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Ultraviolet (280–400 nm)–induced DNA Damage in the Eggs and Larvae of *Calanus finmarchicus* G. (Copepoda) and Atlantic Cod (*Gadus morhua*)¶

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ABSTRACT

In previous work, we evaluated the effects of ultraviolet (UV = 280–400 nm) radiation on the early life stages of a planktonic Calanoid copepod (*Calanus finmarchicus* Gunnerus) and of Atlantic cod (*Gadus morhua*). Both are key species in North Atlantic food webs. To further describe the potential impacts of UV exposure on the early life stages of these two species, we measured the wavelength-specific DNA damage (cyclobutane pyrimidine dimer [CPD] formation per megabase of DNA) induced under controlled experimental exposure to UV radiation. UV-induced DNA damage in *C. finmarchicus* and cod eggs was highest in the UV-B exposure treatments. Under the same spectral exposures, CPD loads in *C. finmarchicus* eggs were higher than those in cod eggs, and for both *C. finmarchicus* and cod embryos, CPD loads were generally lower in eggs than in larvae. Biological weighting functions (BWF) and exposure response curves that explain most of the variability in CPD production were derived from these data. Comparison of the BWF revealed significant differences in sensitivity to UV-B: *C. finmarchicus* is more sensitive than cod, and larvae are more sensitive than eggs. This is consistent with the raw CPD values. Shapes of the BWF were similar to each other and to a quantitative action spectrum for damage to T7 bacteriophage DNA that is unshielded by cellular material. The strong similarities in the shapes of the weighting functions are not consistent with photoprotection by UV-absorbing compounds, which would generate features in BWF corresponding to absorption bands. The BWF reported in this study were applied to assess the mortality that would result from accumulation of a given CPD load: for both *C. finmarchicus* and cod eggs, an increased load of 10 CPD Mb^{-1} of DNA due to UV exposure would result in approximately 10% mortality.

INTRODUCTION

In previous work, we evaluated the effects of ultraviolet (UV = 280–400 nm) radiation on the early life stages of a planktonic Calanoid copepod (*Calanus finmarchicus* Gunnerus) and of Atlantic cod (*Gadus morhua*).

Calanus finmarchicus plays a key role in the pelagic food webs of the North Atlantic because its eggs and nauplii are a predominant prey for larvae of several ecologically and economically important fish species. The first feeding larval stages of some stocks of redfish, cod and haddock feed almost exclusively on *Calanus* sp. eggs and nauplii (1,2). In the estuary and Gulf of St. Lawrence, Québec, Canada, *C. finmarchicus* females release their eggs in surface waters, mostly during spring and summer months (March to September). More than 34% of the eggs are found in the first 4 m of the water column (2, P. Kuhn, unpublished).

Atlantic cod spawn at depth, and their eggs, which are typically positively buoyant, ascend to the surface mixed layer for a period of 2–10 days (3–5). Cod eggs are present in the 0–25 m depth stratum off the Newfoundland Shelf (4), off Greenland and Labrador (6), on southern Georges Bank (7) and in the northern Gulf of St. Lawrence (5). The specific proportion of the egg population present in the surface layer cannot be definitively quantified because the vertical distribution of cod eggs is dependent on a number of variable and interacting factors (egg buoyancy, meteorologic and hydrographic conditions, *etc.*). Nonetheless, when wind speed is low, the highest egg concentrations are observed in the upper 0–10 m of the water column (3). The early larval stages are also typically present and often even closer to the surface (4). Thus, the early life stages of these species are potentially susceptible to UV exposure.

Our previous experiments were conducted outdoors under natural UV (8,9) and in the laboratory under simulated UV (10,11). In the outdoor experiments eggs of both species were incubated with and without the UV-A (320–400 nm) or UV-B (280–320 nm) wave bands. Hatching success in the eggs of *C. finmarchicus* was

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Abbreviations: BWF, biological weighting function; CPD, cyclobutane pyrimidine dimer; ERC, exposure response curve; PC, principal component; PCA, principal component analysis; SS, solar simulator; UV, ultraviolet.

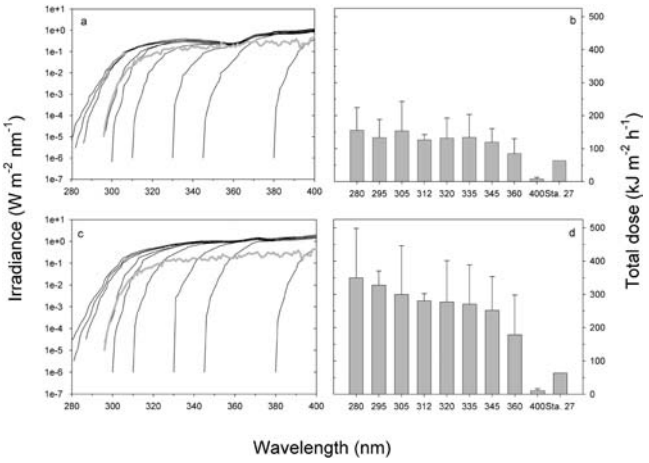

Figure 1. a: Spectral irradiance (280–400 nm) delivered to Atlantic cod (*Gadus morhua*) eggs and larvae under the nine different long-pass cutoff filters in the low total dose 1 h exposure experiment. From left to right, the cuton wavelengths for these filters were 280, 295, 305, 312, 320, 335, 345, 360 and 400 nm. For visualization on this log scale, irradiance values of zero are represented as 10^{-6} W m^{-2}; values of zero were used in all calculations. For comparison, noontime subsurface (10 cm depth) spectral irradiance at Station S27 (located at 59°50′53″N, 49°07′25″W in the Gulf of St. Lawrence, 14) is presented as the gray-tone line. b: Total dose delivered in the low total dose 1 h exposure experiment. c: Spectral irradiance delivered to *Calanus finmarchicus* and Atlantic cod eggs in the high dose exposure experiment (all else as in panel a). d: Total dose delivered under each of the spectral exposure treatments in the high dose experiments. In panels b and d, the total dose delivered at midday over 1 h at Station S27 is presented for comparison.

