CHAPTER - II

Egestion, Excretion and Respiration
RESULTS

Faeces and Mucus (Fe+Mu)

Results on the amount of faeces and mucus (Fe+Mu) produced and percent energy loss in Fe+Mu of control (28°C; starved for 30 days) and experimental *P. granulosa* are presented in tables 3 and 4 and figures 5 and 6 respectively. One-way ANOVA showed significant increase in Fe+Mu with increase in body size at 28°C.

Absorbed energy and absorption efficiency

Results on the amount of absorbed energy (Ab) and absorption efficiency (Ass) of control and experimental *P. granulosa* are presented in tables 5 and 6 and figures 7 and 8 respectively. Absorbed energy increased with increase in body size in both control and experimental groups (Tables 5, 6 and 7) (ANOVA).

Temperature

*Poecilobdella granulosa* of growth phase and inflection point of the growth curve showed no change in Fe+Mu at 18°C but exhibited a significant increase at 38°C (Table 3) compared to that of the control (28°C). However, at reproductive phase *P. granulosa* showed significant increase in Fe+Mu both at 18 and 38°C.

Percentage of consumed energy lost in Fe+Mu showed a gradual increase from growth phase to the reproductive phase at 18, 28 and 38°C (Fig. 5) (ANOVA). But at each life history stage *P. granulosa* acclimated to 18°C showed highest percent loss (GP 15.8%; IP 17.8%; RP 21.2%) in Fe+Mu followed by those acclimated to 38°C (GP 11.7%; IP 14.1%; RP 15.3%) compared to the control group (GP 11.2%, IP 12.0%; RP 13.2%).

Absorbed energy in *P. granulosa* of growth phase decreased significantly at 18°C but did not change at 38°C compared to the control (Student’s t-test) (Table 5). Similar results were found during inflection point and reproductive phase of the growth curve. *P. granulosa* exhibited more than 82% absorption efficiency at all the three life-history stages irrespective of acclimation temperature except at 18°C during reproductive phase where the absorption efficiency was only 79%. At each life history stage absorption efficiency was found to be lowest (GP 84.2%; IP 82.3%; RP 78.8%) in the cold acclimated group compared to either control group (GP 88.7%; IP 88.0%; RP 86.8%) or those acclimated to 38°C (GP 88.3%; IP 86.0%; RP 85.0%) (Fig. 7).
Starvation

Poecilobdella granulosa at growth phase showed significant increase in Fe+Mu with increase in starvation duration from 30 (control) to 60 days but showed insignificant increase with increase in starvation duration from 60 to 90 days (Table 4) (Student’s t-test). Similar results were obtained during inflection point and reproductive phase of the growth curve.

At growth phase 60 day starved P. granulosa showed more percentage loss (11.7%) in Fe+Mu of consumed energy compared to 30 (control) (11.2%) and 90 day starved groups (11.2%) (Fig. 6). At inflection point P. granulosa showed no change in percent energy loss in Fe+Mu after either 60 or 90 day starvation. However, at reproductive phase it showed decreased percent energy loss in Fe+Mu with increase in starvation duration from 60 (12.1%) to 90 (11.5%) days compared to the control group (15.3%).

Poecilobdella granulosa at growth phase, inflection point and reproductive phase showed significant increases in absorbed energy with increase in starvation duration from 30 (control) to 60 days but showed no change with increase in starvation duration from 60 to 90 days (Table 6) (Student’s t-test).

Absorption efficiency was found to be greater than 86% at each of the life-history stages of P. granulosa fed following starvation either for 30, 60 or 90 days (Fig. 8). While no significant changes were observed between the control and starved P. granulosa during growth phase and inflection point, gradual increase with starvation duration occurred during reproductive phase.

Ammonia excretion

Results on the amount of endogenous and exogenous ammonia excretion and percentage of energy loss in excretion in control and experimental P. granulosa are presented in tables 7 and 8 and figures 9 and 10 respectively. ANOVA showed that endogenous and exogenous ammonia excretion increased with increase in body size in both control and experimental groups (Tables 7 and 8).

