

ECOLOGICAL LIFE HISTORY, INCLUDING
LABORATORY RESPIRATORY INVESTIGATION,
OF THE MAYFLY, *AMELETUS TARTERI*
(EPHEMEROPTERA: SIPHLONEURIDAE)*

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INTRODUCTION

Mayflies, such as *Ameletus*, are considered by many to be largely influenced by their environment. This study not only provides information concerning the basic life history of the species in question, but also elucidates the role that environment can play in this life history.

The objectives of this study were: (1) to describe the life history of *Ameletus tarteri*, a newly described species, (2) to investigate the role environment plays in this life history, and (3) to add to the general knowledge concerning mayflies.

TAXONOMY AND DISTRIBUTION

The genus *Ameletus* was first described by Eaton in 1885. The type species was *Ameletus subnotatus*; the type locality was Colorado (Edmunds et al., 1976). There are currently thirty-three nominal species of *Ameletus* from North America. Members of the genus *Ameletus* are found throughout the Holarctic region. According to Edmunds et al. (1976), in the Nearctic region they are most abundant "in the north, extending along the mountains south to California, New Mexico, Illinois, and Georgia."

Ameletus tarteri Burrows was first described in 1987. The holotype was found in Greenbrier County, West Virginia, at Hamrick Run near its confluence with the North Fork of Cherry River. *Ameletus tarteri* was collected by Burrows in Greenbrier County of West Virginia. This species was also identified from Chemung County, New York, and Giles County, Virginia (Burrows, 1987).

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DESCRIPTION OF STUDY AREA

This study was conducted at Carpenter Run, a tributary of the North Fork of Cherry River, Greenbrier County, West Virginia. Carpenter Run originates at an elevation of 3750 feet (1125 m) and flows generally to the southwest to join the North Fork of Cherry River at an elevation of 3200 feet (960 m) for a total fall of 550 feet (165 m). The stream transverses 1.18 miles (1.89 km) and has a drainage area of 0.93 square miles (2.46 m²) (Price and Heck, 1939).

Geologically, the underlying rock of Carpenter Run is of the New River Group, Pottsville series. These Pennsylvanian rocks are composed mainly of sandstones, shales, and coals. Structurally, the Webster springs anticline surfaces along the North Fork of Cherry River. The axis of the Kovan syncline crosses the North Fork of Cherry River between Coats Run and Little Lick Run with outcroppings along the valleys of both the North Fork and the South Fork of Cherry River (Price and Heck, 1939).

MATERIALS AND METHODS

Ameletus tarteri nymphs were collected monthly from November 1986 to November 1987 from Carpenter Run. The nymphs were collected using a small aquarium net, which was gently scraped across the rocks and boulders in the stream. In addition to this, the aquarium net was placed below smaller rocks, which were lifted to dislodge any nymphs. The amount of time spent collecting was recorded. Any nymphs captured were immediately transferred using forceps to a vial containing 70 percent ethanol. Relative abundance of nymphs was determined by dividing the number of nymphs collected by the time spent collecting.

The stream bank was examined weekly from May to July and again in September, dates when pre-emergent nymphs were collected, for the presence of nymphal exuviae. Exuviae were collected, preserved in 70 percent ethanol, and returned to the lab. They were then counted and examined using a Bausch and Lomb dissecting microscope to determine sex.

Attempts were made to trap adults using Ward's insect trap with an ultraviolet light source. Each attempt lasted one hour and was begun at darkness. Seventy percent ethanol was used as a preservative for any insects captured. All captured insects were returned to the laboratory, and any mayflies were removed from the collection.

Water chemistry was tested monthly (excluding January due to adverse weather conditions) using a Hach Ecology Testing Kit—Model AL-360T. The parameters analyzed included dissolved oxygen, alkalinity, acidity, total hardness, carbon dioxide, and pH (November 1986 through May 1987). All of the above parameters listed were measured in mg/L except pH. From June 1987 to November 1987, pH was measured with a Chemtrix Oyster pH meter. Temperature was measured monthly using a Celcius thermometer.

In the laboratory, all *Ameletus tarteri* nymphs were measured with an ocular micrometer in a Bausch and Lomb compound microscope. The increments of the grid were calibrated using an American Optical stage micrometer. This calibration was done at magnifications of 10 \times , 15 \times , and 25 \times so that measurements made at any of these three magnifications could be converted to the nearest 0.01 mm. The body length of each nymph was determined from the anterior tip of the head to the base of the caudal filaments (10 \times). Head width was measured at the widest part of the head (15 \times). A length frequency histogram was completed using increments of one millimeter for body length. A size frequency distribution was also constructed in increments of 0.1 mm for head width.

