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Morphological and chromosomal aberrations during embryonic development in dab *Limanda limanda*

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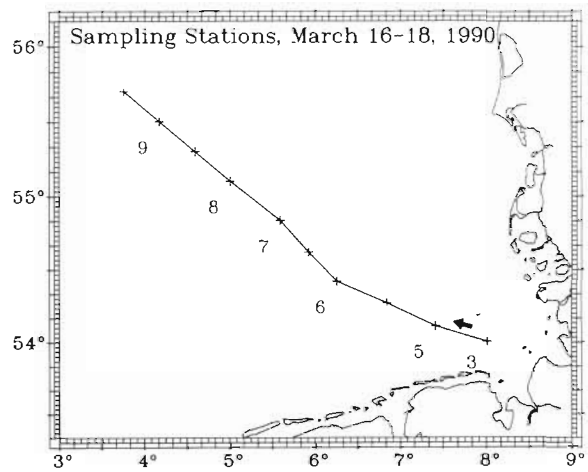
ABSTRACT: During the Bremerhaven Workshop, ichthyoplankton samples were collected with horizontal subsurface hauls at 28 stations from the inner German Bight to the Dogger Bank. Directly after being caught fish embryos were examined alive for morphological developmental defects under a dissecting microscope. Investigations on chromosomal aberrations were confined to preserved blastula stages of dab and were carried out later in the laboratory. Main species sampled were dab *Limanda limanda*, plaice *Pleuronectes platessa*, sprat *Sprattus sprattus* and whiting *Merlangius merlangus*. Because of their widespread distribution and high numerical occurrence, dab embryos were best suited for the detection of regional differences in malformation frequencies. The proportion of malformations in the most sensitive early developmental stage of dab reached 32 % in the inner part of the German Bight and fell to 9 % further offshore, increasing again at the Dogger Bank to values up to 31 %. Data collection was accomplished with a computerized data sampling/evaluation/presentation system, and results concerning the morphological differences of malformation frequencies were available directly after observation. Thus, the method as described below proved to be a suitable approach for biological effects monitoring. Anaphase aberration frequencies in dab embryos reached a peak of 63 % in the inner part of the German Bight, whereas the lowest value (51 %) was detected far offshore at a less polluted station; aberrations on the Dogger Bank were found to be 57 %. Investigations on the chromosomal aberrations in the same material used for the determination of morphological aberrations provided an opportunity to evaluate the potential effects of contaminants at the chromosome level.

INTRODUCTION

Biological effects such as morphological developmental defects and chromosomal aberrations that may reduce the chances of survival of an individual, a population or even a species are suitable parameters for monitoring purposes. Embryonic defects which may result in an impairment in reproduction, one of the most critical activities in the life cycle of a species, should be interpreted as a pollution index of particular significance for affected species in the observed area. Reproduction in many of the commercially important North Sea fish takes place in coastal waters which are generally considered to be the most polluted areas of the North Sea (Kersten et al. 1988, Lohse 1990).

Lipophilic organic contaminants accumulate on their way through the food web, especially in fatty tissue. In the case of fish, the concentration factor with respect to polychlorinated biphenyls (PCB) in the water column may reach 80 000 (Bruggeman et al. 1981). During vitellogenesis, the stored substances may be transported with the lipid reserves from the liver or other somatic tissue to the developing gonads (Dawson & Grimm 1980, Delahunty & de Vlaming 1980, Knickmeyer & Steinhart 1989). Experiments show that concentrations of several chlorinated hydrocarbons, e.g. PCB, DDE and Dieldrin, found in natural flatfish populations lead to malformations during embryonic development which reduce hatching success (Westernhagen et al. 1981, 1989, Hansen et al. 1985, Spies et al. 1988). This is only one group of many potential toxicants that may produce the biological effects which are the focus of this investigation.