Assimilated energy and assimilation efficiency

Results on the amount of assimilated energy (Ass) and assimilation efficiency (AssE) in control and experimental P. granulosa are presented in tables 9 and 10 and figures 11 and 12 respectively. While assimilated energy increased significantly with increase in body size in both
control and experimental groups (Tables 9 and 10) (ANOVA) assimilation efficiency decreased with increase in body size.

**Temperature**

During growth phase, inflection point and reproductive phases *P. granulosa* showed significant decreases in endogenous and exogenous ammonia excretion with decrease in temperature from 28 (control) to 18°C (Table 7) but showed a significant increase in both endogenous and exogenous ammonia excretion with increase in temperature from 28 to 38°C (Student’s t-test). At each life history stage the amount of endogenous excretion was significantly higher than that of exogenous excretion (Table 7). Total amount of energy loss in excretion also increased with increase in body size at each temperature and at each life-history stage (ANOVA) with maximum loss of energy in excretion being found at 38°C.

Percentage of consumed energy loss in excretion decreased at 18°C (GP 7.5%; IP 8.0%; RP 9.8%) and increased at 38°C (GP 10.8%; IP 11.0%; RP 11.6%) compared to control (GP 9.2%; IP 9.8%; RP 10.3%) (Fig. 9). Percent loss in excretion was further found to increase with increase in body size at each temperature.

Assimilated energy at growth phase, inflection point and reproductive phase decreased significantly at 18°C compared to control (28°C) but did not change at 38°C (Table 9) (Student’s t-test). *P. granulosa* exhibited more than 86% assimilation efficiency at all life-history stages (Fig. 11). Assimilation efficiency of *P. granulosa* at growth phase acclimated to 38°C (87.8%) was less than that of either control (89.7%) or those acclimated to 18°C (91.1%). At inflection point no significant change in assimilation efficiency was observed between the control and starved *P. granulosa* (Fig. 11). However, at reproductive phase assimilation efficiency was found to decrease both at 18 (87.6%) and 38°C (86.3%) compared to the control (88.1%).

**Starvation**

*Poecilobdella granulosa* of growth phase, inflection point and reproductive phase showed significant decrease and increase in endogenous and exogenous ammonia excretion respectively with increase in starvation duration from 30 to 60 and from 60 to 90 days (Table 8) (ANOVA). However the magnitude of change was found to be higher following 90 day of starvation compared to 60 days.

Percentage energy loss in excretion increased with increase in body size irrespective of the feeding schedule and increased with increase in the duration of starvation at each life-history stage.
except during reproductive phase where similar percent loss of energy in excretion was observed both at 60 (12.8%) and 90 (12.7%) day starvation (Fig. 10).

*Poecilobdella granulosa* at growth phase showed significant increase in assimilated energy with increase in starvation duration from 30 (control) to 60 days but showed no significant change with increase in starvation duration from 60 to 90 days (Table 10) (Student’s t-test). At inflection point amount of assimilated energy remained unchanged following 60 day starvation but showed a significant increase following 90 day starvation compared to control. However, no significant difference was found in the amount of assimilated energy between 30 and 60 or 60 and 90 day starved *P.granulosa*. During reproductive phase assimilated energy increased significantly with increase in starvation duration from 30 to 60 days or from 60 to 90 days.

Assimilation efficiency was found to be higher than 85% at each of the life-history stages of *P.granulosa* fed following 30, 60 or 90 day starvation (Fig. 12). At growth phase compared to the control assimilation efficiency was found to decrease by 2.85% and 3.63% following 60 and 90 day starvation respectively, while during inflection point a decrease of 2.66% and 3.41% was noticed following 60 and 90 day starvation respectively. However, during reproductive phase assimilation efficiency decreased by 2.65% in 60 day starved group but increased by 6.71% in 90 day starved group.

**Resting and active oxygen consumption and aerobic scope**

Results on the changes in the amount of resting (Rm) and active (Am) oxygen consumption, aerobic scope (As) and respective energy values of control and experimental *P.granulosa* are presented in tables 11 and 12 and percent of ingested energy allocated to maintenance and the amount of energy potentially available for physical activity are presented in figures 13, 14, 15 and 16.