Monthly growth rates were determined by calculating the percent increase in growth from one month to the next. Head width variation was shown using population range diagrams which included mean head width, range, and two standard errors of the mean for each month. Significant differences between means were determined by comparing the overlap of the two standard errors of the mean (0.05 confidence level). The number of instars in the life cycle was determined using the Janetscheck method (Janetscheck, 1967).

The sex of each nymph was determined using two characters. The eyes of the male nymphs were larger and closer together. The male genitalia could be seen developing in the nymphs. Sex was determined for nymphs which had developed to a point at which these two characteristics were clear. All other nymphs were termed "immature." A chi-square analysis was performed to determine if the sex ratio was significantly different from 1:1 at the 0.05 confidence level.

Foregut analysis was performed on five relatively larger nymphs per month using an ocular micrometer (as for the length frequency

distribution). Microdissecting scissors were used to remove the head as well as to make a ventral midthoracic cut. The integument was placed to the side, and the foregut was removed using a stainless steel probe. All five foreguts were placed in 3 ml of water to which 5 drops of iodine solution were added. For August the three foreguts were placed in 1.5 ml of water and 3 drops of iodine were added. After the five foreguts were thoroughly agitated, 1 ml of the mixture was removed using a pipet and placed in a Sedgwick-Rafter cell. This cell was examined under an Olympus compound microscope containing a Whipple grid (200 \times). Ten grids were randomly selected to be examined. This milliliter of fluid was then replaced with a second milliliter of foregut contents from the same month. Ten more grids were then randomly selected for examination. The relative abundance of four food categories was determined by calculating percentages of small grid squares within each field that contained each of the different food items: (1) plant detritus, (2) mineral detritus, (3) filamentous algae, and (4) diatoms.

A Gilson differential respirometer was used to measure the oxygen consumption of the nymphs at three temperatures (7, 11, 14 $^{\circ}$ C) and two pH's (5.0, 7.2). For this test, nymphs were collected from Hamrick Run, a nearby tributary of the North Fork of Cherry River. This stream shares many characteristics of Carpenter Run, including a low pH, and is only 750 m downstream from Carpenter Run. Nymphs were collected in April and May of 1987 and were allowed to acclimate in the lab for no longer than two days before the study was begun. For the pH = 5.0 test, nymphs and stream water were placed in the reaction flask. Nymphs and stream water from Twelvepole Creek in Wayne County, West Virginia, were placed in the reaction vial for the pH = 7.2 test. For each pH test, the water bath was maintained for three hours at each of the following temperatures: 7, 11, and 14 $^{\circ}$ C. At the end of each hour, a reading was taken from the micrometer. For each temperature, these three readings were averaged. Nymphs were sacrificed and heated at 40 $^{\circ}$ C for 24 hours. They were weighed using a Bosch S2000 analytical scale. An average oxygen consumption rate in microliters per milligram dry weight per hour was then calculated. Analysis of variance was used to determine if temperature or pH had a significant influence on oxygen consumption. Data were plotted to determine the influence of body weight on oxygen consumption for both pH's. Slopes of the

two regression lines were compared with a z-distribution to determine if they were significantly different.

Laboratory-reared female subimagos were dissected for egg counts. A midsagittal longitudinal cut was made on the ventral surface to open the body cavity. All eggs were carefully removed and direct egg count made under a Bausch and Lomb dissecting microscope. A regression of fecundity and body length was calculated, and a correlation coefficient was determined for the relationship. Ten percent of the eggs obtained from each female subimago were measured using an ocular micrometer in a Bausch and Lomb dissecting microscope at 25× magnification.

RESULTS AND DISCUSSION

Water chemistry. Dissolved oxygen ranged from 3.7 mg/L (July) to 11.8 mg/L (February) with a mean of 8.7 mg/L. Dissolved carbon dioxide ranged from 2.5 mg/L (November 1987) to 10 mg/L (June) with a mean of 4.4 mg/L. Alkalinity had a mean value of 5 mg/L CaCO₃ with a minimum value of 1 mg/L CaCO₃ in April, May, and September and a maximum value of 15 mg/L in October. Acidity ranged from 8 mg/L (November 1987) to 28 mg/l (July) with a mean equal to 15 mg/L. Total hardness was less variable with a minimum value in March of 3 mg/L CaCO₃ and maximum values of 8 mg/L CaCO₃ in April and November 1987; the mean value was 6 mg/L CaCO₃. The mean hydrogen ion concentration (pH) was 4.5 with a range from 4.2 (November 1987) to 5.0 (November 1986 through February 1987). Detailed water chemistry can be found in Matthews (1988).