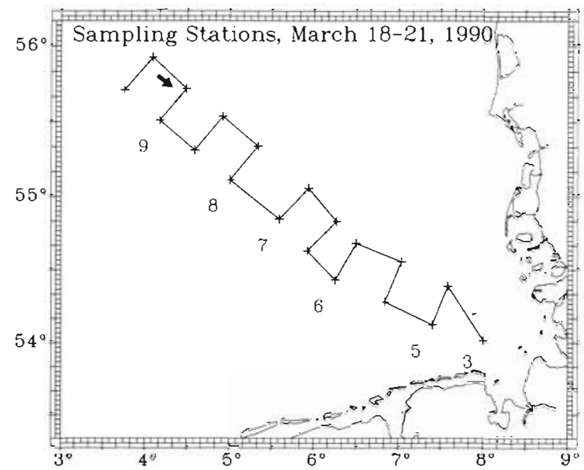


Fig. 1. Sampling stations for the investigation of morphological malformations in pelagic fish embryos in March 1990

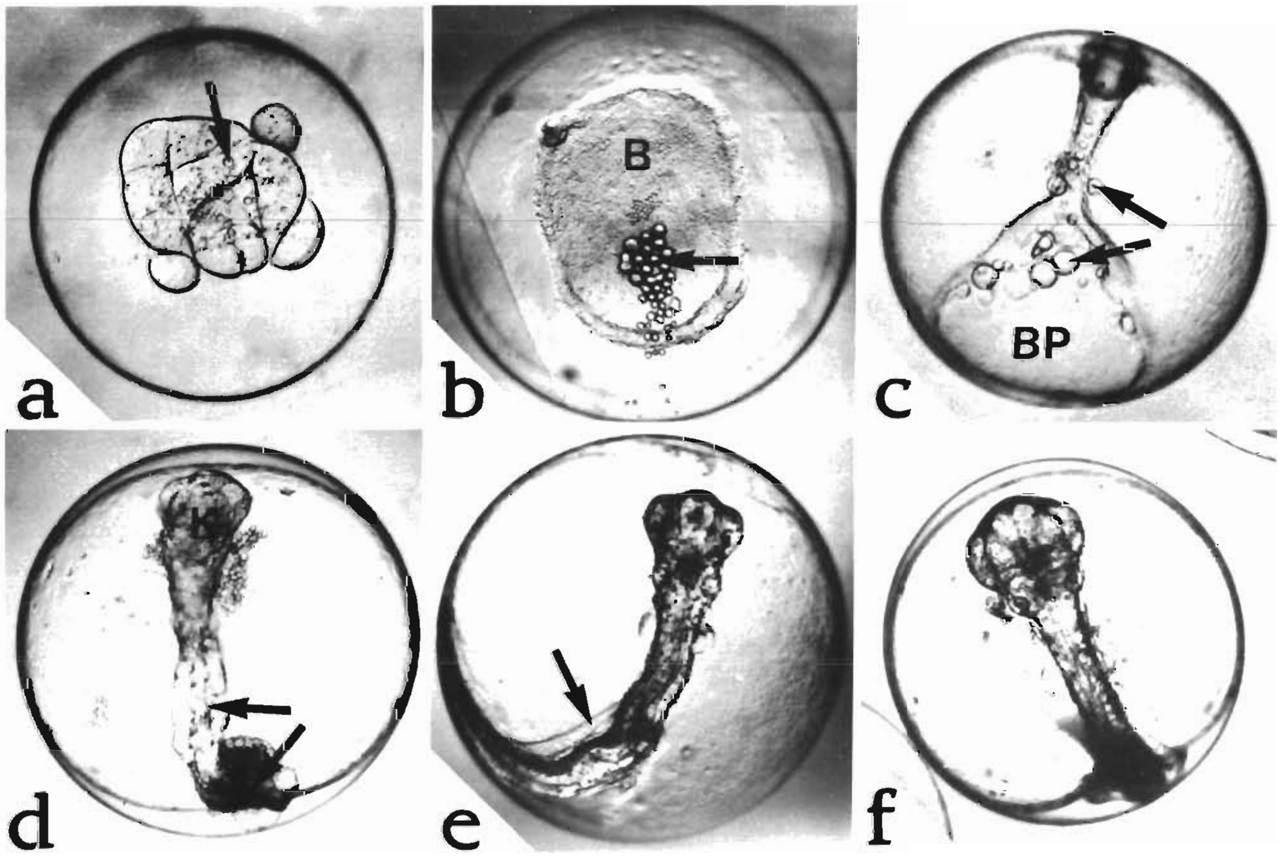


Fig. 2. Malformations during embryonic development of pelagic flatfish eggs. (a to c) Malformations during the early development and the differentiation processes: (a) deformation of blastomeres in 16-cell stage with inner formation of blisters; (b) underdeveloped early gastrulation with abnormal blastodisc (B) shape and severe incorporation of blisters; (c) young embryo with severe blister formation unable to close blastopore (BP). (d to f) Malformations in older embryos: (d) faulty differentiation in the head region, proliferation, twisting of the body and crippled tail; (e) crippled notochord; (f) formation of blisters in further developed fish embryo

MATERIAL AND METHODS

In March 1990, pelagic fish eggs were caught at 28 stations (Fig. 1) in the southeastern part of the North Sea, using a plankton net (CALCOFI, 1 m ∅, 300 µm mesh) towed horizontally just below the surface for 10 min. Because morphological aberrations from the normal embryonic development can only be detected in undamaged embryos, towing speed had to be less than 1 knot, since high towing speeds of 4 knots or more are known to cause damage and up to 92 % mortality in fish eggs (Southward & Demir 1974). Samples were concentrated in the cod end of the net (plankton beaker) and poured into glass vessels containing sea water. After a few minutes, the embryos rose to the surface, where they were sorted for microscopic analysis. At each station, 500 to 1350 eggs per sample were examined on cooled dishes directly after the catch. Morphological aberrations were detected under a dissecting microscope at 25- to 32-fold magnification. After microscopical analysis, the remaining eggs were preserved in 10 % buffered formalin for chromosome analysis.

