THE BIOLOGY
OF THE
MARINE COPEPOD
TIGRIOPUS CALIFORNICUS (BAKER)

by

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Approved for the Graduate Study Committee

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INTRODUCTION

The marine copepod, Tigriopus californicus (Baker), is commonly found in high rocky splash-pools between Alaska and Lower California. In this habitat the animal has the ability of withstanding a great range of temperature and salinity. The body of this work is concerned with experiments designed to investigate the temperature and salinity tolerances of T. californicus. Because this genus is cosmopolitan and is easily maintained under laboratory conditions, it has been the subject of world-wide study. Several of the experiments described in this paper are near replicas of work previously done by Ranade (1957) on the European species, Tigriopus fulvus, and by Takeda (1951) on the Japanese form, Tigriopus japonicus.

This study was begun in June of 1960 and terminated January 1961. During this time data were obtained on T. californicus concerning: population characteristics, reproduction, environmental relationships, morphological characteristics, and temperature and salinity tolerances.

The direct impetus for my interest in this subject has been afforded by my advisor Professor Fred Telonicher of the Humboldt State College Division of Biological Sciences. The author chooses to especially acknowledge Professor Telonicher

for his patient encouragement and stimulation throughout my study. My gratitude is also extended to Dr. James Welsh of the Humboldt Biology Department for additional advice and guidance.

II. LIFE HISTORY OF *Tigriopus californicus*

A. History

The genus *Tigriopus* was described by Norman in 1868. *T. californicus* was described by Baker in 1912 from a specimen taken at Laguna Beach, California, but he erroneously assigned it to the genus *Tisbe*. Cambell, in 1930, again described it and assigned it the name *Tigriopus triangulus*.* Monk, (1941) in his treatment of the California marine harpacticoids, briefly describes the animal investigated in this paper and assigns it the name *T. californicus* (Baker) 1912.

Bozic (1960) in a treatise on the genus *Tigriopus* does not differentiate between *T. californicus* and the Japanese copepod, *T. japonicus*. He bases this finding mainly on the fact that both sexes of *T. californicus* and *T. japonicus* have five bristles on the exopod of their fifth leg as well as several other characteristics. Upon studying sketches of *T. japonicus* by Takeda (1951) and comparing them with sketches of *T. californicus*, the author found no apparent differences in morphology between *T. japonicus* and *T. californicus*. However, there are certain apparent differences in development as reported by Takeda (1951). (see page 9)

Lance and Buzzati-Traverso (1959) have substantiated

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*a secondary reference from Monk (1941).

the view that these two organisms are separate species. Their report is based on morphological studies as well as extensive crosses between available strains of T. californicus and T. japonicus.

B. Key Characteristics

The length of the females from the anterior-most point of the rostral protuberance to the fork of the last urosomal segment varies between 0.90 mm. to 1.2 mm. The length of the males, using the same measuring points, varies between .95 mm. to 1.10 mm. Although this animal has a typical cyclopoid habitus and is described rather ambiguously in Light's Manual (1954), it can be easily identified as a harpacticoid by the shorter antennules, the very heavy antennule of the male, the single egg sac of the female, and the single, major, long seta on each caudal ramus. Ricketts and Calvin (1952) falsely describes the female egg sac by saying:

> "We have taken copulating specimens near Santa Cruz in April and have seen, at the same time, thousands of ovigerous females, each carrying its eggs in two long cylindrical sacs, almost like caudal appendages."

C. Sex Differences

The sexes of adult T. californicus may be distinguished by the unaided eye if one looks for the following characteristics: male - very heavy first antennule; female - first antennule slender, usually carrying a single red or green

egg sac ventrally on the urosome. The male is usually found dorsal to the female in the copulatory position.

Under microscopic examination, the following characteristics may be used to distinguish sexes.

Female. - Metasome elongate ovate, only a little narrowed posteriorly, the first three thoracic segments with lateral lappets, urosome narrower than metasome, the segments diminishing in length posteriorly. The first antennae (figure 1) are nine-segmented, the four basal segments thick and robust, the five distal segments being slender and short, the fourth basal segment having two setae which exceed the length of the five distal segments. The exopod of the second leg has three segments, the distal segment having four setae. (figure 2) The fifth leg has two segments, the distal being ovate with five setae. The basal segment, about three times as large as the distal segment, has six large setae, five medial and one lateral. The sixth leg is rudimentary and is characterized by three medial setae extending from a slight projection on the genital segment of the urosome. (figure 2)

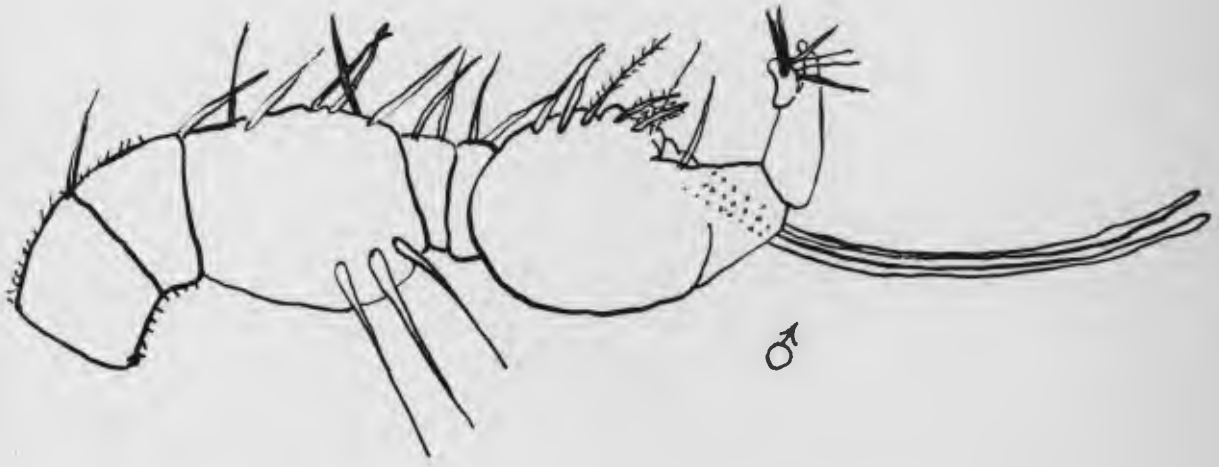
Male. - Metasome and urosome about the same size and shape as the female. First antennae stout, with seven segments, the terminal segment being chelate with a globular hand and a reduced claw-like dactylus. The sixth segments of the first antennae are the largest, being one and one-half times as long as wide and having two setae that are longer

FIGURE 1

SEXUAL DIFFERENCES IN THE FIRST ANTENNULE OF

TIGRIOPUS CALIFORNICUS

FIGURE 1



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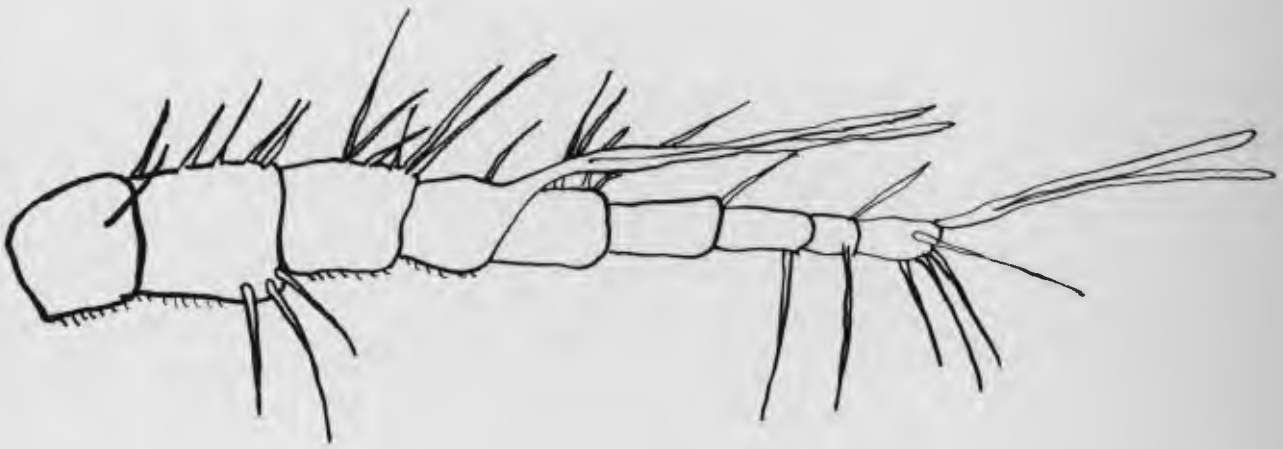

