Marine Biology (1999) 134: 269–284

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Biological weighting of ultraviolet (280–400 nm) induced mortality in marine zooplankton and fish. I. Atlantic cod (*Gadus morhua*) eggs

Received: 2 March 1998 / Accepted: 18 December 1998

Abstract In the Gulf of St. Lawrence, Canada, productivity-determining biophysical interactions occur in the upper 0 to 30 m of the water column. The eggs and larvae of several commercially important marine invertebrates and fishes (e.g. *Gadus morhua* L.) are found in this layer. Measurements of the diffuse attenuation coefficients for ultraviolet-B radiation (280 to 320 nm, UV-B) at various locations in this geographic region indicated maximum 10% depths (the depth to which 10% of the surface energy penetrates at a given wavelength) of 3 to 4 m at a wavelength of 310 nm. This represents a significant percentage of the summer mixed-layer water column: organisms residing in this layer are exposed to UV-B radiation. Laboratory experiments using a Xenon-arc-lamp based solar simulator revealed that cod embryos exposed to UV-B exhibited high wavelength-dependent mortality. The strongest effects occurred under exposures to wavelengths below 312 nm. This susceptibility was also dependent upon developmental stage; mortality was particularly high during gastrulation. At the shorter wavelengths (< 305 nm) UV-B-induced mortality was strongly dose-dependent, and not significantly influenced by dose-rate. The biological weighting function (BWF) derived for UV-B-induced mortality in cod eggs is similar to that reported for naked DNA – suggesting that the mortality is a direct result of DNA damage. There was no evidence of a detrimental effect of ultraviolet-A radiation (320 to 400 nm). Calculations based upon the BWF indicate that, under current noon surface irradiance, 50% of cod eggs located at or very near (within 10 cm) the ocean surface will be dead after 42 h of exposure. Under solar spectral irradiance simulating a 20% decrease in ozone layer thickness, this time drops to 32 h. These are first-order estimates based upon surface irradiance taken at a time of day during which the values would be maximal. Nonetheless, they illustrate the relative changes in UV-B impacts that will result from ozone layer depletions expected over the coming decades. It is also important to point out that variability in cloud cover, water quality, and vertical distribution and displacement of cod eggs and larvae within the mixed layer, can all have a greater effect on the flux of UV-B radiation to which fish eggs are exposed than will ozone layer depletion at these latitudes.

Communicated by R.J. Thompson, St. John's

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Introduction

Over the past 10 to 15 years, levels of solar ultraviolet-B radiation (280 to 320 nm, UV-B) incident at the Earth's surface have increased significantly over mid-latitude areas of the Northern and Southern Hemispheres (Crutzen 1992; Kerr and McElroy 1993; Madronich et al. 1995). These increases in UV-B are linked to reductions of stratospheric ozone (Kerr and McElroy 1993; Madronich 1994; Madronich et al. 1995).

A growing number of studies indicate that UV-B radiation, at current levels, is harmful to aquatic organisms and may reduce the productivity of marine ecosystems (Holm-Hansen et al. 1993; Siebeck et al. 1994; Häder et al. 1995). Such UV-B-induced reductions

in productivity have been reported for phytoplankton, heterotrophs, and zooplankton, the key intermediary levels of marine food chains (Damkaer 1982; Thomson 1986; Cullen and Neale 1994; Chalker-Scott 1995; Smith and Cullen 1995; Booth et al. 1997; Häder 1997). Analogous studies on fish eggs and larvae, although rare, indicate that exposure to levels of UV-B currently incident at the Earth's surface results in higher mortality that may lead to poorer recruitment to adult populations (Pommeranz 1974; Hunter et al. 1981, 1982; Williamson et al. 1997).

In some regions of the Gulf of St. Lawrence, Canada, the late spring and summer water column shows a pronounced thermocline between 10 and 30 m (Petrie et al. 1988; Koutitonsky and Bugden 1991; Runge and de Lafontaine 1996). A cold intermediate layer (CIL, −1 to +1 °C), situated at depths of 30 to 100 m, separates the warm mixed layer near the surface (14 to 16 °C in summer) from the waters at depth (6 °C) (Koutitonsky and Bugden 1991; Runge and de Lafontaine 1996; Gilbert and Pettigrew 1997). As a result of the spring-through-fall presence of this intermediate cold layer, the most important productivity-determining biophysical interactions occur in the upper 0 to 30 m of the water column (Therriault 1991; Ohman and Runge 1994; Runge and de Lafontaine 1996). During summer, the mixed layer in these waters is typically 10 to 15 m deep (Therriault 1991). The eggs and larvae of several commercially important marine invertebrates and fishes are found in this layer (Fortier et al. 1992; Runge and de Lafontaine 1996).

Measurements of the diffuse attenuation coefficients for solar UV-B at various locations in the estuary and Gulf of St. Lawrence indicate maximum 10% depths (the depth to which 10% of the surface irradiance penetrates at a given wavelength) of 3 to 4 m at a wavelength of 310 nm (Kuhn et al. 1999). This represents a significant percentage of the summer mixed-layer water column. In clear tropical ocean waters the 10% depth at 310 nm can be as deep as 15 m (Booth and Morrow 1997). UV-B-induced damage to the DNA of fish eggs and larvae has been detected in samples collected from depths of up to 20 m (Malloy et al. 1997). Thus, the early life history stages of the crustacean and fish species that are present in the shallow mixed layer of the water column may be particularly susceptible to increasing levels of UV-B.

The reproductive season for Atlantic cod (*Gadus morhua*) in the Gulf of St. Lawrence begins early in the spring and continues through late June (Ouellet et al. 1997). Spawning occurs in deep water (>200 m), and cod eggs, which are typically positively buoyant, ascend to the surface mixed layer over a period of 2 to 10 d (Solemdal and Sundby 1981; Anderson and de Young 1995; Ouellet 1997). A significant proportion (10 to 30%) of all cod eggs present in the water column occur in the 0 to 25 m depth stratum off the Newfoundland Shelf (Anderson and de Young 1995), off Greenland and Labrador (Brander 1994), on southern Georges Bank (Lough et al. 1996) and in the northern Gulf of St. Lawrence (Ouellet 1997). However, it is also possible for eggs to become trapped at density barriers (such as the thermocline), and these eggs would likely never reach the surface (Ouellet 1997). Nonetheless, on clear summer days, when wind speed is low, the highest egg concentrations are observed in the upper 0 to 50 m of the water column (Sundby 1983, 1991). The early larval stages are also typically present, and often even closer to the surface (Anderson and de Young 1995).

Here, we investigate potential impacts of ultraviolet radiation (280 to 400 nm, UV-B) on the early life stages of Atlantic cod. The goals of this study were to (1) evaluate the effect of UV radiation on mortality in the eggs of Atlantic cod and determine whether developmental state affects susceptibilty to UV exposure, (2) generate dose-response relationships and test the principle of reciprocity (which states that the UV-B-induced mortality effect on cod eggs will depend on dose, independent of dose *rate*), (3) derive a biological weighting function (BWF) for the effect of UV on mortality in Atlantic cod eggs, and (4) present a preliminary assessment of the potential impact of solar UV-B on the early life stages of cod in the subarctic marine ecosystems of eastern Canada.

Materials and methods

Radiometry and general experimental procedures

Spectral irradiance was measured with an OL754-O-PMT scanning spectroradiometer (Optronic Laboratories, Orlando, Florida). This instrument is based upon a double monochromator design and employs a temperature-controlled photomultiplier detector. The wavelength precision is 0.05 nm, and the wavelength bandwidth for the measurements presented here was <1 nm. The instrument was calibrated against a NIST-traceable 200 W tungsten-halogen standard lamp (OL752-10) immediately prior to any series of measurements. Wavelength and gain accuracy – 0.1 nm and <1%, respectively – were assessed using the OL752-159 Dual Calibration and Gain Check Source Module. Measurements were made during the summer of 1996, on several dates and under varying weather conditions. In-air measurements were made using a 152 mm integrating sphere (OL-IS-640), and underwater measurements were made using a submersible cosine receptor (OL86-T-WP) attached to the end of a 2 m quartz fibre optic probe (OL730-7Q). Additional data on ozone layer thickness and UV-B irradiance were obtained using a Brewer MKIII double monochromator spectrophotometer (Sci-Tec Instruments Inc., Saskatoon, Saskatchewan) deployed on the roof of the Maurice-Lamontagne Institute (MLI: 48°38′25.9″ N; 68°09′21.0″ W).

