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The use of fish by-products in aquaculture

Report of the Scientific Committee on Animal Health and Animal Welfare

Adopted 26th February 2003

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1. GLOSSARY

Anadromous	Fish that migrate up rivers from the sea in order to breed in freshwater.
Biological treatment	Treatment involving the use of microorganisms in a biotechnological process.
Biotechnology	Technology involving biological processes.
Brackish water	Mixture of freshwater and seawater.
By-product	Secondary or incidental product of an industrial or manufacturing process. For the purposes of this report, fish by-products include wild caught fish or parts thereof that are not used for human consumption, and materials (e.g. heads, frames and trimmings) generated from processing wild and farmed fish for human consumption, as well as mortalities from fish farms.
Capture fisheries	Fisheries based on capture of wild fish or shellfish in marine or inland waters and excluding aquaculture (also known as caught fisheries/catch).
Cephalopod	Marine mollusc of the class *Cephalopoda*, characterised by a head and eyes and a ring of sucker-bearing tentacles.
Cfu	Colony forming unit.
Chemoresistance	Resistance to chemical agents.
Copepod	Minute free-living or parasitic crustacean of the subclass *Copepoda* of marine and freshwaters; an important constituent of plankton.
Crustacean	Arthropod of the class *Crustacea,* having an exoskeleton and two pairs of antennae. Examples include prawns, crabs and lobsters.
Cyprinid	Teleost fish of the family *Cyprinidae* e.g. carp, tench, roach, rudd.
D-value	The time in minutes at a given temperature to achieve a 90% reduction in numbers of viable bacteria.
Decanting	Pouring off a liquid from one container to another without disturbing the sediment.
Decimal Reduction Rate	Time required to effect a ten-fold reduction in numbers of viable micro-organisms at a certain temperature (t) (D_t - value).
Demersal fish	Fish that live in deep water or on the bottom of a sea or lake.
DHA	The essential fatty acid docosahexaenoic acid.

Diadromous	Fish that are migratory between fresh- and saltwater.
El Niño	A warming of the eastern tropical Pacific occurring every few years, which disrupts the weather pattern of the region.
Ensilage	The product of ensiling. Fish or fish by-products are usually ensiled using formic and/or propionic acid.
EPA	The essential fatty acid eicosapentaenoic acid.
F value	The holding time at a particular temperature to which a heating process is equivalent.
Fermentation	A chemical reaction that causes an organic molecule to split into simpler molecules by a process involving microorganisms, which may be carried out under aerobic or anaerobic conditions.
Fermenter	Vessel, container or reactor in which fermentation takes place.
Finfish	Fish that use fins for locomotion and balance.
First-feeder diet	Diet of juvenile fish during the period immediately following hatching from the egg.
Fish	Aquatic vertebrate animal of the class *Pisces*, most of which obtain oxygen through their gills (see also Finfish). For the purposes of this document 'fish' is used in a generic sense to also include shellfish- molluscs and crustaceans.
Gastropod	Mollusc of the class *Gastropoda*, typically having a flattened muscular foot for locomotion and a head that bears stalked eyes, e.g. whelks and limpets.
Hatcheries	Places where eggs are hatched under artificial conditions.
Mean hydraulic retention time	Calculated time in which the whole content of a reactor is replaced by filling and emptying (i.e. if 5% of the volume is exchanged each day, the reactor has a MHRT of 20 days).
Krill	Small shrimp-like marine crustacean of the order *Euphausiacea.*
Mollusc	Member of the Phylum *Mollusca*. Those with a two-piece shell are called bivalve molluscs, e.g. oysters and mussels.
Morts	Fish that have died on fish farms before the end of their production cycle, possibly due to disease caused by infectious agents etc.
Organoleptic	Relating to perception involving the use of sensory organs.
Parr	A young salmon during its first 2 years of life when it lives in fresh water.

Pasteurisation	A treatment method in which the product is heated to and maintained at a certain temperature for a specified time before being cooled. The process is designed to reduce the bacterial content within a product without unduly affecting its taste or appearance.
Pelagic fish	Fish living or occurring in the upper waters of open sea.
Pfu	Plaque forming unit.
Salmonid fish	Fish of the family *Salmonidae.*
Shellfish	Aquatic invertebrate with a shell, including both molluscs and crustaceans.
Sessile	Permanently attached to a substratum.
Sieving	To pass through a wire mesh or closely perforated metal for the purpose of straining/sifting.
Swim bladder	Air filled sac, lying above the alimentary canal in bony fishes, that regulates buoyancy at different depths by a variation in the pressure of the air (also called 'air bladder').
T_{90} - value	The time required to reduce a microbial population by 90%.
Teleost	Bony fish of the subclass *Teleostei*, having rayed fins and a swim bladder. The group contains most of the bony fishes and includes herring, carp, salmon and cod.
Wet diets	Diets containing fish and fish by-products that may be fresh or frozen but otherwise unprocessed.
z value	The temperature change required to effect a 10 fold change in the D-value.

2. TERMS OF REFERENCE

The Scientific Committee on Animal Health and Animal Welfare is asked to carry out a risk analysis on the practice of feeding fish by-products in aquaculture.

The report should cover the following aspects;

1. The practice at the moment - what fish by-products are fed to fish, including the feeding of sea-caught fish or fish offals from plants manufacturing fish products for human consumption, and the use of by-products originating on fish farms (e.g. dead fish);

2. What disease risks (animal or public health) attach to the feeding of fish by-products (viral, bacterial and parasitic);

3. Consider the current and possible treatments methods for these by-products, which could reduce or eliminate any identified risks.

3. BACKGROUND

Animal by-products not destined for human consumption are included in the list of products in Annex I to the Treaty. To ensure rational development in this sector, animal health and public health rules for the products in question should be laid down at Community level.

Council Directive 90/667/EEC (CEC, 1990a), laying down the veterinary rules for the disposal and processing of animal waste, for its placing on the market and for the prevention of pathogens in feedingstuffs of animal or fish origin and amending Directive 90/425/EEC (CEC, 1990b), established the principle that all animal waste, regardless of its source, may be used for the production of feed material following appropriate treatment.

Regulation (EC) 1774/2002 of the European Parliament and of the Council (CEC, 2002a) lays down health rules concerning animal by-products not intended for human consumption.

The basis for Community legislation on health issues is sound science, and to this end, scientific advice is required on the use in aquaculture of by-products originating from fish, as outlined in the terms of reference.

4. PREAMBLE

The Committee interpreted the terms of reference as relating specifically to the feeding of fish by-products to farmed fish (including shellfish, molluscs and crustaceans), and the feeding of fish by-products to other animal species has not been considered within this report. Fish by-products are also utilised for a wide range of other purposes, such as the production of cosmetics and pharmaceuticals and various other industrial and treatment processes. There is concern that when by-products from fish are fed to fish such material might contain pathogens that may cause disease in fish and/or have public health significance (Gill, 2000). If fish by-products are used as raw materials for products that are eventually used on or in animals, there is a theoretical possibility of the transfer of contagious diseases through these products. However, in these cases, the raw material is subjected to several production steps that contribute to a reduction of the risk involved. It should be noted that most of the pathogens applicable to fish (see chapter 7), have optimum

growth temperatures of 15-25°C, and may die or not replicate at normal body temperatures of warm-blooded animals. The marine raw material will, before ending up as an ingredient in cosmetics, biotechnological products or in food production, also undergo several production steps, such as heating, centrifugation, filtration, redox reaction etc. All these steps will, to a varying extent, contribute to a reduction of the number of viable microorganisms in the end-product.

While the material used to feed finfish may be of primary concern, large amounts of other types of fish are also produced by aquaculture in the EU. On a world scale herbivorous fish are an important element of total aquaculture production and may become increasingly important within EU aquaculture production. However, the Committee has concentrated in this report on the carnivorous fish species currently produced by EU aquaculture. This report considers by-products produced by capture fisheries, aquaculture and the fish processing industry, and reviews the sources of material used for feed for farmed fish, as well as the infectious agents likely to affect fish. Various processes used to treat fish by-products and inactivate those infectious agents are also described. Other treatment processes such as alkaline hydrolysis are still under development and have not been specifically considered in this report. Data on world and EU fish production are provided to indicate the scale of the industry and the amount of by-products produced. Environmental issues are also relevant to various aspects of the production and use of fish by-products. However, such environmental implications and issues of sustainability/biodiversity are not within the terms of reference of this report and are not specifically considered. The issue of transmissible spongiform encephalopathies (TSE) is not considered in detail since this has been separately dealt with by an opinion of the TSE/BSE *ad hoc* group of the Scientific Steering Committee (SSC) entitled "The feeding of wild fishmeal to farmed fish and recycling of fish with regard to the risk of TSE".

5. FISH PRODUCTION DATA

5.1. World fish production - capture fisheries and aquaculture

Fisheries and aquaculture continue to be a very important source of food, employment and revenue in many countries (FAO, 2002). As an example in 1999 the value of world total fishery production grew by 7% to an estimated US$ 125 billion (FAO, 2000). World production of fish, crustaceans and molluscs continues to increase and reached 130.4 million tonnes in 2000, an increase from 126.2 million tonnes in 1999 and 117.7 million tonnes in 1998- see Table 1. Production of fish by catching (often referred to as 'capture fisheries') accounted for 73% of the total in 2000 at 94.8 million tonnes, while aquaculture production increased to 35.6 million tonnes to comprise the remaining 27%.

China reported a production of approximately 17 million tonnes in 2000 from capture fisheries while other major capture fish producers were Peru (10.6 million tonnes), Japan (5.0 million tonnes) and Chile (4.3 million tonnes). Since the mid-1980s the contribution to fish production from the catching sector has levelled off while aquaculture has shown dramatic growth (Barlow, 2001).

In 2000 approximately three quarters of the global production of fish, crustaceans and molluscs were utilised for direct human consumption, while the proportion of fish used for reduction to meal and oil continues to increase since 1998. Fish utilised as raw material for the production of animal feed represented a significant proportion of the overall utilisation and the species used for reduction to fishmeal consisted almost entirely of natural stocks of small pelagic fish (FAO, 2002).

In 1999, foreign trade earnings from fish amounted to US$ 52.9 billion, an increase of 3.3% at current values, compared to 1998. Thailand continued to be the leading exporter contributing almost 8% of the total world value (US$ 4.1 billion worth of fish trade). Other major exporters were Norway, China, the US, Denmark and Canada (FAO, 2000).

Most of the fishery exports were destined to go to developed countries. In 1999 industrialised countries accounted for over 80% of the total value of imports of which Japan (US$ 14.8 billion) accounted for almost 26% of the world total, followed by the United States (US) (US$ 9.4 billion) accounting for 16% of the total. The European Union (EU) increased further its dependency on imports for its fish supply. Its share of total world imports reached 36%, although more than 40% of the EU's imports originate from intra-EU trade.

Shrimp is the most important commodity accounting for about 19% in value terms of international trade in 1999. This share remained stable over the past 20 years. Groundfish (i.e. bottom dwelling demersal fish) and tuna (a pelagic fish) followed with an 11% and a 9% share respectively. Exports of farmed salmon have been increasing, and represented 9% in value terms of the total trade in 1999. The relative importance of fishmeal and of cephalopods (squid, cuttlefish and octopus) has decreased over the past number of years to reach 3% and 4%, respectively, in 1999. In 2000 imports of fish and fishery products by Japan, the US and the EU increased by 6%, 12% and 9%, respectively, compared to 1999. Japan continued to be the world's leading importer. Spain was the leading importer of fish and fishery products within the EU, followed by France, Italy, Germany and the United Kingdom (UK). China is becoming one of the largest fishmeal consumers in the world and in 2000, it imported 1.1 million tonnes, almost doubling the imports of the previous year, with 40% of its fishmeal imports being used in aquaculture (FAO, 2002).

5.2. Total fishery production from the catching sector

The world catch figures for the year 2000 totalling 94.8 million tonnes represent a huge increase in the fisheries harvest, from the figure of 60 million tonnes in 1967 (Russell-Hunter, 1970).

Total production from the catching sector dropped from the 93/ 94 million tonnes caught in 1996 and 1997 respectively to 87 million tonnes in 1998. China, Japan, the US, Russia, Peru, Indonesia, Chile and India, in that order were the largest producers and together accounted for more than half of total production (FAO, 2000).

In 1999 the increase in landings from the catching sector occurred as fish stocks in the Southeast Pacific recovered from the effects of the El Niño atmospheric phenomenon, which affected those stocks in 1997/98. Landings of Peruvian anchovy and Chilean jack mackerel, which had decreased to a low of 3.7 million tonnes in 1998, amounted to 10.1 million tonnes in 1999. Marine caught fisheries account for more than 90% of world capture fishery production. The remainder comes from inland freshwater fisheries, which have increased their output by almost 0.5 million tonnes per year since 1994 (FAO, 2000).

5.3. Marine capture fish production

World marine capture fisheries production dropped to 79 million tonnes in 1998 (Table 1), representing an 8% decline in relation to the all–time highs of about 86 million tonnes recorded in 1996 and 1997. The decline appears to be due to climatic factors (FAO, 2000) and most of the decline in the world's marine fisheries landings in 1998 can be attributed to changes in the Pacific, which was severely affected by the El Niño phenomenon in 1997–1998. However, within total fish catches there may also be variation over time in terms of the variety of species caught, and possible over-fishing of certain species that will impact on the range and volumes of species caught.

Table 1. World fisheries production and utilisation 1994–2000 (FAO, 2002)

PRODUCTION	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**
INLAND	*(Million tonnes)*						
Capture	6.7	7.3	7.4	7.6	8.0	8.5	8.8
Aquaculture	12.2	14.0	15.9	17.4	18.5	20.2	21.4
Total inland	**18.9**	**21.3**	**23.3**	**25.0**	**26.5**	**28.7**	**30.2**
MARINE							
Capture	85.0	84.7	86.0	86.4	79.2	84.7	86.0
Aquaculture	8.7	10.4	10.8	11.2	12.0	13.2	14.2
Total marine	**93.7**	**95.1**	**96.8**	**97.6**	**91.2**	**97.9**	**100.2**
Total capture	91.7	92.0	93.4	94.0	87.2	93.2	94.8
Total aquaculture	20.9	24.4	26.7	28.6	30.5	33.4	35.6
Total world fisheries	**112.6**	**116.4**	**120.1**	**122.6**	**117.7**	**126.6**	**130.4**
UTILISATION							
Human consumption	78.1	84.3	88.0	90.8	92.7	94.4	96.7
Reduction to fishmeal and oil	30.2	27.4	27.5	25.9	19.9	25.6	27.4
Miscellaneous uses	4.3	4.7	4.6	5.9	5.1	6.6	6.3

5.4. Inland capture fish production

In 1998, production from inland caught fisheries was 8 million tonnes, which represented a 6% increase over 1997 levels. The top ten countries with regard to inland fisheries production are listed in Table 2. These countries account for 65% of the world's total inland catch. More than 90% of this production in 1998 came from developing countries, and only 3.5% from developed countries.

Table 2. Top ten countries in inland caught fish production (FAO, 2000)

Country	**Production in 1998** ***(tonnes)***	**Percentage of world production** ***(65% for top ten countries)***
China	2,280,000	28.5
India	650,000	8.1
Bangladesh	538,000	6.7
Indonesia	315,000	3.9
Tanzania, United Rep.	300,000	3.7
Russian Federation	271,000	3.4
Egypt	253,000	3.2
Uganda	220,000	2.8
Thailand	191,000	2.4
Brazil	180,000	2.3

5.5. EU fish production from catching sector (marine and inland capture fisheries)

After China and Peru, the EU is the third largest fish producer in the world although it has increased its production by just less than 1% since 1970 (EC, 2001). Pelagic fish such as herring, sandeels, sprat, horse mackerel, mackerel and sardines make up about 50% of the total EU (on a weight basis). However, the most economically important fish are the larger pelagic species and demersal fish such as cod and hake, even though they represent less than 10% of the total catch. Species such as sprat, sandeels and blue whiting are primarily intended for industrial use such as in fishmeal and fish oil production (EC, 2001). The share out of the total EU catch (marine and inland) is shown in Table 3. By means of comparison, in 1999 the catches of Norway and Iceland were 2.6 and 1.7 million tonnes respectively. Over the past ten years, landings in ports of the EU have decreased both in terms of volume and value: the volume of landings has gone down by 27% since 1992 while their value has fallen by 18% over the same period. Landings represent the weight of all fishery products landed in the ports of EU Member States without distinction of the flag flown by the vessels making the landings.

Table 3. Total catches (marine and inland) of EU Member States, Iceland and Norway in 1999 and 2000 (Sources: Eurostat, FAO, 2002)

Member State	Capture production 1999 unit tonnes	Capture production 2000 unit tonnes
Denmark*	1,404,912	1,534,074
Spain	1,187,620	994,739
UK	837,757	746,294
France	675,817	690,469
Netherlands	514,615	495,804
Sweden	351,345	338,537
Italy	294,160	299,955
Ireland	283,921	282,925
Germany	238,921	205,245
Portugal	209,312	187,846
Finland	144,520	156,480
Greece	118,783	99,292
Belgium/ Luxembourg	29,876	29,799
Austria	432	859
Iceland	1,736,267	1,982,522
Norway	2,620,073	2,703,415

*not including catches for Greenland and the Faeroe Islands

5.6. World aquaculture

Aquaculture is the farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants. On a worldwide basis, freshwater aquaculture is of greater importance than marine aquaculture. Inland aquaculture is seen as an important source of food in Asia, particularly in land-locked countries (FAO, 2000). Most freshwater aquaculture is of finfish particularly carp. Brackish water (salt content between that of freshwater and seawater) aquaculture development has been mainly linked to the production of tiger prawns and milkfish. In volume terms, marine aquaculture has been dominated by seaweed (particularly Japanese kelp) and molluscs, mainly the Pacific or cupped oyster. Table 4 contains details of global aquaculture production in 2000 and Table 5 details major species groups produced by global aquaculture in 1998. Aquaculture production from both inland and marine waters

continued to increase in 2000 and the Asian region (particularly China) continued to dominate world production. Asia, the Americas and Europe have seen an expansion in aquaculture production, while Africa has been slow to develop the potential of aquaculture (FAO, 2002).

Table 4. Aquaculture production in 2000: major producing countries (FAO, 2002)

Country	Quantity (thousand tonnes)	Value (US$ millions)
China	24,581	24,117
India	2,095	2,166
Indonesia	789	2,246
Japan	763	3,317
Thailand	707	2,431
Bangladesh	657	1,159
Vietnam	511	1,089
Norway	488	1,357
USA	428	870
Chile	392	1,250
Philippines	388	680

Table 5. Global aquaculture in 1998: major species groups (FAO, 2000)

Major species group	Quantity (thousand tonnes)	Value (US$ millions)
Freshwater fish	17,355	19,737
Molluscs	9,143	8,479
Diadromous fish	1,909	5,907
Crustaceans	1,564	9,234
Marine fish	781	3,396
Other aquatic animals	111	330

5.7. EU aquaculture

Aquaculture production has increased significantly over the last decade and now represents over 30% of the total value of fishery production in the EU (EC, 2001). The increase in aquaculture production has helped compensate for the decrease in the quantities of fish caught at sea and in some Member States aquaculture production exceeds the value of landings of caught fish, e.g. in Finland and Greece (EC, 2001). See the Annex for a listing of the species produced in EU aquaculture in 1999 (as well as data on fish by-products production, imports and exports). Table 6 shows the total aquaculture production for EU Member States and Norway for 1999 and 2000, while Table 7 shows the main species produced by aquaculture in the EU in 1999.

Table 6. Total Aquaculture production for EU Member States and Norway for 1999 and 2000 (EC, 2001; FAO, 2002)

Member State	1999 (tonnes)	2000 (tonnes)
Spain	321,145	312,171
France	264,850	267,767
Italy	210,368	216,525
UK	154,800	152,485
Netherlands	108,785	75,339
Greece	79,474	79,879
Germany	73,567	59,891
Ireland	43,856	51,247
Denmark	42,670	43,609
Finland	15,449	15,400
Portugal	7,022	7,538
Sweden	6,035	4,834
Austria	3,070	2,847
Belgium/ Luxembourg	1,597	1,641
Norway	469,032	487,920

Table 7. Table showing the main species produced by aquaculture in the EU in 1999 (EC, 2001).

Species	Quantities (tonnes)
Blue mussel	479,168
Rainbow trout	227,960
Mediterranean mussel	157,812
Atlantic salmon	146 258
Cupped oyster	142,730
Manila clam	51,397
Gilthead seabream	47,116
Seabass	36,230
Carp	17,649
Eel	10,269

5.8. EU processing sector

In the EU the value of the fish processing sector is nearly twice the value of the catching sector although in some Member States there is no direct link between the size of landings and the importance of the processing sector. For example in 1998 in Germany the value of fish landings represents less than 2% of all EU landings while the value of its processing sector accounts for 12% of the EU total (EC, 2001)- see Table 8.

Table 8. Output value of the EU Processing sector in 1998 (EC, 2001)

Member State	Value (millions euro)
Spain	2,271
France	1,870
Denmark	1,447
Germany	1,270
Portugal	1,017
UK	873
Italy	582
Netherlands	465
Sweden	297
Ireland	271
Belgium/ Luxembourg	237
Greece	90
Finland	80

5.9. Consumption of fishery products in the EU

While fishery products play an important role in the European diet as a valuable source of protein, consumption of fishery products varies greatly within the EU with Portugal having the highest consumption per capita and Austria having the lowest. However, EU consumption is on average higher than the world average. Table 9 shows the per capita consumption of fishery products in the Member States.

Table 9. Per capita consumption of fishery products in the EU, Norway and Iceland in 1997 (EC, 2001)

Member State	**Per capita consumption** (kg/head/year)
Portugal	58.5
Spain	40.9
Finland	33.1
France	27.5
Greece	26.5
Sweden	26.1
Denmark	23.5
Italy	22.2
UK	21.1
Belgium/Luxembourg	19.4
Ireland	15.3
Netherlands	15.1
Germany	12.7
Austria	11.2
Iceland[1]	44.7
Norway[2]	18

[1] Source www.hagstofa.is

[2] Source www.ssb.no

5.10. Shellfish- crustaceans and molluscs

Over recent decades shellfish production has expanded significantly, with larger quantities of crustacean and molluscan waste material being consequently produced (Martin, 1994). In the 1960s and 1970s shellfish processing was catering for a fresh market, the shellfish were marketed locally and processing plants were small. The situation is now very different with shellfish being processed in large facilities all over the world. Fish is traded mostly as a frozen food and shrimp is the main fish trade commodity in value terms, accounting for some 20% of the total value of internationally traded fishery products (FAO, 2000). The most commercially harvested crustacean species are crab, shrimp, prawn, Antarctic krill and crayfish (Martin, 1994).

In the crustacean meal industries, waste material may represent more than 80% of the landing. Shellfish wastes consist mainly of shells, viscera, heads and adhered meat, and they can be partly used in the production of fishmeal for animal consumption (Martin, 1994).

The nutritional value of crustacean shellfish waste protein is high, making recovery attractive and, in the case of certain crustaceans, the protein may contain significant amounts of the natural carotenoid pigment, asthaxanthin. However, the high amount of minerals in shellfish waste, especially calcium carbonate, and its high perishability, makes recovery and disposal difficult (Martin, 1994). In terms of volume, mussels make the largest contribution to output from aquaculture in the EU and the production of the Galician Rias bajas in northwest Spain is among the highest in the world, due to the strong seawater upwelling in the area. This has led to a large aquaculture industry there, mainly based on mussel production (Murado *et al*., 1994). The production of mussels by aquaculture in this area is the highest in Europe at 255,928 tonnes annually (value of 108 million euro) and is second only to China in terms of world production. Part of this production is consumed fresh with increasing amounts being processed by being frozen or canned (Murado *et al*., 1994). Such processing has a common first step involving steam treatment, which releases 300-500 litres of effluent per tonne of raw molluscs treated. These mussel processing waters, rich in organic matter, particularly glycogen, are often discarded into the coastal waters where they constitute a factor contributing to eutrophication. However, some attempts have been made to exploit such processing waters for use as a microbial culture medium (Murado *et al*., 1994).

As another example, information received from Denmark indicates that waste from the mussel industry is ploughed into arable land (mussel meal) or, in the case of mussel shells, are sometimes used in road construction or drainage (Kaergaard, pers. comm.).

