organism in dangerous proportions. The organism can multiply vigorously and produce toxin at temperature near about room temperatures i.e. 30-37°C. Therefore refrigeration of the material during handling and processing is highly essential in preventing further multiplication and toxin production. The toxin once formed will not be destroyed even at 100°C even though the organisms are killed at this temperature. It is thus evident that, once sufficient quantity of toxin is formed in food material before its consumption, food poisoning can follow even though the food material is cooked. Foods most likely to be involved in staphylococcal food poisoning are cooked and processed foods having low number of competing micro-organisms. Large number of *Staphylococcus aureus,* usually more than one million organism/g of food material must be present at one time to produce enough enterotoxin. Less than one microgram of toxin is sufficient to cause illness in a sensitive individual. Staphylococcal food poisoning is caused only by certain well defined strains of *S. aureus*; such strains are known as enterotoxigenic strains. Food-borne out breaks due to coagulase-negative strains of *Staphylococci* are seldom reported. *S. aureus* are known to produce 9 different types of enterotoxins designated as enterotoxin A, B, C1, C2, D, E, F, G and H. This is the most drought resistant pathogenic bacteria and they cannot compete with general bacterial flora.

Disease symptoms

Nausea, vomiting, abdominal pain, diarrhoea and subnormal blood pressure are some of the usual symptoms. It may appear within 1 - 4 h of consumption of contaminated food. Complete cure is possible within 48h.

Effect of freezing and frozen storage

The studies have shown that only 5 - 15% of *S. aureus* cells inoculated to cooked shrimps are killed during freezing at - 40°C. However, during frozen storage at -18°C, there is gradual reduction and in 7 months the viability is completely lost.

Preventive measures

a. Adequate control over the health and hygiene of fish handlers
b. Refrigeration (below 4°C) of the material during handling and processing
c. Minimise time/temperature abuse of seafood, especially after cooking.

Allowable limit - 100/g.

4. *Salmonella* spp.

Salmonella are enteric organisms producing enteric fever and food borne gastroenteritis. More than 2500 serotypes of this organism are known to exist at present and more are added to the list every year. Food poisoning due to salmonella is know as "Salmonellosis" infants, elderly and the under nourished are more susceptible to the disease and in such individuals salmonellosis is known to occur even from one single cell of Salmonella.

Salmonella are non-spore forming, mostly motile (exception *S. pullorum* and *S.* gallinarum) facultative – anaerobic, Gram-negative rods.

Primary habitat

Infected gut of man and animals, especially warm blooded animals, insects, lizards etc.

Source of contamination

a. Fishing from polluted waters
b. Washing the catch with polluted coastal/harbour waters
c. Sorting the catch on contaminated sea beaches or contaminated onboard deck, utensils
d. Use of contaminated water for processing
e. Handling the material by workers who are carriers of Salmonella
f. Improper personal hygiene
g. Entry of flies, rodents and wall lizards in the processing hall

Effect of freezing and frozen storage

All serotypes of salmonella can survive freezing at -40°C and also survive for months together at frozen condition (-18°C) even upto 9 months depending upon the serotype and initial load.

Symptoms

Symptoms start within 12-24 h of consuming infected food. Usual symptoms are nausea, vomiting abdominal pain, restlessness, diarrhoea etc. that last for 2-3 days. *S. typhi* cause typhoid and *S.* paratyphi cause paratyphoid,

Incidence of Salmonella in seafood

Freshly caught marine fish are usually free from Salmonella. However fish from polluted coastal waters are usually contaminated with this organism.

Preventive measures

a. Avoid fishing from polluted waters
b. Avoid washing with coastal/harbour waters
c. Avoid sorting the catch on sea beaches
d. Use only chlorinated water for processing and for ice manufacture
e. Periodical medical check-up for workers
f. All contact surfaces/utensils meant for processing need be cleaned and disinfected before and after use.
g. Avoid the workers who are suffering from diarrhoea/vomiting
h. Before starting the work, all fish handlers may wash and disinfect their hands
i. Entry of rodents, wall lizards, flies etc., to the processing hall may be avoided

Allowable limit - should be absent.

5. Shigella spp.

Not indigenous in foods, the disease caused by *Shigella* is generally known as 'shigellosis', transmitted through food or water contaminated with human excreta.

Shigella are Gram-negative, facultatively anaerobic, non-sporulating, non-motile, rod shaped bacteria. They are the most difficult enteric pathogens to isolate.

