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THE SALT MARSH AMPHIPOD GAMMARUS PALUSTRIS, BOUSFIELD, 1969, AT THE NORTHERN LIMIT OF ITS DISTRIBUTION: I ECOLOGY II TEMPERATURE-SALINITY TOLERANCE

MICHAEL F. GABLE

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THE SALT MARSH AMPHIPOD
GAMMARUS _PALUSTRIS_ BOUSFIELD, 1969,
AT THE NORTHERN LIMIT
OF ITS DISTRIBUTION

I. Ecology
II. Temperature-Salinity Tolerance

by

MICHAEL F. GABLE
B.S. University of Florida, 1967
M.S. University of New Hampshire, 1969

A THESIS

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This thesis has been examined and approved.

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Arthur E. Teeri, Prof. of Biochemistry

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THE SALT MARSH AMPHIPOD
GAMMARUS PALUSTRIS BOUSFIELD, 1969,
AT THE NORTHERN LIMIT
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I. Ecology

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Gammarus Palustris extends from northern Florida to the Great Bay-Piscataqua estuary separating New Hampshire and Maine. In this latter northernmost habitat the species experiences large fluctuations in temperature and salinity seasonally, daily, and often with each tidal cycle. This paper examines the ecology of G. palustris at its northern distributional limits.

The amphipods were found to be year-round obligate epifaunal inhabitants of the Spartina alterniflora marsh. They attained their greatest density between mean tide and extreme high water neap tide. Evasion of extreme temperature variation, coupled with aggregation in regions of optimal temperature range, caused a significant seasonal variation in vertical zonation. Preferred microhabitats, during both low and high water, were at the basal system and on the culm surfaces of <u>Spartina</u> and under rocks. Variations within the population in microhabitat preference were according to size and life stage and were reflected by feeding patterns. In winter and early spring the behavior of the animals in response to temperature caused them to occupy the atypical habitats of marsh pools, run-off streams and shallow standing bodies of water. In doing so, they experienced the lowest salinities and the greatest salinity fluctuations of the year on the marsh. Also, normal microhabitat separation from other closely related species was broken down.

Different mean sizes existed among populations of <u>G</u>. <u>palustris</u> within the estuary system; these size differences were inversely related to the densities of the populations. Over winter the density of the entire population was reduced by 44%.

There were two generations of <u>G</u>. <u>palustris</u> during the year: the spring generation was born during the summer, overwintered, and began breeding by mid-spring; the summer generation was born in spring and began breeding by early summer. Large differences in mean and maximum size attained existed between the two generations. The much larger

females of the spring generation laid many more eggs per brood than the females of the summer generation. Since females of both generations bore a maximum of three broods and were present in the field in nearly equal numbers, the reproductive capacity of the spring generation exceeded that of the summer generation (3.8 to 1.0).

The severity of the physical conditions in this northernmost habitat might be expected to precipitate unusual or noticeably stressful responses in G. palustris, since its geographic range mostly covers an area with more moderate conditions. Such responses are shown for the species: 1) the highly variable seasonal distribution patterns within the marsh; 2) the occupation, in great numbers, of very localized microhabitats during winter; 3) the consequent breakdown in normal microhabitat separation, correlated with possible increased predation and greater osmotic stress, and of possible death by the mechanical action of ice; and 4) a reproductive period which, although obviously allowing year to year populations, is associated with decreased reproductive potential.

I. INTRODUCTION

According to Bousfield (1969), the geographical range of the salt marsh amphipod, *Gammarus palustris* (Fig. 1), is from the mouth of the St. John's River in northern Florida to the Piscataqua River estuary separating New Hampshire and Maine. Collections in principle salt marshes along the Maine coast at Cobscook Bay, Castine, Scarboro and Saco during 1969-1971 failed to turn up *G. palustris*. Thus it is assumed that the Piscataqua estuary is indeed the northern limit of the species. The purpose of this paper is to elucidate the ecology and life cycle of this amphipod in its northernmost habitat. This habitat, although allowing *G. palustris* to maintain year to year populations, at the same time confronts the species with environmental extremes not found in more southerly habitats. In turn, these extremes, along with the response of *G. palustris* to them, hold the clue to an explanation of its distributional limits.

Fig. 1. Gammarus palustris

II. THE SALT MARSH HABITAT

Chapman (1964) describes salt marshes as tracts of land covered with phanerogamic vegetation and subject to periodic flooding by the sea. Because of the inseparable ecological and physiographical processes which make, maintain and alter them, salt marshes take on many forms. Therefore, beyond Chapman's broad definition, further generalized descriptions of salt marshes are difficult to make unless one limits oneself to a specific geographical region.

The salt marshes of New England on the eastern coast of North America are often described as a single unit (Chapman, 1940; Teal, 1969). They are all of glacial origin, are fed by a fresh water component with little sediment, and are usually associated with either estuaries or protected bays with shallow water, rather than with spits or off-shore barrier islands. They are also alike in their flora. Miller and Egler (1950) describe New England salt marshes as consisting of seven basic zones or stages, each distinguished by a particular type of vegetation:

1. Upland forest
2. Shrub border
3. _Panicum virgatum_ upper border
4. _Juncus_ upper slope
5. _Spartina patens_ lower slope

6. Spartina alterniflora lower border
7. Channel area

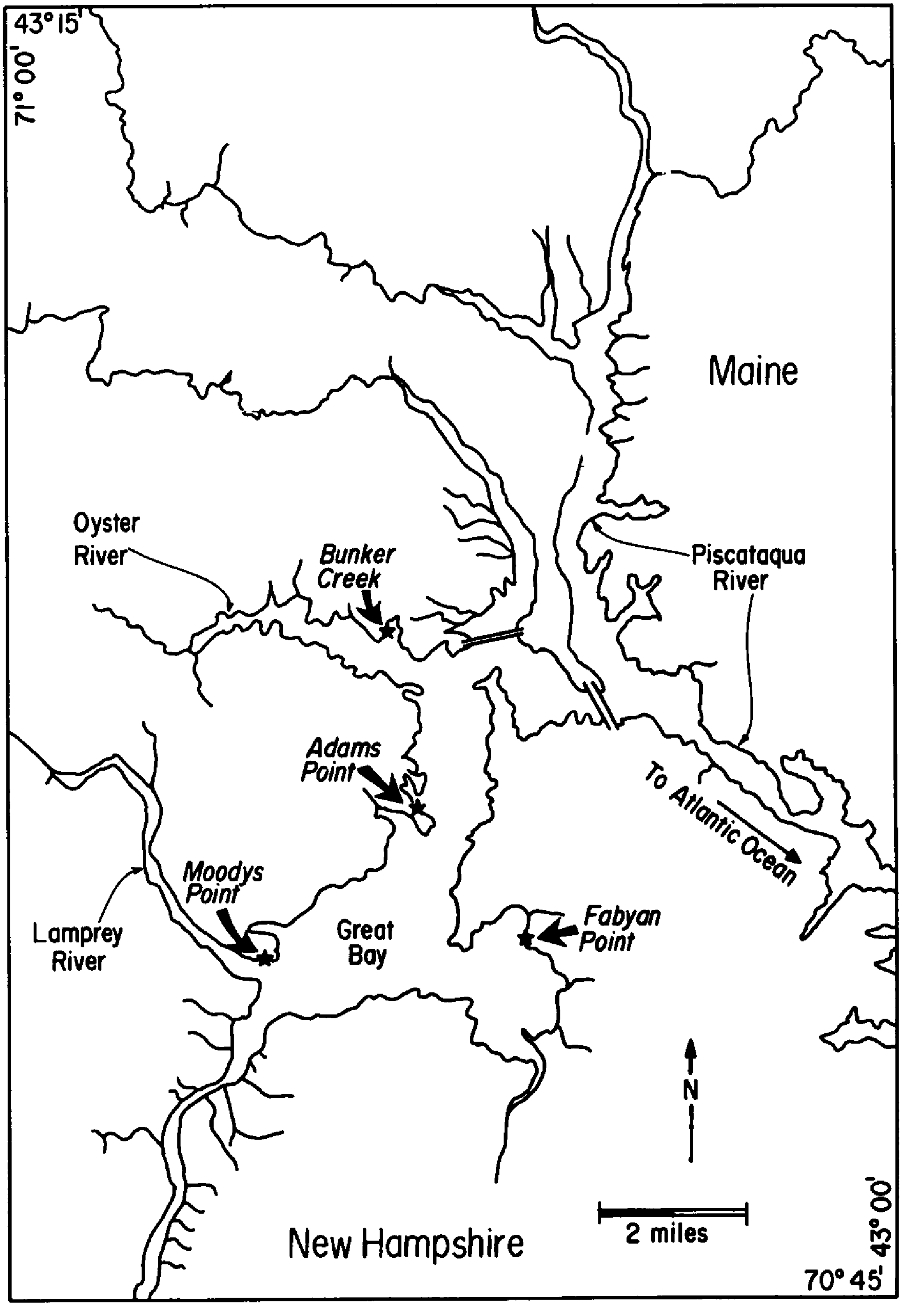
The P. virgatum habitat (3) forms a transitional zone from the uplands (1,2) to the tidal marsh proper, more specifically, to the high marsh (4,5). The S. alterniflora habitat (6), also known as the lower marsh, forms a fringing belt, transitional to the channel area (7). This belt in shallow aggrading areas may be quite wide. As Chapman (1940) points out, however, the communities on any marsh are related to each other in space and time, and thus for many marsh areas only some of the stages will be represented.

This discussion is limited to the area of the lower marsh, the S. alterniflora habitat. In some instances this habitat exists as part of a larger system; in others, it stands more or less by itself. It is the habitat of G. palustris.

1. Description of Collecting Stations

The Great Bay-Piscataqua estuary is a relatively unpolluted tidal system with over 100 miles of shoreline (Fig. 2). Seven rivers empty into the system, each bordered along much of its course, and especially at its mouth, by stands of S. alterniflora. Numerous coves and creeks punctuating these rivers provide additional areas for the growth of the S. alterniflora habitat. Great Bay proper

Fig. 2. Map of Great Bay-Piscataqua Estuary


43° 15'
71° 00'
Maine
Oyster River
Bunker Creek
Piscataqua River
Adams Point
To Atlantic Ocean
Moodys Point
Lamprey River
Great Bay
Fabyan Point
N
2 miles
New Hampshire
43° 00'
70° 45'

(Fig. 2) is bordered along much of its shore by the grass. In addition, the bay is extremely shallow with vast areas of exposed mud flats at low tide. The shoreward parts of these flats provide large areas for the growth of S. alterniflora.

Within this system, four lower marsh communities were selected for the collection and study of G. palustris. Each represents a slightly different habitat and is easily accessible.

Adams Point (Fig. 3). The habitat here consists of a marginal band of S. alterniflora separating a broad cove of Great Bay from a man-made causeway. At the lower border of the S. alterniflora, some Fucus vesiculosus grows on widely spaced granite outcroppings, but a short peat bluff delineates most of the lower border. Above this the S. alterniflora culms are usually individually placed and are separated from each other by a few centimeters. The substratum here is muck and only when this gives way to a firmer, peat-like substratum do the culms begin growing in clusters. Scattered pebbles and small rocks lie among the clustered culms. Above the S. alterniflora are small rocks, boulders and patches of Salicornia sp. Although mentioned nowhere in salt marsh literature, the two zones within the S. alterniflora habitat described here--mucky substratum with single culms; firm substratum with clustered culms--

Fig. 3. Adams Point

Fig. 4. Fabyan Point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are quite noticeable and distinct in almost all the marsh areas of the Great Bay-Piscataqua River system. The only exception exists along steeply bluffed tidal creeks, where the *S. alterniflora* border zone consists of clustered culms. This type of habitat, however, is not frequent in the bay. Therefore, in this discussion, the upper zone of clustered culms will be called the upper *Spartina* bank; the lower zone of single culms, the lower *Spartina* flats. Henceforth, *Spartina* refers to *S. alterniflora*.

Fabyan Point (Fig. 4). Here at the outward tip of Herods Cove, *Spartina* grows as a marginal band on the border of Great Bay proper. Large outcroppings of granite covered with fucoids form the entire lower border of the marsh. The transition from the lower *Spartina* flats to the upper *Spartina* bank is much more gradual than at Adams Point, there being no bluff. The lower flats are also less mucky and the culms of the upper bank, less aggregated. Small rocks and pebbles are abundant throughout the upper bank and continue on past the *Spartina* until a 1.5 to 2.0 meter cliff marks the border of the bay.

Moodys Point (Fig. 5). Here a wide band of *Spartina* separates the tidal flats at the mouth of the Lamprey River from an extensive salt meadow, i.e., high or upper marsh. Single culms begin quite far out on the tidal flats; a

Fig. 5. Moodys Point

Fig. 6. Bunker Creek


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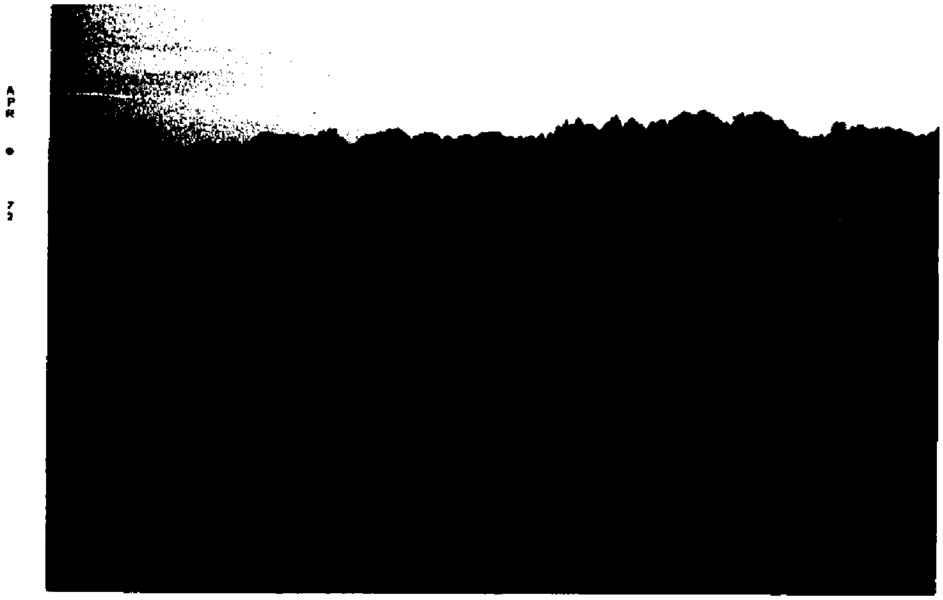

APR • 72

sharp bluff separates them from the upper _Spartina_ bank. The combination of a strong aggregation of culms and the closeness of these aggregations to one another results in a noticeable accumulation of tidal trash throughout much of the year on this upper bank. There are no pebbles or rocks and several old mosquito drainage ditches cross the zone parallel to the shore. The lower marsh at its upper border gives way abruptly to the _S. patens_ of the high marsh.

Bunker Creek (Fig. 6). The _Spartina_ habitat is more extensive here than at the other stations. The marsh is situated in a ravine between two hills of intrusive granite. Formerly, large mud flats extended outward from the creek to narrow bands of _Spartina_ along the shores (Batchelder, 1926). Single-culmed _Spartina_ now covers the entire mud flat. The transition between upper and lower zones is very gradual and covered in many places by a dense mat of _Ascophyllum nodosum_ var. _scorpiodes_ among the bases of the culms. There are no pebbles or stones. The higher portion of the upper bank is covered by small, single culms of _Spartina_ before it gives way to a narrow band of _S. patens_ lying at the base of an escarpment. This is probably indicative of the general succession taking place in the ravine.

III. METHODS

_G_. _palustris_ was sampled to provide data on: relative density, distribution, microhabitat preferences, size, and life cycle phenomena. All animals were collected with forceps. Small plastic containers held the animals alive in the field for either laboratory experiments or later preservation in 5% formalin, buffered with hexamethylenetetramine (approximately 115 g/liter). Collections for data on the life cycle were on a monthly basis at each of the four stations. Frequently the collections were subdivided in the field on the basis of microhabitat or the presence of animals in precopula. On each occasion, observations of biological interest (temperature, salinity, ice cover, general marsh conditions, associated fauna) were noted.

To insure quantification on a relative basis, each collection consisted of the number of animals gathered in two hours. Preliminary transects each month provided information on changes in zonation and microhabitat preferences. Because of a completely different microhabitat preference in winter, quantification on a relative basis was not possible for that season. Also, the highly localized nature of this microhabitat and the thick ice cover made finding _G_. _palustris_ very difficult. Data are therefore available for January and February only at Adams Point.

Animal length (from the anterior end of the head to the telson base) was measured with an optical micrometer to the nearest 0.1 mm. Life stage categories were arranged as follows: 1) Juveniles--no testicular papillae or oostegite buds; 2) Males--testicular papillae; 3) Immature females--oostegite buds or non-bristled oostegites; 4) Ovigerous females--bristled oostegites with embryos or young in marsupium; 5) Post-ovigerous females--bristled oostegites, empty marsupium.

For laboratory investigations, covered finger bowls, 9 cm. in diameter, or capped 6 oz. vials were used to hold the animals. Environair Systems Inc. controlled environment rooms provided constant temperatures, while submersion of the animals within vials on a sea water table with constantly circulating water from Great Bay provided environmental temperatures. The animals, given new water daily or every other day, experienced environmental salinities unless otherwise noted. A supply of detritus and Ulva lactuca, changed weekly, provided G. palustris with food. Pieces of Modiolus demissus adductor muscle were proffered once a week, and if uneaten by the following day, were removed to prevent fouling of the water.

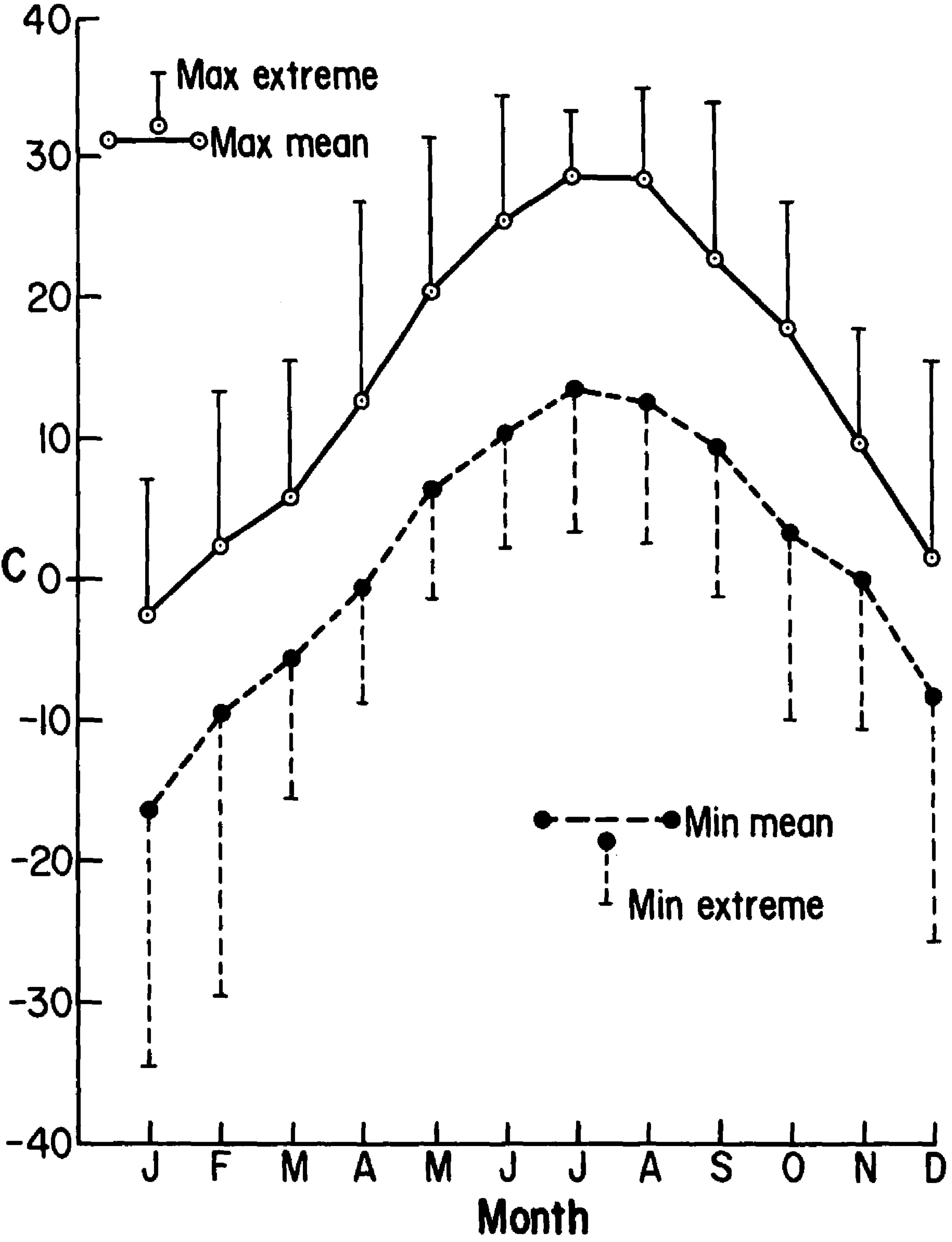
IV. ECOLOGY

1. The Physical Environment

The intertidal zone of the oceans is considered to be quite harsh to its faunal and floral inhabitants, since it demands of them acclimatization to life both in and out of water. The demands are even more severe in estuaries where seasonal, daily and even tidal salinity and temperature variations are more extreme. An even further compounding of the severity may take place where general climatic conditions cause additional stress, for example, in the form of ice cover and very low salinities, when the ice melts. The salt marsh areas of New England, and especially northern New England, are prime examples of the extreme severity of conditions which intertidal estuarine areas can impose on their inhabitants.

Fig. 7 shows the average maximum and minimum air temperatures and the extreme high and low temperatures on a monthly basis as recorded by the official weather station at Durham, New Hampshire. These averages are themselves averages of data for the two year period covering September 1969 to August 1971. Vagenas (1969) showed that temperatures at the Durham station differed little from those recorded at the Hampton-Seabrook, New Hampshire, salt marshes. Therefore, differences between Great Bay marshes and Durham

Fig. 7. Maximum and minimum monthly air temperatures (°C)


40
30
20
10
°C 0
-10
-20
-30
-40
Max extreme
Max mean
Min mean
Min extreme
J
F
M
A
M
J
J
A
S
O
N
D
Month

may be considered negligible.

A Leeds & Northrop Speedomax H temperature recorder and a Honeywell Electronik 15 salinity recorder allowed a daily monitoring of water temperature and salinity in the bay (at the University of New Hampshire's Jackson Estuarine Laboratory). Thus trends and sudden fluctuations could be followed up at the collecting stations. Figs. 8 and 9 represent conditions averaged for both Adams Point and Bunker Creek. The monthly maximum and minimum water temperatures (Fig. 8) and salinities (Fig. 9) at high tide are given for the period of September 1970 to August 1971.

Emery, Stevenson and Hedgpeth (1957) state that the ecologically significant aspect of temperature and salinity in an estuary is not their mean condition but their range on a daily and seasonal basis, and their rate of change through the tidal cycle. In Great Bay, conditions are such that ranges encountered on a given day may nearly equal the range for the same month. For example, summer nights in New Hampshire are often quite cool, and the water temperature at any early morning high tide may well be in the mid-teens. The hot summer days may easily cause the incoming water on the following high tide to be in the mid-twenties.

Figs. 7-9, then, show the seasonal and daily extremes to which animals living in the Great Bay system must be

Fig. 8. Maximum and minimum monthly water temperatures ($^{\circ}C$) at high tide from September 1970 to August 1971.

