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EFFECT OF ULTRAVIOLET IRRADIATION ON EGGS AND LARVAE OF THE NORTHERN ANCHOVY, *ENGRAULIS MORDAX*, AND THE PACIFIC MACKEREL, *SCOMBER JAPONICUS*, DURING THE EMBRYONIC STAGE*

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Abstract—Anchovy and mackerel eggs and yolk-sac larvae were exposed to UV radiation in the bioactive band of wavelengths between 280 and 320 nm, the UV-B region of the spectrum. Irradiation levels were based upon predicted UV-B increases that would result from anthropogenic diminution of Earth's protective ozone shell. Dose–response relationships for mortality and histological and morphological effects were determined for two different spectral energy compositions, using FS-40 sunlamps and two filter combinations. Anchovy were more sensitive than mackerel to UV-B. Data for anchovy were analyzed in terms of DNA-effective doses, i.e. the integrated spectral fluence (in $J/m^2/nm$) with the energy at each nm weighted by its effectiveness relative to the Setlow generalized DNA action spectrum. Fifty per cent of anchovy survived a cumulative DNA effective dose of $1150\,J \cdot m^{-2}$ over a 4-day period. In the surviving larvae, irradiation induced lesions in the brain and eye, caused marked dispersion of pigment within melanophores and retarded growth and development. At the lowest dosage used, 760 $(J \cdot m^{-2})_{DNA\ eff.}$, growth was retarded and brain lesions occurred in anchovy. Calculations of Smith and Baker (in this issue) indicate that in clear ocean water a significant incidence of lesions and retardation of growth in anchovy could occur at the surface at a 25% reduction in ozone and down to 3.5 m at a 50% reduction. Eggs and larvae of anchovy occur at these depths.

INTRODUCTION

Evidence exists for lethal or detrimental effects of UV irradiation on eggs and larvae of fishes. This literature, reviewed by Eisler (1961), describes effects in fresh water fishes, particularly the salmonids, and little information exists on pelagic marine fishes. More recently Marinaro and Bernard (1966) and Pommeranz (1974) showed that pelagic eggs of marine fishes may be quite sensitive to natural UV irradiation. Radiometric measurements in past studies, however, are inadequate for predicting the effects on marine fishes of increased solar UV radiation resulting from diminution of the ozone layer. The objective of this study was to describe the effects of UV-B radiation on eggs and larvae of two pelagic marine fish, the northern anchovy (*Engraulis mordax*) and the Pacific mackerel (*Scomber japonicus*), and to estimate the potential hazard to these species of increased levels of solar UV irradiation.

Both species occur in the egg and larval stages in the upper 1 m of the water column (Ahlstrom, 1959; Ahlstrom and Stevens, 1976), and are, therefore, exposed to significant levels of UV radiation in the sea. These species are more vulnerable to the direct effect of UV irradiation during the egg and larval stage than at any other time because of their near-surface distribution, inactivity, lack of scales or other integument, and the fact that the sensitive processes of organogenesis are taking place.

In this study we examined the effects of UV irradiation during the embryonic period, that is, during the first 4–5 days of life when the fish exist as eggs and small larvae (3–4 mm) subsisting on yolk. At the close of this period, the eyes become pigmented and functional, the jaw becomes functional, nearly all the yolk is exhausted, and feeding begins.

MATERIALS AND METHODS

Apparatus. A temperature-controlled white fiberglass water table, 366 cm long, 122 cm wide and 15 cm deep, was installed on the roof and two were installed in the marine aquarium of the Southwest Fisheries Center at La Jolla, CA. Each table was ruled into 144, 15 cm squares and each square assigned a unique number to identify it for radiometric calibrations and assignment of test containers. An aluminum framework enclosing the rooftop water table (the solarium) provided support for fluorescent UV lamps 122 cm above the water surface (Fig. 1). The frame was enclosed with "Screen Glass®" and air temperature within the enclosure was regulated with an air-conditioner and heater. The Screen Glass transmitted about 20%

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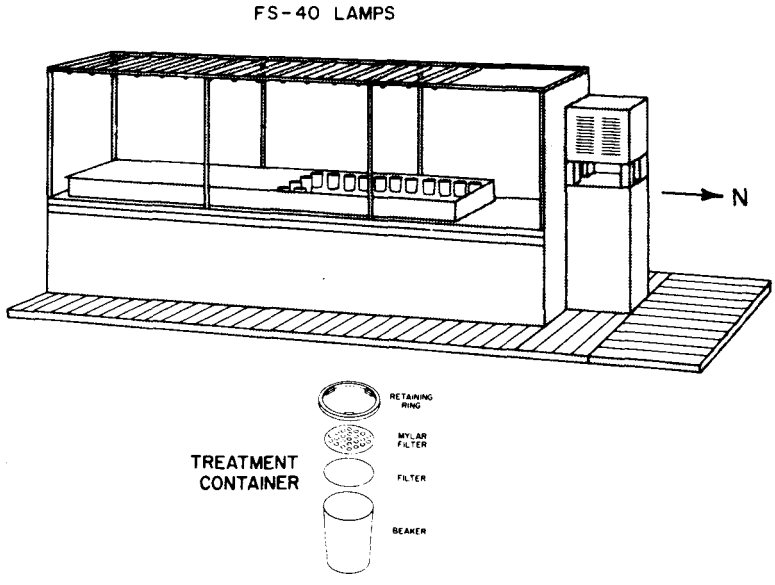

Figure 1. Diagram of roof top facility (solarium) indicating placement of treatment containers, artificial UV-B sources, and north-south orientation of treatment table. Inset shows exploded view of individual treatment container and associated filters.

of the solar UV-B but transmitted 92–94% of the natural radiation between 360 and 700 nm.

The banks of fluorescent lamps used in the aquarium experiments were mounted on large reinforced plywood sheets laminated with mirror-polished aluminum (Alzak®) to provide optimum reflective efficiency for both visible and UV wavelengths. In the solarium, however, the UV-supplementing FS40 lamps were not provided with reflectors, in order to minimize shadows on the treatment table.

In the aquarium apparatus, visible light was provided by "Chroma 50®" fluorescent lamps, which give a fair approximation to the shape of the visible solar spectrum, and the UV energy by FS-40 fluorescent sunlamps. The tubes were mounted on 5.46 cm centers; every third lamp a UV source. The aquarium luminaires were adjusted to either 93 cm or 61 cm above the water surface in the treatment containers. Before use, all lamps were aged for approx. 200 h in order to reach a relatively flat portion of the energy decay curve supplied by the manufacturer.

Irradiance of the treatment tables varied from point to point. Although these differences were not large, they were carefully measured, and taken advantage of by appropriate placement of treatment containers on the tables. Examples of the non-uniform flux distributions from artificial sources are given in Fig. 2 which shows computer-generated plots of the effect for the solarium and one of the aquarium tables. Additional smoothing of the contours was achieved by covering the ceiling and walls of the aquarium room with heavy aluminum foil.

The duration and intensity of artificial irradiation was controlled by switching the fluorescent lamps. In the aquarium studies, the Chroma 50 and FS-40 lamps were switched in accordance with Fig. 3. The rationale for this pattern of onset and offset steps takes crude account of the natural rise and fall of radiant energy on a typical day, as well as the desire to minimize any trauma to the animals. Figure 3 also indicates the approximate course of daily treatment when natural and artificial sources were combined in the solarium. All irradiation schemes were centered on local apparent noon.

