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REPORT OF THE STUDY GROUP ON OCCURRENCE OF M-74 IN FISH STOCKS

Copenhagen, 1 - 3 March 1994

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1 INTRODUCTION

In accordance with C.Res.1993/2:7:9, adopted at the 81st Statutory Meeting of the International Council for the Exploration of the Sea, it was decided to establish a **Study Group on Occurrence of M-74 in Fish Stocks** (SGM74), a "syndrome" causing severe mortality in the early life stages of the Baltic salmon. The Study Group met at the ICES Secretariat in Copenhagen, on 1–3 March 1994.

The Study Group had been assigned the following tasks:

a) to review the available information on M-74 and similar reproductive disturbances;

b) to propose details of scientific studies to investigate the causes and mechanisms of these reproductive disturbances and their effects on reproduction/early life stages of fish;

c) to prepare a report for the Baltic Salmon and Trout Assessment Working Group and the Working Group on Pathology and Diseases of Marine Organisms.

The Study Group also addressed the task:

d) to assess the effect of M-74 on the salmon stocks.

The agenda of the meeting is attached as Annex 1.

Participants at the meeting are listed below and in Annex 2.

B.-E. Bengtsson	Sweden
H. Börjeson	Sweden
I. Brandt	Sweden
G. Bylund	Finland
O. Christensen	Denmark
T. Håstein	Norway
B. Hjeltnes	Norway
E. Ikonen	Finland
H. Lassen	Denmark
S. Mellergaard (Chairman)	Denmark
A. Soivio	Finland
J. Thulin	Sweden
P.J. Vuorinen	Finland

2 STATUS

2.1 Salmon (*Salmo salar*)

2.1.1 Swedish compensatory rearing model

In Sweden hatchery-reared salmon fry were released to rivers as early as the 1860s and rearing to parr size (10–20 g) began in the 1930s. The building of power plants from the 1940s onwards drastically diminished the prerequisites for the natural production of smolt. To compensate for the resulting loss of natural production sites, the Swedish compensatory programme for the release of salmon smolt was developed (Ackefors *et al.*, 1991).

The compensatory programme in Sweden relies on ascending spawners collected in the rivers in order to keep the native river stocks intact. Brood fish are caught in the rivers during summer and autumn (i.e., over the entire ascending period to ensure that they represent the size variation and the time schedule of the whole run) and kept to ripeness in late autumn when they are stripped and the eggs are artificially fertilized. The propagation material is incubated and the offspring reared to two-year-old smolt (40–80 g). The smolt are released into their "home-river" in May to make possible the feeding migration to the Baltic Sea.

The Swedish programme amounts to 1.9 million smolt a year from normal eggs and is concentrated in the rivers entering the Gulf of Bothnia. The hatchery capacity is in maximal use at present. Approximately 2 percent of the released smolt are tagged, recapture rates vary between years and stocks by about 5 to 15 percent.

To compensate for the high yolk sac fry mortality, the number of spawners from compensatory rearing have been increased. Today the annual number of spawners in all rivers except the Ljungan River exceeds 50 pairs. Over 400 pairs are used for the Lule River due to M-74 problems.

2.1.2 Finnish compensatory rearing model

The compensatory model in Finland is totally different from that developed in Sweden. When most of the 18 salmon rivers were dammed for hydroelectric power production or spoilt by pollution in the 1950s, only two natural rivers (the Tornio and the Simo) were left for salmon spawning. The stock of the Ii River was preserved for aquaculture at the Taivalkoski State Aquaculture Station Fish Farm, where a brood stock was created from wild brood fish and descending smolt from the river. This brood stock has been supplemented from eggs collected from ascending brood fish from the restocking sites of this stock. The stocks of the Tornio and Simo rivers have been taken into cultivation and the natural stocks have been supplemented during the last decade.

To supplement the salmon stock of the Oulu River, the Montta Fish Farm was founded in the 1950s. It now releases smolt from a mixed "Montta stock" based on ascending brood fish migrating northwards beyond the mouth of the Oulu River (stocks of the Simo, Ii, and

Tornio rivers). In addition some genetic material has been introduced from the Kalix River in the 1960s.

To compensate for the salmon of the rivers south of the Northern Quark and those running to the Gulf of Finland, the stock of the Neva River was imported as eggs to the Laukaa State Aquaculture and Research Station in the 1970s.

Genetic material has been added to all of these five brood stocks, at least every second or third year, from the wild ascendant spawners caught in the river mouths and sites of release mainly from the early ascendants in the region north of the Northern Quark and from late ascendants from the Neva strain in the mouth of the Kymi River.

From the eggs of brood stocks reared in several fish farms, about 2 million smolt are released into the Baltic Sea. The total Finnish capacity to produce smolt for stocking is about 4 million yearly.

2.2 Clinical and Pathological Symptoms of M-74

The syndrome was first observed in the Swedish compensatory smolt-rearing hatcheries in the early 1970s. Not until 1974, when the procedure of incubating the eggs of each female separately was introduced at the hatchery of Bergeforsen on the Indal River, was it noted that the mortality was linked to certain females. Since that time, this rearing technique has been routinely used in Swedish hatcheries and since 1985 in some Finnish hatcheries, in order to be able to control the egg and the yolk sac fry mortality and to facilitate the management of hatching. Although showing a higher frequency of wriggling behaviour than in females, it was demonstrated that the males had no influence on the occurrence of the M-74 syndrome (Sahlin, personal communication; Johansson *et al.*, 1993). **M** in the name of the syndrome stands for "Miljön" (the Swedish word for environment) and **74** stands for the year it was first observed.

2.2.1 Yolk sac fry

The first signs of M-74 in the yolk sac fry are usually observed 2–3 weeks after hatching, when about 2/3 of the yolk sac has been consumed. When compared to normal yolk sac fry, those affected by M-74 reveal the following symptoms:

- lethargy and weak avoidance reactions (swimming movements are short, uncoordinated rushes leading to quick exhaustion);
- the yolk sac fry are greyish in colour;
- fragility of blood vessels, seen as haemorrhages (primarily in the heart region) and precipitates in the yolk sac close to fat droplets;
- the yolk sac may be swollen and exophthalmus is observed, indicating osmoregulatory disturbances;
- the hepatocytes in yolk sac fry suffering from M-74 show increased vacuolization and lower glycogen content compared to normally developing fry;
- the hepatic cytochrome P-450-dependent 7-ethoxyresorufin-O-deethylase (EROD) activity from M-74 affected yolk sac fry is very high compared to that of the viable fry from hatchery-reared brood fish and 1.7 times the activity in viable yolk sac fry from wild spawners.

The death of the entire family group is more or less total within 3 to 5 days after the observation of the first symptoms.

2.2.2 Behavioral and morphological disorders in brood stock

Behavioral disorders in the wild spawners have been observed since the early 1990s among both males and females. Ascending spawners are continuously caught from July to the end of September on the Swedish coast and from the beginning of June on the Finnish coast. The fish are kept in artificial basins until they are ripe for stripping. In 1991-1993, balance disturbances characterized as "wiggling behavior" were observed in September and October when the fish began to ripen. This wiggling was often associated with mortality prior to ripeness. The tendency for wiggling behavior was higher among males. In 1992, this type of mortality was about 10 percent in Sweden in contrast to less than one percent before the 1990s.

In 1992 a total of 988 females of seven different stocks from the Swedish coast of the Gulf of Bothnia were stripped. Of these, 143 females showed a wiggling behavior and 142 of them produced offspring dying as yolk sac fry due to M-74. The M-74 mortality among the offspring of the other 855 females classified as "normal" was 71 percent (Börjeson *et al.*, in press).

Histopathological examinations of the liver of wild spawners have shown disorganized hepatocytes in females giving M-74 affected offspring (Norrgren *et al.*, 1993).

Börjeson *et al.* (in press) have shown a negative correlation between the pigmentation of the eggs and the frequency of M-74 mortality. In autumn 1993, Eriksson (pers. comm.) demonstrated a correlation between the pigmentation of the skin and the eggs in a sample of 440 females of the Lule River stock.

2.3 Status of M-74

2.3.1 Status in Sweden

In the Torne, Vindel, Ljungan, and Mörrum rivers, spawners of natural origin have been used whereas the other rivers have used spawners originating from reared smolt.