6. RANGE OF USES OF FINFISH AND SHELLFISH BY-PRODUCTS

Fish has a significant capacity for processing and since the early 1990s, there has been a tendency to increase the proportion of fisheries production used for direct human consumption rather than for other purposes. Of the products used for human consumption, fresh fish showed significant growth during the 1990s, complemented by a decline in the use of canned fish. This pattern has

largely been driven by a growth in consumption, which increased the demand for fresh fish and caused a slight decline in other uses (FAO, 2000).

Historically fish by-products were considered to be of low value and were disposed of in the most convenient way. Today, however, in many countries the emphasis is on the possibilities for further utilisation of fish by-products, from aquaculture as well as traditional fisheries, rather than the problem of their disposal. Whilst the production of fishmeal and, to a lesser extent, fish ensilage continue to be the major ways of utilising fish by-products, there is an increasing focus on other uses of raw materials of marine origin. There could also be environmental and economic benefits in utilising by-products of wild fish caught for human consumption, rather than disposing of such material at sea. Figure 1 gives an overview of the utilisation of marine by-products (data collected from RUBIN, 1998).

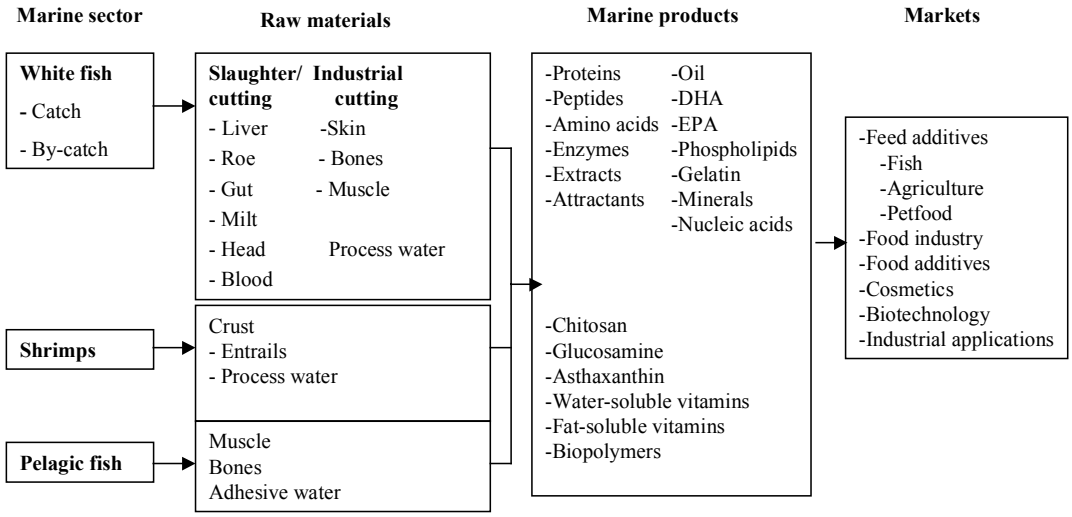


Figure 1 Utilisation of marine by-products (RUBIN, 1998)

Some information from Norway and Spain is presented below as examples of current trends in utilisation of fish by-products, since both countries have significant fishing and seafood industries.

Since 1991 Norway has seen a remarkable change in attitudes towards the extensive use of fish by-products, of which a summary is given in Table 10. From being perceived as a problem, the utilisation of fish by-products has become an important industry and the added value of Norwegian marine by-products in 1999 was approximately 133 million euro. This has led to a growing focus on treating the by-products in a controlled, safe, hygienic manner, similar to that used for the main fish-derived products, as a means of ensuring food safety and high quality. Consumer products represented almost 50% of the added value of processing although the main part of the production

volume of fish by-products was used in low-profit feed-markets (RUBIN, 1998). Table 11 shows the quantity of by-products obtained from cod in Norway for 1996 (RUBIN, 1998).

Table 10. Quantity and utilisation of by-products in Norway for 1996 (tonnes)

	Cod		Herring	Aquaculture	Shrimp	Total
	Ocean fleet	Inshore fleet				
Fishmeal	20,000	23,000	139,000		10,000	192,000
Concentrated ensilage		13,000	42,000	58,000		113,000
Raw ensilage				4,000		4,000
Frozen feed for fur farmed animals		45,000	4,000	2,000		51,000
Fresh feed for fur farmed animals		14,000	1,000			15,000
Human Consumption	2,000	35,000		6,000		43,000
Total utilised	*22,000*	*130,000*	*186,000*	*70,000*	*10,000*	*418,000*
Total disposed	138,000	83,000	6,000	2,000	5,000	234,000
Total utilised/disposed	**160,000**	**213,000**	**192,000**	**72,000**	**15,000**	**652,000**

Table 11. Quantity of by-products from cod produced in Norway for 1996 (tonnes)

By-products	Ocean fleet	Utilised (%)	Inshore fleet	Utilised (%)	Total Produced
Fish entrails	33,000	3.0	30,000	33.0	63,000
Heads	58,000	5.2	62,000	30.6	120,000
Liver	29,000	3.4	25,000	40.0	54,000
Truncate/spines	40,000	12.5	96,000	94.0	136,000
Total	160,000		213,000		373,000

Spain has one of the largest fisheries and fish processing industries in Europe and two of the most important methods of utilising fish by-products are in the production of fishmeal and fish oil. A total of 10 companies were operating in Spain in 2000 with a total production of fishmeal of 32,100 tonnes with an approximate value of 15.5 million euro. However, the imports of fishmeal from other countries, mainly from South America, during the same period were considerably higher (120,870 tonnes with a value of 60 million euro) (Anon., 2001). Some of the raw material for fishmeal production is derived from capture fisheries, although some is also obtained from processing finfish by-products. However, a significant part of the residues from these processing industries are not recycled and are disposed of by being buried, incinerated, composted or disposed of into municipal landfills or publicly owned treatment works, according to national or local regulatory laws. This situation is particularly frequent in enterprises with low or medium production due to the high costs of the facilities required to store fish by-products for prolonged periods.

In most European countries the fishmeal industry is the major receiver of by-products from traditional fisheries, followed in importance by the ensiling of by-products. As an example in Norway, the majority of by-products of cod fishing are used in the fur-industry. The majority of by-products from herring are used in the production of fishmeal, but a small portion is also ensiled. Herring meal is an important ingredient in feed used in aquaculture. Ensiling is also practised in Southern European countries also, but to a lesser extent. In Spain there is only one company producing fish ensilage for use in feed for animals kept for fur production and in this case, ensilage is produced from fish by-products.

With regard to aquaculture, there are two main types of by-products obtained:

1. Fish mortalities, and

2. By-products from slaughter and processing of fish for human consumption

In the past, fish mortalities, which can occur for a variety of reasons, have been disposed of in various ways, including by incineration, biogas production, ploughing into arable land, composting, production of fishmeal, incorporation into petfood or food for animals kept for fur production, ensiling and landfill. By-products from slaughter and processing of healthy fish have also been treated in various ways- by incineration, fishmeal production, incorporation into petfood, food for animals kept for fur production, composting, biogas production, ensiling and landfill. In some countries there are guidelines or legal obligations that fishmeal derived from aquaculture by-products is not recycled for use as fish feed (e.g. BCSFA, 2001). Norway has the largest salmonid aquaculture production in Europe and provides a useful example of how aquaculture by-products are handled. Norwegian data indicates that 97% of by-products from Norwegian aquaculture are utilised. Mortalities are ensiled at the farm, and the ensilage is then treated at dedicated high risk processing plants.

Table 12. By-products from farmed salmon and trout in Norway for 1996 (tonnes)

	Fish entrails	Dead fish	Rejected fish at slaughter
Total amount by-products	48,000	12,000	12,000
Utilised	48,000	10,000	12,000
Disposed	0	2,000*	0

* Cadaverous fish composted and used as fertiliser or fish containing antibiotics destroyed by combustion.

Detailed figures (Tables 10, 11 and 12) from a Norwegian project on recycling of fish by-products can, due to the size of the Norwegian aquaculture industry, indicate the possibility for exploitation of aquaculture by-products (RUBIN, 1998). By-products from processing farmed fish are normally not used for fishmeal production in Norway and instead these by-products are ensiled, and the ensilage has been used as an ingredient for terrestrial animal feed and for fur-animal feed. Over the last 10 years, the processing of by-products has grown into an increasingly important industry in Norway, which focuses on the exploitation of by-products from aquaculture. The aim is to use ensiled or fresh (chilled/frozen) by-products as raw material for production of biotechnological products, or for extraction of oils, and vitamins for further purification and use.

7. PATHOGENS, BIOLOGICAL RISKS AND ZOONOTIC ASPECTS

This chapter deals primarily with bacterial, fungal and viral pathogens as well as with parasites that cause infections in fish and are relevant to the transmission of infectious agents between fish. However, it should also be noted that some shellfish, in particular bivalve molluscs which are filter-feeders, may concentrate human pathogenic bacteria and viruses from water. Crustaceans may also accumulate human pathogens or heavy metals if they live in contaminated water or if they feed on contaminated molluscs (Feachem *et al.,* 1983) although these aspects will not be specifically considered in this report. Molluscs and crustaceans may also have a role in the epidemiology and transmission of fish diseases and cross-order infections may occur between shellfish and fish. For example, infectious pancreatic necrosis (IPN) virus (*Birnaviridae*) may be transmitted between freshwater crayfish (*Astacus astacus)* and trout (Halder and Ahne, 1988), and at least 8 of the aquatic birnaviruses isolated from marine shellfish species have been found to be capable of producing characteristic signs of IPN in experimentally infected rainbow trout (Hill, 1982).

Transmissible spongiform encephalopathies (TSE) are only briefly mentioned in this report since these are covered more comprehensively in an opinion of the SSC concerning the feeding of wild fishmeal to farmed fish and recycling of fish with regard to the risk of TSE. It must be pointed out that in addition to pathogen-related risks, there may be some additional risks linked to the recycling of fish by-products due to toxic organic and inorganic chemical pollutants (e.g. dioxins, heavy metals) if they are carried over into the food chain or released into the environment. This also applies to microbial toxins (e.g. mycotoxins), other contaminants, and any possible residues of antibiotics or anti-parasitic compounds that have been used therapeutically in morts from fish farms, and these risks are considered to be outside the scope of this report. The only toxin considered here is that of *Clostridium botulinum*, which is closely related to the occurrence of toxigenic strains of the organism in the aquatic fauna, with the resulting contamination of fish products and by-products.

Like all animals, wild and farmed fish and shellfish experience disease problems and these may be broadly classified into infectious and non-infectious diseases. However, it is not always easy to make a strict division between these, as non-infectious conditions can stress fish and render them more susceptible to disease agents such as bacteria and viruses. Fish are cold-blooded animals and all of their physiological processes, including their ability to mount an immune response to pathogens, are greatly influenced by water temperature. Indeed heart rate, digestion, growth rate etc. are all affected by water temperature (Roberts, 2001). Similarly, the pathogens that affect fish are also influenced by water temperatures, including the rate of multiplication of the organism. This means that diseases and disease outbreaks often have a seasonality in their occurrence, some diseases occurring during the colder winter months, with others predominantly occurring during the warmer summer months (Roberts, 2001). The critical role of the environment in fish production is also becoming increasingly recognised. Outbreaks of serious diseases are not uncommon in aquaculture and often these outbreaks are related to changes in the physical and chemical environment, especially water temperatures (Roberts, 2001).

There are considerable deficiencies in the data available concerning inactivation of pathogenic agents relevant to fish, and difficulties in extrapolating inactivation data from mammalian pathogens. Often data available regarding inactivation of fish pathogens are not precisely described in terms of D-values (decimal reduction times for a specific pathogen at a particular temperature), specific time-temperature combinations for inactivation, initial and final microorganism concentrations and the media/matrices used. However due to the scarcity of relevant data, the Committee considered it relevant to present such available data for the information of the reader, despite D-values not being provided in all cases.

This chapter will primarily consider those diseases listed under EU fish health legislation in Council Directive 91/67/EEC (CEC, 1991) as well as notifiable and other significant diseases listed in the OIE Diagnostic Manual of Aquatic Animal Diseases (2000). In the OIE categorisation diseases are listed as ‘notifiable’ or as ‘other significant diseases’ while under EU legislation these fish diseases are classified as List I, II or III (CEC, 1991).

Although the overwhelming majority of fish pathogens are not infectious for humans or other mammals, some organisms that have occasionally been reported as opportunistic pathogens of fish will also be considered here because of their zoonotic potential. A list of some important diseases of fish and shellfish is provided in Tables 13-18. Background information on these diseases is provided in Roberts (2001), Woo and Bruno (1999), and the OIE International Aquatic Animal Health Code (2002).

7.1. Viral diseases of fish

Table 13. Some important viral diseases of fish and shellfish

Disease	Causative agent	Susceptible Host	Disease listed by OIE	Disease listed in Directive 91/67	Zoonosis recorded or suspected
Infectious haematopoietic necrosis (IHN)	*Infectious haematopoietic necrosis virus* (IHNV)	Fish	YES, (1)	YES, (4)	NO
Viral haemorrhagic septicaemia (VHS)	*Viral haemorrhagic septicaemia virus* (VHSV)	Fish	YES, (1)	YES, (4)	NO
Spring viraemia of carp (SVC)	*Spring viraemia of carp virus* (SVCV)	Fish	YES, (1)	YES, (5)	NO
Pancreas disease Sleeping disease	*Pancreas disease virus* (PDV) *Sleeping disease virus* (SDV)	Fish Fish	NO NO	NO NO	NO NO
Infectious salmon anaemia (ISA)	*Infectious salmon anaemia virus* (ISAV)	Fish	YES, (2)	YES, (3)	NO
Infectious pancreatic necrosis (IPN)	*Infectious pancreatic necrosis virus* (IPNV)	Fish	YES, (2)	YES, (5)	NO
Viral encephalopathy and retinopathy (VER) and Viral nervous necrosis (VNN)	*Fish nodaviruses*	Fish	YES, (2)	NO	NO
Oncorhynchus masou virus disease	*Type II salmonid herpesviruses* e.g. *Oncorhynchus masou virus* (OMV)	Fish	YES, (1)	NO	NO
Channel catfish virus disease (CCVD)	*Channel catfish virus* (CCV)	Fish	YES, (2)	NO	NO
Eel herpes disease	*Eel herpesvirus* (EHV)	Fish	NO	NO	NO
Koi herpes disease	*Koi herpesvirus* (KHV)	Fish	NO	NO	NO
Epizootic haemotopoietic necrosis (EHN)	*Epizootic haematopoietic necrosis virus* (EHNV) *European sheatfish virus* (ESV) *European catfish virus* (ECV)	Fish	YES, (1)	NO	NO
Red sea bream iridoviral disease	*Red sea bream iridovirus* (RSIV)	Fish	YES, (2)	NO	NO
White sturgeon iridoviral disease	*White sturgeon iridovirus* (WSIV)	Fish	YES, (2)	NO	NO
White spot disease	*White spot virus* (WSV)	Crustaceans	YES, (1)	NO	NO
Taura syndrome	*Taura syndrome virus* (TSV)	Crustaceans	YES, (1)	NO	NO
Infectious hypodermal and haematopoietic necrosis	*Infectious hypodermal and haematopoietic necrosis virus*	Crustaceans	YES, (2)	NO	NO
Yellowhead disease	*Yellowhead virus* (YHV)	Crustaceans	YES, (1)	NO	NO
Nuclear polyhedrosis baculoviruses	*Singly enveloped nuclear polyhedrosis virus* from *Penaeus vannamei* (PvSNPV) *Singly enveloped nuclear polyhedrosis virus* from *Penaeus monodon* (PmSNPV)	Crustaceans	YES, (2)	NO	NO
Spawner-isolated mortality virus disease	*Spawner-isolated mortality virus*	Crustaceans	YES, (2)	NO	NO
Baculoviral midgut gland necrosis	*Baculoviral midgut gland necrosis virus* (BMNV)	Crustaceans	YES, (2)	NO	NO

(1) = notifiable; (2) = other significant disease; (3) = List I; (4) = List II; (5) = List III, (OIE, 2000; CEC, 1991).

The major families of fish viruses may be divided into four groups according to their nucleic acid composition (DNA or RNA) and the presence or absence of an external protein-lipid capsid envelope (Table 14). A comprehensive review of the virology of teleost fish is provided by Smail (2001). There are several important viral diseases of crustacean shellfish, although the causative agents have generally been less well characterised than viruses of finfish and will not be considered further here.

Table 14: Classification of some important fish viruses

GENOME	ENVELOPED	NON-ENVELOPED
RNA	***Rhabdoviridae*** *Infectious Haematopoietic Necrosis Virus* (IHNV) *Viral Haemorrhagic Septicaemia Virus* (VHSV) *Spring Viraemia of Carp Virus* (SVCV) ***Togaviridae*** *Salmon Pancreas Disease Virus* (SPDV) ***Orthomyxoviridae*** *Infectious Salmon Anaemia Virus* (ISAV)	***Birnaviridae*** *Infectious Pancreatic Necrosis Virus* (IPNV) ***Nodaviridae*** *Fish Nodaviruses*
DNA	***Herpesviridae*** *Oncorhynchus masou Virus* (OMV) *Channel Catfish Virus* (CCV) *Eel Herpes Virus* (EHV) *Koi Herpes Virus* (KHV) ***Iridoviridae*** *Epizootic Haematopoietic Necrosis Virus* (EHNV) *Red Sea Bream Iridovirus* (RSIV) *White Sturgeon Iridoviral Disease* (WSIV)	***Adenoviridae*** *Cod adenovirus* *Dab adenovirus* *Sturgeon adenovirus*

Rhabdoviridae

The rhabdoviruses are characterised by a bullet shape (usually 120-380 x 60-90 nm in size) and a helical nucleocapsid enclosed in a lipid envelope bearing surface projections. The genome comprises single-stranded, non-segmented, negative sense RNA. Fish rhabdoviruses include *viral haemorrhagic septicaemia virus* (VHSV) and *infectious haematopoietic necrosis virus* (IHNV), which are important diseases of salmonid and non-salmonid fish species, and *spring viraemia of carp virus* (SVCV) which primarily affects cyprinids. These viruses have a widespread distribution and are found in European, North American and Asian waters (Smail, 2001). Although these viruses have a widespread distribution, they are not ubiquitous and the existence of a number of VHS and IHN free zones within the EU is recognised (CEC, 2002b).

The data available for the inactivation of fish rhabdoviruses is relatively imprecise. For IHNV, Pietsch *et al.* (1977) estimated that the time required to produce a 3 $\log_{10}$ inactivation in minimal essential medium (MEM) containing 10% calf serum was 8 hr at 32°C, 10 days at 21°C and more than 20 weeks at 4°C, although D-values were not calculated. The time required for a 99.9% inactivation of IHNV at pH 5 in buffered water at 21°C was 2.7 days (Pietsch *et al.,* 1977). In the case of VHSV, it was reported that the time required to produce a 3 $\log_{10}$ inactivation was several years at -20°C, several months at 4°C, approximately 4 weeks at 20°C, and less than one min at 70°C. It was reported that in a dry environment, the virus survived for approximately one week at 4°C (Pietsch *et al.*, 1977). VHSV was reported to have survived for 10 min at pH 2.5 and 2 hr at pH 12.2 (Vestergaard-Jørgensen, 1974). A 2 $\log_{10}$ loss of infectivity of SVCV was reported by heating at 45°C for 15 min, and a 3 $\log_{10}$ loss of infectivity following exposure to pH 3 for 15 min (De Kinkelin and Le Berre, 1974). IHNV was reported as being inactivated at 40-60°C, although D-values were not specified (Whipple and Rohovec, 1994). In the milieu of

MEM, IHNV was reported to be not detectable after 5 hours at 35°C, 20 minutes at 40°C, 10 minutes at 45°C, 90 seconds at 50°C or 30 seconds at 55°C. However in this study initial titres were not reported and no D-value was provided.

Togaviridae

Togaviruses are spherical, enveloped viruses approximately 65 nm in diameter. The genome comprises positive sense single-stranded RNA. The aetiology of both pancreas disease in Atlantic salmon (Nelson *et al.,* 1995) and sleeping disease in rainbow trout is attributed to togaviruses of very similar genetic and serological properties (Boucher and Baudin-Laurencin, 1996).

Pancreas disease is widespread in Northern European and American salmon farming and sleeping disease is reported primarily from central European countries. Both diseases usually cause low mortality (<5%) but do not seriously impair other production traits and survivors of pancreas disease are protected against sleeping disease and *vice versa* (Boucher and Baudin-Laurencin, 1996). Limited data are available concerning the inactivation of these viruses, although as enveloped viruses they are inactivated by lipid solvents and ionic or non-ionic detergents. *Bluegill virus*, a toga-like virus reported by Wolf (1988), was found to be labile to acid and heat. It is likely that the inactivation properties of togaviruses are similar to those described for ISAV and rhabdoviruses.

Orthomyxoviridae

Orthomyxovirus virions are pleomorphic, approximately spherical and typically 80-120 nm in diameter. They have a protein-lipid outer envelope studded with projections. The genome is negative sense single-stranded segmented RNA. *Infectious salmon anaemia virus* (ISAV) is a well-characterised orthomyxovirus of fish. ISA is a systemic haemorrhagic disease of Atlantic salmon but the virus may also replicate in other fish (e.g. rainbow trout) without causing disease. The disease has been reported in Canada, Norway, the Faeroe Islands and Scotland (OIE, 2000) and the virus has also been isolated in Chile (Smith, pers. comm.) and Ireland (Geoghegan, pers. comm.).

Torgersen (1997) showed that ISAV is susceptible to most disinfectant/treatment methods relevant for treatment of intake water, wastewater/effluents and organic by-products from the aquaculture industry. The studies also presented results from investigations on the infectivity of trimmings (muscle/fillet) and cut-offs from the head (skull tissue, brain, eyes etc.), and related the infectivity to the major internal organs (kidney, liver and spleen) obtained after slaughtering of fish infected with ISAV. A bioassay was used where test fish were challenged with 1:10, 1:100, 1:1,000, and 1:10,000 dilutions of infective tissue homogenates which were capable of inducing mortalities when injected into salmonid smolts at a dilution of 1:10,000. Due to the nature of this bioassay the precise inactivation rate is not known and D-values could not be calculated. The results showed that the undiluted infective tissue homogenate was still infective after 5 min at 45°C, and after 1 min at 50°C. No detectable infectivity (i.e. at least a 4 $\log_{10}$ reduction in infectivity) was recorded either after treatment at 50°C for 2 min or more, or after treatment at 55°C or 60°C for 1 min or more. With formic acid, the infectivity was reduced after treatment at pH 4.5 and a contact time of 8, and 24 hr. No detectable infectivity (i.e. at least a 4 $\log_{10}$ reduction in infectivity) was recorded after treatment at pH 3.5 or pH 4.0 for 8 and 24 hr respectively.

Birnaviridae

Birnaviruses are spherical non-enveloped viruses with a genome comprising two segments of double-stranded RNA. *Infectious pancreatic necrosis virus* (IPNV), a 60 nm diameter particle, is the most important fish birnavirus and is responsible for serious losses in farmed salmonid fish. IPNV and related aquabirnaviruses have been isolated from numerous freshwater and marine fish species and have a virtually worldwide distribution (Smail, 2001).

IPNV survival both in fresh- and seawater is comparable with a reduction rate of approximately 1 $\log_{10}$ every 5 days at 15 or 20°C (Toranzo and Hetrick, 1982). The virus is sensitive to alkaline conditions (pH 12). However it is resistant to acid conditions, and retains infectivity following exposure to ensilage containing formic and propionic acid at pH 3.8, especially at 4°C (Smail *et al.,* 1993). Under these conditions, Smail *et al.* (1993) reported a reduction in virus titre from 8.3 to 4.2 $\log_{10}$ pfu/ml and from 6.48 to 3.87 $\log_{10}$ pfu/ml with two different isolates over a 147 day period. According to Bylund *et al.* (1993) IPNV survived for several years in ensilage and Vestergaard-Jørgensen (1974) reported that several hours were required to produce a 3 $\log_{10}$ reduction in infectivity at pH 2.5, while the same effect was obtained after 10 min at pH 12.0. Fløgstad *et al.* (1991) found that the use of sodium hydroxide at pH 11.6 produced a 3 $\log_{10}$ reduction in 24 hr, while a reduction of 3 $\log_{10}$ or more was obtained after 6 min at pH 12.0. In the same study, it was found that when formic acid was used, a pH of 2.5 produced a 2 $\log_{10}$ decrease in infectivity in 1 hr, while a 3 $\log_{10}$ reduction was obtained at pH 2.0 in 6 min. IPNV also survives passage in the cow's gastro-intestinal tract at a low pH and at mammalian body temperatures (Smail *et al.,* 1993).

