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Behavioral Changes in Male Diaptomus leptopus
(Copepoda: Calanoida) in Response
to Female Conditioned Water.

H. C. van Leeuwen

A Thesis
in
The Department
of
Biology

Presented in Partial Fulfillment of the Requirements
for the Degree of Master of Science at
Concordia University
Montreal, Quebec, Canada

September 1988

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ABSTRACT

Behavioral Changes in Male Diaptomus leptopus
(Copepoda: Calanoida) in Response
to Female Conditioned Water.

H. C. van Leeuwen

Swimming behavior of male Diaptomus leptopus was recorded prior to and after introduction of either gravid females, nongravid females, or males into one compartment of a two-compartment vessel. The compartments were separated by 110 micron nylon mesh. Swimming behavior (swimming speed and turning behavior) was recorded with a video camera. Swimming behavior of male D. leptopus was altered significantly only after exposure to water conditioned by gravid females. Swimming behavior was not altered in the apparatus where the holding compartment was separated from the other compartment by 5.0 cm but changes in swimming speed were observed when the separation between the two compartments was decreased to 2.5 cm.

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There comes a time when one must name names, to give credit where credit is due. This is one of those times, whether those named below wish to be recognized or not.

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A sincere Thank-you all !

DEDICATION

To my parents for reasons too numerous to mention.

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INTRODUCTION

Position Within Foodwebs

Copepods form an important link within aquatic food chains or webs playing pivotal roles as both predators and prey, transferring energy to higher trophic levels and affecting lower trophic levels by what they consume. They make up the most important planktonic group in marine environments contributing the bulk of the biomass in some seas (Hassel 1986). In most seas they are generally considered to be the most dominant fraction of the zooplankton, making up approximately 70% of the planktonic fauna (Raymont 1983). In fresh water systems copepods along with cladocerans and rotifers are common components of zooplankton communities (Gilbert and Williamson 1983).

Copepods may be herbivorous (Turner 1986), carnivorous (Williamson 1983), or omnivorous (Conley and Turner 1985). In areas studied by Dagg and Turner (1982) they found that copepods consumed approximately 50% of the annual particulate primary production. They feed on a range of phytoplankters (Turner 1986, 1987) and can select prey based on size (Price et al 1983), shape (Gifford et al 1981) and nutritional value (Donaghay & Small 1979, Huntly et al 1983, Paffenhofer & Van Sant 1985). Predatory and omnivorous copepods in fresh water systems can exert

considerable pressure on rotifer prey populations so as to affect the population ecology and community structure of these organisms (Williamson 1983, Williamson and Butler 1986). For instance, copepod predation is thought to be responsible for changes in rotifer communities dominated by soft bodied species in the spring to communities dominated by colonial and evasive species in the midsummer and fall due to differential predation rates (Stemberger and Evans 1984).

Copepods of all developmental stages, eggs through adult, are important prey items for numerous other organisms. A number of fish species consume copepods as part of their diet (Govani et al. 1983, Checkley Jr. 1982, Conway 1980). Zooplankton consumption rate estimates of between 10,000-318,000 metric tons per day have been made for the caplin on the Grand Banks (Vesin et al 1981) with copepods providing a major share of the diet. Chaetognaths and ctenophores also utilize copepods as a significant portion of their diets (Reeve 1980, Sullivan 1980). Copepods also make up a large part of the diet of some marine bird species such as Cassin's auklet (Vermeer et al 1985) or Dovekies where they can comprise 100% of the diet of subadult birds and 99.8% by dry weight of the diet of adult birds in May and June (Bradstreet 1982). In the Bering Sea where copepods are the prime food source during the breeding season for nesting auklets, the numbers and distribution of auklets seem to be related to the

distribution of copepod biomass (Springer and Roseneau 1985).

Nutrient Cycling and Detrital Fluxes

There is a close relationship between grazers and planktonic algae in terms of cycling of nutrients. Nutrient regeneration by grazers provides an important source of nutrients for algae at times when allochthonous inputs and dissolved pools would not support these populations (Lehman 1980, 1984, Korstad 1983). Since copepods can comprise a large part of the zooplankton biomass, they probably play a significant role in terms of nutrient regeneration.

They are also an important component of detrital organic fluxes due to their grazing activities. For example transport of chlorophylls to deep water ecosystems via faecal pellets may represent up to 50% of the flux of chlorophylls between pelagic and benthic ecosystems (Bathman and Liehezeit 1986). Faecal pellets are known to be an important factor in the transportation of organic material to deep sea and benthic systems (Angel 1984).

Sexual Reproduction and Encounter Probability

In light of the integral role played by copepods within pelagic ecosystems, those factors that affect their

production can influence the impact that this group has within the ecosystem. Reproductive ability or potential will be an important factor in regulating their productivity. Reproduction in calanoid and cyclopoid copepods is sexual, therefore male and female copepods must encounter each other to mate (Gilbert and Williamson 1983) in order to maintain their population. While in some species a single mating leads to the production of several clutches (Smyly 1970, Whitehouse and Lewis 1973), remating is required for the production of each clutch in others (Wilson and Parrish 1971, Watras and Haney 1980, Watras 1983a, Williamson and Butler 1987). Hence factors that increase the probability of male-female encounters would tend to increase reproductive success and population levels. Gerritsen and Strickler (1977), using a mathematical model, studied encounter probabilities in zooplankton. Of the factors that affected encounter rates, such as swimming speeds and directions, the encounter radius was found to have the greatest influence on encounter probabilities (Gerritsen 1980a). In mating encounters the encounter radius would be the distance over which the male can detect a conspecific female. The size of this radius will depend greatly on the abilities and sensitivities of the male sensory systems.

Sensory Systems

It has been argued (Strickler 1975) that copepods do not use vision to detect conspecifics due to the fact that light conditions did not affect encounter rates in the laboratory (Strickler 1970 in Strickler 1975). Also, the simple nature of the nauplius eye is such that while copepods can detect changes in light intensity, it is probably not used for image detection (Elofsson 1966). This leaves us with basically two major sensory systems that can be used by copepods to gain information about the location of conspecifics, these are the mechanosensory and chemosensory systems.

The role of mechanoreception has been investigated using schlieren photography (Strickler 1975), which allows one to photograph the disturbances created by a swimming organism. He demonstrated that a zooplankter can detect an approaching organism before coming into contact with it by sensing the displacements of water produced by a moving organism. The antennules of a copepod have been shown to be sensitive to both touch (Gill 1985) and water disturbances produced by a jet of water (Gill and Crisp 1985). Morphological studies have provided evidence (ie. presence of pegs and setae that articulate and are innervated) for the existence of mechanoreceptors on both the body surface and the antennules (Strickler and Bal 1973, Gill 1986).

Essentially the same two lines of evidence support the contention that copepods use chemoreception to sense the environment. Behavioral studies have shown that copepods will exhibit alterations in swimming pattern in the presence of phytoplankton exudate as compared to swimming patterns in filtered sea water (Buskey 1984, Gill and Harris 1987). Evidence presented by Poulet and Ouellet (1982) showed that attraction and swarming behaviors of two different species of copepods could be induced by the presence of specific amino acids, and that each species was attracted to a different group of amino acids. Morphological studies have demonstrated the existence of such features as pegs with openings and the presence of pores on the integument of some copepods. The presence of these structures, in conjunction with the types and numbers of dendrites normally associated with chemoreception, identify some of the structures that are present on the first antennae (Barrientos 1980, Gill 1986) body surface (Elofsson 1971) and mouth parts (Friedman and Strickler 1975, Ong 1969) as being consistent with those that function as chemoreceptors.

Pheromones

Pheromones have been defined as substances secreted from an individual that are received by a conspecific and release a specific behavioral or developmental process

(Karlson and Luscher 1959). If this substance is secreted by one sex and received by the other causing alterations in the behavior of the conspecific in such a manner so as to increase the probability of a successful mating encounter, one may define the substance as a sex pheromone (Dunham 1978).

Anecdotal evidence for the presence of a sex pheromone in copepods was first provided by Parker (1901) who observed changes in the swimming behavior of male Labidocera aestiva when they approached a cylinder containing females as compared to a cylinder containing no females. However Holms (1909) using the same methods could not find evidence for chemically mediated behavior in the copepod Cyclops fimbriatus. Katona (1973) described a type of swimming behavior that was exhibited only by males which consisted of "a very quick and complex swimming pattern, involving somersaults and tight-radius turns and loops". He termed this swimming pattern mate-seeking behavior. This behavior was most often observed when the male was in the presence of a female of its own species and less often when in the presence of a male of the same species or the female of another. Griffiths and Frost (1976) observed the same type of behavior when male copepods were exposed to female conditioned water (water that previously contained recently molted adult female copepods) but rarely saw this behavior when the males were exposed to male conditioned or filtered water. Using

radiotracer methods, they found that dissolved organic substances produced by radioactively labelled females, accumulated at a higher rate by the aesthetes of male antennules than any other part of the male copepods body. This suggests that the aesthetes (elongated, blunt tipped outgrowths of the exoskeleton occurring on the first and second antennae (Pennak 1978)) function as chemoreceptors.

In mating experiments it has been shown that females that are gravid have significantly higher rates of copulation than females that are nongravid (Jacoby and Youngbluth 1983, Watras 1983b). Watras (1983b) suggested two possible mechanisms that would result in a higher copulation rate for gravid females over nongravid females. Gravid females were either more receptive to the males, or released chemicals, sex pheromones, that were then used as cues by the males.

Behavioral Assay

Dunham (1978) described three behavioral response which have been used as evidence for the presence of a pheromone when they were observed. One consists of alterations in swimming behavior such as (chemokinesis); altering swimming speeds (orthokinesis) and /or turning behavior (klinokinesis) upon detection of a chemical substance. A second behavioral response (chemotaxis), involves the movement directly towards or away from a pheromone source.

The location of the pheromone source is determined by sensing the concentration gradient of the pheromone. A third manner involves the detection of the condition of the female thereby releasing specific copulatory or precopulatory behaviors in the male if the female is gravid (releaser reactions). In each case the presence of a chemical substance induces a specific behavior. Observation of this behavior, so long as all other sensory modes are excluded or controlled for, can be used as evidence towards the existence of a pheromone.

