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Behavioral responses of decapod larvae to light, salinity and chlorine

Jerome E. Illowsky
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BEHAVIORAL RESPONSES OF DECAPOD LARVAE

TO LIGHT, SALINITY AND CHLORINE

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A Thesis

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment
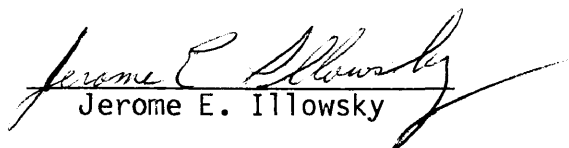
of the Requirements for the Degree of

Master of Science

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by

Jerome E. Illowsky
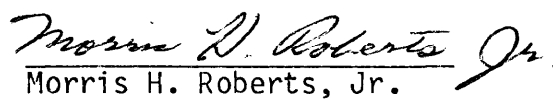
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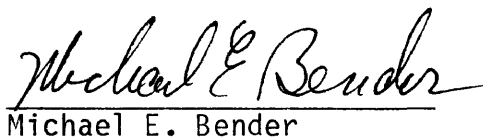
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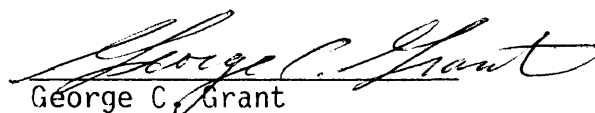
This thesis is submitted in partial fulfillment of the requirements

for the degree of

Master of Science

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ABSTRACT

The phototactic responses of <u>Pagurus longicarpus</u> stage 1 larvae were observed under various light intensities and salinities. No difference was detected in the phototactic response of the larvae when exposed to 240 fc and 24 fc. Larvae tested at their hatching salinity (18 $^O/oo$) swam upward more strongly than larvae hatched in 18 $^O/oo$ water, and then acclimated for 24 hours and tested at 24 $^O/oo$.

An apparatus was designed and developed specifically to test avoidance of polluted water layers by crustacean larvae. In this apparatus larvae are presented with a discontinuity of chlorine or other factor in their natural vertical migration path.

Only starved and light adapted larvae of <u>Palaemonetes</u> sp. were vertically phototactic under the conditions imposed during testing. This observation may demonstrate an important survival mechanism for starving larvae and may be a factor influencing vertical migration.

<u>Palaemonetes</u> sp. stage 2 larvae did not actively avoid or prefer chlorinated water layers of 1.0 to 12.0 mg/l chlorine-produced oxidants (CPO) under the conditions imposed by these experiments. Larvae passively sank to the bottom of the test chambers only after sufficient exposure to chlorine to become immobilized. The rate at which the activity of the larvae declined was in direct relation to concentration and light intensity. Mortality of <u>Palaemonetes</u> sp. larvae cultured in clean water after avoidance testing was directly proportional to CPO concentration.

BEHAVIORAL RESPONSES OF DECAPOD LARVAE

TO LIGHT, SALINITY AND CHLORINE

INTRODUCTION

Chlorine enters the estuarine environment from two major sources, although many minor sources (discussed later) account for significant quantities of chlorine in some local areas. One major source of chlorine pollution is cooling water from power plants. In this case chlorine is used to control fouling organisms and bacterial slime in the cooling and condensing systems of power plants. This is necessary because any restriction in water flows and heat transfer caused by aquatic organisms greatly reduces the efficiency of power production. Chlorine is the primary antifouling agent in use at present because it is cheaper, simpler to use and more efficient than other chemical or mechanical methods (Beauchamp, 1969; Brungs, 1973; Coughlan and Whitehouse, 1978; Draley, 1972; Jolley et al., 1978; White, 1972).

The other major source of chlorine introduced into estuaries is from wastewater treatment plants. Chlorine has many properties which make it the chemical of choice for wastewater disinfection. It has adequate bactericidal properties which effectively control pathogenic organisms. Chlorine also helps reduce biochemical oxygen demand, aids in water clarification, controls flies and reduces odors (Brungs, 1973; Ingols et al., 1953; Morris, 1975; White, 1972).

Various industries use chlorine in many aspects of manufacture, primarily to control pest organisms (insects and bacteria), e.g. in food processing and pulp and paper production. Chlorine is also used in the aluminum industry to oxidize impurities during manufacture (Brungs, 1973; White, 1972).

Toxicity and behavioral studies involving chlorine are difficult to compare because of the complex chemistry of chlorine, especially in seawater. In fresh water, calcium hypochlorite added to water hydrolyzes to form hypochlorous acid and hypochlorite ion.

$$Ca\ (OCl)_2 + 2H_2O \rightleftarrows Ca^{+2} + 2OH^- + 2HOCl \rightleftarrows 2H^+ + 2OCl^-$$
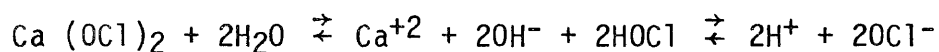
At pH 8.0 the hypochlorous acid (HOCl) to hypochlorite ion (OCl^-) ratio is approximately 1:3. When ammonia or organic amines are present, they react with the chlorine to form chloramines:

$$NH_3 + HOCl \rightleftarrows NH_2Cl + H_2O \qquad \text{monochloramine}$$

$$NH_2Cl + HOCl \rightleftarrows NHCl_2 + H_2O \qquad \text{dichloramine}$$

(urea) $NH_2-CO-NH_2 + 2HOCl \rightleftarrows NHCl-CO-NHCl$ monochloramine (organic)
or
$\rightarrow 2NH_2Cl + H_2CO_3$ dichloramine (organic) (White, 1972)

$$R-C-NH_2 + HOCl \rightarrow R-C-NHCl$$

$$R-C-NHCl + HOCl \rightarrow R-C-NHCl_2 \qquad \text{(Lewis, 1966)}$$
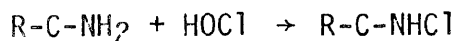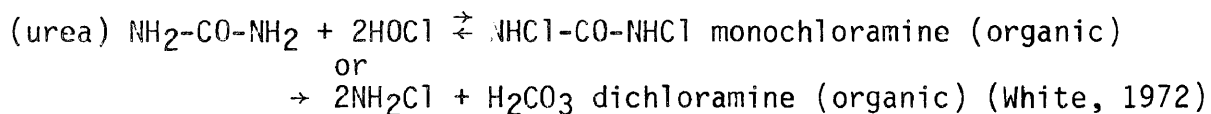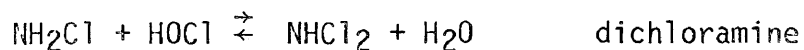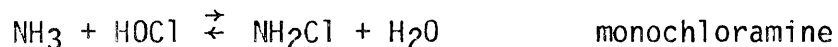
Nitrogen trichloride is also known to exist but only at pH values less than 4. Therefore it is of no concern in environmental work.

The proportions of monochloramine and dichloramine existing at any time depends on the temperature, pH, and chlorine:ammonia ratio.

At a chlorine:ammonia ratio (by weight) of 5:1 the relative percentages of the monochloramine to dichloramine are: at pH 5, 16:84 and at pH 8.0, 85:15 (Lewis, 1966).

Salt water reactions with chlorine are more complex because of the presence of bromide (about 65 ppm in seawater) (Carpenter, 1977; Carpenter and Smith, 1978; Johannesson, 1955; Johnson, 1977; Lewis, 1966; Sugam and Helz, 1977; Wong and Davidson, 1977). At the pH of seawater (about 8.0) hypochlorous acid will react with bromide to form hypobromous acid in the absence of ammonia and other interfering agents:

$$HOCl + Br^- \rightarrow HOBr + Cl^-$$

The hypochlorite ion will also react with bromide to form the analogous hypobromite ion:

$$OCl^- + Br^- \rightarrow OBr^- + Cl^-$$
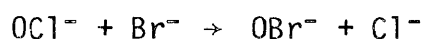
The bromine species react with NH_3 in an analogous manner to chlorine species to form bromamines. Bromamines may coexist with chloramine in varying proportions depending on pH, salinity, ammonia to halogen ratio (Inman and Johnson, 1978; Lewis,1966; Macalady et al., 1977; Sugam and Helz, 1977), and, most importantly, the kinetics of the chlorine and bromine reactions in relation to ammonia (Johnson, 1977).

Dibromamine is the predominant form produced in estuarine and ocean environments (Inman and Johnson 1978). Dibromamine does not persist as long as monochloramine. In some situations it is further oxidized to bromate at a rate that appears to be affected most by

ultra-violet light (Macalady et al., 1977). In other cases dibromamine decays to bromide. When the ammonia concentration is greater than 0.5 mg/l and the chlorine concentration is less than 2.5 mg/l, dibromamine and monochloramine coexist. At ammonia concentrations higher than 1.0 mg/l monochloramine becomes the major component of the total oxidant concentration. At less than 0.4 mg/l ammonia-nitrogen and sufficiently large doses of chlorine, tribromamine and hypobromous acid are the major products. These bromine reactions which occur in estuarine water and seawater upon addition of chlorine have been largely ignored until recently, but appear to be responsible for the predominant forms of oxidants in estuarine water and seawater (Inman and Johnson 1978).

In addition to the inorganic chlorine-produced halogen species mentioned above, many reactions occur between various organics present in the water and the oxidative halogens making identification of potentially toxic organic halogen products extremely difficult (Brungs 1976; Carlson et al., 1978 Christman et al., 1978; Glaze and Peyton, 1978; Hsu and Shimizu, 1977; Jolley, 1973, 1977; Morris and Baum, 1978; Noack and Doerr, 1978; Rockwell and Larson, 1978; Stanbro, 1977). The organic halogen products are probably not produced in high concentrations but some of them are potentially toxic (Jolley, 1973).

The exact halogen species produced upon chlorination of estuarine waters cannot be predicted because of the number of competing reactions possible. For this reason the term chlorine produced

oxidant (CPO) is preferred over total residual chlorine (TRC) in this study when referring to concentration.

The adverse effects of chlorine on aquatic organisms has been well substantiated for a wide variety of taxonomic groups including phytoplankton, protozoans, rotifers, oligochaetes, molluscs, crustaceans and finfish. Brungs (1973, 1976), Mattice and Zittel (1976) and Whitehouse (1975) have reviewed studies on chlorine toxicity. In the review by Brungs (1973), an upper limit of 0.002 mg/l TRC (total residual chlorine) was proposed as appropriate to protect most aquatic organisms. Brungs (1976) revised the estimated safe value to 0.003 mg/l TRC for freshwater organisms. These "safe" values were based mainly on data for freshwater organisms.

Mattice and Zittel (1976) agreed with Brungs (1973) closely in estimating a "safe" chronic toxicity threshold (0.0015 mg/l TRC) but specified that this value was for freshwater organisms. They found salt water organisms to be less sensitive with a threshold of toxicity estimated to be 0.02 mg/l TRC. The levels recommended as safe for freshwater organisms are below the limits of detectability.

Few acute chlorine toxicity tests have been performed on larval decapod species. Roberts (1978) and Roberts et al. (1979) tested the toxicity of chlorinated estuarine water to stage 1 larvae of Panopeus herbstii and Pagurus longicarpus. The 96 hour LC50 values were 0.04 to 0.12 mg/l and 0.062 to 0.102 mg/l for these species, respectively. No chlorine toxicity data were found for Palaemonetes sp. larvae which were used as test organisms in this study.

The toxicity of CPO to <u>Homarus americanus</u> larvae was studied by
Capuzzo <u>et</u> <u>al</u>. (1976). Exposure to concentrations of 2.89 mg/l
residual free chlorine and 0.32 mg/l residual chloramine for periods
up to one hour produced fifty percent mortality after 48 hours.
Respiration was depressed at 0.05 mg/l applied chloramine and 5.0 mg/l
applied free chlorine. These values seem high because the
concentrations in the respiration experiments were measured in terms
of applied dose rather than the residual concentration to which the
larvae were actually exposed.

Authur <u>et</u> <u>al</u>. (1975) examined chronic effects of chlorinated
sewage on freshwater organisms. The most sensitive organism tested
was <u>Daphnia magna</u> which was affected by 0.010 mg/l TRC. No chronic
toxicity tests are reported using chlorine alone or chlorinated sewage
in salt water.

In behavioral tests organisms may detect and react to sublethal
levels of pollutants (Larrick <u>et</u> <u>al</u>., 1978). Observation of
avoidance-preference behavior is one approach that has been used
extensively to assess the response of aquatic organisms when
encountering a pollutant. There are three possible responses an
organism can exhibit when encountering a pollutant: avoidance,
preference or ambivalence. An avoidance reaction to a toxic
concentration of a pollutant would contribute to the survival and well
being of an organism (Larrick <u>et</u> <u>al</u>., 1978). Preference of toxic
levels of a pollutant on the other hand would produce much more

serious effects than might otherwise occur because this behavior would increase the probability of any given animal being affected.

Shelford and Allee (1913) were the first to test avoidance using a countercurrent chamber. This apparatus has subsequently been used extensively, mainly for testing the reactions of freshwater fish to pollutants (Cherry, Hoehn et al., 1977; Cherry, Larrick et al., 1977; Jones, 1947, 1951; Scherer, 1977; Sprague, 1968; Sprague and Drury, 1969). Some estuarine fish have also been tested with this type of apparatus (Lewis and Livingston, 1977; Livingston et al., 1976; Meldrim and Fava, 1977; Meldrim et al., 1974; Middaugh et al., 1977). The double-Y maze is another apparatus that has been used by some researchers to determine avoidance behavior of organisms mainly to pesticides, (Folmar, 1976; Hansen, 1969; Hansen et al., 1972; Hansen et al., 1973; Hansen et al., 1974; Kynard, 1974). Organisms in both apparatuses were exposed to discontinuities of the pollutant in the horizontal plane. The avoidance of organisms to gradients of pollutants has also been studied (Höglund, 1951, 1961; Höglund and Astrand, 1973; Jones et al., 1956; Lindahl and Marcström, 1958).

Avoidance reactions of fish to many kinds of pollutants have been well reviewed by Larrick et al. (1978). Little work has been done on avoidance testing with marine invertebrates and none with invertebrate larvae. Hansen et al. (1973, 1974) tested the avoidance behavior of two shrimp species to pesticides and herbicides. Maciorowski et al. (1977) tested the avoidance of copper by Gammarus lacustris. Costa (1966) showed that Gammarus pulex avoid various concentrations of

chloroform, ethyl alcohol, formalin and various metals. Meldrim et al. (1974) briefly examined the avoidance behavior of Palaemonetes pugio, Crangon septemspinosa and Callinectes sapidus exposed to chlorine residuals. All studies except those of Hansen employed a Shelford-Allee apparatus.

Chlorine avoidance studies are difficult to evaluate and compare because of the complex and dynamic nature of chlorine reactions in water. Many factors in receiving waters affect the form in which chlorine exists in water such as, pH, ammonia concentration, salinity, bromine concentrations, temperature and organic compounds. In seawater the oxidants measured include not only chlorine but also bromine species and related halogenated compounds. There is also disagreement regarding what form of halogen (chlorine) is the most important to measure (Sprague and Drury, 1969; Meldrim et al., 1974).

Most avoidance studies of chlorine for fish have been performed in freshwater with a few studies in estuarine water (Cherry, Hoehn et al., 1977; Cherry, Larrick et al., 1977; Fava and Tsai, 1976; Meldrim and Fava, 1977; Meldrim et al., 1974; Middaugh et al., 1977). Depending on the specific conditions imposed on the fish, the threshold avoidance (THA) concentrations ranged from 0.049 - 0.26 mg/l TRC with 50% avoidance occurring at concentrations from 0.03 to 0.64 mg/l TRC.

Only one study was conducted testing avoidance of chlorinated water by invertebrates. Meldrim et al. (1974) conducted a few chlorine avoidance tests with three decapod species, Palaemonetes

pugio, Crangon septemspinosa and Callinectes sapidus. Threshold avoidance concentrations were approximately 0.04 mg/l, 0.085 mg/l and 0.10 mg/l free residual chlorine, respectively. Approximate values are given because Meldrim et al. (1974) did no data reduction on these tests.

Avoidance studies using invertebrate larvae have been thus far concerned only with salinity (Harder, 1952, 1954, 1957, 1968; Lance, 1962; Roberts, 1971b; Scarratt and Raine, 1967). These studies used a vertical discontinuity apparatus consisting of cylinders in which water of low salinity rested on top of high salinity water. The test protocol of these experiments was similar to the protocol used in the present study.

Responses to natural factors are important considerations when studying avoidance behavior. Light, temperature, pressure, salinity and feeding are factors known to affect larval behavior. Thorson (1964) in a comprehensive review paper recorded that out of 141 species of early stage meroplankton studied, 81% were positively phototactic and migrated to surface water layers, 12% seemed indifferent and 6% of the larvae were negatively phototactic. Subsequent studies have confirmed that the vast majority of early stage zooplankton are positively phototactic (Bardolph and Staun, 1978; Forward, 1974; Forward and Costlow, 1974, Latz and Forward, 1977; Sulkin, 1971).

Thorson (1964) and Forward (1974) report that high light intensities may induce negative phototaxis in normally photopositive

larvae. Temperature increases are thought to induce photonegative responses in larvae although this has not been well studied (Ewald 1912; Thorson 1964). An increase in pressure generally causes upward swimming and increased phototactic response (reviewed briefly by Sulkin, 1971). Salinity decreases have been shown to cause downward movements of larvae even in the presence of light as shown by avoidance studies noted earlier (Thorson, 1964; Latz and Forward, 1977).

Crustacean larvae move primarily in the vertical plane. This behavior is associated with the search for food and with utilization by larvae of the net flows of water layers within estuaries to maintain themselves in an environment favorable for development. Many factors in the environment are known to affect vertical migration. Light intensity and salinity, two major factors of importance, are subjects of this study. In addition to environmental factors, pollutants may affect the behavior and therefore the ability of larvae to survive. Chlorine introduced into estuaries by power plants and sewage treatment plants is generally found in surface plumes and thus may affect the vertical migration of larvae. Reactions of crustacean larvae to chlorinated water layers are also explored in this study.

Crustacean larvae are important to estuarine ecosystems. Some species are of economic significance while others serve as an important step in the food webs of other organisms. Therefore, it is important to study the responses of these organisms to factors they may encounter in the environment.

The primary objectives of this study were 1) to determine the vertical phototrophic behavior of decapod larvae under different conditions of light and salinity; 2) to develop an apparatus that can be used to test the reactions of decapod larvae to non-conservative pollutants such as chlorine in the vertical plane; and 3) to use the apparatus to determine whether decapod larvae can detect and avoid CPO.

MATERIALS AND METHODS

Maintenance of Laboratory Breeding Stock and Test Organisms

Adult <u>Pagurus longicarpus</u> were obtained from the York River near
Gloucester Point, Virginia or from Cedar Island near Wachapreague,
Virginia. Wachapreague crabs were slowly acclimated to the ambient
salinity of the York River at the Virginia Institute of Marine Science
pier (≈ 20 ^{o}/oo) in three equal steps over three days.

Adult crabs were maintained on a sea table receiving a continuous
supply of unfiltered or 10 μm filtered estuarine water. A layer of
subtidal sand was provided as a substrate for the crabs which provided
some food as well as a "normal" environment. Crabs were fed pieces of
frozen fish every other day with Purina Marine Chow® added
periodically as a food supplement. Uneaten food was removed the
following day.

A 14 hour light:10 hour dark photoperiod was maintained by means
of a timer which controlled two 30 W fluorescent bulbs (daylight
white) located directly above the sea table. During the summer the
photoperiod was increased by light entering through windows adjacent
to the sea tables. Water temperature was maintained at a minimum of
20°C. During summer months ambient temperature was used. During the

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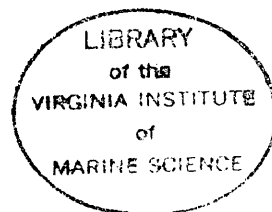
winter the heated water was vigorously aerated to protect the crabs from air embolisms caused by the sudden heating of air saturated water.

Adult crab populations were examined weekly for ovigerous females. Ovigerous crabs were placed in a plexiglass holding tank (Fig. 1A), receiving 1 μm filtered estuarine water (b). Water was discharged through outflow port (c), located near the top of the tank. Hatched larvae in the outflow were trapped in a 254 μm mesh screened basket containing freshly hatched Artemia nauplii. The receiving basket was immersed in a water bath (e). The basket was examined for larvae, stocked with fresh food, and cleaned every morning (hatching usually took place during the night or early morning). Newly hatched zoeae were transferred to 20.3 cm glass finger bowls. Larvae were transferred to clean water and fed daily. Culture techniques were patterned after Roberts (1971a, 1972, 1974).

Palaemonetes sp. adults were collected with a dip net from a marsh near Mumfort Island in Wicomico, Virginia. These shrimp were identified as P. pugio based on rostral spine characteristics (Gosner, 1971). However, using several other diagnostic characters as well, it was later found that the population was a mixture of P. pugio, P. vulgaris and P. intermedius.

Adult shrimp were held in a 379-liter rectangular fiberglass tank. Unfiltered aerated estuarine water flowed through the tank. A minimum temperature of 23°C was maintained during the winter when induced spawning was attempted (Little, 1968). During the summer,
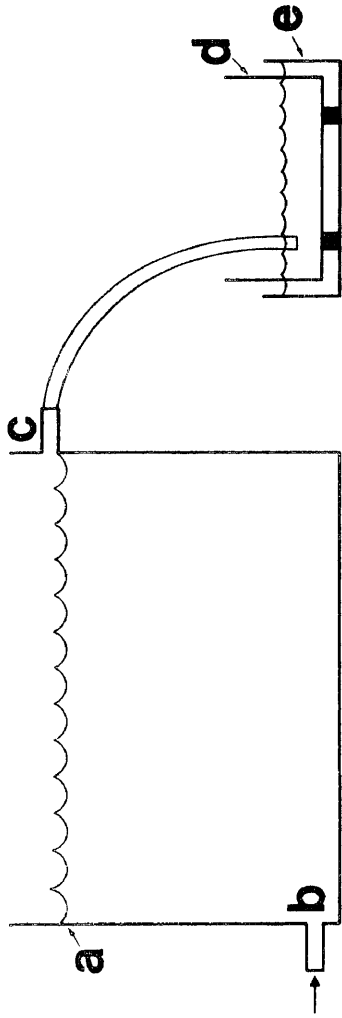
A. a - crab holding tank
    b - 1 µm filtered water inflow port
    c - water and larva exit port
    d - 254 µm screened larva collection basket
    e - water bath for collection basket
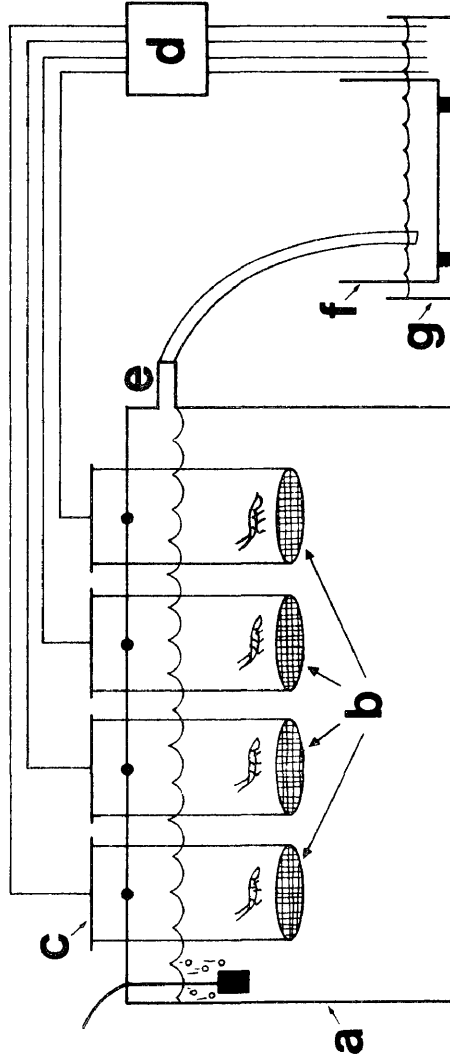
B. a - shrimp holding tank
    b - shrimp holding cylinders with 1 mm
        screened bottoms
    c - black light exclusion covers
    d - peristaltic pump
    e - water and larva exit port
    f - 254 µm screened larva collection basket
    g - water bath for collection basket

Figure 1. Larva hatching and collection apparatus for Pagurus longicarpus (A) and
Palaemonetes sp. (B).

ambient temperature was used. Adult shrimp were fed bits of fish every two days and left-over _Artemia_ nauplii daily. The 14L:10D photoperiod was provided by two 30 W fluorescent bulbs (daylight white) controlled by a timer. During the summer the photoperiod was extended by lighting from nearby windows.

