

AN ABSTRACT OF THE THESIS OF

Christine Rosabelle Dolores Pereira for the degree of Master of

Science in Entomology presented on 7 August 1980

Title: Life History Studies of *Cinygma integrum* Eaton

(Ephemeroptera:Heptageniidae) and Other Mayflies

Associated with Wood Substrates in Oregon Streams

Abstract approved:

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Dr. N. H. Anderson

Wood debris serves an important substrate for macroinvertebrates in streams. Particle size reduction due to feeding and other activities can be an important role of the fauna in wood degradation. In this study the Ephemeroptera associated with wood were investigated, with the objective of assessing their impact in the degradation process. Emphasis was placed on *Cinygma integrum* Eaton which appeared to be the only mayfly that showed a predilection for wood substrates.

The abundance of mayfly larvae on wood at Berry Creek, Benton County, Oregon was compared with that on stones and composite substrates (kick samples) for one year. Nine taxa were encountered. Proportions on wood compared with other substrates were: *C. integrum*, 76%; *Baetis*, 46%; *Ironodes nitidus* (Eaton), 43%. Although *Baetis* was the most abundant species on wood, it was also common on the other substrates. It uses wood for attachment and as an emergence platform--activities that have little effect on wood degradation. *C. integrum* and *I. nitidus* showed feeding habits that are

related to wood decomposition. Their mouth parts are adapted for scraping the aufwuchs on wood substrates. Epeorus (Iron), Cinygmula and Rhithrogena were less associated with wood substrates, apparently exploiting similar food resources on mineral substrates.

Three methods of analysis revealed that C. integrum had a univoltine, slow seasonal (S1) type of life cycle (Hynes, 1970). I. nitidus had a univoltine, fast seasonal (F1) type. These differences are perhaps related to differential effects of temperature on developmental stages. Eggs of I. nitidus were the only stage present in the summer and appeared to undergo a period of quiescence. C. integrum eggs apparently hatched over a longer period. Both species were cool adapted with the majority of growth occurring from November to February.

Three millimeter larvae of C. integrum were reared to adults using a constant temperature of 9°C, 12 hours daylength and a restricted diet of only the aufwuchs present on wood. Success at rearing indicated that this species is perhaps a suitable candidate for future ecological, physiological and biochemical laboratory investigations of wood decomposition.

Mayfly feeding activity appears to be responsible for some biological degradation of wood. Superficial layers of wood that have been partially degraded by microbial activity are ingested and wood particles up to a millimeter in length were present in the guts. Ingestion of wood particles probably accompanies the ingestion of the aufwuchs layer.

Fungal mycelia and spores were commonly ingested. C. integrum showed maximum consumption of fungi with I. nitidus second to it. Aquatic Hyphomycetes, Ascomycetes and Deuteromycetes spores were present in the gut and fecal material, indicating a potential role of spore dispersal. Seasonal periodicity of mycelial ingestion occurred, with a maximum in winter. Small larvae of C. integrum

consumed more detritus than did the mature larvae which had large quantities of fungal material in their guts.

Annual productivity for C. integrum was  $0.28\text{g/m}^2$  (dry weight). Larvae ingested an average of 53% of their body weight of mycelia per day. An annual estimate of mycelial ingestion by the wood-associated mayfly complex was about  $1\text{g/m}^2$ . Mycophagy in wood-associated mayfly larvae thus make a contribution (although an indirect one) to wood processing and mineralization in aquatic habitats. Removal of senescent populations of fungi make available fresh areas for further fungal colonization.

Benefits accrued by mayflies from an association with wood are related to emergence, shelter and feeding. As a result of these biological activities, particularly the latter, this group plays both an indirect and direct ecological role in wood decomposition in aquatic habitats.

Life History Studies of Cinygma integrum Eaton  
(Ephemeroptera:Heptageniidae) and Other Mayflies Associated  
with Wood Substrates in Oregon Streams

by

Christine Rosabelle Dolores Pereira

A THESIS

submitted to

Oregon State University

in partial fulfillment of  
the requirements for the  
degree of

Master of Science

Completed August 7, 1980

Commencement June 1981

APPROVED:

Redacted for Privacy

Professor of Entomology  
in charge of major

Redacted for Privacy

Chairman of Department of Entomology

Redacted for Privacy

Dean of Graduate School

Date thesis is presented 7 August 1980

Typed by D. L. Norvell-Race for Christine Rosabelle Dolores Pereira

## ACKNOWLEDGMENTS

I would specially like to thank my major professor, Dr. N. H. Anderson, for his invaluable assistance and encouragement during the preparation of the thesis. I would also like to express my appreciation to the other members of my Committee, Dr. B. F. Eldridge and Dr. A. K. Ward, for reviewing this thesis, Dr. G. W. Krantz for the photography, Professor C. T. Ingold of Birbeck College, London and Dr. K. L. Suberkropp of New Mexico State University for helping with the identification of fungi, L. M. Roberts and C. Dewberry for their generous help in the field, and my friends who helped me in innumerable ways. This study was supported in part by an NSF grant DEB 78-10594.

I gratefully acknowledge support for study at Oregon State University from the Institute of International Education (Fulbright Scholarship--IGB Partial), Delta Kappa Gamma Society International (World Fellowship) and Oregon State Scholarship Commission (Foreign Student Scholarship).

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LIFE HISTORY STUDIES OF CINYGMA INTEGRUM EATON  
(EPHEMEROPTERA:HEPTAGENIIDAE) AND OTHER MAYFLIES ASSOCIATED  
WITH WOOD SUBSTRATES IN OREGON STREAMS

INTRODUCTION

Large organic wood debris is an important component in streams in western Oregon. This allochthonous material may contribute significant amounts of nutrient material to the stream as a result of physical and biological degradation processes. Wood decomposition is a slow process since a majority of its constituents are highly refractory. Initial breakdown of tissue may be attributed to the microbial population (fungi and bacteria) and results in a conditioning of the wood substrate for subsequent colonization by the macroinvertebrates.

Earlier studies of wood associated macroinvertebrates were designed to investigate the colonization behavior and productivity of these animals (Claflin, 1968; McLachlan, 1970; Nilsen and Larimore, 1973; Benke et al., 1979) and the impact of macroinvertebrate biological activities on the wood degradation process (Anderson et al., 1978). The latter authors reported that the mayflies Cinygma integrum Eaton and Ironodes nitidus (Eaton) were present in significant numbers on wood substrates in some western Oregon streams. Mayflies and other macroinvertebrates utilized this substrate for shelter, pupation, oviposition, case building or feeding.

In the recent past the aquatic entomology program at Oregon State University has centered around studies of wood degradation in streams. It seemed worthwhile to supplement this effort with a study of wood-associated mayfly biologies, insofar as these species appear to be important to wood decomposition and mineralization. In

this way, an attempt could be made to arrive at a preliminary estimate of the relative importance of this group in the processing of wood in aquatic habitats.

This study was undertaken to provide information for further ecological studies on wood degradation in fresh water ecosystems. The objectives were:

- (1) to study the biologies of wood-associated mayflies, particularly the species C. integrum, with emphasis on substrate preference, life cycle and feeding behavior;
- (2) comparison of life histories observed at Berry Creek with that at Flynn Creek and Mack Creek, in order to determine if difference in physical characteristics of the habitats had an effect on the life cycle pattern;
- (3) to study the feeding habits of all wood-associated mayflies at Berry Creek and to assess the impact of mayfly feeding behavior on the wood degradation process in streams;
- (4) to develop techniques for efficient rearing in the laboratory of C. integrum which would be of use in future ecological and physiological studies dealing with this species and related species.

## LITERATURE REVIEW

The quantities of wood in streams and its importance to the biota have been reviewed by Anderson and Sedell (1979) and Triska and Cromack (1980). In the Pacific Northwest, the amount of wood entering the stream is enormous. Oregon streams draining old-growth Douglas fir-hemlock stands had as much as 25 to 40kg/m<sup>2</sup> of wood greater than 10cm in diameter and in second growth stands of mixed hardwood, it varied between 4 to 8kg/m<sup>2</sup>. Wood debris standing crop and input rate into streams are dependent on the forest species composition (type and age of stand) as well as on the physical properties of the channel and the environment (channel morphology, gradient, meteorological conditions).

Wood enters the stream in the form of intact boles and snags; the latter are in a more decomposed state than the boles (Triska and Cromack, 1980). The role of wood debris in streams is both a physical and biological one. Entry of wood into streams creates debris dams and results in sediment retention and alteration of stream flow. This results in the creation of new habitats and favors a more diverse fauna which utilizes the wood substrates for temporary attachment, shelter, pupation, emergence, oviposition and feeding. Metabolically, wood debris aids in nutrient cycling within the aquatic ecosystem as a result of mineralization and biological decomposition which includes microbial and macroinvertebrate activity as well as secondary plant succession (Triska and Cromack, 1980). Turnover rate and nutrient recycling are dependent on wood decay characters which are based on texture, size, shape and proportion of surface area in contact with the substratum. A change in decay class is therefore accompanied by a change in the conditioning of the substrate and thus a shift in the type of fauna that is present (Dudley and Anderson, in prep.).

Triska and Cromack (1980) indicated that wood is a substrate where considerable biological decomposition occurs by the action of

microbes, invertebrate and other aquatic organisms. Claflin (1968), McLachlan (1970) and Nilsen and Larimore (1973) studied the colonization patterns of macroinvertebrates on wood in aquatic ecosystems. Anderson et al. (1978) surveyed the fauna associated with wood in eleven streams in western Oregon. Their study was a preliminary investigation into the role of macroinvertebrates in wood processing. Dudley and Anderson (in prep.) collected a variety of macroinvertebrates from streams in the western states and studied the interactions between the fauna and wood substrate. They attempted to relate the distribution of wood-associated macroinvertebrates with wood texture and consistency. Pereira et al. (in prep.) examined the gut contents of some of these wood-associated organisms and distinguished between those feeding on the aufwuchs layer (fungi, diatoms and detritus) and those that were gouging or tunnelling in wood. Benke et al. (1979) studied the productivity of wood-associated macroinvertebrates on snags in a Georgia river.

Jones and Oliver (1963), Willoughby and Archer (1973) and Sanders and Anderson (1979) studied the colonization patterns of fungi on wood in freshwater ecosystems. Very little progress is being made in this field and studies dealing with successional patterns in microbial communities on wood and their relation to the macrodetritivore and microdetritivore production are very rare.

Few ephemeropteran species which are closely associated with wood in aquatic habitats have been noted in the literature. An exception, however, is the Oriental mayfly, Povilla adusta Navas, which is reported to burrow into submerged wood, wooden boats, aquatic paints, shell fragments and gravel (Petr, 1976; Bidwell, 1979). It is also reported as a pest since it causes considerable damage to wood pilings (Edmunds et al., 1976). Submerged wood, however, serves as one of the many habitats available to mayfly larvae, e.g., Epeorus, Ironodes, Choroterpes, Cinygma, Paraleptophlebia, Leptochyphes, Neoephemera, and Caenis (Edmunds et al., 1976). Flowers and Hilsenhoff (1978) also reported that wood pieces seemed "attractive" to many Stenonema

and Heptagenia species.

Anderson et al. (1978) collected a number of mayfly species on wood in several Oregon streams and indicated that their association was transient or real. Amongst the heptageniids, Cinygma and Iro-nodes were most common on wood substrates. Baetis spp. occurred on wood in considerable numbers but they suggested that its association was incidental. Paraleptophlebia and Ephemerella were also found in sufficient numbers for the authors to suggest a "real" association of these mayflies with wood. Dudley and Anderson (in prep.) indicated that a number of mayflies had a facultative association with wood (Ephemerella, Paraleptophlebia, Heptagenia, Rhithrogena and Epeorus). Cinygma was the only species reported by them to be closely associated with wood. Lehmkuhl (1979) indicated that C. integrum was largely restricted to wood substrates. In seasonal biomass studies of wood-associated macroinvertebrates at Berry Creek (Anderson, unpublished), C. integrum was found to have the highest biomass of all the aquatic insect taxa collected on wood.

The genus Cinygma is comprised of four species and occurs in the Holarctic and Oriental regions (Lehmkuhl, 1979). There are three species in the Northwest with Oregon being the only state in which adults of all three species have been collected (C. integrum, C. dimicki McDunnogh and C. lyriformis (McDunnogh)). The larvae of C. integrum is the only species for which immature stages are known. Jensen (1966) reported larvae of C. integrum from slow to moderately fast flowing waters at elevations of over 1525m in Idaho. He considers this species to be of a "Canadian distributional type." The geographical distribution of the genus Cinygma in North America, Canada and Oregon are shown in Figures 1 and 2.

Knowledge of the life history of C. integrum is limited to general observations and emergence records. This species was first described by Eaton (1885) from a series of adults collected in Washington and Oregon. McDunnough (1933) described the larva of

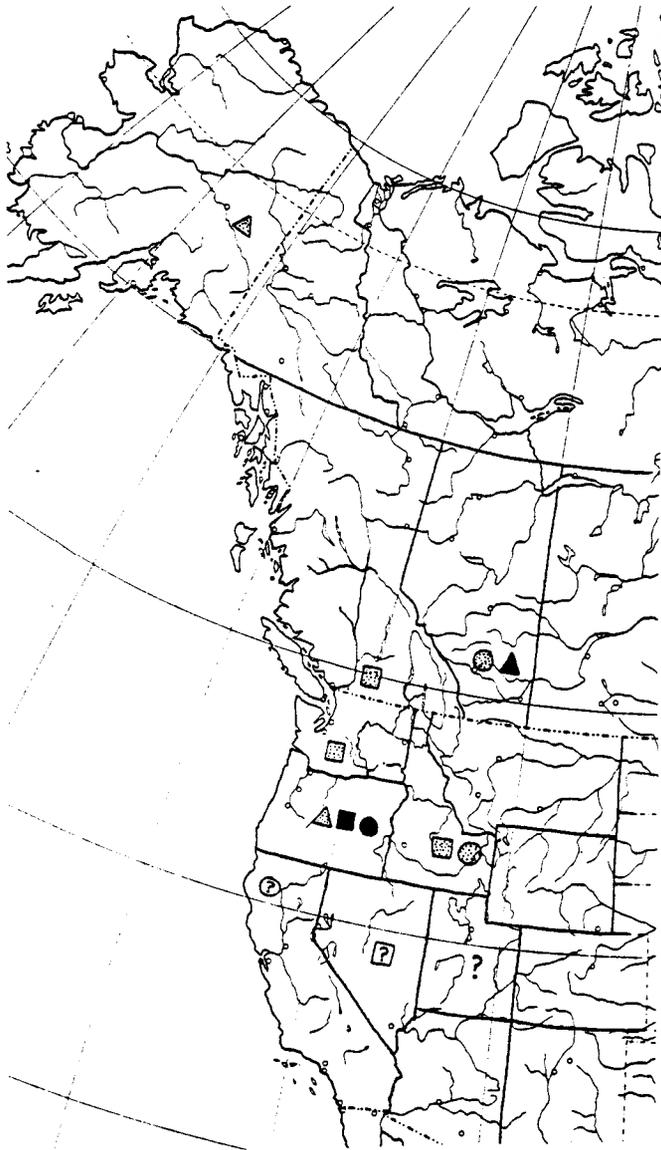


Fig. 1. Geographical distribution of the genus *Cinymsa* in North America and Canada (from Lehnkuhl, 1979). Square: *C. integrum*; Circle: *C. dimicki*; Triangle: *C. lyriformis*. Darkened area indicates type locality.

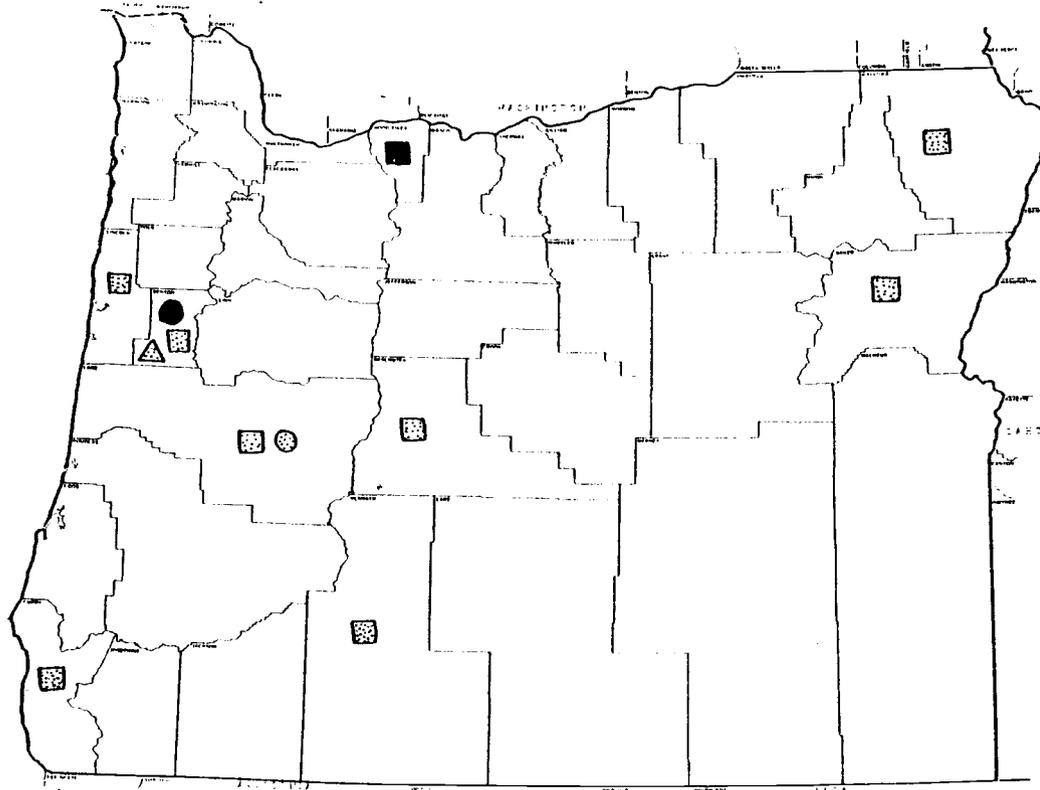


Fig. 2. Distribution of the genus *Cinygma* in Oregon (from Allen, 1955; and collections at Oregon State University, Corvallis). Notation as in Figure 1.

C. integrum. Allen (1955) reported the presence of adults in Oregon during April and May. Lehmkuhl (1979) listed the seasonal occurrence of larval size classes of this species at Oak Creek, Benton County.

McDunnough (1933) described the mouth parts of C. integrum and indicated the functioning of the maxillae as "straining food particles" as well as acting as a "diatom raker." He did not indicate any further functional aspects of the other mouth parts. Merritt and Cummins (1978) class the genus Cinygma as a "scraper, collector-gatherer." The heptageniids, Epeorus, Ironodes, Rhithrogena, and Cinygmula were also classed in this category. Baetis was essentially assigned to the collector-gatherer guild, whilst Paraleptophlebia was placed in the gatherer and shredder functional groups and Ephemerelia included collector-gatherers, scrapers, shredders and predators.

