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Contamination in Shellfish-Growing Areas

Robert E. Croonenberghs

INTRODUCTION

In this chapter the term shellfish will refer only to animals in the phylum Mollusca, class Bivalvia, which means that they are mollusks with two shells such as oysters, clams, mussels, and scallops. Although crabs, crayfish, shrimp, and lobsters have shells, they are members of the phylum Arthropoda, class Crustacea, and are not included as shellfish in this discussion.

Bivalve mollusks feed by filtering microscopically-sized particles out of the water. They pump water via the inhalant siphon through their gills, which sorts out particles of the correct size and "feel," and directs selected particles into their gut. The uneaten particles are collected in mucus, and ejected via the exhalent siphon as pseudofeces, along with the digested gut contents.

It is this filter-feeding mechanism, and the relatively large volume of water the bivalves pump through their siphons, that causes such public health concern about pollutants in shellfish-growing waters. Three primary mechanisms for uptake and concentration of pollutants in bivalves thus exist: particulate ingestion, absorption, and ionic attraction. Depending on the type of pollutant, one or more of these mechanisms may come into play.

Particulate ingestion is the manner in which shellfish concentrate viruses and bacteria from the water. Viruses and bacteria are too small for the shellfish's gills to detect or reject, but due to their small size in relation to such food items as algae and small particles suspended in the water, they tend to attach to these surfaces. The smaller the particle, the larger the surface area relative to its size and, because of the tremendous individual numbers of food items held in the shellfish's gut at any one time, the number of bacteria and viruses concentrated in that location is considerably larger than those present in an equal volume of the surrounding water. Other pollutants of public health concern that shellfish concentrate basically via this mechanism are many types of pesticides, toxic algae, and ions of heavy metals attached to particulate matter.

Shellfish absorb soluble lipophilic ("fat-loving") pollutants directly from the large volumes of water they pump through their bodies. Lipophilic substances have a low polarity; there is almost no electrical charge on the molecule because all ionic charges are balanced. However, water is quite polar: the oxygen end of the molecule has considerable negative charge, and the hydrogen end has considerable positive charge. Pollutants of low polarity (e.g., oils) do not dissolve well in water, so they tend to move out of water and into nonpolar fats when they get the opportunity. Due to the lipid content of shellfish, relatively nonpolar pollutants such as petroleum hydrocarbons, certain organic metallic complexes, and some pesticides are primarily concentrated in this manner.

Ionic substances (such as heavy metals) and highly polar substances are not only ingested when attached to particulate clays, but are also dissolved in the water. Again, due to the high pumping rate of the shellfish, large numbers of these dissolved substances come into contact with their body, and are taken up via an ion exchange reaction in the shellfish.

National Shellfish Sanitation Program

The shellfish sanitation program began at the national level in the United States in 1925 after a series of typhoid epidemics spread by the consumption of raw shellfish threatened the collapse of the industry. The program has changed over the years and is now called the National Shellfish Sanitation Program (NSSP); it is run at the national level by the United States Food and Drug Admin-

istration (FDA). The principles behind this program protecting the consumer from bacterial and viral human pathogens have changed little over the years. Due to the ability of shellfish to concentrate pathogens and pollutants out of the surrounding waters, nearly pristine water is required. For example, the NSSP standard for the average concentration of fecal coliforms in shellfish waters is 14 fecal coliforms per 100 milliliters (ml). This is roughly 14 times more stringent than the 200 fecal coliforms per 100 ml used by many states as a safe swimming standard. The shellfish standard is even more strict because there is a second part to the standard, depending on the method of sample collection. It requires that either no more than 10 percent of the samples exceed 49 most probable number (MPN), fecal coliforms/100 ml for a three-tube test, or that the ninetieth percentile for the data set not exceed 49. As a result, states producing shellfish are required to conduct onshore surveys to check for sources of sanitary waste, and are required to collect numerous growing water samples for analysis of the indicator bacteria used (i.e., fecal coliforms).

Introduction of Pollutants into Waterways

When interpreting fecal coliform concentrations in shellfish-growing areas, one must consider numerous factors controlling the distribution of the bacteria in order to understand what the data mean. Fecal coliforms are used as an indicator of the possible presence of human pathogens, since they originate in the gut of humans and warm-blooded animals. However, pathogens do not necessarily occur in direct proportion to the number of fecal coliforms present. Fecal coliforms may die off at a rate different than that of accompanying pathogens. In addition, there is little documentation to indicate that pathogens from wild animals in the United States have caused human illness through the consumption of raw shellfish. In rural areas, wild animals are quite apt to be the primary source of fecal coliforms found in runoff. As such, an evaluation of the watershed for potential sources is quite important because the use of the fecal coliform indicator alone is not sufficient to assure safe growing waters.

The major human pathogens of concern in shellfish waters usually originate on land, except for sewage discharges from boats and sewage treatment facility (STF) outfalls. In most areas, except those heavily impacted by an STF or a marina, the primary vector transporting fecal coliforms to shellfish waters is runoff from the land. Runoff varies with rainfall, soil types, degree of saturation, etc., and, as such, is periodic. Therefore, one or a few samples in most areas may indicate very little about the pollution potential in that area. Numerous samples must be collected under all weather conditions, or one must sample under conditions that are most likely to transport fecal pollution to the area (e.g., after heavy rains, high tides, etc.).

Generally in estuarine areas most of the runoff enters a tributary through its headwaters. Since the runoff contains most of the bacteriological load, and the larger body of water holds fewer bacteria, the highest concentrations tend to occur in the headwaters. As the influent is carried downstream by the tidal action, the associated mixing steadily reduces the concentrations of fecal coliforms. Of course, where feeder streams enter, concentrations may increase. Also, higher counts tend to occur closer to shore due to runoff from the shore, and these higher counts tend to remain near shore for some distance downstream, since there is rarely a strong cross-channel mixing gradient. Illegal discharges from on-site sanitary facilities into shellfish waters can produce a dramatic local increase in fecal coliform concentrations.

PATHOGENS

Enteric Viruses Associated with Fecal Pollution

In the United States, it is widely believed that viral agents, as opposed to bacteria, have been the primary cause of shellfish-borne disease outbreaks as a result of pollution over approximately the last 20 years (Rippey, 1994). Part of the reason that viruses are considered to be the culprit may well be that sewage is more thoroughly treated now than in the past, and the more resilient viruses survive our sanitation procedures better than do bacteria. Thus, when shellfish become contaminated with fecal pollution, the water tends to have been treated, and primarily only viruses are present.

Shellfish programs in all coastal states have been conducting shoreline surveys to locate and correct sources of human fecal pollution in shellfish waters. In addition, local municipalities have been mandated by the federal government to up-

grade sewage treatment facilities (STF). The result is that in coastal areas the majority of human feces-associated pathogens are either kept in the soil, or are subjected to an STF. While bacteria are quite sensitive to chlorination in a properly operating STF, viruses are significantly more resistant. States usually design permits for STF based on fecal coliform concentrations in the effluent. Thus, large numbers of viruses can be discharged into shellfish waters by a legally operating STF [Lewis et al., 1986; World Health Organization (WHO), 1979].

