Chapter 17
Science, Policy, and Risk Management: Case of Seafood Safety

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Glossary

Action levels	"Action levels and tolerances represent limits at or above which the FDA will take legal action to remove products from the market" – FDA.
An outbreak	Involves two or more ill people – CSPI.
Biological contaminants	In the context of this document, these are pathogenic microorganisms (bacteria, viruses, and parasites) found in seafood.
Chemical contaminants	In the context of this entry, are regrouped under this denomination, all nonbiological contaminants (deleterious chemicals) traceable to seafood.

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Environmental pollutants	Seafood-associated deleterious substances traceable to the environment such as heavy metals and persistent organic pollutants.
Etiological agent	A microorganism responsible for a given disease.
Food safety hazards	According to the Seafood HACCP Regulation, a "food safety hazard" is "any biological, chemical, or physical property that may cause food to be unsafe for human consumption."
Seafood	Edible marine plants and animals (fish and shellfish) are usually grouped under the denomination of seafood in some contexts, these are referred to as "fish and fishery products" [1]. This same term is often given a broader meaning: all edible aquatic plants and animals.
Seafood-associated toxins	Harmful chemical substances produced either by seafood-associated bacterial contaminants, cyanobacteria, or toxic microscopic algae (dinoflagellates and diatoms) on which seafood feed.
Tolerance threshold	Maximum allowable amount of ubiquitous deleterious substance in seafood.

Definition of the Subject

In order to function properly, the human body needs a wide range of essential nutrients, which it gets from food that is ingested on a daily basis. Unfortunately, food also represents a vector for harmful creatures (bacterial, viral, protozoan pathogens) and chemical substances (organic toxins as well as toxic metals and various environmental contaminants). According to the most recent surveys of the Center for Science for Public Interest (CSPI), for more than a decade now, seafood has ranked first as the most likely source of foodborne disease outbreaks of established origin [2, 3]. Based on these surveys, seafood-associated hazards that have caused the largest number of outbreaks are toxins (especially scombrotoxin and ciguatera), followed by bacteria, the most problematic of which are *Vibrio* spp, and finally viruses (especially norovirus). Though food safety is primarily the responsibility of regulatory agencies, several other groups are involved. These include industries, consumers, and the scientific community upon which rests the responsibility of developing cutting-edge technologies capable of eliminating seafood-associated biological and chemical contaminants. The international community also relies on science for the development of revolutionary technologies for a faster, cheaper, easier, and more accurate detection of seafood-associated health hazards; tools without which enforcing laws and regulations set forth by regulatory agencies is virtually impossible. In this entry,

different categories of seafood-associated health hazards as well as a few relevant regulatory and scientific efforts dedicated to reduce the incidence of seafood-borne illnesses are reviewed.

Introduction

Seafood constitutes a significant portion of the world's food supply and is renowned for its delightful taste. It is a critical component of the human diet because of its unique nutritional properties. Fish, for instance, is a good source of protein as its major components are proteins and lipids. All essential amino acids can be derived from fish consumption. Approximately 40% of the lipids found in fish are comprised of highly unsaturated long-chain fatty acids. Other outstanding nutritional qualities are reduced saturated fats and carbohydrates and plentiful essential nutrients. Some fish species are a valuable source of important nutrients such as vitamins A and D, phosphorus, iron, calcium, magnesium, selenium, and iodine [4, 5].

There are numerous reports of health benefits associated with the consumption of seafood. Several of these health benefits have been attributed to seafood's high content of vital nutrients, such as *n*-3 polyunsaturated fatty acids (PUFAs), specifically eicosapentaenoic acid (EPA), and docosahexaenoic (DHA). These health benefits include a reduced risk of developing serious diseases such as depression, [6] myocardial infarction, Alzheimer's [7], dementia [8], and weight loss [9]. A number of reports have associated seafood consumption with a reduced risk of mortality among individuals suffering from coronary heart disease [10] and a reduced risk of developing diseases such as ischemic and thrombotic strokes, colon and intestinal cancers, as well as others [9, 11–13]. These positive effects are counted among the factors that have driven current market trends. There has been a steady increase in the world's per capita fish and fishery products consumption for several decades now [14]. According to the December 2009 Food Outlook Report of the Food and Agriculture Organization (FAO), the annual per capita fish consumption in the world during the years 2007–2009 was estimated at ~17.1 kg. It is important to note that in the 1970s, 1980s, and 1990s, these values were 11.5, 12.8, and 16.4 kg per capita, respectively [15, 16].

As indicated by recent estimates, there has been a net increase in the demand for seafood in countries around the world [17]. In the UK for instance, the seafood retail market has experienced a considerable increase between the years 2003 and 2007, increasing from £2.4 billion (retail price) to an estimated £3.25 billion in 2007 [14]. A significant increase in seafood demand in developing countries has been observed, as well [16]. Millions of tons of seafood are caught each year worldwide to sustain the current demand. There has been a steady increase in the total world fish production since the 1950s, from 19.3 million tons to about 134 million tons in 2002 [18]. According to a 2009 FAO report, the current world production (capture fisheries plus aquaculture) is estimated at 144.1 million tons, divided into 98.8 million from capture fisheries and 54.3 million from aquaculture [15, 16].

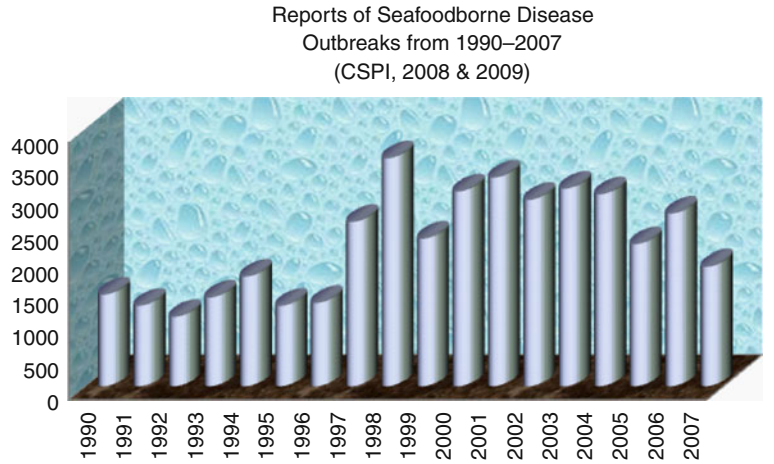

Fig. 17.1 Variation in the number of reported seafood-borne disease outbreaks since the 1990s in the USA

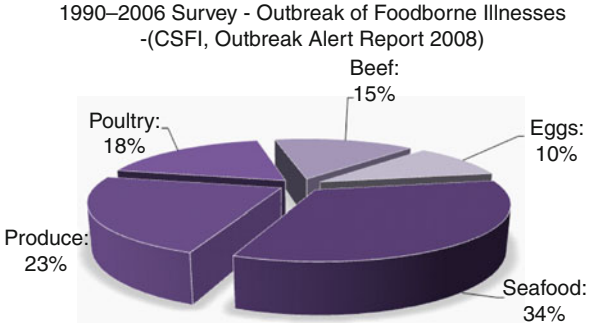

Fig. 17.2 Foodborne outbreaks reported during the years 1998–2007 by category of food in the USA

Unfortunately, seafood consumption is not without risks and food is an important vector of a wide range of health hazards (Fig. 17.1). Foodborne illnesses are a serious public health concern and according to the Centers for Disease Control and Prevention (CDC) roughly 76 million foodborne illnesses corresponding to about 325,000 hospitalizations and 5,000 deaths are recorded in the USA each year [19]. A recent survey conducted by the Center for Science for Public Interest (CSPI) [2] revealed that the food categories that were associated with the largest number of outbreaks in the USA during the period 1990–2006 were seafood, produce, poultry, beef, and eggs. Seafood was responsible for 1,140 out of 5,778 outbreaks and therefore was the most problematic food (Fig. 17.2). Also reported was an increase in the number of seafood-related outbreaks compared to the early 1990s (Fig. 17.1). It is important to mention that the number of reported cases of seafood-borne illness

has remained constant over the years. Though it ranked second as far as number of outbreaks, produce caused the largest number of cases of illness during that same time frame [2].

"Food Safety Hazards" Associated with Seafood

Seafood-associated health hazards can be classified into two main categories: (1) biological contaminants (includes a long list of bacteria, viruses, parasites) and (2) chemical contaminants such as environmental pollutants (pesticides, heavy metals, approved or unapproved drug substances) and finally natural toxins from a variety of structural classes. According to the CSPI's 2008 report, the latter category was associated with the largest number of seafood-borne outbreaks from 1990 through 2006 (Fig. 17.3) [2]. Currently known health hazards associated with seafood are either naturally occurring or from various anthropogenic activities. Seafood becomes contaminated either as a result of feeding on poisonous phytoplankton species or in sewage-contaminated marine environments. Seafood contamination can also arise from inappropriate storage or accidental exposures during handling. Certain types of seafood are more likely vehicles of dangerous substances and pathogenic microorganisms than others.

Categories of seafood that have been associated with greater public safety risks are considered a high priority in sampling and surveillance efforts by the Food and Drug Administration (FDA) [20]. At the top of the FDA's seafood watch list are products such as molluskan shellfish from uncertified sources, refrigerated reduced oxygen packaged products, ready-to-eat seafood, seafood mixes containing cooked, raw, or partially cooked seafood components, as well as, scombrotoxin (histamine)-forming fish, aquaculture-derived seafood, and finally salt-cured or dried uneviscerated finfish [20]. According to the 2008 CSPI report, based on the number of reported outbreaks, finfish (such as tuna and grouper) was the most dangerous

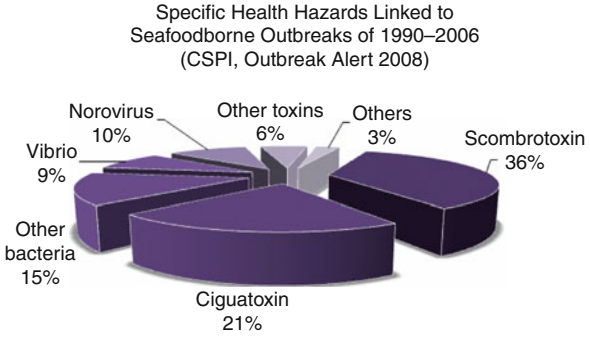

Fig. 17.3 Specific health hazards that caused seafood-borne outbreaks reported during the years 1990–2006 in the USA