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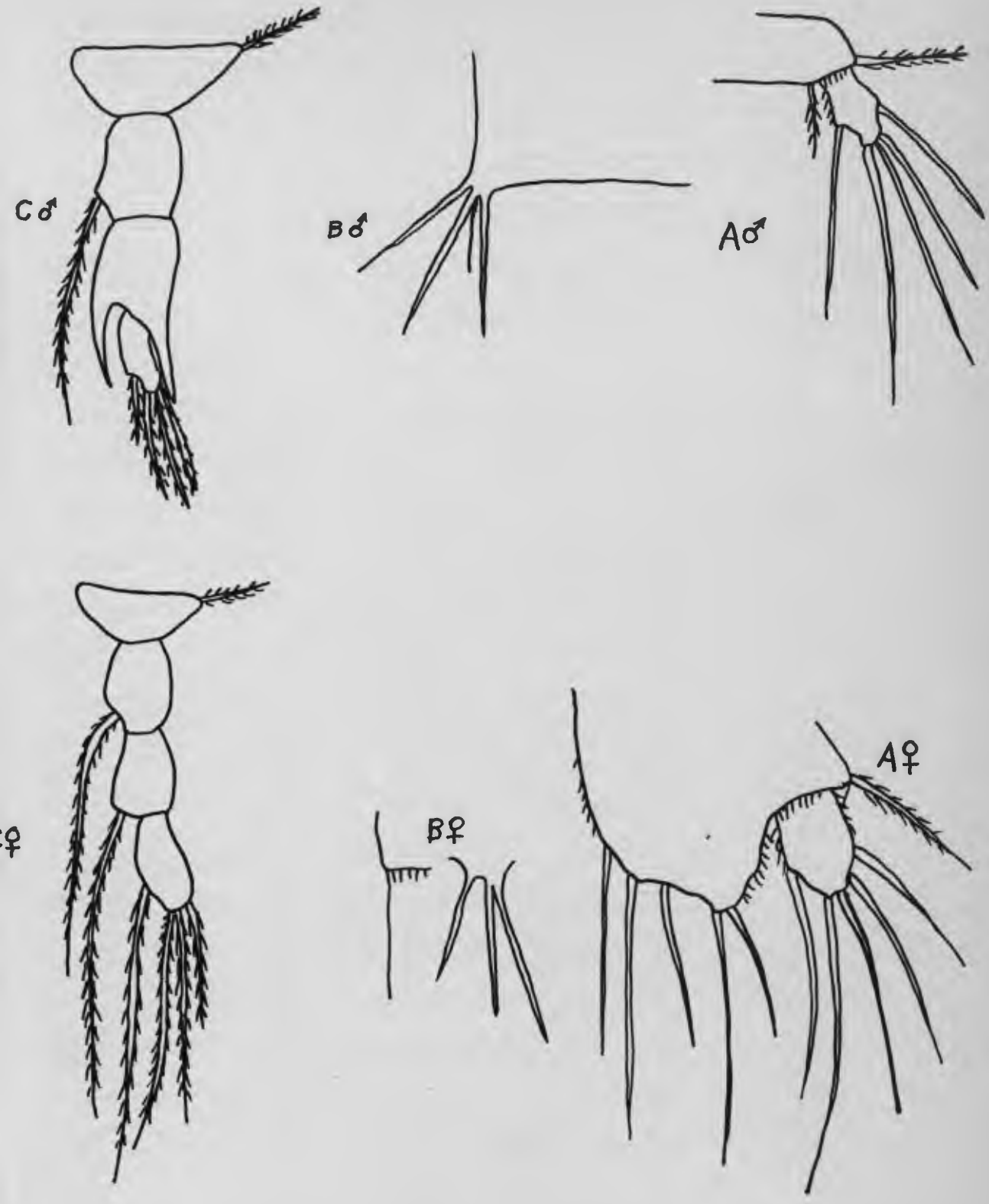
FIGURE 2

SEXUAL DIFFERENCES OF (A) FIFTH LEG (B) SIXTH LEG
(C) ENDOPODITE OF THE SECOND LEG FOR TIGRIOPUS CALIFORNICUS

FIGURE 2



than the basal five segments. The endopod of the second leg has three segments. The second segment has two spines at the distal medial-lateral corners which extend beyond the third segment. The third segment has four setae. The fifth leg is much reduced in size, the inner expansion of the basal segment has disappeared, the terminal segment has five setae. (figure 2). The sixth pair of legs are reduced, as in the female, and are found on the posterior-lateral corners of the genital segment of the urosome.

D. Life Cycle

The development of Tigriopus may be divided into three morphological stages: nauplius stage, copepodid stage, and adult stage. Fraser (1935) describes five distinct stages of nauplius larva for T. fulvus. His five stages are based on molts and the consequent addition of appendages or segments to the appendages. The nauplii in the first stage vary in length from 116 to 126 microns. The nauplii in the fifth stage vary in length from 243 to 256 microns. The length of time for completion of the five stages is thirteen to fifteen days. Fraser also recognizes five distinct copepodid stages, with the copepodids in the first stage varying in length from 360 to 400 microns. The copepodids in the fifth stage vary in length from 770 to 850 microns. The completion of these five stages generally takes one month with the complete development requiring two months. Takeda (1951) recognizes twelve stages from

birth to maturity in T. japonicus; six nauplius and six copepodid stages, the sixth copepodid being the adult stage. He also notes that it takes six to eight days for T. japonicus to go from birth to maturity at 23° C. T. californicus appears to the author to go through five nauplius stages with a marked metamorphosis to the first copepodid stage. (The nauplius larvae are ovate and slightly compressed dorso-ventrally, the copepodid being slightly compressed laterally.) The five nauplius stages are generally completed between nine and eleven days at 20° C. There appear to be five copepodid stages with the mature individuals occurring between twenty-three and thirty days after birth at 20° C. and twenty to twenty-eight days at 25° C. The number of stages have been calculated by observing the number of molts that isolated individuals have undergone.

After these copepods reach maturity they generally copulate within two or three days. Copulation is initiated by the male grasping an unfertilized female laterally on her metasome with his chelated antennules. The copepods will remain in this position from several hours to several days. If a mature male grasps an immature female the copulatory position will be maintained for several days, both using their urosomes for locomotion. Shaw (1938) reported that the copulation period in T. fulvus lasts for only a few seconds. This seems plausible if he is referring

only to the actual transfer of sperm. When the sperm has been transferred to the spermatheca of the female, the female violently struggles to regain her freedom. In populations where males predominate, the males are unusually tenacious and often lose the chela from the terminal segments of their antennae as a result of the post-copulatory struggle. The male's selection of a partner appears to be random in that he will attempt to grasp even a male but usually fails. However, on several occasions the author has observed a male successfully grasping the metasome of another male who is already copulating with a female. The speed and agility of a copulating couple is greatly reduced, therefore facilitating a successful mount of a second male. On one occasion while the author observed the above described three-copepod-chain, the usual struggle took place in a cloud of silt and algae in the bottom of the laboratory jar, from which there emerged three male copepods briefly linked to one female. If, after three or four days of mature life, a female is not successfully fertilized, dark green eggs will appear in her uterus and after a few days her egg sac will fill but the eggs will not develop. The dorsal-medial ovary in the metasome with horned uterus lying laterally can be easily seen through the translucent chitin when eggs are present. An unfertilized female will carry an egg sac of undeveloped eggs for three or four weeks until the egg sac drops off. Then, depending on the age of the female, the egg sac may or may not refill.

If the egg sacs are removed artificially with a needle, the sacs will quickly be reconstructed and filled up to four times by a healthy unfertilized female. The author has never observed a female to copulate after once having a fertile or unfertile egg sac even though subjected to an abundance of males. The literature reviewed by this worker has indicated that parthenogenesis does not exist for any of the species of Tigriopus. In order to verify this finding several dozen female copepodids were individually isolated and observed until death. Although nearly all females produced egg sacs none of the eggs developed. The number of eggs produced varies between ten and thirty with an average of seventeen eggs per female from a random selection of one hundred females at 20° C. Shaw (1938) reports that T. fulvus produces between thirty and ninety-five eggs with an average of sixty-six eggs per egg sac. All fertile females release more than one egg sac during their life. Nichollis (1935) reports that an adult female Longipedia sp. may produce up to nine egg sacs during her reproductive life. In T. californicus the author has observed the appearance of eight egg sacs, seven of which were fertile, producing a total of one hundred and four nauplii with the eighth egg sac being unfertile. (for a complete history of this female see table 1)

Several copepods have been kept alive as long as six months under laboratory conditions. Their longevity

TABLE 1

Life History of a Female *Tigriopus californicus*

Developmental Stages of Female	Date 1960
released from parental egg sac	June 28
first copepodid stage	July 8
copulatory position assumed with a male	July 19
released from copulatory position	July 20
eggs appearing in uterus	July 21
dark green eggs appearing in egg sac, female isolated	July 23
light green eggs appearing in egg sac	July 24
eggs bright red	July 28
released first egg sac, 12 nauplii appear, female again isolated	July 30
egg sac again full	August 1
second egg sac released, 15 nauplii appear, female again isolated	August 4
third egg sac, 14 nauplii appear, female again isolated	August 7
fourth egg sac released, 18 nauplii appear, female again isolated	August 10
fifth egg sac released, 19 nauplii appear, female again isolated	August 13
sixth egg sac released, 17 nauplii appear, female again isolated	August 16
seventh egg sac released, 9 nauplii appear, female again isolated	August 19
eighth egg sac appears	August 21
offspring from first egg sac now copulating	August 23
eighth egg sac released, no nauplii, eggs remaining green	August 29
death of female	September 19

under natural conditions has not been determined.