Life history is defined by Oliver (1979) as those events that govern reproduction and survival of species or populations and are affected by factors such as fecundity, development, longevity and behavioral aspects like habitat selection, feeding and mating. As a primarily life history-oriented project, this study attempted to incorporate all of the above parameters with special emphasis placed on substrate selection and feeding. It was hypothesized that they would be closely associated with the process of wood degradation.

Few studies have been made on microbial-wood-macroinvertebrate interactions, although wood is a major component of headwater streams. This study attempted to investigate the fungal-wood-mayfly interactions. Emphasis was placed on the species C. integrum since it has been reported to be almost restricted to wood substrates and very little is known of its biology.

## MATERIALS AND METHODS

Field studies were restricted to streams in western Oregon and were primarily carried out at Berry Creek, Benton County. Seasonal collections of mayfly larvae from wood substrates were available from Flynn Creek (Lincoln County) and Mack Creek (Lane County). Material from tethered stick samples (Anderson, unpublished) were available and it seemed worthwhile to supplement life history information obtained from Berry Creek with that obtained at the latter two sites. In addition, differences in environmental characteristics (particularly temperature) at the three study sites were examined to see if there were any effects on life cycle patterns.

Description of Study Areas

Berry Creek lies 14.5km north of Corvallis and is one of several east Coast Range streams draining the northern slope of McDonald Forest--a typical second-growth Douglas fir area in Benton County. The South Fork of Berry Creek was the area studied. This, in conjunction with the North Fork, flows into Soap Creek, ultimately draining into the Willamette River by way of the Luckiamute River. The area under study has been brought under controlled flow by means of a diversion dam and a bypass channel (Fig. 3) (Warren et al., 1964). The stream temperatures do not show dramatic fluctuations (Fig. 4) since the study area is almost completely covered by a deciduous canopy of red alder (Alnus rubra) and big leaf maple (Acer macrophyllum). Oregon white oak (Quercus garryana), black cotton wood (Populus trichocarpa), Oregon ash (Fraxinus latifolia) and some second-growth Douglas fir (Pseudotsuga menziesii) and grand fir (Abies grandis) are also present. Other physical and environmental characteristics are given in Table 1.

Flynn Creek is a west Coast Range stream about 16km from the Pacific Ocean. It is a tributary of Meadow Creek which drains into

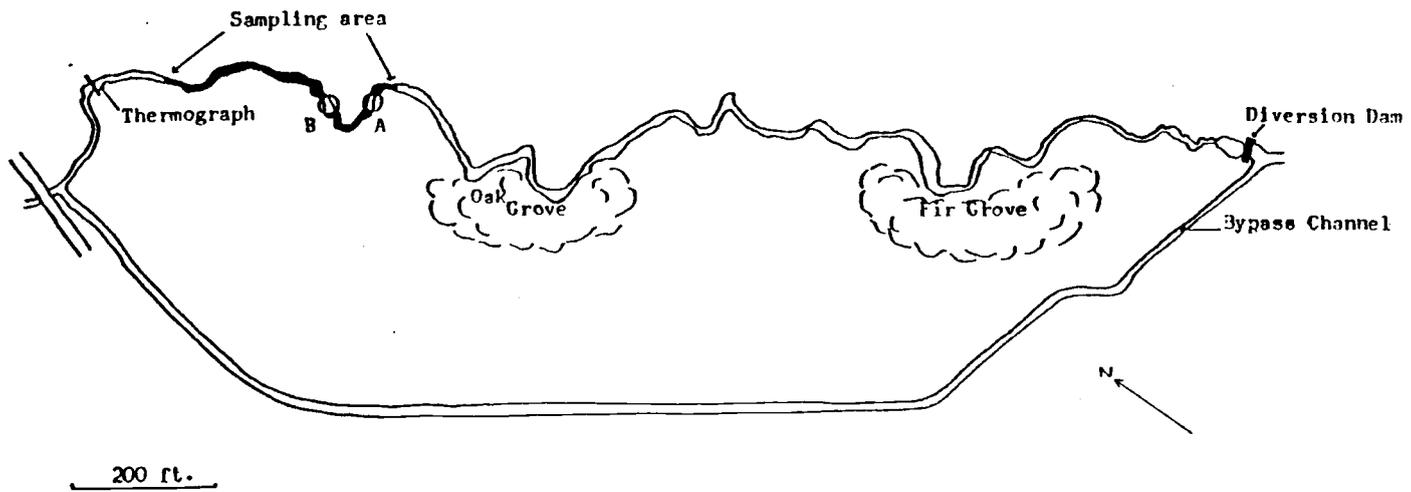


Fig. 3. Berry Creek Study Site. A and B indicate site of emergence traps during 1980.

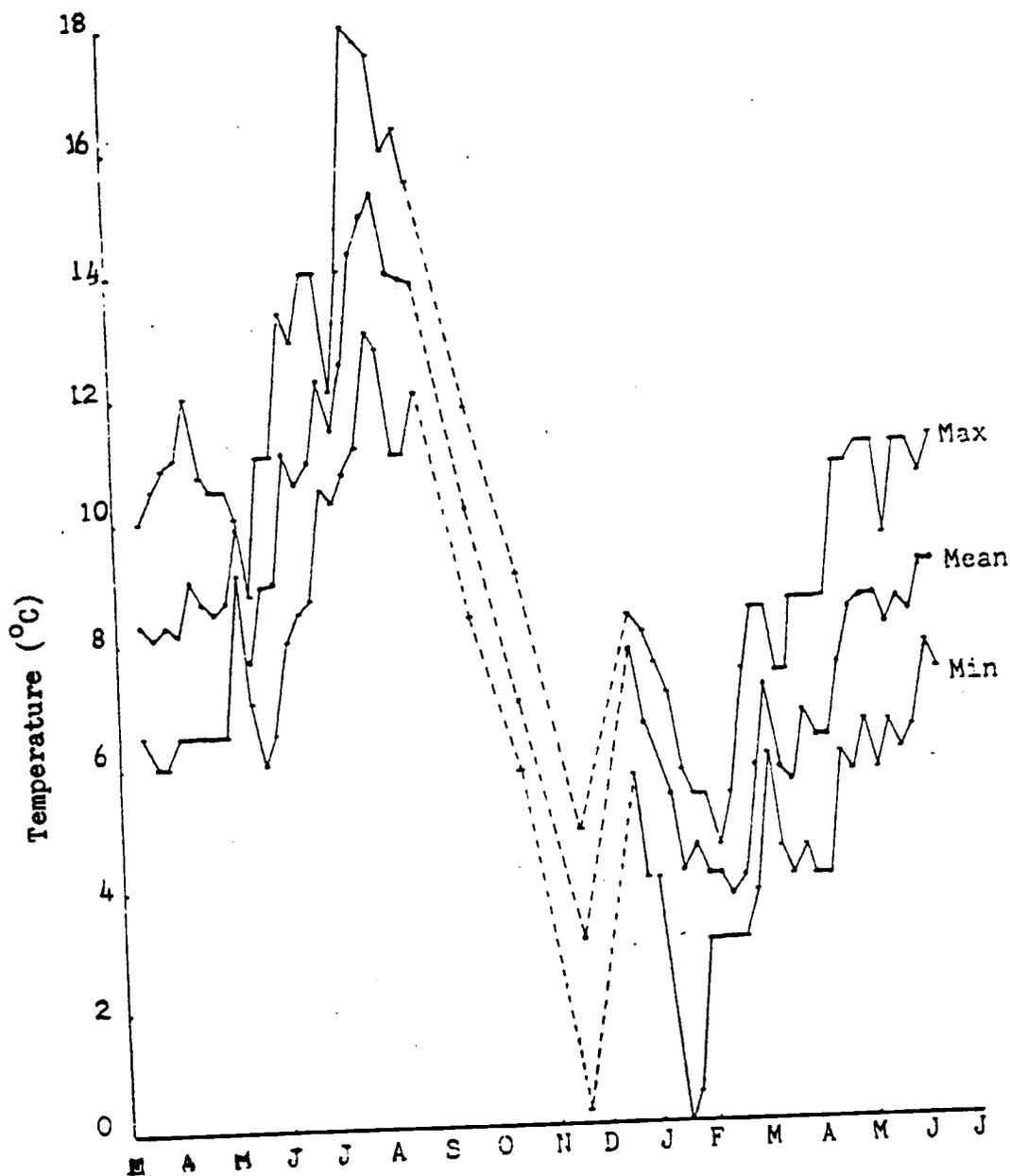


FIG. 4 . Maximum, minimum and mean temperatures at Berry Creek, March 1979 to June 1980. September, October and November stream temperatures are the mean taken for 1960 and 1964.

TABLE 1. Physical and environmental characteristics of the three study sites (from Anderson et al, 1978).

	Berry Creek	Flynn Creek	Mack Creek
Strahler Stream Order	1-2	3	3
Altitude (m)	75	209	830
Drainage Area (km <sup>2</sup> )	3.22	2.02	5.35
Gradient (%)	1.7	2.5	20
Summer Discharge (m <sup>3</sup> )	0.005	0.01	0.10
Winter Discharge (m <sup>3</sup> )	0.03	0.80	2.2
Annual Mean Discharge (m <sup>3</sup> )	0.02	0.12	0.60
Stream Width (m) in July	2.0	3.0	7.0
Annual Mean Rainfall (cm)	120	250	239
Range of Stream Temperature °C	-2 to 20	2 to 16	-2 to 14

Drift Creek and enters the Alsea Bay about 6.5km east of Waldport (Hall and Lantz, 1969). The stream is also densely shaded over most of its length due to a heavy canopy of deciduous and coniferous vegetation. Red alder and Douglas fir are the most common vegetation in the surrounding watershed (Anderson et al., 1978). Table 1 gives other physical and environmental characteristics of this stream.

Mack Creek is one of the three major Cascade Range streams draining the H. J. Andrews Experimental Forest. The stream morphology consists of a series of pools and "free fall zones" (Sedell and Triska, 1975). Mack Creek is different from the other two study sites in that it is at a higher elevation and does not have a very dense canopy throughout its entire length. Temperature fluctuations are much greater at this study site. Other characteristics of this stream are outlined in Table 1.

### Field Studies

Field studies were oriented towards determining the relative abundance of mayflies associated with wood substrates compared to other substrates and studying the seasonal abundance and biologies of the more common wood-associated mayfly species. Berry Creek was the major study site and was selected because of the presence of large amounts of submerged wood debris as well as a high proportion of C. integrum. The area where intensive sampling was carried out had many riffles as well as large amounts of submerged wood (Fig. 3).

Substrates selected for comparison were wood debris (greater than 6cm dia.), stones (greater than 15cm dia.) and "composite substrate" which comprised wood debris less than 2cm dia., stones less than 6cm dia., silt, roots and leaf debris. Monthly samples were taken from July 1979 to June 1980. A minimum surface area of  $1\text{m}^2$  was sampled each time for each substrate. Wood and stones were washed thoroughly into a bucket and the contents filtered through a

seive of mesh size 8meshes/mm. This procedure ensured that small larvae were taken in the sample each time. "Composite substrate" samples (henceforth referred to as kick samples) were collected by disturbing the bottom substrate and collecting all dislodged material with an aquatic net (4meshes/mm) which was held downstream of the disturbed area. Collection of the sample after this was similar to that carried out for wood and stones.

Comparable areas of each substrate type were sampled each month and number/mm<sup>2</sup> collected on each substrate was calculated. The surface area of substrate sampled was determined from the formulae:

$$\text{Surface area of wood} = 2\pi r^2 + \pi 2 rh$$

$$\text{Surface area of stone} = 4\pi r^2$$

Samples were preserved in 70% ethanol and sorted in the laboratory according to species (C. integrum and I. nitidus) and genus for the other mayflies. The proportion of each taxa occurring on wood was expressed as an index (N), calculated as:

$$N = \frac{\text{number collected on wood}}{\text{number collected on wood, stone, and kick sample}} \times 100$$

This value was calculated for each month for all the mayfly taxa collected at Berry Creek. A  $\chi^2$ -test was carried out to determine the significance of these values so that an indication of the association of the species with wood could be determined.

Samples collected from the above three substrates were also used for life cycle studies. The life cycle patterns were studied using both size class and developmental stage data. Environmental factors result in larger larvae that may be physiologically younger, besides there being size differences amongst the mature female and male larvae. Thus, the following developmental stages were used and are a modification of that employed by Lehmkuhl (1969). These

are based on the physiological age of the animal: Stage A:--no wing pads developed and larvae had the full complement of gills at this stage. (No larvae without gills or with fewer than seven gills developed were collected and is thus an indication that the newly hatched individuals were not collected in the samples.) Stage B:--fore wing pads developed but not the hind wing pads. Stage C:--fore and hind wing pads developed but the fore wing pads do not completely cover the hind wing pads. Stage D:--fore wing pads completely cover hind wing pads. Stage E:--larvae with darkened wing pads (referred to as BWP stage by Lehmkuhl, op. cit).

Size classes were all 1mm in range except the first which was 0-2mm. Most of the larvae collected in the 0-2mm range, however, were actually greater than 1mm in body length. Body length was measured with a dissecting microscope and an ocular eyepiece. Measurements were made to the nearest 0.1mm and body length did not include the cerci. The mayfly taxa examined were: C. integrum, I. nitidus, E. (Iron) spp., Baetis spp., Paraleptophlebia spp., Cinygmula sp., Ephemerella spp. and Ameletus spp.

The life cycle patterns of C. integrum and I. nitidus at Berry Creek were compared with the patterns obtained from samples of these two species collected from tethered sticks at Flynn Creek and Mack Creek. The latter two sites were sampled quarterly for wood degradation studies (Anderson, unpublished) from July 1978 to October 1979. Thus, a comparison of life cycle patterns at sites differing in physical characteristics was made.

Emergence traps of the kind shown in Figure 5 (bottom area =  $0.25 \text{ m}^2$ ) were set at Berry Creek from April 1979 to August 1979 and from February 1980 to June 1980. In 1979, twelve traps were set in pools and riffles along the entire length of the stream. In 1980, six traps were placed over wood (greater than 6cm dia.), stone (greater than 15 cm dia.) and "composite substrates." All traps in the 1980 study were placed in riffles (Fig. 3). The 1979 study was

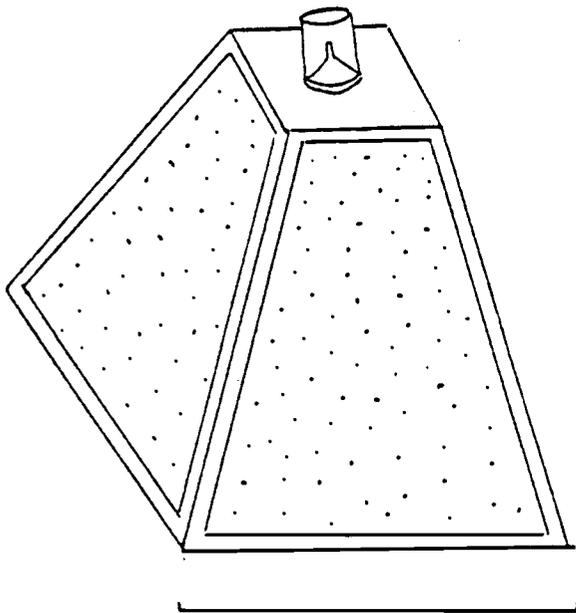


FIG. 5. Emergence trap used at Berry Creek.

designed to study mayfly preference for pool or riffle biotopes prior to emergence. The 1980 study was designed to determine the importance of wood substrates as "emergence platforms" for mayfly species. The collections for the two years were also used to determine the flight period for mayfly taxa at Berry Creek.

Larval growth in the field was determined by plotting head capsule width versus time. Head capsule width is a more reliable measurement than body length as this structure is highly chitinized and less susceptible to variations due to shrinkage (Britt, 1963). The results are plotted using modal points of head capsule width measurements joined to form a growth curve. The mode represents the size attained by the greatest number of larvae and is preferable to the mean since the life cycle of C. integrum appeared to have a delayed period of hatching and larvae of almost all size classes were present through most of the year. Changes in the mode are an indication of slow or rapid growth periods (Elliott, 1967). Absolute growth rates cannot be determined by this method. Average monthly temperatures were also plotted to determine the effects of temperature on growth.

Molting and emergence was observed in the field. Fecundity of the imagoes collected in the field were determined for the imagoes by subsampling the eggs of each adult and using a Sedgwick Rafter Cell (Clifford, 1979). to count the eggs. Sexes of larvae greater than 5mm were noted (according to development of the male genitalia) and used to determine the sex ratio of C. integrum.

Productivity of larvae in the field was calculated according to the method outlined by Hamilton (1969). However, dry weights of larvae collected in the field were determined for each size class so that more accurate productivity values were obtained.

#### Laboratory Rearing

Attempts were made to rear C. integrum larvae in the laboratory

from the first instar stage. This was done by artificially inseminating eggs collected from adults that had emerged in the laboratory from mature larvae collected in the field (BWP stage). The procedure followed was that adopted by Clifford (1979). Eggs were mixed with the sperm directly as well as by placing the eggs in a drop of water and then applying the sperm. The fertilized eggs were transferred to a petri dish containing water and cultured at 20°C, 16°C, and 9°C. None of the eggs hatched from this experiment C. integrum larvae were also reared from larvae in the 3mm size class collected from Berry Creek in late September, 1979.

#### Feeding Behavior and Food Consumption

Gross morphology of the mouth parts was studied by examining preserved specimens with their mouth parts intact, as well as by dissecting the mouth parts and observing them under a compound microscope. Live specimens were also examined under a dissecting microscope to determine the function of each component mouth part in order to study their role in the food gathering process. Those mouth parts that were thought to be important in the scraping and gathering process were examined in further detail at a higher resolution using a scanning electron microscope with a resolution between  $\frac{1}{2}$ cm to 5A°. Thus, the ventral surface of the labial palp and the molar surface of the mandible were examined under a scanning electron microscope.

Feeding habits were studied for all stages of development except Stage E. The latter is the last 3 to 7 days of the final instar prior to emergence of the subimago and the larvae are relatively inactive and do not have much food in the gut. Larvae from monthly wood substrate samples were preserved in 70% ethanol so that no further digestion of the food items could take place during transference from the field to the laboratory. In the laboratory the larvae were dissected and the contents of the fore gut were

emptied onto a microscope slide. This was mounted in Hoyers mounting medium and observed under a compound microscope at a magnification of 450X.