Atlantic cod eggs were irradiated under a custom-designed solar simulator (SS). The SS consisted of two 1-kW Xenon-arc-lamps (SS-1000X, Spectral Energy, Westwood, New Jersey) outfitted with optical feedback amplifiers which maintained their output constant. The output optics of the two Xenon lamps were oriented so that their radiative fields overlapped (Fig. 1). Arc Lamp 1 contained a standard mirror that reflected its entire spectral output (minus the infrared) through to the optics head (Fig. 1a). Arc Lamp 2 contained a dichroic mirror which preferentially reflected wavelengths from 280 to 450 nm (Fig. 1b). The optics heads of the two arc lamps had filter holders into which combinations of optical filters could be inserted, allowing for a variety of spectral exposures and dose rates. The spectral output of these lamps was adjusted using a 280 nm long-pass filter in Lamp 1 (Schott WG-280, quartz

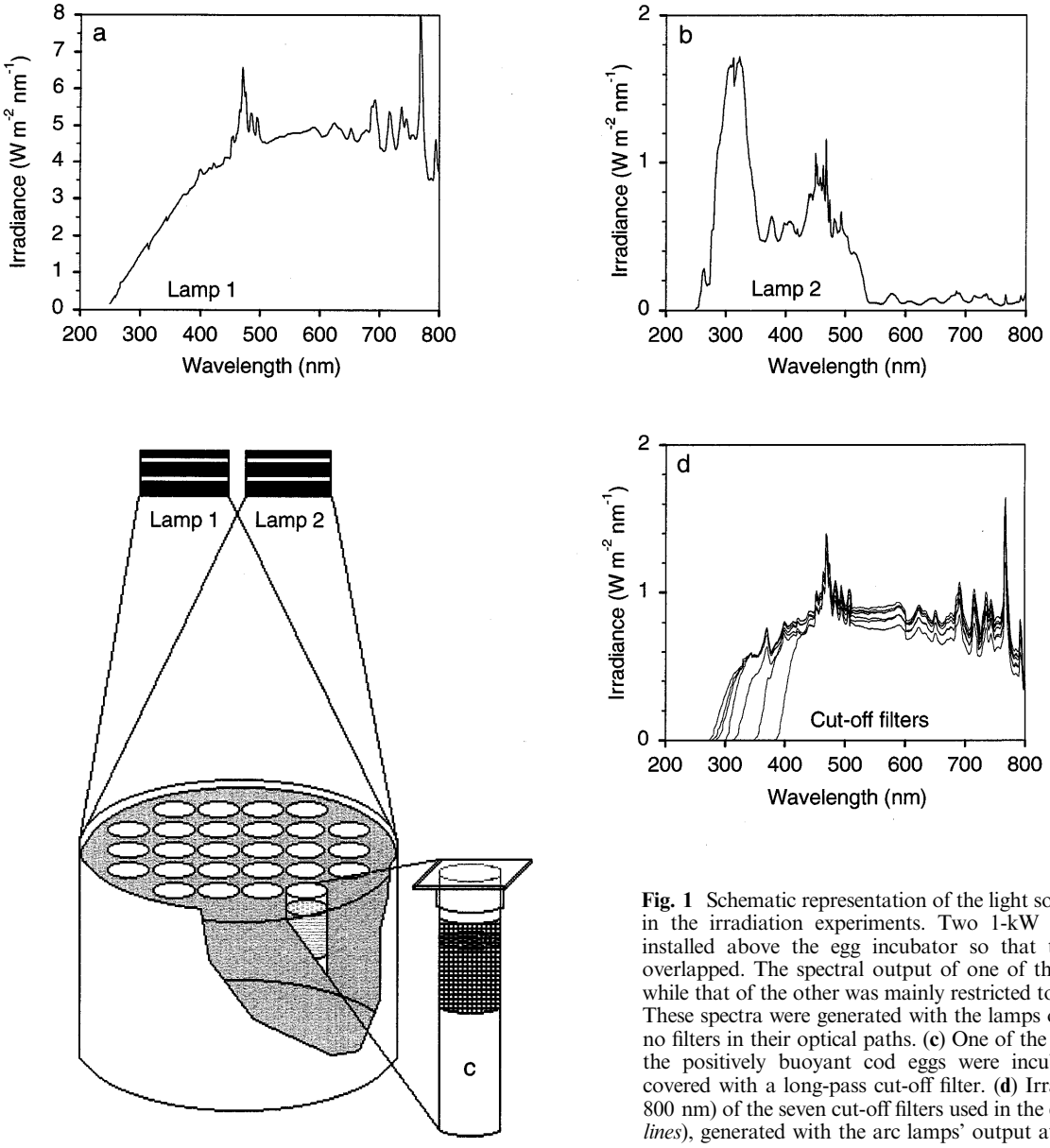

Fig. 1 Schematic representation of the light source and chamber used in the irradiation experiments. Two 1-kW Xenon-arc-lamps were installed above the egg incubator so that their radiative outputs overlapped. The spectral output of one of the lamps was broad (**a**) while that of the other was mainly restricted to the UV waveband (**b**). These spectra were generated with the lamps operating at 1 kW with no filters in their optical paths. (**c**) One of the 34 glass tubes in which the positively buoyant cod eggs were incubated. Each tube was covered with a long-pass cut-off filter. (**d**) Irradiance spectra (250 to 800 nm) of the seven cut-off filters used in the experiments (*continuous lines*), generated with the arc lamps' output at 600 W

silica substrate) and quartz silica substrate neutral density (ND) filters (Lamp 1: ND = 0.3 or 50% transmission; Lamp 2: ND = 0.6 or 25% transmission). With this combination of filters, the mean (±SD) integrated irradiance delivered by the two-lamp SS equalled 450 ± 197 W m^{-2} (280 to 800 nm) and approximated that delivered to the Earth's surface outside MLI during a sunny summer's day (429 ± 164 W m^{-2}, the mean ±SD of three measurements made at 10:00, 12:00 and 15:00 hrs on the same day). However, as compared to sunlight, the spectral irradiance delivered by the SS was higher in the UV-B region and lower in the visible waveband (400 to 700 nm, Fig. 2).

Cod (*Gadus morhua* L.) eggs were incubated in glass cylinders with an inner diameter of 22 mm. These incubation tubes were cut in half and rejoined by fusing a 50 mm long piece of Nytex mesh (500 µm mesh size) between the two sections (Fig. 1c). The height of the tubes was 150 mm. A total of 34 incubation tubes, each containing approximately 400 cod eggs, were immersed in a circular basin filled with re-circulating 0.2 µm filtered seawater at 6 °C and a salinity (28 ± 1 psu) identical to rearing basins from which eggs were collected (see below). This design ensured that all of the eggs in the experiment were incubated under similar water conditions. The bottom of the incubation basin was fitted with a polyethylene holder which contained a slot for each of the incubation tubes. The holder was designed so that it could be positioned at exactly the same orientation under the SS during each exposure. This was important because the SS's output was not spatially uniform. Each incubation tube was covered with a 25 × 25 mm quartz substrate long-pass filter (Fig. 1c). The filters used were Schott WG280, WG295, WG305, WG312, WG335, WG360, and GG400, for which the cut-off wavelength is as specified (Figs. 1d, 2). There were five tubes for each cut-off filter treatment except for the WG360, which had four. Spectral irradiance [E(λ), in W m^{-2} nm^{-1}] was measured at 1 nm intervals (250 to 800 nm) under each of the cut-off filters at all 34 tube positions in the incubator. As a result of the spatially non-uniform output of the SS, each of the 34 tubes received a somewhat different dose rate (Fig. 2) and, since the exposures were always of a set duration, a different dose (J m^{-2}). The combined filters – those in the SS's optics heads and those on top of each tube – eliminated all UV-C radiation (Figs. 1d, 2).

For illustrative purposes, DNA-weighted biologically effective irradiance delivered to the tubes in the WG280, 295, 305, 312 and 335 treatments were calculated as the mean of all the tubes in each