Eggs were determined according to species, developmental stage and morphological aberrations from normal development. Five different stages of embryonic development were differentiated: (Ia) early cleavages until the formation of the blastodisc; (Ib) epiboly, the building of the embryonic shield and the axes; (II) differentiation of the embryo, formation of the head and growth around the yolk (up to 180°); (III) embryo between 180° and 270° around yolk with further differentiation; (IV) embryo between 270° and 360° around yolk, tail free, body movements.

Development was considered defective if stages deviated from normal development and morphological differentiation. Some common abnormalities are shown in Fig. 2. During the first cell divisions incomplete and irregular cleavage may ensue, leading to deformations of the blastomeres and to abnormal shapes of the blastodisc. Wrinkles and blisters inside and on the surface of cells may occur, as well as gastrulae with loose cell aggregates. Differentiation of the embryo may be retarded in certain parts of the body and the closure of the blastopore may be disturbed. In older embryos, blister-like outcrops from body axis, bent notochords, twisting of the tail, deformations of the head region, fin abnormalities, defects of the eyes and pigmentation anomalies may be recognized. The relevance of these registered malformations to embryo survival is known from incubation experiments with egg samples from the western Baltic (Westernhagen et al. 1988). There, low hatching success coincided with high malformation frequencies at the respective stations. In addition, incubation experiments with individual embryos could show that 85 % of the registered malformations were lethal within 5 d (Cameron unpubl.).

For chromosome analysis the blastula-embryos of dab were dissected from chorion and yolk, stained with aceto-orcein and squashed on microscope slides. Anaphase aberrations such as attached and free fragments as well as bridges, leading to an unequal distribution of chromatid material between the dividing cells, were counted at 1000-fold magnification.

