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Ecological life history of Crangonyx pseudogracilis Bousfield (Crustacea: Amphipoda) in the Green Bottom Wildlife Management Area, Cabell County, West Virginia

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Ecological Life History of *Crangonyx pseudogracilis* Bousfield (Crustacea: Amphipoda) in the Green Bottom Wildlife Management Area, Cabell County, West Virginia.

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Dean of the Graduate School

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ABSTRACT

Although the genus *Crangonyx* has been studied in the Appalachian area, most of this work has concentrated on subterranean species. A *Crangonyx pseudogracilis* population from the Green Bottom Wildlife Management Area (GBWMA), Cabell County, West Virginia was sampled weekly from March 1995 through July 1996 to investigate the ecological life history of the amphipod. A total of 1508 amphipods were collected. Amphipods were present in the study sites from November through July, at which time they migrated to deeper waters. *Crangonyx pseudogracilis* had a one year life cycle and bred from November to May. Adult amphipods began to die off in May and were gone by June. Males averaged 5.88 mm and females averaged 8.73 mm in length. There was no significant seasonal variation in sex ratio. Females produced several broods per year and ovigerous females were found from November 2 until May 8. They carried an average of 53.55 eggs and there was a low correlation between fecundity and length. The median tolerance limit (TL_m) for low pH was 4.06, and a 96 hour laboratory test at this value produced a 65 percent survival rate. Plant detritus was the preferred food year round and comprised between 47 and 62 percent of the diet.

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CHAPTER I

INTRODUCTION

Amphipods play an important role in aquatic systems. They provide a crucial link from energy stores available in detritus to the top carnivores in the system (Mathias, 1971). Amphipods also have potential value as bioindicators, but the current confusion in taxonomy prevents their use by biologists in impact surveys (Barnard and Barnard, 1983). Studies of life histories of amphipods were not begun in the United States until the 1940's; therefore, the ecologies and distributions of many species in the United States remain largely unknown. Range extensions are constantly recorded for freshwater amphipods in the United States.

The study of amphipods in West Virginia has focused mainly on subterranean species, and information available on these species is very limited. Both Holsinger (1969) and Culver (1970) have investigated subterranean forms of *Crangonyx* in West Virginia. Sixty-five to seventy-five percent of the North American fauna are thought to be hypogean forms (Holsinger, 1976). *Crangonyx pseudogracilis* is an epigean amphipod found in the Green Bottom Wildlife Management Area in Cabell County, West Virginia. This amphipod had not been previously recorded or studied in West Virginia.

The reported reproductive ecology of this amphipod is very scant. *Crangonyx pseudograilis* was found to have an annual life cycle with ovigerous females from April - September with several broods per female (Bousfield, 1973). Most members of the genus *Crangonyx* only produce one brood before dying; therefore, the reproduction of *C. pseudogracilis* may be somewhat of an exception (Bousfield, 1958). The closely related *Crangonyx gracilis* was reported by Hynes (1955) to produce several broods per year in Britain, while *C. gracilis* in southern Georgia was found to produce only one brood per year (Martien and Benke, 1977). These inconsistencies in the reproduction of *Crangonyx* species raise many questions. *Crangonyx* species appear to be winter and spring breeders, mating when temperatures range 5-15 °C (Bousfield, 1958).

Since information on the ecological life histories of amphipods is widely an unexplored field, especially the study species, the purposes of this study were: (1) to determine the breeding season and the fecundity of the reproductive females and to correlate this with body length of the females, (2) to document size ranges of both sexes, (3) to investigate seasonal sex ratio, (4) to determine life span, (5) to establish low pH tolerance, and (6) to examine the seasonal trends in the diet of *Crangonyx pseudogracilis*.

CHAPTER II

REVIEW OF THE LITERATURE

Although amphipods have been studied in Europe since the 1800's, North American studies were not conducted in depth until the 1940's (Holsinger, 1976). The genus *Crangonyx* was described in 1859 by Bate. The first widespread and detailed account of North American amphipod species was done by Hubricht and Mackin (1940). They described previously unknown North American species and added to the existing information about amphipods. Shoemaker (1942) described a new genus and two new species. Hubricht (1943) greatly increased the knowledge of North American amphipods by describing ten new species and placing the genus *Eucrangonyx* Stebbing into *Crangonyx* Bate. Hoff (1944) examined the habitat of *C. gracilis*. Pennak (1953) presented one of the most in depth general overviews of reproduction, habitat, behavior, taxonomy, diet, and morphology of amphipods. In 1955, Hynes studied the life history of *C. gracilis* in Britain representing one of the most complete ecology studies done at the time. This amphipod was found to produce several broods per year, a variation from the reproduction of most other *Crangonyx* amphipods. Cole (1957) examined the life histories of *C. gracilis*, *C. obliqus*, and *C. shoemakeri* in Kentucky.

Crangonyx pseudogracilis was described by Bousfield in 1958. He also wrote an exhaustive key to the freshwater families, genera and species and included notes on distribution and ecology of the North American freshwater amphipods. In 1961, Bousfield recorded the first *C. pseudogracilis* population west of the Missouri River System in Oregon. Judd (1963) discussed reproduction of *C. rivularis* and *C. richmondensis laurentianus* in Canada. Clifford (1966) examined the life cycle of *C. forebsi*. Sprules (1967) described the life cycle of *C. richmondensis laurentianus*. Mairs (1970) made observations on the life history of *C. r. richmondensis*. Mathias (1971) made notes on the production and diet of *C. r. occidentalis*.

Bousfield (1973) described and named the family Crangonyictidae, and placed *Crangonyx* in this family based on the freshwater members of the genus. Previously, *Crangonyx* had been in the family Gammaridae which contains several brackish and marine species. Bousfield further described the morphology of this family and included notes on the life cycle of *C. pseudogracilis*. The southernmost U.S. population of this amphipod was studied by Thomas (1976), and he noted the low pH (5.1) of these Louisiana waters. Holsinger (1976) presented a collection of morphology, taxonomy and distribution of North American Gammaridae including *C. pseudogracilis*. This respresented the most in depth study of freshwater amphipods after Bousfield. The life cycle and historical zoogeography of the genus *Crangonyx* in Connecticut was recorded by Smith (1977). Martien and Benke (1977) discussed the life cycle and production of *C. gracilis* in southern Georgia. Sutcliffe and Carrick (1981) examined the effects of temperature on *C. pseudogracilis* egg development and growth in the laboratory.

On a worldwide basis, Barnard and Barnard (1983) attempted to present evolutionary patterns and distributions of the freshwater amphipods, including *C.*

pseudogracilis in a two volume work. Extensive studies in the Appalchian area were not begun until 1963 when Holsinger (1969) recognized two species of *Crangonyx* from the region, only two of which were described at the time, *C. antennatus* and *C. dearolfi*. Both of these species are subterranean. Culver (1970, 1971) studied subterranean amphipods *Gammarus minus*, *Styogonectes spinatus* and *S. emarginatus* in Greenbrier County, West Virginia. Crawford and Tarter (1976) examined the life history of *C. forebsi* in a spring-fed cistern in Putnam County, West Virginia.

CHAPTER III

TAXONOMY AND DISTRIBUTION

Taxonomy and Morphology

Kingdom - Metazoa
 Phylum - Arthropoda
  Class - Crustacea
   Subclass - Malacostraca
    Superorder - Peracardia
     Order - Amphipoda
      Suborder - Gammaridea
       Superfamily - Crangonyctoidea
        Family - Crangonyctidae
         Genus - *Crangonyx*
          Species - *pseudogracilis*

Amphipods are difficult to identify for several reasons. According to Holsinger (1976) difficulties arise due to a lack of significance associated with the genitalia, strong sexual dimorphism in some species (as in *C. pseudogracilis*), variation in size classes and instars, and variation in size of taxonomically important structures in aging amphipods. The overlapping distribution of many amphipods also makes taxonomy a greater challenge.

Family Crangonyctidae was separated from the family Gammaridae by Bousfield (1973). The family Gammaridae contains marine and brackish water species while Crangonyx species are entirely freshwater. The freshwater family Crangonyctidae now contains the genera *Bactrurus*, *Allocrangonyx*, *Stygobromus*, *Stygonectes*, *Synurella*, *Synpleonia* and *Crangonyx* (Barnard and Barnard, 1983). Members of this family can be identified by a two segmented accessory flagellum on peduncle anteriorly smooth, head lacking inferior antennal sinus, and large brood plates with many marginal setea. Members of this family are found throughout North America and Eurasia (Bousfield, 1973). The family occupies a wide variety of habitats including caves, seeps, springs, streams, and swamps.

The genus *Crangonyx* was described by Bate in 1859. Members of this genus can be identified by a single segmented accessory flagellum on antenna one with one or more terminal joints. The head is without an inferior antennal sinus and the lower lip is broad with distinct inner lobes. There are typically 7 pectinate spine teeth on the outer plate of the maxilla. Pereopod 4 is longer than peropod 5 and the urosome segments are smooth or nearly smooth. Sternal gills are present and the females are typically larger than the males (Bousfield, 1958).