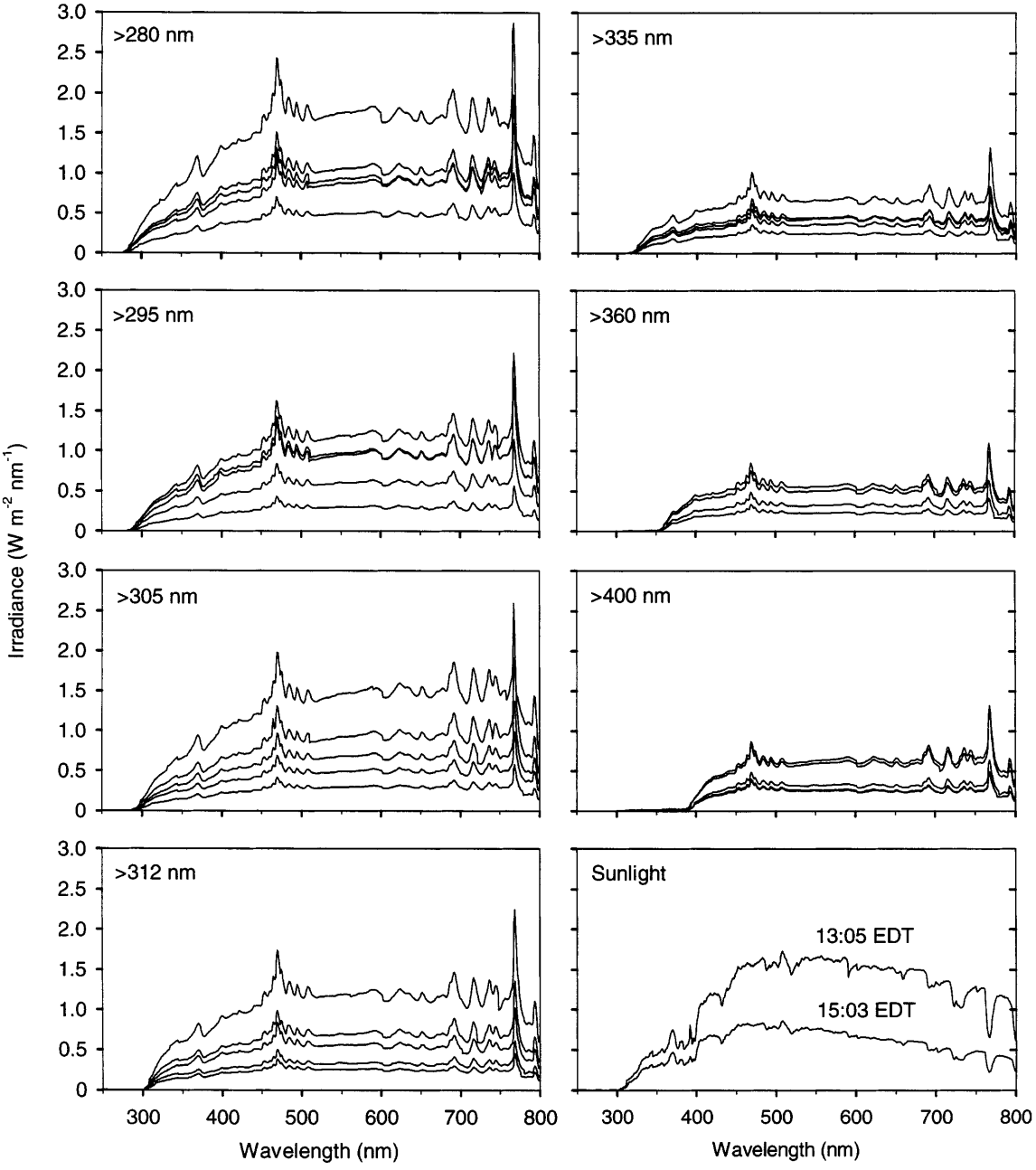

Fig. 2 Spectral irradiance (250 to 800 nm) delivered to cod eggs under the seven different long-pass cut-off filters. Since the radiative field of the Xenon-arc-lamps was non-uniform, irradiance was measured at the position of each and every incubation tube. Note that there were only four tubes for the >360 spectral exposure treatment and that two curves are superimposed in each of the >335 and >400 panels; thus, only four spectra are apparent in these panels. Each frame presents the spectral irradiance for the optical filter whose cut-off wavelength is as specified. The solar spectral irradiance, presented in the bottom right frame, was measured in the air under cloudless skies on 5 August 1996 at 13:05 and 15:03 hrs eastern daylight time (*EDT*) outside the Maurice-Lamontagne Institute, Mont-Joli, Québec, Canada (48°38′25.9″ N; 68°09′21.0″ W)

of these spectral exposure treatments (Fig. 3). The dose rates presented in Fig. 3 were weighted using the Setlow (1974) DNA action spectrum (interpolated linearly in log space), normalized to one at 300 nm, and for which weightings were set to zero for wavelengths greater than 315 nm. These dose rates are compared to those measured on a clear summer day (5 August 1996) at solar noon, and to calculated dose rates that would result from a 20% reduction in ozone layer thickness (Fig. 3).

UV irradiation experiments

Fertilized cod eggs were obtained from a broodstock maintained at MLI for experiments unrelated to UV radiation. The broodstock was divided into two nutritional groups, high ration (fed at 7% of body weight per fish twice each week) and low ration (fed at 2% of body weight per fish once each week) (Dutil et al. 1998). Male–female pairs were kept in separate rearing basins outfitted with egg collectors. Thus, the source of the eggs used in the UV exposure experiments was known. Fertilized eggs were incubated in the dark, at 6 °C, in 60 liter black round-bottom basins from which they were removed immediately prior to an exposure experiment. Eggs were obtained on the basis of availability. Therefore, since only one

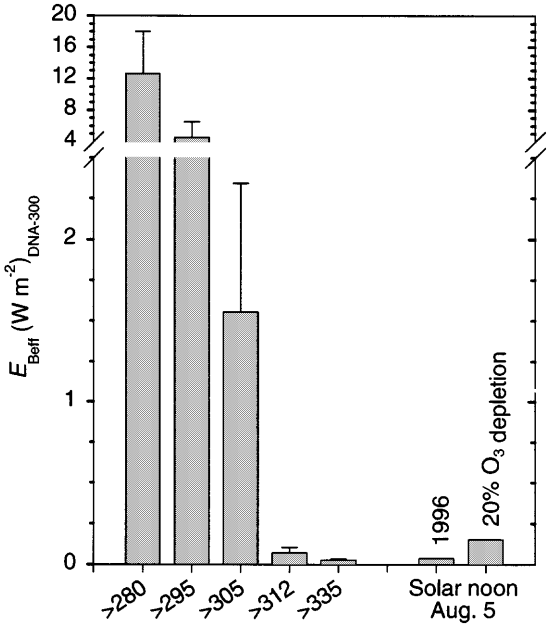

Fig. 3 DNA-weighted biologically effective irradiance (E_{Beff}) delivered by the solar simulator to the WG 280, 295, 305, 312 and 335 nm cut-off filter treatment groups during the irradiation experiments on Atlantic cod (*Gadus morhua*) eggs. Also presented are the analogous values for sunlight, as measured under clear skies, in air, at solar noon on 5 August 1996 at the Maurice-Lamontagne Institute, Mont-Joli, Québec, Canada (48°38′25.9″ N; 68°09′21.0″ W) and as predicted, for these same conditions, but with a 20% reduction in ozone layer thickness over the site. These latter values were obtained by correcting the irradiance for a 20% reduction in ozone using a 50 level delta-Eddington radiative transfer model, which accounts for all major UV attenuants (excluding clouds): ozone, aerosol and Rayleigh scattering (Davies et al. 1999). All values were weighted using the Setlow (1974) DNA action spectrum (interpolated linearly in log space), normalized to one at 300 nm, and for which weightings were set to zero for wavelengths greater than 315 nm. Means (of all tubes in each of these spectral exposure treatments) and standard error bars are plotted

experiment could be run at a time, the eggs used in each experiment were either from different egg batches from the same male–female pair (cod females produce up to 20 egg batches during their annual reproductive cycle), or from different male–female pairs. The choice of which egg group was used for any given experiment was governed solely by availability.

Exposures to UV radiation commenced approximately 4 d post-fertilization (PF), since Atlantic cod eggs are released at depth and will not be exposed to UV radiation until they reach the surface layer several days later. At this point in development, embryos had reached early to middle gastrulation (Stage 3 or 4). All developmental stage numbers referred to in the text are those described by Fridgeirsson (1978). Hatching success and larval survival to 6 d post-hatch (PH) were determined after all experiments.

The first two experiments were conducted to generate a BWF for the effect of UV exposure on mortality in cod eggs and to evaluate the potentially mitigating effect of photorepair on this mortality.

In Experiment 1, eggs collected from a high ration male–female pair were irradiated under the SS for 2 h each day for 7 d: from 4 d PF through 11 d PF, which was just before hatching (Stage 6 – first hatching cells). After each day's exposure, the 34 tubes containing the cod eggs were transferred to an environment-controlled chamber and immersed in freshly filtered (0.2 μm pore size), oxygenated and UV-sterilized seawater at 6 °C. The eggs were held in total darkness until the next day's irradiation. Hereafter, this will be referred to as the without-photorepair experiment. Cod eggs become opaque shortly after dying, making it possible to census mortality by visual inspection. Thus, incubation tubes were examined each morning for egg viability and developmental state, and dead eggs were removed and counted.

In Experiment 2, eggs were collected from a low ration male–female pair and were held under a 12 h light : 12 h dark photoperiod after each daily 2 h exposure. Illumination was provided by two 30 W Vita-Lite (Duro-Test Canada, Inc.) fluorescent tubes placed 1.5 m from the eggs. The spectral output of these lamps includes some long-wave ultraviolet A (320 to 400 nm; UV-A); photorepair mechanisms should have been active under this light regime (Mitchell et al. 1993; Mitani et al. 1996). The photosynthetically active radiation (400 to 700 nm; PAR) output of these lamps was 0.03 W m^{-2}. All other aspects of the methodology were identical to the first experiment. Hereafter, this will be referred to as the with-photorepair experiment.

The next series of experiments investigated whether eggs at different developmental stages were differentially susceptible to UV-B-induced mortality. In Experiment 3, 6 d PF eggs (Late Stage 4, blastopore closed and first pigmentation), from the same female as for Experiment 2 (but a different egg batch), were exposed for 2 h d^{-1} for 3 d. In Experiment 4, 8 d PF eggs (Stage 5, first heart beats), from the same female as for Experiment 1, were exposed for 2 h d^{-1} for 2 d. Eggs from both of these experiments were kept in the dark in between exposures. In Experiment 5, 9 d PF eggs (Stage 6, just prior to hatching), from the same female as for Experiments 2 and 3, were exposed on only 1 d for 2 h. This group was kept in the light after the UV exposure.

Experiment 6 represents an attempt to evaluate whether UV-B-induced mortality in cod eggs is dose and/or dose rate dependent. If the principle of reciprocity is upheld, UV-B-induced mortality on cod eggs resulting from exposure to any given total dose will be the same regardless of the dose rate at which it was received (Smith and Cullen 1995). Stage 4 eggs (mid-gastrulation), collected from a high ration male–female pair, were used in this experiment. One group of eggs (A) was irradiated for 30 min on each of two consecutive days and received two different UV-B doses, a low dose of 23.6 ± 2.8 kJ m^{-2} and a high dose of 58.2 ± 14.3 kJ m^{-2}, at two different dose rates. A second group of eggs (B) was irradiated for 1 h on each of two consecutive days and received the same two UV-B doses but at half the dose rates delivered to Group A eggs. A third group (C) was irradiated for 2.5 h on each of two consecutive days and received the same two UV-B doses but at one-fifth the dose rates delivered to Group A eggs. Thus, there were two different total doses ("low" and "high"), each delivered at three different dose rates (Fig. 4). The different dose rates were obtained by placing combinations of quartz substrate neutral density filters (ND 0.3, 0.6 and 1.0), and cut-off filters (WG280 and WG320), in the optical paths of the SS. There were four tubes for each of the dose/dose rate combinations (a total of 24 tubes). In each of the exposures there were three control tubes: two covered by a GG400 cut-off filter (no UV-B or UV-A) and one sealed with a black stopper (dark). Eggs were maintained in oxygen-saturated seawater at 6 °C under a 12 h light : 12 h dark photoperiod in between exposures. Lighting was provided by 2 × 20 W daylight fluorescent tubes (General Electric Co.) placed 1.5 m from the eggs. Dead eggs were removed and counted each morning.

The null hypothesis that the mortality (on the experiment's last day) in Group A eggs was equal to that in Groups B and C, for both the low and high dose series, was tested by analysis of variance. Student's *t*-test was applied to test the null hypothesis that the overall mortality in the low dose treatment through Day 7 was not significantly different from that in the high dose treatment.