As this study attempted to assess the importance of mayfly feeding activity on wood degradation, the following food categories were selected: (1) detritus, which consisted of wood and leaf material as well as all other unidentifiable material, (2) mineral particles, (3) fungal elements--spores and mycelia, (4) diatoms, and (5) filamentous algae. The feeding habits of mayflies collected on wood were studied to determine the presence of wood in their gut as well as to quantitatively estimate the amount of fungal material. Detritus was subdivided into wood and leaf material but this was inadequate for a quantitative distinction as it was often difficult to decide if the material was wood or non-woody in origin (see Results).

An estimate of the amount of fungi ingested by C. integrum and I. nitidus was obtained by calculating the area and volume of mycelia in the gut content of each species. This was necessary since it was observed that there was a large variation in mycelial length ingested by larvae (range of 0.012mm to 0.96mm). Thus, the length and diameter (assumed to be the breadth) of each mycelium observed in three microscope fields was measured. The total area of mycelial material contained in  $1\text{mm}^2$  of microscope field examined was then calculated and an index (P) obtained, using the following formula:

$$P = \frac{\text{total area of mycelia contained in one microscope field } \times 1\text{mm}^2 \times 100}{\text{area of microscope field}}$$

The index P was obtained for all developmental stages and this was converted to total dry weight of mycelia contained in the gut of a larva at a particular instant. Calculations were made as follows using Alexander's (1977) suggestion that the mean diameter of a mycelium is  $5\mu\text{m}$ , the average density is 1.2 and the water content is 70%.

$$\text{Total area of mycelia in foregut} = \frac{P}{100} \times 112\text{mm}^2$$

Assuming that the area of mycelia in the fore gut = half the area of mycelia in the entire gut:

$$\text{Total area of mycelia in the entire gut} = \frac{P \times 112}{100} \times 2\text{mm}^2$$

$$\text{" length " " " " " " " " } = \frac{P \times 112 \times 2 \times 10^3}{100 \times 5} = P \times 448\text{mm}$$

$$\begin{aligned} \text{" volume " " " " " " " " } &= P \times 448 \times \frac{22}{7} \times \left(\frac{5}{2 \times 10^3}\right)^2 \\ &= P \times 0.0088\text{mm}^3 \end{aligned}$$

1000mm<sup>3</sup> of mycelia weighs 1200 mg.

$$\therefore \text{Total weight of mycelia in the gut} = \frac{P \times 0.0088 \times 1200 \text{ mg}}{1000}$$

$$= P \times 0.0106\text{mg}$$

$$\text{" dry " " " " " " " " } = P \times 0.0106 \times \frac{30\text{mg}}{100}$$

$$= \underline{P \times 0.0032\text{mg}}$$

Food ingested by an animal need not always be utilized by the animal. Fecal material was therefore examined to see if the food (particularly fungal mycelia and wood fibres) were actually digested and assimilated by the animal.

## RESULTS

Substrate Preference

The species of mayflies that have been collected at Berry Creek (Anderson, 1975) are: Siphonurus occidentalis Eaton, Ameletus (nr) connectins McDunnough, A. (nr. or) vancouverensis McDunnough, Heptagenia sp., Cinygma integrum Eaton, Cinygmula reticulata McDunnough, Rhithrogena morrisoni (Banks), Epeorus (Iron) albertae McDunnough, E. (Iron) deceptivus McDunnough, E. (Iron) longimanus (Eaton), Ironodes nitidus (Eaton), Baetis bicaudatus Dodds, B. parvus Dodds, B. tricaudatus Dodds, Baetis sp.?, Centroptilum elsa Traver?, Leptophlebia (Blasturus) pacifica McDunnough, Paraleptophlebia bicornuta McDunnough, P. debilis (Walker), P. gregalis (Eaton), P. temporalis (McDunnough), Ephemerella doddsi Needham, E. flavinea McDunnough, E. hecuba Eaton, E. tibialis McDunnough, Ephemerella sp. Of the above 14 genera reported, nine were collected in the present study. The taxa were, C. integrum, I. nitidus, E. (Iron) spp., Paraleptophlebia spp., Baetis spp., Ephemerella spp., Cinygmula sp., Rhithrogena sp., and Ameletus spp.

A total of 4875 mayfly larvae were collected from the three substrates (wood, stones and kick samples) at Berry Creek during the period July 1979 to June 1980. The proportions of mayflies collected on the three substrates were: 43% on wood, 27% on stones and 30% in kick samples (Table 2). Numerical abundance of the different taxa collected was: C. integrum (756), I. nitidus (864), E. (Iron) spp. (555), Paraleptophlebia spp. (324), Baetis spp. (1413), Ephemerella spp. (388), Cinygmula sp. (428), Rhithrogena sp. (113) and Ameletus spp. (34).

From Table 2 it is evident that C. integrum was the most closely associated with wood at Berry Creek. If there were no restriction of this species to wood, we would expect a mean of 33.3% on wood and the rest equally distributed on the two other substrates

TABLE 2. Proportion of the mayfly population occurring on the three substrate types at Berry Creek, July 1979 to June 1980. Numbers in parenthesis indicate total sample collected on all three substrates for the above period.

	Wood	Stone	Kick Sample	Total
<u>Cinygma integrum</u>	76%(575)	15%(112)	9%(69)	756
<u>Ironodes nitidus</u>	43%(370)	32%(280)	25%(214)	864
<u>Epeorus (Iron) spp.</u>	37%(205)	59%(329)	4%(21)	555
<u>Cinygmula sp.</u>	32%(138)	32%(137)	36%(153)	428
<u>Rhithrogena sp.</u>	21%(24)	72%(81)	7%(8)	113
<u>Baetis spp.</u>	46%(646)	20%(285)	34%(482)	1413
<u>Paraleptophlebia spp.</u>	15%(47)	8%(27)	77%(250)	324
<u>Ephemerella spp.</u>	26%(100)	5%(21)	69%(267)	388
<u>Ameletus spp.</u>	24%(8)	32%(11)	44%(15)	34
Total mayfly population.	43%(2113)	27%(1283)	30%(1479)	4875

sampled. However, the mean percentage determined for C. integrum was 76% which is significantly different from the expected value of 33.3% ( $\chi^2$ -test,  $\alpha = .05$ ). None of the other mayflies showed a mean that was significantly different from the expected value of 33.3% ( $\alpha = .05$ ) (Table 2). Therefore, they were found with equal frequency on the other two substrates or restricted to one of the two. I. nitidus, Baetis spp., Cinygmula sp., Ameletus spp. were found with equal frequency on all three substrates. E. (Iron) spp., and Rhithrogena sp. appeared to be restricted to stones whilst Paraleptophlebia spp., and Ephemerella spp. were mainly in the kick samples. I. nitidus was second to C. integrum in terms of relative abundance on wood. However, it appeared to be found with equal frequency on the other two substrates as well. The other taxa showed no preference for wood although Baetis was more abundant on this substrate than either C. integrum or I. nitidus (Table 2). Thus, we would expect these three taxa to play an important role in wood degradation if their biological activity is closely associated with wood substrates and hence implicated in the decomposition process.

Comparisons of mayfly populations on tethered sticks at Berry Creek with those at Flynn and Mack Creek are given in Table 3. In all three streams C. integrum was a dominant component of the wood fauna, although numerically lower than Baetis at Berry Creek, and Baetis and Ephemerella at Mack Creek. I. nitidus was included with Epeorus and thus data are lacking for I. nitidus. Nevertheless, it was interesting to note that at Flynn Creek, Paraleptophlebia was found to be collected in fewer numbers than I. nitidus, E. (Iron) spp. and Cinygmula sp. At Mack Creek it was also observed that Ephemerella was present in large numbers whereas the numbers of this genus collected at Flynn Creek and Berry Creek were small. The Ameletus population at Mack Creek appeared to be larger and the Paraleptophlebia population smaller than at Berry Creek.

The mature larvae of C. integrum at Berry Creek were more closely

TABLE 3. Numbers per m<sup>2</sup> of mayfly taxa sampled on tethered sticks at the three study sites (Sept. 1978 to July 1979, quarterly samples).

	Berry Creek	Flynn Creek	Mack Creek
<u>Cinygma</u>	1231	1207	1068
<u>Epeorus</u> spp.*	716	502	1004
<u>Cinygmula</u>	164	522	512
<u>Baetis</u>	1514	1082	4863
<u>Paraleptophlebia</u>	511	923	24
<u>Ephemerella</u>	156	70	3553
<u>Ameletus</u>	29	33	297

\* Includes I. nitidus

associated with wood than were the earlier instars (Table 4). Many Stage A larvae were collected on stones but it was apparent that as they matured they moved onto wood substrates. Thus, Stage D larvae had a frequency of 88% on wood whereas the recruiting Stage A larvae was only 23%. In contrast I. nitidus was observed to show less of an association with wood as the larvae matured (Table 5). Stage A larvae were most often found on wood (54%) and stone substrates, but as they matured the Stage D and E larvae appeared to have migrated onto the stones and "composite substrates."

The proportion of larvae in the 0-2mm size class that were collected on wood are compared with those of mature larvae (Stages D and E) and adults for all taxa of mayflies in Table 6. The larvae of C. integrum in the 0-2mm size class occur less frequently on wood (51%) than the mature Stages D and E. This phenomenon might be an indication that either the eggs are scattered in the stream and thus become attached to any substrate, or the eggs are preferentially laid on a particular substrate other than wood so that newly emerged larvae are less commonly found on wood substrates. Sampling techniques might also be a reason why few 0-2mm larvae were collected on wood. The adults of C. integrum were, however, observed to emerge from both wood and stone substrates. This indicates that the species does not selectively choose a particular substrate prior to emergence or they might even move over to stones before they emerge. It must be noted, however, that the number of adults collected in the emergence traps was small and we cannot make any conclusive statements with regard to the choice of substrates as "emergence platforms." Number of adults collected from emergence traps were: wood substrates, 23; stone, 10; "composite substrates," 5.

Analysis of the developmental stages of E. (Iron) spp. indicated that the 0-2mm size class was found mostly on wood substrates and very rarely in the kick samples (Table 6). It was difficult, however, to distinguish the species from that of I. nitidus and as stated earlier, numbers of this size class may be over represented.

TABLE 4. Proportion of each developmental stage of Cinygma integrum on wood substrates from Berry Creek, July 1979 to June 1980. Numbers in parenthesis indicate the ratio of number collected on wood to the total number collected on substrates.

	A	B	C	D	E
	%	%	%	%	%
July	56(41/73)	78(25/32)	50(5/10)	57(4/7)	100(4/4)
August	33(13/39)	83(10/12)	90(9/10)	71(5/7)	100(1/1)
September	54(26/48)	91(10/11)	100(13/13)	100(5/5)	100(1/1)
October	68(48/71)	80(8/10)	0(0/1)	71(11/16)	100(3/3)
November	86(22/26)	93(26/28)	96(22/23)	100(5/5)	-
December	100(3/3)	100(2/2)	83(15/18)	75(3/4)	-
January	25(1/4)	100(1/1)	100(11/11)	100(12/12)	-
February	100(8/8)	100(5/5)	68(2/3)	91(20/22)	-
March	0(0/3)	50(3/6)	80(4/5)	93(40/43)	-
April	100(2/2)	-	100(15/15)	88(14/16)	-
May	50(1/2)	100(2/2)	100(11/11)	75(18/24)	100(4/4)
June	75(12/16)	29(3/7)	83(5/6)	92(32/35)	83(5/6)
Mean	23	78	84	88	95

TABLE 5. Proportion of each developmental stage of Ironodes nitidus on wood substrates from Berry Creek, July 1979 to June 1980. Numbers in parenthesis indicate the ratio of number collected on wood to the total number collected on all three substrates.

	A	B	C	D	E
	%	%	%	%	%
July	0(0/9)	-	0(0/2)	29(2/7)	0(0/1)
August	55(1/2)	-	-	-	-
September	-	-	-	-	-
October	75(118/149)-	-	-	-	-
November	28(21/74)	92(12/13)	82(14/17)	75(3/4)	-
December	41(7/17)	45(5/11)	38(6/16)	50(3/6)	-
January	20(2/10)	26(4/19)	38(18/47)	3(1/3)	-
February	44(4/9)	27(4/15)	29(17/59)	43(6/14)	100(1/1)
March	50(3/6)	40(2/5)	30(17/56)	16(7/43)	0(0/4)
April	50(3/6)	67(6/9)	37(13/35)	32(12/37)	8(1/12)
May	0(0/6)	33(1/3)	68(13/19)	83(29/35)	44(8/18)
June	20(1/5)	100(1/1)	50(1/2)	0(0/9)	0(0/7)
Mean	54	46	39	34	23

TABLE 6. Proportion of 0-2mm size class larvae, Stage D and E larvae and adults collected from wood substrates at Berry Creek, July 1979 to June 1980. Numbers in parenthesis indicate the ratio of absolute numbers collected on wood to the total number collected on all three substrates for the entire sampling period.

	0-2mm	Stage D	Stage E	Adult
	%	%	%	%
<u>Cinygma integrum</u>	51(18/35)	84(166/196)	84(16/19)	44(14/32)
<u>Ironodes nitidus</u>	45(81/181)	38(63/167)	31(10/32)	69(24/35)
<u>Epeorus (Iron) spp.</u>	45(81/181)	63(31/49)	53(8/15)	20(1/5)
<u>Cinygmula sp.</u>	83(85/78)	33(17/51)	33(1/3)	39(9/23)
<u>Rhithrogena sp.</u>	89(8/9)	69(64/93)	50(3/6)	-
<u>Baetis spp.</u>	62(123/199)	43(179/188)	39(34/86)	43(111/260)
<u>Paraleptophlebia spp.</u>	94(84/125)	78(147/188)	95(19/20)	44(17/39)
<u>Ephemerella spp.</u>	67(84/125)	48(59/124)	20(6/29)	14(1/7)
<u>Ameletus spp.</u>	100(1/1)	85(11/13)	100(1/1)	-

there is an apparent migration of these larvae from wood to stone substrates as they became mature. Emergence trap data indicate that emergence took place mainly from stony substrates. According to size class distribution, the small larvae are present from September to January but growth appears to be slow during fall and early winter. Rapid growth was observed in late winter and spring. It is evident that this species does not show the same growth pattern as I. nitidus. Whereas I. nitidus hatched in fall and growth continued through the winter, E. (Iron) spp appeared to hatch mainly in the fall with an apparent delayed growth period until spring. From then onwards growth was rapid with emergence beginning in late spring and continuing until early summer.

Cinygmula sp. at Berry Creek was found with equal frequency on all three substrate types. The 0-2mm size class was commonly found on wood (Table 6) and emergence of adults was observed mainly in traps that were placed over stony substrates. The 0-2mm size class was present throughout the year but was most abundant in late summer and early fall. Mature Stage D larvae were present from September to December and then again from February to June. Emergence of adults was observed in the spring and continued until early summer. It is interesting to note that Kraft (1964) did not collect any adults in emergence traps even though in the present study this taxon was commonly collected in traps.

Similar substrate preference patterns as that observed for Cinygmula was evident for Rhithrogena sp. as well (Table 6). The 0-2mm size class was common on wood (89%) but Stage D and E larvae were most common on stones which indicates that emergence took place from stony substrates rather than from wood.

Baetis larvae in the 0-2mm size class were more abundant on wood substrates than on stones or in kick samples (Table 6). The Stage D and E larvae were, however, commonly collected from stones and kick samples. The preference of Baetis larvae for wood as an

"emergence platform" does not seem to be significant if proportions emerging from the three substrates are compared. If absolute numbers are examined, a considerable number of adults do use this substrate for emergence (Table 6). Baetis species were all grouped together in this study and thus nothing precise can be said about their life cycle patterns.

Paraleptophlebia larvae in the 0-2mm size class appear to be most common on wood substrates (Table 6) which might indicate that considerable hatching takes place either on wood substrates or near them. The 3-5mm larvae were collected mostly from the kick samples and as maturity was attained the larvae were once again observed to migrate back to the wood substrates and emerge from them.

At Berry Creek, C. integrum and I. nitidus were found on wood which has sufficiently rough surfaces with crevices and grooves. This may be a strategy adopted by the larvae to overcome the effects of current. It is also possible that the food resources accumulate in this microhabitat and thus they tend to occur within these grooves or below bark. If bark was absent the larvae of C. integrum were most often observed on the lower surfaces of the wood whereas I. nitidus was often collected from the upper surfaces of wood as well. This may be related to the ability of the latter species to grasp the substrate more efficiently than C. integrum. A study made by Iverson (1972) indicated that I. nitidus was rarely found in the gut contents of fish and he suggested that this was due to the fact that these larvae clung to the mouth of the predator and resisted being swallowed. The tarsal claws of I. nitidus are better developed than that of C. integrum (Fig. 6) and this helps the former to hold onto the substrate more tenaciously than does C. integrum. Thus, in very fast-flowing waters C. integrum was less common, although I. nitidus was present in considerable numbers.

Very rarely were the two species found on wood that was soft and in a highly decomposed state. Larvae were most abundant on wood

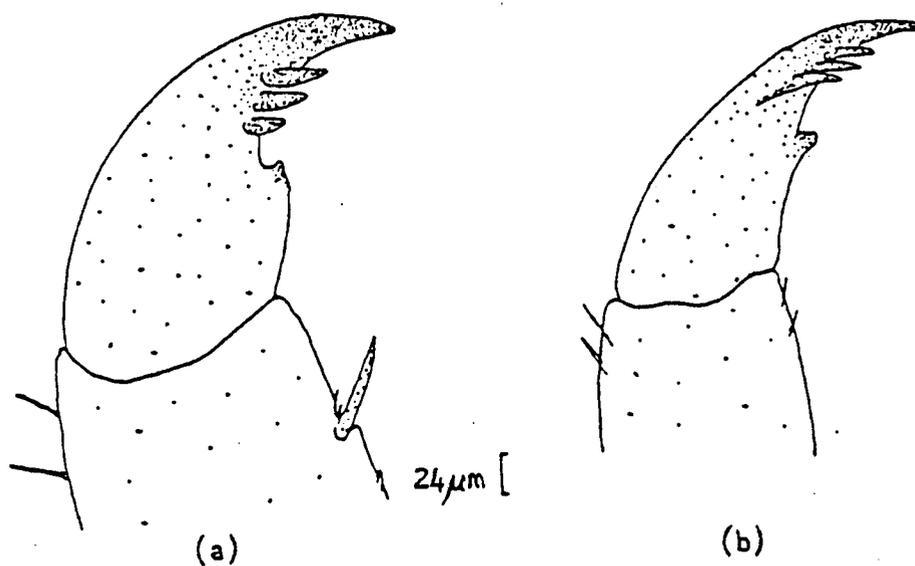


FIG. 6. Tarsal claw of the foreleg of (a)Irónodes nitidus  
(b)Cinygma integrum larva. (Both larvae had a body  
length of 7.5mm and were Stage B larvae).

that was firm but had a grooved outer surface or a layer of bark around it. Similar substrate preferences were observed for Povilla adusta where very soft tissues were least attractive (Petr, 1970), and larvae were most abundant below the bark of wood (Bidwell, 1979). Adaptations for living on wood substrates thus appear to be morphological. The flattened body characteristic of heptageniids enable them to cling closely to the substrate. The dark coloration of C. integrum larvae may also be a protective adaptation for living on wood that is usually dark in color. A simple experiment carried out in the laboratory showed, however, that C. integrum larvae preferred wood for reasons other than just its color. Wood, stones and leaves of a dark color were placed in a white tray with five larvae of different developmental stages. There were three replicates and observations were made five times a day for three days. In the 75 observations made, only three larvae were found on stones and one larva on the leaves. It is likely that the larvae were selectively found on wood because of the food resources present on it. Feeding strategies adopted by these larvae for harvesting the resources found on wood are discussed later.