The status of M-74 mortality is illustrated in Figures 2.1 and 2.2. Figure 2.1 shows the historical situation from 1974 until the present as exemplified by the Bergeforsen hatchery in the Indal River which is representative of the situation in Sweden. The frequency of M-74 mortality has fluctuated from year to year as well as between stocks. The figure also shows winter temperature in the southern parts of the Baltic Sea as a mean for the period from December to April (a factor which will be addressed in Section 3). Efforts have been made to compensate for the M-74 loss of yolk sac fry by increasing the amount of eggs in incubation. However, due to the immense increase in M-74 mortalities in 1992 and 1993 this will not be possible in the future and Sweden will not be able to fulfil its salmon smolt release duty in 1995. Figure 2.2 shows the situation for 1992 and 1993. If this trend continues, the future situation will be very serious and some stocks will be close to extinction. In any case, the risk for loss of genetic diversity due to inbreeding is high when the year-classes 1992 and 1993 return as spawners at the end of the 1990s.

However, a positive prospect for the future is that an increased pigmentation of the spawners stripped in 1993 and their eggs has been observed. Figure 2.3 shows the colour of the eggs for the salmon stocks of the Lule River in the north and the Dal River in the south of the Gulf of Bothnia. It should be noted that the labels 1992 and 1993 indicate the time for stripping and constitute the hatching year-classes 1993 and 1994.

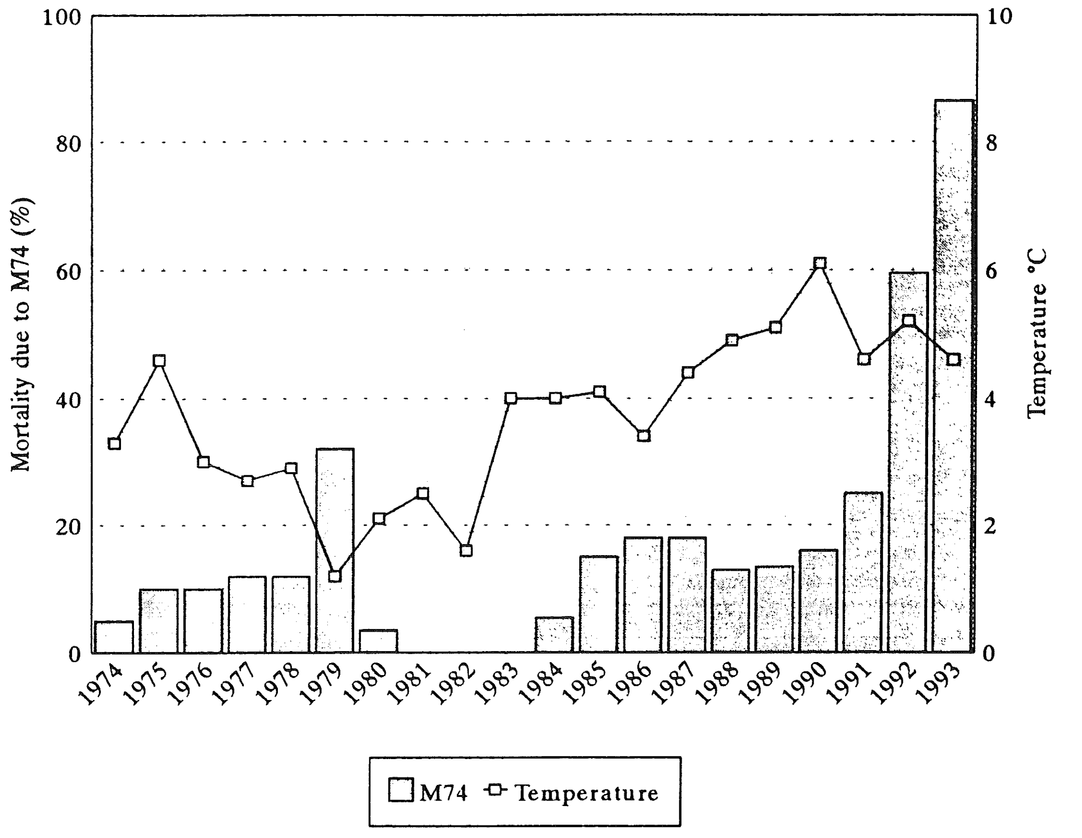


Figure 2.1 Temporal trend in the mortality of Baltic salmon due to M-74 in three major Swedish rivers and the mean water temperature (December–April) in the southern Baltic Sea.

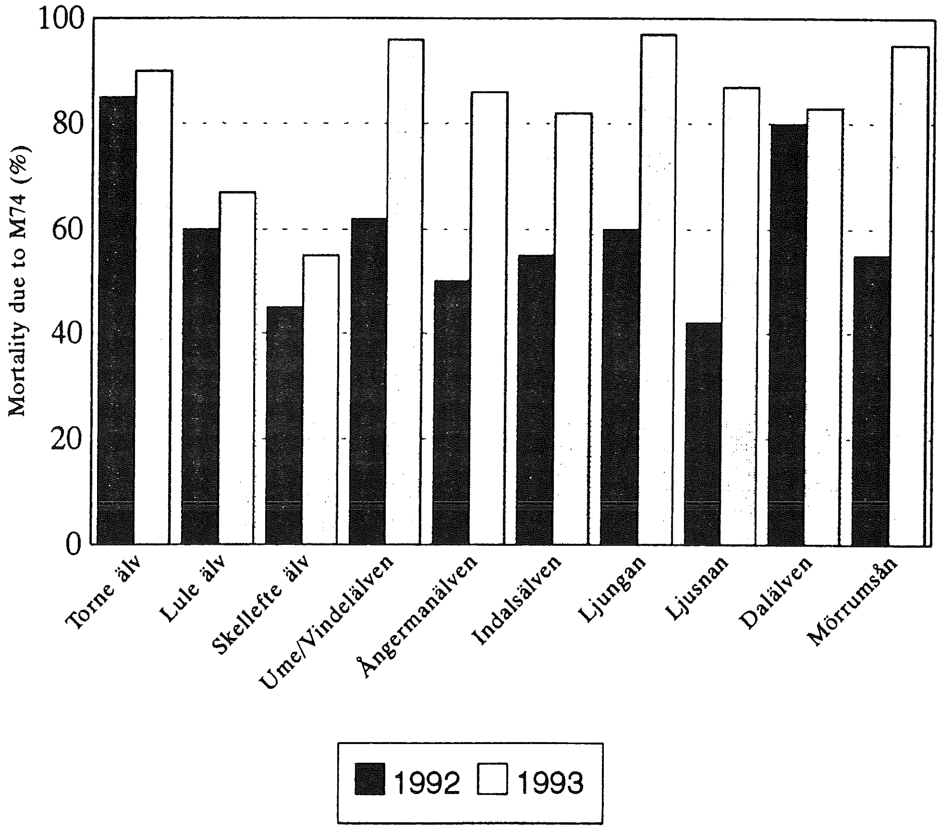
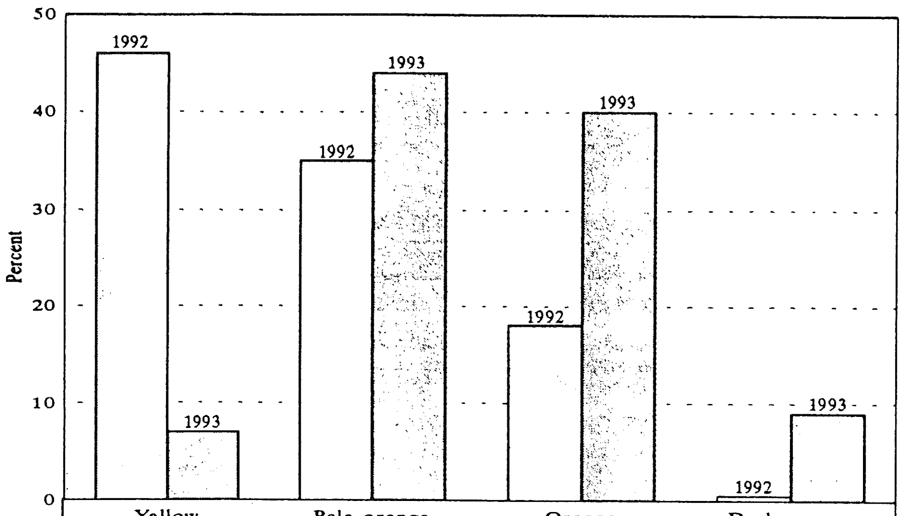


Figure 2.2 M-74-associated mortality in Swedish stocks of Baltic salmon in 1992 and 1993.



	Yellow	Pale orange	Orange	Dark orange
No of females 1992	197	152	79	2
No of females 1993	36	247	220	52

Figure 2.3 Colour of roe from Baltic salmon stocks of the River Lule and the River Dalälven in 1992 and 1993.

