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Three species of copepods were collected within a mile of shore near Newport, Oregon. The copepods were kept in seawater collected at the same time and place, and were immediately transported to the Oregon State University Marine Science Center. They were placed in a 10°C water bath and held overnight. Those which survived were siphoned off and used in temperature shock experiments.

The copepods were placed in small beakers and held at 10°C. Dosing was accomplished by introducing heated seawater to the beakers containing the copepods. Temperature was allowed to decay back to 10°C. After an appropriate period, the vital stain neutral red was used to distinguish live and dead animals.

Percent survival for all species and sexes was generally unaltered by instantaneous (zero duration) shocks to 20° or 25°C ($\Delta T =$ 10° or 15°C). Significant decreases in survival were noted at 30° or 35°C, and few organisms survived a shock to 40°C. Males of all three species appear to be more sensitive to temperature shocks than either females or copepodites.

When exposed for prolonged periods to temperatures of 25° and 30°C, all species and sexes exhibited reduced survival. However, only the males showed significantly reduced survival after extended exposure to 20°C.

The Effect of Short-term Thermal Stresses on the Survival of Nearshore Copepods

by

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THE EFFECT OF SHORT-TERM THERMAL STRESSES ON THE SURVIVAL OF NEARSHORE COPEPODS

INTRODUCTION

The electrical power producing industry has been growing very rapidly in recent years. Estimates indicate a doubling of output every 10 to 15 years (Warren, 1969). An unavoidable by-product of this industry (and many others) is waste heat which is seldom utilized for constructive purposes and may have undesirable environmental effects. Waste heat occurs regardless of whether the source of thermal energy for a power plant is nuclear or fossil fuel, and this excess thermal energy must be dissipated in some manner. Nearly all of the schemes devised for dissipating this heat involve water as either its intermediate or ultimate recipient. This transfer of heat to water may have profound effects on the organisms inhabiting that water.

The literature on the effects of power plants on zooplankton is extensive; however, as will be discussed later, this literature involves the effects of a number of stresses other than temperature. Consequently, the effect of heat alone is difficult to assess. Some data solely on the effect of temperature are available, but there are problems relating this information to conditions existing in Oregon. Most of the studies were conducted on the East Coast where seasonal temperature regimes are very different from those here. Consequently, it is possible that through evolutionary time the East Coast and West Coast populations have adapted to widely differing environmental pressures. Also, those studies which have been done on the Pacific Coast go no farther north than Humboldt Bay in northern California. Consequently, the conditions to which copepods here are acclimated may be very different from those existing in other locations.

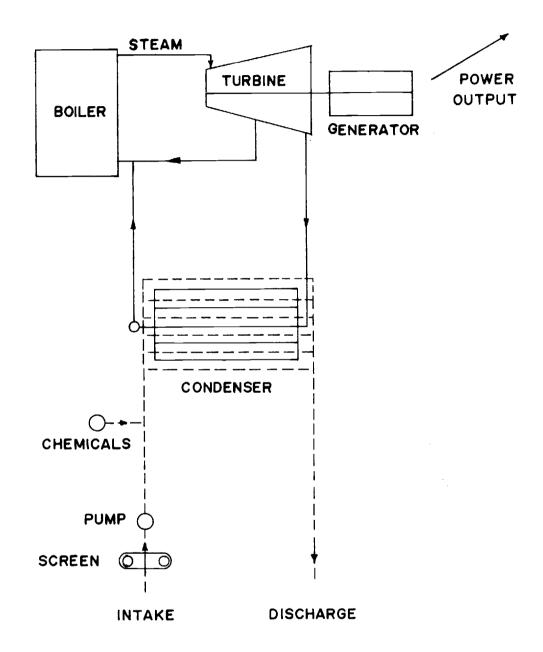
In most published studies, no attention is given to the possibility that one sex may be affected by temperature to a greater extent than the other. This study attempts to examine the interaction of sex with temperature shock. It also provides information on the sensitivity of Oregon copepods to thermal stress.

Heat Dissipation By Power Plants

There are two common types of heat dissipation systems currently employed by power plants. The first, the cooling tower, is a large chimney-like structure. Water is used to accept waste heat from the power plant and to carry it to the tower. Here the heated water is sprayed into the central flue of the unit. Either a natural or a mechanically driven draft blows up through the flue and cools the spray by evaporation. The cooled water falls to the bottom of the tower, collects and is reused. As the water is circulated again and again, it becomes more concentrated in dissolved salts and must eventually be discharged from the plant. This water is discharged little by little rather than all at once, and it is termed "blowdown." Waselkow (1969) provided blowdown rate information for a small, 100 megawatt (MW) plant. Extrapolating from his values, a "typical" 1000 MW coal or gas fired power plant would have a blowdown rate of roughly 0. 15 m³/sec. Cooling towers also lose water to evaporation. The volume of water lost to evaporation is approximately the same as that lost to blowdown. Thus, a crude estimate of water usage by a 1000 MW power plant is 0.30 m³/sec. Because of the nature of the cooling tower system, essentially 100% of the animals which enter it with the cooling water are killed by the time blowdown occurs. Cooling towers, thus, have drastic effects on organisms in the water they use. However, the amount of water they use is relatively small.

The other means of dissipating heat is a "once-through" cooling system. In this, coolant water is brought to the plant via an intake pipe, is passed through tubes in the condenser of the plant where it picks up waste heat, and is then discharged into a river, lake, estuary, or other body of water (Figure 1). This scheme is sometimes modified by having a cooling basin or lake between the power plant and the body of water which ultimately accepts the thermal effluent.

The conditions imposed by once-through cooling systems are perhaps less severe on any given unit of water than those of cooling towers. However, once-through systems require huge volumes of water and, therefore, affect much larger numbers of animals. A



Schematic of a Steam Electric Generating Station Redrawn from Parker and Krenkel 1969

FIGURE I

1000 MW thermal power plant with once-through cooling uses water at a rate of 30-40 m³/sec (Morgan and Bramer, 1969; Chadwick, 1974; Zeller et al., 1969). This is 100-150 times as much water as is necessary for similarly sized plants with cooling towers.

Waste Heat and Water Use

The problem of waste heat begins with the fact that the production of electricity from thermal energy sources (gas, oil, coal, and nuclear reactions) is an inefficient process. Most generating facilities are 30-40% efficient, depending on the size of the units and the source of energy (Tillinghast, 1969). In general, nuclear reactors are nearer the lower end of this range: about 33% for large plants, and 30% for smaller ones. Conventional coal and gas fired steam electric generators tend toward the upper end of the scale, and efficiencies near 40% are not uncommon in newer units.

Thus 60-70% of the energy released at an electric generating station does not go toward the production of electricity. Instead, it is waste heat which must be removed and dissipated. Ninety-five percent of the waste heat produced by nuclear reactors is removed by cooling water. For conventional coal or gas fired generators, the figure is 85%. The difference between these two values is the amount of heat escaping through the chimney of fossil-fuel facilities. Accepting and dissipating this waste energy requires nearly one-third of the water used for all purposes in the United States (Krenkel and Parker, 1969; Warren, 1969; Woodson, 1969).

Even for a single power plant, the volume of water used is large. Facilities run by fossil fuels require an average of about 3.0- 3.5×10^{-2} m³ of cooling water per second per megawatt of output (Zeller et al., 1969). For a typical 1000 MW power station producing at full capacity this means about 33 m³/sec. In a day's time, 2.85 x 10^6 m³ are used. Nuclear power plants require approximately 50% more cooling water than do conventional ones (Morgan and Bramer, 1969). A 1000 MW nuclear plant might, therefore, use 4.3 x 10^6 m³ of water per day.

The volume of water used by a power plant depends on a number of factors. Since the purpose of the water is to remove heat, the volume used can be adjusted by changing the amount of heat that each unit of water removes. The change in the temperature of water as it passes through the plant (Δ T) is a measure of this heat removal. Exactly which balance between water volume and Δ T is employed ultimately depends on such factors as availability of water, heat exchange efficiencies, pumping costs and environmental protection considerations.

Stresses of Entrainment on Zooplankton

Zooplankton are greatly affected by power plants because they

are usually unable to escape from the currents produced by a power plant's water intake, and are therefore sucked into and through the cooling system. In most nearshore areas, including the one in which this study was conducted, the majority of zooplankton both in terms of numbers of individuals and biomass are copepods (Hardy, 1970). These animals make up the bulk of what is commonly called the second trophic level of the oceans. They represent an essential step in the ecological transfer of food energy from phytoplankton to the higher animals including man. Thus, the choice of copepod species as the subjects of this research was appropriate.

The influence of a power plant on the plankton begins when an organism is incorporated into the cooling water flow. This is termed intake entrainment (Coutant, 1974). Clark and Brownell (1973) indicate that as an organism passes the screen at the water intake, it is subjected to turbulent and abrasive forces. Shortly thereafter it may be exposed to biocides, usually chlorine, which are added intermittently to discourage fouling organisms. The organism experiences turbulence and biocides, if present, throughout its passage. Just before reaching the water pump which runs the system, the organism encounters a slight pressure drop. Beyond the pump, pressure increases again as the animal passes into the condenser tubes. Here the plankter is subjected to thermal stress as the water accepts waste heat from the plant. Once out of the condenser tubes the animal passes out of the plant where the pressure drops suddenly to normal. Temperature returns to ambient slowly in the effluent flow.

Direct information on turbulence and abrasion effects are difficult to obtain, but may be inferred from estimates of water speed within a power plant. Millstone Point nuclear power station is a moderately sized (650 MW) facility which uses cooling water at a rate of approximately 25 m³/sec. The water is pumped from Long Island Sound and has a velocity of 0.15-0.3 m/sec at the cooling water intake. By the time the water reaches the screens it is moving 0.5-0.6 m/sec. In the condenser tubes and discharge delivery channel the velocity is 1.8-2.4 m/sec (Clark and Brownell, 1973). Under these conditions severe impact and abrasion damage to an entrained organism seem quite possible.

Pressure changes are due primarily to pumping. As water nears the pump, the pressure drops slightly just ahead of the impeller. Once past the impeller, the pressure builds to $1.7-2.1 \times 10^5$ newtons per square meter (20-30 psi) at the condenser tubes. Beyond the condenser tubes the pressure drops quickly to $1.4-3.5 \times 10^4$ newtons per square meter (2-5 psi) (Clark and Brownell, 1973).

Biocides, when present, are in initial concentrations of 1-15 parts per million (ppm). At coastal and estuarine power facilities concentrations of 4-5 ppm are typical. Chlorine gas is the most common biocide. It is usually administered for about thirty minutes at a time with three or four applications per day. The purpose of biocides is to prevent biological fouling of the cooling system. Other anti-fouling methods such as backflushing with hot water and mechanical scoring are also employed by some power plants (McLean, 1973; Tarzwell, 1972).

Temperature changes are usually 8-12°C, but they can be as high as 16°C. The temperature rise occurs in the condenser tubes over a period of a few seconds to two minutes. The entire journey from the mouth of the intake pipe to the outfall site takes from less than two minutes to nearly fifteen minutes depending on the particular plant involved (Barnett, 1972; Coutant, 1974; Icanberry and Adams, 1974; Zeller et al., 1969).

Effects of Entrainment

The once-through cooling system of an electrical power generating plant presents many hazards to entrained organisms. The organisms may be affected by each of these hazards individually or in combination. Many studies have looked at the overall results on planktonic animals of passage through a cooling system. Others have attempted to isolate the effects of each factor. Unfortunately, there is not close agreement of results among the various studies. This may be due in part to the variety of conditions presented by different power plants at different times.

In an elaborate study involving a nuclear power station on Long Island Sound, Carpenter, Peck and Anderson (1974) found that a large percentage of the entrained copepods were killed by passage through the plant and its receiving pond. They were able to obtain samples during times when there was: 1) temperature shock (ΔT) but no chlorination; 2) chlorination but no temperature shock; and 3) neither temperature shock nor chlorination. Without chlorination or temperature shock mortalities were 71%, 69%, and 48% on different days. This mortality was attributed to mechanical and hydraulic stresses. With no chlorination but with a ΔT of 15.8°C (plus mechanical and hydraulic stresses) mortality was 94%. When no temperature shock was involved but chlorination ranged from 0.2-1.0 ppm, mortalities ranged from 67-83% and were not related to the magnitude of chlorine applied. From this evidence the authors concluded that hydraulic and mechanical stresses were of primary importance in determining copepod survival at that power plant. They did, however, leave open the possibility that other effects could be masked by the magnitude of the mechanically regulated mortality.

These same researchers also found that only about 15% of the copepods were dead as they left the discharge pipes. However, copepods at the discharge pipes sank at 2.5 times the rate that was typical of individuals which had not passed through the condenser tubes. This perhaps indicates that entrained organisms were injured and unable to maintain their positions in the water column. Stratified plankton tows in the receiving pond showed 16 times as many copepods at 25 m as at 2 m. In addition, 60% of the animals at 25 m were dead. Further experiments showed that copepods which passed through the plant died at an accelerated rate regardless of the receiving water into which they were placed. After only 3.5 days 50% of the copepods were dead. In 5.0 days 70% were dead. This compares with only 10% mortality among animals collected at the intake and held 5.0 days under similar conditions.

At the power plant on the Indian River Estuary in Delaware, Davis and Jensen (1974) found no discernible temperature effects $(\Delta T = 6^{\circ}C)$, but found significant effects from chlorination. At chlorine levels of 0.25-1.00 ppm, copepod survival was greater than 60%. When the level exceeded 1.00 ppm, survival dropped to 0-15%. Similar chlorine effects at two other power plants were alluded to by Heinle, Millsaps, and Millsaps (1974). Unfortunately, the data they cite were unpublished. Greater than 90% mortality among <u>Acartia</u> <u>tonsa</u> exposed to 2.5 ppm chlorine for five minutes was reported by McLean (1971). Chlorination would, therefore, seem to be an important factor in copepod survival under some circumstances.

Icanberry and Adams (1974) looked at four power plants on the coast of California. During their sampling periods there was no chlorination so mortalities were due only to temperature, mechanical injuries, and hydraulic stresses. Samples were taken over a span of 10 to 11 months at each power plant. Mean mortalities of adult copepods from the individual stations varied from 0.19% to 13.01%. These numbers included copepods "... appearing to have lost their ability to swim effectively..." as well as those which were totally immobile. The overall mean of all locations combined was 6.20% mortality. The researchers also tested the possibility that entrained organisms died slowly after passage through the condenser. Copepods were collected from the intake and discharge at one of the power plants and were kept at ambient temperature in 1000 ml beakers for 24 hours before counting. No significant difference in mortality was seen. However, organisms kept at the discharge temperature for the same period did show significantly higher mortality.

The lack of consistency among the results of entrainment effects studies probably reflects the variable nature of power plant design and operation as well as differences of approach among investigators. However, mechanical, hydraulic, and chemical (biocidal) stresses are seen to be important under some conditions. By comparison, temperature seems a lesser problem. Nevertheless, it may be important in extreme situations or in augmenting other stresses.

Physiology of Heat Death

In order to examine how heat causes death, it is necessary to

consider literature that does not deal directly with copepods. However, the principles apply to copepods as well as to other animals.

Heat death involves many complicated interrelated physiological processes. Furthermore, the relative importance of each process may depend on the temperature to which the organism is subjected. It may also depend on the organism's genetic constitution, size, stage of development, sex, general physiological fitness, and previous history. Reviews of the subject have been undertaken by Bowler (1963) and de Sylva (1969) among others. Some of the extant ideas on the mechanisms of heat death are presented below.

