

Seafood Toxins

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KEY CONCEPTS

- Seafood poisoning is uncommon in non-endemic regions, but may be responsible for adverse reactions to seafood.
- Marine toxins produce syndromes with primarily acute gastrointestinal and neurological manifestations that frequently masquerade as allergic reactions.
- Seafood poisoning may result in similar symptoms in several individuals who shared the seafood and display an “endemic” nature.
- The absence of prior history of allergy to seafood and its subsequent tolerance point away from an allergic etiology and suggest poisoning.
- Knowledge of specific seafood toxic syndromes is necessary to consider them in the differential diagnosis and obtain the appropriate history and collect specimens to confirm the diagnosis and institute the correct treatment.

Introduction

Fish and shellfish are nutritious foods that constitute desirable components of a healthy diet. However, seafood, including fish, shrimp, lobster, crabs, crayfish, mussels, and clams, are listed among the most frequent causes of food allergy [1–3]. The differential diagnosis of seafood allergy is extensive. It includes true hypersensitivity reactions to non-seafood components, such as peanut or tree nuts, foods that may cross-react with seafood allergens or food contaminants, such as antibiotic residues contained in seafood, adverse reactions to food additives, such as sulfites, monosodium glutamate (MSG), and tartrazine, as well as seafood-associated poisoning (Table 41.1). Seafood poisoning primarily results in acute gastrointestinal and neurological manifestations that frequently masquerade as allergic reactions on presentation to emergency departments and urgent care clinics and are often misdiagnosed [4–8]. Bacteria and bacterial toxins may cause gastrointestinal and systemic symptoms that can also be confused with food allergy. In the United States, seafood poisoning, principally scombroid and ciguatera fish poisoning (Table 41.2), was responsible for about 4% of all reported food-borne disease outbreaks reported by the Centers for Disease

Table 41.1 Differential diagnosis of seafood-associated poisoning

A. Common seafood poisons
1. Fish poisoning
a. Ciguatera
b. Scombroid
c. Tetrodon poisoning
2. Shellfish poisoning
a. Paralytic
b. Neurotoxic
c. Amnesic
d. Diarrhetic
B. Less common seafood poisoning
1. Fish
a. Clupeotoxin
b. Elasmobranch
2. Mollusks
a. Red whelks
C. Infections and bacterial intoxications
1. Bacterial toxins
a. <i>Clostridium botulinum</i>
b. <i>Staphylococcus aureus</i>
2. Bacterial infections
a. <i>Vibrio cholerae</i>
b. <i>Vibrio parahaemolyticus</i>
c. <i>Vibrio vulnificus</i>
3. Viral infections
a. Norwalk and Norwalk-like enteric viruses

Table 41.2 Epidemiology of seafood poisoning in the United States from 1978 through 2004*

Year	Scombroid		Ciguatera		PSP		NSP		AFP		PFP	
	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases
1978	7	30	19	56	4	10	0	0	0	0	0	0
1979	14	134	21	91	1	3	0	0	0	0	0	0
1980	28	151	15	52	5	116	0	0	0	0	0	0
1981	9	93	30	219	0	0	0	0	0	0	0	0
1982	18	58	8	37	1	5	0	0	0	0	0	0
1983	13	271	13	43	0	0	0	0	0	0	0	0
1984	12	53	18	78	0	0	0	0	0	0	0	0
1985	14	56	26	104	2	3	0	0	0	0	0	0
1986	20	60	18	70	0	0	0	0	0	0	0	0
1987	22	98	11	35	0	0	0	0	0	0	0	0
1988	16	65	4	8	1	6	0	0	0	0	0	0
1989	17	80	19	66	0	0	0	0	0	0	0	0
1990	11	194	11	44	2	24	0	0	0	0	0	0
1991	17	40	7	50	2	35	0	0	1	29	0	0
1992	15	135	1	8	0	0	0	0	0	0	0	0
1993	5	21	13	44	0	0	0	0	0	0	0	0
1994	21	83	11	54	3	29	0	0	0	0	0	0
1995	16	91	10	27	1	7	0	0	0	0	0	0
1996	19	55	9	32	0	0	1	3	0	1	1	3
1997	22	92	18	65	2	4	0	0	0	0	0	0
1998	27	124	16	73	1	6	0	0	0	0	0	0
1999	19	59	11	41	0	0	0	0	0	0	0	0
2000	19	73	12	46	3	9	0	0	0	0	0	0
2001	27	126	23	79	0	0	0	0	0	0	0	0
2002	21	59	20	68	1	21	0	0	0	0	0	0
2003	33	187	16	55	1	2	0	0	0	0	0	0
2004	18	48	10	31	2	4	0	0	0	0	0	0
1978–2004	480	2536	390	1576	32	284	1	3	1	30	1	3

*Number of outbreaks and cases (in parenthesis) reported to the Center for Disease Control and Prevention (CDC) (data from [9–11]).

Control from 1988 through 2002 [9,10] (Table 41.3). This was significantly smaller than 17.8% reported for the period 1978–1987 [11]. In Australia from 1995 to 2000, ciguatera and scombroid poisoning were responsible for 11% and 3% of all foodborne outbreaks, respectively [12]. Worldwide ciguatera is the most frequently reported poisoning associated with seafood [13].

National surveillance data on seafood-related poisoning in the United States is based on outbreaks of acute foodborne disease reported by state health departments to the CDC. From 1978 through 2004 there were 480 outbreaks with 2536 cases of scombroid poisoning and 390 outbreaks and 1576 cases of ciguatera poisoning. Thirty-two outbreaks of paralytic shellfish poisoning (PSP) involving 284 people were reported that included two large California outbreaks in 1980. There was one case each of puffer fish poisoning, neurotoxic shellfish poisoning (NSP), and amnesic shellfish poisoning (ASP) during this period [9,10,14,15]. However, these figures are likely to underrepresent the true

incidence of seafood poisoning, since some cases remain undiagnosed and many are not reported to health authorities. For instance, even in the endemic area of Queensland, Australia, it is estimated that only about 20% of ciguatera cases are reported to the local database [16].

Based on the presence or absence of the toxin at the time of capture, fish poisoning can be classified into two categories. In ciguatera and puffer fish poisoning the toxin is present in the live fish, whereas in scombroid it is produced only after the capture in the fish flesh by contaminating bacteria that spoil improperly refrigerated fish. Puffer fish poisoning is associated with a high rate of mortality, as opposed to scombroid and ciguatera that are self-limiting illnesses and resolve spontaneously in the vast majority of cases.

The bulk of shellfish-associated illness is infectious in nature, which can be either bacterial or viral, with the Norwalk virus likely to account for most cases of gastroenteritis. Ingestion of contaminated shellfish results in a wide variety of symptoms, depending on the toxins present, their

Table 41.3 Number of outbreaks and cases associated with seafood toxins and their relative contribution to the foodborne diseases (1988–2002)

Year	Outbreaks of seafood poisoning	Cases of seafood poisoning	Percentage of outbreaks of seafood poisoning	Percentage of cases of seafood poisoning	Total outbreaks of foodborne illness	Total cases of foodborne illness
1988	21	79	4.66	0.502	451	15,732
1989	36	146	7.13	0.92	505	15,867
1990	24	262	4.5	0.136	533	19,231
1991	27	149	5.08	0.99	531	15,052
1992	16	143	3.89	1.29	411	11,083
1993	18	65	3.5	0.46	514	14,080
1994	35	166	5.07	0.997	690	16,995
1995	27	125	4.19	0.927	645	13,497
1996	30	93	4.98	0.603	602	15,421
1997	42	161	5.21	0.856	806	18,802
1998	44	203	3.35	0.76	1314	26,719
1999	30	100	2.23	0.532	1344	18,802
2000	34	128	2.4	0.491	1417	26,043
2001	50	205	4.04	0.819	1238	25,035
2002	42	148	3.15	0.593	1332	24,971
1988–2002	476	2173	3.86	0.734	12,333	277,330

Calculated from [9,10].

concentrations in the shellfish, and the amount of contaminated shellfish consumed. Five different types of shellfish poisonings have been identified including paralytic, neurotoxic, diarrhetic, amnesic, and azaspiracid (AZA) poisonings. PSP may be severe and life threatening, but other shellfish poisonings are usually transient, self-limited, and rarely fatal. Except for scombroid, there is no antidote for seafood poisoning and treatment is primarily supportive.

Toxins responsible for the clinical manifestations are generally produced by microscopic marine algae in the warmer summer months, which are then concentrated in filter-feeding bivalve mollusks, such as clams and mussels. These toxins are retained and concentrated over time. Of the estimated 4000 species of marine algae worldwide, <2% produce toxins [17]. Only about 30 dinoflagellates and a few diatom species are known to cause human illness, and fewer still are potentially lethal [18]. Generally marine toxins do not alter the appearance, taste, or smell of seafood, and are not inactivated by heat or gastric acid. Anthropogenic eutrophication has been incriminated in the higher frequency of harmful algal blooms and increased production of biotoxins by marine dinoflagellates [19]. However, the incidence of shellfish poisoning has been declining, most likely because of careful monitoring, beach closures, and improved public awareness. It is recommended that the public should avoid collecting shellfish from areas where red tides are known to occur and refrain from consumption of suspect shellfish that should be submitted to health authorities for investigation [20].

Seafood poisoning is largely a regional problem and cases are usually concentrated in endemic areas. However, poisonings associated with imported seafood are an exception, since they occur sporadically and do not follow geographic patterns. At present well over half the seafood supply in the United States is imported; and as reef fish are increasingly exported from the tropical areas, seafood poisoning has become a more widespread problem. Most current health risks associated with seafood contamination originate in the environment and should be dealt with by control of harvest or at the point of capture by application of principles of hazard analysis and critical control point (HACCP). Some seafood poisonings, although not a problem in the United States, could become one, as international tourism increases and seafood from different regions of the world becomes available. Thus, knowledge about some of these clinical syndromes is helpful.

Some marine toxins are allelopathic and function in nature to inhibit the growth of other microalgae as an adaptive mechanism. Animals may have evolved to acquire toxicity by sequestration of toxic compounds in their food source, which provides protection from predators that have learned to avoid them. Recently, two new classes of marine toxins that can cause human disease were discovered: azaspiracid and spirolides. The sources of these toxins have also been identified in phytoplanktons that have widespread presence in Atlantic waters. The occurrence of these toxin classes in seafood presents new challenges to the seafood industry and the regulatory agencies. Aquaculture is

gaining an ever-increasing importance in production of seafood, which introduces new challenges to health care and the practicing physician. Use of algicides, antibiotics, and antiparasitic medications that leave detectable residues in farm-raised seafood is a potential human health hazard. Genetic engineering and neo-antigens incorporated into seafood or introduced into other food from a marine origin can present an alternative source of antigen that could potentially lead to allergic sensitization.

