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Male Choice and Sexual Selection in Precopulatory Mate-Guarding Marine *Gammarus* Species (Crustacea: Amphipoda) Found on the Avalon Peninsula of Newfoundland

by

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ABSTRACT

Behavioural aspects of reproduction were studied on five species of mate-guarding *Gammarus* found on the Avalon Peninsula of Newfoundland. These species are *Gammarus setosus*, *Gammarus oceanicus*, *Gammarus duebeni*, *Gammarus lawrencianus*, and *Gammarus obtusatus*. Males chose to form precopulas with females that were close to their next batch of eggs to be fertilized. Females sometimes exhibited reproductive choice by trying to escape the male's grasp. No interspecific precopulas were formed between *G. setosus* and *G. oceanicus*. Size assortative mating occurred in *G. setosus*. The sequences of precopula formation behaviours differed in the successful and unsuccessful precopulas for all five species. Three takeovers by males were observed in *G. oceanicus*, all with egg stage 4 females which are females which are closest to their next batch of eggs to be fertilized. The sequences of precopula formation behaviour contacts differed for successful and unsuccessful takeovers, and were also different from the normal behaviour sequences for *G. oceanicus*. Takeovers were considered to be male-male competition. However, female choice was observed as the females remained passive, which is considered a form of female choice, during both successful and unsuccessful takeovers. Two simultaneous assessments of females occurred with females that were both in the same egg stage (stage.4) in *G. oceanicus*. The sequences of precopula formation behaviour contacts differed for successful and unsuccessful simultaneous assessments and also differed from the normal behaviour sequences for *G. oceanicus*. In the unsuccessful simultaneous assessments, the extra females

sometimes resisted the male's grasp and escaped (female choice) but the original females remained passive. In the successful simultaneous assessments, the original and extra females remained passive. There were successful precopulas formed with both stage 1 females with stage 4 eggs transplanted and with stage 4 females with eggs removed in *G. oceanicus*. The sequences of precopula formation behaviour contacts were different for successful and unsuccessful precopulas with egg manipulation females, and were different from the normal behaviour sequences for *G. oceanicus*. Female choice was observed with egg manipulation females as they sometimes kicked and escaped from the males.

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

1.0 INTRODUCTION

Male choice of a mate puts the male under pressure to make sound behavioural decisions. When males actively choose their mates, an assessment procedure of the potential females must be carried out. A male may use several factors in his assessment of a female as a potential mate. A decision must be made on the acceptance or rejection of the female as a mate. A male can benefit from choosing certain females over others as a mate (Dick & Elwood, 1989a; Halliday, 1983).

Sexual selection results in the evolution of secondary sexual characteristics that confer "an advantage which certain individuals have over other individuals of the same sex and species solely in respect of reproduction" (Darwin, 1871, 256). Sexual selection results in a wide variety of both male and female reproductive strategies. Darwin distinguished two forms of sexual selection: male-male competition and female choice (Andersson, 1994; Adams & Greenwood, 1987; O'Donald, 1983; Birkhead & Clarkson, 1980; Ridley & Thompson, 1979). Competition between males for access to females can be intense and is usually more evident with conspicuous ritualized displays of behaviour than the second form of sexual selection - female choice (Birkhead & Moller, 1993). Female reproductive behaviours usually involve only the adoption or non-adoption of receptive behaviours in the form of postures or movements. Female choice is usually considered to be less important because it is much less obvious than male-male competition (Birkhead & Moller, 1993; Halliday, 1983). However, female choice is currently the most active research area in sexual selection theory and females can benefit from mate choice also.

Crustacean males use one of two strategies for mating: 1. males guard females until they are ready to moult and be fertilized, or 2. the males search for suitable females. Mate-guarding evolves when there is strong competition among males for mates (ie. male-biased sex ratio), female breeding cycles are asynchrous, and the period of female receptivity is short (Conlan, 1991; Adams & Greenwood, 1987; Ward, 1986; Ridley, 1983). Mate-guarding is common in crustaceans that only produce eggs just after a moult, where the moult lasts only for a brief period of time in a female's reproductive cycle (Jormalainen & Merilaita, 1995; Yamamura, 1987; Grafen & Ridley, 1983; Parker, 1974). When males guard females they may either remain near the females (mate-attending) which is common in animals that live in tubes or permanent burrows, or they may carry the females (mate-carrying) (Steele, 1995; Conlan, 1991). Mate-carrying ensures a male is present during the female's short period of receptivity (Conlan, 1991). Precopulatory mate-carrying is common among species of *Gammarus*, a genus of peracarid amphipod crustacean (Conlan, 1991; Dunham, Hurshman & Gavin, 1989; Adams & Greenwood, 1987; Ridley, 1983; Birkhead & Clarkson, 1980).

Male choice and sexual selection in the genus *Gammarus* has been widely studied because the species are widespread, abundant, have overlapping distributions, are similar to one another, and are readily cultured. Several species are common and readily available for study on Newfoundland shores.

In species of *Gammarus* copulation is possible only within a few hours (about 12 hours) after a female's moult (Dick & Elwood, 1989a; Ward, 1983; Birkhead & Clarkson, 1980). Since females do not store sperm, a male must be present shortly after the female has moulted and has released her eggs. Females ovulate within minutes after the moult and the eggs pass into a ventral brood pouch in peracarid Crustacea (Borowsky, 1991). The brood pouch is formed from setose oostegites which form on the bases of the female's peraeopods (Wildish, 1982). The new cuticle of the female is still soft after moulting and the oviducts on somite five are open (Dick & Elwood, 1989a). In *Gammarus*, the male turns the female at right angles to his body with her ventral surface facing his ventral surface. Sperm transfer into the brood pouch occurs from a pair of copulating papillae on the last thoracic somite of the male. Bundles of sperm are thrust near the female's oviduct openings by a vigorous pumping action that lasts less than one minute (Birkhead & Clarkson, 1980). There may be several bouts of copulation (Sutcliffe, 1992). After copulation, the pair separates (Grafen & Ridley, 1983).

In less than 24 hours, the oviducts become hardened and provide a physical barrier for the transfer of sperm (Grafen & Ridley, 1983). The eggs develop and hatch in the brood pouch and the juveniles may remain in the pouch until the female's next moult. The duration varies with species and environmental temperature (Borowsky, 1991;

Grafen & Ridley, 1983; Dick & Elwood, 1989a). The female becomes attractive to males again before the eggs hatch. The mature female produces a new batch of eggs at each successive moult, except when in ovarian diapause (Birkhead & Clarkson, 1980).

While the embryos are developing in the brood pouch, oogonia are simultaneously enlarging and developing in the ovary (Steele & Steele, 1969). Many changes in the female, such as changes in moult physiology and oogonia development in the ovary, are correlated with the embryonic development in the brood pouch (Dunham, 1986). Therefore the stage of egg development in the brood pouch is an indicator of how soon a female will be producing her next batch of eggs for fertilization and how close she is to her next moult.

Female suitability for mating in species of *Gammarus* is determined by how soon she will be releasing a batch of eggs (Grafen & Ridley, 1983). Pairing is initiated when the antennae of the male touch some part of the female. But not all contacts will result in pairs being formed. If the male grabs the female, he holds her to his ventral surface with his antennae at a right angle and touches her dorsal surface with his abdomen. Rapid flexing of the abdomen, termed "beating", then occurs (Hartnoll & Smith, 1978). When there is slow beating of the abdomen, the pleopods also make contact with the female. These are the behaviours the male employs to assess the female's quality. The female is usually passive and tightly curled while the male is performing these actions on her (Dick

& Elwood, 1989a; Dunham, 1986; Birkhead & Clarkson, 1980; Hartnoll & Smith, 1978). However, females sometimes do try to escape the males grasp.

If the female is suitable to the male, he turns the female so that she is now parallel to his ventral surface. The male inserts one dactylus of the smaller anterior gnathopods between the head and the first thoracic segment, and the dactylus of the other anterior gnathopod is inserted between the fifth and sixth segments. Once this position is established, the pair is said to have formed a precopula or pre-amplexus. The male then swims with the female and may stroke the sides of her head with his larger posterior gnathopods. The female sometimes aids the male in swimming while in precopula by straightening her body and beating her pleopods, but usually the female will remain curled underneath the male and offer no assistance (Sutcliffe, 1992; Dick & Elwood, 1989a; Dick & Elwood, 1989b; Borowsky & Borowsky, 1987). If the precopula is maintained until copulation, the female is again turned to right angles to the male while the male deposits sperm and the precopula is terminated a short time later (Miller, 1995; Dick & Elwood, 1989a; Dunham, 1986; Hartnoll & Smith, 1978).

The two forms of sexual selection, male-male competition and female choice, occur among species of *Gammarus*. There is sexual dimorphism between the sexes of *Gammarus*. Males of most species of *Gammarus* are larger in body length than the females of the same species, and the males possess larger posterior gnathopods (Ward, 1988). These enlargements, common to mate-guarders, may reflect an increased amount of aggression between males at maturity. The large posterior gnathopods are not used in carrying the female in precopula but are used in male-male competitions when larger males attempt takeovers of a paired female from its precopula male and also as a defense against takeovers by other males. The gnathopods may take part in the assessment of the female's reproductive state, possibly through contact pheromones (Conlan, 1991). It is also possible that these gnathopods can hold a second female that can then be simultaneously assessed by a male that already has one female in precopula (Dick, 1992).

Females also exhibit reproductive choice. When a male first touches a female, she can either swim away quickly or lay still in order to allow the male to manipulate her. The female can also try and escape from the male after he has been manipulating and assessing her for a period of time by flexing and straightening her body rapidly several times in succession (escape reaction). This may result in the male releasing his hold before precopula is formed and the female will escape. If the female remains passive during these manipulations a precopula can be formed (Hatcher & Dunn, 1997; Jormalainen & Merilaita, 1995; Sutcliffe, 1993a; Dick & Elwood, 1989a; Borowsky & Borowsky, 1987; Robinson & Doyle, 1985).

The male's choice of which female to hold in precopula can depend on the female's body size and her proximity to ovulation (Dick, 1992). Since the enlargement of oogonia in the ovary and the development of the embryos in the brood pouch are correlated, males will attempt precopula formation behaviours with females that bear broods in the later stages of development. This usually occurs when the eggs have already hatched and the juveniles are ready to emerge (Miller, 1995; Borowsky, 1987). Larger females of *Gammarus* produce larger batches of eggs than smaller females and males can be expected to choose females that are larger in order to maximize the number of offspring they produce (Adams, Watt, Naylor & Greenwood, 1989; Birkhead & Clarkson, 1980). Female quality is determined by both her size and how close she is to moulting and producing her next batch of eggs for fertilization. Jormalainen (1998) concluded that the duration of guarding is a compromise between the male and female reproductive strategies.

Removal of a female's embryos and transplanting embryos that are in a different stage may influence the precopula formation behaviours. Embryos from an earlier stage of development may inhibit precopula formation behaviours while embryos from later stages may increase precopula formation behaviours (Borowsky, 1987).

Precopula formations between different species of *Gammarus* have been studied. It was found that these interspecific precopulas can only be formed between certain species combinations but is relatively rare, especially in nature (Dick & Elwood, 1992; Dick, Irvine & Elwood, 1990; Kolding, 1986; Hartnoll & Smith, 1980). Size assortative mating is one of the most common mating patterns in natural populations. Size assortative mating has been found to occur when precopulas are formed. This means there is a positive relationship between male and female lengths (Crespi, 1989; Burley, 1983). In general, it has been found that the ratio of male/female length is around 1.3, meaning the males are approximately 30% larger than the females in the precopula pair (Miller, 1995; Ward, 1988; Birkhead & Clarkson, 1980).

The two species studied most intensively were *Gammarus oceanicus* Segerstråle and *Gammarus setosus* Dementieva. The ecology and distribution of these two species are similar in many respects and they occur in various combinations on the same shores underneath the same rocks and algae.

Gammarus oceanicus Segerstråle is a marine and brackish water species that is found under rocks and algae on sheltered to moderately exposed beaches. This species is confined to the North Atlantic and adjacent seas. Its northern limit is from southern Baffin Island and southeastern Greenland to its southern limit of Long Island Sound, New York. It is the most numerous species found in the intertidal region from Newfoundland to the Gulf of Maine and is the most abundant species of *Gammarus*. The breeding period of this species is long, from November until August, and it produces many successive broods. The adult may live two years and the size range of this species is 15-22 mm (Steele & Steele, 1975; Steele & Steele, 1974; Bousfield, 1973; Steele & Steele, 1972).

Gammarus setosus Dementieva is a marine species found under rocks and algae on sheltered to moderately exposed beaches. This species has a circumpolar distribution in arctic regions and is adapted to live in these arctic conditions. It is found as far north as land extends while its southern limit is the Bay of Fundy, New Brunswick. It is the most abundant species of *Gammarus* north of Newfoundland, but it is less common than *G. oceanicus* on Newfoundland beaches. When *G. setosus* occurs on the same shore as *G. oceanicus*, the former is found slightly higher in the intertidal zone. The breeding period of this species is from August to November with only one brood produced each year. This brood is released in spring or early summer and is related to environmental temperature. The size range of this species is 15-25 mm (Steele & Steele, 1975; Steele & Steele, 1974; Bousfield, 1973; Steele, V.J. & Steele, D.H., 1970).

My previous study described the formation of precopula pairs in four species of *Gammarus* : *Gammarus oceanicus*, *Gammarus duebeni* Lilljeborg, *Gammarus lawrencianus* Bousfield, and *Gammarus obtusatus* Dahl (Miller, 1995). The sequence of precopula formation behaviours was observed and recorded for each species. Two species, *G. oceanicus* and *G. duebeni*, were collected from different sites and there were differences in the average times to form precopulas and the percentage of animals that formed precopulas between the different sites. Interspecific precopulas were formed between *G. oceanicus* and *G. duebeni* but not between any of the other combinations of the four species. Removals of either the first set , second set, or both sets of antennae from the male showed that the second set of antennae are the most important for assessing females. The male must have at least one set of antennae intact in order to form precopulas. Size assortative mating did occur in all four species.

My present work was undertaken to determine significant factors that influence the formation of precopula pairs of species of *Gammarus*. The normal sequence of precopula formation behaviours was observed and recorded for *G. setosus* which was not studied previously. The normal sequence of precopula formation behaviours was compared for 5 species of *Gammarus* that co-occur on Newfoundland shores - *G. oceanicus*, *G. setosus*, *G. lawrencianus*, *G. duebeni*, and *G. obtusatus*. It also investigated *G. setosus* to determine if interspecific precopulas were formed with *G. oceanicus* and if size assortative mating occurred in *G. setosus*.

In addition, further study of the species *G. oceanicus* was conducted. Investigations of which factors influence takeovers of paired females by unpaired males were conducted, including whether a larger unpaired male will displace a smaller paired male, and whether the female's proximity to her next moult affected takeovers by unpaired males. Investigations were undertaken to determine if males would simultaneously assess two females, including comparisons of larger and smaller females in the same stage, and comparison of females closer to or further from moult that are the same size. The effect of egg manipulation on precopula formation behaviours of the male, including removal of eggs from females, and transplanting later stage eggs to females in an earlier stage was also investigated. The role of sexual selection in each of the aspects being investigated will be discussed.

2.0 MATERIALS AND METHODS

Two species of *Gammarus* were collected from Witless Bay on the Avalon Peninsula of Newfoundland. *G. oceanicus* and *G. setosus* were collected on the beach from underneath large rocks in the intertidal zone. *G. setosus* was located a little higher in the intertidal zone, but its distribution overlapped with *G. oceanicus*. Only animals already in precopula were collected. They were transported to the holding tanks in plastic containers with lids, half-filled with sea water from Witless Bay.

G. oceanicus was sampled during its breeding season, February through September and *G. setosus* was sampled during its breeding season, August through December. The animals were kept in aerated holding tanks filled with seawater in a lighted cold room having a temperature of 10°C. The animals were fed tropical and goldfish (WARDLEY ™) food. The holding tanks (35 x 22 x 18 cm) were used to keep the species separated and to prevent overcrowding.

The experimental chamber for the *G. setosus* experiments was a plexiglass chamber (13 x 2 x 13 cm) containing 40ml of seawater. Trial periods were 10 minutes in duration, but if the animals were engaged in precopula formation behaviours at the end of the trial they were allowed to continue until they separated or formed a precopula. Individual animals were left in separate small containers for five minutes before being placed in the experimentation chamber. All physical contacts between the animals were recorded, as well as their sexes, sizes from the base of the first antennae to the telson

(Ward, 1984b), colours, egg stages of the females, initial time of contact between the two animals, and the time of the end of the precopula, if formed. The egg stages that were used are as follows:

1. no eggs visible in the brood pouch
2. eggs clear and just barely visible in the brood pouch
3. eggs darker in colour and well developed in the brood pouch
4. females with hatched eggs

These are modified from Steele & Steele (1969).

2.1 NORMAL BEHAVIOURS OF *Gammarus* SPECIES

2.1.1 *Gammarus setosus*

A total of 69 pairs were tested. The normal precopula formation behaviours for this species were observed and recorded. Before each trial, a precopula pair was separated, taking care not to harm the animals. Both the male and female were then placed into the experimental chamber. Average times to form precopulas were calculated using only data from successful precopulas. Minimum and maximum values were calculated along with standard deviations and standard errors. Percentages of precopulas formed were also calculated. The Mann-Whitney test (Daniel, 1990) was used to determine if there was a difference in average times to form precopulas between morning and afternoon trials. The Proportions test (Welkowitz, Ewen & Cohen, 1982) was used to determine if the percentages of precopulas formed were equal between morning and afternoon trials.

Chi-square analysis (Daniel, 1990) was used to determine if the number of successful and unsuccessful precopulas was equal for each egg stage. Precopula formation behaviours are displayed in figures that show the sequence of contacts in successful and unsuccessful precopulas. Since the behaviours were recorded in sequence, the order of the most common types of contacts could be determined. These comparisons were used to determine if the pattern differed between species and to determine if there was a difference between the sequence of precopula formation behaviour contacts for successful and unsuccessful precopulas.

2.1.2 SPECIES COMPARISONS

The Mann-Whitney test was used to determine if there was a difference in average times to form precopulas between species. The Proportions test was used to determine if the percentages of precopulas formed were equal between species. *G. setosus* was compared with four other species studied in my previous research - *Gammarus oceanicus*, *Gammarus lawrencianus*, *Gammarus duebeni* and *Gammarus obtusatus*.

The sequences of precopula formation behaviours for successful and unsuccessful precopula formations were used to determine if there were differences in the patterns of contacts between the five species.

2.1.3 INTERSPECIFIC PRECOPULAS

A total of 52 combinations were tested. For the interspecific combinations, one male *G. setosus* and one female *G.oceanicus* were placed in the experimental chamber together. Then the other combination was tested. The animals were separated as described earlier.

