

QUALITY MANAGEMENT AND MEASUREMENT IN THE FISH INDUSTRY

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Abstract

The European fish processing industry is facing increasing international competition that is shortening the lifecycle of the products. This development has changed the focus from price to quality and safety. To meet this challenge, quality management systems are now being slowly introduced to the companies. Another trend is the development of new advanced measuring techniques eg. based on microwave techniques and fluorescence spectrometry (two examples described in this paper). Hence, the challenge of the future is to find the right integration of new on-line/at-line measuring methods, information technology and quality management to achieve the optimal use of these systems to the benefit of both the fish processing industry and the consumer.

1. INTRODUCTION

In the early stages of industrialization of food processing, competition was mainly on price. Now quality and safety are the most essential parameters in the modern food industry including the fish processing industry [1]. To survive in the future the fish processing industry will require optimal feed back from the customer relevant to many levels in most steps of the production chain. The fish processing industries' main interest today is to make the production more effective and to raise the efficiency by rationalization. This, in combination with a need for quality, calls for an introduction of quality management systems in cooperation with new measuring methods.

The consumer demands for inexpensive, but still high-quality products, will influence the behavior of the producer. In addition to these demands, increased flexibility of supply, e.g. just-in-time delivery, in combination with the concentration in bigger units within the retail trade, will increase the pressure on the producer to deliver documented high quality products. These challenges call for the introduction of smooth-running quality management systems in the fish industry [2,3].

The introduction of new methodologies for measuring or monitoring results in increasing amounts of available data for use in quality management.

The fish processing industry depends on a few classical measuring methods in the production area and a mixture of classic and new analytical methods in the laboratory. The analytical methods are used for control of biological (pathogenic bacteria and spoilage fungi), chemical, nutritional and sensory characters and physical parameters as well as detection of other substances which can function as markers to check processing and handling.

2. THE MODE OF COLLECTING RELEVANT DATA AND THE QUALITY MANAGEMENT SYSTEMS

The use of information technology in the fish industry has, until now, been mainly in the production process. Information technology can be defined as finding common attributes in data and information, collection or extraction of data and storage of information in a practical way [4]. Monitoring during the processing operation helps prevent expensive rework or disposal of out-of-specification product.

Several measurement techniques are involved in order to control and manage the variables that are responsible for the deterioration of the product by eg. oxidative rancidity or microbial spoilage. The monitoring of temperature profiles is important during storage and in each step of the production chain. Measurement of temperature during heat processing, measurement of pH, water activity, solute concentration, etc. are all important measurements [1]. Table 1 shows that major methods used in the fish industry are very traditional.

The information chain from the fisherman to the consumer is broken nearly every time the commodity goes from one link to the next in the chain, creating information gaps between the links. Only size and species of the fish are registered by all the participants in the production chain, but this registration is being done *de novo* by each participant [5]. Not even a common day measurement such as temperature is registered by all and the information is not transferred from one participant to the next. There are several measurements that are rather important for nearly all links in the chain. Weighing is the most widespread measurement in the fish production chain and sometimes as many as six weighings are done on the same material without any manufacturing being done in between.

The result of the parameters measured on-/at-line does not necessarily coincide with the off-line measurements in the laboratory. Samples taken out of the production line often differ from the actual material in the process. Sometimes the sample changes during transport to the laboratory and sometimes even the preparation alters the composition of the sample, thereby giving a result that is more indicative than exact. When the time taken for a laboratory test exceeds one day, it is often impractical to hold the final product in quarantine until then. More often the laboratory measurements are based on spot tests. Thereby the test becomes a means of checking good manufacturing practice (GMP). The next step towards monitoring and controlling the microbial load, pathogens or toxins in the manufacturing process leads to introduction of HACCP (Hazard Analysis Critical Control Point) system [6].

