# Patterns of Reproduction in Three Species of Littoral Epibenthic Gammarid Amphipods, *Gammarus palustris*, *Gammarus mucronatus*, and *Melita nitida*

by

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# ABSTRACT

The patterns of reproduction of three Gammarid amphipods were studied. Gammarus palustris, Gammarus mucronatus, and Melita nitida were found sympatrically in the littoral zone of Jamaica Bay, New York. Although each species was not present in the littoral zone throughout the year, when all three species were present, their distributions were discrete; G. palustris was found primarily at the high tide mark, G. mucronatus at the mean tide mark and M. nitida at the low tide mark.

The number of juveniles per brood were shown to be least for Gammarus palustris, and greatest for Melita nitida. The lengths of the egg period (the time from ovulation to hatching) and the juvenile period (the time from hatching to juvenile emergence) were determined for the three species at 17° and 21° C. The length of the egg period was fixed for each species. It was longest for G. palustris and shortest for Gammarus mucronatus, at both temperatures. The length of the juvenile period was longest for G. palustris and shortest for M. nitida. Thus, as the typical habitat of the species gets higher in the intertidal zone, the number of juveniles decreases and the length of the juvenile period increases.

The effects of different food regimes, and the effects

of alternating periods of exposure and immersion on the time of juvenile emergence were also studied. It was found that the greater the variety of foods, the longer juveniles tended to remain in the marsupium, and that juveniles emerged at an increased rate a few minutes after presenting mussel meat to females previously fed only Ulva sp. or no food.

The mean day of emergence of broods of females subjected to alternating periods of exposure and immersion was no different from the mean day of emergence of broods kept constantly immersed. However, juvenile emergence was timed to periods of immersion within the day. The rate of emergence was one order of magnitude greater 15 minutes immediately after immersion than it was during the following 3.75 hours of immersion, and the latter rate was one order of magnitude greater than the rate of emergence during the next 8 hours of exposure. Thus, juveniles emerged at the greatest rate shortly after immersion. The relatively long and variable juvenile period of Gammarus palustris may be one factor that permits it to reproduce successfully at the high tide mark.

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The intertidal zone is a stressful environment, yet it is often densely populated by epibenthic macrofauna. Most of these species' immature stages are planktonic, and thus they avoid the stresses of wide fluctuations in temperature, salinity, and periods of exposure during their most vulnerable stage of development (Thorson, 1950; Newell, 1970). Gametes and larvae may be transported over great distances (Scheltema, 1971) but, at a specific stage in their development, the larvae metamorphose into adults, and spend the rest of their lives attached to or associated with the benthos (see Knight-Jones, 1953; Scheltema, 1961, for examples).

The reproductive pattern of the amphipods, one of the most successful epibenthic macrofaunal groups in the littoral zone, (Thorson, 1950; Green, 1968) is at the opposite pole, however. Instead of casting off their offspring at an immature stage, they retain them until the offspring resemble the adults both morphologically and behaviorally (Kaestner, 1970). The amphipods have evolved in the direction of parental care.

Wilson (1975) lists four types of environments which may select for parental care. One of them is the stressed physical environment. Extended care of young means that offspring are protected from the environment until such time as they are ready to meet its demands.

Parental care is not unique to the amphipods. Care of fertilized eggs is found in most crustacea and care of the young after hatching is found in several other crustacean groups as well: the Thermosbaenacea, Leptostraca, Ascothoracica, Ostracoda, and Cladocera (Kaestner, 1970). However, the patterns of parental care in the Peracarida, to which the amphipods belong, are certainly among the most well developed in the crustacea..

The marsupium, or brood pouch, of the female, is the main morphological feature that distinguishes the Peracarida from other crustacea. In the Gammaridea, one of the groups that occupies the littoral zone, the marsupium is composed of four pairs of oostegites, each oostegite attached to one of pereiopods 2-5 (Kaestner, 1970) (Figure 1). Each oostegite is a flat leaf with rather long setae fringing the edges. The setae overlap each other, and all the oostegites together form a basket through which water passes freely, and in which the eggs and juveniles are held loosely (Figure 2).

Several days before the female molts, it is picked up and carried about by a male. The male carries the female by holding onto the dorsal segments of the female's thorax with the gnathopods (Figure 3). This behavior is called precopulation. Within a few minutes after the female's molt, copulation occurs; the male introduces the sperm into the marsupium, and the female ovulates within a few minutes or hours,

Figure 1

Lateral view of male Gammarus mucronatus showing 1) long abundant sensory hairs on second antenna; 2) points between which the head and body lengths were measured; and 3) location of marsupium in the female (from Bousfield, 1973).

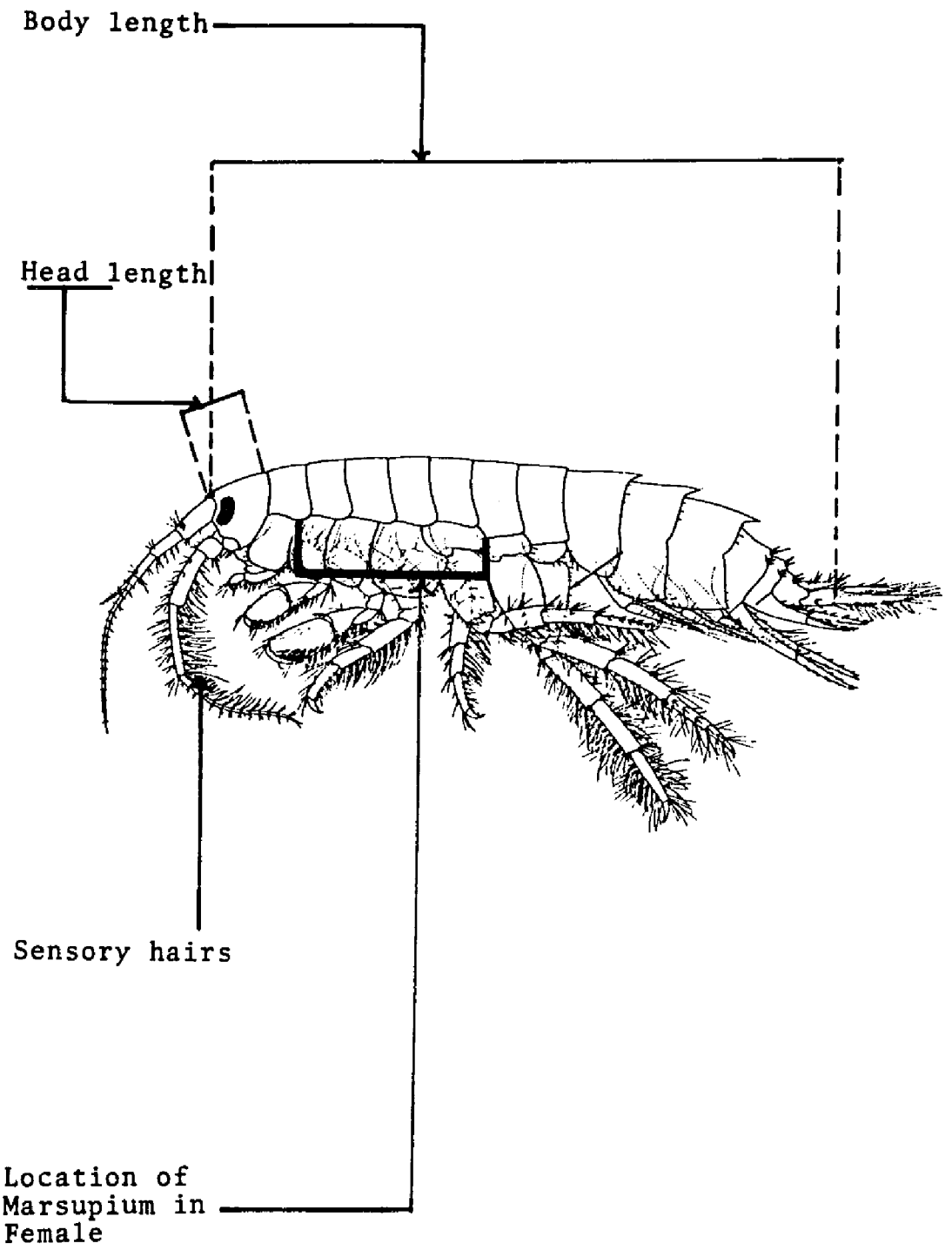

Body length
Head length
Sensory hairs
Location of
Marsupium in
Female

Figure 2

Ventral view of ovigerous Gammarus palustris female showing morphology of marsupium. Taken with a Scanning Electron Microscope, magnified 15 X.

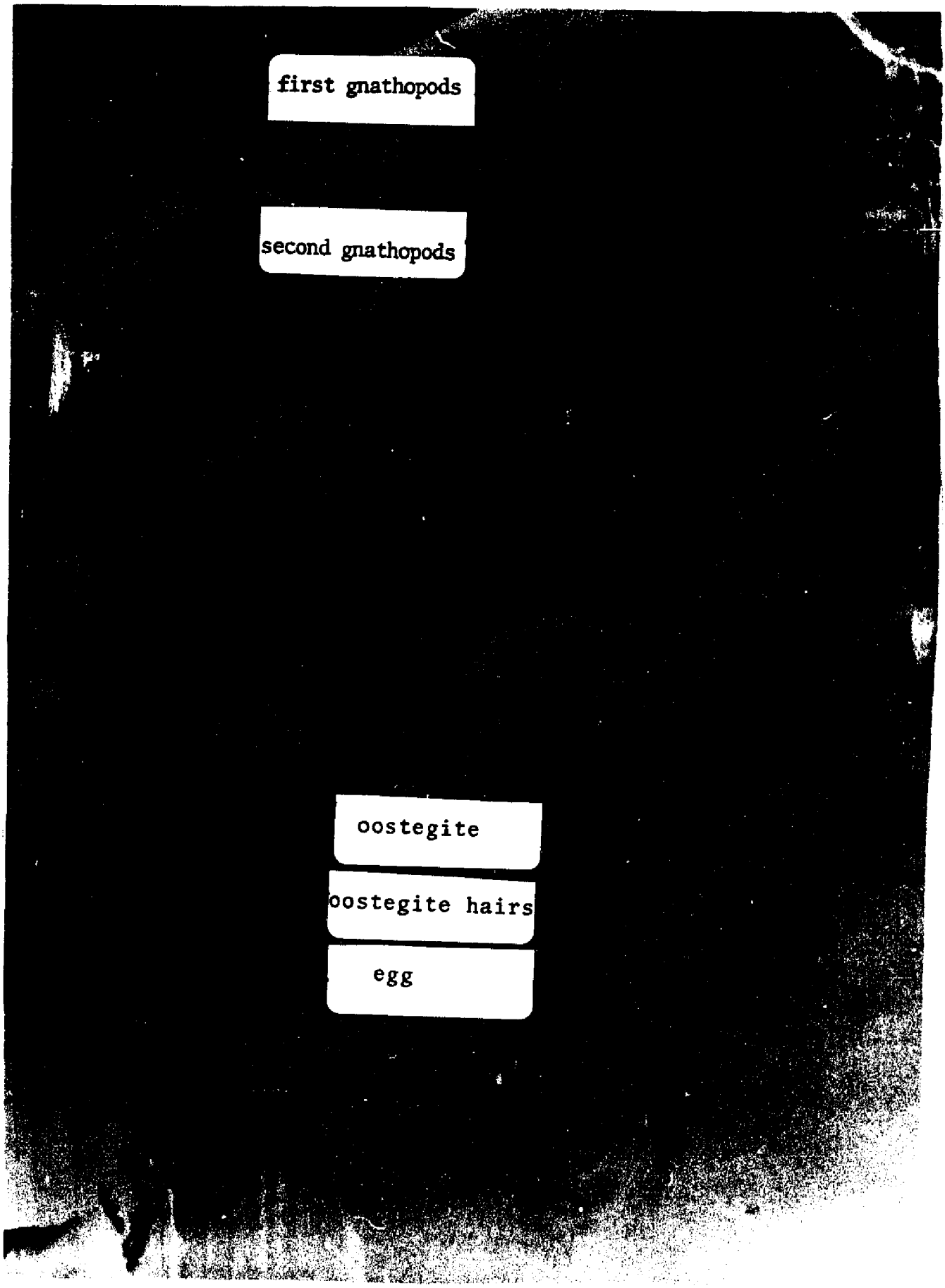
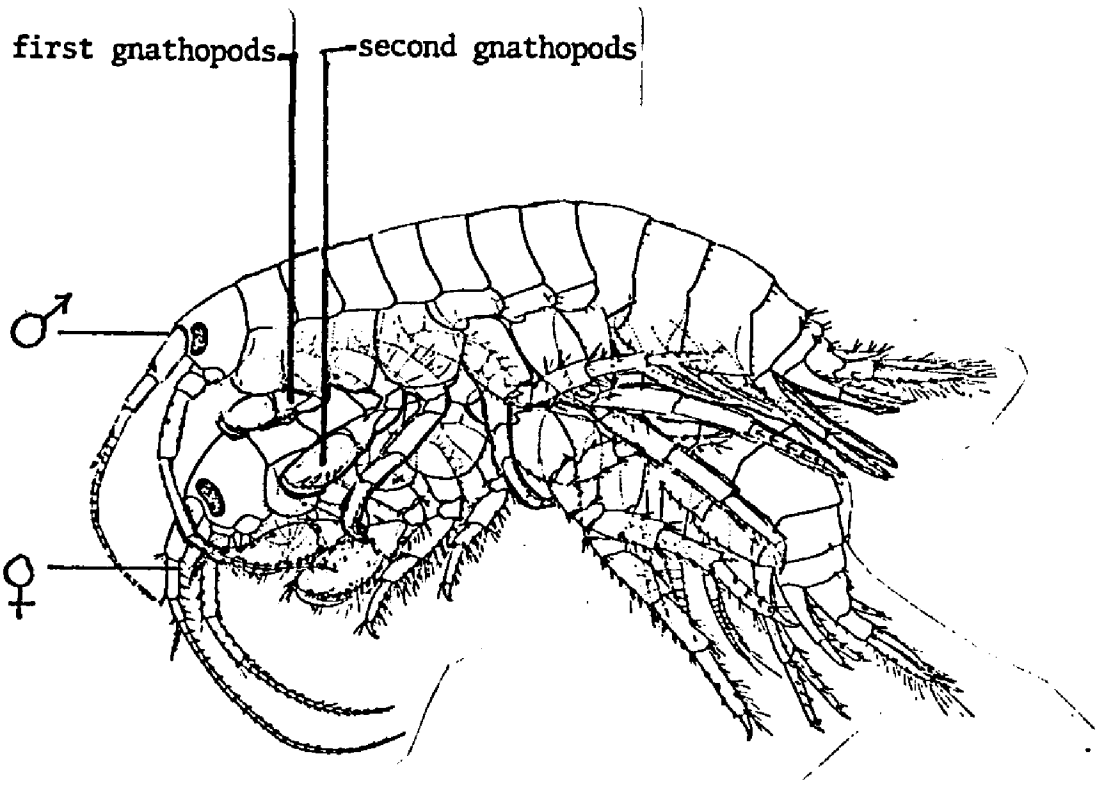

first gnathopods
second gnathopods
oostegite
oostegite hairs
egg

Figure 3

A male and female Gammarus palustris in precopulation (from Bousfield, 1973).


first gnathopods
second gnathopods
♂
♀

depending upon the species. Fertilization probably occurs in the marsupium. The eggs develop and hatch there, and the young emerge as miniature adults. Then the female molts again, copulates and ovulates. There is no terminal molt, and, once mature, a female may molt and produce one to ten broods, depending upon species. The females of Gammarus wilkitzkii and Gammarus setosus, which are confined to the Arctic, produce only one brood, while females of Gammarus tigrinus and Gammarus mucronatus, which extend from the Gulf of St. Lawrence to Florida, may produce up to ten broods (Steele and Steele, 1975). All four species are annual.

The development of a single brood may be conveniently divided into two periods: (1) the egg period, which is the time from oviposition to the time of hatching; and (2) the juvenile period, the time between hatching and emergence of juveniles from the marsupium. Unhatched eggs removed from the marsupium will hatch when maintained in sea water in the laboratory (Gammarus duebeni, Kaestner, 1970; Gammarus obtusatus, Sheader and Chia, 1970), but their survival is poor even when given elaborate care (Sheader and Chia, 1970). It is thought that only eggs retained in the marsupium usually hatch. There is no evidence that nutrients are provided by the female after oviposition (Kaestner, 1970). The egg period varies with temperature;

the colder the temperature, the longer the time until hatching (Hynes, 1954; Kinne, 1959). However, the length of this period seems to be fixed for a specific species at a specific temperature (Kinne, 1970).

On the other hand, the length of the juvenile period is variable, at least in certain species (table 23). In other words, at a specific temperature, such species release their young (which are all the same age, having been ovulated and fertilized on the same day) over the course of many days, whereas other species release all their young on the same day. Variability of the juvenile period is intriguing. What is the selective advantage of this variability? Why do some species exhibit it, while others do not?

Desiccation is a problem for littoral epibenthic amphipods. At low tide, littoral species are found underneath rocks, resting quiescent on the moist substratum or among leafy algae (Martin, 1966; Bousfield, 1973 ; and many others). These are damp to wet environments. Gammarus duebeni and Gammarus oceanicus, littoral amphipods, can detect and travel towards areas of higher humidity. This ability is absent in at least one sublittoral freshwater species, Pontoporeia affinis (Lagerspetz, 1963). The animals are always curled ventrally, head close to uropods, rather than stretched out, when hiding under rocks. Presumably, this attitude decreases evaporation, and also, in the females, permits the retention of water inside the marsupium.

Food may also be a problem. The literature suggests that most epibenthic species are opportunistic feeders, eating anything of suitable size they encounter (Jones, 1951; Martin, 1966; Greze, 1968; Steele, 1975). Sea weeds, diatoms, and detritus are eated. Most Gammarids seem to prefer animal foods (Kinne, 1959; Gammarus tigrinus, Poje, 1977) and some are carnivorous (Gammarus zadachi, Jones and Ericksen, 1951; Gammarus duebeni, Hynes, 1954). Little is known about the nutritional requirements of most species, however. G. duebeni prefers animal foods, but requires fresh algae for normal development (Kinne, 1959). Some boreal species may require filamentous algae for growth (Steele, 1967), but the foods mentioned are patchily distributed both spatially and temporally. Littoral epibenthic amphipods must be able to overcome the problem of food patchiness.

Littoral species must have adaptive mechanisms that protect their juveniles against desiccation and starving shortly after emergence from the female's marsupium. One way of achieving this is to have juveniles emerge at "safe" times and/or places. A "safe" time is likely to be when the animals are submerged (to avoid desiccation). A "safe" place is likely to be near food (to avoid starvation). Epibenthic amphipods are highly mobile, and a female could carry her juveniles to the "safe" place and release them

there. However, this requires some flexibility in the time of emergence.

I hypothesize that littoral species that are subjected to a wide range of environmental extremes have a variable juvenile period that permits them the flexibility to release their juveniles at a relatively "safe" time and/or place. The hypothesis rests upon the assumption that littoral species of amphipods have a more variable juvenile period than supra- and sublittoral species.

Three abundant species of epibenthic amphipods, found at different tide marks at Jamaica Bay, New York, were chosen to test the hypothesis. Preliminary observations had shown that Gammarus palustris occurred primarily at the high tide mark; that Gammarus mucronatus occurred primarily at the mean tide mark, and that Melita nitida occurred primarily at the low tide mark and extended into the sublittoral. The hypothesis tested was that G. palustris would have the most variable, and M. nitida the least variable juvenile periods.

Because it was hypothesized that variability permits juveniles to emerge when relatively "safe" conditions prevail, it had to be shown that emergences varied with environmental conditions that vary in the field. Therefore, experiments were conducted on Gammarus palustris to observe the effects of: 1) different amounts and types of foods and 2) exposure to air on the time of emergence of juveniles.

# MATERIALS AND METHODS

## I. Description of Site of Field Observations

Animals were collected from the littoral zone of the salt marsh Black Bank Marsh, near the North Channel Bridge at Jamaica Bay, Brooklyn, New York (latitude 40°38' N, longitude 73°50' W), from September, 1975 to September, 1977. The Bay has deep water channels maintained by dredging that separate several marshy islands. Municipal wastewater from four sewage treatment plants flows into the bay. Black Bank Marsh is now part of the federally-owned Gateway National Park.

In the sublittoral zone, water temperature ranges from 0° C to about 26° C throughout the year, and salinity from 21 °/oo to 26 °/oo in the deeper areas. According to coliform bacterial count (up to 1100 Most Probable Number/ml; Feuerstein and Maddaus, 1976), the bay is polluted.

The substratum of the collection site was overlain by scattered rocks. Some of these rocks were "natural", but most were either cement or asphalt blocks which fell into the littoral zone when the underlying mud eroded from a road built at the high tide mark. The littoral zone at Jamaica Bay typically has no rocks.

The collection site was constantly disturbed throughout the study. The area is heavily used for recreation, principally for fishing and crabbing. In spite of this disturbance, the three species of amphipods chosen for study

were present in the warmer months throughout the length of this study.

## II. Field Observations

Collections were made at monthly or shorter intervals for two years (62 collection dates) from September, 1975 to September, 1977. Water and air temperatures and salinities were taken on each collection date. Animals were collected by picking them from under rocks, algae or debris with plastic forceps. Collections were always made at low tide during daylight hours. After picking, animals were either fixed immediately in undiluted formalin, or carried to the laboratory in water from the bay.

The exact site of collection within the littoral zone was determined by measurement from a fixed point. There is an abandoned pier at the edge of the collection site, and since the pilings could not be moved, they were used as reference points.

## III. Laboratory and Field Procedures

### A. Stock Cultures

All animals studied were collected in the field and placed in the laboratory within 24 hours of capture, except those used in studying the effects of exposure and immersion on juvenile emergence in Gammarus palustris. Animals used in those experiments were G. palustris taken from stock cultures.

Stock cultures were kept at 21° C and 24 °/oo salinity with a 12L:12D light cycle in a Sherer-Gillette Controlled Environmental Chamber, Model C E L 4-4. Animals were raised in groups of ten to twenty individuals in 20 cm diameter culture dishes. The animals were kept in sea water of 24 °/oo made from Instant Ocean® salts dissolved in tap water and aerated for at least two days. Dishes were covered with glass plates to prevent evaporation, and the water was never changed. The animals were fed (ad libitum) muscle tissue from the ribbed mussel, Modiolus demissus and Ulva sp. thalli with attached aufwuchs. These foods were taken from the collecting site at Jamaica Bay and frozen until placed in the dishes. Thus, Ulva fed to the animals was not from a pure culture, and it was not alive. In addition, the animals ate benthic diatoms and/or blue-green algae which grew on the glass surfaces of the older culture dishes.

B. Treatment of Animals During Laboratory Experiments

During the experiments, animals were maintained individually in ten cm diameter culture dishes, with glass plate covers. The salinity was 24 °/oo, and animals were transferred by a pipette with a diameter of 8 mm with a rubber bulb at the end. Animals were never exposed to air, except in the exposure experiments. All animals were fed mussel meat and Ulva except where noted.

C. Measurement of Animals

All measurements were done on specimens which had been fixed in formalin. When the entire body length was measured, it was taken from the tip of the rostrum of the head to the tip of the telson (Figure 1). In most cases, the animal had been fixed in a curled position, and it was necessary to force the head into a straight line with the telson by pressing the body between the two arms of a forceps.