Protein denaturation has long been considered a factor in heat death. This may be the case at extremely high temperatures, but it is unlikely at more moderate ones. Lehninger (1975) sets the lower limit for denaturation of proteins at 60-70°C. This is far above the temperature at which most organisms succumb.

Coagulation of proteins can occur at temperatures lower than those required for denaturation. This can have serious consequences for the animal. Proteins of the circulatory system can coagulate and partially block blood vessels associated with oxygen exchange. Respiratory malfunction follows. However, relatively high temperatures are required, and de Sylva (1969) indicates that this process probably is not important for marine animals at temperatures below 56°C.

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It is well known that the metabolic rate increases as temperature rises. De Sylva (1969) contends that under conditions of thermal stress this increased metabolism may demand more oxygen than the organism can obtain. As a result the organism goes deeper and deeper into oxygen debt and eventually dies of asphyxiation. If this contention is true, the process could certainly occur at temperatures much nearer the known thermal limits of organisms than could either coagulation or denaturation of proteins.

Still another potential factor in heat death is the failure of nerves to synapse properly. Such failure could cause the loss of pacemaker and smooth muscle functions as well as the complete breakdown of voluntary muscle control. Apparent loss of control over voluntary muscle is a common symptom among thermally stressed organisms, and its occurrence is widely reported in the literature. Indeed, the point at which it first occurs has been considered important enough by several authors, including Gonzalez (1974), to warrant a special name (critical thermal maximum). The fact that muscles falter under conditions of thermal stress does not necessarily mean that synaptic malfunction is the cause. However, it is a possibility which should be considered.

Moore (1976) found that the response of the mussel <u>Mytilus</u> <u>edulis</u> to thermal stress involved changes in the structure and function of membranes. In particular, he found that animals close to death showed a marked loss of "cellular integrity" which he attributed to the "... autolytic activity arising from a catastrophic breakdown of lysosomal regulation."

A closely related phenomenon is the breakdown of the cell's "cation pump." As cellular membranes are altered, an organism's ability to transport ions across them may be impaired. The result is an improper balance of ions (especially sodium and potassium) in various body tissues. This can have serious consequences for the animal, and it can eventually lead to death. As an aside, Bowler (1963) has suggested that "... acclimation to temperature may be brought about by alteration in the enzyme systems which donate energy required in the functioning of tissue 'cation pump'." This alteration could require a simple change in the quantity of the enzymes available, or it might include the production of other isozymes more suited to the new needs of the animal (Somero, 1969). Acclimation to temperature, however, was not a subject addressed by the experiments reported here, and it is mentioned only in passing.

Finally, Fry (1958) suggests that temperature may cause changes in the viscosity of protoplasm which affect the metabolic rate and ultimately the temperature resistance of the cell. The mechanism he proposes is one in which water bound to cellular proteins helps to stabilize those proteins as colloidal structures. Temperature increases release some of this water making the structure less stable. However, the released water decreases the viscosity of the protoplasm thereby increasing the metabolic rate. Heat death may occur when the structure of the colloidal protein-water particles breaks down and the function of the protein is lost.

Death, of course, is not the only effect that temperature shocks can have on an animal. It is simply the most obvious. Less conspicous and more subtle are some of the sublethal effects. Although these are not the subject of this study, they are worth mentioning here. High temperatures increase the rates of metabolism and respiration in animals. This may occur at a time when the oxygen concentration of the water may be decreased due to the new higher temperature. The toxicity of some biocides also increases with temperature. Chlorine and DDT fall into this category (Hargis and Warinner, 1969). Feeding rates, behavior, reproduction, growth rates, distribution, and migration are functions of temperature too. In addition, an organism weakened by exposure to unaccustomed temperatures is probably more vulnerable to attack by predators and diseases. In the final analysis, these sublethal effects may be very important to copepods entrained in cooling water (de Sylva, 1969; Hargis and Warinner, 1969; Mihursky et al., 1970).

MATERIALS AND METHODS

Sample Collection

Zooplankton samples were collected by near surface (0 to 5 m) tows with a Clarke-Bumpus sampling net fitted with 254 μ m nylon mesh. The collections were made in the Pacific Ocean near Newport, Oregon. Sites of sampling varied between one-half and one mile from shore. Sampling occurred at irregular intervals from June 3, 1975 to June 28, 1976.

Ten minute tows at approximately one knot were made in order to minimize physical stresses to the copepods due to the act of collection. Simultaneously, 12 to 16 l of surface seawater were collected in a 19 l polyethylene bucket. The tow samples were placed into the bucket of seawater and were transported immediately to the Oregon State University Marine Science Center.

Water Bath

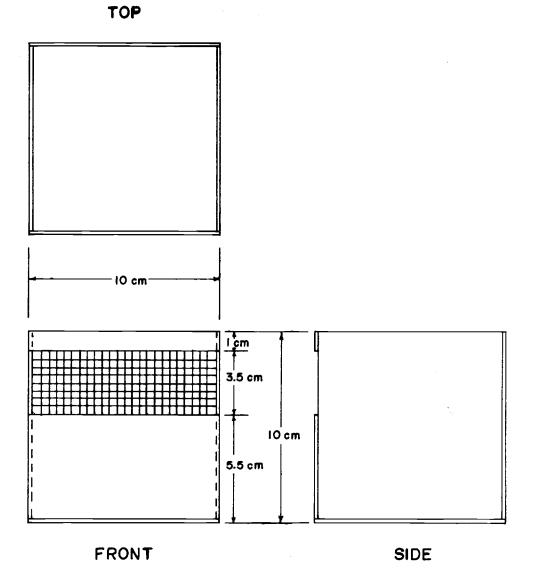
Once at the Marine Science Center, the samples were placed in a 50 l water bath for storage. The bath was maintained at 10°C by an antagonistic heating/cooling system. Cooling was provided by a Bronwill[®] Model CTC-25 chiller. A heater attached to a Versa-Therm[®] Electronic Temperature Controller Model 2149-2 provided heating. This allowed the bath to be controlled to a tolerance of slightly better than ± 0.5 °C. Uniform distribution of temperature was insured by constant stirring using a T-line Laboratory[®] Stirrer Model 107. The 10°C temperature of the water bath was chosen to approximate the temperature of the water from which the copepods were collected. Measured seawater temperatures taken at the time of the collections varied from 8° to 14°C.

Predosing Procedures

Copepods were held in the water bath overnight. This delay allowed those individuals which were injured during the capture or transport phases of the experiment to settle to the bottom of the bucket. Next, 6-7 1 of the collected sample were carefully siphoned from near the surface of the water in the bucket. This portion contained the most actively swimming (and thus the most obviously "alive") copepods. The copepods were concentrated by passing the siphoned water through a sieve fitted with 254 μ m mesh (Figure 2). The seawater thus screened was caught in two one-gallon jars. Copepods were introduced to one of these, and both jars were returned to the water bath.

Thermal Dosing Procedure

The purpose of the dosing procedure was to approximate the temperature conditions in the condenser tubes of a power plant. It was therefore necessary to devise a procedure by which temperature



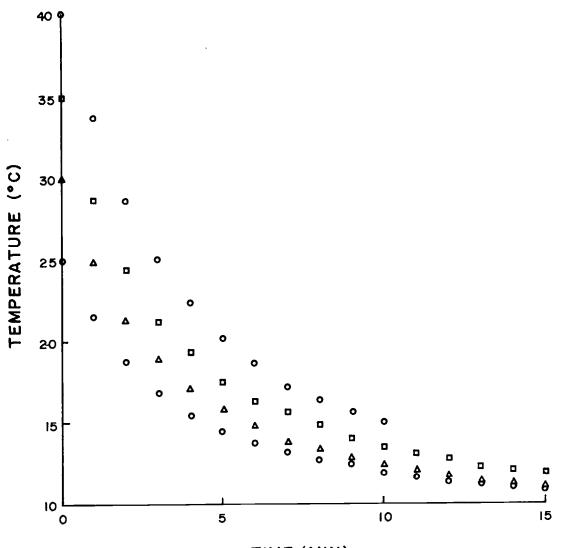


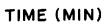
could be elevated quickly and maintained for desired periods of time.

Using a polyethylene turkey baster as a large pipette, portions of the copepod-containing seawater were transferred to 100 ml polyethylene Tri pour beakers and held in the water bath. Meanwhile, seawater from the second jar had been heated on a hotplate. The temperature of the heated water was adjusted so that equal mixtures of it with seawater at 10°C would create the desired shock temperature of 20°, 25°, 30°, 35°, or 40°C. An equal portion of this water was then pipetted into one of the Tri-pour beakers containing copepods. The result was an instantaneous temperature increase. The elevated temperature was maintained by placing the beaker in a water bath of appropriate temperature for the desired duration of exposure. Beakers were then returned to the 10°C bath and the temperature was allowed to decay as shown in Figure 3. For instantaneous or zero duration tests, no elevated temperature bath was necessary, and the beakers were immediately placed back in the 10°C bath. The beaker of heated water was readjusted for the next randomly chosen shock temperature, and the process was repeated. As a control, seawater of 10°C was added to the copepod mixtures which were already at 10°C. The samples thus treated were left in the water bath for an appropriate delay before being scored.

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DECAY OF TEMPERATURE IN EXPERIMENTAL DISHES







Sensitivity of the Method

The heated water was controlled at ± 0.5 °C. The amount of water transferred via the turkey baster in the dosing procedure was determined to average 48.2 ml with extremes of 47 and 50 ml. These are based on 60 trials. If the extremes of volume are combined with the extremes of temperature, a dosage range can be determined as follows:

> The highest possible temperature which was assumed to be 40°C would result from mixing 50 ml of 70° + 0.5° C water with 47 ml of 10° + 0.5° C water. The resulting mixture is 41.43°C. The lowest possible temperature which was assumed to be 40°C would result from mixing 47 ml of 70 - 0.5° C water with 50 ml of 10 - 0.5° C water. The mixture is 39.09°C.

The technique, then, gives the desired temperature $\pm 2^{\circ}$ in the worst possible case. For most temperatures and in most cases the range is probably closer to $\pm 1^{\circ}$ C.

Staining

Live-dead determinations were made using the vital stain, neutral red, as described by Dressel et al. (1972) and Crippen and Perrier (1974). Basically, the method involves introducing stain in a final concentration of 1 to 100,000 to the seawater containing the copepods. The stain itself is not toxic. Living copepods incorporate the stain in their cells and turn red. Dead copepods do not incorporate the stain and remain white or slightly pink. After an hour, the copepods are killed, fixed with 5 ml of 1 M acetic acid per 100 ml of solution, and stored. Storing stained samples in acidic conditions increases the color imparted by the stain.

Theoretically, the process is only slightly more complicated. Neutral red is the salt of a weak dye base and an inorganic acid. The stain (as a chloride salt) dissociates in water to give the positively charged and red colored dye ion. At pH's of 7 or more, this ion is converted to its undissociated hydroxide (ROH) which is apolar and lipid soluble. It is therefore soluble in the plasma membrane and diffuses into the cell. If the stain molecule then encounters an acidic environment within the cell, it dissociates and produces the red colored R^+ ion. This ion, because of its polarity, is unable to diffuse out of the cell. The stain accumulates. Dead cells lack the acidic environments and the selectively permeable membrane necessary for staining (Levitt, 1969).

For the same reasons that dead cells do not accumulate stain, stained cells which are subsequently killed do not retain their color indefinitely. Counting must be done within a matter of days. Acidifying the storage solution of the stained copepods insures that all stain present will be ionized to the visible red form, and it slows the loss of stain from the cells so long as the membrane is intact. All of the samples considered in this study were counted within three days

. . .

after storing.

Counting

Only three species of copepods were present in sufficient quantity to be counted. These were <u>Acartia clausi</u>, <u>Acartia longire</u>-<u>mis</u>, and <u>Centropages</u> sp. Each individual was classified as male, female, or copepodite (immature). Each was also classified as alive or dead.

Nature of Experiments

Table 1 shows the combination of treatments that were observed during these experiments. It can be seen that experiments 1-8 examine the effects of instantaneous or zero duration temperature shocks. Within this group, experiments 1-4 represent an attempt to determine the effect on survival of the time delay before scoring. This delay is the time in hours after exposure to temperature shocks before the copepods are stained. That is, it represents the period during which copepods had to die in order to be scored as dead. Experiments 9-13 look at the effect of the duration of exposure to elevated temperatures. In experiments 5-13 the delay was set at four hours as a matter of experimental convenience.

| Species | Exp. # | Date | Delay Hour s | 10° | | Terr | | ure (°C 0° |) | | | ; | 25° | | | 30° | | 35° | 40° |
|---------------|-----------|-------------------|------------------------|--------------------|---|------|----|---------------|------|---|----|----|-----|-----|---|-----|----|-----|-----|
| | | | | Duration (Minutes) | | | | | | | | | | | | | | | |
| | | | | 0 | 0 | 30 | 60 | 1 2 0 | 1440 | 0 | 15 | 30 | 60 | 120 | 0 | 15 | 30 | 0 | 0 |
| A. clausi | 1 | 6/3/75 | 1 2 | х | х | | | | | х | | | | | х | | | х | |
| A. clausi | 2 | 6/3/75 | 24 | Х | х | | | | | x | | | | | Х | | | Х | |
| A. clausi | 3 | 7 /22 /75 | 1 | Х | | | | | | х | | | | | Х | | | Х | Х |
| A. clausi | 4 | 7/22/75 | 24 | Х | | | | | | х | | | | | Х | | | Х | х |
| A. clausi | 5 | 7 /2 7 /75 | 4 | Х | | | | | | х | | | | | х | | 7 | Х | Х |
| A. longiremis | 6 | 8/5/76 | 4 | Х | | | | | | х | | | | | х | | | Х | Х |
| Centropages | 6 | 8/5/75 | 4 | Х | | | | | | х | | | | | Х | | | Х | х |
| Centropages | 7 | 8/14/75 | 4 | Х | | | | | | х | | | | | х | | | Х | Х |
| A. longiremis | 8 | 8/19/75 | 4 | Х | | | | | | х | | | | | х | | | Х | Х |
| A. clausi | 8 | 8/19/75 | 4 | Х | | | | | | х | | | | | х | | | Х | Х |
| A. clausi | 9 | 9/15/75 | 4 | х | | | | | | х | х | Х | Х | х | х | х | Х | Х | Х |
| A. longiremis | 11 | 5/18/76 | 4 | х | Х | х | Х | х | | х | | х | Х | х | х | х | Х | | |
| Centropages | 11 | 5/18/76 | 4 | х | Х | х | Х | | Х | Х | | Х | Х | х | Х | х | Х | | |
| Centropages | 12 | 6/7/76 | 4 | х | Х | х | | х | | х | | Х | Х | х | | | | | |
| A. clausi | 13 | 6/28/76 | 4 | Х | | | | | | x | Х | х | х | х | х | х | х | х | |

TABLE 1. Temperature-Duration-Delay Treatments Present in Each Experiment.

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Statistics

Data for these experiments are the numbers of live and dead copepods by species, sex and treatment category. From this, the surviving fraction was calculated. The arcsine or angular transformation ($x = \sin^{-1} \sqrt{surviving fraction}$) was applied to the data before statistical analysis. This transformation is suggested by Sokal and Rohlf (1969) in order to prevent the variance being a function of the mean as it would be if fraction surviving were used.