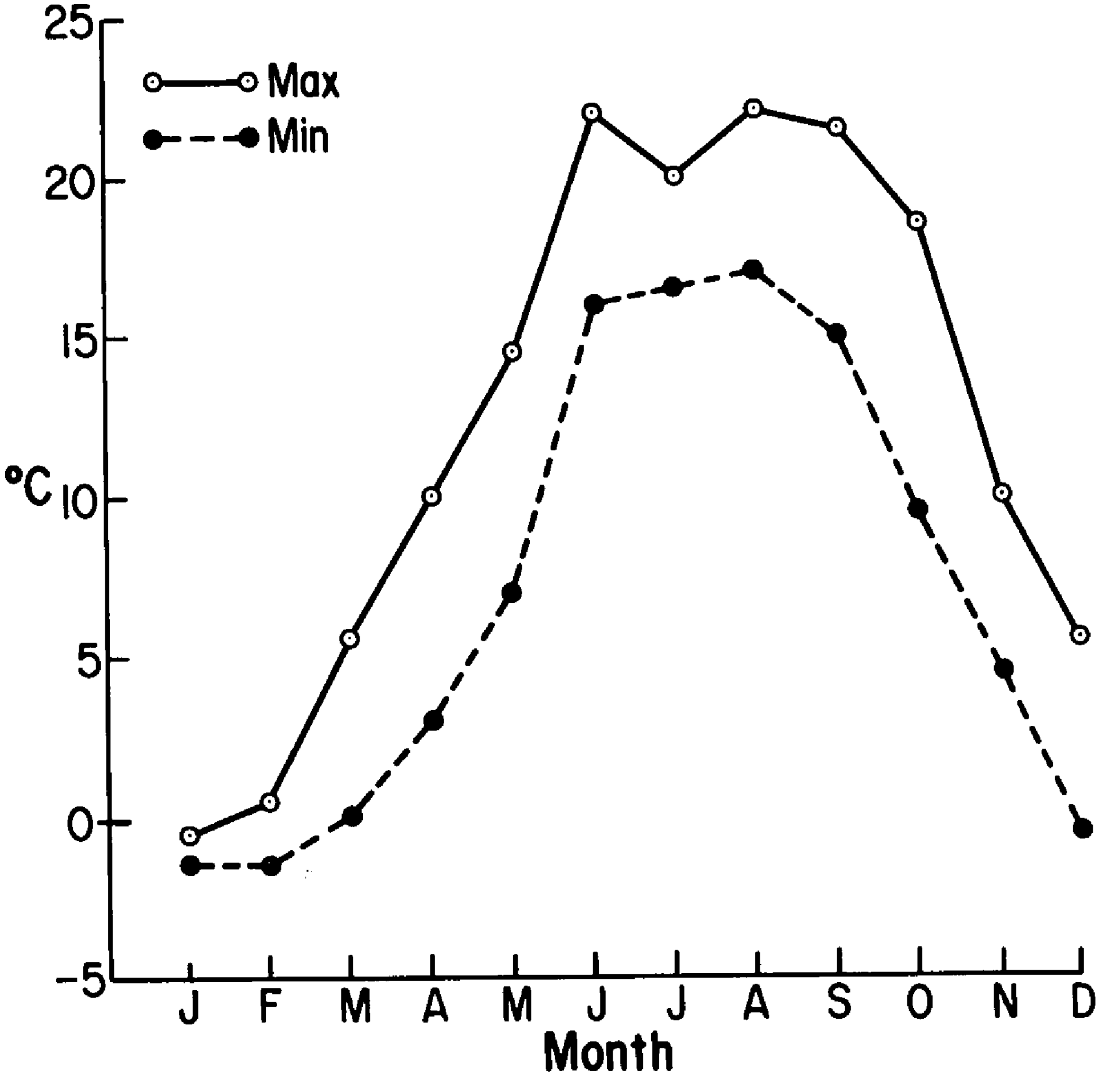
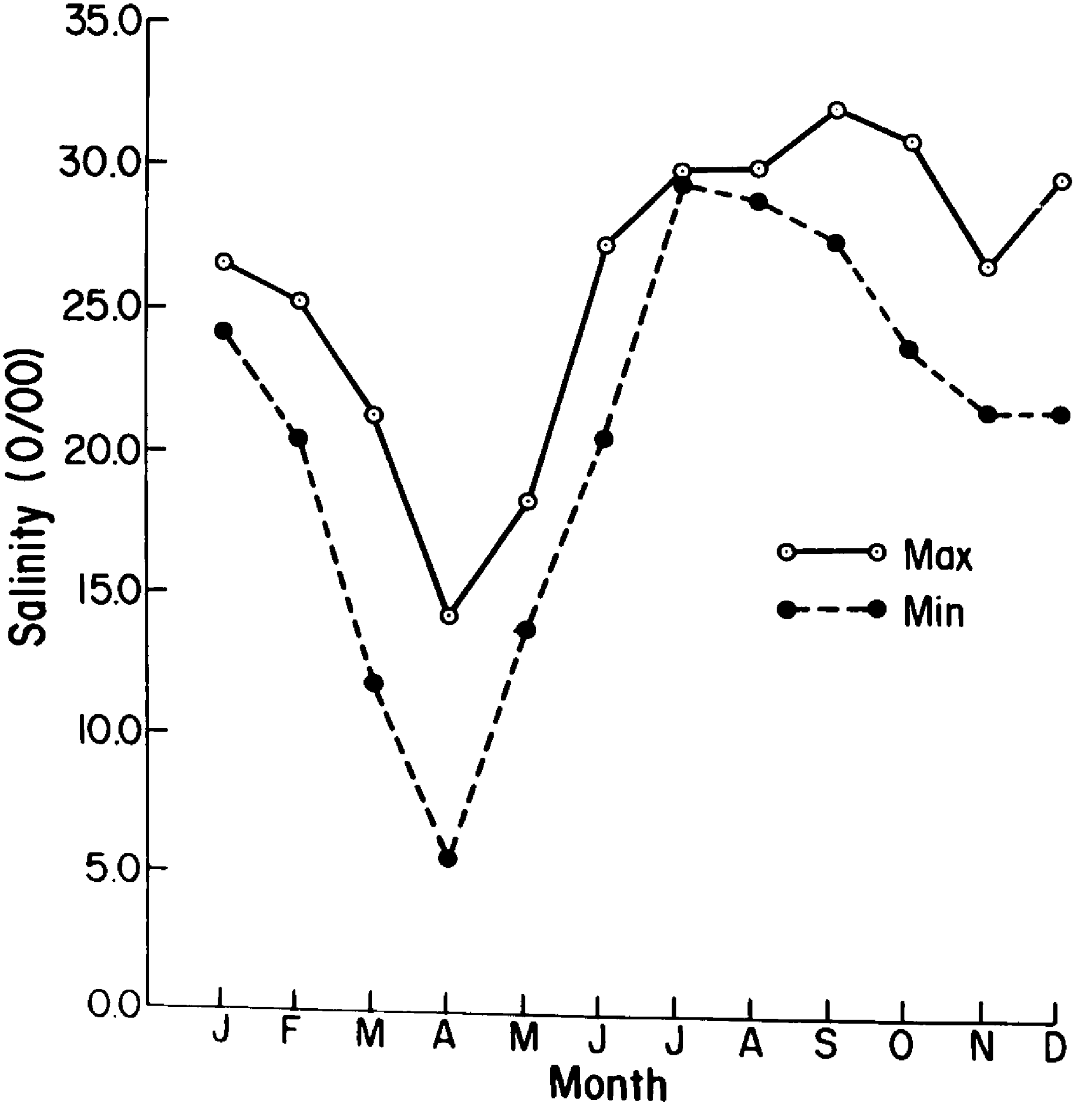

Max
Min
°C
25
20
15
10
5
0
-5
J
F
M
A
M
J
J
A
S
O
N
D
Month

Fig. 9. Maximum and minimum salinities (o/oo) at high tide from September 1970 to August 1971.


Salinity (0/00)
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
J
F
M
A
M
J
J
A
S
O
N
D
Month
Max
Min

able to adjust. But what of shorter time fluctuations, equally important (Emery et al., 1957), experienced during one tidal cycle? Tables 1 and 2 give some indication of these changes. Both tables show the average temperature recorded for each of several microhabitats in the salt marsh at Adams Point from the time they are emersed until immediately after they are again submersed, a period of approximately six hours. A Yellow Springs Tele-Thermometer with remote thermistors was used for temperature measurements. Salinities (silver nitrate titration) and air temperatures are also given in the tables.

Table 1 shows data for a typical, sunny, hot summer day, while Table 2 shows data for a typical early spring day after ice melt. On both days low water occured mid-day. In summer, a sharp rise in temperature, and hence greater extreme variation, occured in some microhabitats (mud surface, culm surface and under those rocks above the Spartina) than in others (base of aggregated culms and under those rocks between the upper bank and the lower flats). Notice how the water on flood tide gained a considerable amount of heat while crossing the wide mud flats. In fact, the microhabitat at the base of the Spartina culms experienced the highest temperature of the day not during exposure to air, but after submersion by the incoming tide.

Table 1. Summer. Temperature (^{o}C)-Salinity (o/oo) profile at Adams Point during low tide on September 10, 1

Location of waters edge	Above *Spartina*	Between upper bank and lower flats	Mud flat	LW 1140 (-0.1)
Time	-3 hrs 40 min	-3 hrs	-1½ hrs	
Location				
Air	20.5	21.5	25.0	29.0
Water	19.0	20.0	23.0	-
Mud surface - lower flats	-	-	23.0	25.2
Base of aggregated *Spartina* culms	19.0 submerged	19.2	21.0	21.2
Culm surface (4-5 cm from bottom)				
shade	-	18.3	20.8	23.5
sun	-	23.2	24.3	31.2

ature (°C)-Salinity (o/oo) profile for selected microhabitats during low tide on September 10, 1971.

ove tina hrs min	Between upper bank and lower flats -3 hrs	Mud flat -1½ hrs	LW 1140 (-0.1)	Mud flat +1½ hrs	Between upper bank and lower flats +3 hrs	Above *Spartina* +3 hrs 40 min
.5	21.5	25.0	29.0	30.0	27.5	27.0
.0	20.0	23.0	-	27.5	27.0	25.5
	-	23.0	25.2	26.2	-	-
.0 erged	19.2	21.0	21.2	21.8	23.2	24.0 submerged
	18.3	20.8	23.5	28.0	26.3	-
	23.2	24.3	31.2	31.8	30.8	-

Under rock (between upper bank & lower flats)	-	20.5	20.2	22.8	24.2	24.
Under rock (highest level of _G_. _palustris_ distribution)	-	20.2	22.2	27.7	27.0	23. shad
Under rock (above distribution of _G_. _palustris_	-	23.0	25.2	29.0	30.8	28.
Water (salinity)	31.2	31.2	31.2	-	30.8	31.

-	20.5	20.2	22.8	24.2	24.3	-
-	20.2	22.2	27.7	27.0	23.8 shade	-
-	23.0	25.2	29.0	30.8	28.3	-
31.2	31.2	31.2	-	30.8	31.0	31.0

Table 2. Early Spring. Temperature (°C)-Salinity (o/oo) p
habitats at Admas Point during low tide on March

Location of waters edge	Above *Spartina*	Between upper bank and lower flats	Mud flat	LW 1408 (0.1)
Time	-3 hrs 40 min	-3 hrs	-1½ hrs	
Air	6.0	10.0	7.5	7.5
Water	1.5	2.0	3.5	-
Mud surface - lower flats	-	-	3.5	4.5
Base of aggregated *Spartina* culms	1.7 submerged	2.5	4.7	4.7
Under mulch	-	2.3	4.5	5.2
Under rock (between upper bank & lower flats)	-	2.5	5.0	5.3

Temperature (°C)-Salinity (o/oo) profile for selected micro-
as Point during low tide on March 23, 1972.

ve <u>ina</u> rs in	Between upper bank and lower flats -3 hrs	Mud flat -1½ hrs	LW 1408 (0.1)	Mud flat +1½ hrs	Between upper bank and lower flats +3 hrs	Above <u>Spartina</u> +3 hrs 40 min
0	10.0	7.5	7.5	6.0	4.0	3.0
5	2.0	3.5	-	2.5	2.0	2.0
	-	3.5	4.5	3.5	-	-
7 rged	2.5	4.7	4.7	4.2	4.0	2.0
.	2.3	4.5	5.2	5.5	4.2	-
.	2.5	5.0	5.3	4.8	4.2	-

Under rock (highest level of distribution)	-	2.7	5.7	6.2	5.5
Under rock (above distribution)	-	8.5	9.0	8.7	8.2
Shallow pool - lower flats	-	-	6.0	9.0	8.0
Water (salinity)	14.1	15.1	15.3	-	11.1

-	2.7	5.7	6.2	5.5	3.2	2.0
-	8.5	9.0	8.7	8.2	3.2	1.7
-	-	6.0	9.0	8.0	-	-
14.1	15.1	15.3	-	11.1	11.2	11.0

In early spring the situation was generally similar, but different in specifics. Again, certain microhabitats (under those rocks considerably beyond the Spartina and in a shallow pool on the lower flats) had greater temperature variations than others (mud surface, under mulch, under rocks between upper bank and lower flats). A comparison of salinities for the two seasons shows a relatively constant condition for summer but a larger variation before and after low water in spring.

During the rest of the year, fluctuations experienced during one tidal cycle probably lie somewhere between the two given examples. There are two exceptions: 1) the undoubtedly colder nights of early spring and the colder nights and days of late fall; and 2) the entire winter, during which the marsh is ice-covered and temperatures beneath the ice are more or less constant for all microhabitats measuring approximately -0.5°C to -2.0°C.

2. Distribution

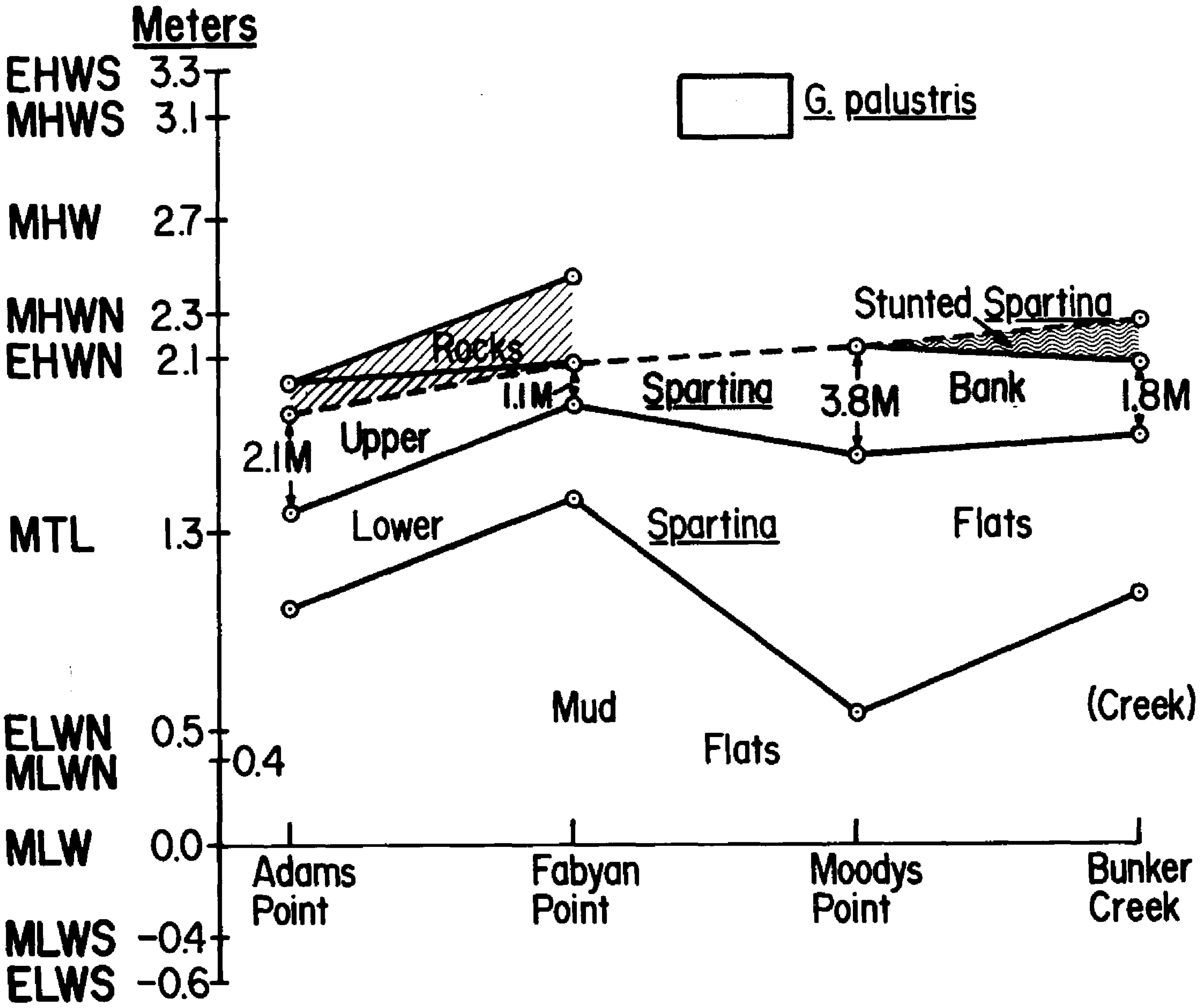
a. The Habitat

In the Great Bay system, *G. palustris* is an obligate inhabitant of the lower salt marsh. Extensive intertidal collecting never turned up an individual outside of the marsh. Nor did plankton tows in river channels and bays, nor dredging in the sublittoral and in the littoral at high water (not directly over Spartina). In addition, as a means of checking possible passive dispersal in the early spring, dislodged pieces of peat and floating strands of severed Spartina were examined for the presence of G. palustris, but the only amphipod found was one Orchestia uhleri. Although always found on the lower marsh, G. palustris reaches its greatest density during most of the year in the zone of the upper Spartina bank, as shown in Fig. 10.

This figure represents the intertidal profile of each station, as measured with a hand-held level and surveyor's pole, and delineates the mud flats, lower Spartina flats and upper Spartina banks. The shoreward border in the zone of maximum density for G. palustris (large shaded area) is precisely at the level of Extreme High Water Neap Tide. The zone is relatively narrow (the width being shown on the figure for each station) and coincides with the entire upper Spartina banks. Exceptions are Bunker Creek, where the Spartina is dwarfed above a certain tidal level and G. palustris does not occur, and Adams Point, where animals may at times extend a short distance beyond the Spartina into the region of pebbles and rocks.

Within this zone of maximum density the preferred microhabitats during time of low tide are: 1) in the basal system of aggregated Spartina culms; 2) on the lower portion

Fig. 10. Profile of stations showing tidal levels of mud flats, lower *Spartina* flats, upper *Spartina* banks, and zone of maximum density (shaded area) of *G. palustris*. For the latter, the horizontal width is indicated.


Meters
EHWS 3.3
MHWS 3.1
MHW 2.7
MHWN 2.3
EHWN 2.1
MTL 1.3
ELWN 0.5
MLWN 0.4
MLW 0.0
MLWS -0.4
ELWS -0.6
G. palustris
Rocks
Stunted Spartina
1.1M
Spartina
3.8M
Bank
1.8M
2.1M
Upper
Lower
Spartina
Flats
Mud
Flats
(Creek)
Adams Point
Fabyan Point
Moodys Point
Bunker Creek

of the culm; 3) in the junctions formed by blades; 4) under rocks; 5) under live vegetation, primarily Ascophyllum nodosum var. scorpioides; 6) under tidal trash, mostly decaying vegetation. The first three microhabitats are available at all four stations; but the fourth, only at Adams and Fabyan Points; the fifth, at Bunker Creek; the sixth, only commonly at Moodys Point.

In the field during low tide, G. palustris moved only when disturbed, and during high tide the species was never observed swimming. This suggested that the observed microhabitat preferences during low tide might also hold during high tide. Laboratory observations showed G. palustris to be quite a sedentary animal. Most individuals kept in plastic containers clung to the sides or bottom; only a few swam with any regularity. When small rocks and tidal trash were added to the container, even fewer animals were seen swimming. For example, 107 well-fed G. palustris were placed in a container and given several hours to adjust. During a two hour period every animal observed swimming was carefully removed. The results appear in Table 3. This shows that the majority of amphipods were sedentary, particularly mature females. Those that left the covering of rocks and trash were mostly males with some juveniles and immature females.

Table 3. Number of swimming and sedentary *G. palustris* according to life stage.

Life stage	No. swimming	No. sedentary
Juvenile	7	8
Male	16	44
Female		
Immature	3	3
Ovigerous	1	16
Post-ovigerous	1	8
Total	28	79

This experiment was confirmed by field work. At high tide, large dip nets forced through the lower sections of the *Spartina* culms, but not disrupting the substratum, seldom caught any *G. palustris* other than some juveniles and a few males. A small plankton net, towed along the water surface at high tide directly over the upper *Spartina* bank caught the following amphipods: 2 *Ampithoe valida*, 9 *G. mucronatus*, 1 *G. oceanicus*, 14 *G. palustris* and 10 *Hyale nilssoni*. Thirteen of the *G. palustris* were juveniles; one, a small male.

It is therefore reasonable to assume that the microhabitat preferences shown by the animals during low tide are also operable during high tide and not merely places of retreat on a falling tide.

b. Seasonal Variation

Seasonal inspection of the habitat by transects and close monitoring of the various microhabitats revealed that *G. palustris* undergoes a clearly discernable yearly cycle of distributional changes. This cycle is dependent upon seasonal changes in the weather and its effect on salt marsh life. The following generalizations are based on field observations made during the years 1969 through 1971, all of which had quite severe, though typical, winters. Observations during the relatively mild, atypical winter of 1972 follow later.

Summer (July, August, September). This is the period of rapid growth and highest rate of productivity in the salt marshes. In Great Bay, *Spartina* grows from 0.5 to 1.7 m tall until, at the end of August or beginning of September, the marsh turns from a summer green to an autumn brown. Although on a daily basis variations in temperature, of both air and water, are the rule rather than the exception, on a seasonal basis summer offers comparatively little variation in temperature extremes (Figs. 7 and 8). Variation in salinity is also comparatively minimal, both daily and seasonally, although the highest salinities of the year occur in late September (Fig. 9).

During summer *G. palustris* is distributed throughout the upper *Spartina* bank and inhabits all available microhabitats. Only a few occasional stragglers are found on the lower *Spartina* flats. The two most favored habitats for the adult amphipods are in the basal system of aggregated culms and under rocks between the upper bank and lower flats.

Autumn (October, November, December). Temperatures in autumn (Figs. 7 and 8) steadily decrease through the end of December until the level of the extreme lows of winter is reached. Unlike summer, great temperature extremes occur on a seasonal basis and large daily temperature variations are still common. Salinities (Fig. 9) fall off somewhat from the highs of late summer and there are apt to be greater fluctuations due to increased amounts of precipitation. The first heavy frost is likely to occur in early October. The *Spartina* then dies and forms a mat on the marsh surface. Patches of non-permanent ice and the first snow falls of late November are harbingers of the permanent ice cover and frequent snows that develop in late December.

With the lowering of temperatures in early October, *G. palustris* shifts its population concentration shoreward. The animals utilize the basal system of aggregated culms at higher tidal levels to a greater degree than during the summer. Fewer animals are found among the culms in the transitional zone between upper bank and lower flats. Rocks lying over the general area of the upper *Spartina* bank serve as shelter more often than during the summer, and fewer amphipods are found under rocks in the transitional zone lower down the shore. At Adams Point the amphipods extend past the upper bank and find protection under the many small rocks covering the area (Fig. 10). This shoreward shift in distribution continues until the beginning of December, when a definite reverse shift begins. By the end of the month all amphipods are in the more moist transitional region between upper bank and lower flats or on the lower flats themselves, often under fucoids, if present.

Winter (January, February, March). Whether considered seasonally, daily, or on the basis of a single tidal cycle, temperature fluctuations during winter are small. A permanent ice cover over the marsh is responsible for these relatively constant temperatures, both during times of low and high tides. However, this same ice cover is responsible for the greatest salinity fluctuations and the greatest changes in marsh physiography of the year.

As mentioned above, the permanent ice cover begins to form in late December. The ice may reach a thickness of slightly more than 1 m. The undersurface is frequently very irregular, and when resting on the marsh during low tide actually only certain projections and edges are in direct contact with the substratum. Among these contacts caverns of various heights and sizes are formed. During a flooding tide the ice is lifted and, since the points of contact have frequently frozen to the marsh surface, pieces of the marsh (vegetation, peat) are dislodged and carried with the ice. Tidal action also frequently causes faulting of the ice with the subsequent formation of huge irregular blocks. When the tide recedes and the ice once again falls on the marsh surface, the points of contact are different. Thus, a constant pitting and scouring takes place which may make the marsh in early spring appear to be one large mud flat.

During January and February the marsh is typically completely covered by ice. The marsh surface beneath the ice is never frozen. Brief thaws may cause some melting, which at low tide can form pools or rivulets under the ice. Salinities up to 10 o/oo below the salinity of the water at the previous high tide were measured for Adams Point. During early March the ice cover may disappear from parts of the lower flats, but not until the end of the month does the upper bank become completely bare. Because of general melting conditions the salinity drops continuously and considerably during the month of March.

With the onset of winter's permanent ice cover, *G. palustris* abandons all of the previously described microhabitats and seeks either an area of thick decaying mulch or a shallow pool. Since these occur infrequently on the lower marsh, vast numbers of animals are concentrated in a very few locations.

At Adams Point *G. palustris* congregates in breaks between the peat banks. These hold a layer of mulch (*S. alterniflora*, *Salicornia* sp., *Zostera marina*, *Fucus* vesiculosus, leaves from deciduous trees, and needles from conifers) and act as run-off channels from the marsh whenever any melting occurs. At Fabyan Point, the animals occupy the few available tide pools. Several of the mosquito ditches at Moodys Point, filled mainly with decaying Z. marina, serve here as the winter retreat. At Bunker Creek, G. palustris occupies the infrequent shallow depressions in the gently sloped lower Spartina flats. Only with the onset of spring will the population leave these winter retreats.

Spring (April, May, June). The relatively constant temperature of the winter season (under the ice) gives way to a picture resembling that of autumn: great temperature extremes on a seasonal basis and large daily temperature variations (Figs. 7 and 8). The combination of continued ice melt, snow melt and the usual heavy precipitation gives April the lowest salinities of the year (Fig. 9). Then, within two short months the salinity returns to near-summer levels.

