

Spring 1979

REGULATION OF THE REPRODUCTIVE RATES OF DIAPTOMID COPEPODS

CARL J. WATRAS

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COPEPODS

University of New Hampshire

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REGULATION OF THE REPRODUCTIVE RATES
OF DIAPTOMID COPEPODS

by

CARL J. WATRAS

B.A. Williams College 1969

M.S. University of New Hampshire 1975

A DISSERTATION

Submitted to the University of New Hampshire

In Partial Fulfillment of

The Requirements for the Degree of

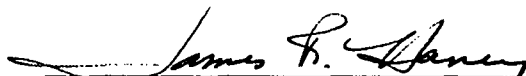
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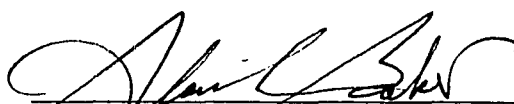
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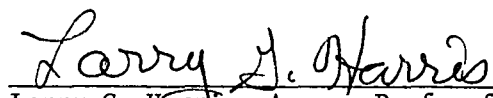
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May, 1979


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ACKNOWLEDGEMENTS

I thank J. Higgins and L. Tabak for technical assistance in the field and laboratory, Drs. J. F. Haney, A. L. Baker, L. G. Harris, R. H. Peters and C. W. Walker for numerous suggestions and constructive criticism and Drs. J. P. Barrett, O. Durgin, M. A. Goddard and, especially, H. F. Bechtell, Jr. for helpful discussions of the analytical mathematics.

This work was supported in part by grants from the University of New Hampshire Research Office (CURF S61 and S95) and the Theodore Roosevelt Memorial Fund of the American Museum of Natural History.

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ABSTRACT

REGULATION OF THE REPRODUCTIVE RATES
OF DIAPTOMID COPEPODS

by

CARL J. WATRAS

Patterns of gamete production, mate-location and embryonic development were investigated with Diaptomus leptopus, D. pygmaeus, D. pallidus and D. dorsalis to clarify the ways environmental and genetic factors influence reproductive rates in populations of diaptomid copepods.

Periodic observations of the reproductive tracts of live, free-swimming females showed that they all oscillated between gravid (dark oviduct) and nongravid (clear oviduct) reproductive conditions. If insemination did not occur during the gravid phase, the contents of the oviducts were voided into the environment and a new batch of oocytes was produced by the ovary. This gametogenic cycle was short relative to a female's lifespan, temperature dependent but generally unaffected by photoperiod. All isolated females tended to cycle independently of each other or any obvious environmental oscillator.

Several relationships between the cycle of gamete production and the regulation of reproductive rates were noted. 1) The duration of the nongravid phase defines the minimal interval between successive ovipositions and can be used to estimate reproductive potential (in terms of clutch production) under various environmental conditions.

2) The cycle period was similar in all females investigated but phase lengths differed between species and these differences may be related to habitat. 3) Interspecific differences in phase length exerted a greater effect on relative rates of clutch production than differences in the duration of embryonic development. 4) Given the average clutch size, the cycle period and phase lengths can be used to calculate upper and lower limits for secondary production by females. 5) Field data indicated that the percentage of gravid females in preserved samples was not a reliable index of reproductive intensity in the population (just as the appearance of a female's oviducts is not a reliable index of sexual maturity). 6) Male Diaptomus mated more readily with gravid than nongravid females, suggesting the operation of a mechanism for mate-selection linked to the gametogenic cycle. Also, successful insemination of a female never occurred during the nongravid phase and reinsemination was required prior to the production of each clutch. 7) Transpecific mating interactions suggested that reproductive interference may effect the synpatric or syntopic occurrence of congeners.

These results point to the importance of considering physiological and behavioral parameters when estimating the reproductive potential of diaptomid populations.

INTRODUCTORY REMARKS

Diaptomid copepods are among the dominant crustacean zooplankters in many lakes. They function as a trophic link in these aquatic systems, grazing on the primary producers, regenerating dissolved nutrients and serving as forage for other heterotrophs like fish. Their importance is a function of population size, which depends, in part, on the rate of reproduction. This thesis deals with some of the factors which affect this rate with four species of Diaptomus.

There are several ways to approach the regulation of reproductive rates in populations of egg-bearing zooplankton. One involves censusing embryonic populations in the field. Hatching rates in the field population are estimated from preserved samples and correlated with changes in environmental variables, such as temperature and phytoplankton density. This approach is simple and direct but, due to a lack of controls, it can tell us little about regulatory mechanisms. A second approach involves defining the biological components of reproductive rates to obtain a comprehensive but workable picture of the reproductive process. Environmental and genetic factors exert their influence through the various components and the system is studied accordingly under controlled conditions.

The second approach was taken in the present study. Five major components of birth rates were defined, one of which was subdivided further. These are:

1. clutch size: average number of embryos per clutch
2. rate of clutch production

- a. carrying time: time from the production of a clutch until it is detached
- b. interclutch time: time from the detachment of an ovisac to the production of the next
 - 1) gametogenic patterns: determine the proportion of time that an individual is gravid
 - 2) mate-location: time needed to find a mate
3. embryo mortality
4. production of resting eggs: temporary loss to the benthos
5. hatching of resting eggs: recruitment from the benthos

This work deals primarily with the second component, the rate of clutch production, and its subdivisions. The other components will form the basis for future investigations. Eventually, this line of research may yield predictive models which will be useful in analyzing the dynamics and distribution of natural populations of Diaptomus.

SECTION I
OSCILLATIONS IN THE REPRODUCTIVE CONDITION
OF DIAPTOMUS LEPTOPUS

Introduction

Several investigators have utilized the appearance of the female reproductive tract as an indication of the reproductive condition of calanoid copepods (Marshall and Orr, 1952; Conover, 1967; Razouls, 1974, 1975; Rigler and Cooley, 1974; Katona, 1975; Moore and Sander, 1976). Although as many as five stages have been described, females with enlarged, darkened oviducts generally have been considered mature and gravid while females with small, transparent ducts have been classified as immature or senescent. The appearance of the ducts results directly from the presence (or absence) and maturation state of primary oocytes.

During a study of mate selection by a planktonic freshwater calanoid copepod, Diaptomus leptopus, regular observations of isolated females indicated short-term fluctuations between the extreme oviducal conditions. The reproductive tract, which was observed through the transparent exoskeleton of live animals (figure 1), at some times appeared dark, at others clear. Instead of reflecting sexual maturity, the condition of the oviducts indicated the gravid (dark phase) and nongravid (clear phase) status of reproductive females. Preliminary investigations also suggested that only females in the dark phase could be successfully inseminated and that insemination was required prior to the production of each clutch.

Given these observations, it seemed that the time between successive dark (gravid) phases might impose a physiological constraint on the rate of egg-clutch production, a major determinant of birth rates in populations of these zooplankton. In other words, the clear phase might delimit the minimal clutch turnover time. Furthermore, the duration of the dark phase might be related to the probability of successful mate location and insemination which impose a behavioral constraint on the rate of clutch production. If isolated individuals oscillated between these reproductive conditions (rather than fluctuating irregularly) the characterization of the oscillations would provide a way to estimate the reproductive potential of these organisms (in terms of clutch production rate) and permit replicate determinations of various treatment effects on reproduction under controlled conditions with individual animals. An alternative to this approach involves the direct observation of clutch production by pairs of males and females swimming together in vials (e.g. Robertson et al., 1974). Such experiments, however, are often hindered by the reluctance of pairs to mate in the laboratory or other container effects and, at best, provide rates which are an ambiguous mixture of behavioral and physiological factors.

The purposes of this section are to 1) demonstrate that the changes in the reproductive condition of D. leptopus are a cyclic phenomenon whose characterization permits insights into the physiological regulation of clutch production rates, 2) describe the fate of eggs during the transition from the dark to clear phases, estimate the amount of egg material involved and discuss the ecological

significance of its fate (especially in relation to secondary productivity and nutrient regeneration), and 3) make comparisons with reproductive processes in other calanoid copepods.

Methods

D. leptopus from a farm pond in Madbury, N.H., were used as experimental animals. To ensure that they were approximately the same age, physiological condition, and fully acclimated to experimental conditions, immature copepodids (CI-CII) were collected during spring, sorted, and individually reared in 30 ml beakers containing 25 ml of filtered pond water. The beakers were kept in a controlled light-temperature chamber ($18 \pm 0.5^\circ\text{C}$; LD 8:16, provided by 10 watt incandescent lamps generating about 7.5×10^{-5} watts cm^{-2} to the water surface). Mixed green algae (Westella, Scenedesmus, Ankistrodesmus, and Chlorella) from xenic cultures were used as food. These genera were all represented in the pond's phytoplankton community, with Scenedesmus being one of the numerically dominant algae from April to June. In an attempt to maintain reasonably constant and sufficient food levels (about 50,000 cells ml^{-1} as measured by hemacytometer counts or $14 \mu\text{g Chl. A l}^{-1}$) equal aliquots of the algal mixture were added to each beaker along with 1 ml aerated, prefiltered pond water three times per week. Algal standing crop in the pond ranged from 3.5×10^3 to 4.5×10^4 cells ml^{-1} during spring. The amounts of detritus, bacteria, and protozoa were not evaluated in either the pond or the food mixture. Sedimented material was removed by pipette and half the volume of each beaker was replaced weekly with fresh, filtered pond water. Variations in

levels undoubtedly occurred, but the digestive tracks of experimental animals appeared full throughout the study indicating sufficient suspended particulate. Dissolved oxygen, (Burke, 1962) and pH measurements ranged from 8.5 to 9.1 ppm and 7.8 to 8.1, respectively. The beakers were examined daily until the animals matured. Survival to maturity was 100% under these conditions.

The pattern of changes in the reproductive systems of the adult females was monitored by inspecting them at approximately 8 h intervals. The state of the oviducts was recorded as either dark or clear. To minimize disturbance, observations were made using a dissecting scope (10x, transmitted light) while the animals swam freely in their beakers and red filters were used during night-time observations.

When a change was observed, the midpoint between the time the new state was observed and the previous observation time was used as an estimate of the time of the change. Regular observations were made from June 2 to June 28, 1976, allowing each female about 10 oviducal changes.

At the conclusion of this observation period, all the females were placed with males and clutch production and oviducal changes monitored simultaneously. In addition, each of twelve virgin females in both phases were placed with three males. After three hours the males were removed and the females observed to see if viable clutches would be produced by both clear and dark phase females. Regular observations were continued for at least four complete phase changes.

Two controls for laboratory conditions were established.

First, field-matured animals were observed in lab for 19 days to determine the effect of lab rearing. Secondly, to estimate the effect of variation in food level between experimental beakers, field-matured females were cultured at two food levels that differed by about an order of magnitude. These females were confined individually in mesh containers that were placed in low plastic aquaria containing 1 l of algal suspension (either 5×10^3 or 5×10^4 cells ml^{-1}). The media were changed every other day and gently aerated to minimize qualitative and quantitative changes. Two aquaria, each with twelve animals, at each food level were kept in a controlled light-temperature chamber.

The amount of material expelled from the oviducts was estimated by comparing the weights of dark phase and clear phase females. Adults were collected in the field and maintained in the lab for one oviducal phase change to ensure they were reproductively active. Live animals of uniform size and oviducal state were placed three at a time on pre-weighed aluminum boats, dried at 110°C for two hours, allowed to cool in a dessicator, and weighed on a Cahn G2 electrobalance. All the animals were allowed to clear their guts in HA Millipore filtered water and prosome length was measured on a drained slide prior to drying. (The weight of eggs from ovisacs was not considered a reliable indicator of primary oocyte weight since substantial changes may occur during embryonic development. Green (1965), for example, reported a 16-25% decrease in Daphnia egg dry-weight during development.)

As an additional control, field-matured animals were isolated

in mesh containers and resuspended in the pond. Observations were made twice daily and the pattern of oviducal changes monitored in situ for nine days.

The pond population was sampled weekly during the ice-free periods of 1976 and 1977 to estimate the proportions of dark phase and clear phase females and to relate these proportions to other measures of reproductive activity. The pond ($A = 2500\text{m}^2$; $Z_m = 2\text{m}$) was divided into 4 quadrats of equal volume; and a short, oblique tow (ca. 5m) with a 30cm net (75 μm mesh) was made in each quadrat. The samples were pooled, preserved in 4% Formalin, and later subsampled to determine the numbers of clear phase females, dark phase females, egg, and in 1977, males. Maximum and minimum water temperatures were recorded daily during 1977; temperatures were taken at the time of sampling on each sample date in 1976. (The pond was generally isothermal.) These water temperatures were used to calculate egg development times from the following regression equation which was developed from egg development times at 24° , 21° , 18° , and 12°C ($N = 62$ clutches):

$$D = 473T^{-1.77} \quad (r = 0.86)$$

where D is the egg development time in days and T is the temperature in degree centigrade. Egg development times were used to compute finite birth rates for each date by using the relation developed by Edmondson (1960)

$$B = \frac{E}{D}$$

where B is the finite birth rate, E is the ratio eggs: females,

and D is the egg development time.

Results

The actual onset of the clear phase was observed twice with virgin lab-matured animals and, both times, the oocytes were discarded rather than resorbed. The eggs were extruded in pairs through the gonopore directly into the medium. No egg sac was produced. These eggs were elongate and opaque at first, but soon became spherical and translucent. The ducts emptied in less than thirty seconds and the eggs disintegrated within 2 or 3 minutes after extrusion. The onset of the clear phase is essentially an instantaneous event, explaining its infrequent observation.

Field-matured females deposited an amorphous mass of unfertilized oocytes into an ovisac rather than expelling them directly into the environment. No distinct eggs were visible within the sacs. Some of these sacs were dislodged within minutes after formation, others were carried for up to 2 days; all disintegrated soon after being dropped. Upon re-isolation, lab-matured females which had previously produced a clutch of viable eggs also extruded an amorphous mass of egg material into an ovisac. Apparently the reproductive history of the animals influenced the way unfertilized oocytes were discarded. Somehow copulation stimulated egg sac production. The length of time for which ovisac production continued after re-isolation was not determined.

In contrast to the sudden onset of the clear phase, the dark phase develops gradually. Considering Ishikawa's (1891) description of oogenesis in Diaptomus sp. which was based on serial sections of

preserved adults, it seems that a new batch of primary oocytes enters the ducts after clearing and begins vitellogenesis. As vitellogenesis proceeds the oocytes become progressively larger and more opaque. In this study the dark phase was considered to commence when the oviducts first became visible as opaque bands. A more quantitative criterion was not possible without disturbing the free-swimming adults.

Records of the alternation between oviducal states for the lab-matured females are shown on figure 2. Visual inspection gives the impression that all the females were behaving similarly; undergoing oscillations between the dark (gravid) and clear (nongravid) phases.

The mean cycle period and phase durations characterize the oscillations, and a single factor analysis of variance was used to test for differences between animals. No significant differences between individuals in the duration of the clear phase ($F = 1.12$; 6,29 df; $\alpha = 0.05$), or the cycle period ($F = 2.81$, 6,28 df; $\alpha = 0.05$) were indicated. The duration of the dark phase did vary significantly between animals, however ($F = 4.44$; 6,29 df; $\alpha = 0.05$). Individual variation of this type does not seem unusual (cf. variation in mammalian menstrual or estrous cycles, Asdell (1968)). However, given the indication of significant variation between individuals, the cycle characteristics for the population were estimated by calculating grand means. As shown on Table 1, the oscillations are characterized by a mean period of 4.4 days, with a dark phase duration of 3.4 days, and a clear phase of 0.9 days.

As an additional check on the regularity of the fluctuations, a spectral analysis was performed. Spectral analysis partitions the total variance of time-series data into periodic and aperiodic components (Wastler, 1969). The results confirm the estimates obtained by calculating mean values. The power spectra for all seven females appear on figure 3. Each shows a major peak between periods of 3.3 to 5.0 days, indicating that most of the variation in these records can be attributed to a dominating period of this magnitude. This estimate of the cycle period is of the same order as that obtained by computing a 95% C.I. for the mean cycle period obtained from Table 1.

The animals appeared to be oscillating independently of each other showing no indication of synchrony or constant phase relationships (figure 2). Plots of the percentage of the lab population in either phase vs. time reinforce this conclusion of random phase relations between animals (figure 4). Correlogram analysis of this record indicates that these fluctuations are random rather than truly oscillatory (insert A, figure 4; see Pielou, 1974).

In addition, there was no detectable temporal synchrony in the cycle records; no tendency for the estimated onset time of either cycle phase to occur within a particular observation interval. All thirty-five onsets of the clear phase and forty-one of the dark phase were distributed randomly between the three daily observation periods ($\chi^2_{\text{clear}} = 5.57$; $\chi^2_{\text{dark}} = 2.27$; 2 df and $\alpha = 0.05$ both cases). Furthermore, the mean cycle period of 4.4 days does not correspond to the period of any obvious environmental oscillator such as photoperiod.

Lab rearing or observation under laboratory conditions

produced no obvious effects. Field-matured females observed in lab and in situ all oscillated between the two oviducal states, indicating that the records depicted on figure 2 were not artifactual. Animals at two food levels differing by an order of magnitude showed no significant differences in the cycle period ($t = 0.347$; 35 df) or phase duration ($t_{\text{clear}} = 0.592$, 37 df; $t_{\text{dark}} = 1.037$, 33 df). This indicated that the differences in food level between beakers probably were not a major cause of cycle variation between animals. Also, lab-matured females placed with males all produced viable clutches comparable in size to those found attached to field-collected females of similar body size. Apparently, lab rearing had not dramatically altered their reproductive capacity.

The effect of mating on the cycle was examined by comparing the clear phase durations of isolated females with those of females cultured with males. Neither dark phase duration nor cycle period were compared since mating results in the clearing of the ducts and clutch production soon after fertilization. As shown on Table 2, estimates of the clear phase of isolated and mating females are similar, suggesting that mating does not significantly affect this reproductive parameter. A single factor analysis of variance was used to compare durations of the four clutch production rate parameters listed on Table 2. Since unequal variances and non-normality were evident in the data, a \log_{10} transformation was employed and the analysis performed on the transformed variables. The analysis of variance indicated significant differences between the estimates ($F = 22.07$; 116 df). Subsequently, a Student-Newman-

Keuls test showed that the two clear phase estimates were not significantly different; but the mean interclutch time (the time between the detachment of one ovisac and the production of the next) was significantly shorter and the embryonic development time significantly longer than all the other estimates ($\alpha = 0.05$). The clear phase, then, remains constant regardless of mating status. The biological significance of the relative sizes of the other estimates will be described below (see Discussion).

Attempts to mate both dark phase and clear phase females showed that only females mated in the dark phase would produce viable clutches. None of the clear phase females produced viable eggs, even though spermatophores had been attached. These females progressed through their cycles normally for at least two cycle periods; in some cases amorphous egg masses in ovisacs attached to the females were observed. Copulation had stimulated ovisac production without resulting in fertilization. In contrast, 50% of the dark phase females reproduced successfully after being with males for three hours.

The weight difference (ΔW) between dark phase and clear phase females was calculated by fitting the model

$$\log_{10} W = b_0 + b_1 \log_{10} L + b_2 X + b_3 \log_{10} L \cdot X \quad (1)$$

to the length-weight data, where W = dry wt. (μg), L = prosome length (mm), and X is an indicator variable which equals 0 if clear phase or 1 if dark phase (figure 5). Fitting one regression with an indicator variable provides more precise estimates of the regression coefficients and more sensitive tests of differences in slope and

elevation than fitting two separate regressions, (Neter and Wasserman, 1974). The above model was chosen because the relationship between body weight and length generally is considered well described by a power function and it produced a better fit than either a simple linear or exponential model. Equation 1 reduces to

$$\log_{10} W = b_0 + b_1 \log_{10} L \quad (2)$$

when $X = 0$, and to

$$\log_{10} W = (b_0 + b_2) + (b_1 + b_3) \log_{10} L \quad (3)$$

when $X = 1$.

The interaction term proved insignificant ($t = -1.57$; 26 df). Given $b_3 = 0$, equation 3 reduces further to

$$\log_{10} W = (b_0 + b_2) + b_1 \log_{10} L \quad (4)$$

Thus, fitting Equation 1 without the interaction term amounts to fitting equations 2 and 4, two lines with a common slope, and b_2 is an estimate of the difference in weight (ΔW) between the two types of female over all body lengths considered.

For the data collected,

$$\log_{10} W = 0.873 + 4.28 \log_{10} L + 0.097 X \quad (R^2 = 82.7)$$

so, from equation 4,

$$\log_{10} W_{\text{dark}} = 0.970 + 4.28 \log_{10} L$$

or, in the original units,

$$W_{\text{dark}} = 9.33L^{4.28}$$

and, from equation 2,

$$\log_{10} W_{\text{clear}} = 0.873 + 4.28 \log_{10} L$$

or

$$W_{\text{clear}} = 7.46L^{4.28} \quad (6)$$

The ninety-five percent C.I. for b_2 (the weight difference) is

$$0.053 \leq b_2 \leq 0.141.$$

Taking the antilog of these limits yields

$$1.13 \leq b_2 \leq 1.38$$

indicating that the mean dry-weight of dark phase females was between 13% and 38% greater than the mean dry-weight of clear phase females over all body lengths. On the average, 25% of the dry-weight of an adult female was expelled during each onset of the clear phase. For several reasons (discussed below) this only can be considered a first estimate.

A turnover rate was calculated by combining this estimate with the mean cycle period estimate. The turnover rate is

$$25\% \text{ body weight (dry)}/4.4 \text{ days} = 5.6\% \text{ b.w. (dry) day}^{-1}$$

at 18°C. (7)

In the absence of reproduction, germinal cells are being produced and

eliminated at this rate. Since secondary production equals the sum of somatic and germinal tissue growth and since copepods generally do not molt after reaching sexual maturity (i.e. somatic growth = 0, although tissue mass may fluctuate with nutrient conditions), this oocyte turnover rate represents a baseline productivity for female D. leptopus. For a given mass of oocytes, secondary production increases from this baseline value via mating and subsequent clutch production to an upper limit which equals the maximum clutch production rate possible under a particular set of environmental conditions. It can be shown that the upper physiological bound on clutch production is imposed by the duration of the clear phase (see Discussion).

Sufficient data was collected to obtain estimates of the field population's gametogenic status, the egg: female ratio (E), and the finite birth rate (B) for thirteen dates during both years. The major objectives were to estimate the magnitude of fluctuations in the proportion of dark phase females and to determine whether this proportion was a reliable indicator of the reproductive activity in the population. Previous authors have attempted to define the breeding seasons of calanoid populations by plotting temporal changes in the percentage of females with dark oviducts. Most of this effort has been applied to marine copepods, (e.g. Moore and Sander, 1976). In the present study, the appropriateness of this approach for diaptomid populations was examined: i.e. was there any evidence of a strong correlation between the proportion of dark phase females and other estimates of reproductive activity, such as the egg: female ratio (E) and the finite birth rate (B). For example, in marine systems a

direct relation is generally proposed. If this proved true for diaptomids as well, one would expect peaks in the percentage of dark phase females, E and B to occur together.

Fluctuations in the proportion of dark phase females for 1976 and 1977 are shown on figure 6. The magnitude of the fluctuations is high but no consistent relationship between the percentage of dark phase females and either E or B is visually apparent. Low direct correlation coefficients confirm this impression (Table 3), and there is no reason to suspect a lag response. These data indicate that this static picture of the population's gametogenic condition is not by itself a reliable index of breeding intensity, just as a single observation of the condition of an individual's oviducts is not a reliable index of sexual maturity.

Discussion

Oscillations in the reproductive condition of Diaptomus leptopus make the extraction of meaningful information from preserved samples more difficult. However, the characterization of these oscillations can provide insights into the regulation of reproductive rates, especially the rate of clutch production. This rate is a function of two sub-components: 1) clutch carrying time, and 2) the interval between successive clutches, interclutch time. For subitaneous eggs¹, carrying time is usually equated with embryonic development time since the ovisac often remains attached to the female until hatching. This has led to the use of embryonic development times to estimate

¹ eggs which hatch directly, without an intervening resting or diapausing stage

maximum clutch production rates, (e.g. Corkett and Zillioux, 1975). Implicit in this approach is the generally untested assumption that fertilization can occur as soon as an ovisac is detached (i.e. minimum interclutch time = 0), as well as the assumption that carrying time and embryonic development time are equivalent. The gross characterization of gametogenic patterns provides an opportunity to test the first assumption (interclutch time = 0) and to examine the effect of varying clutch carrying time on clutch production rates.

Interclutch time has behavioral and physiological determinants. The behavioral factor includes the time necessary for mate location and sperm transfer. (As mentioned earlier, D. leptopus females require insemination prior to the production of each clutch.) The time needed for the production of ripe oocytes constitutes the physiological factor. If a reproductive male and ripe oocytes are available readily after ovisac detachment, the interclutch time is negligible and clutch production depends only on carrying time. The clear phase duration is an estimate of the physiological factor and its magnitude in relation to carrying time tells us how soon successful insemination can occur after ovisac detachment. Since for D. leptopus at 18°C subitaneous egg development time is longer than the clear phase duration (Table 2), there is no delay between the hatching of a clutch and the appearance of ripe gametes. In this case the duration of interclutch time is behaviorally determined, and when the embryos are carried until hatching, the maximum clutch production rate is equivalent to the embryonic development rate.

The relation between D. leptopus gametogenic cycle and clutch

production at 18°C is represented diagrammatically on figure 7. Clutch carrying time is equated with embryonic development time and the effect of insemination at three points after ovisac detachment on interclutch time is depicted. Although the actual interclutch time is a variable that depends on mate location time, note that it is not a continuous function of mating time but a discontinuous function with steps of 0.9 days (clear phase duration) occurring at each clear phase onset. This is because fertilization cannot occur during the clear phase. Also note that most of the dark phase passes during the clutch carrying interval.

