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ORIGINAL ARTICLE

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Influence of solar ultraviolet-B on pelagic fish embryos: osmolality, mortality and viable hatch

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Abstract Eggs of dab (*Limanda limanda*) and plaice (*Pleuronectes platessa*) were experimentally exposed to ultraviolet-B (UV-B) radiation in a solar radiation simulator. The experimental design tried to simulate present and future conditions with reference to increased UV-B exposure due to northern hemisphere ozone loss, employing mainly two scenarios, a reduction to 270 (S1) and to 180 (S2) Dobson units (DU) in single or repetitive exposures of 2, 4 or 6 h. Depending on the total dose of UV-B irradiation and the developmental stage, exposed eggs displayed loss of buoyancy as a sublethal effect, as well as increased embryo mortality and reduced viable hatch. In the single exposure experiments only under conditions of 180 DU for 6 h were effects apparent. Double exposure under conditions of 270 DU did not lead to lasting effects. At the sublethal effect level, i.e. loss of buoyancy, considerable photorepair was observed. It was concluded, that under the present general weather conditions in spring and at the present levels of environmental ozone, allowing for a reduction to 180 DU, the embryonic development of North Sea spring spawning fish is not endangered by UV-B radiation.

Introduction

The atmospheric ozone situation in the northern hemisphere is much more complex than in Antarctica and is characterized by the disturbed arctic vortex. For the area of the North Sea, which is of interest in the present study, continuous observations from the Total Ozone Mapping Satellite (TOMS) show high short-term ozone variations in spring time when the total column ozone ranges from 250 up to 450 Dobson units (DU) (Fig. 1). This leads to significant variations of solar ultraviolet-B (UV-B) (280–315 nm) radiation at the ground and in the surface layer of the sea during the peak reproductive period of pelagic spring-spawning fish. Since after fertilization eggs will float to the surface they are exposed to sunlight depending on the turbulence and optical characteristics of the water, the solar zenith angle and the cloud cover.

The intensification of UV-B irradiation at the sea surface as a consequence of ozone depletion (Calkins and Thoradottir 1980) led to concern about the exposure of living organisms to increased levels of UV light (UV-B), which is known to affect certain compartments of marine ecosystems (Häder et al. 1995). Much of the work on the effects of elevated UV-B irradiation on aquatic organisms has been concentrated on phytoplankton (Holm-Hansen et al. 1993; Vincent and Roy 1993; Häder 1993) and on zooplankton (Damkaer 1982; Williamson et al. 1994; Zagarese et al. 1994; Ringelberg et al. 1984), but also on amphibians (Blaustein et al. 1994; Kiesecker and Blaustein 1995). Work on fish has mainly been concerned with sunburn phenomena and respiratory impairment in marine flatfish (Bullock 1982; Berghahn et al. 1993; Blazer et al. 1997; Freitag et al. 1998; Kouwenberg et al. 1999). Impact of UV-B on the reproduction of fish has been studied by Pommeranz (1972) working on effects of UV-B on embryos of plaice, by Valcárcel et al. (1994) investigating the effects on spermatozoa of the catfish, *Rhamdia sapa*, and by Don and Avtalion (1993) on tilapia spermatozoa. Strähle and Jesuthasan (1993) studied the impairment of epiboly in zebrafish (*Brachydanio rerio*) embryos by ultraviolet irradiation. Hunter et al. (1981), Vetter et al. (1999) and Steeger et al. (1999) stressed the sensitivity of the embryonic stages of anchovy (*Engraulis mordax*) and plaice (*Pleuronectes*

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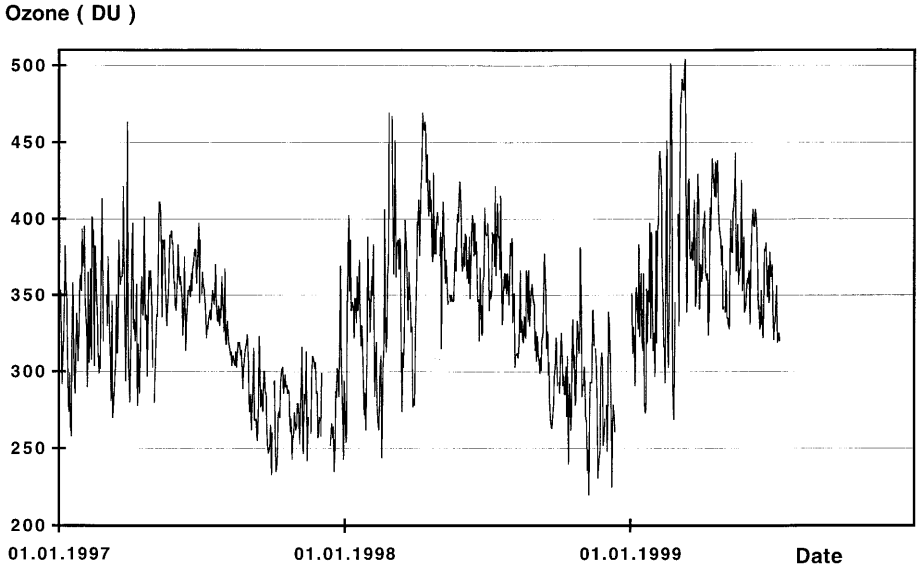


Fig. 1 Total column ozone of the Helgoland area January 1997–July 1999 (Total Ozone Mapping Satellite data). DU Dobson units

platessa) towards solar radiation. For anchovy larvae, a 25% increase in irradiation intensity would elevate annual losses by 13–18%. When presenting a preliminary estimate of UV-induced loss of larval anchovy standing stock in Californian coastal waters, Hunter et al. (1981) concluded that there was evidence that anchovy and other larval fish populations may be under some UV stress today; yet predicted future levels of ozone diminution will probably not have a major effect on stocks of marine fish larvae.

Despite numerous investigations on the damaging effects of UV radiation on marine ecosystems little work has focussed on real ozone-depletion conditions and actual radiation dosages with regard to season and location. The present study was triggered by the possibility to simulate ozone depletion and solar radiation with a new programmable sunshine simulator (SONSI), which provided a good approximation of real solar irradiance at the ground and in the water column.

Materials and methods

Determination of biological effects

The aim of this study was to estimate lethal and sublethal thresholds of actual and possibly future dosages of UV-B under controlled conditions for developing fish embryos. The experiments were done with eggs of dab (*Limanda limanda*), because this species constitutes the dominant component of the spring ichthyoplankton in the southern North Sea (Dethlefsen et al. 1996). Some preliminary work was done with plaice (*Pleuronectes platessa*) eggs.

