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Descriptive Studies of Mortality and Morphological Disorders in Early Life Stages of Cod and Salmon Originating from the Baltic Sea

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Abstract.—The reproductive success of cod *Gadus morhua* from the Baltic Sea and the Barents Sea was compared. The offspring of 17 family pairs from the Baltic Sea and 12 family pairs from the Barents Sea were investigated during the embryonic and larval development stages. Frequencies of mortality over time and frequencies of different disorders at hatch were analyzed. The results indicated that the reproductive success of cod from the Baltic Sea was seriously impaired. The Baltic cod showed high mortality before hatch. In newly hatched larvae, different kinds of disorders were seen, such as vertebrae deformity, disrupted yolk sac or subcutaneous edema in the yolk sac, and precipitate in the yolk. To compare mortality and early developmental abnormalities in Baltic cod and Baltic salmon *Salmo salar*, the offspring of 20 salmon family pairs, caught in the River Dalälven in Sweden, were investigated analogically. The results showed that the majority of the salmon offspring experienced a thiamine deficiency-dependent mortality at different stages of larval development and that five family pairs experienced high mortality before hatch. In salmon, different kinds of disorders were also seen at hatch, such as vertebrae deformity, blood disorders, subcutaneous edema in the yolk sac, and precipitate in the yolk. The disorders at hatch were not correlated to later thiamine deficiency-dependent mortality. Aliquots of newly fertilized salmon eggs were injected with thiamine by the nanoinjection method. This treatment had only a minor effect on the frequency of disorders at hatch, but it protected the salmon larvae almost completely from later thiamine deficiency-dependent mortality. This indicates that factors other than thiamine deficiency are involved in the developmental disorders. In both salmon and cod from the Baltic Sea, the mortality and disorders among the offspring were mainly correlated to the female, and in both species some females produced offspring that experienced high mortality before hatch. Both salmon and cod also showed disorders that might have similar biochemical mechanisms, because the formation of precipitates and edema in the yolk sac occurs in both species.

The Baltic Sea is a brackish-water sea that contains a young ecosystem compared with other marine ecosystems. The Baltic Sea consists of three main basins: the Bothnian Bay, the Bothnian Sea, and the Baltic proper. The Baltic proper opens into the North Sea via a narrow outlet, the Kattegat. The species that invaded after the last glacial period were marine species, with wide salinity tolerance from the North Sea, and limnic species, with salinity tolerance from the glacial lakes. Today, the Baltic Sea is inhabited by few but relatively abundant species, and several are living close to their osmotic tolerance limit. The salinity in the surface water decreases from about 20‰ in Kattegat, to 6–8‰ in the Baltic proper, to 2–4‰ in the northern part, the Bothnian Bay. The deep water has a higher salinity and is only partly mixed with the surface water. The exchange of surface water depends mainly on irregular inflows of marine water, which are dependent on factors such as low and high atmospheric pressure and heavy storms working in concert. Oxygen deficiency is common in some parts of the Baltic Sea below the halocline during stagnation periods.

Today, the Baltic Sea is surrounded by urban industrial countries, which results in anthropogenic effects such as eutrophication and discharges of inorganic and organic pollutants. The hydrographic characteristics of the Baltic Sea, notably its low water turnover rate (about 40 years), low water temperatures, and pronounced thermoclines and haloclines, contribute to an accumulation of persistent organic compounds and heavy metals in the sediments and biota. Certain compounds, such as DDT, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane, and PCBs, have decreased in some species, probably as a result of their being banned, but many "new" and "old" chemicals continue to enter the ecosystem with known and unknown toxicological effects (Bignert et al. 1993).

Several fish species in the Baltic Sea show signs of reproductive disorders. The most obvious occurs in the Atlantic salmon *Salmo salar* and is designated the M74 syndrome, but there are indications of reproductive failure also in sea trout *Salmo trutta* (Bengtsson et al. 1994), cod *Gadus morhua* (Westernhagen et al. 1988; Larsson 1994; Åkerman et al. 1996a), eel *Anguilla anguilla* (Moriarty 1990; Larsson et al. 1991), and perch *Perca fluviatilis*. The reproductive disorders in perch show a geographical connection to the pulp mill industry (Karås et al. 1991). In burbot *Lota lota*, a high frequency of individuals with retarded gonadal development has been reported in the Gulf of Bothnia (the northern part of the Bothnian Bay; Pulliainen et al. 1992).

The underlying reasons for the reproductive disturbances among all of these fish species in the Baltic Sea are not known. Because the Baltic Sea is characterized by low diversity and simple food chains, the observed reproductive disturbances represent a more serious threat here than in other ecosystems.

Cod is one of the most important species for Swedish fisheries. There are five separate cod populations in Swedish waters, but the largest and most important one is the eastern Baltic cod population. This population has adapted, to a certain degree, to the low salinity of brackish water. Salinity levels sufficient for reproduction are present in the deeper parts of the Baltic proper. However, because of the increased eutrophication, the oxygen concentration is often too low for developing offspring. Long stagnation periods, therefore, have led to serious declines in the population. Recently, during the years 1993–1994, large inflows of marine water, attributable to meteorological events, improved the salinity and oxygen conditions in the spawning areas. However, despite the favorable conditions for reproduction, no abundant year-classes of the cod have been found (Larsson 1994).

During the last 23 years, high mortality has occurred among salmon yolk sac larvae (M74 syndrome) in Swedish hatcheries, where reproduction is based on wild broodstock, with the exception of the years 1980–1984, when no or very low mortality was reported. Around 90% of the Baltic salmon stock is reared in hatcheries to compensate for the damming of rivers for hydroelectric power stations. However, larvae from wild spawning salmon have also been found to be affected in Sweden as well as in Finland (Soivio 1994; Karlström 1995). During the years 1992–1995, 57–87% of the female spawners at some Swedish hatcheries produced offspring with very high larval mortality. Just before death, the larvae exhibit lack of coordination, irregular swimming patterns, lethargy, and darkening of the skin. These signs have been correlated to low concentrations of thiamine (vitamin B_1) in the salmon eggs and larvae (Amcoff et al. 1996; Koski et al. 1996).

In North America, the early mortality syndrome (EMS) in several salmonid species in some of the Great Lakes (Lakes Michigan, Ontario, and Erie) and the Cayuga syndrome in landlocked Atlantic salmon in the Finger Lakes of New York State demonstrate similarities with the M74 syndrome in the Baltic salmon. The syndromes show similar clinical signs in the period before death. In Canada, in 1991, Fitzsimons conducted experiments during which he discovered that the mortality is related to low thiamine levels (Fitzsimons 1995). Affected larvae in these different locations have been treated successfully (as measured by lower mortality) with thiamine at the egg or larval stage (Bylund and Lerche 1995; Fitzsimons 1995; Åkerman et al. 1996b; Amcoff et al. 1996; Fisher et al. 1996; Koski et al. 1996). Thus, thiamine deficiency appears to be a common factor in these syndromes. The underlying reason for the low levels of thiamine is a crucial and important question that still needs to be answered.

In a previous report, comparisons were made of the reproductive success of cod from the Baltic Sea and the Barents Sea (Åkerman et al. 1996a). The results from that study indicate that the reproductive success of Baltic Sea cod was seriously impaired as a result of high mortality of the eggs and embryos. Elevated levels of DNA adducts were found in the cod offspring from the Baltic Sea before feeding, indicating a maternal transfer of xenobiotics (Ericson et al. 1996). In the present study, the results from a comparison of reproductive success are presented in more detail. Mortality rates of offspring from different females are presented, and frequencies of disorders in the larvae are illustrated. A comparison with mortality and disorders early in development in salmon offspring from the River Dalälven in Sweden, where the salmon are affected by the M74 syndrome, is also presented. Salmon were also treated with thiamine, by bathing of larvae or by nanoinjection of the newly fertilized eggs.

Materials and Methods

Cod

During the 1994 spawning season, comparative studies of the reproductive outcome from North East Arctic cod and Baltic cod were performed. North East Arctic cod were caught in the

TABLE 1.—Weight, length, condition factors, percentage of fertilized eggs, and number of fertilized eggs studied in Atlantic salmon during the 1994–1995 season (females A–F) and the 1995–1996 season (females G–L).

Female/male	Female weight (kg)	Female length (cm)	Condition factor[a]	Percentage of fertilized eggs	Number of fertilized eggs studied
A/1	10.8	101	1.05	43	77
A/2				44	88
B/1	12.8	103	1.17	98	210
B/2				99	192
B/3				98	196
B/4				98	253
C/3	8.2	89	1.16	96	158
C/4				96	130
D/4	10.2	96	1.15	96	211
D/5				6	14
E/5	6.8	88	1.00	29	65
E/6				63	192
F/6	8.2	91	1.09	21	47
F/7				99	201
G/8	13.5	108	1.07	81	116
H/9	6.9	85	1.12	87	126
I/10	7.2	82	1.31	92	133
J/11	6.2	84	1.05	95	136
K/12	9.5	93	1.18	87	153
L/13	10.5	98	1.12	64	111

[a] (Total weight in grams) ÷ (Total length in cubic centimeters) × 100.