2.1.4 SIZE ASSORTATIVE MATING

Size assortative mating was also investigated. The lengths of males and females of *G. setosus* that were separated for experimentation (N=69) were used to determine if size assortative mating occurred in this species. The ratios of male length to female length were calculated and indicate how much larger a male is compared to a female in precopula. The correlations (Moore & McCabe, 1993) of male and female lengths were calculated to determine if male length increased with increasing female length. Male and female lengths were plotted against one another and a regression line, with regression coefficient, shows if male length increases as female length increases.

All of the following experiments were carried out using *G. oceanicus*.

2.2 TAKEOVERS

For the takeover experiments, the extra males that were required were separated from another precopula. One male and female pair still in precopula and an extra male were placed in the experimental chamber. No takeovers or takeover attempts occurred in initial observations (N=39) in the plexiglass experimental chamber, so a new setup was devised to determine if takeovers were possibly occurring in *G. oceanicus*. Twenty-four separate chambers (10 x 10 x 7.5 cm), each with 40ml of seawater, contained a pair in precopula and a separate male of different size or colour (for easy distinction). Chambers were observed at intervals of 30 minutes for 6 hours. During these trials, switching of the original male (defender) with the separate male (intruder) did occur in thirteen of the chambers. This indicated that a takeover had occurred but none were actually observed in progress. None of these results were used in the final analysis. Results for final observations (N=143) were obtained from four chambers setup together, each containing a precopula pair and an intruder, that were simultaneously observed for 10 minute trials. All contact/takeover behaviours were recorded for all four setups.

The average times for takeover to occur were calculated using only successful takeover data. The minimum and maximum values, standard deviations, standard errors, and percentages of intruders to successfully takeover a female and attempts to takeover a female already in precopula were calculated. Mann-Whitney test was used to determine if average times spent attempting unsuccessful takeovers differed depending on whether intruders were larger or smaller than defenders. Mann-Whitney test was used to determine if there was a difference between the amount of time to form precopulas in normal *G. oceanicus* trials and unsuccessful takeover trials. Mann-Whitney test was also used to determine if female size differed when attempting unsuccessful takeovers whether intruders are larger or smaller than defenders.

Chi-square analysis was used to determine if the number of unsuccessful takeovers and the number of no takeover attempts were equal for each egg stage. The ratios of intruder size to defender size were calculated for successful takeovers, unsuccessful takeovers, and no takeover attempts. Correlations were used to determine if there was a relationship between intruder and defender size for unsuccessful takeovers and no takeover attempts. Male and female lengths were plotted against one another and a regression line, with regression coefficient, shows if male length increases as female length increases for unsuccessful takeovers (with successful takeovers included) and no takeover attempts. Correlations were also used to determine if time spent attempting unsuccessful takeovers would increase as female quality (female egg stage and female size) increases. Unsuccessful takeover times and female egg stage were plotted against one another and a regression line, with regression coefficient, shows if times attempting unsuccessful takeovers increase as female egg stage changes and the female becomes closer to her next ovulation. Also, unsuccessful takeover times and female size were plotted against one another and a regression line, with regression coefficient, shows if times attempting unsuccessful takeovers increase as female size increases.

Takeover behaviours are displayed in figures that show the sequence of contacts in successful and unsuccessful takeovers. These can be compared to the normal precopula formation behaviours for *G. oceanicus*.

2.3 SIMULTANEOUS ASSESSMENTS

For the simultaneous assessment experiments, the extra females that were required were separated from another precopula. One male and female still in precopula and one extra female were placed in each chamber. The four chamber setup used in the takeover experiments was also used for the 10 minute trials. The setups consisted of either same stage females of different sizes, or same size females of different stages. All simultaneous assessment behaviours were recorded. A total of 147 setups were observed; 85 were same stage females and 62 were same size females.

The average times for simultaneous assessments to end were calculated. Minimum and maximum values, standard deviations, standard errors, and percentages of males engaging in simultaneous assessments were calculated.

Mann-Whitney tests were used to determine if average times to form precopulas in normal *G. oceanicus* trials were equal to average times in unsuccessful simultaneous assessments for both same stage females and same size females. Mann-Whiney test was also used to determine if average times attempting unsuccessful simultaneous assessments were equal for same stage females and same size females. Chi-square analysis was used to determine if the number of unsuccessful simultaneous assessments and no simultaneous assessment attempts are equal for larger and smaller extra females and also if equal for closer to moult and further from moult for the extra females.

Simultaneous assessment precopula behaviours are displayed in figures showing the sequence of contacts in successful simultaneous assessments and unsuccessful simultaneous assessments. These can be compared to the normal precopula formation behaviours for *G. oceanicus*.

2.4 EGG MANIPULATIONS

In order to remove eggs or transplant eggs, the female must be anesthetized. This was accomplished by placing the females in a separate holding tank (10 x 5 x 5 cm) that was placed in ice until the temperature dropped to approximately 1°C and the females became immobilized. Embryos or juveniles were removed from and transplanted to a brood pouch with a small soft-bristled artist's brush. Females were allowed to recover in the separate holding tank in the cold room for one day before experiments were carried out. The plexiglass experimental chamber was used for these experiments. A total of 194 pairs were observed; 90 were stage 1 egg manipulation females and 104 were stage 4 egg manipulation females.

For the egg removals, females of stage 4 had all juveniles removed, but none replaced. Females of stage 1 had juveniles from stage 4 females placed into their brood pouch. Females were used for a maximum of 5 days to ensure they stayed in the same stage physiologically during the experiments.

Average times for successful precopulas or unsuccessful precopulas were calculated. Minimum and maximum values were calculated along with standard deviation and standard error. Percentages of precopulas formed and unsuccessful precopulas were also calculated. The Mann-Whitney test was used to determine if there was a significant difference between average times to form successful precopulas in stage 1 egg manipulation and stage 4 egg manipulation females. Mann-Whitney was also used to determine if there was a significant difference between average times spent attempting unsuccessful precopulas in stage 1 egg manipulation and stage 4 egg manipulation females. The Proportions test was used to determine if the percentage of successful precopulas was equal between stage 1 egg manipulation and stage 4 egg manipulation females and also if the percentage of unsuccessful precopulas was equal between stage 1 egg manipulation and stage 4 egg manipulation females.

Mann-Whitney test was used to determine if there was a significant difference in average times to form successful precopulas between *G. oceanicus* normal precopulas with stage 1 and 4 females and stage 1 egg manipulation and stage 4 egg manipulation females. The Proportions test was used to determine if the percentages of successful precopulas were equal between *G. oceanicus* normal precopulas with stage 1 and 4 females and stage 1 egg manipulation and stage 4 egg manipulation females.

Chi-square analysis was used to determine if the number of successful and unsuccessful precopulas were equal for stage 1 egg manipulation and stage 4 egg manipulation females. The ratios of male to female size were calculated for successful and unsuccessful precopulas for stage 1 egg manipulation and stage 4 egg manipulation females. Correlations were used to determine if there was a relationship between female and male size for successful and unsuccessful precopulas for stage 1 egg manipulation and stage 4 egg manipulation females. Male and female lengths were plotted against one another and a regression line, with regression coefficient, shows if male length increases and female length increases for successful and unsuccessful precopulas of stage 1 egg manipulation and stage 4 egg manipulation females.

Precopula formation behaviours are displayed in figures showing the sequence of contacts in successful and unsuccessful precopulas for stage 1 egg manipulation and stage 4 egg manipulation females. These can be compared to the normal precopula formation behaviours for *G. oceanicus*.

3.0 RESULTS

3.1 NORMAL BEHAVIOURS OF *Gammarus* SPECIES

3.1.1 *Gammarus setosus*

The general mating behaviours typical of other species of *Gammarus* previously studied (Miller, 1995) and recorded in the literature (Dick & Elwood, 1989a; Dunham, 1986; Birkhead & Clarkson, 1980; Hartnoll & Smith, 1978) occurred in *G. setosus*. Female choice was also evident as females sometimes swam away after first contact by a male (7.25%) or the female rapidly flexed and escaped after the male had been assessing her for a period (1.45%). Males sometimes also assessed a female for a period of time and then released her, thereby not forming a precopula (2.90%).

There was no significant difference between the times to form precopulas for the morning and afternoon observations of *G. setosus* (Table 3.1.1) ($W = 183.0$, $p = 0.12$, $\alpha = 0.05$). There was also no significant difference between the percentages of pairs that formed precopulas in the morning and afternoon ($z = 0.3507$, $z_{0.025} = 1.96$, $p = 0.7264$, $\alpha = 0.05$).

For *G. setosus* (Table 3.1.2), there was a significant difference in the number of precopulas formed with females in different egg stages ($\chi^2 = 19.217$, d.f. $= 3$, $p = 0.000$, $\alpha = 0.05$). More precopulas were formed with females in later stages (3 and 4) than with

Table 3.1.1. Summary of morning, afternoon, and combined morning and afternoon data giving the percentage of animals that formed precopulas, average amount of time to form precopulas, and descriptive statistical calculations for *G. setosus* from the normal behaviour data.

Time of Day	Morning	Afternoon	Combined
Number Tested	43	26	69
Number Formed Precopula	28	18	46
Percent Formed Precopula	65.12	69.23	66.67
Average Time (min)	6:51	5:52	6:28
Minimum (min)	2:33	1:46	1:46
Maximum (min)	9:53	10:15	10:15
Standard Deviation (min)	1:56	2:27	2:11
Standard Error (min)	00:22	00:35	00:19

Table 3.1.2. Summary of number of successful and unsuccessful precopulas for each female egg stage for *G. setosus*.

Egg Stage	1	2	3	4
Successful Precopula	4	6	13	23
Unsuccessful Precopula Attempt	10	3	7	3

* see p.12 for description of egg stages

females in earlier stages (1 and 2). The most precopulas formed were with stage 4 females. However, there was no significant difference between the number of unsuccessful precopulas in each egg stage ($\chi^2 = 6.043$, d.f. = 3, p = 0.110, $\alpha = 0.05$).

The general pattern of precopulatory formation behaviours for a successful precopula for *G. setosus* (Figure 3.1.1) consisted firstly of the male's gnathopods touching the female's sides or back; the next contact was the male's gnathopods touching the female's sides or the male's antennae touching the female's sides; and next the male's antennae touched the female's sides.

However, the general pattern of precopula formation behaviours for an unsuccessful precopula (Figure 3.1.2) consisted of the male's gnathopods touching either the female's back or sides. Then the male either touched the female's sides with his antennae or his tail touched the female's tail. Next the male's gnathopods touched the female's sides. Failure occurred when the male stopped performing precopulatory formation behaviours on the female and released her, or in some cases the female flexed and straightened rapidly in succession and escaped the male's grasp.

3.1.2 SPECIES COMPARISONS

The average times to form successful precopulas and the percentages of animals that formed successful precopulas for all five species studied are presented in Table 3.1.3.

Figure 3.1.1. The frequency and sequence of precopula formation behaviours initiated by males during successful normal precopula formations with females in *G. setosus*. The sequence of precopula formation behaviours from first to last is in ascending order.

Legend:

g-b = male's gnathopods touching female's back
a-s = male's antennae touching female's side
g-s = male's gnathopods touching female's sides
tb = male beat tail against female side rapidly
t-t = male's tail touches female's tail
a-b = males' antennae touches female's back
a-h = male's antennae touches female's head
o = any other touches made by male contacting the female

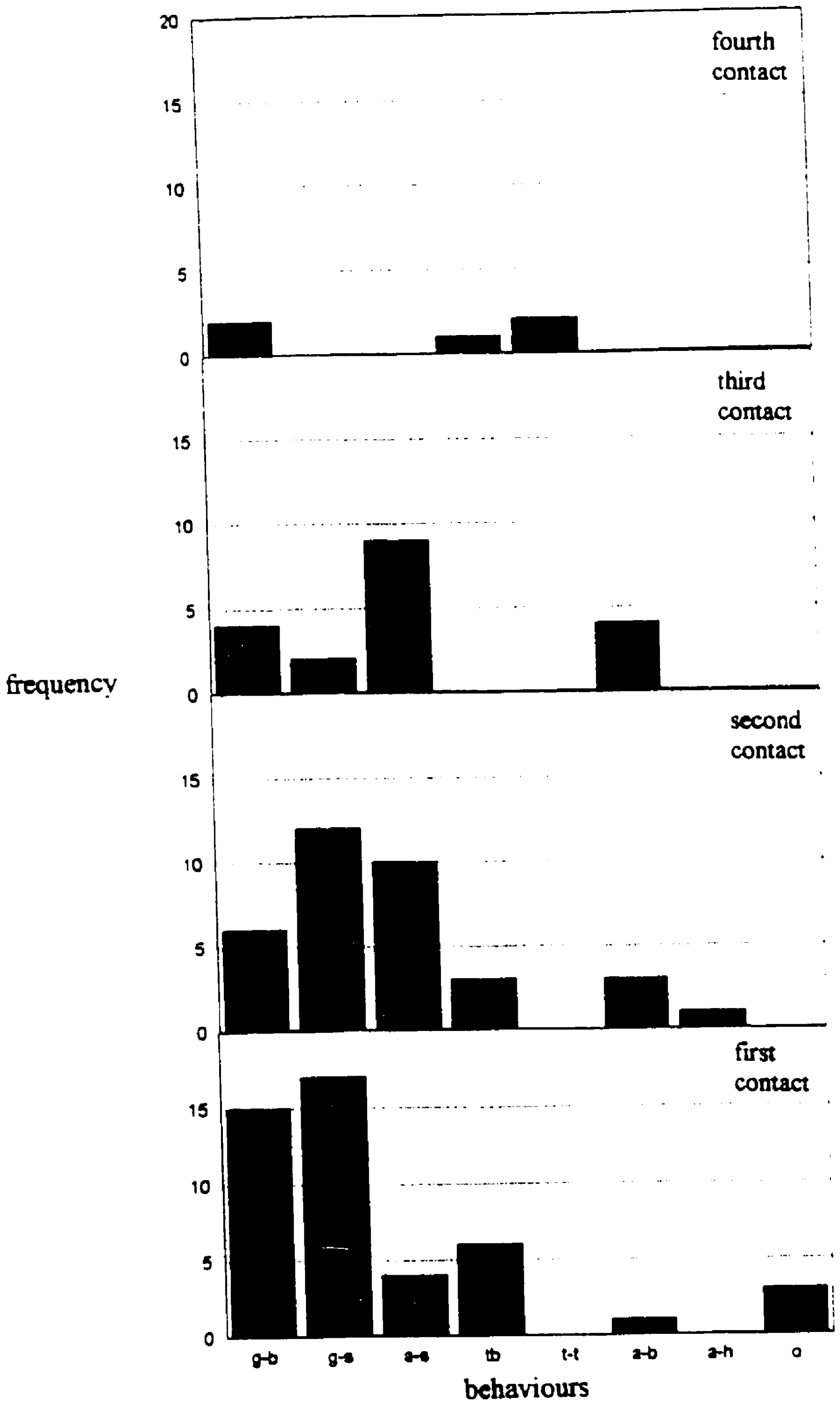

fourth contact
third contact
second contact
first contact
frequency
0
5
10
15
20
g-b
g-s
a-s
tb
t-t
a-b
a-h
o
behaviours

Figure 3.1.2. The frequency and sequence of precopula formation behaviours initiated by males during unsuccessful normal precopula attempts with females in *G. setosus*. The sequence of precopula formation behaviours from first to last is in ascending order. Legend found with Figure 3.1.1.

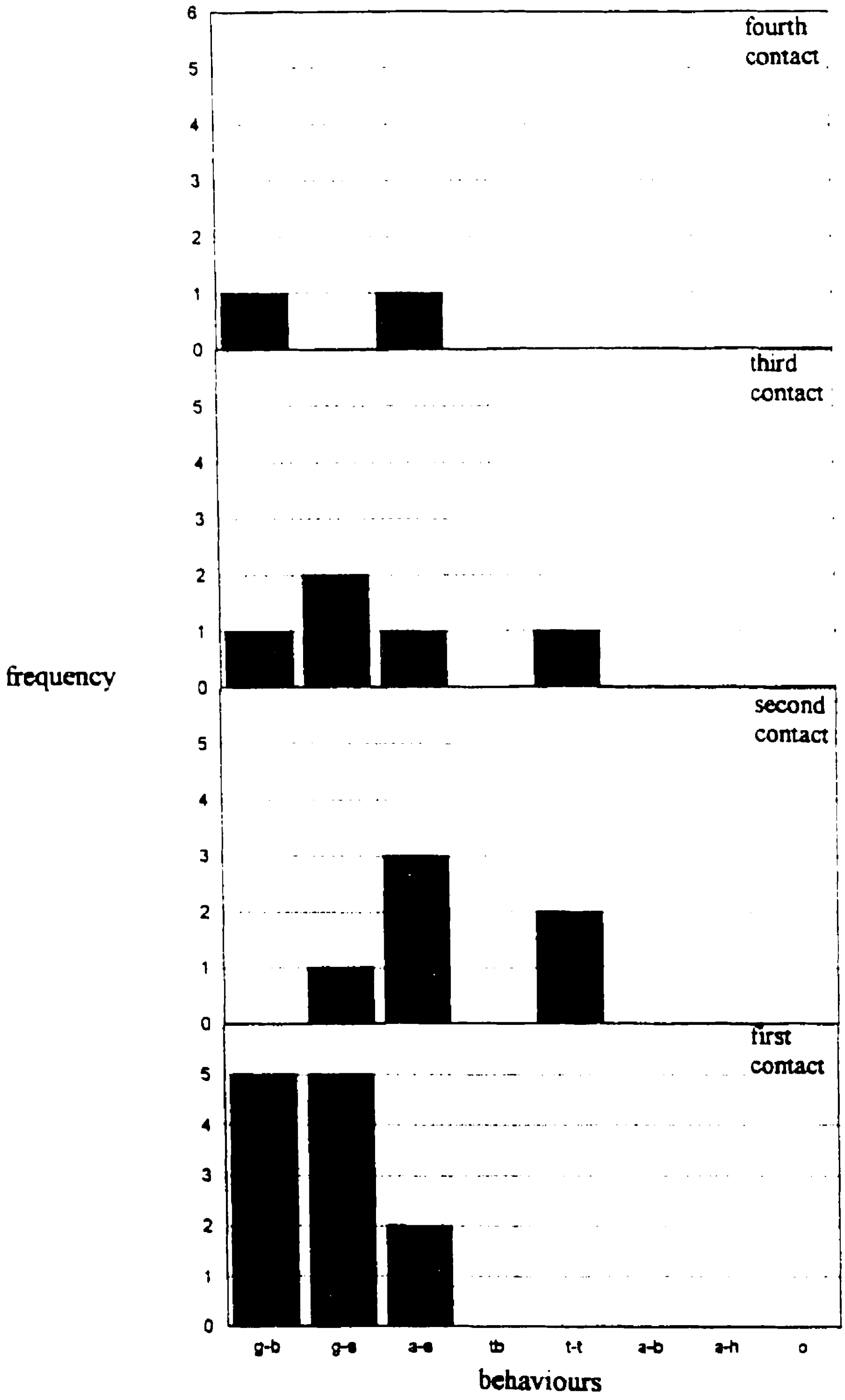


Table 3.1.3. Average times to form precopulas and percentages of animals that formed precopulas for the four previously studied species - *G. oceanicus*, *G. duebeni*, *G.lawrencianus*, *G. obtusatus* (Miller, 1995), and *G. setosus*.