When head sizes were measured, the shed casts were used. This proved quite satisfactory, for an accurate measurement of the animal could be obtained without killing the animal. Further, since it involved the measurement of a single segment, it was less subject to the error inherent in straightening out the body of the entire animal. The head measurement was the straight line distance between the tip of the rostrum and the most posterior dorsal tip of the segment (Figure 1).

D. Statistical Analyses

Parametric statistics were employed for all analyses, except when there were ten or fewer measurements per group, or when there was some question about whether the data were distributed normally. In the latter case, non-parametric statistics were employed. In all tests, the level of significance was taken at 0.05 or less.

When differences among the means of three or more groups were tested, the parametric analysis of variance test (Sokal and Rohlf, 1969) was employed. When differences between the means of two groups were tested, either the parametric Student's t Test (Sokal and Rohlf, 1969) or the non-parametric Mann-Whitney U test was employed (Siegel, 1956). When the extent of association between two sets of attributes were tested, the Chi Square 2 x 2 contingency table was employed (Siegel, 1956).

The correlation coefficient was calculated when the extent to which two independent variables varied together was determined; the regression coefficient was calculated when the extent of the relationship between one dependent and one independent variable was determined (Sokal and Rohlf, 1969).

IV. Reproductive Characteristics of Gammarus palustris, Gammarus mucronatus, and Melita nitida.

Parental care is considered one way to adapt to stressful environments. Two aspects of parental care are: (1) relatively few offspring per brood; and (2) a relatively long time spent with the parent. These two aspects were investigated in the three species.

All animals studied were field-collected. The first group was collected on May 21, 1976, and kept at 17° C until

the animals were fixed to permit measurement of their lengths. On that date, the water temperature and salinity were 11° C and 23.5 °/oo, respectively. The second group was collected in June, 1977, and kept at 21° C until fixed. Individuals in the 1977 group were selected, by eye, for similar size. The water temperature and salinity on June 3 and 25 were both 21° C and 26.5 °/oo respectively. Some females were carrying unhatched broods in their marsupia when brought to the laboratory. These broods will be called field broods hereafter. Each female was placed in a dish with a male. Juveniles hatched and emerged in the presence of the male. Information on the following was obtained: (1) date of hatching; (2) number of juveniles emerging each day after hatching; (3) dates of female's succeeding molt, copulation, and ovulation.

Each female not carrying a brood was placed in a dish with a male until she molted, copulated and ovulated in the laboratory. The male was then removed. The following information was obtained for these broods (called laboratory broods): (1) the date of ovulation; (2) the date the eggs hatched; (3) the number of juveniles that emerged after hatching; and (4) the date the female next molted. After this molt, the females and their shed casts were fixed in formalin, and the body lengths were measured (in mm). Note that females entering the laboratory with broods produced laboratory broods as well as field broods, and were treated exactly as were females that only produced laboratory broods.

The number of juveniles that emerged each day was observed by transferring the female to a new dish with new food, and counting the number of juveniles that remained in the old dish.

## V. The Effects of Food on Juvenile Emergence in Gammarus palustris.

### A. Food Provided Ad Libitum

About one day before the laboratory broods of field-caught females hatched, the eyespots of the embryos became visible. At that time, each female was placed, alone, in a fresh dish and fed in one of the following ways: (1) no food; (2) mussel meat only; (3) Ulva only; (4) mussel meat and Ulva, accompanied by daily transfer of females into fresh sea water and food; or (5) mussel meat and Ulva without transfer of females. Each female (except those in group 5) was transferred daily to a fresh dish and provided with fresh food of the designated type(s) until it molted. The juveniles that remained in the old dish were counted. The day the female molted was noted, and the shed cast was fixed in formalin. The head of the cast was measured.

### B. Observations on the Variety of Food Consumed in the Field

Although gut and fecal content studies usually contain little information (Edmondson, 1957), examinations of laboratory-cultured Gammarus palustris suggested that fecal color might indicate what the animals had eaten. Thus, examination of the feces of field-collected animals might reveal the compo-

sition of field-ingested material.

Preliminary personal observations had indicated that the types of foods provided to a female with hatched juveniles in the marsupium influenced the time of emergence of the juveniles. If amount and variety of food ingested by the female influences the time of juvenile emergence in the field, an examination of the feces of field-collected females, which may indicate what's being consumed, could yield information on juvenile retention time within the marsupium. It was necessary to study precopulating couples because earlier observations had shown that almost all females with hatched broods were in precopulo.

Precopulating couples were obtained from the collecting site in the spring of 1977, and each pair was placed, within one hour, in a dish with fresh sea water. No food was provided. The animals remained in this clean water from 8 to 24 hours. The animals were removed from the water, and the feces classified according to length and color.

C. The Effects of the Introduction of Mussel Meat on Juvenile Emergence

Field-caught females were maintained at 17° C and fed mussel meat and Ulva ad libitum until the day before hatching of the first laboratory brood. Some females were not fed, beginning on the day before hatching (unfed group), and some females were fed Ulva ad libitum, beginning on the day before

hatching (fed group). The fed and unfed groups were each divided into three sub-groups: (1) broods tested on the day of hatching (Day 0); (2) broods tested the day after hatching (Day I); and (3) broods tested on the second day after hatching (Day II).

Beginning the day before hatching and continuing thereafter, all females were transferred daily until the test day, either into fresh sea water without food (unfed group) or into water with newly defrosted Ulva (fed group). To determine the effect that pipetting had on juvenile emergence, the number of juveniles that emerged during the first 15 minutes after the daily transfer were counted and removed from the new dish. The number of juveniles that remained in the old dish was counted. This was the number of juveniles that had emerged during the time since hatching or since the last observation.

Each female was tested once. On the designated test day of a particular female, the following procedures were performed. First, the female was transferred to a fresh dish. Second, the number of juveniles that emerged during the first 15 minutes after transfer were counted and removed. Third, immediately after the first 15-minute period, a piece of mussel meat, about one quarter the size of the female, was placed in its dish. Fourth, the number of juveniles that emerged 0.5, 1.5, 6.5, and 21.5 minutes after the female began to feed were counted and removed. The total observation period was 21.5 minutes. The observation times were suggested

by an earlier preliminary observation on the rates of emergence after the presentation of mussel meat. A juvenile was considered "emerged" only if it left the body of the female. Fifth, the number of juveniles that remained in the old dish was counted. After the test day, all females were transferred, fed mussel meat and Ulva ad libitum, and the number of emerged juveniles was counted daily until the female molted. The length of the head of the cast was measured.

## VI. The Effects of Exposure and Immersion on the Emergence of Juveniles of Gammarus palustris.

### A. The Effects of Exposure on Zero to One Day Old Juveniles

Before determining whether exposure to air had any effect on the time a juvenile emerged, it was thought necessary to determine whether exposure to the air has a harmful effect upon the juveniles, and, if it has, whether the effect can be avoided by residence within the marsupium.

Animals from stock cultures were used. On the day the brood hatched, the female was placed on a bed of crushed frozen sea water of 24 $^{o}/oo$ . This immobilized the female sufficiently to permit the removal of all the juveniles from the marsupium. The juveniles were removed by passing a dissecting needle through the marsupium and pushing them out between the oostegites.

All the juveniles and the female were placed in sea water at room temperature for a ten-minute recovery period. Then the juveniles of each brood were divided into two groups: one-third were placed in fresh sea water until tested, and two-thirds were placed with the mother in fresh sea water in a plastic bottle cap (3 cm diameter, 2 cm deep) in the dark. After two hours, some juveniles had re-entered the marsupium and some had not. Thus, there were three categories of removed juveniles tested: (1) those never replaced with the mother; (2) those replaced with the mother that did not re-enter the marsupium; and (3) those replaced with the mother that did re-enter the marsupium.

Preliminary observations had shown that juveniles exposed to the air for six minutes did not survive. Juveniles in categories 1 and 2 were placed on paper towels (which absorbed all water attached to the juveniles) for six minutes. The mother with the re-entered juveniles was placed on a paper towel for six minutes. The number of females and juveniles within each of the three categories that survived longer than 24 hours after exposure were recorded.

B. The Effects of Alternating Periods of Exposure and Immersion on Juvenile Emergence.

Animals from stock cultures were used and the tests were conducted at room temperatures ($18^{o} \pm 1.7^{o}$ C). The females were divided into four test groups: (1) those exposed

and fed; (2) those fed but not exposed; (3) those unfed and exposed; and (4) those unfed and not exposed. All females were fed and kept immersed until one day before hatching. At that time, each female was placed in a clean dish with a glass microscope slide (which served as a "rock" to hide under when the water was removed). Beginning from the hour of hatching, animals were observed four times a day: at 7 a.m., 11 a.m., 7 p.m., and 11 p.m.

The two groups that were not exposed were either pipetted into fresh sea water with no food at 7 a.m. and 7 p.m. (group 4) or pipetted back into the old dish (group 2). The number of juveniles that had emerged since the last observation was counted and removed.

The other two groups were exposed to the air for two eight-hour periods each day: from 11 a.m. to 7 p.m. and from 11 p.m. to 7 a.m., until the female molted. At 11 a.m. and 11 p.m., the water was pipetted from the female's dish, leaving the female behind. At 7 a.m. and 7 p.m., the water from the dishes of females of group 1, which had been retained, was pipetted back into their dishes. Fresh sea water was pipetted into group 3 females' dishes at those times. The number of juveniles that emerged during the next 15 minutes was counted and removed. The number of juveniles that emerged during the next 3.75 hours was counted and removed

at 11 a.m. and 11 p.m. The number of juveniles that emerged during each eight-hour exposure period was counted and removed at 7 a.m. and 7 p.m.

All females were treated as described until they molted. The casts were then fixed, and the casts' head lengths measured.

# RESULTS

## I. Field Observations

### A. Salinity and Water Temperature

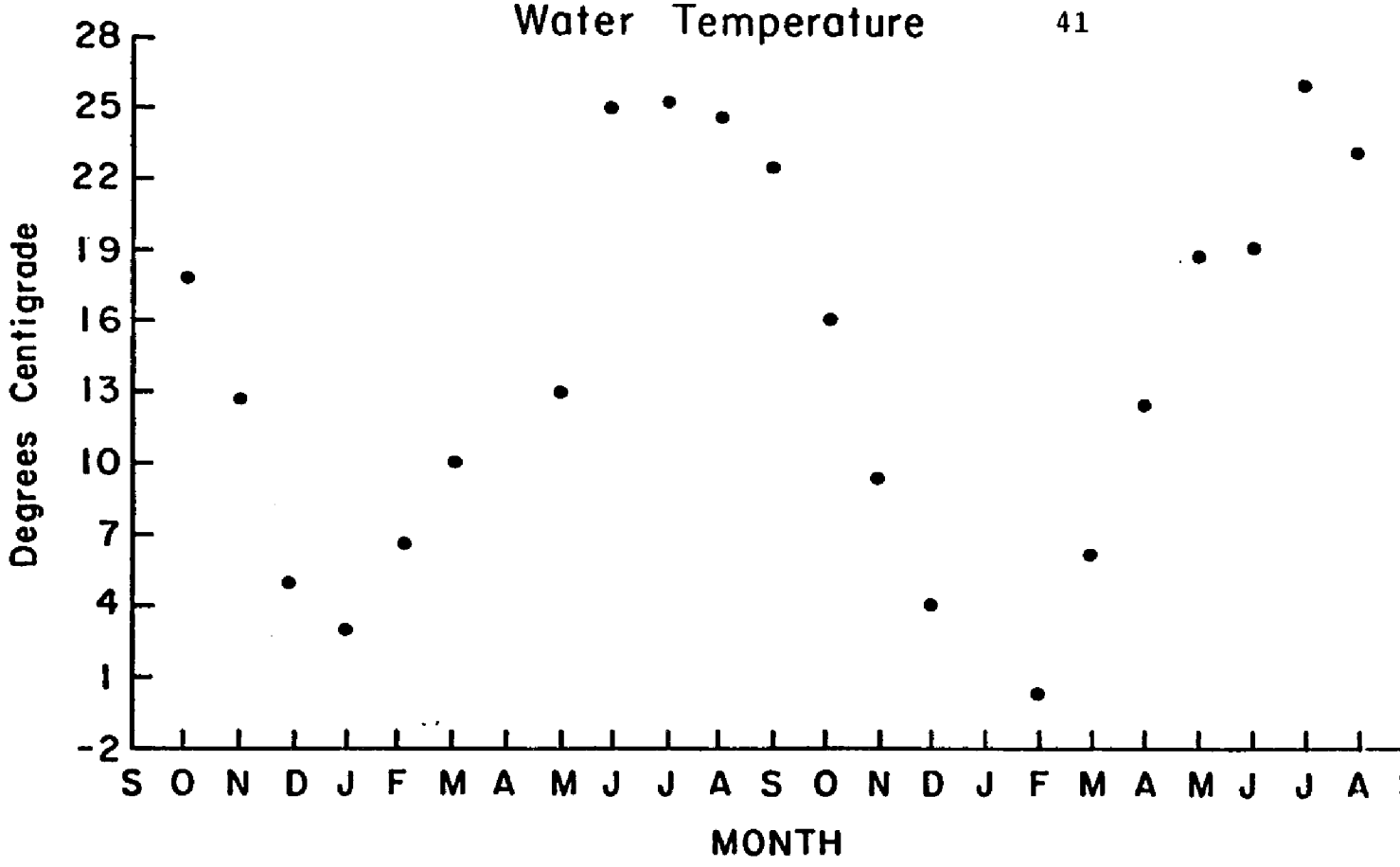
Salinity and water temperature, taken at low tide from September, 1975 to September, 1977, were lowest in the winters, and highest in the summers (Figure 4). The range of salinities was narrow (21.5 $^{o}/oo$ - 29 $^{o}/oo$). During the winter of 1976-1977, there was a thick layer of ice in the littoral zone from early January to mid-February which prevented collections from being made, but there was no ice during those months in the previous winter.

### B. Epibenthic Amphipods Found in the Littoral Zone At Collecting Site.

A list of all species of epibenthic amphipods collected from September, 1975 to September, 1977 in the littoral Zone of the collecting site is given in Table 1. The most abundant

Figure 4

Average monthly water temperature and salinity at low tide at the collecting site.


Water Temperature
Degrees Centigrade
28
25
22
19
16
13
10
7
4
1
-2
S O N D J F M A M J J A S O N D J F M A M J J A
MONTH

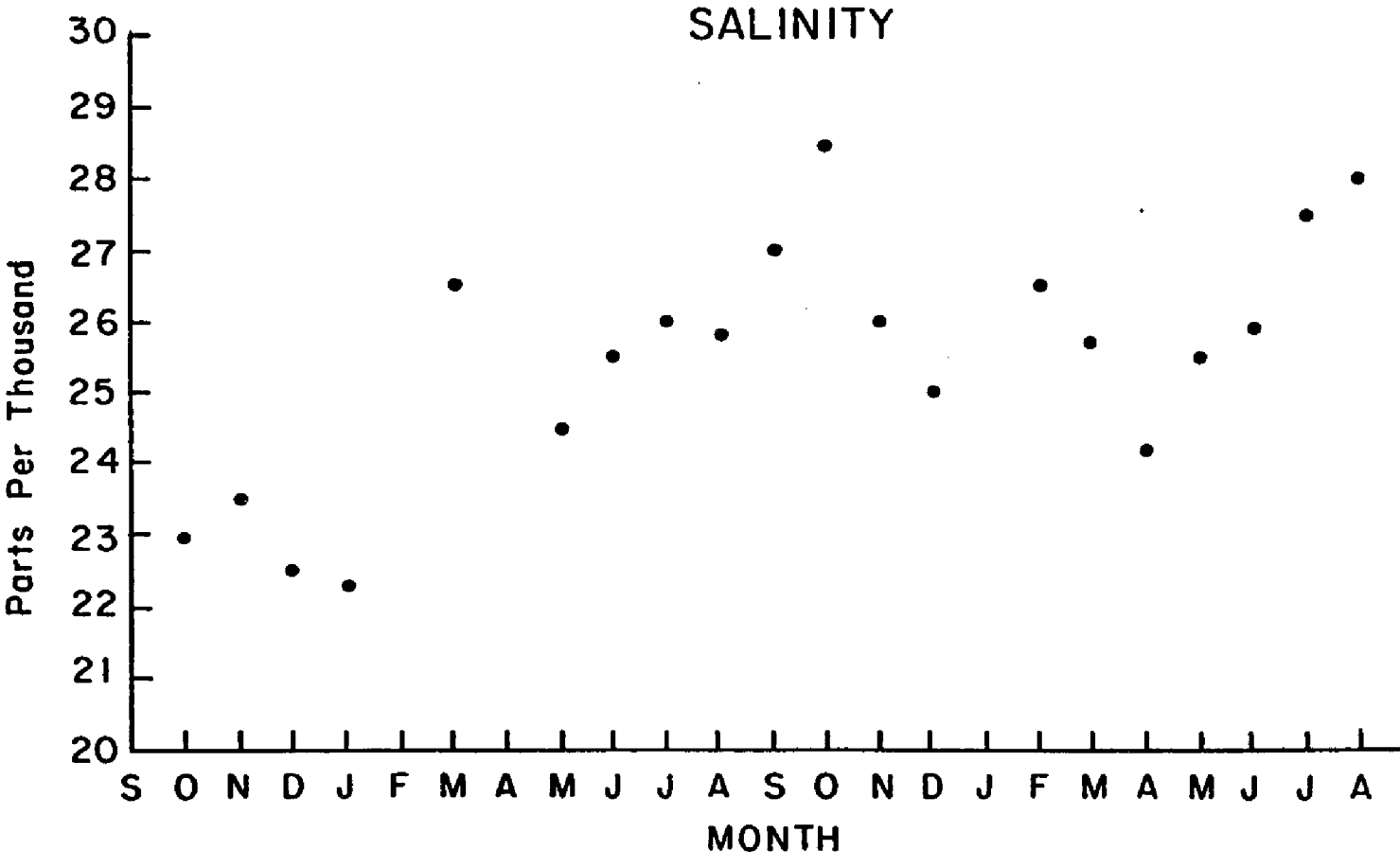

SALINITY
Parts Per Thousand
30
29
28
27
26
25
24
23
22
21
20
S O N D J F M A M J J A S O N D J F M A M J J A
MONTH

TABLE 1

IDENTIFYING CHARACTERISTICS OF THE COMMON SPECIES OF EPIBENTHIC AMPHIPODS FOUND IN THE LITTORAL ZONE AT JAMAICA BAY

| SPECIES | TIDE LEVEL | HABITAT | BEHAVIOR | COLOR |
|---|---|---|---|---|
| Ampithue valida | Low-sublittoral | Builds tubes on Ulva | Quiescent within tube | Bright green; occasionally brown |
| Micodeutopus gryllotalpa | Low-sublittoral | Builds tubes on rocks | Quiescent within tube | Brown |
| Unicola sp. | Low-sublittoral | Insufficient data | Insufficient data | White with thin red markings |
| Gammarus lawrencianus | Low | Under solid substrata or sea weeds; wet areas | Similar to Gammarus mucronatus | Glassy-transparent, with thin black vertical lines; black mouthparts |
| Gammarus mucronatus | Mean to Low | Under solid substrata or sea weeds | Sidles rapidly until it achieves cover | Transparent with thin to thick black stripes and dots |
| Gammarus palustris | High | Under solid substrata in barely damp areas | May sidle, may freeze, may walk upright, using antennae as supports | Transparent to salmon pink, to red; light brown to caramel; the brown distributed in large irregular patches |
| Melita Nitida | Low-sublittoral | Under solid substrata in wet areas | Usually freezes when rock overturned | Pale gray to slate gray; the majority, slate gray |

TABLE 1 (CONTINUED)

IDENTIFYING CHARACTERISTICS OF THE COMMON SPECIES OF EPIBENTHIC AMPHIPODS FOUND IN THE LITTORAL ZONE AT JAMAICA BAY

| SPECIES | TIDE LEVEL | HABITAT | BEHAVIOR | COLOR |
|---|---|---|---|---|
| Elasmopus levis | Low-sublittoral | Insufficient data | Insufficient data | Brown |
| Orchestia grillus | High-supra-littoral | Under solid substrata or dry litter in humid areas | Walks rapidly, does not lean on antennae; map leap into air many times its length | Transparent to pale orange to bright orange; also pale gray and slate gray |

species were Orchestia grillus, Gammarus mucronatus, Gammarus palustris, and Melita nitida. Most species exhibited a wide range of colors, and much overlap of habitat and behavior among the species occured; however, these characteristics, taken together, permitted an accurate field identification of the species listed in Table 1.

C. Temporal Distributions of Melita nitida, Gammarus mucronatus, and Gammarus palustris

The temporal distributions of Melita nitida and Gammarus mucronatus suggest that seasonal migrations of adults occurred into and out of the subtidal region (Figures 5 and 6). Occupation of the littoral zone in the spring occurred gradually, the lowest tide levels being occupied first, the highest levels being occupied last. Both species occupied the largest area of the littoral zone in the summer and the smallest area in winter. Although both species were found occasionally in the littoral zone in the warm winter of 1975-1976, they were absent during the colder winter of 1976-1977, and did not reoccupy the littoral zone until some time after the disappearance of ice; G. mucronatus in late April, and M. nitida in late May.

The temporal distribution pattern of Gammarus palustris was different. From December, 1975, to February, 1976, G. palustris was found on eight of nine collection dates. During

Figure 5

Temporal and spatial distribution of Melita nitida in the intertidal zone of the collecting site.

Legend:

● indicates that no individuals were found anywhere in the intertidal zone on that date.

Δ indicates that at least one individual was found in the intertidal zone on that date.

✗ indicates that at least one individual was found at that tide mark on that date.