determined after 2–3 days of exposures (9). UV-exposed eggs exhibited low percent hatching compared with those protected from UV. Although percent hatching in UV-B–exposed eggs was not significantly lower than that in eggs exposed to UV-A only, the effect of UV-A for 2–3 days was so great that a significant incremental effect of UV-B could not have been observed: that is, our data did not allow us to conclude that UV-B does not have an even greater impact. In analogous experiments with Atlantic cod eggs (mortality was evaluated daily), exposure to UV-B produced a significant negative effect (8). However, unlike for *C. finmarchicus* UV-A did not negatively affect cod eggs. These outdoor exposure experiments demonstrate that both *C. finmarchicus* and cod embryos suffered mortality when exposed to natural UV and that *C. finmarchicus* eggs were more sensitive to UV-A than were cod embryos.

Experiments in the laboratory resolved the magnitude and spectral dependence of UV effects on *C. finmarchicus* and cod eggs (10,11). Biological changes were measured after controlled exposures to irradiance spectra that included different parts of the UV wave band. The resulting biological weighting functions (BWF) derived for UV-B–induced mortality in the eggs of *C. finmarchicus* and cod were similar to the action spectrum for UV-B effects on naked DNA (10,11); each showed that UV-B wavelengths were much more damaging than UV-A. Consistent with the outdoor experiments, *C. finmarchicus* eggs were much more sensitive than cod eggs. Furthermore, DNA damage exhibited a wavelength dependence similar to the mortality effect, with *C. finmarchicus* eggs consistently exhibiting higher damage than cod eggs (see fig. 7 in Browman *et al.* [12]). This suggests that UV-induced mortality in *C. finmarchicus* and cod eggs is a direct result of DNA damage.

To further describe the potential impacts of UV exposure on the early life stages of these two species, we measured the wavelength-specific DNA damage (cyclobutane pyrimidine dimer [CPD] formation) induced under controlled experimental exposure to UV radiation. Specifically, the work reported in this study (1) presents the first BWF for UV-induced DNA damage in these organismal groups; (2) clarifies the relative effects of the UV-A *vs* UV-B wave bands in inducing DNA damage; and (3) evaluates the possibility of relating UV-induced mortality directly to DNA damage.

MATERIALS AND METHODS

Source of live material. *Calanus finmarchicus* females were collected from the St. Lawrence estuary by vertical haul (250–0 m) using a 1 m diameter zooplankton net (333 μm mesh) deployed at midday. Immediately after capture, gravid females were placed in 10 L buckets (50 copepods bucket^{-1}) filled with newly filtered seawater (0.2 μm pore size, salinity of 28 ± 1 psu), where they released their eggs. The buckets were maintained in a temperature-controlled chamber at 6.5°C. Females were fed once per day on the diatom *Skeletonema costatum*, and the filtered seawater in the buckets was replaced every 2 days. This culture of *S. costatum* is not detrimental to hatching success in *C. finmarchicus* (13). Eggs were collected 24–30 h after release by siphoning most of the water from the buckets through a Tygon hose fitted with a 70 m mesh Nytex filter on its intake. The remaining water, containing the concentrated eggs, was collected by gently pouring the contents of the bucket into a beaker. Samples were then transferred from the beaker to petri dishes. Eggs were sorted with a glass pasteur micropipette under a binocular microscope and introduced into the incubation tubes for the exposure experiments (see below).

Fertilized cod eggs were obtained from a broodstock maintained at the Maurice-Lamontagne Institute for experiments unrelated to UV radiation. 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—all eggs used in any single experiment were the progeny of the same male–female pair. Fertilized eggs were incubated in the dark, at 6°C, in 60 L black round-bottom basins from which they were removed immediately before an exposure experiment.

Solar simulator and incubation system. *Calanus finmarchicus* and cod eggs were irradiated under a solar simulator (SS) consisting of two 1 kW Xenon arc lamps. The spectral output of these lamps was adjusted, using various combinations of optical filters, to produce a spectral profile as close to solar as possible (Fig. 1a,c). However, as compared with sunlight, the spectral irradiance delivered by the SS was higher in the UV-B region and lower in the visible wave band (400–700 nm). Full details of the optical characteristics of this SS are available in Kouwenberg *et al.* (10).

Eggs were irradiated in incubation tubes immersed in a circular basin filled with recirculating filtered seawater at 6°C and a salinity of 28 ± 1 psu (see Kouwenberg *et al.* [10], Fig. 1). The bottom of the incubation basin was fitted with a polyethylene holder that contained a slot for each of the incubation tubes. The holder was designed so that it remained in the same position and orientation under the SS during each exposure. Each incubation tube was covered with a 25 × 25 mm quartz-substrate long-pass filter. The filters used were Schott WG280, WG295, WG305, WG312, WG320, WG335, WG345, WG360 and GG400, for which the 50% cutoff wavelength (nm) is approximately that specified (Fig. 1a,c). There were three replicate tubes for each cutoff filter treatment, and a control group was incubated under the SS in the dark (tubes were wrapped in aluminum foil).

