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Ecological life history of Caenis amica Hagen (Emphemeroptera: caenidae) from the mitigated area of Green Bottom Wildlife Management Area, Cabell County, West Virginia

Gail Lynn Perrine

ECOLOGICAL LIFE HISTORY OF *CAENIS AMICA* HAGEN (EMPHEMEROPTERA: CAENIDAE) FROM THE MITIGATED AREA OF GREEN BOTTOM WILDLIFE MANAGEMENT AREA CABELL COUNTY, WEST VIRGINIA

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Advisor Dr. Donald Fortr
Department of Biological Sciences

Leonard J. Deutsch
Dean of the Graduate College

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ABSTRACT

The ecological life history of a population of *Caenis amica* Hagen from the Green Bottom Wildlife Management Area was studied from November 1996 to November 1997. Water quality data and 2,505 larvae were collected from the newly mitigated area of Green Bottom Swamp. Total body lengths of larvae were measured using a computer-digitizing program. These results indicated a bivoltine life cycle. Most larvae were found in the spring and summer months of April, June, and July. The highest peak of larvae (1,035) occurred in July. The pH values ranged from 7.0 to 8.0 with a mean of 7.6. Temperatures ranged from 2.0 to 25.7^{0}C in February and July, respectively. Head width averages of the larvae were used to determine percent growth for each month. The greatest amount of growth (67.5%) occurred between March and April. This growth coincided with the first emergence at the end of April. A second growth period (37.2%) occurred between July and August. Larvae consumed mainly plant detritus. At least 50 percent detritus was present throughout the year. Plant detritus made up the highest percentage (90%) in the spring (April). Filamentous algae and diatoms were also present. Low pH tolerance of *C. amica* larva was determined in a 96 hr. acute static bioassay. The straight line graphical interpolation method gave a value of 4.3 and the linear regression value was 5.2. Using the linear regression value of 5.2, a 60 percent mortality rate was determined after an additional 96 hours. Egg counts of *C. amica* ranged from 325 to 690 eggs per individual female; the average number of eggs per female was 423. The fecundity-length regression equation was $y = 78.1 + 85.8x$ ($r = 0.657$). Adults emerged from April 30, 1997 to September 11, 1997. There

were two emergence peaks, April 30 (1,418 adults) and June 1 (1,833 adults). A chi-square test showed a highly significant difference from the 1:1 sex ratio at 0.05 confidence level.

CHAPTER I

INTRODUCTION

Mayflies are one of the most important groups of aquatic insects. They spend most of their life as larvae in water, only leaving it to mate and lay eggs. Mayflies frequently tolerate only a very small range of environmental conditions and may be very sensitive to small changes in their current environment (Hubbard and Peters, 1978). They are one of the most sensitive groups of aquatic insects to acidification. The number of species decreases as pH decreases. Mayflies can become infrequent or absent under such conditions even though other groups may still be thriving. Acid waters normally have fewer species, lower abundance, and lower biomass of benthic invertebrates than nonacidic waters (Resh and Rosenberg, 1984).

Mayflies have interested biologists for centuries because of their short adult lives. Most mayflies have an adult life of only two or three days. This briefness is indicated in the name of the order Ephemeroptera, since the winged stages are truly ephemeral. The shortness of the adult stage is possible because the only function of the adult is to reproduce. Males form swarms which differ with the species from small companies of dancing individuals to incredible clouds of flying insects. Females enter the swarms, and mating almost always occurs in flight. They deposit the eggs in water after a few minutes or at most a few hours. Adults don't feed. Every adaptation of the mayfly is directed towards reproduction. Mouthparts are vestigal remnants, and in some species, the legs are also vestigal, except for the male forelegs, which are adapted to grasp the female during flight (Edmunds et al., 1976).

Most mayfly larvae are herbivorous, feeding on fine organic detritus or algae. Occasionally, a species is specialized as a filter-feeder or a carnivore.

Ephemeropterans are unusual because they have two winged stages. The first winged stage, the subimago, emerges from the last nymphal instar and is normally not sexually mature. Most species go through a final terrestrial ecdysis before transformation to the imago stage.

Mayfly life cycles range from two or more generations per year (multivoltine) to one generation every two years (semivoltine). Univoltinism is the most common life cycle of mayflies in temperate areas (Ward, 1992).

The role of aquatic insects as pollution indicators has received increasing attention recently. It is almost certain that large mass emergences of mayflies are a result of unknowing interference of people with the environment. People have enriched streams and lakes with sewage from cities, manure and fertilizers from farms, and natural nutrients from eroding soils.

Mayflies also help to remove pollutants from water and return them to the terrestrial environment. The self-cleansing of a river involves the degradation and removal of the organic material. Carbon escapes by respiration, but phosphates and nitrates are mainly removed by being incorporated into the bodies of emerging insects (Edmunds et al., 1976).

Mayfly larvae are strictly aquatic, except for a semiterrestrial South American baetid. Ephemeropterans occur exclusively in freshwater, with the exception of a very few species that venture into brackish waters. Numerous species occupy a wide variety of lotic and lentic habitats. The most distinct ephemeropteran found normally occurs in warm lotic habitats (Ward, 1992).

The primary objective of this investigation was to determine the ecological life history of *Caenis amica* in a mitigated wetland. This investigation is the first study of *C. amica* in West Virginia.

CHAPTER II

REVIEW OF THE LITERATURE

Smith (1935) studied the eggs and oviposition in mayflies. Moon (1939) discussed the developmental cycle of *Caenis horaria* (L.). Judd (1949, 1950) studied the emergence of a phalaenid moth population from an Ontario marsh. Berner and Pescador (1950) looked at the mayflies of Florida, in particular the order Ephemeroptera and the family Caenidae. Judd (1953) studied a population of emerging insect adults, including *C. simulans*, from a marsh in Ontario. Thew (1960) completed a revision of the genera in the family Caenidae, modifying the genera of Caenidae known at the time and supplying a lot of the information on North American *Caenis*. Koss (1968, 1969) studied the taxonomy and morphology of Ephemeroptera eggs. Landa (1968) studied the developmental cycles of central European Ephemeroptera and their interrelations, including *Caenis pseudorivulorum* Keffermuller, *C. undosa* Tiensuu, *C. horaria* (L.), and *C. robusta* Eat. Clifford and Boerger (1974) studied the fecundity of mayflies of a stream in Alberta, Canada. Cloud and Stewart (1974) studied the drift of mayflies in the Bravos River, Texas. Harper and Harper (1976) made records and descriptions of ephemeropterans in a lake in Quebec. Edmunds et al. (1976) discussed the family Caenidae, along with its adult and larval characteristics. Sweeney (1976) looked at the response of *C. simulans* to thermal variation. Faulkner and Tarter (1977) provided records of the family Caenidae in West Virginia. Berner (1977) described the patterns of distribution of southeastern mayflies. Kondratieff and Foster (1977) documented the presence of *C. amica* in Tennessee. Sweeney and Vannote (1978) discussed the size and distribution of hemimetabolous aquatic insects. Merritt and Cummins (1996) studied the aquatic insects of North America, including the Ephemeroptera. McCafferty and Provonsha (1978) reported *C. amica* from northwestern Arkansas. This species is also known to occur in Missouri. Mackey (1978) provided information on the emergence patterns of *Caenis macrura* Steph., *C. horaria* (L.), and *C. robusta* Etn. Bradbeer and Savage (1980) observed the distribution and life history of *Caenis robusta* Eaton in Cheshire and North Shropshire, England. Harper and Harper (1981) discussed the records and descriptions of Northern Canadian mayflies. Clifford (1982) described the life cycles of mayflies with special reference to voltinism. MacFarlane and Waters (1982) described the annual production of mayflies, including *C. simulans* in a western Minnesota plains stream. Rodgers (1982) determined the effects of increased temperatures on the production of *Caenis*. Rodgers (1983) discussed the fecundity of *Caenis* in elevated water temperatures. Weiderholm (1984) studied the responses of aquatic insects to environmental pollution, including the Ephermoptera. Corkum (1984) described the movements of marsh-dwelling invertebrates, including *C. simulans* McDunnough in central Alberta, Canada. Corkum (1985) also discussed the life cycle patterns of *C. simulans* McDunnough in a Canadian marsh. Novotny (1985) described the effects of a

Kentucky flood-control reservoir on *Caenis* in the tailwater. Poff and Matthews (1985) recorded the replacement of *Stenonema* spp. by *Caenis diminuta* Walker as the numerical dominant in the mayfly collection of a thermally-stressed stream. Provonsha and McCafferty (1985) described a new Neartic genus of Caenidae, *Americaenis*. Provonsha (1986) revised the genus *Caenis* in North America. Corkum (1989) studied the habitat characterization of the morphologically similar mayfly larvae, *Caenis* and *Tricorythodes*. Punzo and Thompson (1990) discussed the effects of temperature and acid stress on hatching, survival capacity, metabolism, and posture reflexes in caenid mayflies. Christman and Voshell (1992) determined the life history, growth, and production of ephemeropterans in experimental ponds in Virginia. Fjellheim and Raddum (1992) studied the recovery of acid-sensitive species of Ephemeroptera in a southern Norway river after liming. Pinder et al. (1993) studied the diel periodicities of adult emergence of a caenid mayfly at a western Australian wetland. Wang et al. (1997) discussed the sister relationship of the families Neoephemeridae and Caenidae.

Taxonomically, only the adults are known with any degree of certainty. The following works deal with the North American species in depth: McDunnough (1931) and Needham et al. (1935), and Edmunds et al. (1976) updated the North and Central American species accounts. The following thesis projects involving aquatic insects have been completed at Green Bottom Swamp: Emery (1994), Mullins (1994), Johnson (1995), and Wilhelm (1997).

CHAPTER III

TAXONOMY AND DISTRIBUTION

Taxonomy and Morphology

Kingdom - Metazoa
  Phylum - Arthropoda
    Class - Insecta
      Order - Ephemeroptera
        Suborder - Pannota
          Superfamily - Heptagenioidea
            Family - Caenidae
              Genus - *Caenis*
                Species - *amica*

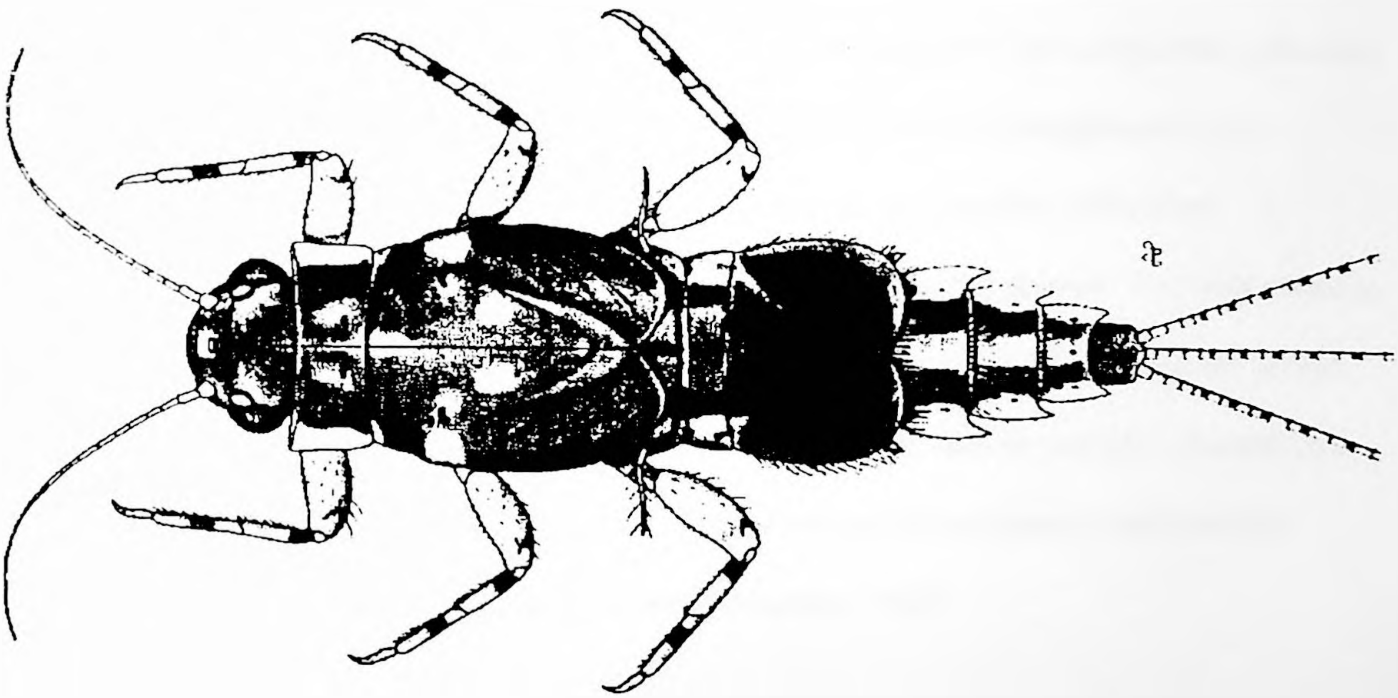
Based on the work of Provonsha (1986), larvae of the family Caendiae are recognized by the following combination of characteristics: "Forewing pads pannote in form; hind wing pads absent; gills on abdominal segment 1 filiform, originating from lateral margin of segment; gills on segment 2 subquadrate, operculate; gills on 3-6 single, with margins fringed and filiform tufts wanting; median terminal filament well developed."

Larvae of the family Caenidae have a strong thorax and a moderately to strongly flattened abdomen without hind pads. The abdominal segments 3-6 are shortened. Quadrate gills are found on the opercular second segment, which is not fused at the midline. Gills are also found on segments 3-6 with fringed margins and no fibrilliform tufts. Three caudal filaments are present. Adults of both sexes have eyes that are small and obscure. Thorax is muscular with a medionotal membrane and the abdomen is relatively small and short. There is one pair of broad wings present, with veins MP_2 and IMP almost as long as vein MP_1. Anal area of the wings is expanded and broad. The hind margins of imago wings have setae. Genital foreceps of the male are one-segmented. There are three caudal filaments, which are very long in the male and short in the female (Edmunds et al., 1976).

A dorsal view of a *C. amica* larvae is shown in Figure 1. Body length of *Caenis* larvae varies between 2 and 7 mm. Head is lacking tubercles and the labial and maxillary palpi are three-segmented. Forelegs are nearly as long as the middle and hind legs, with short claws that are curved somewhat apically. Prominent posterolateral spines are found on the middle abdominal segments, but are not upcurved. Wing length of the adult varies between 2 and 5 mm. The antennal pedicel is twice as long as the scape. Prosternum is two or three times as long as broad and triangular in shape. Four coxae are closely estimated (Edmunds et al., 1976).