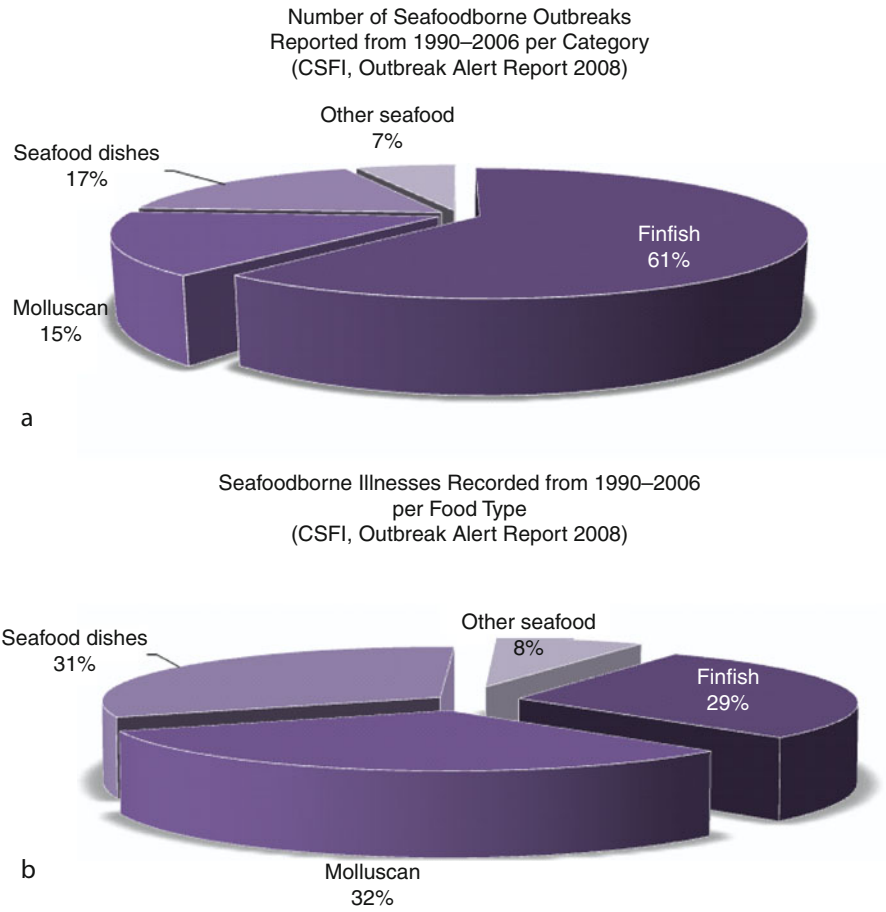

Fig. 17.4 Seafood-borne, outbreaks (**a**) and cases of illnesses (**b**) reported in the USA during the years 1990–2006 (by category of seafood)

type of seafood between the years 1990 and 2006. Finfish was responsible for 61% of all reported seafood-borne outbreaks during this period, followed by molluskan shellfish (15%) (Fig. 17.4a). The largest number of cases of seafood-borne illness was attributable to mollukcan shellfish (Fig. 17.4b) [2].

Seafood-Associated Toxins

Seafood-associated toxins, especially scombrotoxin and ciguatoxin, have been linked to the majority of seafood-borne outbreaks that occurred during the last decade and as such, could be viewed as the most dangerous seafood-associated

"food safety hazard" [2, 3]. Scombrotoxin and ciguatoxin alone were responsible for 57% of all seafood-related outbreaks in the USA reported from 1990 to 2006 [2].

Seafood-associated toxins have been linked to a wide variety of intoxications. These include, poisoning associated with shellfish consumption, namely diarrheic shellfish poisoning (DSP), paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP), neurologic shellfish poisoning (NSP), aZaspiracid shellfish poisoning (AZP), and poisonings associated with fish consumption. The latter include ciguatera fish poisoning (CFP) and puffer fish poisoning [21]. It is necessary to point out that some seafood-associated biotoxins, namely ciguatoxin and toxins responsible for PSP, NSP, and ASP, can be lethal [22]. Seafood-associated toxins are generated either by bacterial contaminants that freely proliferate when seafood is improperly stored or by cyanobacteria and toxic microscopic algae (dinoflagellates and diatoms) on which the seafood feed. The blooms of these latter organisms, which occur from season to season, forming red tides (Harmful Algae Blooms [HAB]) are a subject of public health and environmental concerns, affecting the tourism and fishing industries [23].

Ciguatera Fish Poisoning

Ciguatera fish poisoning has been reported in countries around the world (Europe, Africa, America, Asia, and Oceania). This form of poisoning is frequent between the months of April through August and has been linked to the consumption of certain fish. Some examples are shark, barracuda, snapper, hogfish, horse-eye jack, red grouper, gray triggerfish, Spanish mackerel, narrowhead gray mullet, chinamanfish, swordfish, and amberjack. Ciguatera fish poisoning is caused by a set of heat-resistant polyether toxins known as ciguatoxins (Fig. 17.5), which are the product of in situ gambiertoxin biotransformation [23, 24]. Ciguatoxin, maitotoxin, palytoxin, and scaritoxin are members of this group. These toxins are produced by a variety of organisms; these include *Gambierdiscus toxicus*, *Gymnodinium sangieneum, G. polyedra, Ostreopsis lenticularis, Prorocentrum concavum, P. mexicanum,* and *P. rhathytum*, among others [21, 25]. Symptoms of ciguatera fish poisoning are mainly gastrointestinal and neurological. A few therapeutic approaches in case of poisoning include, but are not limited to, antihistamines, antiemetics (droperidol, prochlorperazin, metoclopramide), atropine, as well as intravenous hydration [23].

Scombrotoxic Fish Poisoning

Scombroid fish poisoning differs from other types of toxin-mediated seafood poisonings because the responsible toxin is not produced by a microalgae. Instead, it is generated under improper storage conditions (temperature $> 20°C$). This toxin is the result of a catalytic reaction involving the conversion of in situ histidine into histamine (Fig. 17.5). The enzyme responsible for this conversion, histidine decarboxylase, can be produced by several types of bacteria. These include various

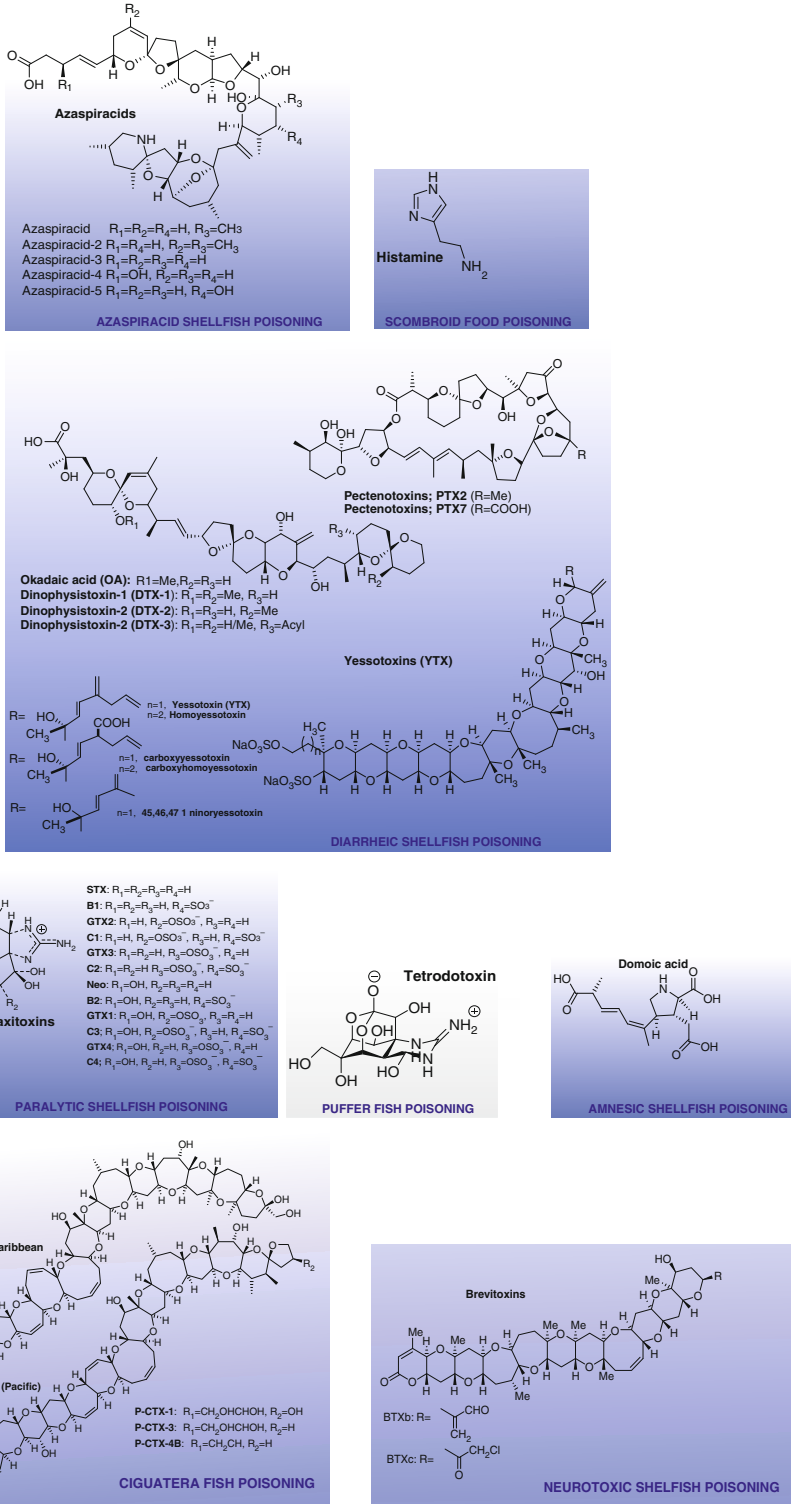

Fig. 17.5 Toxins involved in seafood-borne intoxications

Vibrio sp. *Clostridium, Enterobacteriaceae* (such as *Morganella morganii* and *Klebsiella pneumoniae* and *Hafnia alvei*) and *Lactobacillus* sp. [26]. Scombrotoxin is stable to both heat and cold conditions. Scombroid fish (fish containing a high level of free histidine) and non-scombroid fish have been implicated in this form of poisoning. These include fish such as amberjack, abalone, tunas, sardines, mackerel, bonito, and bluefish, just to name a few [25]; associated symptoms rank from mild and self-limiting to severe. Groups at risk for developing the severe form of this disease are people with respiratory and cardiac conditions or those on medication such as isoniazid and doxycycline that slow histamine degradation [23, 25, 27]. Symptoms of scombrotoxic fish poisoning include headache, abdominal cramps, nausea, diarrhea, and palpitations, among others. As far as pathophysiology, bioamines other than histamine are believed to play a critical role; a few examples are spermine, cadaverine, agmatine, and putrescine [25, 28]. There are several therapeutic approaches for this type of food poisoning. These include administration of activated charcoal, diphenhydramine, cimetidine, and famodine [27].

Other Major Seafood-Borne Poisonings

Paralytic shellfish poisoning (PSP) can be caused by a wide range of tetrahydropurine type toxins (carbamate, *N*-sulfo-carbamoyl, decarbamoyl, and deoxydecarbamoyl) [24] collectively called saxitoxins (Fig. 17.5). These are neurotoxins that act by blocking sodium channels, causing symptoms like numbness, paralysis, and disorientation. Saxitoxins are produced by dinoflagellates and blue-green algae. Dinoflagellates that have been linked to this form of poisoning include *Pyrodinium bahamense, Gymnodinium catenatum,* as well as several organisms belonging to the genus *Alexandrium* [21, 24, 29]. Various types of seafood can serve as vectors; these include clam, crabs, cockles, oysters, salmon, mackerels, scallops, and whales to name a few [21, 23]. PSP has been reported on all continents. Regrettably, there is no antidote for PSP, and therapeutic approaches are mostly supportive and include respiratory support in a life-threatening situation, gastric emptying, dialysis and enhancing renal clearance [23].