2.3.2 Status in Finland

A prerequisite for observing the M-74 syndrome is the possibility to incubate the eggs of individual brood fish separately. This method was adopted in the state hatchery of the Simo River in autumn 1985 and M-74-like symptoms were, for the first time, observed during the seasons 1986-1987 and 1987-1988. The first clear cases were found from 1992 onwards and the mean mortality increased in 1993 to over 90 percent in both the Simo and the Tornio rivers. At the same time, the mortality of fry from hatchery-produced eggs was below 8 percent during the yolk sac stage and no signs of M-74 could be observed (Rytilahti, pers. comm.). In the spring of 1993, a massive mortality of yolk sac fry from wild salmon females was reported by the Guttorp Fish Farm on Åland Island.

In the wild salmon stocks of the Simo and Tornio rivers, the year-classes after 1991 have collapsed indicating the existence of M-74 syndrome there.

2.3.3 Status in other Baltic countries

The situation in other Baltic countries is not clear.

In Estonia, the M-74 syndrome has been definitely noticed in the Kunda River (the only river out of the five rivers contributing to wild salmon stock monitored by electrofishing in 1993). Kangur (pers. comm.) reported that the abundance of year-class 1992 in the Kunda River was at a standard level but the year-class 1993 was totally absent. However, total lack of year-classes has been observed on other occasions during the last ten years.

From Latvia, Mitans (pers. comm.) reported slight increases in yolk sac fry mortality during recent years, which could be associated with M-74. The total mortality reached 10.2% in 1992.

Information from Lithuania was not available on either salmon production or the existence of the M-74 syndrome.

The natural salmon stock in Poland apparently disappeared from the River Vistula in the late 1960s. Tagged Vistula salmon have been reported to migrate all over the Baltic Sea but were mostly caught in the Gulf of Gdansk and the Vistula estuary (Jokiel and Bartel, 1984). The last salmon spawners in the Drawa River were observed on spawning grounds in 1985 (Chelkowski, 1986). In 1987, attempts to catch Drawa salmon spawners were unsuccessful, and only seven fingerlings were caught (Chelkowski, 1988). In the Polish salmon re-establishing programme, the brood fish reared from eyed eggs imported from the Daugava River are reared in cages in the Gulf of Gdansk. From the eggs of these fish (about 1 million/year) 150,000 fingerlings have been reared in one hatchery in 1993. So far, M-74 has not been reported from Poland.

3 POSSIBLE FACTORS INVOLVED IN M-74

3.1 Rearing Environment

The model of compensatory rearing of Baltic salmon smolt has been developed since the early 1950s and has worked well. The technique, with slight variations, is used in Finland and Sweden both for the Baltic stocks and for the Swedish west coast stocks. Furthermore, it has been in use when producing smolt of land-locked salmon stocks of Lake Vänern and Lake Saimaa.

Only Baltic salmon has been threatened by M-74 mortality. Except for the hatchery of the River Mörrum, the smolt production of Baltic salmon in Sweden has mainly been concentrated in areas where acidification of the river water is only a minor problem compared to the hatcheries rearing the Swedish west coast and Vänern smolt. Thus it seems evident that acidification cannot be the cause of M-74.

The results of electrofishing in rivers with natural salmon reproduction show a very strong decrease in 0+ parr in 1992 and 1993, which indicates that naturally reproducing salmon are also affected by the M-74 mortality (Romakkaniemi *et al.*, unpublished report, 1994). Thus the rearing technique itself does not seem to be involved in the M-74 mortality.

Formalin treatment of the spawners is necessary in order to keep them alive during the period from catch to stripping. Formalin treatment is used for spawners on the western coast of Sweden and for the farmed brood stocks in Finland without any adverse effect on eggs or yolk sac fry.

Malachite green is commonly used for treatment and prevention of *Saprolegnia* (fungal) infections on the salmon ova of both wild and hatchery origin. In certain hatcheries, dead ova have been picked up by hand from sparsely loaded incubators instead of using the malachite green treatment. Also these hatcheries have had high M-74 mortality (Uppman, pers. comm.). Börjeson (pers. comm.) investigated the possible effect of malachite green on an outbreak of M-74 by comparing the mortality due to M-74 in treated and untreated aliquots of salmon eggs from 60 females without being able to observe any difference.

Thus, there are no indications that the rearing environment or other abiotic factors in it (such as chemicals used in normal hatchery practise or river water acidification) have any influence on the M-74 syndrome.

3.2 Nutritional Factors

Nutritional factors seem to be involved in the M-74 syndrome. So far, M-74 has not been observed in the

Finnish salmon brood stocks utilized in the Finnish compensatory program (Section 2.1.2) as these are kept under farming conditions and fed with pelleted feed. The fish components in this feed originate from the North Sea or the North Atlantic. Only eggs from ascending spawners seem to develop the M-74 syndrome.

Sprat, along with herring, is the main food of salmon in the main basin of the Baltic (Christensen and Larsson, 1979). The mortality due to M-74 recorded in the Umeälven, Indalsälven, and Mörrumsån rivers (Börjeson *et al.*, in press) correlates significantly with the spawning stock biomass of sprat in the Baltic (Mellergaard, unpublished data) (Figure 3.1).

The contaminant level (in extractable fat) in sprat is approximately the same as in herring (Roots and Aps, 1993). However, the contaminant data presented by Roots and Aps (1993) are not in accordance with the data from salmon (Thurén *et al.*, in press; Vuorinen *et al.*, 1993). Figure 3.2 illustrates the observation that the fat content in sprat is constant throughout the year at a level of 12-15 % and does not exhibit seasonal variations, while herring have a low fat content in spring (2-6 %) and a high level in autumn (7–11 %) (source: Vestkust Fisk SVC, AB, Göteborg).

As sprat constitute one of the main food items for salmon in the Baltic main basin, the contaminant uptake will be considerably higher when sprat are eaten instead of herring, particularly during winter when the herring fat content is comparatively low. This is because most organic contaminants are lipophilic substances.

Parmanne and Ikonen (unpubl.) have shown that the average age of herring eaten by salmon is now 1-2 years older than that reported earlier due to a decreased growth rate in herring in recent years. Therefore, the uptake of chemical contaminants, e.g., DDT and PCBs, in salmon has increased because the level of environmental contaminants in fish increases with age.

The occurrence of mild winters may also be important here. As demonstrated in Figure 2.1 (Section 2.3.1), there also seems to be a correlation between the average winter temperature of the surface water in the main basin of the southern Baltic Sea and the occurrence of M-74. At low winter temperatures, below 3 °C, salmon reduce or cease food intake. However, above this temperature salmon are believed to have a comparatively high food intake and the transfer of lipophilic contaminants especially from sprat may be increased.

Another nutritional factor that may be involved is the carotenoid astaxanthin. The red colour of salmonid flesh results from deposition of the carotenoid astaxanthin. Salmon, like other fish species, is not able to synthesize astaxanthin itself and the major source in nature is from crustaceans which obtain the carotenoids from phytoplankton primary production.

In fish farming, astaxanthin or the closely related compound canthaxanthin is added to the diets. Biological functions of astaxanthin have recently been reviewed by Torrissen (1990). Recent studies have shown that astaxanthin is essential for growth and survival of Atlantic salmon fry (Christiansen *et al.*, in press). The requirement for optimal growth and survival during the start-feeding period has been determined to be between 1 and 5 mg astaxanthin/kg dry diet (Christiansen, pers. comm.). Similar effects on growth and survival have been observed in red tilapia (*Oreochromis niloticus*) (Boonyaratipalin and Unprasert, 1989).

Astaxanthin has been found to act as a provitamin A in rainbow trout and Atlantic salmon (Al-Khalifa and Simpson, 1988; Schiedt *et al.*, 1985). It is also known to act as an antioxidant and may play an important role in protecting lipid tissue from peroxidation (Burton, 1989). Studies on Atlantic salmon fry where fish have been fed diets with and without astaxanthin supplements have shown an effect on the levels of vitamin A, vitamin E, and ascorbic acid in muscle and liver (Christiansen, pers. comm.).

There is increasing evidence that carotenoids act as chemopreventive agents against certain types of cancer in mammals (Krinsky, 1989) and that carotenoids enhance the non-specific and the specific immune response system (Bendich, 1989). In a study on the effect of astaxanthin on the immune response system in salmon, higher survival rates were observed in groups fed diets supplemented with astaxanthin in the fish challenged with *Aeromonas salmonicida* (Christiansen, pers. comm.).

