FAO
FISHERIES
TECHNICAL
PAPER

444

ISSN 0429-9345

Assessment and management of seafood safety and quality

Cover photographs:
Background: Canning sardines at a fish processing factory in Morocco. FAO/G. Bizzarri
Inset top: Testing frozen prawns in Italy. FAO/R. Faidutti
Inset bottom: A variety of cooked shellfish. FAO/FIIU

Assessment and management of seafood safety and quality

FAO
FISHERIES
TECHNICAL
PAPER
444

by
H. H. Huss
Danish Institute for Fisheries Research
Department of Seafood Research
Denmark
L. Ababouch
Fish Utilization and Marketing Service
FAO Fisheries Department
and
L. Gram
Danish Institute for Fisheries Research
Department of Seafood Research
Denmark

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 2004

ISBN 92-5-104954-8

PREPARATION OF THIS DOCUMENT

A document entitled "Assurance of Seafood Quality" was published by the Food and Agriculture Organization of the United Nations (FAO) in 1995 (Huss, 1995). This document was based on a series of lecture notes used at workshops and training activities organized by the FAO/Danish International Development Agency (DANIDA) Training Project on Fish Technology and Quality Control (GCP/INT/391/Den).

By the end of 2000 it became clear that this document required updating. New ideas and developments, particularly in the presentation of the Hazard Analysis Critical Control Point (HACCP) concept, needed to be included. In early 2002, I was requested by FAO to prepare an updated and expanded version of the 1995 document including available information on fish safety and quality, especially as it pertains to:

- fish and seafood-borne illnesses: ecology of causative agents and control measures;
- fish safety and quality management systems, including HACCP, monitoring programmes and risk analysis.

Extensive and significant changes have been made compared with the first document. For this reason a new title was chosen: "Assessment and Management of Seafood Safety and Quality". A number of colleagues, all eminent scientists, some with practical experience, have contributed to this new version and I wish to thank them all for their willingness to assist in completing this project within a reasonable time. First of all I wish to thank my co-editors and co-authors, Professor Lone Gram, DIFRES[1] and Professor Lahsen Ababouch, Chief, Fish Utilization and Marketing Service, FAO[2], Rome for their contributions. Very special and sincere thanks to Professor Gram for her skilful and high quality work in editing the text and contributions from a variety of authors.

For valuable contributions, I wish to thank:

Dr John Ryder, Director of FAO/Eastfish[3] Copenhagen, Denmark
Dr Paw Dalgaard, Senior scientist, DIFRES[1], Denmark
Dr Marco Frederiksen, Scientist, DIFRES[1], Denmark
Dr Peter Karim Ben Embarek, WHO[4], Geneva
Mr Alan Reilly, Deputy Chief Executive, Food Safety Authority[5], Ireland

I also wish to thank Dr Maria Rasch from DIFRES for great assistance in editing and proofreading, and Birgitte Rubæk and Valeriu Popesco for providing excellent drawings.

The Danish Institute for Fisheries Research provided secretarial assistance and other resources (stationary, photocopies, etc.) for the project, which was valuable and very appreciated. Special thanks to librarian Søren Tørper Christensen without whom we would not have managed to write the book.

Lyngby 6 January 2003

Hans Henrik Huss (HHH)

Danish Institute for Fisheries Research
Department of Seafood Research

[1] DIFRES: Danish Institute for Fisheries Research, Department of Seafood Research, c/o Technical University of Denmark bldg. 221, DK-2800 Lyngby, Denmark
[2] Fish Utilization and Marketing Service, Food and Agriculture Organization of the United Nations (FAO), Viale delle Terme di Caracalla, 00100 Rome, Italy
[3] FAO/Eastfish, Midtermolen 3, DK-2100 København Ø, Denmark
[4] World Health Organization (WHO), Avenue Appia 20, CH - 1211 Geneva 27, Switzerland
[5] Food Safety Authority of Ireland, Abbey Court, Lower Abbey Street, Dublin 1, Ireland

Huss, H.H; Ababouch, L; Gram, L.

Assessment and management of seafood safety and quality
FAO Fisheries Technical Paper. No. 444. Rome, FAO. 2003. 230p.

ABSTRACT

This paper compiles the state of knowledge on fish safety and quality with the view to provide a succinct yet comprehensive resource book to risk and fish quality managers. After an introduction about world fish production and consumption and the developments in safety and quality systems, it provides a detailed review of the hazards causing public health concerns in fish and fish products. It devotes several Chapters to risk mitigation and management tools, with a detailed description of the requirements for the implementation of Good Hygienic and Manufacturing Practices (GHP/GMP), of the Hazard Analysis and Critical Control Point (HACCP) system and of the monitoring programmes to control biotoxins, pathogenic bacteria and viruses and chemical pollutants. Chapters on the use of microbiological criteria, the use of the HACCP approach to target quality aspects other than safety matters, predictive microbiology, traceability and examples of food safety objectives complete the document.

Distribution:
FAO Members and interested organizations
FAO Regional and Subregional Fisheries Officers
FAO Fisheries Department

CONTENTS

1 INTRODUCTION (Hans Henrik Huss)

Food quality, including safety, is a major concern facing the food industry today. A number of surveys have shown that consumer awareness about quality of their food is increasing. The extensive coverage in the daily press of food safety issues such as the BSE crisis, concerns about genetically modified foods, use of growth promoters, existence of pesticide and dioxin residues in food, the *Salmonella* problem, transfer between micro organisms of resistance to commonly used antibiotics add to consumers' fear and unease about what they eat.

The situation is further complicated by the fact that many consumers suffer from a serious lack of knowledge on simple food safety issues. Thus, less than one percent of US and Canadian consumers met minimum criteria for acceptable safety practices in a North America audit of food preparation behaviour, in which 106 consumers agreed to be watched while preparing food (Daniels, 1998). In a similar study, only 4.7% of UK consumers fully implemented appropriate food safety control practices (Griffith *et al.,* 1998). Furthermore, most consumers exhibit a general disbelief in the importance of good handling practices and a great resistance to effective protective treatment such as chemical preservation or irradiation. As a consequence, there is an increasing demand for more fresh or even raw food with enhanced natural flavours and produced with less or no use of salt and other preservatives.

A great number of socio-economic changes such as increased urbanization (crowding), migrations and population demographics are further contributing to the safety of foods. The population of highly susceptible persons is expanding worldwide because of ageing, malnutrition, HIV infections and other underlying medical conditions with a weakened immune system.

To meet these challenges, food manufacturing is becoming a highly complex business, particularly since raw material is sourced on a global scale and new processing technologies are used to produce a vast array of products. Much research is needed to evaluate new techniques and to consider food safety issues at all stages, from production of raw materials to sale of final product.

Despite great efforts in research, food-borne diseases continue to present a major problem of both health and economic significance. The cost of food-borne disease is high. Although the full economic impact is not known, preliminary estimates in the United States in 1994 placed the cost between US$ 10-83 billion (FDA, 1997). Some of this huge cost is borne by the food-producing company – and loss of consumer confidence may even cause bankruptcy – but the great majority is borne by the government. It has become overwhelmingly clear that all countries need an adequate food control programme to ensure a safe food supply to protect and promote the health of the consumer.

Yet, food safety is not only a consumer concern, but also at the very root of a properly functioning market. Food safety as a prerequisite for protecting consumer health also serves the interest of producers and those involved in processing and marketing foodstuffs. The production and consumption of food is central to any society and has a wide range of economic, social and in many cases environmental consequences.

Food control includes all activities carried out to ensure the quality and safety of food. Every stage from initial production to processing, storage, marketing and consumption must be included in a food quality and safety programme. The overall goal is to provide a systematic approach to all control and inspection activities through a managed programme based on proper scientific principles and appropriate risk assessment, leading to careful targeting of inspection and control resources. Furthermore, the risk assessment must be transparent, i.e. it must be carefully documented, including any constraints that may have affected the quality of the risk estimate and fully available to independent assessors. Sufficient financial and personnel resources must be made available. However, it must be emphasized that no management system can offer zero risk in terms of consumer health protection.

Fish and fishery products are in the forefront of food safety and quality improvement because they are among the most internationally traded food commodities. In 2001, fish trade amounted to US$ 54 000 million, of which approximately 50 percent originated in developing countries.

The first part of this publication provides some of the information required to make risk assessment for seafood products. It shows that in many situations the essential data needed to perform a formal quantitative risk assessment are currently not available. However, in most cases, semi-quantitative risk assessments are more than sufficient to allow for appropriate control action.

The second part outlines the risk management strategies used in seafood processing today. The prerequisite to use the HACCP system and the HACCP system itself are outlined in detail as examples of risk management programmes.

The management of other quality parameters such as spoilage and shell life of seafood, chemicals and physical quality aspect are discussed in a final Chapter.

The present publication is an update and expansion of an earlier document by Hans Henrik Huss (1994) *Assurance of Seafood Quality*. FAO Fisheries Technical Paper No. 334.

References[*]

Daniels, R.W. 1998. Home food safety. *Food Technology* 52, 54-56.

FDA (Food and Drug Administration) 1997. *Food Code*. US Department of Health and Human Services, Public Health Service, FDA, Washington DC, USA.

Griffith, C., D. Worsfold & R. Mitchell 1998. Food preparation, risk communication and the consumer. *Food Control* 9, 225-232.

[*] All references in this Technical Paper have been left in the authors' bibliographic style

2 WORLD SEAFOOD PRODUCTION AND CONSUMPTION (Lone Gram)

World fish production (catches of wild fish plus production in aquaculture) has increased steadily to approximately 120 million tonnes in recent years (Figure 2.1) (FAO, 2000). Declines in captured fish were seen in 1998 (Figure 2.2), mainly due to decreased catches of small pelagic fish in Chile and Peru, caused by the "El Niño". This decline affected mainly fish meal production, while food fish production stayed the same. In 1999 and 2000 fish production recovered and returned to pre-El Niño level. China is the top producer with some 41.6 million tonnes in 2000. Peru was the second major fishing nation with catches of 10.7 million tonnes. The importance of aquaculture continues to expand, especially for freshwater species such as carp, and almost one third of fish used for human consumption are now produced in aquaculture (FAO, 2000).

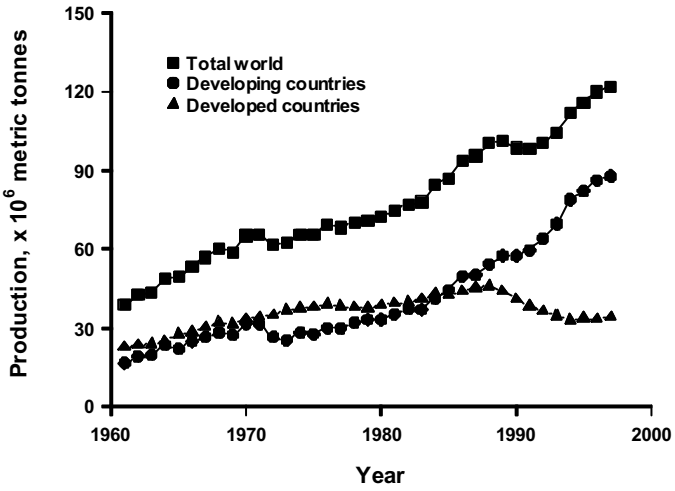

Figure 2.1
Total world fish production from 1961 to 1997 divided between developed and developing countries (FAO, 2000).

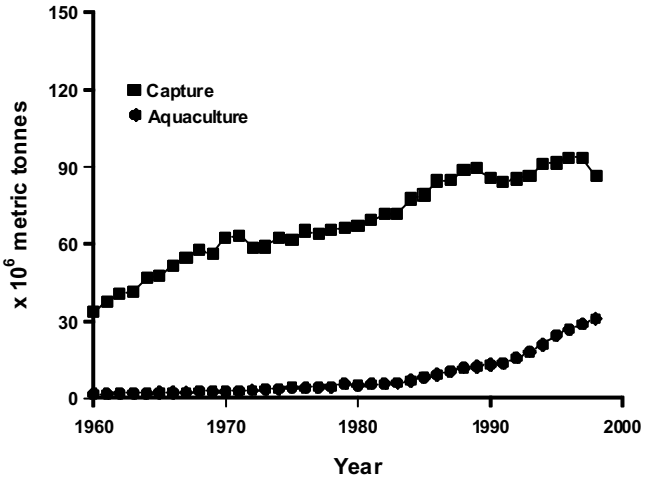

Figure 2.2
Total world fish catches and aquaculture production from 1960 to 1998 (FAO, 2000).

While aquaculture has been increasing for the last 20 years, the increment has dropped during the last five years. The total value of aquaculture and catches by 2000 was approx US$ 130 000 million and total world trade of fish and fishery products increased in 2000 to r each US$ 54 000 million for exports. Thailand is the main exporting country with US$ 4 300 million. China experienced a sharp increase in its export performance. It is now number two among all fish exporting countries with US$ 3 700 million. The Chinese fisheries exporting industry is specializing in re-processing of imported raw material, creating a strong value-addition in this process. Norway, which used to be number two fish exporter in previous years, reported lower export values. This is in part due to lower salmon prices, but also caused by low value of the euro – the currency of the

main trading area for Norwegian fish. Almost two thirds of the total world production is produced by or caught in developing countries (Figure 2.1).

Developed countries accounted for more than 80% of total imports of fishery products in 2000 in value terms. Japan was the biggest importer of fishery products, accounting for some 26% of the global total. The European Union (EU) has increased its dependency on imports for its fish supply. The United States, besides being the world's fourth major exporting country, was the second biggest importer. Imports were growing in 2000, mainly due to expanding shrimp imports. Shrimps and prawns are increasingly produced in aquaculture especially in Southeast Asia. A significant increase has been seen in countries such as Thailand (Figure 2.3).

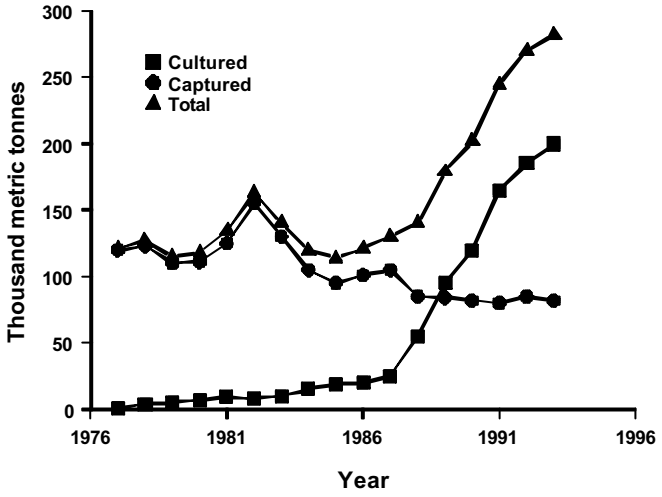

Figure 2.3
Cultured and wild-captured shrimp production in Thailand (Dierberg and Kiattismkul, 1996; cf FAO/NACA, 1995).

Between 20 and 30% of the total world production of fish is used to manufacture animal feeds (Figure 2.4). The greater tonnage comes from processing whole fish that are not suitable for human consumption because they are too bony, too oily, or otherwise unsatisfactory; these fish are sometimes called “industrial fish”. Examples of fish used for fishmeal include capelin, menhaden (*Brevoortia* spp.), sand eel, sprat, Norway pout, blue whiting, horse mackerel, Atlantic herring (*Clupea* spp.), anchovy (*Engraulis* spp.), pilchard and related species. In the USA, for example, the entire menhaden catch goes to rendering. Some of these fish, e.g. Atlantic herring, could be used for direct consumption and the EU prohibits use of Atlantic herring for fish meal production. A secondary source is the waste (offal) from fish and shellfish operations. South America, especially Peru and Chile are big producers of fishmeal with a yearly catch between 5 and 15 million tonnes of industrial fish. Amounts have fluctuated partly due to the El Niño. European countries (Denmark, Norway, Iceland and others) process approximately 6 million tonnes per year and the USA process 1 million tonnes. The vast majority of fishmeal (50%) and fish oil (90%) is used for aquaculture feeds.

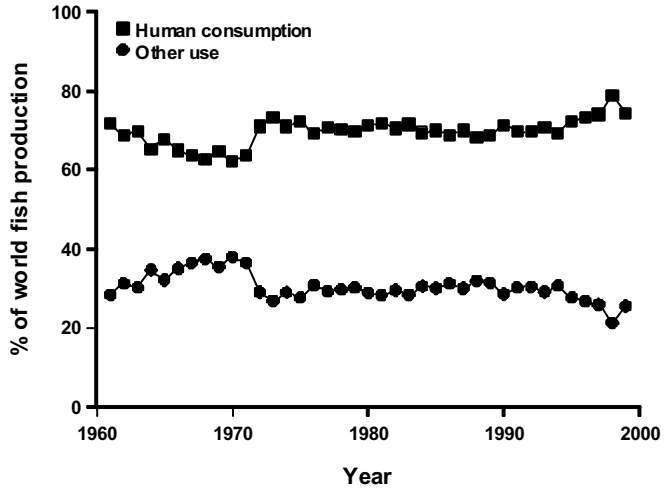

Figure 2.4
Use of world fish production for human consumption and other use (FAO, 2000).

