AN ABSTRACT OF THE THESIS OF

Linda Kay Chalker for the degree of Master of Science

in General Science presented on December 19, 1980

Title : Sensitivity of the Marine Copepod Tigriopus californicus to Ultraviolet-B (290-320 nm) Radiation

Abstract approved: Redacted for Privacy

Henry Van Dyke

Due to the presence of the earth's ozone layer, radiation of the shorter solar wavelengths is reduced during penetration of the atmosphere. Increases in chlorofluoromethanes and nitrogen oxides may significantly decrease the ozone concentration, resulting in shorter wavelength penetration, most notably UV-B (290-320 nm) radiation. Various studies have found UV-B radiation to be harmful to life forms.

The marine harpacticoid copepod, Tigriopus californicus, is a successful colonizer of supralittoral splash pools from Vancouver, British Columbia, to Baja California, Mexico. Since these pools are subject to abundant amounts of direct solar radiation, it is of interest to determine the sensitivity of Tigriopus californicus to UV-B radiation.

Tigriopus was raised under diurnal conditions in the laboratory and was fed a mixture of unicellular algae and bacteria. Both larval and adult stages of the copepod were irradiated on a rotating turntable under cool white fluorescent lamps and sunlamps. Radiation

procedure utilized cellulose acetate filters and Mylar filters to control the ultraviolet exposure. Following irradiation, the animals were returned to the culture area where they were checked daily for survival. Similar experimentation was also performed to compare the survival of Acartia clausii to that of Tigriopus. Simple extraction and identification of the pigment responsible for the bright orange color of Tigriopus was also performed.

Results indicate that all life stages of Tigriopus are significantly resistant to enhanced UV-B radiation. Additionally, there may be a sex-ratio shift in the younger developmental stages following irradiation. Previous work indicates that the pigmentation of Tigriopus californicus is probably due to the carotenoids astaxanthin and astaxanthin ester. These pigments, which are located in the carapace, may function to absorb UV radiation and protect internal structures from photochemically-induced damage.

Sensitivity of the Marine Copepod *Tigriopus californicus* to Ultraviolet-B (290-320 nm) Radiation

by

Linda Kay Chalker

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1981

APPROVED:

Redacted for Privacy

Professor of General Science (Biology)
in charge of major

Redacted for Privacy

Chairman of Department of General Science

Redacted for Privacy

Dean of Graduate School

Date thesis is presented December 19, 1980

Typed by Marcia Griffin for Linda Kay Chalker

ACKNOWLEDGEMENTS

Throughout the preparation of this thesis, I was fortunate enough to have the help and advice of many good friends and colleagues. For the times I was too distracted to say "Thank you," I say it now:

My deep appreciation to my major professor, Henry Van Dyke, for his endless advice and moral support which was generously given 'irregardless' of my mental state;

My gratitude to Bob Worrest for his assistance with technical procedures, and my admiration for feats accomplished at the Golden Bee;

Special thanks to Chris Bayne for his insightful comments and suggestions, and to Frank Dost, both of whom gave up their Friday afternoon to serve on my committee;

I am especially grateful to Jim Karanas, whose assistance with Acartia and statistical procedures was greatly appreciated, as was his unique humor, and to Sister Pam, whose encouraging support helped me through my minor crises;

My thanks also to my friends in the General Science Department, who not only made my years here enjoyable, but also made side bets on how long I would be here;

And finally, my greatest appreciation to my parents for their moral and financial support, and to Jim Scott, without whose assistance, knowledge, and endless patience I would have given up a dozen times.

This research was funded in part by the National Aeronautics and Space Administration: Contract Number NAS 9-14860, and a Grant-in-Aid of Research from Sigma Xi, the Scientific Research Society of America.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

SENSITIVITY OF THE MARINE COPEPOD *TIGRIOPUS CALIFORNICUS* TO ULTRAVIOLET-B (290-320 nm) RADIATION

I. INTRODUCTION

Ozone Formation and Evolution

The relationship between "light and life" was apparently as important in primeval times as it is today. One of the most significant aspects of this relationship was the nature of the solar irradiance impinging upon the earth's atmosphere, which is believed to have been primarily composed of methane, carbon dioxide, ammonia, hydrogen sulfide, and water vapor (Sagan 1973; Giese 1976). These early atmospheric conditions were such that some ultraviolet wavelengths (40-320 nm) reached the earth's surface in amounts that would be highly detrimental to many forms of plant and animal life (Nachtwey 1975). With the advent of photosynthesis and oxygen production, penetration of biologically harmful radiation through the atmosphere was decreased relative to longer wavelength radiations. Prior to this time, life is thought to have been restricted to aquatic areas which attenuated ultraviolet radiation. As organisms began using radiant energy to drive internal mechanisms, oxygen was released into the atmosphere where it effectively absorbs ultraviolet radiation below 246 nm (Chapman 1930) with a peak at 150 nm (Dauvillier 1965). The energy associated with these wavelengths is sufficient to split oxygen in the following manner:

$$O_2 \xrightarrow[150\ nm\ (maximum)]{UV\text{-}C\ (40\text{-}280\ nm)} 2\ O^*$$

These reactive singlet oxygen molecules can then combine with molecular oxygen to form ozone (O_3):

$$O^* + O_2 \longrightarrow O_3$$

Part of the ozone then absorbs longer UV-C and UV-B wavelengths and is degraded to atomic and molecular oxygen:

$$O_3 \xrightarrow[260\ nm\ (maximum)]{UV\text{-}C,\ UV\text{-}B} O^* + O_2$$

The consequence of these reactions was the slow formation of a thin layer of ozone which, if compressed at standard temperature and pressure, would be approximately 3 mm thick (Caldwell 1979). The actual amount of ozone present at any time in a particular area fluctuates as a function of latitude and season. However, only a fraction (.01) of the present atmospheric levels of oxygen and ozone was necessary for the proliferation and speciation of terrestrial life forms (Berkner and Marshall 1965). These early forms presumably developed shielding mechanisms to avoid UV damage of internal structures (Sagan 1973; Margulis et al. 1976), but as the ozone concentration increased organisms became less exposed to harmful radiation and more recent life forms may not have retained these protective mechanisms.

Depletion of the Ozone Layer

Ozone concentrations are held in a delicate equilibrium by a series of checks and balances. Although photosynthesis could theoretically continue to increase the amount of ozone in the atmosphere, the nitrogen cycle acts as a natural method of ozone control. Nitrous oxide (N_2O) is released to the atmosphere by denitrifying bacteria found in soils and aquatic systems (Giese 1976). As the nitrous oxide diffuses into the stratosphere, it may react with photolytically-produced oxygen atoms in this manner

$$N_2O + O^* \longrightarrow 2\ NO$$

where the end product is nitric oxide (Seliger 1977). Nitric oxide can then combine with ozone, which results in the production of oxygen and nitrogen dioxide:

$$NO + O_3 \longrightarrow NO_2 + O_2$$

By a further series of reactions involving atomic oxygen, water and nitrogen dioxide, nitric oxide is regenerated. Nitric oxide can be removed from the atmosphere in the form of nitric acid (HNO_3), thus preventing a buildup in nitric oxide concentrations (Giese 1976). In a natural system, then, ozone concentrations are balanced by nitrogen oxides from bacteria, and to a lesser degree nitrogen oxides from volcanoes and lightning.

Anthropogenic interference with this stable equilibrium may have resulted in increased ozone decomposition. Attention was drawn to this matter in the early 1970's when researchers (Johnston 1971; Crutzen 1972; McElroy et al. 1974) discovered that nitrogen oxides from the exhaust of supersonic transport planes (SSTs) could possibly reduce the ozone concentration. Other possible sources of nitrogen oxides were identified and these included nuclear weapons (Koslow 1977) and nitrogen fertilizers (Liu et al. 1977). Though later improvements of the predicting models showed the effect of SST exhaust on the ozone layer to be limited (NAS 1979a), the alert did serve to begin an intensive study by the Climatic Impact Assessment Program of the Department of Transportation and by the National Academy of Sciences to assess human impact on the stratosphere.

These and other studies found that yet another catalytic cycle was at work in the stratosphere and this was seen to be even more efficient in ozone depletion than nitric oxide (Seliger 1977). Although the presence of these ozone depletion agents had been previously reported (Lovelock 1971), it was not until the research of Molina and Rowland (1974) that the significance of chlorofluoromethanes (CFMs) was realized. CFMs are commonly used as refrigerants, aerosol propellants, and industrial solvents (McCarthy et al. 1977). The catalytic cycle of these "inert" compounds begins in the stratosphere where they are split by UV-C radiation to form free chlorine atoms. As described by Stolarski and Cicerone (1974), these chlorine atoms can react with ozone to form oxygen

molecules and chlorine oxide:

$$Cl + O_3 \longrightarrow ClO + O_2$$

Chlorine oxide can then react with oxygen radicles from the natural photolysis of ozone to regenerate chlorine atoms:

$$ClO + O^* \longrightarrow Cl + O_2$$

Recent study by the National Academy of Sciences has predicted that the release of CFMs into the atmosphere at 1977 levels may significantly decrease the ozone concentration by as much as 16.5% (NAS 1979b). Although world levels of CFMs have dropped to pre-1973 amounts due to the U.S.A. ban of their use in aerosol sprays, production outside the U.S.A. has increased (Maugh 1979). The lack of CFM regulation in other countries appears to exemplify the "wait and see" attitude of some researchers. In the absence of further regulation, however, world levels of CFMs are expected to increase by as much as 57% in the next 20 years (Maugh 1979).

Implications of Increased UV-B Radiation

In theory, a decrease of ozone will lead to an increase in UV-B radiation reaching the earth's surface. Although UV-B has some benefits such as initiating vitamin D production in humans (Epstein 1977), an increase in biologically harmful wavelengths would probably cause more damage than good. Both proteins and nucleic acids are extremely sensitive to, and selectively absorb, UV radiation, with peaks at 278 nm and 260 nm, respectively (Seliger 1977). Although

increased UV-B levels would not include these higher energy wavelengths, the amount of UV-B available and absorbed by living organisms would be significantly increased over present amounts. UV-B absorption leads to the formation of pyrimidine dimers, protein denaturation and cross-linkage, and chain breakage (Smith 1977). Fortunately, most cells and organisms are capable of repairing damaged nucleic acids and proteins by various methods involving enzymatic activity such as excision of damaged material. It should be noted, however, that if these repair mechanisms are "overloaded" (i.e. there is too much damage and not enough time for repair), the damaged DNA may be replicated causing the cell to mutate or die.

An increase in UV-B radiation, then, could have deleterious effects on terrestrial and some aquatic ecosystems. At the human level, the NAS (1979a) has predicted an increase in both non-melanoma and melanoma type skin cancers. This is apparent since the action spectrum for skin cancers peaks at 297 nm, well within the UV-B region (Seliger 1977). Also of great importance are the predicted effects upon members of the lower trophic levels of food chains. For instance, agricultural and non-agricultural plants are expected to decrease in productivity as a direct result of ozone depletion (NAS 1979a). Aquatic systems would also be susceptible with an increased killing of microorganisms and important zooplankton and phytoplankton organisms.

These findings have spurred research dealing with the impact of enhanced UV-B on various organisms, including those in marine

and estuarine ecosystems. Worrest et al. (1978) found a significant decrease in chlorophyll a concentrations, biomass, and community diversity of attached algae in an estuarine ecosystem following UV-B irradiation. Similarly, Thomson et al. (1980) noted a significantly depressed growth rate in a dominant estuarine diatom Melosira nummuloides after exposure to enhanced UV-B, as did Wolniakowski (1980) using the marine phytoplankton species Dunaliella tertiolecta. Working with bacterioplankton assemblages, Moehring (1980) found a decrease in heterotrophic activity of these marine decomposers. Karanas et al. (1979) exposed the economically important planktonic copepod Acartia clausii to enhanced UV-B radiation and correlated this exposure to decreased survival and reproductive potential of this crustacean. Apparently then, there is a basis for concern about ozone depletion and the consequent increased penetrance of UV-B radiation, especially in relation to ecologically and economically important members of food chains.

Importance of Copepods

Research involving a variety of planktonic marine copepods has increased over the years due to the importance of these crustaceans in the transfer of energy from phytoplankton to fish in oceanic food webs and ecosystems. In contrast with planktonic forms, benthic copepods, such as harpacticoids, primarily feed upon bacteria, detritus, and benthic algae (Barnes 1974; Dethier 1980). These activities may vitally influence the exchange of matter and energy

between the bottom community and water column (Kinne 1977).

Since copepods occur in vast numbers, a decrease in population numbers could strongly affect marine food chains. Harpacticoids of the genus Tigriopus have been examined by previous investigators with regard to their life history and ecology (Fraser 1936; Monk 1941; Bradford 1967; Ito 1970; Koga 1970; Huizinga 1971; Vittor 1971; Dethier 1980), genetic make-up (Ar-rushdi 1958; Burton et al. 1979), sex ratio (Takeda 1950; Belser 1959; Egloff 1967), egg production (Comita and Comita 1966; Harris 1973), nutritional needs and economic value (Provasoli et al. 1959; Rothbard 1976), and resistance to such factors as salinity and temperature (Ranade 1957; Kontogiannis 1973), oil pollution (Kontogiannis and Barnett 1973), and hydrostatic pressure (Vacquier 1962; Norton 1962; Vacquier and Belser 1965). The present study dealt with yet another environmental factor - UV-B radiation - and its effect upon the survival, fecundity, and larval development of the local Tigriopus species, T. californicus.

Life History of Tigriopus californicus

Members of the genus Tigriopus occur globally in supra-littoral tidepools of rocky coastlines. This bright orange copepod is represented by seven known species: T. angulatus and T. raki reported from the southwest Pacific along New Zealand and the Antarctic Ocean; T. fulvus, T. minutus and T. brevicornis reported from England, Scotland, and other European countries; T. japonicus from the warmer waters off Japan, and T. californicus from the Pacific

coast of North America. Differences among the species are based on morphological traits such as number of setae, spines and bristles on the legs of the organism; for a taxonomic key, Bradford's article (1967) is most helpful.

Originally identified as Tisbe californica by Baker in 1912, Tigriopus californicus is a benthic, free-swimming harpacticoid copepod found from Baja California, Mexico to Vancouver B.C. (Belser 1959), although recently Dethier (1980) has identified specimens from as far north as Torch Bay, Alaska. The success of these crustaceans in colonizing supralittoral pools is remarkable since environmental conditions can become quite stressful. By definition, supralittoral pools are never submerged by tides; the only water they receive is from high tide and storm splash, and from precipitation. Therefore, these habitats experience extreme and rapid changes in temperature and salinity due to variations in air temperature, wind, precipitation, and incident solar radiation (Burton et al. 1979). This is presumably why most supralittoral animals are semi-terrestrial (Ricketts and Calvin 1968), a generalization for which Tigriopus is an exception although 24 hour periods of desiccation can be tolerated (Egloff 1967).

The apparent resistance of Tigriopus species to various environmental factors has been well documented by previous research. It is both eurythermal and euryhaline as it can withstand and remain active in temperatures from 4°C (Vittor 1971) to over 40°C (Ranade 1957) and salinities from 0‰ (Vittor 1971) to 102‰ (Egloff 1967).

These limits are much narrower if the factors are considered separately; Ranade (1957) demonstrated that with increasing salinity, temperature tolerance increases. At extremely high salinities (334‰), Tigriopus will become inactive but will recover when the salinity is reduced (Egloff 1967). Vittor's study (1971) found that many T. californicus features including body size, population density, and brood size were independent of pool environment conditions such as salinity, temperature, and O_2 concentration. He also noted that natural pH changes from 5.0 to 8.6 (over a thousand-fold increase in alkalinity) had no effect on the copepods' survival.

Resistance to more unusual environmental factors has been noted as well. Vacquier (1962) found that the adults were insensitive to increased hydrostatic pressure, although the larvae exhibited an apparent "sex conversion" of male to female animals under increased pressure. Effects of crude oil on the water surface of Tigriopus pools were observed by Kontogiannis and Barnett (1973); Tigriopus californicus was detrimentally affected by the oil (which is not only toxic but acts as a physical barrier as well), but survived better than did Acartia and Othana sp. which were also tested.

