AN ABSTRACT OF THE THESIS OF

Archie Lee Vander Hart (Name) for the degree Master of Science (Degree)

in Oceanography (Major) presented on May 10, 1976 (Date)

Title: FACTORS INFLUENCING SEX RATIO IN THE ESTUARINE COPEPOD GENUS EURYTEMORA

Abstract approved: Redacted for privacy

Charles B. Miller

An investigation was conducted on factors influencing sex ratio in Eurytemora. Fifteen generations of brother/sister mating have produced family lines with a high proportion of either males or females of Eurytemora affinis. On this basis, a polygenic mechanism of sex determination is proposed for E. affinis. Laboratory experiments on the effect of salinity and temperature variation indicate that these factors act only on the parents to influence the sex ratio of their progeny but are not effective after the stage of egg-laying. The proportion of males produced increased with increases in salinity and temperature. Food density acts on the developmental stages of E. affinis to influence the sex ratio. More females were produced at higher food densities. Water quality and food type had no effect on sex ratio during development. Food type did have a strong effect on survival. How these factors interact in natural populations is unknown, since field data were available only for E. americana.

Factors Influencing Sex Ratio in the Estuarine Copepod Genus Eurytemora

by

Archie Lee Vander Hart

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1976

APPROVED:

Redacted for privacy

Associate Professor of Oceanography
in charge of major

Redacted for privacy

Dean of the School of Oceanography

Redacted for privacy

Dean of Graduate School

Date thesis is presented May 10, 1976

Typed by Margie Wolski for Archie Lee Vander Hart

ACKNOWLEDGEMENTS

I owe Charlie Miller a big "thanks." He has provided intellectual stimulation and financial support throughout this study. His critical comments on several drafts of this thesis greatly improved it. Through our disagreements and discussions I learned a lot about science.

I thank Pete Dawson and my committee members (Larry Small, Roger Petersen and Carroll De Kock) for taking time to discuss with me this small contribution to science. Ron Hill made time-saving suggestions on figure drafting. Thanks, Ron, and thanks, too, for keeping me involved in sports.

Enrique Carillo and Craig Wiese introduced me to culture work. I thank them for helping me get it off the ground. Craig, Ken Johnson and Julie Ambler cared for my beasties when I needed a vacation. I thank them for it, and for fruitful discussions on advancing the art of copepod culture. Bill Peterson, Barb Dexter and Percy Donaghay helped a lot by playing sounding board for my ideas, and by providing moral support. Joan Flynn and Barb Sullivan also shared my ups and downs. Thanks, all of you.

My beloved wife was a constant source of encouragement. She also typed the last-minute draft for the exam. More importantly, she has worked to make our relationship a beautiful thing. I love you

dearly, Leanne.

Without a doubt, the fine books of Rachel Carson (which put me in love with the sea long before I ever saw it) were the impetus for me to study ocean life.

Thanks to Almighty God, I have enjoyed four years of good health, good friends and good times as I sought knowledge here at OSU. Wherever He leads next, these years have been worthwhile. To Him be praise forever.

I thank Sea Grant for providing support for this study under NOAA Contract 04-6-158-44004. Without such support, my education in oceanography would not have taken place.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

FACTORS INFLUENCING SEX RATIO IN THE ESTUARINE COPEPOD GENUS EURYTEMORA

INTRODUCTION

General Considerations

Investigations on the ecology of populations of Eurytemora spp. (Copepoda, Calanoida) in Yaquina Bay, Oregon, indicate large fluctuations in the sex ratio (see Figure 1). Sex ratios differing from 1:1 may occur in natural populations for a variety of reasons. Maly (1970) suggests that sex-selective predation may alter the adult sex ratio of copepods in cases where there is size dimorphism. Bogorov (1939) gives early death of males as the reason for the predominance of females in open ocean catches of copepods. Such differential natural mortality of the two sexes has been documented for Calanus by Raymont and Gross (1942). Cannibalism of males by females has been observed for Macrocyclops albidus, M. fuscus, and Acanthocyclops viridis (Smyly, 1965). Sex ratios are also directly affected by environmental factors (Bacci, 1965) and by the genetic sex determination mechanism (Eshel, 1975).

This thesis reports an attempt to investigate which of these factors were important influences on the sex ratio of Eurytemora spp. During laboratory studies on this copepod over a two year period, I observed no cannibalism on males, nor was there any evidence that

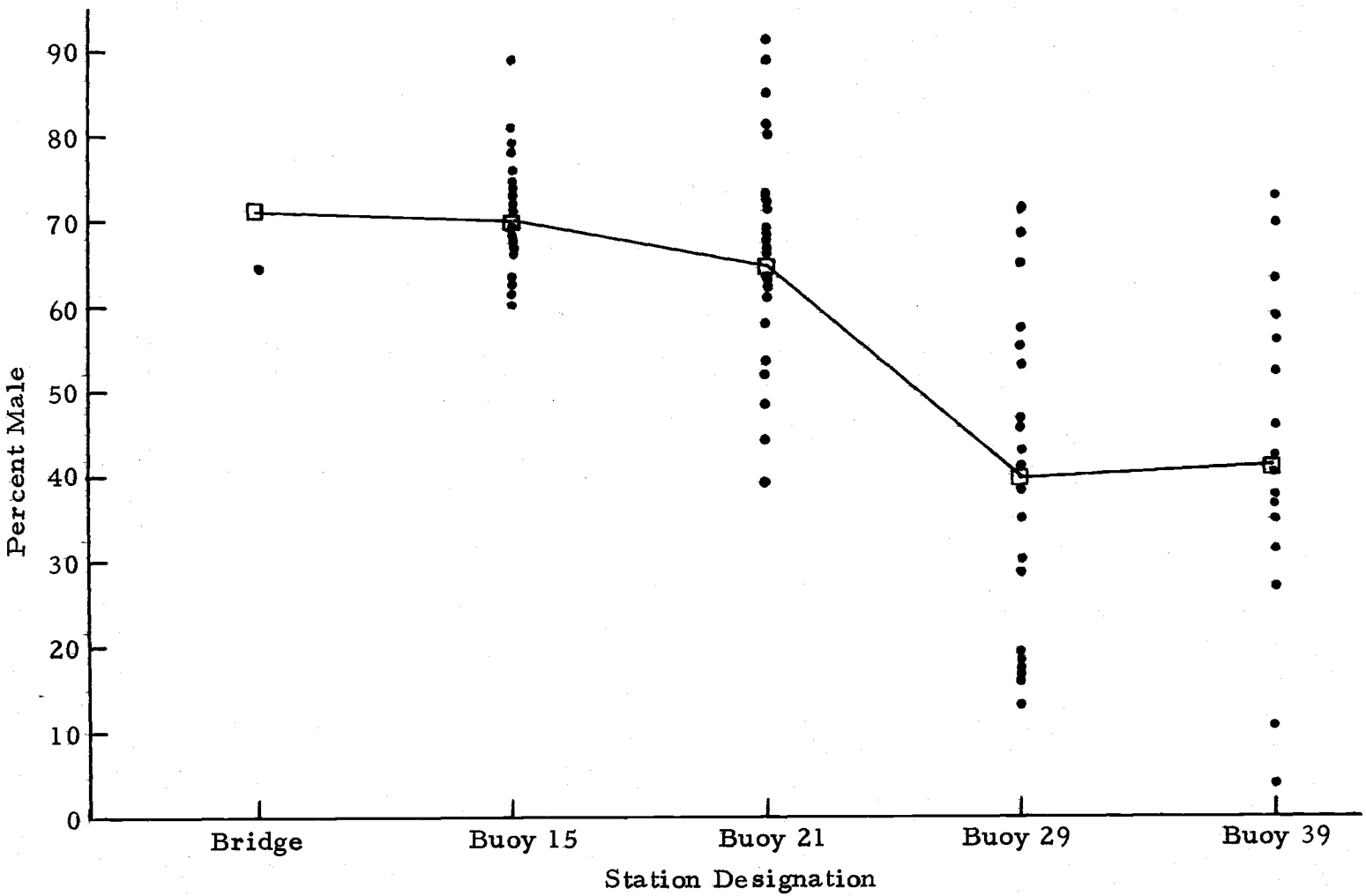

Figure 1. Sex ratios of Eurytemora population at five stations in Yaquina estuary. Individual points are all samples for which $n \geq 30$. Line connects means (□) over all samples for which $n \geq 6$.

males had shorter lifespans than females. Although there is a size dimorphism between sexes in adults of all species of Eurytemora, the sex-selective predation hypothesis could not be tested, since the major predators of Eurytemora spp. are unknown. Therefore, I conducted studies to determine whether the genetic sex determination mechanism or environmental factors could influence the sex ratio in Eurytemora spp.

All experiments were carried out using animals taken from a stock culture of Eurytemora spp. maintained in the laboratory since April 3, 1974. At that time only Eurytemora americana Williams had been reported from Yaquina Bay (Frolander et al., 1973). In August 1975 Eurytemora affinis (Poppe) was found in some plankton samples taken earlier. Examination of the stock culture yielded only E. affinis. Re-examination of plankton samples from Yaquina Bay has shown that E. affinis is much less abundant than E. americana (Joan Flynn, personal communication). Therefore, the stock culture may have contained E. americana before August 1975. This possibility must be taken into account in the interpretation of experiments conducted between April 1974 and August 1975.

When the second Eurytemora sp. was first discovered in Yaquina Bay, it was identified as E. hirundoides (C. B. Miller, personal communication). Gurney (1931) discussed the taxonomic confusion surrounding E. affinis and E. hirundoides. He found no

justification for distinguishing E. hirundoides as a separate species from E. affinis. Probably all North American records of E. hirundoides are E. affinis (Wilson and Yeatman, 1959). Therefore, until the taxonomic question is resolved I will refer to my organisms as E. affinis.

Literature Review

The sex determination mechanism in most copepod groups has not been determined. The problem has been approached by cytological studies looking for sex chromosomes. A summary of this cytological work is given by Beerman (1954). Despite the extensive work on copepod chromosomes in the early 1900's (see for example Braun, 1909; Matschek, 1910; Amma, 1911; Kornhauser, 1915; Heberer, 1932), there is little that can be said about copepod sex chromosomes. These workers did not do complete cytological studies on both sexes, nor on all stages of gametogenesis and zygote formation. This criticism also holds for Harding's (1963) suggestion of an XY sex determination mechanism in Calanus.

Kornhauser (1915) interpreted his results on spermatogenesis in Hersilia apodiformis (Caligulidae) as evidence for a male XY heterogamety. Beerman, however, points out that Kornhauser did not adequately study oögenesis to determine whether the observations on heterochromosomes might also hold for the female. A study of

spermatogenesis in Centropages typicus led Heberer (1932) to argue for a male XO heterogamety in the Calanoida. However, he was not able to duplicate his initial observation, that some sperm have one less chromosome than others, in subsequent studies on the same species. Beerman attributes this to the difficulty of working with cut preparations as opposed to the squash technique.

Beerman (1954) found conclusive evidence for a chromosomal XO heterogamety in female Ectocyclops strenzkei. He cites the work of Braun (1909) and Matschek (1910) on other members of the subfamily Eucyclopinae as evidence that a female XO heterogamety is general in this group. Beerman concludes that nothing can be said with certainty about the chromosomal sex determination mechanisms of the harpacticoid, calanoid or parasitic copepod groups because of the lack of adequate cytological studies. In fact, he states that the question of chromosomal sex determination is still open for subfamilies of the Cyclopoida other than Eucyclopinae (that is, the Cyclopinae and Mesocyclopinae).

Beerman points to Matschek's (1910) study of oögenesis in Diaptomus castor as possible evidence for a female XY heterogamety in Calanoida. Matscheck found fourteen normal tetrads and one ring composed of three conjoint tetrads during metaphase I. Heberer (1932) however, established that this pairing configuration occurs only in the meiosis of females, and only in populations from southern

Germany. The pairing configuration in females from northern and central German populations is seventeen normal tetrads. Males from all populations had seventeen normal tetrads. Beerman concludes that the ring formation is evidence for sex chromosomes. However, while the ring formation is sex linked, this does not make it a sex chromosome, since the genes controlling ring formation may be on chromosomes not found in the ring. This evidence for sex determination by a female XY heterogamety in Calanoida is not strong, especially since this particular pairing configuration only occurs in certain populations of Diaptomus castor.

A word of caution in the interpretation of cytological evidence for or against chromosomal sex determination mechanism comes from Bacci (1965). He states that sex chromosomes may exist even if no heterochromosomes are found cytologically. Similarly, heterochromosomes are not proof that sex is determined solely, or at all, by "sex chromosomes" since autosomal sex control is found in some organisms that may at times override the sex as determined by heterochromosomes.

The best confirmation of a particular mode of chromosomal sex determination in a copepod comes from the work of Rüsch (1958) and Metzler (1955, 1957). Rüsch cytologically determined that a female XY heterogamety existed in Cyclops viridis (Mesocyclopinae). Metzler carried out an extensive laboratory study on the influence of

environmental factors on the sex ratio of this copepod. She found that sex was determined shortly before egg laying. The sex ratio of a clutch of eggs was influenced by environmental factors impinging on the female only during the time of polar body formation. High temperatures, ultraviolet radiation, changes in pH, and decreased food rations all produced a higher proportion of males than did control conditions, presumably because the Y chromosomes is passed more frequently into a polar body. This environmental influence on chromosomal sex determination is similar to that found in the moth Talaeporia tubulosa. In T. tubulosa the orientation of the sex chromosomes on the spindle is strongly influenced by temperature so that high temperatures produce more females (61%) than low temperatures (39%) (Seiler, 1920, cited in Bacci, 1965).

The sex determination mechanism in the cyclopoid forms that have been studied appears to be of the "advanced" (Bacci, 1965) XO or XY types with sex being determined in the egg. The same cannot be said for the two genera which have been studied in the Harpacticoida (Tigriopus and Tisbe). Takeda (1950) investigated the effect of factors speeding or slowing the development of Tigriopus japonicus. He found that sex was not determined until the onset of the first copepodite stage. The application of growth speeding factors (temperature or chemicals) during the naupliar stages yielded a higher proportion of males than did control conditions. These same factors had

no effect on the sex ratio when applied after the molt to first copepodite. Egami (1951) confirmed these results.

Vacquier and Belser (1965) subjected nauplii and copepodities of Tigriopus californicus to increased hydrostatic pressure. They were able to shift the sex ratio to a higher proportion of males at hydrostatic pressures above atmospheric. As in Takeda's study on T. japonicus, the effect on sex ratio could be obtained only if the treatment was applied to the naupliar stages. The results on Tigriopus spp. indicate that some enzyme mediated reaction may be involved in sex determination in the genus.

Ar-Rushdi (1958) conducted inbreeding experiments on Tigriopus sp. and concluded that, since he was able to select for family lines having a high proportion of males and for lines with a high proportion of females, sex was determined by a polygenic system. His work seems to have been stimulated by the observations of Provasoli et al. (1959) that extreme changes in sex ratios occurred in laboratory cultures of T. californicus but not in T. japonicus. Unfortunately Ar-Rushdi does not indicate which species he studied.

Battaglia (1958) found that while selection for a high proportion of males in inbreeding studies on Tisbe gracilis was successful, he could not select for family lines with a high proportion of females. Inbreeding without selection always led to increased proportions of males. He proposed a system of polygenic sex determination involving

several factors for femaleness (F) which are dominant, and whose expression is conditioned by the heterozygous state, and several factors for maleness (m) which are recessive. These factors are non-allelic and spread over several chromosomes so that sex is determined by the degree of heterozygosity of the F and m loci. Thus inbreeding leads to increased homozygosity and an increased proportion of males (Battaglia, 1963). The rarity of intersex individuals indicates a very precise switch mechanism which allows the appearance of normal male and female phenotypes even in cases of near balance betwen the male and female determiners (Bacci, 1965). According to Kosswig (1964) this is a common feature of polyfactorial sex determination systems.

