Ingestion rate of the burrowing mayfly Hexagenia limbata as determined with ^{14}C

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Abstract

The effects of nymph size and temperature on the ingestion rate of *Hexagenia limbata* were investigated using ^{14}C labelled algae mixed into sediment. Ingestion rate increased with nymph length and temperature. Daily ingestion rates of large 19 mm nymphs burrowing in silt averaged 58 and 192% of the dry body weight at 16° and 21 °C, respectively. Ingestion of seston due to filter feeding in artificial burrows was insignificant compared to that ingested by burrowing nymphs.

Introduction

Ingestion rates of deposit-feeding macroinvertebrates are prerequisites involved in determining energy transformation as well as nutrient or contaminant recycling by members of the benthic community. Likewise the feeding activities of benthic invertebrates are of interest as they contribute prominently to the redistribution of surface sediments (Krezoski *et al.* 1978), as well as enhancing microbial activity in the sediments (Briggs *et al.* 1979).

The burrowing mayfly *Hexagenia limbata* (Serville) when abundant, constitutes an important component of the benthic fauna as well as a major prey item of the fish community. The nymphs ingest large quantities of fine sediments and accompanying organic matter (Fremling 1967). The feeding rates of mayfly nymphs have been estimated visually, by Zimmerman & Wissing (1978), & McCullough *et al.* (1979) based on the passage of dark sediment along the gut. Ingestion and assimilation rates have been calculated for various surface feeding detritivores using radio tracers involving single (Hargrave 1970) or dual-labelled food sources (Calow & Fletcher 1972; McCullough *et al.* 1979).

The present study examined the measurement of the ingestion rate of burrowing *Hexagenia* using sediments containing algae labelled with ^{14}C, following a similar procedure used for zooplankton (Haney 1971; Heisey & Porter 1977). The influences of nymph size and temperature on the feeding rate of *Hexagenia limbata* were examined.

Methods

Nymphs of *Hexagenia* were collected from a variety of fine grained sediments at depths of 3–12 m in Batchawana Bay, eastern Lake Superior; within 48 h prior to each experiment. The nymphs were maintained in the predominant sandy-silt sediments and lake water under low light levels and ambient temperatures. Feeding experiments utilized a homogeneous silt of high organic content (23%) collected previously from a depth of 5 m, screened to remove any organisms or leaf debris larger than 0.2 mm. The average dry weight of the silt used was 0.13 mg μl^{-1}. Nymphs were acclimated to this sediment 12 h prior to their use in the feeding experiments. Mature nymphs with black wing pads as well as dying nymphs which lose their equilibrium do not feed and were used as non-feeding controls to determine the adsorption of labelled material onto the body cuticle.

Log phase *Chlorella,* grown axenically in Bristol's medium were labelled by adding 1 m Ci [^{14}C]

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$NaHCO_3$/ 50 ml of culture and incubated for 2 h at pH 4. (J. M. Cooley & J. E. Moore unpublished methodology). The labelled algae were centrifuged, rinsed, then resuspended in 20 ml fresh Bristol's medium. Cell counts and cell activity per ml algae suspension were estimated from 10 μl samples. ^{14}C activity was maintained at over 10^8 CPM per ml of algal suspension to ensure a high counting accuracy when mixed into the silt.

Approximately 0.1 m Ci (2.0 ml) of labelled *Chlorella* suspension was added to 9 cm diameter jars containing 100 ml of silt slurry. Before and after the feeding experiments the silt was thoroughly mixed, with dual aliquots collected using a 200 μl Eppendorf pipette with tips having an enlarged bore, the samples placed in 15 ml of PCS solution (Amersham Corp.). Separate samples were placed on pre-weighed glass filters, dried at 60 °C for 24 h and ashed at 475° for 4 h to determine the dryweight and organic content.

After allowing the silt to settle for ½ h leaving less than 3 mm of free water overlying the sediments; five nymphs of similar size were added to each jar and allowed to feed under subdued light at the ambient water temperatures during August, September and October. Small nymphs of less than 9 mm length were allowed to feed in 25 ml of silt in petri dishes to which 0.5 ml of the algal suspension was added.