Table 1
The most common on-/at-line measurements and other accessible information used for the quality management in the Danish fish sector.

| | Producer, the fisherman | | | Retail trade or fish mongers | |
|--------------------|-------------------------|-----|-----|------------------------------|--|
| Weight | + | + | + | + | |
| Volume | + | | | | |
| Size | + | + | + | + | |
| Temperature | + | | + | + | |
| Quality value | (+) | (+) | + | | |
| Fat (pelagic fish) | + | + | + / | | |
| Ice | + | + 1 | | | |
| Amount of fish | | + | | | |
| Species | + | + | + | + | |
| Storage age | | | + | + | |
| Catching area | | | + | | |
| Sensory parameter | | | + | | |
| Fillet yield | | | + | | |
| Durable costs | | | +. | | |
| Profit margins | | | + | + | |

Quality management is often used as synonym for systems as GMP (Good Manufacturing Practice), HACCP, ISO9000, BS7750, TQM (Total Quality Management), BPR (Business Process Reengineering) etc. although the quality management part of these systems varies .

The illustration in figure 1 shows the connection between increasing complexity of the products and increased supervision. As shown, the different management systems have a common core in Good Manufacturing Practice and voluntary standards and control based on legislation and regulation. It is essential that this part of the management system should function before moving on to introducing more complicated systems. On the other hand, increasing complexity without a competent management system could lead to the ruin of the company.

3. TWO EXAMPLES OF NEW MEASUREMENTS TECHNIQUES FOR USE IN QUALITY MANAGEMENT SYSTEMS

The following two examples of experimental tests shall be considered as both new evidence of the necessity to take new measurements into consideration to solve specific problems and as evidence to look into new possibilities to find a more optimal integration between measurements and quality management systems.

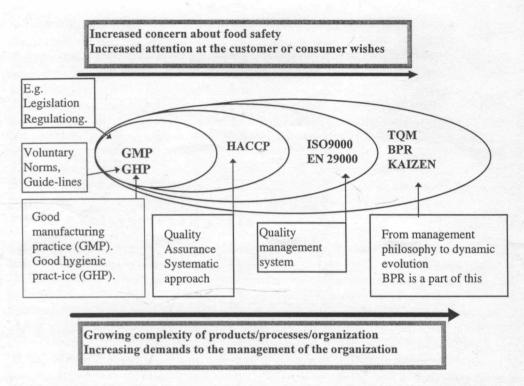


Figure 1. Illustration of the complexity and the connections between the different quality systems and their association to the surrounding environment. HACCP: Hazard Analysis Critical Control Point; TQM: Total Quality Management; BPR: Business Process Reengineering; KAIZEN: Japanese system for gradual continuous improvements [7]).

3.1. Measurement of fat content in herring

Herring is a fish species of great importance to Denmark as well as other European countries. The herring is used for marinated products, but also a significant share is exported semi-manufactured. The dominating herring stocks caught and processed by the Danish industry are from the nearby seas, the North Sea, the Baltic etc.

The important parameters when buying a catch from a fishing vessel are the storage age onboard, the fish size, the sensorial overall impression, and last but not least, the fat content. The fat content is estimated by fishermen who use their long-time experience in this determination. The result is used as an average fat content on the whole catch because by tradition one expects that herring living in schools are of the same age and have the same feeding opportunities. This should result in a very small variation in fat content in the individual fish. The fat content plays a major role in the processing industry, when choosing the proper treatment leading to a good and homogeneous product.

Experiments were done with a Torry Fish Fatmeter which uses microwave technique for measuring the fat content in herring [8]. The instrument measures the fat content in a quick

non-destructive way and was used to measure large samples from commercial catches of herring in 1993 [9].

Figure 2 shows 10 examples of commercial herring catches which have been measured by taking samples from the catch at a factory producing marinated herring.

