Chemicals From Marine Fish Farms

Monitoring of chemicals from marine fish farm in Nordic environments - veterinary medicines, biocides and persistent organic contaminants

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Content

Introduction

Fish from Nordic fish farms have been criticized for containing “too high” concentrations of persistent organic pollutants (POPs) among others dioxin and PCB. These allegations have caused debate and may have a negative effect on the sale of products from Nordic fish farms in addition to giving a generally bad reputation that affects more or less all Nordic fish farms.

It is generally agreed that the relatively high concentrations of POPs detected in fish from Nordic fish farms are caused by the high concentrations of POPs in the fish feed produced and used in the Nordic countries.

Beside potential health effects caused by high POP contents in the fish fillet, the surrounding environment may be impacted as well, not only by excreted POPs but also by veterinary medicines used in the fish farms. In Nordic fish farms, veterinary medicines are mainly used therapeutically against different infectious diseases. The amount of veterinary medicines used varies from year to year and in some years, considerable amounts have been used. The major environmental concern in relation to the use of veterinary medicines is the potential occurrence of antibiotic resistance in the naturally occurring micro flora.

In relation to aquaculture, the major strategy in the Nordic countries and in the EU is thus to ensure an environmentally sound industry. It is therefore important to reduce potential environmental impacts of aquaculture and to keep the production as ecologically sustainable as possible. Fulfilling theses requirements will ensure a good reputation in relation to the environmental impact of the industry.

The overall objective of the present project is to assess the use, release and environmental impact of the veterinary medicines, biocides and persistence organic pollutants (POPs) that are dispersed in connection with fish farming in the Nordic countries.

The project included the following activities:

- Inventory of use of veterinary medicines and antifouling agents in Nordic fish farms
- Emission of POPs to the environment from Nordic marine fish farms
- Analyses of antibiotics and POPs in sediments and biota near a model fish farm
- Assessment of potential antibiotic resistance in sediment bacteria
- Recommendations for environmental monitoring programmes around fish farms

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Summary and main conclusions

Below, the main conclusions from the evaluations are summarised for each activity.

Inventory of use of veterinary medicines and antifouling agents in Nordic fish farms

For each of the Nordic countries assessed in this study, the consumption of antibiotics has been below 1,500 kg/year for the last 5 of the 6 years reviewed. The consumption in Faroe Islands decreased drastically from about 4,000 kg in 1999 to 1,100 kg in 2000, and declined steadily from 2001 to 0 kg in 2004. The consumption in Norway was steady at between 500 and 700 kg per year in 1999 to 2001 but increased to between 800 and 1,200 kg from 2002 to 2004. Also in Denmark, there was an increase by 60% from 2001 to 2002, but there was a 50% decrease in consumption from 1,400 kg in 2003 to 700 kg in 2004.

In Norway, the consumption of antibacterial agents has remained stable at 1.2-1.4 g/ton fish produced, with the increase in 2002 and 2004 mainly related to the increase in biomass of farmed cod. The vaccines developed against vibriosis in cod do not give sufficient protection, and antibiotics are used therapeutically at outbreaks.

In Faroe Islands, the consumption of antibiotics has decreased steadily from 93 g/tons produced fish to 5 g/tons in 2003. In 2004, there was no consumption of antibiotics. In Faroe Islands, the relative numbers of outbreaks of BKD has been much higher than in Norway and may explain the big difference in consumption of antibiotics from 1999 to 2002.

The ratio of the relative consumption of antibiotics to the amount of fish produced in Denmark ranges from 87 to 176 g/tons in the period assessed. The consumption of antibiotics was directly related to the water temperature during the summer in Denmark. Whereas a warm summer gives high consumption due to outbreaks for furunculosis, a cold and rainy summer results in low consumption. Despite vaccination, outbreaks appear when temperatures increase above 20°C. In these cases, even vaccinated fish get furunculosis. In addition to furunculosis that is considered the most common disease, yersinosis and vibriosis also appear in the different sites.

In Iceland, cod and salmon are reared in net cages. Consumption of antibiotics is generally low and in 2004, there were only few cases of atypical furunculosis (*Aeromonas salmonicida* ssp. *achromogenes*) in cod

farms, which were therapeutically treated with antibiotics. There have also been outbreaks of vibriosis (*Listonella* (*Vibrio*) *anguillarum*) in two cod farms in the sea.

For the last five years of the period assessed, the bacterial diseases, which are therapeutically treated with antibiotics, have been furunculosis, winter ulcers and bacterial kidney disease.

Ectoparasitic agents

The agents administered via the feed in 1999-2002, teflubenzurone (Ektobann) and diflubenzurone (Lepsidon) were replaced by the more effective emamectine (slice premix), and this is one of the main reason for the drastic decline in consumption in the beginning of the period from 1999-2004. The agents used today are emamectine (slice premix), which is administered via the feed and cis-cypermetrin (Betamax) and deltametrin (Alpha-max), which are used for immersion.

The administration of ectoparasitic agents is mainly against salmon lice. The relative consumption of ectoparasitic agents was higher in Faroe Islands than that in Norway in 1999 but subsequently decreased to the same level. The degree of infestations is mainly related to the environmental conditions of the site, i.e. degree of exposure, distance from land, management and monitoring routines, use of wrasses etc.

Emission of POPs to the environment from Nordic marine fish farms

The occurrence of POPs incl. dioxins in fish is not a particular farmed salmon or trout problem - it is a highly relevant issue in general, e.g. in the Baltic where the wild fish (salmon and herring) do not meet the EU standards for being marketed. Therefore, in relation to farmed fish, it should be aimed to keep the POP content as low as possible and to use available knowledge and technology for fulfilling this requirement.

POPs are not a necessary nor a constant part of the fish feed. As described, the POP content depends largely on the origin of the feed oil and protein base, whether produced from fish and if so, what species and from what area the fish are taken. This is clearly shown by the study performed by Hites et al. 2004, who showed that highest POP concentrations were obtained in fish farmed in the North Atlantic region.

In the described model study, it has been shown that the retention of dioxins and dioxin-like PCBs in whole fish ranged from 63% to 93%. These values were in the same range as those found in previous projects (EU CRAFT project, 2002) where retention of dioxins in whole fish after 30 weeks of exposure ranged from 54-59% and for dioxin-like PCB, retention ranged from 83-87%. Altogether, the results clearly indicated that

the dioxin and the content of other POPs in the fish reflect the level present in the feed.

The release of dioxin from Nordic fish farms is significant and the bioavailability must be regarded as high. By way of comparison, the release corresponds to up to 10% of the dioxin bound in the fish biomass in the Baltic.

At the model fish farm Bakkafrost in Gøtuvík, solely fish feed from Faroe Islands was used (P/F Havsbrún, Fuglafjørð). Based on the input data from thorough investigation performed by Magnussen and Vang (2006) (e.g. concentration in fish feed; accumulation rate; concentration in fillet etc), it is relatively easy to make a rough estimate of uptake in the fillet and release to the environment for other feed types as well, provided that data on the POP content in feed are available. This could be a valuable tool for pre-estimation of the potential POP content in farmed fish.

In relation to protection of both health and the environment, it is recommended that initiatives are taken to control that fish feed with as low content of dioxins and other POPs as possible is used.

Analyses of antibiotics and POPs in sediments and biota near a model fish farm

One of the purposes of the present project was to study the potential environmental impact of a fish farm with salmon production. The main emphasis was on pollutants imported along with the fish feed or antifouling agents, and on oil-derived pollutants including combustion products and other pollutants stemming from the normal running of the facilities. The influence of these pollutants will mainly be elucidated through quantification of the substances in sediments in and around the fish farm.

The focus of this study on pollutants in sediments near fish farms is the organochlor pesticides and other persistent and lipid-soluble man-made pollutants like PCB, dioxin, brominated flame retardants and even PAH and the antifouling compound TBT. In addition, the metals commonly associated with fish farms were included in the analytical scheme along with markers of increased input of organic material.

In the present study, sediment samples were taken at two fish farms located in a bay on the eastern coast of Faroe Islands. It has thus been established that the sea bottom beneath and around fish farms are marked with elevated concentrations of nutrients, metals, persistent organic pollutants as PCB and pesticides, and antibiotics. Elevated pollutant concentrations in this setting do not necessarily mean that the sites were severely or markedly polluted but it means that the chemical concentration was elevated above what was detected at another station. Whether biologically relevant to the point where negative impact may occur is another matter that must be seen in relation to environmental criteria (EQC).

What can be noted from the comparison of the measured concentrations to the EQC is that only for TBT, the concentrations are raised to a level, at which biological negative impact is likely. The elevated TBT concentrations are found at the reference station and at two fish farm stations. Given that the reference station is situated in the middle of the ship lane in and out the bay, it is easy to understand why the reference station should be the most impacted by this pollutant that has been used in antifouling treatment of ships for many years. It is also apparent from the comparisons that all copper concentrations are within the lower and upper boundary of a provisional EQC(OSPAR), even at the reference station. Taking a closer look, however, reveals that some sites have higher copper concentrations than others do, and they occur at stations that are simultaneously impacted by other pollutants. It appears that copper is a bit special in that it probably occurs in relatively high natural background concentrations in Faroe Islands, and at the same time is a pollutant that may be added by anthropogenic activity - in this instance via the fish farming.

An example of a group of pollutants that is not naturally occurring and does not have other sources in the area is the antibiotics. Analyses have revealed that the antibacterial agents remain in the environment for years after administering them to fish via feed. The antibiotics thus appear to be persistent in the sediments where they also may retain their antibacterial activity. However, the concentrations measured were low in comparison to effect concentrations and are not assumed to pose an imminent environmental threat.

It is also important to note that the study indicated that environmental impact may extend beyond the present day reference stations applied in the environmental monitoring around the farm, which is required by the regulations. These findings of a wider impact area indicate that it is necessary to have reference values or classification criteria from an area that may be located outside the fjord or bay in question when evaluating results from the fish farm environmental monitoring.

Assessment of potential antibiotic resistance in sediment bacteria

Fish Feed

The number of culturable bacteria in the examined fish feed F6 was very low and is unlikely to contribute significantly to the resistance found below the fish farms in Faroe Islands.

Oxytetracycline

It can be concluded that the measurements of oxytetracycline resistance showed a very high percentage of resistant bacteria both below the fish farms and at the "reference" stations, up to 900 m away from the fish farms, indicating that oxytetracycline resistant bacteria are abundant in fjords with fish farms. However, there is some doubt whether the activity of oxytetracycline in the test agar was sufficiently high. Further studies to determine background levels are suggested. In case of high abundance of resistant bacteria, a more thorough investigation including molecular and epidemiological studies is suggested.

Bearing the risk of transfer of resistance genes between aquaculture and human pathogens in mind, the use of oxytetracycline should be reconsidered for use in aquaculture.

Oxolinic acid

The occurrence of oxolinic acid resistance seems to be low and is not considered to be a large problem in Faroe Islands.

On the other hand, the use of oxolinic acid in Norway and Denmark may be problematic since cross-resistance to other quinolones and fluoroquinolones may evolve. Oxolinic acid is a first generation quinolone and better options may exist.

It is suggested to examine the occurrence of cross-resistance to other quinolones and fluoroquinolones in locations where oxolinic acid is in use. In addition, a review of the effectiveness of oxolinic acid and potential alternative antibiotics should be considered.

Sulfadiazine/trimethroprim

Taking the risk of transfer of sulfonamide and trimethoprim resistance between the aqueous and human environments into consideration, its use should be reconsidered.

Recommendations for environmental monitoring programmes around fish farms

It may be concluded from the present assessment that in addition to pollutants already included in the monitoring schemes presently applied in Faroe Islands and Norway, pollutants like PCB and pesticides of the POP type as well as PAHs and antibiotics are found in elevated concentrations in sediments around a fish farm. Although the concentrations of these pollutants may be correlated to parameters already included in the monitoring schemes, this does not mean that it will be possible to extrapolate from these nutrients and metals parameters to the ambient POPs concen-

tration. The reason for this is that POPs are neither a necessary nor a constant part of the fish feed but the POP content depends to a large extent on the origin of the feed oil and protein base, whether produced from fish and if so, what species and from what area were the fish taken. A study of dioxin and PCB in pelagic fish, which are typically used in the production of fish feed from the northeast Atlantic, revealed that in some species the dioxin content exceeded the EU limits for feed (Mundell et al,. 2003) and would therefore not be applicable in feed production without some measures to cleanse or dilute the dioxin content in the fish oil base.

It is obvious that the current monitoring parameters required by regulation in Faroe Islands and according to the Norwegian Standard for Environmental monitoring of marine fish farms (NS 9410) by no means cover the pollutants that are added to the environment in connection with fishfarming activities. However, it is realized that adding extra parameters to the analyses-to-do list, also increases the costs. Therefore, the question could perhaps be reduced to one of; what do we need to know?

In the EU directive 96/23/EC on residue monitoring in food of animal origin in the member states, the list of “need to know” chemicals includes stilbenes and steroids, chloramphenicol and other antibacterial substances like oxolinic acid, oxytetracycline and flumequine, anthelmintics like cypermetrine, deltametrine and emamectine, organochlorine compounds like PCB, DDT, HCB and dioxin, organophosphorus compounds like dichlorvos and azametiphos, metals as cadmium, lead and mercury, mycotoxines as aflatoxines, dyes and other compounds potentially used in the farming activities. In the EU Water framework directive (2000/60/EC), there is a list of priority substances (Annex 10) that contains 33 pollutants that need to be monitored. For these 33 compounds plus an additional 8, the EU Commission has adopted a proposition that there be assigned environmental quality standards, EQS, for these pollutants in surface water (COM (2006) 397 final), where the term surface water includes coastal water where marine aquaculture takes place. In this adopted proposition, which will become a new directive, the EQS are defined in water and are as such probably quite a challenge for the chemical analysts. However, the purpose is unmistakably to protect the natural environment and it does not seem unlikely that analytical methods, which could even rely on analyses on sediments, will be developed to meet the demands of the new directive. On the EU water framework directive list of prioritized compounds, we find members of the PAHs, the PBDE, cadmium, lead, mercury and nickel, HCB, γ-HCH (lindane) and TBT, which have been identified as pollutants that likely accompany a fish farm either by direct measurements or by knowledge of source and route of exposure. If the additional eight substances that are included in the directive for EQS are included, it means that also analyses of DDT and dieldrin would be of common interest both to a fish farm monitoring programme and to the implementation of the EU regulation. Thus, there

is ample room for rationalizing by combining efforts and means so that the monitoring of fish farms both provides a more complete analysis of the pollutants, which is likely to accompany the farming activity and at the same time, provide some of the data needed to assess environmental status as demanded by the EU Water framework directive and daughter directives.

However, the antibiotics and other therapeutic agents will not be included by such a lean onto the EU Water framework directive strategy but require specific and targeted analyses. Whether a regular monitoring is required, the following screening studies need to be established, in which the extent of the antibiotics concentration in the surrounding environment is assessed, and following risk analyses, in which the possible impact of the measured concentrations is assessed. In the present study, some steps were taken towards such screening and risk assessments for three selected antibiotics but the extent and scope of the study did not allow a conclusion to be drawn on whether antibiotics used in fish farms in fjords constitute a significant threat to the ecosystem. At present, the conclusion is thus that antibiotics and even other therapeutic agents depending on use should be monitored until a sufficient data basis is available for the final decision to be made.

1. Inventory of veterinary medicines and antifouling agents in Nordic fish farms

1.1 Introduction

The Nordic countries produced about 750,000 tons of salmon and trout in 2004. The diseases causing the greatest losses vary between the countries, but in Norway and Faroe Islands, salmon lice and virus diseases were the main causes. In Denmark, it is furunculosis caused by the bacteria *Aeromonas salmonicida*, whereas the problems related to diseases in the salmonid marine cage farming is relatively insignificant in Iceland.

The fishfarming industry is using a number of compounds, which are retrieved through different retailers and not registered. Data on consumption of the most important agents with respect to the environment such as antibiotics, endo- and ectoparasitic agents are obtainable through governmental veterinary offices and/or fishfarming organisations. Data on consumption of antifouling agents such as copper is available through governmental pollutant authorities.

Antibiotics are mainly used therapeutically against classical furunculosis (*Aeromonas salmonicida*), winter ulcer (e.g. *Moritella viscosa*) and bacterial kidney disease (BKD) (*Renibacterium, salmoninarum*) but there is also an increased consumption of antibiotics against Vibriosis (*Listonella anguillarum*) in cod farming. The veterinary medicines are implemented in the feed by the feed producers.

Salmonids are often infected with endoparasites such as cestodes, and the most used veterinary medicines for treatment against the cestode *Eubothrium sp*. infestations are praziquantel and fenbendazol. These medicines are also administered via the feed.

Salmon lice (*Lepeophtheirus salmonis*) infestations are among the factors causing the heaviest losses in salmon fish farming, and both immersion and feed-associated veterinary medicines are used. The immersion agents are the organophosphate azametifos (Salmosan) and the pyrethroids cis-cypermetrin (Betamax) and deltametrin (Alpha-max). Among the veterinary medicines administered via the feed, the most common are teflubenzurone (Ektobann), diflubenzurone (Lepsidon) and emamectine (slice premix). In the immersion treatment, the net cage volume is decreased and is enclosed by a plastic tarpaulin in order to maintain the concentration of the agent. Oxygen is supplied during the treatment. After the treatment, the tarpaulin is removed and the net cage volume increased.

More information about the veterinary medicines is available at e.g. http://www.fiskehelse.no/.

1.2 History of disease outbreaks

The known bacterial diseases, vibriosis, coldwater vibriosis (*Vibrio salmonicida*), furunculosis, yersinosis (*Yersinia ruckeri*), bacterial kidney disease (BKD), winter ulcers and parasitic problems such as lice infestations appeared once the salmon farming commenced during the mid 70s. This was accompanied with the occurrence of virus diseases such as infectious salmon anaemia (ISA), infectious pancreas necrosis (IPN) and pancreas disease (PD). Vibriosis, IPN and coldwater vibriosis appeared during 1975-80, BKD in 1980, ISA in 1984 and yersinosis and furunculosis in 1985 (Rødseth et al., 1991).

The most serious diseases in the early days were vibriosis (*Vibrio anguillarum*), cold-water vibriosis and furunculosis. Due to lack of alternatives, treatment with antibiotics was the way of controlling these infections. This caused the enormous increase in consumption of antibiotics during the 80s with a peak in 1987-1988 due to frequent outbreaks of cold-water vibriosis whereas furunculosis was the main cause of the high consumption in 1988-1993. Furunculosis was introduced from Scotland by smolts in 1985 to the Trøndelag area in mid Norway and spread north and south from 1985 to 1991. Development of effective vaccines in combination with prophylactic operational routines, optimal nutrition and good water quality have resulted in a significant reduction in the consumption of antibacterial agents, especially from 1993-1994 (Samuelsen, 2003). The same history was more or less the case for Faroe Islands. The furunculosis was introduced to Iceland in 1995/96.

1.3 Development of vaccines

Commercial vaccines against the bacterial diseases were available on the market from the late 80s to the early 90s. Whereas the vaccines against vibriosis, coldwater vibriosis and yersinosis were introduced in 1987, the oiladjuvant vaccine against vibriosis, coldwater vibriosis and furunculosis was introduced on the market in 1992.

1.4 Future

The viral diseases, infectious salmon anaemia (ISA), pancreas disease (PD), heart- and skeletal muscle inflammation (HSMI) and infectious pancreas necrosis (IPN), seem to be the main reasons for loss in the in-

dustry in Norway and Faroe Islands. In Faroe Islands, the ISA virus outbreak in 2003 almost terminated the fishfarming industry but it came through and in 2008, a production volume of salmon and trout of approx. 45,000 tons is anticipated. Vaccination against IPN is tried to a varying degree without conclusive effect in the field.

The most important bacterial disease seems to be winter ulcer due to *Morticella viscosa* infections. Vaccination against other bacterial diseases such as furunculosis, vibriosis and cold-water vibriosis has shown good results although it seems that outbreaks of furunculosis still is a problem in vaccinated rainbow trout in Denmark, especially at high temperatures (above 20°C). Much remains unknown about the cause of HSMI, CMS and proliferative gill inflammation (Kvellestad et al., 2005). Furthermore, a vaccine against salmon lice is being developed but it not is known when it will be available on the market. In Norway, there is also increasing problems with cestodes.

