AN ABSTRACT OF THE THESIS OF

James John Karanas for the degree of Master of Science

in General Science (Biological Science) presented on March 7, 1978

Title: EXPOSURE TO ULTRAVIOLET-B RADIATION: SENSITIVITY EVALUATION OF AN IMPORTANT ECOLOGICAL PARAMETER OF THE MARINE COPEPOD ACARTIA CLAUSI

Abstract approved: Redacted for Privacy

Henry Van Dyke

The ozone present in the atmosphere filters incident solar ultraviolet (UV) radiation such that only the longer wavelengths of the total UV spectrum emitted by the sun reach the earth's surface. Atmospheric increases in nitrogen oxides and chlorofluoromethanes represent a threat to the existence of this protective screen. As the ozone concentration is decreased, there will be a concomitant increase in the mid-wavelength (UV-B) ultraviolet radiation penetrating the atmosphere. The UV-B waveband (286-320 nm) is detrimental to most biological systems.

Acartia clausi was observed to be the most common zooplanktonic organism encountered in Yaquina and Netarts Bays, Oregon. It is an herbivorous, calanoid copepod important in coastal and estuarine zooplankton communities throughout the northern hemisphere. Determination of the sensitivity of A. clausi to UV-B radiation was the focus of this investigation.

Organisms collected from Yaquiha Bay were maintained in laboratory cultures. Life stages were subjected to varying degrees of UV-B radiation. Irradiation took place on a rotating turntable beneath equal numbers of FS40 sunlamps and "deluxe white" fluorescent lamps. UV-B and visible fluence rates were determined with a Gamma Scientific 2900 SR Spectroradiometer System.

Prior to irradiation copepods were randomly assigned to treatments (dosages) utilizing a randomized complete block design. Analysis of variance indicated lack of significant difference between replications permitting their combination and a more accurate estimate of surviving fractions. Least squares regression analysis was performed between surviving fraction and total dose for all treatments administered to each age group, and between the offspring/survivor ratio and total dose for treatments administered to the oldest age group composed of equal numbers of males and females. Survival was not consistent throughout the age groups, the younger organisms being the more sensitive.

Approximate weighting values determined from analytical representations of two generalized action spectra permitted calculation of biologically effective surface irradiances for both laboratory and natural conditions. The effective (i.e., weighted) irradiances could then be compared between these conditions.

Results indicate possible sensitivity of some age groups to present levels of UV-B, suggesting it as an important environmental parameter limiting natural populations. Reproductive capability of the surviving organism was also negatively affected.

Exposure to Ultraviolet-B Radiation: Sensitivity Evaluation of an Important Ecological Parameter of the Marine Copepod *Acartia clausi*

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1978

APPROVED:

Redacted for Privacy

Associate Professor of Biology
in charge of major

Redacted for Privacy

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Date thesis is presented March 7, 1978

Typed by Leona M. Nicholson for James John Karanas

ACKNOWLEDGEMENTS

The author wishes to express his deepest appreciation to his family for all financial and personal support, without which this thesis could not have been completed. Εὐχαριστῶ.

Special thanks to Henry Van Dyke, who functioned more than effectively as my major advisor and rather reluctantly as a second father.

My colleagues Bob Worrest and Bruce Thomson were invaluable in answering the myriad questions presented to them.

I would also like to thank Paula Kanarek for devoting much time to making statistics both understandable and interesting.

I cannot thank all the OSU zooplankton people individually, simply because I received so much help from so many. Two deserve specific mention: Archie Vander Hart, who gave me my first copepod and Ken Johnson, who never refused me time and/or energy and was a major source of background information.

Leona Nicholson deserves particular recognition, not only for her constant support and friendship, but also for her capacity as information wellspring on University B.S. procedure.

Keith King supplied photographic materials, methods, and more jam-packed 30-second explanations than I could ever hope to assimilate, for all of which I am very thankful.

A general acknowledgement must be extended to the General Science Department and staff whose tolerance of my behavior knew almost no bounds.

This thesis would never have been completed without the time, support, understanding, and parallel structure of my dearest friend and companion, Joan Kent.

Last, but certainly not least, appreciation for comic relief must be extended to Christopher.

This thesis was supported in part by a grant from the Society of Sigma Xi and a contract with the National Aeronautics and Space Administration.

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INTRODUCTION

Trophic Dynamics

An interacting system of organisms, together with the environmental factors with which they interact, is termed an ecosystem. This term was proposed by Tansley (1935) who sought to bring together two ideas: a characteristic community of organisms is associated with and dependent on a specific habitat, and the habitat is modified to a greater or lesser extent by the activities of the organisms living there. In its fundamental aspects, an ecosystem involves the transformation and accumulation of energy and matter by living things. One of the first attempts to study the energy budget of an actual biological system was that of Juday (1940), who accumulated data on the mean standing crops of organisms in Lake Mendota (WI) and converted this information into estimates of production through consideration of the life histories of the organisms and their probable turnover times. From this were produced both physical and biological energy budgets for that lake. There was no mention of energy flow. However, Juday referred to photosynthesis as "the primary accumulation or storage of the energy derived from subsurface illumination" and to those forms which ingest plants as "a secondary stage in the storage of the energy accumulated by the aquatic plants."

Energy transfer from one component of the ecosystem to another is the focus of the trophic dynamic model first proposed by Lindeman

(1942). This highly significant paper provided a fundamental conceptual framework and consequently most modern ecological research directs its efforts toward the study of energy flow within a system. Lindeman proposed that organisms should be formally grouped according to their mode of obtaining energy: producers (autotrophs), primary consumers (grazers), secondary consumers (predators of herbivores) and so on. Each group represented a trophic level and he explained that each had its own energy content which was in a constant state of flux, receiving energy from the previous trophic level and passing it to the next trophic level or dissipating it as heat during metabolism or decomposition. The development by growth and reproduction at each of these levels is termed a production process, and the rates are productivities. Early attempts to describe energy flow and the dynamics of whole ecosystems in the marine realm were made by Clarke (1946) and Harvey (1950).

Productivity seems the most significant single attribute of a natural community. A knowledge of the magnitude of primary production—the rate at which energy is bound or organic material created by photosynthesis and chemosynthesis—is essential to an understanding of any ecosystem. It is most often expressed as dry organic matter in $g/m^2/yr$ or energy in $kcal/m^2/yr$. The rate of primary production in a given water mass changes with the diurnal and seasonal cycles, with changes in temperature, illumination and depth, with relative amounts of phytoplankton and zooplankton present in the water, and with the species present.

Each trophic level contains at any one time a certain amount of living material composed of a number of kinds of organisms. This is the standing crop, most often expressed as the number per unit area or biomass (living weight) per unit area (Smith, 1974). A major factor affecting the standing crop and, hence, production of any particular level is consumption by the next trophic level, e.g., zooplankton grazing greatly affects the primary productivity of the phytoplankton in a given area. The grazer is the important organism transferring energy from the autotrophic to the heterotrophic realm. Thus its feeding efficiency and standing crop are extremely important to all heterotrophic levels in terms of energy available for their consumption.

Discussion in the past has focused upon the intriguing relationship between phytoplankton and zooplankton populations (Nielsen, 1958). Periods of fluorishing phytoplankton alternate with periods in which the biomass of phytoplankton is consumed by the herbivorous zooplankton. Harvey (1955) regarded the sea as a place "where the animals enjoy alternating periods of overeating and starvation" and where "an equilibrium between standing crop of plants, herbivores and carnivores is continually passing in and out of balance."

The relationship between plant and grazer populations must be carefully considered. There is evidence now that low level grazing actually enhances primary productivity. Hargrave (1970) experimentally varied numbers of the herbivorous amphipod Hyalella azteca in sediment cores. The productivity of the sediment microflora was stimulated with increased amphipod numbers within the range of

natural densities. Above this level algal productivity declined as number of amphipods increased. Results suggested that increased grazing, within the range of natural grazing intensities, serves to increase the primary productivity of benthic microflora.

Deason (1975) measured the state of a natural estuarine phytoplankton population, with and without zooplankton present. Large plastic containers were suspended in the estuary to isolate the plankton populations. Evidence suggested, among other things, that excretion by grazers provided a higher level of nutrients in the zooplankton bag, while nutrient depletion occurred in the bag containing only phytoplankton. Cells in the zooplankton bag were richer in carbon, nitrogen and chlorophyll a and the C/N ratios were lower. The species composition within the two containers was also different, due most likely to selective grazing. Thus, the structure of the algal community may indeed be closely related bioenergetically to the nutrition of consumer organisms.

A Marine Planktonic Copepod

The term zooplankton includes a diverse group of animals, with representatives from almost every animal phylum. The factor which unites all these planktonic animals is their relative inability to orient themselves independently of the movements of the water which they inhabit.

Zimmerman (1971) found that zooplankton populations of Yaquina Bay (OR) are dominated by copepods. Copepods are perhaps the most abundant animals in the world. They form the bulk of most zooplankton hauls and inhabit the vast expanse of the oceans, possibly outnumbering all other kinds of animals, even insects, which may have more species but fewer individuals (Marshall, 1973). Zimmerman observed Acartia clausi (Fig. 1) to be the most common organism in his Yaquina Bay study. It accounted for over 60% of the zooplankton organisms collected.

Acartia clausi is an important copepod in coastal and estuarine zooplankton communities throughout the northern hemisphere (Landry, 1976). Due to its wide geographical distribution and population numbers, a substantial body of literature has accumulated concerning its activities (Marshall, 1949; Conover, 1956; Jeffries, 1962; Landry, 1976). This species is generally a dominant member of the planktonic community during the warmer months of the year and is not commonly found outside nearshore, coastal areas (Wilson, 1942).

A. clausi is an herbivorous filter feeder of the suborder Calanoida. Pelagic calanoid copepods can be classified into two groups, the Amphascandria and the Heterarthrandria (Brodskii, 1967). A. clausi is included among the Heterarthrandria, members of which are characterized by males actively feeding throughout their adult life thus enabling them to survive for a substantial portion of the female life span. Males are then available for mating with females as soon

Figure 1. (A) Adult female Acartia clausi.

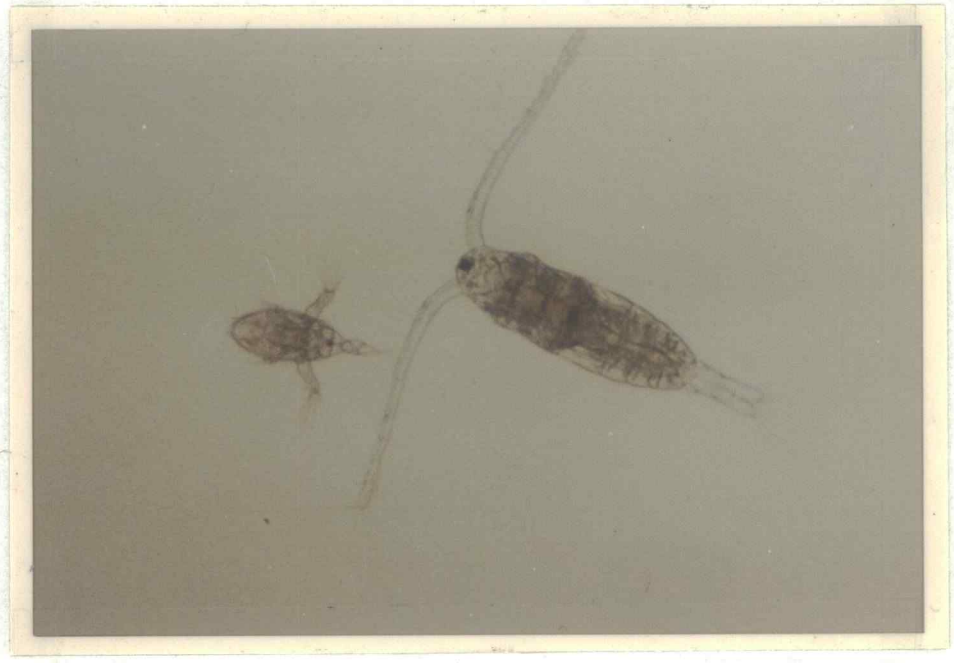


Figure 1. (B) Fifth naupliar and third copepodite larvae.

as the latter molt to reproductive maturity. This life cycle feature facilitates experimental studies requiring large numbers of individuals.

The life cycle of A. clausi (Fig. 2) commences with hatching from the egg as a characteristic nauplius, a larval form typical of many crustaceans. Initially the body is oval with three pairs of limbs used for swimming. These will later become the antennae and mandibles of the adult. The first nauplius and all subsequent life stages possess a single conspicuous antero-dorsal eye. Development includes six naupliar stages prior to the first copepodite molt at which time the organism assumes the appearance of a miniature adult. This molt is accompanied by a large increase in both mass and linear dimensions (Landry, 1976). There are five copepodite stages before the molt to the adult stage, capable of reproduction.

Diurnal vertical migration is a striking feature of the life of many marine organisms. The usual pattern is to submerge during daylight hours and move toward the surface as the light decreases. Not all species migrate and not all life stages of migrating species migrate. The latter situation seems to be the case with A. clausi. Landry (1976) reported such diurnal vertical migration of A. clausi in Jakle's lagoon (WA) for two 48-hour periods. His first study, June 25-26, 1973, indicated that the nauplii were primarily distributed in the top two meters throughout the day and night. This is illustrated in Figure 3. Copepodite stages appeared to reside in the bottom one or two meters during the day and migrate toward the top of the water column at night. The later stages seem to maintain

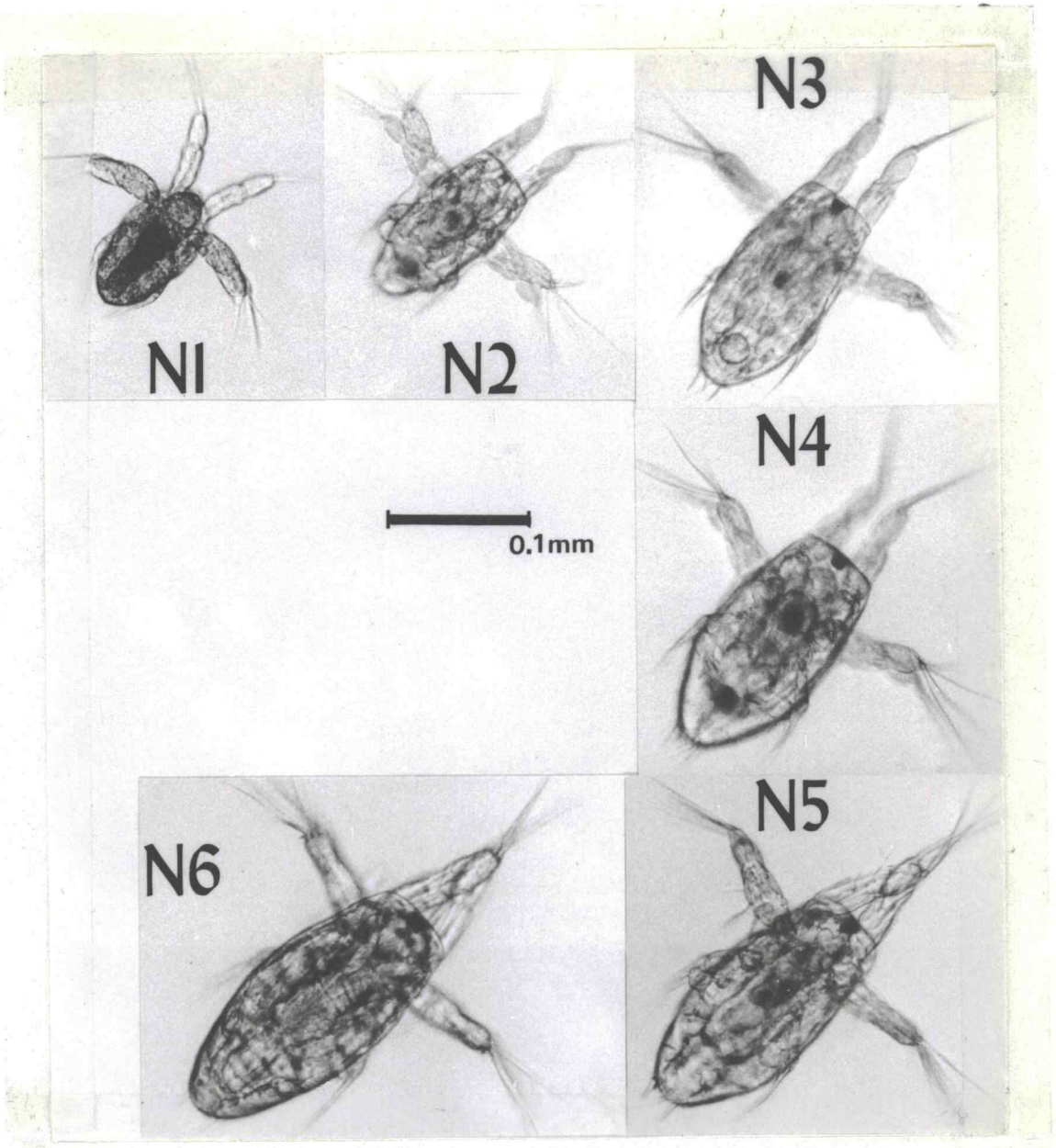


Figure 2. (A) Naupliar stages all to the same scale.