The bovine spongiform encephalopathy (BSE) scare has had an impact on the fish meal market particularly in Europe in 2001. In early 2001 the EU prohibited the use of animal proteins in all animal feeds with the exception of milk powder and fish meal. The use of the latter was prohibited in ruminant's diets only. Fish oil is mostly used for fish feed, although a minor amount is used for human consumption. The demand for fish oil is high and competing vegetable oils seem to be in shorter supply than initially forecast for 2001, and their prices are expected to move up. As a result, a further increase in fish oil prices is likely.

A small – and declining – amount of the fish produced is used for food aid. In 2000, some 7 600 tonnes were donated which compares to 25 800 tonnes in 1989. Canned fish is the main product, while edible fat reported a dramatic decline in recent years. Norway continues to be the main supplier of fish for food aid, and reported a sharp decline in 1998. Developing countries are practically not tapped as a source of fish for food aid.

2.1 Fish utilization

Since 1994, more and more fish has been used for direct human consumption rather than for other purposes (see Figure 2.4). Of the products used for human consumption, fresh fish showed significant growth during the 1990s, and almost 50% of fish used for human consumption is sold fresh (Figure 2.5). This change has been accompanied by a decline in the use of cured and canned fish. Also, the proportion sold as frozen fish is declining. This pattern has largely been driven by growth in consumption.

Fish has a significant capacity for processing and almost two thirds of the catch (in 1998) were used for further processing. A large fraction, approximately 30%, of the fish used for human consumption was frozen, approximately 14% canned and approximately 12% cured. The remaining 45% was sold fresh (Figure 2.5).

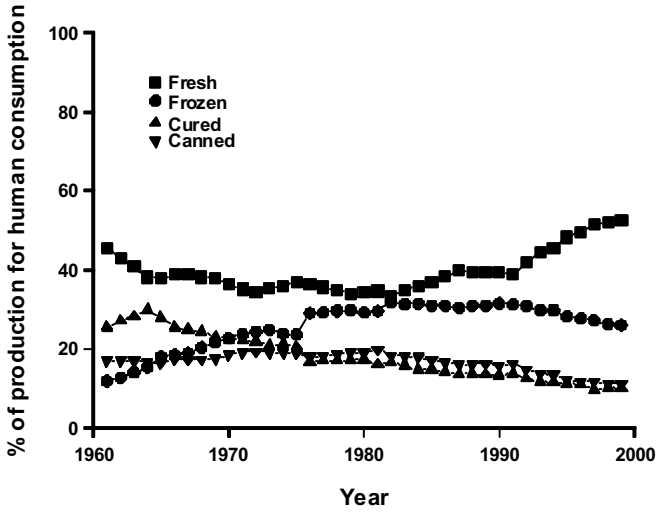

Figure 2.5
Utilization of fish for human consumption (FAO, 2000).

Different regions of the world have very different eating habits with respect to seafoods. Demersal fish such as cod are much preferred in northern Europe and North America, and cephalopods are consumed in several Mediterranean and Asian countries, but to a much lesser extent in other regions. Despite the fast-growing contribution of aquaculture to production, crustaceans are still high-priced commodities and their consumption is mostly concentrated in affluent economies (FAO, 2000).

References

Dierberg, F.E. & Kiattismkul, W. 1996. Issues, impacts and implications of shrimp aquaculture in Thailand. *Environmental Management* 20, 649-666.

FAO (Food and Agriculture Organization). 2000. *The State of World Fisheries and Aquaculture*. FAO, Rome, Italy.

FAO/NACA (Food and Agriculture Organization of the United Nations/Network of Aquaculture Centres in Asia-Pacific). 1995. Regional study and workshop on the environmental assessment and management of aquaculture development (TCP/RAS/2253) *NACA Environment and Aquaculture Series* No. 1. Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand.

3 DEVELOPMENTS IN FOOD SAFETY AND QUALITY SYSTEMS

3.1 Traditional quality control (Hans Henrik Huss/John Ryder)

The traditional quality control program was based on establishing effective hygiene control. Confirmation of safety and identification of potential problems was obtained by end-product testing. Control of hygiene was ensured by inspection of facilities to ensure adherence to established and generally accepted Codes of Good Hygiene Practices (GHP) and of Good Manufacturing Practices (GMP).

Traditional Quality Control
Codes of GHP/GMP
Inspection of facilities and operations
End-product testing

Codes of GHP/GMP are still the basis of food hygiene as outlined in Chapter 7. However, codes – although being essential – only provide for the general requirements without considering the specific requirements of the food and the processing of specific foods. Also the requirements are often stated in very imprecise terms such as "satisfactory", "adequate", "acceptable", "suitable", "if necessary", "as soon as possible" etc. This lack of specifics leaves the interpretation to the inspector, who may place too much emphasis on relatively unimportant matters. He may fail in distinguishing between "what is nice and what is necessary" and consequently increase the cost of the programme without reducing the hazards.

Perhaps one of the most common mistakes that many inspection services and some food companies make is to rely on end-product testing. Very often this has been the only quality and safety assurance system applied. Samples have been taken randomly from the day's production, and examined in detail in the laboratory. There are several problems related to this procedure:

- is costly. A well equipped laboratory will be needed as well as trained personnel. The running costs of a laboratory is high. Also, the cost of products "lost" to testing may be very high;
- the results are retrospective, and all cost and expenses have already been incurred if any hazards are identified in the end-product testing programme. What is needed is a preventive system, where safety hazards are anticipated and safety is built into the product right from the start;
- it may take several days before results from end-product testing are available;
- the chances of finding a hazard will be variable, but most often very low (see below). Nevertheless, the hard work of sampling and testing will give a sensation of "being in control" and create a strong but false sense of security.

It is important to understand the ineffectiveness and limitations in using end-product sampling and testing to ensure product safety. In most cases there is no test that give an absolutely accurate result with no false positives and no false negatives. This is certainly the case for all microbiological testing. Furthermore, there are the principles of sampling and the concept of probability to consider.

3.1.1 Principles of sampling

The number, size and nature of the samples taken for analysis greatly influence the results. In some instances it is possible for the analytical sample to be truly representative of the "lot" sampled. This applies to liquids such as milk and water. However, in cases of lots or batches of food this is not the case, and a food lot may easily consist of units with wide differences in (microbiological) quality. Even within the individual unit (i.e. a retail pack) the hazard (i.e. the

presence of pathogens) can be very unevenly distributed, and the probability of detecting may be very low (Table 3.1).

Table 3.1 Detection probabilities – end-product testing of milk powder contaminated with *Salmonella* (Mortimore and Wallace, 1998).

	Contamination rate	Number of random samples	Probability of detection[1]
Homogenously contaminated	5 cells/kg	10	71%
	1 cell/kg	10	22%
Heterogeneously contaminated	5 cells/kg in 1% of batch	10	<2%
	10^4 cells/kg in 1% of batch	10	<15%

1. Assuming detection test is 100% effective (most are <90%)

In this example, a contamination rate of *Salmonella* at 5 cells/kg and assuming the contamination is restricted to 1% of the batch, the probability of detecting the hazard by taking 10 samples of 25 g would be lower than 2%. If the contamination with *Salmonella* is homogeneously distributed at the same rate, probability of detection would increase to 71%.

A sampling plan (Attributes plan) can be based on positive or negative indications of a micro organism. Such a plan is described by the two figures "n" (number of sample units drawn) and "c" (maximum allowable number of positive results). In a 2-class attributes sampling plan, each sample unit is then classified into acceptable or non-acceptable. In some cases the presence of an organism (i.e. *Salmonella*) would be unacceptable. In other cases, a boundary is chosen, denoted by "m", which divides an acceptable count from an unacceptable. The 2-class sampling plan will reject a "lot" if more than "c" out of "n" samples tested are unacceptable.

In a 3-class sampling plan "m" separates acceptable counts from marginally acceptable counts and another figure "M" is indicating the boundary between marginally acceptable counts and unacceptable counts as shown in Figure 3.1.

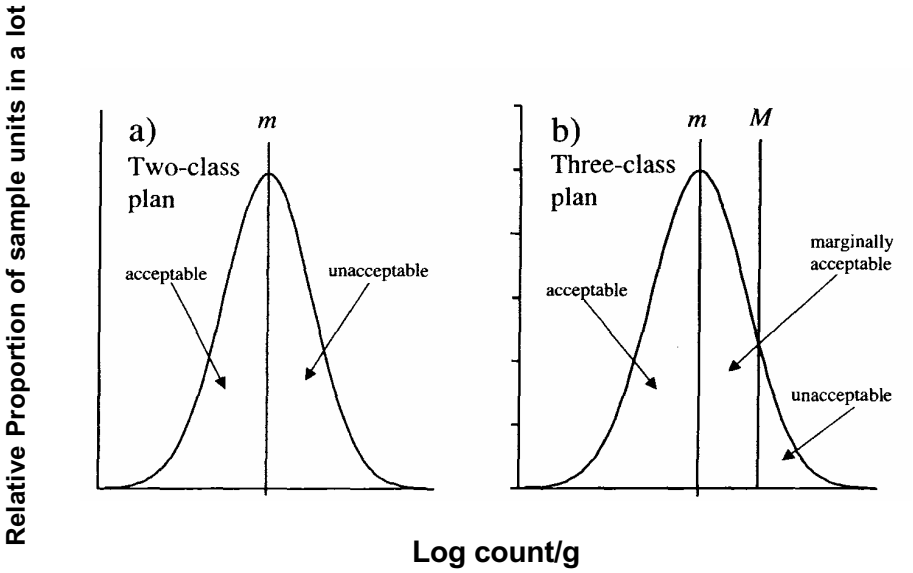

Figure 3.1 Two- and three–class attributes plans (based on ICMSF, 2002).

3.1.2 The concept of probability

The safety which can be obtained with such sampling plans depends on the figures chosen for "c" and "n". This can be illustrated with the so-called operating characteristic curves which are demonstrating the statistical properties of such plans (Figure 3.2).

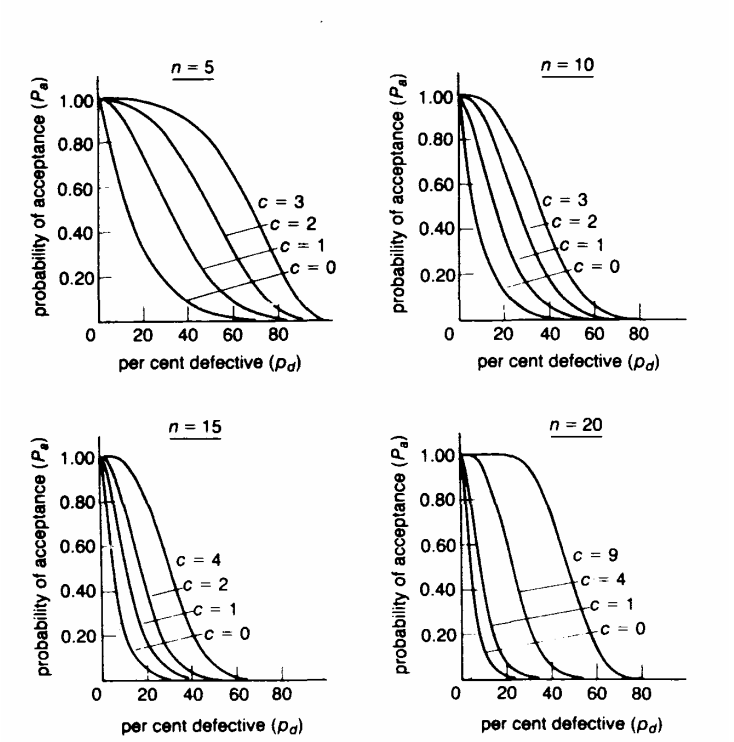

Figure 3.2
Operating characteristic curves for different sample sizes (n) and different criteria of acceptance (c) for 2-class attributes plan (ICMSF, 1986).

The figures show that the greater the number of defective units (P_d), the lower is the probability of acceptance (P_a) of the lot. It is further demonstrated, that high value of "n" and low value of "c" reduces the risk of accepting lots with same number of defective units. It can be seen that testing of foods for the presence of contaminants offers very little protection even when large numbers of samples are examined as also shown in Table 3.2.

Table 3.2 Effect of lot quality (% defective in a lot) on the probability of acceptance (%) for different 2-class sampling plans (based on EC, 1998).

% defective samples in lot	probability of acceptance (%) given sampling plans with a total of "n" samples and allowance of "c" defect samples			
	n=1, c=0	n=5, c=0	n=10, c=0	n=60, c=0
1	99.0	95.1	90.4	54.7
2	98.0	90.4	81.7	30.0
5	95.0	77.4	59.9	4.6
10	90.0	59.1	34.9	0.18
20	80.0	32.8	10.7	0.00015

Table 3.2 clearly shows, that lot testing is not effective when defect rates are low. A product safety defect rate of 1% is absolutely intolerable in many food operations. Potentially, it represents 10 000 unsafe units per one million units manufactured. More than 3 000-5 000 units would need to be sampled and tested in order to detect a 1% defect rate with 95% or 99% probability (Corlett, 1998).

It is evident, that even the most elaborate sampling and testing of end-product cannot guarantee safety of the product. There is no way to avoid some degree of risk and error in each acceptance and each rejection of lots unless the entire lot is tested, in which case no edible food will be left.

3.2 Modern safety and quality assurance methods and systems (Hans Henrik Huss/John Ryder)

To the uninitiated, and also the initiated, there may seem to be a whole host of different options or methods for ensuring the safety and quality of food products. The situation is not helped by the acronyms arising from these methods i.e. ISO, GMP, GHP, HACCP, TQM, etc. seeming to have a life of their own and coming into modern usage as words in themselves, and sometimes used without an understanding of what they mean.

This brief section tries to succinctly define what each of these methods are and what they were designed to achieve.

While this book focuses on the technical aspects of managing quality including safety, it is important to note that companies are also managing other aspects of quality in their companies, which, for instance, could be categorized under managerial and environmental concerns. These are expanded upon in the table below (Table 3.3). The table is more indicative than exhaustive and merely serves to highlight the main items that need to be considered in managing quality in a company. It is maybe obvious to state that it is vital to ensure that all these factors are managed effectively and efficiently in order for companies to survive in today's competitive environments. Unfortunately, it is not uncommon to find companies ignoring these principles.

Table 3.3 Categorization of items to be managed in a company.

Management concern	Items to be managed
Technical	Intrinsic quality of fish (taste, smell and texture); safety; spoilage/ freshness; grading; packaging; nutritional; authenticity; shelf life, etc.
Managerial	Administrative systems; customer relations; promotion; delivery commitments; invoicing and payment, etc.
Environmental	Waste and water management; noise pollution; odeurs; pollutants, etc.

3.2.1 Methods to manage quality and safety

So, what is there in existence to manage quality and safety, and how do they relate to each other?

Below are listed the most well known methods to manage quality and/or safety, and these will be briefly discussed individually and then how they integrate with each other.

- Good Hygienic Practices (GHP) / Good Manufacturing Practice (GMP) or Sanitation Standard Operating Procedures (SSOP) or prerequisite programmes
- Hazard Analysis Critical Control Point (HACCP)
- Quality Control (QC)
- Quality Assurance (QA) / Quality Management (QM) - ISO standards
- Quality Systems
- Total Quality Management (TQM).

The food safety tools and their relationship is shown in Figure 3.3.

Good Hygienic Practices / *Good Manufacturing Practices*

The terms GHP and GMP basically covers the same ground as discussed in Chapter 7. They refer to measures and requirements which any establishment should meet to produce safe food. These requirements are prerequisites to other and more specific approaches such as HACCP, and are often now called prerequisite programmes. In recent years the term Standard Sanitary Operating

Procedures (SSOP) has also been used in the US to encompass basically the same issues, i.e. best practices.

Hazard Analysis Critical Control Point

Hazard Analysis Critical Control Point (HACCP) is a systematic approach which identifies, evaluates, and controls hazards which are significant for food safety (CAC, 1997). HACCP is discussed in great detail throughout this book. In the context of this section, HACCP ensures food safety through an approach that builds upon foundations provided by good manufacturing practice. It identifies the points in the food production process that require constant control and monitoring to make sure the process stays within identified limits. Statistical Process Control systems are relevant to this operation.