Other factors may also contribute to the predominance of viruses in recent United States shellfish-associated disease outbreaks. Once discharged into marine waters, human enteric viruses can survive for extended periods of time (La Belle and Gerba, 1982; Feachem et al., 1981). Some viruses are extremely infectious; for example, Norwalk virus has a theoretical infectious dose of from one to ten particles. Hard clams (*Mercenaria mercenaria*) have been shown to bioconcentrate hepatitis A virus 900 times over the concentration in water (Goswami et al., 1993); in addition, they show a marked seasonal increase in the concentration of bacteriophages (viruses that attack bacteria) in the spring, which may indicate a propensity to do so for other viruses (Burkhardt et al., 1992). Viruses can be retained within actively pumping shellfish much longer than bacteria (Enriquez et al., 1992; De Mesquita et al., 1991; Power and Collins, 1990a & b; Lewis et al., 1986). Viruses can be absorbed intracellularly into shellfish, as opposed to merely being held in the gut, and as such are much more difficult for the shellfish to eliminate (Hay and Scotti, 1987). Once harvested and refrigerated, Coxsackie B3 and polioviruses have been shown to survive basically unreduced for 28 days at 5°C (Tierney et al., 1982; Metcalf and Stiles, 1965). Other work has shown gradual inactivation up to approximately 90 percent for poliovirus over 30 days when refrigerated, and over 12 weeks when frozen (DiGirolamo et al., 1970). Many enteric human viruses are fairly heat-resistant (hepatitis A and Norwalk); even if partially cooked, the viruses may survive (Millard et al., 1987; Truman et al., 1987; Morse et al., 1986; Peterson et al., 1978).

Thus, viruses represent a class of human pathogens that are legally discharged in large quantities into coastal waters; they can be long-lived in the environment; and, when taken up by shellfish, they can remain in the animal for long periods of time after the pollution event and then again after harvesting. Many viruses tend to survive mild cooking and are able, in some cases, to cause infection with only a few particles. It is no wonder that viruses cause such a high percentage of the United States shellfish-related disease outbreaks.

Human pathogenic viruses cannot replicate in shellfish, so those present in shellfish at the time of harvest will not increase, despite temperature abuses that might occur. Therefore, when widespread viral-associated outbreaks occur due to shellstock (as opposed to shucked, which are more intensely handled), the growing area immediately becomes the prime suspect.

Hepatitis A

Hepatitis is the most serious viral disease associated with the consumption of raw shellfish, and the hepatitis A virus (HAV) is the most common agent. HAV is a 27-nanometer (nm) picornavirus, and the incubation period is 15 to 50 days, with an average of 28 to 30 days. The disease causes fever, malaise, anorexia, nausea, and abdominal discomfort, followed by jaundice. It is usually fairly mild and lasts one to two weeks, though it can be severely disabling and last for months. Complete recovery is often prolonged. The fatality rate is 0.6 percent.

HAV is spread via the fecal-oral route. The period of maximum shedding of the viral particles by infected persons appears to be during the latter half of the incubation period and into the onset of jaundice (Benenson, 1995; Hackney et al., 1992). In developing countries, children usually contract a mild form of the disease, which then provides them with a lifelong immunity. Incidence of hepatitis A due to the consumption of raw or undercooked shellfish has been steadily decreasing in the United States over the past 15 years, though in the period from 1961 through 1980, more than 1000 cases due to shellfish were reported along the Gulf and East Coasts (Glatzer, 1999; Rippey, 1994).

HAV can survive long periods of time in the estuarine environment. Though it survives longer in freshwater than estuarine water, it has been shown in the laboratory to survive three months

in estuarine water. HAV is considerably longer-lived in water and other varied environmental conditions than is the polio virus, which has often been used as a model for HAV. Cooler temperatures (5°C) tend to enhance survival over warmer temperatures (25°C), as does particulate matter in water. HAV has been shown to survive in dried feces held at 5°C and 25°C for one month (Sobsey et al., 1988). HAV is quite heat stable and can survive 140°F (60°C) for four hours (Kilgen and Cole, 1991).

HAV appears to be one of the slower viruses to depurate from shellfish (Hackney et al., 1992). Greater than 10 percent of HAV taken up by the eastern oyster (*Crassostrea virginica*) remains after five days of depuration, though less than one percent of polio virus remains (Sobsey et al., 1988). Mussels (*Mytilus chilensis*) have been shown to concentrate HAV 100 times over that in the water, and to retain the virus for seven days under optimal feeding and filtration conditions, with longer retention under poorer feeding conditions (Enriquez, 1992).

Hepatitis E

Hepatitis E disease has also been linked to shellfish, and the active agent is called hepatitis E virus (HEV). The symptoms are very similar to hepatitis A. The incubation period is 15 to 64 days, though the average has varied from 26 to 42 in different epidemics. While the fatality rate is 0.1 to 1 percent, in pregnant women it may reach 20 percent in the third trimester (Hackney et al., 1992; Benenson et al., 1990).

Norwalk, Norwalk-like, and Other Viruses

Gastroenteritis is the now most common disease in the United States caused by the consumption of raw or undercooked shellfish from sewage-contaminated waters (Rippey, 1994). The disease is normally self-limited, with symptoms of nausea, vomiting, diarrhea, and abdominal pain that last normally 24 to 48 hours. Norwalk virus is one of the most common agents of this disease, and is a small, 27 to 32nm calicivirus. Norwalk viruses are extremely infectious, and may infect with as few as one to 10 particles; an outbreak in 1993 was traced to one harvester dumping his feces and vomitus overboard in a remote and wide-open harvest area (Kohn et al., 1995). Norwalk viruses are also quite heat resistant. Other viruses implicated in shellfish-associated illnesses that cause similar symptoms are Norwalk-like viruses, small round viruses (SRV) (Gray and Evans, 1993; Haruki et al., 1991), and Snow Mountain agent (Truman et al., 1987). The incubation period is normally 24 to 48 hours, although volunteer studies with Norwalk virus have shown 10 to 50 hours. Some immunity develops after exposure, but is variable in duration (Benenson, 1990), and some people may be naturally immune (Blacklow et al., 1979).

Enteric Bacteria Associated with Fecal Pollution

Salmonella

Typhoid fever was the dominant recognized form of United States shellfish-borne disease in the first half of this century. Indeed, an outbreak of 1,500 cases in 1924 and another outbreak the following year were the impetus for the development of the precursor to the NSSP. Typhoid fever is caused by *Salmonella typhi*, and though it has not been associated with shellfish since 1954, approximately 500 cases occur in the United States each year, mainly due to importation from endemic areas outside the United States; therefore, a slight potential for a shellfish-borne outbreak still exists. Symptoms include sustained headache, fever, malaise, and anorexia. Incubation typically lasts one to three weeks (Benenson, 1990).