Derivation of the BWF for UV-induced mortality in cod eggs

The BWF for UV-induced cod egg mortality was derived following Rundel (1983) as modified and described by Cullen and Neale (1997). Only an overview of the derivation is presented here.

Data on the differential survival of cod eggs exposed to varying amounts of UV radiation were fit to a simple exponential model

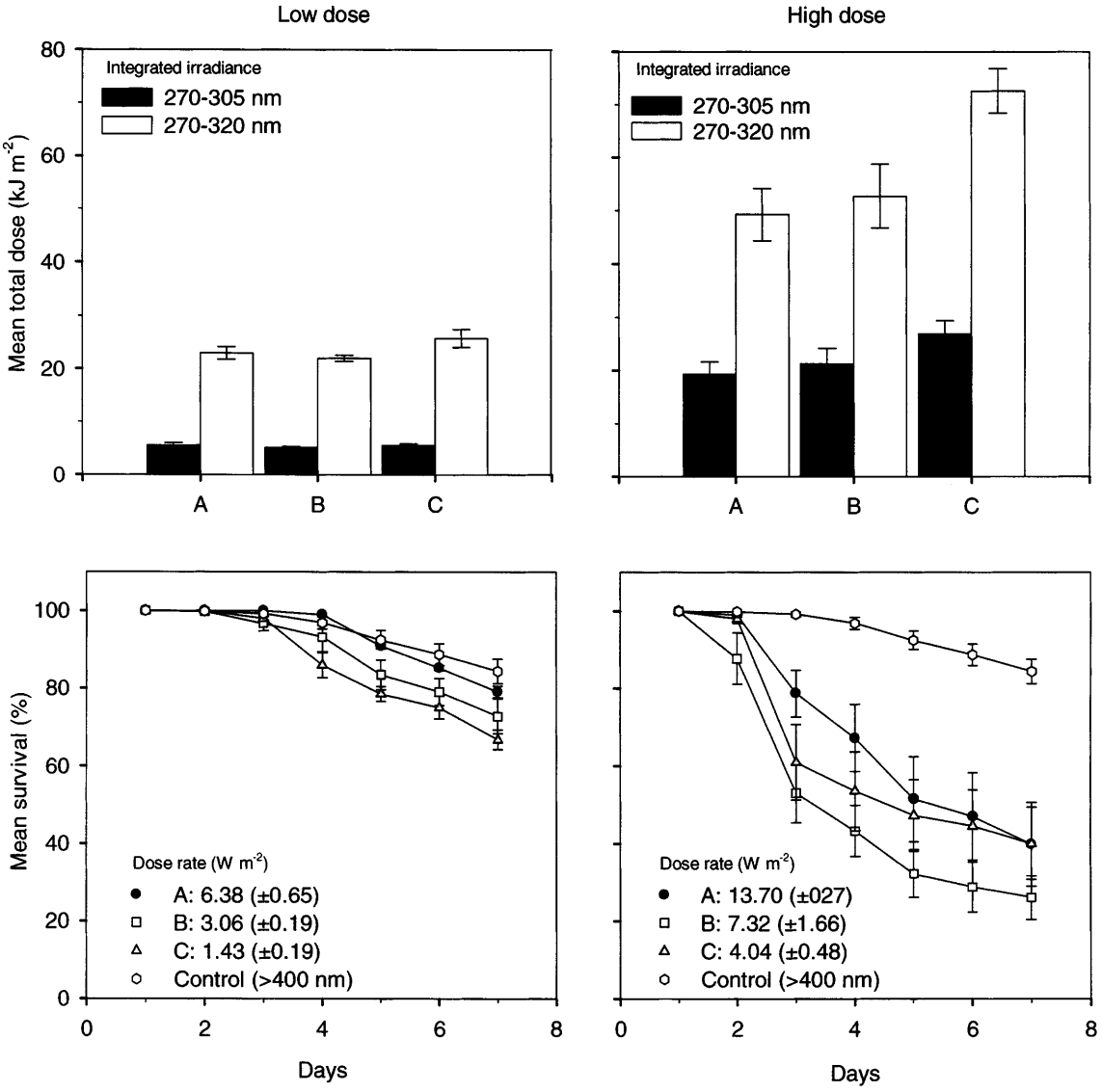

Fig. 4 *Upper panels* present the UV-B irradiance (integrated over wavelength and time), separated into two wavebands (270 to 305 and 270 to 320 nm), delivered to Atlantic cod (*Gadus morhua*) eggs during the reciprocity experiments. *Lower panels* present the mean survival of cod eggs after exposure to various dose rates of UV-B radiation, at low and high doses. Doses for Egg Group A were delivered in 1 h, for Egg Group B in 2 h, and for Egg Group C in 5 h. Means and standard error bars are plotted

using a least-squares minimizing subroutine from MatLab (The Mathworks Inc., Natick, Massachusetts, USA). The subroutine is the Gauss–Newton algorithm with Levenberg–Marquardt modifications for global convergence (Press et al. 1992). The final form of the model is:

$$\frac{\mathrm{Egg}(d)}{\mathrm{Egg}(0)} = \mathrm{e}^{-(H^*+M\cdot T)} \quad , \tag{1}$$

where Egg(0) is the number of live eggs in any one tube on Day 0, Egg(d) is the number of live eggs in that tube on Day d, H^* (dimensionless) is the sample's biologically weighted radiant exposure, M (s^{-1}) is a fitted parameter for non-irradiance dependent mortality and T (s) is the total length of the experiment.

Biologically weighted radiant exposure was calculated according to Rundel (1983) and Cullen and Neale (1997):

$$H^* = t_{\mathrm{uv}} \cdot \sum_{\lambda=250}^{800} \varepsilon_H(\lambda) \cdot E(\lambda) \cdot \Delta\lambda \quad , \tag{2}$$

where t_{uv} (s) is the total time of exposure to incident spectral irradiance, $\varepsilon_H(\lambda)$ (J $m^{-2})^{-1}$ is the biological weighting coefficient for radiant exposure and $E(\lambda)$ (J m^{-2} s^{-1} nm^{-1}) is the incident irradiance at wavelength λ (nm).

$\varepsilon_H(\lambda)$ was calculated according to:

$$\varepsilon_H(\lambda) = C \cdot \mathrm{e}^{\{-[m_1+m_2\cdot(\lambda-290)]\}} \quad , \tag{3}$$

where m_x are fitted parameters and C (J $m^{-2})^{-1}$ is a proportionality constant, here equal to one (Cullen and Neale 1997).

Data generated in Experiments 1 and 2 provided the number of eggs surviving in each of the 34 treatment tubes for each day of the experiment, the corresponding spectral irradiance (250 to 800 nm) for that tube's position (and cut-off filter) under the SS, and the duration of the daily exposure. To avoid including irrelevant data in the analysis (i.e., responses of 100% non-viable eggs to incremental radiation) the ratio of Egg(d)/Egg(0) was set to 0.001 (valid for inclusion in an exponential model) at the first occurrence of no more surviving eggs in any one tube. Thereafter, data for that tube were excluded from the analysis. The ratio Egg(d)/Egg(0) on Day 0 (i.e., 1.0) was not included in the analysis, since the model (Eq. 1) was forced through one.

The BWF analysis produces a weighting for each wavelength, constrained only by the exponential form of Eq. 3; it is not restricted to the UV-B waveband. Further, results from every tube – and not the mean of the four or five tubes from each spectral exposure treatment – are included in the analysis.

Results

Cod egg survival

In the without-photorepair experiment, cod eggs exposed to the shorter UV-B wavelengths (280, 295 and 305 nm cut-off filter treatments) exhibited rapidly declining survival (Fig. 5a). For example, in the 280 and 295 nm long-pass filter treatment groups only a small percentage of the eggs survived to the end of the experiment's fourth day. Thus, exposures in these two treatments were discontinued, although the tubes were still examined daily until 100% mortality: of the 104 eggs alive on Day 4, only five survived to hatching and these died at 2 d PH. In contrast, 65% (±6.8 SD) of the eggs in the 312 nm long-pass filter treatment group, and 76% (±11.6 SD) from the 335, 360 and 400 nm long-pass filter groups survived to hatching (Fig. 5a). There was no significant difference (Student's *t*-test, $p > 0.05$) in the survival of eggs exposed to both UV-A radiation and visible light (335 and 360 nm treatment groups) and those exposed to visible light only (>400 nm treatment group).

Survival was generally lower in cod eggs from the low ration female (Experiment 2) compared to eggs from the high ration female (Experiment 1) (Fig. 5a, b). Despite this, eggs exposed to the shorter UV-B wavelengths (280, 295 and 305 nm cut-off filter treatments), but with photorepair between exposures (Experiment 2), appeared to exhibit higher mean survival, and a lower mortality rate, than the without-photorepair groups (Fig. 5a, b). After 5 d of exposure to wavelengths above 280 and 295 nm, 12.6% of the eggs were still alive. Although this is 10.1% more than for the without-photorepair experiment, the two results were not statistically discernible (Student's *t*-test, $p > 0.05$). Exposures for the without-photorepair treatments were discontinued after 4 d; most of the surviving eggs died at Developmental Stage 6 (just before hatching). The 305 and 312 nm long-pass filter treatments exhibited mean egg survivals of 20.6 and 52%, respectively (Fig. 5b). The control groups in this experiment exhibited lower mean survival than those in the without-photorepair experiment.

To further assess the remediating effect of photoreactivation, the ratio of cod egg survival in each of the cut-off filter treatment groups compared to that in the 400 nm (no UV) treatment group was calculated for the with- and the without-photorepair experiments. For all of the short-wave UV-B-exposed treatments (280, 295 and 305 nm), these ratios were substantially higher for the with-photorepair experiment (Table 1). These ratios also illustrate the lack of a negative effect of UV-A on egg survival.