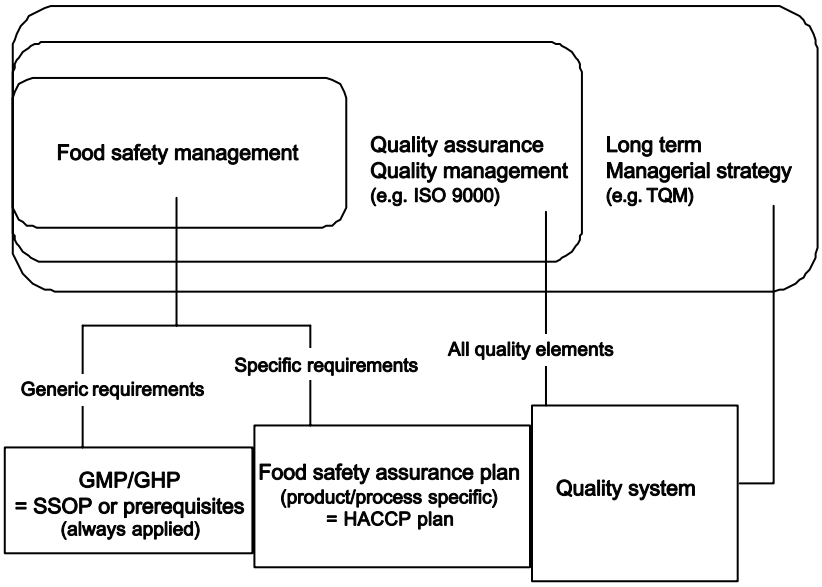

Figure 3.3 Food safety tools: an integrated approach (modified from Jouve *et al.,* 1998).

HACCP is legislated in many countries, including the USA and the European Union. The combination of GHP/GMP and HACCP is particularly beneficial in that the efficient application of GHP/GMP allows HACCP to focus on the true critical determinants of safety.

Quality Control

Quality control (QC)
Can be defined as the operational techniques and activities that are used to fulfil quality requirements (Jouve *et al.,* 1998)

It is an important subset of any quality assurance system and is an active process that monitors and, if necessary, modifies the production system so as to consistently achieve the required quality.

It can be argued that QC is used as part of the HACCP system, in terms of monitoring the critical control points in the HACCP plan. However, traditional QC is much broader than purely this focus on critical control points for safety systems. The pitfalls of relying on QC procedures, more importantly as end product testing, have been detailed in section 3.1 and will not be expanded upon here.

Quality Assurance / Quality Management

This can be defined as all the activities and functions concerned with the attainment of quality in a company. In a total system, this would include the technical, managerial and environmental aspects as alluded to above. The best known of the quality assurance standards is ISO 9000 and for environmental management, ISO 14000.

The term quality management is often used interchangeably with quality assurance. In the seafood industry, the term quality management has been used to focus mostly on the management of the technical aspects of quality in a company, for instance, the Canadian Quality Management Programme which is based on HACCP but covers other technical issues such as labelling.

ISO Standards

The International Organization for Standardization (ISO) in Geneva is a worldwide federation of national standards bodies from more than 140 countries.

The mission of ISO is
to promote the development of standardization and related activities in the world with a view to facilitating the international exchange of goods and services, and to developing cooperation in the spheres of intellectual, scientific, technological and economic activity (www.iso.org)

ISO's work results in international agreements which are published as International Standards. The vast majority of ISO standards are highly specific to a particular product, material, or process. However, two standards, ISO 9000 and ISO 14000, mentioned above, are known as generic management system standards.

Over half a million ISO 9000 certificates have been awarded in 161 countries and economies around the world and in 2001 alone over 100 000 certificates were awarded, 43% of which were the new ISO 9001:2000 certificate.

Historically, the ISO 9000 series of standards of relevance to the seafood industry included:

- ISO 9001 Quality systems - Model for quality assurance in design/ development, production, installation and servicing
- ISO 9002 Quality systems - Model for quality assurance in production and installation.

More recently, the new ISO 9001:2000 certificate is the only ISO 9000 standard against whose requirements a quality system can be certified by an external agency and replaces the old ISO 9001, 9002 and 9003 with one standard.

It is important to note that the ISO 9000 standards relate to quality management with customer satisfaction as the end point, and that they do not specifically refer to technical processes only. ISO 9000 gives an assurance to a customer that the company has developed procedures (and adheres to them) for all aspects of the company's business.

ISO 14000 is primarily concerned with environmental management. Introduced much later than the ISO 9000 series, there are now over 35 000 ISO 14000 certificates awarded in 112 countries or economies of the world. During 2001, nearly 14 000 certificates were awarded, around 40% of the total awarded since the introduction of the standard.

In most countries, implementation of ISO 9000 quality management systems or ISO 14000 environmental systems are voluntary.

Quality Systems

This term covers organizational structure, responsibilities, procedures, processes and the resources needed to implement comprehensive quality management (Jouve *et al.* 1998). They are intended to cover all quality elements. Within the framework of a quality system, the prerequisite programme and HACCP provides the approach to food safety.

Total Quality Management (TQM)

TQM is an organization's management approach, centred on quality and based on the participation of all its members and aimed at long-term success through customer satisfaction and benefits to the members of the organization and to society (Jouve *et al.* 1998). Thus TQM represents the organizations' "cultural" approach and together with the quality systems provides the philosophy, culture and discipline necessary to commit everybody in the organization to achieve all the managerial objectives related to quality.

3.3 Risk analysis, food safety objectives (Lone Gram)

The management and control of (sea)food borne diseases is carried out by several groups of people. It involves experts assessing the risk, i.e. providing the epidemiological, microbiological and technological data about the pathogenic agent, the food, the host etc. It involves risk managers who at government level have to decide what level of risk society will tolerate and risk managers in both industry and government that have to implement procedures to control the risk. At industry level this is done using GHP and HACCP procedures as described below.

The term "risk analysis" it the process underlying development of food safety standards (FAO/WHO, 1997). It consists of three separate but integrated parts, namely risk assessment, risk management and risk communication. The risk analysis process must be open and at every step all stakeholders should be allowed to participate and comment. It has been seen as important that there is a separation between the risk management and the risk assessment (FAO/WHO, 1995). The risk assessment is a science based evaluation whereas risk management (at government level) also involves a range of societal issues.

The objective of the rules that govern international trade with food, the WTO/SPS[1] agreement, is to permit countries to set certain safety measures for their population and ask that imported foods allow the same level of public health protection. To justify and compare the levels of public health protection and food safety measures, risks must be analysed using the risk assessment techniques described by Codex (CAC, 1999).

Analysis of risk includes the following steps:

- identification of a food safety problem
- assessment of the risk
- establish a public health goal, e.g. expressed as a food safety objective
- implement risk management decisions
- establish performance criteria
- establish process and product criteria
- establish acceptance criteria
- communication of risk.

[1] *The rules were agreed during the Uruguay Round of Trade Negotiations and apply to members of the World Trade Organization (WTO). Food safety matters are ruled by the Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS agreement).*

Identification of a food safety problem

A food safety problem may be identified either through a sudden change in disease frequency, i.e. epidemiological data indicate a sudden rise in a particular disease, or the hazard analysis carried out as part of the HACCP system may indicate reason for concern. This could be caused by implementation of new processing technologies, or by changes occurring in population composition.

Assessment of the risk

Evaluating the risk associated with the problem involves estimating the severity of the disease and the likelihood of occurrence. Basically, the magnitude of the problem to public health is being determined. This evaluation of risk can be done by just one or two experts, by an expert panel or a so-called quantitative risk assessment may be conducted. Whether one or the other is chosen depends on the urgency of the matter – sometimes a risk management decision has to be made immediately – and of the complexity and its implications for international trade.

The term ”quantitative risk assessment” can be a bit misleading, since any evaluation of risk requires considerations of quantitative aspects. However, it has recently been used to describe a lengthier and structured process in which the impact of different factors from farm to fork that contribute to risk are quantified. Typically this process involves the use of mathematical modelling at several steps using Monte Carlo simulations. An example of a quantitative risk assessment is the FAO/WHO work on *Listeria monocytogenes* in ready to eat foods (FAO/WHO, 2001). One result of the risk assessment is the graphical representation of dose-response curve in which the likelihood of disease is presented as a function of levels of *L. monocytogenes* consumed (Figure 3.4).

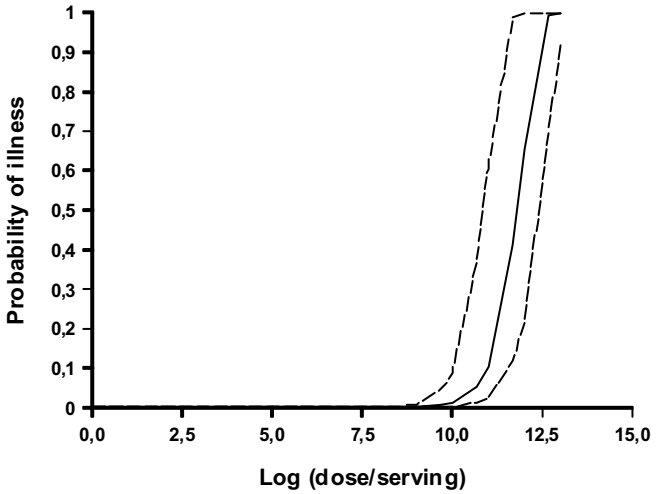

Figure 3.4
Simulated dose-response function for *Listeria monocytogenes* in ready to eat foods for consumers in the high risk group. Based on FAO/WHO (2001).

The graph clearly demonstrates that the risk of disease is related to consumption of high numbers of the organism. However, if the risk is expressed as the log value it becomes evident that there is no threshold value below which the risk disappears but even a few cells do carry some, albeit very low, level of risk (Table 3.4). This curve can be used to determine how many cases a particular level of consumption of a pathogen leads to. Based on the consumption pattern and data from the FDA/FSIS risk assessment as well as the risk characterization curve from the same study (FDA/FSIS, 2001), one can predict how many cases are the result of different levels at point of consumption (FAO/WHO, 2001).

The data in Table 3.4 are based on the US situation. The numbers add up to approximately 2 100 comparable to the reported number of cases of approximately 2 500 per year (in a population of a total of 280 million people). Two things are apparent: i) that it is especially the high doses that cause the problem and ii) that even the lowest number of cells carry a low risk of disease.

Establish a public health goal

When determining a public health goal, risk is most often expressed as a number of cases of illness per capita per year. For instance, the level of listeriosis cases in the US is 0.5 per 100 000 of the population per year and recently, the White House announced that this had to be reduced to 0.25 cases per 100 000 of the population per year.

Several terms exist for such public health goals. Ideally, the goal would be to reduce all (sea)food borne diseases to "zero risk", however, this is technically and financially not possible. It is important to understand that there is no such thing as "absence of risk". Therefore, the public health goal is expressed using different terms such as "appropriate level of protection" (ALOP). Realising that no risk is really ever appropriate, the ICMSF (2002) has suggested to use the term "tolerable level of risk" (TLR).

Table 3.4 Baseline number of cases of listeriosis from ready-to-eat foods as predicted by the FDA/FSIS dose-response model (after FAO/WHO 2001).

Maximum log dose at consumption (log CFU/serving)	Number of servings at the specified dose	Number of cases[1] per year attributed to a specified dose level	Comment
-1.5	5.93×10^{10}	0.01	1 case per 100 years
-0.5	2.50×10^{9}	0.005	1 case per 200 years
0.5	1.22×10^{9}	0.02	1 case per 50 years
1.5	5.84×10^{8}	0.1	1 case per 10 years
2.5	2.78×10^{8}	0.5	1 case per 2 years
3.5	1.32×10^{8}	2.4	2.4 cases per year
4.5	6.23×10^{7}	11.5	etc.
5.5	2.94×10^{7}	54.4	
6.5	1.39×10^{7}	25.7	
7.0	3.88×10^{6}	228	
7.5	2.67×10^{6}	1 580	
8.0 and above	very few	227	
	6.41×10^{10}	2 130	Total

1. The number of cases is predicted based on the dose and the number of servings containing that dose.

Food Safety Objective

Levels of disease attack rate are difficult to measure and target by food managers in government and industry and therefore the term **Food Safety Objective** (FSO) has been introduced. The FSO translates risk into a measurable goal and is expressed as the concentration or frequency of a hazard in a food [at point of consumption] that is considered "safe" or meeting the level of protection/risk set by society. The FSO has been used in broad terms by several (Jouve, 1996; Hathaway, 1997) but was explicitly defined by the ICMSF (van Schothorst, 1998).

Food Safety Objective
Concentration or frequency of a hazard in a food [at point of consumption] that is considered safe or meeting the level of protection set by society

If a quantitative risk assessment has been conducted, the FSO is simply the translation for the Y-axis (with disease risk or cases) to the X-axis (with the number or frequency of the pathogen).

FSOs can – and are often – set even when quantitative risk assessments and the risk characterization curve are not available. Investigations of food borne diseases, epidemiological surveillance programmes, industry records and knowledge of the influence of food processing parameters can (and has for decades) provided information about which foods cause adverse

health effects, which pathogens are implicated, and, to some extent, which levels of pathogens are involved. In effect, the setting of microbiological criteria for foods has been and is an indirect way of setting an FSO – and thus implies a desired public health goal. Many examples of this are present. One is the standard for *Staphylococcus aureus* in cooked crustaceans (n=5, c=2, m=100/g and M=1000/g). This criteria contains an evaluation of the risk related to the concentration of the hazard (growth and high concentrations are required to produce the amount of enterotoxin causing disease) (FAO/WHO, 2002).

It is important to realise that FSOs are not equivalent to microbiological criteria but that, if appropriate, criteria can be derived from FSOs. An FSO is a public health goal whereas a microbiological criteria defines acceptability of a food product or a lot of foods and should indicate sampling plan, method, number of units that must conform etc. (see Chapter 13). An example of an FSO is a concentration of 100 *L. monocytogenes* per gram at point of consumption for ready-to-eat-foods (van Schothorst, 1998; ICMSF, 1994). Criteria for *L. monocytogenes* at earlier points in the chain will typically be lower than the 100 cfu/gram.

It must be evaluated if the FSO as expressed by risk managers is achievable. If not, it must be decided (i) if changes in the industry has to be enforced, (ii) if the product should be taken off the market or (iii) if the product should be labelled as carrying a risk. Examples of such procedures are (i) the mandatory pasteurisation of milk, (ii) the ban of tetrodotoxin containing fish species for the EU market and (iii) the notice by restaurants in several US states that eating raw oysters may be detrimental to health. Examples of FSOs are shown in Chapter 12.

Implement risk management decisions

When a public health goal has been set, it is the responsibility of risk managers in industry (and government) that measures are taken to control the risk. With respect to food-borne pathogens, the risk can in principle be controlled at three levels:

- the initial level of the pathogen
- reducing the level of the pathogen or
- preventing increase of the pathogen.

The primary tools available to the food industry to control safety risks are GHP and HACCP programmes. Incorporated into these programmes may be various processes and criteria that ensure that the FSO (ultimately) is met.

A *performance criteria* describes the outcome of a process or step. This can for instance be that a canning procedure should ensure a 12D kill of *C. botulinum* spores or that only 3% of freshly produced cold-smoked salmon must contain *L. monocytogenes*.

Process and *product criteria* are statements of values for specific processes, such as time x temperature combinations during hot-smoking, or values such as NaCl-% and pH in the product. For instance, the control of *C. botulinum* in lightly preserved fish is not carried out by sampling and testing for *C. botulinum* but by ensuring that the combination of salt and temperature is sufficient to prevent growth.

Acceptance criteria are measurements or statements of conditions that distinguish acceptable from non-acceptable products. These may be based on sensory evaluations, on chemical measurements and may in some cases be *microbiological criteria*. These should specify the agent to be measured, the number of samples and the method used. As described later (Chapter 13), sampling and microbiological testing is best used for detection of high concentrations or frequencies of microorganisms.

Overall the interaction between government's and industry's roles in food safety activities can be described as below (Figure 3.5).

Risk communication

An integral, and very important step, in all stages of a risk analysis is the communication of risk to stakeholders, including industry and consumers. An important part of the risk communication is using the findings of the risk assessment for training purposes and in the process of setting specifications.