If clutches of eggs were normally carried until they hatched, it would seem more efficient to synchronize the dark phase onset and hatching than to begin the dark phase so soon. The energetic costs of maintaining ripe oocytes would be reduced and the fertilizable period would be extended to include most of the dark phase. This extension of the fertilizable period would increase the probability of successful insemination prior to the next clear phase onset. An advantage to the shorter clear phase is apparent when one considers the possibility of ovisac detachment before the completion of embryonic development. The premature dislodgement of subitaneous clutches and the characteristically shorter carrying time of resting clutches (Robertson et al., 1974) could result in the enhancement of clutch production rates predicted from embryonic development times. To estimate the maximum enhancement, the ratio embryonic development time:clear phase can be evaluated. For D. leptopus at 18°C, this ratio equals 3.33, indicating that clutch turnover could be 233%

higher than that predicted from embryonic development time, if clutches were dropped at the dark phase onset. In effect, then, the shorter clear phase duration which delimits the minimum clutch turnover time could have a substantial impact of fecundity, population growth and productivity. Earlier (see Results) a baseline productivity of 5.6% dry wt. day⁻¹ was calculated for normally oscillating adult females. Substituting the clear phase duration, 0.9 days, for the cycle period in equation 7 yields

$$25\% \text{ b.w. (dry)}/0.9 \text{ days} = 28\% \text{ b.w. (dry) day}^{-1}$$

as an estimate of an individual female's productivity if clutches were produced at the maximum rate. Note that both of these production estimates are based on an average clutch size. Given this restriction, production levels between these limits reflect intermediate rates of clutch production. For example, if successful insemination occurred directly after the hatching of subitaneous eggs (3 day embryonic development time at 18°C), production equal to 8.1% b.w. (dry) day⁻¹ would result.

In terms of resting egg production, the short clear phase and decreased carrying time seems a useful strategy. As environmental conditions deteriorate, reproductive effort could be dramatically increased to rapidly enlarge the diapausing portion of the population. In small ponds which dry in summer and/or freeze to the bottom in winter (a common habitat for D. leptopus) this ability to shift reproductive rates could be instrumental in maintaining the population.

Without a knowledge of gametogenic patterns, estimating interclutch times and potential egg production under controlled

conditions can be problematic. In the mating experiments one female was placed with two males in 25 ml containers. The mean interclutch time was 1 day (Table 2). Unless the cycle of gamete production is known, erroneous interpretations of this average time are possible. Given the male:female ratio and high effective density, one might conclude that 1 day was a reasonable estimate of the minimal interclutch time. Combined with data on the gametogenic cycle, however, the data strongly suggest that the high mean resulted from a few extreme values which reflect anomolous mating behavior. The frequency histogram of interclutch times was highly skewed to the right. This evidence indicates that direct estimates of average interclutch times by themselves may be inherently ambiguous due to an inability to control for container effects. Isolating the behavioral and physiological components by examining gamete production and investigating them separately provides more meaningful information about reproduction potential.

The observations on D. leptopus permit comparisons with previously documented changes in the reproductive systems of other calanoids. At any time, the oviducts of Diaptomus contain oocytes which are approximately the same age and state of maturity (Ishikawa, 1891) and which are shed simultaneously. In contrast, the oviducts of several marine species contain cells in a variety of maturation states (Conover, 1967; Razouls, 1974, 1975). After the ripest are released, younger oocytes develop into mature gametes. Changes in the gross appearance of the ducts are apparently subtle. This staggered pattern of gamete maturation within the oviducts has at least two

implications. First, combined with the larger reproductive tracts of some species (Hilton, 1931; Lowe, 1935) it may enable the continuous recruitment of ripe oocytes needed for multiple layings from a single insemination and enhance reproductive rates. Landry (1975) mentioned the osmotic problems which might inhibit sperm storage and preclude this strategy in freshwater. Secondly, the proportion of dark phase females in field populations may be less subject to random fluctuations and more useful in defining breeding seasons. However, since gametogenic patterns have been described for few species, this approach may be premature. To assess the breeding condition of populations of species which cycle like D. leptopus, isolated adults must be examined for evidence of cyclic behavior and, subsequently, the ratio of active to senescent females computed.

Although discarding unfertilized, non-viable eggs seems energetically wasteful, it does not seem unusual in view of the literature on copepods. Robertson et al (1974), Marshall and Orr (1952), Jacobs (1961), Eckstein (1964), Corkett and McLaren (1969) and Katona (1975) all described similar phenomena for various calanoids. Although none calculated turnover rates or described cycles of extrusion, most of these reports implied that oocyte extrusion occurred repeatedly when females were isolated from appropriate mates.

Hall (1964) reported a high incidence of non-viable eggs in Daphnia populations and mentioned the importance of avoiding their inclusion in egg:female ratio estimates which are used for population projections. Sacs of unfertilized D. leptopus eggs are easily distinguished as an amorphous mass, but Eckstein's (1964) work suggests

the release of normal-appearing, unfertilized oocytes by Eudiaptomus. Acartia tonsa, (Heinle, 1969; Wilson and Parrish, 1971), Calanus hyperboreus, (Conover, 1967), and Pseudodiaptomus coronatus (Jacobs, 1961) also apparently discard durable unfertilized oocytes which could be confused with normal eggs. The resolution of this difficulty in identifying non-fertile eggs requires further observations of the egg production cycles of isolated females. Investigating the copulatory stimulation of ovisac and egg membrane production could be useful here.

Some investigators conclude that oocyte extrusion is probably a rare event under natural conditions (e.g. Robertson et al., 1974). No direct evidence exists which relates to this hypothesis. The frequency of oocyte extrusion depends upon the probability of locating a suitable mate. Although little empirical evidence exists, a theoretical approximation of mate location frequency can be obtained. Assuming that copepods interact like individual gas molecules outside a certain reactive space, the frequency of encounter (Z) between a given female and males can be calculated as

$$Z = nR_{mf}^2 \pi \frac{3U_m^2 + U_f^2}{3U_m}$$

where n is the density of females (here equal to the density of males), R_{mf} is the reactive distance of males to females, and U_m and U_f are the mean swimming speeds of males and females (Kauzmann, 1966; Katona, 1973). The number of encounters per female in a given time interval is $Z \cdot t$ and the probability that a particular female will be encountered can be estimated as $p = 1 - e^{-Z \cdot t}$ (Moismann, 1958), where t is the length of the time interval.

This model assumes a uniform distribution of particles, (either within patches or the lake as a whole), which does not seem unreasonable in light of studies by Comita and Comita (1957) on Diaptomus microdistribution. Average swimming speeds were estimated in lab: males = 2.5 mm sec.⁻¹, females = 1.5 mm sec.⁻¹ at 18°C. Since males seldom initiated mate locating behavior in lab when more than 1 or 2 body lengths from a female it seems reasonable to define 1.5 to 3.0 mm as limits on the male's reactive distance (R_{mf}). Although Katona (1973) reported much greater reactive distances for marine calanoids, observations by Kerfoot (1978) on the reactions of predatory copepods suggest that one to two body lengths is a reasonable estimate for freshwater species. The gravid or fertilizable period was used as (t) and estimated two ways: 1) simply as the dark phase duration, 3.4 days; and, since fertilization cannot occur while an ovisac is being carried, 2) as the portion of dark phase left after dropping a subitaneous clutch, cycle period minus embryonic development time = 1.4 days.

Assuming that 10% of all encounters result in insemination,² the probability of oocyte extrusion (p') was plotted as a function of population density for four combinations of reactive distance and fertilizable period (figure 8). The probability of extrusion (p') equals one minus the probability of insemination prior to clear phase onset (given as p above). The curves indicate that oocyte extrusion is very likely to occur in nature under certain conditions.

²Since a male apparently must approach a potential mate caudally, less than 50% of all the possible approach angles produce mate seeking behaviors; and roughly 20% of the correct approaches resulted in successful insemination under laboratory conditions.

The calculated weight of extruded oocytes is a first estimate since controls for nutritional history or age were lacking. Undetected variation in the fullness of the ducts may be responsible for much of the variability of the data. It was not possible to count cells in the ducts, and neither oviduct distension nor opacity were rigorously quantified. Due to their increased volume, variability of this sort is likely to be highest with large animals (provided changes in egg diameter are not compensatory). Both the increasing scatter with body length seen on figure 5 and previous reports of calanoid clutch sizes are consistent with this idea. Generally, the largest clutches and the highest variation in clutch size are associated with the biggest animals (see figures in Roen, 1955; Ravera, 1955; Ravera and Tonolli, 1956; McLaren, 1965; Smyly, 1968; Corkett and McLaren, 1969).

Comparing only weights of the small animals might minimize this variability. Fitting model 1 to these data on figure 5 provided a more precise estimate of the oocyte mass: 36% to 47% of the body weight (dry) were the 95% confidence limits (insert A, figure 5). Again no difference in slope was detected, so the percent loss was constant over all body sizes. More data are needed to test the accuracy of this estimate.

The conclusion of a constant percent loss over all body sizes implies a linear relation between clutch size and body length. This is consistent with the results of previous studies (Ravera and Tonolli, 1956) although McLaren (1963) argued that a power function more adequately described the relationship. Smyly (1973) estimated that egg volume was roughly 20% of the thoracic volume of well fed

cyclopoids regardless of body length. This tends to support the findings of the present study.

The exponent relating prosome length to body weight (equations 5 and 6) suggests that the copepods become relatively stouter or more dense as their length increases. (An exponent greater than 3 indicates that the ratio length:weight decreases with increasing length, see Winberg, 1971). McLaren (1965) reported this phenomenon for Pseudocalanus. In that instance, shorter females were more slender. Measurements on 86 D. leptopus females showed that the relationship between prosome length and width is well described by the equation

$$\text{width} = 0.38 \text{ length}^{1.32} \quad (r = 0.87)$$

and that the exponent is significantly larger than unity ($p < 0.0005$), so the larger females were more stout. In addition, this relationship implies that body volume is proportional to prosome length raised to the power 3.64, an exponent which falls within the 95% C.I. for the exponent in equations 5 and 6.

Considering the mass of material involved and its rapid disintegration, oocyte extrusion could liberate substantial amounts of such nutrients as phosphorus into lake water under both experimental and natural conditions. The rate of phosphorus release due to oocyte extrusion (expressed in $\mu\text{g mg}^{-1} \text{ hr}^{-1}$) can be calculated using the formula

$$\text{oocyte-P} = \frac{(\text{oocyte wt. } (\mu\text{g}) \times \text{P-fraction}) / \text{cycle period (hrs)}}{\text{female wt (mg)}}$$

Although a suitable estimate of the phosphorus concentration in diaptomid eggs is not readily available, given the range of recorded

values for other arthropods it seems reasonable to assume that the concentration lies between 1% and 5% of the egg dry weight (e.g. Baudouin and Ravera, 1972; Wyatt, 1959). Applying these limits to D. leptopus with an egg mass equal to 25% adult dry weight and a cycle period of 4.4 days, oocyte-phosphorus excretion rates of 0.024 to 0.12 mg mg⁻¹ hr⁻¹ result. These rates constitute 6% to 20% of the total phosphorus excretion of D. leptopus females determined by Peters (1975).

In experimental situations, phosphorus excretion rates in excess of 100% of those reported by Peters could be attributed to oocyte phosphorus alone, if 1 animal in 18 voided its oviducts during his 90 minute experiments. This estimate suggests that oocyte phosphorus may be an unforeseen factor contributing to the high variability normally associated with excretion rate estimates. Of primary importance here is the probability that extrusion would occur during such short assays. Assuming that all 18 females were gravid and that they represented a random sample from a uniform distribution within the dark phase, the probability of at least one animal shedding oocytes is $1-(1-E(Y))^n$, where

$$E(Y) = \frac{\text{experiment duration}}{\text{dark phase duration}}$$

and n is the number of gravid females. At 18°C the probability of extrusion in a 90 minute assay with 18 females is 0.28 or, roughly, 1 in every 4 times.

Females initiating vitellogenesis (clear phase females) could affect P excretion rate determinations by sequestering phosphorus.

Marshall and Orr (1961) reported a 58% difference in the phosphorus content of gravid and nongravid female Calanus finmarchicus. They also reported the rapid uptake of ^{32}P by the ovary and oocytes and reduced phosphorus excretion by females actively laying eggs (Marshall and Orr, 1955b, 1961). These observations suggest that a phosphorus excretion cycle may exist which reflects the gametogenic cycle. It should consist of a gradual increase in P excretion as vitellogenesis nears completion followed by a major peak at oocyte extrusion and a return to a baseline level while clear phase females rapidly shunt phosphorus into developing oocytes (provided ingestion rates were not sufficiently enhanced).

Many of the above considerations require further information for evaluation, but they point to the potential importance of considering the gametogenic patterns of animals such as Diaptomus in several areas of zooplankton ecology.

To summarize, the results of section I indicate that female D. leptopus oscillate between nongravid and gravid reproductive conditions. Once characterized, these oscillations can be used to examine constraints on rates of egg-clutch production under controlled conditions. At 18°C, the duration of the nongravid phase is so short that clutches could be produced at a rate more than triple that predicted from the duration of embryonic development. This may be particularly important for the rapid production of large numbers of resting eggs as environmental conditions deteriorate. In section II, the effects of certain environmental factors on this cycle of gamete production and, subsequently, on reproductive potential will be examined.

Bibliography

- Asdell, S. A. 1964. Patterns of Mammalian Reproduction. Ithaca, N.Y.: Cornell University Press.
- Baudoin, M. F. and O. Ravera. 1972. Weight, size and chemical composition of some freshwater zooplankers: Daphnia hyalina (Leydig), Limnol. Oceanogr. 17, 645-650.
- Burke, J. D. 1957. Determination of oxygen in water using a 10 ml syringe. J. Elisha Mitchell Sci. Soc. 78, 145-147.
- Comita, G. W. and J. J. Comita. 1957. The internal distribution patterns of a calanoid copepod population, and a description of a modified Clarke-Bumpus plankton sampler. Limnol. Oceanogr. 2, 321-332.
- Conover, R. J. 1967. Reproductive cycle, early development, and fecundity in laboratory populations of the copepod, Calanus hyperboreus. Crustaceana 13, 61-72.
- Corkett, C. J., and I. A. McLaren. 1969. Egg production and oil storage by the copepod Pseudocalanus in the laboratory. J. Exp. Mar. B. 3, 90-105.
- Corkett, C. J. and E. J. Zillioux. 1975. Studies on the effect of temperature on the egg laying of three species of calanoid copepods in the laboratory. Bull. Plankton. Soc. Japan 21, 77-85.
- Eckstein, H. 1964. Untersuchungen über den Einfluss des Rheinwassers auf die Limnologie des Schluchsees. Arch. Hydrob./Suppl. 28, 118-182.
- Edmondson, W. T. 1960. Reproductive rates of rotifers in natural populations. Memorie Ist. Ital. Idrobiol. 12, 21-77.

- Green, J. 1965. Chemical embryology of the Crustacia. Biol. Rev. 40, 580-600.
- Hall, D. J. 1964. An experimental approach to the dynamics of a natural population of Daphnia galeata mendotae. Ecology 45, 94-110.
- Heinle, D. R. 1969. The effects of temperature on the population dynamics of estuarine copepods. Ph.D. Thesis, College Park, Md.: Univ. Maryland.
- Hilton, I. F. 1931. The oogenesis of Calanus finmarchicus. Quart. J. Microsc. Sci. 74, 193-222.
- Ishikawa, C. 1891. Studies of reproductive elements. I. Spermatogenesis, ovogenesis, and fertilization on Diaptomus sp. J. Fac. Tok. 5, 1-34.
- Jacobs. 1961. Laboratory cultivation of the marine copepod Pseudodiaptomus coronatus Williams. Limnol. Oceanogr. 6, 443-446.
- Katona, S. K. 1973. Evidence for sex pheromones in planktonic copepods. Limnol Oceanogr. 18, 574-583.
- _____. 1975. Copulation in the copepod Eurytemora affinis (Poppe, 1880). Crustaceana 28, 90-95.
- Kauzmann, W. 1966. Kinetic theory of gases, Vol. I. New York: Benjamin.
- Kerfoot, W. D. 1978. Combat between predatory copepods and their prey: Cyclops, Epischura and Bosmina. Limnol. Oceanogr. 23, 1089-1102.
- Landry, M. R. 1975. Seasonal temperature effects and predicting development rates of marine copepod eggs. Limnol. Oceanogr. 20, 434-440.

- Lowe, E. 1935. On the anatomy of a marine copepod, Calanus finmarchicus (Gunnerus). R. Soc. Edinb. 58, 561-603.
- Marshall, S. M. and A. P. Orr. 1952. On the biology of Calanus finmarchicus. VII. Factors affecting egg production. J. Mar. Biol. Ass. U.K. 30, 527-547.
- _____. 1955a. The biology of a marine copepod, Calanus finmarchicus. Edinburgh, Oliver and Boyd.
- _____. 1955b. On the biology of Calanus finmarchicus, VIII, Food uptake, assimilation and excretion in adult and stage V Calanus. J. Mar. Biol. Ass. U.K. 34, 495-529.
- _____. 1961. On the biology of Calanus finmarchicus XII. the phosphorus cycle: excretion, egg production, autolysis. J. Mar. Biol. Ass. U.K. 41, 463-488.
- Mclaren, I. A. 1963. Effects of temperature on growth of zooplankton and the adaptive value of vertical migration. J. Fish. Res. 20, 685-727.
- _____. 1965. Some relationships between temperature and egg size, body size, development rate, and fecundity of the copepod Pseudocalanus. Limnol. Oceanogr. 10, 529-538.
- Moore, E. and F. Sander. 1976. Quantitative and qualitative aspects of the zooplankton and breeding patterns of copepods at two Caribbean coral reef stations. Estuarine Coastal Mar. Sci. 4, 589-607.
- Mosimann, J. E. 1958. The evolutionary significance of rare matings in animal populations. Evolution 12, 246-261.
- Neter, J. and W. Wasserman. 1974. Applied linear statistical models. Homewood-Arundel-Georgetown: Irwin.

- Peters, R. H. 1975. Phosphorus regeneration by natural populations of limnetic zooplankton. *Verh. Int. Ver. Limnol.* 19, 273-279.
- Pielou, E. C. 1974. *Population and Community Ecology*. New York-Paris-London: Gordon and Breach.
- Ravera, Oscar. 1955. Seasonal variation of the reproductive rate in pelagic copepods of Lake Maggiore. *Verh. Int. Ver. Limnol.* 12, 436-447.
- _____ and V. Tonolli. 1956. Body size and number of eggs in Diaptomids, as related to water renewal in mountain lakes. *Limnol. Oceanogr.* 1, 118-122.
- Razouls, S. 1974. Maturite sexuelle et fecundite chez les femelles de Temora stylifera, Copepode pelagique (Copepods; Calanoida). *Arch. Zool. Exp. Gen.* 115, 387-399.
- _____. 1975. Fecundite, maturite sexuelle et differentiation de l'appareil genital des femelles de deux Copepodes planctoniques: Centropages typicus et Temora stylifera.
- Rigler, F. H. and J. M. Cooley. 1974. The use of field data to derive population statistics of multivoltine copepods. *Limnol. Oceanogr.* 19, 636-655.
- Robertson, A., C. W. Gehrs, C. B. Hardin, and G. W. Hunt. 1974. *Culturing and ecology of Diaptomus clavipes and Cyclops vernalis*. Washington, D.C.: U. S. Environmental Protection Agency (EPA-600/3-74-006).
- Roen, U. 1955. On the number of eggs in some free-living copepods. *Verh. Int. Ver. Limnol.* 12, 447-454.
- Smyly, W. J. P. 1968. Number of eggs and body-size in the fresh water copepod Diaptomus gracilis Sars in the English Lake

District. Oikos 19, 323-338.

_____. 1973. Clutch-size in the fresh water cyclopoid copepod, Cyclops strenuus abussorum Sars in relation to thoracic volume and food. J. Nat. Hist. 7, 545-549.

Wastler, T. A. 1969. Spectral analysis: applications in water pollution control. Washington, D.C.: U. S. Federal Water Pollution Control Administration (CWT-3).

Wilson, D. F. and K. K. Parrish. 1971. Remating in a planktonic marine calanoid copepod. Marine Biol. 9, 202-204.

Winberg, G. G. 1971. Methods for the estimation of production of aquatic animals (trans. by Annie Duncan). London-New York: Academic.

Wyatt, G. R. 1958. Phosphorus compounds in insect development. In: Proc. 4th Int. Cong. Biochem., Vienna, (L. Levenbook, ed), New York: Pergaman.

Figure 1. Reproductive system of female D. leptopus as viewed through the exoskeleton during the dark phase of the gametogenic cycle (ca 25X, transmitted light). dt, dorsal tract which has an upper and lower canal (cf anterior diverticula of Lowe (1935) and Marshall and Orr (1955a); diverticule anterieur of Razouls (1975). tt, transverse tract. vt, ventral track (cf posterior part of Lowe (1935) and Marshall and Orr (1955a); diverticule posterieur and tractus ventral of Razouls (1975). lb, lateral bulges (cf short diverticula, temporary extensions and secondary pockets of Marshall and Orr (1952, 1955a). ov, ovary (although not visible through the exoskeleton at this magnification, its position is indicated).

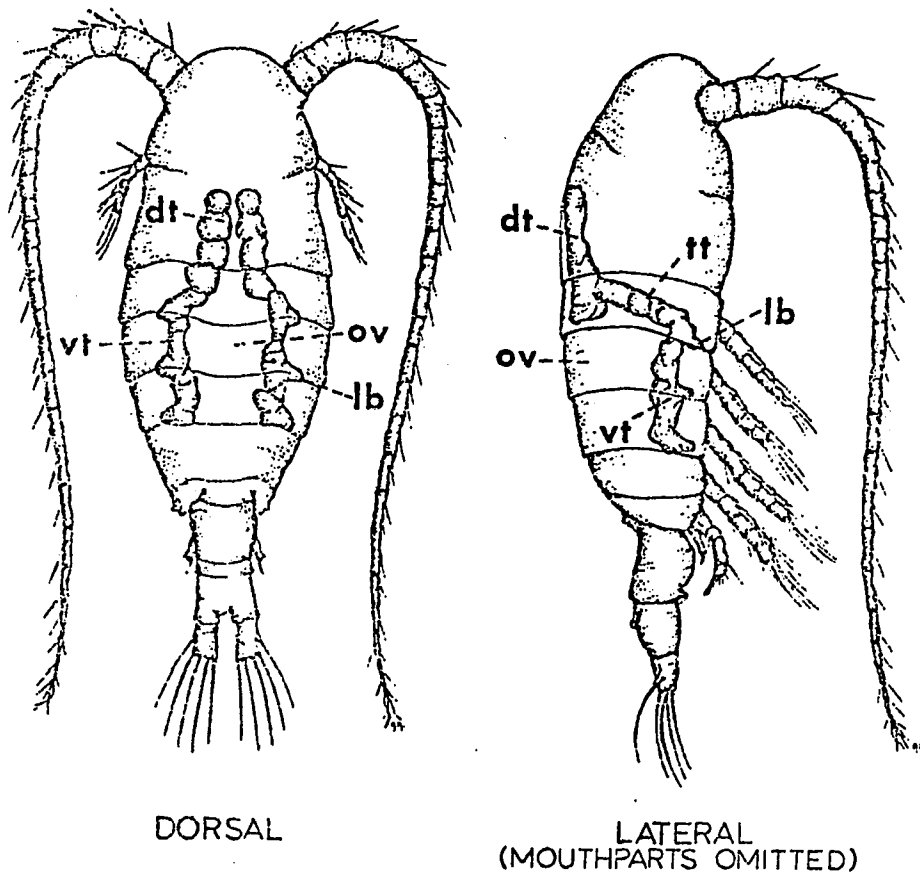


FIGURE 1

Figure 2. Pattern of oviducal changes for seven lab-reared D. leptopus females under controlled laboratory conditions (18°C; LD 8:16). For each adult, the upper horizontal line represents time in the dark phase (d); the lower horizontal line, time in the clear phase (c); the vertical lines are estimated phase onset times. Hash-marks indicate inspection times. Data to right of records are mean durations (days) with standard error in parentheses for each animal.

FIGURE 2

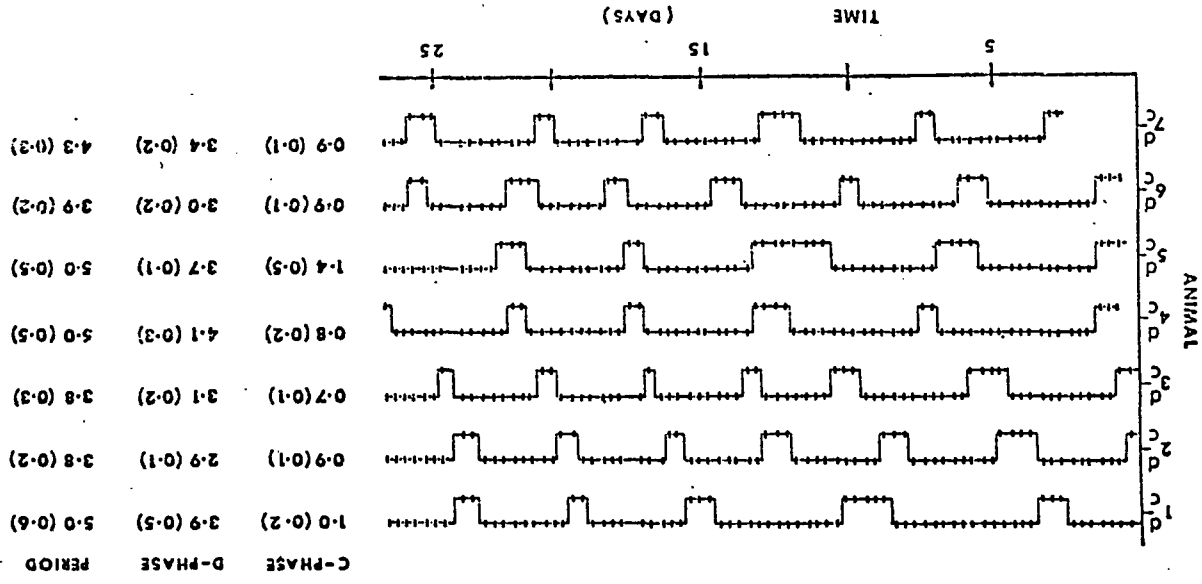


Figure 3. Power spectra of oviducal changes for seven female
D. leptopus, calculated from records shown on fig. 2. (O), 1
(●), 2; (□), 3; (■), 4; (△), 5; (▲), 6; (○), 7.