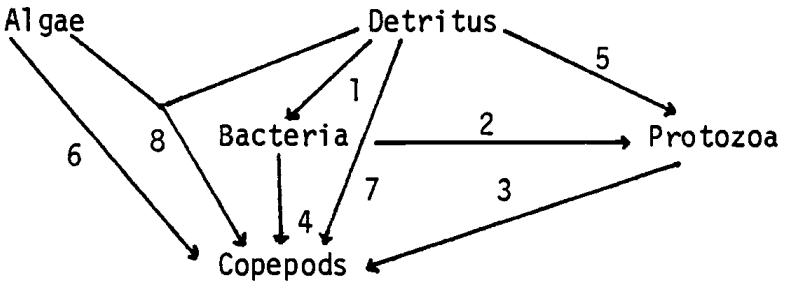
A typical pool containing Tigriopus californicus will show seasonal variation in population numbers as well as environmental components. Described by Vittor (1971) as an opportunistic species, T. californicus of both sexes is abundant in the spring and summer when algal productivity is high, while the population dwindles in winter and consists mainly of females. These, often extreme,

oscillations in density are mediated by fluctuations in food concentrations or other adverse factors. A final element in determining Tigriopus density in any pool is surf splash and runoff from rainfall. Although Tigriopus will cling to the substrate when the water is disturbed, they are still transported to other pools by water activity and to some extent on the carapace of the crab Pachygrapsus crassipes (Egloff 1967). Interestingly, T. californicus has never been found in plankton tows (Vittor 1971; Dethier 1980), though it is apparently not physiologically limited to high pools. Instead, as Dethier (1980) suggests, the orange pigmentation of T. californicus makes it more vulnerable to visual predators, such as cottid fish and crustaceans. In its natural habitat, Tigriopus has no predators and few competitors; when transplanted to lower tidepools, the animals survive and reproduce only in the absence of predation (Dethier 1980).

The evident selection of available Tigriopus by predators such as sculpins and crustaceans has prompted researchers to use T. japonicus as the choice food organism for many species of sea fish (Rothbard 1976). In fact, Kinne (1977) states that T. japonicus is the most promising food-organism candidate for Japanese fisheries, which is one of Japan's most important industries.

Tigriopus has a varied diet and seems to utilize all possible sources of nourishment. Tigriopus is often mistaken for a benthic herbivore using a filter-feeding apparatus (Huizinga 1971). Egloff (1967) has shown that the appendages of T. californicus are not

suited for filter-feeding; a drawing of these appendages compared to those of Acartia, a filter-feeder, is shown in Figure 1. Stomach content studies have revealed that natural populations utilize many food sources, including green and blue-green algae, bacteria, protozoans such as Euplotes, diatoms, and particular matter (Egloff 1967; Huizinga 1971). Egloff (1967) also found naupliar and copepodite exoskeletons in the gut of adults, indicating that the animal is also cannibalistic, especially in densely populated pools. Most recent investigators agree that Tigriopus species feed on detritus and benthic algae (Marshall and Orr 1960; Vittor 1971; Dethier 1980) and their associated bacterial and protozoan fauna. Heinle et al. (1977), using another benthic harpacticoid copepod Scottolana canadensis, suggest that non-algal carbon (e.g. detritus and bacteria) is an important component in the diet of benthic copepods and developed an energy flow chart to depict possible relationships:



The importance of any of these possible food sources should not be overlooked; algae appear to help in egg production and supply necessary carotenoids for pigment production, while detritus, in which ciliated protozoans and bacteria are important in transferring the energy to the organism, may function as a main energy source for

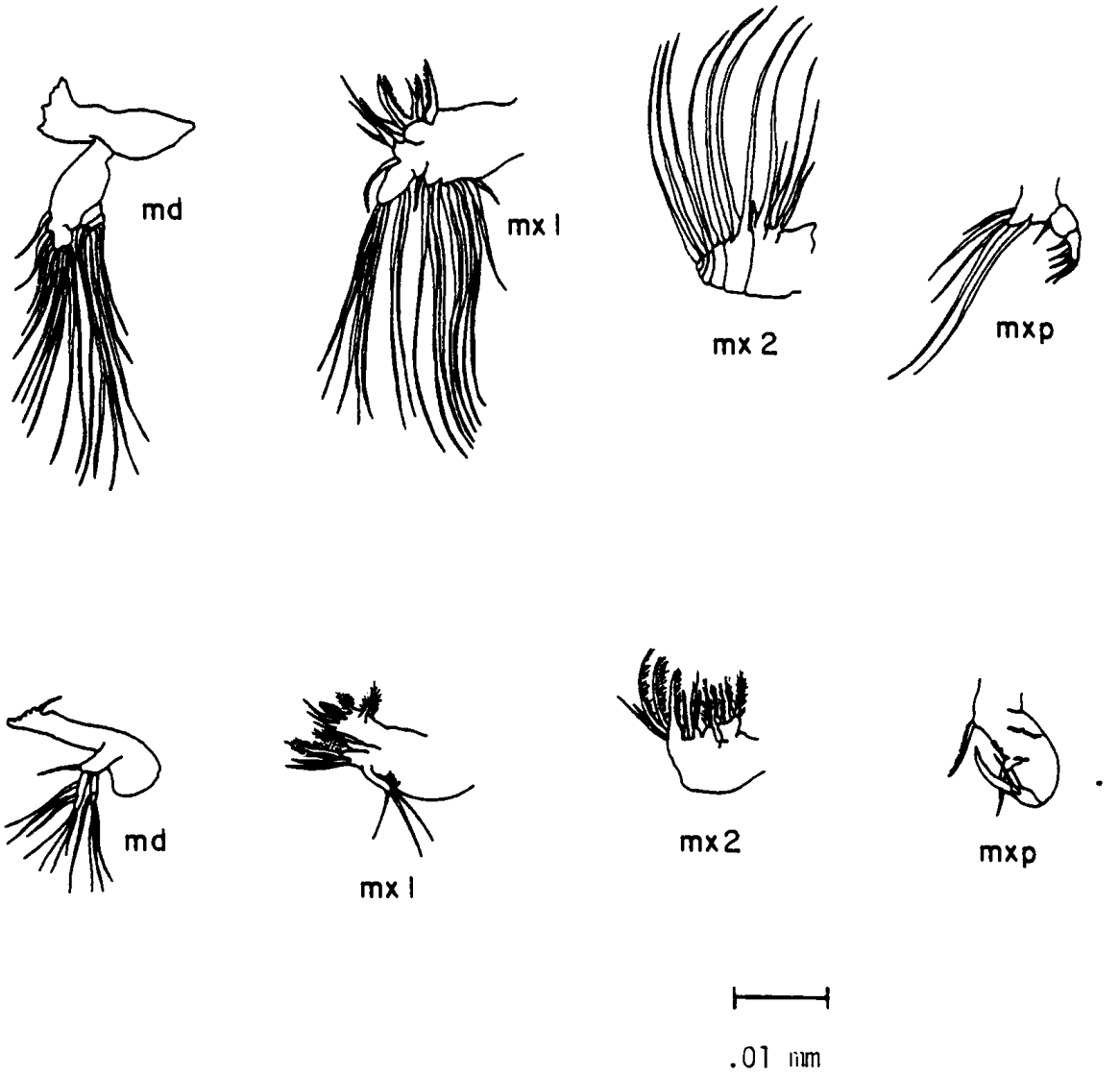


Figure 1. The feeding appendages of Acartia tonsa (top line) and Tigriopus californicus (bottom line). md = mandible; mx 1 = first maxilla; mx 2 = second maxilla; mxp = maxilliped. (From Anraku and Omori 1963 and Egloff 1967).

benthic harpacticoid copepods (Marshall and Orr 1960; Heinle et al. 1977). The significance of bacteria is realized when laboratory cultures of T. californicus are grown; permanent bacteria-free cultures cannot be obtained, as the copepod requires some bacterial nutrient for prolonged culturing (Provasoli et al. 1959; Marshall and Orr 1960). Personal observations found that copepods fed a mono-algal diet were not as hardy as those receiving a mixed algal diet; often a mixed diet is required for the organisms to reach sexual maturity (Belser 1959).

Other nutritional resources include cannibalism of young, which has been observed in this study as well as by Egloff (1967) and Rothbard (1976). Finally, coprophagy was observed during this experimental study, but has not been previously reported in the literature, although this behavior has been observed in other copepods. Presumably, T. californicus ingests feces and thus extracts from them bacteria to be utilized as food.

Members of the genus Tigriopus reproduce sexually in a manner which follows the general procedure for Crustacea (Charniaux-Cotton 1960). Reproduction occurs throughout the year (Dethier 1980), although reproductive activity peaks around April (Ricketts and Calvin 1968). Adult male copepods can be seen mating with various copepodites and mature females, a process that can last up to five days (Vittor 1971). Successful mating (i.e. sperm retention by the female) will occur only after the terminal molt, however; in immature females, the seminal receptacle is lost at ecdysis (Egloff

1967). An interesting observation made by Egloff (1967) was that the immature partner mating with the adult male always developed into a female, although the copepodite was sexually unidentifiable to the researcher at the time of mating.

After a successful mating, females produce up to 12 egg sacs from the original fertilization (Vittor 1971). The number of eggs per sac may vary from 18 (Huizinga 1971) to 60 (Rothbard 1976) depending on such conditions as temperature (Comita and Comita 1966), food abundance, female body size (Vittor 1971) and, possibly, photoperiod. Within a few days, the greenish eggs develop the characteristic orange pigmentation (Figure 2), then give rise to the first naupliar larvae. The nauplii mature through several stages, the exact number of which is a subject of disagreement. Although Huizinga (1971) claims that there are four naupliar and five copepodite stages, most other investigators (Egloff 1967; Vittor 1971; Burton et al. 1979) agree on six naupliar and six copepodite stages, the sixth copepodite being the adult. These numbers are in accordance with the general description of harpacticoid copepod life histories.

The development time from egg to adult is very temperature dependent; generally, the colder the temperature, the longer it takes the young to hatch and develop (Fraser 1936; Comita and Comita 1966; Vittor 1971; Dethier 1980). Figure 3 depicts time in days from egg to adulthood against temperature. Development time appears to decrease under uncrowded laboratory conditions with fairly warm temperatures (21-23°C) and an abundance of diverse food organisms.

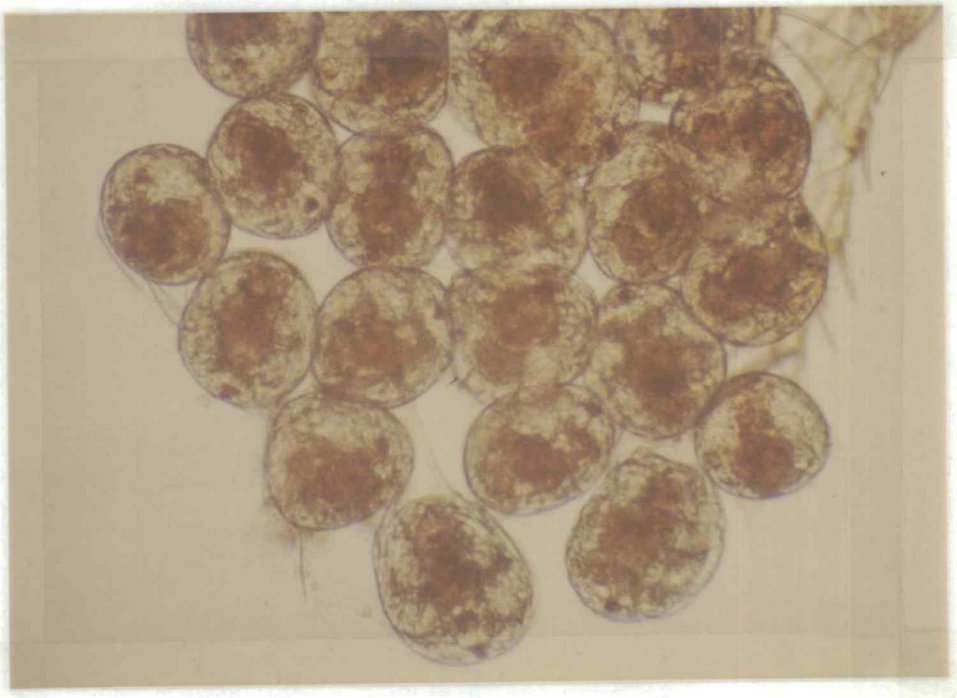
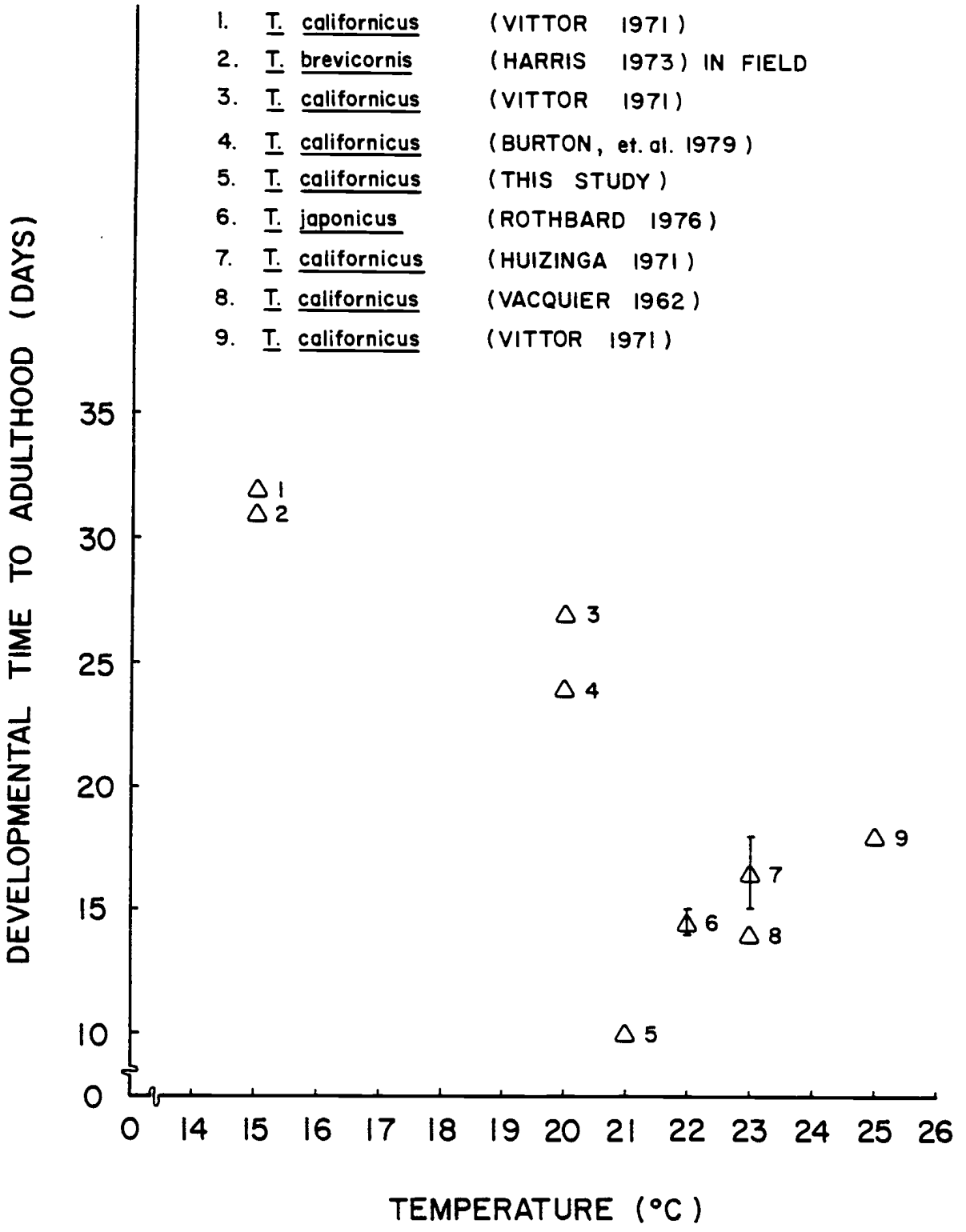


Figure 2. Mature egg sac of T. californicus, showing characteristic orange pigmentation (400x).

Figure 3. Developmental time to adulthood of *Tigriopus* vs. temperature (°C).


1. T. californicus (VITTOR 1971)
2. T. brevicornis (HARRIS 1973) IN FIELD
3. T. californicus (VITTOR 1971)
4. T. californicus (BURTON, et. al. 1979)
5. T. californicus (THIS STUDY)
6. T. japonicus (ROTHBARD 1976)
7. T. californicus (HUIZINGA 1971)
8. T. californicus (VACQUIER 1962)
9. T. californicus (VITTOR 1971)
DEVELOPMENTAL TIME TO ADULTHOOD (DAYS)
TEMPERATURE (°C)
0
10
15
20
25
30
35
0 14 15 16 17 18 19 20 21 22 23 24 25 26

The life span of adult Tigriopus is also temperature dependent; Vittor (1971) noted that longevity increased from 75 days at 25°C to 130 days at 15°C.

