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REVIEW ARTICLE
Shellfish toxicity: human health implications of marine algal toxins

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SUMMARY

Five major human toxic syndromes caused by the consumption of shellfish contaminated by algal toxins are presented. The increased risks to humans of shellfish toxicity from the prevalence of harmful algal blooms (HABs) may be a consequence of large-scale ecological changes from anthropogenic activities, especially increased eutrophication, marine transport and aquaculture, and global climate change. Improvements in toxin detection methods and increased toxin surveillance programmes are positive developments in limiting human exposure to shellfish toxins.

Key words: Food safety, toxic fish and shellfish poisoning, toxins.

INTRODUCTION

Shellfish are a rich source of protein, essential minerals and vitamins A and D and they feed mainly on marine microalgae. The importance of algae in the food chain arises from the fact that they are the only organisms that can readily make long-chain polyunsaturated fatty acids (PUFAs) and the potential beneficial role of shellfish and finfish in the human diet has been attributed to the presence of oils that are rich in PUFAs [1]. Bivalve molluscs filter large volumes of water when grazing on microalgae, and can concentrate both bacterial pathogens and phycotoxins [2]. A range of human illnesses associated with shellfish consumption have been identified as being due to toxins that are produced by marine microalgae. When algae populations increase rapidly to form dense concentrations of cells they may form visible blooms, the so-called 'red tides' (Fig. 1), but blooms are not always visible as they may not be coloured and they can proliferate well below the surface. The term 'harmful algal blooms' (HABs) is preferred and these events can have negative environmental impacts including oxygen depletion of the water column and damage to the gills of fish. Moreover, toxin-producing algae can cause mass mortalities of fish, birds and marine mammals and human illness via consumption of seafood. It is estimated that only 60–80 species of about 4000 known phytoplankton are potentially toxin-producing and capable of producing HABs [3]. Maximum toxin levels permitted in shellfish are controlled by national and international regulations and

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Fig. 1. A dramatic algal bloom (red tide) in the South China Sea. This bloom, *Noctiluca scintillans*, was non-toxic. (Reproduced with permission of Springer SBM NL. In: Okaichi T, Fukuyo Y, eds. *Red Tides*, Berlin, Heidelberg: Springer, 2004.)

new analytical methods have been developed for the determination of toxins in shellfish, especially liquid chromatography–mass spectrometry (LC–MS). These methods have recently been reviewed and will not be discussed in detail [4]. The European Food Standards Agency has recently published a scientific opinion on marine biotoxins with proposals to lower some toxin limits and other measures that will hasten the replacement of mouse bioassay (MBA) methods that have traditionally been used to monitor toxin levels in shellfish for human consumption [5]. Unfortunately, the lack of clinical testing methods has led to a large underestimation of the incidence of human poisonings due to algal toxins, especially since many of the symptoms are similar to viral and bacterial infections. In addition, only acute intoxications due to algal toxins are recognized and there is very little knowledge of the human impacts due to chronic exposure to these toxins. The high potency and target specificity that many of these marine toxins possess has led to their exploitation as research tools [6].

The main vectors of algal toxins to humans are filter-feeding bivalve molluscs and herbivorous finfish that ingest toxic algae (Fig. 2). The bivalve molluscs that are mainly affected with algal toxins include mussels, clams, scallops and oysters. Although crustaceans can also be contaminated with toxins, the extent of toxicity is generally low and the incidences of human intoxications due to crustacean consumption are rare. Other significant environmental impacts of HABs include major fish kills and large mortalities to birds and marine mammals [7, 8]. One of the most dramatic events involving sea mammals was the extensive mass mortalities to sea lions in California due to domoic acid (DA) intoxication where the main vector was anchovy [9]. Figure 2 summarizes the interrelationships and potential vectors for toxins arising from HABs but the toxic impact to humans is predominantly from shellfish consumption. Bivalve shellfish graze on algae and concentrate toxins, if present, very effectively.