E. Physical Characteristics of the Habitat

T. californicus is commonly found in high, rocky splash pools along the entire coast of California. The author has found this copepod in saline potholes twenty-five feet above the mean low tide mark one mile north of Trinidad Head, California. In view of this habitat, T. californicus must be able to withstand abnormally high salinities and temperatures. The surface temperatures of ocean waters adjacent to the vicinity of the study ranged from 9° C. to 11° C. The normal salinity for coastal waters is 34 o/oo. One half mile north of Trinidad Head this copepod has been collected in shallow black basalt-rock tide pools that had a temperature of 32° C. and a salinity of 90 o/oo. During the summer months in northern California when there are no storms, the salinity of most highly situated tide pools ranges from 34 o/oo to 50 o/oo, with the temperature during most sunny days varying between 15° C. and 25° C. During the winter months the temperature of the tide pools in the Trinidad area drops to 6° C. and the salinity to 20 o/oo. On several occasions the copepods have been seen living normally in shallow pools whose periphery was almost completely lined with a layer of salt crystals. These pools, if not supplied with sea spray, usually were subjected to complete desication within a few days. Those pools in which large populations of copepods could always

be found were usually over twelve inches in depth, and had salinities near 40 o/oo and a temperature close to 20° C. during the summer months. The temperature of the shallow pools, those less than six inches in depth, usually did not vary more than 1° C. between the surface and the bottom. In pools deeper than two feet, the temperature did not vary more than 3° C. between surface and bottom. A thermocline has not been detected in any of the tide pools that have been investigated.

F. Ecology of Habitat

The deep permanent tide pools in the vicinity of Trinidad, California, that are inhabited by T. californicus are generally lined with the green filamentous alga, Chaetamorpha. In most pools where there was an admixture of fresh water the yellow-green filamentous alga, Enteromorpha was also present. The most common animal that is found in close association with T. californicus is the gammarid Allorchestes angustus. This amphipod is almost always found in pools containing Chaetamorpha. Allorchestes angustus has been kept alive under laboratory conditions for six months in jars containing both T. californicus and Chaetamorpha. One laboratory specimen reached a length of 1.5 cm. As far as can be determined, this gammarid feeds only on algae. In the tide pool located at College Cove (figure 3) the following animals have been found living in common with T. californicus:

Pachygrapsus crassipes
Littorina scutulata
Mitella californicus
Acmaea digitalis
Tegula funebralis
Pagurus sp.

Littorina scutulata is generally the only animal found in association with T. californicus in temporary, shallow pools with extremely high temperatures and salinities.

G. Population

Populations of copepods taken during the summer months yield quite different sex ratios. In counting the sexes of five hundred randomly selected copepods, taken from the College Cove tide pool on August 13, 1960, it was found that there were 340 females, (125 of which were carrying egg sacs) and 160 males, an approximate 2:1 sex ratio. There were 85 copulating couples included in the five hundred. On August 18th a sample of the population in a tide pool 150 yards from the end of the North Jetty at the entrance of Humboldt Bay was taken. From a random sample of 200 individuals, 78 females and 127 males were counted, yielding a ratio of 0.6:1. The copepods at both locations were in tide pools that had large colonies of Chaetamorpha and salinities close to 40 o/oo. However, the temperature of the tide pool at the North Jetty, because of its shallow depth, is subject to a much greater range of temperature than is the deeper tide pool at College Cove. On August 13, 1960, the population of the College Cove tide pool was estimated

to be approximately 0.5 million copepods. This estimation is based on 5,000 copepods that were counted in a one-gallon sample from the tide pool multiplied by an estimated 100 gallons that the pool contained.

FIGURE 3

TIDE POOL CONTAINING TIGRIOPUS CALIFORNICUS

LOCATED AT THE NORTH END OF COLLEGE COVE,

ONE MILE NORTH OF TRINIDAD HEAD, CALIFORNIA

FIGURE 3

III. METHODS AND MATERIALS

A. Source of material

The T. californicus used for experimentation were collected from a high splash-pool at the north end of College Cove, one-half mile north of Trinidad, California. Because of the position of this pool it is completely protected from the prevailing northwest summer swells. This pool (figure 3) is 12 feet above the mean low tide level, 12 feet in length, and reaches a depth of 3 feet. On August 13, 1960, the surface temperature was 19° C., bottom temperature was 16° C., and the salinity was 40 o/oo. (salinities were derived by titration with silver nitrate) On August 17, 1960, the surface temperature was 26° C., the bottom temperature was 22° C., and the salinity was 40 o/oo. This pool is vulnerable to fall and winter storms and on October 19, 1960, the population of copepods had practically disappeared because of storm surf. Some of the copepods used in the experiments dealing with differences in sex ratios were taken from shallow pools on the North Jetty at the entrance to Humboldt Bay. The copepods were always collected with a large 250 cc. glass basting syringe, and transported to the laboratory in clean glass jars.

B. Maintenance and Feeding

The stock copepods were kept in the laboratory in wide mouth, one-gallon jars. The jars were filled from one-half to three-quarters with filtered sea water of a salinity of 34 o/oo. The filtered sea water was kept in a tightly sealed 10 gallon polyethylene container. Because this sea water, which will be referred to as normal sea water, was used in nearly all the physiological experiments the salinity was periodically checked to insure that it remained at 34 o/oo. Glass plates were used to cover the mouths of the jars to prevent evaporation. The stock jars were kept on a north-facing window sill and never subjected to direct sunlight. The temperature of the laboratory room remained between 18.5° C. and 21.5° C. (The most common temperature was 20° C.)

All culture jars were supplied with large sheets of the blue-green alga, Phormidium. The algal cells are carried into and along a funnel formed by the maxillary setae until they reach the mouth where they are formed into pellets and ingested. Cast off fecal pellets can be and usually are used for food by Tigriopus when there is a food shortage. Yeast stained with congo red was also introduced into a few culture dishes and was found to be ingested and defecated in the form of red pellets. Because of the copepods' use of Phormidium for food, this alga was used as the standard food for all experiments that required feeding.

On several occasions it was observed that the number of newly hatched nauplii were greatly diminished overnight when there was an abundance of adults present in the culture dish. The author has not witnessed cannibalism by Tigriopus but the repeated disappearance of the nauplii when not immediately isolated suggests that the adults eat their offspring. Provasoli, et. al. (1959) report the occurrence of cannibalism in Tigriopus, especially by males.

C. General Experimental Procedures

1. Changes in salinity

Several thousand T. californicus were collected from the high splash-pool at College Cove and transferred to normal sea water to overcome the effects of the fluctuating salinity of the tide pool. A series of twelve solutions were made up with salinities ranging from 0.00 o/oo (distilled water) to 100 o/oo. The twelve solutions were:

0 o/oo	17 o/oo	70 o/oo
2 o/oo	34 o/oo	80 o/oo
4 o/oo	50 o/oo	90 o/oo
8 o/oo	60 o/oo	100 o/oo

Those salinities below 34 o/oo were obtained by diluting normal sea water with distilled water. Those salinities above 34 o/oo were obtained by evaporating 1000 cc. of sea water down to 340 cc. with a resulting salinity of 100 o/oo. Intermediate salinities were obtained by cutting the 100 o/oo solution with distilled water. No precipitation occurs when sea water is evaporated to this concentration. Another method of obtaining high salinities is to add a predetermined weight of dry sea salt to normal sea water. This dry sea salt may be obtained by evaporating sea water, in plastic trays, to complete dryness. This method was not used because of the inaccuracy introduced by certain salts, probably carbonates, which do not go back into solution when added

to normal sea water. Approximately fifty specimens of T. californicus were transferred into 100 cc. of each solution and left for a period of two weeks. Copepods treated in this manner will be referred to as conditioned. The transfer from the stock cultures to the individual solutions were made by filtering off the normal sea water, from a volume of water containing fifty copepods with a course filter paper and a glass funnel. The filter paper with the adhering copepods was touched to the surface of the new solution and usually all of the copepods would free themselves from the paper. The solutions were contained in 125 cc. culture stacking dishes. The alga Phormidium was introduced to the cultures on the first and eighth days as a food supply. The entire experiment was conducted at 20° C. (± 1.5° C.)

2. Procedure for inducing salt shock with high salinities and distilled water

Approximately 100 copepods, that had previously been conditioned in normal sea water, were transferred by filter paper to solutions of the following salinities:

100 o/oo	125 o/oo
110 o/oo	150 o/oo
115 o/oo	175 o/oo
120 o/oo	0 o/oo

(100 cc. of each solution in culture dishes, salinities derived by the volumetric evaporation method described in experiment 1.) Almost immediately upon

coming in contact with solutions of salinities over 90 o/oo and distilled water copepods will go into salt shock. (see discussion, page 56) Every fifteen minutes for three hours, five "salt-shocked" copepods from each high salinity solution and distilled water were removed by an eye dropper and placed in syracuse dishes containing normal sea water. After a period of three hours, five "shocked" copepods were transferred to normal sea water every six hours for two days. The period of time beginning with the introduction of "shocked" copepods into normal sea water and ending when more than half of the copepods recovered was recorded as the recovery period. The copepods that recovered were observed for several days to insure that they resumed normal activity. This experiment was conducted at 20° C. (± 1.5° C.)

