Effect of solar ultraviolet radiation (280–400 nm) on the eggs and larvae of Atlantic cod (*Gadus morhua*)

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Abstract: In the Gulf of St. Lawrence, Canada, solar ultraviolet B radiation (UV-B, 280–320 nm) penetrates a significant percentage of the summer mixed-layer water column: organisms residing in this layer, such as the eggs of Atlantic cod (*Gadus morhua*), are exposed to UV-B. In outdoor exposure experiments, Atlantic cod eggs were incubated in the presence versus the absence of UV-B and (or) UV-A (320–400 nm). We tested two hypotheses: H_1, UV-B induces mortality in Atlantic cod eggs, and H_2, UV-A either exacerbates or mitigates any such UV-B-induced mortality. Hypothesis H_1 was supported: there was a significant mortality effect on Atlantic cod eggs exposed to UV-B at the surface and at a depth of 50 cm. Hypothesis H_2 was not supported: there was no effect of UV-A. These experiments indicate that Atlantic cod eggs present in the first metre of the water column (likely only a small percentage of the total egg population) are susceptible to UV-B. However, UV-B must be viewed as only one among many environmental factors that produce the very high levels of mortality typically observed in the planktonic early life stages of marine fishes.

Résumé : Dans le golfe du Saint-Laurent (Canada), le rayonnement solaire ultraviolet B (UV-B, 280–320 nm) pénètre une partie importante de la couche de mélange estivale. Les organismes présents dans cette partie, comme les oeufs de morue franche (*Gadus morhua*), se trouvent exposés aux UV-B. Dans des essais extérieurs d'exposition, des oeufs de morue franche ont été incubés en étant exposés ou non aux UV-B et (ou) aux UV-A (320–400 nm). Nous avons testé deux hypothèses : H_1, les UV-B entraînent une mortalité chez les oeufs de morue franche, et H_2, les UV-A accentuent ou atténuent l'éventuel effet létal des UV-B sur les oeufs. L'hypothèse H_1 a été corroborée : on a observé une mortalité significative des oeufs exposés aux UV-B à la surface et à une profondeur de 50 cm. L'hypothèse H_2 a été rejetée : les UV-A n'avaient pas d'effet. Ces expériences montrent que les oeufs de morue franche présents dans le premier mètre de la colonne d'eau (qui ne constituent probablement qu'un faible pourcentage de la population totale d'oeufs) sont sensibles aux UV-B. Cependant, les UV-B ne sont qu'un des nombreux facteurs environnementaux responsables des très forts taux de mortalité habituellement observés chez les premiers stades planctoniques des poissons marins.

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Introduction

The work of Marinaro and Bernard (1966), Pommeranz (1974), and Hunter et al. (1979, 1982) provided clear evidence of the detrimental effect of solar ultraviolet B radia-

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tion (UV-B, 280–320 nm) on the planktonic early life stages of fishes. Hunter et al. (1979), working with the eggs and larvae of northern anchovy (*Engraulis mordax*) and chub mackerel (*Scomber japonicus*), reported that exposure to surface levels of UV-B could be lethal. Significant sublethal effects were also reported: lesions in the brain and retina and reduced growth rate. The study concluded that up to 13% of the production of northern anchovy larvae could be lost as a result of UV-B-related mortality (Hunter et al. 1982). In the time since these results were published, very little additional information has been generated for the effects of UV-B on ichthyoplankton (but see Malloy et al. 1997; Williamson et al. 1997; Vetter et al. 1999). The goal of this study was to evaluate the effects of UV (280–400 nm) on the eggs and larvae of Atlantic cod (*Gadus morhua*).

Since 1981, levels of solar UV-B incident at the earth's surface have increased significantly at midlatitudes of the Northern and Southern hemispheres (Kerr and McElroy 1993). These increases in UV-B are linked to reductions of stratospheric ozone (Kerr and McElroy 1993). A growing number of studies indicate that UV-B, at current levels, is

harmful to aquatic organisms and may reduce the productivity of marine ecosystems (Häder et al. 1995; Häder 1997). Such UV-B-induced reductions in productivity have been reported for phytoplankton, heterotrophs, and zooplankton, the key intermediary levels of marine food chains (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, which may lead to poorer recruitment to adult populations (Pommeranz 1974; Hunter et al. 1982; Williamson et al. 1997).

The few analagous studies on UV-A (320–400 nm) have yielded mixed results. While the role of UV-A in inducing photorepair of DNA damage is well documented (e.g., Mitani et al. 1996), only a small number of studies have reported deleterious effects (e.g., Winckler and Fidhiany 1996a, 1996b; Bass and Sistrun 1997; Williamson et al. 1997).

In early summer, the mixed layer in the northern Gulf of St. Lawrence and Labrador Shelf comprises the upper 0–15 m of the water column and is separated from an intermediate layer of colder water (<1°C) by a sharp thermocline (Petrie et al. 1988; Therriault 1991). This underlying cold water, and the steep density gradient associated with it, effectively confines biological productivity to the much warmer surface mixed layer (Therriault 1991). Measurements of the diffuse attenuation coefficients for UV-B at various locations in the Gulf of St. Lawrence indicate maximum 10% depths (the depth to which 10% of the surface energy penetrates at a given wavelength) of 3–4 m at a wavelength of 310 nm (Kuhn et al. 1999). UV-A penetrates these waters to even greater depths: 10% depths of 7–10 m were observed at a wavelength of 360 nm (Kuhn et al. 1999). These depths represent a significant percentage of the summer mixed-layer water column. 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; Vetter et al. 1999). Thus, the early life history stages of the crustacean and fish species that are present in the shallow mixed layer of the water column in this geographic region may be susceptible to UV-B.