Replotted data from Experiments 1 and 2 show an inverse relationship between cumulative dose (integrated across wavelength to 800 nm) and survival in all treatment groups (Fig. 6). The decline in survival was most severe in the 280, 295 and 305 nm treatments. In these

Fig. 5 *Gadus morhua*. Mean survival of Atlantic cod eggs exposed to various spectral wavebands (those presented in Fig. 2). Each curve represents the mortality induced by exposure to radiation greater than the cut-off wavelength indicated. Open symbols denote treatments that received UV-A and visible light, or visible light only. Filled symbols denote treatments that received radiation in the UV-B+ UV-A+ visible wavebands. **a** Mean egg survival in the without-photorepair experiment. **b** Mean egg survival in the with-photorepair experiment. Mean values ± standard error bars

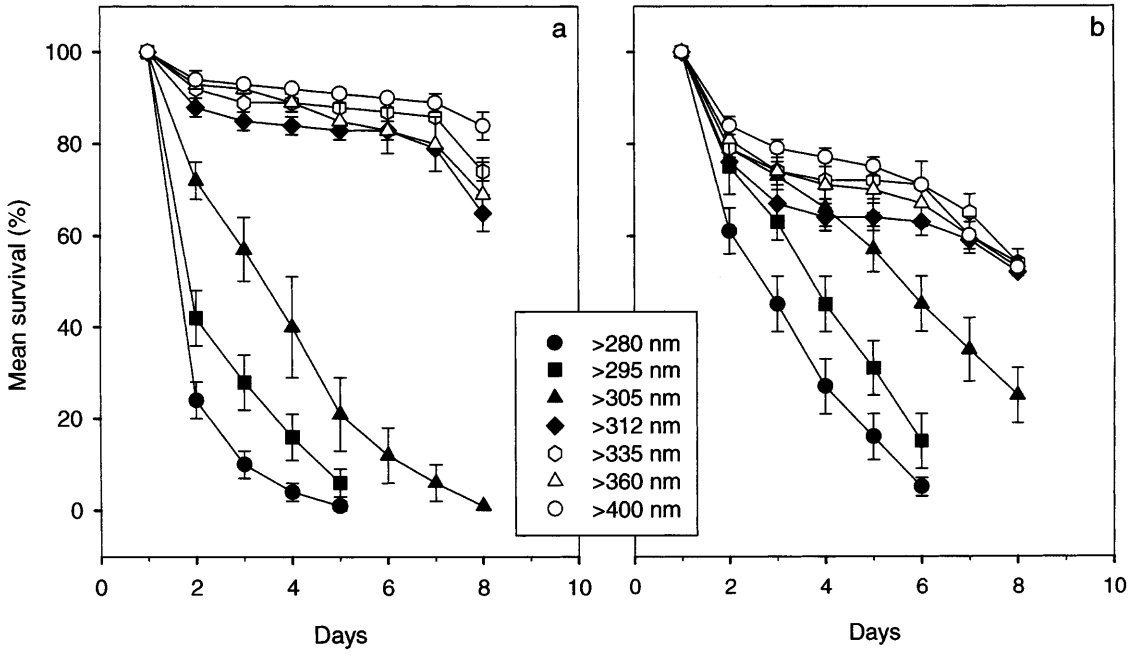

Table 1 *Gadus morhua*. Survivorship in Atlantic cod eggs from the various cut-off filter treatment groups in the with- and without-photorepair experiments. Data are expressed as a ratio of survivorship to Day 5 in each of the cut-off filter treatments compared to that in the >400 nm (no UV) treatment

Treatment group	With-photorepair (at Day 5, no UV = 75% survival) ratio	Without-photorepair (at Day 5, no UV = 91% survival) ratio
>280 nm	0.21	0.01
>295 nm	0.41	0.07
>305 nm	0.76	0.23
>312 nm	0.85	0.91
>335 nm	0.96	0.97
>360 nm	0.93	0.93

groups, survival of the eggs held in the dark in between exposures (without-photorepair) was consistently lower than those provided with photoreactivating light in between exposures (Fig. 6). This trend was reversed in the 312, 335, 360 and 400 nm treatment groups (Fig. 6).

Eggs first irradiated early in embryogenesis (early Stage 4 – from Experiment 1) were highly sensitive to UV-B – the 280 and 295 nm treatment groups exhibited low survival (Fig. 7a). Survival of eggs first irradiated at Stages 4 or 5 (from Experiments 3 and 4) was significantly higher in these same treatment groups (Student's *t*-test, $p < 0.05$) (Fig. 7b, c). Eggs first irradiated at Stage 6 (from Experiment 5) exhibited very low survival after only 2 h of exposure, and this despite having been held in the light immediately thereafter (Fig. 7d). Egg survival in the 360 and 400 nm treatment groups was consistently higher than in the UV-B-exposed groups in all experiments (Fig. 7a–d).

Hatching success and larval survival

In the UV-B-exposed treatment groups (280, 295 and 305 nm), survival to hatching of eggs first irradiated at early Stage 4 (Experiments 1 and 2) was low and larvae that hatched did not survive (Table 2). These indicators were higher for the 312 nm and the 335, 360 and 400 nm treatment groups, although larval survival through Day 6 PH was always ≤50% (Table 2).

Survival to hatching was better for eggs first exposed at Stage 4 and Stage 5 (Experiments 3 and 4), compared to eggs first exposed earlier in embryogenesis (Table 2). However, larval survival to Day 6 PH was still low in the UV-B treatment groups.

For eggs first exposed just prior to hatching (Experiment 5), both survival to hatching and larval survival to Day 6 PH were low in the UV-B treatment groups and higher in the 335, 360 and 400 nm groups (Table 2).

Reciprocity

Survival to 7 d from initial exposure was independent of dose rate (ANOVA, α was set at 0.05, and the *p*-values obtained were higher than this) for either the "low" or "high" dose series (Fig. 4). Survival was generally lower in the "high" dose exposures (Student's *t*-test, $p < 0.0005$), particularly as compared with the control groups (Fig. 4). Survival of larvae (to Day 6 PH) hatching from Groups A, B, and C was statistically indiscernible, although the values for larval survival were consistently higher in the control groups (Table 2).

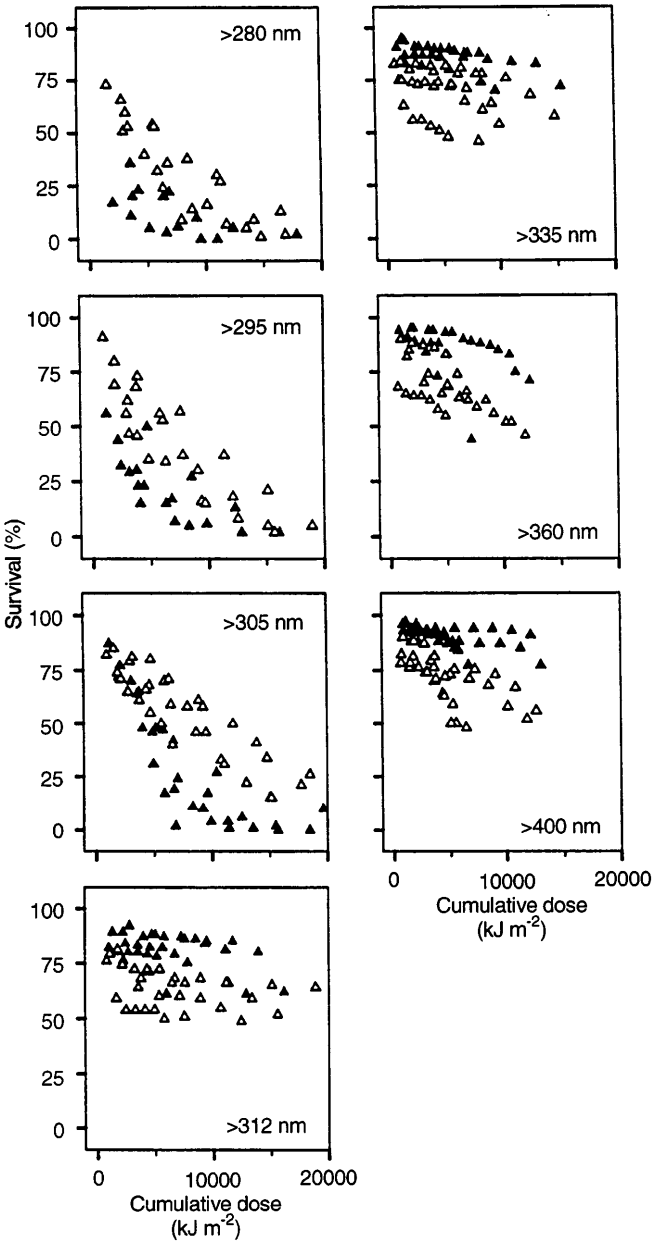

Fig. 6 *Gadus morhua*. Survival of Atlantic cod eggs as a function of cumulative dose (unweighted) delivered during the course of the 7 d without-photorepair and with-photorepair experiments. Each frame presents egg survival for a treatment group that received radiation above the wavelength specified. Open symbols denote results from the with-photorepair experiment, filled symbols results from the without-photorepair experiment. Cumulative doses were calculated by integrating the spectral irradiances, across wavelength, out to 800 nm