Lofoten area by trawling during the spawning period (23–24 March). Mature cod were stripped by a slight pressure to the abdomen, and the eggs were fertilized immediately on the boat. Six females and 3 males were used in 12 different female–male combinations. Each egg batch contained eggs from one female fertilized by one male only. Immediately after fertilization and water hardening, the eggs were transported to the laboratory, where they were held in 34‰ seawater prepared from synthetic sea salt (hw Marinemix, Wiegandt, Germany) and distilled water. The use of synthetic seawater decreased the risk of contamination by biotic factors. The salinity used is the well-known concentration in the spawning area in Lofoten; at this salinity, fertilized eggs float and unfertilized and dead eggs sink to the bottom. From the eggs that remained buoyant at the blastula stage, 300 eggs were used for analyses. The egg batches were kept in a volume of 1 L and at a temperature of 6.5°C. The water was changed daily.

Baltic cod were caught by trawling during two sampling sessions (14 June and 11 July) north of the island Bornholm in the Baltic Sea. Mature cod were stripped and the eggs were fertilized immedi-

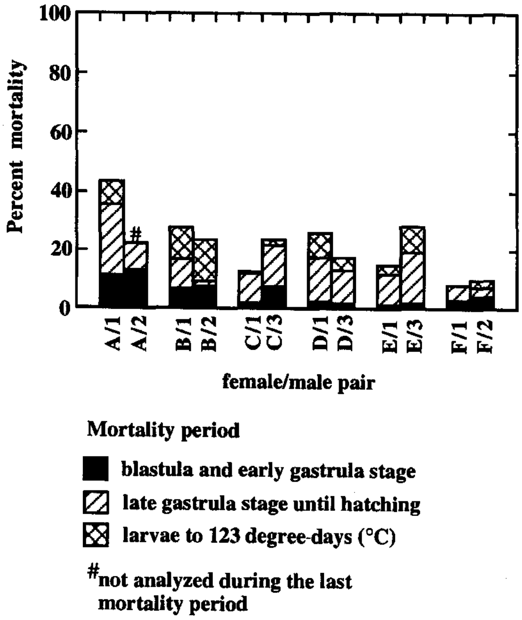

FIGURE 1.—Mortality at different periods in the offspring of six cod females (A–F) and three males (1–3) originating from Lofoten, Norway. The bars represent cumulative percentages of mortality at the different periods, calculated from the number of dead animals recorded during each period as a percentage of the number of fertilized eggs at the start (day 1).

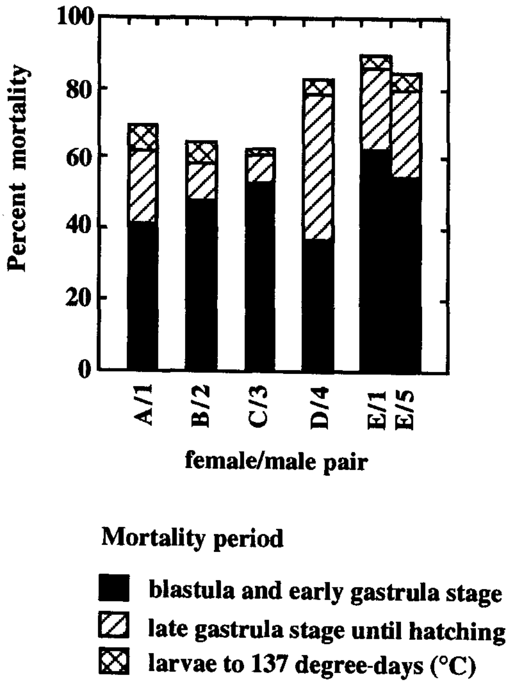

FIGURE 2.—Mortality at different periods in the offspring of five cod females (A–E) and five males (1–5) originating from our first sampling in the Baltic Sea. The percentages are calculated as described for Figure 1.

ately on the boat in the manner described above. The eggs were fertilized and held in 17‰ synthetic seawater prepared as described above. This salinity is optimal for Baltic cod egg development (Nissling and Westin 1991; Westin and Nissling 1991). From the first sampling session, 5 females and 5 males were investigated in 6 different batches; from the second sampling session, 11 females and 7 males were investigated in 11 different batches. Each egg batch contained eggs from one female fertilized by one male only. The number of eggs used in the investigation and the handling of eggs during development were the same as in the Lofoten investigation; the temperatures were 5.8 and 7.0–8.4°C, respectively, for the two sampling sessions.

Dead eggs, embryos, and larvae were removed daily and counted. When hatching was completed, the larvae were examined under a stereomicroscope (Wild M8, Heerbrugg, Switzerland; 6–50× magnification) and frequencies of disorders were recorded. Baltic cod larvae from the second sampling session were examined 2 d after hatching was completed, at 122 degree-days. Variables analyzed were severe disorders (larvae arrested in development combined with vertebrae and eye deformity), vertebrae deformity, disrupted yolk sac or edema in yolk sac, precipitate in yolk, and deformed or opalescent muscle. In total, 8,472 fertilized eggs were investigated; the survivors (3,542 individuals) were examined at 122 degree-days (newly hatched), and 205 were examined at 137 degree-days after fertilization.

Salmon

Studies of salmon reproductive outcome were performed during two seasons, 1994–1995 and 1995–1996. The eggs and sperm were obtained by stripping salmon from the River Dalälven, Sweden (Älvkarleby Fisheries Research Station). During these two reproductive seasons, 47 and 52%, respectively, of the mature female fish showed signs of the M74 syndrome. Hybrid fish (salmon and trout) were detected by starch gel electrophoresis of diagnostic enzymes (glucose-6-phosphate isomerase and phosphoglycometase) and were omitted from the experiments.

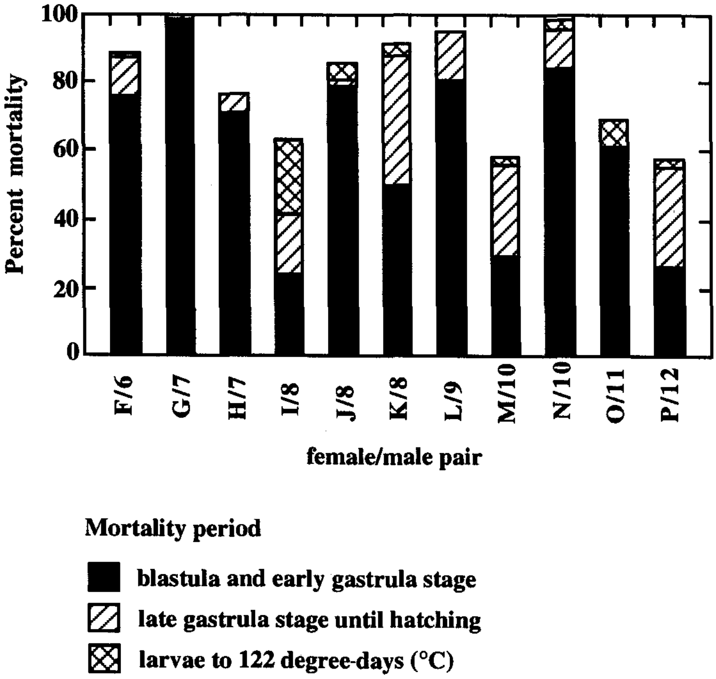

FIGURE 3.—Mortality at different periods in the offspring of 11 cod females (F–P) and 7 males (6–12) originating from our second sampling in the Baltic Sea. The percentages are calculated as described for Figure 1.

During the season 1994–1995, the offspring of six females and seven males were studied. The egg batch from each female was divided into two aliquots and fertilized with milt from two different males, one in each aliquot, except for one female whose egg batch was divided into four aliquots and fertilized with milt from four different males. After fertilization, the 14 egg batches, each containing around 200 eggs, were kept in separate aquariums supplied with running charcoal-filtered tap water from a nearby freshwater lake at an ambient temperature of 4.5–8.5°C until the 6 last weeks, when the temperature was increased to 9–13°C. Each aquarium held 2 L of water and had a water flow of 17 mL/min; thus, the turnover rate was 2 h. Mortality was recorded every second day during egg, larval, and juvenile development until 1,720 degree-days. During week 19 after fertilization, feeding with commercial food was started (Ewos EST 90, Sodertalje, Sweden).

Four weeks after hatching (100 degree-days), aliquots of larvae (30–40 larvae in each) from four family groups were treated by bathing once in thiamine hydrochloride (Sigma T-4625, Sigma Chemical Co., St. Louis, Missouri) at 400 mg/L for 1 h. Another four aliquots from the same family groups were treated for 2 h every third day for 3 weeks in thiamine hydrochloride at 200 mg/L. The pH change during these treatments was less than 0.1.

