Photochemistry and Photobiology Vol. 34, pp. 477 to 486, 1981
Printed in Great Britain.

0031-8655/81/100477-10$02.00/0

EFFECTS OF SOLAR AND ARTIFICIAL ULTRAVIOLET-B RADIATION ON LARVAL NORTHERN ANCHOVY, *ENGRAULIS MORDAX**

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(*Received* 17 *February* 1981; *accepted* 27 *March* 1981)

Abstract—Northern anchovy larvae were exposed to various amounts of both natural (global solar) and artificial (sunlamps filtered by cellulose triacetate) UV-B energy over a 12-day period. Dosage was determined on the basis of a weighting function for biological effectiveness. The action spectrum on which this biologically effective dose for anchovy mortality is based was developed using broad-band spectroscopy. These experiments indicated that biologically adverse conditions exist near the sea surface. Larvae exposed in shallow containers to global solar UV for 12 days suffered significant UV mortality from February to October. Larvae surviving all solar and artificial UV doses were smaller than those not exposed to UV. [Lowest dosage = 398 $J \cdot m^{-2}_{(bio.eff.)}$] Fifty percent of the larvae survived a cumulative dose of 605 $J \cdot m^{-2}_{(bio.eff.)}$, or 50 $J \cdot m^{-2} \cdot day^{-1}_{(bio.eff.)}$. Dose reciprocity did not hold; when a similar cumulative dose was given in the first 4 days of 12, there were about one-half as many survivors.

One meter below the sea surface the daily solar UV dose (corrected for average cloud cover) is equal to or greater than 50 $J \cdot m^{-2}_{(bio.eff.)}$ (33 N, 118 W), the daily equivalent of the LD_{50}, for 7 months of the year (March to September) at moderate chlorophyll-a concentrations (0.5 $mg \cdot m^{-3}$). These data suggest that larvae in the sea may be subject to some UV-B stress at present. Owing to their seasonality of spawning and vertical distribution, anchovy populations may not be gravely endangered by moderate degrees of stratospheric ozone diminution.

INTRODUCTION

Studies of over 60 aquatic microorganisms, protozoa, algae and small invertebrates indicate that most are sensitive to current levels of ultraviolet-B (UV-B) radiation incident upon the water surface (NAS, 1979)‡. More recent studies, better quantified, indicate that at doses comparable to daily levels of UV-B energy presently incident on the water surface, the growth of chain-forming diatoms is reduced (Thomson *et al.*, 1979), as is the biomass and specific diversity of attached marine algae (Worrest *et al.*, 1978) and survival of coral reef epifauna (Jokiel, 1980). Similarly, a UV-induced mortality of a marine pelagic copepod (Karanas *et al.*, 1979) and pelagic crab and shrimp larvae (Damkaer *et al.*, 1980) could occur at present UV-B levels. Hunter *et al.* (1979) examined the sensitivity of northern anchovy (*Engraulis mordax*) during the 4-day embryonic period (egg and yolk-sac stages) and found that brain and eye lesions as well as growth retardation could occur on larvae at or near the ocean surface if the ozone layer were reduced by 25%.

All of these investigations indicate that a high sensitivity to UV-B occurs in some small marine organisms, but the effect on natural populations of increased UV radiation due to ozone reduction remains unclear owing to technical uncertainties and lack of data. The technical uncertainties result from imperfect solar simulation in the laboratory. Problems include the use of artificial sources whose spectra differ greatly from natural solar radiation, the use of unrealistically high fluence rates, failure to establish whether photorepair mechanisms were fully activated in the laboratory, and the arbitrary use of UV action spectra to weight laboratory data with subsequent extrapolation therefrom to conditions in the sea. Assessment of the effects of UV on natural populations is difficult without data on the change in abundance of species with depth, the depth of penetration of UV in specific marine habitats, and appropriate weighting of the effects data by the seasonal abundance of the species of interest.

Our objective was to improve our previous estimates of UV effects on northern anchovy larvae by reducing some of these technical uncertainties, and to address the problem of assessment of UV effects of larval populations in the sea. Anchovy larvae needed to be tested to determine if the relation between cumulative dose and mortality shown in the 4-day experiments would hold for longer periods of exposure. In the sea, UV-B exposure effects might be

*Work supported in part by the U.S. Environmental Protection Agency.

‡*Abbreviations*: bio.eff., biologically effective; Chl *a*, chlorophyll *a*; CI, confidence interval; CTA, cellulose triacetate; LD_{50}, lethal dose to 50% of the organisms; NAS, National Academy of Sciences, USA; SL, standard length.

cumulative over as many as 23 days. [After about 23 days at 16°C, the anchovy swim bladder becomes functional and in daylight the larvae migrate vertically to depths beyond the reach of significant UV-B penetration (Hunter and Sanchez, 1976).]

In our past work larvae were tested under three different artificial UV-B spectral irradiance distributions, all substantially different from natural global solar irradiance. The energy from artificial sources was weighted by the DNA action spectrum of Setlow (1974) and the relation between the DNA-weighted dose and mortality was used to forecast effects in the sea under various ozone optical depths. This procedure remains open to question until the findings can be verified by experiments conducted under natural global solar irradiance. Consequently, our specific goals became: (1) to determine whether the same mortality in larval anchovy occurs when the total cumulative dose received in 4 days is, instead, spread over a longer period at lower fluence rates (12 days); (2) to test larvae under natural global solar radiation in order to verify the apparent UV-B sensitivity of anchovy larvae as well as the adequacy of the DNA weighting procedure; and (3) to make a preliminary estimate of the effect of ozone decrease on natural larval populations.