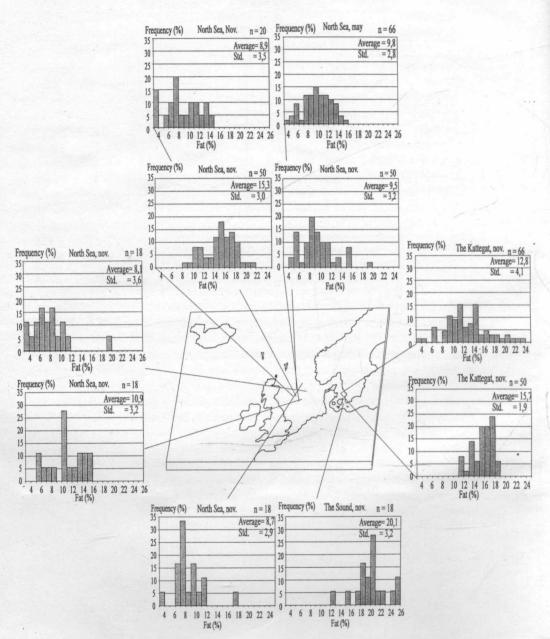


Figure 2. Examples of fat content measured in Danish commercial catches of herring.

The figure shows that there are large variations of the fat content within each catch. All catches but one are from the main season in the autumn where the schools are expected to be homogeneous with respect to fat content. Also there are differences in mean fat content from catch to catch, from 8.1% fat to 15.3% fat even when they are caught in the same area and time of the year.

This is an example of a surprising result obtained using an quick at-line instrument. The large variations in fat content shows that the herring industry are using a variable raw material that can cause problems with product inhomogeneity leading to manufacturing variabilities and consumer complaints.

The natural perspective following these results is the development of an equipment for online measurement of fat content and size and a subsequent individual sorting device so that the process may be adjusted appropriately.

3.2. Fluorescence Spectroscopy as On-line/At-line Measurement Method in the Fish Industry

Fluorescence spectroscopy is widely recognized as a powerful analytical tool in chemical, biological and environmental science due to its sensitivity as well as its selectivity. Modern fluorescence spectroscopy is capable of analyzing a large amount of samples containing several compounds at low concentrations in complex biological samples. These properties make fluorescence an excellent tool for monitoring quality of fish and fish products [10].

Lipid oxidation status is an important quality parameter in pelagic fatty fish. Secondary reaction products of lipid oxidation in fish muscle cause mainly the development of off-flavour compounds but also changes in nutritional quality, a modified texture and changes in colour [11]. Fluorescent compounds from reactions between lipid oxidation products and proteins, as well as changes in concentrations of fluorescent respiratory coenzymes like NADH, might serve as a useful measure of lipid oxidation. The use of fluorescence spectroscopy based on this approach to monitor lipid oxidative status of fish products can lead to the development of an on-line measurement method for industrial use.

The laboratory experiments are being carried out to correlate fluorescence spectra of fish extracts with various parameters representing lipid oxidation status (H. Zappey, E. Larsen and L. Munck, unpublished results). Chloroform/methanol (Bligh & Dyer) extracts were prepared of rainbow trout (*Oncorhynchus mykiss*) muscle fillets. In the extraction procedure both the lipid- containing chloroform phase as well as the water/methanol phase were collected. Both phases were screened for potential informative excitation-emission wavelength pairs by recording auto (primary) fluorescence emission spectra at a large number of excitation wavelengths. All fluorescence spectra were combined to form an excitation-emission matrix (EEM), representative of the fluorophores present in the extract. These excitation emission matrices were collected for a series of fillets stored on ice for periods of 5 to 20 days.