Historically, there have been sporadic reports of shellfish poisoning; one fatal incident that occurred in British Columbia in 1793 was reported by Captain Vancouver and the earliest scientific reference to shellfish poisoning appeared in 1851 [10]. Prohibitions regarding the consumption of shellfish are found in several cultures and, together with religious beliefs, this has limited the role of shellfish as a potential food source. Such prohibitions are found in the Old Testament:

> These ye shall eat of all that are in waters: all that have fins and scales shall ye eat: And whatsoever hath not fins and scales ye may not eat; it is unclean unto you. (Deuteronomy 14: 9–10; King James Version)

In this review, five major human toxic syndromes caused mainly by the consumption of bivalve molluscs contaminated by algal toxins are discussed (Table 1), together with the identification of the increased risks to humans of shellfish toxicity.

SHELLFISH TOXIC SYNDROMES

Paralytic shellfish poisoning (PSP)

Mild symptoms include a tingling sensation or numbness around the lips which gradually spreads to the face and neck, accompanied by a prickly sensation in fingertips and toes. Greater intoxications induce headache, nausea, vomiting and diarrhoea with increasing muscular paralysis and pronounced respiratory difficulty. In the absence of artificial respiration there is a high risk of death as a consequence of acute PSP intoxication [7]. The onset of symptoms of PSP in humans is dose dependent and can occur rapidly (within 30 min) after the consumption of shellfish. PSP toxins are collectively called saxitoxins (STXs) and at least 21 analogues of these cyclic guanidines are known in shellfish, with saxitoxin (Fig. 3*a*) being the most common toxin. STXs exert their effect by a direct binding on the voltage-dependent sodium channel blocking the influx of sodium and the generation

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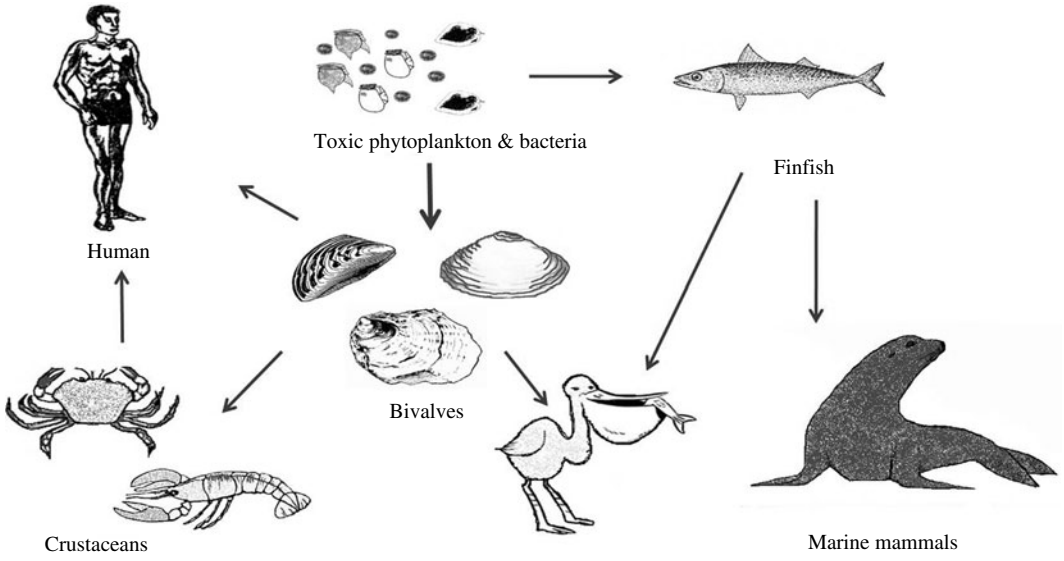

Fig. 2. The toxin cycle: diagram illustrating the interrelationships between harmful algae and shellfish, finfish, birds and mammals.

of action potentials in nerve and muscle cells, leading to paralysis [11]. The primary site of action of STXs in humans is the peripheral nervous system. The lethal dose in humans is 1–4 mg STX, or equivalent STXs, and since levels up to 100 μg STX equivalents/g shellfish have been reported, consumption of only a few contaminated shellfish have proved fatal in these rare cases. However, hospitalization of affected individuals is critical to deal with respiratory paralysis and STXs clear from the blood within 24 h leaving no organ damage or long-term effects [12]. Saxitoxin has reached notoriety by being included, along with ricin, in the Schedule 1 list of the Chemical Weapons Convention. Detection and control of PSP toxins in shellfish is less problematic than the control of lipophilic toxins. PSP toxins are efficiently extracted from shellfish tissues using a strong acid and a MBA has been validated as an official method by AOAC International [13].