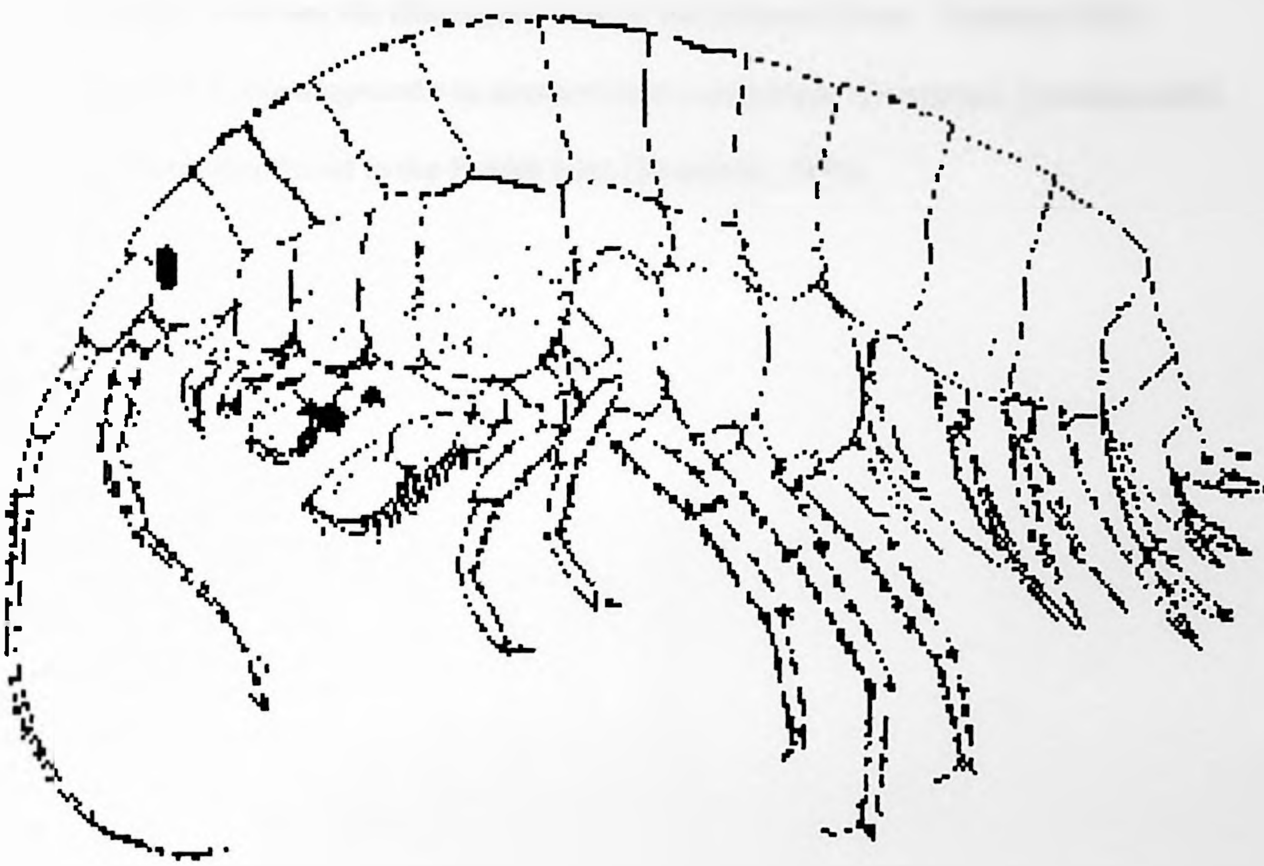
Crangonyx contains 21 nearctic species and four palearctic species. There are approximately 25 species of this genus still undescribed. Most of the group were found in what is now southern Canada during the Mesozoic and Cenozoic and were forced south by Pleistocene glaciation (Bousfield, 1958). *Crangonyx* is found throughout the United States with the exception of the very arid southwestern states and 3 species are found west of the Rocky Mountains. *Crangonyx gracilis* has been introduced into Great Britain from the United States. Amphipods of this genus inhabit both epigean and subterranean habitats, including lakes, rivers, streams, seeps, springs, swamps and caves (Barnard and Barnard, 1983).

Crangonyx pseudogracilis was described by Bousfield in 1958. The type locality is Napanee River at Napanee, Lennox, and Addington County, Ontario, Canada (Holsinger, 1976). Identification of this species can be quite difficult because it is often confused with *Crangonyx gracilis* and C. *pseudogracilis* may, in fact, be a species complex. *Crangonyx pseudogracilis* may be differentiated from *C. gracilis* by a row of comb spines on the outer ramus of the second uropod (Holsinger, 1976).

Crangonyx pseudogracilis is a mid-sized laterally compressed amphipod (Fig. 1). Individuals are brown to gray in color, but shades of red and blue are also found. According to Bousfield (1973: 68-69), *C. pseudogracilis* may be separated from other species in the genus *Crangonyx* by the following characters:

> "Length. 10mm. female. Coxal plates moderately deep, 1 shallower than head. Eye relatively large, especially in male. Antenna 2, peduncle segments 4 and 5 with 3-4 posterior groups of setae. Maxilla 2, inner plate with facial row of setae. Gnathapod 1 (females) weak, not broadest distally, posterior marginal setae inserted, segment 5, posterior lobe narrow, dactyl inner margin smooth, palmar teeth weak, unevenly spaced. Gnathapod 2 (females) slightly stronger, segment 6, superior lateral setae singly inserted, posterior palmar angle with one strong spine and 2 short spines posterior to it. Gnathapods (males) strongly subchelate, palmar spines strong, regular. Peraeopods 3-7, dactyls simple, not elongate with one inner marginal seta. Peraeopod 7, basis posterior margin with about 10-20 serrations. Pleon side plates 2 and 3, hind corners acute, that of 2 slightly produced. Uropod 1, peduncle strong, posterior margins strongly spinose. Uropod 2, outer ramus shorter, simply spinose in female, slightly recurved and distally armed with numerous comb spines in male. Uropod 3, outer ramus short, not more than 2 times length of peduncle. Telson cleft about half to base, lobes apically three-spined."

Figure 1. Female *Crangonyx pseudogracilis*.



The United States distribution of *C. pseudogracilis* is not completely known but appears to range from Canada to the southern United States (Fig. 2). Populations are recorded from southern Canada through Mississippi (Holsinger, 1976). The first record of a population west of the Rocky Mountains was documented by Bousfield in 1961, in

Oregon. This was the first record west of the Missouri River. Thomas (1976) reported *C. pseudogracilis* in southernmost Lousisiana. *Crangonyx pseudogracilis* has been introduced to the British Isles (Bousfield, 1973).

Figure 2. North American distribution of *Crangonyx pseudogracilis*.


= C. pseudogracilis

CHAPTER IV

DESCRIPTION OF STUDY AREA

The Green Bottom Wildlife Management Area (GBWMA) is located 16 miles (25.7 km) north of Huntington, West Virginia (Figs. 3, 4). Lying between State Route 2 and the Ohio River in Cabell and Mason Counties (82°14'00" West, 35°00'30" North), the GBWMA encompasses 338 hectares (WV DNR, 1991). This area is a riparian floodplain which supports swamps in various stages of succession (Stark, 1993).

The GBWMA is a project venture of the U.S. Army Corps of Engineer (USCOE), the U.S. Fish and Wildlife Service (USFWS) and the West Virginia Division of Natural Resources (WVDNR). The mitigation plan established by these organizations serves the purpose of increasing wetland habitat, thereby increasing wildlife and habitat diversity, increasing hunting and fishing opportunities, and providing preservation of historical and archeological sites. The mitigation project has added at least 100 new acres (247 ha) of swamp to GBWMA (WV DNR, 1991).

The GBWMA is a dynamic wetland environment. The wetland acreage of the swamp is constantly being expanded by both human construction and beaver activity. Four types of wetlands are found in the GBWMA: (1) seasonally flooded basins, (2) inland open water, (3) shrub swamp, and (4) wooded swamp (Stark, 1993).

Summer months are warm in this area, with an average temperature of 22.8°C and the maximum of above 38°C. Winters are cold, averaging 2°C and

Figure 3. Map of the Green Bottom Wildlife Management Area, Cabell County, West Virginia (Stark, 1993).