In this chapter, special emphasis is placed on important aspects of the clinical picture, the marine species most commonly involved, and their general geographic distribution; information that we hope will be helpful in recognizing these reactions, making the correct diagnosis, and differentiating them from seafood allergy. Current knowledge on mechanisms of toxicity and methods of detection and quantification of various seafood toxins are reviewed and general treatment and preventive measures are discussed.

Common intoxications associated with fish

Scombroid (histamine poisoning)

A constellation of gastrointestinal, neurological, cardiovascular, and cutaneous symptoms such as nausea, vomiting, diarrhea, abdominal cramping, throbbing headache, palpitations, flushing, tingling, burning, itching, hypotension, urticaria, and angioedema characterize scombroid. In severe cases and in persons with asthma, bronchospasm may develop. The most frequent symptoms are tingling and burning sensations around the mouth, gastrointestinal complaints, and a skin rash. Patients sometimes describe a peppery or bitter taste to the fish, but often the fish tastes completely normal. In general the onset of symptoms is rapid, usually within 10–30 minutes of ingesting fish. Physical signs may include a diffuse blanching erythema, tachycardia, wheezing, and hypotension or hypertension. Immediate reactions may be indistinguishable from anaphylaxis and scombroid is often misdiagnosed as an allergic reaction [4–8]. Scombroid intoxication results from ingestion of fish containing high levels of free histamine. Since histamine is resistant to heat, cooking the fish and even high temperatures used in canning process will not prevent scombroid poisoning [21]. Because the symptoms are usually self-limited and resolve in the vast majority of cases within 4–10 hours without any sequelae, there is often no need for specific treatment. However, H1 and H2 antihistamines ameliorate the symptoms in severe cases [22]. The mildness and transient nature of scombroid contribute to underreporting of the disease.

Initially, the disease was associated with consumption of scombroid fish. Scombroid means like mackerel (*Scomber*); fish belonging to the Scombroidea family that are found in temperate and tropical waters including tuna, mackerel, bonito, and saury. More recently, other non-scombroid species have been identified as causing the intoxication, including

mahi-mahi, bluefish, jack, mackerel, amberjack, herring, sardine, and anchovy. Some of these species constitute highly commercialized marine products and have been among the most valuable resources of the canning industry [23]. In the United States between 1978 and 1999, scombroid poisoning owing to mahi-mahi, tuna, and bluefish accounted for the majority of the cases reported to CDC [9–11].

The histamine is not present when the fish are caught, but is later produced during spoilage by decarboxylation of free histidine, which is naturally present at high levels in species of fish implicated in scombroid [24]. The production of histamine is due to the action of histidine decarboxylase, an enzyme produced by bacteria growing on the fish. The enteric bacteria *Morganella morganii*, *Klebsiella pneumoniae*, and *Hafnia alvei* are most frequently implicated. These organisms are not considered as natural flora of living fish and contamination probably occurs during catching and handling [25]. This reaction occurs optimally between 20°C and 30°C and is prevented by refrigeration or chemical decontamination. Experimental studies have shown that histamine formation is negligible in fish stored at 0°C [26].

Even though histamine levels may not be correlated with any obvious signs of decomposition, histamine content may be used as an index of spoilage in certain fish. Fresh fish normally contain histamine levels of <10 ppm or 1 mg/100 g of fish flesh. Laboratory confirmation of scombroid is based on demonstrating elevated histamine levels >50 ppm in the muscle tissue of incriminated fish using an enzyme-linked immunosorbent assay (ELISA) [27,28].

Although histamine was first suggested as the causative toxin over 50 years ago, it was not until 1991 that urinary excretion of histamine, in quantities far exceeding those required to produce toxicity, was documented *in vivo* in humans in association with the clinical syndrome [29]. Subsequently, elevated plasma histamine levels were demonstrated in scombroid [30]. Various hypotheses have been put forward to explain why histamine consumed in spoiled fish is more toxic than pure histamine taken orally; one postulates a role for other heat-stable substances produced in fish by putrefactive bacteria that inhibit the metabolism of histamine by intestinal flora and permit absorption of a more substantial portion of the ingested histamine. A second hypothesis suggests that urocanic acid, another imidazole compound derived from histidine in spoiling fish, may induce mast cell degranulation, and endogenous histamine release may augment the exogenous histamine consumed in spoiled fish [31]. There is still controversy about the exact mechanism and none has proved totally satisfactory.

Scombroid is preventable by proper handling and prompt refrigeration of fish at the time of capture and during subsequent storage, processing, and distribution until it is preserved or cooked. Fish should be chilled rapidly to temperatures below 10°C within 4 hours after capture and stored at 0–4°C to keep bacterial numbers and histamine

levels low. Despite the huge expansion in trade in recent years, great progress has been made in ensuring the quality and safety of fish products. This is largely the result of the introduction of international standards of food hygiene and the application of HACCP principles [31].

Ciguatera

Ciguatera fish poisoning is a clinical syndrome that presents after consumption of ciguatoxic fish with characteristic gastrointestinal, neurological, and, occasionally, cardiovascular symptoms [32]. The onset of the symptoms ranges between 30 minutes and 12 hours after ingestion of contaminated fish, depending on the severity of intoxication. Nausea, vomiting, watery diarrhea, and abdominal pain usually develop within 3–6 hours and typically last 12–24 hours. Neurological symptoms develop over 24 hours and tend to be the most distinctive and enduring. They include paresthesias that initially involve the lips, tongue, and throat, which later may extend to the extremities, hypoesthesia, dysesthesias, pruritus, generalized weakness, and anxiety. Cold allodynia (burning dysesthesia or sensation of heat upon touching cold water or objects) is almost pathognomonic for ciguatera and is often incorrectly referred to as “temperature reversal” [16,30]. Paresthesias do not follow dermatome patterns [33,34]. Neurological symptoms are often aggravated by alcohol consumption, stress, and physical activity [35]. Other less common symptoms include diaphoresis, chills, dizziness, headache, blurred vision, prostration, myalgias, dry mouth, taste disturbances or a metallic taste, and pain or a loose sensation in the teeth. Weakness may last for 1–7 days. Mean duration of acute illness is typically 8.5 days, although it is not unusual for neurological symptoms such as paresthesias or cold allodynia to periodically reoccur for a month or longer. Diminished or increased reflexes and dilated pupils may also be noted which usually resolve in 2–3 days. Cardiovascular symptoms are found in 10–15% of cases, most commonly in individuals previously exposed to the toxin but, when present, bradycardia or hypotension may require urgent management [36]. In cases of severe intoxication, seizures, coma, and respiratory paralysis may occur and which, in the absence of adequate life support, may be fatal [33,35]. Ciguatera fish poisoning is usually a self-limiting disease, but symptoms may be extremely debilitating, resulting in extended periods of disability.

Current estimates place the annual number of ciguatera cases at 50,000 worldwide [37]. This poisoning spans the globe and generally is observed in warm waters between latitudes within 35° of the equator [38]. It is the most common type of fish poisoning in the Caribbean [39]. In the United States during the period from 1978 through 2004, 390 outbreaks of ciguatera involving 1576 persons were reported to CDC. No ciguatera-related deaths were reported [9,10,15]. In Hawaii the average annual incidence of ciguatera was 8.7/100,000 population based on 150 outbreaks involving

462 individuals that were reported to the State Department of Health during a 5-year interval from January 1984 through December 1988 [41]. These figures however are substantially higher than the CDC statistics which accounted only for 75 outbreaks and 295 cases in the US during that period. Of the 297 outbreaks between 1983 and 2004, 236 were reported from Hawaii and 40 from Florida [10,40]. Reported outbreaks in other states have been related, in most cases, to travel to the endemic areas, or from eating fish caught in endemic ciguatera areas; and there is concern that many cases are not recognized by mainland US physicians [40,42]. Despite its exceedingly low incidence outside endemic areas, as the domestic fish industry expands its sources of supply, the diagnosis of this “tropical” disease must also be considered in areas where coral reef fish are not native.

Ciguatoxins (CTX), the toxins responsible for ciguatera, are produced by *Gambierdiscus toxicus*, a marine dinoflagellate that belongs to the family of benthic macroalgae. They usually grow attached to dead coral and are ingested by small herbivores off the reef [32]. They are lipid-soluble polyether toxins, which when ingested by certain subtropical and tropical finfish can accumulate in their tissues. Biotransformation of CTX in fish increases their polarity and thus their toxicity. The toxins and their metabolites are concentrated when carnivorous reef fish (e.g. barracuda, grouper, and amberjacks) prey on smaller herbivorous fish. Thus, the toxic effect is amplified in large predatory fish that become the most toxic to humans at the end of the food chain [36]. Factors influencing the concentration of CTX that accumulate in fish include the rate of dietary intake, the efficiency of assimilation, the degree and nature of any toxin biotransformation, the rate of depuration, and the rate of growth of fish [43]. More than 400 species of fish can be vectors of CTX, but generally only a relatively small number of species of reef fish belonging to the family Serranidae are regularly incriminated in ciguatera. The fish most commonly implicated include amberjack, snapper, grouper, barracuda, and goatfish. The toxin may be most concentrated in the head, liver, intestines, testes, ovaries, and roe. CTX activate voltage-dependent sodium channels, causing cell membrane excitability and instability [44]. *In vitro* studies suggest that CTX causes a nerve conduction block after initial neural stimulation [45].

Maitotoxins (MTX) are water-soluble polyether phytotoxins also produced by *G. toxicus*, which are distinct from CTX. MTX induce severe pathological changes involving the stomach, heart, and lymphoid tissues of experimental mice and rats when injected intraperitoneally [46]. They also display hemolytic and ichthyotoxic activities. MTX-induced hemolysis is dependent on calmodulin and phospholipase A2 activity. Toxicity to fish is dependent on pH and Ca^{2+} concentration [47]. MTX are potent activators of a voltage-independent, non-selective cationic channel that results in elevation of the intracellular Ca^{2+} concentration, which is ultimately responsible

for toxicity [48]. MTX have low oral potency and poor ability to accumulate in fish flesh, consequently they are unlikely to play a significant role in causing human illness. To date, no compelling evidence exists to support a role for water-soluble toxins, including MTX, in ciguatera [16].