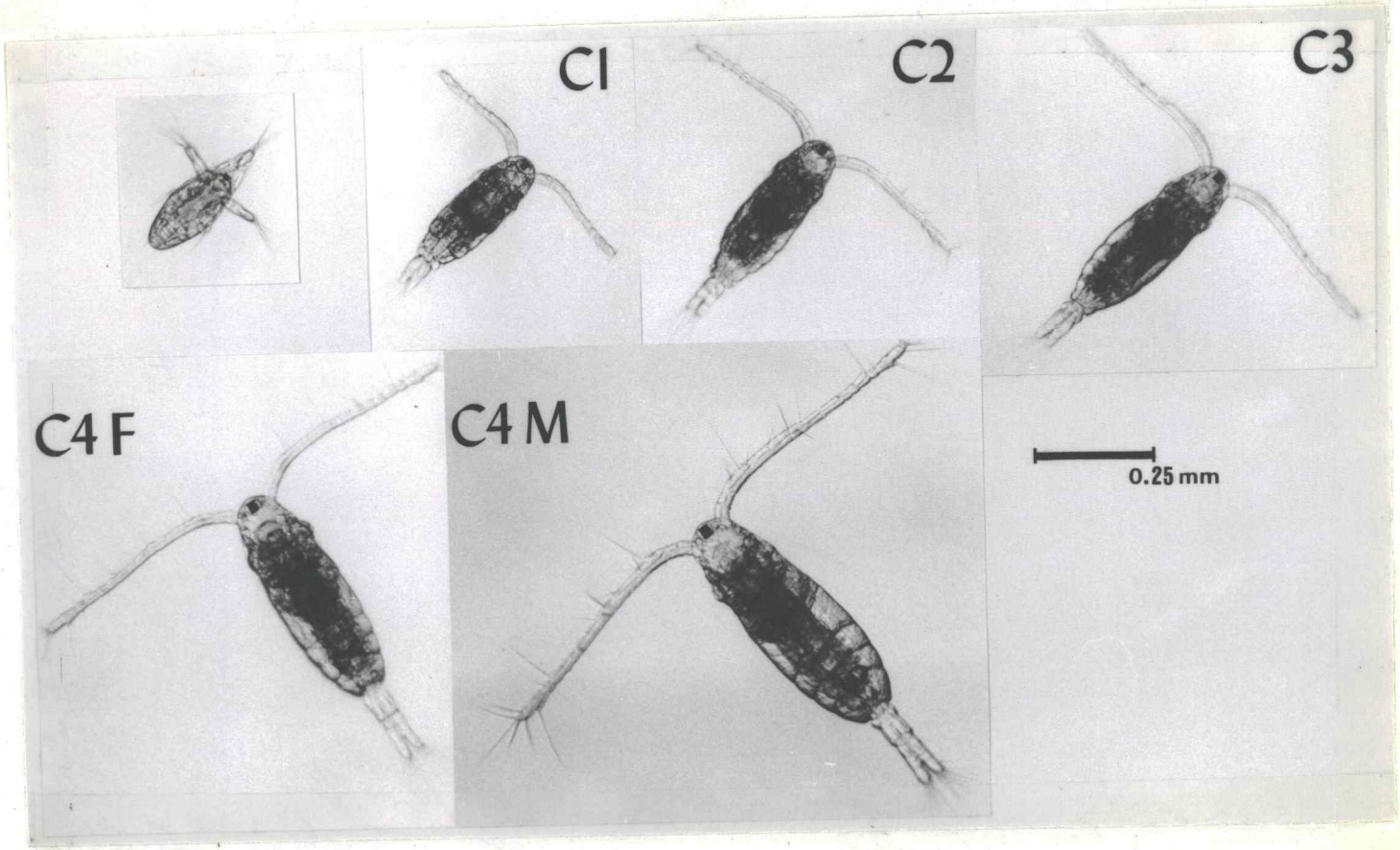


Figure 2. (B) Copepodite stages 1-4 all to the same scale.

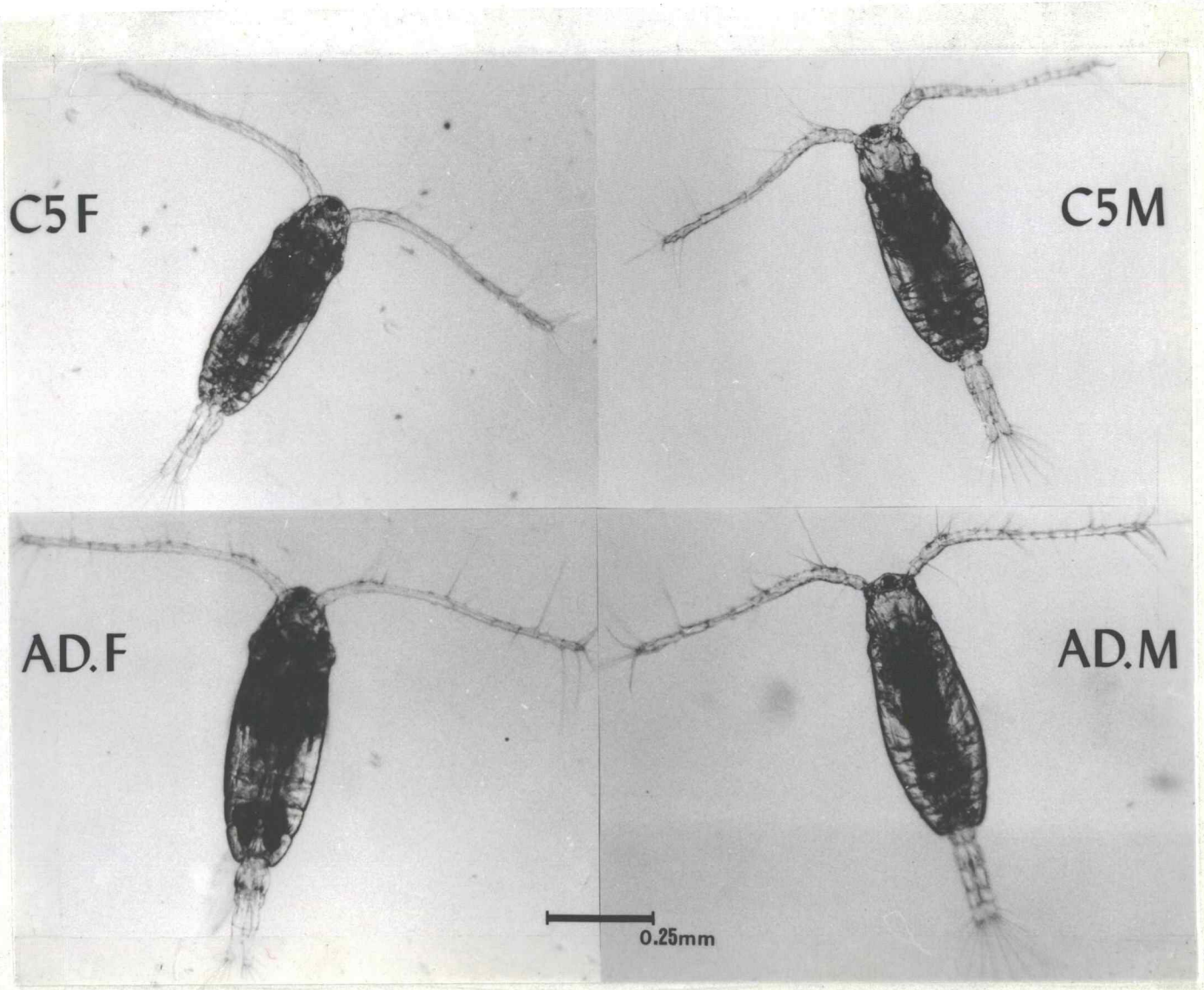


Figure 2. (C) Fifth copepodite and adult stages, all to the same scale.

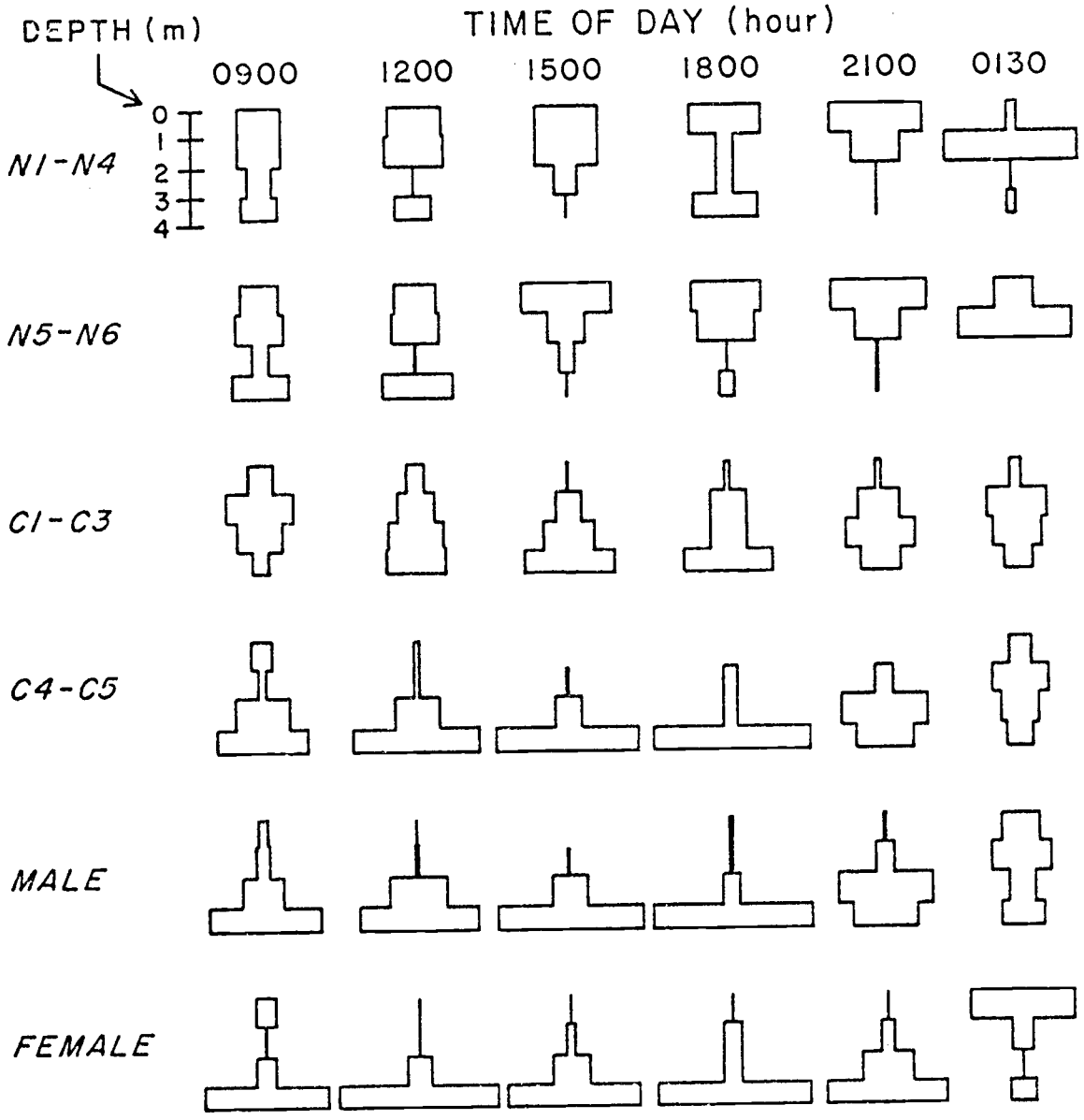


Figure 3. Diel changes in the vertical distribution of A. clausi in Jakle's lagoon on June 25-26, 1973. After Landry, 1976.

a closer association with the bottom and underwent a more extensive migration at night.

The vertical distribution differed somewhat during his second sampling period on June 11-12, 1974 (Fig. 4). Previously the difference between the behavior of the early stages and that of the later stages was quite distinct. In this sampling period there was no marked difference, but rather a gradual transition in the extent of the migration. Early naupliar stages were still primarily located in the upper two meters; however, late nauplii appeared to migrate over a short distance at night. The more mature stages made the most extensive migrations.

A number of selective advantages for vertical migration have been speculated. Evasion of predators by the organisms remaining in regions of lower illumination is a widely discussed hypothesis critically reviewed by McLaren (1963). Zaret and Suffern (1976) have recently produced evidence that visually-orienting predators can contribute significantly to plankton mortality, thus supporting the theory of predator avoidance as a major selective advantage of vertical migration. McLaren (1963) discusses the additional advantage that food uptake is most efficient at the higher temperatures of surface waters, while growth is more efficient at the lower temperatures of deeper waters. Large terminal size and greater fecundity is generally attained by animals growing at lower temperatures (McLaren, 1963). Hardy (1956) proposed that vertical migration may aid herbivores in exploiting denser patches of phytoplankton through horizontal displacement. McAllister (1969) has

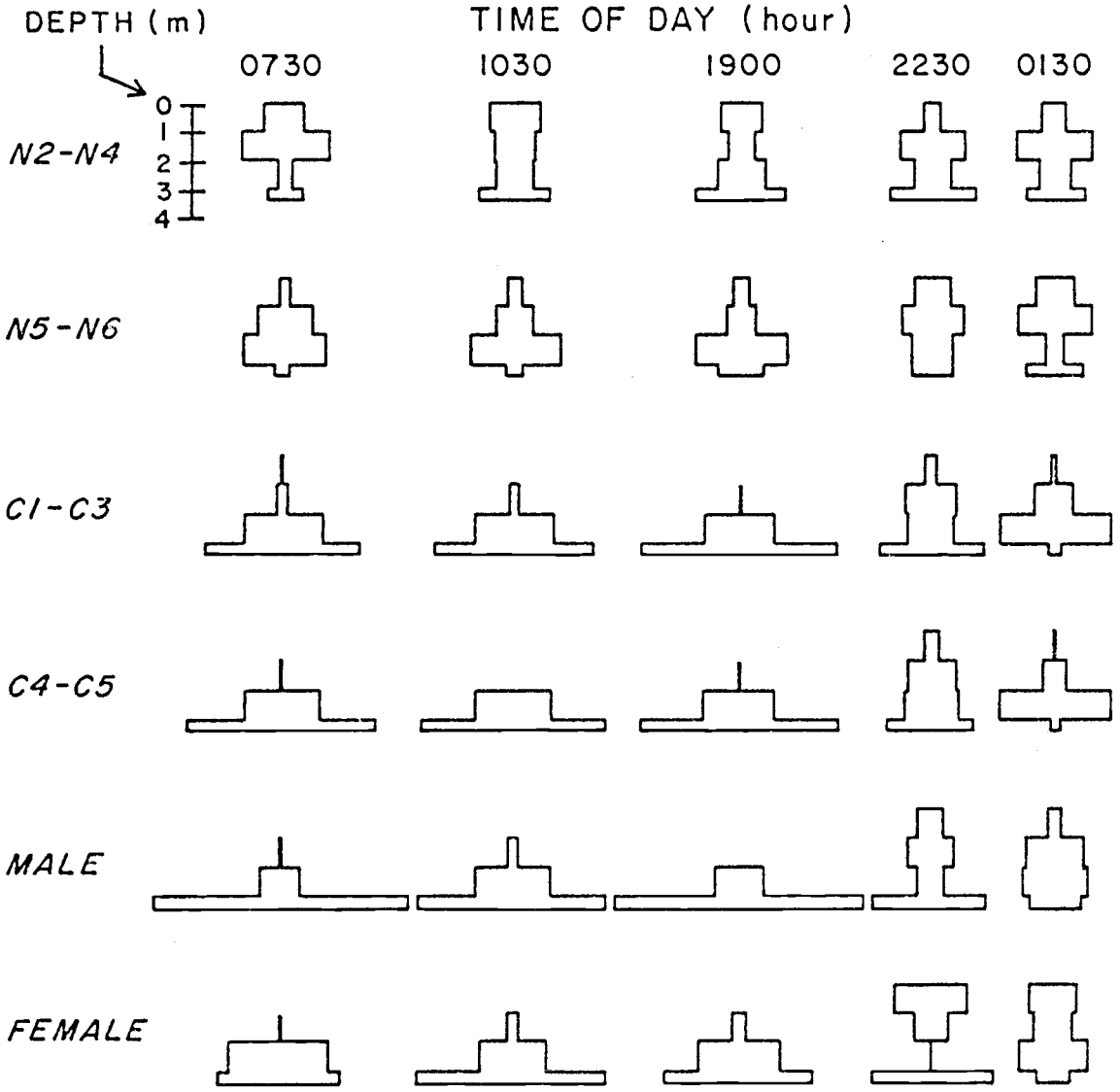


Figure 4. Diel changes in the vertical distribution of A. clausi in Jakle's lagoon on June 11-12, 1974. After Landry, 1976.

suggested that herbivores, which vertically migrate, make better use of the growth potential of the phytoplankton by permitting unimpeded photosynthesis during daylight hours. Kerfoot (1970, 1972) similarly related migrations to feeding strategies. He proposed that the vertical distribution of zooplankton is related to sustained equilibrium production of their food supply. This hypothesis has been recently reviewed by Miller et al. (1972) and McLaren (1974). More recently, Enright and Honegger (1977) have shown that nighttime grazing by herbivores, coupled with vertical migration, can permit the grazers to obtain a greater net gain in energy for growth and reproduction than could be obtained by nonmigrating species continuously feeding in the upper regions of the water column. Another possible advantage of vertical migration, of particular interest to this study, is lessened exposure to downwelling solar radiation which may have deleterious effects upon zooplanktonic organisms. Early experimentation in this area was performed by Huntsman (1924), Klugh (1929, 1930), Harvey (1930) and Marshall et al. (1935). All showed that naturally occurring solar radiation may be harmful to the large, oceanic copepod Calanus. Huntsman and Klugh noted similar mortality with A. clausi. Hairston (1976) offered new experimental support for this hypothesis. Individuals of the fresh water copepod Diaptomus nevadensis containing high concentrations of carotenoids survived significantly better when exposed to natural intensities of visible light than less pigmented copepods of the same species. A review of Hairston's paper and its possible implications as a selective advantage of vertical

migration is dealt with by Enright (1977).

Fundamentals of UV Radiation and Ozone Perturbation

Most activity within the biosphere depends upon the utilization of energy from the sun. Various regions of the solar spectrum have differential effects on life in the biosphere. The UV portion of this incident energy has an impact on life, both useful and damaging. The ultraviolet spectrum reaching the outer surface of the earth's atmosphere has been divided into three regions (Giese, 1976). UV-C spans from the shortest ultraviolet wavelength emitted by the sun (less than 100 nm) to 286 nm. No wavelengths shorter than 286 nm have been detected on the surface of the earth (Giese, 1976). These wavelengths are highly biocidal. Wavelengths of 286 to 320 nm (UV-B region) reach the earth's surface in small quantities. One of the pronounced effects produced by UV-B is erythema of human skin (commonly called sunburn in Caucasians). It also kills bacteria and is important in the synthesis of vitamin D for many vertebrate organisms (Daniels, 1974). UV-A, the span between 320-390 nm, is considered beneficial but can be damaging if received in large doses. It is primarily responsible for pigment darkening of human skin. This appears to be a mechanism for protecting the skin by screening out shorter wavelengths, the more detrimental ultraviolet. An increase in short wavelength UV reaching the earth may have harmful effects on the biosphere. Life tolerates the present level of UV by the use of protective screens, avoidance behavior, and repair processes that reverse much

of the damage inflicted by the radiation (Barenboim, 1973; Bawden and Kleczkowski, 1955; Cook, 1970; Davies, 1969; Harm, 1966). Such repair mechanisms might be overwhelmed by a substantial increase in short UV radiation reaching the earth's surface. The sensitivity of A. clausi to ultraviolet-B radiation is unknown. Determination of the tolerance of this copepod to such radiation was the focus of this investigation.

The ozone in the atmosphere filters incident solar ultraviolet radiation such that only the longer wavelengths of the total UV spectrum emitted by the sun reach the earth's surface. Ozone is presently diffused throughout the stratosphere in relatively small amounts, varying in thickness at STP from 2.4 to 2.6 mm at the equator to 3.1 to 4.3 mm at 70° North latitude. The concentration of ozone can vary with time of day, season and even variations in the sun's activity (Gibson, 1973).

Ozone is formed from molecular oxygen high in the atmosphere by absorption of short wavelength UV-C (peak absorption at 150 nm) radiation; molecular oxygen, after absorption of this highly energetic radiation, is decomposed to atomic oxygen which can combine with another molecule of oxygen to form ozone. Ozone absorbs somewhat longer wavelengths of UV (210-320 nm) than molecular oxygen and becomes altered to form both molecular and atomic oxygen. These can recombine reforming ozone. The absorption of very short wavelength ultraviolet radiation by oxygen, and mid-wavelength UV by ozone prevents the passage of UV-C to the earth's surface and attenuates the UV-B radiation (Johnston, 1971). Present life on earth has

evolved under the protection of ozone. Life in shallow water and on land was probably not possible until after the appearance of photosynthetic activity which liberated oxygen into the atmosphere initiating the development of the ozone layer (Berkner and Marshall, 1964).

