Chapter 11

MUSSELS AND PUBLIC HEALTH

SANDRA E. SHUMWAY

INTRODUCTION

"As at present carried on" (i.e....., the shellfish trade) "nobody who realizes the meaning of clean food could possibly recommend shellfish for eating, and I venture to think that did the buying public fully realize the conditions the consumption of this commodity would fall considerably." (Holden, 1925, in Dodgson, 1928).

Dodgson went on to comment that shellfish are, "at best, a dirty food, and may be, and frequently are, a dangerous one". Fortunately, conditions have improved in most areas and shellfish are now a popular and relatively safe commodity although, by virtue of their estuarine and near-coastal habitats, they are commonly exposed to sewage and land run-off. While clams and oysters have been the major concern of health agencies, the filtration process generally lacks specificity and selectivity; thus, rendering all filter-feeding bivalves as potential vectors for infection from water-borne agents (including bacteria, viruses, pesticides, industrial chemical and radioactive wastes, toxic metals and hydrocarbon derivatives of oil spills).

We have come a long way since the 1900s, when Asiatic cholera and typhoid fever (enteric fever) were commonly associated with the consumption of polluted shellfish; however, identifiable diseases such as typhoid, cholera and infectious hepatitis, along with gastroenteritis of unknown etiology, are all still associated with consumption of contaminated shellfish. It has long been recognized that 'mussel poisoning' includes at least three pathological conditions: allergic, infectious (bacterial and viral food poisoning (fulminant infection)) and toxic (paralytic shellfish poisoning) (Dodgson, 1928; Acres and Gray, 1978; Eastaugh and Shephard, 1989). To this list we can now add diarrhetic and amnesic shellfish poisoning, all of which have been associated with mussel consumption (see Shumway, 1990 for review; Sindermann, 1990).

The following review focuses on public health issues specifically related to consumption of mussels. It must be remembered, however, that mussels are likely to serve as vectors of *any* water-borne disease or contaminant, and these public health problems will continue to play an important role in molluscan aquaculture development and product marketing.

DISEASES TRANSMITTED BY MUSSELS

Bacterial Infections

Marine mussels are often subject to faecal contamination from domestic sewage discharges, which typically contain pathogenic bacteria such as *Salmonella* sp., *Shigella* sp., *Clostridium* and nonpathogenic *Escherichia coli* (Wood, 1972). These bacteria are concentrated to levels far in excess of the surrounding water by filter-feeding shellfish such as mussels (Ayres et al., 1975). The pathogens are then transmitted to humans after consumption of the shellfish.

While some diseases associated with consumption of contaminated shellfish such as typhoid fever and paratyphoid associated with *Salmonella* are no longer common, the presence of other harmful organisms remains a problem for public health officials worldwide. Even with today's relatively high standards of sewage treatment, significant quantities of potentially harmful micro-organisms may be discharged into areas of shellfish harvest and/or culture.

Most bacteriological diseases can be avoided through depuration and/or adequate cooking or marinating of shellfish (van den Broek et al., 1979). Although mussels are usually eaten cooked, it is the custom in some countries to consume them raw, which increases the potential for disease transmission. It is also a common practice in some areas to cook mussels only until the shell valves are open, which is not adequate to kill all bacteria present.

Because of the associated disease hazards to humans due to bacterial contamination, *Mytilus edulis* has been investigated by bacteriologists for over a hundred years (Dodgson, 1928; Al-Jebouri and Trollope, 1981). Estimates of accumulation of contaminants (bacteria and viruses) are based on indicator organisms, most notably the non-pathogenic *Escherichia coli*, the standard accepted indicator of faecal contamination (Escherich, 1885; Bernard, 1973, 1989). The unreliability of this standard as an indicator of other pathogens, especially viruses, will be discussed later.

Numerous species of bacterial and viral contaminants have been identified from various species of mussels (Table 11.1). This list is not intended to be all-inclusive and it should be remembered that *any* water-borne contaminant is a potential hazard. Brisou et al. (1962) isolated 44 strains of vibrios from *Mytilus galloprovincialis* from the Algerian coast, only some of which are considered pathogenic, and there is clear evidence that some strains of naturally-resident aquatic bacteria are capable of causing gastroenteritis, systemic infections and intoxications in man.

Contamination from pathogens associated with terrestrial soil, fresh and marine waters include bacteria of the genus *Vibrio*. The most important of these are *V. vulnificus*, *V. cholerae* non-01 (NAG *Vibrio*) and *V. cholerae*) group 1 and other

Table 11.1. Known contaminants of mussels. Species names are as they appear in original publications.

Species	Contaminant	Disease	Location	Reference
Aulacomya ater *Mytilus edulis*	*Vibrio parahaemolyticus*	Gastroenteritis	U.S.A., Japan, Argentina, Netherlands	Kampelmacher et al. ,1972; Casellas et al.,1977; van den Broek et al. ,1979
Aulacomya ater *Mytilus platensis*	*Vibrio alginolyticus* *Vibrio parahaemolyticus*		Argentina	Casellas et al., 1977
Crenomytilus grayanus	*Photobacterium* *Aeromonas* *Vibrio* *Flavobacterium* *Pseudomonas* Enterobacteriaceae *Bacillus* Coryneforms Mycelial fungi; *Trichoderma, Penicillium*		U.S.S.R.	Mikhailov et al., 1988
Mytilus edulis	not isolated	Hepatitis A	England	Bostock et al., 1979
Mytilus edulis	Hepatitis–A virus	Acute viral hepatitis	Australia	Deinstag et al., 1976
Mytilus edulis	*Klebsiella pneumoniae* *Enterobacter cloccae* *Enterobacter agglomerans* *Escherichia coli* *Shigella dysenteriae* *Yersinia enterocolitica*		U.K.	Al–Jebouri and Trollope,1978
Mytilus edulis	*Citrobacter* *Enterobacter* *Escherichia coli* *Salmonella* *Yersinia* *Klebsiella*		Spain	Ledo et al., 1983
Mytilus edulis	*Proteus* *Serratia*			
Mytilus edulis	*Escherichia coli* *Streptococcus faecalis* *Salmonella anatum*		France	Plusquellec et al., 1990
Mytilus edulis	*Bacillus subtilis* *Serratia marcescens*		U.K.	Al–Salihi and Trollope, 1978
Mytilus edulis	*Aeromonas hydrophila*		U.K.	Trollope, 1984