Ciguatera often affects only a discrete region of a reef, with flare-ups of ciguatera being both temporally and spatially unpredictable [41]. While low levels of *G. toxicus* are found throughout tropical and subtropical waters, the presence of bloom numbers is unpredictable and patchy. Only certain genetic strains produce CTX, and environmental triggers for increasing toxin production are unknown [49]. However, there is concern as to whether disruptions in the reef ecosystem may shift the balance toward a higher rate of toxin-producing *G. toxicus* and an increased incidence of ciguatera poisoning [50].

CTX are heat stable, so are not inactivated by either cooking or freezing. They are not affected by gastric acid and are harmless to the fish itself. Since they are odorless, colorless, and tasteless, ciguateric fish look, taste, and smell normal, and detection of toxins in fish remains a problem. A radioimmunoassay (RIA) and subsequently a stick-enzyme immunoassay and a solid-phase immunobead assay have been developed to detect even negligible amounts of toxins in suspect fish flesh [51–53]. The stick-enzyme immunoassay has been improved and has become a simple, rapid, sensitive, and specific test for CTX [54]. A kit (Cigua-Check, Oceanit Test Systems, Inc) using this detection method is available for use by sports fisherman that could screen fish for CTX. However, its cost and lack of awareness remain an obstacle to its utilization. Because there is no approved assay for CTX in human tissues, the diagnosis is based on a history of recent consumption of potentially ciguateric fish, clinical findings, and by the detection of toxin in samples of fish. Thus, any uneaten portions of fish should be saved in a freezer and submitted to state or local public health officials when suspected cases are reported to assist with the investigation and control of a possible outbreak [55].

There is no immunity and no known antidote for CTX poisoning. Treatment is primarily supportive and for relief of symptoms; however, intravenous mannitol may be effective early in the course of illness in reducing the associated neurological and muscular symptoms [56–58]. The initially promising results with mannitol were not confirmed in a more recent randomized, placebo-controlled trial; thus it cannot be endorsed as a general therapeutic recommendation [59]. To prevent ciguatera, persons living in or traveling to areas where ciguatera toxin is endemic should follow these general precautions [55]:

- 1 Avoid consuming large, predatory reef fish, especially barracuda and amberjack.
- 2 Avoid eating the head, viscera, or roe of any reef fish.
- 3 Avoid eating fish caught at sites with known ciguatera toxins.

Puffer fish (tetrodon) poisoning

Symptoms begin with paresthesias 10–45 minutes after ingestion, initially usually a stinging of the lips, tongue, and inner surface of the mouth. Common symptoms that follow include headache, lightheadedness, dizziness, vomiting, diaphoresis, pallor, weakness, malaise, and feelings of doom [59]. Some patients may experience a floating sensation, salivation, muscle twitching, and pleuritic chest pain. Depending on the amount of tetrodotoxin (TTX) ingested, the patient may experience ataxia, dysphagia, aphonia, and convulsions. Severe poisoning is indicated by hypotension, bradycardia, depressed corneal reflexes, and fixed dilated pupils. An ascending paralysis may develop and death can occur within 6–24 hours secondary to respiratory muscle paralysis [11]. Petechial hemorrhage, blistering and desquamation, and hematemesis have also been reported. Prognosis is good if the patient survives the first 24 hours [59].

Diagnosis is based on clinical symptoms and a history of recent consumption of suspect fish. Treatment is supportive, including active airway management and ventilatory and circulatory support as needed. To minimize the amount of toxin absorbed, gastric lavage and activated charcoal may be beneficial soon after the ingestion. There is no specific antitoxin for human use; however 4-aminopyridine has been reported to effectively reverse neuromuscular blockade and cardiorespiratory depression in a guinea pig model of TTX poisoning [60].

Puffer fish poisoning is rare in the United States and since 1951 only 10 cases have been reported, including three fatalities [61,62]. It is far more common in Japan where 20–100 fatal cases occur each year [11]. The mortality rate is high and approaches 60% [36].

Puffer fish poisoning results from ingestion of the flesh of certain species of fish belonging to the order Tetraodontidae that includes ocean sunfishes, porcupine fishes, and fugu, which are among the most poisonous of all marine life [63,64]. These fish get their name because they characteristically inflate to several times normal size by swallowing air or water when feel threatened. The liver, gonads, intestines, and skin of these fish contain TTX; but the flesh is edible if cleaned and prepared properly, and considered a delicacy by some persons in Japan, who may pay the equivalent of 400 US dollars for one meal. Rigid public health standards including training and certification of fugu chefs have decreased the incidence of puffer fish poisoning; but it has not eliminated the risk associated with consumption of fugu, which remains a common cause of fatal food poisoning in Japan. All puffer species in US waters, including *Sphoeroides maculatus*, *Sphoeroides annulatus*, and *Arothron hispidus*, have been implicated in fatalities and it would seem prudent to consider them potentially toxic. Note the Food and Drug Administration (FDA) has permitted fugu to be imported and served in Japanese restaurants by certified fugu chefs on special occasions. A cooperative agreement with the Japanese Ministry of Health and Welfare ensures

that fugu is properly processed and certified safe for consumption by the government of Japan before export [62].

TTX is a heat-stable alkaloid that blocks sodium conductance and neuronal transmission in skeletal muscle. TTX is also present in several other marine and terrestrial species such as blue-ringed octopus, some newts and toads [65,66]. TTX concentration in puffer fish fluctuates drastically with the reproductive cycle, reaching a peak around the spawning season; and is considerably higher in the female than the male [59]. TTX is not present in cultured puffer fish, nor is it found in all puffer fish of the same species caught in the wild. These observations and the marked individual, regional, and seasonal variability in TTX concentration suggest that all TTX-bearing animals do not themselves produce the toxin, but harbor TTX-producing microorganisms within their bodies. This hypothesis was confirmed when the natural source of TTX was identified in marine *Vibrio* species that are part of puffer fish microflora, and it was proven the fish itself merely accumulated the toxin in its tissue [67–69]. Several other TTX-producing bacteria have since been isolated from various marine organisms including *Alteromonas*, *Bacillus*, and *Pseudomonas* species [70]. The consumption of as little as 10g of the toxic tissue may be fatal and 1–4mg of TTX constitutes a lethal dose for humans [11].

Common intoxications associated with shellfish

Paralytic shellfish poisoning

PSP, which is caused by saxitoxins (STX), is the best known of the shellfish poisonings and causes the most severe symptoms. It is a serious illness in which neurological symptoms predominate. The first and most consistent symptoms are numbness, tingling, and/or burning of the lips, tongue, and throat that begin within 30 minutes of ingestion. Paresthesia spreads to the face and neck and often to the fingertips and toes. This precedes muscular weakness that affects the upper and lower limbs and in more severe cases is followed by dysphonia, dysphagia, and ataxia. Paralysis may follow within 2–12 hours, and may persist for as long as 72 hours. The sensation of floating in air, dizziness, weakness, drowsiness, headache, salivation, intense thirst, and throat tightness are commonly described. Diaphoresis, nausea, vomiting, diarrhea, tachycardia, and temporary blindness may also occur. Reflexes may be normal or absent, and most patients remain calm and conscious throughout. Death can result from paralysis of the respiratory muscles within 2–24 hours, depending on the dose. Prognosis is good for individuals surviving past 12 hours. The duration of the illness may be from a few hours to a few days, but occasionally muscular weakness can persist for weeks following recovery [20].

Diagnosis is based on characteristic symptoms and on a history of recent ingestion of shellfish. There is no specific laboratory diagnostic test for a patient with PSP. However,

examination of water samples for toxic algae and laboratory tests on the suspect food can provide supportive evidence.

Treatment is symptomatic. Gastric emptying has been advocated by some authors as an early treatment and activated charcoal has generally been recommended to help block further absorption of the toxins. Airway management and ventilatory support is the mainstay of treatment and can be life saving. However, larger doses of poison may result in death despite this treatment. Fluid therapy facilitates renal excretion of the toxin and intravenous administration of sodium bicarbonate may be beneficial to correct possible acidosis. Since the half-life of elimination of the toxin from the body is about 90 minutes, 9 hours should be adequate in most cases for physiological reduction of toxin concentration to relatively harmless levels. There is no immunity to PSP and the second attack may be more severe than the first. No effective antidote is available, but experimental results with 4-aminopyridine are promising in a guinea pig model of PSP [60].

In the United States, PSP is a problem primarily in the New England states on the East Coast and in Alaska, California, and Washington on the West Coast. Most disease incidents involve mussels and clams gathered and eaten by recreational collectors, often from closed areas, reflecting the effectiveness of current testing and control measures for commercially produced shellfish. The CDC listed 32 outbreaks involving 284 people with four fatal cases during 1978–2004 suggesting a mortality rate of <2% [9–11,14,15]. The case-fatality rate has been quoted at about 8.5% [71], but at present it is probably <1% in developed countries [72]. Although PSP is an extremely dangerous disease that can cause death, there is reason to believe that mild cases due to consumption of marginally toxic clams by recreational diggers are never reported to health authorities or are misdiagnosed.

The first case of PSP was described in 1793 as poisoning by mussels in explorers of coastline of British Columbia, Canada [73]. The dinoflagellate, *Alexandrium catenella* (then called *Gonyaulax catenella*), was identified as the actual cause about 1927 [74]. Bivalve mollusks, such as mussels, clams, and oysters, assimilate and temporarily store STX, a complex of neurotoxins produced by dinoflagellates; and thus they function as vectors for the toxin. The primary sources of STX include three morphologically distinct genera of saltwater dinoflagellates: *Alexandrium* spp. (previously *Gonyaulax*), *Pyrodinium* spp., and *Gymnodinium* spp. [75]; and four species of freshwater blue-green algae: *Aphanizomenon flos-aquae*, *Anabaena circinalis*, *Lyngbya wollei*, and *Cylindrospermopsis raciborskii* [20]. The STX are a family of water-soluble alkaloids that consist of various sulfonated and hydroxylated derivatives that contain the basic structure of a tetrahydropurine skeleton and two guanidinium groups. They are among the most potent neurotoxins known. More than 20 STX analogs have been described. The positively charged guanidinium group of the toxins binds specifically to a negatively charged site of the sodium (Na^+) channel on the extracellular side of plasma membrane of nerve and muscle cells, thus blocking

the flow of Na^+ through the channel. As Na^+ entry through the nerve cell membrane is essential for impulse transmission, blockage interferes with signal transmission and results in paralysis [75]. Most shellfish contain a mixture of several STX, depending on the species of algae, geographic area, and type of marine animal involved. Biotransformation of the toxin results in generation of more toxic forms. The higher the net charge, the greater is the toxicity. The potency of STX is expressed in mouse units per milligram (MU/mg). One MU is the amount of toxin required to kill a mouse weighing 20g in 15 minutes after intraperitoneal injection and is equivalent to 0.18 μg STX. Toxicity of the STX is generally expressed in terms of saxitoxin equivalents (STX eq) per 100g of shellfish meat. There is great variation in individual susceptibility and children are thought to be more susceptible. As little as 120–180 μg of STX can induce moderate symptoms in adults, and fatalities have been associated with levels of 0.3–12mg [20]. Although normal steaming or boiling will not inactivate the toxins, exposure of toxic shellfish to high temperatures (e.g. in the sterilization step of the canning process) substantially reduces STX concentrations. However, the effectiveness of canning as a means of reducing STX levels below the statutory limit depends on the initial toxicity and must be used with caution [76].

