The seasonal variation of different metals in zooplankton and salinity and pH of the habitat water are shown in Figure 1. Among the metals studied, the concentration of Fe was highest (av. 5102.5 ppm) in zooplankton, while a lowest (av. 12.41 ppm) value observed for Ni. Fe content varied from 1470 ppm to 12,051 ppm. The trend of variation of Fe and Cd through seasons showed higher values during monsoon (July–October), a period of low salinity (mean salinity 7.99‰) and pH. On the other hand, the metal content showed decreasing value during premonsoon (March–June), a period of high salinity (mean salinity 17.74‰) and pH (>8.0). Bryan and Uystal[4] suggested that salinity was an important factor governing the availability of metals to the organisms. The negative correlation (Table 1) between metals concentration in zooplankton and salinity and pH of the habitat water suggests that salinity and pH might play an important role on the availability of metals to the organisms. The annual average of Fe obtained was 5102.5 ppm. George and Kureishy[1] while studying with the metals in zooplankton from Bay of Bengal observed 1139 ppm of Fe. Generally, the river mouths contain large amounts of Fe both in dissolved and particulate states and the source of the metal is in flowing water. Dissolved and particulate iron at the river mouths is almost three times more than offshore stations[5]. The higher primary productivity[6] in this zone during pre- and postmonsoon may be one of the reasons for minimum value of Fe in zooplankton during that period. It has been reported[7] that phytoplankton utilizes considerable amount of iron during photosynthetic process and lowers the concentration of iron in the surface water.

Similar results were observed for Cd also which ranged from 6 ppm to 96 ppm. The annual average of Cd was found to be 33.92 ppm. Literature survey revealed that these values are comparable with those reported earlier[8,9] at different water masses. The absence of the metal in January to March may be due to the high salinity and pH values as evidenced from the high negative correlation (Table 1) between metal concentration in zooplankton and salinity and pH of the habitat water. The relatively higher value (75 ppm) of Cd during monsoon may be due to the considerable amount of industrial effluent carried by monsoonal run off.

Among the metals studied only Ni showed completely different trend of seasonal variation, showing a decreasing order from premonsoon to monsoon and to postmonsoon. Ni content ranged from 4 ppm to 29 ppm. The annual average of Ni found in the samples was 12.41 ppm. Levels of Ni observed in the present study are comparable in magnitude to the concentrations reported by Subrahmanyam[8] in zooplankton collected from Visakhapatnam.

Though other workers[1,9,10] in different regions observed Pb and Co in zooplankton but these two elements were not detected in any sample throughout the season.

Table 1. Correlation coefficient values between metal concentrations in zooplankton and salinity and pH of the habitat water.

	Fe	Cd	Ni
Salinity	−0.439	−0.532	0.561
pH	−0.153	−0.816*	0.265

*Significant at 1% level of probability.

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A method for the estimation of food consumption by chironomids

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Considering the gut clearance time (GCT) and gut content weight of *ad libitum*-fed larva, as well as the energy content of the nutrient provided in the medium at the beginning of the experiment and that left behind at the end of the experiment, a method has been developed to estimate food consumption of the chironomid larva, *Kiefferulus barbitarsis* (Kieffer). Validity of the proposed method was tested by the isotopic tracer method. Food energy ingested by the larva from hatching to pupation as estimated by the present method was 26.6 J compared with 28.4 J by the isotopic tracer method.

REVIEWS on feeding and transformation of food in insects[1–4] indicate selective predominance of studies on

herbivorous insects and paucity of information on carnivores, detritivores and sanguivores. The dearth of information on detritivores such as chironomid larvae is perhaps due to the problems encountered in the estimation of one or the other of the bioenergetics components. Some of the problems are:

1. As in the flour beetle *Tribolium castaneum* and the dung beetle *Aphodius rufipes*, the food and the habitat of the aquatic detritivores are one and the same. Consequently, with increasing feeding duration (under laboratory conditions), the food is progressively diluted by mixing up with the nutritionally poor faeces. 2. The gravimetric method of Waldbauer[1] usually followed for herbivorous and carnivorous insects cannot be followed as such for the detritivorous insects because, separation of faeces from the unfed remains is not possible. Hence, estimation of food consumption is rendered difficult.