Dinoflagellates that produce STXs belong to three genera; *Alexandrium*, *Gymnodinium* and *Pyrodinium* and HABs involving blooms of these dinoflagellates occur in both Northern and Southern Hemispheres [8] (Table 2). It has been estimated that there are 2000 human intoxications per year and PSP outbreaks are seasonal [14, 15]. Although there is anecdotal evidence of human intoxications associated with shellfish for centuries, a PSP outbreak that occurred in northern California in 1927 led to a major investigation of this phenomenon. Poisoning of 102 individuals from mussel consumption caused six deaths [16]. PSP outbreaks have occurred on both the eastern and western coastlines on North America, with Alaska being particularly badly affected and toxic events have been reported for more than 130 years [17, 18]. Large marine mammals have also been affected by PSP and 14 humpback whales died in Cape Cod Bay in 1987 from exposure to STXs where mackerel was suspected to be the main vector [19].

Although STXs are detected in the coastal waters and shellfish in many European countries, human intoxications are rare. In the 1970s, there were several PSP intoxications involving 80–120 individuals, caused by mussels produced in Spain, Portugal and the UK [20–22] but implementation of good regulatory control has effectively eliminated further major outbreaks. There have been repeated PSP outbreaks in Chile and Argentina during the past 40 years, with 21 PSP deaths reported in Chile since 1991 [23], and these investigations included one of the rare identifications of toxins in the body fluids of victims [24]. In the Philippines, there have been an estimated 2000 cases of PSP between 1983 and 1998, with 115 deaths [25]. Blooms of *Pyrodinium* spp. were the main cause of these intoxications and these blooms have spread throughout the tropical Pacific region. Climate change has been implicated with an apparent correlation between these HABs and the occurrence of El Niño Southern Oscillation events [26]. PSP events in geographically remote locations cause higher death

Table 1. *Confirmed outbreaks of human poisonings due to shellfish toxins*

Toxic syndrome	Location of outbreak (year)	Shellfish species	Number of poisonings	Ref.
PSP	USA – California (1927–1936)	Mussels	>100 (6 deaths)	[16]
	USA – Alaska (1973–1992)		117	[14]
	USA (1998–2002)		43	[123]
	Canada (1880–1970)		187	[17]
	Spain	Mussels	120	[20]
	UK (1968)	Mussels	78	[22]
	Norway (1901–1992)		32 (2 deaths)	[124]
	Portugal (1994)		9	[21, 23]
	Chile (1991–2002)	Mussels, oysters	21 deaths	[24, 26]
	Philippines (1988–1998)		877 (44 deaths)	[25]
DSP	Japan (1976–1984)	Mussels, scallops	>1000	[39, 40]
	France (1980–1987)	Mussels	7600	[41]
	Denmark (1990–2002)	Mussels	800–900	[43, 47]
	Norway (1984–1985)	Mussels	>400	[125]
	Spain (1978–1981)	Mussels	>5000	[43]
	Portugal (2002)	Mussels	58	[126]
	UK (1997)	Mussels	49	[45]
	Ireland (1984–1994)	Mussels	?	[46]
	Canada (1990)	Clams, mussels	16	[127]
	Chile (1970–1991)	Mussels, cholgas	>100	[128]
	Argentina (2000)	Mussels	40	[129]
	New Zealand		13	[59]
NSP	USA – North Carolina (1987)	Oysters	48	[60, 130, 131]
	USA – Florida (1996–2006)	Whelks, clams	23	[56, 61]
	New Zealand (1993)	Green mussels, cockles, oysters	186	[58, 132]
ASP	Canada (1987)	Mussels	107 (3 deaths)	[66, 67]
	USA – Washington State (1991)	Razor clams	24	[73, 77]
AZP	The Netherlands (1995)	Mussels	8	[85, 133]
	Ireland – Arranmore Island (1997)	Mussels	20–24	[86]
	Italy (1998)	Mussels	10	[89]
	France (1998)	Mussels	20–30	[89, 94]
	UK (2000)	Mussels	16	[93]
	France (2008)	Mussels	200	[134]

PSP, Paralytic shellfish poisoning; DSP, Diarrhoetic shellfish poisoning; NSP, Neurotoxic shellfish poisoning; ASP, Amnesic shellfish poisoning; AZP, Azaspiracid poisoning.

rates due to the lack of hospital facilities with respiratory support equipment.