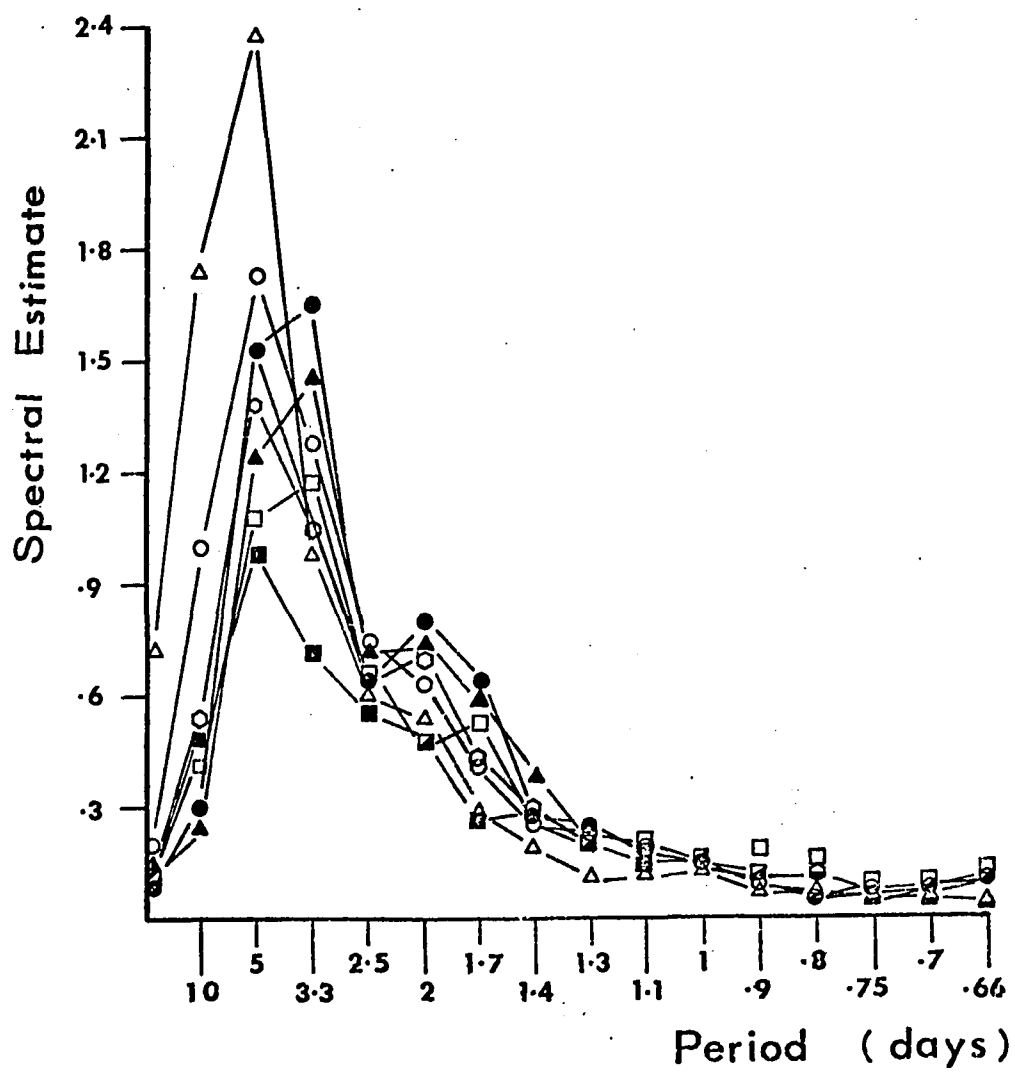


FIGURE 3

Figure 4. Changes in the percentage of the laboratory population in either phase of the gametogenic cycle with time. (●—●) pattern computed from records on figure 2, beginning at time of first dark phase observation of animal 7. (.....) pattern expected if synchrony was high. Insert A shows correlogram of these data (S, lag in days; r_s , autocorrelation).

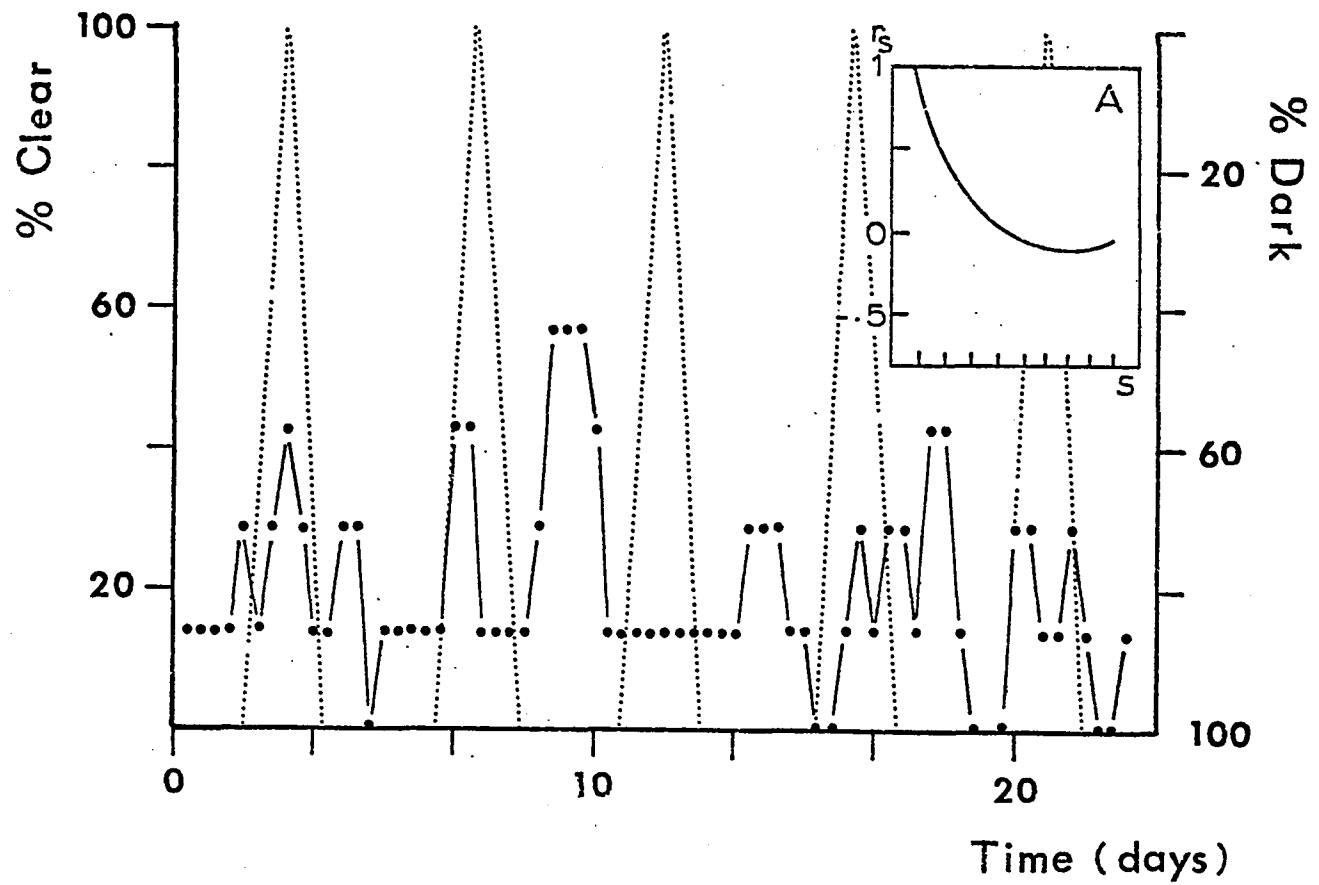


FIGURE 4

Figure 5. Relationship between dry wt (ug) and body length (prosome in mm) for dark phase (solid circles) and clear phase (open circles) D. leptopus females. Note \log_{10} axes. ΔW shows average weight difference over all body lengths. Insert A shows relation for small animals only.

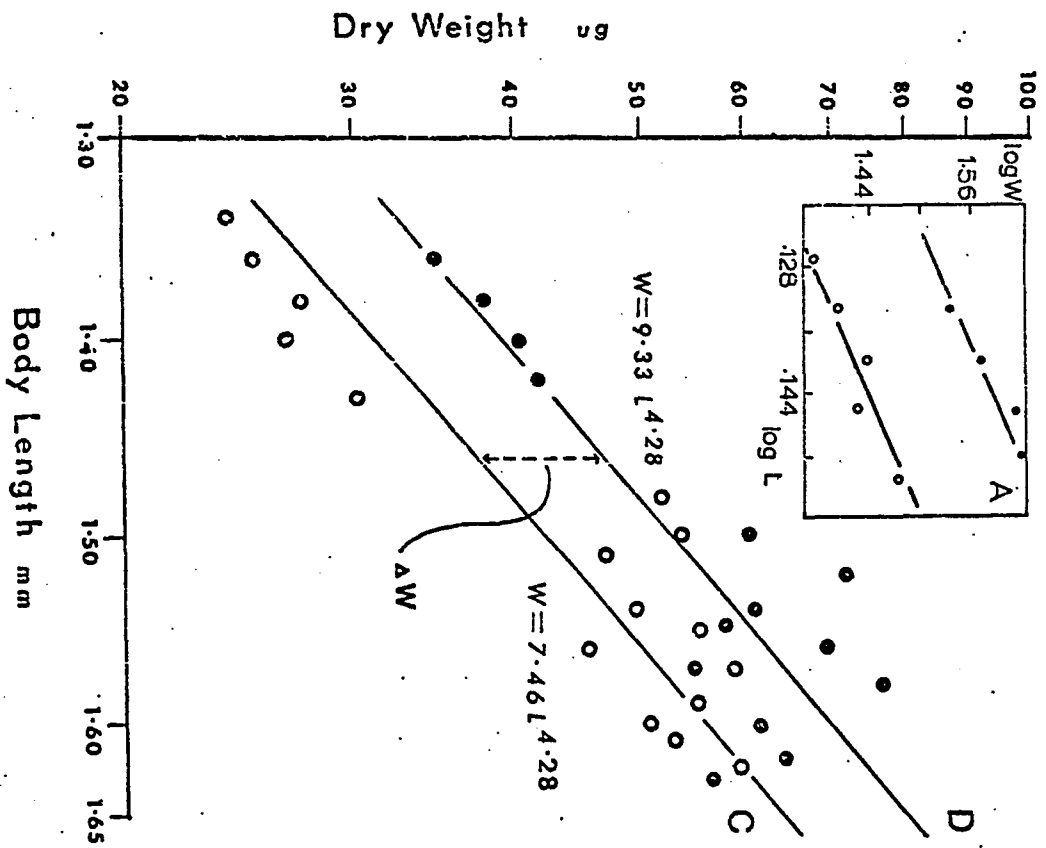


FIGURE 5

Figure 6. Changes in % dark phase females, (d), egg ratio, (e), and finite birth rate, (b), for field populations of D. leptopus during 1976 and 1977. (2) indicates that e and b fell on or near same point. Correlations shown on Table 3.

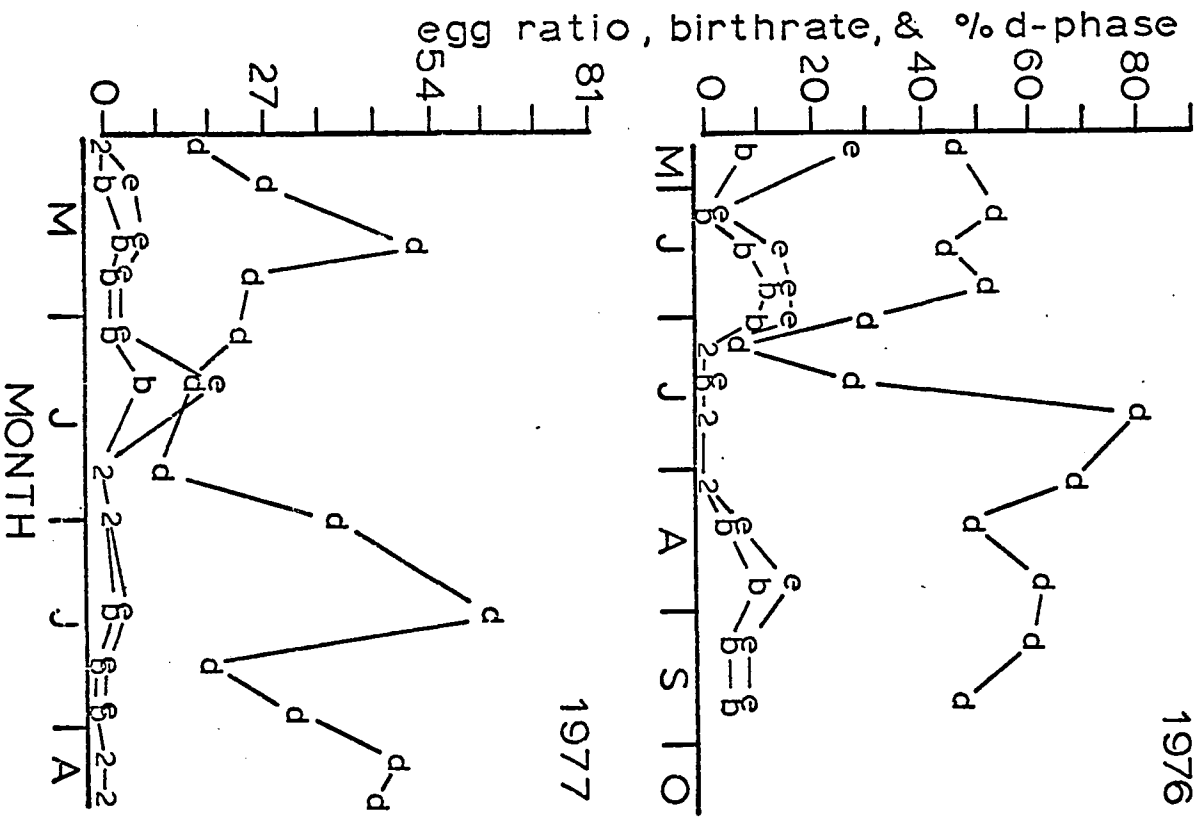


FIGURE 6

Figure 7. Pattern of clutch production resulting from insemination at different points of the gametogenic cycle at 18°C. Closed circles indicate production of a clutch. Open circles indicate detachment of clutch from female. In this example carrying time equals embryonic development time. Case 1): insemination follows immediately after ovisac detachment; interclutch time is zero. Case 2) insemination occurs a short time after ovisac detachment but prior to clear phase onset; interclutch time is greater than zero and rate of clutch production declines. Case 3) insemination occurs after clear phase onset; no subsequent clutch is produced and since successful reinsemination cannot occur until dark phase onset, rate of clutch production decreases further. Note that when a clutch is carried until hatching, less than 50% of the dark phase remains, reducing the probability of insemination before clear phase onset.

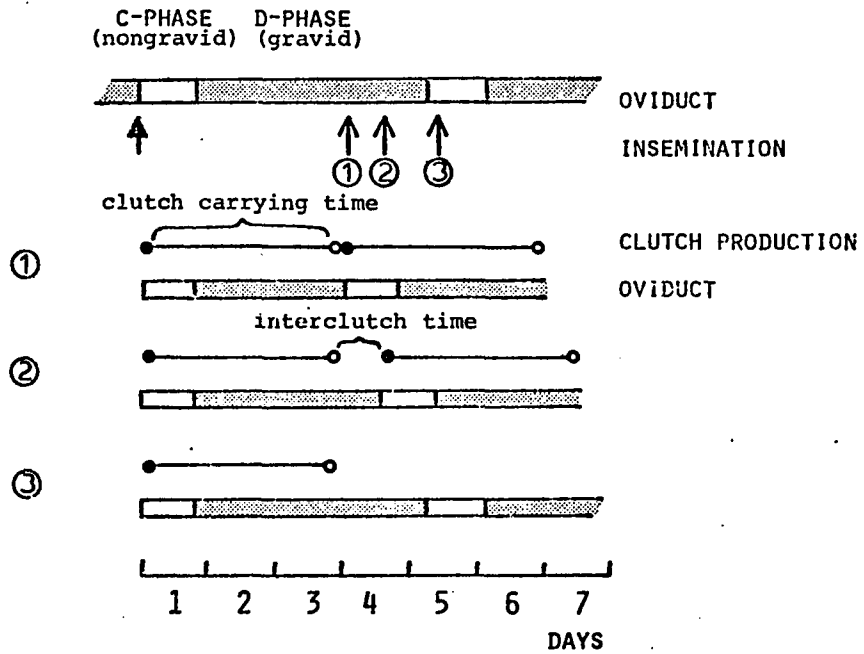


FIGURE 7

Figure 8. Probability of oocyte extrusion, (P') under natural conditions. (See text for explanation of symbols.)

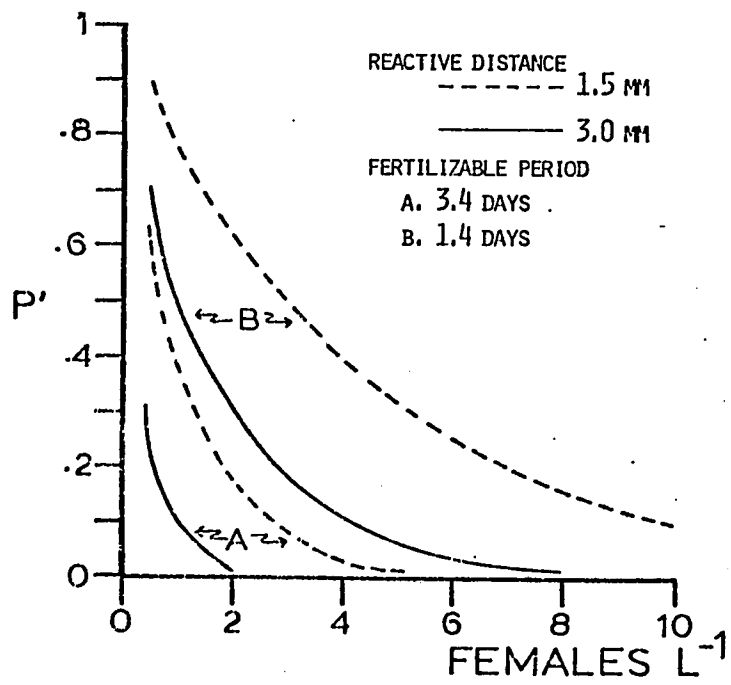


FIGURE 8

Table 1. Characteristics of D. leptopus' gametogenic cycle. (18°C; LD 8:16). Durations are grand means \pm 95% confidence limits, based on n females with number of onsets/female in parentheses (range).

characteristic	n	duration (days)
clear phase (nongravid)	7 (4-6)	0.9 \pm 0.2
dark phase (gravid)	7 (4-6)	3.4 \pm 0.4
cycle period	7 (4-6)	4.4 \pm 0.5

Table 2. Estimates of some parameters which effect rates of clutch production by D. leptopus at 18°C. Durations are means for the raw data (arithmetic) and \log_{10} transformed data (geometric) in days, with standard errors in parentheses.

Parameters	n	Duration	
		arithmetic mean	geometric mean
clear phase (isolated)	36	0.9 (0.07)	0.8 (1.06)
clear phase (mating)	35	1.4 (0.16)	1.1 (1.11)
interclutch time	35	1.0 (0.17)	0.5 (1.22)
embryonic development time (D)	14 ^a	3.0 (0.06)	3.0 (1.02)

^a number of clutches

Table 3. Correlations between the proportions of dark phase (gravid) females and other indicators of the field population's reproductive activity. Data from figure 6. (E, egg:females ratio; B, finite birth rate; M:F, males:females ratio; NA, data not available). None of the correlations significantly different from zero at $\alpha = 0.05$.

Percent dark phase females		
	<u>1976</u>	<u>1977</u>
E	-0.020	-0.004
B	-0.040	-0.262
M:F	NA	0.140

SECTION II
THE EFFECTS OF TEMPERATURE AND PHOTOPERIOD ON
THE CYCLE OF GAMETE PRODUCTION AND POTENTIAL
REPRODUCTIVE RATES OF DIAPTOMUS LEPTOPUS

Introduction

Investigators working with marine calanoids which produce ovisacs (Corkett and McLaren, 1969; Corkett and Zillioux, 1975) have maintained that maximal rates of egg-clutch production are determined by the duration of embryonic development. This assertion is based on the observations that ovisacs are generally carried until hatching and that a new clutch cannot be produced while another is still attached to a female. The investigations with Diaptomus leptopus described in Section I suggest that this rule may not hold for freshwater species and, perhaps, should be re-evaluated for all calanoids.

D. leptopus undergoes a cycle of gamete production which is weakly analagous to the menstrual cycle of some mammals. The relationship between this cycle and rates of egg-clutch production is shown diagrammatically on figure 9. Three cases are illustrated.

In the first, the duration of the nongravid phase and embryonic development are equivalent and the clutch is carried until hatching. In this case, the maximal rate of clutch production (i.e. when vectors A and D are approximately zero) is equal to the rate of embryonic development. Note, however, that the duration of the nongravid phase actually determines when successful insemination can recur.

In the second case, the female is gravid before the completion of development of her embryos. Ovisac detachment prior to hatching (symbolized by the broken line) would result in enhanced rates of clutch production relative to the rate of embryonic development. This would apply in the case of resting egg clutches, which are carried for a shorter time than subitaneous clutches and the premature detachment of subitaneous clutches which frequently occurs in lab without otherwise effecting development (Robertson et al., 1974). As described in Section I, Case II applies with D. leptopus at 18°C (photoperiod = 8:16, LD).

In the third case shown on figure 9, the female is not gravid until some time after her embryos hatch. This results in a physiologically mandated interclutch period (time between the detachment of one ovisac and the production of the next) which would result in decreased rates of clutch production relative to rates of embryonic development.

The purpose of the investigations described in Section II was to determine whether the relationship between gamete production and embryonic development was affected by environmental conditions. The effects of two abiotic factors, temperature and photoperiod, and one biotic factor, male-female interaction, were examined.

All three of these environmental factors have been shown to influence the development and reproductive cycles of a variety of organisms. For example, temperature is considered the dominant factor affecting rates of embryonic development in Diaptomus (Elster, 1954; Eckstein, 1964). But many biological cycles (most

notably circadian rhythms) are affected only slightly by temperature changes (Brown, 1972). So, it seemed possible that the gametogenic cycle might be influenced by temperature in a manner different from embryonic development, if at all. The literature on photoperiodism is enormous, but two papers are particularly relevant to the growth and reproduction of copepods. Spindler (1971) offered evidence that photoperiod influences the molting rhythm of Cyclops and Mullen (1968) concluded that light played an important role in egg-release by Calanus. Similarly, the onset-times and durations of D. leptopus' reproductive phases might also be related to photoperiod. Social interactions also have been shown to affect developmental and reproductive cycles. Whitten (1966) reported that the estrous cycles of female mice could be accelerated by the presence of males. In the present study, the effect of males on the duration of Diaptomus' nongravid phase was examined.

Methods

The methods used to maintain and observe animals in the laboratory were similar to those described in Section I. Immature D. leptopus copepodids (CI-CII) were collected from a small pond in Madbury, N.H., isolated in 30 ml. vials and reared to maturity under controlled laboratory conditions. Aliquots of mixed green algae from xenic cultures were added three times/week along with enough aerated, pre-filtered pond water to maintain the 25 ml volume with about 50,000 cells ml^{-1} . Sedimented cells and fecal material were removed by pipette, and half the volume of each vial was replaced with fresh, filtered pond water each week. The animals were

raised at six temperatures, ranging from 12° to 27°C ($\pm 0.5^\circ\text{C}$). Up to four different photoperiods were maintained at each temperature (LD 8:16, 12:12, 16:8, and 24:0). Illumination was provided by 7.5 or 10 watt incandescent bulbs suspended above the culture vials. Light intensity at the surface of the vials averaged about 8.5×10^{-5} watts cm^{-2} with light on and was not measurable with lights off. Survival to maturity was always 85% or higher.

Upon maturation the females were inspected with a dissecting scope at regular 8 or 12 h intervals, and the condition of the oviducts recorded. The oviducts of gravid females are filled with yolk-laden oocytes. Under low magnification (25x), they appear as a pair of dark bands lateral to the digestive tract. The oviducts of nongravid females are reduced and transparent. The animals were observed while swimming in their vials; none were removed or excessively disturbed during inspection. Red filters were used during night-time observations.

When a change in the oviducal state was noted, the point midway from the previous observation was designated the time of the change. The observations were continued until each female had progressed through three or more changes in the oviducal state (three or more gametogenic cycle phase-onsets). Mean cycle phase and period durations were computed for each animal and expressed to the nearest 0.1 day.

After the initial observation period, the females cultured at 24°, 21°, 18°, and 12°C were placed with males acclimated to the same physical conditions to estimate mating effects.