Species	Average Time (min)	Percent Formed Precopula
G. oceanicus	1:50	83.45
G. duebeni	3:31	69.51
G. lawrencianus	2:17	72.94
G. obtusatus	4:08	17.14
G. setosus	6:28	66.67

There were no differences in average times to form successful precopulas (Table 3.1.4) between *G. oceanicus* and *G. lawrencianus* ($W = 3095$, $p = 0.126$, $\alpha = 0.05$) and *G. duebeni* and *G. obtusatus* ($W = 139$, $p = 0.454$, $\alpha = 0.05$).

All other combinations compared were significantly different in the average times to form successful precopulas. When comparing *G. setosus* and *G. oceanicus*, *G. setosus* and *G. lawrencianus*, *G. setosus* and *G. duebeni*, and *G. setosus* and *G. obtusatus*, *G. setosus* had the longest average time to form precopulas than all of the other four species. When comparing of *G. oceanicus* and *G. obtusatus*, *G. lawrencianus* and *G. obtusatus*, and *G. duebeni* and *G. obtusatus*, *G. obtusatus* had the longest average time to form precopulas. When comparing *G. oceanicus* and *G. duebeni*, and *G. lawrencianus* and *G. duebeni*, *G. duebeni* had the longer average time to form precopulas.

There were no significant differences in the percentage of animals that formed successful precopulas for comparisons between *G. setosus* and *G. lawrencianus* ($z = 0.845$, $z_{0.025} = 1.96$, $p = 0.3954$, $\alpha = 0.05$), *G. setosus* and *G. duebeni* ($z = 0.3732$, $z_{0.025} = 1.96$, $p = 0.7114$, $\alpha = 0.05$), *G. oceanicus* and *G. lawrencianus* ($z = 1.887$, $z_{0.025} = 1.96$, $p = 0.0588$, $\alpha = 0.05$), and *G. lawrencianus* and *G. duebeni* ($z = 0.4893$, $z_{0.025} = 1.96$, $p = 0.6242$, $\alpha = 0.05$).

Table 3.1.4. Summary of statistical comparisons of average times to form successful precopulas (Mann-Whitney) and percentages of animals that formed successful precopulas (Proportions Test) between the five species of *Gammarus* - *G. setosus*, *G.oceanicus*, *G. lawrencianus*, *G. duebeni*, and *G. obtusatus*.

Combination	Mann-Whitney			Proportions Test		
	W	p-value	Significant	Z	p-value	Significant
G. setosus - *G. oceanicus*	332	0	Yes	2.746	0.0142	Yes
G. setosus - *G. lawrencianus*	277.5	0	Yes	0.845	0.3954	No
G. setosus - *G. duebeni*	550	0	Yes	0.3732	0.7114	No
G. setosus - *G. obtusatus*	60	0.025	Yes	4.772	0	Yes
G. oceanicus - *G. lawrencianus*	3095	0.126	No	1.887	0.0588	No
G. oceanicus - *G. duebeni*	2178	0	Yes	2.429	0.015	Yes
G. oceanicus - *G. obtusatus*	109	0.005	Yes	7.657	0	Yes
G. lawrencianus - *G. duebeni*	1343.5	0.024	Yes	0.4893	0.6242	No
G. lawrencainus - *G. obtusatus*	77	0.018	Yes	5.602	0	Yes
G. duebeni - *G. obtusatus*	139	0.454	No	7.882	0	Yes

All other combinations compared were significantly different in the percentages of animals that formed successful precopulas. When comparing *G. setosus* and *G. oceanicus*, *G. oceanicus* and *G. duebeni*, and *G. oceanicus* and *G. obtusatus*, *G. oceanicus* had the highest percentage of animals that formed precopulas. For the comparison of *G. setosus* and *G. obtusatus*, *G. setosus* had the higher percentage, for *G. lawrencianus* and *G. obtusatus*, *G. lawrencianus* had the higher percentage, and for *G. duebeni* and *G. obtusatus*, *G. duebeni* had the higher percentage.

The only combination that had no significant differences between the two species for both the average times to form successful precopulas and the percentages of animals that formed successful precopulas was *G. oceanicus* and *G. lawrencianus*.

The comparisons of *G. setosus* and *G. oceanicus*, *G. setosus* and *G. obtusatus*, *G. oceanicus* and *G. duebeni*, *G. oceanicus* and *G. obtusatus*, and *G. lawrencianus* and *G. obtusatus* were the only combinations that were significantly different between the two species in both the average times to form successful precopulas and the percentages of animals that formed successful precopulas.

Overall, the sequences of precopula formation behaviours were different among all five species in both the sequences for successful precopula formations and unsuccessful precopulas (Table 3.1.5). However, there were several similarities. For successful precopula formations the first contact behaviour always included the male's

<u>Table 3.1.5.</u> The sequence of normal successful and unsuccessful precopula formation behaviours for five species of *Gammarus* studied - *G. oceanicus*, *G. duebeni*, *G.lawrencianus*, *G. obtusatus* and *G. setosus*. Legend found with Figure 3.1.1.

Species		First Contact	Second Contact	Third Contact	Fourth Contact	Fifth Contact
G. oceanicus	Successful	g-b	a-s	a-s t-t	a-s	g-s tb
	Unsuccessful	g-b g-s	a-s	o	a-s	N/A
G. duebeni	Successful	g-b	a-s tb	a-s tb	a-s	N/A
	Unsuccessful	g-b	g-s	tb	a-s tb	N/A
G. lawrencianus	Successful	g-b	a-s g-b g-s	tb a-s	tb	a-s
	Unsuccessful	g-b	g-b g-s	a-s	a-s	g-s tb o
G. obtusatus	Successful	g-b tb	a-s g-s a-b	g-b t-t	g-b	N/A
	Unsuccessful	g-b	g-s	a-s o	N/A	N/A
G. setosus	Successful	g-s g-b	g-s a-s	a-s	g-b t-t	N/A
	Unsuccessful	g-b g-s	a-s t-t	g-s	g-b a-s	N/A

gnathopods touching the female's back. The second contact behaviour was also similar among the species, which always included the male's antennae touching the female's side in successful precopula formations.

There were several differences as well among the species in the first contact in successful precopulas. Along with the common first contact of the male's gnathopods touching the female's back, *G. setosus* included the male's gnathopods touching the female's sides and *G. obtusatus* included tail beating. The second contact also was different among the species in successful precopulas. Although all the second contacts for all five species included the male's antennae touching the female's side, the second contact included another type of contact for all species except *G. oceanicus*. The third contact behaviour for successful precopula formations was where the five species varied greatly and no common precopula formation behaviours were found.

As in successful precopulas, unsuccessful precopula attempts had the first contact behaviour as the male's gnathopods touching the female's back but also included the male's gnathopods touching the female's sides in both *G. oceanicus* and *G. setosus*. However, this first contact behaviour was where the similarities ended and no more common precopula formation behaviours were found.

The only similarity found between the precopula formation behaviours for successful and unsuccessful precopulas was that the first contact behaviour was the male's gnathopods touching the female's back.

3.1.3 INTERSPECIFIC PRECOPULAS

No interspecific precopulas were formed between *G. setosus* and *G. oceanicus*. Either the animals did not have any physical contact at all (30.77%), the male swam away after the first contact (46.15%), the female swam away after first contact (17.31%), or both swam away (5.77%).

3.1.4 SIZE ASSORTATIVE MATING

G. setosus had a ratio of 1.6311 for male:female length for successful precopula formations. There was a positive correlation ($r = 0.492$, $p = 0.001$, $\alpha = 0.05$) whereby as male size increased, so did female size and there was a significant difference between the sizes of males and females of *G. setosus* in precopulas (Figure 3.1.3). The male was always larger than the female.

3.2 TAKEOVERS

There were three successful takeovers (2.10%) and 53 unsuccessful takeovers (37.06%) (Table 3.2.1) in *G. oceanicus*. An unsuccessful takeover occurred when the intruder grabbed the female with his gnathopods and tried to take the female, but did not succeed.

Figure 3.1.3. Relationship between male and female length of *G. setosus* that successfully formed precopulas (r = 0.492). the diagonal broken line indicates equal lengths for males and females.

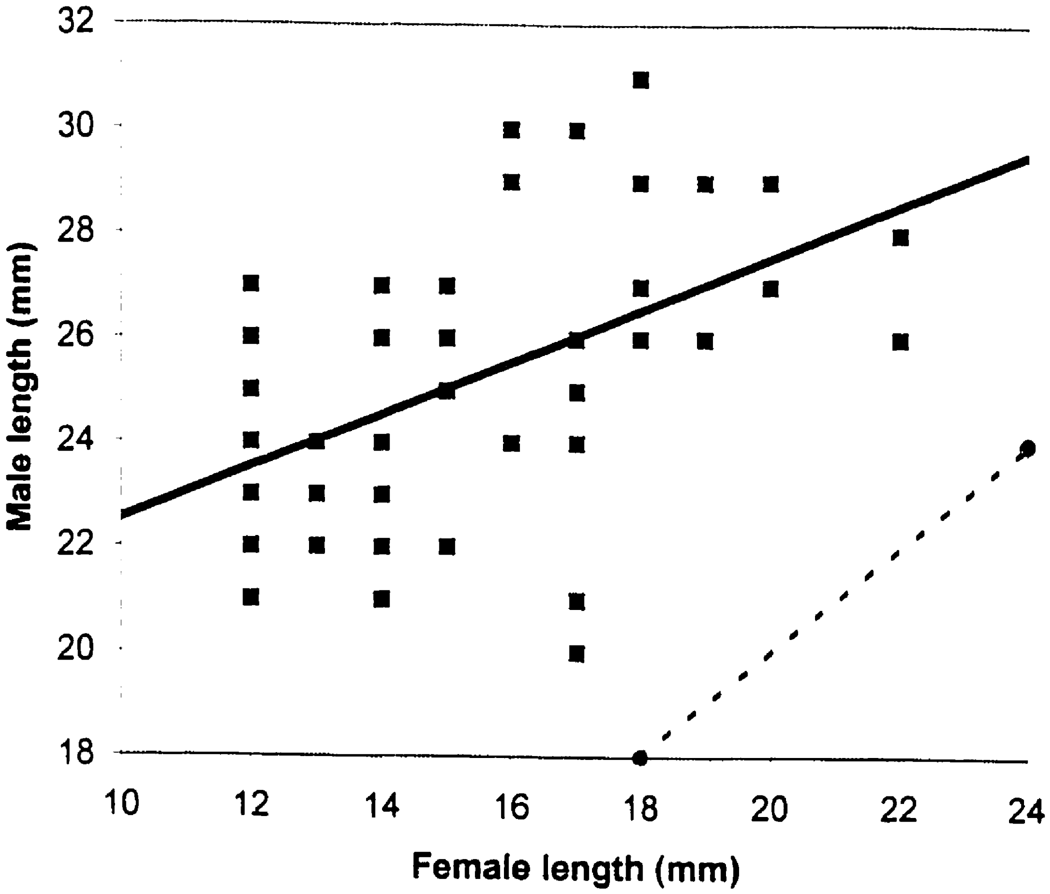


Table 3.2.1. Summary of successful takeovers and unsuccessful takeovers data giving the percentage of animals that had successful takeovers or unsuccessful takeovers, average amount of time engaged in takeover formation behaviours, and descriptive statistical calculations for *G. oceanicus* from a total of 143.

Condition	Successful Takeovers	Unsuccessful Takeovers
Number Successful or Unsuccessful	3	53
Percentage	2.10	37.06
Average Time (min)	0:37	0:23
Minimum Time (min)	0:02	0:03
Maximum Time (min)	1:16	2:24
Standard Error (min)	0:21	0:04
Standard Deviation (min)	0:37	0:26

All three successful takeovers occurred with females that were in stage 4 whereas the unsuccessful takeovers occurred with females of all stages (Table 3.2.2). There was a significant difference between the number of unsuccessful takeovers in each egg stage ($\chi^2 = 50.47$, d.f. = 3, $p = 0.000$, $\alpha = 0.05$) where more unsuccessful takeovers were attempted with females in stage 4. However, there was no significant difference between the number of females in each egg stage that had no attempts made to be taken over by an intruder ($\chi^2 = 6.10$, d.f. = 3, $p = 0.107$, $\alpha = 0.05$).

There was no significant difference in average times spent attempting unsuccessful takeovers between intruders that were larger than the defenders and intruders that were smaller than the defenders ($W = 178.0$, $p = 0.650$, $\alpha = 0.05$) (Table 3.2.3). There was also no significant difference in female size between intruders that were larger than the defenders and intruders that were smaller than the defenders ($W = 159.5$, $p = 0.368$, $\alpha = 0.05$).

The ratio of intruder to defender length of *G. oceanicus* (Table 3.2.4) for successful takeovers was 1.12, meaning that the intruders were 12% larger than the defenders. The ratio was 1.09 for unsuccessful takeovers (intruders 9% larger than defenders) and a ratio of 1.05 for no takeovers attempted (intruders 5% larger than defenders).

Table 3.2.2. Summary of number of successful takeovers, unsuccessful takeovers, and no attempts at takeovers for each female egg stage for *G. oceanicus*.

Stages	1	2	3	4	Total
Successful Takeovers	0	0	0	3	3
Unsuccessful Takeovers	1	8	9	35	53
No Attempt at Takeovers	27	16	16	28	87
Total	28	24	25	66	143

Table 3.2.3. Summary of mean average times and female sizes for intruders that were larger then defenders and intruders that were smaller than defenders in unsuccessful takeovers in *G. oceanicus*.

Conditions	Average Times (min)	Average Female Size (mm)
Intruder Larger than Defender	00:24	00:18
Intruder Smaller the Defender	00:15	00:17

Table 3.2.4. Ratios and correlation values of intruder lengths to defender lengths of *G. oceanicus* for successful takeovers, unsuccessful takeovers, and no attempts at takeovers.

Condition	Ratio	Correlation Value (r)	p-value
Successful Takeovers	1.12	N/A	N/A
Unsuccessful Takeovers	1.09	0.168	0.230
No Attempts at Takeovers	1.05	0.211	0.050

Intruder versus defender lengths of *G. oceanicus* in unsuccessful takeovers (Figure 3.2.1 with successful takeovers also included) and no attempts at takeovers (Figure 3.2.2) were plotted and regression calculated. There was a non-significant positive correlation (Table 3.2.4) of intruder to defender size ($r = 0.168$, $p = 0.230$, $\alpha = 0.05$) for *G. oceanicus* unsuccessful takeovers. There was also a non-significant positive correlation of intruders to defenders size ($r = 0.211$, $p = 0.050$, $\alpha = 0.05$) for *G. oceanicus* making no attempts at takeovers.

Correlation coefficients were also calculated to determine if time spent attempting unsuccessful takeovers increased as female quality increased (Table 3.2.5). There was a positive correlation for time attempting unsuccessful takeovers and female stage ($r = 0.124$, $p = 0.376$, $\alpha = 0.05$) for *G. oceanicus* but there was no significant difference between the times spent attempting unsuccessful takeovers and female stage. The times and female stages were plotted with a regression line (Figure 3.2.3).

There was also a non-significant positive correlation for time attempting unsuccessful takeovers and female size ($r = 0.060$, $p = 0.671$, $\alpha = 0.05$) for *G..oceanicus*. The times and female sizes were plotted with a regression line (Figure 3.2.4).

The general pattern of behaviours for successful takeovers (N=3) of female *G. oceanicus* by intruding males (Figure 3.2.5) consisted firstly of the intruder's gnathopods touching the female's back and sides; the next contact was the intruder

Figure 3.2.1. Relationship between intruder and defender length of *G. oceanicus* for unsuccessful takeovers (r = 0.168) and successful takeovers. The diagonal broken line indicates equal lengths for intruders and defenders.

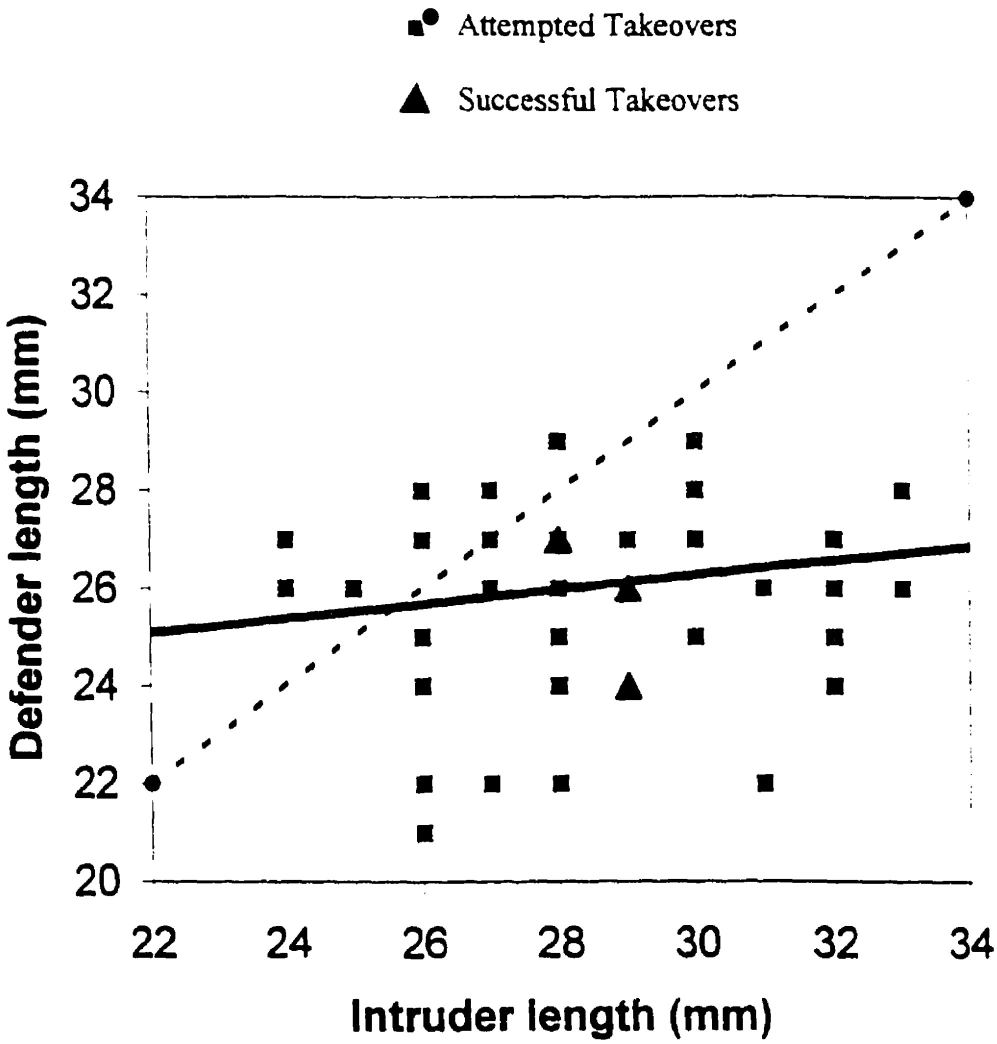


Figure 3.2.2. Relationship between intruder and defender length of *G. oceanicus* that made no attempts at takeovers (r = 0.211). The diagonal broken line indicates equal lengths for intruders and defenders.