In late April the young green blades of Spartina start dotting the exposed mud and peat. Mats of Enteromorpha plumosa and Sphacelaria britannica cover the substratum of much of the lower marsh and last through June. In Great Bay, Spartina grows to a height of approximately 0.5 m by the end of spring.

At Adams Point and Fabyan Point, in the early part of April and continuing through the month, G. palustris leaves its winter retreats and inhabits the lower flats and the transitional zone between these and the upper bank. This movement out onto the flats does not occur at Moodys Point and Bunker Creek until much later in the month. By the beginning of May the amphipods have once again returned to the microhabitats they left at the end of December. By mid-month only a few stragglers are left on the lower flats and by June the animals have completely spread over the upper bank and, in fact, have a distribution similar to that of early autumn. So, once again, with the advent of summer temperatures, a slight downward movement on the upper bank will take place and G. palustris completes the cycle and returns to its normal summer distribution.

Winter of 1972. Much milder than the typical New Hampshire winter, the winter of '72 afforded the opportunity to observe possible differences in the distributional patterns of G. palustris with a change in climate. Ice cover was never permanent for more than a two week period. The only change noticed in the distributional patterns of the species was a departure from its winter retreats two to three weeks earlier than in previous years.

c. Variation within the Population

In addition to the seasonal cycle in distribution and microhabitat selection just described, differences in microhabitat selection also occur within the populations. At Adams and Fabyan Points those animals collected under rocks were placed in separate containers from those collected in association with the *Spartina*. In Figs. 11 and 12 the mean monthly size of the adult population is plotted for each habitat. It is clear that once the amphipods have departed from their winter retreats and established themselves over the upper *Spartina* bank (May), more of the larger members of the population seek shelter under rocks and more of the smaller members seek shelter in the *Spartina*. This separation holds true not only for the population in general, but also for the sexes when considered separately. In other words, the mean size of males found under rocks from May to December is always greater than the mean size of males found in the *Spartina*, and similarly for females.

Juveniles at these two stations have habitat preferences also, as shown in Fig. 13. Here the percentage of juveniles found under rocks and in association with *Spartina* at Adams and Fabyan Points, is plotted for the months May through December. (In May and June at Fabyan Point

Fig. 11. Mean monthly length of adult _G_. _palustris_ at Adams Point.

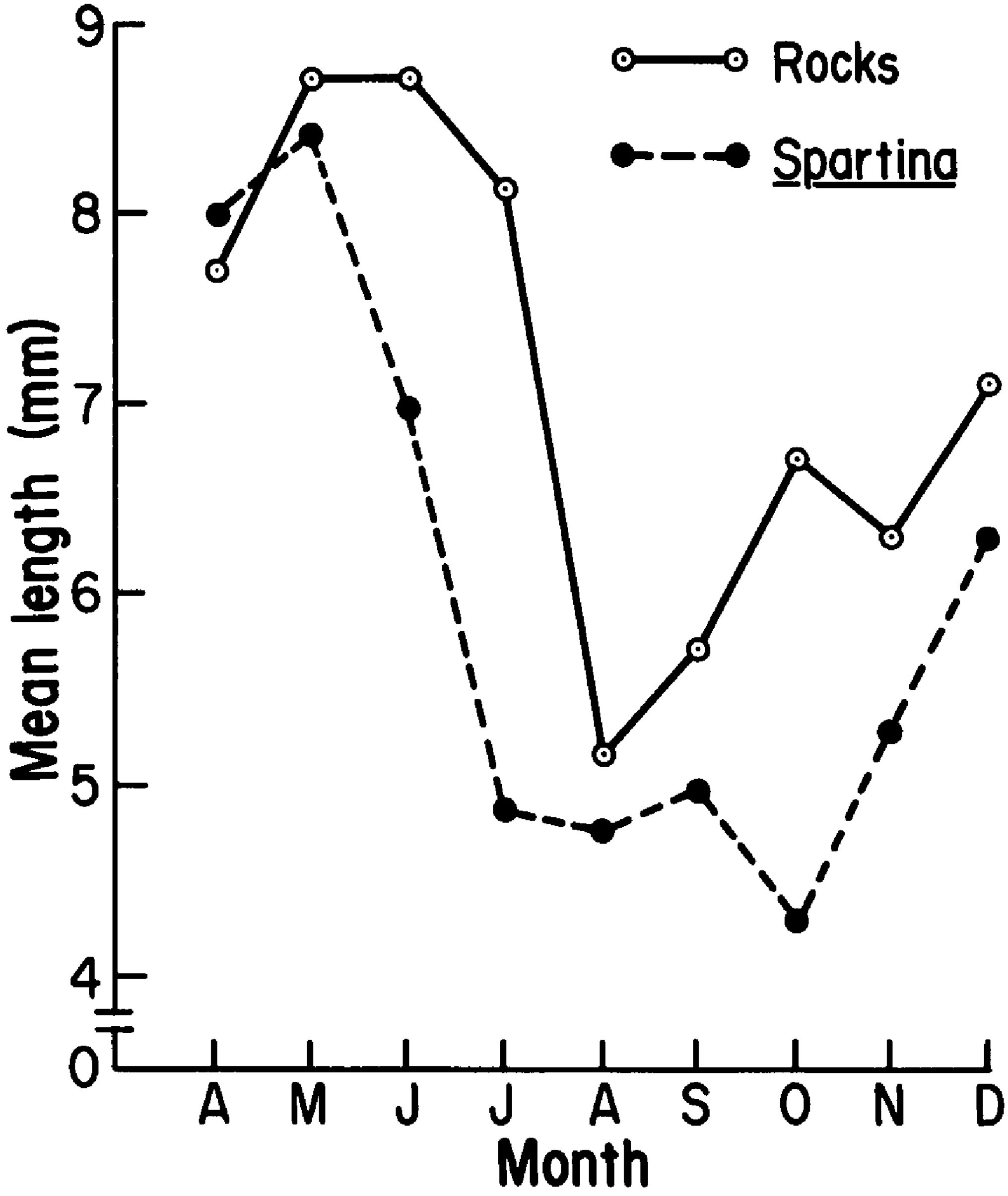

Mean length (mm)
9
8
7
6
5
4
0
A
M
J
J
A
S
O
N
D
Month
Rocks
Spartina

Fig. 12. Mean monthly length of adult _G_. _palustris_ at Fabyan Point.

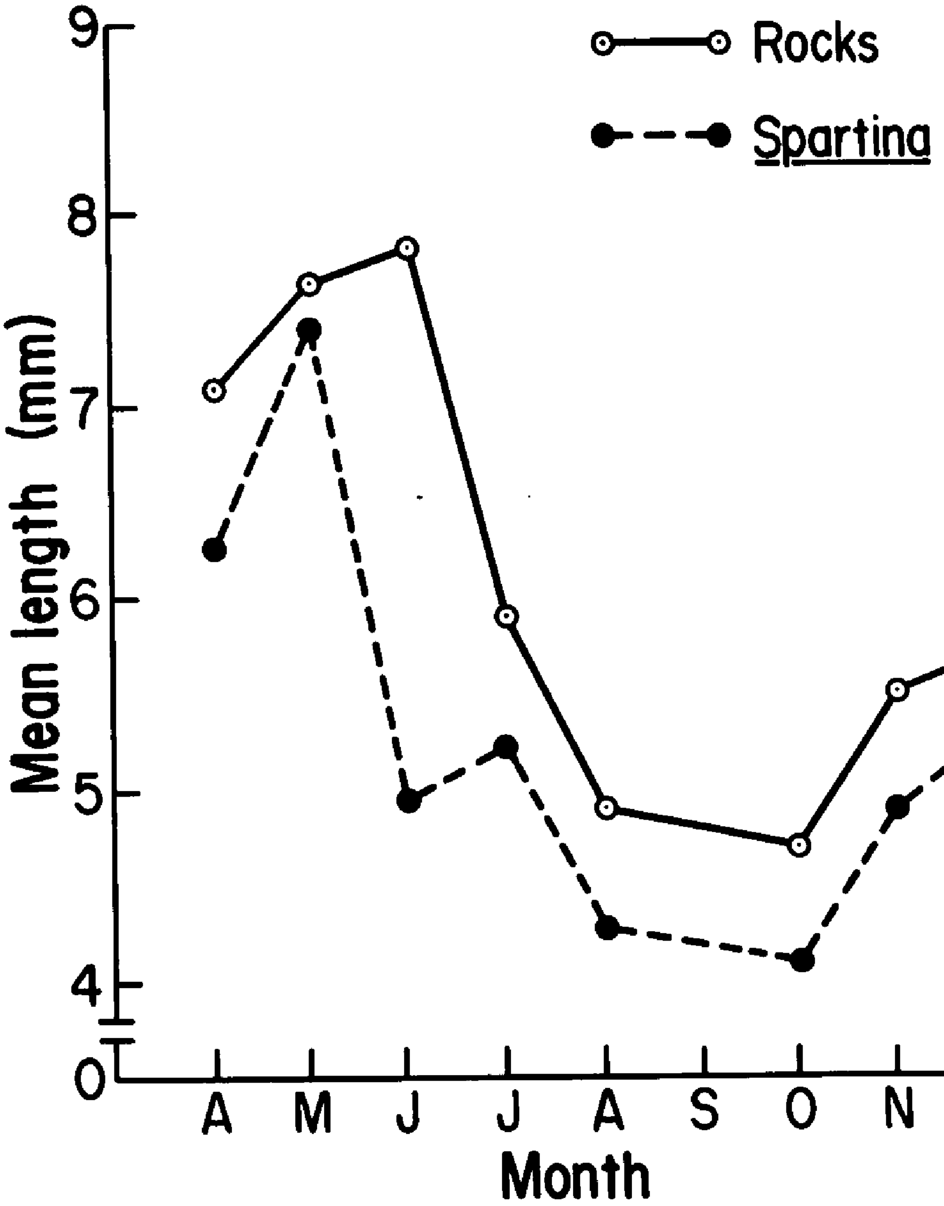
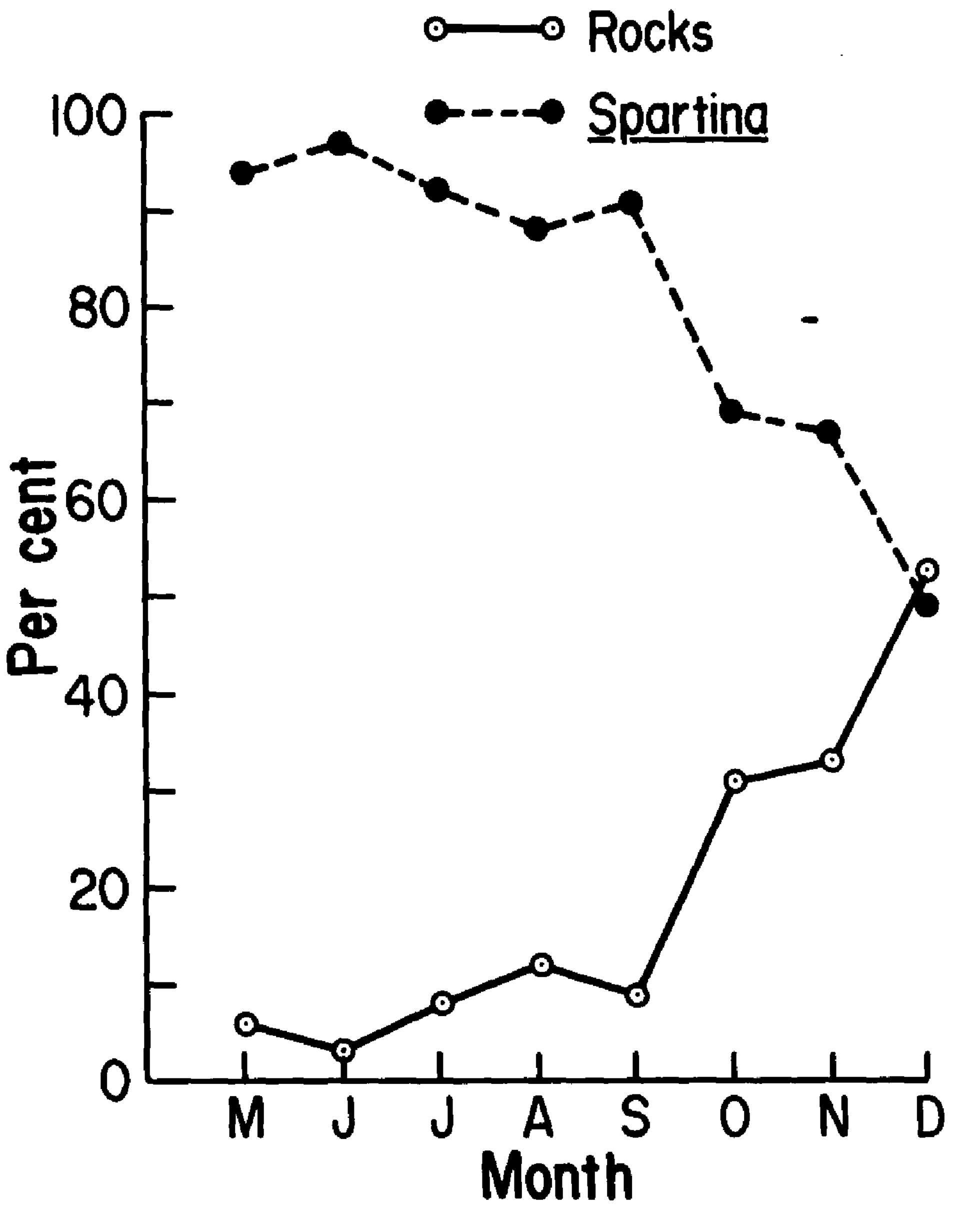

Rocks
Spartina
Mean length (mm)
9
8
7
6
5
4
0
A
M
J
J
A
S
O
N
D
Month

Fig. 13. Per cent of monthly juvenile population associated with either *Spartina* or rocks.


Rocks
Spartina
Per cent
100
80
60
40
20
0
M
J
J
A
S
O
N
D
Month

juveniles were often found in association with the _E. plumosa_ mats. The values for these two months represent the combined numbers found in the mat and in association with _Spartina_). The obvious difference in habitat preference held strongly through the spring and summer and broke down only in autumn. During autumn, the juveniles were much larger than in spring or summer, since they were all born before summer's end. The autumn breakdown was therefore correlated with growth of the juveniles.

But what of those habitats, Moodys Point and Bunker Creek, in which rocks are not found? As for differences in microhabitat distribution based on size, only field observations are available. In general, the larger members of the population were found in the basal system of the largest and most compact aggregated _Spartina_ culms or, at Bunker Creek, under the dense mat of _A. nodosum_ var. _scorpioides_. As for differences based on life stage, the data of Table 4 are the result of a late June collection at Moodys Point. These reinforce subjective observations made throughout spring, summer and part of autumn. These data show that the larger individuals (large males, ovigerous and post-ovigerous females) inhabit the basal system of the aggregated _Spartina_ culms. The juveniles are most often found on the clum surface not more than 5 cm from the substratum.

Table 4. Microhabitat preferences by life stage for that portion of the *G. palustris* population associated with *Spartina*.

Life Stage	Microhabitat of Culm			
	Base	Surface (bottom to 5 cm)	Surface (above 5 cm)	Junction with blade
Juvenile	2	26	3	2
Male (< 5.5mm)	26	13	4	4
Male (> 8.5mm)	30	--	-	-
Female-immature	7	3	1	1
Female-ovigerous	24	--	1	1
Female-post-ovigerous	3	--	-	-

The small males and immature females are more or less divided equally between the basal system and the other three microhabitats. Observations indicate that this microhabitat preference also occurs at Adams and Fabyan Points among that part of the population associated with *Spartina* rather than with rocks. This pattern of microhabitat distribution, though highly complex, is clearly discernible.

3. The Biological Environment

a. Associated Fauna

The fauna of salt marshes is a mixture of fresh water- and estuarine-derived animals with comparatively low species diversity. Terrestrial animals are also represented. Table 5 shows the macrofaunal inhabitants of the *S. alterniflora* habitat of the Great Bay system and gives the relative seasonal abundance of each species. Only a dozen or so species were somewhat abundant year-round, and most of the species occurred only in selected seasons. Most of the terrestrial species (spiders and insects) are probably much more abundant in the high marsh, but they must not be considered mere wanderers into the low marsh, since they were found at Adams and Fabyan Points where no *S. patens* exists. The estuarine components were primarily mollusks and crustaceans and of the 17 species of crustaceans listed, 11 are amphipods. The relationship between *G. palustris* and

Table 5. Associated fauna of the lower marsh, including hig seasonal abundance indicated (A = abundant, M = mo R = rare or scarce). Arrows denote presence only season.

Taxon	Associated Fauna
Cnidaria-Anthozoa	*Edwardsia elegans*
Mollusca-Gastropoda	*Aldaria* sp.
	Elysia sp.
	Hydrobia totteni
	Littorina littorea
	L. obtusata
	L. saxatilis
	Nassarius obsoletus
	Pyramidella fusca
	Melampus bidentatus
-Bivalvia	*Modiolus demissus*
Annelida-Polychaeta	*Nereis virens*
Anthropoda-Pseudoscorpionidae	*Lamprochernes oblongus*
-Araneae	*Lycosa* sp.
	Epeiridae
-Acari	mites (5? species)
-Insecta-Collembola	*Anurida maritima*
-Coleoptera	Carabidae
	Curculionidae
	Dytiscidae
	Dytiscid larvae

ıuna of the lower marsh, including high tide migrants, with
ıdance indicated (A = abundant, M = moderately abundant,
scarce). Arrows denote presence only in early or late part of

ciated Fauna		Winter	Spring	Summer	Autumn
	Edwardsia elegans			R	
	Aldaria sp.		A		
	Elysia sp.		A		
	Hydrobia totteni		M	M	M
	Littorina littorea			M	M
	L. obtusata	A	A	A	A
	L. saxatilis	A	A	A	A
	Nassarius obsoletus		M	R	R
	Pyramidella fusca	M	A	A	A
	Melampus bidentatus	A	A	A	A
	Modiolus demissus	A	A	A	A
	Nereis virens	R→		R	
ionidae	*Lamprochernes oblongus*			R	
	Lycosa sp.	A→	A	A	A
	Epeiridae		R	M	
	mites (5? species)	A	A	A	A
lembola	*Anurida maritima*	A	A	A	A
eoptera	Carabidae		A	A	
	Curculionidae		R	R	
	Dytiscidae		A	A	
	Dytiscid larvae		M	M	

-Diptera	*Tabanus* sp. larvae	M→	
	Dolichopodid larvae		
-Hymenoptera	*Crematogaster* sp.		
-Hemiptera	Saldidae		
-Crustacea	*Leptochelia rapax*		
	Jaera marina	A	
	Ampithoe valida	M	
	Gammarus mucronatus	A→	
	G. oceanicus	R	
	G. tigrinus	M→	
	Hyale nilssoni	A	
	Jassa falcata	R→	
	Marinogammarus finmarchicus		
	M. obtusatus		Se
	Melita nitida	R→	←
	Orchestia grillus	R	
	O. uhleri	A	
	Carcinus maenas		
	Rithropanopeus harrisi		

High Tide Migrants

Limulus polyphemus	
Palaemonetes pugio	
Pagurus sp.	
Apeltes quadricus	
Fundulus heteroclitus	A→

-Diptera	*Tabanus* sp. larvae	M→	A	A	←M
	Dolichopodid larvae			R	
-Hymenoptera	*Crematogaster* sp.		R	R	
-Hemiptera	Saldidae				
:acea	*Leptochelia rapax*		R	R	
	Jaera marina	A	A	A	A
	Ampithoe valida	M			
	Gammarus mucronatus	A→	A	M	
	G. oceanicus	R		R	
	G. tigrinus	M→			
	Hyale nilssoni	A	A	A	A
	Jassa falcata	R→			
	Marinogammarus finmarchicus		M	M	M
	M. obtusatus		See text		
	Melita nitida	R→	←R		
	Orchestia grillus	R	R	R	R
	O. uhleri	A	A	A	A
	Carcinus maenas			R	
	Rithropanopeus harrisi		R	R	
	High Tide Migrants				
	Limulus polyphemus		A		
	Palaemonetes pugio		M	A	
	Pagurus sp.			A	
	Apeltes quadricus		R	R	
	Fundulus heteroclitus	A→	A	A	A

these 11 species was examined.

Table 6 lists the amphipods with their normal microhabitat when they occur on or near the marsh. *G. palustris* maintains a separation from most of these amphipods simply through its own microhabitat preferences. Furthermore, because of its low mobility, this separation is probably maintained to a great extent during both high and low tides. For *O. grillus* and *O. uhleri*, behavioral patterns probably serve in lieu of microhabitat distribution. For *M. obtusatus* the situation is more complex, even though a slight difference in microhabitat preference is indicated. Near the coast in high salinity marshes, *G. palustris* and *M. obtusatus* co-occur, but infrequently. Usually only *M. obtusatus* is found.

A seasonal breakdown in microhabitat separation from the remaining species (except *M. finmarchicus*) occurs in winter and early spring, when *G. palustris* has left its normal microhabitats. During this time several species not usually found on the marsh (*A. valida*, *J. falcata*, *M. nitida*) extend their distribution onto areas of the lower *Spartina* bank. These species, along with others more often found (*G. oceanicus*, *G. tigrinus*, *H. nilssoni*, and particularly *G. mucronatus*) will then be found along with *G. palustris* in shallow pools, beneath piles of tidal trash and under

Table 6. Microhabitat preference of amphipod species found on or near the lower marsh.

Species	Microhabitat
Ampithoe valida	In tidal trash on mud flats
G. mucronatus	In marsh pools; in tidal trash or algae on mud flats and lower *Spartina* flats
G. oceanicus	Under *F. vesiculosus*
G. tigrinus	Fresh water streams
Hyale nilssoni	On *F. vesiculosus*
Jassa falcata	In tidal trash on mud flats
Marinogammarus obtusatus	At bases of *Spartina* on mud; high salinity marshes
M. finmarchicus	In pools or very moist parts of upper *Spartina* bank; under rocks on lower flats
Melita nitida	In tidal trash and under shell fragments on mud flats
Orchestia grillus	Tunnels in *S. patens*; occasional wanderer into lower marsh during low tide
O. uhleri	Similar to *G. palustris*; active during low tide

F. *vesiculosus*. Such a breakdown affords opportunities for behavior normally precluded by the microhabitat separation. Evidence of this is a pair of amphipods found in precopula in early April at Adams Point--a *G*. *mucronatus* male and a *G*. *palustris* female. A subsequent laboratory experiment in which 10 males of each species were placed in separate containers with 10 females of the other species, yielded another mixed-species pair in precopula--this time a *G*. *palustris* male and a *G*. *mucronatus* female.

b. *Parasites*

Two parasites were found in association with *G*. *palustris*. The first was a parasitic ciliate, *Gymnodinioides* sp. (Ciliophora, Apostomatida). Chatton and Lwoff (1935) state that this parasite exists in a sessile encysted stage (the phoront) on the host's gills. When *G*. *palustris* molts, excystation occurs and the newly released trophonts swim into the molt and devour the remaining fluid and small particles present. The trophonts then swim away, encyst (tomont), divide often within the cysts, and finally excyst as tomites. These tomites are the infective stage for the amphipods.

G. *palustris* is also the host for the metacercaria of an unknown trematode. The encysted metacercariae occur mostly in the last three segments of the ventral thoracic

Table 7. Percentage of *G. palustris* parasitized with trematode metacercariae according to life stage during late summer.

Life Stage	Number	Per cent infected
Juvenile	28	14
Male	142	59
Female	68	53
Total	238	52

body cavity, but sometimes occur laterally, or in the abdominal body cavity. Occasionally they may displace internal organs such as the digestive glands. The cysts were noted sporadically throughout the year except for late summer. At this time the infection rate was high. Table 7 shows percentage infection of animals collected during the first two weeks of September. Percentage infection was over 50 for the adult population; juveniles had a much smaller percentage infection. The number of cysts per animal ranged from 1 to 11, yet the average number per animal was only 2.7, since over half the hosts had one or two cysts.

c. Feeding

Laboratory observations and experiments showed that *G. palustris* feeds only when submerged and then only for short irregular periods of time. Detritus and bits of vegetation (*U. lactuca*, *E. plumosa*, *Z. marina*) seemed to be the preferred food. Larger animals would also take pieces of *M. demissus* muscle. Although detritus consumers, the smaller animals and especially the juveniles more frequently scraped the surface of the algae rather than feeding on it directly. Table 8 gives the results of gut content analysis for adult and juvenile *G. palustris* at three different times of the year. Animals were preserved in a 5% buffered formalin solution with copper sulfate added to retain the green

Table 8. Gut analysis of G. palustris adults and juveniles. Numbers refer to the number of guts with the indicated contents according to relative abundance. (S = scarce, M = moderate, A = abundant).

	Winter		Spring (late)				Summer (late)			
Contents	Adults		Adults		Juveniles		Adults		Juveniles	
	S-M	A	S-M	A	S-M	A	S-M	A	S-M	A
Detritus	-	24	-	18	-	22	-	24	-	18
Dinophyceae	12	1	8	-	5	16	2	14	2	16
Rhodophyceae	1	-	-	-	-	-	2	-	-	-
Chlorophyceae (nonfilamentous)	8	8	2	6	-	-	1	4	-	-
Cyanophyceae and Chlorophyceae (filamentous)	-	-	1	-	5	14	2	16	3	15
Number of guts examined	24		18		22		24		18	

color of any algae present (Minckley and Cole, 1963). Only the mid gut was examined.