The mouse bioassay has been the classical method for analysis of STX. It is a standardized procedure in which mice are injected with toxin extracts, and their responses are compared with known amounts of toxin. It is rather insensitive with a detection limit of only 40 μg STX eq/100g shellfish meat [77]. High-performance liquid chromatography (HPLC) is quite rapid and has been considered as a possible replacement for the mouse bioassay. HPLC detection limits are generally an order of magnitude lower than that of the mouse bioassay [78]. A direct enzyme immunoassay (EIA) has been available for determination of STX in shellfish that correlates closely with mouse bioassay [79]. A lateral flow immunochromatographic (LFI) assay has been developed and is termed the MIST Alert dipstick test for PSP. It can detect toxic shellfish (i.e. STX >80 μg /100g) with 100% sensitivity and detects 95% for samples in the range 32–80 μg /100g. It has a false-positive rate of 15% at <32 μg /100g, which is below detection limit of the mouse bioassay [80]. MIST Alert has also been evaluated for the rapid identification of PSP toxins in the water column and benthos. PSP toxins are detected at 100 cells per sample with no false-negative responses. It appears to be an effective tool for broad scale monitoring of algal toxins in coastal waters and has the potential to replace existing surveillance techniques [81]. In addition, the tests for the detection of STX in shellfish tissue have shown a sensitivity of 100% above 80 μg STX eq/100g, which is the current FDA tolerance level. MIST Alert detected reliably STX above 40 μg STX eq/100g and identified the majority of extracts above 32 μg STX eq/100g, which is the mouse bioassay detection limit with a false-positive rate of 6% below 20 μg STX eq/100g [82,83]. STX is a potent inhibitor of

the membrane depolarizing effects of the sodium channel activator veratridine. Based on this property, a membrane potential assay using mouse brain synaptoneurosomes has been developed. PSP toxins contained in shellfish extracts can be detected by inhibition of veratridine-induced depolarization using the fluorescent probe rhodamine 6G. This technique has yet to be validated in field tests to determine its sensitivity and specificity [84].

In the United States, the toxigenic dinoflagellates causing PSP are *A. catenella* and *A. tamarensis*; the first being most dominant on the West Coast responsible for PSP outbreaks in the Pacific and the second on the East Coast associated with New England outbreaks [85]. When the dinoflagellates proliferate or “bloom,” they often give the water a red or reddish-brown discoloration, giving rise to a “red tide.” Outbreaks of PSP tend to cluster from shortly before, up to several weeks after, the appearance of red tide [86]. It should be noted that some *Alexandrium* species do not produce toxins and not all red tides are caused by toxic algae [87]. Conversely, shellfish may also become toxic in the absence of red tide [33]. Anthropogenic eutrophication has been incriminated to result in a higher frequency of red tides or harmful algal blooms and increased production of biotoxins by marine dinoflagellates [19,88].

STX persist in shellfish for varying periods, depending on the shellfish and the tissue involved [85]. Mussels become highly toxic within a few hours to a few days of the onset of a red tide, but lose their toxin rapidly. Clams and oysters generally do not become as toxic as mussels. They require more time to accumulate high levels of toxins and longer to cleanse themselves. The Alaska butter clam, once contaminated, may never be safe for consumption as it retains paralytic shellfish toxins for years [74]. Sea scallops can take up large amounts of STX, even in the absence of algal blooms, but generally do not pose a threat because their adductor muscle, the only part of the scallop that is usually consumed, does not accumulate toxins. Gastropods can also accumulate significant amounts of STX, and in Spain levels as high as 44ppm have been recorded in meat of abalone. Even though paralytic shellfish toxins have been reported in the viscera of rock lobsters and crabs, STX do not appear to accumulate in significant amounts in muscle tissue. Similarly, they can accumulate up to 50ppm in intestine, liver, and gills of Atlantic mackerel, but not to any extent in muscle. Therefore, crustaceans and finfish do not appear to present a threat of PSP unless consumed whole or unless livers are consumed [20].

The rate of accumulation and loss of toxin differ between marine species. Thus, even though mussels, once they are in non-contaminated waters, can lose their toxicity within weeks, it can take the Alaskan butter clam as long as 2 years or more to lose toxicity after the initial accumulation of toxin from a red tide. Shellfish containing STX cannot be detoxified by depuration, and the toxins can persist within shellfish at dangerous levels for weeks or months after the algae are no longer present in the growing waters. Seafood

containing STX looks and tastes normal and cooking or steaming only partially destroys the toxins [71]. The most effective way of protecting consumers is to establish and maintain comprehensive monitoring programs for toxic algal blooms and toxins in shellfish in all growing areas. When toxic algal species are present in significant numbers, seafood products must be tested for toxicity and withheld from marketing if necessary. The FDA and European “alert level” for STX is 0.8ppm (80 μ g/100g) in shellfish meat [89,90]. Commercial shellfish harvesting in United States and European Union must be suspended if higher concentrations are detected in routine monitoring programs. Toxin levels can exceed 10mg/100g mussels [89]. As illness has been reported to occur in adults at a total oral dose of only 120 μ g and death at 300 μ g, this maximum permitted level is not particularly conservative. The best way to prevent PSP is to adhere to the public health agency guidelines on harvesting, processing, and consumption of shellfish. To further minimize the risk of PSP, the public should avoid collecting shellfish from areas of known red tides and refrain from consuming suspect shellfish. In addition, since the toxins are water soluble, they can dissolve and concentrate in the cooking broth, which should be discarded after cooking or steaming [20].

Neurotoxic shellfish poisoning

NSP is characterized by both gastrointestinal and neurological symptoms. The illness resembles a mild case of ciguatera or PSP, but with neuroexcitation rather than flaccid paralysis. The onset is rapid and symptoms occur within 3 hours following the ingestion of contaminated shellfish. Symptoms include numbness of the lips, tongue, and throat; and paresthesias, initially circumoral, which then spread to other parts of the body, “temperature reversal,” myalgias, vertigo, headache, nausea, vomiting, diarrhea, and abdominal pain. Less commonly, victims may experience a feeling of inebriation, burning pain in the rectum, dysphagia, ataxia, tremor, decreased reflexes, mydriasis, and bradycardia. The intoxication is usually self-limited and resolves spontaneously within a few hours. Treatment is supportive and generally all patients recover within a few days with no after effects. No fatalities have been reported. There are no known antidotes for the toxin [91]. From 1978 through 2004 the CDC reported only a single small outbreak involving three members of a family that consumed toxic small clams harvested from Sarasota Bay, Florida, in June 1996 [10]. The diagnosis was confirmed by detection of the causative toxins, brevetoxins (BTX), in the urine of the patients and in extracts of shellfish collected from the same location by RIA and by receptor-binding assay (RBA) [92]. A competitive ELISA has been developed that detects BTX in body fluids such as urine and serum, seawater, and shellfish extract, with a detection limit of 2.5 μ g/100g shellfish meat. It appears to be a useful tool for monitoring shellfish and seawater, and for diagnostic investigations [93].

Unlike other shellfish toxins, BTX can aerosolize by surf and wave action along the beach during red tides. Irritant toxin aerosols produce a syndrome characterized by conjunctival irritation, sneezing, and rhinorrhea that resembles an allergic response. Shortness of breath, non-productive cough, and wheezing due to bronchospasm are also triggered in individuals with underlying asthma or chronic obstructive pulmonary disease. The syndrome is self-limited and treatment of the bronchospastic episodes due exposure to aerosolized toxins is symptomatic. *In vitro* data indicate that BTX produce contraction of human lower airway smooth muscle via stimulation of cholinergic nerve fibers through activation of sodium channels [94]. In 1987 during a red tide off the coast of North Carolina, 48 individuals were reported that experienced upper and/or lower respiratory symptoms [95].

Karenia brevis (formerly *Gymnodinium breve*) is the dinoflagellate that synthesizes BTX, a group of related, heat-stable toxins responsible for clinical manifestations of NSP. BTX are lipid-soluble polyether toxins of unique structure and pharmacological function. They are active *in vivo* in the nanomolar to picomolar concentration range. Their excitatory effect is mediated by the enhancement of cellular Na⁺ influx through the voltage-sensitive sodium channel [96]. Filter-feeding bivalve mollusks, such as oysters, clams, and mussels, that consume *K. brevis* concentrate the toxins in various organs and become toxic to humans but remain unaffected. NSP in the United States is generally associated with the consumption of shellfish harvested along the coast of the Gulf of Mexico from Florida to Texas, and, sporadically, along the southern Atlantic coast. This is identical with the geographic distribution of *K. Brevis* blooms or “red tides.” These red tides occur in many areas within the Gulf of Mexico and may result in massive fish kills. The earliest record of fish kills, later attributed to a *K. brevis* bloom, was in 1844 off the West Coast of Florida, where they still occur most frequently, but may be carried north in the Gulf Stream, affecting the coastline of adjacent states. Red tides occur throughout the world and there was a significant outbreak of NSP in New Zealand involving 186 cases, as well as reported outbreaks in the coastal waters of Japan [89,97].

K. Brevis blooms are initiated on the continental shelf or at the shelf edge, over 40 miles offshore, rather than near the shore where they produce the most deleterious effects. Bloom initiation is characteristically associated with intrusion of deeper, offshore waters onto the shelf. Once dense blooms move inshore, they cannot be sustained without maintaining a minimum nutrient level. Thus human inputs of nutrients could be responsible for extending the duration and impacts of red tides when blooms enter the near-shore waters [98]. These blooms on the Southwest Florida shelf served as a source for cells inoculating the Florida East Coast and North Carolina in 1987–1988 [99]. Concern has

been raised that human activity may increase the frequency of harmful algal blooms and disseminate *K. Brevis* and other toxic phytoplanktons to non-indigenous waters and result in globalization [100,101].

