Seafood Safety

Food-borne illness is not limited to seafood, but is a common concern of all food industries. The recent media attention to seafood has led to an increase in public awareness and a number of misconceptions about the safety of eating seafood.

Between 1973 and 1987, shellfish accounted for 2.8% of the cases of food-borne illness reported to the Centers for Disease Control (CDC), and finfish accounted for 2.2% of the cases. These statistics may seem high at first glance, but they are somewhat misleading. For example, 37% of the cases of seafood-borne illness in the U.S. between 1977 and 1981 were attributed to ciguatera, a toxin found only in tropical and subtropical fish. An additional 37% of the cases during the same time period were attributed to scombroid poisoning, a toxin produced in the flesh of some species of fish when improperly stored at high temperatures. Therefore, the statistics reported by the CDC are skewed by illnesses which either affect only a small geographical area, or only occur with mishandling of fish.

The incidence of illness attributed to seafood can be reduced if the public is better informed, understands the risks, and most importantly, learns to prevent seafood-borne illness. When handled properly, finfish and shellfish are as safe to eat as any other source of protein. For healthy individuals, the nutritional benefits of seafood far outweigh the safety concerns. Persons with compromised immune systems, such as those with liver disease, can also benefit from eating seafood but should follow a few precautionary measures when preparing seafood.

CONTENTS

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I. General Bibliography

The following references are general. For more specific references, see bibliography under each contaminant.

Barnett, J. 1988. Seafood safety: the facts. Seafood Business. September/October. pp. 122-135.

DuPont, H.L. 1986. Consumption of raw shellfish - is the risk now unacceptable? New England Journal of Medicine. 314(11):707-708.

Greenberg, A.E. & D.A. Hunt (eds). 1984. Laboratory Procedures for the Examination of Seawater and Shellfish, 5th edition. American Public Health Association. Washington, DC

Horwitz, M.A. 1977. Specific diagnosis of foodborne disease. Gastroenterology. 73(2):375-381.

Leonard, D.L., M.A. Broutman & K.E. Harkness. 1989. The quality of shellfish growing waters on the East Coast of the United States. NOAA, National Estuarine Inventory. Rockville, MD

Liston, J. 1989. Current issues in food safety - especially seafoods. Journal of the American Dietetic Association. 89(7):911-913.

U.S. Food and Drug Administration, Division of Microbiology. 1984. Bacteriological Analytical Manual, 6th edition. Association of Official and Analytical Chemists. Arlington, VA

II. Viral Contaminants

A. General information on viruses

General Viruses - Description

There are more than 110 different viruses known to be excreted in human feces, collectively called the "enteric viruses" (Goyal, 1984). Viruses survive better at low temperatures and are inactivated at high temperatures (Lo et al., 1976, as cited in Goyal et al., 1984). As a result, most outbreaks of hepatitis occur during winter and early spring. Viruses can remain viable for long periods of time in seawater and have been shown to survive as long as 17 months in marine sediment (Goyal et al., 1984). Viruses associated with sediment are as infectious to animals as those that are freely suspended. Marine sediment acts as a reservoir of viruses, which may be resuspended by any kind of turbulence, such as boating, storms and dredging (LaBelle et al., 1980). Rainstorms can also increase viral concentration in the water by increasing land runoff (Gerba et al., 1979) and by release of sewage from overburdened treatment plants (Goyal, 1984).

Virus Uptake & Elimination by Shellfish

Viruses have been isolated from hard clams, oysters, mussels, soft clams, crabs, cockles, lobster and conch. In filter-feeding mollusks, the viruses can become concentrated at a level higher than the surrounding water. The viruses do not multiply in bivalves, but accumulate in the liver-like digestive gland.

Carnivorous shellfish, such as, crabs and lobster can accumulate viruses by contact with contaminated seawater and/or by consuming contaminated bivalves (Hejkal and Gerba, 1981) Viruses are generally present in crabs at a level below that of the water. The highest concentrations of viruses are found in the inedible portions of crabs (Goyal et al., 1984). However, the potential health hazard should not be overlooked since tissue contamination could occur when crabs are prepared for consumption.

A number of experiments on the efficiency of viral depuration have been conducted and have resulted in a range of conclusions, although the more recent studies generally do not support the use of depuration for viruses. One of the earlier studies, using artificially infected soft shell clams, reported that most viruses are purged within a 24-48 hour period, and low levels of viruses are depurated more rapidly than high levels (Metcalf et al., 1979). A more recent study (Hay and Scotti, 1986) using insect picornavirus and *Crassostrea gigas*, showed that viruses were present in the oyster tissue even after 64 hours of depuration. In a related experiment (Scotti, et al., 1983), both uptake and elimination of viruses were shown to be variable even when bacterial depuration appeared to be normal. These researchers concluded that bacterial depuration rates can not accurately predict viral contamination levels. Finally, an Australian study (Grohmann et al., 1981) using naturally infected oysters, indicated that norwalk virus is not completely depurated after 48 hours. In this study, some of the volunteers, who were fed depurated oysters (which met bacteriological standards), become ill with viral gastroenteritis (60% of illnesses occurred during periods of heavy winter rain).

General Viruses - Detection & Prevention

Fecal coliforms are used as indicator bacteria to predict the possible presence of viruses and other pathogens in shellfish. The water standard for harvesting mollusks is 14 fecal coliforms or less per 100 ml of water (NSSP 1989). However, it is generally accepted that coliforms do not accurately indicate the presence or absence of viruses (Goyal and Gerba, 1978; Gerba et al., 1979; LaBelle et al., 1980; Goyal et al., 1984). Generally, bacteria do not live as long as viruses in the marine environment (LaBelle et al., 1980). Therefore, it is possible for viruses to be present in water which is free of bacteria. In a Texas Gulf coast survey, enteroviruses were detected 35% of the time in waters which met acceptable standards for shellfish harvesting (Gerba et al., 1979). A Similar study of shellfish beds open to harvesting in the Great South Bay, Long Island, NY, resulted in enterovirus recovery in 37.5% of the water and shellfish samples (Vaughn and Landry, 1977, as cited in Gerba and Goyal, 1985). Also, outbreaks of hepatitis A have been associated with oysters harvested from certified grounds (Mackowiak et al., 1976; Portnoy et al., 1975).

Fecal coliform standards only apply to filter-feeding mollusks. The regulations do not apply to commercial harvesting of crabs and lobsters. Although viruses accumulate in the nonedible portions of crabs and lobsters, they have caused viral illness due to contamination of edible tissues while cooking (Goyal et al., 1984). Mobile shellfish, such as crabs and lobsters, also present a problem since they can accumulate viruses in polluted waters and move to cleaner areas and act as vectors of viral disease.

Some cases of illness have been linked to insufficiently cooked shellfish (Feingold, 1973). Most viruses (excluding Hepatitis A) are inactivated when the internal temperature of the mollusk reaches 140° F, which requires 4 to 6 minutes of steaming (Koff and Sear, 1967; Giusti and Gaeta, 1981). A common cooking practice is to steam mollusks only until the shell opens. It has been demonstrated that shells open after only about 1 minute of steaming, which is not sufficient time to inactivate all of the viruses.

Selected Bibliography

Carter, T.H. & F.R. Cantelmo. 1989. Efficacy of commercial depuration in the elimination of enteric viruses and *Clostridia* from the hard clam. Marine Technology Society Journal. 23(1):14-20.

Gerba, C.P. & S.M. Goyal. 1978. Detection and occurrence of enteric viruses in shellfish: a review. Journal of Food Protection 41(9): 743-754.

Gerba, C.P., S.M. Goyal, R.L. LaBelle, I. Cech & G.F. Bodgan. 1979. Failure of indicator bacteria to reflect the occurrence of enteroviruses in marine waters. American Journal of Public Health. 69(11):1116-1119.

Gerba, C.P., J.B. Rose, and S.N. Singh. 1985. Waterborne gastroenteritis and viral hepatitis. Critical Reviews of Environmental Control. 15(3):213-236.

Giusti, G. and G.B. Gaeta. 1981. Doctors in the kitchen: experiments with cooking bivalve mollusks. New England Journal of Medicine. 304(22):1371-1372.

Goyal, S.M. 1984. Viral pollution of the marine environment. Critical Reviews of Environmental Control. 14(1):1-32.

Goyal, S.M., W.N. Adams, M.L. O'Malley & D.W. Lear. 1984. Human pathogenic viruses at sewage sludge disposal sites in the Middle Atlantic region. Applied and Environmental Microbiology. 48(4):758-763.

Goyal, S.M., C.P. Gerba & J.L. Melnick. 1979. Human enteroviruses in oysters and their overlying waters. Applied and Environmental Microbiology. 37(3):572-581.

Grohmann, G.S., A.M. Murphy, P.J. Christopher, E. Auty and H.B. Greenberg. 1981. Norwalk virus gastroenteritis in volunteers consuming depurated oysters. Australian Journal of Experimental Biology and Medical Science. 59:219-228.

Hay, B. and P. Scotti. 1986. Evidence from intracellular absorption of virus by the Pacific oyster, *Crassostrea gigas*. New Zealand Journal of Marine and Freshwater Research. 20:655-659.

Hejkal, T.W. & C.P. Gerba. 1981. Uptake and survival of enteric viruses in the blue crab, *Callinectes sapidus*. Applied and Environmental Microbiology. 41(1):207-211.

Koff, R.S. and H.S. sear. 1967. Internal temperature of steamed clams. New England Journal of Medicine. 276:737-739.

LaBelle, R.L., C.P. Gerba, S.M. Goyal, J.L. Melnick, I. Cech & G.F. Bogdan. 1980. Relationships between environmental factors, bacterial indicators, and the occurrence of enteric viruses in estuarine sediments. Applied and Environmental Microbiology. 39(3):588-596.

Lo, S., J. Gilbert and F. Hetrick. 1976. Stability of human enterovirus in estuarine and marine waters. Applied and Environmental Microbiology. 32:245-249.

Larkin, E.P. and D.A. Hunt. 1982. Bivalve mollusks: control of microbial contaminants. Bioscience. 32(3):193-197.

Landry, E.F., J.M. Vaughn, T.J. Vicale & R. Mann. 1983. Accumulation of sediment-associated viruses in shellfish. Applied and Environmental Microbiology. 45(1):238-247.

Mackowiak, P.A., C.T. Caraway and B.L. Portnoy. 1976. Oyster-associated hepatitis: lessons from the Louisiana experience. American Journal of Epidemiology. 103(2):181-191.
Metcalf, T.G., B. Mullin, D. Eckerson, E. Moulton & E.P. Larkin. 1979. Bioaccumulation and depuration of enteroviruses by the soft-shelled clam, *Mya arenaria*. Applied and Environmental Microbiology. 38(2):275-282.
NSSP Manual of Operations. 1989. Part I, Section C. United States Food and Drug Administration, Shellfish Sanitation Branch, Washington, DC.
Richards, G.P. 1988. Microbial purification of shellfish: a review of depuration and relaying. Journal of Food Protection. 51(3):218-251.
Scotti, P.D., G.C. Fletcher, D.H. Buisson and S. Fredericksen. 1983. Virus depuration of the Pacific oyster (*Crassostrea gigas*) in New Zealand. New Zealand Journal of Science. 26:9-13.
Vaughn, J. and E.F. Landry. 1977. Virus study. Interim report series:6. Nassau-Suffolk Regional Planning Board. Hauppauge, NY.

B. Hepatitis A

Description

Hepatitis A is 27 nm in diameter and has single-stranded RNA (Gerba et al., 1985). The first outbreak of seafood-borne (oysters) hepatitis A occurred in Sweden in 1955 (Lindberg-Braman, 1956). Hepatitis B has never been associated with shellfish consumption, although hepatitis B antigen was recovered from clams near a hospital sewage outlet along the coast of Maine (Mahoney et al., 1974, as cited in Portney et al., 1975). In temperate climates, peaks in hepatitis outbreaks occur in the late fall and early winter (Gerba et al., 1985).

Contaminated Species

Both raw and steamed hard clams (Feingold, 1973), oysters (Mackowiak et al., 1976; Portnoy et al., 1975), mussels (Dienstag et al., 1976, as cited in Gerba and Goyal, 1978) and soft clams (Grady et al., 1965, as cited in Gerba and Goyal, 1978), have been implicated in outbreaks of hepatitis A.

Symptoms & Treatment

Symptoms of hepatitis A infection usually begin within 4 weeks (range: 2 - 6 weeks) of exposure to the virus. The initial symptoms are usually weakness, fever, anorexia, nausea, fever, malaise and abdominal epigastric pain. As the illness progresses, the individual usually becomes jaundice, and may have dark urine. The severity of the illness ranges from very mild (young children are often asymptomatic), to severe, requiring hospitalization. The fatality rate is low (<0.1%), and deaths primarily occur among the elderly and individuals with underlying diseases (Anonymous, 1989; Bryan, 1986; Feingold, 1973).

Statistics

Residence of coastal states have a higher incidence of infection than inland states (Goyal et al., 1979; Goyal, 1984). The CDC reported 4 outbreaks of hepatitis A traced to seafood consumption between 1977 and 1981 (USFDA, 1984).

Detection and Prevention

Hepatitis A appears to be more resistant to heat than other viruses. A laboratory study by Peterson et al. (1978, as cited in Gerba et al., 1985) showed that hepatitis A viruses in infected oysters were inactivated after heating at 140°F for 19 minutes. Therefore, mollusks which are steamed only until the shells open (a common cooking practice) are not exposed to heat long enough to inactivate hepatitis A viruses.

Selected Bibliography

Anonymous. 1989. Epidemiology of hepatitis A in North Carolina in 1988. Epi Notes, Epidemiology Section, Division of Health Services, North Carolina. Report No. 89-1.
Bryan, F.L. 1986. Seafood-transmitted infections and intoxications in recent years. In: D.E. Kramer and J. Liston (eds), Seafood Quality Determination Elsevier Science Publishers, Amsterdam. pp 319-337.

Dienstag, J.L., I.D. Gust, C.R. Lucas, D.C. Wong and R.H. Purcell. 1976. Mussel-associated viral hepatitis, type A: serological confirmation. Lancet. i:561-564.
Feingold, A.O. 1973. Hepatitis from eating steamed clams. Journal of the American Medical Association. 225(5):526-527.
Grady, G.F., T.C. Chalmers and Boston Inter-Hospital Liver Group. 1965. Viral hepatitis in a group of Boston hospitals. New England Journal of Medicine. 272:662-666.
Lindberg-Braman, A.M. 1956. Clinical observations on the so-called oyster hepatitis. American Journal of Public Health. 53:1003-10011.
Mackowiak, P.A., C.T. Caraway & B.L. Portnoy. 1976. Oyster-associated hepatitis: lessons from the Louisiana experience. American Journal of Epidemiology. 103(2):181-191.
Mackowiak, P.A., C.T. Caraway, J.A. Walker, T.W. McKinley & C.A. Klein. 1975. Oyster-associated hepatitis failure of shellfish certification programs to prevent outbreaks. Journal of the American Medical Association. 233(10):1065-1068.
Mahoney, P., G. Fleischner, I. Millman et al. 1974. Australia antigen: detection and transmission in shellfish. Science. 183:80-81.
Portnoy, B.L., P.A. Mackowiak, C.T. Caraway, J.A. Walker, T.W. McKinley & C.A. Klein. 1975. Oyster-associated hepatitis failure of shellfish certification programs to prevent outbreaks. Journal of the American Medical Association. 233(10):1065-1068.
USFDA. 1984. Bacteriological analytical methods, 6th edition. Association of Official Analytical Chemists. Arlington, VA. p. 28.03.

C. Norwalk Virus

Description

Norwalk virus was first recognized as a pathogen during an outbreak of gastroenteritis in Norwalk, Ohio in 1968 (Adler and Zicki, 1969, as cited in Gerba et al., 1985). It is now considered a major cause of non-bacterial gastroenteritis. From 1976 to 1980, the CDC reported that 42% of the outbreaks of non-bacterial gastroenteritis were caused by Norwalk virus (Kaplan et al., 1982, as cited in Gerba et al., 1985).

Contaminated Species

Illness from norwalk virus has been associated with eating clams (both raw and steamed) (Morse et al., 1986; Porter et al., 1987), oysters (Gunn et al., 1982; Eyles et al., 1981) and cockles (Appleton and Pereira, 1977, as cited in Gunn et al., 1981).

Symptoms & Treatment

Norwalk virus causes nausea, vomiting, diarrhea, abdominal cramps and occasionally fever in humans. Symptoms of gastroenteritis usually begin within 40 hours (range 12 - 72 hours) of consuming contaminated food. Gastroenteritis caused by norwalk virus is a self-limiting illness which usually persists < 48 hours, but can last as long as 1 week (Grohmann et al., 1981; Gunn et al., 1982; Bryan, 1986; Morse et al., 1986 and Porter et al., 1987).

Selected Bibliography

Adler, J. and R. Zickl. 1969. Winter vomiting disease. Journal of Infectious Disease. 119:668.
Appleton, H. and M.S. Pereira. 1977. A possible virus aetiology in outbreaks of food poisoning from cockles. Lancet. 1:780-781.
Bryan, F.L. 1986. Seafood-transmitted infections and intoxications in recent years. In: D.E. Kramer and J. Liston (eds), Seafood Quality Determination. Elsevier Science Publishers, Amsterdam. pp 319-337.
Eyles, M.J., G.R. Davey & E.J. Huntley. 1981. Demonstration of viral contamination of oysters responsible for an outbreak of viral gastroenteritis. Journal of Food Protection. 44(4):294-296.
Grohmann, G.S., A.M. Murphy, P.J. Christopher, E. Auty & H.B. Greenberg. 1981. Norwalk virus gastroenteritis in volunteers consuming depurated oysters. Australian Journal of Experimental Biology and Medical Science. 59:219-228.
Gunn, R.A., H.T. Janowski, S. Lieb, E.C. Prather & H.B. Greenberg. 1982. Norwalk virus gastroenteritis following raw oyster consumption. American Journal of Epidemiology. 115(3):348-351.
Kaplan, J.E., G.W. Gary, R.C. Baron, N. Singh, L.B. Schonberger, R. Fieldman and H.B. Greenberg. 1982. Epidemiology of Norwalk gastroenteritis and the role of Norwalk virus in outbreaks of acute nonbacterial gastroenteritis. Annals of Internal Medicine. 96:756.

Morse, D.L., J.J. Guzewich, J.P. Hanrahan, R.Stricof, M. Shayegani, R. Deibel, J.C. Grabau, N.A. Nowak, J.E. Herrmann, G. Cukor & N.R. Blacklow. 1986. Widespread outbreaks of clam- and oyster-associated gastroenteritis: role of norwalk virus. New England Journal of Medicine. 314(11):678-681.

Porter, J. & W. Parkin. 1987. Outbreaks of clam-associated gastroenteritis in New Jersey: 1983-1984. New Jersey Medicine. 84(9):649-651.