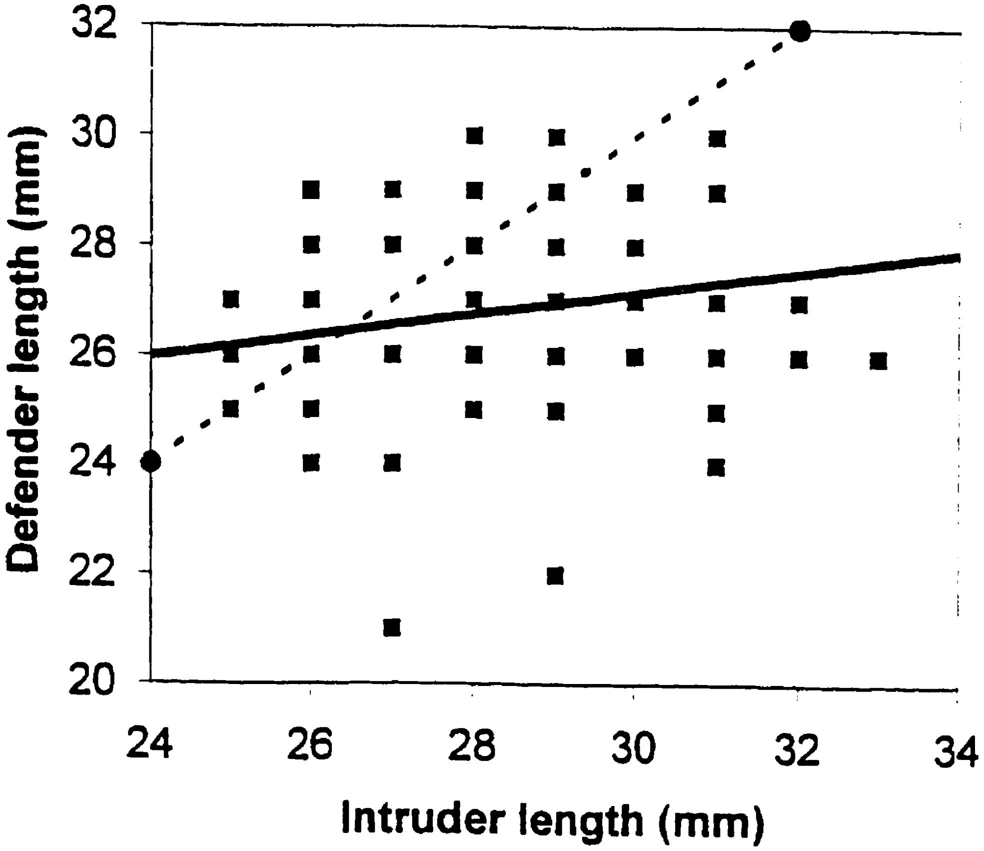


Table 3.2.5. Correlation values of time spent attempting takeovers in relation to both female egg stages and female size of *G. oceanicus*.

Condition	Correlation Value (r)	p-value
Time and Female Stage	0.124	0.376
Time and Female Size	0.060	0.671

Figure 3.2.3. Relationship between time spent attempting unsuccessful takeovers and female egg stage of *G. oceanicus* (r = 0.124).

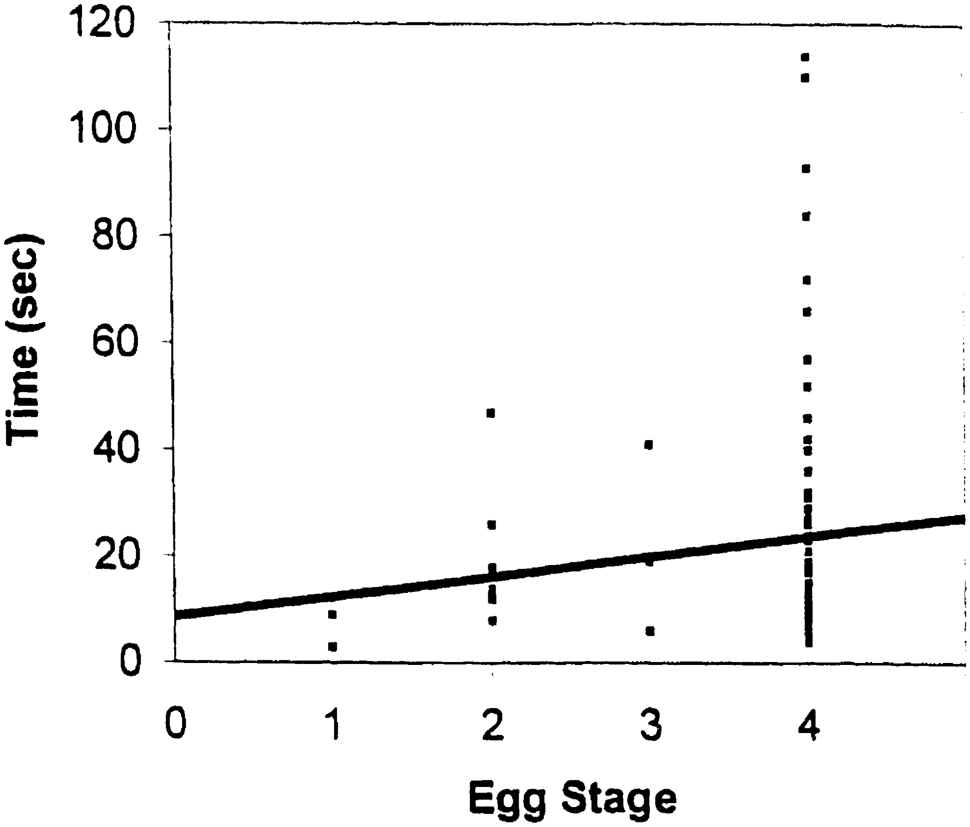


Figure 3.2.4. Relationship between time spent attempting unsuccessful takeovers and female size of *G. oceanicus* (r = 0.060).

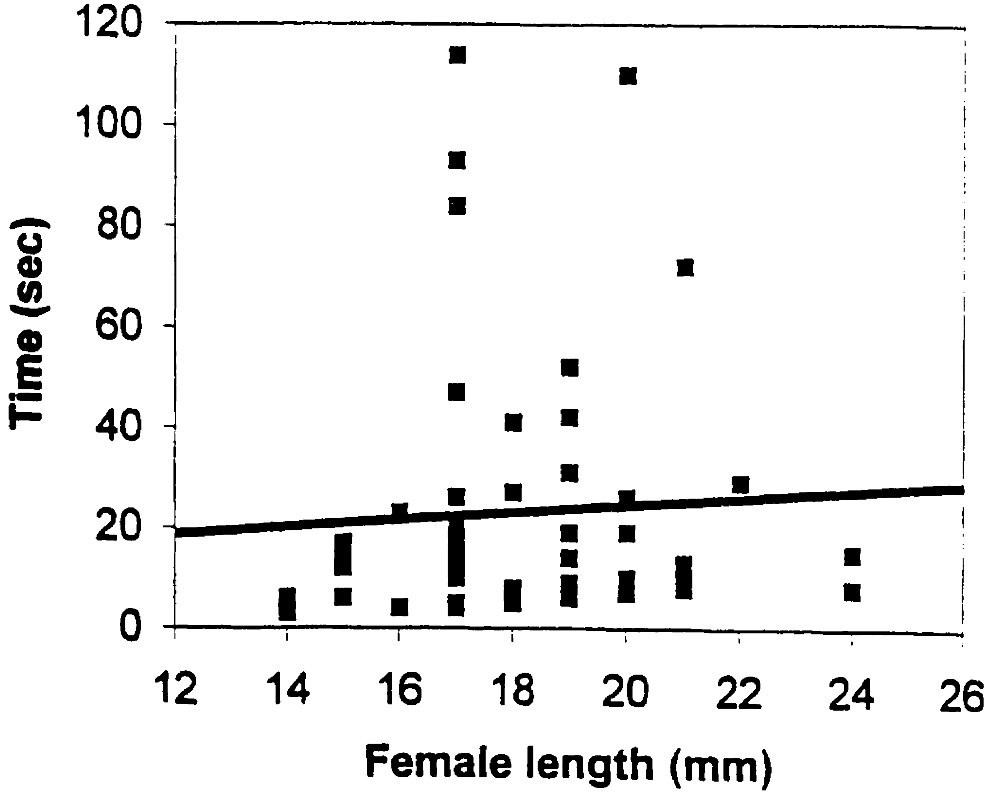
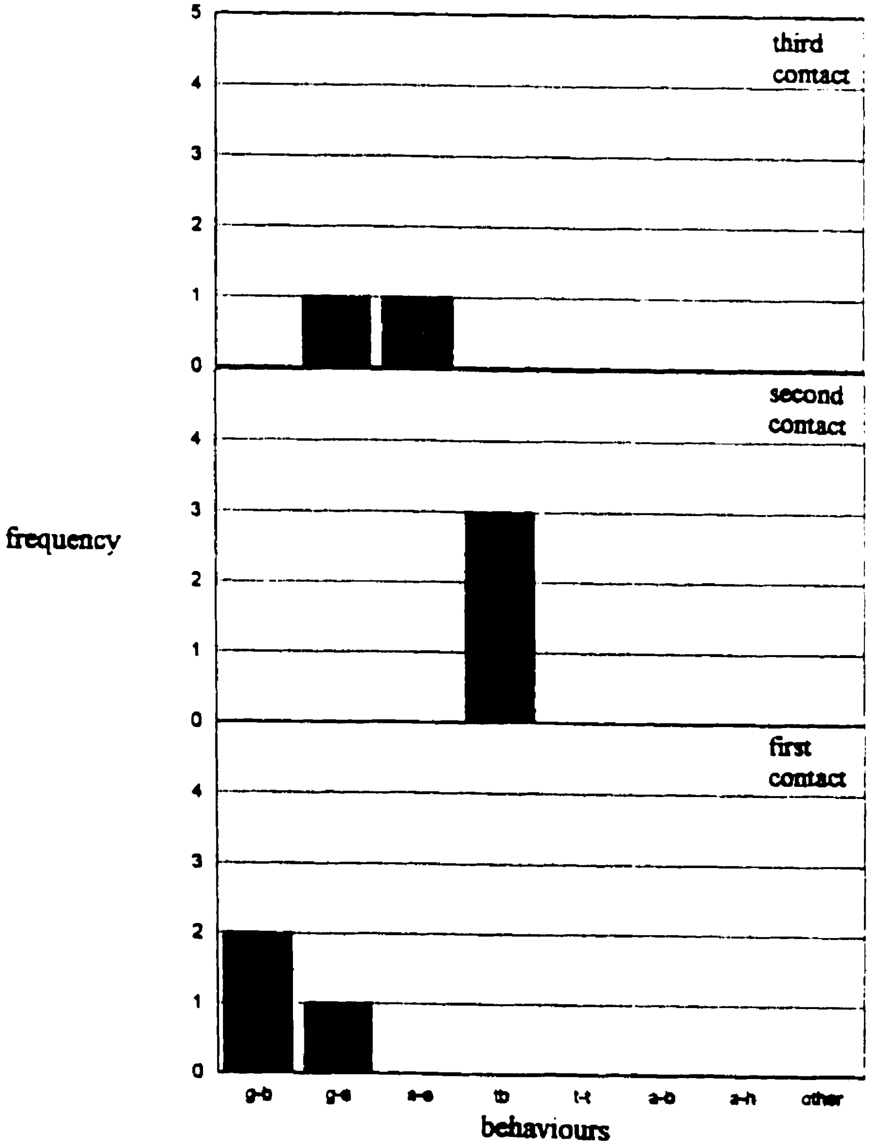


Figure 3.2.5. The frequency and sequence of behaviours initiated by intruding males during successful takeovers in *G. oceanicus*. The sequence of takeover behaviours from first to last is in ascending order. Legend found with Figure 3.1.1.



engaging in tail beating on the female; and next the intruder's antennae or gnathopods touched the female's sides. There was no contact by the intruder with the defender in successful takeovers.

However, the general sequence of behaviours for unsuccessful takeovers of *G. oceanicus* females by intruding males were much more complicated than for successful takeovers. The intruding males made contacts with both the females and defending males (Figure 3.2.6). The first contact was either the intruder touching the defender's back or the female's back with his gnathopods. Next the intruder contacted the female's side with his antennae and then the intruder engaged in tail beating of the female.

Female choice occurred as females did not resist takeovers in any trial, and remained passive in both the successful takeovers and unsuccessful takeovers.

3.3 SIMULTANEOUS ASSESSMENTS

There were two simultaneous assessments (1.36%) and 16 unsuccessful simultaneous assessments, where the extra female was grabbed and either escaped by the normal escape response or the male released her immediately after grabbing (10.88%) in total (Tables 3.3.1 & 3.3.2). The two simultaneous assessments occurred with stage 4 females with both of the extra females being larger than the paired female. The first male that simultaneously assessed both females kept the original female, which was smaller

Figure 3.2.6. The frequency and sequence of behaviours initiated by intruding males during unsuccessful takeovers in *G. oceanicus*. The sequence of takeover behaviours from first to last is in ascending order. Legend found with Figure 3.1.1.

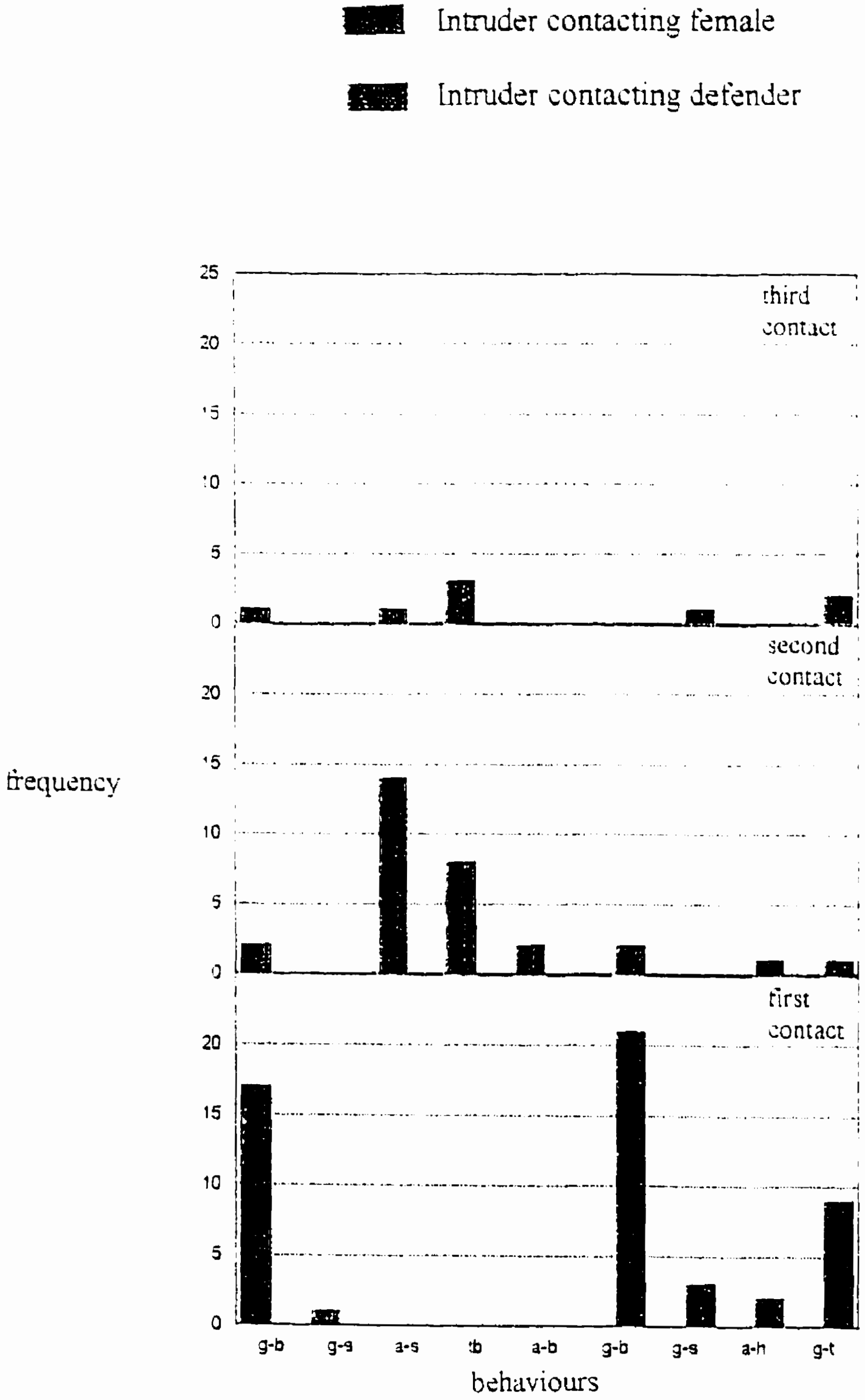


Table 3.3.1. Summary of number of successful simultaneous assessments, unsuccessful simultaneous assessments, and no simultaneous assessment attempts for same stage females and same size females in *G. oceanicus*.

Condition	Same Stage Females	Same Size Females	Total
Successful Simultaneous Assessments	2	0	2
Unsuccessful Simultaneous Assessments	8	8	16
No Simultaneous Assessment Attempts	75	54	129
Total	85	62	147

Table 3.3.2. Summary of successful simultaneous assessment data giving the percentage of animals that had successful simultaneous assessments or unsuccessful simultaneous assessments, average amount of time engaged in simultaneous assessment behaviours, and descriptive statistical calculations for both setups of same stage females and same size females for *G. oceanicus*.

Condition	Successful Simultaneous Assessments (Same Stage)	Unsuccessful Simultaneous Assessments	
		Same Stage	Same Size
Number Successful/ Unsuccessful	2	8	8
Percentage	1.36	5.44	5.44
Average Time (min)	0:52	0:17	0:17
Minimum Time (min)	0:33	0:03	0:02
Maximum Time (min)	1:10	0:51	0:44
Standard Error (min)	0:19	0:06	0:05
Standard Deviation (min)	0:26	0:16	0:14

than the extra female, and released the extra female. Conversely, the second male that simultaneously assessed both females released the original female, which was smaller than the extra female, and kept the extra female.