Green Bottom Wildlife Management Area

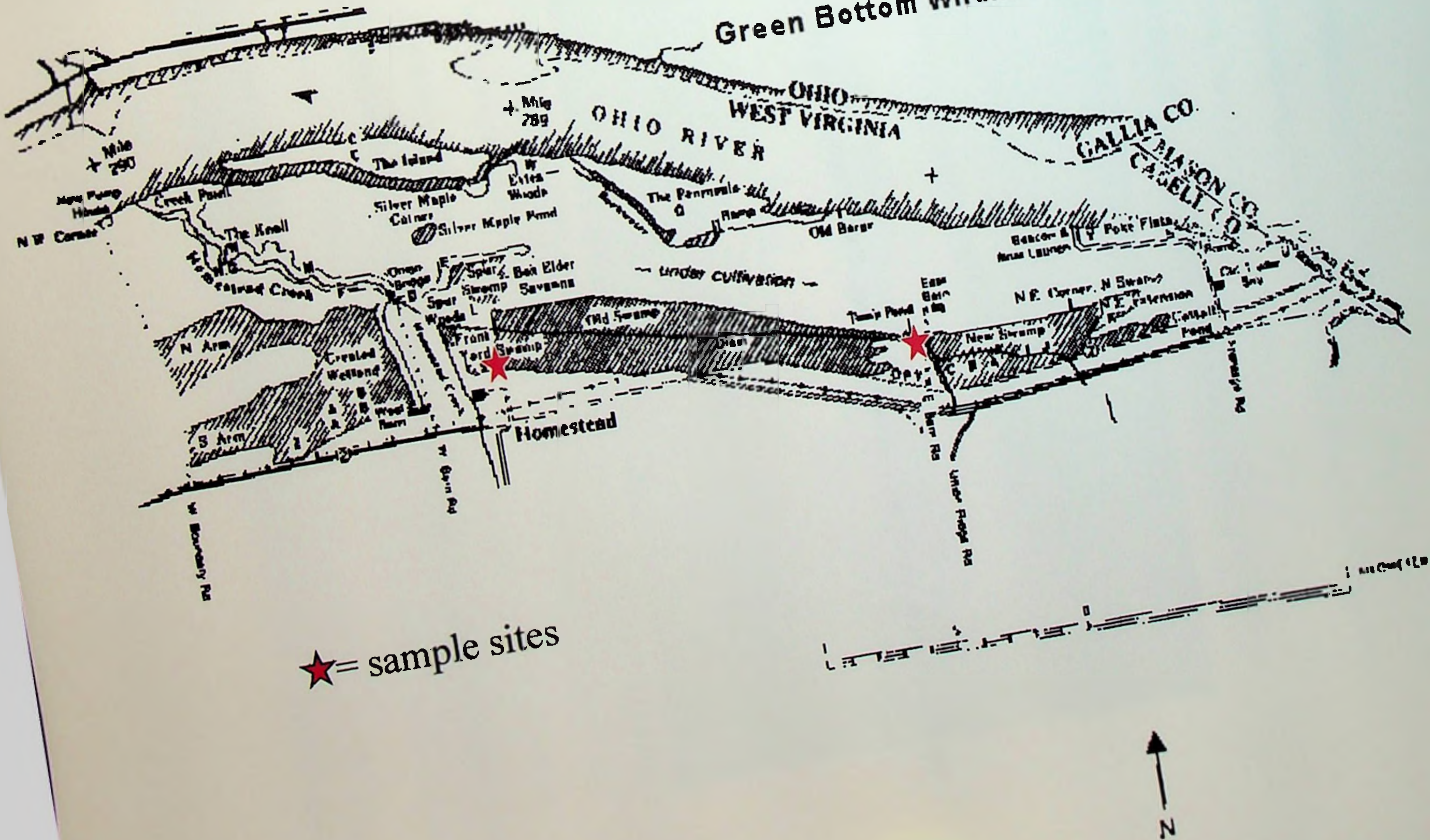


Figure 4. Green Bottom Wildlife Management Area, Cabell County, West Virginia.

66 cm of snowfall annually. Overall, the average annual precipitation is 105 cm and the average temperature is 12.8°C. Humidity ranges from an average of 80 percent at dawn to 60 percent at midafternoon (Cole, 1989).

The GBWMA supports a vast array of plant and animal life. Woody trees include *Acer negundo*, *A. saccharinum*, and *A. saccharum*. Buttonbush (*Cephalanthus occidentalis*) is a small shrub that dominates the wettest areas. Dominant aquatic vegetation are watermeal, liverwort, duckweed, coontail, cattail, mallows and various sedges (WV DNR, 1991).

The GBWMA also provides habitat for many other organisms from insects to large mammals. A wide variety of insects is found in the swamp, including mayflies, damselflies, dragonflies, and water boatmen. Amphibians found in this area include the spotted salamander (*Ambystoma maculatum*), Jefferson salamander (*Ambystoma jeffersonianum*), red spotted newt (*Notopthalmus v. viridescens*), wood frog (*Rana sylvatica*), pickerel frog (*Rana palustris*), leopard frog (*Rana pipiens*), bull frog (*Rana catesbiana*), and spring peeper (*Pseudacris crucifer*). Reptiles include the midland painted turtle (*Chrysemys picta marginata*) and the snapping turtle (*Chelydra serpentina*). Birds found in the GBWMA include red-winged blackbirds (*Agelaius phoniceus*), Canadian geese (*Branta canadensis*), wood ducks (*Aix sponsa*), green heron (*Butorides striatus*) and the great blue heron (*Ardea herodias*). Larger animals found within the GBWMA include muskrat (*Ondatra zibethicus*), beaver (*Castor canadensis*) and white-tailed deer (*Odocoileus virginianus*) (Evans and Allen, 1995).

The first photographic record of GBWMA was taken in 1934. From this photograph, it is known that the swamp was drained and the land used as farmland and pasture. In 1950, the swamp was reestablished due to the construction of a farm road, the building of State Route 2, and beaver colonization. In 1975, 3.24 hectares were lost due to the construction of State Route 2. Beaver activities caused over 85 percent of the bottomland to be dead or dying trees due to inundation by 1985 (WV DNR, 1991).

The GBWMA has a rich anthropological history. The land was inhabited by hunting Paleo-Indians 12,500 years ago, followed by North American Indians 5,000 years ago. During this time, the Eastern Agricultural Complex was developed including crops such as maygrass, little barley, gourds and sunflower. By 1200 A.D., large Fort Ancient Villages dominated the area, raising crops such as beans, corn and squash. The first European, Thomas Hannan, settled the land in 1776. In 1825, the area was purchased by William A. Jenkins, and upon his death, his sons inherited the property. The land was split three ways and Albert G. Jenkins received the house. Albert G. Jenkins served as General in the Confederate Cavalry in the Civil War. The Jenkins homestead was listed on the National Register of Historic Places and was used as the residence for the DNR area manager (WV DNR, 1991). The house is currently being turned into a museum for public view. In all there are 18 recorded archeological sites in the GBWMA, including the Clover Site which dates back to 1600 (WV DNR, 1991).

The preservation of GBWMA presents a unique opportunity for research, nature observation, outdoor recreation, and wildlife education.

CHAPTER V

MATERIALS AND METHODS

Field Studies

Sampling

Specimens of *Crangonyx pseudogracilis* were collected weekly from March 1995 through July 1996 from six sites in Green Bottom Swamp. Quantitative collections were made using a modified Gerking sampler (19" x 19" x 31") (Gerking, 1957) and a standard D-shaped dredge (Fig. 5). The sampler was pressed through the vegetation and into the substrate. The dredge was inserted into the sampler and drawn across the substrate and through the vegetation. Samples were rinsed and transferred from the dredge net into bottles containing 70 percent ethanol. After transport to the laboratory, samples were sorted on a white enamel pan and the amphipods were preserved in vials containing 70 percent ethanol.

Water Quality and Temperature

On each weekly collection date, temperature (°C) was recorded from a maximum-minimum thermometer. On the first collection date of each month, water quality parameters were measured using a Hach Kit, Model AL-36-WR. Tests were conducted for dissolved oxygen (mg/L), alkalinity (mg/L $CaCO_3$), CO_2 (mg/L), total hardness (mg/L $CaCO_3$), total acidity (mg/L) and pH. Water quality tests and temperature were taken from the same location each time.

Figure 5. Modified Gerking Sampler and D-shaped dredge.

Laboratory Studies

Length Measurements

Amphipods were measured in length to the nearest 0.01 mm using a Java computer digitizing system. Measurements were made from the anterior tip of the head to the end of the telson. Size measurements were then categorized into 1 mm size classes using Excel. Histograms were made for each collection month in order to determine the life cycle of the amphipod.

Sex Determination and Reproduction

Mature amphipods were sorted as to sex using a dissecting microscope. The criteria used in determining sex were: (1) males with proportionately larger gnathapods, smaller at maturity, and with genital papillae projecting below the seventh segment and, (2) females of all ages with distinct brood plates that become fringed with setae at the peak of sexual maturity (Hynes, 1955).

Females collected in samples were removed and ovigerous females, those with eggs in the brood pouch, were separated from the gravid females, those with hatched young in the brood pouch. Eggs were removed from the marsupium of each ovigerous female and counted. Ovigerous females were stored in individual vials and measured for length. Linear regression analysis was performed using the SigmaStat spreadsheet program to investigate the relationship between number of eggs versus length of the females. Young from the gravid females were not counted, as most of the marsupia had ruptured and the young escaped during collection.

Low pH Tolerance

Low pH tolerance of *Crangonyx pseudogracilis* was determined by an acute static bioassay. One-hundred amphipods were collected from Green Bottom Swamp using a D-shaped dredge. They were placed in live wells for transport to the lab and acclimated for 24 hours in an environmental chamber. The chamber was maintained at 4°C with a 12 hour dark/light photoperiod. Ten amphipods were placed in two bowls at each value (1.5, 3.0, 4.5, 6.0 and a control of about 7.0). The pH value was checked with an Oakton pH 4 tester and adjusted with sulfuric acid (12N) every two hours from 8 am until midnight. Mortalities were removed every 24 hours. At the end of the 96 hour period, the median tolerance limit (TL_m) was calculated by linear regression analysis using the SigmaStat computer program. Forty more amphipods were then collected and the 96 hour trial repeated using the theoretical TL_m value.

Gut Analysis

Ten mature amphipods were taken per month, except for those months without adults. Microdissection scissors were used to remove the head and then the gut was extracted from the body cavity with forceps. Ten guts were placed in a vial containing three milliliters of water and three drops of iodine. One milliliter of the homogenate was then transferred to a Sedgewick-Rafter cell with a micropipet and examined under a compound microscope. Ten grids from a Whipple occular were chosen at random three times, for a total of 30 grids per month. The different food items were divided into five categories: (1) plant detritus, (2) filamentous alga, (3) diatoms, (4) animal detritus, and (5) mineral. In an attempt to determine seasonal feeding trends, the relative abundance of each item was calculated by comparing the percent of grid squares that contained each food item. No attempt was made to quantify the components of the diet.

CHAPTER VI

RESULTS AND DISCUSSION

Field Studies

Sampling

1508 amphipods were collected from February 1995 through July 1996. Amphipods were taken from the highly vegetated areas in shallower water and seemed to utilize *Potamegeton crispus* as habitat. Amphipod abundance in the sample sites peaked in March and tapered off through July. No amphipods were present in the six sample sites in August, September and October. Amphipods returned in November and numbers steadily increased until a peak once again in March. The abundance also diminished beginning in July the following year (Fig. 6). This disappearance coincide with the increase of water temperature in the summer and the death of the parent generation; therefore, it is possible that the immature amphipods are migrating to cooler, deeper waters (Fig. 7). Aditional collections were taken from deeper areas of the swamp dominated by buttonbush in August and amphipods were present in these areas. This migration has been observed in *Crangonyx richmondensis laurentianus*. Immature males of this species have been shown to leave shallow nursery waters in mid-June followed by females in late-June (Lindeman et al., 1993; Judd, 1963). *Crangonyx forebsi* also has a seasonal migration. These winter breeders travel downstream in fall to breed and move upstream to springs and brooks in the spring (Pennak, 1953).