Ovigerous females were transferred to a 38 liter aquarium equipped with an aquarium filter. Freshly hatched _Artemia_ nauplii were introduced daily. Adult shrimp with eggs in an advanced stage of development (eyespots visible) were transferred to a larva collector (Fig. 1B). The larva collector used for _Palaemonetes_ sp. differed slightly from the one used for _P. longicarpus_. Shrimp will eat eggs off the pleopods of other shrimp or newly hatched larvae. This collector prevents loss of larvae or eggs through cannibalism. It consists of a plexiglass tank (a) with four cylinders suspended from the top (b). The bottoms of the cylinders have 1 mm nylon mesh screens attached.

One gravid shrimp was placed in each basket. The top of the basket was covered with black plexiglass (c) to exclude light, thereby reducing the tendency for newly hatched larvae to swim upward in the tube. Water pumped into the top of the baskets at a rate of about 20 ml/min provided circulation through the tubes which helped flush out hatched larvae. Water and larvae exited the holding tank via a port (d) located at the top of the tank which led to a submerged screened basket identical to the one used to collect _P. longicarpus_ larvae except that the water was recirculated to the baskets holding

adult shrimp. The baskets were rinsed with hot tap water (55°C) daily and washed with Alconox℞ along with the tubes and tank every 2 days.

Shrimp larvae were cultured in a similar fashion to those of P. longicarpus (Floyd, 1977; Provenzano and Goy, 1976). Culture temperatures ranged from 20° to 25°C and ambient salinity was used except during acclimation for tests.

Behavioral Testing Apparatus

The apparatus used to evaluate the response of larvae to chlorinated estuarine water layers was developed specifically to deal with the factors imposed by the non-conservative nature of chlorine and the normal tendency of most decapod larvae to move in a vertical plane. A two-layered flowing water column was produced in the apparatus with chlorine added to the upper layer. Chlorine "decays" rapidly and must be replenished constantly in order to maintain constant CPO levels throughout a test period. Larvae introduced into the lower CPO-free layer were induced to swim towards the upper chlorinated water layer by an overhead light source where they encountered the chlorinated water. Visually observed reactions of the larvae to the concentration discontinuity were recorded at regular intervals during each experiment.

The final design for the apparatus is shown in Figure 2. Rhodamine B and Fluorescene dyes were used to demonstrate that the two water layers remained discrete. Distribution of dye in the test

a – inflow port for water bath surrounding test chamber
b – outflow port for water bath surrounding test chamber
c – upper layer inflow port
d – lower layer inflow port
e – upper layer mixing chamber
f – upper layer diffuser plate
g – upper layer propagation chamber
h – diffuser plate for chambers g and n
i – diffuser plate for chambers j and o
j – upper layer outflow chamber
k – upper layer outflow port
l – lower layer mixing chamber
m – lower layer diffuser plate
n – lower layer propagation chamber
o – lower layer outflow chamber
p – lower layer outflow port
q – separator plate between outflow chambers

TOP VIEW
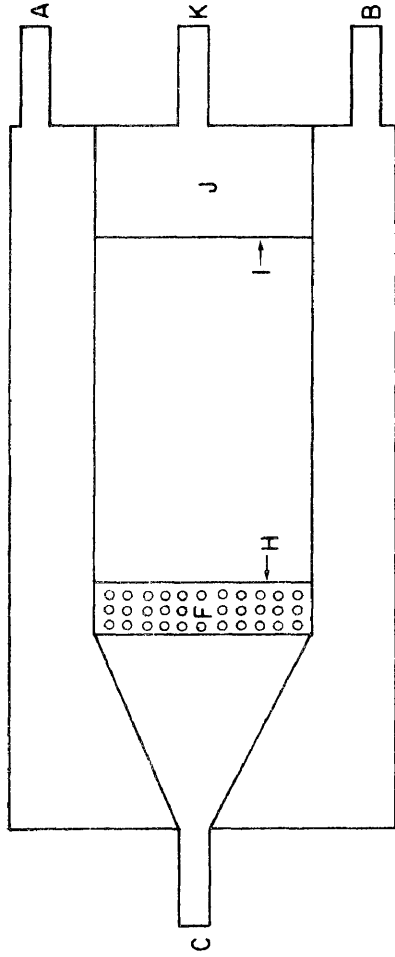
SIDE VIEW
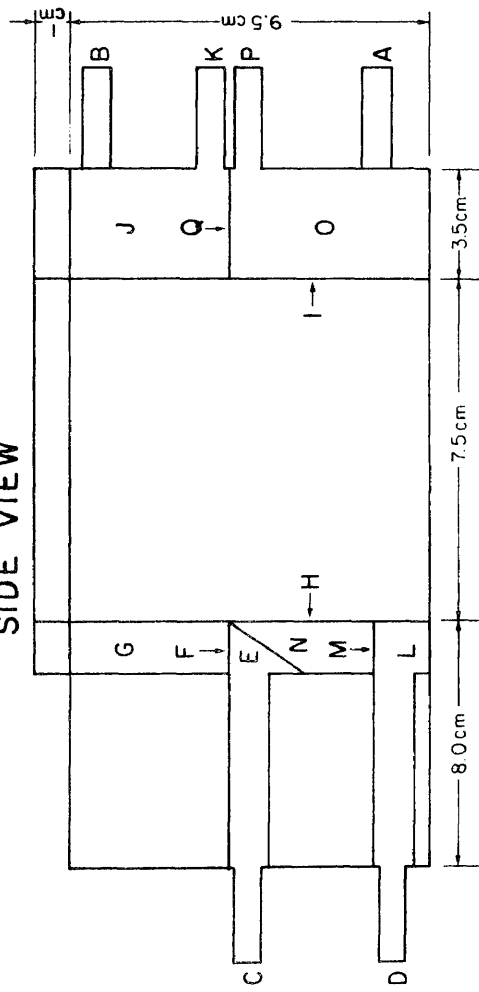
END VIEW
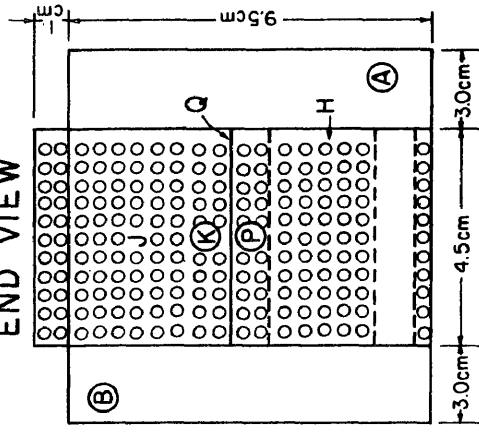
Figure 2. Apparatus developed to test avoidance behavior of crustacean larvae to chlorinated water layers.

chamber was observed for periods exceeding one hour with no significant mixing (Fig. 3). Visual observations were checked by analyzing water samples from each water layer using a Cary-14 scanning spectrophotometer to detect small amounts of dye.

Each test chamber (Fig. 2) was jacketed in a water bath to provide thermal insulation. Temperature control was found to be critical to maintenance of discrete water layers. Water entered the water bath through a port (a) at one end and exited through a port (b) at the same end after passing around the test chamber. Water entered the test chamber through two inflow ports (c and d), one for the upper layer and one for the lower layer. Incoming water to the upper layer passed from a chamber (e) through a diffuser plate (f) into a second chamber (g) and thence into the test chamber through a diffuser plate and nylon mesh screen (h, 254 µm mesh size). Incoming water to the lower layer followed a similar path from a chamber (1), through a diffuser plate (m) into another chamber (n) and then through a diffuser plate and nylon mesh screen (h). The layers flowed across the test chamber and out through a nylon mesh screen and diffuser plate (i) into chambers j (upper layer) and o (lower layer) and outlet ports (k) and (p) respectively. The bottom, back and top edges of the test chambers were painted dull black to prevent undesired scattering of light. The chamber backs were divided horizontally into quarters so that larval position could be accurately determined. Paralax error resulting from the fixed observation port was assumed to be insignificant although this was not tested.
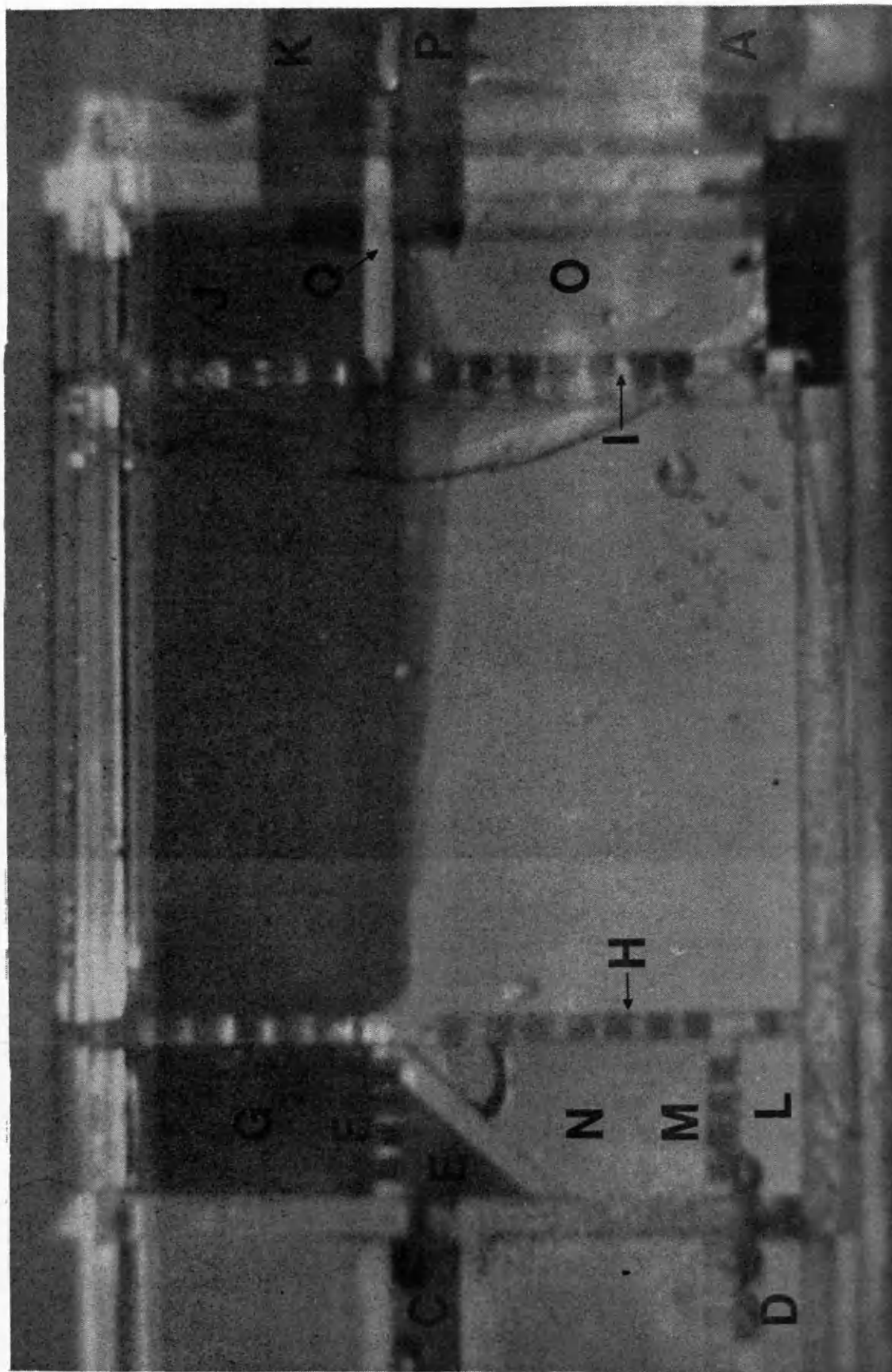
Figure 3. Avoidance apparatus after 80 minutes during dye testing confirming the discreteness of the two flowing water layers (see Fig. 2 for further clarification).

Two identical test chambers were enclosed in a light proof chamber which eliminated outside influences that might affect larval behavior (Fig. 4). Two 30 W fluorescent tubes (cool white or daylight white) provided light to attract larvae into the upper layers of the test chambers (a). Light was directed into the chambers (k) through light "tunnels" (b) to minimize reflections off the sides of the test chambers which might modify the orientation of the larvae. Neutral density filters (c) were placed over the tunnels to reduce light intensity when necessary. A black baffle (d) excluded light from the rest of the apparatus. The test chambers rested on a black platform (e) supported by four rods (f). A blower which circulated air within the enclosure to maintain temperature was attached beneath the platform (g) with a black flexible hose to eliminate vibration.

The enclosure cover was gasketed and all water lines entering the light-proof enclosure were covered with opaque tubing to exclude extraneous light. The enclosure cover was fitted with two observation ports (h) which were plugged when not in use.

Water Delivery System

Filtered (1 μm) estuarine water was delivered to the test chambers from a storage tank (a, Fig. 5). Salinity was adjusted when necessary to 20 O/oo salinity using well water or Instant Ocean® Sea Salts. The water was aerated and used within two days. Water was pumped (b) through a heat exchange water bath (c) into a header tank (d) which contained a heat sensor that controlled the heat exchanger.
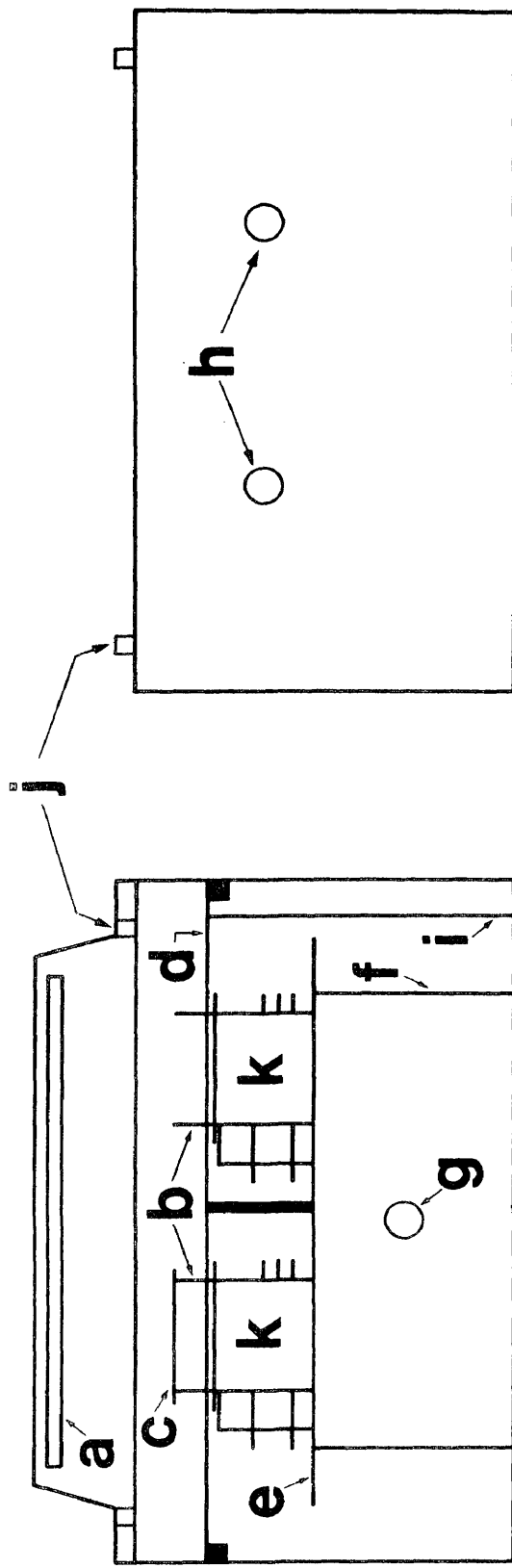
Figure 4. Light proof chamber in which test chambers were located.

a – 30 W fluorescent tubes
b – black plastic light "tunnels"
c – neutral density filter
d – black light baffle
e – platform supporting test chambers
f – support rods for platform

g – air blower opening
h – observation ports
i – lateral light baffle
j – screen window hanging fittings
k – testing chambers with tubing left off for
    clarity (see Fig. 2 and Fig. 5 (g and h) for
    tubing connectors)
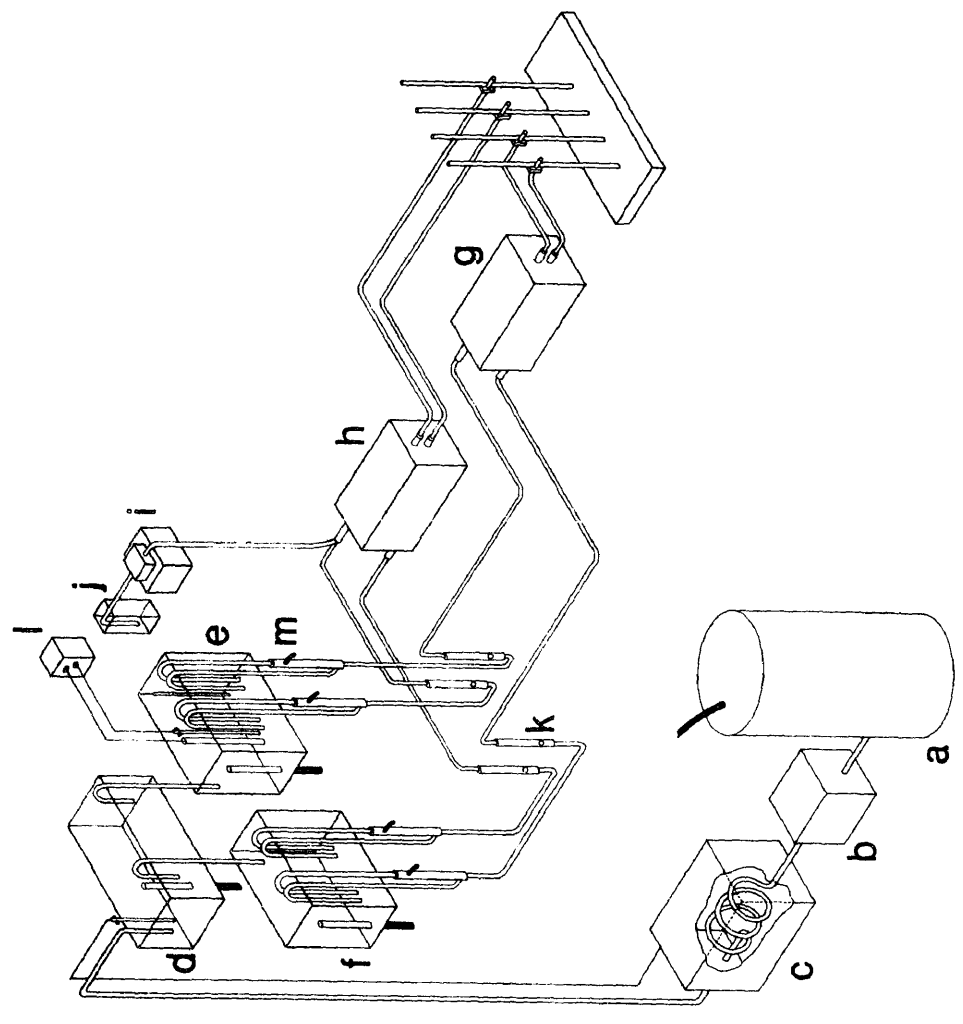
Figure 5. Water delivery system for testing chambers.

a – water storage tank
b – sealed greaseless impeller pump
c – heat exchange water bath
d – header tank containing sensor for water bath
e – upper water layer supply tank containing
    temperature sensor, heater and stirrer
f – lower water layer supply tank

g – control testing chamber
h – chlorinated testing chamber
i – peristaltic pump
j – calcium hypochlorite stock solution
k – flow meters
l – thermoregulation apparatus
m – water temperature maintenance tubing sleeves
n – laboratory tubes supporting water exit tubes

Excess water in the header tank was returned to the storage tank (a). This configuration not only conserved water during experiments but allowed water to circulate in a closed loop for 12 hours prior to experiments allowing accurate temperature adjustment ($\pm$ 0.25°C).

Water from header tank (d) flowed via siphons to two subsidiary header tanks (e and f). A thermoregulator sensor and Vycor® heater, both attached to controller (1), were immersed in header tank (e) which supplied water for the upper layers of the test chambers. The heater was used to raise the temperature of the upper water layer 0.5°C above temperature of the lower water layer which insured layer separation. Water in header tank (e) was stirred to insure a homogeneous temperature throughout the tank. Header tank (f) was identical to (e) except that it did not contain a heater or stirrer. Excess water entering tanks (e and f) exited through constant level standpipes and flowed back to storage tank (a).

Water supplying the upper and lower water layers of the test chambers flowed from their respective supply tanks through jacketed siphons and tubes (m) and through flow meters (k). Flow rates of water entering the test chambers were controlled by means of screw clamps located just outside the light-proof enclosure and by varying the relative heights of the water supply tanks (e and f). A stock solution of calcium hypochlorite was stored in a light-proof container (j). A peristaltic pump (i) injected the stock solution through a 10 gauge hypodermic needle located just before the water supply tubing entered the light-proof enclosure.