The young pass through each naupliar stage quickly, often in as little as 18-20 hours (Koga 1970). Although it is possible to determine different stages by appendage differences, it is easier to compare sizes, as each stage is significantly larger than the previous one (Egloff 1967). Naupliar stage number four is pictured in Figure 4. After the sixth naupliar stage is reached, the young metamorphose into the first copepodite stage, which resembles the adult. By the fourth copepodite stage (Figure 5), the sex of the copepodite can be visually determined (Fraser 1936; Egloff 1967), as the females are usually darker and their appendages and antennae differ from those of the males. The adult copepods are easily differentiated as the females are slightly larger (Monk 1941) and darker (Figure 6), while the males have the first set of appendages modified for clasping the female during copulation (Figure 7).

Environmental conditions such as temperature not only affect development time and life span of Tigriopus, they may also affect sex determination (Takeda 1950; Charniaux-Cotton 1960; Egloff 1967; Vittor 1971; Burton et al. 1979) by as yet unknown mechanisms. Deviation from the expected 1:1 sex ratio is conspicuous and has warranted study by several geneticists.

Sex determination in Tigriopus species is polygenic; there are no sex chromosomes (Ar-rushdi 1958; Burton et al. 1979). Researchers

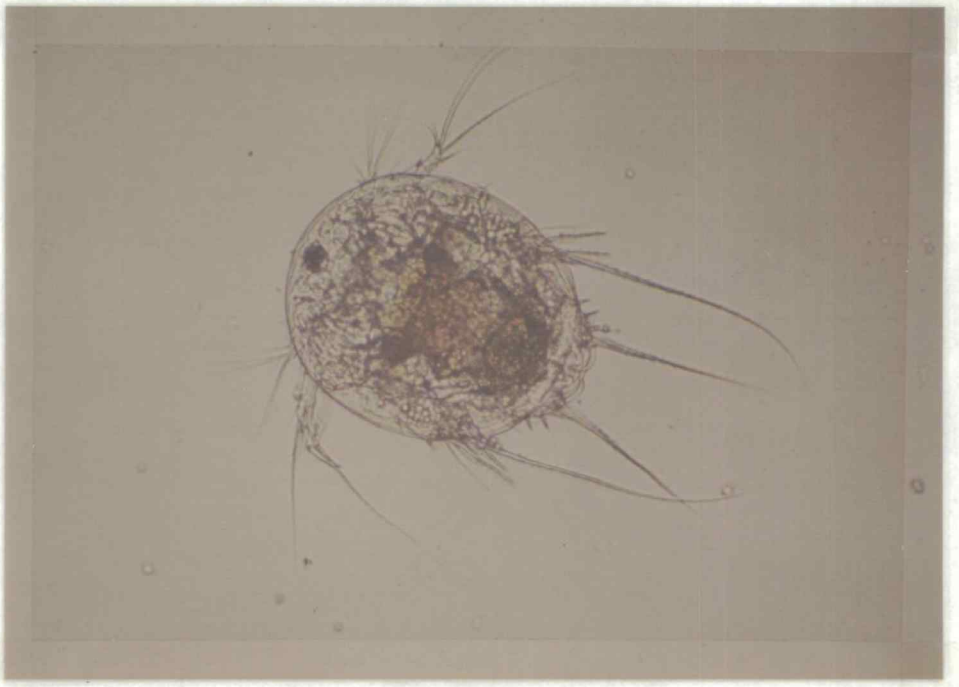


Figure 4. Fourth naupliar stage of T. californicus (100x).

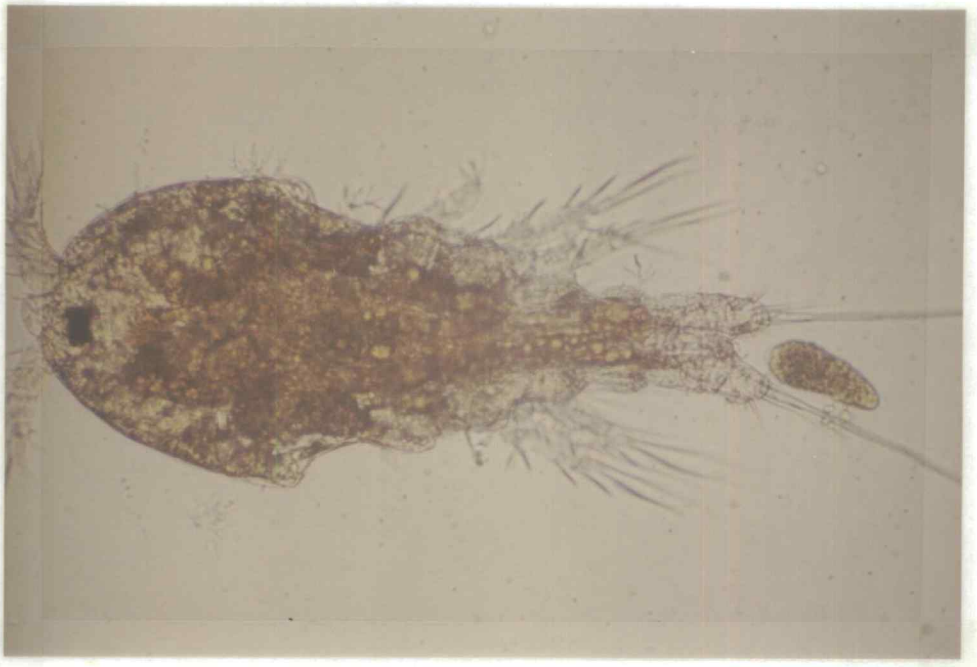


Figure 5. Fourth copepodite stage of T. californicus (female) at 100x.

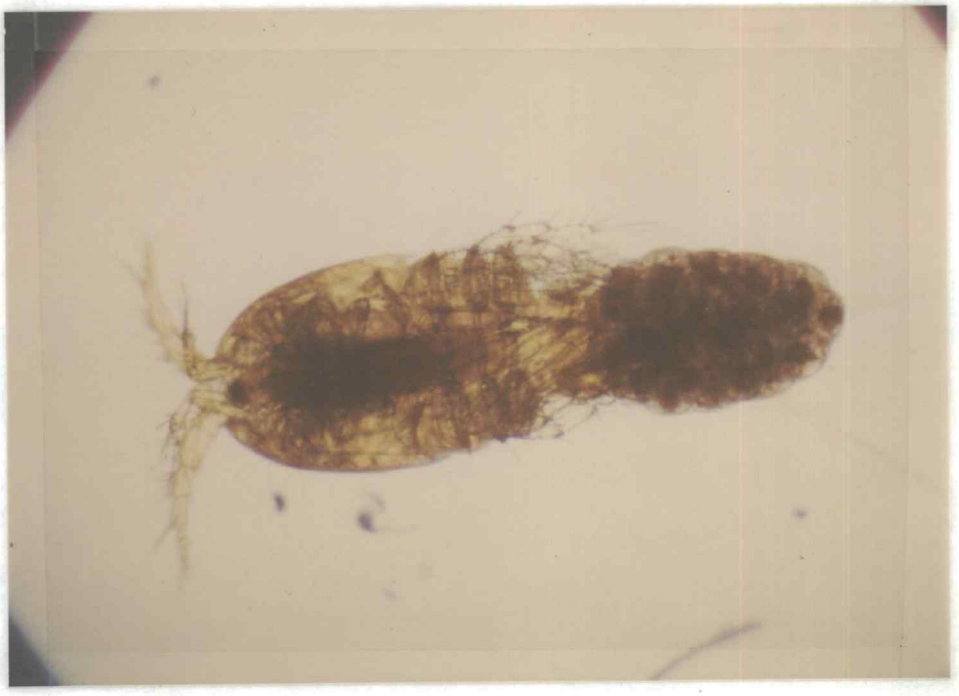


Figure 6. Adult female T. californicus with egg sac (40x).

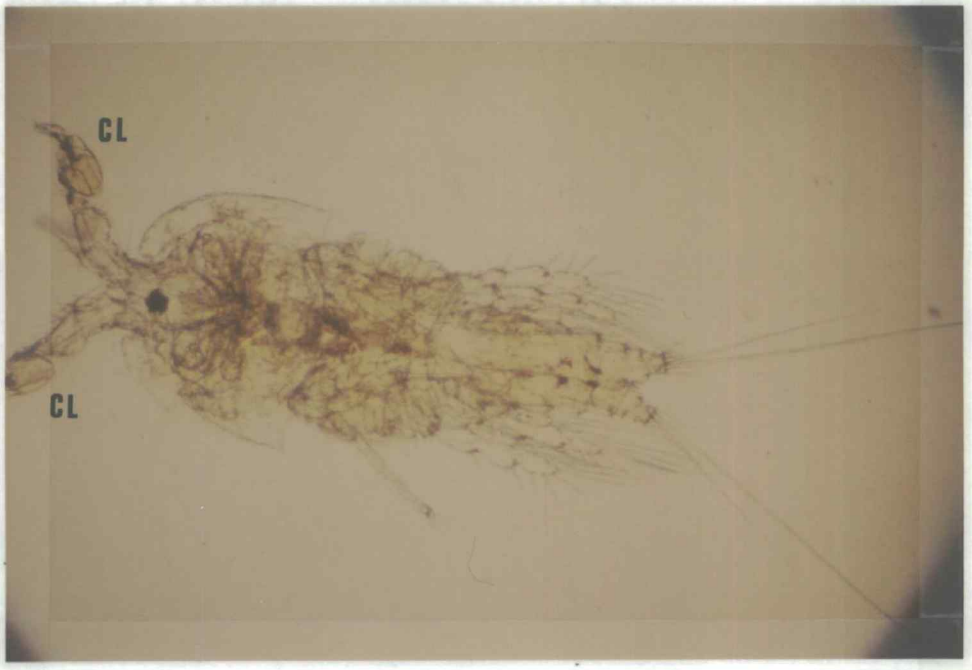


Figure 7. Adult male T. californicus exhibiting lighter pigmentation and modified claspers (CL) (40x).

disagree as to the time of sex determination; according to Takeda (1950) the larvae are sexually neutral before the first copepodite stage and their sexuality depends on the rate of development of the last naupliar stage. Takeda (1950) and others (Egloff 1967; Vittor 1971) found that an increase in temperature resulted in a larger percentage of males in the population. The dependence of sexuality on temperature may help explain why natural populations of Tigriopus show seasonal shifts in sex ratio (Takeda 1950; Egloff 1967); spring and summer months show an increase in males, while during fall and winter females predominate. Natural and laboratory-grown populations show the same tendency for males to increase in number under higher temperatures, but the underlying reasons for the shift may differ. Egloff (1967) suggests that under natural conditions differential mortality is responsible for the decrease in males during unfavorable conditions, since they have a lower resistance (Igarashi 1960). Conversely, laboratory grown populations show sex ratio changes due to phenotypic and polygenic shifts, which could include "sex conversion." This concept of conversion of larvae from one sex to another was also postulated by Vacquier (1962) and Vacquier and Belser (1965), who found more females developed under high hydrostatic pressures. Statistical interpretation of these experiements by Norton (1962) indicated that there was "no reason to doubt sex conversion, as the probability of otherwise is $p > .02$." Apparently, there are several factors which may be capable of shifting sex ratios.

Because Tigriopus lives in a habitat that is exposed to abundant

solar radiation, it is therefore likely to have special protective mechanisms. If its tolerance of UV-B radiation is shown to be greater than that of other marine organisms (e.g. planktonic copepods), the mechanisms of protection may be worthy of study as we face the prospect of increased UV-B radiation due to present and anticipated anthropogenic alterations to the ozone layer.

II. MATERIALS AND METHODS

Collection and Maintenance of Organisms

Tigriopus californicus was collected from supralittoral tidepools in the basalt cliffs at Seal Rock, Oregon (Figure 8). Organisms were collected using a leached plastic syringe and plastic Thermos jugs, although other methods appear to be successful as well (Monk 1941; Kontogiannis and Barnett 1973; Burton *et al*. 1979). In addition to copepods, pool water and detritus were obtained to be used in laboratory maintenance of the cultures. Pool temperatures and salinity were determined at each collection.

Stock cultures were maintained under diurnal laboratory conditions (17L:7D) in a water bath with a temperature of 19°C (±1°C). Illumination was provided by four Vitalite Durotest fluorescent lamps located 45 cm above the culture area, which is shown in Figure 9. The fluence rate of these lamps in the 400-800 nm range was determined to be 9.448 Wm^{-2}. The containers used were sterilized 2-liter Pyrex culture bowls, which later were replaced with 3-liter sterilized glass jars. Best culturing results were obtained when *Tigriopus* was initially maintained in tidepool water. This procedure was continued for a week to allow the organisms to acclimate to laboratory conditions; then sterilized seawater from the Oregon State University Marine Science Center at Yaquina Bay, Oregon, and food items were added. A variety of food sources have been utilized for rearing *Tigriopus* species; bacterized uni-algal cultures of *Platymonas*,

Figure 8. Supralittoral tidepools containing *T. californicus* in basalt cliffs at Seal Rock, Oregon (N 44°40' W 124°05').

Figure 9. Culture area for T. californicus and food organisms Platymonas and Isochrysis.

Isochrysis, or Phaeodactylum species (Provasoli et al. 1959; Comita and Comita 1966; Egloff 1966; Kinne 1977); the crushed seaweed Ulva (Rothbard 1976); shrimp powder (Takeda 1950; Kontogiannis 1973); Purina rat chow (Huizinga 1971); and Tetramin fish food (Burton et al. 1979). In my study, the hardiest copepod populations were those maintained on a mixture of bacterized cultures of Platymonas sp. and Isochrysis galbana and a small amount of Tetramin flaked fish food. Both Platymonas and Isochrysis were grown in f/2 seawater culture medium (Guillard and Ryther 1962) under the same laboratory conditions as Tigriopus. At weekly intervals, Tigriopus was fed the above food items in concentrations high enough to turn the culture water slightly green - an optimum condition for Tigriopus growth (Huizinga 1971). Hemacytometer counts determined the algal concentrations in the Tigriopus culture bowls to be approximately 1.86×10^6 cells ml^{-1}.

Aeration of the copepod cultures was not necessary (Huizinga 1971); in fact, those cultures that were aerated were less useful as the copepods tended to cling to the aeration stone, making removal difficult and usually harmful. The containers were filled only to 60% of their capacity to allow for adequate gas transfer over the water surface, and were loosely covered; any evaporated water was replaced with glass-distilled water to maintain the original salinity. Transfer of organisms was accomplished every 14 days to avoid overcrowding and consequent decrease in population growth. This was done simply by stirring the dish contents and pouring half into a sterile dish; in both dishes, the fluid level was brought up to the

original amount with the addition of sterile seawater and food organisms. Cross culturing among the dishes was done at this time to avoid the emergence of genetically differentiated populations.

Experimental Preparation

Prior to irradiation of Tigriopus californicus, stock cultures of the various larval stages were prepared in addition to the adult populations. To isolate groups of larvae at the same stage of development, the following method was employed. Gravid females from stock cultures were isolated and transferred to several smaller (350 ml) culture dishes with adequate food and water supplies. No more than 20 females were assigned to any one bowl, as overcrowding the containers often leads to cannibalism of nauplii (Egloff 1966). Every day the females were transferred to new bowls, leaving behind nauplii whose ages varied by no more than 24 hours. Another method of obtaining larvae of the same stage was attempted based on the work of Provasoli et al. (1959) and Comita and Comita (1966); this involved using a glass needle to separate red egg sacs from the female. In some cases, however, the female was inadvertantly damaged; in others, unusually high naupliar mortality was observed. Additionally, it was observed that although the nauplii may hatch simultaneously, their later development was not always concurrent and resulted in larvae of two contiguous stages. For these reasons, artificial egg separation was not employed.

Larval cultures were maintained under laboratory conditions

until observation revealed them to be at the desired larval stage. This procedure was followed for all naupliar and copepodite experimentation. Adult copepod experimentation was originally conducted using organisms from the stock cultures, but better results were obtained utilizing laboratory-reared animals since they belonged to the same age class.