Feeding times were generally less than half the estimated gut filling time, with 20 min allowed for burrow construction (Zimmerman & Wissing 1978). Following feeding for a period of 2–4 h the mayflies were removed from the silt, then rinsed for 2 min in pumped water to remove the labelled particles adhering to the gills and body joints. The mayflies were narcotized by warming in water to 40 °C, fresh weight measured prior to preservation in sugared 5% formalin. The rate of gut filling and the initiation of feeding at 15 °C was determined by following the uptake of activity by the nymphs over time, using jars with similar levels of activity per volume of silt. Nymphs were sacrificied at each of 0.5, 1, 2, 2.5, 4, 8, 12 and 15 h after the addition of the nymphs to the jars.

To examine the effect of settling on the distribution of the labelled algae in the silt, labelled *Chlorella* was mixed into the silt of three jars, then allowed to settle over a period of 4 h. Three 20 μl Eppindorff samples of the overlying water were then taken as well as the silt at 1, 5, 10 and 15 mm below the sediment-water interface. The activity of these samples were compared to that of the silt collected immediately after mixing.

Body lengths were measured from the anterior of the head process to the base of the cerci. The nymphs were dissected using a sterioscope, the gut removed to a scintillation vial cover where the outer tissues were then stripped off. The guts plus contents and dissected bodies were digested separately in 1 ml NCS (Amersham Corp.) for 72 h, neutralized with 1 ml acetic acid, then 13 ml PCS scintillation solution added. All samples were dark-adapted for 24 h, then vortexed for 2 min prior to counting in a liquid scintillation counter. Background activity of the lake water, silt and mayflies were measured, quench standards were prepared for the silt and for the mayflies. The CPM data were converted to disintegrations per minute (DPM).

The feeding rates of the nymphs were calculated as:

$$\text{Feeding rate } (\mu\text{l nymph}^{-1}\ \text{day}^{-1}) = \frac{\text{DPM (gut)}}{\text{DPM} \cdot \mu\text{l}^{-1}\ \text{(silt)}} \times \frac{24\ \text{h day}^{-1}}{\text{Feeding time h}}$$

Estimations were made of possible filter feeding on suspended particles carried through the burrows by the respiratory currents, utilizing nymphs held in artificial burrows. Glass tubes of 5 mm ID were bent into an elongated U, 60 mm long and 20 mm deep. One nymph was placed in each water filled tube, the tubes buried underwater in ashed sand with both open ends above the sand-water interface. Following acclimation of 1 h at 20 °C, 2 ml of labelled *Chlorella* suspension were added to the water resulting in concentration of 0.013 mg algae · ml^{-1}; the water being mixed by aeration. Filter feeding rates were calculated for a feeding time of 4 h following the procedure for silt, with controls set up without the glass tubes, since the nymphs were unable to burrow into the loose sand.

Results and discussion

In the jars where the silt was allowed to settle over a period of 4 hours in the absence of nymphs, no difference existed between the activity per volume of the silt collected at 5–15 mm below the sediment-water interface and that of the initial mixed silt. However the activity due to the labelled

Table 1. Percent of total ^{14}C activity on large (18–22 mm) *Hexagenia* nymphs at feeding times of 0.5–12 h; means with SE in brackets. N = number of replicates. Zero times are for non-feeding nymphs maintained in sediment over 4 h.

	T	N	Gills & Cerci %	Body %	Gut %	Total DPM
On sand	0	9		93.9(1.8)	5.8(1.9)	15 972
In silt	0	15	23.1(2.3)	72.7(3.6)	4.2(1.1)	1 745
	0.5	7		29.4(4.3)	70.5(3.3)	2 881
	1	6		27.8(1.5)	72.1(1.3)	3 322
	2	12	11.7(2.0)	28.5(4.6)	59.7(6.2)	3 022
	4	7	10.1(0.2)	34.6(2.4)	55.2(2.5)	9 120
	8	5		43.3(3.1)	56.6(3.6)	31 912
	12	7	4.8(0.6)	42.6(3.5)	53.3(4.1)	17 886

Chlorella in the overlying water and in the silt at a depth of 1 mm were significant less ($p < .001$, $df = 14$) than that of the mixed silt. The labelled *Chlorella* are entrapped by the settling sediment particles so that the activity of the initial mixed silt does not differ from that at the depth where the nymphs burrow.

The distribution of total activity on the nymphs is displayed in Table 1. In spite of the extensive rinse, the radioactivity on the body surface of the non feeding nymphs averaged 1 650 DPM, of which the biramous gills and cerci filaments accounted for less than half the percent activity. Dissection of the gut plus contents from the body, was employed to separate the activity due to ingestion and external adhesion.