Figure 6. Number of *Crangonyx pseudogracilis* collected and monthly water temperature.

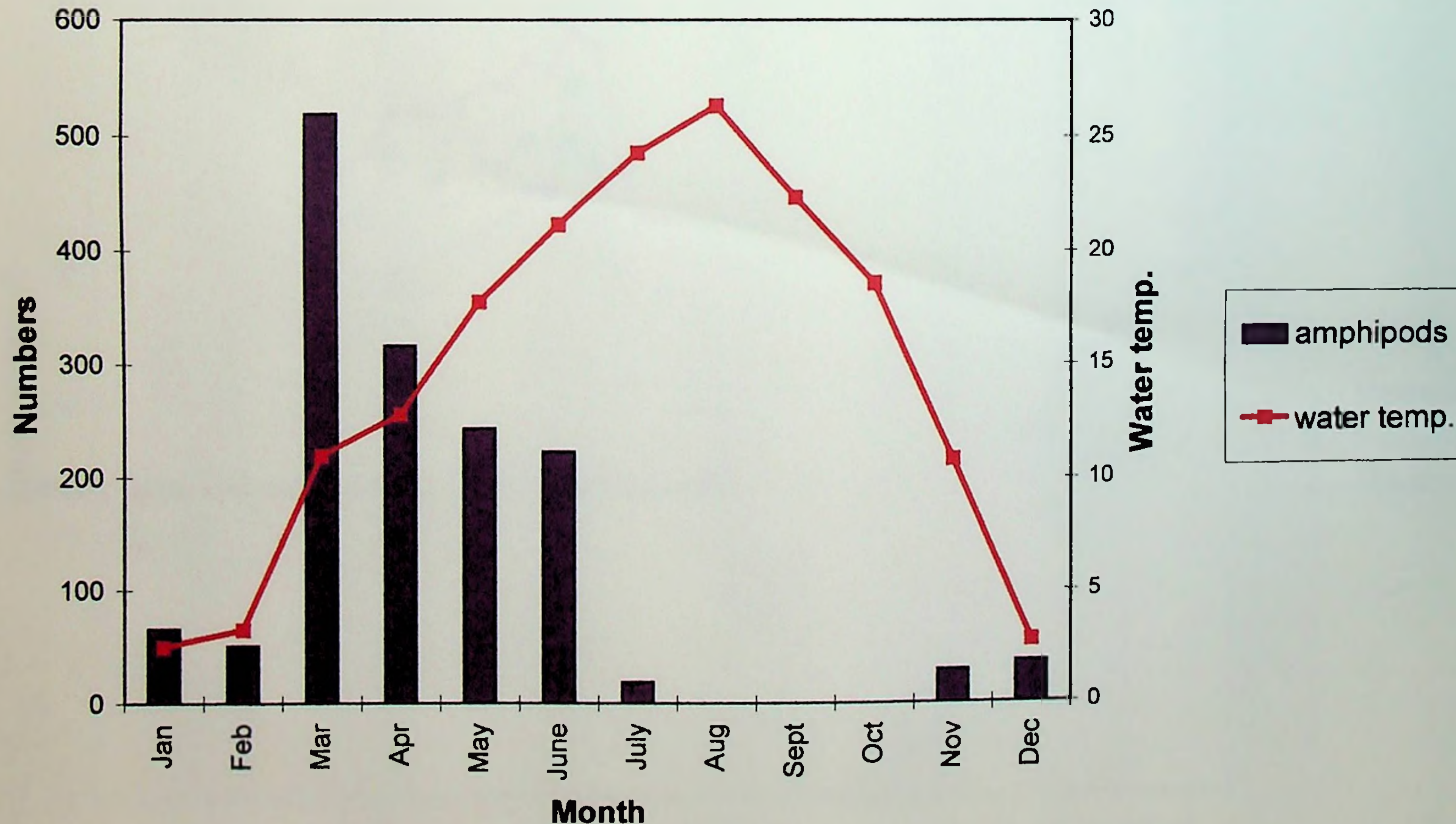
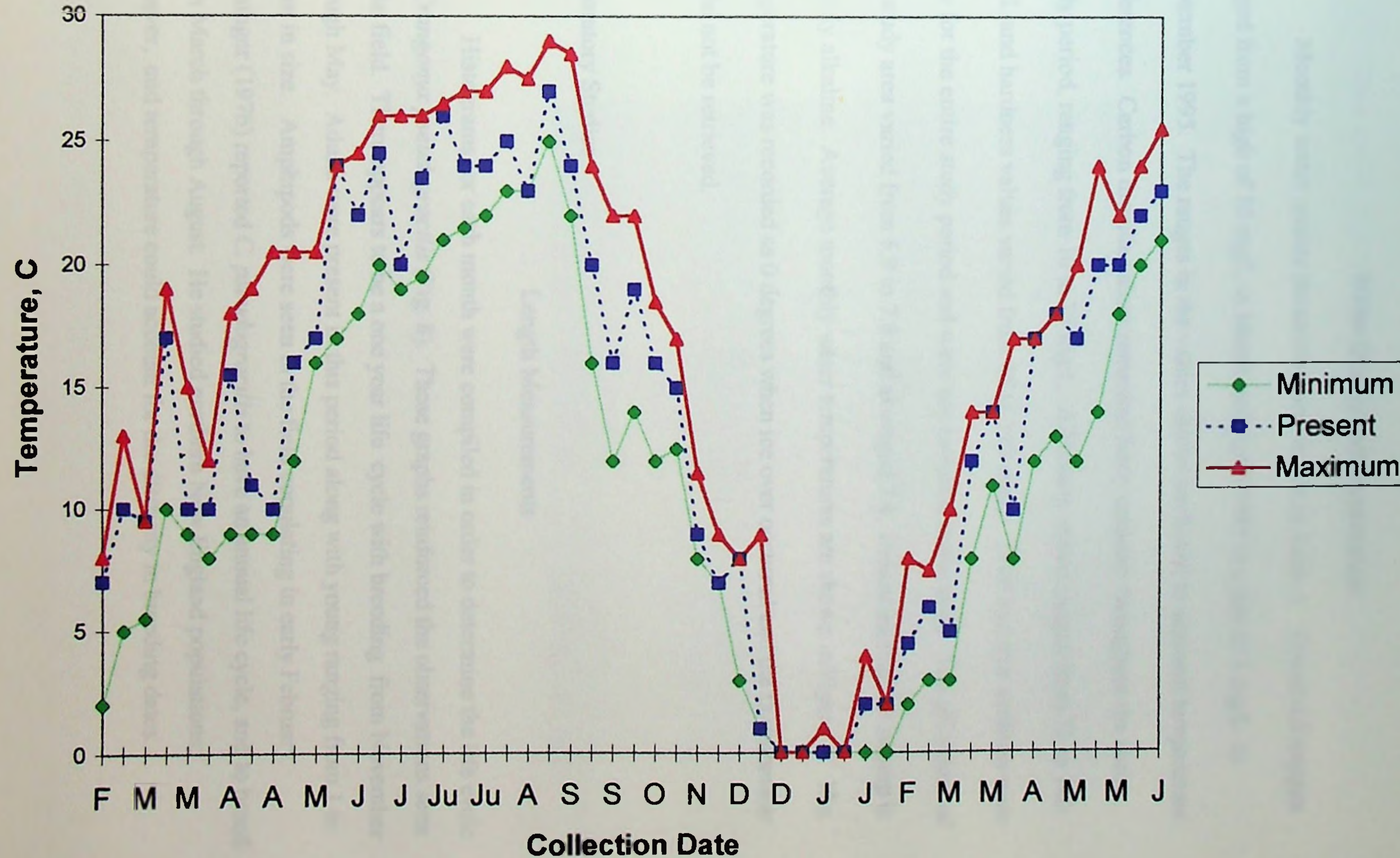

600
500
400
300
200
100
0
Numbers
30
25
20
15
10
5
0
Water temp.
Jan
Feb
Mar
Apr
May
June
July
Aug
Sept
Oct
Nov
Dec
Month
amphipods
water temp.

Figure 7. Water temperature data (°C) for Green Bottom Swamp.



Temperature, C
30
25
20
15
10
5
0
F M M A A M J J Ju Ju A S S O N D D J J F M M A M M J
Collection Date
Minimum
Present
Maximum

Water Quality and Temperature

Monthly water quality parameters are shown in Table 1. Dissolved oxygen ranged from a high of 10 mg/L in March and April 1995 to a low of 3 mg/L in September 1995. The ranges in the values can be attributed to seasonal temperature differences. Carbon dioxide values remained fairly constant throughout the entire study period, ranging from 10 to 25 mg/L. Alkalinity values ranged from 51 to 140 mg/L and hardness values varied from 85 to 153 mg/L. Total and free acidities were zero for the entire study period and were not included in the table. The pH values of the study area varied from 6.9 to 7.8 and averaged 7.4, indicating that the swamp is slightly alkaline. Average monthly water temperatures are shown in Figure 6. The temperature was recorded as 0 degrees when ice over occurred and the thermometer could not be retrieved.