Diarrheic shellfish poisoning (DSP) has also been reported worldwide. Diarrhea, nausea, cramps, and vomiting are common signs of DSP. It is usually associated with consumption of contaminated clams, mussels, oysters, and scallops. This syndrome is caused by a group of acidic (okadaic acid and related dinophysistoxins) and neutral toxins (pectenotoxin). Yessotoxins have also been reported to cause this form of poisoning (Fig. 17.5) [24, 30]. These toxins are produced by a variety of marine dinoflagellates including *Dinophysis* spp. (*D. acuta, D. acuminate, D. caudate, D. mitra, D. norvegica*) as well as *Protoceratium* spp., *Prorocentrum* spp., *Gonyaulax* spp., and *Phalacroma* spp [21].

Amnesic shellfish poisoning (ASP) has been reported in various areas around the world including Europe, North, Central, and South America, Asia, and Oceania.

Several types of seafood, including anchovies, clams, crabs, oysters, mussels, mackerels, lobsters, scallops, and gastropods are potential vectors. ASP is caused by a marine biotoxin called domoic acid (Fig. 17.5). This toxin is produced by a red-brown marine diatom called *Pseudo-nitzschia pungens*. Diarrhea, nausea, and abdominal pain are examples of symptoms that indicate amnesic shellfish poisoning [21].

Neurotoxic shellfish poisoning (NSP) is caused by brevetoxins and its analogs (Fig. 17.5). Occurrences have been reported in countries around the world. This form of poisoning has been associated with the consumption of contaminated clams, mullets, mussels, oysters, tunas, and whelks. *Fibrocapsa japonica, Gymnodinium breve* (*Karenia brevis*), *Raphidophyceae* sp., and *Chattonella marina,* among others, are examples of organisms that produce these toxins [21, 24].

Puffer fish poisoning has been linked to the most potent and lethal marine neurotoxin: tedrodotoxin (Fig. 17.5). It is produced by a variety of animals including the California newt, trumpet shell, the blue ringed octopus, and puffer fish, especially a species known as fugu (present in the liver). Puffer fish is a delicacy in Japan and is the main vector for this form of poisoning. Once again, there is no antidote. The first case in Europe occurred in 2009 and involved an individual that had consumed trumpet shellfish (*Charonia sauliae*) harvested from the Atlantic Ocean in Southern Europe [31]. The main therapeutic approach upon poisoning is supportive and includes respiratory support (life-threatening circumstances). Activated charcoal, atropine, anticholinesterase agents, and alpha agonists, among others, are also recommended [32].

AZaspiracid shellfish poisoning (AZP). Mussels and oysters are known vectors of toxins responsible for azaspiracid shellfish poisoning. AZP has been reported in countries around Europe, namely Norway, Portugal, the UK, and Ireland. AZP is caused by marine toxins known as azaspiracids (Fig. 17.5), which are produced by *Protoceratum crassipes* and *Protoperidinium*. Nausea, vomiting, and diarrhea are a few symptoms of azaspiracid shellfish poisoning [21, 24].

Several other marine biotoxins not listed above have been reported. A few examples able to impair human health are gymnodimine, neosurugatoxin, prosurugatoxin, polycavernoside, and debromoaplysiatoxin [21]. It is also important to note that a number of biotoxin producers (dinoflagellates) have been associated with massive fish mortality and thus represent a major issue to the seafood and tourism industry worldwide. Examples in this case are *Pfiesteria piscicida* and *Karlodinium veneficum*; however, there is growing evidence that *P. piscicida* associated fish kills in the past may indeed have been *K. veneficum* derived. Blooms of *K. veneficum* have been linked to various episodes of massive fish kill around the world. In this particular case, a set of toxins believed to be the etiological agents and regrouped under the denomination karlotoxins or KmTxs has been isolated [33, 34]. Examples of such toxins include the karlotoxin-1 (KmTx-1), the 10-*O*-sulfo-KmTx-1, the KmTx-3, the 64-*E*-chloro-KmTx-3, the 10-*O*-sulfo-KmTx-3, the 65-*E*-chloro-KmTx-1, and, finally, the KmTx-2 (Fig. 17.6) [35], for which the relative and absolute configurations were assigned

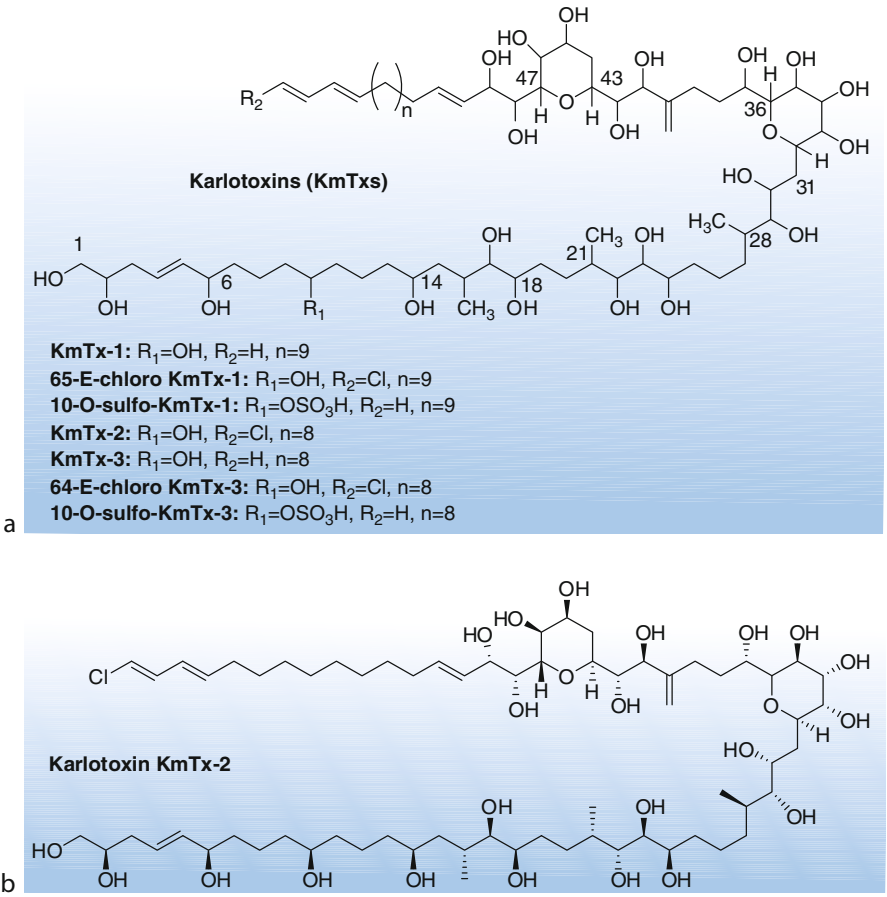

Fig. 17.6 (**a**) Structure of biotoxins produced by *Karlodinium veneficum*. (**b**) The absolute configuration of KmTx-2

only recently [36]. Ongoing work in this area actually began with research by Abbott and Ballantine in the 1940s and 1950s [37]. Karlotoxins kill fish by osmotic cell lysis, a result of the alteration of the ion transport system of the cell membrane. The fish dies as a result of damage to its vital gill epithelial tissues. These toxins are environmental pollutants and detectable in water during fish kill episodes. The ecological role of these toxins was investigated recently and because the toxins possess an allelopathic inhibitory effect on competitors as well as a prey immobilization it was established that the organism produce these toxins in order to facilitate feeding and control of competition during a bloom [38, 39].

Microbial Pathogens

Following chemical toxins, pathogenic microorganisms were the most likely cause of seafood-borne disease outbreaks throughout 1990–2006 (Fig. 17.3). The CSPI's survey associated bacteria to 24% of reported outbreaks during this period, trailed by norovirus (10%) [2].

Pathogenic Bacteria

Seafood's bacterial pathogens can be found either in their GI system (bivalve mollusks) or on their surface (crustaceans). These bacteria have been linked to various infections and intoxications. Pathogens accumulate in the digestive track of bivalve mollusks (cockles, mussels, oysters, clams) as a result of filter feeding in heavily contaminated water. Seafood-associated bacterial pathogens are either indigenous to the marine environment (case of *Vibrionaceae,* a source of greater concern) or nonindigenous (resulting from fecal contamination). Members of the first category are pathogenic *Vibrio* spp. such as *V. cholerae, V. parahaemolyticus, V. vulnificus, Clostridium botulinum* (non-proteolytic types B, E, F), *Aeromonas hydrophila, Plesiomonas shigelloides,* and *Listeria monocytogenes*, just to name a few [22, 26, 40]. Diarrhea is a common symptom of infection caused by several of these microorganisms. *V. parahaemolyticus, V. vulnificus, V. hollisae,* and *V. cholera* non O–1 have been also associated with more serious conditions such as septicemia. These microorganisms are most prolific during summer months as the water temperature rises. The second category includes bacteria such as *Salmonella* (nontyphoidal), *Shigella, Campylobacter, Staphylococcus aureus,* and *Escherichia coli* [26].

Proper seafood storage is sufficient to protect against diseases caused by several of these bacteria. It has been established that their concentration in seafood is normally low (below the minimum infective dose) and will remain so providing that the seafood is stored in conditions that are not conducive to bacterial growth, multiplication, or toxin production (refrigerated (4°C) and frozen (−18°C)). It is important to note that this does not apply to mollusks and their predators [28, 41]. While, in several cases, a low concentration in seafood is tolerated, FDA regulations become more stringent when it comes to bacteria such as *L. monocytogenes, V. vulnificus, Salmonella, C. botulinum*, and toxigenic 01 *V. cholera*. Current regulations require that these be undetectable in seafood requiring minimal cooking before consumption, for instance [1].

Vibrio spp

Vibrio spp., especially *V. parahaemolyticus* and *V. vulnificus*, are a cause of significant concern in the USA and several Asian countries (Japan, Taiwan, India,

and China). *V. parahaemolyticus* (Vp), for instance, has been associated to the vast majority of seafood-borne gastroenteritis in the USA. This bacterium has been associated with fewer outbreaks in Europe [40, 41]. The pathophysiology of Vp is centered on several virulence factors; a few examples in this case are the thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH), which are encoded by tdh and trh genes, respectively [40]. Healthy individuals are also at risk of developing Vp associated infection [25]. The FDA can take legal action when seafood products are found to contain a Vp count $\geq 1 \times 10^4$/g [1]. Raw or improperly cooked fish and shellfish are potential vectors [25]. The minimal infective dose for this pathogen is $>10^6$/g [26]. *V. vulnificus* (Vv) infections can also result from consumption of raw or undercooked seafood. In addition, transmission can occur via wound infection. Though Vv is not a major issue in healthy individuals, in certain groups, Vv can cause serious infections or death. People suffering from alcoholic cirrhosis, hemochromatosis/cirrhosis, chronic hepatitis, postnecrotic cirrhosis, as well as diabetics and alcoholics are at higher risk. *V. vulnificus* is the second leading cause of seafood-related fatality in the USA [42–44]. Vv infections that occur as a result of consumption of contaminated seafood (especially raw oysters) are primarily septicemia and gastroenteritis. One therapeutic approach is the use of antimicrobial agents (tetracycline and intravenous doxycycline with ceftazidime) [43].