K. brevis is well adapted and is able to out-compete or otherwise exclude other phytoplankton species. Low concentrations (<1000 cells/l) of the organism occur in off-shore waters throughout the year and can be detected microscopically. Typically in late summer and fall when nutrients are abundant, and physical, chemical, and biological conditions are favorable, *K. brevis* grows rapidly, gradually building high densities that in 2–8 weeks reach bloom concentrations ($1\text{--}25 \times 10^5$ cells/l).

During severe blooms, fish die rapidly from the neurotoxic effects and do not survive to accumulate high toxin concentrations in their tissues. However, fish exposed to sub-lethal concentrations may accumulate these toxins. Such bioaccumulation in fish eaten by marine mammals, such as dolphins and manatees, results in their demise due to BTX exposure and may also affect human health.

Chlorophyll in *K. brevis* results in discoloration of surface water at $10\text{--}100\text{ mg/m}^3$ and is a good surrogate for biomass. It can be detected by satellite color sensors at densities three orders of magnitude less than when water discoloration is visible to human eye, at about 10^6 cells/l. However, it cannot detect deep patches or distinguish *K. brevis* from other algae, which limit the utility of this technology as an early warning system for a ban on shellfish harvest and beach closure. Local authorities may close shellfish harvesting to industries and the public. The basis for closure is the occurrence of more than 5000 *K. brevis* cells per liter of seawater, and reopening of harvest is dependent on demonstrated absence of BTX in shellfish meat [102,103]. The FDA has established a guidance level for BTX at 0.8 ppm ($80\text{ }\mu\text{g}/100\text{ g}$) BTX-2 equivalent (20 mouse units/100g) in shellfish meat, and shellfish harvesting is banned if higher concentrations are detected in monitored areas [89]. The small number of cases of NSP testifies to the effectiveness of the surveillance and closure systems operated by the states.

Amnesic shellfish poisoning

ASP presents initially with vomiting, diarrhea, and abdominal cramps within 24 hours post-ingestion of contaminated shellfish. In some cases, varying degrees of neurological dysfunction follow within 48 hours, including confusion, loss of memory, and disorientation. Other neurological symptoms are headache, hyporeflexia, hemiparesis, ophthalmoplegia, and abnormalities of arousal ranging from agitation to coma, seizures, and myoclonus, especially affecting the face. The acute symptoms are milder compared with PSP. Loss of short-term memory is unique among the marine poisonings, hence the name ASP [104]. It is the most persistent symptom and can be permanent.

The syndrome was first described in a series of outbreaks in individuals that had eaten mussels cultivated in the river estuaries of Prince Edward Island in Canada from November through December 1987 [105]. In this cohort, the frequency of acute symptoms were vomiting (76%), abdominal cramps (50%), diarrhea (4%), severe headache (43%), and loss of short-term memory (25%). Gastrointestinal symptoms were present in all but 7 of the 107 cases. Onset of symptoms after mussel ingestion ranged from 15 minutes to 38 hours, with a median of 5.5 hours. Nineteen patients (18%) were hospitalized, of whom 12 required intensive care because of seizures, coma, profuse respiratory secretions, or unstable blood pressure. Severity of the disease and permanent neurological sequelae, especially cognitive dysfunction, are associated with age over 60 years, male sex and with pre-existing illnesses, as well as the amount of mussels consumed. Three elderly patients died directly and one died indirectly from the intoxication. Neuropathological studies in these four fatal cases showed neuronal necrosis in the hippocampus and amygdala [106]. The clinical records of 14 more severely affected patients that displayed neurological manifestations were reviewed. All 14 patients reported confusion and disorientation within 1.5–48 hours after ingestion and exhibited a variety of neurological abnormalities including coma [9], mutism [11], seizures [8], purposeless chewing and grimacing [6], and uncontrolled crying, or aggressiveness [6]. In neuropsychological testing performed in those 14 patients several months after the acute episode, 12 had severe anterograde-memory deficits, with relative preservation of other cognitive functions. Eleven from the 14 individuals had clinical and electromyographic evidence of pure motor or sensory motor neuronopathy or axonopathy. The maximal neurological deficits were seen 4 hours post-ingestion in the least affected patients and 72 hours in those most affected, with maximal improvement 24 hours to 12 weeks post-ingestion. Acute coma was associated with the slowest recovery. Seizures ceased by 4 months but were frequent up to 8 weeks [107]. Relative preservation of intellect and higher cortical function appears to distinguish ASP from Alzheimer's disease, and the absence of confabulation with well-preserved frontal lobe function differentiates it from Korsakoff's syndrome.

In mussels left uneaten by the patients, as well as mussels harvested later from the same estuaries, the toxic agent was isolated and identified as domoic acid (DA). Its concentration ranged from 31 to $128\text{ mg}/100\text{ g}$ of mussel meat that suggested an estimated ingestion of 60–290 mg of DA per patient [104].

Diagnosis is based on a recent history of shellfish ingestion and is made on clinical grounds. It is confirmed by demonstration of DA in shellfish samples. At this point, the treatment of ASP is symptomatic and supportive. Seizures respond well to parenteral benzodiazepins and phenobarbital. There is no antidote and immunity does not develop.

The source of DA in the Prince Edward Island outbreak was subsequently identified as the phytoplanktonic diatom *Pseudo-nitzschia multiseries*, formerly known as *Nitzschia pungens* [107]. ASP is the only shellfish poisoning caused by diatoms. Ten isomers of DA (isodomoic acids) have been identified in marine samples, but are minor constituents in ASP relative to DA [108]. DA is a potent neurotoxin that accumulates in mussels and clams that feed on toxic planktons during a bloom. On the Pacific Coast, DA is produced by *P. multiseries* and two other species *P. australis* and *P. pseudo-delicatissima* that bloom in late summer and fall. DA is water soluble and heat stable, similar in structure and function to another excitatory neurotoxin known as kainic acid (KA), which is found in the Japanese seaweed, *Digenea simplex*. DA and KA both appear to produce neurotoxic effects by activating the glutamate receptors [109]. These receptors are ligand-gated, voltage-dependent Ca^{2+} channels that are activated by glutamic acid, mediating a fast excitatory synaptic transmission in the mammalian central nervous system (CNS). Persistent activation of KA receptors results in elevated levels of intracellular calcium (Ca^{2+}) that causes neurotoxicity with subsequent lesions in areas of the brain where glutaminergic pathways are heavily concentrated [110]. The observations that the glutamate receptors are present within the cardiac conducting system, intramural ganglia, and cardiac nerve fibers could explain some of the clinical manifestations such as the arrhythmia described with DA intoxication in humans. Hence individuals with pre-morbid cardiac conditions may be at higher risk of the toxic effects of these excitatory compounds [111]. In animals, DA is 3 times as potent as KA and 30–100 more potent than glutamic acid [112].

DA poisoning first became a noticeable problem in the West Coast of the United States in September 1991 when it was reported that brown pelicans had died after eating anchovies in Monterey Bay off the coast of California. It was subsequently found that the death of these pelicans was due to the bloom of *P. multiseries* that produced high levels of DA [113]. Since this time and until December 2004, 29 cases of ASP have been reported to the CDC, all of which occurred in November 1991 and were caused by razor clams harvested in Washington [10]. No fatalities have occurred in the United States; however, mortality rate was 3.7% in the 1987 Canadian outbreak.

Traditionally a mouse bioassay has been used for detection of DA, which is the same assay as for PSP, however the relative potency of DA appears to be less than STX. There are several newer methods used to detect DA in seawater and shellfish such as HPLC, immunoassay, and an RBA. Also, two indirect competitive EIA for measurement of DA in shellfish and seawater have been developed. One utilizes polyclonal ovine antibodies and the other uses monoclonal murine antibodies. They have a working range of 0.15–15 $\mu\text{g/l}$ and 0.15–10 $\mu\text{g/l}$, respectively, and a quantification limit of <4 $\mu\text{g/100g}$ of shellfish flesh [114,115]. The RBA measures the competitive displacement of radiolabeled KA bound to a cloned

glutamate receptor (GluR6) by DA in a sample. A comparison of the latter two methods showed that the RBA has a larger working range whereas EIA is more sensitive. The detection limit and working range are 3.1 and 5–100 $\mu\text{g/l}$ for the RBA and 0.01 and 0.15–15 $\mu\text{g/l}$ for the EIA, respectively. RBA and EIA yield statistically equivalent results for detection of DA in seawater [116]. An LFI assay, the MIST Alert dipstick test for ASP, is a newer assay and has a detection limit of approximately 8–12 $\mu\text{g/g}$ DA in shellfish extracts, which is about half the regulatory limit a sensitivity approaching 100% [80].

In Canada, to prevent future outbreaks of ASP, sacks of mussels are now labeled with respect to time and place of harvesting; in addition both water column and shellfish are monitored for the presence of *Pseudo-nitzschia* and DA, respectively. Since an estimated concentration of 20 mg/100 g wet weight DA has affected some consumers, applying a safety factor of 1/10, Canadian surveillance authorities have set 2 mg/100 g (20 ppm) as the threshold level above which shellfish commercial operations are suspended.

On the Pacific Coast, DA poisoning has been a serious problem affecting razor clams and Dungeness crabs in Washington; and oysters, bay and razor clams, and mussels in Oregon. Authorities in Washington, Oregon, and California now randomly analyze samples of commercially harvested or cultivated shellfish for DA. The FDA and the European Union have adopted the level of 20 ppm (2 mg/100 g) for DA and when higher levels are detected in seafood closure of beds is enforced [89,90]. The viscera of Dungeness crabs are an exception, where 30 ppm is permitted [89,117]. States in the northeastern United States also monitor shellfish for DA, which is present at low levels that do not necessitate quarantine.

Diarrhetic shellfish poisoning

Diarrhetic shellfish poisoning (DSP) is the mildest and most benign of the toxic shellfish poisonings. Clinical features are generally limited to the gastrointestinal tract and include diarrhea (92%), nausea (80%), vomiting (79%), abdominal pain and cramps (53%). Chills, fever, or headache may also be present in up to 10% of cases [118]. The symptoms usually manifest in a period ranging from 30 minutes to 6 hours after ingestion of contaminated shellfish and persist on average for 36 hours. No known fatalities have occurred, and total recovery is expected within 3 days. Due to the transient nature of the illness and its spontaneous resolution, often patients do not seek medical attention, however the duration could be shortened with charcoal, which reduces the bioavailability of the toxins and its repeated administration interrupts their enterohepatic recirculation. Treatment, if required, is limited to alleviation of symptoms.