Salmonellosis due to shellfish consumption is not very common, and has tended to occur sporadically, with only eight cases in the interval 1984 to 1993 (Rippey, 1994). Sufficient cells are needed to cause the syndrome, usually between 10^5 and 10^8, though the infective dose in high-fat foods can be much less. It is not uncommon to find salmonellae in shellfish, but the typical concentrations of approximately 2/100g of shellfish are believed to be too low to cause illness, and may represent part of the free-living microflora of shellfish (Hackney et al., 1992).

Symptoms of salmonellosis include headache, abdominal pain, diarrhea, nausea, and occasionally vomiting. Dehydration can be dangerous, especially in children, and can last several days. The incubation period can last from six to 72 hours, but is usually 12 to 36 hours (Benenson, 1995). All serotypes of *Salmonella* are considered to be pathogenic, but *S. typhimurium* and *S. enteritidis* are the

most often reported types associated with the disease. *S. infantis* was associated with two cases of shellfish-borne disease in Maine (Rippey, 1991). Hackney et al. (1992) conclude from the literature that *Escherichia coli* (a type of fecal coliform) is a suitable indicator for the presence of *Salmonella*.

Campylobacter

Campylobacteriosis is a major form of food poisoning, and of late is being reported more commonly as a shellfish-borne disease, probably as a reflection of improved analytical capability. Symptoms include diarrhea, abdominal pain, malaise, fever, nausea, and vomiting. Bloody stools are common. The incubation period normally lasts three to five days, and the illness frequently ends within two to five days, though it can be prolonged in adults.

Although cattle, swine, sheep, goats, cats, and dogs all carry *C. jejuni*, poultry have some of the highest carriage rates. *C. jejuni* is a leading cause of gastroenteritis and is transmitted via food and water. Ingestion of only a few hundred cells can cause infection, and often illness (Benenson, 1995; Doyle, 1990a). Therefore, fecal pollution of shellfish waters by animal waste is a concern with this disease (Hackney et al., 1992). In the interval 1984 to 1993, there were 12 cases of shellfish-borne illness attributed to *Campylobacter* in the United States, though none from 1994 to 1999 (Glatzer, 1999; Rippey, 1994).

Shigella

Shigellosis associated with shellfish is not particularly common, though there have been four United States outbreaks and several individual cases in the past 25 years (Glatzer, 1999; Rippey, 1994). Symptoms of the disease include diarrhea, fever, nausea, and occasionally toxemia, vomiting, cramps, and tenesmus. The bacteria invade the intestinal tract, and can cause the discharge of blood, mucus, and pus (dysentery) with the stool. As few as 10 to 100 organisms can cause the disease, which has a usual incubation period of one to three days, and generally lasts four to seven days (Benenson, 1990). *Shigella* rarely occurs in animals; it is a disease of man and higher primates. Convalescent carriers are a major reservoir, and usually shed the bacteria for three to five weeks after symptoms subside (Doyle, 1990b). Outbreaks due to shellfish have been traced to sewage pollution, though the bacteria do not survive well in the environment (Hackney et al., 1992). One shellfish-borne outbreak was traced to the overboard disposal of feces from harvest boats (Reeve et al., 1989).

Escherichia

In their review of pathogenic *Escherichia coli* (*E. coli*), Hackney et al. (1992) indicate that there are four pathogenic strains: enterotoxigenic, enteropathogenic, enteroinvasive, and hemorrhagic. The first three strains are usually associated with human feces, but the hemorrhagic is usually found due to farm animals. The hemorrhagic strain is the most dangerous, sometimes causing death in children and the elderly, and is caused by the type *O157:H7*. The authors report that pathogenic *E. coli* have been isolated from oysters and mussels, and that when analyzed in certain environmental samples, the concentrations correlated well to the total number of *E. coli* present. No United States cases of shellfish-borne illness are attributable to these organisms (Glatzer, 1999; Rippey, 1994).

Vibrio cholerae O1

Vibrio cholerae exist in the estuarine environment both as a result of human fecal pollution and as a naturally-occurring organism. This species is normally classified as two serogroups, O1 and non-O1 *V. cholerae*, though there is a third serogroup, atypical O1, which does not produce cholera toxin. Epidemics of cholera and large-scale shellfish-borne outbreaks are due to fecal pollution of serogroup O1, though this group probably also exists naturally in estuarine waters.

V. cholerae O1 contains two biotypes, classical (*cholerae*) and *El Tor*, both of which contain toxigenic and non-toxigenic strains. These two biotypes can be further divided into serotypes Inaba, Ogawa, and Hikojima. The toxigenic strains produce cholera toxin or similar toxins, and it is these toxins that cause the illness. Toxigenic O1 does not cause localized infections outside of the intestines. The non-toxigenic strains can cause diarrhea and extraintestinal infections. *El Tor* is the current biotype of concern in the Americas, and individual organisms can live in water for long periods of time. *V. mimicus* is closely related and some strains can elaborate a toxin identical to *V. cholerae* (Roderick, 1991; Benenson, 1990; Doyle and Cliver, 1990; Blake, 1983). There have been only a few

sporadic cases of shellfish-borne *V. cholerae* O1 in the United States (Glatzer, 1999; Rippey, 1994).

Symptoms of cholera include a sudden onset of profuse and painless, watery diarrhea, occasional vomiting, rapid dehydration, acidosis, and circulatory collapse. Asymptomatic infection is much more common than clinical illness, and mild cases with only diarrhea are common. The incubation period can be as little as a few hours to five days, though the average is two to three days (Benenson, 1990). The infective dose is estimated to be 10^8-10^9 cells, though antacids and medication to lower stomach acidity and lower the infective dose to 10^3 cells for some strains of *El Tor* (Doyle and Cliver, 1990).

Enteric Bacteria not Associated with Fecal Pollution

There are a number of species of bacteria that naturally occur in estuarine waters that can, on occasion, be pathogenic to humans. The largest number of these opportunistic pathogens occurs in the family Vibrionaceae, and most occur seasonally in largest numbers when the water is warm.

Vibrio cholerae Non-O1

Vibrio cholerae Non-O1 usually accounts for a few individual cases of shellfish-borne illness in the United States each year, occasionally causing an outbreak in a few people (Rippey, 1994). This sickness is characterized by diarrhea, often with abdominal pain and fever (Morris and Black, 1985), but to call this "cholera" is inaccurate (Benenson, 1990). Morris and Black (1985) indicate that less than 5 percent of the Non-O1 serogroup members produce cholera toxin. Members of the Non-O1 serogroup have not been associated with large epidemics or pandemics. Hackney et al. (1992) report that almost all the cases of Non-O1 illness in the United States have been due to the consumption of raw oysters. Non-O1 organisms occur most often in marine waters of a salinity reduced to between four to 17 parts per thousand (ppt) (Colwell and Kaper, 1978). While *V. cholerae* Non-O1 appear to survive and multiply in the estuarine environment, it is not clear whether the strains that cause human illness are spread by human feces or not (Blake et al., 1980).