The reproductive season for Atlantic cod in the Gulf of St. Lawrence begins early in the spring (April) and continues through midsummer (July) (Ouellet et al. 1997). Spawning occurs in deep water (>200 m), and Atlantic cod eggs, which are typically positively buoyant, ascend to the surface mixed layer over a period of 2–10 days (Solemdal and Sundby 1981; Anderson and de Young 1995; Ouellet 1997). A significant proportion of all Atlantic 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, when wind speed is low, the highest egg concentrations are observed in the upper 0–10 m of the water column (Solemdal and Sundby 1981). The early larval stages are also typically present and often even closer to the surface (Anderson and de Young 1995).

Following from the hydrographics of this region, and given the planktonic character of the early life stages of Atlantic cod and many other fish and crustacean species, this system offers an appropriate opportunity to assess the impacts of UV on temperate-latitude marine ecosystems. As one step towards meeting this goal, we undertook a series of experiments in which Atlantic cod eggs were incubated under the sun, with and without the UV-B and (or) UV-A wavebands, in outdoor exposures. We tested two hypotheses: H_1, UV-B has a negative impact on Atlantic cod egg survival, and H_2, UV-A either exacerbates or mitigates any such UV-B-induced mortality. The results of these experiments are reported here.

Materials and methods

Animal husbandry
Fertilized Atlantic cod eggs were obtained from a broodstock maintained at the Maurice Lamontagne Institute. Male–female pairs were kept in separate tanks outfitted with egg collectors (see Dutil et al. (1998) for complete details). Thus, the source of the eggs used in the UV-B exposure experiments was known.

Egg production by these fish occurred several weeks later into the spring–summer than is the case for wild populations in this geographic region. Thus, in our August and September experiments, Atlantic cod eggs were exposed to solar UV-B slightly later in the season than they would be in the wild. However, daily exposure to UV-B was similar in all experiments (with the possible exception of that on 2–12 September) (see Fig. 3a). This strongly supports the validity of our results within a realistic ecological framework for the Atlantic cod spawning cycle in this geographic region.

Fertilized eggs were incubated in the dark at 6 ± 1°C in 60-L black round-bottom basins (the indoor rearing basins). Eggs were removed from these basins immediately prior to an exposure experiment. To minimize the risk of bacterial contamination, newly collected eggs were disinfected by immersion in a solution of 1% Aquiodyne (Aquipro, St-Apollinaire, Que.) after which they were thoroughly rinsed in seawater. Further, at the time the eggs were transferred to the incubation tanks, they were treated with an antibiotic solution (0.075 g streptomycin·L^{-1} and 0.01 g penicillin·L^{-1}). The bottom of the basin was cleaned and the seawater renewed each day.

General protocols and experimental design
Exposures to UV-B commenced about 4 days postfertilization, since Atlantic cod eggs are released at depth and will not be exposed to UV-B until they reach the surface layer several days later. The time required for Atlantic cod eggs to reach the surface is highly variable; therefore, the choice of a 4-day delay in beginning the exposures was arbitrary. At this point in development, embryos had reached stages 14–18. All developmental stage numbers referred to in the text are those described by Friðgeirsson (1978). This index of development is nonlinear; there are more discrete stages early in embryonic development than later. Thus, stage 14–18 embryos have not reached the halfway point of the embryonic period (which consists of a total of 32 stages).

Eggs were removed from the indoor rearing basins using a glass pipette (disinfected prior to each use) and placed into quartz tubes 10 cm long with an inner diameter of 2.2 cm (Quartz Scientific, Fairport Harbour, Ohio). One end of these tubes was sealed with a rubber stopper that had a small central hole. The hole was covered with 470-μm Nitex^{TM}. The other end of the tube was sealed with Nitex^{TM}. This arrangement allowed water to circulate in the tubes during the exposure experiments: removal of the rubber stopper