In 1996 preliminary exposure experiments with naturally spawned plaice eggs and artificially inseminated dab eggs were conducted in order to pinpoint potentially important effects on exposed embryos. The main experiments were conducted in February/March 1997–1999 during three cruises into the southern North Sea between 52°00′ and 55°30′ latitude with the research vessel Walther Herwig III. Eggs of dab were obtained from running ripe fish after stripping and artificial insemination. At the beginning of each campaign the necessary number of running ripe females and males of dab were captured in the centre of the German Bight (06°00′–07°00′E/54°00′–54°30′N). The fish were stripped individually, eggs from one female were inseminated with sperm of two males. Each of the individual batches was quality controlled. Only batches with malformation rates <2.5% and fertilization success >90% were used for further experiments.

Twenty-four hours after fertilization all batches fulfilling the above quality requirements were combined to serve as pooled samples for the forthcoming experiments. For the exposure experiments embryos were transferred to exposure vessels (quartz-glass cylinder of 150 ml volume with a lateral sleeve closed by a silicon rubber plug) containing 60 ml sea water which was exchanged daily. Before and after each exposure and during the time left until hatching, mortality and rate of malformation were determined daily. Eggs containing dead embryos were removed and the number of floating and non-floating eggs was counted. The salinity of the incubating water was 32.2 psu, and the temperature varied between 4°C and 5°C. Oxygen in test vials and reservoirs was measured regularly and was always found to be at saturation levels.

Exposure conditions, radiation measurements and simulation

The instrumental set up used in the present study for the measurement and simulation of solar radiation is depicted in Fig. 2. Calibration was achieved with a 1,000 W quartz-halogen light source in the laboratory of the Alfred-Wegener-Institut (AWI) before and after each cruise.

During all cruises the atmospheric UV radiation between 280 and 322 nm was monitored continuously by means of a spectroradiometer based on a modified Bantham double-monochromator (DM 150) with a multichannel plate detector. The instrument operated as an array spectrometer with 32 separate photon-counting channels. It was placed on deck in a waterproof and temperature-stabilized housing. One complete spectrum was taken each second and the data averaged over intervals of 5 min. An optical resolution of about 1 nm was necessary due to the spectral cut-off and unknown action spectra. The multichannel detection mode is helpful to obtain accurate solar dosage measurements even under quickly varying cloud conditions or rain. Moreover, it allows a quick estimation of stratospheric column ozone by forming irradiance ratios (e.g. at 300–320 nm). In the absence of tropospheric ozone this ratio only depends on the solar zenith angle and total stratospheric ozone and is independent from clouds.

The same type of spectroradiometer was used in a pressure housing to measure UV-B in the water column. The main difference between both instruments is their input optics. While the at-

Table 1 Exposure schedule for 1998 experiments. *S1* 270 Dobson units (DU), S2 180 DU, *C* controls

Date	23 February 1998	24 February 1998	25 February 1998	26 February 1998	27 February 1998	28 February 1998	1 March 1998	2 March 1998	3 March 1998	4 March 1998	5 March 1998
Stage	Ibβ	Ibβ	Ibγ–δ	IIδ	IIIα	IIIα					
Exposure	C	C	C	C	C	C					
Exposure	6 h S1, S2	6 h S1, S2	6 h S1, S2	6 h S1, S2	6 h	6 h S1, S2	S1, S2				
Stage						Iaγ	Ibγ	IIα–β	IIγ–δ	IIIα–β	IIIα
Exposure						C	C	C	C	C	C
Exposure						6 h S1, S2	6 h S1, S2	6 h S1, S2	6 h S1, S2	6 h S1, S2	6 h S1, S2
Stage							Ibβ	Ibβ	Ibγ–δ	IIδ–IIIα	IIIα–β
Exposure							C	C	C	C	C
Exposure							6 h S1, S2	6 h S1, S2	6 h S1, S2	6 h S1, S2	6 h S1, S2

mospheric instrument uses a cos-corrected diffusor, intended to fit to the plane surface of the earth, the underwater unit has a 2π input characteristic, which allows the measurement of scalar irradiance by using the instrument in an upright as well as in top-down position. Both, the atmospheric and the underwater spectroradiometer were running parallel with a time uncertainty of only 1 s. Spectroscopic underwater measurements were only made at a wind speed lower than 3 kn at selected stations.

Parallel to the UV-B instruments an additional spectroradiometer from Kruse, originally developed for the comparison with SEAWIFS satellite data, was used in the water column and in air for the wavelength range 320–700 nm. It was equipped with a diode-array detector and could store up to 80 spectra.

Outdoor radiation conditions were simulated with the sunshine simulator SONSI, developed at the AWI. Two simulators, S1 and S2, adjusted to different total ozone values, were employed simultaneously. The instrument is based on a 400-W discharging lamp (type MSR 400 HR from Philips) containing rare elements, so that their numerous emission lines lead to a pseudo-continuum resembling that of the sun. The emitted light from the bulb is collimated by a parabolic mirror and passes a metal net and three stacked liquid filters with quartz windows. The extinction can be varied by changing the filter thickness or the concentration of the liquids. A combination of the three solutions KNO_3 (6 g/l), K_2CrO_4 (60 mg/l) and $CuSO_4$ (15 g/l) allows the simulation of most ozone conditions. After the light has passed an optional quartz diffusor plate it illuminates homogeneously an area of about 15 cm in diameter. A cooled glass beaker contains the incubation chamber with the biological material to be irradiated.

The last instrument shown in Fig. 2 is a commercially available scanning spectroradiometer of type IS 320 D from Instrument Systems operating in the wavelength range of 280–800 nm. It was used as a reference inside the radiometric system of different instruments and had the following functions: (1) stability control of the atmospheric and underwater spectroradiometers, (2) measurement of spectral solar irradiance onboard under defined conditions (sky situation, solar zenith angle) to set the simulators closest to the outdoor spectra, and (3) control and adjustment of the sunshine simulators, because the transmission of the liquid filters changed slowly under UV light conditions.

The exposure conditions of the SONSIs (S1, S2) were changed in the course of the 3 years of experimenting, making use of the experience accumulated from the preceding year. The conditions were as follows:

1. 1997: The simulators (S1, S2) were adjusted to a model atmosphere of blue sky, airmass 2 (solar zenith angle 60°), irradiance at sea level, with 360 DU ozone for S1 and 180 DU for S2. With these input parameters the program SONSI 96 computed the corresponding solar spectral irradiance in the wavelength range 280–700 nm as well as the attenuation factor and thickness of the three filters for a best fit to the computed model atmosphere. For both simulators this radiation level independent of wavelength was reduced by a factor of 0.5 in a second series of experiments and increased by a factor of 2 in a third series. All embryos were exposed only once (6 h in series 1 and 2, 12 h in series 3) at different stages of development and then incubated without any further irradiation. Five hundred eggs were kept under subdued daylight as controls in jars containing 60 ml seawater.
2. 1998: A real solar spectrum which was taken with the calibrated spectroradiometer IS 320 onboard Walther Herwig III on 9 March 1997. The UV-B dose for this day was 8000 J/m² (see