Radiometry. Spectral irradiance, E(λ) (W m^{-2} nm^{-1}), was measured at 1 nm intervals (280–800 nm) under each of the cutoff filters at all tube positions in the incubator. Measurements were made using a scanning spectroradiometer (OL-754, Optronic Laboratories Inc., Orlando, FL) for which the full width at half maximum was set to 1 nm. Before each measurement series, the instrument was calibrated against a NIST-traceable 200 W tungsten-halogen standard lamp (OL-752-10), and its wavelength and gain accuracy were verified using a dual source calibration module (OL-752-150). For each filter with a nominal cutoff ⩾305 nm, a wavelength of essentially zero transmission was determined by inspection of the spectra. Readings for wavelengths shorter than this were set to zero, thereby eliminating a small amount of spurious variability due to instrument noise and stray light. The small offset at the nominal cutoff wavelength was

subtracted from readings at longer wavelengths. A full characterization of this instrument is available in Kuhn *et al.* (14).

UV irradiation experiments. Calanus finmarchicus and cod eggs were irradiated under SS for 1 h, placed in the dark and preserved in anhydrous alcohol. This ensured that there was little time to repair any UV-induced damage to the DNA molecule, even by mechanisms other than photoreactivation (which typically act on much longer time scales than does photoreactivation). Because dead eggs sink, floating (live) eggs were collected, preserved and then sorted under a dissecting microscope with a UV-blocking filter placed over the light source. The following five exposure experiments (all with nine spectral exposures and a dark control, ×3 replicates each) were conducted: (1) cod eggs (24 h before hatching), low total dose; (2) cod eggs (24 h before hatching), high total dose; (3) cod larvae (12 h after hatching), low total dose; (4) *C. finmarchicus* eggs (48 h after fertilization), high total dose; and (5) newly hatched *C. finmarchicus* nauplii, high total dose. There was no low dose exposure for *C. finmarchicus*. Experiments were run on late-stage eggs and on newly hatched larvae to assess whether the egg membrane provides these embryos with any protection from UV damage. The appropriate total dose to deliver was determined from preliminary CPD assays—we were forced to balance an attempt to deliver an ecologically realistic dose/dose rate combination with the need to generate a measurable amount of DNA damage for a short exposure. The difference between the low and high dose exposures was generated by using neutral density filters or by varying the output of the power supplies (or both). The irradiance spectra are presented in Fig. 1a,c. The UV-B doses delivered in the low and high total dose exposures are presented as integrated hourly irradiance from 280 to 400 nm (kJ m^{-2} h^{-1}) in Fig. 1b,d.

DNA damage assay. UV-specific DNA damage was evaluated using a chemiluminescent antibody detection system for the presence of CPD in DNA (15,16 and references therein). The detection system is sensitive enough to measure DNA damage in a single fish egg or larva (but a minimum of 10 copepod eggs) exposed to natural amounts of sunlight (15,17). Although many forms of DNA damage can occur, CPD appear to be formed by the direct absorption of photons into the DNA helix. In the simplest case two adjacent thymines on the same strand of DNA break their hydrogen bonds with the complementary adenines of the adjacent strand and bond with each other. Hence, the name pyrimidine, or in this case, thymine dimer (18). Thus, the concentration of CPD in an egg or larva gives a good indication of UV-B–induced DNA damage.

Total nucleic acids were extracted from eggs and larvae, and the DNA was purified and eluted into trietholamine buffer (TE). DNA concentration in each sample was determined with a DNA fluorometer. Before blotting, 50 ng of DNA from each egg extraction was denatured, the volume adjusted with ammonium acetate and vacuum blotted onto nylon membranes that were baked at 80°C for 1–2 h. CPD were measured by a chemiluminescent immunoblot assay capable of measuring CPD in samples as small as 50 ng of DNA. The assay and methodology are thoroughly described by Vetter *et al.* (15).

Derivation of the BWF for UV-induced DNA damage. The effects of UV on biological processes often reflect simultaneous and sometimes competing wavelength-dependent processes. Thus, wavelength dependence of the net effect in nature cannot be described with an action spectrum derived from a series of monochromatic exposures. Rather, it should be quantified under a series of polychromatic exposures (19) to derive a BWF (reviewed by Neale [20]). The spectral weightings in a BWF are needed to specify biologically weighted exposure in an exposure response curve (ERC, 21), which describes the kinetics of damage. For example, if UV-induced damage is quantified, and repair is insignificant, damage will be a function of cumulative exposure to biologically weighted UV, H^* (dimensionless or J m^{-2}_{Beff}, see Neale [20]). However, if repair processes rapidly counter UV-induced damage, the net effect can be a function of biologically weighted UV fluence rate, E^* (dimensionless or W m^{-2}_{Beff}). Experimental determination of a BWF requires *a priori* specification of the ERC; spectral weightings are optimized statistically to minimize variability around a functional relationship between exposure and net effect.

To describe the spectral dependence of net CPD formation during 1 h polychromatic exposures, we initially used a very simple ERC in which the UV-dependent accumulation of CPD, D (CPD Mb^{-1} of DNA), is a linear function of biologically weighted exposure, H^* (dimensionless):

$$D = D_d + kH^*, \tag{1}$$

where D_d accounts for an initial concentration of dimers and any possible net change that occurs in the dark and k is a scaling factor that we set to 1.0 CPD Mb^{-1} so that damage could be related directly to spectrally weighted cumulative exposure through the BWF:

$$H^* = \sum_{\lambda=280\text{ nm}}^{400\text{ nm}} \varepsilon_H(\lambda) H(\lambda) \Delta\lambda. \tag{2}$$

Here, unweighted cumulative exposure, $H(\lambda)$, with units of J m^{-2} nm^{-1}, is $\int E(\lambda)dt$, where $E(\lambda)$ is spectral irradiance (W m^{-2} nm^{-1}). The BWF is $\varepsilon_H(\lambda)$, with units of (J m^{-2})$^{-1}$.