In Denmark, occasional outbreaks of viral hemorrhagic septicaemia (VHS) results in mortalities up to 100%. A suggestion for a plan to extinct VHS in Denmark has been developed.

1.5 Data material

The available data are annual consumption of veterinary medicines used as treatments against salmon lice and endoparasites such as cestodes and antibacterials used for aquaculture. These data are available through the national veterinary authorities. The consumption of copper is not easily available but can be obtained through the national pollution authorities.

The data collected from Iceland and Denmark on consumption of different compounds only represent the use in marine cage farming. The data collected from Norway and Faroe Islands include the use in smolt farms. However, the compounds used only in the smolt farms, such as antibacterials given by injection to the brood stock and bronopol for fungus treatment, are not included. It is not possible to separate the remaining consumption of antibacterials and compounds for cestode used in smolt farms from those used in the marine farms. However, it may be estimated that less than 10% of the total consumption of the latter are used by smolt farms but no adjustments have been made for this in this report.

1.6 Consumption of veterinary medicines and chemicals in Iceland

The production of salmon in Iceland increased from 2,900 tons in 1999 to 6,620 tons in 2004. There was a drop in production in 2002 when 1,471 tons

was produced. The use of veterinary medicines has been very low (Table 1.1). Oxolinic acid was the only agent used during the 6-year period.

Table 1.1 Consumption of veterinary medicines and chemicals in marine cage farming in Iceland from 1999 to 2004 (kg of active component). N.A. = not available.

Year	1999	2000	2001	2002	2003	2004
Production of salmon (round weight in tons)	*2,900*	*2,593*	*2,645*	*1,471*	*3,710*	*6,620*
Antibacterial agents						
Oxytetracycline	0	0	0	0	0	0
Oxolinic acid	24	0	5	0	0	7
Flumequine	0	0	0	0	0	0
Florfenicol	0	0	0	0	0	0
Sulfadiazine+trimethoprim	0	0	0	0	0	0
Amoxicillin	0	0	0	0	0	0
Erythromysin	0	0	0	0	0	0
Enrofloxasin	0	0	0	0	0	0
Sum	**24**	**0**	**5**	**0**	**0**	**7**
Endoparasitic agents						
Paziquantel	0	0	0	0	0	0
Fenbendazol	0	0	0	0	0	0
Sum						
Ectoparasitic agents						
Cis-cypermetrine	0	0	0	0	0	0
Deltametrine	0	0	0	0	0	0
Teflubenzuron	0	0	0	0	0	0
Emamectine	0	0	0	0	0	0
Sum	**0**	**0**	**0**	**0**	**0**	**0**
Antifouling agents						
Copper	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.

1.7 Consumption of veterinary medicines and chemicals in Norway

In Norway, the production of salmon and trout increased from 470,000 in 1999 to 630,000 tons in 2004. The consumption of antibiotics was stable from 1999 to 2000 but increased in 2002 and 2004. Oxolinic acid contributes most to the total consumption of antibiotics, and the peaks in 2002 and 2004 are caused by use of oxolinic acid. The second most consumed antibiotic is florfenicol (Table 1.2).

Table 1.2 Consumption of veterinary medicines and chemicals in marine cage farming in Norway from 1999 to 2004 (kg of active component). N.A. = not available.

Year	1999	2000	2001	2002	2003	2004
Production of salmon and trout (round weight in tons)	*473,845*	*488,840*	*506,883*	*546,054*	*578,475*	*629,079*
Antibacterial agents						
Oxytetracycline	25	15	12	11	45	9
Oxolinic acid	494	470	517	998	546	1,035
Flumequine	7	52	7	5	60	4
Florfenicol	65	148	109	205	154	111
Sulfadiazine+trimethoprim	0	0	0	0	0	0
Sum	**591**	**685**	**645**	**1,219**	**805**	**1,159**
Endoparasitic agents						
Praziquantel	239	109	100	152	232	412
Fenbendazol	12	35	21	8	2	23
Sum	**251**	**144**	**121**	**160**	**234**	**435**
Ectoparasitic agents						
Cis-cypermetrine	19	73	69	62	59	55
Deltametrine	11	23	19	23	16	17
Teflubenzurone	231	62	28	0	0	0
Diflubenzurone	50	12	0	0	0	0
Emamectine	4	11	12	20	23	32
Azamethiphos	14	0	0	0	0	0
Sum	**329**	**181**	**128**	**105**	**98**	**104**
Antifouling agents						
Copper	206	306	203	225	210	N.A.

The drastic decline in consumption of ectoparasitic agents is due to decreased use of the veterinary medicines administered via the feed, e.g. teflubenzurone (Ektobann) and diflubenzurone (Lepsidon). The use of these veterinary medicines was terminated in 2002 and 2001, respectively. These veterinary medicines were replaced by the pyrethroids, cis-cypermetrine (Betamax) and deltametrine (Alpha-max) that are used for immersion and by emamectine (slice premix) that is administered via the feed. The consumption of copper was stable at about 200 tons/year.

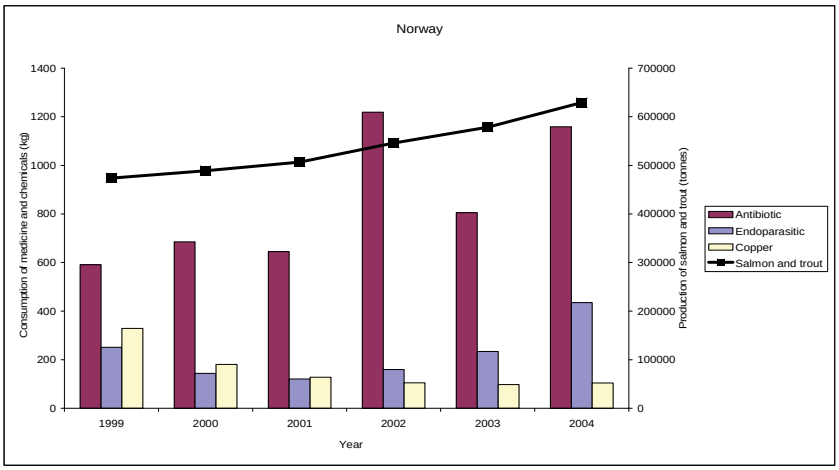


Figure 1.1 Development in Norway in the consumption of veterinary medicines and biocides in relation to the production of salmon and trout (tons)

1.8 Consumption of veterinary medicines and chemicals in Faroe Islands

The production salmon and trout in Faroe Islands in 2008 is predicted to be approx. 45,000 tons having increased from a low of 18,574 tons in 2006[1]. The decline started with outbreaks of infectious salmon anaemia (ISA) in 2003. The consumption of antibiotics in the six-year period 1999-2004 decreased from about 4,000 kg in 1999 (mostly due to BKD) to about 300 kg in 2003. In 2004, there was no consumption of antibiotics. Oxytetracycline has been the most consumed antibiotic agent (Table 1.3).

The veterinary medicines against cestodes (praziquantel and fenbendazol) have not been consumed since 1996. Problems associated with cestodes have been insignificant in Faroe Islands from 1999 to 2004.

The drastic decline in consumption of ectoparasitic agents is due to decreased use of the veterinary medicines administered via the feed, particularly diflubenzurone (Lepsidon). The use of this veterinary medicine was terminated in 2000. These veterinary medicines were replaced by the pyrethroids, cis-cypermetrine (Betamax) and deltametrine (Alpha-max) that are used for immersion and by emamectine (slice premix) that is administered via the feed.

Table 1.3 Consumption of veterinary medicines and chemicals in marine cage farming in Faroe Islands from 1999 to 2004 (kg of active component). N.A. = not available.

Year	1999	2000	2001	2002	2003	2004
Production of salmon and trout (round weight in tons)	*42,544*	*34,823*	*49,140*	*56,102*	*62,746*	*46,079*
Antibacterial agents						
Oxytetracycline	3,834	881	1,083	695	322	0
Oxolinic acid	2	13	0	0	0	0
Flumequine	136	201	77	43	1	0
Sulfadiazine+trimethoprim	0	0	0	0	0	0
Amoxicillin	0	0	0	0	0	0
Sum	**3,972**	**1,095**	**1,160**	**738**	**323**	**0**
Endoparasitic agents						
Paziquantel	0	0	0	0	0	0
Fenbendazol	0	0	0	0	0	0
Sum	**0**	**0**	**0**	**0**	**0**	**0**
Ectoparasitic agents						
Cis-cypermetrine	5.1	6.7	9.3	16.6	12.9	8.6
Deltametrine	2.3	2.0	2.4	1.3	2.4	1.9
Teflubenzurone	0.1	0	0	0	0	0
Diflubenzurone	104.7	0	0	0	0	0
Emamectine	0.05	1.5	1.87	3.81	1.91	1.15
Azamethiphos	17	0	0	0	0	0
Sum	**129.2**	**10.2**	**13.6**	**21.7**	**17.2**	**11.7**
Antifouling agents						
Copper	N.A.	N.A.	31	N.A.	N.A.	N.A.

[1] Føroya Havbúnaðarfelag, 2008. Ársfrágreiðing 2007. Føroya Havbúnaðarfelag, Smærugøta 9A. FO-100 Tórshavn. pp. 25. Available at www.industry.fo

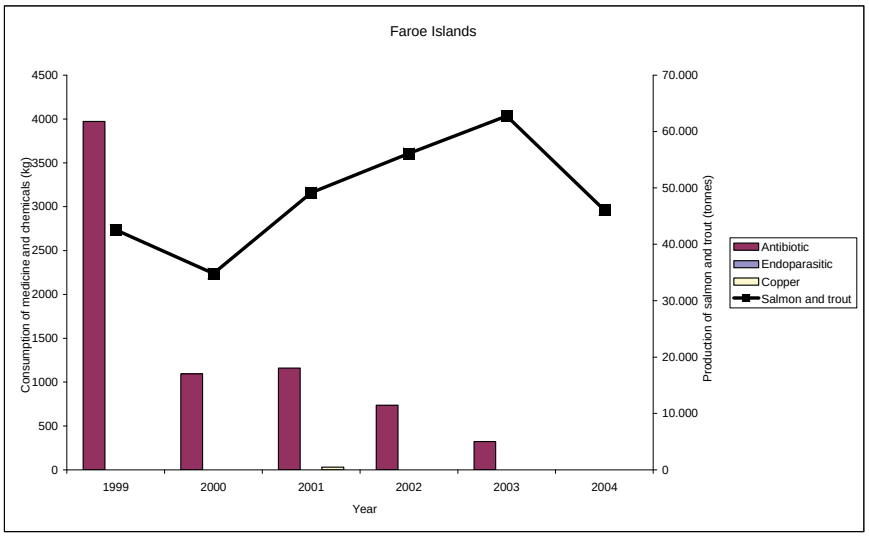


Figure 1.2 Development in Faroe Islands in the consumption of veterinary medicines and biocides in relation to the production of salmon and trout (tons)

1.9 Consumption of veterinary medicines and chemicals in Denmark

From 1999 to 2004, the production of trout was stable at between 7,000 and 8,000 tons/year. Data for 2001 to 2004 are given in Table 1.4. The consumption of antibiotics increased from 850 kg in 2001 to 1,400 kg in 2003, but decreased by 50% to 700 kg in 2004 (Table 1.4). Oxolinic acid and sulfadiazine + trimethoprim and were the antibiotics used, with the latter contributing most to the total consumption.

There are no problems related to cestode infections or salmon lice infestations in Denmark. The copper consumption data are not available.

Table 1.4 Consumption of veterinary medicines and chemicals in marine cage farming in Denmark from 1999 to 2004 (kg of active component). N.A. = not available.

Year	1999	2000	2001	2002	2003	2004
Production of trout (round weight in tons)	*7,000*	*7,264*	*7,000*	*7,331*	*7,236*	*7,995*
Antibacterial agents						
Oxytetracycline	0	0	0	0	0	0
Oxolinic acid			184	320	128	102
Flumequine	0	0	0	0	0	0
Florfenicol	0	0	0	0	0	0
Sulfadiazine+trimethoprim			667	1,031	1,279	597
Amoxicillin	0	0	0	0	0	0
Sum			**851**	**1351**	**1407**	**699**
Endoparasitic agents						
Paziquantel	0	0	0	0	0	0
Fenbendazol	0	0	0	0	0	0
Sum						
Ectoparasitic agents						
Cis-cypermetrine	0	0	0	0	0	0
Deltametrine	0	0	0	0	0	0
Teflubenzurone	0	0	0	0	0	0
Diflubenzurone	0	0	0	0	0	0
Emamectine	0	0	0	0	0	0
Azamethiphos	0	0	0	0	0	0
Sum	**0**	**0**	**0**	**0**	**0**	**0**
Antifouling agents						
Copper	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.

1.10 Discussion and conclusions

Antibiotics

For each of the Nordic countries assessed in this study, the consumption of antibiotics was below 1,500 kg/year for the last 5 of the 6 years reviewed. The consumption in Faroe Islands decreased drastically from about 4,000 kg in 1999 to 1,100 kg in 2000, and declined steadily from 2001 to 0 kg in 2004. The consumption in Norway was steady at between 500 and 700 kg per year in 1999 to 2001, but increased to between 800 and 1,200 kg from 2002 to 2004. Also in Denmark, there was an increase by 60% from 2001 to 2002, but there was a 50% decrease in consumption from 1,400 kg in 2003 to 700 kg in 2004 (Figure 1.3).

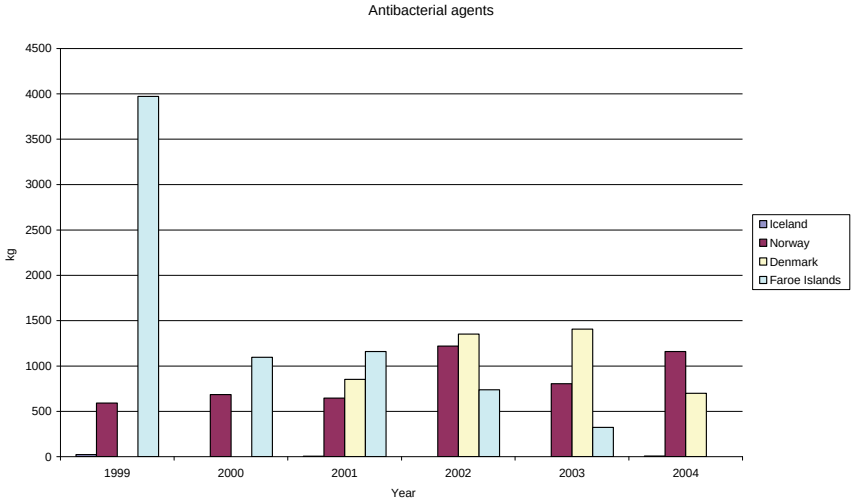


Figure 1.3 Yearly consumption of antibiotics in the 1999-2004-period given for each participating country (kg of active component/year)

In Norway, the consumption of antibacterial agents has remained stable at 1.2-1.4 g/ton fish produced, with the increase in 2002 and 2004 mainly related to the increase in biomass of farmed cod (
Figure 1.4). The vaccines developed against vibriosis in cod do not give sufficient protection and antibiotics are used therapeutically at outbreaks.

In Faroe Islands, the consumption of antibiotics has decreased steadily from 93 g/ton produced fish to 5 g/ton in 2003. In 2004, there was no consumption of antibiotics. In Faroe Islands, the relative number of outbreaks of BKD has been much higher than in Norway and may explain the big difference in consumption of antibiotics from 1999 to 2002 (Table 1.5 and Figure 1.5).

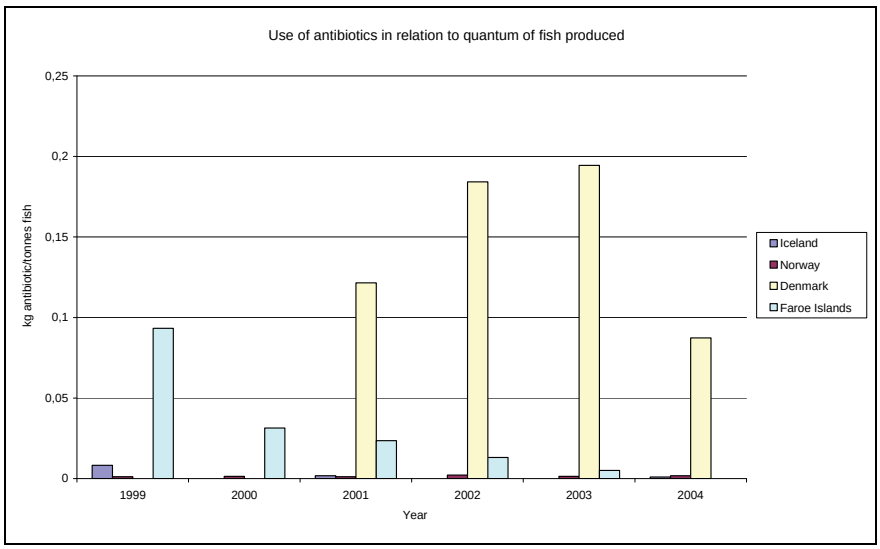


Figure 1.4 Consumption of antibiotics in relation to amount of fish produced from 1999-2004 (kg of active component)

The ratio of the relative consumption of antibiotics to the amount of fish produced in Denmark ranges from 87 to 176 g/ton in the period assessed. The consumption of antibiotics was directly related to the water temperature during the summer in Denmark. Whereas a warm summer gives high consumption due to outbreaks for furunculosis, a cold and rainy summer results in low consumption. Despite vaccination, outbreaks appear when temperatures increases above 20°C. In these cases, even vaccinated fish get furunculosis. In addition to furunculosis,which is considered the most common disease, yersinosis and vibriosis also appear at the different sites.

In Iceland, cod and salmon are reared in net cages. The consumption of antibiotics is generally low and in 2004, there were only few cases of atypical furunculosis (*Aeromonas salmonicida* ssp. *achromogenes*) in cod farms, which were therapeutically treated with antibiotics. There have also been outbreaks of vibriosis (*Listonella* (*Vibrio*) *anguillarum*) in two cod farms in the sea.

For the last five years of the assessed period, the bacterial diseases, which are therapeutically treated with antibiotics, habr been furunculosis, winter ulcers and bacterial kidney disease.

Table 1.5 Number of farm sites with registered outbreaks of different diseases in Norway and numbers per 10,000 tons of fish produced from 1999 to 2004 (kg of active component). N.A. = not available.

Year	1999	2000	2001	2002	2003	2004
Values totally						
Furunculosis (B)	2	6	3	0	2	3
BKD (B)	3	3	3	1	1	1
Piscirickettsiose (B)	6	0	1	17	5	0
Winter ulcers (B)	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
IPN (V)	-	-	-	174	178	172
ISA (V)	14	23	21	12	8	16
PD (V)	10	11	15	13	23	44
HSMI (V)						54
Number of ourbreaks/10.000 tons of fish produced						
Furunculosis (B)	0.042	0.123	0.059	0.000	0.035	0.048
BKD (B)	0.063	0.061	0.059	0.018	0.017	0.016
Piscirickettsiose (B)	0.127	0.000	0.020	0.311	0.086	0.000
Winter ulcers (B)	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
IPN (V)	N.A.	N.A.	N.A.	3.186	3.077	2.734
ISA (V)	0.295	0.471	0.414	0.220	0.138	0.254
PD (V)	0.211	0.225	0.296	0.238	0.398	0.699
HSMI (V)	0.000	0.000	0.000	0.000	0.000	0.858

Table 1.6 Number of farm sites with registered outbreaks of different diseases in Faroe Islands and numbers per 10,000 tons of fish produced from 1999 to 2004 (kg of active component). N.A. = not available.

