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Effects of temperature on gut-loading and gut-clearing times of the burrowing mayfly, *Hexagenia limbata*

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SUMMARY. The effect of temperature on gut-loading times, gut-clearing times, and the calculated ingestion rates, egestion rates, and consumption indices of the deposit-feeding burrowing mayfly, *Hexagenia limbata*, were investigated in laboratory experiments. Rates of movement of two natural sediments of differing colour through the digestive tract were monitored to quantify feeding intensity when ambient water temperatures approached 5, 10, 15, 20, and 25°C.

At each temperature, gut-loading times (GLT) and gut-clearing times (GCT) increased as nymph length increased. Mean GLT and GCT values decreased as temperature increased from 5 to 20°C but were longer at 25°C than at 20°C. Relationships between GLT, GCT, and length of nymphs and temperature were best described by multiple regression equations. No diel variation in gut-clearing times was observed. Low water temperatures resulted in lower ingestion and egestion rates and consumption indices. At most temperatures nymphs ingested over 100% of their dry body weights per day.

Introduction

Nymphs of burrowing mayflies, genus *Hexagenia*, ingest large quantities of sediment (Neave, 1932; Morgan and Grierson, 1932; Walker, 1970), and have been described as 'mud eaters' (Hunt, 1953; Fremling, 1967). Cummins (1973) has classified such forms as 'collectors', with the major food source being detritus which is ingested along with varying amounts of inorganic material.

Though data are available on the feeding intensity of a variety of deposit-feeding invertebrates (Hargrave, 1970a, b, 1972; Fenchel, Kofoed & Lappalainen, 1975), little is known of this aspect of nutrition in the deposit-feeding aquatic insects. Cummins (1973) has noted that gut-loading times and ingestion rates have been determined for only a few species of aquatic insects. Such information is essential in assessing the impact of these forms on the various food sources available in the aquatic environment.

The present study was designed to determine gut-loading and gut-clearing times of nymphs of *Hexagenia limbata* Serville at different temperatures. Predicted rates of ingestion and egestion were calculated and compared with similar estimates for other species of deposit-feeding invertebrates. Approximations of these parameters have been made with radio-tracers or by the monitoring of an inert material added to the food so that progress of a bolus through the digestive tract could be followed (Duncan & Klekowski, 1975). In this study, the rates of movement of two natural sediments of

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differing colour through the digestive tract were utilized to quantify feeding relationships.

Materials and methods

During August–December 1975 and February–April 1976 nymphs and sediments were collected periodically for feeding experiments from Brandenburg Pond (Butler County, Ohio) with an Ekman grab. The morphometry of the pond is described in Rutter & Wissing (1975).

Two sediment types were collected on the same date. The first (A) is found in well defined areas around the periphery of the pond and is composed of yellow-clay and gravel. This sediment is usually inhabited by large numbers of *Hexagenia limbata* nymphs. The second (B), found in the deep centre of the pond, is a black-detritus mud which is normally devoid of *Hexagenia* (Zimmerman, Wissing & Rutter, 1975). Each sediment type was homogenized by hand-stirring and partitioned into two groups of small plastic containers prior to the feeding experiments. One group was supplied with sediment A and the other with sediment B. Organic contents of sediments were determined by loss of weight on ignition (550°C for 3 h). Caloric contents of dried (60°C for 48 h) homogenized sediments were determined with the modified iodate sulphuric acid wet oxidation method of Karzinkin & Tarkovskaya (1964) as described in Hughes (1969a). The modified method requires a correction for unoxidized protein which is determined with the standard Kjeldahl nitrogen technique. No attempts were made to examine changes in sediment characteristics during the course of the feeding experiments.

Prior to each experiment, pond water was filtered through silk bolting cloth (mesh size = 76 μm) and aerated for 24 h. Each container was then filled with filtered pond water and sediment (260:90 ml). During the experiments, dissolved oxygen content of the water in the containers never fell below 3 mg l^{-1}. All observations were made in an environmental chamber with a 12-h light (969 lux, cool-white fluorescent bulbs), 12-h dark photoperiod.