D. Poliovirus

Description

Some of the more frequently recovered viruses from shellfish are the polioviruses because of the common practice of immunizing American children against polio (Larkin and Hunt, 1982). The vaccine consists of live attenuated viruses that replicate in the intestine but produce few or no clinical symptoms. Children who have been immunized excrete viruses (from 1000 to 1,000,000 viruses/gram feces) for several days after the vaccine is administered. An examination of 20% of the polioviruses isolated from the Texas Gulf showed that all were of vaccinal origin. Since the viruses in the vaccine are modified, they present no health hazard if consumed by humans.

Selected Bibliography

Goyal, S.M., C.P. Gerba & J.L. Melnick. 1979. Human enteroviruses in oysters and their overlying waters. Applied and Environmental Microbiology. 37(3):572-581.

Larkin, E.P. and D.A. Hunt. 1982. Bivalve mollusks: control of microbial contaminants. Bioscience. 32(3):193-197.

III. Bacterial Contaminants

A. *Listeria monocytogenes*

Description

In the early 1900's *Listeria monocytogenes* was recognized as a bacterium which caused illness in farm animals. More recently it has been identified as the causative agent of listeriosis in humans.

Listeria is ubiquitous in nature and has been isolated from soil, vegetation, marine sediments and water (Peters, 1989). It is a gram-positive, non-spore forming, motile rod (Gellin and Broome, 1989). This facultative anaerobe, can grow between 1 - 45°C (Peters, 1989), with an optimal growth temperature of 30 - 37°C (USFDA, 1987). The pH range for growth of *Listeria* is 5.0 - 9.6 (Peters, 1989). *Listeria* is also tolerant to salt (Peters, 1989).

Contaminated Species

The greatest threat of listeriosis is from ready-to-eat products which do not require further cooking at home. *Listeria* in raw food is less of a concern to the food industry since the bacteria are killed when cooked thoroughly. *Listeria* has been isolated from dairy products (MMWR, 1989), vegetables (Hughey and Johnson, 1987), seafood (Lennon et al., 1984), beef and poultry (Peters, 1989). Seafood that have tested positive for *Listeria* include: raw fish (NFI, 1989), cooked crabs (Anonymous, 1987), raw and cooked shrimp (Anonymous, 1987), raw lobster, surimi and smoked fish (NFI, 1989).

Although the USFDA has isolated *Listeria* from seafood, listeriosis has not been directly associated with the consumption of finfish or shellfish. It is not understood why *Listeria* has been recovered from seafood but has not caused illness. The USFDA has proposed a number of theories to explain this phenomenon (USFDA, 1987). It is possible that cases of listeriosis have occurred from seafood but have been unreported or misdiagnosed. It has also been suggested that *Listeria* may not be virulent in all foods. Seafood may contain components that reduce the virulence of *Listeria*; or conversely milk and vegetables may contain components that enhance the virulence.

Geographical Area

Listeria is a contaminant introduced to foods during processing. Therefore, no particular geographic areas are especially susceptible to contamination. Listeriosis is reported to occur most commonly in the summer months, but a consistent seasonality has not been observed in systematically collected data (Gellin and Broome, 1989).

Symptoms & Treatment

The incubation period of *Listeria* is estimated to be between 4 days and 3 weeks (Gellin and Broome, 1989). Exposure to the bacteria does not constitute disease. Pathogenic strains of *Listeria* have been recovered from the gastrointestinal tract of asymptomatic individuals (Lamont and Postleth-Waite, 1986, as cited in Gellin and Broome, 1989). Most healthy individuals are either unaffected by *Listeria*, or experience only mild flu-like symptoms (Peters, 1989).

Victims of severe listeriosis are usually immunocompromised. Those at highest risk of contracting listeriosis include: cancer patients, individuals taking immunosuppressive drugs, alcoholics, pregnant women, patients with diminished gastric acidity (Ho et al., 1986, as cited in Gellin and Broome, 1989) and individuals with AIDS (Mascola et al., 1988, as cited in Gellin and Broome, 1989). Severe listeriosis can cause meningitis, abortions, septicemia, encephalitis, endocarditis, abscesses and local purulent lesions, malaise, fever, vomiting, violent or bursting headache and convulsions (Lennon et al., 1984; Gellin and Broome, 1989).

Statistics

Since 1981 there have been three major outbreaks of listeriosis in North America (Gellin and Broome, 1989). The three outbreaks were traced to contaminated coleslaw (occurred in Nova Scotia, Canada), milk and Mexican-style cheese. Aside from these major outbreaks, listeriosis is generally a sporadic illness (Gellin and Broome, 1989). Recently there was an isolated case (a female cancer patient in Oklahoma) of listeriosis which was traced to contaminated turkey hot dogs (Anonymous, 1989). A recent epidemic of perinatal listeriosis in New Zealand was loosely linked to the consumption of shellfish and raw fish, but a definitive connection to seafood could not be drawn (Lennon et al., 1984).

Detection & Protection

Sterile-site cultures can be used to detect *Listeria monocytogenes*. This method requires 10 days for negative results and 14 days for presumptive positive results (USFDA, 1988). More rapid results are now possible with commercial DNA hybridization kits (Gene-Trak) (King et al., 1989; Klinger et al., 1988; USFDA, 1989), or ELISA kits (Organon Teknika) (Mattingly et al., 1988; USFDA, 1988).

Listeriosis can be prevented by thoroughly cooking food, and by preventing cross contamination once the food is cooked.

Selected Bibliography

Anonymous. 1987. FDA checking imported, domestic shrimp, crabmeat for *Listeria*. Food Chemistry News. August 17, pp 7-8.

Anonymous. 1989. The flurry over *Listeria* in franks. Food Protection Report. 5(6):2-3.

Gellin, B.G. & C.V. Broome. 1989. Listeriosis. Journal of the American Medical Association. 261(9):1313-1320.

Ho, J.H., K.N. Shands and G. Friedland. 1986. An outbreak of type 4b *Listeria monocytogenes* infection involving patients from eight Boston hospitals. Archives of Internal Medicine. 146:520-524.

Hughey, V.L. and E.A. Johnson. 1987. Antimicrobial activity of lysozyme against bacteria involved in food spoilage and food-borne disease. Applied and Environmental Microbiology. 53(9):2165-2170.

King, W., S. Roposa, J. Warshaw, A. Johnson, D. Halbert and J.D. Klinger. 1989. A new colorimetric nucleic acid hybridization assay for *Listeria* in foods. International Journal of Food Microbiology. 8:225-232.

Klinger, J.D., A.Johnson, D. Croan, P. Flynn, K. Whippie, M.Kimball, J. Lawrie and M.Curiale. 1988. Comparative studies of a nucleic acid hybridization assay for *Listeria* in foods. Journal of the Association of Official Analytical Chemists. 71:669-673.

Lamont, R.J. and R. Postlethwaite. 1986. Carriage of *Listeria monocytogenes* and related species in pregnant and non-pregnant women in Aberdeen, Scotland. Journal Infect. 13:187-193.

Lennon, D., B. Lewis, C. Mantell, D. Becroft, B. Dove, K. Farmer, S. Tonkin, N. Yeates R. Stamp & K. Mickleson. 1984. Epidemic perinatal listeriosis. Pediatric Infectious Disease. 3(1):30-34.

Mascola, L., L. Lieb and J. Chiu. 1988. Listeriosis: an uncommon opportunistic infection in patients with acquired immunodeficiency syndrome, a report of five cases and review of the literature. American Journal of Medicine. 84:162-164.

Mattingly, J.A., B.T. Butman, M.C. Plank and R.J. Durham. 1988. A rapid monoclonal antibody-based ELISA for the detection of *Listeria* in food products. Journal of the Association of Official Analytical Chemists 71:679-681.

MMWR. 1988. Update - listeriosis and pasteurized milk. Morbidity and Mortality Weekly Report. 37(49):764-766.

NFI. 1989. Some considerations for control of *Listeria*. National Fisheries Institute. Washington, DC.

Peters, J.B. 1989. *Listeria monocytogenes*.: a bacterium of increasing concern. Washington Sea Grant, Seafood Processing Series. Seattle, Washington.

USFDA. 1987. A brief description of bacteria in the FDA surveillance program. pp 10-11.

USFDA. 1988. Bacteriological Analytical Manual. Chapter 29, *Listeria* isolation, revised method of analysis. In: Federal Register. 53(211): 44148-44153.

B. 01 *Vibrio cholerae*

Description

Vibrio cholerae is an autochthonous bacteria of brackish water, estuaries, and salt marshes of temperate zone coastal areas (Hood and Ness, 1982; Colwell et al., 1981). Unlike the other vibrios, *Vibrio cholerae* (and Vibrio mimicus) does not require salt for growth (Blake, 1983). It is a Gram negative, curved, rod-shaped bacterium which is actively motile (Morris and Black, 1985). It has been suggested that *V. cholerae* exists in association with copepods since the bacteria produce chitinase and exhibit similar seasonal fluctuations (Shandera et al., 1983). Higher densities of *V. cholerae* are recovered during warmer months (Blake, 1983).

Contaminated Species

The following seafoods have been implicated in cases of cholera in the U.S.: oysters (Klontz et al., 1987), crabs (Davis and Sizemore, 1982), and shrimp (MMWR, 1986).

Laboratory experiments, using shellfish naturally infected with Vibrios demonstrate that the bacteria do not depurate well from shellfish (Eyles and Davey, 1984). The following case of cholera in Colorado supports these laboratory results. A Colorado resident become ill with cholera after consuming oysters which were harvested from approved Gulf of Mexico waters, trucked to Colorado, and stored for several days in recirculation, disinfected artificial seawater (MMWR, 1989).

Geographic Area

Outbreaks of cholera have been associated with seafood harvested from the Gulf of Mexico. The type endemic to the Gulf of Mexico (*V. cholerae* 01, serotype Inaba, biotype El Tor), is far less pathogenic than its Asian counterpart (Morris and Black, 1985). The bacterium has been recovered from Chesapeake Bay water (Colwell et al., 1981), although no illness has been reported from this area.

Symptoms & Treatment

Symptoms of cholera can begin within 6 hours to 5 days of contact with bacteria (Morris and Black, 1985). Victims initially experience anorexia, abdominal discomfort and mild diarrhea. As the illness progresses, the symptoms may include: watery diarrhea, often grey in color with mucus (called "rice water"), abdominal cramps, vomiting and dehydration (Shandera et al., 1983; Klontz et al., 1987). Victims may have as many as 16 stools/day.

Ingestion of 10,000 - 100,000,000 *Vibrio cholerae* bacteria has been shown to cause illness in humans (Cash et al., 1979, as cited in Davis and Sizemore, 1982). Susceptibility to cholera is enhanced in persons who have had gastric surgery or take antacids, and person who have type O blood tend to experience more severe cases (Morris and Black, 1985).

El Tor infections (the type endemic to the Gulf of Mexico) are less severe than other strains (Morris and Black, 1985). For every El Tor case which requires hospitalization, there are 40 other milder cases (Bart et al., 1970, as cited in Morris and Black, 1985). Death can occur.

Cholera is treated by aggressive replacement of fluids and electrolytes, orally and/or intravenously.

Statistics

Cholera was first recognized in the U.S. in 1832. Since there were no reported cases of cholera between 1911 and 1973, it was believed to be eradicated. However, in 1973 a case of cholera was reported in the U.S., the first in over 60 years (Shandera et al., 1983). There were 31 cases of seafood-borne cholera reported to the CDC from 1973 to 1986 (Adams et al., 1988). The first outbreak, involving 11 cases, occurred in Louisiana in 1978 and was associated with eating undercooked crabs (Blake et al., 1980, as cited in Shandera et al., 1983).

Detection & Prevention

Vibrio cholerae is a naturally occurring bacterium and is not detected by the presence of traditional indicator bacteria (Hood and Ness, 1982; Colwell et al., 1981). Prevention of illness can be accomplished by cooking seafood thoroughly (Boutin et al., 1982). Freezing is ineffective in killing the bacteria.

Selected Bibliography

Adams, L.B., M.C. Henk and R.J. Siebeling. 1988. Detection of *Vibrio cholerae* with monoclonal antibodies specific for serovar 01 lipopolysaccharide. Journal of Clinical Microbiology. 26(9):1801-1809.

Bart, K.J., A. Huq, M. Khan and W.H. Mosley. 1970. Seroepidemiologic studies during a simultaneous epidemic of infection with El Tor Ogawa and classical Inaba *Vibrio cholerae*. Journal of Infectious Disease. 121:(suppl)517-524.

Blake, P.A. 1983. Vibrios on the half shell: what the walrus and the carpenter didn't know. Annals of Internal Medicine. 99(4):558-559.

Blake, P.A., D.T. Allegra, J.D. Snyder, T.J. Barrett, L. McFarland, C.T. Caraway, J.C. Feely, J.P. Craig. J.V. Lee, N.D. Puhr and R.A. Feldman. 1980. Cholerae - a possible endemic focus in the United States. New England Journal of Medicine. 302:305-309.

Boutin, B.K., J.G. Bradshaw & W.H. Stroup. 1982. Heat processing of oysters naturally contaminated with *Vibrio cholerae* serotype 01. Journal of Food Protection. 45(2):169-171.

Cash, R.A., S.J. Music, M.I. Snyder, P.R. Wenzel and R.B. Hornick. 1979. Response of man to infection with *Vibrio cholerae*: clinical, serologic, and bacteriologic responses to a known inoculum. Journal of Infectious Disease. 29:45-52.

Colwell, R.R., R.J. Seidler, J. Kaper, S.W. Joseph, S. Garges, H. Lockman, D. Maneval, H. Bradford, N. Roberts, E. Remmers, I. Huq & A. Huq. 1981. Occurrence of *Vibrio cholerae* serotype 01 in Maryland and Louisiana estuaries. Applied and Environmental Microbiology. 41(2):555-558.

Davis, J.W. & R.K. Sizemore. 1982. Incidence of *Vibrio* species associated with blue crabs (*Callinectes sapidus*) collected from Galveston Bay, Texas. Applied and Environmental Microbiology. 43(5):1092-1097.

Eyles, M.J. and G.R. Davey. 1984. Microbiology of commercial depuration of the Sydney rock oyster, *Crassostrea commercialis*. Journal of Food Protection. 47(9):703-706.

Hood, M.A. & G.E. Ness 1982. Survival of *Vibrio cholerae* and *Escherichia coli* in estuarine waters and sediment. Applied and Environmental Microbiology. 43(3):578-584.

Hood, M.A., G.E. Ness & G.E. Rodrick. 1981. Isolation of *Vibrio cholerae* serotype 01 from the eastern oyster, *Crassostrea virginica*. Applied and Environmental Microbiology. 41(2):559-560.

Klontz, K.C., R.V. Tauxe, W.L. Cook, W.H. Riley & I.K. Wachsmuth. 1987. Cholera after the consumption of raw oysters: a case report. Annals of Internal Medicine. 107(6):846-848.

MMWR. 1986. Title unknown. 35:606-607.

MMWR. 1989. Title unknown. 38(2):18-19.

Morris, J.G. & R.E. Black. 1985. Cholera and other Vibrios in the United States. New England Journal of Medicine. 312(6):343-350.

Shandera, W.X., B. Hafkin, D.L. Martin, J.P. Taylor, D.L. Maserang, J.G. Wells, M. Kelly, K. Ghandi, J.B. Kaper, J.V. Lee & P.A. Blake. 1983. Persistence of cholera in the United States. American Journal of Tropical Medicine and Hygiene 32(4):812-817.

Simonson, J.G. & R.J. Siebling. 1988. Coagglutination of *Vibrio cholerae*, *Vibrio mimicus* and *Vibrio vulnificus* with anti-flagellar monoclonal antibody. Journal of Clinical Microbiology. 26(10):1962-1966.

C. Non 01 *Vibrio cholerae*

Description

Non-01 *Vibrio cholerae*, a bacterium which primarily causes gastroenteritis, is biochemically similar to the epidemic strains of *V. cholerae*, but does not agglutinate in *V. cholerae* 0-group 1 antiserum (Morris et al., 1981). (This bacterium has also been referred to as, non-agglutinable vibrio and non-cholerae vibrio.) Some strains of non-01 produce an enterotoxin similar to cholerae toxin, and some strains appear to produce more than one toxin (Yasumoto et al., 1983, as cited in Morris and Black, 1985).

Non-01 *V. cholerae* is commonly found in estuaries, bays and brackish waters (Blake et al., 1980; Hood and Ness, 1982). Bacterial numbers usually increase during the summer months (DePaola et al., 1983; Blake et al., 1980). A survey in the Chesapeake Bay recovered non-01 *V. cholerae* from water with salinities between 4 and 17 ppt (Kaper et al., 1979). In contrast, a study of non-01 *Vibrio cholerae* levels in the Gulf of Mexico showed an inverse relationship between salinity and a direct relationship with water temperature (DePaola et al., 1983). In the gulf study, *V. cholerae* was recovered from seawater samples with salinities ranging from 0 to 30 ppt, with highest levels found at salinities less than or equal to 5 ppt.

Contaminated Species

Non-01 *V. cholerae* illness is usually associated with consumption of raw oysters (Morris et al., 1981), and the bacterium has also been isolated from crabs (Davis and Sizemore, 1982). A 1979 Food and Drug Administration study found non-01 *V. cholera* in 14% of the raw oysters screened (Twedt et al., 1981, as cited in Morris and Black, 1985).

Geographic Area

Non-01 *Vibrio cholerae* is primarily found in the Gulf of Mexico (Colwell et al., 1981), but has also been recovered from the Atlantic (Colwell, et al., 1981) and Pacific Oceans (Blake et al., 1980).

Symptoms & Treatment

Non-01 *V. cholerae* primarily causes gastroenteritis. Symptoms can begin within 48 hours of consuming contaminated shellfish (Blake et al., 1908). The most common symptoms include: diarrhea (as many as 20-30 stools/day), abdominal cramps and fever. Nausea, vomiting and bloody diarrhea have also been reported (Morris et al., 1981; Morris and Black, 1985; Blake et al., 1980). Symptoms can persist for 2 to 12 days (Morris et al., 1981). Patients with toxigenic isolates have more severe illness that nontoxigenic isolates. Most strains isolated from ill persons in the U.S. are nontoxigenic (Morris et al., 1981). Gastroenteritis is treated by oral and/or intravenous rehydration.

Non-01 *V. cholerae* can also cause septicemia and wound and ear infections (Blake et al., 1980). Cases of septicemia usually involve individuals with a preexisting immunocompromising disease. Although the significance is unknown, non-01 *V. cholerae* has been isolated from a number of human sources other than feces including: bile/gallbladder, sputum, appendix, peritoneal fluid, and cerebrospinal fluid (Blake et al., 1980).

Statistics

In 1979, there were 9 cases of seafood-borne non-01 *Vibrio cholerae* gastroenteritis acquired in the U.S. reported to the CDC (Morris et al., 1981).

Detection & Prevention

Since non-01 *V. cholerae* is a naturally occurring bacterium, it cannot be detected by the presence of traditional indicator species (Hood and Ness, 1983; Colwell et al., 1981; Eyles and Davey, 1984). Illness can be prevented by thoroughly cooking shellfish. Freezing shellfish before consumption is ineffective in preventing illness.

Selected Bibliography

Blake, P.A., R.E. Weaver & D.G. Hollis. 1980. Diseases of humans (other than cholera) caused by Vibrios. Annual Review of Microbiology. 34:341-367.