Inside the enclosure water flowed through the test chambers (h and g) and exited through tubes attached to laboratory stands (i). The outflow rates matched the inflow rates ($\approx$ 50 ml/min) which were controlled by adjusting the height of the exit tubes on the laboratory stands.

Measurement of Chlorine Concentration

The concentration of chlorine stock solutions was measured by the iodometric titration method described in APHA (1975). The chlorine concentration in both layers of the test chamber were measured by amperometric titration using a Sargent Welsh Model P Amperometric Titrator in conjunction with a rotating mercury-platinum electrode and a calomel reference electrode. A strip chart recorder (1 mv full scale) was used to amplify the signal during the titration which facilitated end point detection.

A 50 ml aliquot of water was used for each titration. One ml of 5% KI followed by 1 ml of pH4 buffer was added to the sample. The sample was immediately titrated with 0.005 N phenyl arsine oxide until no change in current was noted on the strip chart recorder (Standard Methods, 1975).

Protocols for Salinity and Light Intensity Tests

Three sets of experiments were conducted using stage 1 larvae of P. longicarpus. Experiments 1 through 4 were conducted to insure that larvae in both test chambers behave similarly when exposed to identical conditions of light, temperature and salinity. In

experiments 5 and 6 the response of the larvae under different intensities of light was measured. The light intensities used throughout the present experiments were 240 and 24 fc which are equivalent to 2583 and 258.3 Lumens m^{-2}, respectively. In experiments 7 through 12 the effect of salinity on the response of larvae was evaluated.

These tests were conducted under static conditions. Test chambers were rectangular with the same dimensions as the actual test chamber of the flow-through chambers described previously, placed in the same light box. Water temperature during all the tests was held constant at 24 ± 1°C.

Newly hatched larvae were placed in 20.3 cm finger bowls (≃100/bowl) at the appropriate salinities. Larvae were fed Artemia nauplii at a density of 15 to 20 per ml until two hours prior to testing. Larvae in clean water without food were dark-adapted for 2 hours prior to testing.

Thirty actively swimming larvae (1 day old) were introduced into the bottom of each test chamber through a canula. Larvae were observed for one hour. The number of larvae in the upper half of each chamber was recorded every five minutes. At the end of the experiment larvae were recovered, counted and examined for physical damage. Two experiments could be conducted in a day, one between 1030 and 1130 EST and one between 1345 and 1445 EST. Replicate experiments were not conducted on the same day and the treatments within experiments were alternated between the test chambers.

Protocol To Test Effects of Illumination and Feeding Regimes

Newly hatched Palaemonetes sp. larvae were normally positively phototactic in the horizontal, but not in the vertical plane. Light adaption and starvation seemed to induce larvae to become vertically photopositive. Several experiments were conducted to confirm this.

The two-layered chambers were used for these experiments. The testing procedures were identical to those used in chlorine tests described in the chlorine avoidance section (see page 28). Flow rates for the upper and lower water layers were approximately 54 ml/min. Salinity was adjusted to 20 ^{o}/oo and temperature was maintained at 24 $\pm$ 1°C. All tests were initiated between 0700-0800 to avoid possible effects of diurnal rhythms.

Approximately 12 hours before the test 50 larvae were transferred to each of two 10 cm finger bowls. Larvae in one bowl were fed Artemia, at a density of 20/ml, while larvae in the other bowl were not fed. Both bowls were placed under fluorescent lights until the experiment was started ($\approx$11 hours light adaption). Each experiment was conducted twice at each light intensity (240 and 24 fc). The effect of dark adaptation vs light adaptation was tested in a single experiment. The test procedure for dark vs light adaption was similar to the starvation experiments except all larvae were starved 12 hours prior to testing and one set of larvae was dark adapted for 12 hours, the other set light adapted for 12 hours to 240 fc.

Protocol for Chlorinated Water Tests

Water for each test was filtered to 1 μm, adjusted to a salinity of 20 ± 1 °/oo, aerated for 24 hours and kept at 24 ± 0.25°C for all experiments.

All experiments were initiated between 0800 and 1000 hours to eliminate effects of diurnal rhythms. At the start of each experiment the inflow rates were measured by flow meters which were calibrated by direct measurements with a graduated cylinder. The exit tube flow rates were adjusted to match the measured inflow rates for each water layer ± 1 ml. Flow rates for each water layer were approximately 55 ml/min.

When all flow rates were adjusted the siphons supplying the water baths surrounding the test chambers were started. The test system was operated for 15 minutes to allow the water temperature in the upper water layer to equilibrate. Chlorine was introduced into the upper layer of the test chamber and allowed to reach a constant concentration over 5-20 minutes. Constant CPO concentration was considered achieved when two consecutive concentration measurements (taken every 5 minutes) were equal. Chlorine concentration of the test water was measured in samples collected from the outflow ports. Concentrations tested were 1.0, 5.0 and 11.0 mg/l CPO. The retention time for the water in the test chambers was calculated to be 10 minutes based on inflow rates and the volume of the test chambers. Chlorine concentration of both water layers were measured at the beginning, middle and end of each experiment.

To start an experiment, 30 actively swimming stage 2 larvae of Palaemonetes sp. were transferred by pipette into each of two 10 ml beakers. Larvae were slowly poured into test and control chambers through a Nalgene® funnel extended with a glass tube to the bottom of each chamber. This method of introduction produced less mechanical damage to larvae than introduction through a canula.

Two minutes was allowed for acclimation before the first observation was recorded. The number of larvae in each horizontal quarter of test and control chambers were recorded every five minutes for one hour along with observations regarding type of behavior, especially telson flips, active swimming and passive sinking (Roberts, 1971b).

At the conclusion of the experiments the number of larvae recovered from each chamber was recorded, and in many cases, the larvae were staged to confirm that they were stage 2. After some experiments, the chambers were operated without chlorine infusion before the larvae were recovered. The recovered larvae were cultured to determine whether the brief chlorine exposure increased the mortality rate. Chlorine tests were replicated two to three times at each concentration and light intensity.

Data Analysis

In experiments to test the reactions of P. longicarpus larvae to salinity and light intensity, the proportion of larvae in the upper halves of the chambers were plotted over time. A Wilcoxon Sign Rank

Test (WSRT) (Sokal and Rohlf, 1969) was used to compare the differences in response of the larvae to each treatment. This method could not be used to compare replicates because the responses of the larvae were obviously different between replicate tests. An important assumption of the WSRT is that each observation is independent of all others. In these experiments the position of a larva at a specific time is dependent on its previous position. This method of analysis is not strictly appropriate but is the best statistical test found.

In experiments to examine the responses of larvae of <u>Palaemonetes</u> sp., histograms were constructed to show the detailed distribution of larvae in each vertical quarter of chambers over time. Larvae resting on the bottom of the chambers were not counted. Also shown on the histograms are the total number of larvae counted (to the left of each histogram) and the number of larvae recovered from each chamber (to the right).

Activity and orientation indices were used by Roberts (1971b) to describe larval behavior in tests in which larval distribution was the measure of response. Both indices are used here to analyze reactions to chlorinated water layers. The activity index (I_A), was used to determine the number of active swimming larvae in the test chambers over time. I_A is the number of larvae counted in all four quarters of the testing chambers divided by the number of larvae recovered from the chambers at the termination of the experiment multiplied by 100. The orientation index (I_0) devised by Lagerspetz and Mattila (1961) is defined by the expression:

$$I_O = \frac{N_U - N_L}{N_U + N_L} \times 100$$

where N_U = the number of larvae in the upper two quarters of the chambers

N_L = the number of larvae in the lower two quarters of the chambers

The orientation index has a range of +100 to -100. Preference for the upper half of the chamber is indicated by positive values (+100 indicates 100% preference) and avoidance of the upper half of the chamber is indicated by negative values.

I_A was plotted over time for each experiment. A least squares line was fitted to the data only to show general trends of larval activity and is not meant to imply that the activity trend is strictly linear. The indices, I_A and I_O, were plotted against each other in order to characterize the behavior of control and experimental larvae in tests. The numbers next to each point on the plots correspond to the sequential observation order. An observation was taken every 5 minutes over the course of each experiment.

Means of I_O and I_A for each experiment ($\bar{I}_O$ and $\bar{I}_A$) were compared between experiments. Interexperimental comparisons were also made by comparing the differences between test and control indices and are represented by $\Delta\bar{I}_O$ and $\Delta\bar{I}_A$ where:

$$\Delta\bar{I}_O = \bar{I}_O \text{ control} - \bar{I}_O \text{ test}$$

and

$$\Delta\bar{I}_A = \bar{I}_A \text{ control} - \bar{I}_A \text{ test}$$

Positive values of the $\Delta \bar{I}_O$ and $\Delta \bar{I}_A$ indices indicate a greater

larval response in the control treatment index than in the

corresponding test treatment index. The separation of points for test

and control treatments was confirmed by discriminant analysis

(Tatsuoka 1971). The Wilkes Lambda (Λ) portion of discriminant

analysis compares the relative distances of the centroids of two data

groups (test and control). The Chi squared (x^2) test associated with

(Λ) statistically determines the discreteness of two data groups.

Cumulative mortality curves were constructed for experiments in

which recovered larvae were cultured.

RESULTS

Effect of Test Chambers

Under all conditions tested, when light intensity and salinity were the same in both chambers there were no significant differences detected in the phototactic response of P. longicarpus larvae. Duplicate experiments conducted at 240 fc and 18 ^{o}/oo salinity showed no significant difference within experiments in overall phototactic response (α = 0.01) using the Wilcoxon Sign Rank Test (WSRT) (Table 1). A difference was detected between experiments in the response of larvae in the left hand chambers (WSRT = 4.1**), but not in the right hand chambers (WSRT = 19.4 ns). This demonstrates that the repeatability of the experiments is poor due to the difference in response between batches of larvae. There is no distinct temporal pattern of response of larvae over the 65 minute test period (Fig. 6A). An average of 60% of the larvae were in the upper half of the chambers throughout these experiments.

Duplicate experiments were also conducted at 24 fc and 18 ^{o}/oo salinity in both chambers. The results were similar to those of the 240 fc 18 ^{o}/oo salinity experiments. There was no significant difference between the responses of larvae in the two chambers within the experiments (Table 1). Highly significant differences were found

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Table 1. Wilcoxon Sign Rank Test Values for the Effects of Light
Intensity, Chamber Effects and Salinity on Stage 1
P. longicarpus Larvae. -

Exp.	Light Intensity (fc)	Temp (°C)	Salinity (⁰/oo)	Left or Right Chamber	WSRT[1]
1	240	24	18	L	19 ns
	240	24	18	R	
2	240	24	18	L	15 ns
	240	24	18	R	
3	24	24	18	L	12.5 ns
	24	24	18	R	
4	24	24	18	L	26.0 ns
	24	24	18	R	
5	240	24	18	R	0**
	24	24	18	L	
6	24	24	18	L	10.0 ns
	240	24	18	R	
7	240	24	18	L	0**
	240	24	24	R	
8	240	24	18	R	1.0**
	240	24	24	L	
9	240	24	18	R	24.0 ns
	240	24	24	L	
10	24	24	18	L	24.0 ns
	24	24	24	R	
11	24	24	18	R	1.0**
	24	24	24	L	
12	24	24	18	L	1.5**
	24	24	24	R	

[1]The significance level for all WSRT values as α = .05 is 12.

A

B

Figure 6. Percent of P. longicarpus stage 1 larvae in the upper half
of the test chambers versus time (min) when both chambers
were exposed to 240 fc in 18 O/oo salinity (A) and 24 fc in
18 O/oo salinity (B).

between experiments (WSRT = 0.0** for both chambers). As can be seen from Figure 6B, over 65% of the larvae were in the upper half of the chambers after 30 minutes in one experiment (with an increasing response over time) while in the duplicate experiment less than 55% of the larvae were in the upper chamber half.

Effect of Light Intensity

The percentage of larvae in the upper half of the test chambers was greater in the 24 fc 18 ^{o}/oo treatments than the 240 fc 18 ^{o}/oo treatments (Fig. 7). In experment 5 (Fig. 7) highly significant overall differences in the percentage of larvae in the upper chamber halves between light intensities were found (Table 1) with the 24 fc treatment producing a greater phototactic response than the 240 fc treatment. In experiment 6 there was a non-significant difference between percentage of larvae in the upper layer between 24 fc and 240 fc light intensities (Table 1). The percentage of the larvae in the upper layer in experiment 6 was greater than in experiment 5 (69 and 79% mean response for experiment 6, 46 and 23% for experiment 5). In experiment 6, the percentage of larvae in the upper layers in the 24 fc treatment and 240 fc treatment was almost equal at the beginning of the experiment. The response of larvae exposed to 240 fc was greater than that of larvae exposed to 24 fc for the first 37 minutes. After 37 minutes the percentage of larvae in the upper layer exposed to 24 fc surpassed that of larvae exposed to 240 fc and remained higher until the termination of the experiment.

Figure 7. Percent of P. longicarpus stage 1 larvae in the upper half of the test chambers versus time (min) when exposed to 240 fc and 24 fc in 18 °/oo salinity.

Effect of Salinity

Larvae did not respond to differences in salinity in a consistent manner. Three experiments (experiments 7, 8, and 9) were performed comparing the response to salinities of 18 o/oo and 24 o/oo at a light intensity of 240 fc. In two experiments (experiments 7 and 8) there were significant differences in the percentage of larvae in the upper layer (Table 1), with the 18 o/oo treatment producing a greater response (Fig. 8A). In experiment 9 there were no significant differences in larval responses to the treatments (Table 1). Although more larvae were in the upper layer when exposed to 18 o/oo than 24 o/oo salinity during the initial 38 minutes of the test, the reverse was true during the remainder of the test.

In experiments 10 through 12 larvae were tested at 18 o/oo and 24 o/oo under a 24 fc light intensity regime. Larvae in two experiments (experiments 11 and 12, Fig. 8B) exhibited significant differences in responses to salinity (Table 1) with the larvae exposed to 18 o/oo treatments having a greater percentage of larvae in the upper layer than those exposed to 24 o/oo salinity. The larvae in experiment 10 had a greater initial response in the 18 o/oo treatment but after 28 minutes the reverse was observed.

In an effort to more clearly characterize the response of P. longicarpus larvae to environmental conditions, results from all similar experiments were pooled by averaging the percentage of larvae in the upper layer for each 5 minute observation period (Fig. 9). No clear larval response to light intensity was seen. The larvae had a
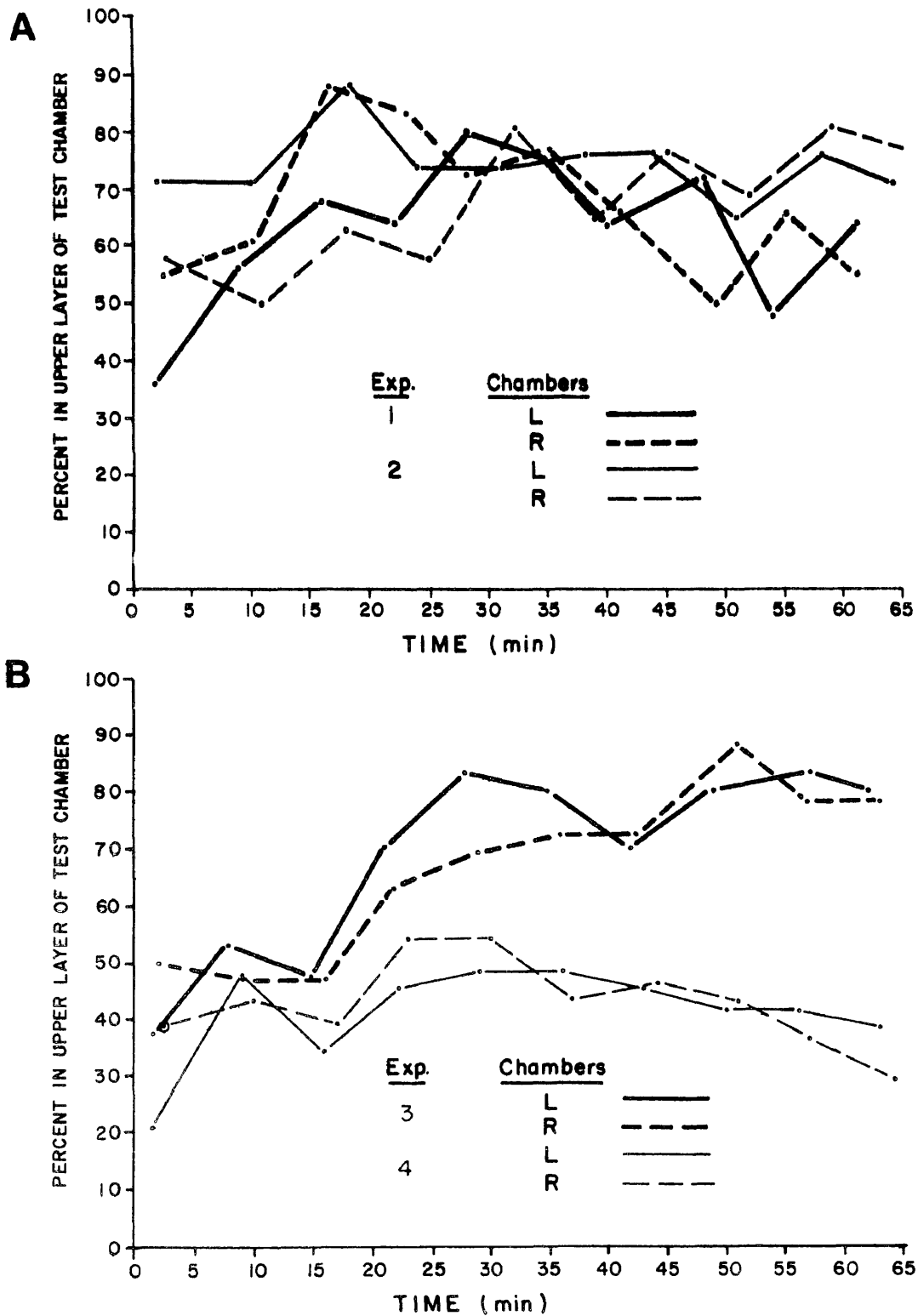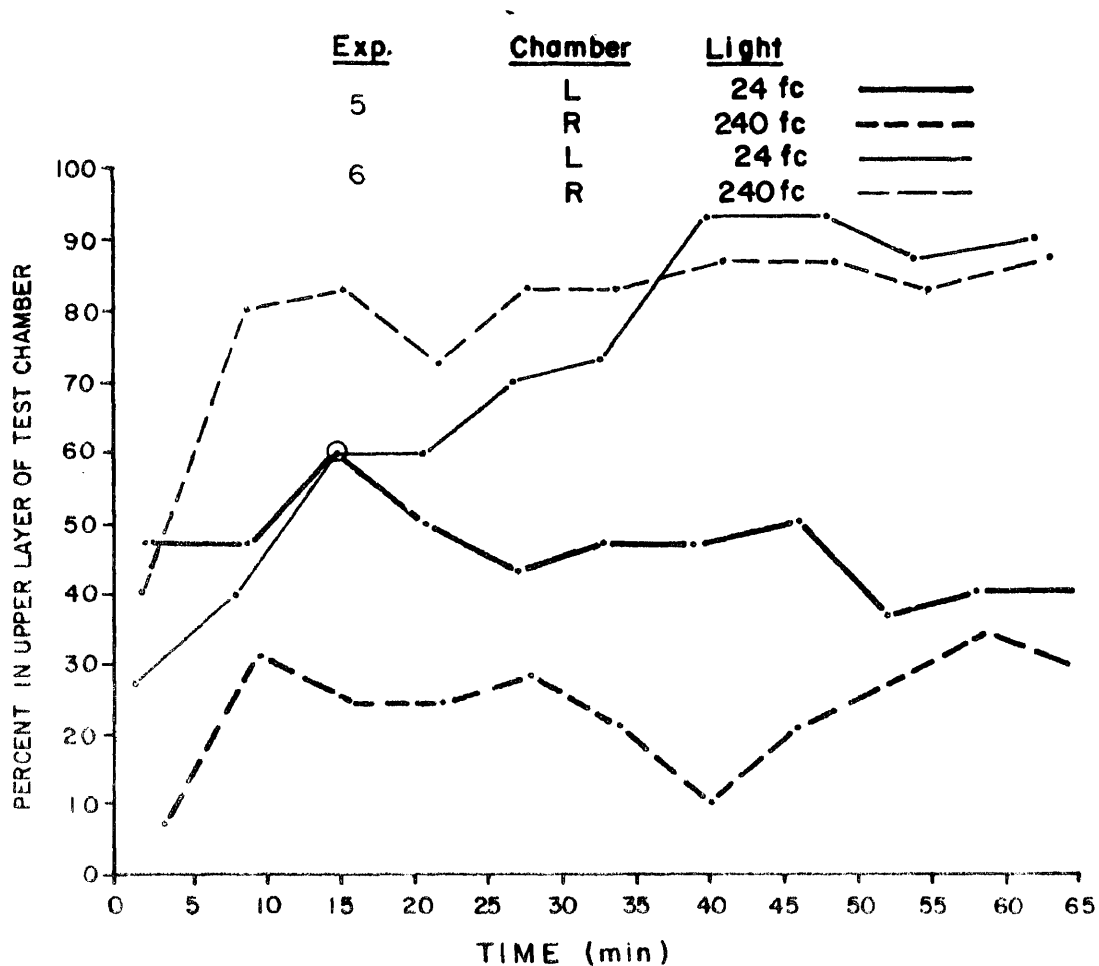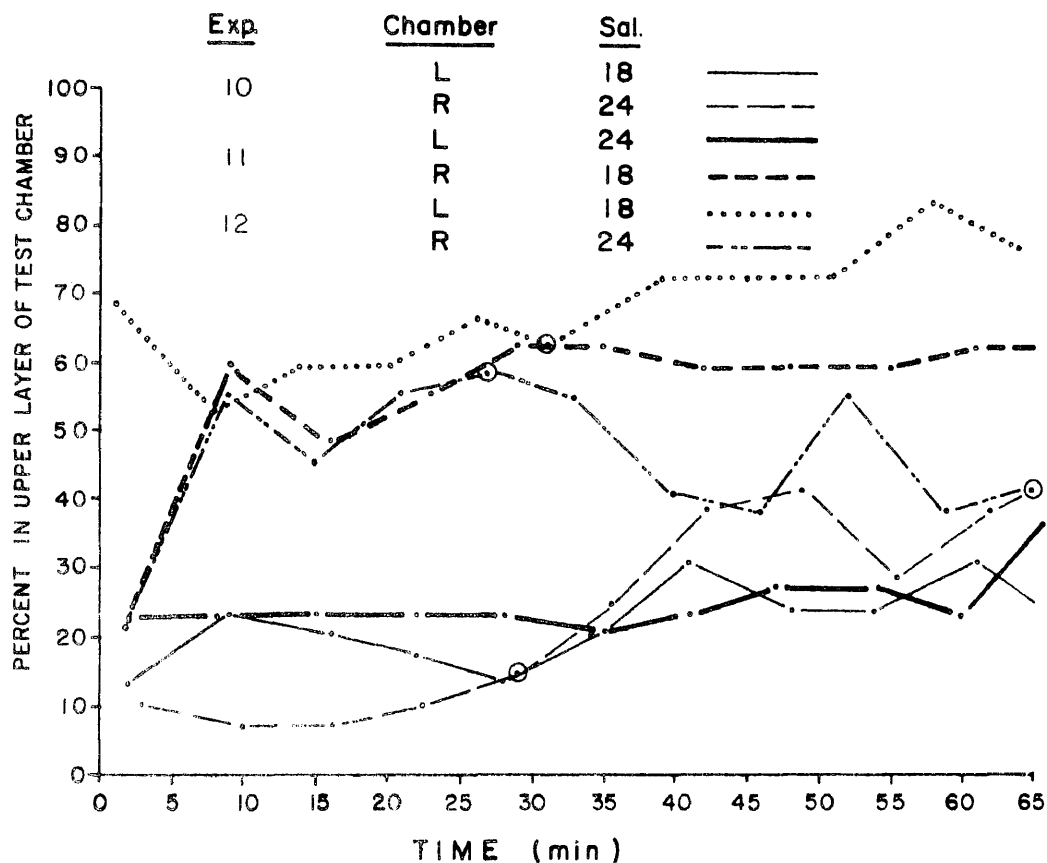
Figure 8. Percent of P. longicarpus stage 1 larvae in the upper half of the test chambers versus time (min) when exposed to 18 °/oo and 24 °/oo at 240 fc (A) and 24 fc (B).
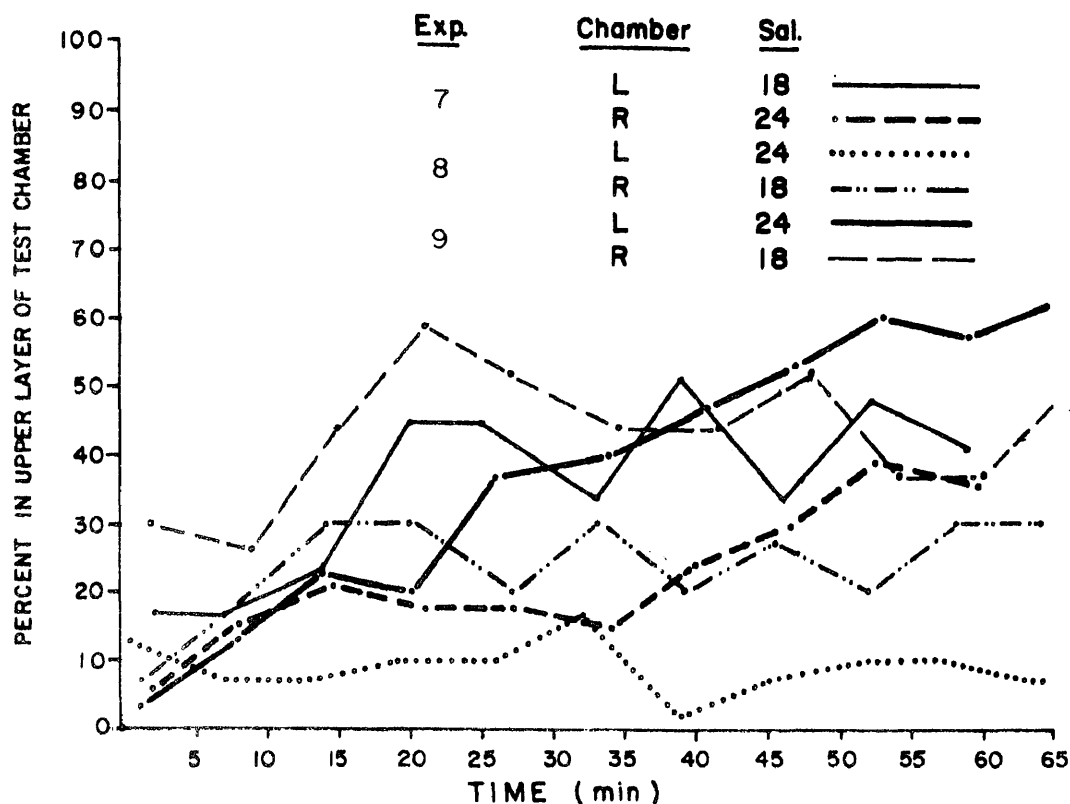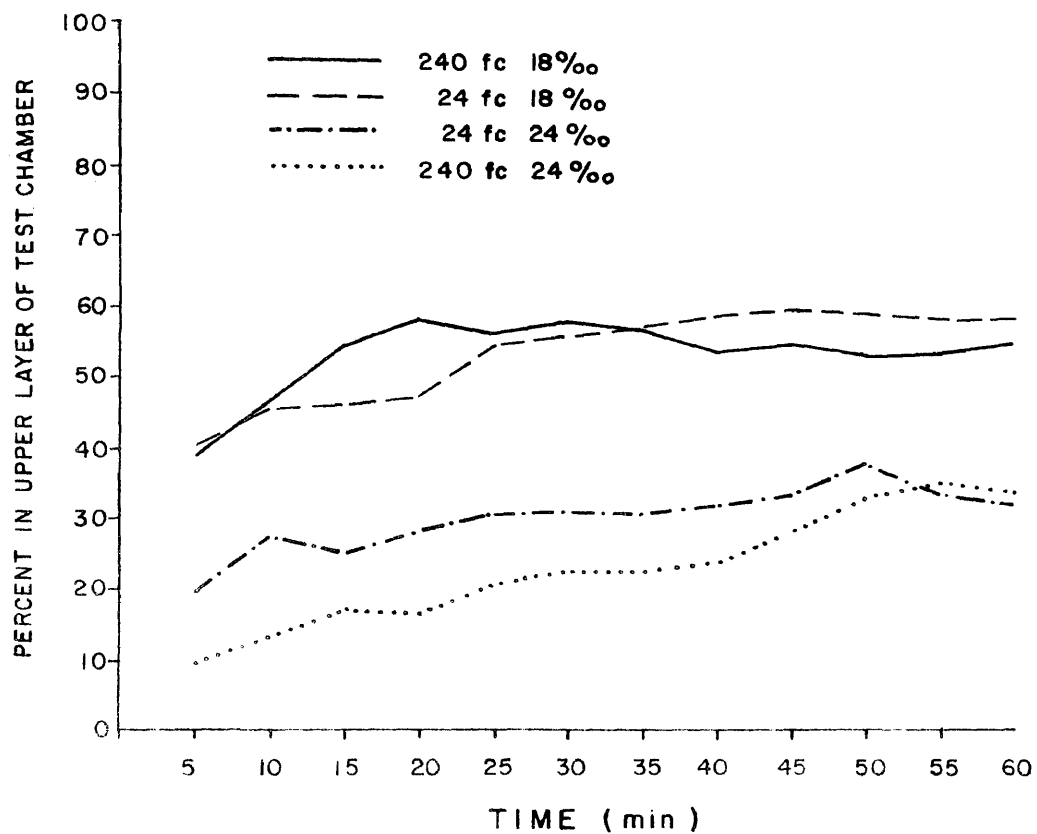
Figure 9. Mean percent of P. longicarpus stage 1 larvae in the upper
half of the test chambers versus time (min) for all
experiments at each light intensity-salinity combination.