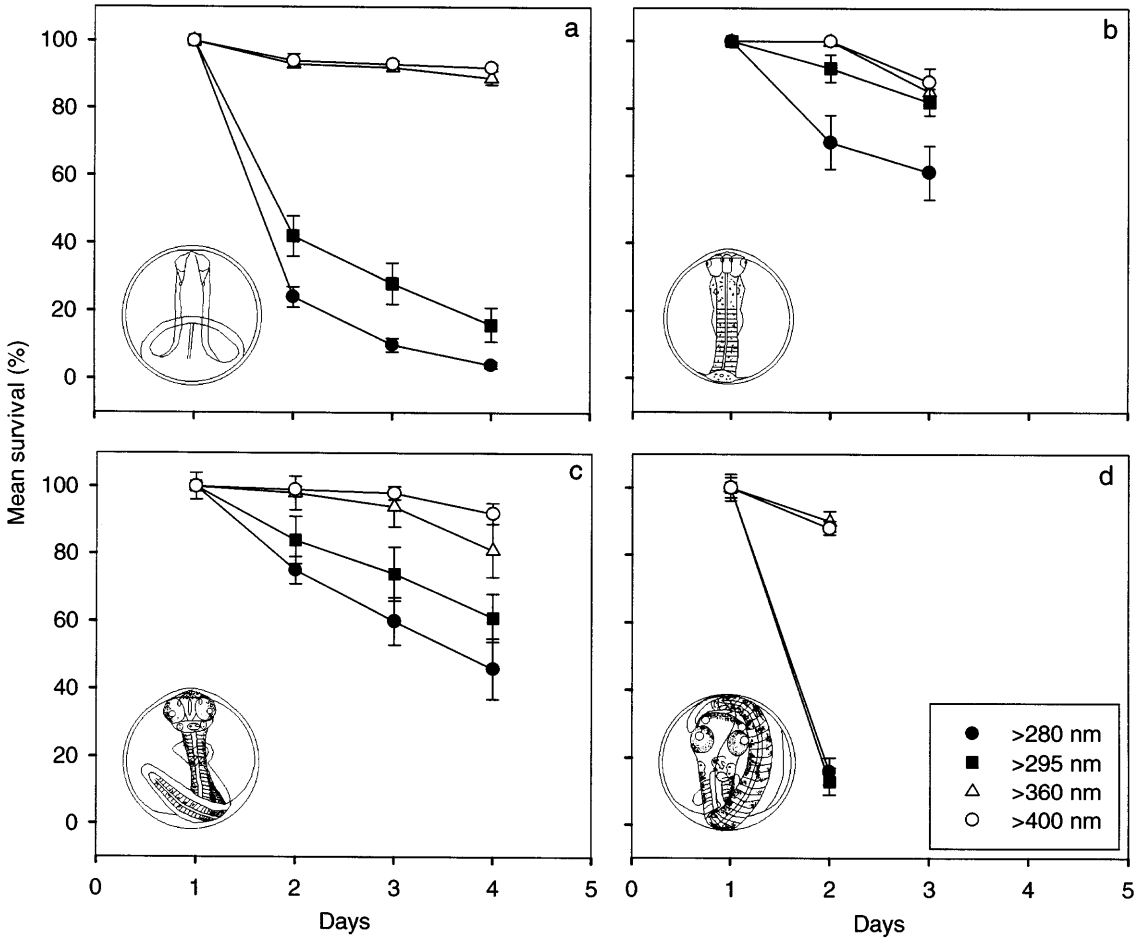

Fig. 7 *Gadus morhua*. Mean survival of Atlantic cod eggs exposed to various spectral wavebands. Each curve represents the mortality induced by exposure to radiation associated with the cut-off wavelength indicated (those presented in Fig. 2). Open symbols denote treatments that received UV-A and visible light, or visible light only. Filled symbols denote treatments that received radiation in the UV-B+ UV-A+ visible wavebands. **a** Data generated in Experiment 1: exposure began at 4 d post-fertilization (PF), Embryonic Stage 4 (mid-gastrulation). UV-B exposures were 2 h d^{-1} for the four days plotted, without photorepair in between. **b** Data generated in Experiment 3: exposure began at 6 d PF, Embryonic Stage 4 (blastopore closed). UV-B exposures were 2 h d^{-1} for 2 d without photorepair in between. **c** Data generated in Experiment 4: exposure began at 8 d PF, Embryonic Stage 5 (beating heart). UV-B exposures were 2 h d^{-1} for 3 d without photorepair in between. **d** Data generated in Experiment 5: exposure began at 9 d PF, Embryonic Stage 6 (hatching stage, chorion breaks up). UV-B exposure was 2 h on 1 d with photorepair afterwards. Mean values ± standard error bars

BWF for cod egg mortality

The BWF for UV-induced mortality in cod eggs exhibits a typically steep decline against wavelength: its impact is almost two orders of magnitude higher at 300 nm than at 320 nm (Fig. 8). Although only the BWF derived from the without-photorepair results is presented in Fig. 8, the BWF derived from the with-photorepair results has a similar shape (see coefficients in Table 3). Weightings in the UV-A waveband were essentially non-existent.

The principle of parsimony was applied in the BWF analysis: neither irradiance-independent mortality (M), nor a second-order term in the wavelength-dependent mortality (m_3), significantly improved the fits. Thus, they were not included in the final formulation (Eq. 3). Their exclusion is further justified in that M was very low, 6.74E-8 s^{-1} (Table 3), suggesting that ancillary mortality was insignificant. For completeness, we present the BWF parameters for the simplest form of the model, for the model with a mortality term, and for both the without-photorepair and the with-photorepair fits (Table 3). Comparison of the coefficients for these two BWFs reveals that the difference between them is approximately 10%.

Discussion

The work of Marinaro and Bernard (1966), Pommeranz (1974), and Hunter et al. (1979, 1981, 1982) provided clear evidence of the detrimental effect of UV-B on the planktonic early life stages of marine fishes. Hunter et al. (1979), working with northern anchovy (*Engraulis mordax*) and mackerel (*Scomber japonicus*) embryos and larvae, reported that exposure to surface levels of UV-B could be lethal. Significant sub-lethal effects were also reported: lesions in the brain and retina, and reduced

Table 2 Overview of all major parameters from the different exposure experiments on Atlantic cod (*Gadus morhua*) eggs (– not analyzed)

Experiment		Nutritional history of parents	Egg stage at first exposure	Photo-repair	Cut-off wavelength (nm)	Initial no. of eggs	Egg survival to hatching (% ± SD)	Larval survival to 6 d post-hatch (%)
No.	Type							
1	Cod egg survival	High ration	4 (mid-gastrulation)	No	280 + 295 + 305	4 092	few	0
					312	1 615	65 (± 7)	–
					335 + 360 + 400	5 289	76 (± 12)	–
2	Cod egg survival	Low ration	4 (mid-gastrulation)	Yes	280 + 295 + 305	12 487	12 (± 13)	0
					312	5 528	52 (± 4)	25
					335 + 360 + 400	13 124	54 (± 6)	50
3	Cod egg survival	Low ration	4 (blastophore closed)	No	280 + 295 + 305	7 428	54 (± 19)	0
					312	1 615	65 (± 7)	–
					335 + 360 + 400	5 289	76 (± 12)	–
4	Cod egg survival	High ration	5 (first heart beating)	No	280 + 295 + 305	2 494	76 (± 16)	0
					312	1 639	90 (± 7)	–
					335 + 360 + 400	1 735	87 (± 4)	80
5	Cod egg survival	Low ration	6 (just before hatching)	Yes	280 + 295	6 664	15 (± 9)	0
					305	4 623	21 (± 14)	0
					312	5 528	52 (± 4)	25
					335 + 360 + 400	13 124	54 (± 6)	50
6	Reciprocity (low dose series)	High ration	4 (mid-gastrulation)	Yes	280 + 295 + 305 + 312 + 320	7 420	73 (± 8)	20
					400 + dark	5 564	84 (± 10)	50
	Reciprocity (high dose series)	High ration	4 (mid-gastrulation)	Yes	280 + 295 + 305 + 312 + 320	5 683	35 (± 17)	20
					400 + dark	5 564	84 (± 10)	50

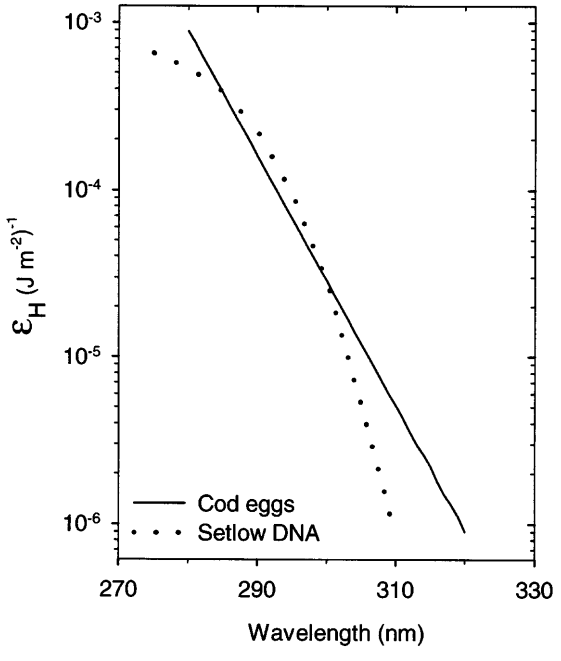

Fig. 8 Biological weighting function (BWF) for egg mortality in Atlantic cod (*Gadus morhua*) (*continuous line*) – derived from the without-photorepair mortality data – and wavelength-dependence of damage to the naked DNA molecule (data drawn from Setlow 1974) (*dotted line*). The Setlow curve has been normalized against the BWF's value at 300 nm for ease of comparison

growth rate. The study concluded that, under some conditions, 13% of the annual production of northern anchovy larvae could be lost as a result of UV-B related mortality (Hunter et al. 1981, 1982).

Since then, little additional information has been published on the lethal effects of UV-B on planktonic fish eggs and larvae (although see Malloy et al. 1997; Williamson et al. 1997; Vetter et al. 1999). The results presented here substantiate these earlier studies and provide the first BWF, and only the second assessment of the reciprocity principle, generated for a marine fish.