All guts, regardless of season or life stage, contained a large amount of non-identifiable detritus. In winter months the adults also ingested diatoms (Dinophyceae) and some nonfilamentous green algae (Chlorophyceae). The same was observed for the adults in late spring, but the juveniles had ingested filamentous green and blue-green (Cyanophyceae) algae rather than nonfilamentous green algae. They also took in more diatoms. In late summer the juvenile ingestion patterns continued to resemble those of spring, while the adult ingestion patterns were similar to those of juveniles.

d. Predation

The list of associated fauna (Table 5) includes migrant animals found moving over the marsh only during high tide. The killifish *Fundulus heteroclitus* is by far the most abundant of these animals and was therefore considered to be the most likely predator. Gut contents of the shrimp *Palaemonetes pugio*, the hermit crab *Pagurus* sp., and the four-spined stickleback, *Apeltes quadracus* yielded no *G. palustris*. Table 9 gives the result of the gut analysis of *F. heteroclitus* at various seasons. Except for winter, when harpacticoid copepods constituted the complete

Table 9. Gut analysis of *Fundulus heteroclitus*. Numbers refer to the number of fish with the indicated food item.

Food Item	Late Winter	Late Spring	Late Summer
Mixed vegetation		6	8
Ulva lactuca			3
Foraminifera		2	5
Nereis sp.		1	1
Littorina obtusata		13	1
Pyramidella fusca			8
Gemma gemma			7
Mya arenaria		1	
Limulus polyphemus		1	
Lycosa sp.		3	1
Mites		4	1
Saldidae		2	2
Dytiscid larvae		1	
Carabidae			1
Crematogaster sp.		3	
Tabanus sp. larvae		1	1
Harpacticoid copepods	11	6	6
Leptochelia rapax			1
Ampithoe valida			1
Corophium sp.		1	
G. mucronatus		1	3
Hyale nilssoni		1	
Orchestia uhleri		1	
Non-gammarid amphipods (unident.)		1	2
Fish eggs			3
Small *Fundulus*		1	

diet, the killifish was quite nonspecific in its prey selection. In fact, the number of prey species listed account for almost one half of the associated fauna, including many species seen only rarely. At no time were any G. palustris found in the killifish guts.

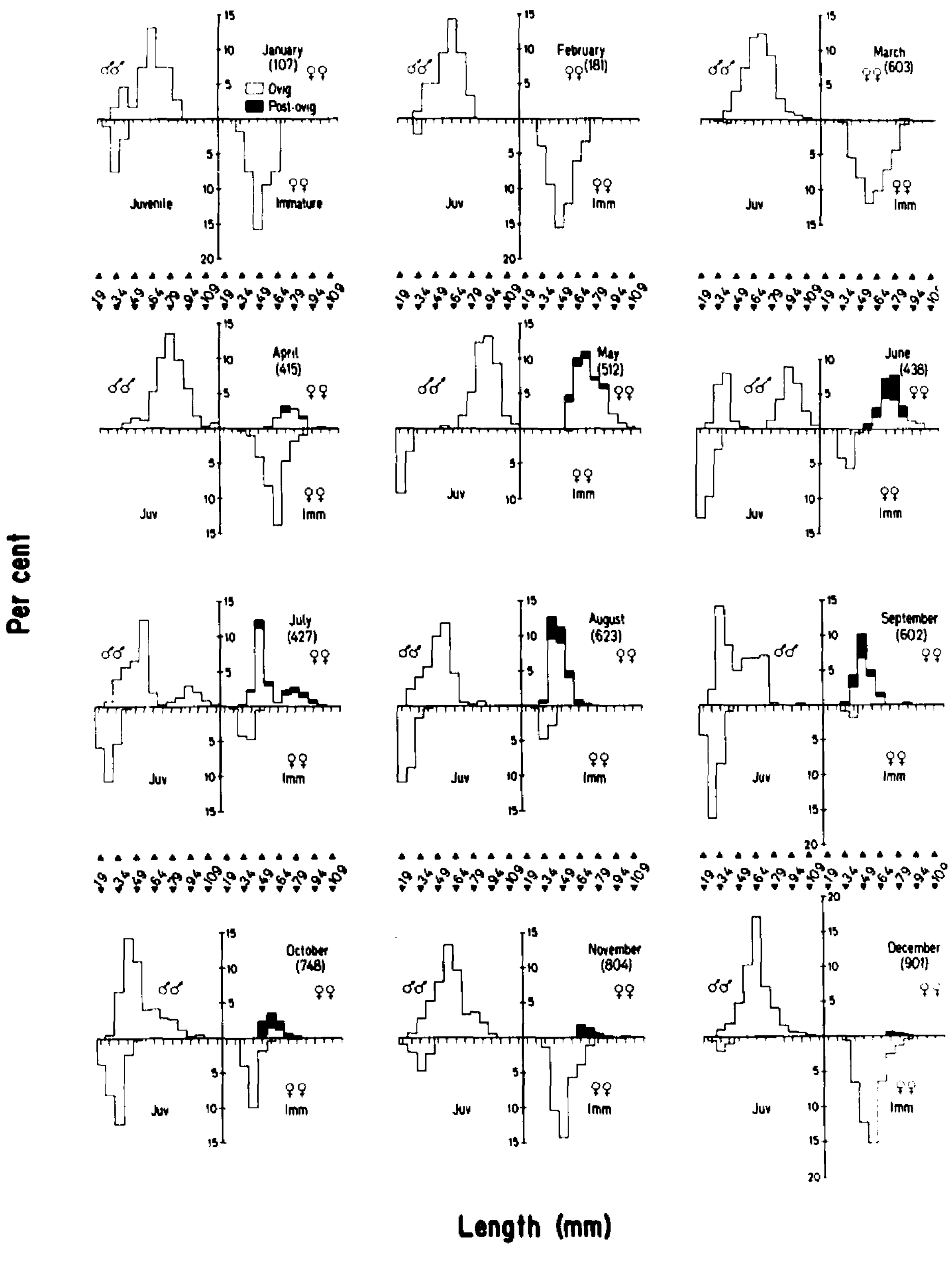
V. LIFE CYCLE

1. <u>Population Structure and Density</u>

Monthly collections of *G. palustris* provided material for a detailed study of the life cycle. The data are combined for the four stations and are presented in Fig. 14. The life stage and size classes are expressed as percentages of the total population for each month. Males always appear in the upper left-hand quadrant; juveniles, in the lower left-hand quadrant; ovigerous and post-ovigerous females, in the upper right-hand quadrant; and immature females, in the lower right-hand quadrant.

During winter the population was quite uniform, consisting almost entirely of males and immature females. Not until late April was there any differentiation. At that time some of the "overwintering generation" had reached sexual maturity and slightly less than 10% of the females were ovigerous. But by mid-spring the population structure had changed drastically. In May there were virtually no more immature females--all were ovigerous or post-ovigerous; and the first newly born juveniles of the year had made their appearance. By season's end there were two distinct generations: the overwintering generation, or the "spring breeders," consisting entirely of large, obviously mature males and large ovigerous and post-ovigerous females; the

Fig. 14. Per cent monthly composition of G. *palustris* population by life stage. Number in ( ) indicates total number collected that month.


January
(107)
February
(181)
March
(603)
April
(415)
May
(512)
June
(438)
July
(427)
August
(623)
September
(602)
October
(748)
November
(804)
December
(901)
♂♂
♀♀
Ovig
Post-ovig
Juvenile
Immature
Juv
Imm
0
5
10
15
20
19
34
49
64
79
94
109
Per cent
Length (mm)

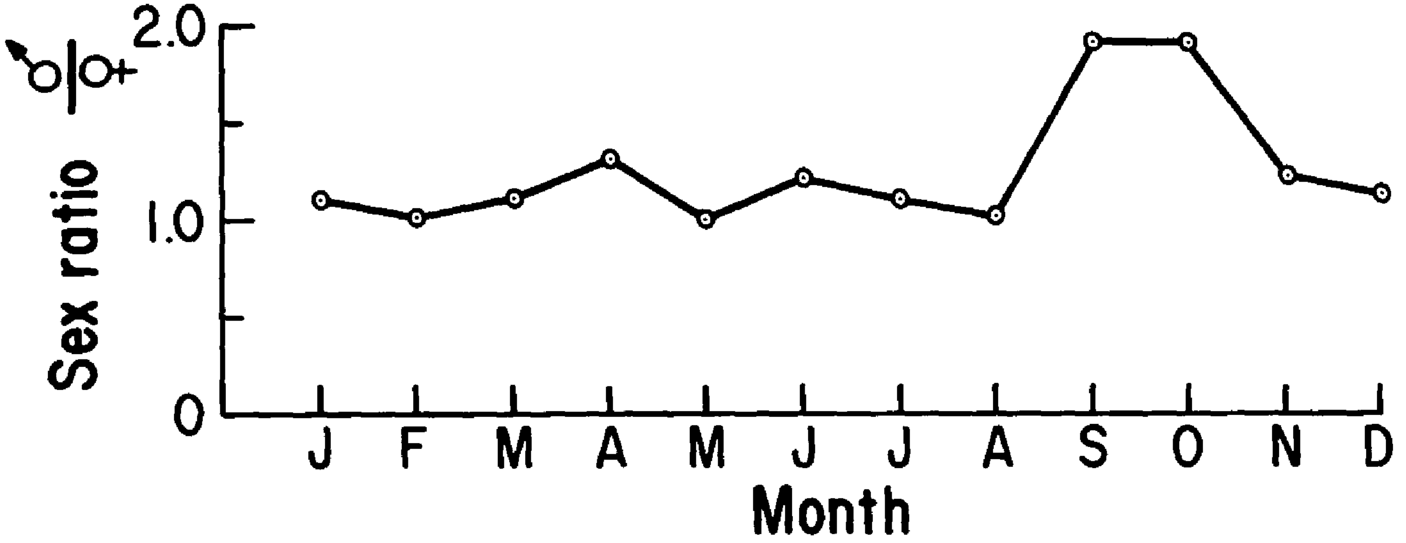
"summer generation" or the "summer breeders," consisting of juveniles, immature females and small, probably immature, males. Each generation accounted for approximately half of the June population.

In July the summer generation began to reproduce. The overwintering generation was also still breeding, but its contribution to the total population had fallen to below 20%. By mid-summer the population contained virtually only the summer breeders and their progeny. No change in structure occurred until autumn.

By October the ovigerous females of the summer generation had essentially disappeared from the population, although older, post-reproductive members of the summer generation still persisted. Juveniles, small males and immature females dominated. During the remainder of the season, animals of the summer generation experienced continued mortality. Juveniles became increasingly sexually identifiable, though not until they had attained a larger size than in summer. They then joined the ranks of the immature male and female _G. palustris_ as the next overwintering population.

Fig. 15 gives the monthly sex ratio for the four stations combined. Although males were always more abundant, they were significantly so only in September and October.

Fig. 15. Monthly sex ratio



Sex ratio ♂/♀
2.0
1.0
0
J
F
M
A
M
J
J
A
S
O
N
D
Month

Mention should be made here of intersexes. Only seven G. palustris were found to possess both male and female characteristics; all were members of the overwintering generation and at least one was found at each station.

Relative density estimates of G. palustris populations were made except during winter. Fig. 16 shows the number of amphipods collected seasonally at each of the four stations, each point representing the average number collected per month per season. There were obviously differences in abundance among the four stations in the sequence Fabyan Point > Moodys Point > Adams Point >Bunker Creek. At each station, the abundance of amphipods in autumn was much greater than in the following spring. This was not due to life cycle phenomena, as shown in Fig. 17. Here the combined totals of animals captured in November and December and of those captured in April and May are plotted for each station. Because of the presence of considerable numbers of the summer generation in October and June, data for these months were omitted. In addition, the combined totals have been corrected for the few, though significant, members of the summer generation present in the population: post-ovigerous females in November and December and an equal number of males and juveniles in May. Therefore the values represent only the overwintering animals, and the reductions

Fig. 16. Seasonal relative densities of G. palustris.

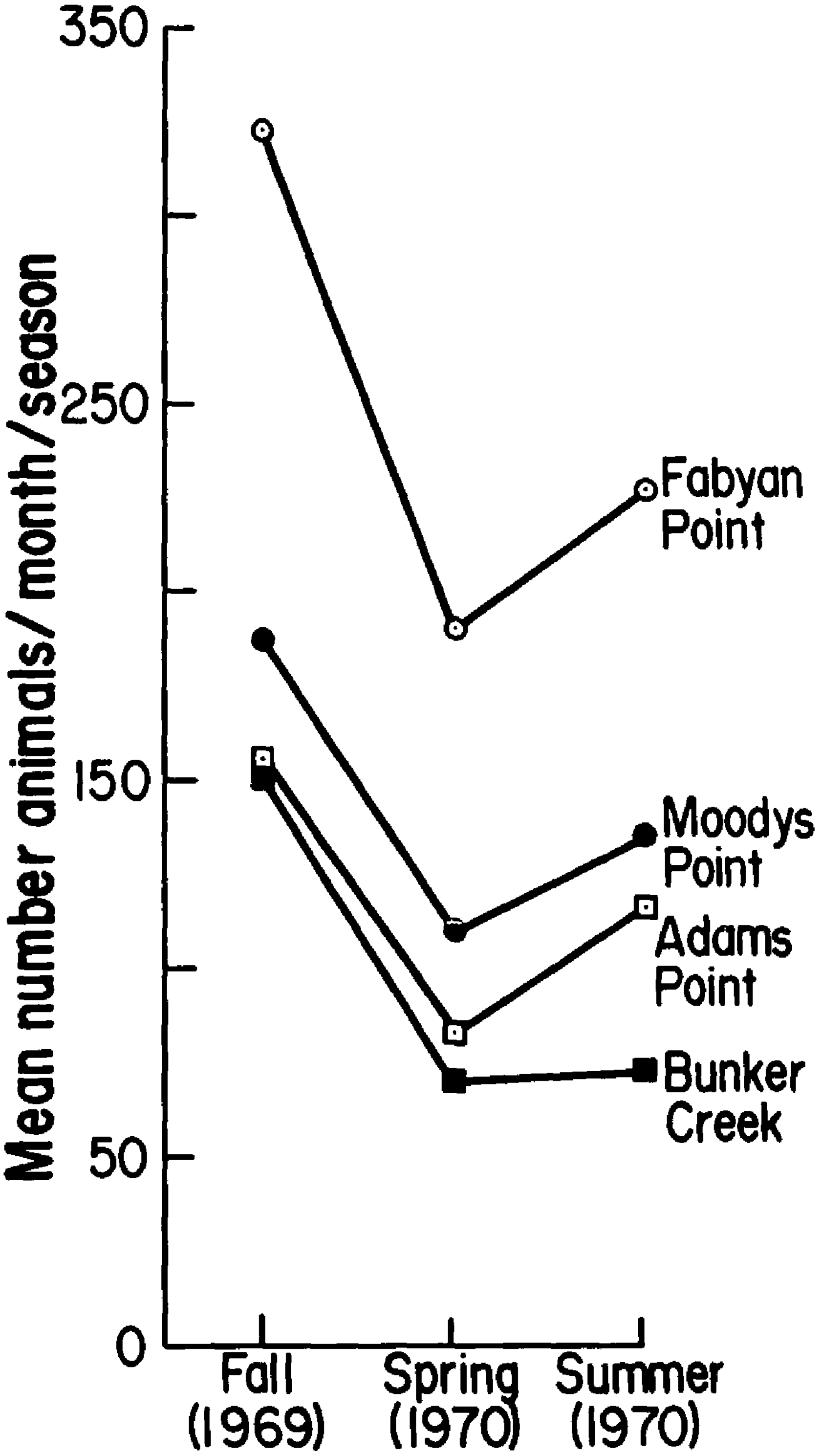
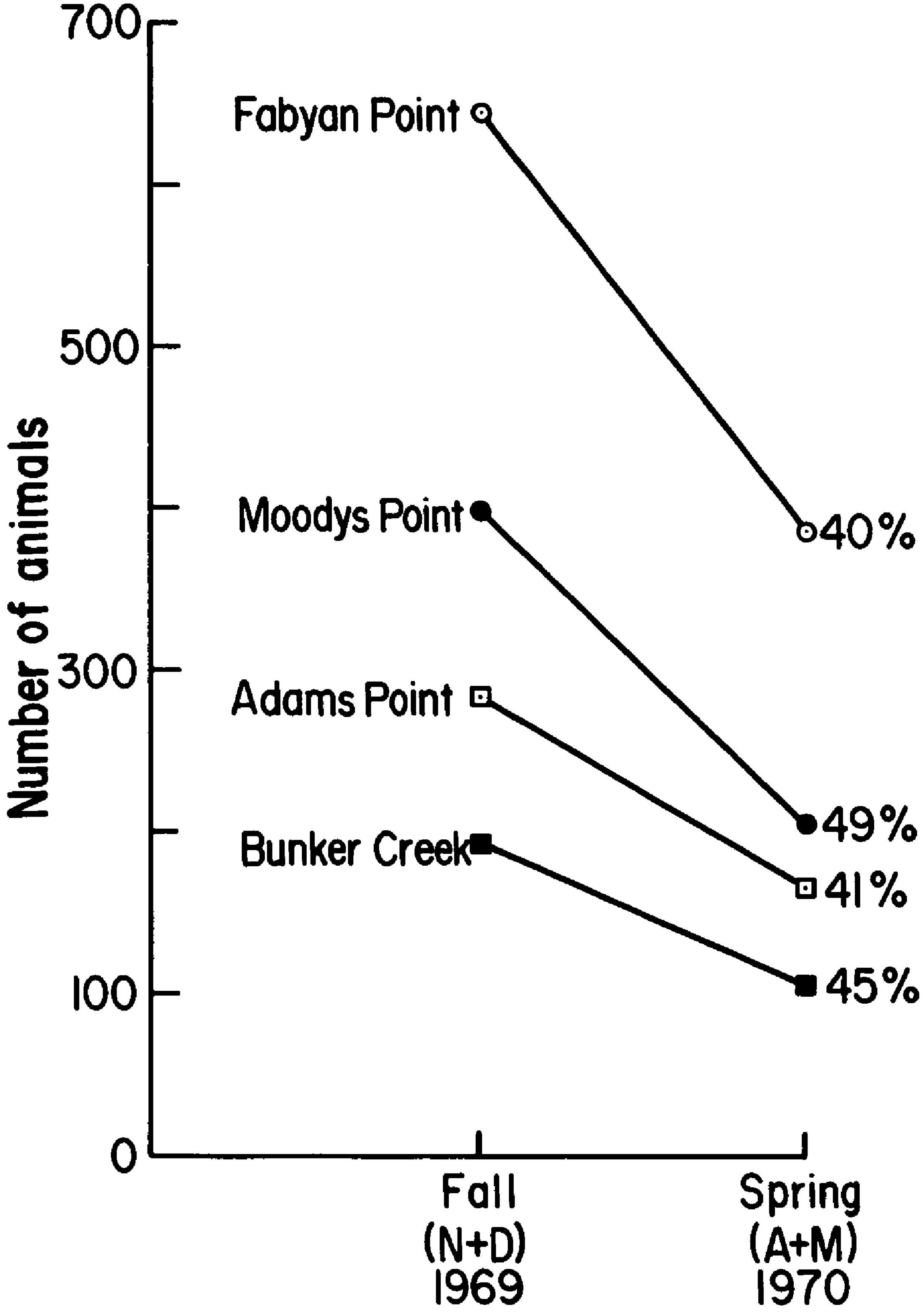

350
250
150
50
0
Mean number animals/ month/season
Fabyan Point
Moodys Point
Adams Point
Bunker Creek
Fall (1969)
Spring (1970)
Summer (1970)

Fig. 17. Density reduction from fall to spring of overwintering generation at each station.


Number of animals
700
500
300
100
0
Fabyan Point
Moodys Point
Adams Point
Bunker Creek
40%
49%
41%
45%
Fall (N+D) 1969
Spring (A+M) 1970

in abundance must have been caused by either physical or biological factors, or both. The reduction was substantial, averaging 44% for the four stations.

One final aspect of the population structure of G. palustris is a comparison of the relative density and the size of the animals. Figs. 18 and 19 show the average monthly length of the males and females respectively in the population at each of the four stations. For the months represented, the average length of the animals was generally in the sequence Bunker Creek > Adams Point > Moodys Point > Fabyan Point. The differences were less distinct in summer and early fall, since at this time the population was quite heterogeneous in life stage composition. Even here, however, animals from the Bunker Creek and Adams Point populations considered together, were decidedly larger than the Moodys and Fabyan Points animals. In late winter and early spring the differences were quite distinct among all four stations. During these periods the population was extremely homogeneous and the animals had experienced several months of growth with no reproduction, enabling them to reach a maximum size. Recalling Fig. 16 it is obvious that the sequence in mean animal length at the four stations is the direct opposite of their relative density sequence.

Fig. 18. Mean monthly lengths of males

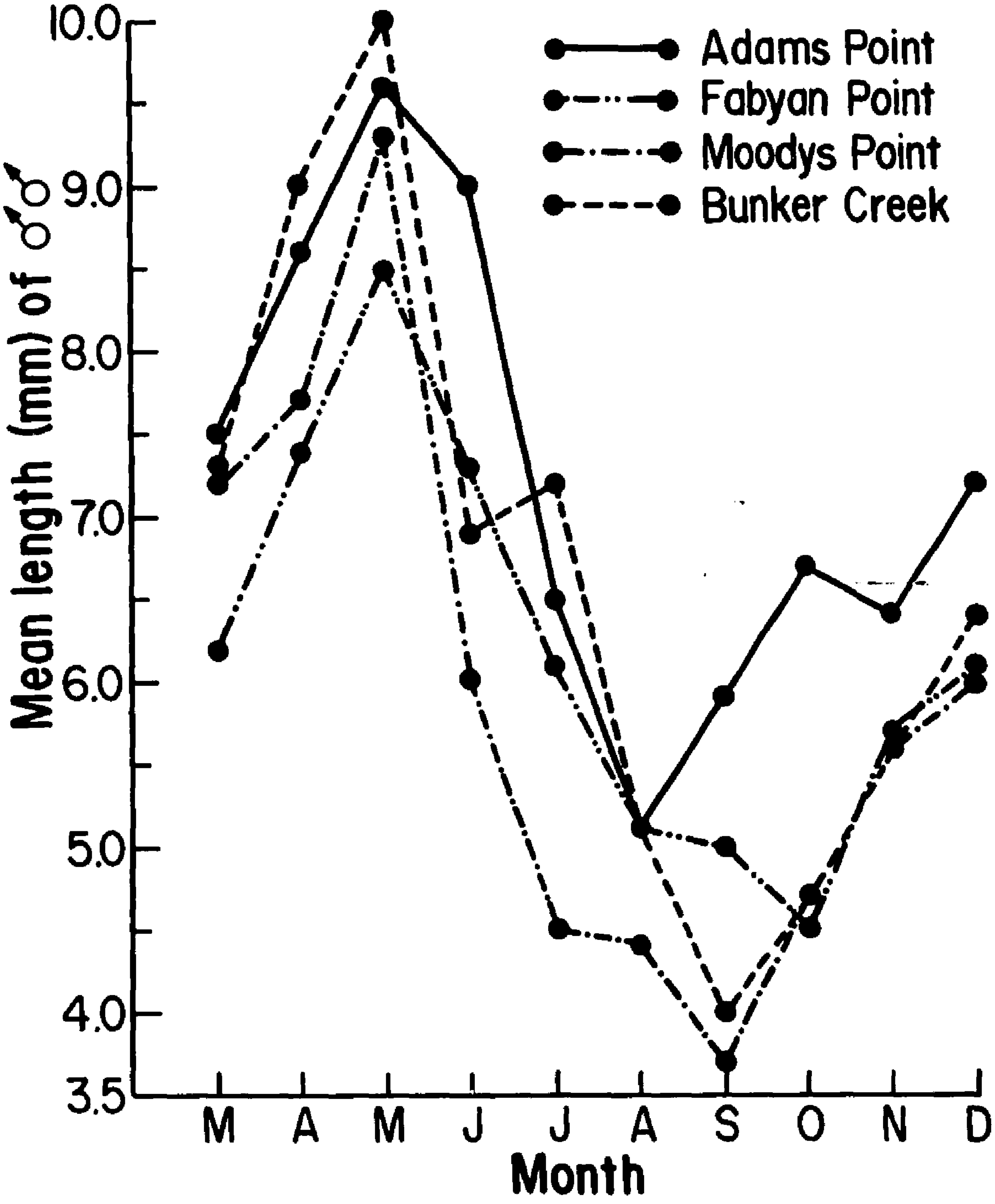
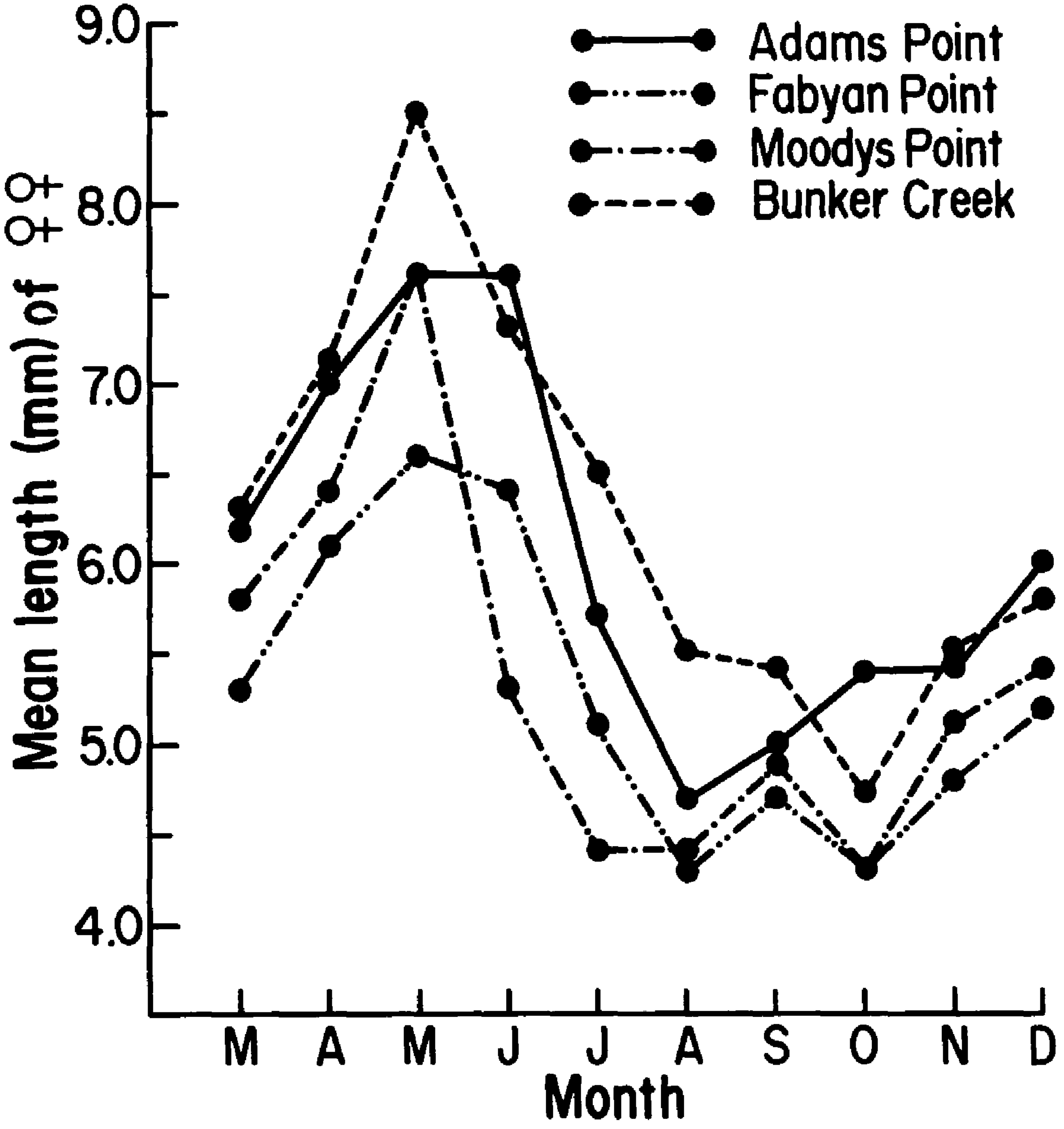