Colwell, R.R., R.J. Seidler, J. Kaper, S.W. Joseph, S. Garges, H. Lockman, D. Maneval, H. Bradford, N. Roberts, E. Reemers, I. Huq and A. Huq. 1981. Occurrence of *Vibrio cholerae* serotype 01 in Maryland and Louisiana estuaries. Applied and Environmental Microbiology. 41(2):555-558.

Davis, J.W. and R.K. Sizemore. 1982. Incidence of Vibrio species associated with blue crabs (Callinectes sapidus) collected from Galveston Bay, Texas. Applied and Environmental Microbiology. 43(5):578-584.

DePaola, A., M.W. Presnell, M.L. Motes, R.M. McPhearson, R.M. Twedt, R.E. Becker & S. Zywno. 1983. Non-01 *Vibrio cholerae* in shellfish, sediment and waters of the U.S. Gulf coast. Journal of Food Protection. 46(9):802-806.

Eyles, M.J. and G.R. Davey. 1984. Microbiology of commercial depuration of the Sydney rock oyster, Crassostrea commercialis. Journal of Food Protection. 47(9):703-706.

Hood, M.A. and G.E. Ness. 1982. Survival of *Vibrio cholerae* and Escherichia coli in estuarine waters and sediment. Applied and Environmental Microbiology. 43(3):578-584.

Kaper, S.H., H. Lockman, R.R. Colwell and S.W. Joseph. 1979. Ecology, serology and enterotoxin production of *Vibrio cholerae* in Chesapeake Bay. Applied and Environmental Microbiology. 37:91-102.

Morris, J.G. & R.E. Black. 1985. Cholera and other Vibrios in the United States. New England Journal of Medicine. 312(6):343-350.

Morris, J.G., R. Wilson, B.R. Davis, I.K. Wachsmuth, C.F. Riddle, H.G. Wathen, R.A. Pollard & P.A. Blake. 1981a. Non-0 group 1 *Vibrio cholerae* gastroenteritis in the United States. Annals of Internal Medicine. 94(5):656-658.

Twedt, R.M., J.M. Madden, J.M. Hunt et al. 1981. Characterization of *Vibrio cholerae* isolated from oysters. Applied and Environmental Microbiology. 41:1475-1478.

Yasumoto, K., Y. Takeda, T. Miwatani and J.P. Craig. 1983. Evidence that a non-01 *Vibrio cholerae* produces enterotoxin that is similar but not identical to *cholerae* enterotoxin. Infect. Immunity. 41:896-901.

D. *Vibrio parahaemolyticus*

Description

Vibrio parahaemolyticus is part of the normal flora of estuarine and other coastal areas throughout most of the world. The optimal temperature for growth of *V. parahaemolyticus* is 37°C, although it will grow well at 25-44°C (Blake et al., 1980).

In most areas, bacterial densities increase during the warmer months, and as a result, most outbreaks of *V. parahaemolyticus* illness in the U.S. occur during the summer (Watkins and Cabelli, 1985). Seasonal variation of *V. parahaemolyticus* in the Gulf of Mexico is not as evident. Studies which investigated *V. parahaemolyticus* levels in Galveston Bay blue crabs (Davis and Sizemore, 1980) and Louisiana oysters (Paille et al., 1987) showed increased concentrations during the summer months. However, seasonal variation was not observed in *V. parahaemolyticus* levels in Galveston Bay oysters (Thompson and Vanderzant, 1976a).

Pathogenic strains of *V. parahaemolyticus* cause hemolysis on Wagatsuma agar (the Kanagawa phenomenon). It has been reported that over 95% of the isolates from individuals with gastroenteritis are hemolytic, or Kanagawa positive (Joseph et al., 1983, as cited in Morris and Black, 1985). However, only 0.18% (Thompson and Vanderzant, 1976b) to 1% (Joseph et al., 1983, as cited in Morris and Black, 1985) of the environmental isolates are K+. A number of theories have been suggested to explain the greater proportion of K+ strains from gastroenteritis isolates than from environmental isolates. It is possible that the present isolation methods do not favor the detection of K+ strains, and are therefore underestimating the number of hemolytic strains in the environment (Hackney et al., 1980). It has been suggested that a small number of pathogenic strains exist in the environment among a large number of nonpathogenic strains (Nolan et al., 1984; Thompson and Vanderzant, 1976b). However, a study which investigated the survival patterns of K- and K+ strains of *V. parahaemolyticus* in the environment found no selective advantage of K- strains in the natural environment (Karunasagar et al., 1987). And finally, the organisms may acquire the hemolysin(s) in the intestinal tract of humans (Thompson and Vanderzant, 1976b). However, studies in which human volunteers ingested K- strains of *V. parahaemolyticus* did not become ill with gastroenteritis (Senyal and Sen, 1974, as cited in Thompson and Vanderzant, 1976b).

The generation time of *V. parahaemolyticus* has been reported to be as short as nine minutes under ideal conditions (Katoh, 1965, as cited in Blake et al., 1980). Barker (1974, as cited in Bachman et al., 1983) calculated that at this rapid rate of replication, 10 bacteria would lead to 1 million bacteria within 3 to 4 hours.

Contaminated Species

V. parahaemolyticus illness has been associated with consuming contaminated crabs, oysters, shrimp and lobster (Thompson and Vanderzant, 1976a). One outbreak of *V. parahaemolyticus* gastroenteritis was traced to depurated oysters (Barrow and Miller, 1969, as cited in Richards, 1988), supporting the laboratory evidence that vibrio bacteria do not depurate well (Eyles and Davey, 1984).

Geographical Area

V. parahaemolyticus has been isolated from the Atlantic (Watkins and Cabelli, 1985; Hackney, et al., 1980), Pacific (Nolan et al., 1984) and Gulf Coasts (Thompson and Vanderzant, 1976a).

Symptoms & Treatment

Gastroenteritis caused by *V. parahaemolyticus* is generally mild to moderate in severity. The onset of symptoms is usually within 4 to 96 hours of consuming contaminated seafood (Morris and Black, 1985). The most commonly experienced symptoms include: diarrhea, abdominal cramps, nausea, vomiting and headache. Fever and chills are less frequently reported (Bryan, 1987; Morris and Black, 1985; Blake et al., 1980). Gastroenteritis caused by *V. parahaemolyticus* is usually a self-limited illness, lasting a median of 3 days (Morris and Black, 1985).

Vibrio parahaemolyticus can also cause septicemia, and ear and wound infections (Blake et al., 1980). The one reported case of septicemia involved an individual who had a preexisting immunocompromising disease (cirrhosis).

Statistics

V. parahaemolyticus was first recognized as pathogen in Japan in the early 1950's (Blake et al., 1980). In 1969, there were several unconfirmed outbreaks of gastroenteritis in the U.S. that were thought to be caused by *V. parahaemolyticus* (USDHEW, 1969, as cited in Thompson and Vanderzant, 1976a). From 1977 to 1981 there were nine outbreaks of seafood-borne *V. parahaemolyticus* illness reported to the CDC (USFDA, 1984).

Detection & Protection

Traditional indicator species do not accurately detect the presence of *V. parahaemolyticus*, since it is a naturally occurring bacterium (Hackney et al., 1980; Thompson and Vanderzant, 1976a). Illness can be prevented by thoroughly cooking shellfish and by bandaging open wounds to prevent exposure to seawater.

Selected Bibliography

Bachman, B., W.P. Boyd, S. Lieb & G.E. Rodrick. 1983. Marine noncholera *Vibrio* infections in Florida. Southern Medical Journal. 76(3):296-299.

Barker, W.H. 1974. *Vibrio parahaemolyticus* outbreaks in the United States. Lancet. 1:551-554.

Barrow, G.I. and D.C. Miller. 1969. Marine bacteria in oysters purified for human consumption. Lancet. 2:421-423.

Blake, P.A., R.E. Weaver & D.G. Hollis. 1980. Diseases of humans (other than cholera) caused by Vibrios. Annual Review of Microbiology 34:341-367.

Boutin, B.K., A.L. Reyes, J.T. Peeler & R.M. Twedt. 1985. Effect of temperature and suspending vehicle on survival of *Vibrio parahaemolyticus* and *Vibrio vulnificus*. Journal of Food Protection. 48(10):875-878.

Bryan, F.L. 1986. Seafood-transmitted infections and intoxications in recent years. In: D.E. Kramer and J. Liston (eds), "Seafood Quality Determinations", Elsevier Science Publishing. pp. 319-337.

Davis, J.W. & R.K. Sizemore. 1982. Incidence of *Vibrio* species associated with blue crabs (*Callinectes sapidus*) collected from Galveston Bay, Texas. Applied and Environmental Microbiology. 43(5):1092-1097.

Eyles, M.J. and G.R. Davey. 1984. Microbiology of commercial depuration of the Sydney rock oyster, *Crassostrea commercialis*. Journal of Food Protection. 47(9):703-706.

Hackney, C.R., B. Ray & M.L. Speck. 1980. Incidence of *Vibrio parahaemolyticus* in the microbiological quality of seafood in North Carolina. Journal of Food Protection. 43(10):769-773.

Joseph, S.W., R.R. Colwell and J.B. Kaper. 1983. *Vibrio parahaemolyticus* and related halophilic vibrios. CRC Critical Reviews of Microbiology. 10:77-124.

Kaneko, T. & R.R. Colwell. 1975. Adsorption of *Vibrio parahaemolyticus* onto chitin and copepods. Applied Microbiology. 29(2):269-274.
Karunasagar, I., I. Karunasagar, M.N. Venugopal & C.N. Nagesha. 1987. Survival of *Vibrio parahaemolyticus* in estuarine and sea water and in association with clams. Systematic Applied Microbiology. 9:316-319.
Katoh, H. 1965. Japanese Journal of Bacteriology. 20:94-99.
Morris, J.G. & R.E. Black. 1985. Cholera and other Vibrios in the United States. New England Journal of Medicine. 312(6):343-350.
Nolan, C.M., J. Ballard, C.A. Kaysner, J.L. Lilja, L.P. Williams Jr, and F.C. Tenover. 1984. *Vibrio parahaemolyticus* gastroenteritis: an outbreak associated with raw oysters in the Pacific Northwest. Diagnostic Microbiology and Infectious Disease. 2:119-128.
Richards, G.P. 1988. Microbial purification of shellfish: a review of depuration and relaying. Journal of Food Protection. 51(3):218-251.
Paille, D., C. Hackney, L. Reily, M. Cole and M. Kilgen. 1987. Seasonal variation in the fecal coliform population of Louisiana oysters and its relationship to microbiological quality. Journal of Food Protection. 50(7):545-549.
Sanyal, S.C. and P.C. Sen. 1974. Human volunteer study on the pathogenicity of *Vibrio parahaemolyticus*. In: T. Fujino, G. Sakaguchi, R. Sakazaki and Y. Takeda (eds), "International Symposium on *Vibrio parahaemolyticus*". Saikon Publishing Company, Tokyo. p. 227.
Thompson, C.A. Jr., C. Vanderzant. 1976. Effect of processing, distribution and storage on *Vibrio parahaemolyticus* and bacterial counts of oysters (*Crassostrea virginica*). Journal of Food Science. 41:123-127.
Thompson, C.A. Jr., C. Vanderzant. 1976. Relationship of *Vibrio parahaemolyticus* in oysters, water and sediment, and bacteriological and environmental indices. Journal of Food Science. 41:118-122.
Thompson, C.A. Jr., C. Vanderzant. 1976. Serological and hemolytic characteristics of *Vibrio parahaemolyticus* from marine sources. Journal of Food Protection. 41:204-205.
USDHEW. 1969. Foodborne outbreaks. Annual Summary. Centers for Disease Control. Atlanta, GA.
USFDA. 1984. Bacteriological analytical methods, 6th edition. Association of Official Analytical Chemists. Arlington, VA. p. 28.03.
Watkins, W.D. & V.J. Cabelli. 1985. Effect of fecal pollution on *Vibrio parahaemolyticus* densities in an estuarine environment. Applied and Environmental Microbiology. 49(5):1307-1313.

E. *Vibrio vulnificus*

Description

Vibrio vulnificus, originally thought to be *V. parahaemolyticus*, is a naturally occurring, lactose fermenting bacterium. It requires salt and is commonly isolated at salinities of 7-16 ppt (Kelly, 1982). Sampling in the Gulf of Mexico showed that the organism is seldom found in water temperature <25°C, and that the incidence of recovery increases steadily as water temperatures rise. Highest densities in the Gulf are found after water temperatures exceed 25°C for several months. Laboratory studies demonstrate an optimal growth temperature of 37°C (Kelly, 1982).

Contaminated Species

Cases of *V. vulnificus* sepsis have been associated with the consumption of oysters and blue crabs (Blake et al., 1980).

Geographic Area

Vibrio vulnificus is primarily found in the Gulf of Mexico (Kelly, 1982), but has also been isolated from the Atlantic (Oliver et al., 1983, as cited in O'Neill et al., in press) and Pacific Oceans (Kaysner et al., 1987, as cited in O'Neill et al., in press). In the Gulf, cell densities are highest during the warmer months, usually April through October (Blake, 1983; Kelly, 1982). The organism has been recovered from shellfish harvested as far north as Maine (O'Neill, et al., in press).

Symptoms & Treatment

Vibrio vulnificus can cause sepsis in individuals who consume contaminated shellfish, and can also cause wound infections in individuals who expose open sores to contaminated water.

Symptoms of septicemia usually begin within 24 - 48 hours of consuming contaminated seafood (Morris and Black, 1985). The most common symptoms include: bullous skin lesions (>70% of patients have lesions, Morris and Black, 1985), fever,

chills and nausea (Bachman et al., 1983; Tacket et al., 1984). Hypotension, abdominal pain, vomiting and diarrhea are less frequently reported. The mortality rate in various studies ranges from 46% (Blake et al., 1980) to 61% (Tacket et al., 1984).

Most patients with sepsis are either immunocompromised (75%, Oliver, 1981; Bachman et al., 1983; Blake et al., 1980; Morris and Black, 1985), and/or male (72%, Tacket et al., 1985; to 90%, Oliver, 1981). The following immunocompromising conditions make individuals more susceptible to sepsis:

- Liver disease (cirrhosis and haemchromatosis)
- Alcohol abuse
- Cancer (especially persons treated with anticancer drugs and radiation)
- Diabetes mellitus
- Chronic kidney disease
- Inflammatory bowel disease (especially persons treated with immunosuppressive drugs)
- Steroid dependency (for treatment of asthma)
- Achlorhydria (condition in which normal stomach acidity is reduced or absent)
- AIDS
- Pregnancy
- Epilepsy

It has been suggested that the increased susceptibility among persons with liver disease could be caused by an increase in iron stores, commonly found in patients with alcohol-related liver disease (Tacket et al., 1984; Morris and Black, 1985). Furthermore, women may have lower iron stores than men, which may explain why men are more commonly affected than women (Tacket et al., 1984). This hypothesis was supported in laboratory experiments in which the median lethal dose of *V. vulnificus* decreased from 1 million cells to slightly >1 cell in iron loaded mice (Wright et al., 1981, as cited in Morris and Black, 1985).

V. vulnificus may also cause septicemia in individuals who have not consumed shellfish. In one case, a man who nearly drowned in the Gulf of Mexico developed pneumonia and died from a lactose + *Vibrio*, indicating that septicemia may develop via the lungs (Kelly and Avery, 1980). A second case involved a 3 day old infant who acquired gastroenteritis and tested positive for *V. vulnificus* (Bachman et al., 1983). It is possible that the illness was transmitted to the infant by the mother, who had a mild flu-like illness 72 hours before delivery but no definite infection was identified.

Individuals who expose cuts, sores, burns and abrasions to contaminated seawater are at risk of developing wound infections. Symptoms usually within 12 hours of contact with the water (Blake et al., 1980; Oliver 1981). Wound infections commonly cause fever, chills, inflammation and occasionally, gastroenteritis (Tacket et al., 1984). The mortality rate for individuals with wound infections is approximately 7% (Blake et al., 1980).

Statistics

There were 32 *Vibrio vulnificus* isolates received by the CDC between 1981 and 1982; 18 were primary sepsis, 9 were wound infections, and 3 were cellulitis with no apparent wounds (Tacket et al., 1984).

In the U.S., most cases occur when water temperature is warm, from May to October (Blake et al., 1980; Blake, 1983; Bachman et al., 1983; Tacket et al., 1984).

Detection & Protection

Vibrio vulnificus is a naturally occurring bacterium which is not found in association with sewage, and therefore is not detected by the presence of traditional indicator bacteria. Infection can be prevented by thorough cooking of shellfish. Individuals in the "high risk" should be especially careful to cook shellfish properly and should avoid exposing open wounds to seawater.

Selected Bibliography

Bachman, B., W.P. Boyd, S. Lieb & G.E. Rodrick. 1983. Marine noncholera *Vibrio* infections in Florida. Southern Medical Journal. 76(3):296-299.

Blake, P.A., R.E. Weaver & D.G. Hollis. 1980. Diseases of humans (other than cholera) caused by Vibrios. Annual Review of Microbiology. 34:341-367.

Boutin, B.K., A.L. Reyes, J.T. Peeler & R.M. Twedt. 1985. Effect of temperature and suspending vehicle on survival of *Vibrio parahaemolyticus* and *Vibrio vulnificus*. Journal of Food Protection. 48(10):875-878.
Davis, J.W. & R.K. Sizemore. 1982. Incidence of *Vibrio* species associated with blue crabs (*Callinectes sapidus*) collected from Galveston Bay, Texas. Applied and Environmental Microbiology. 43(5):1092-1097.
Kaysner, C.A. et al., 1987. Virulent strains of *Vibrio vulnificus* form estuaries of the United States West Coast. Applied and Environmental Microbiology. 53:1349-1351.
Kelly, M.T. 1982. Effect of temperature and salinity on *Vibrio* (*Beneckea*) *vulnificus* occurrence in the Gulf Coast environment. Applied and Environmental Microbiology. 44(4):820-824.
Kelly, M.T. & D.M. Avery. 1980. Lactose-positive *Vibrio* in seawater: a cause of pneumonia and septicemia in a drowning victim. Journal of Clinical Microbiology. 11(3):278-280.
Oliver, J.D. 1981. The pathogenicity and ecology of *Vibrio vulnificus*. Marine Technology Society Journal. 15(2):45-52.
Morris, J.G. & R.E. Black. 1985. Cholera and other Vibrios in the United States. New England Journal of Medicine. 312(6):343-350.
Oliver, J.D., R.A. Warner and D.R. Cleland. 1983. Distribution of *Vibrio vulnificus* and other lactose-fermenting vibrios in the marine environment. Applied and Environmental Microbiology. 45:985-998.
O'Neill, K.R. S.H. Jones, T.L. Howell and D.J. Grimes. in press. Occurrence of *Vibrio vulnificus* in water and shellfish from Maine and New Hampshire. In: Proceedings of the First International Conference on Molluscan Shellfish Depuration. Orlando, FL. November 5-9.
Tacket, C.O., F. Brenner & P.A. Blake. 1984. Clinical features and an epidemiological study of *Vibrio vulnificus* infections. Journal of Infectious Diseases. 149(4):558-561.
Wright, A.C., L.M. Simpson and J.D. Oliver. 1981. Role of iron in the pathogenesis of *Vibrio vulnificus* infections. Infectious Immunology. 34:503-507.