MATERIALS AND METHODS

Apparatus. Test containers were maintained under artificial UV-B in temperature-controlled water tables in the aquarium of the Southwest Fisheries Center and under global solar radiation on the roof of the Center. The containers were of opaque black plastic (36 cm in diam. and 17.5 cm deep) containing 10 l of filtered (2 μm) seawater and covered with a filter top. All treatments and controls consisted of 10 containers each. Banks of fluorescent lamps (Westinghouse FS-40 Sunlamps® for UV energy and General Electric Chroma 50® lamps for visible energy) were all aged for 400 h before use, and were suspended over the aquarium water tables in an aluminum foil-lined room. The aquarium system had a 12-h daily light–dark cycle with 7 h of UV radiation centered on the 12-h light period. The roof system used only natural solar energy.

Filters. Filter tops for test containers in the aquarium consisted of 0.13 mm thick pre-solarized cellulose triacetate (CTA) and 0.13 mm Mylar® plastic. The CTA eliminated UV-C energy from the sunlamps and brought the energy distribution closer to the solar UV spectrum; the Mylar® eliminated UV-B in control containers. (See Hunter *et al.*, 1979, for transmittance of these filters.)

In the solar experiments, 1–3 sheets of 0.13 mm thick Teflon® plastic were used to attenuate UV and visible radiation. One sheet of Teflon® transmitted 67%, two sheets 46%, and three sheets 38% of the incident UV radiation (integral from 290–380 nm), whereas the visible radiation (integral from 380–800 nm) was attenuated to 79, 55 and 48% for one, two, and three sheets of Teflon®, respectively. The ratio of transmitted UV (290–380 nm) to visible (380–800 nm) radiation was about 0.83. We do not believe that this minor change had a significant effect on the results of the solar experiments since the level of photorepair light was more than that needed in these experiments (Kaupp and Hunter, 1981).

The UV treatment containers had only Teflon® tops, but were accompanied by a set of containers having the same thickness of Teflon® and an additional sheet of Mylar®. This control group provided a measure of the combined effects of UV-A and visible radiation independent of UV-B effects. Another solar control had container tops which held three sheets (0.39 mm) of Teflon®, black screen cloth (53% transmission) and Mylar®; these filters in total transmitted only 6% of incident radiation (UV-A + visible radiation). This level of illumination approximated that for the aquarium control containers. Teflon® was used in all solar experiments because it diffused incident radiation. One sheet of Teflon® reduced the UV-B shadowing at the bottom of the containers to within 35% of peak irradiance under extreme sun conditions (clear sky and low sun angle).

Radiometry and the control of UV-B intensity. We varied the intensity of UV-B radiation in the aquarium system by covering the sunlamps with opaque aluminized Mylar® tape, changing the diameter of perforations in Mylar® sheets in the treatment container tops (Hunter *et al.*, 1979) and by adjusting the height of the lamp banks. Solar UV-B dosage was varied by changing the number of Teflon® sheets in the container tops, and by taking advantage of the natural variation in solar radiation.

In the aquarium, UV radiation was continuously recorded with a Norris UV-B radiometer (Optronics model 725) and in the solar system it was recorded continuously with a UV-B radiometer and a Robertson–Berger meter (Berger, 1976). Visible energy was measured continuously in the solar system with a deck cell illuminometer. At intervals during an experiment, measurements were made with a spectroradiometer (Optronics Model 741-V) which was calibrated against a radiometric standard traceable to the National Bureau of Standards. The spectral irradiance measurements were used to convert the continuous records of the radiometers to dosage. For additional details of the apparatus, filters, and radiometry see Hunter *et al.* (1979).

Experimental design. At noon, about 1.5 days after spawning, 100 anchovy eggs were stocked in each test container; eggs hatched about 24 h later (larval age 0 day). The yolk-sac stage ended 5 days after hatching (larval age 4 days) and feeding began. From age 3–5 days, larvae were fed the dinoflagellate, *Gymnodinium splendens*, maintained at a density of 100–200 cells/ml and from age 5–12 days the rotifer, *Brachionus plicatilis*, maintained at a density of 10–30 *Brachionus*/ml (Lasker *et al.*, 1970; Hunter, 1976). Experiments ended at age 12 days: at this time larvae had experienced 12 daily UV exposures. In the aquarium system total cumulative exposure was about 82 h, but in the solar experiments total hours of exposure and dosage varied seasonally and with cloud cover. At the end of each experiment, the surviving larvae were counted and preserved for subsequent length measurements and histological analysis. The survival in treatment containers was normalized to that of the companion control by dividing the number of survivors in a treatment by the number surviving in the control (Table 1), and a dose-response line was calculated for the normalized data using probit analysis (Finney, 1952).

RESULTS

Action spectrum

The relationship between UV-B dose (unweighted) and survival for a 12-day exposure to global solar UV differed by two orders of magnitude from that under artificial UV radiation in the aquarium (Fig. 2). The LD_{50} dose for solar UV, in the integral from 290–320 nm, was 1060 $kJ \cdot m^{-2}$ (95% CI, 871–1280 $kJ \cdot m^{-2}$), whereas that for artificial UV-B, in the integral from 285–320 nm, was 11.0 $kJ \cdot m^{-2}$ (95% CI, 10.1–11.8 $kJ \cdot m^{-2}$). Thus interpretation of all

Table 1. Cumulative UV-B dose and survival for anchovy larvae exposed 12 days to artificial and solar UV-B