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Fig. 1. (*a*) Irradiance delivered to the Quartz, Mylar, and OP-2 spectral exposure treatments under which Atlantic cod eggs were incubated in an outdoor reservoir. Also presented is spectral irradiance measured just below the surface and at 50 cm depth in the outdoor reservoir under cloudless skies on 5 August 1996 at 13:05 EDT outside the Maurice Lamontagne Institute, Mont-Joli, Qué., Canada (48°38′25.9″N, 68°09′21.0″W). (*b*) Spectral irradiance (in the UV) at the surface and at 50 cm depth in the outdoor reservoir (from Fig. 1*a*). Note that irradiance is now plotted on a common log scale and that the units have changed from mW to W.
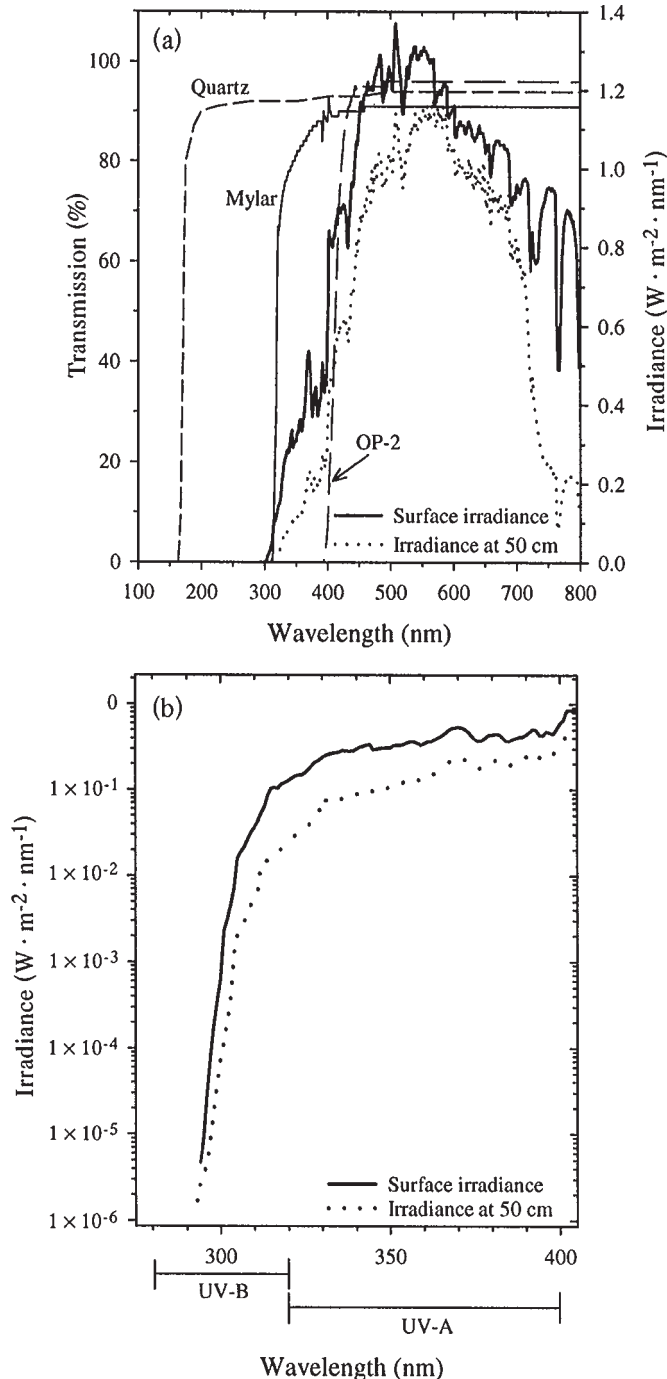
permitted access to the contents. About 100 eggs (range 90–141) were placed into each quartz tube.

The quartz tubes filled with Atlantic cod eggs were immersed in a seawater reservoir (the outdoor reservoir) located on the grounds of the Maurice Lamontagne Institute (48°38′25.9″N, 68°09′21.0″W). The reservoir was made of gray double-walled polyethylene and contained 1.08 m^3 of finely filtered seawater (renewed each hour) drawn from the St. Lawrence estuary. The water intake pipe at the Maurice Lamontagne Institute, and the source of water for the experimental reservoir, is located 100 m offshore at a depth of 15 m. The physical characteristics of the water at this location are unstable: they are affected by the tides and by freshwater runoff from nearby streams. Thus, the salinity and temperature in the reservoir were variable.

Five experiments were conducted: the first between 10 and 20 July 1996, the second between 27 July and 6 August 1996, the fourth between 19 and 29 August 1996, and the fifth between 2 and 12 September 1996. The results of the third experiment in the series are not presented because all of the eggs, in all of the treatments, died soon after being placed in the outdoor reservoir.

Tubes were suspended underwater, at depths of 3 and 50 cm, under three light regimes as follows. (*i*) UV-B + UV-A + PAR (photosynthetically active radiation, 400–700 nm): eggs in this treatment (Quartz), incubated in quartz tubes, were exposed to the complete solar spectrum. (*ii*) UV-A + PAR: in this treatment (Mylar), UV-B was excluded by wrapping the quartz tubes with Dupont 0.05-mm-thick type D Mylar®. (*iii*) PAR only (OP-2): UV-A and UV-B were eliminated by placing the quartz tubes under a 3-mm-thick piece of the acrylic sheet material OP-2® (Cyro Industries). There was no OP-2 treatment in experiment 1 because we had not yet received the material from the manufacturer. The spectral irradiance measured under these materials verified their effectiveness in producing the desired exposure treatment (Fig. 1*a*). There were a total of 42 quartz tubes in each experiment (except for experiment 1, in which there were 28): seven for each light regime at each of the two depths. Incubations were run for 10 days.

Physical variables

The temperature (degrees Celsius) and salinity (precision salinity units) of the water in the outdoor reservoir was recorded during each day of the experiments using a conductivity/salinity/temperature probe (model 130, Orion Research Inc., Beverly, Mass.).