Laboratory Studies

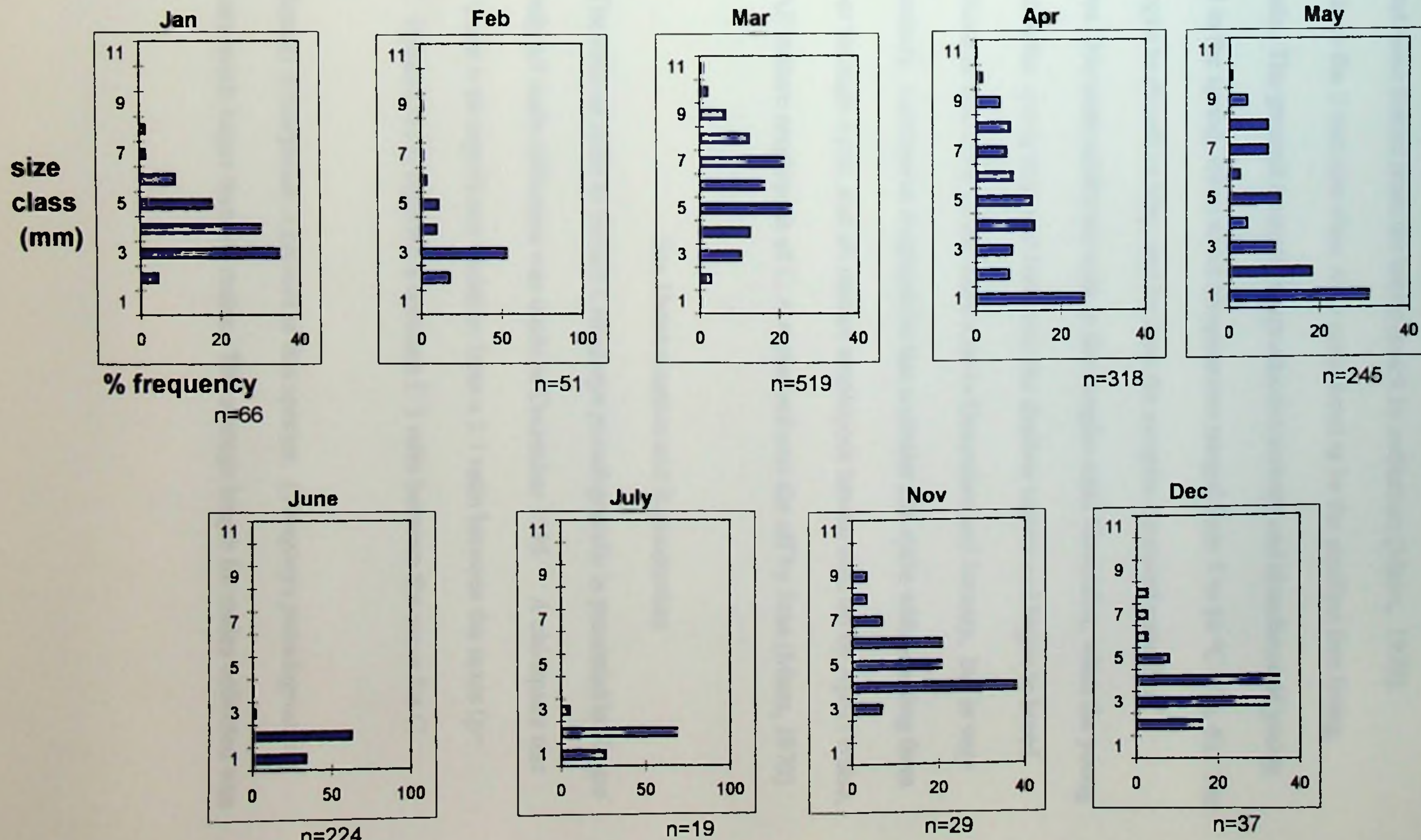
Length Measurements

Histograms for each month were compiled in order to determine the life cycle of *Crangonyx pseudogracilis* (Fig. 8). These graphs reinforced the observations seen in the field. There appears to be a one year life cycle with breeding from November through May. Adults were present in this period along with young ranging from 1 to 3 mm in size. Amphipods were seen in the field copulating in early February. Holsinger (1976) reported *C. pseudogracilis* to have an annual life cycle, and to breed from March through August. He studied northern New England populations; however, and temperature could account for the disparity in breeding dates.

Table 1. Water quality data for Green Bottom Swamp (1995 - 1996). All units expressed as mg/L except pH.

Dates	D.O.	CO_2	Alkalinity	Hardness	pH
March 1995	10	10	119	119	7.1
April	10	15	51	123	7.3
May	7	15	51	123	7.6
June	5	10	140	102	7.2
July	4	15	140	119	7.2
August	4	15	51	119	7.7
September	3	15	51	153	7.8
October	5.5	20	85	119	7.8
November	5	20	85	102	7.6
December	7.5	10	51	102	7.5
Jan. 1996	8	15	85	119	7.1
February	9	15	102	85	7.2
March	7	15	102	85	7.5
April	7	20	85	123	7.5
May	6.5	25	119	119	6.9
June	5	20	119	119	7.2
July	5	15	102	102	7.3
MEAN	6.4	15.9	85	113.7	7.38

Figure 8. Length frequency histograms of *Crangonyx pseudogracilis*.



size class (mm)
% frequency
Jan
n=66
Feb
n=51
Mar
n=519
Apr
n=318
May
n=245
June
n=224
July
n=19
Nov
n=29
Dec
n=37

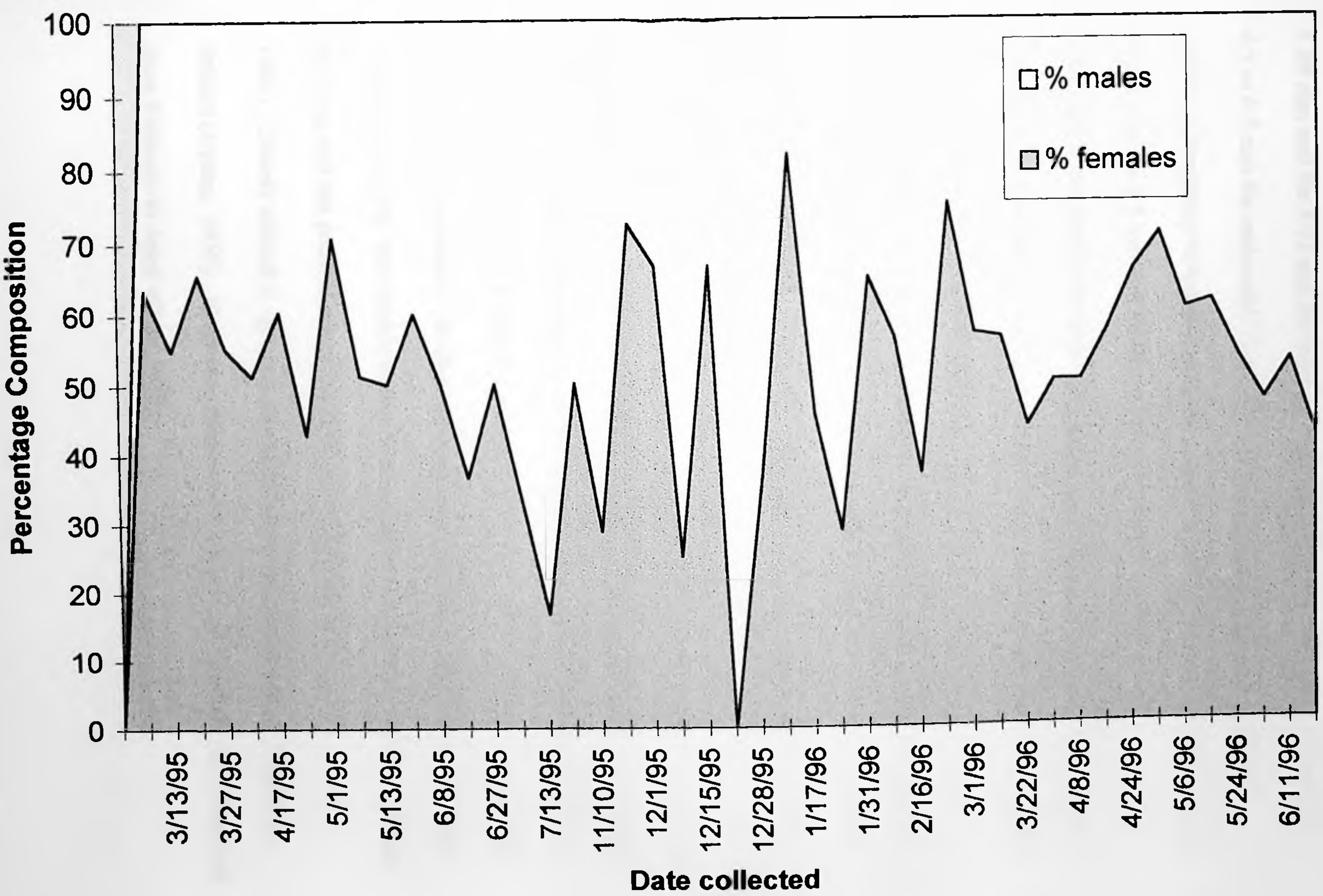
It is also possible that some amphipods in the 1 mm size class were not yet free living, but were forced from the brood pouch by collection (Mairs, 1970). Amhipods in the 2 mm size class were considered to be the smallest free living individuals. The greatest amount of reproductive activity and abundance of young occurred in the spring, when water temperatures ranged from 5 to 10 °C (Fig. 6). The adults began to die off in May, and by June the samples consisted entirely of immatures. No more adults are seen in the samples until November, when the young produced in the spring migrated back into the shallow waters and began to breed. Two millimeter size class young were seen in December and January, but in very small numbers. *Gammarus troglophilus* has a similar life cycle with breeding from November through April, and all mature amphipods having died off by April (Weise, 1953). All mature amphipods of *C. r. richmondensis* die off by June (Mairs, 1970).

Sex Determination and Reproduction

The ratio of male to female *Crangonyx pseudogracilis* is presented in Figure 9. The only all male collection was made in December 1995. A chi-square test showed there is no significant variation from a 1:1 ratio between the sexes ($p= 0.2681$). Hynes (1955) reported a constant 1: 1 ratio between the sexes for *C. gracilis*.

Sexual dimorphism is present in this species. *Crangonyx pseudogracilis* females are much larger than the males. The average length for males collected was

Figure 9. Sex ratio of *Crangonyx pseudogracilis* from each collection date.