To obviate these problems, Holter[5] used chromic oxide as a marker with food and estimated food consumption (C) of the terrestrial detritivore *A. rufipes*. For the estimation of C or other bioenergetics components of aquatic detritivores like *Chironomus plumosus*, *Simulium austeni* and *Hexagenia limbata*, Johannsson[6], Ladle and Hansford[7] and Dermott[8] followed the isotopic tracer method. Food consumption of terrestrial detritivores like *Rhopaea verreauxi*[9], *Costelytra zealandica*[10] and *Tomocerus minor*[11] was indirectly estimated as the sum of $F+U+P+R$, where F is the faeces egested, U the nitrogenous excretion, P the growth and R the energy expended on metabolism.

In the present study, food consumption was estimated by considering the gut clearance time (GCT) and the faeces egested by a fully-fed larva as well as the energy content of the nutrient medium provided at the beginning of the experiment and that left behind at the end of the experiment. The final energy content of the medium in the experimental trays in which the larvae were reared included not only the energy content of the unfed remains, but also the egesta of the larvae. In order to estimate the weight of unfed remains, weight of faeces egested by the larvae was calculated as follows:

Using the larvae fed on chosen nutrient medium, gut clearance time i.e. the time required for unloading the contents of the fully loaded gut was noted for each size group separately. The faeces egested by these larvae was collected, dried and weighed in a monopan balance to 0.01 mg accuracy (see ref. 12). Considering the weight of faeces egested by an *ad libitum*-fed larva (5, 10 and 15 mm) and the time required for the egestion (GCT), weight of the faeces that could be egested by a larva in a day was calculated for each size class. Multiplying the mean daily defecation (mg faeces/larva/day) of the different size classes of the larva with respective larval duration, faeces egested by the larvae in the experimental trays during the complete larval period was calculated. Subtracting the dry weight of faeces from that of the unfed remains and faeces mixture, dry weight of the unfed remains was calculated. The difference between the energy content of the nutrient medium provided and that of the unfed remains at the time of completion of larval development represented food ingested during the larval development. The formulae given below have been followed for the estimation of detritus ingestion rate of *K. barbitarsis*:

$$\text{Faeces egested (mg dry weight/larva) during the entire larval period } (X) = \frac{24 \times Fw \times d}{\text{GCT}}, \quad (1)$$

where 24/GCT is the frequency of gut clearance/day, Fw the dry weight of faeces egested or gut content weight of an *ad libitum*-fed larva and d the larval duration in days.

$$\text{Dry wt. of unfed remains} = \text{wt. of unfed remains and faeces} - \text{wt. of faeces} \quad (2)$$

$$\text{Energy content of the unfed remains } (Y) = (UF - X) \times E_1, \quad (3)$$

where UF is the dry weight of the unfed and faeces mixture left over in the medium at the time of completion of the larval development and E_1 is the energy content of the food in the control medium (into which the larvae were not introduced) at the time, when the larvae in the experimental medium have completed larval development. This value is considered to represent the energy content of the unfed remains in the experimental trays, at the time of completion of larval development.

$$\text{Detritus ingested } (C) = \frac{E_{T1} - Y}{N}, \quad (4)$$

(J/larva)

where E_{T1} is the energy content of the detritus at the commencement of the experiment and N, the number of larvae.

$$\text{Faecal energy input into the medium (J/larva) in the entire larval period } (Z) = ET_0 - Y, \quad (5)$$

where ET_0 is the energy density of the unfed and faeces mixture left over in the medium at the completion of the larval development.