Man is the only known natural host for *Shigella*. The organisms pass the acid barrier of the intestine, multiply in the gut and produce ulceration of large intestine followed by dysentery. The transmission of the organism from one individual to another is by the following means.

a. Through contaminated food, water, ice etc.
b. Through contaminated contact surface
c. Through flies
d. Through food handlers who are carriers of this organism

Symptoms

Abdominal pain, fever, watery diarrhoea, blood and mucus

Four serological groups i.e. A, B, C, and D

A. *Shigella dysenteriae*
B. *S. flexneri*
C. *S. boydi*
D. *S. sonnei*

S. dysenteriae causes the most severe illness

They survive longest when food holding temperatures are 25°C or lower. This organism has not, so far, been isolated from frozen seafood of the expert trade.

Preventive measures

a. Strict maintenance of workers personal hygiene
b. Good sanitary and handling practice
c. Avoid time/temperature abuse
d. Identify and avoid carriers
e. Prevent entry of flies
f. Use properly chlorinated water for processing

6. Vibrio cholerae

It is the causative agent of cholera. The current definition of *V. cholerae* consists of the classical (non-hemolytic) and El Tor (hemolytic) biovars. The El Tor *vibrios* are generally more infectious than the classical *V. cholerae* serotypes and it can survive longer in the environment.

Serotypes

***V. cholerae* 01** Ogawa, Inaba and Hikojima

Interest has now shifted to those strains which are biochemically identical to *V. cholerae* 01, but don't posses the 01 somatic antigens. These strains are now collectively known as non-01 *V. cholerae*, previously known as non-agglutinable vibrios (NAG'S) or non-cholerae vibrios (NCVs). Non-01 *V. cholerae* produce mild diarrhoea.

V. cholerae 0139 Bengal

This organism appears to be a hybrid of the 01 strain and the non-01 strain. The synonym, "Bengal" is in recognition of the origin of this strain. Unlike *V. cholerae* 01, this organism produces a polysaccharide capsule.

V. cholerae are Gram-negative, coma shaped aerobic, non-spore forming, motile rods.

Habitat

The only natural habitat of *V. cholerae* is man.

Source of contamination

Contamination occurs through food, water, flies and contaminated hands.

Symptoms

Nausea, vomiting, profuse diarrhoea, etc., develop after an incubation period of 1 - 4 days. The stool resembles 'rice water' and contains mucus, epithelical cells and a large number of *Vibrios.*

Preventive measures

a. Proper disinfection of contact surface.
b. Avoid cross contamination of cooked products
c. Strict personal hygiene of seafood handlers.

Allowable limit - should be absent.

7. *Vibrio parahaemolyticus*

V. parahaemolyticus is a marine pathogen present in marine and brackish-water environments. It can cause food poisoning when it is consumed in large numbers (more than 10^5/g of Kanagawa-positive strains), along with food materials. This type of food poisoning is more in countries like Japan, where there is a habit of eating un-cooked seafood. In recent years, the incidence of *V. parahaemolyticus* infection has been increasing in many parts of the world, and this has been attributed to the emergence of a new clone of the 03:K6 serotype carrying only the *tdh* gene. The onset of symptoms is within 12 h of eating infected food.

They are Gram-negative, rod shaped bacteria which are non-sporulating, halophilic, motile, and oxidase-positive.

Symptoms

Abdominal pain, vomiting, diarrhoea, and fever are the usual symptoms.

Effect of freezing and frozen storage

99% of the organisms are destroyed during freezing at - 40°C and during storage at - 18°C complete destruction takes place within few days.

The generation time of these organisms is only 7-10 minutes; two types of *V. parahaemolyticus* are known to exist.

Kanagawa - positive (Pathogenic)

Kanagawa-negative (Non-pathogenic)

Isolates from clinical sources are Kanagawa- positive whereas those from marine sources are Kanagawa - negative.

Icing the material immediately after catch, washing with potable water and improvement of hygiene are considered as remedial measures.

8. *Vibrio vulnificus*

It is an emerging pathogen, phenotypically similar to *V. parahaemolyticus.*

And is the etiological agent for 3 syndromes.

a. primary septicemia b. skin infections c. acute diarrhoea

Entry through two portals

a. Ingestion of raw sea foods

b. Exposure of skin lesions

Mortality is up to 60%. It is the part of the normal bacterial flora of estuarine and marine waters.