Other Seafood-Associated Bacteria

Clostridium botulinum – This bacterium is responsible for a condition known as botulism, the responsible agent being a toxin. Lightly preserved, semi-preserved, and fully preserved smoked, fermented, salted, and pickled fish products are likely vectors. Cold-smoked and fermented fish products are of greater risk. Chilling, autoclaving, and salting are approaches used to prevent botulism [26, 28]. *Listeria monocytogenes* – Infections caused by this other bacterium can, at worst, result in septicemia spreading to several organs and even, in the case of pregnant women, to the fetus. This type of infection can be fatal. Groups most at risk are pregnant women, neonates, fetuses, and immunocompromised patients. Shrimp is an important vector [26, 28, 45]. *S. aureus* has also been isolated from seafood. This bacterium is, as *C. botulinum,* a toxinogenic species. It produces toxins that are resistant both to enzymes degradation and heat. It is introduced in seafood as a result of environmental contamination or transferred from an infected worker involved in seafood handling [26, 28]. *Enterobacteriaceae* such as *Salmonella, Shigella*, and *E. coli* have also been reported in seafood. *Enterobacteriaceae* usually occurs in seafood as a result of fecal contamination. *Salmonella* is responsible for salmonellosis and is especially problematic for the shrimp industry [45]. Compared to other food categories, seafood is a less likely vector of *Salmonella* [25, 26]. The minimum infective dose for *Salmonella* sp has been estimated to be in the range of $<10^2$–$>10^6$. Non-bloody diarrhea, fever, abdominal pain and nausea, just to name a few, are indicators of infection by this

Table 17.1 Safety levels set by FDA for several seafood-associated bacteria

Hazards	FDA & EPA thresholds	Analytical approach	Targeted seafood	Higher risk populations
Salmonella sp.	Presence of organism[a]	Conventional culture methods	All fish	Severe in the elderly, infants, AIDS patients
E. coli	MPN of 230/100 g[b] APC – 500,000/g	Hemorrhagic colitis agar – direct plating method	Imported fresh and frozen clams and oysters	All people – most susceptible are young children and the elderly
S. aureus	Presence of staphylococcal enterotoxin, or a load $\geq 10^4$/g (MPN)[c]	Specific precipitation with antiserum	All fish	All people
C. botulinum	Presence of viable spores or vegetative cells or toxin[c]	Mouse neutralization test	All fish	All people

Compliance policy/programs
[a]Sec 555.300 Compliance Policy Guide
[b]Sec 560.600 Compliance Policy Guide
[c]Compliance Program 7303.842

pathogen. Symptoms of a *Shigella* infection, on the other hand, are bloody stools, severe abdominal cramps, fever, and dehydration. The minimum infective dose in this case has been estimated at 10^1–10^2. This is similar to what has been reported for *E. coli,* for which the minimal infective dose is 10^1–10^3 (Table 17.1) [26, 28].

Seafood-Associated Viruses

Seafood can also serve as a vector of viruses. Non-A, non-B enteral hepatitis viruses, hepatitis A virus (HAV), poliovirus, and norovirus, among others, have been associated with seafood-borne outbreaks [25, 26, 46, 47]. Viruses end up in seafood as a result of fecal contamination of the marine environment or when handled by an infected worker. Viruses are one of the most serious seafood-associated threats. So far, reports of seafood-borne infection outbreaks linked to viruses have emerged from countries around the world. HAV was associated with the largest seafood outbreak ever reported. This outbreak, which occurred in 1998, in the Chinese city of Shanghai, was linked to the consumption of contaminated clams and *over 292,000 cases were reported* [47, 48]. Another virus, namely norovirus, a single-stranded nonenveloped RNA virus, is currently responsible for roughly 50% of all foodborne outbreaks of gastroenteritis according to the CDC [49]. Norovirus was reported by CSPI as the most problematic seafood-associated virus during the period 1990–2006 (Fig. 17.3), as it caused 10% of all reported seafood outbreaks during that time [2]. It is important to point out that of the five genogroups of norovirus, GII has been linked

to the majority of infections. Filter-feeding bivalve shellfish are important vectors [43]. Norovirus gastroenteritis-associated symptoms are vomiting, watery stools, non-bloody diarrhea with abdominal cramps, and nausea. It is a fairly resistant virus that can survive harsh conditions such as chlorine treatments (10 ppm), heating to 60°C (4 h), or freezing [49].

Seafood-Associated Parasites

Seafood (raw or undercooked) can also serve as a vector of pathogenic parasites. These include nematodes, cestodes, and trematodes. *Anisakis simplex, Pseudoterranova dicipiens, Gnathostoma* sp., *Capillaria* sp., and *Angiostrongylus* sp. are examples of seafood-associated nematodes. Examples of tapeworms that can be isolated from seafood include *Diphyllobothrium latum* and *D. pacificum; Clonorchis* sp., *Opisthorchis* sp., *Metagonimus yokagawai, Heterophyes* sp., *Paragonimus* sp., and *Echinostoma* sp., on the other hand, are a few examples of seafood-associated trematodes [26].

Several parasite-infected seafood dishes such as sushi, crab, sashimi, herring roe, and undercooked grilled fish have been associated with illnesses [1]. Compared to bacteria and viruses, however, parasites are of lesser concern. Trematodes (such as *Paragonimus westermani),* cestodes (such as *Diphyllobothrium latum, D. pacificum*), and nematodes (such as *Angiostrongylus cantonensis, Contraceacum osculatum)* have been associated with domestic fish and shellfish. Several other organisms have been associated with imported products instead. These include *Clonorchis sinensis, Heterophyes heterophyes, Metagonimus yokogawai, Opisthorchis felineus*, and *Gnathostoma spinigerum* [49]. Some nematodes, cestodes, and trematodes are of greatest concern as far as seafood safety. These include *Anisakis simplex*, *Pseudoterranova* spp.*, Eustrongylides* spp.*, Gnathostoma* spp.*, Opisthorchis* spp.*, Chlonorchis sinensis*, *and Paragonimus* spp., just to name a few [1, 49].

Toxic Heavy Metals

Heavy metals are a threat to the environment and public health and are problematic in regard to their long-term persistence. A variety of heavy metal contaminants has been reported in seafood. These elements originate from natural occurrences (marine volcanism, and geological and geothermal events) and anthropogenic activities. Several categories of anthropogenic activities threaten the marine environment. These include, activities that take place in tanneries, steel plants, battery industries, thermal power plants, and farms, especially those farms using heavy metal containing fertilizers and pesticides. Runoff from roadways has also been recently cited as an important source of contamination [4, 50, 51].

Heavy metals found in seafood include antimony, arsenic, cadmium, chromium, lead, mercury, nickel, copper, iron, manganese, selenium, zinc, aluminum,

silver, strontium, thallium, and tin. Of these heavy metal contaminants, those of greatest concern are antimony, arsenic, cadmium, chromium, lead, mercury, and nickel. It is important to note that elements such as copper, selenium, iron, and zinc (known essential micronutrients) are toxic only at high concentrations [4].

Arsenic can be present under a variety of forms: toxic (inorganic) and nontoxic (organic). Arsenic is an extremely potent poison in its trivalent form and can cause a wide range of acute and chronic illnesses. A few examples include cancer, nephritis, hepatomegaly, peripheral symmetrical neuropathy, and palmar hyperkeratosis, among others. In seafood, arsenic is mainly present in a nonpoisonous form known as arsenobetaine or arsenocholine [46]. A compilation of data (about 100,000 results) received from 15 European countries revealed that seafood is among the food commodities with the highest arsenic levels [52].

Methyl mercury is a neurotoxic contaminant. Due to its potential effects on the fetus, it is one of the most regulated seafood-associated toxic metals. There are several related FDA recommendations to nursing/pregnant women, women of childbearing age, or children when it comes to seafood consumption. CH_3Hg^+ is present in nearly all seafood, but some types of fish such as shark, swordfish, king mackerel, and tilefish are believed to have a higher content. The FDA and the US Environmental Protection Agency (US EPA) have recommended being very selective of the type of fish consumed, limiting uptake to ~12 oz/week as a healthy approach for groups at risk [5]. This has been supported by scientific data [12, 53, 54]. Several reports have shown that moderate to high consumption of fish species containing only a low amount of CH_3Hg^+ during pregnancy has a positive effect on fetal brain development. However, it is also important to note that several other studies, Myers et al. (2003) for instance, did not support the fact that exposure of pregnant women to methyl mercury through fish consumption could have deleterious effects on fetal development [13, 55].

Other toxic heavy metals include nickel, chromium, cadmium, selenium, and lead. Cadmium tends to be bioaccumulated by crustacea and bivalves. Clinical signs of cadmium poisoning include osteoporotic and osteomalacic disease, as well as kidney damage. Lead poisoning, on the other hand, has been associated with anemia, convulsions, paralysis, and proteinuria. This metal tends to accumulate in cortical and trabecular bone, kidney, lung, as well as the CNS. Edema, hepatitis, and hemorrhage are conditions that can result from selenium poisoning, which is also known, to result in congenital malformations and infertility. Arsenic (As), nickel (Ni), and chromium (Cr) are carcinogens [46].

Other Chemical Environmental Contaminants

Organochlorine compounds. Until recently, organochlorine compounds were widely used. A wide range of polychlorinated substances can be found in seafood. These include various insecticides, agrochemicals, industrial chemicals, and by-products. Examples of pesticides traceable to seafood include endrin, heptachlor, dieldrin, benzene hexachloride (BHC), chlordane, dichlorodiphenyltrichloroethane (DDT)

and lindane. Polychlorinated biphenyls (PCBs) and dioxins, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, are example of industrial chemicals that contaminate seafood. Prior to 1970, PCBs were widely used industrially [56]. Because they are non-biodegradable, toxic in nature, and tend to bioaccumulate in seafood, organochlorine compounds now constitute a major environmental and public health concern. Several of these substances are known carcinogens (dioxins and polychlorinated biphenyls); but because seafood-associated health benefits outweigh potential risks, seafood consumption is recommended [13]. The level of these substances in fish is truly minimal. However, it is important to note that people whose diet is predominantly made of seafood are still at risk [56]. Reports on farm raised salmon (especially European farms) containing higher levels of these contaminants compared to wild-type salmon is believed to have had a serious impact on the consumer acceptance of this type of seafood [57, 58].

Antimicrobial drug contaminants of seafood. This is a problem mainly associated with aquaculture, as farmers rely more and more on various drugs and antimicrobial agents to deal with specific seafood diseases (bacterial, fungal, viral), to improve the quality of water, and to manage pest-related issues.

There are quite a few reasons why there are concerns over drug residues being present in seafood. Several antimicrobial agents used in aquaculture have been linked to various adverse health effects, such as hypersensitivity reactions. In vivo studies, in animals, have established several others as carcinogens; these include nitrofuran, malachite green, gentian violet, and fluoroquinolones. Fluoroquinolones have been also associated with antibiotic resistance. There are several drugs used to this end in the USA that have been approved by the Center for Veterinary Medicine (CVM) (Table 17.2). Substances such as ciprofloxacin, erythromycin, tetracyclines, chloramphenicol, nitrofurans, malachite green, gentian (crystal) violet, and fluoroquinolones have been frequently reported in seafood imported into the USA in recent years [20]. A short, recent survey by the FDA has shown that imports from China test positive for the presence of a variety of unapproved substances, such as gentian violet, malachite green, nitrofuran, and fluoroquinolones. Consequently, these products are the subject of great concern and the focus of regulatory surveillance [59].