A standard analysis of variance (ANOVA) with "F" tests was performed on the transformed data. This was done on the CDC 3300 computer system at Oregon State University. Because of limitations of the method, an analysis of variance cannot be performed on experiments which have unequal numbers of data points in the various treatments. Since there were missing data in several of the experiments, the computer would not handle the data as they existed. The various schemes for estimating missing data were not appropriate because of the large number of missing points in some experiments. Wherever possible, the problem was circumvented by eliminating the missing data points and randomly deleting data points from the other treatments in order to create a balanced set. For example, in an experiment with six replicates at each of eight treatments, there might be no data for one replicate of treatment number two. In this case, that missing point would be dropped along with a randomly selected point from each of the other treatments. The result would be a set of experimental data with eight treatments as before but only five instead of six replicates of each treatment. This procedure causes some data to be excluded. However, so long as the lost data points are chosen randomly, the method is statistically valid. Where and to what extent this method was applied is indicated in the next section.

The analysis of variance tables indicate which factors represent a significant source of variation. They do not, however, indicate the point at which a factor such as temperature becomes significant. For this purpose, "t" tests were employed. In the case of instantaneous temperature shocks, the "t" test evaluates the difference between the mean of the 10°C control and the mean of the other selected treatment. Where the shocks were prolonged, the "t" test compares the mean for the instantaneous (zero duration) treatment at that temperature with the mean for some specified duration. Tabulated results are presented in Appendix I.

RESULTS

Summed Data

The data for each treatment and sex category are summed over the 13 experiments and are presented as Table 2. The table provides the mean percent survival for each treatment as well as the number of individuals (N) on which this percent is based.

Effect of Time Before Scoring ("Delay")

Experiments one through four were designed to test the effect of time delay before scoring on the survival rate of the copepods. Experiments one and two tested the difference between delays of 12 hours and 24 hours, respectively. Both experiments were run simultaneously using copepods collected from a single sampling trip. Delays of one hour and 24 hours were examined in experiments three and four. Again, these were run simultaneously. The results are presented graphically in Figure 4. Analysis of variance tables are provided in Table 3, and "t" tests comparing the means of treatments with controls are given in Appendix I. It should be noted at this point that all statistical calculations were performed on arcsine transformed data ($\sin^{-1}\sqrt{\text{fraction surviving}}$). However, for clarity of presentation, all graphs show the data as percent survival.

Delay before scoring is not a statistically significant source

| | Treatment | Male | s | Femal | e s | Copepodit | es. |
|-----------|-----------------|------------|-------------|------------|------------|------------|-------------|
| Species | (Temp-Duration) | % Survival | N | % Survival | N | % Survival | N |
| A. clausi | 10-0 | 82 | 399 | 98 | 614 | 88 | 221 |
| | 20-0 | 98 | 188 | 100 | 134 | 100 | 75 |
| | 25-0 | 81 | 43 2 | 98 | 746 | 96 | 305 |
| | 25-15 | 53 | 36 | 96 | 93 | 94 | 77 |
| | 2 5 - 30 | 3 | 35 | 49 | 98 | 38 | 55 |
| | 2 5 - 60 | 6 | 35 | 41 | 66 | 14 | 35 |
| | 25-120 | 3 | 38 | 15 | 97 | 11 | 53 |
| | 30-0 | 30 | 378 | 85 | 607 | 77 | 2 47 |
| | 30-15 | 19 | 37 | 57 | 60 | 72 | 65 |
| | 30-30 | 8 | 37 | 19 | 62 | 28 | 57 |
| | 35-0 | 12 | 353 | 40 | 663 | 42 | 2 64 |
| | 40-0 | 1 | 126 | 3 | 376 | 1 | 153 |
| | | | | | | | |

TABLE 2. Sums of Data From all Experiments.

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| | Treatment | Male | s | Female | S | Copepod | lites |
|---------------|-----------------|------------|-----|------------|-------------|------------|-------|
| Species | (Temp-Duration) | % Survival | N | % Survival | N | % Survival | N |
| A. longiremis | 10-0 | 77 | 140 | 96 | 308 | 87 | 62 |
| | 20-0 | 90 | 20 | 99 | 73 | 100 | 21 |
| | 20-30 | 10 | 21 | 63 | 89 | 59 | 17 |
| | 20-60 | 0 | 18 | 87 | 91 | 71 | 17 |
| | 20-120 | 0 | 19 | 34 | 76 | 32 | 19 |
| | 25-0 | 90 | 143 | 99 | 409 | 95 | 110 |
| | 25- 30 | 20 | 20 | 74 | 31 | 17 | 12 |
| | 25-60 | 8 | 12 | 58 | 31 | 77 | 13 |
| | 25-120 | 33 | 18 | 16 | 32 | 20 | 20 |
| | 30-0 | 2 6 | 138 | 86 | 367 | 70 | 83 |
| | 30-15 | 6 | 35 | 11 | 70 | 29 | 1′ |
| | 30-30 | 9 | 22 | 14 | 37 | 24 | 1 |
| | 35-0 | 0 | 83 | 15 | 2 50 | 10 | 8 |
| | 40-0 | 0 | 56 | 2 | 256 | 0 | 8 |

TABLE 2. continued.

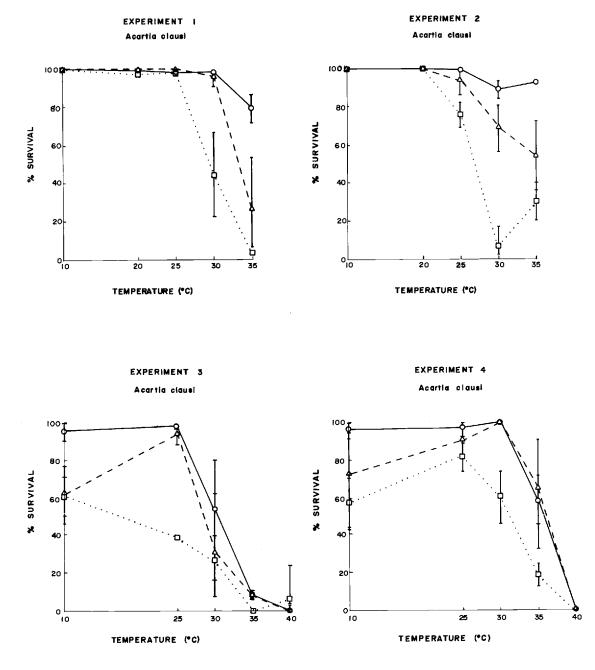
. . . .

| | Treatment | Mal | es | Female | s | Copepodi | tes |
|-------------|-----------------|------------|-------------|------------|------|------------|-----|
| Species | (Temp-Duration) | % Survival | N | % Survival | N | % Survival | N |
| Centropages | 10-0 | 92 | 238 | 98 | 129 | 94 | 88 |
| | 20-0 | 95 | 73 | 100 | 71 | 100 | 32 |
| | 20-30 | 93 | 55 | 96 | 51 | 100 | 30 |
| | 20-60 | 58 | 38 | 98 | 47 | 100 | 15 |
| | 20-120 | 11 | 36 | 100 | . 15 | 86 | - |
| | 20-1440 | 50 | 28 | 76 | 38 | 50 | (|
| | 2 5-0 | 96 | 2 80 | 99 | 125 | 96 | 94 |
| | 2 5 - 30 | 66 | 76 | 93 | 56 | 81 | 3 |
| | 25- 60 | 72 | 67 | 93 | 73 | 91 | 3 |
| | 25-120 | 22 | 54 | 78 | 58 | 55 | 2 |
| | 30-0 | 57 | 183 | 96 | 110 | 85 | 9 |
| | 30-15 | 7 | 43 | 17 | 47 | 23 | 1 |
| | 30-30 | 3 | 34 | 17 | 42 | 32 | 3 |
| | 35-0 | 1 | 100 | 25 | 57 | 37 | 7 |
| | 40-0 | 0 | 133 | 8 | 53 | 0 | 4 |

TABLE 2. continued.

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| Source of Variation | DF | Mean Square | F |
|-----------------------|----|------------------|-----------|
| Delay | | 276,78 | 2.67 n.s. |
| Temperature | 4 | 7416.36 | 71.67 ** |
| Delay*Temperature | 4 | 954.37 | 9.22 ** |
| Sex | 2 | 3970 .2 1 | 38.36 ** |
| Delay*Sex | 2 | 48.91 | 0.47 n.s. |
| Temperature*Sex | 8 | 912.81 | 8.82 ** |
| Delay*Temperature*Sex | 8 | 81.38 | 0.79 n.s. |
| Error | 60 | 103.49 | |
| Total | 89 | | |
| | | | |

TABLE 3. Analysis of Variance for Arcsine Transformed DataExperiments 1 and 2, A. clausi.

Experiments 3 and 4, <u>A. clausi</u>

| Source of Variation | DF | Mean Square | F |
|-----------------------|----|-------------|-----------|
| Delen | 1 | 3071.61 | 11.11 ** |
| Delay Temperature | 4 | 12447.10 | 45.01 ** |
| Delay*Temperature | 4 | 2187.87 | 8.91 ** |
| Sex | 2 | 2464.87 | 0.82 n.s. |
| Delay*Sex | 2 | 226.37 | 7.91 ** |
| Temperature*Sex | 8 | 478.23 | 1.73 n.s. |
| Delay*Temperature*Sex | 8 | 454.65 | 1.64 n.s. |
| Error | 60 | 276.54 | |
| Total | 89 | | |
| | | | |

Experiment 5, <u>A.</u> <u>clausi</u>

| Source of Variation | DF | Mean Square | F |
|---------------------|----|-------------------|----------|
| Sex | 2 | 2681.02 | 11.49 ** |
| Temperature | 4 | 19047 .2 0 | 81.64 ** |
| Sex*Temperature | 8 | 758.20 | 3.25 ** |
| Error | 75 | 233.31 | |
| Total | 89 | | |

| Experiment 6, <u>A. longiremis</u> | | | | |
|------------------------------------|----|-------------|-----------|--|
| Source of Variation | DF | Mean Square | F | |
| Sex | 2 | 4169.64 | 30.23 ** | |
| Temperature | 4 | 17140.20 | 124.26 ** | |
| Sex*Temperature | 8 | 835.47 | 6.06 ** | |
| Error | 45 | 137.94 | | |

TABLE 3. continued

Total

Experiments 6 and 7, <u>Centropages</u>, male and female only - no 40°C.

59

| Source of Variation | DF | Mean Square | F | |
|------------------------|---------|-----------------|-------------------|--|
| Experiment | 1 | 8,23 | 0.03 n.s. | |
| Sex | 1 | 7144.69 | 30 .2 1 ** | |
| Experiment*Sex | 1 | 59 2. 38 | 2.50 n.s. | |
| Temperature | 3 | 18040.50 | 76.27 ** | |
| Experiment*Temperature | 3 | 311.34 | 1.32 n.s. | |
| Sex*Temperature | 3 | 515.76 | 2.18 n.s. | |
| Experiment*Sex*Temp | 3 | 116.63 | 0.49 n.s. | |
| Error | 48 | 236.53 | | |
| Total | 63 | | | |
| | | | | |

| Source of Variation | DF | Mean Square | F |
|---------------------|----|-------------|-------------------|
| Sex | 2 | 4190.26 | 14.90 ** |
| Temperature | 4 | 19315.10 | 68 . 72 ** |
| Sex*Temperature | 8 | 563.21 | 2.00 n.s. |
| Error | 60 | 281.07 | |
| Total | 74 | | |

Experiment 8, A. clausi

TABLE 3. continued

Experiment 8, <u>A.</u> longiremis

| Source of Variation | DF | Mean Square | F |
|---------------------|----|-------------|-----------|
| Temperature | 4 | 27446.80 | 186.99 ** |
| Sex | 2 | 1620.03 | 11.04 ** |
| Temperature*Sex | 8 | 431.58 | 2.94 ** |
| Error | 75 | 146.78 | |
| Total | 89 | | |
| | | | |

Experiment 9, <u>A. clausi</u>, instantaneous shock

| Source of Variation | DF | Mean Square | F |
|--------------------------------|--------|--------------------|--------------------|
| Sex | 2 | 618.24 | 5.11 * |
| Temperature Sex*Temperature | 4 8 | 10616.10 303.68 | 87.69 ** 2.51 * |
| - | | 121 07 | |
| Error | 30 | 121.07 | |
| Total | 44 | | |
| | | | |

Experiment 9, <u>A.</u> <u>clausi</u>, duration at 25°C

| Source of Variation | DF | Mean Square | F |
|---------------------|----|-------------|----------|
| Duration | 4 | 10256.40 | 56.33 ** |
| Sex | 2 | 1563.05 | 8.58 ** |
| Duration*Sex | 8 | 494.81 | 2.72 * |
| Error | 30 | 182.07 | |
| Total | 44 | | |

| TABLE | 3. | continued |
|-------|----|-----------|
|-------|----|-----------|

Experiment 9, A. clausi, duration at 30°C

| | | Mean Square | F |
|---------------------|------------|------------------|-----------|
| Source of Variation | DF | | |
| Duration | 2 | 2038.68 | 11.17 ** |
| Sex | 2 | 1 224. 43 | 6.71 ** |
| Duration*Sex | 4 | 191.56 | 1.05 n.s. |
| Error | 18 | 182.51 | |
| Total | 2 6 | | |
| | | | |

Experiment 11, A. longiremis, duration at 20°C

| Source of Variation | DF | Mean Square | F |
|---------------------|----|-----------------|-----------|
| Duration | 3 | 2772.90 | 21.55 ** |
| Sex | 2 | 12999.90 | 101.05 ** |
| Duration*Sex | 6 | 1123.41 | 8.73 ** |
| Error | 24 | 1 2 8.65 | |
| Total | 35 | | |
| | | | |

Experiment 11, <u>A. longiremis</u>, duration at 25°C

| Source of Variation | DF | Mean Square | F |
|---------------------|----|-----------------|----------|
| Duration | 3 | 11675.10 | 89.72 ** |
| Sex | 2 | 2 693.81 | 20.70 ** |
| Duration*Sex | 6 | 650,92 | 5.00 ** |
| Error | 24 | 130.13 | |
| Total | 35 | | |

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| Source of Variation | DF | Mean Square | F |
|---------------------|------------|------------------|----------|
| Duration | 2 | 6957 .42 | 56.77 ** |
| Sex | 2 | 1689.05 | 13.78 ** |
| Duration*Sex | 4 | 1 22 3.52 | 9.98 ** |
| Error | 18 | 1 22. 56 | |
| Total | 2 6 | | |

TABLE 3. continued

Experiment 11, A. longiremis, duration at 30°C

Experiment 11, Centropages, duration at 20°C

| Source of Variation | DF | Mean Square | F |
|---------------------|--------|---------------------------|-----------------------|
| Duration Sex | 3 2 | 2 970.37 124.72 | 14.68 ** 0.62 n.s. |
| Duration*Sex | 6 | 300.51 | 1.49 n.s. |
| Error | 24 | 202.32 | |
| Total | 35 | | |
| | | | |