Established multivariate procedures, described by Cullen and Neale (22) and reviewed by Neale (20), were used to determine the BWF for DNA damage in eggs and larvae of cod and *Calanus*. Principal component analysis (PCA) was used in the calculations; this approach differs from the somewhat simpler and more commonly used but less powerful Rundel (19) method in that no *a priori* assumptions must be made about the spectral shape of the BWF (*e.g.* an exponential decrease with increasing wavelength). The BWF for eggs of *C. finmarchicus* and cod reported by Kouwenberg *et al.* (10,11) were constrained to a single spectral slope because the number of observations was too small for the PCA method. Briefly, the 27 irradiance spectra for experimental exposures were normalized to the value at 400 nm, so variations in spectral shape could be characterized. The normalized spectra were then subjected to PCA, which generates a small number of statistically independent spectral shapes (principal components [PC]) that describe most of the variation in normalized treatment spectra. For the analysis, two or three PC were retained; they described between 80% and 94% of the variability in spectral shapes. The third component was retained only if its inclusion significantly improved the prediction of D as a function of H^* (described below) using an F-test on fits for three *vs* two PC at $\alpha = 0.01$. Spectral irradiance (280–400 nm) in each of the $i = 1$ to 27 exposures was thus described by (1) factor scores ($c_{i,1}$, $c_{i,2}$ and $c_{i,3}$) for the two or three PC; (2) the PC themselves (component weights for 121 wavelengths from 280 to 400 nm); and (3) spectral irradiance at the normalization wavelength, $E(400)_i$ (22).

Nonlinear curve fitting was used to minimize error in a relationship describing damage to DNA as a function of biologically weighted exposure, as estimated with weighted factor scores, irradiance at 400 nm and time (T, in s):

$$D_i = D_d + TE(400)_i\left(m_0 + \sum_{k=1}^{z} m_k c_{i,k}\right). \tag{3}$$

Here, z is the number of PC. The statistically determined parameters of the model are D_d, m_0 and m_1 through m_z. The coefficient m_0 quantifies the influence of the mean spectral shape and a residual weight that represents any experimental effect that is not dependent on UV. Coefficients m_1 to m_z quantify the influences of the spectral shapes represented by PC 1 to z. Measurements of CPD for the three dark incubations were included in the analysis to help constrain the relationship by including D_d (net CPD production in the dark). The contributions of the PC, m_i, are transformed back to a spectral weighting, $\varepsilon_H(\lambda)$, as described by Cullen and Neale (22), and consistent with the equivalence of Eq. 3 with Eqs. 1 and 2. These weightings constitute the BWF.

For each experiment, the analysis yielded a BWF with confidence limits ($\varepsilon_H(\lambda)$ ± 95% CL). The BWF is used to generate the ERC, *i.e.* net production of CPD as a function of weighted exposure, H^* (Eq. 1). For some of the experiments, residuals about the ERC were negative at low and high values of H^* and positive in the middle, consistent with curvature in the underlying relationship. Consequently, the analysis was run again with the following nonlinear ERC in place of Eq. 1:

$$D_i = D_d + D_{max}\left(1 - e^{-(kH_i^*/D_{max})}\right). \tag{4}$$

This ERC describes measured CPD production as a simple saturating function of weighted irradiance, approaching a maximum dimer concentration, D_{max}. The term kH^* is scaled as in the linear model (Eq. 1), and the normalization to D_{max} in the exponent makes kH^* equal to the initial slope of the relationship between D and H^* as H^* approaches zero. Estimates of H^* from the nonlinear model can therefore be compared

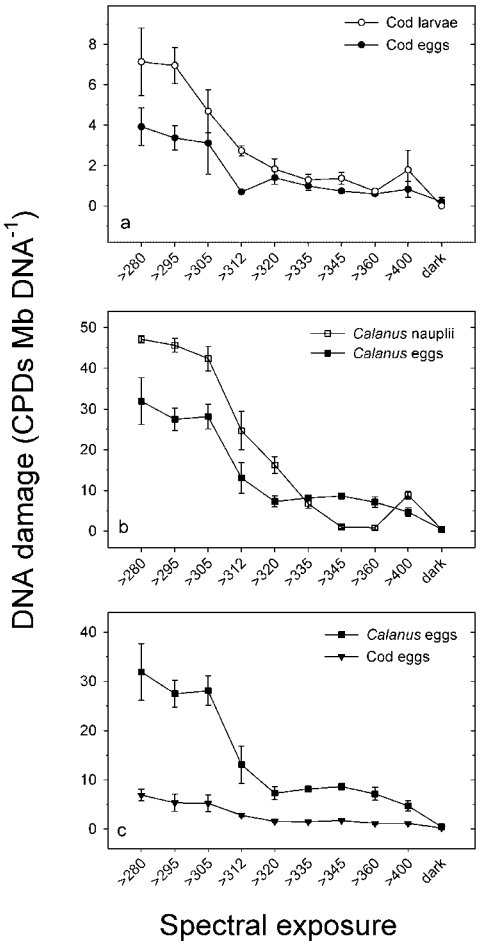

Figure 2. Mean (± standard error) DNA damage in *Calanus finmarchicus* and Atlantic cod (*Gadus morhua*) eggs and larvae exposed to various spectral wave bands. a: Data from low total dose exposures. b,c: Data are from high total dose exposures.

directly with estimates from the linear model, as can the corresponding BWF.