Spectral irradiance was measured, at both incubation depths, using an OL754-O-PMT scanning spectroradiometer equipped with a submersible detector (OL-86T-WP) (Optronic Laboratories, Orlando, Fla.). This instrument provides spectroradiometric data from 250 to 800 nm, with a resolution of 1 nm. Spectral transmission profiles for the screening materials used in the exposure experiments were also measured with this instrument.
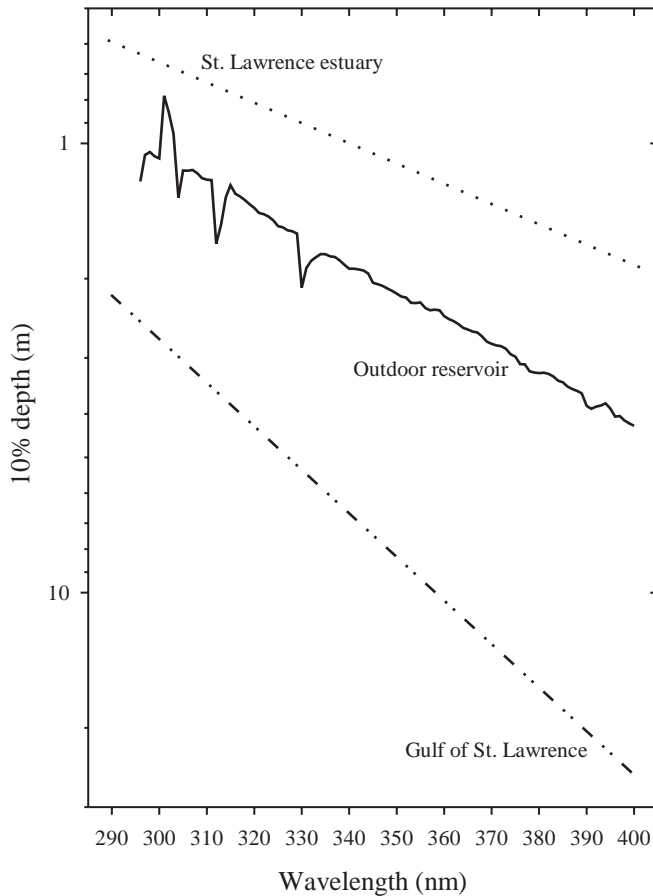
Wavelength-specific diffuse attenuation coefficients ($K_{d\lambda}$) were derived from the spectral irradiance scans made at the two incubation depths. Ten percent depths for the UV waveband were calculated from these $K_{d\lambda}$ values. These calculations are detailed in Kuhn et al. (1999).

Data on total daily UV-B energy delivered to the earth's surface at this location were obtained using a Brewer MKIII double monochromator spectrophotometer (Sci-Tec Instruments Inc., Saskatoon, Sask.) deployed on the roof of the Maurice Lamontagne Institute. Brewer data were not available for all days because of instrument malfunction.

Biological variables

Atlantic cod eggs become opaque shortly after dying, making it possible to census mortality by visual inspection. Thus, the number of dead eggs in each of the incubation tubes was counted daily throughout each experiment. To minimize handling, dead eggs

Fig. 2. Ten percent depths (the depth to which 10% of the surface energy penetrates at a given wavelength), plotted on a common log scale, for the water in the outdoor reservoir on the south shore of the St. Lawrence estuary (49°05′35″W, 67°05′83″N; see Station R5 on the map in Kuhn et al. 1999) and in the Gulf of St. Lawrence at a location near Atlantic cod spawning sites (49°07′25″W, 59°50′53″N; see Station S27 on the map in Kuhn et al. 1999).

tal stage was determined following the numerical descriptive system proposed by Friðgeirsson (1978).

Statistical analysis

Cumulative mortality of Atlantic cod eggs was derived from the daily counts of dead eggs in each tube. These data were $\log(x)$, $\arcsin \sqrt{x}$, or $1/\sqrt{x}$ transformed to obtain homogeneity of variance and normally distributed error terms (Sokal and Rohlf 1994). A two-factor repeated measures design ANOVA was performed on the transformed data to determine the overall effect of spectral treatment and depth on egg mortality. A Tukey–Kramer multiple comparisons test was performed (on cumulative mortality) when the ANOVA identified a statistically discernible effect. This allowed determination of which treatments differed from one another. The Tukey–Kramer test could not be applied to experiment 1 because it requires a minimum of three levels and there were only two in that experiment, Quartz and Mylar.

Larval corporeal pigmentation, standard length, and hatching success data were $\log(x)$, $\sqrt{x}$, or power transformed (as necessary) before being evaluated by single-factor ANOVA (Sokal and Rohlf 1994). Data for larvae from the indoor rearing basins were not included in these analyses because they were not held under the same conditions as those exposed outdoors.

Results

Spectral irradiance

The PAR spectral irradiance delivered to the tubes in the outdoor reservoir was similar at both depths (Fig. 1a). However, irradiance in the UV waveband was several times higher at the surface than at 50 cm (Fig. 1b). Longer-wave radiation (>700 nm) was also rapidly attenuated (Fig. 1a).

The 10% depth for UV in the outdoor reservoir was about 1 m at 300 nm, 1.5 m at 320 nm, and 4.25 m at 400 nm (Fig. 2). Since the water in the reservoir was finely filtered, these 10% depths were greater than those for waters on the south shore of the St. Lawrence estuary (49°05′35″W, 67°05′83″N; see Station R5 on the map in Kuhn et al. 1999) (Fig. 2). However, 10% depths in the Gulf of St. Lawrence, at a location near Atlantic cod spawning sites, were higher than those in the reservoir (49°07′25″W, 59°50′53″N; see Station S27 on the map in Kuhn et al. 1999) (Fig. 2).

Egg mortality

The cumulative mortality of Atlantic cod eggs incubated at the surface was highest in the Quartz treatment on most days (Fig. 3). Although less pronounced, this was also true at 50 cm; at this incubation depth, eggs in the Quartz treatment died at a higher rate (Fig. 3b). Within any given treatment, cumulative mortality was generally lower at 50 cm depth than at the surface. In two of the experiments, cumulative mortality in the Quartz treatment at 50 cm depth was similar to that in the surface groups that were not exposed to UV-B (Mylar and OP-2) (Fig. 3, 19–29 August experiment). These differences were statistically discernible (Tables 1 and 2).