Vibrio parahaemolyticus

Vibrio parahaemolyticus also regularly accounts for a few individual cases of shellfish-borne illness in the United States every year, and occasionally for outbreaks (Rippey, 1994). In Japan it accounts for a significant portion of the bacterial food-borne illness, and is due to consumption of raw seafood in the summer months. The symptoms typically include diarrhea, abdominal cramps, nausea, vomiting, headaches, fever, and chills. The infective dose is normally from 10^5 to 10^7 cells, though a reduction of gastric acid may reduce the threshold for infection. The onset time is nine to 25 hours, and normally lasts 2.5 to three days. The actual symptoms are due to heat-stable toxin production that can occur either in the food prior to consumption, or in the intestines (Hackney et al., 1992; Benenson, 1990; Blake et al., 1980).

Once contracted via oyster consumption, *V. parahaemolyticus* can cause death, accounting for six deaths in the interval 1984-1993 in the United States (Rippey, 1994). *V. parahaemolyticus* also causes extraintestinal infections (Armstrong et al., 1983; Blake, 1983). Levine et al. (1993) have pointed out the danger of *V. parahaemolyticus* to healthy persons, noting the high incidence of otherwise healthy persons contracting gastroenteritis from raw shellfish.

V. parahaemolyticus occurs in estuarine waters around the world (Blake et al., 1980), being present in highest numbers during the summer months. Though it is common in seafood, isolates pathogenic to man (Kanagawa positive) are rare. Estuarine and seafood isolates are predominantly Kanagawa negative (Hackney et al., 1992; Blake et al., 1980).

Recently, however, shellfish-borne disease outbreaks in the United States due to *V. parahaemolyticus* have become more prevalent and have raised concerns for this naturally occurring pathogen in oysters. In 1997 one outbreak occurred on the West Coast. In 1998 three outbreaks occurred, one on each coast of the United States, and in 1999 a small outbreak also occurred in British Columbia (Glatzer, 1999). One strain previously known only in Asian countries, serotype O3:K6, was identified for the first time in 1998 as a cause of two (Northeast and Gulf coasts) of the three U.S. outbreaks. The emergence of this strain as a major

cause of outbreaks in Japan and the United States in recent years, along with other serotypes such as O4:K8, also prevalent in Japan, have raised concerns about the pathogenicities and abilities of such strains to cause outbreaks. A thermostable direct hemolysin (tdh) is produced by virtually all pathogenic strains of *V. parahaemolyticus*, and the presence of the causative gene currently is being used to screen isolates from shellfish to indicate the presence of pathogenic strains. Some data from the recent U.S. outbreaks suggest that the number of pathogenic cells required to cause illness may be as low as 100 to 1,000 cells; however, there remains much uncertainty about an infectious dose. In at least one instance, though, illness resulted from the consumption of a single contaminated oyster (Watkins, 1999).

Vibrio vulnificus

In the past decade, no other bacterium has raised more controversy with respect to shellfish than *Vibrio vulnificus*. From 1988 to 1999 approximately 263 people contracted *Vibrio vulnificus* infections due to the consumption of shellfish, of which 132 people died (Glatzer, 1999). Persons at highest risk are those with pre-existing liver disease, especially alcoholic cirrhosis, and also persons with iron-storage disorders (hemochromatosis, hemocirrhosis), immunosuppression, renal failure, and diabetes. Persons with decreased stomach acidity, steroid-dependent asthma, or rheumatic disorders are also at a significantly greater risk than the general population (Whitman, 1994; Levine et al., 1993; Klontz et al., 1988; Morris, 1988; Tacket et al., 1988; Blake et al., 1979).

Typically, *V. vulnificus* infects a person either through consumption with resulting primary septicemia, or through a preexisting wound. Primary septicemia is the most dangerous form of infection, and the disease can rapidly progress into intractable shock; more than 50 percent of these cases result in death. Bulbous skin lesions characteristically appear. *V. vulnificus* may occasionally cause gastroenteritis (Morris, 1988; Morris and Black, 1985). The infectious dose is not known, but in high-risk populations, fatal infections have occurred due to consumption of only one oyster (Whitman, 1994). Recent limited work indicates that infectious doses may be far less than earlier believed, on the order of 1,000 cells/gram (g) of oyster tissue (Tamplin, 1994a).

V. vulnificus occurs naturally throughout estuarine areas of the United States, preferring temperatures of 20° to 30°C and salinities of five to 25 ppt. Tamplin (1994b) examined water, sediment, and shellfish throughout the United States and found the lowest concentrations on the Pacific Coast, probably due to colder temperatures and higher salinities; the Northern Atlantic estuaries had the next highest concentrations, followed by the Southern and Middle Atlantic, with the Gulf Coast estuaries having the highest concentrations. As part of this work, Tamplin reported the concentrations of the more virulent opaque strains (encapsulated) to be 10 times or more prevalent than the weakly-virulent translucent strains (unencapsulated) in Gulf Coast waters. The encapsulated strains may be more virulent due to the protection provided to the bacteria by the capsule (Morris, 1994; 1988). Many of the wild strains of *V. vulnificus* have the capability to switch back and forth between encapsulated and unencapsulated forms (Morris, 1994).

Temperatures of approximately 13°C or greater are needed for *V. vulnificus* to grow, whether in shell oysters or in a growth medium. At summertime temperatures in the Gulf Coast, *V. vulnificus* can grow significantly in shell oysters in four hours, though icing shell oysters at the harvest site does prevent growth (Tamplin, 1994c). Icing of shellstock has been shown to reduce slowly, over the course of seven to 14 days, the levels of culturable cells (Cook and Ruple, 1992).

At temperatures below 13°C, *V. vulnificus* begins to enter a dormant state, where the cells are alive but cannot be grown on media. This state is called viable-but-non-culturable (VBNC). During the winter months, the number of *V. vulnificus* cells appears to decrease in the water, sediment, and oysters, but conclusive research now reveals that these cells, rather than dying, are simply in the VBNC state. These cells, when placed in warm conditions, revert back to normal culturable cells in one to two days, and apparently do so in shellfish as well. Encapsulated cells retain their capsules when they enter the VBNC state, a factor that aids in their resistance to the human immune system. Experiments with mice made susceptible to *V. vulnificus* show that these VBNC cells do kill

the mice, though the longer the cells have been in the VBNC state, the more cells are required. Whereas seven to eight cells of recently transformed VBNC *V. vulnificus* can kill susceptible mice, 10^6 cells are needed once they have been in the VBNC state for seven days or more. This may help explain why human deaths are so reduced during the cold months (Oliver, 1994), and partially explains why iced, shucked oysters are less of a hazard.