IPNV is relatively resistant to heating. However, there is conflicting information on precise thermal inactivation rates and D-values are not available. Smail and co-workers reported that 2 hr at 60°C is necessary to reduce infectivity of IPNV in silage by 2 $\log_{10}$ (Smail *et al.,* 1993). However, Fløgstad *et al.* (1991) reported a reduction of more than 4 $\log_{10}$ in 1 min when IPNV was heated to 65°C in the presence of organic material. It has also been reported that the virus did not replicate at temperatures above 40°C, and heating to 60°C produced a 4 $\log_{10}$ decrease in infectivity in 30 min (Fløgstad and Torgersen, 1992). Whipple and Rohovec (1994) reported survival times for IPNV of 8 hr at 60°C, 2 hr at 70°C and 10 min at 80°C in MEM. However initial titres and D-values were not calculated.

Nodaviridae

Nodaviruses are small, non-enveloped, icosahedral viruses approximately 25-35 nm in diameter. The genome comprises positive sense single-standed RNA. Nodaviruses have been reported to cause disease in a number of marine and anadromous fish species including sea bass, halibut and Atlantic salmon. Various names have been given to diseases of fish caused by nodaviruses, the best known being Viral Encephalopathy and Retinopathy (VER) and Viral Nervous Necrosis, which are characterised by lesions in the brain, spinal cord, retina and other tissues. Nodavirus diseases of fish have a virtually worldwide distribution (Smail, 2001). It was reported by Arimoto *et al.* (1996) that striped jack nervous necrosis virus, a fish nodavirus, was inactivated by exposure to 60°C for 10 min in PBS and by exposure to pH 12 for 10 min at 20°C. The virus was reported to be stable at

pH 3 under similar conditions, although D-values were not calculated and titre reductions were not provided in this study.

Herpesviridae

Herpesviruses cause a wide variety of diseases in man and animals including fish. The herpes virion is a spherical enveloped particle 120-200 nm in diameter and the genome comprises double-stranded DNA. Two of the most important herpesviruses of fish are *Oncorhynchus masou virus* (OMV) and *Channel catfish virus* (CCV). Characterisation of immediate-early genes in the CCV genome suggests that the virus is most closely related to the alpha sub-family of *Herpesviridae* (Silverstein *et al*., 1995). OMV is an oncogenic virus that causes skin ulcerative conditions in salmonid fish in Japan and the virus may also be present in eastern Asia (OIE, 2000). CCV causes a systemic disease in channel catfish in the USA (OIE, 2000). Neither OMV nor CCV have been reported in Europe, although two other important herpesviruses are found in Europe- *koi herpesvirus* (KHV) and *eel herpesvirus* (EHV).

It is reported that the fish herpesviruses are heat and acid labile (Robin and Rodrigue, 1980). CCV can be recovered from decomposing fish stored on ice for up to 14 days but could not be isolated from fish stored at 22°C for 48 hr (Plumb, 1989). The virus is reported as being inactivated by exposure to 60°C for 1 hour (Robin and Rodrigue, 1980). Smail (2001) reported that OMV is also heat labile and inactivated at pH 3.0 although additional details are not available.

Iridoviridae

Iridoviruses are large isometric viruses with icosahedral symmetry of diameter 130-300 nm. The genome comprises double-stranded DNA. Fish iridoviruses include *Epizootic Haematopoietic Necrosis virus* (EHNV), *Red Sea Bream Iridovirus* (RSIV) and *White Sturgeon Iridovirus* (WSIV). Epizootic Haematopoietic Necrosis (EHN) is a systemic infection of redfin perch, rainbow trout, sheatfish and European catfish. At present EHN has a geographical distribution limited to Australia. Red sea bream iridoviral disease (RSIVD) affects several fish species including red sea bream, sea bass, and yellowtail. The disease is currently restricted to Japan although a genetically related virus has been isolated in Thailand (OIE, 2000). White sturgeon iridoviral disease (WSIVD) affects white sturgeon in North America and Russian sturgeon in Europe (OIE, 2000).

Langdon (1989) reported that EHNV can survive in fish tissues for at least 7 days at 4°C but infected tissue culture medium was reported to be inactivated by altering the pH to 12 or 4 for 1 hour, and by heating at 60°C for 15 min or 40°C for 24 hr. However titre reductions and D-values were not provided.

Summary Comment

Although systematic data on thermal inactivation kinetics of fish viruses are lacking, from the available data it would appear that the probable infective titres of most fish pathogenic viruses are likely to be reduced to a negligible level by treatment at 60°C for 60 min. However, data indicate that IPNV is more thermoresistant than other fish viruses and may require several hours at this temperature for complete inactivation. Most fish pathogenic viruses are inactivated at low pH although data are lacking for the *Nodaviridae* and, within the *Birnaviridae,* IPNV appears relatively stable under acidic conditions.

7.2. Bacterial pathogens of fish

Table 15. Some important bacterial diseases of fish and shellfish

Disease	Causative agent	Susceptible Host	Disease listed by OIE	Disease listed in Directive 91/67	Zoonosis recorded or suspected
Bacterial kidney disease (BKD)	*Renibacterium salmoninarum*	Fish	YES, (2)	YES, (5)	NO
Furunculosis	*Aeromonas salmonicida*	Fish	NO	YES, (5)	NO
Enteric septicaemia of catfish	*Edwardsiella ictaluri*	Fish	YES, (2)	NO	NO
Edwardsiella septicaemia	*Edwardsiella tarda*	Fish	NO	NO	YES
Motile Aeromonad septicaemia	*Aeromonas hydrophila* Other *Aeromonas spp.*	Fish	NO	NO	YES
Mycobacteriosis	*Mycobacterium marinum* *Mycobacterium fortuitum* *Mycobacterium chelonae*	Fish	NO	NO	YES
Enterococcosis	*Lactococcus garvieae* *Enterococcus seriolicida*	Fish	NO	NO	YES
Streptococcosis	*Streptococcus iniae* Other *Streptococcus spp.*	Fish	NO	NO	YES
Botulism	*Clostridium botulinum* toxin	Fish	NO	NO	YES
Yersiniosis/Enteric redmouth disease (ERM)	*Yersinia ruckeri*	Fish	NO	YES (5)	NO
Vibriosis	*Vibrio anguillarum* *Vibrio ordalii* *Vibrio salmonicida* *Vibrio vulnificus* biotype 2 Other *Vibrio spp.*	Fish	NO	NO	NO*
Piscirickettsiosis	*Piscirickettsia salmonis*	Fish	YES, (2)	NO	NO
Brown Ring Disease (BRD)	*Vibrio tapetum*	Molluscs (clams)	NO	NO	NO
Vibriosis	*Vibrio spp.*	Molluscs	NO	NO	NO
Rickettsia of molluscs	*Rickettsia-like organism*	Molluscs	NO	NO	NO
Chlamydia of molluscs	*Chlamydia-like organism*	Molluscs	NO	NO	NO
Gaffkaemia	*Aerococcus viridans* var *homari*	Crustacea (Lobster)	NO	NO	NO

(1)= notifiable; (2) = other significant disease; (3) = List I; (4) = List II; (5) = List III, (OIE, 2000; CEC, 1991).
**V. vulnificus* biotype 1 and *V. parahaemolyticus* are zoonoses

Renibacterium salmoninarum

Renibacterium salmoninarum is a fastidious, aerobic, non-motile, non-spore forming, Gram-positive short rod or diplobacillus. This bacterium is the causative agent of Bacterial Kidney Disease (BKD), a chronic condition first recorded in wild salmonid fish in the 1930s (Mackie *et al.*, 1933). The causative organism is slow growing and has an optimum growth temperature of approximately 15°C (OIE, 2000). The disease is widely distributed throughout North and South America, Europe and Japan. Disease transmission is thought to occur both horizontally via infected water or infected food and vertically from parent to progeny via infected ova. *R. salmoninarum* has not been reported to cause infection in humans or other mammals.

Whipple and Rohovec (1994) demonstrated that a suspension of 10^5 or 10^6 cfu/ml *R. salmoninarum* in phosphate buffered saline (PBS) was not completely inactivated by heating for 4 hr at 50°C, for 3 hr at 55°C, and for 15 min at 65°C. The authors stated that titres decreased after a few minutes at these temperatures but that low numbers of bacteria survived for the periods stated. However precise data on titre reduction or D-values were

not supplied. The authors also reported that from an initial titre of 10^5 or 10^6 cfu/ml *R. salmoninarum* could not be detected in fish silage (pH 3.8-4.3) after 1 min at 55°C

R. salmoninarum (>1x10^8 cfu/ml) was reported to survive for less than 30 min in fish silage (Smail *et al*., 1993), although no D-values were given and the precise inactivation rate is not clear.

Edwardsiella tarda

Edwardsiella tarda, a facultatively anaerobic, non-spore forming, Gram-negative rod is a member of the *Enterobacteriaceae*. It is a pathogen of warmwater fish, particularly catfish and eels in the USA and Japan. The organism, which is frequently found in organically polluted water, grows at temperatures between 25°C and 37°C. *E. tarda* has been reported to cause infection in humans (Jordan and Hadley, 1969; Bockemuhl *et al.*, 1971), and has been isolated from domestic animals, rats, birds, frogs, turtles and healthy fish (Roberts, 2001). No specific data on thermal inactivation or on inactivation with acid have been found regarding this pathogen.

Edwardsiella ictaluri

Edwardsiella ictaluri is the most fastidious of the *Edwardsiella* species and is biochemically less active than *E. tarda.* It is primarily a pathogen of channel catfish and has been reported in most parts of the world where catfish are farmed, causing septicaemic disease during the summer months when water temperatures are between 18-28°C (OIE, 2000). The organism can survive for several months in ponds without fish. *E. ictaluri* has not been reported to cause infection in humans or other mammals. No specific data on thermal inactivation, or on inactivation with acid, have been found for this pathogen

Aeromonas salmonicida

Aeromonas salmonicida, a facultatively anaerobic, non-motile, non-spore forming, Gram-negative rod, is the causative agent of furunculosis in salmonid fish and a range of ulcerative and other conditions in non-salmonid fish species. The organism, which was first isolated in the 19th century, has a virtually worldwide distribution and is a major source of losses in wild and farmed fish (Roberts, 2001). Relatively effective vaccines are now available for salmonid fish. Infection is transmitted horizontally via infected water or ingestion of infected food and the organism can survive in water and sediments for periods of weeks or months. *A. salmonicida* has not been reported to cause infection in humans or other mammals.

A. salmonicida was reported to be rapidly inactivated in commercial fish silage (Smail *et al.*, 1993). The authors reported that from an initial viable count of 5x10^8 cfu/ml the bacterium could not be re-isolated after 30 minutes, although no D-values were provided. Similar results were provided by Whipple and Rohovec (1994) who reported that from an initial viable count of approximately 10^7 cfu/ml the bacterium could not be re-isolated from fish silage (pH 3.8-4.3) after 3 min at 22°C. These authors also reported that a suspension of approximately 10^6 cfu/ml in PBS was completely inactivated after 2 min at 50°C, although no D-values were provided (Whipple and Rohovec, 1994).

Aeromonas hydrophila

Aeromonas hydrophila, a facultatively anaerobic, motile, non-spore forming, Gram-negative rod, is widely distributed in the aquatic environment and forms part of the intestinal flora of healthy fish. It is believed to have a worldwide distribution. *A. hydrophila* is an opportunistic pathogen, causing haemorrhagic septicaemia, particularly in freshwater fish which have been subjected to overcrowding, poor environmental conditions or other forms of stress (Roberts, 2001). Apart from fish, *A. hydrophila* infections have been reported in frogs, alligators, turtles, shrimp and humans, with human infections often being linked to the consumption of infected or contaminated raw fish (Janda and Abbott, 1996).

A. hydrophila was reported to be inactivated at 60°C with a decimal reduction time of 0.026 to 0.040 min (Schumann *et al.*, 1997).

Mycobacterium spp.

Mycobacteria are slow growing, non-motile, Gram-positive, acid-fast rods. These organisms have a worldwide distribution and many species of freshwater and marine fish are susceptible to mycobacterial infection. Three species have been mainly associated with disease in fish: *Mycobacterium marinum, M. fortuitum* and *M. chelonae* (Roberts, 2001). Aquarium fish are particularly vulnerable to the slowly developing chronic disease, but infection has also been reported in wild and farmed fish including cod, halibut, mackerel, salmonid fish and penaeids. Fish may be infected by ingesting contaminated feed and water but transmission may also be linked to invertebrates such as arthropods, freshwater snails or freshwater prawns. In the past, mycobacterial infections in Pacific salmon hatcheries were associated with unpasteurised feedstuffs, but levels of infection fell dramatically when pasteurisation of feed was consistently applied (Ross *et al.*, 1959). Human infections with *M. marinum, M. fortuitum* and *M. chelonae* have been reported (Brown *et al.*, 1977; Cruz, 1938; Blacklock and Dawson, 1979). Sources of infection in humans are not always known but in a number of cases infection by *M. marinum* is believed to have been acquired from swimming pools or from handling tropical aquarium fish.

Fish pathogenic mycobacteria are reported to be inactivated by heating at 76°C for 30 min (Thoen and Schliesser, 1984). It has been reported that the heat susceptibility of *M. marinum, M. fortuitum* and *M. chelonae* is of a similar order to *Legionella pneumophila*, and the following D-values in Table 16 have been reported for mycobacteria in sterile water of standardised hardness (Schulze-Roebbecke and Bucholtz, 1992).

Table 16. D-values in seconds for mycobacteria in sterile water of standardised hardness (Schulze-Roebbecke and Bucholtz, 1992)

Species	D-value in seconds at a temperature of:			
	50°C	**55°C**	**60°C**	**70°C**
M. chelonae	10,130	1,360	260	5
M. fortuitum	6,330	1,520	220	2
M. marinum	4,510	750	60	<10

It has also been reported that a suspension of 7.5 x 10^6/ml of *M. chelonae* in phosphate buffered saline (PBS) was inactivated by heating at 40-60°C, complete inactivation requiring 24 hr at 40°C, 60 min at 50°C, and 2.5 min at 60°C (Whipple and Rohovec, 1994).

Lactococcus garvieae and *Enterococcus seriolicida*

Lactococcus garvieae and *Enterococcus seriolicida* are closely related facultatively anaerobic, non-motile, non-spore forming, Gram-positive ovoid bacteria that form short chains (Domenech *et al.,* 1993). Enterococcal infections of fish have been reported in Japan, Europe, Australia and South Africa. A wide range of freshwater and marine fish species are susceptible to these organisms including yellowtail, turbot, eel and rainbow trout. Disease outbreaks, which usually manifest as a haemorrhagic septicaemia, are most common in the summer months when water temperatures are highest. *L. garvieae* has been associated with endocarditis in humans (Fefer *et al*., 1998).

No data on thermal inactivation, or on inactivation with acid have been found regarding this pathogen.

Yersinia ruckeri

Yersinia ruckeri is a facultatively anaerobic, non-motile, non-spore forming, Gram-negative rod. This bacterium is the causative agent of Enteric Redmouth Disease (ERM), also called Yersiniosis. The causative organism is fast growing and has an optimum growth temperature range of 22-25°C, although it can grow in the temperature range 9-37°C. The disease is widely distributed in North and South America, Europe and Japan. Disease transmission is thought to occur by way of horizontal transmission via infected fish/carriers, via infected waters or by the use of infected fish as bait by anglers. This bacterium is considered to be relatively resistant to disinfectants and other chemical/physical treatments. Gjevre (1988) reported that *Y. ruckeri* may survive for 12 days under pH 4 (formic acid), and for up to 2 days at a pH of 3.5 (formic acid). Jacobsen *et al.* (1989) found that a suspension of 6.6 x 10^7 cfu/ml *Y. ruckeri* in filtered fish slaughterhouse wastewater was inactivated after 1 min at 60°C and after 15 seconds at 72°C. Similar findings were reported by Fløgstad *et al.* (1991) and Fløgstad and Torgersen (1992), who used *Y. ruckeri* and IPN virus as indicator organisms in experiments conducted in order to establish methods for treatment of effluents from salmon slaughterhouses in Norway.

Streptococcus iniae

There are many reports of streptococcosis in marine and freshwater fish, including rainbow trout, golden shiner, striped mullet and stingray (Woo and Bruno, 1999). Disease outbreaks, which can be caused by different species of *Streptococcus*, have been reported in different parts of the world, including Japan, the USA, South Africa and Europe. *Streptococcus iniae* is a facultatively anaerobic Gram-positive coccus which mostly occurs in long chains. High water temperatures and poor water quality predispose fish to disease and the gross pathology of *S. iniae* infection is similar to that of enterococcal infection. *S. iniae* has been associated with invasive infection in humans linked to skin injuries and contact with freshly killed fish (Weinstein *et al*., 1997).

No data on thermal inactivation, or on inactivation with acid have been found concerning this pathogen.

Clostridium botulinum

Clostridium botulinum has a widespread distribution in the environment, particularly in decaying and anaerobic conditions. Outbreaks of disease in fish, due to the consumption of the neurotoxin produced by the organism, have been reported in rainbow trout in Europe

and in Coho salmon in the USA (Roberts, 2001). *Cl. botulinum* is an anaerobic, motile, spore-forming, Gram-positive rod-shaped bacterium. Botulism has only been recorded in freshwater reared fish in earth ponds, and the psychotrophic *Cl. botulinum* type E has been the causative organism in all cases (Roberts, 2001). Disease probably occurs through ingestion of neurotoxin produced when the bacterium grows in the decomposing tissues of dead fish or waste feed at the bottom of the pond. Disease outbreaks in fish have generally been associated with poor husbandry in overstocked or underfed ponds. *Cl. botulinum* is an important pathogen from a human health standpoint.

Cl. botulinum spores are readily inactivated under normal rendering conditions i.e. at 133º C, at 3 bar absolute pressure for 20 min, and the toxin produced by the organism is heat-labile. Decimal reduction times for various Clostridial spores are provided in Table 22 in Chapter 9.

Vibrio spp.

Vibriosis is one of the most important groups of bacterial diseases of marine fish with a worldwide distribution. Several species of Vibrio have been associated with disease in fish, notably *V. anguillarum, V. ordalii, V. salmonicida* and *V. vulnificus* (Roberts, 2001). *Vibrio spp.* are facultatively anaerobic, often motile, non-spore forming, rod-shaped bacteria. Some *Vibrio spp.*, such as *V. anguillarum*, tend to cause disease at higher water temperatures, while others, such as *V. salmonicida*, only cause disease at low water temperatures (Woo and Bruno, 1999). *V. vulnificus* is primarily a pathogen of eels and has been associated with disease outbreaks in Japan and Europe. *V. vulnificus* biotype 1 is an important human pathogen (Blake *et al.,* 1980) whereas the predominant biotype isolated from eels is *V. vulnificus* biotype 2 (Woo and Bruno, 1999). However, a case was reported of *V. vulnificus* infection in a person associated with contact with an infected eel through an open wound (Veenstra *et al.,* 1992). It should be noted that *V. parahaemolyticus* and *V. vulnificus* biotype 1 are important human pathogens (SCVPH, 2001).

A 4 $\log_{10}$ reduction of *V. anguillarum* has been reported following exposure of the organism to a temperature of 47.5°C for 2 min in seawater (Jacobsen and Liltved, 1988). Decimal inactivation values of 1.3 ± 0.09 and 0.41 ± 0.01 min have been reported for *V. vulnificus* in oyster homogenates *in vitro* at 46°C and 48°C respectively (Dombroski *et al.*, 1999).

Rickettsiaceae

Piscirickettsia salmonis

Piscirickettsiosis is caused by *Piscirickettsia salmonis,* an obligate intracellular organism and member of the order *Ehrlichieae*, family *Rickettsiaceae*. These are non-motile 0.4-1.5 µm, coccoid or spherical, sometimes polymorphic, non-capsulated Gram-negative organisms. Several salmonid fish species, especially coho salmon, are susceptible to Piscirickettsiosis, which has been reported in various countries including Chile, Canada, Ireland, and Norway (Lannan *et al.*, 1999). In Chile *P. salmonis* is considered to be the most important pathogen in salmonids reared in seawater (Lannan and Fryer, 1993). Affected fish may have haemorrhagic skin lesions and raised scales, and gills are usually pale. Internally, there may be cream-coloured focal subcapsular nodules in the liver, splenomegaly, swollen kidney and ascites. Petechial haemorrhages are also frequently

observed in the swim bladder, perivisceral fat and pancreatic tissue and in the digestive tract (Bruno and Poppe, 1996).

Transmission of disease occurs horizontally via infected water and possibly by ingestion of infected faeces (Lannan *et al.*, 1999). There is circumstantial evidence of vertical transmission but this has not been proven. It is not known if any vectors are involved in transmission.

As an obligate intracellular organism, the survival of the organism outside the cell is low and it appears not to survive in freshwater. In mimimum essential medium (MEM) with 10% foetal calf serum or in saltwater the organism was reported to survive for 14 days at 5ºC, 10 days at 10ºC or less than one week at 20ºC (Fryer *et al*., 1992; http://www.bacterio.cict.fr/bacdico/pp/piscirickettsia.html). *P. salmonis* presents no known health risk to humans. As replication does not occur at temperatures of 25ºC or above, replication is consequently precluded at human body temperatures (Lannan *et al*., 1999).

P. salmonis replication does not occur at temperatures above 25ºC in tissue culture and the organism is sensitive to both elevated and freezing temperatures (Lannan *et al*., 1999). Dessication is likely to also be a powerful method of inactivation while freshwater seems to prevent *P. salmonis* transmission.

Aerococcus viridans* var *homari

Aerococcus viridans is a non-motile, facultatively anaerobic, tetrad-forming Gram-positive coccus. The bacterium is responsible for Gaffkaemia, or red tail disease, that affects the lobsters *Homarus americanus* and *Homarus gammarus*. The disease is prevalent in North America but outbreaks have also been reported in Europe including in Norway and the UK. Wild lobsters and other crustaceans may serve as reservoirs of infection, which is transmitted through puncture wounds or lesions, and the disease is often fatal (Stewart, 1993). Mortalities occur predominantly when lobsters are injured, overcrowded and at high water temperatures (e.g. 20ºC).

No data on thermal inactivation, or on inactivation with acid, have been found on this pathogen.

Summary Comment

Although systematic data on thermal inactivation of bacterial fish pathogens are lacking, on the basis of the limited data available it would appear that treatment at 60°C for 15 minutes would reduce the probable infective load of most bacteria pathogenic to fish to a negligible level. However, Gram-positive organisms such as *Renibacterium salmoninarum* may require longer holding times and spores of *Clostridium botulinum* will require considerably higher temperatures.

7.3. Parasitic infestations of fish

Table 17. Some important parasitic diseases of fish and shellfish

Disease	Causative agent	Susceptible Host	Disease listed by OIE	Disease listed in Directive 91/67	Zoonosis recorded or suspected
Protozoan infestations	*Bonamia ostreae*	Molluscs	YES, (1)	YES,(4)	NO
	Other *Bonamia spp.*	Molluscs	YES, (1)	NO	NO
	Marteilia refringens	Molluscs	YES, (1)	YES, (4)	NO
	Marteilia sydneyi	Molluscs	NO	NO	NO
	Perkinsus marinus	Molluscs	YES, (1)	NO	NO
	Perkinsus olseni	Molluscs	YES, (1)	NO	NO
	Haplosporidium costale	Molluscs	YES, (1)	NO	NO
	Haplosporidium nelsoni	Molluscs	YES, (1)	NO	NO
	Mikrocytos mackini	Molluscs	YES, (1)	NO	NO
	Ichthyobodo necatrix	Fish	NO	NO	NO
	Myxosoma cerebralis	Fish	NO	NO	NO
	Tertracapsula bryosalmone	Fish	NO	NO	NO
	Ichthyopthirius multifilis	Fish	NO	NO	NO
	Kudoa thyrsitis	Fish	NO	NO	NO
Trematode infestations	*Gyrodactylus salaris*	Fish	YES	YES	NO
	Metagonimus yokogawai	Fish	NO	NO	NO
	Haplorhis yokogawai	Fish	NO	NO	NO
	Nanophyetus salmonicola	Fish	NO	NO	YES
	Heterophyes heterophyes	Fish	NO	NO	YES
Cestode infestations	*Diplogonoporus grandis*	Fish	NO	NO	YES
	Diphyllobothrium latum	Fish	NO	NO	YES
	Diphyllobothrium graciale	Fish	NO	NO	YES
Nematode infestations	*Anisakis marina*	Fish	NO	NO	YES
	Pseudoterranova decipiens	Fish	NO	NO	YES
	Contracaecum spp.	Fish	NO	NO	YES
	Gnathostoma spingerum	Fish	NO	NO	YES
Crustacean infestations	*Lepeopthirus salmonis*	Fish	NO	NO	NO
	Argulus spp.	Fish	NO	NO	NO

(1)= notifiable; (2) = other significant disease; (3) = List I; (4) = List II; (5) = List III, (OIE, 2000; CEC, 1991).