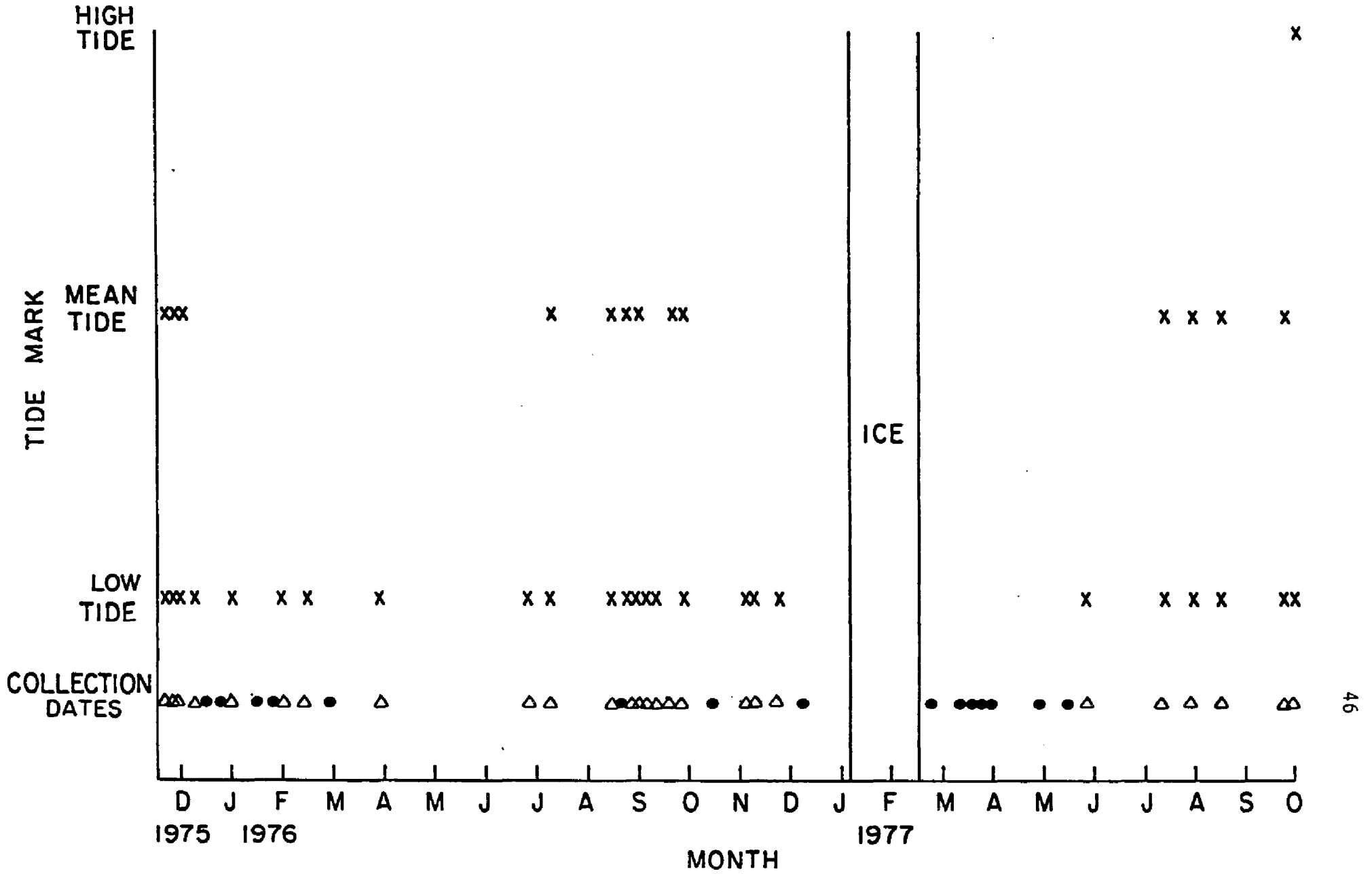

HIGH TIDE
MEAN TIDE
LOW TIDE
TIDE MARK
COLLECTION DATES
ICE
D J F M A M J J A S O N D J F M A M J J A S O
1975 1976
1977
MONTH

Figure 6

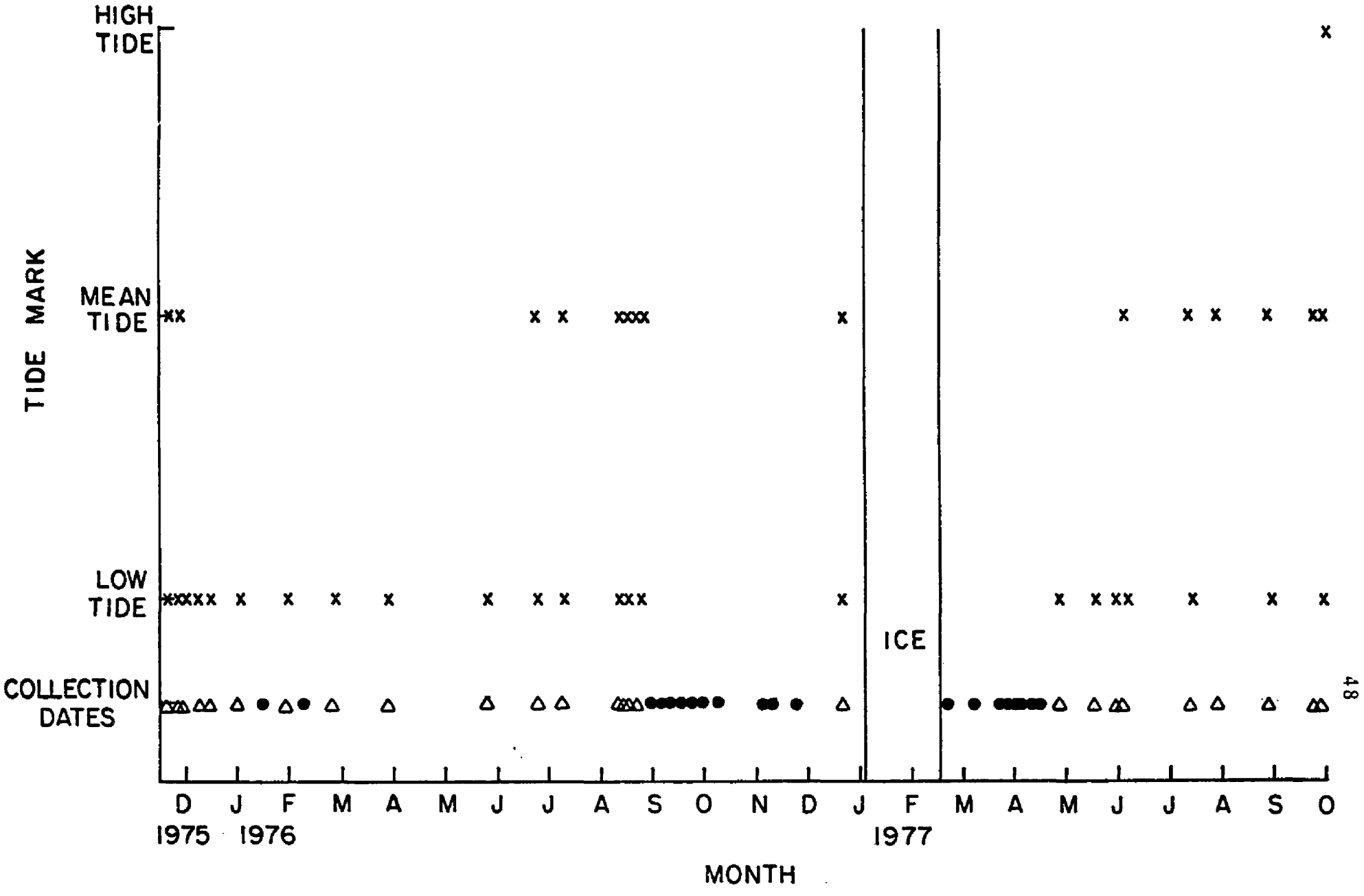
Temporal and spatial distribution of Gammarus mucronatus in the intertidal zone of the collecting site.

Legend:

● indicates that no individuals were found anywhere in the intertidal zone on that date.

△ indicates that at least one individual was found in the intertidal zone on that date.

✕ indicates that at least one individual was found at that tide mark on that date.


HIGH TIDE
MEAN TIDE
LOW TIDE
TIDE MARK
COLLECTION DATES
ICE
D J F M A M J J A S O N D J F M A M J J A S O
1975 1976 1977
MONTH

this time, Melita nitida was found on four of nine, and Gammarus mucronatus, found on five of eight collection dates. Thus, individuals of G. palustris were present more often during the winter months than the other two species. During the winter of 1976-1977, ice covered the littoral zone and no collections were possible. G. palustris, however, was collected immediately before and after the presence of the ice (Figure 7).

Gammarus palustris was present throughout the littoral zone during early spring, 1977. In May, it abandoned the low and mean tide marks for the high tide mark; this coincided with the occupation of the lower and middle parts of the littoral zone by Gammarus mucronatus (Figures 6 and 7)

All three species were collected on only 14 of the 62 collection dates. On the 7 of the 14 collection dates that vertical distances were recorded, Gammarus palustris tended to cluster in the highest; Gammarus mucronatus in the middle; and Melita nitida in the lowest positions of the littoral zone (Table 2).

D. Reproduction of Gammarus palustris in the Field

The number of precopulating couples and the stage of development of broods of Gammarus palustris were observed from November, 1976 through September, 1977. No reproductive

Figure 7

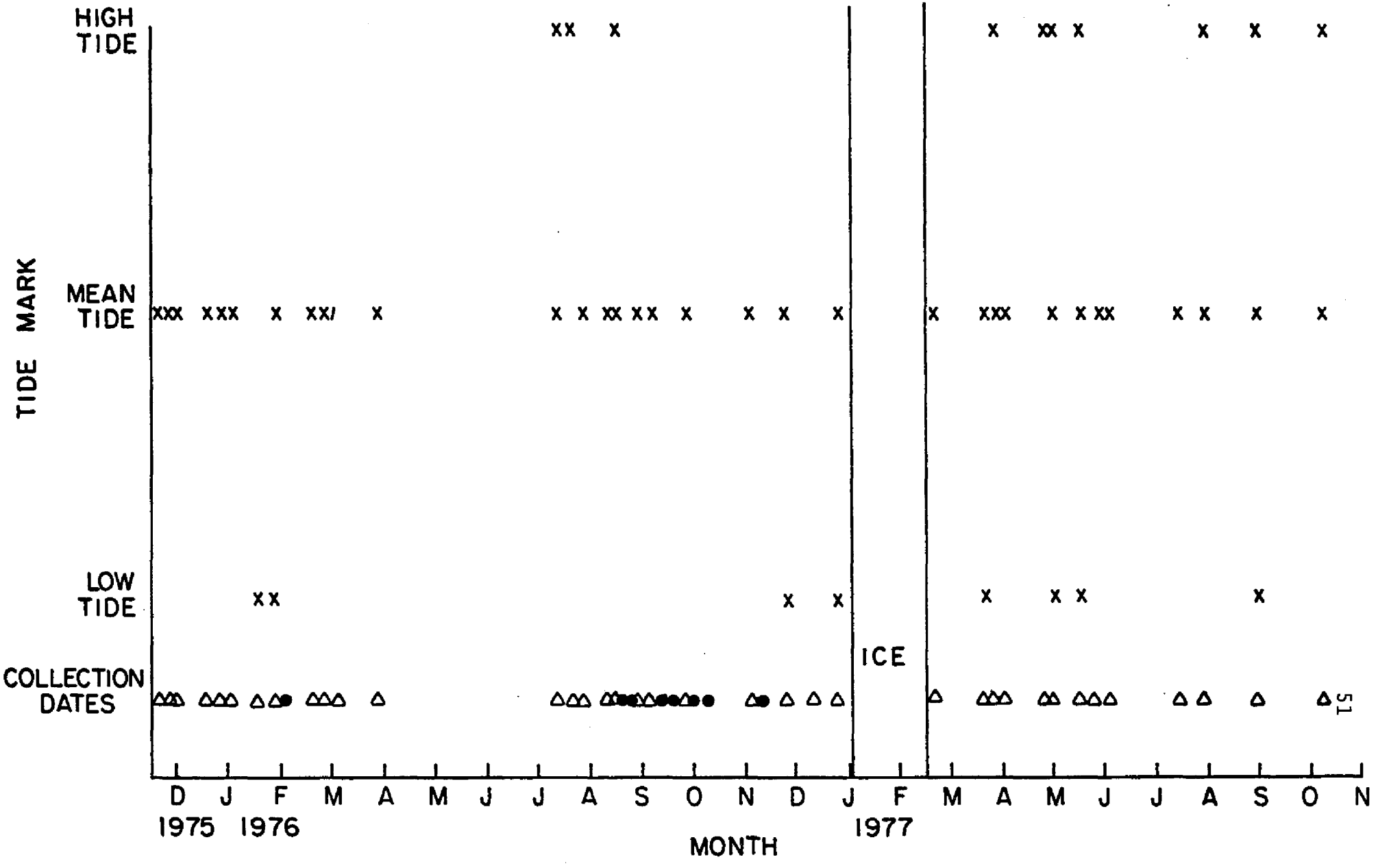
Temporal and spatial distribution of Gammarus palustris in the intertidal zone of the collecting site.

Legend:

● indicates that no individuals were found anywhere in the intertidal zone on that date.

▲ indicates that at least one individual was found in the intertidal zone on that date.

✕ indicates that at least one individual was found at that tide mark on that date.


HIGH TIDE
MEAN TIDE
LOW TIDE
COLLECTION DATES
TIDE MARK
ICE
D J F M A M J J A S O N D J F M A M J J A S O N
1975 1976
1977
MONTH

## TABLE 2

PERCENT ABUNDANCE OF GAMMARUS PALUSTRIS (G.p.), GAMMARUS MUCRONATUS (G.m.), AND MELITA NITIDA (M.n.), AT THE LOW TIDE (LT), MEAN TIDE (MT), AND HIGH TIDE (HT) POSITIONS ON DATES WHEN ALL THREE SPECIES WERE PRESENT

| | 11/16/75 | | | 11/23/75 | | | 6/22/76 | | | 7/7/76 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gp | Gm | Mn | Gp | Gm | Mn | Gp | Gm | Mn | Gp | Gm | Mn |
| %HT | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 5 | 27 |
| %MT | 0 | 94 | 50 | 0 | 100 | 67 | 0 | 56 | 0 | 0 | 86 | 12 |
| %LT | 0 | 6 | 50 | 0 | 0 | 33 | 0 | 44 | 100 | 0 | 10 | 61 |

TABLE 2 (CONTINUED)

PERCENT ABUNDANCE OF GAMMARUS PALUSTRIS (G.p.), GAMMARUS MUCRONATUS (G.m.), AND MELITA NITIDA (M.n.), AT THE LOW TIDE (LT), MEAN TIDE (MT), AND HIGH TIDE (HT) POSITIONS ON DATES WHEN ALL THREE SPECIES WERE PRESENT

| | 8/9/76 | | | 8/10/76 | | | 8/31/76 | | | Means of 7 Days | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gp | Gm | Mn | Gp | Gm | Mn | Gp | Gm | Mn | Gp | Gm | Mn |
| %HT | 100 | 20 | 0 | 100 | 53 | 31 | 89 | 0 | 0 | 98.4 | 11.1 | 8.3 |
| %MT | 0 | 35 | 0 | 0 | 0 | 65 | 0 | 40 | 5 | 0 | 58.7 | 28.4 |
| %LT | 0 | 45 | 100 | 0 | 47 | 4 | 11 | 60 | 95 | 1.6 | 30.3 | 63.3 |

activity occurred during the winter. Of the 97 animals collected between November 2, 1976 and March 18, 1977, none were in precopulation, and no females carried broods. Precopulating couples were captured beginning March 25 and females with broods were captured beginning April 1. Precopulating couples and females with broods were found on most dates through September 30 (Table 3).

Precopulating females tended to have broods in the later stages of development. Laboratory observations showed that the approximate age of a developing brood can be determined by examination of the brood through the transparent oostegites. The newly ovulated eggs look like black spheres. Each egg then separates into a clear area and a black area. The black area narrows into a single thick line, which then differentiates into two thick lines. The two thick lines eventually become thin. Red eyespots appear about a day before hatching. Hatched juveniles remain inside the marsupium for zero to eight days.

Thus, six stages of development can be clearly distinguished through the transparent brood pouch in Gammarus palustris: (1) the black sphere stage; (2) the black sphere with a clear area stage; (3) one thick line stage; (4) two thin lines stage; (5) eyespot stage; and (6) hatched juveniles stage. It is interesting that Sheader and Chia (1970) divided the development of Marinogammarus obtusatus broods into six stages as well: (1) early cleavage, in which all cells are pigmented; (2) the development of the germinal disc, which is pigment-free; (3) the formation of the caudal furrow;

## TABLE 3

### STAGE OF REPRODUCTION OF FEMALES OF FIELD-CAUGHT GAMMARUS PALUSTRIS BETWEEN 3/25/77 AND 9/30/77

| DATE | TOTAL MATURE FEMALES | SINGLE FEMALES | | | | | PRECOPULATING FEMALES | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No Brood | Black Balls | 1 Thick Line | 2 Thin Lines | Hatched Juveniles | No Brood | Black Balls | 1 Thick Line | 2 Thin Lines | Hatched Juveniles |
| 3/25/77 | 8 | | | | | | 8 | | | | |
| 3/27 | 11 | | | | | | 11 | | | | |
| 4/01 | 7 | | 2 | | | | 5 | | | | |
| 4/09 | 22 | | 8 | | | | 14 | | | | |
| 4/27 | 10 | | 1 | | | | 1 | | 5 | 2 | 1 |
| 4/29 | 22 | 1 | 4 | 1 | | | | | 7 | 5 | 4 |
| 5/06 | 27 | 1 | 9 | 1 | | | 1 | | 3 | 10 | 2 |
| 5/13 | 26 | 1 | 1 | 1 | | | | | 5 | 11 | 7 |
| 5/20 | 17 | | 6 | 2 | | | | | 1 | 2 | 6 |
| 5/28 | 12 | | 2 | 4 | | | 1 | | | | 5 |
| 6/03 | 19 | | 13 | | | | 5 | | | | 1 |
| 6/10 | 6 | 1 | | 1 | | | 1 | | | 3 | |
| 8/31 | 5 | 1 | 1 | 3 | | | | | | | |
| 9/21 | 2 | | 2 | | | | | | | | |
| 9/30 | 3 | 1 | | 1 | | | 1 | | | | |

(4) the widening of the caudal furrow, with the embryo assuming a more oval shape; (5) the appearance of red pigment spots on the eye rudiments; and (6) hatched juveniles. Although the two classification systems are not exactly the same, it is clear that the sequence of developmental stages is similar for the two species, and that they can be clearly distinguished.

The eggs of females in the field exhibited the same appearances as the stages described for the laboratory animals. Assuming that they followed the same sequence of development as laboratory broods, broods in the black sphere and one thick line stage can be classed as early stages of development, and broods with two thin lines, eyespots, and hatched juveniles classed as late stages in development. Females carrying late stage embryos were in precopulation significantly more often than females carrying early stage embryos. (Chi Square two by two contingency test; $\chi^2=70.25$, d.f. = 1, $p < .01$). (Females with empty marsupia were not included because it was not known whether the juveniles had emerged, or whether the female had had no brood at all). Thus, precopulation is likely to occur shortly before the female is ready to molt.

In Gammarus palustris, reproduction (as indicated by precopulating adults or the presence of a brood) was not restricted to its usual habitat, the high tide mark. From

March 18 until May 28, animals were found throughout the littoral zone. After May 28 they were confined to the high tide mark. Reproductive activity began on March 25, and continued until September 30. Thus, animals were reproducing at all tide marks during the spring, but only at the high tide mark in the summer and early fall.

## II. Reproductive Characteristics of Gammarus palustris, Gammarus mucronatus, and Melita nitida.

### A. The Fecundity of Laboratory Maintained Animals

The percent of the females that had broods in their marsupia when captured, and subsequently ovulated in the laboratory, was about the same for all three species (Table 4). However, the fecundity of Melita nitida was lower than Gammarus palustris and Gammarus mucronatus in the laboratory. First, the percent of females that ovulated in the laboratory, survived to molt, but aborted the ovulated brood before it hatched was greatest for M. nitida and least for G. palustris (Table 5). Second, the average number of juveniles in laboratory broods was 24% less for M. nitida, but 24% more for G. palustris and 34% more for G. mucronatus.

### B. Differences in Several Reproductive Characteristics Among Species

#### 1. The Number of Juveniles per Brood

In 1977, there was a considerable range in female sizes

TABLE 4

NUMBER OF FEMALES OF GAMMARUS PALUSTRIS, GAMMARUS MUCRONATUS, AND MELITA NITIDA THAT PRODUCED FIELD BROODS AND SUBSEQUENTLY OVULATED IN THE LABORATORY

| Species | Laboratory Temperature | (A) Number of Females with Field Broods | (B) Number of Females from (A) That Subsequently Ovulated in the Laboratory | Percent B/A |
|---|---|---|---|---|
| Gammarus | 17° C | 25 | 18 | 72% |
| palustris | 21° C | 24 | 24 | 100% |
| | Combined | 49 | 42 | 85.7% |
| Gammarus | 17° C | 23 | 15 | 65.2% |
| mucronatus | 21° C | 18 | 18 | 100% |
| | Combined | 41 | 33 | 80.5% |
| Melita | 17° C | 6 | 5 | 83.3% |
| nitida | 21° C | 24 | 21 | 87.5% |
| | Combined | 30 | 26 | 86.7% |

## TABLE 5

NUMBER OF FEMALES OF *GAMMARUS PALUSTRIS*, *GAMMARUS MUCRONATUS*, AND *MELITA NITIDA* THAT OVULATED IN THE LABORATORY BUT SUBSEQUENTLY ABORTED THE BROOD BEFORE HATCHING

| Species | Temperature | (A) Number of Females That Ovulated in the Laboratory | (B) Number of Females From (A) That Subsequently Aborted Broods | Percent B/A |
|---|---|---|---|---|
| *Gammarus palustris* | 17°C | 24 | 2 | 8.3% |
| | 21°C | 25 | 3 | 12.0% |
| | Combined | 49 | 5 | 10.2% |
| *Gammarus mucronatus* | 17°C | 22 | 1 | 4.5% |
| | 21°C | 30 | 6 | 20 % |
| | Combined | 52 | 7 | 13.5% |
| *Melita nitida* | 17°C | 4 | 2 | 50 % |
| | 21°C | 30 | 19 | 63.3% |
| | Combined | 34 | 21 | 61.8% |

among the three species. Individuals of each species, whose sizes were not significantly different (analysis of variance $F_{2,22} = 2.782$) were tested to determine whether the number of juveniles in their broods were significantly different. There was a significant difference in the number of juveniles in the broods among the three species (analysis of variance $F_{2,22} = 3.442$) (Table 6). The average number of juveniles per brood of Gammarus palustris females was 12, of Gammarus mucronatus 27, and of Melita nitida 30. The differences between the number of juveniles per brood of G. palustris and G. mucronatus, and between G. palustris and M. nitida were significant (Student's t test; t=2.27, d.f.=18; t=3.34, d.f.=14, respectively), but there was no significant difference between G. mucronatus and M. nitida (t=0.22, d.f.=12). Thus, G. palustris had significantly fewer offspring per brood than did the other two species. The number of juveniles of laboratory broods was not compared because it had been shown that laboratory conditions affected the fecundity of each species differently (see Results section II A)

2. The Lengths of Development from Ovulation to Hatching

For each species there was little variation in the number of days from ovulation to hatching (Table 7). The development of the broods of all three species was about four days faster at 21° C than at 17° C, but at both temper-

TABLE 6

FIELD BROODS OF *GAMMARUS PALUSTRIS*, *GAMMARUS MUCRONATUS*, AND *MELITA NITIDA*; SIZES OF FEMALES (mm) AND NUMBER OF JUVENILES PER BROOD

Female Size (mm)

| | G. palustris | G. mucronatus | M. nitida |
|---|---|---|---|
| N | 14 | 10 | 8 |
| Range | 6.00-8.57 | 6.43-10.00 | 5.00-8.71 |
| $\bar{x}$ | 7.50 | 8.06 | 6.89 |
| SD | 0.70 | 1.27 | 1.26 |

Number of Juveniles

| | G. palustris | G. mucronatus | M. nitida |
|---|---|---|---|
| N | 14 | 10 | 8 |
| Range | 8-21 | 1-57 | 5-51 |
| $\bar{x}$ | 12.4 | 27.4 | 30.0 |
| SD | 4.1 | 21.4 | 15.0 |

---

* N = number of females

$\bar{x}$ = mean

SD = standard deviation

TABLE 7

LABORATORY BROODS OF GAMMARUS PALUSTRIS, GAMMARUS MUCRONATUS, AND MELITA NITIDA; MEAN NUMBER OF DAYS BETWEEN OVULATION AND HATCHING

| Species | Temperature | N | Range | $\bar{x}$ | SD | Difference Between 17° and 21° |
|---|---|---|---|---|---|---|
| Gammarus palustris | 17°C | 10 | 11-12 | 11.1 | 0.30 | 4.1 days |
| | 21°C | 22 | 7-8 | 7.1 | 0.21 | |
| Gammarus mucronatus | 17°C | 4 | 8-9 | 8.3 | 0.43 | 3.7 days |
| | 21°C | 23 | 4-5 | 4.6 | 0.50 | |
| Melita nitida | 17°C | 1 | - | 10.0 | - | 4.1 days |
| | 21°C | 9 | 5-6 | 5.9 | 0.31 | |

* N = number of broods

$\bar{x}$ = mean

SD = standard deviation

atures tested, Gammarus palustris had the longest, and Gammarus mucronatus the shortest time from ovulation to hatching.