Shucking, washing, blowing (agitation with compressed air bubbles), and then packing oysters (without additional fresh water) does not appear to decrease the number of *V. vulnificus* in oysters. However, keeping shucked oysters on ice usually reduces the number of culturable cells by a 1-log and 2-log unit over three and seven days respectively (Ruple and Cook, 1992). Commercially blowing oysters for extended periods of time and adding substantial amounts of fresh water to packed oysters may disrupt the osmotic pressure and kill significant quantities of *V. vulnificus* (Kilgen, 1994). Freezing, whether individually quick frozen (IQF), carbon dioxide (CO_2) at -30°C, blast freezing at -23°C, or conventional freezing at -18°C, reduces *V. vulnificus* but does not eliminate it. *V. vulnificus* is very heat-sensitive and normal cooking will eliminate it, but steaming oysters to the point of gaping will not totally eliminate it (Cook, 1994). Heating oysters to 50°C for 10 minutes will kill *V. vulnificus;* the process does not impart a noticeable cooked appearance or taste to the oysters, and may prove to be an acceptable method for eliminating the bacteria from oysters (Cook and Ruple, 1992). Depuration has not proven successful in eliminating *V. vulnificus* from oysters, because the cells are so tightly associated with the tissue. Rather than decrease in a depuration system, *V. vulnificus* has consistently grown (Tamplin, 1994d). Some interesting work in New Hampshire suggests that relay to high salinity waters of 25 ppt may help reduce *V. vulnificus* levels in oysters (Jones, 1994). Irradiation of shellstock oysters, though effective in killing *V. vulnificus*, shortens the shelf life of the product too much for commercial application (Tamplin, 1994d). It should be noted that recent work on the irradiation of oysters may have developed an acceptable approach to solve the shelf life problem (Martin, 1996).

The VBNC state and the ability for *V. vulnificus* to resist depuration from oysters have significant implications for the public health aspects of the transplantation of oysters. Since particular areas (Florida and Louisiana) account for most of the deaths due to *V. vulnificus*, it is not unreasonable to suspect more virulent strains exist in certain areas. Therefore, transplantation at any time is of concern. We now know that the individual *V. vulnificus* cells in an oyster do not die during the winter, but simply enter the VBNC state. Since the cells bind so tightly to the tissue of the oyster, it is quite possible that winter harvested and apparently "safe" oysters containing particularly virulent VBNC cells will grow out the virulent strains months later in warm weather. Therefore, to move these oysters from one area to another, even from one region to another, and to leave them for months may not decrease their potential for causing disease. If this practice continues, we may well see deaths attributable to oysters from previously believed-innocuous areas.

Other Vibrios

Numerous other species of the genus *Vibrio*, which occur naturally in the estuarine environment, cause some shellfish-borne gastroenteritis. These species include *V. alginolyticus*, *V. fluvialis*, *V. furnissi*, *V. hollisae*, and *V. mimicus*. As mentioned above, *V. mimicus* is very similar to *V. cholerae*, and can elaborate a toxin identical to *V. cholerae*. In addition, *V. mimicus*-like *V. cholerae* can live in freshwater. The other species of *Vibrio* mentioned here require some salt, probably prefer less than full-strength seawater, and probably exist in higher concentrations in warmer water. *V. furnissi* used to be classified as a biovar of *V. fluvialis*, and most of the shellfish-borne illnesses attributed to these two species may be due to *V. furnissi* (Hackney et al., 1992). One death in 1990 is attributed to *V. fluvialis* from the consumption of shellfish (Rippey, 1994). *V. metschnikovii* has not yet been associated with shellfish-borne disease, but since it is widely distributed in estuaries and has been shown to cause diarrhea (Roderick, 1991), it is a potential candidate. In their review of Rippey's (1994) data, Wittman and Flick (1995) indicate that 95 percent of the deaths attributable to shellfish-borne disease are attributable to the non-cholera vibrios (including *V. vulnificus*).

Pleisomonas shigelloides

Pleisomonas shigelloides is a member of the family Vibrionacae, and it occurs seasonally during the warmer months. Unlike most vibrios, it primarily occurs in freshwater, but does occur in marine waters. Symptoms of the disease resulting from consumption of food include diarrhea, abdominal pain, nausea, chills, fever, headache, and vomiting. The onset time is normally 24 to 50 hours, and usually lasts 24 to 48 hours. They survive well in shell oysters held at refrigeration temperatures (Hackney et al., 1992). There have been 23 shellfish-borne illnesses attributed to *P. shigelloides* over the years 1984 to 1993 (Rippey, 1994) and none from 1994 to 1999 (Glatzer, 1999). However, Roderick (1991) indicates that it is not a proven cause of gastroenteritis, since the disease has not been induced in human volunteers or provided a positive reaction in animal tests.

Aeromonas hydrophila

Aeromonas hydrophila is not accepted as an enteric pathogen, though it has been associated with many cases of diarrhea. It may well be an opportunistic pathogen, but the epidemiological data is conflicting (Roderick, 1991). In the past 15 years, two shellfish-borne outbreaks were attributed to *A. hydrophila*: one involved six persons and the other seven persons in 1984 (Glatzer, 1999; Rippey, 1994). In 1982 a shellfish-borne outbreak involving 472 persons was listed as being of an unknown cause (Rippey, 1994), though *A. hydrophila* was isolated from frozen samples and no other diarrhetic shellfish poison nor pathogens were found. *A. hydrophila* is widely distributed in freshwater and saltwater.

Clostridium perfringens

In their review of *Clostridium perfringens*, Hackney et al. (1992) indicate that although this bacteria is widely distributed in the environment and has often been isolated from shellfish, it is of little importance as a seafood-borne pathogen. Approximately 10^6 to 5 X 10^8 are needed to cause illness, and levels accumulated by shellfish from the environment are apparently less than that. *C. perfringens* are readily depurated from shellfish, though their spores survive well in the environment, and for these reasons have been proposed as an excellent indicator for depuration systems (Hackney et al., 1992). Vegetative cells do not survive long in the environment, and since they are much more likely to be present in elevated concentrations in fresh sewage, they have been suggested as an indicator (Kator and Rhodes, 1991). There are no recorded cases of shellfish- borne sickness attributed to *C. perfringens* in the United States (Glatzer, 1999; Rippey, 1994).

Bacillus cereus

Bacillus cereus is a common food-borne pathogen in Europe, but it is rarely reported in the United States. The bacteria produce two types of toxins: one that is heat stable and induces nausea and vomiting, while the other type (heat-labile) causes colic and diarrhea. Levels of $>10^5$ or $>10^6$ are needed in food to induce sickness. The onset time for vomiting is one to six hours, and is six to 24 hours for cases of diarrhea. Sickness usually lasts no more than 24 hours. It is a ubiquitous soil organism. Problems with cooked food generally occur after it has been held at ambient temperatures and thus allows proliferation of the bacteria (Benenson, 1995; Hackney et al., 1992). No cases of shellfish-borne illness attributed to *B. cereus* are listed by Rippey (1994) or by Glatzer (1999); however, Hackney et al. (1992) indicate a concern for the diarrheal-type sickness attributable to bacterial growth when shellstock are held out of water at warm temperatures.

Klebsiella pneumoniae

Klebsiella pneumoniae has been implicated as an enterotoxigenic agent causing diarrhea in people, though apparently not often; only some strains are potent histamine producers (Stratton and Taylor, 1991). In their evaluation of the entero-pathogenicity of environmental strains of *K. pneumoniae* from Louisiana oysters, Boutin et al. (1986) determined that even relatively high levels of the organism (3 X 10^3 CFU/100g) in oysters would be safe for noncompromised persons. No cases of shellfish-borne illness due to *K. pneumoniae* have been reported (Glatzer, 1999; Rippey, 1994).