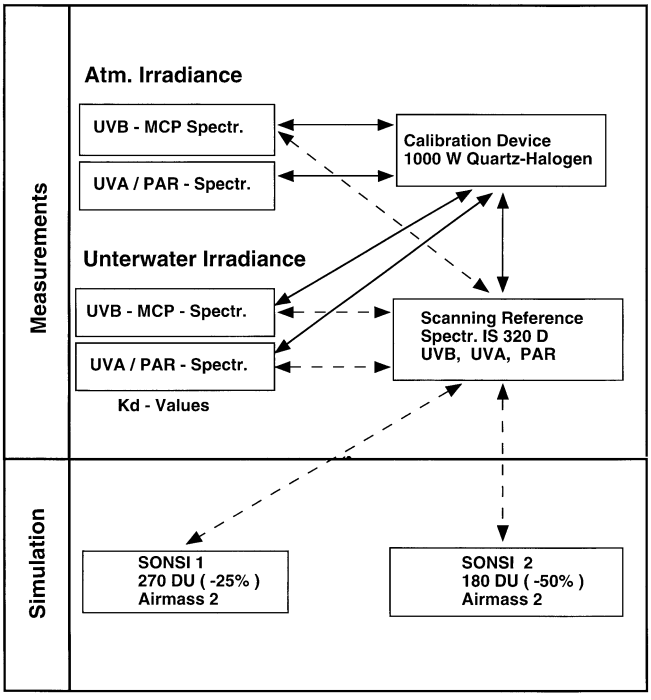


Fig. 2 Instrumentation to measure irradiation on deck, in the water column and in the sunshine simulators (*SONSI*) *UV* Ultraviolet, *PAR* photosynthetically active radiation

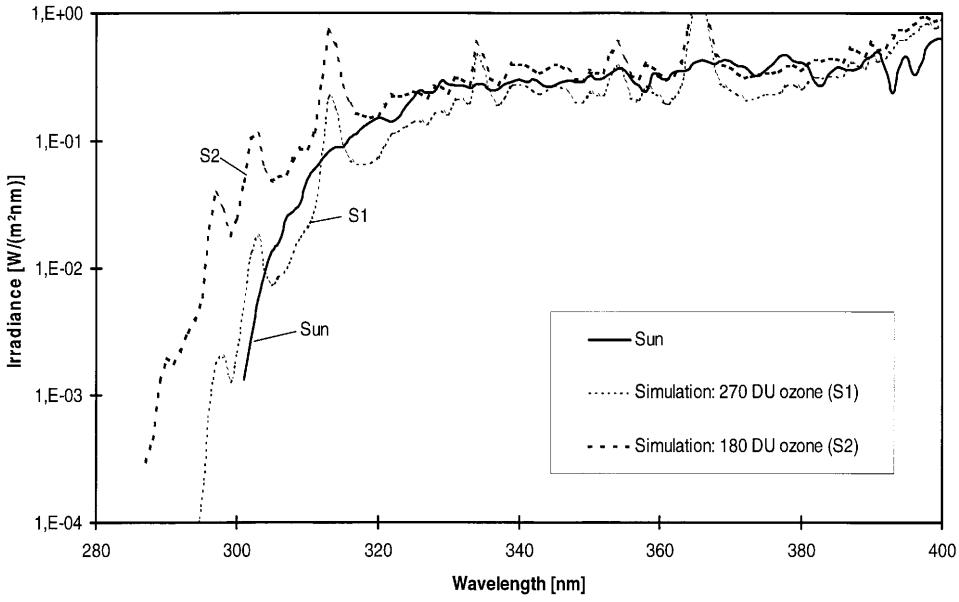


Fig. 3 Sunshine simulator (*S1*, *S2*) adjustment in 1998 and 1999 compared to the solar reference spectrum measured onboard RV Walther Herwig III on 9 March 1997 at 1205 UT (54.5 N, 7.0°E)

below). The spectrum was used as a reference for the adjustment of simulator S1. The conditions were blue sky, solar air mass 2.0 and 270 DU, which yields a typical low ozone value for the study area in spring time (see TOMS data in Fig. 1). S2 was adjusted to the same spectrum but to a reduced ozone value of 180 DU as a possible future scenario. Figure 3 shows the measured solar spectrum together with those of S1 and S2, all taken with the IS 320. The UV-B dose rate was 0.98 W/m² for S1 and 3.63 W/m² for S2. Simulator spectra were checked daily and the filter thickness adjusted if necessary. In contrast to 1997, embryos were exposed for 6 h in overlapping series (S1, S2) for 5 and 6 consecutive days with an 18-h period at 5°C in the dark between exposures (Table 1). Controls were as described for 1997.

3. 1999: Simulator conditions were the same as described for 1998, but exposure time was 2, 4 and 6 h in S1 and S2, respectively applied once and twice (Table 2) commencing at stages Iaβ-Ibα and IIIα-γ respectively (Westernhagen 1970). Five hundred eggs were exposed in one exposure vessel filled with 60 ml seawater. After exposure the embryos were kept under subdued daylight in open vessels until hatching. Controls were as described for 1997. The developing embryos were examined microscopically each day for aberrations. Experiments were terminated 3 days after the onset of hatching when straight (viable), bent and dead larvae and embryos unable to hatch were quantified.

During all three cruises parallel exposure experiments were also conducted under natural daylight conditions on deck using cut-off filters to create the following radiation conditions: (1) natural daylight, (2) UV-A and photosynthetically active radiation (PAR), (c) PAR only. As no significant effects were found (low irradiance level, overcast sky) these experiments will not be referred to further in the Results section.

Apart from visual inspections for abnormal development and behaviour, osmolality of eggs was measured before and after exposure.

Osmotic measurements

Plaice eggs

In plaice eggs osmolality of the perivitelline fluid was used as a marker. The perivitelline fluid was extracted from developing embryos using a glass capillary with an outer diameter of 0.1–0.2 mm. In order to introduce the capillary, the chorion had to be pierced with a micro-scalpel. The osmolality of the perivitelline fluid of single plaice embryos was than determined with the aid of a nanolitre osmometer (Clifton Technical Physics), which was calibrated for the range 0 (double distilled water) and 1,000 mosmol (32.2 g NaCl/l). Readings were given in milliosmol per kilogram H_2O.

Dab eggs

Due to the small size of dab eggs osmolality had to be determined from batches of 500 embryos. Embryos were gently homogenized using a teflon potter in 2-ml Eppendorf caps. After homogenization in ice the homogenate was centrifuged at 5,000 *g* and osmolality measured in a subsample of 100 µl of the supernatant, employing a digital micro-osmometer 5B (Roebling) with Peltier cooling, which was calibrated against a 300-mosmol and 450-mosmol calibration standard. Readings were given in milliosmol per kilogram H_2O.