Table 11.1. continued

Species	Contaminant	Disease	Location	Reference
Mytilus edulis	*Acinetobacter calcoaceticus* var. *lwoffi* *Bacillus* spp. *Campylobacter jejuni* *Clostridium perfringens* *Corynebacterium* sp. *Escherichia coli* *Enterobacter cloacae* *Erwinia herbicola* Faecal streptococci *Flavobacterium* sp. *Klebsiella pneumoniae* *Kurthia* sp. *Micrococcus* sp. *Pasteurella* spp. *Pseudomonas* spp. *Salmonella hadar* *Serratia* sp. *Shigella dysenteriae* *Staphylococcuss* sp. *Vibrio parahaemolyticus* *Yersinia enterocolitica*			
Mytilus galloprovincialis	faecal coliform		Yugoslavia	Krstulović and Šolić, 1988
Mytilus galloprovincialis	Echo virus 5,6,8,12 Coxsackie virus A18		Italy	Bendinelli and Ruschi, 1969
Perna canaliculus	Coxsackie virus B4 CB5 virus Polio viruses 1,2 and 3		New Zealand	Lewis et al., 1986
Mussels	*Vibrio alginolyticus* *Vibrio parahaemolyticus*		Netherlands	Kampelmacher et al., 1972
Mussels	Faecal coliforms; *Escherichia coli*		cosmopolitan	see: Dodgson, 1928; Wood, 1957; Volterra and Tosti. 1983; Power and Collins, 1990
Mussels	Faecal coliforms; *Escherichia coli*		cosmopolitan	see: Dodgson, 1928; Wood, 1957; Volterra and Tosti, 1983; Power and Collins, 1990

Table 11.1. continued

Species	Contaminant	Disease	Location	Reference
Mussels	*Vibrio cholerae*	Cholera	Italy	Baine et al., 1974
Mussels	Polio virus 3		Italy	Petrilli and Crovari, 1965
Mussels	Echo virus 3,9 and 13		Italy	Bellelli and Leogrande, 1967
Mussels	Coxsackie virus A18		France	Denis, 1973
Shellfish	*Salmonella typhi* *Salmonella paratyphi* *Shigella* spp. *Vibrio cholerae* *Vibrio cholerae* O-group 1	Typhoid Dysentary Cholera	U.K. Europe	Ayres et al., 1975

Vibrio species (Eastaugh and Shepherd, 1989). The presence of these types of bacteria is not associated with faecal contamination from human or animal sources, and they are not detected by standard methods of monitoring for bacterial contamination. Furthermore, vibrios are not always eliminated from shellfish using standard commercial decontamination techniques (Richards, 1985; Eastaugh and Shepherd, 1989).

There have been few studies concerned with the uptake of bacteria by mussels. Obviously, mussels located nearest to sewage outfalls contain the highest numbers of bacteria; however, bacteria have been noted in animals considerable distances from outfall areas. Trollope and Al-Salihi (1984) further demonstrated that mussels from the seabed contained higher numbers of *E. coli* than did mussels immersed just below the surface. It has also been shown that *E. coli* seems to accumulate in *M. edulis* to a smaller extent than other coliforms (Webber, 1982). In general, the uptake of bacteria by mussels, held in areas subject to constant levels of water-borne sewage pollution, will be greater during the summer than the winter.

Bernard (1989) showed that rates of uptake and elimination of bacteria by bivalve molluscs are species-specific and temperature dependant and further, that there is little correlation with ambient loading. Of four bivalve species, *M. edulis* attained the highest accumulations of coliforms, at a higher rate than the other species, and also eliminated them more effectively (Fig. 11.1). In a more recent study, Plusquellec et al. (1990) followed bacterial contamination of *M. edulis* both in the laboratory and under natural conditions. They showed that concentration of bacteria by mussels is influenced by the bacterial species, particle density in the surrounding seawater and season (Fig. 11.2).

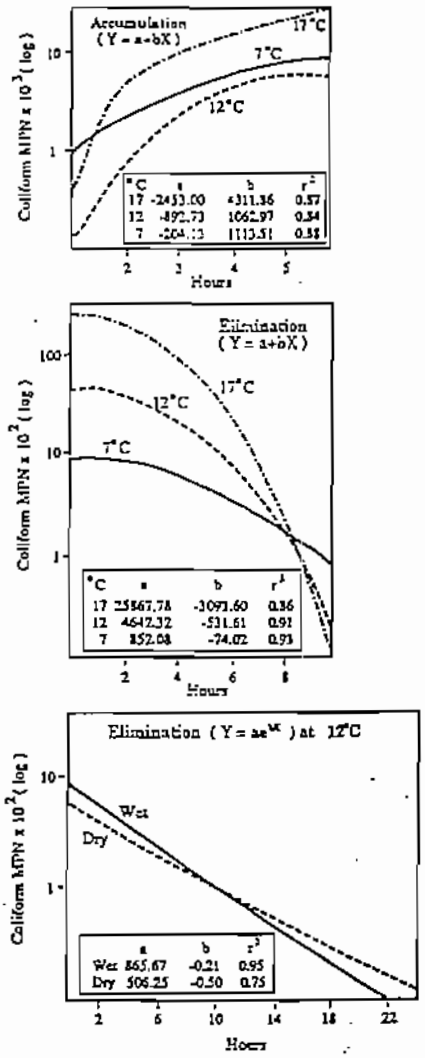

Fig. 11.1. Accumulation and elimination of *Escherichia coli* per g dry meat weight for *Mytilus edulis* as a function of time at three different temperatures and during wet and dry storage. Regression equations and constants are given in the figures; r^2 is the coefficient of determination; MPN = most probable number/100mL. (After Bernard, 1989).

It has been demonstrated that the bacterial load and retention values within mussels vary between tissues, with the digestive gland containing more than 75% of the bacteria (Al-Jebouri and Trollope, 1979). In a later study these authors showed that dissection of the bacteriologically rich digestive gland significantly increased the sensitivity of the *E. coli* detection (3–6 fold enhancement), even when lightly polluted mussels were used. Trollope and Webber (1977) reported a range of 10–87% retention values and a mean of 60 ± 25.35%. Retention by individual tissues also varies:

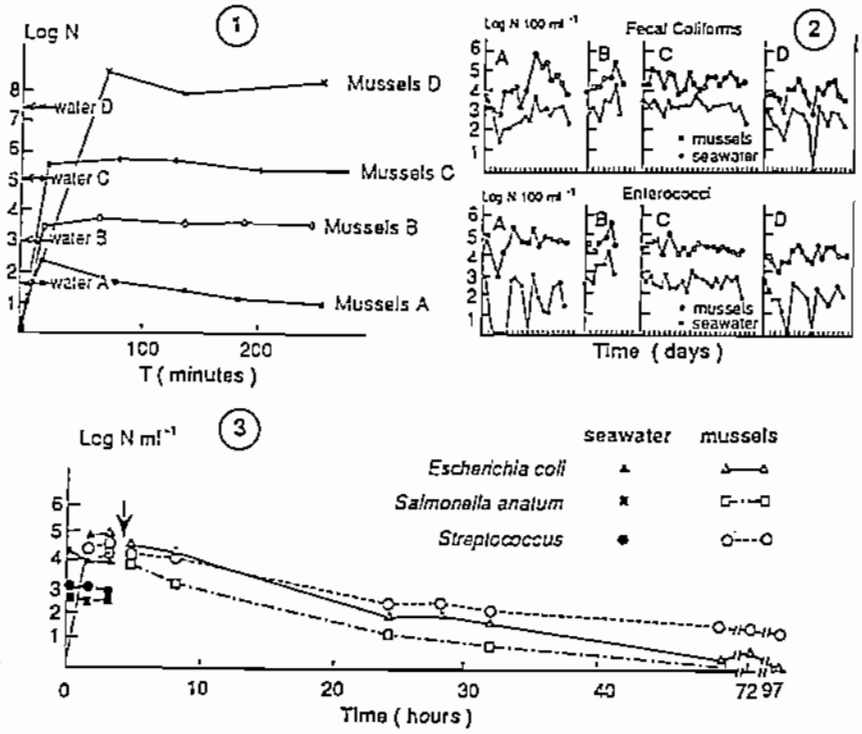

Fig. 11.2. 1: Influence of *Escherichia coli* density in seawater on mussel flesh contamination. (A, B, C, D). 2: Results of daily counts of faecal coliforms and Enterococci in seawater and mussel flesh. A: May–June 1984; B: October–November 1984; C: February 1985; D: May–June 1985.
3: Elimination of bacteria from contaminated mussel tissue. ↓ indicates the transfer to pure running seawater. (After Plusquellec et al., 1990).

intestine, > stomach, > residual tissue, > gill, ≅ mantle, ≅ labial palp, > foot (Al-Jebouri and Trollope, 1978) and Minet et al. (1987) also demonstrated that greater amounts of bacteria are always found in the hind gut. While this differential distribution may provide interesting data for microbiologists, it is of little consequence to the consumer since mussels are consumed whole. In another effort to increase the applicability of *E. coli* tests to public health monitors, Krstulović and Šolić (1988) demonstrated no essential difference in faecal coliform concentration in shellfish flesh alone and those in flesh plus intervalvular fluid. They recommended the use of flesh plus fluid since (1) the coefficient of correlation with the growing water is slightly higher, especially in more polluted waters; (2) the method is simpler since the flesh does not have to be separated from the fluid, and (3), both flesh and fluid are normally consumed.

Viruses

Metcalf (1978) pointed out that at least 66 enteroviruses of human origin might be expected in shellfish-growing areas. These include 3 polioviruses, 24 coxsackievirus A types, 6 coxsackievirus B types and 33 echoviruses. A total of more than 100 viruses might be involved if enteroviruses of animal origin are included. Gerba and Goyal (1978) estimated the number to be more than 100 in human faeces alone including enteroviruses (polio, coxsackie, echo), reoviruses, adenoviruses, infectious hepatitis and rotavirus. Theoretically, any virus excreted in faeces or urine, and capable of producing infection when ingested, could be transmitted by inefficiently treated water (IAWPRC, 1983) and consequently, accumulated by filter-feeding shellfish. These viruses can cause such illnesses as fever, paralysis, meningitis, respiratory disease, diarrhoea and others, and can range from the trivial to the fatal (Feacham et al., 1982). The role of shellfish as vectors of human enteric diseases has been well-documented (Gerba and Goyal, 1978) and luckily, only a few viruses have been shown epidemiologically to be transmitted by shellfish. These include hepatitis A, non-A, non-B hepatitis, Norwalk, Snow Mount agent, astroviruses, coxsackievirus and small round viruses.

Hepatitis A and Norwalk viruses are of chief concern to public health officials, although many others are present and pose potential health hazards and are most commonly associated with oysters (Portnoy et al., 1975; Murphy et al., 1979; Noble, 1990). Nearly all outbreaks of disease associated with excreted viral contamination of shellfish are outbreaks of hepatitis A or viral gastroenteritis (Feacham et al., 1982). To date, no outbreaks of hepatitis B associated with shellfish have been reported, although shellfish in Maine (U.S.A.) waters have been found to carry hepatitis B antigen (Konno et al., 1982). Dienstag et al. (1976) confirmed (both epidemiologically and serologically) acute viral hepatitis from incompletely cooked mussels (*M. edulis*) in Australia. Again, while adequate cooking will destroy most viruses, the habit of eating raw shellfish increases the likelihood of disease outbreak.

Viruses can survive for extended periods of time outside an animal host (see Akin et al., 1975), and can remain infectious for several weeks or longer after discharge into receiving waters. Once inside a shellfish, their survival appears to be further prolonged (Metcalf and Stiles, 1965). Enteric viruses can survive from a few days to over 130 days in marine water (see Akin et al., 1975) and it has been demonstrated that viruses survived for longer periods when in raw sewage (Metcalf and Stiles, 1965). Virus survival in seawater is dependent on temperature, salinity, type of virus, bacterial antagonism, suspended solids and pollution (Gerba and Goyal, 1978); and survival of enteroviruses in sea water is generally reported to be shorter than in fresh water, but they do survive longer in seawater than do coliform bacteria (Feacham et

al., 1982). Temperature appears to be the prime determining factor in viral survival in seawater, with increased inactivation in warmer waters.

Lack of correlation between the depuration of viruses and bacteria has been clearly demonstrated for several species of shellfish (Scotti et al., 1983). It has been calculated that enteric viruses can survive in mussel tissue 3–6 times longer than coliform bacteria, and, in several studies, enteroviruses have been isolated from shellfish otherwise having a satisfactory coliform index (Gerba and Goyal, 1978). Gerba et al. (1975, 1979) reported that no correlation could be found between the presence of entroviruses in marine waters and indicator bacteria; often enteroviruses were found in waters that met current bacteriological standards. In one instance, shellfish (oysters) harvested from a 'clean' area caused a hepatitis A outbreak (Portnoy et al., 1975).

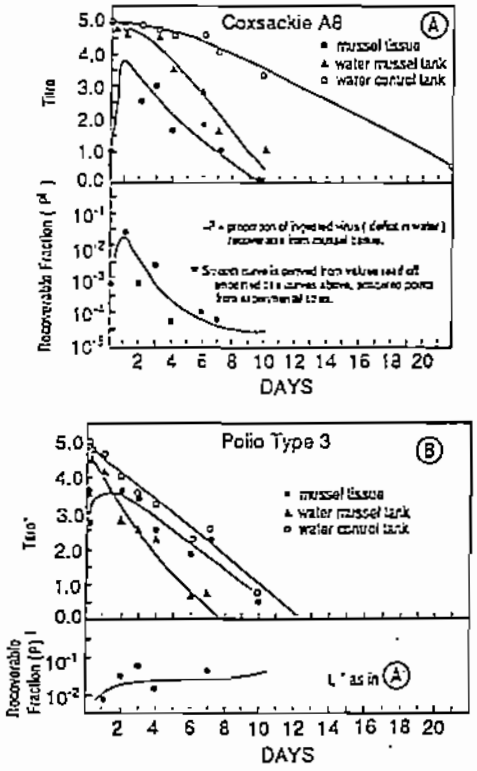

Fig. 11.3. Changes in titres for Coxackie A8 (A) and Polio Type 3 (B) in *Mytilus edulis aoteanus*. A: Titres are expressed as dose concentrations in suckling mice LD_{50s} per 0.03mL. B: Titres are expressed as dose concentrations in tissue culture ID_{50s} per 0.2mL. When virus was just recoverable but not titratable, the point is plotted just below the abscissa. (After Duff, 1967).