V. vulnificus is Gram-negative, halophilic, lactose-positive, rod shaped bacteria.

All strains are pathogenic; infection dose is not known! Infection is associated with the consumption of raw seafood particularly oysters. Ten minutes treatment at 50°C proved adequate to reduce *V. vulnificus* to a non detectable level. Icing is very effective to reduce the load of the organism. This organism is closely associated with oyster tissues and is not removed fully by controlled purification methods such as UV light assisted depuration

No effective means commercially exist for elimination of the health hazard in oyster intended for raw consumption and so, it is advised to avoid raw seafood completely.

9. *Listeria monocytogenes*

It is generally accepted as a food borne pathogen. Outbreak of disease is very rare but considered to be very serious due to high rate of mortality.

This organism is Gram-positive, micro-aerophilic, non-spore forming, motile rods. It can survive freezing and thawing; if the load is more than $5x10^4$ /ml. in milk it can withstand pasteurization. *L. monocytogenes* grows in refrigerated temperatures (even 1° C) and it can survive both acidic and alkaline pH. This is the most heat resistant pathogenic bacteria among non-spore formers.

Habitat

L. monocytogenes is widely distributed in nature. A variety of animals can serve as hosts for this organism. The bacterium is often associated with the intestinal tract of domestic animals, birds and humans. About 1% of human population is known to carry *L. monocytogenes.*

Incidence in foods

Studies have indicated that *L. monocytogenes* is present in various kinds of foods. Dairy products are top in the list. Once present in the food the organism can successfully compete with other microflora and grow. 57% incidence in fresh and frozen poultry meat has been reported. Very little is known about the incidence of this organism in fish products. U.S.F.D.A. has reported the isolation of *L. monocytogenes* from 15 out of 57 seafood samples tested. The samples include raw shrimps, cooked shrimps, cooked crab meat, lobster tail etc.

Symptoms

"Listeriosis" is the general name of the disease caused by this organism. Mortality rate is as high as 30%, but in untreated cases, it may go up to 70%. In some cases the infection produces only mild flu-like symptoms, but in certain other cases it can be even a threat to life particularly in pregnant women, new-borns and adults with compromised immune system.

Allowable limit - As per USFDA should be absent.

10. *Campylobacter jejuni*

Campylobacter jejuni is widely distributed in the intestinal tract of' poultry, live-stock and warm blooded domestic animals. They are very small, Gram-negative, microaerophilic, curved thin rods with corkscrew motility.

Source of contamination

Contaminated food including raw clams, mussels and oysters, person to person contact, cross contamination of food by dirty food contact surface etc. Incubation period is 3-5 days.

Symptoms

Profuse diarrhoea, abdominal pain, headache and fever and meningitis in neonates. Infective dose ranges from 500 to 10,000 cells. This organism survives refrigeration and freezing.

11. *Bacillus cereus*

Food poisoning due to this organism may occur when foods are prepared and held without adequate refrigeration for several hours before serving. *B. cereus* is an aerobic spore forming bacteria that is commonly found in soil, on vegetables and many raw and processed foods. Consumption of food that contains 10^6 *B.* cereus/g may result in food poisoning. Two types of illness have been attributed

a) Abdominal pain, diarrhoea with an incubation period of 4 - 16 h and symptom last 12 - 24 hours

b) Acute attack of nausea and vomiting, occurs with in 1-5 h after ingestion

12. *Clostridium botulinum*

Clostridium botulinum is a dangerous food poisoning organism and it produce a very deadly, exotoxin when grows in food. The food poisoning is known as 'botulism".

It is an anaerobic, Gram-positive, spore-forming rod. The spores are highly heat resistant. Eight different toxins i.e. A, B, C_1, C_2, D, E, F & G known to exist. Type- E is present in sea mud and is mostly involved in botulism food poisoning in seafood. Food poisoning is due to the ingestion of toxin.

Symptoms develop within 12-24 h of consuming infected food. Nausea, vomiting, fatigue, headache, paralysis, difficulty to talk, double vision and sound in the ear are the usual symptoms. Death occurs due to respiratory failure. Mortality rate is high (30 - 65%). Botulism is the problem in home canned foods or canned foods that are improperly sterilized. Proper processing under hygienic conditions and adequate retorting are suggested as remedy.