Experiment 11, Centropages, duration at 25°C

| Source of Variation | DF | Mean Square | F |
|---------------------|----|-------------|----------|
| Duration | 3 | 4031.93 | 14.79 ** |
| Sex | 2 | 5114.88 | 18.81 ** |
| Duration*Sex | 6 | 799.13 | 2.94 * |
| Error | 24 | 271.91 | |
| Total | 35 | | |

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| , | | | |
|---------------------|------------|-------------|-------------------|
| Source of Variation | DF | Mean Square | F |
| Duration | 2 | 11919.00 | 58 .2 7 ** |
| Sex | 2 | 632,36 | 3.09 n.s. |
| Duration*Sex | 4 | 111.62 | 0.55 n.s. |
| Error | 18 | 204.54 | |
| Total | 2 6 | | |
| | | | |

TABLE 3. continued

Experiment 11, Centropages, duration at 30°C

Experiment 12, <u>Centropages</u>, duration at 20°C

| Source of Variation | DF | Mean Square | F |
|---------------------|--------|-------------------|---------------------|
| Duration | 2 | 1576.42 | 15.59 ** |
| Sex Duration*Sex | 2 4 | 4077.37 769.89 | 40.33 ** 7.62 ** |
| Error | 18 | 101.09 | |
| Total | 26 | | |
| | | | |

Experiment 12, <u>Centropages</u>, duration at 25°C

| Source of Variation | DF | Mean Square | F |
|---------------------|----|-------------|-----------|
| Duration | 3 | 1261.85 | 5.65 ** |
| Sex | 2 | 2569.07 | 11.50 ** |
| Duration*Sex | 6 | 500.71 | 2.24 n.s. |
| Error | 24 | 223,32 | |
| Total | 35 | | |

| TABLE 3. continued Experiment 13, <u>A. clausi</u> , instantaneous shock | | | |
|---|-------------|------------------------------|------------------------------------|
| Source of Variation | DF | Mean Square | F |
| Temperature Sex Temperature*Sex | 3 2 6 | 6504.47 1229.98 116.69 | 122.91 ** 23.24 ** 2.20 n.s. |
| Error | 24 | 52,92 | |
| Total | 35 | | |

Experiment 13, <u>A. clausi</u>, duration at 25°C

| Source of Variation | DF | Mean Square | F |
|---------------------|----|-----------------|-----------|
| Duration | 4 | 11599.80 | 97.60 ** |
| Sex | 2 | 24 31.95 | 20.46 ** |
| Duration*Sex | 8 | 118,00 | 0.99 n.s. |
| Error | 30 | 118.85 | |
| Total | 44 | | |
| | | | |

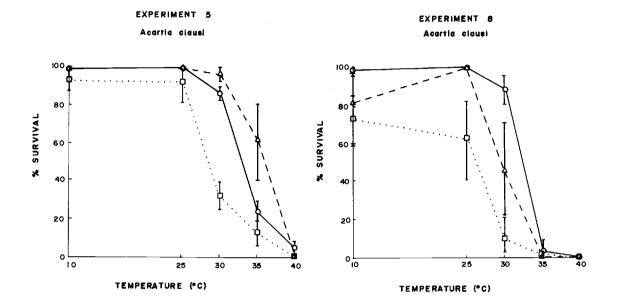
Experiment 13, <u>A. clausi</u>, duration at 30°C

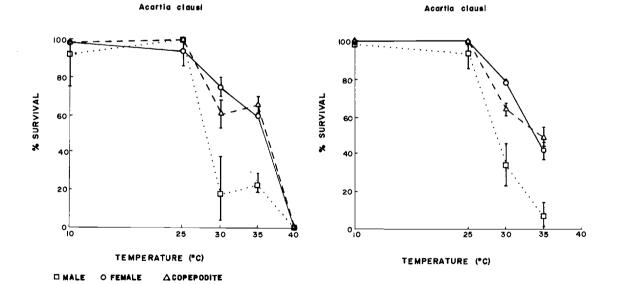
| Source of Variation | DF | DF Mean Square | |
|---------------------|----|-----------------|-----------|
| Duration | 2 | 2305.28 | 26.89 ** |
| Sex | 2 | 2 155.13 | 25.12 ** |
| Duration*Sex | 4 | 227.17 | 2.65 n.s. |
| Error | 18 | 85.74 | |
| Total | 26 | | |
| | | | |

of variation between experiments one and two (12 hrs and 24 hrs). For experiments three and four (1 hr and 24 hrs), the effect of delay is significant at the 99% confidence level. Using "t" tests to compare the responses to the various temperature treatments between the experiments shows that there are four cases in which the means are statistically different at the 95% level or above. These cases are males at 25°C, males at 35°C, females at 35°C, and copepodites at 30°C. In each of these cases the survival at 24 hours is higher than at one hour. This could be a real effect only if the staining technique used to determine survival is affected by delay. Careful examination at each temperature of percent survival versus delay (1 hr, 4 hr, 12 hr, and 24 hr) shows that this is not the case. It therefore seems likely that some other factor such as handling, dosing, or sampling error is responsible for the apparent effect of delay.

Instantaneous (zero duration) temperature shocks on <u>Acartia</u> <u>clausi</u> can be seen graphically in Figure 5. Analysis of variance tables are again given in Table 3, and "t" tests comparing treatments to controls are provided as Appendix I. Note that experiment eight has five replicates per treatment while the others have three. Note also that experiment 13 has no 40°C treatment.

None of the sexes in any of these four experiments shows a significant difference between the survival at 25°C and the 10°C controls. By 30° or 35°C significantly lower survival percentages





EXPERIMENT 13

EXPERIMENT 9

FIGURE 5

occur in all experiments. There are obvious differences among the sexes. These, however, will be addressed later.

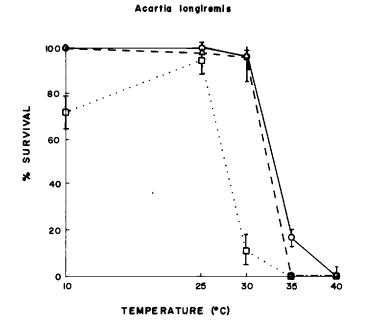
Only two experiments, numbers six and eight, had enough <u>Acartia longiremis</u> to analyze. These are shown in Figure 6. Experiment six originally had six replicates per treatment but was reduced to four replicates because of missing data. Experiment eight had six replicates.

As with <u>A. clausi</u>, survival percentages became significantly lower than controls at temperatures of 30° or 35° ($\Delta T = 20°$ or 25°C). One anomalous reading occurred in experiment six. Survival in males at 25°C was significantly higher (95% confidence level) than in the control. This was the only instance in which this particular anomaly occurred, and it probably represents random error rather than a real event.

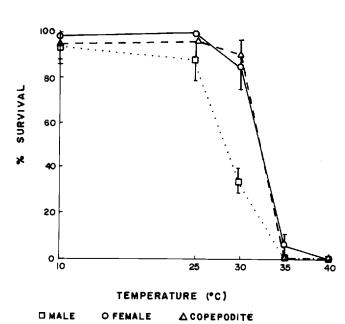
Of the runs which tested the effect of instantaneous temperature shocks only experiments six and seven contained <u>Centropages</u> sp. in sufficient quantity to be analyzed. Even these did not contain enough copepodites to include them in the analysis. Consequently, the analysis of variance tables in Table 3 and the graphs in Figure 7 contain data from males and females only. Because of missing data, both experiments were reduced from six replicates per treatment to four.

The results are similar to those for A. clausi and A. longiremis.

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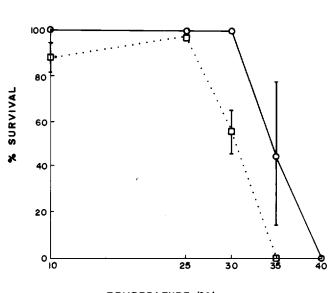


EXPERIMENT 6



EXPERIMENT 8 Acartia longiremis

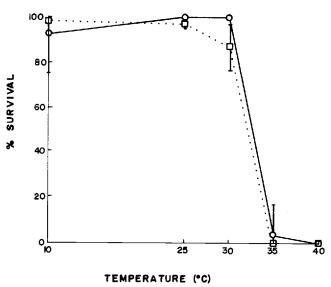
FIGURE 6

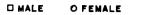


EXPERIMENT 6 Centropages sp.



EXPERIMENT 7 Centropages sp.







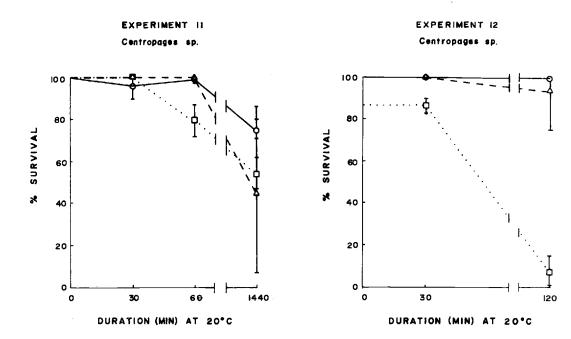
No reduction in survival occurs until the shock temperatures reach 30° or 35°C.

Duration of Exposure

Experiments 9-13 examined the effect of prolonged exposure to temperatures which were generally within the organism's ability to survive during instantaneous shocks. These temperatures were 20°, 25°, and 30°C.

Figure 8 shows the results of extended exposure at 20°C. Two species are represented by the graphs: <u>A. longiremis</u> and <u>Centropages</u> sp. Note that the scale of the ordinate is not continuous. For <u>A. longiremis</u>, only males showed a significant change in survival over 120 minutes of exposure. <u>Centropages</u> males had a highly significant (99% level) drop in percent survival after 60 minutes in experiment 11 and after 120 minutes in experiment 12. Females showed a highly significant drop in survival after 1440 minutes (24 hrs) in experiment 11. Copepodites did not show significant mortality at any duration of 20°C.

Four experiments (9, 11, 12, and 13) including all three species covered the effects of prolonged exposure to 25°C. These are given in Figure 9. <u>Acartia clausi</u> males exhibited markedly lower survival after only 15 minutes at 25°C. Copepodites and females followed suit after 15 to 30 minutes. All classifications of <u>Acartia</u>





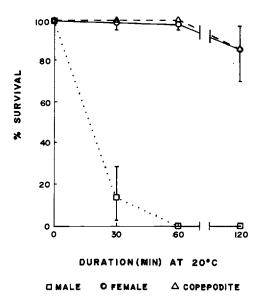
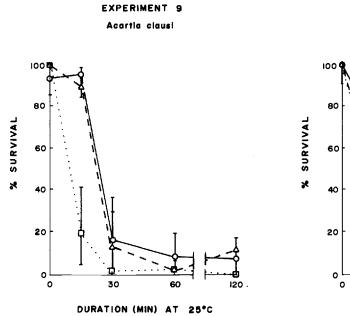
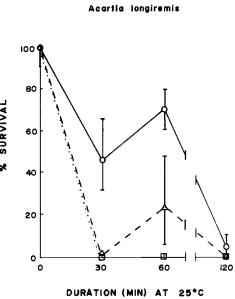


FIGURE 8





EXPERIMENT 11



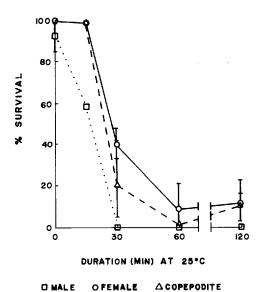


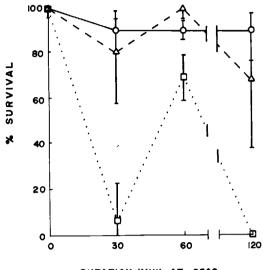
FIGURE 9

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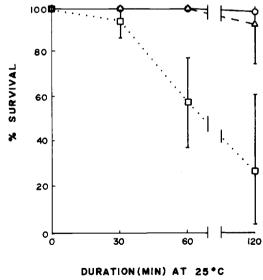


Centropages sp.



DURATION (MIN) AT 25°C

EXPERIMENT 12 Centropages sp.



OFEMALE

△COPEPODITE

FIGURE 9B

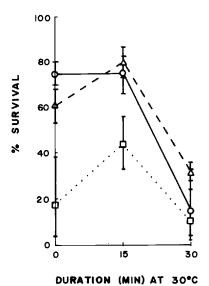
D MALE

<u>longiremis</u> had significantly lower survivals after 30 minutes, but females fared better than copepodites or males. Males of <u>Centropages</u> sp. also appeared to be more susceptible to temperature than females or copepodites. They showed significant differences after 30 to 60 minutes while females and copepodites showed no differences after 120 minutes.

Three of the four experiments which examined the effects of duration at 25°C also looked at the effects of duration at 30°C. These were experiments 9, 11, and 13. The results are graphed in Figure 10. Few copepods of any of these three species survive for 30 minutes at 30°C. Males again seem to fare less well than females or copepodites.

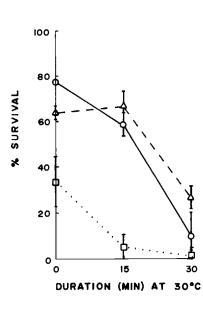
The Influence of Sex on Survival

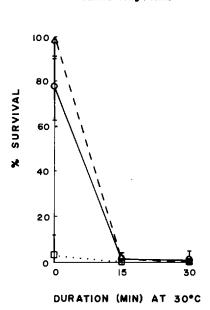
An examination of the analysis of variance data in Table 3 shows that in nearly every experiment sex is a highly significant (99% confidence level) source of variation. There are three exceptions to this. In one case, sex is significant only at the 95% level. The other two cases show no significant variation due to sex. These latter two cases both occur in experiment 11 with <u>Centropages</u> sp. One is the duration exposure to 20°C. The other is duration exposure to 30°C. The preponderance of evidence, however, shows that sex is an important source of variation, and one which should be considered



EXPERIMENT 9

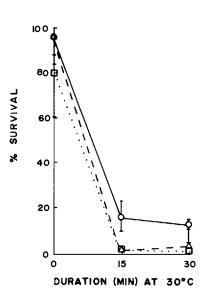
Acartia clausi





O FEMALE

DMALE



Acartia longiremis

EXPERIMENT II

EXPERIMENT II

Centropages sp.

 $\Delta \, \text{COPEPODITE}$

FIGURE 10



Acartia clausi

in experiments of this type.

Seasonality

It is possible that there may be a seasonal component to the ability to survive temperature extremes. Such circumstances are widely reported in the literature for East Coast species which must acclimate to large seasonal temperature differences. These experiments, however, were not designed to address this problem specifically. There is a great deal of variation between experiments in this data, but if a seasonal component does exist, it is not obvious.

DISCUSSION AND CONCLUSIONS

At the very best, it is difficult to relate the results of this study to those of studies reported in the literature. There are problems other than the fact that the various studies represent animals from different geographic areas and environmental conditions. Studies involving operating power plants cannot isolate the effect of temperature from those of mechanical and hydraulic stresses. Laboratory studies vary in the methods used to produce temperature shocks, in the duration of exposure to elevated temperatures, and even in the definition of what constitutes a "dead" animal. Nevertheless, some attempt to compare this study with others is desirable.

In general, this study shows that all three species of copepods tested succumb in significant numbers to instantaneous exposure to temperatures in the range of 30° to 35°C. These animals were acclimated to approximately 10°C in both the ocean and the laboratory. These results are comparable to the results of other studies.