Date	Dose $Jm^{-2}_{bio.eff.}$	Mean number of survivors[1] $\bar{X}$	SD	Percent survival normalized to control
		Artificial UV-B[2]		
25.05.78	0	48.2	15.9	100.0
	1109	10.3	8.5	22.9
	1522	0.5	0.8	1.0
07.06.78	0	55.8	30.6	100.0
	726	28.3	16.9	47.9
	1336	0.1	0.3	0.2
22.06.78	0	71.5	21.1	100.0
	517	58.0	26.9	81.1
	679	33.2	10.7	46.4
11.07.78	0	70.0	11.3	100.0
	587	47.3	21.0	67.6
	788	24.1	7.5	34.4
06.09.78	0	49.4	15.3	100.0
	559	31.3	7.4	63.4
	777	1.9	1.7	3.8
28.06.79[3]	0	31.7	14.8	100.0
	642	9.9	6.3	26.7
		Solar UV-B[4]		
05.10.78	0	38.3	17.3	100.0
	1505	1.0	1.6	2.6
15.02.79	0	44.8	7.8	100.0
	385	23.3	11.5	51.8
23.02.79	0	17.8	8.4	100.0
	1277	0.2	0.6	1.1
20.03.79	0	36.9	13.3	100.0
	630	14.6	5.4	39.6
19.04.79	0	10.2	7.0	100.0
	662	2.0	3.4	19.6
03.05.79	0	60.0	12.7	100.0
	569	46.2	14.3	77.0
17.05.79	0	47.6	10.6	100.0
	599	21.9	18.8	46.0
01.06.79	0	51.6	18.1	100.0
	606	31.1	12.3	60.3
14.06.79	0	25.7	14.1	100.0
	669	23.1	14.9	89.9
28.06.79	0	33.6	21.4	100.0
	753	12.0	12.8	35.7
12.07.79	0	21.1	10.6	100.0
	697	14.2	8.9	67.3
26.07.79	0	39.6	12.0	100.0
	1098	3.8	3.5	9.6
16.08.79	0	14.8	9.1	100.0
	1005	0.8	1.1	5.4
30.08.79	0	23.9	12.3	100.0
	975	1.2	2.1	5.0
13.09.79	0	16.8	17.6	100.0
	873	2.8	3.3	16.7
21.09.79	0	39.6	21.8	100.0
	544	19.1	13.4	48.2
	790	10.9	7.5	27.5
12.10.79	0	31.4	12.2	100.0
	539	4.7	4.6	15.0
25.10.79	0	67.0	10.1	100.0
	398	42.0	10.0	62.7

[1] Stocking density was 100 eggs per container, and means are for 10 containers.

[2] To approximate unweighted Jm^{-2} over 285–320 nm, multiply by 18.2

[3] This dose was given in the first 4 days of the experiment, i.e. eggs, 0-, 1- and 2-day-old larvae.

[4] To approximate unweighted Jm^{-2} over 290–320 nm, multiply by 1750.

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results pivots on the choice of an appropriate action spectrum for weighting UV-B effects. Hunter *et al.* (1979) weighted their dose-response data (4-day exposure) by the DNA action spectrum of Setlow (1974) using the analytical fit of Green and Miller (1975), and brought along common line dose-response data for three different artificial UV-B spectra. When we used the same technique to equate effects under solar and artificial UV for a 12-day exposure, the data clearly did not converge. The LD_{50} for a 12-day exposure to artificial UV-B when weighted by the Green and Miller equation underestimated effects under solar UV-B by 66%.

We re-examined the action spectrum of Green and Miller (1975) and derived an action spectrum that would converge the 12-day exposure data for artificial and solar UV-B doses. This action spectrum gives more weight to wavelengths greater than 300 nm relative to the Green and Miller fit to the Setlow DNA action spectrum. The greatest difference occurs at 320 nm, where approximately a tenfold difference exists (Fig. 1b). This change in the form of the action spectrum greatly affected the weighting of the solar data (increase of 66%), but had little effect on data for artificial UV sources (reduction of less than 3%). Thus, use of our action spectrum did not alter the relation among the three weighted UV dose-response curves for a 4-day exposure (Hunter *et al.*, 1979), because all three were based on modifications of the spectral irradiance from FS-40 Sunlamps®. This action spectrum is an adequate weighting function for solar, as well as for three different artificial spectra. Henceforth in this paper, we use this action spectrum to weight all dosages.

Our action spectrum for anchovy mortality and that for DNA damage presented by Setlow (1974) (and modeled by Green and Miller, 1975) is within the 95% confidence intervals of the data used for determination of action spectra for the killing of phage, bacteria, and several vertebrate cell lines (depicted in Fig. 1b). Our broad band spectroscopy has a variance larger than that associated with the data presented for comparison. Our action spectrum cannot be statistically separated from the Green and Miller fit to the data of Setlow (1974), but we will refer to our weighting as 'biologically effective' (i.e. bio. eff.). Our weighted dose can be converted to an approximate unweighted dose using coefficients in Table 1. Representative spectral irradiance distributions for artificial and solar sources are given in Fig. 1. The spectrum of solar UV radiation varied continuously in our experiments, but the conversion is adequate for making rough comparisons with other studies.

Survival

Fifty per cent of anchovy larvae (12-day solar and aquarium experiments combined) survived a cumulative dose of 605 $J \cdot m^{-2}_{bio.eff.}$ (95% CI = 541–676 $J \cdot m^{-2}_{bio.eff.}$) or a mean daily dose of 50 $J \cdot m^{-2}_{bio.eff.}$. This daily dose is within the range of daily UV fluence we

have measured in La Jolla, California over the last 2 years of daily monitoring. On clear days, it has ranged from 25 J·$m^{-2}_{bio.eff.}$ in December to 215 J·$m^{-2}_{bio.eff.}$ in July. These data indicate that biologically adverse conditions exist near the sea surface at present ozone concentrations. There is no reason to doubt this conclusion since larvae suffered significant mortality from February through October when exposed in shallow containers to the La Jolla sun for 12 days. In all solar experiments, larvae were exposed to levels less than the actual solar radiation because the containers were always covered with Teflon® which attenuated UV-B as well as the energy at other wavelengths (see the Methods section for the degree of attenuation).