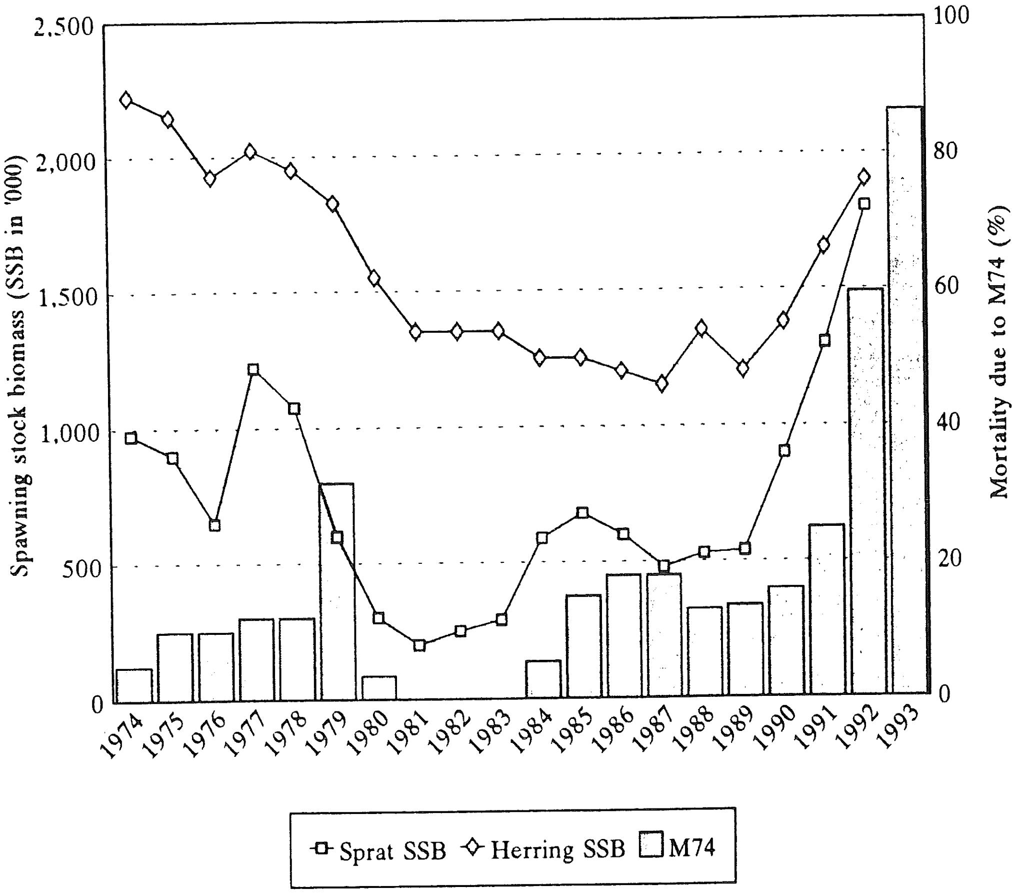


Figure 3.1 Temporal trend in spawning stock biomass of sprat and herring in the Baltic Sea and the mortality due to M-74 in three major Swedish rivers.

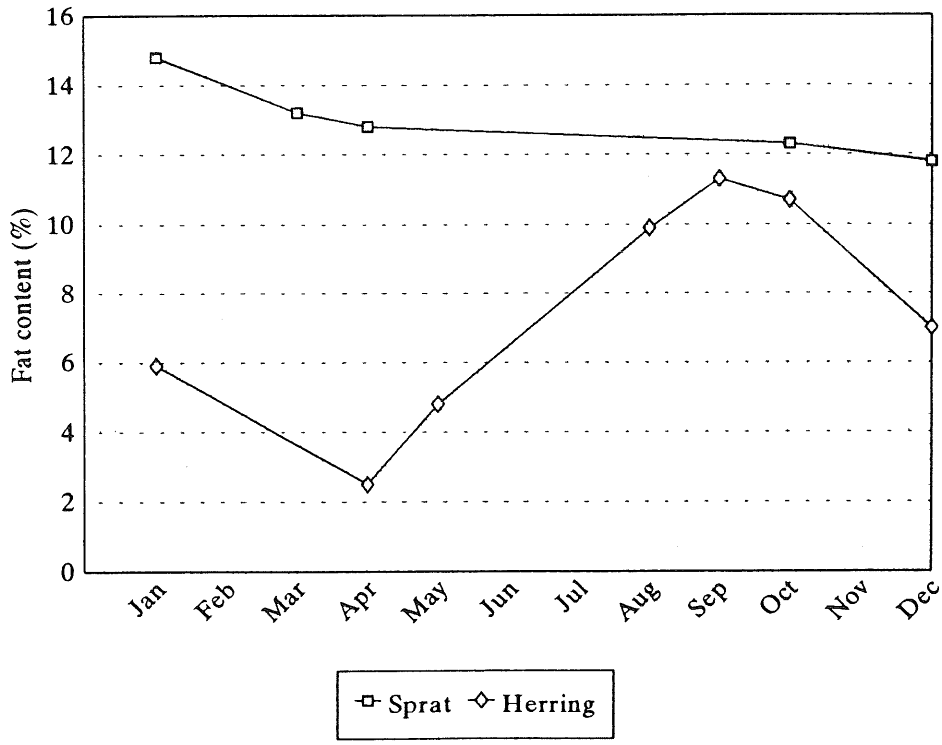


Figure 3.2 Annual trend in fat content in sprat and herring from the Baltic Sea.

3.3 Genetic Factors

The Baltic salmon (*Salmo salar* L.) is the same species as the Atlantic salmon but is genetically isolated from the population living in the North Atlantic and is confined to the Baltic Sea drainage area (Ackefors *et al.*, 1991). There is further genetic differentiation, with reproductive isolation between and within different drainage areas (Ståhl, 1983; 1987). Genetic factors may influence the tolerance of fish and mammals to environmental pollutants (Cooper, 1994). A high level of heterozygoty within an animal population seems to be very important for an effective immune defense system (Gonzales and Nebert, 1990). Koljonen (1989) has demonstrated that the level of heterozygoty in salmon depends on the effective population size and that culturing and isolation diminishes variation by decreasing the effective population size.

The difference in M-74 mortality (42-90%) in Swedish hatcheries in 1992 (Johansson *et al.*, 1993) might be a result of, for example, a different exposure history of the brood stocks. It cannot, however, be excluded that the different mortality figures also reflect the influence of genetic components, i.e., a difference in tolerance to M-74 between Baltic salmon populations. In Finland, genetic selection may partly be due to the possibility of favouring the biggest females from the ascendants caught or in use in the hatchery when supplementing the brood stock.

3.4 Infectious Agents

Until now, there have been no indications that infectious agents are involved in the etiology of the M-74 syndrome. Parasitological, bacteriological, and virological examinations performed on affected brood stock and fry in Finland as well as in Sweden have so far given negative results.

It is evident that further screening, especially with electron microscopy and refined virological techniques, has to be performed before an involvement of a transmittable agent in the etiology is conclusively excluded.

3.5 Chemical Contaminants

It is still not clear whether chemical substances, endogenous or exogenous, inorganic or organic, are responsible for the development of the M-74 syndrome. Both analytically oriented and experimental biological response studies should be undertaken to further elucidate such a connection.

Substances or groups of substances with possible effects on the reproductive process and which are persistent (e.g., polychlorinated hydrocarbons such as PCB, DDT, PCDD/PCDF) or are of intermediate persistence (e.g., polycyclic aromatic hydrocarbons, PAHs) are of primary interest. To this group, substances that have been intentionally dumped in the Baltic Sea should be added (Johansson *et al.*, 1993). These are reactive alkylating substances like mustard gas and substances that are part of product formulations (e.g., a number of substituted benzenes).

Unconditioned analysis of anthropogenic substances does not seem to be meaningful at the present stage of knowledge. Interaction between groups of substances for enhancement of effects on reproduction cannot be excluded but might be difficult to reveal without very high analytical costs.

With the exception of some observations made on local fish populations living in industrial effluent areas (Sandström *et al.*, 1992; Pulliainen *et al.*, 1992), no evidence that links these effects to anthropogenic (pollution) factors exists. The chemical characterization of the pollution load in the Baltic Sea is far from complete and it is, therefore, not possible to exclude a pollution factor behind the M-74 syndrome and other reproductive disturbances in Baltic Sea fish. However, indications of correlations between the mortality of salmon offspring and the concentrations of several organochlorine compounds have been detected (Thurén *et al.*, in press; Vuorinen *et al.*, 1993)

3.5.1 Heavy metals

In Swedish samples, heavy metal concentrations in eggs from M-74-affected and normal females did not demonstrate any significant differences (Norrgren *et al.*, 1993). Heavy metal analyses have not been conducted in spawning salmon from the Finnish rivers. However, analyses for heavy metals (Cd, Cu, Hg, Pb, and Zn) in salmon caught in the Gulf of Finland in 1987 demonstrated low levels, although the concentrations of Cd, Cu, and Pb were slightly higher than those in sea trout from the Gulf of Bothnia sampled in 1990 and 1991 (Vuorinen *et al.*, 1993; in press). Due to their low levels, heavy metals are unlikely to contribute significantly to the M-74 syndrome.