greater initial response at 240 fc, 18 °/oo and 24 fc, 24 °/oo but larvae in 24 fc, 18 °/oo and 240 fc, 24 °/oo, respectively, became more photopositive after a period of time.

Larvae were more strongly photopositive at 18 °/oo than 24 °/oo regardless of light intensity.

The effect of time of day on larval response was also examined. Responses to the treatments 240 fc, 18 °/oo and 24 fc, 18 °/oo were analyzed for temporal effects. Tests were always started between 1030 to 1100 hours (AM) or 1330 to 1400 hours (PM). The larvae in the 240 fc, 18 °/oo treatments showed a greater overall response, in the AM (60.0%) as opposed to PM (46.5%) while the larvae in the 24 fc, 18 °/oo treatments showed the reverse (AM=43.3%, PM=60.5%).

Effect of Feeding

In preliminary experiments, Palaemonetes sp. larvae did not respond to vertical photostimulation. Changes in light intensity, wavelength, salinity, larval stage and water quality did not induce positive phototropism in the vertical plane. In several extended tests (≈24 hours) it was noticed that the larvae gradually become photopositive. It was hypothesized that food deprivation was the main reason for this behavioral change. Tests were conducted to confirm this hypothesis. At both light intensities, unfed larvae demonstrated a greater photopositive response than fed larvae. More unfed larvae than fed larvae entered the upper half of the water column at 240 fc (Fig. 10) than 24 fc (Fig. 11). Most larvae in the upper half of the
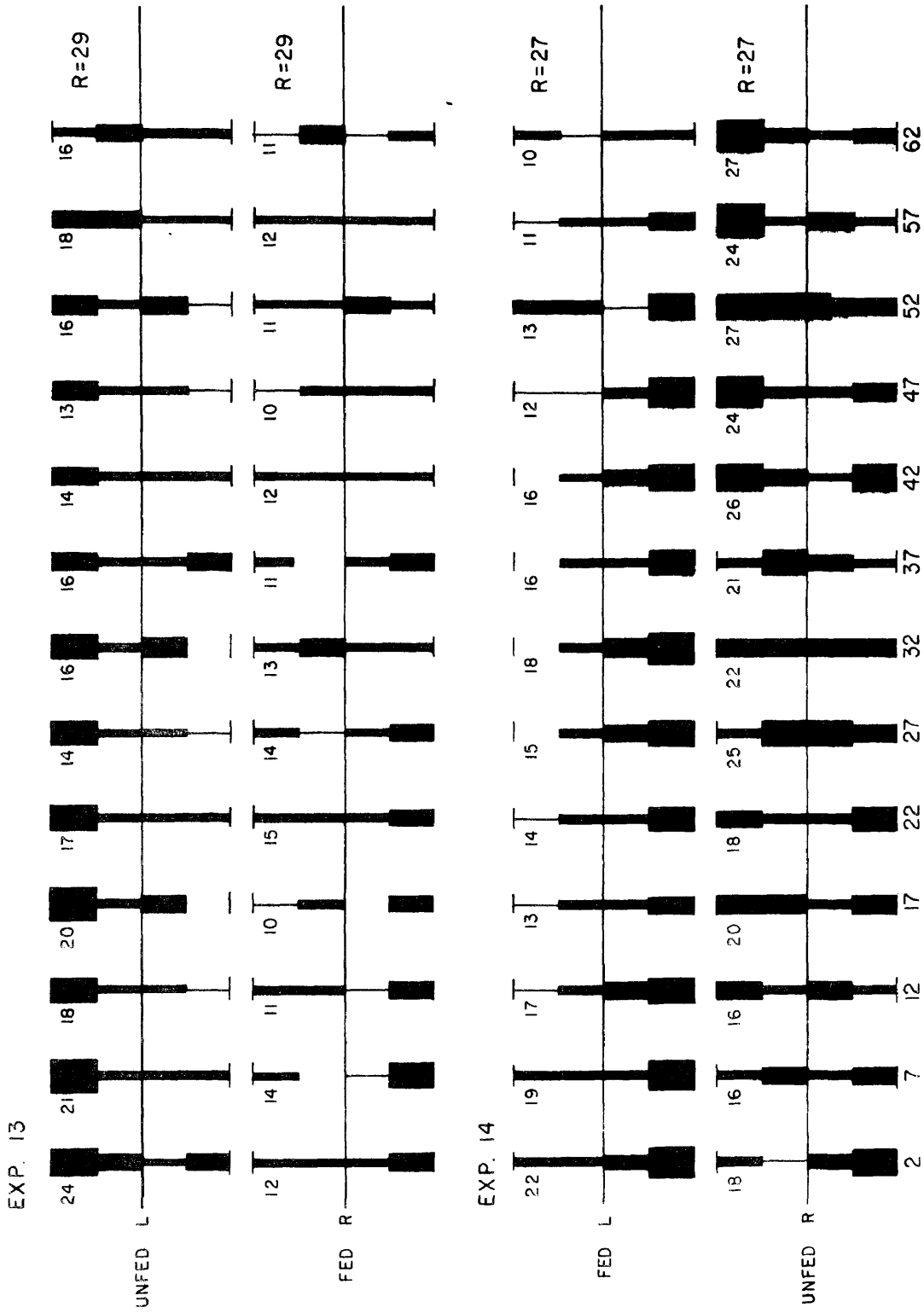
Figure 10. Distribution of fed and unfed stage 2 Palaemonetes sp. larvae at 240 fc.
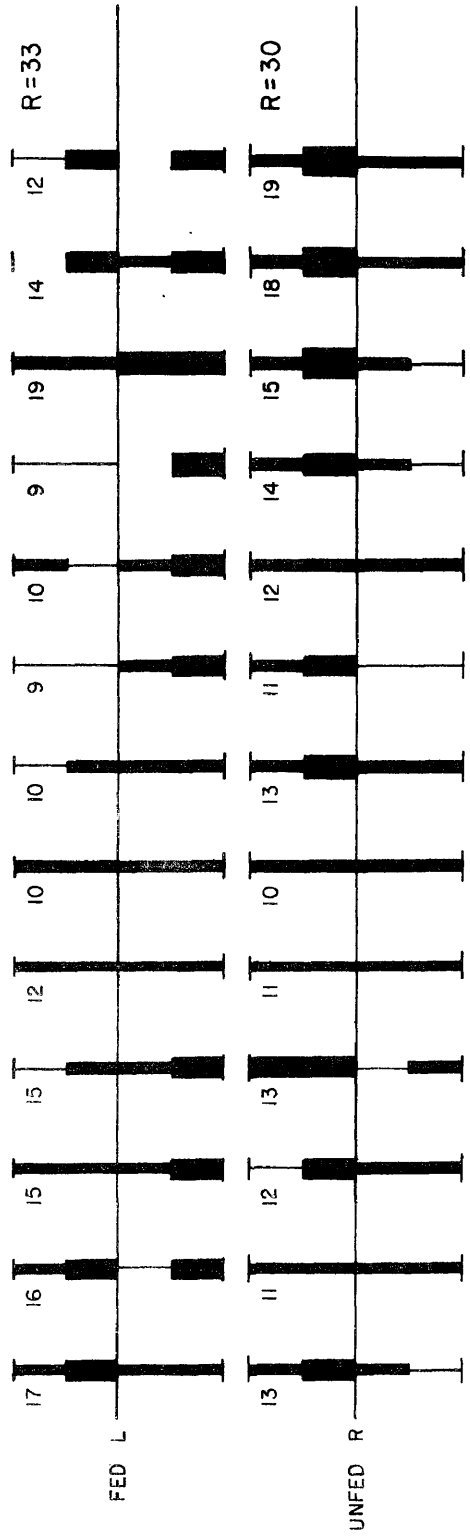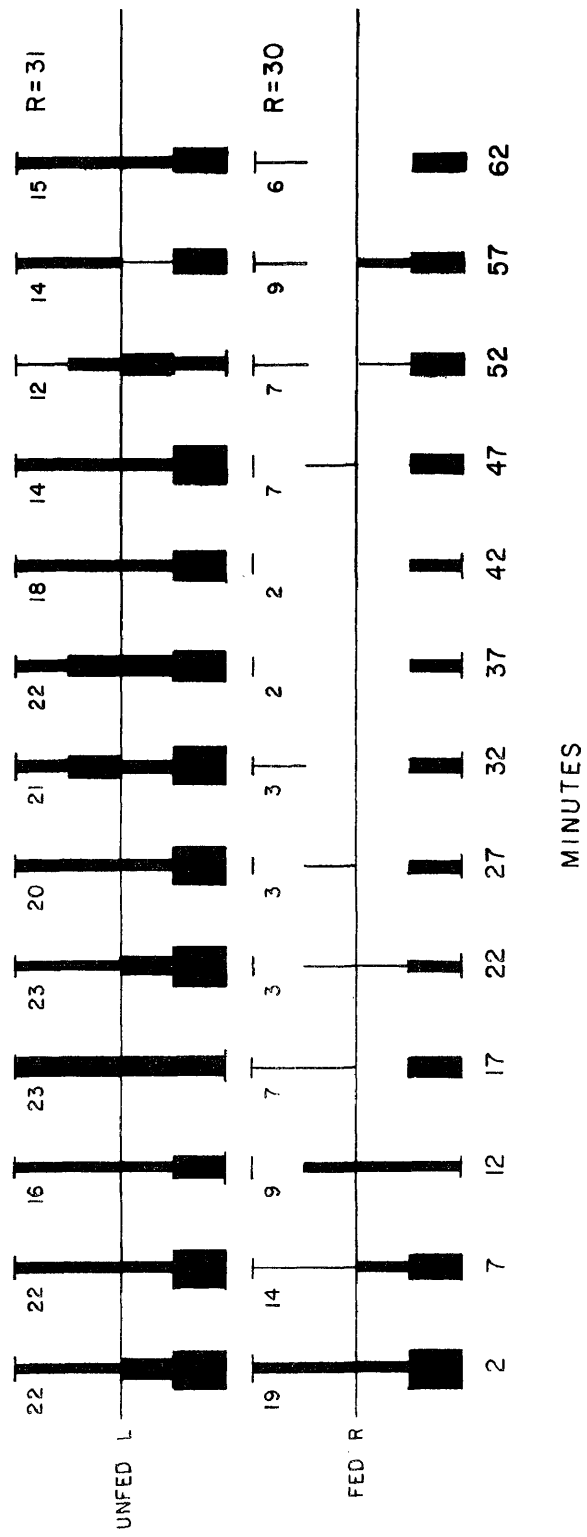
Figure 11. Distribution of fed and unfed stage 2 _Palaemonetes_ sp. larvae at 24 fc.

chamber at 240 fc were concentrated in the upper quarter while at 24 fc the larvae were more evenly distributed throughout the upper half of the test chamber. There was no consistent relationship in the precentage of unfed larvae active with time at either light intensity (Fig. 12). In experiments 13 (240 fc) and 16 (24 fc) the activity of unfed larvae decreased over time while in experiments 14 (240 fc) and 15 (24 fc) the activity of unfed larvae increased over time. The activity of fed larvae decreased over time in all experiments. At 240 fc the activity of unfed larvae was always greater than that of fed larvae after approximately 11 minutes (Fig. 12A) and at 24 fc the same is true after 23 minutes (Fig. 12B).