Protozoan parasites of finfish

Flagellates

This group of protozoan parasites includes skin and gill ectoparasites such as *Ichthyobodo necatrix, Oodinium* and *Amyloodinium,* parasites of the digestive tract such as *Hexamita,* and blood parasites like *Trypanoplasma* and *Cryptobia*. These organisms live in aquatic environments as ectoparasites, in the digestive tract of fish or in biological fluids such as blood, and therefore are susceptible to dessication. Some have different types of life cycles but none of them are found as resistant forms within the fish. In addition, temperatures higher than 35ºC have been found to destroy some of these organisms (Noga, 2000).

Ciliates

Ciliate fish parasites are motile or non-motile (sessile) ectoparasites affecting gills or skin. They are common parasites of fish, and include *Trichodina*, *Tripartiella*, *Chillodonella*, *Brooklynella*, *Apiosoma*, *Epistylis*, *Scyphidia* or *Ambiphrya.* Some of them such as *Ichthyophthirius*, *Cryptocaryon*, *Uronema* or *Tetrahymena* can also affect tissues deeper than the gills or skin. As obligate aquatic living organisms, they are very sensitive to

dessication and to temperatures higher than 40ºC, as described for *Cryptocaryon* (Cheung *et al*., 1979; Noga, 2000). Some of them present different life cycles (Lom and Dykova, 1992) but none of them form cysts or resistant forms within the fish.

Amoebae

Most of the *Amoebae* are free-living organisms, but some of them can also be parasites. They can be ectoparasites (gill parasites such as *Paramoeba*) or in some cases, systemic (Woo and Poynton, 1995). The viability of these parasites, as in the previous groups, is very limited when exposed to salinity changes (Woo and Poynton, 1995) dessication or temperatures of 40ºC or higher.

Apicomplexans

Apicomplexans are intracellular parasitic protozoans showing sequences of proliferative, sexual and infective stages. These infective stages are usually spore-forming stages called sporocysts or oocysts (Lom and Dykova, 1992). They include genera like *Goussia*, *Eimeria, Isospora, Cryptosporidium* and *Haemogregarina*. Non-spore stages can be considered very sensitive to temperature and dessication but spores are more resistant. No data concerning inactivation are available from piscine apicomplexans, but as a reference there is considerable data available for the pathogen *Cryptosporidium parvum.* This is another *Apicomplexa* that may affect humans and other mammals, depending on the subtype, and that is also commonly present in contaminated water (Current and Garcia, 1991). However piscine cryptosporidia have to date not been associated with infections in humans.

Myxosporidian parasites

Myxosporidia are metazoan organisms, from a zoological point of view. However these organisms have traditionally been classified as protozoan. These groups of diseases are caused by Myxozoan parasites, special types of obligate parasites characterised by complex life cycles and spore formation (Lom and Dykova, 1992). They have a worldwide distribution. Many different fish diseases are associated with different species of these parasites (e.g. *Sphaerospora, Myxobolus, Ceratomyxa, Myxidium, Henneguya, Kudoa)*. These organisms can be found affecting wild or farmed fish species causing important economic losses (*Ceratomyxa shasta*, *Myxobolus cerebralis, Kudoa thyrsistes*) or simply living in organ cavities (gall bladder, swim bladder or urinary bladder) causing no apparent detrimental effects to the fish (Lom and Dykova, 1992). In the specific case of *Myxobolus cerebralis*, one of the most typical and well-known Myxosporidian parasites causing severe diseases in fish, spores can survive in sediments for several years and are resistant to drying but can be inactivated by heat at 60ºC (Noga, 2000).

Microsporidian parasites

Microsporidia, in a strict sense, are not protozoa: these organisms should be considered as fungi or basal metazoan.

Microsporidians, another group of widely distributed parasites of fish, are intracellular parasites, with direct life cycles and they also form spores. Some species form macroscopical xenomas (cyst-like structures). Different species have been described in this group (*Glugea, Pleistophora, Loma, Tetramicra, Microsporidium* etc.) causing important pathological problems in different farmed and wild fish species (Lom and Dykova, 1992). Recently, significant interest has been centred on Microsporidian parasites as potential,

although opportunistic, pathogens for humans (Mathis, 2000; Weiss, 2001). Spores can also be inactivated by heat. In the particular case of *Glugea,* spores can be destroyed on holding for several min at 50ºC (Egusa, 1992).

Protozoan parasites of Molluscan Shellfish

Bonamia ostreae

Bonamiosis is a disease of the native European oyster and has been found from the Atlantic coast of Spain to Denmark, including in Ireland, England and France (Bower *et al*., 1994). The disease is of great economic importance in Europe (Wood and Fraser, 1996), causes massive mortalities, cannot be treated and is extremely difficult to eradicate (Wood and Fraser, 1996; FAIR, 1990; ScVC, 1996).

The causal organism *Bonamia ostreae* is a protozoan parasite thought to be a member of the Phylum *Acetospora*, although its exact taxonomy is uncertain. It is an intracellular parasite that invades haemocytes of oysters leading to death (Wood and Fraser, 1996), particularly in 3-4 year old oysters. There is no information available on inactivation parameters concerning this parasite.

Marteilia refringens

Marteiliosis has caused serious mortalities of the European oyster, *Ostrea edulis,* in Europe since 1967 (Wood, 1996). The disease is also known as Aber Disease and caused significant mortality in these flat oysters (*Ostrea edulis*) in France in the 1970s (Grizel, 1979). It is caused by the parasite *Marteilia refringens,* affects the digestive gland of oysters and has been identified in oysters along the Atlantic coast of Europe from the southern UK to Portugal (Bower *et al*., 1994). There is no information available on inactivation parameters for this parasite.

Perkinsus marinus

Perkinsus marinus causes a disease known as Perkinsosis which affects a number of molluscan shellfish species, particularly *Crassostrea virginica*, the American oyster, in which it causes a significant disease known as "Dermo" (*Dermocystidium*) disease in the USA (Bower *et al*., 1994). This disease is characterised by severe emaciation, gaping, and pale appearance of the digestive gland as well as retarded growth and the formation of abscess lesions. The parasite also affects clams, causing Clam Perkinsus disease, which occurs in Spain, Portugal and the Mediterranean and can cause high mortalities (Bower *et al*., 1994).

Perkinsus spp. also cause Perkinsus disease of Japanese scallops (*Patinopecten yessoensis*) and *Perkinsus karlssoni* causes Scallop Perkinsus disease in *Argoppecten irradians* on the Atlantic coast of Canada and eastern US. *Perkinsus olseni* causes Perkinsus disease of abalone in southern Australia (Bower *et al*., 1994). No specific inactivation data was found regarding this parasite.

Haplosporidium nelsoni

Haplosporidium nelsoni primarily affects *Crassostrea virginica*, the American oyster, and causes a disease known as Delaware Bay disease or MSX (Multinucleate Sphere X), leading to serious mortalities (Bower *et al*., 1994).

Another haplosporidian species, *Haplosporidium costale* also affects the American oyster, *Crassostrea virginica*, in which it causes a significant, but less serious disease than MSX (Bower *et al*., 1994). Precise data concerning inactivation parameters are not available for this parasite.

Microcytos mackini

Microcytos mackini primarily affects *Crassostrea gigas* and *Crassostrea virginica* and causes a disease known as Denman Island disease or Microcell Disease of Pacific Oysters (*Crassostrea gigas*) on the west coast of Canada and specific localities around Vancouver Island (Bower *et al*., 1994). Detailed inactivation data are not available for this parasite.

Metazoan parasites

Trematoda

Monogenean trematodes

Monogenean parasites are aquatic (marine or freshwater) trematodes (*Platyhelminthes*) with direct life cycles, not involving an intermediate host (Soulsby, 1968). Eggs laid by the adult parasite hatch to release free-swimming ciliated larvae known as *oncomiracidia* which for only a few hours retain the ability to infect suitable host fish (Roberts, 2001).

Commonly known as gill and skin flukes, these are parasites of cold-blooded aquatic vertebrates (fishes, amphibia and reptiles). Two of the most common representatives of this group are *Gyrodactylus* and *Dactylogyrus*. The detrimental effect caused by these parasites is due to the attachment of the haptor on the host external tissues and the tissue damage due to the feeding activity. These organisms are strictly aquatic and are not able to survive dessication or temperatures higher than 40ºC, the upper tolerance temperature for most tropical fish (Noga, 2000).

Gyrodactylus salaris

Gyrodactylosis of Atlantic salmon *(Salmo salar)* is caused by the viviparous freshwater monogenean *Gyrodactylus salaris* (Malmberg, 1957). The presence of the parasite in Swedish hatcheries has been known since the beginning of the 1950s, but it was regarded as harmless until its introduction into Norway in the mid-1970s. It was introduced via salmon parr transported from the native range of the parasite, the rivers draining into the Baltic (Malmberg,1989).

Gyrodactylus salaris is restricted in its distribution to Europe and it has been found in farmed Atlantic salmon or farmed rainbow trout *(Oncorhynchus mykiss)* in several (mainly northern) European countries. The parasite has been found in wild salmonids, mainly Atlantic salmon parr, from rivers in Russia, Sweden and Norway (OIE, 2000). *Gyrodactylus salaris* is much more common in farmed rainbow trout than previously thought, and is likely to be present in more countries than those currently known. Great Britain and Ireland are considered to be free from the parasite.

Gyrodactylus salaris survive and reproduce in several salmonids, such as rainbow trout *(Oncorhynchus mykiss),* Arctic char *(Salvelinus alpinus),* North American brook trout *(S. fontinalis),* grayling *(Thymallus thymallus),* North American lake trout *(S. namaycush)* and brown trout *(Salmo trutta)* (in declining order of susceptibility). In experiments, Atlantic salmon from a Scottish river have shown equal susceptibility to *G. salaris* as Norwegian salmon, while Atlantic salmon from the Baltic River Neva have shown significant resistance to the parasite (OIE, 2000).

Although *G. salaris* is a freshwater parasite, it has an almost normal reproduction at 5 ppt (parts per thousand) salinity. The survival time at higher salinity may be significant, for example *G. salaris* survives for up to 240 and 42 hr at 10 and 20 ppt salinity respectively (Soleng and Bakke, 1995). No data was found for this parasite concerning inactivation by temperature or pH treatments.

Digenean trematodes

The life cycles of digenean trematodes can require one, two or more intermediate hosts (Soulsby, 1968). These parasites are also flatworms with complex life cycles in which fish can act as definitive or intermediate hosts. Different stages of the life cycle, metacercariae in the case of being intermediate hosts or adult trematodes in the case of definitive hosts, can be found in different tissues of the fish (metacercariae) or in the digestive tract (adult trematodes). There is little danger of adult trematodes being transmitted from fish to man, as fish trematodes are very species-specific.

Metacercariae: The damage produced by metacercariae is due to massive invasion of the fish tissues by cercarial larvae. Some of the best known pathologies due to digenean trematodes are infections by *Diplostomum* (eye fluke) or by *Cryptocotyle* (Noga, 2000). Mullet is known to act as a second intermediate host for a number of species of heterophyid trematodes, with the infective metacercariae encysted in the musculature of this fish species. Several heterophyids are known to be transmitted to mammals, including man. These include *Heterophyes heterophyes*, *Metagonimus yokogawai*, *Haplorhis yokogawai* and others. These trematodes use a freshwater or estuarine gastropod as the intermediate host. The cercariae escaping from the molluscan host penetrate mullet or other estuarine species and encyst as metacercariae in the fish's musculature. When man eats these metacercariae, the adult worm becomes established in the intestine. Human heterophyidiasis is not confined to the intestinal tract and eggs deposited by adult worms can be carried in the circulation to the brain, heart and other vital organs sometimes leading to death. Heterophyids are most common in the Near and Far East but several cases have occurred in Hawaii and also Texas and Florida. One of the most well known trematodes transmissible to man by marine fish is the so-called salmon poisoning fluke, *Nanophyetus salminicola*. This parasite occurs in the Pacific Northwest part of the US. In addition, this parasite acts as a vector for the rickettsial organism, *Neorickettsia helminthoeca* that causes 'salmon poisoning' in dogs that have eaten the entrails of salmon harbouring the trematode (Cheng, 1973).

Adult stages: Adult stages living in the digestive tract usually do not generate significant detrimental effects on their hosts.

These parasites do not present true resistance structures and only metacercariae in some cases can be encysted by the host response (Ferguson, 1989). No specific data are available

concerning thermal resistance of these organisms, but due to their nature and ecology, their resistance to heat or dessication may be comparable to the resistance of cestoda.

Cestoda

Cestodes are opaque white flatworms with indirect life cycles involving one or two intermediate hosts. The adults are usually ribbon-like and segmented, and the scolex may have suckers while the larvae are usually smaller and unsegmented. Cestodes can cause infestations in salmon and trout and some of the largest and most harmful parasites of the body cavity of fish are cestode plerocercoids. Fish can act as final hosts, intermediate and final hosts or solely as intermediate hosts. Cestodes can be found as adults in the intestine of some fish species or as plerocercoids if the fish acts as an intermediate host.

Plerocercoid stage: The main problem is associated with plerocercoid infections. Plerocercoids can damage different tissues of the fish host and can also be transmitted to the definitive host if those tissues containing the plerocercoid are ingested (Roberts, 2001). One of the best-known problems related to cestodes is the infection due to plerocercoids of *Ligula*. *Diphyllobothrium* is also a well-known pathogen due to the ingestion of fish infested with plerocercoids but in this case humans can be affected if they eat raw fish, although few cases occur and the disease is never fatal (Ko, 1995). However, normal cooking temperatures (higher than 55ºC) are sufficient to destroy these plerocercoids.

Adult stages: Adult parasites such as *Bothriocephalus* and *Khawia* can also be found in the intestines of some fish species, producing different degrees of spoilage. Adults of mammalian cestodes can also be destroyed by temperatures higher than 55ºC. As metazoan parasites have a high percentage of water in their bodies and lack special resistance structures it can be assumed that adults and plerocercoids cannot survive dessication.

Only three cestodes transmissible to man from eating raw fish are known: *Diplogonoporus grandis*, *Diphyllobothrium latum*, and *Diphyllobothrium graciale* (Sinderman, 1970). The biology and pathology of *Diphyllobothrium latum* is very well known (Cheng, 1973) and humans become infested primarily from eating infective plerocercoids in freshwater fish. Several species of this worm can occur in salmonids, although the species most often recognised are *D. dendriticum* or *D. ditremum*. The adult worm is found in birds (final host) and the intermediate stage encysts in the viscera of fish (intermediate host).

Members of the pseudophilidean genus *Eubothrium sp*. are commonly found in wild and farmed salmonids in North America and Europe. The life cycle may only involve a copepod intermediate host although sometimes an additional fish host may be involved (Roberts, 2001). *Eubothrium* is typically a parasite of fish in lakes or reservoirs, where copepod hosts are often abundant (Roberts, 2001).

Nematoda

Also known as roundworms, nematode parasites of fish are a group of parasites with complex life cycles involving two different intermediate hosts. Fish can act as secondary or definitive hosts. Larval stages of nematodes can invade the viscera and muscles of the fish and this is how they can be transmitted to other fish or in some cases to man. Some of the better known nematode species affecting fish are *Camallanus*, *Philometra*, *Anguillicola* (a particular type of nematode infecting eel swim bladder), *Contracaecum*, *Eustrongyloides*, *Pseudoterranova* and *Anisakis* (Roberts, 2001). *Anisakis, Phocanema* and *Terranova* can cause particular public health problems (larval migrans) when ingested by humans eating

raw or undercooked fish (usually wild fish). Larvae are usually destroyed by freezing (-20ºC for more than 60 hr) and it is reported that all nematodes were killed when heated to 55°C for 1 min (Huss, 1994).

Some nematodes can cause public health problems from eating fish such as the 'herring worm' *Anisakis marina* and the 'cod worm' *Pseudoterranova decipiens*. Although original cases of anisakiasis were due to eating herring harbouring larval *Anisakis*, other species of fish such as mackerel and salmon can also carry the parasite. The final hosts of this parasite are the grey and common seals.

Pseudoterranova decipiens ('Cod worms') have also been found in other species apart from cod such as haddock, plaice and other species of marine fish. The final host is a marine mammal. Several other species of anasakid nematodes, such as *Porrocaecum* and *Contracaecum* are also believed to be potentially pathogenic to man.

Acanthocephala spp, also called spiny-headed or thorny-headed worms, are small endoparasites that live in the intestinal tract of invertebrates, especially fish, and present complex life cycles. They are cylindrical worms having a thick cuticle and a retractible proboscis equipped with spines or hooks (Soulsby, 1968). Adults usually live attached to the intestinal mucosa and larval stages are occasionally observed in the mesentery. Due to the nature and biological cycle of this parasite, the risk posed by transmission through fish by-products is very low.

Crustacean parasites

Different groups of crustaceans occur as fish parasites, including *Branchiura*, *Copepoda* and *Isopoda*. Members of all of these groups can act as ectoparasites in the skin and gills of fish. *Argulus* is perhaps the most common parasite of the group of *Branchiura*. *Lernaea* (anchor worm), *Lepeophtheirius* (sea louse, an important parasite of farmed fish), *Ergasilus* (gill maggots), *Salmincola* and *Caligus* are also common representatives of the group of *Copepoda* (Roberts, 2001). The last group, *Isopoda*, is represented by species such as *Anilocra* and *Gnathia.* Crustacean parasites do not present any specific resistance structures and therefore it can be assumed that, as with other crustaceans such as shrimps, they can be killed easily and cannot resist normal freezing, cooking or drying procedures.

Summary Comment

In principle, parasites are generally more heat-sensitive than bacteria or viruses, and consequently will be inactivated by the treatments applied to kill these other pathogens. Although very little inactivation data is available for the parasites of fish, and no specific inactivation data is available for some of them, the risk of their transmission to other fish or to humans can be assumed to be low after dessication and/ or appropriate heating to over 65ºC.

7.4. Fungal pathogens of fish

Table 18. Some important fungal diseases of fish and shellfish

Disease	Causative agent	Susceptible Host	Disease listed by OIE	Disease listed in Directive 91/67	Zoonosis recorded or suspected
Epizootic ulcerative syndrome	*Aphanomyces invadans*	Fish	YES, (2)	NO	NO
Crayfish plague	*Aphanomyces astaci*	Crustaceans	YES, (2)	YES, (5)	NO
Saprolegnia spp.	*Saprolegnia diclina* *Saprolegnia parasitica* *Saprolegnia ferax* Other *Saprolegnia spp.*	Fish	NO	NO	NO
Icthyophonus hoferi	*Icthyophonus hoferi*	Fish	NO	NO	NO

(1)= notifiable; (2) = other significant disease; (3) = List I; (4) = List II; (5) = List III, (OIE, 2000; CEC, 1991).

Aphanomyces invadans

Epizootic ulcerative syndrome (also known as mycotic granulomatoses, red spot disease) is caused by *Aphanomyces invadans* and other related species. Some Rhabdoviruses and Gram-negative organisms have also been associated with the syndrome as secondary invaders while *Pfeisteria piscicida* can also be implicated in this syndrome (Noga, 2000). *A. invadans* form groups of aseptate tubular and branched hyphae 7-30 µm width, with or without granular cytoplasm. Sporangia have a typical shape containing numerous zoospores. Susceptible fish species include: Atlantic menhaden, Southern flounder, Ayu, Striped bass, Gizzard, Shad, Grey mullet, Barramundi and Snakeheads. The disease has been reported in estuarine and freshwater areas of Japan, Australia, New Guinea, South East and South Asia and the Western US. To date it has not been reported in Europe.

Aphanomyces can survive in freshwater and brackish waters, but not in seawater. As with other oomycetes, dessication is the best inactivation method. There is a lack of data on thermal inactivation of the organism although it presents no known health risk to humans.

Aphanomyces astaci

Crayfish plague is caused by the oomycete *Aphanomyces astaci* (Alderman and Polglase, 1986; OIE, 2000). This organism forms groups of aseptate tubular and branched hyphae 5-10 µm wide, with or without granular cytoplasm. Sporangia have a typical shape containing numerous zoospores. Susceptible hosts include crayfish of the following species: *Astacus astacus, Astacus leptodactylus, Austropotamobius pallipes* and *Austropotamobius torrentium*. The crayfish *Procambarus clarki* and *Pacifastacus leniusculus* are not susceptible, or are only susceptible under specific conditions, but may act as reservoirs or asymptomatic carriers. The disease has been reported in Europe and North America and can have a significant impact on wild crayfish populations, some of which have been seriously endangered by this disease and transmission of disease is horizontal from crayfish to crayfish.

As with other Oomycetes, *Aphanomyces astaci* can only survive in freshwater. Dessication is the best method of inactivation because oomycetes are water moulds and hyphae and zoospores cannot survive without a high percentage of water. *Aphanomyces astaci* presents no known health risk to humans.

Saprolegnia spp.

This is the most important fungal pathogen of freshwater salmon and trout aquaculture and also affects other freshwater fish species (Woo and Bruno, 1999). It has a worldwide distribution and affects the skin of all ages of fish (including eggs). *Saprolegnia spp*. often cause secondary skin infections on salmonids, and may sometimes be lethal. This fungus is reported to be able to grow at temperatures of between 5-37°C, with optimum growth at 25°C (Bruno and Wood, 1999). The fungus can only survive in freshwater and will be inactivated by dessication.

Ichthyophonus hoferi

Ichthyophonus hoferi can cause an important disease of wild and farmed fish and has a wide host range and geographical distribution (McVicar, 1982). It causes granulomatous lesions in a variety of internal organs.

Ichthyophonus is reported as not being able to resist salinities above 4% and being destroyed at –20ºC. It was also reported as being inactivated at 40ºC for a period of 3 min (Spanggaard and Huss, 1996). The fungus can grow at pH between 3 and 9.

Summary Comment

Most of the fungi affecting fish are strictly aquatic and cannot survive outside an aqueous environment. They generally have a low capacity to survive under conditions of low humidity and their infectivity is rapidly reduced to negligible levels at temperatures above 40°C. Therefore, treatments already described to reduce the infective titres and loads of viruses, bacteria and parasites are also likely to reduce the infectivity of pathogenic fungi to a negligible level.

7.5. Transmissible spongiform encephalopathies

Transmissible spongiform encephalopathies (TSEs) are terminal diseases that affect animals and man and are associated with a build up of an abnormal (more chemically resistant) form of normal prion proteins within cells of affected animals. Resistance to inactivation procedures commonly used for bacteria and viruses is a feature of the agents responsible for TSEs. Accumulation of this altered form of the normal proteins is associated with death of cells and vacuolation in the central nervous system (CNS), ultimately resulting in changes in temperament, locomotor disturbances, wasting and eventual death (Haywood, 1997).

Many species of fish are carnivorous and cannibalistic in the wild and hence have the potential to recycle TSEs if present in fish. Likewise the farming of many species results in their being fed with feed containing processed fish by-products.

PrP immune reactivity with an antibody that detects several mammalian PrPs has been reported in salmon (Gibbs and Bolis, 1997) and Suzuki *et al.* (2002) found a candidate PrP-like gene in pufferfish (*Fugu rubripes*), based on a partial nucleic acid sequence homology. However, Joly *et al.* (2001) concluded from their studies of PrP primary sequence that the PrP from fish is different from that in mammals and would be unlikely to share the pathological properties of mammalian PrPSc. The limited transmission studies that are currently in progress (see SSC opinion for details) have so far not provided evidence of TSE disease or infectivity replication in fish.