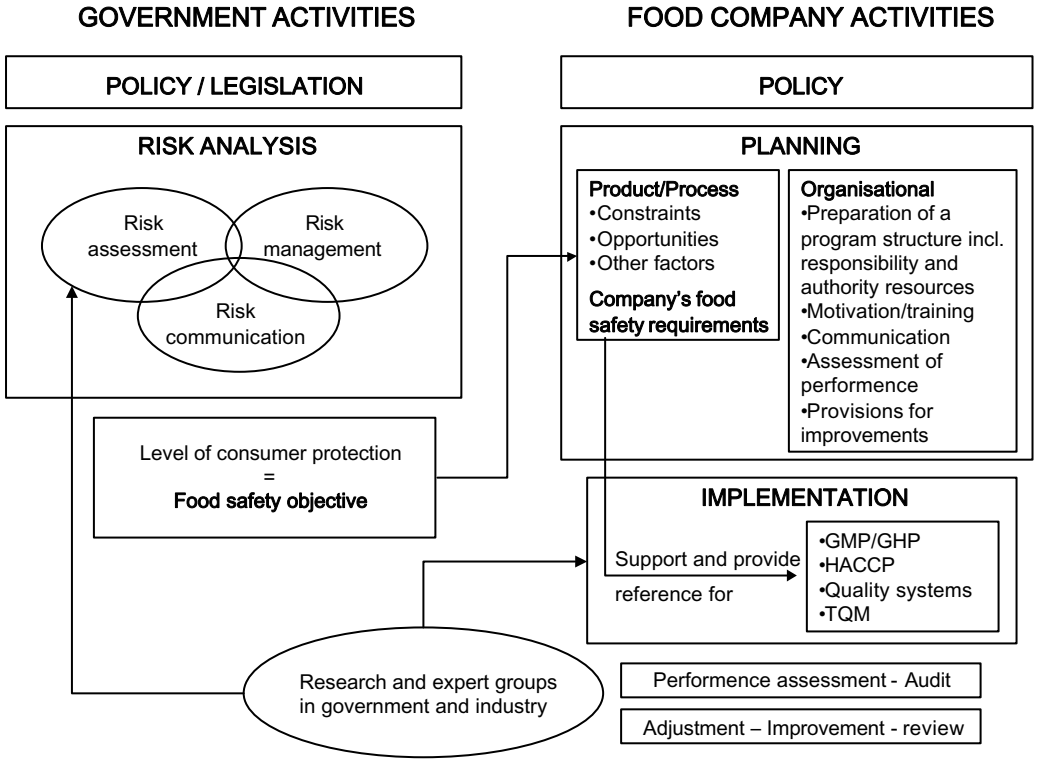

Figure 3.5 Interaction between the government's and industry's food safety activities (modified from Jouve 2000, Jouve *et al.,* 1998).

References

CAC (Codex Alimentarius Commission) 1999. *Principles and Guidelines for the Conduct of Microbiological Risk Assessment*. CAC/GL-30. Food and Agriculture Organization / World Health Organization, Rome, Italy.

CAC (Codex Alimentarius Commission) 2001. *Food Hygiene. Basic Texts*. 2nd ed. Food and Agriculture Organization / World Health Organization, Rome, Italy.

Corlett, Jr. D.A. 1998. *HACCP Users Manual*. An Aspen Publication, Gaithersberg, Maryland, USA.

EC (European Commission) 1998. Food – Science and Techniques. Reports on tasks for scientific cooperation. Microbiological criteria. Collation of scientific and methodological information with a view to the assessment of microbiological risk for certain foodstuffs. EUR 17638.

FAO/WHO (Food and Agriculture Organization/World Health Organization) 1995. Joint FAO/WHO Expert Consultation on the application of risk analysis to food safety standards. 13-17 March, Geneva, Switzerland.

FAO/WHO (Food and Agriculture Organization/World Health Organization) 1997 Joint FAO/WHO Expert Consultation on risk management and food safety. 27-31 January, Rome, Italy.

FAO/WHO (Food and Agriculture Organization/World Health Organization) 2001. Risk Assessment of *Listeria monocytogenes* in ready-to-eat foods. Preliminary report. Authors Buchanan, R., R. Lindqvist, T. Ross, E. Todd, M. Smith and R.C. Whiting. FAO/WHO, Rome, Italy.

FAO/WHO (Food and Agriculture Organization/World Health Organization) 2002. Joint FAO/WHO Expert Consultation on risk the elaboration of Principles and guidelines for incorporating

quantitative risk assessment in the development of microbiological food hygiene standards, guidelines and related texts. 18-22 March, Kiel, Germany.

FDA/FSIS (US Food and Drug Administration/Food Safety and Inspection Services) 2001. Draft Assessment of the Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods. Washington DC, USA.

Hathaway, S.C. 1997. Development of risk assessment guidelines for foods of animal origin in international trade. *Journal of Food Protection* 60, 1432-1438.

ICMSF (International Commission on Microbiological Specification for Foods) 1986. *Microorganisms in Foods 2. Sampling for Microbiological Analysis: Principles and Specific Applications*. University of Toronto Press, Toronto, Canada.

ICMSF (International Commission on Microbiological Specifications for Foods) 1994. Choice of sampling plans and criteria for *Listeria monocytogenes International Journal of Food Microbiology* 22, 89-96.

ICMSF (International Commission on Microbiological Specifications for Foods) 2002. *Microorganisms in Foods 7. Microbiological testing in food safety management*. Aspen Publishers, Inc., Gaithersberg, Maryland, USA.

Jouve, J.L. (ed) 1996. *La Qualité Microbiologique des Aliments: Maitrise et Critéres*. 2nd ed. CNERNA/CNRS, Paris, France.

Jouve, J.L. 2000. Good manufacturing practices, HACCP and quality systems. In: Lund, B.M., T.C. Baird Parker and G.W. Gould (eds) *The Microbiological Safety and Quality of Foods*. Aspen Publishers. Gaithersberg, Maryland, USA. pp.1627-1655**.**

Jouve, J.L., M.F. Stringer & A.C. Baird-Parker 1998. *Food safety Management Tools*. ILSI Europe Risk Analysis in Microbiology, Brussels, Belgium.

Mortimore, S. & Wallace, C. 1998. *HACCP. A Practical Approach*. Aspen Publishers Inc. Gaithesberg, Maryland. USA.

van Schothorst, M. 1998. Principles for the establishment of microbiological food safety objectives and related control measures. *Food Control* 9, 379-384.

Part I: Aspects of Seafood Risk Assessment

4 IDENTIFICATION OF HAZARDS IN SEAFOOD

4.1 Statistics on seafood-borne diseases (Lone Gram)

The true incidence of diseases transmitted by foods is not known. There are many reasons for this. In most countries there is no obligation to report on food borne diseases to public health authorities. In the few countries which have a reporting system there is severe underreporting. It has been estimated that as few as 1% of the actual cases of food-borne diseases are reported (Mossel, 1982). This is because neither the victim nor the physician are aware of the etiological role of foods. Furthermore, the food responsible is often not available for analysis and the true vehicle for the disease agent is not identified. The statistics presented should therefore be used as indications of trends and areas of concern.

The Centre for Disease Control (CDC) in Atlanta compiles all information on food-borne disease in the US. Between 1993 and 1997, 2 751 outbreaks involving 86 000 people were reported (Table 4.1). In only 1/3 of the outbreaks was a food vehicle identified. Seafoods were often implicated in disease but did not, as opposed to some other foods, result in deaths. As products such as meat and poultry are consumed in much larger amounts, the number of cases traced to seafood is rather alarming.

Table 4.1 Food implicated in food-borne disease in the US 1993-1997 (modified from Olsen *et al.,* 2000).

Food	Outbreaks		Cases		Deaths	
	Number	%	Number	%	Number	%
Meat	66	2.4	3 205	3.7	4	13.8
Pork	28	1.0	988	1.1	1	3.4
Poultry	52	1.9	1 871	2.2	0	0.0
Other meat	22	0.8	645	0.7	2	6.9
Shellfish	47	1.7	1 868	2.2	0	0.0
Fish	140	5.1	696	0.8	0	0.0
Egg	19	0.7	367	0.4	3	10.3
Dairy products	18	0.7	313	0.4	1	3.4
Ice cream	15	0.5	1 194	1.4	0	0.0
Bakery goods	35	1.3	853	1.0	0	0.0
Fruits and vegetables	70	2.5	12 369	14.4	2	6.9
Salads	127	4.6	6 483	7.5	2	6.9
Other	66	2.4	2 428	2.8	0	0.0
Several foods	262	9.5	25 628	29.8	1	3.4
Total known foods	967	35.2	58 908	68.5	16	55.2
Total unknown food	1 784	64.8	27 150	31.5	13	44.8
TOTAL	2 751	100.0	86 058	100.0	29	100.0

In the USA, the etiological agent was identified in approximately 50% of the outbreaks caused by shellfish (both molluscan shellfish and crustaceans) whereas the cause of disease was identified in almost 90% of the outbreaks related to fish (Olsen *et al.*, 2000). It is likely that several of the outbreaks caused by molluscan shellfish for which a cause was not identified were indeed viral. This could, in part, be explained by the lack of methods for detecting foodborne virus.

Outbreak Alert (CSPI, 2001) lists outbreaks/cases in which an etiological agent has been identified. From 1990 to 1998, more than 5 000 cases of seafood borne diseases were linked to a cause.

Molluscan shellfish, although being responsible for a much lower number of outbreaks than fish, caused the double the number of cases.

Table 4.2 Number of outbreaks and cases related to seafood in the US from 1990 to 1998. Listed are only outbreaks for which an etiological agent has been identified (CSPI, 2001).

Seafood group	Outbreaks	Cases
Fish	263	1 661
Molluscan shellfish	66	3 281
Other shellfish	8	146
Total	337	5 088

A total of 1 661 cases were caused by consumption of "fish" (Table 4.2). The majority of cases were caused by scombroid or ciguatera intoxication (Table 4.3). Also, several outbreaks of botulism were recorded as were more than 300 cases of salmonellosis. These outbreaks were, however, not universally distributed. Thus the vast majority of ciguatera outbreak occurred in Hawaii or in Florida where the consumption of tropical reef fish is high. Similarly, three fourth of the botulism cases were registered in Alaska and were attributed to the consumption of various fermented seafood preparations.

Etiological agents were identified in more than 3 000 cases of disease caused by molluscan shellfish (Table 4.4). Bacteria indigenous to the marine environment, e.g. *Vibrio* spp. did cause several cases, but organisms from the human-animal reservoir were the dominant causes. This included the major cause of disease, viral gastroenteritis, in particular Norwalk virus, but also *Salmonella* and *Shigella* were responsible for outbreaks.

Shellfish other than molluscan shellfish also caused disease. Etiological agents were identified in 146 cases of food-borne disease from 1990 to 1998 (CPIS, 2001). These were caused by Norwalk virus (one outbreak, 46 cases), *Salmonella* (one outbreak, 45 cases), *Campylobacter* (one outbreak 32 cases), *Vibrio parahaemolyticus* (one outbreak, 7 cases), *Staphylococcus aureus* (one outbreak, two cases) and 3 outbreaks of *V. cholerae* (14 cases).

Table 4.3 Seafood borne diseases traced to "fish" in the USA from 1990 to 1998. Outbreaks and cases for which the etiological agent has been identified (CSPI, 2001).

	Outbreaks					Cases				
Agent	**total**	**%**	**Hawaii**	**Florida**	**Alaska**	**total**	**%**	**Hawaii**	**Florida**	**Alaska**
Scombroid	131	50	46	10	0	759	47	287	55	0
Ciguatera	98	37	73	16	0	394	24	260	82	0
Botulism	14[1]	5	1	0	10	43	3	3	0	30
Salmonella	11	4				305	18			
Haff disease[2]	2	1				6	-			
S. aureus	1	-				2	-			
E. coli O157	1	-				3	-			
V. cholerae	1	-				26	2			
C. perfringens	1	-				25	2			
Norwalk	1	-				37	2			
Tetrodotoxin	1	-				3	-			
"chemical"	1	-				58	4			
Total	263	100				1 661	100			

1. *One outbreak in New Jersey (salted whitefish) and two in California (both home-canned tuna)*
2. *Haff disease is an unexplained rhabdomyolysis (the breakdown of muscle fibres with leakage of potentially toxic cellular contents into the systemic circulation) in a person who ate fish in the 24 hours before onset of illness.*

Table 4.4 Seafood borne diseases traced to "molluscan shellfish" in the USA from 1990 to 1998. Outbreaks and cases for which the etiological agent has been identified (CSPI, 2001).

	Outbreaks		Cases	
Agent	**total**	**%**	**total**	**%**
V. parahaemolyticus	18	27	733	22
Norwalk / virus	15	23	2 175	66
PSP / toxin	14	20	92	3
Salmonella	6	9	183	6
Scombroid	2	3	4	-
Ciguatera	3	5	5	-
Shigella	2	3	17	0.5
Campylobacter	2	3	6	-
V. vulnificus	1	-	2	-
V. alginolyticus	1	-	4	-
C. perfringens	1	-	57	2
Giardia	1	-	3	-
Total	66	100	3 281	100

Between 1992 and 1999, 1 425 foodborne outbreaks of Infectious Intestinal Disease (IDD) were reported in the UK (Gillespie *et al,.* 2001). This represented one third of all infectious intestinal disease outbreaks reported (Table 4.5). Ten percent of the 1 425 foodborne outbreaks were caused by seafoods. Of the 148 outbreaks traced to seafood, 47% were traced to finfish and most were caused by scombroid toxin. These outbreaks typically occurred in the warm summer months. Molluscan shellfish were responsible for one third (36%) of the outbreaks and these were typically associated with viral infections from live oysters. The last major cluster was outbreaks caused by crustaceans (11%) which typically involved viral pathogens or salmonellae. Salmonellae were also involved in four outbreaks traced to finfish.

Table 4.5 Etiological agents of foodborne outbreaks in UK associated with seafood (Gillespie *et al.,* 2001).

Agent	**No. of outbreaks 1992-1999**							
	Total[1]	**Food-borne**	**Seafood**		**Fish**	**Molluscs**	**Crustaceans**	**Other**
			suspected	**confirmed**				
Scombrotoxin				47	47	0	0	0
DSP				1	0	1	0	0
Virus				26	0	21	3	2
Salmonella				14	7	1	4	2
Campylobacter				3	1	0	1	1
S. aureus				1	0	0	1	0
B. cereus				1	1	0	0	0
C. perfringens				3	1	0	1	1
Unknown				52	12	31	7	2
Total	4 603	1 425	181	148	69	54	17	8

1 Total number of reported intestinal disease outbreaks

4.2 Detentions and rejections of seafood in international trade (Lone Gram/Lahsen Ababouch)

Seafoods constitute a major commodity in international trade and despite the introduction of quality assurance schemes in the sector, various sampling and control analysis of end products are carried out, particularly of imported foods at port of entry. Section 4.1 on seafood borne diseases gives indications of the health consequences of biological hazards, but results from import controls may also point to areas of concern.

In the USA, the Food, Drug and Cosmetic Act authorizes FDA to detain a regulated item that appears to be out of compliance with the act (FDA, 2002). This covers a vast range of commodities: foods, beverages, drugs, cosmetics, animal feed, chemicals, orthopaedic equipment etc. Each month, the import refusal report (IRR) is published based on data generated by the FDA's Operational and Administrative Import Support (OASIS). The data are available by country or by product commodity. Approximately 1/10 of the refused products are seafood products (Table 4.6).

The most common reason for import refusal is "filthy" which describes that the product appears to consist in whole or in part of a filthy, putrid or decomposed substance. Although details are not given for the individual products, it is assumed that microbial spoilage is the major reason for the refusal. Second in terms of rejection reason is the detection of *Salmonella*. Both cooked, ready-to-eat products and raw, frozen products are rejected if *Salmonella* is detected. Although *Salmonella* has its niche in the gastrointestinal tract of birds and mammals, it is a common bacterium in ponds in tropical areas and its detection may not indicate hygienic failure. Whether or not the detection in raw foods constitute a health hazard is debatable.

The category "other" covers a vast range of different reasons such as mis-labelling, lack of description of the process, or lack of verification of a HACCP plan.

Table 4.6 Seafood import refusals by US FDA from July 2001 to June 2002 (FDA, 2002).

Year	Month	No. refused		No. of seafood import refusals according to reason					
		Total	Seafood	Filthy	*Salmonella*	*Listeria*	Histamine	Poison	Other
2001	July	1497	122	74[1]	20	5	2	4	21
	Aug	954	146	79	40	3	3	4	25
	Sep	906	59	27	14	7	0	2	11
	Oct	1082	136	59	50	2	3	4	26
	Nov	1079	121	51	39	4	0	1	26
	Dec	826	83	57	18	2	2	5	7
2002	Jan	1452	177	84	71	2	6	1	42
	Feb	1569	184	84	35	12	4	0	64
	Mar	1630	213	90	38	8	4	4	73
	Apr	1381	126	60	20	0	0	5	43
	May	1621	174	72	41	1	1	5	64
	Jun	1525	143	80	41	3	2	2	34

1. Number of rejections where "filthy" is stated as a reason. Note that for some products several reasons, e.g. both "filthy" and "Salmonella" are given as reason for rejection.