Developmental stage, hatching success, and larval viability

The progress of embryonic development was unaffected by spectral treatment, at either incubation depth (Table 3). By the end of the experiments, almost all embryos had reached embryonic stage 32 (the last embryonic stage before

were not removed from the tubes until the end of the experiment. Tubes were retrieved from the outdoor reservoir at the end of each experiment and their content, live and (or) dead eggs and larvae, was examined under a binocular microscope. Hatching success, for experiments 4 and 5 only, was expressed as a percentage of the total number of eggs originally placed in each vial.

Live larvae, retrieved from the tubes at the end of experiments 4 and 5, were placed in petri plates in a temperature-controlled room at 6°C under a 12 h light :12 h dark photoperiod. Images of these yolk-sac larvae ($n = 1$–12), from the different light treatments and depths, were photographed or videotaped 24 h later. Yolk-sac larvae from the indoor rearing basin (the source of the eggs in each of these experiments) were also examined. Larvae were positioned on their left side. These images were used to quantify (i) standard length and (ii) the degree of corporeal pigmentation (the surface area covered with pigment as a function of the total surface area, excluding the eye and digestive system). These measurements were made using a Bioquant System IV imaging system (R&M Biometrics Inc., Nashville, Tenn.).

The progress of embryonic development was followed in experiments 1 and 2. Four or five eggs were removed every day from two of the vials in each treatment and at both depths. Embryos were viewed under a dissecting microscope and their developmen-

Fig. 3. Percent cumulative mortality (mean ± SE) in Atlantic cod eggs incubated outside the Maurice Lamontagne Institute, Mont-Joli, Qué., Canada (48°38′25.9″N, 68°09′21.0″W) between 10 July and 12 September 1996. Incubations were carried out at two depths: (*a*) just below the surface and (*b*) 50 cm depth. Superimposed on the left-hand panels is the daily UV-B integrated irradiance (vertical bars). Superimposed on the right-hand panels are the salinity (precision salinity units) and temperature of the water in the outdoor reservoir throughout the experiments.
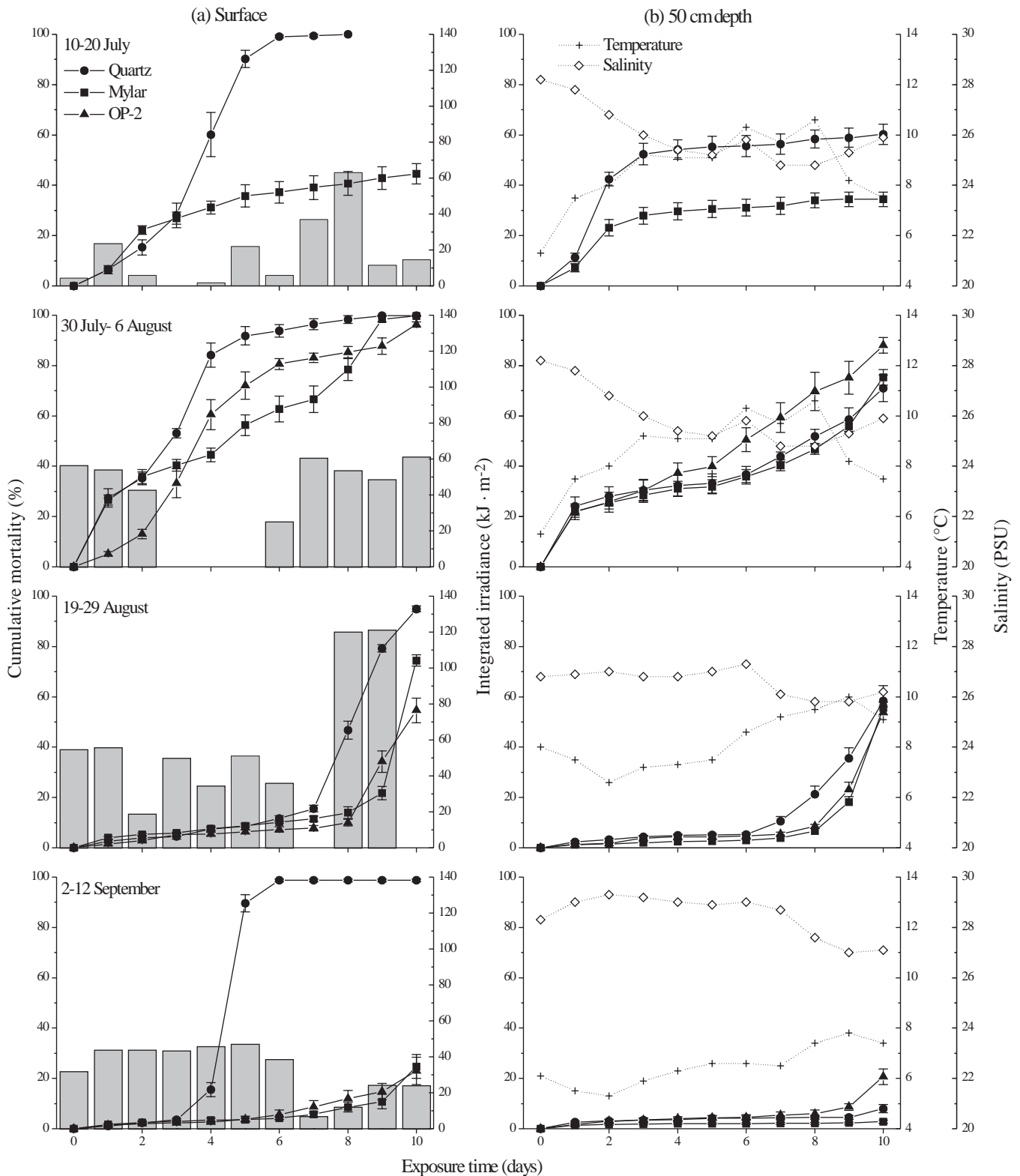
Table 1. Summary of the two-factor repeated measures design ANOVA's performed on the cumulative mortality of Atlantic cod eggs.