DSP is associated with the consumption of mussels, scallops, clams, and oysters contaminated with biotoxins produced by toxic marine dinoflagellates during their blooms in the summer. *Dinophysis* and *Prorocentrum* species have been identified as the source of DSP toxins that are heat stable

and not denatured by normal cooking. Although to date there has been no documented DSP outbreak in the United States, toxin-producing *Dinophysis* species are present in US waters and in 1990 caused an outbreak in eastern Canada. The disease occurs worldwide in temperate waters. It is common in Japan, where over 200 cases are reported annually and has become a public health problem in Europe. Sporadic outbreaks have also been documented in Southeast Asia, Chile, Australia, New Zealand, and eastern Canada [89].

At least 10 different toxins have been isolated from dinoflagellates and shellfish in association with DSP. The major toxins are high molecular weight polyethers including okadaic acid (OA), dinophysistoxins (DTX), and several of their metabolites, as well as pectenotoxins (PTX) and yessotoxin (YTX) [119]. OA is most commonly encountered in Europe where *Dinophysis acuminata* is the usual agent, whereas in Japan mixtures of OA, DTX, and PTX are detected usually involving *D. fortii* [120]. OA is a highly selective inhibitor of protein phosphatases that causes dramatic increases in phosphorylation of numerous proteins and can act as a potent tumor promoter. It induces diarrhea by increasing paracellular permeability of intestinal epithelial lining without inducing cytotoxicity [121]. DTX are structurally related to OA and cause in laboratory experiments highly similar intestinal lesions that appear within 5 minutes of dosing and resolve completely within 2 days [122]. PTX, although non-diarrheagenic, are potently cytotoxic and have been found to be tumor promoters in animals [123]. YTX is only a weak cytotoxin, and is not orally lethal to mice. It does not cause accumulation of intestinal fluid or inhibit protein phosphatase and has no diarrheagenic or hemolytic effects suggesting that it should not be classed as a DSP toxin [124]. The DSP toxins, particularly, OA and some DTX, are potent microalgal inhibitors. They are probably an evolutionary adaptive mechanism and are produced by toxic dinoflagellates to create a survival advantage against other competing microalgae [125].

A mouse bioassay is the standard method for DSP surveillance; however, it is non-specific and lacks sensitivity. HPLC is an alternative technique and has a low detection limit of 26 µg/100g of shellfish for both OA and DTX, but is cumbersome and requires calibration [126]. Most of the recent developments in rapid screening methods for OA detection are based either on the use of specific antibodies or on its ability to inhibit protein phosphatase coupled with use of fluorescence substrates. The fluorimetric assay achieves a detection limit of 1 µg/100g OA in mussel tissue, which is well below 20 µg/100g that has been established by FDA as toxicity threshold level [89,127]. The European Union has adopted a level of 160 µg of OA eq/kg (16 µg/100g) for the total amount of OA, DTX, and PTX, whereas the alert level for YTX is 1 mg/kg [90]. Commercially available EIA kits detect OA and some of its metabolites but not all DSP toxins. Thus EIA kits underestimate total toxin present in crude toxic shellfish [128,129]. Both the HPLC and protein phosphatase

inhibition (PPI) assays correlate with each other and with the standard mouse bioassay. Although EIA does not accurately and consistently detect low DSP toxin concentrations, it offers advantages of rapidity and ease of use [126].

At present, for the US consumer, the risk of DSP is limited to imported products and should be controllable by import regulations that permit import of shellfish only from countries that test it for the presence of toxins. Nevertheless, because *Dinophysis* does occur in US coastal waters, regulatory agencies in the United States should be alert for the possibility of an outbreak [130].

Less common seafood poisonings

Clupeotoxism

Clupeotoxism is a potentially fatal form of human intoxication due to ingestion of clupeidae (herring-like fish). It is a rare poisoning, occurring in the tropics only during the warm summer months. Like ciguatera, it occurs sporadically and over an extensive area of the tropical Pacific and Indian Ocean coasts. A few cases have been reported from Madagascar related to eating sardines, including one fatality in 1994. Symptoms include nausea, vomiting, diarrhea, abdominal pain, headache, dry mouth, sweats, chills, light-headedness, and paresthesia; and in one report one of the two patients with clupeotoxism died [131]. Physical findings may include tachycardia, hypotension, tachypnea, and cyanosis. Treatment is supportive and no specific antidote is available. The causative toxin was identified as palytoxin, one of the most potent phycotoxins known [132]. It is tasteless and odorless and not inactivated by cooking. Palytoxin has been reported to induce rhabdomyolysis after eating parrotfish. Patients presented with weakness and myalgia within 5 hours of ingestion and recovered without complications or fatalities. In one case, myocardial damage was also present as indicated by electrocardiographic changes and elevation of cardiac enzymes [133,134]. A more recent report of 11 cases, also from Japan, described typical features of rhabdomyolysis including myalgia, back pain, dark discoloration of urine, and elevated serum creatine kinase [135]. The benthic dinoflagellate *Ostreopsis siamensis* has been identified as the source of palytoxin [132]. *O. siamensis* is found in African, Caribbean, and Indo-Pacific Coastal waters and plankton feeders such as herring, pilchard, tarpon, and anchovies that ingest it can become toxic. In most cases, the fish have been captured close to shore, indicating that the toxin was obtained from benthic algae in the bottom sediments.

Palytoxin alters the function of excitable cells and acts as a hemolysin. Its hemolytic activity against sheep erythrocytes can be inhibited by polyclonal rabbit anti-palytoxin antibodies. The mechanism of toxicity of palytoxin has been elucidated, and it was shown to bind to Na⁺/K⁺ pump and convert it into a non-selective cation channel [136]. Consequently it results in reduction of the membrane potential and depolarization, which triggers secondary

activation of voltage-dependent Ca^{2+} channels and results in neurotransmitter release by nerve terminals and contractions of striated and smooth muscle cells [137].

Elasmobranch poisoning

Elasmobranch poisoning is caused by the ingestion of contaminated meat or liver from several species of sharks, most notably the Greenland sleeper shark [138]. The disease is characterized by gastrointestinal and neurological symptoms including nausea, vomiting, diarrhea, abdominal pain, headache, and perioral paresthesia. Malaise, weakness, muscle cramps, ataxia, and visual disturbances may also develop. Severe progressive respiratory distress, hyporeflexia, and coma usually precede death. The onset of symptoms is 30 minutes to 5 hours following ingestion. The shark and its meat do not display any unusual characteristics. The toxicity is not affected by cooking. Trimethylamine has been proposed as the cause of this poisoning [138]. In November 1993, a large outbreak involving 188 people who ate the meat from a single shark was reported from Madagascar. The patients presented within 5–10 hours after ingestion with neurological symptoms. Ataxia was almost universally present and of moderate to severe intensity. Gastrointestinal symptoms, like diarrhea and vomiting, were rare. The attack rate approached 100% and the overall case mortality was close to 30%. Carchatoxin-A and -B, two novel lipid-soluble toxins were isolated from the shark's liver, which were distinct from other known marine toxins [139].

Red whelk poisoning

In red whelk poisoning symptoms develop within 30–120 minutes, which include headache, dizziness, blurred vision or diplopia, paresthesia, dry mouth, muscular twitching or cramps, ataxia, weakness, and collapse. Nausea, vomiting, and diarrhea may also be present in some patients [140]. The red whelk (*Neptunea antiqua*) is a gastropod species common in Japan and Northern European waters and is distinguished from the edible whelk (*Buccinum undatum*), by its larger size and its smooth shell that has a distinctive pale orange coloration [141]. It contains a heat-stable water-soluble toxin, tetramine that is present in the salivary gland [142]. The concentration of tetramine shows significant seasonal fluctuations [143]. Tetramine possesses curare-like effect and produces short-lived symptoms that resolve rapidly and recovery is complete within 24 hours. As a result few people are likely to seek medical attention and intoxication is rarely reported. It is notably more common in Japan [141].

Newly discovered marine biotoxins

Azaspiracid

AZA is a structurally novel phycotoxin that contains a unique spiro ring assembly found to be responsible for outbreaks of diarrhetic food poisoning associated with consumption of contaminated shellfish in Europe. The first outbreak was

reported in November 1995 in the Netherlands following the consumption of mussels harvested on the west coast of Ireland and initially was mistaken for DSP; but was subsequently proven to be azaspiracid shellfish poisoning (AZP). Since then, outbreaks have been reported in other European countries, including France, Italy, Ireland, Norway, and the United Kingdom [144]. The onset is 12–24 hours after consumption of mussels and the symptoms of the illness include severe diarrhea, vomiting, nausea, abdominal cramps, headaches, and chills, which resolve in 2–5 days [145]. Scallops have been also reported to cause AZP; and in several instances toxin levels in crabs harvested in Norway have exceeded the safety levels of the European Unions [90,146,147].

There are up to 10 forms of AZA and these toxins are not affected by heat or freezing [148]. The causative organism is *Protopteridinium crassipes*, a dinoflagellate found in North Atlantic waters. While AZA was initially classified as a DSP toxin, it was subsequently re-classified into a new poisoning category known as AZA poisoning. AZA has a number of unique properties that set it apart from the “classic” DSP toxins, OA, DTX, and YTX. In animal experiments AZA administered orally, induces pronounced neurotoxic effects and causes necrosis in the lamina propria of the small intestine, liver, and lymphoid tissues in the Peyer's patches, spleen, and thymus; whereas toxic effects of OA are limited to the gastrointestinal mucosa [149].

In mice, AZA leads to progressive paralysis, and is rapidly fatal within 5–60 minutes; while OA and DTX cause convulsions and prostration and ultimately death over a longer period of time. OA, DTX, and YTX are known to be located exclusively within the hepatopancreas (HP) of the shellfish, while AZA may initially concentrate in HP but eventually distributes throughout the body and migrates also into the flesh. Since depuration occurs in the HP first, mussels contaminated with AZA may take longer to depurate. In addition, surveillance of shellfish toxicity based on the assessment of toxin concentration in HP will underestimate AZA hazard and may allow toxic shellfish to be harvested [150]. Consequently, it has been recommended to determine toxin levels in the whole shellfish and a threshold concentration of 16 µg AZA eq/100g as a regulatory standard in Europe has been successful in preventing AZP outbreaks since 2001 [90,147]. A liquid chromatography–multiple tandem mass spectrometry method for determination of AZA has been developed that is capable of detecting each of the 10 AZA with limit <20 pg within a few minutes and is far more sensitive than the mouse bioassay [151].