Estimates of gut-loading and gut-clearing times were based on the number of abdominal segments filled during successive time intervals. Segments were numbered 1–9, distally. The extent to which the guts were filled appeared as a dark line when a nymph was held against a bright background.

After nymphs had evacuated their digestive tracts in a holding tank, single nymphs were placed in containers of sediment A. Preliminary observations indicated that nymphs did not have to be checked for a period of time subsequent to their introduction into containers. These time intervals (2 h at 25 and 20°C; 4 h at 15°C; and 7 h at 10 and 5°C) provided a period of undisturbed feeding. After these intervals, nymphs were serially examined according to length L, mm (excluding tail cerci). No nymph was left unchecked for longer than 2 h, and no animal had its feeding disturbed more than three times. If the nymph had not completely filled the gut at the time of observation, it was transferred to a new container to avoid anoxia. Gut-loading time was defined as the time needed to completely fill the gut with sediment A. Once the gut was filled with sediment A, nymphs were transferred to sediment B containers. Gut-clearing time (defined as the time required for sediment B to pass from mouthparts to anus) could then be determined by recording the movement of the interface between the yellow (A) and black (B) sediments. Since the colour difference was not easily observed by illumination, the nymphs were serially sacrificed, and their digestive tracts were opened by cutting longitudinally through the sterna from anus to prothorax. Once the gut was exposed, the interface was readily visible.

Measurements were repeated at 5, 10, 15, 20, and 25°C. Nymphs, sediments, and pond water were collected from the pond and transported to the laboratory, when water temperatures approached these values. Twenty nymphs where $L < 15$ mm and 20 where $L \geqslant 15$ mm were used for each feeding series. Final instar nymphs and nymphs where $L < 5$ mm or $L > 32$ mm were not used in the experiments.

A series of measurements was made with nymphs feeding on sediments A and B to determine the effect of sediment type on gut-loading time. In addition, seven gut-clearing experiments were carried out with unstarved nymphs. Two of the latter were run during the nocturnal phase of the experimental photoperiod.

Ingestion rates (mg (GC) nymph^{-1} day^{-1}) were calculated with the equation:

$$I = GC\,(24/GLT) \quad (1)$$

where I = ingestion rate, GC = dry weight of gut contents (mg), and GLT = gut-loading time (h). Values for GC were determined from a regression of dry weight (60°C for 48 h) of gut contents on length of 100 animals collected on 15 May 1976 (water temperature = 20°C). The equation is:

$$\log GC = -1.7180 + 2.2605 \log L \quad (2a)$$

($r^2 = 0.93$ (r is the correlation coefficient); 95% confidence limits of regression coefficient $b = \pm 0.1289$). Organic content of gut material ranged from 22 to 38% of dry weight. Consumption indices (CI, dry weight of food ingested per dry weight of animal per day) were calculated with the formula described in Waldbauer (1968). Mean dry weight of each nymph was determined from a regression of body dry weight (W) on length of 300 animals with empty guts. The equation for this relationship is:

$$\log W = -2.2628 + 2.3863 \log L \quad (2b)$$

($r^2 = 0.86$; 95% confidence limits of $b = \pm 0.1050$). Calculations of egestion rates (mg (GC) nymph^{-1} day^{-1}) were based on the assumption that all inorganic material ingested by the organism is egested, and the observation that 50% of the organic fraction of the ingested material (mean organic = 35%) appears in the faeces (Zimmerman *et al.*, 1975).

Relationships between independent variables (nymph length and temperature) and the dependent variables of gut-loading time and gut-clearing time were established through multiple stepwise regression analyses (Draper & Smith, 1966). Other relationships were established through linear, polynomial, and exponential regression analyses. The Statistical Analysis System (SAS) developed by Barr & Goodnight (1972) was used for the statistical analyses. Comparisons of calculated regression equations were performed with an extension of the dummy variable method of Gujarati (1970) to test for significant differences in slopes and intercepts. Mean separation with Duncan's New Multiple Range Test (Steele & Torrie, 1960) was performed on gut-loading and gut-clearing times to test for significant differences ($P < 0.05$) in mean times at the experimental temperatures.

