

only one generation--had they been followed through subsequent generations, an intermediate level of relatedness (such as between poolmates) may have been shown to produce optimal fitness. These results do, however, show significant costs of inbreeding. Laboratory mate choice did not reflect the fitness differences among matings; mates were chosen at random with respect to relationship. This apparently non-optimal behavior is evaluated. It is suggested that males may not take the time or be able to assess the relationship of available females. There is probably a potential scarcity of virgin females as a result of the fact that females mate only once but males can mate more than once. Such a scarcity may make it more important for males to guard any available immature female rather than choose an adult female of the ideal relatedness.

An Experimental Test of Optimal Outbreeding
in the Harpacticoid Copepod
Tigriopus californicus (Baker)

by

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An Experimental Test of Optimal Outbreeding in the
Harpacticoid Copepod Tigriopus californicus (Baker)

INTRODUCTION

How should animals choose mates? This question is interesting to evolutionary biologists because it is intimately related to questions of animal behavior, genetic structure of populations, and selective pressures on animals. Some authors (i.e. Bateson 1983, Shields 1982) have hypothesized that there is an optimal level of inbreeding/outbreeding: Animals should choose mates that maximize their fitness. To test this hypothesis, one must (1) determine a level of relatedness in a mating that results in highest fitness (as opposed to the null hypothesis: relatedness of mates does not affect fitness) and (2) test for mate choice that reflects this level. The level of relatedness in a mating that results in highest fitness (or highest fitness mating, referred to as 'HFM' from here on) is dependent upon various selective pressures including the costs of inbreeding and the costs of outbreeding. Inbreeding is the mating of individuals that share many genes that are identical by descent; outbreeding is the mating of individuals that share few genes that are identical by descent. The HFM balances any costs associated with inbreeding and with outbreeding.

Previous studies have examined either various aspects of HFM

(such as inbreeding depression or outbreeding losses) or preferences in mate selection. None has investigated both. However, to properly probe optimal outbreeding, both facets must be examined. In this study, I investigate whether a harpacticoid copepod, Tigriopus californicus has optimal outbreeding. More specifically, I attempt to (1) determine a HFM for this organism and (2) test whether mate choice reflects this HFM.

I first review the literature on costs of inbreeding and of outbreeding, then on tests of mate choice in which HFM is assumed but not determined. I discuss characteristics of systems in which optimal outbreeding can be tested and present my system.

The costs of inbreeding have been examined in a wide variety of organisms: Drosophila (Dobzhansky 1948, 1950, Hyde 1924, Maynard Smith 1956, Mukai and Yamaguchi 1974, Ohta 1980), Japanese quail (Sittmann, et.al. 1966), the great tit (Bulmer 1973, Greenwood, et.al. 1978, van Noorwijk 1981), mice (Connor and Bellucci 1979, Festing 1976), voles (Hill 1974), prairie dogs (Hoogland 1982), large primates (Packer 1979), and humans (Schull, et.al. 1970). Inbreeding does not measurably decrease fitness in all cases. For example, land snails (Selander and Hudson 1976), white-crowned sparrows, (Petrinovich and Baptista 1984), and albino rats (King 1918a, 1918b, 1919) show little or no reduced fitness due to inbreeding.

Inbreeding results in an increased level of homozygosity (Falconer 1981). The potential costs associated with this increased homozygosity are (1) the unmasking of detrimental

recessive alleles, (2) decreased variability, which may be important on an evolutionary scale, (Wright 1932), (3) decreased heterosis (Maynard Smith 1978a), and (4) potentially decreased variation among sibs, which could lead to increased competition among them (Bateson 1983).

Although outbreeding costs have not been investigated in as many organisms, they have been suggested for plants (Jain 1976, Levin 1983, Price and Waser 1979), slow-moving, obligate hermaphrodites that live in low densities (Ghiselin 1969), birds (Nottebohm 1972) and Drosophila (Anderson 1969, Templeton 1979, Templeton, et.al. 1976, Vetukhiv 1956, 1957). These studies may suggest that the potential costs of outbreeding could be as detrimental as those of inbreeding.

Outbreeding costs fall into three general categories. (1) Energetic costs may include the cost of producing dispersal attractants, such as petals and scents in plants (Solbrig 1979), or the cost of movement in animals (Bateson 1983). (2) Decreased probability of survival may result from risks encountered during dispersal or from risks during mate selection, particularly if mates are rare. (3) Genetic costs may be due to the loss of inclusive fitness benefit (Cowan 1979, Smith 1979) or to the break-up of adaptive gene groups (Bateson 1983, reviewed extensively by Shields 1982, Solbrig 1979, Templeton, et.al. 1976). Inclusive fitness is increased by mating with related individuals. Adaptive gene groups are groups of genes that evolved in specific habitats and promote highest fitness when intact.

Mating with an individual that shares the same adaptive gene complex preserves the group of genes.

Some theoretical models have been constructed to describe a balance between costs of inbreeding and of outbreeding. Wright (1932) suggests that with some inbreeding, each race within a population has its own 'adaptive peak'; limited outbreeding draws all races "under the influence" of the highest peak. Bengtsson (1978) creates a model balancing the cost of outbreeding (associated with migration) and the cost of inbreeding. Models such as these are helpful in attempts to quantify relative costs of inbreeding and of outbreeding.

Mate preference has been investigated in a few animals in which a HFM was assumed. Farr (1977) found that female guppies preferred phenotypically rare males as mates. Slater and Clements (1981) suggest that zebra finches would choose mates of intermediate relatedness. Gilder and Slater (1978) demonstrate that mice prefer individuals of an intermediate level of relatedness (i.e. nonsibs from the same strain) over siblings and individuals from different strains. Most frequently cited are Bateson's (1978, 1980, 1982, 1983) studies with Japanese quail. He believes that these birds would prefer to mate with intermediately novel quail or first cousins over siblings, third cousins, and unrelated individuals. In all of these cases HFM was assumed but not tested, i.e., the degree of relatedness was known but the resulting fitness correlates of the observed mate choice were not measured. To examine optimal inbreeding/outbreeding, both (1) HFM

and (2) mate choice must be measured.

The concept of ideal mate choice is based upon optimality theory. Since this theory has recently come under serious scrutiny, its basic assumptions warrant close examination. Optimality theory has been applied to a wide variety of areas in ecology and evolutionary biology including territoriality (Hixon 1980), foraging behavior (Krebs and McCleery 1984, Pyke 1984, Pyke, et.al. 1977, Waddington 1983), group size (Pulliam and Caraco 1984), sex ratios (i.e. Fisher 1930, Hamilton 1967), structural design (i.e. Maynard Smith and Savage 1956), and mating behavior (i.e. Parker 1974, Wickler and Seibt 1981). General assumptions of and problems with this theory as well as how they apply to my system will be reviewed in the discussion.

Systems in which an optimal level of outbreeding could be tested must have a HFM that can be determined and mate selection that can be observed. Tigriopus californicus (Class Crustacea, Subclass Copepoda, Order Harpacticoida) is an ideal organism for a test of optimal outbreeding. This species is extremely easy to culture in the laboratory and has a rapid life cycle with readily recognized stages and sexes. Furthermore, there are a priori reasons to believe that there are costs to both inbreeding and to outbreeding in Tigriopus. These four characteristics are elaborated below.

Tigriopus californicus is simple to culture because it is uniquely tolerant of many stresses. This copepod has been active at temperatures ranging from 42°C (Ranade 1957) to 0°C (pers.

obs.) and can withstand salinities ranging from 90‰ (Ranade 1957) to 0‰ (pers. obs.). Tigriopus have a high resistance to ultraviolet-B radiation (Chalker 1981), probably due to its carotenoid pigments (Hairston 1979). Tigriopus can be voracious feeders with individuals consuming up to 2.4×10^3 Platymonas subcordiformis (a unicellular green alga, Class Prasinophyceae) in 48 hours (Lear and Oppenheimer 1962). Yet individuals have a remarkably high efficiency of nitrogen assimilation (Harris 1973) and have lived up to two years in sealed jars (Fraser 1936).