#### Life History Studies of Cinygma integrum

Even though a fine meshed seive was used for collecting the samples at Berry Creek, the smallest larva of C. integrum taken for the entire sampling period was 1.2mm, whereas that for I. nitidus was 0.8 mm. The larvae of C. integrum at this stage had the full complement of gills and were light in color. It is possible that either the small sizes were present on substrates other than those sampled in this study or that the smaller larvae escaped through the seive and therefore the newly emerged larvae were not collected in the samples. As attempts to incubate eggs in the laboratory were not successful, no data are available on the early post-embryonic development of these larvae. Larvae in the 0-2mm size class were

present in almost all months. However, substantial numbers were observed only during the months of June and July and November and December (Fig. 7).

It is difficult to study life cycle phenomena using size classes alone as trends for growth may be apparent, but increase in size may not always be an indication of maturation. Environmental factors (e.g., temperature) may result in larger larvae but not necessarily in a mature stage of development. In addition, as noted earlier, male and female larvae differ in size as they become mature (females being larger than the males). Age classes or developmental stages were thus employed to further supplement conclusions made from the size class data.

Considerable overlap existed in the range of body length of the five stages: Stage A, 0-4.4mm; Stage B, 3.2-5.3mm; Stage C, 4.8-7.7mm; Stage D, 7-12mm; and Stage E, 7.9-13.5mm. Male larvae in the D developmental stage had a body length range of 7-11mm whereas female larvae were larger and ranged between 7-13.5mm. Stage E larvae showed a more distinct size variation between the two sexes. Thus, male larvae with darkened wing pads measured between 7.9-12mm whereas the female larvae were between 9 and 13.8mm. In early spring, Stage E females had a mean length of 11.8mm and males, 10.1mm. In summer, the larvae were on the average smaller and mature Stage E females had a mean body length of 10.3mm, whereas the males had a mean of 8.5mm. Thus, larvae that hatched in early spring and summer attained a larger body size the following spring (maximum observed, 13.8mm) than those that hatched in the fall (maximum observed, 9.6mm).

Ide (1935) found that Epeorus pleuralis (Banks) larvae collected earlier in the year in Ontario were larger and as the emergence season proceeded the individuals collected were smaller with the minimum size attained at the peak of the emergence period. In this study, however, it was observed that at the beginning of the emergence period, the largest larvae were in the 11-12mm size class. As

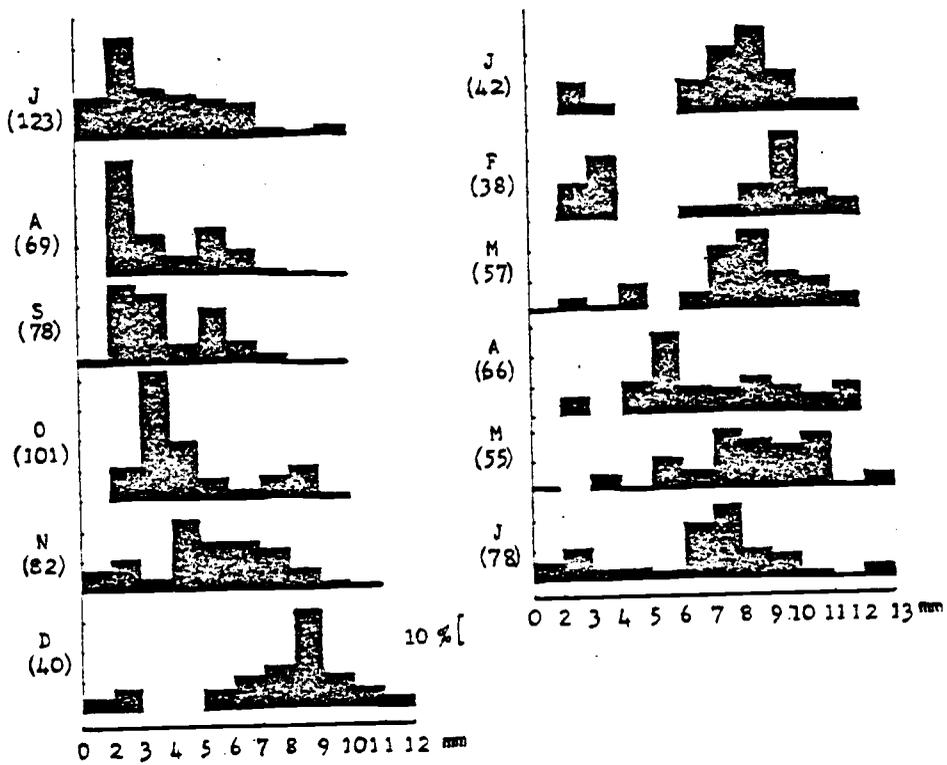


FIG. 7 .Percentage composition of size classes of *Cinygma integrum* larvae at Berry Creek, July 1979 to June 1980. Numbers in parenthesis indicate sample size.

the peak of emergence approached, the body size appeared to increase to the 12-13mm class and decreased once again as the warm summer months approached.

Figure 8 depicts the life cycle of C. integrum based on the percentage composition of the various developmental stages. Although all stages were collected through most of the year, the trend was for Stage A larvae to occur from July to October, followed by a peak in the intermediate Stages B and C in the winter (December, January) and the mature larvae (Stages D and E) from February to June. The extended duration of Stages D and E results in a long emergence period from March to August.

A third approach to the analysis of life cycle pattern of C. integrum is based on a plot of head capsule width versus time (Fig. 9). It was evident that there was an indication of bimodal distribution of the population at least during some months of the sampling period. The waisted histograms in Figure 6 also suggest this interpretation. Therefore, a plot of the first and second modal points were made as shown in Figure 9. The data suggested the presence of a second cohort with Stage A larvae of this cohort observed in November. Stage B and C larvae were common in December and January. Stage D larvae were present from February to October and Stage E larvae were only collected in October.

Based on the above three methods of data analysis, I suggest that C. integrum has a univoltine life cycle. However, there is an extended period of egg hatching and non-synchronous growth rates of larvae (which are perhaps temperature-related). This results in an apparent second small cohort of larvae depicted in Figure 9 by a dotted line. Thus, the life cycle pattern of C. integrum at Berry Creek appears to be similar to Hynes' (1970) slow seasonal (S1) type and lasts between 10-12 months.

It seems probable that the extended occurrence of all size classes of larvae is temperature-related. Eggs appear to hatch

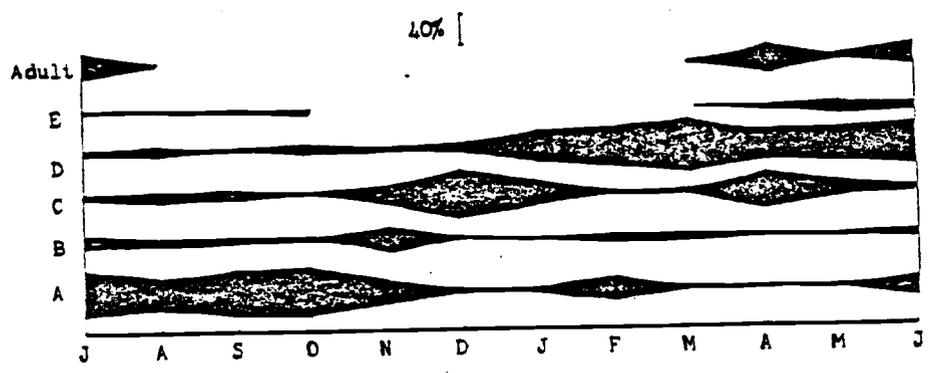


FIG. 8 .Percentage composition of the developmental stages of Cinygma integrum at Berry Creek, July 1979 to June 1980.

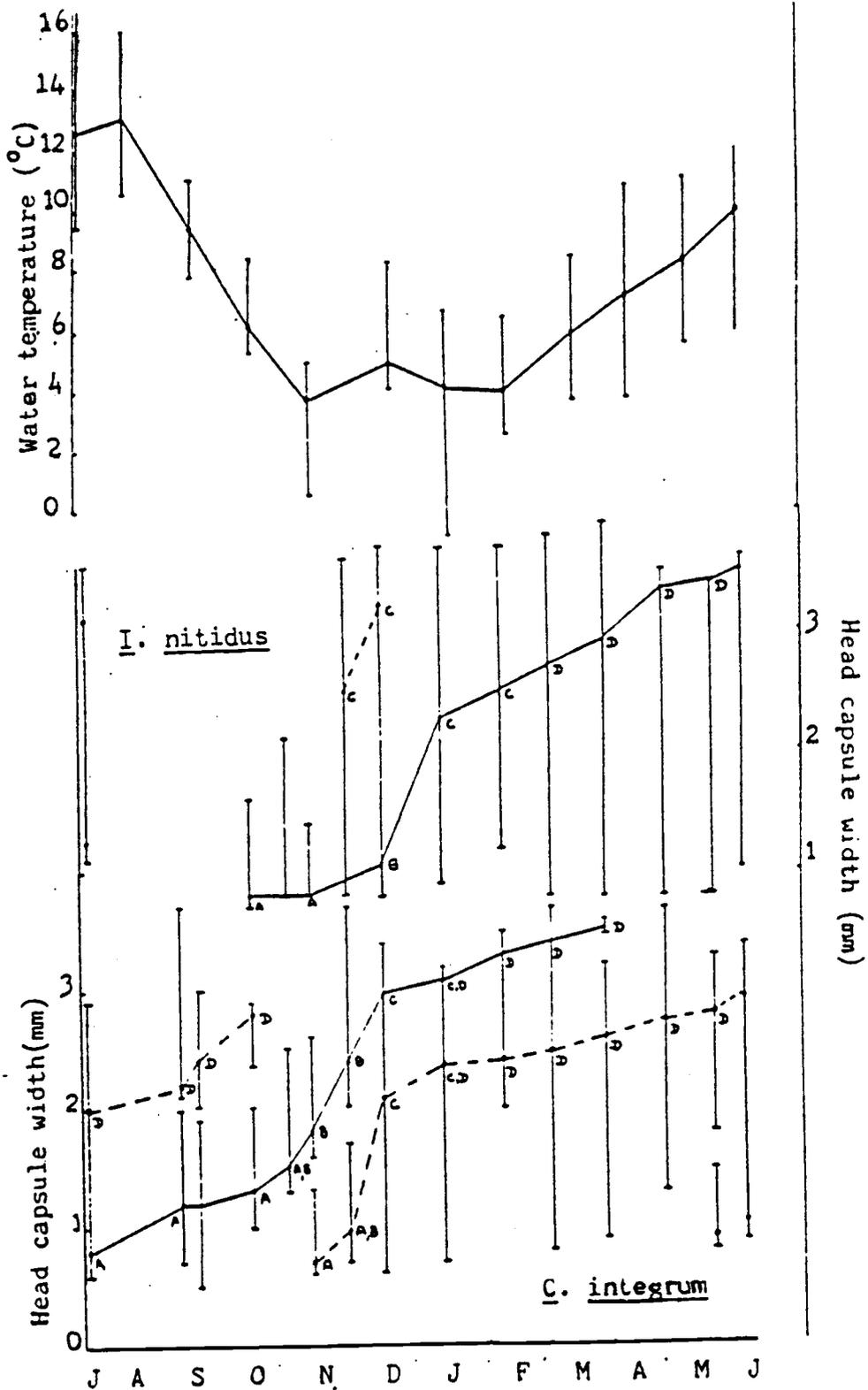


Fig. 9. Range and modal values of head capsule width of Cinygma integrum and Ironodes nitidus at Berry Creek, July 1979 to June 1980. A, B, C, D, E represent the predominant developmental stage at each modal point. Maximum, mean and minimum water temperatures are also given.

TABLE 7. Number of larvae in each developmental stage of Cinygma integrum collected from wood substrates on a seasonal basis, Berry Creek, July 1979 to June 1980. Numbers in parenthesis indicate number collected on all three substrates.

	Stage A	Stage B	Stage C	Stage D	Stage E
	%	%	%	%	%
July-Aug.	66(128)	37(51)	19(26)	41(49)	7(11)
Sept.-Nov.	96(145)	44(49)	35(37)	20(26)	4(4)
Dec.-Feb.	12(15)	8(8)	28(32)	35(38)	-
Mar.-May	3(7)	5(8)	30(31)	72(83)	4(4)

throughout most of the year with maximum hatching in the summer and fall and relatively fewer in winter and spring (Table 7). The major cohort results from the earlier hatch with slow growth when water temperatures are high in the summer months (Fig. 9) and the larvae reach Stage B by the end of fall. The growth rate appears to increase during winter with Stage D occurring by January and February. This stage lasts about 2-3 months so that when the larvae are mature (Stage E), water temperatures have increased (7-12°C) and also air temperature, making adult survival possible. Adults occurred from March to August with a major peak in April and a smaller one in July (Fig. 8). The second larval cohort appears distinct in the early stages but begins to converge with the main cohort by the time Stage D is reached (Fig. 8).

Larvae have darkened wing pads for a week or less and are inadequately sampled in a monthly collecting program, which may explain why a peak of Stage E larvae were not detected before that of the adults in April (Fig. 8). Small numbers of Stage E larvae occurred in July and August, followed by a slight increase in September and October. This pattern could be explained by a hypothesis that high temperatures retard moulting to the Stage E. Thus, if Stage D larvae do not emerge in early summer along with the main cohort, they remain in Stage D (perhaps with additional moults) until water temperatures begin to decrease again in the fall. However, adults were not collected in the field during this period so it is difficult to say if this secondary cohort makes any contribution to the population at all. In the field a subimago was observed to emerge from the sample collected in October which might indicate there is some flight activity of this species in the fall.

Emergence trap data indicate that adult emergence of C. integrum starts in March and continued until August, with two peaks in April and July. Emergence traps set out in pools and riffles suggest that this species showed no significant preference of riffles over

pools (Fig. 8). Kraft (1964) indicated that heptageniids appeared to move to slower waters prior to emergence.

Laboratory rearing of C. integrum helped to substantiate the field data on duration of the life cycle. Preliminary trials in a cool room at 15.6°C resulted in a high mortality and little, if any, growth. However, in a growth chamber at 9°C, 3mm larvae developed to maturity in about nine months. There were differences in the experimental conditions between the cool room and growth chamber. Inadequate aeration, due to the microtubules being often out of the water, appeared to result in high mortality in the cool room. At 9°C, it is possible that the larval feeding rate was not greater than the rate of growth of the aufwuchs. Therefore, the quality and quantity of the food resource did not vary through the week so that the larvae were fed on an adequate diet. It is probable that at 15.6°C as the larvae fed on the aufwuchs there was not an equal amount of food material being replenished. High temperature might have been a factor that reduced the growth of the aufwuchs and, thus, even though at the beginning of the week there was adequate nutrition for the larvae, as the week advanced, the diet became inadequate for growth and survival of the larvae. Therefore, success cannot be totally ascribed to the lower temperature, although 9°C is obviously suitable for both growth and maturity.

Larvae of 3mm body length remained in Stage A for a further 75-90 days at 9°C and averaged four moults. Thus it is likely that the duration of Stage A would be about 4-5 months if the larvae were reared from the first instar. The actual number of moults would have been about 15 moults since Ide (1935b) found that at the 8th instar, all the gills had developed in the heptageniid species Stenonema canadense. Duration and number of moults that were obtained in the laboratory for the other developmental stages are: Stage B, 48-60 days, with an average of 3 moults; Stage C, 60-75 days, with an average of 4 moults; Stage D, 68-82 days, with an average of 3 moults;

Stage E, 3-7 days. Thus, the total duration of the larval stages reared from the first instar at a temperature of 9°C in the laboratory would be between 10 to 12 months, with an average of 25 moults. The duration of the larval stage in the laboratory is comparable to that observed in the field where an approximate period of between 10 to 12 months was determined (Fig. 9). The laboratory-reared adults were of similar size to those emerging in the field. A mean fore-wing length of 13mm was obtained for the laboratory-reared adults (n=3) and 12.5mm for field collected adults (n=3). Laboratory-reared females were found to have an average of 967 eggs (n=3) compared with 1148 for field-collected female imagoes (n=6).

Comparison of life cycle patterns for C. integrum in the three study areas indicated that recruitment at Flynn Creek occurred in late summer and continued through the winter. This was also the case at Mack Creek, except that fewer larvae in developmental Stage A were present in late winter indicating that the hatching period at this site may be shorter than that observed at the other two sites (Fig. 10). Larvae with darkened wing pads were observed at Flynn Creek during September and this might suggest the presence of a prolonged emergence period, or the presence of a second cohort. From Figure 10 it is evident that at Mack Creek, emergence starts in late spring and continues through the summer.