**Temperature**

Both Rm and Am in *P.granulosa* showed significant increase with increase in body size at 18, 28 and 38°C. Aerobic scope decreased with increase in body size (Table 11) (ANOVA). Further *P.granulosa* at growth phase exhibited a significant decrease in Rm, Am and As with decrease in temperature from 28 (control) to 18°C but showed a significant increase with increase in temperature from 28 to 38°C (Student’s t-test). Similar results were obtained during inflection point and reproductive phase of the growth curve.
Poecilobdella granulosa showed a gradual increase in percentage allocation of consumed energy to Rm from growth phase to reproductive phase at 18, 28 (control) and 38°C (Fig. 13). But at each life history stage P.granulosa acclimated to 18°C and 38°C showed lower (GP 19.3%; IP 20.8%; RP 23.3%) and higher percent energy allocation (GP 25.9%; IP 29.1%; RP 30.2%) respectively to Rm compared to that of the control (28°C) (GP 22.8%; IP 23.3%; RP 25.1%).

Poecilobdella granulosa showed a significant decrease in percentage of consumed energy allocated to As from growth phase to reproductive phase at 18, 28 and 38°C (Fig. 14). But at each life history stage P.granulosa acclimated to 18 and 38°C showed lower (GP13.7%; IP 9.2%; RP 5.1%) and higher percent energy allocation (GP 17.3%; IP 10.6%; RP 6.1%) respectively to As compared to that of the control group (28°C) (GP 15.6%; IP 10.1%; RP 5.6%).

**Starvation**

Both Rm and Am increased with increase in body size in P.granulosa fed following 30 (control), 60 and 90 day starvation (Table 12) (ANOVA). In those leeches fed following 30 or 60 day starvation As remained unchanged between growth phase and inflection point but decreased significantly at reproductive phase (Student's t-test). Further P.granulosa fed following 90 day starvation showed a significant increase in As from growth phase to inflection point and inflection point to reproductive phase.

Poecilobdella granulosa of growth phase fed following 30, 60 and 90 day starvation showed highest Rm in 90 day group followed by 60 and 30 day groups. During growth phase Am of P.granulosa fed following 60 and 90 day starvation was found to be similar and significantly higher than that of P.granulosa fed following 30 day starvation. Similar results were obtained during inflection point and reproductive phase. Aerobic scope on the other hand was found to be higher in all three stages of P.granulosa fed following 60 day starvation compared to those fed following 30 or 90 day starvation (Table 12). However, during growth phase, inflection point and reproductive phase P.granulosa fed following 60 day starvation showed higher As than those fed following 90 day starvation (Table 12).

Poecilobdella granulosa fed following 30, 60 and 90 day starvation showed a gradual increase in the percentage allocation of consumed energy to Rm from growth phase to reproductive phase (Fig. 15). At each life-history stage P.granulosa fed following 30 day starvation showed low percent energy allocation to Rm (GP 22.8%; IP 23.3%; RP 25.1%) followed by those fed following 60 (GP 28.1%; IP29.1%; RP 30.9%) and 90 day starvation (GP 32.2%; IP 34.1%; RP 35.0%) (Fig. 15).
Percentage of consumed energy allocated to As was found to decrease with increase in body size in *P. granulosa* fed following 30, 60 or 90 day starvation (Fig. 16). During growth phase *P. granulosa* fed following 30 day and 60 day starvation showed higher percent (15.6%; 15.2%) energy allocation respectively to As compared to those fed following 90 day starvation (8.3%) (Fig. 16). During inflection point *P. granulosa* fed following 60 day starvation showed higher percent energy allocation to As (12.1%) followed by those fed following 30 and 90 day starvation respectively (10.1%; 7.6%). During reproductive phase *P. granulosa* fed following 60 day starvation showed higher percent energy allocation to As (8.3%) than those fed following 30 or 90 day starvation respectively (5.6%; 6.3%).