Results

Dose of irradiance

The natural daily UV-B (280–315 nm) dose, which was measured continuously onboard during the campaigns 1997–1999 is shown in Fig. 4. Start and stop of the integration was triggered automatically by the spectroradiometer at the limit of one photon event per channel; this means, integration time was between about 6:15 and 16:30 UT. Besides a high fluctuation due to variable sky conditions there was a general increase in the UV-B dose with date, which can be attributed to the solar elevation. Minimum air mass was 2.4 on 20 February and 1.6 on 28 March.

Osmolality of the perivitelline fluid of exposed and non-exposed embryos of plaice and dab

The mean of the osmolality of healthy untreated plaice embryos (controls in the dark) was 350 mosmol (SD 42),

Table 2 Exposure schedule for 1999 experiments. For abbreviations, see Table 1

8 March 1999	9 March 1999	10 March 1999	11 March 1999	12 March 1999	13 March 1999	14 March 1999	15 March 1999	16 March 1999	17 March 1999	18 March 1999	19 March 1999	20 March 1999	21 March 1999	22 March 1999
Iaβ	Ibα	Ib α–β	II β–δ	IIδ	IIIα	IIIα	IIIβ–γ	IIIγ	IIIγ–IVα	IVα	Hatch	50% Larvae	End of hatching period	End of experiment
C	C	C	C	C	C	C	C	C	C	C				
2 h	2 h	2 h	2 h	2 h	2 h	2 h	2 h	2 h	2 h	2 h				
S1, S2	S1, S2	S1, S2	S1, S2	S1, S2	S1, S2	S1, S2	S1, S2	S1, S2	S1, S2	S1, S2				
4 h	4 h	4 h	4 h	4 h	4 h	4 h	4 h	4 h	4 h	4 h				
S1, S2	S1, S2	S1, S2	S1, S2	S1, S2	S1, S2	S1, S2	S1, S2	S1, S2	S1, S2	S1, S2				
6 h	6 h	6 h	6 h	6 h	6 h	6 h	6 h	6 h	6 h	6 h				
S1, S2	S1, S2	S1, S2	S1, S2	S1, S2	S1, S2	S1, S2	S1, S2	S1, S2	S1, S2	S1, S2				

that of exposed embryos floating directly below the surface was 388 mosmol (SD 144) (difference not significant: P=0.4335, comparison of two means) (Fig. 5a). The osmolality of the perivitelline fluid of embryos floating in midwater had increased to 635 mosmol (SD 165) (difference with respect to both controls significant; P<0.001), and that of embryos which had sunk to the bottom of the experimental containers was close to that of the surrounding seawater [mean eggs, 825 (SD 168); seawater, 930; difference significant: P=0.0265]. The latter embryos did not display any microscopically visible morphological differences. The osmolality of the perivitelline fluid of live embryos lying on the bottom was comparable to that of dead embryos.

Figure 5b contains information on differences in osmolality of floating, midwater and bottom embryos of dab. The pattern is similar to that found for embryos of plaice (see Fig. 5a), but values in dab were higher ranging from 550 mosmol for embryos from the surface to 775 mosmol for embryos from midwater. Changes in osmolality of non-irradiated dab embryos with developmental time is given in Fig. 6. Despite considerable fluctuations at the beginning of the development, a clear decrease in osmolality was found in later stages.

1997 Experiments (exposure 6 h or 12 h daily)

In the 1997 exposure experiments a time- and irradiation-dependent impact on mortality and buoyancy of embryos of dab and plaice was found. Loss of positive buoyancy became first apparent after 6–8 h of exposure even without ozone reduction, while under conditions of 40% ozone reduction exposed dab eggs already started to lose their buoyancy after 4 h, drifting either in mid water or lying on the bottom of the vessels.

No morphological aberrations were found in early developmental stages of dab (stage Ia, blastodisc), nor in embryos of plaice of developmental stages II and III (von Westernhagen 1970) after 4–18 h of irradiation at an intensity of 180 DU. Yet exposed embryos showed a loss of buoyancy as a function of exposure time. Up to 69% of all embryos were found at the bottom of the exposure chambers at certain exposure conditions after 18 h of radiation. After 24 h of exposure under conditions of S2 a mortality of 48% and loss of buoyancy in 69% of the surviving embryos was registered. Exposure to radiation under S1 conditions resulted in an acute mortality of 4% after 24 h accompanied by a loss of buoyancy of 76% of all embryos. After 36 h all embryos exposed to UV-B had died. In the experiments in which after 24 h a 4% mortality was registered (S1), the mortality rate increased to 18% after 36 h. Fifty percent of the embryos on the bottom of the experimental vials displayed developmental defects such as cavernous vacuoles in the area of the heart and proliferating blisters bilateral to the trunk. These malformations normally lead to mortality in the further course of their development (von Westernhagen et al. 1988). In controls incubated

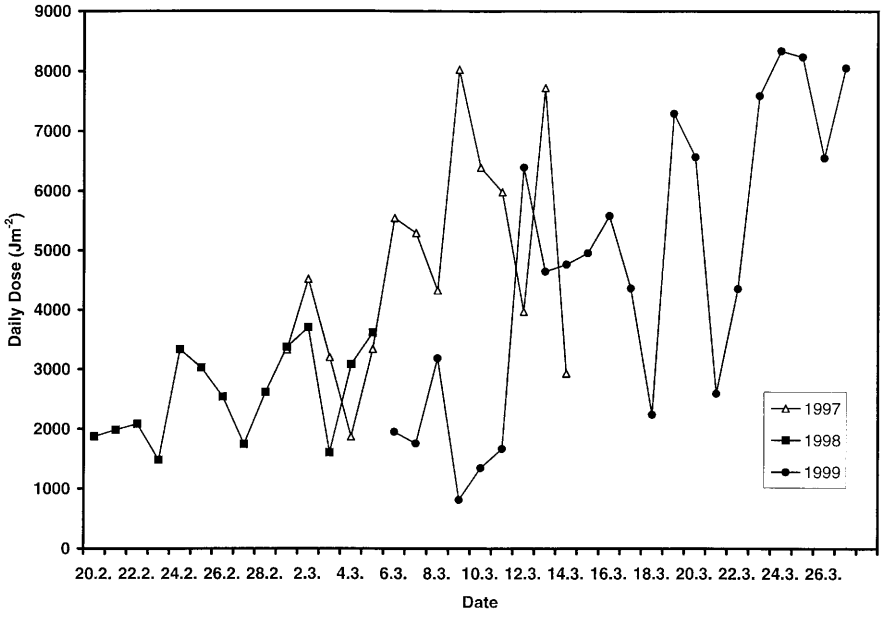
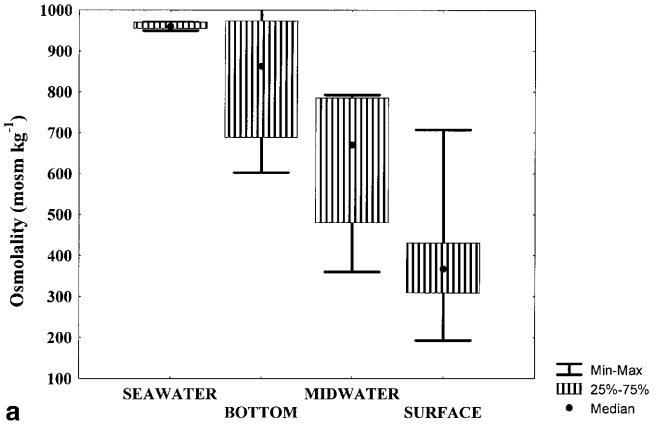