Adams Point
Fabyan Point
Moodys Point
Bunker Creek
Mean length (mm) of ♂♂
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.5
M
A
M
J
J
A
S
O
N
D
Month

Fig. 19. Mean monthly lengths of females



Mean length (mm) of ♀♀
9.0
8.0
7.0
6.0
5.0
4.0
M
A
M
J
J
A
S
O
N
D
Month
Adams Point
Fabyan Point
Moodys Point
Bunker Creek

2. Reproductive Capacity

Reproductive capacity depends on the number of breeding females and their contribution of young to the population. The number of ovigerous females, expressed as percentage of the total population, appeared in the life cycle histograms of Fig. 14. However, it is more informative to consider along with this the numbers of ovigerous females as a percent of the total number of females. Fig. 20 shows the number of immature, ovigerous, and post-ovigerous females as a percent of the total female population each month. For convenience, the numbers at the top of the graph are the percent of females per total population for the corresponding month (as in Fig. 14). Thus, for the spring breeding generation, May was the month with the highest percent of ovigerous females per total population and per total females. In July and August the reproductive capacity of the summer generation was at its peak. April and October marked the beginning and end of the breeding season and each possessed low reproductive capacities. The decrease in reproductive capacities for June and September is clarified in Fig. 21. Here total number, rather than percentages, of immature, ovigerous and post-ovigerous females are plotted for each month. The sharp reduction in numbers of ovigerous females from May to June shows that mortality of

Fig. 20. Monthly number of females as per cent of total female population: immature, ovigerous and post-ovigerous.

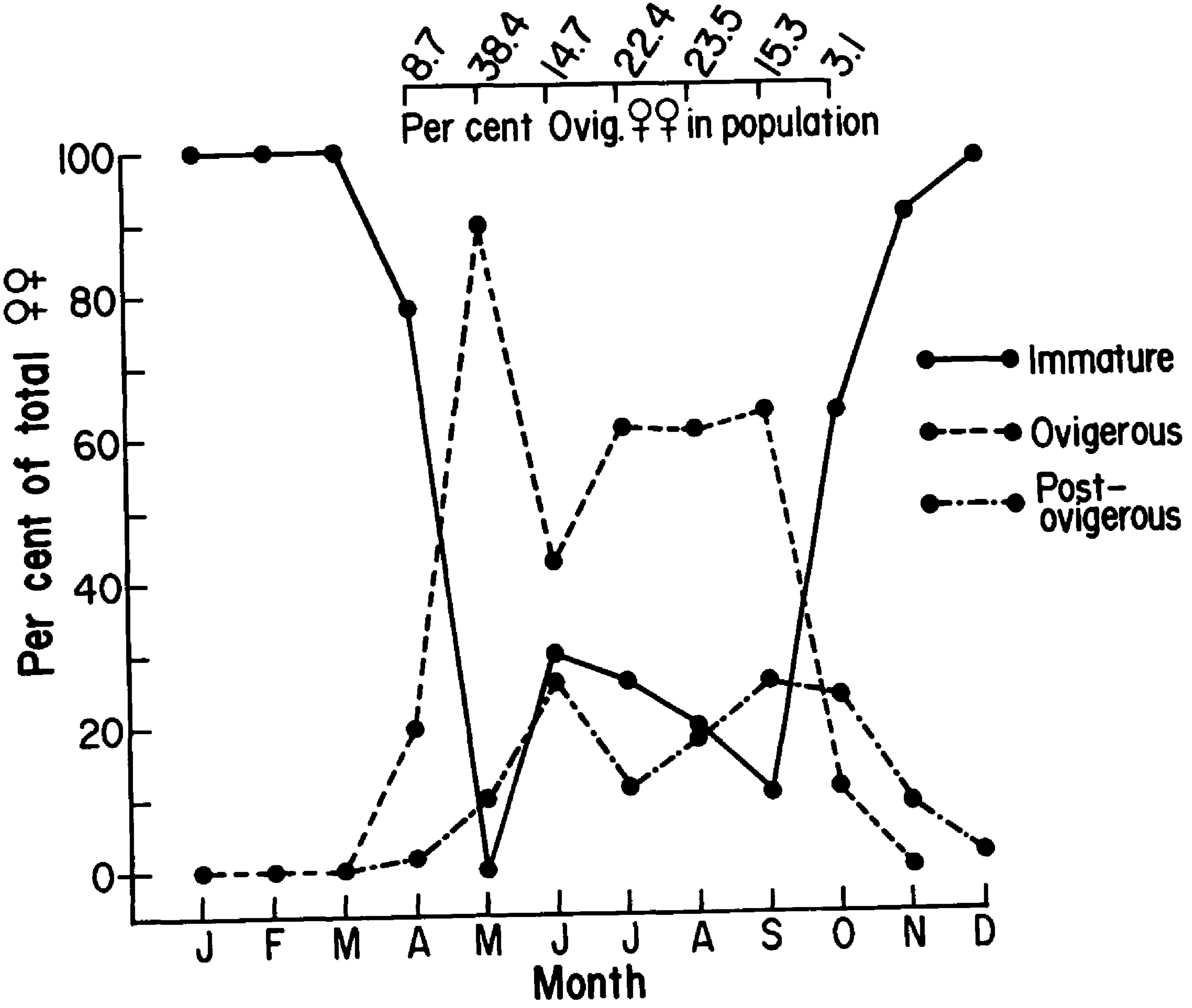
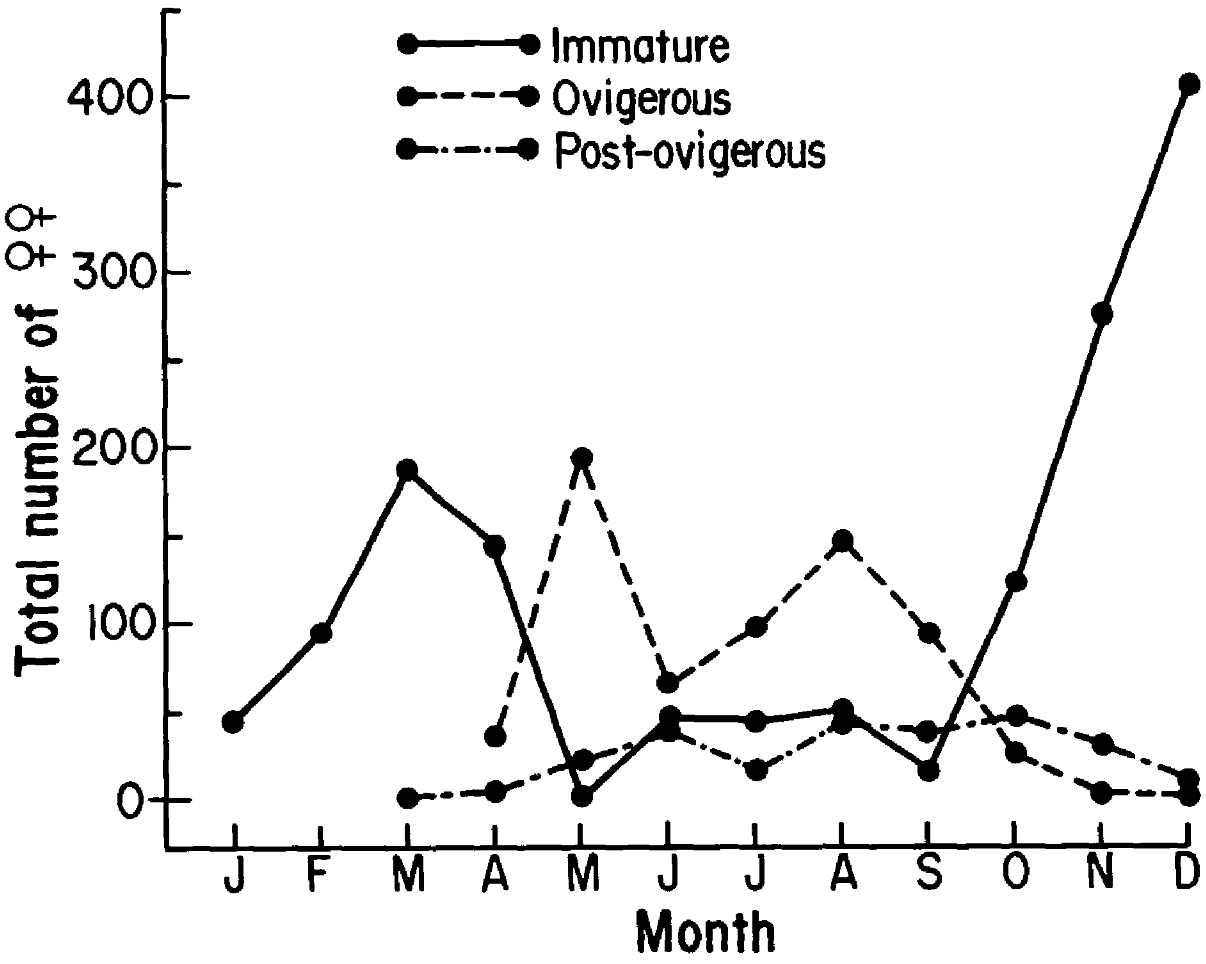

8.7
38.4
14.7
22.4
23.5
15.3
3.1
Per cent Ovig. ♀♀ in population
100
80
60
40
20
0
Per cent of total ♀♀
J
F
M
A
M
J
J
A
S
O
N
D
Month
Immature
Ovigerous
Post-ovigerous

Fig. 21. Monthly totals of females collected: immature, ovigerous and post-ovigerous.

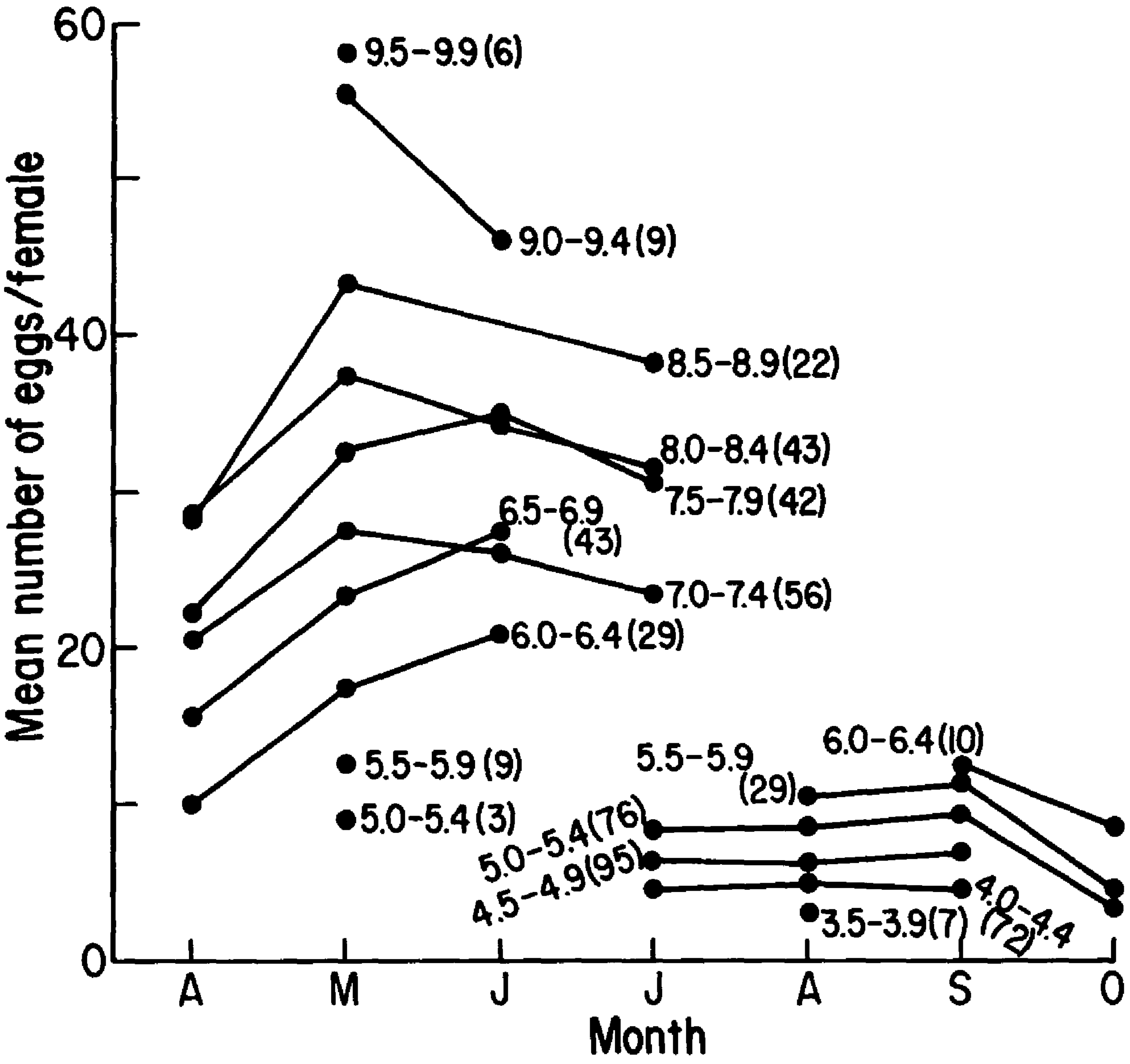

Immature
Ovigerous
Post-ovigerous
Total number of ♀♀
400
300
200
100
0
J
F
M
A
M
J
J
A
S
O
N
D
Month

the overwintering generation had begun. This, combined with the sexually unripe summer breeding generation, accounted for the drop in reproductive potential. Mortality occurring from August to September, combined with a reduced replacement rate of sexually mature females, was responsible for the drop in potential of the summer breeders.

An indication of the reproductive potential of G. palustris appears in Fig. 22. Here the average number of eggs per brood for a particular size class of female is plotted for each month of the breeding cycle. Reproductive potential depends not only on the length of the female, but also on the time of the year. Thus, for example, the spring breeding generation generally peaked in May, lower numbers of eggs per brood coming before and after; the summer breeders showed a definite decrease in numbers of eggs per brood during October. The sharp separation in size of the spring and summer females is very pronounced.

Reproduction potential also depends on the number of broods per female. This is less easily assessed by field studies than other life cycle phenomena, and laboratory investigations must be utilized. The maximum number of broods for G. palustris under laboratory conditions at environmental temperature and salinity was three. This was true for females of both the spring and summer breeding

Fig. 22. Average number of eggs per female during indicated months. Length range of females given next to each curve. Number in ( ) is total number of females in that length range. Spring-breeders on left; summer breeders on right.


Mean number of eggs/female
60
40
20
0
A
M
J
J
A
S
O
Month
9.5–9.9 (6)
9.0–9.4 (9)
8.5–8.9 (22)
8.0–8.4 (43)
7.5–7.9 (42)
6.5–6.9 (43)
7.0–7.4 (56)
6.0–6.4 (29)
5.5–5.9 (9)
5.0–5.4 (3)
5.5–5.9 (29)
6.0–6.4 (10)
5.0–5.4 (76)
4.5–4.9 (95)
3.5–3.9 (7)
4.0–4.4 (72)

generations.

3. Aspects of Reproductive Behavior and Development

Mating of species of *Gammarus* comprises successively precopula, moulting of the females, fertilization, the separation of pairs, and ovulation (Vlasblom, 1969). My observations of *G. palustris* showed these activities to be similar to the description by Kinne (1959) for *G. duebeni*. This reference may therefore be consulted for detailed descriptions.

Laboratory investigations showed that no female *G. palustris* ever deposited eggs in the marsupium without the presence of a male. The broods of females examined during analysis of the monthly collections contained virtually no damaged or stunted embryos. Development of embryos essentially paralleled the sequence outlined by Steele and Steele (1968) for *G. duebeni*. At hatching time for *G. palustris*, observations showed that the third uropods adhered to each other during embryonic development and acted as an "egg tooth." (Sheader and Chia, 1970, reported hatching spines on the urosome of *M. obtusatus*.) Upon hatching, the young *G. palustris* ranged in length from 1.2 to 1.5 mm, the average of 100 newly hatched juveniles was 1.3 mm. The young stayed in the marsupium from two to four days, regardless of temperature, and their stay was terminated by strong flexion of the female rather than by their own activity. The young were often seen feeding, extended almost fully out of the marsupium; yet they never left it. Only rapid, full body contractions by the mother seemed to cause their exit. (Sheader and Chia, 1970, believed thigmotactic and phototactic responses of the young were the deciding factors in release from the marsupium. This difference might be attributable to the much longer stay in the marsupium by *M. obtusatus* young.) Although searched for heartily, no exuviae were ever seen for *G. palustris* juveniles before they left the marsupium. First molts were observed from 7 to 10 days after hatching.

The life cycle histograms of Fig. 14 show that males are sexually distinguishable before females. Forty-six males were in the size class 2.3 to 2.9 mm; the total number of females in this size class was three. Females of the overwintering generation were larger than 5.3 mm when they became ovigerous. Those of the summer breeding generation were from 3.0 to 4.5 mm. Development of fully bristled oostegites coincided with the first oviposition.

Table 10 summarizes several aspects of reproductive behavior and development. Time is expressed in the number of days for length of precopula, length of embryonic development from oviposition to hatching, length between broods

Table 10. Time in days of several life cycle phenomena.

	Precopula			Embryonic Devel.			Between Broods			Between Female Molts					
Brood Number	1	2	3	1	2	3	1	2	3						
Molt Number										1	2	3	4	5	6
Spring Generation															
3.5°C	8	-	-	62	-	-	-	-		59	88				
15.0°C	5	-	-	17	-	-	-	-		-					
20.0°C	2	2	-	8	8	10	9	10		32	19	19	20	20	21
Environmental Temp.	5	3	2	26	15	8	10-20	7		47 77	33 16	20			
Summer Generation															
Environmental Temp.	(Not observed)			8	8	9	8	9		-					

(from the hatching of one brood to the oviposition of the next), and length between female molts. For the latter, Molt number 1 refers to the average length of time after the first observed molt in the laboratory. For the spring generation, 30 immature females, collected at Adams Point on 15 February 1971, were maintained in the laboratory until death: 10 animals each at 3.5°C, 20.0°C and environmental temperatures. The data for 15.0°C (without molting data) are from another set of experiments at a constant salinity of 24 o/oo, and are included only for the sake of comparison. For the summer generation, 10 immature females were maintained in the laboratory at environmental temperatures beginning on 1 July 1971. All the data represent average values except where the individual bits were widely divergent; here data is expressed as a range. As the maximum number of broods per female was three, data is given for each sequential brood.

The first column shows that length of precopula time was inversely proportional to temperature. Comparisons of time spent in precopula both among the data for 3.5°C, 15.0°C and 20.0°C, and among the data for sequential broods at environmental temperature yield this conclusion. The latter set of data represents the seasonal increase in water temperature from mid-April to mid-June, i.e.

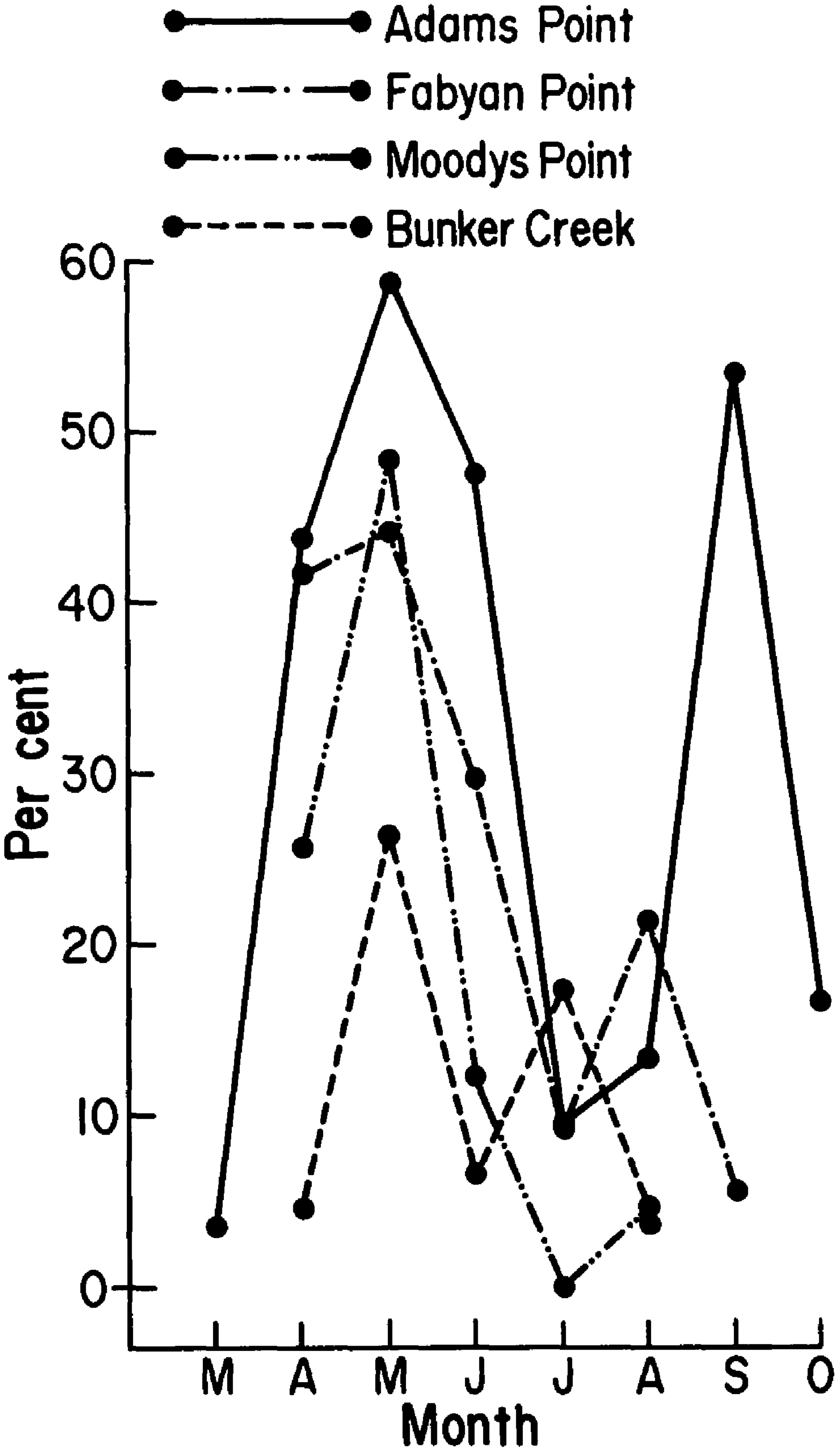
approximately 7.5°C to 20.0°C. Although showing a relationship with temperature, these data are not truly comparable to G. palustris in the field, since the females maintained in the laboratory were not in the constant companionship of males.