The nonlinear model was accepted only if it yielded a significantly better fit than the linear model, as determined with an *F*-test comparing the amount of variance explained:

$$F_{1,n-3} = \frac{([\mathrm{SSR_L} - \mathrm{SSR_N}]/[\mathrm{DF_L} - \mathrm{DF_N}])}{\mathrm{SSR_N}/\mathrm{DF_N}}, \quad (5)$$

where the subscripts L and N refer to the linear and nonlinear models. SSR is the sum of squared residuals, DF is the degrees of freedom, n is the number of exposures (samples) and 3 is the number of variables in the nonlinear model. Possible reasons for the nonlinear functional form are discussed below. Significance was tested at a conservative threshold probability of 0.01 to account for the multiple testing.

An *F*-test was also applied to determine if analyzing the data for low and high exposures of cod eggs separately resulted in any significant improvement over a fit for the combined data. There was no significant improvement, so analysis proceeded for the 60 exposures of cod eggs combined. For analysis of the two experiments combined, a better fit was obtained when the dark production of CPD, D_d, was estimated for each of the two experiments to account for variability unrelated to UV; the *F*-test was adjusted to account for the loss of one degree of freedom.

All our results apply to net production of CPD during 1 h incubations. Repair processes were not assessed (the experiments were designed to minimize the possibility of repair confounding the results), and extrapolation to other time scales is not warranted.

RESULTS

Assessment of DNA damage

UV-induced damage (CPD concentration per megabases of DNA) to the DNA in *C. finmarchicus* and cod eggs was highest in the UV-B exposure treatments (Fig. 2). In most cases, CPD concentration in the UV-A exposure treatments was <10 CPD Mb $^{-1}$ of DNA higher than the background levels observed in the dark controls (Fig. 2). Under the same spectral exposures, CPD loads in *C. finmarchicus* eggs were higher than those in cod eggs (Fig. 2c). For both *C. finmarchicus* and cod embryos, CPD loads were generally lower in eggs than in larvae (Fig. 2a,b).

Quantitative assessment of DNA damage: BWF

The BWF derived from the linear model of DNA damage (Eq. 1) explained much of the variability in CPD production for the experiments, but the nonlinear model provided a better fit in each case (Table 1). The improvement was statistically significant (P = 0.01) for all but the cod eggs. Thus, for cod eggs, the simpler linear model BWF was chosen to represent the wavelength dependence of UV effects on CPD production (Fig. 3).

The nonlinear model explained >90% of the variability in CPD production for eggs and nauplii of *C. finmarchicus* and larvae of cod, and the improvement over the linear model was highly significant in each case (Table 1). The statistical method can reveal no explanation for this behavior. It does, however, yield BWF and ERC that explain most of the variability in CPD production during the 1 h experiments (Fig. 3).

Table 1. Measurements of DNA damage as functions of spectral exposures quantified with PCA (see text). Both a linear model (Eq. 1) and a nonlinear model (Eq. 4) were applied; the result selected is indicated in bold. The nonlinear model was chosen only if it produced a significantly better fit at $\alpha = 0.01$; the degrees of freedom for the *F*-statistic are 1 and 27 for all except cod eggs, where they are 1 and 56 because dark production (D_d in Eqs. 1 and 4) of CPD was estimated for each of the two combined experiments (see text); n is the number of samples, PC is the number of principal components retained for the analysis and P is the probability that the nonlinear model explains no more of the variance than the linear model. DNA damage (CPD Mb^{-1}) is calculated for a modeled exposure to midday solar radiation for 1 h at 1 m depth in clear oceanic water, according to procedures described in Kuhn *et al.* (14). These estimates provide a measure of relative susceptibility to DNA damage, and they illustrate differences between the linear and nonlinear models.

		Linear model			Nonlinear model				
	n	PC	r^2	DNA damage	PC	r^2	DNA damage	F	P
Calanus eggs	30	2	0.679	9.12	**3**	**0.908**	**19.7***	**66.8**	8.85×10^{-9}
Calanus nauplii	30	1	0.550	38.2	**3**	**0.938**	**36.4***	**167.6**	4.29×10^{-13}
Cod eggs	**60**	**2**	**0.835**	**1.30**	2	0.852	1.81	6.20	0.016
Cod larvae	30	2	0.869	2.69	**2**	**0.916**	**4.22**	**15.0**	0.0006

*P = 0.01.