3. The Mean Days of Emergence

An index to describe the average time of emergence after hatching of all the juveniles of a particular brood, called the mean day of emergence (M.D.E.) was calculated for each brood. It is:

$$M.D.E. = \frac{\sum (n_i \times i)}{N}$$

where i = day after hatching, $n_i$ = number of juveniles that emerged on the $i^{th}$ day after hatching, and N = total number of juveniles of that brood.

Because temperature affects many physiological processes in poikilotherms, differences among the species were calculated only within the same temperature. To reduce other variables, field and laboratory broods were analyzed separately.

In field broods at $21^{o}$ C, the average mean day of emergence was different among the three species (analysis of variance $F_{(2,29)} = 12.835$). Gammarus palustris (1.3 days) was significantly longer than either Gammarus mucronatus (0.5 days) or Melita nitida (0.3 days) (Student's t test: t=3.49, d.f.=23; t=4.82, d.f.=25, respectively),but there

was no significant difference between the average mean day of emergence of G. mucronatus and M. nitida (t=0.53, d.f.=22). In laboratory broods at 17° C, the average mean day of emergence was different among the three species ($F_{(2,22)}$=12.409). G. palustris (1.8 days) was significantly longer than either G. mucronatus (1.3 days) or M. nitida (0.4 days) (t=2.40, d.f.=20; t=4.18, d.f.=17, respectively). Further, the average mean day was significantly longer for G. mucronatus than for M. nitida (t=3.21, d.f.=7). In laboratory broods at 21° C, the average mean day of emergence was different among the three species ($F_{(2,54)}$= 25.59). G. palustris (1.7 days) was significantly longer than either G. mucronatus (0.8 days) or M. nitida (0.6 days) (t=5.91, d.f.=44; t=5.04, d.f.=31, respectively), but there was no difference between G. mucronatus and M. nitida (t=1.11, d.f.=33). Thus, after hatching, G. palustris juveniles remained inside the marsupium for the longest time, and M. nitida juveniles for the shortest time.

The differences in the average mean days of emergence among the three species were probably not due to differences in female sizes because the sizes of the females were not significantly different at 21° C (analysis of variance; field broods - $F_{(2,29)}$ = 2.77; laboratory broods - $F_{(2,54)}$= 2.94) (Table 8).

TABLE 8

AVERAGE MEAN DAY OF EMERGENCE, AND AVERAGE FEMALE LENGTH OF GAMMARUS PALUSTRIS, GAMMARUS MUCRONATUS, AND MELITA NITIDA AT 17° AND 21°C

| | Field Broods at 21°C | Laboratory Broods at 17°C | Laboratory Broods at 21°C |
|---|---|---|---|
| G. PALUSTRIS | | | |
| Mean Day (days) | | | |
| N | 14 | 15 | 23 |
| Range | 0.94-2.60 | 0.95-2.96 | 0.48-3.00 |
| $\bar{x}$ | 1.28 | 1.80 | 1.68 |
| SD | 0.53 | 0.21 | 0.59 |
| Female Size (mm) | | | |
| N | 14 | 14 | 22 |
| Range | 6.00-8.57 | 9.29-11.36 | 5.57-8.14 |
| $\bar{x}$ | 7.48 | 9.67 | 7.23 |
| SD | 0.72 | 2.76 | 0.64 |
| G. MUCRONATUS | | | |
| Mean Day (days) | | | |
| N | 10 | 6 | 24 |
| Range | 00.0-1.00 | 0.92-1.83 | 0.0-1.50 |
| $\bar{x}$ | 0.48 | 1.28 | 0.77 |
| SD | 0.44 | 0.43 | 0.08 |
| Female Size (mm) | | | |
| N | 10 | 6 | 24 |
| Range | 6.43-10.00 | 6.54-10.14 | 6.43-10.00 |
| $\bar{x}$ | 8.06 | 8.83 | 7.85 |
| SD | 1.27 | 1.34 | 0.98 |
| M. NITIDA | | | |
| Mean Day (days) | | | |
| N | 13 | 3 | 11 |
| Range | 0.0-1.00 | 0.0-0.78 | 0.0-1.00 |
| $\bar{x}$ | 0.33 | .32 | 0.60 |
| SD | 0.44 | | 0.48 |
| Female Size (mm) | | | |
| N | 8 | 1 | 11 |
| Range | 5.00-8.71 | 6.71 | 6.57-8.57 |
| $\bar{x}$ | 6.89 | | 7.59 |
| SD | 1.26 | | 0.83 |

* N = number of females
$\bar{x}$ = mean
SD = standard deviation

The length of the average mean day of emergence is associated with location of the species in the littoral zone. The higher the species, the longer is the mean day. If all Gammarus palustris females are assigned the number 1, Gammarus mucronatus females number 2, and Melita nitida females number 3, the regression coefficients of mean day against location in the littoral zone are significant for field broods at 21° C, for laboratory broods at 17° C, and for laboratory broods at 21° C ($b = -0.886$, $F_{(1,35)}=22.422$; $b = -0.600$, $F_{(1,22)}=22.798$; $b = -0.678$, $F_{(1,58)}=28.270$, respectively) (Figure 8).

C. Differences in Reproductive Characteristics Within Species

1. Number of Juveniles per Brood

The number of juveniles per brood increased with an increase in female size for each of the three species. In field broods, the correlation coefficient of number of juveniles and female size was significant for Gammarus palustris and Gammarus mucronatus ($r = 0.949$, $t = 12.47$, d.f. = 17; $r = 0.696$, $t = 3.49$, d.f. = 13, respectively), and, though not significant for Melita nitida ($t = 0.751$, d.f. =8), the regression coefficient (0.540) was positive (Figures 9,10,11).

2. Mean Days of Emergence

Within Gammarus mucronatus (Figure 12) and Gammarus palustris (Figure 13), the mean day of emergence was correlated with the length of time between hatching of the brood and the

Figure 8

Regression lines of average mean day of emergence vs. location in the intertidal zone.

Legend:

1 = Gammarus palustris, found primarily at the high tide mark.

2 = Gammarus mucronatus, found primarily at the mean tide mark.

3 = Melita nitida, found primarily at the low tide mark.

O = the average mean day of emergence of field broods at 21° C.

▲ = the average mean day of emergence of laboratory broods at 17° C.

X = the average mean day of emergence of laboratory broods at 21° C.

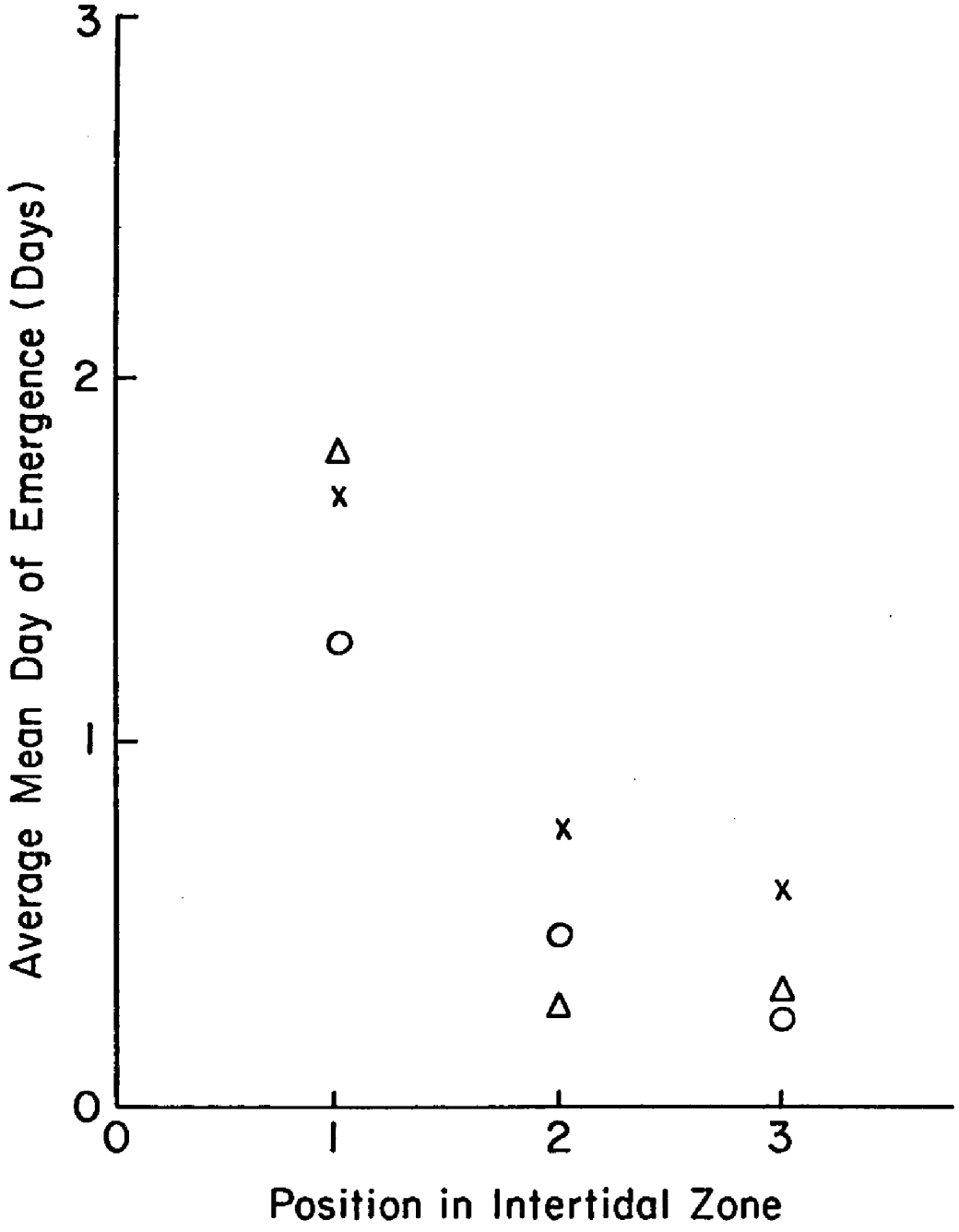

Average Mean Day of Emergence (Days)
3
2
1
0
0
1
2
3
Position in Intertidal Zone

Figure 9

Field broods of Gammarus palustris females: number of juveniles vs. total female length.

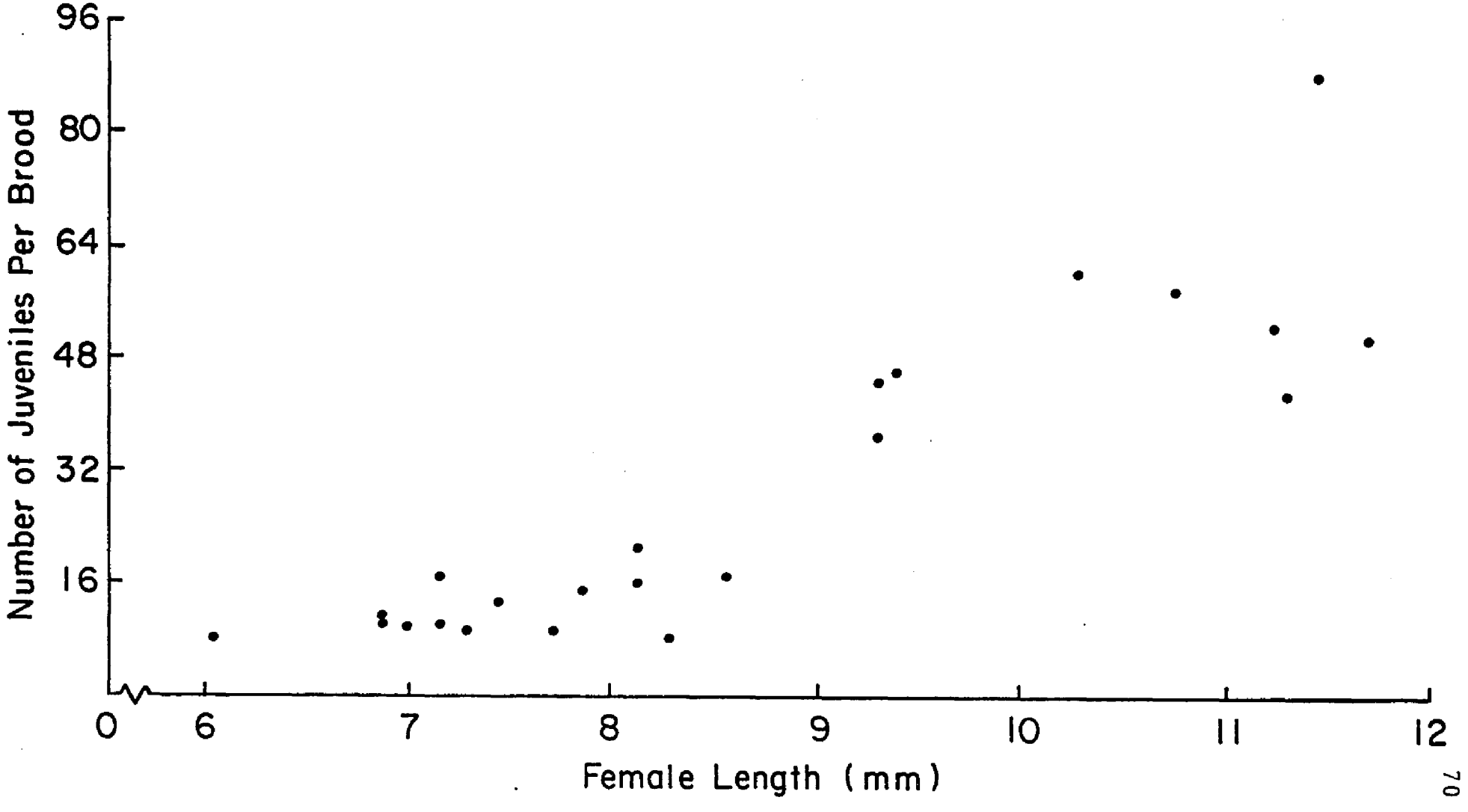

96
80
64
48
32
16
Number of Juveniles Per Brood
0
6
7
8
9
10
11
12
Female Length (mm)

Figure 10

Field broods of Gammarus mucronatus females: number of juveniles vs. total female length.

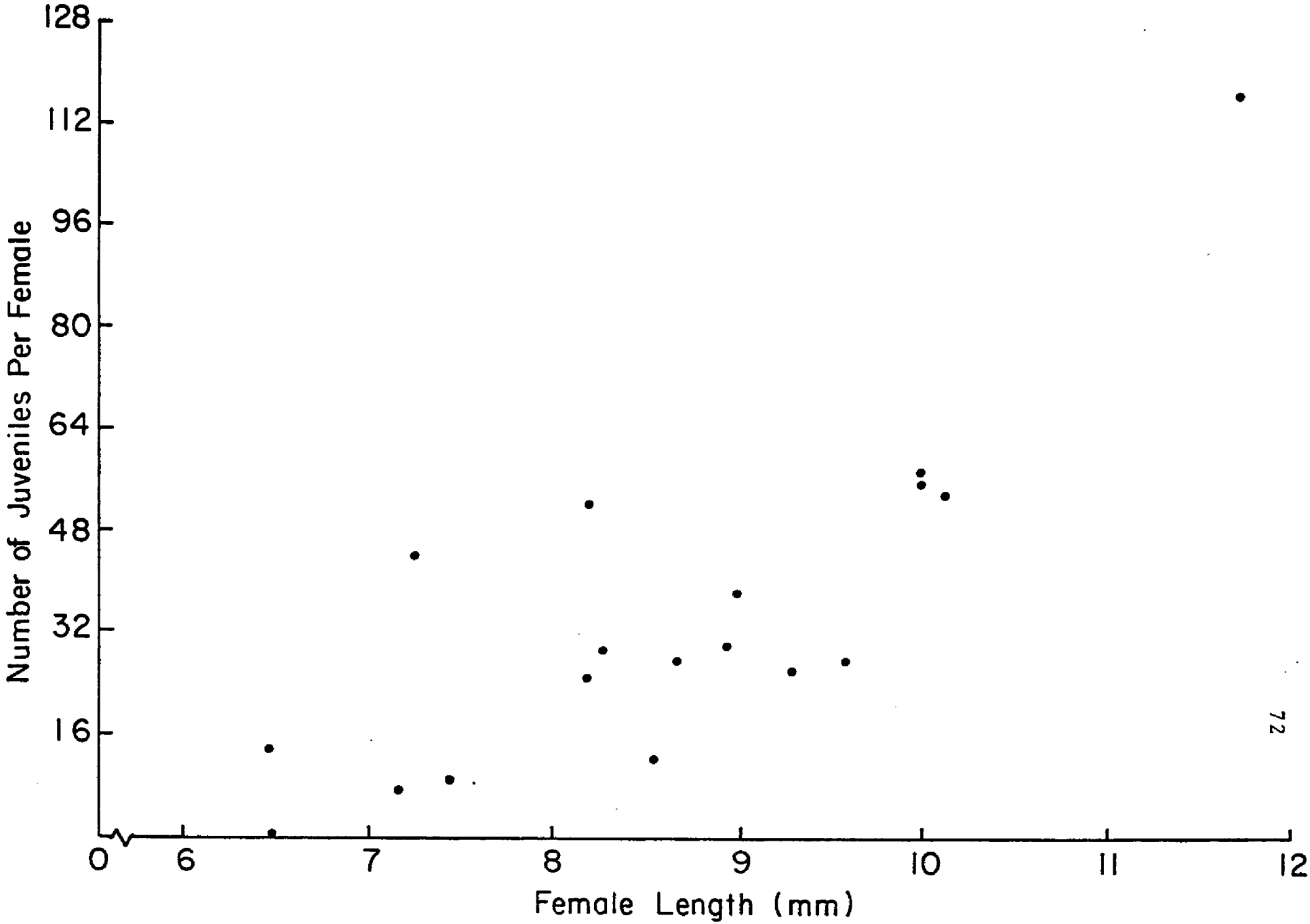

128
112
96
80
64
48
32
16
Number of Juveniles Per Female
0
6
7
8
9
10
11
12
Female Length (mm)

Figure 11

Field broods of Melita nitida females: number of juveniles vs. total female length.

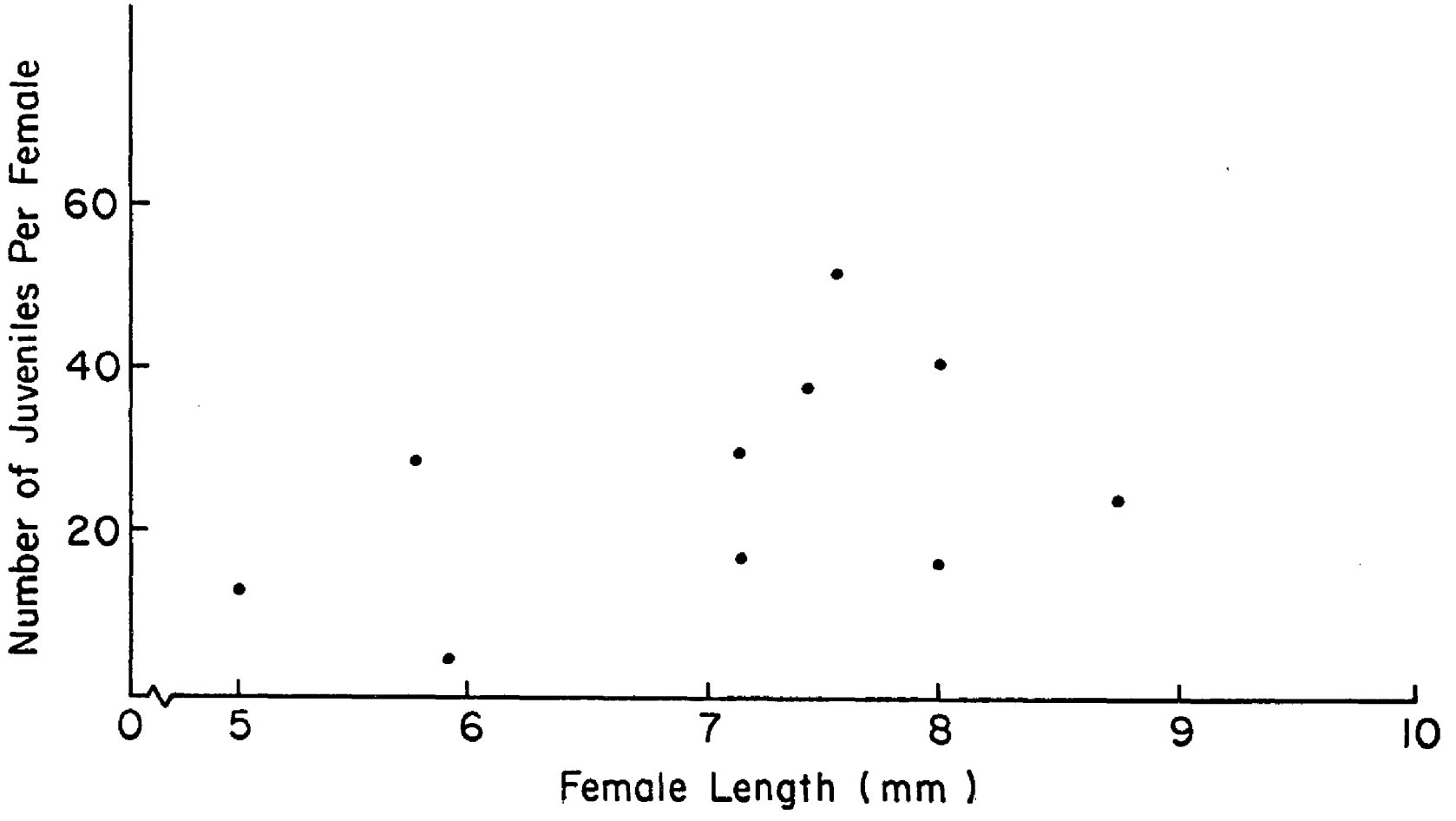

Number of Juveniles Per Female
60
40
20
0
5
6
7
8
9
10
Female Length ( mm )

Figure 12

The mean day of emergence of the brood vs. the day the mother molts in Gammarus palustris.

Legend: The line connects the days of the female molt and indicates the latest time that a juvenile can emerge.