Various authors have commented on the possible influence of environmental factors on the sex ratio of calanoid copepod species under laboratory and field conditions, but the mechanism or timing of these factors has not been elucidated. Paffenhöffer (1970) found that Calanus helgolandicus raised from egg to adult on different algal foods yielded different sex ratios. There was considerable mortality in most instances, but it was not correlated with sex ratio. Heinle (1970) determined that the sex ratio of Acartia tonsa was 1:1 in unexploited (high density) laboratory populations (with one exception), and that there was no differential mortality of the sexes. Laboratory exploitation experiments indicated that the proportion of adult females

increased with an increase in the exploitation rate of cultures or with the decrease in population density. He also suggests there is an inverse correlation between population density and sex ratio in his field studies. Heinle suggests that genotypic males become phenotypic females under these conditions, but the evidence is far from conclusive due to the lack of control of other environmental factors. Lock and McLaren (1970) found that stage III copepodites of Pseudocalanus minutus raised to adulthood in individual containers produced a higher proportion of females (86%) than those raised at densities of 16-20 per container (49%). Conover (1965) noted similar results with Calanus hyperboreus. This suggests that production of males may be regulated by density or the presence of other copepods. Cognard (1973) studied the effect of temperature on Eudiaptomus gracilis. His results showed a "masculinization" of offspring at low temperatures, but the mechanism is obscure due to the very poor survivorship to the adult stage (as low as 6.6% at 4°C and 35% at 18°C). He also found that males spent less time in the fourth copepodite stage than females, and this may provide some insight into the mechanism.

Katona (1970) studied the effect of temperature and salinity on the growth of two species of Eurytemora. The proportion of males was directly correlated with temperature for E. affinis, but the inverse relation held for E. herdmanni. Katona suggested that each species produces more females under stress conditions in order to

bring the population through hard times. However, there was no measure of mortality in the cultures, and differential mortality may account for the result. Woodhead and Riley (1959) found that males of Calanus finmarchicus have a shorter development time in the fifth copepodite stage than females, but their method of sexing immatures has been questioned by Conover (1965). Moriatou-Apostolopoulou (1972) found a positive correlation between population density and the proportion of males in field samples of Centropages typicus and Temora stylifera. Bayly (1965) reported similar results for several estuarine species (Pseudodiaptomus spp., Sulcanus conflictus, Gladioferens pectinatus, Boeckella propinqua).

While a considerable amount of evidence has been accumulated for the sex determination mechanisms of cyclopoid and harpacticoid copepods, such evidence is lacking for the Calanoida. Conover (1965) states that "Although genetic sex determination may occur among calanoid copepods, it is by no means proven."

The study of Egloff (1967) on Tigriopus californicus points out problems in relating sex ratio to environmental conditions. He found a positive correlation between sex ratio and temperature in the field, but a negative correlation existed under laboratory conditions. Thus, field correlations alone should not be used in discussions on the causes of sex ratio fluctuations.

Direction of the Present Work

The successful studies on the causes of sex ratio fluctuations in copepods have been those that investigated the influence of physical factors (Metzler, 1957; Takeda, 1950) or inbreeding (Battaglia, 1958; Ar-Rushdi, 1958) on sex determination. In my studies I utilized the techniques of these successful authors to attempt to determine those factors influencing the sex ratio in the calanoid copepod, Eurytemora affinis. I conducted inbreeding experiments to investigate the possibility of a polygenic sex determination mechanism in this copepod. The majority of the experiments were designed to determine whether, and in what stage of the life cycle, physical factors influenced the sex ratio. By these studies I hoped to elucidate the sex determination mechanism in a calanoid copepod and to investigate the significance of environmental control of the sex ratio.

MATERIALS AND METHODS

Preliminary Work

All copepods used in laboratory experiments were collected on April 3, 1974, at two stations (Buoy 21 and Buoy 29) in the Yaquina River estuary. The stock was maintained in a 9-liter glass serum bottle in sea water diluted to 20‰ with distilled water. Sea water was taken from a sand-filtered sea water system at the Oregon State University Marine Science Center at Newport, and was filtered in the laboratory through a glass fiber filter immediately before use. The stock culture was maintained in a constant temperature room at 16.5°C ± 0.5°C in low light. Continuous aeration was provided to keep phytoplankton in suspension and to provide high oxygen levels. The culture solution was changed every 2 to 3 weeks by pouring the contents through a 52 μm mesh net, and rinsing the contents of the net into fresh culture solution. A food mixture of the diatom Thalassiosira fluviatilis in a concentration of 5,000 cells/ml, the flagellate Rhodomonas sp., 30,000 cells/ml, and the chrysomonads Isochrysis galbana, 40,000 cells/ml and Pseudoisochrysis sp., 50,000 cells/ml was fed to the copepods at each culture transfer. These initial concentrations were maintained by addition of food at 1-3 day intervals as needed. Phytoplankton cultures were maintained using the technique described by Carillo (1974).

The sex ratio of Eurytemora spp. in the field was determined by analysis of samples taken during the years 1967-1972. The samples were part of a multi-year study on the zooplankton of the Yaquina estuary. Sampling techniques and station locations are reported in Frolander et al. (1973). C. B. Miller extracted from the data set all samples for which the total number of adult Eurytemora was 6 or more and calculated the sex ratio for each sample.

Laboratory Experiments

All experiments were conducted as follows, except where noted. 400-500 ml of culture medium was added to 600 ml beakers. The culture solution was glass fiber filtered sea water diluted to approximately 20‰ with distilled water. The standard culture solution included the same four algal species in the same density as was fed to the stock culture. Total food concentration was varied proportionately to the number and size of copepods in the beaker (from 50,000 to 150,000 cells/ml of the four species combined). Stock copepods were screened from the culture as previously described, rinsed into a Petri dish and examined under a dissecting microsope. The desired stages and numbers of copepods were transferred by eye dropper to each experimental container. When eggs were taken from female egg sacs, tricaine methanesulfonate was used to anesthetize the females. The beakers were covered with watchglass covers and

placed on a shelf in the cold room. Light levels were low and uniform. Standard temperature was 16.5 ± 0.5°C. Food was added as needed every 2-4 days using the same technique as described for the stock culture. Experimental beakers were never aerated.

The initial observation on the effect of salinity on sex ratio came from a preliminary experiment on the salinity tolerance of Eurytemora. Five male and five female copepods were placed in experimental beakers at seven salinities ranging from 4‰ to 28‰ on May 6, 1974. These non-replicated beakers were censused on June 10 and June 18 for the number of males and females. By this time healthy populations had been produced at each salinity. Mortality was not measured in this experiment.

Several experiments were then conducted to determine whether temperature, salinity, or animal density had any influence on the sex ratio in Eurytemora. The first (FAC-1) was a 2x3x4 factorial design in temperature (10°C, 16.5°C), animal density (1, 10, 100 eggs/beaker) and salinity (8, 12, 16, 20‰). There were 3 replicates of each treatment. Eggs were removed from stock culture females and placed into experimental beakers on June 14. The 16.5°C beakers were terminated on July 15 and the 10°C beakers on July 22. The purpose of this experiment was to test whether these factors had any effect on sex once the eggs had been laid.

In order to test whether these same factors might affect sex

determination before egg laying occurred (Metzler, 1957) a second experiment (FAC-2) was performed. Adult Eurytemora were treated according to a 2x2x4 factorial design with 3 replicates in animal density, temperature and salinity. The salinity and temperature treatments were the same as in the previous experiment. The two density treatments involved placing 5 breeding pairs into 200 ml or 1200 ml cultures. 1500 ml beakers were used for the latter treatment. The adults were placed into experimental beakers on September 19, 1974. On September 26, nauplii were removed from these beakers and discarded. Fifty eggs were collected from each adult experimental beaker on September 28 and raised under standard conditions. These adults were counted and sexed on October 28.

The third experiment (FAC-3) was designed to look at the sex ratio of progeny when raised from the egg under the same or different conditions than those at which they were laid. The experimental variables were salinity and raising nauplii under the same or different conditions the parents had experienced. Adult copepods were placed into one of four salinity conditions (4, 12, 20, 28‰) on November 21, 1974. Eggs and nauplii were removed on November 26 and discarded. Egg collection for the experiment began on November 30 and continued until December 9. Six hundred eggs were taken from each salinity treatment. Three hundred eggs were divided equally into 3 beakers containing standard culture conditions, and the other 300 were divided

equally into 3 beakers at the same salinity that the parents had experienced. The eggs were raised to adult stage under these conditions, and the number of males and females were counted on December 19.

A fourth experiment (FAC-4) on the effect of temperature and salinity was conducted after the possible occurrence of two species of Eurytemora in the early stock culture was discovered. This was a 2x3 factorial with 4 replicates at each temperature (10, 16.5°C) and salinity (4, 16, 28‰). On July 22, 1975, adult Eurytemora affinis were separated into 2 beakers at the two experimental temperatures. Eggs and nauplii were removed on July 26 and discarded. Stage II-III nauplii were collected from July 26 to August 4. Fifty nauplii were placed into each replicate of each salinity at the same temperature at which they were hatched. They were raised to adulthood, and were counted and sexed on August 25 (16.5°C part) or September 8 (10°C part).

Two experiments were conducted to test whether water quality had any effect on survivorship or sex ratio of Eurytemora. Three types of water treatment were applied: "scrubbing" with activated charcoal, addition of a chelator (EDTA), and ozonization. In both of the experiments the treatments were as follows. "Natural" sea water (N) was glass fiber filtered. Charcoal filtered sea water (CF) was glass fiber filtered, then Darco G-60 activated charcoal was added and allowed to remain for 2 days before removal by glass fiber

filtration. Ozonized sea water (COC) was treated as CF water, then bubbled with ozone for 40 minutes. Twenty-four hours later a second portion of activated charcoal was added to scrub out residual ozone. This water was then glass fiber filtered to remove the charcoal 24 hours later. EDTA at a concentration of 37 mg/l (Neunes and Pongolini, 1965) was added to half of each of the three treatments. This gave 6 water treatments designated as N, CF, COC, NEDTA, CFEDTA, COCEDTA. Twenty eggs from stock culture females were added to each of 4 replicates of each treatment. The eggs were raised to adult stage under the standard conditions of light, food, temperature, and salinity. The first experiment (WQ-1) ran from April 2, 1975 to April 28. The second (WQ-2) ran from April 22 to May 16.

The possible effect of food on the sex ratio of Eurytemora affinis was investigated by two types of experiments. The first (FOOD-1) involved raising E. affinis from early nauplius (stage II-III) to adult on monoalgal foods. The four algal food types used in all other experiments, Isochrysis galbana, Pseudoisochrysis sp., Rhodomonas sp. and Thalassiosira fluviatilis were used individually as the four treatments. Fifty nauplii from the stock culture were placed in each of three replicates of each algal species on October 24, 1975. The adult survivors were counted and sexed on November 12.

The second type of food experiment involved different

concentrations of all 4 algal species combined as the treatment variable. There were 3 experiments of this type (FOOD-2, FOOD-3, and FOOD-4). In each experiment, whenever food was added, it was in the same ratio at each feeding. However, the absolute amount fed at any treatment level varied from one feeding to the next. Culture volume was maintained at 400 ml in each beaker by removal of the same volume of old medium as was added in food that day. FOOD-2 began on September 23, 1975, with 50 stock nauplii (stage IV-V) placed into each of 4 replicates of 4 food levels. The relative rations in FOOD-2 were 4:3:2:1. All cultures were maintained at standard conditions and fed at these levels until FOOD-2 was terminated on October 7. FOOD-3 began on October 7 with 4 replicates of 4 food levels (10:5:2.5:1). Fifty nauplii (stage II-III) were taken from each treatment beaker of FOOD-2 and placed in the corresponding food level of FOOD-3. Thus, nauplii from level "4" of FOOD-2 were placed in level "10" of FOOD-3, and nauplii from level "3" of FOOD-2 were placed in level "5" of FOOD-3. The three highest food levels (10, 5, 2.5) were terminated on October 14, but the lowest level (1) continued until October 28 due to slower growth at this food level. FOOD-4 began on November 10 with 3 replicates of 3 food levels (10:3:1). The adults raised in the "5" and "2.5" levels of FOOD-3 were placed into one large culture vessel with moderate food levels. Fifty nauplii (stage III-V) were taken from this culture on

November 10 and were placed into each treatment beaker of FOOD-4. The two highest food levels (10, 3) were terminated on November 24, but slow growth in the low food (1) beakers delayed these until December 1.

Two inbreeding experiments were conducted to investigate whether family lines could be produced that gave a high proportion of one sex. The first experiment (INBREED-1) was conducted at 22°C in order to decrease the generation time. Whole eggsacs were taken from 20 stock females on February 7, 1974, and placed into individual beakers with standard culture medium. These were placed into a 22°C water bath, and the progeny (F_1 generation) were counted and sexed on February 20. Nineteen eggsacs were taken from females produced in beakers in which the sex ratio deviated from 1:1. These were placed in individual beakers under the same conditions as those of the parental generation. Only 1 of these eggsacs produced progeny. Eleven eggsacs taken for the F_2 generation produced no hatching and the experiment terminated.

In order to investigate the failure of hatching in INBREED-1, an egg hatching experiment was set up at 22°C. One eggsac was placed in each of 5 beakers (E_1-E_5), 2 breeding pairs in each of 5 beakers (A_1-A_5), and 5 unsexed copepodites into each of 5 beakers (C_1-C_5). A total of two eggsacs were taken from adults raised in beakers E_1 to E_5, two eggsacs from F_1 adults in beakers A_1 to A_5, and 13

eggsacs from F_1 adults in beakers C_1 to C_5. This allowed examination of the hatching success of the F_2 generation. The experiment terminated on April 25 due to failure of the temperature control device.

The second inbreeding experiment (INBREED-2) was carried out under standard culture conditions. Eggsacs were taken from 20 stock culture females on April 3, 1975, from which the parental generation was produced. Family lines were selected that produced sex ratios varying from 1:1 in an attempt to produce lines having a high proportion of males or females. At least 20 eggsacs were chosen in each generation by this criteria. By April 23, 1976, 15 generations of brother/sister matings had been obtained from the single surviving original family line "Ø."

During the course of experiment INBREED-2, one of the family lines ("I") produced intersex males having some female characteristics. These intersex individuals were isolated with virgin females on two occasions, but were not observed to copulate with them and left no young. Similar attempts to cross the intersex with normal males also failed.

An interbreeding experiment between Yaquina Bay Eurytemora affinis and Young's Bay (Columbia River) E. affinis was conducted from September 26, 1975 to October 17. These populations must be almost completely isolated from each other genetically, since E. affinis does not appear in the Oregon nearshore plankton (W. T.

Peterson, personal communication). Stage V males and females of each type were isolated from stock cultures. Yaquina Bay males were crossed with Young's Bay females, and Young's Bay males were crossed with Yaquina Bay females. Both crosses produced a fertile F_1 generation.

RESULTS

Field

The sex ratio of Eurytemora spp. in the Yaquina estuary varies from station to station (Figure 1). All samples for which at least 30 adult Eurytemora were found are represented by individual points. There is a positive correlation between sex ratio and salinity (see Figure 2), as might be expected from the upstream/downstream trend. There is no apparent relation between sex ratio and temperature (Figure 3). Samples from 1972 have been completely re-examined. This showed that the population is predominantly Eurytemora americana at all of the stations included in Figure 1.