Stephens (1835) first described the genus *Caenis*. He also made *Caenis macrura* the genotype. Say (1839) described the first North American species, *Caenis hilaris*. Subsequently, *C. diminuta* Walker 1853, *C. amica* Hagen 1861, and *C. latipennis* Banks 1907, were also described. Hagen (1861) listed three species in his *Neuroptera of North America*. However, Eaton (1884) synonymized *C. amica* with *C. diminuta*. McDunnough (1931) reinstated *C. amica* as a valid species, and synonymized *C.amica* Hagen = *C. simulans* McDunnough. McDunnough (1931) also extensively treated the North American species, redescribed and keyed the above species, and added *C. forcipata*, *C. ridens*, *C. punctata*, *C. tardata*, *C. jocosa*, and *C. simulans*. Traver (1935) described and keyed all known species and added *C. anceps* and *C. delicata*. Smith (1935) described and figured the eggs of several North American *Caenis* species. Needham et al. (1935) studied the eggs of *C. hilaris, C. jocosa, C. amica, C. simulans,* and *C. perpusilla*. Koss (1968, 1969) described, figured, and keyed several North American *Caenis*

Figure. 1. Dorsal view of a *Caenis amica* larvae



4.

eggs.

Phylogeny

Adults and larvae of the family Caenidae have many unique characteristics. The family is probably derived from the proto-Neoephemeridae of the Potamanthellus group lineage. Some Neoephemeridae are so analogous to Caenidae larvae that only the lack of hind wing pads and the fibrilliform tuft on the gills of segments 3-6 mark the larvae of Caenidae (Edmunds et al., 1976). In addition, larvae are often confused with those of the family Tricorythidae, which also have operculate gills. However, the caenids have gills on segment 1 and subquadrate operculate gills, whereas the tricorythodes have no gills on segment 1 and have triangular or ovate operculate gills. Larvae have kept many of the primitive characteristics of the proto-Neoephemeridae lineage. However, the adults have become highly derived. The wing venation, the medioscutal membrane, and the one-segmented male genital foreceps differentiate caenid adults from all the other ephemeropteran families. In general, they do resemble Tricorythidae adults and are often misidentified as such. The presence of the medionotal membrane in Caenidae is the easiest way of identification (Provonsha, 1986).

Distribution

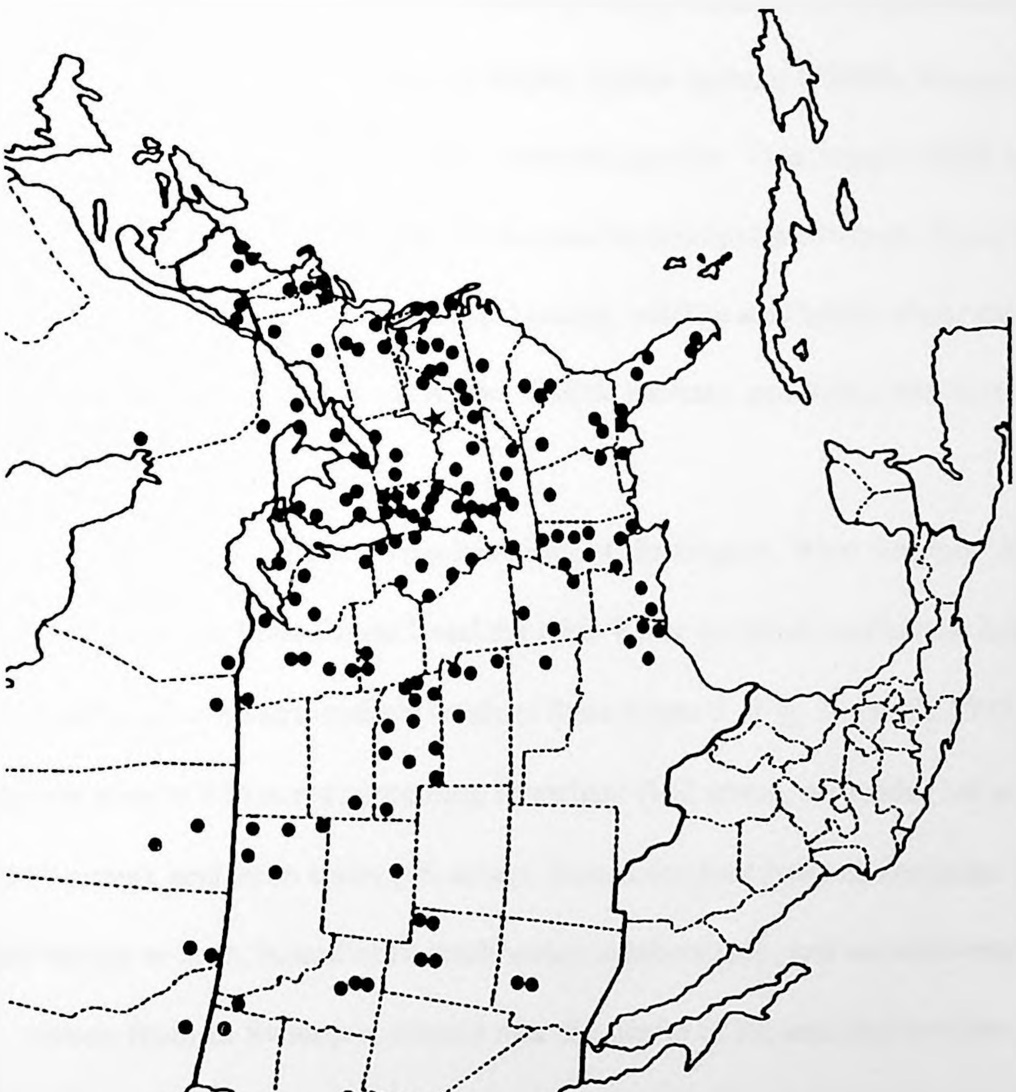
Based on the work of Edmunds et al. (1976), the family Caenidae occurs over most of the earth, and is absent only from New Zealand and most oceanic islands. Larvae appear in a diversity of both lotic and lentic habitats. Although *Caenis amica* is one of the smallest mayflies, they are often found in very large numbers.

There are five species of caenids in West Virginia: *C. amica* Hagen, *C. anceps* Traver, *C. diminuta* Walker, *C. latipennis* Banks, and *C. tardata* McDunnough (Faulkner and Tarter, 1977). The population at Green Bottom Swamp is only the second report of *C. amica* in West Virginia. The first report was at Gandy Creek in Pendelton County, Spruce Knob, VI-22-1973, 1M {USNM}. *C. amica* is found throughout the northeast and southeast United States and sparsely in the central and western states (Fig. 2).

Habitat

Caenis larvae are normally found in quiet bodies of water, but some do inhabit streams. They are often found in rooted vegetation in the bottom of ponds. Larvae are also common among collections of leaf debris and trash and on immersed vegetation such as *Riggia*. They can be found near stream banks, living close to the plant bases. Some larvae inhabit exposed roots of terrestrial plants along stream banks, and stay near substrate. They can occasionally be found in or on anchored debris floating in the water, such as leaves or sticks. Some species are very pollution tolerant. Larvae can also be found in very small fresh water bodies and at lake edges covered with vegetation (Edmunds et al., 1976).

Figure. 2. Distribution of *Caenis amica* in North America



CHAPTER IV

DESCRIPTION OF THE STUDY AREA

The Green Bottom Wildlife Management Area (GBWMA) is a result of joint efforts by the U. S. Corps of Engineers (COE), West Virginia Division of Natural Resources (WVDNR), and the U. S. Fish and Wildlife Service (USFWS). The GBWMA was established to mitigate impacts to wetlands, wildlife, and associated recreation incurred by implementation of the Gallipolis Locks and Dam Replacement Project (Green Bottom Wildlife Management Area Management Plan, 1991). The GBWMA was designed to: 1) increase wildlife and habitat diversity; 2) increase wetland habitat; 3) increase bottomland hardwoods; 4) provide for wildlife related recreation through fishing, hiking, hunting, wildlife and habitat observation; 5) provide opportunities for the study of wildlife and wildlife habitats; and 6) provide for the preservation of archaeological and historical sites.

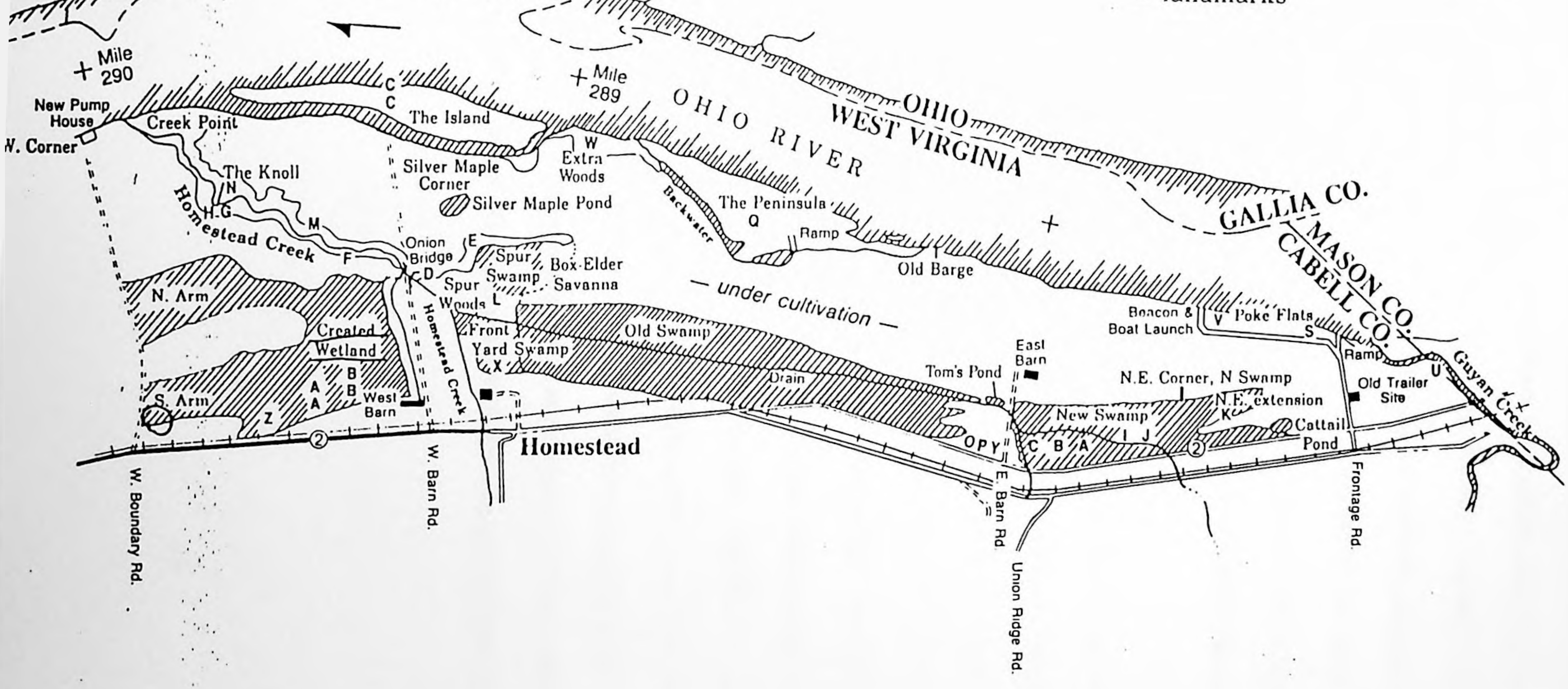
The GBWMA is located 16 miles north of Huntington, West Virginia. Most of the property lies between State Route 2 and the Ohio River in Cabell and Mason counties. About 67 acres located on the eastern end are south of State Route 2 (Fig. 3) (Stark, 1993). The GBWMA includes an area of 836 acres containing forestland (162 acres), wetlands (140 acres), agricultural land (518 acres), and open water (16 acres). It contains four types of wetlands: seasonally flooded basins or flats, inland open fresh water, shrub swamp, and wooded swamp.

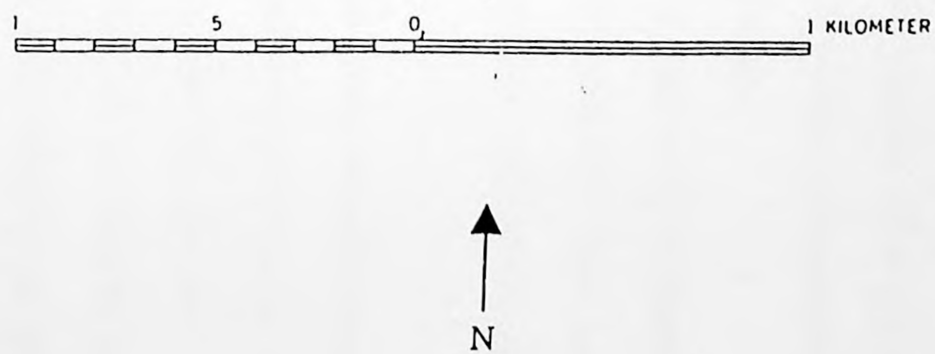
Green Bottom Swamp is located near the center of the area and includes about 100 acres. A 25-acre shrub swamp is located upstream and a 1-acre wooded swamp is located downstream. Dominant trees and shrubs include buttonbush, cottonwood, sycamore, silver maple, black

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

WILDLIFE MANAGEMENT AREA

— landmarks





LEGEND

Shaded areas are inundated.
Capital letters (A) are approximate transect locations.

Landmarks and Transects refer to:

Stark, T. J. 1993. Flora and Vegetation of Green Bottom Wildlife Management Area, W.Va., unpublished master's thesis, Marshall University, Huntington, W.Va.

willow, green ash, honey locust, black cherry, smooth alder, silky dogwood, and multiflora rose. Additional standard wetland plans include watermeal, liverwort, toothcup, water beadgrass, duckweed, loosestrifes, coontail, cattail, mallows, and assorted sedges.

The Ohio River shoreline extends 14,450 feet along the northern border of GBWMA. There are 13, 800 feet of interrupted streams and two embayments of the river. Two small livestock ponds are also found in the area. Excluding the Guyan Creek watershed, the watershed of the rest of the area is less than 2,500 acres.

GBWMA provides habitat for a wide diversity of wildlife. Thirty mammals are known to live there, and an additional sixteen may occur there. One hundred-thirteen bird species, one hundred-five in the fall and forty-seven in the winter, are thought to nest on or near the management area. Bald eagles were first observed in the area in the winter of 1988-89. Twelve species of amphibians and five species of reptiles have also been seen in GBWMA and another thirty-six species may occur there. Fishes common to the Ohio River inhabit the river, embayments, and backchannels of the area. The ichthyofauna of the GBWMA consists of 11 species including the bowfin, central mudminnow, grass pickerel, and bluegill (McGinn et al., 1992). Several benthic macroinvertebrates are found in the swamp including caddisflies, odonates, midges, hemipterans, and amphipods. Three mayflies inhabit the area, including *Caenis amica* (Green Bottom Wildlife Management Area Management Plan, 1991).

CHAPTER V

MATERIALS AND METHODS

FIELD STUDIES

Benthic Sampling

Larvae of *Caenis amica* were collected monthly from November 1996 to November 1997. Collections were made around the 13^{th} of each month. Three sites in thickly vegetated areas in the created wetland were selected based on previous collections of earlier thesis projects (Fig. 4).