Results and Discussion

Photoperiod could exert an influence on several aspects of

Diaptomus' gametogenic cycle either directly or indirectly. For example, by affecting another activity pattern, such as diel feeding rhythms (Haney and Hall, 1975), changes in photoperiod could influence the intake of nutrients and indirectly affect the rate of yolk-deposition and oocyte growth which determines the duration of the nongravid phase. (This possibility is supported by the results of Corkett and McLaren (1969) which showed that low food levels resulted in increased interclutch times with Pseudocalanus.)

In the present investigation, the effects of photoperiod seemed negligible, however. A comparison of cycle phase durations at several photoperiods is shown on figure 10. (At 15°C and 27°C animals were cultured under one light regime only, so information about the influence of photoperiod at these temperatures if not available). Visual inspection of figure 10 suggests that the duration of the cycle's phases is independent on photoperiod. With one exception, statistical analysis of these data confirmed this impression.

T-tests at 24°, 18°, and 12°C and a single factor analysis of variance at 21°C indicated no significant differences in the duration of the gravid phase which could be attributed to photoperiod ($\alpha = 0.05$). The experimental design permitted investigation of a limited set of temperature-photoperiod interactions; two photoperiods, LD 8:16 and LD 16:8, were replicated at 24°, 21°, 18°, and 12°C. No significant photoperiod or interaction effect was indicated by a multiple regression analysis of these data ($\alpha = 0.05$).

Similar results were obtained with the nongravid phase at

24°, 21°, and 12°C. No significant differences in duration due to photoperiod were evident ($\alpha = 0.05$). Unexpectedly, at 18°C a statistically significant difference between the mean durations of the nongravid phase was indicated ($p < 0.05$). However, inspection of figure 10 shows that the magnitude of this difference is small (if real).

Examination of phase-onset times also suggested the lack of any significant influence of photoperiod. The onset of the nongravid phase is instantaneous relative to the onset of the gravid phase. (All the oocytes within the oviducts are voided in a few seconds but vitellogenesis in the new oocytes is a gradual process.) For this reason, only onsets of the nongravid phase were examined for temporal synchrony, the tendency for phase-onset to occur during a particular observation interval.

As shown on Table 4, the observed pattern of onset distribution was generally similar to that predicted by the operation of random events, with two exceptions: LD 8:16 at 24°C and LD 16:8 at 21°C. Since the photoperiod was opposite in these cases, it seems an unlikely causative agent. The strength of conclusions drawn from these data would be greater if the females had been inspected at closer time intervals. But, given this limitation, it appears that photoperiod is not an important factor.

During all the experiments, the females seemed to be cycling independently of each other or any obvious environmental cue. Changes in the percentage of nongravid females in the population over time reinforce this impression of randomness. The data collected at

24°, 18°, and 12°C were extensive and fluctuations in the percentage of nongravid females are shown for two photoperiods at each of these temperatures on figure 11. Oscillations (as opposed to irregular fluctuations) are evident in some instances (12°C, panel C; 18°C, panel B; 24°C, panel C) but since all the animals are cycling at about the same frequency, random phase alignments would tend to persist once established and produce low amplitude oscillations for a time. There is no tendency for these oscillations to be consistently associated with one photoperiod and they show a tendency to damp in all cases. These characteristics are consistent with the conclusion that neither photoperiod or temperature influences phase-relations between animals.

In contrast to the lack of photoperiod effects, temperature had a pronounced influence on gamete production. The mean phase durations and period for each individual are plotted as a function of temperature in figure 12. In these log-log plots, the duration of the gravid phase is an inverse linear function of temperature over the entire range of temperatures examined (figure 12A). This trend is not followed by the nongravid phase, where duration increased at the low and high temperatures (figure 12B). Since the cycle period equals the sum of these two phases, it is not surprising to find a curvilinear trend also in the plot of the cycle period versus temperature (figure 12C).

The linearized form of a power function was fit to the data on the gravid phase from figure 12, yielding the regression equation

$$\log_{10} (\text{gravid phase}) = 2.986 - 1.90 \log_{10} T$$

with the duration of the gravid phase in days and temperature in degrees Celsius. The regression was highly significant ($p < 0.0005$) with a correlation coefficient (r) of 0.93 and no indication of curvilinearity ($p < 0.05$). Transformed to the original variables this regression equation becomes

$$\text{gravid phase} = 968 T^{-1.9}.$$

The same power function model was fit to the data on the nongravid phase and the cycle period. As suggested by visual inspection of the plots, curvilinearity was indicated in both sets of data by a significant reduction in the error-mean-square due to fitting a second order model ($p < 0.0001$).

Fitting a quadratic model to the data on the nongravid phase yielded the relation

$$\log_{10}(\text{nongravid phase}) = 16.01 - 24.76(\log_{10}T) + 9.56(\log_{10}T)^2.$$

The regression was statistically significant ($p < 0.0005$), with a correlation coefficient (R) of 0.62. Transformed to the original variables, the function

$$\text{nongravid phase} = 1.02 \times 10^{16} T^{-24.76} + 9.56 \log T$$

was obtained.

Fitting the same model to the data on the cycle period yielded

$$\log_{10}(\text{period}) = 9.46 - 12.41(\log_{10}T) + 4.32(\log_{10}T)^2$$

or

$$\text{period} = 2.88 \times 10^9 T^{-12.41} + 4.32 \log T$$

Again, the regression was highly significant ($p < 0.0005$) with $R = 0.93$.

From the data shown on figure 12, grand means of each of the three cycle characteristics were computed at every temperature. These data are listed on Table 5. The values obtained for the duration of the nongravid phase seem to indicate an area of temperature independence between 18°C and 24°C. But these results are consistent with the shape of the regression function described above, which would assume a minimum value near 20°C, and do not denote the lack of temperature effect. It will be shown below that 20°C may be an optimal temperature for egg-clutch production by D. leptopus.

After sufficient data were obtained with isolated females, males were added to each vial and the production of gametes and clutches monitored simultaneously. Since every female subsequently produced clutches, rearing conditions had not caused obvious reproductive aberrations. Changes in the duration of the nongravid phase due to mating are shown for three temperatures on figure 13 (data at 12°C were insufficient for reliable comparisons). Visual inspection of these data suggests that mating does not effect the duration of the nongravid phase. At 24° and 18°C no statistically significant differences due to mating status were detectable ($\alpha = 0.05$). However, at 21°C isolated females had a longer non-gravid phase than mating females ($p < 0.05$). Although most of the data collected indicate that interactions between males and females have no influence on the nongravid phase, this exception at 21°C suggests that further investigations might uncover an interesting mate-dependent mechanism.

As shown on figure 13, embryonic development exceeded the

nongravid phase at every temperature examined. To develop this comparison further, the relationship between embryonic development (D, in days) and temperature (T, in °C) was defined by regression techniques. Using the mean times for embryonic development at 24°, 21°, 18° and 12°C, the following equation was obtained

$$\log_{10}D = 2.6 - 1.7 \log_{10}T \quad (r = 0.99)$$

or

$$D = 407T^{-1.7}.$$

This equation was then compared graphically with the function described earlier for the nongravid phase.

As shown on figure 14 females would be gravid before the hatching of a clutch at temperatures between 9° and 26°C. Within this range of temperatures, then, clutches could be produced at a faster rate than that predicted from the duration of embryonic development. This potential increase is greatest at about 16°C, where clutches could be produced about three times faster than embryonic development indicates. Maximal rates of clutch production could occur around 20°C, where the nongravid phase is minimized.

Under laboratory conditions, the average carrying-time for clutches of resting eggs ranged from about 70% to 80% of the development time for D. leptopus' subitaneous eggs. This decrease in carrying time translates into a potential increase of 25% to 43% in rates of clutch production. Minimal carrying times for clutches produced in lab were about 0.3, 1.0, 0.8 and 1.5 days at 24°, 21°, 18° and 12°C, respectively. When compared to the duration of the nongravid phase (Table 5), these data indicate that this species is

capable of reaching its physiological potential in terms of clutch production.

The two curves on figure 14 intersect at 9° and 26°C. Outside these limits, the nongravid phase would exceed embryonic development so females would not be gravid when a clutch hatched (cf. case III, figure 9). Maximal rates of clutch production would be lower than those predicted from the duration of embryonic development because of physiologically mandatory interclutch times. Kuzichkin (1975) reported a similar phenomenon with Leptodora at high temperatures. Embryos in the brood pouch developed at a faster rate than gametes were produced by the ovary.

These predicted changes in interclutch time are supported, in part, by other direct observations. Robertson et al. (1974) reported carrying-times and interclutch times for mating pairs of D. clavipes cultured at several temperatures. Average interclutch times were lowest at intermediate temperatures and increased at the extremes. Rates of clutch production followed the same trend. Similar observations were made with D. leptopus in the present study. Average interclutch times of 2.2, 0.4 and 1.2 days were obtained at 24°, 21°, and 18°C, respectively, and mean rates of clutch production followed the same trend.

However, as mentioned in Section I, such direct determinations tell us little about a species' potential. Since mating is required before the production of every clutch, interclutch time has two components: 1) the time needed to produce a new batch of ripe gametes (i.e. the nongravid phase), which is physiologically determined, and;

2) the time needed for mate-location and successful insemination, which is behavioral. In the present study, even though females were gravid upon ovisac detachment and rapid encounter with a mate was assured (effective density 120 animals liter⁻¹, sex ratio 2:1 (m:f), interclutch time was greater than zero at all temperatures. This may be due to the behavioral component.

Frequency histograms of interclutch times suggest this is the case with D. leptopus and, perhaps, D. clavipes (although data on the physiological component for D. clavipes is unavailable). As shown on figure 15, all the histograms are positively skewed, with the mode at or near the predicted value, zero. Extreme values may be due to behavioral interference; if so, the mean values are artificial. In any case, these direct estimates by themselves do not provide an adequate estimate of physiologically minimal interclutch times or a reliable indication of those likely under natural conditions. As suggested in Section I, examining the physiological and behavioral components separately provides less ambiguous information.

Since Diaptomus does not molt after reaching sexual maturity, all secondary production attributable to the adult population is due to reproductive output (Comita, 1964; Kibby, 1971). With a knowledge of gametogenic patterns, upper and lower bounds on reproductive output can be set. Gametes cannot be released at a rate faster than they are produced, so the reciprocal of the nongravid phase determines the maximal output of adult females. Multiplying this rate by the mass of oocytes in an average clutch (25% adult body weight (dry) was reported as a qualified estimate for D. leptopus in Section I) yields the upper

limit for secondary production. Non-reproducing females would release gametes at a rate equal to the reciprocal of the gametogenic cycle period. Again, multiplying by the clutch size yields an estimate of the lower limit for secondary production. Changes in these limits due to temperature are shown by the upper and lower curves on figure 16. The distance between these two curves indicates the range of potential values for secondary production due to different reproductive schedules.

The intermediate curve shown on figure 16 indicates the maximal level of productivity expected when subitaneous clutches of average size are carried to hatching. Without information on the cycle of gamete production, the distance between this curve and baseline would probably be considered as limits for D. leptopus.

Although the importance of considering gametogenic patterns when examining reproductive rates in populations of Diaptomus can be demonstrated, the effort required to obtain this information may limit its applicability. For this reason, a note on methodology seems justified at this point.

Edmondson (1960, 1965) described a method for estimating the duration of embryonic development which is less time consuming than repeatedly observing egg-production by individual females. Ovigerous females are collected and observed periodically until all the clutches have hatched. The number of ovigerous females remaining in culture is plotted over time and a straight line is fit to the data. The point at which the line intersects the time axis represents the mean duration of development.

Since neither interanimal synchrony nor systematic changes due to aging were observed in the present investigations, the above technique could also be used to determine patterns of gamete production. Gravid and nongravid ovigerous females could be collected and frequently observed as a group until the condition of their oviducts changed and their eggs hatched. Data would be collected and analyzed as described above for embryonic development. Using ovigerous females would ensure that the nongravid animals were sexually mature and that the mature gametes of gravid females had not been fertilized. It would also permit the simultaneous determination of the duration of embryonic development and gametogenic patterns which would simplify comparative studies with this genus.

In conclusion, the results of this section show that D. leptopus' gametogenic patterns are, generally, unaffected by photoperiod or mating status but are strongly dependent on temperature. The nongravid phase of the gametogenic cycle assumed a minimal value near 20°C, increasing at higher or lower temperatures. In contrast, the duration of embryonic development was inversely related to temperature over the range of temperatures examined. Consequently, between 9° and 26°C clutches of subitaneous eggs could be produced faster than they hatch. However, at temperatures outside these limits embryos develop more rapidly than gametes are produced and reproductive potential declines dramatically.

These results point to the importance of considering gametogenic patterns when estimating the effect of environmental factors on the reproductive potential of D. leptopus. In the next section, the gametogenic patterns of three additional diaptomid species will

be examined to establish the generality of reproductive oscillations within the genus and to investigate the effect of genetic factors on reproductive potential.

Bibliography

- Brown, F. A., Jr. 1972. The "clocks" timing biological rhythms. Am. Sci. 60: 756-766.
- Comita, G. W. 1964. The energy budget of Diaptomus siciloides, Lilljeborg. Verh. Int. Ver. Limnol. 15: 646-653.
- Corkett, C. J. and I. A. McLaren. 1969. Egg production and oil storage by the copepod Pseudocalanus in the laboratory. J. Mar. Biol. Ecol. 3: 90-105.
- _____ and E. J. Zillioux. 1975. Studies on the effect of temperature on the egg laying of three species of calanoid copepods in the laboratory. Bull. Plankton Soc. Japan 21: 77-85.
- Eckstein, H. 1964. Untersuchungen unter den einfluss des Rheinwassers auf die limnologie des Schlussee. Arch. Hydrobiol./suppl. 28: 118-182.
- Edmondson, W. T. 1960. Reproductive rates of rotifers in natural populations. Memorie Ist. Ital. Idrobiol. 12: 21-77.
- _____. 1965. Reproductive rate of planktonic rotifers as related to food and temperature in nature. Ecol Monogr. 35(1): 61-111.
- Elster, H-J. 1954. Uber die Populationsdynamic von Eudiaptomus gracilis Sars und Heterocope borealis Fisher im Bodensee-Obersee. Arch. Hydrobiol./suppl. 20: 546-614.
- Geiling, W. T. and R. S. Campbell. 1972. The effect of temperature on the development rate of the major life stages of Diaptomus pallidus Herrick. Limnol. Oceanogr. 17(2): 304-307.

- Haney, J. F. and D. J. Hall. 1975. Diel vertical migration and filter-feeding activities of Daphnia. Arch. Hydrobiol. 75(4): 413-441.
- Kibby, H. V. 1971. Energetics and dynamics of Diaptomus gracilis. Ecol. monogr. 41: 311-326.
- Kuzichkin, A. P. 1975. Effect of temperature on the seasonal population dynamics and fecundity of Leptodora kindti (Focke) (Cladocera) in the vicinity of point of discharge of warm effluent of the Konakouo power plant. Hydrobiol. J. 11(2): 53-56.
- Mullin, C. H. 1968. Egg-laying in the planktonic copepod Calanus helgolandicus (Claus). Crustaceana/Suppl. 1: 29-34.
- Robertson, A., C. W. Gehrs, B. D. Hardin, and G. W. Hunt. 1974. Culturing and ecology of Diaptomus clavipes and Cyclops vernalis. U.S. Environmental Protection Agency, Wash. D.C. (EPA - 660/3-74-006).
- Spindler, K-D. 1971. Untersuchungen über den Einfluss ausser Factorer auf die Dauer der Embryonalentwicklung und den Hautungsrhythmus von Cyclops vicinus. Oecologia 7: 342-355.
- Whitten, W. K. 1966. Pheromones and mammalian reproduction. Advan. Repro. Physiol. 1: 155-177.

Figure 9. Reproductive cycles which show three possible relationships between the production of oocytes and clutches of fertilized eggs. Resultant rates of clutch-production depend on lengths of vectors representing time, as explained in text.

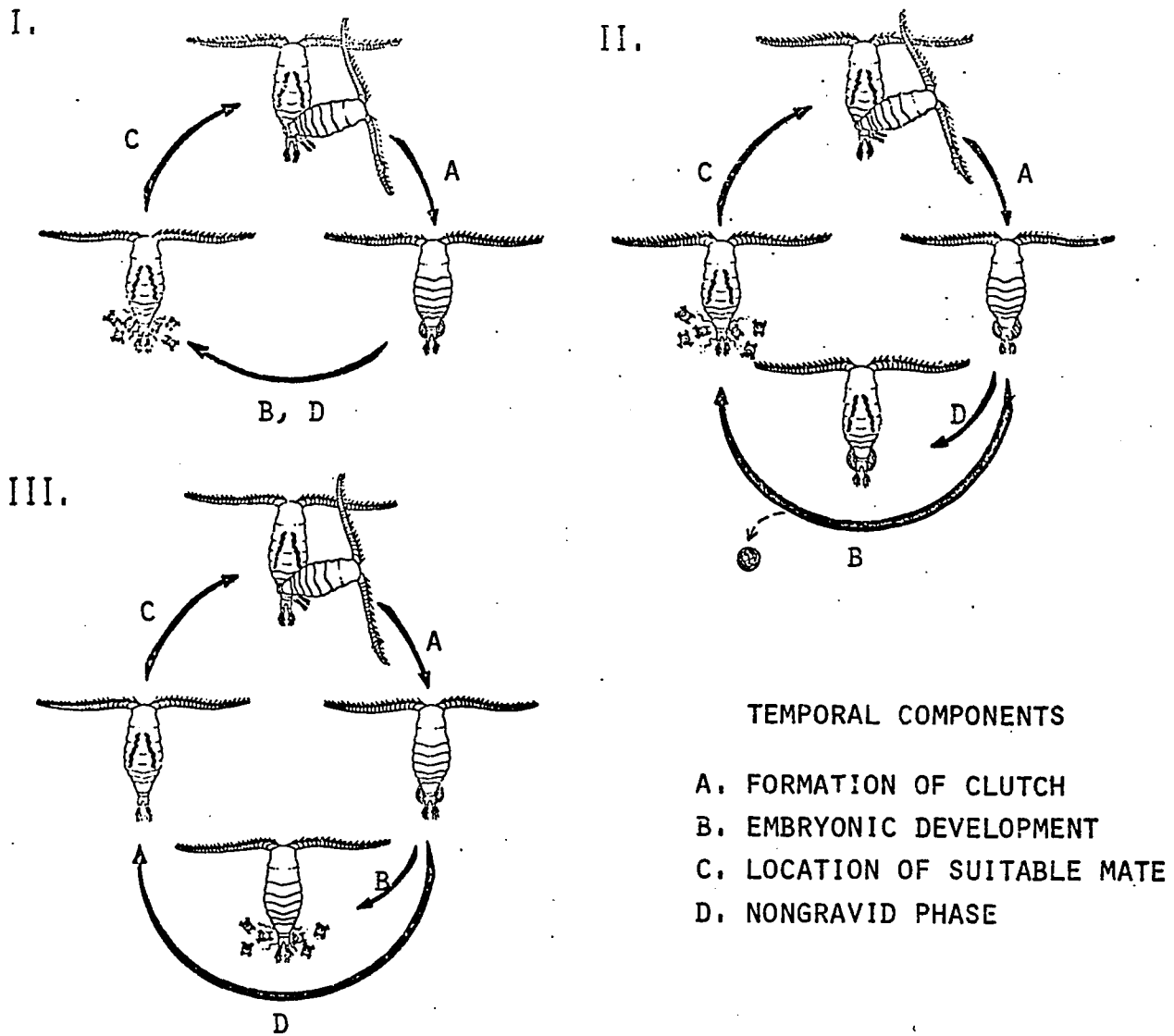


FIGURE 9

Figure 10. Comparison of the duration of the gravid and nongravid phases at different photoperiods (Vertical bars show \pm standard error; photoperiods (L:D): I = 8:16; II = 12:12, III = 16:8, IV = 24:0).

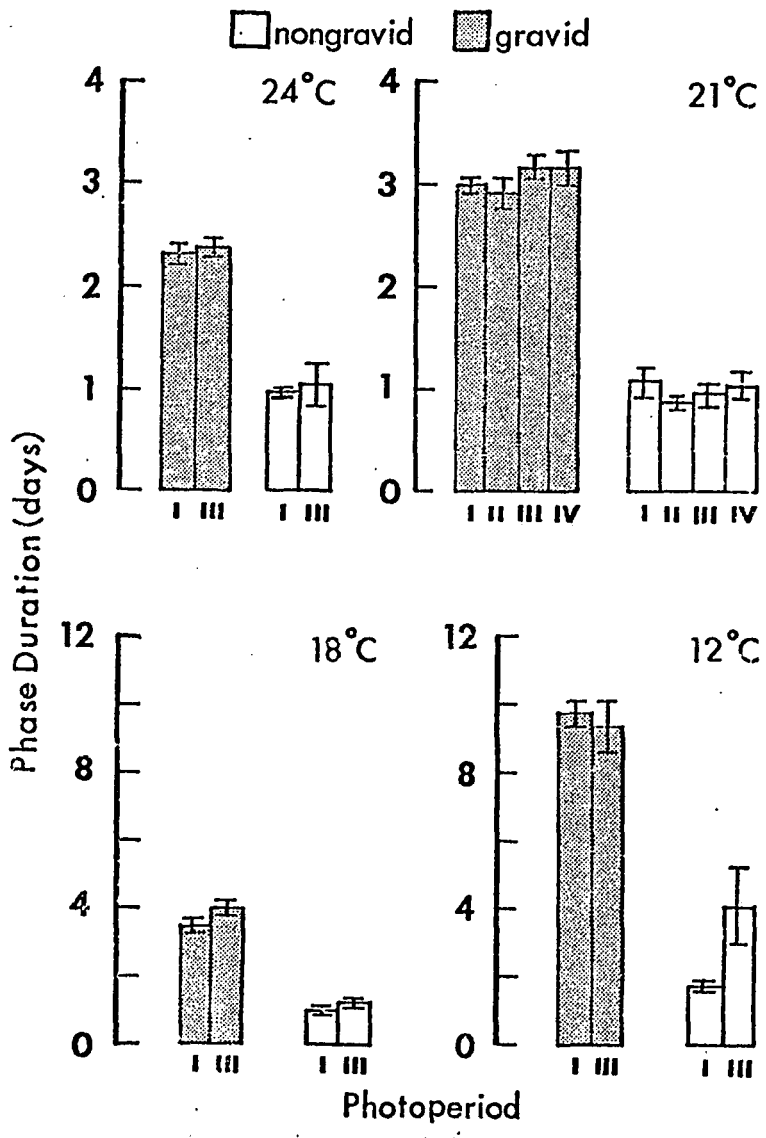


FIGURE 10

Figure 11. Phase relations between isolated D. leptopus females at 12°, 18°, and 24°C. ▼ indicate predicted succession of peaks, given mean cycle period, starting with first peak in record. A = pooled photoperiods; B = LD 16:8; C = LD 8:16.

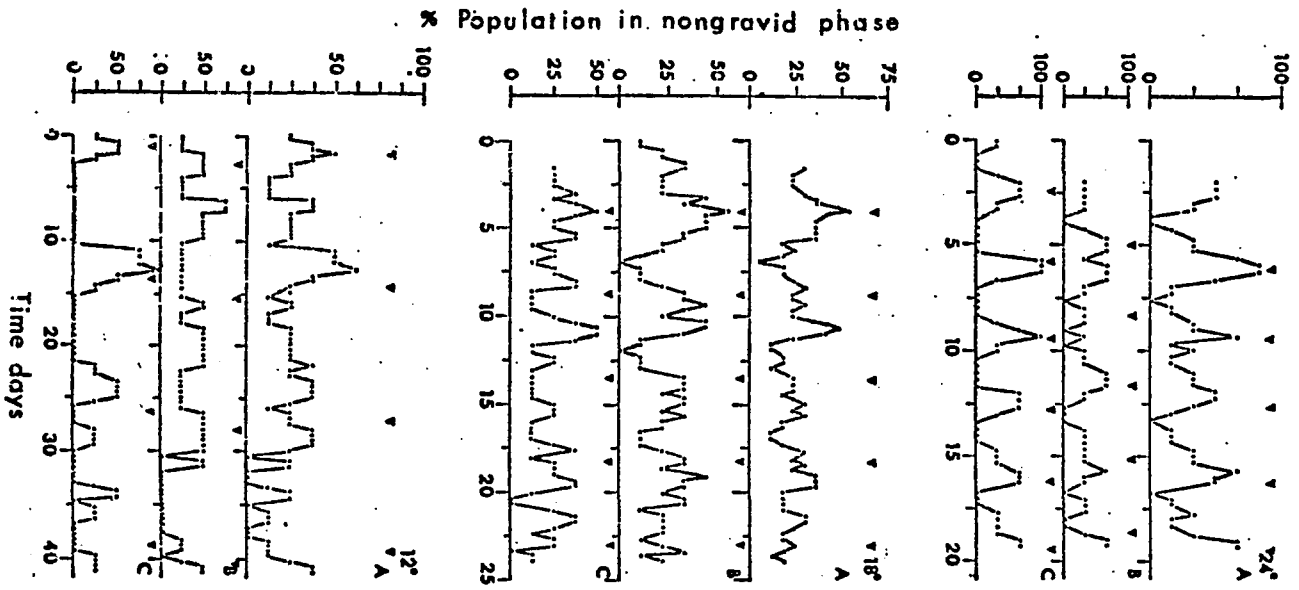


FIGURE 11

Figure 12. Relationship between gametogenic patterns and temperature.
(Solid circles indicate single observations; numerals indicate multiple observations at same point. Each observation is the mean of three or more estimates from a single animal.)

Figure 13. Comparison of observed durations for the nongravid phase (isolated and mating females) and embryonic development. (Bars shown \pm 1 standard deviation.)