Preliminary Observation

The initial laboratory observations on the relation between sex ratio and salinity are given in Table 1. The rank-difference correlation coefficient (Tate and Clelland, 1957) between salinity and percentage of males was -0.79 (N = 7, 95‰ level), which suggests a negative relationship between fraction of males produced and salinity. This relation is opposite in direction to the field observation on Eurytemora (Figure 2).

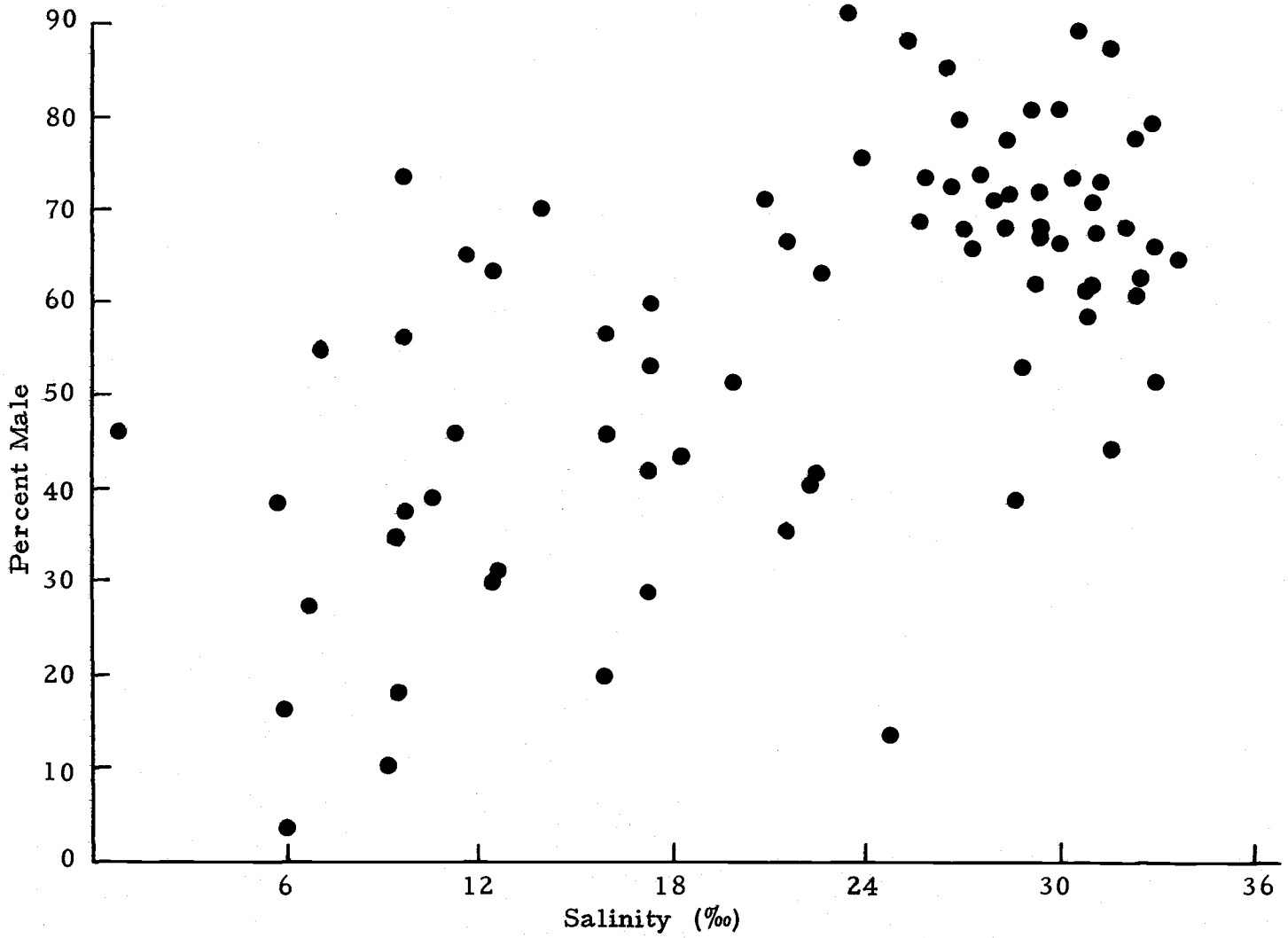

Figure 2. Sex ratio of Eurytemora versus salinity in Yaquina estuary. Points represent all samples for which n $\geq$ 30.

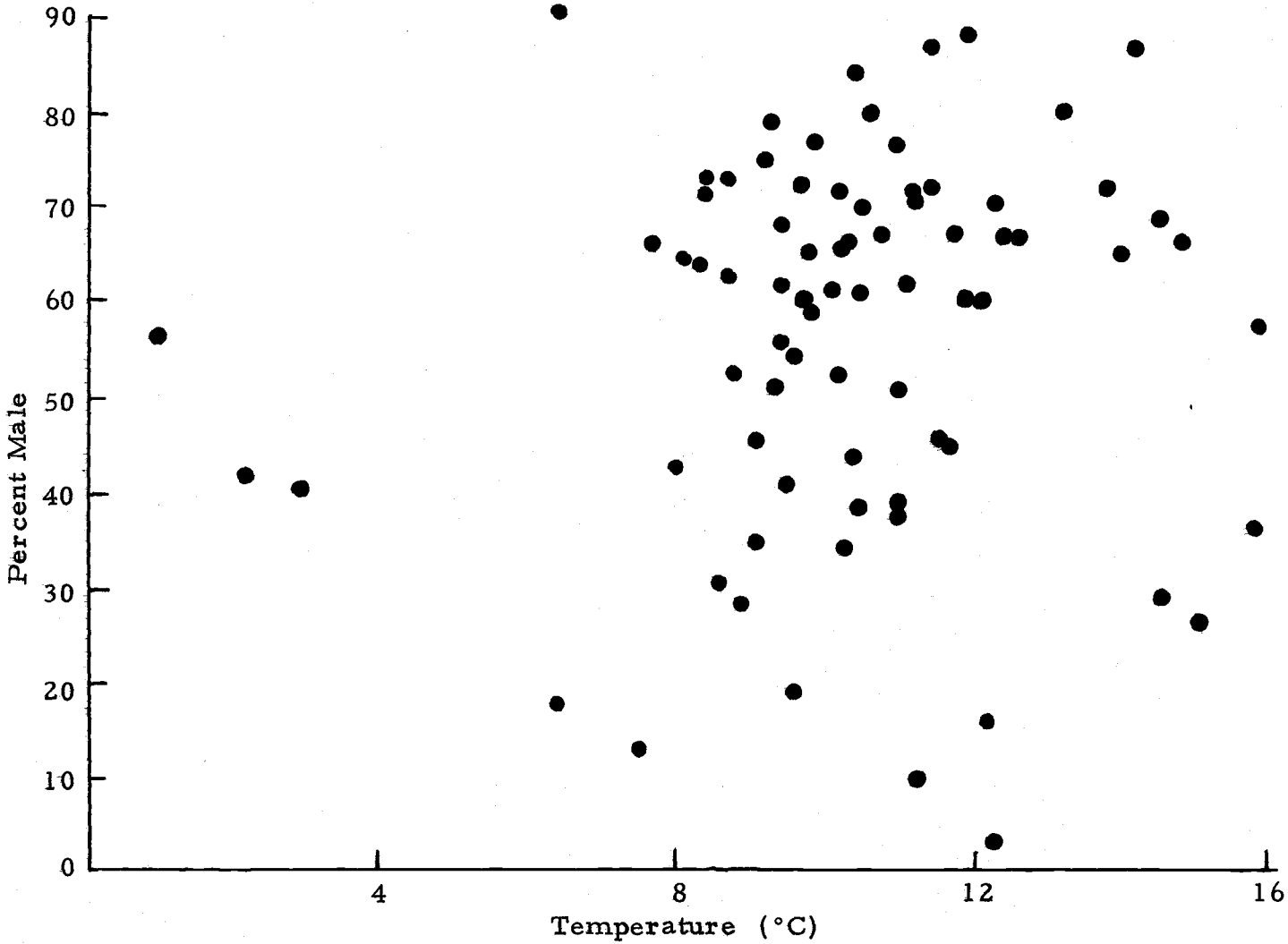

Figure 3. Sex ratio of Eurytemora versus temperature in Yaquina estuary. Points represent all samples for which $n \geq 30$.

TABLE 1. Preliminary Observation on the Effect of Salinity on the sex ratio of Eurytemora spp. Number of males and females are results of a census of the single beaker at each salinity. The temperature was 16.5°C.

Salinity (‰)	No. Males	No. Females	% Males
4	21	6	78
8	17	11	61
12	53	21	72
14	25	25	50
16	13	18	42
20	40	34	54
28	22	28	44

Factorial Experiments

Experiment FAC-1 examined the effects of salinity, temperature, and animal density on sex ratio when applied to eggs and developing copepods only after their production by the parental female. The complete results are presented in Table 2, and the main effect means are summarized in Table 3. There was no significant relationship between any of the treatments and sex ratio. The sex ratio was unrelated to survival. The low survival (63%) of the high density (100 eggs/beaker) treatments may have resulted from crowding. There were large variations in the mean survival rates with salinity, but they did not correlate with salinity in a regular way.

Experiment FAC-2 investigated the effect of these same factors when applied to the parents prior to egg laying. The results are presented in Table 4 and are summarized in Table 5. The proportion of males produced at 17 °C was significantly greater than at 10 °C (proportion test, 99% level). This response of sex ratio to temperature is consistent with Katona's (1970) findings on Eurytemora affinis. The mean proportion of males produced at the two higher salinities is greater than the mean proportion at the lower salinities (proportion test, 94% level). Sex ratio was unaffected by animal density and uncorrelated with survivorship. These results suggest that the salinity and temperature environment of the parents may influence

TABLE 2. Observed sex ratios and survival rates for individual replicates of experiment FAC-1. Different temperatures (10°C, 16.5°C), densities (1, 10, 100 eggs/beaker) and salinities (8, 12, 16, 20‰) were applied over the full egg to adult development. Parents experienced standard conditions (16.5°C, 20‰).

Temperature		10°C											
# eggs/beaker		1				10				100			
Salinity (‰)		8	12	16	20	8	12	16	20	8	12	16	20
Replicate 1	% M	0	100	0	100	30	70	20	67	42	59	14	55
	% S	100	100	100	100	100	100	100	90	7	76	7	95
Replicate 2	% M	100	100	100	50	60	78	20	50	54	58	47	55
	% S	100	100	100	100	100	90	100	100	28	92	17	77
Replicate 3	% M	-	100	100	100	40	63	56	67	60	57	53	62
	% S	0	100	100	100	100	80	90	90	25	88	79	90

Temperature		16.5°C											
# eggs/beaker		1				10				100			
Salinity (‰)		8	12	16	20	8	12	16	20	8	12	16	20
Replicate 1	% M	0	100	0	0	50	50	30	70	61	59	46	56
	% S	100	100	100	100	100	100	100	100	51	90	76	88
Replicate 2	% M	100	100	100	100	67	67	42	60	55	62	52	57
	% S	100	100	100	100	90	90	70	100	76	82	50	53
Replicate 3	% M	0	100	0	-	14	60	28	70	65	56	51	49
	% S	100	100	100	0	70	100	70	100	62	72	77	80

% M = % Male % S = % Survival

TABLE 3. Experiment FAC-1: Main effects on sex ratio and survival of Eurytemora when temperatures, density and salinity treatments are applied over the full egg to adult treatment. Parents experienced standard conditions (16.5°C, 20‰). Percent male and survival values are overall treatment means.

Temperature °C	Males Produced	Females Produced	% Males	% Survival
10	448	358	56	61
17	535	442	55	73

# eggs/beaker	Males Produced	Females Produced	% Males	% Survival
1	15	8	65	92
10	115	108	52	93
100	853	685	56	63

Salinity %	Males Produced	Females Produced	% Males	% Survival
8	170	140	55	46
12	333	239	58	86
16	171	158	52	50
20	309	238	56	82

TABLE 4. Observed sex ratios and survival rates for individual replicates of experiment FAC-2. Different temperatures (10°C, 16.5°C), densities (5 pairs/200 ml, 5 pairs/1200 ml) and salinities (8, 12, 16, 20‰) were applied to parents before egg laying. Each replicate had 50 eggs taken from parental beaker and raised to adulthood.

Temperature		10°C							
# ml with 5 breeding pairs		200				1200			
Salinity (‰)		8	12	16	20	8	12	16	20
Replicate 1	% Male	38	67	55	47	47	34	68	59
	% Survival	64	42	88	38	60	88	68	68
Replicate 2	% Male	70	43	69	57	39	19	72	61
	% Survival	60	42	26	74	76	74	72	56
Replicate 3	% Male	53	94	7	0	40	62	46	55
	% Survival	68	32	92	44	70	42	48	88
Temperature		16.5°C							
# ml with 5 breeding pairs		200				1200			
Salinity (‰)		8	12	16	20	8	12	16	20
Replicate 1	% Male	29	53	78	60	68	60	41	66
	% Survival	68	86	82	94	50	50	68	76
Replicate 2	% Male	56	60	38	57	60	70	57	63
	% Survival	32	100	16	70	84	42	42	16
Replicate 3	% Male	50	47	75	58	39	64	71	60
	% Survival	44	38	48	38	46	50	42	100

TABLE 5. Experiment FAC-2. Main effects on sex ratio and survival rate of progeny when salinity, temperature and density treatments are applied only to parents, and the progeny are reared under standard conditions (16.5°C, 20‰) starting with 50 eggs/beaker. Percent male and survival values are overall treatment means.

Temperature °C	Males Produced	Females Produced	% Males	% Survival
10	354	386	48	62
17	399	292	58	57

5 pairs/# ml	Males Produced	Females Produced	% Males	% Survival
200	359	334	52	58
1200	394	344	53	62

Salinity ‰	Males Produced	Females Produced	% Males	% Survival
8	175	186	48	60
12	178	165	52	57
16	190	156	55	58
20	210	171	55	64

the sex ratio of their progeny. There may be an interaction between the salinity and temperature effects that would confound the treatment means. The variable mortality prevents testing for interaction. Care must be taken in interpretation of these results. This is particularly true of the salinity results, which also involve multiple testing.

The effect of salinity on sex ratio was further examined in experiment FAC-3 (Table 6). As in FAC-2, the proportion of males is greater at higher salinities. The effect of salinity on sex ratio must operate on the adult before egg-laying, since the trend holds whether the progeny were raised in the same or different conditions than the parents experienced (Table 7). Though survivorship was low and variable, it was uncorrelated with sex ratio. The low survivorship may have been caused by crowding resulting from the high (100 eggs/beaker) densities.

The early stock culture which provided animals for experiment FAC-1 may have contained both E. affinis and E. americana. Thus the lack of effect of salinity and temperature on sex ratio in this experiment could be explained by a differential response of these two species to the treatments. Katona (1970), for example, reported that while the proportion of males produced in laboratory cultures of Eurytemora affinis increased with temperature, the opposite result was found for E. herdmanni. For this reason experiment FAC-4 was conducted with animals known to be E. affinis to examine this

TABLE 6. Observed sex ratios and survival rates for individual replicates of experiment FAC-3. Adult Eurytemora experienced one of four salinities (4, 12, 20, 28‰) prior to and during egg-laying. Half of their progeny were raised from egg to adult under the same conditions the parents had experienced, and half of their progeny were raised under the standard salinity of 20‰. Temperature was constant at 16.5°C. Each replicate contained 100 eggs.