The natural production of ozone is considered to be in steady state equilibrium with the natural destruction of the molecule. The equilibrium concentration of ozone is apparently governed, in part, by components of the nitrogen cycle (Johnston, 1972). Naturally occurring oxides of nitrogen serve as catalytic agents accounting for the major portion of ozone breakdown.

If there is indeed an equilibrium, any increase in stratospheric nitrogen oxides will accordingly increase the rate of ozone breakdown. Two of the potential sources for the injection of these substances into the upper atmosphere are the emission from engines of SST aircraft and the formation of oxides of nitrogen from the heat generated in thermonuclear explosions.

Another source of alteration in the ozone layer is the widespread use of chlorofluoromethanes, CF_2Cl_2 and $CFCl_3$. These inert compounds are not affected by life on this planet, nor by any known constituents of the troposphere. They, therefore, appear to accumulate in the troposphere and diffuse upwards into the stratosphere where they are postulated to absorb short wavelength ultraviolet radiation and decompose to release chlorine atoms which catalytically react with ozone to form a chloroxy molecule and oxygen. The chloroxy molecule reacts with oxygen

atoms to form molecular oxygen and releases the active chlorine atoms. The chlorine atoms are now free to initiate the cycle again (Molina and Rowland, 1974; Wofsy and McElroy, 1975).

As the ozone concentration is decreased, there will be a concomitant increase in the UV-B radiation penetrating the atmosphere. As mentioned previously, copepods are the main grazers of the marine ecosystem and A. clausi is one of the more abundant copepods in many temperate estuaries. If a substantial UV-B enhancement occurred the tolerance of this species to UV-B might be considered relevant to the welfare of the ecosystem.

UV Penetration of Natural Waters

The study of the UV-B impact on an aquatic ecosystem raises a major question: To what extent does UV-B penetrate natural water? A scheme of optical classification of ocean water exists (Jerlov, 1968) which distinguishes different marine water types in terms of their transmittance of selected wavelengths of incident solar radiation (Table I). Present among the selected wavelengths is 310 nm, which is within the UV-B range. Jerlov further described the transmission of solar radiation (300-2,500 nm) through these characteristic water types (Table II).

Both Tables I and II illustrate that the coastal water types (1-9) show decreased transmittance as compared with oceanic water types (I through III). Zaneveld (1975) focused upon the 310 nm wavelength radiation and calculated the percent of surface irradiance reaching specific depths of the water types described by Jerlov

TABLE I. Irradiance transmittance for surface water of different water types. After Jerlov, 1968.

Water type	Irradiance transmittance (%/m)															
	Wavelength (nm)															
	310	350	375	400	425	450	475	500	525	550	575	600	625	650	675	700
I	86	94	96.3	97.2	97.8	98.1	98.2	97.2	96.1	94.2	92	85	74	70	66	59
IA	83	92.5	95.1	96.3	97.1	97.4	97.5	96.6	95.5	93.6	91	84	73.5	69.5	65.5	58.5
IB	80	90.5	94	95.5	96.4	96.7	96.8	96.0	95.0	93.0	90.5	83	73	69	65	58.0
II	69	84	89	92	93.5	94	94	93.5	92.5	90.5	87.5	80	71	67.5	63.5	56
III	50	71	79	84	87	88.5	89	89	88.5	86.5	82.5	75	68	65	61	54
1	16	32	54	69	79	84	87.5	88.8	88.5	86.5	82.5	75	68	65	61	54
3	9	19	34	53	66	75	80	82	82	81	78	71	65	62	57	51
5	3	10	21	36	50	60	67	71	73	72	70	67	62	58	52	45
7		5.0	12	22	32	42	50	56	61	63	63	62	58	53	46	40
9		1.5	4.7	9	15	21	29	37	46	53	56	55	52	47	40	33

Table II. Percentage of total irradiance (300-2,500 nm) from sun and sky.[1] After Jerlov, 1968.

Depth (m)	Oceanic water					Coastal water				
	I	IA	IB	II	III	1	3	5	7	9
0	100	100	100	100	100	100	100	100	100	100
1	44.5	44.1	42.9	42.0	39.4	36.9	33.0	27.8	22.6	17.6
2	38.5	37.9	36.0	34.7	30.3	27.1	22.5	16.4	11.3	7.5
5	30.2	29.0	25.8	23.4	16.8	14.2	9.3	4.6	2.1	1.0
10	22.2	20.8	16.9	14.2	7.6	5.9	2.7	0.69	0.17	0.052
20						1.3	0.29	0.020		
25	13.2	11.1	7.7	4.2	0.97					
50	5.3	3.3	1.8	0.70	0.041	0.022				
75	1.68	0.95	0.42	0.124	0.0018					
100	0.53	0.28	0.10	0.0228						
150	0.056			0.00080						
200	0.0062									

[1]For oceanic water the solar altitude is 90°; for coastal water 45°.

(1968). This 310 nm penetration is illustrated in Table III. These calculations give a general view of UV-B penetration through the variety of water conditions. Detection of UV radiation in the 310 nm region may be limited to the upper 15 meters of oceanic water and perhaps the upper two meters of coastal water.

Water and the dissolved mineral components common to most marine water are relatively transparent to UV-B radiation (Hale and Query, 1973; Jerlov, 1968). However, most natural bodies of water are highly absorptive of the mid UV wavelengths. It is the particulate matter, "yellow substance" (humic substances), and organisms themselves which are responsible for a large fraction of the absorption of UV-B in natural water (Jerlov, 1968). Particulates include mostly soil and clay particles, introduced perhaps by upwelling or runoff, and maintained in the water column by turbulent mixing. Humic substance has biological origin, therefore it is clearly possible that the biota of the sea contributes to the UV-B absorption, both indirectly through the synthesis of humic substances and directly through their own absorption properties (Calkins, 1975).

Calkins has investigated the penetration of UV-B through several different natural water conditions. A waterproofed Robertson-Berger Sunburning Ultraviolet Meter (R-B SUV Meter), utilizing a cable modified for noise reduction, was employed. For a more complete discussion of this meter consult Berger (1976). For use of this instrument for underwater measurements see Smith and Calkins (1976). Figure 5 illustrates the diversity of waters sampled by Calkins (1975). A wide variety of water types including

TABLE III. Percent of surface irradiance as a function of depth and water type [λ = 310 nm (UV-B)]. After Zaneveld, 1975.

Water type		I	IA	IB	II	III	1	3	5
Depth (m)	0	100	100	100	100	100	100	100	100
	1	86	83	80	69	50	16	9	3
	2	74	69	64	48	25	2.6	0.8	0.1
	3	64	57	51	33	13	0.4	0.1	-
	4	55	47	41	23	6.3	0.1	-	-
	5	47	39	33	16	3.1	-	-	-
	6	40	33	26	11	1.6	-	-	-
	8	30	23	17	5.1	0.4	-	-	-
	10	22	16	11	2.4	0.1	-	-	-
	12	16	11	6.9	1.2	-	-	-	-
	14	12	7.4	4.4	0.6	-	-	-	-
	16	8.9	5.1	2.8	0.3	-	-	-	-
	18	6.6	3.5	1.8	0.1	-	-	-	-
	20	4.9	2.4	1.2	-	-	-	-	-
	25	2.3	0.9	0.4	-	-	-	-	-
	30	1.0	0.4	0.1	-	-	-	-	-
% Tr in 1 m		86	83	80	69	50	16	9	3
% Tr in 10 m		22	16	11	2	0.1	-	-	-
Depth (m) at which radiation is 10% of surface value		15.4	12.1	10.5	6.2	3.3	1.26	0.96	0.66

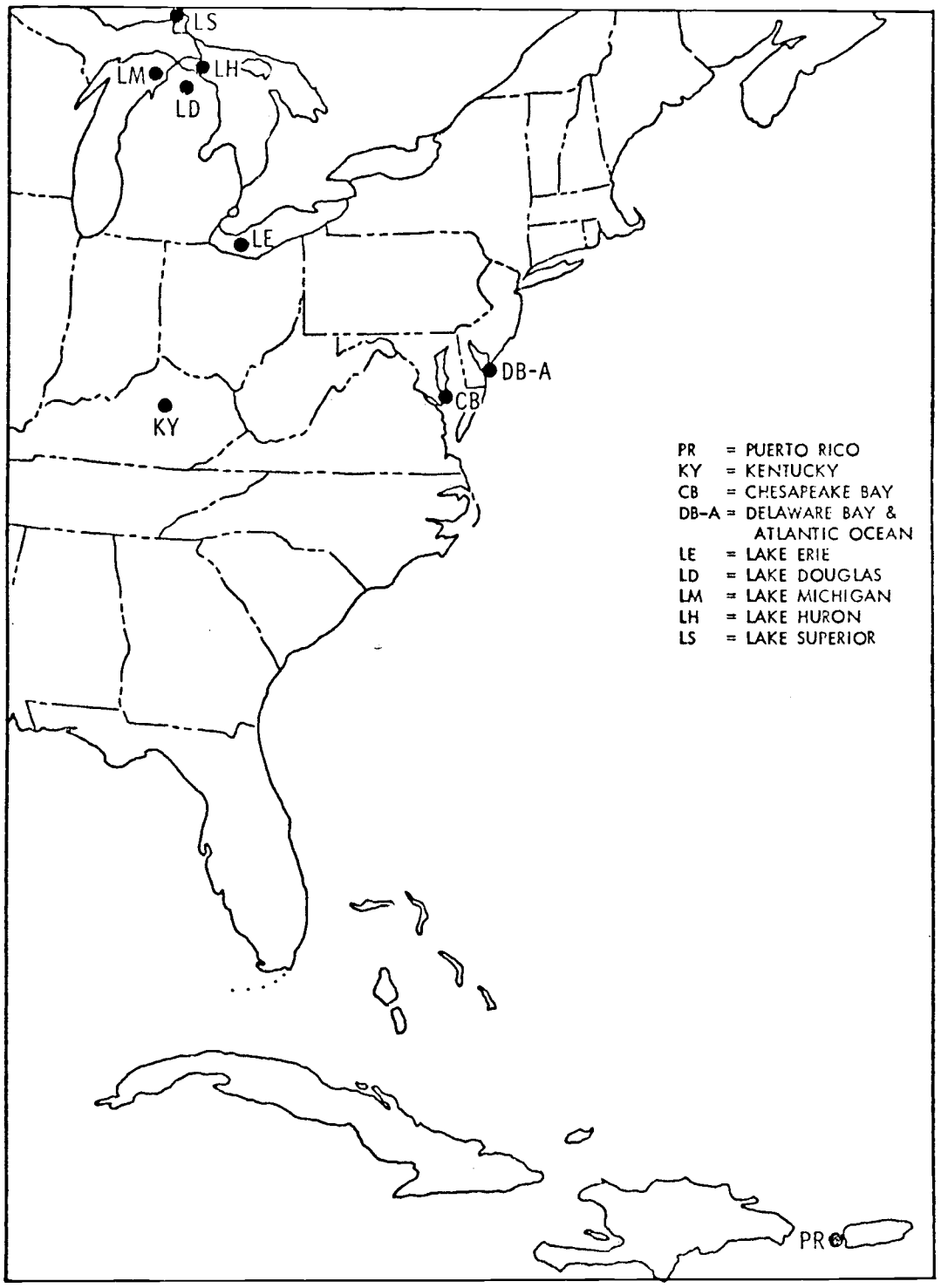


Figure 5. Sites of measurement of water properties. After Calkins, 1975.

oceanic water, bays, estuaries, both relatively clean and eutrophic natural lakes, reservoirs, and small lakes and streams were studied. The sample sites, investigated by Calkins, of interest to this study, are illustrated along with their transmission curves in Figures 6 and 7. These illustrate the penetration of UV-B into natural marine systems. Again it is evident that near-shore stations with higher turbidity showed much less UV-B penetration.

The penetration of UV-B varies widely in various natural waters. However, Calkins (1975) concluded that in all situations, excepting clean lakes with a high content of humic substances, biologically significant levels of UV-B (estimated as 0.01% of the surface value) can occur through most of the euphotic zone.

The complex movements of water in the sea can be separated formally into transport and mixing processes. The large scale transport of water in currents is the best known form of lateral movement and the largest vertical motion of water masses is found in upwelling regions. Turbulent mixing is always present, especially in the surface layers of the sea (Steele, 1959). Through this turbulent mixing organisms normally found deep in the water column may be exposed to increased UV radiation as they are displaced upward. Therefore, the complex movements of water may subject organisms not normally present in the surface water to UV irradiation, though effective levels of UV-B may penetrate only the uppermost layers of the water.

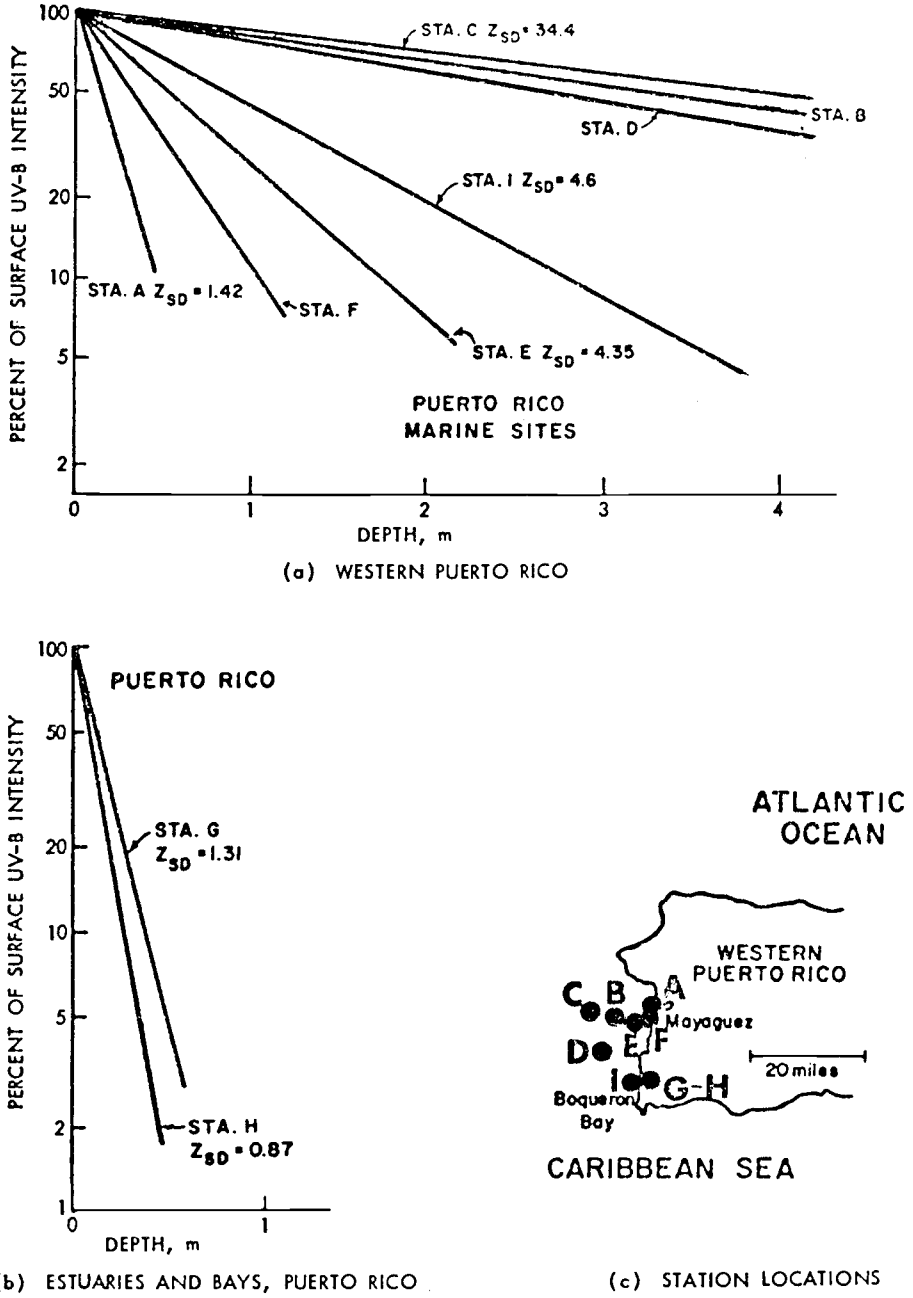
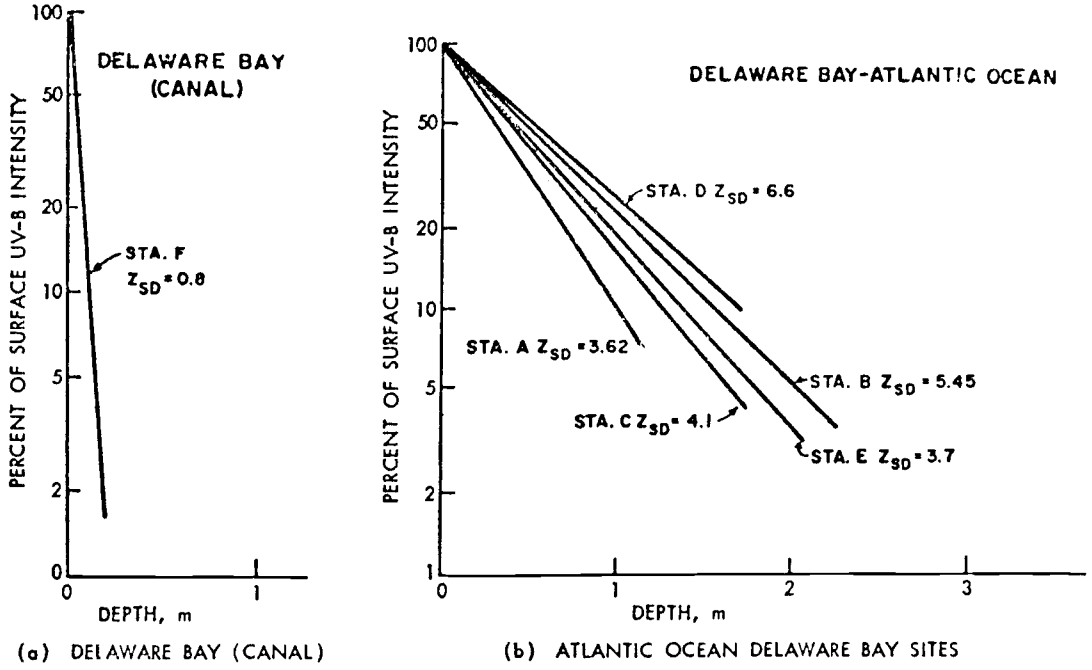
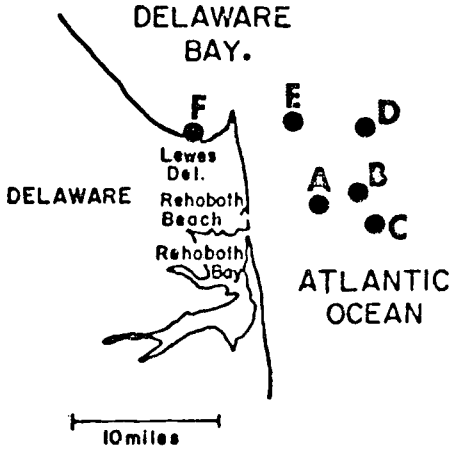


Figure 6. Relative intensity of solar UV-B radiation as a function of depth off western Puerto Rico. Secchi disc depth (in meters) included for all stations except Sta. B and Sta. D at which the bottom was visible (B-8 m, D - 11 m). Depth at Sta. F not indicated. After Calkins, 1975.