Unfed larvae occupied a higher position in the chambers than fed larvae as shown in the plots of I_0 vs I_A (Fig. 13). The differences in mean orientation indices ($\Delta \bar{I}_0 = \bar{I}_0$ unfed - $\bar{I}_0$ fed) were 52.3 (experiment 13) and 55.5 (experiment 14) at 240 fc and 51.8 (experiment 15) and 16.4 (experiment 16) at 24 fc. The difference in the response of the larvae in experiment 16 is probably not significant when considering just I_0 values but because of the $\bar{I}_A$ differences the two treatments are graphically separated very well. The $\bar{I}_A$ values were 17.8 (experiment 13) and 27.4 (experiment 14) at 240 fc compared to 5.4 (experiment 15) and 38.0 (experiment 16) at 24 fc. No consistent effects of light intensity on the response of the larvae was found. The Chi-squared (x^2) values (Table 2) confirm that the responses of fed and unfed larvae were signifiantly different in all experiments.
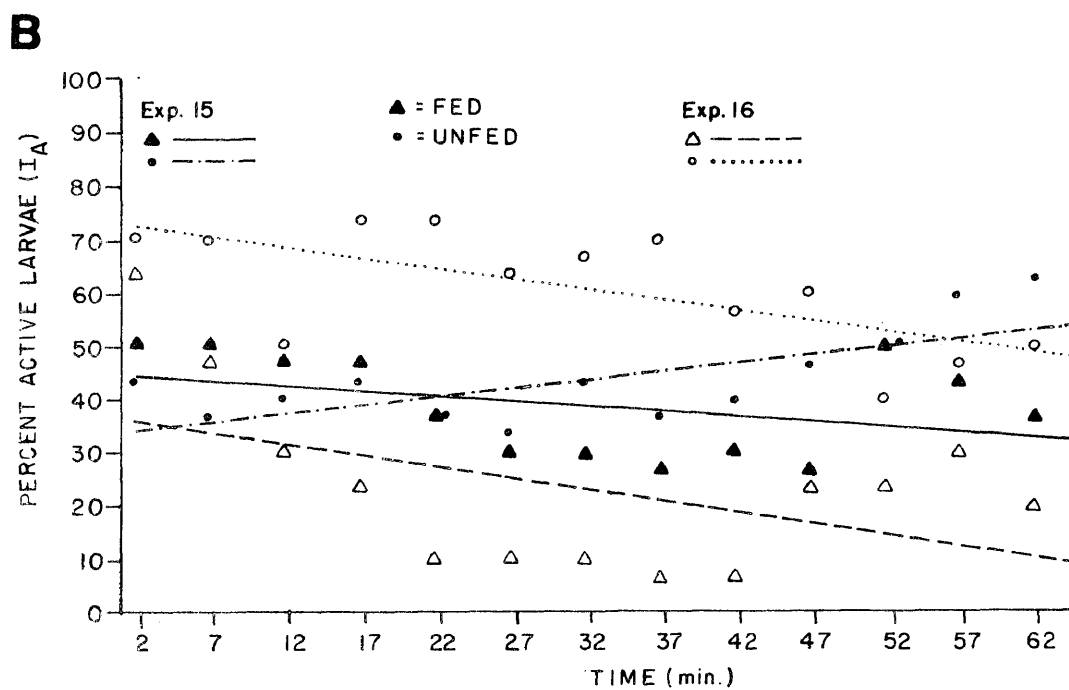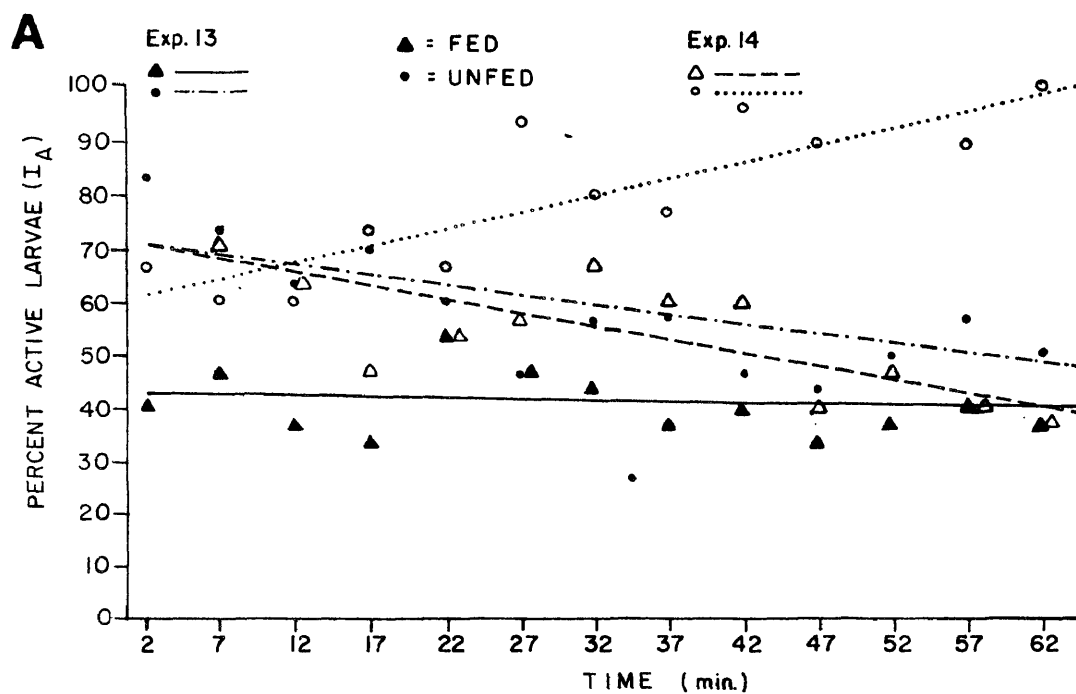
Figure 12. Percent of larvae active over time for fed and unfed stage
2 <u>Palaemonetes</u> sp. larvae at 24° (A) and 24 fc (B).
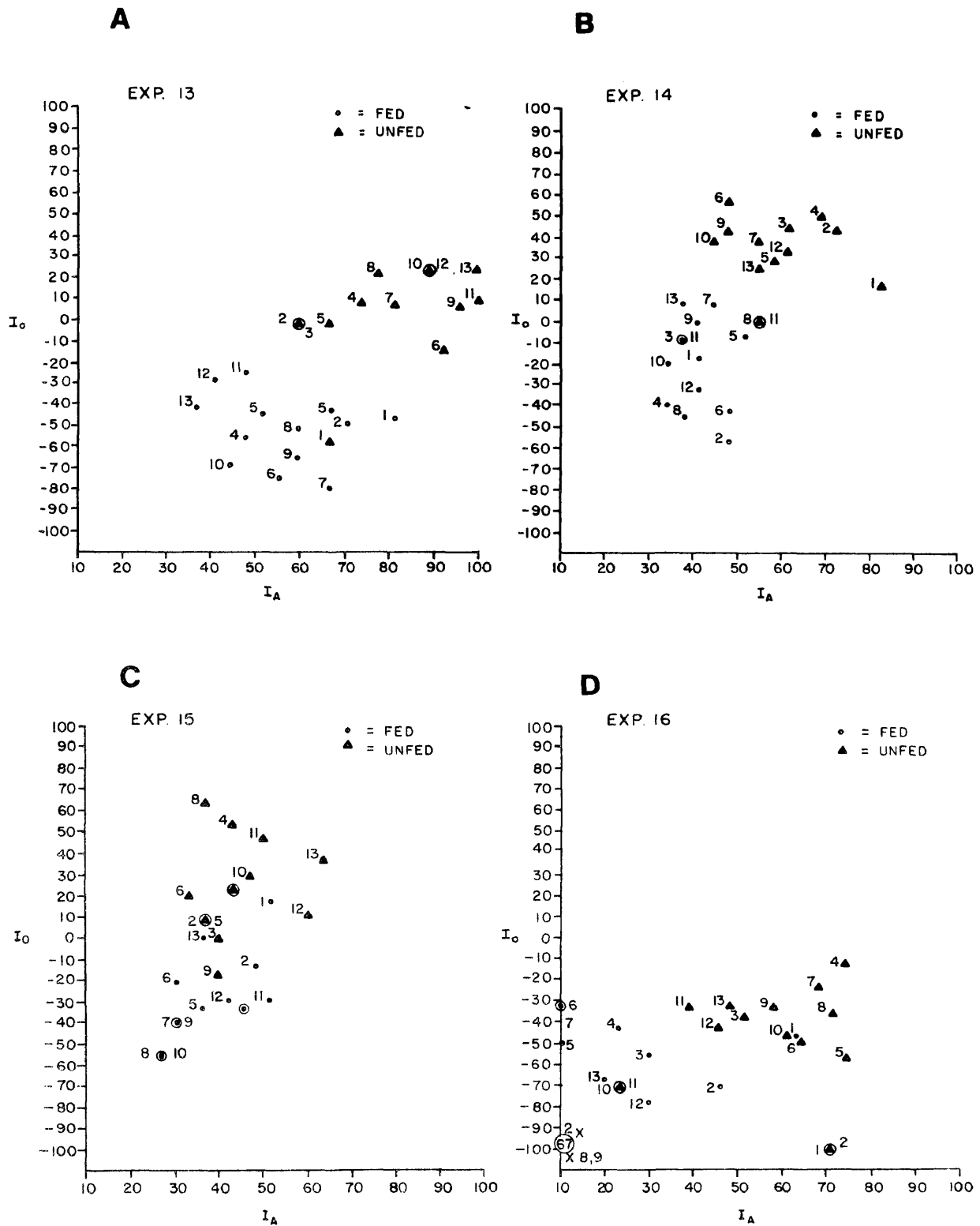
Figure 13. I_O vs I_A plots of unfed and fed stage 2 Palaemonetes sp. larvae under 240 fc (A,B) and 24 fc (C,D).

Table 2.   Wilkes Lambda (Λ) and x^2 Values for Stage 2
<u>Palaemonetes</u> sp. Larvae Experiments $(x^2\alpha = 0.001)$.

Exp.	Mean Dose (mg/l CPO)	L_0(fc)	(Λ)	(x^2)	Df
13	FVU*	240	.227	34.13	2
14	FVU	240	.268	30.30	2
15	FVU	24	.389	22.17	1
16	FVU	24	.312	26.77	2
17	LVD**	240	.385	22.44	1
18	0.886	240	.265	30.56	2
19	1.17	240	.082	58.75	1
20	1.32	24	.260	31.65	1
21	1.37	24	.309	27.02	2
22	4.29	240	.545	13.97	2
23	4.78	240	.178	39.66	2
24	5.77	24	.184	38.96	2
25	6.41	24	.396	21.79	1
26	6.78	24	.216	36.05	1
27	11.54	240	.095	54.08	2
28	12.43	240	.102	52.45	2
29	11.61	24	.201	36.87	2
30	12.07	24	.347	24.36	2

* FVU = Fed versus unfed.
**LVD = Light versus dark adapted.

Effect of Light Adaptation

Based on a single experiment it appears that dark adapted starved larvae are not as photopositive as light adapted starved larvae during a one hour test period (Fig. 14).

The number of larvae active over time is shown in Figure 15A. The activity of dark adapted larvae decreased over time while the activity of light adapted larvae increased slightly over time.

The plot of I_0 vs I_A (Fig. 15B) shows that light adapted larvae were more photopositive and more active than dark adapted larvae. The $\Delta \bar{I}_0$ ($\Delta \bar{I}_0 = \bar{I}_0$ light adapted - $\bar{I}_0$ dark adapted) was 60.6 and the $\Delta \bar{I}_A$ ($\Delta \bar{I}_A = \bar{I}_A$ light adapted - $\bar{I}_0$ dark adapted) was 16.4 between the two treatment groups. The responses of light adapted and dark adapted larvae were significantly different as indicated by the x^2 value in Table 2.

Effect of Chlorinated Water

Representative histograms of the responses of larvae to chlorine and light intensity (Figs. 16, 17 and 18) show that more control larvae were in the upper half of the test chambers than larvae exposed to chlorinated water. This difference was more pronounced at 240 fc than at 24 fc for any given CPO concentration.

The decrease in activity of larvae in the chambers containing the chlorinated water layers over time is shown in Figures 19, 20 and 21. At the lowest concentration tested (1.0 mg/l CPO) at each light
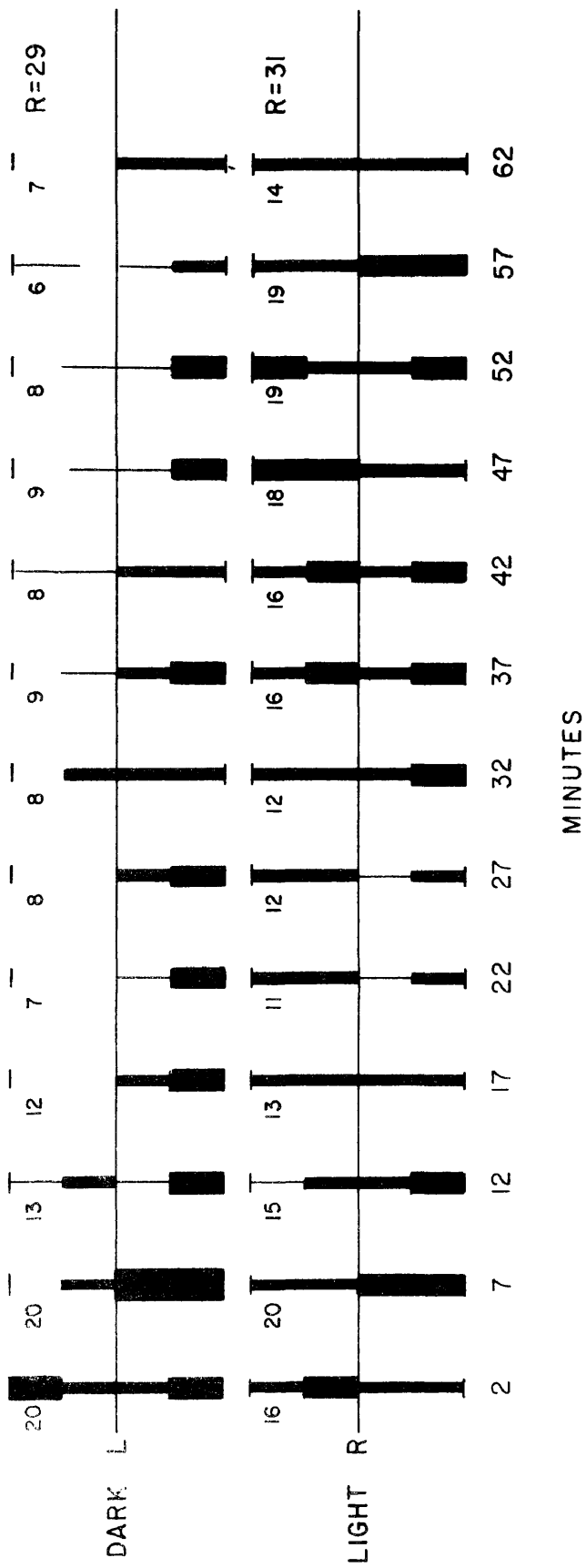
Figure 14. Distribution of light and dark adapted stage 2 Palaemonetes sp. larvae at 240 fc.
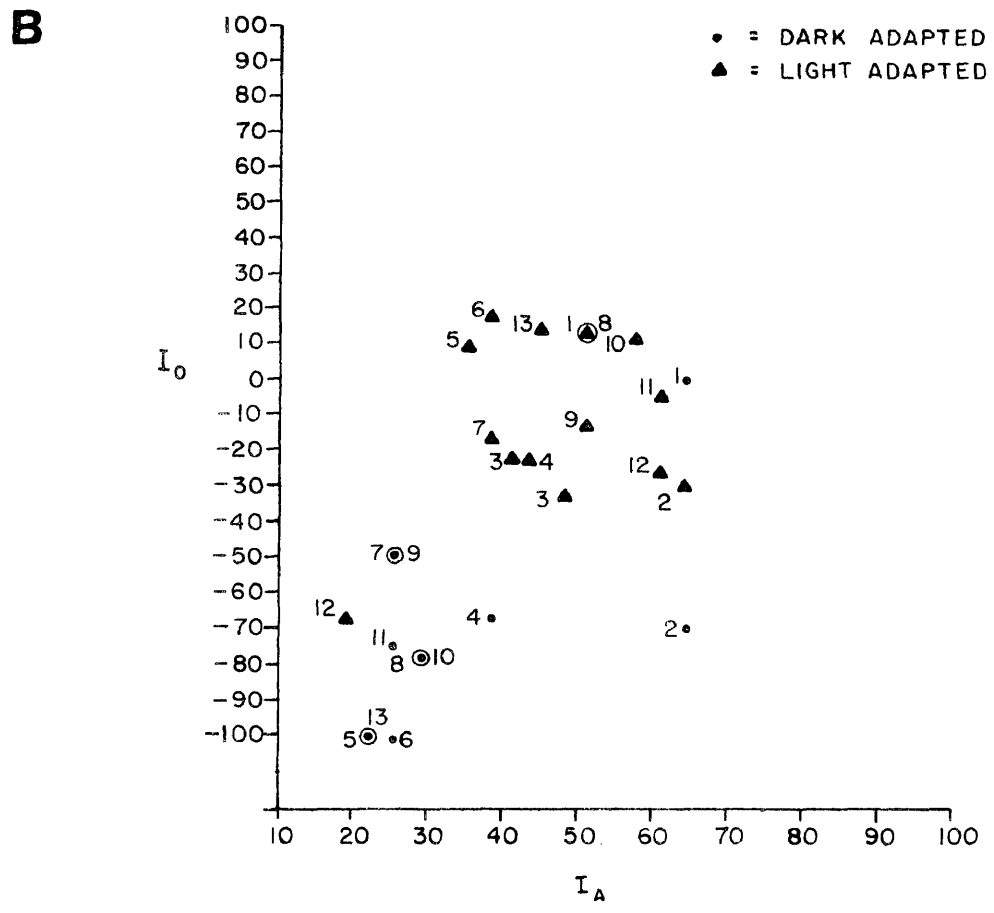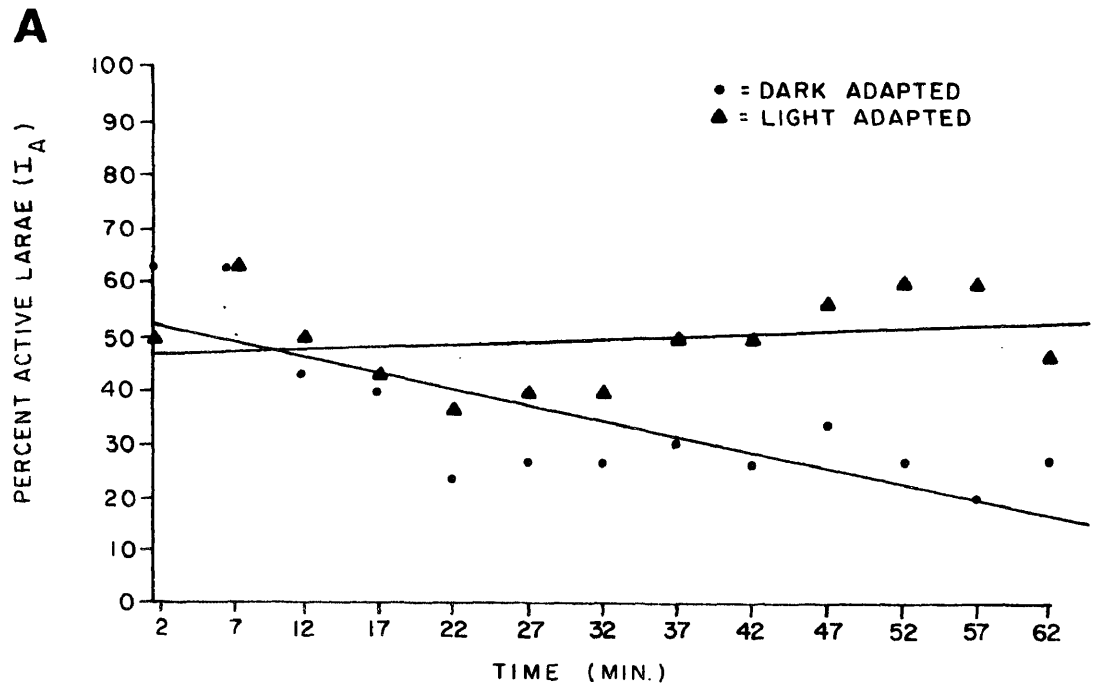
Figure 15. Percent of larvae active over time (A) and I_O vs I_A plot (B) for light and dark adapted stage 2 <u>Palaemonetes</u> sp. larvae under 240 fc.
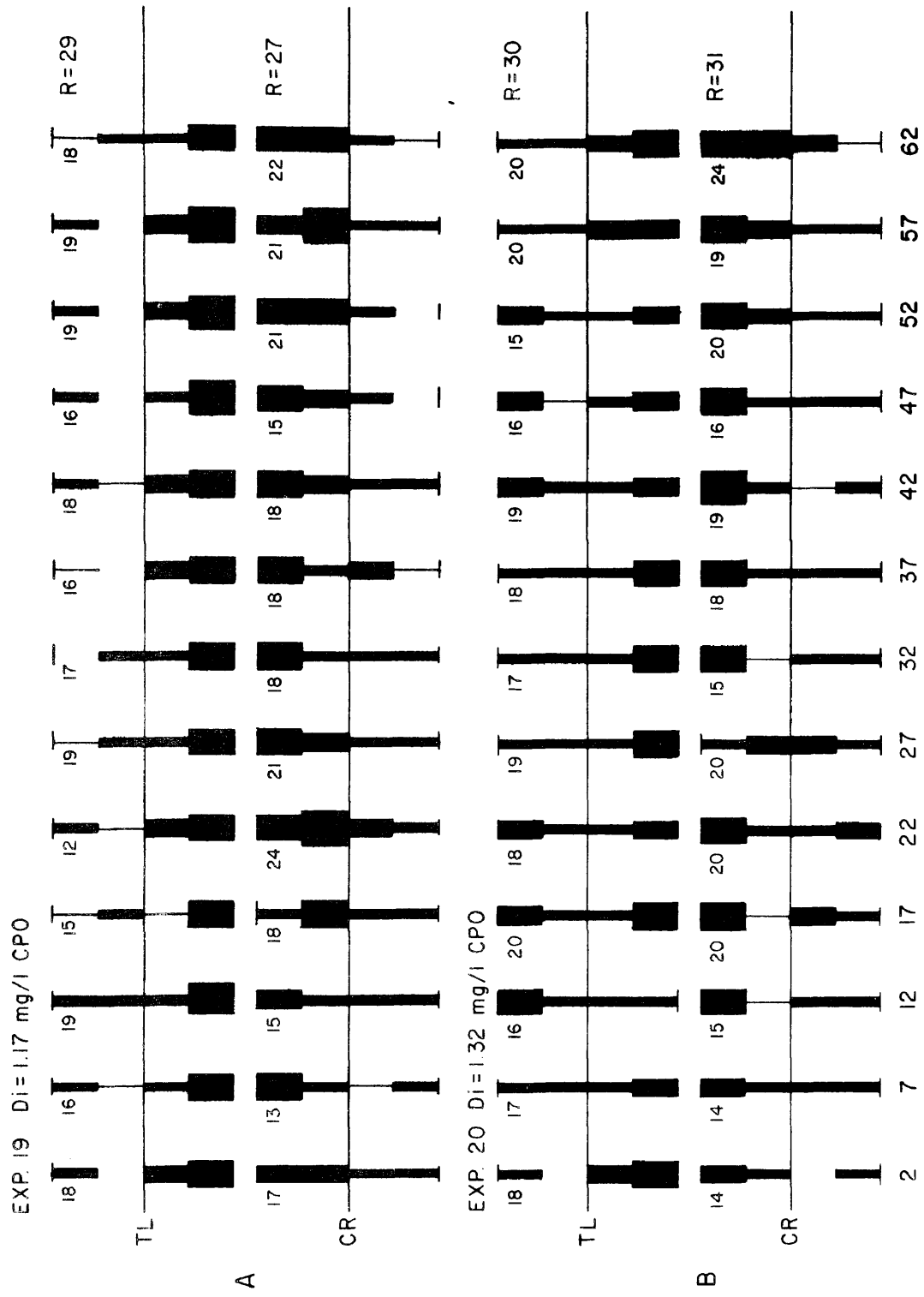
Figure 16. Distribution of representative chlorinated water layer tested (T) and control (C) stage 2 Palaemonetes sp. larvae at $\approx$ 1.0 mg/l CPO under 240 fc (A) and 24 (fc) (B).

Figure 17. Distribution of representative chlorinated water layer tested (T) and control (C) stage 2 Palaemonetes sp. larvae at ≈ 5 mg/l CPO under 240 fc (A) and 24 (fc) (B).
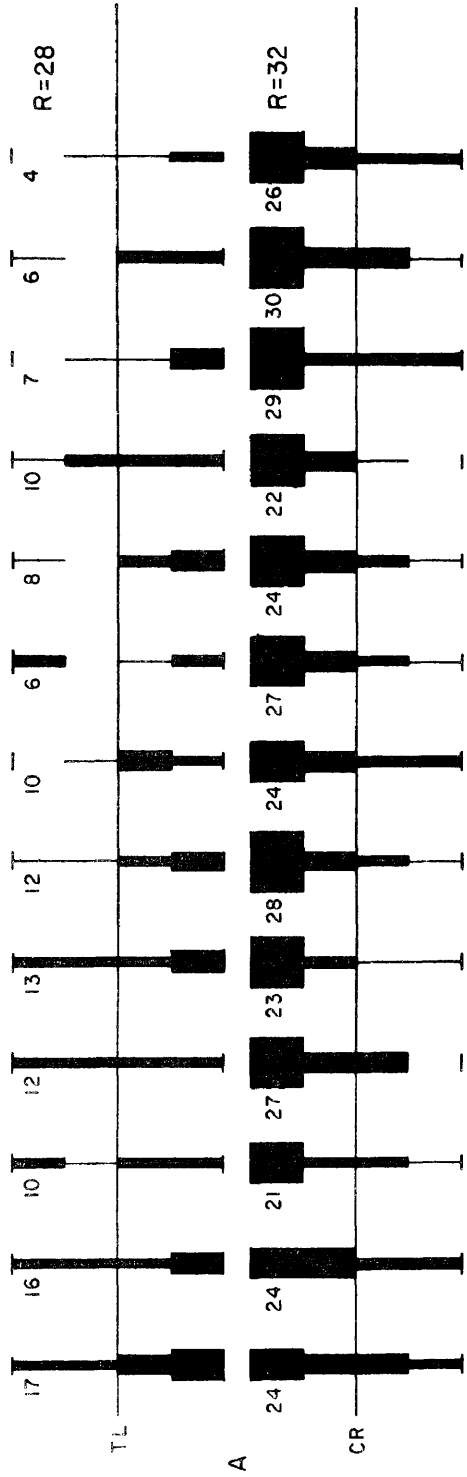
Figure 18. Distribution of representative chlorinated water layer tested (T) and control (C) stage 2 Palaemonetes sp. larvae at ≈ 12 mg/l CPO under 240 fc (A) and 24 (fc) (B).