Salinity (‰) experienced by parents		4	12	20	28	4	12	20	28
Salinity (‰) experienced by progeny		4	12	20	28	20	20	20	20
Replicate 1	% Male	21	50	-	56	62	0	53	59
	% Survival	14	28	0	36	39	1	58	39
Replicate 2	% Male	40	60	100	59	14	45	64	59
	% Survival	10	65	2	37	21	11	50	39
Replicate 3	% Male	46	46	50	68	52	67	60	42
	% Survival	43	26	6	54	21	3	50	48

Table 7. Experiment FAC-3. Main effects on sex ratio and survival of progeny raised under the same or different conditions experienced by their parents. There were 300 eggs/treatment. Percent male and survival values are overall treatment means.

Salinity (‰) experienced by parents	Salinity (‰) experienced by progeny	% Males	% Survival
28	28	62	39
28	20	56	42
20	20	63	3
20	20	55	53
12	12	55	40
12	20	47	5
4	4	40	22
4	20	47	27

possibility. The results are presented in Table 8 and summarized in Table 9. As in FAC-1, there is no evident effect of the treatments on the sex ratio when applied solely to the young. Survival was high in this experiment and not significantly related to sex ratio. The reason for the variation in survival is obscure.

The effect of salinity treatments in the four factorial experiments is summarized in Figures 4 and 5. While different salinities applied to the young had no effect on sex ratio (Figure 4), there is a trend toward an increased proportion of males at higher salinities when the treatments were applied to the parents (Figure 5). This suggests that sex in Eurytemora is determined before egg-laying, as Metzler (1957) found for the cyclopoid copepod Cyclops viridis. This response of sex ratio to salinity agrees with field data (Figure 2), but is opposite to the trend found in the initial observation (Table 1).

Water Quality and Food Type

The results of the water quality experiments are ambiguous. The best survival in WQ-1 (Table 10) was found in the most "purified" treatments (COC and COCEDTA), whereas the best survival in WQ-2 (Table 11) was found in the least treated water (N and NEDTA). The relationship between treatment and sex ratio was also inconsistent between the experiments. As in the previous experiments, survivorship was uncorrelated with sex ratio.

TABLE 8. Observed sex ratios and survival rates for replicates of Experiment FAC-4. The temperature conditions at which the young were raised (10°C or 16.5°C) are the same as parental conditions. The salinity treatment was applied from early nauplius to adult. All parents experienced 20‰ salinity, and eggs were hatched at this salinity. Each replicate contained 50 nauplii.

Temperature		10°C			16.5°C		
Salinity (‰)		4	16	28	4	16	28
Replicate 1	% Male	38	44	46	64	52	52
	% Survival	74	100	100	88	100	62
Replicate 2	% Male	52	56	50	48	50	53
	% Survival	84	100	100	92	100	60
Replicate 3	% Male	60	46	58	57	54	50
	% Survival	86	100	100	28	100	48
Replicate 4	% Male	47	66	60	58	68	56
	% Survival	92	100	100	86	100	28
Totals	% Male	50	53	54.5	56.5	56	53
	% Survival	84	100	100	73.5	100	49

TABLE 9. Experiment FAC-4. Main effects on sex ratio and survival rate of Eurytemora affinis when nauplii are raised to adulthood under the same temperature, but different salinity conditions than their parents experienced. Percent male and survival values are overall treatment means.

Temperature °C	Males Produced	Females Produced	% Males	% Survival
10	295	270	52.2	95
16.5	247	198	55.5	74

Salinity ‰	Males Produced	Females Produced	% Males	% Survival
4	165	147	52.8	79
16	218	182	54.5	100
28	159	139	53.4	74.5

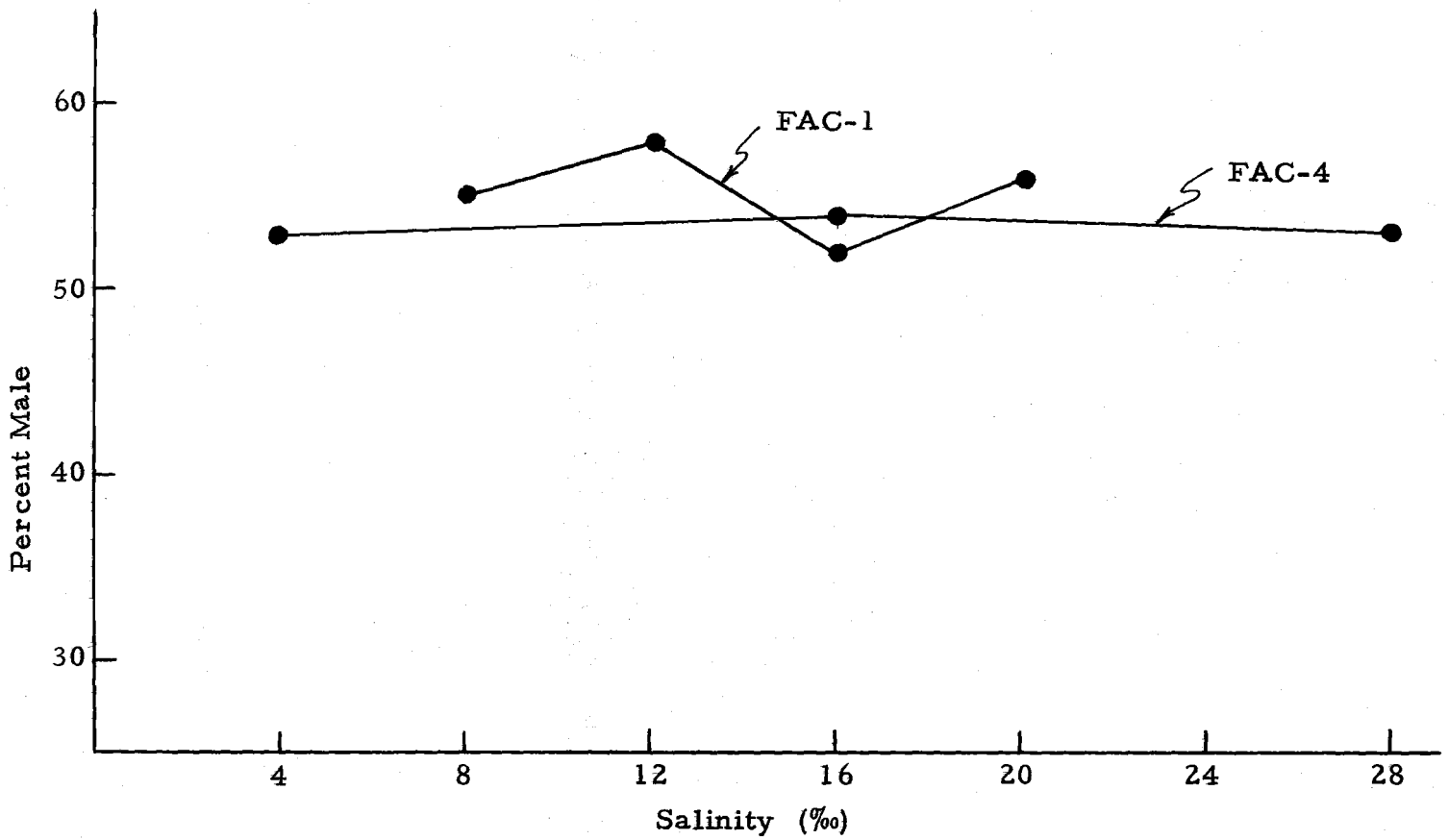

Figure 4. Effect on sex ratio of salinity treatments applied solely to young. Parents experienced standard salinity (20‰). Points are overall salinity treatment means taken from experiments FAC-1 (Table 3) and FAC-4 (Table 9).

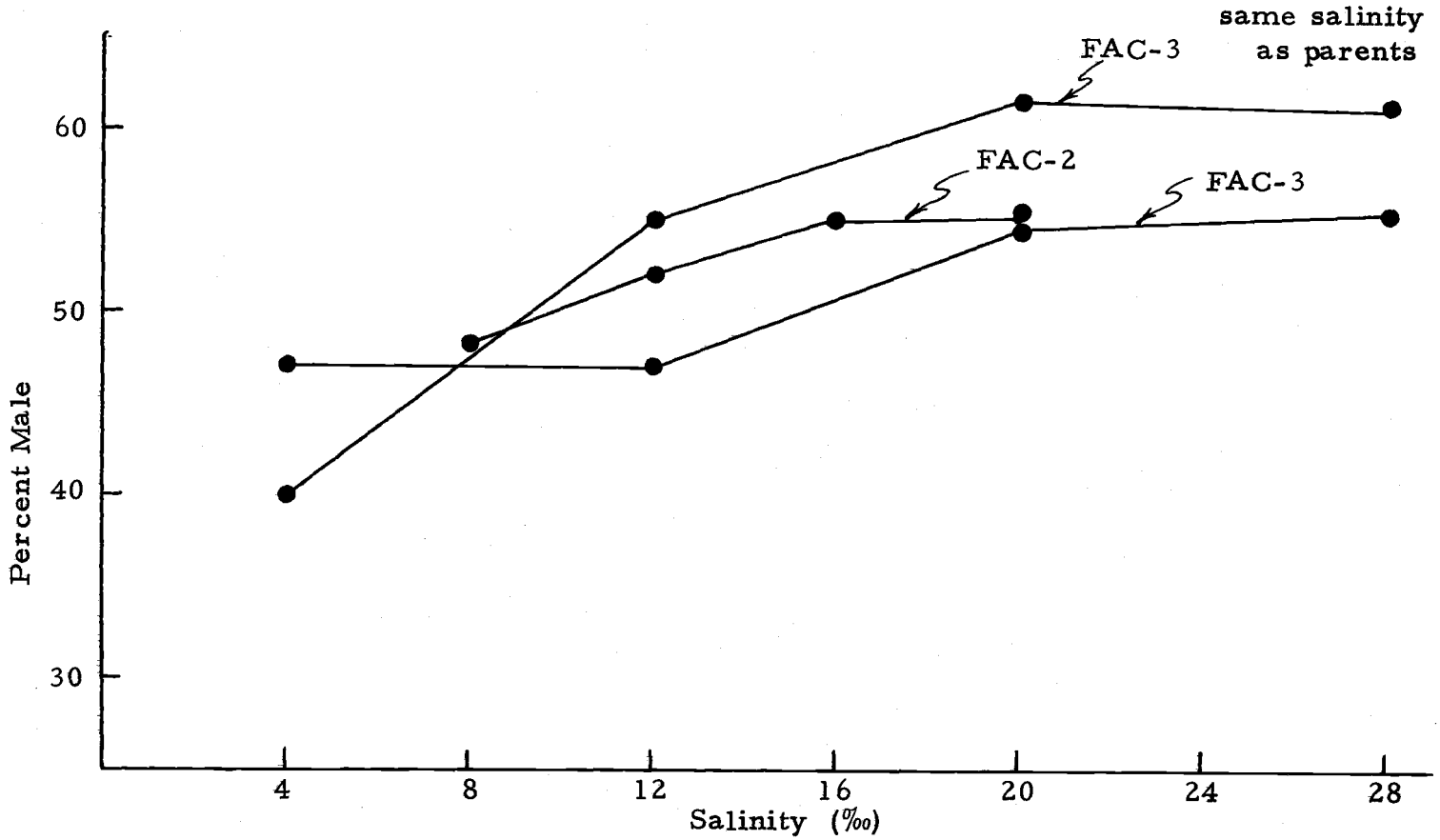

Figure 5. Effect on sex ratio of progeny of salinity treatment applied solely to parents. Points are overall treatment means taken from experiments FAC-2 (Table 5) and FAC-3 (Table 7). Progeny were raised under standard salinity conditions of 20‰ except for the top curve.

TABLE 10. Observed sex ratios and survival rates for replicates of the six water quality treatments in Experiment WQ-1. Each replicate contained 20 eggs from stock culture females. Totals are overall treatment means of percent male and survival. See the Methods section for description of the treatment.

Treatment		N	CF	COC	NEDTA	CFEDTA	COCEDTA
Replicate 1	% Male	77	40	27	67	35	27
	% Survival	65	100	75	60	90	75
Replicate 2	% Male	57	71	19	67	60	45
	% Survival	70	85	80	60	75	55
Replicate 3	% Male	56	100	56	59	44	82
	% Survival	80	40	80	85	80	85
Replicate 4	% Male	50	60	59	53	25	45
	% Survival	30	75	85	75	40	100
Total	% Male	61	62	41	61	43	51
	% Survival	61	75	80	70	71	79

TABLE 11. Observed sex ratios and survival rates for replicates of the six water quality treatments in Experiment WQ-2. Each replicate contained 20 eggs from stock culture females. Totals are overall treatment means of percent male and survival. See the Methods section for description of the treatment.

Treatment		N	CF	COC	NEDTA	CFEDTA	COCEDTA
Replicate 1	% Male	61	55	50	35	42	40
	% Survival	90	100	10	85	60	50
Replicate 2	% Male	56	58	71	39	59	82
	% Survival	90	60	75	90	85	85
Replicate 3	% Male	77	93	67	50	44	29
	% Survival	65	50	60	80	90	70
Replicate 4	% Male	56	90	60	18	57	76
	% Survival	80	50	75	55	35	85
Total	% Male	62	72	65	37	50	60
	% Survival	81	71	55	78	68	73

Experiment FOOD-1 demonstrated that food type has a significant effect on the survival of Eurytemora during development (Table 12). Pseudoisochrysis sp. was the best single food source and Thalassiosira fluviatilis was the worst. The results may reflect the food handling capabilities of Eurytemora nauplii since there was an inverse relationship between food size and survival. However, food type had no effect on sex ratio. Thus, as in the factorial experiments, the developmental environment of the young does not appear to affect the sex ratio.

Food Density

Experiment FOOD-2 (Table 13) produced an increased proportion of females at high food levels. The same relationship holds for experiment FOOD-4 (Table 15). Since survival was nearly perfect in FOOD-4, an analysis of variance on the number of males produced was performed. Food level was a significant factor at the 95% level ($F_{2,6} = 38.9$). The results of experiment FOOD-3 (Table 14) show the same trend in sex ratio, but the values are not significantly different from each other. The reason for the different results in experiment FOOD-3 as compared to FOOD-2 and FOOD-4 is obscure. Sex ratio and survivorship were uncorrelated in each of these experiments. The overall results of the food density experiments suggest that the sex ratio of Eurytemora can be influenced during development

TABLE 12. Observed sex ratios and survival rates of replicates of Experiment FOOD-1. Eurytemora affinis nauplii were raised to adulthood on monoalgal food types. Fifty nauplii from the stock culture were placed in each replicate. Totals are overall treatment means of percent male and survival.

Treatment Food		Pseudoisochrysis sp.	Isochrysis galbana	Rhodomonas sp.	Thalassiosira fluviatilis
Replicate 1	% Male	56	47	55	40
	% Survival	100	72	40	10
Replicate 2	% Male	60	51	43	50
	% Survival	100	82	28	8
Replicate 3	% Male	55	52	62	40
	% Survival	98	84	48	10
Total	% Male	57	50	55	43
	% Survival	99	81	39	9

TABLE 13. Observed sex ratios and survival rates of replicates of Experiment FOOD-2. Eurytemora affinis nauplii were raised to adulthood under one of four relative food densities (4:3:2:1). Each replicate contained 50 nauplii taken from stock culture females. Totals are overall treatment means of percent male and survival. The missing value is due to a dropped beaker. See Methods for details of the food mixtures.

Relative Food Level		4	3	2	1
Replicate 1	% Male	--	44	48	59
	% Survival	--	54	54	74
Replicate 2	% Male	29	53	48	57
	% Survival	82	98	58	60
Replicate 3	% Male	39	31	48	42
	% Survival	66	64	46	62
Replicate 4	% Male	39	63	49	57
	% Survival	66	54	86	60
Total	% Male	35	48	48	52
	% Survival	71	68	61	64

TABLE 14. Observed sex ratios and survival rates of replicates of Experiment FOOD-3. Eurytemora affinis nauplii were raised to adulthood under one of four relative food densities (10:5:2.5:1). Each replicate contained 50 nauplii taken from the corresponding relative food level of FOOD-2. Totals are overall treatment means of percent male and survival.