Burrow construction was completed and feeding initiated within 0.5 h. The percentage of total activity in the gut increased rapidly to maximum at 1 h, then declined significantly ($P < .01$, $df = 9$) to an average of 55% after 4 h. Assimilation during the 1–4 h feeding period did not significantly change the percent activity of the body (Table 1). Not until after a feeding period of 8 h does the percent activity of the body significantly change ($p < .05$, $df = 10$) from that at 0.5 h.

The relationship among feeding rate, nymph length and temperature is displayed in Figure 1, with a multiple correlation of $r = 0.88$ and $df = 43$. The feeding rate of the larger nymphs was significantly greater ($p < .001$) with increased temperature, which has a significant effect on metabolic rate. However the feeding rate of small young of the year nymphs (4–8 mm) at 10 °C was not signifi-

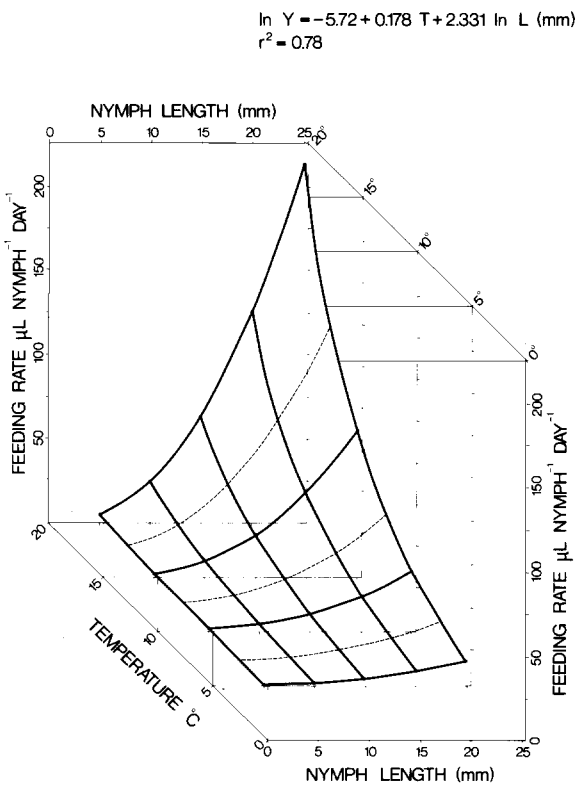

Fig. 1. Relationship among the feeding rate, ambient temperature, and nymph length for *Hexagenia limbata*.

Table 2. Daily ingestion ratios of 5–24 mm *Hexagenia* nymphs fed labelled Chlorella-silt mixture at various temperatures. Ingestion ratios as dry weight and organic weight per nymph weight, or ash free weight (AFW). Feeding times were 2 h at 21 °C and 4 h at 10° and 16 °C.

Temp. (°C)	Size (mm)	Dry weight (mg)	Ingestion mg · mg^{-1} day^{-1}	Organic intake mg · mg^{-1} AFW day^{-1}
	7	0.39	1.13	0.31
	10	1.49	0.36	0.11
10	16	5.23	0.35	0.10
	18	7.40	0.31	0.09
	23	14.31	0.29	0.08
	5	0.25	1.12	0.31
	10	1.44	0.72	0.21
16	16	5.23	0.46	0.13
	19	8.57	0.58	0.17
	24	19.61	0.39	0.12
	6	0.28	5.89	1.61
	11	1.50	2.26	0.64
21	15	4.18	2.05	0.57
	19	8.53	1.92	0.60
	24	18.84	1.38	0.42

cantly different ($p < .05$) from that at 16 °C. Likewise, for each temperature examined the feeding rate was significantly greater ($p < .001$) with larger nymphs. Although total weight ingested increased with larger nymph size, the gut-fraction as dry weight or ash free weight ingested per body weight varied inversely with body size at a given temperature (Table 2).

The accumulation of activity by the nymphs at 15 °C, measured as gut DPM mm^{-1} resulted in a time series uptake curve of ^{14}C accumulation (Sorokin 1966; McCullough *et al.* 1979) given by the regression over 0.5–8 hours.

$$Y = 106.4\ T - 56.7\ r = .96$$

At approximately 8 h, the uptake rate changes to the regression

$$Y = 34.1\ T + 281.9\ r = .58$$

This inflection point is due to the initiation of egestion. These results agree well with that of Zimmerman & Wissing (1978), who used visual methods to determine the gut loading time of similar sized *Hexagenia* nymphs to be 7.1 h at 15 °C. Thus the feeding periods of 2–4 h used in this study in measuring the ingestion rate at 21° and 15 °C ensures that defecation or reingestion of label does not occur.