The methanol/water phase, especially, exhibited an appreciable amount of fluorescence at several excitation-emission wavelength combinations indicating that different fluorophores were present in this phase. The chloroform phase also exhibited fluorescence at other wavelength combinations, albeit at a much lower intensity. This might be caused by the relatively high electron affinity of chloroform. The extracts showed a clearly discernible change in the excitation-emission matrices as a function of the storage time of the fillets on

ice. In order to select the most representative wavelengths and to establish a correlation between the fluorescence spectra and the number of days the fillet was stored on ice, multivariate calibration methods like Partial Least Squares Regression (PLSR) were used [13]. Calibration of the fluorescence data with storage time of the fillets resulted in a number of correlations with correlation coefficients greater than 0.95. It was also possible to extract from the fluorescence emission data specific excitation/emission wavelength combinations which correlated directly with storage time of the fillets on ice. These preliminary results indicated that the use of a fast spectroscopic method like fluorescence has promising potential as a measuring technique in the fish industry and as an integrated part of the a quality management system.

4. DISCUSSION

Quality management is one of the keywords in the future development and rationalization of the food industry.

The Danish fish processing industry was asked in a survey, which subjects were the most essential in the coming years. The industry answered that the greatest challenge was more efficiency and rationalization. Quality management was rated ten times lower [14]. The reason is the very competitive situation in the sector; however, one has to take into consideration that efficiency and rationalization are part of quality management systems. In the same investigation the customers of the fish processing industries represented by the big multinational companies were asked what were their highest values, and the answer was uniform quality and reliability [14]. This answer implies that the processing industry has to take quality management into serious consideration and must integrate it with new measuring techniques.

5. CONCLUSION

The fish processing industry will in the coming years face the introduction of several new measuring techniques and the integration of these techniques into quality management systems.

Measuring methods based on spectrometry techniques seem to have great potential as on-/at-line measurements. Observations such as the variation of the fat content in herring caught from the same school show that the fish processing industries still have practical problems to deal with because of the natural variability in the raw materials.

The challenge is to find the right integration of on-/at-line measuring methods, information technology and quality management to achieve controlled, efficient optimal processing for the benefit of both the fish processing industry and the consumer.

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QUALITY, SAFETY AND NUTRITION Microbiology and quality assurance



MICROBIOLOGY OF FISH AND FISH PRODUCTS

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Abstract

Research on the microbiology of fish and fish products has primarily focused on safety and spoilage, but more recently new preservation techniques such as the use of natural antimicrobial substances including live cultures has also been the subject of much research.

The present paper will summarize some of the recent work carried out at the Danish Institute for Fisheries Research. A number of hazardous pathogens indigenous to the aquatic and general environment have been studied and their ecology in fish products and the environment has been elucidated. Similarly the specific spoilage organisms (SSO) for some fish products have been identified and studied in more detail.

Finally, the trends in consumer demands towards the use of less chemical preservatives and a milder cure of products have lead to studies of new preservation techniques.

While it is not intended to give a general and comprehensive review on the subject, our results will be discussed relative to other published data and suggestions for future research will be presented.

1. UNIQUE ASPECTS OF FISH AS RAW MATERIAL

All food commodities have their own distinctive microbiology. Important factors contributing to the microbiological complexity of seafood are:

- specific as well as non-specific commensal microflora of the live animal, specific and non-specific contamination of products during processing
- growth conditions for microorganisms due to specific intrinsic and extrinsic factors (temperature, a_w, pH, Eh, microbial interactions etc).

The wide range of environmental habitats (freshwater to saltwater, tropical waters to arctic waters, pelagic swimmers to bottom dwellers, degree of pollution) and the variety of processing practices (iced fish products to (sterile) canned products) are all important factors in determining the initial contamination of fish and fish products. The part of the microflora

which will ultimately grow on the products will be determined by the intrinsic and extrinsic parameters. There are several important specific intrinsic factors in fish which greatly influence the microbiology:

- the poikilotherm nature of the fish and its aquatic environment
- the presence of trimethylamine oxide (TMAO)
- · presence of large amounts of non-protein-Nitrogen (NPN)
- low carbohydrate content causing a high post mortem pH in the flesh (usually >6.0)
- · a variable (up to 30%) lipid content

The poikilotherm nature of the fish enables bacteria with a broad temperature spectrum to colonize the surfaces. Particularly when fish are caught in temperate waters, the major part of the bacterial flora is psychrotolerant and, as opposed to the normal mesophilic flora of meat, is very capable of growth at chill temperatures.