(c) STATION LOCATIONS

Figure 7. Relative intensity of solar UV-B radiation as a function of depth in the Atlantic Ocean and Delaware Bay. Secchi disc depth (in meters) included for all stations. After Calkins, 1975.

METHODS AND MATERIALS

Maintenance of Organisms

A. clausi was collected from Yaquina Bay, Newport, Oregon. Cultures were maintained in sterilized 3-liter glass containers (Fig. 8) with a concentration of about 500-600 individuals per jar. The organisms were cultured and irradiated in sterilized seawater obtained from the Oregon State University Marine Science Center, Yaquina Bay, Oregon. Salinity varied seasonally (25-29 °/oo) with changes in salinity of the source water at the intake site for the Marine Science Center. At regular intervals a dense mixture of phytoplankton cells were added to each jar resulting in a concentration of approximately 400,000 cells ml^{-1}. The phytoplankton used as food for the copepods consisted of the chrysomonad Isochrysis galbana (4 μm), the diatom Thalassiosira pseudonana (4 μm), and the green flagellate Platymonas suecica (10-12 μm). Fresh algal food was added to the cultures every 3 days, and on the ninth day the animals were transferred to fresh sterilized seawater. The copepod stock containers were provided with constant aeration to keep the food cells suspended. They were maintained in a constant temperature regime of 15°C (± 2°C S.D.) utilizing approximately 100 lumens/m^2, under a diurnal photoperiod of 8 L:16 D.

A hypotrichous ciliate of the genus Euplotes (Fig. 9), similar to the organism mentioned by Zillioux (1969), was present in the Acartia stock cultures. During microscopic examination the hypotrich

Figure 8. A. clausi stock cultures. 500-600 individuals were maintained in each 3-liter glass container provided with constant aeration.

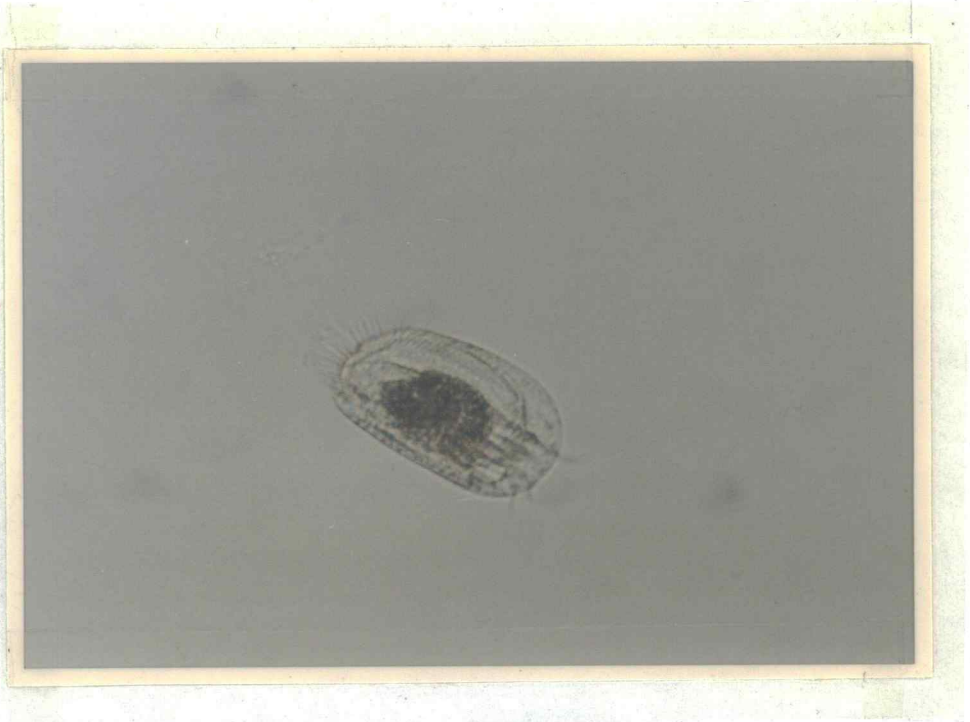


Figure 9. Euplotes sp. A detritus-feeding ciliate present in all copepod stock cultures.

was seen regularly feeding upon detritus, possibly preventing detrimental accumulation. Zillioux (1969) reports that the organism has no adverse effects upon Acartia and was responsible for the reduction in bacterial population and the absence of bacterial slime. A. clausi has also been observed to feed on Euplotes, thus limiting the ciliate population.

Phytoplankton food species were reared in f/2 seawater culture medium (Guillard and Ryther, 1962) at a constant temperature of 12°C under a diel light cycle of 14 L:10 D (~ 5,000 lumens/m^2). Algal culture fluid was periodically removed to be used as copepod food and this volume was replaced with filtered, sterilized f/2. Phytoplankton cell counts were determined with a hemocytometer.

Copepods were transferred by slowly pouring stock from a culture jar through a 53 μm mesh Nitex filter retaining all life stages and hypotrichs but allowing any phytoplankton present to pass through. The organisms remaining on the filter were then gently washed with sterile seawater into a sterilized jar also containing sterilized seawater. Cross-culturing among the populations in different culture jars was carried out at the time of each transfer. This technique was employed to eliminate the development of distinct gene pools within the stock cultures.

All glassware was originally leached for one week through daily changes of distilled water. After use, glassware was washed with distilled water only, avoiding any detergent, and sterilized before reuse. All copepod and phytoplankton stock containers were loosely capped with aluminum foil to permit free exchanges of gases over the

water surface.

Preparation for Irradiation

The various life stages of A. clausi found in Jakle's Lagoon occupy different positions in the water column during the day (Landry, 1976). Therefore, it seems plausible that, within at least this isolated region, these groups would be exposed to varying degrees of UV-B radiation as a function of their distance below the surface. Other examples of differences between developmental stages which may be influential with respect to the amount of radiation received by physiological targets could be cuticular thickness, volume, or even avoidance ability (assuming this is exhibited by A. clausi). It was the intent of this investigation to determine the sensitivity of each life stage to UV-B irradiation. The twelve instars of the life history were divided into five groups: Nauplius 1 and 2 (N1-N2); nauplius 3 and 4 (N3-N4); nauplius 5 and 6 (N5-N6); copepodite 1, 2 and 3 (C1-C3); and copepodite 4 through, and including, the adults (C4-Adults).

Large numbers of the age group to be irradiated were obtained by rearing stock cultures at two temperatures. The enhanced development rate of cold-acclimated A. clausi (Landry, 1976) at higher temperatures is a common acclimation response (Precht, 1958). Conover (1956) found a similar effect for the respiration rate of A. clausi. It was assumed that other metabolic processes might be affected, including egg production and fertilization. Large numbers of unhatched, fertilized eggs were obtained by moving stock cultures

acclimated to 15°C (± 2°C S.D.) to warmer conditions [19°C (± 1°C S.D.)], allowing the culture volume to fully adjust to the new higher temperature, separating a substantial number of adults from these cultures (~200), placing them in sterile seawater at the higher temperature, and providing ample food (200,000 cells ml^{-1} of I. galbana). Frequently the bottom of the beaker that contained the adults was littered with eggs at the end of a twelve-hour period. Another aspect of cold-acclimated A. clausi which could be partially responsible for these large egg yields is that the animals grow larger at colder temperatures. Landry (1976) found the terminal size of A. clausi to be inversely related to temperature and, interestingly, directly related to fecundity.

The eggs collected had been laid over a 12-hour period, placing many at different physiological points of development. Therefore, hatching would not be simultaneous. Since duration time of the early nauplii is relatively short (e.g., N1 = 0.69 days; Landry, 1976), and a large number of copepods at the same stage of development were needed, simultaneous hatching was essential.

Landry demonstrated dark inhibition of egg hatching with A. clausi. Under laboratory conditions, eggs (physiologically developed to the point of being ready to hatch) require light for successful hatching (Fig. 10). Consequently, eggs were placed in a light-secure container and incubated at 19°C for 42 hours. Most of the developing individuals had reached the pre-hatching stage by this time (Fig. 11). The eggs were then placed in the light for 12 hours and at this time a level of 75-80% hatching was achieved of all eggs

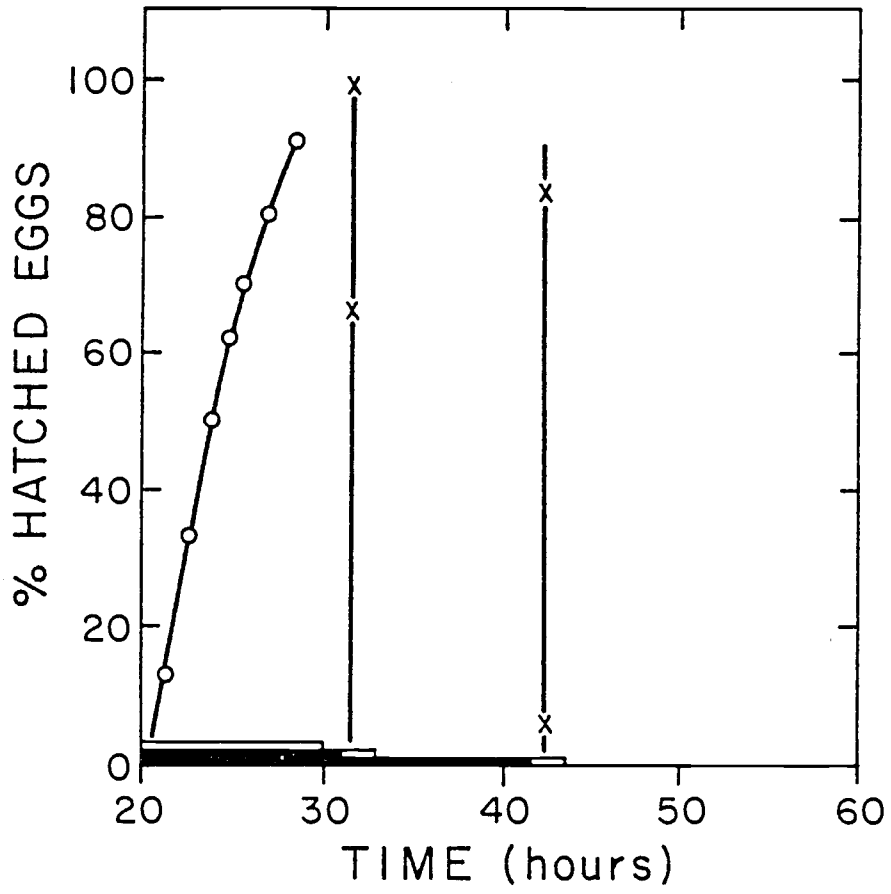


Figure 10. Dark inhibition of hatching of A. clausi eggs. Shaded portion of horizontal bars indicates the duration of dark treatment, X indicates experimental group with some dark treatment, and 0 indicates controls in constant light. After Landry, 1976.

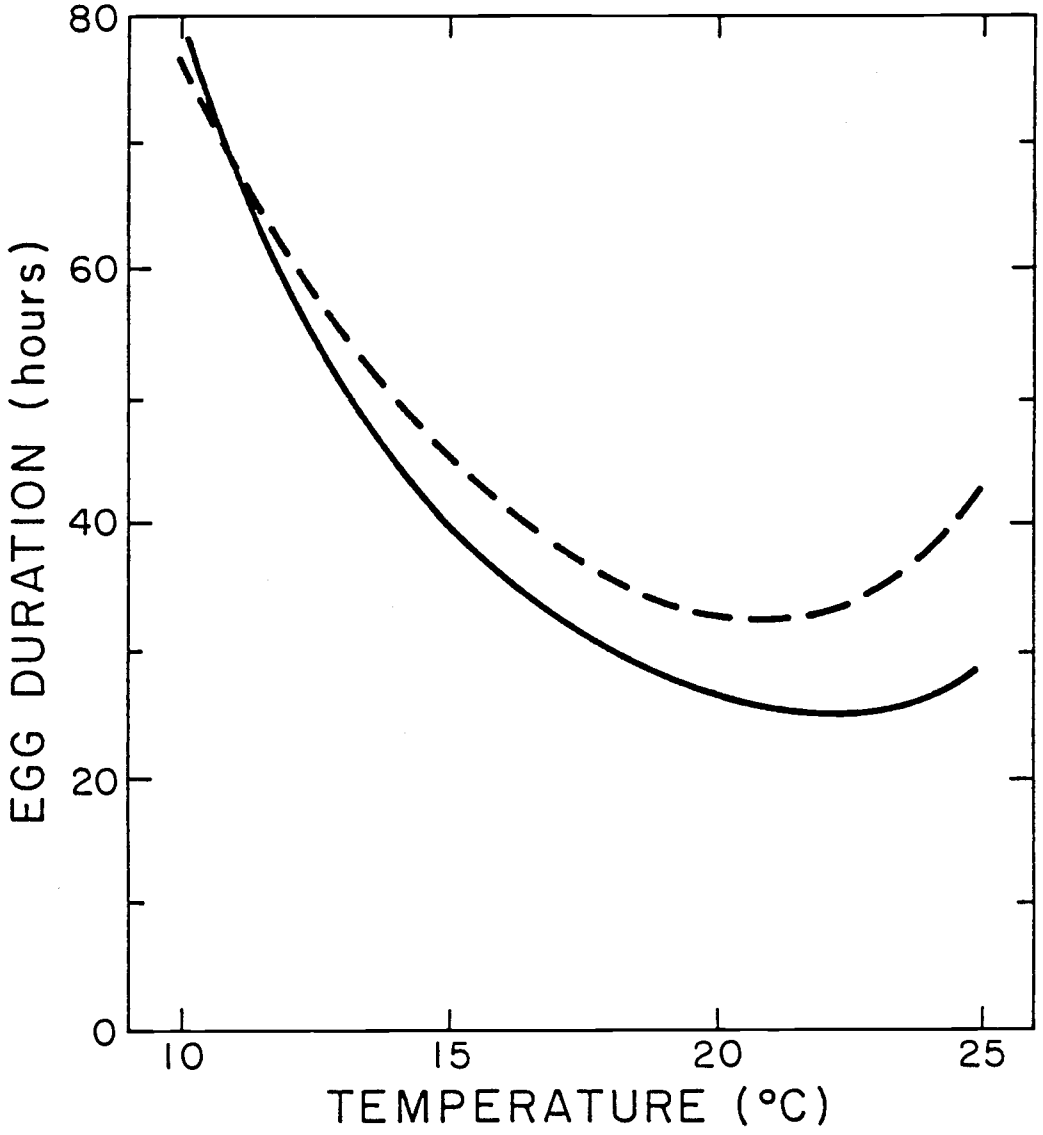


Figure 11. The effect of temperature on development time of A. clausi eggs for two seasonal groups. The curve for animals collected during winter (cold acclimated, 8-10°C) is represented by the solid line and that of animals collected in summer (warm acclimated, 18-20°C) is represented by the broken line. After Landry, 1976.

that eventually hatched. Landry (1976) attained 91-95% hatching in the first half-hour after a 15-minute lag period. This could not be duplicated in the experiments described; however, the method outlined above worked sufficiently well that a substantial number of nauplii, all within the first age group, was obtained. At this point, nauplii were separated from the unhatched eggs.

To acquire large numbers of later stages (age groups through N5-N6) early nauplii were placed in a beaker containing sterile seawater and I. glabana (200,000 cells ml^{-1}). These animals were reared to the desired stage for which the development times were determined by Landry (1976) (Table IV). This technique was not used to obtain organisms for the C1-C3 and C4-Adult age groups. Large amounts of these animals, easier to sort by developmental stage, were extracted directly from cold-acclimated stock cultures for which the water temperature was readjusted to 19°C (± 1°C S.D.) over a 24-hour period. Equal numbers of males and females were separated for the C4-Adult age group.

Prior to irradiation a representative sample (~ 30) of each group of copepods was preserved using 10% buffered formalin. These were examined microscopically to verify the stages of the group being irradiated. Conover (1956) determined the morphological and size changes between successive molts for A. clausi. The size attained by each life stage is directly dependent on developmental temperature and slightly dependent on food (Deevy, 1966). Thus, size will vary according to the conditions during the developmental period. Stages of the samples examined microscopically confirmed the use of

TABLE IV. Predicted minimum development times (DT) and stage durations (D) of A. clausi fully acclimated to 20°C. Development time and duration of any temperature within range of 5 to 20°C are calculated by multiplying the tabulated values by the temperature correction,* where T is temperature (°C). After Landry, 1976.

Stage	DT (days)	D (days)
EGG	0.00	1.37
N1	1.37	0.69
N2	2.06	1.56
N3	3.62	1.09
N4	4.71	1.01
N5	5.72	1.06
N6	6.78	1.16
C1	7.94	1.16
C2	9.10	1.04
C3M	10.14	1.05
C4M	11.19	1.03
C5M	12.22	1.36
M	13.58	
C3F	10.14	1.13
C4F	11.27	1.28
C5F	12.55	1.44
F	13.99	

*Temperature correction = $30.42T^{-1.14}$.

the desired age group for the planned irradiation.

Irradiation Procedure

Figure 12 illustrates the experimental set-up used to irradiate the copepods. The irradiation source consisted of four fluorescent Westinghouse FS40 sunlamps having a peak emission at 310 nm (Fig. 13) which were interdispersed with four "deluxe white" fluorescent lamps (Vitalite, Duro-Test Corp., North Bergen, NJ) to attain a better simulation of the actual solar spectrum. Since the power output of new sunlamps declines rapidly before attaining a slower rate of change (Fig. 14) the tubes were "aged" prior to their use for this investigation.

Output fluctuations were not limited solely to this maturing process of the lamp. Observed fluctuations were also possibly due to ambient temperature changes, the length of time the lamps had been "on," and, most importantly, drains on electrical power in the building. Thus it was difficult to attain the same radiation dose rate for each experiment, however attempts were made to limit this fluctuation: the temperature of the experimental area was kept constant at 19°C (± 1°C S.D.); lamp warm-up time was the same for each experiment; experiments were carried out at the same time of day and the same day of the week to attempt to limit power fluctuations resulting from power drains by other electrical demands in the building. The dose rate was recorded for each experiment prior to irradiation of the organisms. Dose rate measurements were also taken during the exposure period to monitor any major fluctuations in the lamp output.