3.5.2 Organochlorine compounds

A vast number of persistent halogenated hydrocarbons such as DDT-compounds, PCBs, chlorinated dioxins, and dibenzofurans are present in the Baltic ecosystem. In recent years, persistent sulphur-containing metabolites formed from PCBs and DDT in the environment have been identified. Although knowledge about the biological properties of these compounds is far from complete, considerable information on general reproductive toxicity of different persistent chlorinated hydrocarbons is available in the literature. However, to date, information about reproductive disturbances in fish remains limited.

Concentrations of organochlorines increase with salmon size. There exist significant positive correlations between both weight and length and the concentrations of organochlorines in salmon (Vuorinen *et al.*, 1993). Organochlorines have been analysed in the muscle of spawning salmon from the Kemi River in 1982-1986 and from the Simo River in 1988-1992 (Vuorinen *et al.*, unpublished data). DDTs, total PCB, HCB, HCHs, chlordanes, and toxaphene have been analysed in samples of these salmon. Moreover, planar PCBs, polychlorinated naphthalenes (PCNs), chlorinated phenols, and polychlorinated diphenylethers (PCDEs) have been measured, but not in all samples. Some samples have also been analysed for dioxins with low resolution mass spectrometry. Separate incubations of eggs from females taken from the Simo River have been performed in order to compare the concentrations of organochlorines in females with the mortality in their offspring (Vuorinen, pers. comm.).

The levels of PCBs and total DDT compounds in Baltic fish species have exhibited a decrease over time (Olsson and Reutergårdh, 1986; Bignert *et al.*, 1993), and this is also true for salmon during the time period 1982-1992 (Vuorinen *et al.*, 1993). However, concentrations of toxaphene and chlordanes do not show any clear trends.

3.5.2.1 DDTs

Early experimental studies showed that DDT may disturb the implantation of eggs and prolong the estrus cycle in mice (Lundberg, 1973; Lundberg and Kihlström, 1973; Kihlström *et al.*, 1975). The DDT metabolite, DDE, may induce thinning of the egg shell after feeding to birds. The observed egg shell thinning in wild birds has been attributed to high DDE residue levels in the eggs (Ratcliffe, 1970). Despite the fact that residue levels of DDT in the environment have declined, decreased egg shell thickness and quality of guillemot eggs in the Baltic region still seem to occur (Bignert *et al.*, unpublished data). Hydroxylated metabolites of DDT have been implicated in exerting estrogenic effects in rats (Bustos *et al.*, 1988).

The DDT metabolite, DDD, is a toxicant to the adrenal cortex in dogs and humans (Nelson and Woodard, 1949; Bergenstahl *et al.*, 1960). A methylsulphone-containing metabolite of DDT, 3-methylsulphonyl-DDE, has recently been shown to be a highly potent toxicant to the adrenal cortex in mice (Johnsson *et al.*, 1991), and the role of such adrenocorticolytic pollutants in the development of adrenocortical hyperplasia in Baltic seals has been suggested. Recent comparative studies have demonstrated that there are major species differences in the adrenocorticolytic potency of these DDT metabolites, while certain species such as several species of birds seem to be sensitive to both compounds (Brandt *et al.*, 1992). Preliminary results indicate that adrenocorticolytic DDT metabolites may be metabolized to toxic intermediates in glucocorticosteroid-producing cells in cod (Ingebrigtsen and Brandt, pers. comm.).

The M-74 syndrome was not observed in the Simo River until 1991. However, weak positive significant correlations were detected between concentrations of DDT or its metabolites and mortality after fertilization, at eyed stage and mortality of sac fry already in 1988-1990 (Vuorinen *et al.*, 1993) and again in 1991 and 1992. Concentrations of DDTs in 1991 and 1992 were of about the same order as in 1988-1990, but the proportion of DDE was higher than in 1982-1990 (Vuorinen, unpublished results).

3.5.2.2 PCBs

As with DDT, early experimental studies with technical PCB mixtures revealed effects on reproductive performance in experimental animals. Experiments with minnows (*Phoxinus phoxinus*) exposed over 40 days to a technical PCB mixture (Clophen A50), representing a GC-"fingerprint" resembling the content of PCB in Baltic salmon (Jensen *et al.*, 1969), showed that the minnows developed reproductive disturbances (Bengtsson, 1980). The effects included delayed spawning, decreased egg production, increased fry mortality, and deformation more than 7 months after exposure to PCB was terminated.

Coplanar PCBs are known ligands of the Ah-receptor in several species. Hence, induction of CYP1A1 (monitored as ethoxyresorufin-O-deethylase (EROD) activity) has been used as a marker for the presence of coplanar PCBs and other Ah-receptor ligands such as polychlorinated dioxins and dibenzofurans in the tissues of wild-living animal species. Interestingly, studies on the CYP1A1 activity in yolk sac fry from Baltic salmon have revealed higher EROD activity in M-74 affected yolk sac fry than in healthy yolk sac fry. Similarly, the hepatic cytochrome P-450 level in female spawners was higher in those giving rise to M-74 affected progeny than in those giving healthy progeny. Moreover, the EROD activity recorded in yolk sac fry from farmed salmon was lower than that of healthy yolk sac fry from Baltic salmon (Norrgren *et al.*, 1993; Börjeson *et al.*, in press). Assuming that the EROD activity in yolk sac fry from farmed fish represents a level closer to the natural background level, it appears that the amount of coplanar PCBs and other Ah-receptor ligands in Baltic salmon is sufficiently high to cause cytochrome P-450 induction.

In salmon from the Kemi and Simo rivers, PCB concentrations have been reported mainly as total PCBs. Weak positive significant correlations have been observed between concentrations of total PCBs and mortality after fertilization, at eyed stage and mortality of yolk sac fry of Simo River salmon *before* (Vuorinen *et al.*, 1993) and *after* the appearance of M-74. Coplanar PCBs have been analysed from too few samples to allow any correlations with offspring mortality to be made. However, concentrations of coplanar PCBs in salmon muscle are about ten times higher than dioxin concentrations so more data are needed for these compounds.

Edgren *et al.* (1981) observed significantly higher levels of total DDTs and PCBs in perch from the cooling water recipient of a nuclear power plant as compared to values in perch from the intake area.

3.5.2.3 Dioxins

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is the prototype for persistent halogenated aromatic compounds eliciting toxicity following binding to the Ah-receptor. Accordingly, the pattern of toxic effects observed in experimental studies is very similar to those induced by other Ah-receptor ligands such as coplanar PCBs and polychlorinated dibenzofurans. TCDD is known as a potent toxicant affecting reproduction in several laboratory and wild species including fish.

The presence of organochlorine compounds has been proposed to explain early life stage mortality and failure of recruitment seen in lake trout (*Salvelinus namaycush*) (Peterson, in pres). Walker (in press) investigated the toxicity of TCDD on the development and survival of early life stages of lake trout and found that the greatest TCDD-related mortality occurred during the yolk sac fry stage. TCDD was found to cause 0-100% yolk sac fry mortality over a TCDD concentration range in the fertilized eggs of approximately 30-100 pg/g (Peterson and Cook, in press). Egg concentrations of maternally-derived TCDD manifested mortality associated with yolk sac edema, subcutaneous haemorrhages, and arrested growth, resembling blue-sac disease (Symula *et al.*, 1990). However, it should be noted that these pathological changes do not completely correspond to those observed in connection with M-74.

Data on the concentrations of dioxins in spawning salmon from rivers discharging to the Baltic Sea are too limited to allow for any conclusions concerning eventual correlations between dioxins and salmon offspring mortality.

3.5.2.4 Others

Due to their relatively high concentrations in salmon muscle, chlordanes and toxaphene without a doubt contribute significantly to the load of toxicants in salmon. Significant correlations between chlordane and toxaphene concentrations and mortality of early life stages of salmon from the Simo River were observed before, as well as after, the onset of M-74 (Vuorinen *et al.*, 1993).