RESULTS

The species occurring in the winter ichthyoplankton of the southern North Sea were, in descending abundance: dab *Limanda limanda*, plaice *Pleuronectes platessa*, sprat *Sprattus sprattus*, whiting *Merlangius merlangius*, rockling, *Onos* sp., flounder *Platichthys flesus*, cod *Gadus morhua*, long rough dab *Hippoglossoides platessoides* and dragonet *Callionymus lyra*. Eggs of *L. limanda* constituted one-third of all fish eggs in number and occurred at each station. Due to the decreasing sensitivity of the embryos with ongoing development (especially after the closure of the blastopore), and the loss of aberrant embryos due to selective mortality, the observed frequencies of malformations declined as development progressed. Values for *L. limanda* decreased from 22.9 % (stage Ia) to 1.1 % (stage IV), for *P. platessa* from 6.2 % to 1.9 %, for *S. sprattus* from 43.2 % to 6.2 % and for *M. merlangus* from 28.7 % to 6.8 %. In addition, there existed species-specific differences in the occurrence of malformation frequencies. The most severely affected species was *M. merlangus*, showing a mean aberration frequency of 24 %, followed by *S. sprattus* with 20 %, whereas mean malformation frequencies in *L. limanda* and *P. platessa* with 6 % and 5 % respectively were much lower.

When looking for regional differences in malformation frequencies, early stages were preferred as they reflect the condition of the ovary from which the unfertilized egg is released, and as advection effects are minimized. Studies should therefore concentrate on the first cleavage stages before the closure of the blastopore (Ia to II), which takes about 2 d after fertilization depending on water temperature. Furthermore, species with widespread distribution of their early developmental stages are best suited for the detection of regional differences in malformation frequencies. Therefore we chose *Limanda limanda* embryos for comparison of malformation frequencies between different stations.

Malformation frequencies in very young *Limanda limanda* embryos (stage Ia) were found to reach 30.1 %

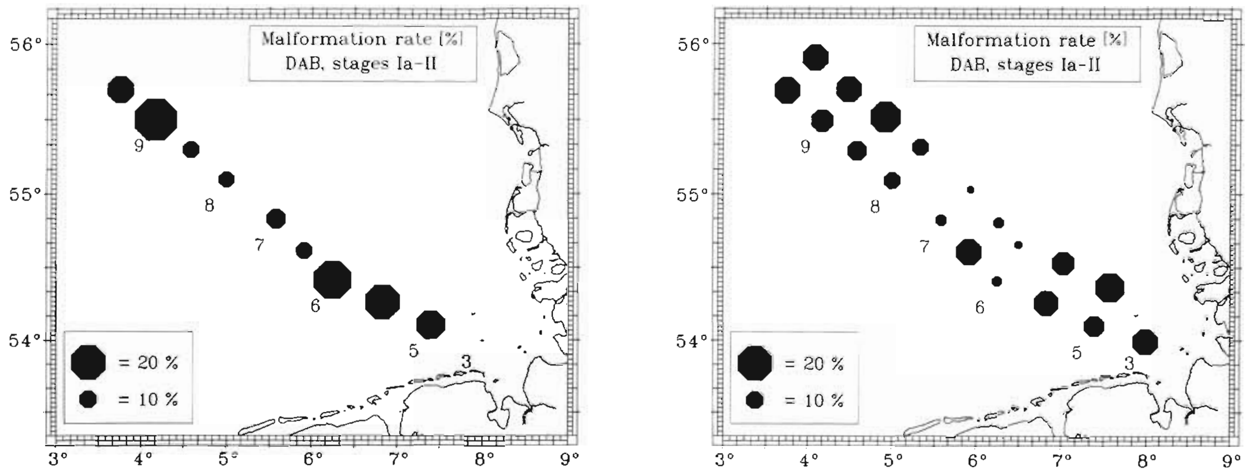


Fig. 3. *Limanda limanda*. Morphological malformation frequencies of dab at developmental stages Ia to II

in the inner part of the German Bight, declining offshore to values of 8.9 % (Stn 8), but rising again to 30.6 % on the Dogger Bank (Stn 9). In order to get a more complete spatial resolution and in order to increase the number of stations with sufficient eggs for evaluation, we took developmental stages Ia to II into account (Fig. 3). The resulting picture was similar with high aberration frequencies (16.5 to 21.8 %) at the inshore stations (Stns 3 to 6), lower values (4.5 to 15.0 %) at the reference Stns 7 & 8, and a higher frequency of 24.6 % at the Dogger Bank (Stn 9). In comparison with the mean malformation frequency of 12.3 %, all the stations southeast of Stn 6 (Elbe influence) as well as northwest of the station between 8 and 9 (Dogger Bank) had higher values, whereas the frequencies at the reference Stns 7 & 8 were below the mean during all 3 transect investigations.