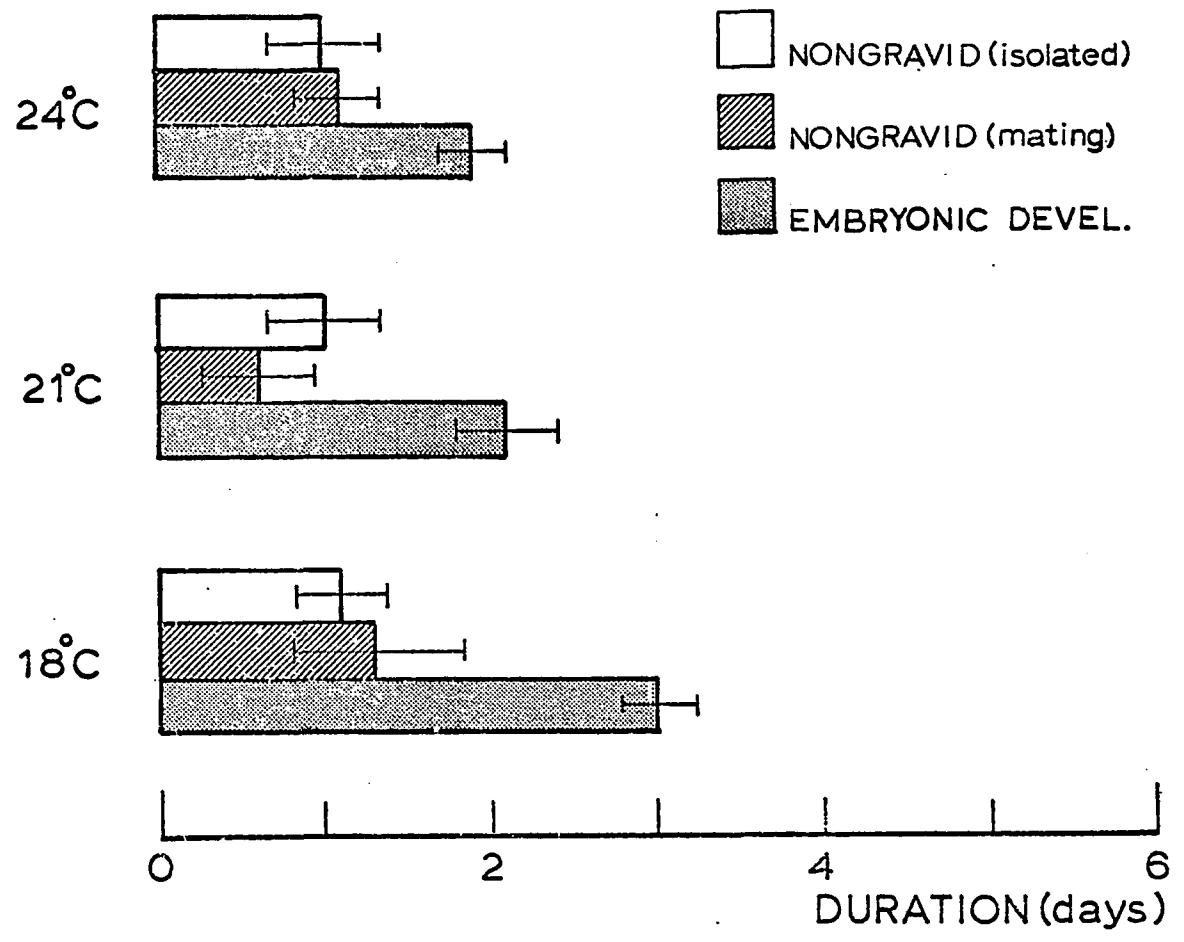


FIGURE 13

Figure 14. Comparison of predicted values for the nongravid phase and the duration of embryonic development. (See text for regression techniques.)

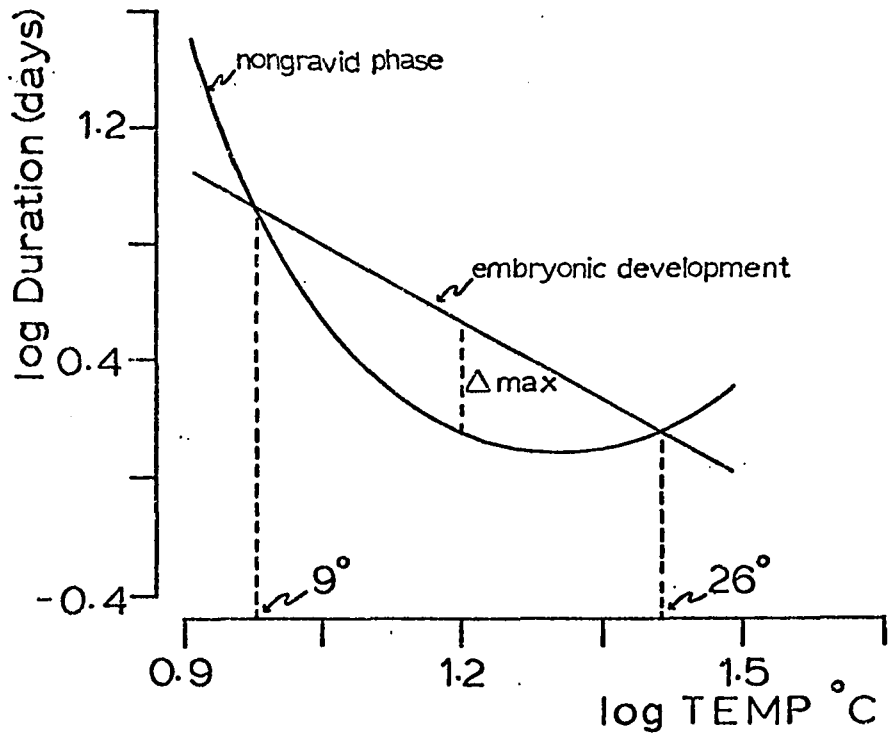


FIGURE 14

Figure 15. Interclutch times estimated directly with mating
D. leptopus (this study) and D. clavipes (data from
Robertson et al., 1974).

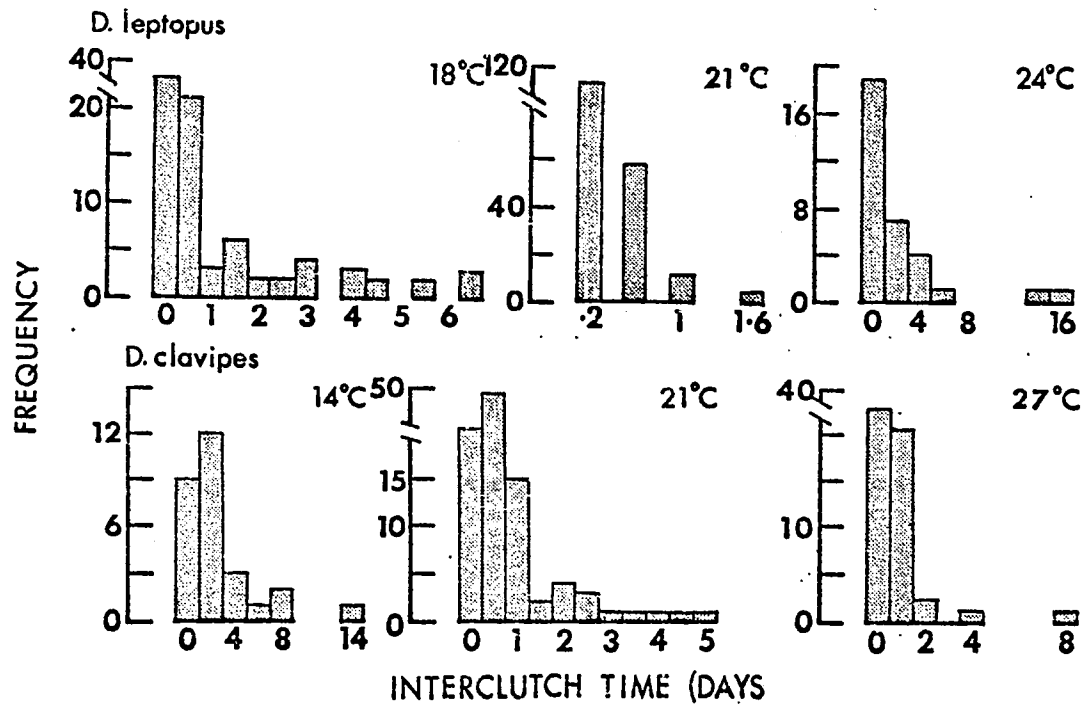


FIGURE 15

Figure 16. Limits on secondary production by female D. leptopus as a function of temperature (derivation of curves explained in text).

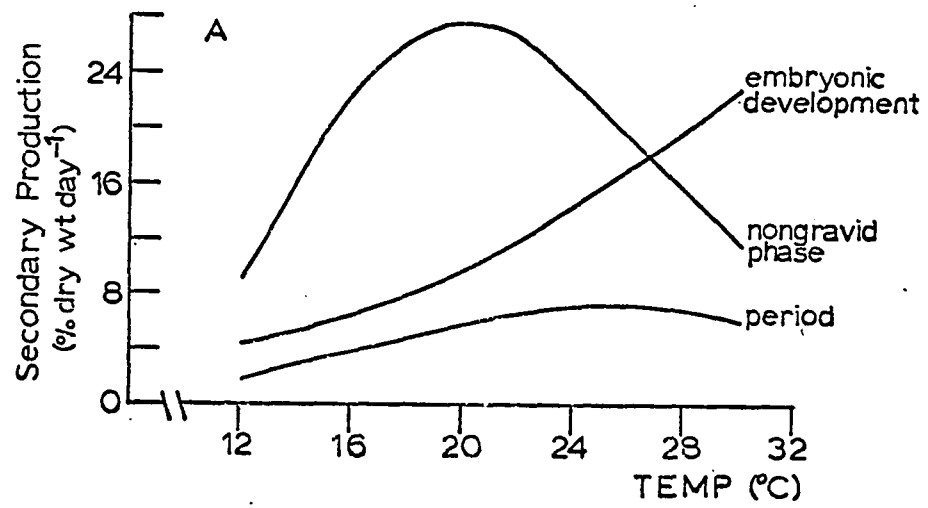


FIGURE 16

Table 4. Temporal distribution of onset-times for the nongravid phase. Inspection times:

3/Day = 0800, 1500, 2200 hrs.; 2/Day = 1000, 2200 hrs.

Temp. (°C)	LD	Inspections /Day	No. Onsets	Distribution		χ^2
				Obs	Exp	
27	16:8	2	29	10:19	14.5:14.5	2.79
24	8:16	3	18	5:1:12	5.25:5.25:7.5	6.17*
	16:8	3	23		6.7:6.7:9.6	1.54
21	8:16	2	16	10:6	8:8	1.00
	12:12	2	30	13:17	15:15	0.53
	16:8	2	21	6:15	10.5:10.5	3.85*
	24:0	2	26	13:13	13:13	0.00
18	8:16	3	38	16:5:17	11.1:11.1:15:8	5.60
	16:8	3	43	18:11:14	12.5:12.5:17.9	3.53
15	16:8	2	20	9:11	10:10	0.20
12	8:16	2	13	5:8	6.5:6.5	0.68
	16:8	2	14	8:6	7:7	0.29

*significant at 0.05

Table 5. Characteristics of D. leptopus' gametogenic cycle at different temperatures (photoperiods pooled.) Durations are grand means \pm 95% confidence limits, based on n females with the number of onsets/female in parentheses (range).

Temp (°C)	n	Duration (days)		
		Nongravid Phase	Gravid Phase	Period
12	8(2 to 4)	3.0 (\pm 1.6)	9.6 (\pm 0.9)	12.5 (\pm 1.4)
15	11(1 to 2)	1.4 (\pm 0.4)	5.4 (\pm 0.5)	6.8 (\pm 0.4)
18	18(2 to 6)	1.1 (\pm 0.1)	3.8 (\pm 0.3)	4.9 (\pm 0.4)
21	34(1 to 3)	1.0 (\pm 0.1)	3.1 (\pm 0.1)	4.0 (\pm 0.2)
24	8(3 to 6)	1.0 (\pm 0.3)	2.4 (\pm 0.1)	3.4 (\pm 0.4)
27	9(1 to 4)	1.7 (\pm 0.5)	1.9 (\pm 0.4)	3.6 (\pm 0.6)

SECTION III
COMPARISON OF THE REPRODUCTIVE CYCLES
OF FOUR DIAPTOMID COPEPODS

Introduction

One factor which determines the size of zooplankton populations is the rate of reproduction. The reproductive rates of planktonic copepods like Diaptomus (which carry their embryos in ovisacs) have two principal components, clutch size and the rate of clutch production. Although the specific mechanisms controlling clutch size in Diaptomus are not entirely clear, several authors have reported the range of clutch sizes produced in field and laboratory populations of various species (Ravera, 1955; Roen, 1955; Ravera and Tonolli, 1956; David, 1961; Cole, 1966; Smyly, 1968; Maly, 1973; Robertson et al., 1974). In contrast, as Chapman (1969) noted, there is still a lack of field or experimental data on rates of clutch production by individual diaptomids. Eckstein (1964) provided limited data on the average time between clutches for D. gracilis and D. vulgaris, but the only extensive investigation of clutch production to date seems to be that of Robertson et al. (1974) who worked with D. clavipes.

One reason for this paucity of data may be the inability to maintain Diaptomus in laboratory cultures. Tonolli (1971) noted that freshwater calanoids are particularly difficult to rear under controlled conditions. In anticipation of this difficulty, Robertson et al. (1974) developed a reasonably reproducible technique for culturing D. clavipes before attempting to examine reproductive parameters.

In the present investigation, a similar technique developed with D. leptopus (Section I and II) has been used successfully with several additional species.

Diaptomus require remating before the production of each clutch (Eckstein, 1964; Robertson et al., 1974) for unlike many marine species, female diaptomids are apparently unable to store sperm for extended periods. As a result, rates of clutch production have both a physiological and behavioral component, i.e. gamete production and mate location. Unless the behavioral component can be controlled, reliable data on the physiological capacity of a species cannot be obtained via the techniques used successfully with parthenogenic zooplankton (e.g. Hall, 1964) and also employed by Eckstein (1964) and Robertson et al. (1974) (i.e. periodically examining a single female - in this case, cultured with males - and recording the presence or absence of embryos).

It may be possible to avoid the problems of mating behavior. Observations of D. leptopus (Sections I and II) showed that the females oscillated between gravid and nongravid reproductive conditions. When a female is gravid, the oviducts contain mature primary oocytes and appear as a pair of dark bands in the prosome. When nongravid, the oviducts are not visible under low magnification. The duration of the nongravid phase represents the time needed to generate a new batch of ripe gametes and, as such, it delimits the minimal time (physiologically) between the production of successive clutches. By examining the effects of environmental factors on the cycle of gamete production, then, constraints on rates of clutch production can be examined without the complications introduced by obligate sexuality.

The generality of this cycle within the genus remains to be demonstrated, however. The objectives of this Section are to 1) describe and compare patterns of gamete production observed with four diaptomid species, 2) show how these patterns relate to reproductive potential, in terms of clutch production rate and, 3) examine the relationship between gametogenic patterns, reproductive potential and habitat.

Methods

To minimize intraspecific variability and ensure acclimation of the adult animals, immature copepodids (CI - CIII) were collected in the field, isolated in 30 ml vials and reared to maturity under experimental conditions. Food levels in the vials were maintained at about 5×10^4 algal cells ml^{-1} (using Westella, Scenedesmus, Ankistrodesmus and Chlorella from xenic cultures). Temperature was controlled and reasonably constant. Illumination was provided by 7.5 or 10 watt bulbs suspended above the vials; light intensity with the lamps on averaged about 7.5×10^{-5} watts cm^{-2} . Dissolved oxygen varied from about 89% - 100% of saturation; pH from 7.8 to 8.2. Survival to maturity was 80% - 100%.

The species compared were D. dorsalis from a clear reservoir near Miami, Florida (collected January, 1976 by Dr. C. Buchanan), D. pallidus from a turbid pond in the Raleigh-Durham area of North Carolina (collected February, 1975 by Dr. J. Haney) and D. pygmaeus collected during May, 1976, from Stonehouse Pond in Barrington, N.H. D. dorsalis and D. pallidus were cultured at 23°C ($\pm 1^\circ\text{C}$) under a single photoperiod (LD 12:12). D. pygmaeus were cultured at 12° , 18° and 24°C

($\pm 0.5^{\circ}\text{C}$) with two photoperiods at each temperature (LD 8:16 and 16:8). Previously reported data (Section II) for D. leptopus are included in this Section to extend the comparisons.

To characterize the gametogenic cycles of these species, the oviducts of free-swimming animals were observed at 8 or 12 h intervals with a dissecting microscope (10x, transmitted light, at night red filters were used). During each inspection the condition of the oviducts was recorded as either dark (gravid) or clear (nongravid). When a change in the condition of the oviducts was noted, the midpoint between the last two observations was designated the time of the change. The duration of each phase and the cycle period was calculated to the nearest 0.1 day.

After collecting sufficient data on the gametogenic cycle, the frequency of egg-clutch production and the duration of embryonic development were determined for comparison with parameters of the cycle. These investigations were performed only with D. pygmaeus. Two males were placed with each female and the three animals were observed as before. The average time from the production of one egg-clutch to the production of a subsequent clutch was determined and the reciprocal of this time used to estimate the frequency of clutch production by individual females. Oviducal changes were also monitored to determine the effects of mating on the duration of the nongravid (clear) phase. The durations of the gravid (dark) phase and cycle period were not recorded. (These two parameters vary with mating frequency, since successful insemination leads immediately to voiding of the oviducts and ovisac formation).

To examine the effects of heavily pigmented oviducts on Diaptomus' ability to avoid visual predators, equal numbers of gravid (dark) and nongravid (clear) copepods were offered to single fish in a small aquarium (25 x 18 x 15 cm with rounded corners). Small Micropterus salmoides³ (35 to 40 mm) were seined locally, maintained in lab at the experimental temperature (16°C) on a diet of mixed plankton (including diaptomids) for 5 to 10 days. The fish were then acclimated to the small aquarium without food for 6 to 12 hours prior to the addition of prey. To simulate a natural background, the experimental aquarium was centered in a chamber of black plastic (2 x 1 x 1m) with a grey stone bottom. Between the black walls and the small aquarium, larger aquaria filled with pond water were placed on three sides. The fourth side remained open for observation. Light was provided by florescent tubes above the aquaria (intensity, 1.6×10^{-4} watts cm^{-2} at mid-depth). Before each trial 25 gravid and 25 nongravid copepods were placed in HA Millipore filtered pond water until their digestive tracts were clear. The copepods were then poured into the experimental aquarium on the side of a clear plastic divider opposite the fish. After a few minutes the divider was removed and the fish allowed to feed until 20% to 30% of the food items were eaten. (Removal of a larger number of prey may so alter the prey ratio that predation pressure is automatically shifted to the less attractive but now more abundant prey item - see Zaret, 1972). Feeding was terminated by tapping on the aquarium;

³Preliminary experiments with Micropterus and Leptomis showed that Micropterus was a more satisfactory experimental predator. Leptomis took longer to acclimate to experimental conditions, was more easily disturbed during feeding and had a higher ratio misses:strikes with copepods; this complicated prey-preference determinations unnecessarily. Furthermore, several studies have shown that young (25-40 mm) largemouth bass feed heavily on copepods (including Diaptomus) in natural situations (e.g. Applegate and Mullan, 1968; Cooper, 1936; Ewers, 1933).

the fish was removed and the remaining copepods counted. Selection was estimated using chi-square tests. Ten trials were done with D. leptopus and six with D. pygmaeus.

Results and Discussion

Several interspecific similarities were noted. First, all the species oscillated between the gravid (dark oviduct) and nongravid (clear oviduct) reproductive conditions, suggesting that this type of reproductive cycling is characteristic of the genus (figure 17). Secondly, photoperiod did not effect the cycle characteristics. As shown previously for D. leptopus (Section II), no significant effect of photoperiod was detected at any temperature with D. pygmaeus (Table 6). Thirdly, the cycle period and phase durations were temperature dependent. Moreover, the data strongly suggest that a single equation can be used to predict the cycle period from temperature regardless of the species.

Since the data for D. dorsalis and D. pallidus are restricted to one temperature a rigorous comparison of periods is not possible. But when plotted together, the cycle periods seem similar for the four species (figure 18c). When all the data are pooled, the relationship between the cycle period (P, in days) and temperature (T, in °C) is well described by the equation

$$\log_{10}P = 10.03 - 13.31 \log_{10}T + 4.68(\log_{10}T)^2 \quad (R = 0.94, \\ \text{SD} = 0.0658, \\ 140 \text{ df})$$

and the coefficients of this equation do not change significantly when the same regression model is fit to data from only one species, e.g.

D. leptopus (even at $\alpha = 0.20$).

At least one important difference between the species was apparent, however. Although the cycle periods may be considered equivalent, the allocation of time between the gravid and nongravid phases differs (figure 18a, 18b). This difference is most striking when D. leptopus and D. pygmaeus are compared. While D. leptopus spends the majority of its cycle in the gravid phase (about 72%), D. pygmaeus is gravid only 44% of the time. D. pallidus is intermediate between these extremes, allocating equal time to the two phases. D. dorsalis tends to partition its time more like D. leptopus (61% gravid).

These interspecific differences in phase duration can have a substantial impact on potential rates of clutch production. Since eggs cannot be fertilized before the onset of the gravid phase, the nongravid phase delimits a species minimal clutch turnover time and shortening this phase can increase potential rates of clutch production. A more complete discussion of this effect follows the examination of embryonic development times which can also limit clutch production.

The relationships between temperature and the duration of embryonic development for D. pygmaeus and D. leptopus are shown on figure 19a. The mean development times for each temperature are shown as well as the curves fit to a total of 155 observations for both species. Separate power functions differing in both the degree of curvature and elevation best describe these data ($p < 0.05$). (No significant improvement in the fit was obtained with second order equations, $\alpha = 0.05$; cf. Bottrell, 1975). Note that the curves inter-

sect at about 12°C. Above 12°C, D. leptopus' embryos develop faster than those of D. pygmaeus ($P < 0.05$). Below 12°C, this trend is reversed.

The differences in the embryonic development curves seem to parallel certain differences in the life cycles of these species. While D. pygmaeus overwinter in the plankton of Stonehouse Pond and ovigerous females are abundant in early spring (February to March), D. leptopus overwinter in the farm pond as resting eggs which do not hatch until around April and reproductive females are absent until May (minimal daily water temperature 10° to 14°C in 1976 and 1977). The reduced slope of D. pygmaeus embryonic development curve may indicate a physiological mechanism which facilitates reproduction in the spring and fall. The shape of D. leptopus' curve would permit faster development during summer.

Since the presence of an ovisac prevents proper spermatophore placement, and since several species of copepods frequently carry a clutch of eggs until they hatch, estimates of maximal clutch production rates are often based on embryonic development times (e.g. Corkett and Zillioux, 1975). Potential rates of clutch production by D. leptopus and D. pygmaeus determined from the embryonic development curves shown on figure 19a are given on Table 7. Over the range of temperatures listed it appears that D. leptopus could turnover clutches up to 31% faster than D. pygmaeus.

Actually, however, the difference between the length of a species' nongravid phase and the duration of embryonic development indicates when a new clutch can be produced relative to hatching. If the nongravid

phase is longer than embryonic development, ripe female gametes will not be available for insemination at hatching. A mandatory interclutch period results.⁴ But if the nongravid phase is shorter than the duration of embryonic development, oocytes could be fertilized prior to clutch-hatch provided the ovisac was detached. The difference between these parameters is depicted graphically for D. pygmaeus and D. leptopus on figure 19b and 19c.

At all temperatures, D. pygmaeus' nongravid phase either approximately equals or exceeds the duration of embryonic development (figure 19b). This results in substantial interclutch time at most temperatures. For example, projected clutch production rates would be maximized at the minimal point on the nongravid phase curve (ca 26°C), but this rate would be about 40% lower than that predicted from the embryonic development time. At this temperature the minimal interclutch time is about 1 day. Given the forms of the two functions, interclutch time would increase geometrically below 16° and above 19°C. Outside this narrow range of temperatures, rates of clutch production based on embryonic development would overestimate the capacity of this species.

Embryonic development and the nongravid phase assume a different relationship with D. leptopus (figure 19c). Between 9° and 26°C the nongravid phase is considerably shorter than the time needed for embryonic development. Potentially, clutches could be turned over at a rate faster than that predicted from the hatching rate. The premature detachment of subitaneous clutches and the shorter carrying time of resting clutches (e.g. Robertson et al., 1974) could result in these

⁴The time between the hatching of eggs in one ovisac and the production of a subsequent clutch.

faster rates.

Considering the influence of the nongravid phase, predicted maximal rates of clutch production by these two species are compared directly on figure 19d. Since D. pygmaeus' nongravid phase is always greater than or approximately equal to embryonic development time, the reciprocal of the nongravid phase was used to estimate maximal rates of clutch turnover. For D. leptopus, estimates based on both the nongravid phase and embryonic development are shown. Figure 19d indicates that D. leptopus can produce clutches at a much faster rate than D. pygmaeus under certain conditions (e.g. about 3.3 times higher at 16°C). These differences in clutch production rate are usually much greater than those obtained when embryonic development times are compared without considering patterns of gamete maturation (cf Table 7).