Tigriopus may tolerate these stresses because they are adapted to an extreme habitat. The genus appears to be restricted to rocky tidepools above the high tide line throughout its range. Tigriopus is found in western Europe (T. brevicornis and T. fulvus) and on the eastern shores of the Pacific (T. californicus), north to Torch Bay, Alaska (Dethier 1980) and south to Baja California (Morris, et.al. 1980). These species differ in only a few morphological characters (Monk 1941) and may, in fact, not be separate species. The pools in which Tigriopus live are subject to extreme fluctuations in both biotic and abiotic parameters. Predators may restrict Tigriopus to this habitat. Dethier (1980) demonstrated that tidepool sculpins (Oligocottis maculosus) and anemones (Anthopleura elegantissima) eat Tigriopus when these predators are transplanted from lower to higher pools. Physical factors may also restrict these animals to high pools. Wave splash in lower pools may be too frequent or too harsh to allow Tigriopus populations to become established.

The life cycle of Tigriopus has been well documented (Egloff 1967, Fraser 1936, Morris, et.al. 1980, Vittor 1971). A female mates only once, probably immediately after her final molt (Ward 1984a, Williams 1907). The male attaches to the female prior to her final molt--that is, while she is still immature. He clasps her using his antennal hooks, which are located on the first pair of antennae (Figure 1). At copulation, the male transfers a spermatophore, which contains two types of sperm, to the opening of the female receptacle (Heberer 1955). From this single mating, the female produces 9-12 broods, each with 20-140 eggs. The size of the brood is dependent upon food level, density, and size of the female (Vittor 1971). The mother carries the egg sac in her brood pouch; at hatching, the nauplii swim out directly from the sac. The nauplia then pass through six naupliar stages and six copepidite stages, molting between each (Figure 2). After the final molt, the individual is sexually mature. Individuals are not sexually differentiated until the late copepidite stages. Sex of an individual may be environmentally determined (Egloff 1967, Vacquier 1962, Vacquier and Belser 1965, Vittor 1971). The total time for development from hatching to adult is temperature dependent--4 weeks at 15°C and only 2 weeks at 23°C.

There is reason to believe that there are costs to inbreeding in Tigriopus. Battaglia (1970) found inbreeding depression in harpacticoid copepods of the genus Tisbe. As the coefficient of inbreeding was increased from 0 (assumed) to .375 in laboratory populations, the total number of offspring produced dropped more

Legends for Figure 1 and Figure 2

Figure 1. Tigriopus californicus body parts (50x)

- a) Male first antennae, showing antennal hooks
- b) Female body, showing labelled parts of body

Figure 2. Tigriopus californicus life cycle (40x)

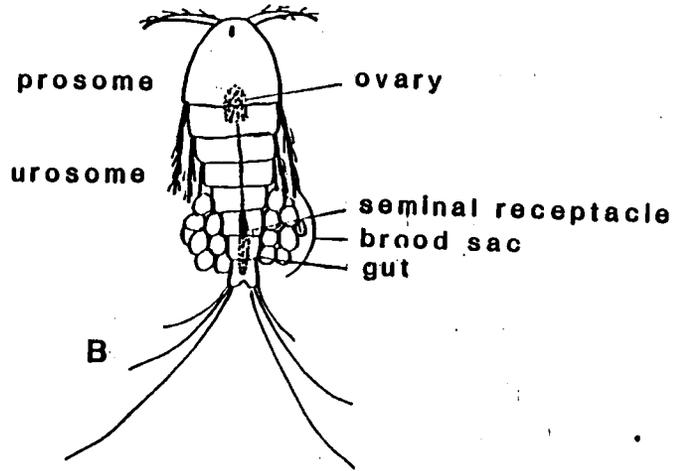


Figure 1.

50x

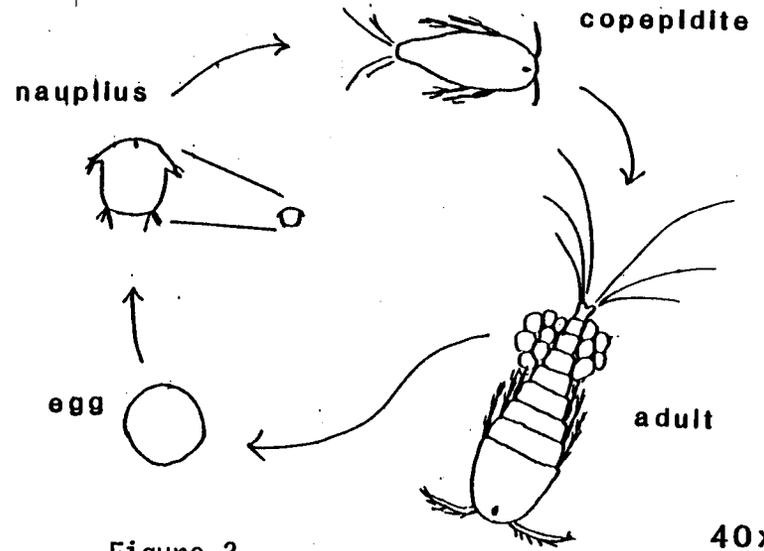


Figure 2.

40x

than 60%. Furthermore, the frequency of survival dropped more than 50% and the net reproductive rate decreased over 90%. Because Tisbe is a closely related genus that lives in an environment similar to that of Tigriopus, it is reasonable to suggest that Tigriopus has similar costs to inbreeding.

There is also reason to believe that there are costs to outbreeding in Tigriopus. I predict that genetic composition of populations varies among distant pools due to (1) high variation in physical and biological parameters among pools and (2) low dispersal of individuals among distant pools. Field studies were performed to measure the degree of variation among pools. Tigriopus disperse among pools probably during high wave splash, on the legs of the shore crab, Pachygrapsus (Egloff 1967), or on streams of water between tidepools, especially those carrying Tigriopus-scented water (Burnett, pers. comm., Cooper 1977). Burton and Feldman (1981) studied genetic composition of Tigriopus pool populations using electrophoresis. They found that genotype frequencies at the loci examined were similar among pools on the same outcrop but different among pools from adjacent outcrops. A later study (Burton and Swisher 1984) provides evidence for high dispersal of Tigriopus among pools on any outcrop but little dispersal between outcrops. Tigriopus with known allele frequencies transplanted to some pools on an outcrop were rapidly dispersed over all pools on that outcrop. Allele frequencies in pools within an outcrop remained constant over time (four years). Burton and Feldman (1982, 1983) suggest that because (1) the amino

acid levels regulated by some of the alleles that differ in frequency among populations are important in osmotic regulation and (2) different pools have varying osmotic fluctuations, these different allele frequencies may represent adaptations to the different microhabitats. Therefore, I predict that matings between individuals from distantly separated pools will result in decreased fitness due to loss of adaptive alleles and break-up of potential adaptive gene complexes.

This background information coupled with the possibility of (1) measuring HFM by counting the number of offspring produced by matings of differently related individuals and (2) determining mate choice make Tigriopus californicus a good system to evaluate hypotheses concerning optimal inbreeding/outbreeding.

METHODS

Field Studies

Field studies were designed to measure environmental heterogeneity among pools. The study site is a basalt point south of Yaquina Head, located 5 km north of Newport, Oregon. Twenty-two tidepools containing Tigriopus were marked and subsequently monitored biweekly from February to May, 1985. The pools are scattered over a vertical range from approximately 3 m to 9 m above mean lower low water and a horizontal range of approximately 75 m. Relative density of Tigriopus (absent, low, medium, or high) and presence of co-occurring macroscopic biota were estimated visually. More accurate density measurements were not possible because of the extremely patchy distribution of animals within any tidepool. Pool volume was approximated by length, width, and depth measurements on the first and last monitoring dates. Temperature was measured at the top and bottom of each pool. Salinity was estimated with a Baume and specific gravity hydrometer (VWR Scientific).

