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**Agenda Item 3** **CX/FFP 03/3-Add.2**

**JOINT FAO/WHO FOOD STANDARDS PROGRAMME**
**CODEX COMMITTEE ON FISH AND FISHERY PRODUCTS**

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# DRAFT STANDARD FOR SALTED ATLANTIC HERRING AND SALTED SPRATS

# RISK PROFILE *CLOSTRIDIUM BOTULINUM* IN SALTED ATLANTIC HERRING AND SPRAT

**(Prepared by Norway with assistance from the Netherlands, the United States and the representatives of FAO and WHO)**

## Introduction

During the 25th Session of the Codex Committee on Fish and Fishery Products the DRAFT STANDARD FOR SALTED ATLANTIC HERRING AND SALTED SPRAT, Section 2.2 "Process definition" was discussed. The Delegations of Norway and Germany expressed the view that this product had a long history of safe use and that no further health protection measures were required. The Committee however was of the view that the matter of protection of public health from *Clostridium botulinum* hazard needed to be addressed in more detail and therefore agreed that the Delegation of Norway with assistance from the Netherlands, the United States and the representatives of FAO and WHO would prepare a risk profile on this matter for consideration by the 26th session. This would assist the Committee in deciding whether a full risk assessment for this hazard in salted Atlantic herring and sprats was needed. Such a risk profile would include an assessment of the information available and the possible need to obtain additional information from research studies.

## Background

Seafood borne disease transmitted by finfish is in the majority of cases related to biotoxins or bacteria (Huss, 1994). Several bacterial species have been associated with disease in humans after the consumption of seafood, of which *Listeria monocytogenes*, *Vibrio* sp. and *Clostridium botulinum* are among the most important examples (Jay, 1992; WHO, 1999). The later organism is found in many parts of the world and in a variety of environments. Most isolated strains are able to produce potent neurotoxic substances during growth in food. Thus the presence of *Clostridium botulinum* is consequently of great concern in the assessment of food safety (Dodds and Austin, 1997). In the following a risk profile for *Clostridium botulinum* in salted Atlantic herring (*Clupea harrengus*) and sprat (*Sprattus sprattus*) is described.

Fundamental to this risk profile are the questions of whether the organism is likely to be present in these fish species and if it can be expected to grow and produce toxins if the fish is inappropriately handled.

The Norwegian delegation poses the questions of whether we should proceed to clarify whether or not this presents a public health issue according to the procedures of the Codex guidelines on new work on microbiological risk assessment/management, CL 2002/X-FH. The delegation also raised the question as to

whether the work group members should conduct literature searches concerning gutted or ungutted fish and botulism, and on salted herrings and sprats and botulism. Furthermore, proposed that it would be appropriate that other countries took samples at landing of Atlantic herring and sprat from other regions.

Scope and Rationale

As for other foods, the safety of seafood is a main concern among consumers and governmental bodies. This topic has gained increased attention in recent years. The scope of the present risk profile is to review the information currently available on the presence and significance of *Clostridium botulinum* in salted Atlantic herring and sprat, in order to give recommendations for risk management actions.

Pathogen –food commodity combination of concern

The present risk profile deals with salted Atlantic herring and sprat. In the following a short presentation of typical handling and processing in Norway of these products, is given.

Immediately after harvest Atlantic herring and sprat caught by Norwegian vessels are transferred to refrigerated seawater (RSW) reaching a temperature of -1ºC (30.2ºF) to
- 0.5ºC (31.1ºF) before landing. Pelagic fish may in a few sporadic cases also be transported on ice. This practice is at present rare.

The time span from harvest to the initiation of salting of herring may under Norwegian conditions vary from a few hours to two days. Typical sizes of Atlantic herring intended for salting are 160 to 500 grams. The herring is salted by adding dry NaCl in typical amount of 220 to 240 grams pr kg fish contained in plastic barrels. The barrels are rotated to obtain a uniform distribution of salt. At regular intervals during the salting process, saturated NaCl brine are added. In the traditional salting process applied for herring in Norway, the salt content will within 10 days reach alt least 12 - 15 % in the water phase through the fish.

Norwegian Atlantic herring is mainly exported in a frozen state, and the most important import countries are Russia, Ukraine, Poland and states within the EU-union. In 2002 only 3 % of the catch volume was exported as salted products, mainly to EU countries.