Using the basic data provided in Table 1, food consumption of *K. barbitarsis* larva in the entire larval period of 12 days was determined as follows:

Energy content of the medium at $T_1 = 818.2$ mg × 14.2 J/mg = 11616.5 J

Table 1. Basic data used for the estimation of food consumption of *K. barbitarsis* larva

Parameter	Size class of the larva (cm) (0–0.5)	(0.5–1.0)	(1.0–1.5)
Duration of the stage (days)	3	4	5
Gut clearance time (min.)	161 8	261 8	464.5
Faeces egested (mg larva⁻¹time)	0.03	0.11	0.15
Faeces egested (mg larva/day)	0 27	0 61	0.47
Total number of larvae*	409	488	330
Total weight of faeces egested (mg wet wt.)	327.60	989 85	775.50† 240.95*
or (mg dry wt.)	58 97	178.20	181.13
	f_1	f_2	f_3

*At the termination of the experiment, out of 409 larvae 79 were still in the size class of <1.0 cm; therefore, faeces weight of the respective size class (0 61 mg) was used for calculating egestion.

Energy content of the medium at $T_0 = 499.3$ mg × 8.7 J mg = 4358.5 J

Energy density of the medium at T_1 (14.2 J/mg) and at T_0 (8.7 J mg) and that of food in the control tray (E_1) (= that of unfed remains in the experimental tray at the time of completion of larval development) were estimated in a Parr semimicro bomb calorimeter (model 1411, Parr Instrument Co., Moline, USA).

Total weight of faeces $= f_1 + f_2 + f_3 = 418.3$ mg

Unfed remains (dry wt.) $= 499.3 - 418.3 = 81.0$ mg (cf. eqn. (2))

Energy content of the unfed remains (Y) = 81.0 mg × 9.1 J/mg = 737.1 J (cf. eqn. (3))

Energy content of the faeces egested/409 larvae = 4358.5 − 737.1 = 3621.4 J (cf. eqn. (5))

Faeces egested/larva = 8.81 J

Food ingested/409 larvae = 11616.5 − 737.1 = 10879.4 J (cf. eqn. 4)

Food ingested/larva = 26.6 J.

The results obtained by this method were tested by isotopic tracer method using ^{14}C labelled *Chlorella* as food for *K. barbitarsis* larva. The larvae of the chosen size classes were starved for 4 to 6 h in glass cavity blocks containing distilled water, and fed on the labelled *Chlorella*. Once in every 20 min, a few larvae were transferred to 5% formalin and after thorough washing in distilled water, the larvae were dried and the radioactivity of the larvae was counted. The time at which maximum count was obtained indicated the gut loading time. Similarly, the fully-fed larvae were transferred to cavity blocks containing distilled water for gut evacuation and the radioactivity of the larvae was counted periodically. The time at which the minimum count was obtained was considered as GCT. Considering the maximum and minimum counts, the quantity of *Chlorella* consumed by the larvae was calculated. Table 2 provides data on the bioenergetics

Table 2. Bioenergetics of *K. barbitarsis* larva fed *ad libitum* with *Chlorella* Each value ($\bar{X} \pm SD$) represents the average of three replicates, each consisting of 409 larvae reared from hatching to pupation

Parameter	
Larval duration (days)	12.0
Consumption (J)	26 57 ± 1 47
Faeces (J)	8.81 ± 0 16
Absorption (J)	17.80 ± 1.4
Excretion (J)	1.73 ± 0.12
Assimilation (J)	16 02 ± 1.0
Production (J)	10.63 ± 0.21
Metabolism (J)*	5.38 ± 1.73
Metabolism (J)**	8 05 ± 0 35
Assimilation efficiency (%)	60.30 ± 1.70
Pe1 (%)	40.00 ± 1 80
Pe2 (%)	66.35 ± 6.10

*Calculated as ($A - P + U$); **Estimated respirometrically.