13 .*Clostridium perfringens*

Food poisoning due to this organism may occur when foods such as meat or poultry are cooked and held without adequate heating or refrigeration before serving. Illness typically occurs 8-15h after ingestion of the contaminated food. The symptoms are intense abdominal

cramps, gas and diarrhoea due to the production of toxin during the spore forming of this organism in the intestine. C. *perfringens* cells lose their viability when foods are frozen or held under prolonged refrigeration.

14. *Aeromonas* spp.

The genera *Aeromonas* comprise Gram-negative, facultatively anaerobic, oxidase-positive, glucose-fermenting rod-shaped bacteria, generally motile. *Aeromonas* species viz. *A. hydrophila, A. sorbia* and *A. caviae* has been described as emerging food-borne pathogens. Besides gastroenteritis *A. hydrophila* may cause cholera like infections. *Aeromonas* spp. are natural members of aquatic environments and is commonly found in fish and fish products of all aquatic environments. *A. hydrophila* is very resistant organism and it can survive in food items stored in cold for long period. Oysters have been implicated in food-borne disease. *Aeromonas* associated diarrhoea has been reported from different parts of India. Some *Aeromonas* spp. are psychrotrophs and some others are enteropathogenic. Studies have shown that very high percentage of the isolates from seafood produced hemolysin (79.2%) and cytotoxin (91.7%). Psychrotrophic *Aeromonas* strains are able to grow at 4-5°C and produce toxin in oysters at 5°C. Combination of chilling, salting and/or acidification is effective means of preventing the growth of *Aeromonas.*

15. *Plesiomonas shigelloides*

The genera *Plesiomonas* comprise Gram-negative, facultatively anaerobic, oxidase positive, glucose fermenting, rod shaped bacteria, generally motile. It is an emerging pathogen, mostly associated with fresh water and seawater in warm months. This organism is predominantly associated with seafood. *P. shigelloides* was implicated as the causative agent for diarrhoea after consumption of seafood in Hong Kong and USA. It cannot grow at chilled condition, but can survive. Growth can be prevented by chilling, moderate salting/acidification.

B. Fungal hazards

The fungi associated with foods are generally yeasts and moulds. The greatest concern for food safety are mycotoxins eg. aflatoxin, fusarin, patulin, etc. which are produced by moulds and may be associated with chronic illness, such as cancer. Fungi needs lesser moisture for growth compared to bacteria. If the water activity (a_w) is less than 0.60 there will not be any growth of fungi or other microorganisms. Water activity of biscuits is 0.30 and sugar is 0.10.

C. Viral hazards

Viruses contaminate the foods same way as bacteria. It reproduces only within susceptible living cells. A ready to eat food containing a pathogenic virus is a health hazard. Viruses don't reproduce in food; it exists in foods without growing, so they need no food, water or air to survive. Viruses don't cause spoilage but may cause illness. It can survive in human

intestine, water, frozen foods etc. for months. Viruses can be found in people who were previously ill. Adequate cooking can destroy it.

Viral pathogens

1. Hepatitis A virus

This virus can survive in seawater and sediments for long periods (over a year). It can also survive better in lower temperature and killed in higher temperature.

Symptoms

Weakness, fever, abdominal pain and the patient gradually get jaundiced. Thorough cooking is the preventive measure.

2. *Norwalk virus*

Considered to be the major cause of nonbacterial intestinal illness (gastroenteritis). Associated with eating raw and steamed clams or oysters etc. cause nausea, vomiting, diarrhoea, abdominal cramps and fever. This hazard can be prevented by thorough cooking and preventing cross contamination of cooked foods.

D. Parasitic Hazards

Parasites are organisms that need 'a host to survive, living on or within it. Human unknowingly consume microscopic and small macroscopic animals with their food. The intestinal tract is inhospitable to most of these organisms, which are either digested or evacuated in the faeces. There are two types of parasites that can infect people through food or water

(a) parasitic worms
(b) protozoan parasites.

Parasitic worms include

1. Round worms (nematodes)
2. Tapeworms (cestodes)
3. Flukes (trematodes)

For most food borne parasites, the food is part of their life cycle. Some parasites may be transmitted through food or water that is contaminated by faecal material shed by infected host.

a. Parasitic Worms

1. *Anisakis simplex*

A nematode commonly called as herring worms, its final hosts are dolphins, porpoises and sperm whales. Larval stages are in fish and squid. Parasites in fish are considered to be a hazard only in case the fish is eaten raw or undercooked. "Anisakiasis" is the human illness caused by this parasite. In other products parasites are considered as filth but not hazard.