A combination of field and laboratory data gives a better picture of naturally occurring precopula. During May, many females with embryos in their marsupium were also in precopula. These females were with either their first or second brood. Since embryonic development for the first brood took an average of 26 days and for the second, 15 days, with an additional period before the next oviposition (Table 10), the animals must have been in precopula much longer in the field than the laboratory results indicated.

The temperature dependency and resultant long length of precopula in the spring explains Fig. 23, where the percentage of the adult population in precopula is plotted per month. The greater percentages in spring reflect only that, since precopula lasts longer, chances are better that it will be seen.

The temperature-precopula relationship also permitted the observance of another phenomenon. In May collections the following data were recorded for all females: whether or not they were in precopula; whether or not they were

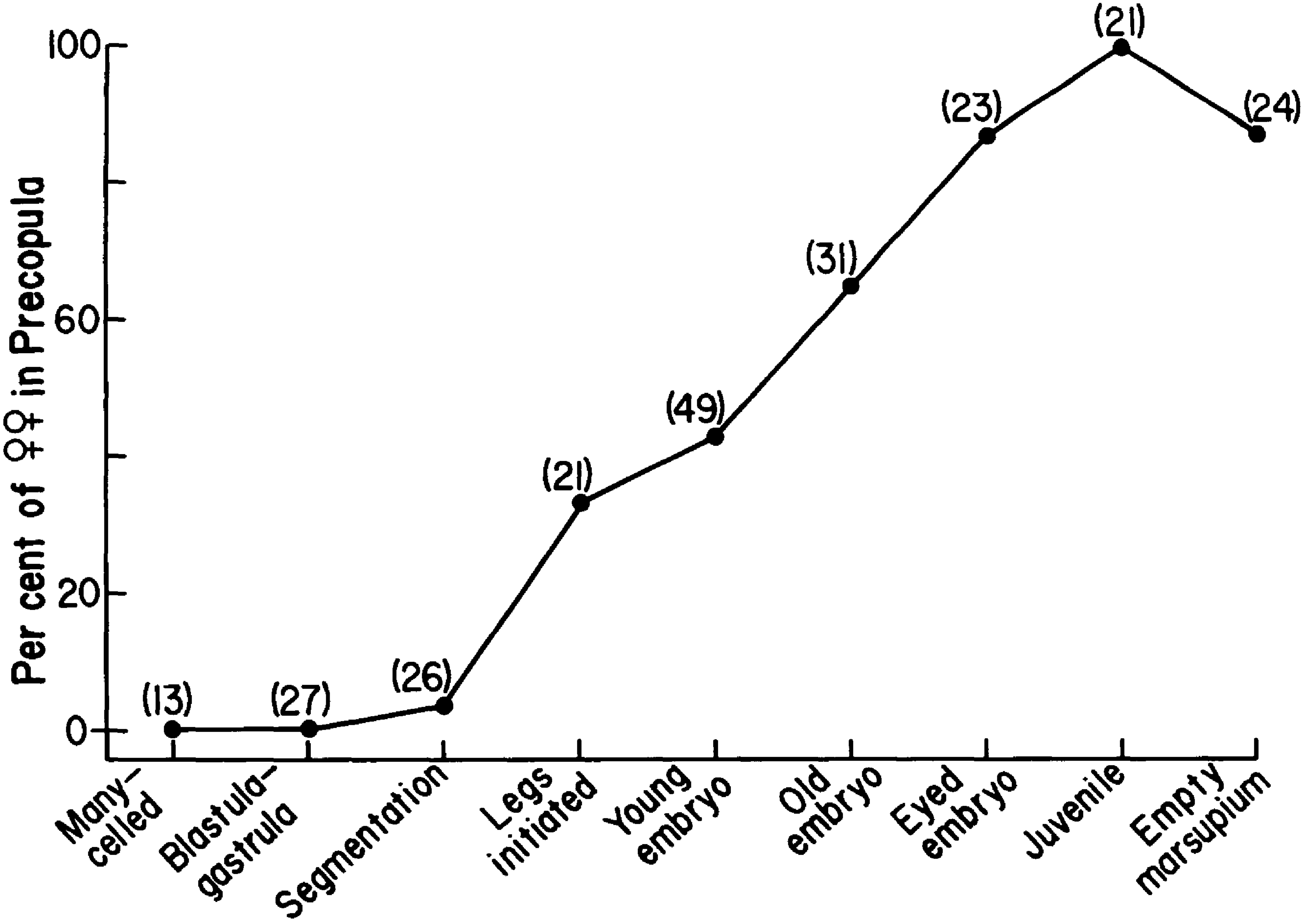
Fig. 23. Monthly number of _G_. _palustris_ in precopula as per cent of total adult population at each station.


Adams Point
Fabyan Point
Moodys Point
Bunker Creek
Per cent
60
50
40
30
20
10
0
M
A
M
J
J
A
S
O
Month

ovigerous (if not, they were most likely between broods); the stage of embryonic development of the brood. Fig. 24 shows this data by presenting the number of females in precopula as a percentage of the total number of females for each of the indicated embryonic developmental stages (through hatching and expulsion from the marsupium). A linear relationship is shown between percentage of females in precopula and stage of embryonic development, i.e. females are more frequently in precopula with an advanced stage brood, or empty marsupium. Therefore precopula must be primarily dependent on some factor or factors of the female reproductive condition, and the length of time of precopula is actually only partly dependent on temperature.

In Table 10 the temperature dependency of embryonic development is clearly evident. Embryos at 3.5°C required two months to hatch; those at 15.0°C, over two weeks; while those at 20.0°C, just over a week to one and a half weeks. The development time for all broods of the summer generation and for the third brood of the spring generation at environmental temperatures was similar to the time for all broods of the spring generation at 20.0°C. Indeed, temperatures in the field for the former two would be approximately 20.0°C. It should be mentioned that almost all broods at 3.5°C were not viable; egg development progressed

Fig. 24. Per cent of females in precopula during May according to stage of embryonic development (from early cleavage through hatching and ejection from the marsupium).


Per cent of ♀♀ in Precopula
100
60
20
0
(13)
(27)
(26)
(21)
(49)
(31)
(23)
(21)
(24)
Many-celled
Blastula-gastrula
Segmentation
Legs initiated
Young embryo
Old embryo
Eyed embryo
Juvenile
Empty marsupium

only to the eyed-stage. Only once did the young hatch, but these were dead two days later.

For spring generation females at environmental temperatures the time between the first and second broods ranged from 10 to 20 days. The rest of the data (similar environmental conditions) shows a range of 7 to 10 days. These figures, when taken in conjunction with the length of embryonic development, are supportive of life cycle data of the natural populations derived from field collections. It was stated earlier that the first ovigerous females of the overwintering population appeared in mid-April; Fig. 20 indicated that some mortality had occurred by mid-June--a period of approximately 60 days. Adding times of embryonic development to times between broods for females maintained at spring environmental temperatures gives a length of 66 to 76 days of reproductive activity for overwintering females. Therefore, those females with three broods would be expected to die between the middle and end of June. Of course, some females, as observed in the laboratory, would probably die after the first or second brood, and these individuals in the field probably would account for most of the mortality indicated by mid-June. The life cycle histograms also showed that some spring breeders (based on female length) were still reproductively active in mid-July. A possible explanation might be indicated by the two sets of average molting times listed for the spring breeders maintained at environmental temperatures. Four females molted (molt times of 48, 49, 41, and 38 days) considerably before three others (molts at 73, 78, and 80 days). If this really indicates a naturally occurring phenomenon, then the later molters would probably be the individuals surviving into July (99 to 106 days).

Similar calculations may be carried out for the summer breeders. The first ovigerous females appeared in the population in mid-July and mortality began between mid-August and mid-September. Adding times of embryonic development to times between broods for the summer generation females at environmental temperatures (Table 10) gives a length of 42 days. Therefore females who started breeding in mid-July and had all three broods would be expected to die sometime in late August.

The data for time between molts of females in Table 10 shows that this time actually decreases during breeding for the overwintering generation. The number of molts beyond three for animals maintained at 20.0°C are attributable to one female, not mated, who lived longer than the rest. Notice how the length of time between molts remained relatively constant rather than increasing with age.

VI. DISCUSSION

A topic of considerable interest in population biology is the distributional pattern of organisms in space and time (Ehrlich and Holm, 1962; Elton, 1966). This study deals with the elucidation of this pattern for the salt marsh amphipod G. palustris and a consideration of associated physical factors.

Newell (1970) discussed at length the factors involved in the determination of zonation for intertidal animals. Naturally foremost were the direct effects of tidal emersion and submersion. The requirement for submersion of G. palustris with every tidal cycle is clear from the shoreward limit of the species, i.e. it was never found above Extreme High Water Neap tide level. But the intricate spatial and temporal relationships shown for G. palustris are best understood by considering an additional factor associated with the tide's ebb and flow, i.e. the extreme temperatures, both maximum and minimum, to which particular intertidal zones are exposed. As shown here, and by Miller and Egler (1950), the temperatures in some salt marsh microhabitats may exceed the ambient temperature. In response to these temperature extremes, the importance of behavior by intertidal animals cannot be underestimated, since it also influences their zonational distribution (Janssen, 1960; Frank, 1965; Micallef, 1966; Newell, 1970).

Microhabitat preferences and temperatures show that _G_. _palustris_ were found in those microhabitats with the least temperature variation during low tide, i.e. under rocks at certain levels and in the basal system of aggregated culms. When the variation was less, _G_. _palustris_ extended its distribution shoreward; when more pronounced, the species moved to rocks and culm bases at a lower tidal level or, in late fall, abandoned the rocks and culms altogether. At this time, and in late winter and early spring, live algae or decaying vegetation were important as protection against temperature extremes (Vagenas, 1969; Newell, 1970). Since temperatures were somewhat similar in all microhabitats under the ice, the winter distributional pattern probably reflected the pattern already established in late fall. Evasion of extreme temperature variation coupled with aggregation in regions of more optimal temperature range, therefore appears to play an important role in the zonational pattern of _G_. _palustris_.

Andrewartha and Birch (1954) stated that temporal population variability is greatest toward the extreme distributional limits of a species. Haven (1971) showed a similar variability, but with a discernible pattern, in the seasonal vertical movements of the intertidal limpets

Acmaea scabra and *A. digitalis*. The present study shows the same for *G. palustris* at the northern geographical limits of its range, i.e. a complex and ever-changing distributional pattern within the marsh, but a clearly discernible one.

From late December through April, however, the response of this amphipod toward temperature extremes did not bring it to the best of all possible worlds. The pools, shallow depressions and run-off channels that either served as or accompanied the winter retreats were also the areas of greatest salinity fluctuation. During a period of heavy spring rain, Nicol (1935) measured fluctuations in the salinity of a marsh pool from 1.0 to 32.2 o/oo during a 2 hour period. Although of somewhat less magnitude, large salinity fluctuations were also observed in the winter retreats of *G. palustris* in Great Bay during low tide. These, together with long periods of extremely low salinities during early spring high tides, created continuous, but still varying, low mesohaline to oligohaline conditions. This combination of low temperatures and low salinities would add considerably to the osmotic burden of an animal (MacIntyre, 1959; Kinne, 1964).

Where rocks and attached fucoids were present, *G. palustris* began to leave its winter retreats with the moderating air temperatures of early spring. With the onset of higher daily temperatures, a larger temperature fluctuation occurred in marsh pools and the regions of optimal temperature range shifted. But at Moodys Point and Bunker Creek there were no rocks, the dead *Spartina* culms were shorn off by ice, and the new shoots would not appear until the end of April. Literally, the animals at these stations were without a place to go and, regardless of the temperature, had to remain in their possibly precarious winter retreats.

Late winter and early spring are the most probable times for the passive dispersal of salt marsh animals. As mentioned, *O. uhleri* was found on a piece of dislodged peat carried by the currents in Great Bay. Wildish (1970) also found evidence for the passive dispersal of the salt marsh amphipods *O. gammarella* and *M. marinus* in floating tidal trash. Because of the small amount of floating debris examined in Great Bay, the possibility of passive dispersal for *G. palustris* still exists.

There was no evidence for the active dispersal of *G. palustris*. On the other hand, Williams and Bynum (unpublished) reported *G. palustris* from nocturnal plankton tows in North Carolina estuaries. Yet the epibenthic nature of this species and its ties to the salt marsh are still indicated, since only 74 animals were netted in 10 years of monthly tows. This may be contrasted with the capture of 2,915 G. mucronatus during the same period.

For Georgia salt marshes, Teal (1962) categorized G. palustris (= G. chesapeakensis) as "an aquatic species with its center of abundance in the estuary, but occurring in streamside marsh." The tenacity with which G. palustris holds to the marsh in Great Bay, and the findings of Williams and Bynum (unpublished) seem to indicate that classification of G. palustris as "an aquatic marsh species living entirely within the marsh" would be more appropriate.

Detailed studies on the differences existing in zonational patterns and microhabitat preferences within a littoral invertebrate species population, whether by sex, size, or life stage, are relatively few. For example, on rocky shores Frank (1965) and Sutherland (1970) demonstrated a differential distribution in size classes of A. digitalis and A. scabra, respectively, with the oldest and largest limpets being found highest in the intertidal zone. For sandy beaches, Dexter (1967) showed that female Neohaustorius schmitzi amphipods were more abundant at upper tidal levels, males at lower tidal levels, while juveniles were concentrated at both mid and low tidal levels. Fielder (1971) described the well-defined aggregations of the sand bubbler crab Scopimera inflata. Female crabs were on the seaward side of an aggregation and males on the landward side. In addition, the larger animals of each sex tended to be more landward with the average size decreasing towards the seaward margin. In salt marshes, Brown (1948) showed that the young of the hemipteran Saldula palustris live lower on the shore than the adults.

The importance of understanding these distributional subtleties within populations was pointed out by Green and Hobson (1970). They stated that population studies cannot be carried out properly in terms of "the intertidal community" without taking the spatial and temporal structure of the population into consideration. They demonstrated that distances as small as 6 m on an intertidal sand flat were accompanied by different mortality rates as well as different densities for a species. Therefore studies on population parameters for G. palustris would probably have to take into account the differences in size class, sex, and life stage among that section of the population under rocks, that section associated with Spartina, and perhaps subsections of the latter associated with different portions of the Spartina plant.

The genus Gammarus forms a complex in which overlap of geographic range of species is often quite large (Dunbar, 1970). Ecological studies have shown that closely related, sympatric species often occupy niches that are non-overlapping in at least one important dimension: e.g. vertical range, microhabitat preference, food preference, or period of reproductive activity (Mayr, 1963; Croker, 1967; Haven, 1971). _G_. _palustris_ in the Great Bay-Piscataqua estuary experiences an overlap of geographic range with five closely related species whose microhabitat preferences seem to suffice most of the time in niche separation. Potential overlap throughout the year would appear to be strongest between _G_. _palustris_ and _M_. _obtusatus_, since _M_. _obtusatus_ is also an epibenthic inhabitant of the lower marsh. But its marked inability to withstand lowered salinities (Hartog, 1964), a slightly different choice of food (Martin, 1966), and a slightly different microhabitat while in the marsh (_at_ rather than _in_ the aggregated culm bases) appear to be sufficient for niche separation. Indeed, as shown here and by Bousfield (1969), the species do co-occur, although only rarely in New Hampshire. Being stenohaline, _M_. _obtusatus_ is absent from Great Bay, but _G_. _palustris_, a euryhaline species (as will be shown later) should be able to exist in higher numbers in the higher salinity coastal marshes. Obviously, other factors are in operation, limiting _G_. _palustris_ mainly to the inner marshes of the bay.

The change in microhabitat experienced by G. palustris from winter to early spring resulted in a microhabitat overlap with G. oceanicus, G. tigrinus and G. mucronatus. Ecologically this is important, e.g. Kinne (1959) reported G. duebeni females were prevented from reproduction in being carried by other Gammarus species. The relationship with G. tigrinus was not pursued in the present study because of the few numbers seen with G. palustris in the field. Nor was the relationship between G. oceanicus and G. palustris, since a different reproductive cycle and life stage distribution of G. oceanicus served as additional niche differences. But with G. mucronatus, the situation was different. Kinne (1954) obtained a rather frequent occurrence of precopula between the closely related (Spooner, 1947) G. salinus and G. zaddachi. If such precopular activity is indicative of close affinity, then Bousfield's (1969) claim of close morphological and systematic allignment between G. palustris and G. mucronatus is supported by my behavioral observations.

In general, the feeding methods and habits of adult G. palustris are very similar to those described by Martin (1966) for M. obtusatus and M. pirloti. However, these marinogammarids are primarily macrophagous algal feeders, and thus frequently employ their gnathopods in holding food, while G. palustris uses only its mouthparts. Since all three species are emersed for long periods during each tidal cycle, it is not surprising that each has the habit of feeding for short periods at frequent time intervals while submersed. Martin maintained that this is preferable from the point of view of efficiency in digestion, rather than refilling the entire alimentary canal rapidly in one meal. He also believed that the gammarid foregut is most efficient when the animals eat small amounts at frequent intervals. The same can undoubtedly be said for G. palustris.

Since adult G. palustris were noticed sorting and selecting detritus particles, they could be classified along with Corophium volutator in that category of feeders which select only the nutritive particles from the deposits in which they live, i.e. true selective deposit feeders (Newell, 1970). Yet Martin (1966) claimed that for the marinogammarids he studied, the potential nutritive value appeared to be of little importance in the selection of food. This situation requires further study.

The microhabitats selected by G. palustris adults are ideal in providing a continuous supply of its main food, detritus. The rigid, erect Spartina is very effective in slowing water currents, and the rate of accumulation of new sediment is greater in Spartina areas than in other parts of an estuary (Green, 1968). Surface layers of the sediments in salt marshes also contain a diversified population of algae, including pennate diatoms, dinoflagelates, and green and blue-green algae (Pomeroy, 1959). As shown, representatives of several of these groups were found in the guts of *G. palustris* adults in addition to detritus.

The difference between adult and juvenile ingestion patterns was first suggested by laboratory observations on methods of feeding. Differences in juvenile microhabitat selection also suggested possible differences in feeding and indeed microhabitat preferences were shown to be correlated with the foods ingested. A considerable number of diatom species attach themselves to solid substrata or form a mat-like growth intermingling with either *Enteromorpha* or blue-green algae (Green, 1968). In addition, at the base of *Spartina* there is a skin of blue-green algae (Pomeroy, 1959). A consideration of juvenile feeding behavior (scraping), microhabitat preference (*E. plumosa* and the bottom 5 cm of *Spartina* culms), gut contents (detritus, diatoms, filamentous green and blue-green algae), and the algal distribution just discussed, provides a trophic picture of the position of *G. palustris* juveniles in the marsh. This trophic position is probably similar to that of the snail *Littorina irrorata*, living on the surface of sediments and on the stems of marsh grass from New Jersey south (Gosner, 1971), and considered as a "detritus-aufwuchs feeder" (Odum and Smalley, 1959). In late summer the similarity of adult and young _G_. _palustris_ gut contents was probably associated with the smaller length of the adults and the greater abundance (Green, 1968) of blue-green algae and diatoms on the marsh.

A comparison of the lower marsh fauna of New Hampshire with the fauna listed by Teal (1962) for Georgia marshes shows that the principle larger detritus-algae feeders of southern marshes are entirely absent from the marshes of Great Bay. Consequently, in New Hampshire, _G_. _palustris_, as one of the few abundant larger detritus-algae feeders of the marsh, would be a necessarily important species in an energy flow analysis.

Fish predation on _Gammarus_ (sens. lat.) is well documented (e.g., Clemens, 1950; Spooner, 1951; Hynes, 1955; Dedju, 1967). The absence of _G_. _palustris_ from the gut of its most likely potential predator, _F_. _heteroclitus_, may be explained by the combination of the amphipods low vagility (particularly of reproductively active females) and protective microhabitat (at least for adults). The basal system of aggregated _Spartina_ culms forms such a tight bastion that any fish would be hard pressed to intrude upon it. This protection is lost, however, in the winter retreats. Because of the highly localized and dense aggregations of _G_. _palustris_, chances of severe population depletion by predators are enhanced during winter and early spring. Killifish probably confine themselves to river channels or stream beds from mid-autumn to mid-spring, but patterns of migration under the ice in near-shore areas of Great Bay are simply not that well known (Sawyer, pers. comm.) to eliminate the possibility of high predation by other fish, especially in early spring before _G_. _palustris_ leaves its winter retreats.

Potential predators not examined were coleopterans, often numerous on the marsh. Green (1968) gave many examples of predation by salt marsh beetles on other marsh animals, including the active hunting of _Talitrus_ _saltator_ by the carabid _Eurynebria_ _complanata_. Both adult carabids and the highly predacious dytiscids were found in Great Bay marshes.

A high rate of parasitic infection in late summer months, as found for _G_. _palustris_, was also found by Hynes (1955) for _G_. _lacustris_ and by Maitland (1966) for _G_. _pulex_. Both of these amphipods were parasitized by acanthocephalans. Hynes found the infected amphipods to be smaller than the non-infected members of the same generation, but no change in length was noticed in the G. palustris infected by trematode metacercariae.

The life cycle of G. palustris was composed of two generations per year, each differing from the other in the mean length and the maximum length of animal achieved. One, the spring-breeding generation, entered the population during the summer, grew to a relatively large size during winter and reproduced throughout the following spring into early summer. The other, the summer-breeding generation, first entered the population in mid-spring and matured by early summer at less than half the length at maturity of the spring-breeders. Steele and Steele (1969) also reported differences in length at maturity between the cold and warm seasons for female G. duebeni, but the mean length at 50% population maturity, though significant, differed only by 1.0 mm. For G. palustris, this difference in length between the generations was sustained throughout the breeding season. The maximum length achieved by the sexually active summer generation just barely overlapped the smallest length at which the spring-breeding generation matured.

The sex ratio throughout most of the year was close to 1 to 1. The rise to almost 2 to 1 for September and October was merely a reflection of two factors: 1) faster maturation of developing males of the overwintering generation; and 2) the sudden, heavy mortality of the summer-breeding females in late summer and early autumn. Even the slight preponderance of males, indicated by the monthly sex ratios for the rest of the year, disappears in the months when reproduction occurred if only mature males and females are considered. (Maturity in males is defined as one length class higher than the smallest ovigerous females, since the male of a pair in precopula is always larger.) Thus there is no reason to assume a departure from amphogeny in _G_. _palustris_, as claimed for _G_. _duebeni_ (Kinne, 1959: Bulnheim, 1967).

Female _G_. _palustris_ had no "resting stage," i.e. where a female once having reproduced loses oostegite setae, but subsequently develops new setae and breeds again (Steele, 1967). The only two other gammarids reported to be without this phenomenon were _G_. _lacustris_ (Hynes, 1955) and _G_. _duebeni_ in Britain (Hynes, 1954). Steele and Steele (1969), however, claimed that the relatively large _G_. _duebeni_ females without oostegite setae, classified by Hynes as immatures, were probably females in a resting stage. Whether or not _G_. _palustris_ and _G_. _lacustris_ really differ from other gammarids in this respect depends on the definition of "resting stage."

For example, Steele, V.J. and Steele, D.H. (1970)

implied an expansion of the definition of resting stage in discussing G. setosus:

> "The animals could be sexed by late July, but the females did not develope setae on their oostegites, even when beyond the stage at which they normally mature (14 mm). While animals of this size were thought to be immature in the past it is evident that they have entered the resting stage immediately before breeding."

Earlier, Hynes (1954) believed the same phenomenon to be present in G. lacustris and G. pulex. Following this reasoning, G. palustris females could also be said to have a resting stage before maturity--the large "immatures" of the overwintering generation. Thus, either the commonly held definition for the resting stage must be expanded, or G. lacustris and G. palustris must be considered different in this respect from their congeners.