Eight unsuccessful simultaneous assessments occurred with females in the same stage while the other eight occurred with females of the same size. An unsuccessful simultaneous assessment occurred when the male grabbed the extra female with his gnathopods and tried to hold the extra female, along with the original female, perpendicular to his body, but was unsuccessful in grabbing and holding the extra female for manipulation. The male may have just simply released the extra female before he attempted any assessment of the extra female or the extra female may have resisted and escaped.

There was no significant difference between the number of unsuccessful simultaneous assessments when the extra female was larger or smaller than the original female in the same stage female trials (Table 3.3.3) (χ^2 = 0.500, d.f. = 1, p = 0.480, α = 0.05). There was also no significant difference between the number of unsuccessful simultaneous assessments when the extra female was further from or closer to moulting than the original females in the same size female trials (χ^2 = 0.000, d.f. = 1, p = 1.000, α = 0.05).

Table 3.3.3. Summary of number of unsuccessful simultaneous assessments for same stage females and same size females in *G. oceanicus*.

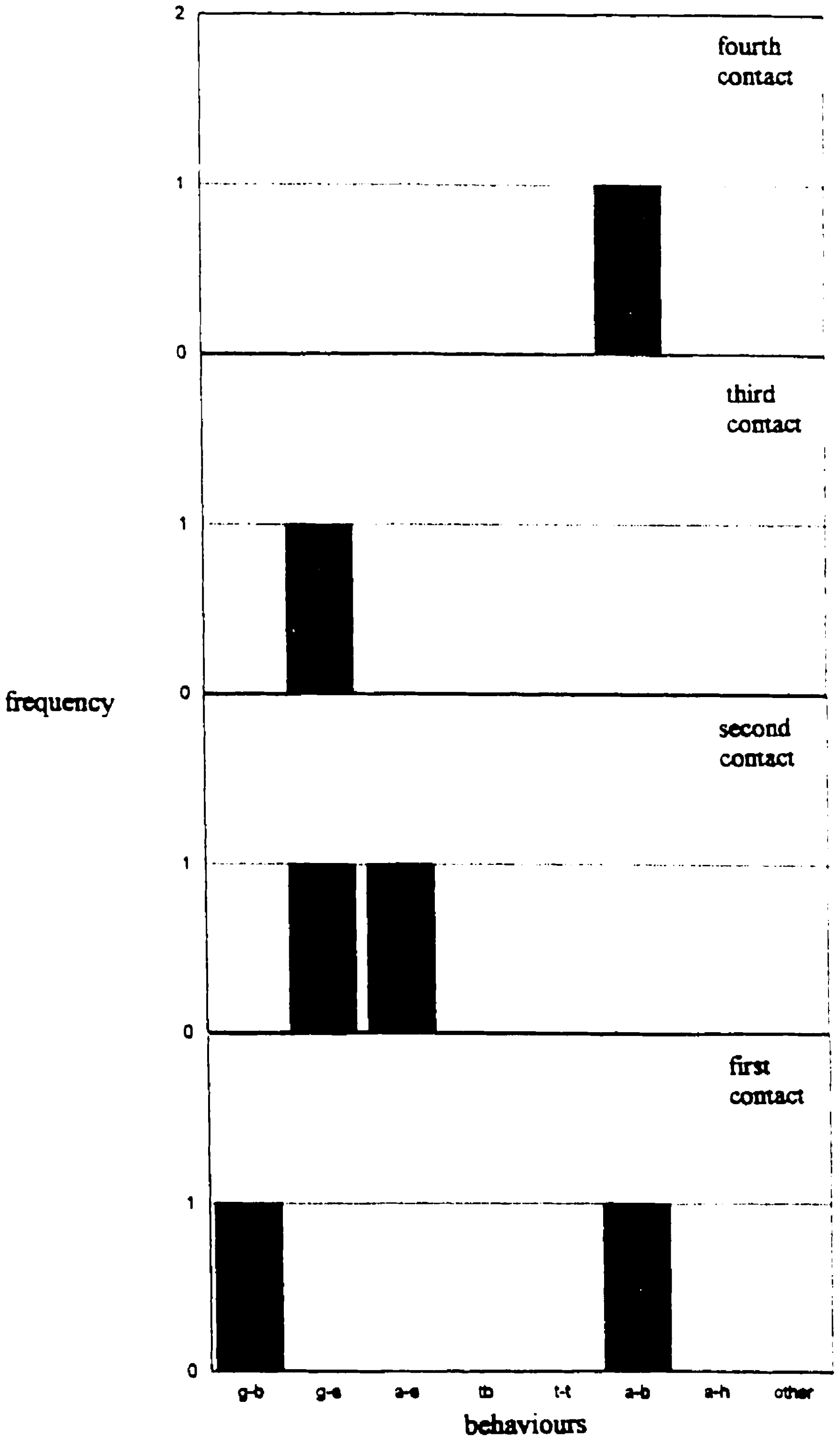
Condition	Same Stage		Same Size	
	Larger	Smaller	Further from Moult	Closer to Moult
Unsuccessful Simultaneous Assessments	5	3	4	4

There was a significant difference between the times to form precopulas in normal *G. oceanicus* trials and unsuccessful simultaneous assessments for same stage females (W = 114.0, p = 0.000, $\alpha = 0.05$). There was also a significant difference between the times to form precopulas in normal *G. oceanicus* trials and unsuccessful simultaneous assessments for same size females (W = 127.5, p = 0.001, $\alpha = 0.05$). The time spent attempting unsuccessful simultaneous assessments for both same stage and same size females were shorter than for normal *G. oceanicus* precopulas. However, there was no significant difference between the average times attempting unsuccessful simultaneous assessments for same stage females and same size females (W = 67.5, p = 0.958, $\alpha = 0.05$).

In both the successful and unsuccessful simultaneous assessments, once an extra female was initially contacted, the male placed the original female perpendicular to his body while still holding her with his smaller anterior gnathopods. The male then grabbed the extra female with his larger posterior gnathopods and proceeded with his sequence of behaviours on the extra female.

The general pattern of behaviours for successful simultaneous assessments of two females for *G. oceanicus* by a male (Figure 3.3.1) consisted firstly of either the male's gnathopods or antennae contacting the extra female's back; the next contact was the male's antennae or gnathopods touching the extra female's side; next the male's

Figure 3.3.1. The frequency and sequence of behaviours initiated by males during successful simultaneous assessments of same stage females in *G. oceanicus*. The sequence of simultaneous assessment behaviours from first to last is in ascending order. Legend found with Figure 3.1.1.



gnathopods touched the extra female's side; and then the male's antennae contacted the extra female's back.

The general sequences of behaviours for unsuccessful simultaneous assessments have been broken down into pattern of contacts for same stage females (Figure 3.3.2) and same size females (Figure 3.3.3). For the same stage females, the male first contacted the extra female's back with his antennae. Next the male's antennae touched either the extra female's side or back, then the male's antennae touched the extra female's side.

For the same size females, the male first contacted the extra female's back with his gnathopods. Then the male's antennae touched the extra female's side, and next either the male's antennae touched the extra female's side or the male performed tail beating on the extra female.

Neither the original females nor extra females resisted during the successful simultaneous assessments. However, one extra female resisted an unsuccessful simultaneous assessment in the same stage trials and two extra females resisted unsuccessful simultaneous assessments in the same size trials.

3.4 EGG MANIPULATIONS

The general behaviours that occur when the female is held at a right angle to the male occurred in *G. oceanicus* for both stage 1 egg manipulation and stage 4 egg

Figure 3.3.2. The frequency and sequence of behaviours initiated by males during unsuccessful simultaneous assessments of same stage females in *G. oceanicus*. The sequence of simultaneous assessment behaviours from first to last is in ascending order. Legend found with Figure 3.1.1.

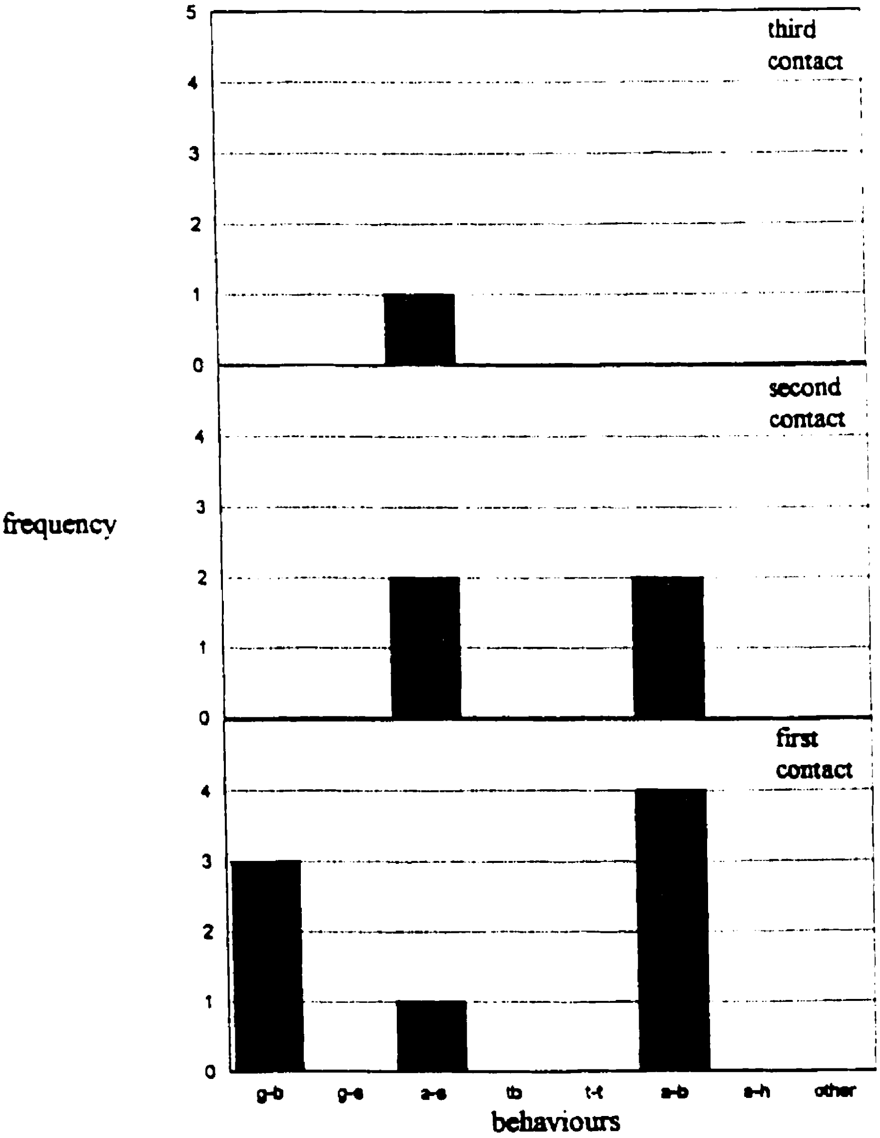
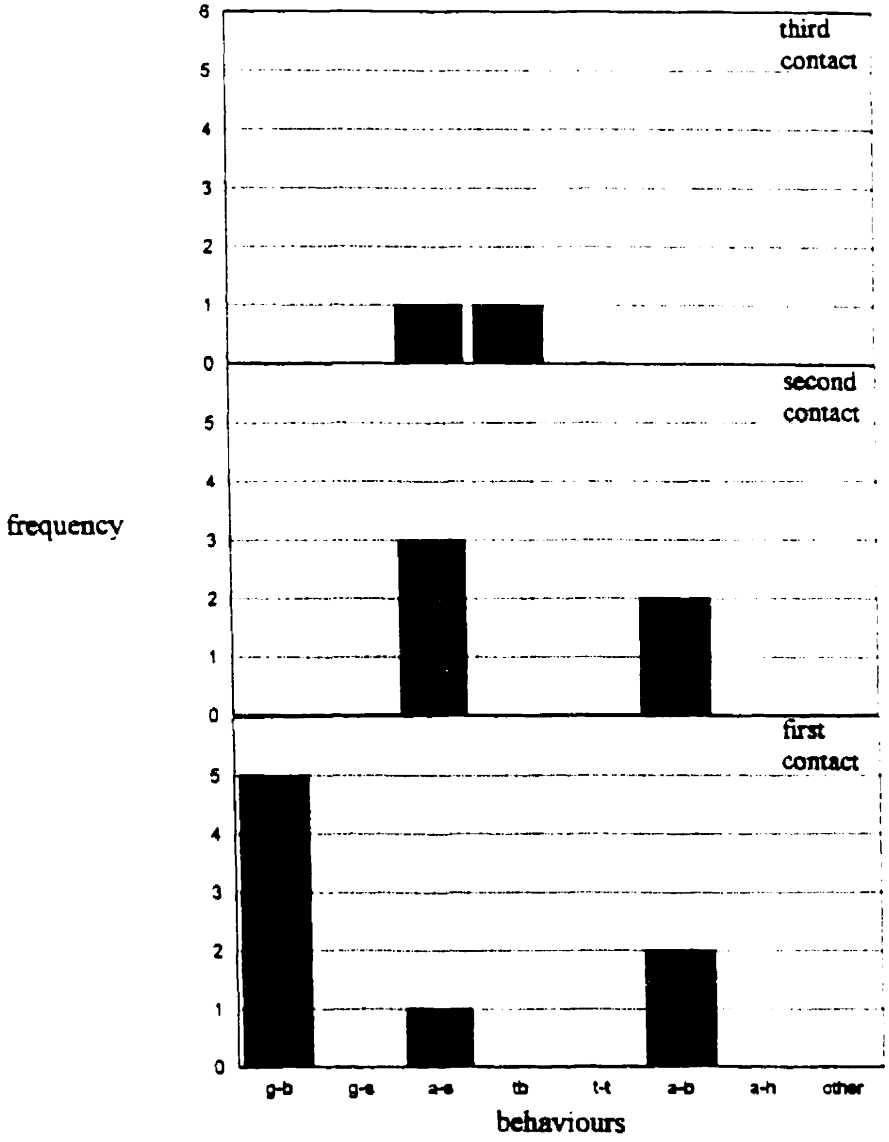


Figure 3.3.3. The frequency and sequence of behaviours initiated by males during unsuccessful simultaneous assessments of same size females in *G. oceanicus*. The sequence of simultaneous assessment behaviours from first to last is in ascending order. Legend found with Figure 3.1.1.



manipulation females. Female choice was evident as females sometimes swam away after the first contact by a male (11.34%) or the female rapidly flexed and straightened in succession and escaped after the male had been assessing her for a period (7.73%).

There was no difference between the times forming successful precopulas for the stage 1 egg manipulation and stage 4 egg manipulation females of *G. oceanicus* (Table 3.4.1) ($W = 545.0$, $p = 0.069$, $\alpha = 0.05$). There was no difference between the times attempting unsuccessful precopulas for the stage 1 egg manipulation and stage 4 egg manipulation females ($W = 281.5$, $p = 0.412$, $\alpha = 0.05$). There was no difference between the percentage of pairs that formed successful precopulas for the stage 1 egg manipulation and stage 4 egg manipulation females ($z = 0.2194$, $z_{0.025} = 1.96$, $p = 0.8258$, $\alpha = 0.05$). However, there was a higher percentage of unsuccessful precopulas in stage 1 egg manipulation than stage 4 egg manipulation females ($z = 2.237$, $z_{0.025} = 1.96$, $p = 0.0178$, $\alpha = 0.05$).

There was no difference between the times forming successful precopulas for the stage 1 egg manipulation females and *G. oceanicus* normal precopulas with stage 1 females ($W = 1688.5$, $p = 0.083$, $\alpha = 0.05$). However, there was a significant difference between the times forming successful precopulas for the stage 4 egg manipulation

Table 3.4.1. Summary of data for stage 1 females with stage 4 eggs transplanted and stage 4 females with eggs removed giving the percentage of animals that formed precopulas and unsuccessful precopulas, average amount of time to form or attempt precopulas, and descriptive statistical calculations for *G. oceanicus* from a total of 90 stage 1 and 104 stage 4 females.

Condition	Stage 1		Stage 4	
	Successful	Unsuccessful	Successful	Unsuccessful
Number Successful/ Unsuccessful Precopulas	36	31	40	21
Percentage	40.00	34.44	38.46	20.19
Average Time (min)	1:10	0:25	0:52	0:21
Minimum (min)	0:05	0:02	0:08	0:01
Maximum (min)	6:16	1:34	6:55	1:09
Standard Deviation (min)	1:14	0:21	1:19	0:18
Standard Error (min)	0:12	0:04	0:12	0:04

females and *G. oceanicus* normal precopulas with stage 4 females ($W = 1443.5$, $p = 0.000$, $\alpha = 0.05$) where it took longer to form successful precopulas in normal *G. oceanicus* than in stage 4 egg manipulation female precopulas.

There was a higher percentage of precopulas formed in normal successful precopulas in *G. oceanicus* than in stage 1 egg manipulation female successful precopulas ($z = 6.800$, $z_{0.025} = 1.96$, $p = 0.000$, $\alpha = 0.05$). There was also a significant difference in the normal successful precopulas in *G. oceanicus* which had a higher percentage than the stage 4 egg manipulation female successful precopulas ($z = 7.245$, $z_{0.025} = 1.96$, $p = 0.000$, $\alpha = 0.05$).

There was no significant difference between the number of successful precopulas formed with stage 1 egg manipulation or stage 4 egg manipulation females (Table 3.4.2) ($\chi^2 = 0.211$, d.f. = 1, $p = 0.646$, $\alpha = 0.05$). There was also no significant difference between the number of unsuccessful precopulas with stage 1 egg manipulation or stage 4 egg manipulation females ($\chi^2 = 1.923$, d.f. = 1, $p = 0.166$, $\alpha = 0.05$).