The time span from harvest to the initiation of brining of Norwegian Atlantic sprat is usually within hours. Typical sizes of sprat may vary between 20 and 35 grams. The sprat is cured by adding dry NaCl, sugar and spices in a typical amount of 240 grams pr kg fish contained in plastic barrels. At regular intervals during the process more salt/sugar/spice are added.

The main portion of Norwegian Atlantic sprat is used in canned products. Salted products are of minor importance, as is the export values of salted sprat products. Some frozen sprat is exported to Sweden for further processing.

Description of the Public Health Problems

The pathogen

*Clostridium botulinum* is a gram positive, anaerobic rod-shaped bacterium able to produce spores (Cato *et al.*, 1986). Spores of *Clostridium* sp. are naturally found in soil, sediments and water on a world-wide basis (Fach *et al.* 2002; Hielm *et al.*, 1998; Huss, 1980; Gram, 2001), and has even been detected in sea-salt intended for fish salting (Fenicia *et al.*, 2002). Most strains within the species of *C. botulinum* are able to produce very potent proteineous toxins during growth, and the presence of such bacteria is consequently of great concern in the assessment of food safety (Dodds and Austin, 1997).

The species *C. botulinum* may be divided into seven types (A to G) based on the serology of the toxins produced. These toxins are thermally unstable, and will generally be inactivated at temperatures above 85°C (185°F) for 5 minutes. However, bacterial toxins in general, including botulinum toxin are stable at high salt concentrations

and low pH (Huss and Rye Pedersen, 1980). Any toxin present or preformed in the raw material may thus be carried over to the final product, necessitating strict growth control from harvest to consumption (Huss, Ababouch and Gram, 2003). Human botulism, *i.e.* infections or intoxications associated with *C. botulinum*, is in the vast majority of cases associated with the types A, B, E and rarely F (Dodds and Austin, 1997). Types C and D gives botulism in animals, and type G has so far not been shown to cause any disease.

The type E *C. botulinum* is together with the types G and F classified as psycrothrophic, and thus able to grow at relatively low temperatures. At otherwise optimal conditions *C. botulinum* type E may grow and produce toxins at a temperature of 3.3ºC (37.9°F), and up to 5 % NaCl (Gram, 2001). Optimal temperature of growth is reported to be 18-25ºC (64-77°F), and the minimum water activity ($a_w$) required is 0.97.

Strains of *C. botulinum* may also be divided in four groups designated by the roman numbers I to IV, depending on physiological properties of which the groups I and II are most relevant in this context. Group I consists of proteolytic strains, have heat resistant spores and a growth minimum of 10ºC (50°F), whereas bacteria belonging to group II are non-proteolyic, having spores with low heat resistance and a growth minimum of 3,3ºC (37.9°F). All type E and some type B strains belong to the latter group.

Reported occurrence in seafood

Several authors have reported on the prevalence of *C. botulinum* in seafood (Cann *et al.*, 1966; Fach *et al.* 2002; Gram, 2001; Hielm *et al.*, 1998; Huss, 1980; Hyytiä *et al.*, 1998; Hyytiä-Trees, 1999). When found in seafood products from cold-water areas such as Scandinavia, Canada, Alaska, Russia and some parts of Japan, *C. botulinum* type E is reported to be the most prevalent type (Huss, 1994).

Johannsen (1963) concluded from available data that *C. botulinum* type E may not occur in the southern hemisphere or in the United States. However subsequent reports of illnesses caused by *C. botulinum* type E toxins in the United States from smoked fish and reported isolation of type E in the U.S. Great Lakes, the Atlantic coast, the Gulf of Mexico coast, the Pacific northwest, the Baltic Sea, and Finnish fish farms demonstrate that the organism is ubiquitous, at least in the northern hemisphere (Telzak, 1990).

The amount of toxin necessary to cause human illness is very small, and may be measured in nanograms. Thus, if a microenvironment favorable to germination and outgrowth of spores from any of the seven types of *C. botulinum* known to produce neurotoxins exists in fish before or during processing, there is a potential risk of illness.

Telzak (1990) reported that out of 32 outbreaks by *C. botulinum* type E reported from 1899 to 1977, 31 were traced to marine products. The spores are reported to be found at highest prevalences in the viscera of fish (Badhey et al.,1986; Telzak, 1990).