Relative Food Level		10	5	2.5	1
Replicate 1	% Male	46	34	42	53
	% Survival	100	100	100	90
Replicate 2	% Male	54	40	44	52
	% Survival	100	100	100	88
Replicate 3	% Male	52	54	38	45
	% Survival	100	100	100	98
Replicate 4	% Male	34	50	54	51
	% Survival	100	100	100	94
Total	% Male	46.5	44.5	44.5	50
	% Survival	100	100	100	92.5

TABLE 15. Observed sex ratios and survival rates of replicates of Experiment FOOD-4. Eurytemora affinis nauplii were raised to adulthood under one of three relative food densities (10:3:1). Each replicate contained 50 nauplii taken from a pooled culture of the middle two food levels (5, 2.5) of FOOD-3). Totals are overall treatment means of percent male and percent survival.

Relative Food Level		10	3	1
Replicate 1	% Male	44	54	54
	% Survival	100	100	100
Replicate 2	% Male	50	50	60
	% Survival	100	100	100
Replicate 3	% Male	44	52	60
	% Survival	100	98	100
Total	% Male	46	52	58
	% Survival	100	99	100

by factors other than differential mortality. This result contradicts the observations from the factorial experiments in which sex ratio appeared to be fixed at the time of egg-laying.

Inbreeding

The first inbreeding experiment (INBREED-1) was conducted at 22°C. Two of the twenty parental generation clutches produced sex ratios significantly different from 1:1 (binomial test, 95% level). Only one of the 19 clutches chosen for the F_1 generation hatched. The single clutch that did hatch did not maintain the sex ratio deviation of the parental generation. There was no hatching in the 11 clutches taken from F_1 females to produce an F_2 generation. The experiment terminated without significant results.

Since 29 of the 30 F_1 and F_2 clutches produced at 22°C in INBREED-1 failed to hatch, a two generation egg hatching study was conducted at this temperature. All but one of the F_1 and F_2 generation clutches hatched at this temperature. Thus, the hatching failure in INBREED-1 cannot be attributed to the high temperature. The successful hatching of F_2 clutches indicates that the hatching failure in INBREED-1 was not due to inbreeding. This is confirmed by many generations of inbreeding in experiment INBREED-2. It is possible that the poor hatching in INBREED-1 was due to unfertilized clutches being chosen. This could occur if, in haste to start the next

generation, clutches were taken from unfertilized females.

Experiment INBREED-2 was conducted at the standard culture temperature (16.5°C), since this was the temperature to which the animals had been acclimated. At the time of writing, 15 generations of brother/sister mating have occurred in the single remaining family line ("Ø"). The results shown are partial since the experiment is still continuing. Table 16 presents the mean sex ratio over all generations for each of the 20 family lines. The overall mean for two lines (I, Ø) shows a significant deviation from 1:1 (binomial test, 95% level). These lines are discussed in detail below. The mean sex ratio over all family lines in each generation is shown in Figure 6. The numeral by each data point represents the total number of adults produced in that generation in the family lines represented. Line "Ø" has been the most successful under the selection criteria, and has produced the greatest number of significant deviations from a 1:1 sex ratio (Table 16). Family lines and sub-lines were terminated for a variety of reasons summarized in Table 17. There are no consistent trends in factors causing termination of lines. Table 16 shows that a total of 51 clutches in INBREED-2 yielded a sex ratio significantly different from 1:1 (binomial test, 95% level). Three of these 51 clutches produced only one sex and thus terminated the family sub-line. One sub-line of "N" ended in 19 males and no females, and one sub-line of "E" ended in 10 males and no females.

TABLE 16. Results of Experiment INBREED-2. Family line means overall generations.

Family Line	No. Generations	No. Adults Produced	Average % Male	No. Clutches with Sex Ratio Significantly Different from 1:1 (α = 0.05)
A	1	2	50	
B	5	106	46	
C	1	1	100	
D	4	71	54	
E	5	117	56	1*
F	13	496	52	
G	1	1	100	
H	5	113	49	1
I	12	837	61	7
J	--	--	--	
K	1	1	0	
L	1	2	0	
M	--	--	--	
N	4	81	56	2*
O	16	2962	53	40*
P	--	--	--	
Q	1	3	33	
R	4	108	53	
S	--	--	--	
T	--	--	--	

* Includes one family subline ending in clutch producing sex ratio significantly different from 1:1 (α = 0.05) and all adults of one sex.

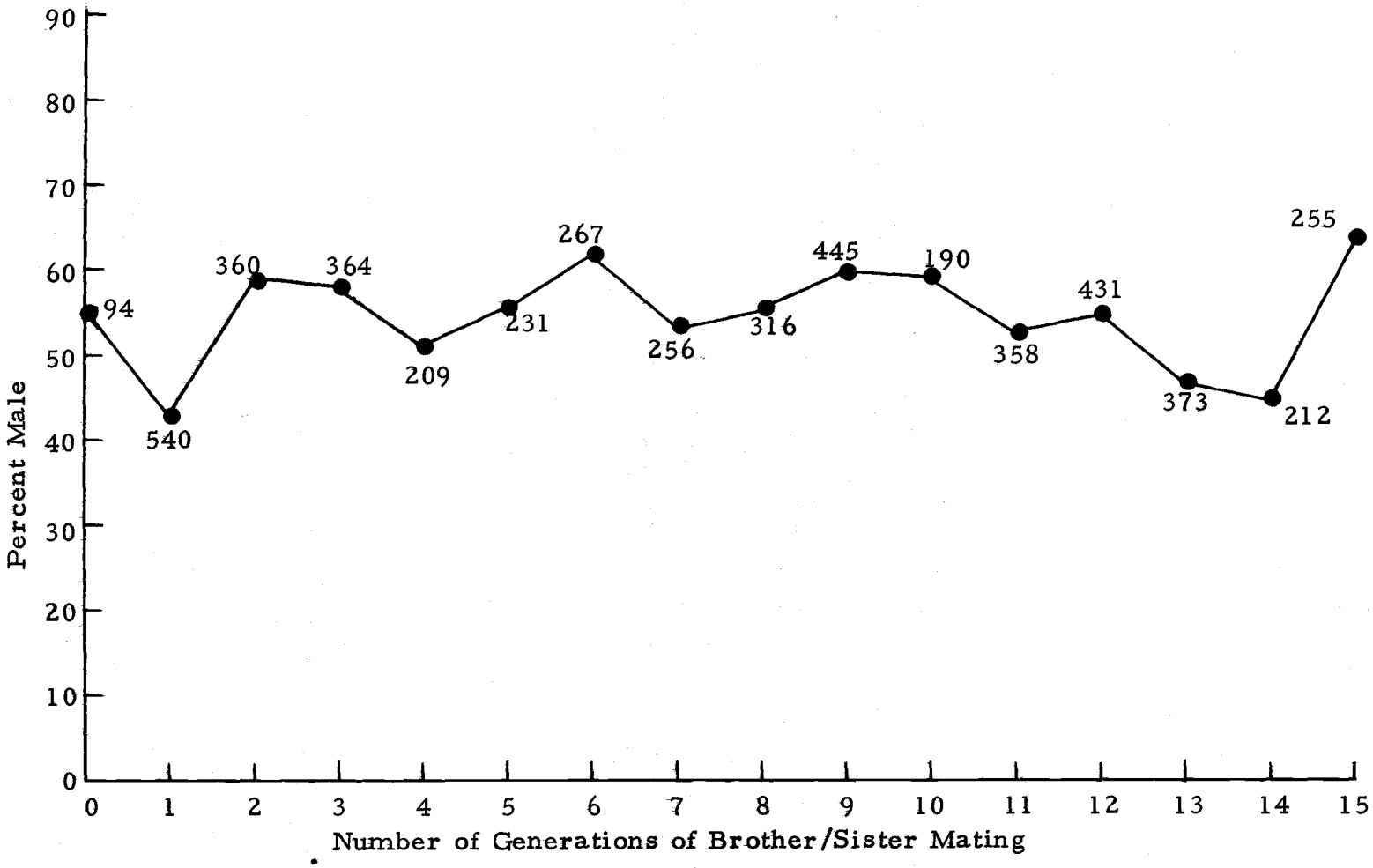

Figure 6. Results of INBREED-2. Points represent mean sex ratio each generation over all family lines. The total number of adult Eurytemora produced each generation is given.

TABLE 17. Results of Experiment INBREED-2. Reason for termination of family sublines. Generation "P" is the parental, "1" is first generation of brother/sister mating.

Generation	Total number of clutches	Reason For Termination: No adults from clutch	No males from clutch	No females from clutch	Few adults produced	Clutch sex ratio close to 1:1	Total no. of terminated lines
P	20	5	2	2		1	10
1	20	1			1		2
2	30	6		1	3	2	12
3	43	9	2	5	2	1	19
4	47	12	5	5	9	4	35
5	20	2	4	1	1	1	9
6	22	2	1	1	1	2	7
7	26	3	1	2	5	1	12
8	25	3	3		2	2	10
9	32	7	2		4	2	15
10	25	7	4	1			12
11	21	3			2	4	9
12	23	2	1	1	3	3	10
13	21	1	5	1	1	1	9
14	20	2	2	1	1	2	8
15	20	8	1				9
Total	415	73	33	21	35	26	188

One sub-line of "Ø" ended in 11 females and no males (Figure 9).

An analysis was made of the 240 clutches producing 6 or more adults (Table 18). Six was chosen since it is the smallest total number of adults at which a statistically significant difference from a 1:1 sex ratio can occur (binomial test, 95% level). Of the 51 clutches producing a significant deviation (Table 16), 36 produced an excess of males, and 15 produced an excess of females (Table 18). The pattern of significant deviations is of interest. If the deviations occur randomly, then selection for a high proportion of one sex has been unsuccessful. If clutches chosen for their deviation in a given direction produce succeeding generations with significant deviations in the same direction, then selection for a high proportion of one sex has been successful.

Selection for a high proportion of one sex has been unsuccessful in most family lines. Lines "I" and "Ø" are possible exceptions to this, and the results for those lines are presented in detail in Figures 7 through 14. In these figures the numerals following the family line designation trace the sub-line genealogy. Figures 8 through 13 represent several sub-lines of "Ø". These sub-lines diverge at generation 7. Figure 8 presents the early generations of these sub-lines. Only generations 7 through 15 are represented in Figures 9 through 13. In the first 8 generations of these lines selection for a high proportion of one sex was unsuccessful. After this there is a trend

TABLE 18. Results of Experiment INBREED-2. Analysis of clutches producing 6 or more adults. Generation "P" is parental, "1" is first generation of brother/sister mating.

Generation	No. of clutches with 6 or more adults	Clutches with 50% male	Clutches with > 50% male	Clutches with < 50% male	Clutches with sex ratio > 50% sig. at 95% level	Clutches with sex ratio < 50% sig. at 95% level
P	6	2	3	1		
1	18	1	8	9		2
2	16	3	9	4	4	1
3	26	2	18	6	2	1
4	16	5	7	4		
5	9	1	6	2	2	1
6	17	2	13	2	3	
7	16	2	10	4	1	1
8	15	2	7	6	2	
9	19	1	14	4	4	
10	11	1	7	3	3	
11	15	2	9	4	2	1
12	18	1	14	3	4	2
13	14	1	4	9	3	3
14	13	1	5	7	1	2
15	11	0	6	5	5	1
Total	240	27	140	73	36	15

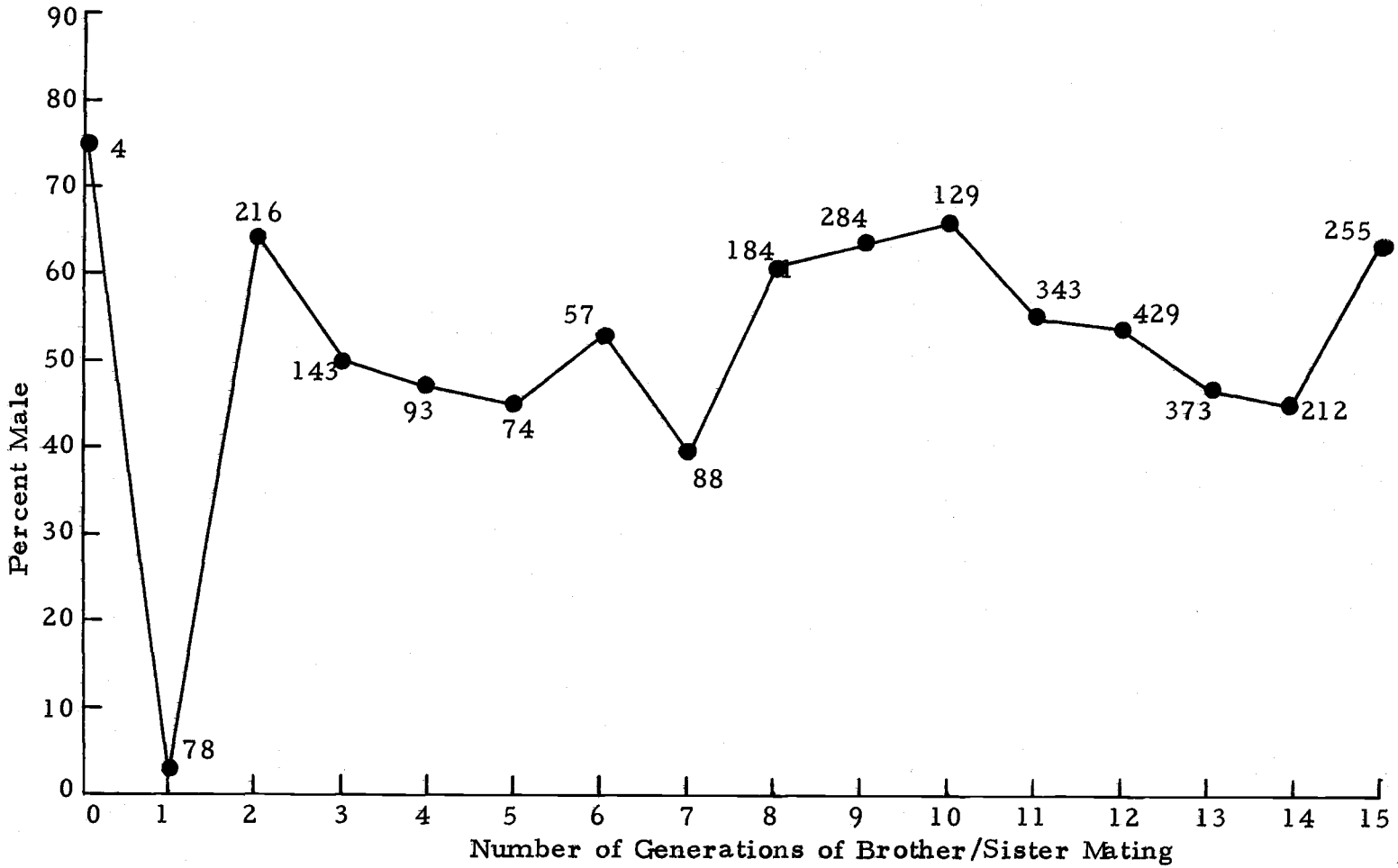

Figure 7. Results of INBREED-2, family line ∅. Points are means over all sub-lines of ∅. Number of adults produced each generation is given.