During the season 1995–1996, the offspring of six females and six males were studied. The entire egg batch from each female was fertilized by one male. After fertilization and water hardening, an aliquot of eggs from each female was placed in a 1% agarose gel in Petri dishes before they were put in separate aquariums as described above, now at an ambient temperature of 4.0–7.5°C. Four square Petri dishes, each holding 36 eggs, were used for each family pair. This method of placing the newly fertilized eggs in holes made in an agarose gel facilitates injections with fine glass capillaries into the eggs early in development and is known as the nanoinjection method (Åkerman and Balk 1995). Equally important, this method facilitates observation of embryonic development until hatching without disturbing the developmental process. The eggs in one Petri dish from each female were injected with 24–31 nL (0.02% of egg volume) of thiamine chloride solution (0.3 mol/L, pH adjusted to 6.4), and the eggs in one Petri dish from three of the females were

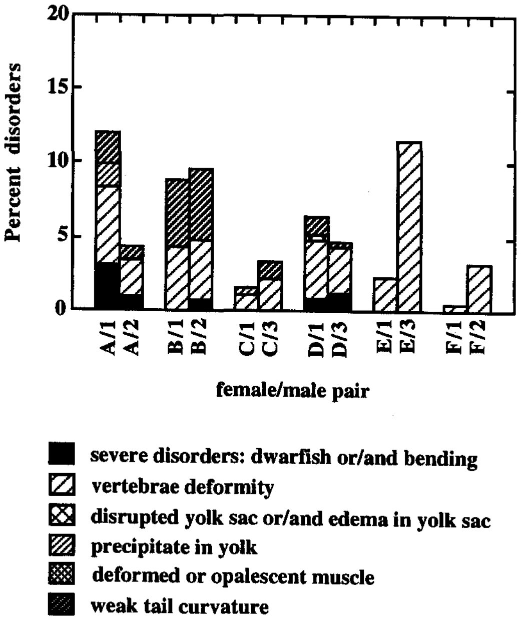

FIGURE 4.—Disorders recorded in newly hatched cod larvae from six females (A–F) and three males (1–3) originating from Lofoten, Norway. Females A–F and males 1–3 denote the corresponding female–male pairs as in Figure 1. The bars represent percentages of hatched larvae with various disorders. Larvae with more than one disorder are presented once, in the first group from below.

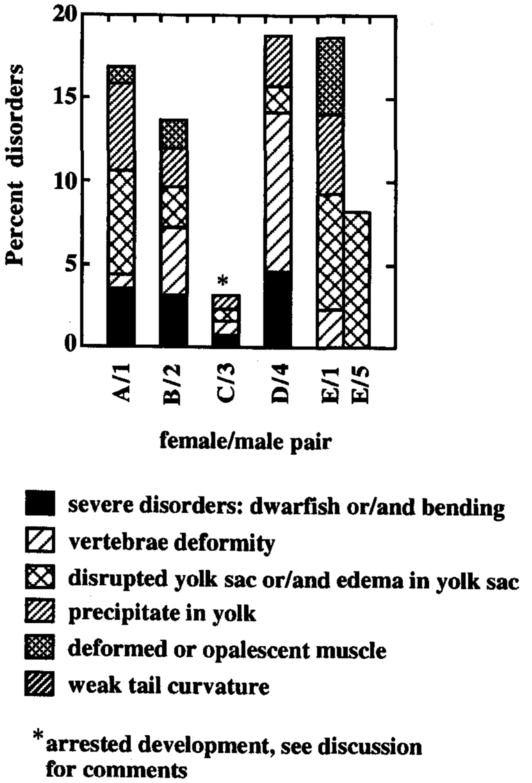

FIGURE 5.—Disorders recorded in newly hatched cod larvae from five females (A–E) and five males (1–5) originating from our first sampling in the Baltic Sea. Females A–E and males 1–5 denote the corresponding female–male pairs as in Figure 2. The percentages are calculated as described for Figure 4.

injected with 61–78 nL (0.05% of egg volume) of the same solution. After injection, the concentrations of thiamine in the eggs were increased by 52 and 130 nmol/g, respectively. Mortality was recorded every second day during egg, larval, and juvenile development until 940 degree-days. In all other respects, the eggs and larvae were handled and observed as described in the preceding season.

Immediately after hatching, the larvae were examined under a stereomicroscope (Wild M8; 6–50× magnification) and frequencies of disorders were recorded. Variables analyzed were vertebrae deformities, precipitate in the yolk, hemorrhages, and subcutaneous edema. All calculations of frequencies were made after disposal of unfertilized eggs.

The basic data for the salmon females used in these studies are shown in Table 1. For each female, the percentage of fertilized eggs with each male and the number of investigated eggs or embryos are also shown. The condition factor was calculated according to the formula [(total weight in grams) ÷ (total length in cubic centimeters) × 100]. In total, 3,878 salmon eggs were investigated for mortality during early development (until 940 or 1,720 degree-days). In addition, the survivors at hatch (2,432 individuals) were examined for disorders at the newly hatched stage.

Results

Cod

Mortality until hatching in the offspring of females and males caught in Lofoten ranged from 7 to 22%, except for one female–male combination whose offspring experienced 36% mortality (Figure 1). Mortality during the larval period ranged from 0.3 to 14%. The majority of deaths occurred during embryonic development. Mortality during the blastula and early gastrula stages was correlated to a greater extent to the female than to the male.

Mortality until hatching in the offspring of females and males caught in the first sampling session in the Baltic Sea ranged from 58 to 86%. Mortality was most

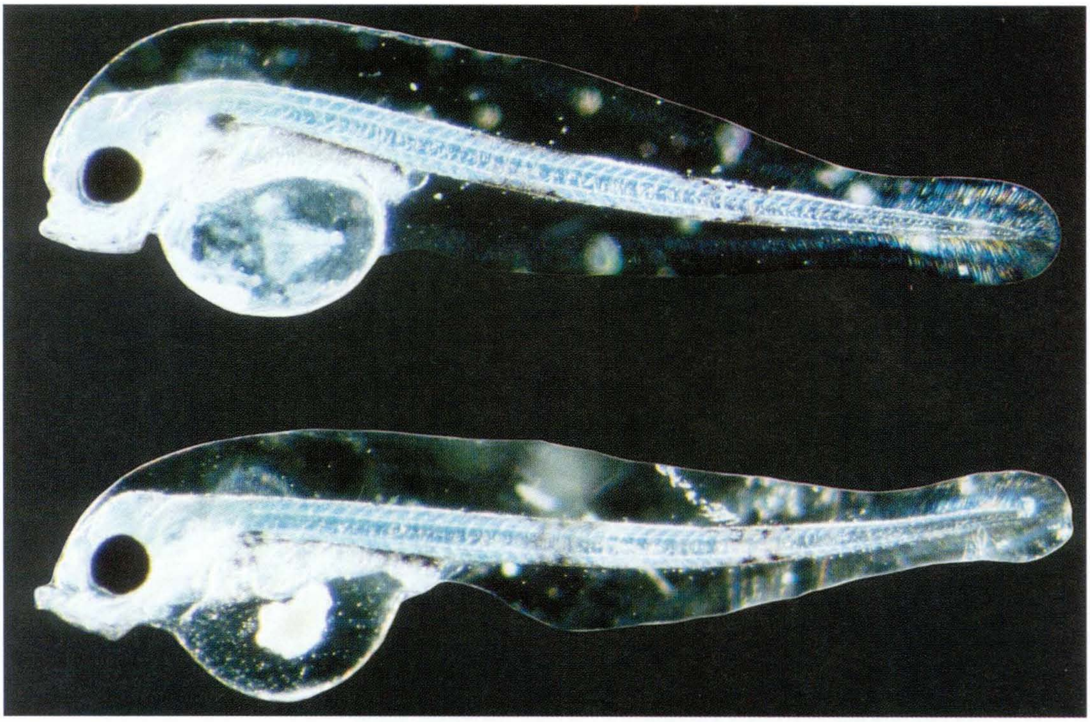

FIGURE 6.—Larvae from cod originating from the Baltic Sea. The upper larva has a precipitate in the yolk and the lower larva has a disrupted yolk sac. These kinds of disorders occur frequently in newly hatched cod larvae from the Baltic Sea (see Figures 5 and 8).

pronounced during the blastula and early gastrula stages (Figure 2). During the embryonic period (late gastrula until hatch), a relatively large difference in mortality was observed between females. The larval mortality was not greater than 7%. Only minor differences in mortality were observed between the egg batches from female E fertilized by male 1 or male 5.

The offspring of the females investigated from the second sampling session in the Baltic Sea showed a mortality range before hatching of 42–100% (Figure 3). As in the first sampling, the highest frequency of mortality occurred during early development, except in the egg batches from three females, I, M, and P, in which the frequencies were less than 30%. At the end of yolk sac consumption, however, the mortality reached almost 60% in these batches.

The major disorders in newly hatched larvae originating from Lofoten were vertebrae deformity and weak tail curvature (Figure 4). Vertebrae deformity was mostly expressed as curvature of the tail that affected swimming behavior. The larvae in the group with weak tail curvature showed normal swimming behavior. The frequencies of these two disorders differed depending on the male used, although egg batches from different females had the strongest influence.