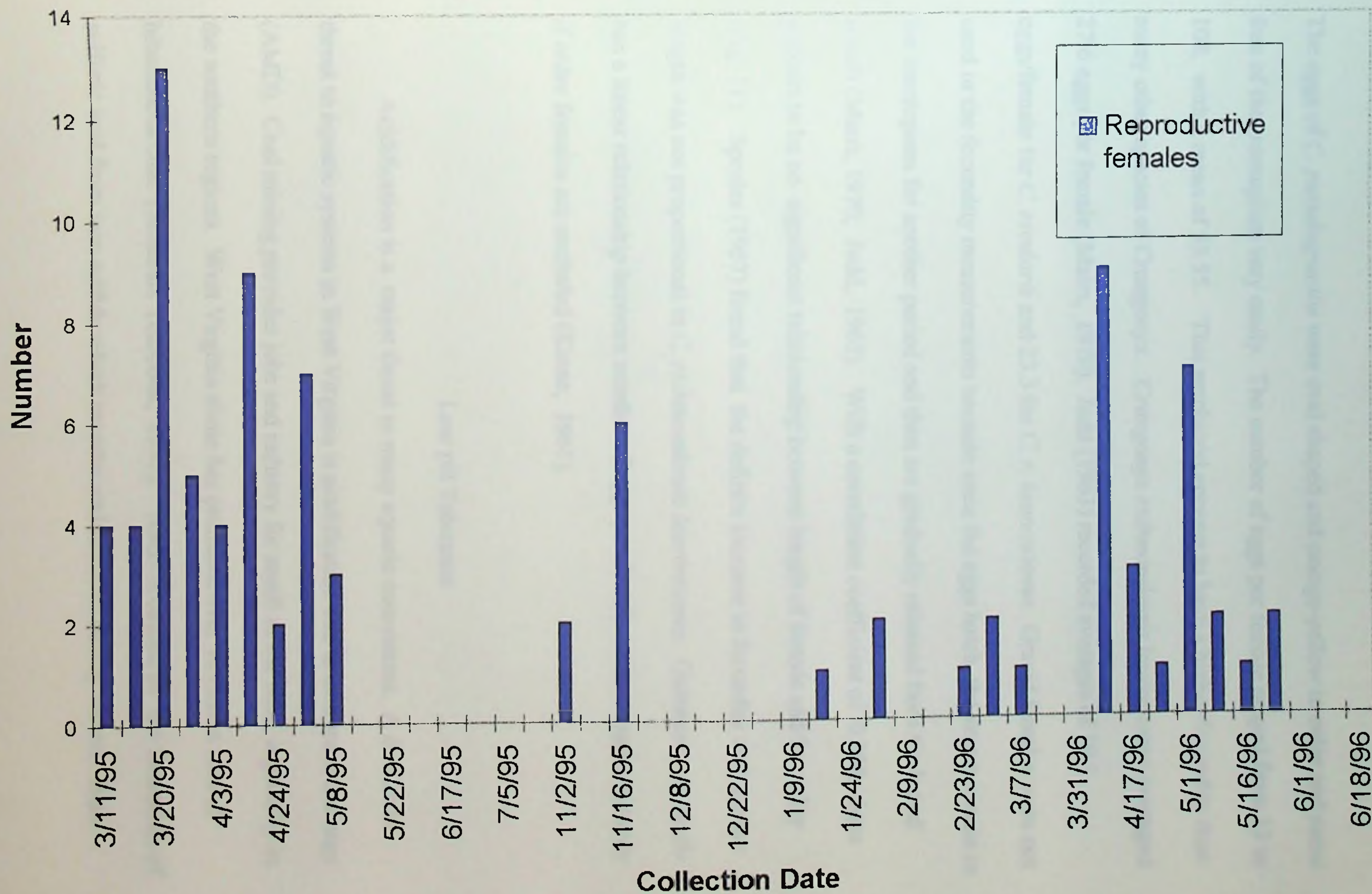


% males
% females
Percentage Composition
100
90
80
70
60
50
40
30
20
10
0
3/13/95
3/27/95
4/17/95
5/1/95
5/13/95
6/8/95
6/27/95
7/13/95
11/10/95
12/1/95
12/15/95
12/28/95
1/17/96
1/31/96
2/16/96
3/1/96
3/22/96
4/8/96
4/24/96
5/6/96
5/24/96
6/11/96
Date collected

5.88 mm and the 8.73 mm for females. These values are within the size ranges of 4.5 to 6.5 mm for males and 7.5 to 10.5 mm for females reported by Holsinger (1976). *Crangonyx richmondensis laurentianus* shows a similar sexual dimorphism, males average 8.5 mm and females 13.8 mm (Sprules, 1967). Ovigerous females were seen in the population from November 2 until May 8 (Fig. 10). The greatest abundance of reproductive females was seen in the spring, which correlates with the great abundance of young seen in the late spring and early summer. Thirteen ovigerous females were taken on March 20, the highest number in any sample. Far fewer reproductive females were taken in the winter months, only 11 in November and December combined. *Crangonyx shoemakeri* also has a long breeding season that occurs from December until May (Cole, 1957).

Crangonyx pseudogracilis produced more than one brood per year per female. Ovigerous females showed size increases throughout the breeding season. The eight ovigerous females in November were the smallest collected with an average size of 6.90 mm, and the March females averaged 8.01 mm. If in fact they produced one brood and died, as does *C. forebsi*, the females would not be larger as the breeding season progresses, but would produce their brood at sexual maturity and die without growing past the point of sexual maturity (Crawford and Tarter, 1976; Clifford, 1966). Closely related *C. gracilis* produces four to eight broods in its lifetime in Britain (Hynes, 1955). Ovigerous females of *C. gracilis* in Kentucky were observed from February to April (Cole, 1957).

Figure 10. Reproductive females of *Crangonyx pseudogracilis* from each collection date.



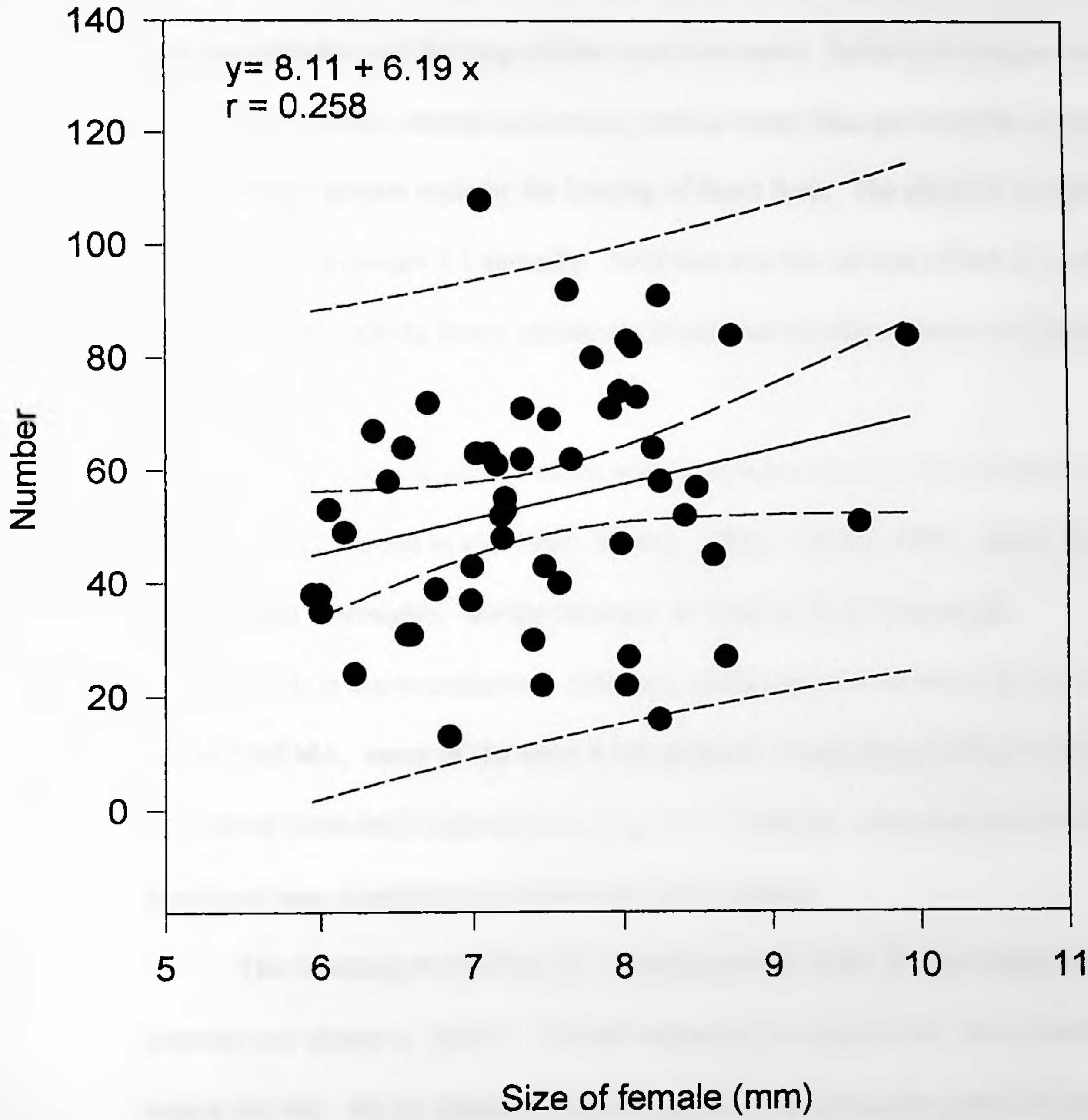
Number
14
12
10
8
6
4
2
0
Reproductive females
3/11/95
3/20/95
4/3/95
4/24/95
5/8/95
5/22/95
6/17/95
7/5/95
11/2/95
11/16/95
12/8/95
12/22/95
1/9/96
1/24/96
2/9/96
2/23/96
3/7/96
3/31/96
4/17/96
5/1/96
5/16/96
6/1/96
6/18/96
Collection Date

The eggs of *C. pseudogracilis* were oval shaped and orange-yellow in color and came free of the marsupium very easily. The number of eggs per female ranged from 13 to 108, with a mean of 53.55. This amphipod appears to have a higher fecundity than many other species of *Crangonyx*. *Crangonyx richmondensis laurentianus* averaged 27.6 eggs per female (Mairs, 1970). Judd (1963) recorded averages of 19.3 eggs/female for *C. rivularis* and 23.3 for *C. r. laurentianus*. Gravid females were not used in the fecundity measurements because once the eggs hatch, the young remain in the marsupium for another period and then are gradually released from the brood pouch (Mairs, 1970; Judd, 1963). With a correlation coefficient of 0.258, there appears to be no significant relationship between length of female and fecundity (Fig. 11). Sprules (1967) found that the definite increase in fecundity with greater length was not proportional in *C. richmondensis laurentianus*. *Gammarus zaddachi* has a linear relationship between number of eggs per female versus length, but only if older females are excluded (Kinne, 1961).