Year	1999	2000	2001	2002	2003	2004
Values totally						
BKD	11	8	9	11	N.A.	N.A.
IPN	0	1	1	0	N.A.	N.A.
ISA	0	1	4	6	N.A.	N.A.
PD	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
HSMI	1	1	1	1	N.A.	N.A.
Winter ulcers	3	5	1	1	N.A.	N.A.
Number of ourbreaks/10.000 tons of fish produced						
BKD (B)	2.586	2.297	1.832	1.961	N.A.	N.A.
IPN	0.000	0.287	0.204	0.000	N.A.	N.A.
ISA	0.000	0.287	0.814	1.069	N.A.	N.A.
PD	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
HSMI	0.235	0.287	0.204	0.178	N.A.	N.A.
Winter ulcers (B)	0.705	1.436	0.204	0.178	N.A.	N.A.

Ectoparasitic agents

The veterinary medicines administered via the feed in 1999-2002, teflubenzurone (Ektobann) and diflubenzurone (Lepsidon), were replaced by the more effective emamectine (slice premix), and this is one of the main reason for the drastic decline in consumption in the beginning of the period from 1999-2004. The veterinary medicines used today are emamectine (slice premix), which is administered via the feed, and cis-cypermetrine (Betamax) and deltametrine (Alpha-max), which are used for immersion.

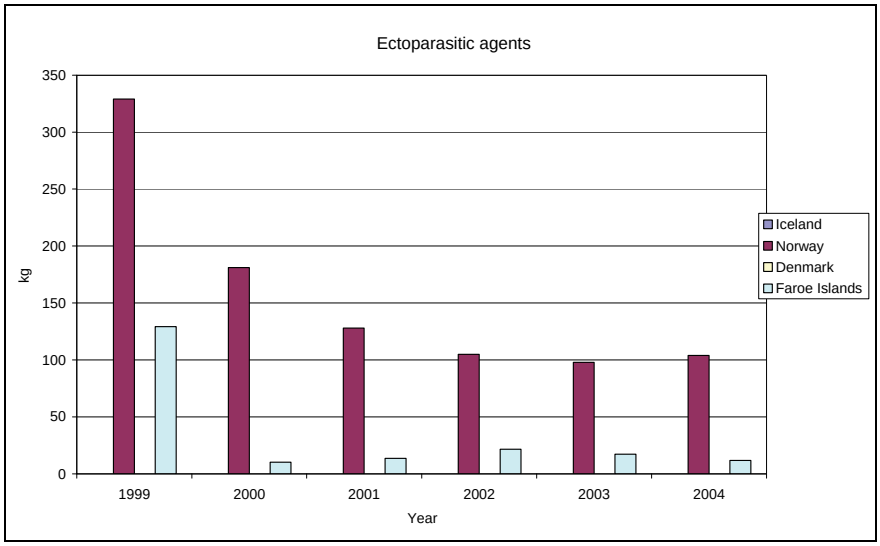


Figure 1.5 Consumption of ectoparasitic agents (kg) in Nordic countries from 1999-2004 (kg of active component)

The administration of ectoparasitic agents is mainly against salmon lice. The relative consumption of ectoparasitic agents was higher in Faroe

Islands than that in Norway in 1999 but subsequently decreased to the same level (Figure 1.6). The degree of infestations is mainly related to the environmental conditions of the site, i.e. degree of exposure, distance from land, management and monitoring routines, use of wrasses etc.

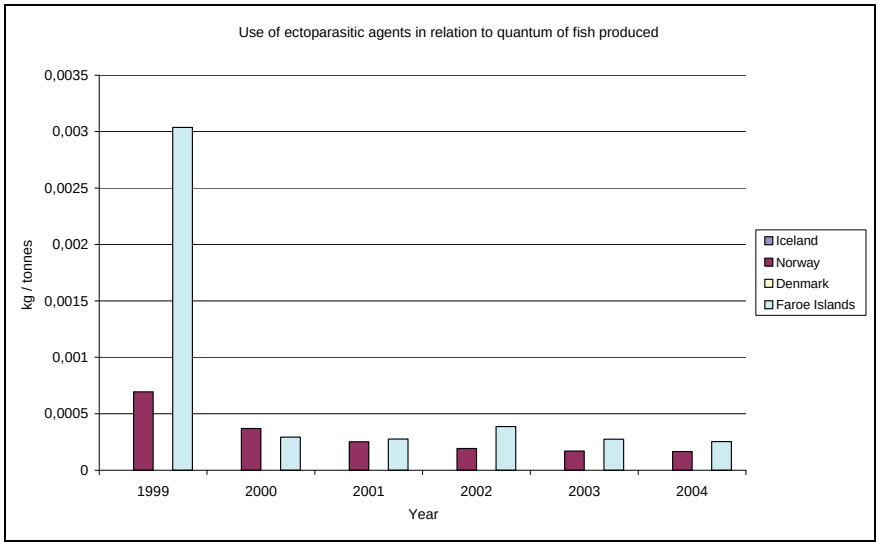


Figure 1.6 Consumption of ectoparasitic agents in relation to amount of fish produced from 1999-2004 in Nordic countries (kg of active component)

2. Emission of persistent organic pollutants from Nordic marine fish farms

2.1 Introduction

During the last two decades, salmon consumption has increased annually at a rate of 14% in the European Union and 23% in the United States. Correspondingly, the annual global production of farmed salmon (predominantly Atlantic salmon (*Salmo salar*)) has risen from about 24,000 to over 1 million metric tons during the same period (Hites et al., 2004).

Accordingly, marine fish farming has maintained its position as an important industry in the Nordic countries.

Farmed salmon and trout have become popular because it is a nutritious and relatively inexpensive source of protein and long-chain omega - 3 fatty acids, which contribute to cardiovascular health and infant brain development. Therefore, health authorities in the EU, FDA and WHO recommend that we should eat fish at least twice a week. However, it is also known that salmon and other fat fish may contain high amounts of persistent organic pollutants, and doubts have been cast about the healthiness of consuming farmed salmon even those raised in “remote” and presumably unpolluted areas as in the North Atlantic. It has to be emphasized that the occurrence of POPs incl. dioxins in fish is not a particular farmed salmon or trout problem - it is a highly relevant issue in general, e.g. in the Baltic where the wild fish (salmon and herring) do not meet the EU standards for being marketed.

Hites et al. (2004) reported levels of persistent organic pollutant (POPs) such as dioxins, PCBs, mirex, DDT, endrin, dieldrin, α-chlordane, γ-chlordane, heptachlor epoxide, lindane, hexachlorobenzene (HCB) and toxaphenes in 246 samples of salmon farmed or catched in different parts of the world. Hites et al. (2004) conclude that in particular farmed salmon from the North-East Atlantic area (Norway, Faroe Islands and Scotland) did have high contents of especially dioxins and PCBs. Therefore, the article by Hites and colleagues was given great attention and it was expected that articles like that may threaten the reputation and marketing of farmed salmon and trout from the Nordic countries.

The problematic POPs are all highly fat-soluble and will therefore accumulate in fish with a high fat content such as salmon and trout. The high content of POPs in the farmed fish from the northeast Atlantic areas

is due to a generally high content of POPs in the fish feed, typically produced and used in northeast Atlantic.

Feed for farmed salmon is primary meal and oil produced of so-called trash fish which among others include herring, sand eel, sprat and blue whiting. It is estimated that more than 20% of this trash fish in the EU exceed the limit values for content of dioxin and dioxin-like PCB in fish (EU Parliament). Especially fish from the Baltic and the North Sea have a high content of POPs and metals.

In order to cope with these different limits, the Icelandic Association of Fishmeal Manufacturers together with P/F Havsbrún started an investigation with the purpose to clarify dioxin and dioxin-like PCB levels in 4 commercially important fish stocks in the northeast Atlantic (Mundell et al., 2003). One of the conclusions from this project was that fish for fish feed production should be caught at the time of the year when the fish are most well-fed as the investigation showed that the POP concentration is lowest when the lipid content in the fish is highest.

Another initiative to cope with the high POP content in trash fish was taken by the Danish fish meal and fish oil producer Triple-Nine that in 2005 started a plant for removal of POPs from fish oil and meal. The removal was performed by the use of huge active carbon filters.

One solution for minimizing the concentrations of POPs in fish feed could be to be more selective when choosing the fish used for the feed production. This would have to be based on a more thorough knowledge of concentrations of POPs in different populations of fish used for the feed production combined with knowledge about the variability over the season. Landing of fish when the concentration of POPs in the fat is lowest possibly combined with cleaning of the produced fish oil and fishmeal will be usable methods for minimizing the concentrations of POPs in fish feed. It is obvious that manufacturers of fish feed, fishermen as well as the governments in the Nordic countries should have common interests to cope with this problem as both sale and reputation of fish feed and farmed fish from the Nordic areas are in question.

Guidance on acceptable levels of different POPs in fish feed is given by the EU. E.g. the EU directive 2001/102/EC dated November 2001 established a maximum permissible level for dioxin of 1.25 ng WHO-TEQ per kilogram fishmeal, and 6.00 ng WHO-TEQ per kilogram fish oil. The actual regulatory limit for the content in fish feed is 2.25 ng dioxin WHO-TEQ per kg feed.

2.2 Estimation of emissions of POPs from the model fish farm Bakkafrost in Gøtuvík, Faroe Islands

Short description of Bakkafrost in Gøtuvík

The fish farm Bakkafrost is placed in Gøtuvík, at the east coast of the fjord. The farm is reached only by boat from Norðagøta habour. For the time being, Bakkafrost fish farm consists of 7 rings containing fish and 2-3 rings, which are presently not in use.

Figure 2.1 Bakkafrost fish farm in Gøtuvik

The water depth at the fish farm location is approx. 40 m. In May 2005, the model ring, which is the field station for the present project as well as a parallel project analysing the uptake of POPs from feed into growing salmon (Magnussen and Vang, 2006), contained approx. 73,350 fish. The fish were placed at the rearing place on 1 May 2004 (74,400 fish) and were ready for slaughtering in autumn (September - October) 2005. At this time, the fish were expected to have a whole-body weight approximating 4-5 kg. During the growth period, a food consumption rate of 1.1-1.2 was expected.

The fish were fed from a ‘feeding boat’, which was anchored at the farm site, and which is manned once daily. The feeding is electronically controlled in such a way that it is possible to monitor different parameters (temperature, oxygen, pH, amount of food applied, expected number of fish, etc.) via a computer.

Figure 2.2 Feeding boat from Bakkafrost fish farm

Estimation of emissions of POPs

More that 3 tons of trash fish are needed for the production of 1 ton farmed salmon or trout (Naylor et al., 2000), and generally a major part of the POPs in the fish feed are accumulated in the farmed fish.

The transfer and bioaccumulation of different POPs from fish feed into fish fillet were investigated by Magnussen and Vang (2006) in collaboration with Bekkafrost fish farm. A tight collaboration between this project and the present project was established in early 2005. Work plan, knowledge and data were discussed and shared. The specific data on estimation of release of POPs from marine fish farms to the environment have primarily been based on the results generated in the POPs transfer and bioaccumulation project by Magnussen and Vang (2006).

During the growth period, a commercial salmon feed from P/F Havsbrún was used. Depending on the size of the salmon, they were fed pellets with a size of 2.5; 3.5; 5.0; 6.0 or 9.0 mm.

The biomass was continuously corrected for dead fish removed from the pen. On 13 December 2004, the calculated biomass in the pen was 98 tons, corresponding to 19.6 kg/m^3 (number of fish 73,795; mean weight 1,325 g). On this day, all fish were transferred to a 15,000 m^3 pen. On 7 September 2005, when slaughtering of the salmon started, the biomass was calculated to 345,702 kg (number of fish 73,087; mean weight 4,730 g) corresponding to a density of 23 kg/m^3 in the pen. The actual harvested amount was within only a few percent of the calculated biomass.

During the growth period, fish were sampled for analysis of lipid and POP content at regular intervals. The sampling scheme, methods and the analytical results are further detailed in Magnussen and Vang (2006).

Based on data by Magnussen and Vang (2006), release of POPs to the environment are estimated on the basis of the difference between the amount of POPs added with feed and the amount accumulated in fish. In the calculation, it is assumed that degradation of POPs in both fish and the environment is negligible.

The concentration of the different monitored POPs when the fish were slaughtered and the average concentration of POPs in the fish feed are summarised in Table 2.1. In total 392,077 kg feed was used during the whole cultivation period to produce 345,702 kg salmon with a feed conversion rate of 1.13.

Table 2.1 Concentration of POPs in salmon and fish feed. Dioxin and dioxin-like PCB results are reported in WHO-TEQ

POP	Mean concentration in fish feed (µg/kg ww)	Amount of POPs added by feed (µg)	Concentration in fish fillet on 7 September 2005 (µg/kg ww)
Dioxin	9.8E-04	384	6.6E-04
Dioxin-like PCBs	3.5E-03	994	3.1E-03
Marker-PCBs	20.4	8,006	21.2
PBDEs	2.9	1,134	2.5
Toxaphene	26.3	10,321	24.4
Dieldrin	7.3	2,793	5.5
Clordane	7.6	2,996	8.0

The values indicate a close relationship between the mean concentration in the feed and the concentration in fish fillet.

In Table 2.2, the relation between the POP accumulation in the fish fillet and the release to the environment is shown.

Table 2.2 Accumulation of POPs in fish (whole fish, fillet, non-fillet parts) and estimated release to the environment

	Accumulation in whole salmon	Accumulation in fillet	Accumulation in non-fillet	Release of chemicals to the environment (in relation to feed added)	
	%	*%*	*%*	*%*	*mg*
Dioxin	63	25	38	37	0.14
Dioxin-like PCBs	93	37	56	7	0.07
Marker-PCBs	98	39	59	2	160
PBDEs	80	32	48	20	227
Toxaphene	90	36	54	10	1,032
Dieldrin	68	27	41	32	893
Clordane	90	36	54	10	300

The release to the environment is only indicative since the estimates are based on the measured concentrations in fillet and only few analyses on whole fish. This measurement indicates that the content in the rest of the fish was 1.5 times the content in the fillet (Magnussen and Vang 2006).

Furthermore, the results indicate that the retention of dioxins and dioxin-like PCBs in whole fish ranged from 63% to 93%, respectively. These values are in the same range as those found in the DAPAFF project (EU CRAFT project 2002), in which retention of dioxins in whole fish after 30 weeks of exposure ranged from 54-59% and for dioxin-like PCBs, retention ranged from 83-87%. Four salmon feeds with graded levels of dioxin were tested in the DAPAFF project.

The results in the DAPAFF project and Magnussen and Vang (2006) clearly indicated that the dioxin content of the fish reflected the level present in the feed.

In the ESFA report from 2005, a study by Karl *et al.* (2003) is quoted, in which the transfer of dioxins from commercial fish feed produced in Norway into the edible part of rainbow trout was studied. This study showed that the mean transfer rates ranged from 11.1% at 6 months of age to 30.7% at 19 months of age, which is a significantly lower transfer/retention than the rates found by Magnussen and Vang 2006 and DAPAFF 2002.

At the model fish farm Bakkafrost in Gøtuvík, solely fish feed from Faroe Islands is used. The estimated emission of POPs is thus based on one feed type. It will easily be possible to make a rough estimate of uptake and release when fish are fed on other food types when input data (POP content in food) are available.

In general, the concentrations of dioxin and dioxin-like PCB in European fish feeds are highly variable and depend strongly on the content in the fish meal and fish oil from which the fish feed is produced (Data from EFSA 2005) (see Table 2.3).

Table 2.3 Variability in the content of dioxin and dioxin-like PCBs in different feeding stuffs in European fish feeds

Feeding-stuff		Dioxins (ng WHO-TEQ/kg)			Dioxin like PCBs (ng WHO-TEQ/kg)		
	No of samples	*Low 5th percentile*	*Media*	*High 97.5th percentile*	*Low 5th percentile*	*Media*	*High 97.5th percentile*
Fishmeal	104	0.10	0.46	1.98	0.13	1.01	4.23
Fish oil	222	0.87	3.17	6.48	2.10	6.75	16.84
Fish feed	188	0.10	0.63	2.01	0.04	1.34	3.30

Fish oil and fishmeal from European productions contain higher levels of dioxin and dioxin-like PCBs than fish oil and fishmeal of South Pacific origin (EC SCAN, 2000). It is concluded that up to 98% of the content of dioxin and dioxin-like PCBs in fish feed for carnivorous fish like salmons comes from the content in the fishmeal and fish oil (EC SCAN, 2000).

In the article by Hites et al. (2004), more than 6 ng dioxin WHO-TEQ per kg feed is reported for fish feed from different areas of the world. The highest concentrations were found for fish feed produced in Scotland.

The fish feed from Scotland is probably comparable with fish feed used and purchase in the Nordic countries.

2.3 Discussion

Results from studies performed under realistic conditions indicate that a significant percentage of the dioxin in the fish feed may be release to the environment. The release of dioxin to the environment will among others depend on the composition of the feed, the amount of feed spill as well as growth and physiological condition of the fish. In the study at Bakkafrost salmon farming, Faroe Islands, it was estimated that about 37% of the dioxin in the feed added during the growth of the salmon over a 17-month period ended up in the environment. Correspondingly, the estimate in the DAPAFF (EU CRAFT project, 2002) study was 41-46%.

As shown in the previous section, the concentration of dioxin in feed for fish is highly variable and depends on the origin of the fish oil and fishmeal used in the production of feeds. Different studies showed that the concentrations of dioxin in fish feed may range between 0.1-2.01 ng dioxin WHO-TEQ per kg feed (EFSA, 2005) and 1-6 ng dioxin WHO-TEQ per kg feed (Hites et al., 2004). In the fish feed used in the study at the Bakkafrost salmon farm, the concentration of dioxin was 0.98 ng WHO-TEQ per kg feed.

In the Nordic countries, the 2004-production of salmon was about 690,000 tons in total. Based on the EFSA data, the release of dioxin to the environment in 2004 from Nordic salmon fish farms is in the range of 29-760 mg dioxin. Based on Hites et al. (2004) data, the release of dioxin to the environment will be in the range of 600-2,100 mg. For comparison, the total amount of dioxin bound in the fish biomass in the Baltic is estimated to be about 20 g or 20,000 mg (Assmunth and Jalonen, 2005).

In relation to environmental risk, it should be realized that in contrast to atmospheric deposition of dioxin and emission of dioxin from industry, the bioavailability of the dioxin released from fish farms must be regarded as being very high. The potential for biological uptake and cycling of dioxin released from fish farms may thus be considerable large.

2.4 Conclusion

The occurrence of POPs incl. dioxins in fish is not a particular farmed salmon or trout problem - it is a highly relevant issue in general, e.g. in the Baltic where the wild fish (salmon and herring) do not meet the EU standards for being marketed. Therefore, in relation to farmed fish, it should be aimed to keep the POP content as low as possible and to use available knowledge and technology for fulfilling this requirement.

POPs are neither a necessary nor a constant part of the fish feed. As described, the POP content depends to a large extent on the origin of the feed oil and protein base, whether produced from fish and if so, what species and from what area the fish are taken. This is clearly shown by the study performed by Hites et al. (2004), which showed that the highest POP concentrations were obtained in fish farmed in the North Atlantic region.

In the described model study, it has been shown that the retention of dioxins and dioxin-like PCBs in whole fish ranged from 63% to 93%. These values were in the same range as those found in previous projects (EU CRAFT project, 2002), in which retention of dioxins in whole fish after 30 weeks of exposure ranged from 54-59% and for dioxin-like PCB, retention ranged from 83-87%. Altogether, the results clearly indicated that dioxin and the content of other POPs in the fish reflect the level present in the feed.

The release of dioxin from Nordic fish farms is significant and the bioavailability must be regarded as high. By way of comparison, the release corresponds to up to 10% of the dioxin bound in the fish biomass in the Baltic.

At the model fish farm Bakkafrost in Gøtuvík, solely fish feed from Faroe Islands was used. Based on the input data from thorough investigation performed by Magnussen and Vang (2006) (e.g. concentration in fish feed; accumulation rate; concentration in fillet etc), it is relatively easy to make a rough estimate of uptake in the fillet and release to the environment for other fish feed types as well, provided that data on the POP content in feed is available. This could be a valuable tool for pre-estimation of the potential POP content in farmed fish.