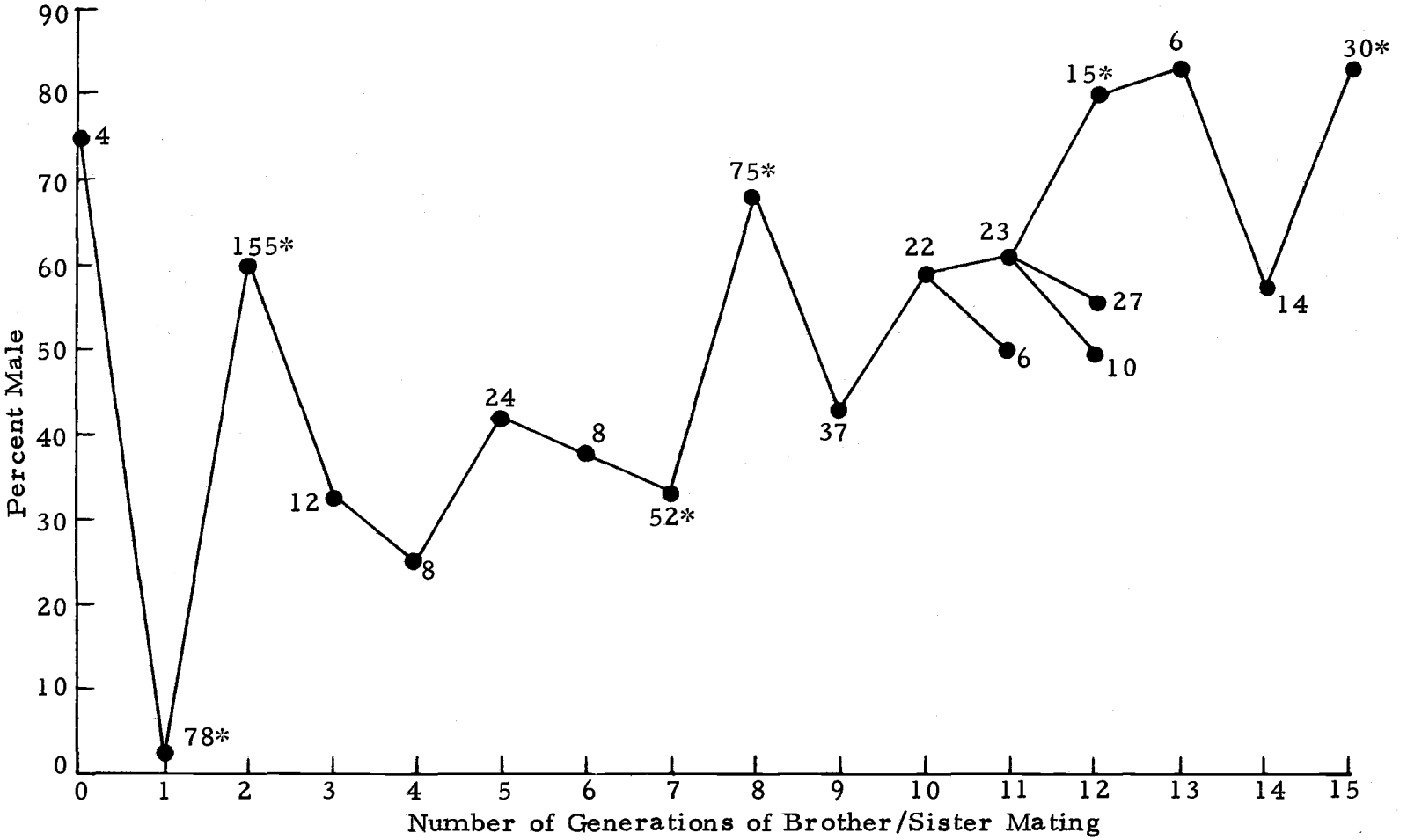

Figure 8. INBREED-2. Family sub-line Ø2192121221. Points are observed sex ratios, and numbers are adults produced in each clutch each generation. * marks values significantly different from 1:1.

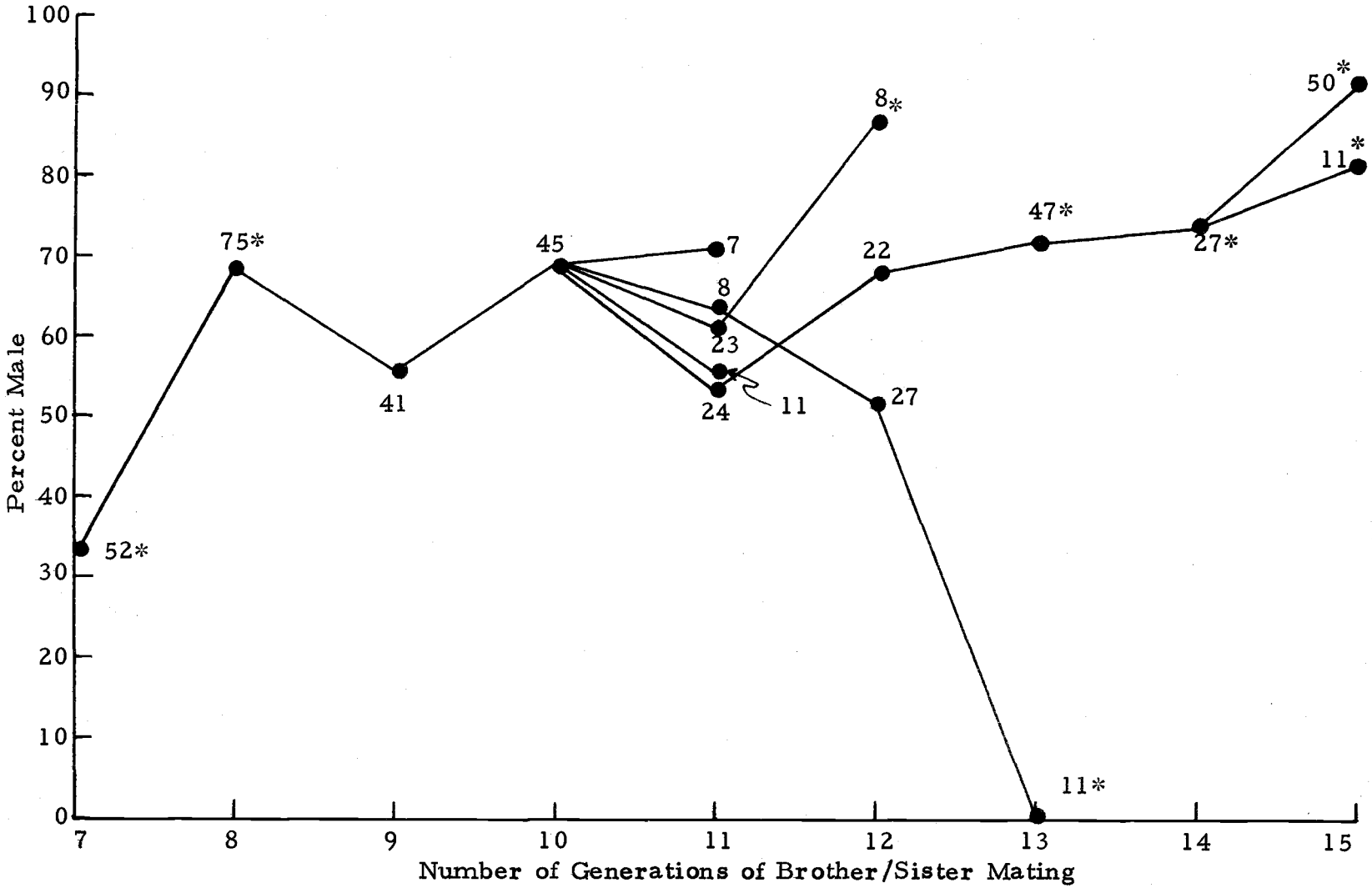

Figure 9. INBREED-2. Family sub-line Ø2192121211 starting at Generation 7. Points are observed sex ratios, and numbers are adults produced in each clutch each generation. * marks values significantly different from 1:1.

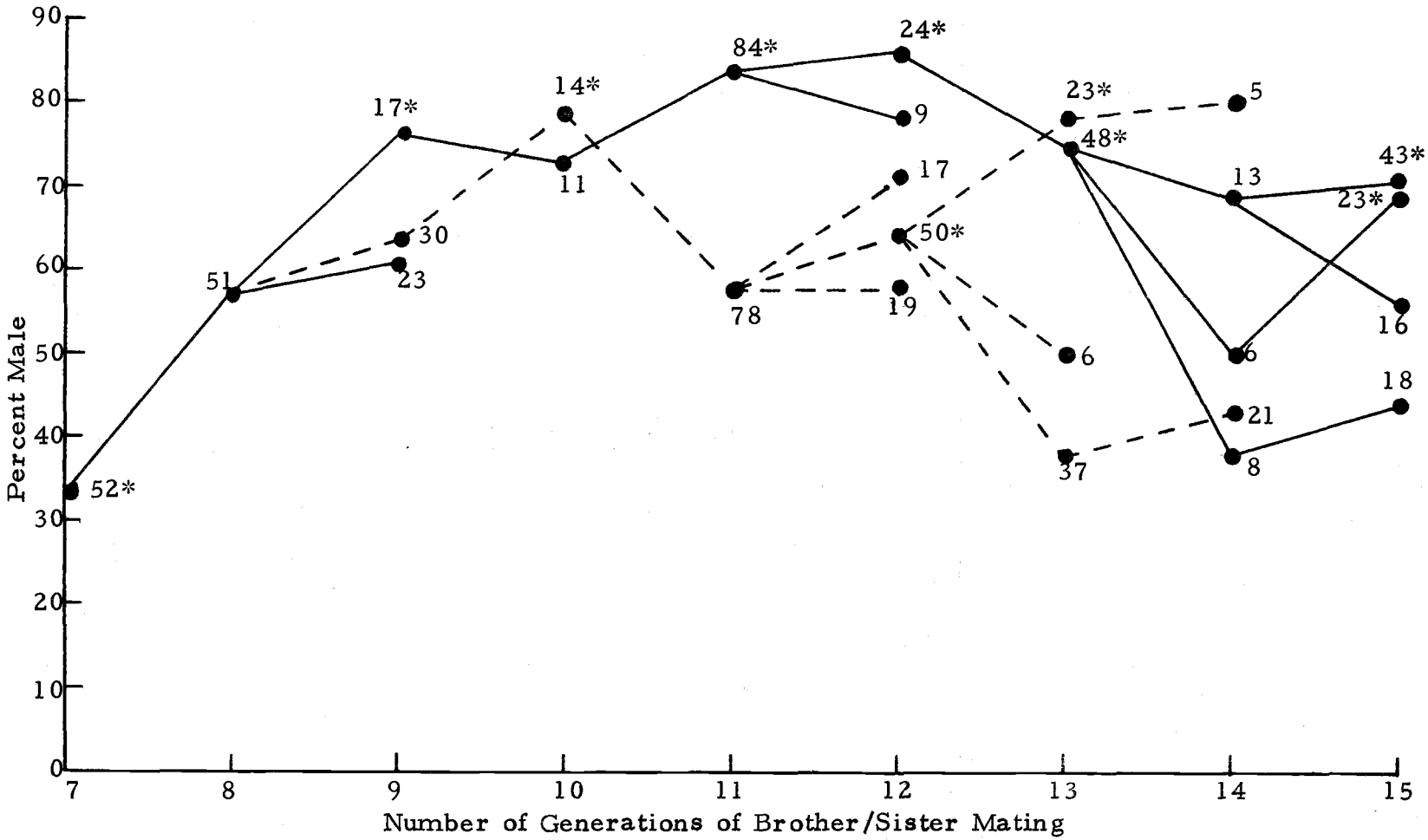

Figure 10. INBREED-2, family sub-line Ø21921211 beginning with generation 7. Points are observed sex ratios, and numbers are adults produced in each clutch each generation. * marks values significantly different from 1:1.

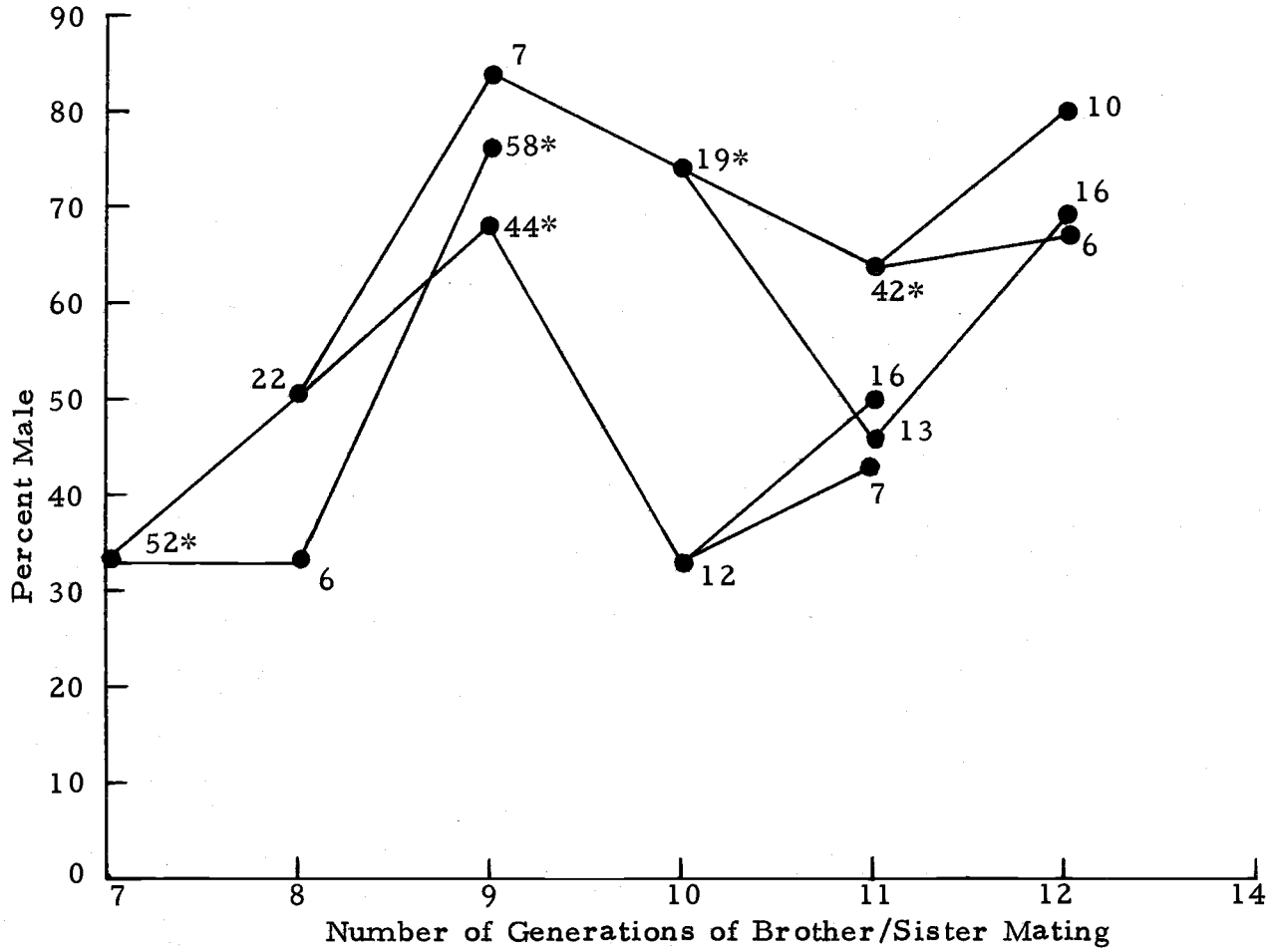

Figure 11. INBREED-2, family sub-lines Ø21921214 and Ø21921213, beginning with generation 7. Points are observed sex ratios and numbers are adults produced in each clutch each generation. * marks values significantly different from 1:1.