Fig. 4 Daily dose of surface UV-B (290–315 nm) between 0615 and 1630 UT expressed in W/m^2 during the three campaigns 1997–1999



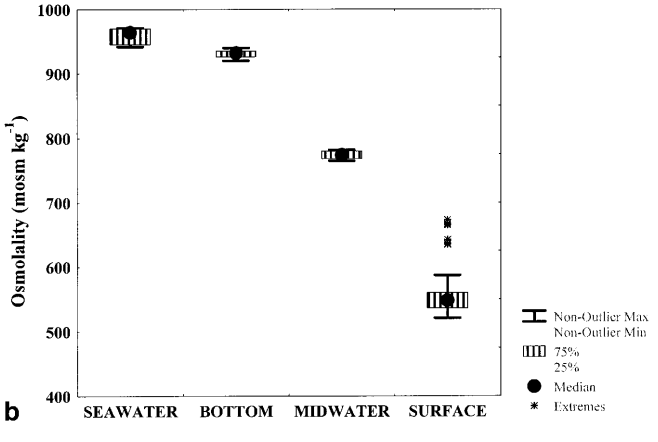


Fig. 5 Box plots of the osmolality of **a** the perivitelline fluid of embryos of plaice (*Pleuronectes platessa*) (no outliers) and **b** total body fluids of dab (*Limanda limanda*) collected at different positions in the water column of the exposure chambers after exposure to UV-B irradiation and ensuing loss of buoyancy

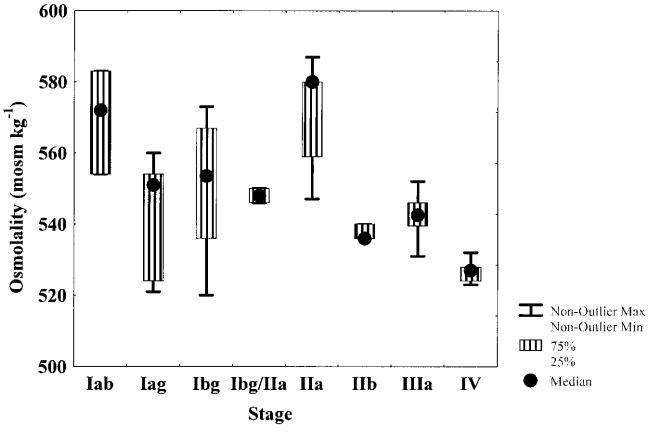


Fig. 6 Osmolality of non-irradiated eggs of dab (*L. limanda*) measured in batches of 500 individuals at different developmental stages. *Iab* Iaβ, *Iag* Iaγ, *Ibg* Ibγ, *IIa* IIα, *IIb* IIβ, *IIIa* IIIα

under subdued natural daylight neither mortality nor other effects were observed (mortality rate, 0%; loss of buoyancy rate, 0%).

1998 Experiments (exposure 6 h, dark 18 h, daily)

Results are summarized from consecutive experiments conducted under identical conditions using eggs from different fertilizations. For a period of 5 days dab embryos were exposed for 6 h and kept in darkness for 18 h, after which exposure was repeated. Control embryos were kept permanently in the dark. The development stage at the beginning of the exposure was Ibβ (Ib stages are designated as the beginning of epiboly, Westernhagen 1970). Significant differences of cumulative mortality between the treatments were encountered after 48 h

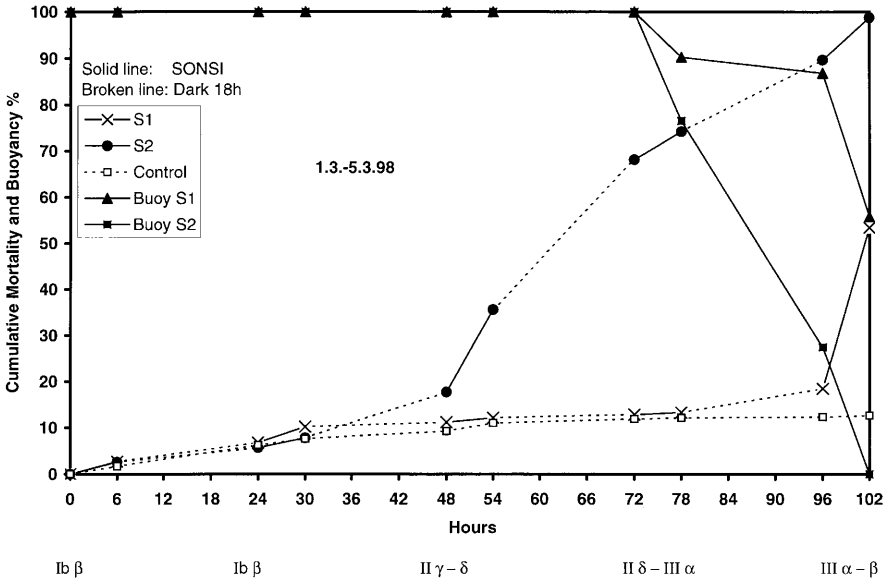


Fig. 7 Cumulative mortality and loss of buoyancy of dab (*L. li-manda*) embryos after consecutive exposure in S1 and S2 in comparison to those of controls