Cod egg survival

UV-B radiation, particularly in the 280 to 312 nm waveband, had a strong negative impact on the survival of Atlantic cod eggs (Fig. 5). This is consistent with observations on several other species (Marinaro and Bernard 1966; Pommeranz 1974; Hunter et al. 1982; Williamson et al. 1997), although these earlier studies do not provide the same spectral resolution. Mortality in the with-photorepair experiment was generally lower than in the without-photorepair experiment, at least for the three UV-B cut-off filter treatments (Fig. 5). This effect was consistent, regardless of dose-rate, across a broad range of cumulative doses, and despite the higher background mortality exhibited by the eggs from the low-ration female (Fig. 6). Since many aquatic organ-

Table 3 Parameter values for the biological weighting function fit to Atlantic cod (*Gadus morhua*) egg mortality data generated in the without-photorepair and the with-photorepair experiments (M^* was set to zero)

Parameter	Lower 95%	Fitted value no photrorepair	Upper 95%	Fitted value photorepair	r^2
Without irradiance-independent mortality					
m_1	8.670	8.740	8.810	9.590	
m_2	0.167	0.172	0.178	0.233	
M^*	0.000	0.000	0.000	0.000	0.938
With irradiance-independent mortality					
m_1	8.700	8.810	8.920	10.560	
m_2	0.160	0.168	0.177	0.121	
M	−1.35E-08	6.74E-08	1.48E-07	9.53E-07	0.938

isms possess mechanisms of DNA repair that are tied to photon absorption (Mitchell and Karentz 1993; Mitchell et al. 1993; Naganuma et al. 1997; Zagarese et al. 1997), we interpret this as evidence for the presence of photorepair mechanisms in cod eggs. This assertion is supported by the observation that cod egg survival in the UV-B-exposed treatments, as a ratio of that in the group not exposed to any UV, was much higher in the with-photorepair experiment (Table 1). Kaup and Hunter (1981) reported similar remediating effects of photorepair on UV-B-induced mortality in northern anchovy larvae. More recently, Mitchell et al. (1993) reported on DNA photorepair in UV-B-exposed platyfish (*Xiphophorus variatus*) and Mitani et al. (1996) observed that exposure to UV-A and blue light induced the production of cyclobutane pyrimidine dimer photolyase (involved in the repair of UV-B-induced DNA damage) in cultured cells of the goldfish (*Carassius auratus*).

The UV-B mortality effect was related to the stage of embryonic development at which irradiation began (Fig. 7). Despite inconsistencies in the total dose delivered in Experiments 2 through 5, and the differing male–female pairs from which the eggs were obtained, embryos appeared to be more susceptible to UV-B exposure during gastrulation (early to late Stage 4) than during later stages (Fig. 7a). Once gastrulation is complete, and the blastopore is closed, the eggs appeared to be more resistant to UV-B (Fig. 7b, c). Embryos just prior to hatching also appeared to be more susceptible to UV-B (Fig. 7d). These observations are consistent with the results reported by Strähle and Jesuthasan (1993), who described how UV-C (250 to 280 nm) exposure impaired epiboly in zebrafish embryos, and with the data generated by Hyodo-Taguchi (1982), who observed that mortality in Japanese medaka (*Oryzias latipes*) eggs irradiated with UV-B was related to the developmental stage at which they were exposed. Further, pigmentation in cod embryos begins to appear shortly after gastrulation (Fridgeirsson 1978), which may reduce their susceptibility to UV-B.

The effect of cumulative UV-B dose on cod egg mortality (Fig. 6) is similar to that reported for northern anchovy (Hunter et al. 1979). At the shorter wavelengths (< 305 nm) UV-B-induced mortality was strongly dose-dependent. Survival of eggs from the without-photorepair experiment was consistently lower than that for eggs from the with-photorepair experiment, at least for the 280, 295 and 305 nm treatment groups. This trend was reversed in the 312, 335, 360 and 400 nm groups (Fig. 6). There are several possible explanations for this. One is that the reversal reflects nothing more than the poorer quality of eggs produced by the low ration female. An alternative interpretation is that there was a detrimental effect (albeit relatively mild) of longer term exposure to the fluorescent lights under which the eggs were incubated in between UV-B exposures. The spectral output of the fluorescent lights under which the cod eggs were incubated in the with-photorepair experiment includes some long-wave UV-A radiation, and it is possible, therefore, that day-long exposure to UV-A is deleterious to cod embryos. However, a visual comparison of the 335 and 360 nm treatment groups with the 400 nm treatment group (Fig. 6) indicates that UV-A alone was not responsible. This is further supported by the observation that cod egg survival in the UV-A-exposed treatments was similar to that in the group not exposed to any UV, and that these ratios were identical in both the with- and the without-photorepair experiments (Table 1). Nonetheless, several recent studies report on negative effects of UV-A: hatching success in the Japanese medaka is negatively affected by UV-A exposure (Bass and Sistrun 1997); long exposures to UV-A radiation had adverse effects on the metabolic performance and survival of the convict cichlid (*Cichlasoma nigrofasciatum*) (Winckler and Fidhiany 1996a, b); in situ exposure to UV-A radiation induced elevated mortality in yellow perch (*Perca flavescens*) eggs (Williamson et al. 1997); UV-A exposure produced a discernible mortality effect, and delayed development, in Atlantic cod eggs (R.D. Vetter, personal communication). Further experiments on the effects of UV-A and visible light – both in inducing mortality and with respect to the balance between photodamage and photorepair – are required to resolve these issues.

Hatching success and larval survival

Mortality in the egg groups that were not exposed to UV radiation was highly variable (Table 2), but this is typical of many marine fishes (Sinclair 1988). Further, egg mortality in the rearing basins – in which the source egg populations were maintained throughout these

experiments – was similar to that for the control groups (unpublished data). The percentage of larvae surviving to Day 6 PH was generally twice as high in the control groups as in the 312 nm treatment groups (Table 2). This indicates that longer wavelength UV-B exposure can produce sub-lethal effects that are only mortal some time after irradiation. This conclusion is consistent with the observation that northern anchovy larvae exposed to UV-B radiation exhibit lower growth rates than non-exposed control groups (Hunter et al. 1979, 1981). Similar results have been reported for amphibians (Blaustein et al. 1997).

Reciprocity

One of the more important fundamental assumptions for construction of an accurate dose-dependent BWF is the principle of reciprocity (de Gruijl et al. 1986; Coohill 1991; Cullen and Neale 1997; Buma et al. 1997). In the context of a UV-B exposure experiment, reciprocity holds if the effect of cumulative dose is the same regardless of the dose rate at which it was delivered. If reciprocity fails, a short intense exposure would result in a different effect than a long weak exposure to the same cumulative dose. In this latter case, evaluations of effect versus cumulative exposure (i.e. dose-dependence) cannot be applied outside the conditions (i.e. time scales) under which they were generated, and BWFs derived from such results would be less reliable and of more limited use.

There was no discernible difference in the UV-B-induced mortality of cod eggs under two cumulative doses delivered at six dose rates (Fig. 4): reciprocity holds. These radiative conditions were the same as those delivered in the experiments used to derive the BWF. Further, reciprocity held despite the fact that the eggs were incubated under fluorescent lamps in between UV exposures; i.e., they were allowed to photorepair. This indicates that the conditions under which the data used to derive the BWFs were generated were ecologically relevant and that the weightings can be applied to realistic exposure scenarios.

To our knowledge, Hunter et al. (1981, 1982) present the only other assessment of the reciprocity principle for a marine fish. For northern anchovy larvae, and under relatively broad dose/dose rate exposures, reciprocity did not hold. The reasons for this inconsistency are unknown. However, one possibility is the difference in the relative duration of intense UV-B exposures versus the time for repair. To the extent that repair dominates damage, reciprocity fails. When damage dominates, repair processes will not significantly compromise reciprocity. It is possible that the experiments reported here were generally consistent with reciprocity because the exposure durations were relatively short – and so damage was dominant – while those of Hunter et al. (1981, 1982) were longer and less intense – and so repair was dominant.

BWF for cod egg mortality

Hunter et al. (1981) related weighted UV-B exposure to the survival of northern anchovy eggs and larvae using several UV-B action spectra. They found that survival was best predicted when the UV-B exposure was weighted by the Setlow (1974) DNA action spectrum. This represents the first attempt to apply a BWF to UV-B-induced mortality in ichthyoplankton. BWFs such as those used by Hunter et al. (1981) yield only relative predictions – they tell us how much more (or less) mortality there will be for one spectral exposure versus another. The BWF reported here for cod eggs was derived from the mortality response itself, as opposed to being chosen as the best predictor of relative mortality. Consequently, the weightings are in absolute units $(J\ m^{-2})^{-1}$ and, thus, egg mortality (in absolute terms) resulting from any given exposure can be predicted using a modified form of Eqs. 1 and 2 (see below). This is the first such BWF generated for ichthyoplankton.

The wavelength-specific sensitivity of UV-induced mortality in cod eggs, as defined in the BWF (Fig. 8), exhibits virtually the same slope as the DNA action spectrum through 310 nm (Setlow 1974). This is consistent with the analysis of Hunter et al. (1981), who found that UV-B-induced mortality in northern anchovy was best fit to the DNA action spectrum. Following from this, it seems likely that UV-B-induced mortality in cod embryos results from DNA damage. There was no relative effect outside of the UV-B waveband; i.e., there was no UV-A effect.

Two BWFs are provided for cod embryos. One was generated for the primary "gross" effect of UV (the "pure" effect, without photorepair) and one for the "net" effect (with photorepair). The BWF coefficients for both cases are presented in Table 3. Comparison of these coefficients reveals that the difference between them is approximately 10%. While this will certainly make some difference in calculations based upon these coefficients, that difference is actually comfortingly small. The BWF for the "gross" effect of UV radiation can be considered a worst case scenario; that for the "net" effect would be more ecologically realistic. Nonetheless, the difference is only 10%, an error level which is very low in the context of ecological studies on ichthyoplankton.