Laboratory Studies

In the lab, I (1) investigated whether a specific level of relatedness in a mating results in highest fitness and (2) determined whether mate preference reflects this HFM. Animals were collected from six of the twenty-two tidepools at Yaquina Head that differed in biotic and abiotic parameters. There should be a very low level of dispersal among these particular pools because they were chosen to be separated by areas of dry, rugged basalt,

were almost always above the wave splash zone, and were not adjacent to any crab-occupied crevices. The Tigriopus populations were maintained in 0.3 l glass jars in an incubator at 20°C and 16:8 L:D and were fed a mixture of nutritional yeast and powdered Tetramin[®] flaked fish food dissolved in sea water. After two weeks equilibration time, twenty-four gravid females from each population were isolated in 2 ml clear plastic culture chambers. The offspring from the first eight females of each population to release nauplii were isolated at early copepodite stages. Early isolation of the young prevented any unexpected matings.

(1) Test of HFM

Females were mated with siblings, poolmates, or non-poolmates. It was assumed that these represent different levels of relatedness in mates. Poolmates may have been as closely related as half-sibs (sharing the same father) or not related at all. Likewise, non-poolmates may have been closely related; though, due to the distance between some of these six pools, this is highly unlikely. I assume that, on average, level of relatedness is sibling > poolmate > non-poolmate. Forty females were used in each cross. All Tigriopus in this test were virgin and approximately the same size and age.

Females were kept in 2 ml chambers with their mates for 9 days after mating was first observed to insure insemination. The gravid females were then transferred to 50 ml vials with abundant food (there was always excess food on the bottom of the vial). Female Tigriopus eat their young when densities are high (Egloff 1967, pers. obs.); the large vials and large food supply were used

to help minimize cannibalism. Three weeks (approximately one generation span) after mating, the number of offspring in each vial was counted. This value is used as an index of fitness. A more accurate measure of fitness would be achieved by counting each female's total number of offspring that survive to reproduce. I could not use this method due to constraints in money, time, and space. However, the index I do use should be robust for a comparative study such as this.

(2) Mate Preference Tests

In mate choice tests, a female and two males of different relatedness to the female were placed in a 2 ml chamber. All animals were virgin adults of approximately the same size and age. The pair of males used in each test was either sibling + poolmate, sibling + non-poolmate, or poolmate + non-poolmate. The numbers of replicate matings were 40, 57, and 40, respectively. When this study was designed, I assumed that females choose mates because females mate only once, whereas males may mate more than once; thus, there may be stronger selective pressures on females to choose mates than vice versa. Because males clasp females, males also probably make mate choices. My design--one female and two males--allows for mate choice by both sexes. To allow me to distinguish between the two males in each test, carmine powder was mixed with the food of half of the males 24 hours before testing, coloring their guts. Behavior did not differ between stained and unstained individuals. The male that first mated in each test was recorded. Pumping of the male urosome over the female receptacle indicated mating.

RESULTS

Field Studies

Both density of Tigriopus and number of taxa differed markedly among pools. The density of Tigriopus varied among pools at any sampling date and within any pool over time (Figure 3). Five pools lost all Tigriopus by mid-May. These pools may contain populations during the winter months only, when pool water is replenished by rain water and storm wave splash. Pool N was dry April 30 but had a low density of Tigriopus May 15. I have found individuals surviving brief dry periods in moist crevices or wet sand in other pools and suspect that this occurred in pool N. No pool maintained high densities throughout the monitoring period. This may be due to occasional population 'blooms', dispersal among pools, or the cryptic behavior of Tigriopus on hot days.

The number of macroscopic taxa (including Tigriopus) inhabiting pools was less variable; some pools had the same number of taxa present for the entire period (Figure 4). The exception was pool Z, in which number of taxa varied from two to six. The most common co-occurring biota were green algae (Enteromorpha), midge larvae, and ostracods. Less frequently observed biota were amphipods, mussels (Mytilus), periwinkles (Littorina), limpets (Collisella), and other seaweeds (i.e. Leathesia).

Abiotic parameters in these pools showed substantial variation, both among pools on the same date and within any pool over time. The volume of the tidepools ranged from $>50 \times 10^3$ cm to $<4 \times 10^3$ cm in early February and from $>50 \times 10^3$ cm to completely dry in mid-May (Figure 5). Tidepool dimensions longer than 100 cm

Legend for Figure 3

Figure 3.
Density of Tigriopus in tidepools

Height of bar represents relative density of animals:

 = high  = medium  = low

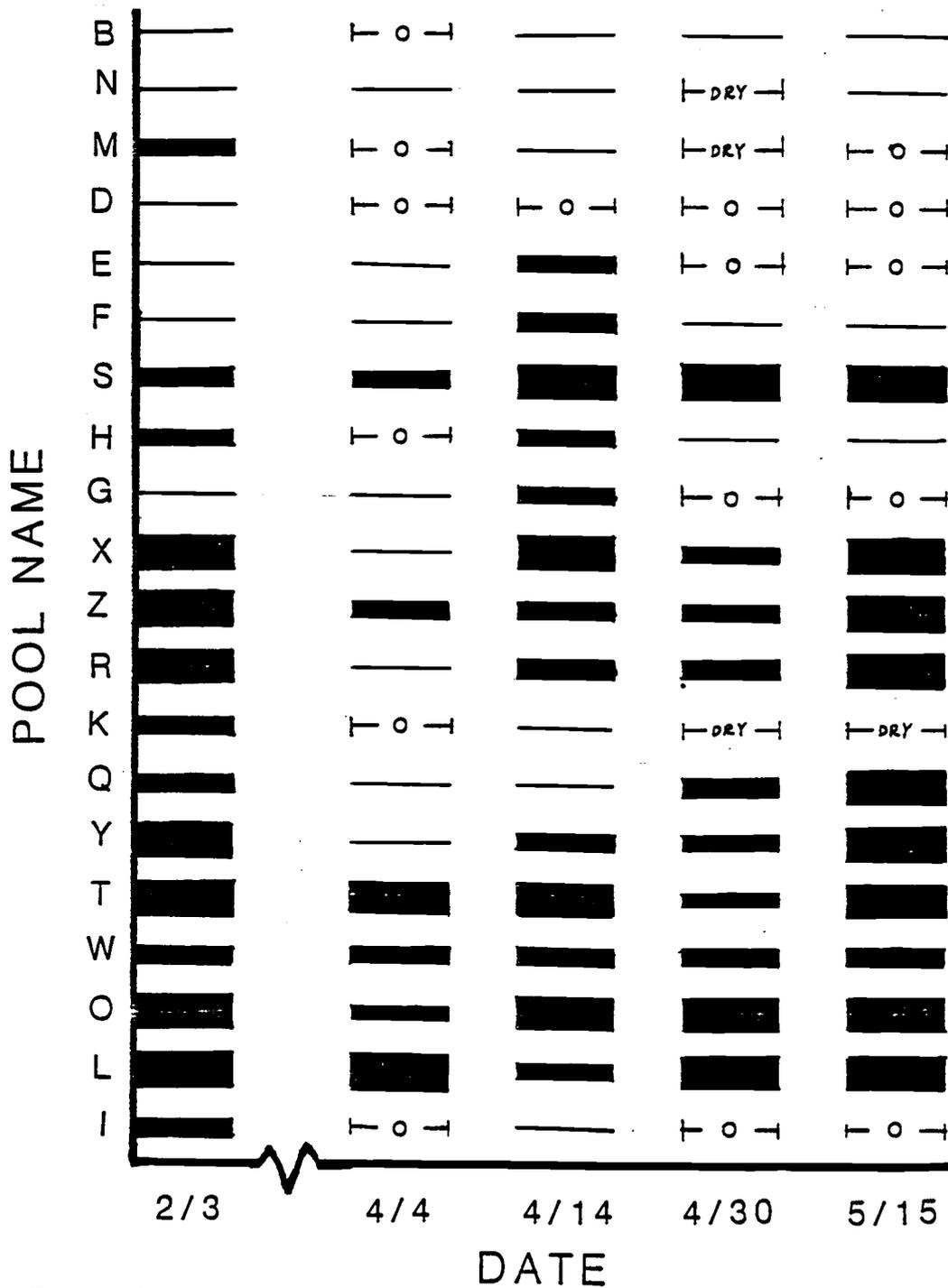


Figure 3.