Experimental Procedure

Experimental protocol was based on a similar study by Karanas (1978) using the calanoid copepod Acartia clausii. The experimental area was located in a temperature controlled room (19°C ± 1°C) 45.5 cm under a lamp bank consisting of four Westinghouse FS40 sunlamps interspersed with eight Vitalite "deluxe white" fluorescent lamps (Figure 10). These lamps were "aged" prior to experimentation to reduce the change in power output. In addition to this precaution, each experiment was conducted at the same time of day to minimize differences in power output due to other electrical activity in the building. Finally, experimentation was carried out on a rotating turntable (16 rpm) to provide equal exposure to all organisms.

Output of the lamps was determined using an Optronic Laboratories, Inc. Spectroradiometer Model 742 with Data Aquisition System Model 755. Using these measurements, which appear in Appendix 1, the irradiance at wavelengths from 290-320 nm could be translated to quantitative units. Under the Mylar filter, the irradiance was 1.697×10^{-2} Wm^{-2} at the water's surface; under cellulose acetate,

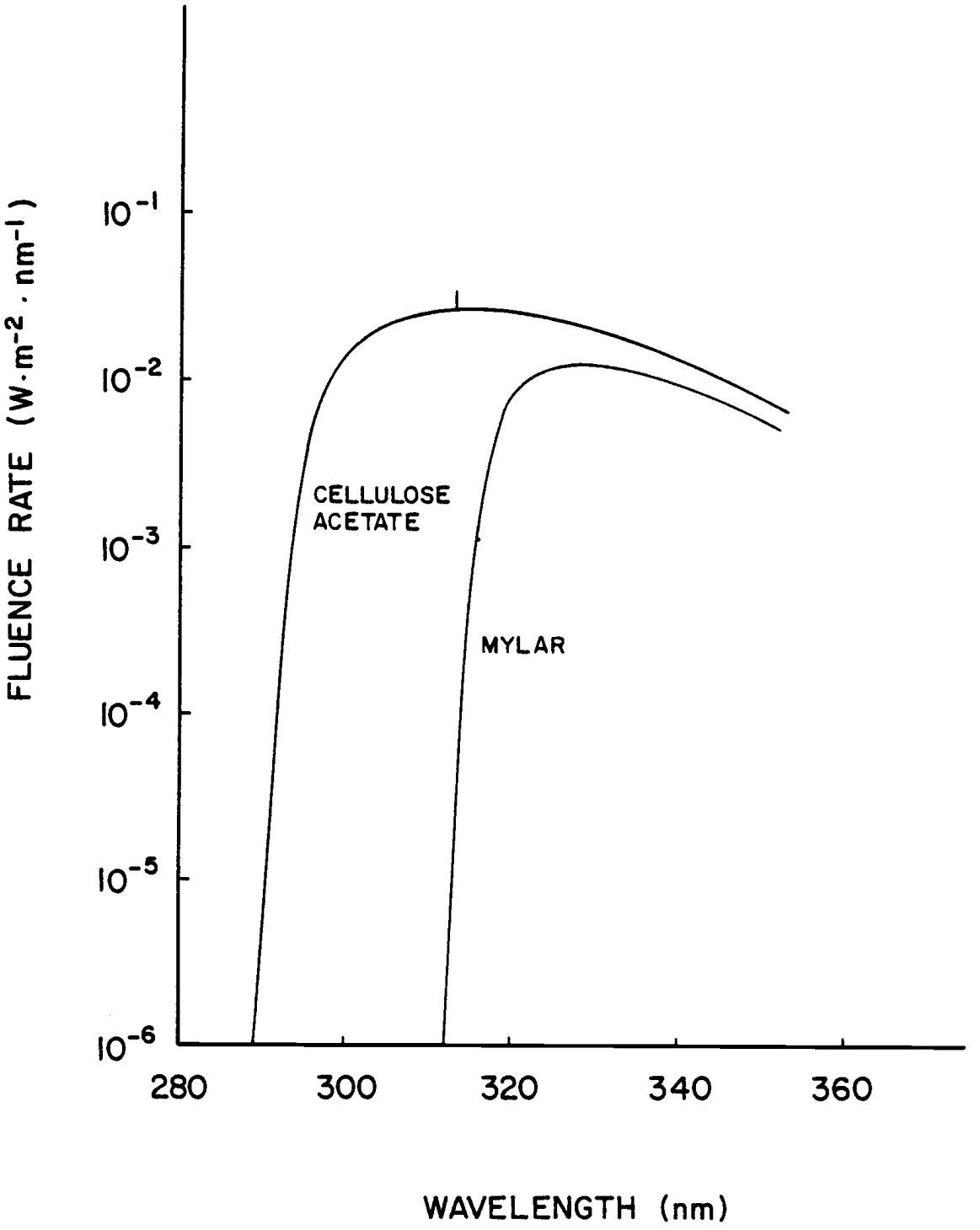
Figure 10. A group of twelve exposure chambers, covered by appropriate filters (Mylar or cellulose acetate), arranged on the experimental turntable.

it was 5.039 x 10^{-1} Wm^{-2} (Figure 11). The filters used for these measurements and those used for experimentation were presolarized under sunlamps for 24 hours to obtain relatively stable transmission properties (Worrest 1975).

The Mylar and cellulose acetate filters furnished the means for control of the wavelengths of exposure. Those organisms to be exposed to enhanced UV-B radiation were placed under squares of 0.25 mm (10 mil) cellulose acetate, while those animals to be screened from UV-B were placed beneath 0.18 mm (7 mil) Mylar squares. The difference between the two treatments is seen in Figure 11; cellulose acetate decreases transmission of wavelengths below 290 nm, while Mylar decreases transmission of wavelengths below 315 nm. The "control" organisms were defined as those under the Mylar sheets, receiving wavelengths above the UV-B region. "Experimental" organisms received the same wavelengths as the controls, plus radiation in the UV-B range.

Larval irradiation involved five separate experiments, each with a specific age group. In each case, 20 individuals were pipetted into each small glass exposure chamber (Pyrex 50 x 12 mm petri dish) with 15 ml sterile seawater and a small amount (49,000 cells ml^{-1}) of Platymonas sp. and Isochrysis galbana. Three chambers were used as replicates for controls and for each level of irradiation. Post-irradiation treatment involved returning the organisms to the culture areas where they were fed Platymonas, Isochrysis and Tetramin in concentrations relatively similar to that which culture dishes

Figure 11. Relative transmittance of the two experimental filters, .18 mm Mylar and .25 mm cellulose acetate.


FLUENCE RATE (W·m⁻²·nm⁻¹)
10⁻¹
10⁻²
10⁻³
10⁻⁴
10⁻⁵
10⁻⁶
CELLULOSE ACETATE
MYLAR
280
300
320
340
360
WAVELENGTH (nm)

received. Organisms were then allowed enough time to reach adulthood, which varied according to the age of the group at the time of irradiation. Survivors at the adult stage were enumerated and sexed.

Irradiation of adults was conducted in the same manner as the larval experiments with a few exceptions. Initially, ten animals were placed into each chamber in equal male:female proportions. Two separate experiments were performed with this age group: one at a "low" fluence and the other at a "high" fluence. Following irradiation the adults were returned to the culture area and were treated in a similar manner as were the exposed larvae. Total survival and differential male:female survival were determined after 15 days of post-irradiation care. In addition, the number of offspring produced per exposure chamber was noted to provide information of copepod fecundity.

Another study was conducted to compare the survival of T. californicus with that of Acartia clausii. Acartia was collected from Yaquina Bay, Oregon and was laboratory acclimated in 0.45 Millipore filtered bay water under diurnal conditions (17L:7D) at 16.3°C $\pm$ 1°C to accomodate the temperature limits of Acartia. Three replicates were used for each of the following experimental groups: Tigriopus under Mylar, Tigriopus under cellulose acetate, Acartia under Mylar, and Acartia under cellulose acetate. After irradiation, the organisms were returned to their respective culture areas for observation.

Since little is known of the pigment content of Tigriopus

californicus, and because females appear to be more deeply colored than males (Figures 6 and 7), a simple pigment analysis was conducted. One hundred males and one hundred non-gravid females were obtained from stock cultures, rinsed with sterile seawater, and placed in separate containers. Each group of animals was then placed in a Millipore filter flask and the liquid was filtered off using a vacuum pump. The two filter papers containing the copepods were assigned to separate homogenizing tubes and five ml of reagent grade 90% aqueous acetone was added to each. Both tubes were homogenized for one minute, after which the homogenate was poured into the two centrifuge tubes. The homogenizing tube and pestle were rinsed with five ml of 90% acetone after each homogenization, and the rinse was added to the respective centrifuge tube. The tubes were left in the dark for 20 minutes, then centrifuged for six minutes.

The liquid portion of each homogenate was poured off into quartz spectrophotometric tubes. A "blank" containing 90% acetone was also poured at this time. All three tubes were then placed in a Shimadzu Recording Spectrophotometer Model MPS-50L and readings were taken from 280 to 800 nm. Results of this recording were compared to previously reported pigment analysis studies.

Statistical Methods

Throughout these experiments, random techniques were utilized to eliminate experimental bias in the results. A randomized block design was used in placing exposure chambers on the turntable after

the method described by Karanas (1978). Using this method one replicate from each irradiation level was placed in each of three "blocks" on the turntable. In addition, individuals were randomly assigned to each exposure chamber. Statistical analysis by Karanas (1978) found that turntable position was not a significant variable and therefore pooling of individual treatment groups was permitted for all blocks.

Within each experiment, t-tests were performed to determine any significant differences in survival between irradiation levels. An F-test was conducted to determine whether the data from the two adult experiments were "identical" and therefore able to be pooled. Finally, linear regression was performed for all age groups to discover the best fit line for each group, and the slope coefficient for each line was analyzed for any significant deviation from zero. The results of these statistical analyses are found in the Appendices.

III. RESULTS

Figures 12-14 illustrate the effect of increased UV-B radiation of the survival of the various life stages of *T. californicus*. In all experiments, there was no significant decrease in survival in relation to increase in effective fluence. (Effective fluence is the product of total fluence values multiplied by a biological weighting function, in this case Setlow weightings.) Appendices 2-3 contain actual data points and t-statistics for these experiments. Although there are seven test pairs that are significant ($p < .1$), five of these show an increase in survival with increased UV-B irradiation. In most cases, fitted regression lines (Figure 15) for all experiments were found to be non-significant, since the slope coefficient (b_1) was not significantly different from zero ($p > .1$). The exception to this is the N_1/N_2 experiment which showed an increase in survival with increased radiation ($.05 < p < .1$). It should be noted that data for the "high fluence" and "low fluence" adult experiments were tested for equality using a general linear test approach for comparing two regression lines. On the basis of this test, the two regressions were found to be not significantly different ($p \approx .5$) and were therefore combined to form one regression function.

Differential survival of adult males and females, and their respective regressions, is illustrated in Figures 16 and 17. Again on the basis of general linear test results, male and female regressions are combinations of data from the "low fluence" and "high fluence" experiments ($p \approx .5$). Although female copepods appear to

Figure 12. Percent surviving fraction vs. effective fluence. Surviving fractions of the N_1/N_2, N_3/N_4, and N_5/N_6 experiments were determined on Day 15, Day 13, and Day 12, respectively, following irradiation. (N = naupliar stage)

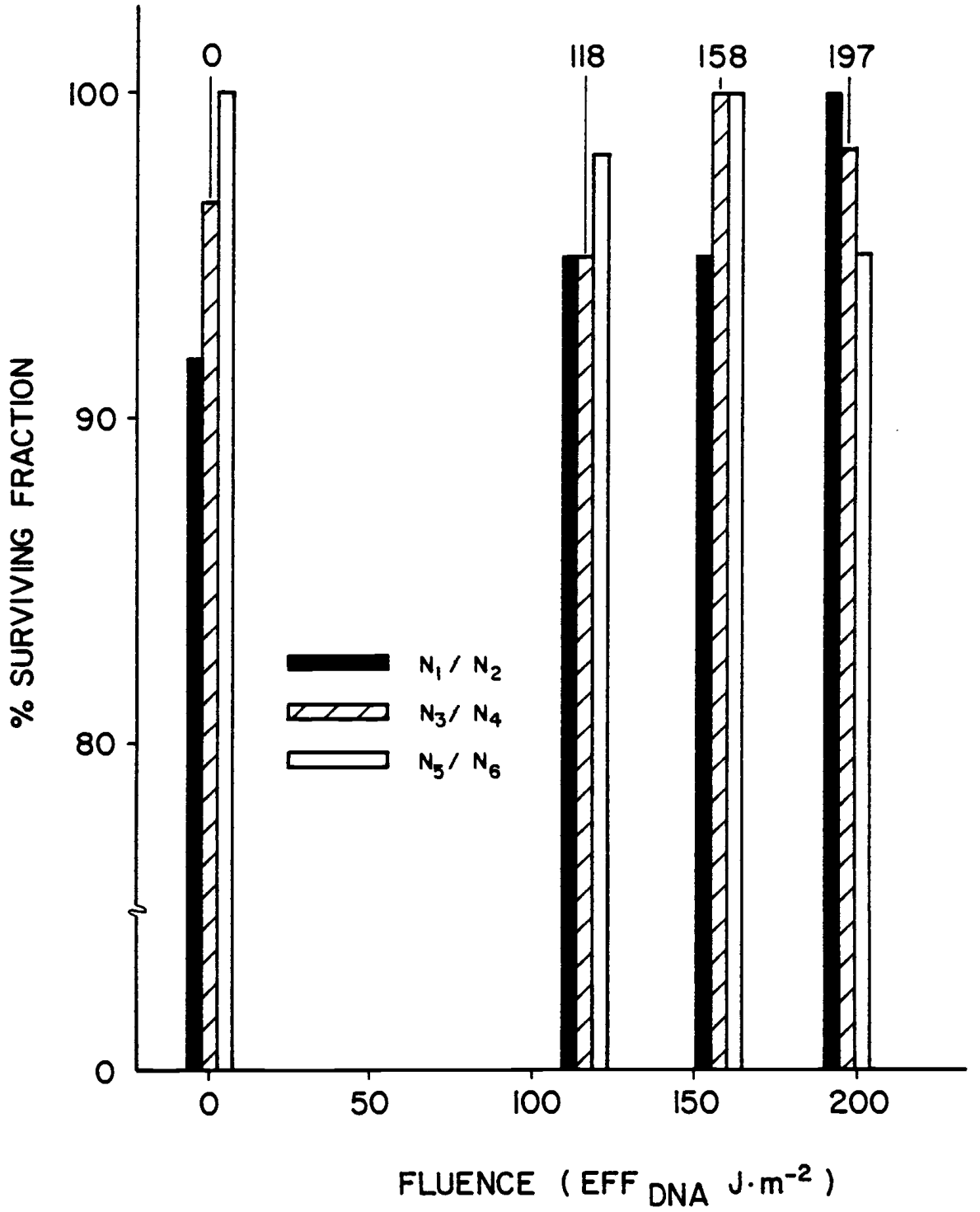

0
118
158
197
100
90
80
0
% SURVIVING FRACTION
N1 / N2
N3 / N4
N5 / N6
0
50
100
150
200
FLUENCE (EFF DNA J·m-2)

Figure 13. Percent surviving fraction vs. effective fluence. Surviving fractions of the C_1/C_2 and C_3/C_4 experiments were determined on Day 12 and Day 10, respectively, following irradiation. (C = copepodite stage)