Monthly water temperature values varied from -1.0°C in January to 18.9°C in July. The mean annual water temperature was 8.5°C.

Habitat observations. The habitat preference of *A. tarteri* is vertical rock surfaces perhaps even slanted beyond the perpendicular for the mature nymphs, while flat rock surfaces in shallow eddies were preferred by the younger nymphs. The larger, more mature nymphs were more often found on the vertical sides of the larger boulders in the stream while the younger nymphs were easily obtained from large horizontal rock surfaces. *Ameletus tarteri* nymphs have been found to have a preference for high elevation streams. Burrows (pers. comm.) found these mayflies at an elevation of 3000 ft. (900 m), North Fork of Cherry River, but not at 2000 ft

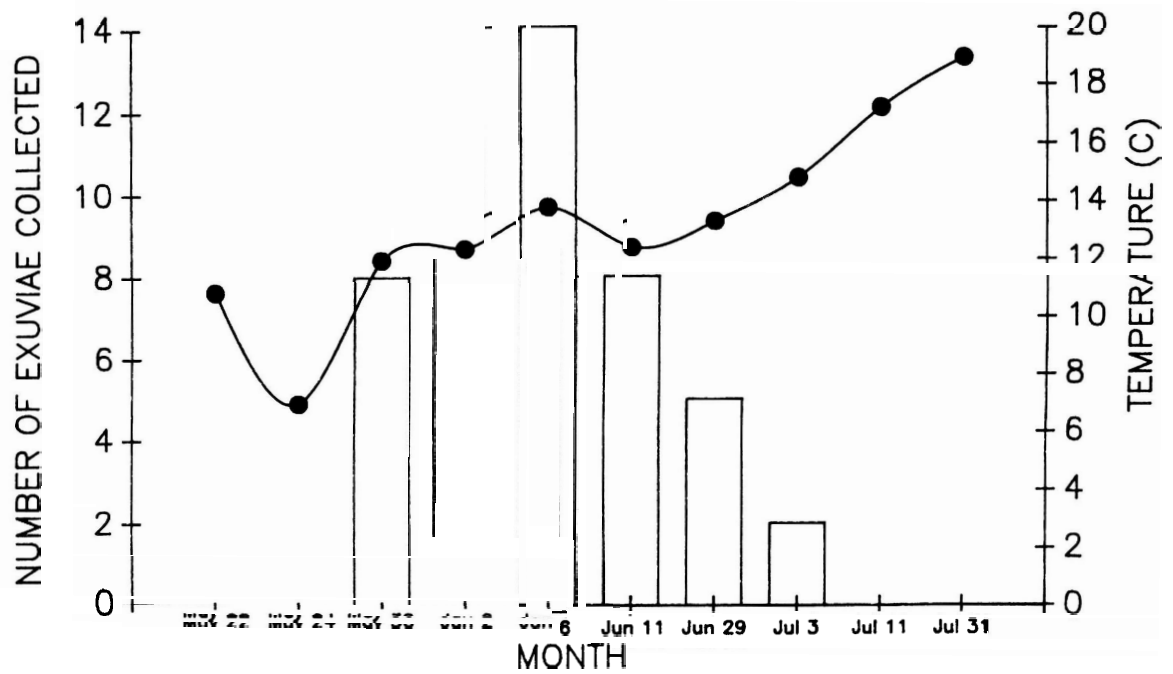


Figure 1. Nymphal exuviae collected, shown with bar graph, from May 22 through July 31, 1987, indicating the pattern of emergence for *Ameletus tarteri* in Carpenter Run, Greenbrier County, West Virginia. The water temperature (C) during the study period is indicated by the line graph.