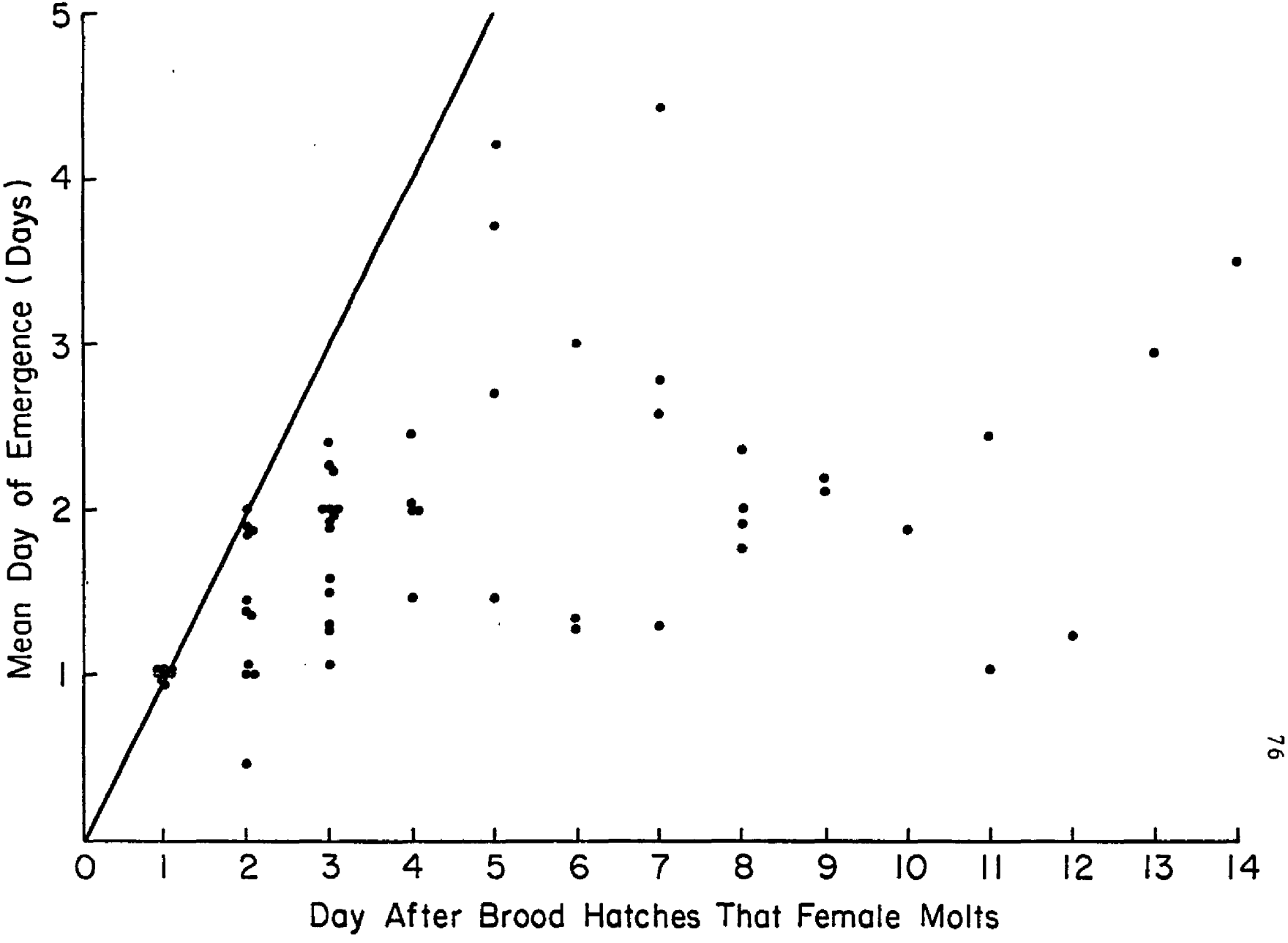
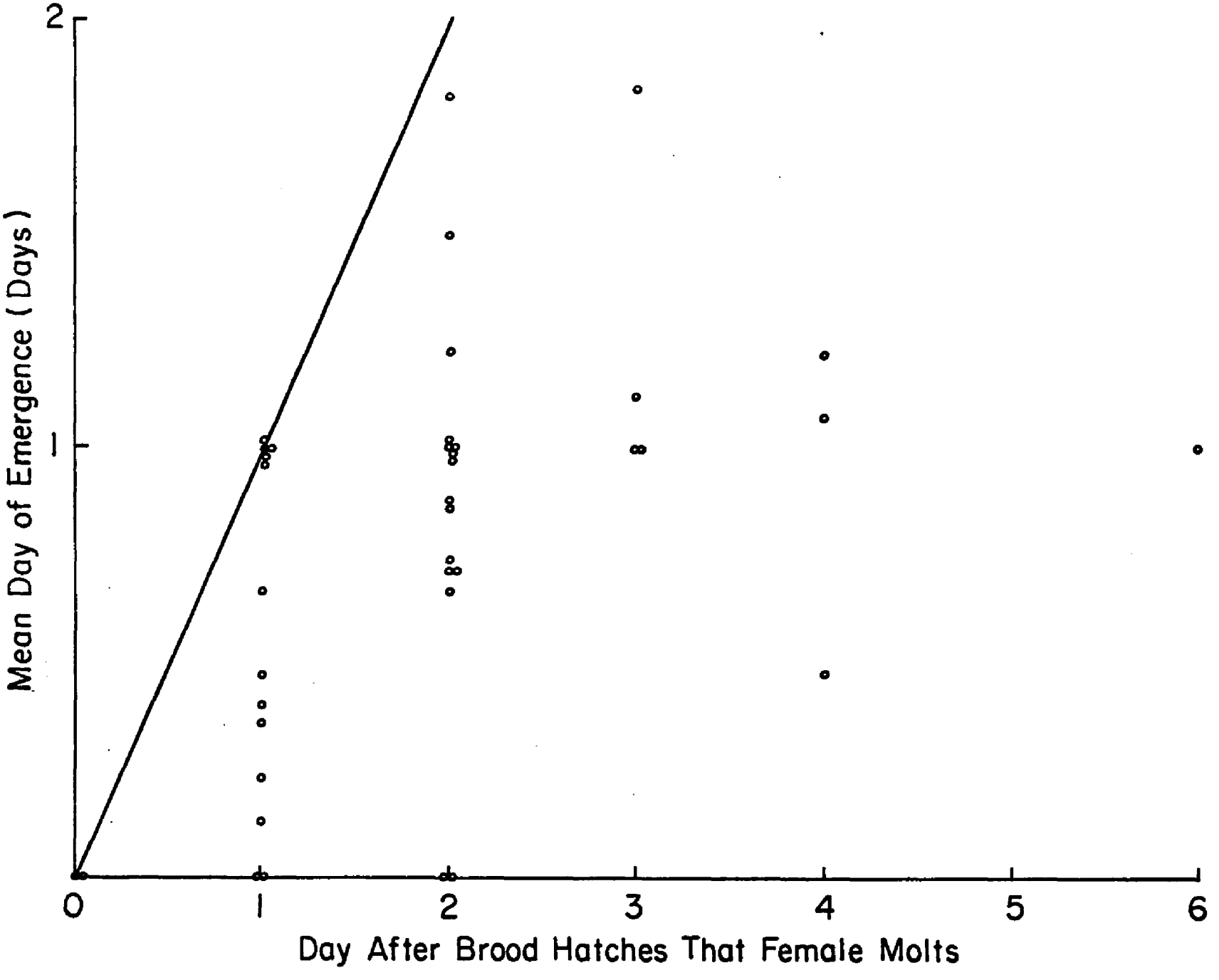

Mean Day of Emergence (Days)
5
4
3
2
1
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Day After Brood Hatches That Female Molts

Figure 13

The mean day of emergence of the brood vs. the day the mother molts in Gammarus mucronatus.

Legend: The line connects the days of the female molt and indicates the latest time that a juvenile can emerge.


2
1
Mean Day of Emergence (Days)
0
1
2
3
4
5
6
Day After Brood Hatches That Female Molts

next female molt. In G. palustris, when all broods were combined, the correlation was significant (r=0.451, t=3.88, d.f.=59). Broods of females that molted on day three or earlier tended to remain in the marsupium until the female molted. If all the broods are divided into two groups: (1) broods whose mothers molted between zero and three days; and (2) broods whose mothers molted between four and 14 days, the correlation is significant for the former (r=0.597, t=4.14, d.f.=31), but not the latter group (r=0.021, t=0.11, d.f.=26).

The average mean day of emergence was 2.26 days for all broods whose mothers molted on day four or later. These data suggest that the average juvenile of Gammarus palustris would remain in the marsupium for 2.26 days if the female's molt did not precipitate premature emergences. The broods whose mothers molted between zero and three days remained within the marsupium until cast out coincidental with the female's molt (Figure 12).

A similar relationship holds for Gammarus mucronatus. When all the broods were combined, the correlation between mean day of emergence and number of days between hatch and the female molt was significant (r=.449, t=3.06, d.f.=37). All the broods are divided into two groups: (1) those whose mothers molted between days zero and one; and (2) those whose mothers molted between days two and six. The correla-

tion was significant for the former group, but not the latter ($r=0.501$, $t=2.17$, d.f.=13; $r=0.124$, $t=.59$, d.f.=22, respectively). In this species, the average mean day of emergence was 0.95 days in females that molted on day two or later (Figure 13).

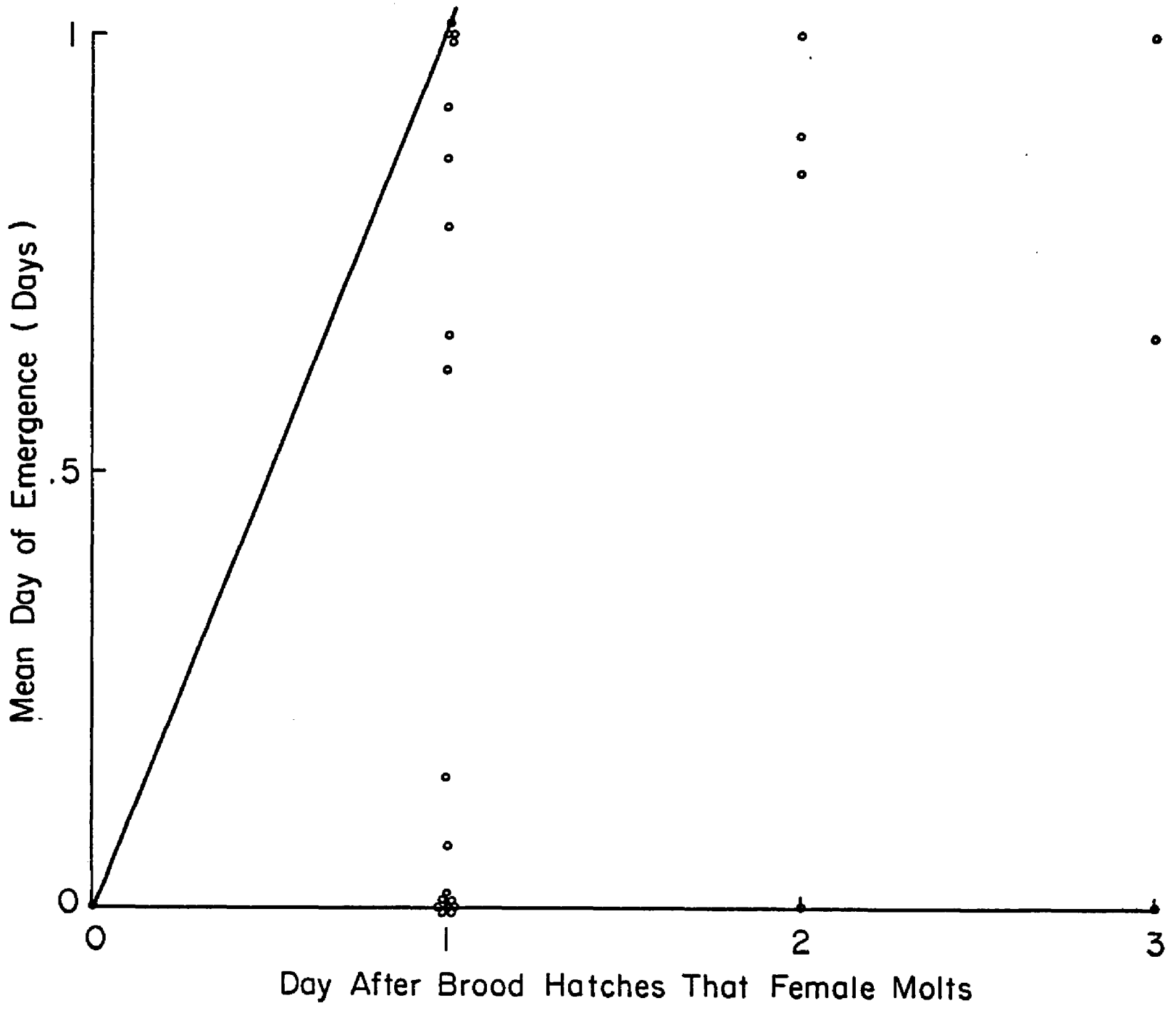
Thus, the relationship between the length of time after hatching that the female molts, and the mean day of emergence, is similar for Gammarus palustris and Gammarus mucronatus. The important difference between the two species is that the mean day of emergence and the length of time from hatching to the female molt was shorter for G. mucronatus than for G. palustris. The relationship between the length of time after hatching that the female molts and the mean day of emergence was different in Melita nitida, however. When all broods were combined, the correlation coefficient was not significant ($r=0.225$, $t=1.16$, d.f.=25). The mean length of time between hatching and the female molt was short; most females molted about a day after the brood hatched (Figure 14).

For Gammarus palustris and Melita nitida there were no significant correlations between mean day of emergence and (1) female size, or (2) number of juveniles in field and laboratory broods. Nor was there a significant difference between the mean days of emergence of laboratory broods raised at 17° C and 21° C (Student's t test, $t=1.41$, d.f.=36; $t=.90$, d.f.=12, respectively).

Figure 14

The mean day of emergence of the brood vs. the day the mother molts in Melita nitida.

Legend: The line connects the days of the female molt and indicates the latest time that a juvenile can emerge.


Mean Day of Emergence ( Days )
1
.5
0
0
1
2
3
Day After Brood Hatches That Female Molts

In Gammarus mucronatus, however, the correlation coefficient between mean day of emergence and female size was significant in laboratory broods but not in field broods (Table 9). The mean days of emergence were significantly longer in laboratory broods at 17° C than at 21° C (Student's t test; t=2.71, d.f.=28). However, because the females were larger at 17° C than at 21° C (t=2.05, d.f.=28), the difference in mean days of emergence may be due either to size, temperature, or both. The data are insufficient to distinguish between the two factors.

Under laboratory conditions, then, the most important factor that influences the mean day of emergence of a brood of Gammarus palustris and Gammarus mucronatus is the length of time from juvenile hatching to the female's molt.

D. Differences in Days of Emergence of Juveniles Within a Brood

In Melita nitida, the time of emergence of a given juvenile within a brood: (1) appears to be relatively fixed; (2) occurs within a day of hatching; and (3) is almost immediately followed by the molt of the mother. Even among those females which molted on Day Two, there were no juveniles inside the marsupium by Day Two (Figure 14). Further, most juveniles of a given brood emerged on the same day; females which molted on Day Two had 83% or more juveniles emerge on the same day (Figure 15). Sixty-eight percent of the females molted on the day after the brood hatched (Figure 16).

Figure 15

Broods of six Melita nitida females that molt on Day Two after their brood hatches: the percent of the total number of juveniles of a brood that emerges each day after hatching.

Legend:

● = the percent of each brood that emerged on that day

$\not{X}$ = the mean of the percents of the six broods that emerged on that day.

Percent of Juveniles That Emerge
0
25
50
75
100
Day After Brood Hatches
1
2

Figure 16

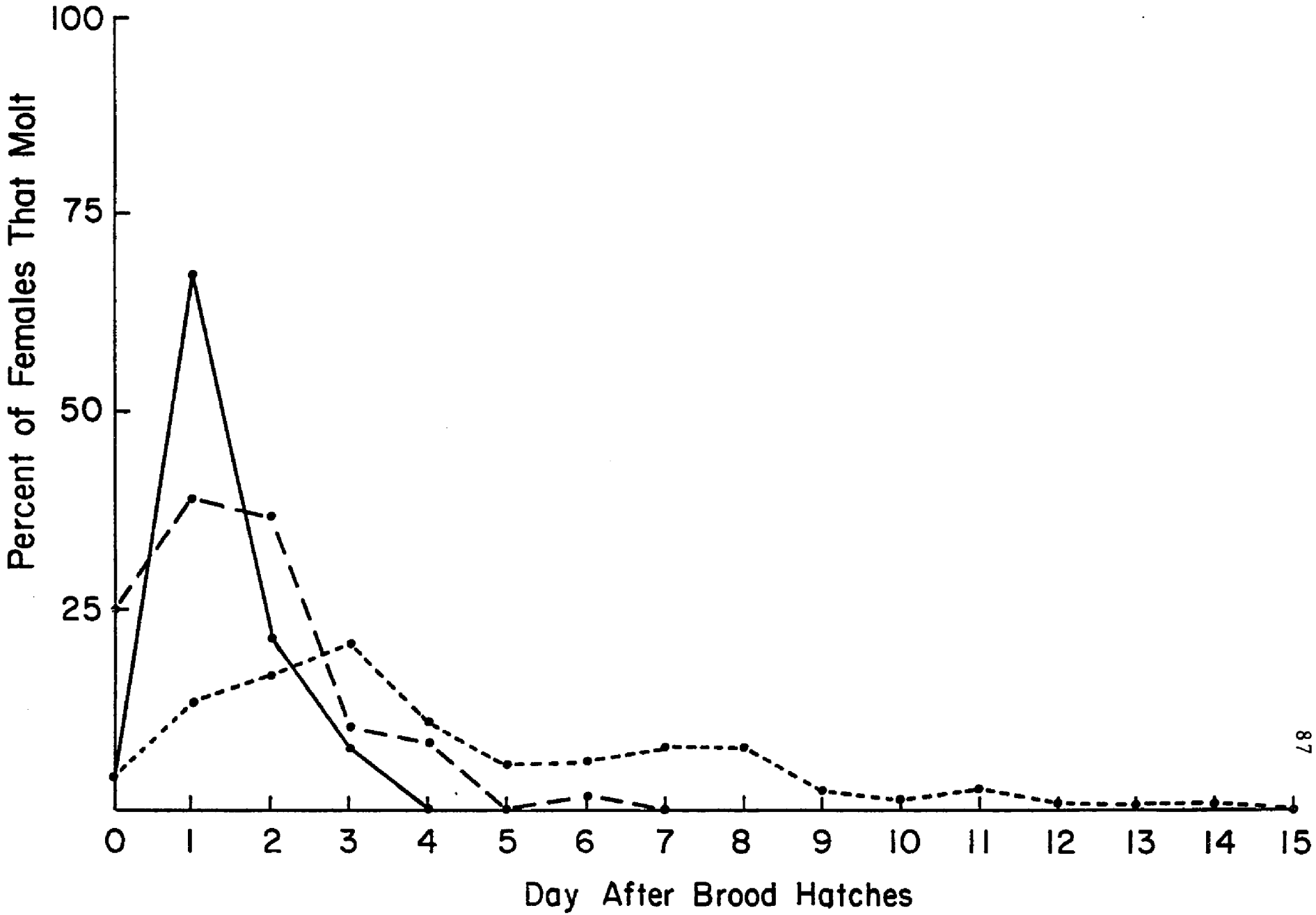
*Melita nitida*, *Gammarus mucronatus*, and *Gammarus palustris* females: the percent of the total number of females that molt each day beginning the day of hatching of their brood.

Legend:

unbroken line: *Melita nitida* females

dashed line: *Gammarus mucronatus* females

dotted line: *Gammarus palustris* females


100
75
50
25
Percent of Females That Molt
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Day After Brood Hatches

## TABLE 9

CORRELATIONS OF: (1) MEAN DAY OF EMERGENCE AND FEMALE SIZE; AND (2) MEAN DAY OF EMERGENCE AND NUMBER OF JUVENILES IN THE BROOD FOR GAMMARUS PALUSTRIS, GAMMARUS MUCRONATUS, AND MELITA NITIDA

| | | GAMMARUS PALUSTRIS | | | GAMMARUS MUCRONATUS | | | MELITA NITIDA | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | A | B | C | A | B | C |
| FEMALE SIZE AND MEAN DAY | r | .092 | -.446 | -.093 | -.152 | .210 | .293 | .230 | sample too small | .035 |
| | t | 0.292 | -0.130 | 0.396 | 0.376 | .429 | 1.439 | .461 | | 0.093 |
| | df | 12 | 12 | 20 | 8 | 4 | 22 | 6 | | 9 |
| NUMBER OF JUVENILES AND MEAN DAY | r | -.215 | -.377 | -.337 | -.285 | .461 | .012 | .091 | | -.301 |
| | t | 0.694 | 0.924 | -1.639 | 0.728 | 0.737 | 0.094 | 0.274 | | 0.835 |
| | df | 12 | 13 | 21 | 8 | 4 | 22 | 11 | | 9 |

* r = correlation coefficient

t = t statistic for significance of difference from 0 of "r"

df = degrees of freedom

A = field broods at 21°C

B = Laboratory broods at 17°C

C = Laboratory broods at 21°C

Gammarus mucronatus exhibited a wider range of juvenile emergence times, as well as a longer mean time between the day the brood hatched and the day the female molted than did Melita nitida. Most emergences occurred on the day after hatching, regardless of how long it took the female to molt (Figures 17 and 18). However, if the female molted relatively late (four to six days), a small number of juveniles remained in the marsupium in some broods (Figure 18). Further, most broods emerged over the course of two or more days; only 13 of 22 females that molted on Day Two or later had 83% or more of their juveniles emerging on the same day. Forty-two percent of the females molted the day after hatching (Figure 16).

Gammarus palustris exhibited a wider range of times a given juvenile might emerge, as well as a longer mean time between the day the brood hatched and the day the female molted than did Gammarus mucronatus. The greatest number of emergences occurred on Days One and Two after hatching (Figures 19 and 20). Yet, some juveniles of females that molted relatively late remained inside the marsupium many days beyond Day Two (Figure 20). The latest that a juvenile was observed to emerge was eight days after hatching. Further, almost all broods emerged over the course of two or more days; only four of the 33 females that molted on Day Four or later

Figure 17

Broods of fourteen Gammarus mucronatus females that molt on Day Two after the brood hatches: the percent of the total number of juveniles of a brood that emerges each day after hatching.

Legend: • = the percent of each brood that emerged on that day

X = the mean of the percents of the fourteen broods that emerged on that day

Percent of Juveniles That Emerge
25
50
75
100
0
1
2
Day After Brood Hatches

Figure 18

Broods of four Gammarus mucronatus females that molt on Days Four and Six after the brood hatches: the percent of the total number of juveniles of a brood that emerges each day after hatching.

Legend: • = the percent of each brood that emerged on that day

X = the mean of the percents of the four broods that emerged on that day

Percent of Juveniles That Emerge
25
50
75
100
0
1
2
3
4
5
6
Day After Brood Hatches

Figure 19

Broods of eight Gammarus palustris females that molt on Day Four after the brood hatches: the percent of the total number of juveniles of a brood that emerges each day after hatching.