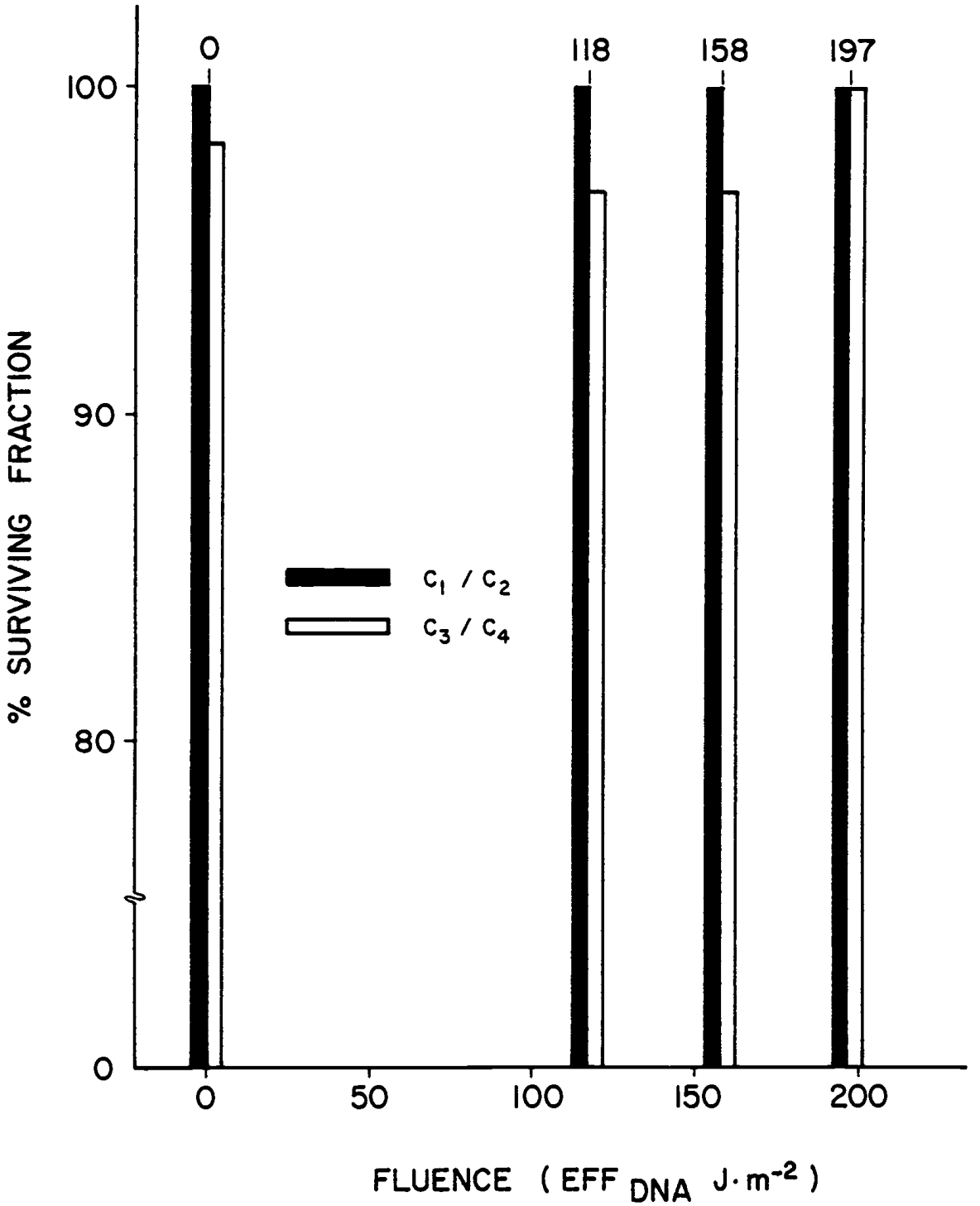

0
118
158
197
100
90
80
0
% SURVIVING FRACTION
C_1 / C_2
C_3 / C_4
0
50
100
150
200
FLUENCE (EFF DNA J·m^{-2})

Figure 14. Percent surviving fraction vs. effective fluence. Surviving fractions of two C_5/adult experiments ("high" and "low" fluences) were determined on Day 15 following irradiation.

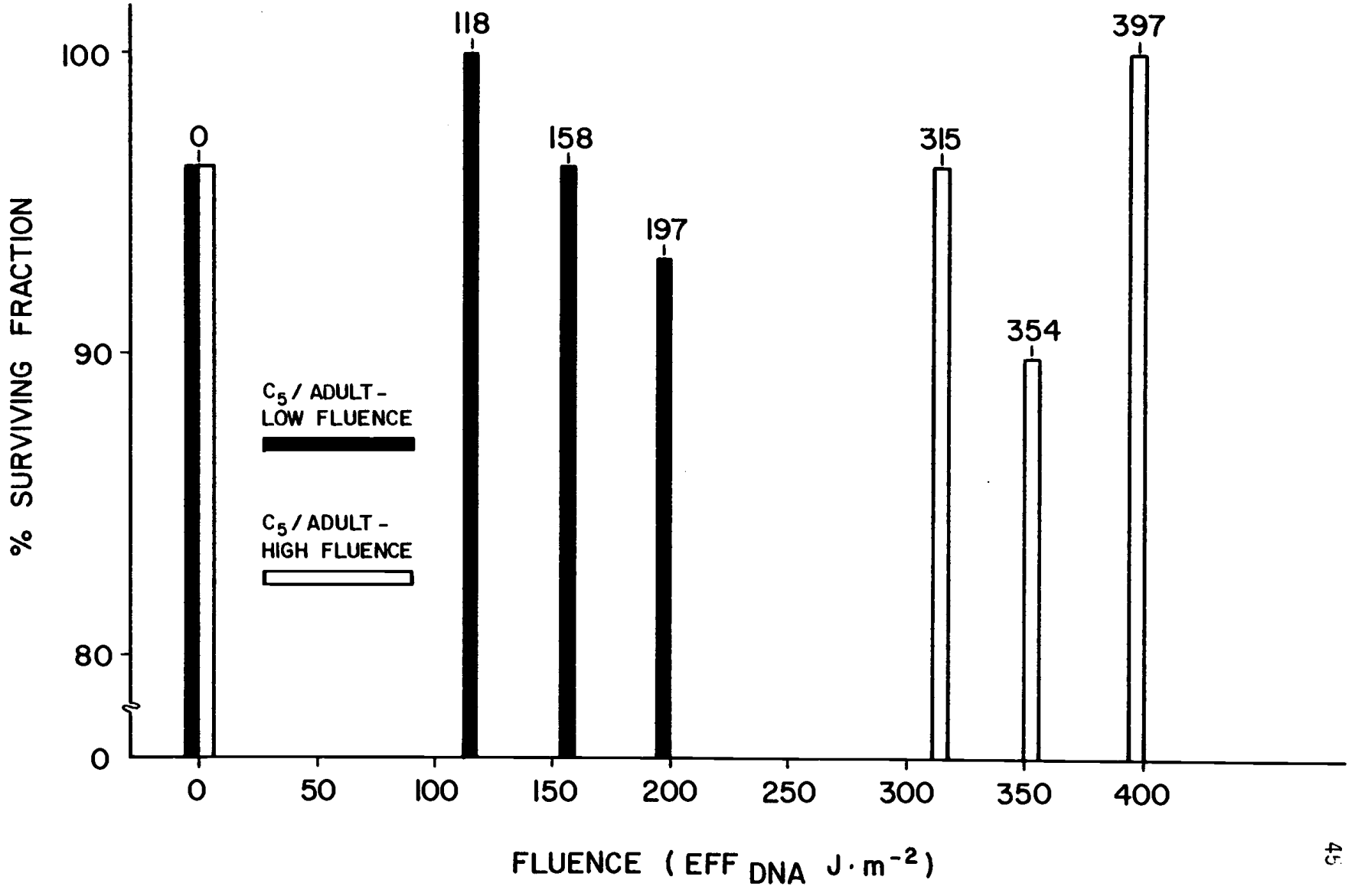

% SURVIVING FRACTION
100
90
80
0
0
50
100
150
200
250
300
350
400
FLUENCE (EFF DNA J · m⁻²)
0
118
158
197
315
354
397
C5 / ADULT - LOW FLUENCE
C5 / ADULT - HIGH FLUENCE

Figure 15. Best fit regression lines for Figure 12 through 14.

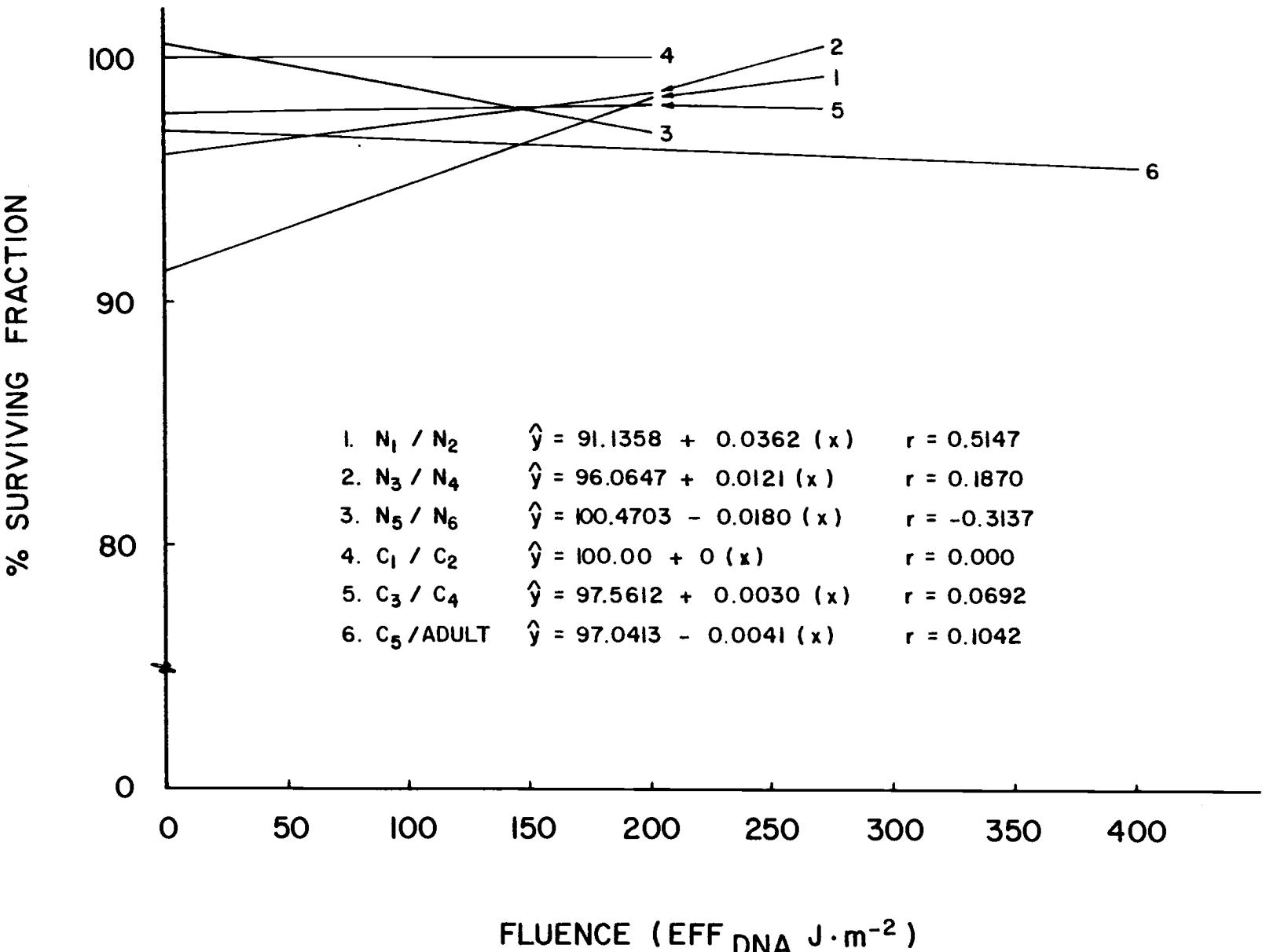

100
90
80
0
% SURVIVING FRACTION
0
50
100
150
200
250
300
350
400
FLUENCE (EFF DNA J·m⁻²)
1. N1 / N2 ŷ = 91.1358 + 0.0362 (x) r = 0.5147
2. N3 / N4 ŷ = 96.0647 + 0.0121 (x) r = 0.1870
3. N5 / N6 ŷ = 100.4703 - 0.0180 (x) r = -0.3137
4. C1 / C2 ŷ = 100.00 + 0 (x) r = 0.000
5. C3 / C4 ŷ = 97.5612 + 0.0030 (x) r = 0.0692
6. C5 / ADULT ŷ = 97.0413 - 0.0041 (x) r = 0.1042
1
2
3
4
5
6

Figure 16. Percent surviving fraction vs. effective fluence. Differential male - female survival using information from Figure 14.

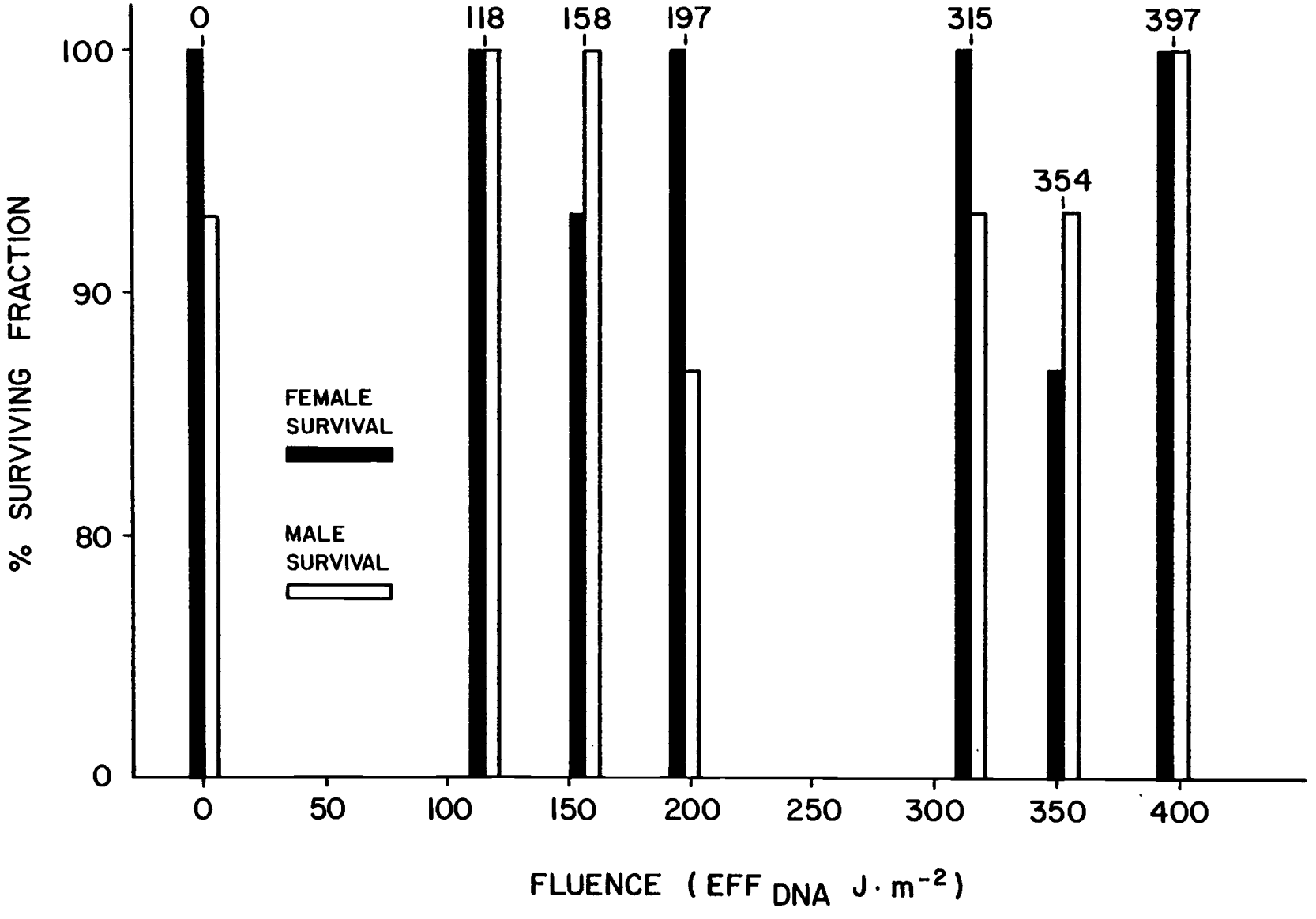
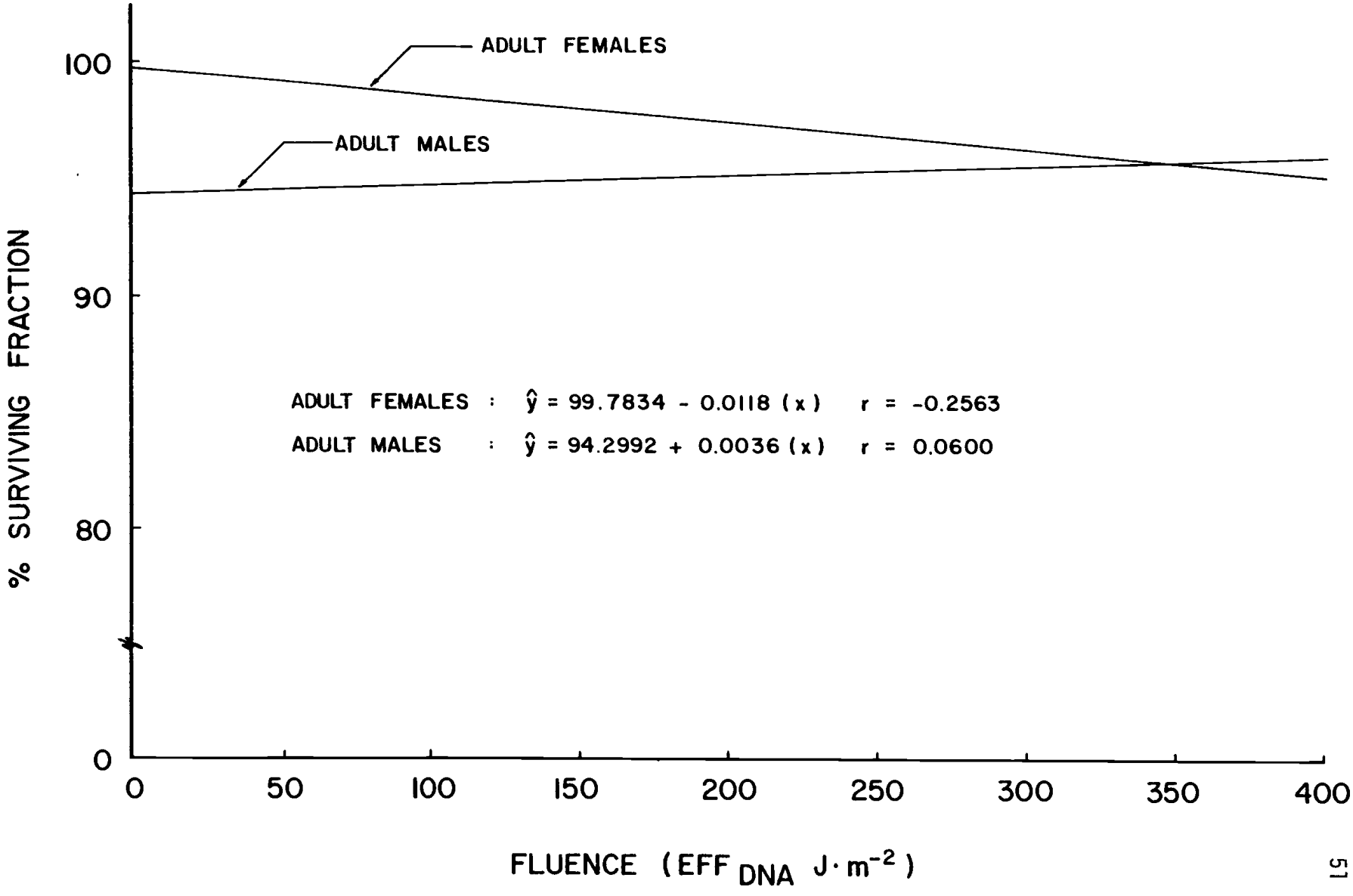