Radiometry, sources, and filters. Various integrating instruments (sunburn meter—Solar Light Company; UV-B radiometer—Optronic Laboratories; deck cell illuminometer) were used for photometric and radiometric determinations during the first months of research because of the lack of a UV-sensitive spectroradiometer. It was later

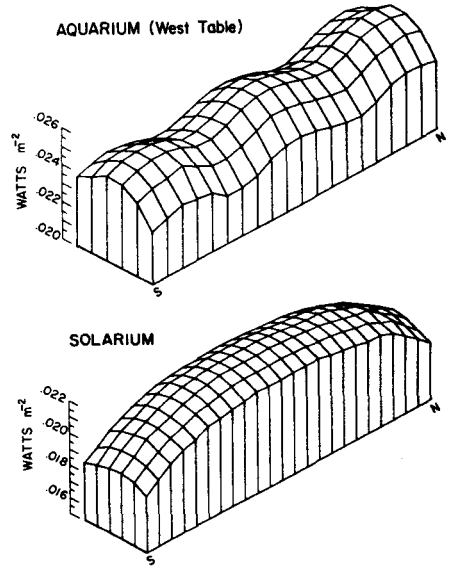

Figure 2. Computer-generated plots showing non-uniformity of irradiance over treatment tables when all UV lamps are lit. For artificial UV-B sources, lamp height was 93 cm in aquarium table and 122 cm in solarium. The aquarium table plot shows the effect of lamp end cooling.

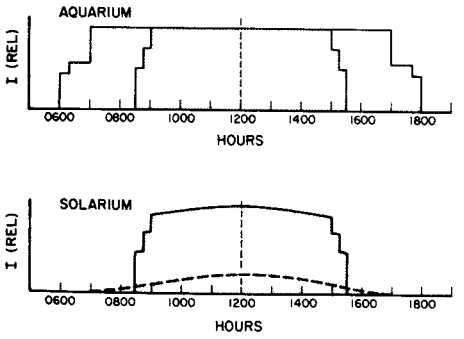

Figure 3. Schematic representation of the irradiation regimen for aquarium and for solarium. Shaded areas show UV-B component alone. In the solarium, area under curve results from switching on UV-B lamps to supplement natural component (area under dashed curve). [Local apparent time.]

possible to reconstruct the test situation and to recalibrate our measurements using a recording spectroradiometer (Optronic Model 741-V). This instrument scanned automatically from 250 to 800 nm with a 1 nm readout from 250 to 360 nm and 2 nm readout at longer wavelengths. Wavelength accuracy using a low-pressure Hg lamp for calibration was ± 0.5 nm and the spectroradiometric accuracy was ± 3%. The dynamic range was seven decades in intensity owing to the use of a logarithmic amplifier. Intensity calibration was done by use of a 1,000 W tungsten–halogen standard lamp operated at 8.000 (± 0.1%) A on a 2-m photometric bench and is traceable to the National Bureau of Standards. Doses were determined by measuring the absolute irradiances (in $W \cdot m^{-2} \cdot nm^{-1}$) integrated from 285 to 300 nm, multiplied by the duration of the experiment in h, and expressed in $J \cdot m^{-2}$.

The eggs and larval fish were irradiated in two ways: either with daylight supplemented by artificial UV-B radiation (solarium system), or by a combination of UV-B and visible light from artificial sources (aquarium system). Natural radiation was monitored, both inside and outside the canopy of the solarium, and its spectral energy distribution determined for different days.

One-ℓ polypropylene beakers, coated with black tape on the outside and containing 800 mℓ of filtered seawater were used as test containers. Each beaker was provided with a cap which held the treatment control filter (or filters) appropriate to a given condition (Fig. 1).

Various plastic materials were used in the cap for control of dosages and to prevent evaporation in the containers. They were: Polystyrene—clear sheet, 0.13 mm thick, Cellulose triacetate—clear sheet, 0.13 mm, Aclar®—clear sheet, 0.13 mm, Polystyrene 666-U—molded discs, 0.97 mm, Mylar—clear sheet, 0.13 mm.

Both polystyrenes and cellulose triacetate were used to provide different shortwave cutoff characteristics in the UV-B spectral region. The Aclar®, which transmits well in the UV-B region, was used to control evaporation, with negligible attenuation of UV-B. Mylar, which transmits little UV-B, was used in solid sheets to cover the control containers (Fig. 4). The integrated transmittance of Mylar in the UV-B band, 285–320 nm, is about 4% of that for CTA under FS-40 lamps and when weighted by the Setlow DNA action spectrum (Green and Miller, 1975), the effective transmittance of Mylar is 0.6% of that for CTA.

The experimental dosage levels (other than zero or 100% of full available intensity) were controlled by use of circular Mylar filters that were perforated by holes of various sizes, as seen in Fig. 1. Transmission of these filters was determined by the hole size with the number of holes and center-to-center distances held constant. When used with an Aclar® filter at one end of the range and a solid Mylar at the other, the range of percentages of incident UV-B flux was: 94.0, 69.6, 49.7, 29.0, 23.5, 18.9, 10.7, ~0

The optical geometry resulting from use of perforated filters produces non-uniformity of irradiation across the water surface. The effect is greatest for the direct solar component of natural UV-B under lower-transmittance filters, but considerably less for extended sources (sky dome and fluorescent luminaires) and larger hole sizes.

Experimental design. Eggs of anchovy and mackerel were obtained from brood stock maintained in reproductive condition throughout the year at the Southwest Fisheries Center (Leong, 1971, 1977). In most experiments, eggs were held for 24 h at 16°C before stocking to reduce incidence of non-viable eggs. Each treatment and control consisted of 15 test containers stocked with 50 eggs each. Areas of similar incident UV-B irradiation on each water table were identified by square number and treatment and control containers were assigned a square within these fields on a random basis. Specific fields were selected for specific treatments to increase uniformity of dosage within a treatment; controls were partitioned equally in different fields but square assignment within a field was random. Areas of high variability such as along the sides and ends of tables or beneath the ends of lamps in the west aquarium table were avoided.

Experiments ended at the close of the embryonic period. Anchovy were exposed for about 6–10 h in the egg stage and 15–20 h as yolk-sac larvae, and mackerel about 6–16 h as eggs and 25–32 h as larvae. Three experimental conditions were used: in the first, we used the solarium enclosed by Screen Glass®, the source of UV energy was FS-40 lamps filtered by polystyrene caps on the beakers, and the sun was the source of visible energy (anchovy exps. 1 and

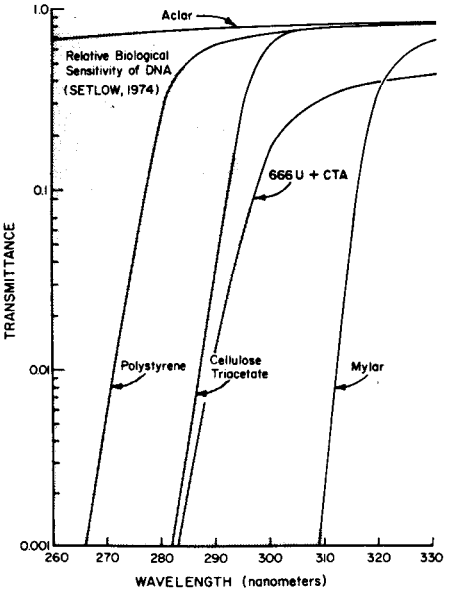

Figure 4. Spectral transmittance curves for the plastic filter materials used in the experiments. The shaded region represents the relative biological sensitivity of DNA as a function of wavelength.