In relation to protection of both health and the environment, it is recommended that initiatives are taken to control that fish feed with as low content of dioxins and other POPs as possible is used.

3. Pollutant concentrations in sediments beneath fish farms in a bay in Faroe Islands

3.1 Introduction

One of the purposes of the present project was to study the potential environmental impact of a fish farm with salmon production. The main emphasis was on pollutants imported along with the fish feed or antifouling agents, on oil-derived pollutants including combustion products and other pollutants stemming from the normal running of the facilities. The influence of these pollutants will mainly be elucidated through quantification of the substances in sediments in and around the fish farm.

The types and amounts of veterinary medicines used in fish farms in Faroe Islands are listed in Table 1.3, from which it is obvious that the usage of veterinary medicines has been very low for the last few years since the outbreak of ISA in 2003 and the following emptying out of the infected fish farms.

The farmed fish are subject to monitoring for veterinary medicines and other unwanted substances in food according to the EU veterinary directive 96/23. Substances such as antibiotics, anthelmintics, organophosphorus compounds, steroid hormones, antibacterial substances, chlorinated organic compounds and heavy metals are included in this monitoring made on fish tissue as is mycotoxines in fish feed. So far, this monitoring has not revealed any traces of the veterinary medicines or other compounds that could have been intentionally - although possibly illegally - added in the course of the production though chlorinated pesticides and unintentionally produced compounds are detected[2], however, in concentrations well below the EU maximum residual limits.

The focus of this study of pollutants in sediments near fish farms is the organochlor pesticides and other persistent and lipid soluble manmade pollutants like PCB, dioxin, brominated flame retardants and even PAH and the antifouling compound TBT. In addition, the metals commonly associated with fish farms have been included in the analytical scheme along with markers of increased input of organic material. Antibiotics that are or have been used in the treatment of fish in farms in Faroe Islands (Chapter 1) have been subject to analysis of a somewhat extended

[2] Reports desribing the finding of this monitoring in Faroe Islands are availabe at www.hfs.fo. See for example FVEA file 20040057-79, Control of residues in live animals and animal products.

area coverage, in that samples from in all 7 Faroese coastal areas have been analysed either as part of this study or as part of a supporting study commissioned by the Faroese Food, Veterinary and Environmental Agency. The environmental impact of antibiotics in the form of a possible occurrence of microbial resistance to antibiotics is treated in Chapter 4.

During the summer of 2005, sediment samples were taken at two fish farms located in Gøtuvík bay on the eastern coast of Faroe Islands (Map 3.1). The sampling was coordinated with the regular monitoring programmes of the fish farms, which are required by the environmental authorities as part of the Environmental permit. Additional sampling for the purpose of analysis of possible bacterial resistance to antibacterial agents and the occurrence of traces of antibiotics in sediments were made in June 2006, in Gøtuvík bay as well as in Fuglafjørð and in Pollurin off Klaksvík.

Map 3.1 Faroe Islands with the sampling sites in Gøtuvík, Pollurin and Fuglafjørður

There are two fish farms in Gøtuvík bay, with Bakkafrost operating the farm situated closest to the northern arm and Luna operating the farm on the southern arm (Map 3.2). The fish farms are thus operated by different companies and their fish feed suppliers are not the same.

The main current direction in the bay is counter clockwise with the inflow of seawater from the Faroe Islands shelf along the northern side of the bay and the outflow along the southern, thus making the transport out the fjord along the northern arm less likely. At the foot of the bay, three villages Syðrugøta, Gøtugjógv and Norðragøta are situated in close vicinity of each other, with a total population of approx. 1,030 inhabitants per 1 January 2005 (www.hagstova.fo). There is no large scale industry in the area but a fish filleting facility producing from 4,000 to 6,000 tons of fish per year (Gregersen, 2006).

3.2 The fish farms and their surroundings

The fish farm Bakkafrost is situated on the north side of the Gøtuvík bay, with 7 pens at approx. 62°11'30'' N, and 6°42'3'' W.

The production at the farm is approx. 70 000 fish per pen. With a full weight at slaughter of approx. 5 kg, and with a feed quotient of 1.2, this gives a total of 420 tons of feed added to the area during approx. 18 months.

The production at the farm has varied over the years. The area was closed in 2003 due to an infectious salmon anaemia, ISA, outbreak and reopened in 2004.

The sediments along the northern arm are mainly silt and sand whereas there are more clay and mud along the southern arm where the Luna farm is situated. The hard bottom along the northern arm means that given an equal input of pollutants, the likelihood of detecting these in the sediments under this northern arm fish farm will be less than in those under the fish farm on the southern arm, because the partitioning of pollutants to sediments is less likely when sediments consist of larger grain particles.

The relative bottom hardness in the Bakkafrost fish farm area is approx. 60% whereas in the Luna fish farm area along the southern arm, the relative hardness is approx 35%. This difference is mirrored also in the loss on ignition, LOI, of sediment samples in these two areas, where an average of 3-4% LOI is found in the Bakkafrost area, and 7% is not uncommon in the Luna area along the southern arm.

The Gøtuvík bay is approx. 3 km wide in its outer range, with the shorter northernmost arm approx. 6 km long. In the inner part of the bay, where the Bakkafrost and the Luna fish farms are located, it is approx. 2 km wide, and the depth where the fish farms are located is approx. 40 m.

Sampling 2005

Descriptions of the sediment sampling locations are given in Table 3.1, and in Map 3.2 the locations are shown along with the extent of the areas that have been assigned to the two fish farm operators. The sampling was originally planned to be in a transect so the extent of the influence area of a fish farm could be described. However, this plan was abandoned during the sampling cruises when it turned out that samples further out the fjord could not be effectively retrieved with the sampling gear applied.

The sampling gear consisted of a stainless steel haps corer with a Plexiglas tube. The Plexiglas tubes had an inside diameter of 4.5 cm and an outside diameter of 4.8 cm and were 60 cm long. Prior to use, the tubes were cleaned with RBS solid (Chem. prod. R. Borghgraef s.a. Brussels) in warm water for approx. 30 min. Thereafter, the tubes were rinsed with tap water and then with 5% Extran (extran MA02 neutro, 1.07553, Merck) and left to soak for 20 min. before rinsed in tap water and Millipore purified water (18 MOhm). Rubber stoppings were soaked in RBS water solution for 1 hour and rinsed in tap water and Millipore water (3 times).

Luna fish farm sampling was made on 22 July 2005 from 1–4 pm.

Stations nos. L 4, L 6, L 8, L 11 and Ref. were sampled, two parallel samples from each site. Samples were left over night at room temperature before subsampling of 0–2 cm, 2–5 cm and 5–10 cm. The segments from the two parallel samples were combined into one jar of heat-treated glass using heat-treated Al-foil under the lid.

Samples for antibiotics resistance were taken from 0–2 and 2–5 segments from the stations L 11 and Ref. The samples were homogenised using a spoon before the subsamples of approx. 5 g were transferred to glass jars.

Bakkafrost fish farm sampling was made on 1 August 2005 from 9-12 am. Stations B 1, MPM (approx. station B 16), B 11 and B 4 were sampled.

All samples from station B 1 were taken using stainless steel cuffs to secure the tube in the sampler. The other three stations were sampled using a brass cuff[3].

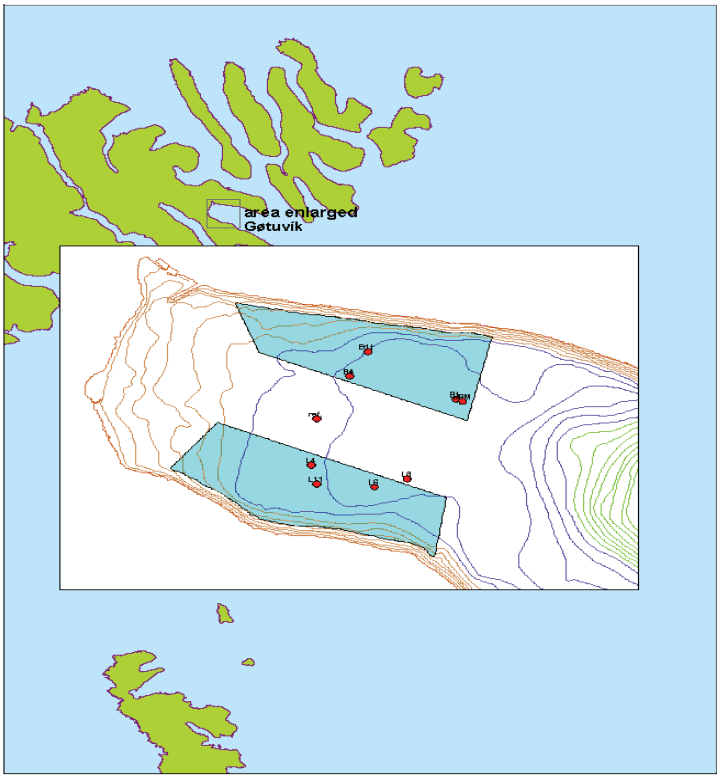
Sampling further out the fjord was attempted but unsuccessful at three sites and thus abandoned.

[3] For these three stations, the results for Cu and Zn in sediments samples do not indicate that the brass manchet has tainted the samples.

Table 3.1 Sediment sampling sites on Gøtuvík fish farms 2005*. The water depth at the sampling sites was approx. 40 m.

Fish farm	Station ID	Latitude, N	Longitude, W	Expected pollution level (1–4, where 1=least polluted)	Station description
Luna	L 4	62°10'68'' N	6°43'11'' W	3	At bay side of the 6 pens
	L 6	62°10'55'' N	6°42'53'' W	2	Out the fjord, approx. 200 m down the current from the pens.
	L 8	62°10'59'' N	6°42'22'' W	1	Further out the fjord, down the current.
	L 11	62°10'58'' N	6°43,06'' W	4	At sea side of the 6 pens
Reference	Ref	62°10'94'' N	6°43'03'' W	1	Reference station situated approx. midway in the bay between the two fish farms Luna and B.
Bakkafrost	B 1	62°11'02'' N	6°41'73'' W	1	Out the fjord, on the up current side of the pens.
	MPM	62°11'01'' N	6°41'67'' W	3	At the down current side of the pen used as a study site for dioxin uptake in salmon. This pen in the outermost in the farm. Sandy sediments with foul smell. Expected pollution level 4 but reduced to 3 due to the coarseness of the sedi-ments.
	B 11	62°11'30'' N	6°42'53'' W	3	At the down current side of the innermost pen. Foul smell, but sandy sediments.
	B 4	62°11'17'' N	6°42'71'' W	2	Silty sediments, the good catch in a grab tempted the taking of this additional station, situated in the outer reaches of the farm area and thus probably quite unpolluted.

* The two-decimal presentation reflects the precision of the station description.



Map 3.2 The sampling sites in Gøtuvík are shown. The sites denoted L are within the Luna farm, the sites denoted B are within the Bakkafrost farm. The common reference site for the two fish farm areas is situated in the middle of the bay.

Sampling 2006

Sediment samples for analyses of antibiotics (oxolinic acid, oxytetracycline and flumequine) and bacterial antibiotics resistance (Activity 4) were taken on 15 June 2006 in Gøtuvík as well as in two fjords with a long history of fish farming. The fjords chosen were Fuglafjørð and Pollurin near Klaksvík, and the sampling locations are given in Table 3.2. The upper 5 cm of the sediment core was analysed.

Table 3.2 Sediment sampling sites on Gøtuvík, Fuglafjørð and Pollurin, Klaksvík, 15 June 2006

Fjord area	Station ID	Latitude, N	Longitude, W	Water depth, m	Station description
Gøtuvík	Reference (pos. 9)	62°10'94'' N	6°43'03'' W	39	Sand
Gøtuvík, Bakkafrost (along northern arm)	B 16	62°11'28'' N	6°42'22'' W	42	Sand
Fuglafjørð	Reference I	62°13'85'' N	6°48'30'' W	24	Clay
Fuglafjørð	19	62°13'88'' N	6°48'53'' W	25	Clay
Pollurin, Klaksvík	Reference (PolRef)	62°14'78'' N	6°36'43'' W	41	Sand
Pollurin, Klaksvík	7 (Ringur 7)	62°15'10'' N	6°35'67'' W	40	Sand/mud

3.3 Methods

Determination of lead, cadmium, copper, nickel and zinc in sediments
The metals were analysed using the method SS-EN 13346mod/SS11885-1.

Determination of PCDD/PCDF, PCB, PBDE, PAH and pesticides in sediments

The entire sample was homogenised and prepared for organic analysis according to the standard operating procedure. Approx. 5 g of the sediment was weighed into a filter tube and placed in a soxhlet equipped with a water-separator. Internal Standards labelled with ^{13}C were added to the soxhlet content before the extraction. The samples were spiked with a mixture of 16 different ^{13}C-labelled PCDD/PCDFs, 12 different ^{13}C-labelled PCBs, 8 different ^{13}C-labelled PAHs and one ^{13}C-labelled hexachlorobenzene and extracted with toluene for 24 hours. The sample extracts consisting of 200 mL toluene were concentrated to 2 mL under vacuum on a rotary evaporator.

Clean-up procedure for removing the interfering compounds by Gel Permeation Chromatography (GPC). Prior to GPC treatment, toluene was replaced with dichloromethane (DCM). The extract pre-concentrated to 1.5 mL was then transferred to a graduated vial and introduced on the GPC system (Gilson). The GPC column was 90 cm long, 1 cm ID, Bio Beads S-X3 (Bio Rad) was a stationary phase and DCM was used as a mobile phase. Fraction between 37 to 90 min was collected in a Zymark

vessel containing a small amount of toluene. DCM volume was reduced to 0.5 mL, the extract was transferred into a vial and diluted to 1,000 µL with toluene for PAH and PBDE determination.

Chromatography and mass spectrometry

Before PBDE and PAH determinations, the extract was spiked with 2D10-fluoranthene used as an injection standard. For PBDE determination, the sample was introduced in a pulsed splitless mode into Agilent 5973 GC/MS/NCI equipped with a capillary column DB5 MS. PAH determination was performed on a 5973 GC/MS in EI mode on DB5 MS capillary column. Before the next step of analysis, the solvent toluene was replaced with n-nonane and the extract was spiked with ^{13}C-labeled TCDD/HxCDD as injection standard. The final volume of the extract was adjusted to 100 µL.

All determinations of PCDD/PCDF, PCB and pesticides were carried out using an Agilent 5973 gas chromatograph equipped with a PTV injector and an AutoSpec Ultima High Resolution Mass Spectrometer (HRMS) from Waters/Micromass. The HRMS was operated at Resolution 10,000 at 10%. All chromatography was performed on 40 m, 0.18 mm DB5 MS capillary column.

The internal standard procedure was used throughout all quantification work. For each batch (n = 10) of samples, a minimum of three different levels of calibration standards was applied. Those standards were used to calculate the relative response factors for all compounds. Blanks and non-extracted verification standard were analysed frequently in order to check for column performance and detector sensitivity. Peaks were identified by their relative retention times (RRT) and their masses. Sample compounds were identified and quantified automatically by a Micromass quantification program MassLynx. The quantifications were performed with the labelled mixture of internal standards, which were added in accordance to the standard operating procedures. Finally, the toxic equivalent quantity (TEQ) was calculated for PCDDs, PCDFs and PCBs using WHO-TEFs.

Limits of quantification (LOQ), all in DW

HCH, chlordane 0.01 ng/g; dieldrin 0.03 ng/g; hexachlorobenzene 0.1 ng/g; PCDD/F individual congeners 2 ng/kg; PCB individual congeners 2 ng/kg; PAH individual compounds 10 ng/g.

Precision

The following precisions are stipulated for the analytical methods used during the study: PAH ±15-25%; PCB7 ±15-30%; PCB WHO ±15-20%; PCDD/PCDF ±30-35%; Pesticides ±30-40%.

Determination of TBT in sediments

TBT as well as TPT (triphenyltin) were analysed by Alcontrol B.V in Holland as project no. 060209 with limits of detection of 1 ng Sn/g DW.

Determination of antibiotics in sediments

The three most used antibiotics in Faroese fish farming during recent years were analysed by Bent Halling-Sørensen, at the Department of Pharmaceutics and Analytical Chemistry, University of Copenhagen, Denmark. The method is described in Appendix 1.

3.4 Results

This section describes the detailed results of all chemical analyses of sediment samples. However, those results involving the antibacterial agents are given in Table 3.7 while the results for the antibacterial agents are given in Table 3.5.

Indicators on increased input of organic material to the sediments

The organic part of the sediments was described as a loss on ignition, LOI, and nutrients were quantified as phosphorus and nitrogen content.

The LOI was on average 63.6 g/kg DW ± 10% with no apparent difference between the reference station and the fish farm stations. Overall, the LOIs at the sampling sites were high compared to those found in other Faroese fjords (Table 3.3), but then the reference fjords (Johansen, 2006) were typically areas with high water exchange. The concentration of phosphorus in the sediments was on average 1.28 ± 0.88 g/kg DW and the concentration of nitrogen was on average 1.44 ± 0.25 g/kg DW. The concentration of phosphorous was markedly elevated at some stations compared to the reference station, whereas there were smaller differences in nitrogen concentrations. Overall, there was a weak correlation between the concentration of phosphorous and nitrogen, with an $R^2 = 0.55$. In the sediments, the highest concentrations of phosphorous were found at the sites L 11 and MPM, which are the sites where the appearance of the sediments indicated a high organic mass input (Table 3.1).

Metals in sediments

The analyses comprised quantification of the six heavy metals cadmium, copper, lead, mercury, nickel and zinc. Concentrations of Ni were uniform in the samples with a mean and relative standard deviation of 41.22 µg/g ± 4%. The widest relative range of concentrations were found for cadmium with a range from 0.073 µg/g to 0.26 µg/g DW around a mean

of 0.13 µg/g ± 48%. Also, copper and zinc concentrations were fairly variable with relative standard deviations of 29% and 30%, respectively. The mean copper concentration was 62.4 µg/g with a range from 49 µg/g to 110 µg/g. The mean zinc concentration was 52 µg/g with the lowest at 35 µg/g and the highest at 73 µg/g. Concentrations of lead was found to be in the range of 2.4 µg/g to 3.5 µg/g with a mean of 3.06 µg/g ± 12%. The highest concentrations of copper were found at stations MPM and L 11 where also the highest concentrations of zinc and phosphorus were found.

In 2006, a range of reference data on metals and some nutrients parameters became available, and a set of classification criteria for these pollutants in Faroese fjords were calculated (Table 3.4) using the methodology described by the Swedish Environmental Protection Agency (NVV 1999). The Classification criteria (Table 3.4) appears to be somewhat skewed towards high copper concentrations, which probably stems from naturally elevated copper concentrations in the reference fjords analysed.

Table 3.4 Classification of sediments at sampling sites from the Gøtuvík bay.

	Unit	L 4	L 6	L 8	L 11	Ref	B 1	MPM	B 11	B 4
LOI	%	V. High	High	V. High	V. High	High	High	V. High	High	High
N	g/kg DW	High	High	High	V. High	High	High	V. High	High	High
Pb	mg/kg DW	High	High	Average	High	High	High	Average	Average	High
Cd	mg/kg DW	High	Average	Low	High	Low	Average	High	Average	Low
Cu	mg/kg DW	Low	Low	Low	Low	Low	Low	High	Low	Low
Ni	mg/kg DW	Low	Low	Low	Low	Low	Low	Low	Low	Low
Zn	mg/kg DW	Low	Low	Low	High	Low	Low	High	High	Low

The classification is based on measured pollutant concentration compared to Faroese reference fjords (Table 3.4). The parameters evaluated were loss on ignition (LOI), nitrogen (N), lead (Pb), cadmium (Cd), copper (Cu), nickel (Ni) and zinc (Zn) in sediments samples. V. High = Very High.