3. Procedure for inducing salt shock using normal sea water and Na^{+}, K^{+}, and Ca^{++}.

A calculated weight of pure NaCl was added to normal sea water to derive salinities of 125 o/oo, 150 o/oo 175 o/oo, and 225 o/oo. Pure KCl was added to normal sea water to derive salinities of 125 o/oo, 175 o/oo, and 225 o/oo. Solutions of the above three salinities also were made by adding $CaCl_2$ to normal sea water. Approximately one hundred conditioned copepods were added, by filter paper, to 100 cc. of each of the above

solutions. As in experiment 2, all of the copepods went into immediate salt shock. Five "shocked" copepods from each solution were transferred by an eye dropper to normal sea water at fifteen minute intervals for three hours. The time was recorded when at least one half of the "shocked" copepods completely recovered in normal sea water. All dishes were covered with glass plates to reduce any evaporation which would tend to increase the salinity. This experiment was conducted at 20° C. (± 1.5° C.)

4. Procedure for determining the lethal temperature at various salinities

The lethal temperature for T. californicus in normal sea water, 34 o/oo, was determined by putting four 2 inch petri dishes each containing 20 cc. of normal sea water and twenty copepods in a regulated water bath. Starting at 28° C. the temperature was increased at the rate of three degrees per hour until three quarters of the copepods assumed a motionless position at the bottom of the dish. The temperature at this point was recorded as the lethal temperature. This death point was determined by immediately transferring the motionless copepods to normal sea water; none of these recovered at room temperature (20° C. ± 1.5° C.).

By the procedure outlined in experiment 1, solutions of the following salinities were made:

2 o/oo	50 o/oo
4 o/oo	60 o/oo
8 o/oo	70 o/oo
17 o/oo	80 o/oo
34 o/oo	90 o/oo

Twenty specimens of conditioned T. californicus were transferred from normal sea water, by filter paper, to 20 cc. of each of the above solutions. Glass petri dishes containing the copepods were placed on a water bath. The temperature of the water bath was increased at a rate of 3° C. per hour. The copepods were observed constantly and the temperature was recorded when 75% of the animals in each dish lay motionless in the bottom of the dish.

5. Procedure for determining the lethal temperature at various salinities using selected salts

By adding a pre-calculated weight of pure NaCl to normal sea water the following salinities were produced:

50 o/oo	80 o/oo
60 o/oo	90 o/oo
70 o/oo	

Another set of solutions of the same salinities were made by the addition of $MgCl_2$. Solutions of the same salinities were made by using KCl and $CaCl_2$ but because the animals went into salt shock immediately upon being introduced into these solutions their lethal temperature could not be determined. The lethal temperature was again recorded when 75% of the copepods lay motionless

in the bottom of the dish.

6. Procedure for altering the sex ratio by artificial means

To examine the effects of chemicals on sexuality, the nauplius larvae from one female egg sac were divided as evenly as possible into two groups and put into separate 150 cc. culture stacking dishes. One group was reared in an artificial medium, while the other was put into normal sea water and used as a control. The artificial medium was made by adding 0.15 gms. of $KClO_3$ to 100 cc. of normal sea water (Takeda 1951). The larvae were transferred to the artificial medium and normal sea water within 24 hours after their release from the female. Females with red egg sacs were isolated in syracuse dishes and usually placed under a bright light over night to induce the release of the larvae from the egg sac. The larvae were transferred to the artificial media or the normal sea water by an eye dropper the aperture of which had been reduced to a small diameter by heating. The other end of the dropper was attached to two feet of rubber tubing that was held in the operator's mouth. The nauplius larvae were provided with enough Phormidium to last several weeks. This experiment was conducted at 25° C. with the aid of an incubator in order to compare the resulting ratios with Takeda's experiment. Upon reaching maturity

the sexes were counted and recorded. If more than two copepods had been lost during the course of the experiment from any one culture dish the entire culture was discarded. Another series of cultures were used in order to determine the effect of temperature on sex ratios, again evenly dividing larvae from one egg sac and placing them into two dishes, but both cultures were raised in normal sea water. One-half of the cultures were kept in the incubator at 25° C. and the other half were kept at room temperature, 20° C. ± 1.5° C. The same experimental procedure was used in isolating, transferring, counting, and recording the specimens. This experiment was repeated at 25° C. and 30° C. All copepods in the temperature control experiments were fed Phormidium.

IV. RESULTS

A. Results of Changes in Salinity on T. californicus (Table 2)

It was found that those T. californicus that were introduced into the solutions of a salinity of 100 o/oo and distilled water, 0 o/oo, immediately went into shock. At least one-half of the animals lived for a period of two weeks in solutions of the following salinities:

2 o/oo	50 o/oo
4 o/oo	60 o/oo
8 o/oo	70 o/oo
17 o/oo	80 o/oo
34 o/oo	90 o/oo

The highest mortality, close to 40%, occurred at salinities of 2 o/oo, 4 o/oo, 80 o/oo, and 90 o/oo. The lowest mortality, less than 5%, was at salinities of 34 o/oo, 50 o/oo, and 60 o/oo. It was found that there was usually a 2% mortality when the animals were transferred due to injury inflicted by the filtering process. This experiment was repeated three times, yielding similar results.

B. Results of Inducing Salt Shock at High Salinities and Distilled Water (figure 4 and 5)

At a salinity of 100 o/oo it was found that T. californicus would undergo reversible salt shock, recovering after two hours without being immersed in normal sea water. However, after six hours in the 100 o/oo solution they again would go into salt shock. The salt shock was irreversible

TABLE 2

% Mortality Rate for *T. californicus* at Various Salinities

o/oo salinity	% mortality
2 o/oo	42 %
4 o/oo	40 %
8 o/oo	20 %
17 o/oo	10 %
34 o/oo	2 %
50 o/oo	5 %
70 o/oo	20 %
80 o/oo	35 %
90 o/oo	40 %

FIGURE 4

RECOVERY FROM SALT SHOCK FOR *TIGRIOPUS CALIFORNICUS*

FIGURE 4

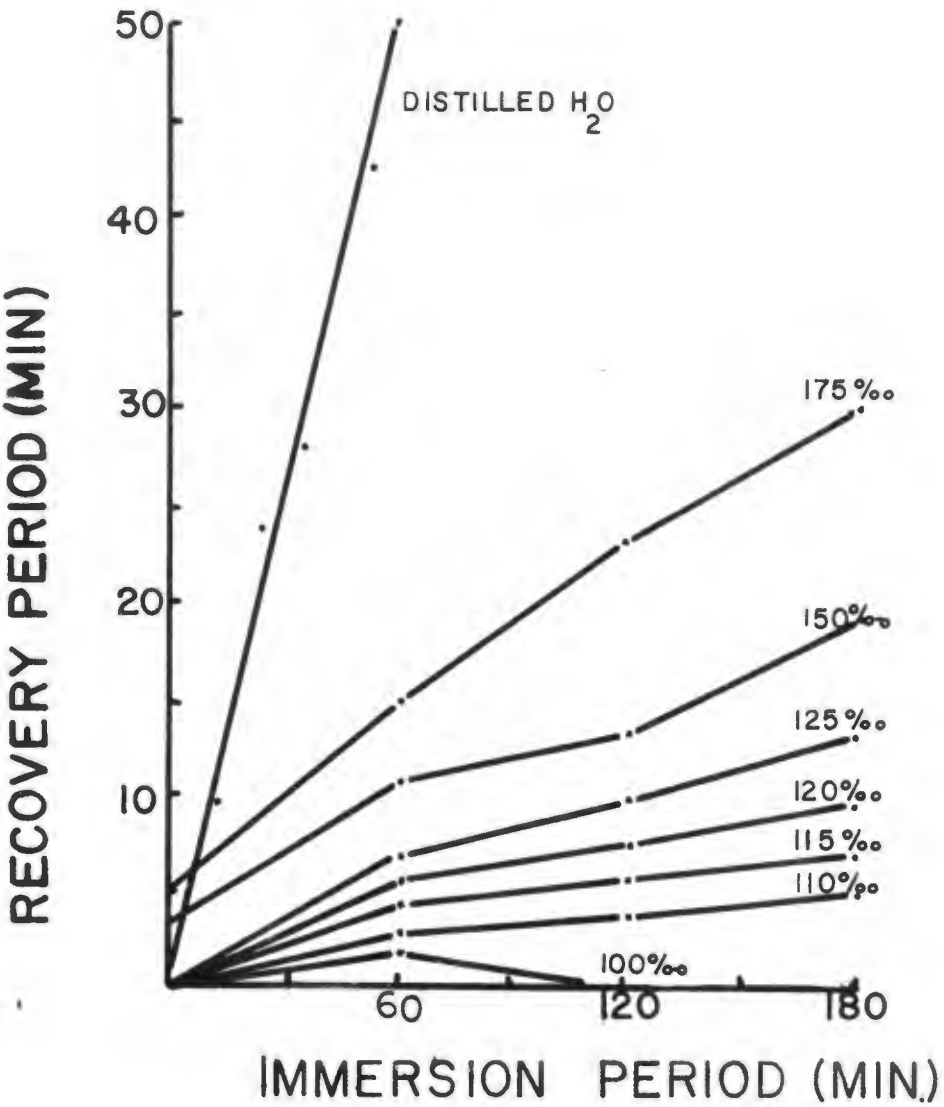
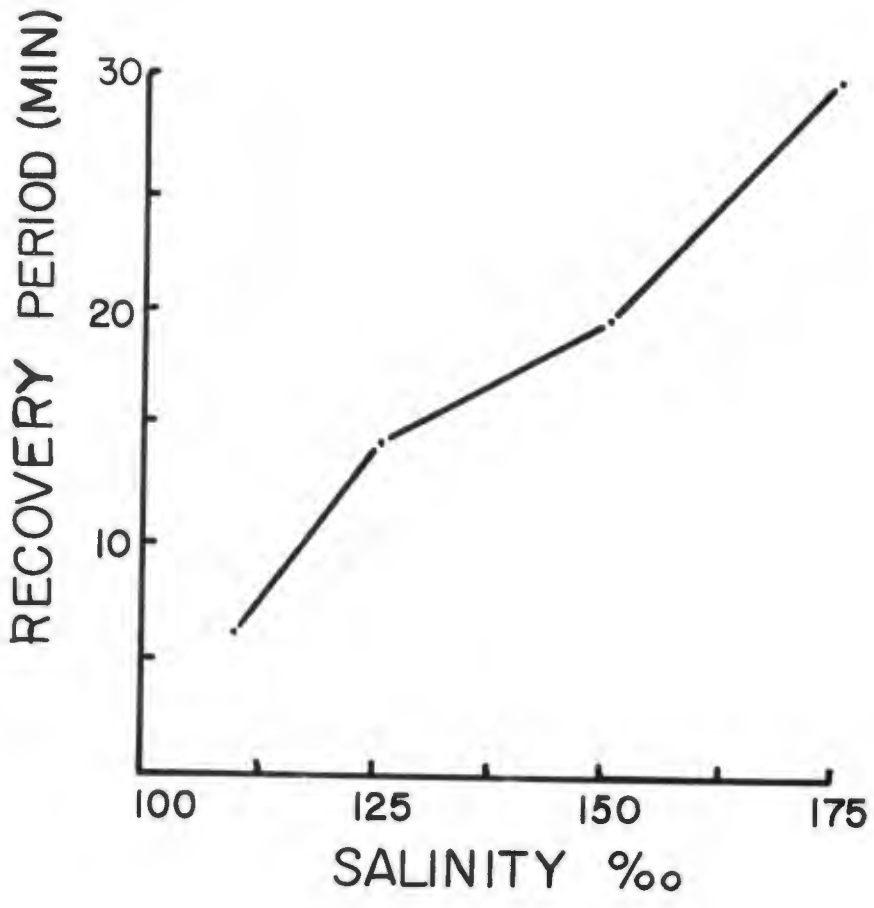