It is recommended that a number of conditions to reduce a potential risk from recycling fish should be fulfilled in accordance with former SSC Opinions (1999a, 1999b). An opinion of the SSC considers "The feeding of wild fishmeal to farmed fish and recycling of fish with regard to the risk of TSE". In summary, it was concluded that potentially TSE infected feed should not be fed to fish and that sourcing of fish by-products (including for their use in fish-derived feed) should not be performed from fish that have been exposed to potentially infected feed. If the cause of death of condemned fish can be identified as a conventional agent, by-products from such condemned fish may be recycled provided the material is treated at 133ºC, at 3 bar absolute pressure for 20 min or a validated equivalent process.

8. NUTRITIONAL REQUIREMENTS OF FISH

8.1. Finfish

In their natural environment most of the fish farmed in the EU lie near the apex of the food pyramid, consequently, they have generally evolved as carnivores; omnivorous or herbivorous habits have developed in very few species. This is especially true of the marine environment, where omnivorous fish are conspicuous by their absence (Cowey and Walton, 1989). However, some fish species of major importance in terms of world aquaculture production are herbivorous (e.g. grass carp) and the importance of these species within the EU may increase in the future. Diets for such species are not considered in this report.

The principal finfish species farmed in Europe are Atlantic salmon, rainbow trout, sea bass, sea bream, turbot, eel and common carp (EC, 2001). Under natural conditions, salmon breed in freshwater and go to sea to feed, whereas trout and carp usually spend all their lives in freshwater, although some migratory strains of rainbow trout also go to sea to feed. Sea bass, sea bream and turbot are exclusively marine species. Salmon spend their first two to four years in freshwater.

Young wild salmon and trout are carnivorous and feed mainly on insect larvae, winged insects and occasional terrestrial organisms such as beetles. In the sea, salmon feed on shrimp-like crustaceans, sandeels, herring and other small fish (Bagenal, 1972). The pink colour of salmon and trout is derived from carotenoid pigments that are synthesised only by plants, including phytoplankton in the sea. The main sources of carotenoids for wild salmon are zooplankton such as copepods and krill, which obtain carotenoid pigments by ingesting phytoplankton. The natural carotenoid taken in by wild salmon is asthaxanthin.

Wild sea bass and sea bream are also carnivorous and feed on crustacea such as small crabs and shrimps, and larger fish feed on sprats, herring, young cod and flatfish. Turbot feed on planktonic crustacea when young and subsequently on young haddock, whiting and sandeels when older.

Most of the fish species farmed in Europe are carnivorous in the wild and under farmed conditions they require high protein diets, most of which is supplied by fishmeal. Fishmeal and fish oil are key constituents of pelleted diets for the intensive production of carnivorous species (Scottish Executive Report, 2002).

The nutritional requirements of salmon and trout are very similar although trout are generally considered less nutritionally fastidious. All salmon and trout feeds are still

produced principally from high-grade fishmeals and fish oils, with added vitamins and minerals. Fishmeal is still generally considered the best source to meet the fish's protein requirements but it is expensive. In first-feeder diets generally all the protein comes from fishmeal. In older fish, particularly trout, the quality of protein does not have to be as high and other sources of protein can be used to some extent although the bulk of protein must still be provided by fishmeal (Laird and Needham, 1988).

Substitutes for fishmeal protein and marine fish oils are continuously being sought, and fish feed substituted with plant meal and oils in particular has already been used commercially in Norway (Scottish Executive Report, 2002).

8.2. Specific nutritional requirements

Dietary Energy

Fish require less than 10% of the energy for maintenance required by birds or mammals of the same size (Smith, 1989). Oil is the richest source of energy in fish diets with protein being the next richest source and carbohydrate being the least rich energy source. Fish normally obtain their energy by using all three sources of energy in the following order of preference: protein, fat and lastly carbohydrate (Laird and Needham, 1988). Carbohydrates are generally poorly utilised by fish (although herbivorous fish have a higher capacity to utilise them). Since protein is expensive it is important that as little as possible be used for energy purposes i.e. it should be spared so that most is used to produce muscle. Consequently, most salmonid diets are high-energy diets, meaning they contain large amounts of fat and as much protein as is required for growth and no more (Laird and Needham, 1988). These diets typically contain about 23% oil, and 44% protein for salmon and somewhat less oil for trout.

Protein

Fish are among the most efficient of animals in converting feed ingredients into body growth because of their low energy requirements for maintenance. Salmonids, the most important finfish species farmed in the EU, are carnivorous and, consequently, their natural diet contains a significant amount of protein. Since they are efficient converters, they sometimes achieve a feed conversion rate (FCR) of 1 particularly after their transfer to seawater when dry pelleted diets are used. However, this is based on comparing feeding a dry feed (10% moisture) to a wet weight gain (70% water). Expressing them both on a dry weight basis this feed conversion is approximately 5:1 (Laird and Needham, 1988). Farmed salmon and trout are fed on artificial diets made up principally of fishmeal supplemented with fish oils, vitamins and minerals. Modern fish diets have a high protein and oil content and a low carbohydrate content. It should be noted that the dietary protein requirements of fish are much higher than those required by most other animals (Smith, 1989).

In mammals most of the nitrogen required by the animal is used for protein synthesis and most of the food nitrogen is present as protein (McDonald *et al*., 1966). Concerning fish, the primary constituent of fish flesh is protein but, unlike mammals, protein can be used very effectively and readily by fish for energy purposes, as well as for growth (Laird and Needham, 1988). Consequently, meeting the protein requirements of fish is essential in the growth and survival of wild and cultured fish. Ideally a diet should contain adequate amounts of protein to meet growth demands with a minimum amount of excess protein for

energy production. Surplus protein, which is not utilised for growth, will be used as an energy source which might be supplied from more economical sources (Skrudland, 1993).

Most of the protein contained in diets of farmed fish is derived from fishmeal. Consequently, any limitations on the availability of fishmeal and fish oil has the potential to limit the growth of those forms of aquaculture which depend on this resource, such as salmon farming (Scottish Executive Report, 2002). The major fishmeals include anchovy, capelin, menhaden, herring, whitefish and salmon. They may be made of whole fish, as is the case with anchovy and capelin, or from processing residue, as is the case with menhaden, herring and salmon. White fishmeal is a by-product of waste from cod and haddock processing plants (Hardy, 1989)

Amino acids need to be supplied through the protein in the diet in amounts and relative concentrations that qualitatively and quantitatively meet the demand for growth and maintenance of fish. Anatomically the muscles of fish are by far the major component of the fish body and fish muscles from different species seem to have very similar composition. Thus the muscles from 10 different species analysed simultaneously for amino acids showed very nearly the same quantitative distribution (Braekkan and Boge, 1962). There are 22 amino acids found in fish muscle protein, about half of which can be synthesised by fish. However, 10 amino acids cannot be synthesised and must be supplied in the diet (Laird and Needham, 1988) and all fish species so far investigated require the same 10 essential amino acids as mammals- arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, trypthophan and valine (Wilson, 1989). However, there are differences between species in the amounts of particular amino acids required.

Protein requirements for fish are influenced by many factors such as water temperature, fish size, species and diet composition. Table 19 shows the protein requirements for a number of fish species. As can be seen, the protein requirements of fish are also species-dependent. An increase in water temperature may increase the protein requirements of fish and the amount and quality of protein required in the diet also decreases with the age of the fish.

Table 19. Recommended protein levels of fish diets (NRC, 1977, 1981)

Species	Fry	Fingerlings	Yearlings
Channel catfish	40%	30–35%	25-35%
Carp	43-47%	37-42%	28-32%
Eel	50-56%	45-50%	N/A
Salmonids	45–50%	40%	35%

In fish, unless other forms of energy are supplied in diets, proteins will readily be used for energy production.

Different protein sources have varying quantities of the essential amino acids. Sources of protein other than fishmeal, such as plant proteins, can be deficient in one or more of the essential amino acids required by fish and therefore can only partially replace fishmeal and must usually be used in combination with fishmeal as a source of protein (Laird and Needham, 1988).

However, substitutes for fishmeal protein are being continuously sought and an EU research project, “Perspectives of Plant Protein usage in aquaculture”, is currently studying this

issue, while research in Norway has been investigating the use of soya meal in feedstuffs for salmonids. Protein substitutes are already used in fish feed in the UK and Norway with up to 25% of the protein in the feed derived from plant origin (Scottish Executive Report, 2002).

Carbohydrates

There is very little carbohydrate in natural diets of carnivorous species, whereas herbivorous species consume much plant material with a high content of carbohydrate (Smith, 1989). There is some controversy concerning how much carbohydrate fish can effectively use. Trout utilise only very small amounts of carbohydrate and no more than 12% has been recommended in the diet, and the inability of fish to use dietary carbohydrate has been demonstrated by glucose tolerance tests. On the other hand, it was found that dextrin at levels as high as 28% of the diet did not cause any digestive or nutritional problems (Halver, 1989). Carbohydrates in the diets of fish are used for energy production and are usually incorporated as raw treated starch and fibre. Carbohydrate is the least expensive source of energy and it is therefore economically advantageous to use as much carbohydrate as possible in fish diets (Smith, 1989).

Lipids/Fats

Lipids provide the major energy source for farmed fish. The lipids in fish are characterised by a high content of polyunsaturated fatty acids. Whereas fatty acids of the oleic (ω-9) and linoleic (ω-6) series dominate in mammals, the linolenic (ω-3) series seems to dominate in fish (Laird and Needham, 1988). When feeding fish oil as a major part of the diet, the requirement for (ω-3) fatty acids is met.

One of the major problems associated with using fish oil is that of oxidation, since the fatty acids are highly unsaturated. Oxidation will lead to inadequate levels of essential fatty acids and rancidity of fats in fish feed can lead to the formation of potentially harmful chemicals which can cause conditions such as lipoid liver degeneration in fish fed with such diets (Laird and Needham, 1988). The addition of antioxidants such as Vitamin E can prevent such oxidation of oils.

Substitutes for fish oil in fish feeds are being sought at present and research has shown that partial replacement of fish oils with rapeseed oil and linseed oils can be used in the farming of Atlantic salmon without significantly influencing growth performance. However, there is evidence that the types of lipids used in fish diets may affect the ultimate characteristics of the lipids found in the fish. An EU funded project, “Researching Alternatives to Fish Oil in Aquaculture” (RAFOA) is studying the effects of substituting fish oils with plant oils on growth performance, fish health and product quality during the entire life cycle of salmon, rainbow trout, sea bass and sea bream. In Norway similar research has also been conducted (Scottish Executive Report, 2002).

Another EU project, “Fish Oil Substitution in Salmonids” (FOSIS) is investigating whether fish oil can be replaced by vegetable oils in the diet without reducing the nutritional value or growth performance of the fish, whilst minimising fat deposition in the flesh. Also, an EU research project is investigating the use of cultivated marine microorganisms as an alternative to fish oil in feed for aquatic animals (Scottish Executive Report, 2002).

Animal fats such as tallow cannot be used to replace fish oils in fish diets since it has been found that under cold-water conditions these hard fats are not digestible by fish (Laird and Needham, 1988)

Minerals

All forms of aquatic animals require inorganic elements or minerals for their normal life processes. Unlike most terrestrial animals fish have the ability to absorb some inorganic elements not only from their diet but also from their external environment. The exchange of ions occurs across the gills and skin and complicates the quantitative determination of mineral requirements (Lall, 1979). Fish have basically the same mineral requirements for metabolism and skeletal structure as mammalian species. They require additional minerals for maintenance of osmotic balance between body fluids and their environment. Mineral requirements for some of the more important species of fish have been published (Lall, 1989).

Most of the minerals required for growth in fish and used in osmoregulation are contained in adequate quantities in the raw materials used for producing feed and also occur in seawater. These requirements can be met from the raw materials used to make fish feed. However, high levels of calcium in fishmeal may inhibit or compete with the uptake of other divalent mineral ions such as zinc and phosphorus (Laird and Needham, 1988). Fish also require all the trace elements and macro elements, which are needed as co-factors as in other animals.

Vitamins

Fish are dependent on having almost the same vitamins supplied in the feed as for mammalian species. However, there are some differences among different species regarding levels of vitamins; for example it is known that warm-blooded species are capable of synthesising vitamin B12 in the intestines, provided that a sufficient level of cobalt is included in the diet (Skrudland, 1993).

In 1957 Halver identified that eleven water-soluble vitamins were required for salmon and trout, and with regard to the fat-soluble vitamins, qualitative requirements have been established and published for vitamins A, E, K and D (Skrudland, 1993).

All the vitamin requirements for good growth and healthy fish can be met in the dietary raw materials used for fish feed prior to processing. However, the processing of the fishmeal raw material used to produce fish feed results in loss of vitamins from the diets. Consequently, vitamins are added at the time of manufacture of fish feeds at required levels, some of these being synthetic vitamins. Some of these vitamins, particularly Vitamin C, are quite labile and will be degraded on storage if conditions are not ideal (Laird and Needham, 1988).

8.3. Feeding of farmed fish and availability of feed supplies

In intensive fish farming artificial feeding is essential for growth and even in extensive farming, some artificial feeding is usually required. Generally, fishmeal is used as the major source of protein in feeds formulated for cold-water farmed carnivorous fish rations. High quality fishmeals supply the major portion of protein (30-40% by weight) in commercial diets for salmonids (Martin, 1994).

Good quality whole fishmeal is rich in highly digestible protein of excellent quality since it contains high levels of essential amino acids in proportions that resemble the requirements of fish. Fishmeal also contains numerous essential nutrients such as polyunsaturated fatty acids, minerals, vitamins and vitamin–like compounds (e.g. choline, inositol), phospholipids and cholesterol. It also possesses excellent organoleptic properties for most fish species (Young Cho and Bureau, 2002).

The issues concerning the use of industrial fishmeal and fish oils in artificial pelleted diets in the fish farming industry are wide-ranging and complex. The increasing emphasis on utilising nutritionally valuable fisheries resources as human foods will mean a decrease in fishmeal supplies. In addition, with the development of aquaculture there has been an increased demand for fish feeds.

Although aquaculture production is predicted to rise significantly over the next decade, catches from industrial fisheries are set to remain static in volume. Forecasts differ, but there are concerns regarding how the finfish aquaculture industry may perform if fishmeal and/or fish oil supplies become limited (Scottish Executive Report, 2002).

As previously discussed, considerable research efforts are being directed at replacing fishmeal and fish oils with vegetable oils and proteins. Using two or three protein sources may reduce the potential negative impacts of relying on using a single protein source that may not be sustainable. Different protein sources may be complementary so that a mixture of them may match or exceed the amino acid requirements of the fish (Young Cho and Bureau, 2002)

Two protein sources with complementary amino acid profiles for salmon and rainbow trout are corn gluten meal and soyabean meal (Young Cho and Bureau, 2002). This has also been utilised for other animal feed industries. Corn and soyabean meal make up the bulk of poultry and pig feeds produced worldwide. Corn gluten meal is also a highly palatable protein source for fish, although it may be marginally deficient in lysine. Supplementation with L-lysine, as is done with poultry and pig feeds, may be possible. There are numerous other economical feed ingredients whose complementarities could be utilised (Young Cho and Bureau, 2002).

An example of the basic composition of a fish feed containing 4,700 Kcal (per kilogram) is contained in Table 20 (Christensen, pers. comm.).

Table 20. Example of the general composition of a fish diet

Ingredient	Content
Fish meal	45%
Oil* added	20%
Soya protein	15%
Wheat	10%
Wheat gluten	4%
Minerals and vitamins	1%

* The composition of added oil is in general terms: 75% fish oil and 25% vegetable oil.

8.4. Types of diets used in salmon and trout farming

Since there are four different types of diets in use, based on their physical characteristics, the production methods differ for each type of diet (Laird and Needham, 1988).

Wet diets are produced by mincing whole marine fish. White fish can be used but the oil content of such fish is quite low, usually only about 2–3%, and fish oils must be added. This type of diet was used mainly in Norway when farmed salmon would not take pelleted feed at low water temperatures during the colder months of the year but this type of feed is now virtually obsolete, although it is still used occasionally to feed newer aquaculture species such as cod and halibut.

It should be noted that there is evidence of a risk of transmission of infectious diseases to farmed fish via the feeding of unpasteurised diets (McArdle, pers. comm.) and in the past major epizootics of mycobacterial disease in Pacific salmon hatcheries were associated with unpasteurised feedstuffs. However, levels of infection decreased dramatically when pasteurisation of feed was consistently applied (Ross *et al.,* 1959). In Japan, Minami (1979) showed a possible link between the feeding of yellowtail with unpasteurised fish diets and the occurrence of outbreaks of streptococcosis. In Scotland, investigations into the source of an outbreak of VHS in farmed turbot in 1994 considered a possible link with the feeding of an unpasteurised diet (Munro, 1996). Although the principal feed source of the farmed fish was a commercial pelleted diet, a moist diet composed of frozen uncooked minced marine fish, mainly haddock from the North Sea, with a binder and a vitamin mineral premix was used when temperatures fell below 10°C. Only fish stocks which were fed this diet suffered disease mortality. VHS virus has been isolated from a number of fish species in the North Sea, including cod and haddock. However, at the time of the disease investigation very little of the diet fed remained and samples taken tested negative for virus. It was concluded that marine wild fish were probably the source of the virus that infected the farmed stocks, although it could not be determined whether the virus entered via the unprocessed diet or the water. There do not appear to have been any recorded outbreaks of fish diseases and fish pathogens being transmitted to fish via the feeding of processed fishmeal/fish feed.

The production of semi-moist feeds involves the mixing of ensiled fish by-products with a mixer meal consisting of mainly fishmeal, oil, vitamins and minerals. Ensiling of fish involves preserving of fish by placing them into an acid environment (pH 4.5 or lower) and suppressing the growth of normal spoilage bacteria. The normal fish enzymes break down the fish into a thick soup silage and pellets are produced from the silage/mixer meal mixture. The pellets are highly perishable and prone to rancidity.

Most of the diets used in the salmon farming industry today are dry pelleted diets made from fishmeal, fish oil and added vitamins and minerals. High-density pellets are made by passing the fishmeal/oil mixture through a ring-die press consisting of holes of various sizes, depending on the size of pellet being made. The process compresses the meal resulting in a hard dense pellet. Additional oil is usually added to the pellets produced by spraying warm oil on to the surface of the pellets and allowing it to soak in. The pellets are usually stable for several months provided they are stored in cool, dry conditions.

Dry diet expanded pellets are similar to these high-density pellets in that they are dry but are less dense. Uncooked starch, usually in the form of wheat is added to the fishmeal mixture/oil mixture. The mixture is placed into a pressure vessel and subjected to steam and heat, causing the water in the starch to vaporise and also cooking the starch, which expands

the pellet. The pellets are then dried and become hard but are less dense than the conventional high density pellet and do not crumble to dust. These pellets also have the advantage that they can absorb more oil than conventional high-density dry pellets. These pellets sink more slowly decreasing the amount of food wastage at feeding.

Pigmentation

The normal red/orange colour of wild salmon and trout is created in farmed fish using carotenoid pigments. The two pigments used are canthaxanthin and asthaxanthin. For consumers, pigmentation is regarded as the most important criterion after freshness for farmed salmonids. According to a recent report of the Scientific Committee on Animal Nutrition (SCAN) there has been a trend towards increased pigmentation in fish diets. Between 1976 and 1982, fish flesh contained up to 2.5 mg carotenoids (mainly added canthaxanthin) per kg of flesh in Norwegian farmed Atlantic salmon. Then between 1982-1988, the concentrations increased up to around 3-5 mg/kg (SCAN, 2002).

Three different pigmentation strategies have been applied in intensive salmonid farming:

- for portion size trout, canthaxanthin/asthaxanthin is added to the complete feedingstuff at a concentration of 80 mg/kg. The feed is often fed to fish for an average of 6 to 8 weeks before slaughtering (1-6 kg liveweight)

- for larger fish, canthaxanthin/asthaxanthin is added at a concentration of up to 80mg/kg feed, depending on the pigmentation regime, from the weight of 150-200g and for the whole life of the fish (up to approximately 6 kg liveweight when slaughtered)

- some salmonid feeds contain a combination of canthaxanthin and asthaxanthin in the diet at a maximum concentration of 100 mg total carotenoids/kg feed

Canthaxanthin is mainly deposited in the muscle of salmonids but carotenoids are also found in the skin mainly along the lateral line. During sexual maturation considerable amounts of carotenoids are transferred to eggs in wild and farmed trout (Sivtseva, 1982). Carotenoids have been shown to improve egg viability by improving respiration at low oxygen concentrations and protecting against sunlight. Improvement of growth rate in early feeding stages of Atlantic salmon fry have also been showed to be related to the content of asthaxanthin. In the EU, canthaxanthin had, up to recently, been authorised for use as a colouring agent up to a level of 80 mg per kg in complete feedingstuffs for salmonids. However, a recent Commission Directive has reduced this to 25 mg/kg complete feeding stuff (CEC, 2003). When combined with asthaxanthin, there is a maximum permitted level of 100 mg total canthaxanthin plus asthaxanthin per kg complete feedstuff (SCAN, 2002; CEC, 2003).

8.5. Production of fish feed

By-products from fish are used to produce fishmeal, which is used in the production of fish feed for aquaculture as well as hydrolysate, and protein concentrate, which is used in producing extruded fish feeds. By-products are also used in alternative and novel feeds for salmonids and several marine species.

Some data are available on the current and prospective use of fishmeal and fish oil and its incorporation into various animal feeds and non-feed products (Barlow, 2001).

Fishmeal and fish oil are important raw ingredients for fish feed. Farmed fish diets are dependent on high quality fishmeal and the conditions of processing fish into fishmeal have an important effect on the feeding value of the resultant product. As well as being fresh, the raw material should be processed as gently as possible. Meals prepared at temperatures below 90°C have been found to have a higher digestibility of protein for salmonids and support better growth. A drying temperature of 75-80°C is usually considered optimal (Laird and Needham, 1988). The process of fishmeal production is described in Chapter 8.6. However, treatment methods used in the production of fish feed are generally unvalidated with regard to their ability to inactivate fish pathogens that may be present in fish by-products used to produce fishmeal.

Raw materials which are mixed together at the initial stages of feed production typically include:

- fishmeal

- carbohydrates and binders such as maize, soya and wheat

- vitamins and minerals

- pigments

Following a preconditioning step, typically involving temperatures of 60-100°C for up to 3 min, the mix proceeds through a cooker extrusion process which raises the temperature to approximately 120°C for a period of up to 1 min and produces pellets of appropriate size. Drying takes place in stages with decreasing temperatures from 120°C down to 60°C over a period of around 30 min. When drying is complete the pellets are coated with fish oil and allowed to cool to ambient temperature before packing.

8.6. Production of fishmeal and fish oil

Fishmeal is the crude flour obtained after milling and drying fish flesh and is produced from either whole fish, deboned fish flesh or other fish by-products resulting from processing. Many different species are used for fishmeal and fish oil production. However, the species of fish used to produce fishmeal is nutritionally less important than the freshness, as most fish have similar amino acid profiles. The first prerequisite for good quality fishmeal is that the fish used are fresh. Since the 1970s it has been calculated that around 30% of the total marine fish catch is of low commercial value and diverted to the production of fishmeal and oil (Mackie, 1974, 1983; Sikorski and Naczk, 1981; Venugopal, 1992). In general, the fish used are mainly pelagic species (the shoaling, surface swimming varieties) such as anchovies, herring, mackerel, menhaden and sardines (del Valle and Aguilera, 1990). These pelagic species have reddish muscle and substantial fat reserves under the skin and within the flesh that gives them their characteristic oily appearance (Mackie, 1983).

The landed low-commercial fish species either come from the intentional catch of pelagic fish or as a by-catch of other important commercial species such as shrimp. These two groups are the main source of raw material for the production of fishmeal (Barzana and Garcia-Garibay, 1994). Another important source of raw material for the production of fishmeal comprises the processing waste from commercial fish species used for human consumption, including those with white lean flesh such as cod, pollock, hake, flounder etc., which contain low fat levels of between 1-3% (Spinelli *et al*., 1975). These by-

products comprise leftovers after the fillets are removed, including offal, skin, heads and backbones (‘frames’). By-products from processed finfish reared in aquaculture facilities are also another significant source of raw material.