0
118
158
197
315
354
397
100
90
80
0
% SURVIVING FRACTION
FEMALE SURVIVAL
MALE SURVIVAL
0
50
100
150
200
250
300
350
400
FLUENCE (EFF DNA J · m⁻²)

Figure 17. Best fit regression lines for Figure 16.


ADULT FEMALES
ADULT MALES
100
90
80
0
% SURVIVING FRACTION
ADULT FEMALES : ŷ = 99.7834 - 0.0118 (x) r = -0.2563
ADULT MALES : ŷ = 94.2992 + 0.0036 (x) r = 0.0600
0
50
100
150
200
250
300
350
400
FLUENCE (EFF DNA J·m-2)

decrease slightly in survival, and males appear to increase, neither of the two slopes are significantly different from zero (for males, $p > .45$; for females, $p > .25$), nor do they differ significantly from each other ($p \simeq .5$).

Results from the fecundity study are shown on Table 1. Although neither of the fitted regressions are significantly different from zero, there is a tendency in the "high fluence" experiment for the number of offspring to decrease with increasing radiation. Combination of the two sets of data was not possible since an F-test showed significant differences between them ($.025 < p < .05$).

Table 2 illustrates the results of the sexual development aspect of larval irradiation. For larval stages N_3/N_4 through C_3/C_4, regressions of both sexes were not significantly different from zero ($p > .4$). While the regression function for female survival did not vary significantly from zero for the N_1/N_2 experiment ($p > .3$), the regression line for male survival significantly increased with increasing radiation ($.05 < p < .1$). Statistical analysis of variation between sexes at each larval stage revealed the following p-values:

Larval Stage	Significance
N_1/N_2	$p<.001$
N_3/N_4	$.005<p<.001$
N_5/N_6	$.05<p<.1$
C_1/C_2	$.05<p<.1$
C_3/C_4	$.1<p<.5$

Table 1. Offspring production from "high fluence" and "low fluence" groups.

Effective Dose*	Offspring	Mean ± S.D.	Effective Dose	Offspring	Mean ± S.D.
0	413, 20, 56	163 ± 217.75	0	256, 606, 380	414 ± 177.46
118	13, 147, 53	71 ± 68.79	315	433, 243, 310	329 ± 96.36
158	346, 167, 160	224 ± 105.42	354	137, 587, 244	323 ± 235.09
197	77, 376, 187	213 ± 151.23	394	393, 174, 274	280 ± 109.64

Regressions:			
	Low fluence group	$y = 132.388 + 0.3015\ (x)$	$r = 0.1652$
	High fluence group	$y = 416.743 - 0.3022\ (x)$	$r = -0.3303$

* Effective dose = $Eff_{DNA} Jm^{-2}$

Table 2. Differential sexual development of irradiated larval stages.

Group	Effective Dose*	Number of Females	Mean ± S.D.	Mean Proportion	Number of Males	Mean ± S.D.	Mean Proportion
N_1/N_2	0	11, 15, 16	14.00 ± 2.65	.76	6, 4, 3	4.33 ± 1.53	.24
	118	18, 10, 10	12.67 ± 4.62	.67	2, 7, 10	6.33 ± 4.04	.33
	158	11, 12, 14	12.33 ± 1.53	.65	8, 7, 5	6.67 ± 1.53	.35
	197	10, 13, 13	12.00 ± 1.73	.60	10, 7, 7	8.00 ± 1.73	.40
	$\hat{y} = 13.9638 - 0.0102\ (x)$		$r = -0.3044$		$\hat{y} = 4.2633 + 0.0175\ (x)$		$r = 0.5398$
N_3/N_4	0	9, 11, 14	11.33 ± 2.52	.59	9, 9, 6	8.00 ± 1.73	.41
	118	14, 14, 12	13.33 ± 1.15	.70	3, 6, 8	5.67 ± 2.52	.30
	158	9, 17, 15	13.67 ± 4.16	.68	11, 3, 5	6.33 ± 4.16	.32
	197	8, 9, 12	9.67 ± 2.08	.49	11, 11, 8	10.00 ± 1.73	.51
	$\hat{y} = 12.2118 - 0.0017\ (x)$		$r = -0.0482$		$\hat{y} = 7.0010 + 0.0042\ (x)$		$r = 0.1118$
N_5/N_6	0	16, 8, 7	10.33 ± 4.93	.52	4, 12, 13	9.67 ± 4.93	.48
	118	11, 9, 11	10.33 ± 1.15	.53	9, 11, 8	9.33 ± 1.53	.47
	158	14, 13, 10	12.33 ± 2.08	.62	6, 7, 10	7.67 ± 2.08	.38
	197	9, 12, 10	10.33 ± 1.53	.54	8, 8, 10	8.67 ± 1.15	.46
	$\hat{y} = 10.4014 + 0.0036\ (x)$		$r = 0.1087$		$\hat{y} = 9.6926 - 0.0072\ (x)$		$r = -0.2193$

(continued on next page)

Table 2. Continued.

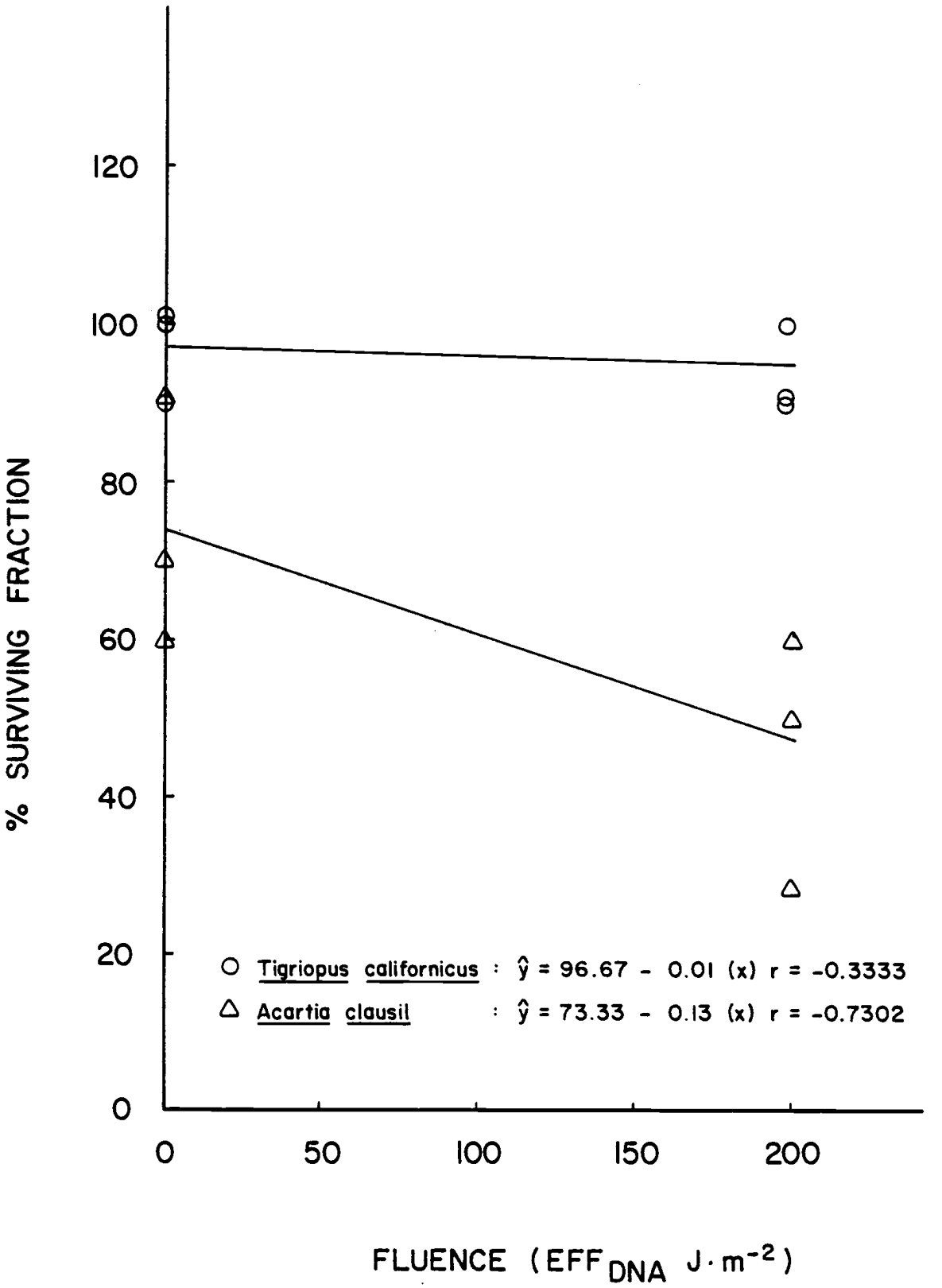
Group	Effective Dose*	Number of Females	Mean ± S.D.	Mean Proportion	Number of Males	Mean ± S.D.	Mean Proportion
C_1/C_2	0	10, 12, 10	10.67 ± 1.15	.53	10, 8, 10	9.33 ± 1.15	.47
	118	17, 7, 13	12.33 ± 5.03	.62	3, 13, 7	7.67 ± 5.03	.38
	158	10, 12, 12	11.33 ± 1.15	.57	10, 8, 8	8.67 ± 1.15	.43
	197	9, 13, 13	11.67 ± 2.31	.58	11, 7, 7	8.33 ± 2.31	.42
	$\hat{y} = 10.9304 + 0.0048\ (x)$ $r = 0.1460$				$\hat{y} = 9.0695 - 0.0048\ (x)$ $r = -0.1460$		
C_3/C_4	0	9, 5, 11	8.33 ± 3.06	.42	11, 15, 8	11.33 ± 3.52	.58
	118	10, 6, 10	8.67 ± 2.31	.45	10, 14, 8	10.67 ± 3.06	.55
	158	6, 12, 9	9.00 ± 3.00	.47	13, 8, 10	10.33 ± 2.52	.53
	197	5, 13, 8	8.67 ± 4.04	.43	15, 7, 12	11.33 ± 4.04	.57
	$\hat{y} = 8.3805 + 0.0024\ (x)$ $r = 0.0688$				$\hat{y} = 11.1317 - 0.0018\ (x)$ $r = -0.0487$		

* Effective dose = $Eff_{DNA} Jm^{-2}$

Figure 18 depicts actual data points and best-fit regressions for the survival of Tigriopus and Acartia at two dose levels (0 and 197 Eff_{DNA} Jm^{-2}). There was a significant difference between the two sets of data ($.001 < p < .005$).

Pigment analysis revealed two absorption peaks at 480 nm and 270 nm. Additionally, in the 280-312 nm range, the pigment extract from males absorbed more radiation than did the pigment extract from females. Conversely, in the visible region female extract absorbed more than the male extract.

Figure 18. Percent surviving fraction vs. effective fluence. Actual data points and best-fit lines are shown for both *Acartia clausii* and *Tigriopus californicus*.


120
100
80
60
40
20
0
% SURVIVING FRACTION
0
50
100
150
200
FLUENCE (EFF DNA J·m⁻²)
Tigriopus californicus : ŷ = 96.67 - 0.01 (x) r = -0.3333
Acartia clausil : ŷ = 73.33 - 0.13 (x) r = -0.7302

IV. DISCUSSION

Response to UV-B Irradiation

The quantity of solar radiation reaching supralittoral tide pools and their inhabitants is relatively more than that reaching estuarine and marine communities. Primarily this increased importance of solar irradiation is due to the shallowness of the pools, the clarity of the usually undisturbed water, and the paucity of "escape hatches" for the pools' inhabitants.

Solar irradiation is at its maximum in the northern hemisphere during the summer months. Similarly, UV-B fluences are also at a maximum (Table 3). Using the information from this table, and additional material not shown, effective fluences were plotted to roughly estimate daily effective fluence at 45°N latitude on June 21st. Interpolation of the data gave a value of approximately 96 effective-DNA Jm^{-2} day^{-1}. Therefore, the maximum UV-B radiation striking the earth's surface at Newport, Oregon, during the year would not normally be greater than 96 Eff_{DNA} Jm^{-2} day^{-1}.

In these experiments, increasing amounts of UV-B radiation were administered to T. californicus members. These levels, and their relative increase over natural daily UV-B levels on June 21st, are listed on page 61.

Table 3. Current levels of solar UV-B irradiation at the surface of the earth.

Latitude	Season	Ozone Concentration (atm-cm)	Absolute Fluence (280-320 nm) (Jm^{-2} d^{-1})	Effective Fluence ($Eff_{DNA}Jm^{-2}$ d^{-1})
40°	Winter	0.318	16,713	4.5
	Spring	0.352	68,891	31.6
	Summer	0.314	145,048	110.4
	Fall	0.281	81,915	53.2
50°	Winter	0.357	4,214	0.8
	Spring	0.395	41,479	12.7
	Summer	0.333	127,696	79.6
	Fall	0.299	52,978	24.3

Season = equinox or solstice during that season

From Nachtwey (1979) and Green et al. (1980)

Fluence Level (Eff_{DNA} Jm^{-2})	% Increase Over Natural Levels
0	----
118	22.9
158	64.6
197	105.2
315	228.1
354	268.8
397	310.4

The fluence rate at which the irradiation was administered is close to the natural rate at 45°N latitude. The solar noon fluence rate on June 21st at 40°N latitude is approximately 5.532 x 10^{-3} Eff_{DNA} Wm^{-2}; at 50°N latitude on the same day it is approximately 3.770 x 10^{-3} Eff_{DNA} Wm^{-2} (Nachtwey, personal communication; Green et al. 1980). The experimental fluence rate was calculated to be 4.1548 x 10^{-3} Eff_{DNA} Wm^{-2}. Therefore, these experiments were conducted at an approximately natural solar noon fluence rate for June 21st, but for extended periods of time resulting in enhanced effective fluences.