50
40
30
20
10
RECOVERY PERIOD (MIN)
DISTILLED H_2O
175‰
150‰
125‰
120‰
115‰
110‰
100‰
60
120
180
IMMERSION PERIOD (MIN.)

FIGURE 5

RECOVERY FROM SALT SHOCK AFTER THREE HOURS IMMERSION IN VARIOUS SALINITIES FOR *TIGRIOPUS CALIFORNICUS*

FIGURE 5



for copepods in a solution of 100 o/oo after seventy-two hours. Those T. californicus that were introduced into distilled water, 0 o/oo, could be revived when reintroduced to normal sea water if they were removed from the distilled water before a period of one hour. After one hour in the distilled water the shock was irreversible. Those copepods immersed in solutions between 110 o/oo and 175 o/oo took a proportionately longer period of time to recover after being reintroduced to normal sea water. (see figure 5) Those animals immersed in salinities of 110 o/oo to 150 o/oo would not recover from salt shock after thirty-six hours. Irreversible salt shock occurred in those copepods immersed in 175 o/oo after twenty-four hours.

C. Results of Inducing Salt Shock at High Concentrations of NaCl, KCl, and $CaCl_2$ (figures 6, 7, 8, 9)

In sea water to which NaCl had been added irreversible salt shock occurred immediately upon introduction to the solution of 225 o/oo, after thirty minutes immersion in 175 o/oo, after three hours in 150 o/oo, and after twelve hours in 125 o/oo. The recovery period took much longer at higher salinities than at lower salinities for the same immersion period. (figure 6)

For KCl and normal sea water irreversible salt shock occurred after ten minutes immersion at 225 o/oo, after one hour at 175 o/oo and after two hours at 125 o/oo. The time required for recovery was much greater at higher

FIGURE 6

RECOVERY FROM SALT SHOCK INDUCED BY IMMERSION IN NORMAL SEA WATER PLUS NaCl FOR *TIGRIOPUS CALIFORNICUS*

FIGURE 6

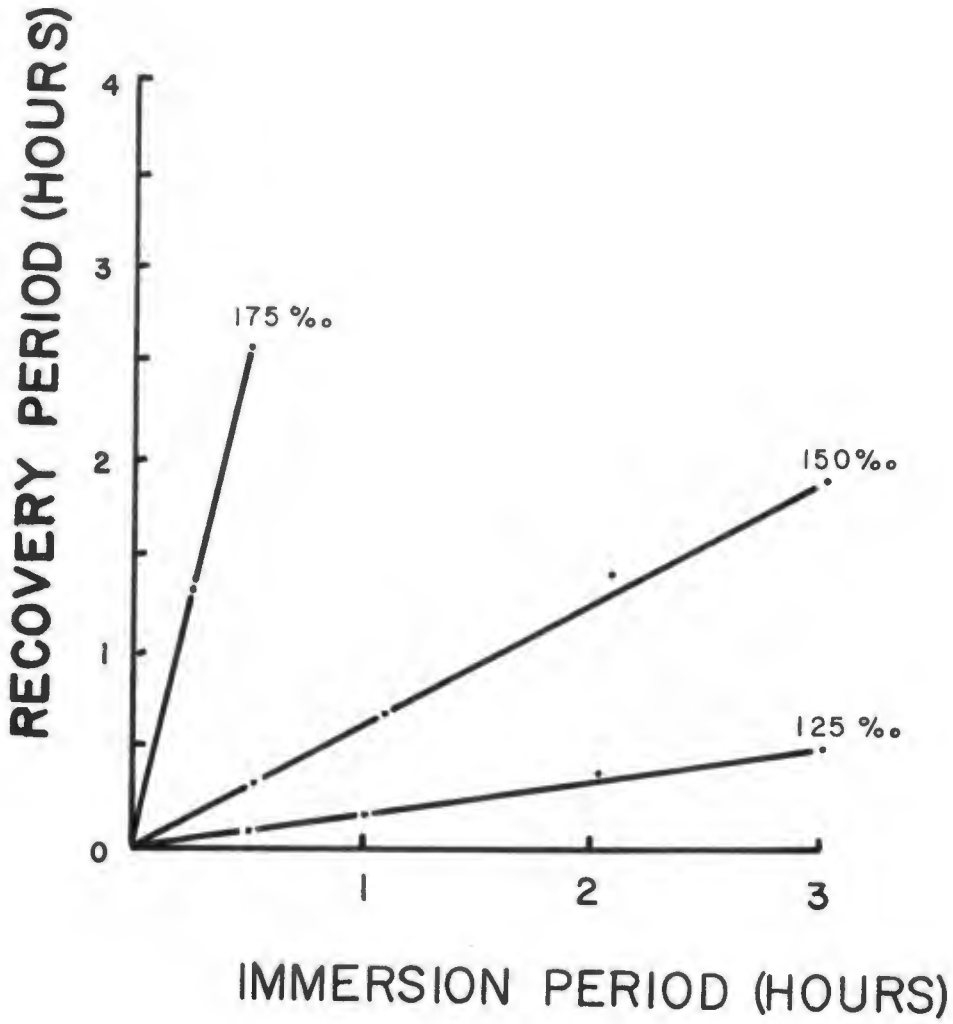

RECOVERY PERIOD (HOURS)
4
3
2
1
0
175 ‰
150‰
125 ‰
1
2
3
IMMERSION PERIOD (HOURS)