Legend for Figure 4

Figure 4. Macroscopic taxa in tidepools

Sampling dates: 1 = February 3, 1985
 2 = April 4, 1985
 3 = April 16, 1985
 4 = April 30, 1985

Taxa legend:

	= <u>Tigriopus</u>		= <u>Enteromorpha</u>		= ostracods
	= midge larvae		= amphipods		= <u>Mytilus</u>
	= <u>Collisella</u>		= <u>Leathesia</u>		= <u>Littorina</u>

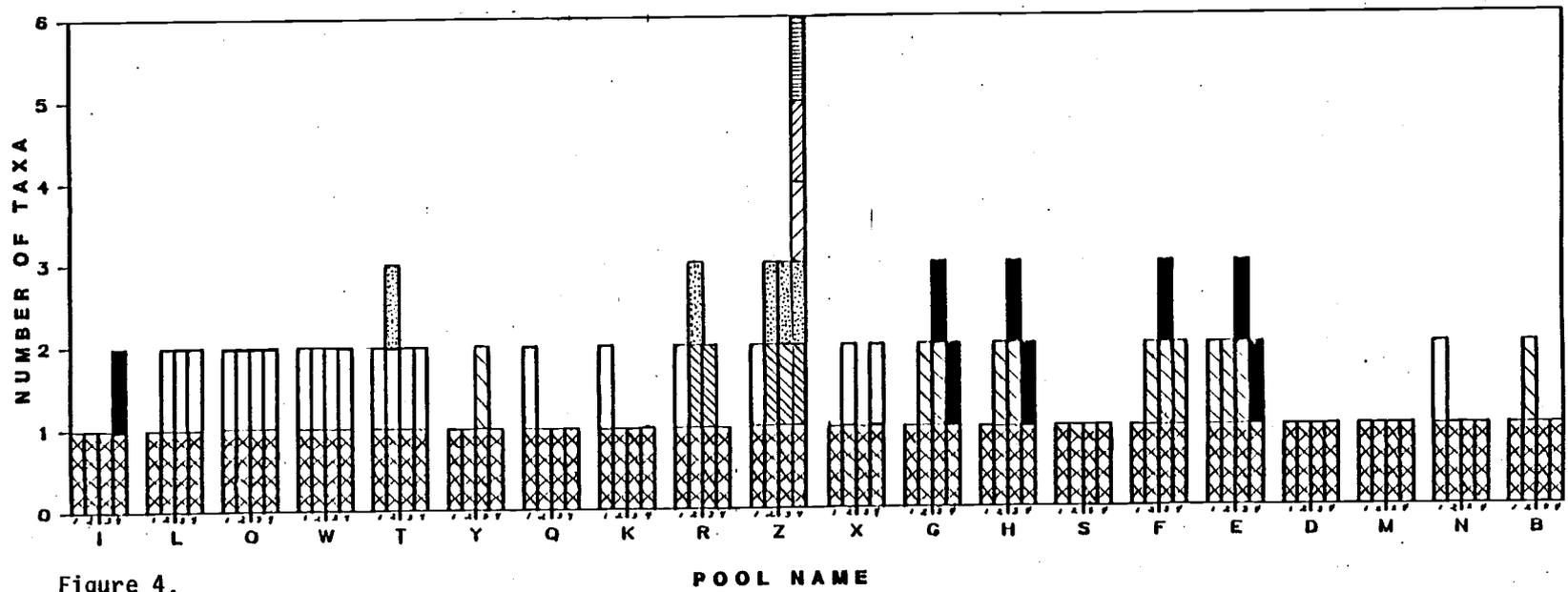


Figure 4.

Legend for Figure 5

Figure 5. Approximate volume of pools

Volume was estimated by length x width x depth.
Lengths and widths greater than 100 cm were estimated;
thus, volumes greater than 50,000 cm³ are not known.

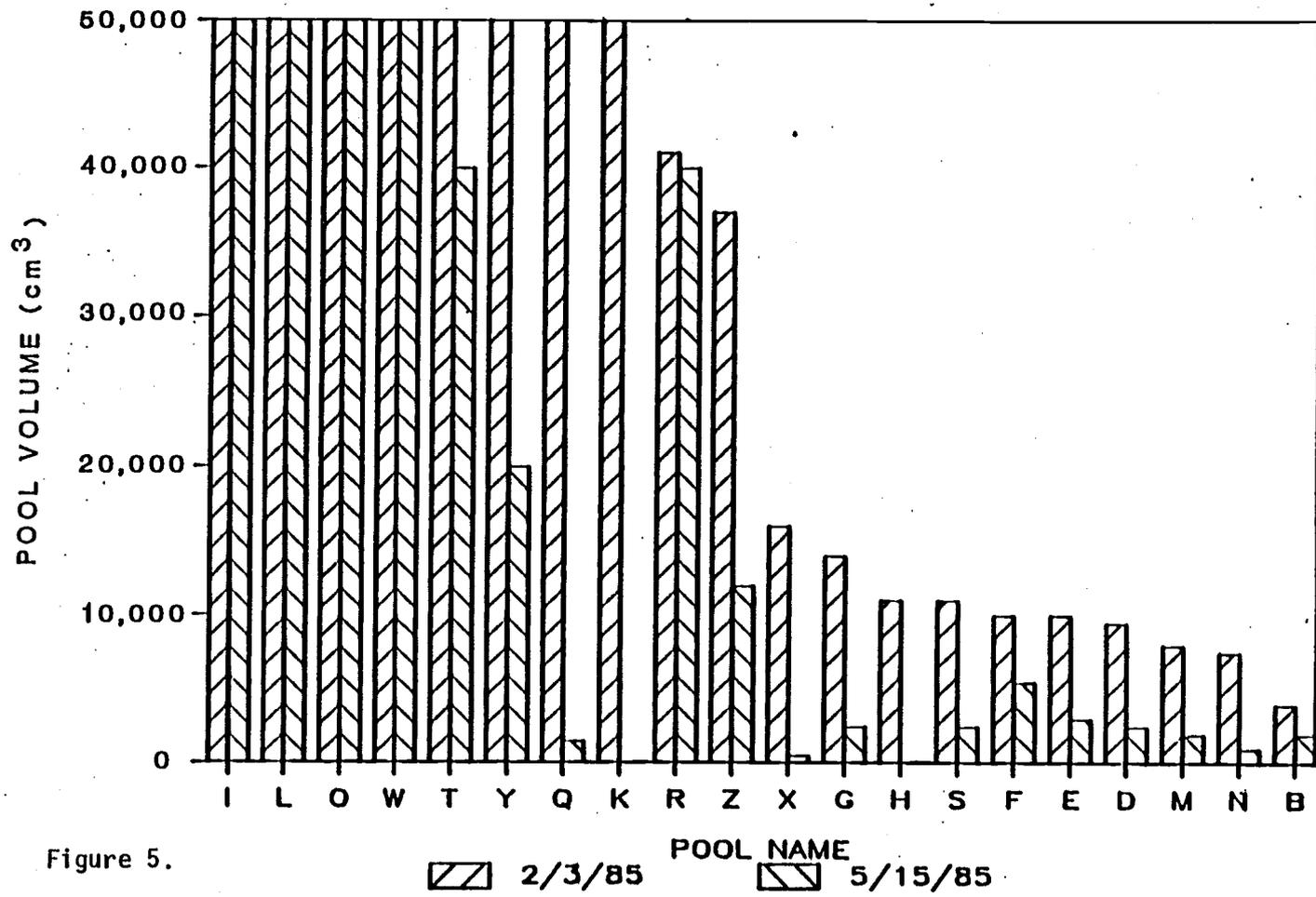


Figure 5.

were approximated; thus, actual volumes greater than $50 \times 10^3 \text{ cm}^3$ are not known. Some pools (i.e. Q, K) dropped in volume by at least $50 \times 10^3 \text{ cm}^3$. Other pools (i.e. R, B) showed very little change in volume. Large, shallow pools in exposed areas tended to change volume more than deep pools in crevices. Pool temperatures often varied greatly, both between and within pools (Figure 6a and 6b). On February 16 (Figure 6a), temperatures among pools ranged from 6°C to 20°C and on three other sampling dates, temperature range was at least 9°C . Temperature range was less on rainy days (March 5 and March 24) than on sunny days. On some days, top and bottom temperatures of pools were the same; for these pools only one point is graphed. Mean:variance ratios of pools (Figure 6b) show that fluctuations within pools differ among the pools monitored. Salinity also fluctuated among and within pools (Figure 7a and 7b). Salinity in most pools at any date (Figure 7a) was between 0% and 30%. Again, rainy days or days following rainy periods were correlated with reduced variation in salinity. Range of fluctuations among pools (Figure 7b) was also great.