Table 3. Comparison of mean daily food consumption, egestion and assimilation of *K. barbitarsis* larvae fed *ad libitum* with *Chlorella* as estimated by three different (isotopic, GCT and gut content weight and gravimetric) methods. Each value ($\bar{X} \pm SD$) represents the average of three replicates each consisting of 409 larvae reared from hatching to pupation

Method	Consumption (J/larva/day)	Faeces (J/larva/day)	Assimilation efficiency (%)
Isotopic method	2 40 ± 0 09[a]	0 87 ± 0 03	63.8 ± 2.2[a]
GCT and gut content methods	2.22 ± 0.12[a]	0 88 ± 0 04	60.3 ± 1 7[a]
Gravimetric method ($P + F + U + R$)	2.42 ± 0.12[a]	—	—

Values in a column superscribed with the same alphabet are not statistically significant (Students t test).

of *K. barbitarsis* as estimated by this method. Assimilation (60.3%) and net production (Pe_2) efficiencies (66.4%) of *K. barbitarsis* are far higher than those reported for other aquatic detritivorous insects fed on nutritionally poor detritus organic matter and the causative factors have been discussed in detail elsewhere[13].

Data on consumption, egestion and assimilation of *Chlorella* detritus obtained by GCT and gut content weight method, gravimetric method ($P+F+U+R$), and isotopic tracer method are provided in Table 3. The differences between the estimates of consumption and assimilation by these methods were less than 7%. Statistical analysis revealed that the differences between these values were not significant ($P>0.5$) (Table 3). Therefore, the proposed method, which considers GCT and faecal energy is recommended for the precise estimation of food consumption of detritivores like *K. barbitarsis*.

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Electron microscopic observations of the Indian isolate of equine infectious anaemia virus grown in equine leucocytes

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We have used electron microscopy to demonstrate the presence of viral particles in the leucocytes of naturally infected active symptomatic and asymptomatic equine infectious anaemia-(EIA) carrier horses as well as in the primary leucocyte cultures obtained from a seroconverted asymptomatic donkey after its experimental infection with the blood, spleen and lymph node materials of asymptomatic-carrier horses. Typical retroviral particles resembling to EIA virus were observed in various developmental stages in both asymptomatic and symptomatic cases.

THE occurrence of equine infectious anaemia (EIA) was reported in India in 1987 in Thoroughbred horses[1]. The disease, first suspected clinically, was confirmed serologically by demonstration of specific antibodies by Coggins' test[1] as well as by C-ELISA[2]. There had been apprehensions in the equine industry regarding the infectiousness of asymptomatic EIA-carrier horses. Therefore, the purpose of the present study was to demonstrate the EIA virus by electron microscopy in the ultrathin sections of the leucocytes of asymptomatic seropositive-carrier horses.

Buffy coat cells from active or asymptomatic seropositive natural cases of EIA in horses and 9 days old *in vitro* primary leucocyte cultures of a donkey infected experimentally with blood, spleen and lumph node material from asymptomatic EIA horses as well as from a EIA seronegative healthy pony were prepared as described elsewhere[3].

For preparation of the material for electron microscopy, the buffy coat cells/leucocytes were centrifuged at 2500–3000 rpm for 30 min in the refrigerated centrifuge (Remi K-70). The pellet obtained was put in 3% glutaraldehyde solution and stored at 4°C. Thereafter, the material was transferred into 2% osmium tetroxide, pH 7.2, for 4–5 h till it became brownish to black. After dehydration in ascending grades of acetone (25, 50, 75 and 100%), the material was kept in 2% uranyl acetate for 15 min for staining.

The material was dehydrated in ascending grades of acetone with embedding mixture (50, 75, 100%) keeping for 30 min in each step. The material was then transferred in the centre of the bottom of gelatin capsule. The capsule was filled with 100% embedding solution and kept at 60°C for 36–48 h. When the preparation became solid, sections of 1–2 millimicron thickness were cut, stained with methylene blue for 30 sec and examined under a light microscope. From the selected portion retained on the block, thin sections (60–80 nm thick) were cut, picked upon 200 mesh