Listeria monocytogenes

Listeria monocytogenes occurs in estuarine and marine waters primarily as runoff from the land, as a result of sewage discharges. However, it is so widespread in waters that one should not link it to pollution in a traditional sense, because most estuarine waters in the United States contain the bacteria (Motes, 1991). *L. monocytogenes* rarely occurs in raw shellfish; this may either be because

the numbers of the bacteria in estuarine waters are fairly low (Embarek, 1994), or because of antimicrobial action by the shellfish, or because the shellfish are a poor substrate for growth.

Listeriosis is usually manifested as meningoencephalitis and/or septicemia, though milder flu-like symptoms and asymptomatic cases occur. Fetuses and newborn infants are highly susceptible; immunocompromised persons, the elderly, and pregnant women are at a higher risk than the general public. The infection is rarely diagnosed in the United States. The disease occurs extremely rarely worldwide as a result of seafood; Embarek (1994) reports three confirmed sporadic cases and two suspected outbreaks. At least one of the suspected outbreaks was due to smoked products (mussels) (Lennon et al., 1984) and is not conclusive (Fuchs and Surendran, 1989). The author could not determine whether any of the cases were linked to raw shellfish.

TOXIC SUBSTANCES

The concentration of lipids in a particular bivalve varies, due not only to species, but also to season, state of the spawning cycle, etc.; this concentration greatly affects uptake of lipophilic substances. The size of bivalves also affects uptake, because smaller individuals pump more water per body weight and may tend to concentrate to higher levels (Stronkhorst, 1992; Roberts et al., 1979). The concentration of the pollutant in the water, sediment, and suspended particulate matter all likewise affect the ultimate body burden of a bivalve.

PESTICIDES

As a general rule in the United States, pesticides in shellfish from growing waters do not pose a significant public health risk for several reasons. Many of the persistent pesticides such as chlordane and DDT have been banned. Also, although pesticides are widely used, a more educated public is keeping most of the pesticides on the land; and when they do run off into the waterways, they are quickly diluted by the typically large volume of water surrounding shellfish beds. When a pesticide is a problem, it is usually in a small local area and often of short duration.

As indicated in the 1993 Revision of Part II of the NSSP, the FDA will take action to exclude shellfish in interstate markets if they exceed the following concentrations: 0.1 parts per million (ppm), mirex; 0.3 ppm, aldrin + dieldrin, chlordane, chlordecone (Kepone^{R}), heptachlor + heptachlor epoxide; 5.0 ppm, toxaphene, DDT+DDE+TDE. These are all long-lasting, chlorinated hydrocarbon pesticides that were of much concern in the 1960s and 1970s. Their use has been either banned or strictly curtailed in the United States, and now they are usually found only in low background concentrations well below regulation levels, though the potential for hot spots exists.

Organochlorine pesticides such as these are rather insoluble in water; as a whole they tend to adsorb onto clay particles and can be fairly rapidly buried in estuarine environments. In the James River of Virginia, Allied Chemical released an estimated 9,070-18,140 kg of Kepone^{R} from the late 1960s until mid-1975. In the downstream estuarine portions of this four-mile-wide river, Kepone^{R} concentrations reached a maximum of 0.5 ppm (wet weight) in oysters with an average value of approximately 0.2 ppm in 1976; by 1987 they averaged well below 0.02 ppm, though one outlier of 0.37 ppm did appear [Virginia Department of Health (VDH) data].

DDT and its breakdown products of DDD and DDE, along with chlordane, are usually the dominant pesticides present in shellfish. United States studies on the West Coast (Phillips and Spies, 1988), Gulf Coast (Sericano et al., 1993; 1990), South Carolina (Markus and Renfrow, 1990), Chesapeake Bay (VDH data, Murphy, 1990), and Long Island Sound (Robertson et al., 1991) all indicate concentrations of DDT and its metabolites, chlordane, and the other persistent organochlorine pesticides to be well below NSSP requirements. Chlorinated hydrocarbons in the Western Mediterranean also appear to be decreasing (Sole et al., 1994).

Chlordane is a resistant chlorinated hydrocarbon pesticide mixture of more than 140 compounds. The primary components are *a*-chlordane, *g*-chlordane, heptachlor, and *trans*-nonachlor. All sales and uses were banned in the United States in 1988; however, due to its widespread use in the past, it is ubiquitous in the marine environment, with higher concentrations near urban areas. Analysis of oysters in the Gulf of Mexico indicates that the dominant components bioconcentrated were *a*-chlordane and *trans*-nonachlor, along with

lesser concentrations of heptachlor and its epoxide. The five-year trend (ending 1991) in the Gulf of Mexico oysters indicates little change in total chlor-dane concentrations, though heptachlor and heptachlor epoxide have been increasing since 1987 (Sericano et al., 1993).

While in the environment, chlorinated pesticides break down slowly into various metabolites. DDT has an estimated environmental half-life of 20 years (Woodwell et al., 1971); DDE, which is its major hydrolysis product, appears even more persistent than that (Wolfe et al., 1977). Aldrin may be degraded into dieldrin, and heptachlor is enzymatically changed to its epoxide by organisms.

Carbamate and organophosphorus pesticides are in wide use today. These pesticides are generally shorter-lived, and have not been found to accumulate to high concentrations in shellfish to date, though the data is limited (Markus and Renfrow, 1990; Hale, 1989). Many types of bacteria and higher-order animals, including the brackish water clam (*Rangia cuneata*) and the hard clam (*Mercenaria mercenaria*), can hydrolyze organophosphate pesticides to nontoxic forms (Landis, 1991).

Polychlorinated Biphenyls

Polychlorinated biphenyls (PCBs) represent a mixture of 209 possible congeners — that is, 209 ways that chlorine atoms can be attached to the biphenyl molecules. Even if world production were to stop, the marine environment would continue to receive inputs from numerous sources (Duursma, 1989). Concentrations of PCBs in shellfish worldwide seem to be either not decreasing (Stronkhorst, 1992; Phillips and Spies, 1988) or reaching a new slower rate of decrease (Sole et al., 1994; Picer and Picer, 1990). These data should be sobering because there is considerable concern about some of the specific congeners present in PCB mixtures.

PCB congeners of particular concern are called non-*ortho*-substituted congeners, of which there are 20 types, and, to a lesser extent, the mono-*ortho*-substituted congeners. With this particular type of chlorine substitution, the molecules can attain a planar structure, which makes them similar to the highly toxic dibenzo-*p*-dioxins and dibenzofurans (de Boer et al., 1993; Hansen, 1987; McKinney et al., 1985). The PCBs of particular concern are the ones with four, five, or six chlorines in the non-*ortho* positions because they mimic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF) (Safe, 1990; Goldstein and Safe, 1989). These planar PCBs are slower to be taken up by shellfish; however, once taken up, they are slower to be depurated than the other PCBs of the same number of chlorine atom substitutions (Sericano et al., 1992; Kannan et al., 1989).