The cumulative UV dose required for 50% mortality in 12 days (605 J·$m^{-2}_{bio.eff.}$) was about half that required for the same mortality in 4 days (1195 J·$m^{-2}_{bio.eff.}$) (Hunter *et al.*, 1979). The 12-day experiments gave a more sensitive assay of UV damage. To survive past yolk absorption (age 4 days), larvae must be able to search and capture live prey, whereas in the 4-day experiments, larvae needed only to metabolize yolk. It seems unlikely that the severely

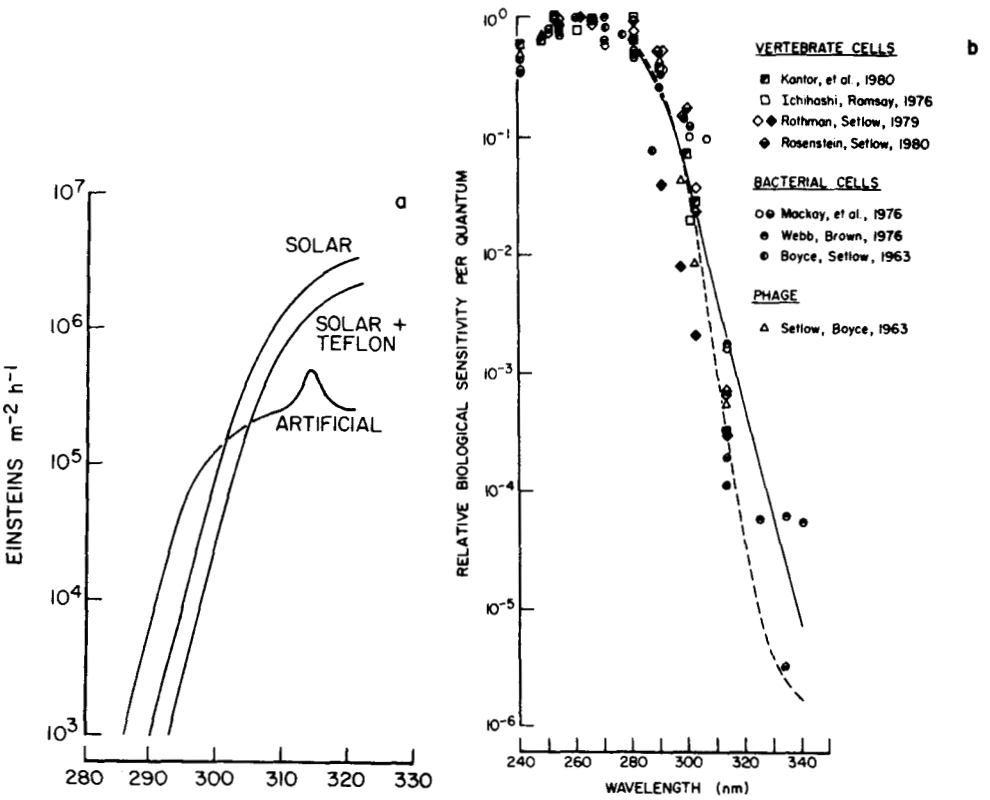

Figure 1. (a) Comparison of solar and artificial UV-B spectra. The solar spectra, measured at local apparent noon, 5 March 1979 (32.5° N, La Jolla, CA), is shown with and without attenuation produced by 0.13 mm of Teflon®. The unattenuated solar spectrum represents 37.8 J·$m^{-2}_{bio.eff.}$ and 25.4 J·$m^{-2}_{bio.eff.}$ attenuated. The artificial spectrum (used in the laboratory experiments) was produced by Westinghouse FS40 Sunlamps® filtered by 0.13 mm of cellulose triacetate and represents 37.4 J·$m^{-2}_{bio.eff.}$ (b) Comparison of DNA action spectrum with data for the killing of vertebrate and bacterial cells, and inactivation of a phage; (---), Green and Miller (1975) fit to the DNA action spectrum of Setlow (1974); (——), empirical action spectrum used to weight dose-response data for anchovy; and points from UV action spectra in literature normalized to the wavelength yielding the maximum response in the particular study. Points for vertebrate cells include: unscheduled DNA synthesis (Ichihashi and Ramsay, 1976; for 10 J·m^{-2} dose) (□), and inactivation (Kantor *et al.*, 1980; average values for normal and DNA exision-repair deficient strains) (◩) of human fibroblast; DNA pyrimidine dimer formation (◇) and killing (◆) of Chinese hamster cells (Rothman and Setlow, 1979); and the killing of haploid frog cells (Rosenstein and Setlow, 1980; average of data with and without photoreactivating light) (⬙). For bacterial cells: killing of *Salmonella typhimurium* (rec A) (○), and *E. coli* (rec A) (⬭) (Mackay *et al.*, 1976; average of stationary phase and exponentially growing cells); stationary phase *E. coli* (B/r and B/r Hcr) (⬬) (Webb and Brown, 1976); of *E. coli* (15 TAU) (◐) (Boyce and Setlow, 1963). For the inactivation of phage T_4B (△) (Boyce and Setlow, 1963).