Selection	Experiment	Source(s)	df error	F_s	p
Entire experiment	1	Spectral exposure	24	53.735	<0.001
		Depth	24	2.054	0.165
	2	Spectral exposure	34	15.902	<0.001
		Depth	34	209.513	<0.001
	4	Spectral exposure	35	9.480	0.001
		Depth	35	31.940	<0.001
	5	Spectral exposure	36	9.662	<0.001
		Depth	36	14.640	<0.001
Surface	1	Spectral exposure	12	34.464	<0.001
	2	Spectral exposure	16	32.173	<0.001
	4	Spectral exposure	18	6.295	0.008
	5	Spectral exposure	18	9.291	0.002
50 cm	1	Spectral exposure	12	22.016	0.001
	2	Spectral exposure	18	7.016	0.006
	4	Spectral exposure	17	7.229	0.005
	5	Spectral exposure	18	4.128	0.033
Quartz	1	Depth	12	2.519	0.138
	2	Depth	12	266.260	<0.001
	4	Depth	12	5.961	0.031
	5	Depth	12	19.845	0.001
Mylar	1	Depth	12	0.645	0.437
	2	Depth	12	56.646	<0.001
	4	Depth	12	34.315	<0.001
	5	Depth	12	5.531	0.037
OP-2	1	Depth	na	na	na
	2	Depth	10	10.305	0.009
	4	Depth	11	2.948	0.114
	5	Depth	12	0.109	0.747

Note: The ANOVA's test for the overall effect of spectral exposure treatment, and depth, on egg mortality. The spectral exposures were UV-B + UV-A + PAR (Quartz), UV-A + PAR (Mylar), and PAR (OP-2). Incubation depths were surface and 50 cm. na, not available.

hatching), but most never hatched (Table 4). Hatching success was highest in the OP-2 treatment (Tables 4 and 5). Those larvae that did hatch were moribund and died soon after exiting the egg.

Corporeal pigmentation and standard length

Neither corporeal pigmentation nor the standard length of larvae was affected by spectral exposure, at either depth (Tables 4 and 5).

Discussion

Direct effects of UV-B

Egg mortality

Atlantic cod embryos exposed to UV-B (Quartz treatment) exhibited higher cumulative mortality than those shielded from UV-B (Mylar and OP-2 treatments). UV-B-induced mortality at the surface was virtually 100%, while that at 50 cm depth was less severe. The mechanism of this mortal-

ity appears to be direct damage to the DNA molecule (Kouwenberg et al. 1999).

Mortality of yellow perch (*Perca flavescens*) eggs, incubated in situ at various depths and with spectral exposure treatments similar to those reported here, was very high (>95%), even at depths up to 0.8 m (Williamson et al. 1997). Observations from the few other studies on UV-B-induced mortality in fish eggs are also consistent with our results (Marinaro and Bernard 1966; Pommeranz 1974; Hunter et al. 1982).

Several studies report on negative effects of UV-A: hatching success in the Japanese medaka (*Oryzias latipes*) is adversely affected by UV-A exposure (Bass and Sistrun 1997), long exposures to UV-A had negative effects on the metabolic performance and survivorship of the convict cichlid *Cichlasoma nigrofasciatum* (Winckler and Fidhiany 1996a, 1996b), and in situ exposure to UV-A induced elevated mortality in yellow perch eggs (Williamson et al. 1997). In the experiments reported here, Atlantic cod eggs were not negatively affected by exposure to UV-A: there were no clear dif-

Table 2. Summary of the Tukey–Kramer multiple comparisons tests on the cumulative mortality of Atlantic cod eggs.

Experiment	Depth	Spectral exposure	Exposure (days)									
			1	2	3	4	5	6	7	8	9	10
2	Surface	Quartz	a	a	b	b	b	b	b	b	a	a
		Mylar	a	a	a	a	a	a	a	a	a	a
		OP-2	b	b	a	a	a	a	a	a	b	b
	50 cm	Quartz	a	a	a	a	a	a	a	ab	ab	a
		Mylar	a	a	a	a	a	a	a	a	a	a
		OP-2	a	a	a	a	a	b	b	b	b	a
4	Surface	Quartz	a	a	a	a	a	a	a	a	a	a
		Mylar	b	a	a	a	a	ab	a	b	b	b
		OP-2	ab	a	a	a	a	b	b	b	c	c
	50 cm	Quartz	a	a	a	a	a	a	a	a	a	a
		Mylar	a	a	b	b	b	b	b	b	b	a
		OP-2	a	a	ab	ab	ab	ab	ab	b	ab	a
5	Surface	Quartz	a	a	a	a	a	a	a	a	a	a
		Mylar	a	a	a	b	b	b	b	b	b	b
		OP-2	a	a	a	b	b	b	b	b	b	b
	50 cm	Quartz	a	a	a	a	a	a	a	a	a	a
		Mylar	a	a	a	a	a	a	a	a	b	b
		OP-2	a	a	a	a	a	a	a	a	a	a

Note: This test identifies under which spectral exposures cumulative mortality was different on each day (within a single depth). The same letter in any given column indicates that there is no discernible difference between those treatments at that depth and on that day. A different letter indicates a discernible difference. The spectral exposures were UV-B + UV-A + PAR (Quartz), UV-A + PAR (Mylar), and PAR (OP-2).