Resting oxygen consumption of fed and unfed *P. granulosa* and apparent specific dynamic action

Results on the changes in resting oxygen consumption of fed (Rm fed) and unfed (Rm unfed) *P. granulosa* apparent specific dynamic action (ASDA) and percentage of ingested energy allocated to ASDA of control and experimental groups are presented in tables 13 and 14 and figures 17 and 18 respectively. Resting oxygen consumption of fed and unfed *P. granulosa* and ASDA increased significantly with increase in body size in both control and experimental groups (Tables 13 & 14) (ANOVA).

Temperature

During growth phase, inflection point and reproductive phase, resting oxygen consumption of fed and unfed *P. granulosa* and ASDA were found to decrease at 18°C and increase at 38°C compared to control (28°C) (Table 13) (Student’s t-test).

Percentage of consumed energy allocated to ASDA followed no particular pattern with increase in body size (Fig. 17). At 28°C the percentage energy allocated to ASDA decreased from 10.7% at growth phase to 9.4% at inflection point and again increased to 10.0% at reproductive phase. At 18°C the percentage energy allocated to ASDA increased from 8.6% at growth phase to 9.8% at inflection point and again decreased to 9.1% at reproductive phase. At all the three life history stages *P. granulosa* acclimated to 38°C showed higher percent energy allocation to ASDA (GP 12.2%; IP 11.8%; RP 12.2%) compared to those acclimated to 18°C (GP 8.6%; IP 9.8%; RP 9.1%) or 28°C (GP 10.7%; IP 9.4%; RP 10.0%).
Starvation

Resting oxygen consumption of fed and unfed *P.granulosa* and ASDA increased significantly with increase in starvation duration from 30 days to either 60 or 90 days at all the three life history stages (Table 14) (Student’s t-test).

Following 60 and 90 day starvation, percentage of consumed energy allocated to ASDA increased from 13.8% at growth phase to 14.4% at inflection point and to 15.3% at reproductive phase (Fig. 18). Further at each life history stage the percentage of energy allocated to ASDA increased with increase in starvation duration and maximum percent energy allocation found following 90 day starvation.
DISCUSSION

Faeces and Mucus (Fe + Mu)

In almost all the animals energy lost in faeces includes not only undigested food material but also sloughed intestinal epithelial cells, mucus, catabolized digestive enzymes and bacteria. Faeces which is a mixture of undigested food components and unabsorbed residues is one of the enzyme – rich waste products eliminated from animals. Due to their inseparable nature both faeces and mucus are usually measured together. For an energy budget the measurement of faeces is essential because it has a separate origin and distinctive chemical composition and thus possess different energy contents. The amount of faeces produced and its composition is influenced by a variety of factors, some intrinsic to the animal and some environmental (extrinsic) in origin.

Herbivores which are specialized seed – eaters may eliminate only 20% of the energy content of their food in egestion whereas sap-sucking insects egest 90% (Brafield and Lewellyn, 1982).

Mucus loss was found to play an important role in the energetics of triclads (platyhelminths: Turbellaria) (Calow and Woolhead, 1977) and annelids (Singhal and Davies, 1987; Dratnal and Davies, 1990; Kalarani and Davies, 1994). Since considerable quantities of energy are often egested through mucus, many organisms exploit this as a food source. Generally mucus plays an important role in respiration of aquatic animals and, on the other hand, it also assists in digestion, excretion and locomotion.

The composition and quantity of faeces depend not only on the nature of the ingested food but also on the extent of digestion and absorption. Hence marked increase in Fe+Mu with increase in body size in control group (Tables 3 and 4) along with increased food ingestion (Chapter 1; Tables 1 and 2) indicate that larger individuals might have lost greater amounts of energy in egestion because of their inability to elevate digestion and / or absorption efficiency on par with the ability to consume large amounts of blood. Faecal material in animals may arise in four ways (Brafield and Lewellyn, 1982). First, some ingested food may not be digested because of absence of required enzymes. Second, food may only partially be digested because the concentrations of required enzymes are less or the retention time of food in the alimentary canal was too short. Third, some of the products of digestion may not be assimilated and fourth, molecules capable of assimilation may not actually be assimilated. Since chemical analysis of faeces and mucus has not been carried out in the present study the composition of Fe+Mu produced is not known. However, the energy content of Fe+Mu obtained provide required information regarding energy loss in egestion. Although no significant effect of biomass on Fe+Mu production has been found in a cyprinid, P. phoxinus (Cui and Wootton, 1988), a linear relationship