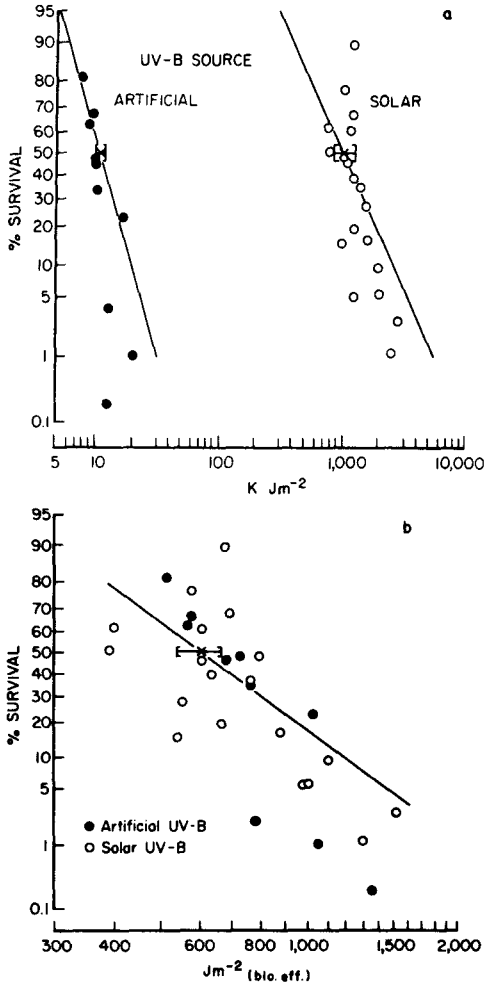

Figure 2. (a) Relation between unweighted UV-B dose ($kJ \cdot m^{-2}$; 285–320 nm) cummulated over 12 days and survival of larval anchovy exposed to artificial (●) and solar (○) radiation. The lines represent the regression of survival probit (Y) on $\log_{10}$ dose (X) where $Y = 4.962\ X - 15.04$ for artificial source, and $Y = 3.467\ X - 1594$ for solar source. Bars and crosses indicate $LD_{50} \pm 95\%$ confidence intervals. (b) Relation between weighted UV-B dose (285–320 nm) cummulated over 12 days for both artificial and solar exposures. The regression of survival probit (Y) on log dose (X) where $Y = 4.275\ X - 6.89$.

damaged larvae that survived the high dosages used in the 4-day experiments (high incidence of eye and brain lesions) could ever feed successfully.

More importantly, dose reciprocity did not hold. In a single aquarium experiment (28.6.79), we exposed eggs and larvae in 10 containers for 4 days to a cumulative dose of $642\ J \cdot m^{-2}_{bio.eff.}$ which is close to the LD_{50} dose for 12 days. Only 26.7% (95% CI, 14.4–39.0%) of these larvae survived by age 12 days. Thus only half the expected number of larvae survived when an LD_{50} dose for 12 days was given at a high dose rate for 4 days. Dose rate, as well as, cumulative dose appear to be important variables. This fact does not alter the interpretation of our dose-response relationships, since we administered dosages over periods comparable to the daily solar cycle or used the actual natural pattern of daily global solar radiation.

We also tested for combined effects of solar UV-A and visible radiation on survival of larvae. Solar energy at wavelengths 315 nm or greater did not show a statistically significant effect on survival after 12 daily exposures. A slight mortality from solar energy above 315 nm may exist, since survival in those groups that received no UV-B was slightly lower than (but statistically inseparable from) control groups maintained under filters that transmitted only 6% of radiation above 315 nm and no UV-B. If real, this effect is probably negligible in the sea.

The extent of photorepair of UV-induced damage could be a significant variable in the interpretation of survival of UV-B radiation. That this is an insignificant factor in the present study is shown by Kaupp and Hunter (1981). They found that the level of photorepair fluence in our laboratory apparatus was sufficient to fully stimulate photorepair of UV damage in anchovy larvae, while the solar photorepair fluence was many times that of the required level.

Growth

Larvae surviving 12 daily exposures to UV-B in the aquarium and in the solar experiments were smaller than survivors in the controls. Significant differences existed ($P = 0.05$) even at the lowest dosages ($517\ J \cdot m^{-2}_{bio.eff.}$ in aquarium experiments; $398\ J \cdot m^{-2}_{bio.eff.}$ in solar experiments). The mean standard length of larvae at age 12 days decreased as a linear function of dosage in the aquarium experiments (Fig. 3). Regression analysis for the solar data was inappropriate because water temperature, which has a marked effect on growth, could not be rigorously controlled.

Temperatures varied only ± 0.2°C over the course of the aquarium experiments but fluctuations of 2–3°C were not uncommon in the solar experiments and the mean temperatures in the latter ranged from 14.3–16.1°C, whereas the means of aquarium experiments were 16.0 ± 0.2°C. Regardless of the temperature problems, the decline in average larval length with dosage followed about the same pattern in both groups of experiments. This analysis indicates that growth was inhibited by exposure to UV-B and the magnitude of the effect was a linear function of dosage. Retardation of growth could translate into additional mortality if survival were to be assessed after the 12th day of life.

PRELIMINARY ASSESSMENT OF EFFECTS OF UV-B ON LARVAL POPULATIONS

Realistic assessment of the effects of UV-B on the anchovy larval populations requires knowledge of

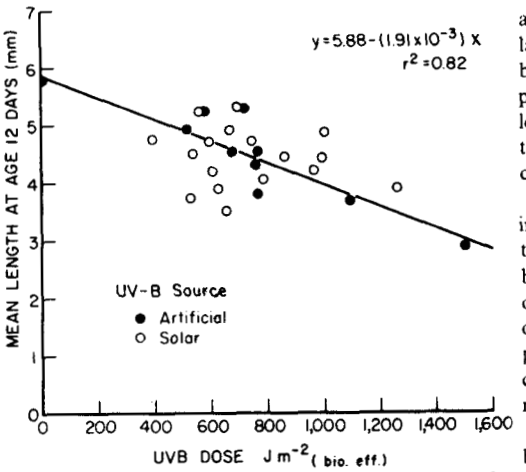

Figure 3. Relation between mean standard length of anchovy larvae (preserved in 4% formaldehyde) after 12 daily exposures of UV-B as a function of cumulative weighted UV-B dose. Regression line is fit only to data from artificial UV-B sources (●).

seasonal changes in larval abundance, incident UV-B, spatial and temporal variation in the depth of penetration of UV-B in the habitat, vertical distribution of larvae in the sea, rates of vertical mixing of eggs and larvae, and natural rates of mortality for eggs and larvae. At present, many of these parameters can only be approximated or are unknown. Nevertheless, a preliminary assessment is useful because it may be a long time before these parameters for natural populations are accurately estimated and because such calculations identify the dominant factors in the analysis.