Table 1. Effect of UV on anchovy during embryonic stage

Expt. no. (date)	UV source	Visible source	Filter[1]	UV lamp exposure egg h	UV lamp exposure larvae h	Total UV lamp exposure h	Total visible dosage meter-candle h	Dosage[2] J·m^{-2}	Number of containers	Mean number of survivors[3] $\bar{x}$	Mean number of survivors[3] s	Percent survival normalized to controls
1 (5–2)	FS40	Solar	M	0	0	0	12,000	0	15	21.67	6.67	100.0
			PS	10.00	15.00	25.00	12,000	13,000	15	19.47	8.53	89.8
			PS	10.00	15.00	25.00	12,000	26,900	15	11.67	8.09	53.9
			PS	10.00	15.00	25.00	12,000	41,100	13	8.77	5.08	40.5
			PS	10.00	15.00	25.00	12,000	69,600	15	0.33	0.72	1.5
2 (6–7)	FS40	Solar	M	0	0	0	9,240	0	16	30.75	7.59	100.0
			PS	10.00	17.00	27.00	9,240	14,200	15	30.26	5.20	98.4
			PS	10.00	17.00	27.00	9,240	29,400	15	19.20	9.32	62.4
			PS	10.00	17.00	27.00	9,240	44,900	15	6.60	3.26	21.5
			PS	10.00	17.00	27.00	9,240	91,100	15	0.33	0.62	1.1
3 (7–25)	FS40	Chroma 50	M	0	0	0	4,400	0	15	25.40	6.51	100.0
			CTA	6.20	21.00	27.20	4,400	68,300	15	23.10	5.12	90.9
			CTA	6.20	21.00	27.20	4,400	94,000	14	9.57	5.81	37.7
			CTA	6.20	21.00	27.20	4,400	121,000	15	3.73	2.49	14.7
			CTA	6.20	21.00	27.20	3,770	162,000	15	0.13	0.52	0.5
4 (8–1)	FS40	Chroma 50	M	0	0	0	3,930	0	15	41.20	2.78	100.0
			CTA	5.25	21.00	26.25	3,930	62,500	15	39.07	2.89	94.8
			CTA	5.25	21.00	26.25	3,930	90,400	15	30.60	6.07	74.3
			CTA	5.25	21.00	26.25	3,930	115,000	15	16.47	6.03	40.0
			CTA	5.25	21.00	26.25	3,360	148,000	15	0.33	1.29	0.8
5 (8–22)	FS40	Chroma 50	M	0	0	0	4,360	0	15	22.93	8.04	100.0
			CTA	6.58	19.75	26.33	4,360	92,000	15	6.53	4.27	28.5
			CTA	6.58	19.75	26.33	4,360	109,000	15	1.47	1.41	6.4
			CTA	6.58	19.75	26.33	4,360	117,000	15	1.13	1.06	4.9
6 (12–19)	FS40	Chroma 50	M	0	0	0	4,300	0	15	40.50	3.46	100.0
			666–U + CTA	6.00	20.33	26.33	4,300	248,000	16	19.3	7.33	47.6

[1]M = Mylar; PS = Polystyrene; CTA = Cellulose triacetate; 666–U = Polystyrene 666–U.
[2]275–320 nm PS experiment; 285–320 nm CTA experiment.
[3]$\bar{x}$ = Mean number survivors per container; s = Standard deviation.

Table 2. Effect of UV on mackerel during embryonic stage

Expt. no. (date)	UV source	Visible source	Filter[1]	UV lamp exposure egg h	UV lamp exposure larvae h	Total UV lamp exposure h	Total visible dosage meter-candle h	Dosage[2] J·m^{-2}	Number of containers	Mean number of survivors[3] $\bar{x}$	Mean number of survivors[3] s	Percent survival normalized to controls
1 (4–25)	FS40	Solar	M	0	0	0	11,400	0	15	43.93	2.60	100.0
			PS	10.00	15.58	25.58	11,400	25,400	15	40.93	4.13	93.2
			PS	10.00	15.58	25.58	11,400	38,400	15	43.27	4.48	98.4
			PS	10.00	15.58	25.58	11,400	65,500	15	23.07	9.85	52.5
			PS	10.00	15.58	25.58	11,400	114,000	15	0.00	0.00	0.0
2 (7–19)	FS40	Chroma 50	M	0	0	0	5,780	0	15	37.26	11.16	100.0
			CTA	16.00	17.50	32.50	5,780	127,000	15	20.07	9.11	53.8
			CTA	16.00	17.50	32.50	5,780	159,000	15	7.13	8.43	19.1
			CTA	16.00	17.50	32.50	4,940	199,000	15	0.27	0.59	0.7
			CTA	16.00	17.50	32.50	5,780	254,000	15	0.00	0.00	0.0
3 (8–9)	FS40	Chroma 50	M	0	0	0	4,730	0	15	39.00	7.14	100.0
			CTA	7.75	17.25	25.00	4,730	65,600	15	38.53	7.38	98.8
			CTA	7.75	17.25	25.00	4,040	91,300	15	43.13	2.69	110.6
			CTA	7.75	17.25	25.00	4,730	94,900	15	43.73	4.04	112.1
			CTA	7.75	17.25	25.00	4,730	120,000	15	31.67	13.96	81.2
4 (8–30)	FS40	Chroma 50	M	0	0	0	4,400	0	15	46.20	2.76	100.0
			CTA	6.00	21.00	27.00	4,400	93,300	15	33.73	9.16	67.5
			CTA	6.00	21.00	27.00	4,400	125,000	14	19.00	14.33	41.1
			CTA	6.00	21.00	27.00	4,400	177,000	15	8.07	8.22	17.5

[1]M = Mylar; PS = Polystyrene; CTA = Cellulose triacetate.
[2]275–320 nm PS experiments; 285–320 nm CTA experiments.
[3]$\bar{x}$ = Mean number survivors per container; s = Standard deviation.