Classification criteria	LOI %	Pb	Cd	Cu	Cr	Hg	Ni	Zn	Tot-N, g/kg DW
Low	3.2	1.1	0.067	77	35	0.005	60	54	0.49
Average	4.1	1.8	0.086	80	43	0.008	86	59	0.72
High	5.1	2.9	0.111	84	54	0.012	122	66	1.07
Very high	6.5	4.6	0.144	88	67	0.018	175	73	1.60

Classification criteria calculated as described by the Swedish Environmental Protection Agency (NVV 1999) using measured pollutant concentrations in reference fjords in Faroe Islands (Johansen, 2006). Concentrations are given in mg/kg DW unless otherwise stated. The analyses of metals were made following a mild acid digestion procedure as in the present study.

Pesticides in sediments

The analytical scheme included dieldrin, DDTs (incl. p,p- and o,p-isomers of DDT, DDE and DDD), HCH (alpha, beta and gamma isomers), alpha- and gamma-chlordane, toxaphene (as parlar 50) and hexachlorobenzene, HCB.

Neither p,p- nor o,p-DDT was detected (at 0.1 ng/g), nor was o,p-DDE or o,p-DDD (at 0.03 ng/g and 0.05 ng/g, respectively). p,p-DDE

was detected in some samples, and p,p-DDD in a single sample with detection limits at 0.05 ng/g and 0.1 ng/g, respectively.

Dieldrin was detected in all but one sample at a detection limit of 0.03 ng/g. HCB and HCH (the three isomers) were detected in one sample only, L 11, at detection limit 0.1 ng/g for HCB and 0.01 ng/g for the HCHs. Chlordane was detected at a few stations, i.e. L 11 and MPM, using a detection limit of 0.01 ng/g and toxaphene (as parlar 50) was detected at one station only using the same detection limit as for chlordane. Appearing in an unfavourable light in these comparisons are the stations named MPM and L 11.

PCB in sediments

PCB was quantified as the seven marker PCBs; CBs 28, 52, 101, 118, 138, 153 and 180. All these marker PCBs were detected in all samples with CB 153 being the most common, at an average of 93 ± 69 ng/kg, with a low of 25 ng/kg (at B 1) and a high of 210 ng/kg (at MPM). The sum of the marker congeners, PCB_7, was on average 292 ± 229 ng/kg with a high of 780 ng/kg at the MPM station.

Dioxin-like PCBs, both the non-ortho (CBs 77, 82, 126 and 169) and the mono-ortho (CBs 105, 114, 118, 123, 156, 157, 167 and 189) congeners, were quantified using a detection limit of 2 ng/kg. The majority of the congeners were detected in a few but not all stations whereas three congeners CB 81, 123 and 126 were not detected in any samples, and CBs 105, 118 and 156 were detected in all samples. The congeners occurring in the highest concentration among the dioxin-like PCBs was CB 118 with an average of 46 ± 38 ng/kg, and a maximum at the MPM station of 115 ng/kg.

Dioxins in sediments

Dioxin (PCDD/PCDF) was analysed in the sediment samples but overall, dioxin was not detected at 2 ng/kg DW, giving an upper-bound sum of PCDD/PCDF of 5.8 pg I-TEQs/g DW at all stations, except for the reference station where the upper-bound PCDD/PCDF concentration was 6.2 pg I-TEQs/g DW. The dioxins detected in the sediment samples from Gøtuvík were 1234678 HpCDD and OCDD, which were found in nearly all samples, and then the latter at the highest concentrations from 6.7 to 22 pg/g DW whereas 123789 HxCDD was detected in one single sample - actually the reference station - at 5.3 pg/g DW. The furans were detected less frequently and only 1234678 HpCDF or OCDF, again with the reference station as one of the few stations where the compounds were detected, at max. 5.4 and 5.1 pg/g DW, respectively. The measured concentrations of these higher chlorinated dioxins and furans are similar to what has been found in deeper layer of sediments from the Gulf of

Finland and may thus be regarded as "background" dioxins (Isosaari 2004) stemming from for example fires. There are no data on dioxin in sediments from Faroe Islands available for comparison. For surficial layers of sediments from the Baltic, a range of dioxin concentrations from 7 pg/g to 350 pg I-TEQs/g have been reported (Jensen, 2003). In sediment samples from the Mediterranean, the background levels ranged from 0.4 to 39 pg/g and in freshwater lakes and rivers in rural areas, a background level of less than 1 pg/g may be typical (Isosaari, 2004 and references therein). The present study in Gøtuvík was not aimed at establishing the background level but to detect pollution from the fish farm activities, thus it is not possible to assess a precise dioxin background level other than in general terms, that the concentration of dioxin in Gøtuvík appears to be low and probably close to the overall background for a Faroese fjord.

TBT in sediments

The antifouling compound, TBT, was detected at four of the nine stations with a detection limit of 1 ng Sn/g, with the highest concentration, 4.9 ng Sn/g (approx. equal to 12 ng/g of TBT), at the reference station, which also happens to be between the two farms and thus in the middle of the ship lane for traffic in and out the fjord.

TBT and imposex have been analysed in blue mussels and dog whelks, respectively, at six location along the Faroe Islands coast in 1996, 2001 and a new survey is being made in 2006. In previous surveys, widespread imposex has been detected at all but one or two sampling sites, and concentrations of TBT in blue mussels in the range 22 to 789 ng Sn/g DW have been recorded (Hoydal and Dam, 2004). According to the Norwegian State Pollution Control authority, blue mussels with TBT concentrations equivalent to 100-500 ng/g are indicative of a moderately polluted area (Molvær *et al.*, 1997; Esperud and Lárusdóttir, 2005).

TBT was analysed in sediment samples from the Torshavn harbour area in 2002 (Dam and Danielsen, 2003). TBT in sediments from the inner harbour area was found to be in the range of 130 to 100,000 ng/g and just outside, it was found to be 42 ng/g. A survey of TBT in sediments was made along the Norwegian coast in 1993-1994 (Espedal and Lárusdóttir, 2005) and the results revealed that at the majority of the sites, the concentration of TBT was within the range of 20-100 ngSn/g.

In marine sediments near Nuuk, Greenland, the concentration of TBT ranged from below the limit of detection of 1 ng Sn/g to 171 ng Sn/g (Jacobsen and Asmund, 2000).

According to the aforementioned Norwegian authority, a sediment TBT concentration of 1 to 5 ng/g is typical of a moderately polluted area, whereas concentrations exceeding 100 ng/g would be found in very badly polluted areas (Molvær *et al.*, 1997).

The provisional OSPAR ecotoxicological assessment criterion for TBT in sediments has been set to be within the range from 0.005 to 0.05 ng/g (OSPAR Commission, 2000). It is easily seen that at the sites where TBT could be detected in this study, the concentration also exceeds the OSPAR ecotoxicological assessment criterin for sediment.

US-EPA has stated that the TBT saltwater criterion to protect aquatic life from chronic effects is 7.4 ng TBT/L or 3.0 ng Sn/L (adapted from EPA, 2003). The concentration of TBT in the water column was not measured, but it may be estimated on the bases of literature data. According to Meador (2000), the organic carbon-normalized partitioning coefficient between sediments and water in marine environments is approx. 32,000 ($\log K_{oc} \sim 4.5$). The organic carbon content in the sediments was not measured, but may be estimated on the basis of the loss on ignition, LOI, to approx. 25 g C/kg sediment when assuming the carbon part of the organic material lost is approx 40%. To produce an organic carbon-normalized sediment TBT concentration, the measured concentration is divided by the organic content. From the organic carbon-normalized TBT concentration, the concentration of dissolved TBT in the water column is estimated on the basis of the above partitioning coefficient to be in the range of 6 ng Sn/L at the most contaminated site, which is thus twice as high as the EPA saltwater criterion.

Polycyclic aromatic hydrocarbons in sediments

Polycyclic aromatic hydrocarbons, PAH, was analysed as 19 individual compounds including naphthalene and the subset often referred to as EPA 16. EPA 16 includes the carcinogenic compounds benz(a)anthracene, benz(a)pyrene, benzo(b)fluoranthene, chrysene/triphenylene, dibenz(a,h) anthracene and indeno(1,2,3-cd)pyrene among other PAHs. The individual compounds were analysed with a detection limit of 10 ng/g, except for naphthalene, which was analysed with a detection limit of 100 ng/g. With benzo(b)fluoranthene and fluorene as exceptions, PAHs were only occasionally detected in the sediment samples. Site L 4 appears to be the one where PAHs are most often found, and then at concentrations either higher than or comparable to what is found at other stations. Again, the reference station does not appear to be especially noteworthy in comparison to the other station regarding PAH load in the sediments.

Sediment samples from Tangafjord, Faroe Islands, in 1994 was analysed for PAH (Granmo, 1996). In this Tangafjord study, benzo(b)fluoranthene and fluoranthene were found in concentrations of 41 ng/g and 21 ng/g, respectively, which is quite similar to the results from Gøtuvík in the present study where these compounds were found at a range from less than the detection limit (10 ng/g for both these compounds) to 44 ng/g and to 22 ng/g, respectively. The total organic carbon in the Tangafjord sediments was 1.93% which may be regarded as comparable to a loss on ignition for the

Gøtuvík sediment samples of between 5.3 to 7.1% assuming that the organic material is primarily present as carbohydrates.

Brominated diphenyl ethers in sediments

Brominated diphenylethers, PBDE, were analysed as the isomers BDE-47, -85, -99, -100, -138, -153, -154 and -209. PBDE was analysed in samples from two stations only, at MPM and B 11, but in neither of these were any of the PBDE isomers detected, at 0.01 ng/g DW.

Both marine and freshwater sediments from Norway have been analysed for PBDE (Fjeld *et al.*, 2004a,b; 2005). The concentration of PBDE in sediments from the Drammensfjord ranged from 6.28 ng/g to 33.37 ng/g as the sum of congeners BDE-47, -85, -99, -100, -138, -153, -154 and -209, with the decabrominated compound BDE-209 making up 95% of the sum of the BDEs. In freshwater sediments from the Drammens River, the concentration of PBDE ranged from a low of 0.03 ng/g to 59 ng/g where BDE-209 made up 92% of the sum of the PBDE. PBDE in sediments from the inner Oslofjord and the Trondheimsfjord, was found to be 2.82 ng/g and 3.03 ng/g. In sediments known to be polluted with PBDE such as off the coastal town Ålesund and in the freshwater lake Mjøsa, concentrations of PBDE as high as 464.03 ng/g and 26 ng/g, respectively, have been recorded.

In marine sediments sampled in Denmark, the lowest concentration of BDE-209 at 1.6 ng/g DW, was found in the Sound with just a little higher concentrations in the Odense bay and off Anholt and Ven, and with the highest concentration in the Copenhagen harbour area at 21.5 ng/g (Christensen and Platz, 2001). In this study, a subset of PBDE isomers consisting of BDE-47, -99, -100 and -153 was analysed in a larger number of samples including coastal areas in Jutland. The sum of these four isomers was found to be within 0.1 ng/g to 1.0 ng/g in most areas including Århus and Randers bays and the Liim Fiord. In the same study, freshwater sediments from mid-Jylland and one lake in Zealand were analysed as well, and the lowest and highest sum of the four isomers were found in Stream Varde in Jutland, at 0.07 ng/g, and in Lake Brabrand near Århus, at 2.58 ng/g, respectively.

Overall, from these comparisons of PBDE in marine and freshwater sediments from other Nordic countries, it is concluded that the concentrations of PBDE in Gøtuvík are low, and that the analyses have been conducted with a detection limit suitable to reveal low to moderate PBDE pollution.

Antibiotics in sediments

Antibiotics were analysed in sediment samples from three bays or fjords with both previous and ongoing fish farming activities. In addition to the

Gøtuvík bay area, sediments were collected from Pollurin off Klaksvík and Fuglafjørð. From each fjord or bay, two samples of sediments were taken for these analyses; one from a site that is or was previously under the influence of farming activities and one from a site that is used as a reference site. The analysed antibiotics were those antibacterial substances that had been most commonly used in the fish farming in Faroe Islands in recent years (Chapter 1). When listed in descending order of therapeutic use volumes since 1987, these most common antibiotics are, oxytetracycline, oxolinic acid and flumequine. Of these, oxytetracycline is by far the most used antibiotic agent both in recent years as well as overall in the period 1987 to 2004 (Chapter 1; Mikkelsen et al., 2002). In the period 1987-2004, the total consumption of oxytetracycline, oxolinic acid and flumequine in Faroese fish farming was 18,835 kg, 12,415 kg and 4,239 kg of active ingredient, respectively.

These antibacterial agents are water soluble with log octanol-water partitioning coefficients in the range from -0.9 for oxytetracycline to 1.6 for flumequine (Chem ID Plus). Thus, these compounds are not prone to bioaccumulate. They have, however, been described as stable and active in sediments (Lunestad *et al.* 1993) meaning that they were not immediately degraded in sediments but remained there and reduced (reversibly) the number of bacteria in the sediments and at the same time, increased the number of antibiotics-resistant bacteria strains. In field studies, the eventual disappearance of the antibacterial agents from the sediments was found to occur with half-lives of 151 days for oxytetracycline and oxolinic acid and 60 days for flumequine in the uppermost sediment layers whereas in deeper sediment layers (5-7 cm), the half-lives were found to be significant prolonged and larger than 300 days for the three compounds (Hektoen et al., 1995). The same authors suggested that the depuration was due to leaching and redistribution rather than degradation. Recent studies of microbial degradation of antibiotics like oxytetracycline have however indicated that degradation by bacteria likely contribute to the decreasing concentration of antibacterials with time (Maki *et al.*, 2006).

Oxytetracycline is a bacteriostatic antibiotic that exerts antibacterial effect by hindering bacterial growth in contrast to the quinolones oxolinic acid and flumequine that kill bacteria; they are bactericidal.

The three antibiotics oxytetracycline, oxolinic acid and flumequine were detected in four, three and one of the six sediment samples, respectively (Table 3.5). The antibacterial agent found in the highest concentration was oxolinic acid, at a mean of 2.9 ng/g DW for the two parallel samples analysed, whereas the highest concentrations of oxytetracycline and flumequine found in these sediment samples were 0.27 and 0.29 ng/g DW, respectively. The highest concentrations of oxolinic acid were found in sediments from Pollurin and Fuglafjørður, both sites that have been extensively used for fish farming for a number of years. It should also be noted that in two of the cases, antibiotics were detected not only at the

site close to previous or present fish farming but also at the reference stations. There are few data available for comparison but the Food, Veterinary and Environmental Agency of Faroe Islands had sediment samples taken in 2003 from six other sites in vicinity of fish farms, i.e. not reference sites, analysed for the same three antibiotics as in the present study. These data have been made available for the current assessment and the results were in brief that only in two out of six samples were antibiotics found, with the limits of quantification defined in Table 3.5, and then one type only at each site. The concentration of oxytetracycline found in the 2003 sample where it could be detected was 0.17 ng/g DW, and in the sample, in which oxolinic acid was detected, it was present at a concentration of 0.66 ng/g DW.

The levels of antibacterial agents in these Faroese fjord samples are low or comparable to levels in various types of fish farms in Italy (Lalumera *et al.*, 2004), and very low compared to sediments around fish farming areas, for instance in Puget Sound, Washington, where concentrations of oxytetracycline has been reported to be around 1 μg/g WW (Herwig and Gray, 1997). In a study of oxytetracycline in sediments below a marine fish farm, concentrations as high as 285 μg/g have been reported (Samuelsen et al., 1992), which would be equivalent to 285,000 ng/g when the same unit as is used for the present analytical data is applied. Minimum effect concentrations (MECs) for retarded growth in river sediment microflora have been estimated for oxytetracycline and oxolinic acid to be 20 μg/g and 0.63 μg/g, respectively, which would be represented as predicted no effect concentrations, PNECs, equal to 10 μg/g and 0.31 μg/g for the sediment type studied (O'Reilly and Smith, 2001). In marine water, the activity of antibiotics is assumed to be only 10% of what it is in freshwater, and interaction between the antibacterial agent and organic material and clay in sediment may reduce the activity of the antibiotics[4]. Thus, applying the suggested freshwater PNECs in the present case should yield a conservative estimate of environmental risk even though the organic content of the sediments (Table 3.7 as loss on ignition) is approx. half of that in the freshwater study, in which the PNECs were estimated (approx. 12% organic content). From such a comparison, it is found that the measured concentrations of oxolinic acid are max. 1% of PNEC and for oxytetracycline, the highest concentration measured was less than 0.01% of PNEC. Flumequine has been found to have a toxicity of 12-15 mg/L as EC_{50} in bioluminescence assays with *Vibrio fisheri* compared to 121-139 mg/L for oxytetracycline (Lalumera et al., 2004). The maximum concentrations of flumequine found in the present study were of similar magnitude as that of oxytetracycline (approx. 0.3 ng/g), and given that the factor 10 higher toxicity can be expressed as a PNEC for flumequine equal to PNEC for oxytetracycline

[4] Studies of antibiotics in marine sediments showed that the antibacterial agents retained their antibacterial activity despite of the interaction with sediments (Hektoen et al., 1995).

× 0.1, then the highest measured flumequine concentration would not exceed 0.1% of PNEC. Thus, for neither of these antibacterial compounds is there any reason for concern for environmental impacts in terms of negative impact on the microbial community.

A study assessed the concentration of oxytetracycline in the water-column immediately above the sediments and found that in the case of the sediments with the 285 μg/g of oxytetracycline, the concentration in the water phase would not exceed 0.11 μg/g (Smith and Samuelsen, 1996). The washing out of antibiotics from sediments has been found to be dependent on the degree of adsorption of the antibiotic to the sediments, which in turn is dependent on sediment characteristics like grain size, humus content etc. If it is assumed that the same ratio of dissolved oxytetracycline to sediment-bound oxytetracycline may be used in the present study as was found in the study of out-washing of the 285 μg/g oxytetracycline, it may be calculated that near a seabed containing 0.3 ng/g oxytetracycline, the water phase concentration would be approx. 0.1 μg/L. A PNEC of 20 μg/L for oxytetracycline has been estimated from ecotoxicological data (Andersson et al., 2006), and thus it is reasonable to assume that the measured oxytetracycline level does not pose any threat to biota in the water masses on site.

An interesting feature to note is that antibacterial agents have hardly been used at the sites where sediments were sampled for the last three years or more prior to the sampling. E.g., oxolinic acid has not been in registered use in fish farms since 2000 when 13 kg was applied, in fact the entire use volume since the ISA outbreak in 1993 has been mere 385 kg. In the three-year period from 1989 to 1991, however, oxolinic acid was the most widely used antibacterial agent in Faroe Islands fish farming, with a total of 9,463 kg of active agent of oxolinic acid applied (Mikkelsen et al., 2002). Thus, it is likely that the antibiotics detected in the present study with sediment sampling in 2006 to a large extent are “legacy” pollutants stemming from usage perhaps more than 10 years ago. In a study of antibacterial agents in sediments around fish farms in Italy (Lalumera et al., 2004), it was likewise found that oxytetracycline and flumequine could be detected in sediments 2 years and 8 months, respectively, after the last therapeutic application of these in the pond culture farm. This is also in accordance with earlier studies by Nygaard et al. (1992), Lunestad et al. (1993) and Hektoen et al. (1995) who have all found that oxolinic acid, oxytetracycline and flumequine were stable, especially in the deeper sediment layers.

Table 3.5 Sediments from sites in three Faroese fjords/bays with either ongoing or previous fish farm activities and from reference sites for the same area were analysed for antibiotics.

ID no.	Sampling location	Replicate no.	Sampling date	Oxytetracycline, ng/g DW	Oxolinic acid, ng/g DW	Flumequine, ng/g DW
1	Fuglafjørður st 19	1	15062006	0.20	2.25	nd
		2		0.35	2.02	nd
		mean		0.27	2.14	nd
2	Fuglafjørður reference station	1	15062006	0.31	0.97	nd
		2		0.13	0.45	nd
		mean		0.22	0.72	nd
3	Gøtuvík North st 16	1	15062006	0.09	nd	nd
		2		0.15	nd	nd
		mean		0.12	nd	nd
4	Gøtuvík, reference station	1	15062006	0.07	nd	nd
		2		0.12	nd	nd
		mean		0.10	nd	nd
5	Pollurin st 7	1	15062006	nd	3.47	0.34
		2		nd	2.33	0.24
		mean		nd	2.91	0.29
6	Pollurin reference station	1	15062006	nd	nd	nd
		2		nd	nd	nd
		mean		nd	nd	nd

The samples were analysed in duplicate, and the mean values are presented along with the two individual replicate results. The limit of quantification (LOQ) for oxytetracycline was 0.07 ng/g DW, LOQ for oxolinic acid was 0.40 ng/g DW and LOQ for flumequine was 0.14 ng/g DW. Results lower that LOQ are listed as ”nd” in the table.