between faecal production and food consumption was found in several other fish earlier (Gerking, 1955; Cui and Wootton, 1988). Blood sucking insects, ticks and leeches generally produce very little faeces because their food is almost completely digestible and so almost all is assimilated (Brafield and Llewellyn, 1982). However, this study has demonstrated that considerable amount of energy loss in egestion occurs even in blood sucking leeches which increases with body size.

The potential amount of energy available for excretion, metabolism and growth depends upon the proportion of food energy lost in egestion. Elliott (1976) found that 25-30% of food energy is lost in egestion in *S.trutta* feeding on *Gammarus* sps. while 6.5% was lost in minnows feeding on white worms (Cui and Wootton, 1988) and 25.6% was lost in the fish, *R.rutilus* feeding on meal worms (Hofer et al. 1985). The proportion of energy loss (11 to 14%) in Fe+Mu (Figs. 5 and 6) indicate that depending upon size 86 to 89% of ingested energy will be available for other life history traits in *P.granulosa*.

**Absorbed energy and absorption efficiency**

In animals absorption occurs through the wall of the intestine after digestion of food. Digestion and absorption mainly occur in the intestine although crop is also used in predatory species for this purpose. Energy absorbed determines the energy available for growth and respiration (Smith and Davies, 1995).

Increase in absorbed energy with increase in body size in both control and experimental *P.granulosa* could be attributed to higher rate of increase in C with biomass (Table 1 and 5) despite decreased absorption efficiency (Fig. 7). Although Elliot (1976) found no effect of body size on absorption efficiency in *S.trutta*, Kelso (1972) found decreased absorption efficiency in *S.vitreum* with increase in body size. In contrast Smith (1973) in *Histrio histrio* (L.), Allen and Wootton (1983) in *G.aculeatus* and From and Rasmussen (1984) in *S.gairdneri* observed increased absorption efficiency with increase in body size.

**Temperature**

Amount of egestion varies as a function of feeding level and temperature in several aquatic species (Kitchell et al. 1974, 1977; Elliott, 1976). During growth phase and inflection point *P.granulosa* acclimated to 18°C showed no change in Fe+Mu compared to the respective controls (Table 3) despite decreased food ingestion (Chapter 1; Table 1). This shows that low temperature not only results in low ingestion but also in the reduction of digestion and/or absorption efficiency. A significant increase in Fe+Mu at 38°C further indicates that high temperature increases food ingestion (Chapter 1; Table 1). However, due to lack of increase in digestion and absorption
efficiencies on par with ingestion high temperatures may cause greater loss of energy through egestion. Increase in faeces with elevation in temperature upto 22°C has been noticed in yellow perch, *P.flavescens* (Kitchell et al. 1977). But in *N.obscura* increase in Fe + Mu with increase in temperature from 5 to 15°C and decrease in Fe + Mu with increase in temperature from 15 to 25°C was found to be an effect of food ration (Kalarani and Davies, 1994). Increased amounts of Fe+Mu secreted by *P.granulosa* during reproductive phase both at 18 and 38°C despite decrease and increase in C respectively (Chapter 1; Table 3) provide evidence that both low and high temperatures lead to reduced efficiency of digestion and/or absorption in animals and the effect could be life history stage specific.