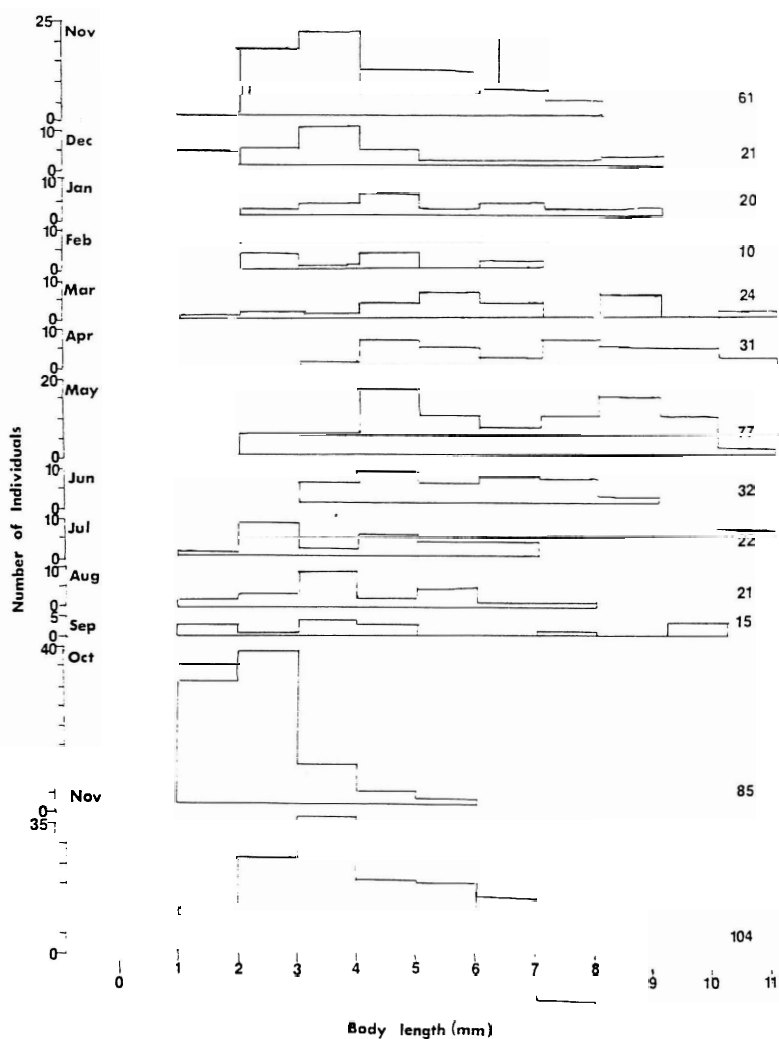


Figure 2. Length frequency distribution showing monthly body length of nymphal *Ameletus tarteri* from Carpenter Run, Greenbrier County, West Virginia. The number of individuals collected per month is at the right.

(600 m) in Cherry River. This preference for high elevation is seen at Carpenter Run; the elevation of this stream is 3200 ft (960 m) (Price and Heck, 1939).

Emergence period. Nymphal exuviae were recovered from vertical rocks along the stream from May 30 to July 3, 1987 (Fig. 1). Exuviae were searched for but not recovered on May 22, May 24, and June 2. A large storm occurred on June 1 which could account for the absence of exuviae on this date. The largest number of exuviae were recovered on June 6. No exuviae were found on July 11, 1987 or July 31, 1987. Because of this, weekly examination for exuviae ceased. A nymph with dark wing pads was found on September 26; examination for exuviae was undertaken, but none were found. Data from this study (nymphs with dark wing pads and cast exuviae) suggest that emergence of *A. tarteri* occurs from late May to early July and again in late September, constituting a bimodal emergence pattern.

Collection of adults. Eleven attempts to collect adults of *A. tarteri* using an ultraviolet light source beginning May 9 and ending July 31 were unsuccessful. According to Edmunds et al. (1976), the adults and subimagos of some species are attracted to lights only at certain times of the night; perhaps *A. tarteri* is one of these species.

Length frequency distribution. A length frequency histogram of body length in 1 mm increments was constructed for the nymphs (Fig. 2). The smallest nymph, measuring 1.1 mm, was found in October. The largest nymph, measuring 11.9 mm, was collected in April. A size frequency distribution of head width in 0.1 mm increments is found in Figure 3. The smallest head width was 0.2 mm and was found in October while the largest measured 1.9 mm and was collected in May.

Data summarized in Figures 2 and 3 indicate a univoltine life cycle. This is also true for *Ameletus inopinatus* Eaton as described by Gledhill (1959). *Ameletus inopinatus* emerged from late May until August with two periods of egg hatching; one in autumn and another in spring. This results in overlapping cohorts and an extended emergence period.