As a part of the elaboration of the present risk profile, samples of herring and sprat have recently been collected from Norwegian fishing grounds along the coast, and analysed with respect to anaerobic spore formers. These examinations have been performed by the District laboratory of The Directorate of Fisheries in Solver and Ålesund and the National Institute of Nutrition and seafood Research in Bergen. Standard methodology was applied including selection of spores in 50% ethanol, anaerobic incubation, plating on specialised media and biochemical characterisation by Rapid ID 32 A (Solomon and Lilly, 2001; Varnam and Evans, 1991).

The viscera and gills from 130 fish samples of herring from 19 landings were individually examined. In this material a total of five samples from two landings harboured *C. botulinum*. The sample material in three of the positive samples was from gills and two were from viscera. The isolated strains of *C. botulinum* have not yet been classified into toxin production groups. In addition five samples harboured *C. acetobutylicum*.

The viscera and head from 25 samples of sprat from five landings were individually examined for the presence of anaerobic spore formers. In this sample material no anaerobic spore formers were detected.

Some authors have commented on the relative rarity of botulism and the low incidence of all types of *C. botulinum* in waters, seafood and sediment (Hackney et al. 1991). However while the incidence may be low, the occurrence is widespread. As reported by Johannsen (1963) *C botulinum* type E is ubiquitous in Sweden and adjacent areas, and the Baltic area is a reservoir for type E organisms that spread to neighbouring waters. He summarizes the literature reporting the presence of *C. botulinum* type E in Japan, British Columbia, Soviet Union, Germany, France, Denmark, and Greenland. Huss (1980) reported the presence of type E organisms in aquatic environments in Denmark, the Faroe Islands, and Greenland. Based on the widespread distribution reported in the present publication, the author considers *C. botulinum* type E to be a true aquatic organism.

The Public Health problems

Currently four categories of botulism in humans have been recognised. Food associated botulism is caused by the ingestion of foods contaminated with preformed botulinum toxin, whereas infant botulism may occur amongst children under the age of one year when eating food containing spores of *C. botulinum*. In the latter case the spores germinate, colonise and produce toxins in the intestinal tract. In addition *C. botulinum* may give wound infections and in some cases a generalised infection amongst adults as a result of intestinal germination and growth, resembling the infant botulism (Dodds and Austin, 1997; Gram, 2001).

In the period from 1899 to 1990 a total of 962 cases of human botulism outbreaks were registered in the United States (Solomon and Lilly, 2001). These outbreaks involved 2320 cases of which 1036 were fatal. Two outbreaks of botulism could be traced back to the product "kapchunka", a whole, smoked and brined whitefish preparation (MMWR, 1987; MMWR, 1987). In 1981 a Californian man was sickened and in 1985 two Russian immigrants died of botulism poisoning from commercially prepared "kapchunka" in New York. Furthermore six Israelis contracted botulism from fish that had been sent from New York as a gift. The U.S. FDA had allowed the continued production of "kapchunka" following the 1981 episode, but stipulated that time, temperature, and brining conditions would have to be strictly controlled. Following the failure to maintain these control measures and the subsequent 1985 illnesses, the U.S. FDA prohibited further production of this type of uneviscerated fish product (FDA, 1988).

Herring has been reported as one of the fish species most liable to sustain the growth and toxin production by *C. botulinum*, and has therefore been recommended as the best fish species to be used in establishing safety margins (Cann, et al., 1965)

The incidence of botulism in Norway is comparatively low. The first verified case was described in 1934, involving consumption of home made cured ham. From 1961 to 2002, a total of 62 cases have been reported. Under Norwegian conditions botulism is, in the vast majority of food borne cases, linked to the consumption of non-vacuumed, lightly salted and fermented trout from freshwater sources ("rakfisk"), or from home produced lightly salted and dried ham. Rakfisk is produced on a small-scale local basis, and has at present no export value.

In the period from 1996 to 1999 no cases of botulism were reported in the Netherlands.
Three cases of human botulism were registered in 2000, of which two cases were linked to consumption of imported, homemade heat-treated vegetables. In the third case the involved food was unknown. In 2001 two cases were reported. The involved food was not confirmed, but no fish products were in question. Two cases of infant botulism were registered in 2002. Consumption of honey was a possible reason, but was not confirmed. By September 2003 one case of botulism was reported probably linked to consumption of imported sterilized meat and vegetable.