G. oceanicus stage 1 egg manipulation females had a ratio of 1.51 for male:female length (Table 3.4.3) for successful precopula formations. The sizes of males and stage 1 egg manipulation females of *G. oceanicus* in successful precopula formations (Figure 3.4.1) were plotted with a regression line. There was a positive correlation (r =

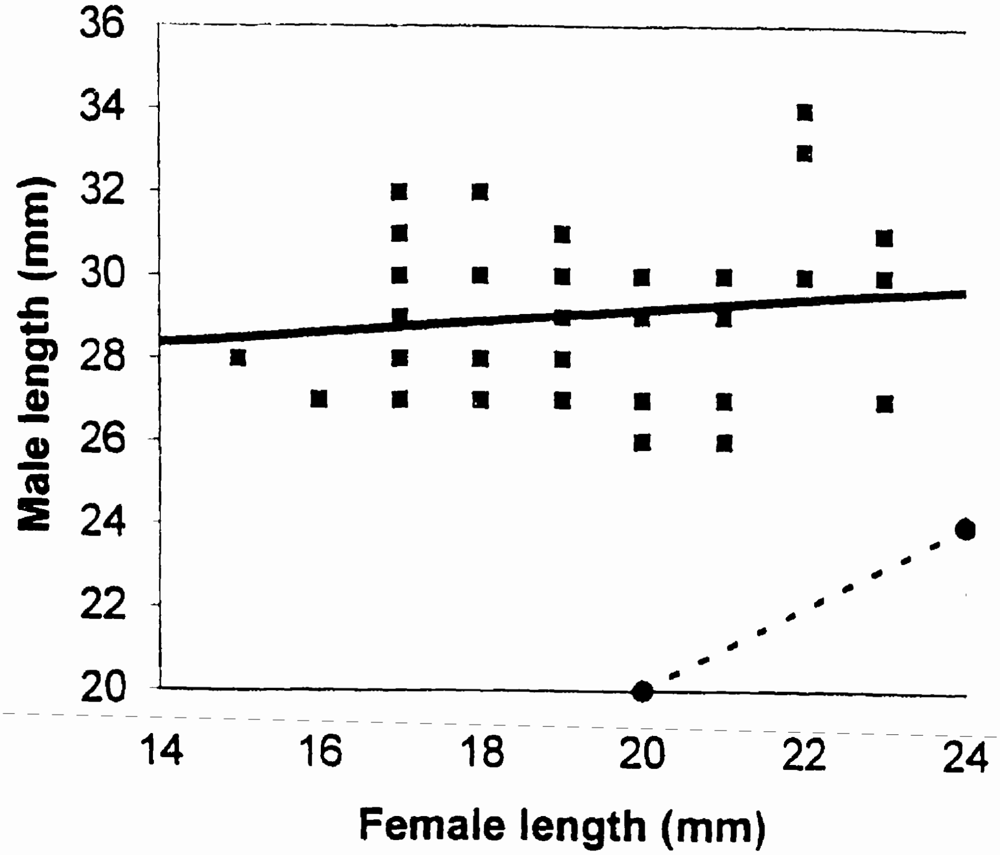
Table 3.4.2. Summary of number of successful and unsuccessful precopulas for stage 1 females with stage 4 eggs transplanted and stage 4 females with eggs removed for *G. oceanicus*.

Stage	1	4	Total
Successful	36	40	76
Unsuccessful	31	21	52
No Attempts	23	43	66
Total	90	104	194

Table 3.4.3. Ratios and correlation values of male length to female length of *G. oceanicus* for successful and unsuccessful precopulas with females in stage 1 with stage 4 eggs transplanted and females in stage 4 with eggs removed.

	Stage 1		Stage 4	
	Successful	Unsuccessful	Successful	Unsuccessful
Ratio	1.51	1.47	1.47	1.49
Correlation Value (r)	0.144	0.306	0.386	0.204
p-value	0.403	0.156	0.014	0.433

Figure 3.4.1. Relationship between male and female length of *G. oceanicus* females in stage 1 with stage 4 eggs transplanted that successfully formed precopulas (r = 0.144). The diagonal broken line indicates equal lengths for males and females.



0.144, $p = 0.403$, $\alpha = 0.05$) whereby as male size increased, so did female size; however, there was no significant difference between the sizes of the males and females.

G. oceanicus stage 1 egg manipulation females had a ratio of 1.47 for male:female length for unsuccessful precopulas. The sizes of males and stage 1 egg manipulation females of *G. oceanicus* in unsuccessful precopulas (Figure 3.4.2) were plotted with a regression line. There was a positive correlation ($r = 0.306$, $p = 0.156$, $\alpha = 0.05$) whereby as male size increased, so did female size; however, there was no significant difference between the sizes of the males and females.

G. oceanicus stage 4 egg manipulation females had a ratio of 1.47 for male:female length (Table 3.4.3) for successful precopula formations. The sizes of males and stage 4 egg manipulation females of *G. oceanicus* in successful precopula formations (Figure 3.4.3) were plotted with a regression line. There was a positive correlation ($r = 0.386$, $p = 0.014$, $\alpha = 0.05$) whereby as male size increased and there was a significant difference between the sizes of the males and females.

G. oceanicus stage 4 egg manipulation females had a ratio of 1.49 for male:female length for unsuccessful precopulas. The sizes of males and stage 4 egg manipulation females of *G. oceanicus* in unsuccessful precopulas (Figure 3.4.4) were plotted with a regression line. There was a positive correlation ($r = 0.204$, $p =$

Figure 3.4.2. Relationship between male and female length of *G. oceanicus* females in stage 1 with stage 4 eggs transplanted for unsuccessful precopulas (r = 0.306). The diagonal broken line indicates equal lengths for males and females.

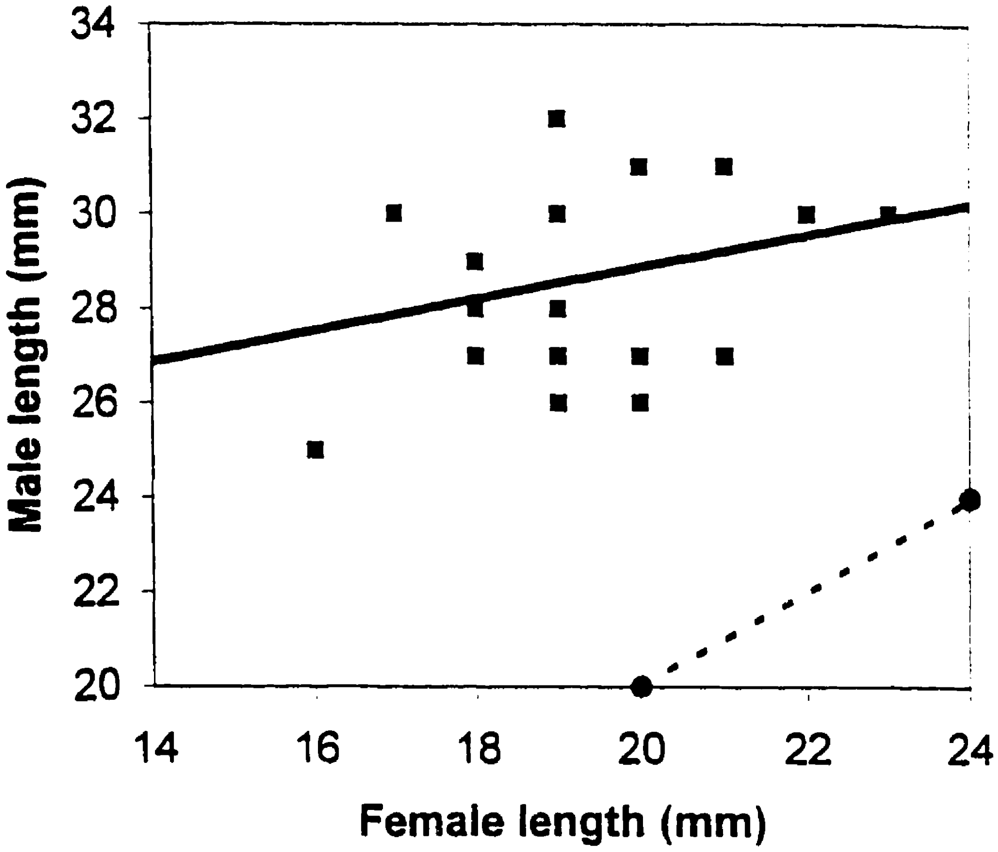


Figure 3.4.3. Relationship between male and female length of *G. oceanicus* females in stage 4 with eggs removed that successfully formed precopulas (r = 0.386). The diagonal broken line indicates equal lengths for males and females.

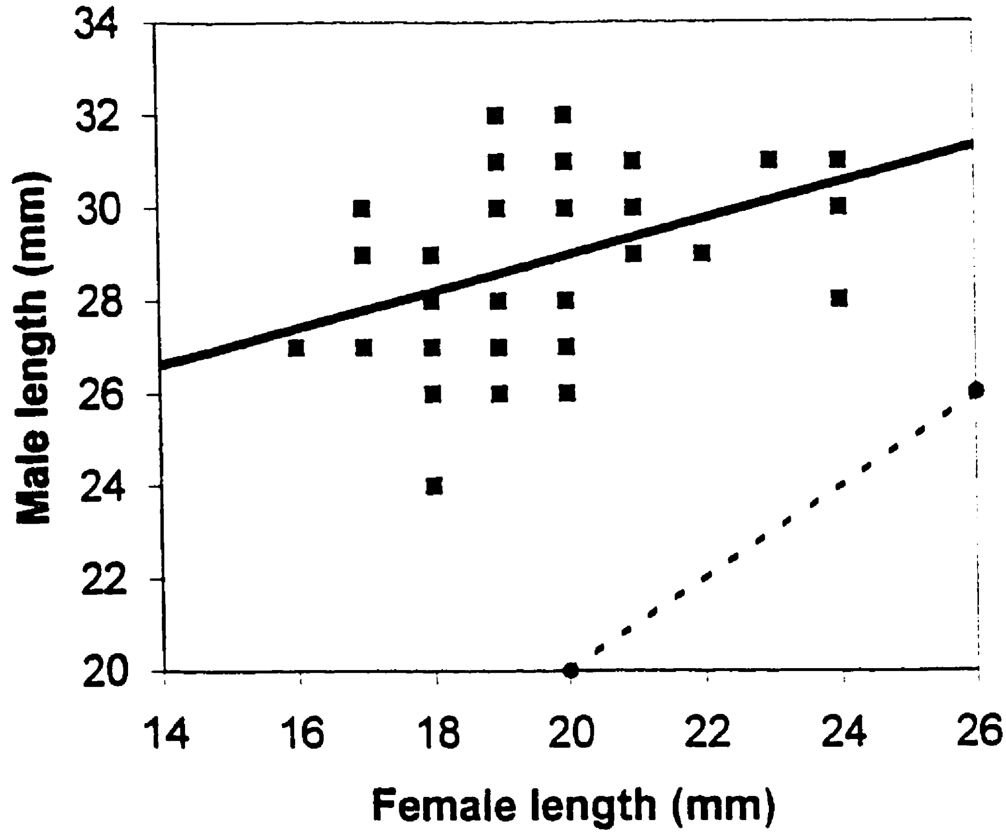
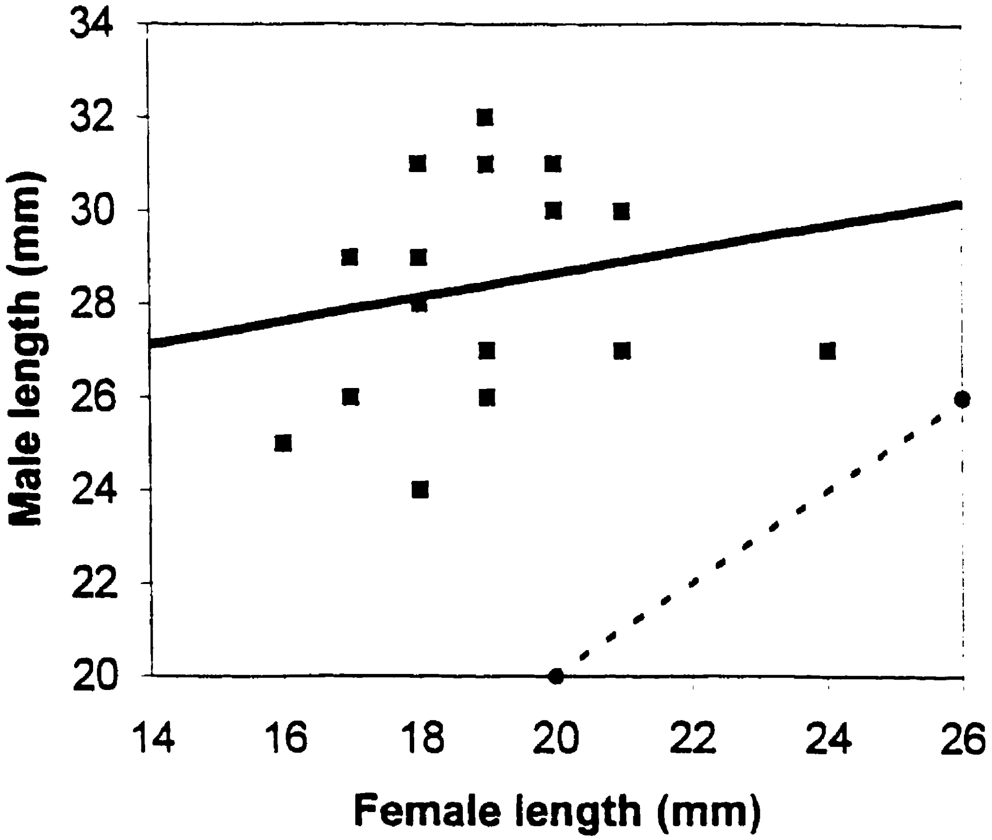


Figure 3.4.4. Relationship between male and female length of *G. oceanicus* females in stage 4 with eggs removed that successfully formed precopulas (r = 0.204). The diagonal broken line indicates equal lengths for males and females.



0.433, $\alpha = 0.05$) but there was no significant difference between the sizes of the males and females.

The general pattern of precopula formation behaviours for a successful precopula in *G. oceanicus* stage 1 egg manipulation females (Figure 3.4.5) consisted firstly of the male's gnathopods contacting the female's back; then the male's antennae touched the female's side; next either the male's gnathopods or antennae contacted the female's sides; and then the male's gnathopods touched the female's back.

The general pattern of precopula formation behaviours for an unsuccessful precopula in *G. oceanicus* stage 1 egg manipulation females (Figure 3.4.6) consisted of the male's gnathopods either contacting the female's side or back; the next contact is the male's antennae touching the female's side; then the male performed tail beating on the female; next the male's gnathopods touch the female's side; and then the male's antennae contacted the female's back.

The general pattern of precopula formation behaviours for a successful precopula in *G. oceanicus* stage 4 egg manipulation females (Figure 3.4.7) consisted of first the male's gnathopods contacting the female's back; next the male's antennae touched the female's side; the male's antennae touching the female's side is again performed as the third contact; and then either the male's gnathopods or antennae contacted the female's sides.

Figure 3.4.5. The frequency and sequence of precopula formation behaviours initiated by males during successful precopulas with *G. oceanicus* females in stage 1 with stage 4 eggs transplanted. The sequence of precopula formation behaviours from first to last is in ascending order. Legend found with Figure 3.1.1.

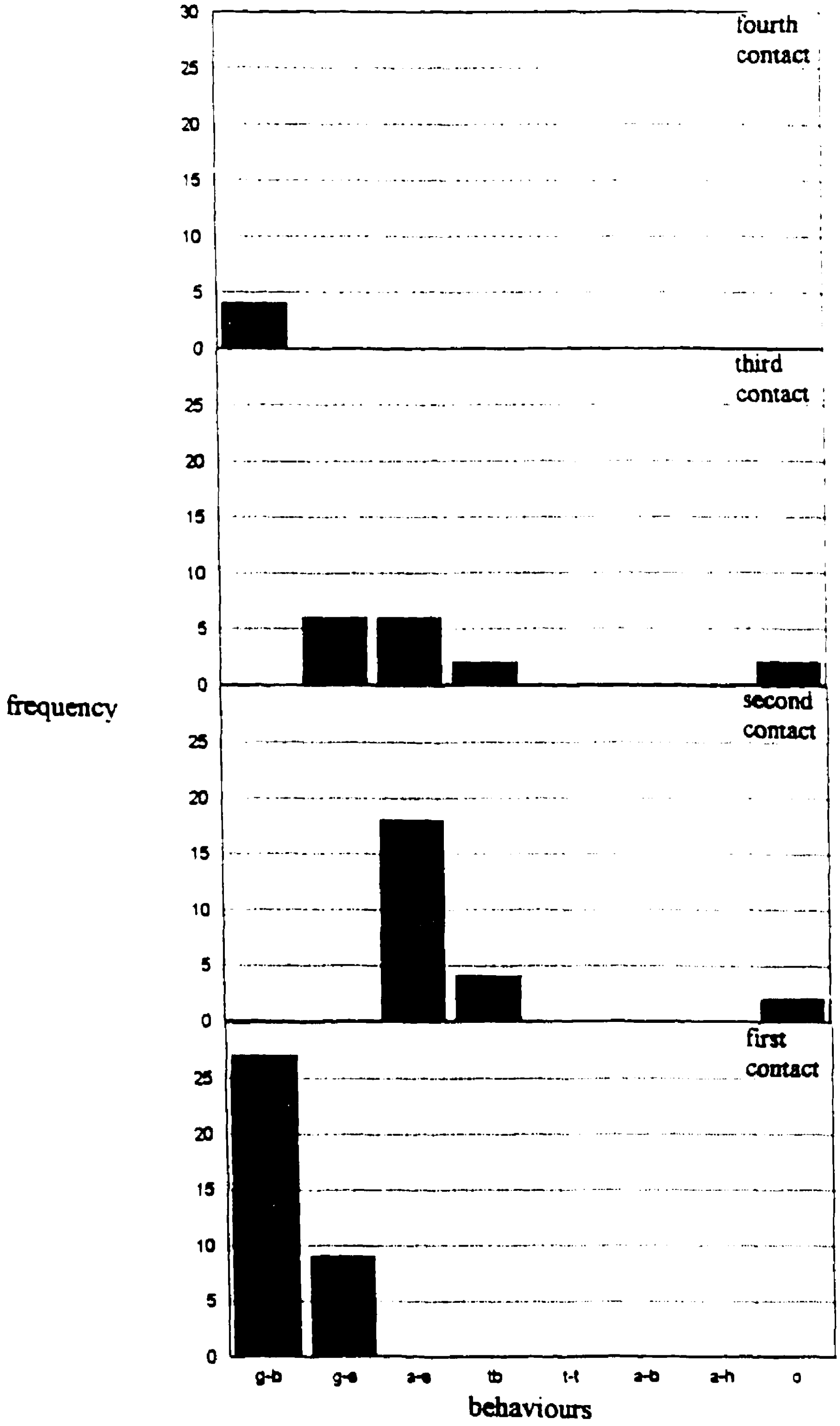


Figure 3.4.6. The frequency and sequence of precopula formation behaviours initiated by males during unsuccessful precopulas with *G. oceanicus* females in stage 1 with stage 4 eggs transplanted. The sequence of precopula formation behaviours from first to last is in ascending order. Legend found with Figure 3.1.1.