Legend: • = the percent of each brood that emerged on that day

× = the mean of the percents of the eight broods that emerged on that day

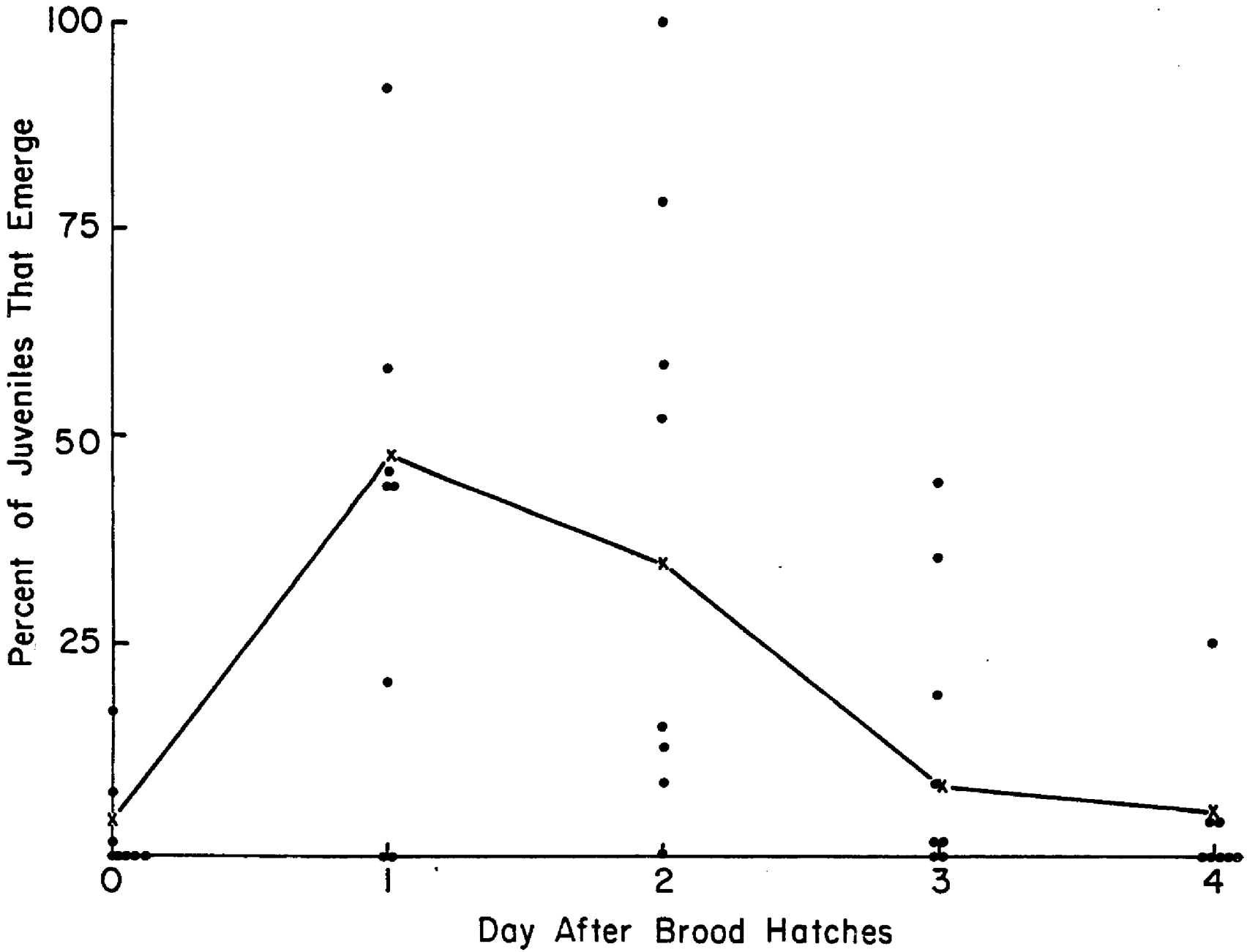
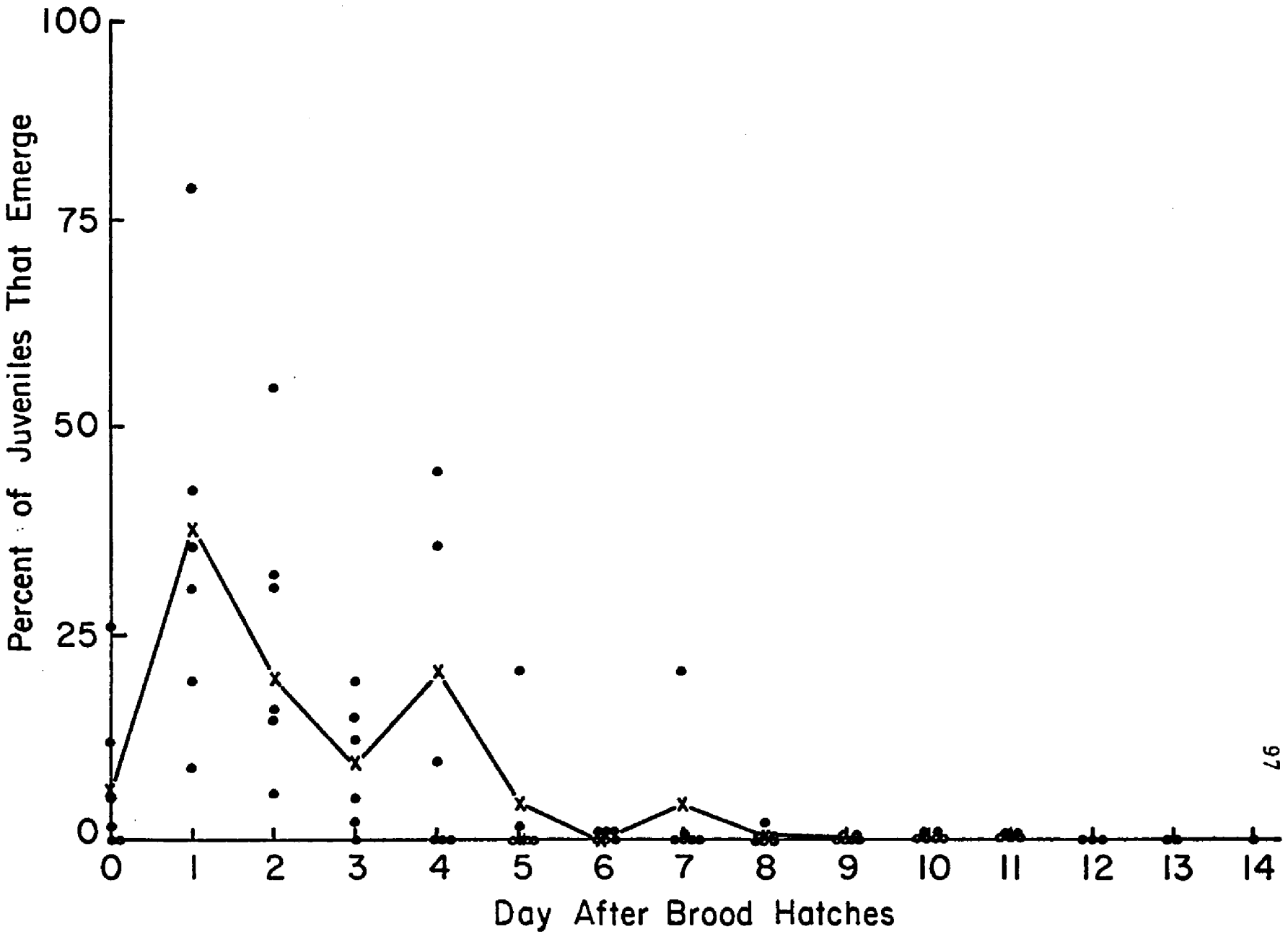

100
75
50
25
0
1
2
3
4
Percent of Juveniles That Emerge
Day After Brood Hatches

Figure 20

Broods of six Gammarus palustris females that molt on Days Ten to Fourteen after the brood hatches: the percent of the total number of juveniles of a brood that emerges each day after hatching.

Legend: • = the percent of each brood that emerged on that day

$\times$ = the mean of the percents of the six broods that emerged on that day


100
75
50
25
0
Percent of Juveniles That Emerge
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Day After Brood Hatches

had 83% of their juveniles emerge on the same day, and only 13% of the females molted on the second day after hatching (Figure 16).

These results show that the time of emergence of a given juvenile is relatively fixed for Melita nitida, is somewhat variable for Gammarus mucronatus, and is most variable for Gammarus palustris. Thus, it was shown that the higher in the littoral zone the species is found, the more variable is the day of emergence of a given juvenile.

## III. The Effects of Food on Juvenile Emergence in Gammarus Palustris

### A. Food Provided Ad Libitum

Since female size, number of juveniles in the brood and number of days from juvenile hatching to female molt might influence the mean day of emergence, these characters were measured. There was no significant difference in female head sizes, number of juveniles in the broods and number of days from hatching of the brood to the female molt among the five feeding groups tested (analysis of variance $F_{(4,29)}=2.057$, 1.179, 1.648 respectively)(Table 10).

The mean day of emergence was related to the variety of foods provided the females. The greater the variety of foods provided, the longer was the mean day of emergence. Although there was no significant difference in the mean days

## TABLE 10

GAMMARUS PALUSTRIS FED AD LIBITUM: FEMALE HEAD SIZES, NUMBER OF JUVENILES IN BROODS, AND NUMBER OF DAYS FROM BROOD HATCHING TO FEMALE MOLT

| | HEAD SIZES (mm) | | | | NUMBER JUVENILES | | | | NUMBER DAYS HATCH TO MOLT (DAYS) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FOOD GROUP | N | Range | $\bar{x}$ | SD | N | $\bar{x}$ | Range | SD | N | $\bar{x}$ | Range | SD |
| Unfed | 6 | 0.95-1.09 | .99 | 0.06 | 7 | 19.0 | 13-28 | 5.5 | 6 | 4.2 | 3-6 | 1.2 |
| *Ulva* Only | 7 | 0.87-1.02 | 0.94 | 0.05 | 7 | 15.4 | 10-20 | 3.9 | 7 | 3.1 | 2-5 | 1.1 |
| Mussel Meat Only | 6 | 0.76-1.09 | 0.92 | 0.12 | 6 | 18.2 | 10-24 | 5.5 | 6 | 3.8 | 2-7 | 1.7 |
| Mussel Meat; *Ulva* No Transfer | 7 | 0.80-0.98 | 0.90 | 0.06 | 7 | 13.3 | 2-22 | 8.8 | 7 | 3.1 | 2-5 | 1.1 |
| Mussel Meat; *Ulva* With Transfer | 7 | 0.91-1.06 | 0.98 | 0.05 | 7 | 14.6 | 9-21 | 3.9 | 7 | 4.6 | 2-7 | 1.9 |

* N = number of females

$\bar{x}$ = mean

SD = standard deviation

of emergence among the five food groups tested (analysis of variance $F_{(4,28)}=1.476$) (Table 11), the regression coefficient of mean day of emergence of foods fed to the female was significant ($b=0.351$, $F_{(2,31)}=6.063$) (Figure 21).

There was no significant difference between the mean days of emergence of groups of females fed the same number of foods. There were two groups of females fed two foods: a group that was transferred daily into fresh sea water, and a group that was kept in the same dishes through the experiment. Both groups were fed Ulva and mussel meat daily, and there was no significant difference between their mean days of emergence (Mann-Whitney U test, $U_{(7,7)} = 22$). Further, there was no significant difference between the mean days of emergence of females fed Ulva only, and females fed mussel meat only ($U_{(6,7)} = 20$). There was a significant difference between the mean days of emergence of the unfed group and the four fed groups combined (Student's t test, $t=1.85$, d.f.=32).

Thus: (1) the presence of any food lengthened the stay of juveniles inside the marsupium and (2) the greater the variety of foods provided to the female after the brood hatched, the longer the juveniles remained in the marsupium.

B. Observations on the Variety of Food Consumed in the Field.

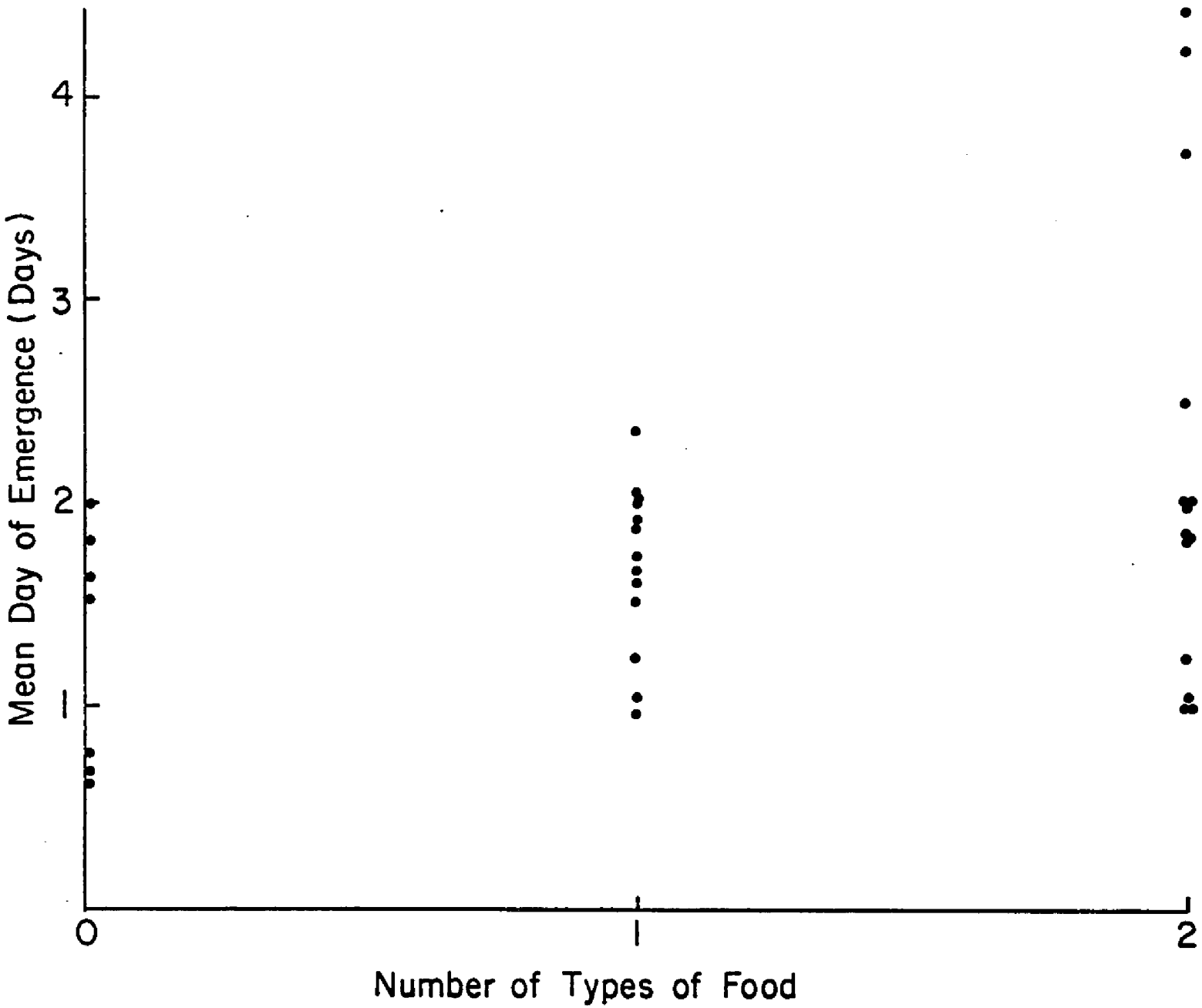
TABLE 11

*GAMMARUS PALUSTRIS* FED AD LIBITUM: MEAN DAY OF EMERGENCE OF BROODS OF FEMALES UNDER DIFFERENT FOOD REGIMES

| Food Group | N | Range | $\bar{x}$ | SD |
|---|---|---|---|---|
| Unfed | 7 | .63-2.00 | 1.29 | .60 |
| *Ulva* only | 7 | .95-2.39 | 1.74 | .46 |
| Mussel Meat Only | 6 | 1.06-2.04 | 1.65 | .41 |
| Mussel Meat and *Ulva* - No Transfer | 7 | 1.00-4.21 | 2.07 | 1.08 |
| Mussel Meat and *Ulva* - With Transfer | 7 | 1.00-4.43 | 2.31 | 1.27 |

Figure 21

Mean days of emergence of broods of females of Gammarus palustris fed different types of foods ad libitum vs. the number of types of foods provided.


Mean Day of Emergence (Days)
4
3
2
1
0
1
2
Number of Types of Food

Laboratory observations showed that fecal color depended upon what the animal had eaten. Animals fed only Ulva produced only Ulva-green feces, and animals fed only mussel meat produced only feces that were clear to slightly milky. Feces from animals cultured in dishes overgrown with blue-green algae and benthic diatoms were a khaki green-brown color.

Feces from field-collected animals were placed in one of six color categories (Figure 22). Variation in fecal color showed that the animals were eating a variety of foods in the field. First, most feces were mixtures of colors. Color I, the greatest proportion of the feces (86%), was itself a mixture of colors. Second, most couples (14 of 18) produced feces of more than one color.

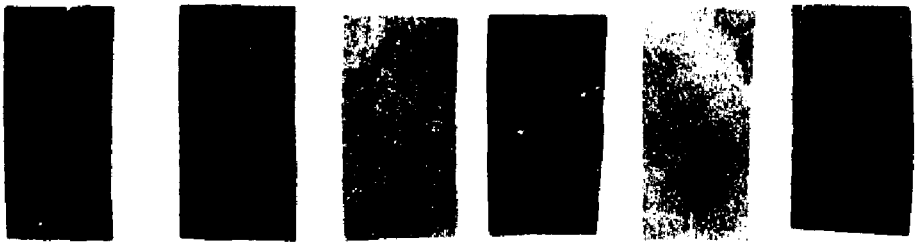
Color I resembles the color of feces of animals grown in dishes with benthic diatoms and blue-green algae. The next most common color was Color IV, which resembles the color of feces of animals grown on Ulva and aufwuchs only. Color V resembled the color of feces of animals grown on mussel meat only. It was only one percent of the total and was only produced by one couple.

## C. The Effects of Introduced Mussel Meat on Juvenile Emergence

Figure 22

Fecal colors of eighteen precopulating couples of Gammarus palustris.

Color




| | I | II | III | IV | V | VI |
|---|---|---|---|---|---|---|
| Length of feces (mm) all couples combined | 126.4 | 2.8 | 4.6 | 9.4 | 0.8 | 3.2 |
| Percent of the total feces | 86.0 | 2.0 | 3.0 | 6.0 | 1.0 | 2.0 |

Forty-one females were divided into test categories as shown in Table 12. There were no significant differences among the head sizes of the females, the number of juveniles in the brood, or the time from hatching of the brood until the females' molt, neither among test day groups nor between fed and unfed females (Tables 12 and 13).

1. The Effects of Transferring the Female

On any test day, there was no significant difference between the number of juveniles that emerged from unfed broods and fed broods after transfer (Students' t test: Day 0, $t = 0.33$, d.f.=39; Day I, $t=1.29$, d.f.=28; Day II, $t=1.29$, d.f.=7). Therefore, data from both food regimes were combined (Table 14).

Transferring the females to new dishes did stimulate emergence. The mean number of juveniles that emerged per minute during the first 15 minutes after transfer was one order of magnitude greater than the number that emerged during the previous 24 hours for both Day I and Day II (Table 15).

2. The Effects of Mussel Meat

Mussel meat was introduced into the dishes of 11 females from the unfed and 21 females from the fed group. Fewer females from the fed group were tested because seven of the original 18 females from the unfed groups, and two of the original 23 females from the fed groups had no juveniles left in their marsupia by the time of the designated test day.

## TABLE 12

### THE EFFECTS OF MUSSEL MEAT ON JUVENILE EMERGENCE IN GAMMARUS PALUSTRIS: FEMALE CHARACTERISTICS

| | HEAD SIZES | | | | NUMBER JUVENILES | | | | NUMBER DAYS HATCH TO MOLT | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| UNFED FEMALES | N | Range | $\bar{x}$ | SD | N | Range | $\bar{x}$ | SD | N | Range | $\bar{x}$ | SD |
| Test Day 0 | 5 | 0.95-1.09 | 0.99 | 0.06 | 6 | 6-26 | 16.7 | 7.3 | 5 | 2-7 | 3.6 | 1.9 |
| Test Day 1 | 5 | 0.91-1.02 | 0.97 | 0.04 | 5 | 12-24 | 18.2 | 4.4 | 5 | 2-10 | 5.6 | 3.6 |
| Test Day 2 | 7 | 0.84-1.02 | 0.95 | 0.06 | 7 | 11-18 | 15.3 | 3.1 | 6 | 2-11 | 5.2 | 3.5 |
| ULVA FED FEMALES | | | | | | | | | | | | |
| Test Day 0 | 8 | 0.76-1.06 | .95 | 0.10 | 8 | 10-31 | 21.5 | 7.5 | 8 | 3-5 | 3.4 | 0.7 |
| Test Day 1 | 7 | 0.80-1.09 | .91 | 0.09 | 7 | 10-26 | 16.0 | 5.2 | 6 | 3-6 | 4.0 | 1.1 |
| Test Day 2 | 8 | 0.91-1.09 | .99 | 0.07 | 8 | 6-30 | 19.1 | 8.3 | 7 | 3-5 | 3.6 | 0.8 |

* N = number of females

$\bar{x}$ = mean

SD = standard deviation

TABLE 13

THE EFFECTS OF MUSSEL MEAT ON JUVENILE EMERGENCE IN *GAMMARUS PALUSTRIS*: RESULTS OF ANOVA's TESTING DIFFERENCES IN FEMALE CHARACTERISTICS

| | Between Fed and Unfed Females | Among the 3 Test Days of the Unfed Females | Among the 3 Test Days of the Fed Females |
|---|---|---|---|
| HEAD SIZE | $F_{(1,40)} = 0.140$ | $F_{(2,21)} = 1.960$ | $F_{(2,16)} = 1.130$ |
| NUMBER OF JUVENILES | $F_{(1,39)} = 1.493$ | $F_{(2,21)} = 1.090$ | $F_{(2,16)} = 0.470$ |
| NUMBER OF DAYS FROM HATCH TO MOLT | $F_{(1,35)} = 2.876$ | $F_{(2,18)} = 0.903$ | $F_{(2,16)} = 1.500$ |

## TABLE 14

THE EFFECTS OF MUSSEL MEAT ON JUVENILE EMERGENCE IN *GAMMARUS PALUSTRIS*: NUMBER OF JUVENILES THAT EMERGED DURING 15 MINUTES AFTER TRANSFER OF FEMALES TO NEW DISHES

| | DAY 0 | | | | DAY 1 | | | | DAY 2 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Range | $\bar{x}$ | SD | N | Range | $\bar{x}$ | SD | N | Range | $\bar{x}$ | SD |
| FED FEMALES | 23 | 0-2 | 0.2 | 0.5 | 15 | 0-12 | 1.9 | 3.2 | 6 | 0-1 | 0.2 | 0.4 |
| UNFED FEMALES | 18 | 0-1 | 0.2 | 0.4 | 9 | 0-1 | 0.4 | 0.5 | 3 | 0-4 | 1.3 | 2.3 |
| ALL FEMALES COMBINED | 41 | 0-2 | 0.2 | 0.5 | 24 | 0-12 | 1.3 | 2.6 | 9 | 0-4 | 0.6 | 1.3 |

* N = number of females
$\bar{x}$ = mean
SD = standard deviation

## TABLE 15

THE EFFECTS OF MUSSEL MEAT ON JUVENILE EMERGENCE IN *GAMMARUS PALUSTRIS*: A COMPARISON OF THE RATE OF EMERGENCE DURING THE 24 HOURS BEFORE TRANSFER AND 15 MINUTES AFTER TRANSFER

| | N | Range | $\bar{x}$ | SD |
|---|---|---|---|---|
| DAY 1 | | | | |
| B | 27 | 0-19 | 5.0 | 5.3 |
| A | 23 | 0-12 | 1.3 | 2.6 |
| B(number/min) | 27 | 0-0.013 | 0.003 | 0.004 |
| A(number/min) | 23 | 0-0.800 | 0.084 | 0.178 |
| DAY 2 | | | | |
| B | 12 | 2-17 | 7.7 | 4.2 |
| A | 9 | 0-4 | 0.6 | 1.3 |
| B(number/min) | 12 | 0-0.012 | 0.006 | 0.003 |
| A(number/min) | 9 | 0-0.267 | 0.037 | 0.089 |

---

* B = Number of Juveniles that emerge during 24 hours after previous transfer

A = Number of Juveniles that emerge during 15 minutes after transfer

N = number of broods

$\bar{x}$ = mean

SD = standard deviation

Fed females behaved differently from unfed females upon encountering the mussel meat. All the unfed females, but only 15 of the 21 fed females, ate the meat. All 11 females from the unfed groups fed throughout the 21.5 minute observation period; but of the 15 fed females that ate during the observation period, four fed for one minute or less, four fed from two to ten minutes, and only seven of the 21 fed females ate for the entire 21.5 minutes. Thus, most females fed Ulva ad libitum ate mussel meat, but ate it for a shorter time than did unfed females.