FIGURE 7

RECOVERY FROM SALT SHOCK INDUCED BY IMMERSION IN NORMAL SEA WATER PLUS KCl FOR *TIGRIOPUS CALIFORNICUS*

FIGURE 7

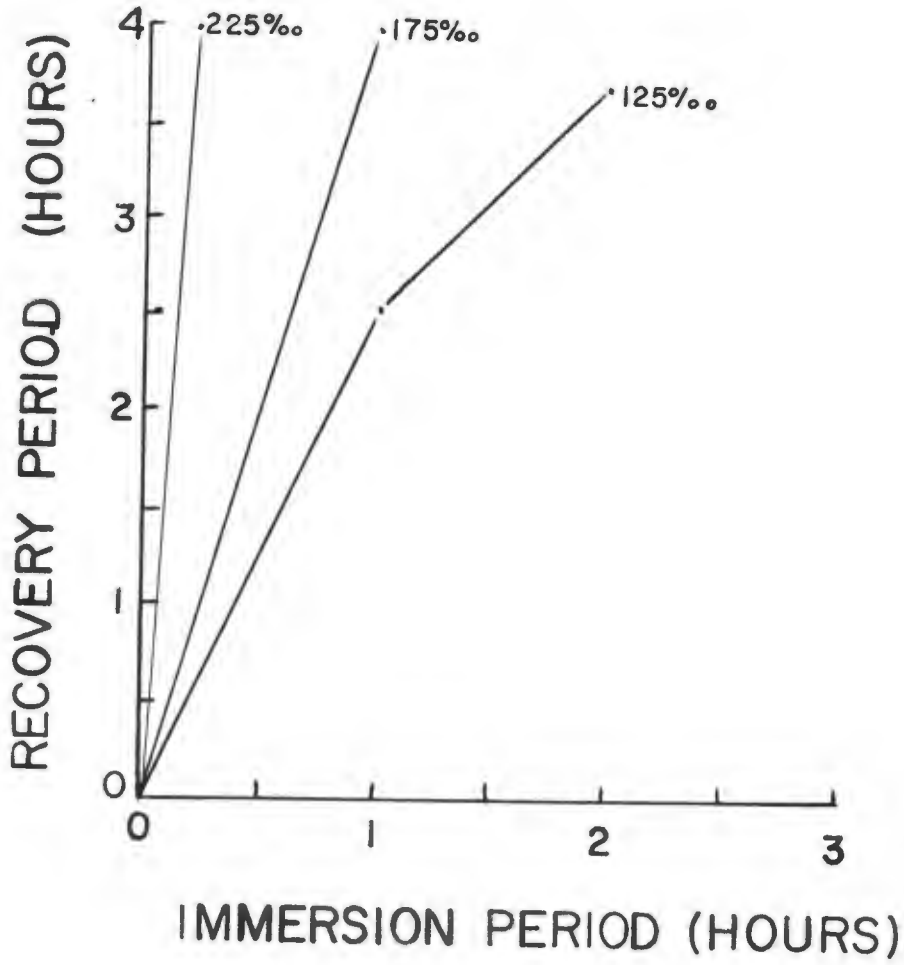

RECOVERY PERIOD (HOURS)
4
3
2
1
0
225‰
175‰
125‰
0
1
2
3
IMMERSION PERIOD (HOURS)

FIGURE 8

RECOVERY FROM SALT SHOCK INDUCED BY IMMERSION IN NORMAL SEA WATER PLUS $CaCl_2$ FOR TIGRIOPUS CALIFORNICUS

FIGURE 8

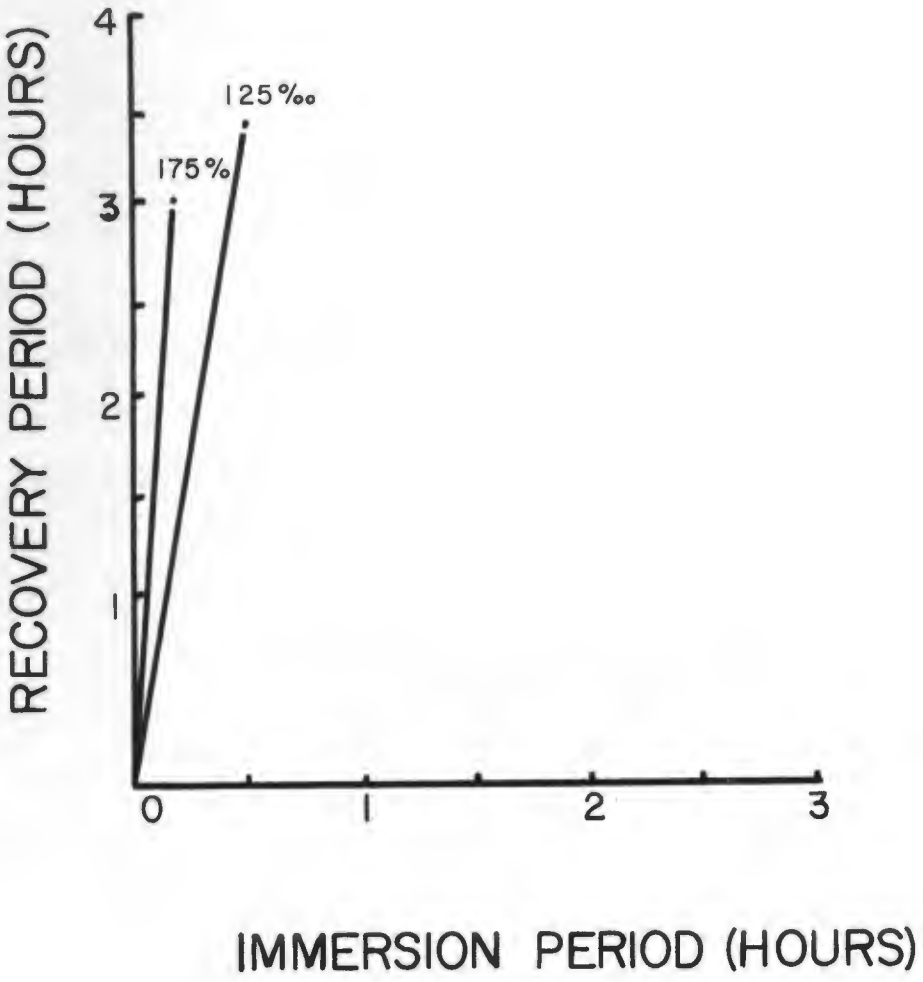
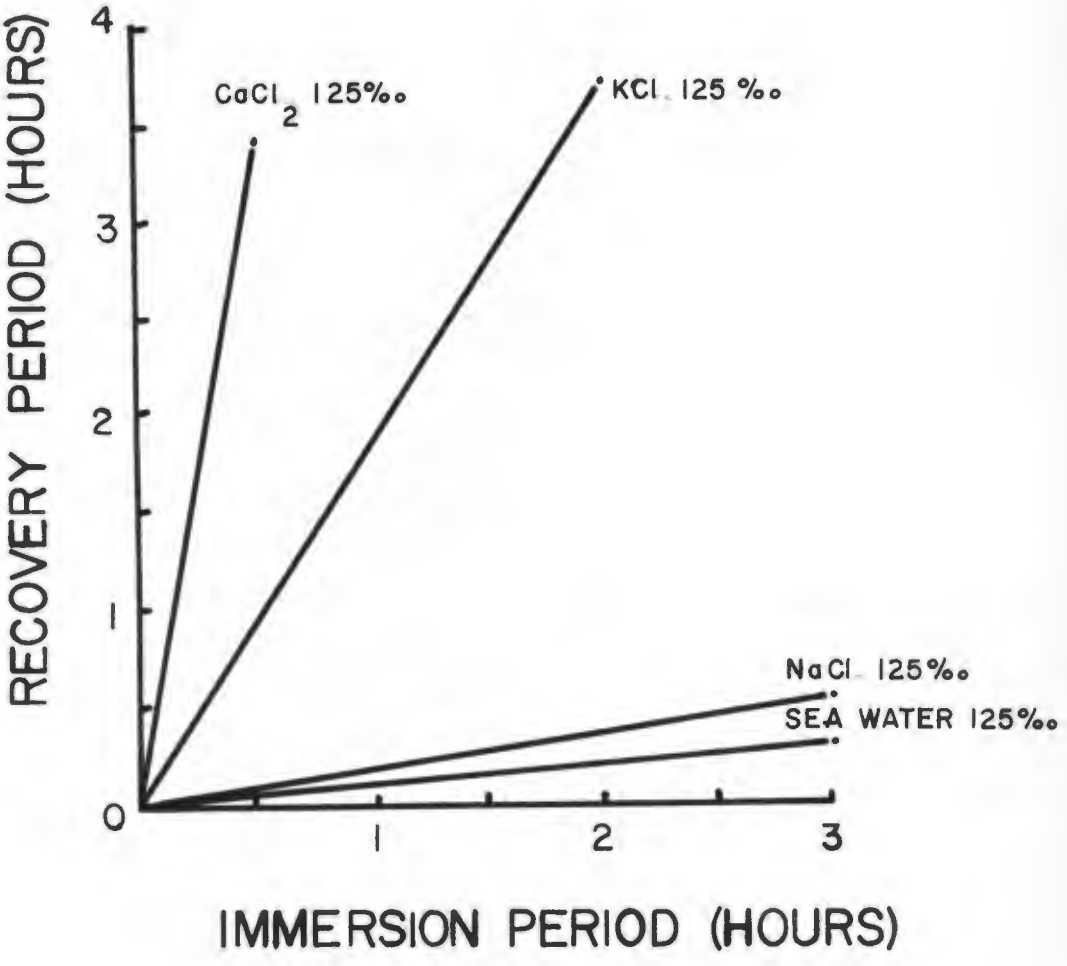

RECOVERY PERIOD (HOURS)
4
3
2
1
0
1
2
3
125‰
175‰
IMMERSION PERIOD (HOURS)

FIGURE 9

RECOVERY FROM SALT SHOCK INDUCED BY IMMERSION IN NORMAL SEA WATER AND IN SEA WATER MADE UP TO 125 0/00 WITH THE ADDITION OF NaCl OR KCl OR $CaCl_2$ FOR TIGRIOPUS CALIFORNICUS

FIGURE 9


RECOVERY PERIOD (HOURS)
4
3
2
1
0
CaCl2 125‰
KCl 125 ‰
NaCl 125‰
SEA WATER 125‰
1
2
3
IMMERSION PERIOD (HOURS)

salinities than at lower salinities for the same immersion period. (see figure 7)

T. californicus exposed to 225 o/oo $CaCl_2$ went into immediate irreversible salt shock. At 175 o/oo irreversibility occurred after ten minutes immersion and after thirty minutes immersion at 125 o/oo. (see figure 8)

For a summary of the relationships of immersion-recovery periods for NaCl , KCl, and $CaCl_2$ at salinities of 125 o/oo see figure 9.