Low pH Tolerance

Acidification is a major threat to many aquatic ecosystems. A dominant threat to aquatic systems in West Virginia is acidification due to acid mine drainage (AMD). Coal mining provides jobs and industry for much of the state, especially in the southern regions. West Virginia alone has produced over 10 billion tons of bituminous coal (Minerals Yearbook, 1991). AMD is caused by the formation of sulfuric acid from iron sulfide which invades and acidifies nearby waters. Both

Figure 11. Linear regression analysis for fecundity and body length of *Crangonyx pseudogracilis*..


y= 8.11 + 6.19 x
r = 0.258
Number
Size of female (mm)
140
120
100
80
60
40
20
0
5
6
7
8
9
10
11

operating and long abandoned mines contribute to the problem. The pH of waters affected by AMD may commonly be as low as 2.0 - 2.5 (Smith and Frey, 1971).

Acid rain is also a potential contributor to acidification, especially in the eastern United States. Acid rain is the result of sulfur and nitrogen oxides combining with precipitation and forming sulfuric and nitric acids. Sulfur and nitrogen oxides are released through natural occurrences such as forest fires and volcanic eruptions but on a much greater scale by the burning of fossil fuels. The pH level of rain in West Virginia averages 4.1 annually. Acid rain also has adverse effects on aquatic ecosystems by leeching heavy metals out of soil and into the environment (Mayer et al., 1984).

The effects of acidification on aquatic systems has been well researched (Havas, 1982; Burton et al., 1982; Lackey, 1938; Warner, 1971). Insect taxa have been studied thoroughly, but the tolerance of amphipods to lowered pH environments is much unknown. Although acidification is not presently a problem in the GBWMA, many of the areas in the range of *Crangonyx pseudogracilis* are affected by both AMD and acid rain (Fig. 12). Therefore, other populations of this amphipod may experience problems with acidification.

The resulting mortalities of *C. pseudogracilis* in the 96 hour acute static bioassay are shown in Table 2. The pH values are averages of the values recorded during the test. All the amphipods survived the entire test in the control pH of 7.3 and 6.0. In the first 24 hour period, there were no survivals at a pH of 1.5 and 55 percent survival at a pH of 3.0. All survived the first day at pH 4.5. After 48 hours,

Figure 12. United States distribution of *Crangonyx pseudogracilis* and acid mine drainage affected areas.

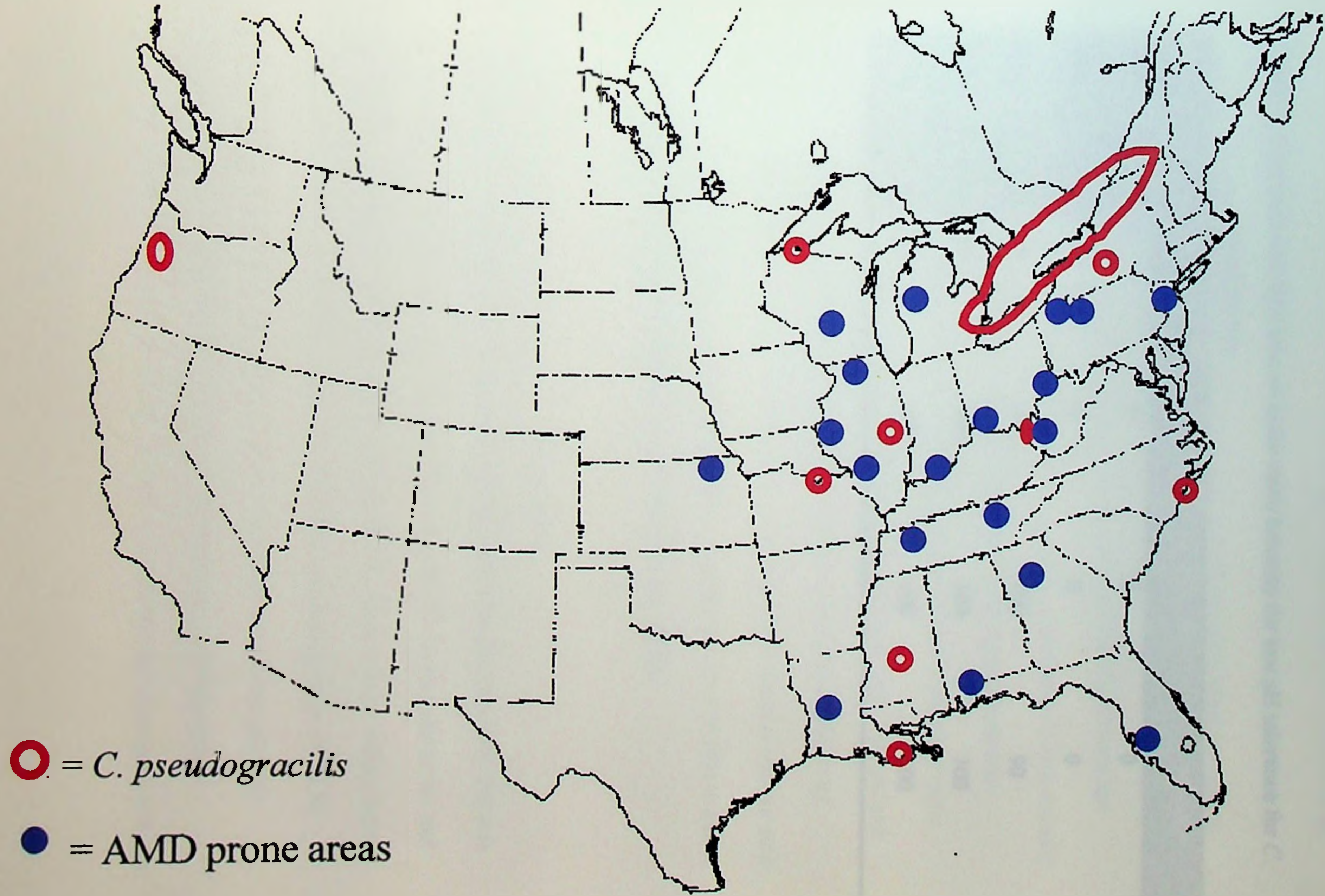

= *C. pseudogracilis*
= AMD prone areas

Table 2. Experimental data from an acute static bioassay for low pH tolerance for *C. pseudogracilis.*

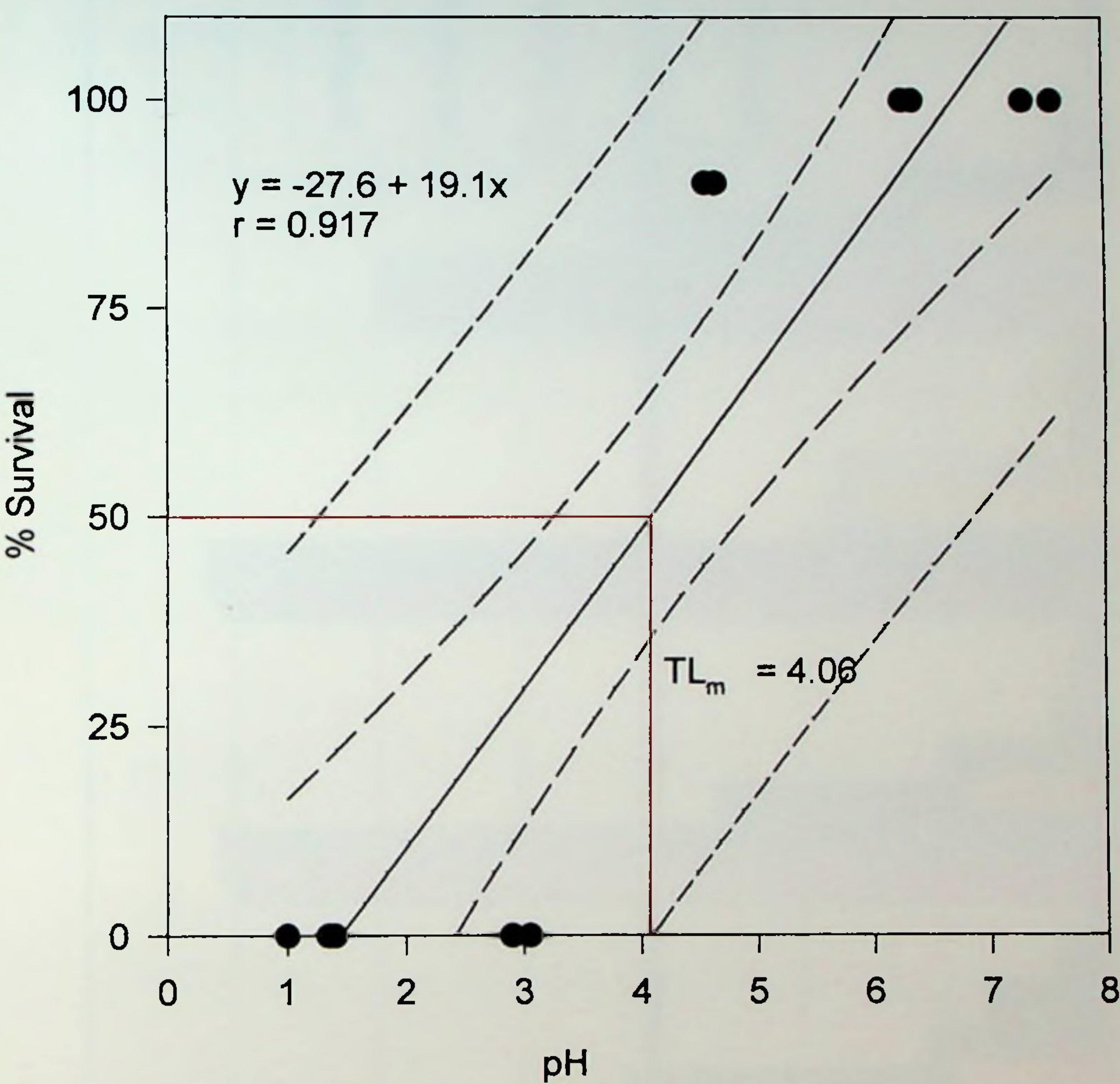
pH	24 hours	48 hours	72 hours	96 hours
1.50	0	0	0	0
3.00	55	0	0	0
4.60	100	100	100	90
6.05	100	100	100	100
7.43	100	100	100	100