It seems likely, therefore, that there are only minor differences in the occurrence and duration of the developmental stages of C. integrum at the three study sites. Stability of the environment due to absence of freshets and flooding at Berry Creek and/or different thermal regimes may contribute to these differences. Lehmkühl (1979) indicated the presence of a second species at Mack Creek (C. dimicki). Thus, accurate life cycle patterns at Mack Creek may have been obscured if the two species were collected and analyzed together for size class and developmental stages. Sex ratio of larvae (greater than 5mm body length) and adults indicate a 2:1 ratio. Of the 3-7

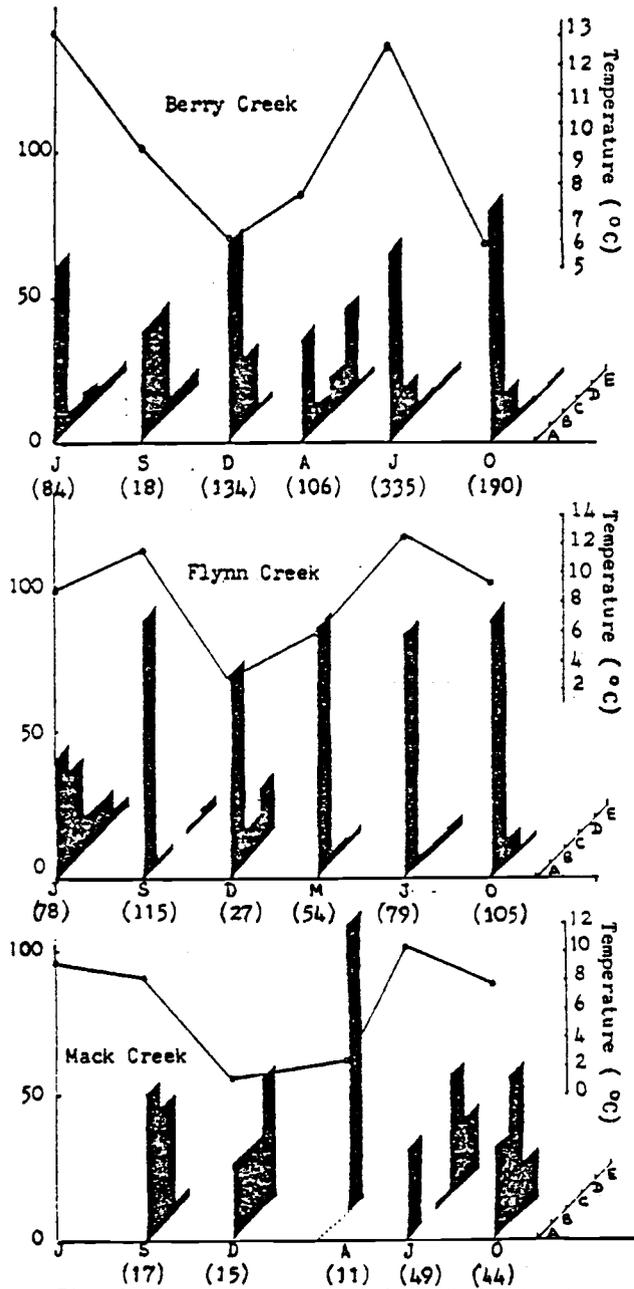


Fig. 10. Percentage composition of the developmental stages of *Cinygma integrum* at the three study sites, from July 1978 to Oct. 1979.

larvae collected in the field, 204 were females and 113 were males. A similar ratio was obtained for the adults where, of the 36 adults examined, 26 were females and 10 were males. A 2:1 sex ratio in favor of females was observed by Hunt (1953) for Hexagenia limbata and he indicated that this might be an adaptation for species in which the males mated more than once. The duration of the imago of C. integrum in the laboratory at temperatures between 9-20°C ranged between 2-10 days with longer duration occurring at lower temperatures. Thus, the adult males live long enough to mate more than once. However, further studies on the mating behavior need to be done before anything conclusive can be said about this matter.

All of the eggs examined were dissected from imagos. The eggs are white, ellipsoidal in shape, and the surface has a rough texture. Dimensions of the eggs were approximately 0.33mm by 0.18mm. By contrast, Needham et al. (1935) give dimensions of only 0.14 by 0.10mm. They indicated that the chorion is covered by very small bumps giving it a granulated appearance, shagreen. They suggest that these bumps are probably adhesive. When eggs in this study were placed in water, they began to adhere to the container after a couple of minutes, indicating an adaptation to prevent them being washed away by the current. Koss (1968) did not include this genus in his taxonomic key of mayfly eggs. He noted, however, that heptageniids lacked polar caps and had an oval-shaped sperm guide. At a magnification of 450X, no sperm guide was observed in this study. This was also noted by him in the genus Stenonema. Koss used various staining techniques which were not used in this study and it might very well be that such structures were not evident since no stains were used.

Attempts to rear C. integrum in the laboratory from artificially inseminated eggs were unsuccessful. A fungus was observed to grow on the eggs after 7-10 days. As a result, further attempts at rearing larvae from the 1st instar were abandoned.

In the field, emergence was observed twice. The subimago emerged from a well-lighted riffle where there was a lot of submerged wood. Emergence took place around 3 p.m. In the laboratory it took place most often in the late afternoon (after 4 p.m.). The larvae attach themselves to pieces of wood or other objects about an inch or two below the water surface. Larvae prior to moulting were observed to become inactive (no feeding) and seemed to prefer well-aerated waters where there was considerable turbulence. The cuticle splits mid-dorsally and the subimago wriggles out of the larval cuticle. The entire process is fairly rapid and takes place between 5 to 10 minutes. The subimago is able to fly as soon as it emerges and usually flies straight up to the ceiling in the laboratory. If subimagoes rest high in the trees, it might explain why no adults were collected in the field with a sweep net. In the laboratory, imagoes were observed to live for 10-15 days at a temperature of 9°C and 5-10 days at a temperature of 16°C. Proper humidity was necessary for emergence of the imago in the laboratory. If the conditions were not humid, the subimago was unable to complete its moult usually because the exuviae did not separate from the wings. Burks (1953) observed that excessive moisture could dampen the wings of the subimago and prevent successful moulting. Excessive drying too, he indicated, was detrimental to the insect and must be guarded against during rearing in the laboratory.

Productivity values of C. integrum at Berry Creek were obtained from specimens collected during the entire sampling period. A total production value of 0.28g/m<sup>2</sup> was calculated using the Hynes-Coleman method as modified by Hamilton (1969)(Table 8). No production studies seem to have been made on any wood-associated insect larvae. Production estimates for other heptageniids have, however, been made by Zelinka (1973) for Rhithrogena semicolorata, 2.8g/m<sup>2</sup> and Ecdyonurus, 1.4g/m<sup>2</sup>. These numbers are high in comparison to the value obtained for C. integrum in this study and perhaps related to food

TABLE 8. Calculation of productivity of Cinygma integrum by the Hamilton Method (1969).

Size Class	Wt. at Loss (mg)	Total No.	Loss	Production x 11
0-3mm	0.06	186	75	51.1
3-4mm	0.20	111	23	22.6
4-5mm	0.38	88	8	33.3
5-6mm	0.80	80	2	63.8
6-7mm	1.04	78	2	81.5
7-8mm	1.91	76	10	145.0
8-9mm	2.58	66	20	170.4
9-10mm	3.26	46	26	149.8
10-11mm	4.16	20	6	83.2
11-12mm	5.37	14	8	75.2
12-13mm	7.00	6	6	42.1
				<u>3094.3mg/11m<sup>2</sup>/year</u>
				Total production= 0.28g/m <sup>2</sup> /yr.

quality. Biomass data for wood-associated insect larvae are available for tethered sticks at Berry Creek (Anderson, unpublished)., and the standing biomass for C. integrum is higher than that of Lara or Heteroplectron (C. integrum, 38.3mg/m<sup>2</sup>; Lara, 17.4mg/m<sup>2</sup>; Heteroplectron, 15.9mg/m<sup>2</sup>). In the present study, biomass of C. integrum was 89mg/m<sup>2</sup>. Petr (1970) found that the wood-associated mayfly Povilla adusta had an average standing biomass of 37g/m<sup>2</sup> in a lake in Africa.

#### Technique for Rearing of Cinygma integrum in the laboratory and percentage survival

Fifty larvae in the 3-4mm size class were collected from Berry Creek in late September and reared to maturity in the laboratory through a period of 9-10 months. The larvae were reared in growth chambers at 9°C and 12 hours daylength. Turbulence and aeration appeared to be necessary for survival and were maintained by a series of hypodermic needles and microtubing connected to an aerator. Pieces of wood were collected from Berry Creek and kept at 9°C and 12 hours daylength in the laboratory. The wood in each chamber was changed each week and replaced with fresh pieces from the stock culture. Fecal material was removed each week with a pipette and fresh water added to the chambers.

Emergence of adults in the laboratory began in March and continued through May. The onset of emergence was similar to that in the field. Mortality was high in the more mature stages (Stages D and E). Mortality occurred most often when the microtubules were accidentally removed from the water and aeration was insufficient. The number of successful emergences was 22 out of a total of 50 larvae (44% survival). This value would have been higher if greater care was taken in seeing that a continuous supply of oxygenated water was available to the larvae. The older, more mature larvae appeared to be most susceptible to deoxygenated waters. Larvae were observed to survive for longer periods of time without aeration

if kept at cooler temperatures than at warmer temperatures. These results indicate that successful rearing of C. integrum larvae in the laboratory is possible. By contrast, other heptageniid species collected in this study did not survive for more than a month under similar laboratory conditions. From the above laboratory rearing technique, it was evident, therefore, that a constant temperature of 9°C and 12 hours daylength was sufficient for growth of larvae that was comparable to that which occurred in the field. Further, it was also apparent that the larvae were able to survive solely on the food resources or the aufwuchs present on the surface of wood.

#### Life History Studies of Ironodes nitidus

I. nitidus shows a distinct univoltine life cycle at Berry Creek. Larvae were absent in September and only two specimens were collected in August. The eggs are laid in spring and summer and appear to undergo a period of quiescence when temperatures are high in the summer. Larvae in the 0-2mm size class are present in October when temperatures become cooler (Fig. 9) and growth occurs during winter and spring (Fig. 11). The smallest larva collected was 0.8mm and the largest was 13.6mm (collected in April). All size classes were present throughout most of the year (Fig. 11). Maximum recruitment was, however, observed mainly in the fall and winter (Table 9; Fig. 12). During winter, growth was rapid with most of the larvae in Stages B and C (Fig. 9). Emergence began in February and continued through the summer (Fig. 12), with peak emergence in May and June.

Maximum recruitment took place in the fall and winter with an average 43 larvae per month (Table 9). In summer and spring the average rate of recruitment was 6 larvae per month. Thus, it appears that the optimal temperature for hatching of I. nitidus eggs occurred during the fall. This was not observed for C. integrum, where maximum hatching occurred in summer and fall. However, even

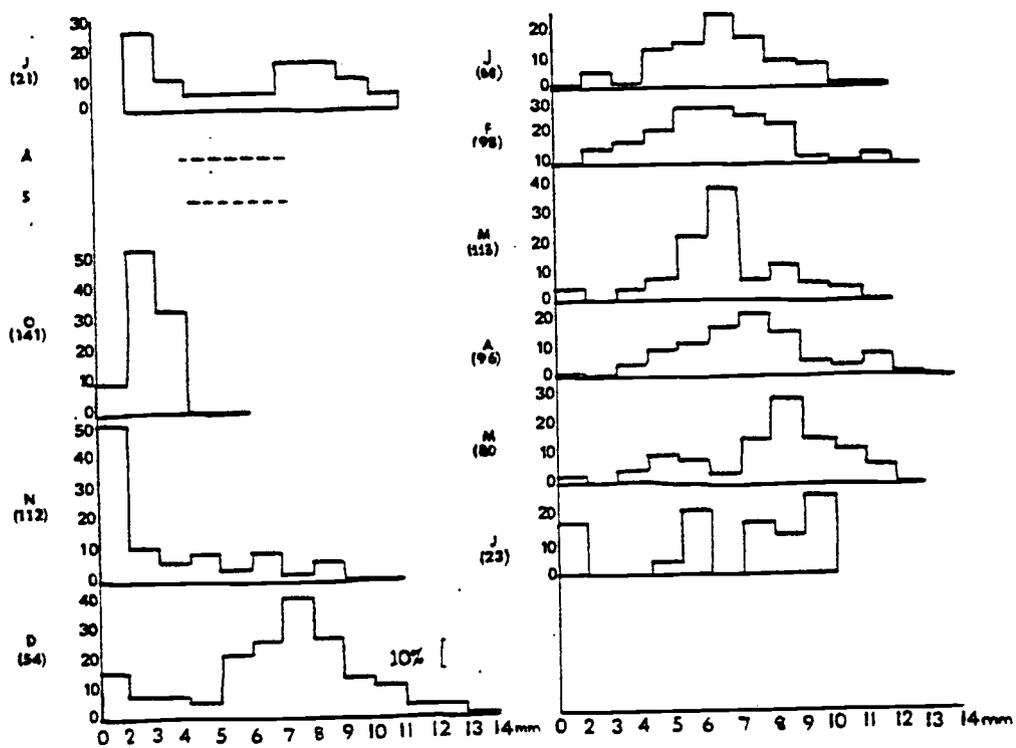


FIG. 11. Percentage composition of size classes of *Ironodes nitidus* larvae at Berry Creek, July 1979 to June 1980. Numbers in parenthesis indicate sample size.

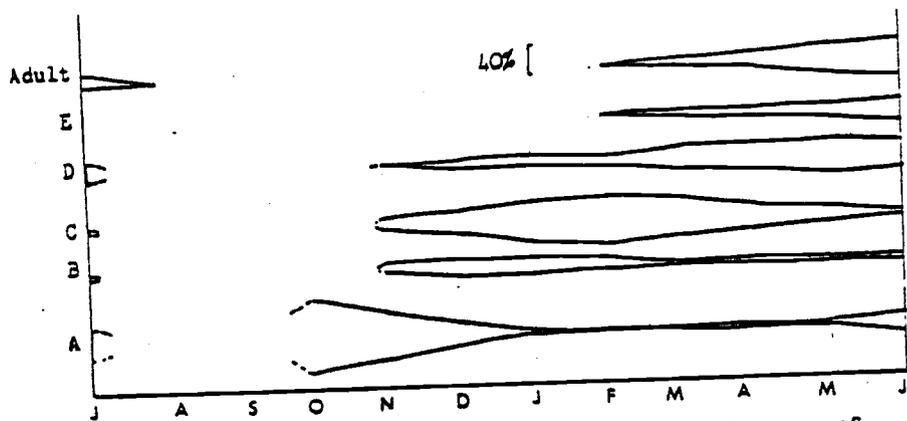


FIG. 12. Percentage composition of the developmental stages of Ironodes nitidus at Berry Creek, July 1979 to June 1980.

TABLE 9. Number of larvae in each developmental stage of Ironodes nitidus collected from wood substrates on a seasonal basis, Berry Creek, July 1979 to June 1980. Numbers in parenthesis indicate total number on all three substrates.

	Stage A	Stage B	Stage C	Stage D	Stage E
June-Aug.	2(17)	0(2)	0(4)	2(11)	0(9)
Sept.-Nov.	106(223)	12(13)	14(17)	3(4)	-
Dec.-Feb.	13(36)	14(45)	41(100)	10(32)	1(1)
Mar.-May	9(18)	9(17)	43(107)	48(115)	9(39)

though hatching begins later in the year for I. nitidus than for C. integrum, growth appears to be more constant for the former species. Emergence begins in February and continues until August. Therefore, it appears that growth rate of I. nitidus is greater than for C. integrum and duration of the larval stage appears to be shorter (6-9 months). The life cycle pattern of I. nitidus is therefore similar to Hynes' (1970) fast seasonal pattern (F1).

Range in body length for the different developmental stages was: Stage A, 0-4.9mm; Stage B, 4.6-5.8mm; Stage C, 5.4-7.5mm; Stage D, 7.2-12.4mm; Stage E, 10.2-13.6mm. Male larvae in the Stage D had a body length range of 7.2-10.9mm and females, 8.3-13.4mm. Larvae with black wing pads ranged as follows: males, 10.2-12.8; females, 10.1-13.6mm.

Growth rates determined from head capsule widths of larvae collected monthly in the field indicated no distinct second cohort even though larvae in Stage C were collected in November and December (Fig. 9). It seems unlikely that these larvae hatched in early fall since a growth rate of at least 1mm per month would have had to occur if this was the case. Thus, it is possible that larvae that hatched earlier in the summer and were unable to complete development before the warmer temperatures occurred and underwent diapause or a period of inactivity in a section of the stream that was not sampled during that period. In general, however, cool temperatures favored larval emergence and growth of I. nitidus. Emergence begins earlier in the season than that observed for C. integrum.

Emergence trap data indicate I. nitidus prefers riffle biotopes to pools prior to emergence. Adults were also observed to select wood substrates more than stone or "composite substrates" as "emergence platforms" (Table 6). Sex ratio of adults collected in the field showed a 1:1 ratio (32 females:39 males). Swarming was observed once in the field in the bypass section. It occurred at about 2:30 p.m. and there were about 25-35 adults on the wing

at an average height of about 3-5m above the surface of the water. In the section of the stream where this was observed, there was hardly any submerged wood and most of the substrate was stones between 6-10cm in diameter.

Attempts to rear I. nitidus larvae in the laboratory were unsuccessful. Larvae seemed to require a highly oxygenated environment as they did not survive for long if the air supply was accidentally removed. The genus Ironodes has been reported to be more adapted to torrential conditions than to slower-moving waters (Edmunds et al., 1976). In the laboratory the larvae were often observed to climb out of the container in which they were reared.

Insufficient data collected at Flynn Creek and Mack Creek for I. nitidus makes it impossible to make detailed comparisons of life cycle patterns (Fig. 13). However, it appears that there are no major differences in the three sites studied and the general trend in the life cycle that was observed at Berry Creek seems to occur at Flynn Creek and Mack Creek.

### Feeding Behavior and Food Consumption

#### Morphology and behavior

A major objective of this study was to determine the relationship of mayflies with wood substrate at Berry Creek. As C. integrum and I. nitidus were shown to be closely associated with wood, it seemed important that a comparison of their mouthpart morphology and behavior were made. For the other genera, a study of the proportions of food types ingested was deemed sufficient to obtain an estimate of the impact of their feeding on wood decomposition and mineralization.