Polychlorinated diphenyl ethers (PCDEs) are another group of compounds that should be considered because they contribute to the toxic load in salmon about equally as much as dioxins when calculated by the toxic equivalency factors given by Safe (1992) (Koistinen *et al.*, 1993). However, these toxicity factors have only been estimated for mammals and there is very little information available about the toxicity of PCDEs to fish.

Concentrations of polychlorinated naphthalenes (PCNs) have been determined to be somewhat higher in the muscle of females giving M-74 offspring than in females giving viable offspring (Thurén *et al.*, in press). PCN concentrations in salmon caught from the Baltic proper were several times higher than concentrations of coplanar PCBs (Tarhanen *et al.*, 1989).

3.6 Other Factors

In recent decades the Baltic Sea has been heavily influenced by natural and anthropogenic factors which have considerably changed the structure and function of its ecosystem. Many of these changes are of a large-scale character and include both abiotic and biotic changes which may directly (e.g., changes in water quality or water temperature) or indirectly (e.g., changes in food web structure) affect the reproduction of fish populations. Therefore, a systematic evaluation of environmental factors inducing specific (e.g., M-74) or other kinds of reproductive disturbances of fish is needed.

4 EFFECT OF M-74 ON BALTIC SALMON STOCKS

4.1 Mortality in Wild and Reared Salmon Stocks

The recruitment of salmon smolt to the Baltic Sea is mainly based on hatchery-reared smolt. In 1992, about 4.9 million smolt originated from rearing stations, while the natural production in the rivers was estimated at about 0.5 million smolt.

Contrary to the Finnish artificial smolt production based on reared brood stocks and, therefore, unaffected by M-74, the smolt production primarily of Sweden and Latvia, amounting to 2.9 million individuals, is based on captures of spawning migrants entering the rivers. Like the natural reproduction, these rearing programmes responsible for around 50% of the total production can be seriously threatened by the syndrome.

Electrofishing surveys in the Torne, Simo, Byske, and Åby rivers in 1992 and 1993 seem to indicate some effect of the M-74 syndrome on the natural reproduction of salmon in the rivers (Karlström, 1993; Romakkaniemi *et al.*, 1993). The year classes of 1992 and 1993 representing 1+ and 0+ parr occurred in the reproduction areas in a conspicuously low number compared to the density in the previous half of the 1980s, considering that the spawning stocks since 1990 have significantly increased. Furthermore, the conditions for electrofishing were poor due to high water discharge. It should also be noted that in the first half of the 1980s the number of 0+ parr per square unit of river bottom were low, too, though M-74 at this time was not supposed to seriously affect the reproduction (Table 4.1).

The hatchery-reared smolt production may be maintained by replacing all captures of breeding fish for reproduction purpose with pond-reared brood stocks. The natural stocks may, however, be endangered if the impact of M-74 remains at the same level as experienced by hatcheries in recent years.

The hatchery production of smolt has not yet decreased due to the syndrome and this also applies to the smolt run 1994, as captures of breeders in 1992 were still sufficient for the stipulated production. Based on electrofishing surveys in the Torne and Kalix rivers in 1993, the age group 2+ salmon seemed to occupy the parr biotopes to a number per square unit not significantly different from those in previous years (Table 4.2). The great majority of this age group is smoltifying in spring 1994.

Referring to the terms of reference of the Baltic Salmon and Trout Assessment Working Group for 1994 to:

"*a) assess the status of Baltic salmon stocks and provide catch options for 1995 (in numbers of fish) within safe biological limits which have been defined to 'safeguard wild Baltic salmon stocks';*"

it is obvious that to meet these requests M-74 will enter into the recommendation of the Working Group as an important item.

Table 4.3 gives a survey of the estimated smolt production in 1992 in the rivers where such information is available, supplemented with information on the basis for the estimates. The rivers are grouped into the four management area categories according to the run-reconstruction model prepared by the Baltic Salmon and Trout Assessment Working Group.

The effect on the hatchery-reared production can be summarized below for the four management areas mentioned in Table 4.3.

In the Gulf of Finland, hatchery-reared smolt production is mostly based on the Finnish releases. In the short term, this production is not affected by M-74-induced mortality. Therefore, releases can be continued at the same level as in previous years.

Table 4.1 Number of 0+ salmon parr per 100 m^2 in the middle and upper part of River Torne and in the upper part of River Kalix 1980–1993 (Anon., 1993).

Year	River Torne		River Kalix	
	Density	No. of sites	Density	No. of sites
1980–1984	0.1	10	0.6	2
1986	0.2	5		
1987	0.5	11	0.4	2
1988	0.8	10		
1989	1.0	12	4.3	7
1990	0.6	20	8.5	4
1991	4.7	24	3.7	7
1992	0.2	16	2.4	7
1993	0.2	-	0.3	-

Table 4.2 Number of 1+ salmon parr per 100 m^2 in River Torne and in River Kalix estimated by electro-fishing surveys in 1980–1993 (Anon., 1993).

Year	River Torne		River Kalix	
	Density	No. of sites	Density	No. of sites
1980–1988	0.6	18	3.4	6
1989-1991	2.1	18	8.3	6
1989	1.9	18	-	-
1990	2.0	18	-	-
1991	2.4	18	-	-
1992	6.5	10	-	-
1993	3.7	-	4.0	-

Table 4.3 Status of natural salmon stocks in 1992.

River	Smolt production	Basis for smolt production estimates
		GULF OF FINLAND STOCKS
Kunda	4	Electro-fishing surveys
Loobu	7	Parr and adult observations
Pirita	1	Parr and adult observations
Keila	2	Parr and adult observations
Vasalemma	1	Electro-fishing surveys
		NORTH-EAST GROUP
Kalix	75	Reproduction area 2 500 ha, electro-fishing surveys, river catch statistics
Torne	100	Reproduction area 5 000 ha, electro-fishing surveys, smolt trapping, river catch statistics
Simo	17	Reproduction area 240 ha, electro-fishing surveys, smolt trapping, river catch statistics
		NORTH-WEST GROUP
Byske	18	Reproduction area 270 ha, electro-fishing surveys, river catch statistics
Vindeln	20	Reproduction area 2 200 ha, fish ladder countings of spawners, electro-fishing surveys
Ljungan	10	Reproduction area 60 ha, electro-fishing surveys
		SOUTHERN GROUP
Em	5	Reproduction area 60 ha
Mörrum	110	River catch statistics
Irbe	10	
Venta	15	Electro-fishing surveys
Saka	10	
Salaca	26	Smolt trapping, electro-fishing surveys
Vitrupe	5	
Peterupe	5	
Gauja	20	
Daugava	5	

*Only those rivers are included where information of the status of smolt production is available.
Source: Report of the Baltic Salmon and Trout Assessment Working Group (C.M.1993/Assess:14).

Minor Estonian and Russian releases are based on spawners entering the rivers and, therefore, they may be affected by M-74. However, no data were available to suggest possible effects of M-74.

Northeast group releases are based entirely on the Finnish releases and, therefore, in the near future releases can be continued on the same level as previously.

The northwest group is based totally on the Swedish releases. Because Swedish hatchery rearing is totally based on the spawners entering the rivers after feeding in the Baltic Sea, they are badly affected by M-74. According to available information (Börjeson, pers. comm.), Swedish releases in 1995 will be about 50% of the 1994 releases.

Data available from the southern stock releases are poor. Releases are partly based on Swedish releases in the River Mörrum, but at the moment this hatchery is closed and therefore Swedish releases and also EU releases which were based on Mörrum hatchery production seem to be zero. However, the European Union has plans to buy salmon smolt from Latvia, but an agreement has not yet been reached.

The effect of M-74 on Latvian hatchery production is not known. Political and economic changes have also had effects on salmon rearing and, therefore, any comparisons to the earlier production are not possible. However, Latvian smolt production is predicted to decrease by 70% in 1995 (Table 4.4). This is a rough estimate based on the fact that salmon feeding in the Baltic main basin is expected to be affected by M-74.

Hatchery-reared smolt production in the Baltic Sea is estimated to decrease by 30% in 1995 due to M-74. In 1996, the decrease will be greater because the mortality of M-74 observed in hatcheries in 1993 was higher than in 1992.

The data on 0+ and 1+ parr density in 1993 in the Torne and Kalix rivers where electrofishing surveys have been performed are not sufficiently conclusive to allow for a proper assessment of the effect of the M-74 syndrome on wild stocks. As the food of salmon has been shown to be among the possible responsible factors for the onset of the syndrome, it is supposed that wild stocks are affected to the same extent as the reared stocks. In order to estimate the wild production in 1995, the same mortality rate due to M-74 as experienced at the rearing stations in 1992 and 1993, i.e., an average of 70%, has been applied.