3.5 Discussion and conclusions

In the above review, it has been established several times that there are elevated concentrations of a range of pollutants at especially two of the sediment sampling sites. At mere sight and sniff during sampling, these sites were identified as very likely marked by the fish faming activity taking place in the water column above. It has thus been established that the sea bottom beneath and around fish farms are marked with elevated concentrations of nutrients, metals, persistent organic pollutants such as PCB and pesticides, and antibiotics. These are the pollutants that were found to be more abundant under the fish farms than at the reference station or at sites more remote from the farming influence. For a few pollutants or groups of pollutants like dioxin, PAH and TBT, the station with the lowest concentration was just as often as not one of the fish farm stations, whereas the reference station could be more impacted. For these pollutants, it can thus be concluded that they were not related to the aquaculture activity as such but to more general pollution sources where traffic is probably the most important.

Elevated pollutant concentrations in this setting means not necessarily that the sites were severely or markedly polluted; it means that the chemical concentration was elevated above what was detected at another station. Whether biologically relevant to the point where negative impact may occur is another matter that must be seen relative to environmental criteria. In Table 3.6, the concentration of pollutants at the various sampling sites in Gøtuvík has been compared to the Environmental Quality

Criteria, EQC, for the compounds for which such criteria have been available. The EQCs have been published either by the OSPAR Commission or by the US-EPA. What can be noted from the comparison of the measured concentrations to the EQC, is that only for TBT, the concentrations are raised to a level, at which biological negative impact is likely. The elevated TBT concentrations are found at the reference station and at two fish farm stations. Given that the reference station is situated in the middle of the ship lane in and out the bay, it is easy to understand why the reference station should be the most impacted by this pollutant that has been used in anti-fouling treatment of ships for many years. It is also apparent from the comparisons (Table 3.6) that all copper concentrations are within the lower and upper boundary of a provisional EQC(OSPAR), even at the reference station. Taking a closer look, however, reveals that some sites have higher copper concentrations than others, and this occurs at stations that are simultaneously impacted by other pollutants. It appears that copper is a bit special in that it probably occurs in relatively high natural background concentrations in Faroe Islands and at the same time is a pollutant that may be added by anthropogenic activity - in this instance via the fish farming. An example of a group of pollutants that is not naturally occurring and does not have other sources in the area is the antibiotics. The analyses have revealed that the antibacterial agents remain in the environment for years after administering this to the fish in feed. The antibiotics thus appear to be persistent in the sediments where they also may retain their antibacterial activity. However, the concentrations measured were low compared with effect concentrations and are not assumed to pose an imminent environmental threat.

It is also important to note that the study has indicated that environmental impact may extend beyond the present day reference stations applied in the environmental monitoring around the farm which is required by the regulations. These findings of a wider impact area indicate that it is necessary to have reference values or classification criteria from an area that may be located outside the fjord or bay in question when evaluating results from the fish farm environmental monitoring.

Table 3.6 The environmental status of sediments sampled in Gøtuvík bay is described in terms of how the measured pollutant concentrations compare to published classification criteria. The classification criteria used are Environmental Quality Criteria, EQC. >EQC means that the measured concentration exceeds EQC (OSPAR)* or EQC (US-EPA); ~ means within the lower and upper boundary of a provisional EQC (OSPAR)

Station	L 4	L 6	L 8	L 11	Ref	B 1	MPM	B 11	B 4
TBT	>EQC	>EQC			>EQC				
PAH	<	<	<	<	<	<	<	<	<
Naphthalene	<	<			<				
Phenanthrene	<	<			<				
Benzo[a]pyrene	<	<			<				
DDE	<	<	<	<	<	<	<	<	<
Dieldrin	<	<	<	<	<	<	<	<	<
PCB7	<	<	<	<	<	<	<	<	<
Cadmium	<	<	<	~	<	<	<	<	<
Copper	~	~	~	~	~	~	~	~	~
Mercury	<	<	<	<	<	<	<	<	<
Lead	<	<	<	<	<	<	<	<	<
Zink	<	<	<	<	<	<	<	<	<

* EQC(OSPAR) is given for a reference content of 1% organic carbon. The estimated organic carbon in the Gøtuvík sediments is approx. 2.5 times this amount. Thus, for the present purposes, the measured concentrations may be divided by 2.5 to be representative in the present comparison.

Table 3.7 Results of the chemical analyses of sediment samples from Gøtuvík fish farms in 2005

ID		06018284	06018285	06018286	06018288	06018289	06018291	06018297	06018299	06018304
Compound	*Unit*	*L 4*	*L 6*	*L 8*	*L 11*	*Ref (L and B)*	*B 1*	*MPM*	*B 11*	*B 4*
Dry weight	%	59.2	63.5	62.3	62.8	62.6	62.7	60.8	63.7	62.8
Remains after ignition	% of DW	92.9	93.7	93.3	93.2	93.6	93.6	93.2	94.7	94.6
Loss on ignition	g/kg DW	71	63	67	68	64	64	68	53	54
Phosphor, P	g/kg DW	0.96	1.2	0.54	2.6	0.58	0.51	2.5	2.1	0.55
Nitrogen, N	g/kg DW	1.6	1.3	1.4	1.7	1.2	1.4	1.9	1.4	1.1
Lead, Pb	mg/kg DW	3.1	3	2.9	3.4	3.5	3.2	2.4	2.6	3.4
Cadmium, Cd	mg/kg DW	0.13	0.12	0.073	0.26	<0.075	0.095	0.12	0.1	<0.076
Copper, Cu	mg/kg DW	58	57	54	64	60	49	110	55	55
Mercury, Hg	mg/kg DW	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
Nickel, Ni	mg/kg DW	42	42	40	44	41	38	42	41	41
Zink, Zn	mg/kg DW	50	46	38	73	43	35	72	70	41
Dieldrin	ng/g DW	0.17	0.03	0.03	0.04	0.09	<0.03	0.22	0.06	0.04
Hexachlorobenzene	ng/g DW	<0.1	<0.1	<0.1	0.06	<0.1	<0.1	<0.1	<0.1	<0.1
DDD-o,p	ng/g DW	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
DDD-p,p	ng/g DW	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.05	<0.1	<0.1
DDE-o,p	ng/g DW	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03
DDE-p,p	ng/g DW	0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.42	0.22	<0.05
DDT-o,p	ng/g DW	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
DDT-p,p	ng/g DW	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
HCH-alpha	ng/g DW	<0.01	<0.01	<0.01	0.023	<0.01	<0.01	<0.01	<0.01	<0.01
HCH-beta	ng/g DW	<0.01	<0.01	<0.01	0.006	<0.01	<0.01	<0.01	<0.01	<0.01
HCH-gamma	ng/g DW	<0.01	<0.01	<0.01	0.015	<0.01	<0.01	<0.01	<0.01	<0.01
Chlordane-alpha	ng/g DW	<0.01	<0.01	<0.01	0.011	<0.01	<0.01	0.057	0.04	<0.01
Chlordane-gamma	ng/g DW	<0.01	<0.01	<0.01	0.02	<0.01	<0.01	0.085	0.08	<0.01
Toxaphene, Par 50	ng/g DW	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.027	<0.01	<0.01
2378 TCDD	ng/kg DW	<2	<2	<2	<2	<2	<2	<2	<2	<2
12378 PeCDD	ng/kg DW	<2	<2	<2	<2	<2	<2	<2	<2	<2
123478 HxCDD	ng/kg DW	<2	<2	<2	<2	<2	<2	<2	<2	<2
123678 HxCDD	ng/kg DW	<2	<2	<2	<2	<2	<2	<2	<2	<2
123789 HxCDD	ng/kg DW	<2	<2	<2	<2	5.3	<2	<2	<2	<2
1234678 HpCDD	ng/kg DW	4.9	<2	3.1	3.3	6.2	2.6	3.4	<2	<2
OCDD	ng/kg DW	15	9.2	13	22	20	8	10	6.7	12
2378 TCDF	ng/kg DW	<2	<2	<2	<2	<2	<2	<2	<2	<2
12378 PeCDF	ng/kg DW	<2	<2	<2	<2	<2	<2	<2	<2	<2
23478 PeCDF	ng/kg DW	<2	<2	<2	<2	<2	<2	<2	<2	<2
123478 HxCDF	ng/kg DW	<2	<2	<2	<2	<2	<2	<2	<2	<2

To be continued

123678 HxCDF	ng/kg DW	<2	<2	<2	<2	<2	<2	<2	<2	<2

ID		06018284	06018285	06018286	06018288	06018289	06018291	06018297	06018299	06018304
Compound	*Unit*	*L 4*	*L 6*	*L 8*	*L 11*	*Ref (L and B)*	*B 1*	*MPM*	*B 11*	*B 4*
123789 HxCDF	ng/kg DW	<2	<2	<2	<2	<2	<2	<2	<2	<2
234678 HxCDF	ng/kg DW	<2	<2	<2	<2	<2	<2	<2	<2	<2
1234678 HpCDF	ng/kg DW	3.8	<2	<2	<2	5.4	<2	<2	<2	<2
1234789 HpCDF	ng/kg DW	<2	<2	<2	<2	<2	<2	<2	<2	<2
OCDF	ng/kg DW	4.2	<2	<2	<2	5.1	<2	2.6	<2	<2
I-PCDD/F-TEQ LB	ng/kg DW	<2	<2	<2	<2	<2	<2	<2	<2	<2
I-PCDD/F-TEQ UB	ng/kg DW	5.8	5.8	5.8	5.8	6.2	5.8	5.8	5.8	5.8
Rec 2378 TCDD	%	88	81	81	81	80	80	78	86	84
Rec 12378 PeCDD	%	91	92	90	92	93	89	98	102	93
Rec123478 HxCDD	%	84	93	89	92	104	92	90	93	106
Rec123678 HxCDD	%	84	77	71	86	75	75	76	79	62
Rec1234678HpCDD	%	55	61	63	53	75	66	44	46	73
Rec OCDD	%	23	36	59	31	63	56	22	36	60
Rec 2378 TCDF	%	86	82	82	85	84	82	83	86	84
Rec 12378 PeCDF	%	97	95	98	101	103	88	102	107	92
Rec 23478 PeCDF	%	98	94	96	129	101	91	96	107	97
Rec 123478 HxCDF	%	99	78	99	100	104	75	104	96	112
Rec 123678 HxCDF	%	62	59	65	75	60	61	66	55	45
Rec 123789 HxCDF	%	56	47	53	70	57	55	67	62	57
Rec 234678 HxCDF	%	78	72	75	88	74	75	79	75	65
Rec 1234678HpCDF	%	52	59	64	55	73	68	46	52	69
Rec 1234789HpCDF	%	52	56	59	42	67	60	34	39	63
Rec OCDF	%	18	28	45	20	51	49	15	23	41
PCB#77	ng/kg DW	<2	<2	<2	5.4	<2	<2	2.8	2.1	<2
PCB#81	ng/kg DW	<2	<2	<2	<2	<2	<2	<2	<2	<2
PCB#105	ng/kg DW	18	9.3	7	41	8.8	4.8	48	35	7.3
PCB#114	ng/kg DW	<2	<2	<2	<2	<2	<2	3.9	2.6	<2
PCB#118	ng/kg DW	38	21	16	83	28	12	115	77	21
PCB#123	ng/kg DW	<2	<2	<2	<2	<2	<2	<2	<2	<2
PCB#126	ng/kg DW	<2	<2	<2	<2	<2	<2	<2	<2	<2
PCB#156	ng/kg DW	7.8	3.2	4.3	23	5.5	2.8	16	18	3.4
PCB#157	ng/kg DW	3.4	<2	<2	3.7	<2	<2	5.5	5.9	<2
PCB#167	ng/kg DW	4.9	<2	2.7	8.6	3	<2	11	11	<2
PCB#169	ng/kg DW	<2	<2	<2	<2	<2	<2	4	6.3	<2
PCB#189	ng/kg DW	<2	<2	<2	2.7	<2	2.7	<2	4.2	<2
PCB WHO-TEQ	ng/kg DW	<0.01	<0.01	<0.01	<0.03	<0.01	<0.01	<0.07	<0.09	<0.01
PCB WHO-TEQ	ng/kg DW	0.23	0.23	0.23	0.25	0.23	0.23	0.27	0.29	0.23
PCB#28	ng/kg DW	22	8.4	8.1	33	13	7.7	33	15	8.1
PCB#52	ng/kg DW	11	4.8	3.1	16	5.4	4.3	37	16	2.9
PCB#101	ng/kg DW	29	16	15	4.5	26	9.5	75	33	15
PCB#118	ng/kg DW	39	20	15	83	28	12	110	77	21
PCB#138	ng/kg DW	52	28	28	130	52	20	170	30	27
PCB#153	ng/kg DW	78	40	43	180	68	25	210	150	39
PCB#180	ng/kg DW	38	33	23	81	12	16	150	17	13
PCB, Sum 7 congeners	ng/kg DW	270	150	130	530	200	94	780	340	130
PCB7/ LOI	ng/g DW	3.80	2.38	1.94	7.79	3.13	1.47	11.47	6.42	2.41
Benzo(a)anthracene	ng/g DW	<10	17	<10	<10	<10	<10	<10	<10	<10
Benzo(a)pyrene	ng/g DW	<10	27	<10	<10	<10	<10	<10	<10	<10
Benzo(b)fluoranthene	ng/g DW	<10	44	<10	12	17	17	13	12	16
Benzo(k)fluoranthene	ng/g DW	23	17	11	<10	<10	<10	<10	<10	<10
Chrysen/trifenylen	ng/g DW	<10	23	<10	<10	<10	12	<10	<10	<10
Dib(a,h)anthracene	ng/g DW	<10	<10	<10	<10	<10	<10	<10	<10	<10
Ind(1,2,3-cd)pyrene	ng/g DW	12	<10	<10	<10	<10	<10	<10	11	10
PAH, sum carcinogenic	ng/g DW	. 35	. 130	. 11	. 12	. 17	. 29	. 13	. 23	. 26
Acenaphthene	ng/g DW	<10	<10	<10	<10	<10	<10	<10	<10	<10
Acenaphtylene	ng/g DW	<10	<10	<10	32	<10	<10	<10	<10	<10
Antracene	ng/g DW	<10	<10	<10	<10	<10	<10	<10	<10	<10
Benz(ghi)perylene	ng/g DW	<10	<10	<10	<10	<10	<10	<10	<10	<10
Phenanthrene	ng/g DW	15	<10	<10	<10	<10	<10	<10	<10	<10
Fluoranthene	ng/g DW	21	22	<10	<10	<10	22	<10	<10	17
Fluorene	ng/g DW	23	25	28	<10	19	21	30	28	25
Naphthalene	ng/g DW	<100	<100	<100	<100	<100	<100	<100	<100	<100
Pyrene	ng/g DW	15	25	<10	<10	11	21	<10	<10	17
PAH, sum	ng/g DW	74	72	28	32	30	64	30	28	59
Benzo(e)pyrene	ng/g DW	<10	23	<10	<10	<10	<10	<10	<10	<10

To be continued

Perylene	ng/g DW	<10	<10	<10	<10	<10	<10	<10	<10	<10

ID		06018284	06018285	06018286	06018288	06018289	06018291	06018297	06018299	06018304
Compound	*Unit*	*L 4*	*L 6*	*L 8*	*L 11*	*Ref (L and B)*	*B 1*	*MPM*	*B 11*	*B 4*
Coronen	ng/g DW	<10	11	<10	<10	<10	<10	<10	<10	<10
Tributyltin	ug Sn/kg DW	1	1.5	<1	<1	4.9	<1	3	<1	<1
TeBDE#47	ng/g DW							<0.01	<0.01	
PnBDE#85	ng/g DW							<0.01	<0.01	
PnBDE#99	ng/g DW							<0.01	<0.01	
PnBDE#100	ng/g DW							<0.01	<0.01	
HxBDE#138	ng/g DW							<0.01	<0.01	
HxBDE#153	ng/g DW							<0.01	<0.01	
HxBDE#154	ng/g DW							<0.01	<0.01	
DecaBDE#209	ng/g DW							<0.01	<0.01	

4. Antibiotic resistance in sediment bacteria

This activity was closely co-ordinated with activity 3: Analysis of the content of veterinary medicines and persistent organic pollutants in sediment and biota at the model fish farm Bakkafrost in Gøtuvík

At the meeting, the content of this task was discussed. It was decided to initiate a literature review on antibiotic resistance as a result of antibiotic treatment.

At the meeting, the general use of antibiotics at the model fish farm Bakkafrost in Gøtuvík was discussed. It seemed that no antibiotics or veterinary medicines in general have been used during the last year, in which the fish have been present. It was thus decided that if the experimental activity should be carried out, the antibiotic resistance should be investigated on seabed samples taken all over Faeroe Islands and thus co-ordinated with the yearly monitoring programme from the Faroese authorities.

4.1 Introduction

The use of antibiotics in marine fish farms for the treatment of bacterial fish diseases may have a number of adverse effects, e.g. contamination of the environment around the marine fish farms with antibiotics, antibiotics-resistant bacteria and development of resistant fish pathogens, which may impede future treatment. Furthermore, the resistant properties may be transferred to humans - either directly via the treated fish or indirectly via the environment - and potentially impede the treatment of human infections.

Presence of antibiotic resistance at marine fish farming

The presence of antibiotic resistance in marine fish farms has been investigated since the 1980's (Torsvik et al., 1988, quoted in Kerry et al., 1995), especially by three research groups from Norway, Ireland and Washington, USA.

Nygaard et al. (1992) investigated the presence of antibiotics-resistant bacteria in sediments, which had been experimentally treated with oxytetracycline and oxolinic acid and laid out on the seabed. They found that the share of bacteria, which were resistant, increased to approx. 16% or about three times higher than the background levels found.

Samuelsen et al. (1992) investigated the sediment under three cages for a period of 18 months after they had been treated with oxytetracycline for ten days. They found that all investigated bacteria were resistant. After 72 days, the share of resistant bacteria was about 20%. The share of resistant bacteria stabilized at a level between 10% and 50%.

Kerry et al, (1994) investigated the presence of resistance towards oxytetracycline in the sediment under two cages (Galway Bay 53°15'95''N, 09°44'3''W). Below one cage, in which 865 g oxytetracycline had been applied per day for ten days, the share of resistant bacteria was not significantly higher than the background level of 1.2 ±1.8%. Under the other cage, which had been treated with 175 kg over 12 days, the share of oxytetracycline-resistant bacteria was 16.0 ± 8.9%, which is considerably higher than the background level. Furthermore, they found that the share of resistant bacteria decreased exponentially with a half-life of 26 days. An increased share of resistant bacteria was observed up to 75 meters from the cages but, after 33 days, increased resistance could only be observed directly under the cages. Increased resistance was also found in sediment samples, in which no oxytetracycline could be detected.

In further investigations of two marine fish farms in the Galway Bay, Kerry et al. (1995) found an increase in resistance of the same magnitude as the one found earlier (Kerry et al., 1994). The investigations showed an unexpected rise 24 days after treatment. An analysis of the fish feed showed that it contained oxytetracycline-resistant. Based on a phenotypic characterization of the resistant bacteria and detection of bacteria in mussels above the sediment, it was rendered probable that the resistant bacteria originated from fish faeces or from the fish feed. Later, Kerry et al. (1997) found that the share of resistant bacteria in the intestine of *Salmo salar* L. was not increased during the antibiotic treatment, and they concluded that the increased presence of resistant bacteria at antibiotic treatment was probably due the bacteria in the not-eaten part of the feed.