Samples were taken using a 19" by 19" by 31" modified Gerking Sampler (Gerking, 1957) and a long handled D-shaped dredge (Fig. 5). The sampler was placed into the vegetation and pushed hard into the substrate. The dredge was then used to gather the benthic substrate and vegetation within the sampler. Each sample was preserved in 70 percent ethanol until a lab analysis could be made. Samples were sorted in a white enamel pan and larvae were preserved in vials containing 70 percent ethanol.

Water Quality and Temperature

Water chemistry was examined using a Model TA-3 Hach kit during each monthly collection. Values measured included: dissolved oxygen (mg/L), free acidity (mg/L), total acidity (mg/L), alkalinity (mg/L $CaCO_3$), carbon dioxide (mg/L), total hardness (mg/L $CaCO_3$), and pH. Temperature data were recorded at the time of each collection using a maximum-minimum thermometer (^oC). Water depths (cm) were also recorded with a meter stick.

Larval Exuviae

The swamp bank was examined weekly from May to September for the presence of larval

Figure. 4. Green Bottom Swamp mitigated area

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

exuviae. Exuviae were occasionally seen on vegetation close to the bank and in samples. Exuviae in the samples were preserved in 70 percent ethanol for each month.

Collection of Adults

A black light (model #29 w 6002) and white sheet were used from April to September to collect emerging adults (Fig. 6). Air and water temperatures were also taken at the beginning, middle, and end of the collection time. The light was turned on a few minutes after dusk. Once *Caenis amica* was seen, the light was left on fifteen minutes longer. After the collection was complete, 70 percent ethanol was added to the trap and taken back to the lab. Contents of the trap were then transferred to a small labeled bottle of 70 percent ethanol.

LABORATORY STUDIES ON LARVAE

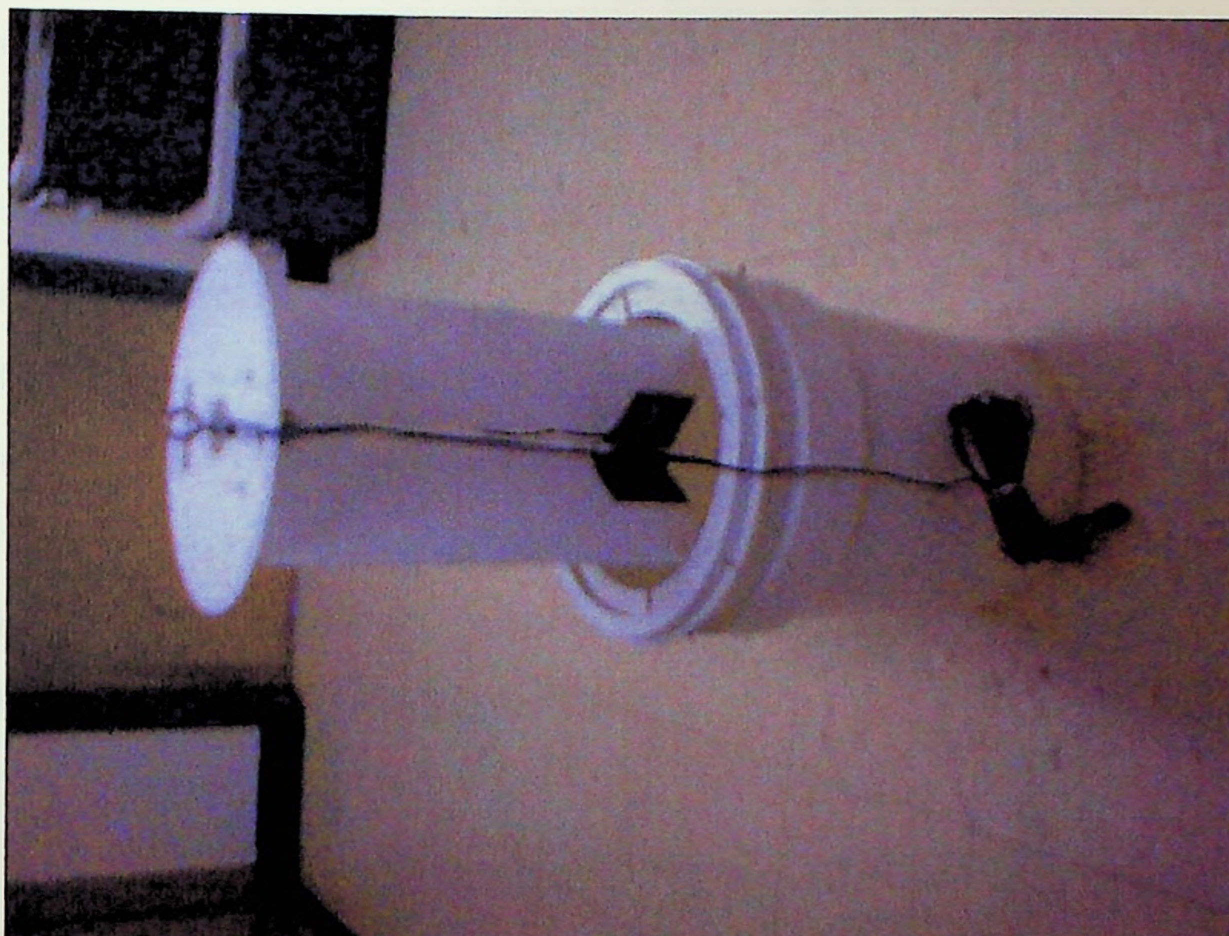
Voltinism

Total body length for all larvae was measured using a Java computer-digitizing program. All measurements were made from the anterior tip of the head to the tip of the last abdominal segment. Then, the size measurements were categorized into 0.01 mm size classes using a SAS program. After the sizes were categorized, length frequency histograms were used to determine the number of generations (voltinism) in the population.

Growth

Larval growth rates were determined monthly by calculating the percent increase in growth from one month to the next of mean head width measurements. Head width was measured to the nearest 0.01 mm with the Java computer system. A population range diagram was used to display monthly head width, range, and two standard errors of the mean. The overlap of two standard errors of the mean were compared to determine the significant

Figure. 6. Model #29 w 6002 emergence light trap



differences between the means (0.05 confidence level). Daily growth rates were determined using linear regression analysis.

Foregut Analysis

Approximately ten of the largest larvae were chosen from each season (April, July, October, and January) for foregut analysis. Foreguts were examined with a Bausch and Lomb dissecting scope after being removed from a mid-thoracic incision with microdissecting scissors. A homogenate was produced by placing 10 foreguts in a dissecting dish containing 3 ml of water. The homogenate was thoroughly agitated, and 1 ml of the mixture was removed with a micropipet and placed in a Sedgewick-Rafter cell. An Olympus compound microscope containing a Whipple ocular grid at 200X was used to examine the cell. Ten grids were randomly chosen to be examined. Thirty grids were examined for each season by twice repeating the previous procedure.

The relative abundance of food items was decided by calculating the percentages of small grid squares within each field that included each of three different items: 1) plant detritus, 2) filamentous green algae, and 3) diatoms.

pH Tolerance

A pH tolerance test was done to calculate the acid tolerance of this species under static bioassay conditions. One-hundred larvae were collected from the mitigated area of Green Bottom Swamp using a D-shaped dredge. They were returned to the lab and permitted to acclimate in an environmental chamber for 24 hours at 15^0C. Ten larvae were put in each of 5 duplicate dishes (8 ¼" x 3 ¼"). Sulfuric acid (12N) was used to adjust five pH values: 1.5, 3.0, 4.5, 6.0 and the control of 8.29. The experiment lasted for 96-hours and pH values were controlled daily with a Cole-Parmer pH microcomputer. Fatalities were indicated every 24 hours during the experiment. The 96-hour TLm (mean tolerance limit) test was used as a measure of acute toxicity to low pH at the end of the experiment. Also, linear regression analysis was used to determine the pH value at which 50 percent of the larvae survived after 96 hours. This pH value was then tested with ten more larvae in two duplicate dishes.

LABORATORY STUDIES ON ADULTS

Fecundity and Egg Size

Fecundity (the number of eggs) was determined by making direct egg counts of females. Female adults captured during emergence were dissected with stainless steel scissors. The body cavity was opened and the eggs were removed. Eggs were counted under a Bausch and Lomb dissecting microscope. A regression of fecundity on body length was calculated and a coefficient of correlation (r) was determined. Diameters of 20 percent of the eggs from 10 females were measured using an ocular micrometer (nearest 0.01mm) in a dissecting microscope. The location and descriptions of the eggs were discussed.

Emergence Period

Using the adults from April to September, the emergence period was determined for the *Caenis amica* population in Green Bottom Swamp.

Sex Ratio

Sex was determined by the size of the eyes and the presence of genitalia. The male has larger eyes, than the female. Also, the male has short genital forceps and longer caudal filaments than the female. In addition, the adult male body length is 2 to 4.5 mm and the adult female body length is 2.5 to 6.2 mm. A chi-square analysis was used to calculate any significant differences from the 1:1 ratio at the 0.05 confidence level.

CHAPTER VI

RESULTS AND DISCUSSION

FIELD STUDIES

Benthic Sampling

A total of 2,505 larvae of *Caenis amica* were collected from November 1996 to November 1997. Most larvae were found in the spring and summer months of April, June, and July. The highest peak was found in July with 1,035 larvae (Fig. 7). Larvae were more commonly found among mud and vegetation in the three sample sites.

Larvae are found in almost any freshwater habitat but are more common in areas with little or no current, like lakes, ponds, and the slower parts of rivers and streams. Larvae live in numerous different substrates such as fine sand, mixed gravel, and submerged debris, but are most commonly found in organically rich silty areas (Provonsha, 1986). They normally occupy ponds, where they grow on the bottom in the zone of submerged vegetation, or among groups of leaf debris and trash (Edmunds et al., 1976).

Water Quality and Temperature

Monthly water quality and temperature data were recorded from November 1996 to November 1997 (Table 1). Data from November 1996 through April 1997 were incorrect because of defective chemicals in the Hach kit. Therefore, data from former theses at Green Bottom were used from December 1977 through November 1997. Averages for these months were used in Table 1. Dissolved oxygen ranged from 4.0 mg/L in July, August, and September, to 10.6 mg/L in February with a mean of 6.5 mg/L. Carbon dioxide values increased from 5.0 mg/L in October to 16.7 mg/L in April. Hardness ranged from 95.6 mg/L $CaCO_3$ in

Figure. 7. Total number of larvae collected from Green Bottom Swamp from November 1996 to November 1997

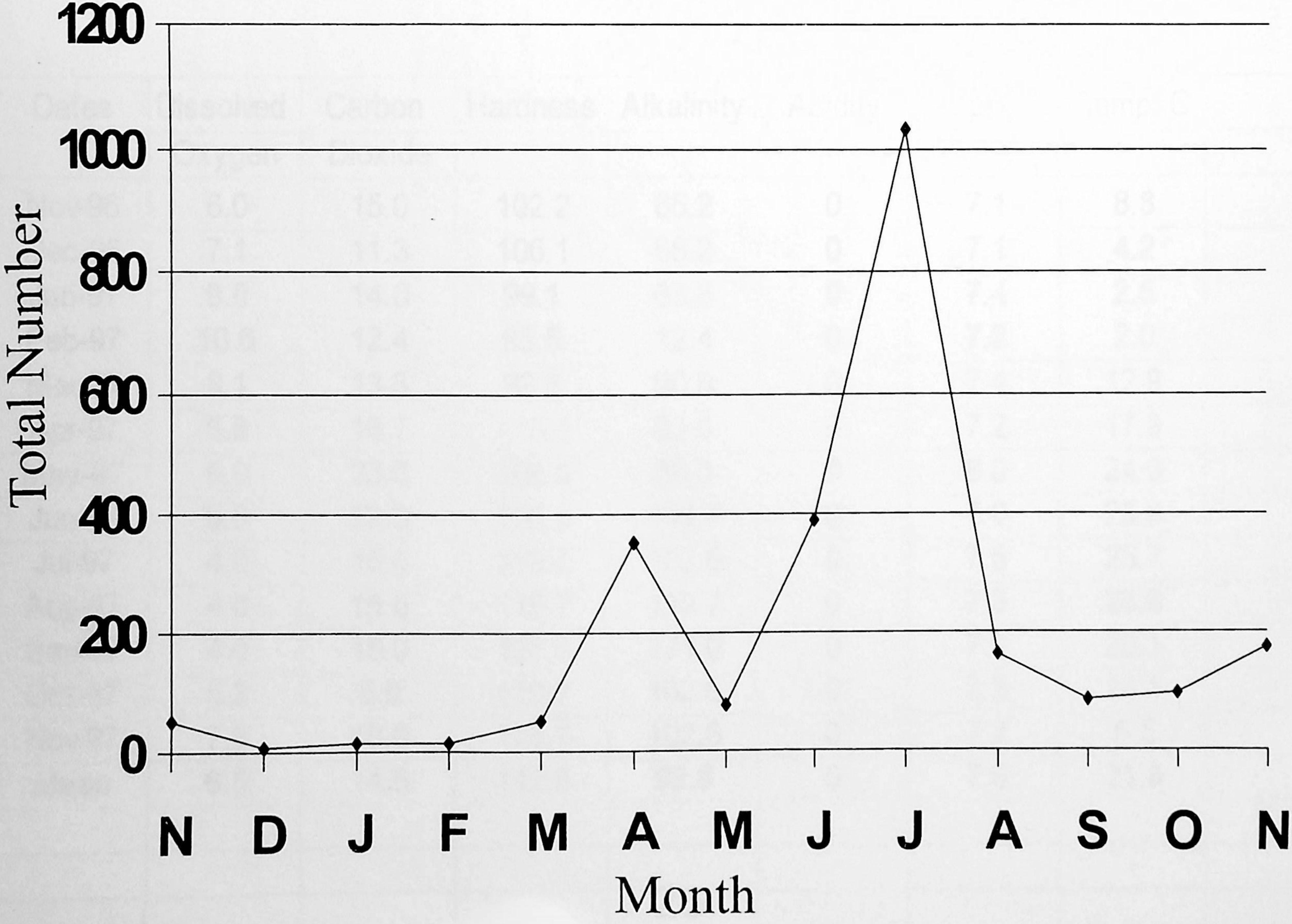

1200
1000
800
600
400
200
0
Total Number
N D J F M A M J J A S O N
Month

Table 1: Water Quality and Temperature

Dates	Dissolved Oxygen	Carbon Dioxide	Hardness	Alkalinity	Acidity	pH	Temp C
Nov-96	6.0	15.0	102.2	85.2	0	7.1	8.8
Dec-96	7.1	11.3	106.1	68.2	0	7.1	4.2
Jan-97	9.8	14.0	99.1	63.8	0	7.4	2.5
Feb-97	10.6	12.4	95.6	12.4	0	7.2	2.0
Mar-97	8.1	13.6	99.8	90.0	0	7.4	12.9
Apr-97	6.8	16.7	113.9	93.0	0	7.2	17.3
May-97	6.9	23.0	108.5	86.0	0	8.0	24.0
Jun-97	5.0	22.0	136.5	109.7	0	7.0	25.6
Jul-97	4.0	15.0	119.7	102.6	0	7.5	25.7
Aug-97	4.0	15.0	119.7	119.7	0	7.3	23.3
Sep-97	4.0	15.0	126.0	171.0	0	7.0	20.1
Oct-97	5.3	5.0	119.7	102.6	0	7.3	14.1
Nov-97	7.0	10.0	119.7	102.6	0	7.2	8.8
Mean	6.5	14.5	112.8	92.8	0	7.6	21.8

February to 136.5 mg/L $CaCO_3$ in June. Alkalinity ranged from 12.4 to 119.7 mg/L $CaCO_3$, February and August, respectively. Free and total acidity were 0 throughout the year. The pH values ranged from 7.0 to 8.0 with a mean of 7.6. Temperature ranged from 2°C in February to 25.7°C in July.