The major disorders in newly hatched larvae originating from the Baltic Sea were vertebrae deformity and different kinds of abnormalities in the yolk sac, such as disrupted yolk sac, edema in the yolk sac, and precipitate in the yolk. Deformed or opalescent muscle was also frequently observed (Figure 5). Examples of abnormalities in the yolk sac are shown in Figures 6 and 7.

The larvae from the second sampling session in the Baltic Sea were investigated at a later stage of development, when only a minor part of the yolk remained. Vertebrae deformity together with disrupted yolk sac or edema were the main disorders (Figure 8).

Only small differences were found in the disorders seen in the fish from the first and second sampling sessions in the Baltic Sea. Offspring of cod originating from Lofoten showed no disrupted yolk sacs or edema and very rare occurrence of precipitate in the yolk, which were observed frequently in the offspring of cod originating from the Baltic Sea.

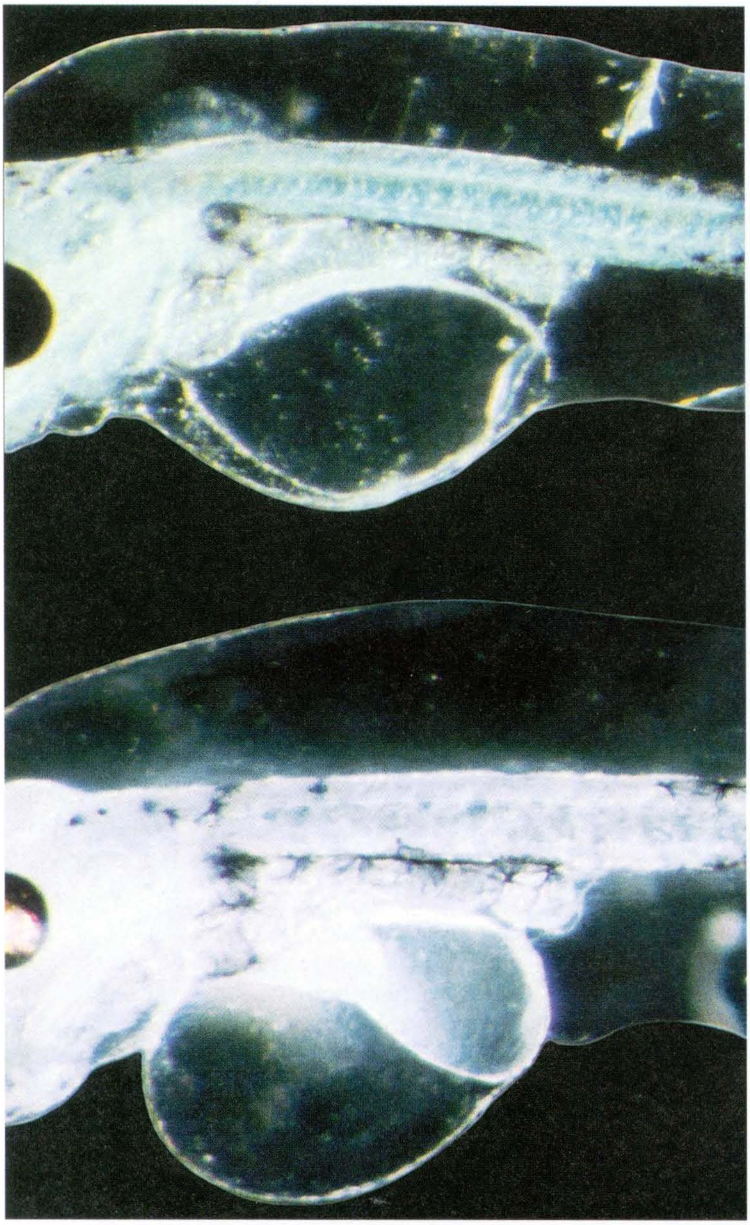

FIGURE 7.—Detail of yolk sacs in cod larvae originating from the Baltic Sea. The upper larva shows a normal yolk sac and the lower larva shows a large edema in the yolk sac. Edema in the yolk sac occurs frequently in newly hatched cod larvae from the Baltic Sea (see Figures 5 and 8).

Salmon

Mortality during different periods in the offspring of six females fertilized by seven males during the season 1994–1995 is shown in Figure 9. Early mortality was seen in the offspring of two females. Approximately 50% of the eggs or embryos from female A died before hatching, that is, about 20% during the first 4 weeks after fertilization and about 30% during embryonic development (weeks 5–12). High early mortality was also seen in the offspring of female E fertilized by male 6. In many embryos that died before hatching, the blastopore was not closed, and the embryos were very small, with severe vertebrae deformities (Figure 10). For two of the females, D and C, the larvae died during the later part of yolk sac absorption after showing signs such as lethargy and abnormal swimming. The larvae from female D experienced almost total mortality just before swim-up, and the larvae from female C experienced almost

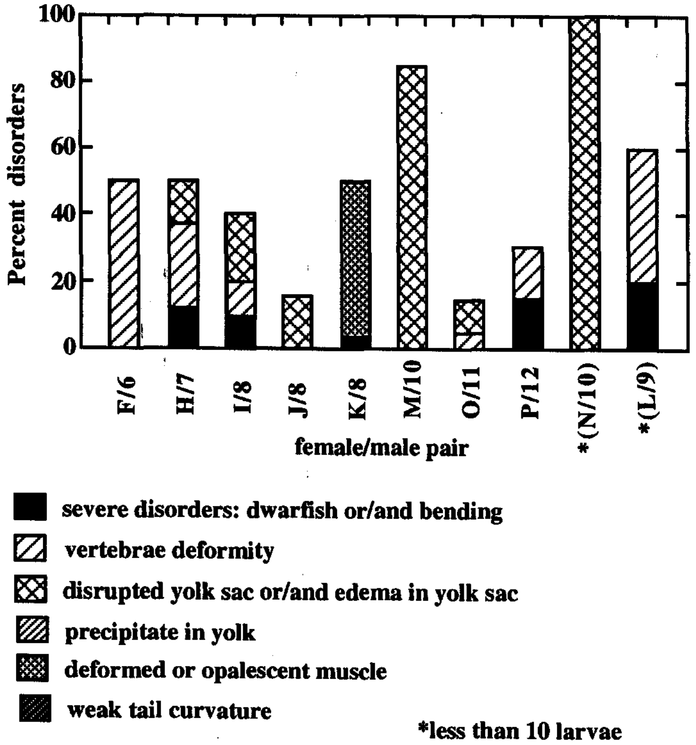

FIGURE 8.—Disorders recorded in cod larvae at 122 degree-days from 10 females (F and H–P) and 7 males (6–12) originating from our second sampling in the Baltic Sea. Females F and H–P and males 6–12 denote the corresponding female–male pairs as in Figure 3. The percentages are calculated as described for Figure 4.

total mortality just after swim-up. In the larval groups from females B and F, mortality started late, at weeks 24–28, which was several weeks after the start of feeding (week 19). Mortality of the remaining offspring of female A and the offspring of female F and male 7 started even later, during weeks 29–33. Before they died, they showed the same signs of lethargy and abnormal swimming that were seen in the larvae of females D and C.

Figure 9 also shows the influence of the different males used. In most cases, the mortality time curve obtained was correlated to the specific female with no or little influence from the male. However, for some family pairings, the male definitely influenced the mortality over time. For instance, for the eggs from female E, male 6 produced drastically earlier mortality compared with male 5. Also, for the eggs from female F, male 6 produced earlier mortality, in this case compared with male 7.

Thiamine treatment by bathing aliquots of larvae from four family pairs during weeks 16–18 delayed the onset of mortality in a dose-dependent manner (Figure 11). Untreated larvae from female D and male 4 experienced their major mortality during weeks 16–18. One treatment (400 mg/L for 1 h) during week 16 shifted the mortality to occur mainly during weeks 19–23. Repeated treatments (200 mg/L for 2 h) during weeks 16–18 shifted the mortality period even further, to weeks 29–33. Larvae from female F and male 7 experienced comparatively late mortality when left untreated, most occurring during weeks 24–28. One treatment shifted the major mortality to the last period studied, weeks 29–33, whereas repeated treatments protected the larvae during this period. The larvae from untreated females B and C experienced mortality at an intermediate period compared with the larvae from females D and F. For larvae from females B and C, only repeated treatments shifted the mortality to a later period, weeks 29–33. This was especially pronounced for larvae from female B.