The differences from the mean frequencies were tested with a 4-squares Chi^2-test ($p < 0.05$). On the way offshore (Fig. 1; March 16 to 18), the station between 5 and 6 as well as Stn 9 had significantly higher values compared to the mean, whereas Stn 8 and the station between 8 and 9 had significantly lower values. The second part of the cruise (Fig 1; March 18 to 21) showed significantly higher malformation frequencies at the stations northwest, northeast and east of Stn 9 (near the Dogger Bank) as well as at Stn 3 and at the stations west and northeast of Stn 5 (inshore). Significantly lower values were detected at Stns 6 & 7 and at the 3 stations northeast of these.

Anaphase aberration frequencies in *Limanda limanda* embryos reached a maximum of 63.4 % in the inner part of the German Bight (Stn 3), then declined to 60.7 % (Stn 5), 58.2 % (Stn 6), 53.5 % (Stn 7) down to the lowest value with 51.0 % at the less polluted Stn 8. Significant differences occurred between Stns 3 & 5, on the one hand, compared to Stns 7 & 8, on the other hand, using a 4-squares Chi^2-test ($p < 0.01$). On the Dogger Bank, values were found to be as high as 56.9 %.

DISCUSSION

An array of conditions can cause malformations in fish embryos. Among the natural factors are high and low water temperature, low salinity and low oxygen (Westernhagen 1970, Alderdice & Forrester 1971, Braum 1973). When comparing the hydrographic data registered during the cruise with the tolerance limits determined for the respective fish embryos, it became apparent that the hydrographic conditions had not been critical for normal embryonic development.

There is a wealth of information on the experimentally determined influence of pollutants such as chlorinated hydrocarbons on the development and the reproductive capacity of fish (Allison et al. 1964, Hogan & Brauhn 1975), and some investigations exist on the impairment of reproduction caused by contaminants occurring in the field (Westernhagen et al. 1981, 1989, Hansen et al. 1985, Spies & Rice 1988). The development of the embryo may be disturbed by contaminants entering the egg during the time of maturation in the female gonads, or pollutants may interfere with development after the release of the spawning product into the surrounding water before and after fertilization. Actual contaminant concentrations in the water column are probably too low to cause acute effects, but the surface microlayer is known to affect the embryonic survival of fish eggs due to several potentially effective substances accumulated in this medium (Kocan et al. 1987, Westernhagen et al. 1987).

Among the contaminants that can be considered as having an influence on reproductive capacity are chlorinated and aromatic hydrocarbons as well as heavy metals. It has been suggested that concentrations of chlorinated hydrocarbons in fish ovaries from the southern North Sea are responsible for an impairment of reproductive capacity due to increased prelarval mortality in whiting *Merlangus merlangus* (Westernhagen et al. 1989), flounder *Platichthys flesus* and dab *Limanda limanda* (Cameron & Westernhagen unpubl.). Breeding experiments with *M. merlangus* also showed a positive relationship between anaphase aberrations in embryos and the contamination of PCB and DDE in parental gonads and liver (Dethlefsen et al. 1987). The data from the analysis of female *L. limanda* liver obtained during the workshop show high values of chlorinated hydrocarbons, especially of the chlorinated biphenyl congeners 138, 153 and 180 from the inner part of the German Bight (Cofino et al. 1992) which could be responsible for the reduction in hatching success of larvae from contaminated parents.

The first quantitative investigation in the field on the occurrence of defective fish embryos with morphological as well as chromosomal deviations from normal development was conducted in the inner part of the Balsfjord in northern Norway (Kjörsvik et al. 1984). During the spawning season in 1983, morphological malformations in cod *Gadus morhua* embryos reached 20 % in young developmental stages. Even embryos which appeared normal sometimes had severe chromosome aberrations. In March 1987, morphological malformation frequencies in the early developmental stage in *Limanda limanda* in the southeastern North Sea varied between 23 and 85 % with maximum values occurring northwest of Helgoland, near the dumping area for wastes from the production of titanium dioxide, as well as off the Rhine estuary (Cameron et al. 1990).