Numbers of *Caenis amica* larvae were graphed against water temperature for the entire collection period (Fig. 8). Generally, number of larvae increased with temperature with the highest peak in July at 1,035 larvae and a water temperature of 25.7°C.

Average water depths are shown in Table 2. These values ranged from 22.1 cm in November 1997 to 67.4 cm in April 1997. The yearly average water depth was 46.8 cm. Water level was highest in the spring and lowest in the fall. There was also a gradual takeover by the lily pads starting in April and lasting through October, when they began to die. In October the water depth was low and muddy.

Larval Exuviae

Small numbers (less than 10) of larval exuviae were found in the samples and noted on the vegetation by the shoreline. In Texas, the greatest exuvial capture of 166 in $10m^3$ was recorded in August and coincide with a time of maximal larval density in the drift of the Brazos River. Most of the *Caenis* exuviae in the drift in June and August after sunset may represent emergence (Cloud and Stewart, 1974).

Collection of Adults

Adults were collected from April 30, 1997 to September 11, 1997. A black light trap was used to collect emerging adults. All collections of *C. amica* adults were made in the first hour or so after dark (Table 3).

Figure. 8. Number of *Caenis amica* larvae versus water temperature (^{o}C)

bars = number of *Caenis amica*
lines = water temperature

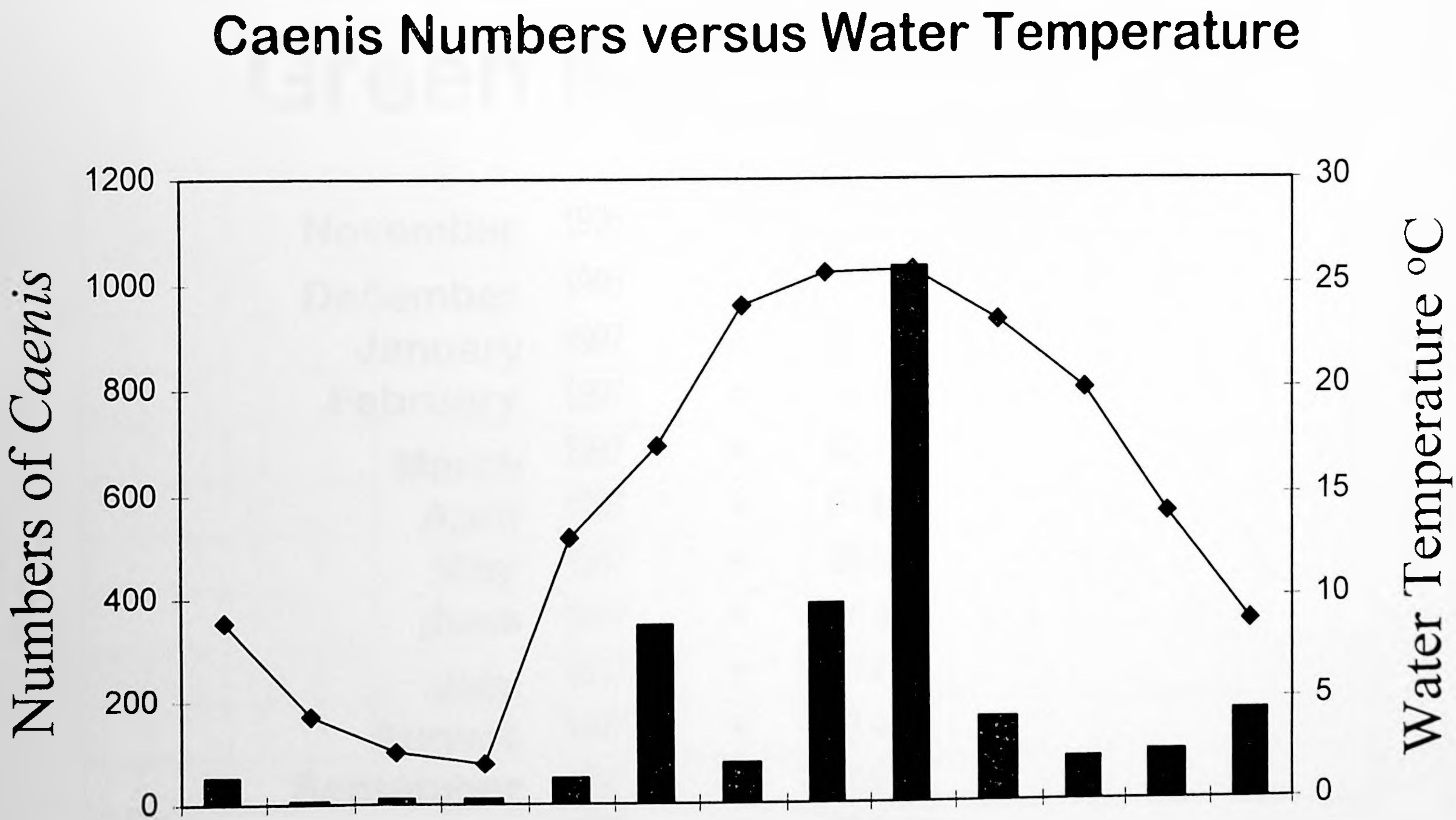

Caenis Numbers versus Water Temperature
Numbers of *Caenis*
1200
1000
800
600
400
200
0
Water Temperature °C
30
25
20
15
10
5
0

Table 2: Water Depths at Green Bottom Swamp

November	1996	=	no data
December	1996	=	no data
January	1997	=	46.2 cm
February	1997	=	no data
March	1997	=	62.4 cm
April	1997	=	67.4 cm
May	1997	=	60.3 cm
June	1997	=	no data
July	1997	=	53.2 cm
August	1997	=	43.4 cm
September	1997	=	37.6 cm
October	1997	=	28.5 cm
November	1997	=	22.1 cm

Laboratory Studies On Larvae

Voltinism

Length-frequency histograms for each collection date were arranged into 0.5 mm size classes to determine voltinism (Figs. 9-12). The smallest larva was 1.04 mm long and was found in June 1997. The largest larva was 6.8 mm long and was found in May 1997. The greatest numbers of large larvae were found in the spring and summer months of April, May, June, July, and August. Larvae were absent in the other months. The length frequency histograms indicated a bivoltine life cycle.

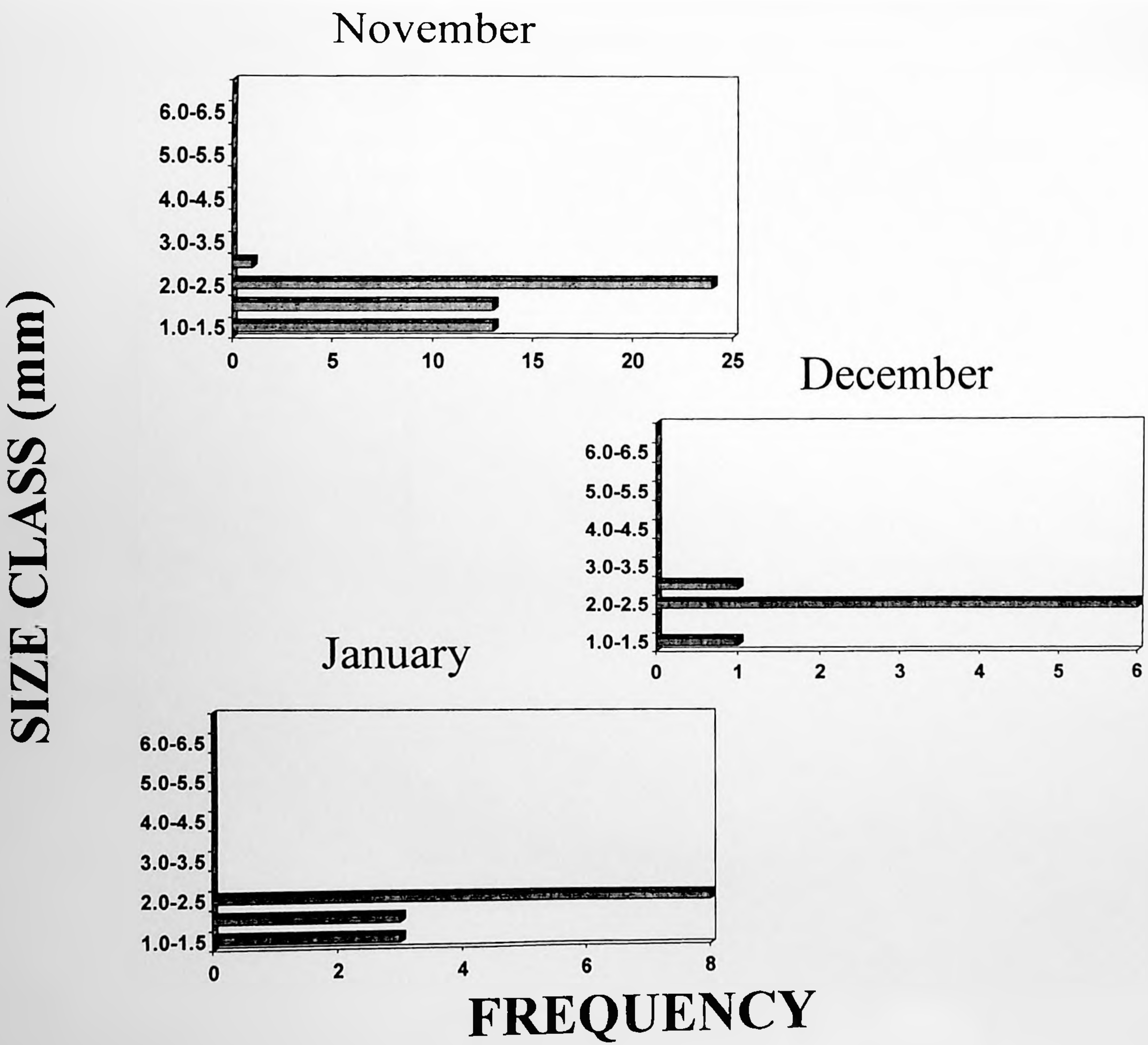
Corkum (1985) determined that *Caenis simulans* (=*C. amica*) had a bivoltine life cycle in an Alberta, Canada marsh. There was an extended emergence period, which occurred for three months and showed two peaks. In Neartic regions, *Caenis* populations are generally univoltine with overwintering larvae, or bivoltine with winter and summer generations (Clifford, 1982). Both univoltine and bivoltine populations have been reported for numerous caenid species including *Caenis horaria*, *C. moesta*, *C. rivulorum*, and *C. robusta* (Moon, 1939, Landa, 1968, Bradbeer and Savage, 1980). Clifford (1982) reported no consistent life cycle for *C. simulans*.

Edmunds et al. (1976) found that *C. amica* has two successive generations per summer. In Pennsylvania and Indiana, *C. amica* may be univoltine with multiple broods (Sweeney, 1976 and Provonsha, 1986). Rodgers (1982, 1983) also determined *C. amica* to be bivoltine when using biothermal channels in Alabama, the southern limit of the range of this species. The total length of larvae at emergence ranged from about 6.0 to 7.5 mm. Length of emergence was almost 100 days except in the +3 channels (above ambient temperature) where *Caenis* emerged for only about 80 days. *Caenis* overwintered as larvae whose range of total length is 1.0-5.75

Table 3. Emergence Data for Summer 1997

DATES	TOTAL MALES	TOTAL FEMALES	GRAND TOTAL
4/30/97	1358	60	1418
5/1/97	0	0	0
5/4/97	35	1	36
5/6/97	153	0	153
5/11/97	44	5	49
5/13/97	2	0	2
5/18/97	451	22	473
5/20/97	175	5	180
5/22/97	290	3	293
5/27/97	2	1	3
5/29/97	61	2	63
6/1/97	1821	12	1833
6/5/97	0	0	0
6/9/97	2	0	2
6/15/97	0	7	7
6/17/97	13	7	20
6/19/97	1	1	2
6/21/97	4	2	6
6/24/97	1	0	1
6/28/97	49	26	75
6/30/97	4	1	5
7/6/97	16	3	19
7/8/97	138	3	141
7/10/97	9	1	10
7/13/97	3	0	3
7/15/97	7	2	9
7/17/97	1	2	3
7/19/97	2	3	5
7/24/97	73	11	84
7/31/97	5	6	11
8/5/97	20	25	45
8/7/97	1	3	4
8/11/97	99	15	114
8/14/97	10	8	18
8/21/97	20	1	21
8/25/97	380	7	387
9/2/97	293	14	307
9/11/97	13	0	13
TOTALS	5556	259	5815

Figure. 9. Length frequency histograms of *C. amica* larvae for November, December, and January at Green Bottom Swamp (1997-1998)



November
December
January
6.0-6.5
5.0-5.5
4.0-4.5
3.0-3.5
2.0-2.5
1.0-1.5
0
5
10
15
20
25
1
2
3
4
6
8
SIZE CLASS (mm)
FREQUENCY

Figure. 10. Length frequency histograms of *C. amica* larvae for February, March, and April at Green Bottom Swamp (1997-1998)