The presence of trimethylamineoxide (TMAO) in all marine and some fresh water fish species [1,2] is well established and this is known to cause a high (positive) redox potential (Eh) in the fish flesh [3,4]. The significance of this is not quite clear, but it has been suggested that the high Eh in lightly salted smoked fish is an additional hurdle to control growth of *Clostridium botulinum* in fish products [5]. In sugar-salted herring, the presence of TMAO and the high Eh was established as the protective mechanism against the most common type of spoilage (sweet-sour, rotten, putrid) as the organism causing this type of spoilage is a strict anaerobe requiring a low Eh for growth [6,7].

The spoilage of fresh fish is also influenced by the presence of TMAO, particularly under conditions where oxygen is excluded. A number of well defined spoilage bacteria (*Shewanella putrefaciens*, *Photobacterium phosphoreum*, *Vibrionaceae*) are able to utilize TMAO as the terminal electron acceptor in an anaerobic respiration resulting in off-odours and -flavours due to formation of trimethylamine (TMA) [8-12].

Another important intrinsic factor related to fish is the very high post mortem pH (>6.0). Most fish contains only very little carbohydrate (<0,5%) in the muscle tissue and only small amounts of lactic acid are therefore produced *post mortem*. The high pH and the presence of large amounts of Non-protein-Nitrogen (e.g. free amino acids) which are readily available for bacterial metabolism, contribute to the generally favourable growth conditions for bacteria on or in fish flesh.

2. PATHOGENIC BACTERIA

Fish and shellfish are common vehicles for transmission of foodborne diseases. The percentage of outbreaks associated with seafood depends on local climate and dietary customs. Seafood is involved in an estimated 11% of foodborne outbreaks in USA [13]. The main concern in some countries is scombroid fish poisoning, while in others the viral infections associated with consumption of bivalve molluscs or intoxications due to the preserve of biotoxins (ciguatera and shellfish poisoning due to toxic algae) may represent the majority of seafood borne diseases.

It is apparent from the statistics in USA [13] that bacterial pathogens are involved in approx. 25% of the outbreaks of seafood borne diseases. Some of these outbreaks are caused by bacteria originating in the animal/human reservoir (Salmonella, Shigella, E.coli, Staphylococcus aureus). This group of bacteria is normally not present on fish, but the fish products may become conta-minated during processing, storage, distribution or preparation for consumption.

In contrast, a number of pathogenic bacteria may represent part of the natural flora on fish, particularly on fish from coastal and estuarine environments as shown in Table 1. While these pathogens are naturally present on fish, other organisms as listed in Table 2 may be present on freshwater fish or fish caught in coastal areas due to contamination of the aquatic environment as a result of water run-off from surrounding land.

It should be emphasized, however, that all the genera of pathogenic bacteria mentioned in Tables 1 and 2 contain non-pathogenic environmental strains. For some organisms it is possible to correlate between certain characteristics and pathogenicity (e.g. the Kanagawa-test for *V. parahaemolyticus*) while in others (e.g. *Aeromonas* spp.) there are no known methods available.

While it is true that all fish and fish products which have not been subject to bactericidal processing may be contaminated with one or more of these pathogens, the level of contamination is normally quite low and it is unlikely that the numbers naturally present in uncooked seafood are sufficient to cause disease in healthy human beings. An exception is when pathogens are concentrated by filtration (molluscs). On the other hand high levels of indigenous bacteria may be found on fish products as a result of growth. This situation constitute a serious hazard with a high risk of causing illness. Growth (and in certain cases toxin production) must therefore be prevented. Some of the growth requirements of bacteria normally occurring on seafood are listed in Table 3.