Herwig et al. (1997) investigated the presence of resistance under three marine fish farms in Puget Sound, Washington. At Farm A, which applied the highest amounts of antibiotics, the share of oxytetracycline-resistant bacteria before dosing was about 1%. The share increased to between 3% and 9% after dosing. Resistance towards Romet 30 (a mixture of ormetoprime and sulfadimethoxine) had a tendency to follow the results of oxytetracycline although Romet 30 was statably only dosed in limited amounts. At Farm B, the share of oxytetracycline-resistant bacteria was between 1% and 10%. At Farm C, which had the lowest consumption of oxytetracycline, the share was 5% or less. The results confirm that the presence of antibiotics-resistant bacteria increases with increasing consumption. Investigations of sediments, which had not been impacted by antibiotics from marine fish farms, showed that the share of oxytetracycline-resistant bacteria was 0.6% on an average.

The conclusion from the investigations is that the antibiotic treatment impacts the environment in and around the marine fish farms as there is a distinct correlation between the application of antibiotics and the presence of oxytetracycline-resistant bacteria. The most obvious explanation of the impact is that the presence of antibiotics offers a competitive advantage to the resistant bacteria, which will thus dominate during antibiotic treatments. Another explanation, which is partly supported by the investigations from Ireland, may be that the resistant bacteria are introduced with the fish feeds. In the quoted investigations, the methods used to isolate and characterize the resistant bacteria have not been able to clarify the causal relation.

Risk of transfer of resistance genes between the aquacultural environment and human/animal environment

Data on impact of human disease treatment lacked in 1994 but Smith et al. (1994) estimated that the impact was very small. However, various more recent investigations, in which molecular biological techniques were applied, have shown that there is a risk of transfer of resistance between aquacultural environments, livestock and humans. Rhodes et al. (2000) showed that genes coding for tetracycline resistance are exchanged between the fish pathogen, *Aeromonas salmonicida*, and the human pathogens, *A. hydrophila*, *A. caviae* and *E. coli*, and that a transport of these genes occurs between the aquacultural environment and humans. Similar results were obtained by Furoshita et al. (2003) for oxytetracycline. Recently, tetracycline resistance (*tet*(C)) was found with the intracellular pathogen *Chlamydia suis. Tet*(C) originates from *Aeromonas salmonicida* (Dugan et al., 2004). Resistance to trimetroprim and sulfonamide is also shared between *Aeromonas salmonicida* and *E. coli/Salmonella*.

Molecular and epidemiological evidence has demonstrated that the antibiotic determinants of *Salmonella enterica* DT104, a multiresistant *Salmonella*, probably originates from aquaculture in the Far East (Cabello, 2006). Also, resistance to quinolones and fluoroquinolones is likely to originate from aquatic bacteria (Robicsek et al., 2006).

It is thus likely that the use of antibiotics in fish farms increases the risk of presence of resistant bacteria in human and livestock and hence is a matter of concern.

4.2 The use of antibiotics in aquaculture

The use of antibiotics in aquaculture in Iceland, Faroe Islands, Norway and Denmark is presented in Tables 1.1-1.4. The use varies among the four countries and between years. Of the five antibiotics used, two, oxytetracycline and trimethoprim/sulfonamide, are also used for treatment of human infections and are therefore those of main concern in relation risk of impeding the treatment of human diseases.

Oxytetracycline is used in aquaculture in Faroe Islands and Norway. The estimated use of oxytetracycline for human treatment (calculated from the human use in Denmark in 2003 multiplied by the population ratio) is 13 kg in Faroe Islands and 1,300 kg in Norway. Hence, in Faroe Islands, the use of oxytetracycline by far exceeded the human use until 2003. In Norway, the human use exceeds the use in aquaculture by a factor of approx. 100.

In Denmark, the use of sulfadiazine/trimethoprim was between 600 kg and 1,300 kg per year between 2001 and 2004. For comparison, the use for human treatment is: Trimetroprim (and derivatives) in combination with sulfoamides 269 kg, sulfonamides, 3,000 kg and trimethoprim (and derivatives) 307 kg. Hence, the aquacultural use of sulfadiazine/trimetroprim is significant compared to the use for human treatment.

Considering the risk of transfer of tetracycline, sulfonamide and trimethoprim resistance between the aqueous and human environments, and the significant uses of these antibiotics, especially in Faroe Islands and Denmark, actions should be taken to reduce the use to a minimum.

The use of quinolones in aquaculture has been totally restricted in the industrialized countries because they are a highly effective group of antibiotics for treatment of human infections and because quinolones may generate cross-resistance among other members of the quinolones (Capello, 2006; WHO, 1998). However, oxolinic acid belongs to the first generation of quinolones and is used in both Norway and Denmark in relatively high amounts, and its use is of concern. It is suggested to examine the occurrence of resistance to other quinolones and fluoroquinolones in locations were oxolinic acid is used. In addition, oxolinic acid may not be the best choice for treatment since it is a first generation quinolone, and its use in aquaculture should be reconsidered.

The quinolones are used extensively in Chile and quinolone resistance has emerged in China as an important public health problem as a result of the unrestricted use of this group of antibiotics in aquaculture and in industrial animal husbandry (Capello, 2006).

Considering the risks associated with the use of antibiotics, a very strict use of antibiotics could be used by the Nordic fish farming industry in the marketing of high quality products.

4.3 Laboratory studies

Based on the concerns raised by the literature review and discussions in the group, it was decided to analyse for the presence of resistance at selected locations at Faroe Islands. Oxytetracycline was selected because it has been used widely, and oxolinic acid, which has been used only to a minor extent. Since the literature suggests that the resistant bacteria may

be introduced via the fish feed, it was decided to analyse antibiotic resistance in fish feed as well.

Materials and methods

Determination of microbial resistance

Samples were taken on 15 June 2006 below the fish farms at the northern arm in Gøtuvík, at Bakkafrost station 16, in Fuglafjørð station 19 and in Pollurin off Klaksvík. The sampling was made by personnel from two parties that normally perform environmental monitoring around fish farms. The sampling was coordinated by the Food- Veterinary- and Environmental Agency of Faroe Islands. The samples were taken by Haps with plastic cylinders with a diameter of approx. 5 cm. Subsamples consisting of approx. half of the upper 5 cm of the sediment core were made in a makeshift laboratory in Fuglafjørð by qualified personnel. Subsamples were stored in PE bags (Minigrip®) at 1-5°C. The samples were delivered on 16 June 2006 at DHI in a cooler box by Maria Dam.

The coordinates of the sampling sites are shown in Table 4.1.

Table 4.1 Sediment sampling sites on Gøtuvík, Fuglafjørð and Pollurin, Klaksvík, 15 June 2006

Fjord area	Station ID	Latitude, N	Longitude, W	Water depth, m	Station description
Gøtuvík, Bakkafrost	"Reference" (pos. 9)	62°10'94'' N	6°43'03'' W	39	Sand
Gøtuvík, Bakkafrost	16	62°11'25'' N	6°42'22'' W	42	Sand
Fuglafjørð	"Reference" I	62°13'85'' N	6°48'30'' W	24	Clay
Fuglafjørð	19	62°13'88'' N	6°48'53'' W	25	Clay
Pollurin, Klaksvík	"Reference" (Pol-Ref)	62°14'78'' N	6°36'43'' W	41	Sand
Pollurin, Klaksvík	7 (Ringur 7)	62°15'10'' N	6°35'67'' W	40	Sand/mud

At Gøtuvík and Pollurin, the "reference" samples were taken approx. 900 m SW of the fish farms, and at Fuglafjørð approx. 200 m SE of the fish farm. As the fish cages are moved from year to year and several cages are located in the same fjords, the "reference" samples cannot be considered as being unaffected by antibiotics. The "reference" samples are therefore in quotation marks.

Sampling pre-treatment

In the laboratory, equal sized aliquots (as judged by eye) from the subsamples were mixed into one composite sample for each sampling site.

Analysis of number of resistant heterotrophic plate count

The composite samples were spread on marine agar 2216 with 25 mg/L of OTC (Kerry et al., 1994) and 10 mg/L of OXA (Smith, 1998) and compared to samples spread on agar 2216 without antibiotics.

Approx. 16 g (WW) were transferred to 90 mL of saline phosphate buffer (pH 7.2, 28 g/L NaCl) and mixed vigorously on a vortex shaker for 1 min. The tube was left for 1 min to allow large particles to sediment. 1 mL was transferred to 9 mL saline phosphate buffer for decimal dilutions. 0.1 mL of the dilutions was spread in duplicate on agar without or without antibiotics. The dry matter content of the samples was determined.

The plates were incubated at 22 ± 1°C and read after 72 ± 4 hours.

Determination of minimum inhibition concentrations (MIC)

The minimum inhibition concentration of oxytetracycline was determined for 60 different bacterial isolates. From each sampling site, 5 bacterial strains were isolated from agar plates with 25 mg oxytetracycline/L and 5 bacterial strains were isolated from agar plates without oxytetracycline on marine agar 2216. The isolates were transferred to a marine bouillon containing 20 g NaCl/L, 5 g peptone/L, 1 g yeast extract/L and 50 mL of seawater from the North Sea to provide minerals. From here, the isolates were transferred to a serial 2-fold dilution of oxytetracycline in the marine bouillon (32 mg/L to 0.06 mg/L) in microtitre plates. The plates were incubated at 22°C for 72 hours. Bacterial growth in the wells of the microtitre plates was detected by visual inspection after 24, 48 and 72 hours. The lowest concentration without growth is defined as the minimum inhibition concentration.

Screening of fish feed for heterotrophic bacterial count

A sample of fish feed F6 was delivered by Magnus Pauli at DHI on 29 May 2006 to the laboratory for initial tests. The fish feed was obtained from Bakkafrost. F6 is widely used as fish feed in Faroe Islands.

Four different agars were tested to determine the optimum conditions for determining the heterotrophic plate count in the fish feed: Marine agar 2216 was tested for comparison with the results from the sediments samples. Beef extract agar was tested because it was considered the commercially available agar with a composition most similar to fish feed. Lab Lemco agar was used as a representative of a common nutrient rich agar. Finally, a fish feed agar was produced by grinding 10 g of fish feed in Millipore water. The fish feed was added to 1 L of Millipore water with 15 g/L of agar. pH was adjusted to 7.4 and autoclaved at 121°C for 15 min.

1 g of fish feed was suspended in 9 mL of saline phosphate buffer (pH 7.2, 28 g NaCl/L) and mixed well until evenly distributed. A row of decimal dilutions to 10^{-4} was produced, and 0.1 mL was spread in dupli-

cate on the four agars. 1 mL of 10^{-1} and 10^{-2} dilutions was spread on 14-cm Petri dishes as well. Plates were incubated at 21°C for 7 days.

4.4 Results

Antibiotic resistance heterotrophic plate count

The results of determination of the antibiotic resistance heterotrophic plate count after 3 days of incubation are shown in Table 4.2

Table 4.2 Heterotrophic plate count and resistance plate count at 25 mg oxytetracycline/L and 10 mg oxolinic acid/L. Incubated in 72 hours at 22°C.

	Total	Oxytetracycline	Oxolinic acid	Oxytetracycline	Oxolinic acid
	CFU/g ww	CFU/g ww	CFU/g ww	% resistant	% resistant
Gøtuvík Bakkafrost st. 16	66,000[1)]	24,000	200[1)]	36	0.3
Gøtuvík duplicate	37,000[1)]	18,000	540	49	1.5
Gøtuvík Bakkafrost st. 16 “reference”	27,000[1)]	26,000	620	96	2.3
Gøtuvík ref duplicate	24,000[1)]	32,000	700	133	2.9
Fuglafjørð st. 19	28,000[1)]	11,000	810	39	2.9
Fuglafjørð st. 19 “reference”	30,000[1)]	15,000	1,300	50	4.3
Pollurin, Klaksvík	30,000[1)]	25,000	720	83	2.4
Pollurin “reference”	160,000	76,000	790	48	0.5

[1)] Based on a low number of colonies.

It can be seen that the total number of culturable bacteria in the sediment is between 24,000 CFU/g and 160,000 CFU/g. The total number of CFU was lower than expected. Therefore, the dilution of the samples was too high, and the number of colonies on the agar plates was below 20. Consequently, the uncertainty of the results is relatively high. This can also be seen on the relatively large difference between the duplicate runs, which were carried out on the samples taken from Gøtuvík Bakkafrost station 16. Duplicate analyses were carried out to get an idea of the analytical variation. The percentage of oxytetracycline-resistant bacteria is between 36% and 133% of the total number while the number of oxolinic acid-resistant CFU is between 4.3% and 0.3%.

Because of the too high dilution, the analysis was repeated on 30 June 2006, two weeks after receipt of the samples. The results of this analysis showed a much higher number of CFUs, a higher percentage of oxytetracycline-resistant bacteria and a lower percentage of oxolinic acid-resistant bacteria. These data are not shown.

Because of the unusually high percentage of oxytetracycline-resistant bacteria, it was decided to characterize the bacteria with respect to the minimum inhibition concentration (MIC). The MIC of a specific bacterial

isolate is defined as the lowest concentration of the antibiotic, which inhibits its growth. A total of 57 isolates were characterized. The isolates had a MIC of 4 µg/mL or lower, except for one isolate with a MIC of 16 µg/mL. Seventeen strains were analysed in duplicate. It was found that 16 of the 17 strains gave the same result or were only different by one dilution. The 17th strain was different by 2 dilutions. There were no difference in MIC values of bacterial strains isolated from agar plates with 25 mg oxytetracycline/L and agar plates without addition of oxytetracycline.

Heterotrophic plate count in fish feed F6

The results of the analyses of heterotrophic plate counts are shown in Table 4.3.

Table 4.3 Results of heterotrophic plate count in fish feed F6

Agar	HPC (CFU/g)
Marine agar 2216	36
Beef extract agar	75
Lab Lemco agar	49
Fish feed agar	18

The heterotrophic plate count in the fish feed is low. The numbers are results of count of very few colonies (less than 10), which means that the standard deviation of the results is high.

4.5 Discussion

In this study, we found between 2.4×10^4 CFU/g and 1.6×10^5 CFU/g of total heterotrophic plate count. Pursell et al. (1996) found heterotrophic plate counts in sediments associated with fish farms in Ireland between 9.8×10^4 and 1.8×10^5 CFU/g, and Kerry et al (1996) found between 4.8×10^4 and 1×10^5 CFU/g. These studies have used the same methods as this study, which allows for direct comparison of the results. The results obtained in this study is therefore well in line with the literature results. It was expected that the heterotrophic plate count would be higher below the fish farms than at “reference” stations. This seems not to be the case.

The numbers of oxytetracycline-resistant bacteria were found to be between 1.1×10^4 and 7.6×10^4 CFU/g corresponding to between 39% and 115%. The percentage of resistant bacteria is relatively high compared to results from literature, which are usually 20% or below (Nygaard et al., 1992; Kerry et al., 1994; Herwig et al., 1997) except for some studies which have shown that all bacteria were resistant following treatment (Samuelsen et al., 1992).

The percentage of resistant bacteria was lower at the "reference" station at Pollurin than it was below the corresponding fish farm whereas they were higher at "reference" stations at Gøtuvík and Fuglafjørð. The high occurence of resistance at the "reference" stations indicated that they have been affected by the antibiotics and cannot be used as true references.

The background levels of the "reference" stations found in this project are very high. Kerry et al. (1994) analysed 153 sediment samples unaffected by antropogenic influence and found 1.2% resistant bacteria as an average, and Nygaard et al. (1992) found a background level of 5%. The reason for the high background levels in our study could be that an excessive amount of oxytetracycline has been used over the years leading to a generally high occurence of resistance in the area.

The agar used in this study is a marine agar with 92 mM Mg^{2+} and 16 mM Ca^{2+}. The high Mg^{2+} and Ca^{2+} content may decrease the activity of oxytetracycline (Lunestad and Goksøyr, 1990) and lead to the high occurence of resistance. The MIC determinations support this view. However, our method is identical to the methods used by Kerry et al. (1994) and the results are expected to be comparable. Considering the concerns raised in the literature review regarding oxytetracycline resistance, it is important to perform further studies to confirm or disconfirm our results. The studies should include determination of the level of resistance in fjords unaffected by antibiotics.

Nygaard et al. (1992) studied the resistance towards oxolinic acid in boxes with test sediments placed at 20 m depths in the Oslo Fjord. The boxes were administered 10 ppm of oxolinic acid and the percentage of oxolinic acid-resistant bacteria were determined after 10 and 12 months on TSA medium with 70% seawater and 10 µg oxolinic acid/L. Plates were incubated for 3 days at 18°C. They found a level of 7% ± 1% resistant before addition of oxolinic acid, 14% ± 2% after 10 months and 20% ± 2% after 12 months. The results of our studies indicate even lower levels of resistance to oxolinic acid than reported by Nygaard et al. (1992). The results are in accordance with the fact that oxolinic acid has not been in use in Faroe Islands since 2000 (Section 1.8).

Four different agars were used to find an optimum medium for the detection of culturable bacteria in the fish feed F6. None of the media yielded significant amounts of culturable bacteria. These results are significantly lower than what others have found. Kerry et al. (1997) found $1.3 \times 10^4 \pm 1 \times 10^4$ CFU per g fish feed on marine agar 2216 and Herwig et al. (1997) found 1.9×10^3 to 4.0×10^3 CFU pr. g fish feed on MHS agar (15°C, 7 days). The low number of cultivable bacteria in the fish feed indicates that bacteria in the fish feed F6 do not contribute significantly to the antibiotic resistance in the sediments below fish farms.

4.6 Conclusions

Fish feed

The number of culturable bacteria in the examined fish feed (F6) is very low and is unlikely to contribute significantly to the resistance found below the fish farms in Faroe Islands.

Oxytetracycline

It can be concluded that the measurements of oxytetracycline resistance showed a very high percentage of resistant bacteria both below the fish farms and at the "reference" stations up to 900 m away from the fish farms, indicating that oxytetracycline-resistant bacteria are abundant in fjords with fish farms. However, there is some doubt whether the activity of oxytetracycline in the test agar was sufficiently high. Further studies to determine background levels are suggested. In case of high abundance of resistant bacteria, a more thorough investigation including molecular and epidemiological studies is suggested.

Bearing the risk of transfer of resistance genes between aquaculture and human pathogens in mind, the use of oxytetracycline should be reconsidered for use in aquaculture.

Oxolinic acid

The occurrence of oxolinic acid resistance seems to be low and is not considered a large problem in Faroe Islands.

On the other hand, the use of oxolinic acid in Norway and Denmark may be problematic since cross-resistance to other quinolones and fluoroquinolones may evolve. Oxolinic acid is a first generation quinolone and better options may exist.

It is suggested to examine the occurrence of cross-resistance to other quinolones and fluoroquinolones in locations where oxolinic acid is in use. In addition, a review of the effectiveness of oxolinic acid and possible alternative antibiotics should be considered.

Sulfadiazine/trimethroprim

Considering the risk of transfer of sulfonamide and trimethoprim resistance between the aqueous and human environments their use should be reconsidered.

5. Development of recommendations for environmental monitoring programmes for veterinary medicines, help substances and persistent organic pollutants in Nordic fish farms including identifycation of relevant indicators

Abstract

The environmental monitoring around fish farms includes a very limited set of pollutants mainly restricted to nutrients and selected metals. A pilot study performed as part of the present assessment included a range of unintentionally added pollutants such as persistent organic pollutants, dioxin and oil-derived pollutants as well as intentionally added antibiotics. Analyses of this kind are expensive and may not be justified as parts of a regular monitoring scheme. The study revealed however, that certain pollutants correlated and it may thus be possible to choose a “cheap” parameter as a proxy for a more expensive one. This simplified monitoring would necessitate an initial study to ascertain inter-pollutants correlations because the dominant sources of pollutants will depend on the origin and composition of e.g. the fish feed and are not necessarily the same from one fish farm to the next. In addition, and quite important, monitoring that complies with the EU water framework directive may comprise the majority of pollutants associated with fish farming especially those unintentionally added to the environment. On the other hand, therapeutic agents will not be monitored in such a scheme but requires a specific effort - first of all, assessment of the magnitude of the potential impact by measurements of the concentrations of the therapeutic agent in the environment and risk assessments of ecosystem effects.