Laboratory Studies

(1) Test of HFM (Highest Fitness Mating)

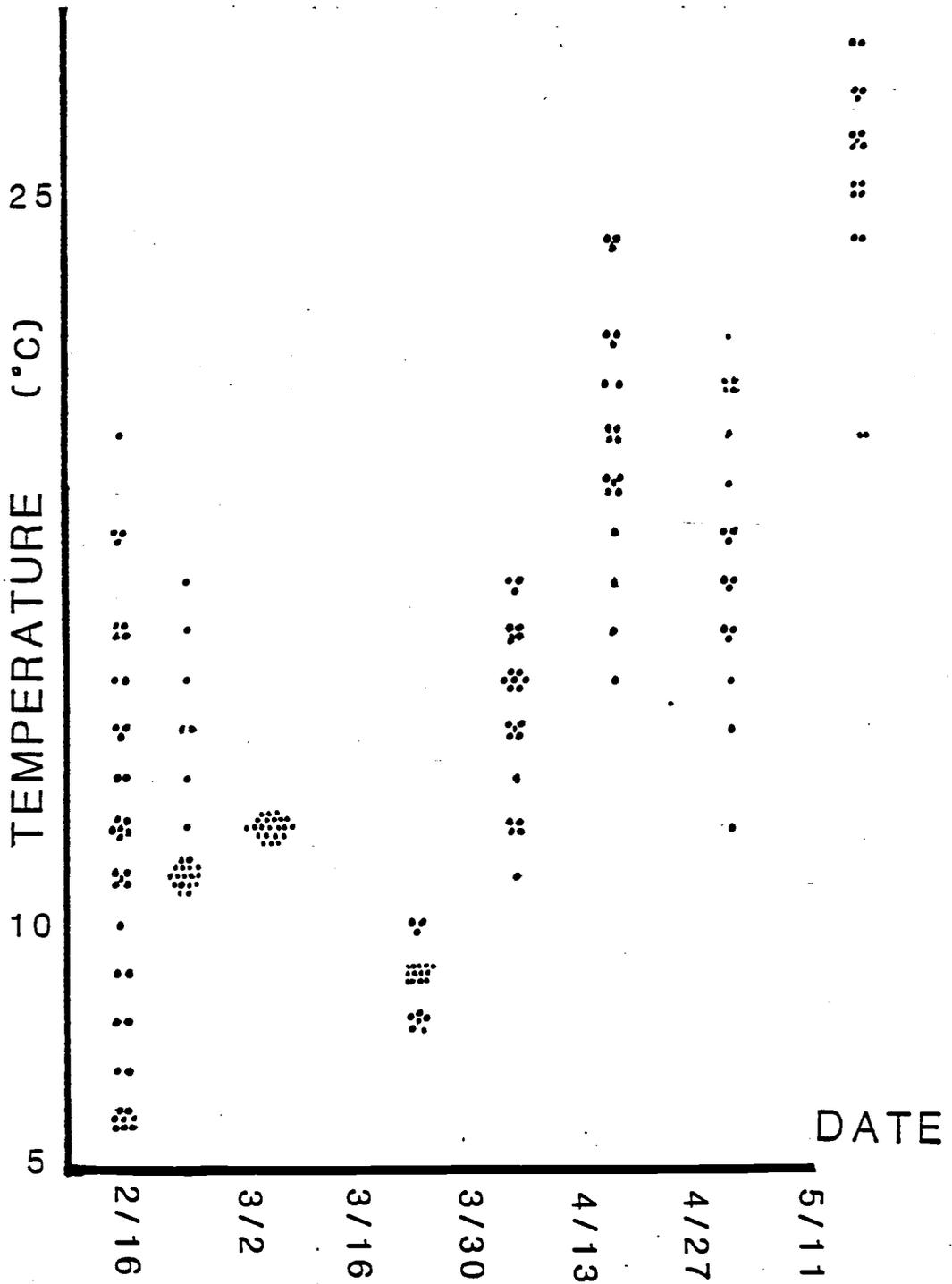
Tigriopus appears to have a HFM (Figure 8): there is a significant difference among numbers of offspring produced by sibling, poolmate, and non-poolmate matings (single classification ANOVA, $F=3.23$, $p<.05$; Sokal and Rohlf 1981). Females mated with poolmates produced significantly more offspring than did females mated with siblings (protected LSD; $t=.05$, $df=99$; Snedecor and Cochran 1980). There is also a strong trend toward more offspring

Legend for Figure 6

Figure 6. Temperatures of pools

- * a) Temperature range among pools at eight sampling dates
- b) Mean:variance ratios of each pool. This value is an index of fluctuation of temperatures within any pool over time.

* See note on page 23A



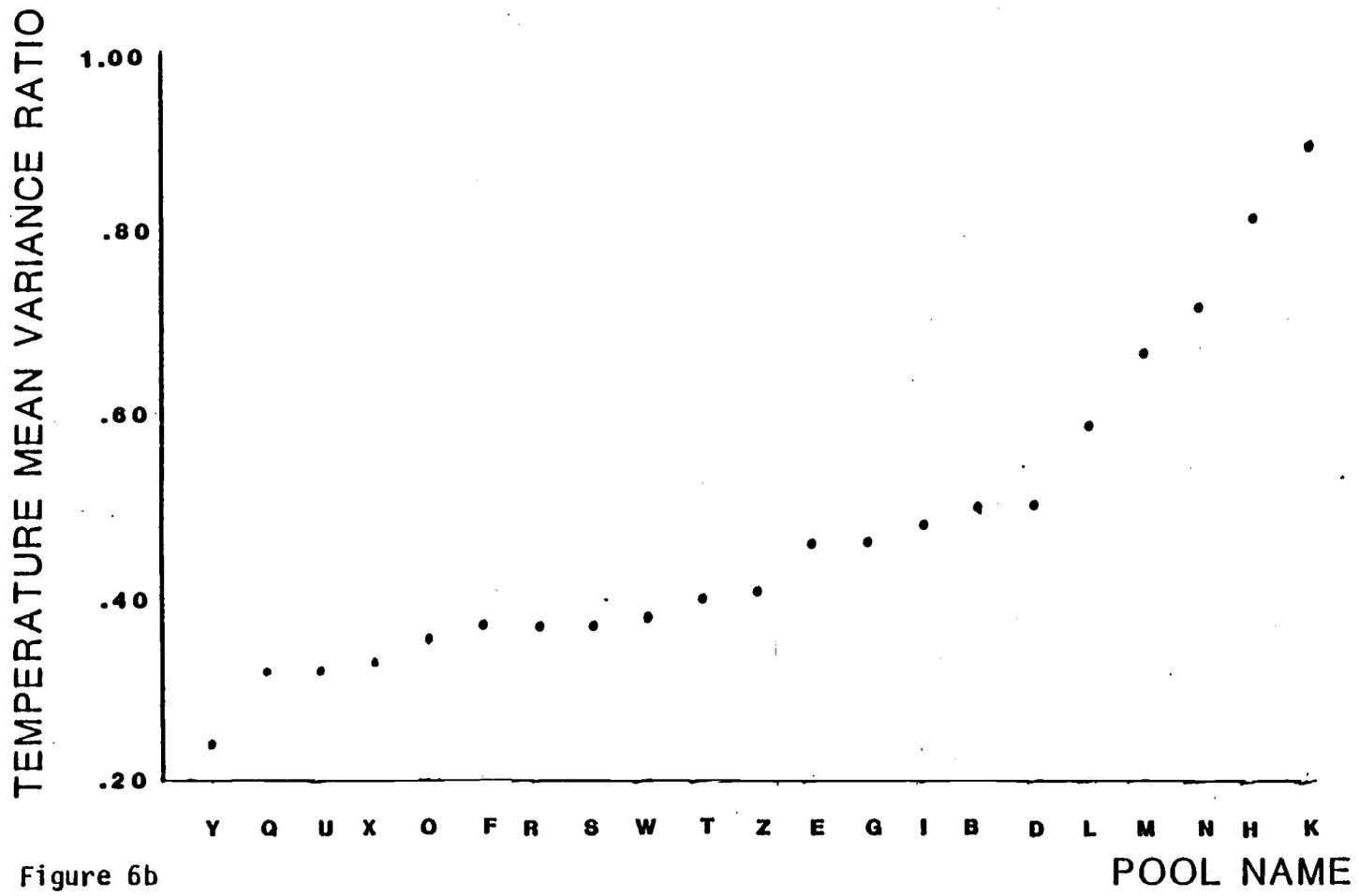


Figure 6b

Legend for Figure 7

Figure 7. Salinities of pools (ppt)