Table 1
Pathogenic bacteria indigenous to the aquatic environment - *naturally* present on fish [14]

| Organism | Primary habitat | Quantative levels |
|--|---|--|
| Clostridium botulinum -non-proteolytic types | temperate and arctic aquatic environment. | generally low (<0.1 spore g ⁻¹ fish) |
| B, E, F | Multiplication in aquatic carrion (type E) | but up to 5.3 spore g ⁻¹ fish |
| Pathogenic Vibrio spp. V.cholerae V. parahaemolyticus V.vulnificus other vibrios | ubiquitous in warm (>15°C) seawater environment | up to 10 ² -10 ³ cfu g ⁻¹ in shellfish up to 10 ⁴ -10 ⁸ cfu g ⁻¹ in intestines of shellfisheating fish |
| Aeromonsa hydrophila | aquatic environment | up to 10 ⁴ cfu ml ⁻¹ in seawater and 10 ⁷ cfu ml ⁻¹ in sewage 10 ⁶ cfu g ⁻¹ in raw seafood ^e) |
| Plesiomonas shigelloides | warm water environment freshwater fish | |

Table 2
Pathogenic bacteria indigenous to the general environment - *frequently* present on fish [14]

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

Table 3
Growth limiting factors and heat resistance of pathogenic bacteria normally occurring on seafood. The Table is adapted from Huss [15]

| Species | Min | | Max | Heat resistance | |
|--------------------------|---------|---------|--------|---|--|
| | Temp | pН | % NaCl | | |
| C. botulinum | A TOTAL | | | | |
| -non-proteolytic (B,E,F) | 3.3 | 5.0 | 3-5 | D82.2 = 0.15-0.2 min in broth $D80 = 4.5-10.5$ min in products with high protein and fat content | |
| -proteolytic (A,B,F) | 10 | 4.0-4.6 | 10 | D121 of spores = $0.1-0.25 \text{ min}$ | |
| Listeria monocytogenes | 1 | 5.0 | 10 | $D_{60} = 2.4-16.7$ min in meat $D_{60} = 1.95-4.48$ min in fish | |
| Vibrio spp. | | | | | |
| V.cholerae | 5 | 5.0 | <8 | $D_{71} = 0.3 \text{ min}$ | |
| V.parahaemolyticus | 5 | 6.0 | 8-10 | $D_{55} = 0.24 \text{ min}$ $60^{\circ}\text{C/5} \text{ min gave 7 log}_{10} \text{ decline}$ | |
| V.vulnificus | - 8 | 4.8 | 5 | oo C/3 mm gave 7 log10 decime | |
| Aeromonas spp. | 0-4 | 4.0 | 4-5 | $D_{55} = 0.17 \text{ min}$ | |
| Plesiomonas spp. | 8 | 4.09 | 4-5 | 60°C/30 min gave no survivors | |

2.1. Clostridium botulinum

The association of certain types of botulism with fish has been recognized for many years and is documented in a number of excellent reviews, the latest one by Hauschild and Dodds [16]. It was clearly demonstrated by Huss [17] that *C. botulinum* type E is indigenous to the aquatic environment in cold and temperate waters and the hypothetical circles shown in Figure 1 provide an explanation for the local and the global distribution of this organism.

It should be emphasized that fish products may be contaminated during proces-sing with the more mesophilic *C. botulinum* types A and B. The growth requirements for these types must therefore also be included in the risk assessment of fish products.

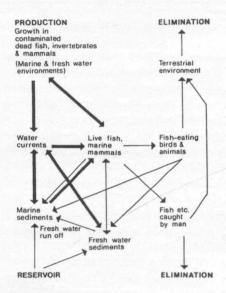


Figure 1. Hypothetical circles of Clostridium botulinum type E [17]

Psychrotolerant non-proteolytic strains of *C. botulinum* (types B, E and F) are able to grow at low temperature as shown in Table 3. Additional hurdles are therefore required to control the growth under these conditions. Cann and Taylor [18] showed that a minimum concentration of 2.5-3.0% water phase salt (WPS) prevented production of toxin in hot smoked vacuum packed trout and mackerel for 30 days at +10°C and recently McClure *et al.* [19] reported data from model experiments showing time to toxin production for the same organisms in soya peptone at +5°C and 3% WPS to be 60 days. At higher temperatures (>10°C) much more salt is required to prevent growth as the proteolytic strains will be able to grow as shown in Table 3.