The European Commission is operating a Rapid Alert system for foodstuffs. The system is used to inform Member States about problems or risks concerning foods which do not meet food safety requirements. The legal basis for the system is Council decision 92/59/EEC (EC, 1992) on general product safety. The principal objective is to prevent the placement on or the recall from the community market foodstuffs which pose a serious risk to the health of the consumer. Member States notify the Commission when

- a foodstuff poses a serious risk to the health and safety of consumers, and
- the probability that the foodstuff is on the market in another Member State.

The data from 1999 were compiled by Huss (unpublished) who concluded that in 1999, 107 seafood products were involved in Rapid Alerts (out of 295 in total). The main products and the main reasons for the Alerts were: chilled and frozen fish (or fish products) were implicated in 75 Alerts. The reason was primarily the presence of pathogenic bacteria (*Vibrio* spp., *Salmonella*, *Listeria monocytogenes*, *Staphylococcus*, Enterobacteriaceae, "aerobic mesophiles"), but also a number of chemical dangers were listed (heavy metals, pesticide-residues) shrimp, cray-fish tails, crab-tails (without specification of whether they were raw or cooked) were implicated in 30 Alerts,

and the reason was always the presence of pathogenic bacteria (pathogenic *Vibrio* spp., *Salmonella*, *Staphylococcus*) tuna-fish products (canned, frozen or fresh) were involved in 6 Alerts: too high content of histamine (3), mercury (1) or presence of *Salmonella* or "aerobic mesophiles" detection of biotoxins, viruses or indicator bacteria (faecal coliforms, *E.coli*) in bivalve molluscs (8) presence of pathogenic bacteria in a number of un-specified seafood.

In an ongoing study, Ababouch and Gandini (unpublished) analysed the EU Rapid Alert System data of interest to Third Countries, i.e. non EU countries exporting fish and fishery products to the EU member states. The analysis encompassed the period from January 1999 to June 2002 (Table 4.7).

These data indicate that the number of alerts has increased steadily during the period January 1999 – December 2001 and basically exploded in 2002. The initial steady increase and the explosion of alerts in 2002 are due to several concurrent facts:

- The alert system has become generalized and fully operational only during the last 12 or 18 months, indicating some underreporting in the initial phase;
- Several safety concerns have emerged during the period 2001-2002 which triggered several additional controls at the entry point to the EU, e.g. analysis of *Vibrio*, analysis of antibiotic residues and other chemical pollutants (polycyclic aromatic hydrocarbons), following the enacting of recent EU regulations to monitor these residues in fish and fishery products marketed in the EU;

Regarding the cause of rejection/detention (Table 4.7), chemical and drug residues (46.4%), followed by microbial contaminants (39.7%) were the main causes for alert during the period 1999-2002. The majority of alerts because of chemical and veterinary drugs residues (74.4%) occurred recently in 2002, with chloramphenicol and nitrofurans representing respectively 54% and 24.5% of the alerts caused by chemical hazards and 39.6% and 18% of the total. Histamine and parasites caused the lowest rates of alerts, respectively 1.3% and 4%;

For microbial contaminants, there was a decrease (from 59.3 % in 1999 to 41% in 2001) of alerts due to the presence of indicator organisms and an increase (from 40.1 % in 1999 to 59.2 % in 2001) of alerts because of the presence of indigenous organisms, especially *Vibrios*. The former indicates improvement in the sanitary and hygienic conditions in handling and processing fish in their countries, probably as a result of the gradual implementation of GHP/GMP and HACCP. The latter reflects more recent decisions of the EU to analyse for indigenous microorganisms, especially *Vibrio* species while awaiting the results of risk assessments of Vibrios in seafood. In the meantime, the temporary EU decisions have led to rejections and detention of consignments that were probably safe to consume and have led to economic losses by exporters.

In fact, a risk assessment commissioned in 2001 by the European Commission (EC, 2001) concluded that:

i) the practice of judging seafood exclusively based on total Vibrio counts as indicative for the presence of pathogenic Vibrios is not appropriate and should be discontinued.

ii) the practice of judging seafood exclusively based on total *V. Paraheamolyticus* counts without consideration of the virulence factors (TDH/TRH (or *tdh*/*trh*) is not appropriate and should be discontinued;

iii) currently available scientific data do not support setting specific standards or microbiological criteria for *V. Vulnificus* and *V. Parahaemolyticus* in seafood. Codes of practice should be established to ensure that GHP has been applied.

Table 4.7 Causes of rejection/detention of seafood imported into the EU during the period January 1999 – June 2002 (Ababouch and Gandini, unpublished)

Cause of detention/rejection	No. of rejections / detentions			
	1999	2000	2001	2002
Microbial	59	53	49	47
V. parahaemolyticus	13	10	19	14
V. vulnificus		2	1	3
V. cholerae	9	8	9	5
Other vibrios		1		
Enterobacteria	6	2	4	6
S. aureus	7	0		
Listeria		0		
Salmonella	20	18	10	12
Hepatitis	1	1		
Total plate count	1	8	4	7
Molds		1	1	
Clostridium		2	1	
Chemicals / residues	13	15	34	158
Biotoxins		1		1[1]
Pesticides	2			
Mercury	4	4	9	8
Cadmium	5	2	3	4
Lead				2
Nitrofurans				39
Histamine	1	4	1	1
Chloramphenicol	1		16	86
Phenols				
Policyclic Aromatic Hydrocarbons		4	3	7
Veterinary drug residues				4
Sulfites			2	2
Benzopyran				1
Malachite green				1
Antimicrobial agents				2
Parasites	1	13	11	7[2]
Others	6	13	18	5
Labelling	3	7	8	2
Sanitary certificate	1	1	3	
Shelflife	1		2	
Interrupted cold chain	1	1		
Insects				1
Import prohibited		2	2	1
Mixing of fish species				1
Uncertified establishment		1		
packaging			2	
Not specified		1	1	
Total	79	94	112	217

1. *DSP*
2. *One cestode*

By region, exporting countries from Asia accounted for 69.8% of the alert cases, followed by Africa (17.8%), the Americas (8.8%), Europe (non-EU) (2.7%) and Oceania (0.9%). This does not reflect the volume of exports by region, which amounted in 2000 for Asia to 14.7 % of the total export from third countries, 19.9% for Africa, 22.7% for the Americas (5.5% for North America and 17.2% for Latin America). These data indicate a need for improving further the sanitary conditions in Africa and Asia throughout the food chain from fish harvesting to export. In addition, there is an urgent need to improve sanitary conditions in aquaculture, especially by generalizing the application of

Good Aquaculture Practices and a strict control on the use of banned drugs such chloramphenicol. These drugs banned for use in aquaculture and animal husbandry are becoming a significant health concern in major markets of Europe and the USA. Obviously, Asia, which produces around 89% of the world aquaculture fish is concerned at the highest level.

References

CSPI (Centre for Science in the Public Interest) 2001. Outbreak Alert. Closing the Gaps in our Federal Food-Safety Net. CSPI, Washington DC, USA.

EC (European Commission) 1992. Council Directive 92/59/EEC of 29 June 1992 on general product safety. *Official Journal of the European Communities* L. 228, 11/08/1992, p. 0024-NBNB

EC (European Commission) 2001. Opinion of the Scientific Committee on veterinary measures relating to public health on Vibrio vulnificus and Vibrio parahaemolyticus (in raw and undercooked seafood). Report adopted 20 September 2001. Health and Consumer Protection Directorate General. 64 Pages.

FDA (Food and Drug Administration) 2002. Introduction to FDA's Import Refusal Report (IRR). http://www.fda.gov./ora.oasis/ora_oasis_ref_intro.html

Gillespie, I.A., G.K. Adak, S.J. O-Brien, M.M. Brett and F.J. Bolton 2001. general outbreaks of infectious intestinal disease associated with fish and shellfish, England and Wales, 1992-1999. *Communicable Disease and Public Health* 4, 117-123.

Mossel, D.A.A. 1982. *Microbiology of Foods*. University of Utrecht. Faculty of Veterinary Medicine, Bittshact 172, Utrecht, The Netherlands.

Olsen, S.J., L.C. MacKinnon, J.S. Goulding, N.H. Bean and L. Slutsker 2000. Surveillance for foodborne-disease outbreaks – United States, 1993-1997. Report CDC Surveillance Summary. *Morbidity and Mortality Weekly* 49,1-62.

5 CHARACTERIZATION OF HAZARDS IN SEAFOOD

The aim of this Chapter is to discuss known data on each hazard and provide information useful in the control of seafood-borne diseases. This includes data on frequency or likelihood of contamination of raw material and/or foods by the hazardous agent. Also, the Chapter discusses changes in the level or frequency of the hazard over time depending on processing, preservation parameters and storage conditions.

Hazard
A biological, chemical or physical agent in, or condition of, food with a potential to cause an adverse health effect (CAC, 2001)

The presence, growth, survival or death of microorganisms or destruction of toxins as influenced by processing, packaging and storage conditions will also be considered. The adverse health effects (the disease), the dynamics of infection or intoxication, host susceptibility, healthy carriers and possible spread of disease through secondary transmission are factors included in the characterization of the hazards. Where relevant, each hazard will be described under the following subheadings:

- The disease (adverse health effect) and epidemiological aspects
- The niche or origin of the organism/agent and its prevalence in fish and fishery products
- Growth and survival in fish and fishery products
- Prevention and control (including critical limits)

It should be noted that the information provided in this Chapter are some of the aspects needed in: "Exposure Assessment" and "Hazard Characterization", which are two of the elements in a Risk Assessment project (see section 3.3). However, in a quantitative risk assessment, much more data than presented here will be required.

5.1 Biological hazards

Biological hazards include pathogenic bacteria (infectious or toxin producing), biogenic amines, viruses, parasites and aquatic biotoxins.

5.1.1 Pathogenic bacteria (Hans Henrik Huss/Lone Gram)

Pathogenic bacteria are defined as those bacteria that that may cause illness in humans. Some pathogenic bacteria are transmitted to humans via food. Food-borne pathogenic bacteria are few among the many different types of seafood bacteria, which are causing no harm to humans. Many microorganisms are even beneficial being used in the production of food and drinks. Others are able to spoil food. Bacterial food-borne pathogens may be grouped into those that cause food intoxication and those that can result in food-borne bacterial infection.

In case of bacterial food poisoning or intoxication the causative organism multiplies in the food where it produces its toxins. A food poisoning is therefore characterized by rapid onset of the illness (typically symptoms are nausea, vomiting) as the toxins are already formed in the food before consumption. Thus ingestion of viable bacteria is <u>not</u> a prerequisite for the induction of the disease. Most often intoxications require that the toxin producing bacteria have grown to high numbers (10^5 – 10^8 cfu/g) in the food before it is eaten.

In contrast, the food merely act as a carrier for the causative organism in food-borne infections. The infectious agent may or may not have multiplied in the food, but the ingested viable bacteria continue to grow within the host's body to produce the typical symptoms (fever, diarrhoea). The number of viable bacterial cells necessary to cause disease (the Minimum Infective Dose, MID) varies considerably between bacterial species. Thus the MID is known to be high (>10^5-10^6 cells)

for pathogenic *Vibrio* spp. (Twedt, 1989) and very low for some *Salmonella typhi* and *Shigella* species (Kothary and Babu, 2001).

Seafood-borne pathogenic bacteria may conveniently be divided into 3 groups according to their ecology and origin as those who are indigenous to:

- the aquatic environment (Table 5.1)
- the general environment (Table 5.2)
- the animal/human reservoir (Table 5.3).

The level of human pathogenic bacteria in fish is generally quite low as shown in Table 5.1. Highest concentrations are found in molluscs and in the intestines of molluscs' predators. The ambient temperature strongly influences the composition (quantitatively and qualitatively) of the natural micro flora present in the environment and on the fish raw material.

Table 5.1 Pathogenic bacteria indigenous to the aquatic environment and naturally present on fish (based on Huss 1997).

Organism	Primary habitat	Quantitative levels
Clostridium botulinum; non-proteolytic types B, E, F	Temperate and Arctic aquatic environment; multiplication in aquatic carrion (type E)	Generally low (<0.1 spores/g fish) but up to 5.3 spores/g fish has been recorded
Pathogenic *Vibrio* spp. incl. *V. cholerae* *V. parahaemolyticus* *V. vulnificus*	Ubiquitous in warm (>15°C) seawater environment	Up to 10^2-10^3 cfu/g in shellfish; up to 10^4-10^8 cfu/g in intestines of shellfish-eating fish
Plesiomonas shigelloides	Warm aquatic environment; Freshwater fish (animals)	
Aeromonas spp.[1]	Aquatic environment	Generally low, but up to 10^4 cfu/ml in seawater; 10^7 cfu/ml in sewage and 10^6 cfu/g in raw seafood

1. The role of Aeromonas spp. in food-borne disease is not resolved

The presence of pathogenic bacteria in the general environment is also low (Table 5.2). Furthermore it should be emphasized that all the genera of pathogenic bacteria listed in Tables 5.1 and 5.2 contain non-pathogenic environmental strains. Thus *V. cholerae* non-01 was detected in six samples (out of 752 samples examined) of warm-water shrimps imported to Denmark, but none of these strains contained plasmids or genes encoding cholera toxins (CT) or heat-stable enterotoxin (NAG-ST) suggesting that these organisms do not constitute a public health problem (Dalsgaard *et al.,* 1996). However, for some organisms, such as *Listeria monocytogenes*, there is no known method available to distinguish between pathogenic and non-pathogenic strains.

Table 5.2 Pathogenic bacteria indigenous to the general environment and frequently present on fish (based on Huss, 1997).

Organism	Primary habitat	Quantitative levels
Listeria monocytogenes	Soil, decaying vegetation ubiquitous in general (temperate) environments	<100 cfu/g in freshly produced fish products
Clostridium botulinum proteolytic type A, B	Soil	Generally low (<0.01 spore/g soil)
Clostridium perfringens	Soil (type A); animals (type B, C, D and E)	10^3-10^4 cfu/g soil
Bacillus spp.	Ubiquitous in general environment (soil, natural waters, vegetation)	10^1-10^3 cfu/g or ml raw, processed food

The pathogenic bacteria found in the animal/human reservoir are shown in Table 5.3. They are found on outer and inner surfaces of diseased or asymptomatic carriers. Contamination of fish products is almost always due to poor hygiene (poor personal hygiene, poor processing hygiene or poor water quality).

It must be emphasized that it is nearly always possible to detect a range of human pathogenic bacteria on any fish or fish product that has not received any bactericidal treatment. Some of these pathogens may constitute part of the natural flora on the fish (pathogens from the aquatic environment) or be there as a result of unavoidable contamination (pathogens from the general environment). It is common for these pathogens that some growth in the fish products is required to produce disease in humans. This applies naturally for the intoxicating types, but as the MID for the infective environmental pathogens is high (- or higher than the natural level found in fish products), some growth is also required for these types. This means that the preventive measure for all these pathogens is prevention of growth of the organisms in the products (see Table 5.4).

Table 5.3 Pathogenic bacteria in the animal/human reservoir.

Organism	Primary habitat	Quantitative levels
Salmonella spp. *Shigella* spp. *Escherichia coli*	Intestines of warm blooded animals/humans	Levels in symptomatic and asymptomatic carriers vary; levels in seafood assumed to be sporadic and low. May accumulate in molluscan shellfish
Campylobacter jejuni and other mesophilic campylobacter	Birds, intestines of warm blooded animals	Sporadic, low levels. Possibly accumulation in molluscan shellfish
Staphylococcus aureus	Outer surface (skin) and mucus membranes (nose)	Transient, but present on 50% of population. Generally <100 cfu/cm^2 skin

The MID for pathogens originating in the animal/human reservoir may be high or as low as < 10 organisms for some *Shigella* and for *E. coli* O157 (Kothary and Babu, 2001). As these bacteria are not normally present in fish and fish products, the main preventive measure is to avoid contamination by applying good hygienic practices (GHP) and good manufacturing practices (GMP) (Table 5.4). However, for some of these bacteria, including *Staphylococcus aureus*, which is a toxin producing pathogen, growth in the products is required to produce disease.

Table 5.4 Seafood-borne pathogenic bacteria and disease.