Results

Organic, caloric and nitrogen contents of homogenized sediments used in the feeding experiments at each of the five temperatures are given in Table 1. In most cases, the values of sediment B are higher than those of sediment A. Particle-size analysis of actual surface sediments taken from the pond in areas corresponding to sediments A and B indicated that sediment B has a higher silt content than sediment A (40 and 10% respectively), and that sediment A contains a higher sand and clay content (68 and 49% sand, 22 and 15% clay, respectively).

Sample sizes, body lengths and mean gut-loading times (GLT, h) and gut-clearing times (GCT, h) for feeding experiments with *H. limbata* nymphs at five temperatures are summarized in Table 2. A significant ($P < 0.05$, Duncan's New Multiple Range Test) decrease in mean gut-loading and gut-clearing times occurred as temperatures increased from 5 to 20°C (GLT from 12.48 to 4.11 h and GCT from 12.90 to 5.01 h). Mean gut-loading and gut-clearing times were longer at 25°C than at 20°C (at 25°C, GLT = 4.33 h and GCT = 5.30 h), but the differences were not significant. Hughes (1969b) observed a decrease in gut-clearing times of the tellinid, *Scrobicularia plana,* between 6 and 24°C. The reduced effect of temperature on gut-loading and gut-clearing times observed at 20 and 25°C suggests that maximal rates of food processing are achieved as water temperature approaches 20°C. Thereafter, the rate of food movement through the gut declines slightly.

The relationship between gut-loading times and length (L, mm) of nymphs at all temperatures (T, °C) is shown graphically in Figure 1. This relationship was best described by the following multiple regression equation:

$$GLT = 11.03 - 0.42\,T + 0.17\,L \quad (3a)$$

($R^2 = 0.92$; 95% confidence limits for $T = \pm 0.0260$, for $L = \pm 0.0280$). The relationship for gut-clearing times was best described by the equation:

$$GCT = 10.79 - 0.38\,T + 0.18\,L \quad (3b)$$

($R^2 = 0.90$; 95% confidence limits for $T = \pm 0.0187$, for $L = \pm 0.0394$). The 95% confidence limits of the partial regression co-

TABLE 1. Mean organic (% dry-weight), mean caloric (cal/g ash-free dry weight) and total organic nitrogen (% dry-weight) contents of sediments used in feeding experiments with *H. limbata* nymphs at five temperatures. Numbers in parentheses represent the range of values for two determinations

Sediment type	Temperature (°C)				
	5	10	15	20	25
A					
Organic content	3.6 (3.5–3.7)	4.5 (4.3–4.7)	5.5 (5.2–5.8)	6.1 (6.0–6.2)	6.8 (6.5–7.1)
Caloric content	1415 (1397–1433)	1865 (1840–1890)	1809 (1768–1850)	1926 (1871–1981)	1916 (1893–1939)
Nitrogen content	0.05	0.08	0.35	0.90	0.99
B					
Organic content	5.5 (5.2–5.8)	5.8 (5.7–5.9)	7.8 (7.7–7.9)	8.0 (7.6–8.4)	8.6 (8.4–8.9)
Caloric content	1895 (1875–1915)	1814 (1804–1824)	2261 (2238–2284)	2096 (2065–2127)	2725 (2706–2744)
Nitrogen content	0.75	0.84	1.05	1.00	0.98

TABLE 2. Sample sizes, body lengths (mm), gut-loading times (GLT, h; feeding on sediment A), and gut-clearing times (GCT, h; feeding on sediment B) for feeding experiments with *H. limbata* nymphs at five temperatures. Results apply only to nymphs with completely full guts (sediment A) and completely cleared guts (sediment B). Unless otherwise indicated*, mean values for GLT and GCT differ from one another at the 0.05 probability level

	Temperature (°C)				
	5	10	15	20	25
Sediment A					
No. with full gut (No. observed)	31 (40)	36 (40)	37 (40)	40 (40)	40 (40)
Mean body length (range)	17 (10–29)	16 (9–28)	16 (9–27)	17 (9–30)	16 (9–26)
Mean GLT (±95% CL)	12.48 (±0.59)	10.08 (±0.36)	7.16 (±0.79)	4.11* (±0.63)	4.33* (±0.87)
Sediment B					
No. with cleared gut (No. observed)	16 (31)	24 (36)	24 (37)	26 (40)	29 (39)
Mean body length (range)	17 (10–29)	17 (9–26)	16 (10–27)	16 (9–30)	16 (8–26)
Mean GCT (±95% CL)	12.90 (±0.81)	10.18 (±0.43)	7.44 (±0.79)	5.01* (±0.78)	5.30* (±0.74)

* No significant difference; Duncan's New Multiple Range Test.