Hard evidence for the existence of a pheromone would require that a number of other criteria be met. These include isolation and characterization of the chemical as well as determination of the functional significance of the pheromone (is the chemical released and detected for the purposes we believe it to be? (Dunham 1988)). For these last criteria to be met an effective behavioral assay is required.

I undertook to develop such an assay and determine if there is behavioral evidence for the existence of a sex pheromone in a calanoid copepod. Copepods have been shown to respond to chemical compounds by altering their swimming speeds and turning behavior (Buskey 1984). Male copepods have also demonstrated alterations in these parameters when near a female copepod, or before encountering her to copulate (Katona 1973, Uchima and Murano 1988). Increases in swimming speeds of one individual relative to another

will increase the probability of encounter (Gerritsen 1980a) and increases in turning behavior are indicative of area-restricted searching (Pyke 1984). Changes in both swimming speed and turning behavior may be expected to occur if female copepods produced a chemical substance and the male copepod could detect it. For these reasons alterations in swimming speed and turning behavior were chosen as indicators of a pheromone.

The specific questions under study are as follows:

- 1) Is the pattern of higher copulation rates for gravid females over nongravid females seen in the laboratory, also observed in the field?
- 2) Is there chemokinetic evidence for the production of a pheromone by gravid females?
- 3) Is the effect of gravid females distance-dependent?

METHODS AND MATERIALS

MATING and REPRODUCTIVE STATE

Laboratory studies have demonstrated that copulatory rates in gravid females are higher than nongravid females (Watras 1983b, Jacoby and Youngbluth 1983, Chow-Fraser and Maly 1988). To determine if this trend also occurs in nature, data from diagonal tows taken with a Wisconsin plankton tow net (80 μ m mesh size) in the summer of 1984 at a number of lakes in the region of Montreal were analyzed (see Appendix I for location of the lakes). Additional data from 1983 for Diaptomus birgei Marsh, 1894, were included to increase the sample size for this species. I took the presence or absence of a spermatophore on the female to reflect recent copulatory activity and the presence or absence of ripe oocytes to reflect the females reproductive condition. Females were considered gravid when the oviducts were dark coloured and nongravid when the oviducts appeared clear (Watras 1983b, Watras and Haney 1980). The total number of gravid and nongravid females for Diaptomus leptopus Forbes, 1882, D. oregonensis Lilljeborg, 1889, D. minutus Lilljeborg, 1889, and D. birgei were tabulated and the number of females with or without a spermatophore was counted for each species.

Fisher's exact test (Zar 1984) was then performed on the tabulated results of each species to determine if the proportion of gravid females with a spermatophore was significantly greater than the proportion of nongravid females with a spermatophore. This test was used since for each species tested greater than 20% of the cells contained expected values that were less than five. These values would bias results if a regular contingency table were used.

BEHAVIORAL ASSAYS

APPARATUS

The basic apparatus (Figure 1.) consisted of an observational vessel positioned on top of a stage. A light source was placed under the stage to provide back lighting. This light passed through a red filter, was reflected by a mirror, then passed through an semiopaque filter. The light intensity was kept at a low level (less than 2.5 ft/cd) since there is evidence (Katona 1973) that high light levels inhibit search behavior. A red filter (approx. 600 nm) was used to further diminish any effect that back lighting may have had since copepods have reduced sensitivities at the red wave lengths (Swift and Forward 1983, Stearns and Forward 1984a) and do not appear to respond in the far-red (770 nm) end of the spectrum

Fig. 1: Diagram of the apparatus.

DIM OVERHEAD LIGHTING

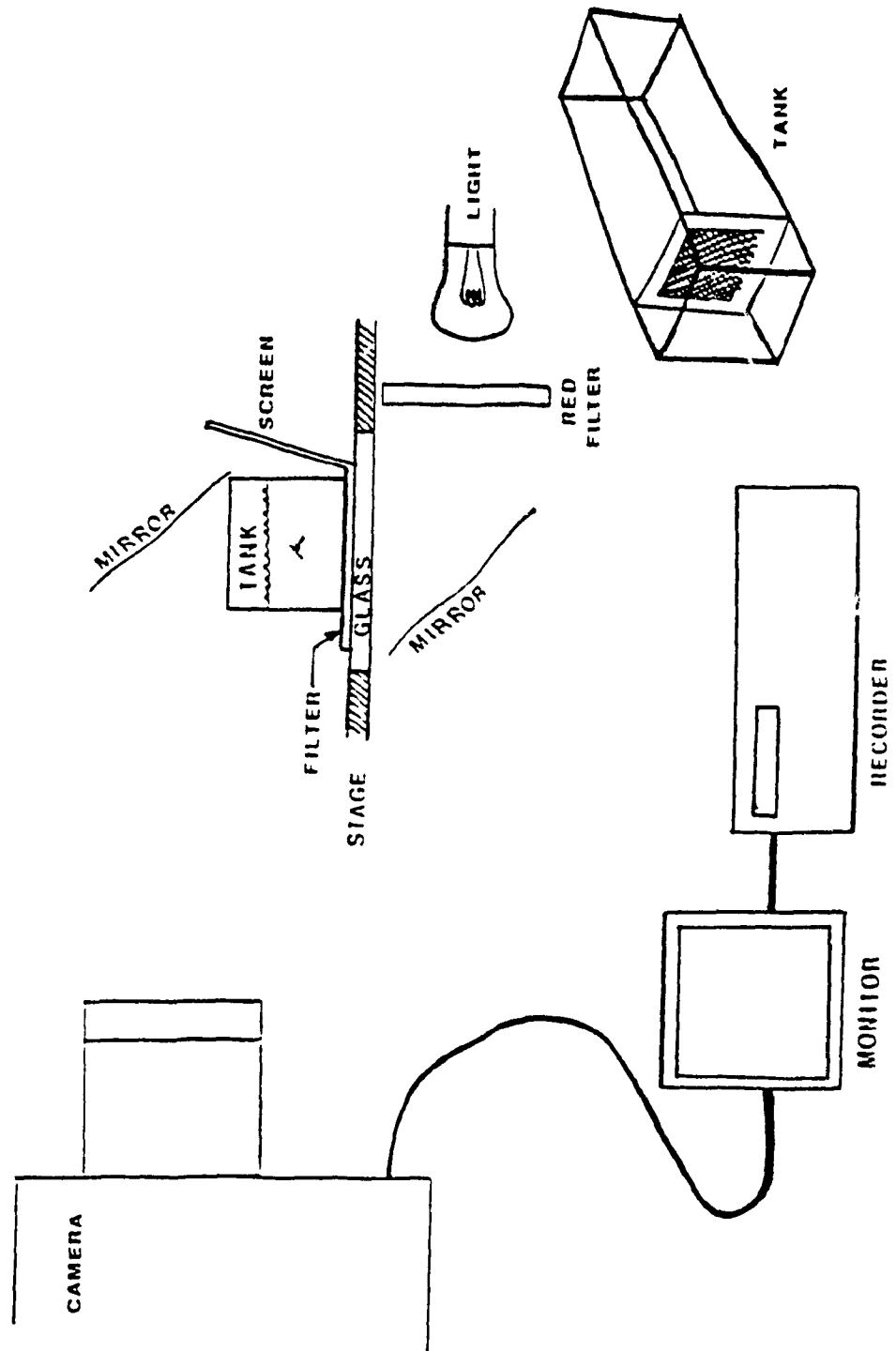


Fig. 1

(Stearns and Forward 1984b). A mirror on top of the tank enabled the camera to observe both the vertical and horizontal view. The behaviors were monitored on a closed circuit television with a video camera, monitor, recorder system. The camera was a Hitachi model HV-17A CCTV camera with a S-4092 Newvicon tube. This tube is sensitive to low levels of light, (0.5-1,500 lx, (0.05-150 ft:cd)), with a standard lens (F 1.9- F 22). The lens used was a 25 mm Apollo high speed lens, F/0.85, which enabled me to tape at light intensities lower than that actually used, assuring a good picture.

The tank or vessel was constructed of plexiglass and divided into two compartments by a 110 μ m mesh screen. The larger or behavioral compartment measured 5 x 2 x 2.5 cm. (length x width x height); the smaller holding compartment measured 1.5 x 2 x 2.5 cm.

A second, larger, vessel was constructed to determine the distances over which a male copepod could detect the presence of the pheromone. The overall dimensions of this tank were 14 x 2.5 x 2.5 cm. The holding compartment measured 2.5 x 1.5 x 2.5 cm. with a screen of 110 μ m mesh on one side. The behavioral compartment, 5 x 2.5 x 2.5, was produced by two moveable separations, one of which had a screen covered by 110 μ m mesh, which allowed me to vary the distance between the two compartments.

COLLECTION AND ISOLATION

D. leptopus were collected from Barbotte 3 or Hill Pond with a Wisconsin plankton tow net before each experiment. Behavioral experiments were carried out between October and March. Samples were transported back to the laboratory in a 20 liter carboy and stored at (10-12 °C). The test animals that were to be used in behavioral studies the next day were isolated to preclude mating. This was done by pouring the contents of the carboy through a cup with nylon mesh (110 μ m) at one end. The cup was then placed into a 1000 ml beaker of filtered pond water. With the aid of a dissecting microscope the copepods were drawn off, isolated, and sorted into three categories: males, non gravid females, and gravid females. Each group of copepods was then placed in a separate 100 ml beaker of pond water that had been filtered through 110 μ m mesh. Experiments were carried out over the subsequent two days.

PROCEDURE

The day after sorting, a single male was introduced into the observational portion of the tank and allowed to acclimate for 10 minutes. Only active copepods were chosen. After the acclimation period, the male copepod's behavior was recorded on videotape for 10 minutes. Ten treatment individuals; gravid females, non gravid females,

or males, were then introduced into the holding portion of the tank and the behavior of the test male was monitored for another 15 minutes. Each male copepod was only used once and is considered a separate trial. The experimental chamber was re-used for each trial after rinsing with hot water. Katona (1973) found that the attractive quality of the chemical was negatively affected by heat. The same treatment individuals were used for each of the six trials that were carried out that day. One treatment was carried out over each two day period. New individuals from the carboy would be sorted for the next day's experiments. The treatments were carried out in the following order: gravid females, nongravid females, males, and gravid females. The volume of water in the tank was kept the same for each trial by filling the container to a height of 2.0 cm with filtered pond water. Experiments were carried out between 1:00 PM and 8:00 PM, however the time of day should not affect capacity to mate since it has been shown that there is no diel periodicity in phase set (presence or absence of ripe oocytes) or egg laying (Williamson and Butler 1987).