Zimmerman & Wissing (1978) using gut loading times determined that *Hexagenia* nymphs ingested over 100% of their dry body weight per day at 10° and 15 °C. Those rates were considerably higher than the present study, where only at 21 °C did larger nymphs ingest over 100% of their body weight per day. Rapid turnover of sediment has been observed in other deposit feeding invertebrates. Hargrave (1970) estimated the amphipod *Hyalella* ingested 60% of its body weight per day at 15 °C, while McCullough *et al.* (1979) found the small stream mayfly *Tricorythodes* to ingest 3.8 times its body weight per day.

As in other deposit feeding invertebrates (Davies 1975; Hylleberg & Gallucci 1975), the organic content of the sediments was lower than that in the alimentary tract of the nymphs. Caution is required, however, in interpreting the organic content of the fecal matter, in order to take into account the contribution due to the gut flora, excretory products, and encasing peritrophic membrane. Zimmerman & Wissing (1978) proposed that *Hexagenia* selectively ingest particles of high organic content. Analysis of the gut contents of *Hexagenia* inhabiting fine sandy sediments in Batchawana Bay, indicated a significant selection ($p < .01$, $df = 18$) of clay sized particles (.004 mm) at the expense of sand size particles. No significant difference existed in the proportion of silt or algal remains in the foreguts of the nymphs versus that in the inhabited sediments. Clay sized particles and associated bacteria (average diameter 1.7 μm) composed 63% of the foregut volume. Silt sized particles (diameter <60 μm) accounted for 15% of the volume while diatoms and other algal remains averaged only 3%. Shapas & Hilsenhoff (1976) reported non-algal detritus to account for 92% by volume in the foreguts of *Hexagenia* collected from Wisconsin streams. *Hexagenia* feed as detritus collectors, selecting particle sizes characteristic of the fine grained sediments inhabited.

The feeding rate of 22 mm nymphs maintained in glass tubes as a result of filter feeding was calculated as 0.58 ml $nymph^{-1}$ day^{-1} ($SE = 0.19$ $n = 9$). An organic weight of .006 mg $nymph^{-1}$ day^{-1} was ingested at 20 °C, representing only 0.07% of the ash free body weight. This was considerably less than the 3.7 mg organic matter ingested per day by 19 mm nymphs feeding in silt; suggesting that the main food source is the sediments and not seston brought into the burrows with the respiratory currents. Surveillance of burrowing nymphs indicated that the incurrent burrow openings shift with time, concurring with Fremling's (1976) observations that the burrows constantly change position due to the feeding activities of the nymphs.

Cammen (1980) postulated that the ingestion rate of aquatic detritivores varied with body size and inversely with the organic content of the food source. In preliminary feeding experiments utilizing fine sandy sublittoral sediments with low organic content (4%), *Hexagenia* nymphs ingested 82 mg day^{-1} compared to an average 13.4 mg day^{-1} silt ingested at 20 °C by similar sized nymphs. However, the total organic matter ingested remained the same, i.e., 3.3 and 3.1 mg day^{-1} respectively, the nymphs apparently increasing the ingestion rate to compensate for the lower food quality of the sandy sediments.

Although Johannsson (1980) & Hargrave (1970) found insignificant adsorption of radioactivity onto the cuticle of the organisms examined, the

large surface area of *Hexagenia* necessitates careful rinsing to remove the high levels of radioactivity from the outside of the nymphs. Caring for the precautions of Conover & Francis (1973), the estimation of ingestion rate by this direct method requires that the activity in the guts alone be measured, using a feeding time considerably less than the gut loading time of the burrowing detritivores.

Summary

Ingestion rates of *Hexagenia limbata* were investigated over a range of temperature and nymph sizes, using ^{14}C labelled algae mixed into natural sediments. The distribution of activity in the sediments indicated that the algae remained well mixed, being entrapped by the sediment particles. The average daily ingestion rate increased with temperature and increase in nymph length. The daily ration ingested per body weight varied inversely with nymph size.

Ingestion rates of 19 mm nymphs averaged 58% and 192% of the body dry weight at 16 and 21 °C respectively. Filter feeding in artificial burrows resulted in a daily ingestion rate representing only 0.08% of the dry body weight. Although a greater weight of low organic sediment than silt was ingested per day, the organic matter ingested remained the same.

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