Gradual increase in the percentage of consumed energy lost in Fe+Mu from growth phase to reproductive phase at 18 and 38°C similar to the control (28°C) group (Fig. 5) indicate that digestion/absorption efficiency decreases as body size increases irrespective of the temperature. Elliott (1976) has conducted the most searching analysis to date, determining the separate and combined effects of temperature, ration and biomass on the faecal energy loss in *S.trutta* feeding on *Gammarus pulex* (L.) and reported that at a given ration (eg. R_max) faecal loss decreased with increase in temperature, falling from approximately 29% at 4°C to 20% at 19°C. At maximum rations (P=1.0), the proportion of egested energy declined with increase in temperature in the yellow perch, *P.flavescens* (Kitchell et al. 1977). In juvenile sockeye salmon of Babene lake the energy loss was found to be 30% of the ingested food throughout the year irrespective of the temperature (Beauchamp et al. 1989). Higher percent losses in Fe+Mu exhibited by *P.granulosa* at each life history stage both at 18 and 38°C than at 28°C further suggests that 28°C could be the optimum temperature which is associated with higher digestion/absorption efficiency and low energy loss in Fe+Mu with potential increase in utilizable energy.

A significant decrease in absorbed energy in *P.granulosa* during growth phase, inflection point and reproductive phase at 18°C (Table 5) indicate that due to depressive effects of low temperature, the abilities of digestion/absorption were decreased. Thus no compensatory phenomenon was found to exist in *P.granulosa* regarding absorption against low food ingestion. Further no change in absorbed energy despite increased food ingestion at 38°C implies that the animal may have a cut off level or maximum capacity to digest or absorb ingested food and hence although food ingestion is high the amount of food absorbed may not increase at higher temperatures.

Decreased absorption efficiency with decrease or increase in temperature (Fig 7) indicates that any deviation or change in the normal range of temperatures affects digestive capacity in leeches. Although temperature was not found to influence absorption efficiency in *S.gairdneri*
From and Rasmussen, 1984), a decrease in absorption efficiency with increase in temperature was found in the minnow, *P. phoxinus* (Cui and Wootton, 1988). However, an increase in absorption efficiency with increase in temperature is common among several fish (Brocksen and Brugge, 1974; Elliott, 1976; Allen and Wootton, 1983).

Greater decrease in absorption efficiency at 18°C than at 38°C compared to the respective controls at each life-history stage further indicate that decrease in temperature is more stressful than an increase. But significantly AbE (more than 82%) exhibited by *P. granulosa* at all the three life history stages irrespective of acclimation temperature (except RP at 18°C) indicate that even at altered temperatures, leeches tend to maintain absorption efficiency closer to the controls.

**Starvation**

In practice, fasting animals avoid small amounts of faeces than the normal animals since the faecal energy of metabolic origin is of little significance for animals receiving normal amounts of food (Guillaum & Summers, 1970). Variation in the digestibility of foods is generally a major factor affecting their usefulness as energy sources to the animal.

Blackburn (1968) found that the efficiency of the digestive process in the largemouth bass, *M. salmoides* feeding daily on guppies improved from 3.2% of energy loss in egestion to only 0.8% loss when fed once every 5 days. Significant increase in Fe+Mu with increase in starvation duration from 30 to 60 days (Table 4) with no further significant increase following 90 day starvation at each life history stage indicate that although starvation results in increased food ingestion (Chapter 1; Table 2) which in turn results in increased Fe+Mu similar trend may not continue following prolonged starvation, perhaps due to limitations in gut capacity and digestion/absorption efficiency. Higher animals like *Megalops cyprinoides* (Broussonet) also exhibited no significant increase in digestion efficiency following starvation from 10 to 40 days resulting in higher egestion levels (Pandian, 1967).

Similar proportions of energy loss in Fe+Mu by starved *P. granulosa* (11 to 13%) compared to the controls (11 to 14%) irrespective of life history stage and duration of starvation (Fig. 6) indicate that with increased food ingestion (Table 2) along with no change in energy loss in egestion, starved leeches may obtain more energy available for life-activities compared to the controls. Also leeches may survive long starvation periods by voiding small proportions of energy in egestion.

Although absorption efficiency decreased with increase in biomass in control group (Fig. 8) following starvation for 60 and 90 days, lower rate of decrease in efficiency or even increased
efficiency with increase in biomass was found to result in corresponding increase in absorbed energy (Table 6). Over compensation in absorption efficiency during reproductive phase following 90 day starvation (Fig. 8) could well be orchestrated as one of the mechanisms exhibited by maturing animals in order to procure enough resources to meet the demands of reproduction.