ANALYSIS OF BEHAVIOR

Analysis of the experimental male's swimming pattern was done at a later date by examining a 3 minute period before introduction of the treatment individuals and a 3 minute period, 10 minutes after the introduction of the

treatment organisms. Parameters monitored were swimming speed and turning behavior.

Swimming speeds were calculated by tracing the path of the male copepod over the 3 minute periods, from the video monitor onto acetates. This was done by taping an acetate onto the screen of the monitor and then tracing the path of the copepod onto the acetate with a marker as it swam. Total path lengths were obtained by following the paths traced on the acetates with a cartographers wheel. This distance was then divided by the time over the given path length to obtain swimming speed. This value was multiplied by a conversion factor to reach a real swimming speed. The conversion factor was derived by taking into account the magnification of the camera. This was done by measuring the actual length of the container then measuring the length of the container as it appeared on the screen of the monitor. The first value was then divided by the second to provide the conversion factor. The path lengths monitored were in the horizontal plane; this was done since vertical displacement was limited and adult copepod movement is primarily (approx. 80%) in the horizontal plane (Gerritsen 1980a).

Turning behaviour was measured as discribed by Buskey (1984), Buskey et al (1983). The shortest distance between the start and finish of a given course of travel (Net path Displacement) was measured. This value was divided by the total path length (Gross Displacement) to obtain a ratio.

This ratio is termed the Net to Gross Displacement Ratio (NGDR). The ratio varies from 0-1 where low ratios indicate a high amount of turning behavior while high ratios indicate a low amount. Thus, values of 0 would indicate that the copepod had moved in a circular pattern ending its path at the point where it had started, while values of 1 would indicate that the copepod was swimming in a straight line, net and gross path length being equal. Since the experimental animal would come into contact with the edge of the container at times, NGDR's were measured for the free swimming periods only, and a mean NGDR was calculated over the trial.

REACTIVE DISTANCE

In order to measure the distance over which the gravid females could be detected essentially the same procedure as that above was carried out. Test males were placed in the observational portion of the tank and allowed to acclimate for 10 minutes. The behavior of the male copepod was recorded for 10 minutes after which 10 gravid females were added to the holding portion of the tank. The behavior of the test male was monitored for another 30 minutes. Two sets of experiments were carried out; one where the nearest edge of the observational tank was 5 cm. away from the holding tank, and another where the nearest edge of the observational tank was 2.5 cm. away. A 3 minute section of

swimming behavior was analyzed for swimming speeds and turning behavior before introduction of the treatment individuals, and another 3 minute section, 27 minutes after their introduction. Alterations in the swimming speeds and turning behaviors were assessed by the Wilcoxon paired-sample test.

STATISTICS

Wilcoxon paired-sample tests were performed on the swimming speeds for each of the treatments to test for significant changes in behavior. Turning behavior was also assessed using the Wilcoxon paired-sample test. In all tests $\alpha = 0.05$. Paired observations of activity patterns were used to take into account individual variations in levels of activity. The Wilcoxon paired-sample test was performed on the data since the differences did not appear to come from a normally distributed population. Spearman rank correlations were performed on the data to see if there was a relationship between the initial behaviors and their subsequent changes.

RESULTS

Field data

Analysis of the field data indicates that the reproductive condition of the females can affect the probability of spermatophore placement. Values for the number of gravid and nongravid females with or without a spermatophore are presented in Table 1. These values, presented as the percentages of gravid versus nongravid females with a spermatophore, demonstrate the differences between gravid and nongravid females in frequency of spermatophore placement (Figure 2). Gravid females were found to have a significantly greater proportion of spermatophores present than nongravid females for each of the species tested ($P < 0.003$, Fisher's exact test).

Behavioral experiments

Swimming Speeds

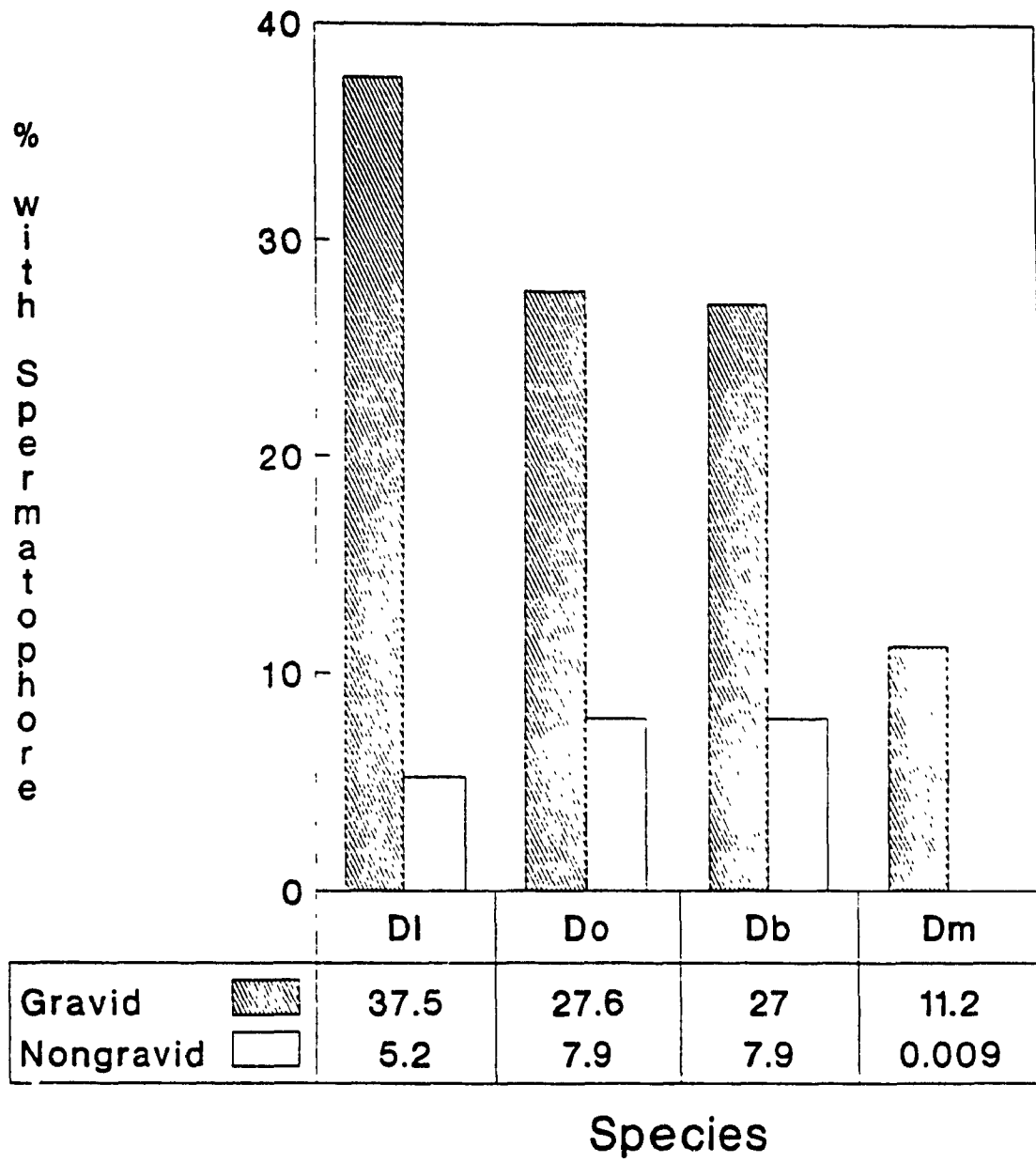
The effects of the different treatments (gravid females, nongravid females, and males) on swimming speeds can be seen in Figure 3 (Appendix II). Each bar on the x-axis represents a different trial and each trial represents the swimming behavior of a single male. The y-axis represents the change in swimming speed of the male

Table 1: The number of gravid and nongravid females with and without a spermatophore.

Species	gravid females		nongravid females		Fisher's exact test
	without	with	without	with	
<u>Diaptomus leptopus</u>	10	6	123	7	$P < 0.001$
<u>Diaptomus oregonensis</u>	21	8	257	22	$P < 0.001$
<u>Diaptomus birgei</u>	89	33	612	53	$P < 0.001$
<u>Diaptomus minutus</u>	63	8	527	5	$P = 0.003$

Fig. 2: Percentage of gravid versus nongravid Diaptomus females with an attached spermatophre. D1 = D. letopus , Do = D. oregonensis , Db = D. birgei , Dm = D. minutus

% Females with Spermatophore Gravid vs. Nongravid



copepod after introduction of the treatment individuals (swimming speed after introduction of the treatment individuals minus the swimming speed before in mm/sec). A significant increase ($P = 0.0025$, Wilcoxon paired-sample test) in swimming velocities was observed in the gravid female treatment (Figure 3a) but not in the treatments consisting of nongravid females and males (Figure 3b,c).

Turning Behavior

Figure 4 (Appendix III) illustrates changes in turning behavior (NGDR). The y-axis represents the change in the NGDR (NGDR after introduction of the treatment individuals minus NGDR before), while the x-axis represents each trial. A significant ($0.05 < P < 0.025$, Wilcoxon paired-sample test) decrease in NGDR's, which is indicative of an increase in turning behavior, was seen upon introduction of the gravid females into the test chamber (Figure 4a). No significant increase in turning behavior was observed however when males or nongravid females were introduced into the test chamber (Figure 4b,c).