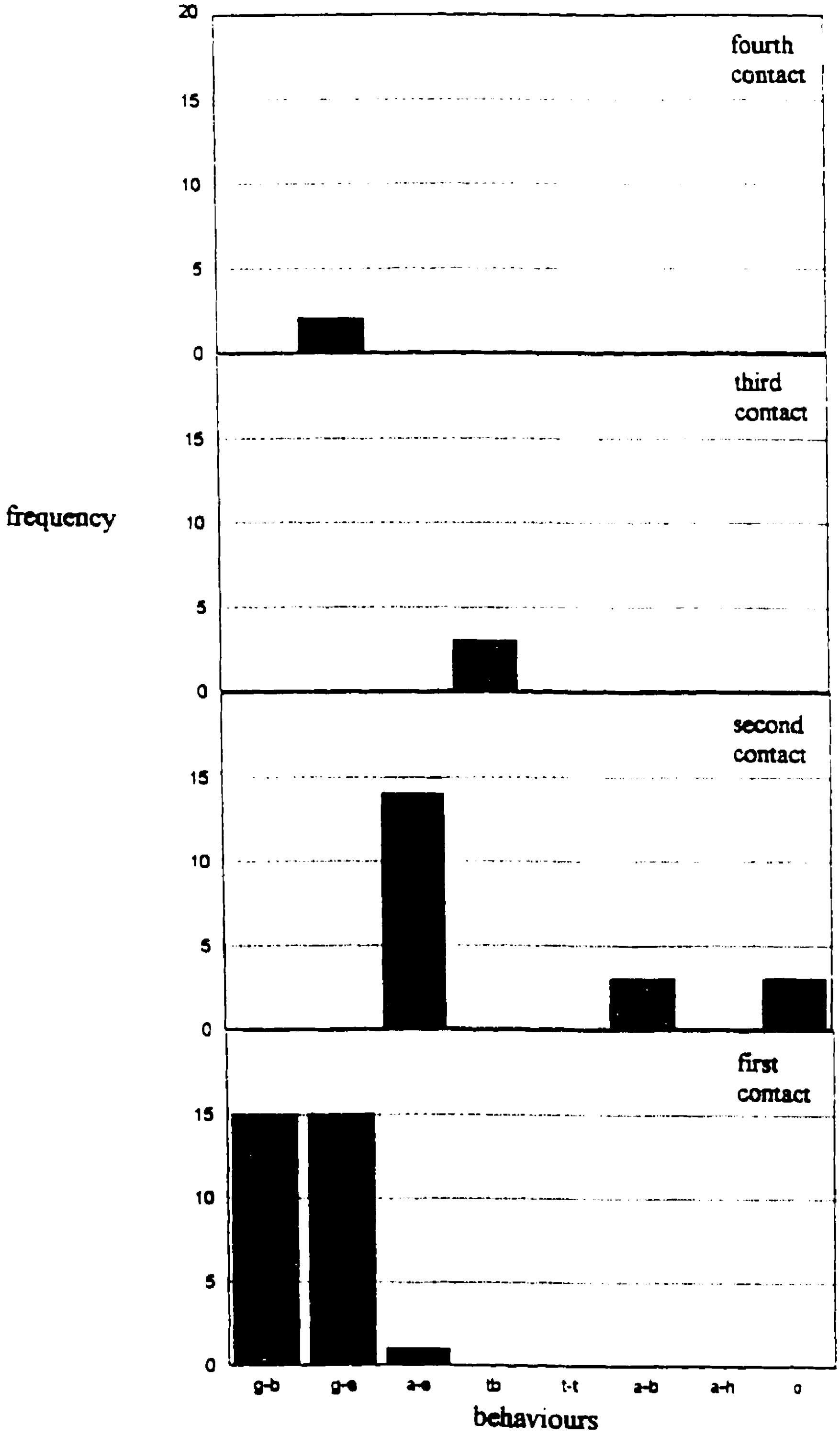
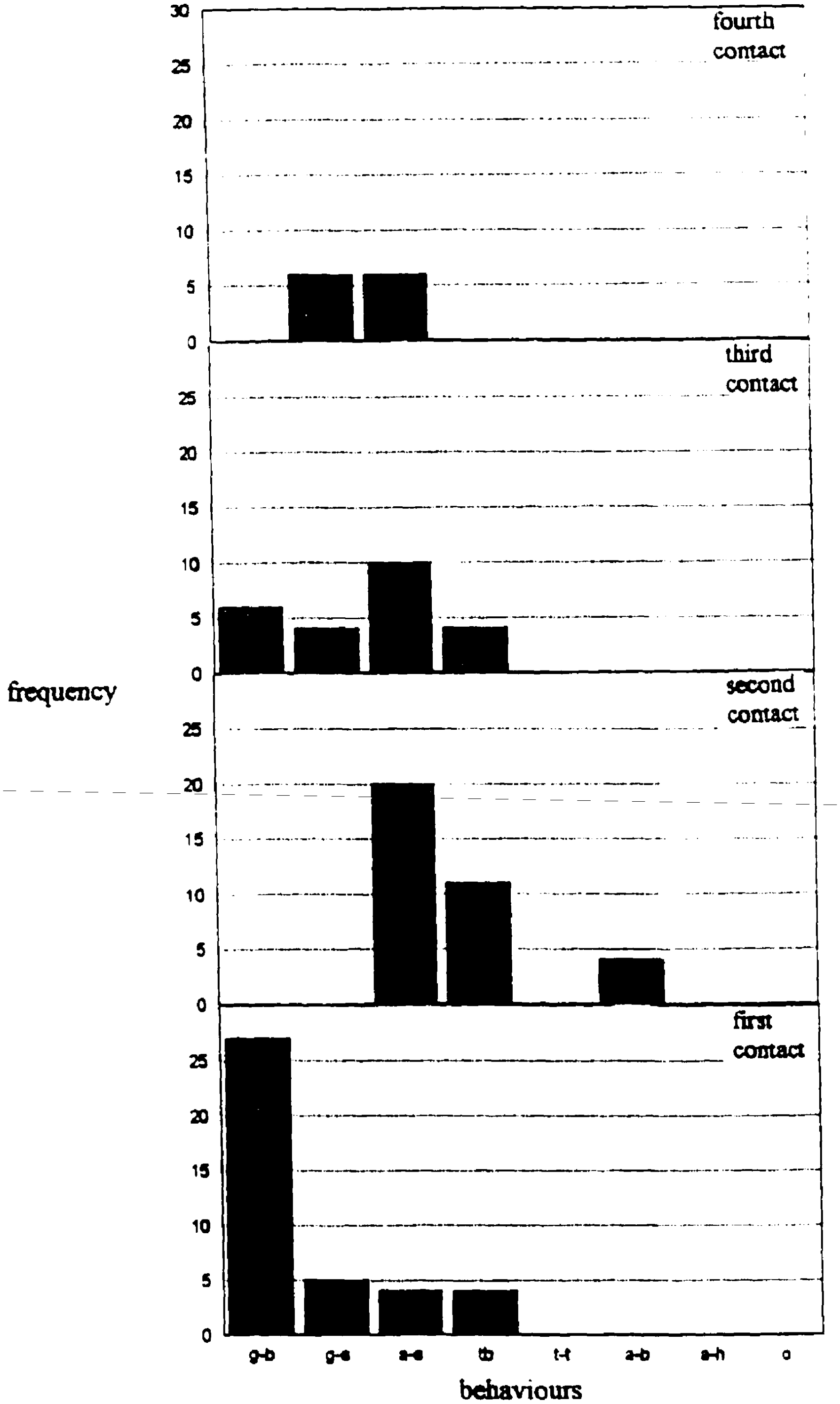
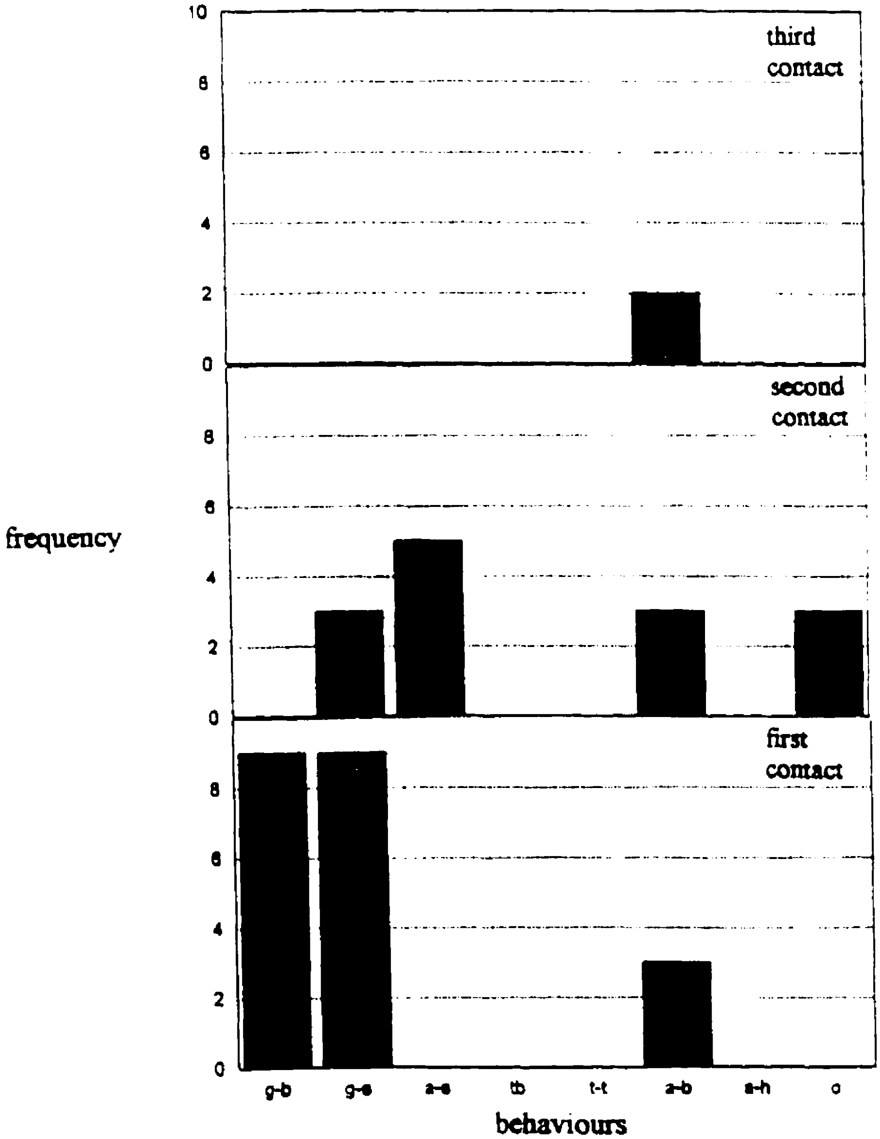


Figure 3.4.7. The frequency and sequence of precopula formation behaviours initiated by males during successful precopulas with *G. oceanicus* females in stage 4 with eggs removed. The sequence of precopula formation behaviours from first to last is in ascending order. Legend found with Figure 3.1.1.



The general pattern of precopula formation behaviours for an unsuccessful precopula in *G. oceanicus* stage 4 egg manipulation females (Figure 3.4.8) consisted of first the male's gnathopods contacting either the female's sides or back; then the male's antennae touched the female's side; and next the male's antennae touched the female's back.

Figure 3.4.8. The frequency and sequence of precopula formation behaviours initiated by males during unsuccessful precopulas with *G. oceanicus* females in stage 4 with eggs removed. The sequence of precopula formation behaviours from first to last is in ascending order. Legend found with Figure 3.1.1.



4.0 DISCUSSION

4.1 NORMAL BEHAVIOURS OF *Gammarus* SPECIES

4.1.1 *Gammarus setosus*

G. setosus was not previously studied but there are similarities and differences between this species and the four species (*G. oceanicus*, *G. duebeni*, *G. lawrencianus*, and *G. obtusatus*) already studied. Times to form successful precopulas and the percentages of animals that formed successful precopulas in *G. setosus* did not differ significantly in the morning and afternoon trials. This was also the finding for the other four species previously investigated.

Egg stages 3 and 4, which are close to the production of the next batch of eggs, have the highest numbers of females that formed successful precopulas indicating that the female is close to moulting and oviposition. In all 5 species there was a positive correlation between the development of the embryos in the brood pouch and the oogonia enlargement and development in the ovary, as was found by Dunham (1986) and Steele & Steele (1969). Males of these five species can determine how close a female is to her next moult which indicates how soon she will produce her next batch of eggs that can be fertilized.

Female choice was evident in *G. setosus*. Sometimes the females resisted precopula formation by flexing and kicking rapidly and escaping from the male's grasp. This was also the case for *G. oceanicus*, *G. duebeni*, *G. lawrencianus*, and *G. obtusatus* (Miller, 1995). Hatcher & Dunn (1997) observed female resistance in *G. duebeni*. Dick & Elwood (1989) and Ward (1984) observed female resistance in *Gammarus pulex*. Robinson & Doyle (1985) and Hunte, Myers & Doyle (1985) observed female resistance in *G. lawrencianus*, and Borowsky & Borowsky (1987) observed female resistance in *Gammarus palustris*.

4.1.2 SPECIES COMPARISONS

Only general descriptions of behaviours that occur during male assessment of females are reported in the literature of precopula formations in *Gammarus* species. The male is described as holding the female, he touches the female's dorsal surface with his abdomen and also makes contact with his pleopods. The male may, after forming a precopula, stroke the female's sides with his gnathopods, which has been suggested as behaviour to pacify the female and is not considered a reproductive behaviour. This is described for several species of *Gammarus* - *G. pulex* (Dick & Elwood, 1989a & 1989b; Birkhead & Clarkson, 1980), *G. lawrencianus* (Dunham & Hurshman, 1991; Dunham & Hurshman, 1990; Dunham, Hurshman & Gavin, 1989; Dunham, 1986; Dunham, Alexander & Hurshman, 1986), *G. duebeni* (Hartnoll & Smith, 1978) and *G. palustris* (Borowsky & Borowsky, 1987). The present study describes in detail the sequence of behaviours involved in successful and unsuccessful precopula formations to provide a more complete picture of the precopula formation behaviours for the five species studied - *G. setosus*, *G. oceanicus*, *G. duebeni*, *G.lawrencianus*, and *G. obtusatus*. Previous studies have not described the precopula formation behaviours in the detail provided in this study. Previous studies have also not compared average times to form precopulas, the percentage of animals that form precopulas, or compared the detailed sequence of precopula formation behaviours between species of *Gammarus*.

The five species studied are morphologically diverse, but do have some resemblance to each other in certain respects. *G. setosus* and *G. oceanicus* are the most closely related morphologically of these five species. The main difference occurs with the setae on the first peduncular segment of the first set of antennae and the length of the peraeopod 7 setae. *G. duebeni* and *G. lawrencianus* are morphologically different from one another and from both *G. setosus* and *G. oceanicus*. The setae on the first peduncular segment of the first set of antennae and the peraeopod 7 setae of *G. duebeni* are somewhat similar to that of *G. oceanicus* and *G. setosus*, whereas the setae of these particular appendages in *G. lawrencianus* are less similar to *G. oceanicus* and *G. lawrencianus*. Also, *G. setosus*, *G. oceanicus*, and *G. duebeni* have a similar size range. However, *G. lawrencianus* is considerably smaller than these three species (Bousfield, 1973).

G. obtusatus is very different morphologically from the other four species. It has been classified, at different times, as *Marinogammarus obtusatus*, *Eulimnogammarus* *obtusatus*, and *Gammarus obtusatus*. The main morphological difference that classifies *G. obtusatus* as either *M. obtusatus* or *E. obtusatus* is its inner ramus of uropod 3 which is very small being less than one third or one half the length of its outer ramus. This compares with the inner ramus of uropod 3 being at least one third or one half the length of the outer ramus in other species of *Gammarus*. The relative sizes of the two sets of gnathopods are also reversed; the anterior set is larger than the posterior set (Lincoln, 1979; Bousfield, 1973).

The sequences of successful precopula formation behaviours for all five species include as the first contact the male's gnathopods contacting the female's back and as the second contact the male's antennae contacting the female's sides. The third contact includes the male's antennae contacting the female's side in all species except *G. obtusatus*. *G. oceanicus* and *G. duebeni* have the most similar sequence of precopula formation behaviours whereas *G. obtusatus* is the most different from the other four species. One possible explanation for the differences between the sequences of successful precopula formation behaviours in *G. obtusatus* is its morphological distinctness from the other four species.

The sequence of unsuccessful precopula formation behaviours includes the male's gnathopods contacting the female's back as the first contact for all five species. The sequences of contacts are quite different for the five species starting with the second contact behaviour.

G. obtusatus is significantly different in all combinations from the other four species for both average times and percentages of precopulas formed, except for the average times between *G. obtusatus* and *G. duebeni*. Again, a possible explanation for the differences between the four species and *G. obtusatus* could be the extreme differences in their morphologies.

Either one or both of average times or percentage of precopulas formed differed for most of the rest of the species comparisons. However, the comparison between *G. oceanicus* and *G. lawrencianus* indicated there were no differences in either the average times or percentages. Overall, the differences in sequences of precopula formation behaviours, average times to form precopulas, and percentages of animals that form precopulas show there are significant differences among these five species. This concurs with the differences in the morphologies of these species.

4.1.3.INTERSPECIFIC PRECOPULAS

There were no interspecific precopulas formed or attempted between *G. setosus* and *G. oceanicus*. Kolding (1986) suggested that *G. oceanicus* should not form any interspecific precopulas with smaller species of *Gammarus* because of premating isolating mechanisms of size since this species is usually larger than other species. However, this does not hold true for this combination of *G. oceanicus* and *G. setosus* since they are of the same relative size. These species can occur together on the same beaches with overlapping distributions but *G. setosus* is found slightly higher on the shore (Steele & Steele, 1974). No interspecific pairs were observed in the natural population (Miller, unpublished observations) These species may be able to recognize each other even without contact.

There were no interspecific precopulas formed between the four previously studied species, with the exception of *G. oceanicus* and *G. duebeni*. Four interspecific precopulas were formed with these two species (Miller, 1995); however, Kolding (1986) found that these two species did not form any interspecific precopulas with one another.

4.1.4 SIZE ASSORTATIVE MATING

Size assortative mating occurred in *G. setosus*. The male/female ratio was 1.63 showing that the males were approximately 60% larger than the females they have formed precopulas with. This is higher than the ratios recorded for *G. pulex* of 1.30 to 1.40 (Ward, 1988, Birkhead & Clarkson, 1980). This ratio is also higher than any found for the other 4 species - *G. lawrencianus* (1.38), *G. duebeni* (1.45), *G. oceanicus* (1.47) and *G. obtusatus* (1.30) (Miller, 1995). This means that *G. setosus* males choose smaller females than the other four species.

Larger females produce larger numbers of eggs than smaller females (Naylor & Adams, 1987; Adams & Greenwood, 1983; Birkhead & Clarkson, 1980). Therefore it is expected that males will choose larger females. However, this is not the case with *G. setosus* since the males are 60% larger than the females. Carrying females in precopula puts constraints on the male's swimming ability, so there is a trade-off between maximizing the numbers of eggs fertilized and maximizing swimming ability (Adams & Greenwood, 1983). *G. setosus* may not be as strong a swimmer as the other four species studied, or just may choose to maximize swimming ability more than the number of eggs fertilized.

Ratios can vary up to a ratio of 1.50 for various species (Sutcliffe, 1993b; Adams & Greenwood, 1987). Size assortative mating has been found in numerous species of *Gammarus* - *G. pulex* (Ward & Porter, 1993; Elwood & Dick, 1990; Ward, 1988; Elwood, Gibson & Neil, 1987; Adams & Greenwood, 1985; Greenwood & Adams, 1984; Thompson & Moule, 1983; Adams & Greenwood, 1983; Birkhead & Clarkson, 1980), *G. duebeni* (Hatcher & Dunn, 1997; Naylor & Adams, 1987; Ward, 1985), *Gammarus pseudolimnaeus* (Mathis & Hoback, 1997), *G. oceanicus*, *G. palustris* and *G. obtusatus* (Adams & Greenwood, 1987).