There is some indication in the literature that PCBs may not accumulate in marine animals in the classic food chain concept, but rather as a direct partitioning among the various compartments, such as water, sediment, particulate matter, and organisms (Mackay, 1991; Duursma, 1989). Mussels have been shown to comply with the concept that they take up PCBs mainly by equilibrium partitioning between PCBs in the surrounding water and the lipid pool of the mussel. As such, they are good indicators of the concentrations of PCBs in water. The uptake of these PCBs is thought to occur primarily through the gills and across the body surfaces, with ingestion of additional amounts in food playing a significantly lesser role. PCBs with fewer chlorine atoms are more hydrophilic, and, as such, reach equilibrium in uptake and depuration quicker in these mussels than the more highly chlorinated and thus more hydrophobic congeners (Bergen et al., 1993; Tanabe et al., 1987). Bivalves tend to accumulate the lesser chlorinated PCBs, especially the 4-, 5-, and 6-chlorine substitutions (Pruell et al., 1993; Sericano et al., 1990). Oysters tend to concentrate PCBs 10 to 20 times that of their surrounding sediment concentrations (Wade et al., 1988). Also, bivalves tend not to metabolize PCBs (Pruell et al., 1993; Sericano et al., 1990; Boon et al., 1989).

The NSSP requires that total PCBs in shellfish be less than 2.0 ppm on a wet-weight basis. In most of the United States, shellfish are substantially below the NSSP limit (Robertson et al., 1991; Murphy, 1990; Sericano et al., 1990; Phillips and Spies, 1988; Steimle et al., 1985; VDH data). However, there are some local areas of considerably higher concentrations.

Dioxins and Furans

Dioxins and furans represent classes of chemical compounds that can be chlorinated at numer-

ous sites on different molecules producing a suite of congeners as in PCBs. These chemicals are regarded as among the most toxic chemicals in the marine environment (Tanabe, 1988b), and dioxins are believed to be about 10 times as toxic as furans (Waldichuk, 1990). Two of these congeners of particular concern due to their toxic effects to mammals are 2,3,7,8-TCDD and 2,3,7,8-TCDF. The FDA has not established advisory limits for dioxins and furans in seafood.

These compounds are released into the estuarine environment by bleached kraft mill effluents, from lumber treated with pentachlorophenol, from PCBs used as heat transfer fluids, and from the combustion of various types of waste. Though the number of isomers of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans are lower in pulp and paper mill effluents, the most toxic isomers 2,3,7,8-TCDD and 2,3,7,8-TCDF are a prominent fraction present (Tanabe, 1988b). There is little research concerning the uptake of these TCDDs or TCDFs by bivalves from the estuarine environment, but two studies (Pruell et al., 1993; Petreas et al., 1992) indicate that clams tend not to accumulate these toxins very much over that in bottom sediments, in contrast to PCBs. Clams apparently reach equilibrium concentrations with their environment within two months. Mollusks may be able to survive in TCDD- and TCDF- contaminated environments better than many other animals since they may not contain a biological receptor that causes the genetic expression of toxic effects (Hahn et al., 1993).

Polyaromatic Hydrocarbons

Hydrocarbons are organic (i.e., containing carbon atoms) molecules, such as oils. They are classified as to such structures as chain types (e.g., alkanes) and ring structures (e.g., naphthenes, aromatics). The simplest of these common ring structures is the six-carbon benzene ring. Benzene is one of the few documented human carcinogens; however, since it is so light, it evaporates from spilled gasoline, etc., and does not accumulate in the estuarine environment. Polyaromatic hydrocarbons (PAHs) basically are a series of these six-carbon benzene rings linked together in the molecule. Other names for PAH are polynuclear aromatics (PNAs), polycyclic aromatic compounds (PACs), etc. Though PAHs of two rings can accumulate in the benthos (Farrington et al., 1986), typically molecular structures of three to five rings are the types that are heavy enough to remain in the aquatic system (Farrington and Quinn, 1973) and are most toxic. PAHs are of particular concern because many of them have the potential to be carcinogenic or mutagenic. Sixteen types of PAHs are listed as priority pollutants by the World Health Organization (WHO) and the United States Environmental Protection Agency (EPA). The FDA has no consumption standards for seafood containing PAHs at this time.

PAHs are ubiquitous in the environment, and are due to the incomplete combustion of organic matter such as fossil fuels and wood and the use of petroleum products (Wade et al., 1988; Neff et al., 1979). PAHs are introduced into the estuary via river transport, nonpoint source runoff, industrial discharges, atmospheric deposition (which can be from remote sources), and spills. The usual low-level background concentrations (Steimle et al., 1985) appear to be due primarily to burning, whereas locally elevated concentrations tend to be due to spills, sewage treatment plant discharges, or urban runoff (Helmstetter and Alden, 1994; Jackson et al., 1992; Pereira et al., 1992; Wade et al., 1988; Hoffman et al., 1984). PAHs from urban highway and industrial areas are a significant source to the estuary; they are similar to PAHs in atmospheric fallout, and may primarily be due to fallout. PAHs from municipal STFs are not weathered like those from atmospheric deposition, and as such contain more, lighter-molecular-weight PAHs such as naphthalenes (Hoffman et al., 1984). The ratio of phenanthrene to anthracene is helpful in distinguishing between petroleum and nonpetroleum sources of PAHs in shellfish (Wade et al., 1988).

When released into the environment, PAHs partition into the various "compartments," both biotic and abiotic, due to their physicochemical properties such as vapor pressure, water solubility, and sediment/water partitioning coefficient (Porte and Albaiges, 1993). Microbial degradation and evaporation may remove most of the lightweight PAHs from the water column, whereas photooxidation and sedimentation may remove most of the heavier PAHs from the water column (Wade et al., 1988). Since PAHs are very hydrophobic, upon entering the estuary they tend to

attach quickly to suspended particulate matter and sediments, which can then settle out on and be incorporated into the benthos. Release rates of PAHs from bottom sediments are inversely related to the organic carbon content, and directly correlated to the water solubility of the individual PAH (Helmstetter and Alden, 1994; Karickhoff et al., 1979).

Originally, mollusks were thought to be unable to metabolize PAHs. However, cytochrome P-450 and associated mixed-function oxidase enzyme systems capable of metabolizing PAHs have been found to operate in many mollusks, though at a slower rate than in fish and crustaceans (Neff et al., 1976), and much slower than in vertebrates. When metabolized, PAHs tend to be changed into more polar compounds which then mix better with water and are more easily eliminated by the organism (Hellou et al., 1993). There is some evidence that oysters may be able to metabolize benzo(a)pyrene into mutagenic or carcinogenic derivatives (Pittinger et al., 1987).