Diarrhoetic shellfish poisoning (DSP)

DSP is a gastrointestinal illness and the main symptoms are diarrhoea followed by nausea, vomiting and abdominal cramps. DSP can occur within 30 min to a few hours after ingestion of contaminated shellfish and complete recovery occurs within 3 days. Since clinical tests are rarely used for DSP toxins, this condition is often confused with bacterial enterotoxin poisoning. DSP is caused by the ingestion of contaminated filter-feeding bivalve molluscs, especially mussels and scallops, where the lipophilic toxins are accumulated mainly in the digestive glands (hepatopancreas) [27].

DSP toxins were originally divided into three different structural classes: (*a*) okadaic acid (OA) (Fig. 3*b*) and its analogues, dinophysistoxins (DTXs), (*b*) pectenotoxins (PTXs) and (*c*) yessotoxins (YTXs) [28]. However, YTXs have now been excluded from the DSP classification because they are not orally toxic and do not induce diarrhoea [29, 30]. PTXs

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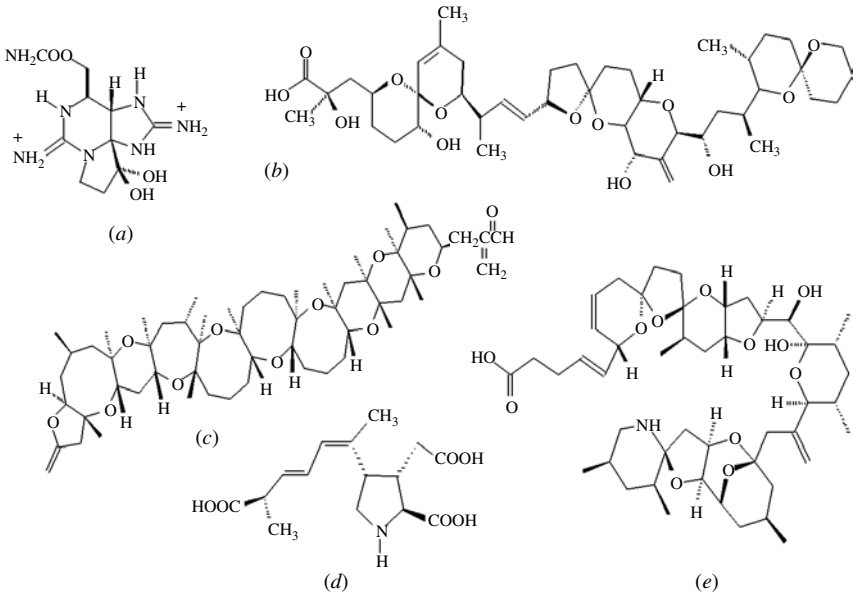

Fig. 3. Structures of the most abundant toxin responsible for each of the five shellfish toxic syndromes; (*a*) saxitoxin (PSP), (*b*) okadaic acid (DSP), (*c*) brevetoxin (NSP), (*d*) domoic acid (ASP), (*e*) azaspiracid (AZP).

and YTXs are toxic to mice upon intraperitoneal injection, which is the official, but primitive, DSP testing procedure. However, no case of human poisoning due to these toxins has been reported. The strange scenario when using the official MBA is that the least toxic substances, YTXs, elicit the highest toxic response [7]. Not only are these lethal bioassays prohibited in several countries, including Germany, The Netherlands and Sweden, alternative methods for toxin determination can only be implemented in the EU when they have been validated against the MBA which itself has never been validated [31]. A recent pronouncement from the European Food Safety Authority belatedly acknowledged the unacceptable current regulatory situation and stated [5]:

> The mouse bioassay (MBA) is the official reference method for lipophilic biotoxins. The Panel on Contaminants in the Food Chain (CONTAM Panel) noted that this bioassay has shortcomings and is not considered an appropriate tool for control purposes because of the high variability in results, the insufficient detection capability and the limited specificity.