no survivors remained in the pH of 3.0. The only other mortalities occurred at 96 hours when there was 10 percent mortality in the pH of 4.5. At the end of this period, a median tolerance limit (TLm) of 4.06 was found using linear regression analysis (Fig. 13). An additional 96-hr. test was then performed with this theoretical TLm. At a pH of 4.06 with 20 amphipods, 65 percent survived the 96 hour test period. These results indicated that the actual TL_m for *C. pseudograclis* may actually be a little lower than 4.06. No references to laboratory pH experiments with amphipods could be found; however, Thomas (1976) reported *C. pseudogracilis* from water of pH 5.1. *Gammarus sp.* were found to survive in West Virginia and Indiana in streams of pH 2.2 and 3.2 (Lackey, 1938). Lake studies in Norway, however, showed *Gammarus lacustris* and *G. pulex* to be very sensitive and not exist in lakes below a pH of 5.7 (Burton et al., 1982). Crustaceans may be able to utilize exoskeletal $CaCO_3$ to aid in buffering body fluids (Havas, 1982).

Gut Analysis

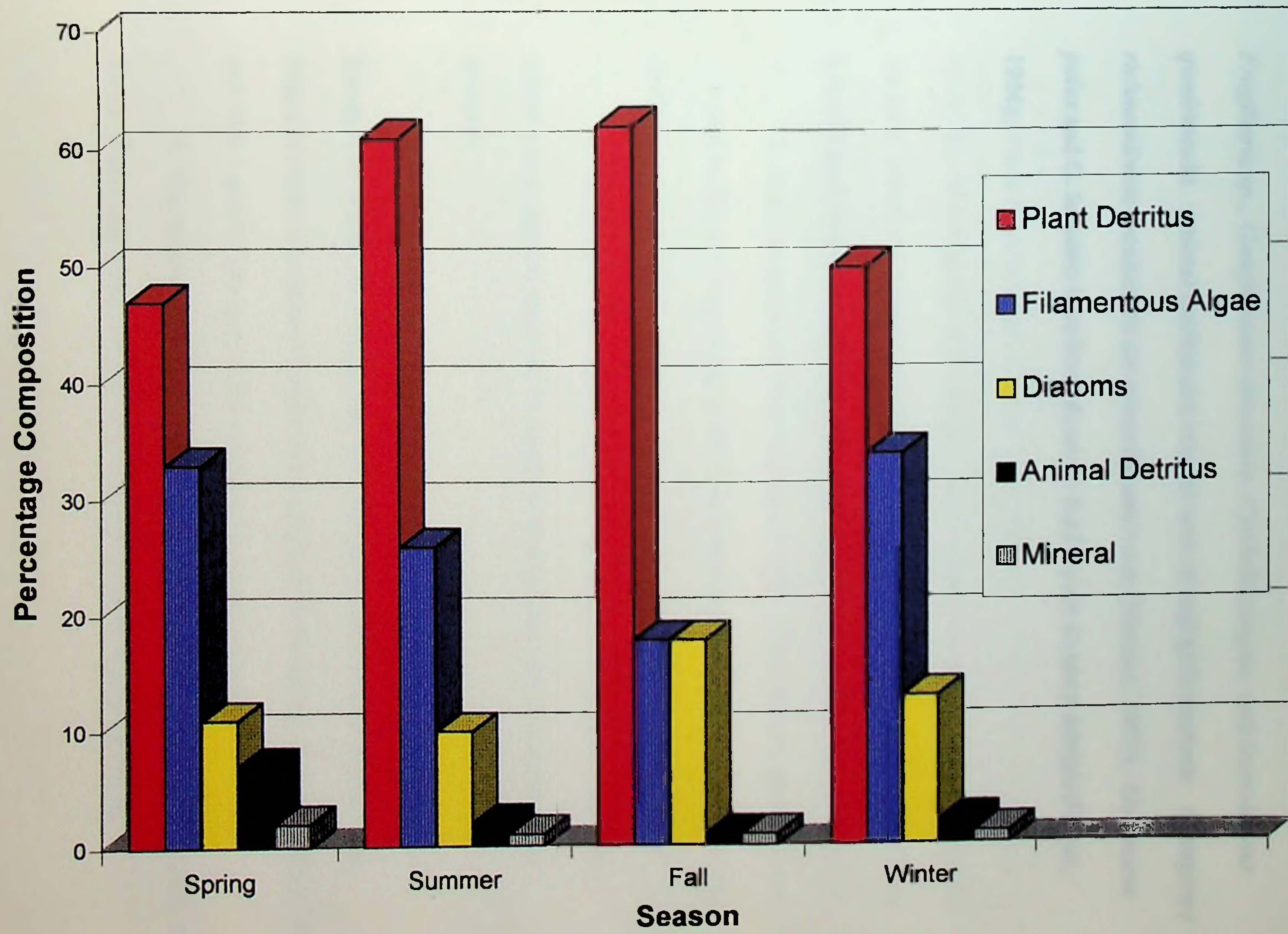
Seasonally preferred food items of *Crangonyx pseudogracilis* indicated that it is primarily a detritivore (Fig. 14). Plant detritus made up the biggest part of the diet in all seasons, from 47 to 62 percent in spring and fall, respectively. Filamentous algae were the second largest component of the diet, comprising from 18 to 34 percent in fall and winter, respectively. Algae commonly seen in the gut were *Lyngbya digueti*, *Hydrodictyon reticulatum*, *Spirogyra sp.*, and *Mougeotia genuflexa*. Diatoms were a consistent, but minor part of the diet, averaging between

Figure 13. Linear regression analysis for low pH tolerance of *Crangonyx pseudogracilis.*



y = -27.6 + 19.1x
r = 0.917
TL$_m$ = 4.06
% Survival
100
75
50
25
0
0
1
2
3
4
5
6
7
8
pH

Figure 14. Seasonal percentage composition of food items from gut analysis of *Crangonyx pseudogracilis*.



Percentage Composition
70
60
50
40
30
20
10
0
Spring
Summer
Fall
Winter
Season
Plant Detritus
Filamentous Algae
Diatoms
Animal Detritus
Mineral

10 and 15 percent of the total diet. Common diatoms were *Synedra acus*, *Fragiliaria sp.*, *Gomphonema olivaceum*, *Cymbella turgida*, and *Scenedesmus quadricauda*. Animal detritus and mineral were of negligible amounts. *Crangonyx richmondensis occidentalis* are primarily carnivores (Mathias, 1971). *Gammarus pulex* and *G. lacustris* have been shown to feed mainly on plant detritus (Hynes, 1954).

CHAPTER VII

CONCLUSIONS AND SUMMARY

1. The life history of the amphipod *Crangonyx pseudogracilis* was investigated from March 1995 through July 1996 in the Green Bottom Wildlife Management Area in Cabell County, West Virginia.

2. Water quality of the sample sites remained within normal parameters for the area. Dissolved oxygen ranged from a high of 10 mg/L in March and April to a low of 3 mg/L in September. The pH values ranged from 6.9-7.8.

3. Water temperatures were recorded weekly. The highest temperatures were recorded in July and August (up to 29°C) and the lowest in December and Januuary (0°C).

4. Amphipods were most abundant in the shallow vegetated areas in the winter and spring and migrated to deeper buttonbush dominated habitats in the summer.

5. Length frequency histograms indicated that *Crangonyx pseudogracilis* breeds from November through May and females produce several broods. The largest number of ovigerous females were collected in the spring, between March 11 and May 1 and the life span of this amphipod is about one year.

6. The number of eggs per females ranged from 13 to 108, with an average of 53.55. There was a weak relationship between fecundity and body length.

7. Sexual dimorphism is present in *C. pseudogracilis*. The average length for *C. pseudogracilis* was 5.88 mm for males and 8.73 for females.

8. There was no significant seasonal variation in sex ratio.

9. In a low pH tolerance bioassay, the TL_m value for this amphipod was determined by using linear regression analysis to be 4.06, and a 96-hr. test at this value produced a 65 percent survival rate, indicating that the TL_m value for this amphipod may be a little lower.

10. Gut analysis indicated that the diet of *C. pseudogracilis* was predominantly plant detritus year round. Filamentous algae were also a consistent part of the diet throughout the year.

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