Special clinical considerations of botulism

Botulism is a rare disease and may be difficult to diagnose (Hackney et al., 1991), particularly when the symptoms are not fatal. Badhey et al. (1986) reviewed the symptoms of botulism in two elderly persons who had eaten "kapchunka". They concluded that the symptoms can be confused with stroke, atypical Guillian Barre syndrome, myasthenia gravis, Eaton-Lambert syndrome, trichinosis, polyneuritis of diphtheria, chemical intoxication, tick paralysis or psychiatric disorder. Gastrointestinal symptoms may be more prominent than neurological signs. The symptoms of one patient could have been attributed to congestive heart failure,

obstructive pulmonary heart disease, or other causes. Badhey et al. (1986) concluded that the true incidence of botulism poisoning is unknown and that single cases may go unrecognized.

On the other hand considering the severity of some cases of botulism, infections and intoxications caused by *C. botulinum* might also be more likely to be reported than disease caused by less pathogenic microorganisms. This may give better epidemiological data, but also more concern among consumers and official bodies involved in food safety.

Food Production, Processing, Distribution and Consumption

Difficulties of safe processing

Provided anaerobic conditions, strains of *C. botulinum* possess the ability to grow at low temperatures and in the presence of relatively high concentrations of NaCl. Consequently these organisms have the potential of growth and toxin production in chilled and lightly preserved seafood products with an extended shelf life.

The sodium chloride concentration is an important factor in controlling the outgrowth and toxin production of *C. botulinum*. It is generally accepted that the inhibitory concentration for nonproteolytic *C. botulinum* is 5.0% water-phase salt (wps), equivalent to a water activity of 0.97. A 10% wps concentration is necessary to inhibit the proteolytic strains. The hurdle principle will apply in such a situation, and it should be stressed that these NaCl limits apply under optimal conditions for bacterial growth, and that other factors as the presence of oxygen, low temperature and high or low pH will reduce the salt tolerance of *C. botulinum* in a practical situation. Huss (1994) concluded that in fish products stored at temperatures below 10ºC (50°F), a water phase salt concentration of 3 % is sufficient to inhibit the growth of *C. botulinum* for at least 30 days. In the traditional salting process applied for herring in Norway, the salt content will within one week reach alt least 12 - 13 % in the water phase through the fish, giving a narrow time window where growth may occur, even if the temperature were favourable for bacterial growth.

However, Eklund et al. (1982) reported on the difficulty of achieving uniform salt concentration in large batches of fish or in sections of an individual fish during the brining operations. This is not unexpected as the fish, especially marine fish in the round, are designed by nature to resist desiccation into the surrounding more saline seawater. They are very impervious to penetration of salt from the outside and to water loss. The gills are the most vulnerable sites and they are shielded by the operculum. The dynamics of brining of fish does not seem to have been studied in detail, however it would be expected that water drawn osmotically from the fish would be a rapid process, with the diffusion of sodium chloride into the fish being a slower process, especially for ungutted fish. To facilitate salt penetration the Norwegian code of praxis for salted herring recommends that each fish should be "nibbed" or "gibbed" before submersion into brine. This praxis involves either removal of gills and parts of the viscera, removal of gills only or the mechanical destruction of the skinny tissue below the ventral parts of the gills. Such treatment is performed on a routine basis during the production of salted herring in Norway.

Eklund et al. (1982) also cautioned that the nonproteolytic nature of type E organisms would not result in the development of odours indicative of spoilage. Thus toxin could be formed with little evidence to the consumer that the fish was spoiled and possibly unsafe. In a 1963 outbreak described by these authors, only 3 of 16 affected people reported any unusual flavours or off odours.

Temperature control is an effective means of controlling the growth and toxin production of *C. botulinum*. The Norwegian quality regulations contain several provisions imposing demands on temperature and time control during harvest, shipment and the processing of fish (Directorate of Fisheries, 2003). The absence of botulism cases that can be traced back to herring and sprat in Norway demonstrates that these provisions, together with the salting regimes traditionally applied, represents effective means of controlling the growth and toxin production of *C. botulinum* in seafood originating from these species.
In some cases however, temperature control may be inadequate and non controllable, especially among the consumers. As noted above for the manufacture of "kapchunka" temperature control was inadequate. Ongoing surveys in the US and published data from a previous survey by Audits International, show that refrigeration

temperatures at retail and consumer levels in the U.S. commonly exceed the recommended refrigeration temperature of 4.4°C (40°F). Twenty seven per cent of product temperatures taken in home refrigerators exceeded 4.4°C (40°F) and 4% exceeded 10°C (50°F). Any failure to have achieved adequate salt concentration in the gut of brined uneviscerated fish could lead to toxin formation from inadequate temperature control at the retail or consumer level.