The mechanism of action of the OA group toxins is via inhibition of serine-threonine protein phosphatase 2A (PP2A) [32], which plays important roles in many regulatory processes in cells. OA probably causes diarrhoea by stimulating phosphorylation of proteins that control sodium secretion in intestinal cells [33]. Protein phosphatase assays are very sensitive and can be readily applied for detecting OA and analogues in shellfish but LC–MS methods are more widely used [34, 35]. Although DSP is not fatal, this type of poisoning deserves attention, because in addition to the severe acute effects, the chronic effects may be important as OA and DTX1 have been shown to be potent tumour promoters [36, 37]. A major risk factor for colorectal cancer from shellfish consumption has been proposed due to the presence of DSP toxins [38].

The first confirmed outbreak of DSP occurred in Japan in the late 1970s with 164 cases of shellfish poisoning [39]. There were 34 outbreaks of DSP in Japan between 1976 and 1984, affecting more than 1000 people [40]. DSP outbreaks have involved large population numbers and have affected the greatest number of individuals compared to the other shellfish toxic syndromes (Table 1). In Europe, DSP outbreaks involving several thousand individuals have been reported since 1978 in France [41, 42], Norway and Denmark [43], Spain [44, 45] and mussels exported from Ireland have caused DSP outbreaks throughout Europe [46]. Despite this DSP monitoring, mussels from Denmark caused DSP intoxications to more than 1000 individuals in Belgium [47]. DSP is now recognized as a worldwide problem and also affects Canada, Chile, Argentina and New Zealand (Table 1).

DSP toxins are produced by the dinoflagellates, *Dinophysis* spp. and *Prorocentrum* spp. (Table 2) and their toxin profiles can vary within a single species

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Table 2. *Seafood toxic syndromes, toxins and the phytoplankton source of toxins*

Toxic syndrome	Toxins	Affected seafood	Toxic algae
1. Paralytic shellfish poisoning (PSP)	Saxitoxin (STX), neosaxitoxin (NEO), gonyautoxin (GTX) and 18 other analogues	Bivalve shellfish, crustraceans	*Alexandrium* spp. [135], *Gymnodinium* spp. [136]
2. Diarrhoetic shellfish poisoning (DSP)	Okadaic acid (OA), dinophysistoxins (DTXs), pectenotoxins (PTXs)	Bivalve shellfish	*Dinophysis* spp. [49, 137], *Prorocentrum* spp. [138, 139]
3. Neurotoxic shellfish poisoning (NSP)	Brevetoxins (PbTx)	Bivalve shellfish	*Karenia brevis* (formerly *Gymnodinium breve* and *Ptychodiscus brevis*) [140, 141]
4. Amnesic shellfish poisoning (ASP)	Domoic acid (DA) and analogues	Bivalve shellfish, finfish	*Pseudonitzschia* spp. [68]
5. Azaspiracid poisoning (AZP)	Azaspiracids (AZAs) and analogues	Bivalve shellfish	*Protoperidinium crassipes* [105], *Azadinium spinosum* [106]

[48–50]. In Europe, OA and its isomer, DTX2, are the predominant DSP toxins and they co-occur in shellfish from Ireland [46], Portugal and Spain [51]. DTX1, the methyl analogue of OA, is the predominant DSP toxin in Japan [49, 52]. The regulatory level for these toxins in Europe is currently 0·16 μg/g.

Neurotoxic shellfish poisoning (NSP)

NSP is a illness caused by the consumption of bivalve molluscs contaminated with neurotoxins that are produced by the marine dinoflagellate, *Karenia brevis* (formerly known as *Gymnodinium breve* and *Ptychodiscus brevis*) [53, 54]. Brevetoxin (Fig. 3*c*) and its analogues can also affect finfish, aquatic mammals and birds and this topic has been recently reviewed [55, 56]. The symptoms of NSP include gastroenteritis and neurological problems [53]. Brevetoxin-producing HABs have caused problems in the Gulf of Mexico for many decades and have been responsible for respiratory problems and eye irritation in humans due to exposure to aerosol sprays along Florida beaches [55]. Brevetoxins have also been responsible for the deaths of large marine animals, including manatees and bottlenose dolphins [57]. In New Zealand, brevetoxins have also caused problems and new analogues have been identified [58, 59]. The first confirmed NSP outbreak in New Zealand occurred in 1993, affected 186 individuals, and caused both gastrointestinal symptoms and respiratory problems due to aerosol inhalation (Table 1) [59].

In humans, the onset of symptoms of NSP occurs within 0·5–3 h after consumption of shellfish and can include gastroenteritis, chills, sweats, hypotension, arrhythmias, numbness, peripheral tingling and, in severe cases, broncho-constriction, paralysis, seizures and coma. NSP symptoms can persist for a few days [53, 60, 61].