In Europe several species are used to produce fishmeal and fish oil and these can be divided into three groups :

1. those species not used for human consumption (e.g. sandeel, capelin, Norway pout)

2. those species having a potential use for human consumption but mainly used for fishmeal (e.g blue whiting, sprat)

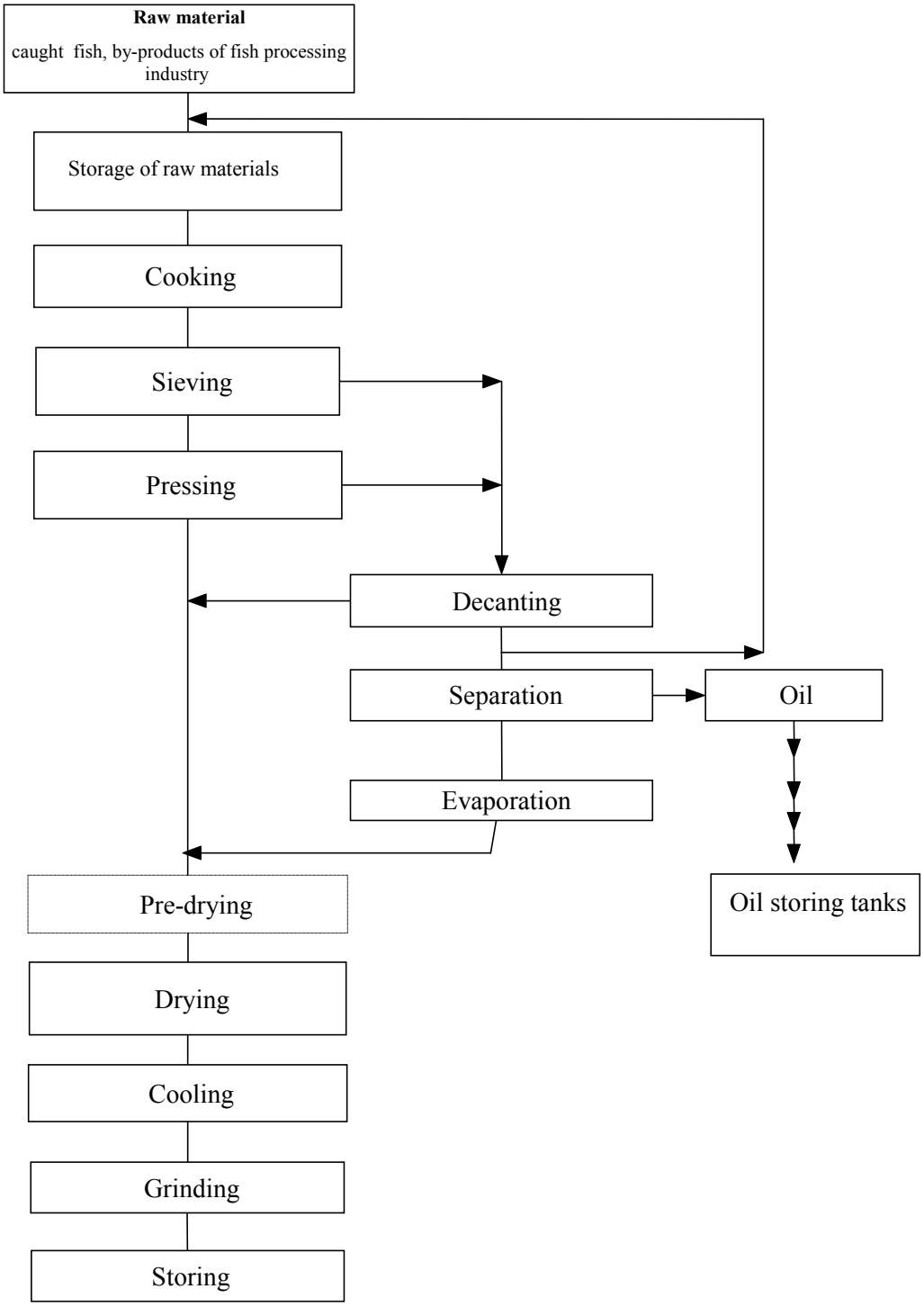
3. those species whose primary use is for human consumption but any surplus may be used for fishmeal (e.g. cod, herring, horse mackerel).

Fishmeal production also provides a major outlet to recycle trimmings from the fish processing sector, which would otherwise be dumped at an extra cost to the environment (IFFO, 2001).

The raw material for fishmeal production is steam-cooked, pressed, dried, cooled and ground down (see Figure 2). The pressing of the cooked fish results in a protein fraction called press cake, and a mixed water and oil fraction containing suspended and soluble proteins. The oil and the water fraction with proteins are separated and the ‘stick’ water (the residual watery phase from the centrifugation of the press liquor) is concentrated through evaporation. The crude fish oil is freed of moisture and suspended solids by high-speed centrifugation and the ‘stick’ water can be concentrated in multiple-effect evaporators to recover soluble ingredients either to be incorporated into the fishmeal stream process before drying or to be used separately for animal feed (Stansby, 1974).

For most of the fishmeal produced, the stick water fraction is integrated into the fishmeal but some ‘press cake meals’ are also produced which do not contain the stick water. Some factories produce fish protein concentrate from the stick water and further details are provided in the following section. Some factories also produce fish protein concentrates by an enzymatic process.

Figure 2 Steps involved in fishmeal production



In the process of fishmeal production, the most widely used technique is the wet reduction process, which is operated continuously and requires large amounts of raw material. The fish is slowly steam cooked at temperatures of 95 to 100°C for 20-30 min, pressed, the press fluid centrifuged and the press cake is then dried. The temperature used, particularly at the drying stage, should not be so hot that it denatures the protein. A drying temperature of 75-80^{o}C is usually considered optimal in order to maintain nutritional quality (Laird and Needham, 1988). As most fishmeal is produced from fatty fish, oil is recovered after pressing and mixed with stick water (Stansby, 1974). The drying of the press cake can be achieved in rotary dryers using either steam or flame-heated air. For smaller factories a batch dry reduction process may be used, in which the fish is cooked and dried as a single operation in a steam-jacketed dryer (Stansby, 1974).

During the process wastewaters are produced at various stages. The 'bail water' is the water used to transport fish by pumping, i.e. it is the carrying fluid in operations such as unloading the catch from the ships, and it contains an average of 2.8% total solids and 1% crude protein. The 'blood water' is the liquid exudate produced in the reduction plant during bulk storage of raw fish, and it contains 4.6-7.3% total solids and 2.2-4.4% crude protein. Finally, the 'stick water', which is produced during the process itself, is the residual watery phase from the centrifugation of the press liquor, and it contains an average of 9.4% total solids and 7.1% crude protein (del Valle and Aguilera, 1990). Waste waters from the cooking and drying processes are collected and sent to waste water treatment plants.

Depending on the species and the equipment used for the production, as well as the production parameters, different qualities of fishmeal and fish oil are achieved. In addition to fishmeal production by shore-based fishmeal plants, there is also some onboard production of fishmeal on Norwegian deep sea fishing vessels.

Two types of fishmeal can also be differentiated based on the temperature treatments used: Low Temperature meal (LT-meal) and High Temperature meal (HT-meal). The differences between these two types of fishmeals are mainly derived from the heat-induced effects on the nutritional quality of the proteins obtained using these two methods.

Severe heat treatments in HT-meals can reduce the protein digestibility and availability, and can also damage other nutrients such as vitamins (Pike *et al*., 1990; Luzzana *et al*., 1995). This is the main reason for an alternative and less aggressive treatment. With advances in technological developments concerning fishmeal production, new methods have been developed in order to reduce the temperatures applied, especially during the drying process. In the case of some LT-meals, temperatures do not exceed 95-100ºC during the first part of the process, avoiding the undesirable (from the nutritional point of view) effects of heating and drying process. Once the moisture content of the product diminishes, the temperature decreases to 65-75ºC. In light of these data it is very important to consider the type of fishmeal and the treatment it undergoes in order to evaluate any associated risks of transmitting fish pathogens.

Specialised types of fishmeal also exist. For example a form of fishmeal, known as shellfish meal, can be obtained from shellfish, particularly crustaceans. The major components in shellfish waste are chitin and protein. Chitin is the principal molecule in crustacean structure and is a polymer of *N*-Acetylglucosamine. Protein, chitin and pigments are valuable constituents in crustacean wastes from the perspective of the food and feed industries. Chitin, residual protein and internal organs are the basis for shellfish meal,

whose protein content is about 45%. Shellfish meal, high in chitin, is used as a supplement in chicken and fish diets.

A specific type of shellfish meal known as shrimp meal is produced solely from shrimps caught at sea around North-West Europe. The shrimp are cooked and after processing the by-products and undersized shrimps are used the same day to produce shrimp meal. The by-products from the shrimp are pressed to remove most of the water, then dried, ground down and sieved.

Some data is given in Table 21 on fishmeal and fish oil production in some EU Member States and for Norway.

Table 21. Fishmeal and fish oil production (unit tonnes) in some EU Member States and Norway in 1999 (FAO, 2002)

Country	Fishmeal Production	Fish oil production
Denmark	379,806	129,267
Spain	83,772	18,246
United Kingdom	56,532	11,644
Sweden	35,500	9,572
The Netherlands	30,000	2,700
Ireland	21,500	4,420
France	21,100	4,000
Germany	18,550	6,045
Portugal	10,081	1,252
Finland	3,000	N/A
Italy	2,800	N/A
Greece	1,618	N/A
Belgium/Luxembourg	400	31
Austria	N/A	100
Norway	265,000	84,740

Fishmeal is also imported from Third Countries into the EU for use in fish feed and Commission Decision 94/344 (CEC, 1994) lays down relevant animal health and certification requirements for the importation from Third Countries of processed animal protein, including fishmeal. The certification requirements for fishmeal include:

- being subject to a heat treatment of 80°C throughout,
- being randomly sampled during storage at the processing plant and shown to comply with the following standards- *Salmonella* : absence in 25g, n=5, c=0, m=0, M=0[3], *Enterobacteriaceae* : n=5, c=2, m=10, M=3x10^2/g,
- Derived from fish or other sea animals except sea mammals, caught in the open sea or from fresh fish offal from plants manufacturing fish products for human consumption,

[3] n= number of units comprising the sample; m= threshold value for the number of bacteria, the result is considered satisfactory if the number of bacteria in all sample units does not exceed m; M= maximum value for the number of bacteria, the result is considered unsatisfactory if the number of bacteria in one or more sample units is M or more; c= number of sample units the bacterial count of which may be between m and M, the sample still being considered acceptable if the bacterial count of the other samples is m or less.

- Not derived from fish which was spoiled,
- Not derived from fish which in the course of the inspection provided for in Community legislation failed to comply with the veterinary requirements for their importation into the Community,
- The end product was examined immediately prior to dispatch by the competent authority by a random sample and found to comply with the following standards- *Salmonella* : absence in 25g, n=5, c=0, m=0, M=0,
- The end product was packed in new packaging materials or in case of dispatch as bulk transport, containers or any other means of transport were thoroughly cleaned and disinfected with a disinfectant approved by the competent authority before use,
- The end product has undergone all precautions to avoid recontamination with pathogenic agents after heat treatment.

In addition Commission Directive 98/88 (CEC, 1998) establishes guidelines for the microscopic identification and estimation of constituents of animal origin in feedstuffs for the official control of feedingstuffs.

8.7. Novel sources of fish feed constituents

BioProteins

At the Agricultural University in Norway, a 5 year research project on Bioprotein production from natural gas has been launched. Production of single cell protein from bacteria utilising natural gas as their source of nutrients (BioProtein) for use in feed for fish and domestic animals has considerable potential and current research status shows that BioProtein has important nutritional and technological properties, and products have already been approved by the EU for use in feed for salmon and to some extent for domestic animals.

An integrated Project called “Production of Aquaculture Feed, Sustainable and Healthy Food Production”, has also been presented by a broad range of European research institutions. The objective is to improve understanding of the role of animal feed, including products containing genetically modified organisms and the use of sub-products of different origins for that feed, to reduce the use of undesirable raw materials and to develop alternative new animal feed sources. The results from this project may reduce the dependence on importing feed ingredients, like soyabean meal, from outside Europe. The proposed project contains three main dimensions: consumers needs, safety of the food supply chain and enhancing competitiveness.

Exploitation of zooplankton resources is a possible way to enhance marine harvest of bio-resources for fish feed and other industrial applications. Salmon feed mainly on krill, copepods, other large zooplankton, and on small fish in the sea. This makes zooplankton an interesting nutritional source for fish feed, and feed in general. New technology is needed, but earlier experiences suggest that lack of suitable technology may only be a temporary hindrance. Environmental considerations, sustainability of resources and maintenance of biodiversity should also be borne in mind. The increasing demands for fish feed for aquaculture may accelerate developments, since lack of such feed resources will become a limiting factor for further aquaculture developments in the coming decades. Agricultural

production is based on selection and long-term plant breeding, both being efficient tools to increase production yield, but also to meet specific nutritional demands for humans or farmed animals. Similar developments have yet to be performed to meet the specific nutritional demands of aquaculture species.

9. RISK-REDUCING BY-PRODUCT TREATMENTS

Fishmeal is obtained from the drying, heating and pressing of dead fish or trimmings of such fish after filleting for human consumption. Although recycled fish in the form of fishmeal is the principal ingredient of food for farmed fish, some codes of good practice recommend that recycled farmed fish are not used as an ingredient of fishmeal used to produce fish feeds for farmed fish (e.g. BCSFA, 2001). It was recognised by previous SSC Reports (1999a, 1999b) that recycling of animal by-products processed into basic biochemical substances such as fat and protein could be an effective way of re-using such materials. These reports consider that intra-species recycling can be acceptable when the material of origin is, from an epidemiological point of view, safely sourced with regard to TSEs and treated appropriately to prevent any spread of conventional diseases.

Procedures to prevent the contamination of animal feed by by-products of terrestrial animals have been developed over the last 20 years using a cumulative series of risk reduction measures. The additive effects of obtaining products from 'safe sources', where possible removing the potentially harmful elements of the feed, treating products to minimise the presence of contaminants (CEC, 1990a) and controlling the use of the end-product (including the prevention of intraspecies re-cycling of animal by-products) are recognised as reducing the risk of feedborne contamination associated with animal by-products.

The standard treatment to 'sterilise' feed products of routine microbiological agents is subjecting the material (which should have particles no bigger than 50mm) to a temperature of 133ºC at 3 bar absolute pressure for 20 minutes. Similar treatments have been reported to be associated with reducing bacterial counts in samples to undetectable levels (Taylor *et al*., 1995). EU Council Directive 90/667 (CEC, 1990a) indicates that numbers of Clostridial spores and numbers of *Salmonella* and *Enterobacteriaceae* in samples of animal by-products that are treated at a temperature of 133ºC at 3 bar absolute pressure for 20 minutes (having a maximal particle size of 50 mm) should be reduced to undetectable levels.

As with the utilisation of other by-products of animal origin, the epidemiological risk depends on the origin and properties of the raw material as well as the ultimate use of the end-product. Even though products may be classed as fit for human consumption, they may still carry organisms that could pose a risk to susceptible species. Recycling of by-products as feed is an economical method of disposal, but also theoretically the most hazardous way of dealing with animal by-products, if appropriate treatment procedures are not applied. Various treatments are applied to by-products from marine caught fish used for the production of cosmetics, and in biotechnology or industrial applications, where by-products of aquatic origin are processed in order to extract various marine ingredients. If fish are classified as being 'fit for human consumption', their by-products could also be considered safe for fish provided that their intervening storage does not enhance the growth of spoilage organisms. Such storage will generally involve chilling (<4°C), freezing, or conservation with formic acid to a pH<4 (ensiling).

The safest way to treat organic by-products of animal origin is 'sterilisation' (with regard to viruses, bacteria and parasites) at 133°C at 3 bar steam pressure for at least 20 min (Strauch and Ballarini, 1994). If this causes technological problems with the fish material (e.g. a reduction in nutritional value) other time/temperature relationships could be applied but such treatments would

need to be validated with relevant organisms, and if a temperature below 100°C is used some bacterial spores may survive the treatment. Clostridial spores are taken as an example of a particularly resistant agent that may occur in fish by-products. In Table 22 D-values for some selected clostridial spores are given as an example.

Table 22. Decimal reduction rates (D_t values) of some selected Clostridial spores (Mitscherlich and Marth, 1984; Hoppe, 1978)

Species	D_t value	Material
Cl. botulinum Type A	D_{100} = 30.7 min	Phosph. Buffer pH 7
	D_{106} = 7.51 min	Phosph. Buffer pH 7
Cl. botulinum Type B	D_{100} = 20.73 min	Phosph. Buffer
	D_{113} = 1.67 min	Phosph. Buffer pH 7
	D_{121} = 0.26 min	Phosph. Buffer pH 7
Cl. botulinum Type E	D_{104} = 11.0 min	Not reported
Cl. botulinum Type F	D_{82} = 16 hr	Crab meat
Cl. perfringens	D_{115} = 0.27 min	Not reported
	D_{90} = 460 min	Phosph. Buffer pH 7

Dry heat is less effective than wet heat as a thermal inactivation procedure, and consequently the ability of the drying process to inactivate microorganisms is often overestimated (Wallhäußer, 1995). Moreover the matrix influences the thermostabilty and consequently every process needs to be validated using the material to be processed and a representative test organism, which has the same heat resistance class and comparable chemoresistance as the target pathogens that are required to be inactivated by the process.

If other heat treatment processes with different time and temperature relationships are used, these need to be validated on a case-by-case basis. In relation to all the other hygienic conditions, the principles of strictly separated clean/unclean areas, hygienic product supervision with validated processes could be applied, in combination with a hazard analysis critical control point (HACCP) system.

Several types of treatments may be used to recycle fish by-products. The aim of the treatment is both to transform the raw material, which tends to convert rapidly by spoiling into a microbiologically stabilised product, and also to protect the product properties for the intended field of application. Processes based on physical, chemical or biotechnological treatments, alone or in combination, may be used. Besides thermal treatment, drying and incineration, aerobic (composting) and anaerobic (biogas production) biotechnological treatment together with stabilisation by acidification are considered below. Each of these treatments leads to a useful end-product, while incineration and landfill, when used, result in complete destruction and disposal.

9.1. Chemical and biological waste treatments

These chemical and biological treatments for fish by-products (often referred to as biotechnological treatments) may be performed either in an aerobic or anaerobic microbiological process leading to a generally stable end-product that may be used as a fertiliser. The anaerobic process (biogas fermentation) may be either a thermophilic (temperature above 50°C) or a mesophilic (temperature above 30°C) system operated continuously or as a batch process (Scherer, 1995; ATV-DVWK, 2002). The process may be run in one single fermenter or in two fermenters (two-stage biogas plant). Thermophilic and mesophilic fermenters may be combined in the case of two-stage biogas plants. Due to

hygienic reasons, a pasteurisation unit is often placed before the fermenter, and occasionally subsequent to it. Sometimes the liquid product of the fermentation is dewatered and the solid phase is composted together with some structure material (material rich in carbon, e.g. paper, cardboard etc.). Aerobic treatment is commonly used for the composting of generally solid organic material, but it may also be used for liquids in specially-designed reactors. Composting may be either performed in the traditional way in windrows or in reactors that are closed to varying extents (ATV-DVWK, 2002).

Material that could contain pathogens of any kind can only be processed in a biotechological process which has been validated with representative test organisms and which is continuously supervised in terms of conforming with the relevant process parameters. If material of animal origin is processed in this way, additional hygienic measures need to be taken in order to protect the environment and the final product from possible contamination (Böhm, 1998).

9.1.1. Ensiling

Ensiling is a process used for the preservation of fish by-products until further processing and is an important means of processing farmed and wild fish by-products. The by-product is usually preserved in acid at a pH of 4.5 or lower, if necessary with an anti-oxidant added to prevent rancidity. The most appropriate acids seem to be formic acid or hydrochloric acid (Wood *et al.,* 1995). Organic acids such as formic acid or propionic acid have antiseptic properties that enable the silage to be stable at a relatively high pH of 4 to 4.5 and can penetrate fish cell walls more effectively than inorganic acids and accelerate the rate of autolysis (Carswell *et al.,* 1992). The most commonly used acid in Europe is formic acid (Torgersen, pers. comm.). Ensiling is not strictly a process due solely to microbial activity but the addition of chemicals is necessary to support the activity of the acidophilic microflora in such a protein-rich substrate. The pH also needs to be monitored to ensure that the process is efficient and a pH of 3.5-4 is maintained throughout.

Ensiling is a very suitable method for intermediate storage of mortalities at the farm. In these cases, the dead fish need to be collected regularly, ground down and ensiled at the farm, and simple systems can be used consisting of small ensiling containers on farms from which the ensilage is regularly collected.

Fish by-products containing a minimum of bones are preferred because of the high content of calcium which may neutralise the acid; thus more acid is required resulting in higher running costs (Carswell *et al.,* 1992). Fish by-products containing bones will result from grinding of the fish by-product and should, where possible, be separated before entering the process. The presence of bone material in fish by-products has been a considerable problem as the bone material may sediment in the ensiled material and be difficult to pump through pipes. The process is effective but expensive and so far no technique to utilise the bone fraction has been developed.

As an example from one country, the market for ensilage in Norway has increased by a factor of four from 1991 to 1998- a total of 140,000 tonnes of ensiled fish was utilised in 1998 (RUBIN, 1998). Ensiled fish by-products are an established product distributed to large markets and the fate of the product is different, depending on the source of the ensilage. Both the oil fraction and the protein concentrate of ensilage from fish caught in open sea, are used in aquaculture. The oil fraction from ensilage made from aquaculture is often used for technical uses or as heating oil. The greater part of the concentrated protein

is used in feed for warm-blooded animals, primarily domestic animals and also for animals kept for fur production.

9.1.2. Biogas production

Biogas production is a process where organic matter in biological waste products raw material (e.g. animal slurry, sewage sludge, fish by-products, fish oil, dairy waste, biological waste of municipal or industrial origin) is fermented under anaerobic conditions, alone or in combination (Scherer, 1995). Fish by-products are usually processed in co-digestion with a liquid substrate, such as slurry. The process itself involves four steps (Zachäus, 1995):

- Hydrolysis of macromolecules (such as cellulose and large molecular weight proteins)
- Fermentation/acidification (main products are butyrate and propionate)
- Acetogenesis to form acetic acid
- Methanogenesis in which methane and carbon dioxide are formed from the acetic acid

The main gases produced are methane (50-75%) and carbon dioxide (25-50%). The energy in biogas is contained in the methane fraction and biogas is used for various energy purposes, e.g. heating of buildings or in power stations (Zachäus, 1995; ATV-DVWK, 2002).

The liquid end-product, consisting of undigested matter, non-decomposable matter and waste products containing methane-producing bacteria, can be used as a fertiliser for arable land. Fish and fish by-products are regarded as high value raw materials in biogas production, especially when the fish oil contents are high and biogas plants are divided into two main categories, mesophilic and thermophilic anaerobic digestion (ATV-DVWK, 2002):

Mesophilic anaerobic digestion

Mesophilic anaerobic digestion is carried out at 33-35°C where the liquid fraction remains for 20-25 days (mean hydraulic retention time) in the biogas fermenter. If the reactor is operated in a continuous or semi-continuous manner, a portion of the end-product is removed every 2-12 hr and new raw material is added. A risk associated with this procedure is that infective pathogens in new waste material, which has only been in the reactor for 2-12 hr, might be transferred into the end-product which may be removed from the system. Consequently infective pathogens could possibly survive and persist in the end-product.

Thermophilic anaerobic digestion

Thermophilic anaerobic digestion is carried out at 52-55°C where the liquid fraction generally has a mean hydraulic retention time in the reactors of 15-20 days. As with mesophilic anaerobic digestion, there is a risk of shunting pathogens from new waste material to end-products being removed if it is operated in a continuous or semi-continuous manner.

Additional heat treatment

Both mesophilic and thermophilic digestion can be performed with or without pasteurisation, which can be done prior to digestion when new material is moved from the pre-storage tank to the digestion tank. Alternatively, the end-product can be pasteurised following digestion just after it is pumped out of the reactor by heating it to 70°C for 1 hour.

The following are some general parameters maintained and monitored in digestion tanks:

pH: In the digestion tank the pH is held between 7 and 8 and in most plants the pH is controlled continuously. If the pH decreases to less than 7, new raw material, which is alkaline in nature, due to the content of ammonia in slurry, will be pumped into the fermenter.