Uptake of viruses by mussels has been clearly demonstrated for three species: *M. edulis* (Dimmock, 1967), *M. galloprovincialis* (Milo, 1971), and *Mytilus edulis aoteanus* (Duff, 1967), now considered to be *M. galloprovincialis* (McDonald et al., 1991). Like bacteria, the majority of viruses are concentrated in the digestive system of

the mussels. Duff (1967) demonstrated that polio type 3 virus was significantly more resistant to inactivation capacity of the mussel tissue than coxsackie A8 virus and that maximum infectivity occurred approximately 18-36h after pollution (see Fig. 11.3).

Finally, several authors have suggested that particle association significantly extends the survival capacity of viruses, and enhances their potential for interaction with local marine organisms (Landry et al., 1980, 1983; Liew and Gerba, 1980). Retention of viruses by gill structures is enhanced by adsorption of viruses to fine particulate matter (Duff, 1967). This may have special significance for mussels which are known to feed heavily on resuspended organic matter (Kiørboe et al., 1980; Lucas et al., 1987; Fréchette et al., 1989). The incidence of viral accumulations in the oyster, *Crassostrea virginica* and the clam, *Mercenaria mercenaria* was increased when the sediments (viruses) were resuspended in the water column (Landry et al., 1983). While increased viral accumulation in mussels has not yet been demonstrated in mussels exposed to resuspended sediments, it seems a likely cause for concern, and public health officials need to be more aware of the potential hazards associated with viral contamination of mussels.

Toxic Algal Blooms

Blooms of toxic algal species are common occurrences in shellfish growing areas worldwide and pose a severe threat to public health. The toxins associated with these algae are potent, and the filter-feeding shellfish accumulate the toxic cells during feeding, thus rendering them vectors in various forms of shellfish poisoning including: paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP) and amnesic shellfish poisoning (ASP). Of all shellfish consumed, mussels probably pose the greatest threat with regard to shellfish poisoning. Very early cases were noted but associated merely with 'toxic action on the nerves'. Many fatalitites were noted (see Dodgson, 1928) and the public was warned against taking mussels from 'foul and stagnant water' as they become 'intensely poisonous', and no association was made with plankton blooms. Both ASP and PSP can prove fatal, whereas DSP is easily confused with gastroenteritis and general stomach upsets associated with eating shellfish or contaminated shellfish. Sensitivity to PSP is highly variable and estimates of the lethal dose for humans range from 0.3–1.0mg of saxitoxin (Tennant et al., 1955; Schantz, 1973; Eastaugh and Shepherd, 1989). Severe symptoms have occurred with ingestion of as little as 124μg of toxin. Death has resulted from ingestion of only 456μg (Music et al., 1973).

Amnesic shellfish poisoning is a novel and devastating form of a previously unknown marine toxin. It has been attributed to a bloom of *Nitzschia pungens* f.

multiseries in Canada (Bates et al., 1988). Acute illness was characterized by gastrointestinal symptoms and unusual neurologic abnormalities, including loss of short-term memory. Some patients required intensive care due to seizures, coma, profuse respiratory secretions or unstable blood pressure (Perle et al., 1990). Domoic acid, which can act as an excitatory neurotransmitter, was identified as the causative agent, and has been described recently by Teitelbaum et al., (1990). An intense monitoring program was established in Canada after the first outbreak (Bates et al., 1988, 1989), and no new cases have occurred since December 1987.

All of these forms of shellfish poisoning have been associated with mussels and the topic has been recently reviewed by Shumway (1990). Table 11.2. summarizes the effects of both toxic and noxious algal blooms and their effects on mussels. No geographic area seems immune from possible blooms of toxic species, and many outbreaks of PSP cases occur in areas where there are no monitoring programmes, or amongst picnickers who ignore posted warnings. Government agencies, including food and drug, fishery and public health groups, have taken measures for many years to prevent toxic shellfish from getting into commercial markets and to warn the general public against collecting for their own use. Since the toxins are not inactivated by cooking and there are no known antidotes, mussels should only be eaten from areas known to be monitored regularly for the presence of toxins by an authorized and recognized public health agency.

Allergy

Halstead and Schantz (1984) described an allergic form of shellfish poisoning manifested by severe allergic reaction. The incubation period is usually short (a few hours) and the symptoms consist of "a diffuse erythema, swelling, and urticaria of the face and neck, but may involve the entire body (the rash may be accompanied by a severe itching); headache, sensation of warmth, conjunctivitis, coryza, gastric distress, dryness of the throat, swelling of the tongue and respiratory distress may be present". Patients usually recover within a few hours, but death may occur.

One of the best accounts of an allergic reaction to mussels is given by Dodgson (1928). The symptoms associated with an allergic reaction to mussels were so well-known that 'musselling' was a common term for any indisposition caused by the consumption of various foods. Symptoms were usually noted within a short period of time ranging from five minutes to several hours, and consisted of a red rash or 'nettle-rash', sometimes accompanied by intense itching. Some patients suffered breathing distress, vomiting and/or diarrhoea while others experienced little discomfort other than rash. Recovery was usually complete in less than 12 hours.

Table 11.2. A summary of toxic and noxious algal blooms and their effects on shellfish. Taxonomic nomenclature is as it appears in the original publications.