In addition to ingestion, the second route of exposure to brevetoxins is by inhalation of sea spray and this affects individuals who are near to a beach. *K. brevis* is a very fragile dinoflagellate and during rough seas this organism readily ruptures releasing toxins into the water. This exposure to aerosols containing brevetoxins can cause irritation of the eyes and nasal membranes, as well as respiratory problems [62].

The mode of action of brevetoxins is by receptor binding to the sodium channels which control the generation of action potentials in nerve, muscle and cardiac tissue, enhancing sodium entry into the cell. This leads to the incessant activation of the cell which causes paralysis and fatigue of these excitatory cells [63]. A recent NSP outbreak in Florida affected 20 individuals, of which seven were hospitalized. Six individuals complained of uncontrolled muscle contractions and psychotic outbursts [56].

The monitoring of shellfish for NSP has traditionally involved MBAs that involve a non-specific extraction process but this test can be effective for control in situations where other lipophilic toxins are not prevalent. The action level is 20 mouse units (MU) per 100 g shellfish tissue which is equivalent to

0·8 μg brevetoxin (PbTx-2)/g tissues [56]. There are a number of sensitive receptor-binding assays that utilize the specific binding of brevetoxins to sodium channels [64]. LC–MS is the only method for identifying individual brevetoxins in seafood [55]. Overall, it can be concluded that NSP is relatively rare [56], it is not geographically widespread and therefore poses the least threat to human health of the five toxic syndromes discussed in this review.

Amnesic shellfish poisoning (ASP)

ASP first came to attention in Canada in 1987 when human fatalities occurred from eating mussels (*Mytilus edulis*) cultivated in Prince Edward Island [65]. In addition to gastrointestinal disturbance, unusual neurological symptoms, especially memory impairment, were observed. Of the 107 cases involved in this ASP event, three individuals died within 18 days after admission to hospital [66]. The neurological symptoms included headache, confusion, disorientation, seizures and coma within 48–72 h. However, the permanent loss of short-term memory in some of the survivors led this toxic syndrome to be named ASP. Epidemiological studies revealed age-dependent responses to ASP. Those aged <40 years were more likely to suffer gastrointestinal problems whereas individuals aged >50 years were more likely to suffer from memory loss [66]. DA was identified as the causative toxin (Fig. 3*d*) [67] and a short time later, marine diatoms of the *Pseudonitzschia* spp. were identified as the source of this toxin [68]. DA was a previously known marine natural product and was originally discovered in seaweed in Japan where the latter was used for its anthelminthic and insecticidal properties [69]. In addition to mussels, DA can enter the food chain through vectors such as scallops, razor clams and crustaceans [70–72]. There was a second report of human intoxications from consumption of razor clams, cultivated in Washington State, USA, but only two individuals experienced slight neurological problems [73].

Although there are many analytical methods for the determination of DA in seafood, liquid chromatography with ultra-violet detection is used by most regulatory agencies. The permitted limit of 20 μg DA/g shellfish tissue has been generally adopted [74]. DA is a tricarboxylic amino acid and analysis is complicated somewhat by the presence of isomers of DA, as well as tryptophan, in naturally contaminated samples [75]. There have been many worldwide reports of DA contamination of seafood and mortalities to marine animals and birds [76]. An event that generated worldwide publicity was when 70 sea lions were washed up onto beaches in California. It was evident that they were suffering from neurological problems including seizures and 47 animals died. DA was identified in faecal samples from these animals and in anchovies collected nearby [9].

In 1991, an outbreak of DA poisoning was reported in Monterey Bay, California, USA, where pelicans and cormorants were behaving strangely, e.g. vomiting, exhibiting unusual head movements, scratching, with many deaths [77]. In this case the vector was the northern anchovy and it is probable that the making of the Alfred Hitchcock film *The Birds* was prompted by a similar event that happened in the summer of 1961, near Santa Cruz in California. Flocks of shearwaters began acting erratically, flying into houses and cars, pecking people, breaking windows and vomiting. These 'strange' events were reported in local newspapers and these clippings were included with Alfred Hitchcock's studio proposal to make the film, based on Daphne du Maurier's novella. In subsequent years, several similar incidents occurred along the same coastline which have been attributed to DA produced by blooms of *Pseudonitzschia* spp. [78].