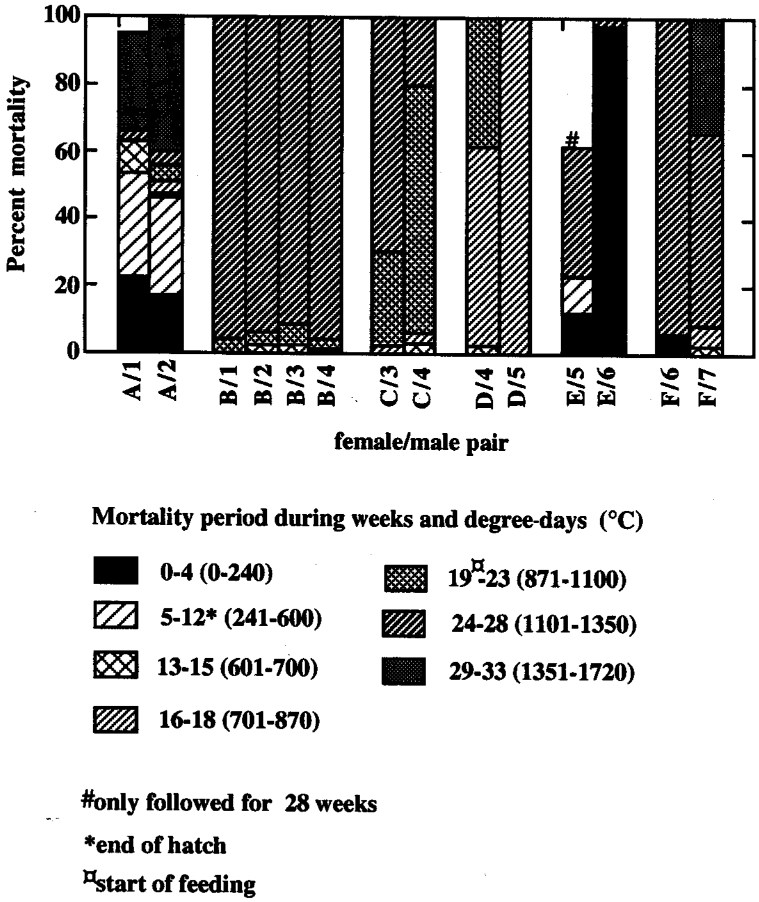

FIGURE 9.—Mortality at different periods in the offspring of six salmon females (A–F) and seven males (1–7) during the 1994–1995 season. The first period starts at fertilization (week 0) and the last period ends after 34 weeks or 1,720 degree-days. The percentages are calculated as described for Figure 1.

During the season 1995–1996, six different family pairs were investigated. In general, the mortality occurred comparatively earlier during development than in the season 1994–1995. The offspring of one female, L, experienced early mortality: 30% during the first weeks after fertilization (weeks 0–5) and another 30% during the embryonic period (weeks 6–16; Figure 12). The larval mortality for females H, I, J, and K occurred after the appearance of signs such as lethargy and abnormal swimming. The offspring of three of these females, H, I, and J, developed the signs when approximately only one-third to one-half of the yolk sac was consumed. For female L, the larval mortality during weeks 19–24 was not preceded by signs of lethargy. These larvae suffered from severe vertebrae deformity (see below). The offspring of female G showed very low overall mortality for the whole study period of 24 weeks. At 24 weeks, almost all of the oil in the yolk had been consumed.

In addition to the mortality in the untreated groups, Figure 12 also shows the results from the thiamine injections into the eggs immediately after fertilization by the nanoinjection method. Two dosages were investigated, 52 and 130 nmol/g. Both concentrations prevented larval mortality until the end (almost all oil consumed) of the experiment for the offspring of females H, I, J, and K. In some of the egg batches, however, thiamine caused a tendency toward increased early mortality. This was especially true for eggs from the relatively unaffected (low mortality) female G at the 130 nmol/g dose of thiamine.

FIGURE 10.—Eggs from Atlantic salmon originating from the Baltic Sea. In the egg at left, the embryo has developed normally. In the egg at right, the embryo has developed severe disorders and will die before hatching. This kind of embryo was seen in the offspring of females A, F, and L (see Figures 9 and 12).

Blood disorders (Figure 13), such as hemorrhage in the head and areas of arrested blood cell circulation in the head or yolk sac vascular system, and precipitate in the yolk sac (Figure 14) were the major disorders in the newly hatched 1995 larvae. For blood disorders, a frequency of around 15–25% seems to be common. Examples of the most frequently seen type of hemorrhage, which occurs in the head, are shown in Figure 15. The larvae from female A showed the highest percentage of blood disorders, affecting 30–50% of the larvae. This was correlated to increased mortality before hatch (see above). The males that were used to fertilize the eggs may influence the frequency of disorders to some extent. Male 3 increased the frequency of blood disorders in larvae from female C and the frequency of edema in larvae from female B compared with male 4. An example of edema in the yolk sac is shown in Figure 16. Male 7 increased the frequency of blood disorders in larvae from female F compared with male 6. Larvae with high frequencies of blood disorders also seem to have high frequencies of precipitate in the yolk. The different males affected the frequency of precipitate by around 20% in the offspring of four females (Figure 14). The precipitate observed immediately after hatching was situated along the blood vessels; later in development, but before larval mortality, the precipitate was observed around the oil droplets situated near the yolk sac membrane (Figure 17).

Blood disorders and precipitate in the yolk were also the major disorders in the newly hatched 1996 larvae. For blood disorders, a frequency of 15–30% was seen in most of the larval groups (Figure 18). The frequency of precipitate was highest in larvae from females G and L (not shown). One group of larvae experienced a high degree (80%) of severe vertebrae deformities. This was correlated to a high level of early mortality (see above).

Injection of thiamine into the eggs at the newly fertilized stage had little influence on total disorder frequencies. A slight but consistent decrease in disorders was observed in larvae injected with thiamine at 52 nmol/g compared with control larvae (except those from female L).

Untreated larvae investigated at 21–24 weeks during the season 1995–1996 that showed signs of lethargy and abnormal swimming before death also exhibited dark pigmentation. Other disorders occurred sporadically within family groups. The most frequent disorder was precipitate in the yolk, but the amount differed between individual larvae in the same family group. Precipitate in the yolk is sometimes described as white precipitate or opalescent yolk. The appearance is different depending on the direction of light (from above or below) during observation or photography (see Figure 19). Exophthalmia was also frequent in all larvae groups

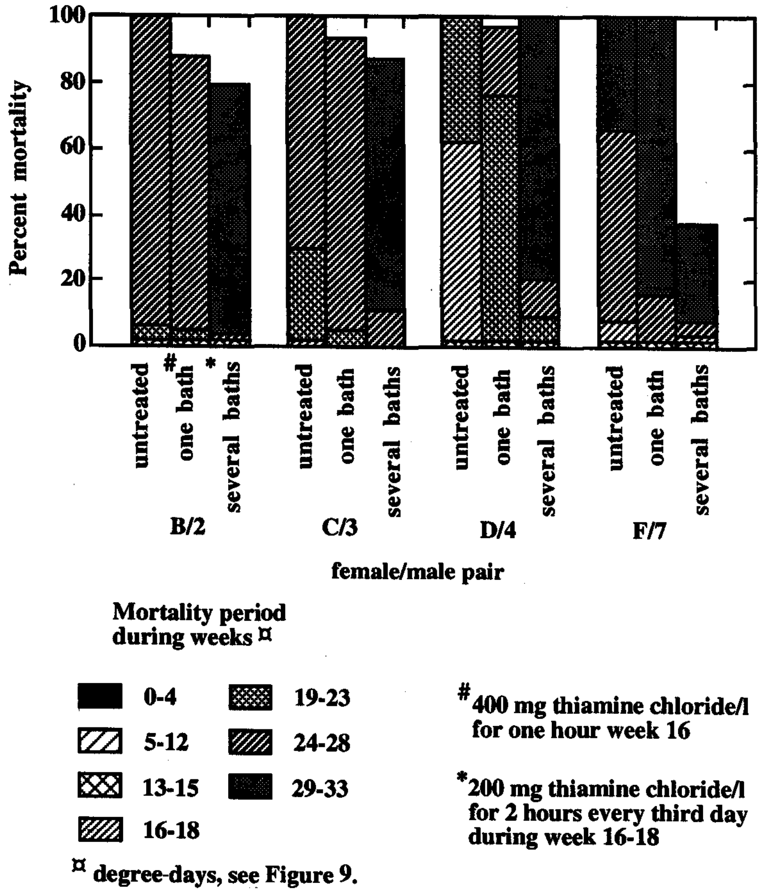

FIGURE 11.—Mortality at different periods after thiamine treatment in the larvae from four female–male pairs during the 1994–1995 season. Treatment consisted of bathing aliquots in thiamine hydrochloride. Females B–D and F and males 2–4 and 7 denote the corresponding female–male pairs as in Figure 9. The percentages are calculated as described for Figure 1.

but to a different extent in different groups. In every family group, some larvae had disorders such as hemorrhages in the head, cessation of blood flow in different tissues, and subcutaneous edema in the yolk sac. Some larvae had pale livers and some had pale yolk oil.

Discussion

The purpose of this investigation was to compare and carefully analyze mortality over time and mortality frequency together with the frequency of various kinds of disorders in both salmon and cod. Disorders were studied with the aim of obtaining data on variables that might be specific for the later development of yolk sac mortality and that might provide clues to the underlying reasons for impairment among offspring. It was decided to investigate the occurrence of disorders just after hatch. This period was selected to avoid disorders that occur near the previously described time of larval death (Bengsston et al. 1994), which could diminish the value of information concerning a cause and effect relationship in larval mortality.