F. *Vibrio mimicus*

Description

Vibrio mimicus was originally misidentified as *V. cholerae*. The bacteria differs from *V. cholerae* in its inability to ferment sucrose. *V. mimicus* is gram-negative, oxidase positive, nonhalophilic and motile by a single flagellum (Shandera et al., 1983). It is most likely a part of the normal marine flora of the Atlantic and Gulf Coasts. Unlike the other Vibrios, *V. mimicus* (and *V. cholerae*) do not require salt for growth (Blake 1983).

Vibrio mimicus can cause both gastroenteritis and ear infections. Gastrointestinal illness is associated with consumption of raw oysters and boiled crawfish (Shandera et al., 1983). Ear infections are associated with seawater exposure. The median time of gastroenteritis onset is 24 hours (Shandera et al., 1983). Diarrhea, nausea, vomiting and abdominal cramps are the most commonly reported symptoms (Morris and Black, 1985). Some infected individuals have also experienced fever, headache and bloody diarrhea. Diarrhea lasts a median of 6 days. Between 1977 and 1981 there were 21 cases (19 gastroenteritis and 2 ear infections) reported to the CDC.

Selected Bibliography

Blake, P.A. 1983. Vibrios on the half shell: what the walrus and the carpenter didn't know. Annals of Internal Medicine. 99(4):558-559.
Morris, J.G. & R.E. Black. 1985. Cholera and other Vibrios in the United States. New England Journal of Medicine. 312(6):343-350.
Shandera, W.X., J.M. Jeffrey, M. Johnston, B.R. Davis & P.A. Blake. 1983. Disease from infection with *Vibrio mimicus*, a newly recognized Vibrio species. Annals of Internal Medicine. 99(2):169-171.

G. *Vibrio hollisae*

Description

Vibrio hollisae previously belonged to enteric group EF-13. It is a naturally occurring, halophilic bacteria. No environmental isolates of *V. hollisae* have been found, although illness is associated with consumption of raw oysters, clams and shrimp (Morris et al., 1982). Between 1971 and 1981, 15 cases of illness were reported to the CDC (Morris et al, 1982). The most common symptoms of *V. hollisae* infection are diarrhea, vomiting, fever and abdominal pain (Blake, 1983; Morris and Black, 1985). Symptoms usually begin within 5 days of ingestion and persist for one day (range: 4 - 13 days).

Selected Bibliography

Morris, J.G. & R.E. Black. 1985. Cholera and other Vibrios in the United States. New England Journal of Medicine. 312(6):343-350.

Morris, J.G., R. Wilson, D.G. Hollis, R.E. Weaver, H.G. Miller, C.O. Tacket, F. W. Hickman, P.A. Blake. 1982. Illness caused by *Vibrio damsela* and *Vibrio hollisae*. Lancet. 1294-1296.

III. Toxins

A. Ciguatera

Description

Ciguatera is the most commonly reported disease associated with consumption of seafood (Morris, 1980). Between 1977 and 1981, 37% of the seafood-borne illness reported to the CDC were attributed to ciguatera (USFDA, 1984). Approximately 80% of the cases are due to weekend fishermen who are unfamiliar with the types of fish commonly ciguatoxic.

By ingesting toxic dinoflagellates, certain species of tropical and subtropical fish can become toxic to humans. The dinoflagellate species most often associated with ciguateric fish is *Gambierdiscus toxicus* (Yasumoto et al., 1977; Adachi and Fukuyo, 1979 as cited in Taylor, 1979). Other algal species which cause ciguatera include *Prorocentrum mexicanum*, *P. concavum*, *P. lima*, and *Ostreopsis lenticularis* (Carlson and Tindall, 1985).

The conditions best suited for toxic dinoflagellate growth are not well understood. There have been a number of conflicting observations concerning optimal growth conditions, some of which are as follows:

(1) *G. toxicus* is negatively influenced by land runoff and high light intensity (Yasumoto et al., 1980 as cited in Carlson and Tindall, 1985).

(2) Dinoflagellates replicate rapidly when disturbed, such as, after major storms, and areas of construction or dredging (Craig, 1980).

(3) Highly toxic sites seem to be toward the leeward side of islands.

(4) Populations of dinoflagellates are affected by rainfall in varying degrees. Moderate rainfall may promote dinoflagellate growth by increasing dissolved nutrients in the water, through increased terrestrial runoff. However, heavy rainfall may inhibit growth by increasing dilution and/or turbidity (Carlson and Tindall, 1985).

(5) *G. toxicus* may specifically associate with macroalgae where high concentrations of nutrients are available for growth (Carlson and Tindall, 1985).

There are at least four known toxins which appear to be concentrated in the viscera, head or central nervous system of affected fish (Tosteson et al., 1988): ciguatoxin, scaritoxin, maitotoxin and ciguaterin. Ciguatoxin, the principal toxin, is lipid soluble (Kantha, 1987). Studies indicate that oral intake of as little as 0.1ug (11MU) of ciguatoxin could cause illness in an adult human (Yasumoto, 1985). Maitotoxin is water soluble and approximately three times more toxic than ciguatoxin (Yasumoto, 1985).

Contaminated Species

Tropical and subtropical coral reef fish can become ciguatoxic. The incidence of poisonous fish, however, is sporadic. All fish of the same variety and caught in the same area may not necessarily be toxic (Hokama et al., 1983). A study done in Hawaii indicated that if fish in one location are toxic, other fish in the vicinity are approximately 60% likely to be toxic. Both herbivorous and carnivorous fish can become toxic. Herbivorous fish become toxic by eating the toxic algae itself. Carnivorous fish become toxic by consuming toxic herbivorous fish. Generally, large fish are more poisonous than small fish because they consume greater amounts of the toxins (Craig, 1980). The fish most often implicated in cases of ciguatera include: barracuda (Olson et al., 1984 as cited in Tosteson et al., 1988); grouper (Craig, 1980; Lawrence et al., 1980); snapper

(Craig, 1980; Lawrence et al., 1980); surgeon fish (Miyahara et al., 1987); jack (Craig, 1980; Hokama et al., 1983; Miyahara et al., 1987); and parrot fish (Bryan, 1986; Bryan, 1988).

Geographic Area

Ciguatera is found world-wide in fish between 35N and 34S latitude (Craig, 1980). It is a problem in the South Pacific, Japan Islands, U.S. and Bahamas. The only areas of the U.S. affected by ciguatera are: Florida, Hawaii, Puerto Rico and the U.S. Virgin Islands. There is evidence that ciguatoxic dinoflagellate populations experience seasonal fluctuations. In Hawaii ( ) and the Virgin Islands (Carlson and Tindall, 1985), the algae exhibits a bimodal pattern of abundance with population maxima occurring in conjunction with the peak periods of rainfall; April to May, and August to October. Studies in Puerto Rico indicate that populations of *Ostreopsis lenticularis* and *Gambierdiscus toxicus* experience a seasonal trend, although densities are highly variable (Ballantine et al., 1988). Peak populations tend to occur during the late summer and fall, and do not appear to be correlated to rainfall. Although additional data are needed, there appears to be a seasonal fluctuation in the toxicity of *Ostreopsis* as well. In a three year period in Puerto Rico, toxicity of *Ostreopsis* ranged from nontoxic to 182 MU/1,000,000 cells. In two of the three years, peak toxicities occurred in October.

Symptoms & Treatment

Ciguatera exhibits both gastrointestinal and neurological symptoms (Lawrence et al., 1980). The time of onset is usually less than 24 hours. Gastrointestinal symptoms, which usually persist for 12 hours (range < 1 hour - 7 days), include: diarrhea, abdominal pain, nausea and vomiting. The most common neurological symptoms include: paresthesia (abnormal or impaired skin sensations), vertigo, ataxia (lack of muscle coordination), cold-to-hot sensory reversal, myalgia (muscular pain), itching (especially during any activity that increases skin temperature and blood flow). Neurological symptoms may recur intermittently with gradually diminishing severity for a long as six months. No deaths have been reported from ciguatera in the U.S. (Morris, 1980), although world-wide the mortality rate of ciguatera is 7-20% (Craig, 1980).

Ciguatera from consumption of herbivorous fish has reportedly been associated with more severe gastrointestinal complaints, whereas neurological and cardiovascular effects often predominate in poisoning by carnivores (Bagnis, 1968 as cited in Miyahara et al., 1987). This observation was supported in a study which demonstrated that different species of ciguatoxic fish accumulate different toxins (Miyahara et al., 1987).

Statistics

World-wide, there may be as many as 50,000 cases of ciguatera per year (Ragelis, 1984). In the U.S., between 1970 and 1980, 94 outbreaks (418 cases) of ciguatera were reported to the CDC, making it the most frequently reported food-borne illness associated with consumption of seafood (Morris, 1980).

Detection & Prevention

MOUSE BIOASSAY (Kimura et al., 1982) - For lack of a better technique, the mouse bioassay is currently the laboratory method used to detect ciguatera. Concentrated lipid extracts of fish tissue are injected intraperitoneally into a 20 g mouse and the mouse is observed for toxic symptoms for 24 - 48 hours. Listed below are a number of general disadvantages of the mouse bioassay to detect marine toxins:

- Need to maintain a mouse colony and have 19 - 22 g mice always available, - limit of sensitivity dependent on mouse strain,
- the onset of toxic symptoms is subjective,
- high incidence of false positives due to other contaminants,
- assay is not linear,
- labor intensive and expensive,
- cannot be used in the field,
- the use of mammals in experiments is becoming controversial with the public,
- poor sensitivity,

A number of other laboratory methods have been suggested as a replacement for the mouse bioassay and are described below:

MOSQUITO BIOASSAY (Chungue et al., 1984) - Toxins are extracted from fish and injected intrathoracically into mosquitoes. The mosquitoes are observed for one hour for signs of death. This technique requires only a small amount of fish tissue and results can be obtained within 2 hours.

IN VITRO GUINEA PIG ATRIUM ASSAY (Miyahara et al., 1979 as cited in Kimura et al., 1982) - Crude lipid extracts of fish are added to an isolated guinea pig atrium. The effects are expressed as the ratio of the amplitude of contraction occurring after the addition of extract as compared to the initial amplitude of untreated atrium.

"STICK" TEST (Hokoma et al., 1985; Hokoma et al., 1987a; Hokoma et al., 1987b; Hokoma et al., 1989) - Bamboo sticks, pretreated to help adsorb the toxin, are stuck into the fish flesh for 1 second. The sticks are then fixed with methyl alcohol and immersed in a solution of blue latex beads and ciguatoxin antibody. A positive result will change the bamboo stick to a dark-blue or purple color within 10 minutes. This procedure does not require extraction of tissue (although it is capable of testing extracted tissue), gives rapid results, and is inexpensive.

RADIOIMMUNOASSAY (Hokama et al., 1977; Kimura et al., 1982) - Sheep anti-ciguatoxin serum coupled with iodine-125 is added to a sample of fish tissue extract. Excess antibody is removed and the samples are analyzed with a scintillation counter. If ciguatoxin is present in the fish flesh, the DPM will be high. If the fish is free of toxin the DPM will be low. This procedure is sensitive and relatively specific, however, it is economically unfeasible for testing fish weighing <9 kg (Hokama et al., 1983).

ENZYME-IMMUNOASSAY (Hokama et al., 1983) - Sheep anti-ciguatoxin serum coupled to horseradish peroxidase is added to a sample of fish tissue extract and incubated at room temperature for 1 hour. The amount of toxin in the tissue is determined by measuring the absorbance at 405 nm.

Ciguatera toxins impart no unusual tastes, odor or color to the fish (Craig, 1980) and ciguateric fish cannot be made safe to eat by cooking, freezing, drying or smoking (Tosteson et al., 1988). Listed below are methods which have been suggested to avoid ciguatera:

- Avoid types of fish often contaminated with toxins. (Because barracuda has been frequently associated with ciguatera, Miami City Code prohibits the sale of barracuda.)
- Avoid eating large fish of the variety that are potentially toxic (Lawrence, 1980).
- Avoid eating the viscera and roe of all reef fishes especially during the reproductive season (Craig, 1980).
- Eat a small piece of the fish and wait several hours in order to determine if any signs of poisoning occur before consuming the whole fish.
- There is some evidence that washing (leaching) the flesh of toxic fish effectively removes some of the ciguaterins (Deichmann, 1977 as cited in Kantha, 1987).
- Persons affected once should avoid eating potentially toxic fish for several months because a second episode might be more severe. Repeated exposure may cause extreme sensitivity to the toxin resulting in the onset of symptoms even when fish containing only trace amounts of toxin are consumed (Lawrence, 1980).

Selected Bibliography

Adachi, R. and Y. Fukuyo. 1979. The thecal structure of a marine toxic dinoflagellate *Gambierdiscus toxicus* gen. et sp. nov. collected in a ciguatera endemic area. Bull. Japan. Soc. Sci. Fish. 45:67-71.

Bagnis, R. 1968. Clinical aspects of ciguatera (fish poisoning) in French Polynesia. Hawaii Medical Journal. 28:25.

Ballantine, D.L., T.R. Tosteson, A.T. Bardales. 1988. Population dynamics and toxicity of natural populations of benthic dinoflagellates in southwestern Puerto Rico. Journal of Experimental Marine Biology and Ecology. 119:201-212.

Bryan, F.L. 1986. Seafood-transmitted infections and intoxications in recent years. In: D.E. Krammer and J. Liston (eds), "Seafood Quality Determination", Elsevier/Amsterdam, pp. 319-337.

Bryan, F.L. 1988. Risks associated with vehicles of foodborne pathogens and toxins. Journal of Food Protection. 51(6)498-508.

Campbell, B., L.K. Nakagawa, M.N. Kobayashi & Y. Hokama. 1987. *Gambierdiscus toxicus* in gut content of the surgeonfish *Ctenochaetus strigosus* (herbivore) and its relationship to toxicity. Toxicon. 25(10):1125-1127.

Carlson, R.D. & D.R. Tindall. Distribution and periodicity of toxic dinoflagellates in the Virgin Islands. In: Anderson, D.M., A.W. White & D.G. Baden (eds), "Toxic Dinoflagellates, Proceedings of the Third International Conference", Elsevier/North Holland, New York, pp. 171-176.

Chungue, E., R. Bagnis & F. Parc. 1984. The use of mosquitoes (*Aedes aegypti*) to detect ciguatoxin in surgeon fishes (*Ctenochaetus striatus*). Toxicon. 22(1):161-164.

Craig, C.P. 1980. It's always the big ones that should get away. Journal of the American Medical Association. 244(3):272-273.

Deichmann, W.B., W.E. MacDonald, D.A. Cubit, C.E. Wunsch, J.E. Bartels and F.R. Merritt. 1977. Pain in jawbones and teeth in ciguatera intoxications. Florida Scientist. 40:227-238.

Hokama, Y., A.H. Banner and D.B. Boylan. 1977. A radioimmunoassay for the detection of ciguatoxin. Toxicon. 15:317.

Hokama, Y., M.A. Abad & L.H. Kimuar. 1983. A rapid enzyme-immunoassay for the detection of ciguatoxin in contaminated fish tissues. Toxicon. 21(6):817-824.

Hokama, Y., A.M. Osugi, S.A.A. Honda & M.K. Matsuo. 1985. Monoclonal antibodies in the detection of ciguatoxin and other toxic polyethers in fish tissues by a rapid poke stick test. Proceedings of the Fifth International Coral Reef Congress, Tahiti. 4:449-455.

Hokama, Y., L.K. Shirai, L.M. Iwamoto, M.N. Kobayashi, C.S. Goto & L.K. Nakagawa. 1987a. Assessment of a rapid enzyme immunoassay stick test for the detection of ciguatoxin and related polyether toxins in fish tissues. Biology Bulletin 172:144-153.

Hokama, Y., S. Honda, M. Kobayashi, L. Nakagawa, J. Kurihara and J. Miyahara. 1987b. Monoclonal antibodies in the detection of ciguatoxin (CTX) and related polyethers in contaminated fish tissues. In: P. Gopalakrishnakone and C.K. Tan (eds), "Progress in Venom and Toxin Research", National University of Singapore, pp. 385-393.

Hokama, Y., S.A.A Honda, M.N. Kobayashi, L.K. Nakagawa, A.A. Asahina and J.T. Miyahara. 1989. Monoclonal antibody (MAb) in detection of ciguatoxin (CTX) and related polyethers by the stick-enzyme immunoassay (S-EIA) in fish tissues associated with ciguatera poisoning. In: IUPAC, Tokyo Japan, Elsevier/Amsterdam, In Press.

Kimura, L.H., Y. Hokama, M.A. Abad, M. Oyama & J.T. Miyahara. 1982. Comparison of three different assays for the assessment of ciguatoxin in fish tissues: radioimmunoassay, mouse bioassay and in vitro guinea pig atrium assay. Toxicon. 20(5):907-912.

Kantha, S.S. 1987. Ichthyotoxins and their implications to human health. Asian Medical Journal 30(8):458-470.

Lawrence, D.N., M.B. Enriquez, R.M. Lumish & A. Maceo. 1980. Ciguatera fish poisoning in Miami. Journal of the American Medical Association. 244(3):254-258.

Lobel, P.S., D.M. Anderson & M. Durand-Clement. 1988. Assessment of ciguatera dinoflagellate populations: sample variability and algal substrate selection. Biology Bulletin 175:94-101.

Miyahara, J.T., C.K. Akau and T. Yasumoto. 1979. Effects of ciguatoxin and maitotoxin on the isolated guinea pig atria. Res. Commun. Chem. Path. Pharmac. 25:177.

Miyahara, J.T., C.K. Kamibayashi, L.K. Shirai and Y. Hokama. 1987. The similarity of toxins in *Ctenochaetus strigosus* and cultured *Gambierdiscus toxicus*. In: P. Gopalakrishnakone and C.K. Tan (eds), "Progress in Venom and Toxin Research", National University of Singapore, pp. 363-371.

Morris, J.G. 1980. Ciguatera fish poisoning. Journal of the American Medical Association. 244(3):272-274.

Olson, D.A., D.W. Nellis and R.S. Wood. 1984. Ciguatera in the eastern Caribbean. Marine Fisheries Review. 46:13.

Ragelis, E.P. 1984. In: E.P. Ragelis (ed), "Seafood Toxins", American Chemical Society, Washington DC, pp. 25-36.

Scheuer. P.J. 1989. Ciguatera - what we know and what we would like to know. In: IUPAC, Tokyo, Japan. Elsevier/Amsterdam.

Taylor, F.J.R. 1979. A description of the benthic dinoflagellates associated with ciguatoxin, including observations on Hawaiian material. In: Taylor, D.L. and H. H. Seliger (eds), "Toxic Dinoflagellate Blooms", Elsevier/North Holland. pp. 71.

Todd, E.C.D. 1985. Ciguatera poisoning in Canada. In: Anderson, D.M., A.W. White & D.G. Baden (eds), "Toxic Dinoflagellates, Proceedings of the Third International Conference", Elsevier/North Holland, New York, pp. 505-510.