Soon after the establishment of monitoring programmes in Europe, DA was found in shellfish from Galicia, Spain [79], Ireland [80], Portugal [81], Scotland [82] and France [83]. In Ireland, only the king scallop (*Pecten maximus*) exhibited high levels of toxin. Although a record high level of DA (2820 μg DA/g) was found in the digestive glands of scallops, the adductor muscle and gonad contained levels below or just over the regulatory limit of 20 μg DA/g [71]. It would therefore be a prudent and simple food safety measure to recommend the non-consumption of the digestive glands of these shellfish to reduce the risk of exposure of humans to ASP. DA has also been found in shellfish from New Zealand, Australia and Chile, but there have been no major toxic incidents involving humans. Further information regarding ASP and DA can be found in a recent review [84].

Azaspiracid shellfish poisoning (AZP)

AZP is the most recently discovered toxic syndrome from shellfish consumption and several analogues belonging to this new class of toxins were identified in contaminated mussels [85–87]. The first confirmed event was in 1995 in The Netherlands and was caused

by the consumption of mussels (*M. edulis*) that were cultivated in Killary Harbour in the west of Ireland. At least eight individuals were affected and the symptoms, nausea, vomiting, diarrhoea and abdominal cramps were similar to DSP. Azaspiracid (AZA1) was isolated from these mussels and the structure was later modified following the total synthesis of AZA1 (Fig. 3*e*) [88]. Several other AZP outbreaks occurred in the following years due to the consumption of mussels cultivated in Ireland (Table 1) [89]. Following the development of sensitive LC–MS methods for their determination [90–92], azaspiracids were identified in five other European countries, including the UK, Norway [93], France, Spain [94] and Denmark [47], as well as throughout the western coastline of Ireland [95]. Azaspiracids have also recently been found in North Africa [96] and Japan [97]. More than 20 analogues of AZA1 have been identified in shellfish [86, 87, 98, 99], which complicates the regulatory control of these toxins as most have not yet been toxicologically evaluated.

Toxicological studies have indicated that azaspiracids can induce widespread organ damage in mice and that they are probably more dangerous than previously known classes of shellfish toxins [100, 101]. AZA1 is distinctly different from DSP toxins as its target organs include liver, spleen, the small intestine and it has also been shown to be carcinogenic. Using oral administration to mice, multiple organ damage was observed; (*a*) fatty change and single-cell necrosis in liver, (*b*) erosion epithelial cells of small intestinal villi and (*c*) lymphocyte necrosis in the thymus and spleen. In the most severe cases, inflammation and oedema in the lungs and stomach occurred. The chronic study showed tumour formation in lungs and malignant lymphomas. All mice used in these studies developed interstitial pneumonia and had shortened small intestinal villi, even at low doses (1 mg/kg) [100, 101]. Cytotoxicity studies using neuroblastoma cells showed that AZA1 disrupts cytoskeletal structure, inducing a time- and dose-dependent decrease in F-actin pools. A link between F-actin changes and diarrhoeic activity has been suggested and this may explain the severe gastrointestinal disturbance in AZP outbreaks. Azaspiracids were found to induce a significant increase in intracellular Ca^{2+} concentration in lymphocytes. Elevation of intracellular Ca^{2+} levels can lead to cell death [102–104].

Azaspiracids have been identified in two dinoflagellates, *Protoperidinium crassipes* [105] and a new species, *Azadinium spinosum* [106]. AZA2 has also recently been found in a sponge (*Echinoclathria* sp.) in Japan, representing the first report of this class of toxins in Asia [97]. Although confirmed reports of AZP have only been associated with mussel consumption, several other types of bivalve shellfish species have been found to accumulate these toxins, including oysters, clams and scallops [95]. The exclusive reliance on the DSP live animal bioassays, recommended by the EU, to monitor azaspiracids contamination of shellfish failed to prevent human intoxications [89]. This was a consequence of poor sensitivity of the assay and the incorrect assumption that azaspiracids were exclusively concentrated in the shellfish digestive glands that were used for testing [107]. Most regulatory agencies in Europe now comply with a strict regulatory control of azaspiracids in shellfish ($<0{\cdot}16\ \mu g/g$ edible tissues) by frequent testing of shellfish using sensitive LC–MS/MS analytical methods, as outlined in recent reviews [4, 108].