Natural habitat of pathogen	Mode of action of disease		
	Infection		Intoxication
	High MID	Low MID	
Aquatic environment	*Vibrio* spp. *(Aeromonas)* *(Plesiomonas)*		*Clostridium botulinum* Type E (non-proteolytic)
General environment	*Listeria monocytogenes*		*Clostridium botulinum* Type A, B (proteolytic) *C. perfringens* *Bacillus cereus*
Animal-human reservoir	*Salmonella* *E. coli* (EPEC, ETEC)[1]	*S. typhi* *Shigella* *E. coli* (EHEC)[1] *Campylobacter*	*Staphylococcus aureus*
Preventive measure	Prevention of growth	Hygiene; GHP/GMP	Prevention of growth

1. EPEC: Enteropathogenic E. coli; ETEC: Enterotoxigenic E. coli; EHEC: Enterohaemorragic E. coli.

The safety concerns related to pathogenic bacteria in seafood is demonstrated in Table 5.5. The mere presence (in low numbers) of pathogens from the aquatic and general environment is of no safety concern, not even in ready-to-eat (RTE) products.

In contrast, the presence of pathogens from the animal/human reservoir is a serious safety concern for products to be eaten without (further) cooking. Growth of pathogens is likewise a serious safety concern for most RTE products. For raw fish products to be eaten raw the safety concern is limited. Growth of these pathogens is only possible at elevated temperatures (>5°C) (Table 5.16), and at this condition spoilage will proceed very rapidly and the fish will probably be rejected due to off-odours and off-flavours long before being either toxic or infective organisms reach high numbers.

Table 5.5 Safety concerns related to pathogenic bacteria in seafood.

Natural habitat of pathogen	State of pathogen	Safety concern[1]		
		Fresh fish to be eaten		RTE[2]
		cooked	raw	
Aquatic environment	Presence	-	-	-
	Growth	-	(+)	+
General environment	Presence	-	-	-
	Growth	-	(+)	+
Animal-human reservoir	Presence	-	+	+
	Growth	(+)	+	+

1. "+" definitely a safety concern; "(+)" limited safety concern; "-" no safety concern
2. Ready-to-eat products see Table 9.5.

Growth of pathogens in raw fish to be cooked is similarly of Ittle safety concern. Only limited growth is possible before spoilage is causing rejection and in borderline cases, cooking will destroy the pathogen. Growth of pathogens from animal/human reservoir is of no direct safety concern in raw fish to be cooked before consumption as described above, but it may constitute a secondary

hazard due to increased spread and contamination of the processing or kitchen environment with these pathogens.

5.1.1.1 Bacteria indigenous to the aquatic and general environment

Control of disease from human pathogenic bacteria occurring in the aquatic or general environment is very often ensured by preventing their growth – or destroying any organisms present. Tables 5.6 and 5.7 give overviews of growth limiting factors and heat resistance of these organisms. The D-value used to determine heat-resistance indicates the length of time (seconds, minutes) which is required at a given temperature to reduce the population to 10% of its initial count (decimal reduction).

Table 5.6 Growth limiting factors of pathogenic bacteria indigenous to the aquatic and the general environment (adapted from Huss, 1994; ICMSF, 1996).

Pathogenic bacteria	Temperature, °C		pH	a_w	NaCl (%)
	minimum	Optimum	minimum	minimum	maximum
Clostridium botulinum					
Proteolytic, type A, B, F	10	35-40	4.6	0.94	10
non-proteolytic, type B, E, F	3.3	25-28	5.0	0.97	3-5
Vibrio spp.					
V. cholerae	10	37	5.0	0.97	< 8
V. parahaemolyticus	5	37	4.8	0.93	8-10
V. vulnificus	8	37	5.0	0.96	5
Plesiomonas shigelloides	8	37	4.0		4-5
motile *Aeromonas* spp.	0-4	28-35	4.0	0.97	4-5
Listeria monocytogenes	0-2	30-37	4.6	0.92	10
Bacillus cereus	4[1]	30-40	5.0	0.93	10
Clostridium perfringens	12	43-47	5.5	0.93	10

1. Most strains of B. cereus are mesophilic with minimum temperature of approximately 8-10°C, however, psychrotrophic variants have been isolated

Table 5.7 Heat resistance of pathogenic bacteria indigenous to the aquatic and the general environment (adapted from Huss, 1994; ICMSF, 1996; Ababouch 1987).

Pathogenic bacteria	Heat resistance
Clostridium botulinum	
proteolytic, type A, B, F	D_{121} (spores) = 0.1 – 0.25 min D_{119} (spores) = 7.44 min in products with high fat content
non-proteolytic, type B, E, F	D_{100} (spores) < 0.1 min; $D_{82.2}$ = 0.5 – 2.0 min (broth); D_{80} (spores) = 4.5 – 10.5 min in products with high fat content
Vibrio spp.	
V. cholerae	D_{55} = 0.24 min
V. parahaemolyticus	D_{60} = 0.71 min
V. vulnificus	D_{50} = 1.15 min (buffer); 0.66 min (oysters)
Plesiomonas shigelloides	All cells killed after 30 min at 60°C
motile *Aeromonas* spp.	D_{55} = 0.17 min
Listeria monocytogenes	D_{60} = 2.4 – 16.7 min in meat products; 1.95 – 4.48 min in fish
Bacillus cereus	D_{121} (spores) = 0.03 – 2.35 min (buffer) D_{95} (spores) = 3.0 – 19 min (milk)
Clostridium perfringens	D_{90} (spores) = 0.015 – 4.93 min (buffer) D_{100} (spores) = 0.31-13.0 min (broth)

Clostridium botulinum *(Hans Henrik Huss)*

Clostridium botulinum is classified into toxin types from A to G. The types pathogenic to humans (types A, B, E and F) can conveniently be divided into two groups:

- the proteolytic types A, B and F, which are also heat resistant, mesophilic, NaCl-tolerant and have the general environment as the natural habitat
- the non-proteolytic types B, E and F, which are heat sensitive, psychrotolerant, NaCl-sensitive and have the aquatic environment as the natural habitat.

a) The disease and some epidemiological aspects

Toxins produced by *C. botulinum* types A, B, E and F are the cause of human botulism. The disease can vary from a mild illness, which may be disregarded or misdiagnosed, to a serious disease, which may be fatal within 24 hours. In most cases, the symptoms develop within 12 to 36 hours. These are generally nausea and vomiting followed by neurological symptoms such as visual impairment (blurred or double vision), loss of normal mouth and throat function (difficulty in speaking and swallowing, dry mouth), lack of muscle coordination and respiratory impairment, which is usually the immediate cause of death.

Type E botulism tends to have most rapid onset of symptoms, while type A botulism tend to be the most severe. Early fatality rates in the first half of the 20th century were about 50% or higher for botulism, but with the availability today of antisera and modern respiratory support systems, they have decreased to about 10% (Austin and Dodds, 2001).

The majority of botulism outbreaks in the northern and temperate regions are associated with fish, and in general type E was the responsible type. Type A and B botulism has generally been associated with meat or meat products, but fish and fish products have also been vehicle for those types. All types of fish products except raw fish to be cooked immediately before consumption

have been involved in outbreaks of botulism, but the majority of outbreaks has been associated with fermented fish (Huss, 1981).

Botulinum toxin is one of the most potent of all poisons, and the amount needed to cause death in humans has been estimated to be as low as 30-100 ng (Lund and Peck, 2000). The toxin is sensitive to heat and pH above 7. For safe inactivation of any botulinum toxin at concentrations up to 10^5 LD/g in foods, time/temperature combinations of 20 min. at 79°C or 5 min. at 85°C has been recommended (Hauschild 1989). Normal household cooking and frying of raw fish products are therefore sufficient to destroy any pre-formed toxin. This may be one of the reasons for the excellent safety record of unprocessed fish with respect to problems from *C. botulinum.*

While botulinum toxin is rapidly destroyed in fish products with pH>7.5, such as spoiling cod, it is extremely stable in a salty and acid environment. Thus botulinum toxin formed in the raw material will be found again or even increase in situ in the final products such as heavily salted, marinated or fermented fish (Huss and Rye Petersen, 1980). This is illustrated by the fact that many outbreaks of botulism have been traced to products, which do not support the growth of *C. botulinum.*

b) Prevalence in fish and fishery products

The spores of the non-proteolytic *C. botulinum* types, particularly type E are widely distributed in the aquatic (marine and fresh water) environment in the temperate and arctic zones. Thus, up to 100% of sediment samples from coastal areas, particularly in closed, shallow fjords and from aquaculture ponds may contain the organisms (Huss, 1980; Dodds, 1993). The distribution patterns of *C. botulinum* type E suggest that this is a true aquatic organism and that multiplication occurs, in situ, particularly in carrion. A much lower prevalence is found in live fish although up to 100% of fish from aquaculture and coastal waters may carry this organism. Fish caught in the high seas are generally free from *C. botulinum*. In warm tropical waters and in fish from these areas, other types than type E are frequently found, see Figure 5.1.

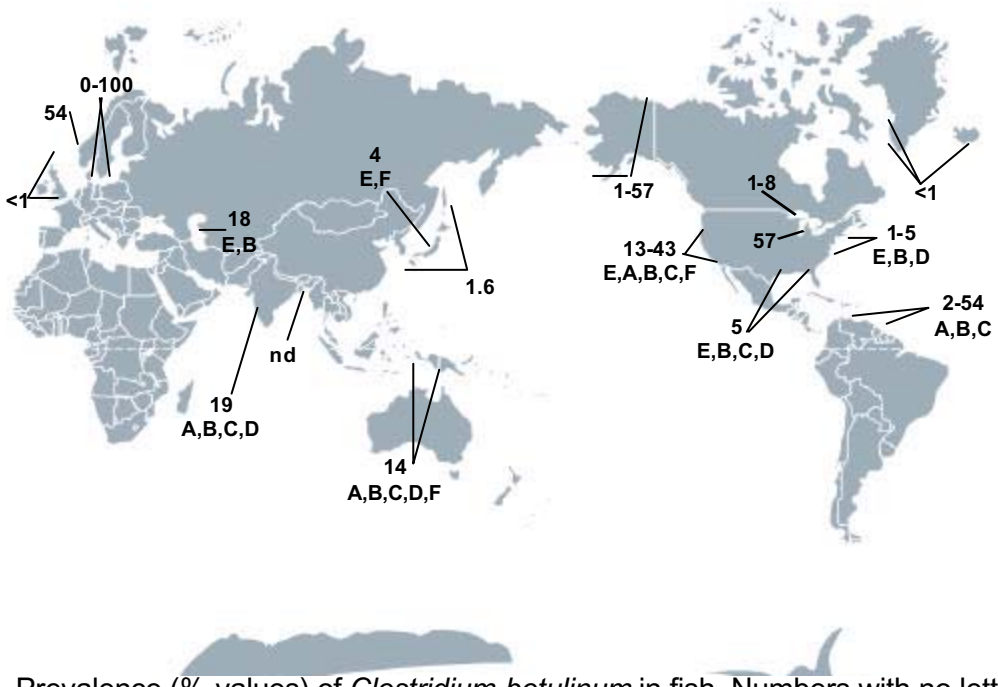

Figure 5.1 Prevalence (%-values) of *Clostridium botulinum* in fish. Numbers with no letter attached refer to type E; otherwise letters indicate *C. botulinum* types detected. ND = Not detected. For references to surveys, see Huss (1980). Cochin data from Lalitha and Surendran (2002).

The proteolytic *C. botulinum* are frequently found in soil and the terrestrial environment (Huss, 1980; Hauschild, 1989; Dodds, 1993). Animals, both vertebrates and invertebrates, have an important role in both the distribution and build up of botulinum spores. Spread of spores from the terrestrial environment to the aquatic environment (coastal waters and fresh waters systems) including the fish in these areas is therefore a distinct possibility as well as spread of spores to the fish processing environment. Being mesophilic, the proteolytic types do not have the same possibilities for multiplication in nature as type E.

c) Growth and survival in fish and fishery products

The main factors that control growth of *C. botulinum* in foods are temperature, pH, water activity (a_w), salt, redox potential and added preservatives. Maximum and minimum limits for these parameters, which would permit growth are shown in Table 5.6.

The figures quoted in Table 5.6 are used in many regulations worldwide. These figures have mostly been established at near optimal conditions in challenge studies, where *C. botulinum* spores have been inoculated in large numbers and as a single organism. There are at least three factors adding to the safety of fish products using the figures from Table 5.6 in the control of *C. botulinum*:

- the natural level of *C. botulinum* in fish is much lower than levels used in most challenge studies. Initiating growth and toxin production will therefore be much delayed at comparable conditions
- the associate flora in fish products may cause spoilage before the product becomes toxic. Some microorganisms may also inhibit *C. botulinum*
- *C. botulinum* is an anaerobic organism preferring a low redox potential (Eh) for growth. The Eh for fish and fish products is high (Huss and Larsen, 1979, 1980) and this may cause delay in growth and toxin production at otherwise comparable condition in bacteriological media.

The presence of an associate (spoilage) micro flora may, however, also add to the risk, as this micro flora may use oxygen and facilitate the growth and toxin production by *C. botulinum* type E. It is clear therefore, that using the figures in Table 5.6 in control of *C. botulinum* does provide considerable safety margin. It should also be emphasized, that those factors seldom function independently. Usually they act in concert often having synergetic and accumulative effects. A few examples are shown in Table 5.8.

Table 5.8 Toxin production in smoked fish inoculated with 10^2 *C. botulinum* type E spores per gram (cold smoked) or using naturally contaminated fish (hot-smoked).

Product	Salt WPS[1]	Storage Temp.	Time to Toxicity	Reference
Cold-smoked salmon	1.7%	8°C	>28d.	Dufresne *et al.,* 2000
Hot smoked trout	3%	10°C	>30d.	Cann and Taylor, 1979

1. *WPS = water phase salt*

The data in Table 5.8 clearly demonstrate, that a combination of salt and low temperature very effectively inhibits toxin production (and growth) of *C. botulinum*. A very detailed review of the effect of growth limiting factors can be found in Lund and Peck (2000) and in Eklund (1993).

Thermal inactivation of *C. botulinum* spores have been extensively studied. The D-value varies considerably among *C. botulinum* types and even among strains within the same type. The spores of the non-proteolytic types are considerably less resistant than the proteolytic types as shown in Table 5.7. The heat resistance of non-proteolytic types is particularly important for mildly heat treated, pasteurised products, where conditions for growth are excellent for surviving spores. The D-values at 82°C for these product may vary from 0.5 to 2 min as shown in Table 5.7. A minimum

heat treatment of 90°C for 10 min should provide a safety factor of 10^6 (a 6-D process or a 6-log reduction of spore count) for non-proteolytic *C. botulinum* as recommended by a number of advisory committees (Martens, 1999).

The spores from proteolytic *C. botulinum* are much more heat resistant. In general, D_{121} values are in the range of 0.1-0.25 min. These spores are a particular concern in the sterilisation of low acid canned foods, and the canning industry has adopted a D-value of 0.2 min at 121°C as a standard for calculating thermal processes. For the most resistant strains, z-values (the temperature change necessary to bring about a 10-fold change in D-value) are approximately 10°C, which has also been adopted as a standard (Austin and Dodds, 2001).

d) Prevention and control

Control of *C. botulinum* in fishery products can be achieved by inactivation of spores or by inhibition of growth. Current guidelines regarding safety with respect to *C. botulinum* includes one of the following procedures (listed by Martens, 1999):

- storage at all times at <3.3°C
- storage at 5-10°C and a shelf life of <5 days
- a heat treatment of 90°C for 10 min combined with chill storage (<10°C)
- a pH ≤ 5.0 throughout the food combined with chilled storage (<10°C)
- a salt-on-water concentration ≥ 3.5% or a_w ≤ 0.97 throughout the food combined with chill storage (<10°C).

It should be noted that products where growth of non-proteolytic *C. botulinum* is completely inhibited (by salt or low pH) or inactivated still has a requirement for chilled storage. The reason is that the proteolytic *C. botulinum* may still be able to grow if temperatures are >10°C. It is a US-requirement, that vacuum packed cold smoked fish contain 3.5% NaCl (water phase salt = WPS) or 3% if combined with 100-200 ppm nitrite. For air packaged fish not less than 2.5% NaCl (WPS) in the loin muscle is required (FDA, 1998).

The canning industry has adopted a 12-D process as a minimum heat process applied to commercial canned low acid foods. The heat required to provide this “botulinum cook” or a 12-decimal reduction in proteolytic *C. botulinum* spores (also called F-value) is therefore equal to 12 x D_{121}-value or 12 x 0.2 = 2.4 min at 121°C. The highest know D_{121}-values is 0.25 min which gives a F-value of 12 x 0.25 = 3. Using F-values between 2.4-3 has led to safe production of canned low acid food for many decades. Often higher F values (e.g. 5) are used in commercial practice.

Refrigeration is often regarded as the primary method of preservation of fresh foods, including seafoods. At temperatures below 10°C there is no risk of toxin production by proteolytic *C. botulinum* types A and B. At higher storage temperature additional preservation or treatment is required to produce safe food as summarised in Table 5.9.

Table 5.9 Control of *Clostridium botulinum* in food.