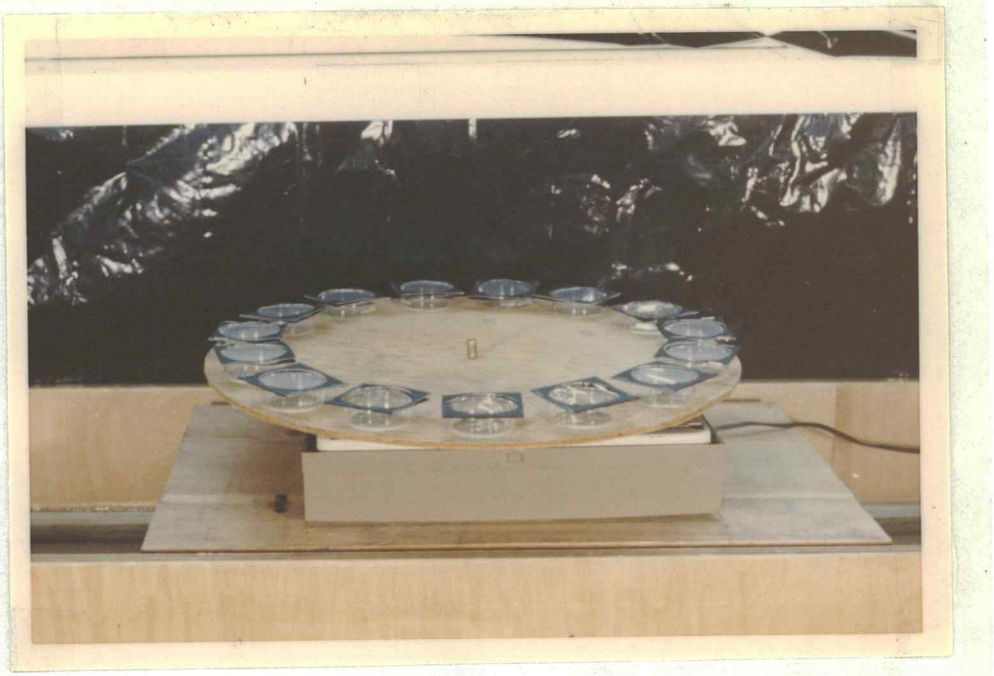


Figure 12. A group of fifteen exposure chambers, covered by appropriate filters (either Kodacel or Mylar), arranged on the experimental turntable. Four "deluxe white" fluorescent lamps interdispersed with four FS40 sunlamps are located 25 cm above the turntable.

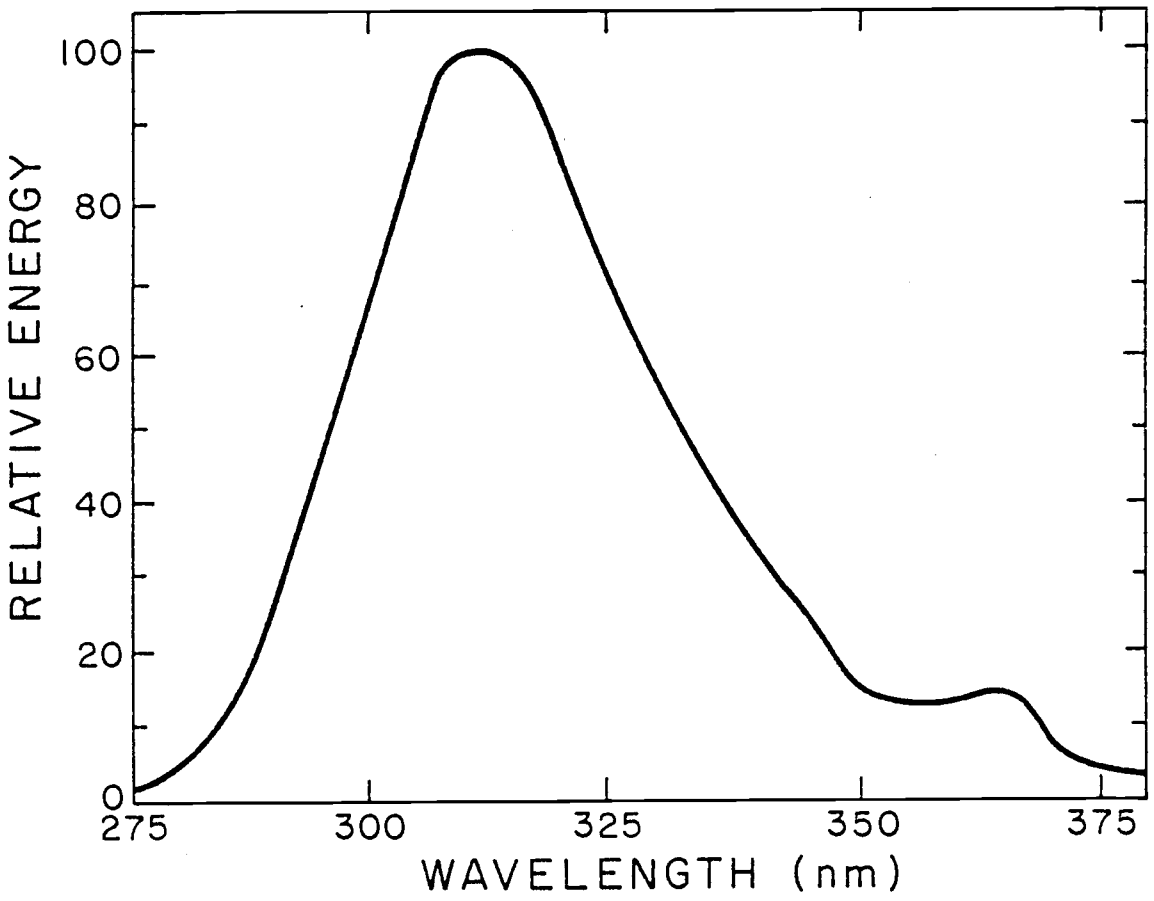


Figure 13. Relative energy distribution of Westinghouse FS40 fluorescent sunlamp. Westinghouse Corp., Data Sheet ASC-504. Major mercury emission line at 366 nm not illustrated.

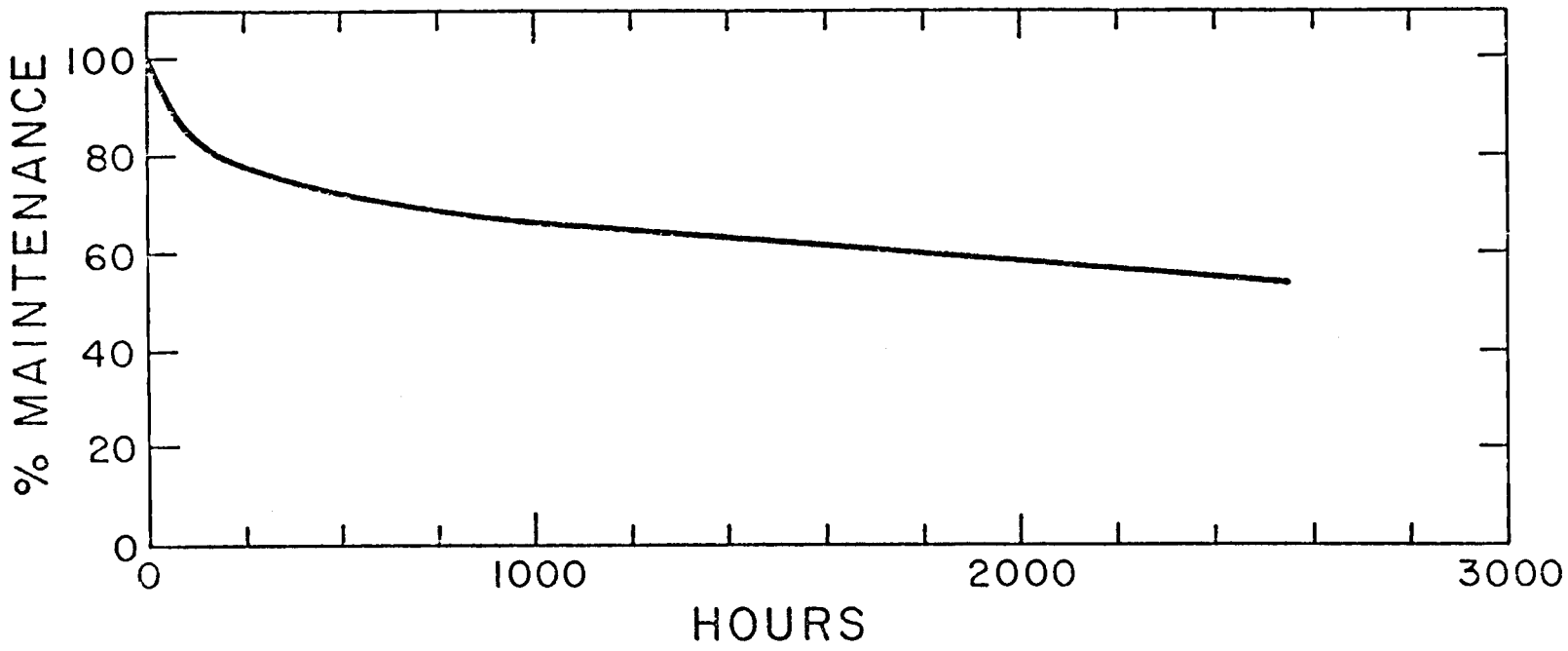


Figure 14. Output throughout life of Westinghouse FS40 fluorescent sunlamp. Westinghouse Corp., Data Sheet ASC-504.

The radiant energy supplied by the FS40 sunlamps was measured with a portable Robertson-Berger Sunburning Ultraviolet (R-B SUV) Meter (Berger, 1976).

Irradiance measurements of the experimental lamp bank were also made with a Gamma Scientific 2900 SR Spectroradiometer System. With the use of this instrument, the output provided by the lamps, at specific wavelengths, can be expressed in quantitative energy units (Fig. 15). The irradiance at the surface of the water in the 290-320 nm waveband under the Mylar filter was 0.02 W/m^2 and under Kodacel it was 1.45 W/m^2.

Organisms to be exposed to the enhanced UV-B levels were placed under a 0.25 mm cellulose triacetate sheet (Kodacel-TA401) for varying times related to the desired exposure. The Kodacel was presolarized (photo-oxidized) to obtain relatively stable transmission characteristics (Worrest, 1975) as illustrated in Figure 16. The FS40 sunlamps provided an emission spectrum from approximately 275 nm to 380 nm. The Kodacel acts to block the transmission of wavelengths shorter than 290 nm providing a spectrum enriched with UV-B radiation. The organisms to be shielded from UV-B were placed under a 0.25 mm thick polyester sheet (Mylar 'S') which transmits only wavelengths longer than 315 nm. Organisms protected by the Mylar filter are designated as the "controls." Those under Kodacel, receiving UV-B, are "experimentals."

Each experiment employed copepods of a specific age group, generally about 30 individuals. Since number of individuals available for irradiation within naupliar age groups was dependent upon

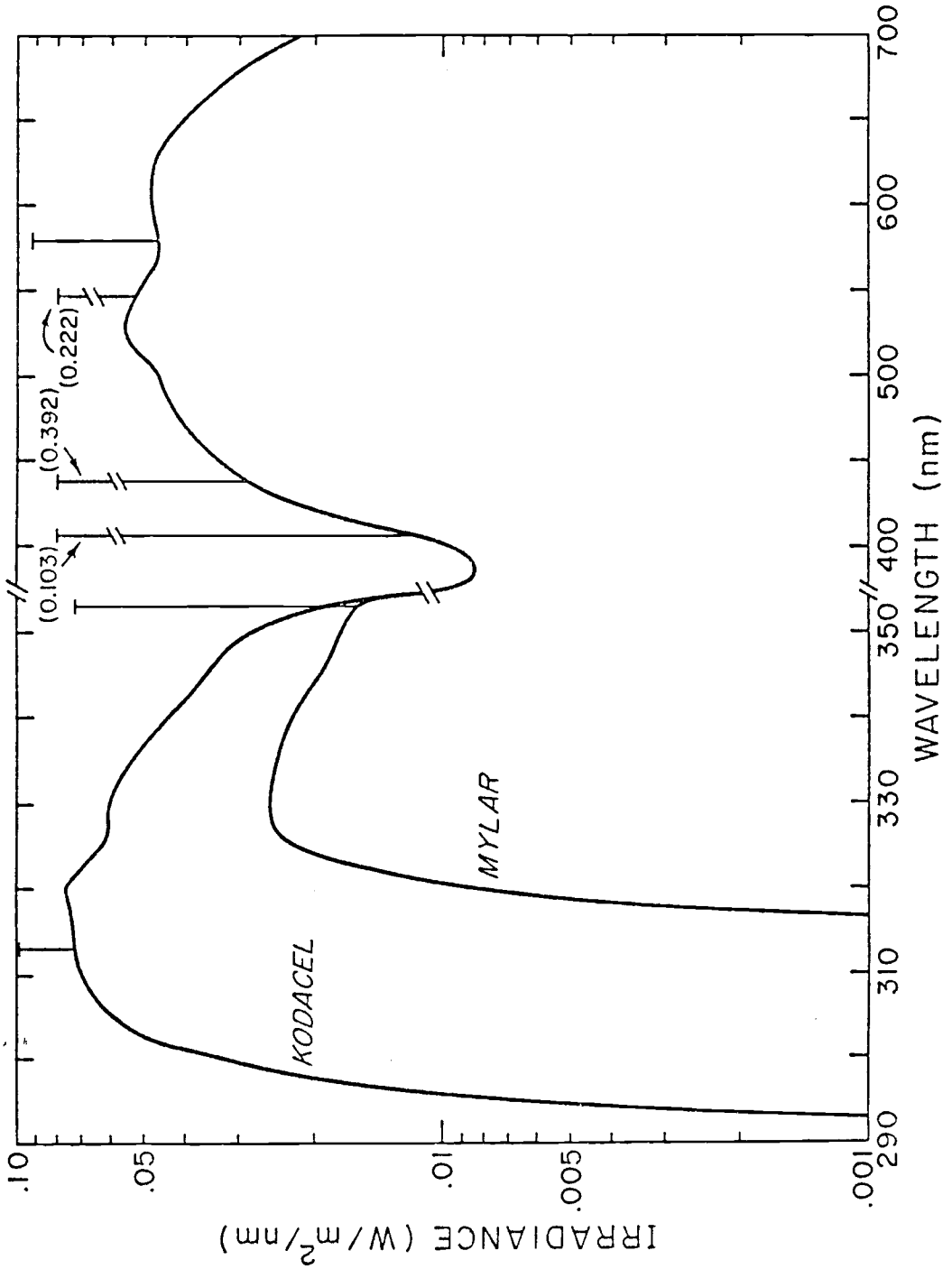


Figure 15. Irradiance (fluence rate) spectra of the experimental set-up. Irradiance was measured at the water surface of the exposure chambers by a Gamma Scientific 2900 SR Spectroradiometer System. Spectral fluence rate in the 290-320 nm waveband under Mylar was 0.02 W/m^2 and under Kodacel it was 1.45 W/m^2. Irradiance of characteristic emission lines of fluorescent lamps used are illustrated.

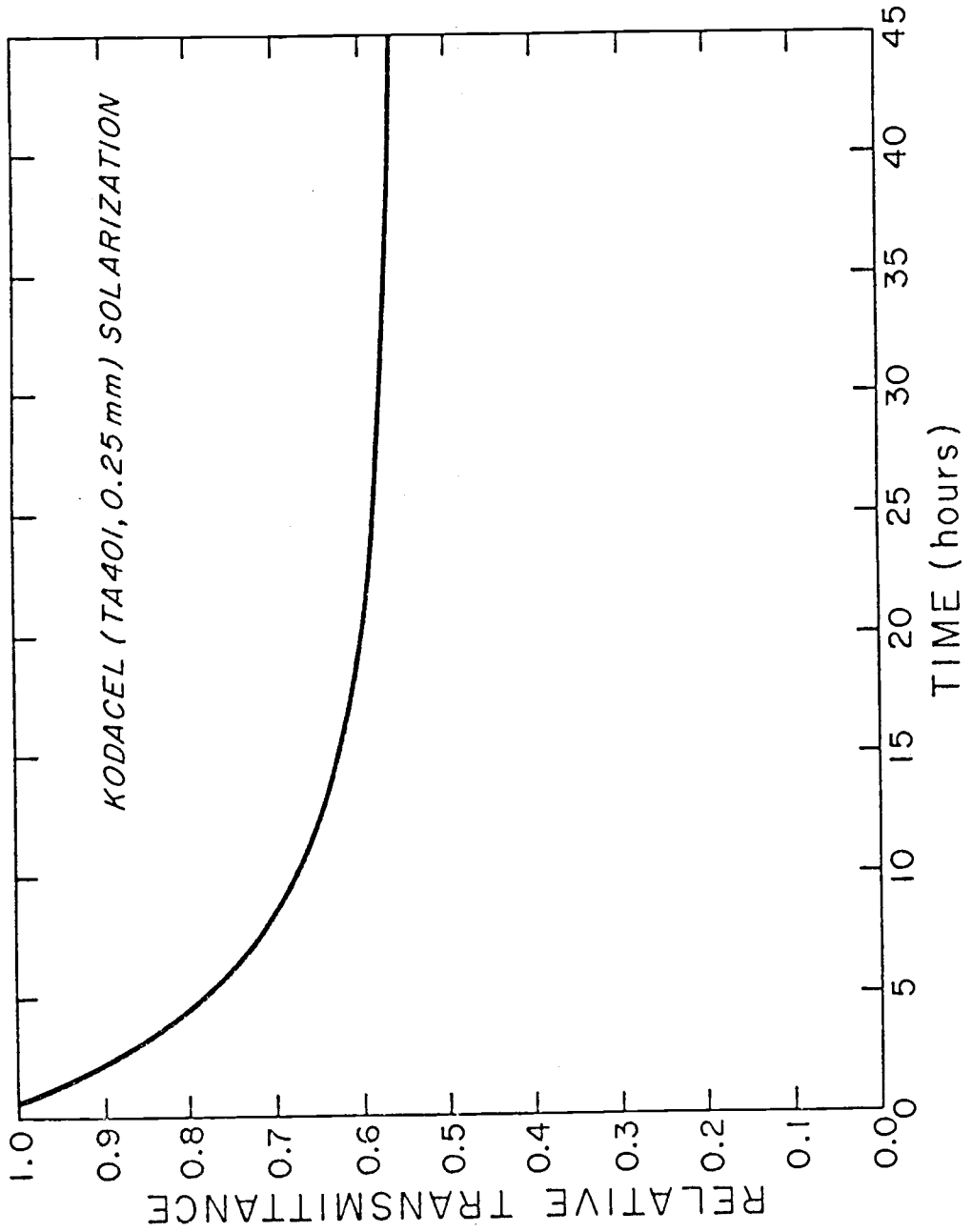


Figure 16. Effect of solarization on the transmission of 0.25 mm cellulose triacetate (Kodacel - TA401). Solarization source as in Fig. 12.

original egg production by the separated adults, percent hatching, and percent surviving, the sample size per exposure varied between experiments. In all cases, the largest possible sample size was used (see data Appendix). Individuals were carefully pipetted into a circular glass exposure chamber (5 cm diameter, 1.5 cm depth) containing 20 ml of sterile seawater and I. galbana (50,000 cells ml^{-1}). Three such containers served as replicates for each level of irradiation. Three controls of equal sample size were included in each experiment. Generally two experiments were performed for each age group. All nauplii were handled without the use of chloroform. Minimal amounts of chloroform were necessary to briefly anesthetize and thus facilitate handling of all copepodites and adults. Full recovery was allowed before irradiation. All organisms were irradiated on a rotating turntable (16 rpm) in an attempt to provide equal exposure.