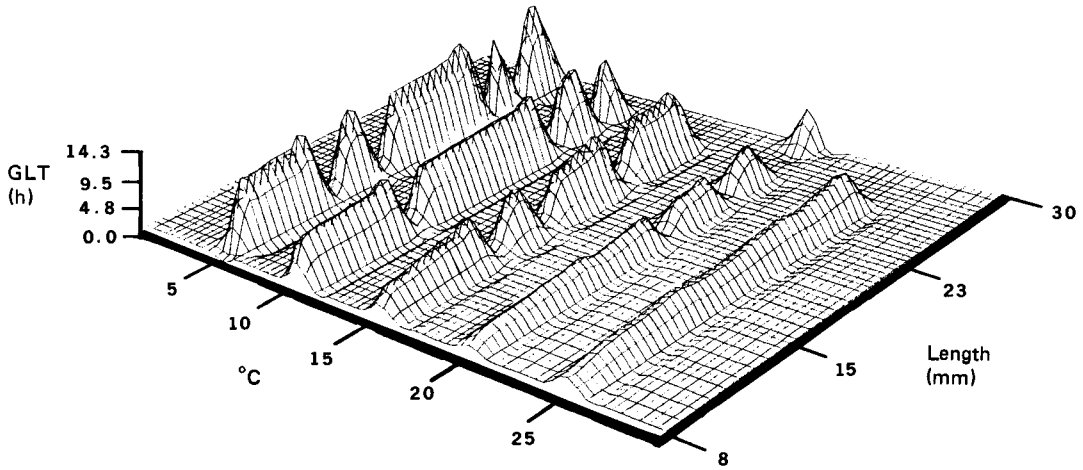

FIG. 1. Three-dimensional graph showing experimental relationships between gut-loading times (GLT) and nymph lengths of *H. limbata* at 5, 10, 15, 20 and 25°C.

TABLE 3. Sample sizes, body lengths (mm), and gut-clearing times (GCT, h) for diurnal (07.00–19.00 hours) feeding experiments with unstarved *H. limbata* nymphs at five temperatures. Results apply only to nymphs with completely cleared guts

	Temperature (°C)				
	5	10	15	20	25
No. with cleared gut (No. observed)	24 (40)	27 (40)	25 (40)	24 (40)	26 (40)
Mean body length (range)	17 (10–26)	18 (10–25)	16 (9–25)	17 (9–26)	20 (9–28)
Mean GCT (±95% CL)	12.70 (±0.45)	10.06 (±0.29)	7.47 (±0.66)	5.00 (±0.45)	5.45 (±0.58)

efficient are given for each term of equations (3a) and (3b); R^2 is the multiple coefficient of determination. Analyses were performed on nymphs which completely filled (equation 3a) or emptied (equation 3b) their guts of sediment A, and prediction of values of GLT and GCT is limited to these size ranges (Table 2). The stepwise selection procedure indicated that temperature is the best single variable predicting these relationships. Partitioning of the R^2 values showed that temperature accounted for 91% of the total R^2 value in equations (3a) and (3b). At each temperature, gut-loading and gut-clearing times increased as nymph length increased. Although gut-clearing times were slightly longer than gut-loading times, no significant differences ($P > 0.05$) were observed among the partial regression coefficients or the intercept values for these two measures of food movement. Thus, a graphical summary of the GCT values is not given.