After breeding, female G. palustris of both generations died, precluding a "resting stage" and subsequent recruitment of young to the population. Since the number of eggs per female was shown to depend in part on the length of the female, the large size difference between the two generations obviously resulted in a much greater fecundity (number of eggs/brood) for the spring breeders. Unless balanced in the summer generation by either greater numbers of broods or greater numbers of reproductive females, the reproductive potential for the spring breeders would have to be greater. For G. palustris, however, the total number of ovigerous and post-ovigerous spring-breeding females collected from April to July in the field nearly equaled the number of the summer-breeding females collected from July to September. Furthermore, both generations had a maximum number of three broods. As a means of quantifying this information, the formula of Wildish (1971) for estimating reproductive potential can be used:

$$R = X(bnp)$$

where R is the reproductive potential, X the absolute number of adults, b the mean number of eggs per female, n the estimated number of broods, and p the percentage of females in the adult population. In this way, the reproductive potential of the spring breeders is in fact 3.8 times that of the summer breeders (21,650/5,690).

Cole (1954) showed that from the point of view of contributions to future population size that the age schedule upon which offspring are produced is more significant than the number of offspring which a female can produce per lifetime. The relatively young age at which summer-breeding G. palustris began to reproduce (two months after hatching) as opposed to the overwintering generation (approximately seven to eight months) could then be a significant factor in understanding the reproductive potential of the population. Yet the theory behind Cole'e statement is based on the idea of continuous breeding and subsequent exponential growth.

The production of a second generation of summer breeders for G. palustris seems unlikely. Even if some of the progeny produced in mid-July were to develop to maturity before summer's end, their contribution of young to the population would be negligible, since breeding effectively ceased by late September. Further support arises from the decreased reproductive potential shown for September, caused, in part, by the lack of smaller, recently matured females entering the population. Therefore, there is little chance for exponential growth, and the decreased reproductive potential of the summer breeding generation is taken to be a significant feature of the life cycle of G. palustris.

The reproductive pattern reported here is for G. palustris at the northern limit of its distribution. This may be expected to differ from populations elsewhere in the species geographic range. According to Steele and Steele (1969), populations of G. duebeni at Holyrood, Newfoundland, and the Kleiner Kiel, Germany, experienced higher summer temperatures than populations on the Isle of Man. They believed that this might allow a second breeding generation for the former two populations before the onset of the resting stage. Sheader and Chia (1970) reported a maximum of six broods for *M. obtusatus* in England, while Steele and Steele (1970a) reported a maximum of three or four broods for the same species in Canada. For *G. lawrencianus*, a species allied with an endemic southern group of Atlantic coastal *Gammarus*, including *G. palustris* (Bousfield, 1956a), Steele and Steele (1970b) reported that the reproductive cycle was delayed in Labrador populations. Similarly, Bousfield (1969) reported several ovigerous females (out of nine) in a late March collection of *G. palustris* from Maryland. This is at least a month before reproduction of the overwintering generation of *G. palustris* in Great Bay begins. Therefore, as Hynes (1954) stated, latitudinal differences in temperature regime are correlated with variations in the life cycle of gammarids, including *G. palustris*.

The decrease in density from fall to spring experienced by the overwintering generation was shown to be unconnected with any aspect of the life cycle. Severe winter mortality caused by a combination of physical and biological factors is documented for several intertidal animals. For example, Frank (1965) referred to the intimate association between the catastrophic winter mortality of *A.*

digitalis and exceptionally severe frosts. Emery, Stevenson, and Hedgpeth (1957) stated that the formation of ice at high latitudes is an important ecological factor, especially in the littoral zone, where it removes the organisms by mechanical action. An avoidance of this by littoral animals could be achieved by subtidal migration or by burrowing. Jenner (1958) and Sindermann (1961) proposed that dense aggregations of intertidal gastropods at the onset of winter in northern latitudes preceeded migration to deeper waters. Bousfield (1956b) suggested the importance of burrowing by the terrestrial Talitridae in avoiding winter icing. G. palustris, however, does not burrow, nor are the dense aggregations seen in late autumn preliminary gatherings to off-the-marsh migrations. As shown, the species is bound to the marsh and must endure winter conditions while remaining a littoral epibenthic form. The precariousness of the species highly localized retreats on the marsh has already been mentioned in relation to increased salinity stress and predation. The mechanical action of the ice either through shearing or merely by coming to a rest on one of the retreats must certainly be considered another possible cause of any mortality during winter.

An inverse relationship between density and size of animals has been reported for motile littoral invertebrate forms. Frank (1965) showed that crowding decreased growth slightly but significantly in the limpet *A. digitalis*. Sutherland (1970) demonstrated lower densities in the high littoral population of *A. scabra* and a resultant greater annual growth and attainment of a larger size and biomass than in the more dense lower littoral population. Foin and Stivens (1970) in studies of the fresh water lotic gastropod, *Oxytrema proxima*, showed that density produced effects on maximum size, total live weight, and to a lesser extent mean size of the populations. The inverse relationship described for *G. palustris* between density and mean length is the first known report of such a phenomenon for the Amphipoda.

The inverse relationship between temperature and both length of embryonic development and length of precopula allowed the observance of a phenomenon in *G. palustris* during May that would go unnoticed during other parts of the year or in other gammarids with differently phased life cycles. This is the linear relationship between the percentage of females in precopula and the stage of development of their embryos. Sheader and Chia (1970) probably observed a less distinct representation of the same phenomenon for *M. obtusatus*, since they stated that a male paired with a female that was carrying or had released her young, or more rarely, it paired with a female that was carrying eggs almost ready to hatch.

Holmes (1903) believed that precopula in Amphipoda resulted from an entirely chance meeting of male and female, with a male recognizing a ripe female by her passivity. Sexton and Matthews (1913) also held that precopula resulted from an original chance meeting, but felt the coupling to be the result of a mutual "appreciation" between the partners. The authors stressed the importance of the antennae in affecting the coupling. More recently Dahl, Emanuelsson and von Mecklenburg (1970) showed the presence of a female sex pheromone in _G_. _duebeni_. Radioactivity from treated liver fed to females was concentrated in calceoli on the males' second antennae. Dahl _et_ _al_. stated that the site of production of the pheromone was still unknown although Ryan (1966) showed, that in the decapod _Portunus_ _sanguinolentus_, prevention of urine outflow from the antennal gland in the female also prevented attraction of males. With this background, and the data presented for _G_. _palustris_, a picture of the chain of events leading to precopula can be proposed.

Laboratory observations showed that initial contact between two _G_. _palustris_ was always accidental. Homosexual contact resulted in repulsion; in heterosexual contact, there was repulsion only some of the time. Any lingering was accompanied by antennal, head to head activity, even if the initial collision was not head on. Although males were always the most active of the two sexes, the females' activity varied from active to passive. After the "antennal sensing" the pair either parted or entered into precopula. Sometimes precopula occurred so rapidly that this chain of events could not actually be observed.

But how would such behavior produce a greater number of pairs in precopula, the more advanced the stage of embryonic development in the females' marsupiums? Faux _et al._ (1969) reported a rising titer of ecdysones during the molt cycle in the decapod _Callinectes_. It is likely that other hormonal compounds, such as those associated with ovarian development, might show a similar rise in titer. Furthermore, Sheader and Chia (1970) showed for _M. obtusatus_ that almost all the growth of the ovary coincided with the incubation period of the previously oviposited eggs (from oviposition to hatching). Therefore, a rising titer of an ovarian-associated hormonal compound, which very conceivably could be present in the female's urine in an amount proportional to the rising titer within her (Tillinghast, pers. comm.), could act as a "variable intensity" sex pheromone. In this way, the greater the amount of pheromone present, the more likely sensory structures in male antennae would pick it up during antennal sensing after a collision, and the more likely precopula would occur. Thus, one would expect an increasing number of pairs in precopula, the more advanced the embryos in the female marsupium, or, more specifically, the more advanced the development of new eggs within the ovary.

In conclusion, the salt marsh habitat is a severe one, especially in northern New England. Much of this severity can be attributed to a combination of large variations in environmental extremes and the large range in these extremes. This environment might be expected to precipitate unusual, or noticeably stressful responses in an animal whose geographic range mostly covers an area to the south with more moderate conditions.

I believe that such responses have been shown for G. palustris. These are: 1) the highly variable seasonal distribution patterns within the marsh; 2) the occupation, in great numbers, of very localized microhabitats during winter; 3) the consequent breakdown in normal microhabitat separation, correlated with possible increased predation and greater osmotic stress, and of possible death by the mechanical action of ice; and 4) a reproductive period which, although obviously allowing year to year populations, is associated with decreased reproductive potential in the latter part of the yearly cycle. These, combined with the 40 to 50% reduction in population size over the winter, provide insight into the ecology of _G_. _palustris_ at its northern limit of distribution in the Great Bay-Piscataqua estuary.

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THE SALT MARSH AMPHIPOD
<u>GAMMARUS</u> <u>PALUSTRIS</u> BOUSFIELD, 1969, AT THE NORTHERN LIMIT OF ITS DISTRIBUTION

II. Temperature-Salinity Tolerance

by

MICHAEL F. GABLE

B.S. University of Florida, 1967
M.S. University of New Hampshire, 1969

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GAMMARUS PALUSTRIS BOUSFIELD, 1969,
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II. Temperature-Salinity Tolerance

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Under the ice cover in winter, and in early spring, the salt marsh amphipod Gammarus palustris occupied atypical microhabitats in pools, run-off streams and shallow standing bodies of water. At this time of the year the lowest salinity conditions on the marsh occurred in these habitats. This paper examines the tolerance of G. palustris to temperature-salinity combinations with particular emphasis on low temperatures and salinities.

Since G. palustris tolerated temperatures from -1.5 to 30.0^{o}C and salinities from 0.0 to 55.0 o/oo in either the laboratory or the field with no mortality, it can be designated strongly eurythermal and euryhaline. To determine tolerance, percent mortality after 24 hours in various temperature-salinity combinations was recorded. The species showed no difference in tolerance with differences in sex, or size within each of two yearly generations (the spring generation was born in summer, overwintered and bred in spring; the summer generation was born in spring and bred in summer). Differences were apparent, however, according to season and life stage. The summer generation adults tolerated higher salinities at all temperatures except 30.0°C than the spring generation adults, and the latter experienced somewhat less mortality at low salinities than the summer generation. Newly hatched juveniles showed much less mortality in low salinities at most temperatures than adults of either generation. Juvenile tolerance to high salinity-temperature combinations, however, was much less. The embryonic stages had considerable tolerance to temperature-salinity combinations which caused high mortality in most other life stages.

The entire G. palustris population, regardless of sex, size, life stage, or seasonal consideration, was shown to be tolerant to almost every temperature-salinity combination which might exist in Great Bay, except the low temperature-low salinity conditions of late winter and early spring. In the laboratory, only the large, overwintering, immature animals of the spring generation could survive the low/low combinations: another example of differences in tolerance with life stage. These animals, nevertheless, were shown to be under more stress at a low temperature, the lower the salinities. Laboratory experiments also showed that the animals survived longer at low temperatures and in low salinities while constantly submersed than when subjected to tidal conditions. Thus, their occupation of constantly submersed habitats in the field, even with the lower salinities experienced during low tide, may be less stressful than alternate submersion in the low salinities of high tide and emersion at low air temperatures. It is concluded that *G. palustris* may well be existing at conditions near its lower lethal tolerances.

I. INTRODUCTION

Gammarus palustris Bousfield, 1969, is a salt marsh amphipod allied with an endemic southern group of North American Atlantic coastal *Gammarus*. The species reaches its northern distributional limit in the Great Bay-Piscataqua River estuary, separating New Hampshire and Maine. In the marshes of this system there is a large seasonal range in water temperature and salinity, the latter approaching low brackish-fresh water conditions in late winter and early spring. During this time, *G. palustris*, in response to severe low temperatures, occupies atypical microhabitats in marsh pools, run-off streams and shallow standing bodies of water and is thus subjected to the lowest salinities on the marsh.

Temperature and salinity are of special importance in the study of aquatic organisms since they represent ecological master factors, i.e. their combined effect bears heavily on the physiological and ecological maintenance of life (Kinne, 1964b). Despite this, the information available for most animal groups is rather limited, or, when available, is often of such a nature (e.g., monofactorial) that meaningful comparisons and resultant generalizations are impossible. Kinne (1970) expressed the hope that future studies would be multifactorial and employ the classical lethal dosage method, where upper and lower lethal limits are expressed in terms of the lethal dose to kill certain percentages of the test population within a given period of time.

This paper attempts: 1) to define the effect of temperature-salinity combinations on _G_. _palustris_, and 2) to relate these findings to the ecology of _G_. _palustris_ in its northernmost habitat, with particular consideration of the low temperature-low salinity combination experienced by the species during late winter and early spring.

II. METHODS

Experimental stock salinities (10.0 o/oo, 20.0 o/oo, etc.) were made from "Instant Ocean" and distilled water; tap water was considered to be 0.0 o/oo salinity. Intermediate salinities were then obtained by simple dilution and neither these nor the stock solutions ever differed by more than ± 0.1 o/oo from the intended concentration (silver nitrate titration). "Instant Ocean" was used rather than natural bay water because preliminary experiments showed higher mortality in the latter at high temperatures than in "Instant Ocean" or sterilized bay water. In addition, bay water contained minute quantities of detritus and would have had to be filtered to prevent animals from feeding during the experiments. Environair Systems Inc. controlled environment rooms and a Sherer incubator provided constant experimental temperatures and darkness.

All specimens of G. palustris were collected with forceps, returned to the laboratory, and kept prior to use in mesh-covered containers on a sea water table with constantly circulating water from Great Bay. The circulating water provided environmental temperature and salinity conditions and also detritus for the animals to feed on. No animals were used for experiments if maintained for more than five days in the laboratory.

To prevent feeding, animals were placed in closed containers submersed on the sea water table 24 hours before acclimation to experimental temperatures. These containers were then placed in either the environmental chambers or incubator and the temperature of the natural water was allowed to equilibrate with the ambient experimental temperature. After equilibration, animals were transferred to individual 4 dram screw cap vials containing 15 ml of the experimental salinities. More than one animal per vial was allowed only for adults at $1.0^{o}C$ and for juveniles. Animals kept in water of environmental salinity served as controls. After 24 hours the condition of each animal was recorded (alive, dead, moribund) in addition to sex and length (from the anterior end of the head to the telson base). Juveniles were not measured. Animals able to move parts of the body, but not able to move from place to place were designated moribund, but considered dead in data analysis since previous experiments showed that these animals rarely recovered. The duration time for experiments was chosen as the shortest time period after which morbidity was identifiable. Usually two replicates of 15 to 20 animals each were conducted at each temperature-salinity combination. In those experiments where animals were maintained for more than 24 hours, water was changed daily.

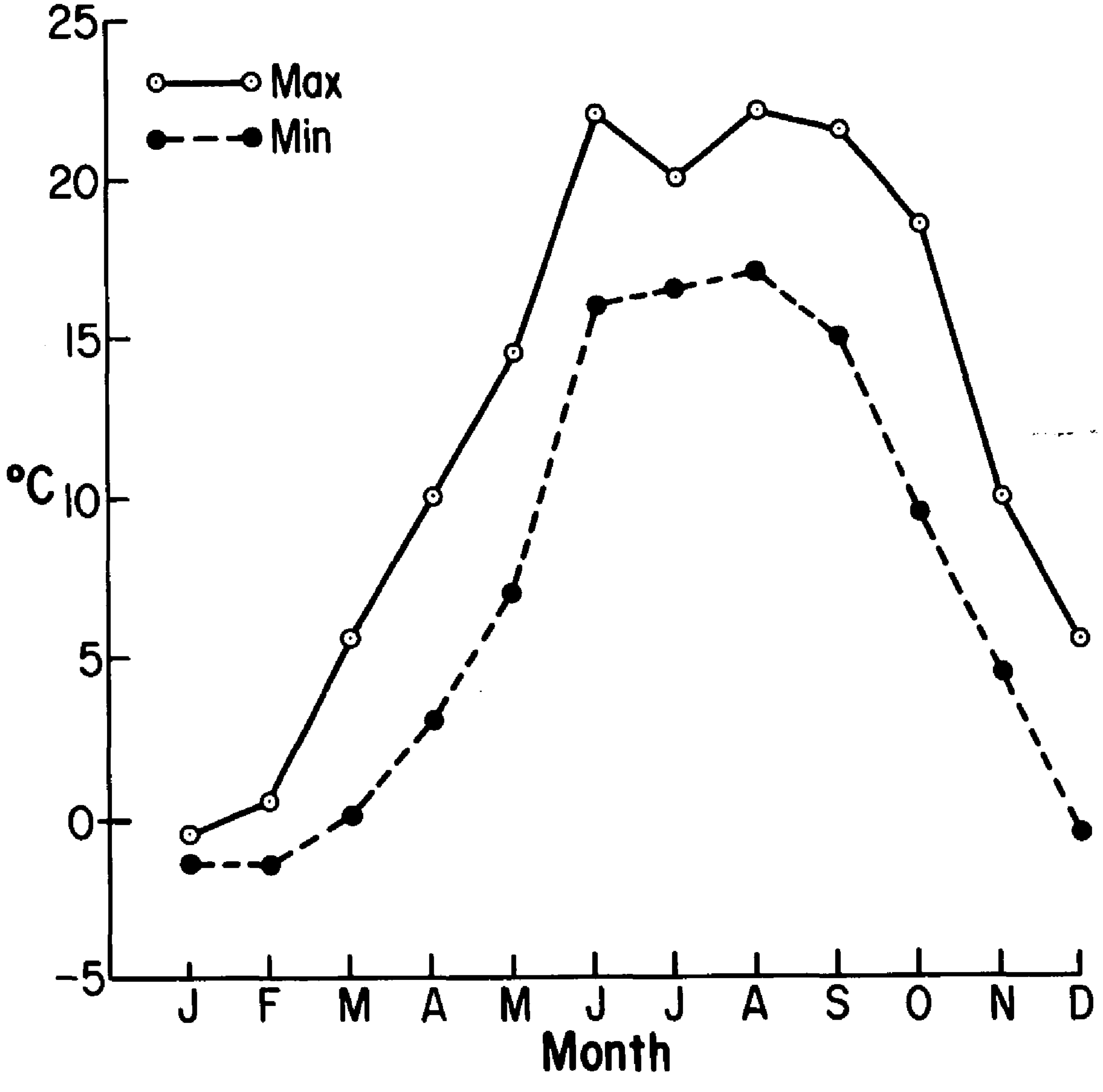
Three groups, each of approximately 200 overwintering immature G. palustris, were acclimated, one group each, to salinities of 0.0, 15.0 and 30.0 o/oo at 3.5^{o}C for 48 hours. After being washed in distilled water and blotted, the animals were macerated and the press mixture was then centrifuged. The osmotic concentration of the decanted press fluid was determined by the use of a Wide-Range Osmometer (Advanced Instrument, Inc.).

III. RESULTS

The monthly maximum and minimum water temperatures and salinities at high tide in the marshes of Great Bay inhabited by G. palustris are given in Figs. 1 and 2 respectively, for the period September 1970 to August 1971. The data represent the average values obtained at two locations in the estuary. The minimum conditions shown for April indicate the low temperatures and salinities experienced in the marshes in the early part of the month. Even lower salinities, i.e. down to 1.5 o/oo, were recorded in the marsh pools, run-off streams and shallow standing bodies of water.

According to Kinne (1964a, b), maximum acclimation to temperature may only be possible at near-optimum salinities, and, conversely, maximum acclimation to salinities may only be possible at near-optimum temperatures. Table 1 shows the maximum acclimation to high temperatures after 24 hours at an optimal salinity of 20.0 o/oo (determined previously) for adults collected in May. Fifty percent mortality occurred at approximately 32°C. Since 30°C was the highest temperature at which no mortality occurred, it was selected as the highest temperature for the bifactorial experiments. Maximum acclimation to low temperatures could not be determined, since adult G. palustris withstood freezing and sub-

Fig. 1. Water temperature at high tide in Great Bay marshes from September 1970 to August 1971.



Max
Min
°C
25
20
15
10
5
0
−5
J
F
M
A
M
J
J
A
S
O
N
D
Month

Fig. 2. Salinity at high tide in Great Bay marshes from September 1970 to August 1971.

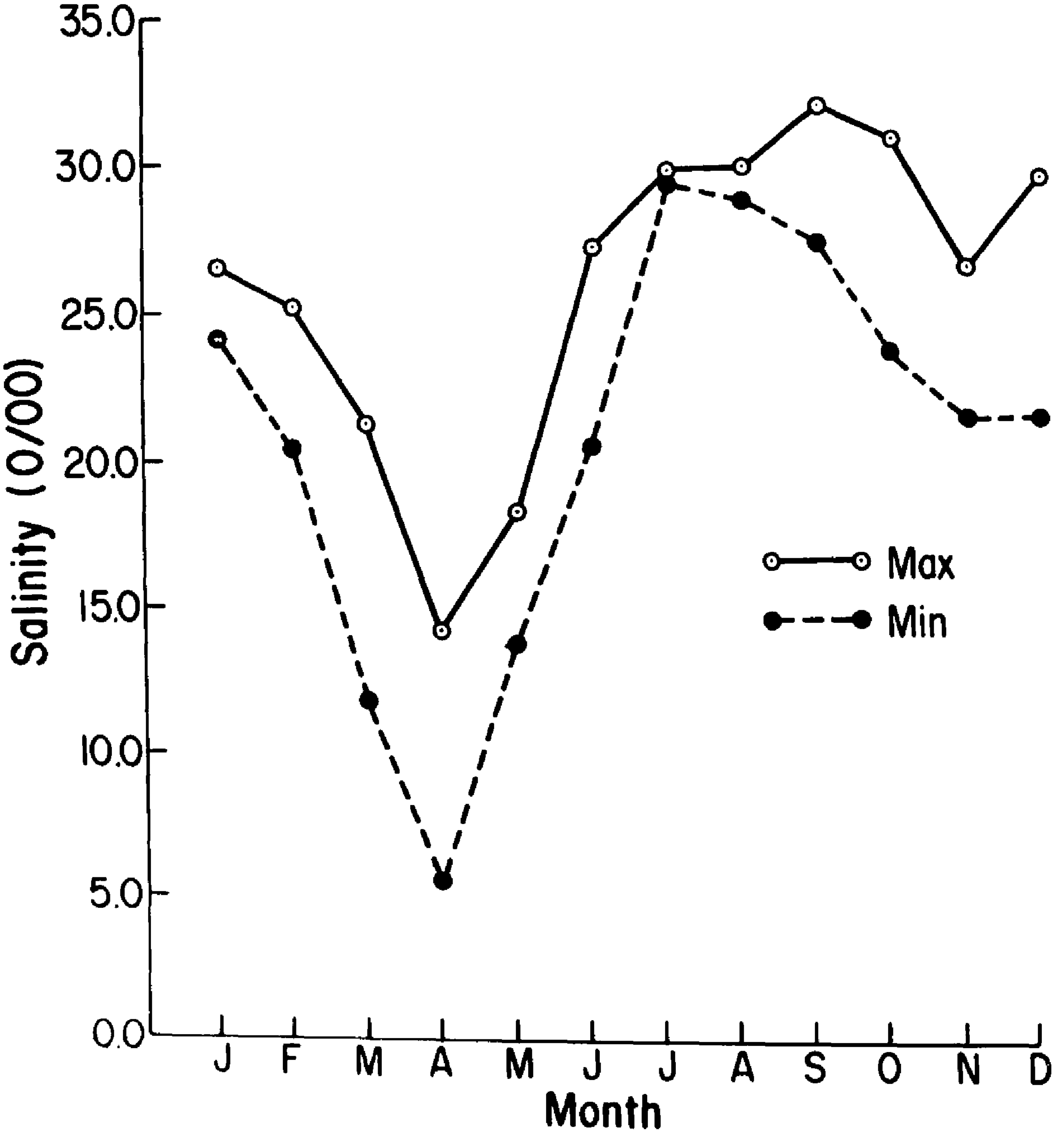

Salinity (0/00)
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
J
F
M
A
M
J
J
A
S
O
N
D
Month
Max
Min

Table 1. Maximum acclimation to high temperatures at an optimal salinity of 20.0 o/oo, as shown by percent mortality. N = 120.

Temperature (^{o}C)	Mortality (%)
30.0	0
31.0	15
32.0	45
33.0	75
34.0	85
35.0	100

sequent thawing at room temperature at a salinity of 20.0 o/oo. The lowest temperature chosen for the bifactorial experiments was 1.0°C; thus freezing of the 0.0 o/oo water was avoided. Intermediate temperatures of 10.0 and 20.0°C were also chosen. Salinities of 0.0, 1.0, 1.5, 2.5, 5.0 and 10.0 o/oo, and of 10.0 o/oo intervals thereafter, were used until mortality began in high salinity experiments. Then smaller salinity intervals were once again used.

The results of the temperature-salinity experiments (Fig. 3) show percent mortality of adults during May and early June of 1971. These adults formed the spring generation of a two generation yearly population of G. palustris. They were born the preceeding summer, overwintered as immatures and began breeding in mid-April. Results for members of the summer generation, at a comparable time during their life span (August to early September, 1971) appear in Fig. 4. These animals were the progeny of the spring generation and had matured and begun to breed by early summer. Their mean and maximum sizes were approximately half those of the spring generation.

For both generations there were no significant differences in mortality between males and females, nor among size classes of the same generation. Therefore, the data presented are for the entire respective adult populations.