Tosteson, T.R., D.L. Ballantine & H.D. Durst. 1988. Seasonal frequency of ciguatoxic barracuda in Southwest Puerto Rico. Toxicon. 26(9):795-801.

Tosteson, T.R., D.L. Ballantine, C.G. Tosteson, V. Hensley, A.T. Bardales. 1989. Associated bacterial flora, growth, and toxicity of cultured benthic dinoflagellates *Ostreopsis lenticularis* and *Gambierdiscus toxicus*. Applied and Environmental Microbiology. 55(1):137-141.

USFDA. 1984. Bacteriological Analytical Methods, 6th edition. Association of Official and Analytical Chemists. Arlington, VA. p. 28.03.

Yasumoto, T. 1985. Recent progress in the chemistry of dinoflagellate toxins. In: Anderson, D.M., A.W. White & D.G. Baden (eds), "Toxic Dinoflagellates, Proceedings of the Third International Conference", Elsevier/North Holland, New York, pp. 259-270.

Yasumoto, T., I. Nakajima, R.A. Bagnis and R. Adachi. 1977. Finding of a dinoflagellate as a likely culprit of ciguatera. Bull. Japan. Soc. Sci. Fish. 43:1021-1026.

Yasumoto, T., A. Inoue, T. Ochi, K. Fujimoto, Y. Oshima, Y. Fukuyo, R. Adachi and R. Bagnis. 1980. Bull. Japan. Soc. Sci. Fish. 46:1397.

B. Scombroid Toxicity

Description

Scombroid toxicity results from ingesting fish which have been improperly handled or stored. The toxin is believed to consist of histamine, and possibly putrescine and cadaverine which potentiate the toxicity of histamine (Taylor and Sumner, 1986). (Putrescine and cadaverine inhibit the histamine-metabolizing enzymes, diamine oxidase and histamine N-methyl-transferase.) Enzymatic decarboxylation of histidine (found in abundance in the free state in dark-fleshed fish) results in histamine (optimal temperature 20-30°C). Putrescine and cadaverine are formed by the decarboxylation of ornithine and lysine respectively (Farn and Sims, 1986; Taylor and Sumner, 1986). The production of histamine can be fairly rapid. In one outbreak, threshold toxin levels were reached after only 3 - 4 hours of storage at room temperature (Kow-Tong and Malison, 1987). Certain bacteria, especially Proteus morganii, are believed to cause histamine formation in fish with scombroid toxicity. Other weak histamine forming bacteria include: *Hafnia alvei, Klebsiella* sp. *Proteus* sp. (Arnold and Brown, 1978; Omura et al., 1978; Eitenmiller et al., 1980; Taylor and Sumner, 1986).

Studies on the production of histamine in mackerel (Murray et al., 1982) showed that fish which were allowed to spoil in ice, had histamine levels which rarely exceeded 5mg/100g of fish, even when it became unfit to eat. However, storage at higher temperatures (especially above 10°C) resulted in high levels of histamine, and production was shown to be exponential. Therefore, levels of histamine over 5mg/100g of fish indicate that the fish has been unnecessarily exposed to high temperatures. The higher the level of histamine, the more abuse there has been.

Contaminated Species

Scombroid toxicity mostly effects fish of the Scomberesocidae and Scombridae families, although toxicity is not limited to these fish families. Between 1978 and 1982, 42% of outbreaks reported to the CDC were associated with non-scombroid fish (CDC, 1982 as cited in Kow-Tong and Malison, 1987). Varieties of fish most often implicated in illness include: mahi mahi (Bryan, 1988; MMWR, 1989; tuna (Murray et al., 1982; MMWR, 1989); bluefish (Bryan, 1988; MMWR, 1898); mackerel (Murray et al., 1982); bonito (Murray et al., 1982) and skipjack (Chen et al., 1988).

Geographic Area

Fish of the temperate and tropical regions have been found to cause scombroid poisoning. Between 1973 and 1986 the states which reported the most cases to of scombroid poisoning to the CDC (in descending order) were: Hawaii, California, New York, Washington and Connecticut (MMWR, 1989).

Symptoms & Treatment

Symptoms of scombroid poisoning can begin 10 minutes to four hours after consuming contaminated fish. The most common symptoms include (Arnold and Brown, 1978; Eitenmiller et al., 1980; Murray et al., 1982; Bryan, 1986; MMWR, 1989): metallic, sharp or peppery taste; nausea, vomiting, abdominal cramps and diarrhea; oral blistering and perioral numbness; facial swelling and flushing; headache, and dizziness; palpitations; hives; rapid and weak pulse; thirst and difficulty in swallowing.

Complete recovery usually occurs within 24 hours. Administration of antihistamines results in immediate improvement of patient condition (Taylor and Sumner, 1986).

The dose of histamine required to cause scombroid poisoning in humans is variable. One experiment in which 100-180mg pure histamine was administered orally resulted in only mild symptoms (headache, nausea, vertigo) (Motil and Scrimshaw, 1979 as cited in Taylor and Sumner, 1986). On the other hand, two cases of scombroid poisoning occurred in New Mexico in 1987 from eating mahi mahi which had a histamine level of only 20 mg/100g fish (MMWR, 1989). The discrepancy between pure histamine resulting in only mild symptoms, while relatively low levels of histamine in fish can result in severe symptoms, may be explained by the presence of potentiators in spoiled fish. Potentiators, such as putrescine and cadaverine, may decrease the dose of histamine required to cause scombroid poisoning in humans (Taylor and Sumner, 1986). Variability

in dosage required to cause illness may also be due to increased susceptibility in individuals with allergies, asthma or peptic ulcers (Blackwell et al., 1969 as cited in Rice et al., 1976).

Statistics

Scombroid toxicity is a common illness associated with seafood. Between the years of 1977 and 1981, scombroid toxicity was responsible for 37% of the seafood-borne illnesses in the U.S. (USFDA, 1984). From 1973 to 1986, 178 outbreaks, affecting 1096 individuals, were reported to the CDC (MMWR, 1989). No deaths have been reported in the U.S.

Detection & Prevention

Cooking, freezing and smoking are ineffective in removing the toxin from fish flesh. The best way to avoid scombroid poisoning is by preventing its production. This can be done by icing or refrigerating fish soon after capture and maintaining the cold temperature until cooking.

The USFDA has established hazard action levels for histamine in fish. For canned tuna the action level is 50mg histamine/100g fish (USFDA, 1982), and for fresh and frozen fish the level is 20mg histamine/100g fish (USFDA, personal communication).

The method most commonly used to detect histamine is a fluorometric assay (Arnold and Brown, 1978; Taylor and Sumner, 1986). There are several different fluorometric procedures which are all based on the condensation of histamine with o-phthalaldehyde to yield a fluorophore. Other histamine detection techniques which are less commonly used include: an enzymatic assay, thin layer, paper, gas-liquid or high pressure liquid chromatography, and guinea pig ileum bioassay.

Selected Bibliography

CDC. 1982. Foodborne disease surveillance, annual summary report, 1978 to 1982. Centers for Disease Control, Atlanta, Georgia.

Anonymous. 1989. Scombroid poisoning - Illinois, South Carolina. Morbidity and Mortality Weekly Review. 38(9):140-147.

Arnold, S.H. and W.D. Brown. 1978. Histamine (?) toxicity from fish products. In: C.O. Chichester, E.M. Mark, and G.F. Stewart (eds), "Advances in Food Research", Academic Press, Inc.

Blackwell, B., L.A. Mabbitt and E. Marley. 1969. Histamine and tryamine content of yeast products. Journal of Food Science. 34:47-51.

Bryan, F.L. 1986. Seafood-transmitted infections and intoxications in recent years. In: D.E.Krammer and J. Liston (eds), "Seafood Quality Determination", Elsevier, Amsterdam. pp 319-337.

Bryan, F.L. 1988. Risks associated with vehicles of foodborne pathogens and toxins. Journal of Food Protection. 51(6):498-508.

Chen, C-M., M.R. Marshall, J.A. Koberger, W.S. Otwell & C.I. Wei. 1988. Determination of minimal temperatures for histamine production by five bacteria. Proceedings of the 12th Annual Conference of the Tropical and Subtropical Fisheries Technology Society of the Americas. Florida Sea Grant College Program.

Eitenmiller, R.R., J.H. Orr and W.W. Wallis. 1980. Histamine formation in fish: microbiological and biochemical conditions. In: "Chemistry and Biochemistry of Marine food Products". pp 39-50.

Farn, G. and G.G. Sims. 1986. Chemical indices of decomposition in tuna. In: D.E. Krammer and J. Liston (eds), "Seafood Quality Determination", Elsevier, Amsterdam. pp. 175-183.

Kantha, S.S. 1987. Ichthyotoxins and their implications to human health. Asian Medical Journal. 30(8):458-470.

Kow-Tong, C. & M.D. Malison. 1987. Outbreak of scombroid fish poisoning, Taiwan. American Journal of Public Health. 77(10):1335-1336.

Motil, K.J. and N.S. Scrimshaw. 1979. The role of exogenous histamine in scombroid poisoning. Toxicology Letter. 3:219-222.

MMWR. 1988. Scombroid poisoning. Morbidity and Mortality Weekly Review. 37(29).

Murray, C.K., G. Hobbs & R.J. Gilbert. 1982. Scombrotoxin and scombrotoxin like poisoning from canned fish. Journal of Hygiene, Cambridge. 88:215-220.

Omura, Y., R.J. Price & H.S. Olcott. 1978. Histamine-forming bacteria isolated from spoiled skipjack tuna and jack mackerel. Journal of Food Science. 43:1779-1781.

Rice, S.L., R.R. Eitenmiller and P.E. Koehler. 1976. Biologically active amines in food: a review. Journal of Milk and Food Technology. 39(5)353-358.

Taylor, S.L. 1986. Histamine food poisoning: toxicology and clinical aspects. Critical Reviews of Environmental Control, Critical Review of toxicology. 17:91-128.

Taylor, S.L. & S.S. Sumner. 1986. Determination of histamine, putrescine and cadaverine. In: Kramer, D.E. & J. Liston (eds), "Seafood Quality Determination", Elsevier, New York, pp 235-245.

USFDA. 1982. Defect action levels for histamine in tuna; availability of guide. Federal Register. 47:40478.
USFDA. 1984. Bacteriological Analytical Methods, 6th edition. Association of Official and Analytical Chemists. Arlington, Virginia. p 28.03.

C. Paralytic shellfish poisoning

Description

Filter-feeding molluscs can become poisonous to humans by consuming toxic dinoflagellates. There are many species of toxic phytoplankton which cause paralytic shellfish poisoning. The species which commonly blooms in the New England area when the water is warm (April through October) is *Protogonyaulax tamarensis* (White, 1988) (= *Gonyaulax tamarensis*, *G. excavata*, *Alexandrium tamarensis* and *A. fundyense*). Some other dinoflagellate species which cause red tides in other parts of the world include: other species of *Gonyaulax*, *Gymnodinium* sp. and *Pyrodinium bahamense* (White, 1988).

PSP can be caused by a combination of any of 18 toxins, depending on the species of dinoflagellate, geographic area and type of shellfish involved. The primary toxins include the carbamate toxins (saxitoxin, neosaxitoxin and gonyautoxin 1, 2, 3, and 4) and the sulfocarbomoyl toxins (B1, B2, C1, C2, C3, and C4). Decarbamoyl toxins (dc-saxitoxin, dc-neosaxitoxin and dc-gonyautoxin 1, 2, 3, and 4), which are derivatives of carbamate or sulfocarbomoyl toxins, can also be present in shellfish (Sullivan and Wekell, 1987; Sullivan, 1988).

Contaminated Species

All filter-feeding molluscs accumulate and depurate paralytic shellfish toxins. Blue mussels become highly toxic within a few days of the onset of a red tide, but also lose their toxin load rapidly (Shumway, 1989). Mussels can become extremely toxic without apparent alert. For example, in Maine (August 1980) mussel toxin levels rose from the detection level to 8000+ ug/100g in 2 days (Shumway et al., 1988). Calculations based on laboratory feeding experiments suggest that during blooms of highly toxic dinoflagellates (ex. *Alexandrium fundyense*) the level of toxins in mussels can exceed acceptable levels in less than 1 hour (Bricelj et al, 1990). Soft-shell clams generally do not become as toxic as mussels. They require more time to accumulate high levels of toxins, and also require longer to cleanse themselves of toxins (White, 1988). Hard clams and oysters do not become as toxic as other molluscs (White, 1988). *Mercenaria mercenaria* exposed to *P. tamarensis* in the laboratory showed a pronounced valve closure (Shumway and Cucci, 1987). Briclej et al. (1990) demonstrated that *M. mercenaria* can ingest *A. fundyense* cells, although only when non-toxic cells are also present.

Scallops can become extremely toxic even during periods when blooms are not evident. However, scallops generally do not pose a threat of PSP since the adductor muscle, the only part of the scallop traditionally sold and consumed in Western society, does not accumulate toxins. Recently there has been pressure in the U.S. to market whole scallops. This practice is strongly advised against because of the high levels of toxins recorded in tissues other than the adductor muscle and the unpredictable nature of toxin levels in scallops.

In the past it was believed that toxic dinoflagellates did not harm or affect shellfish. However, recent evidence has shown that in the presence of *Gonyaulax tamarensis*, molluscs exhibit species specific responses that include (Gainey and Shumway, 1988; Shumway et al., 1985): shell valve activity alteration (Shumway and Cucci, 1987); oxygen consumption increase or decrease; heart rates inhibited excited or unaffected; reduction of byssus production in blue mussels and ribbed mussels (Shumway et al., 1987); filtration rate decrease, increase or remain unchanged (Cucci et al., 1985; Shumway and Cucci, 1987).

Geographic Area

Paralytic shellfish poisoning is a worldwide problem. Blooms have occurred in New England, Canada, Northwestern U.S., England, Norway, Brazil, Argentina, India, Thailand and Japan (Anderson, 1989; White, 1980).

Symptoms & Treatment

Symptoms usually begin within 30 minutes of consumption. The individual initially experiences a numbness, burning or tingling sensation of the lips and tongue, which spreads to the face and fingertips. This leads to general muscular incoordination of arms, legs and neck. Other less commonly reported symptoms include: weakness, dizziness, malaise, prostration, headache, salivation, rapid pulse, thirst, dysphagia, perspiration, impairment of vision or temporary blindness, ataxia with a "floating" sensation, incoherent speech or loss of voice, nausea, vomiting, diarrhea, feeling of loose teeth and

convulsions. Severe cases of PSP can result in respiratory paralysis, and professional medical treatment should be sought. Although rare, PSP can be fatal. If the individual survives beyond 24 hours, total recovery with no lasting effects is expected (Hughes, 1979; Bryan, 1987; Concon, 1988).

Human susceptibility to paralytic shellfish toxins varies with weight, age and health of the individual. Mild cases of PSP have been reported in adults who have consumed 340 ug of the toxin, and ingestion of 1000 ug of the toxin has resulted in death. Due to the difficulty of determining toxin levels ingested by sick persons and the variability among individuals, these dosage levels should be considered rough estimates.

Statistics

Between 1971 and 1977 there were 12 outbreaks of PSP, involving 68 individuals in the U.S. (Hughes, 1979). Only 2 of these outbreaks were attributed to commercially distributed shellfish (Hughes, 1979).

Detection & Prevention

The toxins cannot be destroyed by normal cooking, freezing or smoking. The best prevention of PSP is by detecting the toxins in shellfish and discarding them before they reach the market. The detection method used most often is the mouse bioassay. However, due to numerous disadvantages of this assay, alternate methods are being tested.

MOUSE BIOASSAY - To detect PSP, toxins are extracted from 25g of shellfish digestive gland and injected intraperitoneally into a 20g mouse. The mouse is then observed for 10 minutes for sign of toxicity and/or time of death. Aside from the general disadvantages of the mouse bioassay (see Ciguatera - Detection and Prevention) there are a number of additional problems in using this method for detecting PSP:

- This assay is significantly limited in its minimum detection level of approximately 37ug/100g meat, which is close to the maximum allowed level of 80ug/100g meat (Yang et al., 1987).
- Schantz "salt effect" may cause an underestimate of the toxin concentration by as much as a factor of 3, especially at low toxin levels (Schantz et al., 1958, as cited in Sullivan, 1988).
- Accuracy can be ± 20% (Sullivan and Wekell, 1987).
- A high concentration of free fatty acids can cause false positives (Hamano et al., 1985).

HPLC (Sullivan and Wekell, 1987; Sullivan et al., 1985) - The high performance liquid chromatography method is based on the oxidation of toxins to fluorescent products. Depending on the toxins present, the limit of detection can be as low as 10-30 ug STX/100g, and accuracy can be +/-10%. When toxin levels are approximately 200ug or below by the mouse bioassay, the HPLC method may indicate significantly higher total toxin levels, possibly resulting in false positives (Hurst et al, 1985).

AUTOANALYZER (Sullivan et al., 1985; Jonas-Davis et al., 184) - This technique may be useful for prescreening shellfish since it is rapid and easy. The results of the autoanalyzer would divide shellfish samples into low (<61ug/100g), medium (61-250 ug/100g) and high groups (>250 ug/100g), with only the medium group being subject to a more time consuming and accurate assay.

RADIOIMMUNOASSAY (Yang et al., 1987) - The radioimmunoassay is a competitive assay in which radiolabeled, anti-saxitoxin serum is added to a sample of shellfish tissue extract. Excess antibody is removed and the samples are analyzed with a scintillation counter. If saxitoxin is present in the shellfish, the DPM will be high. If the shellfish is free of toxin the DPM will be low. This technique is very sensitive to saxitoxin. A complete standard curve is mandatory for each run.

COMPETITIVE DISPLACEMENT ASSAY (Davio and Fontelo, 1984; Hall et al., 1985) - Saxitoxin acts by binding to sodium channels in nerve cell membranes. The competitive displacement assay detects saxitoxin by measuring the amount of radiolabeled saxitoxin displaced from a rat brain membrane preparation. This assay is extremely sensitive and selective for saxitoxin.

FLY BIOASSAY (Ross et al., 1985; Hall et al., 1985) - Toxins are extracted from shellfish and injected into a house fly. The fly is then observed for time of death. This method is more sensitive than the mouse bioassay, since flies are not affected by the "Schantz salt effect".

Selected Bibliography

Anderson, D.M. 1989. Toxic algal blooms and red tides: a global perspective. In: E.Graneli, B. Sundstrom, L. Edler and D.M. Anderson (eds), Toxic Marine Phytoplankton. Elsevier, New York. pp 11-16.

Anderson, D.M. & F.M.M. Morel. 1979. Toxic dinoflagellate blooms in the Cape Cod region of Massachusetts. In: Taylor, D.L. & H.H. Seliger (eds), "Toxic Dinoflagellate Blooms", Elsevier/North Holland, New York, pp. 145-150.

Anderson, D.M. & B.A. Keafer. 1985. Dinoflagellate cyst dynamics in coastal and estuarine waters. In: Anderson, D.M., A.W. White & D.G. Baden (eds), "Toxic Dinoflagellates, Proceedings of the Third International Conference", Elsevier/North Holland, New York, pp. 219-224.