Spirolides

Spirolides are a novel family of lipophilic shellfish toxins that were recently isolated from the marine dinoflagellate, *Alexandrium ostenfeldii*. They consist of a spiro-linked tricyclic ether ring system and an unusual seven-membered spiro-linked cyclic iminium moiety; hence the name spirolide

[152]. To date no human disease has been associated with spirolides. However, based on toxicity profile they should be viewed as a potential cause of seafood poisoning. They were discovered in 1991 during routine biotoxin monitoring of shellfish in eastern Canada. Their distinct toxicological and chemical properties differentiate them from other known lipophilic shellfish toxins. Spirolides are macrocyclic imines that were initially labeled as fast-acting neurotoxin, since in mouse bioassay they rapidly resulted in neurological symptoms and death in 3–20 minutes. They appear to activate muscarinic receptors in the brain and particularly affect the brain stem. The rapid lethal action in rodents is probably due to compromise of cardiorespiratory centers in the brain stem [153]. Liquid chromatography–mass spectrometry is a highly sensitive analytical assay that can detect spirolides in concentrations as low as 2 µg/l; and is the method currently used for surveillance of biotoxins in Canada [154].

Potential allergens associated with seafood

Residues of bioactive substances from aquaculture

Aquaculture is an important source of food worldwide and now contributes up to 15% of the US seafood supply [155]. Traditionally, the environmental safety risks of seafood products have been subdivided into natural hazards such as biotoxins, and anthropogenic contaminants such as synthetic chemicals. In aquaculture, the latter hazard becomes more prominent as more synthetic products are used in the seafood industry. The use and misuse of antibiotics to control diseases in aquacultured species appears widespread. Similarly, the improper or illegal use of chemicals to control pond pests and algae can also result in human health hazards. Natural products that are not present in aquatic environments can also become health hazards when misused or abused; for instance raw chicken manure as pond fertilizer may result in the transmission of *Salmonella* from manure to the cultured product [156].

Compounds commonly used in aquaculture include chemicals that might be considered a potential threat to human health include drugs and biologics, pesticides, disinfectants, and water-treatment products. FDA oversees the use of drugs in aquaculture and has approved oxytetracycline, sulfadimethoxine/ormetoprim, formalin, and tricaine for use in various aquatic species [157]. Many more drugs are believed to be used in an off-label fashion in aquaculture. FDA Office of Seafood began a monitoring program for animal drug residues in farmed seafood in 1991 and has detected some instances of residues of chloramphenicol in shrimp and oxolinic acid in salmon [158].

Oxytetracycline is a prototype antibiotic approved by the FDA for use in fish farming to control certain diseases in salmonids and catfish [159]. The normal method of

administration of oxytetracycline to fish is to mix the drug into feed. As a consequence, the concentration of the drug in feed and the composition of feed can influence the disposition of the drug itself [160]. Oxytetracycline is depleted over a period following the completion of the treatment and detectable residues are present in the fish and could be transferred to human if the fish is marketed during that period [161]. In addition, use of other antibiotics as, for example, chloramphenicol in shrimp culture, may similarly result in significant levels in the harvested product. Likewise other bioactive substances, such as antiparasitic agents and algicides, can accumulate in the aquaculture products. For instance, mebendazole and its metabolites have been shown to leave detectable residues in cultured eel [162].

Allergic reactions have been reported following the ingestion of penicillin containing milk in a few previously sensitized patients. Primary sensitization of humans to antimicrobials through the consumption of drug residues in foods has never been clearly documented and evidence suggests that the residue levels in food may be too low to cause sensitization. Drug toxicity, other than allergic reactions, appears not to result from residues of antimicrobial drugs in food [163,164]. Although the available data suggests that these cases are exceedingly rare, they illustrate the continuing need to control antibiotic residues. The human risk can be minimized by the judicious use of antibiotics and observance of washout periods [164].

Genetically engineered neo-antigens

Food biotechnology, the use of recombinant-DNA and cell-fusion techniques to confer selected characteristics on plants and animals used for food, can be used to increase agricultural productivity. The transfer of genes from microbes, plants, or animals into foods raises issues about the unintended consequences of such manipulations. Allergenicity could be one such consequence, since genes encode proteins, which potentially could be allergenic [165]. Even though several bioengineered products have been introduced into the human diet since 1990, they have not yet resulted in any confirmed reported case of food allergy.

An allergen from a food known to be allergenic can be transferred into another food by genetic engineering. This situation occurred when Brazil nut 2S albumin, which is probably one of its major allergens, was introduced into soybeans to improve their nutritional quality. Recognizing the potential problem, the company that had developed the transgenic soybeans discontinued plans to market them [166]. Thus products of food biotechnology should be subjected to a careful and complete safety assessment including the potential allergenicity of the novel proteins introduced into these foods before commercialization [167].

Polar fish produce antifreeze proteins (AFPs), which even at low concentrations decrease the freezing point of

solutions and inhibit ice crystal growth. Transgenic expression of AFP in plants can prevent frost damage to crops and improve the quality of frozen fruits and vegetables. Genes encoding for fish AFPs have been successfully expressed in tobacco and tomato plants [168]; and AFP genes transferred from winter flounder to Atlantic salmon, resulted in functionally effective levels of AFP [169]. Fish AFP do not belong to known fish allergens, but they may acquire allergenic properties when expressed in a different host or consumed in larger amounts. Using the same technique, other genes can be transferred from marine species to other animals or plants that could create neo-antigens and result in allergic sensitization. On the other hand, fish can be recipients of transgenes that enhance disease resistance, increase growth rate and size, improve food conversion ratio, or benefit consumers by enhancing nutritional value or palatability. Transgenic expression of growth hormone has been achieved in commercially farmed fish, such as tilapia, catfish, trout, and salmon [170–172]. Limited data are available on the safety of biotechnology products in aquaculture; however, no post-marketing rise in incidence of seafood allergy has been clearly documented and in a published trial no adverse effects were detected in healthy subjects after the consumption of growth hormone-transgenic tilapia [173].

Poisoning due to bacterial toxins

Botulism

Foodborne botulism is acquired from ingestion of food contaminated with preformed toxin that is produced by *Clostridium botulinum*, a sporulating, anaerobic Gram-positive bacillus. It is characterized by symmetric, descending, flaccid paralysis of motor and autonomic nerves, usually beginning with the cranial nerves, which may be fatal due to respiratory failure. Onset is abrupt, usually 12–36 hours after ingestion of toxin. The first manifestations are often dry mouth, diplopia, blurred vision, blepharoptosis, and photophobia due to loss of papillary light reflex, but may be preceded by gastrointestinal symptoms, such as nausea, vomiting, abdominal pain, and diarrhea. Other common symptoms are generalized weakness, dysphagia, dysarthria, nasal voice, and constipation due to paralytic ileus. Sensory disturbances, fever, and tachycardia are typically absent. The diagnosis of botulism is based on clinical findings; history of exposure to suspect foods; and is confirmed by detection of toxin in serum, stool, or gastric contents of the patient or in leftover fish. The differential diagnosis includes the Guillain–Barre syndrome, myasthenia gravis, basilar meningitis, and stroke [174].

C. botulinum elaborates seven types of toxin. Types A, B, and E are usually involved in human poisoning. Botulism from seafood products is most frequently caused by type E toxin, which is the most predominant type in Alaska and Great Lakes area. Type E spores have been demonstrated in lakeshore mud, coastal sands, and sea bottom silt in

northern latitudes that can contaminate the intestinal tract of fish. Outbreaks of botulism have been reported after eating unviscerated, salted, air-dried whitefish and mullet, known as kapchunka and faseikh, respectively [175,176]. In Alaska, cases have been linked to Alaska Native foods, such as marinated raw fish aged in plastic bags, seal meat stored in oil, and smoked salmon wrapped in seal skins [177].

The spores are highly heat resistant and may not be inactivated by boiling for several hours. However, commercial canning procedures that use moist heat at temperatures above 250°F (121°C) will kill the spores. Although the majority of reported cases of botulism have been associated with the consumption of inadequately processed home-canned food, about 10% of outbreaks have resulted from contamination of commercially canned fish. In these cases, post-processing contamination owing to faulty cans or inadequate heating during the process have been found to be responsible for the outbreaks [174,178]. Toxins, on the other hand, are readily destroyed by heat and are inactivated by boiling for 10 minutes, or by heating at 80°C for 30 minutes. They are, however, resistant to digestive enzymes and are readily absorbed into circulation from the gastrointestinal tract. The toxins are zinc metalloproteinases that cleave specific components of the synaptic membrane docking and fusion complex, which prevents the release of acetylcholine at the neuromuscular junction and autonomic synapses [179].

Treatment includes close medical supervision, supportive care, and early use of trivalent equine antitoxin (types A, B, and E) and GI decontamination. The source of an outbreak must be determined to prevent further cases. Only prompt recognition, therapy, and epidemiological investigation can reduce the death toll from botulism [180]. In Alaska, where approximately 27% of US foodborne botulism cases occur, early diagnosis and antitoxin treatment have contributed to the decline of the case-fatality rate from approximately 31% during 1950–1959 to no deaths since 1994 [181].

Staphylococcal food poisoning

Acute gastroenteritis is caused by the ingestion of food contaminated with preformed staphylococcal enterotoxin. The onset is abrupt and ranges 2–8 hours post-ingestion. Symptoms start with characteristically severe nausea and vomiting. Other symptoms may include abdominal cramps, diarrhea, and occasionally headache and fever. The attack is brief, most often lasting only 3–6 hours, and recovery is complete; but in severe cases it may lead to dehydration, prostration, and shock. Diagnosis is clinical and can be confirmed by demonstration of coagulase-positive staphylococci in the suspected food or vomitus. Treatment is symptomatic [182]. The disease is caused by the enterotoxins produced by *Staphylococcus aureus*, rather than the organism *per se*, which can multiply in a wide temperature range from 39°F to 115°F (4°C to 46°C). Fish, along with cream pastries, milk, processed meat, and mayonnaise, provide excellent media and if

contaminated and allowed to remain at room temperature, these organisms can rapidly multiply and produce toxins. Currently nine enterotoxins have been identified. They are resistant to heat and are only destroyed by prolonged boiling [183].

Bacterial and viral infections

Vibrio species

Nine marine *Vibrio* species have been associated with food-borne disease in humans. *Vibrios* are not detected by standard methods of monitoring coastal waters for bacterial contamination, and standard commercial decontamination techniques do not rid shellfish of these organisms.