A

Exp. 18 $\bar{D}i$ = 0.886 mg / l. CPO

▲ ——————
● —·—·—·—

Exp.19 $\bar{D}i$ = 1.17 mg/l.CPO

△ — — — —
○ ·············

PERCENT ACTIVE LARVAE (I_A)

TIME (min)

B

Exp. 20 $\bar{D}i$ = 1.32 mg / l. cpo

▲ ——————
● —·—·—·—

Exp. 21 $\bar{D}i$ = 1.37 mg/l.CPO

△ — — — —
○ ·············

PERCENT ACTIVE LARVAE (I_A)

TIME (min)
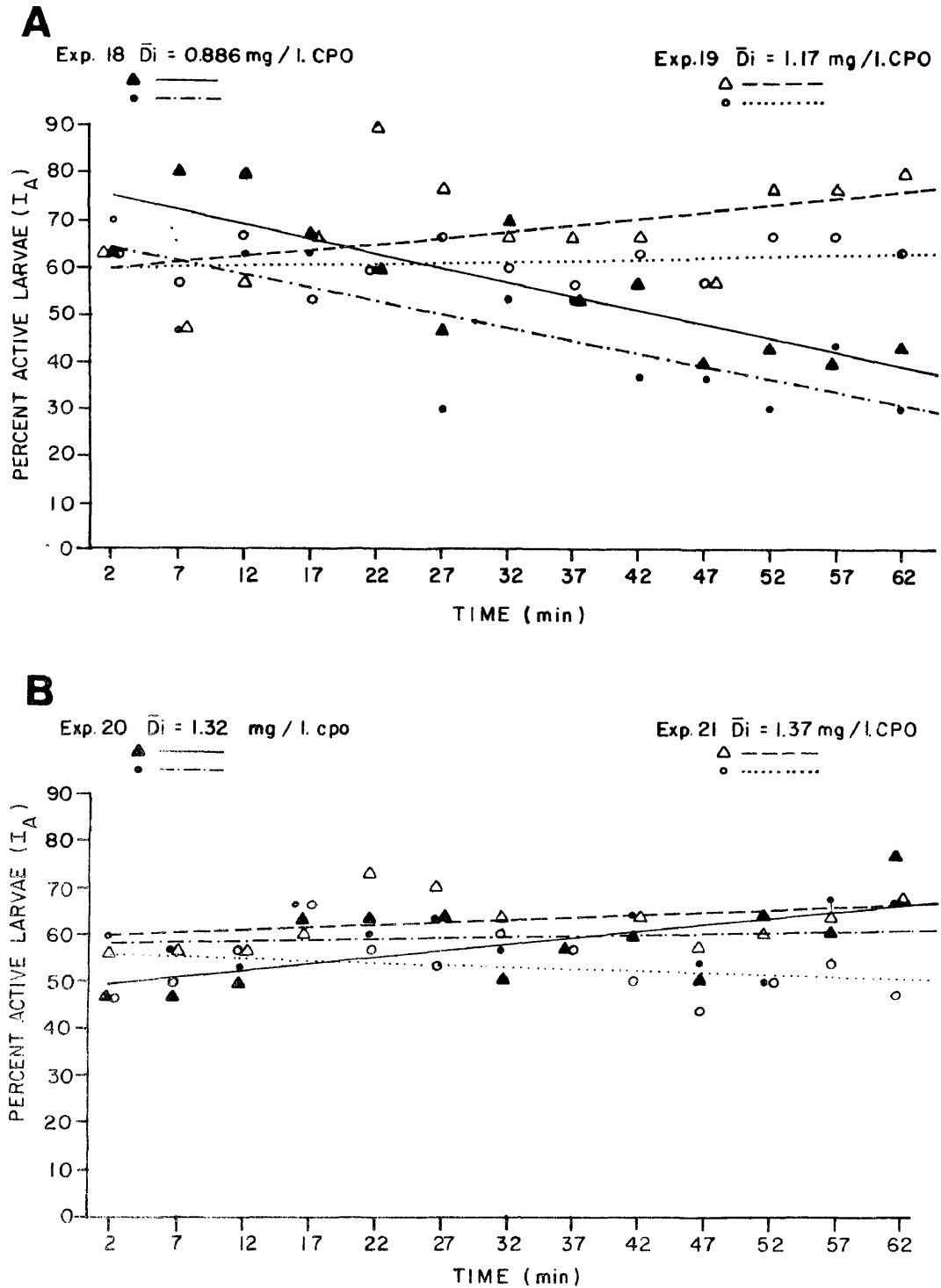
Figure 19. Percent of larvae active over time for test (dots) and control (triangles) stage 1 <u>Palaemonetes</u> sp. larvae at ≈1.0 mg/l CPO and 240 fc (A) and 24 fc (B).

A

Exp. 22 $\bar{D}i$ = 4.29 mg/l. CPO   Exp. 23 $\bar{D}i$ = 4.78 mg/l. CPO

PERCENT ACTIVE LARVAE (I_A)

80
70
60
50
40
30
20
10
0

TIME (min)

2 7 12 17 22 27 32 37 42 47 52 57 62

B

Exp. 24 $\bar{D}i$ = 5.77   Exp. 25 $\bar{D}i$ = 6.41

PERCENT ACTIVE LARVAE (I_A)

70
60
50
40
30
20
10
0

TIME (min)

2 7 12 17 22 27 32 37 42 47 52 57 62

C

90
80
70
60
50
40
30
20
10
0

Exp. 26 $\bar{D}i$ = 6.78 mg/l. CPO

PERCENT ACTIVE LARVAE (I_A)

TIME (min.)

2 7 12 17 22 27 32 37 42 47 52 57 62
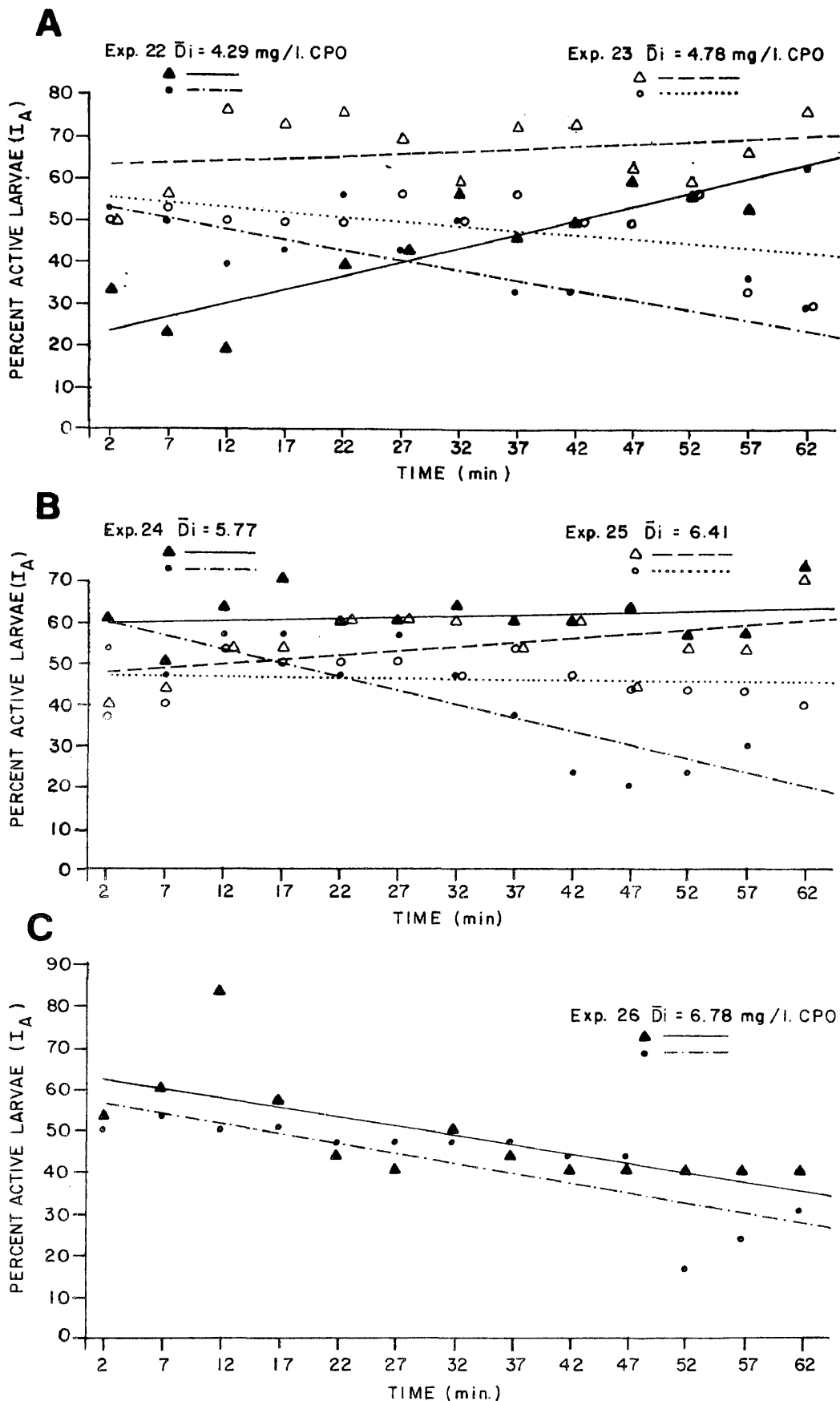
Figure 20. Percent of larvae active over time for test (dots) and control (triangles) stage 1 <u>Palaemonetes</u> sp. larvae at ≈5.0 mg/l CPO and 240 fc (A) and 24 fc (B,C).

A

Exp. 27 $\bar{D}i$ = 11.54mg / l. CPO

Exp. 28 $\bar{D}i$ = 12.43 mg /l. CPO

B

Exp. 29 $\bar{D}i$ = 11.61 mg/l. CPO

Exp 30 $\bar{D}i$ = 12.07 mg/l. CPO
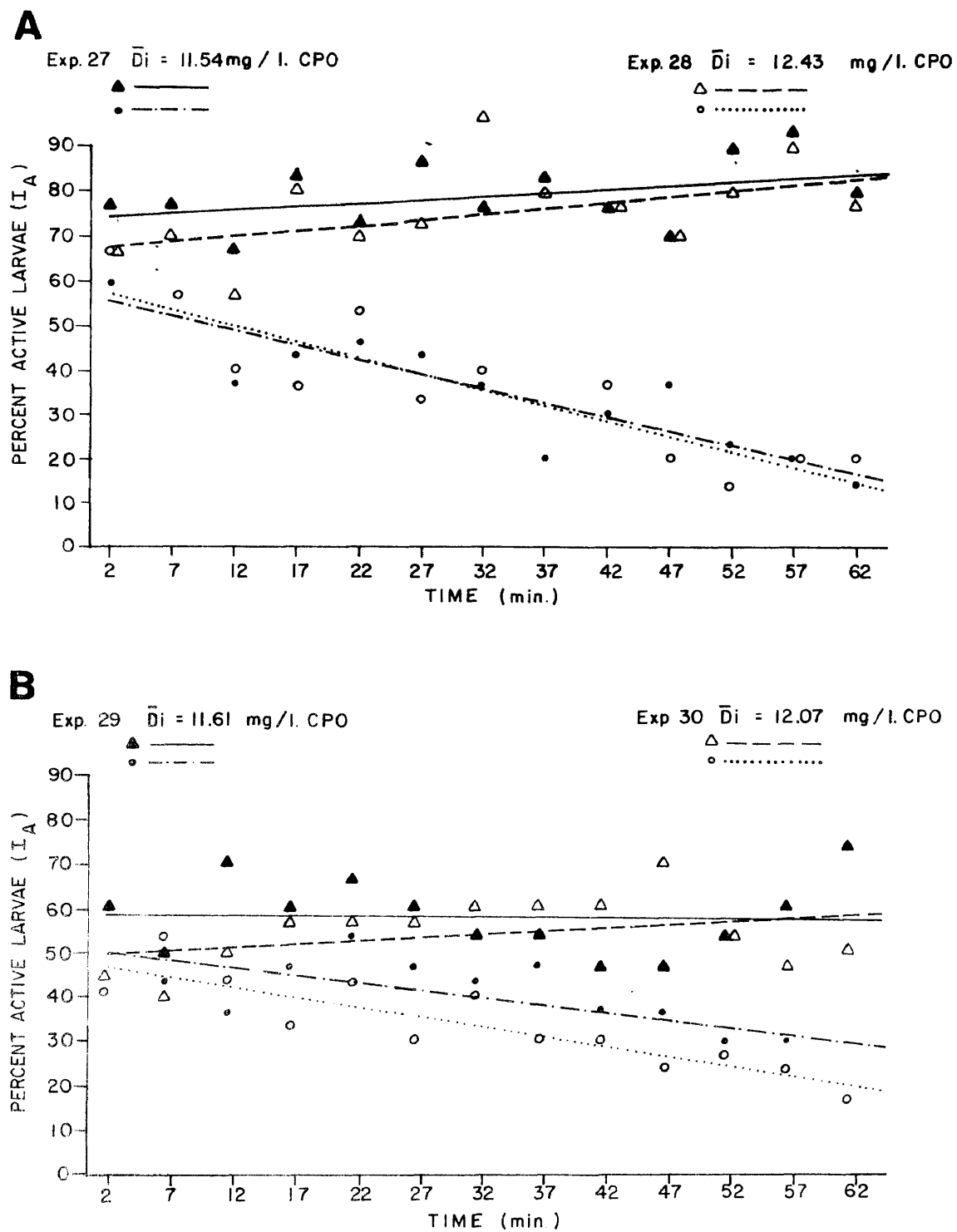
Figure 21. Percent of larvae active over time for test (dots) and
control (triangles) stage 1 <u>Palaemonetes</u> sp. larvae at
≈12.0 mg/l CPO.

intensity (experiments 18 and 20) larvae exposed to chlorine showed slightly increasing activity over time. In the other two experiments at 1.0 mg/l CPO as well as all experiments at 5.0 and 12.0 mg/l CPO larval activity decreased over time in the chlorinated treatments. Only two cases of decreasing activity of control larvae occurred, one at 1.0 mg/l CPO - 240 fc (experiment 18) and one at 5.0 mg/l CPO - 24 fc (experiment 26).

In all cases after 42 minutes, the proportion of active larvae in control chambers exceeded that of larvae in chambers receiving chlorinated water. At the highest CPO concentrations tested and 240 fc (Fig. 21A), there were no cases in which the activity of control larvae was equalled or exceeded by that of larvae exposed to chlorine. At 12 mg/l CPO 24 fc (experiments 29 and 30) the activity of control larvae was not equaled or exceeded by that of test larvae after 3 minutes. However, the difference is more pronounced in the 240 fc tests. This indicates that the higher concentrations of chlorine affected the larvae faster and to a greater degree than the lower concentrations. Further, higher light intensities caused a more rapid decrease in activity when larvae were exposed to CPO than lower light intensities.

Plots of I_0 vs I_A for all of these experiments (Figs. 22-24) show that control larvae occupy a higher position in the test chambers and are more active than the larvae in chambers receiving chlorinated water. Larvae in chlorinated chambers occupied a progressively lower position over time within all experiments. The position of control
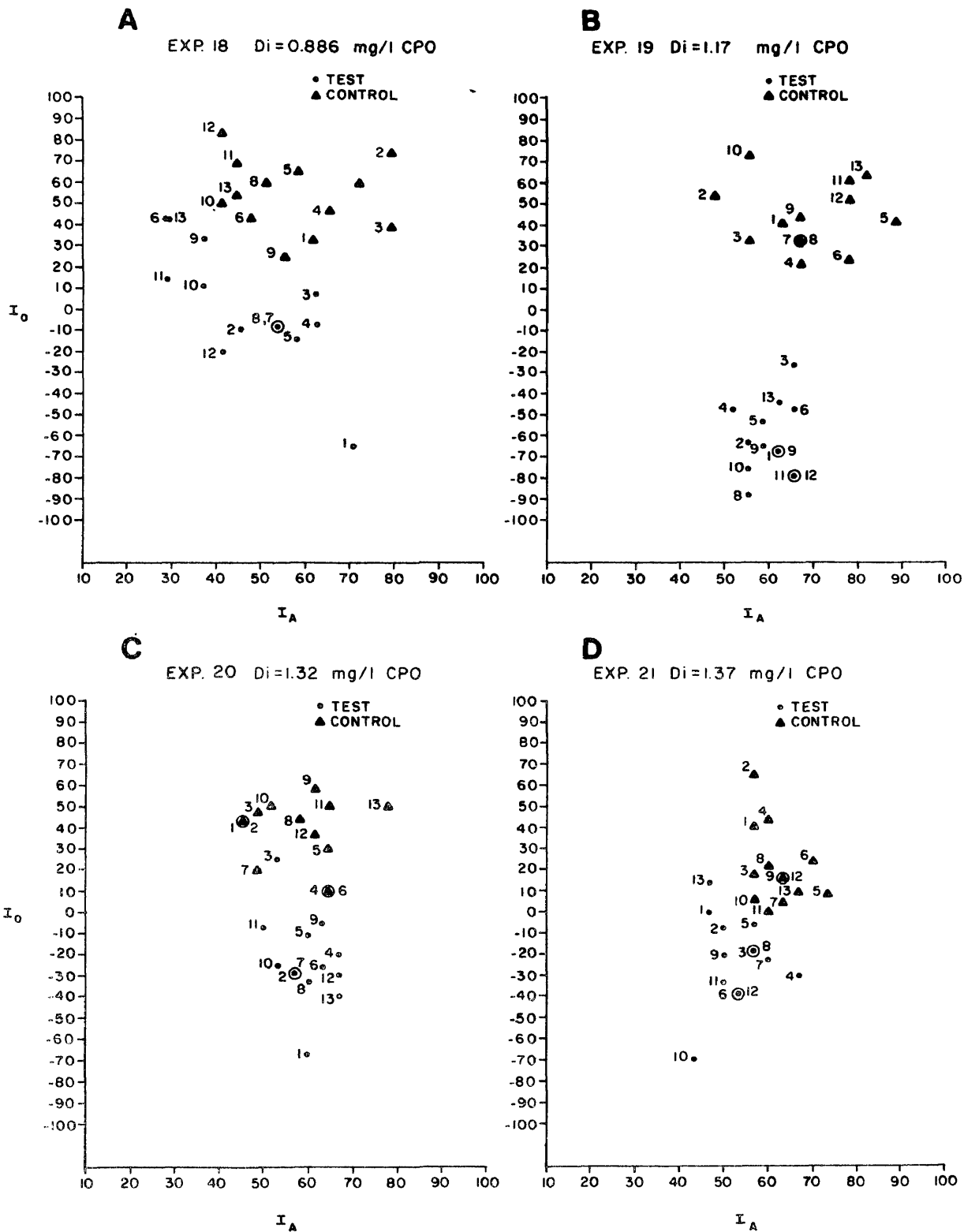
Figure 22. I_0 vs I_A plots of test and control stage 2 Palaemonetes sp. larvae at ≈ 1.0 mg/l CPO at 240 fc (A,B) and 24 fc (C,D) light intensities.