2. *Pseudoterranova decipiens* .

Another parasitic nematode commonly called as cod worm or seal worm. The disease caused by this parasite is also known as "Anisakiasis". The transmission and control of this organism is same as that of *Anisakis simplex.*

3. Diphyllobothrium latum

This parasitic cestode primarily infects freshwater fish. This tapeworm can mature and cause disease in human beings. These worms are transmitted to humans through raw or undercooked fish. Proper cooking can destroy it.

4. *Clonorchi sinensis, Metagonimus yokagawai and Heterophyes heterophyes*

These are members of a large group of trematode parasites with common characteristic of being acquired through eating raw or insufficiently cooked fish. *C. sinensis* can cause inflammatory and fibrotic changes in the bile duct, gall bladder and liver. *M. yokagawai* and H. *heterophyes* are very small flukes (1-2.5 mm in length). They burrow into the mucosa of the small intestine and produce irritation, inflammation, mucus formation and symptoms of diarrhoea and abdominal pain.

b. Protozoan Parasites

These are single celled animals.

1. ***Entamoeba histolytica*** can cause severe disease as classical amoebic dysentery which may be fatal if the parasites invade extra-intestinal tissues, such as liver, lungs, or brain.
2. ***Giardia lamblia*** associated with diarrhoea, constipation and gastrointestinal pains, rarely invade the tissues.
3. ***Toxoplasma gondii:*** Illness caused is "Toxoplasmosis", symptoms are hydrocephalus, blindness in children. Less severe in adults. Source of infection under cooked or raw meats i.e. pork, lamb, beef, poultry & cats. Intermediate host is domestic cat.

4. *Cryptosporidium* spp.

Most frequently diagnosed opportunistic pathogens associated with diarrhoea and wasting syndrome in patients with AIDS. *Cryptosporidium* produces a life-threatening, prolonged cholera like illness in immuno-compromised patients. 'Cryptosporidiosis' is acquired after ingesting food or water contaminated with infective cryptosporidium oocytes. The mean prevalence of *C. parvum* in Europe and the U.S. is between 1 and 3% and it is considerably higher in developing countries.

Bacterial Pathogen Growth and Inactivation

Table – 1 contains information on the minimum water activity (a_w), acidity (pH) and temperature; the maximum pH, water phase salt and temperature: and oxygen requirements that will sustain growth for the bacterial pathogens that are of great concern in seafood processing.

Table – 2 contains information on: the maximum cumulative time/internal temperature combinations for exposure of fish and fishery products that, under ordinary circumstances, will be safe for bacterial pathogens that are of great concern in seafood processing.

Pathogen Survival through Cooking

One of the purposes of cooking is to eliminate vegetative cells of pathogens that may have been introduced to the process by the raw material or by processing that occurs before the cooking step. The process of heating raw fish sufficiently to kill bacterial pathogens is also sufficient to kill parasites. Selection of the target pathogen is critical. Generally *Listeria monocytogenes* is selected because it is regarded as the most heat tolerant food-borne pathogen that does not form spores. Cooking processes are not usually designed to eliminate spores of pathogens. Determining the degree of destruction of the target pathogen is also critical. Generally a reduction of six orders of magnitude (six logarithms) is the level of contamination is suitable. This is called a "6 D" process. 6 D process times for a range of cooking temperatures with L. *monocytogenes* as the target pathogen is given in Table-3.

FDA & EPA Safety Levels

Listing of FDA & EPA levels relating to safety attributes of fish and fishery products published in regulation and guidance with respect to bacterial pathogens is given in Table-4. In many cases, these levels represent the point at or above which the agency will take legal action to remove products from the market.

Table-5 contains information on: minimal infective doses for pathogens, its incubation period, duration of illnesses and fatality rates.