Conclusion

- Spores of *C. botulinum* are widespread in the aquatic environment, especially in the northern temperate zones.
- The ubiquitous nature of *C. botulinum* spores strongly suggests that this organism may be found in and on fish. Although only a few studies have been conducted on the occurrence of such spores in or on herring, this species is generally recognized as suitable for botulism toxin formation. [1]
- Botulism is a rare but potentially fatal disease. Due to the diverse nature of botulism symptoms, the true incidence of sporadic non lethal cases of botulism poisoning is uncertain.
- Some salted or brined seafood products have been associated with botulism.
- Known cases of botulism have not been encountered with traditionally prepared salted herring and sprat in Norway.
- Since toxins from *C. botulinum* is stable at a high salt concentrations and low pH, any toxin present or preformed in the raw material may be carried over to the final product. This hazard can only be eliminated by having strict control over the handling process from harvesting to consumption. Necessary measures includes strict temperature and time control during harvest, shipment and processing of fish, in addition to proper salting giving water phase NaCl levels of 5% or above throughout the fish.
- Botulism from salted Norwegian herring and sprat products does not seem to be a public health issue, and does thus not fulfil the requirements set for the initiation of a "Risk assessment" according to the procedures of the Codex guidelines on new work on microbiological risk assessment/management, CL 2002/X-FH.

Recommendation for Risk Management Actions

Considering the current state of knowledge related to this food borne pathogen, it is recommended that the CCFFP should undertake the following risk management activities;

- Botulism from traditionally prepared Norwegian salted products of Atlantic herring and sprat does not seem to be a public health issue. Even though spores of *C. botulinum* have been detected in such fish, the time and temperature during the transport, the handling, the brining operation and the required concentrations of NaCl in the final product is under strict control, rendering *C. botulinum* unable to grow and produce toxins even in uneviscerated fish.
- For traditionally prepared Norwegian salted products of Atlantic herring and sprat, further Risk Management Actions are not considered required.
- If strict control of time and temperature and the NaCl concentration in the final product can not be undertaken, the need for evisceration of the fish should be considered in a separate risk assessment.
- New herring and sprat products in which a lower NaCl content is to be used, should be carefully evaluated with respect to the potential of sustaining growth of *C. botulinum*.
- To facilitate evaluation on new products, literature search and if necessary research should be conducted to obtain information on salt penetration times in herring of various sizes. This information should be applied in mathematical modelling predicting germination, growth and toxin production of relevant types of *C. botulinum*.

1 The explanation for this statement should be clarified. Is the way of processing of herring the reason or is herring a species specifically liable to sustain the growth and toxin production by *C. botulinum*?

- Atlantic herring and sprat are exported to many counties that apply different processing and conservation of the fish. Information on typical storage and distribution temperatures and the conservation methods applied should be collected and evaluated with respect to food safety.

References


Badhey, H; DJ Cleri; RF D'Amato; JR Vernaleo; V Veinni; J Tessler; AA Wallman; AJ Mastellone; M Giuliana; L Hoschstein. 1986. Two fatal cases of type E adult food-borne botulism with early symptoms and terminal neurological signs. *J. Clin. Microbiol.* 23:616-618.

Cann, DC; BB Wilson; G Hobbs; JM Shewan. 1965. The growth and toxin production of *Clostridium botulinum* Type E in certain vacuum packed fish. *J. Appl. Bact.* 28(3): 431-436.

Cann, D.C., B.B. Wilson, J.M. Shewan and G. Hobbs. 1966. Incidence of *Clostridium botulinum* type E in fish products in the United Kingdom. *Nature*, 211, p. 205-206.

Cato, E.P., W. Lance George and S.M. Finegold. 1986. Genus *Clostridium*, p. 1141 – 1200, in: P.H.A. Sneat, N.S. Mair, M.E. Sharpe and J.G. Holt (eds). Bergeys Manual of Systematic bacteriology, Vol. 2, Williams and Wilkins, Baltimore.

Directorate of Fisheries. 2003. Quality Regulations Relating to fish and fisheries products, available at http://www.fiskeridir.no/english/pages/regulations.html.