Reactive Distance

In the experiment investigating the reactive distance, I found that when the test male was separated from the gravid females by a distance of 5 cm., no significant

Fig. 3: The effect of treatment on male copepod swimming speeds. Treatments consisted of gravid females Fig. 3a, nongravid females Fig. 3b, and males Fig. 3c. Each bar on the x-axis represents a different trial and each trial represents the swimming behavior of a single male copepod. The y-axis represents the change in swimming speed (swimming speed after introduction of the treatment individual minus the swimming speed before introduction of the treatment individuals) where swimming speed is measured in mm/sec.

Changes in Swimming Speed

Gravid Female Treatment

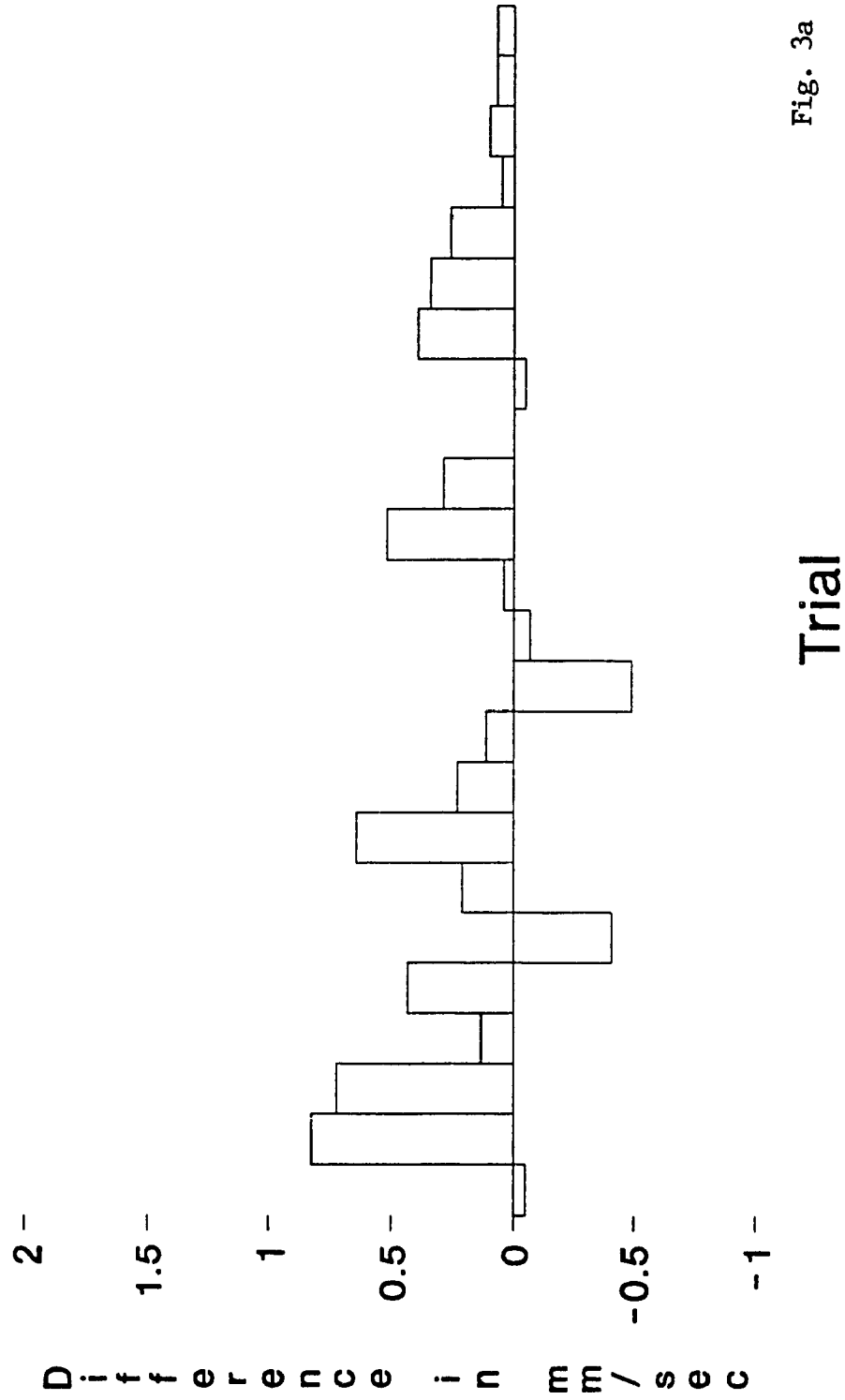


Fig. 3a

Changes in Swimming Speed Nongravid Female Treatment

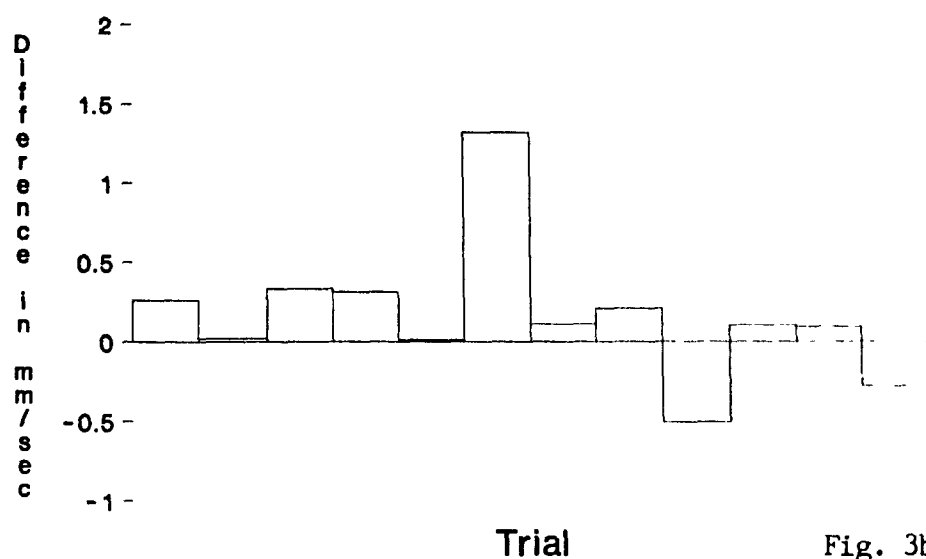


Fig. 3b

Changes in Swimming Speed Male Treatment

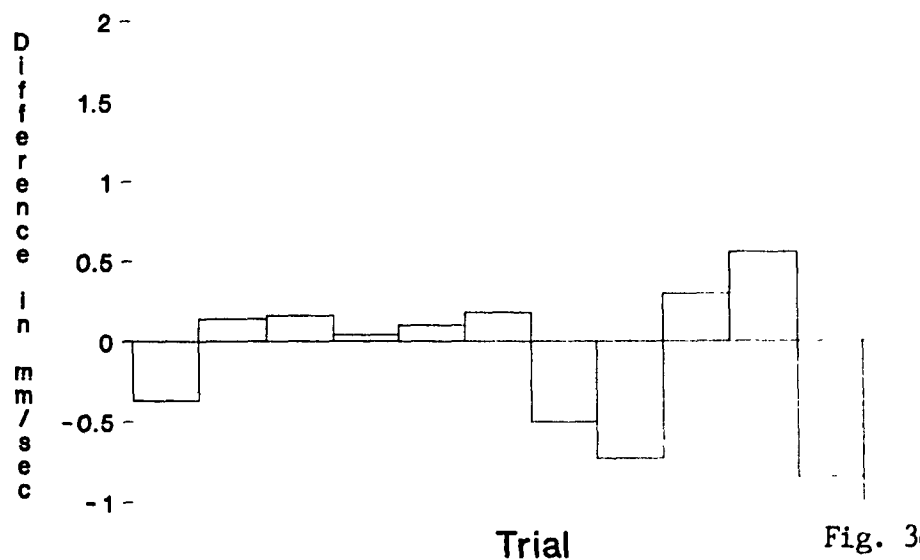


Fig. 3c

Fig. 4: The effect of treatment on male copepod turning behavior. Treatments consisted of gravid females Fig.4a, nongravid females Fig. 4b, and males Fig. 4c. Each bar on the x-axis represents a different trial and each trial represents the turning behavior of a single male copepod. The y-axis represents the change in turning behavior (NGDR after introduction of the treatment individuals minus NGDR before) which is measured as NGDR, Net/Gross Displacement Ratio.