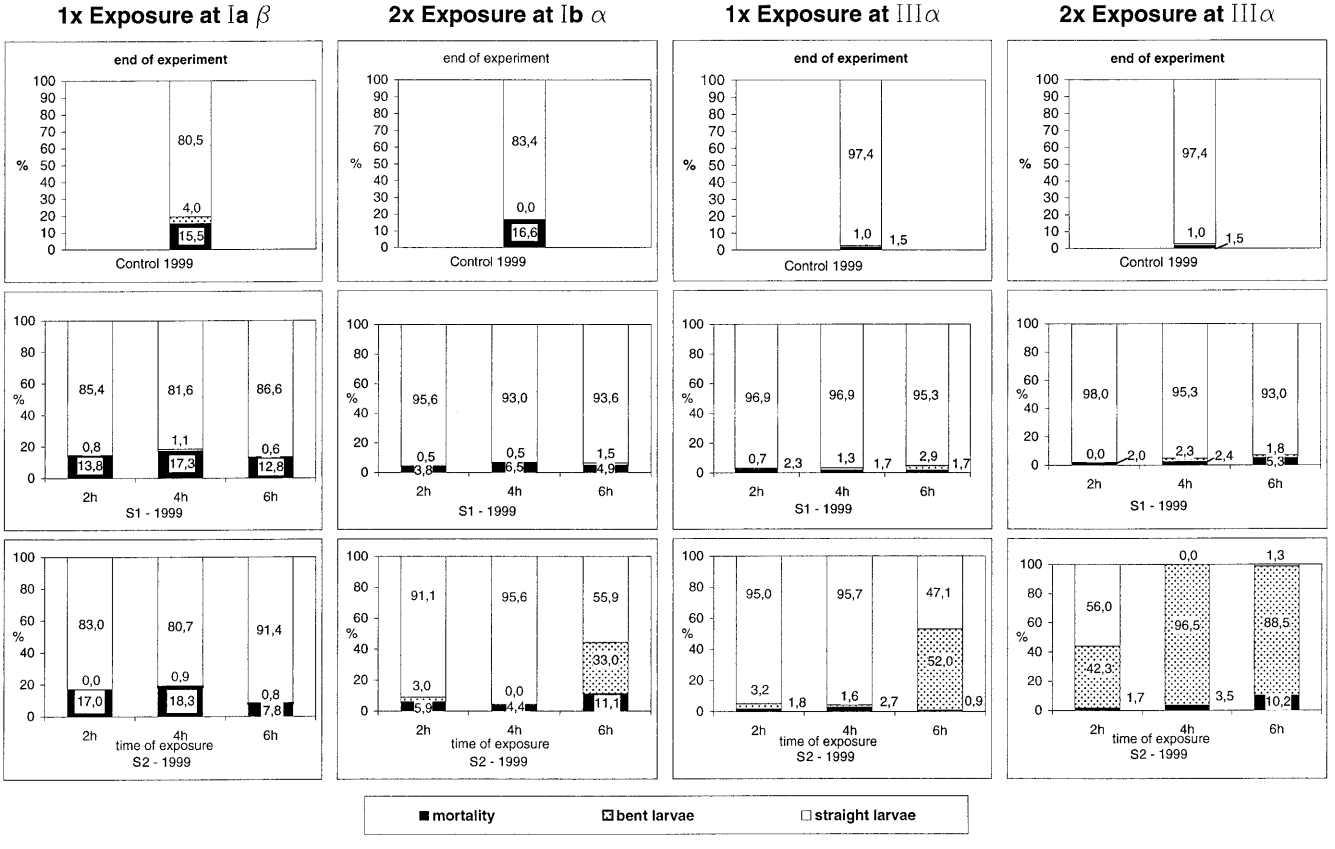


Fig. 8 Mortality of embryos and proportion of straight larvae of dab (*L. limanda*) after single and double exposures for 2, 4, 6 h in S1 and S2 at different developmental stages in comparison to those of the controls (1999)

(Fig. 7). Mortality under S2 conditions had increased and differences between controls and S1 as well as S2 were significant from then on. At the end of the experiment after 102 h (at the development stages IIIα–β) 100% mortality was reached in S2, while in the S1 experiment mortality had risen above 50% and was significantly higher than for the controls. Loss of buoyancy had occurred in S1 and S2 after 72 h (fourth exposure). No embryos were floating in S2 at the end of the experiment and only 56% of the embryos in S1 were still floating after 102 h and five exposures. Buoyancy in control embryos was never affected.

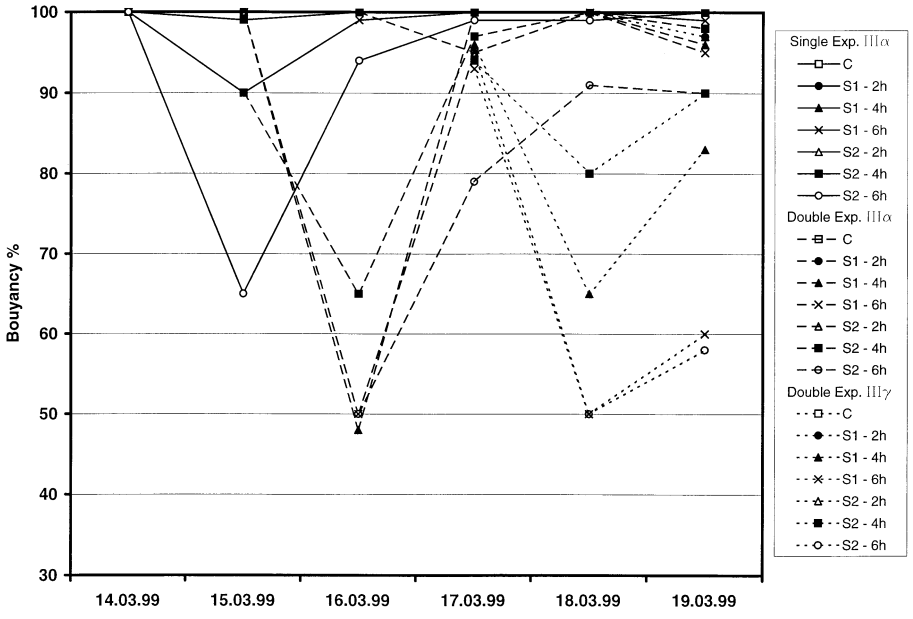


Fig. 9 Loss of buoyancy of embryos of dab (*L. limanda*) after single and double exposure for 2, 4 and 6 h at development stages IIIα and IIIγ in comparison to that of the controls (1999)

1999 Experiments (exposure 2,4 or 6 h, either once ore twice)

Mortality and viable hatch

The exposure schedule is depicted in Table 2. Single exposure experiments began at the developmental stage Iaβ. Results are given in Fig. 8, column 1. There were no differences in the cumulative mortality for S1 and S2 as compared to controls. Likewise, percentages of bent and straight larvae were not different from what was found in controls. In the double exposure experiments (Fig. 8, column 2), starting at stage Ibα, mortality of exposed embryos did not differ from that of control embryos. Yet under the S2 conditions at 6 h the proportion of bent larvae was significantly increased as compared to control embryos or those exposed for 2 and 4 h.

After a single exposure at a more advanced stage, IIIα (Fig. 8, column 3), cumulative mortality and the proportion of straight larvae were not different in S1 as compared to those of controls. Similarly after exposure for 2 and 4 h in S2 no significant differences with respect to S1 were detected. But the proportion of straight larvae in S2 after 6 h of exposure was significantly lower as compared to that of all preceding experiments ($P<0.0001$).