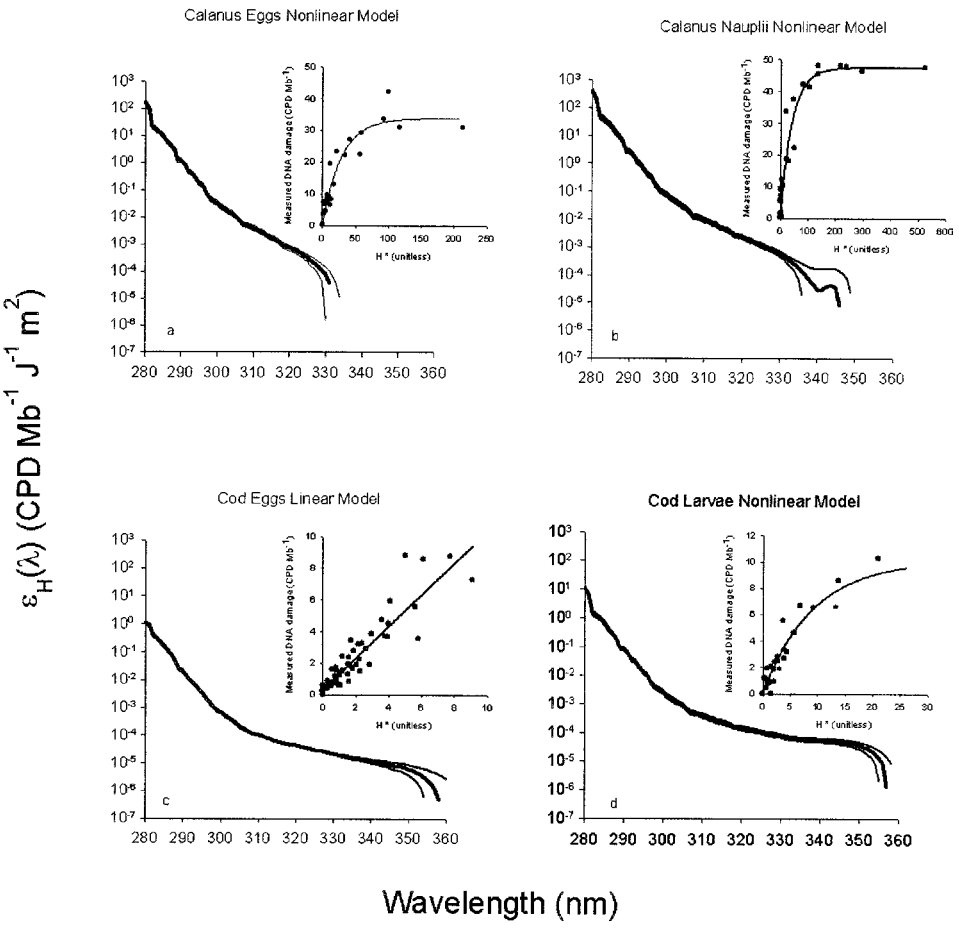

Figure 3. BWF (±95% CL) for DNA damage in (a,b) *Calanus finmarchicus* eggs and larvae and Atlantic cod (*Gadus morhua*) (c) eggs and (d) larvae. All these curves are plotted on the same scale so that results can be compared. Insets are plots of measured DNA damage as a function of calculated biologically weighted exposure (H^*, dimensionless). The solid lines in these inset plots represent the estimated ERC. BWF were calculated to 400 nm; none of the spectral weightings for $\lambda > 360$ nm were significantly greater than 10^{-7} CPD Mb^{-1} J^{-1} m^2, and they are not shown here. Each BWF included slightly negative values at longer wavelengths, and the BWF for both *Calanus* eggs and nauplii show features (decreased sensitivity) consistent with a 10–15 nm absorption band near 355 nm.

None of the spectral weightings for $\lambda > 360$ nm were significantly greater than 10^{-7} CPD Mb^{-1} J^{-1} m^2, and they are not shown in Fig. 3. Each BWF included slightly negative values at longer wavelengths and the BWF for both *Calanus* eggs and nauplii show features (decreased sensitivity) consistent with a 10–15 nm absorption band near 355 nm. The influence of these features on sensitivity to solar irradiance (*e.g.* DNA damage calculated in Table 1) is very small, however.

The four BWF (Fig. 3) are well constrained statistically in the UV-B range and show nonlinear shapes on semilog plots. These spectral shapes could not be produced by the simple Rundel (19) method that was used by Kouwenberg *et al.* (10,11) in their studies of UV-induced mortality. Comparison of the BWF (Fig. 4) reveals consistent differences in sensitivity: *C. finmarchicus* is more sensitive than cod, and eggs are more sensitive than larvae. This is consistent with the raw CPD values displayed in Fig. 2.

Shapes of the BWF are similar (Fig. 4) and consistent with a quantitative action spectrum for damage to T7 bacteriophage DNA that is unshielded by cellular material (23). Agreement with such an action spectrum, determined from monochromatic exposures, supports the interpretation that the PCA method applied here accurately reflects the BWF for damage to DNA in *C. finmarchicus* and cod. Furthermore, the strong similarities in the shapes of the weighting functions are not consistent with photoprotection by UV-absorbing compounds in the UV-B where DNA is most sensitive because absorption by these compounds would alter the shape of the curves relative to that of naked DNA (24).

The BWF for mortality, generated earlier (10,11), can be compared against those presented in this study for DNA damage. When displayed on a semilog scale, the BWF for mortality have no spectral structure (Fig. 5): the Rundel (19) method that was used to generate them yields only a slope and a relative magnitude. Nonetheless, some first-order comparisons are both possible and useful. For both *C. finmarchicus* and cod eggs (Fig. 5), the slope of the exponential function describing mortality is consistent with the BWF for DNA damage. Examination of the scales for the BWF shows that a mortality-weighted exposure of 0.1 (*i.e.* a survivorship of exp[−0.1] = 90.5%; 10,11) corresponds to UV-induced production of about 10 CPD Mb^{-1}.

DISCUSSION

The relationship between CPD concentration and UV-B in naturally exposed organisms is influenced by many factors: the optical properties of the tissues, the orientation of the organism in the water column, the capacity for repair, the time course of exposure and the saturability of the DNA in different cell layers. In fishes, the dynamics of CPD formation and their repair have been well studied in cell lines, embryos and skin, albeit from a clinical perspective (*e.g.* 25–30). CPD formation has also been measured in fish larvae exposed to sunlight, under both natural and artificial conditions (15,17,31–33). These studies reported measurable DNA damage in samples collected from depths as great as 20 m. The diel time course of damage and repair and differences between eggs and

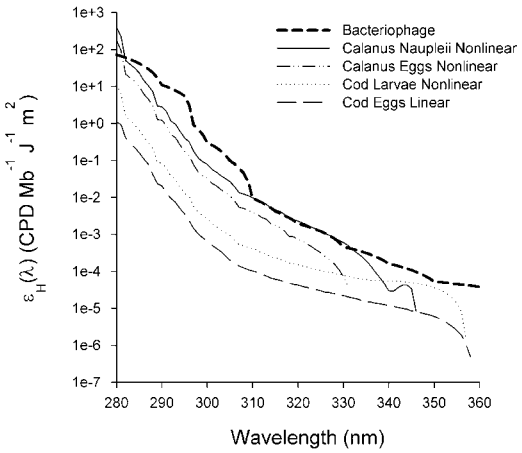

Figure 4. Comparison of the BWF for DNA damage in *Calanus finmarchicus* and Atlantic cod (*Gadus morhua*) eggs and larvae with an action spectrum for CPD formation in the T7 bacteriophage (23).