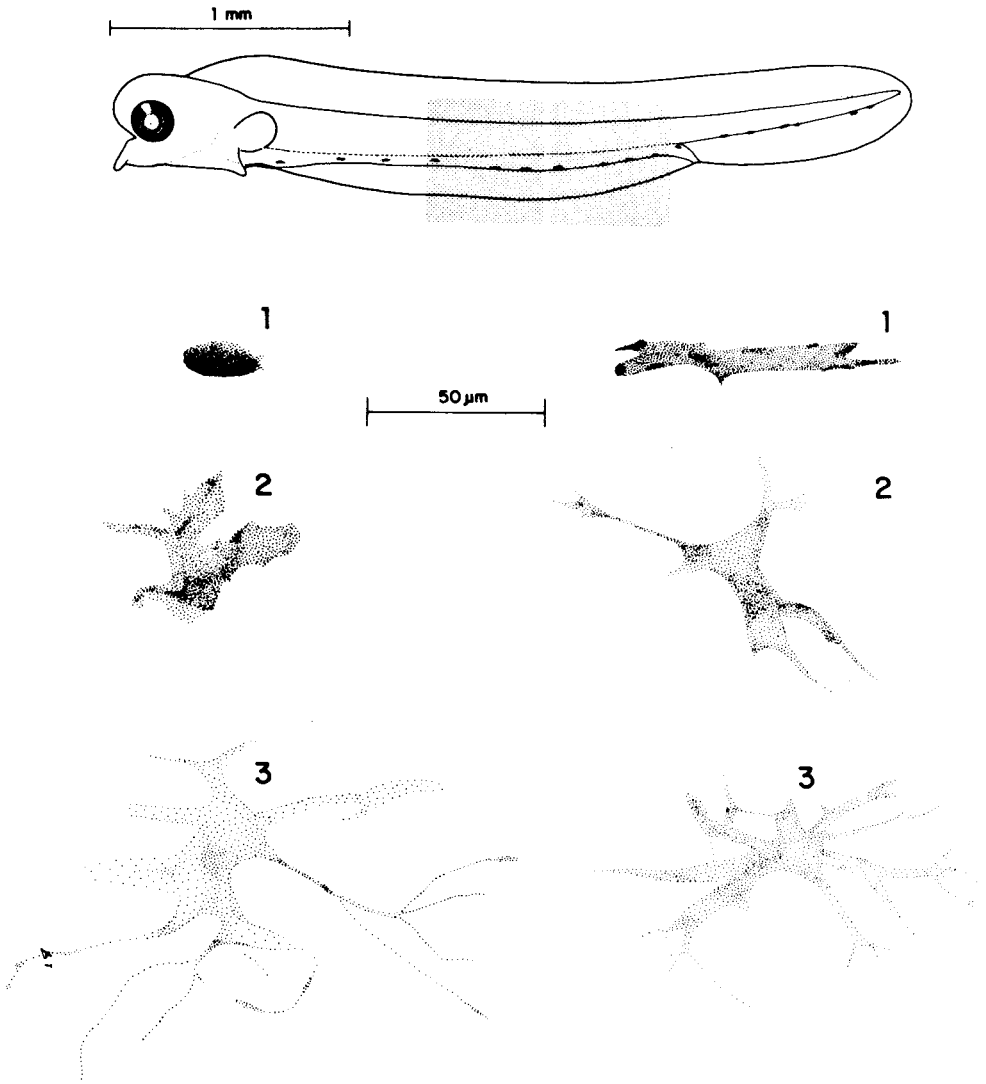

Figure 5. State of melanosome dispersion in Mylar control and UV-B irradiated larvae drawn to scale from photomicrographs. Shaded areas on larvae indicate areas on body used in anchovy for grading. Numbers are grades used to quantify dispersion: grade 1 was typical of controls; grades 2–3 occurred in irradiated specimens. Right and left columns of figure indicate the range of dispersion states included within a grade.

2, Table 1; mackerel exp. 1, Table 2); in the second, aquarium tables were used, the source of UV energy was FS-40 lamps filtered by cellulose triacetate (CTA) and the source of visible energy was Chroma 50® lamps (anchovy exps. 3, 4, and 5; mackerel exps. 2, 3, and 4); the third, the aquarium table was used with FS-40 lamps filtered by polystyrene 666-U and CTA (anchovy exp. 6).

Water temperature in the containers, and UV-B and visible flux were monitored continuously, and salinity, oxygen and ammonia at the beginning and end of each experiment. Salinity varied from 33.0 to 34.0‰, oxygen from 97.3 to 71.2% saturation, ammonia from 0.010 to 0.091 ppm and temperature from 16.3 to 17.0° C. Surviving larvae were counted at the end of an experiment; some fixed in Bouin's solution for histological analysis, and the rest preserved in 3% formalin.

Formalin-preserved specimens from experiments employing CTA filters, were examined to determine effects of UV-B on morphology and pigmentation. We measured the standard length, body depth at the origin of the pectoral fin, maximum depth of the yolk-sac, eye pigmentation, and extent of pigment dispersion in melanophores of 32–85 larvae per treatment. Typically, five larvae per container within a treatment were measured, but numbers varied with the number of survivors. Larvae were assigned a grade of 1 to 3 on the basis of eye pigmentation: Grade

1, only a trace of pigment or pigment distributed around only the outer margin of the eye; grade 2, intermediate pigmentation; and grade 3, eye completely pigmented. The state of dispersion of the melanosomes (pigment granules) within the melanophores was estimated by assigning grade 1 to the completely aggregated condition and grade 3 to the fully dispersed (Fig. 5). In anchovy, two regions along the ventral margin of the body were graded, and in mackerel, because they had more melanophores, four regions were used: the dorsal surface of the head; yolk-sac; hind gut; and ventral margin of the body.

Complete sagittal serial sections cut at 5–6 μm were prepared from each specimen preserved for histological work. Slides from selected experiments were examined for the number of lesions in the brain and nuclear layer of the retina. Larvae were examined without knowledge of dosage to avoid interpretative prejudice. Lesions were defined as consisting of one or more spherical pycnotic nuclei surrounded by an extracellular vacuolated region, frequently containing eosinophilic cytoplasmic debris. To provide an index of damage as a function of dosage, we counted the number of lesions in the brain and eye in every section of a larva and divided the total by the number of sections examined. Incidence was defined as the average number of lesions in a tissue per section. The number of sections examined per larva varied, ranges for anchovy were: eyes, 33–40; brain, 27–43; and for mackerel: eyes, 45–55; brain, 41–50. Typically 12–15 larvae per treatment were examined but fewer in treatments with very low survival.

Survival in the controls varied among anchovy experiments from 43 to 80% and from 74 to 92% in mackerel, because of differences in egg viability between spawns (Tables 1 and 2). To adjust for these differences, we divided the percent survival in the treatments by that of the controls in each experiment. These normalized survival data were used to calculate a dose-response line using probit analysis (Finney, 1952). Adjusting the treatment data in this manner increases the survival probabilities, and thereby decreases the variance. This underestimate was proportional to the reciprocal of the percent survival of the controls and we have corrected the standard deviation by multiplying by this factor.

RESULTS

Survival

In experiments conducted in the aquarium using FS-40 lamps and cellulose triacetate filters (CTA), 50% of the anchovy survived a cumulative UV-B (285 to 320 nm) dosage of 91,200 J·m^{-2} (95% C.I. = 80,700–100,000 J·m^{-2}) over a 4-day period (Fig. 6) and 50% of the mackerel survived a cumulative UV-B dosage of 125,000 J·m^{-2} (95% C.I. = 98,900–148,000 J·m^{-2}) over a 4- to 5-day period (Fig. 7). When the energy from FS-40 lamps was filtered by polystyrene (PS), the LD_{50} occurred at lower dosages. In these experiments 50% of the anchovy survived a cumulative dosage (275–320 nm) of 31,000 J·m^{-2} (95% C.I. = 25,3000–38,200 J·m^{-2}) (Fig. 6), and data from a single experiment suggest that 50% of mackerel are able to survive a dose of about 65,500 J·m^{-2} over a 4-day period (Table 2, exp. 1). Differences in the dose–response relation between experiments employing CTA filters and those using PS filters may be attributed to differences in the spectral transmission of the two filters. The PS filter transmits about 30% of the energy at 280 nm whereas the CTA filter transmits less than 1% (Fig. 4). Thus, larvae and eggs under the PS filter received more energy in the shorter and more actinic wavelengths of the UV band. Under both experimental conditions, anchovy were more sensitive to UV irradiation than mackerel. The difference in the LD_{50} was about 34,500 J·m^{-2} under the two experimental conditions.

The likelihood of biological effects may be seriously underestimated if measurement of dose is based on integrated flux alone. Ozone diminution results in a disproportionately large increase in radiant energy in

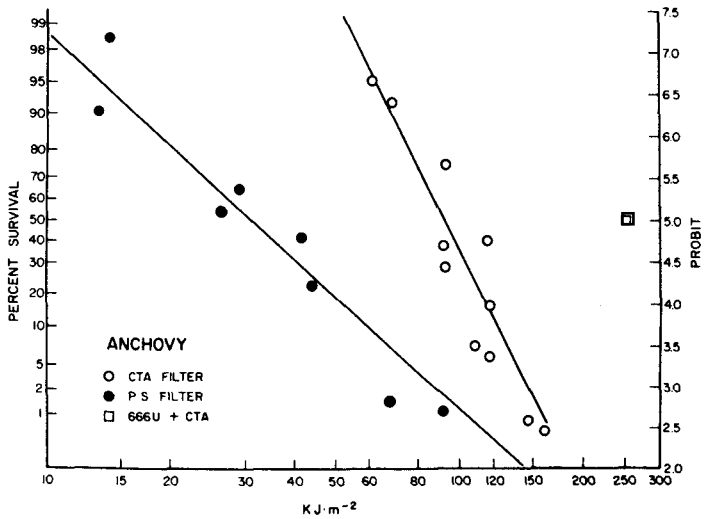

Figure 6. Per cent survival of northern anchovy at the end of the embryonic period (probit scale) and total cumulative dose of UV-B (log scale) in kJ·m^{-2}, for three spectral energy distributions. Energy sources were: FS-40 lamps with cellulose triacetate filters (CTA); polystyrene filters (PS); and polystyrene 666-U + CTA. Lines are regressions of mortality probit on log dose and points are the mean survival for 15 containers.