Shellfish species affected	Algal species	Notes	Location	Reference
Aulacomya ater *Mytilus chilensis*	*Gonyaulax catenella*	toxic	Chile	Guzman and Campodonico, 1978; Avaria, 1979
Modiolus auriculatus *Pinna* sp.	*Pyrodinium bahamense*	toxic shellfish; some human	New Guinea	Maclean, 1973, 1975; Worth et al., 1975
Modiolus sp.	*Pyrodinium bahamense*	toxic	Palau, Micronesia	Harada et al., 1982
Mytilus chilensis	*Amphidoma* sp.	mildly toxic	Chile	Campodonico and Guzman,1974; Avaria, 1979
Mytilus coruscus *Mytilus edulis*	*Protogonyaulax tamarensis* *Protogonyaulax catenella*	toxic	Japan	Anraku, 1984
Mytilus coruscus *Mytilus edulis*	*Dinophysis fortii* *Dinophysis acuminata* *Gymnodinium catenatum* *Protogonyaulax tamarensis*	toxic	Japan	Oshima et al., 1982; Anraku, 1984; Ikeda et al., 1989
Mytilus edulis	*Dinophysis acuminata* *Dinophysis acuta*	highly toxic	Netherlands	Kat, 1983, 1985, 1989
Mytilus edulis	*Dinophysis acuta*	DSP	Sweden	Edler and Hageltorn, 1990
Mytilus edulis	*Dinophysis* spp. including *acuta, acuminata, norvegica*	highly toxic; remained toxic for up to 7mo	Sweden, Norway, Denmark	Krogh et al., 1985; Underdal et al., 1985; Yndestad and Underdal, 1985; ICES, 1988
Mytilus edulis	*Dinophysis* spp. *Prorocentrum* sp.	DSP; first report from area	Wadden Sea, Germany	Meixner and Luckas, 1988
Mytilus edulis	*Prorocentrum micans*	40-50% mortality; probably due to low oxygen.	Brittany	Lassus and Berthome, 1988
Mytilus edulis	*Prorocentrum micans*	toxic; PSP	Portugal	Pinto and Silva, 1956
Mytilus edulis	*Gonyaulax tamarensis*	toxic	U.K.	Ingham et al., 1968

Table 11. 2. continued

Shellfish species affected	Algal species	Notes	Location	Reference
Mytilus edulis	*Gonyaulax tamarensis* *Gymnodinium catenatum*	toxic; PSP	Spain	Campos et al., 1982; Fraga et al., 1984; Blanco et al., 1985; Fraga and Sanchez, 1985
Mytilus edulis	*Gonyaulax acatenella*	several cases of PSP	British Columbia	Prakash and Taylor, 1966
Mytilus edulis	*Gonyaulax excavata*	toxic; shellfish mortalities	Faroe Is.	Mortenson, 1985; Dale et al., 1987; Gaard and Poulson, 1988
Mytilus edulis	*Gonyaulax excavata*	toxic	Argentina	Carreto et al., 1985
Mytilus edulis	*Gonyaulax* sp.	toxic	Uruguay	Davison and Yentsch, 1985
Mytilus edulis	*Nitzschia pungens* f. *multiseries*	highly toxic; over 106 illnesses and 3 human deaths	Prince Edward Is., Canada	Bates et al., 1988, 1989; Subba Rao et al., 1988; Addison and Stewart, 1989; Smith et al., 1990
Mytilus edulis	*Dinophysis* spp.	"probably source of DSP"	New York, U.S.A.	Freudenthal and Jijina, 1988
Mytilus edulis planulatus	*Gymnodinium catenatum*	toxic	Tasmania	Hallegraeff and Summer, 1986
Mytilus edulis *Modiolus modiolus*	*Gonyaulax tamarensis* (*Protogonyaulax*)	highly toxic	Gulf of Maine and E. Canada; Bay of Fundy; St. Lawrence regions	Prakash, 1963; Caddy and Chandler, 1968; Prakash et al., 1971; Hartwell, 1975; Hurst, 1975; Tufts, 1979; Shumway et al., 1988
Mytilus edulis *Mytilus californianus*	*Gonyaulax catenella*	toxic	California and Pacific coast states, U.S.A.	Sharpe, 1981; Nishitani and Chew, 1988
Mytilus edulis galloprovincialis *Mytilus coruscus*	not specified but probably *Protogonyaulax* spp.	toxic	Korea	Jeon et al., 1988
Mytilus sp.	*Dinophysis sacculus* *Gymnodinium catenatum*	DSP; first report from area; PSP outbreaks	Portugal	Franca and Almeida, 1989; Alvito et al., 1990

Table 11.2. continued

Shellfish species affected	Algal species	Notes	Location	Reference
Perna perna	*Protogonyaulax tamarensis* *Gonyaulax monilata*	toxic	Venezuela	Ferraz-Reyes et al., 1985
Perna perna	*Cochlodinium* sp.	symptoms similar to PSP; several fatalities; many illnesses	Venezuela	Reyes-Vasquez et al., 1979
Perna perna	*Gonyaulax tamarensis*	first record from Caribbean; 1 human fatality	Venezuela	Reyes-Vasquez et al., 1979
Perna viridis	*Protogonyaulax tamarensis*[1]	63 cases of PSP; 1 human fatality.	Pran Buri, S. Thailand	Tamiyavanich et al., 1985; Maclean, 1984
Perna viridis	*Pyrodinium bahamense*	highly toxic	Brunei, Philippines	Beales, 1976; Arafiles et al., 1984; Jaafar and Subramaniam, 1984; Gacutan et al., 1985; Gonzales et al., 1989
Perna viridis	*Pyrodinium bahamense*	several human fatalities; mostly juveniles.	Philippines	Estudillo and Gonzales, 1984
Mussels	*Alexandrium minutum*	PSP; first record from area.	France	Nezan et al., 1990
Mussels	*Alexandrium tamarensis* *Alexandrium acatenella*	toxic	Kamchatka, U.S.S.R.	Konovalova, 1989

[1] In a later study (Kodama, 1985) it was demonstrated that the strains of *Protogonyaulax tamarensis* in this area are nontoxic and that the toxicity exhibited by shellfish is due primarily to *P. cohorticula*.

DEPURATION

In many areas depuration is mandatory prior to marketing of shellfish, and in some areas only those shellfish which are cultured, or meant for export, are depurated. In others, depuration needs to be made a routine part of the cultivation process. Some regions are noted for unsanitary measures in the culture and harvesting of mussels, and this has resulted in lack of consumer demand. The two types of depuration processes available to cleanse shellfish contaminated with pathogenic viruses or bacteria are: (1) relaying to clean water and (2), treating with disinfectants, including ultraviolet light (UV), chlorine and ozone (see Blogoslawski, 1983, 1989, 1990; Blogoslawski and Stewart, 1983; Richards, 1988).

The simplest method of cleansing is to move the contaminated shellfish to unpolluted waters (relaying) or to maintain them in sterilized waters under controlled conditions (depuration). Details of plant construction and operation have been given by Furfari (1966), Canzonier (1984), the NSSP Manual, Part II (USDHHS, 1986; USPHS, 1988) and most recently by Howell and Howell (1989). As Dodgson (1928) so aptly put it, "The mussel is as diligent and successful in cleansing itself in favourable conditions as it is in polluting itself in unfavourable ones". Based on this ability to purge themselves, Dodgson established the first mussel purification plant at Conwy, Wales (U.K.), and showed that depuration is an effective method for reducing the microbial flora of contaminated shellfish. Since that time, relaying has been an accepted method for reducing the potential risk of public health hazards (Wood, 1969; Ledo et al., 1983), and this method remains one of the best for reducing public health risks associated with contaminated shellfish. Relaying or self-depuration technology has remained essentially unchanged since first established by Dodgson (Power and Collins, 1989).