Table 3. Embryonic development of Atlantic cod during 10-day exposures to solar radiation.

Experiment	Depth	Light regime	Exposure (days)										
			0	1	2	3	4	5	6	7	8	9	10
1	Surface	Quartz	14	18	21	24	24	28	ad	ad	ad	ad	ad
		Mylar	14	18	21	24	24	28–30	30	31	32	32	32, hatching
	50 cm	Quartz	14	18	21	24	24	30	30–31	31–32	32	32	32, hatching
		Mylar	14	18	21	24	24	30	30	31–32	32	32	32, hatching
2	Surface	Quartz	18	21	24	26	na	na	na	na	32	32	ad
		Mylar	18	21	24	27–28	29	30	30–31	31	32	32	ad
		OP-2	18	21	24	26–27	30	30	30	31	32	32	32, hatching
	50 cm	Quartz	18	21	24	26	30	30	31–32	32	32	32	32, hatching
		Mylar	18	21	24	28	30	30	31	32	32	32, hatching	32, hatching
		OP-2	18	21	24	28	30	30	31	32	32	32, hatching	32, hatching

Note: Measurements were made for embryos incubated under three spectral exposures, UV-B + UV-A + PAR (Quartz), UV-A + PAR (Mylar), and PAR (OP-2), and at two depths, surface and 50 cm. ad, all dead; na, not available.

ferences in mortality, rate of development, pigmentation, or size in the UV-A + PAR treatment relative to the PAR-only treatment (OP-2). This result is consistent with other experiments, conducted at a higher spectral resolution (three discrete spectral treatments within the UV-A waveband as opposed to the one presented here), on UV-induced mortality in Atlantic cod eggs: there was no clear negative effect of UV-A (Kouwenberg et al. 1999). Further experiments on the role of UV-A and visible light are required to resolve this issue.

Mortality in the treatments that were not exposed to UV was high, but egg mortality in the indoor rearing basins, in which the source egg populations were maintained through-

out these experiments, was also high. Nonetheless, some discussion of the elevated mortality exhibited by all of the treatment groups (in all but the last experiment) is necessary.

Fluctuations in water temperature and salinity might have influenced Atlantic cod egg mortality during these experiments. However, mortality of Atlantic cod eggs incubated at 5 and 10°C (Hunt von Herbing and Boutilier 1996) or between 2 and 12°C (Laurence and Rogers 1976) was not discernibly different. The temperatures to which Atlantic cod eggs were exposed in the current experiments fall within this range (Fig. 3). Further, development of Atlantic cod eggs is apparently normal at salinities above 11 precision salinity units (Westin and Nissling 1991). Thus, it is unlikely that

Table 4. Corporeal pigmentation, standard length, and hatching success of Atlantic cod larvae hatched from eggs exposed to solar radiation for 10 days and at two depths.

Variable	Spectral exposure	Experiment 4		Experiment 5	
		Surface	50 cm	Surface	50 cm
Corporeal igmentation (%)	Quartz	na	19.0±5.6 (5)	na	26.1±1.7 (5)
	Mylar	na	23.4±3.1 (9)	na	24.4±3.2 (11)
	OP-2	17.6±9.9 (2)	17.8±4.7 (2)	28.5±5.4 (11)	22.9±2.2 (12)
	Indoor basin	16.8±2.6 (12)		13.3±3.9 (12)	
Standard length (mm)	Quartz	na	3.8±0.2 (5)	na	3.8±0.1 (5)
	Mylar	na	3.9±0.1 (9)	na	3.8±0.2 (11)
	OP-2	3.4±0.3 (2)	3.1±0.1 (2)	3.6±0.1 (11)	3.7±0.1 (12)
	Indoor basin	3.8±0.2 (12)		4.5±0.1 (12)	
Hatching success (%)	Quartz	0.0	1.13±0.45	0.0	2.03±0.55
	Mylar	0.88±0.35	5.91±0.67	0.0	5.76±0.86
	OP-2	17.97±2.32	7.76±0.78	8.39±1.89	7.64±1.38

Note: The spectral exposures were UV-B + UV-A + PAR (Quartz), UV-A + PAR (Mylar), and PAR (OP-2). Also reported are values for larvae hatched in the indoor rearing basins. Values are means ± SE (n in parentheses). na, not available.

Table 5. Summary of the single-factor ANOVA's performed on corporeal pigmentation, standard length, and hatching success of Atlantic cod larvae.