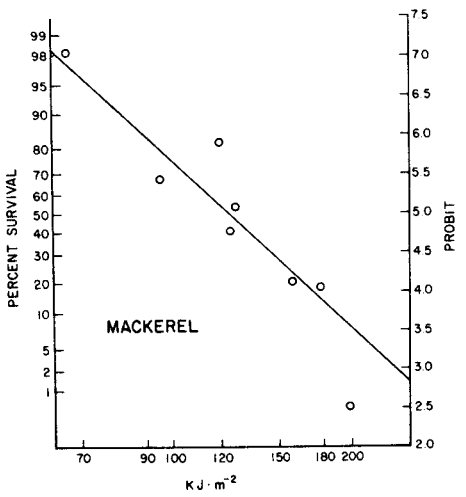

Figure 7. Percent survival of Pacific mackerel at the end of the embryonic period (probit scale) and total cumulative dose of UV-B (log scale) in $kJ \cdot m^{-2}$. Energy source was FS-40 lamps filtered by cellulose triacetate (CTA); points are mean survival for 15 containers.

that part of the UV-B spectral band to which larval fishes are the most sensitive. Thus, an effective dose must be based upon a weighting function that takes account of the wavelength dependency of biological action. Accordingly, we applied several existing weighting functions to the combined data from the CTA and PS experiments: germicidal action spectrum (Kaufman and Christiansen, 1972); Caldwell's generalized action spectrum (Green and Miller, 1975); and Setlow's action spectrum for DNA (Green and Miller, 1975). The spectral energy distribution for each treatment under CTA and PS was weighted by multiplying the energy at each nanometer by the appropriate coefficient from the particular weighting function and the weighted energy for a particular treatment integrated. Table 3 gives the unweighted energy per nanometer for the LD_{50} in the PS and CTA experiments upon which these calculations are based.

The weight that gave the best fit to regression of probit on log weighted dose for the combined data was the Setlow DNA action spectrum (Fig. 8). For example, the LD_{50} estimates for the CTA and PS experiments, when weighted by the Setlow DNA action spectrum, differed by only 8%, whereas they differed by 42% when weighted by the Caldwell action spectrum. As can be seen by comparing Figs. 6 and 8, the Setlow weighting function provided an adequate adjustment for the differences in spectral composition between the two experiments. The LD_{50} for anchovy using the DNA weighted dosages from the two experiments, was 1150 $J \cdot m^{-2}$ (95% C.I. = 990–1400).

As a test of this weighting procedure, we conducted an experiment using a filter combination (polystyrene 666-U + CTA) that produced a cutoff at a longer wavelength than either PS or CTA filters (Fig. 4). In this experiment, 47.7 ± 9.0% of the anchovy larvae survived a dosage of 248,000 $J \cdot m^{-2}$. This dosage is much higher than those producing comparable survival in the other experiments (Fig. 6) but when weighted by the DNA action spectrum it is reasonably close to those values (Fig. 8). Thus, the Setlow DNA action spectrum was a relatively good model for predicting lethal UV-B effects in anchovy larvae under differing spectral energy distributions. This result not only unifies the results from experiments employing different spectral energy distributions but also makes possible calculation of dose levels that would occur under various conditions of ozone diminution.

Histological effects

Lesions occurred in the brain and the eye in anchovy and mackerel larvae surviving exposure to UV irradiation (Fig. 9). Incidence of lesions was higher in the PS experiments than in the CTA experiments as could be expected from the difference in spectral energy composition of UV-B. It required roughly twice the dosage to produce an equivalent response using CTA filters than when PS filters were used. For example, the mean incidence of eye lesions in anchovy was 0.53 ± 0.18 at a dosage of 45,000 $J \cdot m^{-2}$ in the PS experiments whereas it required a dosage of 94,000 $J \cdot m^{-2}$ to produce an incidence of 0.44 ± 0.28 in the CTA experiments. More specimens were examined histologically in the CTA experiments

Table 3. Dose per nm at the LD_{50}[1] of northern anchovy larvae for polystyrene and cellulose triacetate filters under FS-40 lamps

Wavelength (nm)	Dose ($J \cdot m^{-2}$) PS	CTA	Wavelength (nm)	Dose ($J \cdot m^{-2}$) PS	CTA
276	8	—	299	839	2320
277	10	—	300	901	2640
278	14	—	301	939	2890
279	20	—	302	1010	3190
280	30	—	303	1070	3440
281	41	—	304	1070	3510
282	55	—	305	1130	3750
283	71	—	306	1160	3900
284	91	—	307	1190	3980
285	114	—	308	1200	4060
286	148	22	309	1240	4180
287	181	23	310	1280	4370
288	218	26	311	1370	4650
289	259	35	312	1440	4900
290	303	59	313	1460	5010
291	341	109	314	1360	4660
292	387	205	315	1270	4360
293	447	369	316	1200	4170
294	510	604	317	1170	4050
295	582	912	318	1150	3990
296	660	1270	319	1130	3930
297	716	1610	320	1100	3840
298	783	1990			

[1]Sum of values in table are within 2% of integrated dosages given in text.

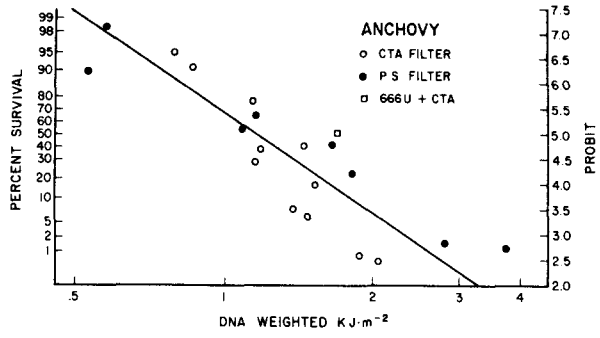

Figure 8. Per cent survival of northern anchovy at the end of the embryonic period (probit scale) and total cumulative dose of UV-B weighted by DNA action spectrum of Setlow (1974) using the analytical fit of Green and Miller (1975). Line is the regression of mortality probit on log dosage for the combined weighted data from the CTA and PS experiments.

and the incidence of lesions was plotted as a function of dosage (Fig. 10). Even at the lowest dosages employed in the CTA experiment, 63,000 $J \cdot m^{-2}$, incidence of lesions in the brain of anchovy was different from the control (t test, $P < 0.01$) and that in the eye was between the 5 and 10% probability levels. At 68,000 $J \cdot m^{-2}$, incidence of lesions in both brain and eye in anchovy were different from the control ($P < 0.01$ for brain; $P < 0.05$ for eye). Thus, significant damage to both brain and eye occurred after only 4 days at our lowest dosages.