Similar issues have been reported in Europe. In fact, during the years 1999–2002, the presence of residues of antimicrobial drugs, mainly nitrofuran and chloramphenicol, was one of the main reasons for detention or rejection of seafood imports into Europe (Fig. 17.7) [28]. A handful of aquaculture drugs approved in Europe are presented in Table 17.3.

What Are the Approaches Used to Assure Public Safety?

Regulatory authorities and the scientific community are two groups with complementary goals that play a key role in ensuring public safety against seafood-associated health hazards.

Table 17.2 Aquaculture drugs approved in the USA and action levels

FDA-approved aquaculture drugs			
Drug name	Tolerance level in the flesh	Type of seafood	Purpose
Unapproved drugs	No trace tolerated[a]	All fish	–
Chorionic gonadotropin[b]		Brood finfish	Reproductive
Formalin solution[c]		Salmon trout, catfish, largemouth bass, and bluegill	Antiparasitic and fungicidal/static
Tricaine methanesulfonate[d]		Catfish, salmon, and trout, pike and perch	–
Oxytetracycline	2.0 ppm[e]	Salmonids, catfish, and lobster	Disease control
Sulfamerazine[f]	Undetectable[g]	Trout	–
Sulfadimethoxine/ Ormetoprim combination[h]	0.1 ppm[i]	Salmonids and catfish.	–

Compliance policy/programs
[a]Sec 615.200 Compliance Policy Guide
[b]21 CFR 522.1081
[c]21 CFR 529.1030
[d]21 CFR 529.2503
[e]21 CFR 556.500
[f]21 CFR 558.582
[g]21 CFR 556.660
[h]21 CFR 558.575
[i]21 CFR 556.640

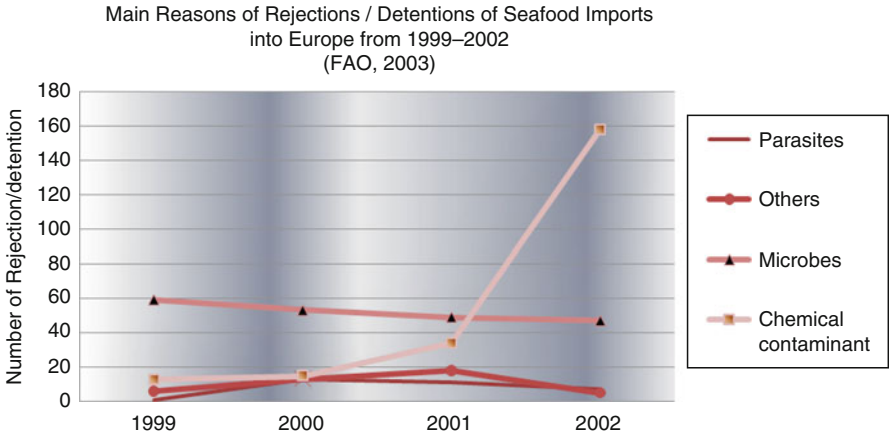

Fig. 17.7 Main causes of rejection or detention of seafood imports into Europe during the years 1999–2002

Table 17.3 Aquaculture drugs approved in Europe and action levels

The Council of the European Communities approved aquaculture drugs		
Drug name	Maximum Residue Limit (MRL) – muscle and skin in natural portion	Type of seafood
Trimethoprim	50 μg/kg[a]	Finfish
Flumequine	600 μg/kg[a]	Finfish
Oxolinic acid	100 μg/kg[a]	Finfish
Sarafloxacin	30 μg/kg[a]	Salmonidae
Tylosin A	100 μg/kg[a]	Finfish
Thiamphenicol	50 μg/kg[a]	Finfish
Colistin	150 μg/kg[a]	Finfish
Deltamethrin	10 μg/kg[a]	Finfish
Emamectin B1a	100 μg/kg[a]	Finfish
Oxolinic acid	300 μg/kg[a]	Finfish
Florfenicol	1000 μg/kg[a]	Fish
Thiamphenicol	50 μg/kg[a]	Finfish
Deltamethrin	10 μg/kg[a]	Finfish

Compliance policy/programs
[a]Council Regulation (EEC) No 2377/90 of 26 June 1990

Regulatory Agencies

Seafood regulatory agencies, via promulgation and enforcement of various laws and regulations, guidance, and recommendations, are usually at the heart of protecting the public from seafood-associated hazards. Numerous programs have been designed over the years to this end. In the USA, the primary responsibility of assuring seafood safety falls to the FDA, which is in charge of setting the maximum safe levels of unavoidable toxic substances in seafood [59]. The FDA has the authority to detain and even refuse import entries into the USA. A recent related event was the June 28, 2007, decision of the FDA to detain Chinese farm-raised catfish, basa, shrimp, and dace until they were cleared from containing unapproved drug substances [59]. As for domestic seafood, the FDA can recommend legal sanctions, which include "warning letters, seizure of products, injunction against further non-compliant practices, or prosecution of an individual or establishment" [20]. There are a few other regulatory agencies; these include the EPA (Environmental Protection Agency) in charge of chemical contaminants such as pesticides and the National Marine Fisheries Service for instance. In Europe, the EU parliament is at the heart of food safety control. This organization promulgates food safety-related laws, regulations, and directives, which are mandatory in all states of the European Union. The EU parliament works in close collaboration with the European Food Safety Authority, which was established by regulation (EC) No.178/2002. This latter organization, more science oriented, is in charge of risk assessment.

There is a long list of guidance and regulations, mostly to seafood industries that protect the public from dangers associated with seafood consumption. Several of these are preventive measures. A few examples in the USA are the National Shellfish Sanitation Program, the Salmon Control Plan, the Low-Acid Canned Food (LACF) Program, the Hazard Analysis & Critical Control Points (HACCP) Program, and the Good Manufacturing Practice regulation. The latter is intended to assure that recommended processing conditions were used. The Salmon Control Program, on the other hand, was designed to assure the safety of salmon consumers and is a cooperative approach involving the FDA, industries, and various associations. As part of the Shellfish Sanitation Program in the USA, the level of various pollutants in coastal water is to be monitored in order to classify each given area as suitable or unsuitable for shellfish harvest. As for the HACCP, it applies to domestic as well as imported seafood. HACCP requires both domestic and foreign processors of fish and fishery products to understand all concepts behind food safety hazards and through a system of precautionary control measures to prevent hazards from occurring [20, 59]. The EU parliaments, as well as regulatory agencies of countries around the world, have issued several similar regulations and directives that apply to domestic and imported seafood. A few examples of seafood-related regulations promulgated by the European Parliament include regulations (EC) No. 852/2004 and No. 853/2004, which established some key hygienic rules for food (including seafood) business operators and regulation (EC) No. 854/2004 in which key exigencies related to the organization of seafood official control programs are determined. Several of these regulations elaborate on the "tolerance threshold" for contaminants present in seafood. Examples of "tolerance threshold" for seafood-associated health hazards, set by regulatory agencies around the world are presented in Tables 17.4, 17.5, and 17.6.

The Scientific Community's Contribution in Ensuring Seafood Safety

Through the development of cutting-edge technologies to solve problems at hand, science has also played a critical role in ensuring public safety against dangers associated with seafood. A simple literature search with a keyword such as "seafood safety" in ScienceDirect shows a significant and steady increase in seafood safety-related research effort since 1991 (Fig. 17.8). The international community relies on science to develop cutting-edge technologies and techniques that can rid seafood from associated biological and chemical contaminants in order to bring a safer product to the market. The development of cutting-edge technologies for faster, cheaper, easier, and more accurate detection methods of seafood-associated health hazards is another way scientists have contributed in the protection of consumers against these dangers.

Table 17.4 Seafood-associated marine biotoxins and action level set by regulatory agencies around the world

Hazards	Detection method (analytical methods for regulatory purposes)	Tolerated thresholds	Targeted seafood
Paralytic shellfish poison	USA – mouse bioassay	0.8 ppm (80 μg/100 g) saxitoxin equivalent[a] 800 ug/kg (live bivalve mollusks)	All fish
	EU – mouse bioassay[b]	80 mg STX eq/100 g of meat[c,d]	Bivalve mollusks
	Africa – mouse bioassay[b]	80 μg STX eq/100 g	mollusks
	Canada – mouse bioassay[b]	<80 mg STX eq/100 g	Mollusks
	Asia – mouse bioassay[b]	400 MU/100 g	Shellfish
	Australia – mouse assay[b]	80 mg STX eq/100 g	Shellfish meat
Amnesic shellfish poison	USA – LC method	20 ppm domoic acid (in general)[a] 30 ppm (in viscera of dungeness crab)[a]	All fish
	Europe – LC method	20 mg/kg of domoic acid[c]	Live bivalve mollusks
	Canada – LC method	20 mg DA/kg	Mussel
	New Zealand – LC method	20 mg DA/kg	Shellfish
Neurotoxic shellfish poison		0.8 ppm (20 mouse units/100 g) (USA)	Clams, mussels, and oysters, fresh, frozen, or canned
Diarrheic shellfish poison	EU – mouse bioassay[b]	160 ug of okadaic acid/kg[c]	Mollusks, echinoderms, tunicates and marine gastropods
	Asia (Japan) mouse bioassay	5 MU/100 g whole meat	Shellfish
	Australia	16–20 μg OA eq/100 g	Shellfish
Azaspiracids	USA – mouse or rat bioassay	160 ug azaspiracid equivalents/kg[c]	Bivalve mollusks, echinoderms, tunicates, and marine gastropods
	Europe – mouse or rat bioassay	160 μg/kg	Live bivalves
Ciguatoxin		Presence[c]	Fishery products
Histamine	USA – extraction coupled to fluorescence spectroscopy	50 ppm	Tuna, mahi mahi, and related fish
	Europe	<200 ppm[e]	Scombridae,clupeidae, engraulidae, and coryphaenidae

Compliance policy/programs

[a]Compliance Program 7303.842 or Sec 540.250 Compliance Policy Guide

[b]FAO (2004) Marine Biotoxins Food and Agriculture Organization of the United Nations Rome, 2004 http://www.fao.org/docrep/007/y5486e/y5486e00.HTM available online, retrieved December 30, 2009

[c]Regulation (EC) No 853/2004 (European standards)

[d]EU Directive 91/492/EEC

[e]Council directives 91/493/EEC

Table 17.5 Seafood-associated toxic heavy metals and action level set by the FDA

Seafood Health Hazard	Tolerance threshold	Targeted seafood
Methyl mercury	1.0 ppm[a]	All fish
Arsenic	86 ppm (76 ppm for crustacea)[b]	Clams, oysters, and mussels
Cadmium	4 ppm (3 ppm for crustacean)[b]	Clams, oysters, and mussels
Chromium	13 ppm (12 ppm for crustacea)[b]	Clams, oysters, and mussels
Lead	1.7 ppm (1.5 ppm for crustacea)[b]	Clams, oysters, and mussels
Nickel	80 ppm (70 ppm for crustacean)[b]	Clams, oysters, and mussels

Compliance Policy/program
[a]Sec 540.600 Compliance Policy Guide
[b]Appendix 5 – FDA & EPA Safety Levels in Regulations and Guidance

Table 17.6 Seafood-associated environmental pollutants and action level set by the FDA