Since gut-loading and gut-clearing times were similar at each temperature, it is doubtful that nymphs overcompensated during initial feeding, as might be expected owing to their starved condition. As a further test, nymphs that had not emptied their guts prior to feeding on yellow sediment (A) for 24 h were transferred to black sediment (B), and gut-clearing times were recorded. Additional observations and measurements were carried out at 20 and 25°C during the dark phase of the photoperiod. Sample sizes, body lengths and mean gut-clearing times of nymphs not previously starved are given in Tables 3 and 4. Mean gut-clearing times of unstarved nymphs and nymphs previously starved (Table 2) are similar (e.g. GCTs at 15°C for previously starved and unstarved nymphs were 7.44 and 7.47 h, respectively). Diurnal gut-clearing times for unstarved animals (Table 3) were best described by the following multiple regression equation:

$$GCT = 10.89 - 0.39\,T + 0.18\,L \qquad (3c)$$

($R^2 = 0.92$; 95% confidence limits for $T = \pm 0.0280$, for $L = \pm 0.0130$). Tests for the equality of the regression coefficients for the diurnal gut-clearing times (equation 3c) and for the gut-clearing times of previously starved nymphs (equation 3b) indicated no significant differences ($P > 0.05$).

Hexagenia nymphs appear to feed continuously and to process sediment throughout the day and night at similar rates. Mean diurnal and nocturnal gut-clearing times (Table 4) at 20 and 25°C were similar (e.g. at 25°C, diurnal mean GCT = 5.45 h and nocturnal mean GCT = 5.30 h). Furthermore, thousands of nymphs of all sizes have been observed during a 6-year study of the Brandenburg Pond population, and all, except for last instar nymphs and nymphs undergoing ecdysis, contained at least some food in their digestive tracts.

Discussion

A factor that probably has a major effect on the rate of food intake in many deposit feeders is food texture. Although there are particle-size differences in pond sediments, the ability of nymphs to feed on homogenized sediments A and B is similar (Table 2). Furthermore, observations of 10 nymphs feeding on sediment A and 10 on sediment B resulted in no statistical differences ($P > 0.05$) among regression coefficients for the relationships of gut-loading time and nymph length (at 20°C), indicating that nymphs of the same size ingest either of the two pond sediments at similar rates. An additional factor that could have influenced gut-loading times observed in this study was the transfer of

TABLE 4. Sample sizes, body lengths (mm), and gut-clearing times (GCT, h) for nocturnal (19.00–07.00 hours) feeding experiments with unstarved *H. limbata* nymphs at two temperatures. Results apply only to nymphs with completely cleared guts

	Temperature (°C)	
	20	25
No. with cleared gut (No. observed)	31 (40)	30 (40)
Mean body length (range)	17 (10–26)	17 (8–28)
Mean GCT (±95% CL)	5.07 (±0.47)	5.30 (±0.53)

nymphs to different containers, if their guts were not full, thereby forcing them to construct new burrows. Construction of new burrows could decrease the gut-loading times because the nymphs would be required to ingest their way into a new burrow, or it could increase the estimate through interruption of feeding. Observations in the laboratory indicated that the burrow is not constructed by ingestion of sediments, but instead is created by the action of the forelegs, body undulations, and gill movements. Average time for burrow construction at 23°C was 0.3 h (n = 15; 95% confidence limits = ±0.21).

Gut-loading times and gut-clearing times derived from our experiments were also used to calculate ingestion and egestion rates and consumption indices (Table 5). Calculations were made for three sizes (10, 15, and 25 mm) of *Hexagenia* nymphs at five temperatures. At each temperature, ingestion and egestion rates increased as the weight of the nymph increased. The consumption index (CI) decreased with an increase in weight of the nymph.

Ingestion and egestion rates and consumption indices were lower at colder temperatures (where $T < 15$°C) than at warmer temperatures (20°C and 25°C). For example, a 15-mm nymph (body dry wt = 8.71 mg) at 20°C ingested 16.22 mg (GC) nymph^{-1} day^{-1}, giving a CI = 1.86, and it egested 11.77 mg (GC) nymph^{-1} day^{-1} (representing 135% of its dry body weight). At 5°C, the corresponding values were 7.32, 0.84, and 5.98 (representing 68% of its dry body weight). Linear relationships between ingestion-egestion rates and temperature have been observed for other deposit-feeding invertebrates: the polychaete, *Pectinaria gouldii* (Gordon, 1966); the oligochaete, *Tubifex tubifex* (Appleby & Brinkhurst, 1970); and the amphipod, *Hyalella azteca* (Hargrave, 1972).