D. Effect of Changes in Salinity and Temperature on T. californicus (figure 10)

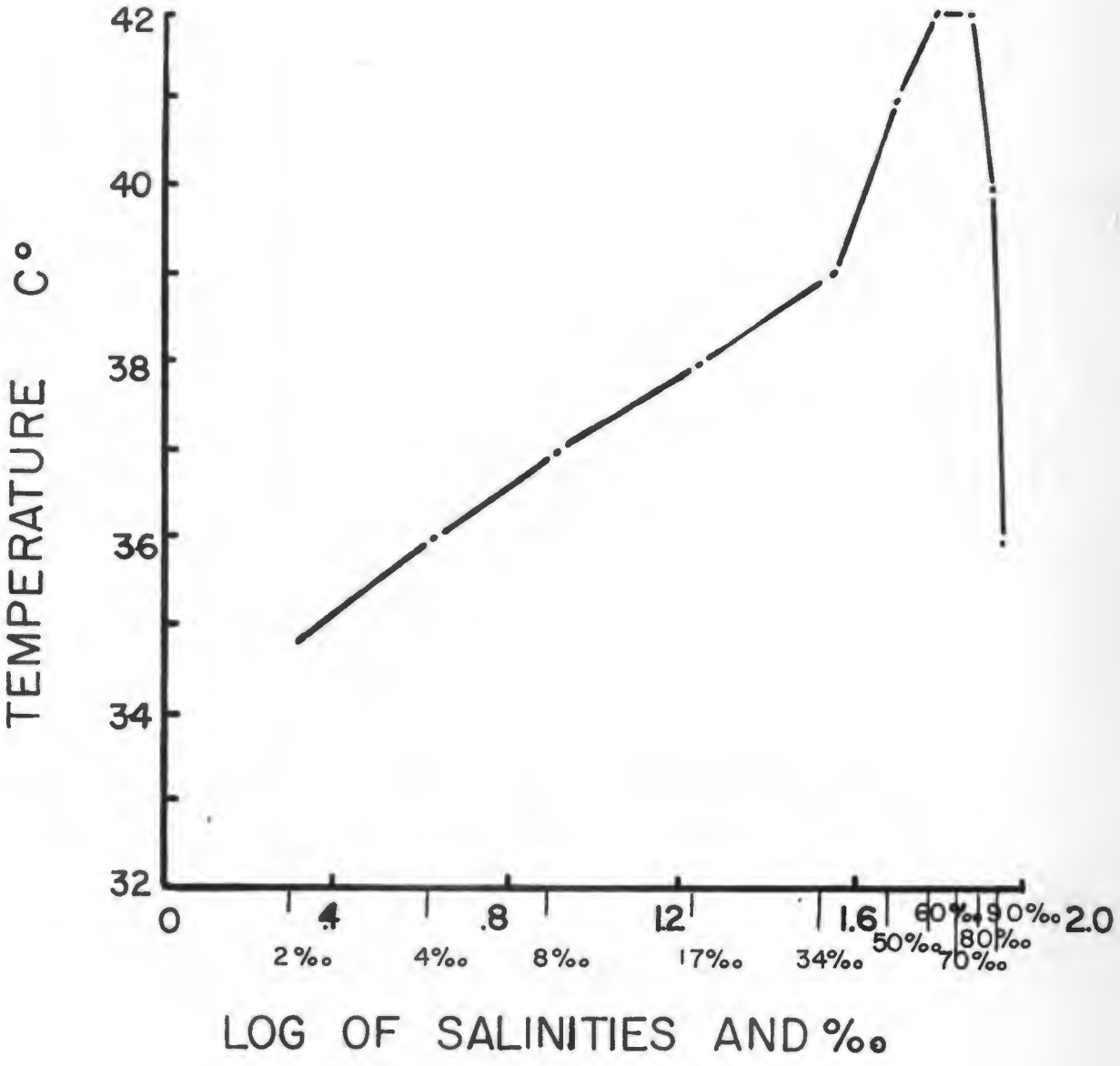
The lethal temperature for T. californicus in normal sea water varied between 38.5° C. and 39.5° C. with the average for four experiments being 39° C. This point was determined when three-quarters of the copepods under examination died almost simultaneously. Thermal shock may be distinguished from salt shock by the cessation of all movement when the lethal temperature is reached. The other one-quarter died within 5 minutes. Therefore it is evident that no appreciable temperature change occurred. As shown in figure 10, the lethal temperature increased from 35° C at 2 o/oo to 42° C. at 60 o/oo and at 70 o/oo. From this point the lethal temperature then decreased as the salinity increased. The lethal temperature at 80 o/oo was 40° C. and at 90 o/oo was 36° C. Higher salinities were not used because the animals went into immediate salt shock.

FIGURE 10

LETHAL TEMPERATURES AT VARIOUS SALINITIES FOR

TIGRIOPUS CALIFORNICUS

FIGURE 10


TEMPERATURE C°
42
40
38
36
34
32
0
.4
.8
1.2
1.6
2.0
2‰
4‰
8‰
17‰
34‰
50‰
60‰
70‰
80‰
90‰
LOG OF SALINITIES AND ‰

E. Results in Changes of Temperature and High Concentrations of Selected Ions on T. californicus (figure 11)

The solutions of sea water and $CaCl_2$ (125 o/oo) induced immediate salt shock at 20°C. The same results were obtained with the solutions of sea water and KCl. Therefore, these two solutions could not be used for further investigation of effects of high temperature.

Solutions of $MgCl_2$ and sea water showed slight heat protection* at salinities of 50 o/oo and 60 o/oo. The lethal temperature at these salinities was 38° C. The lethal temperature at a salinity of 70 o/oo was 37° C. and was 34° C. at 80 o/oo. Solutions of $MgCl_2$ and sea water at 90 o/oo induced salt shock.

Solutions of NaCl and sea water afforded protection to heat death to a greater extent than did $MgCl_2$ and sea water. The lethal temperature for NaCl and sea water at various salinities is: 39° C. at 50 o/oo; 41° C. at 60 o/oo; 40° C. at 70 o/oo; 38° C. at 80 o/oo; and 36° C. at 90 o/oo. For a summary of the relationship of the lethal temperatures of varying salinities of NaCl and sea water to normal sea water at the same salinities, see figure 11.

F. Effect of Temperature and of $KClO_3$ in Sex Ratio of T. californicus (tables 4 and 5)

The effect of placing T. californicus in the artificial

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*see discussion, page 59.

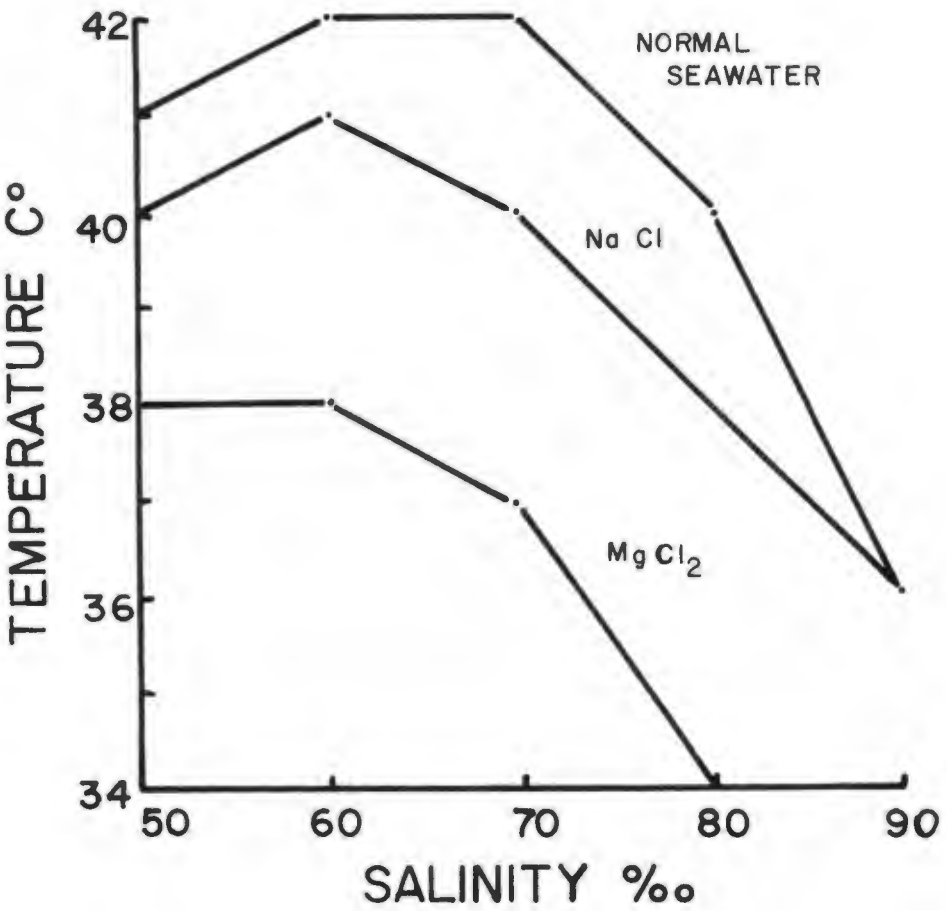
FIGURE 11

LETHAL TEMPERATURE FOR TIGRIOPUS CALIFORNICUS

IN NORMAL SEA WATER AND NORMAL SEA WATER PLUS NaCl

AND NORMAL SEA WATER PLUS $MgCl_2$ AT VARIOUS SALINITIES

FIGURE 11


42
40
38
36
34
TEMPERATURE C°
NORMAL SEAWATER
Na Cl
Mg Cl2
50
60
70
80
90
SALINITY ‰

medium, .15 gms. $KClO_3$/100 cc. sea water, is to accentuate the already great number of females to males. The sex ratio found in the artificial medium was 20% males to 80% females. The sex ratio of the control, normal sea water, was 35% males to 65% females. The discrepancy in the sex ratio found in the artificial media was shown by application of the Chi Square test to be significant (see table 3). The probability of this discrepancy being due to chance alone is five in one hundred ($P < .05$).