Results of the survival experiments suggest that even with large increases of effective UV-B fluences over natural levels, Tigriopus life stages are unusually resistant. This resistance is expressed statistically by the lack of significant slope coefficients (b_1). The exception to this was the slightly significant increase in survival of N_1/N_2 larvae with increased radiation. This anomaly, while not readily explainable, nevertheless suggests the resistance to UV-B radiation of the youngest life stages of Tigriopus. An

interesting observation is the negative survival slope for the N_5/N_6 stages. While not significant, it corresponds with Karanas' (1978) results using Acartia clausii, which showed the most susceptible life stage to be the N_5/N_6 group. Differences in adult survival, which also showed a slightly negative (albeit non-significant) slope, could be due to random variation within the experiment.

Although increased UV-B radiation did not significantly alter egg production in either the "low fluence" or "high fluence" groups, significant differential fecundity was observed between the groups. Increased fecundity is noticed in natural populations during spring and summer months (Egloff 1967). Therefore, the increase in photoperiod from low to high fluences may be sensed by the organisms, which respond by increasing egg production.

The sexual development of irradiated life stages was interesting, especially since the mechanism of sex determination in Tigriopus is still not known. The ratio of developing males to females increased significantly in the N_1/N_2 experiment and in the N_3/N_4 experiment. Later stages showed progressively less difference between the sexes, with a non-significant increase in females. This may support Takeda's (1950) claim that the larvae are sexually neutral before reaching the copepodite stages, and environmental factors can shift sex ratios. Since it has been previously shown that males increase in proportion during summer months (Igarashi 1960; Egloff 1967), which have a higher daily UV-B fluence, the increase of UV-B could

be interpreted by the sexually neutral larvae as the arrival of summertime. In late stages (N_5/N_6 through C_3/C_4), the sex may have already been determined; thus, there is no significant increase or decrease of either sex.

Comparison of Acartia and Tigriopus survival lines indicated that while Tigriopus had 96.6% corrected survival, Acartia had only 63% survival, with a significant decrease from controls to experimentals ($.05<p<.1$). Although Karanas (1978) found a 63% survival at only 122.37 Eff_{DNA} Jm^{-2}, the laboratory care of Acartia has improved since those experiments were carried out. This includes better temperature control both during and following irradiation, and gentler handling of the copepods by the researcher. Clearer results may have been obtained if higher fluences had been used, but there was still a noticeable decrease in the survival of Acartia at 197 Eff_{DNA} Jm^{-2} as compared to nearly 100% survival of Tigriopus.

The resistance of Tigriopus californicus to high levels of UV-B radiation echoes the work of Klugh (1929, 1930) who stated that there is a relationship between the depth at which the organism is found and its susceptibility to short-wave radiation; those organisms that live in shallow pools have a greater resistance to UV radiation than those that live at greater depths. The mechanism of this resistance may be, as Dethier (1980) suggests, the presence of carotenoid pigments that may protect Tigriopus from incident solar radiation.

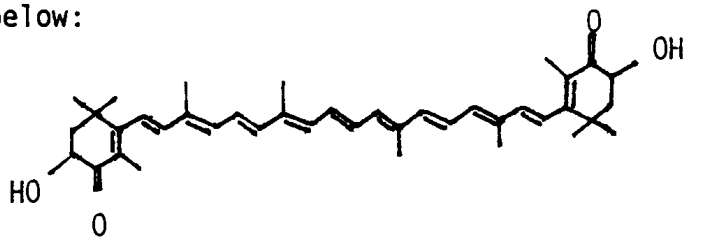
Implications of Carotenoids

An important aspect in the ecology of Tigriopus is the characteristic red-orange pigmentation of the carapace. Orange and red pigments are generally classified as carotenoids; more specifically, they are divided into two groups - oxygenated xanthophylls and oxygen-free carotenes (Goodwin 1952). Although members of both groups are found extensively in bacteria (Matthews 1959), algae (Bold and Wynne 1978), higher plants (Goodwin 1952) and invertebrates (Cheesman et al. 1967), carotenoid synthesis appears to take place only in bacteria and plant tissue (Goodwin 1952; 1960). In order for invertebrates to incorporate carotenoids, they must obtain these pigments from their diet.

In the Crustacea, carotenoids are widely distributed; the most commonly found pigments are the xanthophylls lutein and astaxanthin and the carotene β-carotene (Goodwin 1960). Astaxanthin is of special note as it is nearly always the major component in freshwater and marine Crustacea (Goodwin 1952). It has been isolated in various forms from copepods such as Diaptomus sp. (Palmer 1922; Czeczuga and Czerpak 1966; Hairston 1976, 1979) Calanus finmarchicus (Goodwin 1960), Tisbe furcata (Goodwin 1952), and Tigriopus fulvus (Goodwin and Srisukh 1949; Goodwin 1960). Although sophisticated pigment isolation has not been conducted using T. californicus, the absorption peak of 480 nm in this study falls well within the 470-506 nm range of values for astaxanthin determined by Gilchrist and Lee (1972) using various solvents. Further analysis of T. californicus

is needed, but for purposes of this discussion they are assumed to consist of astaxanthin and its ester as Goodwin and Srisukh (1949) found in T. fulvus.

Astaxanthin is apparently derived from β-carotene (Nakayama 1962), echinenone, or other xanthophylls (Herring 1968a). Its structure appears below:



From Goodwin 1974.

Astaxanthin, however, does not normally appear in algae except for cases such as euglenoid eyespots (Bold and Wynne 1978). The only other common instances in which astaxanthin is found in algal cells is during low nitrogen (Goodwin 1974) or low phosphorus (Kuhl 1974) concentrations. The cells then turn from green to orange until favorable conditions return. It is therefore hypothesized that the copepods assimilate β-carotene from their diet and from it produce astaxanthin (Hairston 1979).

In addition to the free and esterfied forms of astaxanthin found in copepods such as Tigriopus, carotenoprotein complexes are apparently common pigments as well. These complexes are blue or orange-red in appearance and are generally found in the exoskeleton, eggs, and ovaries of some copepods (Cheesman et al. 1967; Hairston 1979). Cheesman and his associates (1967) also determined that astaxanthin and its derivatives are the only carotenoid prosthetic

groups of carotenoproteins thus far identified.

The function of carotenoids, specifically astaxanthin and its aggregates, has been the subject of conjecture for some time. Nearly 60 years ago, Palmer (1922) postulated that carotenoids protect plant cell enzymes against absorbed light energy. Matthews (1959) suggested that the carotenes of photosynthetic bacteria protect the organisms against lethal photodynamic reactions. Nakayama (1962), Jensen (1965), Cheesman et al. (1967), Krinsky (1971), Gilchrist and Lee (1972), and Hairston (1976, 1979) all concur that carotenoids appear to protect bacteria, algae, and crustaceans from radiation which could otherwise mediate potentially harmful photo-oxidation of the cell and its components. The mechanism by which photo-oxidative damage is prevented was recently reported by Rodgers and Bates (1980). They suggest that carotenoids act as energy acceptors where they undergo collisions with chemically reactive singlet oxygen, thereby removing the potential for cellular damage. Carotenoids may also function as heat shields (Gilchrist and Lee 1972) or as food reserves (Hairston 1979).

For astaxanthin and other carotenoids to function protectively, they must first be incorporated and accumulated by the organism involved. Ninneman (1980) found that blue and near-UV wavelengths were effective in inducing carotenoid synthesis in algae, while the crustacean Daphnia magna was observed to accumulate carotenoids when subjected to intense illumination (Herring 1968b). Protection of young would also be of importance and both Daphnia magna (Herring 1968b) and Tigriopus fulvus (Goodwin and Srisukh 1949) females

transfer half of their carotenoids to the eggs. Green (1957) theorized that carotenoids and carotenoproteins of Daphnia act to protect the egg and embryo from ultraviolet radiation. This may also explain why T. californicus females appear darker than the males, as they may produce extra pigment to use in egg production.

The disadvantage of accumulating pigments such as astaxanthin is that an organism would become more visible to predators. Hairston (1979), working with species of the pelagic copepod Diaptomus, discovered that pigmented individuals were selected by predators over pigmentless organisms, but survived twice as long under visible blue light as the non-pigmented species. In his study, Hairston concluded that the advantage of being pigmented outweighed the costs. In the case of Tigriopus, however, there are no predators in the natural environment. This fact, coupled with the intense solar radiation which supralittoral pools receive daily, seems to indicate that accumulation of a protective pigment such as astaxanthin would be highly favorable for an exposed organism such as Tigriopus californicus. If this pigment is also associated with a protein and is carried in the carapace of the copepod, the amount of UV radiation entering the internal structures of the organism would be greatly reduced, as protein complexes have a peak absorbance of 278 nm (Smith 1977).

While much of the previous research assumes visible "light" is responsible for pigment accumulation and death of non-pigmented organisms, in many cases filters were not employed (e.g. Matthew 1959) to insure complete exclusion of the ultraviolet component. For this

reason, and in view of this study's experimental results, it seems logical to assume that the presence of a carotenoid in the carapace of Tigriopus californicus may assist in this organism's hardy survival under conditions of enhanced UV-B radiation.

V. CONCLUSIONS

Experimental results of this study suggest that the supralittoral tide pool copepod, Tigriopus californicus, is significantly resistant to UV-B irradiation. None of the irradiated life stages showed a significant decrease in survival, even when 300% increases over normal maximum fluence were administered. Fecundity was not adversely affected by UV-B radiation; in fact, an increase in offspring was noted at some levels. This increase of egg production may be due to photoperiod changes. Similarly, sex-ratio shifts in young irradiated larvae may be a product of increased radiation.

The pigments found in T. californicus were shown to be most probably the carotenoids astaxanthin and astaxanthin ester. The presence of the pigments in the carapace of the copepod may function to protect internal structures from radiation damage.

Further areas of research could include the study of survival at increased fluence rates and fluences, the effect of UV-B radiation on egg production and sex ratio shifts, and further pigment analysis and its role in photoprotection.

VI. REFERENCES

Anraku, M., and M. Omori. 1963. Preliminary survey of the relationship between the feeding habit and the structure of the mouthparts of marine copepods. Limnology and Oceanography 8: 116-126.

Ar-rushdi, A. H. 1958. The polygenic basis of sex-ratio in Tigriopus. Proceedings of the Xth International Congress on Genetics 2: 8-9.

Barnes, R. D. 1974. Invertebrate Zoology, 3rd Ed. Philadelphia, W. B. Saunders Co.

Belser, W. L. 1959. The research frontier: Where is science taking us? Saturday Review 42(3): 58.

Berkner, L. V., and L. C. Marshall. 1965. On the origin and rise of oxygen concentration in the earth's atmosphere. Journal of Atmospheric Science 22: 225-261.

Bold, H. C., and M. J. Wynne. 1978. Introduction to the Algae. New Jersey, Prentice-Hall, Inc.

Bradford, J. M. 1967. The genus Tigriopus Norman (Copepoda: Harpacticoida) in New Zealand with a description of a new species. Transactions of the Royal Society of New Zealand, Zoology 10(6): 51-59.

Burton, R. S., M. W. Feldman, and J. W. Curtsinger. 1979. Population genetics of Tigriopus californicus (Copepoda: Harpacticoida): I. Population structure along the central California coast. Marine Ecology Progress Series 1: 29-39.

Caldwell, M. M. 1979. Plant life and ultraviolet radiation: Some perspective in the history of the Earth's UV climate. BioScience 29: 520-525.

Chapman, S. 1930. A theory of upper-atmosphere ozone. Memoirs of the Royal Meteorological Society 3: 103-125.

Charniaux-Cotton, H. 1960. Sex determination, p. 411-417. In T. H. Waterman (ed.), The Physiology of Crustacea, Volume 1. New York, Academic Press.

Cheesman, D. F., W. L. Lee, and P. F. Zagalsky. 1967. Carotenoproteins in invertebrates. Biological Reviews 42: 132-160.

Comita, G. W., and J. J. Comita. 1966. Egg production in Tigriopus brevicornis, p. 171-185. In H. Barnes (ed.), Some Contemporary Studies in Marine Science. London, George Allen and Unwin, Ltd.

Crutzen, P. J. 1972. SST's - A threat to the earth's ozone shield. Ambio 1: 41-51.

Czeczuga, B., and R. Czerpak. 1966. Carotenoids in certain Diaptomidae (Crustacea). Comparative Biochemistry and Physiology 17: 523-534.

Dauvillier, A. 1965. The Photochemical Origin of Life. New York, Academic Press.

Dethier, M. N. 1980. Tidepools as refuges: Predation and the limits of the harpacticoid copepod Tigriopus californicus (Baker). Journal of Experimental Marine Biology and Ecology 42(2): 99-112.

Egloff, D. A. 1967. Ecological aspects of sex ratio and reproduction in experimental and field populations of the marine copepod Tigriopus californicus. Doctor's Thesis. Palo Alto, Stanford University. 141 numbered leaves.

Epstein, J. H. 1977. Photomedicine, p. 175-208. In K. C. Smith (ed.), The Science of Photobiology. New York, Plenum Press.

Fraser, J. H. 1936. The occurrence, ecology and life history of Tigriopus fulvus (Fischer). Journal of the Marine Biological Association of the United Kingdom 20: 523-536.

Giese, A. C. 1976. Living with our Sun's Ultraviolet Rays. New York, Plenum Press.

Gilchrist, B. M., and W. L. Lee. 1972. Carotenoid pigments and their possible role in reproduction in the sand crab, Emertia analoga (Stimpson, 1857). Comparative Biochemistry and Physiology 42B: 263-294.

Goodwin, T. W. 1952. The Comparative Biochemistry of the Carotenoids. London, Chapman and Hall, Ltd.

Goodwin, T. W. 1960. Biochemistry of pigments, p. 119-130. In T. H. Waterman (ed.), The Physiology of Crustacea, Volume 1. New York, Academic Press.

Goodwin, T. W. 1974. Carotenoids and biliproteins, p. 181-182. In W. D. P. Stewart (ed.), Algal Physiology and Biochemistry. Los Angeles, University of California Press.

Goodwin, T. W., and S. Srisukh. 1949. Some observations on astaxanthin distribution in marine Crustacea. Biochemical Journal 45: 268-270.

Green, A. E. S., K. R. Cross and L. A. Smith. 1980. Improved analytic characterization of ultraviolet skylight. Photochemistry and Photobiology 31: 59-65.

Green, J. 1957. Carotenoids in Daphnia. Proceedings of the Royal Society Series B 147: 392-401.

Guillard, R. R. L., and J. H. Ryther. 1962. Studies of marine planktonic diatoms. I. Cyclotella nana Hustedt, and Detonula confervacea (Cleve) Gran. Canadian Journal of Microbiology 8: 229-239.

Hairston, N. G., Jr. 1976. Photoprotection by carotenoid pigments in the copepod Diaptomus nevadensis. Proceedings of the National Academy of Sciences 3: 971-974.

Hairston, N. G., Jr. 1979. The adaptive significance of color polymorphism in two species of Diaptomus (Copepoda). Limnology and Oceanography 24(1): 15-37.

Harris, R. P. 1973. Feeding, growth, reproduction and nitrogen utilization by the harpacticoid copepod, Tigriopus brevicornis. Journal of the Marine Biological Association of the United Kingdom 53: 785-800.

Heinle, D. R., R. P. Harris, J. F. Ustach, and D. A. Flemer. 1977. Detritus as food for estuarine copepods. Marine Biology 40: 341-353.

Herring, P. J. 1968a. The carotenoid pigments of Daphnia magna Straus - I. The pigments of animals fed Chlorella pyrenoidosa and pure carotenoids. Comparative Biochemistry and Physiology 24: 187-203.

Herring, P. J. 1968b. The carotenoid pigments of Daphnia magna Straus - II. Aspects of pigmentary metabolism. Comparative Biochemistry and Physiology 24: 205-221.

Huizinga, H. W. 1971. Cultivation, life history and salinity tolerance of the tidepool copepod, Tigriopus californicus Baker 1912, in artificial sea water. Transactions of the Illinois State Academy of Science 64: 230-236.

Igarashi, S. 1960. On the relationship between sex ratio and salinity as a regulator of growth speed in Tigriopus japonicus. Bulletin of the Marine Biological Station at Asamushi 10: 103-108.

Ito, T. 1970. The biology of the harpacticoid copepod Tigriopus japonicus Mori. Journal of the Facility of Science at Hokkaido University (Series 6) 17: 474-500.