Mortality among yolk sac larvae of salmon in a number of different rivers draining into the Baltic Sea has been described in previous studies (Bengtsson et al. 1994; Norrgren et al. 1994). The high mortality occurs at a specific time, that is, at a developmental stage when about two-thirds of the yolk sac is consumed (Norrgren et al. 1994; Bylund and Lerche 1995). In contrast to the disorders that occur near the time of high larval mortality, it has been argued that disorders that occur during the earlier stages of development generally are limited or absent. However, lower levels of the carotenoids, es-

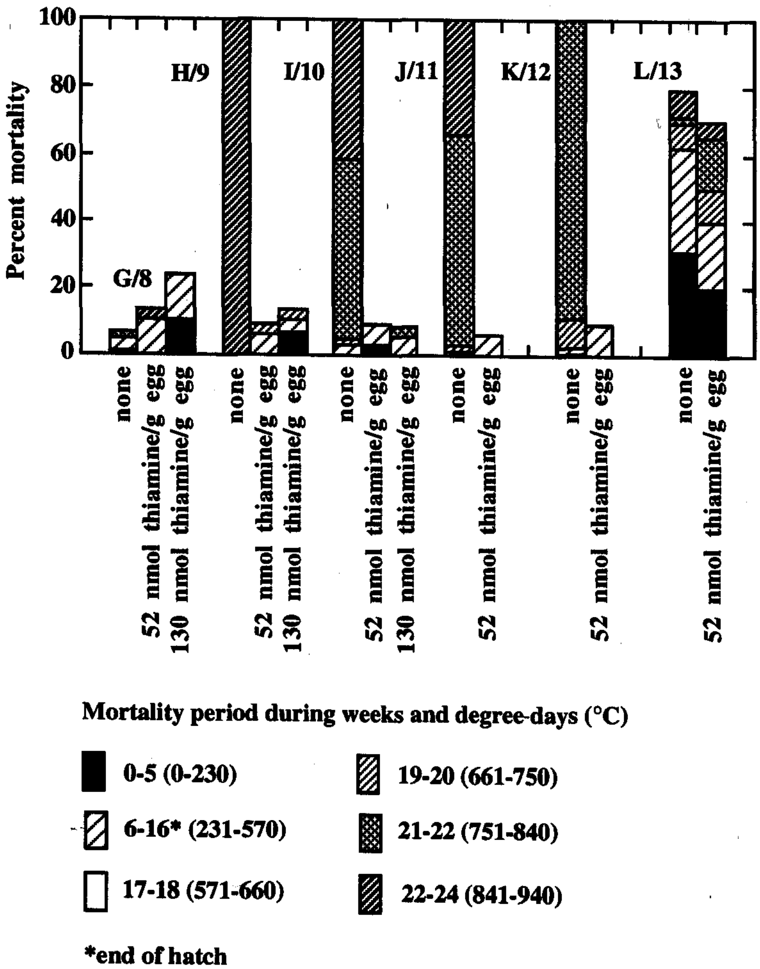

FIGURE 12.—Mortality at different periods in the offspring of six salmon females (G–L) and six males (8–13) during the 1995–1996 season. The second and third bars in each group show mortality in eggs and larvae that were nanoinjected with thiamine (52 or 130 nmol/g) in the yolk immediately after fertilization; the first bar in each group shows mortality in the noninjected control group (none). The first period starts at fertilization (week 0) and the last period ends after 25 weeks or 940 degree-days. The percentages are calculated as in Figure 1.

pecially astaxanthin, have been described in batches of eggs that subsequently develop high larval mortality (Lignell 1994; Pettersson and Lignell 1996).

The reproductive success of the Baltic cod was investigated in parallel with that of cod from the Lofoten area. The reason for this is that we are uncertain if there are healthy cod still to be found in the Baltic Sea. Lofoten is located in the northern part of Norway near the spawning grounds of the North East Arctic cod, which have their feeding areas in the relatively unpolluted Barents Sea. This cod stock is exposed to significantly lower concentrations of anthropogenic substances than the cod living in the Baltic Sea (Jensen et al. 1972; Koistinen 1990; Falandysz 1994; Falandysz et al. 1994). Obviously mature cod were caught in the field during their optimal spawning periods for the two areas (Lofoten and the Baltic Sea). Stripping (with a slight pressure) and fertilization were performed immediately on the boat. This might be a special advantage of this study compared with previous studies, because no selection of parent animals occurred as a result of mortality. Cod caught by trawling exhibit high mortality levels in several handling situations, such as during ship transport to the harbor, during land transport to

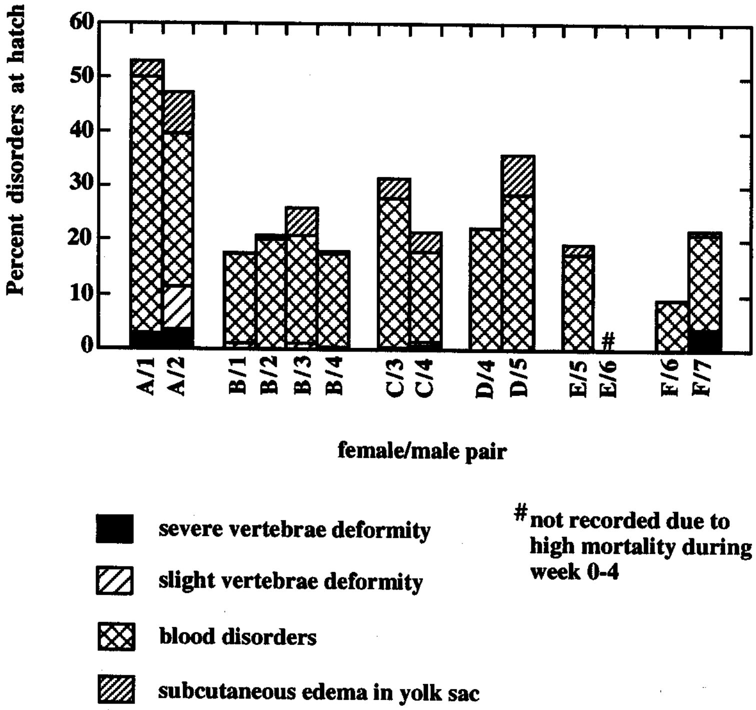

FIGURE 13.—Disorders recorded at hatch in salmon larvae from six females (A–F) and seven males (1–7) during the 1994–1995 season. Females A–F and males 1–7 denote the corresponding female–male pairs as in Figure 9. The percentages are calculated as in Figure 4.

the laboratory, and while staying in basins at the laboratory before producing gametes. This mortality might seriously influence the quality of the offspring produced when the cod are subsequently stripped for mature gametes and artificial fertilization or when they are allowed to spawn freely in the basins. The quality of the offspring in such experiments might be unnaturally high compared with that in the field, because there is a risk that some of the inferior animals will be eliminated as a result of mortality. For instance, fish with low thiamine contents have been reported to show increased sensitivity to handling (Boonyaratpalin and Wanakowat 1993). There is also a possibility that individuals in poor health do not develop mature gonads in the laboratory situation, which would further decrease the influence of affected fish. Together or in isolation, these factors could affect any analysis of the difference in offspring quality when cod originating from different areas are compared.

Cod and salmon are two teleost species very different in several respects, such as reproduction, behavior, and phylogenic origin. It has been hypothesized that substances of anthropogenic origin might be, at least in part, responsible for the observed reproductive impairment in these species. Therefore, it is highly relevant that they both feed, to a large degree, on the same prey in the Baltic Sea (Uzars 1994; Ikonen 1996). Consequently, we looked for possible similarities and dissimilarities between the two species.

The offspring of 3 or 4 of the 12 investigated salmon females experienced mortality during the period previously described as typical for the M74 syndrome (i.e., when around two-thirds of the yolk sac has been consumed) (Bengtsson et al. 1994; Norrgren et al. 1994). The larvae from 3 females experienced mortality when only one-third to one-half of the yolk was consumed, and the larvae from 3 or 4 females experienced mortality rather late in development. Remarkably, the offspring of 3 of the

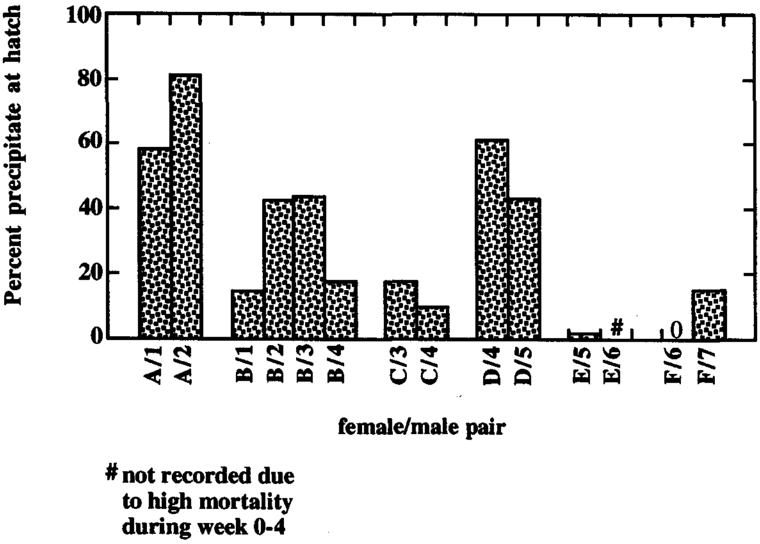

FIGURE 14.—Precipitate in the yolk seen at hatch in salmon larvae from six females (A–F) and seven males (1–7) during the 1994–1995 season. The larvae may also have other kinds of disorders, which are presented in Figure 13. Females A–F and males 1–7 denote the corresponding female–male pairs as in Figure 9.