Dodds, K.L. and J.W. Austin. 1997. *Clostridium botulinum*, p. 288 – 304, in: Doyle, M.P., L.R. Beuchat and T.J. Montville (eds.). Food Microbiology, fundamentals and frontiers, ASM Press, Washington.

Eklund, M.W., G.A. Pelroy, R. Paranjpye, M.E. Peterson and F.M. Teent. 1982. Inhibition of Clostridium botulinum types A and E toxin production by liquide smoke and NaCl in hot-process smoke-flavoured fish. *J. Food. Protect.*, 45, 935-941.

Fach, P., S. Perelle, F. Dillasser, J. Grout, C. Dargaignaratz, L. Botella, J.-M. Gourreau, F. Carlin, M.R. Poppoff and V. Broussolle. 2002. Detection by PCR-enzyme-linked-immunosorbet assay of *Clostridium botulinum* in fish and environmental samples from a coastal area in Northern France. *Appl. Environ. Microbiol*., 68 (12), p. 5870-5876.

FDA. 1988. Salt-cured, air-dried, uneviscerated fish. Compliance Policy Guide; Federal Register 53(215): 44949-44951.

Fenicia, L., F. Anniballi, M. Poushaban, G. Franciosa and P. Aureli. 2002. Presence of *Clostridium botulinum* spores in sea-salt in Italy. Poster presented at the 18th International ICFHM Symposium, Food Microbiology, Lillehammer, Norway, 18. 23. August 2002.

Gram, L. 2001. Potential hazards in cold-smoked fish: *Clostridium botulinum* type E. *J. Food Science*, suppl. vol. 66 (7), p. 1082-1087.

Hackney, CR; TE Rippen; DR Ward. 1991. Principles of pasteurization and minimally processed seafoods, 355-371, In; Microbiology of Marine Products, Ward & Hackney, eds., Van Nostrand Reinhold, New York.

Hielm, S., E. Hyytiä, A.B. Andersin and H. Korkeala. 1998. High prevalence of *Clostridium botulinum* type E in Finnish freshwater and Baltic Sea sediment samples. *J. Appl. Microbiol*., 84, p. 133-137.

Huss, HH. 1980. Distribution of *Clostridium botulinum*. *Appl. Environ. Microbiol*., 39: 764-769.

Huss, H.H. and E. Rye Pedersen. 1980. *Clostridium botulinum* in fish. *Scan. J. Vet. Med.*, 31, 214-221.

Huss, H.H., 1994. Assurance of seafood safety. FAO Fisheries technical papers No. 334, p. 8 – 26, FAO Rome.

Huss, H.H., L. Ababouch and L. Gram. 2003. Assessment and management of seafood safety, FAO Technical Report (in press).

Hyytiä-Trees , E., 1999. Prevalence, molecular epidemiology and growth of *Clostridium botulinum* type E in fish and fishery products, academic dissertation Department of Food and Environmental Hygiene, Faculty of Veterinary Medicine, University of Helsinki, Finland. ISBN 952-45-8684-0.

Hyytiä , E., S. Hielm and H. Korkeala. 1998. Prevalence of *Clostridium botulinum* type E in Finnish fish and fisheries products. *Epidemiology Infect*., 120, 245-250.

Jay, J.M., 1992. Modern Food Microbiology, p. 487 – 500, Chapman and Hall, New York.

Johannsen, A. 1963. *Clostridium botulinum* in Sweden and the adjacent waters. *J. Appl. Bact.* 26:43-47.

MMWR. 1985. Botulism associate with commercially distributed Kapchunka-New York City. 34(35): 546-547.

MMWR. 1987. International outbreak of type E botulism associated with ungutted, salted whitefish. 46(39), 812-813.

Solomon, H.M and T. Lilly, Jr. 2001. *Clostridium botulinum*. Bacteriological analytical manual – online. U.S. Food and Drug Administration, Centre for Food Safety and Applied Nutrition, website: http://www.cfsan.fda.gov/~ebam/bam-17.html.

Telzak, EE; EP Bell; DA Kautter; L Crowell; LD Budnick; DL Morse; S Shultz. 1990. An international outbreak of type E botulism due to unevisceration of fish. *J. Inf. Dis.* 161:340-342.

Varnam, A.H. and M.G. Evans. 1991. Foodborne pathogens, an illustrated text. Wolfe Publishing.

WHO. 1999. Food safety issues associated with products from aquaculture, WHO Technical Report Series, Report 883, Geneva.