Table –1
Limiting Conditions for Pathogen Growth

Pathogen	Min. a_w (using salt)	Min. pH	Max. pH	Max. % water phase salt	Min. temp.	Max. temp.	Oxygen require-ment
Bacillus cereus	.92	4.3	9.3	10	4°C	55°C****	aerobe
Campylobacter jejuni	.987	4.9	9.5	1.5	30°C	45°C	micro-aerophilic*
Clostridium botulinum, type A, and proteolytic B and F	.935	4.6	9	10	10°C	48°C	anaerobe**
Clostridium botulinum, type E, and non-proteolytic B and F	.97	5	9	5	3.3°C	45°C	anaerobe**
Clostridium perfringens	.93	5	9	7	10°C	52°C	anaerobe**
Pathogenic strains of *Escherichia coli*	.95	4	9	6.5	6.5°	49.4°C	facultative anaerobe***
Listeria monocytogenes	.92	4.4	9.4	10	-0.4°C	45°C	facultative anaerobe***
Salmonella spp.	.94	3.7	9.5	8	5.2°C	46.2°C	facultative anaerobe***
Shigella spp.	.96	4.8	9.3	5.2	6.1°	47.1°C	facultative anaerobe***
Staphylococcus aureus – *growth* – toxin	 .83 .85	 4 4	 10 9.8	 20 10	 7°C 10°C	 50°C 48°C	 facultative anaerobe***
Vibrio cholerae	.97	5	10	6	10°C	43°C	facultative anaerobe***
Vibrio parahaemolyticus	.94	4.8	11	10	5°C	45.3°C	facultative anaerobe***
Vibrio vulnificus	.96	5	10	5	8°C	43°C	facultative anaerobe***
Yersinia enterocolitica	.945	4.2	10	7	-1.3°C	42°C	facultative anaerobe***

*requires limited levels of oxygen ** requires the absence of oxygen *** grows either with or without oxygen **** growth significantly delayed (>24hr.) at 55°C

Source: Fish and Fisheries Products Hazards & Control Guidance: Third Edition, FDA (2001)

Table – 2

Time/Temperature Guidance for Controlling Pathogen Growth and Toxin Formation in Seafood

Potentially Hazardous Condition	Product Temperature	Maximum Cumulative Exposure Time
Growth and toxin formation by *Bacillus cereus*	4-6°C 7-10°C 11-21°C above 21°C	5 days 17 hours* 6 hours* 3 hours
Growth of *Campylobacter jejuni*	30-34°C above 34°C	48 hours 12 hours
Germination growth and toxin formation by *Clostridium botulinum* type A. and proteolytic B and F	10-21°C above 21°C	11 hours 2 hours
Germination growth and toxin formation by *Clostridium botulinum* type E and nonproteolytic B and F	3.3-5°C 6-10°C 11-21°C above 21°C	7 days >2 days 11 hours 6 hours
Growth of *Clostridium perfringens*	10-12°C 13-14°C 15-21°C above 21°C	21 days 1 day 6 hours* 2 hours*
Growth of pathogenic strains of *Escherichia coli*	7-10°C 11-21°C above 21°C	14 days 6 hours 3 hours
Growth of *Listeria monocytogenes*	0-5C 6-10°C 11-20°C above 21°C	7 days 2 days 12 hours* 3 hours*
Growth of *Salmonella spp.*	5-10°C 11-21°C above 21°C	14 days* 6 hours* 3 hours*
Growth of *Shigella spp* and toxin	6.1-10°C 11-21°C above 21°C	14 days* 12 hours* 3 hours*
Growth and toxin formation by *Staphylococcus aureus*	7-10°C 11-21°C above 21°C	14 days 12 hours* 3 hours
Growth of *Vibrio cholerae*	10°C 11-21°C above 21°C	21 days 6 hours* 2 hours
Growth of *Vibrio parahaemolyticus*	5-10°C 11-21°C above 21°C	21 days 6 hours* 2 hours
Growth of *Vibrio vulnificus*	8-10°C 11-21°C above 21°C	21 days 6 hours 2 hours
Growth of *Yersinia enterocolitica*	1.3-10°C 11-21°C above 21°C	1 day 6 hours 2.5 hours

*Additional data needed.

Source: Fish and Fisheries Products Hazards & Control . Guidance. Third Edition, FDA (2001

Table –3

Inactivation of *Listeria monocytogenes*

Internal Product Temperature (°C)	Lethal Rate	Time for 6D Process (minutes)
63	0.117	17.0
64	0.158	12.7
65	0.215	9.3
66	0.293	6.8
67	0.398	5.0
68	0.541	3.7
69	0.736	2.7
70	1.000	2.0
71	1.359	1.5
72	1.848	1.0
73	2.512	0.8
74	3.415	0.6
75	4.642	0.4
76	6.310	0.3
77	8.577	0.2
78	11.659	0.2
79	15.849	0.1
80	21.544	0.09
81	29.286	0.07
82	39.810	0.05
83	54.116	0.03
84	73.564	0.03
85	100.000	0.02

Note: z=7.5°C (Z value - Increase in temperature required to reduce the decimal reduction time to one-tenth of its initial value).