Areas used for relaying (depuration in a natural setting (Richards, 1988)) are frequently closed by the regulating agency for various lengths of time until the relayed shellfish are deemed safe to harvest. This method uses waters from approved shellfish harvest areas, and has the advantage of being comparatively inexpensive, and the disadvantage of having a recovery rate of only 50% of the relayed shellfish. The major advantage of this method is that relayed shellfish are only required to meet open area shellfish bacterial standards. The major drawback to relaying is that these systems are labour-intensive and are thus frequently unfeasible (Biogoslawski, 1989). Canzonier (1988) discussed the many drawbacks associated with depuration which include: variable efficacy, unfeasibilty in the case of very heavy bacterial loads, virtual uselessness in reducing contaminants such as hydrocarbons and heavy metals, economic unfeasibility in some cases, lack of control over viral contaminants and potential conflicts with watermen.

Bacterial pathogens can be eliminated from seawater by treatment with UV light, and this system is used commonly to sterilize seawater for depuration of bivalve shellfish. Depuration in the U.S.A. is exclusively with UV light disinfection (Richards, 1988). In U.S. depuration plants the sterilized water must meet drinking water standards for bacteria, i.e. <1 MPN (most probable number)/100mL for coliforms. Water used for depuration in the U.S.A. must come from areas that do not exceed moderate pollution levels i.e. <700 coliforms/100mL. Chlorine has also been used to disinfect seawater which must then be dechlorinated before it can be used to depurate contaminated shellfish. Although this method is more costly than other forms, it is still the method of choice in many depuration facilities (France, Spain, England) because of its reliability. While artificial purification of mussels is widely practiced in Europe, mussels are not currently depurated in the U.S.A.

Table 11.3. Approximate times of contaminant retention for various species of mussels (represents time taken for levels to fall below either quarantine or detection levels).

Species	Source of contamination	Retention time	References
Choromytilus meridionalis	*Gonyaulax catenella*	3mo	Popkiss et al., 1979
Modiolus auriculatus	*Pyrodinium bahamense*	6 weeks	Worth et al., 1975
Modiolus modiolus	*Gonyaulax tamarensis*	up to 60 days[†]	Gilfillan et al., 1976
Mytilus californianus	*Gonyaulax catenella*	<1mo	Sommer and Meyer, 1937; Sharpe, 1981
Mytilus edulis	*Protogonyaulax tamarensis*	10 days–7 weeks up to 50 days	Oshima et al., 1982; Gilfillan et al., 1976; Prakash et al., 1971
	Gonyaulax acatenella	11 weeks 4 weeks	Quayle, 1965 Sharpe, 1981
	Gonyaulax excavata	2–3 weeks	Gaard and Poulsen, 1988
	Dinophysis spp.	1 week	Haamer et al., 1990
Mytilus edulis	*Escherichia coli*	<24h	Bernard, 1989
Mytilus edulis	Polio virus 2	48h	Crovari, 1958
Mytilus edulis	*Escherichia coli* *Salmonella anatum*	4 days	Plusquellec et al., 1990

[†] Dependant on initial level of toxicity

Ozone is another powerful disinfectant that does not leave harmful chemical residues, as does chlorine, and is now the depuration method of choice in major shellfish-cleansing stations in France. While ozone has been tried as a means of detoxifying shellfish exposed to paralytic shellfish toxins, it has not been shown to be effective and there are currently no commercial methods available for ridding shellfish of toxins (Blogoslawski, 1988). Heat processing has also been shown to reduce, but not eliminate, toxin levels (Medcof et al., 1947; Prakash et al., 1971). Mussels usually purge themselves of accumulated toxins after blooms of toxic algae subside. Time taken to reach quarantine levels varies between species and ranges from one week to three months (see Table 11.3).

Although mussels are possibly by volume the most frequently depurated shellfish, there have been few studies on the rate of elimination of bacteria by mussels. Wood (1957) showed that mussels continued to purge themselves of faecal coliforms at

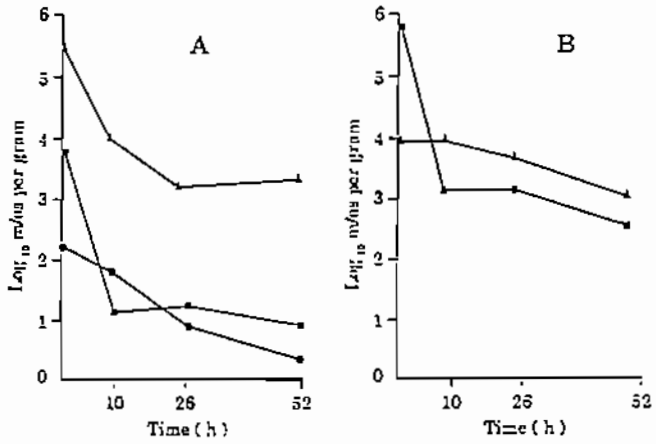

Fig. 11.4. (A) Elimination of poliovirus (plaque forming units (PFU) per gram) (•), *Escherichia coli* 4A (colony forming units (CFU) per gram) (■) and ϕA1-5a (PFU per gram) (▲). (B) Elimination of *E. coli* 4A (CFU per gram) (■) and ϕA1-5a (PFU per gram) (▲) from mussels during depuration in the laboratory-scale system. Environmental conditions during depuration were as follows: temperature, 15.5–19.5°C; salinity, 27–29.3°/oo; dissolved oxygen, >60% saturation; pH, 7.4–8.3. (After Power and Collins, 1989).

temperatures as low as 1–2°C, and Trollope and Webber (1976) demonstrated that immersion for 48h was necessary to ensure 100% removal of *E. coli* from mussels. Volterra and Tosti (1983) noted that streptococci were often in higher concentrations than coliforms, and it has been suggested (Wood, 1976) that faecal streptococci may be retained longer than coliforms. Kueh (1987) also demonstrated rapid uptake of bacteria and elimination via the faeces of *Perna viridis*. More recently, Plusquellec et al. (1990) showed that, while a four-day period is necessary to depurate down to undetectable levels of *E. coli* and *Salmonella anatum* in *M. edulis*, this period is not sufficient for a complete elimination of *Streptococcus faecalis*, and that these differences are more marked *in situ* than in laboratory studies.