Seafood health hazard	Tolerance threshold	Targeted seafood
Polychlorinated biphenyls (PCBs)	2.0 ppm (edible portion)[a]	All fish
DDT, TDE and DDE	5.0 ppm (edible portion)[b]	All fish
Chlordane –	0.3 ppm (edible portion)[b]	All fish
Chlordecone –	0.3 ppm (0.4 ppm in crabmeat)[b]	All fish
Mirex	0.1 ppm[b]	All fish
Diquat	0.1 ppm[c]	All fish
Heptachlor and heptachlor epoxide	0.3 ppm[b]	All fish
Glyphosate	0.25 ppm[d] 3.0 ppm (for Shellfish)	Fin fish
Fluridone	0.5 ppm[e]	Fin fish and crayfish
Simazine	12 ppm[f]	Fin fish
Aldrin and dieldrin –	0.3 ppm[b]	Fin fish and shellfish

Compliance Policy/program
[a]21 CFR 109.30
[b]Sec 575.100 Compliance Policy Guide
[c]40 CFR 180.226
[d]40 CFR 180.364
[e]40 CFR 180.420
[f]40 CFR 180.213

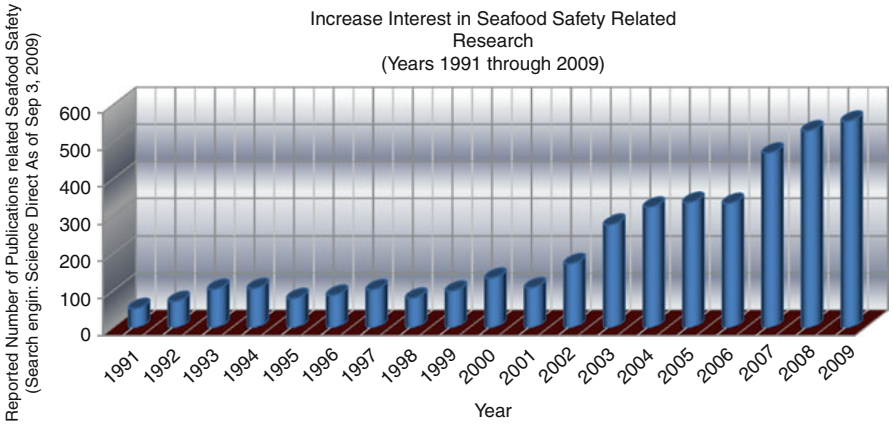

Fig. 17.8 Increased interest in seafood safety related research

Detection Tools for Seafood-Associated Health Hazards

Detection of biotoxins. Years of effort have yielded a wide range of approaches for toxin detection in seafood. These include various bioassays (in vivo and in vitro assays), biochemical techniques (immunoassays), and chemical techniques including fluorometric and colorimetric techniques, chromatographic techniques, electrophoretic techniques, mass spectrometry, and finally biosensor-based techniques (Table 17.7). In countries around the world, the mouse bioassay, despite its numerous shortcomings, and liquid chromatography are the two official methods recommended for biotoxin detection in seafood (Table 17.4).

Chromatographic techniques are at the heart of several effective analytical approaches to biotoxin separation and detection. Compared to animal assays, analytical techniques present the advantages of higher accuracy and sensitivity. While with animal bioassays, it is impossible to clearly determine the nature of contaminants, these techniques, coupled with the appropriate detection methods, will permit not only separation and accurate identification of incriminated toxins, but also, using a standard curve, their quantification. A variety of analytical approaches is currently available for the detection of marine toxins. These include Gas Chromatography (GC), Thin-Layer Chromatography (TLC), Liquid Chromatography (LC), Liquid Chromatography–Mass Spectrometry (LC-MS), and Capillary electrophoresis [21, 59]. In addition to advantages listed above, with chromatography-based detection methods, several toxins can be monitored simultaneously. A new liquid chromatography–tandem mass spectrometry method was developed recently [60]. With this method, up to 28 marine lipophilic toxins can be monitored at the same time. In this case, toxins are separated using a gradient of acetonitrile/water at alkaline pH on a new type of C18 column proven stable under these conditions: a Waters X-Bridge C_{18} (150 mm $\times$ 3 mm, 5 μm) [60].

Biosensor-based detection methods have also been investigated for application in seafood safety programs. They are not only economical, straightforward, and easy to use, but also offer the advantages of high sensitivities/low limit of detection, plus, these technologies are portable [21]. An example of a recent development in this field is a new planar interdigital sensor-based sensing system developed by Syaifudin et al. (2009). This approach involves simple monitoring of variations of reactive impedance of the planar interdigital sensors. Using this approach, as little as 12.6 μg/g of domoic acid in mussel meat, for instance, can be successfully detected [61].

ELISA has also been investigated widely for application in seafood biotoxin detection. An example of a recent development in this field was made by Zhou et al. (2010), who reported a reliable ELISA-based approach to monitor brevitoxin in mollusks with reduced interference from the matrices. Oysters and cockles were used in this experiment. With such a method, the limit of detection of brevitoxin is improved. The main advantage of immunoassays, which are based on antibody–antigen interactions, is high specificity [62].

Table 17.7 Proposed methods for biotoxin detection in seafood [21, 24, 63]

	PSP	DSP	ASP	Ciguatera	NSP	SFP
Bioassays						
In vivo assays	Mouse bioassay	Mouse bioassay	Mouse bioassay	Mouse bioassay	Mouse bioassay	
		Suckling mouse assay		Chicken assay	Fish bioassay	
		Rat bioassay		Mongoose and cat assay		
		Daphnia magna assay		Brine shrimp assay		
		Intestinal loop assays		Mosquito assay		
				Diptera larvae assay		
In vitro assays	In vitro hippocampal slice assay	Cytotoxicity assays (rat hepatocytes, KB cells, fibroblasts)	Receptor binding assays	Sodium channel binding assays for ciguatoxins	Neuroblastoma cell assay	
	Sodium channel blocking assay		Hippocampal slice preparations		Synaptosome binding assay	
					Hippocampal slice assay	
Biochemical techniques	Immunoassay (ELISA)	Immunoassay (RIA or ELISA)	Immunoassay (ELISA)	Immunoassay		(Radioimmunoassay
		Immunoassay (ELISA)			Acid phosphatase assay	Enzyme-linked immunosorbent assay (ELISA)
						Stick tests
						Immunoassays based on monoclonal antibodies
Solid-phase	immunobead assay)					
		TLC,GC, LC				

Chemical/ analytical techniques	Fluorometric and colorimetric technique, MS		Amino acid analysis	Chromatographic detection	Micellar electrokinetic capillary chromatography detection	Chromatography coupled with fluorimetric detection or derivatization techniques
	Chromatographic and	Electrophoretic techniques (TLC, GC,CE, HPLC)	MEKC, MS	TLC, LC, CE, MS	Nuclear magnetic resonance (NMR)/mass spectrometry (MS)	Electrospray LC/MS
					Ionspray LC/MS	
					Mass spectrometry	LC/MS/MS
Capillary zone				electrophoresis	Biosensors based techniques	
Sodium channels based biosensors	Immuno sensors	Optical immuno sensors				
	Enzyme inhibition based biosensors	Molecularly imprinted polymer based biosensors				

Drug residues in seafood. With the rise in demand of fish and shellfish, a large portion of seafood found in market places around the world comes from aquaculture, especially from China. As it is the case with any animal husbandry, veterinary drugs are often heavily used in aquaculture to control pests, infections, or to increase production. Unfortunately, drug residues, often molecules that have proven harmful to humans, are found in edible seafood tissues. As previously mentioned, lately in the USA as in Europe, there have been a significant number of alerts regarding seafood imports contaminated with unapproved drug residues (Fig. 17.7). Over the years, through the dedication of the scientific community, better and more improved detection methods have been made available. An example in this case is liquid chromatography (LC) coupled with triple-quadrupole mass-spectrometry (LC–QqQ-MS). Until recently, in a single run, this sort of method could only analyze related molecular entities. An upgraded and more robust version, a multicomponent quantitative HRLC–ToF-MS, was reported recently. This new approach has proven effective at simultaneously monitoring a wide range of unrelated drugs generally used in aquaculture or found in seafood tissues [64]. Smith et al. (2009) also developed an LC-ion trap mass spectrometry approach, effective at detecting several types of veterinary drugs in fish samples. In this case, the extraction of drug residues from seafood matrixes is completed in acetonitrile and hexane followed by HPLC separation on a phenyl column. In certain cases, imidazoles, macrolides, fluoroquinolones for instance, very low concentrations (0.01 ppm) could be detected using this technique. Other drugs involved in these studies included ionophores, macrolides, nitroimidazoles, benzimidazoles, anthelmintics, penicillins, quinolones, sulfonamides, tetracyclines, amphenicols, and tranquilizers, among others [64, 65].

Detection of microbial pathogens in seafood. The best approaches for the detection of microbes in seafood are usually a combination of culture-based and molecular-based techniques; the latter being often used to assist in bacterial strain identification, while culture is required for enrichment purpose. The kind of medium used to this end is dictated by the type of microorganisms targeted. Over the years, a tremendous amount of effort has been dedicated to the conception of superior media. Recommended Standard Operating Protocols for use in microorganism detection, in seafood, are described in detail in the FDA's 1998 Bacteriological Analytical Manual (BAM) [63]. Targets of quality control programs are numerous. These include various pathogenic bacteria such as *Vibrio*, *Salmonella, S. aureus, L. monocytogenes*, and *E. coli* as well as indicators of fecal contamination [26].

Several molecular and culture-based methods have been developed to assist in the detection of *V. parahaemolyticus* (Vp) and *V. vulnificus* (Vv), which are major issues as far as seafood safety is concerned. Commonly used culture-based approaches, which present the main disadvantages of being time consuming, laborious, and inaccurate, are the MPN and the ISO cultural methods [40]. Quite a few *Vibrio*-specific media has been developed to assist in the isolation of these bacteria from seafood matrixes; these include TCBS agar (for *V. cholera* and *V. parahaemolyticus)* and modified cellobiose polymyxin colistin (mCPC) and

CC agar for *V. vulnificus* [66]. The MPN method, coupled with various techniques to assist in the identification of suspect isolates, is recommended for detection of Vv and Vp in seafood. Examples of approaches used for these identifications are the establishment of a biochemical profile, DNA probes, or PCR. In the case of PCR for instance, DNA primers targeting tdh and *trh* genes are used to detect virulent strains of *V. parahaemolyticus*.

Until the advent of real-time PCR, this approach presented the main drawback of being limited to qualitative evaluation of food samples. Today, faster and quantitative assessment of *Vibrio*'s presence in food samples is possible [40] More sophisticated methods have been introduced. In 2009 for instance, Espiñeira et al. introduced a sequential multiplex PCR system for the detection of *Vibrio* sp. that have been involved in fish and shellfish poisoning, namely *V. cholerae*, *V. parahaemolyticus, V. vulnificus, V. alginoliticus*, and *V. mimicus*. This method, which has been validated, is not only able to detect problematic *Vibrio* species, but it can also, using a fragment analysis, confirm the viable/dead status of these microorganisms and most importantly, probe for the presence of important serogroups and virulence factors [67]. Genetic markers, a precious tool for PCR, have been identified for several other seafood-borne pathogens. These include cytotoxin-hemolysin (*V. vulnificus*), *ctxAB* (*V. cholerae*), *oriC*, chromosomal origin of replication (*Salmonella* spp.), listeriolysin O (*hly*), and the 16 S rRNA (*L. monocytogenes*), polymerase gene (hepatitis A virus), and polymerase gene for norovirus, just to name a few [66].