In *A. tarteri*, there appears to be no egg diapause. Nymphs with dark wing pads were found in May, June, and September suggesting emergence occurred at these times (Fig. 3). Eggs are laid in late May as "physiologically ready individuals" emerge, and immediately

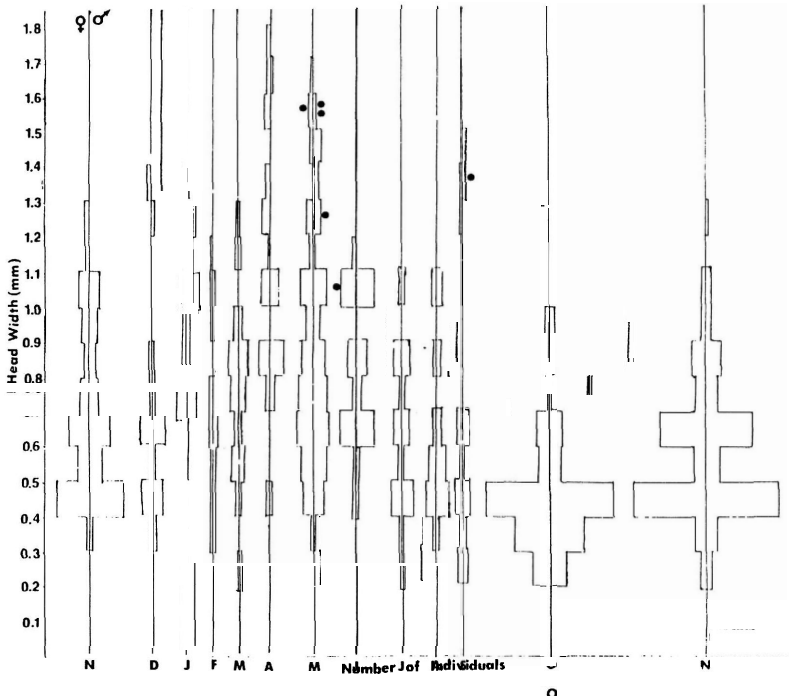


Figure 3. Size frequency distribution for head width of nymphal *Ameletus tarteri* from Carpenter Run, Greenbrier County, West Virginia, from November 1986 to November 1987. Sex is indicated for nymphs with a head width greater than 1.1 mm. Closed circles represent black wing pads.

hatch, accounting for the increased relative abundance at this time. Emergence and hatching continue through June. At this time, the water temperature averaged 12.7°C. During middle July through August the temperature rose to 18.9°C, and the stream flow decreased as the water level dropped; no subimagos emerged. As the temperature dropped to 11.7°C in September, emergence began again (Fig. 3). At this time the remaining individuals of the generation emerged. A large number of eggs hatched and the relative abundance increased. Because there were two emergence periods in this bimodal cycle, a variety of size classes were found throughout the year. This type of emergence pattern is adaptive in periods of

environmental stress such as high temperatures or dryness. Both of these conditions were present at Carpenter Run in the summer of 1987.

Growth. Population range diagrams demonstrate the monthly progression of head width sizes during the study period. This is seen in conjunction with a temperature curve (Fig. 4). The largest amount of growth occurred between October and November 1987 (31.4%) and December 1986 to January (29.3%). The largest decrease in size occurred between June and July (-34.9) and September to October (-37.9). This corresponds with the months following a period of emergence and further supports the bimodal theory of emergence.

Significant amounts of growth, based on amount of overlap between two standard errors of the mean in the population range diagrams, occurred between March and April (prior to emergence) and between October and November 1987. A significant decrease in size occurred between April and May, June and July, and September and October (Fig. 4).

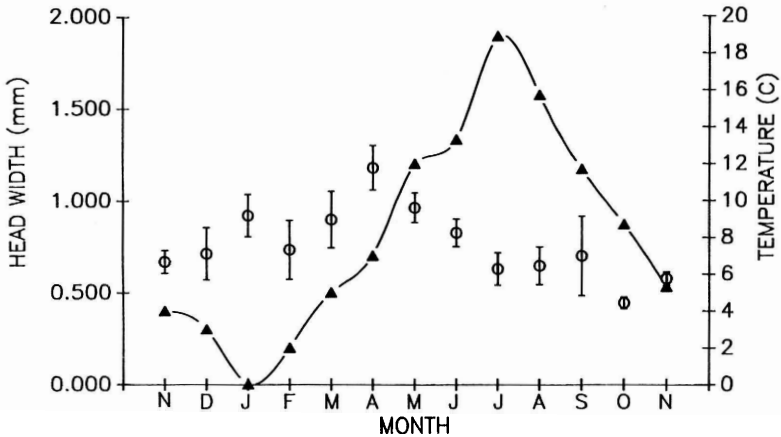


Figure 4. Population range diagram showing head width variations of nymphal *Ameletus tarteri* from Carpenter Run, Greenbrier County, West Virginia from November 1986 to November 1987. Open circles are the means, vertical lines are the two standard errors of the means, and solid line is temperature (C).