The Ultimate Lethal Temperature (ULT) of Gonzalez (1974) is probably comparable to the instantaneous shock temperature of this study. Although the methods of obtaining these temperatures are different, the highest temperature obtained and the length of exposure to elevated temperature are similar. Using East Coast <u>A. clausi</u> acclimated to 10°C, Gonzalez found ULTs of approximately 33°C.

Tarzwell (1972) found similarly acclimated <u>A. clausi</u> were killed by temperatures of 32°C. Information on the sensitivity of <u>A. longiremis</u> and <u>Centropages</u> sp. is not available in the literature. Consequently, direct comparisons cannot be made. However, Drost-Hansen and Thorhaug (1974) provided both experimental evidence and theoretical arguments that many marine organisms of several phyla may have upper temperature limits of about 31°C. It appears, then, that in terms of survival to instantaneous shocks Oregon copepods are not greatly different from East Coast copepods.

This study clearly demonstrates that copepods from Oregon exhibit markedly decreased survival rates when exposed to 25° C for prolonged periods. Some also show lower survival after long periods at 20°C. This latter effect is not nearly so severe as at 25°C, and it appears to be related to sex. There are no studies which are directly comparable to these "duration" experiments. Gonzalez (1974) described Critical Thermal Maxima (CTM) for <u>A. clausi</u> as being 27°-28°C for individuals acclimated to 10°C. The CTM of an animal is the temperature at which it loses muscular control. This may or may not be the same as the temperature below which the individual can survive for extended periods. It is possible that longer exposure to lower temperatures could also cause death. From his data, it is not possible to accurately determine a correlation between his experiments and these. Tarzwell (1972) also reported that for <u>A. clausi</u>, "...the highest temperature which they can survive..." is 25-27°C. However, he did not indicate the duration of exposure to these temperatures which the copepods could survive. If they are able to survive indefinitely at these temperatures, then East Coast copepods may be more thermal tolerant than their Oregon counterparts. This, however, is gross speculation, and much more data are needed on the subject.

Very few of the studies reported in the literature even consider the possibility that thermal tolerance may be dependent upon sex. Carpenter, Peck and Anderson (1974) examined males and females of <u>A. tonsa</u>. The parameter that they measured was the rate of sinking through the water column of the copepods. They used this rate as a measure of the response of copepods to stresses (including temperature) encountered during passage through a power station. No differences were noted between the sexes.

Bradley (1976), however, found that sex was influential in determining the temperature tolerance range of the copepod <u>Eurytemora affinis</u>. Pre-adult stages were more tolerant than adults, and females were as tolerant as or more tolerant than males.

Little else is available on the interaction of sex and thermal tolerance. In the present study, this interaction was examined closely. All analyses of variance involving <u>A. clausi</u> and <u>A. longire</u>-mis showed that sex is a significant (95%) or highly significant (99%)

source of variation in survival of these species. For <u>Centropages</u> sp. sex contributed significant variation in five of seven cases. Generally, speaking, males of all three species appeared to be less tolerant than either females or copepodites. No attempt was made to sex copepodites and determine if this increased sensitivity was present in pre-adult males. Clear differences between the tolerance of females and copepodites were not apparent in this data. The evidence indicates that sexual differences are important and need to be seriously considered in studies of this type.

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APPENDIX I

| Exp # | Species | Treatment | Males | | Females | | Copepodites | |
|-------|------------|-----------|-------|----------|---------|---------|-------------|-------|
| | | Temp-Dur. | Mean | "t" | Mean | "t" | Mean | "t" |
| 1 | A. clausi | 10-0 | 90.0 | | 90,00 | | 90.00 | |
| | | 20-0 | 82.16 | 1.84 | 90.00 | | 90.00 | |
| | | 25-0 | 85.32 | 1,00 | 85,58 | 1.00 | 90.00 | |
| | | 30-0 | 41.87 | 3.73* | 83.96 | 1.00 | 81.14 | 1.00 |
| | | 35-0 | 11.58 | 132.58** | 63.26 | 5.03** | 27.24 | 3.73* |
| 2 A. | A. clausi | 10-0 | 85.46 | | 90.00 | | 90.00 | |
| | | 20-0 | 90.00 | -1.00 | 90.00 | | 90.00 | |
| | | 25-0 | 60.50 | 4.10* | 85.17 | 1.00 | 75.40 | 1.99 |
| | | 30-0 | 15.88 | 7.27** | 70.27 | 5.10** | 56.25 | 4.38* |
| | | 35-0 | 32.92 | 6.64** | 74.48 | 14.77** | 47.30 | 3.98* |
| 3 A. | A. clausi | 10-0 | 51,82 | | 78.42 | | 52,45 | |
| | 11. 010001 | 25-0 | 39.24 | 1.94 | 85.01 | -0.84 | 77.25 | -2.23 |
| | | 30-0 | 31.85 | 2.04 | 48.03 | 4.86** | 34.25 | 0.90 |
| | | 35-0 | 0.00 | 8.07** | 16.99 | 9.33** | 17.58 | 3.76* |
| | | 40-0 | 15.00 | 2.26 | 5.37 | 8.99** | 5.37 | 4.46* |
| 4 | A. clausi | 10-0 | 48.87 | | 78.14 | | 58.41 | |
| | | 25-0 | 65.10 | -1,62 | 79.48 | -0.17 | 72.86 | -0.76 |
| | | 30-0 | 51,14 | -0,20 | 90.00 | -1.97 | 90.00 | -1.87 |
| | | 35-0 | 25,70 | 2,52 | 50.34 | 2.90* | 53.86 | 0.18 |
| | | 40-0 | 0.00 | 6.00** | 0.00 | 13.01** | 0.00 | 3.46* |

"t" TESTS FOR ARCSINE TRANSFORMED DATA

| Exp # | Species | Treatment Temp-Dur. | Males | | Females | | Copepodites | |
|-------|---------------|------------------------|---------------|-------------------------------|---------------|-----------------|-------------|----------------|
| | | | Mean | ^{1†} t ^{1†} | Mean | ''t'' | Mean | "t" |
| 5 | A. clausi | 10-0 | 74.38 | | 86.44 | | 84.34 | |
| | - • - | 25-0 | 73.18 | 0,1 2 | 88.02 | -0 .52 | 85.57 | -0 .2 1 |
| | | 30-0 | 34.33 | 5.98** | 67.67 | 5.0 2 ** | 78.82 | 0.63 |
| | | 35-0 | 2 1.34 | 5.87** | 29.40 | 13 .89** | 51.14 | 2.62* |
| | | 40-0 | 0.00 | 14.59** | 12.52 | 15.17** | 0.00 | 23.20** |
| 6 | A. longiremis | 10-0 | 57.60 | | 90.00 | | 90.00 | |
| | | 25-0 | 76.10 | -2.92* | 87.85 | 1.00 | 82.50 | 0.80 |
| | | 30-0 | 19.02 | 4.61** | 79.17 | 4.46** | 78.75 | 1.00 |
| | | 35-0 | 0.00 | 1 2. 75** | 23.98 | 26.56** | 0.00 | |
| | | 40-0 | 0.00 | 10 .2 0** | 5.47 | 15.44** | 0.00 | |
| 6 | Centropages | 10-0 | 69.52 | | 90.00 | | | |
| | 10 | 25-0 | 80.73 | -1 . 95 | 90.00 | | | |
| | | 30-0 | 48.32 | 2. 95* | 90.00 | | | |
| | | 35-0 | 0.00 | 13,24** | 42.2 1 | 3.10* | | |
| | | 40-0 | 0.00 | 14.63** | 0.00 | | | |
| 7 | Centropages | 10-0 | 81.14 | | 75,00 | | 90.00 | |
| - | | 25-0 | 81.97 | -0.11 | 90.00 | -1.00 | 90.00 | |
| | | 30-0 | 69.30 | 1.07 | 90.00 | -1.00 | 90.00 | |
| | | 35-0 | 0.00 | 14.49** | 12.00 | 3.18* | 15.00 | 5.98** |
| | | 40-0 | 0.00 | 14.49** | 0.00 | 5.00** | 0.00 | - - |

| APPENDIX | I. | continued |
|----------|----|------------|
| | | 001101-404 |

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i.

| Exp # | Species | Treatment | Males | | Females | | Copepodites | |
|-------|---------------|----------------|---------------|--------------|---------|---------------|----------------|----------------|
| | | Temp-Dur. | Mean | "t" | Mean | "t" | Mean | "t" |
| 8 | A. longiremis | 10-0 | 75.08 | | 81.73 | | 77.59 | |
| | 5 | 25-0 | 69.71 | 0.54 | 90.00 | -2. 20 | 78.73 | -0.1 2 |
| | | 30-0 | 35 .82 | 5.02** | 67.09 | 1.89 | 71.31 | 0.52 |
| | | 35-0 | 0.00 | 10.57** | 14.20 | 11.06** | 0.00 | 9.86* |
| | | 40-0 | 0.00 | 10.57** | 0.00 | 21.71** | 0.00 | 9.86* |
| 8 | A. clausi | 10-0 | 58.75 | | 83.08 | | 64.93 | |
| - | | 2 5 - 0 | 52.51 | 0.41 | 86.30 | -0.56 | 85.57 | -1.37 |
| | | 30-0 | 18.49 | 3.30** | 70.46 | 1.51 | 42.61 | 1.08 |
| | | 35 - 0 | 0.00 | 6.29** | 11.36 | 9.90** | 0.00 | 4.10* |
| | | 40-0 | 0.00 | 6.95** | 0.00 | 18.81** | 0.00 | 4.53* |
| 9 | A. clausi | 10-0 | 75.00 | | 84.41 | | 81.47 | |
| · | | 2 5-0 | 90.00 | -1.00 | 75.98 | 0.89 | 90.00 | -1.00 |
| | | 25-15 | 26.75 | 4.63** | 78.68 | -0.28 | 71.28 | 4.42* |
| | | 25-30 | 0.00 | | 24.57 | 3.37* | 21.49 | 5.97* |
| | | 25-60 | 7.40 | 11.16** | 17.40 | 4.95** | 8.86 | 9.16* |
| | | 25-120 | 0.00 | | 16.28 | 5.23** | 20.58 | 14.65* |
| | | 30-0 | 25.00 | 2. 50 | 60.22 | 3.75* | 51.06 | 3.35* |
| | | 30-15 | 41.75 | -1.14 | 59.95 | 0.04 | 63.69 | -1.89 |
| | | 30-30 | 19.16 | 0.35 | 23,08 | 3.03* | 34.58 | 3.57* |
| | | 35-0 | 28.94 | 3.00* | 50.89 | 5.98* | 54 . 12 | 3 .2 8* |
| | | 40-0 | 0.00 | 5.00** | 0.00 | 15.09** | 0.00 | 10.21* |

APPENDIX I. continued

| Exp # | Species | Treatment | Mal | es | Fem | ales | Coper | odites |
|-------|---------------|---------------|---------------|---------|-------|---------|-------|--------|
| | | Temp-Dur. | Me an | "t" | Mean | "t" | Mean | ''t'' |
| 11 | A. longiremis | 20-0 | 81.14 | | 90.00 | | 90.00 | |
| | 0 | 20-30 | 2 1,75 | 4,21* | 83,51 | 1.00 | 90.00 | |
| | | 20-60 | 0.00 | 9.16** | 81.26 | 2.00 | 90.00 | |
| | | 20-120 | 0.00 | 9.16** | 68.07 | 1,90 | 68.25 | 1.98 |
| | | 25-0 | 81.14 | | 84.15 | | 90.00 | |
| | | 25-30 | 0.00 | 9.61** | 44.29 | 3.43* | 0.00 | |
| | | 25-60 | 0.00 | 9.61** | 57.29 | 3.17* | 28.68 | 4.08* |
| | | 25-120 | 0.00 | 9.61** | 12.77 | 8.08** | 0.00 | |
| | | 30-0 | 10.00 | | 61.94 | | 81.14 | |
| | | 30-15 | 0.00 | 1.00 | 4.99 | 5.36** | 5.85 | 7.09** |
| | | 30-30 | 0.00 | 1.00 | 6.49 | 4.86** | 0.00 | 9.16** |
| 11 | Centropages | 20-0 | 90.00 | | 90.00 | | 90.00 | |
| | 1 0 | 20-30 | 90.00 | | 78.11 | 1.93 | 90.00 | |
| | | 20-60 | 63 .52 | 4.65** | 85.01 | 1.00 | 90.00 | |
| | | 20-1440 | 47.09 | 9.00** | 60.84 | 12.28** | 41.75 | 1.84 |
| | | 25-0 | 83.51 | | 90.00 | | 90.00 | |
| | | 25-30 | 14.26 | 4.42* | 71.97 | 1.97 | 63.25 | 1.96 |
| | | 25-6 0 | 56.23 | 3.01* | 71.81 | 4.39* | 83.51 | 1.00 |
| | | 25-120 | 0.00 | 12.87** | 70.64 | 1.92 | 55.77 | 1.96 |
| | | 30-0 | 64.31 | | 80.00 | | 78.25 | |
| | | 30-15 | 5.85 | 4.11* | 23.78 | 5.01** | 0.00 | 6.66** |
| | | 30-30 | 6.49 | 4.95** | 21.05 | 5.77** | 10.00 | 4.42* |

APPENDIX I. continued

| Exp # | Species | Treatment | Mal | es | Fem | ales | Coper | odites |
|-------|-------------|--------------|-------|--------|-------|-----------------|-------|----------------|
| - | | Temp-Dur. | Mean | ''t'' | Mean | "t" | Mean | "t" |
| 12 | Centropages | 20-0 | 69.05 | | 90.00 | | 90.00 | |
| | | 20-30 | 68.57 | 0.13 | 90.00 | | 90.00 | |
| | | 20-120 | 15.04 | 6.55** | 90.00 | | 75.00 | 1.00 |
| | | 2 5-0 | 90.00 | | 90.00 | | 90.00 | |
| | | 25-30 | 76.16 | 1,80 | 90.00 | | 90.00 | |
| | | 25-60 | 49.83 | 3,33* | 90.00 | | 90.00 | |
| | | 25-120 | 31.65 | 2.88* | 82.60 | 1.00 | 75.00 | 1.00 |
| 13 | A. clausi | 10-0 | 84.63 | | 90.00 | | 90.00 | |
| | • - | 25-0 | 75.00 | 1.01 | 90.00 | | 90.00 | |
| | | 25-15 | 50.22 | 3.15* | 85.58 | 1.00 | 85.32 | 1.00 |
| | | 25-30 | 6.90 | 6.51** | 39.48 | 11.98** | 26.64 | 4.5 2 * |
| | | 25-60 | 0.00 | 9.54** | 17.90 | 7 . 72** | 6.14 | 13.65* |
| | | 25-120 | 0.00 | 9.54** | 20.18 | 22.73** | 18.80 | 7.34** |
| | | 30-0 | 35.48 | 5.65** | 62.29 | 24.24** | 52.86 | 19.79** |
| | | 30 - 15 | 13.04 | 2.37 | 50.17 | 3.97* | 55.13 | -0.49 |
| | | 30-30 | 6.49 | 3.07* | 18.23 | 4.78** | 31.44 | 5,63** |
| | | 35-0 | 14.81 | 7.64** | 40.24 | 10.16** | 44.45 | 15.90** |