Spieth (1933) described a generalized labium, mandible and maxilla of mayflies. The labium consists of the fused second maxillae and is made up of two jointed palpi, glossae and paraglossae. The latter two structures are flattened and lined terminally with

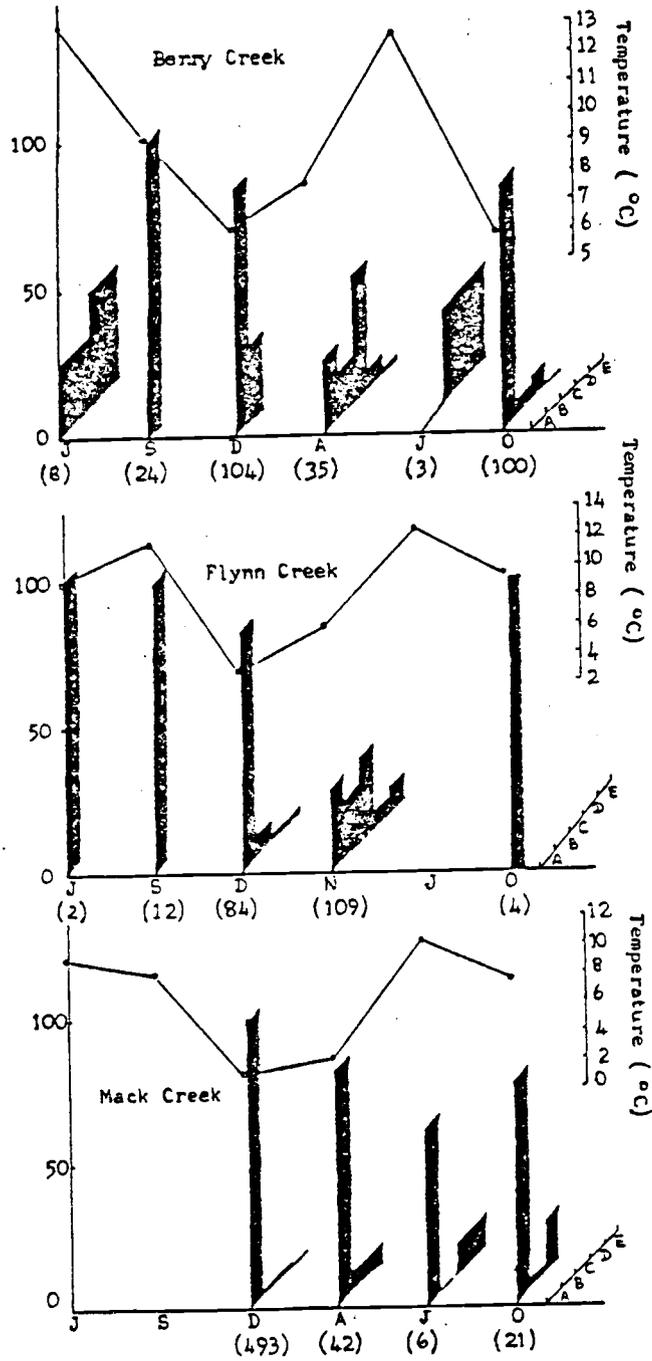


FIG 13. Percentage composition of the developmental stages of *Ironodes nitidus* at the three study sites, from July 1979 to Oct. 1979.

hairs which probably aid in the manipulation of food particles. It is the labial palps however that appear to be most important in the collecting process and are probably the major scraping organs. The palp is a flattened structure, the ventral surface of which is lined with a series of ridges and grooves (Fig. 14). The ridges are actually rows of fine teeth-like structures which are curved ventrally and inwards (Fig. 15). Each ridge is similar to a garden rake with about thirty such ridges per palp. The outer edge of the palp is lined with a dense mat of hairs, which probably prevents the escape of food material out through the grooves. McDunnough (1933) did not attach much importance to the labial palp of C. integrum in feeding and he did not report the structures mentioned above. Strenger (1953), however, observed these teeth-like structures in Rhithrogena.

A generalized mandible consists of a dentation that is divided into an outer, or ventral, incisor area and an inner or dorsal molar surface. The molar surface serves as a grinding organ and is made up of a series of ridges and troughs which alternate with each other (Fig. 16). The incisors are made up of an outer and inner component and probably serve in cutting and grasping the food. In both C. integrum and I. nitidus, the mandibles appear much the same as the generalized form. However, the lacinia mobilis which is a structure present between the incisors and molars are reduced to a few hairs and a seta on the right mandible (Fig. 14). The mandibles are asymmetric. The incisors are located ventral to the molars and each prong bears a number of teeth which probably help in cutting and mastication of food prior to its entry into the mouth.

Each maxilla is made up of a palp and a galea-lacinea. Spieth (1933) describes the distal end of the maxilla as having several "highly chitinized pointed processes referred to as the dentes of the lacinia." C. integrum and I. nitidus have no such chitinized structures though dentes were seen in other heptageniids (Cinygmula,

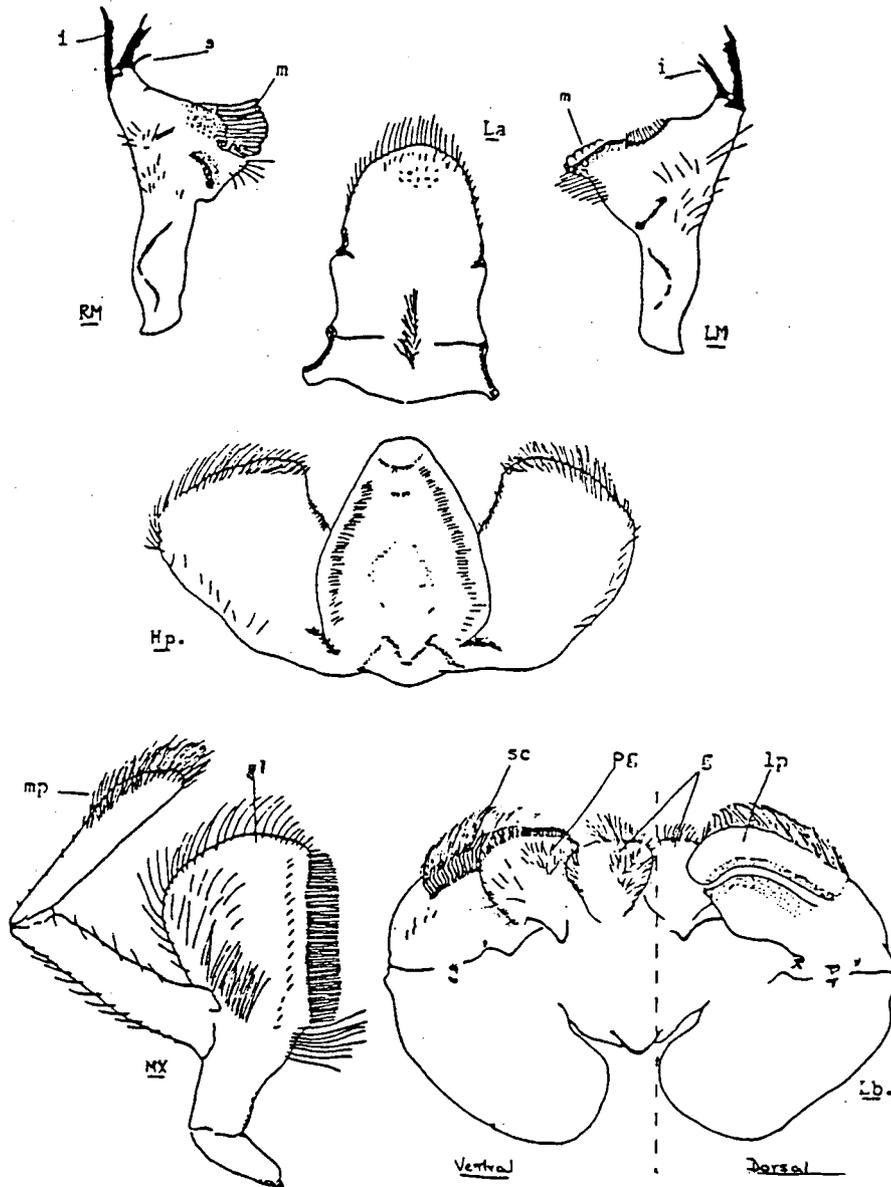


Fig. 14. Mouthparts of *Cinygma integrum*. g=glossae, gl=galea-lacinea, Hp=hypopharynx, i=incisor, La=labrum, LM=left mandible, Lb=labrum, lp=labial palp, m=molar surface, mp=maxillary palp, pg=paraglossa, RM=right mandible, sc=scraper.

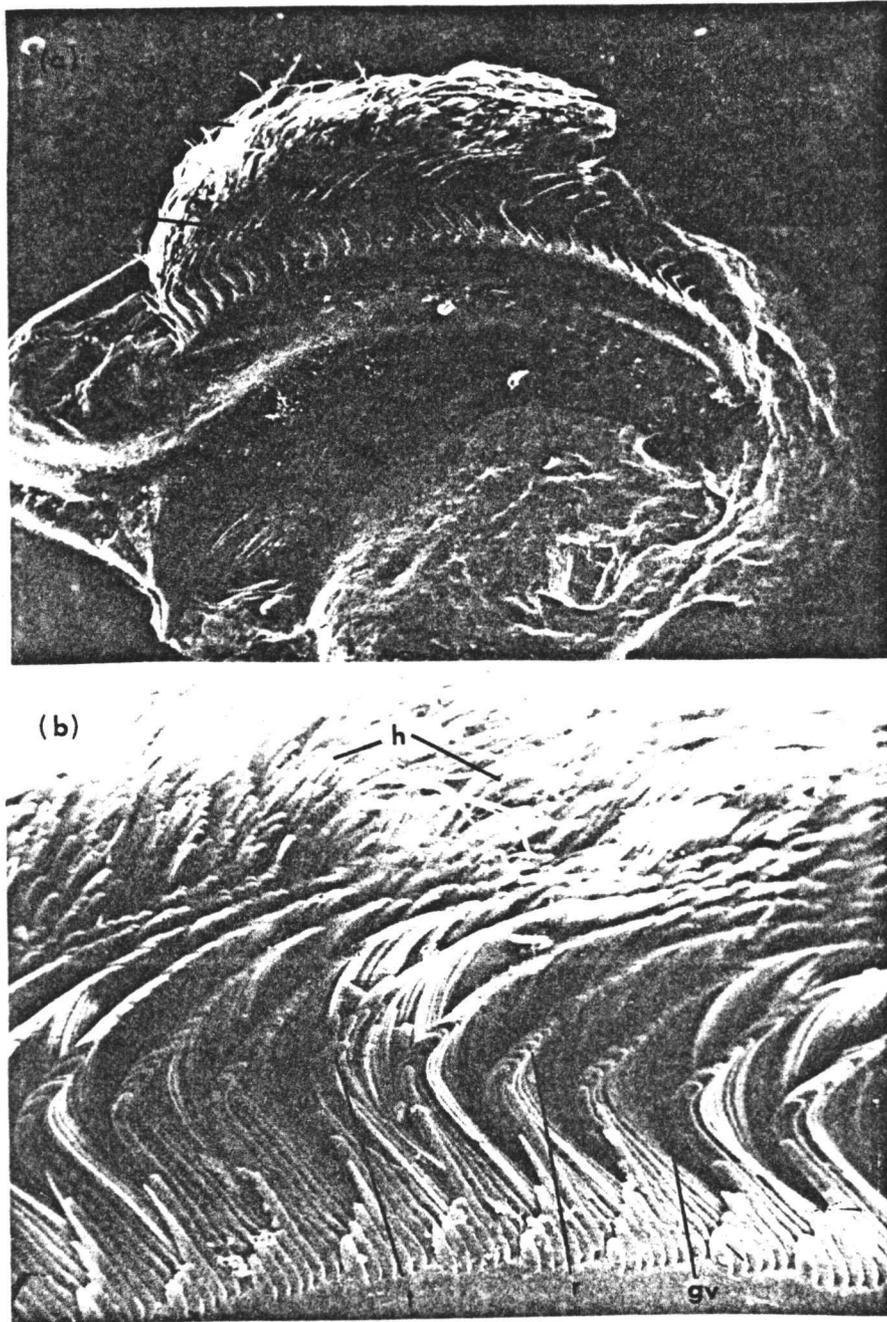


FIG. 15. Scanning electron micrographs of (a) ventral surface of the labial palp (X100) (b) toothed ridges on the ventral surface of the labial palp (X200) of Cinygma integrum.

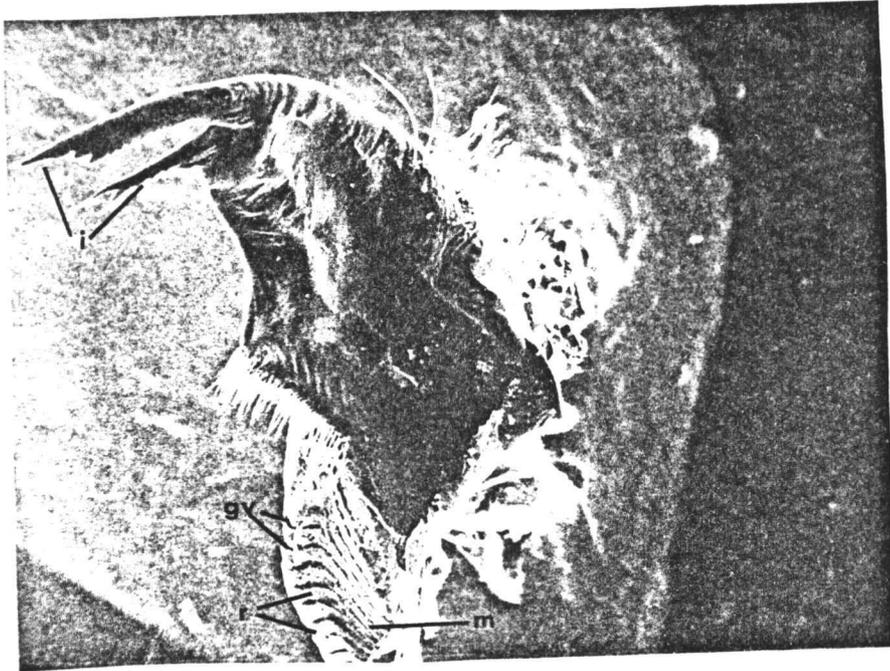


FIG. 16. Scanning electron micrograph of the left mandible of *Cinygma integrum* (X160). g=groove, r=ridge.

E. (Iron). The inner edge of the galea-lacinea bears numerous setae arranged as a strainer for food particles (McDunnough, 1933). It is probable that these setae prevent the entry of food particles that are too large to be handled by the animal without further mastication by the mandibles (Figs. 14, 15). The two-jointed broad, flattened palpi are terminally lined with hairs in both species. In I. nitidus, however, the hairs appear to be more chitinized, shorter and closely arranged and thus, as McDunnough describes (op. cit.), might function as a "diatom raker."

Feeding movements of C. integrum larvae have been observed in the laboratory. The labial and maxillary palps are extended in front of the head and, on making contact with the substrate, the palps make a circular inward movement scraping the food material off the substrate during this process. It is not known if the maxillary palps help in this scraping process. However, it is likely that they propel the material along the hypopharynx (Strenger, 1977). The final guidance to the molars is done by the hypopharynx itself (Strenger, op. cit.) and probably some mastication takes place at the molar surfaces before the food is passed into the foregut. According to Strenger (1977), the food of ephemeropterans is primarily algae and detritus. This material is easily captured with the aid of bristles or hairs that are present over all the appendages that comprise the mouth parts. The function of palps are therefore primarily for guidance of food particles to the mouth. It is evident, however, that in the above two heptageniids studied the mouth parts show modifications for scraping the substrate. In the case of I. nitidus, the anterior margin of the head capsule is lined with a row of closely arranged hairs and this might be an adaptation for collection of food particles. The labrum (Fig. 17) in this species is also much shorter and broader than that of C. integrum, and this structure probably aids in preventing food particles from escaping and also probably helps in manipulation and

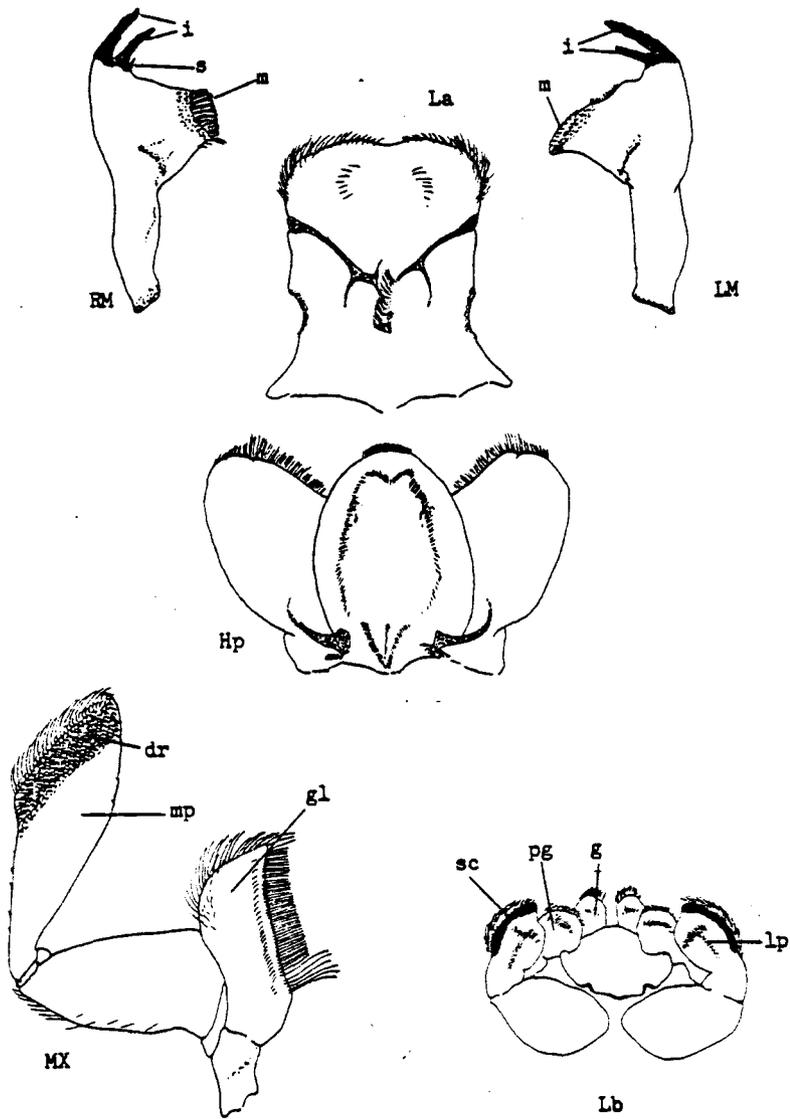


FIG. 17. Mouth parts of *Ironodes nitidus*. Notation as in figure 14. dr=diatom raker.

and transference of food to the mouth.

#### Gut content analysis

A review of the literature revealed that early gut content studies centered mainly around the following food types: diatoms, other algae, detritus and animal material. As this study attempted to assess the importance of mayfly biological activity on the breakdown of wood in aquatic systems, the additional categories of wood and fungus were necessary.

Woody material was distinguished by the presence of tracheids, highly lignified cell walls, pits, and fibers. Unless one of the above characteristics was observed, it was often difficult to say if the detritus was wood or leaf material. However, a rough scan of the entire sample was made and, on the basis of how much wood was present in the entire sample, a decision was made as to whether the particular questionable material was wood or leaf material. This procedure is undoubtedly a weakness in this study since it is often difficult to distinguish between the lignified cells of wood and those present in the veins of leaves. Further, the cortical region of bark can often be mistaken for the parenchymatous cells of leaves.

Fungal mycelia were recognized by the color and consistency of the cell wall and clamp connections. The spores were easily recognized by their thick wall, shape and color. The different types of fungi ingested were not identified (apart from a few which were identified by Professor C. T. Ingold, Birbeck College, England, as no fruiting bodies or perithecia were observed in the gut).