The number of instars for nymphal *A. tarteri* was estimated using Janetscheck method (Janetscheck, 1968) (Fig. 5). When body length in increments of 0.099 mm was used for nymphal *A. tarteri*, 21 instars could be discerned (Fig. 5c).

Sex ratio. A 1:1 sex ratio was not observed for *A. tarteri* nymphs. A chi square test was applied to 71 females and 49 males. The difference was significant at the 0.05 level. The difference could be influenced by sexual dimorphism. The males may be slightly smaller than the females and could have been classified as immature. If the sex of the exuviae recovered from the creek and the sex of the subimagoes that emerged in the laboratory are added to the

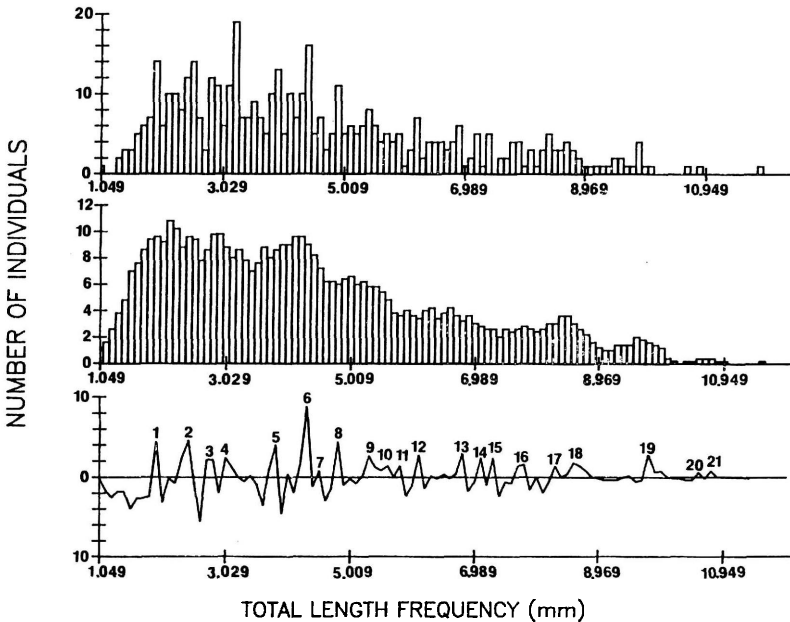


Figure 5. Instar determination for *Ameletus tarteri* from Carpenter Run using the Janetscheck method. a. frequency histogram of total length of nymphs b. trendlines of the population, calculated by the method of gliding means over five successive size-class frequencies at a time c. periodicity of maximum frequency of size

chi-square calculation, then the chi square comparison would be between 99 females and 75 males. When this is done, there is not a significant difference at the 0.05 level, supporting a 1:1 sex ratio.

Foregut analysis. Nymphal *A. tarteri* can be classified as a detritivore; the major factor of its diet is plant detritus (77.3%) (Table 1). Mineral detritus composed 19.0 percent of the diet. Diatoms, composing 2.8 percent of the diet, were seen consistently. Diatoms may not be an essential component of the diet. Diatoms identified include *Eunotia* sp., *Tabellaria* sp., *Frustulia* sp. and *Surirella* sp. *Eunotia* and *Tabellaria* were the most commonly found diatoms, present in virtually every month. *Frustulia* was present only in January and August while *Surirella* was found only in January, March and June. The filamentous algae *Microspora* sp. was found in the foreguts only during March, April and May. This may coincide with an algae bloom in the stream. It is perhaps more likely that *A. tarteri* reverted to this algae as food when the leaves were washed from the stream in the spring. Merritt and Cummins (1978) classified *Ameletus* as collector-gatherers of fine organic particulate matter.