GLOBAL INCREASE IN HABs

There has been an apparent global increase in the occurrence of algal toxins in shellfish, with several new toxin classes identified in recent years. However, the reasons behind the apparent expansion in HABs and shellfish toxicity remain unclear with a number of factors being implicated including, climate change, anthropogenic activities, changes in shellfish cultivation, eutrophication, increased global marine traffic, improved toxin detection and better food control and toxin monitoring programmes [15, 109–112]. Projected increases in ocean temperatures are predicted to change global circulation that may lead to an increase of HABs. Moreover, the increased concentrations of greenhouse gases are expected to reduce pH, increase surface-water temperatures and affect vertical mixing and upwelling [113]. Phytoplankton growth is dependent on the availability of nitrogen. Atmospheric deposition of nitrogen, from agricultural and urban sources, can lead to increased algal blooms [3, 114]. Most marine HABs are comprised of dinoflagellates. The mobility characteristics of dinoflagellates allow them to swim under stratified layers of the water column to access nutrients in deeper layers. This may give dinoflagellates a competitive edge over other phytoplankton that cannot swim [113]. The potential consequences of these changes for HABs have received relatively little attention and are not well understood. Several studies have emphasized the

relevance of coastal eutrophication to increased HABs and this is especially relevant to shellfish production and intoxication [110]. Increased coastal aquaculture activities can lead to local nutrient enrichment and eutrophication which not only increases the growth of toxic algae but also acts as the main vector for increased exposure of humans to toxins. A remarkable example of the positive effects of reducing nutrient loading was in Hong Kong harbour where the frequency of algal blooms declined after several years of nutrient reduction [115]. However, many algal blooms are not due to national anthropogenic activities and toxic algae can be transported from remote oceanic regions to affect coastal regions which have normally pristine waters. Thus, in Europe, the major shellfish toxicity from HABs occurs along the western Atlantic coastline, affecting Scotland, Norway, Ireland, France, Spain and Portugal, but the Mediterranean region which has a high nutrient loading, has a low incidence of such problems. It is therefore prudent to caution against a rush to judgement until there has been an extensive database of algal population flux over an extended period of years.

The emergence of non-indigenous toxic algal species in various geographical locations has been linked to an increase in global marine traffic. In particular, the release of ballast waters has been shown to be responsible for invasions of exotic species, including algae, bacteria and zooplankton. Algal cysts in ballast waters have been identified as the cause of new PSP events in regions of Australia that were previously unaffected and led to new ballast water guidelines to limit exposure to exotic species [8, 116]. Recent evidence of an increased global expansion of HABs includes the first reports of palytoxin and tedrodotoxin in European waters and the discovery of azaspiracids in Japan [97]. An outbreak of respiratory illness in people exposed to marine aerosols occurred in Genoa, Italy, in 2005 and a palytoxin analogue was identified as the probable causative agent [117]. *Ostreopsis* spp. are widely distributed in tropical and subtropical areas, but recently these dinoflagellates have also started to appear in the Mediterranean where they produce palytoxins [118, 119].

Tetrodotoxin is a well known paralytic toxin that is found in pufferfish and causes fatalities in Japan almost annually [120]. Once again, a toxin that is usually found in tropical and sub-tropical waters appeared in a trumpet shellfish (*Charonia sauliae*), harvested from the Atlantic coastline of Portugal. An individual was hospitalized and suffered general paralysis, including the respiratory muscles, a few minutes after the consumption of several grams of this shellfish [121]. The investigation of the extent and implications of these new toxic problems in Europe is currently the subject of a collaborative EU project (ATLANTOX) [122].

CONCLUSIONS

The impact on human health from the consumption of biotoxins in shellfish has apparently increased in recent decades. There is evidence, although not conclusive, that the increase in HABs is a consequence of large-scale ecological changes from anthropogenic activities, especially increased eutrophication, marine transport and aquaculture. Global climate change has also been implicated. Recent improvements in toxin detection methods and increased toxin surveillance programmes are positive developments in limiting human exposure to shellfish toxins. However, there is a requirement for the development of clinical tests to improve the correct diagnosis of shellfish poisoning in humans.

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DECLARATION OF INTEREST

None.

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