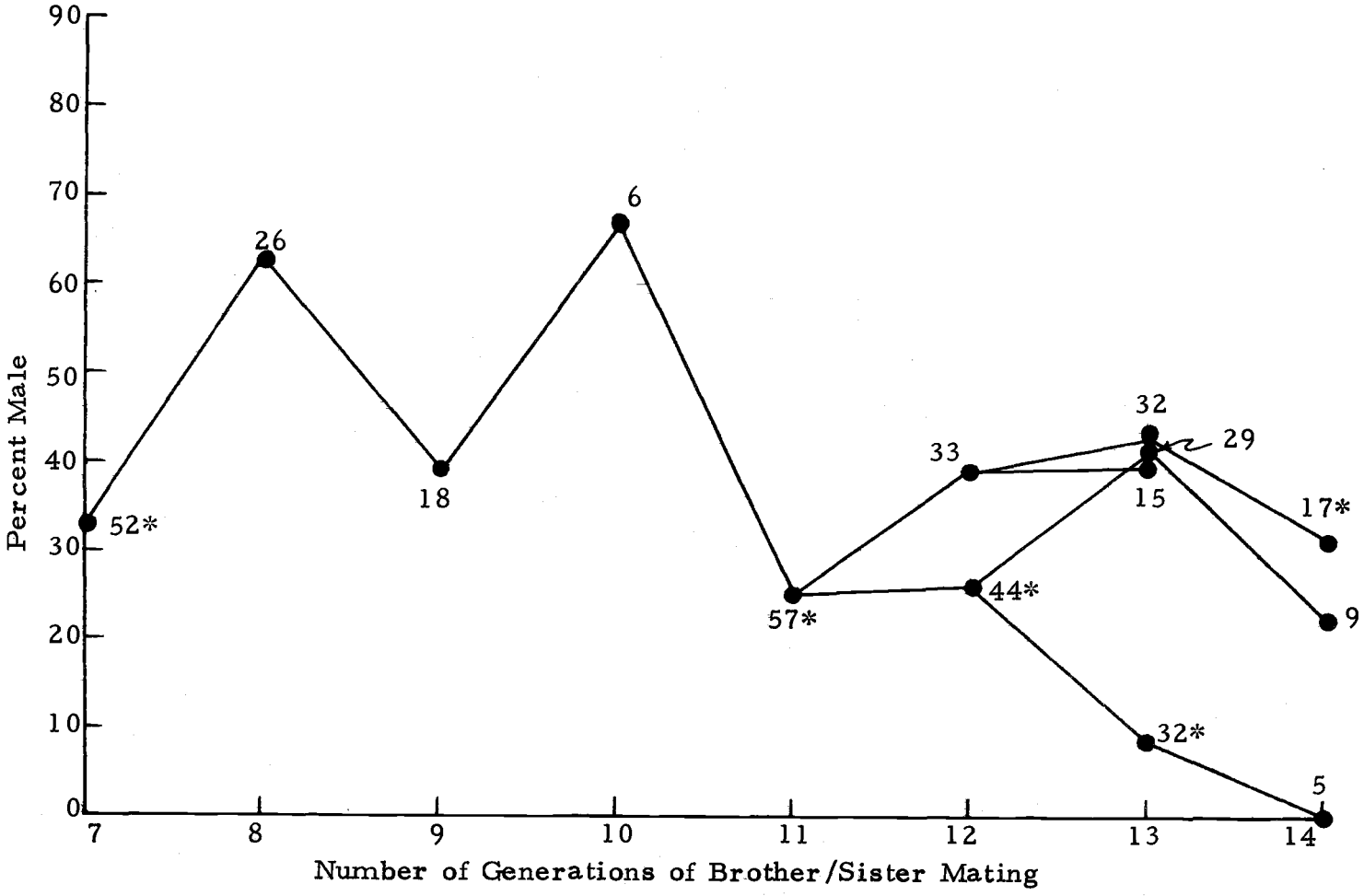

Figure 12. INBREED-2, part of family sub-line Ø21921216111 beginning with Generation 7. Points are observed sex ratios, and numbers are adults produced in each clutch each generation. *marks values significantly different from 1:1.

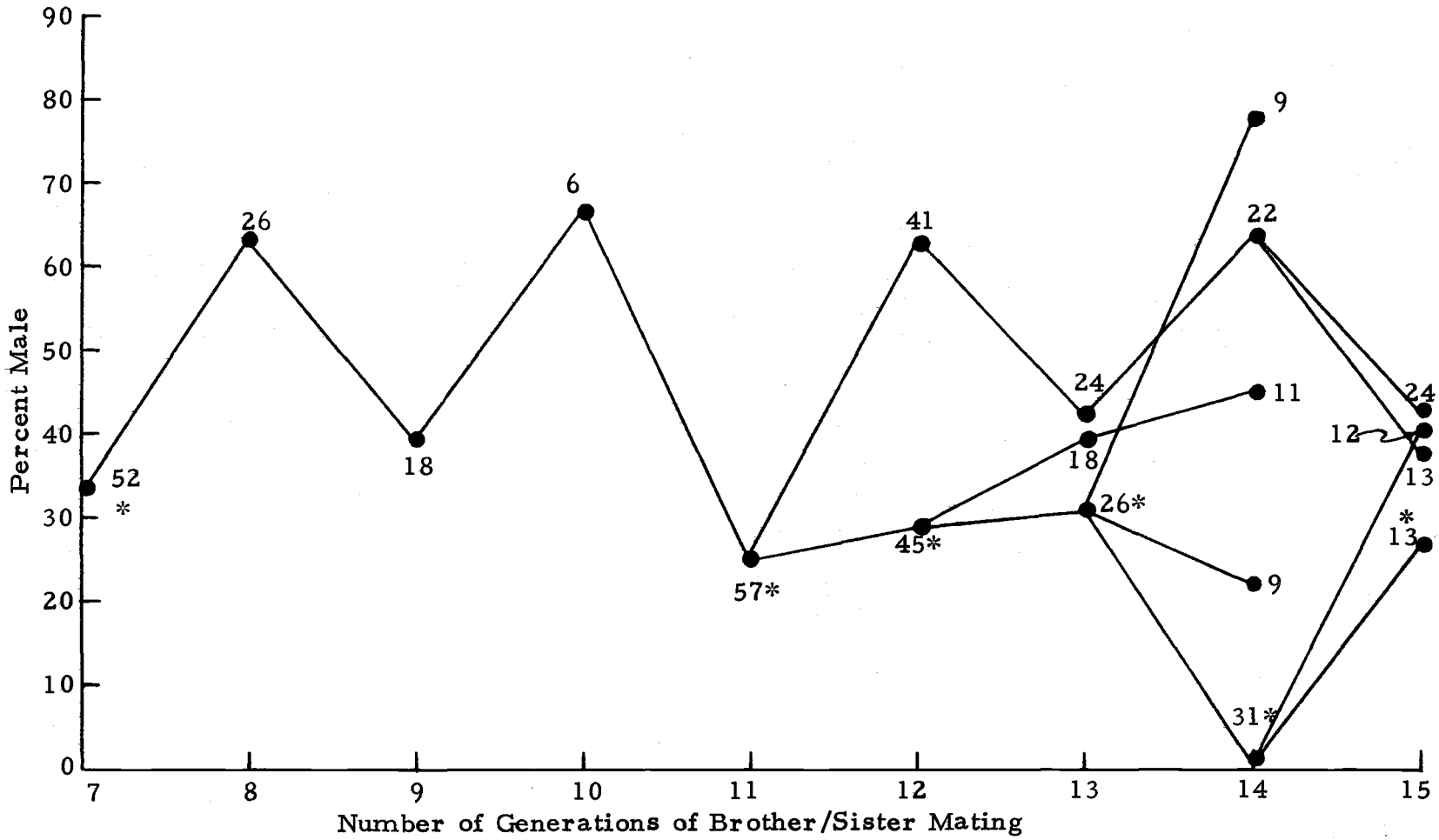

Figure 13. INBREED-2, part of family sub-line Ø21921216111 beginning with generation 7. Points are observed sex ratios, and numbers are adults produced in each clutch each generation. * marks values significantly different from 1:1.

toward a high proportion of males in 5 sub-lines (Figures 8 through 12) and toward a high proportion of females in one sub-line (Figure 13). Further inbreeding will show whether these trends represent successful selection for aberrant sex ratios. Similar results were obtained from family "I" (Figure 14). Selection was unsuccessful for 4 generations, after which significant deviations toward a higher proportion of males appear. This sub-line was also characterized by the appearance of several intersex individuals having a mixture of male and female traits. The intersex will not be described here.

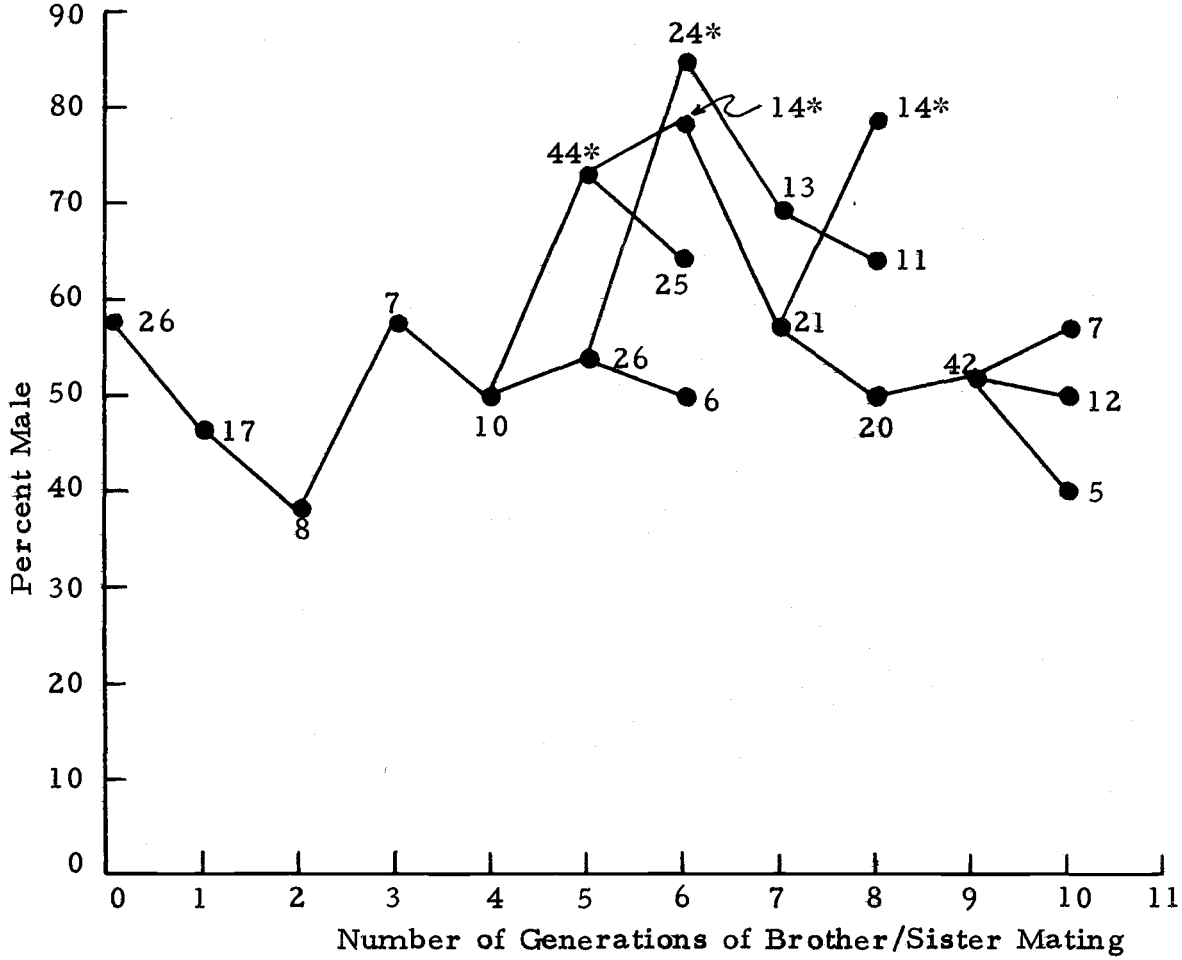

Figure 14. INBREED-2, family sub-line I4122. Points are observed sex ratios and numbers are adults produced in each clutch each generation. * marks values significantly different from 1:1.

DISCUSSION

Sex Ratio Theory

Discussion of the effect of natural selection on sex ratio began with Darwin. He stated, "I formerly thought that when a tendency to produce sexes in equal numbers was advantageous to the species, it would follow from natural selection, but I now see that the whole problem is so intricate that it is safer to leave its solution for the future" (Darwin, 1871, cited in Fisher, 1930). "The future" began with Fisher (1930). He argued that selection will favor an equilibrium sex ratio at the end of the period of parental care. The mechanism is as follows. Suppose that the parental expenditure on an individual male is equal to that on an individual female, and that there are more females than males in the population. Since, on a population-wide basis, males and females contribute equally to the gene pool of the next generation, the average contribution of a male would be greater than that of a female. Therefore genes favoring the production of males will be favored until the population reaches an equilibrium value, in this case, of 1:1. In general, the equilibrium ratio of males to females in the population would be the inverse of the relative parental expenditure on individual males compared to females.

Fisher's verbal arguments have been expressed in mathematical models by many authors (for example MacArthur, 1965; Spieth, 1974;

Charnov, 1975). There have been two basic disagreements in this literature. The first began with Kolman (1960) who argued that, while the mean sex ratio was under the influence of selection, the variance was not. Thus even a population in which half of the breeding pairs produced only males and the other half only females would be stable against selection. Verner (1965), however, showed that this argument depended on the occurrence of a statistically improbable event: either there must be no further variation among individuals or their offspring once a balance is achieved; or all variations must be simultaneously compensated by variations in other individuals. Thus, Verner argued that selection will, in fact, tend to reduce the variance in sex ratio.

The second disagreement concerns the effect of differential mortality after the period of parental care. All of the above authors agree with Leigh (1970) that such mortality cannot influence the primary sex ratio. The exception is Emlen (1968) who states that "it is clear that mortality at all ages will affect the evolution of the primary sex ratio." Intuitively, it seems Emlen may be correct since the sex ratio at reproductive age should be a strong selective factor. For example, suppose a sufficient number of males die between the end of parental care and reproductive age that there remain only a small number of males to fecundate all the females. Then genes favoring production of more males would be favored by selection, and this

could alter the primary sex ratio. This disagreement and confusion about fundamental questions concerning the effect of selection on sex ratio may have precipitated Emlen's (1973) statement that "It is not by any means clear that sex ratio is a useful concept."

Sex Ratio and Sex Determination in Eurytemora affinis

Eshel (1975) stated that, "problems concerning sex ratio are closely related to problems of sex determination." This was recognized by Kalmus and Smith (1960) for the problem of the evolution of sexual differentiation as well. In the present study, the sex ratios observed in experiment INBREED-2 provide insight into the sex determination mechanism of Eurytemora affinis. An important consideration in INBREED-2 is that Fisher's principle does not hold, because inbreeding violates his assumption of population-wide competition for mates (Hamilton, 1967).

The first observation of interest in INBREED-2 is that the frequency of clutches with sex ratios significantly different from 1:1 is higher than expected. Fifty-one out of the 240 clutches presented in Table 18 are significantly different from 1:1, compared to the expected number of 12 (one out of 20). According to Shaw and Mohler (1953) this is evidence for the existence of autosomal control of sex determination. This implies that the sex determination mechanism in Eurytemora affinis may be polygenic.