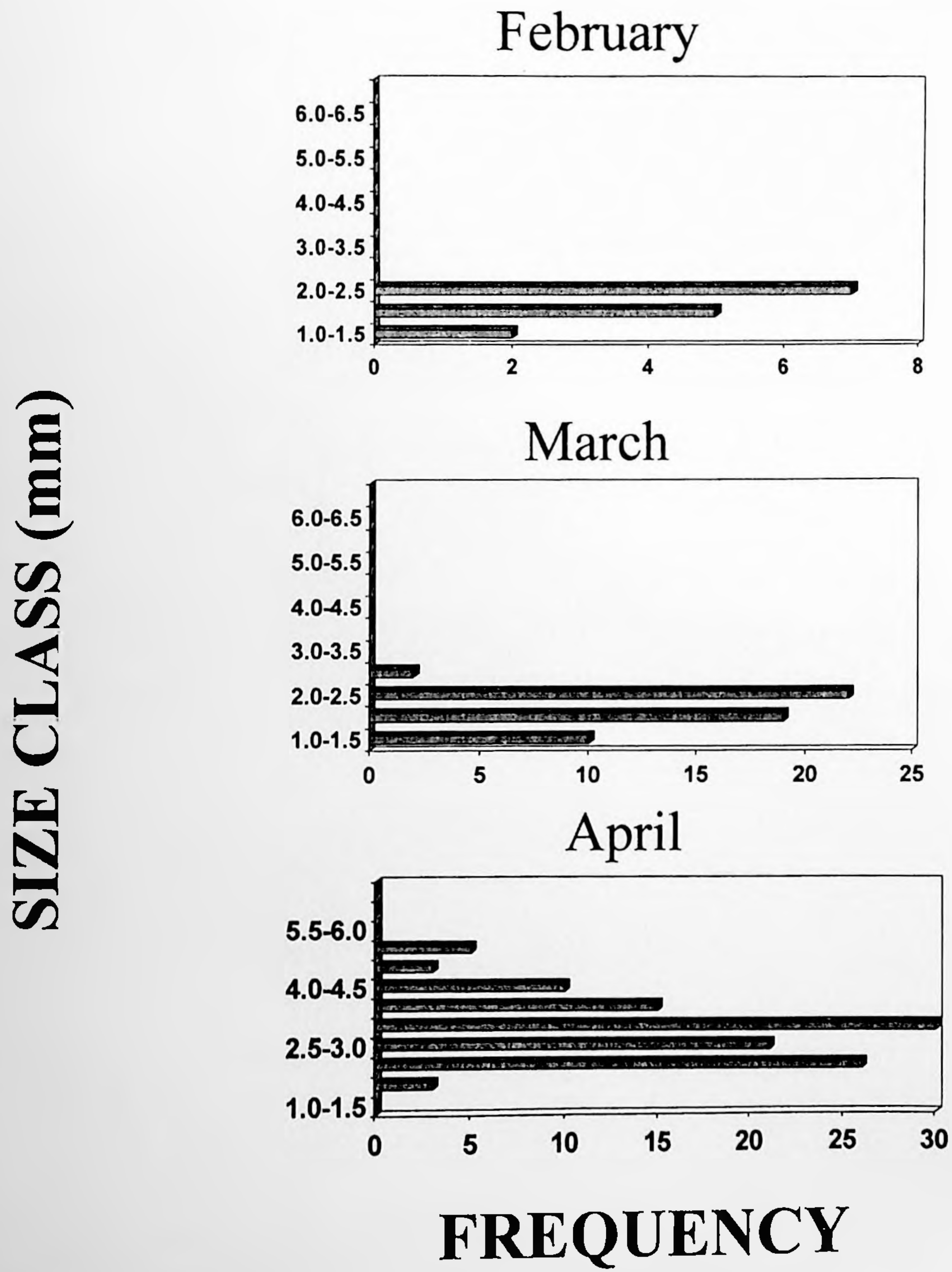

February
6.0-6.5
5.0-5.5
4.0-4.5
3.0-3.5
2.0-2.5
1.0-1.5
0
2
4
6
8
March
6.0-6.5
5.0-5.5
4.0-4.5
3.0-3.5
2.0-2.5
1.0-1.5
0
5
10
15
20
25
April
5.5-6.0
4.0-4.5
2.5-3.0
1.0-1.5
0
5
10
15
20
25
30
SIZE CLASS (mm)
FREQUENCY

Figure. 11. Length frequency histograms of *C. amica* larvae for May, June, and July at Green Bottom Swamp (1997-1998)

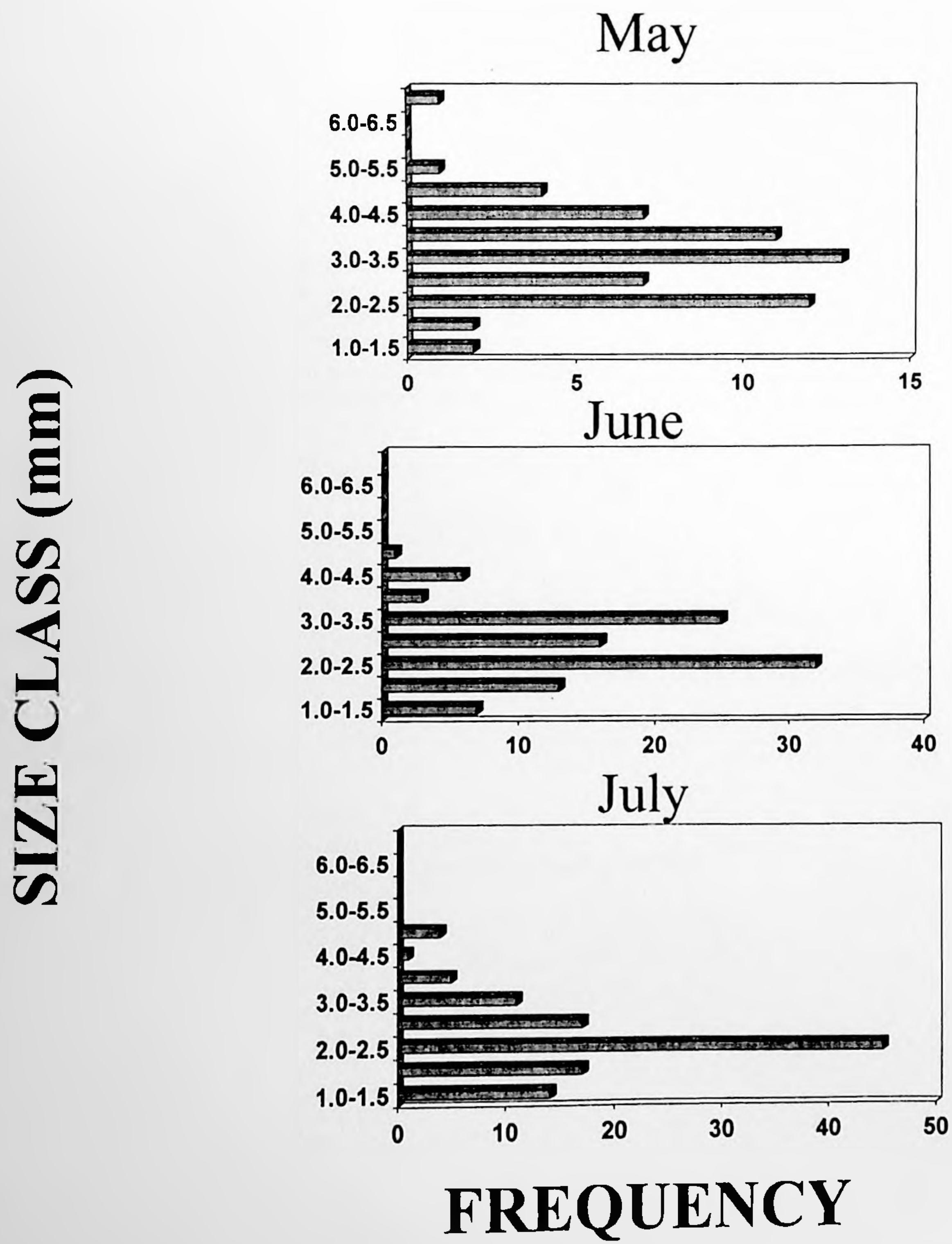
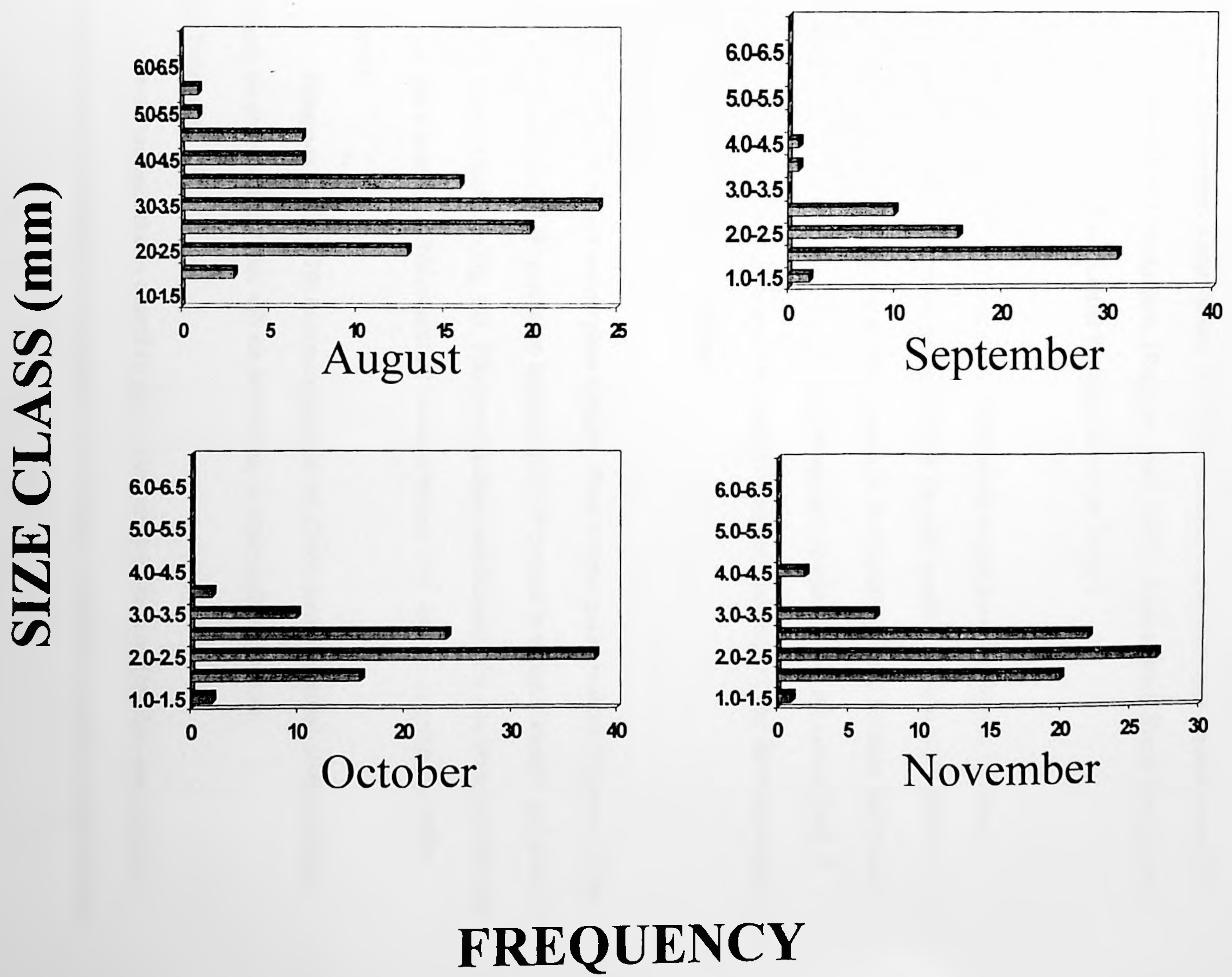

May
June
July
6.0-6.5
5.0-5.5
4.0-4.5
3.0-3.5
2.0-2.5
1.0-1.5
0
5
10
15
20
30
40
50
SIZE CLASS (mm)
FREQUENCY

Figure. 12. Length frequency histograms of *C. amica* larvae for August, September, October, and November at Green Bottom Swamp (1997-1998)


SIZE CLASS (mm)
6.0-6.5
5.0-5.5
4.0-4.5
3.0-3.5
2.0-2.5
1.0-1.5
0
5
10
15
20
25
August
0
10
20
30
40
September
October
November
FREQUENCY

mm, suggesting asynchronism before emergence. This was observed in the long emergence periods in the summer. Large larvae, 7.0 to 7.5 mm total length, were only abundant enough to be collected in +6 and +9 channels (Rodgers, 1982, 1983). A list of the different life cycles of *Caenis* spp. in North America and Europe is shown in Table 4.

Growth

Head width measurements of *C. amica* larvae ranged from 0.69 mm to 1.67 mm. Averages were used to determine percent growth for each month from November 1996 to November 1997 (Table 5). The greatest growth (1.75 percent) occurred between March and April, which would be expected, since the first emergence took place at the end of April. A second growth period (37.2 percent) occurred between July and August, and showed numerous smaller secondary peaks of emergence.

Foregut Analysis

Larvae consumed mainly plant detritus. Plant detritus made up about 71 percent of the food in summer (July), 90 percent in spring (April), 50 percent in winter (January), and about 69 percent in fall (October) (Fig. 13). Filamentous algae and diatoms were also present in the diet. Merritt and Cummins (1996) classified *Caenis simulans* (=*C. amica*) as gatherers and/or scrapers.

Edmunds et al. (1976) determined that larval *Caenis amica* are omnivorous, and feed mainly on plant material, but will eat dead larvae or other organic material.

pH Tolerance

Acid precipitation is caused by the addition of fossil fuels and has become a serious environmental problem in the northeastern United States. Mayflies are among the most sensitive

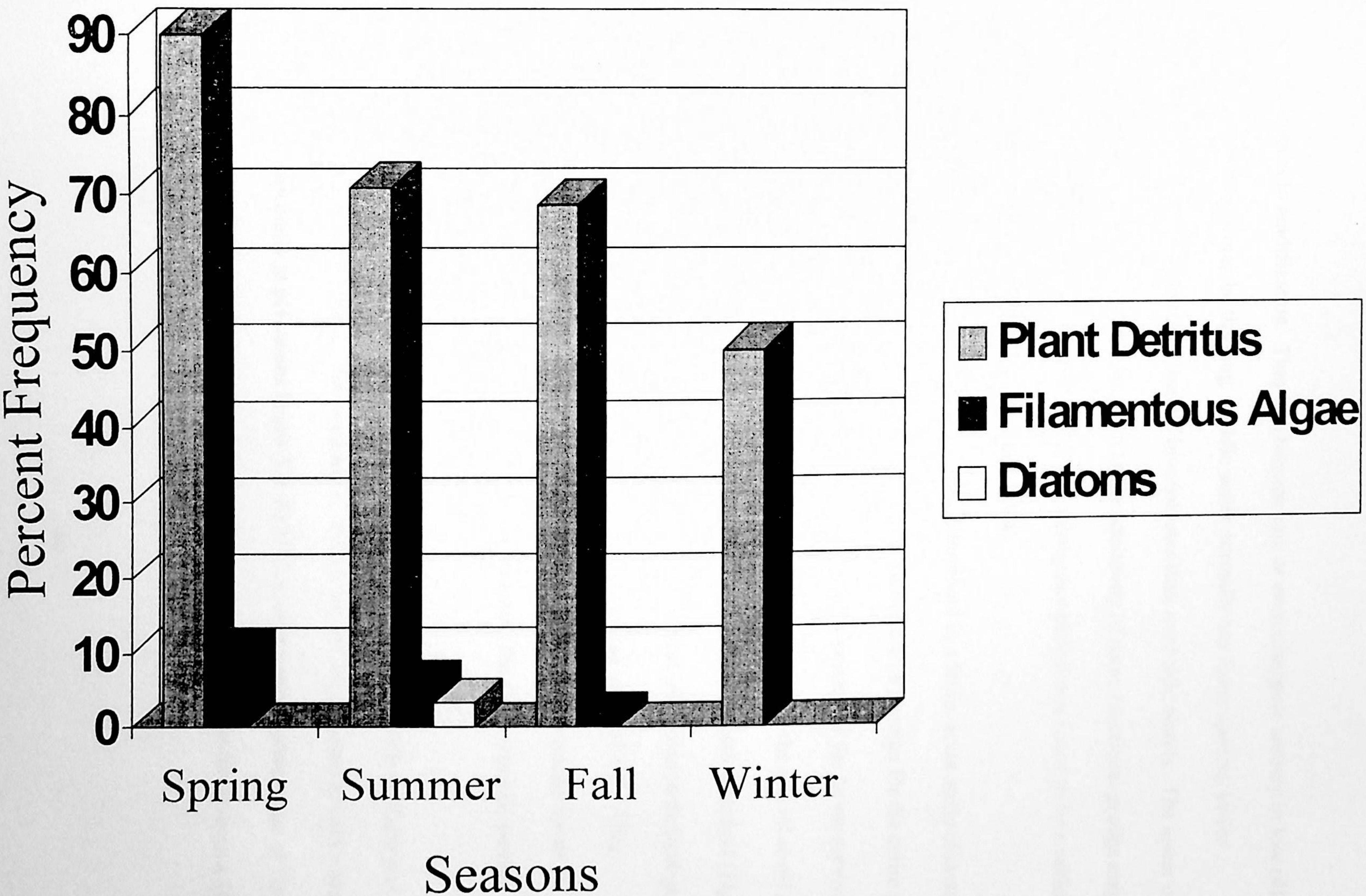
TABLE 4: DISTRIBUTION OF *C. amica* IN THE WORLD