- *a) Salinity range among pools at eight sampling dates
- b) Mean:variance ratios of each pool. This value is an index of fluctuation of salinity within any pool over time.

* In both Figure 6a and Figure 7a, each point represents one reading of temperature or salinity. On each sampling date, readings were taken at both the top and bottom of each pool. When these measurements differed, both values were recorded. When top and bottom values were the same, only one value per pool was recorded.

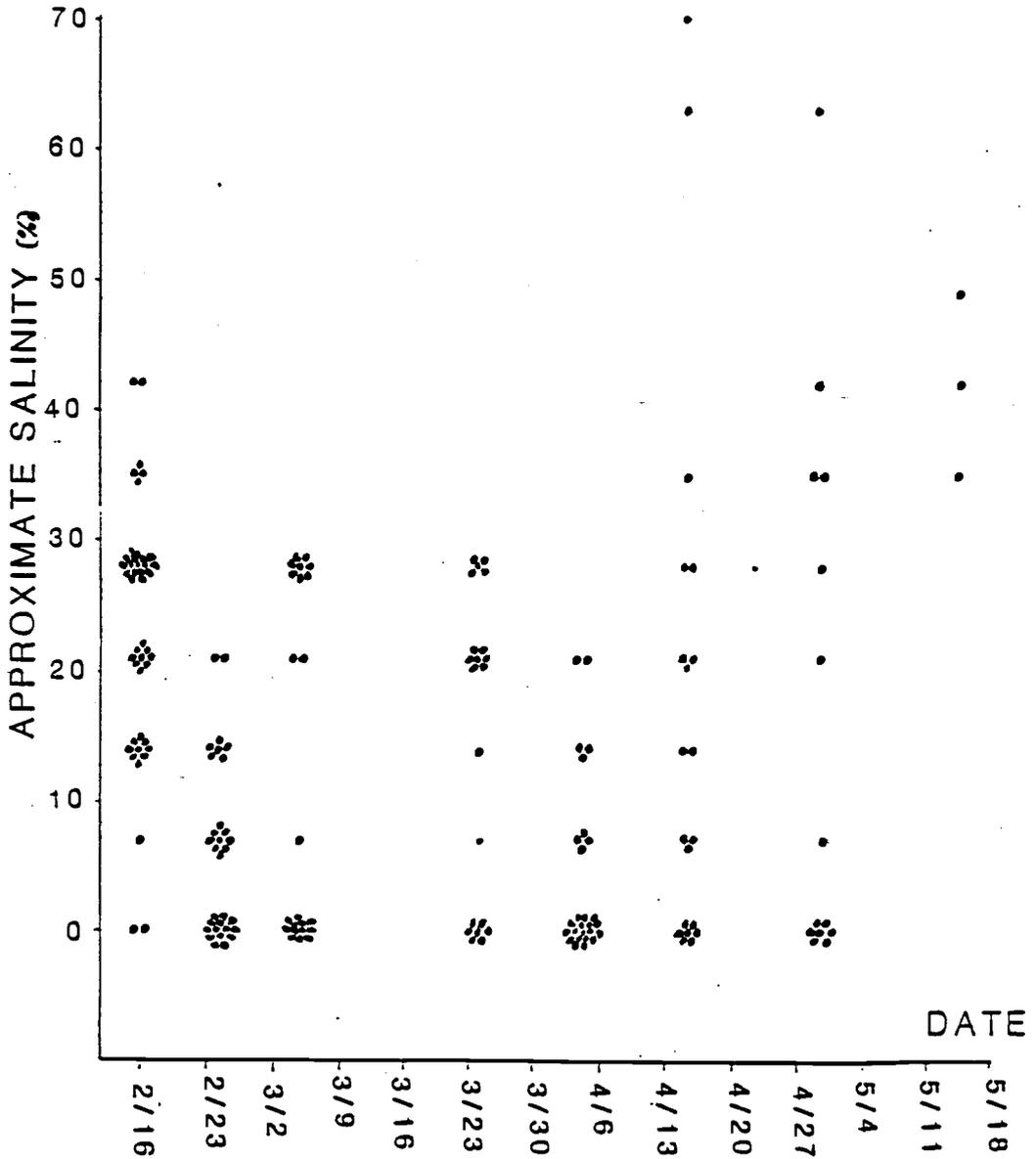


Figure 7a.