Following irradiation the animals in each exposure chamber were placed in sterilized 600 ml beakers containing sterile seawater maintained at 19°C (± 1°C S.D.). Each beaker contained an initial concentration of 200,000 cells ml^{-1} of I. galbana and was aerated by a micropipette (Fig. 17). Control and experimental organisms were then reared to reproductive maturity. Survivors were filtered to a smaller vessel, chloroformed and counted. The time allotted for development to maturity varied according to the age of the group at the time of irradiation. Survivors of the C4-Adult age group were counted nine days after irradiation, which represents one week of development beyond sexual maturity. This permitted the production of

Figure 17. Following UV-B exposure, A. clausi was reared to to sexual maturity in 600 ml beakers aerated by micropipettes.

offspring. In experiments with all other age groups the number of survivors was the only criterion evaluated. However, with the irradiation of equal numbers of males and females in the C4-Adult group, the appraisal was extended to include an examination of the fecundity of both experimentals and controls. Therefore the number of offspring produced per exposure replicate was recorded.

Statistical Methods

In this study, numerous copepods within each age group were exposed to five or six different treatments. A second variable, position of exposure chamber on the turntable, was introduced as a "block variable" to eliminate (or block out) unwanted variation in the variable of interest, i.e., response of the organism to the effects of varying levels of UV-B. The exposure chambers were not placed randomly on the turntable but were distributed equidistant from its center among three sections (designating three levels of blocks). When an age group was to receive five different doses, the exposure chambers around the circumference of the turntable were arranged so that one representative dish of each of the five treatments was placed in each of the three blocks. The copepods within a block were randomly assigned to treatments. Each container exposed to the same treatment received an equivalent dose.

The random assignment of individuals to each treatment in all blocks constitutes a randomized complete block for a two-factor design. The essential feature of a randomized block design is that each treatment (here a dose of UV-B) appears once in each block.

Each block constitutes a replication of the experiment providing an estimate of each treatment effect, as well as a contribution to the analysis of experimental error.

Two-factor analysis of variance was performed, testing the equality of group means within treatments and blocks. Prior to the use of analysis of variance, strict criteria requires a measure of the homogeneity of variances for all groups to be compared. The Hartley test (Neter and Wasserman, 1974) was employed to determine the equality of variances of groups within the two classifications (treatments and blocks). Calculated H-statistics did not differ significantly ($p > 0.05$) from critical values.

Using two-factor analysis of variance, the F-statistics indicating differences between blocks (position on the turntable) were not significant (predicted at the 5% level) for all age groups except N5-N6:

N1-N2	$(0.1 > p > 0.05)$
N3-N4	$(p > 0.5)$
N5-N6	$(0.025 > p > 0.01)$
C1-C3	$(0.5 > p > 0.1)$
C4-Adult	$(0.5 > p > 0.1)$.

This indication of increased differences between blocks for the N5-N6 age group may be a result of the reduced sample size used in those experiments or other biological reasons dealt with in the discussion. Regardless, the N5-N6 group received the same statistical treatment as all other age groups.

These non-significant F-values resulting from a comparison between blocks for all age groups indicate that position on the turntable was not an important variable and that the numbers surviving a particular treatment for all blocks was therefore representative of the same population. This permitted the pooling of these individual treatment groups for all blocks, providing a larger total number of survivors for each treatment and, consequently, a more accurate estimate of the surviving fraction:

$$\frac{\text{Total number of survivors for treatment X in age group Z}}{\text{Total number of control (0.00 dose) survivors in age group Z}}$$

Least squares regression analysis was performed between surviving fraction and total dose for each age group. Blocks were not combined for this analysis but were regressed as replicate observations at particular dose (independent) levels. Repeated trials for the same level of the independent variable (replications) enable the testing of how well the computed linear regression equation fits the data by providing an opportunity to investigate the sampling error at each of these treatment levels.

Replications at independent levels enable the partitioning of the residual sum of square (SSE) of the regression into two components: the pure error component (SSPE) and the lack of fit component (SSLF). The mean square of the pure error component is an unbiased estimator of the random sampling error (error variance) about the regression. This error variance (σ^2) was estimated by the sampling variances at independent levels calculated as a result of

repeated observations. The remaining component is the lack of fit component which is indicative of the amount of remaining deviation between the actual data and the fitted regression line after sampling error is accounted for. If there was a substantial lack of fit, some alteration of the data or the model would be desirable if possible.

For testing the significance of the lack of fit component, an F-statistic is determined for the ratio of the mean squares of both components (MSLF/MSPE). If the data deviates substantially from values predicted by the regression, the lack of fit component will be larger than the estimate of the error variance (determined by the pure error component). Large values of F indicate significant lack of fit by the calculated regression.

Other regression analyses were performed to investigate other variables of interest. The surviving fractions of males and females for each treatment within the C4-Adult age group were regressed separately with dose to compare UV-B sensitivity between sexes. A linear regression model between the offspring to survivor ratio of the C4-Adult age group and total dose was also developed.

Standard data transformations (SQRT, LN, LOG10, EXP, 1/x, 1/y, x^2, y^2) and addition of quadratic terms were performed in an attempt to improve the fit of all regression models.

RESULTS

Survival curves depicted in Figures 18-21 illustrate a significant inverse relationship between total exposure to UV-B radiation and the surviving fraction of A. clausi. Points on each curve represent the ratio between total surviving number for each treatment (dose) for all blocks within an age group and total number of controls surviving for all blocks within the same age group. Ninety-five percent confidence intervals were constructed for these surviving fractions based on random large samples of a binomial distribution (Ingram, 1974). For a 95% confidence interval estimate of $\hat{p}$ (point estimate of proportion of survivors of particular treatments to control survivors of the same age group) $\hat{p} \pm \left(1.96\sqrt{\frac{\hat{p}\ (1 - \hat{p})}{n}}\right)$ was used.

Analysis of variance indicates differences in survival between treatments to be significant ($p < 0.001$) for all age groups. This clearly indicates significant differences in the response of copepods to varying dose levels. However, to identify individual differences between any two particular treatments within any age group would require further testing.

Figure 22 is representative of another way of expressing the negative effects of UV-B upon A. clausi. All regression equations (Fig. 22) have significant negative slope components (b_1) ($p < 0.005$) indicating that, per unit increase in dose, there was a significant decrease in the surviving fraction. Data was regressed in its original form, although a natural log transformation noticeably

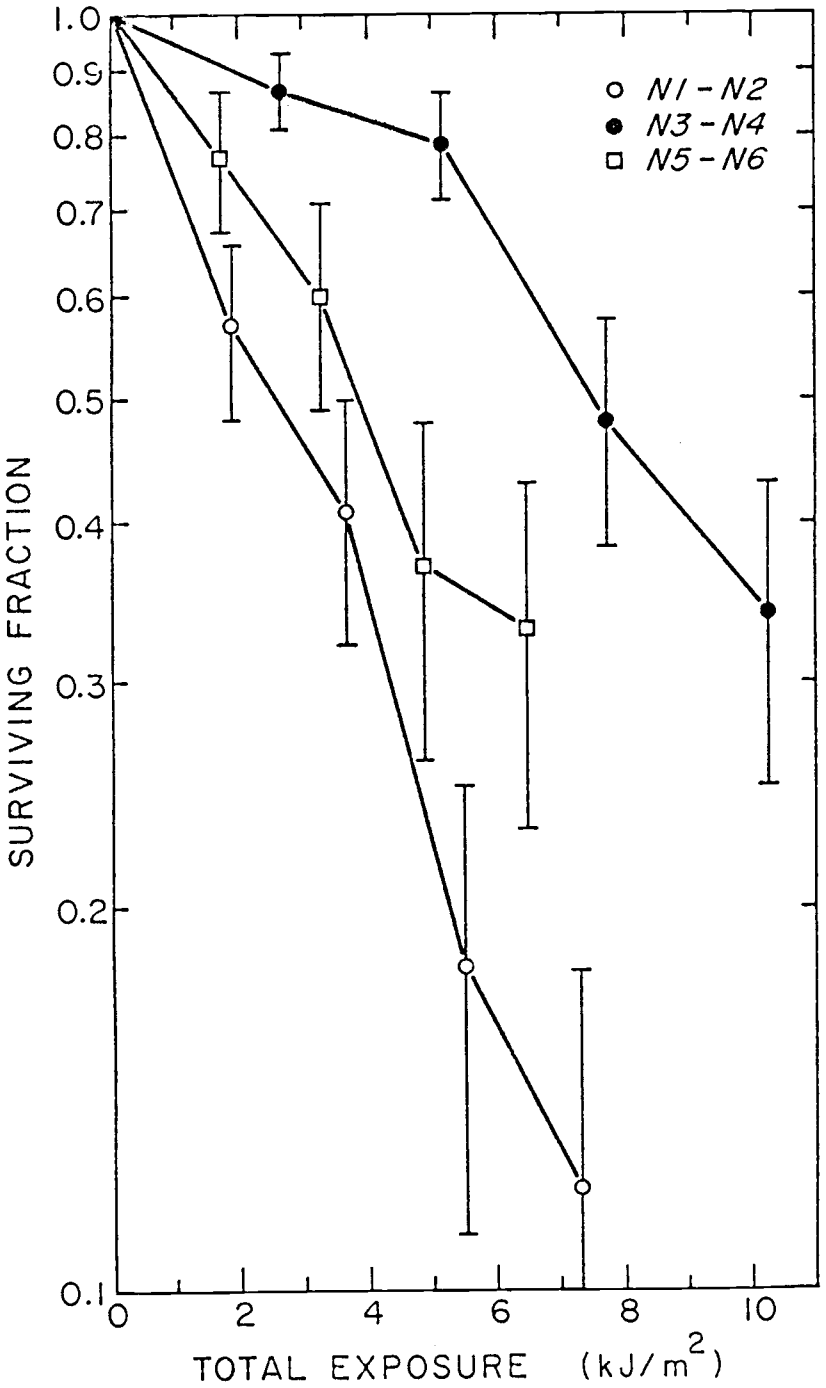


Figure 18. Survival curves for the naupliar stages of development of A. clausi.

UV-B fluence rates were: N1-N2, 1.51 W/m^2;
N3-N4, 1.42 W/m^2;
N5-N6, 1.33 W/m^2.

95% confidence intervals of surviving fractions are shown.

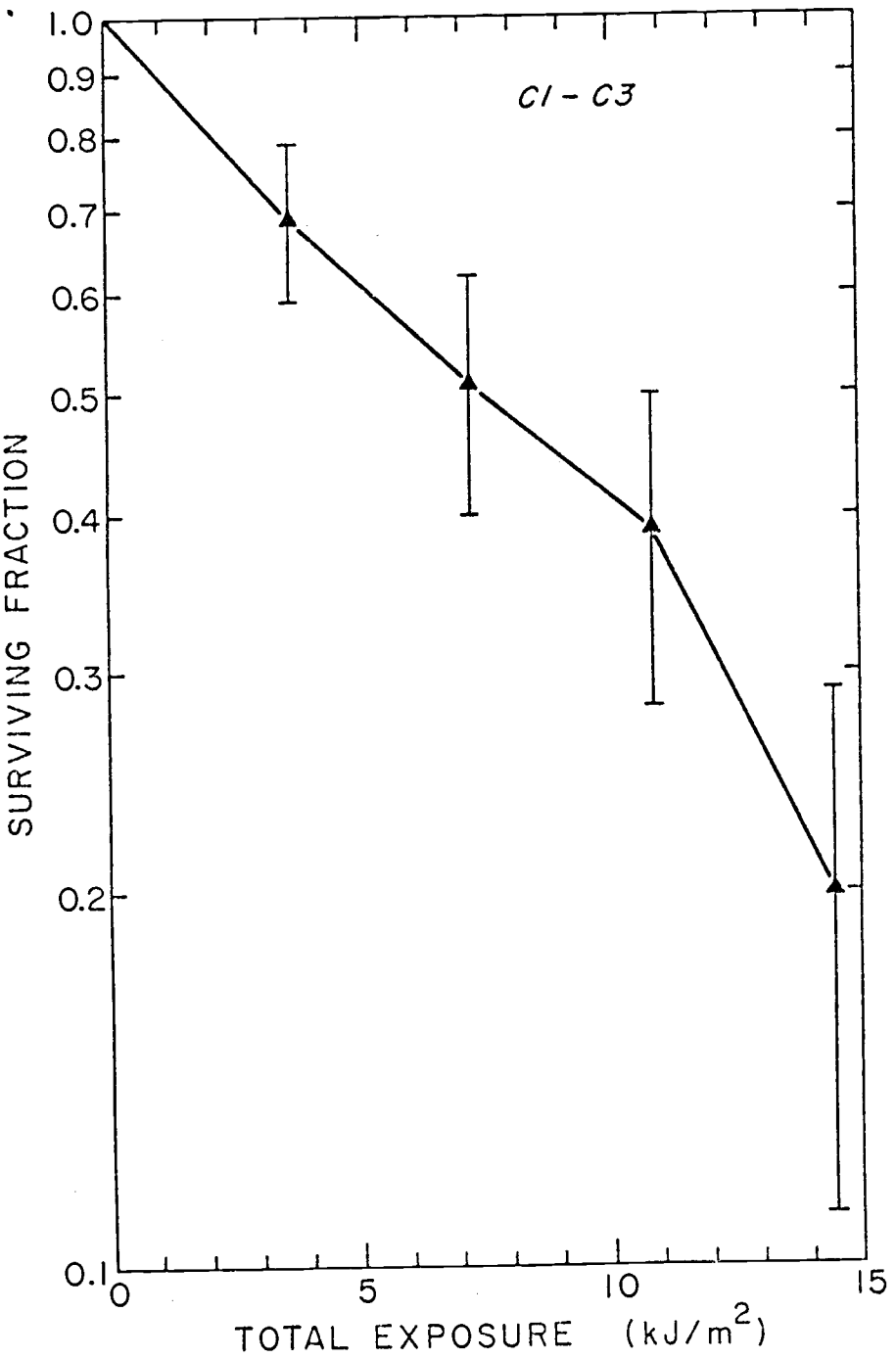


Figure 19. Survival curve for the early copepodite age group of A. clausi. UV-B fluence rate was 1.34 W/m^2. 95% confidence intervals of surviving fractions included.

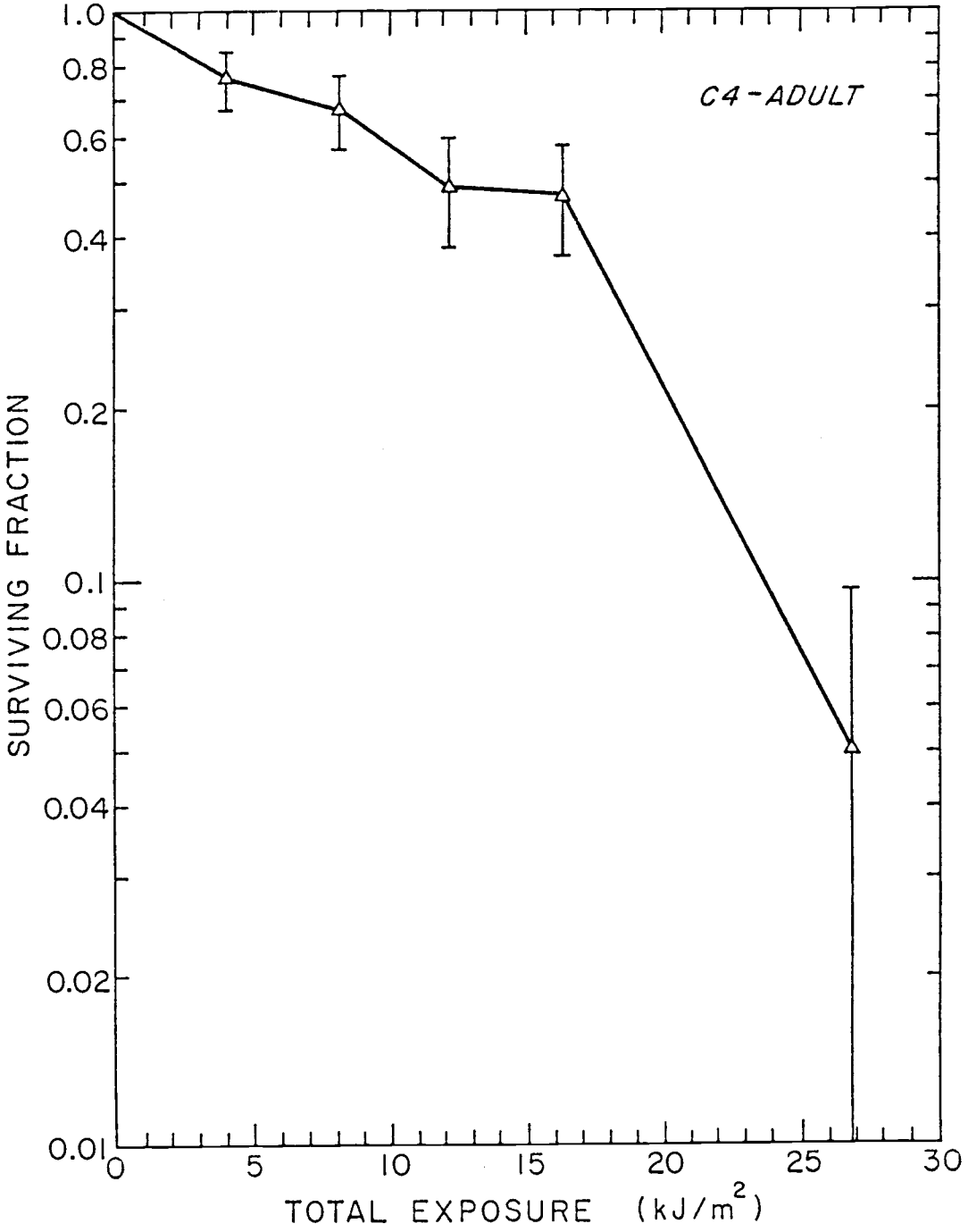


Figure 20. Survival curve for the late copepodite-to-adult age group of A. clausi. UV-B fluence rate was 1.52 W/m^2. Each exposure group contained fifteen males and fifteen females. 95% confidence intervals of surviving fractions are illustrated.