Rates of clutch production observed in lab tend to support the conclusions made from the curves shown on figure 19d. Maximal and average observed rates for the two species are shown as points on this figure. For D. pygmaeus, observed maximal rates correspond well to the predicted maxima. However, average rates are considerably lower, probably due to behavioral factors associated with mate location mentioned previously. For D. leptopus, the observed maxima are intermediate between maxima predicted by the duration of embryonic development and the duration of the nongravid phase. Again average rates were relatively low. For both species, the trend indicated by the observed values closely follows that predicted from the nongravid phase duration. Note, for example, the optima at 20° - 21°C for D. leptopus. These data substantiate the limiting effect and predictive value of gametogenic

patterns. They also show that neither the duration of embryonic development nor rates of clutch turnover determined directly in lab are sufficient estimates of a species' reproductive potential.

The short duration of D. leptopus' nongravid phase may be an adaptation to temporary or highly seasonal environments, since it dramatically increases potential rates of clutch production. The volume of the farm pond from which D. leptopus were collected ($A = 2500 \text{ m}^2$, $Z_{\text{max}} = 2\text{m}$) shows large seasonal fluctuations. By midsummer the mean depth is usually less than 10 cm and it often freezes to the bottom in winter. Such small or shallow lakes (generally without planktivorous fish) are, apparently, typical habitats for this species (see Cole, 1961; Hazelwood and Parker, 1961; Patalas, 1964; Sawchyn and Hammer, 1968; Winner, 1970; O'Brien et al., 1973)⁵. In contrast, the D. pygmaeus used in this study came from a mesotrophic lake ($A = 59,000 \text{ m}^2$; $Z_{\text{max}} = 17\text{m}$) which shows only slight volumetric fluctuations seasonally (Mattson, 1979). The persistence of populations in impermanent environments such as the farm pond may depend on their ability to utilize refugia from lethal conditions. For example, standard recourse for the plankton in a deteriorating environment is to leave the water column and colonize the benthos as resting or diapausing stages. Increasing rates of clutch production would facilitate the dispersal of resistant stages into the benthic zone; decreasing the duration of the nongravid phase is one way to increase these potential rates.

⁵D. leptopus has been reported from larger lakes (e.g. Patalas, 1964) but often these show extreme fluctuations in biologically critical parameters, like dissolved oxygen (e.g. Teraguchi and Northcote, 1966).

Another way of viewing the relationship between the duration of embryonic development and the nongravid phase is illustrated by figure 20. Observed values for the duration of embryonic development are plotted against the nongravid phase duration at the same temperature. The diagonal in this figure depicts a 1:1 relationship between these two reproductive parameters. It divides the figure into two zones in which the maximal rate of clutch production is either less than (lower right) or greater than (upper left) the development rate of embryos. This construct could be used to separate species on the basis of potential reproductive output and may prove helpful in analyzing patterns of distribution and abundance. For example, one might hypothesize that points for copepods from impermanent environments would fall in the upper zone while those from more constant environments would fall on or below the diagonal. This type of separation between D. leptopus and D. pygmaeus can be seen on the figure.

Other parameters, such as clutch size, are important in determining the reproductive output of a species and could be included in the above model. In the present study the largest clutches from field-collected D. leptopus contained almost five times the number of eggs in the largest D. pygmaeus clutches (99 and 22 eggs, respectively). Combined with a faster rate of clutch production, this could confer a substantial reproductive advantage to D. leptopus.

However, gametogenic patterns and embryonic development may prove to be more stable and, therefore, more useful biological predictors. For example, the variability of clutch-size between individuals or populations of a species is well documented (e.g. Ravera, 1955; Roen,

1955; Ravera and Tonolli, 1956; Davis, 1961; Smyly, 1968; Maly, 1973). In this study, the range was 22 to 3 eggs clutch⁻¹ for D. pygmaeus and 99 to 8 eggs clutch⁻¹ for D. leptopus. (Sawchyn and Hammer (1968) reported a maximum of 158 eggs for D. leptopus in a Canadian pond.)

The usefulness of figure 20 depends, in part, on the existence of similar embryonic development times between species. Otherwise, points for copepods with relatively high potential rates of clutch production due to very rapid embryonic development could fall below the diagonal. Although statistically distinguishable, the curves describing embryonic development shown on figure 19a tend to support the assumption of interspecific similarity. In fact, when only mean values are compared (i.e. the points shown on figure 19a) no statistically significant difference between D. leptopus and D. pygmaeus can be demonstrated (at $\alpha = 0.05$).

To further examine this assumption, previously published data on the mean duration of embryonic development of several other diaptomid species were plotted along with the data from D. leptopus and D. pygmaeus (figure 21). The relationship between temperature (T, in °C) and embryonic development (D, in days) is quite similar between species. Furthermore, a single regression equation fit to all these data yielded the following function which fit the data well,

$$\log_{10} D = 1.496 - 0.6528(\log T)^2. \quad (\text{SD} = 0.0528 \\ 129 \text{ df})$$

Approximately 97% of the variation in the data set was accounted for by

the regression.⁶

A more detailed analysis (multiple regression with species indicators) shows statistically significant differences ($\alpha = 0.05$) between several species and separate equations may best describe the data shown on figure 21 (see Appendix I). But as pointed out by Chapman (1969) and Bottrell *et al.* (1976) it is difficult to determine whether these differences are real, since techniques often vary substantially between studies. At present, interspecific variability seems sufficiently low to justify the approach suggested by figure 20.

The enhancement of reproductive rates in seasonal environments is only one of several hypotheses which can be erected to explain the ecological basis for interspecific differences in gametogenic patterns. Two others are indicated on Table 8. Here it is suggested that a relatively short nongravid phase could result in elevated metabolic costs (due to the maintenance of ripe oocytes for long periods) and decreased ability to avoid visual predators (due to more conspicuous oviducts) as well as enhanced rates of clutch production. A longer nongravid phase would lower the metabolic costs, increase the ability to avoid predation but lower the rates of clutch production. The advantage of such trade-offs would depend on environmental circumstances.

Empirical evidence relating to the metabolic advantage are not available, so this hypothesis will not be discussed further. Extant

⁶Bottrell (1975) and Bottrell *et al.* (1976) also plotted the embryonic development times of several diaptomids and noted similarities, but cautioned against describing these data with equations which are not statistically justified. It should be noted that the data from Elster (1954) and Eichhorn (1964) used in these studies came from curves fit by the original authors rather than from their original observations. For this reason, the appearance of interspecific differences may be exaggerated.

data do pertain to the predator avoidance hypothesis, however.

Several studies have suggested that the transparency of zooplankton may be related to the avoidance of visual predators. Zaret (1972) and Zaret and Kerfoot (1975) developed a "visibility hypothesis" which postulated that the selective predation by fishes on Cladocera was a function of eye pigmentation. Confer and Blades (1975) reported that fish were more responsive to Daphnia magna than similar sized D. pulex with less pigmented carapaces.

Since the oviducts of gravid Diaptomus appear as heavily pigmented bands, it seemed that they might serve as an important visual cue for predatory fish. If so, the proportion of time that a species allocated to the gravid phase might be related to the probability being eaten by fish in its habitat. (In the present study, the lake from which D. pygmaeus were collected contained planktivorous fish; D. leptopus' pond did not.)

The prey-selection experiments with Micropterus were conducted as an initial test of this hypothesis. It should be noted that such experimental determinations of prey-preference are complicated by several factors, such as light intensity, contrast, prey density, alternative prey and the physiology of the predator (see Ivlev, 1961; Vinyard and O'Brien, 1976; Jacobs, 1979). Therefore, extensive investigation may be needed to realistically interpret results obtained under a particular set of conditions.

With this in mind, the results of these experiments are shown on Table 9. Only one of ten trials with D. leptopus indicated significant selection for gravid females (Table 9a). In two of six experiments with

D. pygmaeus chi-square tests indicated selective feeding, but the direction of selection was opposite in these cases (Table 9b).

These results are consistent with the findings of previous studies which suggest that other characteristics of the prey, such as body-size, may override the influence of pigmentation (e.g. Vinyard and O'Brien, 1975). Since gametogenic patterns show no strong relationship to body-size (Table 10), the results also suggest that the enhancement of reproductive rates may be the principal advantage of the short nongravid phase observed with some diaptomid species.

In summary, the results of section II showed that all the female Diaptomus oscillated between gravid and nongravid reproductive conditions with a similar frequency. However, important differences between species were noted. Although the period of their cycles was similar, the allocation of time to the gravid and nongravid phases was different. These interspecific differences exerted a substantial effect on relative rates of clutch production. Species with short nongravid phases have high reproductive potential and, consequently, may be well suited for existence in impermanent or ephemeral ponds where persistence of the population may depend on the rapid production of resting eggs.

This section concludes investigation of gametogenic patterns in Diaptomus. In section IV another component of reproductive rates, mate location and selection, will be examined and a behavioral correlate of the gametogenic cycle will be described.

Bibliography

- Applegate, R. L. and J. W. Mullan. 1967. Food of young largemouth Bass in a New and Old Reservoir. *Trans. Am. Fish. Soc.* 96: 74-77.
- Bottrell, H. H. 1975. The relationship between temperature and duration of egg development in some epiphytic Cladocera and Copepoda from the River Thames, Reading, with a discussion of temperature functions. *Oecologia* 18: 63-84.
- _____, A. Duncan, Z. M. Gilwicz, E. Grygierek, A. Herzig, A. Hillbrict-Ilkowska, H. Kurasawa, P. Larsson and T. Weglenska. 1976. A Review of Some Problems in Zooplankton Production Studies. *Norw. J. Zool.* 24: 419-456.
- Chapman, A. 1969. The bionomics of Diaptomus gracilis Sars (Copepoda: Calanoida) in Loch Lomond, Scotland. *J. Anim. Ecol.* 38: 257-283.
- Cole, G. A. 1961. Some calanoid copepods from Arizona with notes on congeneric concurrences of Diaptomus species. *Limnol. Oceanogr.* 6: 432-442.
- _____. 1966. Contrasts among Calanoid copepods from permanent and temporary ponds in Arizona. *Am. Midl. Nat.* 76(2): 351-368.
- Confer, J. L. and P. I. Blades. 1975. Omnivorous Zooplankton and planktivorous fish. *Limnol. Oceanogr.* 20: 571-579.
- Cooley, J. M. 1970. The effect of temperature on the development of the egg, first, and second naupliar stages, and vertical migration in Diaptomus oregonensis Lillj. (Copepoda: Calanoida). M.S. Thesis, Univ. of Toronto.

- Cooper, G. P. 1936. Food habits, rate of growth and cannibalism of young largemouth Bass in state-operated rearing ponds in Michigan during 1935. *Trans. Am. Fish. Soc.* 66: 242-266.
- Corkett, C. J. and E. J. Zillioux. 1975. Studies on the effect of temperature on the egg laying of three species of calanoid copepods in the laboratory (Acartia tonsa, Temora longicornis, and Pseudocalanus elongatus). *Bull. Plankton Soc. Japan* 21(2): 77-85.
- Davis, C. C. 1961. Breeding of calanoid copepods in Lake Erie. *Verh. Int. Ver. Limnol.* 14: 933-942.
- Eckstein, H. 1964. Untersuchungen über den einfluss des Rheinwassers auf die limnologie des Schluchsees. *Arch. Hydrobiol./suppl.* 28: 118-182.
- Eichhorn, R. 1957. Zur Populationsdynamik der calanoiden Copepoden in Titisee und Feldsee. *Arch. Hydrobiol./suppl.* 24: 186-246.
- Elster, H-H. 1954. Über die Populationsdynamik von Eudiaptomus gracilis Sars und Heterocope borealis Fischer im Bodensee-Obersee. *Arch. Hydrobiol./suppl.* 20: 546-614.
- Ewers, L. A. 1933. Summary Report of crustacea used as food by the fishes of the western end of Lake Erie. *Trans. Am. Fish. Soc.* 63: 379-390.
- Geiling, W. T. and R. S. Campbell. 1972. The effect of temperature on the development rate of the major life stages of Diaptomus pallidus Herrick. *Limnol. Oceanogr.* 17(2): 304-307.
- Hall, D. J. 1964. An experimental approach to the dynamics of a natural population of Daphnia galeata mendotae. *Ecology* 45(1): 94-110.

- Hazelwood, D. H. and R. A. Parker. 1961. Population dynamics of some freshwater zooplankton. *Ecology* 42(2): 266-274.
- Ivlev, V. S. 1961. Experimental ecology of the feeding of fishes. Yale Univ. Press. New Haven, Conn.
- Jacobs, J. 1979. The influence of prey size, light intensity and alternative prey on the selectivity of plankton feeding fish. *Verh. Int. Ver. Limnol.* 20: 2467-2476.
- Maly, E. J. 1973. Density, size and clutch of two high altitude diaptomid copepods. *Limnol. Oceanogr.* 18(6): 840-848.
- Mattson, M. 1979. Distribution Patterns and Population Dynamics of Polyphemus pediculus (L.) (Crustacea, Cladocera) in a small New England lake. Ph.D. Thesis, Univ. New Hampshire, Durham.
- Munro, I. G. 1974. The effect of temperature on the development of egg, naupliar, and copepodite stages of two species of copepods, Cyclops vicinus Uljanin and Eudiaptomus gracilis Sars. *Oecologia* 16: 355-367.
- O'Brien, F. I., J. M. Winner and D. K. Krochak. 1973. Ecology of Diaptomus leptopus S.A. Forbes 1882 (Copepoda:Calanoida) under temporary pond conditions. *Hydrobiol.* 43(1): 137-155.
- Patalas, K. 1964. The crustacean plankton Communities in 52 lakes of Different Altitudinal Zones of Northern Colorado. *Verh. Int. Ver. Limnol.* 15: 719-726.
- Ravera, O. 1955. Seasonal variation of the reproductive rate in pelagic copepods of lake Maggiore. *Verh. Int. Ver. Limnol.* 12: 436-447.
- _____ and V. Tonolli. 1956. Body size and number of eggs in

- diaptomids, as related to water renewal in mountain lakes. *Limnol. Oceanogr.* 1: 118-122.
- Robertson, A., C. W. Gehrs, B. D. Hardin, and G. W. Hunt. 1974. Culturing and ecology of *Diaptomus clavipes* and *Cyclops vernalis*. U. S. Environmental Protection Agency, Wash. D.C. (EPA - 660/3-74-006).
- Roen, U. 1955. On the number of eggs in some free-living copepods. *Verh. Int. Ver. Limnol.* 12: 447-454.
- Sawchyn, W. W. and U. T. Hammer. 1968. Growth and reproduction of some *Diaptomus* spp. in Saskatchewan ponds. *Can. J. Zool.* 46: 511-520.
- Smyly, W. J. P. 1968. Number of eggs and body-size in the freshwater copepod *Diaptomus gracilis* Sars in the English Lake District. *Oikos* 19(2): 323-338.
- Teraguchi, M. and T. G. Northcote. 1966. Vertical distribution and migration of *Chaoborus flavicans* (Meigen) larvae in Corbett Lake, British Columbia. *Limnol. Oceanogr.* 11: 164-176.
- Tonolli, L. 1971. Allomorphic relationship of antenna to body length in *Eudiaptomus vulgaris* Schmeil. *Limnol. Oceanogr.* 16(2): 467-472.
- Vinyard, G. L. and W. J. O'Brien. 1975. Dorsal light response as an index of prey preference in bluegill sunfish (*Lepomis machrochirus*). *J. Fish. Res. Bd. Can.* 32: 1860-1863.
- _____. 1976. Effect of light and turbidity in the reactive distance of Bluegill (*Lepomis machrochirus*). *J. Fish. Res. Bd. Can.* 33(12): 2845-2849.

- Wilson, M. S. 1959. Calanoida. p. 738-794 in W. T. Edmondson (ed.)
Fresh-water Biology. Wiley.
- Winner, J. M. 1970. An ecological study of the calanoid copepod
Diaptomus leptopus. Ph.D. Thesis, Univ. Mass., Amherst.
- Zaret, T. M. 1972. Predators, invisible prey, and the nature of
polymorphism in the Cladocera (class Crustacea). Limnol.
Oceanogr. 17(2): 171-184.
- _____ and W. C. Kerfoot. 1975. Fish predation of Bosmina
longirostris: body-size selection versus visibility selection.
Ecology 56: 232-237.

Figure 17. Oscillations in the reproductive condition of three species of Diaptomus. (stippled zone, time spent in gravid phase (dark oviducts); non-stippled zone, time spent in nongravid phase (clear oviducts). Records for D. pygmaeus from photoperiod 16:8 (LD).

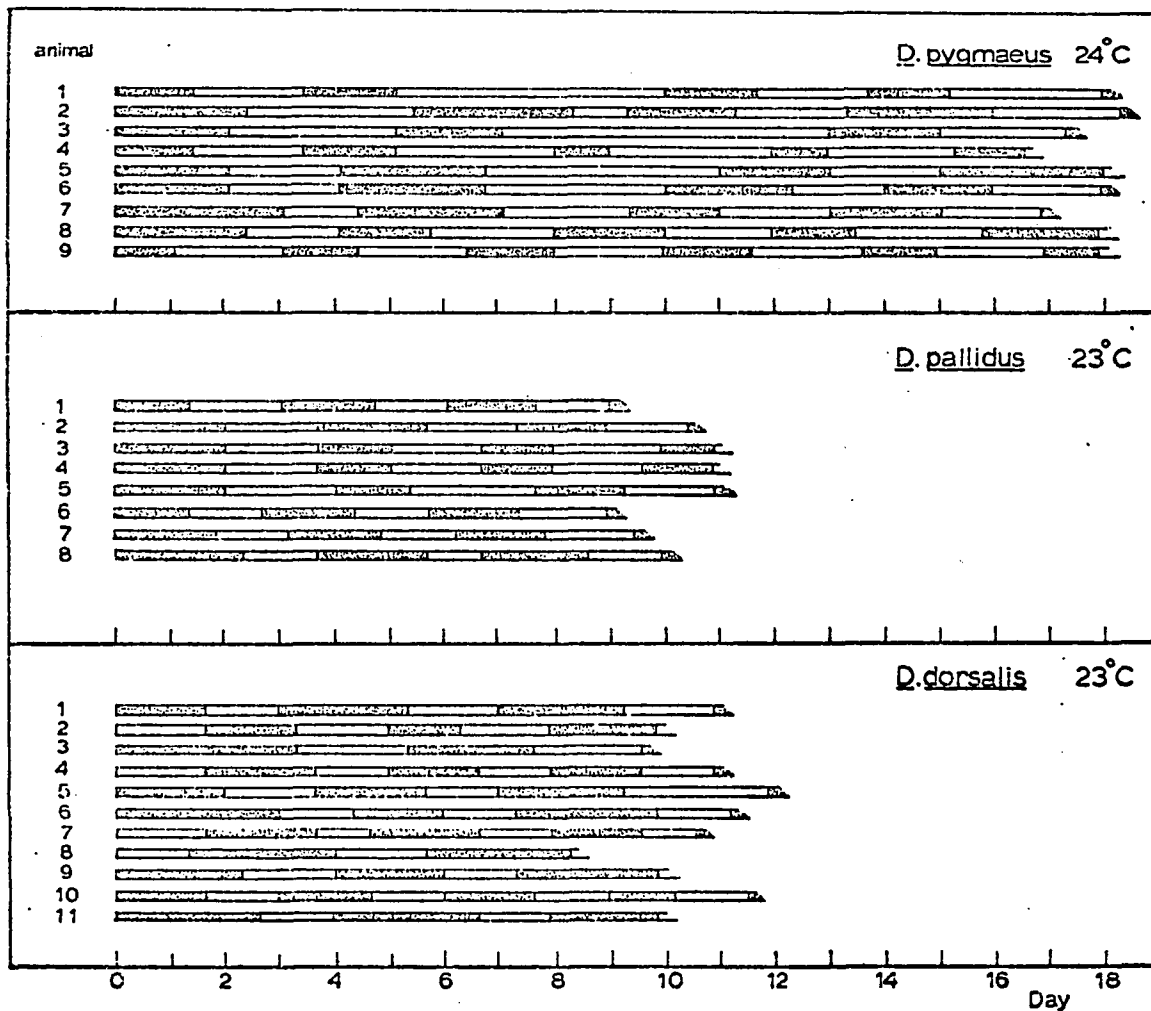


FIGURE 17

Figure 18. Relationship between temperature and the gametogenic patterns of four species of Diaptomus. (Points indicate mean values, bars show one standard deviation. Curves fit by eye to show trends).

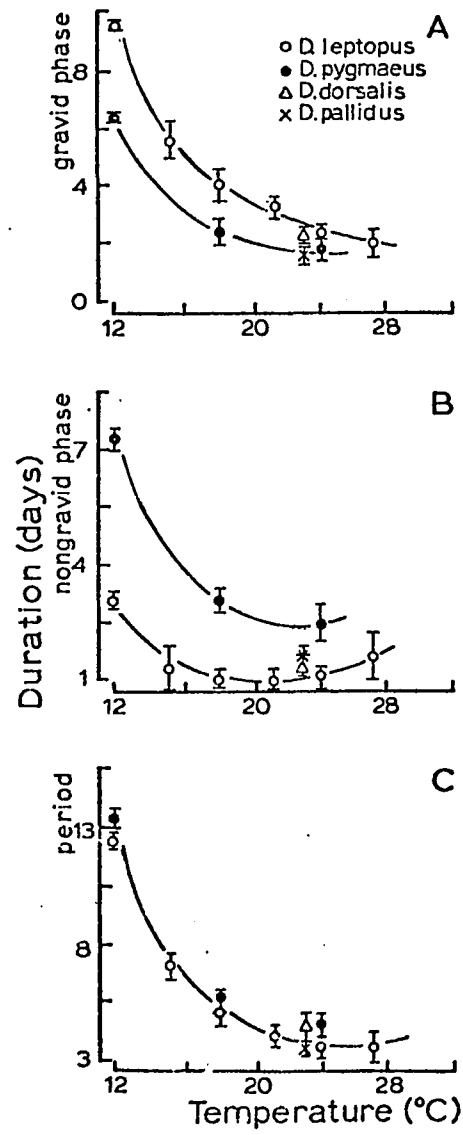


FIGURE 18

Figure 19. Comparison of reproductive parameters of D. leptopus and D. pygmaeus. A, relationship between temperature and the duration of embryonic development. (D. leptopus: solid line, open circles; D. pygmaeus: broken line, closed circles. Points are mean values, shown for reference only; curves fit to individual observations.) B, comparison of the duration of embryonic development and the nongravid phase of D. pygmaeus. ($\log_{10}(\text{nongravid}) = 8.02 - 10.72 \log T + 3.77(\log T)^2$, $R = 0.88$, $SD = 0.0988$, $n = 36$; points indicate mean phase duration, for reference only). C, same as above for D. leptopus. ($\log_{10}(\text{nongravid}) = 16.01 - 24.76 \log T + 9.56(\log T)^2$, $R = 0.63$, $SD = 0.157$, $n = 88$, points indicate mean phase duration, for reference only). D, comparison of potential and observed rates of clutch production.

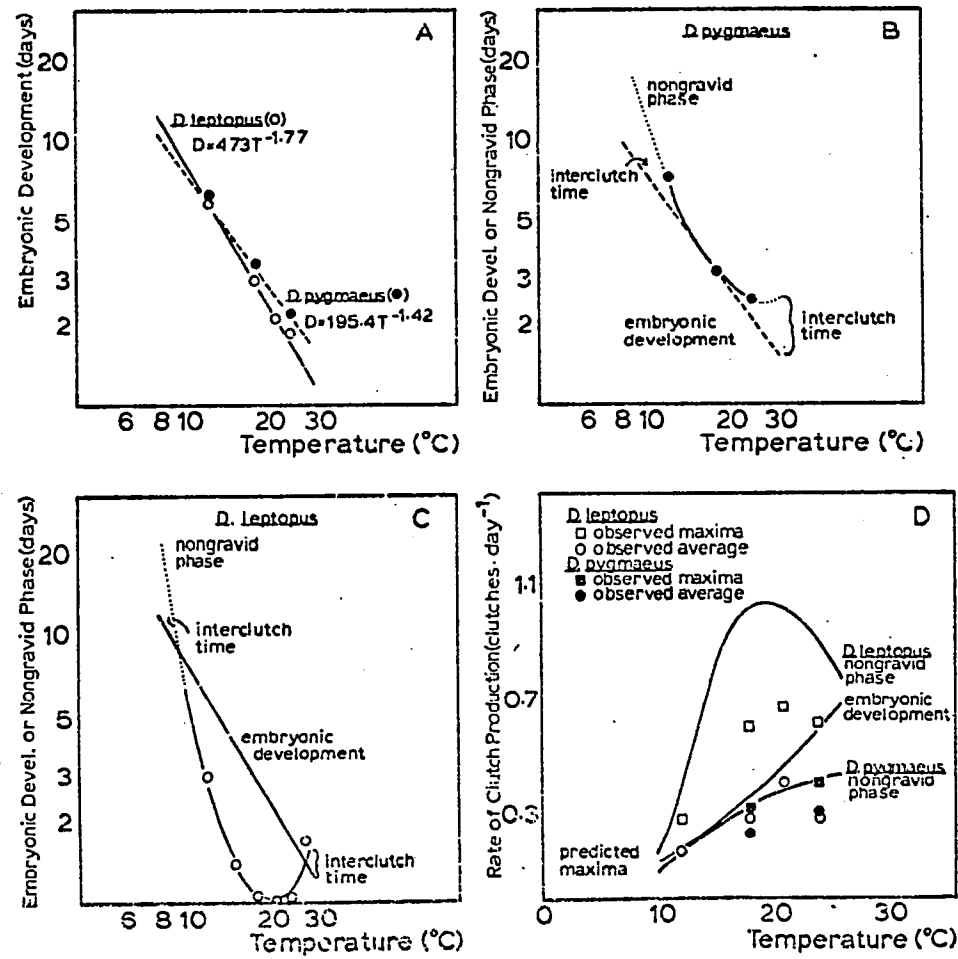


FIGURE 19

Figure 20. Separation of diaptomid copepods on basis of nongravid phase duration (i.e. minimal time for clutch turnover) relative to the duration of embryonic development at the same temperature. (Points are means of observed values for each species; a, data for embryonic development from Geiling and Campbell, 1972).