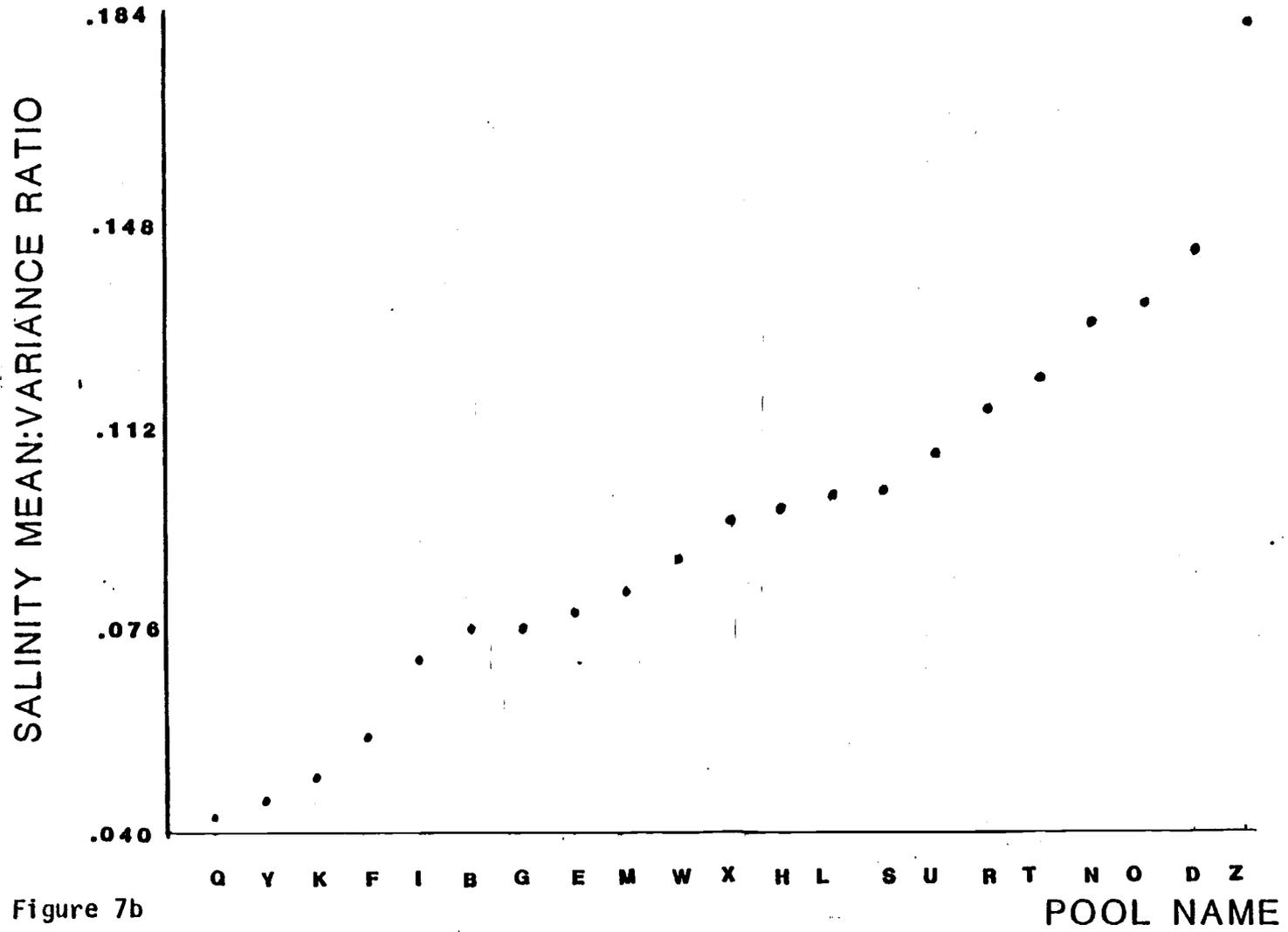


Figure 7b

Legend for Figure 8

Figure 8. Effect of mate relationship on number of offspring produced by each female

Bars represent one standard error.

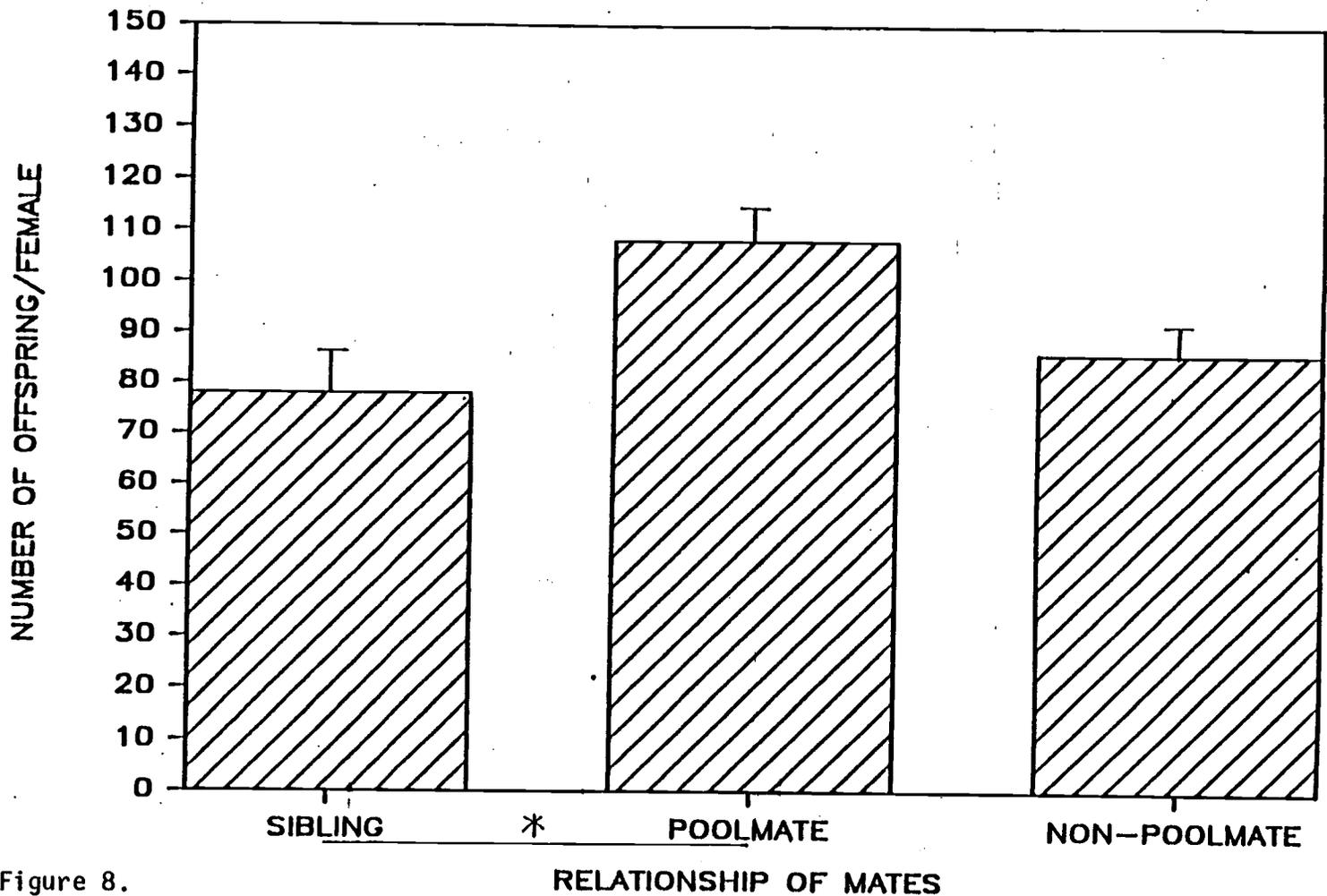


Figure 8.

produced by poolmate matings than by non-poolmate matings ($p=.07$). Females produced a mean number of 78.87 offspring when mated with a sibling ($N=30$), 107.54 offspring when mated with a poolmate ($N=37$), and 86.57 offspring when mated with a non-poolmate ($N=35$). The original sample size ($N=40$ for each test) was reduced due to handling accidents by humans.

(2) Mate Preference Test

Mate preference, however, does not appear to reflect this HFM (Table 1). The null hypothesis that mating occurs at random with respect to relationship cannot be rejected (χ^2 test, $p < .05$ in all cases). Staining did not have a significant effect on mate choice (χ^2 test, $p < .05$ in all cases). A preference of 2:1 would be needed in this particular experiment for the deviation from random to be significant.

Results of Mate Choice Tests

Female ChoiceRelation EffectStain Effect

1. Sibling and poolmate

	sibling	poolmate	Σ
stained	13	13	26
unstained	9	5	14
Σ	22	18	40

$$\chi^2 = .40$$

$$p > .5$$

$$\chi^2 = 3.6$$

$$p > .05$$

2. Sibling and non-poolmate

	sibling	non-poolmate	Σ
stained	15	18	33
unstained	13	11	24
Σ	28	29	57

$$\chi^2 = .02$$

$$p > .9$$

$$\chi^2 = 1.4$$

$$p > .05$$

3. Poolmate and non-poolmate

	poolmate	non-poolmate	Σ
stained	12	11	23
unstained	10	7	17
Σ	22	18	40

$$\chi^2 = .40$$

$$p > .5$$

$$\chi^2 = 0.9$$

$$p > .05$$

Table 1.

DISCUSSION

The results suggest that Tigriopus californicus has costs of inbreeding and perhaps costs of outbreeding as well. Fewer offspring are produced by matings between siblings than by matings between poolmates. Poolmate crosses presumably represent an intermediate level of relatedness in which combined costs of inbreeding and of outbreeding are minimized. Poolmate matings may even result in a maximum number of offspring produced. In evaluating laboratory studies, it is important to remember that the laboratory environment is different from the natural habitat, and thus the number of offspring produced or behaviors observed may not exactly reflect actual or 'natural world' values. In spite of this possible shortcoming, this study still provides more insight into optimal mating behavior than previous studies.

Although significant effects of inbreeding depression were observed in the F1 generation in this system, costs of inbreeding might be more pronounced in later inbred generations, as has been found in Tisbe (Battaglia 1970). Reduction in the number of offspring produced in the F1 generation reflects reproductive failure at fertilization or in the stages prior to when I counted number of offspring. A decreased number of offspring produced in subsequent inbred generations might reflect these fertilization or developmental problems in addition to other factors, such as decreased mating success, reduced production of viable eggs or sperm, or decreased probability of survival to adult. Thus, the

costs of inbreeding in Tigriopus revealed by this study are most likely quite conservative--actual costs are may be much greater.