APPENDIX I. continued

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APPENDIX II

RAW DATA

| Exp. # | Species | Delay | Temp. | . Duration | | ales / Dead | Females Alive / Dead | | Copepodites Alive / Dead | |
|--------|-----------|-------|-------|------------|----|----------------|-------------------------|---|-----------------------------|---|
| 1 | A. clausi | 12 | 10 | 0 | 29 | 0 | 16 | 0 | 6 | 0 |
| | | 12 | 10 | 0 | 37 | 0 | 27 | 0 | 7 | 0 |
| | | 12 | 10 | 0 | 22 | 1 | 12 | 0 | 8 | 0 |
| | | 12 | 20 | 0 | 41 | 1 | 27 | 0 | 18 | 0 |
| | | 12 | 20 | 0 | 44 | 3 | 16 | 0 | 15 | 0 |
| | | 12 | 20 | 0 | 33 | 0 | 37 | 0 | 15 | 0 |
| | | 12 | 25 | 0 | 51 | 0 | 41 | 0 | 22 | 0 |
| | | 12 | 25 | 0 | 41 | 0 | 18 | 1 | 9 | 0 |
| | | 12 | 25 | 0 | 32 | 2 | 30 | 0 | 14 | 0 |
| | | 12 | 30 | 0 | 23 | 4 | 15 | 0 | 4 | 0 |
| | | 12 | 30 | 0 | 12 | 29 | 51 | 0 | 12 | 3 |
| | | 12 | 30 | 0 | 5 | 22 | 28 | 3 | 10 | 0 |
| | | 12 | 35 | 0 | 1 | 27 | 1 2 | 7 | 0 | 7 |
| | | 12 | 35 | 0 | 1 | 26 | 25 | 4 | 2 | 2 |
| | | 12 | 35 | 0 | 2 | 39 | 27 | 4 | 4 | 3 |
| 2 | A. clausi | 24 | 10 | 0 | 17 | 0 | 18 | 0 | 6 | 0 |
| | | 24 | 10 | 0 | 17 | 1 | 23 | 0 | 5 | 0 |
| | | 24 | 10 | 0 | 28 | 0 | 16 | 0 | 10 | 0 |
| | | 24 | 20 | 0 | 24 | 0 | 22 | 0 | 11 | 0 |
| | | 24 | 20 | 0 | 22 | 0 | 11 | 0 | 6 | 0 |
| | | 24 | 20 | 0 | 20 | 0 | 16 | 0 | 8 | 0 |
| | | 24 | 25 | 0 | 27 | 5 | 28 | 0 | 17 | 0 |

| Exp. # | Species | Delay | Temp. | Duration | Ma Alive | les / Dead | Fem Alive | | Copep Alive / | odites / Dead |
|--------|-----------|-------|-------|----------|-------------|---------------|--------------|----|------------------|------------------|
| 2 | A. clausi | 24 | 25 | 0 | 14 | 4 | 21 | 0 | 7 | 1 |
| 2 | | 24 | 25 | 0 | 14 | 8 | 15 | 1 | 11 | 2 |
| | | 24 | 30 | 0 | 0 | 22 | 20 | 1 | 6 | 8 |
| | | 24 | 30 | 0 | 4 | 34 | 17 | 4 | 15 | 4 |
| | | 24 | 30 | 0 | 6 | 20 | 14 | 2 | 14 | 3 |
| | | 24 | 35 | 0 | 5 | 10 | 16 | 1 | 7 | 7 |
| | | 24 | 35 | 0 | 2 | 14 | 20 | 2 | 2 | 6 |
| | | 24 | 35 | 0 | 6 | 7 | 14 | 1 | 11 | 2 |
| 3 | A. clausi | 1 | 10 | 0 | 10 | 13 | 16 | 1 | 2 | 2 |
| 5 | | 1 | 10 | 0 | 9 | 6 | 14 | 0 | 4 | 5 |
| | | 1 | 10 | 0 | 4 | 1 | 7 | 1 | 8 | 1 |
| | | 1 | 25 | 0 | 9 | 13 | 28 | 2 | 7 | 1 |
| | | 1 | 25 | 0 | 9 | 15 | 20 | 0 | 10 | 1 |
| | | 1 | 25 | 0 | 5 | 7 | 12 | 0 | 7 | 0 |
| | | 1 | 30 | 0 | 4 | 11 | 11 | 5 | 3 | 4 |
| | | 1 | 30 | 0 | 1 | 8 | 1 | 10 | 0 | 3 |
| | | 1 | 30 | 0 | 4 | 4 | 16 | 2 | 7 | 2 |
| | | 1 | 35 | 0 | 0 | 16 | 3 | 24 | 1 | 10 |
| | | 1 | 35 | 0 | 0 | 22 | 2 | 44 | 1 | 15 |
| | | 1 | 35 | 0 | 0 | 8 | 1 | 8 | 1 | 7 |
| | | 1 | 40 | 0 | 0 | 12 | 0 | 12 | 0 | 14 |
| | | 1 | 40 | 0 | 1 | 1 | 2 | 24 | 1 | 12 |
| | | 1 | 40 | 0 | 0 | 13 | 0 | 21 | 0 | 13 |

APPENDIX II. continued

| Exp. # | Species | Delay | Temp. | Duration | | ales / Dead | | nales / Dead | Copepe Alive / | |
|--------|------------------|------------|-------|----------|----|----------------|------------|-----------------|-------------------|---|
| 4 | A. clausi | 24 | 10 | 0 | 8 | 14 | 16 | 2 | 2 | 5 |
| | | 2 4 | 10 | 0 | 7 | 7 | 24 | 2 | 7 | 4 |
| | | 24 | 10 | 0 | 22 | 5 | 31 | 0 | 14 | 0 |
| | | 24 | 25 | 0 | 10 | 1 | 20 | 0 | 11 | 0 |
| | | 24 | 25 | 0 | 7 | 1 | 1 2 | 1 | 13 | 2 |
| | | 24 | 25 | 0 | 11 | 6 | 13 | 1 | 6 | 2 |
| | | 24 | 30 | 0 | 4 | 2 | 21 | 0 | 9 | 0 |
| | | 24 | 30 | 0 | 12 | 3 | 16 | 0 | 6 | 0 |
| | | 24 | 30 | 0 | 2 | 4 | 10 | 0 | 3 | 0 |
| | | 24 | 35 | 0 | 2 | 14 | 9 | 13 | 1 | 4 |
| | | 24 | 35 | 0 | 6 | 13 | 18 | 4 | 12 | 0 |
| | | 24 | 35 | 0 | 2 | 12 | 10 | 9 | 7 | 7 |
| | | 24 | 40 | 0 | 0 | 10 | 0 | 17 | 0 | 2 |
| | | 24 | 40 | 0 | 0 | 8 | 0 | 21 | 0 | 6 |
| | | 24 | 40 | 0 | 0 | 9 | 0 | 13 | 0 | 4 |
| 5 | <u>A.</u> clausi | 4 | 10 | 0 | 7 | 2 | 35 | 1 | 15 | 1 |
| | | 4 | 10 | 0 | 9 | 1 | 38 | 0 | 13 | 0 |
| | | 4 | 10 | 0 | 11 | 2 | 10 | 0 | 8 | 1 |
| | | 4 | 10 | 0 | 5 | 1 | 23 | 1 | 6 | 0 |
| | | 4 | 10 | 0 | 6 | 0 | 19 | 0 | 9 | 0 |
| | | 4 | 10 | 0 | 5 | 0 | 28 | 0 | 10 | 0 |
| | | 4 | 25 | 0 | 10 | 0 | 35 | 0 | 6 | 0 |
| | | 4 | 25 | 0 | 16 | 1 | 45 | 2 | 13 | 0 |
| | | 4 | 25 | 0 | 7 | 0 | 14 | 0 | 8 | 0 |

APPENDIX II. continued

| Exp. # | Species | Delay | Temp. | Duration | Ma Alive | les / Dead | | ales / Dead | Copep Alive | |
|--------|---------------|-------|-------|----------|-------------|---------------|----|----------------|----------------|----|
| 5 | A. clausi | 4 | 25 | 0 | 5 | 3 | 23 | 0 | 13 | 0 |
| 5 | | 4 | 25 | 0 | 8 | 0 | 28 | 0 | 8 | 0 |
| | | 4 | 25 | 0 | 3 | 4 | 35 | 0 | 4 | 1 |
| | | 4 | 30 | 0 | 3 | 5 | 13 | 2 | 6 | 0 |
| | | 4 | 30 | 0 | 3 | 3 | 19 | 1 | 12 | 0 |
| | | 4 | 30 | 0 | 1 | 5 | 18 | 8 | 7 | 1 |
| | | 4 | 30 | 0 | 5 | 6 | 32 | 4 | 13 | 3 |
| | | 4 | 30 | 0 | 1 | 9 | 11 | 3 | 7 | 1 |
| | | 4 | 30 | 0 | 5 | 8 | 34 | 4 | 8 | 0 |
| | | 4 | 35 | 0 | 0 | 4 | 4 | 5 | 0 | 3 |
| | | 4 | 35 | 0 | 1 | 7 | 7 | 37 | 10 | 2 |
| | | 4 | 35 | 0 | 2 | 2 | 2 | 13 | 4 | 3 |
| | | 4 | 35 | 0 | 4 | 10 | 10 | 19 | 7 | 3 |
| | | 4 | 35 | 0 | 0 | 3 | 3 | 8 | 2 | 2 |
| | | 4 | 35 | 0 | 1 | 3 | 2 | 12 | 4 | 0 |
| | | 4 | 40 | 0 | 0 | 1 | 0 | 21 | 0 | 2 |
| | | 4 | 40 | 0 | 0 | 6 | 1 | 22 | 0 | 7 |
| | | 4 | 40 | 0 | 0 | 4 | 2 | 20 | 0 | 4 |
| | | 4 | 40 | 0 | 0 | 2 | 3 | 14 | 0 | 10 |
| | | 4 | 40 | 0 | 0 | 2 | 0 | 17 | 0 | 4 |
| | | 4 | 40 | 0 | 0 | 4 | 1 | 7 | 0 | 6 |
| 6 | A. longiremis | 4 | 10 | 0 | 1 | 1 | 10 | 0 | 0 | 0 |
| | ¥ | 4 | 10 | 0 | 6 | 1 | 15 | 0 | 2 | 0 |
| | | 4 | 10 | 0 | 6 | 2 | 22 | 0 | 1 | 0 |

APPENDIX II. continued

| Exp. # | Species | Delay | Temp. | Duration | Ma Alive / | les / Dead | Fem Alive | | Copepo Alive / | odites ′Dead |
|--------|---------------|-------|-------|----------|---------------|---------------|--------------|----|-------------------|-----------------|
| 6 | A. longiremis | 4 | 10 | 0 | 2 | 3 | 8 | 0 | 0 | 0 |
| v | | 4 | 10 | 0 | 5 | 2 | 22 | 0 | 1 | 0 |
| | | 4 | 10 | 0 | 6 | 3 | 12 | 0 | 1 | 0 |
| | | 4 | 25 | 0 | 7 | 0 | 43 | 0 | 3 | 0 |
| | | 4 | 25 | 0 | 3 | 0 | 17 | 0 | 1 | 0 |
| | | 4 | 25 | 0 | 7 | 2 | 2 3 | 0 | 3 | 0 |
| | | 4 | 25 | 0 | 4 | 1 | 19 | 1 | 1 | 1 |
| | | 4 | 25 | 0 | 10 | 3 | 54 | 0 | 10 | 0 |
| | | 4 | 25 | 0 | 2 | 0 | 27 | 0 | 1 | 0 |
| | | 4 | 30 | 0 | 4 | 8 | 41 | 1 | 4 | 0 |
| | | 4 | 30 | 0 | 2 | 6 | 22 | 1 | 1 | 0 |
| | | 4 | 30 | 0 | 2 | 7 | 22 | 2 | 1 | 1 |
| | | 4 | 30 | 0 | 0 | 11 | 42 | 2 | 0 | 0 |
| | | 4 | 30 | 0 | 0 | 4 | 14 | 1 | 0 | 0 |
| | | 4 | 30 | 0 | 1 | 7 | 31 | 0 | 1 | 0 |
| | | 4 | 35 | 0 | 0 | 5 | 3 | 11 | 0 | 0 |
| | | 4 | 35 | 0 | 0 | 4 | 2 | 12 | 0 | 5 |
| | | 4 | 35 | 0 | 0 | 3 | 3 | 16 | 0 | 2 |
| | | 4 | 35 | 0 | 0 | 7 | 5 | 14 | 0 | 3 |
| | | 4 | 35 | 0 | 0 | 5 | 3 | 12 | 0 | 0 |
| | | 4 | 35 | 0 | 0 | 5 | 1 | 18 | 0 | 4 |
| | | 4 | 40 | 0 | 0 | 2 | 5 | 12 | 0 | 3 |
| | | 4 | 40 | 0 | 0 | 2 | 0 | 42 | 0 | 7 |
| | | 4 | 40 | 0 | 0 | 0 | 0 | 9 | 0 | 2 |
| | | 4 | 40 | 0 | 0 | 0 | 0 | 24 | 0 | 7 |

APPENDIX II. continued

| Exp. # | Species | Delay | Temp. | Duration | Ma Alive / | les / Dead | | nales / Dead | Copepo Alive / | |
|--------|-----------------|-------|------------|----------|---------------|---------------|---|-----------------|-------------------|---|
| 6 | A. longiremis | 4 | 40 | 0 | 0 | 3 | 0 | 2 3 | 0 | 4 |
| ÷ | | 4 | 40 | 0 | 0 | 1 | 0 | 21 | 0 | 8 |
| 6 | Centropages sp. | 4 | 10 | 0 | 17 | 0 | 3 | 0 | 0 | 0 |
| • | | 4 | 10 | 0 | 4 | 1 | 3 | 0 | 0 | 0 |
| | | 4 | 10 | 0 | 25 | 2 | 3 | 0 | 0 | 0 |
| | | 4 | 10 | 0 | 15 | 2 | 4 | 0 | 2 | 0 |
| | | 4 | 10 | 0 | 21 | 6 | 2 | 0 | 0 | 0 |
| | | 4 | 10 | 0 | 10 | 4 | 2 | 0 | 0 | 0 |
| | | 4 | 25 | 0 | 43 | 1 | 3 | 0 | 1 | 0 |
| | | 4 | 25 | 0 | 24 | 0 | 4 | 0 | 2 | 0 |
| | | 4 | 25 | 0 | 18 | 0 | 2 | 0 | 1 | 0 |
| | | 4 | 2 5 | 0 | 33 | 4 | 2 | 0 | 0 | 0 |
| | | 4 | 25 | 0 | 18 | 1 | 4 | 0 | 0 | 0 |
| | | 4 | 25 | 0 | 15 | 1 | 2 | 0 | 0 | 0 |
| | | 4 | 30 | 0 | 11 | 1 | 4 | 0 | 1 | 0 |
| | | 4 | 30 | 0 | 6 | 5 | 5 | 0 | 2 | 0 |
| | | 4 | 30 | 0 | 6 | 7 | 4 | 0 | 1 | 1 |
| | | 4 | 30 | 0 | 7 | 13 | 6 | 0 | 0 | 0 |
| | | 4 | 30 | 0 | 10 | 7 | 4 | 0 | 1 | 0 |
| | | 4 | 30 | 0 | 7 | 10 | 3 | 0 | 0 | 0 |
| | | 4 | 35 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| | | 4 | 35 | 0 | 0 | 9 | 0 | 2 | 0 | 0 |
| | | 4 | 35 | 0 | 0 | 16 | 1 | 0 | 0 | 0 |
| | | 4 | 35 | 0 | 0 | 19 | 2 | 1 | 0 | 2 |