4.2 TAKEOVERS

Overall, few studies have been conducted with respect to takeovers. The percentage of successful takeovers in *G. oceanicus* is low (2.10%), compared to results of takeover experiments for other species of *Gammarus*. *G. pulex* is the most commonly studied species with respect to takeovers. Ward (1983) found 9% successful takeovers for *G. pulex* while Birkhead & Clarkson (1980) found 10.7% successful takeovers for *G. pulex* and Elwood, Gibson & Neil (1987) found 9.6% successful takeovers, also for

G. pulex. In all three of these experiments, no takeovers were actually observed as the animals were not continuously observed but were checked after a few hours or days. However, in the experiments of Elwood, Gibson & Neil (1987) the setups were observed for a continuous period of 10 minutes before performing a final check after two hours to determine if takeovers had occurred. Again no takeovers were directly observed. It may therefore be that some pairs may have separated of their own accord before the female moults and the second male (intruder) may form a precopula with the now separated female in the normal fashion (Birkhead & Clarkson, 1980). The percentages recorded as successful takeovers may not be takeovers at all; thus the percentages may be rather high estimates of possible successful takeovers. The differences may also be partly explained by the fact that all of these takeover experiments were carried out on a different species (*G. pulex*) than the *G. oceanicus* of this experiment.

Dick & Elwood (1990) also carried out takeover experiments on *G. pulex* using the same 10 minute observations as in the takeover experiments with *G. oceanicus*. No takeovers were observed when a pair, with the female in various egg stages, was placed with an extra male (intruder) of varying sizes for *G. pulex*. However the same setup with a newly moulted female had 14.8% takeovers (Dick & Elwood, 1990). These results are comparable to the *G. oceanicus* takeover experiments of this paper since the setup was the same and three takeovers were actually observed. The higher percentages are likely due to differences between the two species. All of the three successful takeovers for *G. oceanicus* occurred with females in stage 4 that corresponds closely to the newly moulted females in the *G. pulex* experiments of Dick & Elwood (1990). The majority of unsuccessful takeovers for *G. oceanicus* were also of stage 4 females. Females in egg stage 4 are the closest to producing their next batch of eggs for fertilization (Dunham, 1986; Steele & Steele, 1969) therefore it was expected that successful and unsuccessful takeovers would occur the most with stage 4 females.

No significant difference was found for *G. oceanicus* in average times spent attempting unsuccessful takeovers between intruders larger than defenders and intruders smaller than defenders which was also found by Dick & Elwood (1990) for *G. pulex* unsuccessful takeover attempts. The size of the intruder, in relation to the defender size, does not make any difference to the amount of time attempting unsuccessful takeovers.

The ratio of intruder length to defender length of *G. oceanicus* was highest for successful takeovers (1.12), compared to an unsuccessful takeover ratio of 1.09 and a ratio of 1.05 when no attempts were made. These results suggest that in order for takeovers to occur in *G. oceanicus*, there must be a ratio higher than 1.09. Iribarne, Fernandez & Armstrong (1996) found that *E. oclairi* must have a minimum ratio of 1.10 for takeovers to occur, while Ward (1983) found a ratio of 1.11 for successful takeovers in *G. pulex* which corresponds to the ratio of successful takeovers for *G. oceanicus*. Intruding males of *G. oceanicus* must be 12% larger than defending males in order for takeovers to occur.

The correlations of intruder to defender size for both unsuccessful and no takeover attempts were low and there was no significant difference between the sizes of intruders and defenders of *G. oceanicus*. However, no analysis could be carried out for the successful takeovers since only three occurred. There may actually be a high positive correlation with a significant difference between intruder and defender sizes for successful takeovers, but a greater number of takeovers would have to be observed to determine this.

There was no significant difference between either the times spent attempting unsuccessful takeovers and female egg stage or female size in *G. oceanicus*. Males can assess female quality quickly and do not need increased time to determine if the female is suitable. This is advantageous for the intruding male since time attempting a takeover decreases the time the male has available to search for a female not already in precopula. A quick assessment of a paired female maximizes the time left to the intruder to find another female.

In general, the sequence of successful takeover behaviours for *G. oceanicus* differed from the sequence of unsuccessful takeover behaviours and the normal precopula formation behaviours of *G. oceanicus*. The main difference in the successful takeover behaviour sequence of contacts was the male engaging in tail beating of the female as the second contact. In normal successful precopula formation behaviours of *G. oceanicus* the second contact is the male's antennae contacting the female's side (Miller, 1995).

The sequence of unsuccessful takeover behaviours for *G. oceanicus* is quite different from the successful takeover behaviours since the intruder does engage in some contacts with the defender as well as with the female. Ward & Porter (1993), in a simulation study, suggest that if a female is already paired then the male (intruder) will assess the paired male (defender). They suggest this is behaviour by the defender to get between the intruder and the female to prevent contact between the two. As discussed earlier, in *G. oceanicus* the intruders were 12% larger than the defenders when successful takeovers occurred. The intruder may be assessing his size in relation to the defender, along with assessing the quality of the female, to determine if a successful takeover can occur. If the intruder determines the defender is too large in relation to his own size, or the female quality is not acceptable, then no takeovers will occur.

The most common first contact in unsuccessful takeovers is the intruder's gnathopods contacting the defender's back. The intruder also uses his gnathopods to contact the female's back as a first contact quite often. Borowsky (1984) observed in *G. palustris* that an intruder will use his smaller anterior gnathopods at the usual attachment points on the female and uses the larger posterior gnathopods to pry off the smaller anterior gnathopods of the defender while pushing the defender away from the female with his uropods. These observations agree with the first contact in successful takeovers in *G. oceanicus* but only partly agree with the first contact in unsuccessful takeovers since the most common first contact is the intruder's gnathopods contacting the defender's back. The intruder does attach to the female at the usual attachment points with his anterior gnathopods in *G. oceanicus*, but there was no actual observation of the use of the larger posterior gnathopods to pry off the defender's anterior gnathopods from the female or the pushing away of the defender by the intruder's uropods as in *G. palustris*.

Male-male competition is present in *G. oceanicus* in the form of takeovers. Numerous attempts occur but actual takeovers are rare. The majority of the defenders retained their female even if they were smaller than the intruder. Dick & Elwood (1990) suggest there is a positional advantage of the defender since the defender has a tighter grip on the female already in its possession than an intruder would on the same female which is supported by the results with *G. oceanicus*.

Female choice was also evident in *G. oceanicus* in successful or unsuccessful takeovers as the female remained passive and did not resist during any successful or unsuccessful takeovers. However, Elwood, Gibson & Neil (1987) did observe female resistance (choice) in *G. pulex*. *G. oceanicus* females do sometimes resist during normal precopula formation (Miller, 1995).

4.3 SIMULTANEOUS ASSESSMENTS

The percentage of successful simultaneous assessments in *G. oceanicus* is low (1.36%) compared to results of simultaneous assessment experiments for other species. Dick & Elwood (1989a) found 5.3% successful simultaneous assessments for *G. pulex* and 25.3% unsuccessful simultaneous assessments, which is higher than the percentages of 1.36% for successful simultaneous assessments and 10.88% for unsuccessful simultaneous assessments in *G. oceanicus*. Dick (1992) also found 9.3% successful simultaneous assessments in a later experiment with *G. pulex*. The differences in percentages could be attributed to differences between species.

These experiments with *G. pulex* found that the majority of males chose females that were closer to moult and larger - 85.7% (6 out of 7) (Dick, 1992) and 100% (Dick & Elwood, 1989a). Neither of these experiments with *G. pulex* tried to separate the two main determinants of female quality - time until next moult and female size. These two experiments, both with *G. pulex*, have been the only experiments conducted on simultaneous assessments with species of *Gammarus*.

The two successful simultaneous assessments in *G. oceanicus* both occurred with same stage females, where one male kept the original female, which was smaller than the extra female, and the other male switched and took the extra female, which was larger than the original female. These females were both in stage 4 and close to moult, therefore even though one male kept a smaller female, his choice was still sound since the female he was originally holding was close to moult. The male should also want to choose a larger female since larger females will produce a greater number of eggs (Adams, Watt, Naylor & Greenwood, 1989; Birkhead & Clarkson, 1980), especially since the two females present were both in stage 4. However, Elwood, Gibson & Neil

(1987) found that although larger females offer a greater reproductive success due to the greater number of eggs produced, it comes at a cost to the male of carrying a larger female. When females close to moult are encountered, size may not play as important a role as when females are further from moult.

In the same stage female trials for *G. oceanicus* there was no significant difference between the number of larger extra females or smaller extra females that males attempted simultaneous assessments with. In the same size female trials for *G. oceanicus* there was no significant difference between the number of extra females closer to moult or further from moult that males attempted simultaneous assessments with. This indicates that males will sometimes attempt to grab extra females that they encounter regardless of the quality of the extra female. *G. oceanicus* males grab and manipulate females which allows proper assessment of female quality. This supports Dick & Elwood (1989a) who suggest from their study of simultaneous assessments in *G. pulex* that initial touching without grabbing and manipulations of the females does not allow males to properly assess female quality.

There was no significant difference between the average times of unsuccessful simultaneous assessments in *G. oceanicus* for same stage females and same size females which means that males can assess both of these indicators of female quality in the same time period.

The sequence of successful simultaneous assessment behaviours for *G.oceanicus* differs from the sequence of unsuccessful simultaneous assessment behaviours, both same stage and same size, and the normal precopula formation behaviours of *G. oceanicus*. The main difference in the successful simultaneous assessment behaviour sequence of contacts is the male's gnathopods touching the extra female's side as the third contact. In normal precopula formation behaviours of *G. oceanicus* for successful precopulas, the third contact is the male's antennae contacting the female's side or the male's tail contacting the female's tail (Miller, 1995).

The main difference in the sequence of unsuccessful simultaneous assessment behaviours of same stage females by *G. oceanicus* from the normal precopula formation behaviours for unsuccessful precopulas was the first contact. The male's antennae contacts the extra female's back whereas in the normal precopula formation behaviours of *G. oceanicus* the first contact is the male's gnathopods touching the female's back (Miller, 1995). This first contact is also different from the successful simultaneous assessments and unsuccessful simultaneous assessments of same size females, which is also the male's gnathopods touching the female's back. The main difference in the unsuccessful simultaneous assessment behaviour sequence of same size females by *G. oceanicus* from the normal precopula formation behaviours for unsuccessful precopulas was the third contact. The male's antennae touched the extra female's side or the male performed tail beating on the extra female whereas in the normal precopula formation behaviours of *G. oceanicus* the third contact also is the male's antennae touching the female's side but it also includes the male's tail touching the female's tail (Miller, 1995). The extra behaviour of tail beating of the extra female is another difference between unsuccessful simultaneous assessments with same size females.

Females of *G. oceanicus*, both the original and extra female's, did not resist simultaneous assessments and remained passive during the successful simultaneous assessments. However, three extra females resisted unsuccessful simultaneous assessments but all of the original females remained passive during the unsuccessful simultaneous assessments. Female choice is evident in *G. oceanicus* in simultaneous assessments by the extra female. This corresponds to the normal precopula formation behaviours for *G. oceanicus* where females sometimes resisted when grabbed by a male attempting precopula formation (Miller, 1995). Dick & Elwood (1989) also observed the extra female resisting with rapid kicking motions in *G. pulex* in simultaneous assessment experiments.

4.4 EGG MANIPULATIONS

Precopulas were formed in *G. oceanicus* with both stage 1 females with stage 4 eggs transplanted (stage 1 egg manipulation females) and stage 4 females with eggs removed (stage 4 egg manipulation females). There was no significant difference in the times forming successful precopulas or unsuccessful precopulas between the stage 1 egg manipulation females and stage 4 egg manipulation females of *G. oceanicus*. This suggests that since the stage 4 eggs are transplanted into the stage 1 females, it makes them as attractive as stage 4 females without eggs. There was no significant difference between the percentage of pairs that formed successful precopulas in *G. oceanicus* for stage 1 egg manipulation females and stage 4 egg manipulation females. But there was a significant difference between the percentage of pairs that attempted unsuccessful precopulas for stage 1 egg manipulation females and stage 4 egg manipulation females. This suggests that stage 1 egg manipulation females, who had a higher percentage of unsuccessful precopulas than stage 4 egg manipulation females, initially seemed more attractive to the male, but other factors than just brood pouch contents (eggs) are also involved in male's decisions on female quality. Dunham (1986), from his work with *G. lawrencianus*, also suggests that other factors influence the male's decision about female quality in addition to the female's brood pouch contents. Dunham's (1986) experiment is the only one conducted with respect to egg manipulations in species of *Gammarus*.

There were significant differences between successful normal behaviour and successful egg manipulation average times to form precopulas and percentages of animals that form precopulas for stage 1 and stage 4 females. However, there was no significant difference in stage 1 egg manipulation females and stage 1 normal behaviour females in the average times to form precopulas. The average times and percentages were higher for the normal behaviours than the egg manipulations, which indicates that males form fewer precopulas with the egg manipulation females. The brood pouch contents may indicate to the males that the female is in a different stage but it is possible that there may be other factors that influence the male's decision about the female's quality. The male may be receiving conflicting information and this is possibly the reason why the percentages of precopula formation were lower with egg manipulation females than with normal females.

There was no significant difference between number of successful or unsuccessful precopulas between stage 1 egg manipulation females and stage 4 egg manipulation females which supports the theory that both brood pouch contents and other factors play a role in male's assessment of female quality.

The ratios of male to female length in successful and unsuccessful precopulas with stage 1 egg manipulation females and stage 4 egg manipulation females were all equal to or above the ratio found in normal *G. oceanicus* (1.47) (Miller, 1995). Size assortative mating occurs with the egg manipulation females as with normal females.

In general, the sequence of successful and unsuccessful precopula formation behaviours for *G. oceanicus* stage 1 egg manipulation females and stage 4 egg manipulation females differ from the sequence of behaviours of the normal *G. oceanicus*. The first contact in normal precopula formations is the male's gnathopods contacting the female's back (Miller, 1995) which is also the case in both the successful precopulas with stage 1 egg manipulation females and stage 4 egg manipulation females. It is also the case for both the stage 1 and stage 4 egg manipulation unsuccessful precopulas; however, the males also used their gnathopods to contact the female's sides as the first contact. This is the same for normal *G. oceanicus* unsuccessful precopulas.

The differences arise with the third contact. In normal *G. oceanicus* successful precopulas, the third contact is either the male's antennae contacting the female's side or the male's tail contacting the female's tail. For stage 1 egg manipulation females forming successful precopulas, the third contact is either the male's gnathopods or antennae touching the female's sides and for stage 4 egg manipulation females forming successful precopulas, the third contact is the male's antennae contacting the female's side.

In normal *G. oceanicus* unsuccessful precopulas, the third contact is other. For stage 1 egg manipulation females unsuccessful precopulas, the third contact consists of the male performing tail beating on the female, and for stage 4 egg manipulation females unsuccessful precopulas, the third contact is the male's antennae touching the female's back.

The differences in the sequences of precopula formation behaviours for egg manipulation females and normal females indicate the male must use a different sequence to properly determine the female's quality because of the conflicting information of the brood pouch contents and other factors.

Females of both stage 1 with stage 4 eggs transplanted and stage 4 with eggs removed sometimes resisted precopula formation. Female choice is evident in both the successful and unsuccessful precopulas. This corresponds to the results of normal *G. oceanicus* precopulas where females sometimes resist precopula formation (Miller, 1995).

5.0 SUMMARY

1. *G. setosus* males chose to form precopulas more often with females that were closer to producing their next batch of eggs to be fertilized. This indicates that males use brood pouch contents as a factor to determine a female's quality.
2. Sexual selection, in the form of female choice, was present in *G. setosus*.
3. There were similarities in the first contact in the sequence of precopula formation behaviours in successful and unsuccessful precopulas for all five species studied. However, the overall sequence differs among the species for both successful and unsuccessful precopulas indicating that the males of each species must use a different sequence to determine the female's quality.
4. There were significant differences among average times and percentages of precopulas formed for all five species, which corresponded to the differences in morphology of the species.
5. *G. obtusatus* was the most different in the sequences of precopula formation behaviours, average times and precopulas formed from the other four species. This may be explained by the extreme differences in morphology between *G. obtusatus* and the other four species.
6. No interspecific precopulas were formed or attempted between *G. setosus* and *G. oceanicus*, although both are found on the same beaches with overlapping distributions. This indicated that these species might be able to recognize each other even without contact.

7. Size assortative mating occurred in *G. setosus*. The males of this species, however, chose slightly smaller females than the other four species studied. This may be a tradeoff between size of the female and the male's swimming ability.
8. Takeovers occurred in *G. oceanicus*, all with stage 4 females which were the closest to producing their next batch of eggs to be fertilized.
9. The size of the intruder, in relation to the defender, made no difference to average times attempting takeovers in *G. oceanicus*.
10. The sequences of precopula formation behaviours of successful and unsuccessful takeovers were different from each other and from the normal behaviour sequences for *G. oceanicus*.
11. Sexual selection, in the form of male-male competition as takeovers and female choice as passiveness, occurred in *G. oceanicus*.
12. Simultaneous assessments occurred in *G. oceanicus* with females that were both in the same egg stage - stage 4 which are the closest to producing their next batch of eggs to be fertilized.
13. There were no differences between the average times in unsuccessful simultaneous assessments for same size and same stage trials in *G. oceanicus* indicating males can assess both of these indicators of female quality within the same amount of time.
14. The sequences of precopula formation behaviours were different for successful and unsuccessful simultaneous assessments and were also different from the normal behaviour sequences for *G. oceanicus*.

15. Sexual selection, in the form of female choice, was evident in extra females during unsuccessful simultaneous assessments in *G. oceanicus*, where extra females sometimes kicked to escape from the male's grasp. The original females in unsuccessful simultaneous assessments and the extra and original females in successful simultaneous assessments remained passive, which is also a form of female choice.
16. There were no significant differences in the average times forming successful or unsuccessful precopulas between stage 1 egg manipulation females and stage 4 egg manipulation females. This indicated that stage 1 females with stage 4 eggs transplanted were as attractive as stage 4 females with eggs removed in *G. oceanicus*.
17. *G. oceanicus* males chose females based on other factors than just brood pouch contents when determining female quality.
18. The sequence of successful and unsuccessful precopula formation behaviours with egg manipulation females differed from the sequences of normal *G. oceanicus*. This indicated that the male must use a different sequence to properly determine the female's quality because of the conflicting information of the brood pouch contents and other factors.
19. Sexual selection, in the form of female choice, was present in the *G. oceanicus* egg manipulation females.

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