Ammonia excretion

Many of the simple products of digestion which are absorbed by the gut are broken down sooner or later. The waste products of carbohydrate and fat metabolism are carboxydiol and water, but in the case of protein some nitrogenous wastes are produced as well, which contain some energy. The commonest among such waste products are ammonia, urea and uric acid derived either from absorbed food or from body tissues and are mainly composed of carbon, hydrogen, oxygen and nitrogen. Nitrogen containing molecules are especially important for animals because they are essential for making proteins and nucleic acids, necessary for maintenance and growth.

The proportion of energy loss through nitrogenous excretion was found to be insignificant in temperate freshwater aquatic organisms and generally not measured (Brafield, 1985). *N. obscura* which is widely distributed in North America was found to lose less than 5% of total energy acquired in nitrogenous wastes (Kalarani and Davies, 1994). Aquatic organisms are generally ammonotelic. Ammonia is the major excretory product in fresh water invertebrates since it is readily eliminated from the body (Pandian, 1987; Randall and Wright, 1987; Zebe et al. 1986). Nitrogen excretion has two components: Endogenous excretion and exogenous excretion. Endogenous excretion is defined as the minimum value of nitrogen excretion and is estimated after complete starvation or fed without proteins (Savitz, 1971; Savitz et al. 1977). It also corresponds to the degradation of body proteins after the utilization of reserves. Exogenous nitrogen excretion refers to the excretion of nitrogen from the food source and is mainly influenced by food consumption (Elliott, 1976; Savitz et al. 1977; From and Rasmussen, 1984).

Increased endogenous and exogenous ammonia excretion with increase in body size in *P. granulosa* could be correlated to increased food ingestion and thus to the rate of protein catabolism. The excretion rate in copepodes was found to increase allometrically with individual length (Nival et al. 1974; Ikeda, 1985) while the excretion rate per unit weight decreases with (Corner et al. 1965) or is independent of individual length (Kremer, 1977; Morand et al. 1987). Gerking (1955) demonstrated a weight dependent relation for endogenous nitrogen excretion (approx. \(W^{0.54}\)) in bluegill sunfish following the well known relationship of a decrease in the rate of metabolism with increase in body size.
In *S. trutta* Elliott (1976) found little effect of body weight on energy loss in excretion. Nelson et al. (1977) also found a positive correlation in *M. rosenbergii* between ammonia production (mg NH4/hr) and biomass although unit ammonia production showed a negative trend. Wickins (1985) and Wajsbrot et al. (1989) found a decrease in the rate of ammonia excretion with increase in body size in *P. monodon* and *Penaeus semisulcatus* (De Haan) respectively. Further in *P. chinensis* ammonia – N excretion had a negative power relationship within the range of biomass from 0.29 to 11.19 g indicating a positive correlation between total excretion and body size a general trend observed in several aquatic organisms (Gerking, 1955; Iwata, 1970; Chen et al. 1991) although fractional loss of excretion was found to be independent of weight (4 to 300g range) in gammarids (Elliott, 1976).

**Assimilated energy and assimilation efficiency**

Movement of energy through an animal depends on the efficiency with which it assimilates energy from its food and the efficiency with which this assimilated energy is transformed into production. The energy actually assimilated by an animal is the difference between the energy absorbed and energy lost in excretion. Petrushewicz and Macfadyen (1970) regarded assimilation as the energy channeled into only respiration and production.

A significant increase in assimilated energy (Ass) with increase in body size in both control and experimental *P. granulosa* (18 and 38°C) (Tables 9 and 10) along with an increase in food ingestion indicate that the amount of energy assimilated is primarily an effect of the amount of food ingested while the later is the direct effect of body size at any given temperature (Tables 1 and 2). Further gradual decrease in assimilation efficiency with increase in body size (Fig. 11) indicates that although larger individuals have greater ability to assimilate higher amount of consumed food only a limited proportion of food energy gets assimilated at each body size or temperature. Assimilation efficiency was found to be constant with increase in body size (Scheerboom and Geldof, 1978) and food quantity in the pond snail, *Lymnaea stagnalis* L. (Scheerboom, 1978). But an inverse relation between feeding rates and efficiencies has been noticed in several organisms (Calow, 1984).