Bricelj, V.M., J.H. Lee, A.D. Cembella & D.M. Anderson. in press. Uptake of *Alexandrium fundyense* by *Mytilus edulis* and *Mercenaria mercenaria* under controlled conditions. In: Granelis, E., B. Sundstrom, L. Edler & D.M. Anderson (eds), "Toxic Marine Phytoplankton", Elsevier, New York.

Bryan, F.L. 1987. Seafood-transmitted infections and intoxications in recent years. In: D.E. Kramer and J. Liston (eds), Seafood Quality Determination. Elsevier Science Publishing, the Netherlands, pp 319-337.

Cucci, T.L., S.E. Shumway, R.C. Newell and C.M. Yentsch. 1985. A preliminary study of the effects of *Gonyaulax tamarensis* on feeding in bivalve molluscs. In: D.M. Anderson, A.W. White and D.G. Baden (eds), Toxic Dinoflagellates. Elsevier, New York, pp 395-400.

Davio, S. R. & P.A. Fontelo. 1984. A competitive displacement assay to detect saxitoxin and tetrodotoxin. Analytical Biochemistry. 141:199-204.

Davio, S.R., J.F. Hewetson & J. E. Beheler. 1985. Progress toward development of monoclonal antibodies to saxitoxin; antigen preparation and antibody detection. In: Anderson, D.M., A.W. White & D.G. Baden (eds), "Toxic Dinoflagellates, Proceedings of the Third International Conference", Elsevier/North Holland, New York, pp. 343-348.

Franks, P.J.S., D.M. Anderson & B.A. Keafer. 1989. Fronts, Upwelling and Coastal Circulation: spatial heterogeneity of Ceratium in the Gulf of Maine. In: Okaichi, Anderson & Nemoto (eds), "Red Tides: environmental science, and technology", Elsevier, New York, pp. 153-156.

Gainey, L.F. Jr. & S.E. Shumway. 1988a. A compendium of the responses of bivalve molluscs to toxic dinoflagellates. Journal of Shellfish Research. 7(4):623-628.

Gainey, L.F. Jr. and S.E. Shumway. 1988b. Physiological effects of *Protogonyaulax tamarensis* on cardiac activity in bivalve molluscs. Comparative Biochemistry and Physiology. 91(1):159-164.

Hall, S., Y. Shimizu, J.J. Sullivan, B. Underal, R. Bagnis, S.R. Davio, M.R. Ross & D.G. Baden. 1985. Toxin analysis and assay methods. In: Anderson, D.M., A.W. White & D.G. Baden (eds), "Toxic Dinoflagellates, Proceedings of the Third International Conference", Elsevier/North Holland, New York, pp. 545-548.

Hamano, Y., Y. Kinoshita and T. Yasumoto. 1985. Suckling mice assay for diarrhetic shellfish toxins. In: D.M. Anderson, A.W. White and D.G. Baden (eds), Toxic Dinoflagellates. Elsevier, New York. pp 383-387.

Hughes, J.M. 1979. Epidemiology of shellfish poisoning in the U.S., 1971-1977. In: Taylor, D.L. & H.H. Seliger (eds), "Toxic Dinoflagellate Blooms", Elsevier/North Holland, New York, pp. 23-28.

Hurst, J.W., Jr. 1979. Shellfish monitoring in Maine. In: Taylor, D.L. & H.H. Seliger (eds), "Toxic Dinoflagellate Blooms", Elsevier/North Holland, New York, pp. 231-233.

Hurst, J.W.R. Selvin, J.J. Sullivan, C.M. Yentsch & R.R.L. Guillard. 1985. Intercomparison of various assay methods for the detection of shellfish toxins. In: Anderson, D.M., A.W. White & D.G. Baden (eds), "Toxic Dinoflagellates, Proceedings of the Third International Conference", Elsevier/North Holland, New York, pp. 427-432.

Jonas-Davies, J., J.J. Sullivan, L.L. Kentala, J. Liston, W.T. Iwaoka & L. Wu. 1984. Semiautomated method for the analysis of PSP toxins in shellfish. Journal of Food Science. 49:1506-1509.

Martin, J.L. & A. White. 1988. Distribution and abundance of the toxic dinoflagellate *Gonyaulax excavata* in the Bay of Fundy. Canadian Journal of Fisheries and Aquatic sciences. 45(11):1968-1975.

Nishitani, L. and K. Chew. 1988. PSP toxins in the Pacific coast states: monitoring programs and effects on bivalve industries. Journal of Shellfish Research. 7(4):653-669.

Ross, M.R., A. Siger, B.C. Abbott & A. Hancock. The house fly: an acceptable subject for paralytic shellfish toxin bioassay. In: Anderson, D.M., A.W. White & D.G. Baden (eds), "Toxic Dinoflagellates, Proceedings of the Third International Conference", Elsevier/North Holland, New York, pp. 433-438.

Schantz, E.J., E.F. McFarren, M.L. Schafer and K.H. Lewis. 1958. Purified poison for bioassay standardization. Journal of the Association of Official Analytical Chemists. 41:160-168.

Shumway, S.E. 1989. A review of the effects of algal blooms on shellfish and aquaculture. World Aquaculture. 20(4):65-74.

Shumway, S.E. and T.L. Cucci. 1987. The effects of the toxic dinoflagellate *Protogonyaulax tamarensis* on the feeding and behavior of bivalve molluscs. Aquatic Toxicology. 10:9-27.

Shumway, S.E., T.L. Cucci, L. Gainey & C.M. Yentsch. 1985. A preliminary study of the behavioral and physiological effects of *Gonyaulax tamarensis* on bivalve molluscs. In: Anderson, D.M., A.W. White & D.G. Baden (eds), "Toxic Dinoflagellates, Proceedings of the Third International Conference", Elsevier, New York, pp. 389-394.

Shumway, S.E., F.C. Pierce and K. Knowlton. 1987. The effect of *Protogonyaulax tamarensis* on byssus production in *Mytilus edulis* L., *Modiolus modiolus* Linnaeus, 1758 and *Geukensia demissa* Dillwyn. Comparative Biochemistry and Physiology. 87a(4):1021-1023.

Shumway, S.E., S. Sherman-Caswell & J.W. Hurst. 1988. Paralytic shellfish poisoning in Maine: monitoring a monster. Journal of Shellfish Research. 7(4):643-652.

Sullivan, J.J. 1988. Methods of analysis for DSP and PSP toxins in shellfish - a review. Journal of Shellfish Research. 7(4):587-596.

Sullivan, J.J., J. Jonas-Davis & L.L. Kentala. 1985. The determination of PSP toxins by HPLC and autoanalyzer. In: Anderson, D.M., A.W. White & D.G. Baden (eds), "Toxic Dinoflagellates, Proceedings of the Third International Conference", Elsevier, New York, pp. 275-280.

Sullivan, J.J. and M.M Wekell. 1987. The application of high performance liquid chromatography in a paralytic shellfish poisoning monitoring program. In: D.E. Kramer and J. Liston (eds), Seafood Quality Determination. Elsevier, New York. pp 357-371.

White, A.W. 1988. Blooms of toxic algae worldwide: their effects on fish farming and shellfish resources. Proceedings of the International Conference on Impact of Toxic Algae on Mariculture. Aqua-Nor '87 International Fish Farming Exhibition. Trondeim, Norway.

White, A.W. 1988. PSP: poison for Fundy shellfish culture. World Aquaculture. 19(4):23-26.

Yang, G.C., S.J. Imagire, P. Yasaei, E.P. Ragelis, D.L. Park, S.W. Page, R.E. Carlson & P.E. Guire. 1987. Radioimmunoassay of paralytic shellfish toxins in clams and mussels. Bulletin of Environmental Contamination and Toxicology. 39:264-271.

Yasumoto, T. Recent progress in the chemistry of dinoflagellate toxins. In: Anderson, D.M., A.W. White & D.G. Baden (eds), "Toxic Dinoflagellates, Proceedings of the Third International Conference", Elsevier/North Holland, New York, pp. 259-270.

D. Amnesic shellfish poisoning

Description

Shellfish can become toxic to humans by consuming large quantities of the diatom, *Nitzschia pungens* (Bird and Wright, 1989; Duerden, 1989). *N. pungens* is a common coastal water alga of the Atlantic, Pacific and Indian Oceans and ranges between 62N and 41S latitude (Bird and Wright, 1989). It has a broad thermal tolerance and can thrive in the low salinities of estuaries. *N. pungens* was considered an innocuous alga until 1987, when a bloom off the coast of Prince Edward Island produced the toxin, domoic acid (also called acidic amino acid). Observations of natural *N. pungens* populations show that appreciable quantities of domoic acid are only produced when the alga is present at high densities. This is confirmed by laboratory cultures which only produce the toxin once the culture has reached the stationary phase. It is also possible that not all forms of *N. pungens* are capable of producing domoic acid (Bird and Wright, 1989).

Contaminated Species

It should be assumed that all filter feeding molluscs are capable of accumulating domoic acid. However, the only shellfish implicated in cases of ASP have been mussels (Grey, 1988; Bird and Wright, 1989; Duerden, 1989; Shumway, 1989).

Geographic Area

To date, cases of ASP have only been associated with mussels from Prince Edward Island, Canada. Domoic acid has been found in the digestive gland of some sea scallops in the New England area (J. Hurst, personal communication), although there have been no reported cases of ASP in the U.S.

Symptoms & Treatment

ASP causes both gastrointestinal and neurological symptoms. In the early stages of ASP, the individual usually experiences gastrointestinal symptoms. Severe ASP can cause a facial grimace or chewing motion, short term memory loss, excessive bronchial secretions and difficulty breathing. Death can occur. Autopsies have shown brain lesions (Grey, 1980; Shumway, 1989).

Statistics

The first documented case of ASP occurred in 1987, and to date, has not occurred outside of Canada (Duerden, 1989). The blooms of 1987 and 1988 resulted in approximately 130 illnesses and 2 deaths in Canada (Grey, 1988).

Detection & Prevention

HPLC is primarily used to detect domoic acid (the mouse bioassay can be used as a qualitative test) (Shumway, 1989). A standard level of 20 ppm of domoic acid has been set (Duerden, 1989).

Selected Bibliography

Bird, C.J. & J.L.C. Wright. 1989. The shellfish toxin domoic acid. World Aquaculture. 20(1):40-41.
Duerden, C. 1989. Domoic acid probably annual event, says DFO. Canadian Aquaculture. March/April, p. 11.
Grey, C. 1987. Canadian Medical Association Journal. 138:350-351.
Shumway, S.E. 1989. A review of the effects of algal blooms on shellfish and aquaculture. World Aquaculture. 20(4):65-74.

E. Neurotoxic shellfish poisoning

Description

Pytchodiscus brevis (formally, *Gymnodinium breve*) (Concon, 1988), was first recognized as the causative agent for NSP in the mid 1960's (Gervais, 1985). Blooms of this unarmored or "naked" dinoflagellate are usually associated with fish kills, but can also make shellfish toxic to humans (Yasumoto, 1985; Lutz and Incze, 1979). The blooms generally begin offshore and are transported inshore (Gervais, 1985). *P. brevis* produces 3 known toxins: brevetoxin B, brevetoxin C and GB-3 (Yasumoto, 1985).

Contaminated Species

Oysters and clams are the only shellfish which have been associated with NSP illness (Hughes, 1979). However, it should be assumed that all filter-feeding molluscs are capable of accumulating neurotoxic shellfish toxins.

Geographic Area

NSP is primarily limited to the Gulf of Mexico along the West Coast of Florida (Concon, 1988). There was however, a red tide which occurred in Onslow Bay, North Carolina in 1987 (Pietrafesa et al., 1987). The North Carolina bloom is believed to have been caused by the transportation of *P. brevis* cells out of the Gulf of Mexico and north by the Gulf Stream.

Symptoms & Treatment

SP resembles a mild case of ciguatera or PSP. Symptoms begin within 15 minute to 3 hours of consuming contaminated shellfish (Hughes, 1979) and usually include: tingling of the face and spreading to other parts of the body, cold-to-hot sensory reversal, bradycardia, dilation of the pupils, and a feeling of inebriation. Less commonly, victims may experience: prolonged diarrhea, nausea, poor coordination and burning pain of the rectum (Hughes, 1979; Concon, 1988). Complete recovery is expected within 48 hours (Hughes, 1979).

Statistics

Neurotoxic shellfish poisoning is rare and mostly confined to Florida. In 1974 there was 1 case, and in 1975 there were 3 cases reported in Florida (Concon, 1988). No deaths have been reported from NSP in the U.S. (Lutz and Incze, 1979).

Detection & Prevention

The state of Florida monitors harvesting areas for toxic dinoflagellates and areas are closed when cell counts exceed 5000 cells/l. Two weeks after dinoflagellate concentrations drop below 5000 cells/l, mouse bioassays are run on shellfish. The area is reopened to harvesting when levels are below 20 MU/100g (Gervais, 1985; Hunt and Tufts, 1979).

Selected Bibliography

Baden. D.G., T.J. Mende & L.E. Brand. 1985. Cross-reactivity in immunoassays directed against toxins isolated from *Ptychodiscus brevis*. In: Anderson, D.M., A.W. White & D.G. Baden (eds), "Toxic Dinoflagellates, Proceedings of the Third International Conference", Elsevier/North Holland, New York, pp. 363-368.

Hunt, D. and N. Tufts. 1979. Monitoring programs and epidemiology. In: D.L. Taylor and H.H. Seliger (eds), Toxic Dinoflagellate Blooms, Elsevier/North Holland, New York, pp 489-492.

Lutz, R.A. and L.S. Incze. 1979. Impact of toxic dinoflagellate blooms on the North American shellfish industry. In: D.L. Taylor and H.H. Seliger (eds), Toxic Dinoflagellate Blooms, Elsevier/North Holland, New York. pp 476-483.

Pietrafesa, L.J., G.S. Janowitz, D.S. Brown, F. Askari, C. Gabriel and L.A. Salzillo. 1988. The invasion of the red tide in North Carolina coastal waters. UNC Sea Grant College Program. Raleigh, NC.

Yasumoto, T. 1985. Recent progress in the chemistry of dinoflagellate toxins. In: Anderson, D.M., A.W. White & D.G. Baden (eds), "Toxic Dinoflagellates, Proceedings of the Third International Conference", Elsevier/North Holland, New York, pp. 259-270.

F. Diarrhetic shellfish poisoning

Description

Diarrhetic shellfish poisoning was recognized as a pathogen in Japan within the last decade. Several species of dinoflagellates have been associated with DSP including: *Dinophysis fortii* (Yasumoto, et al., 1980, as cited in Yasumoto 1985), *D. acuminata* (Kat, 1983), and possibly, *D. acuta* (Yasumoto, 1985) and *D. norvegica* (Kat, 1985; Freudenthal and Jijina, 1985). In some areas, DSP dinoflagellates have co-occurred with PSP dinoflagellates, making monitoring and management of shellfish harvesting areas difficult (Freundenthal and Jijina, 1985).

To date, eight lipid soluble toxins have been isolated which are involved in causing DSP. The acidic toxins are okadaic acid, dinophysistoxin -1 and -3 and the neutral toxins are pectenotoxin -1, -2, -3, -4 and -5 (Yasumoto, 1985). Epidemiological data indicate that as little as 12MU of dinophysistoxin - 1 or okadaic acid is sufficient to cause illness in humans (Yasumoto et al., 1980, as cited in Stamman et al., 1987). Laboratory experiments with mice indicate that both okadaic acid and dinophysistoxin -1 may be potent tumor promoters (Suganuma et al., 1988).

Contaminated Species

Filter-feeding molluscs can accumulate toxins in their hepatopancrease even at dinoflagellate concentrations below that necessary to discolor the water. Mussels (Kat, 1983), oysters, hard clams and soft-shell clams (Freudenthal and Jijina, 1985) have been implicated in cases of DSP. Contaminated scallops have caused cases of DSP in Japan (Yasumoto et al., 1980, as cited in Yasumoto, 1985), but the likelihood of scallops causing illness in this country is greatly reduced since the whole scallops are not typically consumed in the U.S., as they are in Japan.

Geographic Area

DSP is a global health hazard. Outbreaks have been reported in Japan, (Yasumoto, 1985) the Netherlands (Kat, 1983), Chile, Spain, France, Sweden, Thailand and Norway (Yasumoto, 1985). There is circumstantial evidence that cases of DSP have occurred in the U.S. (Freudenthal and Jijina, 1985; Freudenthal and Jijina 1988).

Symptoms & Treatment

As the name implies, the symptoms of diarrhetic shellfish poisoning are gastrointestinal in nature. Symptoms usually begin within 3 to 7 hours (range: 30 minutes - 15 hours) of consuming contaminated shellfish and include: diarrhea, nausea, vomiting, moderate to severe abdominal pain and cramps and chills (Stamman et al., 1987; Freudenthal and Jijina, 1985; Freudenthal and Jijina, 1988).

No known fatalities have occurred and total recovery is expected within 3 days, with or without medical assistance (Yasumoto, 1985).

Statistics

Although DSP has not been definitively documented in the U.S., there have been reports of cases in the Mid Atlantic region which are suggestive of DSP (Freudenthal and Jijina, 1985; Freudenthal and Jijina, 1988). These probable cases are based on symptoms, time of onset, negative results from conventional testing, and correlation with seasonal and spatial distribution of *Dinophysis* from monitoring data in the harvesting areas.

Detection & Prevention

Toxins in shellfish cannot be destroyed by normal cooking, freezing or smoking. The best prevention of DSP is by detecting the toxins in shellfish before they reach the market. The following are methods which are currently used, or are being developed, for the detection of DSP:

RAT BIOASSAY (Hagel, 1990) - This technique is currently used in the Netherlands to monitor DSP. White rats are fed the hepatopancrease of suspect shellfish and observed. Consistency of the feces and refusal to consume the shellfish are used to test for the presence of DSP toxins.

SUCKLING MOUSE ASSAY (Humano et al., 1985) - The suckling mouse assay is based on the fluid accumulation in the mouse intestine as a reaction to the acidic toxins (okadaic acid and dinophysistoxin -1 and -3). Shellfish extract is intragastrically administered to 4 - 5 day old mice. After 4 hours the mice are sacrificed, the intestine removed and the fluid accumulation ratio (expressed as the ratio of intestinal weight to remaining body weight) is determined. This method is more sensitive to DSP toxins and less influenced by free fatty acids and other contaminants than the mouse bioassay. However, it requires maintaining a mouse colony and it is difficult to obtain a quantitative estimate of the amount of DSP toxin present (Sullivan, 1988).

CYTOTOXICITY ASSAY (Underal et al., 1985, as cited in Sullivan, 1988) - This procedure is based on measuring the leakage of lactate dehydrogenase from rat hepatocytes following treatment with an extract of shellfish tissue.

HPLC (Lee et al., 1987) - Shellfish extracts are esterified with 9-anthryldiazomethane (ADAM) and analyzed with an HPLC. This method is most effective when okadaic acid and Dinophysistoxin -1 are the principal toxins, and is limited in its ability to detect the other toxins.

STICK TEST - The stick test used to detect DSP is based on an enzyme immunoassay procedure and can detect nanogram levels of okadaic acid.