Examples of Recent Technological Breakthroughs in Efforts to Free Seafood from Associated Contaminants

In recent years, outstanding breakthrough techniques and cutting-edge technologies to aid in freeing seafood from associated contaminants have been developed. Molluskan shellfish and associated pathogens (especially *V. vulnificus* and *V. parahaemolyticus*), scombrotoxin, fish safety (especially of cold-smoked salmon), and bio-preservation of seafood are a few examples of highly investigated topics.

Molluskan shellfish and associated pathogens. Nowadays, seafood regulatory authorities face major issues with molluskan shellfish. For years now, molluskan shellfish has been classified as "high-risk" seafood by the FDA. They were responsible for the largest number of seafood-borne illnesses during 1990–2006 (Fig. 17.4). Because of their filter-feeding habits, they tend to accumulate a wide range of etiological agents (pathogenic bacteria, parasites, and viruses) as well as biotoxins. The emergence of innovative FDA-approved Post-Harvest Processing (PHP) technologies such as Individual Quick Freezing (IQF), Heat-Cool Pasteurization (HCP), and High Hydrostatic Pressure (HHP) has revolutionized the industry of seafood, particularly in regard to oysters. These technologies, which are currently commercially available, have made it possible to bring raw and healthy products to consumers. An end product of high quality (fresh taste and superior

appearance) is the major advantage of IQF, HCP, and HHP. These processing techniques reduce the level of *Vibrio* bacteria to undetectable levels [67]. Though HHP is already approved by the FDA, several attempts to perfect this technology are currently on the way. In 2008 for instance, using a pressure-resistant strain of Vp (MLT 403) to picture the worst-case scenario, Kural et al. proposed better pasteurizing conditions. Under such conditions, a 5-log reduction in the load of the pressure-resistant Vp in live oysters could be achieved. These conditions were as follows: a 2-min treatment at pressure $\geq$350 MPa (1–35°C) and a 2-min treatment at 40°C (pressure $\geq$300 MPa) [68].

As far as seafood safety is concerned, HHP processing is an especially promising technology. Its inactivating effect on a variety of pathogenic agents isolable from seafood has been documented. These agents include viruses, parasites, and several other types of seafood-associated bacterial pathogens. The ability of HHP to inactivate oyster-associated viruses has been extensively studied [69–72]. A 5-min HHP treatment at pressure 400-MPa and 0°C was established as an effective approach to bring murine norovirus-1 to undetectable levels in oysters [71]. HAV can also be effectively inactivated from oyster tissues using HHP. Calci et al. (2005) could achieve a PFU reduction >3 $\log_{10}$ with a 1-min treatment at 400 MPa [69]. It is important to note that temperature, matrices' pH, and salinity have a great effect on the efficiency of pressure-mediated HAV inactivation [70].

Another recent development in the field of molluskan shellfish safety is the application of super critical CO_2 ($scCO_2$), a known antibacterial substance widely used in the food industry to reduce the load of oyster-associated bacteria. Two conditions, 100 bar and 37°C for 30 min and at 172 bar and 60°C for 60 min, were reported as able to induce 2-log and 3-log reductions in the oysters' aerobic plate count, respectively. No significant change in the physical appearance, smell, or texture was recorded (Fig. 17.9) [73].

As previously stated, *V. vulnificus* and *V. parahaemolyticus* are major seafood-associated health concerns. In recent years, there has been a tremendous amount of effort to design approaches to reduce their load, especially in molluskan shellfish. One of the latest innovations in this field is the introduction of a "weak acidic electrolyzed water"-based approach [74]. Quan et al. (2010) have demonstrated that weak acidic electrolyzed water possesses outstanding antibacterial potency against Vv and Vp, especially when compared to sodium hypochlorite (NaClO), a commercial sanitizer [74].

A chlorine dioxide (ClO_2)-based approach was also recently introduced. According to Wang et al. (2010), a 6-h treatment with 20 mg/L of ClO_2 is enough to disinfect oyster tissues contaminated with Vp. These authors particularly recommend this method because it is cost effective; it also has the potential of increasing seafood's shelf life ($\sim$12 days) [75]. In addition, *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes*, other pathogens of importance regarding seafood safety, have proven sensitive to this type of treatment.

Scombrotoxin. Because of the large number of associated outbreaks, scombrotoxin can be viewed as the most dangerous seafood-associated health hazard [2, 3]. To store

Fig. 17.9 Appearance of oysters before and after a 40-min exposure to $scCO_2$

fish at low temperatures is generally considered sufficient to prevent the growth of causative bacteria. Unfortunately, such conditions cannot always be respected, especially during retail processes. Phuvasate and Su (2010) proposed an alternative to low temperature storage applicable under these circumstances, namely the use of Electrolyzed Oxidizing (EO) water and ice. According to these authors, the load of several histamine-producing bacteria on food surfaces and fish skin can be significantly reduced simply by using electrolyzed oxidizing water and ice. Conditions reported as effective using salmon and tuna's skin were, for EO water, 100 ppm chlorine for 120 min, and for EO ice, 100 ppm chlorine for 24 h, respectively. Experts now agree that electrolyzed oxidizing water possesses a great potential as far as seafood safety. Its use is recommended not only because it is environmentally friendly, safe, and cost effective, but also because its application is quite straightforward. Its antibacterial effects against several other seafood-associated bacteria have

been reported. A few examples are *E. coli* O157:H7, *L. monocytogenes*, as well as *Salmonella enteritidis*, *Campylobacter jejuni*, *Enterobacter aerogenes*, and *S. aureus* [76].

Bio-preservation of seafood. To add various chemicals to seafood in order to reduce its bacterial load or inhibit the growth of unwanted bacteria is an option that many have proposed as a solution to some seafood safety issues. Regrettably, consumer acceptance of these products is not always guaranteed. As chemical/preservative-free, ready-to-eat seafood products are gaining in popularity alternatives to the use of chemicals have emerged, and an example is bio-preservation. Recently, there have been a few innovations in this field. An example in this case is the proposed use of *Carnobacterium divergens* M35 and divergicin M35 in an effort to rid seafood from one of its most persistent bacterial contaminants, namely *L. monocytogenes* [77]. Matamoros et al. (2009) characterized several strains of lactic acid bacteria that can be used to this end as well. These bacteria (*Lactobacillus fuchuensis, Leuconostoc gelidum, Lactococcus piscium,* and *Carnobacterium alterfunditum*) showed inhibitory potential against seafood spoiling and pathogenic bacteria [78]. Pinto et al. (2009) also reported two bacteriocins produced by lactic acid bacteria (*Enterococcus faecium* and *Pediococcus pentosaceus*) that can be used to this end [79].

Irradiation and other recent breakthroughs. Several other cutting-edge technologies designed to help deal with seafood-associated health issues have been introduced in recent years. A few examples are X-ray, gamma ray, and electron beam irradiation-based technologies. Gamma irradiation (0.5, 1, 2, and 5 kGy) and electron beam irradiation have recently proven an effective non thermal approach to reduce the load of *V. parahaemolyticus* as well as several other seafood-associated contaminants, namely *L. monocytogenes* and *S. aureus*, in a raw seafood dish (oyster *Jeotkal*). Organoleptic properties were not negatively affected by the irradiation. In this particular case, gamma irradiation appears a better alternative to electron beam irradiation [80].

The beneficial antibacterial effects of electron-beam irradiation applied to another seafood dish (salted and seasoned short-necked clam) were reported in 2009 by Kim et al. It is important to note that, in this case, no change in sensory qualities was observed. A significant microbial inactivation (coliform bacteria, aerobic bacteria, yeast and mold) was reported [81]. Similar results were achieved with cold-smoked salmon [82]. This technique appears to be a better alternative to HHP in regards to sanitization of cold-smoked salmon. In fact, while both approaches (irradiation at 2 kGy and HPP: 450 MPa for 5 min) yielded a safer final product, the visual aspect of HHP-treated, not irradiation-treated, salmon was negatively affected. The microbial load of both products did not exceed 6 $\log_{10}$ cfu/g after 35 days storage at 5°C [82].

X-ray irradiation has also been investigated for use in improving seafood microbiological quality. Several recent publications have demonstrated its beneficial effects on *V. parahaemolyticus*, *V. vulnificus* (shrimp contaminants). It is important to note that several other shrimp-associated bacterial contaminants, namely *E. coli*, *Salmonella enterica*, and *Shigella flexneri*, were inactivated as well [83, 84].

High Hydrostatic Pressure (HHP) was also reported as an excellent alternative to reduce the level of *Listeria* in fish. According to Gudbjornsdottir et al. (2010), a 700–900 MPa treatment of 10 s is sufficient to reduce the load of *L. innocua* to undetectable levels. This experiment was completed with cold-smoked salmon. In this case, though there was no lipid oxidation, some variations in color and microstructure of the final product were noted [85]. The potential of HHP to rid mackerel from parasites, such as the nematode *Anisakis simplex*, was also recently reported. In this particular case, a complete inactivation of the larvae in the fish tissue was achieved at 300 MPa after a 5-min exposure [86].

Another recent report has proposed using CO_2 in packaging fish. Schirmer et al. (2009) proposed using CO_2 combined with various organic acids: citric acid (3% w/w, pH 5) and acetic acid (1% w/w, pH 5) in packaging fresh fish, as an effective way to improve its quality and shelf life. This combination has proven efficient at completely inhibiting bacterial growth in naturally contaminated salmon stored at 4°C for 14 days. Monitored bacteria were *Enterobacteriaceae*, lactic acid bacteria, and sulphur reducing bacteria. Sensory analysis was not completed by these authors [87].

A better alternative to rid shrimp from *V. parahaemolyticus* was recently introduced. The use of chlorine is the current approach to reduce the level of shrimp-associated pathogenic bacteria. Unfortunately, health issues such as bronchitis and pulmonary edema in workers have been reported. Norhana et al. (2009) proposed an even simpler approach to deal with shrimp-associated acterial pathogens. These authors show that washing shrimp with acidic fruit juice, namely bilimbi (*Averrhoa bilimbi*) or tamarind (*Tamarindus indica* L.) was also an effective way to significantly reduce its load of bacteria [88]. In 2007, Chaiyakosa et al. reported another safer alternative to reducing the load of *V. parahaemolyticus* in shrimp, namely the use of Chitosan [89]. Another author reported this same substance as an effective means of bringing safer salmon to consumers. Packing cold-smoked salmon in chitosan-coated plastic films containing 4.5 mg/cm^2 sodium lactate or either a combination of 4.5 mg/cm^2 sodium lactate plus 0.6 mg/cm^2 potassium sorbate or 2.3 mg/cm^2 sodium lactate plus 500 IU/cm^2 nisin was found to be beneficial. It was established that for seafood conditioned using this approach and stored at low temperature (~4°C), *L. monocytogenes*' growth was inhibited for at least 6 weeks [90].