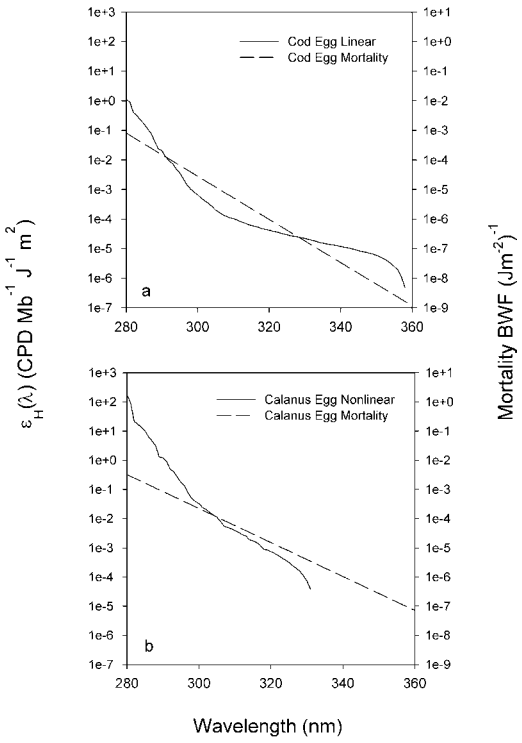

Figure 5. BWF for DNA damage (solid lines) are compared directly with BWF for mortality (dashed lines). a: CPD production in cod eggs (linear model) compared with a BWF for mortality (no photorepair, no irradiance-independent mortality) as reported by Kouwenberg *et al.* (10). b: CPD production in *Calanus* eggs (nonlinear model) compared with a BWF for mortality (no photorepair, no irradiance-independent mortality) as reported by Kouwenberg *et al.* (11). The logarithmic scales for mortality and DNA damage have been adjusted to be roughly comparable: each span a factor of 10^{10}, so equal slopes on the plots correspond to the same relative change. The maximum values on the mortality axes were adjusted, so the BWF for DNA damage and mortality were of similar magnitude in UV-B range, where environmental effects are greatest. As a result of these adjustments, the weighting for DNA damage (CPD Mb^{-1} J^{-1} m^2) at any point in the graph is 100× greater than the weighting for mortality (J m^{-2})$^{-1}$, that is, an exposure that would cause 10 CPD Mb^{-1} corresponds to a mortality of 0.1 (survival of e$^{-0.1}$), *i.e.* 90.5% survival.

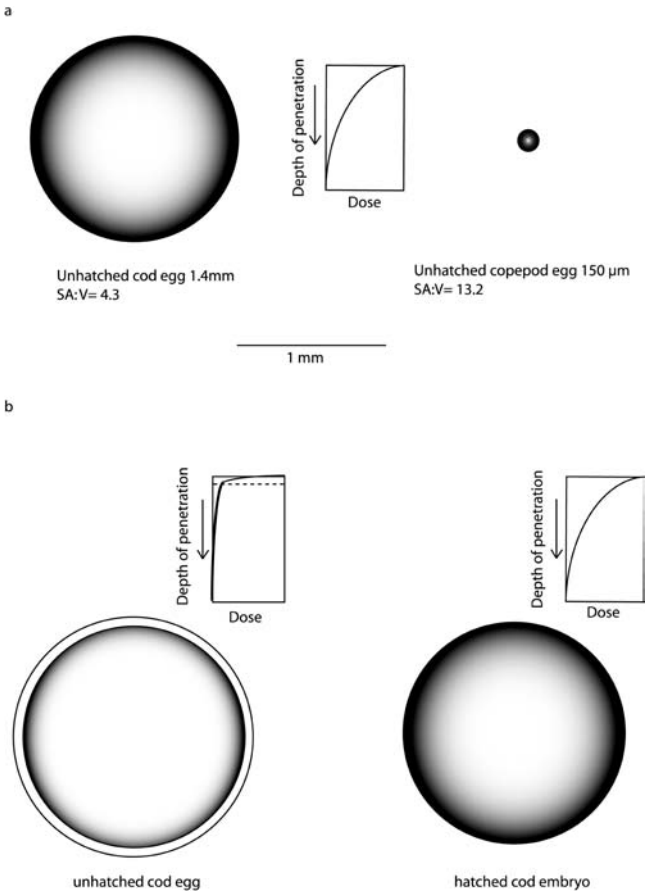

Figure 6. a: An explanation of differences in CPD concentration per unit of extracted DNA of cod and copepod eggs. In this example eggs are free to rotate, and spectral absorption properties of both species are considered similar. We also assume that UV has already penetrated the protective chorion layer and that UV is exponentially attenuated in cells in a broadband manner similar to its attenuation in seawater. Higher CPD per megabases of DNA would be due only to surface to volume ratio differences. b: An explanation of linear *vs* nonlinear exposure–damage relationships due to saturation effects at high exposures. In this example we assume that the cells in the egg and chorion-free embryo (larvae) are the same but that the chorion provides additional protection. The egg contains a layer of cells at the surface that are saturated with CPD, resulting in a flattening of the exposure–damage relationship at high exposures (Fig. 3). The gray scale shows the intensity of DNA damage, dark to light represents more damage to less damage, respectively.

larvae have also been investigated (15,17, R. D. Vetter, unpublished). Far fewer evaluations of either DNA damage or repair exist for zooplanktonic crustaceans (31,34). The results reported above—and discussed below—allow further development of CPD measurements as a predictor of environmentally induced damage in the early life stages of marine crustacean zooplankton and fishes.