Temperature: The temperature is continuously monitored, and kept stable within the mesophilic or thermophilic temperature range. If a decrease in temperature occurs, the material in the digestion tank is additionally heated to the relevant temperature by heat supplied from an external source. In case of an excessively high temperature, the appropriate temperature is regained by simply stopping the heating process.

To ensure hygiene and prevent cross-contamination, separation between clean and unclean sections of the plant is required. To avoid transport of infective pathogens from fresh raw material to the end-product, the traffic within the plant area is one-way, and plant facilities are constructed so that cross-contamination is avoided. Usually the end-product is carried away from the plant in the same container trucks that delivered the raw material, and for this reason after delivery of the raw material the container trucks need to be appropriately disinfected with a validated disinfectant (e.g. a 2% solution of sodium hydroxide).

Flow of Material within Biogas Plants

The following is a general outline of the process in a plant using slurry with fish by-products as co-fermentate/co-digestate (Jensen, pers. comm.; Paamand, pers. comm.).

Arrival station and mixing

Slurry is delivered by container trucks and unloaded at the arrival station. The slurry is mixed with other biological waste products in a mixing tank at the arrival station.

Pre-storage tanks

The mixed material is kept in buffer tanks (pre-storage tanks) and stored until needed in the subsequent process. The buffer tanks make it possible to receive raw material discontinuously/ irregularly without causing stops in gas production or overloading of the facilities. To avoid emissions of odorous gases from the pre-storage tanks these gases are collected and treated in one of the following ways:

- Destruction using a biofilter,
- Destruction by UV-irradiation,

- Absorption using activated charcoal, or by
- Combustion (either by its own content of inflammable gases, by the addition of biogas, or using a thermostatically controlled electric combustion at 900°C).

Pre-heating of raw material

The biological digestion in the biogas reactors is sensitive to changes in temperature. To avoid disturbance of the biogas process, the fresh raw material is often heated to up to 40°C, before it is moved into the biogas reactor. The pre-heating is carried out in a heat exchanger, which makes it possible to recycle heat from the digested end product to the cold raw material.

Digestion in reactors

In the biogas reactors, methane-producing bacteria digest the organic matter in the raw material (e.g. a reactor may have a capacity of 5,000 tonnes).

Biogas storage

The biogas produced is moved from the reactors to gas storage tanks (e.g. these may have a capacity of 2,000 cubic metres). Excessive biogas is burned in a torch-tower when the production exceeds requirements or the capacity of the gas-storages.

Pasteurisation tanks

The fresh raw material or the digested end product is pasteurised in specially constructed tanks or heaters and this pasteurisation is performed by heating to 70ºC for 1 hour.

End-product

In order to recycle heat from the end-product to the pre-heating process, cooling takes place in heat exchangers and the cooled end-product is stored in end-storage facilities until it is ploughed into arable land as a fertiliser.

9.1.3. Composting

Composting is a triphasic microbial process and in the first, the mesophilic phase, the easily degradable nutrients are metabolised by certain populations of microorganisms. Due to their metabolic activity the temperature rises and populations of thermophilic microorganisms further degrade the remaining nutrients in the second phase, both steps requiring the presence of oxygen (aerobic conditions). The material to be composted needs to have a carbon/ nitrogen (C:N) ratio of between 20:1 and 40:1 for example, a water content of between 40% and 65% and an air pore volume of above 50% (ATV-DVWK, 2002; Zachäus, 1995). Therefore fish by-products, which contain a lot of nitrogen and have a relatively compact and moist structure, have to be mixed with so-called 'structure materials', which are rich in carbon (e.g. paper, cardboard, wood chip, sawdust, dead leaves). Using this technique all fish by-products can be composted successfully.

Once the microorganisms have consumed most of the decomposable by-products, the third phase of cooling down ensues and the composting process will slow down. At this stage the primary composting process is finished and the material has to be 'cured', i.e. it has to be stored until it is inactive and ready for use as a final product, for example as determined by

maturity testing (Bundesgütegemeinschaft Kompost, 1994). Compost maturity is a term used to express characteristics that are frequently associated with unfinished or poor quality compost. Compost which undergoes adequate decomposition will normally result in a product that favours plant growth, improves soil fertility and potentially suppresses soil-borne pathogens. Under some circumstances, however, compost may not fully mature, meaning that it does not undergo sufficient breakdown to become stable and growth promoting. Maturity testing includes establishing a maturity index for the compost used at two application rates as well as pH and electrical conductivity analysis (Bess, 1999).

Aerobic thermophilic treatment can also be achieved by aerating a liquid organic substrate such as slurry or sewage sludge in an insulated tank. The so-called 'Liquid composting' is performed at temperatures ranging between 50°C and 60°C in which inactivation of pathogens can be achieved (Philipp *et al*., 1992, Strauch *et al*., 2000). Various other organic materials such as fish by-products can be added to the substrate as additional carbon sources and wet composting is a possible method of handling dead fish. Animal slurry is low in nitrogen and nitrate and by adding fish ensilage the nitrogen level is elevated and the product is more suitable for use as a fertiliser. Adding 15-25% fish ensilage was found to be optimal and fish by-products can be composted together with manure, slurry, sludge or food waste, for example. The results of a Norwegian study (RUBIN, 1998) suggested that *A. salmonicida* and the *infectious salmon anaemia virus* were not detected in stable compost following the process of composting. Compost acts as a fertiliser, soil enhancer and also suppresses a wide spectrum of microorganisms and diseases (Hoitink and Fahy, 1986).

In Maine, USA, the process of composting has helped the aquaculture industry to solve their waste disposal problem. It has also assisted other industries with waste disposal, as the composters frequently use horse or poultry manure as additional sources of material to compost with fish by-products (Mathies, 2002).

Composting provides a cost-effective approach to handling fish processing by-products and can be performed using a variety of systems with varying levels of sophistication. Technology employed needs to conform to regulations in force (CEC, 2002a). Open windrow composting is being discontinued and closed windrow composting or in-vessel technologies will need to be implemented. When carried out properly, composting can produce a soil enhancer/fertiliser that is odour-free, stable and easily stored. If materials such as fish by-products are composted, access of vectors such as rats/birds has to be avoided, therefore 'in-vessel' composting is preferred. The same hygienic precautions must be followed in running the composting plant as outlined for the anaerobic treatment.

9.1.4. Microbiological safety of the end-products from various treatment processes

Products coming out of a complex aerobic or anaerobic degradation process are not sterile products and if they are to be used as fertilisers they should satisfy certain hygienic requirements which can vary depending on the field of application. These requirements may also differ from the standards applicable to the use of such material for the production of feed. Nevertheless various indicator organisms may be used to monitor the end-product, depending on the aerobic and anaerobic treatments applied as well as the use of ensiling and the raw materials used. In general, biological by-products of plant and/or animal origin should only be treated in processes and technical equipment (e.g. pasteurisation device, reactors) which have been validated with representative test organisms. Otherwise the relevant pathogens may not be inactivated by the processes applied. Monitoring and recording of relevant process data are also required and restrictions may also be applied to

the eventual use of the final product (Böhm, 2002). For example, such a strategy is described in the German Biowaste Ordinance (1998) and could be adapted to the special conditions related to processing of fish by-products. Similar requirements are laid down in EU legislation (CEC, 2002a). Additional validation and monitoring measures could be taken in the framework of a HACCP system.

Hygienic safety following composting of residuals and by-products from fish

Depending on the type of composting (e.g. windrows, closed vessel) and the raw materials used, as well as the climatic conditions, the temperature parameters of the process and the heat distribution in the material may be different. An example is given in the German Biowaste Ordinance (1998) which specifies that composting plants should operate with a material having a moisture content of 45-50% at a pH of approximately 7. When held in windrows, the entire material needs an exposure time of at least two weeks at 55°C, while in closed vessels exposure to 65°C for one week is required. Previous recommendations of 1hr at 70°C require updating based on available scientific knowledge and technical/hygienic aspects. In theory, many types of fish pathogens can be inactivated in a validated composting process, apart from spores of spore-forming bacteria. Even if systematic investigations with fish pathogens have not yet been performed, it may be possible to extrapolate from the behaviour of other similar pathogens of warm-blooded animals, as well as of relevant indicator organisms, that a validated process will be safe from the hygienic point of view. However, the data presented by Smail *et al*. (1993) highlights the robustness of IPN virus and its ability to survive this process. Consequently it is necessary to consider the capacity of individual fish pathogens to survive various treatment processes. Data concerning inactivation of viral pathogens in relation to some possibly comparable fish pathogens are presented in Table 23.

Table 23. Inactivation times during semi-technical in-vessel composting of selected mammalian viral pathogens in a temperature range between 25°C and 65°C (Moss and Haas, 2000)

Type of virus	Pathogens tested	Range of titre reduction	Range of exposure times required	Comparable fish pathogens
Non-enveloped	SVD Virus, FMD Virus	4-5 log $TCID_{50}$ 4-8 log PFU	27-72 hr [1] 12-144 hr	Nodaviruses, TSV, Adenoviruses, IPNV
Enveloped	CSF Virus, AD Virus, ASF Virus	4-5 log $TCID_{50}$ 4-5 log $TCID_{50}$ 3-5 log $TCID_{50}$	12-144 hr 20-192 hr 24-168 hr [1]	EHNV, RSIV, WSIV, IHNV, VHSV, SVCV, ISAV, YHV, OMV, CCV

1. Insufficient inactivation within 27hr if at least 55°C had not been reached

TCID: Tissue Culture Infective Dose, PFU: Plaqe Forming Units, SVD: Swine Vesicular Disease, FMD: Foot-and-Mouth Disease, CSF: Classical Swine Fever, AD: Aujezsky's Disease, ASF: African Swine Fever, TSV: *Taura syndrome virus*, EHNV: *Epizootic haematopoietic necrosis virus*, RSIV: *Red sea bream virus*, WSIV: *White sturgeon iridovirus*, IPNV: *Infectious pancreatic necrosis virus*, IHNV: *Infectious haematopoietic necrosis virus*, VHSV: *Viral haemorrhagic septicaemia virus*, SVCV: *Spring viraemia of carp virus*, ISAV: *Infectious salmon anaemia virus*, YHV: *Yellowhead virus*, OMV: *Oncorhynchus masou virus*, CCV: *Channel catfish virus.*

Australian officials recommend not to compost crustaceans and molluscs, due to the resistance of the shells and the exoskeleton (Aquavetplan, 2002), but experiments with

bones show that if the particle size is low enough, this will not inhibit effective composting (Breitenfeld, 2000).

Process validation and supervision (combined with product supervision in order to assure hygienic safety of products) can be used as part of overall GMP and HACCP systems for process and product control (Evans and Lindsay, 1996; Pearson and Dutson, 1995). End-products from such aerobic or anaerobic treatment of organic by-products have been used as fertilisers or soil improvers (Strauch *et al*., 2000).

Hygienic safety following anaerobic treatment of aquaculture by-products

Although thermophilic anaerobic treatment may be designed as a batch process, in most cases the heat treatment (e.g. 1 hr at 70°C) is performed either before or after the anaerobic digestion is carried out. Preheating is preferred in order to have a microbiologically stable product after the fermentation- if the material was heated after the biogas fermentation step there would be a higher risk of recontamination and microbial multiplication in such heated material with easily available nutrients. Instead of pasteurisation, composting of the solid phase in a validated process may be performed after dewatering. If the process is a mesophilic one or if it is run continuously, separate composting is always necessary. Pasteurisation for 1hr at 70°C is sufficient in most cases, although if catering wastes are processed in codigestion pasteurisation needs to be performed at 90°C for 1hr (Böhm, 2002). Additional hygienic measures (e.g. strict separation of raw material and end-product) must also be taken in such cases. Inactivation data for fish pathogens in validated thermophilic anaerobic batch processes are not available, but it may be concluded from Tables 24 and 25 (which contain comparable data on pathogens of warm-blooded animals and indicator organisms) that under comparable circumstances similar fish pathogens will also be inactivated. In Table 24 the longest survival times are given without taking the exposed matrix (virus suspension or virus adsorbed to a membrane) into account and in both tables some fish pathogens are listed which may be comparable to the viruses and bacteria investigated.

Table 24. Ranges of T_{90} values (hr) of a variety of viral pathogens and indicator organisms determined in a semi-technical co-digestion plant (catering waste and slurry) at several temperatures (Moss and Haas, 2000).

TYPE OF VIRUS	INVESTIGATED VIRUSES	30 °C	35 °C	50 °C	55 °C	SIMILAR FISH PATHOGENS
DNA NON ENVELOPED	BPV	N.R.	180.5 hr to 318 hr	10.5 hr to 31 hr	5.5 hr to 8 hr	Adenoviruses
DNA ENVELOPED	ASF virus AD virus	1.43 hr to 2.63 hr	1.21 hr to 4.55 hr	0.13 hr to 1.11 hr	0.05 hr to 0.21 hr	EHNV, WSIV, RSIV, EHV, OMV, CCV, WSV, PvSNPV, PmSNPV, BMNV
RNA NON ENVELOPED	ERV ECBO virus POLIO virus SVD virus FMD virus	11.5 hr to 43.4 hr	5 hr to 25.2 hr	0.06 hr to 1.38 hr	0.02 hr to 0.54 hr	Nodaviruses, TSV, IPNV
RNA ENVELOPED	CSF virus	0.73 hr to 0.9 hr	0.43 hr to 0.46 hr	0.12 hr to 0.15 hr	0.05 hr to 0.06 hr	IHNV, VHSV, SVCV, ISAV, YHV

N.R.: not reported, BPV: *Bovine parvovirus*, ASF: African Swine Fever, AD: Aujezsky's Disease, ERV: *Equine rhinovirus*, ECBO: *Enteric cytopathogenic bovine orphan virus*, Polio: *Poliomyelitis virus*, SVD: Swine Vesicular Disease, FMD: Foot-and-Mouth Disease, CSF: Classical Swine Fever, EHNV: *Epizootic haematopoietic necrosis virus*, WSIV: *White sturgeon iridovirus,* RSIV: *Red sea bream iridovirus,* EHV: *Eel herpes virus*, OMV: *Oncorhynchus masou virus*, CCV: *Channel catfish virus,* WSV: *Whitespot virus*, *PvSNPV: Singly enveloped nuclear polyhedrosis virus* from *Penaeus vannamei* (PvSNPV), PmSNPV: *Singly enveloped nuclear polyhedrosis virus* from *Penaeus monodon* (PvSNPV), BMNV: *Baculoviral midgut gland necriosis virus*, TSV: *Taura syndrome virus*, IPNV*: Infectious pancreatic necrosis virus*, IHNV: *Infectious haematopoietic necrosis virus*, SMV: *Spawner-isolated mortality virus*, VHSV: *Viral haemorrhagic septicaemia virus*, SVCV: *Spring viraemia of carp virus*, ISAV: *Infectious salmon anaemia virus*, YHV: *Yellowhead virus*, T_{90}= Time necessary to inactivate 90% of the original microbial population.

Table 25. Decimal reduction rates T_{90}-values (hr) found for several bacteria in co-digestion (catering waste and slurry) at different temperatures (Hoferer *et al.*, 2000; Hoferer, 2002)

TYPE OF BACTERIA	INVESTIGATED SPECIES	30 °C	35 °C	50 °C	55 °C	SIMILAR FISH PATHOGEN
Gram-negative	*Salmonella senftenberg, Escherichia coli O157:H 7*	41.76 h to 78.48 h	25.20 h to 27.60 h	0.40 h to 0.60 h	0.02 h to 0.11 h	*Edwardsiella tarda Edwardsiella ictaluri Aeromonas spp. Yersinia ruckeri. Vibrio spp.*
Gram-positive	*Enterococcus faecium*	74.40 h to 186.24h	93.36 h	7.48 h to 11.26 h	1.64 h to 1.70 h	*Renibacterium salmoninarum Lactococcus garvieae Enterococcus seriolicida Streptococcus iniae*

The inclusion of a pasteurisation step, by heating to at least 70°C for 1 hour before or at the end of the fermentation process, is required. The pasteurisation process inactivates vegetative bacteria, but not bacterial spores and this also applies if that solid phase is separated and composted in a validated process to inactivate the relevant pathogens. Well-established procedures for separation of raw material and end-products are essential.

The use of the end-products as fertiliser, preferably on arable land, should be regarded as safe when used without the risk of contamination of the aquatic environment (e.g. not used on frozen land or when a risk of flooding exists).

Hygienic safety following ensiling

Organic acids such as formic acid act bacteriostatically at pH values of between 5 to 6 and below 5 slow but distinct bactericidal action occurs. Trauzettel (1993) reported that most

bacterial pathogens except *Mycobacteria* and bacterial spores are inactivated within two hours at concentrations above 4% of formic acid. In this regard, considerable data regarding inactivation of Gram-negative bacteria are available with inactivation being pH dependent. At a temperature of 5°C, *Salmonella spp*. were shown to survive for between 19 and 49 days at a pH of 3.9 to 4.1, between 1 and 27 days at a pH of 3.0-3.1, and for less than 1 day at a pH of 2.1-2.6 (Mossel and de Bruin, 1960). On the other hand some viruses are relatively resistant to inactivation by acids: in experiments carried out regarding the ensiling of fish wastes in Norway, 1 x 10^8 cfu of *A. salmonicida* were inactivated using the procedure described by Bjoeru (1996). ISA virus was shown to be inactivated within 1 day at a pH of 4.0 or less, although the inactivation of IPN virus during ensiling was questioned. Indeed Bylund *et al*. (1993) showed that IPN virus could survive in fish ensilage for at least 4 years. Consequently additional research is urgently required to determine under what conditions ensiling is capable of inactivating IPN virus.

9.2. Thermal treatments

Thermal treatment of organic material may be carried out in various ways depending on time/temperature relationships during treatment. Either dry or moist heat treatments will not only inactivate microorganisms and viruses but will also influence the properties of the product. Thermal treatment may also be combined with chemical treatment in order to produce synergistic inactivation effects or modify the eventual end-products. Pasteurisation and sterilisation procedures are described below as well as incineration, which results in complete destruction of the organic material.

9.2.1. Pasteurisation

Heat treatment at temperatures below 100°C can be considered as pasteurisation and will only have limited inactivating effects on microorganisms. Heat resistant spores of mesophilic or thermophilic sporeformers (heat resistance classes III and IV respectively-see Table 26) will generally survive this procedure or will only be inactivated after extremely long exposure times (Borneff, 1977; Konrich and Stutz, 1963). The advantage of such moderate heat treatment is that product quality is maintained, especially with regard to easily hydrolysed proteins that are found in raw materials originating from fish.

Table 26. Classification of the resistance of microorganisms to moist heat (Borneff, 1977; Konrich and Stutz, 1963)

Resistance Class	Representatitive organisms or groups of organisms	Resistance against moist heat
I	Vegetative bacteria (e.g. *Staphylococcus aureus*), fungi and fungal spores, viruses	80°C secs to min F^6_{80} <20 min
II	Spores of *Bacillus anthracis*	100°C 8-15 min F^6_{100} <20 min
III	Mesophilic native soilborne spores	100°C 10-20 hr 121°C 10-15 min F^6_{121} <20 min
IV	Thermophilic native soilborne spores	100°C 40-50 hr 121°C several hr 140°C 5 min F^6_{133} <20 min

F^z_t : t is reference temperature, z is z-value (Lewis and Heppell, 2000).

Pasteurisation may be performed in a heating device run as a continuous or batch process and the heat transfer may be achieved either by indirect or direct heating. Often heat exchangers are used to heat the material before pasteurisation, in order to conserve energy. For indirect heating systems, steam or hot water is used to heat the walls of a double-walled heating device. Direct heating may be performed by placing an electric heater into the liquid or by blowing steam into the material. The construction of the heating devices can vary, in that it may either be constructed as a pipe heater or as a pasteurisation tank. In the latter, stirring improves the heat transfer and heat distribution.

Any time/ temperature relationship that has been validated with the relevant organisms may be used for pasteurisation. For materials likely to contain high numbers of pathogens (excluding bacterial spores) pasteurisation at 90°C for 1 hr is often used. For materials with a low pathogen load, such as slurry or sewage sludge, 70°C for one hour is often applied. Thermal inactivation of pathogens also depends on the size of exposed particles if the material to be pasteurised contains solid material, such as animal tissues. Often a maximum particle size of 50 mm is recommended for heating at 90°C/ 1 hr, and a lower particle size of below 30 mm for heating at 70°C/1 hr. Batch treatment is preferred to safeguard the microbiological safety of the process and end-product.

9.2.2. Sterilisation

The standard procedure for 'sterilising' animal-derived materials that may contain a high number of pathogens (viruses, bacteria and parasites), or mesophilic/thermophilic sporeformers, is treatment at 133°C with a steam pressure of 3 bars for at least 20 min (Riedinger *et al*., 1975). The particle size should not exceed 50 millimetres and the material needs to be continuously stirred. This process is only safe in a batch procedure when the shortest exposure time is determined to be over 20 min in a tracer experiment in a continous process (Riedinger, 1980; Heubl, 1995). Recording thermographs are needed at the critical point of the heating process to monitor the heat treatment and ensure that the required temperature is achieved throughout. Other systems of heat treatment may be used provided that they offer equivalent guarantees with regard to microbiological safety. Microbial standards, which are, in principle, not relevant for supervision of the heat treatment itself (*Salmonella* and *Enterobacteriaceae),* are used as indicators of re-contamination and the general hygienic quality of the product. However spores of *Bacillus stearothermophilus* could also be used as a suitable bioindicator.

Processing of fish material alone in the process described (133°C, 3 bars for 20 min) will lead to technical problems and a product which cannot be used as feed or fertiliser due to glue formation and hydrolysis of proteins. From the technological point of view practical experiences have shown that up to 5% of fish material may be added to material originating from warm-blooded animals in order to avoid technological difficulties. However, this will result in a product with a characteristic fish smell.

9.2.3. Incineration and burning

In the framework of this report incineration and burning are defined as considered in the scientific opinions of the SSC (1999a, 2003). More general information may be accessed in reference texts (see for example Porteus, 1992).

Incineration is a carefully controlled burning process (normally using forced air to ensure good oxidation) carried out in an authorised and tested device. There are, however, several classes of incinerator, depending upon parameters such as the temperature conditions,

security of handling, residence time, risk materials being processed, emission clean-up, etc. and some also cater for the recovery of heat. It involves a thermal destruction process of organic material in specially designed combustion chambers with filtering systems to reduce emissions such as chlorinated dioxins. The destruction process is continuously maintained in an incineration chamber at temperatures of between 750 and 1200°C. However, incinerators designed for the disposal of animal carcasses usually operate at the lower temperatures of 750-850°C leaving a residue of ash material. Treatment at 850°C for 2 seconds is considered sufficient to inactivate TSEs (SSC, 1999a).

Burning is a simple method for the thermal destruction of organic material. Burning may be as effective as incineration for destroying many hazardous materials but typically it is less well controlled than incineration with respect to a number of important parameters for assuring complete oxidation, (e.g. temperature, retention time, air supply or emissions). Processing conditions may show high variability and the degree of destruction and the temperature reached varies in relation to moisture content available oxygen and external conditions and is often below 800°C, but may also be above 1000°C. The fire is generated by the carcass itself and additional solid or liquid fuels are sometimes used in the outdoors or in simple burning devices. Power stations and cement production plants represent more sophisticated methods of burning, but the process may be less controlled than dedicated incinerators. Typically commercial incinerators and commercial burning plants will mix the animal-derived material with other raw materials. Indeed animal-derived material tends to have too high a carbon content to be used as the sole fuel and usually the water contents of unprocessed by-products from aquaculture are so high that their calorific value is zero, or negative (meaning that it costs energy to burn it). Both incineration and burning inevitably leave a residue which has to be disposed of (approximately 10% of the original volume) and if there is a residual risk the disposal of this ash residue should be by controlled landfill. If there is no residual risk, the residue could be used for example as a building material etc. Ashes are continuously removed from the bottom of the incinerator or burning device to a storage area, which is separate from areas where raw material destined for incineration is handled. The ashes can be used as stabilising material at road constructions etc.

9.3. Hygienic safety of heat treatment processes

While relevant experimental data are lacking concerning the impact of these processes on fish pathogens, all relevant fish pathogens including *Clostridium botulinum* will be destroyed by sterilisation, burning and incineration, since the most resistant *Clostridium sporogenes* spores have a D_{121} -value of less than one min (Alcock and Brown, 1985). Incineration must be regarded as a means of destroying aquaculture by-products, with well-established separation required between incoming material and the residual ashes.