12 females experienced a high level of mortality very early in development, before hatch. In summary, among the investigated females, mortality was found to occur from the first period (0–250 degree-days) to a period of more than 1,720 degree-days, which was several weeks after the start of feeding. These results are not in agreement with those of previous reports (Börjeson et al. 1994) on the occurrence of mortality in offspring of salmon originating from the Baltic Sea. However, they are in agreement with a recent presentation (Lundström et al. 1996), in which parts of these results were also presented in preliminary form (Åkerman et al. 1996b). Early mortality is not reported from Swedish hatcheries but is reported in connection with EMS in coho salmon *Oncorhynchus kisutch* from Lake Michigan (Marcquenski 1996).

No correlation of mortality to female size ($r = -0.61$) or condition factor ($r = 0.21$) could be seen among the 12 investigated salmon females. The sample size, however, might be too small to observe such a correlation. Karlsson et al. (1996) observed no differences in mortality among females of different ages during the 1994–1995 season. For female cod, no correlation between mortality and size or condition factor was observed, as previously presented (Åkerman et al. 1996a).

The larval mortality associated with the M74 syndrome is considered to be female dependent. The results from the season 1994–1995, when each female was fertilized by two or four different males, indicate some influence from the male with regard to both time when mortality occurred and the number and degree of disorders. The male might influence the expression of enzyme levels for metabolism of anthropogenic substances as well as exert other genetic influences.

The total frequency of disorders in salmon offspring at hatch was not correlated to mortality during the different periods. However, a high percentage of disorders, more than 40%, was correlated to a high mortality before hatch.

Injection of thiamine at 52 or 130 nmol/g had a very pronounced effect in newly fertilized eggs that otherwise showed 100% mortality during the larval period. A concentration of 52 nmol/g was found to be enough to prevent mortality almost completely. This rather high concentration corresponds to a level reported to occur in coho salmon eggs (65.8 nmol/g) from Lake Superior that do not develop EMS (Marcquenski 1996). However, for the egg batch from female L, which showed high mortality during the embryonic stage, the effect of 52 nmol/g was minor. The egg batches from females G, H, I, J, and K showed a decrease in disorders at hatch of only

FIGURE 15.—Larvae from Atlantic salmon originating from The Baltic Sea. The arrows show hemorrhages in the head. This type of blood disorder occurs frequently in newly hatched salmon larvae (see Figures 13 and 18).

15–50% compared with noninjected eggs. The higher dose, 130 nmol/g, which was used in only a few egg batches, did not result in a decreased incidence of disorders at hatch.

The 12 cod family pairs from the Lofoten area showed a mortality of around 20%. Such low mortality was not found in any of the investigated family pairs from the Baltic Sea, where the mortality in most cases was around 80%. The high mortality recorded in the Baltic cod eggs agrees with results reported by Wieland et al. (1994), Pickova et al. (1996), and Mellergaard (1996). Wieland et al. reported that the average survival until hatching was around 30%. The spawning cod in that investigation were caught in the Bornholm basin, and incubation of the eggs started immediately on the boat, as in the present investigation. High rates of malformed embryos have been recorded in pelagic fish eggs (cod, plaice *Pleuronectes platessa*, flounder *Platichthys flesus*) sampled by plankton net in different areas of the Baltic Sea (Grauman 1986; Westernhagen et al. 1988). Some of the malformations reported in these investigations, such as a variety of vertebrae curvatures and irregular cell cleavages, resemble those seen in our Baltic cod egg batches before death.

In cod offspring from Lofoten, the total frequency of disorders at hatch was around or, in most cases, less than 10%. In the offspring from the Baltic Sea analyzed at hatch, the corresponding frequency was higher (Student's t-test, $P < 0.05$), around 15%, except for one group that showed a frequency of less than 5% (female C and male 3). However, when all of the larval groups were examined

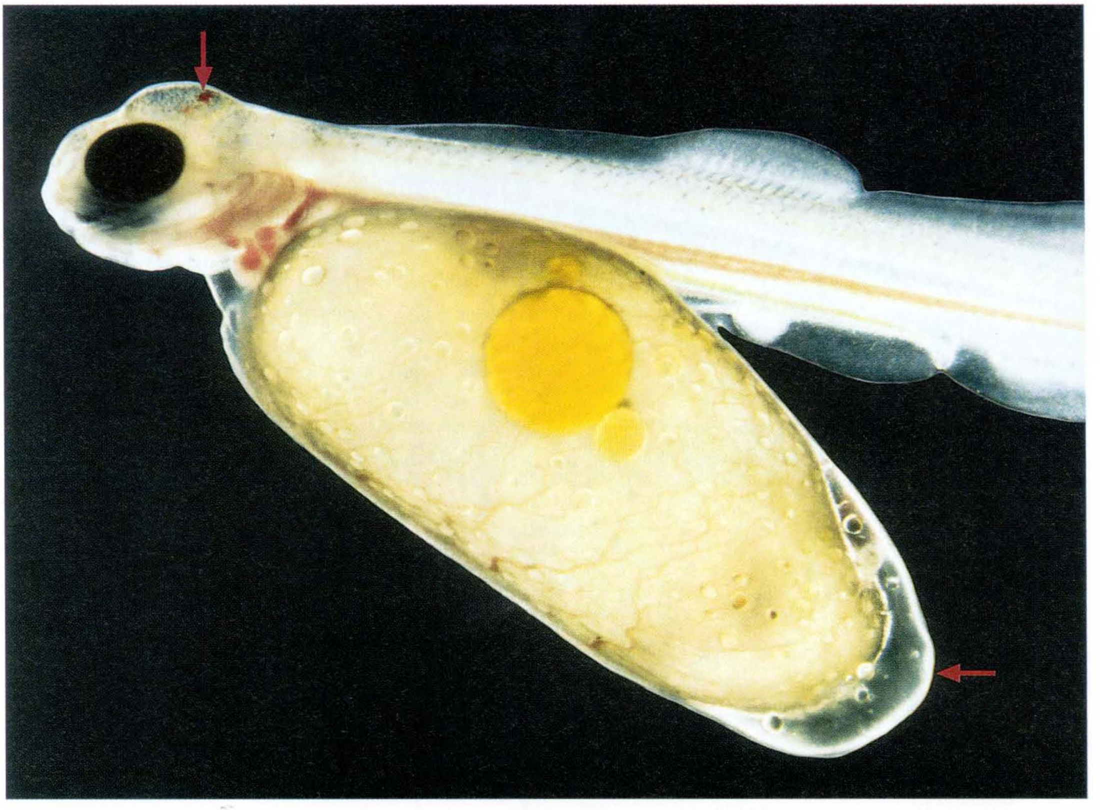

FIGURE 16.—A larva from Atlantic salmon originating from the Baltic Sea. The arrows show a small hemorrhage in the head and a subcutaneous edema in the yolk sac. This kind of disorder occurs frequently in newly hatched salmon larvae (see Figures 13 and 18).

after consumption of the yolk (results not presented), this low-frequency group was arrested in development and had consumed only a minor part of the yolk.

The frequencies of mortality during different periods and the kinds of disorders found in salmon and cod in this study differ in many respects. However, there are also some striking similarities, despite the large "biological" difference between these two species. For instance, larvae from the salmon females A and L experienced mortality during developmental stages similar to larvae from the cod females I, M, and P. In salmon, the results strongly indicate that there might be more than one kind of reproductive disorder. One is connected to thiamine deficiency and occurs at various stages of yolk sac consumption. The other is not directly connected to a thiamine deficiency and occurs mainly at earlier stages of development. This type shows similarities with reproductive disorders occurring in cod in the Baltic Sea. The prevailing hypothesis is that anthropogenic substances could be the underlying cause of the reproductive disorders in both of these species. It is tempting to speculate that what we are observing might be different responses on the dose axis among salmon females and that cod in general are in the more exposed or more sensitive response "area" relative to salmon.

The conclusions of the present study are summarized below.

1. Both salmon and cod from the Baltic Sea are affected by mainly female-associated mortality and disorders among their offspring.
2. Females of both salmon and cod from the Baltic Sea produce offspring that experience early mortality.