As expected from previous studies on isolated DNA and bacteriophage (23,35), UV-induced damage to the DNA in *C. finmarchicus* and cod was highest in the spectral exposures that received shortwave UV-B radiation (Figs. 2–4). There was no evidence of wavelengths greater than 360 nm having a strong effect on CPD formation. This is not consistent with the observations of Lesser *et al.* (32), who reported significant CPD production associated with exposure of Atlantic cod embryos to UV-A. The reason for this disagreement is unclear, although the UV-A exposure treatment in Lesser *et al.* (32) had a 50%

transmission cuton wavelength of 315 nm. Thus, it is possible that it was the shortest wavelength in the Lesser *et al.* (32) UV-A exposure treatment that caused the DNA damage observed.

Calanus finmarchicus was much more susceptible to CPD formation than cod. In both species, eggs were less sensitive than hatched larvae (Figs. 2–4). Despite these differences between the two species and between the eggs and larvae, the shapes of the four BWF—to each other and to that for T7 bacteriophage DNA (Figs. 3 and 4)—are remarkably similar in the UV-B wave band. This is noteworthy, particularly in the context of evaluating the hypothesized protection from UV damage associated with the presence of ''screening'' compounds (*e.g.* 24,36). The observed greater UV sensitivity in larvae than in embryos suggests that the egg membrane (chorion), or the fluid-filled perivitelline space (or both), provides embryos with some protection from UV-B–induced DNA damage (Fig. 2a,b). These structures and the developing embryo can contain low–molecular weight UV-blocking compounds, such as gadusol, that absorb UV radiation (*e.g.* 36,37). Although these compounds may serve other functions (38), it has been proposed that they protect cellular function against UV damage in the developing embryos of some marine organisms (39). Also, cod eggs typically float yolk side up (as was the case in these experiments) so that the rapidly dividing cells of the embryo may be protected from downwelling UV radiation by the more inert oil globule and yolk proteins. However, differential sensitivity in eggs and larvae is not a property of all fish eggs. The egg membrane does not appear to protect Northern anchovy (*Engraulis mordax*) embryos: there are similar levels of CPD in eggs and newly hatched larvae (15, R. D. Vetter, unpublished). The influence of UV-blocking compounds has been demonstrated by showing a significant depression in a BWF (greatly reduced sensitivity) corresponding to the absorption wave band of purported UV-protective compounds (24). However, the similarity in the shapes of the BWF reported here with that of naked bacteriophage DNA (Fig. 4) indicates that screening compounds, if present, are not providing any significant (wave band specific) relief from damage at the sensitive wavelengths <330 nm for either species. The principal differences between the four BWF are in relative sensitivity at all wavelengths; this could possibly be due to differences in AT content.

DNA damage saturated as a function of biologically weighted exposure in *C. finmarchicus* eggs and nauplii but to a much lesser extent in cod eggs and larvae (Fig. 3). Species- or life-stage–specific differences in the content of broadband UV-blocking compounds might explain this observation. However, it might also result from differences in surface area to volume ratios. In a large organism, DNA in cells at the surface may be saturated with CPD, whereas those in the core receive no exposure to UV light and have little or no damage. Because the CPD assay is based on total DNA extraction, it is possible to observe saturation kinetics even though some cells (in deeper tissue) sustain little or no damage. The larger the egg or larvae, the greater the region of cells at the core that receives little or no UV, the greater the proportion of unexposed DNA and the lower the CPD load per unit DNA. Following this line of reasoning, *C. finmarchicus* eggs (which are one-tenth the diameter of cod eggs) would sustain damage to a far greater number of cells than would cod eggs (Fig. 6a). An added attenuation due to the presence of a chorion can explain the differences between eggs and newly hatched larvae (Fig. 6b). This is consistent with our earlier observation that cod larvae exhibited higher CPD loads and higher mortality when incubated under the same exposure conditions (Fig. 13.3 in Browman and Vetter [17]). With the additional protection of the chorion, cod eggs were not saturated, even for the DNA in cells at the surface of the egg. Hence, the linear relationship between dose and damage (Fig. 6b).

The BWF reported in this study can be applied to assess the mortality that would result from accumulation of a given CPD load: for both *C. finmarchicus* and cod eggs an increased load of 10 CPD Mb^{-1} would result in approximately 10% mortality (Fig. 5). This is consistent with the results of Mitchell and Karentz (18) and Yasuhira *et al.* (40) on the levels of DNA damage resulting in death of goldfish cells in culture. Although there are many complications inherent in this comparison, it is the best that is currently available. It also confirms the earlier work of Hunter *et al.* (41), who predicted that a significant proportion of the eggs and larvae of pelagic fishes are routinely damaged by sunlight. For some species, repair mechanisms appear sufficient to eliminate CPD loads under most conditions (31), whereas for other species they do not (15, R. D. Vetter, unpublished).

Our results illustrate how ozone layer thinning and concomitant changes in UV spectral irradiance at the Earth's surface (and underwater) will have the same spectrally dependent effects on eggs or larvae of *C. finmarchicus* and cod but that the magnitude of these effects are species specific. The extent to which these BWF can be applied more broadly will depend on a more accurate quantification of repair capacity in these species and of the time scales over which both DNA damage and repair operate.

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