Variable	Experiment	Depth	Spectral exposure	df error	Fs	p
Corporeal pigmentation	4	50 cm	Quartz, Mylar	12	1.271	0.282
	5	Surface, 50 cm	OP-2	21	0.772	0.390
	5	50 cm	Quartz, Mylar, OP-2	25	0.262	0.772
Standard length	4	50 cm	Quartz, Mylar	12	0.566	0.466
	5	Surface, 50 cm	OP-2	21	0.490	0.491
	5	50 cm	Quartz, Mylar, OP-2	25	0.902	0.418
Hatching success	4	50 cm	Quartz, Mylar, OP-2	17	18.767	<0.001
	5	50 cm	Quartz, Mylar, OP-2	18	12.033	<0.001

Note: The ANOVA's test for the overall effect of spectral exposure treatment, or depth, on these variables. The spectral exposures were UV-B + UV-A + PAR (Quartz), UV-A + PAR (Mylar), and PAR (OP-2).

temperature or salinity alone would have caused the mortality observed. Other factors (bacterial or viral infection, high light intensity (across the entire spectrum), poor egg group quality, or some synergy among all of these) may have been responsible for the non-UV-B-related mortality observed.

Since all of the tubes in each of the experiments were incubated under the same water quality conditions, any differences in mortality were most likely the result of the spectral exposure treatments. Thus, the high mortality exhibited by many of the treatments does not obviate the conclusion that UV-B negatively affects Atlantic cod eggs: the UV-B-exposed group had the highest cumulative mortality, or reached 100% mortality soonest, in all of the experiments (Fig. 3).

Corporeal pigmentation and standard length

Melanosome dispersion in northern anchovy larvae exposed to UV-B was more pronounced than in those groups shielded from UV-B (Hunter et al. 1979). Further, UV-B-exposed larvae were darker than larvae reared indoors. These observations were interpreted as a shielding response that would protect the larva's tissues from damage due to absorption of high-energy UV-B photons (Hunter et al. 1979). Melanin content in the epidermis of juvenile hammerhead sharks increased in response to the higher levels of

solar radiation to which they are exposed when they migrate to the near-surface waters of Hawaii (Lowe and Goodman-Lowe 1996). It appears, therefore, that pigments play an important photoprotective role in aquatic vertebrates, as they do in zoolankton (Dey et al. 1988). Despite this, there was no discernible effect of UV-B exposure on the pigmentation of Atlantic cod larvae in our experiments. This was most likely due to the small number of larvae that survived the experiments: given the small sample sizes, it is not possible to say definitively that UV-B exposure does not affect corporeal pigment coverage in Atlantic cod larvae.

There was no consistent difference in the size of Atlantic cod larvae reared under the different spectral exposures. This is contrary to the observations of Hunter et al. (1979), who reported that UV-B-exposed northern anchovy larvae were smaller than those shielded from UV-B. However, as was the case for pigment content, the small numbers of larvae available to be measured prevent any definitive conclusion.

Indirect effects of UV-B

Indirect effects of UV-B on Atlantic cod early life stages, and other ichthyoplankton, are also possible. UV-B can be a potent immunosuppressive agent in adult fishes (Salo et al. 1998); it is not known whether this is also true for em-

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bryos 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; Chalker-Scott 1995; Häder et al. 1995; Williamson et al. 1997). 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 that themselves take up these fatty acids from the microalgae upon which they graze. One of the microalgal fatty acids that is negatively influenced by UV-B exposure, docosahexaenoic acid (Wang and Chai 1994), also appears to be important for normal visual and behavioural development in fish larvae (Bell et al. 1995; Masuda et al. 1998). All such indirect effects have yet to be evaluated; these will be a focus of our future efforts.

Ecological context

The experiments reported here indicate that Atlantic cod eggs, at least those present in the first metre of the water column, are susceptible to UV-B. The 10% depths for UV penetration in the outdoor reservoir were less than those for regions of the Gulf of St. Lawrence where Atlantic cod spawn (Fig. 2). This suggests that the impact of UV-B reported here is an underestimate of that which would be observed in the wild. This conclusion, however, must be carefully qualified.

Although the available information on the vertical distribution of Atlantic cod eggs in this region is limited, it appears that most are not present in the upper 4 m of the water column (Ouellet 1997). Even if most Atlantic cod eggs were present in the 10- to 15-m-deep mixed layer of the northern Gulf of St. Lawrence water column, they would be in circulation and their daily residence time in the upper 4 m would depend on meteorological and hydrographic conditions (among other things; see Solemdal and Sundby 1981). Short residence times, which appear likely, would further reduce the population-level impact of UV-B on Atlantic cod eggs.

A more complete quantitative assessment of direct UV-B effects on the population dynamics of Atlantic cod early life stages requires further information and analysis. Specifically: (*i*) detailed vertical distribution of eggs in the mixed layer of the water column (with high resolution in the upper 10 m), (*ii*) surface UV-B irradiance during the spawning period and subsurface spectral irradiance for waters supporting such eggs (see Kuhn et al. 1999), (*iii*) a biological weighting function, which explicitly considers the possibility of photorepair (and therefore the absence of reciprocity), for the effect of UV-B on Atlantic cod egg mortality (see Kouwenberg et al. 1999), and (*iv*) 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 meteorological and hydrographic conditions. All of these components would have to be incorporated into a broader simulation model that would provide an assessment of UV-B effects on a population of eggs distributed (and circulating) throughout the mixed layer (e.g.,

Neale et al. 1998). We are currently developing such a model for Atlantic cod eggs.

Finally, although UV-B can have negative impacts on ichthyoplankton populations, it must be viewed as only one among many environmental factors that produce the very high levels of mortality typically observed in the planktonic early life stages of marine fishes.

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