Our data for *Hexagenia* nymphs can be compared with literature values for two other feeding indices. Cummins (1973) compared CI values for a number of aquatic insects belonging to three trophic categories (collectors, grazers, and shredders), but did not correct for temperature or size differences. Both temperature and size affect the CI values of *H. limbata* nymphs. The range of values for *Hexagenia* are higher than those listed by Cummins, except for the caddisfly-grazer *Neophylax concinnus*, which has an index range of 0.80–1.60 (Sedell, 1971). Hargrave (1972) discussed the relationships between dry body weight and egestion reported for various aquatic deposit-feeding invertebrates. The only aquatic insect listed by Hargrave was the stonefly, *Pteronarcys scotti*, studied by McDiffett (1970). A 10-mg stonefly fed on dead leaves (at 15°C) egested 1.8 mg (GC) nymph^{-1} day^{-1}, corresponding to 18% of its dry body weight. In contrast, an 8.71 mg *Hexagenia* egested 8.89 mg (GC) nymph^{-1} day^{-1} (at 15°C), corresponding to 102% of its dry body weight. Hargrave (1970a, b) also observed variation in ingestion-egestion rates and assimilation efficiencies for the amphipod, *Hyalella azteca*, fed on different food materials.

Rapid food-turnover rates have been reported for a number of deposit-feeding invertebrates (Gordon, 1966; Hargrave, 1971). At most temperatures *H. limbata* nymphs ingested over 100% of their dry body weight per day. In *Hexagenia*, this might serve in part as a mechanism to ensure an adequate diet for the nymphs, since the adults do not

TABLE 5. Ingestion and egestion rates (mg (GC) nymph^{-1} day^{-1}) and consumption indices (CI = mg (GC) dry wt nymph^{-1} day^{-1}) for three sizes (length, mm) of *H. limbata* nymphs at five temperatures (°C). Calculated dry weights (mg), from equation (2b), for 10, 15, and 25-mm nymphs are 3.48, 8.71, and 27.6, respectively

Temperature	Length	Ingestion rate	CI	Egestion rate
5	10	2.98	0.86	2.45
	15	7.32	0.84	5.98
	25	21.54	0.78	17.49
10	10	3.74	1.08	3.00
	15	8.93	1.03	7.15
	25	25.62	0.93	20.39
15	10	3.99	1.15	3.82
	15	9.49	1.09	8.89
	25	27.70	1.00	24.42
20	10	7.37	2.12	5.28
	15	16.22	1.86	11.77
	25	41.27	1.50	34.05
25	10	14.31	4.11	8.45
	15	27.27	3.13	17.37
	25	59.39	2.15	40.45

feed and depend entirely on food reserves accumulated during the immature stages. The question still remains, however, regarding what fraction of the ingested material serves as food for *Hexagenia,* as well as for other deposit-feeders. Hunt (1953) concluded that, since the contents of the alimentary tracts of nymphs always contained large amounts of inorganic mud, some nutritional benefit must be derived from this material. Studies of a variety of organisms (caterpillars, dragonflies, snails and sticklebacks) have shown that these forms can assimilate a portion of the ash fraction in their food (Pavlyutin, 1970). Of more importance perhaps are the observations that deposit-feeders glean the microbiota attached to particle surfaces and remove any absorbed colloidal organic matter in the particles as they pass through the gut (Newell, 1965; Kaushik & Hynes, 1968; Hargrave, 1970a,b). Evidence suggesting the mode of feeding in *Hexagenia* is derived from the observation that nymphs seldom leave their burrows to feed but will occasionally approach the mouth of the tube and dislodge surrounding material (Hunt, 1953). It is also possible that gill movements generate water currents which carry seston into the burrow. Other data (Zimmerman *et al.*, 1975) indicate that the caloric and organic contents of surface sediments, detritus, and faeces are lower than those of gut contents in *Hexagenia,* implying that nymphs selectively ingest organic matter of high caloric content. Ingestion of food of high nutritive value, coupled with continual feeding, would enable the animal to meet its metabolic needs and still maintain optimal growth during the spring and summer months.

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