In this calculation we assume that no vertical mixing of eggs and larvae exists. There are no data on the vertical mixing of fish larvae, though the possibility certainly exists. It is important to recognize that our assumption of a static vertical distribution (without vertical mixing) presents the most extreme case of potential UV damage. Vertical migration and mixing could greatly reduce our estimate of the effect of UV radiation.

Hunter and Sanchez (1976) investigated the possibility that anchovy larvae vertically migrate. Using the original data of Ahlstrom (1959), they compared day and night catches of larvae less than 11.75 mm (standard length, SL) to those equal to or greater than 11.75 mm (Ahlstrom did not give larval length in his paper, and his original data must be used to make the calculation). Hunter and Sanchez (1976) found evidence for a diel change in depth distribution in larvae greater than 11.75 mm, but no evidence for a diel change in depth distribution in larvae less than

Table 2. Proportion of northern anchovy larval population affected by UV-B radiation at present (0.32 cm) and at 25% reduction (0.24 cm) in ozone concentration

	0.32 cm Ozone (ambient)				0.24 cm Ozone (25% reduction)			
			Larvae at or above LD_{50}[3]				Larvae at or above LD_{50}	
Months	Daily fluence UV-B ($J \cdot m^{-2}_{bio.eff.}$)[1]	Depth of LD_{50}[2] (m)	Percent[4]	Percent weighted by relative larval abundance[5]	Daily fluence UV-B ($J \cdot m^{-2}_{bio.eff.}$)[1]	Depth of LD_{50}[2] (m)	Percent	Percent weighted by relative larval abundance
J	25	0	0	0	40	0	0	0
F	52	0.11	3.76	0.62	82	1.34	13.32	2.17
M	94	1.74	15.09	2.53	149	2.95	19.65	3.29
A	150	3.03	19.90	3.96	238	4.21	23.48	4.67
M	158	3.17	20.37	2.70	250	4.35	23.85	3.16
J	167	3.32	20.85	1.65	265	4.50	24.28	1.92
J	162	3.24	20.59	1.16	257	4.42	24.06	1.36
A	147	2.97	19.72	0.37	233	4.16	23.32	0.44
S	111	2.20	16.96	0.30	176	3.40	21.09	0.37
O	65	0.72	9.73	0.11	103	1.95	15.98	0.18
N	30	0	0	0	48	0	0	0
D	20	0	0	0	32	0	0	0
Σx				13.40				17.56

[1] The sea surface irradiance is taken from Baker *et al.* (1980) coefficients for the model of Green *et al.* (1974) for 33°N, and adjusted for 18-yr average cloud cover (1961–1978) for the Los Angeles Bight (30–35°N, 115–120°W; Renner, 1979) using the equation of Bener (1964) and then weighted by an empirical fit to the DNA action spectrum.

[2] Depth in sea where the daily UV-B fluence is equivalent to the LD_{50}. The depth of UV-B penetration was calculated for moderately productive water (0.5 mg Chl *a* m^{-3}) using the downwelling irradiance diffuse attenuation coefficients of Baker and Smith (personal communication); $K_T = 0.3630$ for 0.32 cm ozone and $K_T = 0.3702$ for 0.24 cm ozone.

[3] 50 $J \cdot m^{-2}_{bio.eff.}$.

[4] The monthly percent of larvae at or above the LD_{50} depth was calculated using the vertical distribution data for northern anchovy larvae (< 11.75 mm standard length; Hunter and Sanchez, 1976) of Ahlstrom (1959) adjusted to unit sea surface (Smith and Richardson, 1977) and fitted by the equation, $y = 11.44\ x^{0.5}$, $r^2 = 0.99$, for the data from the surface to 28 m, where y is the cumulative percent of larvae from the surface to depth x, in meters.

[5] 10-year average larval abundance from Lasker and Smith (1977) was used to weight the percent of larvae affected by UV-B for each month; see Fig. 2.

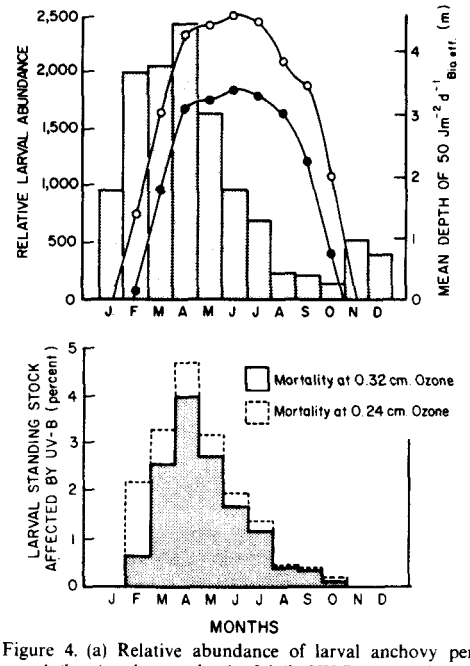

Figure 4. (a) Relative abundance of larval anchovy per month (bars) and mean depth of daily UV-B penetration of $50\,J \cdot m^{-2}_{bio\cdot eff.}$ in the sea per month at present (●) and at 25% reduction in ozone (○). $50\,J \cdot m^{-2}_{bio\cdot eff.}$ is the daily equivalent of the LD_{50} for anchovy larvae cumulated over 12 days. Data sources are given in footnotes to Table 1. (b) Percent of annual larval anchovy standing stock affected by UV-B ($50\,J \cdot m^{-2} \cdot d^{-1}_{bio\cdot eff.}$) per month at present ozone concentration [0.32 cm optical ozone depth (——)] and at 25% reduction in ozone [0.24 cm optical ozone depth (---)] according to calculations given in Table 1.