As shown in Table 4.4, a prognosis of the smolt recruitment to the Baltic Sea in 1994 and 1995 has been attempted, with all possible reservations. The table shows the total smolt production distributed over wild and hatchery production, affected and unaffected by M-74 in the four management areas (Anon., 1994) for the years 1992 and 1993 according to Anon. (1993) and as a precautionary estimate for the years 1994 and 1995.

Table 4.4 Salmon and smolt production in four management areas (Anon., 1994) and the effect of M-74 (number of salmon in '000). ("Wild M-74" and "Hatchery reared M-74" indicate that smolt production is estimated to be affected by M-74 induced mortality from 1995).

	Year			
	1992	1993	1994[1]	1995[2]
GULF OF FINLAND				
Wild M-74	15	15	15	5
Hatchery-reared M-74	13		13	
Hatchery reared	402	400	400	400
Total	**430**	**415**	**428**	**405**
NORTH-EAST GROUP				
Wild M-74	192	192	190	57
Hatchery reared M-74				
Hatchery reared[a]	1,711	1,708	1,700	1,700
Total	**1,903**	**1,900**	**1,890**	**1,757**
[a]Includes enhancement.				
NORTH-WEST GROUP				
Wild M-74	48	48	48	14
Hatchery reared M-74	1,860	1,985	2,000	1,069
Hatchery reared				
Total	**1,908**	**2,033**	**2,048**	**1,083**
SOUTHERN GROUP				
Wild M-74	211	211	211	63
Hatchery reared M-74	1,136	1,245	1,200	360
Hatchery reared				
Total	**1,347**	**1,456**	**1,411**	**423**

[1] Figures estimated on the basis of the previous year.
[2] Decrease in wild smolt production and hatchery production of the southern group is estimated to 70% based on the mortality figures from Finnish and Swedish hatcheries.

Source: Report of the Baltic Salmon and Trout Working Group (C.M.1993/Assess:14).

4.2 Genetic Diversity Consequences

The present compensatory breeding of Baltic salmon (e.g., as practised in Finland and Sweden) in combination with the occurrence of high mortalities of yolk sac fry owing to M-74 has created a genetic bottle-neck which threatens the genetic diversity of the Baltic salmon. In addition to loss of genetic adaptation to the local conditions in the various drainage areas where salmon spawn and spend their first years of development, there is also a risk of decreased immune system defense (as discussed in Section 3.3). When creating and supplementing brood stocks in hatcheries in Finland, special care has been taken to avoid "sister and brother" breeding by crossing subsequent age classes and by using cryo-preserved milt. The size of the brood stock has also been kept big enough to prevent the loss of heterozygoty.

5 REPRODUCTIVE DISORDERS IN OTHER BALTIC FISH SPECIES

The identification of the M-74 syndrome may be due to the fact that salmon is thoroughly surveyed earlier in the life cycle than other species. Therefore, the presence of the observed syndrome or similar conditions in other fish species cannot be excluded. The question should, therefore, be asked whether the reproductive disturbances in salmon reflect a more general reproductive failure in Baltic Sea fish in general and, if so, whether such disturbances are caused by anthropogenic substances, by naturally occurring diseases or simply represent natural fluctuations in the population.

Reproductive disorders in fish species from the Baltic Sea have been described for flounder (*Platichthys flesus*) (Westernhagen *et al.*, 1981), sprat (*Sprattus sprattus*) and cod (*Gadus morhua*) (Grauman and Sukhorukova, 1982), spring spawning herring (*Clupea harengus*) (Hansen, 1985), perch (*Perca fluviatilis* L.) and roach (*Rutilus rutilus* L.) (Sandström *et al.*, 1988), cod, plaice (*Pleuronectes platessa*) and flounder (Westernhagen *et al.*, 1988), perch (Karås *et al.*, 1991), burbot (*Lota lota* L.) (Pulliainen *et al.*, 1992), eel-pout (*Zoarces* *viviparus*) (Jacobsson *et al.*, 1992) and cod (Larsson, in press; Åkerman *et al.*, in press; Prince, pers. comm.). Parasitic infestations in cod larvae (Pedersen *et al.*, 1993) and in turbot larvae (*Scophthalmus maximus*) (Pedersen, 1993) seem to cause mortality. High mortality in hatchery-reared cod larvae due to different injuries and infections was reported by Buchmann *et al.* (1993).

The study of Westernhagen *et al.* (1981) was made on running ripe flounder (caught in a bottom trawl). The eggs were stripped and artificially inseminated, and incubated in Baltic Sea water at 6.8°C at a salinity of 27.5‰. Viable hatch varied between 1 and 96%. Ovary and liver samples from female parental fish were collected and analysed for chlorinated hydrocarbons and heavy metals. The viable hatch was significantly affected at ovarian PCB levels higher than 120 ng/g wet weight. Other chlorinated hydrocarbons and heavy metals measured did not show any correlation between ovary or liver contamination and viable hatch.

In sprat and cod eggs collected from Bornholm to the Gulf of Finland, embryo abnormalities increased from 13% in 1979 to 40-50% in 1981 (Grauman and Sukhorukova, 1982). Additionally, Grauman and Sukhorukova (1982) also found abnormal sprat and flounder larvae.

Hansen (1985) made an investigation similar to that of Westernhagen *et al.* (1981) but on Baltic spring spawning herring. He found a significant correlation between viable hatch of herring eggs and ovary concentrations of DDE higher than 18 ng/g wet weight and PCBs higher than 120 ng/g wet weight.

Westernhagen *et al.* (1988) examined developmental defects in pelagic fish embryos from the southwestern Baltic. Aberrant development was observed in 18-32% of cod eggs, in 24-28% of plaice eggs, and in 44% of flounder eggs. The authors considered the numbers of malformations rather high and to be associated with environmental toxicants. Investigations of viable hatch in eggs caught in horizontally-towed plankton nets demonstrated very high mortality, 96-99%, for all species investigated. However, considering the fragility of fish eggs, high egg mortality should be expected when eggs caught by plankton nets are used for hatching.

Reduced gonadal development was observed especially in perch affected by bleached pulp mill effluents in an area along the Swedish east coast (Sandström *et al.*, 1988). In roach, the effect was more diffuse. Furthermore, this study indicated a change in the energy allocation system in fish exposed to bleached pulp mill effluent, as growth was faster in exposed fish.

The work of Karås *et al.* (1991) focused on the effect of bleached pulp mill effluent on the recruitment of perch in the same area as the latter paper. The bleached pulp mill effluent seriously affected the recruitment, as larval and fry abundances were low. The problem was not due to a reduced number of eggs in the exposed area. No increased egg mortality was observed, but close to hatching approximately 10% of the embryos were found malformed. Sampling of larvae provided evidence for high mortality at or very close to hatching. Two causes for the problem were suggested: chronic failure of the parental reproductive system or acute embryo or early larval mortality.

In an area along the northern coast of the Bothanian Bay, a serious lack of sexual maturation of burbot was observed during the late 1980s (Pulliainen *et al.*, 1992). Nearly all burbot (87-98%) off Tornio and Kemi were found sterile while the figures for two neighbouring areas were in the level of 29-55%. No disease agents could be isolated from the fish and histopathological examinations did not reveal any pathological changes that could have been responsible for the phenomenon. Analysis of burbot liver and muscle samples for some heavy metals and chlorinated hydrocarbons did not reveal any correlation between the contaminant level and the lack of maturity. However, it is proposed that there might be some relationship between some pollutants and the problem. The phenomenon was, however, described by Segerstråhle as early as 1945 (Segerstråhle, 1945), which indicates that other "natural" factors may also be involved.

Jacobsson *et al.* (1992) demonstrated that effluent water from a bleaching paper mill and chlorinated effluent water from an electrical power plant seriously affected the growth and survival of eel-pout fry. The pollutants did not affect the female fish but only the progeny.

Hatching and rearing experiments with cod have been carried out in Sweden with the aim of producing cod for sea ranching in the Baltic (Larsson, 1993). These experiments have demonstrated reduced hatching and larval survival in Baltic cod compared to Skagerrak cod. Similar observations have been made in Danish experiments with cod production for sea ranching purposes in the Baltic (Prince, pers. comm.)

A protistan endoparasite was observed in the yolk sac in up to 30% of Baltic larval cod (Pedersen *et al.*, 1993) and in up to 40% of Baltic turbot larvae (Pedersen, 1993). In cod, the parasite, which occurred in numbers of 1-3 per larvae, seemed to kill the larvae. The same is expected to happen for the turbot larvae.