Detritus, which includes leaf and wood material as well as other unidentifiable material, was an important component in the diets of almost all the mayflies studied (Table 10), which has also been reported in previous studies (Chapman and Demory, 1965; Gilpin and Brusven, 1970; Shapas and Hilsenhoff, 1976; Flowers and Hilsenhoff, 1978). As was mentioned earlier, no quantitative studies of

TABLE 10. Percentage composition of food types in the diet of wood-associated mayflies collected on wood substrates (expressed as an annual estimate for all size classes).

	Size range (mm)	Fil. Algae	Diatoms	Fungi	Detritus	Min. Part.	No. in sample
<u>C. integrum</u>	2.9-10.7	2.8	8.4	32.9	41**	16.5	156
<u>I. nitidus</u>	3.2-9.4	1.8	7.2	28.9	47.1**	15.3	104
<u>E. (Iron) spp.</u>	2.4-5.8	9.5	6.0	22.2	52.2*	10.3	10
<u>Cinygmula sp</u>	3.0-6.4	4.0	16.2	20.3	28.7*	21.2	22
<u>Rhithrogena sp.</u>	3.2-6.7	17.3	0.2	24.5	34.6Tr	23.1	4
<u>Baetis spp.</u>	1.9-8.2	2.4	56.1	9.5	22.4*	9.0	34
<u>Paraleptophlebia spp.</u>	2.1-8.4	3.6	9.7	25.3	44.3*	17.4	38
<u>Ephemerella spp.</u>	3.0-6.5	5.4	28.4	17.8	37.6*	7.0	30
<u>Ameletus spp.</u>	3.5-7.2	3.1	8.8	14.5	35.2*	28.9	10

Tr=Trace

\* =Presence of wood

\*\*=Abundance of wood

the wood component were made due to difficulties often encountered in distinguishing leaf and wood material. However, Table 10 indicates that all mayflies, except Rhithrogena, had wood particles in their gut contents, the latter species having only traces of it. C. integrum, I. nitidus and Paraleptophlebia spp. had large amounts of wood material in their guts (Fig. 18), whereas in the other taxa wood particles were commonly observed but were not in great abundance. Therefore, mayflies probably ingest wood to varying degrees, although it is unknown if they selectively ingest such particles or if ingestion is only incidental to intake of other material. Nevertheless, it is evident that mayfly feeding activity does play a part in the conversion of wood CPOM to FPOM. E. (Iron) spp. and Paraleptophlebia showed proportions of detritus, but the latter species had mostly woody material whilst the former had mainly leaf material. Ameletus spp., Ephemerella and Cinygmula most often appeared to ingest large proportions of leaf material rather than wood. Petr (1970) observed that the larvae of Povilla adusta feed upon the planktonic and periphytic algae as well as the detritus. He, however, did observe pieces of wood in the gut contents which he presumed originated from their burrows.

Gut content samples also contained a variety of fungal spores. Tetraradiate spores belonging to the genus Flabellospora (identified by Prof. Ingold) were commonly observed during the period December to March. This could be correlated with the low temperatures that have been observed to induce sporulation (Koske and Duncan, 1974). Other common spores observed were Lemonniera sp?. (fungi imperfecti) (January), Ascospores (common throughout the year), and Deuteromycetes species (February). Many of the spores were, however, unidentifiable (Fig. 19). Amongst the spores that were identified by Prof. Ingold were Dictyosporium, Epicoccum, Dactylosporium and Leptosphaeria (?) (all belonging to the fungi imperfecti).



FIG. 18. Fine particulate wood material in the gut contents of Cinygma integrum (X450).



FIG. 19. Fungal spora and mycelia in the gut contents of Cinygma integrum.

In addition to the wood particles in the guts of mayflies, a considerable amount of fungal mycelia was observed (Table 11) (Fig. 20). C. integrum showed the highest percentage of this food type (Table 11) with I. nitidus second. The origin of this fungal material could be either from leaf or wood tissues. However, since only those larvae sampled from wood substrates were dissected, it is probable that most of the fungi was from wood. In addition, most of the fungal material observed was not closely associated with the detrital tissue and appeared not to be pervasive fungi which are more characteristic of leafy detritus where they ramify through the matrix of the leaf tissue. Larvae of other wood-associated mayflies showed varying degrees of mycelial ingestion (Table 10). The percentages were, however, not comparable with that observed for C. integrum and I. nitidus. Baetis showed the least amount of this food category.

Variations in the percentage of fungal elements ingested by C. integrum and I. nitidus showed seasonal periodicity (Figs. 21, 22), with maximum ingestion observed during winter when temperatures in the stream were low (0-7°C). It is possible that most of these were aquatic hyphomycetes which grow well at cooler temperatures (Thornton, 1953). It is not known if these mayfly larvae selectively feed upon these fungi or if ingestion is incidental. Nevertheless, it is evident that the amount of fungal material ingested is related to seasonal variations either in the growth and abundance of mycelial material in the stream or, conversely, the amount of algae (diatoms) present. It is interesting to note, however, that the amount of fungal component consumed is greater than that of components resulting from primary production (diatoms and other algae) (Table 11, Figs. 21, 22).

Considerable variation in mycelial length was observed and ranged between 0.12mm to 0.96mm. Therefore, a plot of percentage fungal particles versus time may not be a suitable method of studying seasonal patterns of fungal ingestion. An index P (percentage

TABLE 11. Percentage composition of food types in the gut contents of Cinygma integrum and Ironodes nitidus from samples taken at Berry Creek, July 1979 to June 1980.

	Fil. Algae		Diatoms		Fungi		Detritus		Min. Particle		No. in Sample	
	C.i.	I.n.	C.i.	I.n.	C.i.	I.n.	C.i.	I.n.	C.i.	I.n.	C.i.	I.n.
June-Aug.	6.2	0	12.0	0	25.4	12.0	35.1	80.3	22.1	7.8	30	6
Sept.-Nov.	2.1	1.8	9.2	4.9	33.3	25.9	35.2	30.3	26.4	25.2	42	18
Dec.-Feb.	3.5	3.2	9.4	2.5	50.4	59.4	25.8	25.1	12.2	9.3	34	40
Mar.-May	0.4	1.3	6.6	9.9	24.1	18.5	65.8	60.1	7.3	10.1	50	40
Mean	3.1	2.1	9.3	5.8	33.3	28.9	40.5	49.1	17.0	13.1		

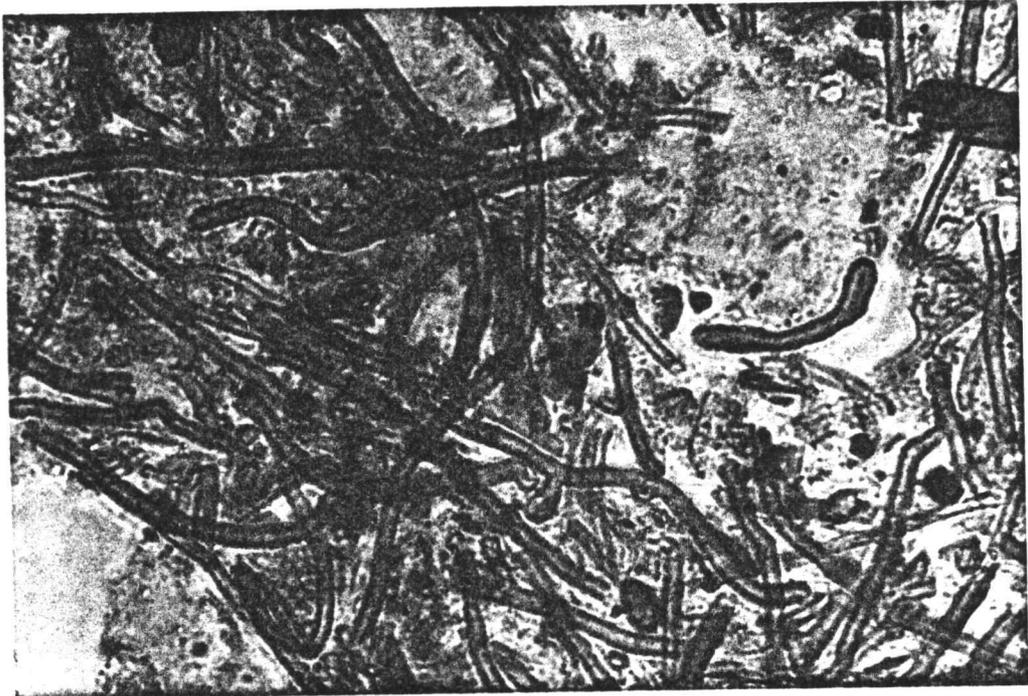


FIG. 20. Fungal mycelia in the gut contents of Cinygma integrum (Upper, X450; Lower, X100).

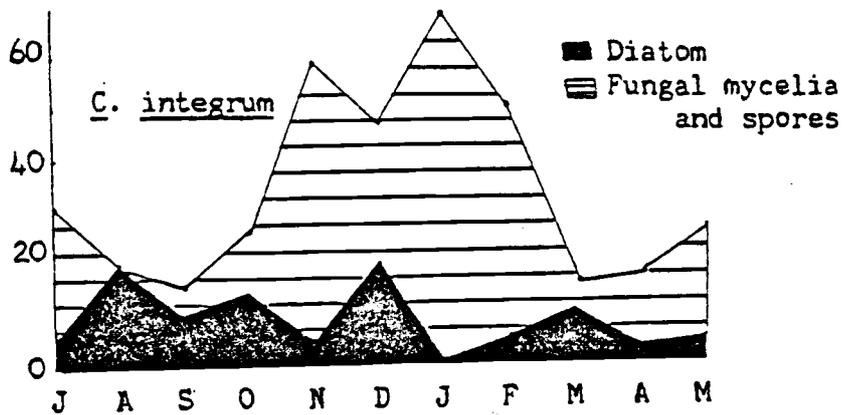


Fig. 21

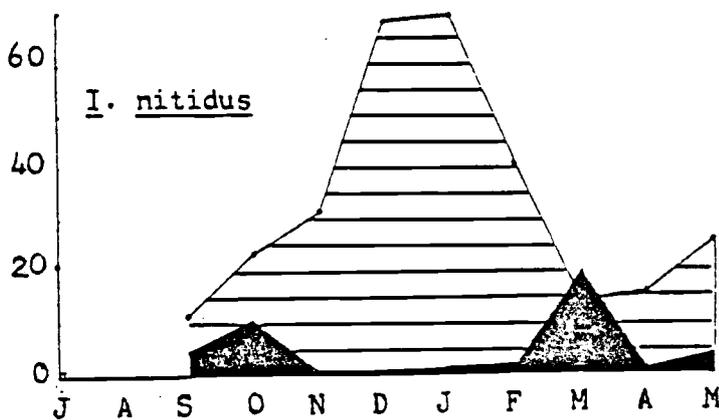


Fig. 22.

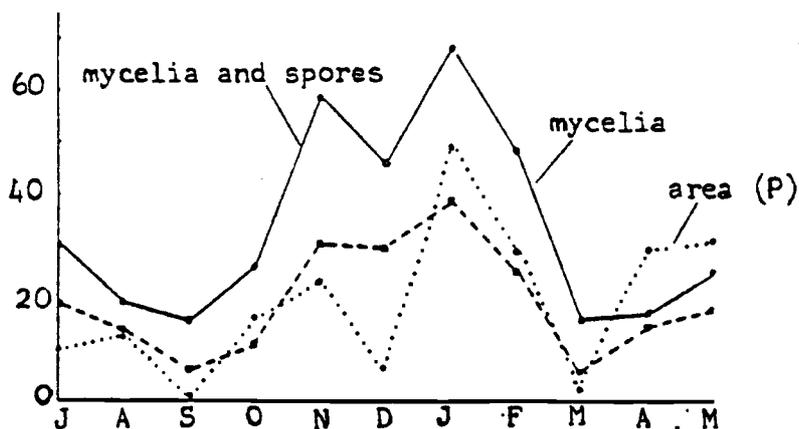


Fig. 23

Figs. 21 and 22. Percentage composition of fungal particles and diatoms in the gut contents of (a) *Cinygma integrum* and (b) *Ironodes nitidus* from larvae collected at Berry Creek, July 1979 to May 1980.

Fig. 23. Percentage composition of fungal elements, mycelia and area of mycelia ingested per  $1\text{mm}^2$  of microscope field (P) for *Cinygma integrum*.

area of fungal mycelia ingested per  $\text{mm}^2$  area of microscope field) was used (see Materials and Methods) and a plot of P versus time for C. integrum (Fig. 23) showed the same general trend of seasonal ingestion of fungal mycelia as that obtained for percentage fungal particles. Figure 22 also shows that the number of spores ingested by the larvae was almost equal to the number of mycelia, which is an indication that these larvae are surface feeders.

Amongst the different developmental stages of C. integrum, the smallest Stage A larvae showed the least ingestion of fungal mycelia ( $0.09\text{mm}^2/\text{m}^2$ , Table 12). The Stage D larvae were found to contain  $0.326\text{mm}^2/\text{mm}^2$  of microscope field examined. This indicates that, either Stage A larvae were unable to graze efficiently on the mycelial mat or they selectively choose detritus and diatoms as food. The mean area of mycelial material ingested by all stages on an annual basis was  $0.18\text{mm}^2/\text{mm}^2$  of microscope field (Table 12).

In order to determine the impact of fungal consumption on wood degradation, the area of fungal mycelia ingested (P) was converted to biomass (dry weight) using Alexander's assumptions (1977) that the average density of mycelia is 1.2, mean diameter is  $5\mu\text{m}$  and the water content is 70% (see Materials and Methods). Thus, from Table 12, larvae are found to contain 5.3% of their body weight at a particular instant of time. Considering that the average gut retention time observed in the laboratory for C. integrum was 2-3 hours, it is probable that the larvae ingest approximately ten times this amount for one day and, thus, a consumption of fungal mycelia equivalent to 53% of its body weight per day.

The gut contents of C. integrum larvae collected from wood were compared with the gut contents of larvae collected on stones during October and March. The fungal material observed in their diet was approximately the same in quality and quantity. This observation might indicate that these larvae were washed onto this substrate or had drifted temporarily onto stones and actually do not selectively choose rock substrates for feeding on the organic layer present on

TABLE 12. (a) Seasonal analysis of percentage area of mycelia ingested per mm<sup>2</sup> of microscope field (P) for each developmental stage. (b) Calculation of mean percentage mycelia (dry weight) per body weight of Cinygma integrum larvae examined.

	A	B	C	D	Total
(a) June-Aug.	5.2	11.4	16.1	14.3	11.7
Sept.-Nov.	5.8	13.7	7.2	30.0	13.8
Dec.-Feb.	2.7	24.6	34.0	63.0	26.3
Mar.-May	22.5	0.4	17.6	23.2	19.3
Mean	9.1	12.5	18.7	32.6	17.8
<hr/>					
(b) Mean dry weight of larva (mg)	0.09	0.38	0.81	3.30	
Dry weight of mycelia ingested (mg)	0.015	0.04	0.06	0.104	
Percentage mycelia/body weight	16	11	7	3	
Mean percentage mycelia/body weight (instantaneous) = 5.3%					
Gut turnover time = 2 hours					
Mean percentage mycelia/body weight (per day) = 53%					

the surface of the substrate. This might be an indication that the association of C. integrum with wood substrates is partly due to the fungal component comprising the aufwuchs. On the other hand it is possible that the fungi present in the organic layer on stones (Madsen, 1972; Karlstrom, 1978) were the same as that observed in the guts of the larvae examined. Until further studies are made of the fungal component on stones and wood, nothing very precise can be said with regard to the feeding of fungal elements from stone and wood substrates.

Larvae of I. nitidus collected from stones, however, showed a considerable amount of diatoms in their gut contents. It is therefore apparent that I. nitidus does not show a preference for fungi over the other food types present on other substrates. The presence of fungi in the guts of specimens collected on wood might indicate that this species is a generalist and feeds on whatever is available in its surroundings.

Exceptionally high proportions of diatoms in the gut contents of Baetis spp. were observed and often the entire field was filled with diatoms. Baetis larvae apparently scrape diatoms off the wood substrate without removing wood particles as well from the surface. Ephemerella and Cinygmula also had considerable quantities of diatoms in their diet (Table 12). The more commonly observed diatoms were Cocconeis, Navicula, Rhoicosphenia, Gomphonema and Synedra. In early spring, Cyclotella was also observed.

A very small proportion of filamentous algae was present in the gut sample of almost all the mayflies examined (Table 10). There were, however, large proportions observed in the diet of Rhithrogena sp. Recognizable algae included Spirulina? Arthrospira (December to April) and desmids (November, December and March). The latter group was included with the filamentous algae even though it belongs to a separate group.

## DISCUSSION

Physical and biological factors influence the distribution and ecology of mayflies. Amongst the physical factors, current speed (Hynes, 1970) and temperature (Ide, 1935a) were reported to be important in determining the habitats of these animals. Linduska (1942) suggested that bottom type may be an important consideration. Conditions of the microhabitat also influence the distribution and depend on the environmental requirements of the species. Distribution thus depends on a combination of factors and includes temperature, water velocity, bottom type, light intensity, water chemistry, food availability and interspecific and intraspecific competition. Thus, morphological, behavioral and physiological strategies have been adopted by the larvae for living in a particular geographical region, habitat and on a particular substrate.

Substrate Preference

At Berry Creek, the distribution of mayflies on the three different substrate types indicated that C. integrum was found mostly on wood and that its association with this substrate was almost obligatory. In the case of I. nitidus, the association with wood substrates was more facultative. This flexibility or non-selectivity of substrate may be partly due to its morphological adaptation for movement from one substrate to another more easily than C. integrum since it is able to resist strong water currents due to the more well-developed tarsal claws. In the case of C. integrum, mobility seems to be more limited as it is not able to withstand high degrees of turbulence. Microdistribution differences between these two species indicated that C. integrum was found in less abundance on the upper surfaces of wood substrates whereas I. nitidus was commonly found in this microhabitat and thus was able to exploit the resources found on the surface of wood as well. C. integrum

appeared to be restricted to the cracks and crevices on wood substrates and was common below the bark as well.

All other mayflies collected on wood substrates showed a facultative association with wood. Baetis was the most abundant taxon on wood but was ubiquitous on all three substrates sampled. This might be explained by the fact that this genus is a highly active swimming form and is reported to be a major component in the drift (Lehmkuhl, 1972; Anderson and Lehmkuhl, 1968). Paraleptophlebia larvae were most frequently associated with "composite substrates." They are adapted to backwater habitats where wood settles out and may use this substrate for purposes of feeding and emergence. Other heptageniids with characteristic flattened bodies would enable them to exploit wood substrates in fast-flowing waters. Low relative abundance of E. (Iron) and Rhithrogena on wood substrates is perhaps due to the fact that stones may seem more "attractive" to them as far as food resources are concerned. It would be interesting to make a study of the aufwuchs layer on stones and compare it with that found on wood in a particular biotope. Comparison of the proportions of these components (fungi, bacteria, detritus and diatoms) on the two substrates and gut analysis of the associated fauna might indicate why some substrates seem more attractive than others to the macro fauna in an ecosystem. These differences could be due perhaps to food preferences but factors such as competition and physical characteristics (oxygen, temperature) need to be considered as well.