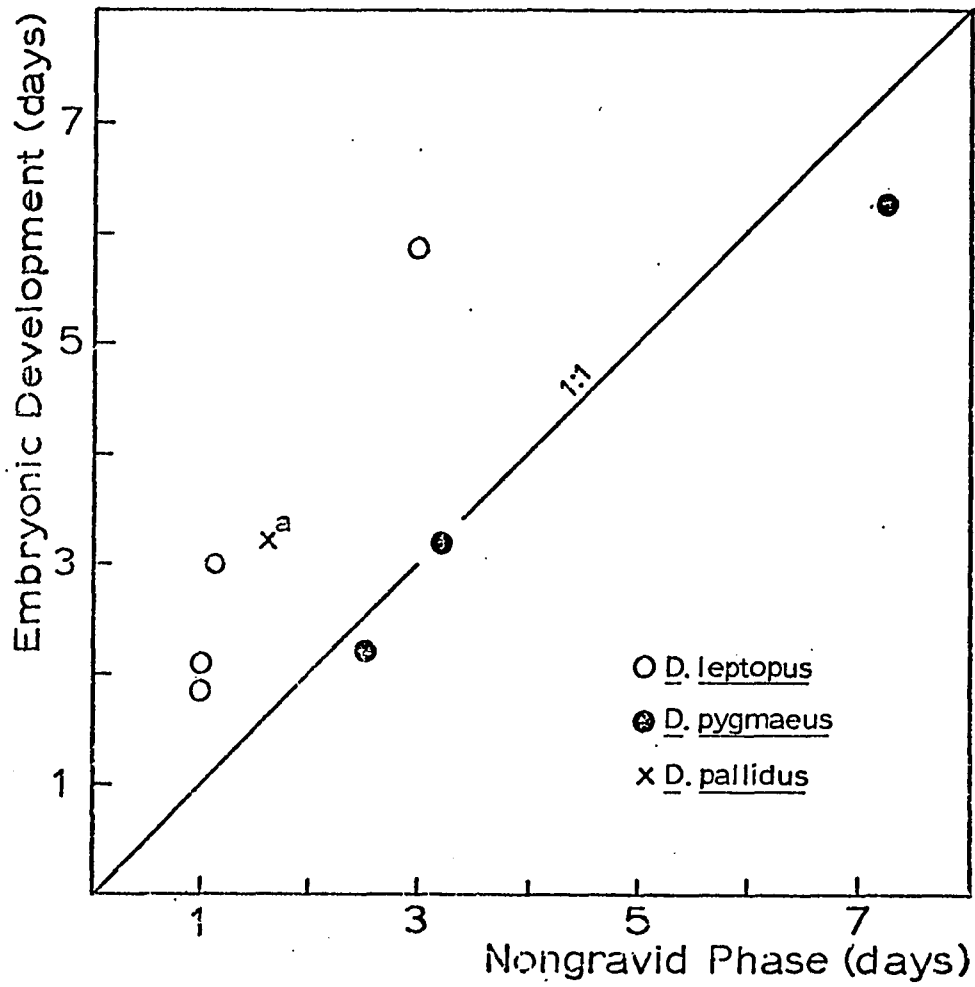


FIGURE 20

Figure 21. Relationship between temperature and the duration of embryonic development for several species of Diaptomus (numerals indicate number of overlapping points).

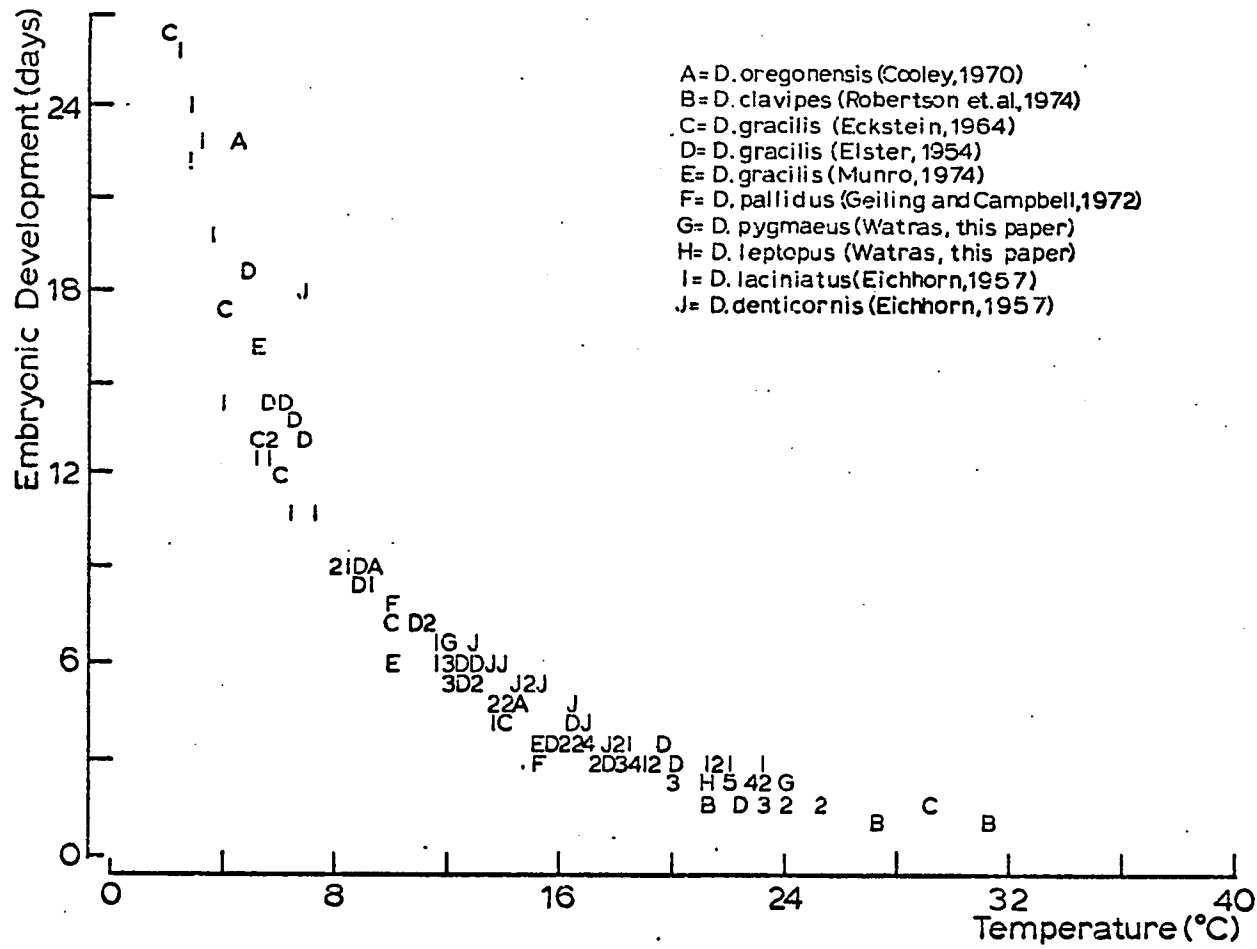


FIGURE 21

Table 6. Effect of photoperiod on the reproductive cycle of D. pygmaeus. (Data are mean durations, in days, with SD in parentheses.)

Temp (°C)	Gravid Phase				Nongravid Phase				Period			
	photoperiod (L:D)		t	p	photoperiod (L:D)		t	p	photoperiod (L:D)		t	p
	8:16	16:8			8:16	16:8			8:16	16:8		
24	1.7 (0.35)	1.9 (0.42)	0.970	>0.20	2.6 (0.35)	2.5 (0.61)	0.400	>0.50	4.3 (0.26)	4.4 (0.69)	0.346	>0.50
18	2.3 (0.75)	2.2 (0.15)	0.000	-	3.0 (0.33)	3.2 (0.26)	1.628	>0.10	5.2 (0.72)	5.5 (0.38)	0.800	>0.20
12	6.2 (2.32)	6.5 (2.17)	0.200	>0.50	6.9 (2.66)	7.4 (2.89)	0.300	>0.50	13.1 (2.84)	14.0 (2.58)	0.490	>0.50

Note: Analysis by multiple regression also failed to detect any effect of photoperiod at $\alpha = 0.05$

Table 7. Comparison of rates of clutch production based on functional relationship between the duration of embryonic development and temperature.

Temp (°C)	Rate of Clutch Production (Clutches·day ⁻¹)		Difference (%)
	<u>D. leptopus</u>	<u>D. pygmaeus</u>	
12	0.17	0.17	0
14	0.23	0.22	5
16	0.29	0.26	12
18	0.35	0.31	13
20	0.42	0.36	17
22	0.50	0.41	22
24	0.58	0.46	26
26	0.68	0.52	31

Table 8. Three possible adaptive advantages of different gametogenic patterns.

Criterion	Duration of Nongravid Phase	
	short	long
Metabolic cost	high	low
Avoidance of visual predators	low	high
Rate of clutch production	high	low

Table 9A. Selective feeding by Micropterus on gravid (D) or nongravid (C) D. leptopus

Expt.	Prey Avail.	Prey Ratio D:C	Time Exposed (min)	Prey Eaten	Selection Ratio		X ²
					Expected	Observed	
1	50	1:1	10	14	7:7	7:7	0.00
2	50	1:1	15	21	10.5:10.5	13:8	1.19
3	50	1:1	3	18	9:9	8:10	0.25
4	50	1:1	3	13	6.5:6.5	5:8	1.60
5	50	1:1	2	14	7:7	7:7	0.00
6	50	1:1	10	10	5:5	7:3	1.60
7	50	1:1	3	12	6:6	6:6	0.00
8	50	1:1	25	14	7:7	9:5	1.14
9	50	1:1	3	14	7:7	11:3	4.57*
10	50	1:1	20	5	2.5:2.5	0:5	-

* p < 0.05

Table 9B. Selective feeding by Micropterus on gravid (D) or nongravid (C) D. pygmaeus

Expt.	Prey Avail.	Prey Ratio D:C	Time Exposed (min)	Prey Eaten	Selection Ratio		χ^2
					Expected	Observed	
1	50	1:1	5	15	7.5:7.5	13:2	8.06*
2	50	1:1	25	13	6.5:6.5	2:11	6.23*
3	50	1:1	3	15	7.5:7.5	8:7	0.06
4	50	1:1	6	12	6:6	3:9	3.00
5	50	1:1	3	12	6:6	7:5	0.33
6	50	1:1	2	10	5:5	5:5	0.00

*p < 0.05

Table 10. Relationship between female body-length and percentage of time spent in gravid phase of reproductive cycle.

Species	Body-length (prosome in mm)	Duration of gravid phase Cycle Period x 100%
<u>D. leptopus</u>	0.95 to 1.7 ^a	72%
<u>D. pallidus</u>	0.84 to 0.96 ^b	50
<u>D. dorsalis</u>	0.73 to 0.86 ^c	61
<u>D. pygmaeus</u>	0.70 to 0.90 ^d	44

- a. range for ice-free season in small farm pond, 1976 and 1977 (weekly samples, n = 798)
- b. 70% and 80% of total length (1.2 mm) reported by Wilson (1959)
- c. range for one sample from reservoir near Miami, Florida, 1975 (n = 24)
- d. range for 1975 in Stonehouse Pond (12 monthly samples, n = 260).

SECTION IV

MATE-LOCATION AND SELECTION BY DIAPTOMUS:

BEHAVIORAL CORRELATES OF A REPRODUCTIVE CYCLE

Introduction

Mate-location and selection are behavioral determinants of reproductive rates for zooplankton which reproduce sexually. In this investigation, behavioral interactions between diaptomid copepods were observed, classified and evaluated quantitatively in an attempt to clarify the mechanisms which regulate mating in natural populations.

The sequence of events during mating has been described previously for several calanoid genera (e.g. Wolf, 1905; Gauld, 1957; Jacobs, 1961, Roff, 1972; Katona, 1975; Blades, 1979). Fleminger (1967) and Lee (1972) have stressed the importance of morphological variation in reproductive structures for maintaining genetic isolation.

However, the behavioral mechanisms which facilitate mate-location within the planktonic community have not been studied as carefully. Katona (1973) described the responses of male Eurytemora and Pseudodiaptomus to tethered "potential mates" and concluded that pheromones were involved in estuarine and marine interactions. He also noted that mating females were usually gravid. No comparable studies exist for freshwater calanoids.

In previous experiments with Diaptomus (Sections I-III), it was noted that females oscillated between gravid and nongravid reproductive conditions. Since only gravid females could be successfully

inseminated, totally random mate-selection seemed unlikely, even though males had been observed mating with nongravid females and, occasionally, other males.

The purpose of the present section is to present evidence that male Diaptomus mate more readily with gravid females than with other potential mates and to discuss the physiological mechanisms which may mediate the process of mate-location and selection. In addition, transpecific interactions and the potential for hybridization will be examined briefly, and reproductive interference (i.e. the misplacement of spermatophores) will be developed as a mechanism regulating the coexistence of congeners.

Methods

Observations were made on four species of Diaptomus: D. leptopus and D. pygmaeus from southern New Hampshire, D. pallidus from North Carolina and D. dorsalis from southern Florida. To ensure that the animals were in similar physiological condition, all were reared to the adult stage in lab.

Falcon tissue-culture flasks (25cm^2 ; ca. 40 mm wide, 20 mm deep) were used as experimental containers when quantifying behavioral interactions or making general observations. Observations were recorded on videotape as the animals swam freely in the flasks. Illumination was provided by over-head florescent lights (1.4×10^{-4} watts cm^{-2}). Swimming speeds (D. leptopus only) were estimated in an 80 x 80 x 7 mm gridded plexiglas container using the method described by Hairston (1976).

Behavioral interactions were quantified by observing isolated pairs of copepods for pre-specified intervals. Male D. leptopus were

paired with conspecific males, gravid females and nongravid females. The occurrence or absence of a particular behavior was recorded. The frequency of occurrence was then calculated as the percentage of experiments (or trials) in which the behavior was observed.

Males of the other three species were paired with conspecific gravid or nongravid females (omitting male-male interactions). These experiments were designed to test for selectivity in mating; the number of trials which resulted in mating was recorded during an observational interval of 30-60 min. depending on the species (see Table 15). Transpecific pairings of males and gravid females were also conducted with these species to examine the potential for interbreeding.

To estimate the average remating interval (i.e. time between subsequent matings) for male D. leptopus, single males were isolated with groups of five gravid females and the interactions were recorded continuously for three hours.

Results

General observations on the swimming patterns of Diaptomus showed the following. When moving horizontally, all four species of Diaptomus oriented their ventral surface upward (unlike such genera as Epischura which swim with the dorsal side up). Most swimming movement consisted of a steady cruising powered by the first antennae. Quick jumps or changes of direction (apparently due to the action of thoracic appendages) occurred sporadically, often with no apparent stimulus.

Also, males swam more actively than females, which often moved only slightly while hanging in a vertical position. Swimming speeds for male and female D. leptopus are compared on Table 11. Gravid and

nongravid females moved at similar speeds, as indicated by the overlap of 95% confidence intervals. (Observations on ovigerous females were not made.) The average velocity of males was about 1.6 times that of nonovigerous females. In addition, males tended to move in the horizontal plane more frequently than females. However, directional coordinates were not systematically recorded.

Five classes of behavioral interaction were observed with Diaptomus. These were: avoidance, following, searching, precopula and copula. Four of these behaviors are diagrammed on figure 22. The relative frequency of occurrence for male-male and male-female encounters with D. leptopus is also shown. A condensed description of each behavior along with previous reports for other species is given on Table 12. A more detailed account follows.

When two diaptomids approached each other frontally or laterally, mutual avoidance occurred before the pair collided. Generally, both animals executed a quick leap moving them in opposite directions.

If a male approached another copepod from behind, he would begin pursuit when within one or two body-lengths. Both animals usually increased velocity and coordinated turns were often executed. Frequently, the male made short, quick movements toward the leading copepod, presumably attempting to attain precopula. The most extensive pursuit occurred between two males and continued over a distance of about 4 cm involving three tandom turns and repeated attempts at precopula. For quantitative purposes, males were required to follow for at least 1 cm and execute a coordinated turn or grasping movement before the interaction was classified as pursuit.

If outdistanced by his target, a male often moved in small circles or swiveled for a few seconds. This type of behavior was classified as searching. Females never searched or pursued another copepod.

Precopula occurred when a male grasped the caudal setae, furcae, or urosome of the leading copepod with his prehensile right antennule. A series of turns and loops which were too rapid for precise resolution usually followed. Based on previous accounts of calanoid mating (see Introduction) it seems likely that movement into copula occurred when the male secured the other's urosome with the exopod and claw of his right fifth leg and released his antennular hold. A spermatophore was transferred by the left fifth leg. This was determined when copula was interrupted before transfer was complete and the male continued to hold the spermatophore while swimming (see Appendix 2).

The duration of copula varied with species and the reproductive condition of the female (Table 13). The average time in copula was always longer with gravid females. These gravid females generally produced ovisacs 15-30 min. after copula, or not at all. Even though spermatophores were successfully deposited on nongravid females, none produced an ovisac. Their gametogenic cycles proceeded normally and, if not remated during the subsequent gravid phase, the oocytes were discarded.

Spermatophores often remained attached to females for substantial lengths of time. With D. leptopus, (Appendix 3) gravid females which failed to produce an ovisac occasionally carried a spermatophore for up

to an hour after copula. With nongravid females, spermatophores remained attached for up to 20 h.

After depositing a spermatophore, male D. leptopus did not attempt to remate for an additional 45-60 min. (Appendix 2). This interval may reflect the time required to form a new spermatophore, since males only carry one at a time.

In a series of experiments with D. leptopus, single males were isolated with one other adult copepod and the frequency of interactions was recorded. Other males, nongravid females and gravid females were used as the "targets" in these experiments. As shown on figure 22 and Table 14, behavior was strongly dependent on the sex and reproductive condition of the target.

Avoidance reactions occurred more frequently when the target was another male than either a gravid or nongravid female. Males tended to avoid females with equal frequency, regardless of reproductive condition.

The incidence of pursuit was not distinguishable between the three types of target. Although males pursued all other copepods, pursuit resulted in precopula significantly more often with gravid females than with male or nongravid females.

Likewise, copula was attained more frequently with gravid females than with either other target. In this series of experiments, males were never able to deposit a spermatophore on another male.

These results suggest the existence of a mechanism for selecting appropriate mates in this species. Only gravid females can produce a clutch of embryos upon insemination; and, although male D. leptopus

pursued and tried to mate with copepods regardless of sex or reproductive condition, success was significantly higher with gravid females.

The generality of this selective behavior was examined by repeating this experiment with three other species of Diaptomus. In these cases, the incidence of copula with gravid and nongravid females was compared. (Preliminary experiments showed that males of all three species would pursue any other copepod).

As shown on Table 15, male Diaptomus consistently mated with gravid females more readily than nongravid females. This tendency, then, seems characteristic of the genus.

Males were then isolated with gravid females of another species. These transpecific experiments showed that males would readily pursue and copulate with gravid females of other species (Table 16). However, the transition from precopula to copula was often difficult. In one instance, the female's thoracic appendages were damaged. At least once, a spermatophore was successfully attached to the female. But viable embryos were never produced as the result of a transpecific mating.

Discussion

The general observations on the locomotor behavior of Diaptomus are consistent with previous observations for calanoid copepods. The two modes of swimming (cruising and rapid jumps) are characteristic of most calanoids (see Gauld, 1966). Sexual differences in the level of locomotor activity are also common; males are usually more active than females (Jacobs, 1961; Farenbach, 1962; Lillelund and Lasker, 1971; Katona, 1973). However, sexual differences in the principal plane of

movement (i.e. horizontal vs. vertical dominance) are not well documented. A study by Gerritson (1979) suggests that such differences could function to increase the probability of encounter. He shows mathematically that predatory or mate-seeking animals can increase the frequency of collision by moving in a plane perpendicular to that of the potential prey or mate.

Although important differences in the morphology and alignment of body-parts can be shown, the basic sequence of events from precopula to deposition of a spermatophore is similar for all calanoids with prehensile antennules. The mating behavior described for Diaptomus in the present paper corresponds closely to that described previously for Diaptomus (Wolf, 1905), Centropages, Temora, Eurytemora, and Acartia (Gauld, 1957; Katona, 1975), Pseudodiaptomus (Jacobs, 1961), and Limnocalanus (Roff, 1972).

These similarities suggest that the potential for mistakes in mating may be high. Holmes (1909) reported that male cyclopoids seized anything they collided with, including other males. Precopula and copula between male calanoids has also been reported (Jacobs, 1961; Sawchyn and Hammer, 1968; Katona, 1975). Katona (1975) also reported the deposition of about 31 spermatophores on a single female Eurytemora. In the present study, 25-30 spermatophores were attached to a male isolated with nine other males in a 75 ml vial. None of the others had spermatophores attached. As shown on Table 16, interspecific mating interactions are also possible with Diaptomus.

Holmes (1909) concluded that the mating of cyclopoids was a function of random collisions; the frequency of mating with other

males was retarded simply by their ability to evade capture. Katona (1975) came to a similar conclusion about male-male encounters with Eurytemora. Male Diaptomus are also successful at avoiding other males. This success may be due to their increased swimming speed and the reduction in area occupied by their caudal setae, which extend directly back from the caudal furcae rather than fanning-out as they do on females. Thus, there is less area for a pursuing male to grasp.

Unlike Holmes (1909), most studies indicate that copepods are capable of detecting and responding to potential mates and other objects before collision. For example, Schroder (1961) examined avoidance-responses by observing Mixodiaptomus when it approached barriers in a small tank. He concluded that copepods sensed objects in their path by echo-location, effective with hard, reflective surfaces but not with those that absorbed or scattered information.

Although a conclusive test of Schroder's hypothesis has not been made, several other studies indicate that copepods can respond to mechanical disturbance. Strickler and Bal (1973) used cinematography to document the avoidance of encounter by male cyclopoids. As found in the present study with Diaptomus, they executed avoidance jumps before colliding. In addition, Strickler and Bal offered electron micrographs of longitudinal sections through an antennular seta (showing structures which appear to be microtubules) as evidence of the mechanoreceptor. Similar conclusions about the sensory nature of antennular setae were reached in early investigations using light microscopy (see Esterly, 1906). Lillelund and Lasker (1971) proposed that vibrations from the beating tails of larval fishes served as cue for prey-detection

by Labidocera. These calanoids ignored motionless larvae or eggs but readily attacked when a larval tail moved. Kerfoot (1978) has made similar observations with copepods preying on invertebrates.

Most investigators agree that visual discrimination is an unlikely basis for the avoidance or pursuit responses of copepods. The copepod eye is a simple light-gathering organ, not an image-forming structure (Farenbach, 1964).

However, several investigations suggest that olfactory discrimination may be involved in mate-location or selection. Parker (1901) and Katona (1973) report behavioral observations which point to the importance of chemical cues, although neither study entirely eliminates the potential influence of vibrational stimuli. Fleminger (1967) and Griffiths and Frost (1976) provide evidence for the existence of chemosensory structures along the antennules of males. Ong (1969), Elofsson (1971), Friedman and Strickler (1975) and Poulet and Marsot (1978) also provide evidence that copepods are capable of receiving chemical stimuli.

Results of the present investigation indicate that, within a certain reactive space, the location of mates by male Diaptomus is oriented rather than the result of random collisions. Orientation was not highly specific, however. Males pursued free-swimming copepods regardless of their sex or species. Katona (1973) observed a similar lack of specificity in the reactions of male Eurytomora to tethered copepods.

The exact nature of this orienting mechanism remains unclear but, since pursuit is executed only when a male is behind another copepod,

one might hypothesize that it is related to a generalized mechanical disturbance or chemical cue produced by the lead copepod. Orientation could involve a rheotactic response to the swimming current, acoustical signals or a wake-effect reducing drag on the pursuer. A pheromone may also be involved (e.g. Katona, 1973).

For example, a chemical cue may supplement the mechanical stimulus which determines whether a male's response will be avoidance or pursuit. This dual mechanical-chemical stimulation could explain the selective behavior of males toward gravid females. As the internal chemistry of a female changes during gametogenesis, excretory products would also vary. Levels of attractant might be highest during the gravid phase, stimulating males to make more frequent and aggressive attempts to mate. A similar phenomenon has been observed with decapods, where the production of pheromones changes with the molting cycle (Ryan, 1966; Kitteridge *et al.*, 1971).

The results of previous studies tend to support this hypothesis. Male Eurytemora responded positively to cotton fibers soaked in extracts of "attractive" females, provided the medium was gently swirled (Katona, 1973). Also, "attractive" female Eurytemora were generally gravid (Katona, 1975). Griffiths and Frost (1976) noted no substantial changes in the swimming patterns of male Calanus or Pseudocalanus when males and females in a tank were separated by a mesh barrier. However, when together in small vials positive mating responses were observed.

Alternative hypotheses remain to be tested, however. For example, males could play a passive role in mate selection. Physiological changes during gametogenesis may alter the behavior of females, so they are more

receptive to the approach of males. Such behavioral changes are often correlated with physiological rhythms; Tamm and Cobb (1978) correlated changes in the aggressive behavior of lobsters with phases of their molting cycle. The explanation for the low incidence of precopula between males provided by Holmes (1909) and Katona (1975) is consisted with this hypothesis of changing receptivity.

The importance of investigating such behavioral mechanisms can be seen when one considers an ecological problem concerning differences in species abundance and species composition of copepods in lakes.