Those copepods from the same brood which were divided evenly and one half raised at 20° C. and the other half raised at 25° C. gave almost identical sex ratios, 30% males to 70% females at 20° C. and 31% males to 69% females at 25° C. (see table 5). Those specimens that were raised at 30° C. died during the first copepodid stage.

TABLE 3

Calculation of Chi Square Value for Sex Ratios

in T. Californicus

	♂ ♂	♀ ♀
Artificial	17	66
Control	29	55
$x^2 = \frac{N\,(ad-bc)^2}{(a+b)(c+d)(a+c)(b+d)}$		
$x^2 = 4.15$	$P < .05$	

TABLE 4

Effects of $KClO_3$ on the Sex Ratio of T. californicus

Culture No.	Artificial Medium		Control	
	♂♂	♀♀	♂♂	♀♀
1	1	4	2	4
2	2	9	5	6
3	1	4	1	4
4	2	7	4	6
5	3	5	2	5
6	1	7	3	6
7	-	6	3	3
8	2	8	2	7
9	-	8	3	6
10	3	4	1	4
11	2	4	3	4
Total	17	66	29	55
Percent	20 %	80 %	35 %	65 %

Artificial medium .15 gms. $KClO_3$/100 cc. sea water.
Experiment conducted at 25° C.
Phormidium was used as standard food.

Copepods for each culture were divided as evenly as possible from the offspring of one isolated female. The naupiius larvae were transferred to the media within 24 hours after their release from the parental egg sac.

TABLE 5

Effects of Temperature on the Sex Ratio of T. californicus

Culture No	20° C.		25° C.	
	♂ ♂	♀ ♀	♂ ♂	♀ ♀
1	3	2	4	6
2	4	3	2	5
3	1	6	2	5
4	4	6	3	6
5	3	6	2	6
Total	13	29	13	28
Percent	30 %	70 %	31 %	69 %

V. DISCUSSION AND CONCLUSION

On warm summer days it has been observed that small, shallow tide pools near Trinidad, California, containing copepods will be subjected to a decrease in volume by evaporation, with an accompanying increase in salinity. The copepods in these desicated pools, which are usually lined with salt crystals, are found lying motionless at the bottom of the pool. Upon returning to the same tide pool on the following morning, (during the interim the pools had been replenished with an abundance of normal sea spray from the evening tide) the previously motionless copepods were again actively moving about.

The phenomenon that I would like to call salt shock, was first described in 1914 by Issel, "..a few weeks of periodical observations of the pools since 1912 have drawn my attention to a phenomenon worth studying: as soon as the density of the water reaches a certain degree the copepod T. fulvus falls into a state of apparent death, from which it can awake even after a very long time and regain normal activity when the water is sufficiently diluted." (from Issel, 1914, as quoted in Ranade, 1957) The author has chosen to describe the above phenomenon as "salt shock" because of its descriptive nature rather than the more commonly used "apparent state of death."

From the experimental results presented in this work, it is evident that T. californicus can tolerate salinity changes from 2 o/oo to 90 o/oo. It is also apparent that this copepod can recover from salt shock induced by salinities higher than 90 o/oo when placed in normal sea water (34 o/oo). In order to better understand the mechanism involved in this phenomenon the author conducted a series of experiments introducing the copepods into several solutions whose salinities were varied by adding NaCl to normal sea water, KCl to normal sea water, and $CaCl_2$ to normal sea water. The salinities of these three artificial media were the same as those used for the investigation of salt shock at high salinities of sea water alone. The recovery period for copepods immersed in the artificial media in salinities between 125 o/oo and 225 o/oo were compared with the recovery period, at the same immersion periods, to the same high salinities of sea water alone. The recovery periods of NaCl and sea water showed the greatest similarity to the recovery periods in sea water alone. These results might be expected, because of the high concentration of NaCl in normal sea water.

No apparent movement can be detected in a salt-shocked copepod. It has been suggested that the nervous system or possibly the musculature may be temporarily paralyzed by the high concentration of salt. This idea seems fairly feasible. With what we know about the transmission of

arthropod nerve impulses and the importance of the Na^+ ion and the Cl^- ion at the nerve cell membrane along with the results yielded by the immersion-recovery rates of high salinities of NaCl and sea water it seems that the Na^+ ion and the Cl^- ion may play an important part in this copepod's ability to recover after periods of high salinities when the pools are again replenished with surf spray.

There is no evidence available to indicate whether these animals are entirely stenohalinic or euryhalinic. The almost straight line relationship (shown in figure 5) of the recovery periods in normal sea water at various salinities, after three hours immersion, seems to indicate that recovery may be a matter of simple diffusion of the salts from the inside to the outside. The internal salinity of this copepod, has not been investigated, but if one were to investigate this internal salinity at various phases throughout the recovery period, one might be able to determine if the movement of salt is by simple diffusion or by an active transport system. Osmoregulation probably occurs at the thin inter-segmental septa because of the relatively impermeable nature of the chitinous exoskeleton of this organism at other points.

Salinity changes in the tide pools are correlated with temperature increases. It was found that the lethal temperature of T. californicus at 2 o/oo sea water is 35° C. and 36° C. at 90 o/oo, with apparent protection

afforded at 60 and 70 o/oo because death did not occur until the temperature had increased to 42° C. Experimentation was undertaken to determine if there was a specific salt responsible for the appearance of thermal protection at salinities of 60 and 70 o/oo. Salinities of 50, 60, 70, 80, and 90 o/oo were made by adding $MgCl_2$ to normal sea water and NaCl to normal sea water. (KCl and $CaCl_2$ were not used in this experiment because they induce salt shock at these salinities) It was again found that NaCl and sea water afforded protection to high temperature at moderately high salinities similar to the protection afforded by high salinities of sea water alone. This ability to withstand high temperature is a characteristic which is not called into play here on the Northern California coast because of the normally low temperatures of the tide pools during the various seasons ranging between 20° C. and 32° C. However, this characteristic of heat tolerance may be of great survival value to Tigriopus in Southern California because of the higher temperatures there. Although no evidence has been compiled concerning Tigriopus activities at salinities between 40 o/oo and 60 o/oo other than evidence of heat protection at these salinities and the fact that these animals are generally found in tide pools of salinities greater than 40 o/oo, it appears to the author that this animal is better or equally adapted to an environment with salinities greater than normal sea water.

Takeda (1951) found that substances or conditions that accelerated the development of T. japonicus induced masculinization and those conditions which decreased the development induced feminization. These conditions had to be invoked while the copepods were still in the nauplius stage. After repeating one of Takeda's experiments aimed at decreasing development to induce feminization in T. californicus, the author found that feminization was induced using $KClO_3$ in normal sea water at 25° C. Takeda found that rearing copepods in normal sea water at 23° C. accelerated growth and induced masculinization, and copepods raised in normal sea water at 18° C. induced feminization. It was found that raising T. californicus at 20° C. and 25° C. did not induce masculinization but yielded a sex ratio of two females to one male.

The means by which sex determination can be altered in different aquatic invertebrates are quite varied. For example, Brown and Banta (1932) raised daphnids (Crustaceans) at various high temperatures and found the results to be observable in the next generation. Shull and Ladoff (1916) reared Hydatina (Rotifer) under various conditions using solutions of amonium salts and solutions of sodium hydroxide and found the effects on the sex ratio to appear in the second generation. It appears as though $KClO_3$ may affect the undifferentiated gonad of T. californicus during the nauplius stage and that temperatures between 20° C. and 25° C.

have the same effect - a tendency toward feminization. Lance and Buzzati-Traverso (1959) report that sex determination is genotypic and depends upon a system of polygenes. They have, by means of selection experiments, been able to shift the sex ratio in favor of females or males so that either sex was present only in 1% of the total population. The differences in sex ratios that are found in tide pools throughout the year are probably due to a combination of selection and certain other unknown external agencies.

SUMMARY

Under experimental conditions, Tigriopus californicus has been found to tolerate salinities between 2 o/oo and 90 o/oo and temperatures up to 39° C. It has also been shown that T. californicus will go into salt shock when introduced into solutions with salinities between 90 o/oo and 175 o/oo and will recover and resume normal activity after being transferred to normal sea water. NaCl added to sea water to yield a salinity of 125 o/oo has shown similar results to those obtained with normal sea water at 125 o/oo with respect to immersion-recovery periods. This copepod has shown that it can tolerate higher temperatures when found in environments with salinities between 60 o/oo and 70 o/oo. When T. californicus is reared in sea water to which $KClO_3$ has been added, there is a significant increase in the number of females produced.

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* a secondary reference taken from Ranade (1957).

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