Fig. 3. Percent mortality of spring generation adults at various temperature-salinity combinations. N = 960.

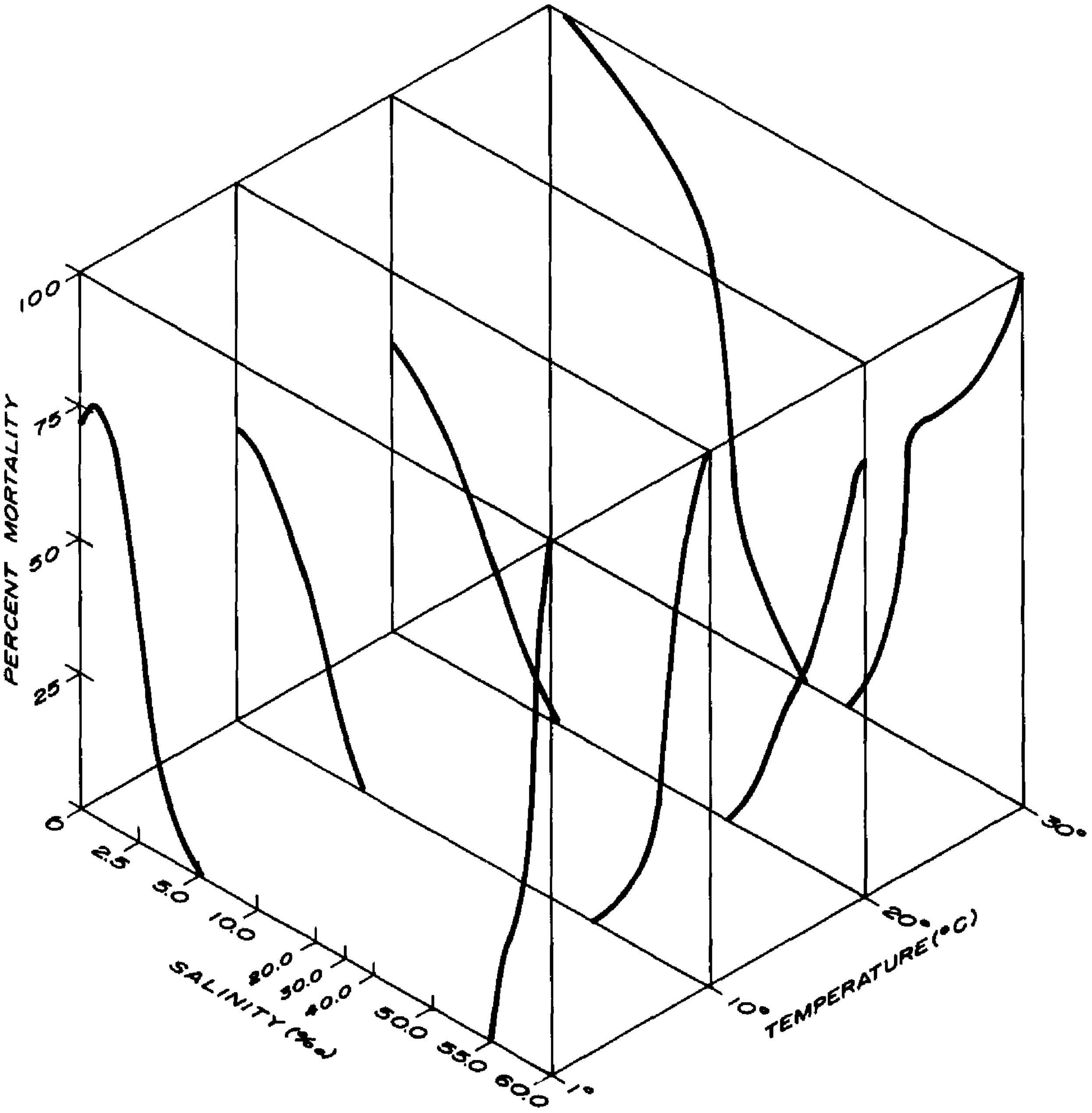
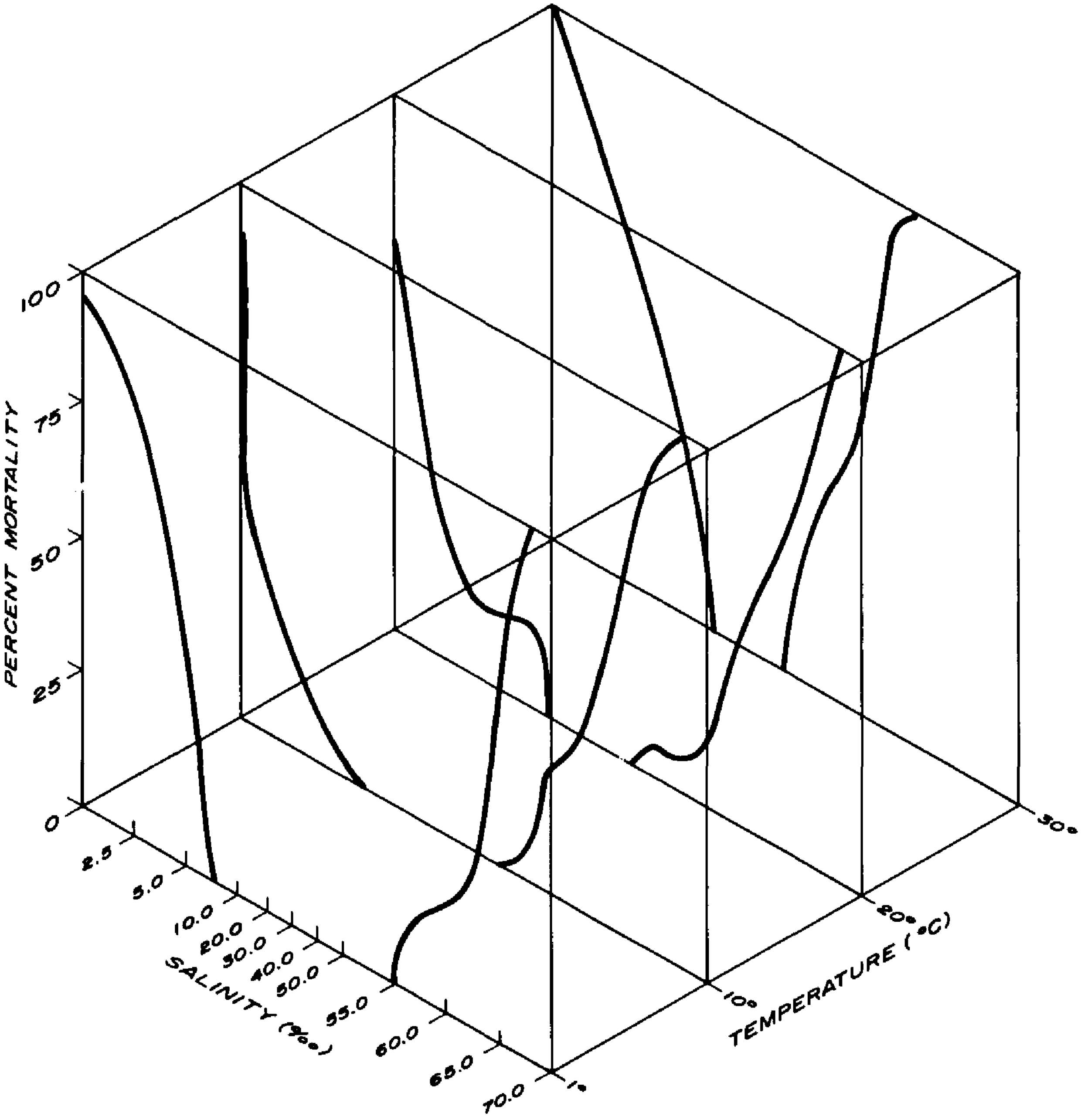

PERCENT MORTALITY
0
25
50
75
100
SALINITY (‰)
2.5
5.0
10.0
20.0
30.0
40.0
50.0
55.0
60.0
TEMPERATURE (°C)
1°
10°
20°
30°

Fig. 4. Percent mortality of summer generation adults at various temperature-salinity combinations. N = 1,477.

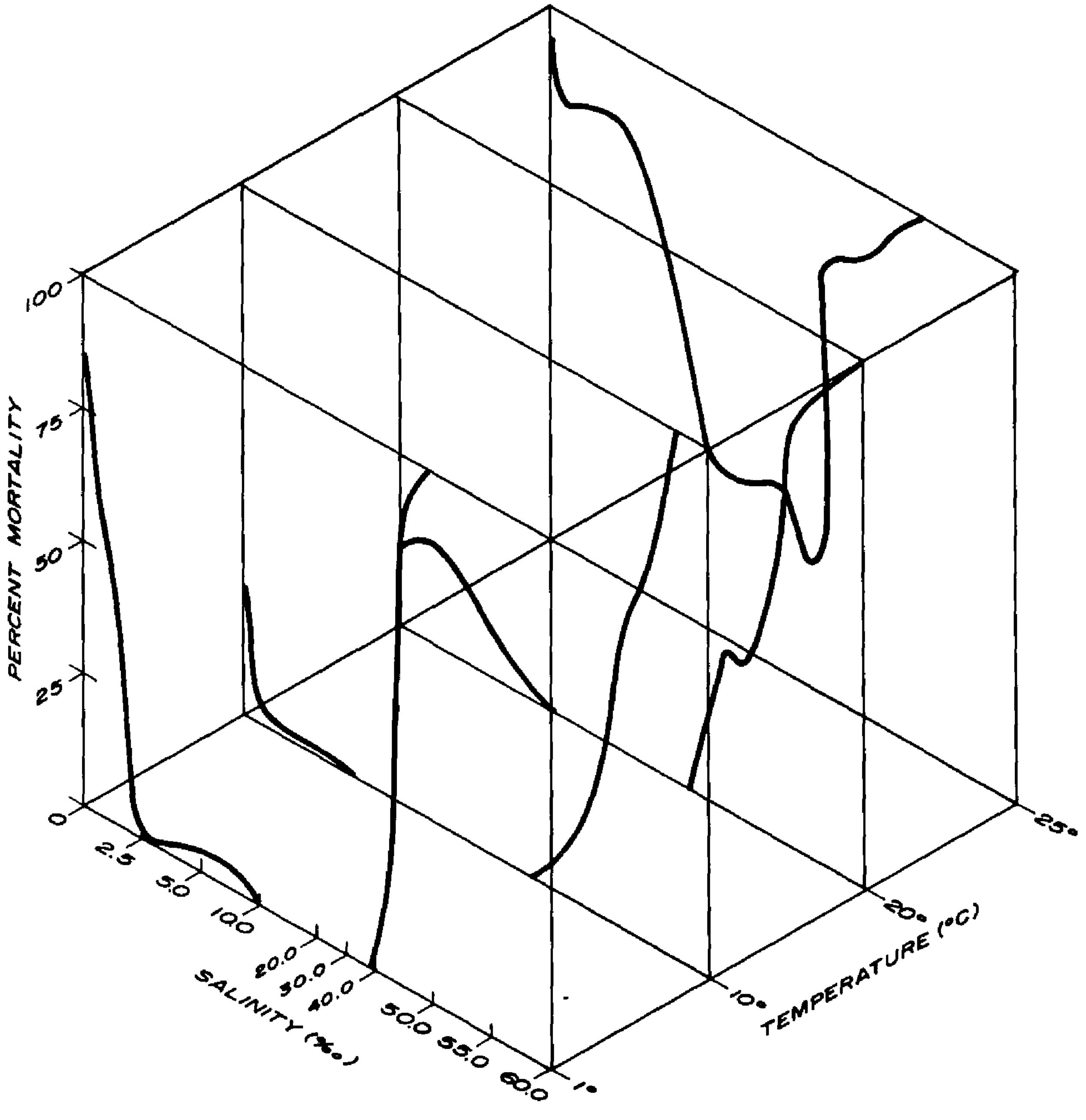

PERCENT MORTALITY
100
75
50
25
0
2.5
5.0
10.0
20.0
30.0
40.0
50.0
55.0
60.0
65.0
70.0
SALINITY (‰)
1°
10°
20°
30°
TEMPERATURE (°C)

The results appear generally similar for both generations, e.g., the smallest tolerated salinity range was at 30.0°C; and optimum temperature-salinity relationships were at 10.0 and 20.0°C and at 1.0°C in high salinities. There were, however, some noticeable differences. The summer generation tolerated higher salinities at all temperatures except 30.0°C than the spring generation, and the latter experienced somewhat less mortality at low salinities than the summer generation.

It should be mentioned that the results of step-wise acclimations to low or high salinities at experimental temperatures showed no significant differences from the data presented here. This was true whether acclimation proceeded over several days or within a 24 hour period.

Fig. 5 shows the results of the temperature-salinity combination experiments on juveniles of the spring generation. The large size of the spring breeding females and consequent large brood permitted collection of the many juveniles needed for the experiments. All juveniles used were recently hatched, none being older than five days. Compared to the adults, the juveniles experienced less mortality at low salinities and temperatures of 1.0, 10.0 and 20.0°C. Mortality began, however, at lower high salinities (40.0 o/oo) at the same three temperatures.

Fig. 5. Percent mortality of newly hatched spring juveniles at various temperature-salinity combinations. N = 2,274.


PERCENT MORTALITY
100
75
50
25
0
2.5
5.0
10.0
20.0
30.0
40.0
50.0
55.0
60.0
SALINITY (‰)
1°
10°
20°
25°
TEMPERATURE (°C)

Notice that at the highest temperature (25.0°C) to which juveniles were exposed, mortality occurred even at the optimal salinities of 20.0 and 30.0 o/oo.

To investigate the relationship between G. palustris and the low temperatures and salinities encountered in the field during late winter and early spring, animals were collected at various times throughout the year and subjected in the laboratory to low temperature (1.0 and 3.5°C) and salinity (0.0 and 2.5 o/oo) combinations for 24 hours. The 3.5°C temperature and 2.5 o/oo salinity were chosen as representative of field conditions in early spring. The results are shown in Table 2. The large immatures of the spring generation experienced the least overall mortality. Juveniles experienced mortality only at 0.0 o/oo salinity at both 1.0 and 3.5°C, but the mortality was much greater at the former temperature. May adults (immatures of one and two months earlier) experienced considerable mortalities at the lower temperature, and August adults experienced high mortalities at all combinations. The immatures of December experienced considerable mortality only at the 0.0 o/oo salinity.

To give an indication of the stress possibly experienced by G. palustris at these low temperature-salinity combinations, the following experiment was conducted. In

Table 2. Per cent mortality throughout the year in low temperature - low salinity combinations. N = 492.

		1.0°C		3.5°C	
Life stage	Month	0.0 o/oo	2.5 o/oo	0.0 o/oo	2.5 o/oo
Juv.	May	83	0	36	0
Imm.	March	25	0	0	0
	April	20	0	0	0
Adults	May	72	40	21	0
	Aug.	96	80	87	47
Imm.	Dec.	100	0	80	5

early April, 160 large immature animals were maintained without food until death at 3.5°C in salinities of 0.0, 2.5, 5.0 and 15.0 o/oo. Half the animals were constantly submersed; the other half experienced tidal conditions by being alternately emersed and submersed every six hours. During emersion, a wetted sponge in the vial cap insured high humidities. The results are shown in Fig. 6. Except for a slight overlap in 95% confidence intervals at 5.0 o/oo there was generally a significant difference in mean number of days of survival between the tidal and non-tidal animals in salinities above 0.0 o/oo. As the figure shows, survival time was shorter in the extreme low salinities as compared to 15.0 o/oo salinity.

The osmotic concentration of the press fluids of _G. palustris_ in three salinities (0.0, 14.7, 30.2 o/oo) at 3.5°C is given in Table 3. The animals were osmoregulators at all three salinities and maintained a much steeper gradient at 0.0 o/oo than at the other salinities.

Fig. 6. Mean number of days of survival for spring generation immatures at 3.5°C and various salinities. N = 160.

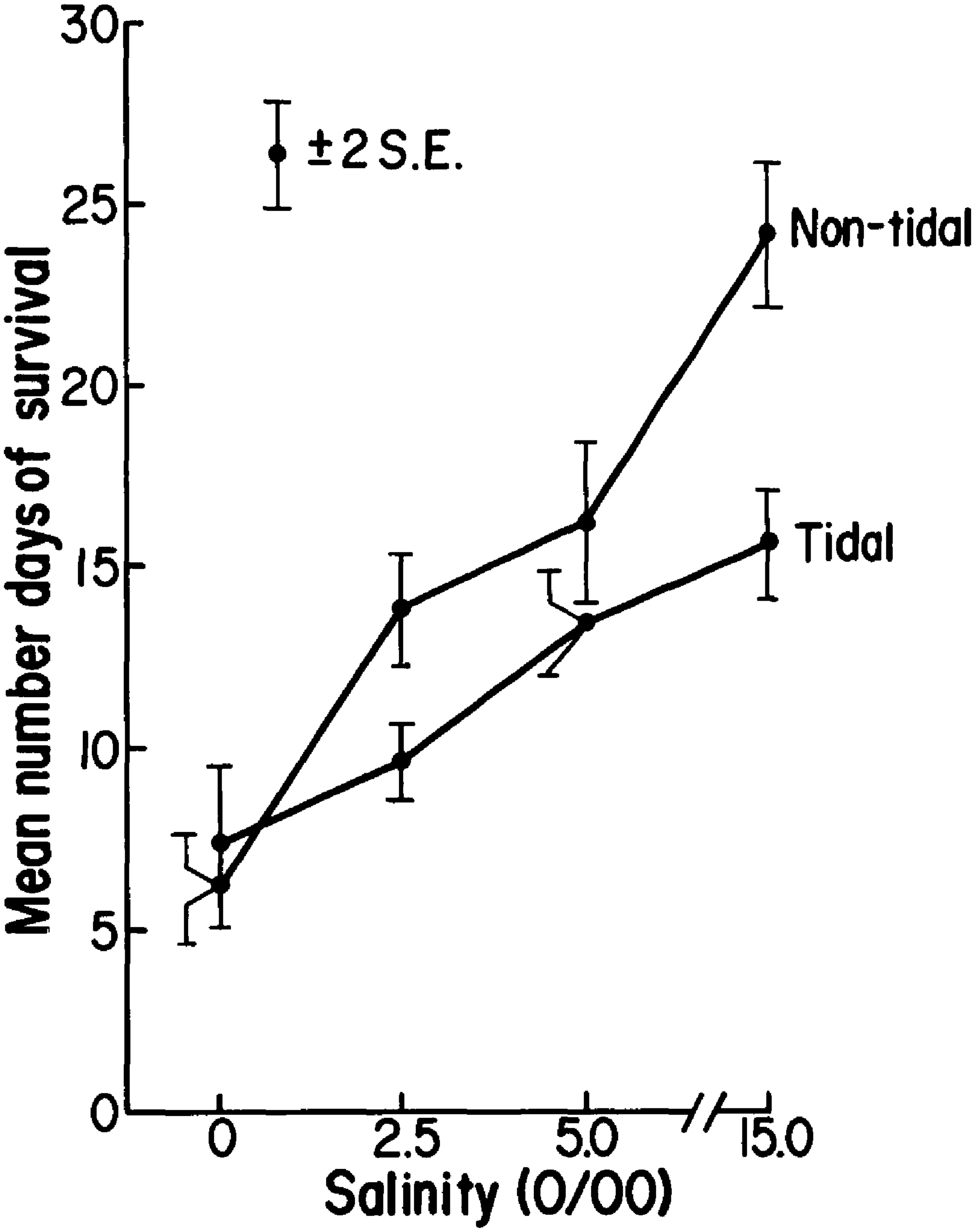

±2 S.E.
Non-tidal
Tidal
Mean number days of survival
30
25
20
15
10
5
0
0
2.5
5.0
15.0
Salinity (0/00)

Table 3. Osmotic concentration of overwintering immature *G. palustris* at 3.5°C after 48 hours of acclimation.

Salinity		
o/oo	milliosmoles	*G. palustris* press fluids (milliosmoles)
0.0	26.6	411.8
14.7	408.8	675.6
30.2	870.2	1012.1

IV. DISCUSSION

The active life of most marine and brackish water animals is restricted to temperatures between 0° and 35°C, although this range is usually much narrower for any given species (Kinne, 1963). Likewise, the maximum range in salinity tolerance is usually limited to 10 to 15 o/oo, and very few species, termed holoeuryhaline, can exist in the whole range from pure fresh water to sea water (Kinne, 1964b). Since G. palustris can tolerate temperatures from -1.5 to 30.0°C and salinities from 0.0 to 55.0 o/oo in either the laboratory or the field with no mortality, it can certainly be designated as both eurythermal and euryhaline. However, as the species probably does not exist in fresh water in the field, the term holoeuryhaline should not be applied.

The temperature-salinity combination experiments on adult G. palustris showed that temperature and salinity tolerances are reciprocally related: the range of temperatures tolerated tends to be widest in optimal salinities and the range of salinities tolerated tends to be widest at optimal temperatures. This was also reported by Kinne (1964b) for G. duebeni. The seemingly anomalous results at 1.0°C and high salinities probably may be explained by the oxygen supply (Kinne, 1970) and lower activity of the animals at lower temperatures.

For crustaceans, Kinne (1964b, 1966) and Haefner (1969) showed that temperature-salinity tolerances may differ with sex, size or life stage of the animal, or with the season. G. palustris showed no difference in tolerance with differences in sex, or size within each generation. But the differences noted between the spring and summer generations could be attributable to size or, more probably, to seasonal acclimations.

Differences according to life stage were shown by the tolerances of newly hatched juveniles. Kinne (1964b) stated that G. duebeni juveniles, during early growth, tolerated a wider salinity range than older mature individuals. In low salinities at 1.0, 10.0 and 20.0°C, G. palustris juveniles showed much less mortality than adults of either generation. Their tolerance to high salinity-temperature combinations, however, was much less. This may again be related to seasonal acclimation. As stated by Kinne (1970), it has been shown for many invertebrates that their upper lethal temperatures are only slightly above normal habitat extremes. In Great Bay, juvenile G. palustris are the only members of the population that may be in this position. Even in the optimal salinities of 20.0 and 30.0 o/oo, mortality occurred at 25.0°C, a temperature not far above that of water at summer high tides and exceeded for brief periods, as during submersion by incoming tidal waters heated in their passage over the mud flats.

In a number of estuarine animals, sensitivity to temperature and salinity extremes reach maximum values during embryonic development (Kinne, 1963, 1966). Vlasblom (1971) showed that the egg membranes of *Marinogammarus marinus* and *Orchestia gammarella* provided only slight protection against dilution of the medium, since isotonicity of the extraembryonic fluid with the medium is reached fairly rapidly. In the eyed-stage embryos of *G. palustris*, however, 89% of the embryos (N = 277) were with beating hearts after 24 hours at temperature-salinity combinations that had killed the ovigerous females. These combinations were also not tolerated in the experiments on newly hatched juveniles. In addition, the hearts continued to beat up to one hour after the embryos were placed in formalin. Thus, for *G. palustris*, the embryonic stages have considerable tolerance to adverse temperature-salinity combinations.

The entire *G. palustris* population, regardless of sex, size, life stage, or seasonal consideration, is tolerant to almost every temperature-salinity combination which might exist in Great Bay, except the low temperature-low salinity conditions of late winter and early spring.

This fact is emphasized by the data of Table 2. Only the large, overwintering, immature animals of the spring generation could survive the low/low combinations: another example of differences in tolerance with life stage and possibly with size. The sudden reduced tolerance of the May adults can be attributed to their reproductive activities (Kinne, 1966, 1970); and the reduced tolerance of the August adults, to the same in addition to possible seasonal acclimations and possibly to differences in size. The difference in tolerance between the December and March or April immatures, suggests that the greater tolerance of the latter either suddenly appeared or was gradually acquired. In any event, at low temperatures the large overwintering immature _G. palustris_ were shown to be under greater stress, the lower the salinity (Fig. 6). The greater ability of this life stage to cope with the increasing stress of low/low combinations as compared with other life stages (Table 2) may be explained by Kinne's (1963) report on _G. duebeni_. Kinne reported an increase in blood osmoconcentration at low temperatures, especially in salinities significantly below that of normal sea water. An indication of this for _G. palustris_ is shown by the osmotic concentrations of overwintering immature animals (Table 3).

Very few studies on temperature-salinity tolerance have taken into account the effects on intertidal animals of emersion and submersion by the tides (Kinne, 1970). The present study (Fig. 6) showed that in low salinities above 0.0 o/oo and at 3.5°C, G. palustris survived longer with constant submersion than with simulated tidal conditions. Submersion during low tide, even with its consequent extremely low salinities, may, in the field, be less stressful to the species than alternate submersion in the low salinities of high tide and emersion at low air temperatures.

As stated before, it has been shown for many invertebrates, and here for newly hatched G. palustris juveniles, that they exist in the field at conditions quite close to their upper lethal temperature tolerances. The demonstration of a similar phenomenon at lower lethal temperature tolerances has seldom been accomplished (Kinne, 1970). The differences shown between responses at 1.0 and 3.5°C (Table 2) to low salinities, especially during March, April and May, indicate that G. palustris as a species population in Great Bay, may well be existing at conditions near its lower lethal tolerances.

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