Bivoltine *C. simulans*	marsh in Alberta, Canada
Univoltine *C. simulans*	w.Minnesota plains stream
Bivoltine *C. amica*	Tennesse River in Alabama
Bivoltine *C. amica*	VA Polytechnic Institute and State Univ. experimental pond facility
Univoltine *C. simulans*	Illinois
Unvoltine with multiple broods	Pennsylvania and Indiana
Bivoltine *C. amica*	northeastern and southeastern US
Univoltine *C. simulans*	Pennsylvania stream
Unvoltine *C. robusta*	England
Bivoltine *C. robusta, C. horaria, C. moesta*	central Europe
Asynchronous Multivoltinism *C . diminuta*	Savannah River, South Carolina
Bivoltine (seasonal fluctuations) Multivoltine (constant water temp) *Tricorythodes minutus*	Deep Creek Curlew Valley, Idaho-Utah

Table 5. Monthly Percent Growth for Larval *Caenis amica*

Date	Larval Average Lengths	Difference (mm)	Percent Growth
Nov-96	1.882941	0.320809/1.882941	17.04%
Dec-96	2.20375	0.2886607/2.20375	-13.01%
Jan-97	1.917143	0.031429/1.917143	-1.64%
Feb-97	1.885714	0.005795/1.885714	0.31%
Mar-97	1.891509	1.277341/1.891509	67.53%
Apr-97	3.16885	0.149116/3.16885	4.71%
May-97	3.317966	0.68473/3.317966	-20.63%
Jun-97	2.633203	0.233377/2.633203	-8.86%
Jul-97	2.399826	0.893543/2.399826	37.23%
Aug-97	3.293369	1.184352/3.293369	-35.96%
Sep-97	2.109017	0.0305874/2.109017	14.50%
Oct-97	2.414891	0.032106/2.414891	-1.33%
Nov-97	2.382785		

Figure. 13. Percent composition of food items from foregut analysis of *C. amica* larvae


Percent Frequency
90
80
70
60
50
40
30
20
10
0
Spring
Summer
Fall
Winter
Seasons
Plant Detritus
Filamentous Algae
Diatoms

aquatic insects to acidification. They can become rare or even disappear entirely in low pH, even though other insects may be thriving. Acidic water normally has fewer species, lower abundance, and lower biomass of benthic invertebrates than nonacidic waters. The lower overall high number of benthic invertebrates and a larger sensitivity of some functional groups may affect the metabolism of acidic ecosystems. The emergence period was found to be a critical time in relation to acidification (Weiderholm, 1984).

Low pH tolerance of *C. amica* larvae was determined in a 96 hr. acute static bioassay. Results of the pH study are shown in Table 6. The pH values are an average for the entire study. In pH 1.50 and 3.15, all larvae were dead after 24 hours. Sixty percent of the larvae survived after 96 hours in pH 4.59 and 6.38. All larvae survived after 96 hours in the control at pH 8.29. A straight line graphical interpolation method (Fig. 14) and a linear regression method (Fig. 15) were used to calculate the final values. The straight line graphical interpolation method gave a final value of 4.3, while the linear regression method gave a final value of 5.2. After determining the LR value of 5.2, 10 additional larvae were put into two separate bowls to determine if this calculated TL_{50} was accurate. After 96 hours, the result was a 60 percent survival rate.

In Florida, annual average pH values less than 4.7 are common in the northern part of the state. Results showed that *C. diminuta* and *C. hilaris* are not very tolerant to cold water temperature, especially at pH values below 5.0. At pH 3.5, over a temperature range of 10-30^{o} C, *Caenis diminuta* and *C. hilaris* mortality was 100 percent for embryos and larvae over a 10-day exposure period (Punzo and Thompson, 1990).

Table 6. Experimental data from acute static bioassay involving low pH tolerance

pH	24 hours	48 hours	72 hours	96 hours
1.5	0%	0%	0%	0%
3.15	0%	0%	0%	0%
4.59	100%	80%	80%	60%
6.38	100%	80%	80%	60%
8.29	100%	100%	100%	100%

Figure. 14. Straight Line Graphical Interpolation Method for low pH tolerance of *C. amica* larvae

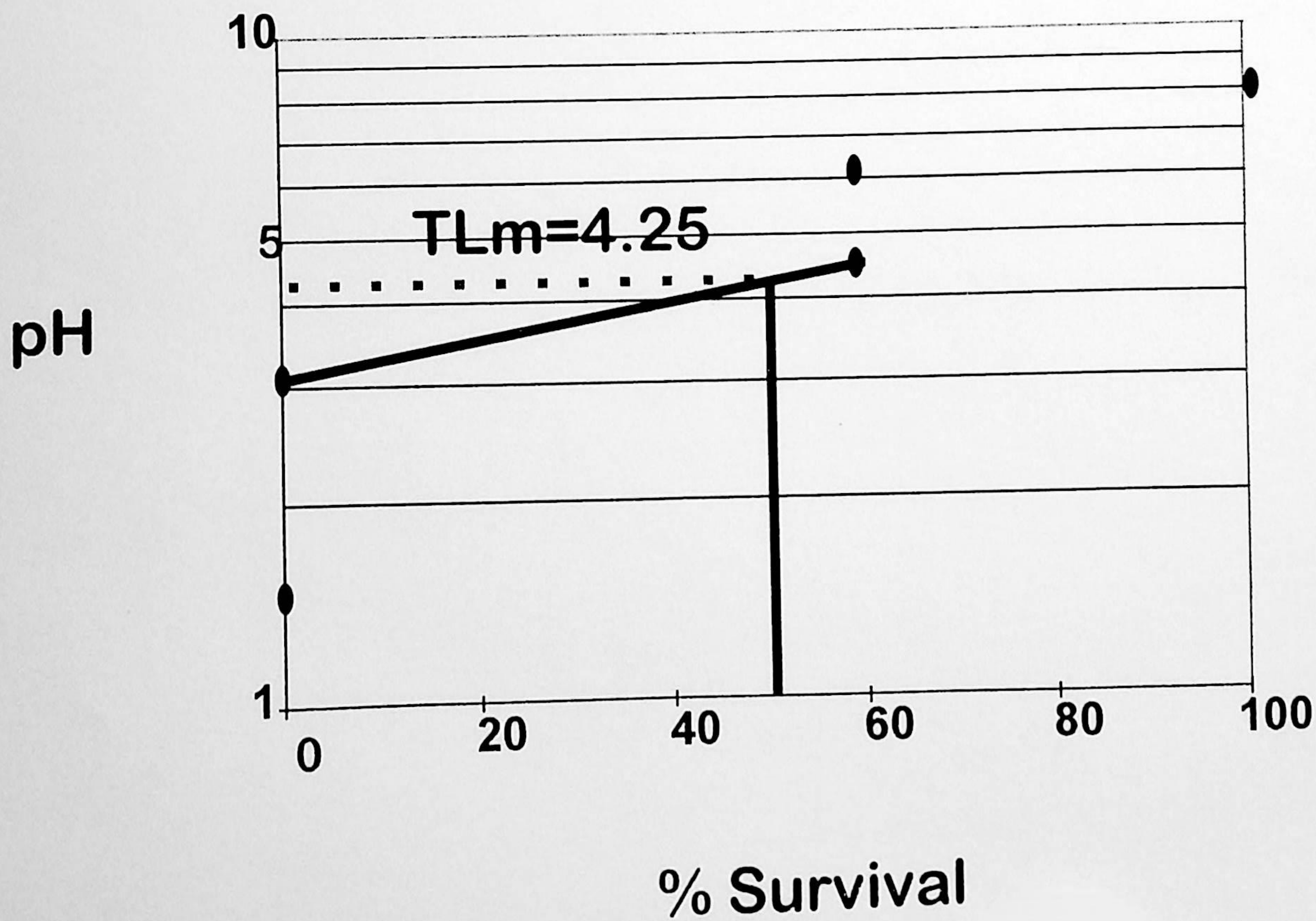
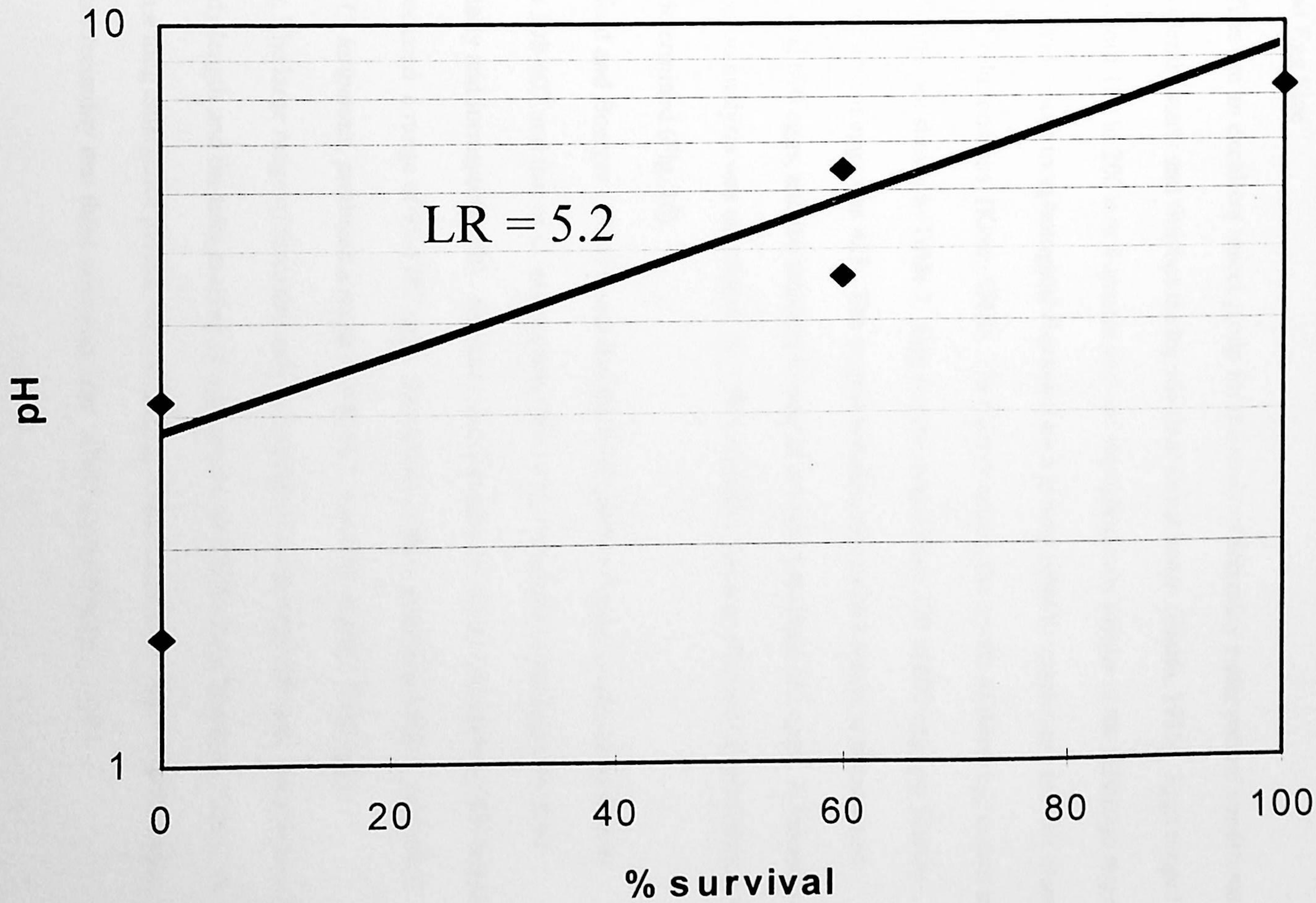