Little is known of the depuration of viruses by mussels and the literature appears to be limited to three studies. Power and Collins (1989) monitored the elimination of sewage effluent-associated poliovirus, *E. coli* and 22nm icosahedral coliphage by *M. edulis* under both laboratory- and commercial-scale recirculating, UV depuration systems. Their results are summarized in Figure 11.4, and suggest that the organisms are eliminated from mussels by different mechanisms during depuration under stable conditions. The relative rates of elimination during depuration were: *E. coli* 4A >ϕ A1-5a > poliovirus type 1, regardless of experimental conditions. Spawning appeared to have little effect on the elimination of poliovirus. They also demonstrated slow elimination of viruses from nondigestive tract tissues. Again, these data indicate that conventional depuration practices are inappropriate for efficient elimination of

viruses from mussels. Lewis et al. (1986) showed that no significant reduction in viral numbers had occurred after eight days of depuration in *Perna canaliculus*. They also showed no significant correlation between viral and faecal coliform numbers, supporting the contention of many others (Gerba and Goyal, 1978; Ellender et al., 1980; La Belle et al., 1981; Lewis et al., 1985) that faecal coliform numbers are unreliable indicators of the presence of human enteroviruses, i.e. the absence of faecal coliforms is not sufficient to ensure the safe consumption of shellfish (Power and Collins, 1986, 1989).

Properly used, depuration produces high-quality shellfish but lack of understanding of the depuration process has produced shellfish that have caused illnesses. While depuration can increase the marketability of shellfish (Blogoslawski, 1989), it must be remembered that viruses are not necessarily removed by purification procedures (Bryan, 1980, 1986; Power and Collins, 1987). With the increased market for shellfish worldwide, further studies are needed to develop rapid, sensitive analyses for viral contaminants in shellfish.

MONITORING AND REGULATIONS

With the continuing increase in international shipment of shellfish, especially mussels, strict attention must be paid to quality control. Constant surveillance by public health authorities is a necessity if the safe marketing of shellfish is to be assured. Most countries recognize the importance of quality control; however, inadequate sanitary and processing facilities are often the nemesis of shellfish operations, especially in developing countries (see Davy and Graham, 1982 and papers therein).

Any shellfish sanitation programme should include an infrastructure responsible for monitoring, culture and harvesting activities, and should provide adequate surveillance. This infrastructure is usually composed of public agencies. An administrative system, which coordinates the activities of the various agencies responsible to enjoin the prosecution of violators of the programme is the other major component.

Methods employed for sanitary control of molluscan shellfish have been reviewed by several authors (See Wood, 1972; AOAC, 1984; Richards, 1988) and references therein) and will not be dealt with here.

Many agencies work in cooperation with each other, and with the increased international trade in live shellfish, many countries have become involved in cooperative programmes. The United Nations Programme (UNEP) inititated the Regional Seas Programme in 1974. This programme was designed to assess the state of marine pollution, the sources and trends of the pollution and the impact of pollution on human health, marine ecosystems and amenities (UNEP/WHO, 1983a,

Table 11.4. Action levels, tolerances and other values for poisonous or deleterious substances in seafood (from NSSP Manual Part 1, Appendix C 1986)

Deleterious Substances	Level	Food Community	Reference
Aldrin/Dieldrin	0.30ppm	Fish and shellfish	CPG 7120.23–A
Chlordane	0.30ppm	Fish only	CPG 7120.23–C
DDT, DDE, TDE	5.00ppm	Fish only	CPG 7120.23–D
Endrin	0.30ppm	Fish and shellfish	CPG 7120.23–F
Heptachlor/Heptachlor Epoxide	0.30ppm	Fish and shellfish	CPG 7120.23–H
Kepone	0.30ppm	Fish and shellfish	CPG 7120.23–I
	0.40ppm	Crabmeat	CPG 7120.23–I
Mercury	1.00ppm	Fish and shellfish	CPG 7108.07
Mirex	0.10ppm	Fish only	CPG 7120.23–K
Paralytic shellfish poison	80µg/100g of meat	Fresh, frozen and canned clams, mussels and oysters	CPG 7108.20
Polychlorinated biphenyls (PCBs)	20ppm	Fish and shellfish	21 CR 109.30
Ptychodiscus brevis toxins	20 Mouse Units/100g	Shellfish	APHA Lab. Procedures (17)
Toxaphene	5.00ppm	Fish only	CPG 7120.23–L

b; 1988). A set of reference methods and guidelines for marine pollution studies (faecal coliform) have been developed and have been recommended for adoption to governments participating in the Regional Seas Programme .

The National Shellfish Sanitation Programme (NSSP) was established in the U.S.A. in 1925 to establish sanitary control mechanisms for prevention of further outbreaks of shellfish-borne typhoid fever and other diseases of bacterial origin (Hunt, 1972), and to insure that shellfish shipped interstate would not be the cause of communicable disease. In the 1940s, steps were taken to protect the public against paralytic shellfish poisoning, and in 1957 radionuclides were added to the list of possible contaminants of shellfish. In the 1960s and 1970s it became apparent that shellfish also concentrate other poisonous and/or deleterious substances, including metals, pesticides, hydrocarbons and others, to potentially unsafe levels (see Chapters 8 and 9). It is the responsibilty of each individual State to supervise the growing, harvesting, relaying and transportation of the shellfish. NSSP is a voluntary programme which

encourages states to adopt shellfish sanitation regulations based on federal agency recommendations. Table 11.4. gives the action levels and tolerance values allowed for poisonous or deleterious substances for which standards exist in the U.S.A. (NSSP Manual, USPHS, 1988). At this time, bacterial standards for depurated mussels have not been set by NSSP. When a standard is established, it can be expected to be similar to those set for softshell clams, quahogs and oysters. Routine testing, or monitoring of viruses in shellfish or their waters, is not carried out or recommended due to the technical complexity, time required, high cost and limitations of the detection and recovery methods (USDHHS Manual, 1986; USPHS Manual, 1988). Conspicuous by its absence from this list is okadiac acid or diarrhetic shellfish poisoning (DSP). This is a recent phenomenon and individual countries have set their own tolerance levels and accepted methods of analysis (Table 11.5). Although DSP has not been positively identified from U.S. waters (Stamman et al., 1987), its presence is suspected (see Freudenthal and Jijina, 1988; Shumway, 1990). Acceptable methods for determinating levels of contamination have not as yet been arrived at.

The NSSP was reorganized in September 1982 and is now titled Interstate Shellfish Sanitation Conference (ISSC). The ISSC establishes guidelines for shellfish sanitation standards, which are published in a Manual of Operations (NSSP, 1989a, b), available to all interested parties. The Manual describes in detail how a programme should be operated in any member state, describes the interrelation of member state programmes, and cites the criteria to be applied by the Food and Drug Administration (FDA) in evaluating the programmes (Canzonier, 1988).

ISSC is a state, federal and industrial cooperative and includes shellfish sanitation control agencies in 22 States of the U.S.A., Canada, the Hiroshima Prefecture of Japan, and also shellfish industry organizations in these countries. It is administered by the FDA's Shellfish Sanitation Branch (Hunt, 1972).