No juveniles emerged from females that were not eating. In other words, for emergence to occur, the physical proximity of the meat was necessary. On the other hand, juveniles did not emerge from every female that fed. There was no significant difference between the number of fed females and the number of unfed females from which juveniles emerged while they were feeding (nine of 15 and six of 11 females, respectively: Chi Square Test: $\chi^2 = .113$, d.f. = 1).

After the female began to feed on the meat, it lay quiescent on the bottom of the dish. It was possible to observe the mechanism of juvenile emergence at that time. Observations were made through a binocular microscope. The female grasped the food with the gnathopods, thus holding it over the marsupium while feeding. The juveniles moved about

actively inside the marsupium, and many extended their heads out of the marsupium. Juveniles inside and outside the marsupium fed on the meat while the female held it. Juveniles emerged by crawling out between the oostegites without assistance from the female.

The data from the females of both feeding groups were combined because there was no significant difference between the groups with respect to: (1) the number of juveniles that emerged; (2) the percent of the total juveniles in the brood that emerged; or (3) the percent of juveniles remaining in the marsupium that emerged (Mann-Whitney U Test: $U_{(11,15)}=59$; $U_{(11,15)}=55$; $U_{(11,15)}=58$, respectively) (Table 16). Further, there was no significant difference in the number of juveniles that emerged among the three test days ($F_{2,23}=0.837$) (Table 17).

The female's feeding on meat has an immediate effect upon emergence; the mean number of juveniles that emerged during the first 1.5 minutes of observation (1.3) was significantly higher than during the next 20 minutes (0.8) (all days combined - $U_{(15,15)}=11.5$)(Table 17). Finally, the number of juveniles that emerged after transfer (0.3) was significantly less than the number that emerged after the female was fed on meat (1.6). This was determined by comparing the number of juveniles that emerged during the 15-minute period after the transfer of a given female in the absence of meat with the number of juveniles that emerged during the first 6.5

TABLE 16

THE EFFECTS OF MUSSEL MEAT ON JUVENILE EMERGENCE IN GAMMARUS PALUSTRIS: NUMBER AND PERCENT OF JUVENILES EMERGING WHILE FEMALES FED ON MUSSEL MEAT

| | Total Number of Juveniles in Brood | | | | Percent of Total Juveniles in Brood that Emerge | | | | Percent of Juveniles Remaining in Marsupium | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Range | $\bar{x}$ | SD | N | Range | $\bar{x}$ | SD | N | Range | $\bar{x}$ | SD |
| FED FEMALES | 15 | 0-7 | 2.7 | 2.7 | 15 | 0-50.0 | 6.1 | 6.2 | 15 | 0-53.8 | 19.1 | 19.7 |
| UNFED FEMALES | 11 | 0-3 | 1.2 | 1.3 | 11 | 0-11.8 | 14.8 | 16.3 | 11 | 0-25.0 | 8.3 | 9.2 |
| COMBINED | 26 | 0-7 | 2.0 | 2.3 | 26 | 0-50.0 | 11.1 | 13.5 | 26 | 0-53.8 | 14.5 | 16.8 |

* N = number of females
$\bar{x}$ = mean
SD = standard deviation

## TABLE 17

THE EFFECTS OF MUSSEL MEAT ON JUVENILE EMERGENCE IN *GAMMARUS PALUSTRIS*: NUMBER OF JUVENILES THAT EMERGE BETWEEN 0 AND 1.5, AND BETWEEN 1.5 AND 20 MINUTES AFTER MEAT IS INTRODUCED

| | DAY 0 | | | DAY 1 | | | | DAY 2 | | | | ALL DAYS COMBINED | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Range | $\bar{x}$ | SD | N | Range | $\bar{x}$ | SD | N | Range | $\bar{x}$ | SD | N | Range | $\bar{x}$ | SD |
| Total Number of Juveniles That Emerge During 21.5 Minutes | 11 | 0-5 | 1.5 | 1.9 | 8 | 0-7 | 3.4 | 2.3 | 7 | 0-7 | 1.4 | 2.6 | 26 | 0-7 | 2.0 | 2.3 |
| Number That Emerge During First 1.5 Minutes | 11 | 0-4 | 1.0 | 1.8 | 8 | 0-4 | 1.8 | 1.9 | 7 | 0-5 | 1.1 | 3.0 | 26 | 0-5 | 1.3 | 1.5 |
| Number That Emerge During Last 20 Minutes | 11 | 0-3 | 0.5 | 0.8 | 8 | 0-5 | 1.0 | 1.8 | 7 | 0-2 | 0.3 | 0.5 | 26 | 0-5 | 0.8 | 1.4 |

* N = number of broods

$\bar{x}$ = mean

SD = standard deviation

minutes of the observation period following the introduction of meat to that same female (Paired t Test, all days combined; t=3.35, d.f. = 25) (Table 18).

## IV. The Effects of Exposure and Immersion on Juvenile Emergence in Gammarus palustris

### A. The Effects of Exposure on Zero to One Day Old Juveniles

In order to test the effects of exposure to air on newly hatched juveniles, a total of 134 juveniles from the broods of ten females were divided into groups as shown in Table 19. Of the 91 juveniles placed with the mothers, 27 (30%) reentered the marsupium after two hours. Forty-three juveniles were not replaced with their mothers. Thus, 27 juveniles were exposed to the air while within the female's marsupium, and 107 were exposed to the air outside the marsupium. Of the 27 within the marsupium, 24 survived to emerge, one died, and two were missing. Of the 107 exposed outside the female, only one survived beyond 24 hours. All of the females survived to their next molt. Thus, juveniles between zero and one day after hatching survive exposure to the air better inside the marsupium than outside it.

TABLE 18

THE EFFECTS OF MUSSEL MEAT ON JUVENILE EMERGENCE IN GAMMARUS PALUSTRIS: NUMBER OF JUVENILES THAT EMERGE (A) DURING THE FIRST 15 MINUTES AFTER TRANSFER, AND (B) DURING THE 6.5 MINUTES AFTER FEMALES BEGIN FEEDING ON INTRODUCED MUSSEL MEAT

| Number That Emerge 15 Minutes After Transfer | Number That Emerge 6.5 Minutes After the Introduction of Mussel Meat |
|---|---|
| 0 | 0 |
| 0 | 0 |
| 0 | 3 |
| 0 | 2 |
| 0 | 0 |
| 0 | 1 |
| 0 | 1 |
| 0 | 3 |
| 1 | 0 |
| 0 | 0 |
| 0 | 2 |
| 0 | 0 |
| 0 | 0 |
| 1 | 0 |
| 2 | 5 |
| 0 | 2 |
| 2 | 5 |
| 1 | 3 |
| 0 | 2 |
| 0 | 0 |
| 0 | 5 |
| 0 | 0 |
| 0 | 0 |
| 0 | 0 |
| 0 | 7 |
| 1 | 1 |
| N = 26 | N = 26 |
| Range = 0-2 | Range = 0-7 |
| $\bar{x}$ = 0.3 | $\bar{x}$ = 1.6 |
| SD = 0.6 | SD = 2.0 |

* N = number of broods
$\bar{x}$ = mean
SD = standard deviation

## TABLE 19

THE EFFECTS OF EXPOSURE ON ZERO TO ONE DAY OLD JUVENILES IN *GAMMARUS PALUSTRIS*: NUMBER OF JUVENILES PLACED IN EACH TEST GROUP

| Female | Total Juveniles in Brood | Number Not Replaced With Female | Number Replaced With Female That Re-entered Marsupium | Number Replaced With Female That Did Not Re-enter Marsupium |
|---|---|---|---|---|
| 1 | 10 | 4 | 1 | 5 |
| 2 | 10 | 2 | 4 | 4 |
| 3 | 18 | 9 | 3 | 6 |
| 4 | 10 | 5 | 2 | 3 |
| 5 | 8 | 1 | 2 | 5 |
| 6 | 10 | 2 | 2 | 6 |
| 7 | 3 | 0 | 1 | 2 |
| 8 | 24 | 8 | 6 | 10 |
| 9 | 27 | 9 | 4 | 14 |
| 10 | 14 | 3 | 2 | 9 |
| TOTALS | 134 | 43 | 27 | 64 |

B. The Effects of Alternating Periods of Exposure and Immersion on Juvenile Emergence

Because feeding was shown to affect mean days of emergence, females were divided into two food treatment groups. Each of the food treatment groups was divided into two exposure treatment groups. Each of the four test groups contained 11 females. To evaluate the effects of alternating periods of exposure and immersion, it first had to be determined whether there were any differences among the four groups in their key reproductive characteristics. Analyses of variance performed on head sizes, number of juveniles per brood and mean days of emergence were not significantly different, but the length of time from the broods' hatching to the females' molt was significantly different among the four test groups (Table 20).

The length of time from hatching of the brood to the female's next molt was significantly shorter for the fed group ($\bar{x}$ = 4.00 days) than for the unfed group ($\bar{x}$ = 6.55 days) (Student's t test; t=3.82, d.f.=42). However, for exposed and unexposed groups, the lengths of time were not significantly different ($\bar{x}$ = 5.55 and 5.00 days, respectively; t=0.71, d.f.=42). Although the average mean day of emergence of the fed group was longer than for the unfed group (2.32 vs. 1.94 days, respectively), the difference was not signif-

TABLE 20

THE EFFECTS OF ALTERNATING PERIODS OF EXPOSURE AND IMMERSION ON JUVENILE EMERGENCE IN GAMMARUS PALUSTRIS: DIFFERENCES IN FEMALE CHARACTERISTICS AMONG TEST GROUPS

| | HEAD SIZE | | | | TOTAL NUMBER OF JUVENILES PER FEMALE | | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | A | B | C | D |
| N | 11 | 10 | 11 | 11 | 11 | 11 | 11 | 11 |
| RANGE | 0.69-1.13 | .69-1.02 | 0.80-1.06 | 0.69-1.09 | 2-22 | 5-25 | 7-25 | 4-17 |
| $\bar{x}$ | 0.94 | 0.89 | 0.90 | 0.91 | 12.5 | 12.7 | 13.5 | 11.3 |
| SD | 0.15 | 0.10 | 0.08 | 0.12 | 6.8 | 7.0 | 6.6 | 3.9 |
| Value of ANOVA | $F_{(3,39)} = .324$ | | | | $F_{(3,40)} = .235$ | | | |

* A = Group constantly immersed and fed
  B = Group constantly immersed and unfed
  C = Group exposed 8 hours and immersed 4 hours and fed
  D = Group exposed 8 hours and immersed 4 hours and unfed

N = number of females
$\bar{x}$ = mean
SD = standard deviation

TABLE 20 (CONTINUED)

THE EFFECTS OF ALTERNATING PERIODS OF EXPOSURE AND IMMERSION ON JUVENILE EMERGENCE IN GAMMARUS PALUSTRIS: DIFFERENCES IN FEMALE CHARACTERISTICS AMONG TEST GROUPS

| | MEAN DAY OF EMERGENCE | | | | NUMBER DAYS HATCH TO FEMALE MOLT | | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | A | B | C | D |
| N | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| RANGE | 1.00-6.88 | 1.40-3.28 | 1.00-3.90 | 1.25-2.44 | 2-5 | 1-11 | 2-8 | 5-9 |
| $\bar{x}$ | 2.34 | 2.05 | 2.29 | 1.83 | 3.5 | 6.5 | 4.5 | 6.5 |
| SD | 1.57 | 0.62 | 1.00 | 0.44 | 1.3 | 2.9 | 2.4 | 2.0 |
| Value of ANOVA | | $F_{(3,40)} = .617$ | | | | $F_{(3,40)} = 5.2163$ | | |

* A = Group constantly immersed and fed

B = Group constantly immersed and unfed

C = Group exposed 8 hours and immersed 4 hours and fed

D = Group exposed 8 hours and immersed 4 hours and unfed

N = number of females

$\bar{x}$ = mean

SD = standard deviation

icant ($t = -1.28$, d.f. = 42, $p = .10$). Thus, although the length of time between hatching of the brood and the female's molt is shorter in fed than unfed females, the juveniles emerge later in fed females.

There was no significant difference between the mean days of emergence of exposed and unexposed broods ($t = -0.46$, d.f. =42). Thus, juveniles subjected to alternate periods of exposure and immersion did not emerge on an earlier or later day than juveniles kept immersed continuously.

No female molted during a period of exposure, even though exposure periods were twice as long as immersion periods. Further, no locomotion or feeding was ever observed during periods of exposure.

In the group of females subjected to alternating periods of exposure and immersion, most juveniles emerged during periods of immersion (251 of 272). Ninety-six of the 251 emerged during the first 15 minutes after water was introduced into the dishes, and 139 emerged during the succeeding 3.75 hours of immersion. Sixteen juveniles emerged during immersion, but I don't know whether it was during the first 15 minutes after immersion or later. A greater number of juveniles emerged per minute during the first 15 minutes after immersion than during the eight hour exposure periods (0.06 and 0.0005 per hour, respectively) (Table 21). There was a significant difference between the number of

## TABLE 21

EFFECTS OF ALTERNATING PERIODS OF EXPOSURE AND IMMERSION ON JUVENILE EMERGENCE IN GAMMARUS PALUSTRIS: NUMBER OF JUVENILES AND NUMBER OF JUVENILES PER MINUTE THAT EMERGED DURING DIFFERENT PERIODS FROM 22 FEMALES

| | Total Number Emerged in First 15 Minutes | Number Per Minute During First 15 Minutes | Total Number Emerging in 3.75 Hours; Constant Immersion | Number Per Minute During 3.75 Hours; Constant Immersion | Total Number Emerged in 8 Hours; Constant Exposure | Number Per Minute During 8 Hours; Constant Exposure |
|---|---|---|---|---|---|---|
| RANGE | 0-10 | 0-0.178 | 0-13 | 0-0.016 | 0-9 | 0-.0042 |
| $\bar{X}$ | 4.2 | 0-0.056 | 6.8 | 0.006 | 1.0 | 0.0005 |
| SD | 3.6 | 0-0.061 | 3.9 | 0.004 | 2.0 | 0.0010 |

* $\bar{X}$ = mean

SD = standard deviation

juveniles per minute that emerged during the first 15 minutes after immersion and the number per minute that emerged during the succeeding 3.75 hours (Student's t test; t=3.89, d.f.=42), and between the latter group and the number per minute that emerged during periods of exposure (t=5.64, d.f.=42). However, among the females kept constantly immersed, there was no significant difference between the number of juveniles per minute that emerged during the four hour periods (t=0.232, d.f.=42) (Table 22).

Thus, although the mean day of emergence is not altered by the mother's experiencing periods of exposure and immersion, the timing of emergences within the day is determined by whether or not the female and her brood is immersed, and by the length of immersion.

TABLE 22

EFFECTS OF ALTERNATING PERIODS OF EXPOSURE AND IMMERSION ON JUVENILE EMERGENCE IN GAMMARUS PALUSTRIS: NUMBER OF JUVENILES AND NUMBER OF JUVENILES PER MINUTE THAT EMERGED DURING 4 AND 8-HOUR PERIODS AMONG 22 FEMALES KEPT CONSTANTLY IMMERSED

| | Total Number Emerged in 8-Hour Periods | Number Per Minute During 8-Hour Periods | Total Number Emerged in 4-Hour Periods | Number Per Minute During 4-Hour Periods |
|---|---|---|---|---|
| RANGE | 3-20 | 0-0.012 | 2-12 | 0-0.010 |
| $\bar{X}$ | 7.2 | 0.003 | 4.6 | 0.004 |
| SD | 5.3 | 0.003 | 3.7 | 0.003 |

* $\bar{X}$ = mean

SD = standard deviation

# DISCUSSION

## I. Seasonal Changes in Distribution

During the winter months, the number of Gammarus palustris individuals found in the littoral zone is smaller than at any other time of the year, and individuals of Gammarus mucronatus and Melita nitida are absent. There are two possible explanations for the reduction in numbers of the three species in the winter: (1) burrowing in the substratum in the littoral zone, or (2) migrating into deeper waters. It is also possible that they migrate to deeper waters and bury themselves in the substratum there. Seasonal migrations have been reported for several species of littoral amphipods (Gammarus oceanicus, Gammarus salinas, Gammarus duebeni, Kinne, 1955). Van Dolah et al (1977) suggested that G. palustris migrates seasonally in Maryland, but Gable and Croker (1977) found no evidence of horizontal migrations or burrowings of the same species in New Hampshire.

Gammarus palustris stops reproducing in the colder months at Jamaica Bay. This has also been reported in New Hampshire (Gable and Croker, 1977) and Maryland (Rees, 1975), and for other epibenthic amphipods as well (Steele, 1967). In temperate climates, females enter a "resting stage"; the females molt in the fall, and with that molt, the oostegites become non-functional because they lose their

overlapping hairs (Steele, 1967).

II. Spatial Distribution

When all three species are present in the littoral zone, their vertical distributions are distinct. Assuming all three species would occur throughout the littoral zone, if possible, one can speculate on the factors that restrict them to their respective vertical positions. First, because Gammarus palustris is the only species found at the high tide mark, it is possible that this species can withstand environmental stresses such as long periods of exposure to the air, wide temperature ranges, and desiccation, better than the other two species. Second, Gammarus mucronatus may exclude G. palustris from the mean and low tide marks. G. palustris reproduces equally well under conditions of constant immersion and under conditions of alternating periods of immersion and exposure. Thus, it is to be expected that G. palustris would fare as well at the mean and low tide marks as at the high tide mark. In fact, in the early spring, when only G. palustris is in the littoral zone, it is found at all tide marks, with reproductive activity occurring throughout the littoral zone. As G. mucronatus gradually migrates into the littoral zone, however, G. palustris becomes gradually confined to the high tide mark. The absence of G. palustris from the mean

and low tide marks during the warmer months may be due to the presence of G. mucronatus. The non-overlapping distribution of the two species was also found in July, 1977, at Eastern Point and Fairhaven, Connecticut, and in October, 1977, at Sherwood Island, Connecticut (personal observation). The vertical distribution of the two species is therefore not peculiar to Jamaica Bay.

The present study has shown that Gammarus mucronatus is more fecund than Gammarus palustris. The former species has a greater number of juveniles per brood, and has broods more often than the latter species (Tables 6 and 7). The pressure of ever increasing numbers of G. mucronatus may drive G. palustris into the higher parts of the littoral zone, a habitat suitable for G. palustris, but not for G. mucronatus.

Competition for space occurs between two other intertidal crustacea, Chthamalus stellatus and Balanus balanoides at Millport, Scotland (Connell, 1961). B. balanoides has a greater population density and grows faster so it eliminates most C. stellatus by crowding it out. C. stellatus can exist higher on shore mainly as a result of its greater tolerance to heat and/or desiccation.

Another possible explanation for the differences in the distribution of Gammarus palustris and Gammarus mucronatus may be differential predation as occurs among the three

barnacles, Balanus glandula, B. cariosus and Chthamalus fissus at San Juan Island, Washington. Connell (1970) stated that the rates of feeding of the two snails that prey upon B. glandula, Thais lamellosa and Thais emarginata, could account for the reduced numbers of B. glandula at low shore levels in mid and late summer.

Finally, the distribution of the two species may be the result both of differential predation and interspecies competition for space, as occurs among the barnacles in at least one rocky intertidal community (Dayton, 1971).

III. Reproductive Characteristics of Gammarus palustris, Gammarus mucronatus and Melita nitida.

The length of the egg period at the two temperatures tested, 17° C and 21° C, was fixed for each of the three species (Table 7). This has been reported for other amphipods as well (Kinne, 1970). At both temperatures tested in this study, broods of Gammarus palustris had the longest (11 and 7 days) and Gammarus mucronatus the shortest (8 and 5 days) time of development from ovulation to hatching. This is surprising for two reasons. First, G. palustris and G. mucronatus are morphologically much more similar to each other than either is to Melita nitida. One would expect their physiological processes to be more similar to each other as well, but they are not. Second, the habitat of

G. mucronatus is between that of G. palustris and M. nitida. If relative lengths of time of egg development is an adaption entirely to habitat, one would expect the egg development time of G. mucronatus to be between that of the other two species.

If the average mean day of emergence is added to the mean egg development time for each species at each temperature tested, the relationship still holds; Gammarus palustris is longest, and Gammarus mucronatus shortest. At 17° C, the mean total development time of G. palustris, G. mucronatus, and Melita nitida are 12.9, 9.6, and 10.4 days respectively. At 21° C, they are 8.6, 5.3, and 6.4 days respectively. Van Dolah (1975) plotted the "egg development times", which I assume to be the sum of the egg and juvenile periods of seven species of epibenthic amphipods (including G. palustris), against temperature, and found they were significantly correlated. Using his formula ($Y=77.43\ e^{-.097x}$), where x is temperature, the total "egg development time" of the species he considered should be 14.9 days at 17° C, and 10.1 days at 21° C. All the development times observed in the present study were shorter than the formula predicted, the differences at 17° C were 2.0, 5.3, and 4.5 days for G. palustris, G. mucronatus and M. nitida respectively, and the differences at 21° C were 1.5 , 4.8, and 3.7 days respectively (Figure 23).