Oysters serve as excellent sentinel organisms for PAH pollution since they do not move, and they readily accumulate and release PAHs in close correlation with the amount in their environment. When transplanted into a more highly contaminated area, or when moved from a contaminated area to a cleaner area, oysters reach their new equilibrium within 14 days (Pittinger et al., 1987) to about a month (Jackson et al., 1994; Bender et al., 1987). Oysters have been shown to accumulate PAHs from around marinas, and areas of creosote pollution (Elder and Dresler, 1988; Bender et al., 1987; Marcus and Stokes, 1985). Oysters and the brackish water clam *Rangia cuneata* do not tend to highly concentrate PAHs over that found in the adjacent sediments, but concentrate from less than one to approximately four times that of the sediment (in contrast to organochlorine pesticides and PCBs) (Pendoley, 1992; Wade et al., 1988; Bender et al., 1987, 1986). However, particular PAHs can be significantly bioaccumulated (Bender et al., 1987). Blue mussels (*Mytilus edulis*) have been shown to rapidly take up PAHs from contaminated sediments, and to depurate them with half-lives ranging from 12 to 30 days depending on the type of aromatic present (Pruell et al., 1986). However, hard clams (*Mercenaria mercenaria*) do not appear to depurate certain PAHs even after 45 days or more (Tanacredi and Cardenas, 1991; Boehm and Quinn, 1976). Mollusks tend to have the highest concentrations of PAHs in the visceral mass, which represents the net balance between the uptake and elimination of the contaminants. The lowest levels of PAHs in mollusks occur in the muscular tissue, which reflects only uptake.

Variations in concentrations of PAHs among individuals of a molluscan species in nearby areas occur due to many factors. Smaller organisms tend to accumulate to higher concentrations, possibly due to the increased pumping of water and increased gill size in relation to their body. Organisms with large numbers of gametes may attain higher concentrations. Other factors include differences in pumping rate, filtration rate, lipid content, and the concentration of the PAHs in the sediment in the immediate vicinity of the organism (Hellou et al., 1993; Bender et al., 1986).

Heavy Metals

Generally, heavy metal pollution in United States coastal areas is restricted to local areas, and most United States shellfish are not above FDA advisory levels (Morse et al., 1993; Robertson et al., 1991; Turgeon et al., 1991; Lauenstein et al., 1990; Murphy, 1990; Farrington et al., 1983; Croonenberghs, 1974; VDH data). The primary source of heavy metals to marine waters is runoff. Due to the low solubility of metals in water, metals tend to attach to sediments and settle out locally around areas of high input. Sediments tend to concentrate heavy metals by three to five orders of magnitude above amounts in water.

Even though local areas may receive elevated inputs of heavy metals, shellfish in the area may not contain significantly increased amounts due to the complexity of factors that come into play. As a general rule, shellfish do reflect concentrations of metals in their environment, but these concentrations are the net result of many biological and chemical processes.

Most metals exhibit several pathways for uptake by bivalves. Metals will partition among dissolved phases (in several oxidation states) and phases associated with particles such as clays. They are often sequestered by either organic ligands (chelated) or precipitated out of the water

by iron sulfides, again in several oxidation states. In addition, some metals are alkylated by bacteria into more organically soluble forms (e.g., methyl mercury) that can greatly increase the potential for uptake by organisms.

The biology of the bivalve also greatly affects its body burden of heavy metals. As is true for many other contaminants, smaller animals of a species tend to concentrate metals to higher levels. Different species concentrate metals to varying levels; as a general rule, oysters, with their high pumping rate, have higher concentrations of metals than other bivalves. Deposit-feeding bivalves (e.g., *Mya arenaria, Macoma balthica*) ingest sediment and therefore are exposed to different concentrations and forms of metals than suspension feeders (*Crassostrea virginica, Mytilus edulis*).

Following the lead of Bryan and Langston (1992) in their detailed review, the following brief summation of the different heavy metals in relation to shellfish will center on those generally perceived to be of concern in the estuarine environment. They are listed in order of their appearance in the periodic table for ease of comparison with their behavior (bioavailability, toxicity) and chemical similarity: copper (Cu), silver (Ag) (Group IB); zinc (Zn), cadmium (Cd), mercury (Hg) (Group IIB); tin (Sn), lead (Pb) (Group IVA); arsenic (As) (Group VA); selenium (Se) (Group VIA); chromium (Cr) (Group VIB).

Copper

Copper (Cu) is generally considered to be of little concern to humans in concentrations found in shellfish. However, copper is toxic to marine organisms and as such is widely used in antifouling paint on boat hulls. As such, one would expect to find locally elevated concentrations around marinas. Copper dissolved in water as the Cu^{2+} ion is the most available and toxic form, and oysters readily accumulate it in this form (Zamuda et al., 1985; Zamuda and Sunda, 1982). However, the dissolved ion is easily chelated by organic compounds and made less available for uptake. Also, the ionic form seems to compete with calcium (Ca) and magnesium (Mg) in seawater, such that less is taken up in higher salinity waters by the soft clam (*Mya arenaria*) (Wright and Zamuda, 1987). The eastern oyster (*Crassostrea virginica*) accumulates significantly higher Cu concentrations in lower salinity water than in higher salinity (Phelps et al., 1985; Huggett et al., 1973; VDH data), and tends to concentrate to higher levels in smaller organisms (Phelps et al., 1985). Oysters have been shown to be capable of taking up Cu incorporated into algae. In sediments, Cu is associated with iron oxides and humic acids (Bryan et al., 1992). Copper concentrations in bivalves may be increasing slightly in the United States (Stephenson and Leonard, 1994; Turgeon and O'Connor, 1991). The FDA has not established advisory limits for Cu in shellfish.

Silver

Silver (Ag) probably occurs primarily in seawater as $AgCl_2$, though the most available form to biota may be as the neutral monochloro-complex AgCl. In remote estuarine areas, Ag in oxidized sediments is associated with humic acids which seem to interfere with uptake of the metal by the biota. In sewage effluents, the organics present also bind the Ag, but extracellular polymers released by bacteria may increase the availability for uptake. Oysters seem to take up silver primarily from water since they do not ingest significant quantities of sediment, and since once incorporated into algae it is too tightly bound by organics for incorporation into the oyster. The deposit-feeding bivalve *Macoma balthica* is reported to accumulate Ag from sediments. In solution Ag is regarded as one of the most toxic of heavy metals (Bryan and Langston, 1992; Abbe and Sanders, 1990). The FDA has not set a specific advisory level for Ag in shellfish.

Zinc

Zinc (Zn) is most available to biota as the Zn^{2+} ion, and this form is often the most abundant of the dissolved species. Bryan and Langston (1992) conclude that loosely-bound Zn in sediments and dissolved Zn in interstitial water (among sediment grains) and overlying waters are important sources to biota. Oysters do not regulate the uptake of Zn, and depuration is minimal, although the mussel *Mytilus edulis*, with its more active kidney, strongly regulates Zn concentrations. The short-term uptake of Zn by the oyster is probably a function of its pumping rate, and its total body burden increases over its lifetime, although the rate of increase decreases as the oyster grows (Mo and Neilson, 1993). The levels of Zn in oysters is