A

EXP. 22 Di = 4.29 mg/l CPO

● = TEST
▲ = CONTROL

I_0

I_A

B

EXP. 23 Di = 4.78 mg/l CPO

● = TEST
▲ = CONTROL

I_A

C

EXP. 24 Di = 5.77 mg/l CPO

● TEST
▲ CONTROL

I_0

I_A

D

EXP. 25 Di = 6.41 mg/l CPO

● TEST
▲ CONTROL

I_0

I_A
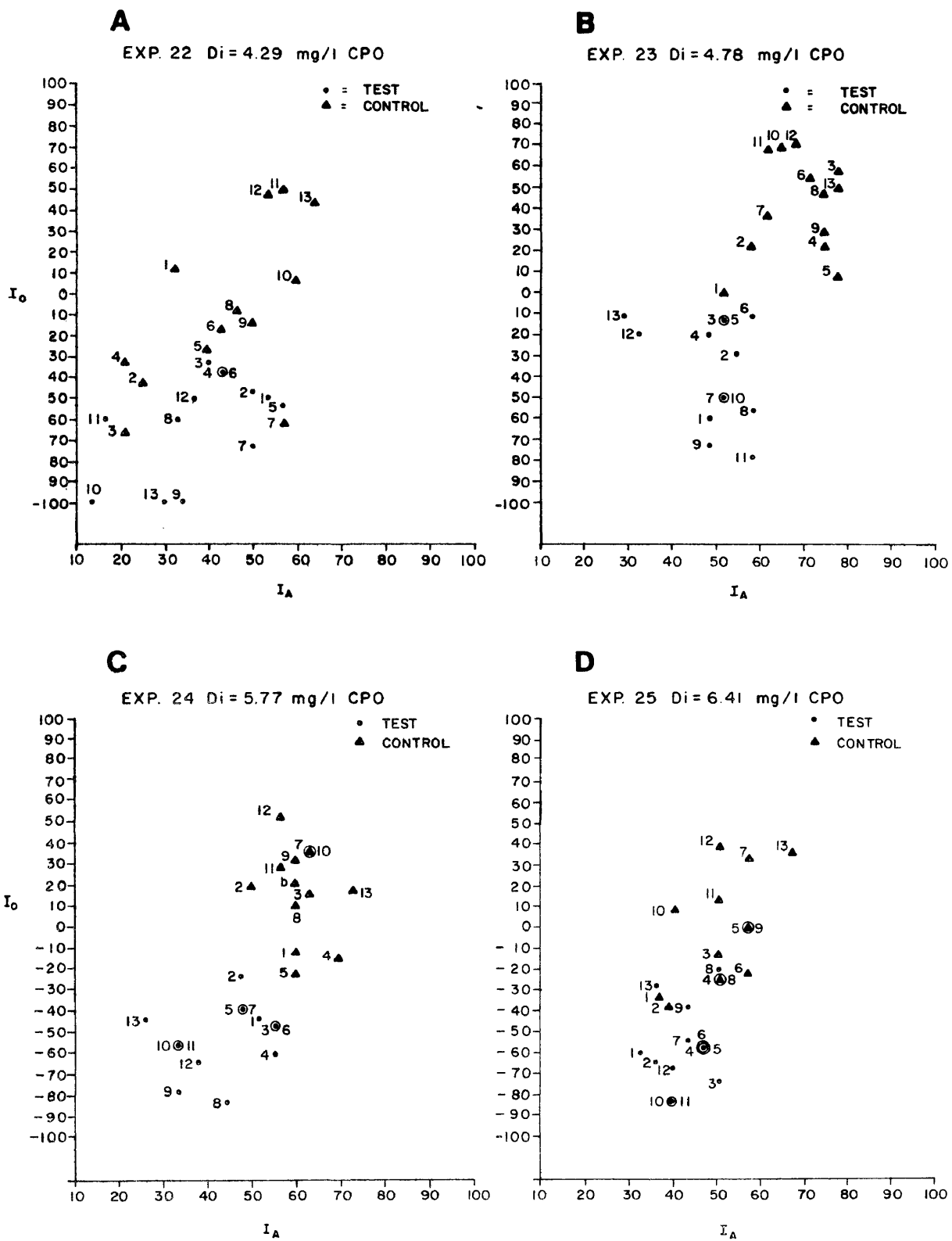
Figure 23. I_0 vs I_A plots of test and control stage 2 Palaemonetes sp. larvae at ≈ 5.0 mg/l CPO at 240 fc (A,B) and 24 fc (C→E).
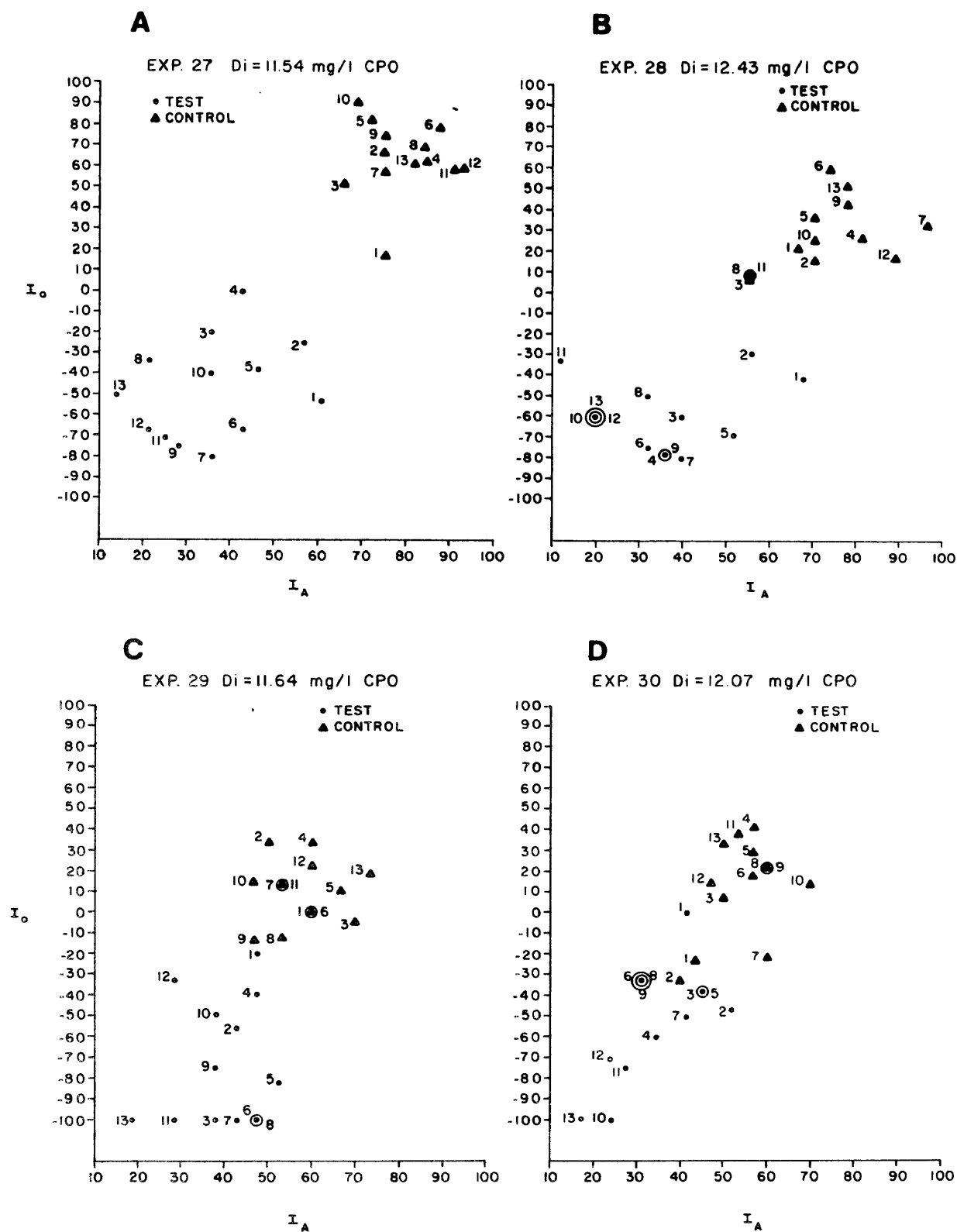
Figure 24. I_0 vs I_A plots of test and control stage 2 _Palaemonetes_ sp.
larvae at ≈ 12 mg/l CPO at 240 fc (A,B) and 24 fc (C,D).

larvae in the chambers was in most cases higher at the end of the test than at the beginning.

At both light intensities I_0 is more important than I_A in separating the responses of the test and control larvae at the lowest CPO concentration. I_A becomes more important than I_0 at the two higher CPO concentrations. This indicates that larvae in the test chambers were only slightly affected by chlorine at the lowest concentration since they did not swim upward as actively as control larvae. The number of active larvae decreased with increasing concentration indicating that larvae were being affected by CPO as opposed to actively avoiding it.

There is no correlation between test concentration and absolute I_0 or $\bar{I}_0$ values at either light intensity (Table 3). The $\Delta\bar{I}_0$ values (Table 3) ranged from 43.1 (1.37 mg/l CPO, 24 fc, experiment 21) to 111.9 (11.54 mg/l CPO, 240 fc, experiment 27) indicating that there is a weak relationship between CPO concentration and the degree of photopositive response of the larvae in chlorinated water. The $\Delta\bar{I}_A$ values are highest at the highest CPO concentrations. This indicates the differences in activities of the larvae between the chlorinated water layer and control treatments within experiments increase with increasing CPO concentration. No constant relationship was observed between $\Delta\bar{I}_A$ and the two lower concentrations at either light intensity. No relationship was found between light intensity and I_0 (Figs. 22-24), $\bar{I}_0$ or $\Delta\bar{I}_0$ (Table 3). At the highest concentration tested, the relative activity ($\Delta\bar{I}_A$) of the 24 fc treatments is nearly

Table 3. Mean Orientation Index (I_0) and Activity Index (I_A), Relative Mean Orientation Index ($\Delta\bar{I}_0$), Mean Activity Index ($\bar{I}_A$) and Relative Mean Activity Index ($\Delta\bar{I}_A$) for Stage 2 Palaemonetes sp. Larvae in the Chlorination Experiments.

Experiment No.	Actual Test Concentration (mg/l CPO)	Lo (fc)	Test $\bar{I}_0$	Test $\bar{I}_A$	Control $\bar{I}_0$	Control $\bar{I}_A$	$\Delta\bar{I}_0$	$\Delta\bar{I}_A$
18	0.886	240	1.5	47.1	54.0	57.1	52.5	10.0
19	1.17	240	-61.5	60.2	44.4	68.6	105.9	8.4
22	4.29	240	-61.7	38.5	-8.8	43.9	52.9	5.4
23	4.78	240	-37.2	49.4	44.7	68.5	81.9	19.1
27	11.54	240	-47.6	36.0	64.3	79.1	111.9	43.1
28	12.43	240	-59.5	35.7	27.5	72.3	87.0	36.7
20	1.32	24	-22.8	59.7	37.8	58.1	60.6	-1.6
21	1.37	24	-21.9	53.1	21.2	62.1	43.1	9.0
24	5.77	24	-52.0	44.1	17.6	61.3	69.6	17.2
25	6.41	24	-56.9	45.6	-2.4	54.1	54.5	8.5
26	6.78	24	-48.1	42.0	42.2	48.8	90.3	6.8
29	11.64	24	-70.1	39.9	9.5	57.9	79.6	22.8
30	13.07	24	-52.1	33.7	12.3	54.1	64.4	20.4

double that of the 240 fc treatment. The Chi-squared (x^2) values for all experiments are all highly significant (Table 2) indicating the discreteness of the behavior of test and control larvae within experiments. There is no strong relationship between Wilkes Λ and dose as can be seen from Figure 25A. In general, Λ decreases with increasing concentration at both light intensities (the lower the Λ value, the greater the relative distance between two groups).

Larvae from one experiment at each CPO concentration at 24 fc were retained and cultured in unchlorinated water in order to evaluate the conditon of larvae after testing. Larvae exposed to high CPO concentrations had greater mortality rates than those exposed to low concentrations or controls (Fig. 25B). At the two lower concentrations 50% mortality occurred after approximately 62 hours while 50% mortality for larvae tested in the highest concentration occurred between 24 and 48 hours. The time it took for fifty percent of the larvae to die after CPO avoidance testing was approximately the same for the two lower concentrations (1.32 and 5.77 mg/l CPO) but the cumulative mortality for the lower concentration at the end of the culture period was lower. All larvae exposed to the highest concentration (11.61 mg/l) died without molting (100% mortality within 100 hours). These data show that the larvae were physiologically affected by the chlorinated water and did not effectively avoid the chlorine at any concentration.

No distinct avoidance behavior was observed during the tests. No telson flip responses were observed at any CPO concentration or light
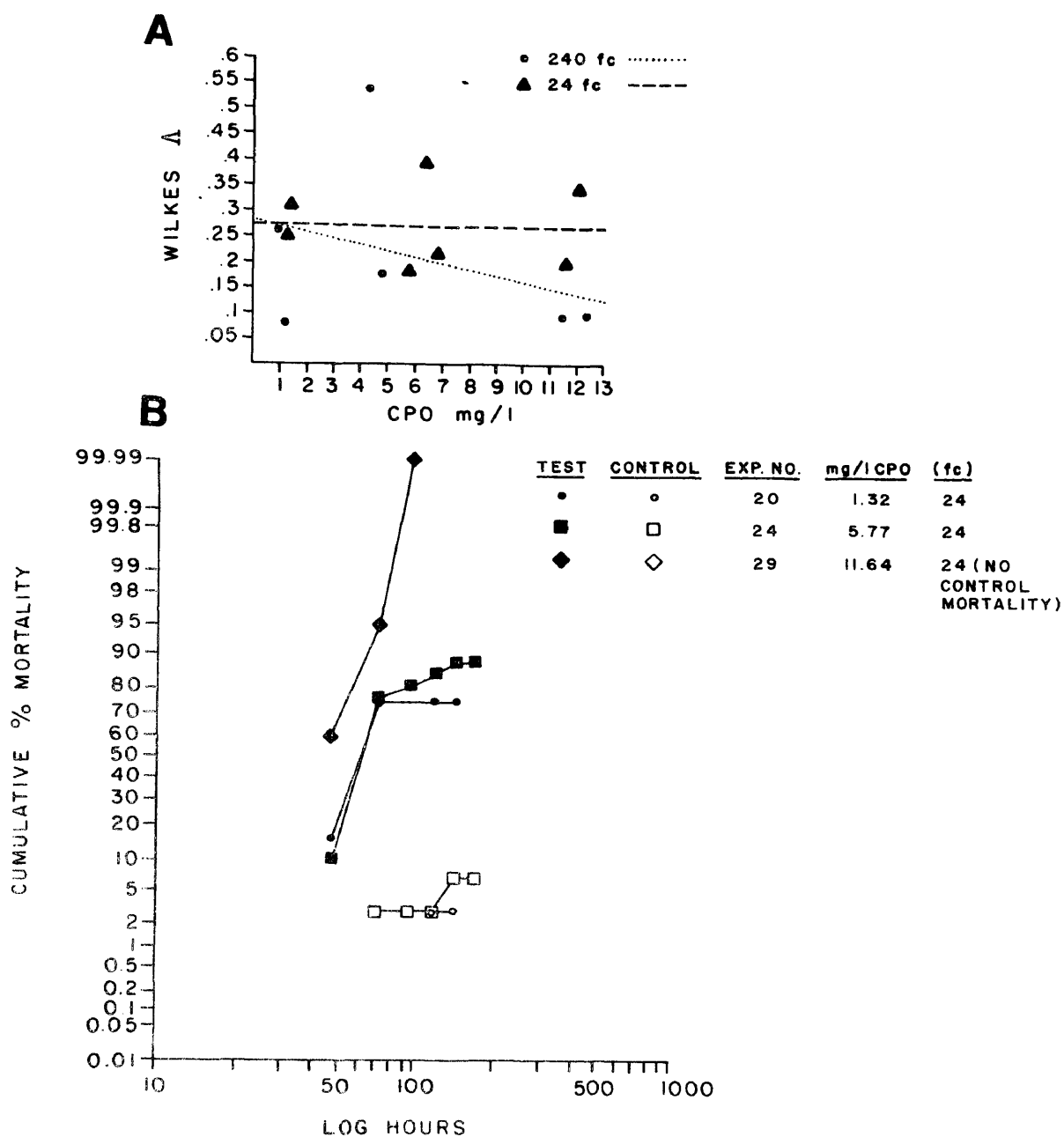
Figure 25. Wilkes Lambda (Λ) values for all chlorine tests plotted against chlorine test concentrations (A) and cumulative % mortality of larvae retained after chlorine avoidance testing at 1.32, 5.77 and 11.61 mg/l CPO under 24 fc light intensity (B) for stage 2 Palaemonetes sp. larvae.

intensity. Downward swimming was occasionally observed at the low CPO

concentration ($\approx$ 1.0 mg/l CPO) at 24 fc light intensity. Observed

distributions resulted from passive sinking which was frequently

observed. Passive sinking seemed to increase as the CPO concentration

increased irrespective of light intensity although this was not

quantified.

DISCUSSION

Effect of Environmental Variables

Stage 1 larvae of P. longicarpus were positively phototactic in the vertical plane under the conditions imposed on them during testing. The percent of larvae active in the chamber in the present experiments agrees closely with the results of Roberts (1971b) where 60 to 63% of the larvae were active under similar conditions. The higher I_0 found in this study than in Roberts (1971b) is probably due to chamber depth (9.5 cm this study, 46 cm Roberts [1971b]).

There were no significant differences found in the response of larvae betwen the two chambers when tested under identical conditions. Therefore in tests in which each chamber had different treatments, any difference in response is concluded to be due to the treatment.

There was great variability in the response of larvae to the same sets of conditions between tests. One contributing factor to the variability of response could be that a small number of P. intermedius and P. vulgaris larvae were used along with the P. pugio larvae. Other factors could be the genetic variability within species, the different developmental rates for larval batches or past nutritional and physiological history of the adults before collection. Culture technique or slight variations in testing methodology might also account for some of the variation found but this was not tested.

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No difference was detected between the responses of the larvae exposed to different light intensities in these experiments. No information has been found dealing with the effects of light intensity on vertical phototactic responses of crustacean larvae in the laboratory. In experiments on larvae of Rhithropanopeus harrisii tested in the horizontal plane Forward and Costlow (1974) found that intensities between 0.01 and 20 W/m^2 produced the greatest positive phototaxis with lesser responses at higher and lower intensities. These intensities are close to the light intensities used in these experiments (240 fc $\approx$ 3.8 W/m^2, 24 fc $\approx$ 0.38 W/m^{-2}).

Positive vertical phototropism in Palaemonetes sp. larvae had not been studied in the laboratory prior to these tests. Lyon (1906) found that Palaemonetes larvae were most photopositive to blue light and hardly sensitive to red light in the horizontal plane.

White (1924) working with Palaemonetes vulgaris larvae agreed with Lyon and quantified larval response to two light sources of different intensities positioned at right angles to each other in the horizontal plane.

In the present experiments Palaemonetes sp. larvae were not naturally positively phototactic in the vertical plane. This lack of vertical response in the laboratory was also observed by Thorne et al. (1979) working with Macrobrachium novaehollandiae larvae. He speculated that this behavior is an adaptive mechanism for retention of larvae in the estuary.

Baylor and Smith (1957) found that Daphnia magna when hungry respond differently to light than well fed Daphnia. They do not mention any effect of light adaption. They did state that vertical illumination elicited food searching in the horizontal plane and horizontal illumination elicited searching in the vertical plane.

Burton (1979) observed that newly hatched larvae of Emerita analoga were photopositive for the first four hours and then became strongly photonegative. When the larvae were fed Artemia nauplii they remained photonegative, but when starved they became photopositive. He states, however, that the photopositive response occured only in the horizontal plane and not in the vertical plane. Burton (1979) also found that larvae under increased hydrostatic pressure were photopositive regardless of the light orientation. He suggested that nutritional state aids in maintaining pressure sensitivity which affects depth regulation.

It was found in the present experiments that light-adapted, starved larvae were strongly photopositive in the vertical plane. Larvae that were light-adapted and fed and larvae that were dark-adapted and starved were not photopositive in the vertical plane to any appreciable extent. This behavior may have important survival value. Larvae that are unable to find enough food in the lower water layers of an estuary would, after a period of light adaptation, swim toward the surface of the water. In the upper water layer of the

estuary the larvae would stand a good chance of encountering food or be dispersed to another area with a better supply of food by the net downstream flow of the estuarine water. This starvation-light adaption behavior may also play a part in the diurnal migration of larvae.

Cronin and Forward (1979) found that laboratory-reared R. harrisii larvae exhibit only weak circadian rhythms while field collected larvae exhibited strong circatidal rhythms. Larvae in their experiments were fed on a random schedule. Therefore, the laboratory-reared larvae may not have been conditioned to a set rhythm based on light and hunger (they were maintained on a 12 L:12D light regime). Field collected larvae on the other hand may have been conditioned to migrate to the surface in a tidal rhythm possibly associated with food availability.

Pearre (1973) studied Sagitta elegans in the field and laboratory. He concluded that the diel migration pattern detected in the field was related to the degree of satiation which in some way influenced the depth control mechanics of this chaetognath.

Lang et al. (1980) found no major change in photopositive and photokinetic response of stage 2 larvae of Balanus improvisus that were starved for 24 hours.

In the present study, larvae of Pagurus longicarpus exhibited a greater overall response in the vertical plane at 18 °/oo than at

24 O/oo salinity regardless of light intensity. This is contrary to what might be expected from the work of Roberts (1971b). Work on species other than P. longicarpus by Harder (1952a, b, 1954, 1957, 1968), Lance (1962), Lagerspertz and Matilla (1961), Scarratt and Raine (1967), and Latz and Forward (1977) also appear to disagree with the present study.

The response of larvae in the present experiments may have been influenced by embryonic irreversible adaptation to 18-20 O/oo salinity in the larval hatching system (Rosenberg and Costlow, 1979). This irreversible salinity adaptation could be responsible for the lower activity of larvae at 24% salinity. Latz and Forward (1977), however, found that Rhithropanopeus harrisii larvae exposed to decreases in salinity recovered their positive vertical phototaxis after ten minutes. The responses of larvae subjected to salinity increases, such as those in the present experiments, have not been studied in the vertical plane. In the horizontal plane salinity increases caused Balanus perforatus larvae to become photopositive (Ewald 1912).

A difference in larval response of Pagurus longicarpus at different times of the day was only weakly apparent in these experiments. Cronin and Forward (1979) found that laboratory-reared R. harrisii larvae had weak diel rhythms while field-caught larvae had strong circatidal rhythms. The larvae used in the present experiments were obtained from females induced to spawn in January. These results tend to agree with Cronin and Forward (1979) in that weak diel rhythms appear to be present in laboratory reared populations.

Effect of Chlorinated Water on Vertical Movement of Larvae

There is no clear evidence of active avoidance of chlorinated water by decapod larvae. Active avoidance behaviors such as telson flips and downward swimming as described by Roberts (1971b) for Pagurus longicarpus larvae exposed to salinity discontinuities were never observed. The differences in the number of larvae observed in the upper layers of test and control chambers suggest avoidance of the chlorinated water layers by the larvae. Direct observation reveals these results are not the product of active avoidance behavior but rather passive sinking after the larvae were adversely affected by the chlorine in the upper water layer. There were more larvae in the upper layers of control chambers than test chambers regardless of light intensity and CPO concentrations. Further, the activity index for larvae exposed to CPO was less than that of control larvae which means that the larvae were not simply retreating to the lower unchlorinated layer, but were sinking to the bottom of the chambers. There was also no accumulation of larvae near the CPO discontinuity as might be expected if larvae were actively avoiding the upper chlorinated layer.

The number of active larvae in the chlorinated test chambers (Figs. 19-21) decreased over time while the activity in control chambers increased or remained constant. The difference showed up most clearly at 12.0 mg/l CPO.

A greater difference between the activity of control and test larvae occurred at 240 fc, than at 24 fc light intensity. This may be

due to increased attraction of larvae toward the light source at the higher intensity. This would cause the larvae at the high light intensity to become affected more rapidly and to a greater degree than the larvae at the low light intensity.

Roberts (1971b) used activity (I_A) and orientation (I_O) indices to describe the reactions of Pagurus longicarpus larvae to salinity discontinuities. He examined these indices separately and these two components of larval response in his narrative. The two indices are plotted against each other in the present study in order to characterize larval response to each treatment much like a temperature-salinity diagram is used to characterize water masses. Graphically this method of comparison appears to separate test and control responses extremely well. This was confirmed by the Wilkes-Lamda and X^2 discriminant analysis method for comparing means and variances, respectively, for discreteness of grouped data. This graphical method also easily indicates whether the activity of larvae or their position in the water column contributes most to the separation of the data for the two groups.

The decreasing I_O values for larvae in the chlorinated chambers over time indicate that larvae did not initially avoid the chlorinated water but occupied a lower position in the chambers as they became increasingly affected by the chlorine. The I_O values averaged ($\bar{I_O}$) for each experiment show no pattern relating to concentration or light intensity between experiments but this is most likely due to difference in the inherent activity between batches of larvae. The

difference between averaged control and test I_0 values ($\Delta \bar{I}_0$) was positive, which indicates that larvae in control chambers occupied a higher position in the water column. Differences in the relative position of control and test larvae ($\Delta \bar{I}_0$) increased with concentration. A slight effect of light intensity may exist but this is not clear. The $\Delta \bar{I}_0$ values would seem to indicate weak larval avoidance, but this is most likely an artifact resulting from counting CPO affected (sluggish) larvae in the lower two quarters of the test chamber which could not maintain higher positions in the testing chambers.

The I_A values for larvae exposed to chlorinated water decrease over time. The difference in activity between test and control larvae became more apparent with increasing concentration. The activity of control larvae (I_A) generally increased or stayed the same over time. The averaged I_A values ($\bar{I}_A$) of the test larvae decreased with increasing CPO concentrations. The relative differences between $\Delta \bar{I}_A$ values for test and control larval activity indicate that larvae exposed to 240 fc were in general affected more rapidly and to a greater extent than larvae tested at 24 fc especially at 12.0 mg/l CPO where the relative activity of larvae exposed to 240 fc was only half that of the larvae at 24 fc.

The differences between treatments within experiments increased significantly with increasing concentration in all experiments as evidenced by the Wilkes Lambda (Λ) values (Fig. 25A). There also appeared to be an interaction of light and CPO concentration at 240 fc

producing greater differences in response between the test and control larvae than at 24 fc.

Since post-exposure mortality was proportional to CPO concentration and since no distinct active avoidance behavior was observed, it is concluded that larvae in these experiments did not avoid chlorinated water layers but swam into them. Larvae became increasingly affected by the CPO as concentration and time increased.