Yannopoulos & Yannopoulos (1981) described lethal morphological abnormalities in eggs of pilchard *Sardina pilchardus* (12 %) and anchovy *Engraulis enchrasicolus* (9 %) in the Aegean Sea. Investigations in the New York Bight (Longwell et al. 1984) on the cytologic and cytogenetic status of mackerel embryos *Scomber scombrus* showed elevated malformation frequencies up to 87 % in early developmental stages with up to 86 % mitotic abnormalities, which was attributed by the authors to pollution of the sea surface water. High values of aromatic and chlorinated hydrocarbons as well as of heavy metals could be determined as the main reasons for morphological embryonic malformations as well as for mitotic abnormalities in all developmental stages (Chang & Longwell 1984). For the years 1979 to 1982, Graumann & Sukhorukova (1986) discovered severe morphological anomalies in older cod *Gadus morhua* (36 %) and sprat *Sprattus sprattus* (25 %) embryos in the central and eastern Baltic. Oil pollution of the Baltic was discussed as a potential causative agent. In spring 1983 and 1984, a survey of pelagic fish eggs was conducted by Westernhagen et al. (1988) in the western Baltic. There a considerable amount of all embryos studied displayed aberrant morphological development, ranging from 28 % in plaice *Pleuronectes platessa* over 32 % in cod *Gadus morhua* to 44 % in flounder *Platichthys flesus*. Anthropogenic factors were discussed as possible reasons for the occurrence of defective fish embryos.

Looking at the overall situation of fish recruitment in the southern North Sea, no stock-endangering impairment of recruitment appears to exist at present, despite the fact that at certain sites high malformation frequencies have been observed. It is well known that natural mortality in fish embryos and larvae is generally considerable (Harding & Talbot 1973, Koslow et al. 1987, Westernhagen et al. 1988), and populations have to deal with relatively high variability in egg and larvae production and survival. Since year-class size is not a simple function of egg production (see Sinclair 1988), the effects of additional losses of eggs on recruitment due to teratogenic effects are difficult to assess. A potential effect on recruitment cannot be excluded, particularly if contaminant concentrations increase. Calculations of mortality rates in cod *Gadus morhua* embryos from the Baltic (Westernhagen et al. 1988) revealed a decrease of newly hatched larvae by 50 % due to additional mortality caused by embryonic malformations. Even mortalities up to 100 % in the case of the mackerel stock off the North American coast do not necessarily lead to the extinction of the population, because recruitment is assured by drifting and migration of eggs and larvae from unpolluted areas (Longwell et al. 1984).

Because of their great sensitivity towards pollutants, fish embryos and larvae have widely been used in experimental toxicity studies (Rosenthal & Alderdice 1976, Westernhagen 1988). In this study it could be shown that fish embryos also respond to a deteriorating environmental situation in the field, displaying higher malformation frequencies in areas where Lohse (1990) and Schmidt (1988) found high concentrations of several contaminants. The same reaction by fish eggs could be shown for some areas of the Baltic as well as for the New York Bight (Longwell et al. 1984, Graumann 1986, Westernhagen et al. 1988). Thus, fish eggs may very well be used as indicators of water quality, and the investigations on morphological malformations of pelagic fish embryos are well suited for biological effects monitoring. The investigations on chromosomal aberrations in the same material as used for the determination of morphological aberrations provide the additional opportunity to evaluate the potential effects of contaminants on the chromosomal level, whereas its practical use is limited by the time-consuming work. In particular, dab *Limanda limanda* with its wide distribution in the southeastern North Sea and a long spawning season is adequately suited to follow man-made changes in this marine environment, even though *L. limanda* may not be the most sensitive species as far as the frequency of embryo malformations is concerned.

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