APPENDIX II. continued

| Exp. # | Species | Delay | Temp. | Duration | | lles / Dead | Fem Alive / | | Copepo Alive / | odites ′Dead | | | | | | | | | | | | |
|--------|------------------------|-------|-------|----------|---|----------------|----------------|---|-------------------|-----------------|--|--|--|---|----|---|---|---|---|---|---|---|
| 6 | Centropages sp. | 4 | 35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | | | |
| Ŭ | <u>oomrepages</u> spr | 4 | 35 | 0 | 0 | 15 | 1 | 5 | 0 | 0 | | | | | | | | | | | | |
| | | 4 | 40 | 0 | 0 | 17 | 2 | 1 | 0 | 2 | | | | | | | | | | | | |
| | | 4 | 40 | 0 | 0 | 19 | 0 | 6 | 0 | 0 | | | | | | | | | | | | |
| | | 4 | 40 | 0 | 0 | 12 | 0 | 1 | 0 | 0 | | | | | | | | | | | | |
| | | 4 | 40 | 0 | 0 | 20 | 0 | 2 | 0 | 1 | | | | | | | | | | | | |
| | | 4 | 40 | 0 | 0 | 22 | 0 | 1 | 0 | 0 | | | | | | | | | | | | |
| | | 4 | 40 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | | | | | | | | | | | | |
| 7 | Centropages sp. | 4 | 10 | 0 | 8 | 2 | 4 | 0 | 4 | 0 | | | | | | | | | | | | |
| • | <u>centropages</u> sp. | 4 | 10 | 0 | 4 | 1 | 2 | 0 | 1 | 0 | | | | | | | | | | | | |
| | | 4 | 10 | 0 | 3 | 0 | 2 | 0 | 1 | 0 | | | | | | | | | | | | |
| | | | | | | | | | | | | | | 4 | 10 | 0 | 4 | 0 | 2 | 0 | 2 | 0 |
| | | 4 | 10 | 0 | 3 | 0 | 2 | 0 | 0 | 0 | | | | | | | | | | | | |
| | | 4 | 10 | 0 | 3 | 0 | 0 | 1 | 0 | 0 | | | | | | | | | | | | |
| | | 4 | 25 | 0 | 6 | 0 | 4 | 0 | 1 | 0 | | | | | | | | | | | | |
| | | 4 | 25 | 0 | 7 | 0 | 4 | 0 | 0 | 0 | | | | | | | | | | | | |
| | | 4 | 25 | 0 | 5 | 1 | 2 | 0 | 2 | 0 | | | | | | | | | | | | |
| | | 4 | 25 | 0 | 7 | 0 | 2 | 0 | 2 | 0 | | | | | | | | | | | | |
| | | 4 | 2.5 | 0 | 7 | 0 | 5 | 0 | 2 | 0 | | | | | | | | | | | | |
| | | 4 | 25 | 0 | 5 | 1 | 5 | 0 | 2 | 0 | | | | | | | | | | | | |
| | | 4 | 30 | 0 | 6 | 0 | 3 | 0 | 1 | 0 | | | | | | | | | | | | |
| | | 4 | 30 | 0 | 4 | 1 | 2 | 0 | 0 | 0 | | | | | | | | | | | | |
| | | 4 | 30 | 0 | 6 | 1 | 4 | 0 | 0 | 0 | | | | | | | | | | | | |
| | | 4 | 30 | 0 | 2 | 4 | 1 | 0 | 1 | 0 | | | | | | | | | | | | |

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APPENDIX II. continued

| Exp. # | Species | Delay | Temp. | Duration | Ma Alive / | | Fem Alive / | | Copepo Alive / | dites Dead |
|--------|------------------------|-------|-------|----------|---------------|---|----------------|---|-------------------|---------------|
| 7 | Contropagos sp | 4 | 30 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| (| <u>Centropages</u> sp. | 4 | 30 | 0 | 4 | 0 | 1 | 0 | 0 | 0 |
| | | 4 | 35 | 0 | 0 | 3 | 0 | 1 | 0 | 0 |
| | | 4 | 35 | 0 | 0 | 4 | 0 | 4 | 0 | 2 |
| | | 4 | 35 | 0 | 0 | 2 | 3 | 1 | 1 | 1 |
| | | 4 | 35 | 0 | 0 | 6 | 0 | 3 | 0 | 3 |
| | | 4 | 35 | 0 | 0 | 8 | 0 | 8 | 0 | 0 |
| | | 4 | 35 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| | | 4 | 40 | 0 | 0 | 1 | 0 | 1 | 0 | 0 |
| | | 4 | 40 | 0 | 0 | 4 | 0 | 4 | 0 | 2 |
| | | 4 | 40 | 0 | 0 | 7 | 0 | 4 | 0 | 3 |
| | | 4 | 40 | 0 | 0 | 5 | 0 | 2 | 0 | 2 |
| | | 4 | 40 | 0 | 0 | 1 | 0 | 1 | 0 | 1 |
| | | 4 | 40 | 0 | 0 | 5 | 0 | 6 | 0 | 1 |
| 8 | A. clausi | 4 | 10 | 0 | 2 | 2 | 9 | 1 | 5 | 2 |
| 0 | A. Clausi | 4 | 10 | 0 | 7 | 1 | 16 | 0 | 4 | 0 |
| | | 4 | 10 | 0 | 3 | 0 | 14 | 0 | 2 | 0 |
| | | 4 | 10 | 0 | 8 | 4 | 20 | 0 | 7 | 2 |
| | | 4 | 10 | 0 | 1 | 3 | 11 | 2 | 0 | 1 |
| | | 4 | 10 | 0 | 4 | 1 | 15 | 0 | 4 | 0 |
| | | 4 | 25 | 0 | 3 | 1 | 20 | 0 | 4 | 1 |
| | | 4 | 25 | 0 | 1 | 1 | 21 | 0 | 9 | 0 |
| | | 4 | 25 | 0 | 14 | 1 | 36 | 0 | 11 | 0 |
| | | 4 | 25 | 0 | 2 | 0 | 13 | 0 | 8 | 0 |

APPENDIX II. continued

| Exp. # | Species | Delay | Temp. | Duration | Ma Alive / | les / Dead | | nales / Dead | Copep Alive | odites / Dead |
|--------|---------------|-------|-------|----------|---------------|---------------|----|-----------------|----------------|------------------|
| 8 | A. clausi | 4 | 25 | 0 | 0 | 3 | 12 | 2 | 4 | 0 |
| | | 4 | 25 | 0 | 1 | 1 | 13 | 0 | 6 | 0 |
| | | 4 | 30 | 0 | 4 | 3 | 18 | 0 | 2 | 2 |
| | | 4 | 30 | 0 | 0 | 5 | 9 | 8 | 4 | 2 |
| | | 4 | 30 | 0 | 0 | 4 | 11 | 2 | 0 | 1 |
| | | 4 | 30 | 0 | 0 | 4 | 16 | 0 | 4 | 0 |
| | | 4 | 30 | 0 | 3 | 6 | 19 | 2 | 5 | 1 |
| | | 4 | 30 | 0 | 1 | 4 | 12 | 5 | 0 | 1 |
| | | 4 | 35 | 0 | 0 | 2 | 2 | 10 | 0 | 0 |
| | | 4 | 35 | 0 | 0 | 6 | 4 | 10 | 0 | 2 |
| | | 4 | 35 | 0 | 0 | 6 | 0 | 15 | 0 | 5 |
| | | 4 | 35 | 0 | 0 | 0 | 0 | 17 | 0 | 6 |
| | | 4 | 35 | 0 | 0 | 5 | 0 | 12 | 0 | 7 |
| | | 4 | 35 | 0 | 0 | 10 | 1 | 2 3 | 0 | 8 |
| | | 4 | 40 | 0 | 0 | 4 | 0 | 11 | 0 | 4 |
| | | 4 | 40 | 0 | 0 | 4 | 0 | 7 | 0 | 5 |
| | | 4 | 40 | 0 | 0 | 1 | 0 | 13 | 0 | 5 |
| | | 4 | 40 | 0 | 0 | 5 | 0 | 13 | 0 | 4 |
| | | 4 | 40 | 0 | 0 | 4 | 0 | 11 | 0 | 3 |
| | | 4 | 40 | 0 | 0 | 7 | 0 | 9 | 0 | 6 |
| 8 | A. longiremis | 4 | 10 | 0 | 8 | 1 | 15 | 0 | 5 | 0 |
| | | 4 | 10 | 0 | 4 | 0 | 13 | 0 | 3 | 0 |
| | | 4 | 10 | 0 | 8 | 0 | 16 | 2 | 2 | 1 |
| | | 4 | 10 | 0 | 5 | 3 | 24 | 2 | 4 | 0 |

| Exp. # | Species | Delay | Temp. | Duration | Ma Alive / | | | n al es / De a d | Copepo Alive | |
|--------|---------------|-------|-------|----------|---------------|---|----|-----------------------------------|-----------------|---|
| 8 | A. longiremis | 4 | 10 | 0 | 5 | 2 | 16 | 1 | 6 | 4 |
| - | | 4 | 10 | 0 | 3 | 0 | 10 | 0 | 2 | 0 |
| | | 4 | 25 | 0 | 4 | 2 | 12 | 0 | 4 | 0 |
| | | 4 | 25 | 0 | 4 | 1 | 8 | 0 | 5 | 1 |
| | | 4 | 25 | 0 | 14 | 2 | 47 | 0 | 12 | 0 |
| | | 4 | 25 | 0 | 11 | 0 | 6 | 0 | 8 | 1 |
| | | 4 | 25 | 0 | 3 | 2 | 11 | 0 | 5 | 1 |
| | | 4 | 25 | 0 | 4 | 0 | 19 | 0 | 11 | 0 |
| | | 4 | 30 | 0 | 2 | 2 | 19 | 4 | 3 | 0 |
| | | 4 | 30 | 0 | 2 | 3 | 4 | 4 | 3 | 2 |
| | | 4 | 30 | 0 | 1 | 6 | 12 | 1 | 3 | 0 |
| | | 4 | 30 | 0 | 1 | 2 | 10 | 0 | 4 | 0 |
| | | 4 | 30 | 0 | 6 | 8 | 17 | 1 | 7 | 1 |
| | | 4 | 30 | 0 | 2 | 5 | 13 | 8 | 3 | 5 |
| | | 4 | 35 | 0 | 0 | 3 | 0 | 3 | 0 | 5 |
| | | 4 | 35 | 0 | 0 | 3 | 1 | 14 | 0 | 6 |
| | | 4 | 35 | 0 | 0 | 7 | 0 | 10 | 0 | 7 |
| | | 4 | 35 | 0 | 0 | 4 | 2 | 7 | 0 | 6 |
| | | 4 | 35 | 0 | 0 | 7 | 1 | 7 | 0 | 6 |
| | | 4 | 35 | 0 | 0 | 6 | 2 | 13 | 0 | 8 |
| | | 4 | 40 | 0 | 0 | 2 | 0 | 9 | 0 | 2 |
| | | 4 | 40 | 0 | 0 | 4 | 0 | 10 | 0 | 5 |
| | | 4 | 40 | 0 | 0 | 6 | 0 | 23 | 0 | 3 |
| | | 4 | 40 | 0 | 0 | 5 | 0 | 15 | 0 | 9 |
| | | 4 | 40 | 0 | 0 | 9 | 0 | 8 | 0 | 7 |

APPENDIX II. continued

| Exp. # | Species | | Temp. | Duration 0 | Males Alive / Dead | | Fem Alive | ales / Dead | Copepo Alive / | |
|---------------|---------------|---|-------|---------------|-----------------------|---|--------------|----------------|-------------------|----|
| 8 | A. longiremis | | 40 | | 0 | 6 | 0 | 11 | 0 | 8 |
| 9 | A. clausi | 4 | 10 | 0 | 2 | 0 | 11 | 1 | 5 | 1 |
| , | | 4 | 10 | 0 | 1 | 0 | 10 | 0 | 3 | 0 |
| | | 4 | 10 | 0 | 1 | 1 | 5 | 0 | 1 | 0 |
| | | 4 | 25 | 0 | 9 | 0 | 19 | 0 | 8 | 0 |
| | | 4 | 25 | 0 | 2 | 0 | 8 | 2 | 8 | 0 |
| | | 4 | 25 | 0 | 6 | 0 | 13 | 1 | 8 | 0 |
| | | 4 | 25 | 15 | 3 | 3 | 15 | 1 | 22 | 1 |
| | | 4 | 25 | 15 | 2 | 4 | 8 | 1 | 8 | 2 |
| | | 4 | 25 | 15 | 0 | 3 | 4 | 0 | 10 | 1 |
| | | 4 | 25 | 30 | 0 | 4 | 0 | 6 | 4 | 6 |
| | | 4 | 25 | 30 | 0 | 7 | 3 | 10 | 2 | 9 |
| | | 4 | 25 | 30 | 0 | 4 | 7 | 7 | 0 | 6 |
| | | 4 | 25 | 60 | 1 | 6 | 1 | 6 | 1 | 4 |
| | | 4 | 25 | 60 | 0 | 1 | 0 | 2 | 0 | 8 |
| | | 4 | 25 | 60 | 0 | 4 | 1 | 3 | 0 | 3 |
| | | 4 | 25 | 120 | 0 | 5 | 2 | 7 | 1 | 3 |
| | | 4 | 25 | 1 2 0 | 0 | 7 | 0 | 9 | 1 | 14 |
| | | 4 | 25 | 1 2 0 | 0 | 2 | 1 | 7 | 1 | 11 |
| | | 4 | 30 | 0 | 2 | 2 | 3 | 1 | 3 | 1 |
| | | 4 | 30 | 0 | 0 | 3 | 10 | 2 | 5 | 4 |
| | | 4 | 30 | 0 | 1 | 3 | 2 | 1 | 2 | 2 |
| | | 4 | 30 | 15 | 2 | 1 | 7 | 1 | 9 | 2 |
| | | 4 | 30 | 15 | 1 | 2 | 7 | 3 | 9 | 1 |