The incidence of lesions in brain and eye was much higher in anchovy than in mackerel. At dosages near the LD_{50} for each species the incidence of eye lesions was about three times and brain lesions five times higher in anchovy than in mackerel. Eye lesions ranged from 5 to 48 μm (maximum dimension) in anchovy whereas in mackerel they were somewhat smaller, usually ranging from 5 to 10 μm and occasionally to 24 μm. Lesions also occurred in the olfactory bulb in both species but were not evaluated. The treatments affected the rate of development of melanistic pigment in the eye. The proportion of larvae examined histologically with partially pigmented or unpigmented eyes increased in both species with dosage. This effect is described in more detail from morphological examination and is discussed in the next section.

Morphological effects

During the yolk-sac period, the yolk supply decreases as larvae increase in length and body depth; during the last day, the eye becomes progressively more pigmented, until at the end of the period eye pigmentation is complete and the yolk supply is nearly exhausted. At the end of our experiments, all larvae in the controls were at this later stage of development. Treated larvae were smaller in length and depth, had more yolk, and less eye pigmentation than the controls (Figs. 11 and 12). Thus, UV-B irradiation retarded development in larvae and the extent of retardation was related to dosage. At the lowest dosage in our experiments (63,000 $J \cdot m^{-2}$) length, body depth, eye pigmentation, and yolk diameter were different from the controls (t test $P < 0.001$) indicating a significant effect of UV-B irradiation on growth and development even at the lowest dosage.

The melanosomes in the melanophores of anchovy and mackerel larvae were dispersed in those exposed to UV-B radiation, whereas they were aggregated in the controls (Fig. 13). The extent of dispersion as indicated by our grading system increased with dosage. Melanosomes in the fully dispersed condition (grade 3, Fig. 5) occurred commonly at dosages at and above 90,000 $J \cdot m^{-2}$ and less commonly at lower dosages, but even at the lowest dosage (63,000 $J \cdot m^{-2}$) pigment in anchovy melanophores was more dispersed than the controls (t test, $P < 0.001$).

The literature on effects of UV irradiation on pigment movement within melanophores of fishes is limited to effects of energy from germicidal lamps. In contrast to our findings, these studies indicate that UV irradiation at 254 nm induces some aggregation rather than dispersion of melanosomes (Fujii, 1973; Fujii *et al.*, 1973). In addition, Fujii *et al.* (1973) demonstrated that UV irradiation caused a depression of the response of the melanophore to pigment aggregating substances (norepinephrine and melatonin) which was proportional to dosage. They suggested the UV-induced inhibition of melanosome mobility was caused by UV-induced lesions in the neuro-effector system. To determine if the changes we observed in response to UV-B were reversible or were caused by neural damage would require further experimentation and submicroscopic analysis.

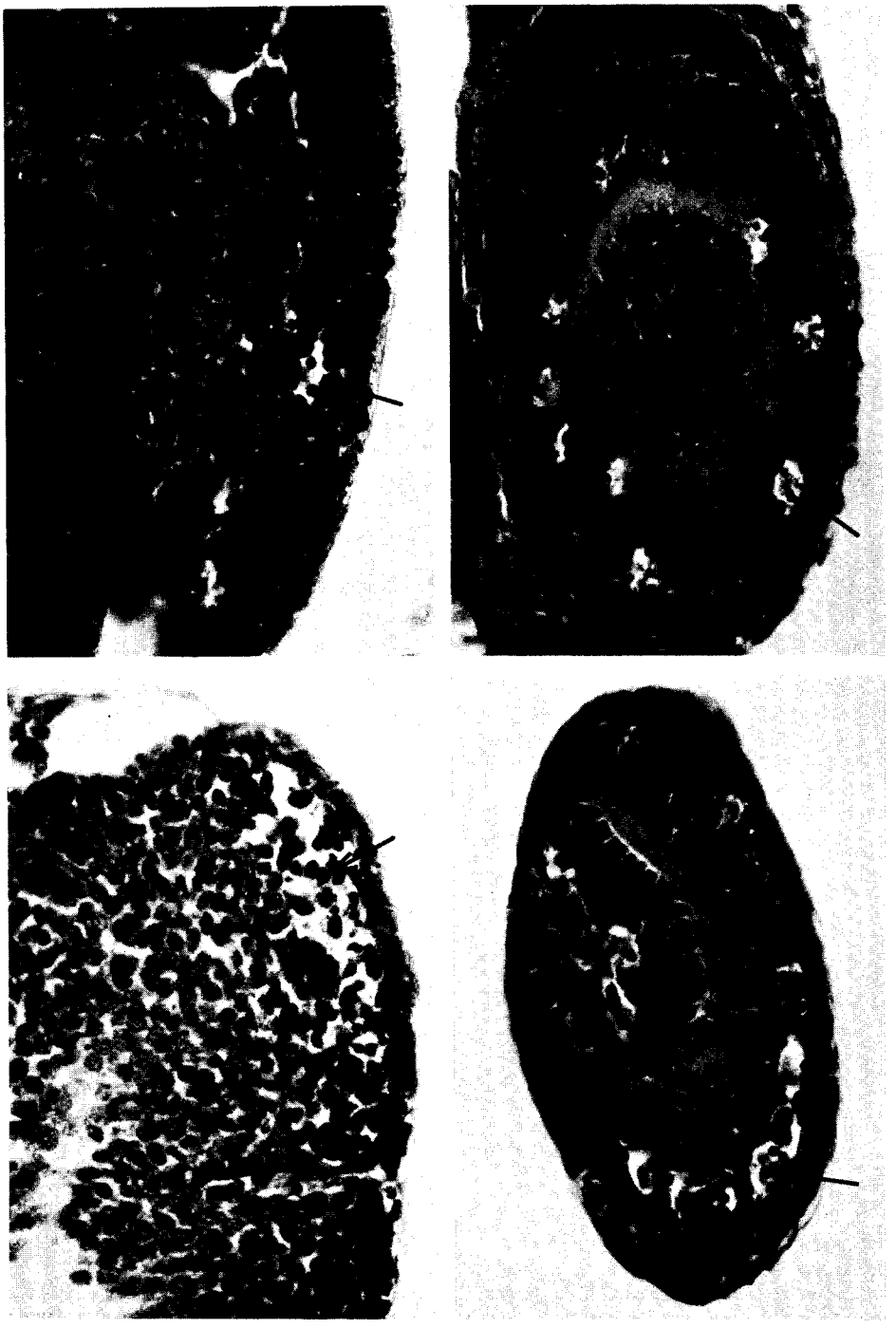

Figure 9. Eye (top left) and brain (top right) of northern anchovy larvae and eye (bottom left) and brain (bottom right) of Pacific mackerel larvae showing lesions (arrows) produced by UV irradiation with CTA filters. Irradiations were 109 k $J \cdot m^{-2}$ (top left), 115 k $J \cdot m^{-2}$ (top right), 199 k $J \cdot m^{-2}$ (bottom left), 199 k $J \cdot m^{-2}$ (bottom right). Sagittal sections stained with H and E, 400X.