Storage temperature		Preservation				Heat treatment
t < 3.5°C						
3.5°C < t < 10°C	AND	(pH < 5.0	OR	WPS[1] > 3.5%	OR	90°C,10 min)
t > 10°C	AND	(pH < 4.5	OR	WPS > 10%	OR	121°C, 2.4-3 min)

1. WPS = Water phase salt

***Vibrio* species** (Lone Gram)

Vibrio species belong to the Vibrionaceae family. All species are typical of marine and/or estuarine environments and most require NaCl (2-3%) to grow. Since the marine environment is their natural niche, *Vibrio* species are commonly isolated from fish and crustaceans. Most of the species are mesophilic and their numbers tend to increase during the warm seasons. The genus comprises 34 species of which 13 species can cause human disease, including wound infections, septicemia and gastroenteritis (Kaysner, 2000; FAO/WHO, 2001). Seafood-borne diseases are primarily caused by *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio cholerae* (Oliver and Kaper, 1997). *V. parahaemolyticus* and *V. cholerae* both cause gastrointestinal disease whilst *V. vulnificus* causes a septicemic condition.

Vibrio species are indigenous to the aquatic environment and their presence and numbers are influenced by factors such as temperature, salinity and algal density. There is no correlation between their occurrence or numbers and faecal human pathogens or indicators of faecal human pathogens.

Vibrio parahaemolyticus *(Lone Gram)*

a) The disease and some epidemiological aspects

V. parahaemolyticus may cause gastroenteritis in humans and the disease has exclusively been linked to consumption of seafood, in particular raw or inadequately cooked seafoods. The incubation period ranges from 8 to 72 hours and the onset of disease is very sudden with explosive diarrhoea. Other symptoms include nausea, vomiting, headache, fever and chills (Kaysner 2000). Symptoms typically subside within 48 to 72 hours but may last up to a week and treatment of most cases primarily include rehydration. Volunteer feeding trials suggest that ingestion of 2×10^5 to 3×10^7 cells is required to cause disease. In these feeding trials, antacid treatment was administered to the volunteers and this probably protected the bacteria. Recent US data using epidemiological evidence indicate that doses of approximately 10 times more are required (FDA, 2000). The genus is one of the leading cause of gastroenteritis in Japan and eastern Asian countries whereas the occurrence in other countries is much lower (Table 5.10). This difference could be linked to seafood consumption patterns as the disease is mainly associated with consumption of raw seafoods.

Table 5.10 European and Japanese gastroenteritis cases caused by *Vibrio parahaemolyticus* (EC, 2001; CAC, 2002)

Country	Period	Cases
UK and Wales	1995-1999	115
Northern Ireland	1997	44
Scotland	1994-1999	6
Spain	1995-1998	19
France	1995-1998	6
	1997	44[1]
Sweden	1992-1997	350[2]
Norway	1999	4
Denmark	1980-2000	2
Japan	1991-2000	64 000

1. Associated with seafood imported from Asia
2. Associated with crayfish imported from China

The exact virulence mechanisms of *V. parahaemolyticus* are not known. However, at least four haemolytic components are produced. Of these, two components: a thermostable direct hemolysin (TDH) and a TDH-related hemolysin (TRH) are strongly correlated with virulence. TDH positive strains causes hemolysis of human red blood-cells and this phenomenon is known as the Kanagawa reaction. Some strains, which are TDH-negative but TRH-positive have been reported

to cause gastroenteritis (EC, 2001). An elaborate serotyping system has been developed relying on O-antigens (12 types) and K-antigens (65 types) and this system is widely used in Japan. It is important to realise that the majority of environmental strains (approximately 95-99%) are not pathogenic whilst 99% of strains isolated from human cases are Kanagawa positive. Due to its mesophilic nature, incidents of *V. parahaemolyticus* are clearly correlated with temperature and the vast amount of cases/outbreaks occurs during the warmer months (Figure 5.2).

b) Prevalence in fish and fishery products

V. parahaemolyticus is commonly isolated from seafood products, especially in bivalve molluscs. Levels fluctuate with temperature, especially in the temperate zones, with the higher numbers being isolated in the warmer months. During colder months, the organism probably survives in sediments and is then released into the water with zooplankton when the temperature rises (EC, 2001). Also, salinity affects occurrence and the highest numbers are seen at 20-25 ppt salinity (FAO/WHO, 2001). The incidence seems to be highest in molluscan shellfish, followed by crustaceans, and lowest in finfish (Sumner *et al.,* 2001). Numbers in oysters may range from less than one per gram to 10^4 cfu/g but is typically less than 10 cfu/g. *V. parahaemolyticus* occurs less frequently in European fish and shellfish probably due to the relatively low water temperatures. During summer months, e.g. from August to October, 25 of 91 Dutch shellfish samples were positive for *V. parahaemolyticus* (Tilburg *et al.,* 2000).

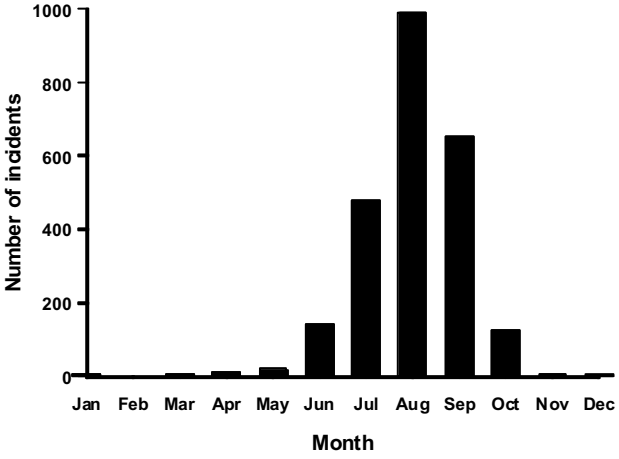

Figure 5.2
Number of *Vibrio parahaemolyticus* incidents per month in Japan (CAC, 2002).

c) Growth and survival in fish and fishery products

Being a mesophilic, halotolerant bacteria, *V. parahaemolyticus* will grow well in seafoods stored at ambient temperature. The very low generation time at high temperatures (e.g. 12-18 minutes at 30°C) allows the organism to proliferate rapidly. The bacteria is also capable of proliferation in live oysters during storage. Thus numbers increased 50 fold when oysters were stored at 26°C for 10 hours and almost 800 fold after 24 hours storage. Subsequent cooling to 3°C reduced numbers by almost 10-fold during 14 days of storage (Gooch *et al.,* 2002). In general, low temperature storage will cause numbers to decrease, however, the extent of decrease depends on food matrix, salinity and other factors.

V. parahaemolyticus is very heat sensitive and easily destroyed by cooking. D-values at 50-60°C are in the range of 0.3-0.8 min (Kaysner, 2000). Growth limits with respect to NaCl-%, temperature and pH are indicated in Table 5.6.

d) Prevention and control

Numbers of *V. parahaemolyticus* may be high in some live bivalves during warm months and a recent US risk assessment (FDA, 2000) demonstrated that the (initial) level in raw oysters was the most significant risk factor. However, the high infectious dose indicates that mostly growth has to take place in the product for the organism to reach hazardous levels. Thus, it is the high levels

(growth) of *V. parahaemolyticus* and not its mere presence (in low numbers) that is the hazard. Rapid and efficient cooling (time x temperature control) is one of the most important control parameters in prevention of *V. parahaemolyticus* gastroenteritis. Cooling to 5°C will prevent growth. High NaCl-concentrations (>10% NaCl in water phase) or acidification as used in several semi-preserved products can prevent growth. Good Hygienic Practices (GHP) programmes should ensure that cooked products are not cross-contaminated. Depuration of molluscan shellfish has no significant effect on the level of *Vibrio* that may even multiply in depurating shellfish (FDA, 2000; Eyles and Davey, 1984).

Vibrio vulnificus

a) The disease and some epidemiological aspects

V. vulnificus can cause wound infections in humans and a range of fish diseases, however, it may also cause a very serious infection transmitted by seafood. As opposed to the other seafood-borne *Vibrio* diseases, this is a bacteremia and a septicemia not a gastrointestinal disease. Seafood-borne *V. vulnificus* infections are almost exclusively caused by consumption of raw bivalve molluscs such as oysters. Infections with *V. vulnificus* are not common in Europe but have for some years been a safety issue in the Gulf Coast area of the USA. The disease is an invasive disease causing primary septicemia, i.e. with no infectious focus. Common symptoms are fever, chills, and nausea. Symptoms occur approximately 38 hours after consumption. The disease primarily affects people in specific risk groups with underlying medical conditions such as chronic cirrhosis, hepatitis or a history of alcohol abuse (EC, 2001). Liver dysfunction is typical of several of these conditions and iron overload (typical in liver conditions) appears to facilitate infection. In particular males above 40 that have a history of alcohol consumption (and eat live oysters) are at risk (Kaysner, 2000). Mortality in risk groups may be as high as 60%.

V. vulnificus produce an extracellular cytotoxin and a battery of hydrolytic enzymes. These are probably responsible for the rapid degradation of muscle tissue seen during infection. The presence of a polysaccharide capsule is essential for infection.

Three different biotypes of *V. vulnificus* have been identified. Approximately 85% of strains isolated from human clinical cases are biotype 1 whereas biotype 2 mainly causes infections in eels. Biotype 3 was identified recently (Bisharat *et al.,* 1999) and was associated with seafood mediated bacteremia.

Disease – and numbers of *V. vulnificus* – fluctuate with the water temperature. Most cases occur during the warm summer months. The infectious dose is not known, but shellfish with levels of 10^3 *V. vulnificus* per gram have been implicated in disease. Using data on numbers in oysters, modelling growth between harvest and consumption, estimating number of servings based on landings and comparing this to the reported number of cases per months (Table 5.11) it becomes clear that especially high levels are likely to result in disease (FDA, 2000).

b) Prevalence in fish and fishery products

Isolation of *V. vulnificus* from the environment can be difficult, however, it is frequently isolated from warmer marine or estuarine waters. It appears to be associated with the Gulf Coast of the USA, although it has been isolated from other areas such as the East coast of the USA (Oliver *et al.,* 1983) and from the Italian Adriatic coast (Barbieri *et al.,* 1999). *V. vulnificus* accumulates in oysters up to 10^4 cfu/g and can be found in levels of up to 10^6 cfu/g in intestines of fish feeding on oysters. Just as *V. parahaemolyticus,* the occurrence in both water and oysters follows a seasonal pattern with high numbers (and disease) being detected in the summer-months – and 90% of the cases in the US occurring between April and October. Motes *et al.* (1998) found that the density in oysters was approximately 10^4 per gram when the water was 25-30°C but dropped to below 100 per gram when the temperature decreased below 15°C. Also salinity affects its occurrence with optimal salinity at 17 ppt. *V. vulnificus* may multiply within the live animal and each oyster may shed up to 10^6 bacteria per day (Tamplin and Capers, 1992).

Table 5.11 Environmental and epidemiological data for *Vibrio vulnificus* in the USA (modified from FDA, 2000)

Month	Water-temp. °C	Mean log Vv/g at harvest[1]	Mean log Vv/g at consumption	Servings for at risk individuals	Log of mean Vv per serving dose	Average # cases in month
Jan	12.5	-0.03	-0.34	62 000	2.45	0
Feb	15	0.76	0.61	63 000	3.40	0
Mar	17.5	1.45	1.51	73 000	4.30	0.2
Apr	22.5	2.52	2.96	63 000	5.75	1
May	26	3.04	3.75	53 000	6.54	3
Jun	28.5	3.28	4.19	51 000	6.98	2.5
Jul	30	3.38	4.41	47 000	7.20	2.5
Aug	30	3.38	4.41	42 000	7.20	3.5
Sep	28	3.24	4.11	48 000	6.90	3
Oct	23	2.61	3.12	61 000	5.91	3
Nov	18	1.57	1.68	70 000	4.46	1.5
Dec	15	0.76	0.61	72 000	3.40	2

1. Vv = Vibrio vulnificus

c) Growth and survival in fish and fishery products

V. vulnificus is a mesophilic bacterium and grows poorly below 15°C (minimum temperature approximately 13°C) and disease seems to be correlated to temperatures above 20°C. In seafood products stored at ambient temperature it grows rapidly and numbers can in live oysters increase with a factor 100 (2 log units) during 14 hours of storage at 24 to 33°C. Growth limiting parameters are indicated in Table 5.6.

d) Prevention and control

V. vulnificus is very sensitive to a range of food-relevant treatments. It dies rapidly during heating with D-values of approximately 78 sec at 47°C. The EU directive (EC, 1991) requires that shellfish from so-called Class C areas are heat treated at 90°C for 90 sec (or equivalent). Class C areas are areas where there is a microbiological limit on the shellfish of < 60 000 faecal coliforms/100g and the shellfish must be relayed for at least 2 months (see Chapter 11). It is more sensitive to cold-storage than *V. parahaemolyticus* and declines with approximately 0.04 log units per day under "normal" cold storage (FAO/WHO, 2001). The bacterium is relatively sensitive to low pH and does not grow below pH 5 (Little *et al.,* 1997). Thus products such as pickled fish do not constitute a risk. Storage at refrigerated temperatures or below 0°C results in reduction of counts of *V. vulnificus*. This is either attributed to a die-off of the organism or to entrance into a so-called viable-but-non-culturable state. Frozen storage (-40°C) can result in a 4-5 log reduction over a 3 week period.

The bacterium is not removed from oysters by normal depuration and the bacterium may, as *V. parahaemolyticus*, actually multiply in depurating animals. In contrast, relaying in waters of high salinity does decrease numbers. Heat treatment is a very efficient way of reducing numbers. For animals with an initial low number of *V. vulnificus*, rapid and efficient cold-storage is crucial in preventing proliferation.

Vibrio cholerae

a) The disease and some epidemiological aspects

V. cholerae may be sub-typed into more than 130 serotypes. Of these only serotype O1 and O139 are associated with epidemic and pandemic cholera. Both produce the cholera toxin. The O1 may be further subdivided into the serogroups Ogawa or Inaba or Hikojima which is an uncommon type. O1 types may also be subdivided into two biotypes: classical and El Tor of which the latter is hemolytic. O139 strains resemble the El Tor types being also hemolytic (Kaysner, 2000).

Cholera affects only humans and the main source of the bacteria during epidemics are the faeces of acutely infected people. However, the bacteria persists in the environment and is often found attached to plankton (Chiavelli *et al.,* 2001). *V. cholerae* non-O1 and non-O139 are as the other *Vibrio* species, ubiquitous in marine and estuarine waters. Some non-O1 and non-O139 may be pathogenic to man, causing mainly gastroenteritis, but they are not associated with the epidemic diseases. Water contaminated with sewage is the main cause of spread of cholerae but also seafood products being contaminated with cholera-containing waters have been the cause of disease. The largest recent out-break of cholera, the pandemic South American outbreak in the early 1990s was partially caused by ceviche, a raw, marinated fish product, for which contaminated water or fish was used in the preparation. This was a O1-outbreak and caused more than 400 000 cases.

Cholera is a gastrointestinal disease characterized by diarrhoea and passage of watery, voluminous (so-called rice-water) stools leaving the patient dehydrated. Treatment with salt- and sugar-water is required. One of the major virulence factors is the production of the cholerae toxin secreted by O1 and O139 serotypes. The infective dose is believed to be approximately 10^6 cells (Kaysner, 2000) although some authors state that ingestion of as much as 10^{11} cells are required to make up for the rapid reduction by gastric acids (Stewart-Tull, 2001).

b) Prevalence in fish and fishery products

As mentioned, an association between phyto- or zoo-plankton and numbers of *V. cholerae* has been observed. Water temperature and salinity also affect the occurrence and persistence of *V. cholerae*. Thus the highest numbers are observed at lower salinities of 2-5 ppt (Kaysner, 2000) and the natural niche of *V. cholerae* is estuarine waters (Oliver and Kaper, 1997). *V. cholerae* survives for long periods of time in river waters (FAO/WHO, 2001). Toxigenic *V. cholerae* have been isolated from the hindgut of crab (Huq *et al.* 1996) and it is believed that their chitinolytic activity, which also explains their preference for plankton aggregates, is the cause of this adherence. *V. cholera* is not common on fresh fish, thus none of 748 samples of warm water shrimp imported into Denmark were positive (Dalsgaard *et al.* 1996), nor were 131 fresh and brackish water prawn samples from Bangladesh (Balakrish Nair *et al.,* 1991). However, in some areas of the world it may be more prevalent. *V. cholerae* O1 has been isolated from 3.5-18.3% of fresh fish in Mexico (Torres-Vitela *et al.,* 1997).

c) Growth and survival in fish and fishery products

V. cholerae is very sensitive to heat, acid and cooling. Therefore, it is either eliminated by food processing treatments or its growth in foods is prevented. The majority of cases in which cholera has been linked to seafoods have involved raw products, often molluscs. Due to the involvement of ceviche in the South American epidemic, its survival in slightly acidified products has been studied and a 2-3 log reduction is seen over a 24 hour period. Limits for growth are given in Table 5.6.

d) Prevention and control

Inadequate sanitation and lack of safe water are the major causes of cholera epidemics. Therefore, cholera can only be reliably prevented by ensuring that all populations have access to adequate excreta disposal systems and safe drinking water. WHO has issued recommendations for water supply and sanitation (Table 5.12).