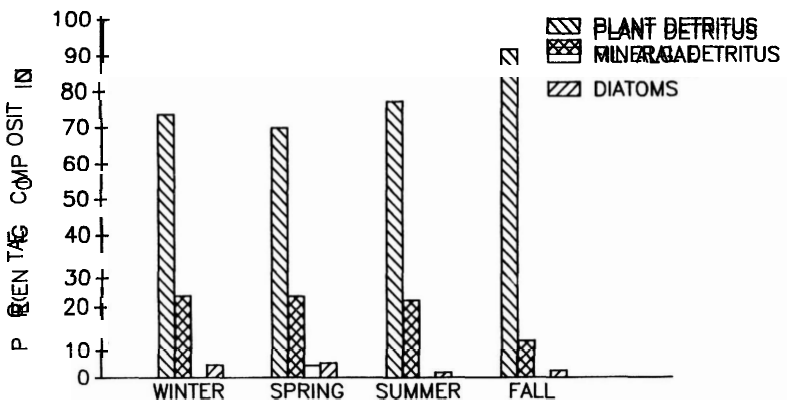


Figure 6. Seasonal foregut content of nymphal *Ameletus tarteri* from Carpenter Run, December 1986 to November 1987.

Table 1. Relative foregut contents of nymphal *Ameletus tarteri* at Carpenter Run, November 1986–November 1987.

Month	Plant	Mineral	Algae	Diatoms
Nov	71.12	21.71	0.00	7.17
Dec	75.87	21.16	0.00	2.97
Jan	66.58	26.55	0.00	6.87
Feb	77.24	21.57	0.00	1.19
Mar	74.53	15.88	1.42	8.17
Apr	66.87	21.33	9.11	2.69
May	65.76	33.40	0.63	0.21
Jun	74.35	24.22	0.00	1.43
Jul	77.93	20.88	0.00	1.19
Aug	86.43	10.71	0.00	2.86
Sep	86.23	11.37	0.00	2.40
Oct	83.98	14.06	0.00	1.99
Nov	92.25	6.42	0.00	1.34
MEAN	77.34	18.96	0.93	2.78

If the foregut data are examined with regard to season (Fig. 6), it can be seen that the spring diet and the fall diet are considerably different. In spring there was an increase in algae consumption and a decrease in plant detritus consumption. In fall, the consumption of plant detritus increases correspondingly with an increase in the leaf litter in the stream. There is a decrease in the consumption of mineral detritus and diatoms. Algae is absent from the fall diet.

Respiratory studies. Analysis of variance was used to evaluate the effects of temperature and pH on oxygen consumption. Temperature and pH each exerted a significant influence on oxygen consumption. There was no significant interaction between the two parameters. Using *Ameletus* spp. and *Eccopteura xanthenes* (Newman), Doherty and Hummon (1980) found that the low pH of acid mine drainage did not consistently alter the oxygen consumption rate. Mayflies in general are considered to be the most sensitive order of aquatic insects to low pH. *Ameletus tarteri* seems to have developed adaptations to low pH. At a lower pH, oxygen consumption is decreased at a time when oxygen demand is higher (Havas, 1980).

Figure 7 shows the relationship between dry weight and oxygen consumption. At pH 5.0, there exist very small nymphs (less than 1

mg) that have an extremely high oxygen consumption. As they acclimate during their life cycle, their oxygen consumption rates return to a level closer to the control.

Independent of pH, there exists a relationship between oxygen consumption and body size (Fig. 7). A negative correlation of -0.503 was found between these two factors for $\text{pH} = 5$ and of -0.536 at $\text{pH} = 7.2$. Both of these correlation coefficients are significant at the 0.05 level. A curvilinear relationship exists between oxygen consumption and biomass (Knight and Gaufin, 1966; Knight and Simmons, 1975a,b). Knight and Gaufin (1966) theorized that this could be attributed to a lower metabolic rate found in older organisms when compared to younger ones perhaps due to differences in relative amounts of active tissue with older organisms having a higher lipid content.

The two slopes of the regression lines were compared using a z-distribution and were found to be significantly different at the 0.05 level. This supports the theory that the relationship between oxygen consumption and body weight is different at the two pH levels.

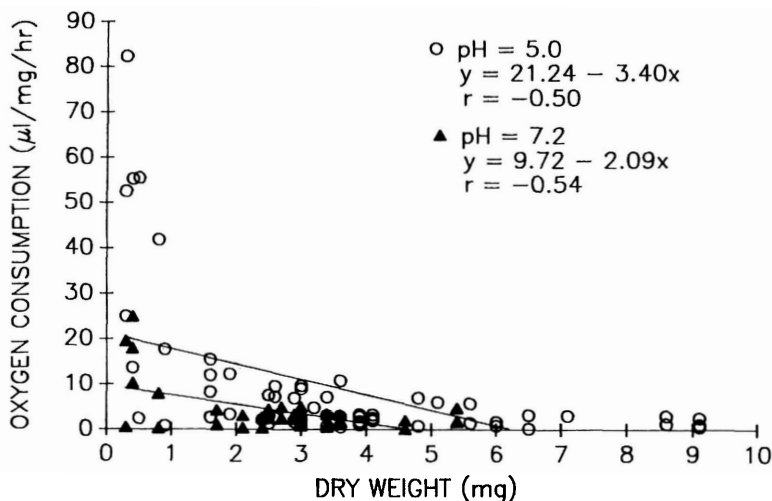


Figure 7. Graph of body weight of nymphal *Ameletus tarteri* and oxygen consumption. Open circles are organisms tested at $\text{pH} = 5$ and closed triangles are organisms tested at $\text{pH} = 7.2$.