#### Seasonal Life Histories

This study is the first comprehensive investigation made of the life history of C. integrum. At Berry Creek, this species has a univoltine life cycle and could be categorized in the seasonal slow (S1) type of life cycle (Hynes, 1970). The eggs apparently hatch over a long period of time. The 0-2mm larvae were abundant in June

and July and November and December. There was a continuous recruitment of Stage A larvae and these larvae grow slowly in the summer with their major growth period occurring in winter and spring. An extended emergence period from March to August was observed, with peaks in April and July.

The long hatching period and/or the staggered growth periods of larvae appeared to be responsible for the presence of two cohorts at the main study site. These phenomena are perhaps related to temperature regimes. Successful rearing in the laboratory from 3mm larvae to adults was obtained at 9°C and 12 hours daylength. The adults obtained from the laboratory rearing were similar in size to those collected in the field. Comparable duration of life cycles both in the field and laboratory (10-12 months) indicate that C. integrum is a cool adapted species.

The presence of two cohorts at Berry Creek of C. integrum could be either due to stability of the environment or to thermal regime. The second cohort arising from eggs that were laid in the fall is able to survive the winter and continue growth through the summer. The study site at Berry Creek was in a controlled flow section, therefore drastic effects such as flooding were not observed. The newly recruited larvae are thus able to survive the winter period because high mortality due to flooding does not occur. Kraft (1964) observed a second cohort for Paraleptophlebia heteronea at Berry Creek. He attributes this to the fact that temperatures in October were similar to those in spring and thus favored the emergence of this species again in October.

At Berry Creek, I. nitidus had a univoltine life cycle and it was evident that it was a fast seasonal (F1) type (Hynes, 1970). There was an extended emergence period which started in February and continued until August, with the peak of emergence in May and June. Hatching did not commence until after the temperatures had become cooler in fall. A distinct absence of larvae in the hot summer

months of August and September is an indication that eggs underwent a period of quiescence when stream temperatures were high. Practically all the larval growth occurred during the months of November to January--a period when the stream is at its coldest. This species, too, appears to be cool-adapted as was observed for C. integrum.

A comparison of the two above heptageniid species thus showed differences in the range of substrate utilized, microdistribution on wood substrates, abundance on wood, as well as differences in life cycle patterns. Thus, interspecific competition between the two species for space and food appears to be minimal.

Temperature, nutrition and competition have been suggested as potential factors controlling growth of larvae (Ulfstrand, 1969; Radford and Hartland-Rowe, 1971). In this study it was evident that temperature was responsible at least in part for the differences in life cycles and growth rates of C. integrum and I. nitidus. Clifford (1970) indicated that the length of duration in which a stage (egg, larva) remained before transforming into the next stage depended on environmental conditions. He also stated that these conditions might not necessarily be the same ones or act to the same degree on each different stage. Thus, it appears that the two heptageniids species studied have differences in the life cycle pattern which are related to the differential effects of temperature on the eggs and larvae.

The egg stage has been shown to be the most resistant stage to high temperatures for many mayflies (Ide, 1935; Macan, 1960). It is apparent that high summer temperatures are not detrimental to eggs of either C. integrum and I. nitidus. Embryonic development of C. integrum did not appear to be affected by high temperatures in the summer since 0-2mm larvae were present during the summer, which is an indication of hatching occurring even at these temperatures. On the other hand, it appeared that the eggs of I. nitidus

were affected by the summer temperatures and no 0-2mm larvae were present in the months of August and September. This phenomenon has been observed for Epeorus pleuralis (Banks) (Ide, 1935), where the egg stage was the only one present during the warm summer months. Bohle (1972) indicated that even in the egg stage, different stages of embryonic development were sensitive to temperature and determined when hatching took place. Therefore, it is possible that the eggs of I. nitidus were all held at a certain stage of suspended development during the summer and hatched in a more synchronized manner when temperatures became cooler. In contrast there was a delayed hatching period for C. integrum but synchronized hatching was not evident.

Humpesch (1978) observed that embryonic development of heptageniids was temperature-dependent and larvae hatch from eggs at different times depending on whether the eggs were laid at different seasons. Larval growth is also dependent on temperature; if larvae emerge when temperatures are optimal for growth, development will be rapid. Thus, in the case of I. nitidus, duration of the life cycle was between 6 to 9 months and was faster than that observed for C. integrum. This could perhaps be explained by the larvae being present at a time of the year when temperature was optimal for growth of the immature stages. Variations in the duration of the life cycle of the two cohorts of C. integrum appear to be a result of these temperature effects.

It is also obvious that different developmental stages of larvae have different optimal temperatures for growth. In both C. integrum and I. nitidus being cool-adapted species, the young larval stages showed rapid growth during winter. This has also been observed for R. semicolorata and E. pleuralis (Macan, 1960; Ide, 1935; Maxwell and Benson, 1963). Warm temperatures, on the other hand, appear to be more favorable for transformation into the mature stage and growth of adult structures (wings and genitalia). Thus, there is some synchronization of emergence

to periods when air temperatures are favorable for adult survival. Earlier emergence of I. nitidus in the season may indicate the tolerance of cooler temperatures by the adults than for C. integrum.

Field data suggest that differences in the life cycle of these two species are dependent at least partly on thermal regime. Additional laboratory and field studies on embryonic development at various temperatures need to be conducted before more conclusive statements can be made.

#### Mayfly-Microbe-Wood Interactions

The decomposition of wood occurs mainly through the combined activity of fungi and macroinvertebrates in terrestrial ecosystems (Ingles, 1933; Savely, 1939; Wallace, 1953; Larkin and Elbourne, 1964; Elton, 1966; Fager, 1968). In the aquatic ecosystem, however, quantitative studies of these interactions between microbial and macroinvertebrate populations have received little attention. Preliminary studies on the qualitative aspects of the macroinvertebrate interactions on wood have been made recently (Anderson et al., 1978; Dudley and Anderson, in prep.; Pereira et al., in prep.). Decomposition of leaf litter, on the other hand, through activities of microbial and macroinvertebrate activities have been the subject of considerable research in aquatic habitats (Kaushik and Hynes, 1968, 1971; Triska, 1970; Barlocher and Kendrick, 1973a,b, 1975, 1976; Hargrave, 1970; Cummins et al., 1973; Mackay and Kalff, 1973; Kostalos and Seymour, 1976; Rossi and Fano, 1979).

Fungi have the capacity to concentrate the important elements present in wood substrates. Martin (1978) observed that the mycelia have low levels of nitrogen (1-7%) compared to the spores. The major component of mycelia is carbohydrate (50% of which are polysaccharides). Proteins account for 20-40% (dry weight); lignins, 1-11% of fungal dry weight. Relatively small amounts of vitamins and micronutrients are also present. Kaushik and Hynes

(1968, 1971) showed that the protein content of leaves can be doubled due to the growth of fungi. In the case of C. integrum, fungal mycelia and detritus (particularly wood particles) were present in its gut in large quantities. Wood is a seemingly low nutritive material and quite unlikely to be of nutritive value to the animal if ingested alone. However, the fungal component associated with it is of value in increasing the nutrient content for C. integrum. It is probable, therefore, that besides the benefits incurred from occupying a substrate where competition with other heptageniids is low, restriction to wood (although on its own may be low in nutritive value) is beneficial to the animal when the food resources present on it are considered.

The biochemical requirements of mycophagy are thus the possession of enzymes which could change the available fungal macronutrients to more assimilable forms. It is unlikely that C. integrum and other mayflies have the full complement of enzymes (Monk, 1976) and this is further evident from the fact that the fecal material of C. integrum contained fungal mycelia and spores. Thus, the strategies that these animals could adopt are: (1) utilize the extracellular fungal enzymes for the digestion of wood particles and fungal hyphae; (2) utilize only the minor dietary components--vitamins and micronutrients; (3) mechanically break down the cell walls of spores and mycelia with the scrapers on the labial palps and molar edges of mandibles, and utilize only the labile, "free" components, e.g., amino acids present within the cell and spore. This mechanical breakdown would also release polysaccharides and other substrates within the cell which are acted upon by the extracellular fungal enzymes. Thus, breakdown of these substrates is possible and they can become available to the animal in the form of more easily assimilable nutrients. Which of these strategies are actually adopted, however, could only be determined with future physiological and biochemical experiments.

The microdistribution and ultrastructure of the aufwuchs present on the surface of wood has not been studied. Karlstrom (1978) and Madsen (1972) observed the organic layer on stones and other artificial substrates as being in the form of a multi-storied layer. The algal and microbial components first colonize the substrate and, due to their mucoid secretions, detrital particles are entrained in this layer, thus forming multiple layers of these three components. It is possible that all three components are present on wood as well with fungi dominating the substrate initially, and detritus (FPOM) being entrained later. Thus, the aufwuchs layer increases the nutritional level of wood substrates and the association of macroinvertebrates for purposes of feeding will have an indirect effect (via the fungi) and a direct effect (particle size reduction) on the degradation process.

In this study, very little emphasis was placed on the contribution made by bacteria to wood degradation as a food resource for macroinvertebrates because this component could not be identified with the methods employed. It must be noted, however, that bacteria are closely associated with detritus (Bradnam and Baker, 1970) and may thus form a part of the nutrition of mayflies which consume detritus. Thus, in actual fact, the aufwuchs on wood comprises fungi, diatoms and detritus with associated bacteria. Table 10, however, does not include the bacterial component and its relationship with macroinvertebrates since this study did not deal with this component at all.

Cummins and Klug (1979) noted that food resource categories correspond with the morpho-behavioral adaptations of invertebrate functional feeding groups. Mayflies, which are either scrapers or collectors, are able to exploit the resources on wood substrates. However, partitioning of the habitat has resulted in associations with wood substrates such that they could be separated on the basis

of obligatory, facultative or chance association.

Fungi are apparently the most important microbial component on wood surfaces and in the superficial layers. The mouth parts of C. integrum and I. nitidus are well-adapted to utilizing this resource. The scraper on the ventral surface of the labial palps, though characteristic of the heptageniids is especially well-developed in these two species. In the process of scraping and raking, the surface inhabiting biota into the mouth, a considerable amount of wood particles is also ingested.

The main food item of small C. integrum larvae was detritus; their gut contained considerably smaller proportions of mycelia than was found in larger larvae. A possible explanation for this is that the mouth parts of small larvae are less chitinized and the ridges are less well-developed for a scraping type of feeding.

All mayflies had fungal material in their gut contents. The extent to which this component is nutritionally beneficial to the animals is unknown. C. integrum and I. nitidus were found to ingest large quantities of fungal mycelia in comparison to the other mayfly species. Cummins et al. (1964) showed that the heptageniid Stenonema (classed as a collector) lost weight on a diet strictly of fungal hyphae. They considered that high mortality was due to either a nutritional inadequacy in the diet or to mechanical problems associated with larval feeding on the fungal mat that was provided in the experiment. It is unknown if a diet consisting solely of fungi would be adequate for C. integrum. However, the laboratory rearing demonstrated that survival was possible solely on the resources found on wood.

Seasonal periodicity of fungal ingestion was observed for C. integrum and I. nitidus and was highest during the winter with decreases in the warmer summer months. In the summer, decreases in fungal growth are likely (Thornton, 1963; Webster et al., 1976), and thus these animals revert to detritivory at this time. Detritus

was the most common component present in the diets of most of the mayflies studied. Dominance of detritus seemed a common observation in most mayfly feeding studies (Gilpin and Brusven, 1970; Chapman and Demory, 1965; Brown, 1960, 1961; Flowers and Hilsenhoff; 1978). The exploitation of this food category requires the least expenditure of energy since it is a component that is present in significantly high proportions in the environment.

In the case of C. integrum, between 0.15 and 1.04mg of mycelia (dry weight) was consumed by a larva per day. This is comparable with that observed for Gammarus pseudolimnaeus (Barlocher and Kendrick, 1973) where daily consumption per animal was between 0.024 to 1.07mg. How significant a comparison of these two animals is seems uncertain but there are no other studies on consumption rates of mycelia by wood-associated aquatic insects. Considering that an average larva on an annual basis consumes 193.45mg ( $.053 \times 10 \times 365$ ) per mg body weight, and annual production of C. integrum at Berry Creek was  $2.8\text{mg}/\text{m}^2$ , an approximate dry weight of 542 mg of mycelia per  $\text{m}^2$  area of substrate is consumed by the C. integrum population annually. This estimate is for C. integrum only. The impact of the entire mayfly complex would be at least double this figure, so a conservative annual estimate of fungal consumption at Berry Creek would be approximately  $1\text{g}/\text{m}^2$  of wood surface. The role of wood degradation, therefore, appears to be more of an indirect one, but is significantly less than typical gougers such as Lara avara and Heteroplectron californicum (Anderson et al., 1978), which consume large quantities of wood and thus have a direct role in wood degradation.

Feeding activity by wood-associated mayflies thus appears to be significant to wood decomposition. Mechanical breakdown by the scraping and "vacuum cleaning" type of feeding behavior results in the conversion of CPOM to FPOM of wood substrates. Mycophagy observed in all the mayfly larvae contributes quite significantly

to the mineralization process since large areas of senescent colonies may be grazed upon, thus making way for further colonies of fungi to degrade the wood substrate further. Depending on whether the association of these larvae are facultative or obligatory, this could perhaps be the major role played by mayflies in wood degradation. Considering the large amount of fungal spores that pass through the gut of C. integrum, it seems possible that spore dispersal is another role that may be attributed to the larval mayfly population in aquatic systems.

Whilst the role of mayflies in wood degradation seems to be a direct one of mechanical breakdown as well as an indirect one of mineralization via the microbial fauna, the benefits gained by these larvae as a result of their association with wood substrates is also important. Mayflies utilize this substrate for purposes of shelter and protection, feeding, emergence and minimizing interspecific competition.

This study has been a preliminary investigation into the interactions of the mayfly population the microbiota (fungi) and wood. Further studies in this direction need to be done before we can understand the complex interactions that occur in wood decomposition. It must be mentioned, however, that studies on the microbial fauna that is present on wood requires extensive work. In addition, biochemical and physiological studies dealing with mycophagy in the aquatic environment need to be explored further if we are to understand more fully the interactions of the microbial and macroinvertebrate populations and their impact on wood degradation and mineralization.

## SUMMARY AND CONCLUSIONS

1. Heptageniids were the dominant mayfly component associated with wood substrates at Berry Creek. The densities of C. integrum and I. nitidus collected on wood were  $48/m^2$  and  $31/m^2$ , respectively, whereas that for Baetis was  $54/m^2$ .
2. In comparing wood substrates with stones and composite substrates (kick samples), the proportions of mayflies on wood in riffle areas were: C. integrum, 76%; Baetis, 46%; I. nitidus, 43%; E. (Iron) spp., 37%; Cinygmula sp., 32%; Ephemerella spp., 26%; Ameletus spp., 24%; Rhithrogena sp., 21%; Paraleptophlebia spp., 15%.
3. At Berry Creek, C. integrum was found to be restricted to wood substrates whereas I. nitidus was found with equal frequency on all three substrates sampled. Cinygmula, Baetis and Ameletus were equally distributed on all three substrate types whereas E. (Iron) and Rhithrogena were more restricted to stones. Paraleptophlebia and Ephemerella appeared to be more commonly in the kick samples.
4. The restriction of C. integrum to wood substrates, the flexibility of substrate preference shown by I. nitidus, and differences of life cycle patterns (which appeared to be related to temperature) may be factors that minimize competition between C. integrum and I. nitidus for space and food.
5. The mature larvae of Baetis, Paraleptophlebia, C. integrum and I. nitidus appeared to show a preference for wood substrates and used it as an emergence platform.
6. The life cycle of C. integrum at Berry Creek was interpreted as being univoltine. The majority of larvae began development in the summer, but there was also a smaller cohort that began

development in fall. This phenomenon of two cohorts in the life cycle appeared to be related to temperature regime and stability of the environment at this study site. The life cycle was a slow seasonal (S1) type (Hynes, 1970) and lasted between 10-12 months.

7. A univoltine life cycle was observed for I. nitidus at Berry Creek with the absence of any larval stage during August and September. The life cycle pattern appeared to be a fast seasonal type and larval duration varied between 6-9 months.

8. Both C. integrum and I. nitidus are cool adapted with most growth occurring between November and February. Maturation, as evidenced by increased rate of development of wing pads and genital structures, becomes apparent with the increase in water temperature in the spring.

9. Successful rearing of C. integrum larvae in the laboratory from the 3mm size class was achieved at a temperature of 9°C, 12 hours daylength and on a diet solely of the aufwuchs present on wood substrates. Larvae collected in late September began to emerge in the laboratory in March which was similar to the field emergence pattern. Laboratory-reared adults were similar in size to field-collected specimens.

10. The food resource categories of the two heptageniid species studied appeared to correspond well with their morpho-behavioral characteristics. Thus, the scraping and vacuum-cleaning type of mouth parts (particularly the labial palps) seemed to be well adapted for harvesting the aufwuchs found on wood substrates. Each labial palp consists of a series of ridges and recurved teeth. The aufwuchs layer appeared to consist of fungi, diatoms, detritus (wood, leaf and unidentifiable material), mineral particles, filamentous algae and bacteria. The latter food type was, however, not considered in this gut analysis study.

11. Seasonal periodicity of fungus consumption was observed, with maximum intake occurring during the winter months. This may be related to the abundance of fungi when water temperatures are low (0-7°C). Stage A and B larvae were found to feed mostly on detritus. The older larvae consumed greater proportions of fungal material than did Stage A and B larvae.
12. Ingestion of fungal spores was observed for all wood-associated mayflies but very few perithecia or fruiting bodies were observed. Fungi identified from spores only were: Flabellospora, Lemonniera, Dactylosporium, Dictyosporium, Epicoccum and Leptosphaeria (?).
13. On an annual basis, a C. integrum larva consumed approximately 0.5mg of mycelia. Extrapolation based on gut content analysis and on populations of the mayflies suggest that the total mayfly complex at Berry Creek would ingest approximately  $1\text{g}/\text{m}^2$  of fungal mycelia from wood substrates.
14. The large proportion of fungal material ingested and the presence of spores and mycelia in the feces indicated that larvae play a role in the mineralization of wood. This takes place by removal of senescent colonies and clearing of fresh areas for further colonization by fungi as well as by dispersal of spores to fresh habitats.
15. Considering that wood is common in headwater streams, mayfly species in other geographical locations may occupy the same ecological niche and probably exhibit morpho-behavioral and physiological strategies similar to those observed in this study. Biological activities of these organisms may then indirectly or directly contribute to the degradation of wood in streams.

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