Vibrio cholera infection is most prevalent during the summer months. It is characterized by abrupt onset and watery diarrhea; vomiting occasionally occurs [34]. It is endemic in the coastal waters of the Indian Ocean. The largest outbreak in the United States in a century involving 18 persons was reported in 1986 in Louisiana. It was associated with undercooked crabs, which are the most important vehicle for *V. cholerae* infection in the United States; but shrimp and oysters can also transmit the disease. A persisting reservoir along the Gulf Coast may continue to cause sporadic cases [184].

Vibrio parahaemolyticus is found in coastal waters throughout the world. This agent is the leading cause of acute diarrheal disease in Japan, presumably because of the frequency of ingestion of raw seafood. In the United States, it has been related to inadequately cooked seafood, usually shrimp, and was recently reported to be associated with crayfish consumption [185]. *V. parahaemolyticus* damages the intestinal mucosa and the stool may be bloody. Diarrhea develops 12–48 hours after ingestion of contaminated food, and is associated with abdominal cramps. Chills and fever are observed in over half the cases. Between 1973 and 2004, 60 outbreaks of *V. parahaemolyticus* infections were reported to the CDC, and involved about 1200 individuals [10,186]. Most of these outbreaks occurred during the warmer months and were attributed to seafood, particularly shellfish. Of patients with acute *V. parahaemolyticus* gastroenteritis, 88% reported having eaten raw oysters during the week before their illness occurred. The median attack rate among persons who consumed the implicated seafood was 56% [186].

Although quite rare, infection of immunocompromised persons with *V. vulnificus* can be associated with high mortality (50%). It appears to be part of the normal bacterial flora of estuaries along the United States, Gulf, Atlantic, and Pacific coasts. The septicemia induced by *V. vulnificus* is associated with eating raw oysters. Of patients with primary septicemia, which accounts for approximately half of the cases, 96% consumed raw oysters and 61% died, usually in association with underlying liver disease. Oysters harvested in the Gulf of Mexico grown and water temperature exceeding 72°F (22°C) closely correlated with the infection [187].

Norwalk virus

In the United States, about 55% of reported shellfish-related incidents are registered as unknown etiology, but are believed to be due mainly to Norwalk, Norwalk-like, or human enteric virus infections, with a smaller proportion caused by *Vibrio* bacteria [188]. The first documented shellfish-associated gastroenteritis involving Norwalk virus was in Australia in 1979, with more than 2000 cases [189]. Since then, many outbreaks of Norwalk or Norwalk-like viral gastroenteritis have been reported in the United States. Incubation periods were generally 24–48 hours. The most common symptoms were nausea (100%), vomiting (83%), diarrhea (50%), and abdominal cramps; and were of brief duration and resolved within 24–48 hours [190,191]. The diagnosis is clinical, with typically unrevealing bacterial studies on stool and shellfish specimens. It can be confirmed by demonstration of seroconversion and the formation of IgM antibody to Norwalk virus. In addition, Norwalk virus was identified by RIA in clam and oyster specimens. The reported incidents have increased in the last decade.

Shellfish-borne disease occurs mostly from mollusks consumed raw or lightly heated. In a confirmed outbreak of Norwalk virus gastroenteritis, 83% of persons who ate raw oysters became ill versus only 7% of people who did not, suggesting a relative risk, 11.9% for consumption of raw oysters. The outbreak was caused by contamination of oysters in the oyster bed by stool from ill harvesters who routinely dispose their sewage overboard [192]. Steaming clams to open the shells takes about 1 minute, but to inactivate viruses it takes between 4 and 6 minutes [193]. These organisms do not multiply once released into the marine environment, but remain infectious in the presence of organic material in the water and temperatures below 50°F (10°C) [194].

Finally, marine organisms such as oysters may concentrate microorganisms including hepatitis A [195]. Contamination occurs through collection of shellfish grown in sewage-polluted waters, by contaminated waters used in irrigation and through infection of foods by food handlers.

Conclusions

This review presents the more common clinical syndromes produced by the ingestion of natural seafood toxins. For the practicing allergist, knowledge of this wide array of toxic syndromes is important for the proper differential diagnosis of seafood allergy (Table 41.4). A careful history and physical examination are essential to establish the diagnosis on clinical grounds, which can be confirmed by detection of toxins either in remnants of the seafood or in specimens collected from the patient. The history should include symptoms and their severity, time of onset with respect to ingestion of seafood, number and frequency of reactions, whether others became ill, previous history of food allergy, types of marine species ingested and where they were captured, and

Table 41.4 Summary of common toxic syndromes associated with naturally occurring toxins in seafood

Type of poisoning	Type of toxins	Source	Symptom onset	Clinical syndrome
Scombroid	Histamine	Tuna, mahi-mahi, bonita, marlin, bluefish, wahoo, mackerel, and salmon	Minutes to 4 hours	Severe headache, dizziness, nausea, vomiting, flushed skin, urticaria, and wheezing
Ciguatera	Ciguatoxins	Coral reef fish: amberjack, snappers, grouper, goat fish, barracuda, sea bass, surgeon fish, ulua, and papio	30 minutes to 4 hours	Abdominal pain, diarrhea, vomiting, paresthesias, cold-to-hot sensory reversal, weakness, and myalgias
Puffer fish poisoning	Tetrodotoxin	Ocean sunfishes, porcupine fishes, and fugu	10–45 minutes	Paresthesias headache, vomiting, diaphoresis, and respiratory paralysis
Paralytic shellfish	Saxitoxins	Mussels, clams, and oysters	5–30 minutes	Vomiting, diarrhea, facial paresthesias, and respiratory paralysis
Neurotoxic shellfish	Brevetoxins	Mussels and clams	30 minutes to 3 hours	Diarrhea, vomiting, abdominal pain, myalgias, paresthesias, and ataxia
Amnesic shellfish	Domoic acid	Mussels, clams, crabs, and anchovies	15 minutes to 38 hours	Vomiting, diarrhea, headache, myoclonus, loss of short-term memory, seizures, coma, and hemiparesis
Diarrhetic shellfish	Okadaic acid, Dinophysistoxins, Pectenotoxins, Yessotoxin	Mussels, clams, and scallops	30 minutes to 6 hours	Diarrhea, nausea, vomiting, and abdominal pain

the quantity of food consumed, and the way in which it was prepared. Whether the food was eaten at a restaurant, the patient was traveling, alcohol was consumed, or medications were taken by the patient should be recorded.

Presence of similar symptoms in other individuals who shared the seafood meal and the “endemic” nature of the syndrome are paramount in alerting the physician to possible seafood poisoning. The absence of prior reactions to the same seafood and its subsequent tolerance without symptoms point away from an allergic etiology and should be considered as corroborative evidence in support of a toxic syndrome. Since histamine mediates the symptoms of both scombroid and type I hypersensitivity reactions, clinical manifestations of scombroid may be virtually indistinguishable from seafood allergy. History of a “peppery” taste and type of fish consumed, as well as suspected improper refrigeration, are helpful in reaching the proper diagnosis.

Neurological symptoms associated with an allergic reaction are the result of hypoperfusion of the CNS and correlate with the severity of cardiovascular involvement and hypotension in anaphylaxis. This may help the physician to distinguish ciguatera, PSP, NSP, and ASP, where neurological impairment is commonly present in the absence of hypotension. In ciguatera, knowledge of the type of fish and whether it is imported from or consumed in endemic areas, such as Caribbean, Hawaii, and Pacific Islands, will provide clinical information to differentiate it from seafood allergy. Likewise in puffer fish poisoning, consumption of fugu, a delicacy of Japanese cuisine; and in shellfish poisoning, the location where seafood was caught, for instance

Pacific Coast in cases of PSP and ASP, are crucial pieces of information. The seasonal association with algal blooms and presence of high levels of biotoxins or toxic algae that are reported by authorities surveying coastal waters should increase the index of suspicion for physicians practicing in endemic areas. In the majority of these toxic syndromes, the causative toxin does not alter the taste and appearance of the seafood and is not inactivated by normal cooking.

Treatment is supportive, with active early respiratory support, especially in cases where neurological involvement could lead to respiratory paralysis. Upper respiratory reactions in individuals with no history of atopy and exacerbation of chest symptoms in asthmatics are caused by aerosolized NSP toxins. These irritant reactions are usually associated with a red tide and should not be mistaken for allergic respiratory symptoms.

Viruses, bacteria, and bacterial toxins may cause gastrointestinal and systemic symptoms that can be confused with food allergy. Raw or lightly steamed shellfish and raw fish are potential sources of infection with hepatitis A and Norwalk virus and *Vibrio* sp. Botulism is a hazard associated with consumption of home-canned, vacuum-packed smoked, or unviscerated salt-dried fish. If alternative diagnoses cannot be ruled out and seafood allergy remains a likely diagnosis, skin prick test and oral food challenge are diagnostic procedures of choice that may be employed in the evaluation of the patient.

Most current health risks associated with seafood contamination originate in the environment and should be dealt with by control of harvest or at the point of capture using HACCP principles. The most effective way of protecting

consumers is to establish and maintain comprehensive monitoring programs for toxic algae and toxins in shellfish in all growing areas. Developing a better understanding of factors that promote harmful algal blooms and lead to production of toxins by marine algae is crucial to control human exposure and deleterious environmental effects. Further research is needed in most areas of seafood poisoning. Easy, accurate, and cost-effective methods for detection of toxins in seafood, monitoring shellfish for viral and bacterial contamination, and surveillance of coastal waters for harmful marine algae and their toxins are needed. Knowledge gained from research on the mechanism of action of marine toxins should lead to more specific treatment modalities that would limit morbidity and mortality of seafood intoxications. The following general preventive measures could greatly reduce the incidence of poisoning outbreaks that are associated with seafood:

- 1 Avoid eating raw seafood.
 - 2 Avoid eating lightly steamed and undercooked shellfish.
 - 3 Adhere to the public health agency guidelines on harvesting, processing, and consumption of shellfish and avoid shellfish from areas of frequent red tides.
 - 4 Promptly refrigerate the catch of sport fishermen.
 - 5 Avoid eating large, predatory reef fish usually implicated in ciguatera poisoning, especially barracuda, amberjack, and snapper.
 - 6 Avoid reef fish caught in ciguatera endemic areas, especially the head, viscera, and roe.
 - 7 Promptly report the suspected outbreaks of seafood poisoning to local health departments.
 - 8 Submit left-over seafood or uncooked portions of the fish or shellfish to local health departments for analysis to establish nature and amount of contaminating toxin.
- Finally, the informed physician can be of great help in public health prevention through public education and involvement with the local and public agencies that deal with these health issues.

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