Health monitoring in hatchery-reared yolk sac larvae of Baltic cod showed high prevalences of bacterial and mycotic infections (up to 50%) as well as other injuries such as yolk sac herniae, scratches of primordial fins, and skeletal deformities (Buchmann *et al.*, 1993).

6 REPRODUCTIVE DISORDERS IN FISH IN OTHER GEOGRAPHIC AREAS

High mortality in hatchery-reared lake trout fry in Lake George was described by Burdick *et al.* (1964). The symptoms appeared at the time of first feeding just after the resorption of the yolk sac. The problem was associated with the female fish. Analyses of DDT in extracts from eggs compared with the development of the syndrome revealed a limit concentration of 2.9 mg/kg other-extracted-oil DDT. Eggs containing more than 2.9 mg/kg other-extracted-oil DDT developed the syndrome while eggs with values below did not develop symptoms.

An association between DDT and fry mortality was described in steelhead fry (*Oncorhynchus mykiss*) in Lake Michigan hatcheries (Johnson and Pecor, 1969). The mortality began in the fourth or fifth week after hatching, increased to peak numbers in the sixth or seventh week and had generally ended by the eighth week. The symptoms were loss of equilibrium and erratic swimming at the surface. The affected fry generally weakened, sank to the bottom, and died within a few days. The stomach of affected fry usually contained some yolk but no food. No external or internal lesions were observed, with the exception of a few fish showing degenerative changes in the kidney and liver. Chemical analyses of DDT of the last fraction of yolk present in the gut showed concentrations that were 6-12 times higher than those in the body tissue. Significantly higher concentrations were found in affected fry than in unaffected fry from the same female. The absorption of the relatively high concentrations of DDT from the gut is suggested as an explanation for the mortality.

In North Sea whiting (*Merlangius merlangus*), Westernhagen *et al.* (1989) demonstrated a correlation between hatching success and organochlorines in samples collected in 1984. A significant negative correlation existed between total hatch and DDT, Σ DDT, dieldrin and Σ of all measured organochlorines. Viable hatch also showed a significant negative correlation with Σ DDT and Σ of organochlorines.

Due to the observation of increased rearing mortality in chinook salmon (*Oncorhynchus tshwytscha*) in the State of Michigan, Giesy *et al.* (1986) investigated the contaminant level of DDT and PCBs in chinook salmon eggs. The investigation revealed a negative correlation between the survival of fry to the swim-up stage and two principal residue components which contained primarily toxaphene and PCB.

In the beginning of the 1980s recruitment failure was observed in the lake trout (*Salvelinus namaycush*) stock in Lake Michigan (Willford *et al.*, 1981; Walker and Peterson, 1991). Mac *et al.* (1985) examined the possible role that organic contaminants and the overall water quality in Lake Michigan might play in this reproductive failure. The investigation demonstrated an almost total mortality of fry from eggs of the southeastern Lake Michigan lake trout. The problem was associated with the source of eggs and/or sperm, not the water quality. The contaminant analysis gave some indications of an association between PCBs and DDE and the syndrome, but it was concluded that these two components alone are not the only factors responsible for the syndrome. Reproductive disturbances of lake trout have been suspected to be caused by organochlorine compounds released from waste dumping grounds and from pulp mills with chlorine bleaching (Whittle *et al.*, 1992). Further investigations by Mac and Schwartz (1992) demonstrated that embryonic mortality was greatest during epiboly and just before hatching, whereas post-hatching mortality was greatest shortly after swim-up. Hatching showed a significant negative correlation with the total PCB content in eggs.

In an attempt to explain an observed reduction in the recruitment of charr (*Salvelinus alpinus* L.) in Lake Geneva, Monod (1985) investigated the correlation between egg mortality and the contaminant burden in the eggs. The total mortality (from fertilization up to yolk sac resorption) was highly variable (5-76%) and seemed to be independent of the age of the females. The investigations demonstrated a significant and positive correlation between PCB and DDT levels in the eggs (expressed on a lipid weight basis) and the total mortality. When egg development was separated into four different developmental stages, a positive correlation between PCB and DDT levels in the eggs (expressed on a lipid weight basis) and the first two developmental stages, e.g., stage 1: eggs without embryonic development, and stage 2: eggs with young embryos.

Investigation of the possible implications of dioxins (as TCDD-EQ) and in PCB-containing extracts from flesh and eggs of chinook salmon in Lake Michigan on reproductive success was carried out by Ankley *et al.* (1991). These investigations showed a statistically significant negative correlation between the total concentrations of PCBs in the eggs and the hatching success of the fish, with an effect concentration that corresponded to approximately 100 pg TCDD-EQ/g egg.

Williams and Giesy (1992) conducted a study to investigate possible correlations between concentrations of PCBs or dioxins (TCDD-EQ) and mortality in early life stages of chinook salmon in Lake Michigan. These authors were not able to establish any correlations between TCDD-EQ, individual PCB congeners or total PCBs in the eggs and the egg and fry mortality.

7 RECOMMENDED RESEARCH EFFORTS

There is an urgent need for an interdisciplinary cooperative research programme to prevent the disappearance of wild salmon stocks in the Baltic Sea.

Research efforts on the M-74 syndrome in salmon should be developed as a separate and goal-oriented project aimed at reducing or eliminating the occurrence of the syndrome.

The most relevant research needs in such a programme are:

- a follow-up of the wild salmon stock status, electrofishing, and confirmation of the relation between the ratio of spawning fish and smolt production;

- a confirmation of criteria for diagnosis: syndrome symptomatology and pathology should be defined;

- a compilation of environmental factors in the artificial breeding that may influence the occurrence of the syndrome: breeding technology factors, opportunistic infections, pollutants;

- an epidemiological description of the syndrome: importance of, e.g., sex, age, size, color, climate, dwelling area in the Baltic Sea;

- a preliminary search for toxic components, starting in affected yolk sac fry and going back to the parental fish, followed by an experimental confirmation;

- the use of biological markers to measure impacts on the reproduction of salmon. Such markers should mirror immunological and/or hormonal functions as well as nutritional status and exposure to environmental pollutants, e.g., via measuring the induction of ethoxyresorufin-O-deethylase (EROD).

<u>Examples of possible markers</u>:

- Measurements of relevant nutritional factors such as astaxanthin in salmon. Changes in tissue levels should either reflect a changed metabolism in the fish or a more profound influence on the ecosystem where these factors are produced.

- Induction of drug-metabolizing enzymes in fish. Such changes can reflect the presence of environmental pollutants or be an effect caused by or causing the reproductive disturbance.

- Changed levels of sex hormones or other hormones, which cause or reflect an observed reproductive disturbance in the fish.

- The presence of morphological changes associated with reproductive disturbance. Such changes could include reduced organ weights (e.g., gonad weight), histological or ultrastructural changes in critical tissues.

- Development of other relevant markers could, within limited financial resources, provide the possibility for screening for environmentally induced changes in the fish.

During recent decades, the Baltic Sea has been heavily influenced by natural and anthropogenic factors which have considerably changed the structure and function of its ecosystem. The effects of these changes, abiotic as well as biotic, on the occurrence of reproductive failures in fish should be studied in relation to:

- abiotic (water quality, oxygen level, temperature, etc.) and/or biotic changes (quality and quantity of food, changes in condition and physiology of the fish, genetic aspects, etc.) from a historical perspective;

- the magnitude and effect of anthropogenic influences from local, regional, and large-scale points of view;

- the magnitude and effect of changed natural conditions from local, regional, and large-scale points of view;

- changes in hydrographic and sedimentological patterns.

In addition, future research programmes should contain components such as preventive fishing regulations and establishing gene banks for threatened stocks.

Most of the above-mentioned elements identified for research on M-74 in salmon may be used in the research on reproductive disturbances in other fish species.

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ANNEX 1

STUDY GROUP ON OCCURRENCE OF M-74 IN FISH STOCKS

Copenhagen, 1–3 March 1994

AGENDA

1. Opening of the meeting
2. Adoption of agenda
3. Presentation of participants
4. Appointment of rapporteurs
5. Presentation of background papers
    - H. Börjeson: What is M-74? and the status of M-74 in Sweden
    - A Soivio: The status of M-74 in Finland
    - E. Ikonen: Assessment of Baltic salmon stocks and the possible implications of M-74
6. Proposed table of contents of the report
7. Distribution of tasks for the preparation of the report
8. Reproductive disorders in other Baltic fish species
9. Reproductive disorders in fish in other geographic areas
10. Possible factors involved in M-74
11. Effects on Baltic salmon stocks
12. Recommended research efforts
13. Recommendations

ANNEX 2

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