Ecological context

A number of factors make it difficult to predict the biological effect of UV-B radiation on aquatic organisms. For example, (1) the spectral composition and intensity of light reaching the Earth's surface are highly variable, being affected by weather conditions, the thickness of the ozone layer, and air pollution, among other things (Varotsos et al. 1994; Graedel and Crutzen 1995; Madronich et al. 1995; Németh et al. 1996). This variability has daily, seasonal and annual scales. (2) The underwater light field

is further affected by the wavelength-specific diffuse attenuation coefficients of water bodies, themselves highly variable, geographically, seasonally and annually (Piazena and Häder 1994; Laurion et al. 1997). (3) Photon absorption by the DNA molecule, by proteins, by tissues and by whole organisms, is strongly wavelength dependent, dropping off steeply above 300 nm (see the UV-B action spectra reported by Setlow 1974; Coohill 1991; Cullen and Neale 1997, among others). Since the biological effectiveness of UV photons is inversely related to wavelength, and short-wave photons are strongly absorbed by organic molecules and seawater, relatively small changes in UV-B irradiance can lead to large changes in biological effect. (4) Ozone layer depletion will not affect the entire UV-B waveband equally. Rather, increases in UV-B associated with a thinning ozone layer will be mainly restricted to the 295 to 312 nm waveband: the most damaging wavelengths (Kerr and McElroy 1993; Graedel and Crutzen 1995; Madronich et al. 1995). Following from this, any attempt to assess the impact of UV-B radiation on planktonic marine organisms requires that the wavelength-dependent biological effect of UV-B photons be known. That is, a relevant BWF – like that presented here for cod egg mortality – must be available (see Cullen and Neale 1997 for a more complete presentation of this issue).

Biologically weighted radiant exposure – H^* (Eq. 2) – is obtained by combining spectral irradiance and exposure time with the BWF for cod egg mortality. Using several spectra of solar irradiance – for a late summer day in the air and the same day after 20% ozone depletion (Fig. 9, Curves A and C, respectively) – allows a visual and quantitative comparison of the biologically effective irradiance that would be produced under each of these conditions (compare Curves B and D in Fig. 9). The salient feature of this graph is the large increase in biologically effective irradiance that results from only a small change in UV-B irradiance.

A more ecologically meaningful analysis is possible from Eq. 1 which, since the second-order mortality term (M) is zero, reduces to

$$\frac{\mathrm{Egg}(d)}{\mathrm{Egg}(0)} = \mathrm{e}^{-H^*} \quad . \tag{4}$$

Under each of the above spectral irradiance conditions, the H^* that yields, for example, $\mathrm{Egg}(d)/\mathrm{Egg}(0) = 0.5$, is calculated. This value then allows Eq. 2 to be solved for t_{uv}, the exposure time resulting in 50% egg mortality. Under current noon surface irradiance, 50% of cod eggs located at the ocean surface (0 to 10 cm) will be dead after 42 h of exposure. Under solar spectral irradiance simulating a 20% decrease in ozone layer thickness, this time drops to 32 h for eggs at the surface. These are, of course, first-order estimates based upon surface irradiance taken at a time of day during which the values would be maximal. Nonetheless, they illustrate the relative changes in UV-B impacts that will result from ozone layer depletions expected over the coming decades (Kerr and McElroy 1993; Graedel and Crutzen 1995).

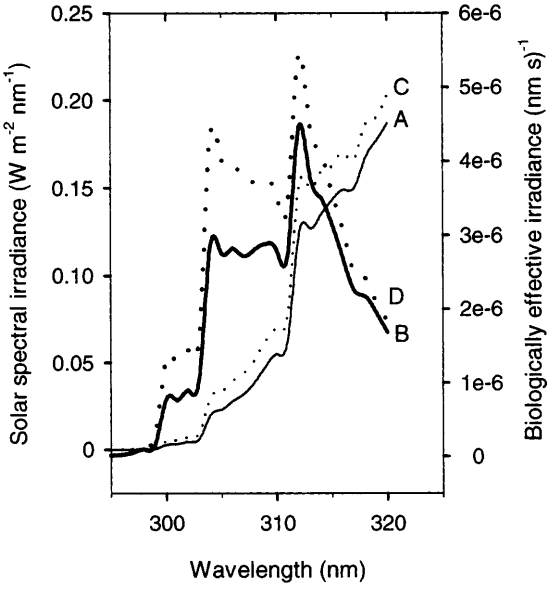

Fig. 9 An illustration of how the biological weighting function (BWF) for Atlantic cod (*Gadus morhua*) egg mortality can be combined with measurements of spectral irradiance to estimate biologically effective irradiance at each wavelength [*A* solar spectral irradiance measured outside the Maurice-Lamontagne Institute, Mont-Joli, Québec, Canada (48°38′25.9″N; 68°09′21.0″W) on 5 August 1996 under cloudless skies; *B* biologically effective irradiance under the spectral conditions illustrated in A; *C* solar spectral irradiance for 5 August 1996 corrected for a 20% ozone depletion (using a 50 level delta-Eddington radiative transfer model, Davies et al. 1999); *D* biologically effective irradiance under the spectral conditions illustrated in C]

The depth at which eggs are suspended in the water column – and the diffuse attenuation coefficient for UV-B wavelengths exhibited by the water – will also affect their susceptibility to UV-B. We calculated (as above) that 50% of cod eggs incubated under 50 cm of water from the maritime estuary of the St. Lawrence River would be dead after 83 h of exposure to the noon-time sun – double the time calculated for the surface. In an outdoor exposure experiment at MLI during August 1996, approximately 60% of cod eggs died after 4 d of incubation in quartz tubes held at 5 cm depth (Béland et al. 1999). In a similar exposure experiment, approximately 90% of yellow perch eggs were dead after 6 d (Williamson et al. 1997). These values are of the same order of magnitude as the above estimates, indicating that the calculations are at least reasonably realistic.

Indirect effects of UV-B on ichthyoplankton are also possible. UV-B radiation can be a potent immunosuppressive agent in adult fishes (Salo et al. 1998); it is not yet known whether this is also true for embryos or larvae. For species that spawn in the surface layer, UV-B may affect sperm quality (Don and Avtalion 1993; Valcarcel et al. 1994). There is also the potential effect of UV-B on food availability – the protozoan and zooplankton prey of fish larvae are also negatively affected by UV-B (e.g. Karanas et al. 1981; Williamson et al. 1994; Chalker-Scott 1995; Häder et al. 1995). Further,

food quality might be negatively impacted by UV-B. For example, UV-B exposure reduces the omega-3 fatty acid content of some microalgae (Wang and Chai 1994). Fish larvae require these fatty acids in their diets and obtain them from the zooplankton that they ingest – zooplankton which themselves take up these fatty acids from the microalgae upon which they graze. One of the microalgal fatty acids which is negatively influenced by UV-B exposure – docosahexaenoic acid (Wang and Chai 1994) – also appears to be important for normal visual function in fish larvae (Bell et al. 1995). All such indirect effects have yet to be evaluated.

This study represents a first step in generating an ability to predict the ecological significance of UV-B radiation on cod early life stages in the Gulf of St. Lawrence. A more rigorous and realistic quantitative assessment of direct UV-B effects on the population dynamics of marine ichthyoplankton requires additional data on the vertical distribution of eggs in the mixed layer of the water column (with a greater resolution in the upper 10 m than currently exists), surface UV-B irradiance during the spawning period, and subsurface spectral irradiance for waters supporting such eggs. A model to predict the vertical position of passive particles (such as eggs) in the mixed layer, and particularly their daily residence time near the surface under various weather conditions, is also necessary. All of these components would have to be incorporated into a simulation model that could then provide an assessment of UV-B effects on a population of eggs distributed throughout the mixed layer (e.g. Neale et al. 1998). We are currently developing such a model.

It is also important to point out that variability in cloud cover, water quality, and vertical distribution and displacement within the mixed layer, can all have a greater effect on the flux of UV-B radiation to which fish eggs are exposed than will ozone layer depletion at these latitudes. Given the vertical distribution of cod eggs, it is unlikely that the majority of eggs released during a spawning event will reach the upper 1 to 4 m of the water column. For other species, whose eggs are routinely found close to the surface, the scenario might be different. Thus, although UV-B radiation can have negative impacts on ichthyoplankton populations, it must be viewed as only one amongst many environmental factors that produce the very high levels of mortality typically observed in the planktonic early life stages of marine fishes.

Acknowledgements We are grateful for the assistance of the following friends and colleagues: C. Alonso Rodriguez, F. Béland, M. Bélanger, R. Gagnon, C. Hazzi, P. Joly, P. Kuhn, B. Nieke and H. ten Napel. M. Castonguay, P. Kuhn, I. Novales. Flamarique and P. Ouellet provided helpful comments on earlier versions of the manuscript. Suggestions made by C. Williamson and M. Arts, who reviewed the submitted manuscript, significantly improved the clarity of the presentation. The biological impact components of this project were supported by grants in aid of research from the Department of Fisheries and Oceans Canada, the St. Lawrence Vision 2000 Program, and the Natural Sciences and Engineering Research Council of Canada (NSERC) to H.I. Browman and J.A. Runge. The optical and meteorological components were supported from these sources and also by grants in aid of research from Environment Canada's Atmospheric Environment Program to B. McArthur. J. Kouwenberg was supported by a visiting postdoctoral fellowship to the Department of Fisheries and Oceans Canada's Maurice-Lamontagne Institute. J.J. Cullen and R. Davis were supported by the NSERC Research Partnership Program.

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