Shellfish sanitation and the guarantee of a safe product for human consumption is an international problem. To this end, the U.S. Department of Health and Human Services, the Public Health Service and the FDA regularly publish an interstate certified shellfish shippers list. The shippers listed have been certified by regulatory authorities in the U.S.A., Australia, Canada, Japan, the Republic of Korea, Iceland, Mexico, England and New Zealand under the uniform sanitation requirements of the National Shellfish Programme, under the terms of the shellfish sanitations agreements with the governments of these countries (USPHS, 1990). Control measures of the states are evaluated by the FDA.

'Interstate Certified Shellfish Shippers List' (ISSN 0364–7048) is published monthly for the information of, and use by, food control officials, seafood industry and other interested persons (USPHS, 1990). The publication is distributed under authorities of the authorities of the Public Health Service Act and Food, Drug and Cosmetic Act by the U.S. Food and Drug Adminstration, 200 'C' Street, Washington, D.C. 20204.

Table 11.5. Diarrhetic shellfish poisoning (DSP) tolerance in various countries (from Krogh, 1992).

Country	Tolerance	Method of Analysis
Denmark	No detectable amount	Mouse bioassay: rat bioassay[a]
Germany	No detectable amount	Rat bioassay
France	0.2–0.4MU/g digestive glands	Mouse bioassay[b]
Ireland	No detectable amount	Rat bioassay (HPLC for confirmation)
Japan	5MU/100g soft tissue	Mouse bioassay
Netherlands	No detectable amount	Rat bioassay
Norway	5–6MU/100g soft tissue	Mouse bioassay
Portugal	No detectable amount	Mouse bioassay
Spain	No detectable amount	Mouse bioassay[c]
Sweden	60µg/100g soft tissue	HPLC mouse bioassay[c]

[a] Employed for export commodities of shellfish to countries requiring this method of analysis

[b] A modified version, with shorter observation period, so the Mouse Units (MU) cannot be compared to those of the original Japanese method used in other countries

[c] No clean-up with either of the shellfish extract

[d] Okadaic acid + DTX-1

[e] Employed for export shellfish commodities

Shellfish programmes vary from state to state (see Broutman and Leonard, 1988; Leonard et al., 1989; Leonard and Slaughter, 1990) and country to country; however, the concern of all is the provision of a safe product. Any state which plans to ship their products interstate must conform to the ISSC (NSSP). Currently, the ISSC (NSSP) manual for interstate shipment stipulates that all growing areas must be certified, commercial shellfish harvesters must be licensed, and the processors and distributors be certified. All products can then be traced to their point of origin by either harvester number or distributor number on the shipping tag (Hungerford and Wekell, 1992). While the ISSC has generally provided a safe shellfish market there is still a need for more effective measures for monitoring the safety of shellfish products, particularly with reference to the presence of viruses (Subcommittee on Microbiological Criteria et al., 1985).

Table 11.6. Microbiological standards for shellfish and shellfish growing areas fixed by Italian regulation (DM, 1978) (from Bonadonna et al., 1990)

Areas	Shellfish		Water	Shellfish destination
	E. coli mL^{-1}	*Salmonella*	*E. coli* $100mL^{-1}$	
Approved	4	absent–25 mL^{-1}	2 (10% of samples 7)	Purification treatment
Conditionally approved	39		34 (10% of samples 49)	Food preservation industry
Prohibited	>39		>34	

The Subcommittee on Microbiological Criteria, the Committee on Food Protection, the Food and Nutrition Board and the National Research Council (1985) have proposed that the Hazard Analysis Critical Control Point system (HACCP) be implemented to provide a "more specific and critical approach to the control of microbiological hazards in foods than that provided by traditional inspection and quality control approaches". First introduced at the 1971 National Conference on Food Protection (APHA, 1971), this system consists of three units: (1) identification and assessment of hazards associated with growing, harvesting, processing, marketing, preparation and use of a given raw material or food product; (2) determination of critical control points to control any identifiable hazard and (3), establishment of systems to monitor critical control points. Strong emphasis on the application of the HACCP system was given by the WHO Expert Committee on Microbiological Aspects of Food Hygiene (1976), and it has been suggested that discussion of the HACCP system be incorporated into appropriate WHO training programmes (WHO/ICMSF, 1985).

Other countries have establish their own criteria for shellfish water standards. Italian regulations (DM, 1978) distinguish three classes of areas where shellfish may be harvested and collected (Table 11.6). A microbiology network dates from April 1989 in France, and is focused on assessing the level and tendencies of bacteriological contamination in the marine environment as measured in shellfish used for integration and in consumer protection (Berthome, unpublished results). In addition, France has a monitoring and warning network for phytoplankton consisting of 100 sampling stations, 38 of which are sampled systematically throughout the year, the remainder being supplemental monitoring stations if toxic species occur (Berthome, unplublished results). France's extensive monitoring programme has been reviewed by Furfari and Hunt (1981).

Monitoring programmes for PSP, DSP and ASP are well-established in many countries. The United States' programme has been recently reviewed by Hungerford and Wekell (1992). Other countries with extensive monitoring programmes include France, Spain, Japan, Canada and most recently, Tasmania (see Shumway, 1990). More monitoring programmes are urgently needed, especially in developing countries, where primitive culture facilities are common and technical assistance may be lacking.

CONCLUSIONS

As the global culture and transportation of mussel species continues to grow, the public health aspects cannot be ignored. While improved inspection systems, laws and regulations governing the shellfishing industry, and monitoring programmes have all contributed to the decline in shellfish-borne illnesses, many outbreaks still occur. Cooking is not always sufficient to insure complete inactivation of infectious particles from heterogeneous and homogeneous populations (Milo, 1971), and it is impossible to be confident in our ability to certify filter-feeding molluscs which are to be consumed raw. In addition, viruses can be present in shellfish that have been certified 'clean' based on *E. coli* measurements. More outbreaks occur from home collections and consumption in private homes and beaches than from commercial suppliers, and no amount of regulation can stop these incidences. To prevent unnecessary outbreaks of shellfish-borne diseases, shellfish should be obtained only from approved, certified sources and never harvested from waters contaminated with raw sewage. The shellfish should be thoroughly cooked, not 'quick-steamed', as destruction of the viruses may not be complete otherwise. Increased public education and awareness are a must if disease outbreaks are to be curtailed.

As summarized by Richards (1988), more research is urgently needed to: (1) develop more sensitive, reliable and universally accepted assay techniques for virus anaylses; (2) delineate the role of environmental parameters on the depuration process; (3) reassess the usefulness of indicator organisms (e.g. *E. coli*) as predictors of overall shellfish quality and safety and (4), standardize depuration research to include internal viral and/or bacterial controls coupled with inter- and intralaboratory comparisons. To this list we would add implementation of the HACCP system to assure the quality of shellfish and provide a uniform means of testing and reporting contamination levels.

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Edited by

ELIZABETH GOSLING

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