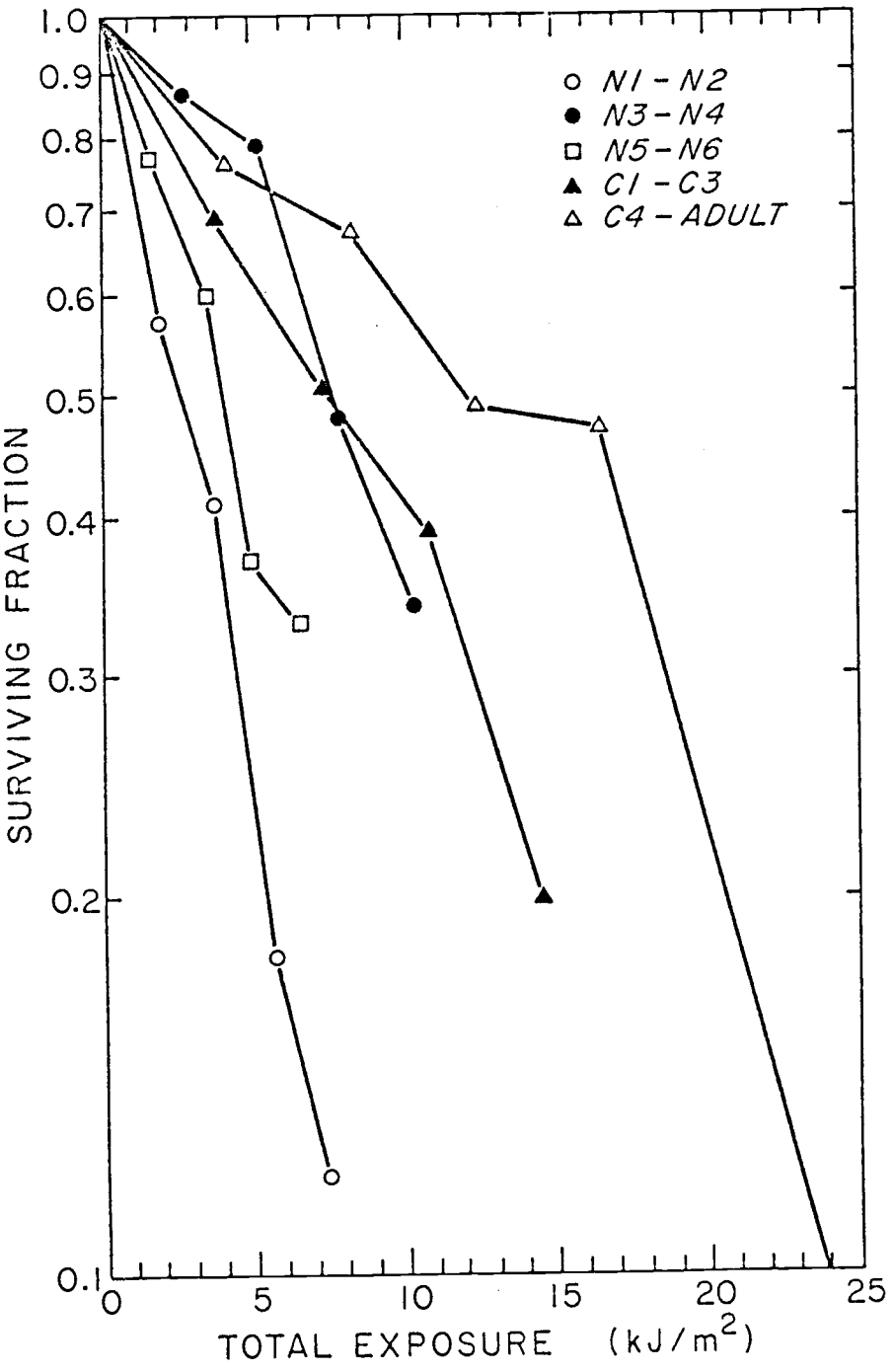
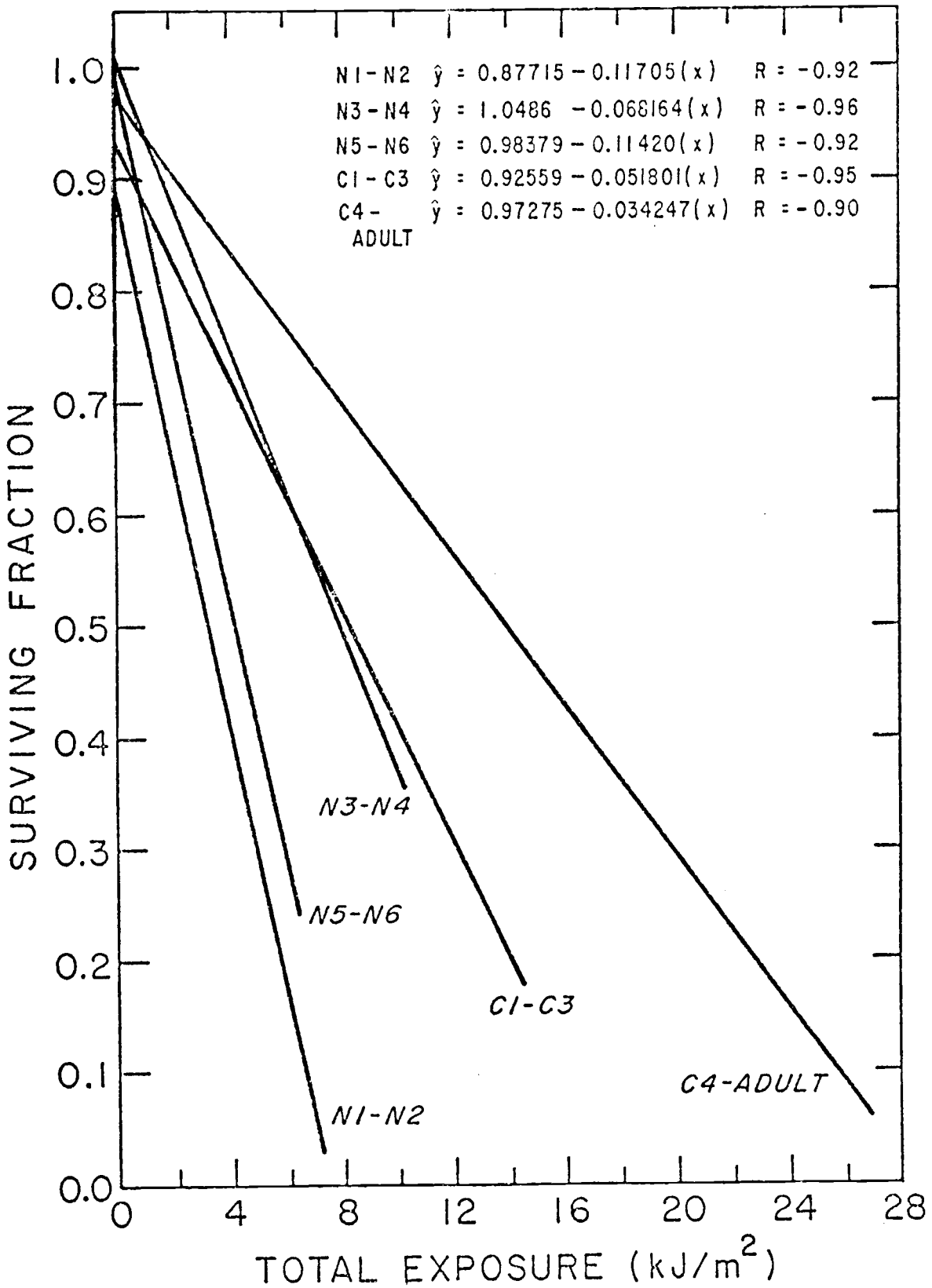


Figure 21. Survival curves for five different developmental phases of A. clausi. Fluence rate in the 290-320 nm waveband, 1.43 ± 0.09 W/m^2.

Figure 22. Least squares regressions between surviving fraction and total dose for five developmental phases of A. clausi. Regression model with correlation coefficient shown for each age group. Slopes of all regressions are significant ($p < 0.005$).


N1-N2 ŷ = 0.87715 − 0.11705(x) R = −0.92
N3-N4 ŷ = 1.0486 − 0.068164(x) R = −0.96
N5-N6 ŷ = 0.98379 − 0.11420(x) R = −0.92
C1-C3 ŷ = 0.92559 − 0.051801(x) R = −0.95
C4-ADULT ŷ = 0.97275 − 0.034247(x) R = −0.90
N3-N4
N5-N6
C1-C3
C4-ADULT
N1-N2
SURVIVING FRACTION
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
4
8
12
16
20
24
28
TOTAL EXPOSURE (kJ/m²)

increased the coefficient of determination (R^2) and increased the randomness of residual plots for the N1-N2 group. A square root transformation worked equally well for the N3-N4 group but for all other age groups the regressions fit best with untransformed data. Therefore, for the purpose of simplicity and comparison, data for these five regressions was not transformed.

A visual comparison of slopes is easiest when all originate from the same point. There were only two significant differences between all the possible combinations of any two of the five regression intercepts (b_0):

N1N2--N3N4 ($0.01 > p > 0.005$)

C1C3--N3N4 ($0.05 > p > 0.025$)

All other combinations ($p > 0.1$).

Therefore, all age groups were combined within a common regression equation. This forced the slopes of all five regressions through a common intercept for better visual comparison. With this technique, statistical testing indicated significant changes in the slopes of some age groups. This was probably a result of the previously noted differences in intercepts. Therefore, all regressions could not be validly forced through a common intercept.

Comparison of slopes of the individual regressions (Fig. 22) indicate significant differences between all combinations of any two of the five regressions:

N3N4--C1C3 ($0.025 > p > 0.01$)

All other combinations ($p > 0.005$).

There is one exception, the slope of N1-N2 and N5-N6 show no

statistical difference ($0.9 > p > 0.8$). While Figures 18 and 21 indicate an apparent greater resistance to increasing dose for the N5-N6 age group than with the N1-N2, Figure 22 illustrates that this may not be the case. The differential sensitivity throughout the age groups in Figure 22 appear consistent with that of Figures 18-21. As the organism ages, its sensitivity decreases. The only contradiction seems to be the N3-N4 group which appears less sensitive than the N5-N6. A possible biological explanation for this is considered in the discussion.

Tests of lack of fit were performed for the regression equations of the five age groups. N1-N2 and N3-N4 both showed significant lack of fit:

N1-N2 ($0.01 > p > 0.005$)

N3-N4 ($0.05 > p > 0.01$).

All other age groups indicated no significant lack of fit:

N5-N6 ($p > 0.5$)

C1-C3 ($0.5 > p > 0.1$)

C4-Adult ($p > 0.5$).

The significant deviation from the data demonstrated by the N1-N2 and N3-N4 models was not unexpected. As mentioned earlier, data transformations adequately improved both regressions.

The irradiation of equal numbers of males and females for each treatment within the C4-Adult age group provided the opportunity to test for differential sensitivity between sexes. Percent of the original fifteen animals (male or female) surviving from each exposure chamber at the time of counting was regressed with total dose. This

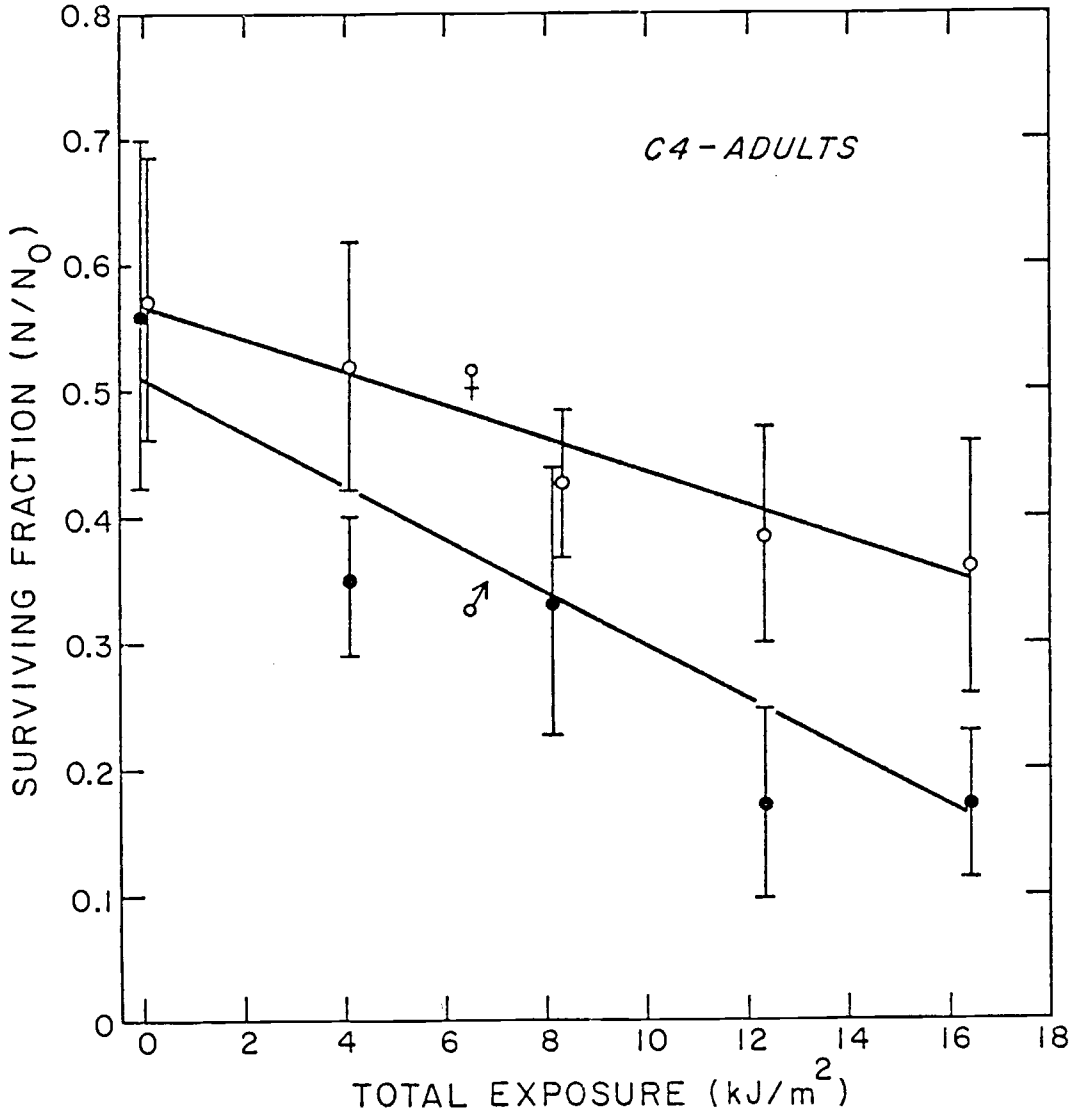


Figure 23. Least squares regressions between male or female surviving fractions and dose after exposure of the late copepodite-to-adult age group. Regression intercepts are not significantly different ($0.2 > p > 0.1$). Slopes of the regressions (males = - 0.023, females = - 0.014) differ significantly ($0.05 > p > 0.025$). Mean and standard deviation of original data are shown. UV-B fluence rate, 1.52 W/m^2.

provided the two least squares regressions illustrated in Figure 23:

$$\text{Male:} \quad y = 0.506 - 0.023\ (x)$$

$$\text{Female:} \quad y = 0.566 - 0.014\ (x).$$

The coefficients (b_0, b_1) of these regressions were compared to zero and to each other to test for significant differences. Both slopes were significantly negative ($p < 0.005$). No significant difference existed between the regression intercepts ($0.2 > p > 0.1$) (the surviving fraction at 0.00 dose), which indicates that there was no significant difference in survival between the males and females protected from the UV-B radiation. However, a statistical difference between the slopes of the two regressions was evident ($0.05 > p > 0.025$), which may demonstrate that per unit increase in UV-B dose the males are more sensitive than the females. Partitioning of the residuals was performed for both regressions indicating no significant lack of fit [male ($0.5 > p > 0.1$), female ($p > 0.5$)].

Another negative response that occurred with supplemental UV-B irradiation involved reduced offspring production. Figure 24 illustrates a least squares regression between the offspring/survivor ratio for three of the five C4-Adult blocks and total dose. Analysis of variance between all five blocks indicated no significant difference ($p > 0.5$). However, the information within the last two blocks was obtained from an experiment performed at a different time from the first three. All experimental variables were essentially the same except the temperature at which the test organisms were reared. Since the temperature to which the animals are acclimated is a highly significant factor when considering fecundity (Landry, 1976),

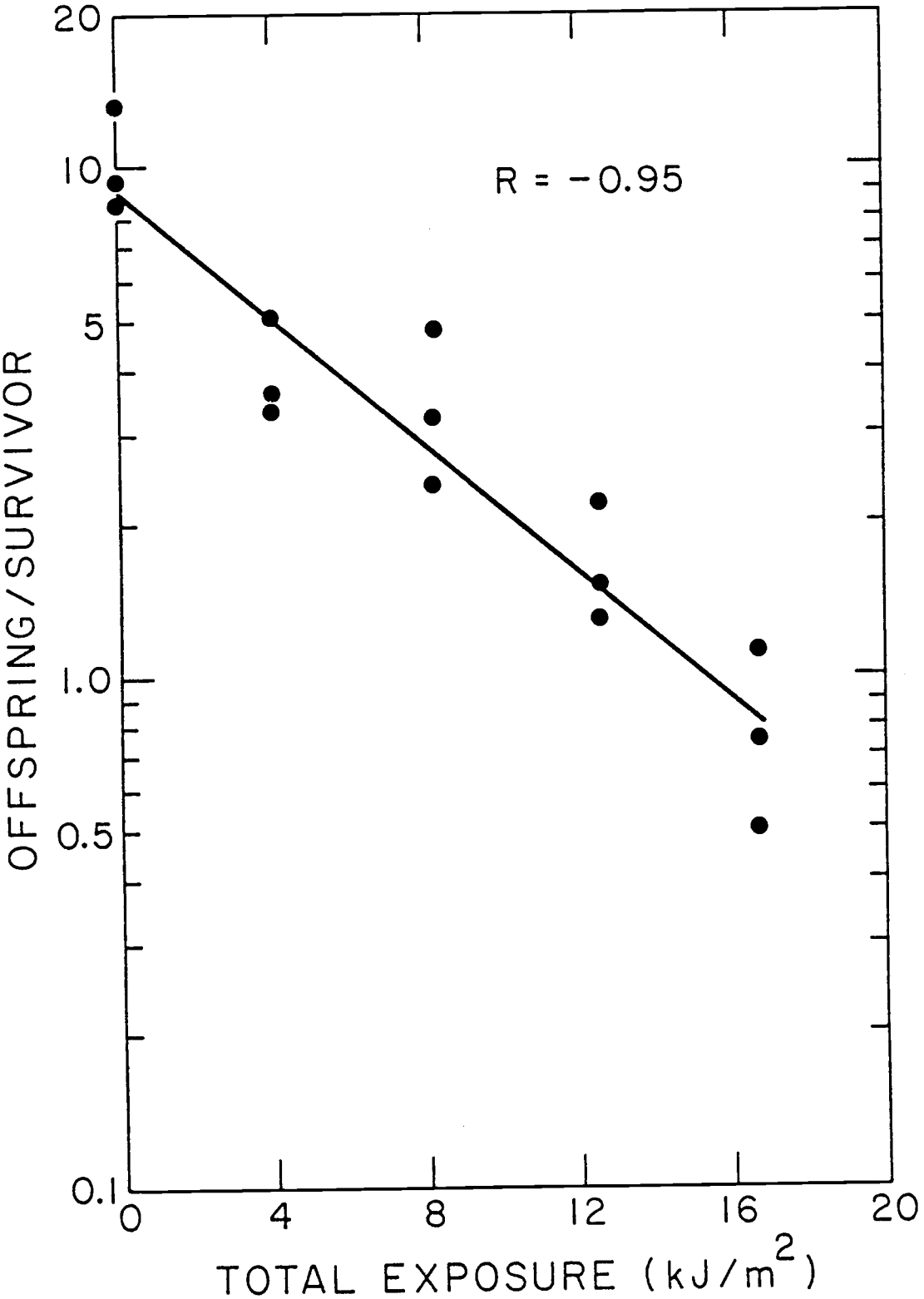


Figure 24. Relationship between the natural log of the number of offspring produced per survivor of the irradiated C-4-Adult age group and total dose as fitted by a least squares regression.

the data within the last two blocks was omitted from this regression.