After two exposures at an advanced stage, IIIα (Fig. 8, column 4), mortality in the 6-h exposure in S1 was slightly but significantly higher as compared to that of controls ($P<0.0001$). Yet in S2 the proportion of bent larvae in all experiments was significantly increased as compared to that of controls ($P<0.001$). In the exposures lasting 4 and 6 h the proportion of viable larvae was reduced to 0 and 1.3%, respectively. More than 95% of the hatched larvae were either bent or severely crippled and not viable.

Loss of buoyancy

The loss of buoyancy in percent of still floating eggs (single exposure in IIIα) is given in Fig. 9. Under the strongest radiation regime (S2, 6h) up to 35% of the eggs were lying on the bottom of the incubating jars on the second day. On day 3 the effect was less marked and not detectable from day 4 on.

After two exposures at IIIα a loss of buoyancy (Fig. 9) was detected in 35–50% of the embryos in S1 (4 h) and S2 (4 h and 6 h) on the first day after exposure. One day later buoyancy was normal for all embryos in the experiment with the exception of those exposed for 6 h in S2. Of the latter assay, 10% of embryos never recovered until hatching. After two exposures in a still later stage (IIIγ, Fig. 9) loss of buoyancy was even more pronounced and was observed also after exposure for 4 and 6 h under the conditions of S1.

In all experiments an exposure-dependent recovery from the loss of buoyancy was observed after termination of the exposures. The recovery was less marked when embryos were exposed at a late developmental stage. Particularly after a double exposure under conditions of S1, 4h; S1, 6h; S2, 4h; and S2, 6h, recovery was never complete until hatching and between 10 and 40% of the exposed eggs had lost their buoyancy permanently.

Discussion

There are a few papers dealing with the influence of UV-B irradiation on fish embryos. Hunter et al. (1981) found brain and eye damage and reduced growth of 4-day-old embryos and larvae of *Engraulis mordax*, after UV-B exposure when the ozone concentration was reduced by 25% at the water surface. Pommeranz (1972) found a

high sensitivity of embryos of plaice. Strähle and Jesuthasan (1993) showed that the epiboly of embryos of zebrafish was impaired by UV irradiation. Steeger et al. (1999) and Kouwenberg et al. (1999) could show that marine fish embryos (plaice, cod) reacted with reduced survival when exposed to UV-B radiation during early development.

The results of the present exposure experiments allow some conclusions as to the potential effects of UV-B radiation on pelagic fish eggs. From what is known about UV-B protective compounds in planktonic organisms, the most common protecting pigments such as carotenoids and cuticular melanin are largely absent in young embryos. Yet other substances that could serve as UV protection such as mycosporine aminoacids with absorbance maxima between 320 and 360 nm (Chioccara et al. 1980) or the compound gadusol (Plack et al. 1981, absorbance maximum 296 nm) are usually found in pelagic eggs of scombrids, gadoids and flatfish. Nevertheless, the unrealistically high UV-B dosages employed in our 1997 experiments produced 100% mortality in embryos of dab and plaice. UV-B irradiation, which was only slightly higher as compared to field conditions, still produced dose-dependent mortality and an impact on the buoyancy of embryos.

Loss of positive buoyancy

The buoyancy of pelagic fish eggs is achieved by decreasing their specific weight below that of the surrounding seawater. For this purpose either lipids or oil globules with a low specific weight can be incorporated into the yolk of the embryo. In addition, the perivitelline fluid of the healthy pelagic egg is kept hypoosmotic (Fig. 6) as compared to the surrounding medium (Holliday and Jones 1967), thus reducing the overall weight of the egg. As a consequence, under calm weather conditions many pelagic fish eggs float directly below the water surface. Making use of the semipermeability of the chorion, the embryo can also actively change the density of the perivitelline fluid to a certain extent. This feature becomes particularly important at the end of the egg phase, when lipids have been used in ontogenetic processes and positive buoyancy has to be achieved by a reduction of osmolality. Thus for the survival of the pelagic egg the maintenance of a positive buoyancy is of crucial importance.

In the present exposure experiments changes in osmolality of the perivitelline fluid led to the observed loss of buoyancy in irradiated eggs. The observed total loss of buoyancy presents a severe threat to the eggs because sinking to the bottom will inevitably lead to high mortality in the developmental stages of pelagic fish embryos, since the thin chorion cannot protect the pelagic egg sufficiently against diverse attacks from bacteria, fungi or active predators of the benthic realm. On the other hand, reduced buoyancy could serve as an avoidance mechanism, causing the egg to sink deeper into the water column, thus avoiding the strong radiation at the surface and initiate repair mechanisms as described by Kaupp and Hunter (1981) for anchovy eggs and larvae (*Engraulis mordax*) and Applegate and Ley (1988) for fathead minnows (*Pimephales promelas*), which would work even in deeper layers.

Since the results indicated that relatively small doses of repetitive UV-B irradiation could cause high percentages of exposed embryos to lose their buoyancy irretrievably, this effect may be of ecological significance. When looking at the results as far as loss of buoyancy is concerned, both mechanisms, recovery and a total loss of buoyancy, have been experienced. In Fig. 7 we show that a loss of buoyancy with SONSI 2 occurred only after four exposures together with a substantial mortality (>70%) of the egg population which was exposed at a rather early stage (Ib). The fact that, due to repetitive irradiation, virtually no recovery was experienced gives strong support to the assumption that we are looking at a sublethal effect rather than at a “survival mechanism” for a mildly affected organism.

In Fig. 9 the reactions towards single and double exposures at stage IIIα are depicted. They differ from the reactions described above in the sense that in these assays a pronounced recovery was visible giving reason to assume the existence of substantial repair mechanisms, as already described by Kaupp and Hunter (1981) for anchovy embryos. Both assays (1998 vs. 1999) varied in that only during the consecutive exposures (1998) were embryos kept in the dark, which was insufficient for photo repair. In the later experiments (1999), embryos were kept under a 12 h/12 h light/dark regime (subdued light) after exposure, and thus profited from photorepair as described by Vetter et al. (1999) for northern anchovy. Applegate and Ley (1988) calculated that 50% of the produced pyrimidine dimers (a measure of DNA damage) after UV radiation were removed within 9 h after exposure.

Exposure at a later stage (IIIγ) also shown in Fig. 9 apparently had more severe effects on buoyancy. Although repair had still occurred, the embryos exposed for 6 h still suffered a substantial loss of buoyancy after the second day.