Future Directions and Conclusions

From pathogenic bacteria, parasites, and viruses of all sorts, to life threatening biotoxins, the range of chemical and biological contaminants present in seafood is broad in scope and challenging to manage. Despite years of efforts from regulatory authorities and the scientific community, the public at large is still at risk of dangers associated with seafood consumption. For several years now, seafood has ranked

foremost as the most significant source of food-borne disease outbreaks of known origin. Contributing factors are numerous and represent key points upon which urgent action, from regulatory authorities and the scientific community is required. Special attention should be paid to deleterious agents that have been associated with the largest number of outbreaks in the past years. In the USA for instance, major threats were scombrotoxin, responsible for 36% of all reported seafood-borne outbreaks from 1990 to 2006, followed by ciguatera (responsible for 21% of outbreaks), bacteria (especially *Vibrio* spp) responsible for 24% and finally noroviruses, which cause about 10% of all outbreaks reported during this same period [2]. It is important to note that these same agents pose serious problems in other parts of the world, as well. Scombroid fish poisoning, for instance, is also a serious problem in countries like Japan and the UK [28] *Vibrio* spp, especially *V. parahaemolyticus,* have been reported as a major problem in several Asian countries [40]. A recent study, a 2005–2008 survey of shellfish (mussels, clams and oysters), showed that norovirus is a problem in Italy, as well [91].

The task of ensuring consumers protection against the dangers of seafood is shared by several groups. These include (1) regulatory authorities, which depend heavily on the scientific community, and are in charge of the promulgation and enforcement of laws and regulations, (2) establishments involved in the harvest, processing, storage, and retail of seafood, and (3) consumers themselves. Failures and flaws at various levels of the current safety management system explain why seafood has been a persistent issue for the past few years. A certain number of defects in the seafood regulatory system of the USA for instance are presented in a 2008 report of the Center for Science in the Public Interest (CSPI). Mentioned shortcomings are the "voluntary recall" approach currently in place, added to financial issues. CSPI sees in the limited budget allocated to the FDA (Fig. 17.10), a serious hindrance to its efficiency. This can be a serious hindrance, for instance, when it comes to law enforcement. CSPI reports an extremely low inspection rate of food processing companies by the FDA, a rate that is insignificant compared to the USDA's [2]. Currently, in the USA, there are approximately 13,400 seafood-processing establishments. The FDA reports having inspected only 3,066, 2,830, and 2,456 during the fiscal years 2004, 2005, and 2006 respectively.

Financial limitations are not the only obstacle to full efficiency of regulatory agencies. The unavailability of effective technological tools that could either help rid seafood from hazardous entities/substances, or assist regulatory authorities in effective risk assessment and management during various control programs is also critical. Without effectual detection methods, efficient law enforcement is nearly impossible. Though numerous approaches applicable to virtually all seafood-associated health hazards have been proposed so far, several gaps remain.

This is the case of biotoxins for instance, which alone were responsible for 63% of all reported seafood-borne diseases from 1990 to 2006. Because they are extremely resistant to various post-harvest processing techniques, as far as assuring seafood safety, preventive measures are a better option. More effective detection tools are thus critically needed in order to reduce the current incidence of biotoxin-linked seafood-borne illnesses. Presently, in this field, there is still a need for cost-effective,

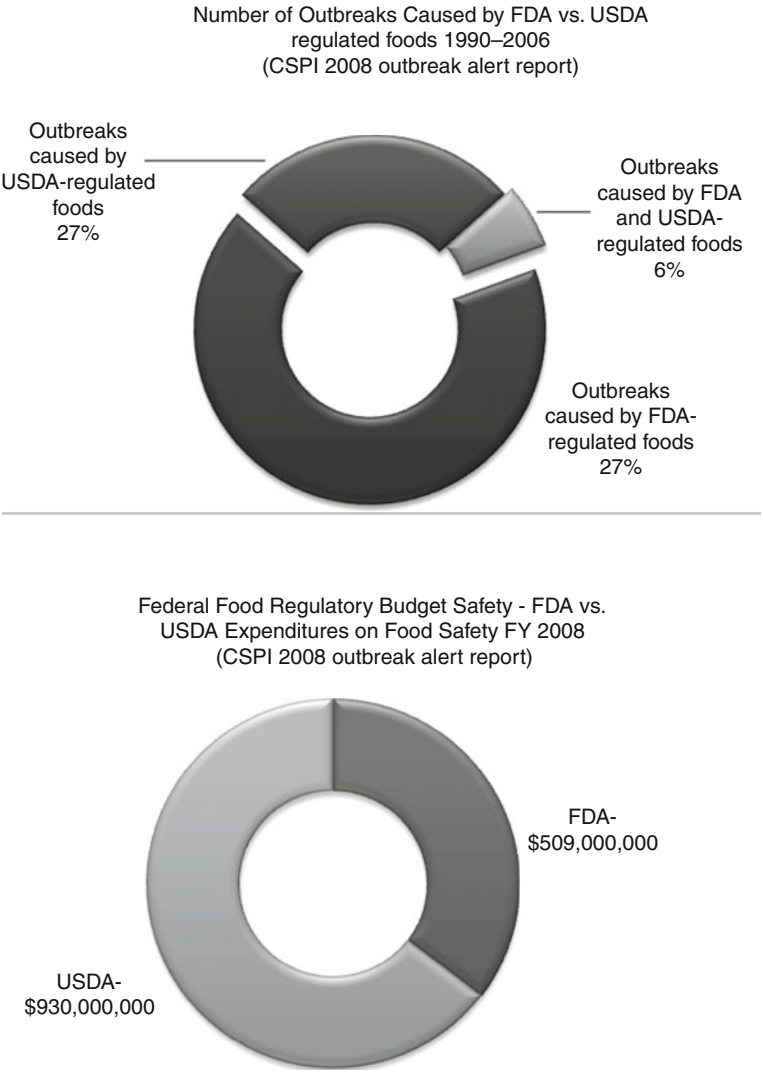

Fig. 17.10 Graphical representation of some limitations in the current federal food safety regulatory system with significant impact on seafood safety

rapid, sensitive, specific, and straightforward methods that can be operated by untrained personnel on a routine basis.

Though several approaches to toxin detection in seafood have been proposed so far, and even significant progress made recently (Table 17.7), animal-based assays and liquid chromatography, techniques that have their share of shortcomings, are still the official methods (Table 17.4). Animal bioassays for instance, are fit to assess the overall toxicity of a sample but cannot give insight on

the nature of the toxin(s) involved. Moreover, these assays are time consuming, of limited accuracy and controversial (ethics). As stated previously, liquid chromatography, on the other hand, when coupled with effectual detection methods, is valuable at accurately identifying the nature and concentration of the incriminated toxin(s). However, it is important to note that, chromatography-based methods are lengthy and not cost-effective; heavy equipments and trained specialists are needed. Better methods have been proposed, but, none has so far been approved by regulatory authorities for routine use. Though these new methods present some clear advantages, it is important to point out that they also possess some weaknesses. Among these are difficulty of application on a routine basis, the necessity for heavy equipment, and the complexity of protocols and generated data, just to name a few.

There are flaws in current approaches to control scombroid fish poisoning and ciguatera. In the case of scombroid fish poisoning for instance, there is still a need for more effective alternatives to control Histamine-Producing Bacteria (HPB). Current recommendations of the FDA involve rapid cooling ($\leq$40°F) after visceration (for larger fish) upon death. Unfortunately, this approach possesses a few shortcomings. It is important to note that not all HPB are mesophiles. Several studies showed that histamine can be produced even at low temperature by physchrophilic HPB [28, 92]. Ciguatera was second to scombrotoxin as the most likely cause of seafood-borne disease outbreak from 1990 to 2006. Though ciguatera is such an issue in the USA, "there are neither standards, nor an official method" that applies to Ciguatera Fish Poisoning (CFP) in this country [24]. Innovations, regulation-wise, in this regard are obviously critical. Current efforts with respect to CFP prevention involve various toxin-monitoring programs, education, alongside with bans on the sale and capture of fish most likely to cause poisoning (Europe, Australia).

Bacteria were next to toxins as the most prevalent cause of seafood-borne illnesses during the past decade. Fortunately, in this case, there are currently several approved Post-Harvest-Processing (PHP) approaches aimed at reducing their load in seafood. These included IQF (Individually Quick Frozen), HCP (Heat Cold Pasteurization), and HHP (High Hydrostatic Pressure). Though these techniques have revolutionized the industry of oysters, for instance, there is still room for improvement, especially because oysters do not survive such processing [93]. In terms of shelf life, this can be an issue. To store, HHP-, IQF- and HCP-treated oysters at low temperatures is, unfortunately, not enough to solve the issue. Prapaiwong et al. [94], studying variations in the bacterial load (total aerobic bacterial counts) of HHP-treated oysters stored at 4°C for instance, determined that the bacterial count of processed products can rise quickly during storage and can even reach $\sim 10^7$ CFU/g in just 7 days [94]. Moreover, long-term storage presents the disadvantage of increasing the final production cost. Post-harvest-processing techniques non lethal to oysters would be, without the shadow of a doubt, a better alternative. Mahmoud and Burrage (2009c) reported X-ray irradiation as a better approach for reducing the load of oysters-associated *V. parahaemolyticus* because oysters are able to survive even extremely high X-ray

doses [95]. Oysters have also been shown to be able to survive $scCO_2$ exposure, making it an attractive tool for further exploration [73].

Norovirus is another major seafood-associated health hazard. Major gaps in the current system are regulatory and scientific. Despite the clear threat posed by this virus, not much effort has apparently being exerted regulation-wise. As mentioned by Terio et al. [91], "there is no virological standard for bivalve shellfish in European legislation" [91]. Though several approaches to the detection of norovirus in seafood have been proposed so far, much still needs to be done; an area in need of improvement is the development of more efficient methods of viral RNA extraction. RNA extraction is a critical step in several virus detection protocols. RNeasy Kit was recently presented by Husman et al. (2009) as a most useful alternative for viral RNA extraction after comparing five such approaches side by side. A modified paramagnetic silica-based guanidium extraction based technique was also developed recently [96, 97].

Other challenges faced in norovirus detection are related to the virus' genetic variability and scarcity in samples. Interferences of seafood matrix, mainly, the presence of inhibitory substances, also represents a serious obstruction [97]. An effective TaqMan RT-PCR based approach for quantification of genogroups I and II norovirus, which presents the advantage of overcoming background inhibitory effects, was introduced recently by Gentry et al. (2009) [98]. An effective and more sensitive multiplex RT–PCR-based approach to norovirus and rotavirus detection in oysters was also introduced recently [99].

There is no doubt that the development of effective technologies able to surmount each and every one of these challenges will aid efforts to reduce outbreak incidents of seafood-borne infections attributable to viruses.

Compliance Policy/Regulation Related Citations

Compliance Program 7303.842
Sec 560.600 Compliance Policy Guide
Sec 555.300 Compliance Policy Guide
21 CFR 522.1081
21 CFR 529.1030
21 CFR 529.2503
21 CFR 556.500
21 CFR 558.450
21 CFR 558.582
21 CFR 556.660
21 CFR 558.575
21 CFR 556.640
Council Regulation (EEC) No 2377/90 of 26 June 1990
Sec 615.200 Compliance Policy Guide

21 CFR 556.660
Compliance Program 7303.842 or Sec 540.250 Compliance Policy Guide
Sec 540.600 Compliance Policy Guide
21 CFR 109.30
Sec 575.100 Compliance Policy Guide
40 CFR 180.226
Sec 615.200 Compliance Policy Guide
21 CFR 556.660
40 CFR 180.364
40 CFR 180.420
40 CFR 180.213
Sec 540.525
Regulation (EC) No 853/2004 (European standards)
EU Directive 91/492/EEC
Council directives 91/493/EEC

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