APPENDIX II. continued

| Exp. # | Species | Delay | Temp. | Duration | Mal Alive / | | Fen Alive / | nales / Dead | Copepo Alive / | odites / Dead |
|--------|---------------|-------|-------|----------|----------------|-----|----------------|-----------------|-------------------|------------------|
| 9 | A. clausi | 4 | 30 | 15 | 1 | 2 | 3 | 2 | 6 | 3 |
| 7 | A. Clausi | 4 | 30 | 30 | 0 | 3 | 0 | 5 | 3 | 6 |
| | | 4 | 30 | 30 | 1 | 6 | 2 | 3 | 3 | 6 |
| | | 4 | 30 | 30 | 1 | 2 | 2 | 6 | 3 | 7 |
| | | 4 | 35 | 0 | 1 | 6 | 13 | 9 | 10 | 5 |
| | | 4 | 35 | 0 | 2 | 5 | 8 | 5 | 8 | 3 |
| | | 4 | 35 | 0 | 2 | 5 | 12 | 8 | 8 | 6 |
| | | 4 | 40 | 0 | 0 | 7 | 0 | 11 | 0 | 11 |
| | | 4 | 40 | 0 | 0 | 8 | 0 | 13 | 0 | 9 |
| | | 4 | 40 | 0 | 0 | . 7 | 0 | 11 | 0 | 7 |
| 11 | A. longiremis | 4 | 10 | 0 | 3 | 0 | 14 | 0 | 2 | 0 |
| 11 | In Iongiround | 4 | 10 | 0 | 2 | 0 | 9 | 0 | 2 | 0 |
| | | 4 | 10 | 0 | 2 | 0 | 12 | 0 | 2 | 0 |
| | | 4 | 20 | 0 | 4 | 1 | 17 | 0 | 4 | 0 |
| | | 4 | 20 | 0 | 7 | 0 | 13 | 0 | 4 | 0 |
| | | 4 | 20 | 0 | 3 | 0 | 21 | 0 | 2 | 0 |
| | | 4 | 20 | 30 | 1 | 3 | 6 | 0 | 3 | 0 |
| | | 4 | 20 | 30 | 0 | 1 | 10 | 0 | 4 | 0 |
| | | 4 | 20 | 30 | 1 | 2 | 8 | 1 | 3 | 0 |
| | | 4 | 20 | 60 | 0 | 4 | 12 | 0 | 1 | 0 |
| | | 4 | 20 | 60 | 0 | 3 | 17 | 1 | 4 | 0 |
| | | 4 | 20 | 60 | 0 | 5 | 20 | 1 | 3 | 0 |
| | | 4 | 20 | 120 | 0 | 1 | 6 | 4 | 2 | 1 |
| | | 4 | 20 | 120 | 0 | 1 | 4 | 1 | 1 | 0 |

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APPENDIX II, continued

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| Exp . # | Species | Delay | Temp. | Duration | Mal Alive / | | | nales / Dead | Copep Alive / | |
|----------------|---------------|-------|------------|--------------|----------------|---|------------|-----------------|------------------|----|
| 11 | A. longiremis | 4 | 20 | 120 | 0 | 3 | 11 | 0 | 3 | 1 |
| | | 4 | 2 0 | 1440 | 0 | 1 | 8 | 5 | 0 | 0 |
| | | 4 | 20 | 1440 | 0 | 0 | 9 | 5 | 0 | 0 |
| | | 4 | 20 | 1440 | 0 | 0 | 7 | 10 | 0 | 0 |
| | | 4 | 25 | 0 | 4 | 1 | 11 | 0 | 3 | 0 |
| | | 4 | 25 | 0 | 3 | 0 | 8 | 0 | 4 | 0 |
| | | 4 | 25 | 0 | 6 | 0 | 10 | 1 | 3 | 0 |
| | | 4 | 25 | 30 | 0 | 4 | 5 | 20 | 0 | 3 |
| | | 4 | 25 | 30 | 0 | 1 | 9 | 9 | 0 | 3 |
| | | 4 | 25 | 30 | 0 | 4 | 10 | 3 | 0 | 1 |
| | | 4 | 25 | 60 | 0 | 2 | 8 | 2 | 0 | 1 |
| | | 4 | 25 | 60 | 0 | 2 | 1 2 | 3 | 3 | 2 |
| | | 4 | 25 | 60 | 0 | 2 | 3 | 3 | 1 | 2 |
| | | 4 | 25 | 1 2 0 | 0 | 4 | 2 | 12 | 0 | 2 |
| | | 4 | 25 | 1 2 0 | 0 | 2 | 0 | 19 | 0 | 5 |
| | | 4 | 25 | 1 2 0 | 0 | 6 | 1 | 12 | 0 | 4 |
| | | 4 | 30 | 0 | 0 | 1 | 4 | 1 | 4 | 1 |
| | | 4 | 30 | 0 | 1 | 3 | 20 | 1 | 5 | 0 |
| | | 4 | 30 | 0 | 0 | 3 | 5 | 5 | 2 | 0 |
| | | 4 | 30 | 15 | 0 | 2 | 1 | 14 | 0 | 3 |
| | | 4 | 30 | 15 | 0 | 7 | 0 | 19 | 0 | 3 |
| | | 4 | 30 | 15 | 0 | 2 | 0 | 19 | 0 | 2 |
| | | 4 | 30 | 30 | 0 | 2 | 1 | 8 | 0 | 2 |
| | | 4 | 30 | 30 | 0 | 3 | 0 | 8 | 0 | -3 |
| | | 4 | 30 | 30 | 0 | 3 | 0 | 4 | 0 | 2 |

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APPENDIX II. continued

| Exp. # | Species | Delay | Temp. | Duration | Mal Alive / | | Fema Alive / | | Copepo Alive / | dites Dead |
|--------|-----------------|--------|------------|----------|----------------|-----|-----------------|---|-------------------|---------------|
| | | 4 | 10 | 0 | 13 | 0 | 7 | 0 | 2 | 0 |
| 11 | Centropages sp. | + 4 | 10 | 0 | 10 | 0 | 14 | 0 | 2 | 0 |
| | | 4 | 10 | 0 | 11 | 0 | 16 | 0 | 4 | 0 |
| | | 4 | 20 | 0 | 17 | 0 | 19 | 0 | 4 | 0 |
| | | 4 | 20 | 0 | 12 | 0 | 2 0 | 0 | 5 | 0 |
| | | 4 | 20 | 0 | 13 | 0 | 16 | 0 | 4 | 0 |
| | | 4 | 20 | 30 | 3 | 0 | 14 | 1 | 6 | 0 |
| | | 4 | 20 | 30 | 12 | 0 | 7 | 1 | 8 | 0 |
| | | 4 | 20 | 30 | 7 | 0 | 13 | 0 | 4 | 0 |
| | | 4 | 20 | 60 | 6 | · 1 | 12 | 0 | 3 | 0 |
| | | 4 | 20 | 60 | 5 | 3 | 16 | 0 | 4 | 0 |
| | | 4 | 2 0 | 60 | 8 | 1 | 14 | 1 | 5 | 0 |
| | | 4 | 20 | 120 | 0 | 0 | 1 | 0 | 1 | 0 |
| | | 4 | 20 | 120 | 0 | 0 | 2 | 0 | 0 | 0 |
| | | 4 | 20 | 120 | 0 | 1 | 5 | 0 | 0 | 0 |
| | | 4 | 20 | 1440 | 4 | 2 | 7 | 2 | 2 | 0 |
| | | 4 | 20 | 1440 | 5 | 8 | 9 | 4 | 0 | 1 |
| | | 4 | 20 | 1440 | 5 | 4 | 13 | 3 | 1 | 2 |
| | | 4 | 25 | 0 | 10 | 0 | 17 | 0 | 5 | 0 |
| | | 4 | 25 | 0 | 8 | 1 | 8 | 0 | 4 | 0 |
| | | 4 | 25 | 0 | 10 | 0 | 12 | 0 | 3 | 0 |
| | | 4 | 25 | 30 | 0 | 8 | -6 | 2 | 2 | 0 |
| | | 4 | 25 | 30 | 0 | 7 | 10 | 2 | 1 | 1 |
| | | 4 | 25 | 30 | 6 | 7 | 13 | 0 | 2 | 1 |
| | | 4 | 25 | 60 | 11 | 2 | 27 | 1 | 8 | 1 |

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APPENDIX II. continued

| Exp. # | Species | Delay | Temp. | Duration 60 | Males Alive / Dead | | Fem Alive | | Copep Alive | |
|--------|-----------------|-------|-------|----------------|-----------------------|----|--------------|----|----------------|---|
| 11 | Centropages sp. | 4 | 25 | | 7 | 3 | 9 | 2 | 4 | 0 |
| | | 4 | 25 | 60 | 3 | 3 | 9 | 1 | 5 | 0 |
| | | 4 | 25 | 120 | 0 | 9 | 10 | 2 | -3 | 0 |
| | | 4 | 25 | 120 | 0 | 7 | 11 | 5 | 2 | 5 |
| | | 4 | 25 | 120 | 0 | 3 | 6 | 0 | 1 | 1 |
| | | 4 | 30 | 0 | 2 | 1 | 3 | 1 | 1 | 0 |
| | | 4 | 30 | 0 | 6 | 0 | 9 | 0 | 3 | 0 |
| | | 4 | 30 | 0 | 5 | 4 | 11 | 0 | 2 | 1 |
| | | 4 | 30 | 15 | 0 | 7 | 5 | 11 | 0 | 4 |
| | | 4 | 30 | 15 | 0 | 19 | 2 | 17 | 0 | 2 |
| | | 4 | 30 | 15 | 1 | 10 | 1 | 9 | 0 | 3 |
| | | 4 | 30 | 30 | 1 | 8 | 2 | 9 | 0 | 2 |
| | | 4 | 30 | 30 | 0 | 10 | 1 | 9 | 0 | 4 |
| | | 4 | 30 | 30 | 0 | 15 | 2 | 16 | 2 | 6 |
| 12 | Centropages sp. | 4 | 10 | 0 | 17 | 0 | 5 | 0 | 2 | 0 |
| | <u> </u> | 4 | 10 | 0 | 17 | 0 | 8 | 0 | 3 | 0 |
| | | 4 | 10 | 0 | 12 | 0 | 10 | 0 | 1 | 0 |
| | | 4 | 20 | 0 | 10 | 1 | 6 | 0 | 1 | 0 |
| | | 4 | 20 | 0 | 6 | 1 | 1 | 0 | 3 | 0 |
| | | 4 | 20 | 0 | 11 | 2 | 2 | 0 | 5 | 0 |
| | | 4 | 20 | 30 | 4 | 1 | 5 | 0 | 5 | 0 |
| | | 4 | 20 | 30 | 12 | 2 | 4 | 0 | 1 | 0 |
| | | 4 | 20 | 30 | 13 | 1 | 6 | 0 | 6 | 0 |
| | | 4 | 20 | 60 | 2 | 4 | 2 | 0 | 2 | 0 |

APPENDIX II. continued

| Exp. # | Species | Delay | Temp. | Duration 60 | Males Alive / Dead | | Fem Alive | ales / Dead | Copepo Alive / | |
|--------|-----------------|-------|------------|----------------|-----------------------|----|--------------|----------------|-------------------|---|
| 12 | Centropages sp. | 4 | 20 | | 1 | 5 | 0 | 0 | 1 | 0 |
| | | 4 | 20 | 60 | 0 | 2 | 2 | 0 | 0 | 0 |
| | | 4 | 20 | 1 2 0 | 1 | 10 | 5 | 0 | 1 | 1 |
| | | 4 | 20 | 1 2 0 | 3 | 11 | 1 | 0 | 2 | 0 |
| | | 4 | 2 0 | 120 | 0 | 10 | 1 | 0 | 2 | 0 |
| | | 4 | 25 | 0 | 16 | 0 | 3 | 0 | 1 | 0 |
| | | 4 | 2 5 | 0 | 7 | 0 | 4 | 0 | 3 | 0 |
| | | 4 | 25 | 0 | 5 | 0 | 3 | 0 | 3 | 0 |
| | | 4 | 25 | 30 | 14 | 1 | 6 | 0 | 1 | 0 |
| | | 4 | 25 | 30 | 12 | 3 | 5 | 0 | 3 | 0 |
| | | 4 | 25 | 30 | 15 | 0 | 7 | 0 | 1 | 0 |
| | | 4 | 2 5 | 60 | 11 | 5 | 4 | 0 | 2 | 0 |
| | | 4 | 2 5 | 60 | 11 | 2 | 4 | 0 | 1 | 0 |
| | | 4 | 25 | 60 | 1 | 4 | 4 | 0 | 3 | 0 |
| | | 4 | 25 | 120 | 7 | 1 | 1 | 0 | 1 | 0 |
| | | 4 | 2 5 | 1 2 0 | 0 | 7 | 6 | 1 | 1 | 1 |
| | | 4 | 2 5 | 120 | 3 | 13 | 6 | 0 | 2 | 0 |
| | | 4 | 30 | 0 | 5 | 11 | 8 | 0 | 3 | 1 |
| | | 4 | 30 | 0 | 8 | 9 | 8 | 0 | 2 | 0 |
| | | 4 | 30 | 0 | 2 | 1 | 3 | 0 | 1 | 0 |
| 13 | A. clausi | 4 | 10 | 0 | 8 | 0 | 12 | 0 | 3 | 0 |
| | | 4 | 10 | 0 | 12 | 1 | 16 | 0 | 1 | 0 |
| | | 4 | 10 | 0 | 6 | 0 | 16 | 0 | 4 | 0 |
| | | 4 | 2 5 | 0 | 9 | 1 | 15 | 0 | 4 | 0 |

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APPENDIX II. continued

| Exp. # | Species | Delay | Temp. | Duration | Ma Alive / | les / Dead | | nales / Dead | Copep Alive | |
|--------------|-----------|--------|-------|----------|---------------|---------------|----|-----------------|----------------|-----|
| 13 <u>A.</u> | A. clausi | ausi 4 | 25 | 0 | 4 | 0 | 12 | 0 | 4 | 0 |
| | | 4 | 25 | 0 | 4 | 1 | 6 | 0 | 3 | 0 |
| | | 4 | 25 | 15 | 4 | 3 | 18 | 1 | 16 | 1 |
| | | 4 | 25 | 15 | 3 | 2 | 10 | 0 | 6 | 0 |
| | | 4 | 25 | 15 | 3 | 2 | 11 | 0 | 0 7 | 0 |
| | | 4 | 25 | 30 | 0 | 4 | 4 | 6 | 2 | 5 |
| | | 4 | 25 | 30 | 0 | 7 | 4 | 10 | 6 | 5 |
| | | 4 | 25 | 30 | 1 | 7 | 8 | 7 | 0 | 3 |
| | | 4 | 25 | 60 | 0 | 3 | 2 | 12 | 0 | 3 |
| | | 4 | 25 | 60 | 0 | 8 | 0 | 4 | 1 | 9 |
| | | 4 | 25 | 60 | 0 | 6 | 3 | 8 | 0 | 3 |
| | | 4 | 25 | 120 | 0 | 8 | 3 | 16 | 0 0 | 4 |
| | | 4 | 25 | 120 | 0 | 9 | 1 | 8 | 2 | 5 |
| | | 4 | 25 | 120 | 0 | 5 | 2 | 11 | 1 | 5 |
| | | 4 | 30 | 0 | 3 | 3 | 4 | 1 | 2 | 1 |
| | | 4 | 30 | 0 | 1 | 6 | 9 | 3 | 4 | 3 |
| | | 4 | 30 | 0 | 2 | 5 | 4 | 1 | 4 | 2 |
| | | 4 | 30 | 15 | 0 | 6 | 8 | 4 | 9 | 4 |
| | | 4 | 30 | 15 | 1 | 7 | 6 | 4 | 7 | 2 |
| | | 4 | 30 | 15 | 1 | 9 | 2 | 2 | 7 | - 5 |
| | | 4 | 30 | 30 | 1 | 8 | 3 | 12 | 2 | 6 |
| | | 4 | 30 | 30 | 0 | 7 | 0 | 6 | 3 | 5 |
| | | 4 | 30 | 30 | 0 | 7 | 2 | 7 | 2 | 8 |
| | | 4 | 35 | 0 | 1 | 6 | 10 | 11 | 4 | 6 |
| | | 4 | 35 | 0 | 0 | 7 | 8 | 10 | 4 | 3 |
| | | 4 | 35 | 0 | 1 | 6 | 7 | 14 | 2 | 2 |

| APPENDIX | II. | continued |
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|----------|-----|-----------|