Mortality and reduction of viable hatch

The mortality and reduction in viable hatch which was experienced after moderately high UV-B exposure was strongly dependent on the total UV-B doses applied, but also on the developmental stage. No mortality occurred after a single exposure at stages Iaβ and Ibβ, neither after the second exposure in the repetitive exposure assay (Fig. 8, column1), nor in the double exposure assay under S1 conditions (Fig. 8, column 2). Increased mortality and reduced viable hatch were only apparent after the third consecutive exposure or under S2 conditions in the double exposure assay (Fig. 8, column 3) in embryos exposed at an early stage. Later embryos (stage IIIα) were more sensitive, showing significantly elevated non-viable hatch at the longest exposure times of 6 h (Fig. 8,

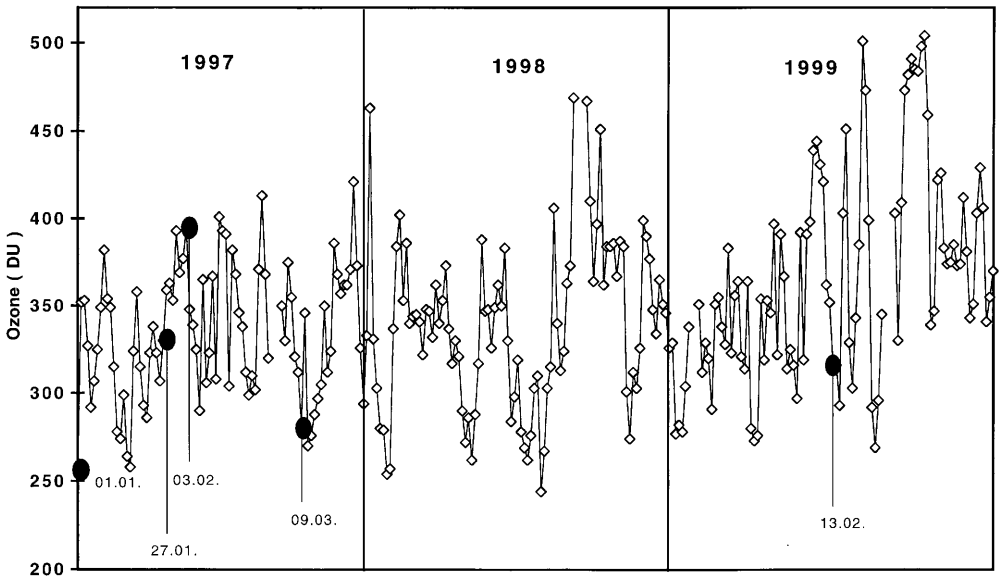


Fig. 10 Ozone conditions (DU) in the southern North Sea during the first quarters of 1997–1999. *Black spots* mark days with meteorological conditions necessary for UV-B effects

columns 3, 4). The mechanisms underlying the observed reactions are probably related to the capacity of aquatic organisms for photorepair, as described by Kaupp and Hunter (1981) and Vetter et al. (1999) for embryos of northern anchovy (*Engraulis mordax*) and Siebeck and Böhm (1989) for *Daphnia* sp.

On the other hand, although apparently repair had occurred concerning the capacity for osmoregulation (see Fig. 8), considerable mortality was the result at a later developmental stage (IV), i.e. embryos were either severely crippled so that they did not hatch at all and remained in the shell, or the hatched larvae were extremely bent and moribund.

Do present ozone conditions bear ecological significance for the survival of pelagic fish eggs?

With the background information on considerable direct mortality after UV irradiation and the remarkable permanent loss of positive buoyancy, which ultimately may lead to a loss of reproductive potential in North Sea fish, it is of high interest to know whether the experimental conditions leading to these effects are relevant in view of the actual situation in the North Sea.

From the results of our experiments we know that in order to cause significant damage to the developing embryo in stage IIIα either a single 6-h dose at radiation conditions of S2 (180 DU) was required, or similar effects could be triggered by a double exposure (2 consecutive days) of 4 h under conditions of S1 or S2. Both experimental setups resulted in a considerable percentage of eggs losing their buoyancy and an increased reduction of viable hatch between 47% (one exposure of 6 h, S2) and 0% (two exposures of 4 h, S2) as compared to between 80% and 97% in controls.

Since under field conditions during the period under question (January–March) the necessary conditions for significant UV-B radiation-induced injury do not occur permanently and are rather the exception than the rule, we attempted a calculation of the probability and the degree of damage that can be expected in future UV-B scenarios found in the southern North Sea.

For an estimation of the potential effects of increased UV-B exposure on pelagic fish eggs, the ozone situation, the meteorological conditions (sunshine duration, cloud cover, wind speed) and the light attenuation in the water column has to be taken into account. As potentially effective daytime the hours 0900 and 1500 between 1 January and 31 March of the years 1997–1999 will be considered. In order to keep the embryos in the upper surface layer, vertical mixing of the sea water has to be low and the wind speed must not exceed 7 kn. The vertical attenuation coefficient for downward irradiance (K_d) varies with location and wavelength. In the Helgoland area, which is considered in the present paper, the mean K_d value is 3.6 m^{-1} at 300 nm and 2.3 m^{-1} at 320 nm, which is about one magnitude higher than for clear ocean water. Thus, the loss of UV-B at a depth of only 30 cm is in the range of 55% (at 320 nm) to 70% (at 300 nm) including about 5% reflectance at the water surface for the sun at 30° elevation. As a consequence, in order to suffer from UV-B effects, fish eggs are required to stay permanently in the upper 30 cm of the water column during daylight exposure.

In order to fulfil the above-mentioned requirements, only days with the following conditions were selected (meteorological data collected at the Helgoland weather station by Deutscher Wetterdienst, Hamburg): (1) cloud cover <4/8, (2) sunshine duration 3, 4 and 5 h, (3) wind speed <7 kn.

As shown in Fig. 10, these requirements were met on 5 days. On only 2 days (1 January 1997 and 9 March 1997) the radiation level due to ozone depletion came close to those of S1. Based on our experimental results, we can assume a minimum requirement of 2 consecutive days under S1 conditions necessary to inflict any damage

in late-stage embryos. Also for previous years the statistical probability – not regarding ozone depletion – is rather low. From 1983 to 1999 under the above conditions the mean number of days per year with >3 h sunshine was 2.2±1.6; for >4 h, 1.6±1.3; and for >5 h, 0.8±1.1. For a consecutive exposure of 2 days during the past 17 years calculations were made for a total of only six occasions over the whole period.

After consideration of all the above factors, the likelihood that under the present situation of stratospheric ozone, weather conditions and water characteristics pelagic fish embryos are damaged by UV-B radiation is very low.

Yet for fish embryos in the central North Sea the outcome of our investigations may be helpful for the development of future scenarios regarding further ozone depletion, or the effects of a change in weather conditions due to a global climate effect. Assuming a future situation in which only the mean ozone value for January–March will be decreased by 50 DU, it can be derived from Fig. 10 that about 20 of 90 days would meet the S2 radiation conditions, leading to p_r=0.2 for a damage event. Keeping the weather conditions unchanged (2 of 90 days fulfil the above meteorological preconditions) the probability for the same time interval is p_m=0.02. A damage event for a single exposure therefore is given by the product p=4×10^{-3}, which is still low and will hardly affect the UV-B situation for pelagic fish eggs in the North Sea.

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