5.1 Introduction

The previous chapters of this report has treated various aspects of impact of fish farms on the surrounding environment, both those that may be

anticipated - through e.g. the addition of feed with or without added veterinary medicines to a fjord ecosystem - and those that are actually detected - like pollutants in vicinity of fish farms.

The pollutants accompanying fish farms are connected to the feed added, they are connected to the veterinary medicines and bactericidal/fungicidal and cleaning agents used – including those for pen maintenance - and to the traffic accompanying the running of the fish farms. In addition to this pollution, there is the potential for pollution with infectious agents that follows when boats sail from one farm area to another or when entire pens are towed to processing plants probably located in an other fjord and in several other situations when fish, fish-offal or water recently occupied by fish are being transported in the process of transforming fish in farms to fish in a dish. Also, the potential for genetic pollution carried by escapees from farms is a threat that continues to be a problem even though the number of salmon escaping has been kept at bay in many areas, but the issue needs renewed consideration when more escape-prone fish species are being raised in pens. Keeping track of how many fish that escape and from where should be regarded as a minimum of monitoring measures to follow the genetic pollution threat, but in the following discussion, only monitoring that is established for clear-cut environmental purposes will be discussed. Thus, the potential pollution, which may be described as spreading of diseases and parasites, is not part of the following either, other than as an issue of chemical pollution, which actually accompanies the warfare against bacteria and other disease-carrying agents when this is done by chemical means.

Restricting therefore this discussion to the chemical pollution, three source categories needs to be considered:

1) Fish feed
2) Veterinary agents including “cleaning” and antifouling chemicals
3) Transport

The addition of fish feed to a pen adds not only nutrient like nitrates and phosphates to the fjord ecosystem but also persistent organic pollutants, POPs, if the feed is fishmeal-based. In certain areas this import of POPs which follows the feed, can be quite marked, especially when fish used for the fishmeal production are taken in polluted areas like in European coastal waters. Also when fish from the North Sea is imported to low POP-background areas, the addition of POPs can be substantial. This import of pollutants may be reduced in future as fish producers pursue products with lower POPs concentrations following the general public demand for healthier seafood. It is no secret that, in particular, fish has been frequently in media focus, not only for its beneficial effects but also for the unquestionable negative effects of mercury and POPs, which are often found in elevated concentrations in certain fish species. However,

as regulation implementation and restriction in use limit the ambient marine environment POP level, it is anticipated that the import of POPs with feed to a farm area may decrease given that the production of fish mass remains the same. On the other hand, the addition of nutrients is likely to remain more or less constant when the ratio of feed to fish produced has reached the targeted low level. The importance of the added nutrients in terms of increasing the trophic status of the recipient may vary extremely from site to site depending on the water exchange rates and on the relative input of fish farm nutrients to those carried by the water masses into the fjord from natural sources in surrounding waters.

Present regulation in Norway is based on a set of environmental criteria and a standard environmental monitoring programme (Nolsøe, 2006). The regulation defines a guideline for environmental monitoring around fish farms as mandatory, being the Norwegian standard (NS 9410: 2000). The regulation is based on a tiered monitoring scheme and a definition of environmental condition index, in which the demand for monitoring in terms, of which parameters should be included and how frequently is defined as e.g. a function of environmental condition indices for a given area.

There are three environmental monitoring tiers; A, B and C, where Tier A is voluntary and, basically, a measurement of sedimentation of feed and faeces underneath the pens. Tier B is monitoring on sensory parameters and simple but descriptive physiochemical parameters to be performed on sediments just below or a few metres away from the fish farm. Tier C is a quantitative biodiversity study and monitoring of chemical parameters, which are intended to describe the overall environmental status and should include sampling stations also outside the immediate surrounding of the farm. In addition to defining the frequency of sampling, the guideline (NS 9410: 2000) also describes the number of samples to be taken, the general position of these sampling sites in relation to the main current direction in the farm, and requirements of quality assurance and control that must be met by the party performing the various tiers in the monitoring. In addition to defining the monitoring, the standard also defines what levels of environmental condition are acceptable and what are not, and thus defines the points at which regulatory measures must be taken to mitigate the unacceptable environmental condition.

The parameters included in the B module includes pH and redox potential (Eh), and sensory parameters like smell, sediment appearance with description of colour, general character, presence of gas bubbles, thickness of mud layer, whether fish feed and faeces are present and whether there are live animals present or not. These parameters are well suited for a first-glance assessment of environmental condition and provide a good indication of the extent of impact of the farming activities on the immediate area surrounding the farm.

The chemical analyses included in the C module include parameters like loss on ignition, total organic carbon, phosphorus, nitrogen, zinc and

copper in addition to particle size distribution, all analysed in sediment samples.

In Faroe Islands, the monitoring around fish farms is based on similar principles as the Norwegian regulation although in a more simplified version and includes a similar set of parameters to be analysed in sediment samples taken in the vicinity of the fish farms in addition to a reference station (Olsen and Hansen, 2003).

5.2 Pollutants found in a Faroese fish farm pilot study

In the present assessment, a pilot study on pollutants occurring in sediments in the vicinity of a fish farm in Gøtuvík, Faroe Islands, was performed (see Chapter 3). The study mainly incorporated the persistent organic pollutants, POPs, in addition to nutrients and other general physiochemical analyses (Table 5.1). Sensory parameters were not included other than as a basic guidance to assumed pollution level in the various samples. In addition, some samples were analysed for antibiotics-resistant bacteria and quantitative chemical analyses of the most commonly used antibacterial agents in Faroese fish farms in recent years (Chapters 3 and 4). Simultaneously, a detailed POPs uptake study was conducted on fish in one pen in the Gøtuvík northern arm fish farm where POPs were monitored in salmon as they grew from smolt to consumer size and in feed that were administered to these (Chapter 2).

The parameters analysed (Table 5.1) may be linked to various sources in the present Gøtuvík context although in another setting, these sources may not be the most prominent.

Due to budget limitations, most analyses were made using detection limits that would signal if a compound was present in marked elevated concentrations but not necessarily report the ambient concentration if this was at or close to background concentrations. At most locations sampled, pesticides were not detectable in the sediment. However, when they were detected, they tended to occur simultaneously. Thus, monitoring one of these pesticides or pesticide metabolites, e.g. the one occurring at highest concentrations, p,p'-DDE, would be a good proxy for the occurrence of legacy pesticides in the sediments. This method will of course only be pertinent if the pesticides in question have the same source, as in the present case where fish feed is the likely source. If this is not the case, like when traces of therapeutically used pesticides are the sought after pollutants, the assumption of a correlation to p,p-DDE would, of course, not be relevant. Dieldrin was exceptional in that it was detected in all but one sample, and then in concentrations at most being half of that of p,p'-DDE. Thus, it was the low detection limit of the analytical method that allowed dieldrin detection, not the magnitude of the concentrations as such.

Table 5.1 The compounds analysed in the pilot project focussed on the Gøtuvík fish farms of Faroe Islands in Activity 3.

Class of compound	Compounds analysed	Most probable prominent source in current context
Basic physiochemical parameters	Loss on ignition and dry mass	Fish feed and fish faeces
Nutrients	Phosphorus and nitrogen	Fish feed
Metals	Lead, cadmium, copper, mercury, nickel and zinc	Fish feed and pen maintenance
Pesticides and by-products or metabolites	o,p'- and p,p'-DDT, DDE and DDD, dieldrin, α-, β- and γ-HCH, α- and γ-chlordane, toxaphene (as parlar 50)	Fish-based feed
Chemicals and (unwanted) chemical by-products	Dioxin (2,3,7,8-substituted PCDD/DF), HCB, PCB both non- and mono-ortho substituted (i.e. the dioxin-like) and the seven congeners often denoted marker PCBs. Also, the flame-retardant PDBE was analysed in two samples	Fish-based feed
Oil-related compounds	The selection of PAH often denoted EPA-16, incl. naphthalene, plus perylene and coronene	Transportation (sailing)
Antifouling compounds	TBT and degradation products	Transportation (sailing)
Antibiotics	Oxytetracycline, oxolinic acid and flumequine	Therapeutic use

PCB concentrations in sediments around fish farms tended to follow pesticides as well but PAHs did not. Neither did TBT correlate with pesticides and PCBs. In fact, the highest concentration of TBT occurred in the reference station situated along the ship lane for traffic in and out the bay. Even dioxin concentrations tended to be higher at the reference station than in closer vicinity of the fish farms, indicating that fish farms are not a major dioxin source in this area even though it is a well-established fact that the dioxin content in fish feed may lead to elevated dioxin levels in farmed fish. Flame retardants, PBDE, share many features with the other POPs like PCBs and DDT and will occur in similar matrices as POPs. Samples from two stations with high PCB concentration were analysed for PBDE but none of the congeners were detected at 0.01 ng/g DW.

Analysing the contaminants that could be quantified reveals that the content of organic matter was not significantly correlated to the pollutants, meaning that other drivers than adsorption surface alone are determining the pollutants concentrations. Phosphorus was correlated to PCB both as CB 153 and as the sum of the 7 congeners often denoted marker PCBs. Phosphorus was also correlated to fluorene, one of the PAHs that were detected in all but one sample. Copper and nickel were correlated, and zinc and PCB and zinc and fluorene. Dieldrin was correlated to PCB. Dioxin, as OCDD, was not correlated to other detected pollutants.

The nutrients and metals that were found in elevated concentrations near the fish farms relative to background sites were phosphorous, nitrogen, lead, cadmium, copper and zinc.

Data on POPs in sediments in Faroese fjords in general, which could have been compared to the findings in the fish farm study, are very scarce. Thus assuming that the reference station may be seen as not affected by the aquaculture, it may be stated that in the vicinity of the fish

farm, the concentration of PCB was definitely elevated as were the contrations of pesticides including dieldrin, DDTs, HCBs, chlordane and toxaphene.

Also the concentrations of PAHs tended to be elevated near fish farms compared to the reference station although as already mentioned, it was not found to be correlated to the POPs.

TBT was found at concentrations exceeding environmental quality criteria (EQC) issued by the Oslo-Paris Convention for protection of the seas (OSPAR) or US-Environmental Protection Agency, but the source could not be linked to fish farms specifically. Copper concentrations were generally high compared to OSPAR EQC but except for one site, these concentrations were not markedly elevated compared to regional background values and this observation along with those of other studies indicate that a high copper background concentration is most likely a natural geological feature of the area. At the site with elevated copper concentration, also concentrations of other pollutants like POPs were markedly elevated.

Partly as part of the present project (Chapter 3) sediments from a number of fjords with either ongoing or a previous history of fish farming activity were analysed for antibiotics most commonly used in Faroe Islands (Chapter 1). The antibiotics analysed were oxytetracycline, oxolinic acid and flumequine, where the three antibiotics were detected in 5, 4 and 1 of the total 12 sediment samples, respectively, with detection limits of 0.07, 0.4 and 0.14 ng/g DW sediment for the three antibiotics, respectively. The antibiotics found at the highest concentration was oxolinic acid, at 2.9 ng/g DW, and the highest concentrations of oxytetracycline and flumequine found in these sediment samples were 0.27 and 0.29 ng/g DW, respectively. The highest concentrations of oxolinic acid were found in sediments from sites, which have been extensively used for fish farming for a number of years. Compared to the therapeutic dose concentrations of the antibiotics when administered to fish, the concentrations of antibiotics in sediments are low. Nonetheless, the results indicate that antibiotics remain in the environment surrounding the fish farm also years after farming activities have been discontinued. The present results are thus in accordance with those of earlier studies indicating that the degradation of antibiotics like oxolinic acid is very much slowed down in environments with reduced access to oxygen compared to degradation in anoxic sediments layers.

However, the results also disclose that the reference stations being used in some fjords are not sufficiently remote from the farming activities as to be rendered unaffected as traces of antibiotics were also detected at two reference stations. Whether this is due to re-suspension or current-mediated transport of fish feed is not known at present but the net effect is the same and the implication is that the interpretation of the monitoring results is flawed. Discussing distance of influence of a fish farm in terms

of metre is not relevant in partly strong current regimes as the Faroese fjords and sounds although in these cases, it can soon be established that the reference stations should have been placed further away from the farms. However, with the rather limited extents of these fjords and with the other criteria to a reference station restricting the choice of site - e.g. that of suitability for sampling soft bottom - it is hardly likely that a better reference site could have been found within these particular fjords.

5.3 Conclusion

It may be concluded from the present assessment that in addition to pollutants already included in the monitoring schemes presently applied in Faroe Islands and Norway, pollutants like PCB and pesticides of the POP type as well as PAHs and antibiotics are found in elevated concentrations in sediments around a fish farm. Although the concentrations of these pollutants may be correlated to parameters already included in the monitoring schemes, this does not mean that it will be possible to extrapolate from these nutrients and metals parameters to the ambient POPs concentration. The reason for this is that POPs are neither a necessary nor a constant part of the fish feed but the POP content depends to a large extent on the origin of the feed oil and protein base, whether produced from fish and if so, what species and from what area were the fish taken. A study of dioxin and PCB in pelagic fish, which are typically used in production of fish feed from the northeast Atlantic, revealed that in some species the dioxin content exceeded the EU limits for feed (Mundell et al., 2003) and would therefore not be applicable in feed production without some measures to cleanse or dilute the dioxin content in the fish oil base.

It is obvious that the current monitoring parameters required by regulation in Faroe Islands and according to the Norwegian Standard for Environmental monitoring of marine fish farms (NS 9410) do not by any means cover the pollutants that are added to the environment in connection with fishfarming activities. It is, however, realized that adding extra parameters to the analyses-to-do list, also increases the costs. Therefore, the question could perhaps be reduced to one of; what do we need to know?

In the EU directive 96/23/EC on residue monitoring in food of animal origin in the member states, the list of “need to know” chemicals includes stilbenes and steroids, chloramphenicol and other antibacterial substances like oxolinic acid, oxytetracycline and flumequine, anthelmintics like cypermetrine, deltametrine and emamectine, organochlorine compounds like PCB, DDT, HCB and dioxin, organophosphorus compounds like dichlorvos and azametiphos, metals as cadmium, lead and mercury, mycotoxines as aflatoxines, dyes and other compounds potentially used in the farming activities. In the EU Water framework directive (2000/60/EC), there is a

list of priority substances (Annex 10) that contains 33 pollutants that need to be monitored. For these 33 compounds plus an additional 8, the EU Commission has adopted a proposition that there be assigned environmental quality standards, EQS, for these pollutants in surface water (COM (2006) 397 final), where the term surface water includes coastal water where marine aquaculture takes place. In this adopted proposition, which will become a new directive, the EQS are defined in water and is as such probably quite a challenge for the chemical analysts. The purpose is, however, unmistakably to protect the natural environment and it does not seem unlikely that analytical methods, which could even rely on analyses on sediments, will be developed to meet the demands of the new directive. On the EU water framework directive list of prioritized compounds, we find members of the PAHs, the PBDE, cadmium, lead, mercury and nickel, HCB, γ-HCH (lindane) and TBT, which have been identified as pollutants that likely accompany a fish farm either by direct measurements or by knowledge of source and route of exposure. If the additional eight substances that are included in the directive for EQS are included, it means that also analyses of DDT and dieldrin would be of common interest both to a fish farm monitoring programme and to the implementation of the EU regulation. Thus, there is ample room for rationalizing by combining efforts and means so that the monitoring of fish farms both provides a more complete analysis of the pollutants that are likely accompanying the farming activity and at the same time, provides some of the data needed to assess environmental status as demanded by the EU Water framework directive and daughter directives.

However, the antibiotics and other therapeutic agents will not be included by such a lean onto the EU Water framework directive strategy but require specific and targeted analyses. Whether a regular monitoring is required, needs to be established following screening studies, in which the extent of the antibiotics concentration in the surrounding environment is assessed, and following risk analyses, in which the potential impact of the measured concentrations is assessed. In the present study, some steps were taken towards such screening and risk assessments for three selected antibiotics but the extent and scope of the study did not allow any conclusion to be drawn on whether antibiotics used in fish farms in fjords constitute a significant threat to the ecosystem. At present, the conclusion is thus that antibiotics and even other therapeutic agents depending on use should be monitored until a sufficient data basis is available for the final decision to be made.

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Appendix 1: Method antibiotics

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Sample preparation

The sediment samples were frozen (-18°C) and stored until analysis if needed at -18°C. The sediment sample was mechanical homogenised. Before extraction each samples were spiked with surrogate internal standards resembling the compounds to be analysed. Chlortetracycline (mz = 479/444), ciprofloxacin (mz = 332/314) and erythromycin (mz = 360/342) was used as surrogate internal standard and 2 µg/L of each compound was spiked to each sample. Approx. 10 g sediment was dried at room temperature and extracted by pressurised liquid extraction (PLE), performed with an ASE 200 system equipped with a solvent selector, both from Dionex Sunnyvale, California, USA). The extraction procedure was optimised with regard to extraction solvent, pressure, temperature and number of cycles. Optimum extraction conditions were found to be 2,500 psig and 75°C and a three-step extraction procedure using two different extraction buffers - 0.2 mol L^{-1} aqueous citric acid buffer with pH adjusted to 4.7 with NaOH (two cycles) then 50% methanol:acetone (one cycle). 5 mL of both buffers was applied. The program for each cycle of the PLE-procedure was: 5 min heat (no pre-heat), 5 min static, 50% flush volume, and 60-s purge.

The two extracts were mixed and diluted to 500 mL with Millipore water, adjusted to pH 3 by addition of 1.5 mL formic acid, and then vacuum-filtered through GF-3 glass fibre filters (0.6 µm retention; Macherey–Nagel, Germany). Finally, the samples were concentrated using SAX-HLB tandem SPE. In brief, the SAX cartridge was placed on top of the HLB cartridge and both columns were conditioned with 3 × 1 mL methanol and 3 × 1 mL 0.04 mol L^{-1} citric acid buffer (pH 3). The samples were passed through both cartridges at approximately 5 mL min^{-1}, washed with 5 mL Millipore water, and, finally, dried under vacuum for 15 min. The SAX cartridge was then discarded and the antibacterial agents were eluted from the HLB cartridge with 2 mL methanol. Before LC–MS–MS analysis, the methanol extracts were diluted 1:1 with Millipore water.

LC–ESI-MS–MS analysis

Analysis of the sediment extracts was performed by LC–ESIMS–MS with an Agilent Technologies (Palo Alto, CA, USA) 1100 series HPLC system equipped with a degasser, a cooled autosampler (4°C), and a cooled column oven (15°C) and coupled to a Sciex API 2000 triple quadrupole mass spectrometric detector with an electrospray source (ESI) (Applied Biosystems, Foster City, CA, USA). Collection and data treatment were performed with Analyst 1.4 Software (Applied Biosystems).

HPLC gradient separation of the antibacterial agents was achieved on Xterra MS-C18 guard and analytical columns (10 mm × 2.1 mm and 100 mm × 2.1 mm, respectively; particle size 3.5 μm). Mobile phases A and B consisted of 95% and 20% methanol, respectively, in Millipore water at pH 3 (adjusted with formic acid). The antibacterial agents were separated by use of a 25 min gradient running from 5 to 75% mobile phase A, then 15 min equilibration.

The mass spectrometer was operated in positive-ion mode Chromatograms showing the extracted ion-counts (XIC) for MRM analysis of the individual analytes. The following m/z was used: oxytetracycline (mz = 461/425), flumequin (mz = 262/244) and oxolinic acid (mz(1) = 262/216) and mz(2) = 262/244).

The limit of detection (LOD) and limit of quantification (LOQ) for the entire method were determined as signal to-noise ratios (S/N) of 3 and 10, respectively. S/N values were calculated by correlation with the sample concentration determined by standard addition, by use of the software tool Analyst 1.4.

Quantification of antibiotics

The amounts of antibiotics were quantified by standard addition. Each sample furnished 2 mL methanol extract; this was divided into six sub-samples of 250 μL and the remaining 500 μL was saved for potential further analysis. Each sub-sample was subsequently diluted 1:1 with aqueous standards to create a standard addition plot with six concentration levels (0-250 μg L^{-1}) added with the aqueous standards.