The one most obvious explanation for the lack of avoidance behavior by the larvae is that they cannot detect chlorine in the water. Alternately larvae may prefer chlorinated water. In this case I_0 values would have been high initially and decreased over time as larvae became incapacitated. This was not what occurred. A third possibility is that all chlorine concentrations were so high that upon initial contact with the chlorine the receptors of the larvae were incapacitated and hence the larvae could not respond to CPO. Another explanation for lack of avoidance behavior is that the method used to induce the larvae to become vertically photopositive, (starvation and light adaption), overrode the avoidance response the larvae might otherwise have exhibited.

Studies of avoidance of chlorine by fish indicate that fish detect and avoid CPO (Cherry, Larrick et al., 1977; Cherry, Hoehn et al., 1977; Middaugh et al., 1977; Meldrim and Fava, 1977; Meldrim et al., 1974; Fava and Tsai, 1976). Meldrim et al. (1974) reported avoidance reactions by adult Palaemonetes pugio, Crangon septemspinosa and Callinectes sapidus. However, if the data for the invertebrate

species were tested statistically it is possible that no significant avoidance occurred. The fact that fish avoid CPO and a decapod larva does not, demonstrates that you cannot make general ecological statements about the behavior of all aquatic organisms by studying a few. Much more work on invertebrate avoidance behavior needs to be done.

CONCLUSIONS

1. An apparatus to test avoidance of polluted water by larval decapods in a flowing system was designed, built and tested. This apparatus is especially suited to test larval responses to non-conservative pollutants such as chlorine.

2. No difference in phototactic response was detected when larvae of P. longicarpus were exposed to 240 fc and 24 fc at 18 ^{o}/oo salinity.

3. Stage 1 larvae of P. longicarpus gave a greater phototactic response in the morning at 240 fc than at 24 fc. In the afternoon the larvae gave a greater phototactic response at 24 fc than at 240 fc light intensity.

4. Stage 1 larvae of P. longicarpus were more positively phototactic at their hatching salinity of 18 ^{o}/oo after 24 hours than when acclimated for 24 hours to 24 ^{o}/oo salinity after hatching in 18 ^{o}/oo water regardless of light intensity.

5. Larvae of Palaemonetes sp. must be starved and light adapted in order to induce them to become photopositive in the vertical plane.

6. Under the conditions imposed by these experiments, larvae of Palaemonetes sp. neither avoided nor preferred chlorinated water

layers of 1.0 to 12.0 mg/l CPO. Larvae gradually decreased in activity and occupied lower positions within the CPO treated chambers as time and CPO concentration increased.

7. The effects of light intensity on CPO avoidance by Palaemonetes sp. larvae were difficult to evaluate because of the variability in response of larvae between experiments. There is some evidence to indicate that larvae tested at 240 fc became affected by the CPO more rapidly and to a greater extent than larvae tested at 24 fc intensity at the same CPO concentration.

8. Exposure of larvae to CPO during the course of the chlorine avoidance experiments resulted in post-exposure mortality proportional to the test concentration.

LITERATURE CITED

Arthur, J. W., R. W. Andrews, V. R. Mattson, D. T. Olson, G. E. Glass, B. J. Halligan and C. T. Walbridge. 1975. Comparative toxicity of sewage-effluent disinfection to freshwater aquatic life. U.S. EPA Duluth, Minn. Ecological Research Series EPA 600/3-75-012. 62 pp.

A.P.H.A. 1975. Standard Methods for the Examinations of Water and Wastewater. 14th Ed. pp. 874.

Bardolph, M. and R. H. Staun. 1978. Polarized light sensitivity in the stage 1 zoea of the mud crab Panopeus herbstii. Mar. Biol. 46:327-333.

Baylor, E. R. and F. E. Smith. 1957. Diurnal migration of plankton crustaceans. In: B. T. Sheer (ed.) Recent Advances in Invertebrate Physiology. Univ. Oregon, Eugene, Oregon. pp. 21-35.

Beauchamp, R. S. A. 1969. The use of chlorine in the cooling water system of a coastal power station. Chespeake Sci. 10:280.

Brungs, W. A. 1973. Effects of residual chlorine on aquatic life. J. Water Poll. Contr. Fed. 45:2180-2193.

Brungs, W. A. 1976. Effects of wastewater and cooling water chlorination on Aquatic Life. U.S. EPA Duluth, Minn. Ecological Research Series. EPA 600/3-76-098 pp. 45.

Burton, R. S. 1979. Depth regulatory behavior of the first stage zoea larvae of the sand crab Emerita analoga (Stimpson) (Decapoda: Hippidae). J. exp. mar. Biol. Ecol. 37:255-270.

Capuzzo, J. M., S. A. Lawrence and J. A. Davidson. 1976. Combined toxicity of free chlorine chloramine and temperature to stage I larvae of the American lobster Homarus americanus. Water Res. 10:1093-1099.

Carpenter, J. H. 1977. Problems in measuring residuals in chlorinated water. Chesapeake Sci. 18:112.

Carpenter, J. H. and C. A. Smith. 1978. Reactions in chlorinated sea water. In: R. L. Jolley, H. Gorchev and D. H. Hamilton, Jr. (Eds.) Water Chlorination Environmental Impact and Health Effects, Ann Arbor Science, Michigan. pp. 195-208.

Carlson, R. M., R. Caple, A. R. Oyler, K. J. Welch, D. L. Bodenner and R. Liukkonen. 1978. Aqueous chlorination products of polynuclear aromatic hydrocarbons. In: R. L. Jolley, H. Gorchev and D. H. Hamilton, Jr. (Eds.) Water Chlorination Environmental Impact and Health Effects, Ann Arbor Science, Michigan. pp. 59-66.

Cherry, D. S., R. C. Hoehn, S. S. Waldo, D. H. Willis, J. Cairns, Jr. and K. L. Dickson. 1977. Field-laboratory determined avoidances of the spotfin shiner and the bluntnose minnow to chlorinated discharges. Water Res. Bull. 13:1047-1055.

Cherry, D. S., S. R. Larrick, K. L. Dickson, R. C. Hoehn and J. Cairns, Jr. 1977. Significance of hypochlorous acid in free residual chlorine to the avoidance response of spotted bass (Micropterus punctulatus) and rosy face shiner (Notropis rubellus). J. Fish. Res. Bd. Canada 34:1365-1372.

Christman, R. F., J. D. Johnson, J. R. Hass, F. K. Pfaender, W. T. Liao, D. L. Norwood and H. J. Alexander. 1978. Natural and model aquatic humics: reactions with chlorine. In: R. L. Jolley, H. Gorchev and D. H. Hamilton, Jr. (Eds.) Water Chlorination Environmental Impact and Health Effects, Ann Arbor Science, Michigan. pp. 15-28.

Costa, H. H. 1966. Responses of Gammurus pulex (L.) to modified environment. I. Reactions to toxic solutions. Crustaceana 11:245-256.

Coughlan, J. and J. Whitehouse. 1977. Aspects of chlorine utilization in the United Kingdom. Chesapeake Sci. 18:102-111.

Cronin, T. W. and R. B. Forward, Jr. 1979. Tidal vertical migration: An endogenous rhythm in estuarine crab larvae. Science 205:1020-1022.

Draley, J. E. 1972. Treatment of cooling waters with chlorine. Argonne Natl. Laboratory, Argonne, Illinois. ANL ES-12. 11 pp.

Ewald, E. F. 1912. On artificial modification of light reactions and the influence of electrolytes on phototaxis. J. Exp. Zool. 13:591-612.

Fava, J. A., Jr., and C. F. Tsai. 1976. Immediate behavioral reactions of blacknose dace Rhinichthys atratulus to domestic sewage and its toxic constituents. Trans. Am. Fish. Soc. 105:430-441.

Floyd, W. R., III. 1977. The effects of temperature and salinity on the larval development of the grass shrimp Palaemonetes pugio Holthuis reared in the laboratory. Masters Thesis, Old Dominion University, Norfolk, Virginia. 145 pp.

Folmar, L. C. 1976. Overt avoidance reaction of rainbow trout fry to nine herbicides. Bull. Environ. Contam. Toxicol. 15:509-514.

Forward, R. B., Jr. 1974. Negative phototaxis in crustacean larvae possible functional significance. J. exp. mar. Biol. Ecol. 16:11-17.

Forward, R. B., Jr. and J. D. Costlow, Jr. 1974. The ontogeny of phototaxis by larvae of the crab Rhithropanopeus harrisii. Mar. Biol. 26:27-33.

Glaze, W. H., and G. R. Peyton. 1978. Soluble organic constituents of natural waters and wastewaters before and after chlorination. In: R. L. Jolley, H. Gorchev and D. H. Hamilton, Jr. (Eds.) Water Chlorination Environmental Impact and Health Effects, Ann Arbor Science, Michigan. pp. 3-14.

Gosner, K. L. 1971. Guide to identification of marine and estuarine invertebrates. Cape Hatteras to the Bay of Fundy. Wiley-Interscience, New York. 693 pp.

Hansen, D. J. 1969. Avoidance of pesticides by untrained sheepshead minnows. Trans. Am. Fish. Soc. 98:426-429.

Hansen, D. J., E. Matthews, S. C. Nall and D. P. Dumas. 1972. Avoidance of pesticides by untrained mosquito fish, Gambusia affinis. Bull. Environ. Contam. Toxicol. 8:46-51.

Hansen, D. J., S. C. Schimmel, and J. M. Keltner, Jr. 1973. Avoidance of pesticides by grass shrimp (Palaemonetes pugio). Bull. Environ. Contam. Toxicol. 7:129-133.

Hansen, D. J., S. C. Schimmel, and E. Matthews. 1974. Avoidance of Aroclor^R 1254 by shrimp and fishes. Bull. Environ. Contam. Toxicol. 12:253-256.

Harder, W. 1952. Einige Gerate zur Herstellong von Wasserschichten verschiedener Dichte. Kurze Mitt. Fischbiol. Abt. Max. Planck Inst. Meeresbiol. Wilhelms 1:22-27.

Harder, W. 1954. Weitere experimentelle Untersuchungen uber das Verhalten von Copepoden gegenüber Sprungschichten. Kurze Mitt. Fischbiol. Abt. Max-Planck. Inst. Meeresbiol. Wilhelms 4:1-47.

Harder, W. 1957. Verhalten von Organismen gegenüber Sprungschichten. Année Biol. 33:227-232.

Harder, W. 1968. Reactions of plankton organisms to water stratification. Limnol. Oceanogr. 13:156-168.

Höglund, L. B. 1951. A new method of studying the reactions of fishes in gradients of chemical and other agents. Oikos 3:247-267.

Höglund, L. B. 1961. The reactions of fish in concentration gradients. Fish. Bd. of Sweden, Inst. Freshwater Res., Drottningholm. Rep. No. 43 1-147.

Höglund, L. B. and M. Astrand. 1973. Preferences among juvenile char (Salvelinus alpinus L.) to intraspecific odours and water currents studied with the fluviarium technique. Fish. Bd. Sweden, Inst. Freshwater Res., Drottningholm, Rep. 53:21-30.

Hsu, R. Y. and Y. Shimizu. 1977. Phenylpropanoids in chlorination. Chespeake Sci. 18:129.

Ingols, R. S., H. A. Wyckoff, T. W. Kethley, H. W. Hodgden, E. L. Fincher, J. C. Hilderbrand and J. E. Mandel. 1953. Bactericidal studies of chlorine. Indust. Eng. Chem. 45:996-1000.

Inman, G. W., Jr. and D. Johnson. 1978. Effect of ammonia concentration on the chemistry of chlorinated sea water. In: R. L. Jolley, H. Gorchev and D. H. Hamilton, Jr. (Eds.) Water Chlorination Environmental Impact and Health Effects, Ann Arbor Science, Michigan. pp. 235-252.

Johannesson, J. K. 1955. Note on the chlorination of water in the presence of traces of natural bromide. New Zealand J. Sci. Tech. 36B:600-602.

Johnson, J. D. 1977. Analytical problems in chlorination of saline water. Chesapeake Sci. 18:116-118.

Jolley, R. L. 1973. Chlorination effects on organic constituents in effluents from domestic sanitary sewage treatment plants. Ph.D. Dissertation, University of Tennessee, Knoxville. 374 pp.

Jolley, R. L. 1977. Identification of organic halogen products. Chesapeake Sci. 18:122-125.

Jolley, R. L., H. Gorchev and D. H. Hamilton, Jr. (Eds.). 1978. Water Chlorination: Environmental Impact and Health Effects, Vol. 2. Proceedings of the Second Conference on the Environmental Impact of Water Chlorination, Ann Arbor Science Pub., Ann Arbor, Mich. 909 pp.

Jones, B. F., C. E. Warren, C. E. Bond, and P. Doudoroff. 1956. Avoidance reactions of salmonid fishes of pulp-mill effluents. Sew. Ind. Wastes 28:1403-1413.

Jones, J. R. E. 1947. The reactions of <u>Pygosteus</u> <u>pungitius</u> L. to toxic solutions. J. Exp. Biol. 25:22-34.

Jones, J. R. E. 1951. The reactions of the minnow <u>Phoxinus</u> <u>phoxinus</u> (L.) to solutions of phenol, orthocresol and paracresol. J. Exp. Biol. 28:261-270.

Kynard, B. 1974. Avoidance behavior of insecticide susceptible and resistant populations of mosquito fish to four insecticides. Trans. Am. Fish. Soc. 103:557-561.

Lagerspetz, K. and M. Mattila. 1961. Salinity reactions of some fresh and brackish water crustaceans. Biol. Bull. 120:44-53.

Lance, J. 1962. Effects of water of reduced salinity on the vertical migration of zooplankton. J. Mar. Biol. Ass. U.K. 42:131-154.

Lang, W. H., M. Marcy, P. J. Clem, D. C. Miller and M. R. Rodelli. 1980. The comparative photobehavior of laboratory-hatched and plankton-caught <u>Balanus</u> improvisus (Darwin) nauplii and the effects of 24-hour starvation. J. exp. mar. Biol. Ecol. 42:201-212.

Larrick, S. R., K. L. Dickson, D. S. Cherry and J. Cairns, Jr. 1978. Determining fish avoidance of polluted water. Hydrobiologia 61:257-265.

Latz, M. I. and R. B. Forward, Jr. 1977. The effect of salinity upon phototaxis and geotaxis in a larval crustacean. Biol. Bull. 153:163-179.

Lewis, B. G. 1966. Chlorination and mussel control. I. The chemistry of chlorinated sea water: A review of the literature. Central Electric Research Labs. RD/L/N 106/66. 10 pp.

Lewis, F. G. III and R. J. Livingston. 1977. Avoidance of bleached kraft pulpmill effluent by pinfish (Lagodon rhomboides) and Gulf killifish (Fundulus gradis). J. Fish. Res. Bd. Canada 34:568-570.

Lindahl, P. E. and A. Marcstrom. 1958. On the preference of roaches (Leuciscus rutilus) for Trinitrophenol, studied with the fluviarium technique. J. Fish. Res. Bd. Canada 15:685-694.

Little, G. 1968. Induced winter breeding and larval development in the shrimp Palaemonetes pugio Holthuis (Caridea, Palaemonidae). Crustaceana Suppl. 2 pp. 19-26.

Livingston, R. J., C. R. Cripe, R. A. Laughlin and F. G. Lewis, III. 1976. Avoidance responses of estuarine organisms to storm water runoff and pulpmill effluents. In: Martin Wiley, Ed., Estuarine Processes, Vol. I. Uses, stresses and adaptations to the estuary. Academic Press, New York. pp. 313-331.

Lyon, E. P. 1906. Note on the heliotropism of Palaemonetes larvae. Biol. Bull. 12:23-25.

Macalady, D. L., J. H. Carpenter and C. A. Moore. 1977. Sunlight induced bromate formation in chlorinated seawater. Science 195:1335-1337.

Maciorowski, H. D., R. McV. Clarke, and E. Scherer. 1977. Use of avoidance-preference bioassays with aquatic invertebrates. Proc. 3rd Toxicity Workshop (1976) Environmental Protection Service Tech. Rept. No. EPS-5AR-77-1, Halifax, Canada. pp. 49-58.

Mattice, J. S. and H. E. Zittel. 1976. Site specific evaluation of power plant chlorination. J. Water Poll. Contr. Fed. 48:2284-2308.

Meldrim, J. W., and J. A. Fava. 1977. Behavioral avoidance responses of estuarine fishes to chlorine. Chesapeake Sci. 18:154-157.

Meldrim, J. W., J. J. Gift and B. R. Petrosky. 1974. The effect of temperature and chemical pollutants on the behavior of several estuarine organisms. Ichth. Assoc. Bull. No. 11. 129 pp.

Middaugh, D. P., J. A. Couch and A. M. Crane. 1977. Responses of early life history stages of the striped bass Morone saxatilis, to chlorination. Chesapeake Sci. 18:141-153.

Morris, J. C. 1975. Chlorination and disinfection state of the art. Am. Waterw. Assoc. J. 63:769-774.

Morris, J. C. and B. Baum. 1978. Precursors and mechanisms of haloform formation in the chlorination of water supplies. In: R. L. Jolley, H. Gorchev and D. H. Hamilton, Jr. (Eds.) Water Chlorination Environmental Impact and Health Effects, Ann Arbor Science, Michigan. pp. 29-48.

Noack, M. G. and R. L. Doerr. 1978. Reactions to chlorine, chlorine dioxide and mixtures thereof with humic acid: An interim report. In: R. L. Jolley, H. Gorchev and D. H. Hamilton, Jr. (Eds.) Water Chlorination Environmental Impact and Health Effects, Ann Arbor Science, Michigan. pp. 49-58.

Pearre, S., Jr. 1973. Vertical migration and feeding in Sagitta elegans Verrill. Ecology 54:300-314.

Provenzano, A. J. and J. W. Goy. 1976. Evaluation of a sulphate lake strain of Artemia as food for larvae of the grass shrimp, Palaemonetes pugio. Aquaculture 9:343-350.

Roberts, M. H., Jr. 1971a. Larval development of Pagurus longicarpus Say reared in the laboratory II. Effects of reduced salinity on larval development. Biol. Bull. 140:104-116.

Roberts, M. H., Jr. 1971b. Larval development of Pagurus longicarpus Say, reared in the laboratory. III. Behavioral responses to salinity discontinuities. Biol. Bull. 140:489-501.

Roberts, M. H., Jr. 1972. Culture techniques for decapod crustacean larvae. In: Walter L. Smith and Matoira H. Chanley (Ed.). Culture of Marine Invertebrate Animals. Plenum Press, N.Y. pp. 209-221.

Roberts, M. H., Jr. 1974. Larval development of Pagurus longicarpus Say reared in the laboratory. V. Effect of diet on survival and molting. Biol. Bull. 146:67-77.

Roberts, M. H., Jr. 1978. Effects of chlorinated seawater on decapod crustaceans. In: R. L. Jolley, H. Gorchev and D. H. Hamilton, Jr. (Eds.) Water Chlorination Environmental Impact and Health Effects, Vol. 2. Ann Arbor Science Pub., Ann Arbor, Michigan. pp. 329-340.

Roberts, M. H., Jr., C. E. Laird and J. E. Illowsky. 1979. Effects of chlorinated seawater on decapod crustaceans and Mulinia larvae. U.S. EPA Gulf Breeze, Fla. Environmental Research Series EPA 600/3-79-031. 110 pp.

Rockwell, A. L. and R. A. Larson. 1978. Aqueous chlorination of some phenolic acids. In: R. L. Jolley, H. Gorchev, and D. H. Hamilton, Jr. (Eds.) Water Chlorination Environmental Impact and Health Effects, Vol. 2. Ann Arbor Science Pub., Ann Arbor, Michigan. pp. 67-76.

Rosenberg, R. and J. D. Costlow, Jr. 1979. Delayed response to irreversible non-genetic adaptation to salinity in early development of the brachyuran crab Rhithropanopeus harrisii and some notes on adaptation to temperature. Ophelia 18:97-112.

Scarratt, D. J. and G. E. Raine. 1967. Avoidance of low salinity by newly hatched lobster larvae. J. Fish. Res. Bd. Canada 24:1403-1406.

Scherer, E. 1977. Behaviroal assays-principles, results and problems. Proc. 3rd Aquatic Toxicity Workshop, Halifax, Nova Scotia, 2-3 November 1976. Environ. Prot. Serv. Tech. Rep. No. EPA-5AR-77-1. pp. 33-40.

Shelford, V. E. and W. C. Allee. 1913. The reactions of fishes to gradients of dissolved atmospheric gases. J. Exp. Zool. 14:207-266.

Sokal, R. R. and F. J. Rohlf. 1969. Biometry - The principals and practice of statistics in biological research. W. H. Freeman and Co., San Francisco. 776 pp.

Sprague, J. R. 1968. Avoidance reactions of rainbow trout to zinc sulphate solutions. Water Res. 2:367-372.

Sprague, J. B. and D. E. Drury. 1969. Avoidance reactions of salmonid fish to representative pollutants. In: S. H. Jenkins, (Ed.) Advances in Water Pollution Research. Pergamon Press, Oxford and New York. pp. 169-179.

Stanbro, W. D. 1977. The chemistry of amino acids and peptides in power plant cooling towers. Chesapeake Sci. 18:126-128.

Sugam, R. and G. R. Helz. 1977. Speciation of chlorine produced oxidants in marine waters: theoretical aspects. Chesapeake Sci. 18:113-115.

Sulkin, S. 1971. Behavioral responses of the development stages of two xanthid crabs to light gravity and pressure under laboratory conditions. Ph.D. Dissertation, Duke University. 141 pp.

Tatsuoka, M. M. 1971. Multivariate analysis: Techiques for education and psychological research. John Wiley and Sons, New York, New York. 310 pp.

Thorne, M. J., D. R. Fielder and J. G. Greenwood. 1979. Larval behaviour of Macrobrachium novaehollandiae (De Man) (Decapoda, Palaemonidae). Australian J. Mar. Freshwater Res. 30:11-23.

Thorson, G. 1964. Light as an ecological factor in the dispersal and settlement of larvae of marine bottom invertebrates. Ophelia 1:167-208.

White, G. C. 1972. Handbook of Chlorination. Litton Educational Publishing, Inc., Van Nostrand Reinhold Co., New York. 744 pp.

White, G. M. 1924. Reaction of the larvae of the shrimp Palaemonetes vulgaris and the squid Loligo pealii to monochromatic light. Biol. Bull. 47:265-273.

Whitehouse, J. W. 1975. Chlorination of cooling water: A review
of literature on the effects of chlorine on aquatic organisms.
Laboratory Memorandum No. RD/LM 496, Central Electricity Research
Laboratories Leatherhead, Surrey, England. 22 pp.

Whitehouse, J. W. 1978. Continuous low-level chlorination for marine
fouling control at power stations in the United Kingdom. In:
R. J. Jolley, H. Gorchev, D. H. Hamilton, Jr. (Eds.) Water
Chlorination: Environmental Impact and Health Effects, Vol. 2.
Ann Arbor Science Pub., Ann Arbor, Michigan. 368 pp.

Wong, G. T. F. and J. A. Davidson. 1977. The fate of chlorine in
seawater. Water Res. 11:971-978.

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