Kosswig (1964) described some characteristics of polygenic sex determination systems. He stated that "In cases of polymeric sex-determination, any prediction of the sex ratio in a given cross is highly doubtful." That this is clearly the case for E. affinis is illustrated in Figure 8. While the sex ratio in the first generation of brother/sister mating was significantly less than 1:1, it was significantly higher than 1:1 in the second generation. The same unpredictability holds for generations 7 and 8. A more extreme example was produced in which, during the course of successful selection for a high proportion of males, one clutch produced all females (Figure 9).

The unpredictability of a particular cross makes proof of a polygenic mechanism difficult. According to Kosswig (1964), "The only way to prove the correctness in principle of the interpretation lies in the possibility of producing lines rich in males and others rich in females by selection continued over many generations." Figures 8 through 12, and 14 are evidence that lines rich in males are being produced in E. affinis, and Figures 12 and 13 are evidence for female-rich lines.

On the basis of the above evidence, it seems highly probable that E. affinis has a polygenic sex determination system. This appears to be the first documentation of the method of sex determination in a calanoid copepod. If these results hold for other calanoids, they will join harpacticoid copepods in having polygenic sex

determination.

It is not possible at this time to elucidate detailed features of the polygenic mechanism in E. affinis. The number of pairs of alleles is probably large, since selection for a high proportion of one sex was difficult (see Kosswig, 1964). Whether the male and female determining genes are allelic in some or all cases, and what proportion of each is necessary to produce one sex or the other cannot yet be determined. It is possible that the proportion of male-determining factors needed to produce a male is not constant. For example, environmental factors may influence the expression of male and female determiners in the zygote or later developmental stages. Whatever the mechanism, the system must be characterized by a switch mechanism that produces normal males and females instead of intergrades. This is seen by the rarity of intersex individuals, and is a characteristic of polygenic sex determination (Kosswig, 1964).

Environmental influence on sex ratio modifier genes (Shaw, 1958) could explain the results of the food density, salinity and temperature effect on sex ratio. The food density experiments (FOOD-2, FOOD-3, FOOD-4) indicated that the sex ratio of E. affinis can be influenced during development (Tables 13 and 15). The factorial experiments using salinity and temperature treatments on the adults (FAC-2 and FAC-3) indicated that these factors may act on the egg or early zygote to influence sex ratio. Evidently, these factors

alter the balance between the male and female determiners, perhaps by acting on the switch mechanism, to shift the sex ratio. In fact, the switch mechanism may be totally under environmental control, except for cases of extreme predominance of either male or female determiners in an individual.

Thus, what has been interpreted as environmental influence on sex determination may result from these factors acting on the components of a polygenic system of sex determination (Kosswig, 1964). A polygenic mechanism could account for Conover's (1965) observation that male Calanus hyperboreus may be produced only when needed. The presence of virgin females would lower the threshold ratio of male/female determiners needed to produce a male. It could also account for the production of phenotypic females from "genotypic" males as proposed by Heinle (1970) for Acartia tonsa.

Ecological Significance of Copepod Sex Ratios

The ecological significance of the various sex ratios observed for copepods has been discussed by several workers. Mednikov (1961) argued that there was a trend toward a higher proportion of females in less productive environments. He stated that the prevalence of females was an adaptation for increasing the fecundity of a population having a restricted food supply. Heinle (1970) suggested that "genotypic" males became phenotypic females at low population

densities to increase fecundity at these levels. Katona (1970) argued that both species of Eurytemora, which he studied, produced more females under stress conditions to carry the population through hard times. Conover (1965) has suggested that males of Calanus hyperboreus may be produced only when needed for fertilization of females. On the other hand, populations of Tisbe and Tigriopus produce more males under stress conditions, such as low food availability. Ar-Rushdi (1958) argues that this is adaptive since females are energetically expensive to maintain compared to males. Battaglia (1963) notes that the prevalence of males at low population densities may be an adaptation to insure fertilization of all females. Thus, while most authors argue about the adaptiveness of the sex ratio under stress conditions, there is not agreement as to which "strategy" is adaptive.

Sex Ratio in the Present Study

Discussion about the ecological significance of sex ratio in Eurytemora in the present study is difficult. The field data predominantly concern E. americana, for which there are no laboratory studies. The laboratory data are for E. affinis for which there are inadequate field data (for reasons given in the Introduction). There are several hypotheses to explain the higher proportion of E. americana females at upstream stations in Yaquina estuary (Figure 1).

First, if sex ratio in E. americana has the same response to salinity as in E. affinis, the field data might be explained as environmental influence on sex ratio. The relationship between sex ratio and salinity in the field (Figure 2) was in the same direction as the laboratory studies (Figure 5). The initial laboratory observation (Table 1) was, for unknown reasons, in the opposite direction. A second hypothesis is that salinity in the field only correlates with some other factor, such as food availability, that directly influences sex ratio. A third hypothesis is that the swimming behaviors of the two sexes are causal distributional factors. In the laboratory, males of both E. affinis and E. americana are more active than females. Females spend more time resting on surfaces (see also Katona, 1973). The observed downstream displacement of males relative to females (Figure 1) may result from preferential physical displacement of one sex due to the different amounts of time they spend in the water column. A fourth hypothesis is that males and females have differential mortality rates along the stream axis. This could result from different intensities of sex-selective predation (Maly, 1970) made possible by the size difference between the sexes of adult Eurytemora. It was not possible to determine, on the basis of the study, which of these hypotheses, if any, is correct.

The same problems exist in interpretation of the laboratory data on E. affinis. It is found rarely in the sampled area of Yaquina

estuary, presumably because the bulk of the population resides upstream of the sample area. Since there were no field data to compare with the laboratory observations, it is not easy to discuss the ecological significance of the influence of environmental factors on sex ratio in E. affinis. The results of the factorial experiments on salinity and temperature indicate that these factors may affect sex ratio in E. affinis. It may be that the salinity or temperature regime of the parents acts as a long range predictor of conditions to which the sex ratio of the progeny must be adapted. The food density experiments (Tables 13 through 15) suggest that E. affinis may be able to respond to high food levels by producing a high proportion of females in order to increase population growth rates. Since food density influences the sex ratio of the progeny during their development, it may be a more proximate indicator of conditions late in the life cycle than salinity or temperature. How these factors interact in natural populations of E. affinis requires further study.

Conclusions

Interpretation of the experimental results on Eurytemora affinis as evidence for polygenic sex determination is consistent with present knowledge of such systems (see Kosswig, 1964). Polygenic sex determination can allow changes in the sex ratio of the population in response to environmental cues, and therefore may be an advantage for

organisms in the seasonal cycle of estuarine conditions. Natural selection operating on the outbreeding natural population would act to prevent extinction by favoring the increase of those genotypes which favored the less numerous sex (Fisher, 1930). Thus, once a skewed sex ratio had been established as an adaptive response to environmental conditions, Fisher's principle would act to restore balance between the sexes in a few generations.

BIBLIOGRAPHY

Amma, K. 1911. Über der Differenzierung der Keimbahnzellen bei den Copepoden. Archiv für Zellforschung 6: 497-576.

Ar-Rushdi, A. H. 1958. Polygenic basis of sex-ratio in Tigriopus. Proceedings of the International Congress of Genetics 10(2): 8.

Bacci, G. 1965. Sex determination. International Series of Monographs in Pure and Applied Biology. Division: Zoology. Volume 26. Pergamon Press, Oxford, England. 306 p.

Battaglia, B. 1958. Sex ratio in Tisbe gracilis, a marine copepod. Proceedings of the International Congress on Zoology 15(3): 288-290.

Battaglia, B. 1963. Advances and problems of ecological genetics in marine animals. Proceedings of the International Congress of Genetics 11(2): 451-463.

Bayly, I. A. E. 1965. Ecological studies on the planktonic Copepoda of the Brisbane River estuary with special reference to Gladiofierens pectinatus (Brady) (Calanoida). Australian Journal of Marine and Freshwater Research 16(3): 315-350.

Beerman, W. 1954. Weibliche Heterogametie bei Copepoden. Chromosoma 6: 381-396.

Bogorov, V. G. 1939. Sex ratio in marine Copepoda. Akademii Nauk SSSR, Doklady 23(7): 705-708.

Braun, H. 1909. Die spezifischen Chromosomenzahlen der einheimischen Arten der Gattung Cyclops. Archiv für Zellforschung 3: 449-482.

Carillo, E. 1974. Experimental interbreeding between Atlantic and Pacific populations of the marine calanoid copepod Acartia clausi Giesbrecht. M.S. thesis, Corvallis, Oregon State University. 47 numb. leaves.

Charnov, E. L. 1975. Sex ratio selection in an age-structured population. Evolution 29(2): 366-368.

Cognard, C. 1973. Influence de la température sur les développements embryonnaire et post-embryonnaire du Copépode Diaptomide Eudiaptomus gracilis (Sars, 1863). Crustaceana 24: 323-331.

Conover, R. J. 1965. Notes on the molting cycle, development of sexual characteristics and sex ratio in Calanus hyperboreus. Crustaceana 8: 308-320.

Darwin, D. 1871. The descent of man, and selection in relation to sex. John Murray, London. p. 399.

Egami, N. 1951. A note on the sex differentiation of the marine copepod Tigriopus japonicus. Annotations zoologicae japonenses 24: 131-136.

Egloff, D. A. 1967. Ecological aspects of sex ratio and reproduction in experimental and field populations of the marine copepod Tigriopus californicus. Ph. D. thesis. Stanford University. 150 numb. leaves.

Emlen, J. M. 1968. A note on natural selection and the sex ratio. American Naturalist 102: 94-95.

Emlen, J. M. 1973. Ecology: An evolutionary approach. Addison-Wesley, Reading, Massachusetts. 483 p.

Eshel, I. 1975. Selection on sex-ratio and the evolution of sex-determination. Heredity 34(3): 351-361.

Fisher, R. A. 1930. The genetical theory of natural selection. Clarendon Press, Oxford, England. 272 p.

Frolander, H. F., C. B. Miller, M. J. Flynn, S. C. Myers, and S. T. Zimmerman. 1973. Seasonal cycles of abundance in zooplankton populations of Yaquina Bay, Oregon. Marine Biology 21: 277-288.

Gurney, R. 1931. British Fresh Water Copepoda, 1. Ray Society Publication, London. 236 p.

Hamilton, W. D. 1967. Extraordinary sex ratios. Science 156: 477-488.

Harding, J. P. 1963. The chromosomes of Calanus finmarchicus and C. helgolandicus. Crustaceana 6: 81-88.

Heberer, G. 1932. Die Spermatogenese der Copepoden II. Zeitschrift für wissenschaftliche Zoologie 142: 14-253.

Heinle, G. 1970. Population dynamics of exploited cultures of calanoid copepods. Helgoländer wissenschaftliche Meeresuntersuchungen 20: 360-372.

Kalmus, H. and C. A. B. Smith. 1960. Evolutionary origin of sexual differentiation and the sex-ratio. Nature 186: 1004-1006.

Katona, S. K. 1970. Growth characteristics of the copepods Eurytemora affinis and E. herdmani in laboratory cultures. Helgoländer wissenschaftliche Meeresuntersuchungen 20: 373-384.

Katona, S. K. 1973. Evidence for sex pheromones in planktonic copepods. Limnology and Oceanography 18(4): 574-583.

Kolman, W. A. 1960. The mechanism of natural selection for the sex ratio. American Naturalist 94: 373-377.

Kornhauser, S. J. 1915. A cytological study of the semi-parasitic copepod Hersilia apodiformis (Phil.), with some general considerations of copepod chromosomes. Archiv für Zellforschung 13: 399-445.

Kosswig, C. 1964. Polygenic sex determination. Experientia 20: 190-199.

Leigh, E. G. 1970. Sex ratio and differential mortality between the sexes. American Naturalist 104: 205-210.

Lock, A. R. and I. A. McLaren. 1970. The effect of varying and constant temperatures on the size of a marine copepod. Limnology and Oceanography 15(4): 638-640.

MacArthur, R. H. 1965. Ecological consequences of natural selection. In: Theoretical and Mathematical Biology, ed. by T. H. Waterman and H. J. Morowitz. Blaisdell, New York. p. 388-397.

Maly, E. J. 1970. The influence of predation on the adult sex ratios of two copepod species. Limnology and Oceanography 15(4): 566-573.

Matscheck, H. 1910. Über Eireifung und Eiblage bei Copepoden. Archiv für Zellforschung 5: 37-111.

Mednikov, B. M. 1961. On the sex ratio in deepwater calanoida. Crustaceana 3: 105-109.

Metzler, S. 1955. Über die Beeinflussbarkeit des Geschlechtsverhältnisses bei Cyclops viridis Jurine. Die Naturwissenschaften 42: 517-518.

Metzler, S. 1957. Über die Beeinflussbarkeit des Geschlechtsverhältnisses von Cyclops viridis Jurine durch Aussenfaktoren. Zoologische Jahrbucher, Abteilung für Allgemeine Zoologie und Physiologie der Tiere 67: 81-110.

Moriatou-Apostolopoulou, M. 1972. Sex ratio in the pelagic copepods Temora stylifera Dana and Centropages typicus Kroyer. Journal of Experimental Marine Biology and Ecology 8: 83-87.

Neunes, H. W. and G. F. Pongolini. 1965. Breeding a pelagic copepod, Euterpina acutifrons (Dana) in the laboratory. Nature 208: 571-573.

Paffenhöfer, G. A. 1970. Cultivation of Calanus helgolandicus under controlled conditions. Helgoländer wissenschaftliche Meeresuntersuchungen 20: 346-359.

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

Raymont, J. E. G. and F. Gross. 1942. On the feeding and breeding of Calanus finmarchicus under laboratory conditions. Proceedings of the Royal Socity of Edinburgh 61: 267-287.

Rüsch, M. E. 1958. Weibliche Heterogametie bei Cyclops viridis Jurine. Die Naturwissenschaften 45: 69.

Seiler, J. 1920. Geschlectschromosomenuntersuchungen an Psychiden. I. Experimentelle Beeinflussung der Geschlectsbestimmenden Reifeteilungen bei Talaeporia tubulosa. Archiv für Zellforschung 15: 249-268.

Shaw, R. F. 1958. The theoretical genetics of the sex ratio. Genetics 43:149-163.

Shaw, R. F. and J. D. Mohler. 1953. The selective significance of the sex ratio. American Naturalist 87: 337-342.

Smyly, W. J. P. 1970. Observations on rate of development, longevity and fecundity of Acanthocyclops viridis (Jurine) (Copepoda, Cyclopoida) in relation to type of prey. Crustaceana 18: 21-36.

Spieth, P. T. 1974. Theoretical considerations of unequal sex ratios. American Naturalist 108: 837-849.

Takeda, N. 1950. Experimental studies on the effect of external agencies on the sexuality of a marine copepod. Physiological Zoology 23(4): 288-301.

Tate, M. W. and R. C. Clelland. 1957. Nonparametric and shortcut statistics. Danville, Interstate. 171 p.

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

Verner, J. 1965. Selection for sex ratio. American Naturalist 99: 419-421.

Wilson, M. S. and H. C. Yeatman. 1959. Free-living copepoda. In: Freshwater Biology, ed. by W. T. Edmondson. Wiley and Sons, New York. 1248 p.

Woodhead, P. M. J. and J. D. Riley. 1959. Separation of sexes of Calanus finmarchicus (Gunnerus) in the fifth copepodite stage, with comments on the sex ratio and duration in this stage. Journal du Conseil, Conseil Permanent International pour l'Exploration de la Mer 24(3): 465-471.