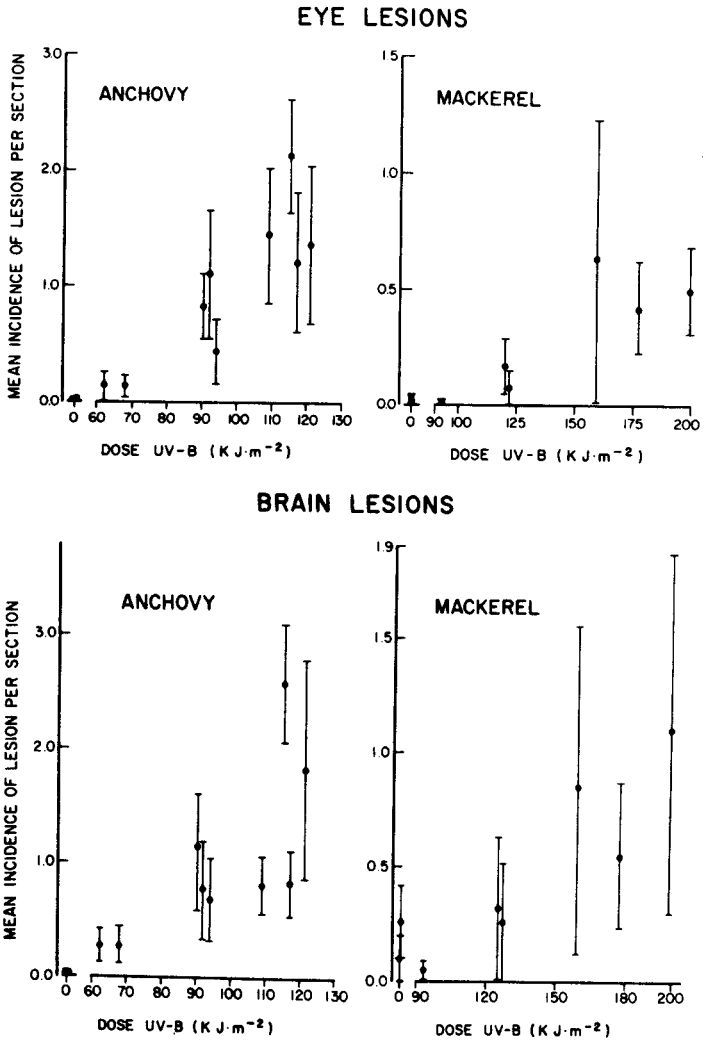

Figure 10. Mean incidence of lesions in the eye and brain of northern anchovy and Pacific mackerel larvae that survived various unweighted doses of UV-B irradiation. UV energy source was FS-40 lamps filtered by CTA; controls, ~0 UV-B, had Mylar filters; points are means for treatments, and vertical bars are two standard errors.

DISCUSSION

Our approach in these experiments was to simulate natural conditions and we did not evaluate photoreactivating repair of UV-induced damage. In the aquarium system white lights were turned on 2.5 h before and continued for 2.5 h after UV-B exposure (Fig. 4). The flux in the region for photoreactivation, however, was somewhat lower in the aquarium system than under natural conditions and this could have affected the results. That the DNA-weighted dose–response relation was about the same in experiments using natural solar irradiation supplemented with UV (anchovy PS experiments) as in the aquarium experiments suggests that our results were not affected by the lower level of photoreactivating energy in the aquarium system.

A striking feature of these results was the occurrence of marked and clearly defined damage after an exposure of only 4 days. The damage included lesions in the brain and retina, marked retardation of growth and development and abnormal dispersion of melanosomes. Significant damage occurred not only in those larvae surviving a 50% mortality dose but in the survivors of all dosages. It seems unlikely that any of the damaged survivors, regardless of dosage, would

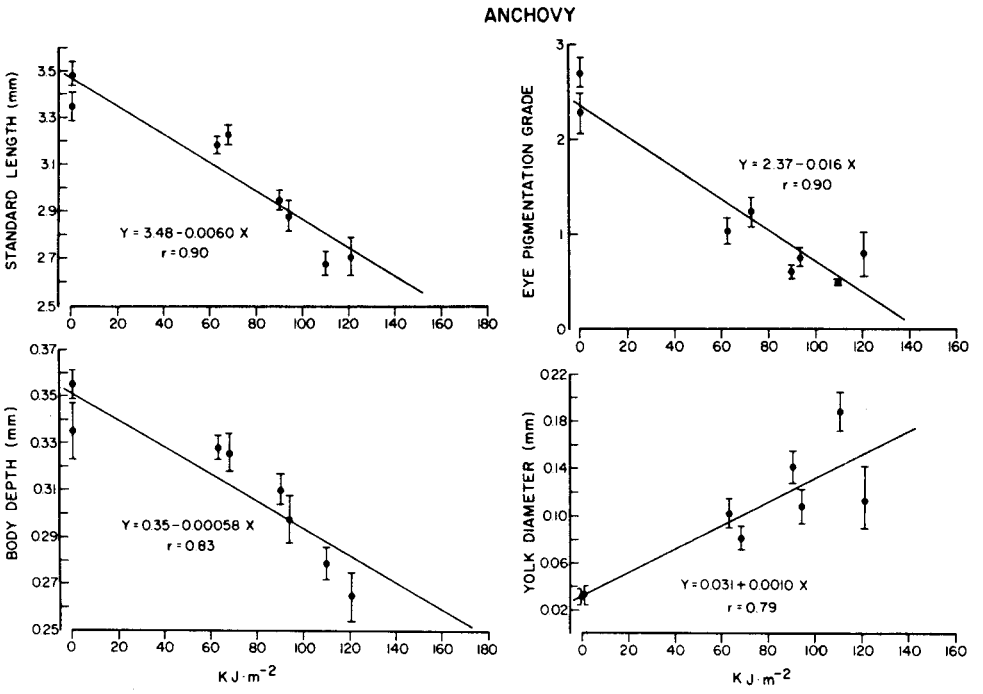

Figure 11. Standard length, body depth, eye pigmentation grade, and yolk diameter of larval northern anchovy surviving various doses of UV-B (FS-40 lamps, CTA filters). Mylar-filtered controls are shown as ~0 dose; points are treatment means; and bars, two standard errors.

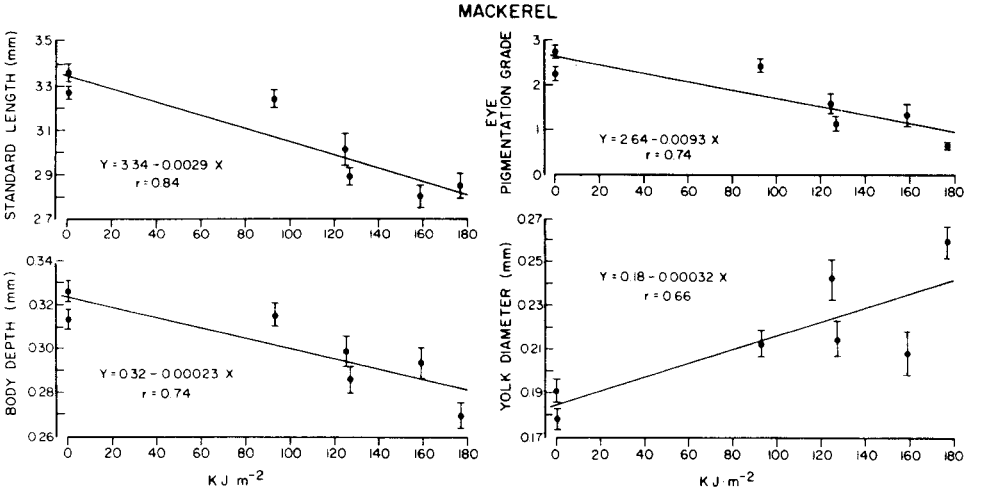

Figure 12. Standard length, body depth, eye pigmentation grade and yolk diameter of larval Pacific mackerel surviving various doses of UV-B (FS-40 lamps; CTA filters). Mylar filtered controls are shown as ~0 dose; points are treatment means; and bars, two standard errors.