11.75 mm. As the maximum size of larvae at the end of our experiments was 6.5 mm SL, we used Ahlstrom's data for larvae less than 11.75 mm SL to calculate the vertical distribution, using techniques outlined by Smith and Richardson (1977). Smaller larvae may migrate vertically but the change in their vertical distribution is not detectable using Ahlstrom's original data. The equation fitted to Ahlstrom's data, and other documentation for these calculations, are given as footnotes in Table 2. We assume that larvae of the size considered here (SL ≤ 6.5 mm) do not migrate vertically and have a depth distribution similar to that given in Table 2.

We also assume that all larvae at those depths with UV energies equal to the LD_{50} ($50\,J \cdot m^{-2} \cdot day^{-1}_{bio\cdot eff.}$) will die, while those receiving lesser dosages will survive. This criterion was chosen because of the symmetry of the dose-response curves about the LD_{50} value. It does not take into account sublethal damage, nor the fact that UV-B radiation decreases with depth faster than does the anchovy larval abundance. This use of the LD_{50} limits the conclusions from the calculation of effects of enhanced UV-B to an indexing of effect. These effects will alter the absolute value of our index of mortality, but they will have little or no effect on the rate of change of the index in response to ozone depletion.

Estimates of daily UV-B energy entering the sea were derived from Baker *et al.* (1980) adjusted for average cloud cover using the equation of Bener (1964). Average cloud cover data were 18-yr monthly means (Renner, 1979) for an area in the Southern California Bight (30–35°N, 115–120°W), which is the center of the anchovy spawning region (Ahlstrom, 1967). To estimate the depth of penetration of UV-B in the sea at various ozone concentrations, we used the equations and the attenuation coefficients of Smith and Baker (1979) for moderately productive ocean water ($0.5\,mg\,Chl\,a\,m^{-3}$) weighted by our action spectrum for anchovy mortality. This chlorophyll concentration is typical of the Southern California Bight during the spawning season (Owen and Sanchez, 1974).

The proportion of larvae in the water column affected by UV-B (proportion of larvae at or above the depth where the daily UV-B dose = $50\,J \cdot m^{-2}_{bio.eff.}$) is highest in June (21% at 0.32 cm ozone; 24% at 0.24 cm ozone) and declines with the seasonal changes in UV radiation (Fig. 4; Table 2). Although the predicted rate of UV mortality is highest in June, the abundance of anchovy larvae (shaded area, Fig. 4, upper) is relatively low (Lasker and Smith, 1977). When the loss rate per month at present ozone levels is weighted by the monthly larval abundance, the months that contribute most to the total annual UV mortality are March, April and May (Fig. 4, lower). The principal effect of a 25% reduction of ozone is to advance the period of UV vulnerability one month in the spawning season; the period of vulnerability would thus begin in February instead of March and remain at elevated levels through the peak months of spawning.

Our calculations indicate that at the present time about 13% of the annual production of larvae could be lost because of UV-B mortality and this may increase to about 18% at a 25% reduction in ozone (Table 2). The increase in the estimated UV-B mortality for the larval population is nearly a linear function of ozone concentration with mortality increasing about 0.2% for each percentage decrease in ozone (Fig. 5). Thus ozone decline produced only a minor increase in predicted UV-B mortality for the population, but a loss of 13% of larval standing stock at present ozone levels seems high. If such a UV-B mortality exists, it may have little effect on the population because natural rates of mortality from other causes are high; the rates decline from about 53% per day for eggs (Smith and Lasker, 1978) to about 17% per day for 12-day-old anchovy larvae (Zweifel and Smith, 1981).

The estimated effect of ozone diminution on larval anchovy populations is low for several reasons. Each year UV radiation increases during the peak months of spawning (January–April) at a rate equivalent to about a 25% reduction in ozone every month

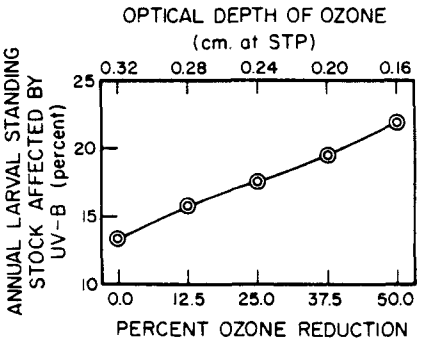

Figure 5. Estimated annual loss of larval anchovy standing stock (%) as a function of optical depth of ozone. The method of calculation is illustrated in Table 1.

(Table 2). This condition (decreasing spawning during increasing UV-B) reduces the relative impact of ozone loss in our calculations. Impact is also reduced because anchovy larvae exist below depths where significant UV effects occur as well as within such depths. A 25% reduction in ozone in moderately productive waters (0.5 Chl a m^{-3}) is equivalent to about a 1 m increase in the depth of penetration of the biologically effective dose. This increase in depth of penetration does not have a major effect on populations that are distributed over many meters of depth.

Some factors could completely change our assessment, viz.: an extreme change in the form of the vertical distribution of larvae, or seasonal changes in larval vertical distribution; seasonal changes in UV-B diffuse attenuation coefficients in the habitat; elevated UV sensitivity at a specific life stage of the larvae; or vertical mixing or movement of the larvae. These considerations suggest that the life history traits that would greatly increase vulnerability of a marine planktonic organism to ozone decline are: (1) the entire population is confined to a very limited depth range—perhaps a few meters—so that an increase in the depth of UV-B penetration of 1 m or less could have important consequences; or, (2) the period of vulnerability is confined to a short span in the spring or winter (about 1 month at our latitude), or to the summer months so that adaptation to UV is limited to a relatively constant fluence rate.