Fecundity. Nine subimagoes were dissected. The mean number of eggs counted was 715 with a range of 382 to 1286. The mean body length of the female was 9.64 mm. A positive correlation of 0.85 was calculated for female body length versus number of eggs (Fig. 8).

Egg size. The eggs of *Ameletus tarteri* were found to be flattened ovoids. The length and width of 645 eggs were determined. The mean length of the eggs was calculated to be 0.25 mm with a range of 0.16 to 0.32 mm. The mean width was 0.16 mm with a range of 0.08 to 0.22 mm. Needham et al. (1935) described *Ameletus* eggs as flattened ovoids; eggs of *A. lineatus* Traver measured 0.32 by 0.19 mm.

SUMMARY

An ecological life history study was conducted on the mayfly *Ameletus tarteri* Burrows from Carpenter Run, Greenbrier County, West Virginia, from November 1986 to November 1987. Additionally, laboratory studies were conducted to determine the nymphal mayflies' respiratory response to temperature and pH changes. A total of 504 nymphs was collected with the highest relative abun-

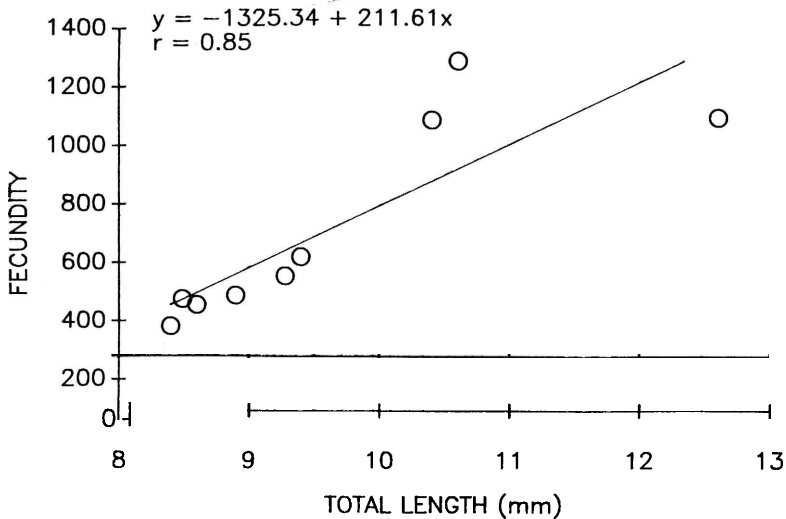


Figure 8. Regression line of total length of subimago of *Ameletus tarteri* and number of eggs produced.

dance occurring in November 1987. Older nymphs were found most often on vertical rock faces while the younger nymphs preferred horizontal rocks. Nymphal exuviae were recovered from May 30 to July 3. Length frequency distributions indicate a univoltine life cycle. A bimodal pattern of emergence was indicated with the first emergence period beginning in late May and continuing to early July and second emergence period in September. An estimate of 21 instars was made using the Janetscheck method. The lack of a 1:1 sex ratio in nymphs may be attributed to sexual dimorphism. Monthly foregut analysis indicates that nymphal mayflies are detritivores with 77.3 percent of their diet composed of plant detritus. Mineral detritus and diatoms are consistent components of the diet. Studies using the Gilson respirometer indicate that oxygen consumption decreases as temperature increases and that as pH is lowered, oxygen consumption increases. The correlation coefficient between oxygen consumption and body weight at pH 5.0 was -0.503 . The correlation coefficient for oxygen consumption and body weight at pH 7.2 was -0.536 . When the slopes of the two regression lines were compared, they were found to be statistically different (0.05 confidence level), supporting the hypothesis that the relationship between these two variables is different at the two pH values. Direct egg counts ranged from 382 to 1286 eggs/female with a mean of 715. A correlation coefficient of 0.85 was calculated for female body length versus number of eggs. Mean egg measurements were 0.25 mm in length and 0.16 mm in width.

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