One likely cost of outbreeding in Tigriopus may be due to the break-up of adaptive gene complexes. There are two reasons to believe that individuals from different tidepools may have different groups of adaptive genes. First, Burton and Feldman (1981) showed that genotype frequencies of Tigriopus populations differ among separated pools and are constant over time within a pool. The Yaquina Head populations, which inhabit an environment similar to that of Burton and Feldman's Tigriopus populations, would be expected to show the same variation. Second, my field studies demonstrate that both biotic and abiotic parameters differ among and within pools. Thus, different pool populations may have different adaptive gene groups. Matings between non-poolmates (individuals that presumably do not share the same gene complexes) would break up the adaptive gene group and therefore reduce fitness. The costs of outbreeding estimated in this study may also be conservative. Vetukhiv (1956) found that matings between Drosophila from geographically separated areas resulted in some heterosis in the F1 generation but a decrease in the number of eggs produced in the F2 generation. Thus, significant outbreeding depression in Tigriopus may not be observed until subsequent outbred generations.

Tigriopus appear not to show optimal outbreeding behavior. There is a presumed level of relatedness in a mating that results in highest fitness. Yet Tigriopus choose mates at random rather

than selecting poolmates, the HFM. How can this non-optimal behavior be explained? Before addressing this question, I review structure and assumptions of optimality theory. Then, I examine how optimality theory can be applied to mating behavior of Tigriopus and propose an explanation for the apparent non-optimal outbreeding.

The general structure of optimality theory is the same in all applications (see Maynard Smith 1978b, Oster and Wilson 1978 for good reviews). The form consists of a system in which individuals behave, optimizing a given currency within a set of constraints. Systems have parameters such as size, age, and level of interactions among individuals (Oster and Wilson 1978, Real 1983) that affect how individuals behave. The currency being optimized is assumed to be correlated with fitness (Maynard Smith 1978b, Waddington 1983). Examples of currencies are energy intake, time usage, or number of offspring produced. The constraints may be in time or space or restrictions on physiological, morphological, or genetic potentials.

Optimization theory may be used to generate predictions about systems (Maynard Smith 1978b); however, this use has been criticized by Oster and Wilson (1978) and Rothstein (1982). These authors suggest that use of optimization theory should be limited to increasing our knowledge about patterns in evolution and organization of systems. A major problem associated with optimality theory is that in order to test models of how organisms behave optimally, we must first assume that they do, in fact,

behave in an optimal manner. Unfortunately, the basic assumption that organisms behave optimally cannot be tested. In light of this problem, I restrict use of optimality theory to helping to understand the mating patterns in Tigriopus.

Optimization theory is based on numerous assumptions (see Maynard Smith 1978b). First, we must assume that the currency chosen for study is actually of primary importance to the organism. We may design a model describing optimal foraging of a honey bee maximizing nectar intake but not be sure that minimizing time away from the hive isn't the more important currency. Second, we must assume that the constraints defined are correct. We may theorize that it is physiologically impossible for the bee to fly faster between flowers when lack of visual acuity in recognizing nectar sources may be an equally important constraint. Third, we must assume that the organism is omniscient. It is very likely that the bee is not able to determine the quality of each flower in the field in order to 'plan' the most efficient route among the best plants. Fourth, we must assume that the behavior is heritable. The foraging patterns of the honey bee may be learned through trial and error and have little heritable genetic components. Finally, we must assume that the behavior we are studying is adaptive. Gould and Lewontin (1979) have criticized strongly this "adaptationist programme". They point out that not all traits are necessarily adaptive, even if we assume that they arose via natural selection. The trait in question may have been adaptive in another environment or in another era but not in the

present habitat. For example, honey bee foraging may have evolved in a colder habitat with scarcer resources. The length of time since habitat change may have not been sufficient for more efficient foraging patterns to have evolved.

To apply optimality theory to mating systems of Tigriopus, I assume that the currency being optimized is number of offspring produced. Because there is a trend toward maximum number of offspring produced from poolmate matings, to optimize the currency Tigriopus should choose poolmates as mates. I assume that there are constraints in neither (1) physiological ability to recognize individuals of the ideal level of relatedness nor (2) time to find and choose mates. I assume that Tigriopus are able to evaluate all other individuals in an area and compute the ideal mate given the relationship of all potential mates. Moreover, I assume that this ability to choose is heritable. Finally, I must assume that the behavior observed is adaptive. In stating this assumption, I am claiming that any observed non-optimal behavior is an indication that my assumptions are false rather than that Tigriopus actually behave non-optimally.

To evaluate this optimization argument, all assumptions must be examined (see Maynard Smith 1978b). The assumption that maximizing the number of offspring produced is the important currency is valid if this is a good index of fitness. Although number of offspring produced in three weeks is not a perfect measure of fitness, I believe that it is the most robust index that I can calculate, as discussed earlier.

The constraints (recognition ability and time) that I assume do not affect mating behavior have been investigated in other arthropods. Sweat bees are able to recognize nestmates (Buckle and Greenberg 1981, Greenberg 1979), Hemilepistus (Isopoda) can recognize offspring (Linsenmair 1972), and Gonodactylus (Stomatopoda) can recognize dominant individuals (Caldwell 1979). Linsenmair (1985) suggests that this recognition may have evolved so that individuals could choose mates that were neither too closely related nor too distantly unrelated. Tigriopus have demonstrated some ability to recognize conspecifics. Migrating individuals can detect water that contained other Tigriopus (Burnett, pers. comm., Cooper 1977). Males are able to distinguish between virgin and non-virgin females--no attempts are made to mate with gravid females. Nonetheless, Tigriopus may not have evolved the ability to recognize individuals to as fine a level as have some other arthropods. Thus, Tigriopus may not be able to distinguish among siblings, poolmates, and non-poolmates.

Time needed to find a mate may be an important constraint if virgin females are a scarce resource. The scarcity of females may be the reason that Tigriopus exhibit precopula behavior. Adult males clasp immature females prior to their final molt; females are not released until after insemination. This behavior has been observed in other crustacea such as amphipods (Hartnoll and Smith 1980, Ward 1984b) and isopods (Manning 1975, Thompson and Manning 1981). Precopula behavior (mate guarding) presumably evolves when females are a limiting resource and the costs of guarding mates

are less than the gain in fitness (Manning 1975, Parker 1974, Wickler and Seibt 1981). Males that attempt to find females of the ideal level of relatedness may be at a disadvantage if all other males are guarding immature females.

The final three assumptions cannot be tested. That organisms behave as if omniscient must be assumed in all deterministic optimality arguments. Heritability of optimal mating behavior may be a valid assumption in this animal that has no familial associations after hatching. Finally, the adaptiveness of mate choice is robust (to an adaptationist) because the habitat in which Tigriopus live is probably stable over evolutionary time; the behaviors that evolved in the recent past should still be adaptive today.

Thus, if I assume that Tigriopus behave optimally but my experiment demonstrates that they behave non-optimally, I must re-examine the assumptions of my model. The discrepancy may lie in the assumption that there are no constraints in recognition ability or time to pick mates by males. Virgin females appear to be scarce, as evidenced by (1) the fact that females mate only once and (2) precopula behavior. Tigriopus males probably maximize fitness by clasping any available female, regardless of relationship. The cost of mating with an individual of 'non-optimal' relatedness is most likely less than the risk of not finding any mate. Thus, selective pressures on males to evolve recognition of siblings, poolmates, and non-poolmates are probably much weaker than selective pressures on males to evolve

recognition of immature females that are close to the final molt. This might explain why Tigriopus males choose adult females at random. The most optimal breeding behavior may result from securing a female at the 'right' age (closest to final molt) at which costs associated with guarding would be minimized. A model for optimal mate choice by males could be constructed that incorporates factors such as cost of guarding, number of available virgin females, number of competing males, of number of previous matings by the male. Such models have been put forward by Parker (1974), Thompson and Mann (1981), and Wickler and Seibt (1981) that also incorporate other variables such as cost of searching and size of female. Thus, not only relationship of potential mates may influence 'optimal' mate choice behavior in Tigriopus.

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