Figure 23

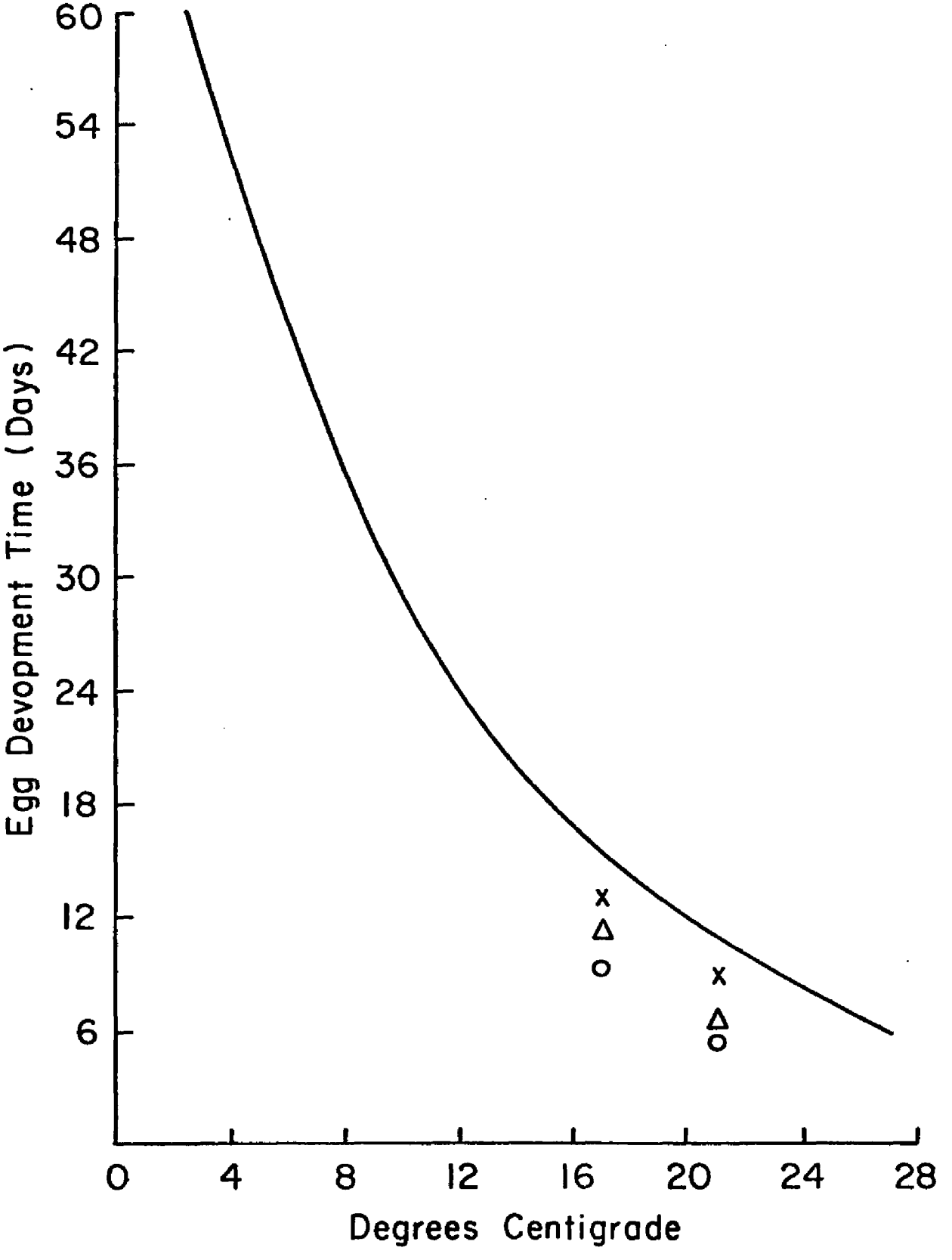
Total development time of broods of Gammarus palustris, Gammarus mucronatus and Melita nitida determined in this study plotted on the curve calculated by Van Dolah, et al, (1975). Van Dolah, et al, used data from seven species, one of which was Gammarus palustris.

Legend:

X indicates the mean total development time of Gammarus palustris, present study

• indicates the mean total development time of Gammarus mucronatus, present study

△ indicates the mean total development time of Melita nitida, present study


60
54
48
42
36
30
24
18
12
6
Egg Devopment Time (Days)
0
4
8
12
16
20
24
28
Degrees Centigrade
x
Δ
o
x
Δ
o

It is possible that the data used by Van Dolah were obtained under significantly different conditions than were the data of the present study. At this time it may be an error to conclude, as did Van Dolah, that all species of epibenthic amphipods have similar development times at a given temperature.

Gammarus palustris had the fewest juveniles per brood, the longest egg period, and the longest mean day of emergence among the three species. Thus, G. palustris invests a longer period of time on the development of fewer offspring than do the other two species. G. palustris has evolved in the direction of greater parental care, one of the solutions to the problems of reproduction in species occupying a stressful physical environment.

Parental care has also evolved in decapod crustacea that have adapted to another stressful environment, fresh water. In the decapod crustacea, adaptation to fresh water has been accompanied by an increase in size and reduction in number of eggs. Further, whereas marine decapods typically hatch as immature larvae and develop in the plankton, fresh water species such as Parathelphusa guerini develop entirely within the egg and hatch as miniature adults (Bliss, 1968).

One of the reasons that the littoral zone is so stressful is that it is subject to a wide range of environmental extremes. Gammarus palustris has the widest and Melita nitida the narrowest range of mean days of emergence. This

coincides with their relative habitats in the littoral zone. G. palustris, found highest, is most subject to environmental fluctuations, and M. nitida, found lowest, is least subject to these fluctuations. This suggests that the variability of the juvenile period is an adaptation to the variability of the environment.

The benefit of a variable juvenile period for a littoral species is that it permits a juvenile to emerge early or late, depending upon environmental conditions. A juvenile of a species occurring in the sublittoral or sublittoral fringe zone does not need this leeway, for environmental conditions are likely to be about the same no matter when the juvenile emerges within the possible range of time a juvenile of a given brood can emerge.

The relative variability of the juvenile period has been shown to be related to habitat in other species of amphipods. In Table 23, those species of epibenthic amphipods whose juvenile periods are known are arranged according to their vertical position in the littoral zone. It may be seen that the juvenile periods of littoral species are more variable than are sub-or supra-littoral species.

Both the egg and the juvenile periods are fixed for each species, suggesting that they are genetically determined. However, different environmental factors have different modifying effects on the two periods. The lengths of the egg

## TABLE 23

### LENGTHS OF EGG AND JUVENILE STAGES IN SALT WATER EPIBENTHIC AMPHIPODS

| Habitat | Species | Salinity | Number Days Egg Stage | Temp°C | Number Days Juv. Stage | Temp°C | Reference |
|---|---|---|---|---|---|---|---|
| Terrestrial Semi-terrestrial | Orchestia | No data | No data | No data | 1-3 | No data | Wildish, 1972 |
| Rock pools supra-littoral | Gammarus duebeni | Euryhaline | 24 | 12 | 1-5 | 12.5 | Kinne, 1959 Hynes, 1955 |
| High Tide Mark | Gammarus palustris | Polyhaline | 11.1<br>7.1 | 17<br>21 | 0-8<br>0-6 | 17<br>21 | Personal Observation |
| Mean-Low Tide Mark | Gammarus mucronatus | Polyhaline | 8.3<br>4.6 | 17<br>21 | 0-4<br>0-2 | 17<br>21 | Personal Observation |
| Mean-Low Tide Mark | Gammarus obtusatus | Polyhaline | 12-14<br>13.1 | summer temps<br>7-9 | 4-17<br>3-14 | summer temps<br>7-9 | Sexton & Spooner, 1940 Sheader & Chia, 1970 |
| Low Tide Mark-sublittoral | Gammarus locusta | Marine | 11-12 | room temps | 1-4 | room temps | Blegvad, 1922 |
| Ditches Low Tide | Gammarus chevreuxi | Brackish | 12-14 | room temps | 1-2 | room temps | Sexton & Matthews |

periods were different at 17° C and 21° C, but the mean days of emergence were not. (There may have been a difference in mean days of emergence of Gammarus mucronatus due either to temperature or female size; the mean day of emergence was positively correlated with female size and temperature.) It is possible that a wider range of experimental temperatures would have produced a significant difference in mean days of emergence, considering that the intermolt periods are temperature-dependent in other species (Koch-Kallnbach, 1977), and that the mean day of emergence is determined in part by the time the female molts. It is clear from data from this study that the egg period is more modified by temperature than is the juvenile period. The length of the egg period was about four days longer at 17° C than at 21° C for all three species (Table 7), while the difference in the average mean day of emergence between 17° C and 21° C was 0.17, 0.51 and 0.28, days for Gammarus palustris, G. mucronatus and Melita nitida respectively.

IV. Factors that Affect the Time a Juvenile Emerges.

In Gammarus palustris and Gammarus mucronatus, when the female molted early, juveniles were cast out prematurely. If the molt occurred relatively late, some juveniles chose to remain in the marsupium longer. Thus, the mean day of emergence of a brood in these two species was determined by the

interaction of the optimum length of time the juveniles remained in the marsupium and the time the female molted. Under similar laboratory conditions, however, the time a Melita nitida female molted had little influence on the mean day of emergence, because both the mean days of emergence and the female molt occurred shortly after hatching of the brood.

Kinne (1959) showed that the presence of a male shortens the time from hatching to the female molt in Gammarus duebeni. Presumably, females can delay the molt for a time until contact is made with a male. Although field data presented here showed that females with hatched juveniles are almost without exception found in precopulation with males, females in this study were maintained in the laboratory without males. This was done to prevent juveniles from being eaten by the males. Thus, it is possible that in nature the length of time from hatching to the female molt, and thus, the mean day of emergence, is shorter for all three species than was found in the laboratory.

Two factors in addition to the time of the female's molt were shown to affect the time a particular juvenile emerged in Gammarus palustris broods: (1) the type of food provided for the female after the brood hatched; and (2) whether the female was immersed or not. When the food was

provided ad libitum, juveniles emerged later from females provided with the greatest variety of foods. Juveniles emerged relatively early from females fed suboptimally. If, however, a brood fed only Ulva sp. was presented with a piece of mussel meat, some juveniles emerged immediately. This sudden enrichment of their diet shortened, rather than prolonged, their stay in the marsupium.

This seeming contradiction may be resolved in the following way. In a food-rich environment, a juvenile is likely to have ready access to food whenever it emerges. Therefore, it would tend to remain in the marsupium for a relatively long period of time to take advantage of the physical protection offered by the marsupium. In a food-limited environment, the scarcity of food might be caused by one or both of the following: (1) the female is unable to get food although it is abundant; (2) the food is scarce in the environment. In the first case, it may be best for the juvenile to abandon the female as soon as possible to try to find its own food. In the second case, it is good for the juvenile to abandon the female as soon as she carries it to a source of food. The juveniles may perceive that food is limited because they will have been hatched for a time, but have been unable to feed. As soon as food becomes available, then, they will take steps to secure it. Therefore, in a food-limited environment, juveniles emerge early,

and are stimulated to emerge immediately upon the female's finding and feeding upon some preferred food.

The hypothesized strategy is supported by three observations made during this study. First, juveniles emerged only from females that were actually feeding. This is as would be expected by the hypothesis; the presence of meat in the dish does not guarantee its accessibility. Juveniles may trade off the advantages of protection of the marsupium for the advantage of eating a preferred food only if the latter is guaranteed. Second, most juveniles emerged unassisted by the female. Thus, food as a stimulus for emergence acted directly upon the juveniles, permitting them the option of emergence. Third, the activities of the emerged juveniles, as well as those that remained in the marsupium were directed at the meat itself. The actual number of juveniles that emerged is only one indication of the increased activity of the juveniles while the female fed. Many emerged from the marsupium and then re-entered it. Juveniles moved about actively within the marsupium in an effort to reach the meat. Emerged juveniles and those within the marsupium were observed feeding on the meat.

There are three ways a juvenile can emerge from the marsupium. It can be forced out by other juveniles; it can be forced out by the female; or it can emerge on its own.

After presentation with meat, it is possible that a few were forced out by the activities of the other juveniles. However, most emergences were seen to be a result of the individual's own movements. After transfer, females swam actively about while juveniles were emerging. Thus, there were no observations possible on the mechanism of juvenile emergence after the female was transferred.

The presentation of meat caused more juveniles to emerge than did transfer from one dish to another. This is particularly interesting because meat was introduced 15 minutes after the female was transferred, and is further evidence that the mechanism of emergence after transfer is different from the mechanism of emergence after presentation with meat.

In Gammarus palustris, food is more important in determining when a juvenile emerges than is the length of time between hatching of the brood and the female's molt. It was shown that the length of time from hatching to the female's molt was longer in unfed than in fed females. Crustacea slow their growth and delay their molt when food is scarce. This has been shown in Calanus finmarchicus (Raymont and Gross, 1942) and for many crabs (Warner, 1977). However, the absence of food actually shortens the time from hatching to emergence of juveniles in G. palustris.

It is likely that in Gammarus palustris the optimal

time of emergence of a juvenile is close to the optimal time of the female molt, because under good food conditions, the mean day of emergence should be relatively long, and the mean length of time from hatch to the female molt relatively short. This system is most energetically conservative. If emergence and molt are close in time, just when the juveniles are mature enough to emerge, and thus, free up the marsupium, the female is ready to fill it with a new brood. This would permit the constant use of the marsupium throughout the reproductive life of the female in the warmer months.

The third factor that influenced the time a juvenile emerged was exposure to air. The mean day of emergence of broods subjected to alternating periods of exposure to air and immersion did not differ from the mean day of emergence of broods kept constantly immersed. However, the time of emergence within the day was determined by whether or not the female was covered with water, and when the female had been covered by water.

During periods of exposure, the female lay between the glass slide and the dish, which had a thin layer of water trapped between the two glass surfaces. Generally, the female was curled ventrally, and the pleopods were still. Such a position might protect the juveniles against exposure by forming an inner and outer protective shield around them.

The inner shield is formed when the body is flexed, forcing the oostegites closer together and closing off the spaces between them, thereby trapping a droplet of water within the marsupium. The outer shield is formed by the coxal plates of the thoracic segments surrounding the marsupium when the body is flexed ventrally. The juveniles are protected by these shields in two ways. First, water may be trapped in the marsupium, and thus, the juveniles remain essentially "immersed" even when the tide is out. Second, juveniles are physically prevented from emerging from the marsupium when the female is exposed.

Amphipods under rocks in the littoral zone during low tide are invariably found curled ventrally. Even when the female is not curled up, however, juveniles are protected by the marsupium. In this study, females with juveniles in their marsupia were exposed to the air for six minutes; they walked actively about the paper towel, and most juveniles survived (p.116). Newly emerged juveniles were more adversely affected by exposure to air than adults, but juveniles within the marsupium were protected against these adverse effects. Thus, emergences are timed to periods of immersion.

The adverse effects of air on juveniles might be due to inability to respire atmospheric oxygen and/or inability to withstand desiccation.

How can newly emerged juveniles remain at the high tide mark during periods of low water? The probable answer is that the juveniles are never exposed to such severe desiccation as they were in this experiment. They may hide under rocks or within the folds of the green algae that grow on rocks. Here they may remain in a thin film of water, or, at worst, under conditions of relatively high humidity. The terrestrial amphipods have become independent of large bodies of water, but not of high humidity. They are only able to survive on land by living in moist environments, such as leaf litter (Hurley, 1968). Terrestrial isopods also prefer humid environments (Edney, 1968).

Many other factors will certainly be shown in the future to be important in determining the time at which a juvenile emerges. The most important ones will probably be salinity, light intensity, pollutants, oxygen tension, and female pheromones.

V. Re-entry of Juveniles into the Marsupium.

Re-entry of juveniles into the marsupium deserves some discussion. Zero to one day old Gammarus palustris juveniles re-entered the marsupium. This has been reported for at least three other species of amphipods: Marinogammarus obtusatus and Marinogammarus finmarchicus (Sheader and Chia, 1970); and Neohaustorius schmitzii, a sand-burrowing species

(Croker, 1968). Although most juveniles of M. obtusatus re-entered the marsupium (unfortunately, the number of juveniles of M. finmarchicus and N. schmitzii were not counted) most juveniles of G. palustris did not. Earlier personal observations of G. palustris had shown that even fewer juveniles would re-enter if the animals were placed in larger dishes and in the light.

In both Sheader and Chia's study and the present study, the juveniles that re-entered the marsupium had been removed by the investigator. The meaningful question to ask is whether juveniles in the field, once emerged, re-enter the marsupium. Sheader and Chia report finding re-entered juveniles in the marsupiua of a small number of field-caught females of Marinogammarus obtusatus. In the three species studied here, no instance of a re-entered juvenile from field-caught females was found. It is tempting to speculate that re-entry is a common occurrence, for the adaptive value is obvious; return to the marsupium for protection if danger is imminent. The evidence suggests, however, that the frequency of re-entry in the field is low, at least for epibenthic species, and that re-entry should not be considered a typical behavior pattern for juveniles.

The re-entry of juveniles is an act not directed at the marsupium specifically. In the study of Marinogammarus

obtusatus, juveniles preferred to enter the marsupia of females who had been carrying older broods. Sheader and Chia suspected that this was because the oostegites spread apart as the brood became older, which would have made it easier for a juvenile to pass between them back into the marsupium. In all studies to date, the female has not been observed to assist the re-entry of juveniles. In Gammarus palustris, juveniles were seen not only within the marsupium, but also outside it, and other parts of the female's body as well. The best interpretation of this is that juveniles seek a hiding place on a solid substratum, and, in an empty dish, the female's marsupium is the only one available.

## VI. Laboratory Culture of Gammarus palustris, Gammarus mucronatus, and Melita nitida.

In amphipods, the size of the individual increases with each molt if conditions are favorable, and the number of eggs per brood increases with female size. The number of juveniles per brood increased in the second broods of Gammarus mucronatus and Gammarus palustris females, but decreased in Melita nitida females, probably because the particular laboratory conditions were not conducive to the growth of M. nitida. Additional evidence that laboratory conditions were not conducive to M. nitida growth comes from the fact that no juvenile of this species was ever raised to sexual maturity (defined as the ability to reproduce in the laboratory).

Both Gammarus palustris and Gammarus mucronatus have been raised through many generations. However, the latter species required more care than the former for successful culturing; each individual requires a greater volume of water and more frequent feeding than does G. palustris. The requirement of a larger culture dish for culturing G. mucronatus suggests that more oxygen may be necessary because more oxygen can dissolve across the relatively greater surface area of the larger culture dish; this, plus the necessity of more frequent feeding, suggests that G. mucronatus may have a higher metabolic rate than does G. palustris.

VII. Intraspecific color variation.

Most of the species of amphipods found at Jamaica Bay exhibited a range of colors, and some, such as Orchestia grillus and Gammarus palustris had wide ranges of color.

In Gammarus palustris, the richest and most varied colors were present in the spring. In the fall and winter, animals tended to be pale pink to glassy colored. Fecal color of animals collected in the spring suggested that the animals were eating a variety of foods. A comparison of the colors of feces of field-collected animals with those of laboratory cultured individuals suggested that the former were primarily eating benthic diatoms and/or blue-green algae. Gable and Croker (1977) have provided direct support for this conclusion. They examined the gut contents of G. palustris

individuals from a New Hampshire salt marsh. They found that diatoms, blue-green and green algae were abundant in juvenile and adult guts in summer, and in juvenile guts in the spring. Other algae became relatively more abundant in other seasons.

The carotenoids, which "are responsible for most of the bright colors of the crustacea" (Nicol, 1967), cannot be manufactured by animals, but must be ingested (Hoar, 1975). Different species of phytoplankton have different chemical compositions (Parsons, et al, 1961). It is likely that different types of benthic algae also have different chemical compositions, and, therefore, different amounts of carotenoids. At Southhampton, a salt marsh about 100 miles from Jamaica Bay, seasonal changes in benthic algae assemblages have been observed (Lee et al, 1975). I suspect that the typical diet in the spring, the bulk of which is probably benthic diatoms and blue-green algae, contains sufficient carotenoids to permit the expression of the full range of colors of Gammarus palustris. Since Jamaica Bay is a salt marsh similar to Southhampton, assemblages of benthic algae must change seasonally as well. It is therefore likely that as the seasons change, amphipods at Jamaica Bay feed on different algae, and thus consume different amounts of carotenoids.

## VIII. Precopulation

Precopulating females of Gammarus palustris had broods

in later stages of development in the field. The timing of precopulatory behavior with the imminence of the female's molt has been reported for Gammarus pulex and Gammarus roeselii in the laboratory (Koch-Kallnbach, 1977), and several species of isopods (Asellus aquaticus, Kaestner, 1970; and Helleria brevieornis, Mead and Gabouriaux, 1977). To my knowledge, the present study is the first verification of this for a species of amphipod in the field.

Because the male must be present when the female molts, it is essential that precopulation begin before then. In Gammarus duebeni the female's pre-molt condition is signalled by the excretion of a pheromone beginning a few days prior to the molt. The male is attracted to the female by this pheromone (Dahl, et al, 1970). But precopulating couples are more awkward and less mobile than individual animals, particularly when out of the water. This may be why females in early stages of development of the brood do not copulate. In the later stages of development, the disadvantages of awkwardness and decreased mobility are outweighed by the advantages of precopulation.

Precopulation is a fascinating phenomenon. It assures a female of a male's presence and assures the male access to the female's eggs at the optimal time for fertilization to be effected. At the same time, it permits the animals to move

about and feed without losing their guarantee of fertilization.

An interesting question arises: the current brood of a precopulating female probably belongs to a different male than the one to which she's coupled. The new male is thus probably carrying about the brood of another male, and is providing the hatched juveniles with food and protection while providing it for the female and himself. In the three species studied here, the male does the swimming and walking during precopulation, and the female is entirely passive. This is unusual. If the males of a species protect any offspring, they usually protect their own. This enhances the male's fitness, for it enhances the chances of survival of his genotype. For example, male lobsters (Homarus americanus) guard the female and her newly fertilized eggs until the female's molt hardens, thus decreasing the chances of another male's fertilizing her eggs (Nelson and Hedgecock, 1977). Males of some species will even destroy the offspring of another male if given the opportunity (Hrdy, 1977), thus increasing their own fitness by decreasing the fitness of other males. Do amphipod males, then, contrary to what is expected, improve the fitness of another male?

I do not think so. In the amphipods, the number of eggs increases, sometimes logarithmically, with the length of the female (Myers, 1971; Steele, 1975; Van Dolah, et al,

1975; and present study). Amphipods increase in length with each molt. Thus, the number of eggs that the female produces for the present male to fertilize is going to be greater than the number of eggs produced for the previous male. This automatically increases the present male's fitness.

If this explanation is correct, a logical prediction follows. Males should precopulate with the largest females available (limited, of course, by their own length and strength to carrying a female smaller than themselves). In fact, non-quantitative observations of couples in the field and in the laboratory showed that large males did precopulate with large females and small males with small females when there was a range of adult sizes available. This remains to be confirmed however.

## IX. Conclusion

The results of these experiments indicated that amphipods whose typical habitat is high in the littoral zone have a more variable juvenile period than amphipods whose habitats are low in the littoral zone. The results supported the hypothesis that the variability of the species at the high tide mark is an adaptation which permits the animals to emerge when environmental conditions are propitious.

Many questions have been raised by the results of this study which warrant further research. For example, what is

the mechanism that keeps the three species at different tide marks in the littoral zone? Is it each species' preference for different environmental factors or is it interspecies competition, or both? What is the mechanism of juvenile emergence? Do juveniles emerge using their own energy or does the mother somehow contribute to their emergence? Most important, however, it remains to be determined whether the response of juveniles to different food regimes and alternating periods of exposure and immersion that was observed in the laboratory also occurs in the field.

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