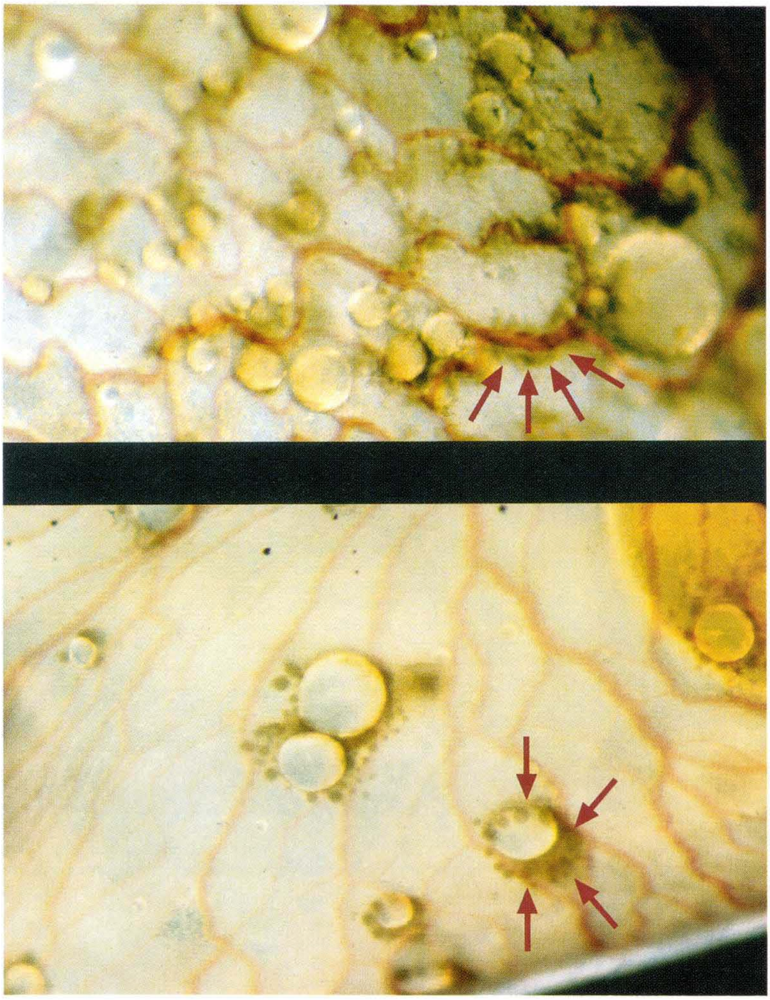

FIGURE 17.—Detail of yolk sacs in larvae from Atlantic salmon originating from the Baltic Sea. The upper larva has precipitate situated along the blood vessels (arrows). This kind of precipitate occurs frequently in newly hatched larvae (see Figure 14). The lower larva was photographed later in development but before larval mortality. At this stage, the precipitate (not recorded in these studies) can be observed around the oil droplets situated near the yolk sac membrane (arrows).

3. A major proportion of the salmon experience thiamine deficiency-dependent mortality. However, this mortality is not correlated to a specific stage of yolk sac consumption.

4. Cod exhibit a number of disorders at hatch, such as vertebrae deformity, disrupted yolk sac or subcutaneous edema in the yolk sac, and precipitate in the yolk.

5. Salmon exhibit a number of disorders at hatch, such as vertebrae deformity, blood disorders, subcutaneous edema in the yolk sac, and precipitate in the yolk.

6. Disorders at hatch in salmon offspring are not correlated to later thiamine deficiency-dependent mortality. Treatment of newly fertilized eggs by thiamine injection had only a minor influence on the frequency of disorders at hatch, whereas the treatment completely protected the larvae from later thiamine deficiency-dependent mortality. This indicates that other factors, in addition to thiamine deficiency, are involved in the disorders during development.

7. Both salmon and cod from the Baltic Sea exhibit disorders that might have similar biochemical mechanisms, because the formation of precipitates and edema in the yolk sac occurs in both species.

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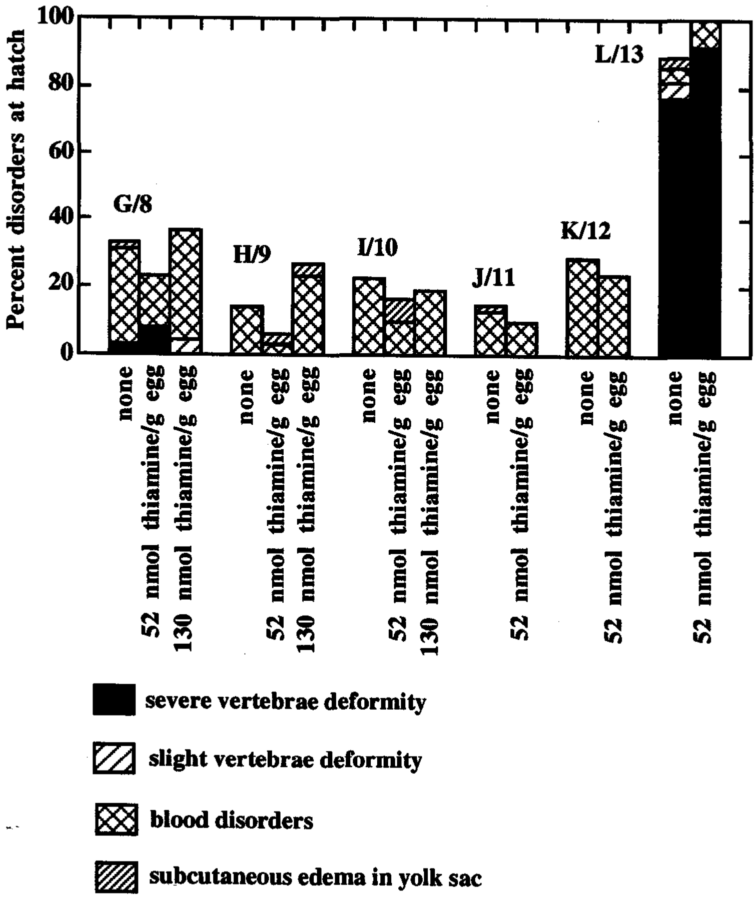

FIGURE 18.—Disorders recorded at hatch in salmon larvae from six females (G–L) and six males (8–13) during the 1995–1996 season. The second and the third bars in each group show disorders in larvae that were nanoinjected with thiamine (52 or 130 nmol/g) in the yolk immediately after fertilization; the first bar in each group shows disorders in the noninjected control group (none). Females G–L and males 8–13 denote the corresponding female–male pairs as in Figure 12. The percentages are calculated as in Figure 4.

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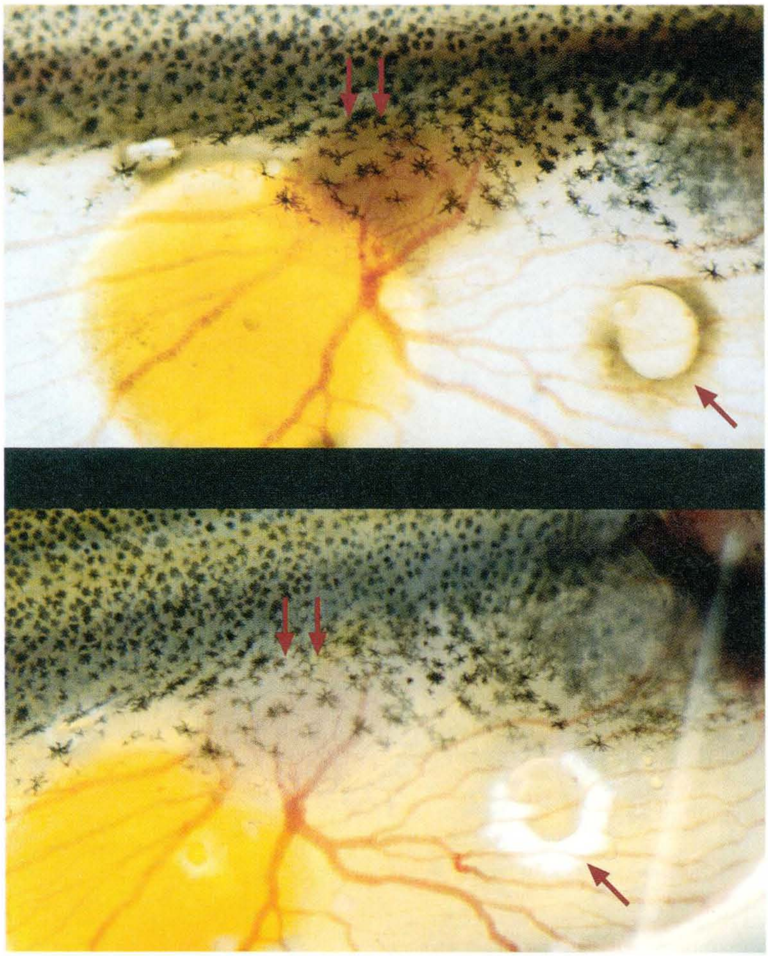

FIGURE 19.—The same salmon yolk sac photographed with light from two different directions (from above and below). The liver (arrows) has quite a different appearance, and different interpretations might occur. Precipitates in the yolk (arrow) are sometimes described as white precipitates, opalescent yolk, or opalescences in the yolk. It is obvious that identical disorders can be described and interpreted differently.

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