Selected Bibliography

Freudenthal A.R. & J.L. Jijina. 1988. Potential hazards of *Dinophysis* to consumers and shellfisheries. Journal of Shellfish Research. 7(4):695-701.

Freudenthal A.R. & J.L. Jijina. 1985. Shellfish poisoning episodes involving or coincidental with dinoflagellates. In: Anderson, D.M., A.W. White & D.G. Baden (eds), "Toxic Dinoflagellates, Proceedings of the Third International Conference", Elsevier/North Holland, New York, pp. 461-466.

Hagel, P. 1990. Monitoring program for DSP in Dutch shellfish growing areas. Abstracts of the 82nd Annual Meeting of the National Shellfisheries Association, Williamsburg, VA, April 1-5, 1990. pp 441.

Hamano, Y., Y. Kinoshita & T. Yasumoto. 1985. Suckling mice assay for diarrhetic shellfish toxins. In: Anderson, D.M., A.W. White & D.G Baden (eds), "Toxic Dinoflagellates, Proceedings of the Third International Conference", Elsevier/North Holland, New York, pp. 383-387.

Kat, M. 1983. Dinophysis acuminata blooms in the Dutch coastal area related to diarrhetic mussel poisoning in the Dutch Waddensea. Sarsia. 68:81-84.

Kat, M. 1985. Dinophysis acuminata blooms, the distinct cause of Dutch mussel poisoning. In: D.M. Anderson, A.W. White and D.G. Baden (eds), Toxic Dinoflagellates. Elsevier/North Holland, New York. pp 73-77.

Lee, J.S., T. Yanagi, R. Kenma & T. Yasumoto. 1987. Fluorometric determination of diarrhetic shellfish toxins by high-performance liquid chromatography. Agricultural and Biological Chemistry 51(3):877-881.

Stamman, E., D.A. Segar & P.G. Davis. 1987. A preliminary epidemiological assessment of the potential for diarrhetic shellfish poisoning in the Northeast United States. NOAA Technical Memorandum NOS OMA 34.

Suganuma, M., H. Fujiki, H. Suguri, S. Yoshizawa, M. Hirota, M. Nakayasu, M. Ojika, K. Wakamatsu, K. Yamada & T. Sugimura. 1988. Okadaic acid: an additional non-phorbol-12-tetradecanoate-13-acetate-type tumor promoter. Proceedings of the National Academy of Science, USA. 85:1768-1771.

Maranda, L. & Y. Shimizu. 1987. Diarrhetic shellfish poisoning in Narragansett Bay. Estuaries. 10(4):298-302.

Sullivan, J.J. 1988. Methods of analysis for DSP and PSP toxins in shellfish - a review. Journal of Shellfish Research. 7(4):587-596.

Underal, B., M. Yndestad and T. Aune. 1985. DSP intoxication in Norway and Sweden, Autumn 1984- Spring 1985. In: D.M. Anderson, A.W. White and D.G. Baden (eds), Toxic Dinoflagellates. Elsevier/North Holland, New York. pp 489-494.

Yasumoto, T. 1985. Recent progress in the chemistry of dinoflagellate toxins. In: Anderson, D.M., A.W. White & D.G. Baden (eds), "Toxic Dinoflagellates, Proceedings of the Third International Conference", Elsevier/North Holland, New York, pp. 259-270.

Yasumoto, T., Y. Oshima, W. Sugawara, Y. Fukoyo, H. Oguri, T. Igarash and N. Fujita. 1980. Identification of *Dinophysis fortii* as the causative organism in diarrhetic shellfish poisoning. Bull. Jap. Soc. Sci. Fish. 46:1405-1411.

G. Puffer fish toxicity

Description

Puffer fish, also called fugu or blowfish, contain the potent toxin, tetrodotoxin. It is unclear whether the fish itself produces the toxin, or like ciguatera, it is introduced to the fish by ingestion of toxic algae. Tetrodotoxin acts on both the central and peripheral nervous systems (Kantha, 1987).

Contaminated Species

There are approximately 80 species of puffer fish which are known to contain tetrodotoxin (Kantha, 1987). The domestic species of puffer, sometimes called sea squab, is much less poisonous than the Japanese species, but should be handled by experienced chiefs only.

Geographic Area

Puffer fish are found in the Pacific, Atlantic and Indian Oceans. Cases of puffer fish poisoning occur most commonly in Japan where fugu is considered a delicacy.

Symptoms & Treatment

Symptoms of poisoning usually begin within 10 minutes of consuming puffer fish. The victim first experiences numbness and tingling of lips, tongue and inner surfaces of the mouth. This is followed by weakness, paralysis of limb and chest muscles, decreased blood pressure, and quickened and weakened pulse. Death can occur within 30 minutes (Horwitz, 1977; Kantha, 1987).

Cases of puffer fish poisoning should be treated by maintaining adequate respiration, circulation and renal functions. There is some evidence that recovery of muscle power is accelerated by administration of an anticholinesterase (Torda et al., 1973 as cited in Kantha, 1987).

Statistics

From 1972 to 1974, there was one outbreak, involving two cases, of puffer fish poisoning reported to the CDC (Horwitz, 1977). According to USFDA reports there are an average of 19 fatalities from puffer fish poisoning in Japan each year (Anonymous, 1989). Other sources report that puffer fish toxicity is the number 1 cause of fatal food poisoning in Japan and is responsible for approximately 100 fatalities/year (Anonymous, 1989).

Detection & Prevention

In Japan, chefs are required to have at least three years experience before they are allowed to handle fugu. According to Japanese reports, no cases of poisoning have been attributed to puffers prepared by certified chefs. Problems only occur when inexperienced individuals try to prepare fugu (Anonymous, 1989).

The USFDA has recently lifted an import ban on one type of puffer fish, the tiger fish. Unlike some species of puffer, which may have toxins in the entrails, liver, ovaries, skin and muscle; tetrodotoxin is only found in the liver of the tiger fish (Anonymous, 1989). Before importation to the U.S., the FDA requires that all tiger fish are cleaned, toxic tissues are removed and fish are laboratory certified to be tetrodotoxin-free. Japanese chefs preparing the fish for export to the U.S. are required to have at least 13 years of experience in handling fugu.

Selected Bibliography

Anonymous. 1989. FDA lifts ban on importing puffer fish. Food Protection Report. 5(4):3-4.
Horwitz, M.A. 1977. Specific diagnosis of foodborne disease. Gastroenterology. 73(2):375-381.
Kantha, S.S. 1987. Ichthyotoxins and their implications to human health. Asian Medical Journal. 30(8):458-470.
Liener, I.E. 1974. Toxic Constituents of Animal Foodstuffs. Publisher? pp 73-110.
Torda, T.A., E. Sinclair and D.B. Ulyatt. 1973. Puffer fish (tetrodotoxin) poisoning, clinical record and suggested management. Medical Journal of Australia. 60(1):599-602.n

IV. Parasites

A. *Anisakis simplex* or herring worm

Description

Anisakis simplex, commonly called herring worm, is a parasitic nematode whose final hosts are dolphins, porpoises and sperm whales (Oshima, 1972, as cited in Pinkus et al., 1975). In the larval stage, the stage which infects fish, the parasite is usually 18-36 mm in length, 0.24-0.69 mm in width and whitish in color (Pinkus et al., 1975). *Anisakis* larvae are more prevalent in the Pacific than the Atlantic since the Pacific has a large population of whales, one of the final hosts of *Anisakis* (Myers, 1979; Schantz, 1989).

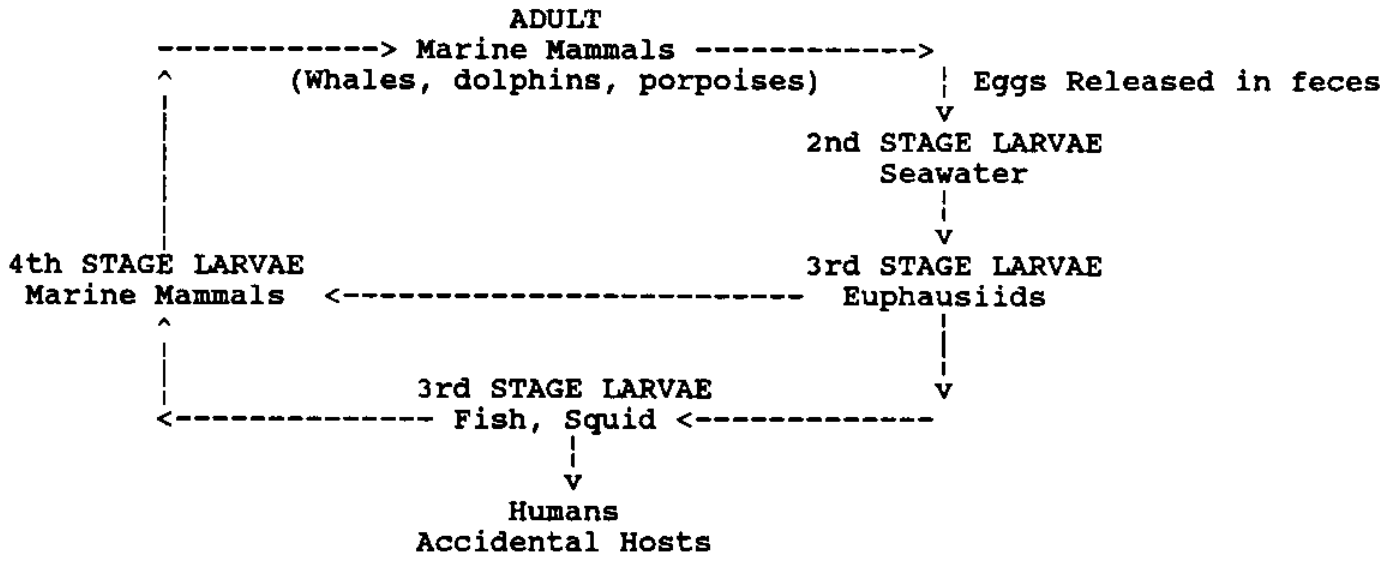
Humans are accidental hosts of *Anisakis* larvae. Anisakiasis, the illness caused by *Anisakis simplex*, is associated with the presence of a marine mammal population and eating raw or undercooked fish (sushi, sashimi, lomi lomi, ceviche, sunomono, Dutch green herring, marinated and cold smoked fish).

Contaminated Species

Generally, both prevalence and intensity of *Anisakis* infection increase with size and age of fish (Smith and Wootten, 1978; McGladdery and Burt, 1985 as cited in McGladdery, 1986). Those species which feed primarily on benthic organisms have a lower incidence of *Anisakis* contamination, while fish that predominantly feed on other fish generally have a high incidence of contamination (Myers, 1979). The types of fish commonly infected with *Anisakis* larvae include: herring (Hauck, 1977; Smith, 1984; Schantz, 1989); Atlantic and Pacific cod, (Myers, 1979; Rodrick and Cheng, 1989; Schantz, 1989); Pacific salmon (Deardorff et al., 1986; McKerrow et al., 1988); sole (Myers, 1979; and Cheng, 1976 as cited in Roderick and Cheng, 1989); mackerel (Schantz, 1989; Smith, 1984); pollock (Smith, 1984); whiting (Smith, 1984); bonito (Pinkus et al., 1975); squid (Pinkus et al., 1975; Olson, 1986; Oshima, 1987); and Pacific rockfish (Schantz, 1989; McKerrow et al., 1988).

Life Cycle

The life cycle of *Anisakis* was described by Oshima (1972 as cited in Pinkus et al., 1975; Oshima, 1987). Adult *Anisakis* worms live in the stomach of various marine mammals including: whales, dolphins and porpoises. The eggs are passed in the feces of the final hosts into seawater where they molt and develop to second-stage larvae. The second-stage larvae are consumed by euphausiids (krill), where they molt to the third stage. The infected euphausiids are consumed by either whales, where they develop to the adult stage; or by fish and squid, where they migrate to the viscera and musculature and remain in the third larval stage. Fish and squid, infected with the third stage larvae, are consumed by piscivorous marine mammals. In the mammals, the larvae attach to stomach and develop to fourth-stage larvae and finally to the adult stage. Humans are infected by consuming raw or undercooked fish and squid infected with third-stage larvae (Deardorff et al., 1986).



Symptoms & Treatment

Symptoms of anisakiasis have been detailed by a number of authors (Deardorff et al., 1986; Pinkus et al., 1975; Olson, 1986). When ingested by humans the larvae often die or are passed out of the body without affecting the individual. The incidence of anisakiasis appears to be low in relation to the number of larvae consumed. Larvae which persist in the body can invade the mucosa, causing severe abdominal pain (may be spasmodic) sometimes accompanied by nausea, vomiting, and occasionally fever. The onset of symptoms varies depending on the location of larval penetration. Penetration in the stomach usually causes symptoms within 1 - 12 hours of eating infected fish. The onset of intestinal anisakiasis usually occurs within 1 - 5 days of eating infected fish.

If the larva penetrates the mucosa, surgical removal is necessary. No detrimental effects to the patient have been documented after the larva has been removed. Anthelminthic drugs have not been useful in treating cases of anisakiasis (Schantz, 1989). Since the nematodes do not develop to full maturity and produce eggs in humans, stool examination is not useful for diagnosis (Deardorff et al., 1986; Schantz, 1989).

Statistics

Anisakiasis is associated with the consumption of raw or undercooked fish. Most documented cases of anisakiasis have occurred in areas where raw fish is commonly eaten, such as, Japan, the Netherlands and Western U.S. (Hawaii, Alaska, and California).

Since the enactment of the Marine Mammal Protection Act of 1972, the population of marine mammals has increased (Bonnell et al., 1983 as cited in McKerrow et al. 1988). The number of human *Anisakis* infections has also increased. Between 1980 and 1988 there was a 70% increase in the number of reported cases (McKerrow et al., 1988). However, it is unclear whether the recent increase in human infections is due to the increase in the marine mammal population, or if it is due to better diagnosis and reporting of the disease.

Documentation and reporting of anisakiasis is ambiguous. *Anisakis simplex* looks very similar to Pseudoterranova decipiens, or cod worm, and the symptoms of both infections are similar. As a result, cases are sometimes misdiagnosed or incorrectly reported. Also, anisakiasis is not reportable to the CDC, so there is no definitive record of the number of cases.

The majority of *Anisakis* infections are acquired from dishes prepared at home, restaurants have been incriminated only rarely (Schantz, 1989). The first case of anisakiasis in the U.S. was reported in 1958 (McKerrow et al., 1988). Since then there have been 30 to 50 documented cases in this country (Schantz, 1989).

Detection & Prevention

Processing plants often use candling techniques to detect larvae in fillets. This technique is not completely effective in detecting *Anisakis* since the larvae are small and whitish in color, making it difficult to differentiate between the larvae and the flesh of light fish. One study indicated that candling efficiency could be improved 16% by bleeding fish. Another study suggested that detection efficiency could be improved by scoring or stripping the fillets, a method which would result in loss of product quality. Research has been done on acoustic detection of larvae, but this technique is still not commercially viable (Margolis, 1977).

Since there are no infallible methods of detecting and removing larvae, the best prevention is to ensure that the larvae are killed before eating the fish. Cooking fish to a temperature of 140°F will kill *Anisakis* larvae (USFDA, 1987). If the fish will be eaten raw it is advisable to freeze it first. Commercial blast-freezing to at least -35°C for 15 hours kills 99% of *Anisakis* larvae and those that survive are presumed damaged beyond the ability to cause human illness (Deardorff and Throm, 1988). For home freezing fresh fish, the temperature and time required to kill *Anisakis* larvae can depend on the species of fish, the depth of penetration, and the physiological condition of the larvae. The USFDA recommends freezing fish at -10°F for at least 168 hours (7 days) (USFDA, 1987).

Storing ungutted fish on ice after capture may result in post-mortem migration of *Anisakis* larvae from the viscera to the flesh of some species of fish (Myers, 1979). Post mortem migration of larvae is evident in herring and mackerel, but not in pollock, whiting or blue whiting, suggesting that migration occurs in "fatty" fish species, but not in "non-fatty" species (Smith, 1984). For this reason it appears that the length of time between the catch of the fish and actual gutting may be an important factor

in governing the number of larvae in some fish flesh. The incidence of *Anisakis* in fillets could possibly be reduced if "fatty" fish are processed more quickly or gutted at sea.

Selected Bibliography

Cheng, T.C. 1976. The natural history of anisakiasis in animals. Journal of Milk and Food Technology. 39(1):32.

Deardorff, T.L., T. Fukumura & R.B. Raybourne. 1986. Invasive anisakiasis: a case report from Hawaii. Gastroenterology. 90:1047-1050.

Deardorff, T.L. & R. Throm. 1988. Commercial blast-freezing of third-stage *Anisakis simplex* larvae encapsulated in salmon and rockfish. Journal of Parasitology. 74(4):600-603.

Hauck, A.K. 1977. Occurrence and survival of the larval nematode *Anisakis* sp. in the flesh of frozen, brined and smoked pacific herring, *Clupea harengus* pallasi. Journal of Parasitology. 63(3):515-519.

McGladdery, S.E. 1986. *Anisakis simplex* (Nematoda: Anisakidae) infection of the musculature and body cavity of atlantic herring (*Clupea harengus* harengus). Canadian Journal of Fisheries and Aquatic Science. 43:1312-1317.

McGladdery, S.E. and M.D.B. Burt. 1985. Potential of parasites for use as biological indicators of migration, feeding and spawning behavior of northwestern Atlantic herring (*Clupea harengus*). Canadian Journal of Fisheries and Aquatic Science. 42:1957-1968.

McKerrow, J.H. & T.L. Deardorff. 1986. Anisakiasis: revenge of the sushi parasite. New England Journal of Medicine. 319(18):1228-1229.

Meyers, B.E. 1979. Anisakine nematodes in fresh commercial fish from waters along the Washington, Oregon and California Coasts. Journal of Food Protection. 42(5):380-384.

Olson, R.E. 1986. Marine fish parasites of public health importance. In: D.E. Kramer and J. Liston (eds), "Seafood Quality Determination", Elsevier Science Publishers, Amsterdam. pp 339-355.

Oshima T. 1987. Anisakiasis - Is the sushi bar guilty? Parasitology Today. 3(2):44-48.

Oshima, T. 1972. *Anisakis* and anisakiasis in Japan and adjacent area. In: Progress in Medical Parasitology in Japan, Vol 4. Meguro Parasitological Museum, Tokyo, Japan. p. 301.

Pinkus, G.S. & C. Coolidge. 1975. Intestinal anisakiasis: first case report from North America. American Journal of Medicine. 59:114-120.

Rodrick, G.E. and T.C. Cheng. 1989. Parasites: occurrence and significance in marine animals. Food Technology. 43(11)98-102.

Schantz, P.M. 1989. The dangers of eating raw fish. New England Journal of Medicine. 320(7):1143-1145.

Smith, J.W. 1984. The abundance of *Anisakis simplex* L3 in the body cavity and flesh of marine teleosts. International Journal of Parasitology. 14(5):491-495.

Smith, J.W. and R. Wootten. 1975. Experimental studies on the migration of *Anisakis* sp. larvae (Nematoda: Ascaridia) into the flesh of herring *Clupea harengus* L. International Journal of Parasitology. 5:133-136.