Vacuum-packaging has been shown to enhance toxin production by *C. botulinum* [20,21]. This is of great concern in some countries, e.g. USA, where the National Marine Fisheries Service (NMFS) recommends (but does not require) that fisheries products should not be Modified Atmosphere (MA) or vacuum-packed if they are to be stored under refrigeration [21,22].

2.2. Listeria monocytogenes

The primary habitat of *Listeria monocytogenes* is probably soil and decaying vegetation where the bacterium leads a saprophytic existence [23]. Dillon and Patel [24] and Ben Embarek [25] have summarized recent data on occurrence and biology of *L. monocytogenes* in the environment and in seafood. Although also frequently isolated from surface water, its

presence in this environment may be as a result of contamination (sewage or land-run off). Thus there is some evidence that clear unpolluted water (seawater, fresh water) and fish from such areas do not harbour this organism (Table 4).

Table 4
Prevalence of *Listeria* in cold smoked fish and the environment [26]

| Samples | Num | ber of | % Positive for | |
|------------------------|---------|---------|----------------|-----------------|
| | surveys | samples | Listeria | L.monocytogenes |
| Sediments (freshwater) | 3 | -79 | 20-30 | 0-17 |
| Seawater | | | | |
| - unpolluted | 3 | 121 | 0-3 | 0 |
| - polluted | 3 | 32 | 25-52 | 14-33 |
| Freshwater | | | | |
| - spring | 1 | 24 | 0 | 0 |
| - surface water | 4 | 236 | 33-100 | 0-62 |
| Live fish - reared in: | | | | |
| spring or ground water | 1 | 60 | 21 | 0 |
| - surface water | 1 | 30 | 3 | 30 |
| - unpolluted seawater | 1 | 30 | 0 | 0 |
| Final product | | | | |
| - cold smoked salmon | 10 | 986 | 0-80 | 0-75 |

To prevent the occurrence of *L. monocytogenes* in the final products (e.g. cold smoked salmon) it is important to identify the sources of contamination. Eklund *et al.* [27] found the raw material to be the primary source. However, using multilocus enzyme electrophoresis (MEE) as a discriminatory typing system, Rørvik *et al.* [28] found different types of *Listeria* on raw material and final product.

While *C. botulinum* can be controlled in most seafoods by traditional means, this is not the case with *L. monocytogenes*. Huss *et al.* [26] reviewed the situation for cold smoked fish and found that at conditions prevailing ($t \le 5^{\circ}$ C, WPS 3-4%, pH >6 vacuum packaging) *L. monocytogenes* multiplied at a rate of 1-2 log units per week. Current cleaning and sanitation methods do not effectively eliminate the organism although the incidence can be dramatically reduced as shown by Garland [29].

L. monocytogenes is one of the more heat resistant of the non-sporeforming pathogens. However, vastly different recommendations on listericidal heat-treatments have been issued ranging from 70°C in 0.08 min. to 70°C in 2 min. [25]. D_{60} -values for different strains of L. monocytogenes were found to vary between 1.95 to 4.48 min. in cod and salmon fillets respectively [30]. Thereby a reduction of 4 log units which is the recommended value for pasteurized food [31] would require a heating time at 60°C from 8-17 min depending on the product. However, 37 min would be required for cod containing 4% NaCl [30].

2.3. Vibrio spp.

In late January 1991, cholera appeared in Peru. By August 1992, 19 countries had reported 640.000 cholera cases and 5.600 deaths. Although the primary source of infection is