pH
10
5
1
TLm=4.25
0
20
40
60
80
100
% Survival

Figure. 15. Linear Regression Analysis for low pH tolerance of *C. amica* larvae



10
1
pH
LR = 5.2
0
20
40
60
80
100
% survival

Laboratory Studies On Adults

Fecundity and Egg Size

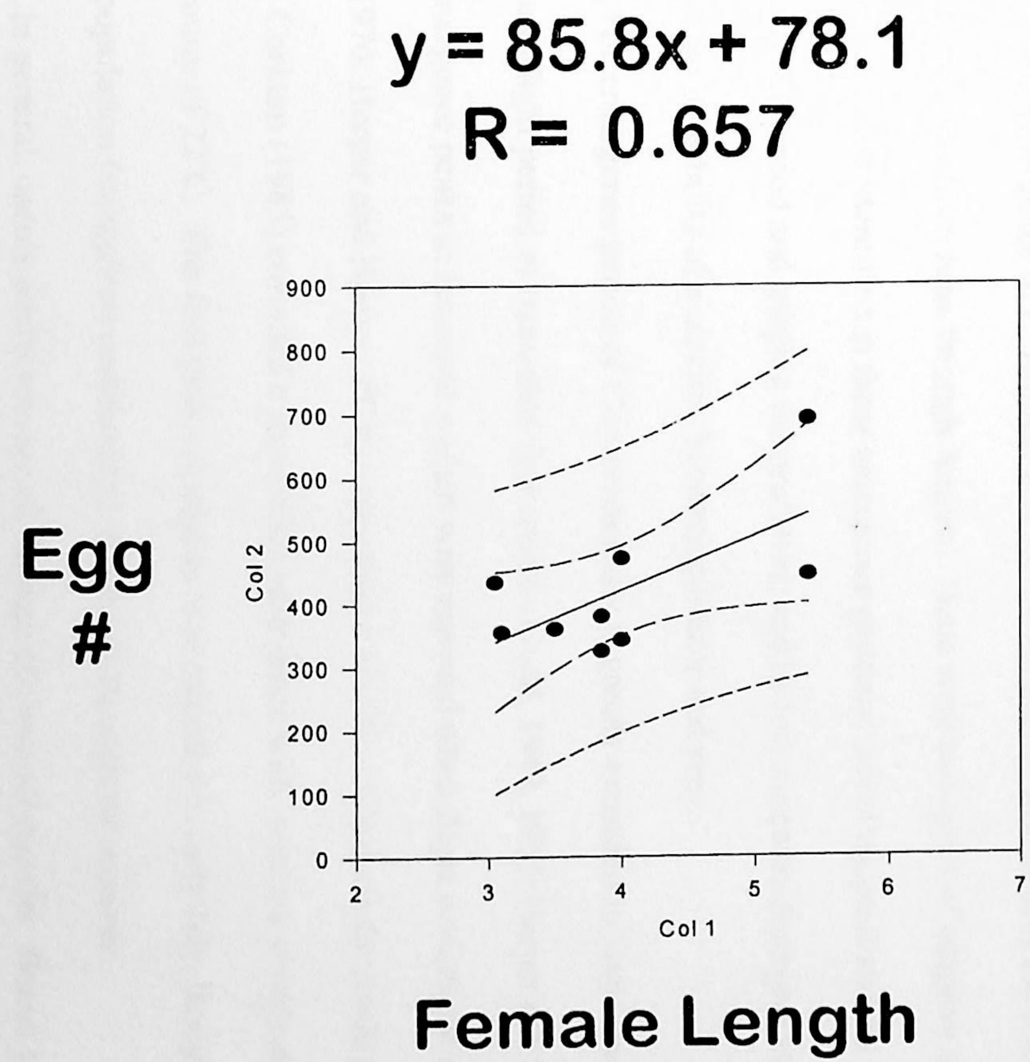
Mayflies are an excellent insect group for the study of fecundity under natural conditions. Ovulation evidently starts and finishes during the final larval instar (Smith, 1935). Eggs ranged in size from about 100 to 200 u in diameter and are physiologically mature at the subimago stage. They often provide more morphological characteristics to help identify species and provide clues to phylogenetic relationships (Koss, 1968). In *Caenis amica*, the results of direct egg counts of nine adult females are shown in Table 7. Egg counts ranged from 325 to 690 eggs per female; the average number of eggs was 423. The largest females, measured 5.4 mm, and contained between 446 and 690 eggs, and the smallest female of 3.1 mm contained 355 eggs. A fecundity-length regression analysis was calculated ($y = 78.1 + 85.8x$) and a coefficient of correlation ($r = 0.657$) was determined (Fig. 16).

Clifford and Boerger (1974) found that the total number of eggs produced by *Caenis horaria* was 508-607 and that of *C. moesta* was 765-1103. Fecundity changes greatly both interspecifically and intraspecifically. Sweeney and Vannote (1978) and Rodgers (1983) found *C. amica* produced a range of 95-1787 eggs. Provonsha (1986) determined that *C. anceps*, *C. hilaris*, and *C. latipennis*, produced a range of 448-567, 414-806, and 812-2163 eggs, respectively. The large range of fecundity may be explained by the fact that there is a relationship between body length and the total number of eggs produced (Clifford and Boerger, 1974). In species with a long emergence period, early emerging insects seem to be larger and therefore have a greater fecundity that those emerging later (Clifford and Boerger, 1974).

Table 7. Female length and number of eggs of *Caenis amica*

Length of female	Number of Eggs
3.05mm	435
4.00mm	472
3.85mm	380
4.00mm	342
5.40mm	690
5.40mm	446
3.10mm	355
2.50mm	360
3.85mm	325
Average length of female = 4.2mm	Average number of Eggs = 423

Figure. 16. Fecundity length regression analysis of *C. amica* adult female length and egg number


y = 85.8x + 78.1
R = 0.657
Egg #
900
800
700
600
500
400
300
200
100
0
Col 2
2
3
4
5
6
7
Col 1
Female Length

Emergence Period

Adults emerged from April 30, 1997 to September 11, 1997. There were 5,556 males and 259 females, for a total of 5,815 adults (Fig. 17). The largest emergence peak (1,833 adults) recorded for *C. amica* in this study was on June 1, with an air temperature of 20°C and a water temperature of 22°C (Fig. 18). Another large emergence peak (1,418 adults) occurred on April 30.

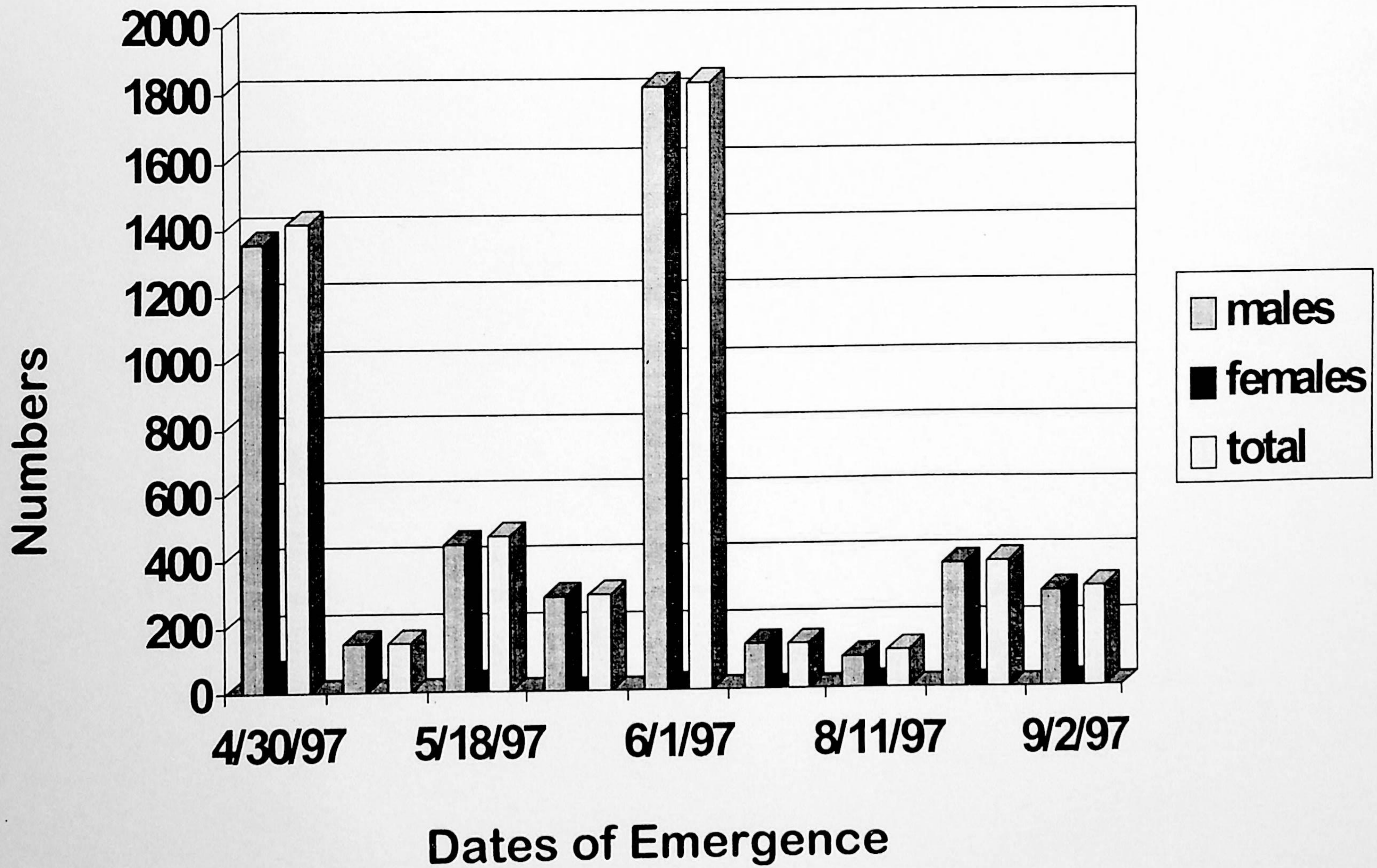
In England, Mackey (1978) found *Caenis macrura* and *C. horaria* had two emergence peaks, but the emergence of *C. robusta* was somewhat evenly spread over the whole emergence period, which was from June through August. There were two types of adaptive strategies for avoiding predation identified in these emergence patterns: avoiding predation by synchronizing the emergence period and gorging the predators, and by low numbers emerging at any one time to decrease the possibility of a meeting between predator and prey.

The emergence period of *Caenis simulans* is greatly extended in lentic areas, with a maximum flight period of more than four months (Judd, 1949, 1953; Harper and Harper, 1976). Two emergence peaks in June and August were reported when flight periods are extended (Hall et al., 1970; Harper and Harper, 1976) suggesting a bivoltine life cycle for lentic populations.

Corkum (1985) collected a mass adult emergence with overcast, cloudy skies and a water temperature of 22°C. The first peak emergence was recorded in early July, though some of the larval population (stragglers) continued to mature all through the summer.

In general, caenis adults emerge all through the warmer months. Berner (1977) noted adult collections from March to November in the southeast. Emergence time is more confined in the northern latitudes. Adult emergence occurs from dusk until about 1 hour after dark.

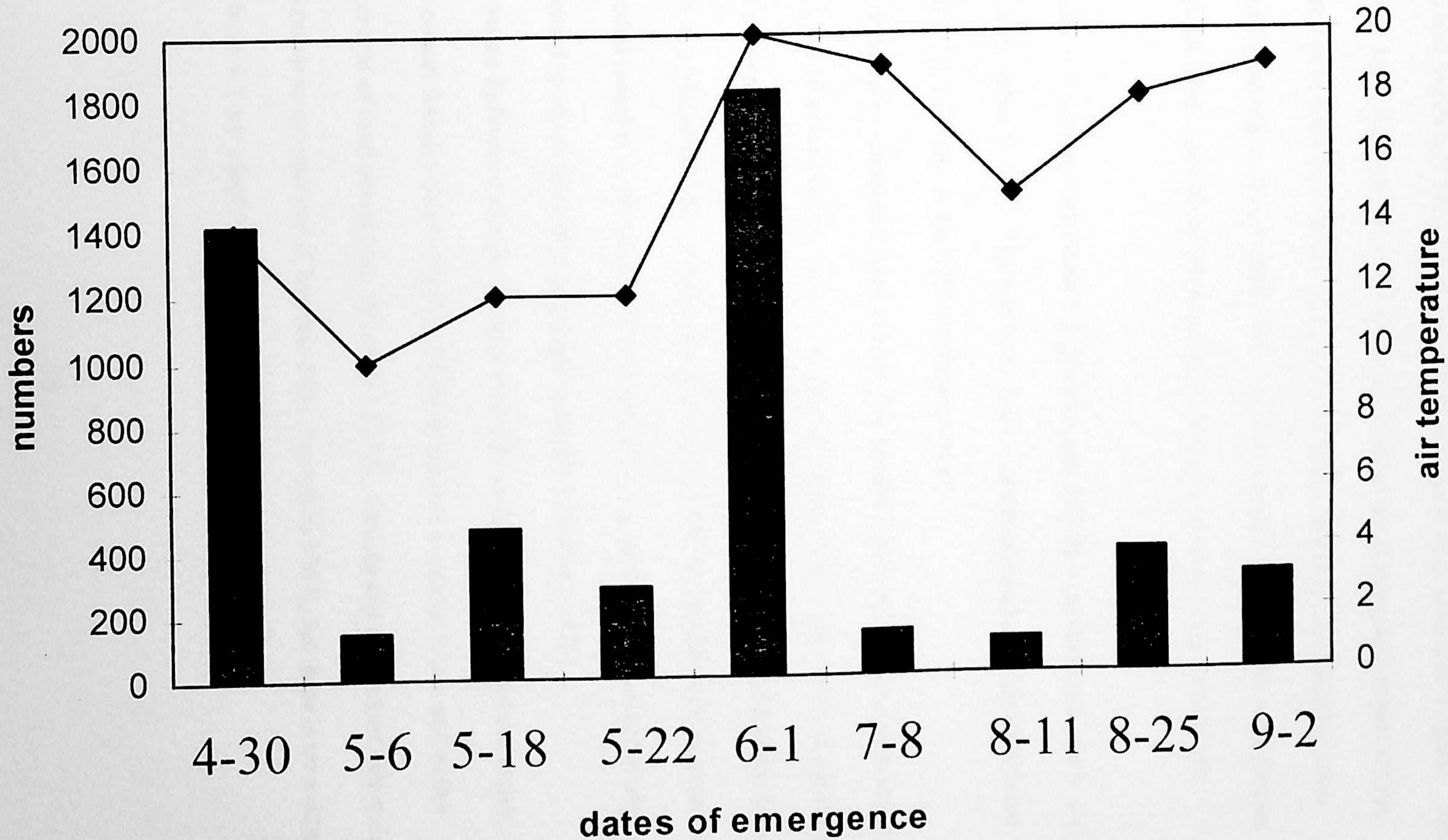
Figure. 17. Emergence data showing peaks of emergence from April 30, 1997 to September 2, 1997 for *C. amica* adults



Numbers
2000
1800
1600
1400
1200
1000
800
600
400
200
0
4/30/97
5/18/97
6/1/97
8/11/97
9/2/97
Dates of Emergence
males
females
total

Figure. 18. Emergence data versus air temperature (°C) for *C. amica* adults

bars = number of *C. amica*
lines = air temperature


numbers
2000
1800
1600
1400
1200
1000
800
600
400
200
0
air temperature
20
18
16
14
12
10
8
6
4
2
0
4-30
5-6
5-18
5-22
6-1
7-8
8-11
8-25
9-2
dates of emergence

Christman and Voshell (1992) found a bivoltine life cycle of *C. amica* in Virginia. These mayflies emerged in June, July and August. There was a slow developing winter generation, which overwintered in several size classes and peaked in June. This was followed by a fast developing summer generation , which peaked in July and August. Early instars were present from June through October, indicating an extended period of emergence and recruitment.

Sex Ratio

A chi-square test was performed on 5,556 males and 259 females collected yearly, from May 30, 1997 to September 9, 1997. The test on *C. amica* adults showed a highly significant difference from the 1:1 sex ratio at the 0.05 confidence level.