The regression of the offspring/survivor ratio with total dose fit best after a natural log transformation of the original data. The resulting regression equation was $(y) = 9.003\ e^{-0.143(x)}$ (solved on the log scale), the slope being significantly different from zero ($p < 0.005$). Lack of fit tests indicated no significant deviations between the data and the model.

DISCUSSION

Investigations assessing the impact of solar UV-B upon aquatic ecosystems are presently handicapped by inadequate knowledge concerning the transmission of UV-B radiation through the several categories of natural water as defined by Jerlov (1968). This inhibits adequate approximation of the amount of potentially lethal UV-B present at various levels in a water column for given aquatic conditions. Thus, it is difficult to develop reliable comparisons between the quantity of radiant energy administered to a particular facet of an aquatic ecosystem under laboratory conditions and the amount of similar radiation actually received under natural conditions.

It is possible, nonetheless, to estimate the amount of radiant energy per unit wavelength of UV-B received at the surface of the earth throughout the year at a variety of latitudes. Johnson et al. (1976) tabulated values of the daily global UV irradiance (in Joules/m^2) at the earth's surface as a function of wavelength, latitude, and season, for clear sky and seasonally- and latitudinally-averaged ozone concentration. The most important variables determining the amount of UV radiation received at any location are the solar zenith angle, the atmospheric ozone content, and the transparency of the atmosphere, including the degree of cloudiness (Johnson et al. 1976). Mathematical corrections for cloudiness are referenced and easily introduced.

These tabulations can be combined to estimate the UV-B radiant energy received at selected surface locations for a clear sky at any

time of the year. It is possible to estimate, albeit unreliably, the amount of energy present in the UV-B waveband at some water depth for particular ecological conditions by use of two factors: (1) the Zaneveld (1975) approximation of the transmission of 310 nm wavelength radiation through the water types characterized by Jerlov (Table III) and (2) the relative intensity of UV-B radiation as a function of depth for several geographic locations as set forth by Calkins (1975, Fig. 5-7).

This estimate still does not permit, however, the comparison of treatments given under laboratory conditions to radiation actually received under natural conditions, even though the quantities of radiation have been calculated in comparable energy units (J/m^2). Expressing the radiation dose in terms of total energy (J/m^2) for a defined waveband does not specify the amount of radiation present for each wavelength within the waveband, which is a characteristic dependent upon the radiation source. The energy distribution over the UV-B wavelengths differs when the FS40 and solar spectral radiations are compared. In the 290 nm region there is greater irradiance under FS40 sunlamps than under solar radiation at 45°N latitude. For UV-B hazard analysis, this nonequivalent distribution of energy between wavelengths must be carefully considered, since each wavelength within the UV-B waveband differs in its degree of effectiveness in producing a specific biological endpoint (in the case of this investigation, the death of A. clausi).

The sensitivity of A. clausi to the individual wavelengths comprising the UV-B range can be described by an action spectrum

(specific to the biological endpoint desired). An action spectrum (biological sensitivity function) is the relationship between the biological effectiveness and wavelength of monochromatic radiation. The biological effectiveness is usually expressed as the reciprocal of the dose required to elicit a particular quantitative biological response (Nachtwey, 1975). Once this action spectrum is developed, an analytic representation can be assigned to it in order to predict the dose needed at a specific wavelength to produce the endpoint. The analytic equation can then be used to weight the spectral irradiance values for the sun or the experimental radiation source. Weighting the irradiance at a particular wavelength by its relative effectiveness over all UV-B wavelengths yields the effective surface irradiances and permits comparison of the two radiation conditions.

This approach presupposes knowledge of the action spectrum of interest, which, in the case of A. clausi, is not known. Approximate weighting values developed by the Green and Miller (1975) analytic representation of two generalized action spectra can be employed. These are: (1) the long-wavelength tail of a DNA action spectrum compiled by Setlow (1974),

$$\varepsilon_{DNA}\ (\lambda) = \exp\left\{k\left[\frac{1}{1 + \exp\ [(\lambda - \lambda_o/\lambda_f)] - 1}\right]\right\},$$

where $k = 13.82$, $\lambda_o = 310$, and $\lambda_f = 9$, and (2) Caldwell's (1968) generalized action spectrum,

$$\varepsilon_c\ (\lambda) = A\left[1 - (\lambda/\lambda_c)^n\right] \exp - \left[(\lambda-\lambda_o)/\lambda_f\right],$$

where A = 2.618, n = 2, λ_c = 313.3, λ_o = 300, and λ_f = 31.08. Caldwell compiled data on inhibition of photosynthesis, mutation in spores and a fungus, frequency of endosperm deficiencies, a germicidal action spectrum, epidermal cell damage, and induction of chromosomal aberrations.

These analytic equations were used to obtain a weighting value for the spectral irradiance of each UV-B wavelength received from both the sun on an average, clear August day at 45°N latitude and from the experimental FS40 sunlamps used for copepod irradiation. Restrictions occur with the analytical model of the Caldwell action spectrum, since weighting values can be determined only for wavelengths in the band 290-313 nm. Therefore, only these wavelengths were compared when the Caldwell biological sensitivity function was used. The Setlow analytical expression provided weighting values for the full UV-B range (290-320 nm).

Two comparisons were made between the amount of effective (i.e., weighted) simulated solar radiation required to kill 50% of the laboratory population of each of the five age groups and the effective natural solar radiation received by the surface of the water at 45°N latitude on an average August day.

The total amount of effective radiation required to produce an LD_{50} (using Fig. 22) of each of the five age group populations, using the Setlow weighting function is:

N1-N2, 26.23 Effective J/m^2;

N3-N4, 63.73 Eff J/m^2;

N5-N6, 37.74 Eff J/m^2;

C1-C3, 71.94 Eff J/m^2;

C4-Adult, 103.70 Eff J/m^2.

Employing the Setlow weighting values, the amount of effective radiation striking the earth's surface at 45°N latitude on a clear average day in August is 78.42 Effective J/m^2. When effective radiation conditions are compared, 1.93 hours under the experimental set-up is equivalent to a daily solar dose of UV-B in August.

According to Caldwell, the amount of effective radiation required to produce an LD_{50} for the population of each age group is:

N1-N2, 220.32 Eff J/m^2;

N3-N4, 525.26 Eff J/m^2;

N5-N6, 316.99 Eff J/m^2;

C1-C3, 604.26 Eff J/m^2;

C4-Adult, 871.04 Eff J/m^2.

The amount of effective radiation striking the earth's surface on a clear August day at 45°N latitude is (according to Caldwell) 1213.97 Eff J/m^2. Using the Caldwell model, 3.55 hours under the experimental set-up is equivalent to a daily solar dose of UV-B in August.

With the Zaneveld (1975) prediction for transmission of the 310 nm wavelength (Table III), as the only existing quantitative estimate of the transmission of the entire waveband, present levels of UV-B appear capable of potential damage to A. clausi within the upper meters of the water column. There is definite error in using the 310 nm wavelength as a guideline for UV-B penetration. Not only do the separate wavelengths of the UV-B band vary in biocidal effectiveness and amounts of energy, but they also exhibit unequal

attenuation by water. The smaller, more biologically detrimental wavelengths, are attenuated to a greater extent than the longer wavelengths of the UV-B bandwidth. The extent of this attenuation also varies with water type.

Knowledge of both the differential attenuation of the separate wavelengths with depth for the variety of water types of interest and the relative biological effectiveness of each wavelength for the action of interest would make it possible to evaluate the effects of UV-B upon any facet of an aquatic ecosystem.

Results of this investigation indicate an inverse relationship between age of the organism and sensitivity to UV-B. The only exception to this is the N5-N6 group. The observed, increased sensitivity for this group may be a result of both biological and methodological factors. Most copepod irradiation took place about midday of the diurnal light cycle of the copepods on the same day of the week for fifteen consecutive weeks, in an attempt to limit lamp output fluctuations (see Methods and Materials). However, a major portion of the N5-N6 copepods had to be irradiated during their nocturnal period as a result of uncontrollable events. This creates problems with data interpretation. Survival was slightly reduced for the copepods irradiated at night. This may indicate a time of increased sensitivity in a circadian rhythm. After daytime irradiation, when the organisms were returned to their normal diurnal light cycle, several hours of light still remained to promote photo-repair processes. After nighttime irradiation, the organisms were returned to the nocturnal period thus inhibiting photoreactivation

and possibly decreasing survival. These factors complementing the reduced sample size used for those experiments no doubt increased the statistical variability of the results and are possibly responsible for the significant F-statistic indicating differences between blocks observed for this group.

Regardless, the N5-N6 nauplii may actually be the second most UV-B sensitive age group as a result of the organism's physiological state prior to molting. During crustacean growth, the physiological processes involved in molting do not cease during the intermolt period but continue until the organism either dies or attains sexual maturity (Barnes, 1968). The process of molting is often hazardous. Russell-Hunter (1969) claims the molt cycle may affect population dynamics of arthropods such that a survival curve over time for a population might resemble a series of steps indicating increased chance of mortality at each molt. This contrasts with a more smooth concave curve of survival seen for many non-arthropod invertebrates. The only major morphological transformation in the life of A. clausi occurs between the last naupliar and first copepodite stage, the preparatory phase of which may place a greater strain on the organism than do all other molts. This may sensitize animals of this stage to environmental stress to a greater degree accounting for their increased mortality under UV-B exposure. Gehrs (1975) observed high mortality occurring for the sixth naupliar stage of laboratory populations of Diaptomus clavipes, a freshwater calanoid copepod with a life history similar to that of A. clausi. Diaptomus undergoes an N6-C1 molt as does Acartia. Gehrs speculated the increase in

mortality at this stage of the organism's life cycle possibly being associated with the morphological changes occurring when the organism molts from naupliar to copepodite form.

It appears that current natural levels of UV-B radiation, as well as enhanced levels, may inhibit the development of A. clausi under certain natural conditions. The vertical distribution of A. clausi in Jakle's lagoon, as described by Landry (1976), places the major portion of the early stages in the upper two meters of the water column throughout the day where they could possibly receive damaging amounts of UV-B radiation.

The differences in vertical distribution of the several age groups in Jakle's lagoon may be a result of the particular physical conditions of the lagoon. It is a semi-isolated shallow body of saline water bounded on three sides by a dense stand of Douglas fir, with no apparent source of freshwater inflow, although some runoff from the surrounding hills is likely. Exchange of ocean water occurs only through a narrow tidal channel (Landry, 1976). Because it is isolated from the major effects of tidal mixing, has no substantial freshwater inflow and is surrounded by trees protecting it from the wind, movement and mixing of the water column is most likely reduced enough to allow for the vertical distribution of animals which occurs. For whatever reason this occurs and even though such water is generally highly productive, limiting deep UV penetration, the proximity of these organisms to the surface may place them in a zone where potentially lethal levels of radiation may occur. The physical parameters of Jakle's lagoon are not representative of estuarine

conditions where A. clausi is generally found.

A similar pattern of vertical distribution of A. clausi has not been recorded in any major estuaries, probably due to the increased turbulent mixing. Bar-built estuaries (Pritchard, 1967) though considerably larger than Jakle's lagoon, are not much deeper. Pritchard describes them as the least turbulent estuaries. Though tidal action is reduced, turbulent mixing can still occur as a result of freshwater inflow and wind. Vertical distribution of zooplankton in representative bar-built estuaries has not been discussed in the literature. Zimmerman (1971) recorded A. clausi as the most abundant organism in Netarts Bay (OR), which is similar to a bar-built estuary although it differs in some of the defined properties of this category and is referred to as a coastal lagoon. The bay is described as being very shallow with a maximum depth of 14 feet at low tide. Though the A. clausi may not be differentially stratified as a result of turbulent mixing, the shallow depths of this estuary may result in unfavorable conditions throughout a major portion of the water column.

Drowned river valleys, or coastal plain estuaries (Pritchard, 1967) are perhaps the most widespread estuaries. These are considerably deeper than bar-built estuaries and exhibit a greater degree of turbulent mixing. Detrimental levels of UV-B occur most likely in only the upper meters of these estuaries. Zooplankton, as a result of turbulent mixing, may be placed in biologically-detrimental regions for intermittent periods of time.

If the sensitivity of A. clausi is at all indicative of the sensitivity of other species of copepods, many marine species may

presently be receiving detrimental levels of UV-B radiation. Oceanic water permits much greater transmission of the biocidal UV-B wavelengths than does estuarine water. Calkins (1975) has reported that a pathlength of nearly 15 meters may be required to reduce the UV-B radiation to the 10% level in oceanic water. Zooplankton also have a worldwide distribution, and according to Johnson et al. (1976), the daily UV irradiance at the earth's surface, for certain wavelengths, may exhibit magnitude differences with variations in latitude, as a result of varying stratospheric ozone concentration.

Among the groups of copepods exposed to UV-B there were marked differences in the number of second generation organisms produced per surviving adult (Fig. 24). This suggests that even when a copepod survives exposure to UV-B radiation, the ability to reproduce suffers. The combination of reduced survival and lessened reproduction indicates that a potential exists for an adverse effect on this facet of the marine ecosystem.

Current levels of UV-B radiation may prove to be an important ecological parameter, possibly limiting many marine populations. Landry (1976) suggests, as a reasonable goal of ecological research, "The understanding of how organisms interact and are affected by and adapted to their environment so that the effects of natural perturbations and manipulations of environmental variables can be predicted with confidence." The major functional unit of the ecosystem is the population, which occupies a certain functional niche related to the role of the species in energy flow and cycling of nutrients (Smith, 1974).

Analysis of the dynamics of natural populations depend on detailed knowledge of processes affecting population birth rates and death rates (Landry, 1976). Landry's investigation provides extensive documentation of processes affecting the annual cycle of population growth and decline for A. clausi. Four factors were investigated in an attempt to account for the observed pattern of mortality of A. clausi in Jakle's lagoon: tidal outflow, physiological death, cannibalism, and fish predation. Present levels of UV-radiation may be a previously unconsidered but significant factor in the mortality of this species, thereby implicating UV-B as an important ecological parameter to possibly be incorporated in future discussions of population dynamics.

Since present levels of UV-B radiation may be ecologically significant, the possibility of enhanced levels of this radiation as a result of ozone depletion may pose a serious threat to the welfare of many marine ecosystems. What needs to be determined more precisely is whether UV-B substantially affects natural population levels at present. If not, it is conceivable that organisms may presently survive in nature through a combination of mechanisms for tolerating the detrimental effects of UV-B. In such a case, subsequent investigations need to determine if these mechanisms would be able to mitigate enhanced levels of this radiation. Adequate approximations of UV-B fluence rates under natural waters would aid predictions immeasurably.

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APPENDIX

Group	Sample size	Total dose (kJ/m^2)	Number of survivors	Mean ± SD	Mean proportion
N1-N2	30	0.00	27, 23, 18, 22, 24	23 ± 3	0.77
		1.82	14, 16, 10, 16, 9	13 ± 3	0.43
		3.61	13, 10, 7, 10, 7	9 ± 3	0.30
		5.43	3, 5, 4, 5, 4	4 ± 1	0.13
		7.25	4, 2, 2, 2, 4	3 ± 1	0.10
N3-N4	40	0.00	33, 35, 35	34 ± 1	0.85
		2.55	33, 28, 29	30 ± 3	0.75
		5.10	30, 26, 25	27 ± 3	0.68
		7.66	15, 16, 18	16 ± 2	0.40
		10.21	11, 10, 14	12 ± 2	0.30
N5-N6	22	0.00	19, 18, 17, 19	18 ± 1	0.82
		1.62	17, 12, 13, 14	14 ± 2	0.64
		3.24	15, 11, 9, 9	11 ± 3	0.50
		4.86	6, 9, 5, 7	7 ± 2	0.32
		6.47	9, 6, 3, 3	6 ± 2	0.27
C1-C3	30	0.00	28, 24, 22	25 ± 3	0.83
		3.61	14, 19, 18	17 ± 3	0.57
		7.22	16, 11, 11	13 ± 3	0.43
		10.81	10, 11, 8	10 ± 2	0.33
		14.42	6, 5, 4	5 ± 1	0.17
C4-Adult	30	0.00	12, 15, 19, 21, 18	17 ± 4	0.57
		4.11	12, 13, 11, 13, 16	13 ± 2	0.43
		8.24	10, 8, 14, 12, 13	11 ± 2	0.37
		12.35	10, 10, 5, 7, 10	8 ± 2	0.27
		16.46	9, 8, 8, 6, 9	8 ± 1	0.27
		26.89	0, 2, 0, 1, 1	1 ± 1	0.03

Table A1. Survival blocks for the five irradiated age groups.

C4-Adult

Total dose (kJ/m^2)	Number of female survivors	Mean ± SD	Number of male survivors	Mean ± SD
0.00	6, 8, 9, 10, 10	9 ± 2	6, 7, 10, 11, 8	8 ± 2
4.11	8, 8, 6, 7, 10	8 ± 1	4, 5, 5, 6, 6	5 ± 1
8.24	6, 5, 7, 7, 7	6 ± 1	4, 3, 7, 5, 6	5 ± 2
12.35	7, 7, 4, 5, 6	6 ± 1	3, 3, 1, 2, 4	3 ± 1
16.46	6, 5, 7, 3, 6	5 ± 2	3, 3, 1, 3, 3	3 ± 1

Table A2. Female and male survival blocks for the late copepodite-to-adult age group. Each exposure chamber contained fifteen males and fifteen females.

C4-Adult

Total dose (kJ/m^2)	Number of offspring	Mean ± S.D.
0.00	103, 141, 196 | 104, 96	126 ± 44
4.11	62, 47, 37 | 42, 32	44 ± 12
8.24	49, 26, 33 | 30, 25	33 ± 10
12.35	13, 15, 11 | 12, 15	13 ± 2
16.46	10, 6, 4 | 4, 8	6 ± 3

Table A3. Number of second generation organisms produced by survivors of the irradiated late copepodite-to-adult age group. Blocks to right of broken line were omitted from the regression in Figure 24.