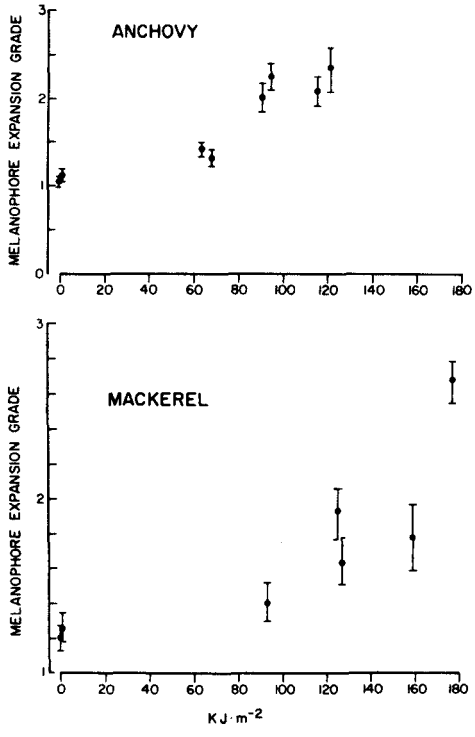

Figure 13. Relation between average melanosome dispersion grade and unweighted dosage in anchovy and mackerel larvae. Source of irradiation was FS-40 lamps with CTA filters. Mylar controls are shown as ~0 dose; points are means for treatments; and bars, two standard errors. Grade 1 is completely aggregated condition; and grade 3 completely dispersed (see Fig. 5).

be able to feed successfully. Retardation of development is another potential source of mortality under natural conditions because it prolongs the stage of highest vulnerability. Natural mortality in the sea during the embryonic period (egg through yolk-sac stages) is generally 3–10 times higher than after the larvae begin feeding (Jones and Hall, 1974).

We estimated from solar irradiance measurements made in September–November that the DNA weighted dose for 4 days in June in La Jolla would be 439 $[J{\cdot}m^{-2}{\cdot}(4\ day)^{-1}]_{DNA\ eff.}$, assuming the daily dose to be about six times incidence at solar noon. Assuming a 6% loss through the sea surface gives a value of 413 $[J{\cdot}m^{-2}{\cdot}(4\ day)^{-1}]_{DNA\ eff.}$ for the DNA weighted dose just beneath the surface. This estimate differed by only 2% from the value 422 $[J{\cdot}m^{-2}{\cdot}(4\ day)^{-1}]_{DNA\ eff.}$ calculated by Smith and Baker (in this issue) using Green *et al.*'s (1974) semi-empirical analytic formula with coefficients fit to multiscattering data to obtain the total daily dose just beneath the sea surface in mid-June at La Jolla for the "ambient" ozone depth of 0.32 cm. Thus, to relate our experimental results to effects of ozone depletion, Green's analytic formula for downward spectral irradiance ($[W{\cdot}m^{-2}{\cdot}nm^{-1}]$) was transmitted through sea surface, then weighted by the Setlow DNA action spectrum and integrated to obtain the biologically effective downward irradiance ($[W{\cdot}m^{-2}]_{DNA\ eff.}$). This result was then integrated, as a function of sun angle, over the course of a day and multiplied by four to obtain the total 4-day biologically effective dose ($[J{\cdot}m^{-2}{\cdot}(4\ day)^{-1}]_{DNA\ eff.}$). These calculations, expressed as the total cumulative, Setlow DNA weighted, UV flux in $J{\cdot}m^{-2}$ for 4 days in June are compared to our results for anchovy below:

$[J{\cdot}m^{-2}{\cdot}(4\ day)^{-1}]_{DNA\ eff.}$	Event
422	0.32 cm ozone ("ambient")
564	0.28 cm ozone (13% reduction)
758	0.24 cm ozone (25% reduction)
760	First incidence of lesions and retardation of growth in anchovy
1150	LD_{50} for anchovy
1434	0.16 cm ozone (50% reduction)

These calculations indicate that incidence of lesions and retardation of growth in anchovy larvae occur at dosages that could be expected to occur at the surface in 4 days in June at our latitude provided that the optical ozone depth is reduced about 25%. Fifty % mortality could be expected to occur at the surface in 4 days at a level between a 25 and 50% reduction. Smith and Baker (in this issue) have carried the calculations a step further and have estimated the DNA weighted dosage as a function of depth in the sea for various water types. Their calculations indicate that in clear ocean water a significant incidence of lesions and retardation of growth could occur only at the surface at a 25% reduction in ozone and down to 3.5 m at a 50% reduction. Eggs and larvae of anchovy commonly occur at these depths but an assessment of the potential damage to pelagic fish populations would require additional work. These estimates depend on the assumption that the Setlow DNA weighting function adequately expresses the relation between wavelength and dosage in anchovy, and on statistical and radiometric uncertainties.

It must be reiterated that our experiments to date apply only to the first 4 days of life; the fate of surviving larvae, particularly those who exhibit lesions, who must now begin to seek food and avoid predators, remains to be studied. If we assume that approximate reciprocity holds, as it appears to for the relatively short periods we have studied (4–5 days), then lower dosage rates over a longer time period could produce the same effects found at higher rates for a few days. The effects of long-term chronic dosage, must be evaluated as such studies probably would alter not only our present projections but could be of great significance in understanding the course of pathological changes induced by UV exposure. Induction of tumors might be one of the characteristic pathological changes revealed by longer term studies. For example, Hart and Setlow (1975) have shown that single exposures of cells of the fish *Poecilia formosa* to UV irradiation (254 nm) resulted in neoplastic transformation after 3–9 months.

The level of UV-B enhancement needed to produce structural anomalies and retard development argues for maintaining the integrity of the ozone shell. Whether or not the animals would or, in fact, could use evasive tactics (such as swimming deeper) is moot, and it is unlikely that adaptive evolutionary changes could be effected in time.

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REFERENCES

Ahlstrom, E. (1959) *Fish. Bull. U.S.* **60,** 107–146.
Ahlstrom, E. and E. Stevens (1976) *Calif. Coop. Oceanic Fish. Rep.* **18,** 167–180.
Eisler, R. (1961) *Growth* **25,** 281–346.
Finney, D. (1952) *Probit analysis: A Statistical Treatment of the Sigmoid Response Curve.* 318 p. Cambridge University Press, London.
Fujii, R. (1973) In *Responses of Fish to Environmental Changes* (Edited by W. Chavin), pp. 342–361. Charles Thomas, Springfield.
Fujii, R., T. Nakazawa and Y. Fujii (1973) *Pigment Cell* **1,** 195–201.
Green, A. and J. Miller (1975) In *CIAP Monograph* 5, Part 1, Chap. 2, pp. 60–70. U.S. Dep. Transportation, Climatic Impact Assessment Program, Washington.
Green, A. E. S., T. Sawada, and E. P. Shettle (1974) *Photochem. Photobiol.* **20,** 473–482.
Hart, R. and R. Setlow (1975) In *Molecular Mechanisms for Repair of DNA*, Part B (Edited by P. C. Hanawalt and R. B. Setlow) pp. 719–724. Plenum Publishing, New York.
Jones, R. and W. Hall (1974) In *The Early Life History of Fish* (Edited by J. Blaxter) pp. 87–102. Springer-Verlag, New York.
Kaufman, J. and J. Christiansen (Editors) (1972) *IES Lighting Handbook*, Sec. 25, pp. 1–24. Illuminating Engineering Soc., New York.
Leong, R. (1971) *Fish. Bull. U.S.* **69,** 357–360.
Leong, R. (1977) *Fish. Bull. U.S.* **75,** 205–211.
Marinaro J. and M. Bernard (1966) *Pelagos* **6,** 49–55.
Pommeranz, T. (1974) In *Early Life History of Fish* (Edited by J. Blaxter) pp. 397–416. Springer-Verlag, New York.
Setlow, R. (1974) *Proc. Natl. Acad. Sci. U.S.* **71,** 3363–3366.
Smith, R. and K. Baker (1978) *Photochem Photobiol.* **29,** pp. 311–323.