Emergence samples contained large numbers of insects and were collected in a way that avoided the influence of sexual differences in emergence timing, sometimes showed significantly more females or more males. Flannagan and Lawler (1972) found a majority of females for most caddisfly species in a Manitoba lake, whereas males of the mayfly *Stenonema interpunctatum canadense* exceeded females by about 3:1. A 2:1 ratio favoring males of mature larval and adult *Tipula sacra* seemed to result from higher female mortality (Pritchard 1976).
Collections of mature *Ephemera danica* nymphs normally included 60 to 65 percent males, although in one cohort females showed better ability to survive a sudden freeze and males dropped to 45 percent of total emergence (Svensson 1977). Genetically determined sex-ratio distortions are known in mosquitoes (Clements 1963; Hamilton 1967), but there is no evidence that deviations from 1:1 are adaptive for aquatic insects (Williams, 1979).

CHAPTER VII

CONCLUSIONS AND SUMMARY

An ecological life history on *Caenis amica* was investigated at Green Bottom Wildlife Management Area from November 1996 to November 1997. Most larvae were found in the spring and summer months of April, June, and July. The highest peak occurred in July with 1,035 larvae. Larvae were more commonly found among mud and vegetation in the three sample sites. Water temperature ranged from 2.0 to 25.7^{0}C, February and July, respectively. Number of larvae generally increased with water temperature. The pH ranged from 7.0 to 8.0 with a mean of 7.6. Water depth values ranged from 22.1 cm in November 1997 to 67.4 cm in April 1997.

Length-frequency histograms indicated a bivoltine life cycle. The largest larva for *C. amica* was 6.8 mm long and was found in May 1997. The smallest larva was 1.04 mm long and was found in June 1997. The greatest amount of larval growth (67.5%) occurred between March and April. The greatest number of large larvae were found in the spring and summer months of April, May, June, July, and August, followed by their absence in the other months. Head width measurements ranged from 0.69 to 1.67 mm.

Larvae consumed primarily plant detritus, which made up at least 50 percent of the diet year round. Plant detritus comprised the highest percentage (90%) in spring (April). Filamentous algae and diatoms were also present in small amounts.

Low pH tolerance of *C. amica* was determined in a 96 hr. acute static bioassay. Results gave a SLGIM value of 4.3 and a LR value of 5.2. After a second 96 hours in the pH of 4.3, the result was a 60 percent survival rate. Egg counts ranged from 325 to 690 eggs per individual female; the average number of eggs per female was 423. The largest females, measuring 5.4 mm, contained between 446 and 690 eggs, and the smallest female of 3.1 mm contained 355 eggs. A fecundity-length regression was used to compare the number of eggs and length of the female. The fecundity-length regression equation was $y = 78.1 + 85.8x$ ($r = 0.657$). Adults emerged from April 30, 1997 to September 11, 1997. There were 5,556 males and 259 females, for a total of 5,815 adults. There were two emergence peaks, in April (1,418 adults) and June (1,833 adults), respectively. A chi-square test showed a highly significant difference from the 1:1 sex ratio at the 0.05 confidence level.

LITERATURE CITED

Banks, N. 1907. A new mayfly of the genus *Caenis*. Ent. News 18:13-14.

Berner, L. 1977. Distributional patterns of southeastern mayflies (Ephemeroptera). Bull. Florida St. Mus., Biol. Sci. 22:1-56.

--------- and M.L. Pescador. 1950. The mayflies of Florida. Univ. Florida Press, Gainesville, 267pp.

Bradbeer, P.A. and A.A. Savage. 1980. Some observations on the distribution and life history of *Caenis robusta* Eaton (Ephemeroptera) in Cheschire and North Shropshire, England. Hydrobiologia 68(1):87-90.

Christman, V.D. and J.R. Voshell, Jr. 1992. Life history, growth, and production of Ephemeroptera in experimental ponds. Ann. Entomol. Soc. Am. 85(6):705-712.

Clements, A.N. 1963. The physiology of mosquitoes. Pergamon Press, Oxford. 393pp.

Clifford, H.F. 1982. Life cycles of mayflies (Ephemeroptera), with special reference to voltinism. Quaest. Entomol. 18: 15-90.

--------------, and H. Boerger. 1974. Fecundity of mayflies (Ephemeroptera), with special reference to mayflies of a brown-water stream in Alberta, Canada. Can. Entomol. 106: 1111-1119.

Cloud, T.J., Jr. and K.W. Stewart. 1974. The drift of mayflies in the Brazos River, Texas. J. Kans. Ent. Soc. 47(3): 379-395.

Corkum, L.D. 1984. Movements of marsh-dwelling invertebrates. Freshw. Bio. 14:89-94.

-----------. 1985. Life cycle patterns of *Caenis simulans* McDunnough (Caenidae: Ephemeroptera) in an Alberta, Canada, marsh. Aqu. Ins. 7(2):87-95.

-----------. 1989. Habitat characterization of the morphologically similar mayfly larvae, *Caenis* and *Tricorythodes* (Ephemeroptera). Hydrobiologia 179:103-109.

Eaton, A.E. 1883-1887. A revisional monograph of recent Ephemeridae or mayflies. Trans. Linn. Soc. London, Sec. Ser. Zool. 3:1-352.

Edmunds, G.F., Jr., S.L. Jensen, and L.Berner. 1976. The mayflies of North and Central America. Univ. of Minn. Press Minn. 330pp.

Emery, E.B. 1994. Seasonal variation in the diversity of the macrobenthos in the Green Bottom Wildlife Management Area, Cabell County, West Virginia, in relationship to the water quality and vegetation types. 89pp.

Faulkner, G.M. and D.C. Tarter. 1977. Mayflies, or Ephemeroptera, of West Virginia with emphasis on the nymphal stage. Ent. News. 88(7&8):202-206.

Fjellheim, A. and G.G. Raddum. 1992. Recovery of acid-sensitive species of Ephemeroptera, Plecoptera and Trichoptera in River Audna after liming. Env. Poll. 78:173-178.

Flannagan, J.F. and G.H. Lawler. 1972. Emergence of caddisflies (Trichoptera) and mayflies (Ephemeroptera) from Heming Lake, Manitoba. Can. Ent. 104: 173-183.

Gerking, S.D. 1957. A method for sampling the littoral macrofauna and its application. Ecology 38(2):219-225.

Green Bottom Wildlife Management Area Management Plan, 1991. Wildlife Resources Section, West Virginia Division of Natural Resources. 45pp.

Hagen, H. 1861. Ephemeroptera. In: Synopsis of the Neuroptera of North America, with a list of South American species. Smith. Misc. Coll. 38-55.

Hall, D.J., W.E. Cooper,and E.E. Werner. 1970. An experimental approach to the production dynamics and structure of freshwater animal communities. Limnol. Oceanogr. 15:839-928.

Hamilton, W.D. 1967. Extraordinary sex ratios. Science. 156:477-488.

Hamilton, H. 1979. Food habits of ephemeropterans from three Alberta, Canada streams. MSc thesis, Dept. of Zoology, Univ. of Alberta, Edmonton. 207p.

Harper, F. and P.P. Harper. 1976. Inventaire et phenologie des Ephemeropteres du lac Saint-Louis, Quebec. Ann. Soc. ent. Quebec. 21:136-143.

---------- and -----------. 1981. Northern Canadian mayflies (Insecta: Ephemeroptera), records and descriptions. Can J. Zoo. 59(9):1784-1789.

Hubbard, M.D. and W.L. Peters. 1978. Environmental requirements and pollution tolerance of Ephemeroptera. United States Environmental Protection Agency. EPA-600/4-78-061

Johnson, B.R. 1995. Ecological life history of *Callibaetis fluctuans* (Walsh) (Ephemeroptera: Baetidae) from the Green Bottom Wildlife Management Area, Cabell County, West Virginia. 57pp.

Judd, W.W. 1949. Insects collected in the Dundas Marsh, Hamilton, Ontario, 1946-47, with observations on their periods of emergence. Can. Ent. 81(1):1-10.

---------. 1953. A study of the population of insects emerging as adults from the Dundas Marsh, Hamilton, Ontario, during 1948. Amer. Mid. Nat. 49(3):801-824.

Kondratieff, B.C. and J.W.S. Foster III. 1977. Some mayflies (Ephemeroptera) of middle and east Tennessee. J. Tenn. Acad. Sci. 52(3):112.

Koss, R.W. 1968. Morphology and taxonomic use of Ephemeroptera eggs. Ann. Entomol. Soc. Am. 61:696-721.

---------. 1969. Ephemeroptera eggs and their contributions to phlogenetic studies of the order. Ph.D. Thesis. Univ. Utah, Salt Lake City. 240pp.

Landa, V. 1968. Developmental cycles of central European Ephemeroptera and their interrelations. Acta. Ent. Bohemoslov. 65:276-284.

MacFarlane, M.B. and T.F. Waters. 1982. Annual production by caddisflies and mayflies in a western Minnesota plains stream. Can. J. Fish. Aquat. Sci. 39:1628-1635.

Mackey, A.P. 1978. Emergence patterns of three species of *Caenis* Stephens (Ephemeroptera: Caenidae). Hydrobiologia 58(3): 277-280.

McCafferty, W.P. and A.V. Provonsha. 1978. The Ephemeroptera of mountainous Arkansas. J. Kan. Entomol. Soc. 51:360-379.

McDunnough, J. 1931. New North American Caeninae with notes (Ephemeroptera). Can. Entomol. 63:254-268.

McGinn, K., T. Hayes, T. Jones, J. Wirts, D. Tarter. 1992. Ichthyofauna of the Green Bottom Wildlife Area, Cabell County, West Virginia, with preliminary observations on the spawning activities of the bowfin, *Amia calva* Linnaeus. Proc. W. Va. Acad. Sci. 64(1):45.

Merritt, R.W. and K.W. Cummins. 1996. An introduction to the aquatic insects of North America. Kendall/Hunt Publishing Co., Dubuque, IA. 441pp.

Moon, H.P. 1939. The growth of *Caenis horaria* (L.), *Leptophlebia vespertina* (L.), and *L. marginata* (L.)(Ephemeroptera). Proc. Zool. Soc. London, A, 108:507-512.

Mullins, L.A. 1994. Behavioral and physiological ecology of dragonflies (Odonata: Anisoptera) at the Green Bottom Wildlife Management Area, Cabell County, West Virginia, with special reference to *Erythemis simplicicollis* and *Pachydiplax longipennis*. 81pp.

Needham, J.G., J.R. Traver, and Yin-Chi Hsu. 1935. The biology of mayflies with a systematic account of North American species. Comstock, Ithaca, N.Y. 759pp.

Novotny, J.F. 1985. Effects of a Kentucky flood-control reservoir on macroinvertebrates in the tailwater. Hydrobiologia 126: 143-153.

Pinder, A.M., K.M. Trayler, J.W. Mercer, J. Arena, and J.A. Davis. 1993. Diel periodicities of adult emergence of some chironomids (Diptera: Chironomidae) and a mayfly (Ephemeroptera: Caenidae) at a western Australian wetland. J. Aust. Ent. Soc. 32:129-135.

Poff, N.L. and R.A. Matthews. 1985. The replacement of *Stenonema* spp. by *Caenis diminuta* Walker as the numerical dominant in the mayfly assemblage of a thermally-stressed stream. J. Freshw. Ecol. 3(1):19-26.

Pritchard, G. 1976. Growth and development of larvae and adults of *Tipula sacra* Alexander (Insecta: Diptera) in a series of abandoned beaver ponds. Can. J. Zool. 54:266-284.

Provonsha, A.V. 1986. A revision of the genus *Caenis* in North America (Ephemeroptera: Caenidae). Trans. Amer. Ent. Soc. 116(4):801-804.

--------------- and W.P. McCafferty. 1985. *Amercaenis*: new Neartic genus of Caenidae (Ephemeroptera). Int. Quart. Entomol. 1:1-7.

Punzo, F. and D. Thompson. 1990. Effects of temperature and acid stress on hatching, survival, capacity, metabolism and postural reflexes in caenid mayflies (Ephemeroptera). Comp. Biochem. Physiol. 95A(1):69-72.

Resh, V.H. and D.M. Rosenberg. Editors. 1984. The ecology of aquatic insects. Praeger Publishers. New York, NY. 625pp.

Rodgers, E.B. 1982. Production of *Caenis* (Ephemeroptera: Caenidae) in elevated water temperatures. Freshw. Invert. Biol. 1(2):2-16.

------------. 1983. Fecundity of *Caenis* (Ephemeroptera: Caenidae) in elevated water temperatures. J. Freshw. Ecol. 2(3):213-218.

Say, T. 1839. The complete writings of Thomas Say on the entomology of North America, I and II. A.E. Foote, Philadelphia.

Smith, O.R. 1935. Part I, Chap. VII, eggs and oviposition. p.67-89. In: J.G. Needham et al. The biology of mayflies. Comstock, Ithaca, N.Y.

Stephens, J.F. 1835. Illustrations of British entomology, or a synopsis of indigenous insects: containing their generic and specific distinctions. Baldwin Cradock, London.

Stark, T.J. 1993. Flora and vegetation of Green Bottom Wildlife Management Area, W. Va., unpublished master's thesis, Marshall University, Huntington, W.Va.

Svensson, B. 1977. Life cycle, energy fluctuations, and sexual differentiation in *Ephemera danica* (Ephemeroptera), a stream-living mayfly. Oikos. 29:78-86.

Sweeney, B.W. 1976. The response of aquatic insects to thermal variation. Ph.D. Thesis, Univ. Pennsylvania, Philadelphia. 203pp.

------------, and R.L. Vannote. 1978. Size variation and the distribution of hemimetabolous aquatic insects: two thermal equilibrium hypotheses. Science, 200:444-446.

Thew, T.B. 1960. Revision of the genera of the family Caenidae (Ephemeroptera). Trans. Amer. Entomol. Soc. 136:187-205.

Traver, J.R. 1935. Part II, Systematic. pp.239-739. In: J.G. Needham et al. The biology of mayflies. Comstock, Ithaca, N.Y.

Walker, F. 1853. List of neuropterous insects in the British Museum. III. Termites and Ephemeridae. British Museum (Natural History), London, pp.535-585.

Wang, T.Q., W.P. McCafferty, and Y.J. Bae. 1997. Sister relationship of the Neoephemeridae and Caenidae (Ephemeroptera: Pannota). Ent. News. 108(1):52-56.

Ward, J.V. 1992. Aquatic insect biology 1. biology and habitat. John Wiley and Sons, Inc. New York, NY. 438pp.

Weiderholm, T. 1984. Responses of aquatic insects to environmental pollution, p530-535. In V.H. Resh and D.M. Rosenberg (eds), The Ecology of Aquatic Insects, Praeger Publishers, New York.

Wilhelm, E. 1997. A taxonomic study of the class Insecta in the Green Bottom Wildlife Management Area with notes on the life history of *Belostoma lutarium* (Stal), and *Hesperocorixa lucida* (Abbott), Cabell County, West Virginia. 59pp.

Williams, G.C. 1979. The question of adaptive sex ratio in outcrossed vertebrates. Proc. Royal Soc. London. B. 205:567-580.