Changes in Turning Behavior

Gravid Female Treatment

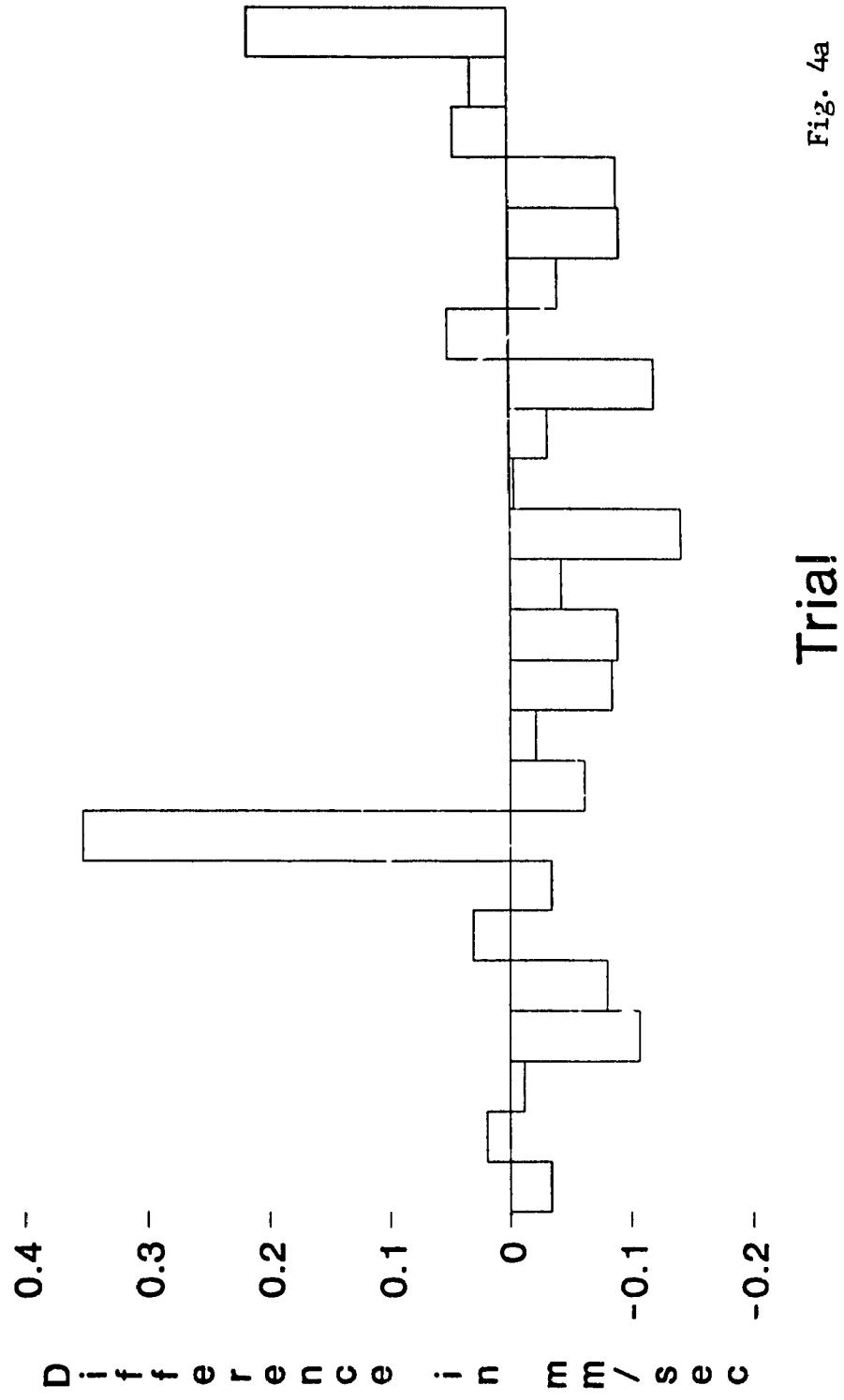
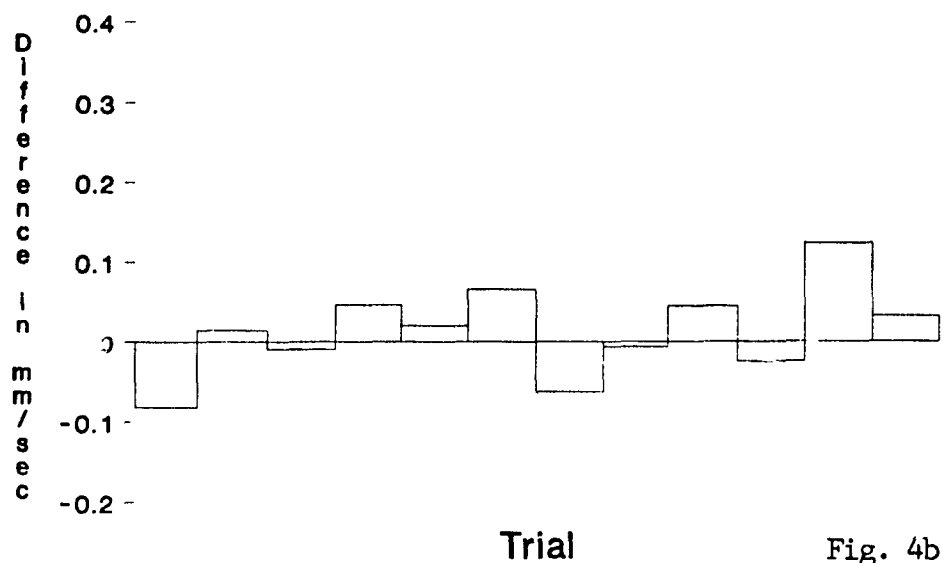


Fig. 4a

Changes in Turning Behavior Nongravid Female Treatment



Changes in Turning Behavior Male Treatment

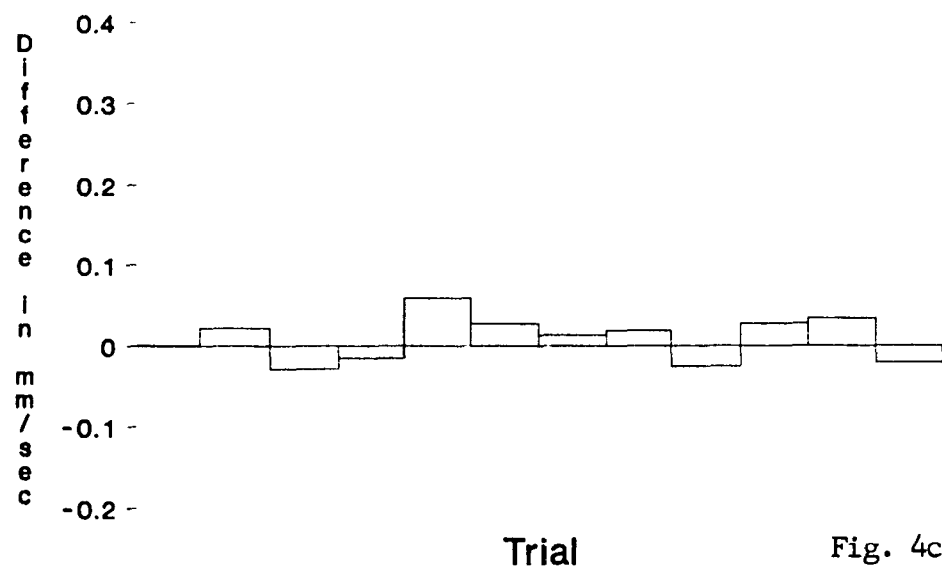
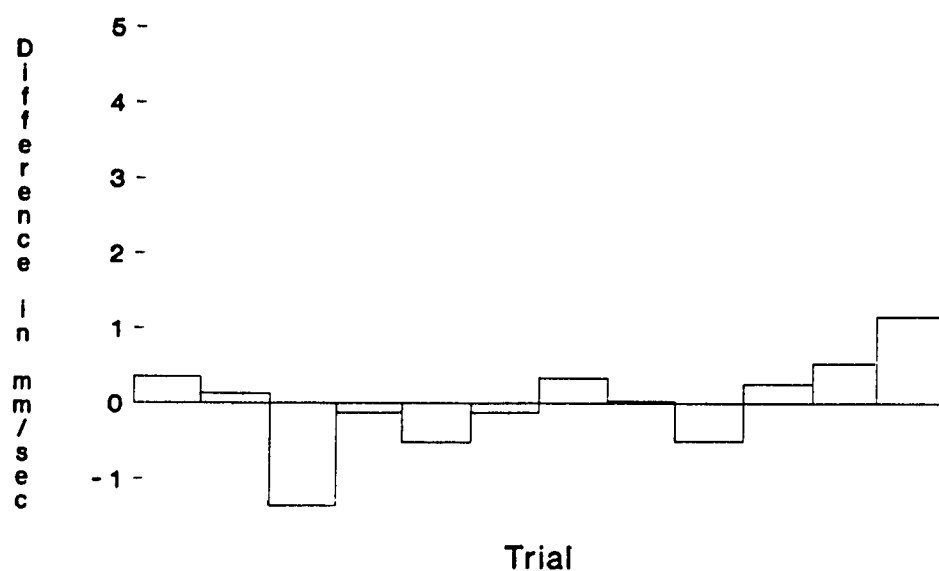


Fig. 5: The effect of distance on male copepod swimming speeds. Fig. 5a represents the change in swimming speed at a distance of 5.0 cm. Fig. 5b represents the swimming speed at a distance of 2.5 cm. Each bar on the x-axis represents a different trial, each trial being a different male. The y-axis discibes the change in swimming speed (mm/sec.). The treatment individuals are gravid females in both Fig. 5a,b.