Data from systematic investigations of pasteurisation relevant to fish pathogens are rare. Compilation of data from the Australian Aquatic Animal Diseases Veterinary Emergency Plan (2002) is given in Table 27 (Thoen and Schliesser, 1984; Jacobsen *et al*., 1989; Humphrey *et al*., 1991; Fløgstad and Torgersen, 1992; Whipple and Rohovec, 1994; Arimoto *et al*., 1996; Falk *et al*., 1997; Hine and MacDiarmid, 1997; Fraser, 1999; Schumann *et al.*, 1997; Torgersen, 1997; Dombroski *et al*., 1999).

Table 27. Selection of published values of temperature and treatment times required for the inactivation of pathogens of aquatic animals*

PATHOGEN	TEMPERATURE (°C)	TIME OF TREATMENT (min)
Aeromonas salmonicida	55	30
Aeromonas hydrophila	60	D_{60} = 0.04*
Renibacterium salmoninarum	35	300
Renibacterium salmoninarum	65	15
Vibrio ssp.	50	30
Vibrio vulnificus	48	D_{48} = 0.41*
Yersinia ruckeri	60	1
Aphanomyces astaci	30	1800
Ichthyophonus hoferi	40	3
Mycobacterium ssp.	65	10
Myxobulus cerebralis	80	10
Perkinsus marinus	40	60
Baculovirus penai	90	10
Baculoviral midgut gland necrosis virus	30	180
Channel catfish virus	60	60
Epizootic haematopoietic necrosis virus	60	15
Erythrocytic necrosis virus	60	15
Infectious haematopoietic necrosis virus	45	10
Infectious pancreatic necrosis virus	80	15
Infectious salmon anaemia virus	56	30
Pike fry rhabdovirus	45	15
Rhabdovirus of penaeid shrimp	37	720
Salmon pancreas disease virus	50	30
Spring viraemia of carp virus	56	30
Striped jack nervous necrosis virus	60	30
Viral erythrocytic necrosis virus	60	15
Viral haemorrhagic septicaemia virus	60	60
Yellowhead virus	80	10

* in some cases a D-value is quoted

9.4. Landfill

Burial (SSC, 1999a) refers to the general practice of burying animals on farms or other premises (possibly combined by covering the carcase with quicklime). Burial may be a controlled/regulated process, with the site having previously been authorised on the basis of a risk assessment concerning animal and public health and environmental protection. Whereas landfills may be very large, burial sites tend to be quite small scale and rarely is there any formal containment barrier. Moreover burial is generally relatively close to the surface. There is no particular reason to assume that the microbial degradation in a burial site differs from that in a landfill unless the material is very close to the surface.

Landfill involves the disposal of waste by burying it under layers of earth following certain principles (Porteus, 1992) and this method has been used for the disposal of farmed fish mortalities. There is a trend towards moving away from this waste disposal option and the EU Landfill Directive (CEC, 1999) requires a reduction by 65% of the quantity of organic matter going to landfill and contains tight controls on general waste policy and limits the feasability of this disposal option.

Controlled landfill on the other hand is performed on previously authorised sites, selected following an assessment of the characteristics of the site and a risk analysis with respect to human and animal health and the environment. The nature of the landfill is consequently

dictated by the type of wastes it receives (e.g. municipal, industrial, inert, hazardous, non-hazardous, putrescible). A contained site is one that prevents leachate from escaping from the site and the more modern sites often use plastic liners for containment. A contained site may or may not also have gas collection facilities and leachate treatment on site can vary from spraying the leachate in the air (resulting in its oxidation) to a full secondary and tertiary treatment in a waste-water treatment plant. Commonly, materials will be buried many metres under the eventual surface. Some estimate of microbial action can be made from the rate of gas production- microbial action commences rapidly but methane generation will not occur for some time.

9.5. Model organisms

Generally model organisms can be used for two different purposes:

- Validation of a treatment process to determine if it is capable of inactivating pathogens that could otherwise be transmitted via use of the resulting end-product,

- Monitoring of the end-product to determine if it contains relevant pathogens that may have survived the applied treatment, or contaminants that may be harmful if the product is handled and used as intended.

In the context of this report, two different fields of application have been considered- the utilisation of fish by-products in fish feed or recycling the material for non-feed purposes such as fertiliser.

The validation of processes used for the treatment of fish by-products and microbiological testing of the end-products should encompass fish pathogens (which may be specifically used for fish by-products and of epidemiological significance), and spoilage organisms if the raw material is heavily spoiled (such spoilage organisms may also be applicable to other forms of biological waste). A model organism used for the validation of treatment processes needs to cover the resistance of all the relevant pathogens and absence of the relevant spoilage organisms should assure the product's microbiological quality and safety. Moreover the organism should be safe and easy to handle and capable of being reliably recovered from the relevant matrix.

9.5.1. Model organisms for the validation of the production of feed from fish by-products

Under current legislation (CEC, 2002a), only fish by-products from fish deemed fit for human consumption and excluding mortalities could be used for the production of feed. For such processes endospore-forming bacteria will not be considered as model organisms and the following fish pathogens are suggested as model organisms for the validation of the treatment process:

- *Mycobacterium chelonae*

- IPN - virus

Although fish pathogenic organisms are the first choice as model organisms in this context, it must be borne in mind that there may be technical problems regarding their recovery from the material and reliably quantifying the isolates. Therefore non-fish pathogens should also be considered and *Enterococcus faecalis* and Bovine Parvovirus (BPV) are proposed as

alternatives, since approved re-isolation techniques are available for each of these pathogens (Hoferer, 2002).

For hygienic supervision of end-products, general hygienic parameters may be applied, such as absence of *Salmonella* in 50 g, or setting total bacterial count levels for Clostridia, faecal coliforms or *Enterobacteriaceae*.

9.5.2. Model organisms for the assessment of the hygienic safety of fertilisers

A GMP or HACCP-based approach can be used to achieve hygienic safety, especially when processing fish by-products in composting or biogas plants.

With respect to validation of processes in which fish by-products are used to produce fertilisers, (chapters 9.1-9.3.1), the use of fish related model organisms needs to be considered (see Table 25). *Salmonella senftenberg* (W775, H2S negative), *E. coli* and *Enterococcus faecalis* are considered in this context. A model organism used for the validation of treatment processes should cover all the relevant pathogens in terms of resistance. If comparative experiments show that those organisms will not address fish pathogenic organisms such as IPN virus, another easily-handled representative organism could be selected (e.g. BPV). From the existing data it seems that BPV could fulfil all those requirements while at least a three $\log_{10}$ reduction in the exposed contaminated material would need to be achieved by the treatment process. Infectious Bursal Disease (IBD) virus of birds is another birnavirus for which some inactivation data are available (Alexander and Chettle, 1998).

It must be noted that only limited data exist concerning the inactivation of fish pathogenic bacteria, fungi, parasites and viruses in the environment, in biotechnological treatments and with regard to their heat resistance parameters. Until such data are available, well-established parameters such as those for BPV should be used in process validation. The parameter *Enterobacteriaceae* is not applicable to products resulting from mesophilic or thermophilic biotechnological processes composting because even a product produced in a validated process may contain betwen 10^1 to 10^7 *Enterobacteriaceae* per g (Breitenfeld, 2000). The parameter of absence of *Salmonella* is, in principle, applicable, although a more representative sample size of 50g would be preferable (a mixed sample taken from different locations within the bulk material).

10. CONCLUSIONS

1. Production of fish and shellfish by aquaculture comprises a significant and increasing proportion of world and EU fish production. Fish differ from other farmed animals in that most species of fish presently farmed in the EU are carnivorous and naturally eat other fish. Due to the nutritional requirements of fish for specific oils and high protein levels, fish oil and fishmeal are major constituents of the diets of farmed finfish.

2. Fishmeal and fish oil are generally made from fish deliberately caught for that purpose and from by-products of fish caught for human consumption. Novel sources of feed for farmed fish (such as bioproteins) that allow the reduction of the fishmeal content of fish feed are currently being investigated.

3. Outbreaks of disease have not been reported in farmed fish linked to the transmission of fish pathogens via thermally treated/processed fishmeal and fish feed. However, there are risks of transmission of infectious agents to farmed fish (and possibly subsequently to humans in the case of zoonotic organisms) if farmed fish are fed untreated fish by-products. The highest risk of disease transmission results from feeding untreated by-products from clinically diseased farmed finfish or mortalities from fish farms ('morts') to farmed finfish, and feeding untreated by-products of diseased farmed invertebrates to farmed invertebrates

4. Codes of good fish farming practice exist which recommend that by-products from farmed fish are not fed to farmed fish.

5. Some bacterial and parasitic agents affecting fish are potentially zoonotic, although methods commonly used to treat fish by-products can reduce or eliminate zoonotic risks.

6. The majority of those fish pathogens for which scientific data on inactivation are available appear to be relatively sensitive to heat. Infectious Pancreatic Necrosis (IPN) virus is more thermoresistant than other fish pathogenic viruses, although this virus is not a zoonosis.

7. It is likely that the time/temperature treatments normally applied in the production of fishmeal would result in sufficient inactivation to reduce to negligible the risk of disease from the majority of conventional fish pathogens, based on available data. However, there are major knowledge gaps in the scientific literature concerning inactivation parameters for many fish pathogens. In addition, current treatment methods used in the production of fishmeal/ fish feed are generally not yet validated with regard to the elimination of viruses, bacteria, fungi and parasites that may originate from fish by-products used to produce fishmeal. A number of indicator viruses and bacteria could be used to validate processes used in fishmeal/ fish feed production.

8. Based on available data it is likely that the process of ensiling fish by-products at an approximate pH of 3.5 would result in sufficient inactivation to reduce to negligible the risk of transmission of disease from the majority of conventional fish pathogens, with the exception of some relatively pH stable fish pathogens such as Infectious Pancreatic Necrosis (IPN) virus.

9. A number of biotechnological and heat treatments are suitable for the treatment of fish by-products used for non-feed purposes and a variety of treatments are used to handle mortalities from fish farms ('morts'). However, these treatments require validation of their capabilities for the

inactivation of pathogens and verification of the microbiological safety of the end-products produced.

11. RECOMMENDATIONS

1. In order to limit any risk of the transmission of fish diseases to fish or humans via the feeding of fish by-products processed into fishmeal/fishfeed, and in light of the issue of intra-species recycling, it is recommended that:

- The by-products of farmed finfish should not be fed to farmed finfish,
- The by-products of farmed invertebrates should not be fed to farmed invertebrates.

2. The feeding to fish of 'wet' diets containing fresh or frozen but otherwise unprocessed fish by-products is not recommended.

3. Processes used for the production of feed or fertilisers from by-products of wild or farmed fish should be validated with regard to their ability to inactivate representative model organisms.

4. Current procedures used to process mortalities from fish farms ('morts') should be validated in terms of their ability to inactivate fish pathogens and also in terms of the microbiological safety of the end-product.

12. FUTURE RESEARCH

Research projects are required to establish the persistence capacities of fish pathogens in differing environments and matrices such as fresh- or seawater, naturally degrading material etc. Additional data on the survival capacities of agents when treated with chemical processes (alkaline or acid environments) is needed.

The inactivation capacities of processes such as ensiling, biogas production, composting etc. need to be established using reference organisms that are appropriate for fish by-products. It is important that D-values be established for representatives of major families of fish pathogens when subjected to such treatment processes. Examples of such organisms are:

- Viruses: *Viral haemorrhagic septicaemia virus, Infectious pancreatic necrosis virus.*

- Bacteria: *Renibacterium salmoninarum, Mycobacterium chelonae, Yersinia ruckeri.*

Comparison of D-values of representative fish pathogens with those of similar pathogens of mammals or birds is needed, to facilitate the carrying out of comparative laboratory work using mammalian or avian pathogens. This should consider inactivation parameters in biotechnological processes, chemical and physical treatments using comparative experiments.

Epidemiological and/or experimental studies should be carried out to identify any possible role that the feeding of fishmeal plays in disease transmission and the role, if any, of fish intra-species re-cycling in disease transmission to fish or mammals, including humans. Until such studies have been performed, the recommendation that the by-products of farmed invertebrates or finfish should not be fed to farmed invertebrates or finfish respectively should apply.

Simple methods for the on-farm disposal of by-products and farmed fish mortalities (‘morts’) need to be developed and validated with regard to their ability to inactivate microorganisms. Novel sources of feed for farmed fish, which provide appropriate oil and protein nutritional requirements for farmed fish should be investigated and applied. This would reduce any possible risk of transmission of fish diseases to fish via the feeding of fish by-products.

13. EXECUTIVE SUMMARY

The Scientific Committee on Animal Health and Animal Welfare was requested to prepare a report on the use of fish by–products in aquaculture, considering the types of fish by-products fed to fish, any disease risks (viral, bacterial or parasitic) associated with this practice, and treatment methods to reduce or eliminate any risks identified.

The report provides background data on world and EU fish production and how fish by-products are utilised, including their use in the production of fishmeal and fish feed. Environmental issues were not considered since these were not within the terms of reference of the report. In the EU most of the species farmed in aquaculture are carnivorous, although herbivorous fish are important in terms of world aquaculture and in the future may become more important within the EU. Various viral, bacterial, parasitic and fungal agents that are pathogenic to fish are discussed, as well as data concerning their inactivation either by changes in temperature or pH. The nutritional requirements of fish are briefly discussed as these will impact on the constituents used in fish feed. Various alternative methods of treating fish by-products to reduce or eliminate any risk of pathogen transmission are also described. Such treatment processes include ensiling, biogas production, composting, thermal treatments and landfill. The use of model organisms is also considered to either validate the pathogen inactivation capacity of a processing treatment or monitor the microbiological safefy of an end-product.

The report concludes that there are risks of transmission of infectious agents to farmed fish (and possibly subsequently to humans in the case of zoonotic organisms) if farmed fish are fed untreated fish by-products. The highest risk of disease transmission results from feeding untreated by-products from clinically diseased farmed finfish or mortalities from fish farms (‘morts’) to farmed finfish, and feeding untreated by-products of diseased farmed invertebrates to farmed invertebrates. It is concluded that it is likely that time/temperature treatments normally applied in the production of fishmeal/ fish feed would reduce to negligible the risk of disease from the majority of conventional fish pathogens, based on available data. However, scientific data concerning inactivation are lacking for many pathogens and current treatment methods used in the production of fishmeal/ fish feed are not yet validated with regard to their pathogen inactivation capacities. When considering inactivation processes, special attention should be given to Infectious Pancreatic Necrosis (IPN) virus since it is more pH stable and more thermo-resistant than other fish pathogenic agents.

In order to limit any risk of the transmission of fish diseases to fish or humans via the feeding of fish by-products processed into fishmeal/fishfeed, and in light of the issue of intra-species recycling, it is recommended that the by-products of farmed finfish should not be fed to farmed finfish, and the by-products of farmed invertebrates should not be fed to farmed invertebrates. The feeding of ‘wet’ diets (containing fish by-products that are fresh or frozen but otherwise unprocessed) is not recommended. It is recommended that processes used for the production of feed or fertilisers from by-products of wild or farmed fish should be validated with regard to their ability to inactivate representative model organisms. Current procedures used to process mortalities from fish farms (‘morts’) should also be validated in terms of their ability to inactivate fish pathogens and in terms of the microbiological safety of the end-product. A number of areas requiring additional research are also highlighted, including further investigation of novel feed sources that satisfy appropriate oil and protein nutritional requirements of fish, and would avoid any possible disease transmission risks linked to the feeding of fish by-products.

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15. ANNEX

Table 28. Aquaculture Species In EU Member States (Eurostat's New Cronos Database).

SPECIES	1999		SPECIES	1999	
	Value[4]	Quantity[5]		Value[1]	Quantity[2]
Blue mussel – *Mytilus edulis*	239 701	479 168	Rainbow trout - *Salmo gairdneri*	622 251	227 960
Mediterranean mussel – *Mytilus galloprovinciali*	90 424	157 812	Sea trout - *Salmo trutta*	9 367	3 175
Pacific cupped oyster – *Crassostrea gigas*	222 496	142 730	Brook trout - *Salvelinus fontinalis*	1 828	509
Cupped oysters nei – *Crassostrea spp*	5 924	4 079	Gilthead seabream - *Sparus aurata*	230 657	47 116
European flat oyster – *Ostrea edulis*	25 315	6 443	White seabream - *Diplodus sargus*	570	112
Flat and cupped oysters – *Ostreidae*	570	754	Seabass - *Dicentrarchus labrax*	213 219	36 230
Grooved carpet shell – *Tapes decussatus*	38 093	2 949	Atlantic salmon - *Salmo salar*	410 230	146 258
Carpet shell – *Tapes pullastra*	17 490	2 336	Salmonoids nei – *Salmonoidei*	136	48
Common cockle – *Cardium edule*	8 313	5 212	Chars nei - *Salvelinus spp*	1 826	386
Japanese(=Manila)clam – *Venerupis japonica*	14 654	2 776	Arctic char - *Salvelinus alpinus*	383	102
Venus clams – *Veneridae*	57	16	Common carp - *Cyprinus carpio*	31 834	17 649
Donax clams – *Donax spp*	424	129	Carp - Cyprinids nei – *Cyprinidae*	73	26

[4] Total value expressed in 1000 euro,
[5] Total quantity expressed in tonnes live weight (: indicates that data are not available)

Clams nei – *Bivalvia*	11	4	Grass carp – *Ctenopharyngodon idella*	78	31
Flathead grey mullet - *Mugil cephalus*	10 345	3 430	Silver carp – *Hypophthalmichthys molit*	47	19
Mullets nei – *Mugilidae*	282	90	Bighead carp – *Hypophthalmichthys nobil*	13	3
Periwinkles nei – *Littorina spp*	1 952	800	Rudd - *Scardinius erythrophthalmus*	704	300
Common scallop – *Pecten maximus*	2 808	716	Bleak - *Alburnus alburnus*	12	5
Crayfishes – *Astacus spp+ Cambarus spp*	532	22	Turbot - *Psetta maxima*	34 416	4 093
Danube crayfish – *Astacus leptodactylus*	281	15	Striped venus - *Venus gallina*	369	123
Signal crayfish – *Pacifastacus leniusculus*	957	51	Common sole - *Solea vulgaris*	169	19
Red swamp crawfish – *Procambarus clarkii*	66	5	Tunas nei – *Thunnini*	:	3 347
Palaemonid shrimps – *Palaemonidae*	276	98	North African catfish - *Clarias lazera*	3 387	1 504
Kuruma prawn – *Penaeus japonicus*	1 033	82	Wels (=Som)catfish - *Silurus glanis*	1 923	343
Octopuses – *Octopus spp*	120	32	Roach - *Rutilus rutilus*	5 864	2 500
European eel – *Anguilla anguilla*	77 349	10 275	Tench - *Tinca tinca*	3 878	1 376
Sturgeons nei – *Acipenseridae*	3 124	552	Northern pike - *Esox lucius*	1 976	316
Siberian sturgeon – *Acipenser baeri*	4 105	350	Pike-perch - *Stizostedion lucioperca*	518	204
Marine fishes nei – *Osteichthyes*	11 549	2 042	European perch - *Perca fluviatilis*	246	101
Freshwater fishes nei – *Osteichthyes*	3 621	1 667	Pollan(=Powan) - *Coregonus lavaretus*	208	66
Freshwater gobies – *Gobiidae*	75	32	Tilapias nei - *Oreochromis+Saratherodon*	911	271
Meagre – *Argyrosomus regius*	281	30	Goldfish - *Carassius auratus*	16	7
Largemouth black bass – *Micropterus salmoides*	23	9	***TOTALS***	***2 359 360***	***1 318 905***

Table 29. Production, Imports and Exports of meals and similar feedingstuffs of aquatic animal origin (FAO, 2002) unit thousand tonnes

Country	Production 1998	Production 1999	Production 2000	Imports 1998	Imports 1999	Imports 2000	Exports 1998	Exports 1999	Exports 2000
Peru	832,093	1,769,532	2,241,529	0	0	54	671,752	1,471,300	2,352,208
China	692,555	707,433	806,423	420,035	634,298	1,189,252	2,288	1,898	2,712
Chile	646,718	1,004,868	880,744	755	226	40	546,195	592,657	537,581
Japan	392,884	386,273	362,600	329,627	346,329	338,140	9,647	10,848	14,881
Denmark	404,952	375,906	388,685	94,878	139,768	131,057	302,795	319,756	301,966
Iceland	226,403	242,068	253,328	140	79	185	232,939	241,457	253,219
Norway	301,596	242,808	265,000	99,753	144,688	185,032	153,982	153,157	87,530
UK	51,858	53,432	54,000	238,302	220,896	240,840	19,474	17,920	15,984

Table 30. Production, Imports and Exports of fats and oils of fish and aquatic mammals- other than liver oils (FAO, 2002) unit thousand tonnes

Country	Production 1998	Production 1999	Production 2000	Imports 1998	Imports 1999	Imports 2000	Exports 1998	Exports 1999	Exports 2000
Peru	122,956	514,818	587,312	0	1	0	34,926	258,653	456,448
Chile	106,693	201,376	180,199	12,034	60,294	94,617	4,466	64,546	16,146
Japan	75,698	68,784	59,974	26,585	24,863	49,812	659	391	242
Denmark	135,940	129,195	139,968	23,576	21,264	28,369	75,589	94,206	113,512
Iceland	88,430	86,000	76,795	269	125	4,848	88,430	85,952	76,790
Norway	98,000	68,900	84,700	156,787	219,083	234,460	40,938	54,038	59,644
UK	9,619	9,600	9,700	45,965	43,841	41,417	3,047	2,043	1,530

Table 31. Fish By-Products production and marketing in EU Member States: Flours; meals and pellets of fish or of crustaceans; molluscs or other aquatic invertebrates; unfit for human consumption (Source Eurostat)

DATATYPE		1995	1996	1997	1998	1999
Sold production	Quantity[6]	667 748 737	769 283 219	:	742 870 851	534 910 449
	Value[7]	289 352 924	356 757 415	333 567 650	425 549 178	283 968 648
Imports Intra EU	Quantity[1]	342 166 000	301 814 700	331 275 800	306 622 100	291 752 700
	Value[2]	155 559 450	160 426 440	194 295 120	211 232 110	146 716 320
Imports Extra EU	Quantity[1]	866 752 700	820 941 200	872 712 800	702 918 300	727 377 900
	Value[2]	320 762 430	392 954 000	471 761 590	451 962 750	323 380 230
Exports Intra EU	Quantity[1]	356 602 700	365 653 500	379 854 500	342 878 700	323 277 600
	Value[2]	152 880 230	188 331 090	217 416 530	233 576 890	161 075 040
Exports Extra EU	Quantity[1]	381 813 100	310 632 700	275 050 300	278 646 000	288 070 800
	Value[2]	177 194 360	164 938 470	163 045 850	188 795 950	152 523 470
Domestic market	Quantity[1]	1 138 251 637	1 215 752 919	:	1 130 886 551	942 692 649
	Value[2]	435 600 214	556 868 295	619 161 897	666371 198	440 466 688

Table 32. Fish By-Products production and marketing in EU Member States: Inedible fish products (Source Eurostat)[8]

DATATYPE		1995	1996	1997	1998	1999
Imports Intra EU	Quantity[1]	309 308 400	276 848 100	334 786 800	351 697 600	343 498 900
	Value[2]	31 409 920	35 100 540	51 334 360	56 200 770	46 289 040
Imports Extra EU	Quantity[1]	103 532 700	120 189 300	167 346 300	195 495 800	229 361 000
	Value[2]	23 493 470	26 412 240	33 188 570	41 001 280	41 091 130
Exports Intra EU	Quantity[1]	156 369 800	87 815 100	98 453 000	99 360 100	68 590 400
	Value[2]	28 139 190	21 861 960	22 309 970	24 748 740	19 552 840
Exports Extra EU	Quantity[1]	42 477 700	78 683 200	102 685 100	85 103 400	27 993 000
	Value[2]	13 085 170	17 221 520	22 537 740	18 765 850	10 718 530

[6] Quantity expressed in kilograms
[7] Value expressed in ecus
[8] Including fish by-products, excluding whalebone and whalebone hair, coral and similar materials, shells and cuttle-bone, unworked or simply prepared/natural sponges.

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