USFDA. 8/21/1987. Food preparation - raw, marinated or partially cooked fishery products. In: "Retail Food Protection Program Information Manual", part 6, chapter 1, number 2-403. Center for Food Safety and Applied Nutrition, Retail Food Protection Branch.

B. *Pseudoterranova decipiens* or cod worm

Description

Pseudoterranova decipiens (formally *Phocanema*, *Porrocaecum* and *Terranova*) (Olson, 1986), commonly called "codworm" or "sealworm", is a parasitic nematode. The final hosts of *Pseudoterranova* are grey seals, sealions and walruses. In the larval stage, the stage that infects some fish, codworms are is 5-58 mm in length, 0.3-1.2 mm in width and yellowish, brownish or reddish in color (Hafsteinsson and Rizvi, 1987). *Pseudoterranova* larvae are mostly found in Atlantic Ocean fish since the Atlantic has a large population of grey seals (Myers, 1979).

Humans are accidental hosts of sealworms and infections are associated with eating raw or undercooked fish (sushi, sashimi, ceviche, sunomono, green herring, marinated and cold smoked fish).

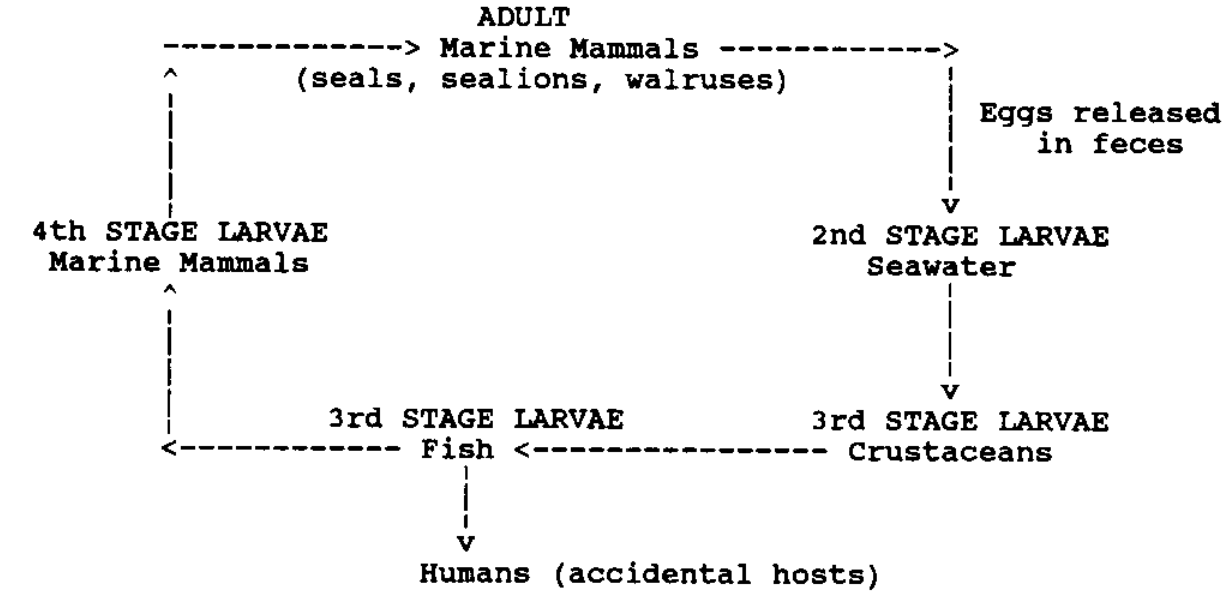
Contaminated Species

The incidence of sealworm infection in fish generally increases with length, weight and age of the fish host (Scott and Martin, 1857; Templeman et al., 1957; Young, 1972; as cited in Hafsteinsson and Rizvi, 1987). Codworms are mostly found in temperate and polar fish and are more common in bottom dwelling fish than pelagic fish (Margolis, 1977). The infection rate in fish can be highly variable, but often especially high in cod. In one study of the Gulf of St. Lawrence the infection rate of cod was estimated to be 70-90% (Margolis, 1977). The species of fish most often infected with sealworm include: cod (Margolis, 1977; Oshima, 1987); pollock (Oshima, 1987); halibut (Margolis, 1977; Oshima, 1987); greenling (Oshima, 1987); squid (Oshima, 1987); and flatfish (Oshima, 1987).

Life Cycle

The life cycle of codworm has been described by a number of authors (Margolis, 1977; Hafsteinsson and Rizvi, 1987; Oshima, 1987). Adult *Pseudoterranova* live in the digestive tracts of seals (especially grey seals), sealions and walruses (Schantz, 1987). The eggs are passed in the feces and develop to second stage larvae in seawater. The larvae are ingested by crustaceans where they molt to the third larval stage. Fish become infected by consuming infected crustaceans, and by serially passing the larvae from one fish to another. When infected fish are eaten by marine mammals, the larvae attach to the stomach and develop to fourth stage larvae and finally to the adult stage.

Humans are accidental hosts. They are infected by consuming raw or undercooked fish contaminated with third stage larvae.



Symptoms & Treatment

Pseudoterranova infections and Anisaksis infections are often misdiagnosed or incorrectly reported. As a result, the symptoms of sealworm infection may, in some documented cases, be exaggerated. It is generally accepted that sealworm infections are less severe than Anisaksis infections (Olson, 1986; McKerrow et al., 1988; Schnatz, 1989). According to one report, there has only been one case in the U.S. where tissue penetration has occurred, and 12 cases in which a larva was either coughed up or manually removed from the mouth (Hafsteinsson and Rizvi, 1987). Other reports indicate more severe symptoms caused by sealworm infections including, epigastric pain (sometimes spasmodic and occurring every 3-10 minutes), sometimes accompanied by nausea, vomiting and abdominal discomfort (Margolis, 1977). Animal experiments support that *Pseudoterranova* is capable of penetrating the gastric mucosa, however, penetration is usually superficial and involves only the head end of the worm (Young and Lowe, 1969 as cited in Margolis, 1977).

Cases which require surgical removal of the parasite result in complete recovery of the patient within hours to days. Anthelminthic drugs have not been useful in treating codworm infections (Schantz, 1989). Since the nematodes do not develop to maturity and produce eggs in humans, stool examinations are not a useful diagnostic method (Deardorff et al., 1986; Schantz, 1989).

Statistics

Documentation and reporting of codworm infection is ambiguous. *Pseudoterranova* decipiens look very similar to *Anisakis*, and the symptoms of both infections are similar. As a result, cases are sometimes misdiagnosed or incorrectly reported. Also, codworm infection is not reportable to the CDC, so there is no definitive record of the number of cases. Prior to 1987, there have been 13 reported cases of codworm infection in this country (Hafsteinsson and Rizvi, 1987).

Detection & Prevention

Candling is often used in processing plants to detect codworm larvae in fillets. This technique is both time consuming and difficult since the larvae are small and often blend in with the fish flesh. Studies have shown that candling does not detect sealworms embedded deeper than 6mm in the fish musculature (Hafsteinsson and Rizvi, 1987). Acoustic detection is currently being developed, but it is still not commercially viable (Hafsteinsson and Rizvi, 1987).

Freezing or cooking are the most effective methods of codworm prevention. If the nematode is dead when consumed, it poses no threat to humans. Cooking the fish to 140°F is sufficient to kill codworm larvae (USFDA, 1987). If the fish will be eaten raw it is advisable to freeze it first. For home freezing fresh fish, the temperature and time required to kill codworm larvae can depend on the species of fish, the depth of penetration, and the physiological condition of the larvae. The USFDA recommends freezing fish at - 10°F for at least 168 hours (7 days) to kill codworm larvae (USFDA, 1987).

Selected Bibliography

Deardorff, T.L., T. Fukumura and R.B. Raybourne. 1986. Invasive anisakiasis: a case report from Hawaii. Gastroenterology. 90:1047-1050.

Hafsteinsson, H. & S.S.H. Rizvi 1987. A review of the sealworm problem: biology, implications and solutions. Journal of Food Protection. 50(1):70-84.

Margolis, L. 1977. Public health aspects of "codworm" infection: a review. Journal of the Fisheries Research Board of Canada. 34:887-898.

Meyers, B.E. 1979. Anisakine nematodes in fresh commercial fish from waters along the Washington, Oregon and California Coasts. Journal of Food Protection. 42(5):380-384.

Olson, R.E. 1986. Marine fish parasites of public health importance. In: D.E. Kramer and J. Liston (eds), Seafood Quality Determination. Elsevier Science Publishers, Amsterdam.

Oshima T. 1987. Anisakiasis - Is the sushi bar guilty? Parasitology Today. 3(2):44-48.

Rodrick, G.E. and T.C. Cheng. 1989. Parasites: occurrence and significance in marine animals. Food Technology. 43(11):98-102.

Schantz, P.M. 1989. The dangers of eating raw fish. New England Journal of Medicine. 320(7):1143-1145.

Scott, D.M. and W.R. Martin. 1957. Variations in the incidence of larval nematodes in Atlantic cod fillets along the Southern Canadian mainland. Journal of the Fisheries Research Board of Canada. 14:975-996.

Templeman, W., H.J. Squires and A.M. Fleming. 1957. Nematodes in the fillets of cod and other fishes in Newfoundland and neighboring areas. Journal of the Fisheries Research Board of Canada. 14:831-897.

USFDA. 8/21/1987. Food preparation - raw, marinated or partially cooked fishery products. In: "Retail Food Protection Program Information Manual", part 6, chapter 1, number 2-403. Center for Food Safety and Applied Nutrition, Retail Food Protection Branch.

Young, P.C. 1972. The relationship between the presence of larval anisakine nematodes in cod and marine mammals in British home waters. Journal of Applied Ecology. 9:459-486.

Young, P.C. and D. Lowe. 1969. Larval nematodes from fish of the subfamily Anisakinae and gastrointestinal lesions in mammals. Journal of Comparative Pathology. 79:301-313.

C. Eustrongylides

Description

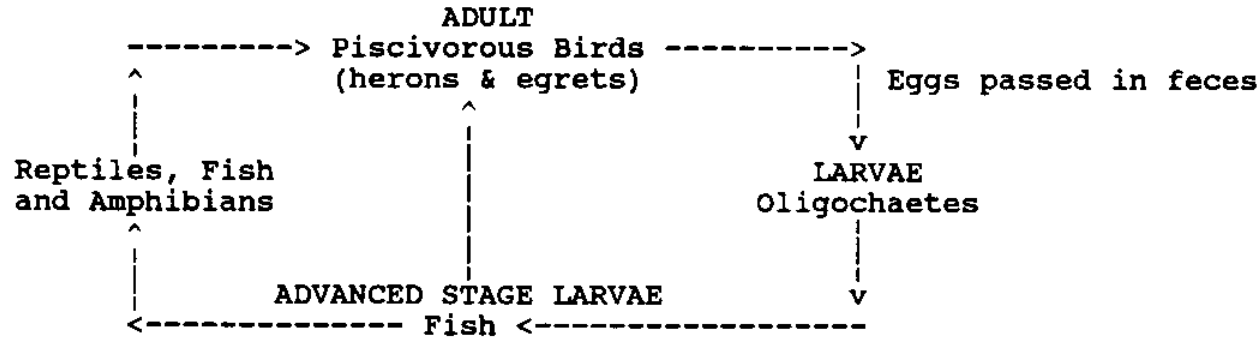
Eustrongylides is a parasitic nematode, whose adult form parasitizes the gastrointestinal tract of fish-eating birds. In the advanced larval stage *Eustrongylides* is pinkish-red in color and can be as large as 40 mm in length and 1 mm in width (Wittner et al., 1989).

Contaminated Species

Eustrongylides has not been reported in species of fish commonly eaten by humans (Schantz, 1989). It is highly prevalent in brackish and freshwater bait fish from Florida to Maine (Gunby, 1982). Minnows were examined for *Eustrongylides* in two Baltimore area studies. One study reported a 33% infection rate (Shirazian et al., 1984), the other study reported a 48% infection rate (Gunby, 1982).

Life Cycle

The life cycle of *Eustrongylides* is not completely known. In the adult stage, the nematode is found in the digestive tract of fish eating birds, frequently great blue herons and egrets. Eggs are passed in the feces and ingested by aquatic oligochaetes, the first intermediate host. Many species of fish serve as the second intermediate host and a variety of amphibians, reptiles and fish are the paratenic, or transport hosts. *Eustrongylides* develop to advanced stage larvae in the body cavity and flesh of fish, amphibians and reptiles (Cooper et al., 1978 as cited in Shirazian et al., 1984).



Symptoms & Treatment

Intestinal perforation by *Eustrongylides* results in severe abdominal pain, gradually increasing with time. One individual has been successfully treated with drugs (Shirazian et al., 1984), the other four reported cases required surgery (Gunby, 1982; Schantz, 1989; Wittner et al., 1989).

Statistics

To date there have only been five reported cases of *Eustrongylides* infection in humans. In 1982, three Baltimore area fishermen became ill after consuming live bait fish (a practice of some anglers when the fish are not biting) (Gunby, 1982; Shirazian et al., 1984). Two more cases were reported in 1989, involving a New Jersey fisherman who ingested live bait fish, and a New York man who ate homemade sushi of uncertain fish variety (Schantz, 1989; Wittner et al., 1989).

Detection & Prevention

Human infection of *Eustrongylides* can be avoided by not consuming live bait fish. No precise information on temperature tolerance of *Eustrongylides* is available (Wittner et al., 1989).

Selected Bibliography

Cooper, C.L., J.L. Crites and D.J. Sprinkle-Fastzkie. 1978. Population biology and behavior of larval *Eustrongylides tubifex* (Nematoda: Dioctophymatida) in poikilothermous hosts. Journal of Parasitology. 64:102-107.

Gunby, P. 1982. One worm in the minnow equals too many in the gut. Journal of the American Medical Association. 248(2):163.

Schantz, P.M. 1989. The dangers of eating raw fish. New England Journal of Medicine. 320(17):1143-1145.

Shirazian, D., E.L. Schiller, C.A. Glaser & S.L. Vanderfecht. 1984. Pathology of larval *Eustrongylides* in the rabbit. Journal of Parasitology. 70(5):803-806.

Wittner, M., J.W. Turner, G. Jacquette, L.R. Ash, M.P. Salgo & H.B. Tanowitz. 1989. Eustrongylidiasis - a parasitic infection acquired by eating sushi. New England Journal of Medicine. 320(17):1124-1126.

D. *Diphyllobothrium latum* tapeworm

Description

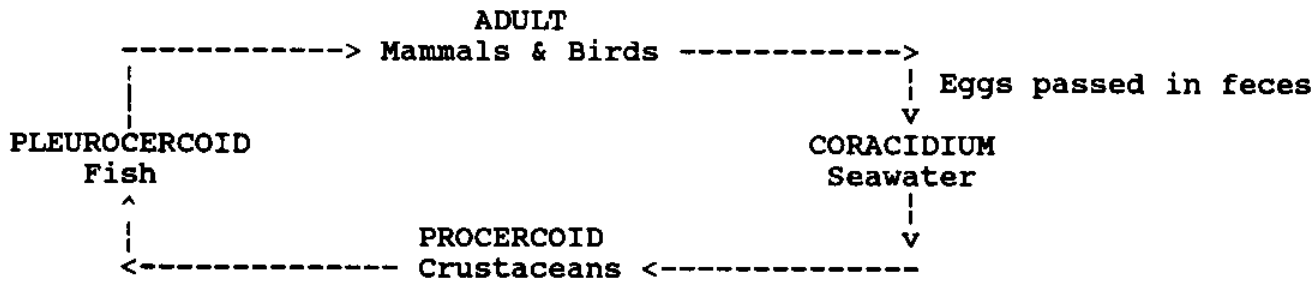
Diphyllobothrium latum is a cestode, or tapeworm, which parasitizes a variety of piscivorous mammals of the upper northern latitudes (Olson, 1986). Cestodes are flatworms which have an anterior attachment structure, called the scolex, and body segments, called proglottides. Cestodes found in infected fish range from a few millimeters to several centimeters in length (Olson, 1986).

Contaminated Species

Most *Diphyllobothrium* tapeworms infect freshwater fish, however, anadromous salmonid fish can also carry the parasite (Schantz, 1989; Olson, 1986). *Diphyllobothrium* tapeworms are usually found unencysted and coiled in musculature, or encysted in viscera.

Life Cycle

Adult *Diphyllobothrium* live in the intestine of a variety of fish-eating mammals and birds. Eggs are passed in the feces and develop to coracidium, or ciliated larvae, in the water. The larvae are consumed by crustaceans, usually copepods. In the digestive tract of the crustaceans, the larvae develop to the first tapeworm stage, called the procercoid. Fish become the second intermediate hosts by consuming infected crustaceans. The pleurocercoid, or second tapeworm stage, develops in the fish. Birds and mammals (including humans) become the final hosts by consuming fish containing the pleurocercoid (Olson, 1986).



Symptoms & Treatment

Most cases of human *Diphyllobothrium* infection are asymptomatic (Schmidt and Roberts, 1985 as cited in Olson, 1986). However, some individuals experience abdominal pain, diarrhea, constipation and occasional megaloblastic anemia. Examination of eggs and proglottides in the feces of affected individuals provides diagnosis of tapeworm genus only. In order

to determine the species, it is necessary to examine the scolex (Matsui et al., 1985 as cited in Olson, 1986). Cases of human infection have been successfully treated with anthelminthic drugs (Schantz, 1989).

Detection & Prevention

Salmonid fish should be thoroughly cooked to avoid infection. No precise information on the freezing temperature tolerance of *Diphyllobothrium* is available.

Selected Bibliography

Matsui, T., M. Iida, M. Murakami, T. Kimura, M. Fujishima, T. Yao and M. Tsuji. 1985. Intestinal anisakiasis: clinical and radiologic features. Radiology. 157:229-302.

Olson, R.E. 1986. Marine fish parasites of public health importance. In: D.E. Kramer and J. Liston (eds), "Seafood Quality Determination", Elsevier Science Publishers, Amsterdam. pp 339-355.

Schantz, P.M. 1989. The dangers of eating raw fish. New England Journal of Medicine. 320(17):1143-1145.

Schmidt, G.D. and L.S. Roberts. 1985. In: "Foundations of Parasitology", Times Mirror/Mosby College Publishing, St. Louis.

VI. Seafood Safety Computer Bulletin Board

This information on seafood safety was obtained from the Seafood Safety Electronic Bulletin Board developed by the New England Fisheries Development Association. To access the bulletin board you will need a computer, modem, communications software and a SCIENCEnet mailbox from Omnet (617/265-9230). The following communications software settings are used to communicate with SCIENCEnet: 1200 baud, 8 data bits, 1 stop bit, no parity, full duplex. Once you are connected to SCIENCEnet, type "COMPOSE SEAFOOD.SAFETY" to access the bulletin board. For information about the seafood safety bulletin board, call the New England Fisheries Development Association at: (617) 542-8890.

This bulletin board has been established to provide information on seafood contaminants and to act as a forum for seafood safety issues. The New England Fisheries Development Association encourages you to leave questions, comments and suggestions in the forum section of the bulletin board, or directly in their mailbox.

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