Source: Fish and Fisheries Products Hazards & Control Guidance. Third Edition, FDA (2001)

Table 4
FDA & EPA Safety Levels in Regulations and Guidance

Product	Level	Reference
Ready to eat fishery products (minimal cooking by consumer)	Enterotoxigenic *Escherichia coli* (ETEC)- $1x10^5$ ETEC/g. LT or ST positive	Compliance Program 7303.842
Ready to eat fishery products (minimal cooking by consumer)	*Listeria monocytogenes* – presence of organism.	Compliance Program 7303.842
All fish	Salmonella species *presence of organism*	See 555 300 Compliance Policy Guide
All fish	*Staphylococcus aureus* 1. positive for staphylococcal enterotoxin or 2. *Staphylococcus aureus* level is equal to or greater than 10^4/g (MPN)	Compliance program 7303.842
Ready to eat fishery products (minimal cooking by consumer)	*Vibrio cholerae* – presence of toxigenic 01or non 01.	Compliance Program 7303.842
Ready to eat fishery products (minimal cooking by consumer)	*Vibrio parahaemoliticus*-levels equal to or greater than $1x10^4$/g. (Kanagawa positive or negative)	Compliance Program 7303.842
Ready to eat fishery products (minimal cooking by consumer)	*Vibrio vulnificus* – presence of pathogenic organism	Compliance Program 7303.842
All fish	*Clostridium botulinum- 1.* Presence of viable spores or vegetative cells in products that will support their growth or 2. Presence of toxin	Compliance Program 7303.842
Clams and oysters, and mussels fresh or frozen-imports	Microbiological – 1. E. coli - MPN of 230/100 grams (average of subs or 3 or more of 5 subs): or 2. APC – 500,000/gram (average of subs or 3 or more of 5 subs).	Sec 560.600 Compliance Policy Guide
Clams and oysters, 7303.842 and mussels fresh or frozen-domestic	Microbiological – 1. *E. coli* or fecal coliform – 1 or more of 5 subs exceeding MPN of 330/100 grams or 2 or more exceeding 230/100 grams or 2. APC – 1 or more of 5 subs exceeding 1,500,000/gram or 2 or more exceeding 5,00,000/gram.	Compliance Program

Source:Fish and Fisheries Products Hazards & Control Guidance. Third Edition, FDA (2001).
FDA = Food and Drug Administrations. EPA = Environmental Protection Agency.

Table –5

Minimal Infective Doses for Pathogens, its Incubation Period, Duration of Illnesses and Fatality Rate

Name of organism	Infective dose	Incubation period	Duration of illness	Fatality rate
Bacillus cereus (toxin)	10^6	4-16 h	12-24 h	0
Campylobacter (infection)	400 – 500	2-5 days	7-10 days	rare 1/100
Clostridium botulinum (toxin)	few nanogram of toxin	18-36 h	Months	10-50%
Clostridium perfringens(toxin)	10^6-10^7	8-22 h	12 – 24 h	rare
Pathogenic *E. coli* (infection and toxin)	10^6-10^8	12 h – 4 days	2-9 days	rare to 1%
Listeria monocytogenes (infection)	Unknown	3 days – 3 weeks		30%
Salmonella (infection)	10 s– 1000 s	12 – 24 h	1-7 days	upto 10%
Shigella (infection)	10 – 100	12 – 96 h	4 – 7 days	rare
				<1/1000
Staphylococcus aureus (toxin)	10^6	2 – 6 h	6-24 h	rare
Streptococcus (infection)	10^3-10^7	2h – 3 days	variable	0
Vibrio parahaemolyticus (infection)	10^6-10^7	4 – 96 h	4 – 7 days	<10%
Vibrio cholerae 01(infection)	10^3	6h – 5 days	hours to days	<1%
Vibrio cholerae non-01 (infection)	10^6	8h – 3 days	6 – 7 days	
Vibrio vulnificus((infection)	Unknown	12 – 72 h		40%
Yersinia(infection)	Unknown	3 – 7 days	1 day to 3 weeks	rare
Giardia lamblia (protozoa)	10-100 cysts			
Entamoeba (protozoa)	10 cysts			
Cryptosporidium (protozoa)	10 cysts			
Ascaris (round worm)	1-10 eggs			
Hepatitis A virus (infection)	1-10			
Norwalk virus (infection)	1-10			