Several investigations have noted the lack of sympatry between populations of diaptomid species with similar body-sizes (see Hutchinson, 1967). In lakes where such populations co-occur, they are usually not syntopic (e.g. Sandercock, 1967). Competitive exclusion based on similar modes of feeding has been involved as the mechanism preventing co-existence in space or time (Hutchinson, 1967).

However, no study has satisfactorily addressed the problem of competition for food between zooplankters. One wonders, for example, why diaptomids of similar size don't co-occur in eutrophic systems or why competition between immature stages doesn't prevent the co-existence of two populations in which adults are not the same size.

Historical factors are undoubtedly important determinants of distributional patterns in nature. In the case of Diaptomus, however, reproductive interference may prove to be a valid mechanism restricting the sympatric or syntopic occurrence of species and a useful alternative to hypotheses based on dietary overlap. Mistakes in mating between large and small species would be restricted in the same way that successful encounters between males are reduced (i.e. enhanced probability of

escape). But with species of similar size, the transpecific deposition of spermatophores could lead to progressively lower rates of reproduction for one species (i.e. females cannot be successfully inseminated while carrying the wrong spermatophore), resulting eventually in local extinction. Colonization of an environment could also be prevented in this way, with relative density serving as the principal determinant in most cases.

The results of experiments examining transpecific mating interactions reported in the present and previous papers (esp. Katona, 1973) strongly suggest that reproductive interference may occur between calanoid populations in nature. In the following discussion, this will be examined on an intuitive level and the basis for a more quantitative evaluation will be established.

Since hybridization appears unlikely⁷, for each incorrect mating encounter, the male's population would suffer a loss of gametes and a reduction in the potential rate at which it's own females are successfully mated (since the male would be lost from the mating population during copula and recovery). The female's population would also experience a reduction in the rate of successful mating, since the number of available females would be decreased by one during copula and the time that a spermatophore was carried. As shown later, gametes may also be lost from the female's population.

As an initial question, one might ask which population is likely to experience the greater negative effect. Here, consideration will be

⁷Hybridization never occurred in this study. Eckstein (1964) reported similar results with Eudiaptomus gravilis and E. vulgaris. Differences in reproductive morphology (Fleminger, 1967; Lee, 1972) or the incompatibility of gametes may contribute to this genetic isolation.

given to the effects on reproductive rates; energetic factors (i.e. gamete loss) will not be considered.

Simple population statistics suggest that a female may have a higher reproductive value than a male. Although the ratio males:females is approximately one in many field populations of Diaptomus (see Sawchyn and Hammer, 1968), the ratio males:gravid, nonovigerous females (i.e. fertilizable females) will be higher since females carry ovisacs for long periods after fertilization. The effective surplus of males which results would tend to increase the relative reproductive value of a female.

Also, females may be lost from their populations for a longer interval than males. Both copepods would spend equal time in copula (e.g. 0.5-13 min., Table 13). An additional interval may pass before the male actively seeks another mate (45-60 min. for D. leptopus; Appendix 2). The production of a new spermatophore would occur within this time. Similarly, the female would also be unable to remate while carrying a spermatophore. In conspecific interactions, this interval ranged from 1-20 h (Appendix 3); in transpecific interactions, it may increase. For example, Phillips (1976) reported that female Tortanus were unable to remove spermatophores deposited by Centropages. Furthermore, while carrying a spermatophore, the gametogenic cycle of a female diaptomid would proceed normally and the nongravid phase may onset, further increasing the nonreproductive interval and causing the loss of female gametes. In addition, during transpecific interactions females were occasionally damaged (i.e. appendages broken) resulting in their permanent loss. This was never observed with males.

For these reasons, it seems likely that the female's population would experience the greater negative effect due to transpecific mating interactions. It remains to be shown that transpecific mating could result in the elimination of one species from a given habitat. On a small scale, one might be able to demonstrate this experimentally. The problem could also be approached theoretically using simulation techniques. A framework for such simulation is presented below but further examination of this procedure is beyond the scope of this section. The purpose here is simply to show that the problem is tractable and worthy of consideration.

The process of mating between copepods is strongly analogous to the process of invertebrate predation as described by Holling (1959, 1961, 1963, 1966). The male functions as a special type of predator and the female as prey. Using a form of Holling's disc equation, the number of mating interactions in a population can be approximated as

$$N_M = \alpha [T_E - N_M(T_H + T_R)]N_0 \quad (1)$$

where N_M is the number of successful mating encounters, α is the instantaneous rate of successful encounter, T_E is the total time available for mating, T_H and T_R are, respectively, handling time (time spent in copula) and recovery time (spermatophore reconstruction, etc.) which result from each encounter, and N_0 is the population density.

The instantaneous rate of successful encounter, α , is the product of the rate of encounter between males and females (Z) and the ratio successful:total encounters (θ). So

$$\alpha = \theta Z \quad (2)$$

where θ is a constant and Z is a complex function of reactive distance

and relative swimming speeds.

Using a formulation described by Kauzmann (1966) and applied to zooplankton populations by Katona (1973), Z can be approximated as

$$Z = \pi R_{mf}^2 \frac{3U_m^2 + U_f^2}{3U_m} \quad (3)$$

where R is the reactive distance for males and U_m and U_f are, respectively, the swimming speeds of males and females.⁸

Modeling the process of reproduction in this or a similar fashion would then permit simulation of the effects of varying the values of critical parameters. Relative rates of reproduction and population growth could be generated and the effects of reproductive interference on the co-existence of calanoid populations examined. Results of such simulations could be used as a basis for testible hypotheses and predictions in natural situations. This approach will serve as the basis for future investigations of the effects of behavioral interactions on the dynamics of diaptomid populations.

To summarize, the results of Section IV show that mate-location by Diaptomus is both an oriented and selective phenomenon. The exact nature of the mechanism for mate-selection remains obscure but it is clearly related to the cycle of gamete production in females. The results also suggest that reproductive interference may be an important mechanism regulating the co-occurrence of diaptomid species.

This section concludes a series of investigations designed to clarify the ways that reproductive rates are regulated in populations

⁸ As discussed in Section I, equation 3 assumes random movement. If sexual differences in the principal plane of movement exist, a correction factor may be needed (see Gerritson, 1979).

of Diaptomus. The approach has been experimental and mechanistic and the results indicate that this approach is a necessary compliment to the analysis of data from preserved samples of natural populations.

Bibliography

- Blades, P.I. 1979. Ritualized Mating Behavior in Copepods. *Limnol. oceanogr.* Special Symposium: In Press.
- Eckstein, H. 1964. Untersuchungen über den einfluss des Rheinwassers auf die limnologie des Schluchsees. *Arch. Hydrobiol./suppl.* 28: 118-182.
- Elofsson, R. 1971. The ultrastructure of a chemoreceptor organ in the head of copepod crustaceans. *Acta Zoologica* 52: 299-315.
- Esterly, C. O. 1906. Some observations on the nervous system of Copepods. *Univ. Calif. Publ. in Zoology* 3(1): pp. 1-12.
- Fahrenbach, W. H. 1962. The biology of a harpacticoid copepod. *La Cellule* 62(3): 303-376.
- _____. 1964. The fine structure of a nauplius eye. *Z. Zellforsch.* 62: 182-197.
- Fleminger, A. 1967. Taxonomy, distribution and polymorphism in the *Labidocera jollae* group with remarks on evolution within the group. *U.S. Nat. Mus. Proc.* 120: 1-61.
- Friedman, M. M. and J. R. Strickler. 1975. Chemoreceptors and feeding in calanoid copepods (Arthropoda:Crustacea). *Proc. Nat. Acad. Sci. USA.* 72(10): 4185-4188.
- Gauld, D. T. 1957. Copulation in calanoid copepods. *Nature* 180: 510.
- _____. 1966. The swimming and feeding of planktonic copepods in H. Barnes (ed.), *Some Contemporary Studies in Marine Science*, pp. 313-334.
- Gerritson, J. 1979. Adaptive responses to encounter problems. *Limnol. oceanogr.* Special Symposium: In Press.

- Griffiths, A. M. and B. W. Frost. 1976. Chemical communication in the marine planktonic copepods Calanus pacificus and Pseudocalanus sp. *Crustaceana* 30(1): 1-9.
- Hairston, N.G., Jr. 1976. Photoprotection by carotenoid pigments in the copepod Diaptomus nevadensis. *Proc. Nat. Acad. Sci. USA* 73: 971-974.
- Holling, C. S. 1959. The components of predation as revealed by a study of small mammal predation of the European pine sawfly. *Can. Ent.* 91: 293-320.
- _____. 1961. Principles of insect predation. *Ann. Rev. Ent.* 6: 163-182.
- _____. 1963. An experimental component analysis of population processes. *Mem. Ent. Soc. Can.* 32: 22-32.
- _____. 1966. The functional response of invertebrate predators to prey density. *Mem. Ent. Soc. Can.* 48: 3-83.
- Holmes, S. J. 1909. Sex recognition in Cyclops. *Biol. Bull.* 16: 313-315.
- Hutchinson, G. E. 1967. *A Treatise on Limnology*, vol. 2, Wiley.
- Jacobs, J. 1961. Laboratory cultivation of the marine copepod Pseudodiaptomus coronatus Williams. *Limnol. Oceanogr.* 6: 443-446.
- Katona, S. K. 1973. Evidence for sex pheromones in planktonic copepods. *Limnol. Oceanogr.* 18(4): 574-584.
- _____. 1975. Copulation in the copepod Eurytemora affinis (Poppe, 1880). *Crustaceana* 28(1): 90-95.
- Kauzmann, W. 1966. *The Kinetic Theory of Gases*, vol. 1. Benjamin.
- Kerfoot, C. W. 1978. Combat between predatory copepods and their prey: Cyclops, Epischura and Bosmina. *Limnol. Oceanogr.* 23: 1089-1102.

- Kittredge, J. S., M. Terry, and F. T. Takahashi. 1971. Sex pheromone activity of the molting hormone, crustecdysone, on male crabs.
- Lee, C. M. 1972. Structure and function of the spermatophore and its coupling device in the Centropagidae (Copepoda:Calanoida). Bull. Mar. Ecol. 8: 1-20.
- Lillelund, K. and R. Lasker. 1971. Laboratory studies of predation by marine copepods on fish larvae. Fish. Bull. 69(3): 655-667.
- Ong, J. E. 1969. The fine structure of the mandibular sensory receptors in the brackish water calanoid copepod Gladioferens pectinatus (Brady). Z. Zellforsch. 97: 178-195.
- Parker, G. H. 1901. The reactions of copepods to various stimuli, and the bearing of this on daily depth migrations. Bull. U.S. Fish. Comm. 21: 103-123.
- Phillips, D. 1976. The biology of the predatory calanoid copepod Tortanus discaudatus (Thompson and Scott) in a N.H. Estuary. Ph.D. Thesis, University of New Hampshire, Durham.
- Poulet, S. A. and P. Marsot. 1978. Chemosensory grazing by marine calanoid copepods (Arthropoda:Crustacea). Science 200: 1403-1405.
- Roff, J. C. 1972. Aspects of the reproductive biology of the planktonic copepod, Limnocalanus macrurus Sars, 1883. Crustaceana 22: 155-160.
- Ryan, E. P. 1966. Pheromone: evidence in a decapod crustacean. Science 151: 340-341.
- Sandercock, G. A. 1967. A study of selected mechanisms for the co-existence of Diaptomus spp. in Clarke Lake, Ontario. Limnol. Oceanogr. 12: 97-112.

- Sawchyn, W. W. and U. T. Hammer. 1968. Growth and reproduction of some Diaptomus spp. in Saskatchewan ponds. *Can. J. Zool.* 46: 511-520.
- Schroder, R. 1960. Echoorientierung bei Mixodiaptomus laciniatus. *Naturwissenschaften* 23: 548-549.
- Strickler, J. R. and A. K. Bal. 1973. Setae of the first antennae of the copepod Cyclops scutifer (Sars): their structure and importance. *Proc. Nat. Acad. Sci. USA.* 70(9): 2656-2659.
- Tamm, G. R. and J. S. Cobb. 1978. Behavior and the crustacean molt cycle: changes in aggression of Homerus americanus. *Science* 200: 79-81
- Wolf, E. 1905. Die Fortpflanzungsverhältnisse unserer einheimischen Copepoden. *Zool. Jahrbucher Abth. f. Syst.* 22: 101-280.

Figure 22. Frequency of occurrence for behavioral interactions
with D. leptopus.

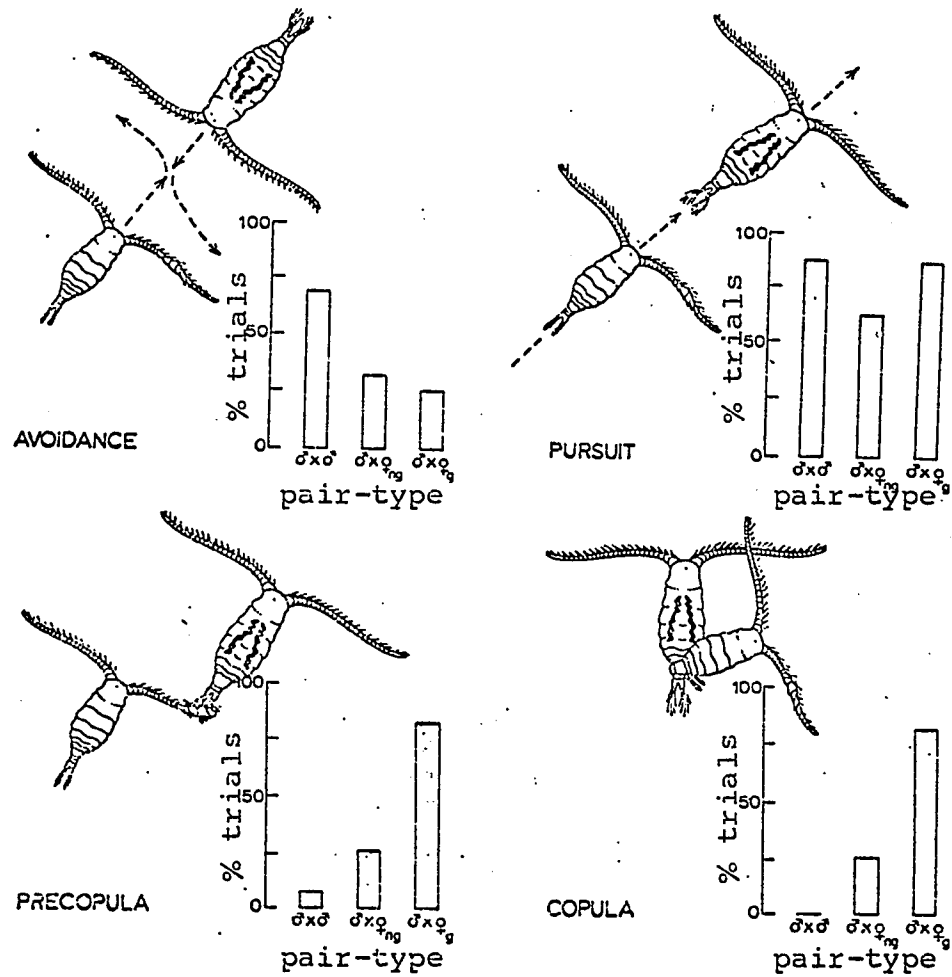


FIGURE 22

Table 11. Comparison of swimming-speeds for male and female D. leptopus.

Sex	<u>n</u>	swimming-speed (mean <u>±</u> 95% CL)
male	20	14.9 (<u>±</u> 1.86) cm min ⁻¹
female (gravid)	27	10.7 (<u>±</u> 1.05)
female (nongravid)	27	8.4 (<u>±</u> 0.72)

Table 12. Inventory of Social Behaviors Exhibited by Diaptomid Copepods.

<u>Behavior</u>	<u>Description</u>	<u>Previous Reports</u>
Avoidance	males and females; frontal-lateral approach; quick leap away	Strickler (1973)
Pursuit	males only; caudal or caudolateral approach; increased swimming velocity; coordinated turns; attempts to grasp lead copepod	Jacobs (1961); Fleminger (1967); Katona (1973)
Searching	males only; small circles and loops, generally while hanging vertically	Parker (1901); Katona (1973)
Precopula	males only: grasp caudal setae, furcae, or urosome with geniculate antennule	Wolf (1905); Gauld (1957); Felminger (1967); Blades (1979); Katona (1975)
Copula	males only; clasps urosome with endopod of right 5th leg; releases antennular grip on caudal furcae; deposits spermatophore on females genital segment with left 5th leg	Wolf (1905); Gauld (1957); Lee (1972); Blades (1979); (Katona, 1975)

Table 13. Data from mating interactions between male Diaptomus and conspecific gravid or nongravid females (data pooled from all experiments).

Species	Reproductive Condition of female	Total no. of copulations observed	Time spent in copula (mean, range in minutes)	No. ovisacs produced	Average time to production of ovisac (post copula) (mean, range in minutes)
<u>D. leptopus</u>	gravid	26	6.2 (1.2 to 19.5)	exact data not available	15.5 (13.4 to 20.1)
	nongravid	8	4.2 (4.0 to 7.0)	0	(n = 3)
<u>D. pygmaeus</u>	gravid	8	1.5 (0.6 to 2.0)	7	data not available
	nongravid	3	0.7 (0.5 to 0.8)	0	
<u>D. pallidus</u>	gravid	13	1.3 (0.5 to 1.8)	8	25.4 (20.5 to 38)
	nongravid	2	0.5 -	0	
<u>D. dorsalis</u>	gravid	9	13.8 (9.0 to 15.5)	4	data not available
	nongravid	3	10.6 (10.0 to 11.8)	0	

Table 14. Results of chi-square tests comparing reactions of male D. leptopus to conspecific adults (data from Figure 22).

Behavior	Comparison ("targets")		
	gravid female vs. nongravid female	gravid female vs. male	nongravid female vs. male
Avoidance	0.15 ^a	6.15**	4.50*
Pursuit	2.67 ^a	0.000 ^a	2.67 ^a
Precopula	10.16***	18.29****	2.13 ^a
Copula	10.16***	21.89****	4.57*, ^a

* 0.025 < p < 0.05

** 0.005 < p < 0.01

*** 0.001 < p < 0.005

**** p < 0.001

a. expected value of 2 cells less than 5.

Table 15. Evidence for selection of gravid females by conspecific male Diaptomus

Species	Temp. °C	Duration of each trial (min)	Reproductive condition of female	No. of trials	No. of trials ending in copula	Chi ²	P
<u>D. leptopus</u>	21 (+ 1.5)	10	gravid nongravid	16 16	13 4	10.16	<0.005
<u>D. pygmaeus</u>	20 (+ 0.5)	30	gravid nongravid	12 12	8 3	4.20	<0.05
<u>D. pallidus</u>	22 (+ 0.5)	60	gravid nongravid	16 15	13 2	14.30	<0.001
<u>D. dorsalis</u>	21 (+ 1.0)	60	gravid nongravid	17 15	9 3	5.00	<0.05

Table 16. Transpecific mating interactions with Diaptomus

Male	Female		
	<u>D. dorsalis</u>	<u>D. pallidus</u>	<u>D. pygmaeus</u>
<u>D. dorsalis</u>	-	Trials: 3 Copula: 1	Trials: 2 Copula: 1
<u>D. pallidus</u>	Trials: 2 Copula: 0	-	Trials: 3 Copula: 2
<u>D. pygmaeus</u>	Trials: 4 Copula: 4	Trials: 2 Copula: 1	-

Appendix I. Relationship between the duration of embryonic Development (D, in days) and Temperature (T, in °C) for several species of Diaptomus. Data are means (rounded to nearest 0.1 day or °C) from indicated sources. Functions fit by least-squares regression, R and r are correlation coefficients.

Species	Source	D	T	Function	R or r
<u>D. oregonensis</u>	(Cooley, 1970)			$\log D = 1.836 - 0.454 \log T -$	1.00
				$0.491 (\log T)^2$	
		23.0	4.2		
		8.9	9.1		
		4.6	14.2		
		2.8	19.0		
		2.8	19.1		
		2.1	22.9		
		2.0	23.0		
		2.0	23.1		
2.0	23.2				
<u>D. clavipes</u>	(Robertson <u>et al.</u> , 1974)			$\log D = 6.533 - 7.727 \log T +$	1.00
				$2.280 (\log T)^2$	
		4.7	14.0		
		2.0	21.0		
		1.4	27.0		
1.2	31.0				
<u>D. gracilis</u>	(Eckstein, 1964)			$\log D = 1.555 - 0.2678 \log T -$	0.99
				$0.4674 (\log T)^2$	
		26.5	2.0		
		17.4	4.0		
		13.1	5.0		
		11.8	6.0		
		9.1	8.0		
		6.9	10.0		
		5.3	12.0		
		4.2	14.0		
		3.3	16.0		
		2.9	18.0		
		2.5	20.0		
		2.2	22.0		
		2.1	23.0		
2.0	24.0				
2.0	25.0				
1.5	29.0				

Species	Source	D	T	Function	R or r
<u>D. gracilis</u>	(Elster, 1954)			$\log D = 1.866 - 0.6516 \log T - 0.3448 (\log T)^2$	0.99
		18.8	4.8		
		14.6	5.6		
		13.3	5.7		
		14.2	6.1		
		14.0	6.3		
		13.0	6.8		
		8.5	8.8		
		8.7	8.9		
		7.1	10.6		
		6.9	11.2		
		6.9	11.3		
		5.6	11.8		
		5.6	11.9		
		5.7	12.0		
		5.5	12.3		
		5.7	12.5		
		5.8	12.6		
		4.5	13.7		
		4.5	13.9		
		3.9	15.4		
		3.9	15.8		
		4.0	16.2		
		3.8	16.3		
		3.3	16.5		
		3.6	16.6		
		3.4	16.7		
		3.4	16.8		
		3.5	16.9		
		3.2	17.0		
		3.0	17.6		
		3.1	18.5		
		3.3	19.4		

Species	Source	D	T	Function	R or r
		2.8	19.8		
		2.6	21.9		
		2.0	22.5		
		2.3	22.6		
		2.2	22.8		
		2.2	22.9		
		2.3	23.1		
<u>D. gracilis</u>	(Munro, 1974)			$\log D = 2.207 - 1.435 \log T +$	1.00
		16.3	5.0	$0.0076 (\log T)^2$	
		5.8	10.0		
		3.3	15.0		
		2.2	20.0		
<u>D. pallidus</u>	(Geiling and Campbell, 1972)			$\log D = 5.230 - 6.211 \log T +$	0.99
		7.9	10.0	$1.875 (\log T)^2$	
		3.2	15.0		
		2.2	20.0		
		1.6	25.0		
<u>D. lacinatus</u>	(Eichhorn, 1957)			$\log D = 1.646 - 0.4911 \log T +$	0.99
		26.0	2.5	$0.311 (\log T)^2$	
		22.0	2.7		
		23.7	2.9		
		22.5	3.2		
		19.8	3.5		
		14.5	3.9		
		12.8	5.3		
		12.9	5.6		
		13.3	5.7		
		11.0	6.3		
		10.8	7.0		
		8.8	8.1		
		9.1	8.3		
		8.3	9.3		

Species	Source	D	T	Function	R or r
		6.4	11.5		
		5.8	11.7		
		6.2	11.9		
		5.5	12.8		
		5.1	12.9		
		4.9	13.6		
		4.4	13.7		
		2.9	17.2		
		3.6	17.8		
		3.4	18.1		
		3.3	18.2		
		3.2	18.3		
		3.0	18.4		
		3.2	18.5		
		3.0	18.6		
		3.1	21.2		
		2.9	21.6		
		3.1	21.7		
		2.9	21.9		
		2.6	22.0		
		2.6	22.1		
		2.7	23.1		
<u>D. denticornis</u>	(Eichhorn, 1957)	18.0	6.7	$\log D = 2.596 - 1.61 \log T$	0.99
		6.7	12.9		
		6.0	13.2		
		5.9	13.5		
		5.3	14.5		
		5.1	14.7		
		5.1	14.9		
		5.3	15.1		
		4.8	16.5		
		4.3	16.7		
		3.6	17.5		
		2.6	22.0		

Species	Source	D	T	Function	R or r
<u>D. pygmaeus</u>	(This study)	6.3	12.0	$\log D = 2.441 - 1.528 \log T$	0.99
		3.2	18.0		
		2.2	24.0		
<u>D. leptopus</u>	(This study)	5.9	12.0	$\log D = 2.600 - 1.698 \log T$	0.99
		3.0	18.0		
		2.1	21.0		
		1.9	24.0		

Appendix 2. Data from remating experiments with D. leptopus. In each experiment, the interactions between one newly-matured male and five newly-matured, gravid females were recorded continuously on video-tape

Expt. #	Time to 1st copula (min.)	Interval between subsequent attempts to mate (min.)			Time spent in copula (min.)			
		2nd	3rd	4th	1st	2nd	3rd	4th
1	2.3 ^a	50.7	50.3	53.5	19.5	3.9	5.9	7.4
2	8.6 ^b	44.5 ^d			3.6	1.2		
3	14.4 ^c	59.1 ^e			7.9	10.1		
4	3.3	61.7			11.9	2.0		
5	9.2	51.3			5.3	-		

a, b, c Time to appearance of ovisac (postcopula): 13.4, 20.1, 13.0 min.

d, e Unsuccessful transfer of spermatophore, carried by male: 5.2, 4.0 min.

Appendix 3. Supplementary data from mate-selection experiments (see Figure 1) with D. leptopus

Target	Most extensive pursuit	Searching behavior	Time spent in copula (mean and range in minutes)	% matings which yielded ovisacs	maximum time spermato-phore attached (hrs.)
gravid females	20 cm, 2 turns	yes	5.5 (3 to 7)	33%	1
nongravid females	20 cm, 2 turns	yes	4.2 (4 to 7)	0%	20
males	40 cm, 3 turns	yes	-	-	-