Our estimate of the effects of ozone diminution on larval anchovy standing stocks is patently simplistic, having many uncertainties and assumptions, and should therefore be regarded only as an index of the possible losses due to enhanced UV-B radiation. The final estimated loss to the annual standing stock is an index of effects, and should not be confounded with actual mortality rates, which are size-specific. Despite these shortcomings we believe the analysis demonstrates that ozone loss of 25% or less may not have a great effect on the larval anchovy population, at least in the first 12 days of life. The possible effects of sublethal radiative doses in the eventual survival and reproductive ability of anchovies has yet to be investigated.

DISCUSSION

An important ecological implication of this research is that UV radiation is probably a major factor in the early life history of many pelagic marine fish larvae. The important life history characteristic of the northern anchovy is the restriction of intense spawning to periods of low UV radiation. Fish larvae that exist only at the surface during the months of most intense UV radiation probably have evolved mechanisms to cope with high fluence rates. Such larvae are often deeply pigmented (H. G. Moser, unpublished manuscript, NMFS, Southwest Fisheries Center, La Jolla, CA) or may have other protective mechanisms, seemingly, as does the Pacific mackerel which spawns intensively in June, has little apparent pigmentation, but is much more resistant to UV radiation than are anchovy (Hunter *et al.*, 1979). Obviously, the vertical distribution of larvae and their vertical migration are also important adaptations.

High sensitivity to UV radiation may be a common trait among many pelagic clupeoid fish worldwide. The period of maximum spawning in many of the major stocks of clupeoid fish does not coincide with the period of maximum UV-B fluence (Table 3). Clupeoids that spawn very near shore or in bays, e.g. *Harengula jaguana* and *Opisthonema oglium* (Table 3) do not follow this pattern, but such species seldom form large fish stocks. Such inshore species could be as sensitive to UV-B radiation as northern anchovy because the inshore and bay habitat may provide protection from UV radiation owing to high Chl concentrations and turbid conditions of such shallow waters. Inshore areas and bays in southern California typically have Chl concentrations as high as 5 mg Chl a m^{-3} (Brewer *et al.*, 1980). This value is very high relative to the one used in our calculations, 0.5 mg Chl a m^{-3}, which is representative of offshore areas. Such a difference in Chl concentration would reduce the depth of penetration of a biologically effective dose by a factor of about 5.

We do not wish to imply a direct cause and effect relationship between seasonality of spawning and UV-B fluence. Spawning seasons probably evolved to coincide with optimum larval feeding conditions and not to avoid UV-B radiation. That spawning intensity is low when UV-B intensity is the highest in many clupeoids indicates that they may be less resistant to UV-B radiation than other fish that spawn during high UV-B fluences at the same latitude. Our calculations for northern anchovy might apply to many of the world's clupeoid stocks that spawn pelagic eggs, because they show similar spawning patterns in relation to the annual UV cycle. Our present analysis indicates that these stocks may be subject to some UV stress today, but owing to their spawning season, vertical distribution, and high natural rate of mor-

Table 3. Relation between seasonal UV-B maxima and spawning maxima in various clupeoid fish

Species/location	Years of data collection	Latitude of sampling	Months (winter / summer / winter; north: J F M A M J J A S O N D; south: J A S O N D J F M A M J)	Portion of annual equatorial UV-B_{BE}	Source
Engraulidae					
Engraulis mordax Southern California and Baja California	1951–60	32 N	UV[1]; eggs and larvae[2] (66%)	0.50	Ahlstrom, 1967
Engraulis capensis South Africa	1978–79	21 S	UV; eggs (78%)	0.78	Butterworth, 1979
Engraulis ringens Peru	1966–74	6–14 S	UV; UV; eggs (47%); eggs (20%)	0.92	Santander and Castillo, 1976
Cetengraulis mysticetus Gulf of Panama	1956–57	9 N	UV; UV; eggs (99%)	0.92	Simpson, 1959
Clupeidae					
Brevoortia tyrannus Atlantic coast of USA	1965–66	34–41 N	UV; eggs (70%)	0.40	Kendall and Reintjes, 1973
Sardinops melanosticta Southern Japan	1949–51	35 N	UV; eggs (79%)	0.45	Nakai and Hattori, 1962
Sardinella anchovia Gulf coast of Florida	1971–74	24–30 N	UV; eggs (70%)	0.60	Houde *et al.*, 1979
Etremus teres Gulf coast of Florida	1971–74	24–30 N	UV; eggs (88%)	0.60	Houde *et al.*, 1979
Herengula jaguana Gulf coast of Florida	1971–74	24–30 N	UV; eggs (97%)	0.60	Houde *et al.*, 1979
Opisthonema oglinum Gulf coast of Florida	1971–74	24–30 N	UV; eggs (90%)	0.60	Houde *et al.*, 1979

[1] The line represents the months spanned by the most intense half of the annual UV-B flux for the given latitude.
[2] The spawning "season" (dashed line) was taken from net tow data cited in the reference given on the right-hand column. The percentage given refers to the portion of annual production represented by the spawning "season".

tality, they may not be greatly affected by ozone diminution. For the northern anchovy this seems to be the most tenable conclusion.

To confirm our assessment of UV effects on anchovy larval populations would require: better data on vertical distribution of larvae in the sea; measurement of seasonal pattern of Chl concentration (to estimate penetration of UV in the habitat); laboratory studies on the relative resistance to UV-B of various life stages of larval anchovy; and studies of the long-term effects of short-term exposure to UV-B radiation.

Acknowledgements—This research represents a team effort, and would have been impossible without the able and

dedicated assistance of all members. We wish to thank Beverly Macewicz for her assistance with rearing the larvae, and her expertise in microtechnique and histological analysis; Eric Lynn for so graciously keeping us supplied with ample food for the larvae; and Roger Leong for the maintenance of adult anchovy rearing stocks and anchovy eggs upon demand. The cooperation of Raymond Smith and Karen Baker (Visibility Laboratory, University of California, San Diego) was indispensible in the extrapolation of our laboratory results to environmental effects.

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