Changes in Swimming Speed Effective Distance 5cm



Changes in Swimming Speed Effective Distance 2.5cm

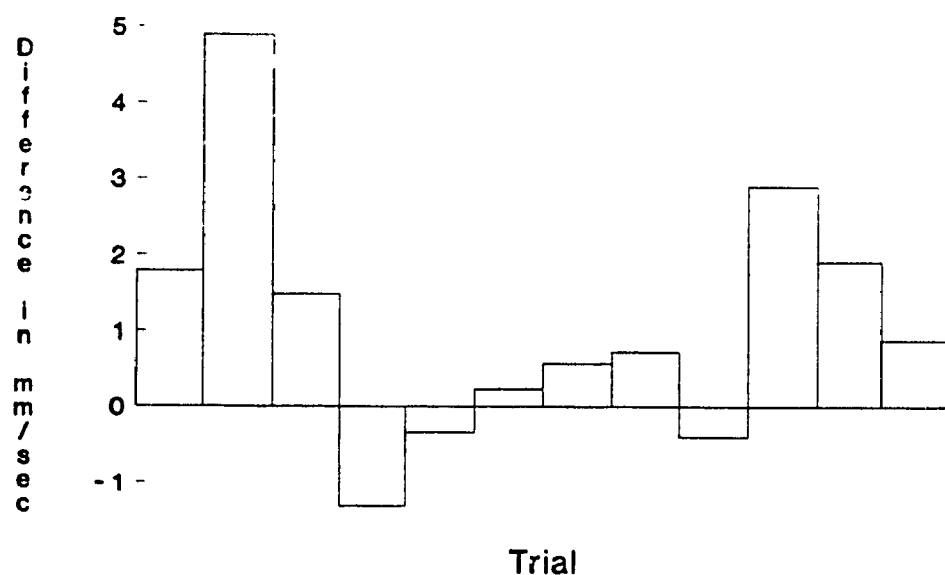
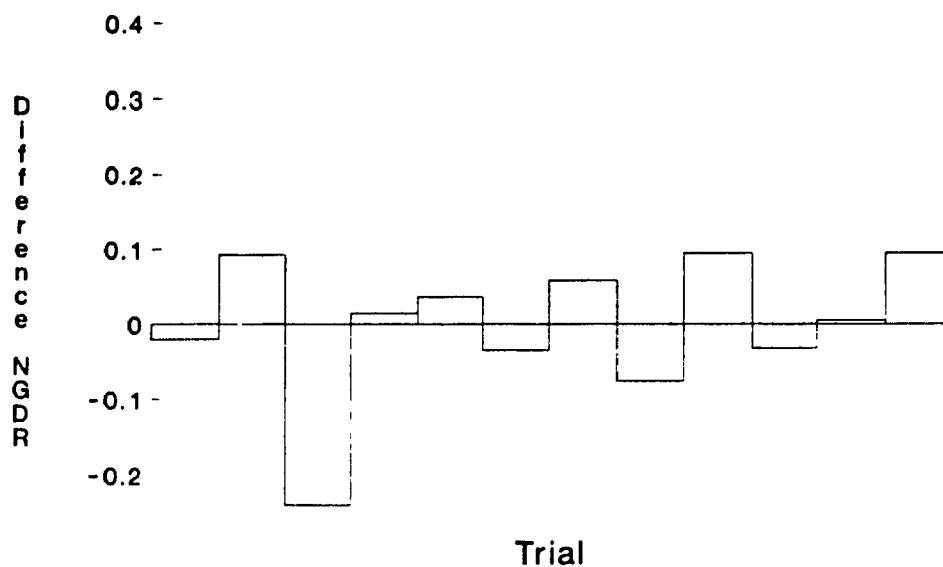
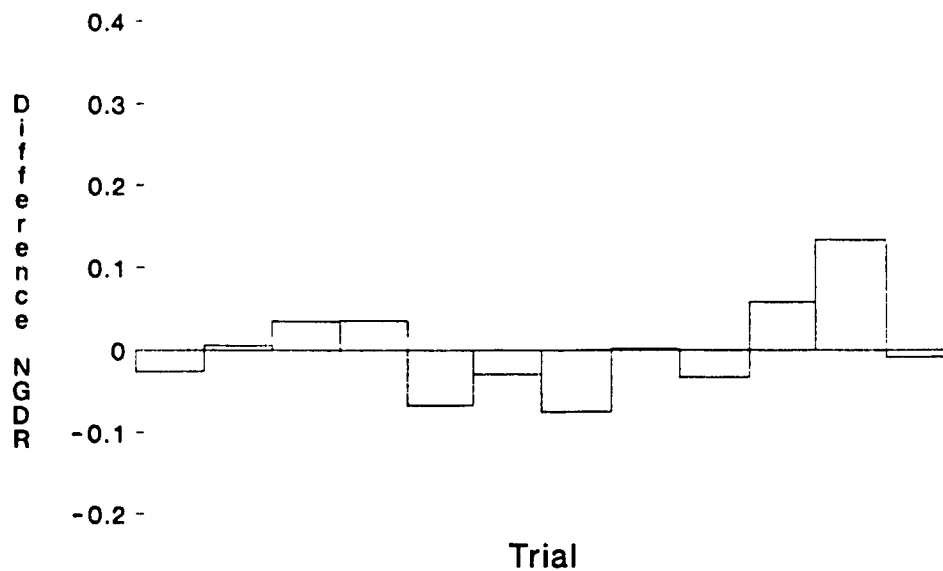


Fig. 6: The effect of distance on male copepod turning behavior. Fig. 6a represents the change in turning behavior at a distance of 5.0 cm. Fig. 6b represents the change in turning behavior at a distance of 2.5 cm. Each bar on the x-axis represents a different trial, each trial being a different male. The y-axis describes the change in turning behavior (NGDR). The treatment individuals are gravid females in both Fig. 6a,b.

Changes in Turning Behaviour Effective Distance 5cm



Changes in Turning Behaviour Effective Distance 2.5cm



alteration in the swimming speed (Figure 5a, Appendix IV) or turning (Figure 6a, Appendix V) was observed. However when the separation between the test male and the gravid females was decreased to 2.5 cm., a significant increase ($0.025 < P < 0.01$, Wilcoxon paired-sample test) in the swimming speed was observed (Figure 5b, Appendix IV). There was no concurrent significant increase in turning behavior however (Figure 6b, Appendix V).

Though there is no significant increase in turning behavior the alteration in turning behavior was greater at the 2.5 cm distance ($\bar{x} = -0.0019$, $sd = 0.0576$) than the 5.0 cm distance ($\bar{x} = -0.0008$, $sd = 0.0942$).

CONTAINER EFFECTS

Since due to the procedure used there is the possibility of container effects, the mean number of times that the test copepods came into contact with the sides of the container were tabulated (Table 2, Appendix VI). A Wilcoxon paired-sample test was performed to determine if the initial and final number of contacts with the container were different. In each of the treatments, excepting for the 2.5 gravid female treatment ($0.05 < P < 0.02$), there was no significant difference between the initial and final number of contacts. While there is a significant increase in the number of contacts in the 2.5 gravid female treatment, a rank Spearman correlation test between initial

Table 2: Mean and standard deviation of initial and final number of contacts with the side of the container for each treatment.

Treatment	Separation	Initial	sd	Final	sd
gravid female	0.0 cm	21.08	13.44	18.83	8.85
nongravid female	0.0 cm	22.17	13.13	23.67	11.44
male	0.0 cm	23.67	7.95	23.42	6.23
gravid female	2.5 cm	34.67	11.24	45.58	6.47
gravid female	5.0 cm	9.33	3.47	9.42	4.66

contacts and change in swimming speed for this treatment, found a significant negative correlation ($0.005 < P < 0.002$). This indicates that the copepods with the lower number of contacts tended to increase their swimming speeds more than those with higher initial number of contacts. Furthermore a rank Spearman correlation test between final number of contacts and change of swimming speed found that there was no correlation between these two variables, once more indicating that increases in swimming speed are not related to contacts with the container.

Table 3 is a summary of the results of the Spearman rank correlation tests examining the relationship between initial swimming speed and the degree of change in swimming speed upon introduction of the treatment individuals. Strong negative correlations were found between the initial swimming speed and degree of change in speed in the 0.0 cm gravid female and 2.5 cm gravid female treatments indicating that the individuals that were traveling at the slower speeds had the highest increases in swimming speed. Mean initial swimming speeds are essentially the same in each of the tests with the exception of the 2.5 gravid female trial. A nonparametric multiple comparison test (Zar 1984) found that there was no significant difference between the 0.0 gravid female, 0.0 nongravid female, 0.0 male, and 5.0 gravid female tests. There was a significant difference between the 2.5 gravid female test and all other tests except the 0.0 male test ($P < 0.001$ for the 0.0 cm

Table 3: Treatment, separation between trial male and treatment individuals, mean initial swimming speed for each treatment, and correlation between initial swimming speeds and change in swimming speed observed.

Treatment	Separation	Initial swimming speed (mm/sec.)	Correlation
gravid female	0.0 cm	2.40	- 0.419 P < 0.05
nongravid female	0.0 cm	2.87	- 0.293 NSD
male	0.0 cm	2.69	- 0.377 NSD
gravid female	2.5 cm	5.10	- 0.804 P < 0.005
gravid female	5.0 cm	2.03	0.226 NSD

and 5.0 cm gravid female treatments; $0.05 < P < 0.02$ for the 0.0 cm nongravid female treatment)

In terms of turning behavior (Table 4) there are three treatments where there is a significant correlation between initial turning behavior and subsequent turning behavior; 0.0 cm gravid female, 0.0 cm nongravid female, and 5.0 cm gravid female. However no significant correlation was observed in the 0.0 cm male and 2.5 cm gravid female treatment. Hence there appears to be no consistent pattern between initial and subsequent turning behaviors and therefore cannot attribute the significant change in turning behavior observed to container effects.

The possibility that aberrant behavior by a few individuals may have adversely affected the interpretation of the results or their statistical outcome was investigated. Values that were considered outliers (values greater than 3 standard deviations from the mean) and may have altered the significance of the various treatments were discarded and the data reanalyzed. I found that while the removal of the outliers did alter the level of significance of the tests (Table 5), their removal did not alter the conclusions that were drawn.

Table 4: Treatment, separation between trial male and treatment individuals, mean initial turning behavior, and correlation between initial turning behavior (NGDR) and the change in turning behavior observed.

Treatment	Separation	Initial turning behavior (NGDR)	Correlation
gravid female	0.0 cm	0.7536	- 0.428 P < 0.05
nongravid female	0.0 cm	0.8188	- 0.629 P < 0.05
male	0.0 cm	0.7910	0.126 NSD
gravid female	2.5 cm	0.8570	- 0.482 (0.1 < P < 0.2)
gravid female	5.0 cm	0.7562	- 0.795 P < 0.005

Table 5: Effect of removal of outliers (values greater than 3 sd away from the mean) on the results of statistical analysis by the Wilcoxon paired sample test. The values given are means of each treatment with and without the outlier and the changes in the levels of significance due to its' removal.

Treatment	Separation	Behavioral parameter	With outlier	Without outlier	Change in significance
nongravid female	0.0 cm	speed	0.165	0.06	P 0.1 to P 0.25
gravid female	0.0 cm	turning	- 0.0138	- 0.0298	P 0.05 to P 0.01

Discussion

Chemical cues

As mentioned previously, reproduction in calanoid and cyclopoid copepods is sexual, therefore male and female copepods must encounter each other to mate to produce offspring (Gilbert and Williamson 1983). Female copepods oscillate between periods when they are nongravid (oviducts clear) and gravid (oviducts dark) (Watras and Haney 1980, Watras 1983a, Katona 1975). In some calanoid copepods the female must be in the gravid state in order to produce a viable clutch (Chow-Fraser and Maly 1988, Watras 1983a). Under conditions where male densities are low (either in real terms; the number of males/m³, or relative terms; males per female) it would be advantageous for the female to release cues that would enhance encounter probabilities and communicate her condition to potential mates. Likewise if a male could detect the presence of a female and her reproductive condition, he also would increase his probability of encountering her and would not waste time and energy pursuing nonreproductive females.