Table 5.12 WHO recommendations for water supply and sanitation with respect to cholera control (WHO, 1992).

Recommendation	
Water supply	Sanitation
• Drinking water should be adequately disinfected, procedures for disinfection in distribution systems and rural water systems should be improved • Tablets releasing chlorine or iodine may be distributed to the population with instructions on their use • Where chemical treatment of water is not possible, health educators should stress that water for drinking (as well as for washing of hands and utensils) should be boiled before use • Water quality control should be strengthened by intensifying the surveillance and control of residual chlorine, and the conduct and analysis of bacteriological tests, in different points in production and distribution systems	• Quality control in sewage treatment plants should be strengthened • The use of treated waste water for irrigation should be carefully controlled, following national and international guidelines • Large-scale chemical treatment of waste water is very rarely justified, even in emergencies, because of the high cost, uncertain effect, and possible adverse impact on the environment and health • Health education should emphasize the safe disposal of human faeces: o All family members should use a latrine or toilet that is regularly cleaned and disinfected o Faeces of infants and children should be disposed of rapidly in a latrine or toilet, or by burying them

Low temperature storage may reduce numbers of *V. cholerae* (Mitcherlich and Marth 1984, Table 5.13) but must never be relied on as a preventive measure.

Table 5.13 Survival of *Vibrio cholerae* (culturable cells) (Mitcherlich and Marth, 1984).

Food	Survival times, days
Fish stored at 3-8°C	14-25
Ice stored at –20°C	8
Shrimp, frozen	180
Vegetables, 20°C	10
Carrots	10
Cauliflower	20
River water	210

Listeria monocytogenes *(Lone Gram)*

Listeria monocytogenes is a Gram-positive, motile bacteria that grows well at 37°C at human body temperature but which at the same time is psychrotolerant and halotolerant (Table 5.6). Seven species of *Listeria* are known and of these only *L. monocytogenes* is pathogenic to humans (Farber and Peterkin, 2000). *Listeria* species are closely related to the lactic acid bacteria. *L. monocytogenes* is divided into 13 serovars on the basis of somatic (O) and flagellar (H) antigens, however, most isolates involved in human disease belong to three serotypes. From an epidemiological point of view, DNA-based methods such as random amplification of polymorphic DNA (RAPD), ribotyping or amplified fragment-length polymorphism (AFLP) are more discriminatory and have allowed tracing of outbreaks and contamination sources in the food industry.

a) The disease and some epidemiological aspects

Listeriosis is in its most known form an invasive disease transmitted by food products. Listeriosis is a rare disease and mostly affects people in particular risk groups where the immune defence

system is reduced. This is typically elderly people, people with HIV infection, transplant patients but also pregnant women (where the immune defence is reduced to avoid rejection of the foetus). The disease infects the central nervous system and often manifests itself as meningitis. The bacterium multiplies within the macrophages and "shoots" itself from cell to cell using a tail of actin polymers. The fatality rate in the risk group is high; typically 20-40%. In infected pregnant women, listeriosis typically results in abortion. The incubation period is very variable ranging from one to 91 days and since most people do not remember their food consumption three months ago, it is often difficult to trace the food that was the source of the pathogen. If diagnosed, the disease can be treated with standard antibiotics. The incidence of listeriosis is approximately 0.5 cases per 100 000 inhabitants in the western countries. Neonates are infected since *L. monocytogenes* can cross the placenta and whilst the pregnant women suffer only a mild flu-like disease, the foetus is seriously affected. Recently, it has been documented that *L. monocytogenes* may also cause a non-invasive febrile gastroenteritis in otherwise healthy people that have eaten smoked trout (Miettinen *et al.,* 1999). The incidence of this type of listeriosis is not known.

Listeriosis is typically caused by processed, industrialized foods that have extended shelf lives at chill temperatures and that are ready-to-eat (RTE). Thus there is no final heat treatment by the consumer. Due to its widespread occurrence, *L. monocytogenes* is easily isolated from several types of RTE foods. The disease was noticed initially from soft cheeses made from raw milk but has since been caused by a range of products such as paté, frankfurters, salads and RTE fish products (cold-smoked trout). Several risk assessments (Buchanan *et al.,* 1997; FAO/WHO, 2001a; FDA, 2001) have concluded that although even low number of cells carry some risk of infection, the majority of cases (>99%) are caused by food products with high levels of the bacterium (Figure 5.3). Thus, the real risk is the growth of the organism in the product rather than its mere presence. Despite this knowledge and the understanding that low levels are unlikely to cause disease, several countries, including the United States, have regulation asking so-called zero tolerance, i.e. that the organism must not be detected in 25 grams of food.

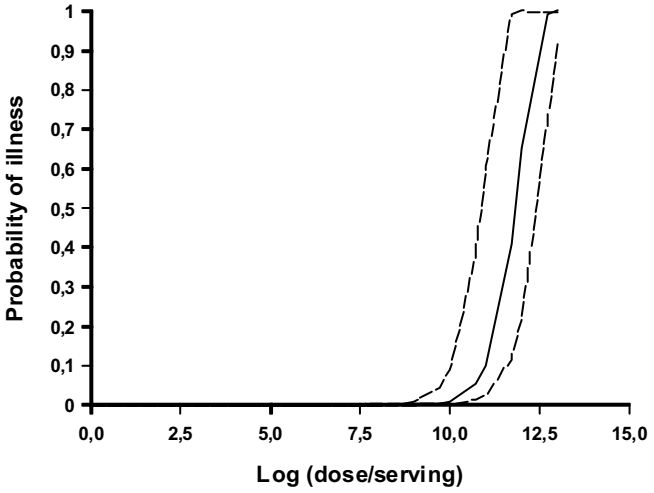

Figure 5.3
Simulated dose-response function for *Listeria monocytogenes* in ready to eat foods for consumers in the high risk group. Based on FAO/WHO (2001).

Epidemiological evidence suggests that listeriosis has been associated with smoked mussels (Brett *et al.,* 1998), "gravad" trout (Ericsson *et al.,* 1997), and smoked trout (Miettinen *et al.,* 1999). In the latter case the outbreak was not the classical invasive listeriosis, but cold-smoked trout was associated with febrile gastroenteritis in five healthy people.

b) The niche and prevalence in fish and fishery products

As indicated, *L. monocytogenes* is an organism indigenous to the general environment where it is typical of decaying plant material. Also, it occurs in the gastrointestinal tract and 2-6% of humans are healthy carriers. It is not typical of aquatic and marine environments. Thus the organism cannot be isolated from free open waters nor from fish caught or cultured in such waters (Table 5.14) . In contrast, water close to agricultural run-off harbour the organism and in principle the bacterium

must be assumed to be present, albeit in low levels on raw fish (Gram, 2001; Huss *et al.,* 1995). In contrast to the low levels or absence on raw fish, *L. monocytogenes* can easily be isolated from processed fish products. Thus 3-40% of RTE seafoods are positive for *L. monocytogenes* (Table 5.15), but in some smoke houses as much as 80% of the samples are positive.

Table 5.14 Prevalence of *Listeria* spp. and *Listeria monocytogenes* in live or newly slaughtered fish (modified from Gram (2001)).

Sampling location	No. of samples	% positive for	
		***Listeria* spp.**	***L. monocytogenes***
Freshwater			
skin of live trout (Switzerland)	45	33	11
channel catfish (USA)	4	100	nd
slaughtered trout (Switzerland)	27	22	15
Seawater			
salmon, at harvest (Norway)	10	0	0
salmon, at processing plant (Norway)	18	0	0
salmon (Faroe islands)	18	nd[1]	1
frozen salmon (received at plant) (USA)	65	nd	34
salmon (USA, Chile, Norway, Canada, Scotland)	32	nd	10

1. nd = not determined

Table 5.15 Prevalence of *Listeria* spp*. and Listeria monocytogenes* seafood products (modified from Farber and Peterkin (2000) and Gram (2001)).

Product	No. of samples	% positive for	
		***Listeria* spp.**	***L. monocytogenes***
Fresh shrimp	74	nd[1]	11
Fresh shrimp	178	nd	17
Slaughtered fish	50	2	0
Ceviche	32	75	9
Cold-smoked salmon	61	nd	0
Cold-smoked salmon	100	nd	24
Smoked salmon	65	11	11
Hot-smoked fish	142	25	5
Seafood salads	37	32	16
Cooked blue crab	126	10	8

1. nd = not determined

The bacterium is isolated at much higher frequency from RTE seafood products than from raw materials. Several studies have demonstrated that the processing environment is an important niche for *L. monocytogenes* (Autio *et al.,* 1999; Fonnesbech Vogel *et al.,* 2001). Thus using DNA-typing methods it has been shown that both slicers and salt brine harbours the types found in the product. Also, Table 5.15 shows that the bacterium is detected in heat processed products subjected to a listericidal process. Post-process contamination is the likely cause of this contamination. Cleaning and disinfection may temporarily remove the organism which is often found in more permanent niches in drains or floor mats.

c) Growth and survival in fish and fishery products

Listeria monocytogenes is halo- and psychrotolerant and can grow well in refrigerated foods. It is difficult to control it in RTE seafood products where there is no listericidal processing step and where *L. monocytogenes* can grow at the temperature / a_w / atmosphere conditions prevailing in the products. Several studies have demonstrated that it grows (rapidly) in brined shrimp and cold-smoked fish. Most – if not all – of these experiments were conducted with inoculated samples and

growth in naturally contaminated products appear much slower. This may partly be explained by the so-called Jameson effect where the presence of a competitive associate micro flora depresses the maximum cell density of the bacterium (Figure 5.4).

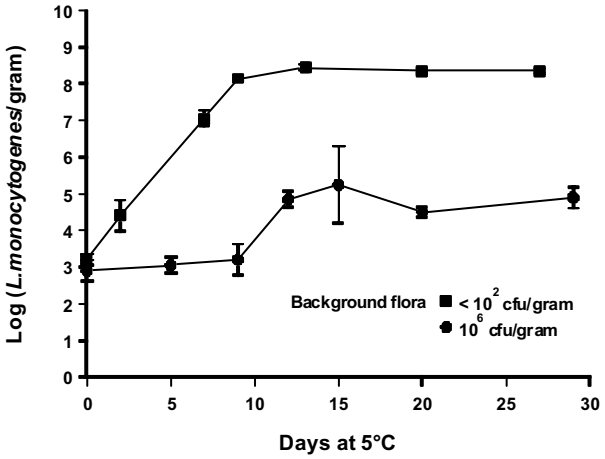

Figure 5.4
Growth of *Listeria monocytogenes* (mixture of 6 strains) on vacuum-packed cold-smoked salmon (5°C) when initial background flora is low or high (Huss *et al.,* 2000).

The NaCl concentration is critical when evaluating growth potential, as the bacterium may grow rapidly at 3-4% NaCl but much slower with 7-8% NaCl. *L. monocytogenes* is of little importance in semi-preserved seafood products where 2.5% acetic acid is used. Also, use of citric acid can be used to clean floors and drains and eliminate the organism from processing environments. Nitrate, lactate, di-acetate and bacteriocins inhibit or delay growth. The limited growth illustrated in Figure 5.4 can be used deliberately as preservation by adding a bio protective competitive lactic acid bacterial flora that inhibits *L. monocytogenes* (Nilsson *et al.,* 1999).

Listericidal processing consists primarily of heat treatment. The heat resistance of *L. monocytogenes* has been extensively studied in milk and dairy products (ICMSF, 1996). The thermal death time curve for *L. monocytogenes* in cod and salmon was studied by Ben Embarek and Huss (1993). The heat resistance of the bacterium is higher in salmon than in cod with D_{60} values being 4.5 min and 1.8 min, respectively. It is assumed that the higher lipid content (approximately 13%) of salmon protected the bacterium.

d) Prevention and control

Control of listeriosis can be achieved using HACCP and GHP. A critical control point occurs when processing can include a step where *L. monocytogenes* is eliminated. This can only be guaranteed in products that after packaging are subjected to a listericidal process, typically a heat treatment. In many products, low levels of *L. monocytogenes* will occur regularly or sporadically. Control of growth in products where a listericidal process is not used can be done in several ways. Freezing of products will eliminate growth, and sufficient levels of acid and NaCl will also prevent growth. Sorbate (0.05-0.1%) or the combination of lactate (2%) and di-acetate (0.1%) has been shown to eliminate growth in frankfurters (Tompkin, 2001). As mentioned, the addition of live lactic acid bacteria may inhibit growth in some products.

Critical control points cannot be identified in the processing of a number of RTE seafood products. Therefore, control of level of contamination using GHP is of outmost importance. *L. monocytogenes* is sensitive to common cleaning and disinfecting agents and both chlorine, iodine, acid, anionic and quaternary ammonium-type sanitizers are effective against *L. monocytogenes* at concentrations of 100 ppm, 25-45 ppm, 200 ppm and 100-200 ppm, respectively. *L. monocytogenes* often hides in niches in the processing environment and great care must be taken to clean such niches. The processing plant must have a *Listeria* surveillance programme installed and procedures to be implemented when the organism is detected.

As several risk assessments have shown that low levels of *L. monocytogenes* are consumed daily with no adverse effect, a limit of 100 cfu/g has been suggested as a food safety objective (van

Schothorst, 1998). A microbiological criteria involving 20 samples with m = 100 cfu/g and c = 0 is used has been suggested for RTE products where there is a potential for growth of the organism (van Schothorst, 1996).

Other clostridia and bacilli *(Lone Gram)*

Clostridium perfringens is an anaerobic, Gram-positive mesophilic spore-former widely distributed in the environment where it may be found at levels of 10^3-10^4 per gram soil. It can also be isolated from water and sediments and from faeces of healthy individuals (Adams and Moss, 2000).

If high levels of vegetative cells are eaten, a sufficient number may survive the gut passage and sporulate in the small intestine. The sporulating cells produce an enterotoxin of approximately 35 kilo Dalton (kDa). This results in nausea, abdominal pain, diarrhoea and, sometimes, vomiting 8-24 hours after ingestion. In the US, approximately 7 annual cases of *C. perfringens* are reported with links to seafood and it is estimated that approximately 200 seafood-caused cases occur every year (Feldhusen, 2000).

C. perfringens is typically associated with heated meat products or dishes which are temperature abused or heated slowly for long time. Due to its anaerobic nature, it prefers food with low redox potential.

C. perfringens does not grow at chill temperatures and grows only slowly below 20°C. The vegetative cells are sensitive to acid (minimum pH of 5), salt (maximum 6%) and do not grow at water activities below 0.95. Therefore controlling proliferation in seafoods is not complicated. Observing proper time-temperature conditions and avoiding cross-contamination to heated foods is essential.

Bacillus cereus strains are aerobic, Gram-positive spore-forming bacteria. As *C. perfringens* they are widely distributed in the environment. The spores are resistant to drying and are easily spread with dust. *B. cereus* can easily be isolated from many foods but typically occurs only in low numbers especially in raw foods (Granum and Baird-Parker, 2000). Heat processing will select for the spore formers.

B. cereus causes two types of disease, both caused by toxin formation. One is characterized by abdominal pain and profuse watery diarrhoea and symptoms occur 8-16 hours after ingestion. This type resembles the *C. perfringens* intoxication described above. The other, the so-called emetic type, has a shorter incubation period (½ to 5 hours) and nausea and vomiting are typical effects. This resembles the *S. aureus* gastroenteritis. The diarrhoeal type is associated with toxin formation in the gut whereas the emetic type is caused by a toxin preformed in the food. The toxin is produced in the late exponential to stationary phase and thus high numbers of *B. cereus* are a prerequisite for disease. The emetic type is typically related to rice, dough or other starchy products.

Most strains of *B. cereus* are mesophilic and do not grow below 10-15°C. However, psychrotrophic, toxin-producing strains have been isolated from foods stored at 4-6°C. Such strains must be considered for instance in the production of *sous-vide* products, where a mild heat-treatment is combined with subsequent cold-temperature storage. Although vacuum-packed, *Bacillus* species have been isolated in high numbers from *sous-vide* cod fillets stored at 5°C (Ben Embarek, 1994). Except for the few psychrotrophic strains, control of *B. cereus* is efficiently obtained by chilling.