Jensen, S. L. 1965. Biosynthesis and function of carotenoid pigments in microorganisms, p. 163-179. In C. E. Clifton, S. Raffel, and M. P. Starr (eds.), Annual Review of Microbiology, Volume 19. Palo Alto California, Annual Reviews, Inc.

Johnston, H. S. 1971. Reduction of stratospheric ozone by nitrogen oxide catalysts from SST exhaust. Science 173: 517-522.

Karanas, J. J. 1978. Exposure to ultraviolet-B radiation: Sensitivity evaluation of an important ecological parameter of the marine copepod Acartia clausi (sic). Master's Thesis. Corvallis, Oregon State University. 79 numbered leaves.

Karanas, J. J., H. Van Dyke, and R. C. Worrest. 1979. Mid-ultraviolet (UV-B) sensitivity of Acartia clausii Giesbrecht (Copepod). Limnology and Oceanography 1104-1116.

Kinne, O. 1977. Cultivation of animals, p. 761-766. In O. Kinne (ed.), Marine Ecology. New York, John Wiley and Sons.

Klugh, A. B. 1929. The effect of the ultraviolet component of sunlight on certain marine organisms. Canadian Journal of Research 1: 100-109.

Klugh, A. B. 1930. The effect of the ultraviolet component of the sun's radiation upon some aquatic organisms. Canadian Journal of Research 2: 312-317.

Koga, F. 1970. On the life history of Tigriopus japonicus. Journal of the Oceanographic Society of Japan 26(1): 11-21.

Kontogiannis, J. E. 1973. Acquisition and loss of heat resistance in adult tide-pool copepod Tigriopus californicus. Physiological Zoölogie 45: 50-54.

Kontogiannis, J. E., and C. J. Barnett. 1973. The effect of oil pollution on survival of the tidal pool copepod, Tigriopus californicus. Environmental Pollution 4: 69-79.

Koslow, E. E. 1977. An aposematic statement of nuclear war: Ultraviolet radiation in the post-attack environment. Bio-Science 27: 409-413.

Krinsky, N. I. 1971. Function, p. 669-716. In O. Isler (ed.), Carotenoids. Basel, Birkhäuser.

Kuhl, A. 1974. Phosphorus, p. 640-641. In W. D. P. Stewart (ed.), Algal Physiology and Biochemistry. Los Angeles, University of California Press.

Liu, S. C., R. J. Cicerone, T. M. Donahue, and W. L. Chameides. 1977. Sources and sinks of atmospheric N_2O and the possible ozone reduction due to industrial fixed nitrogen fertilizers. Tellus 29: 251-263.

Lovelock, J. E. 1971. Atmospheric fluorine compounds as indicators of air movements. Nature 230: 379.

Margulis, L., J. C. G. Walker, and M. Rambler. 1976. Reassessment of roles of oxygen and ultraviolet light in Precambrian evolution. Nature 264: 620-624.

Marshall, S. M., and A. P. Orr. 1960. Feeding and nutrition, p. 245-247. In T. H. Waterman (ed.), The Physiology of Crustacea, Volume 1. New York, Academic Press.

Matthews, M. M. 1959. Function of carotenoid pigments in non-photosynthetic bacteria. Nature 184: 1892-1893.

Maugh, T. H. 1979. The threat to ozone is real, increasing. Science 206: 1167-1168.

McCarthy, R. L., F. A. Bower, and J. P. Jesson. 1977. The fluorocarbon-ozone theory - I. Production and release - world production and release of CCl_3F and CCl_2F_2 (fluorocarbons 11 and 12) through 1975. Atmospheric Environment 11: 491-497.

McElroy, M. B., S. C. Wofsy, and Y. L. Yung. 1974. The nitrogen cycle: Perturbations due to man and their impact of atmospheric N_2O and O_3. Philosophical Transactions of the Royal Society of London: Series B 277: 159-181.

Moehring, M. P. 1980. Influence of ultraviolet-B radiation on the heterotrophic activity of estuarine bacterioplankton. Doctor's Thesis. Corvallis, Oregon State University. 112 numbered leaves.

Molina, M. J., and F. S. Rowland. 1974. Stratospheric sink for chlorofluoromethanes: Chlorine atomc-atalysed (sic) destruction of ozone. Nature 249: 810-812.

Monk, C. R. 1941. Marine harpacticoid copepods from California. Transactions of the American Microbial Society 60: 75-103.

Nachtwey, D. S. 1975. Linking 254 nm UV effects to UV-B effects via 280 nm equivalents, p. 3-54 - 3-73. In D. S. Nachtwey, M. M. Caldwell, and R. H. Biggs (eds.), Impacts of Climatic Change on the Biosphere, Part 1: Ultraviolet Radiation Effects (CIAP Monograph 5). Springfield, Virginia, U. S. Department of Transportation.

Nachtwey, D. S. 1979. Life Sciences Officer, NASA-Johnson Space Center, Houston, Texas. Personal communication to Worrest.

Nakayama, T. O. M. 1962. Carotenoids, p. 409-420. In R. A. Lewin (ed.), Physiology and Biochemistry of Algae. New York, Academic Press.

National Academy of Sciences. 1979a. Protection against Depletion of Stratospheric Ozone by Chlorofluorocarbons. Committee on Impacts of Stratospheric Change. Washington, D. C., National Academy of Sciences.

National Academy of Sciences. 1979b. Stratospheric Ozone Depletion by Halocarbons: Chemistry and Transport. Panel on Stratospheric Chemistry. Washington, D. C., National Academy of Sciences.

Ninnemann, H. 1980. Blue light photoreceptors. BioScience 30(3): 166-170.

Norton, H. W. 1962. Sex conversion in the copepod. Science 137: 951.

Palmer, L. S. 1922. Carotenoids and Related Pigments. New York, The Chemical Catalog Company, Inc.

Provasoli, L., K. Shiraishi, and J. R. Lance. 1959. Nutritional idiosyncrasies of Artemia and Tigriopus in monoxenic culture. Annals of the New York Academy of Science 77: 250-261.

Ranade, M. R. 1957. Observations on the resistance of Tigriopus fulvus (Fischer) to changes in temperature and salinity. Journal of the Marine Biological Association of the United Kingdom 36: 115-119.

Ricketts, E. F., and J. Calvin. 1968. Between Pacific Tides, 4th Edition. Stanford, Stanford University Press.

Rodgers, M. A. and A. L. Bates. 1980. Kinetic and spectroscopic features of some carotenoid triplet states: Sensitization by singlet oxygen. Photochemistry and Photobiology 31: 533-537.

Rothbard, S. 1976. Experiments in mass culture of the marine copepod Tigriopus japonicus (Mori) on a bed of crushed seaweed Ulva petrusa (Kjelman). Bamidgeh 80-105.

Sagan, C. 1973. Ultraviolet selection pressure on the earliest organisms. Journal of Theoretical Biology 39: 195-200.

Seliger, H. H. 1977. Environmental photobiology, p. 143-174. In K. C. Smith (ed.), The Science of Photobiology. New York, Plenum Press.

Smith, K. C. 1977. Ultraviolet radiation effects on molecules and cells, p. 113-142. In K. C. Smith (ed.), The Science of Photobiology. New York, Plenum Press.

Stolarski, R. S., and R. J. Cicerone. 1974. Stratospheric chlorine: A possible sink for ozone. Canadian Journal of Chemistry 52: 1610-1615.

Takeda, N. 1950. Experimental studies on the effect of external agencies on the sexuality of a marine copepod. Physiological Zoölogie 23: 288-301.

Thomson, B. E., R. C. Worrest, and H. Van Dyke. 1980. The growth response of an estuarine diatom (Melosira nummuloides (Dillw.) Ag.) to UV-B (290-320 nm) radiation. Estuaries 3: 69-72.

Vacquier, V., Jr. 1962. Hydrostatic pressure has a selective effect on the copepod Tigriopus. Science 135: 724-725.

Vacquier, V. D., and W. L. Belser. 1965. Sex conversion induced by hydrostatic pressure in the marine copepod Tigriopus californicus. Science 150: 1619-1621.

Vittor, B. A. 1971. Effects of the environment on fitness-related life history characters in Tigriopus californicus. Doctor's Thesis. Eugene, University of Oregon. 115 numbered leaves.

Wolniakowski, K. U. 1980. The physiological response of a marine phytoplankton species, Dunaliella tertiolecta, to mid-wavelength ultraviolet radiation. Master's Thesis. Corvallis, Oregon State University. 101 numbered leaves.

Worrest, R. C. 1975. Effect of enhanced mid-ultraviolet radiation (290-315 nm) on development and survival of Boreal toad (Bufo boreas boreas) tadpoles. Doctor's Thesis. Corvallis, Oregon State University. 119 numbered leaves.

Worrest, R. C., H. Van Dyke, and B. E. Thomson. 1978. Impact of enhanced simulated solar ultraviolet radiation upon a marine community. Photochemistry and Photobiology 27: 471-478.

APPENDICES

Appendix 1. Spectroradiometer readings for experimental area (290-320 nm).

Wavelength (nm)	.25 mm Cellulose Acetate		.18 mm Mylar	
	Absolute Fluence ($W\ m^{-2}$)	Eff_{DNA} Fluence ($W\ m^{-2}$)	Absolute Fluence ($W\ m^{-2}$)	Eff_{DNA} Fluence ($W\ m^{-2}$)
290	4.740×10^{-6}	1.2274×10^{-6}	--	--
291	2.323×10^{-5}	5.2205×10^{-6}	--	--
292	9.607×10^{-5}	1.8499×10^{-5}	--	--
293	3.493×10^{-4}	5.6853×10^{-5}	--	--
294	9.371×10^{-4}	1.2707×10^{-4}	--	--
295	2.263×10^{-3}	2.5184×10^{-4}	--	--
296	3.927×10^{-3}	2.5322×10^{-4}	--	--
297	6.032×10^{-3}	4.3082×10^{-4}	--	--
298	8.317×10^{-3}	4.6546×10^{-4}	--	--
299	1.056×10^{-2}	4.5477×10^{-4}	--	--
300	1.281×10^{-2}	4.1790×10^{-4}	--	--
301	1.547×10^{-2}	3.7614×10^{-4}	--	--
302	1.728×10^{-2}	3.0824×10^{-4}	--	--
303	1.845×10^{-2}	2.3799×10^{-4}	--	--
304	1.962×10^{-2}	1.8050×10^{-4}	--	--
305	2.063×10^{-2}	1.3378×10^{-5}	--	--
306	2.145×10^{-2}	9.6974×10^{-5}	--	--
307	2.210×10^{-2}	6.9013×10^{-5}	--	--
308	2.273×10^{-2}	4.8724×10^{-5}	--	--
309	2.332×10^{-2}	3.4147×10^{-5}	--	--
310	2.371×10^{-2}	2.3654×10^{-5}	--	--
311	2.694×10^{-2}	1.8317×10^{-5}	--	--
312	3.475×10^{-2}	1.6139×10^{-5}	3.599×10^{-6}	1.6717×10^{-9}
313	3.462×10^{-2}	1.1034×10^{-5}	1.970×10^{-5}	6.2804×10^{-9}
314	2.785×10^{-2}	6.1333×10^{-6}	1.059×10^{-4}	2.3326×10^{-8}
315	2.436×10^{-2}	3.7406×10^{-6}	4.227×10^{-4}	6.4896×10^{-8}
316	2.386×10^{-2}	2.5810×10^{-6}	1.074×10^{-3}	1.1615×10^{-7}
317	2.360×10^{-2}	1.8209×10^{-6}	2.317×10^{-3}	1.7882×10^{-7}
318	2.345×10^{-2}	1.3085×10^{-7}	3.856×10^{-3}	2.1515×10^{-7}
319	2.305×10^{-2}	9.4372×10^{-7}	5.615×10^{-3}	2.2991×10^{-7}
320	2.279×10^{-2}	6.9549×10^{-7}	7.109×10^{-3}	2.1693×10^{-7}
290-320 nm	5.039×10^{-1}	4.1548×10^{-3}	1.697×10^{-2}	1.0531×10^{-6}

Appendix 2. Survival Blocks for the five irradiated age groups.

Group	Sample Size	Effective Dose*	Proportion of Survivors	Mean Proportion ± S.D.
N_1-N_2	20	0	.85, .95, .85	0.917 ± 0.0058
		118	1.00, .85, 1.00	0.950 ± 0.087
		158	.95, .95, .95	0.950 ± 0.000
		197	1.00, 1.00, 1.00	1.000 ± 0.000
N_3-N_4	20	0	.90, 1.00, 1.00	0.967 ± 0.058
		118	.85, 1.00, 1.00	0.950 ± 0.087
		158	1.00, 1.00, 1.00	1.000 ± 0.000
		197	1.00, 1.00, .95	0.983 ± 0.029
N_5-N_6	20	0	1.00, 1.00, 1.00	1.000 ± 0.000
		118	1.00, 1.00, .95	0.983 ± 0.029
		158	1.00, 1.00, 1.00	1.000 ± 0.000
		197	.85, 1.00, 1.00	0.950 ± 0.087
C_1-C_2	20	0	1.00, 1.00, 1.00	1.000 ± 0.000
		118	1.00, 1.00, 1.00	1.000 ± 0.000
		158	1.00, 1.00, 1.00	1.000 ± 0.000
		197	1.00, 1.00, 1.00	1.000 ± 0.000
C_3-C_4	20	0	1.00, 1.00, .95	0.983 ± 0.029
		118	1.00, 1.00, .90	0.967 ± 0.058
		158	.95, 1.00, .95	0.967 ± 0.029
		197	1.00, 1.00, 1.00	1.000 ± 0.000
C_5-adult	10	0	1.00, .90, 1.00 .90, 1.00, 1.00	0.967 ± 0.058
		118	1.00, 1.00, 1.00	1.000 ± 0.000
		158	1.00, 1.00, .90	0.967 ± 0.058
		197	1.00, .90, .90	0.933 ± 0.058
		315	1.00, .90, 1.00	0.967 ± 0.058
		354	.80, 1.00, .90	0.900 ± 0.100
		394	1.00, 1.00, 1.00	1.000 ± 0.000

*Effective dose = $Eff_{DNA} Jm^{-2}$

Appendix 3. Differential survival between fluence levels within each life stage experiment.

Group	Test Pairs	t-value	Significance
N_1-N_2	0 - 118	0.555	n. s.
	0 - 158	0.999	n. s.
	0 - 197	2.499	.025<p<.05
	118 - 158	0.000	n. s.
	118 - 197	0.999	n. s.
	158 - 197	-----	p<.001
N_3-N_4	0 - 118	0.277	n. s.
	0 - 158	0.999	n. s.
	0 - 197	0.447	n. s.
	118 - 158	0.999	n. s.
	118 - 197	0.632	n. s.
	158 - 197	0.999	n. s.
N_5-N_6	0 - 118	0.999	n. s.
	0 - 158	0.000	n. s.
	0 - 197	0.999	n. s.
	118 - 158	0.999	n. s.
	118 - 197	0.632	n. s.
	158 - 197	0.998	n. s.
C_1-C_2	0 - 118	0.000	n. s.
	0 - 158	0.000	n. s.
	0 - 197	0.000	n. s.
	118 - 158	0.000	n. s.
	118 - 197	0.000	n. s.
	158 - 197	0.000	n. s.
C_3-C_4	0 - 118	0.447	n. s.
	0 - 158	0.707	n. s.
	0 - 197	0.999	n. s.
	118 - 158	0.000	n. s.
	118 - 197	0.999	n. s.
	158 - 197	1.999	.05<p<.1

(Continued on next page)

Appendix 3. Continued

Group	Test Pairs	t-value	Significance
C_5-Adult	0 - 118	0.999	n. s.
	0 - 158	0.000	n. s.
	0 - 197	0.707	n. s.
	0 - 315	0.000	n. s.
	0 - 354	0.499	n. s.
	0 - 394	0.999	n. s.
	118 - 158	0.999	n. s.
	118 - 197	1.999	.05<p<.1
	118 - 315	0.999	n. s.
	118 - 354	1.732	.05<p<.1
	118 - 394	0.000	n. s.
	158 - 197	0.707	n. s.
	158 - 315	0.000	n. s.
	158 - 354	0.499	n. s.
	158 - 394	0.999	n. s.
	197 - 315	0.707	n. s.
	197 - 354	0.499	n. s.
	197 - 394	1.999	.05<p<.1
	315 - 354	0.499	n. s.
	315 - 394	0.999	n. s.
	354 - 394	1.732	.04<p<.1