Results from field data indicate that there is a significant relationship between the reproductive condition of the female and the presence or absence of an attached

spermatophore (Fig. 2). In each of the four species of diaptomids investigated in the field study the gravid females had a significantly ($P < 0.01$) greater proportion of attached spermatophores than nongravid females. This finding is consistent with observations made in the laboratory where a number of researchers have also found that the copulatory rates of gravid female copepods is significantly higher than nongravid females (Chow-Fraser and Maly 1988, Jacoby and Youngbluth 1983, Watras 1983b). In both the laboratory and the field male copepods are placing spermatophores onto gravid females more often than nongravid females.

Since in copepods the male copepod pursues the female and initiates copulatory behavior (Katona 1973, Katona 1975, Blades 1977, Uchima and Murano 1988), the differential rates of spermatophore placement between gravid and nongravid females suggests that the males are able to detect the reproductive condition of the females.

Swimming Behavior

Buskey (1984), in investigations on the role of sensory systems in the recognition of food in the copepod Pseudocalanus minutus, found that when the copepod was presented with seawater that contained phytoplankton, the average swimming speed was lower than that observed when the copepods were presented with filtered seawater. When

the copepods were presented with chemical stimulation (filtered phytoplankton exudate) alone however, increases in swimming speed and number of swimming speed bursts were noted. Alterations in the beat frequency of the first maxilla, which are used for both swimming and feeding activities, have been used to make inferences about a copepods ability to detect the presence of different amino acids (Gill and Poulet, Poulet and Gill 1988). They found that some combinations of amino acids that they tested were more stimulatory than others as evidenced by increases in the beat frequencies.

The consistent increases in swimming speeds by the male Diaptomus leptopus in the gravid female treatment but not in male or non-gravid female treatments indicate that the male copepods were able to detect the reproductive states of the females (Fig. 3). The significant increases observed in the swimming speeds of the test males under the gravid female treatment (Fig. 3a) would concomitantly result in an increase in encounter rates since an increase in swimming speeds will enhance the probabilities of encounter (Gerritsen and Strickler 1977).

As well as increasing encounter rates by increasing their swimming speeds, it has been suggested (Buskey 1984, Gill and Poulet 1988) that increasing the water flow past a copepod's receptors may enhance the copepod's ability to detect changes in chemical concentration allowing them to detect concentration gradients. This statement is based on

evidence that chemoreception may be enhanced by increasing water flow over chemoreceptors (Schmitt and Ache 1979).

TURNING BEHAVIOR

Increases in the amount of turning once the male detects that a gravid female is near will cause a copepod to stay in the same region. This type of behavior has been termed "area restricted" searching (Pyke 1984). Detection of a signal in the water by the male would indicate that a gravid female is in the vicinity encouraging the male to search that area. The significant increases in turning behavior (decreases in the NGDR), would be indicative of area restricted searching and was seen when the male Diaptomus leptopus were exposed to treatments consisting of gravid females but not observed when the treatments consisted of non-gravid females or males (Fig. 4). These results are indicative of the release and detection of a chemical by the copepods and that the release of the chemical is dependant upon the reproductive state of the female.

Area restricted search patterns such as looping or turning behaviors will keep one individual in the vicinity of the other (Pyke 1984). Williamson (1981) found that the looping behavior of the copepod Mesocyclops edax increased with increasing prey densities. Uchima and Murano (1988) found that when the male copepod Oithona

davisae passed through "water immediately vacated by the female" the male initiated a "spiraling" swimming pattern, the radius of which decreased as the male approached the female. The significant increases in turning behavior observed in my experiments are consistent with spiraling and looping behavior that has been described as mate seeking behavior (Uchima and Murano 1988, Katona 1973).

DISTANCE

In the distance experiments the male copepod did demonstrate alterations in swimming behavior at the 2.5 cm distance but not at the 5.0 cm distance (Fig 5). The significant increase in the swimming speed observed at the 2.5 cm distance in conjunction with a greater degree of turning behavior at the 2.5 cm distance than the 5.0 cm distance (Fig 6) is suggestive of a searching pattern which is similar to that discussed above but less restrictive in nature. The change in the degree of turning, as evidenced by changes in the NGDR, was not as great as that seen when the separation between the gravid females and the test male copepod was minimal (Fig 4a), however there was a higher level of turning than at the 5.0 cm distance. This observation suggests that the male copepods may be able to distinguish differences in stimulus concentration and use this information to adjust its search behavior. A copepod would therefore search a greater or smaller area depending

on stimulus concentration thereby producing a search pattern appropriate to the perceived females proximity. A search behavior similar to this has been observed in the marine copepod Oithona davisae (Uchima and Murano 1988). They found that the mate-pursuit behavior of the male copepod consisted of a turning behavior they termed spiraling movements, the radius of which decreased as the male approached the female.

Confounding Variables

The container used in each experiment was exactly the same so that we would expect that alterations in behavior, if seen, are the result of the effect of the treatment and not that of the container. In fact, if there are significant container effects, they would tend to obscure changes in behavior due to different treatments since the behaviors of the copepods would be forced into conformity by the tank, regardless of the treatment offered.

Perhaps the initial behavioral state of the copepods can affect the way in which they perceive the container and hence affect subsequent behavioral states. To test this I compared some of the initial parameters measured to see if any particular patterns emerged. Looking at the mean initial swimming speeds (Table 3) I found that there is a significant difference between the 2.5 cm gravid female treatment and each of the other treatments excepting the

0.0 male treatment. I do not believe that the high initial swimming speed observed in this treatment would result in a further increase in swimming speeds for the following reasons:

(1) If they are moving at or near their maximum speeds they would not be able to accelerate further.

(2) The negative correlation seen in Table 3 between initial and change in swimming speeds (0.0 cm gravid female and 2.5 cm gravid female treatments) suggests that the slower individuals change their swimming speeds more than the faster moving individuals. Hence increases observed are due to treatment and not container effects.

It was also found that the rates at which the copepods of this trial came into contact with the side of the container was higher than that of any other trial (Table 2). It might be thought that this may induce stress in the copepods causing escape reactions to occur, thus resulting in an increase in the observed swimming speed. However, I found this not to be the case since a Spearman rank correlation between the initial number of contacts and the change in swimming speeds found a significant inverse relationship. This indicates that the individuals that came into contact with the container frequently increased their swimming speeds less than those individuals that did not. I therefore cannot infer that the increase in swimming speeds observed are due to the stress caused by bumping into the sides of the container. Similarly,

alterations in the degree of turning illustrated by the male copepods in the different treatments cannot be attributed to differences in the initial contact rates since both the treatment with the highest number, and the treatment with the lowest number of contacts (Table 2) were found to have no significant alteration in turning behavior. Hence contact with the container did not affect turning behavior.

The alterations in behavior observed in the above experiments are consistent with the production of a chemical signal or stimulus and not a mechanical one for the following reasons:

(1) The screen separating the treatment individuals from the test individuals would greatly reduce the distance that a mechanical signal could travel. Kirk (1985) found that fluid disturbances caused by Daphnia pulex attenuated at a rate faster than d^{-14} where d equals the distance from the center of the animal in μm . Kirk fit his data to the following formula ($U_d = b \times d^m$, where U_d is the maximum speed (mm/sec) of a flow pulse at a distance d (μm), m and b are regression parameters). We can see that at the attenuation rates described (d^{-14}), disturbances produced by the Daphnia dissipate very quickly. If a mechanical disturbance came into contact with a screen, we can expect that the signal would decay even more rapidly.

(2) Mechanical signals certainly could not be responsible for the behavioral changes observed at the

2.5 cm treatment. Kirk (1985) found that fluid disturbances produced by Daphnia pulex dissipate over a distance of mm. He also found that the Daphnia can produce flow signals with velocities of 182 mm/sec while a diaptomid copepod only demonstrated a maximum flow velocity of 3.1 mm/sec. Fluid disturbances could not cover the 2.5 cm distance to the observational portion of the vessel and be responsible for the changes in behavior observed.

(3) The distance over which a mechanical signal can travel is related to the strength of the signal. Male copepods swim faster than female copepods and then would be expected produce a stronger mechanical signal. However no behavioral alterations were seen in the 0.0 cm male treatment.

(4) Even if a mechanical signal did manage to cross the screen, Strickler (1974) found that turbulence patterns produced by swimming zooplankton were only specific down to the level of Order. If any information relating to the sex and reproductive condition of the treatment copepods were produced, this information would certainly be lost as it passed through the screen.

Conclusion

Alterations of behavior consistent with search behavior, increases in swimming speed and turning behavior, were only found in the gravid female treatments. The

results suggest that male copepods are able to detect the reproductive condition of a female from a distance suggesting that the gravid females are producing a sex pheromone. The production of a pheromone would greatly increase the encounter radius of a male therefore increasing the probability of him encountering a female that is ready to mate. This would be consistent with the higher rates of spermatophore placement on gravid over nongravid females found in the field in this study and in the laboratory by others.

Since a male and female copepod must encounter each other in order to mate and reproduce there is a minimum population density (critical density) below which the population will not be able to maintain itself. This critical density is proportional to the square of the encounter radius (Gerritsen 1980b). While my experiments did not establish the exact size of this radius the distance experiments demonstrated that it is in the order of cms. As the encounter radius increases, the critical density required to maintain or establish a population decreases. The production of a pheromone extends the area over which a copepod can detect a mate reducing the critical density. This can be critical for freshwater species of copepods since the lakes and ponds that they live in are essentially isolated environments. Species of copepods such as D. leptopus often live in small ponds that are in the process of disappearing. If they are to

establish new populations in new environments they would be better able to do so if they produced a pheromone because then a lower number of colonizing copepods are then required to start the population.

Evidence that crustaceans do produce and detect pheromones is accumulating (Gleeson et al. 1984, but for review see Dunham 88). This study demonstrates that male copepods are able to distinguish the reproductive state of the female and probably do use pheromones to enhance rates of encounter. The next step is to develop a reliable method of isolating and concentrating the pheromone and repeating the behavioral assay to substantiate its activity.

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APPENDIX I

Table I): Location of lakes and ponds sampled.

Species	Lake or pond	Location
<u>Diaptomus leptopus</u>	Hill Pond	45 25'50" N, 73 56'45" W
<u>Diaptomus birgei</u>	St. Joachim B4 (Nun's Island)	45 27'15" N, 72 31'20" W 45 27'15" N, 73 33'30" W
<u>Diaptomus oregonensis</u>	Nick du Moulin Seigneurial	45 12'50" N, 72 19'36" W 45 32'30" N, 73 19'30" W 45 32'42" N, 73 19'24" W
<u>Diaptomus minutus</u>	Sally's (Knowlton) Brome Libby's Choiniere Roxton Lauzon des Sables Trap Grand Truite Rouge	45 10'15" N, 72 26'00" W 45 14'30" N, 72 31'23" W 45 16'45" N, 72 22'24" W 45 25'45" N, 72 35'18" W 45 27'48" N, 72 39'00" W 46 21'00" N, 74 30'46" W 46 25'14" N, 74 24'42" W 46 26'36" N, 74 21'32" W 46 27'48" N, 74 15'20" W

APPENDIX II

Table II: Effect of treatments on male swimming speedsTable II (a): Gravid female treatment.

Swimming speeds (mm/sec)			
Trial #	Before (B)	After (A)	Difference (A-B)
1	4.74	4.69	- 0.05
2	1.06	1.89	0.83
3	2.38	3.10	0.72
4	2.06	2.19	0.13
5	4.56	4.95	0.43
6	3.19	2.77	- 0.42
7	2.73	2.93	0.20
8	0.97	1.61	0.64
9	4.68	4.90	0.22
10	5.14	5.52	0.11
11	4.18	3.69	- 0.49
12	2.89	2.82	- 0.07
13	2.15	2.19	0.04
14	1.64	2.16	0.52
15	0.80	1.08	0.28
16	1.69	1.69	0.00
17	1.84	1.79	- 0.05
18	1.31	1.70	0.39
19	1.47	1.81	0.34
20	1.70	1.95	0.25
21	1.67	1.72	0.05
22	1.77	1.87	0.10
23	2.07	2.14	0.07
24	0.93	1.00	0.07

Table II (b): Nongravid female treatment.

Swimming speed (mm/sec)			
Trial #	Before (B)	After (A)	Difference (A-B)
1	6.58	6.83	0.26
2	6.10	6.11	0.02
3	1.10	2.33	0.33
4	2.54	2.85	0.31
5	1.36	1.37	0.01
6	2.06	3.38	1.32
7	1.55	1.66	0.11
8	2.22	2.44	0.21
9	3.12	2.61	- 0.51
10	2.70	2.18	0.10
11	1.87	1.95	0.09
12	3.28	3.00	- 0.28

Table II (c): Male treatment.

Swimming speed (mm/sec)			
Trial #	Before (B)	After (A)	Difference (A-B)
1	3.08	2.71	- 0.37
2	1.76	1.89	0.14
3	2.18	2.33	0.16
4	2.29	2.32	0.04
5	1.99	2.09	0.10
6	3.44	3.62	0.18
7	3.28	2.78	- 0.50
8	2.48	1.75	- 0.73
9	2.30	2.61	0.30
10	2.70	3.26	0.56
11	2.71	1.86	- 0.85
12	4.08	3.08	- 0.99

APPENDIX III

Table III: Effect of treatments on turning behavior.Table III (a): Gravid female treatment.

Turning behavior (NGDR)			
Trial #	Before (B)	After (A)	Difference (A-B)
1	0.7757	0.7411	- 0.0346
2	0.4773	0.4975	0.0202
3	0.7993	0.7876	- 0.0117
4	0.6485	0.5417	- 0.1068
5	0.9149	0.8355	- 0.0794
6	0.6804	0.7115	0.0311
7	0.6883	0.6534	- 0.0349
8	0.4126	0.7432	0.3525
9	0.8263	0.7649	- 0.0614
10	0.8768	0.8557	- 0.0211
11	0.8580	0.7746	- 0.0834
12	0.8092	0.7208	- 0.0884
13	0.8589	0.8160	- 0.0429
14	0.8720	0.7320	- 0.1401
15	0.5824	0.5793	- 0.0031
16	0.7911	0.7590	- 0.0321
17	0.8119	0.6920	- 0.1200
18	0.7800	0.8310	0.0510
19	0.7869	0.7461	- 0.0409
20	0.8770	0.7851	- 0.0919
21	0.7560	0.6661	- 0.0899
22	0.8190	0.8650	0.0460
23	0.8680	0.9001	0.0320
24	0.5159	0.7330	0.2180

Table III (b): Nongravid female treatment.

Turning behavior (NGDR)			
Trial #	Before (B)	After (A)	Difference (A-B)
1	0.9293	0.8475	- 0.0818
2	0.8925	0.9066	0.0141
3	0.7801	0.7701	- 0.0100
4	0.7652	0.8117	0.0465
5	0.6042	0.6235	0.0193
6	0.7896	0.8558	0.0662
7	0.8757	0.8129	- 0.0628
8	0.8993	0.8925	- 0.0068
9	0.8780	0.9225	0.0445
10	0.8954	0.8708	- 0.0246
11	0.7065	0.8295	0.1230
12	0.8099	0.8423	0.0324

Table III (c): Male treatment.

Turning behavior (NGDR)			
Trial #	Before (B)	After (A)	Differences (A-B)
1	0.7683	0.7691	0.0008
2	0.7822	0.8041	0.0219
3	0.6830	0.6541	- 0.0289
4	0.7723	0.7571	- 0.0152
5	0.7375	0.7962	0.0587
6	0.8119	0.8397	0.0278
7	0.7726	0.7855	0.0129
8	0.8797	0.8988	0.0191
9	0.7909	0.7649	- 0.0260
10	0.7819	0.8093	0.0274
11	0.8240	0.8574	0.0334
12	0.8873	0.8666	- 0.0207

APPENDIX IV

Table IV: The effect of distance on pheromone perception. (Behavioral alterations in swimming speed)

Table IV (a): 5.0 cm distance between experimental and treatment organisms.

Swimming speeds (mm/sec)			
Trial #	Before (B)	After (A)	Difference (A-B)
1	2.52	2.87	0.35
2	1.86	1.99	0.13
3	1.60	0.24	- 1.36
4	2.48	2.36	- 0.13
5	2.24	1.73	- 0.52
6	1.99	1.86	- 0.13
7	2.11	2.44	0.33
8	2.33	2.35	0.03
9	2.01	1.52	- 0.50
10	2.16	2.41	0.26
11	0.76	1.29	0.53
12	2.08	3.23	1.15

Table IV (b): 2.5 cm distance between experimental and treatment organisms.

Swimming speeds (mm/sec)			
Trial #	Before (B)	After (A)	Difference (A-B)
1	5.72	7.51	1.79
2	2.65	7.55	4.89
3	5.79	7.27	1.48
4	7.51	6.20	- 1.31
5	3.92	3.58	- 0.34
6	5.98	6.20	0.23
7	5.87	6.45	0.57
8	4.68	5.40	0.72
9	6.50	6.09	- 0.40
10	2.90	5.79	2.89
11	3.90	5.82	1.91
12	5.76	6.64	0.88

APPENDIX V

Table V: The effect of distance on pheromone perception (analysis of turning behavior).

Table V (a): 5.0 cm distance between experimental and treatment organisms.

Turning behavior (NGDR)			
Trial #	Before (B)	After (A)	Difference (A-B)
1	0.7855	0.7650	- 0.0205
2	0.6905	0.7828	0.0923
3	0.8763	0.6360	- 0.2403
4	0.7217	0.7355	0.0138
5	0.6810	0.7168	0.0358
6	0.7553	0.7203	- 0.0350
7	0.8106	0.8693	0.0587
8	0.8747	0.7990	- 0.0757
9	0.5816	0.6764	0.0948
10	0.8276	0.7952	- 0.0324
11	0.7857	0.7904	0.0047
12	0.6845	0.7793	0.0948

Table V (b): 2.5 cm distance between the experimental and treatment organisms.

Turning behavior (NGDR)			
Trial #	Before (B)	After (A)	Difference (A-B)
1	0.8872	0.8614	- 0.0258
2	0.8381	0.8432	0.0051
3	0.8389	0.8744	0.0355
4	0.8588	0.8228	0.0360
5	0.8460	0.7789	- 0.0671
6	0.8971	0.8677	- 0.0294
7	0.8495	0.7750	- 0.0745
8	0.9251	0.9261	0.0010
9	0.9135	0.8805	- 0.0330
10	0.8303	0.8886	0.0583
11	0.7307	0.8643	0.1336
12	0.8683	0.8593	- 0.0090

APPENDIX VI

Table VI: Number of contacts with the side of the container before and after introduction of the treatment individuals.

<u>Gravid female treatment</u>		

Number of Contacts		

Trial #	Before	After

1	55	43
2	15	17
3	22	29
4	14	14
5	33	33
6	28	27
7	21	27
8	6	13
9	47	39
10	39	34
11	43	25
12	29	21
13	13	10
14	14	24
15	6	9
16	15	20
17	14	11
18	14	17
19	12	16
20	18	18
21	14	11
22	21	17
23	9	5
24	5	10

<u>Nongravid female treatment</u>		

Number of Contacts		

Trial	Before	After

1	50	51
2	47	36
3	19	16
4	19	21
5	9	6
6	14	23
7	12	16
8	19	24
9	23	22
10	23	23
11	11	16
12	20	30

<u>Male treatment</u>		

Number of Contacts		

Trial #	Before	After

1	26	21
2	13	12
3	21	19
4	23	23
5	18	20
6	40	35
7	29	31
8	12	27
9	20	18
10	22	28
11	28	22
12	32	25

Table VI: Number of contacts with the side of the container before and after introduction of the gravid females for the 5.0 and 2.5 cm distance experiment.

<u>5.0 cm Distance experiment</u>		

Number of Contacts		

Trial #	Before	After

1	15	17
2	5	6
3	13	8
4	11	10
5	7	8
6	11	14
7	10	8
8	9	10
9	11	9
10	3	4
11	11	17
12	6	2

<u>2.5 cm Distance Experiment</u>		

Number of Contacts		

Trial #	Before	After

1	31	45
2	19	50
3	35	53
4	53	47
5	27	32
6	36	46
7	45	43
8	32	39
9	51	43
10	21	50
11	24	33
12	42	42