**Temperature**

Exponential increase in specific excretion rate with temperature is a common phenomenon in copepods (Nival et al. 1974). Anderson and Nival (1986) and Kremer (1975) also found similar trends in *Salpa fusiformis* (Cuvier) and the ctenophore, *Mnemiopsis leidyi* (Agassiz). A significant decrease in endogenous and exogenous ammonia excretion in *P. granulosa* during GP, IP and RP at 18°C (Table 7) could be attributed to low metabolic and deamination activities. Guirin – Ancy
obtained stable endogenous ammonia excretion in the fish, *Dicentrarchus labrax* (Linnaeus) after 6 days at 20°C and after 8 days at 16°C. Although there was some evidence to show that temperature had a non-linear effect on N – excretion, regression analysis carried out by Cui (1987) showed that the rate of excretion was mainly determined by the rate of food consumption. This explanation could well be extended to the present study also because *P.granulosa* showed decreased energy loss not only in total excretion but also in exogenous ammonia excretion (Table 7) with decrease in food consumption at 18°C (Table 1) while those at 38°C showed increased food consumption (Table 1) and also increased total and exogenous ammonia excretion (Table 7). A significant increase in both endo and exogenous ammonia excretion with increase in temperature at 38°C (Table 7) suggest increased catabolism and utilization of proteins due to higher energy demands. Wieser (1972) attributed higher production of ammonia in *Ligia beaudiana* (Milne Edwards) in September than in October to higher temperature. Increased ammonia excretion with elevation in temperature has also been noticed in a ribbed mussel, *G.demissa* (Wilbur and Hilbish, 1989). Kalarani and Davies (1994) also observed that elevated temperature causes an increase in endogenous and exogenous ammonia excretion in *N.obscura*. Higher amounts of endogenous excretion than exogenous excretion at each life history stage in *P.granulosa* (Table 7) indicate higher proportion of body protein utilization compared to food protein utilization.

Increased proportion of consumed energy loss in excretion at each temperature (Fig. 9) with increase in body size in *P.granulosa* could well be a combined effect of the levels of both food ingestion and maintenance utilization of body proteins. Decrease in percent energy loss in excretion at 18°C and increase at 38°C at each life-history stage compared to the control (Fig. 9) further indicates that the lower the food intake the greater will be the percent loss in excretion. In gammarids also nitrogenous excretory products account for about 4 to 6% loss in energy at 4°C (Elliot, 1976) and about 11 to 15% loss at 20°C (Brett and Grooves, 1979).

It is clear that during GP, IP and RP *P.granulosa* at 18°C showed a significant decrease in assimilated energy and this could be an effect of low food ingestion (Tables 1 & 9). However an increase in temperature upto 38°C was found to cause no significant effect on the amount of energy assimilated along with food ingestion. While the larvae of *Pericalla ricini* (Fabricius) showed one and half times higher rate of feeding and assimilation at high temperature (32°C) over those at low temperature (26°C), the adult *P.ricini* were found to show increased food consumption and assimilation at lower temperature (26°C) than at higher temperature (35°C) (Krishnan and Chockalingam, 1988). Further the rate of food consumption, assimilation and conversion in *P.ricini* were found to attain optimum level at about 32°C. Mathavan and Pandian (1975) and Rawlins and Leaderhouse (1981) also made similar observations in other lepidopterans like the monarch
butterfly, *Danaus chrysippus* (L.) and the monarch caterpillar, *Danaus plexippus* (Linnaeus) respectively. However the larvae of *D.chrysippus* were found to have higher total food consumption and assimilation rates at cold temperature (19°C) than at warm temperature (37°C). Decreased assimilation efficiency with increase in temperature (Fig. 11) in *P.granulosa* at each life history stage provided the best evidence for the direct effect of temperature on the ability of assimilation.

In contrast, Brockson and Brugge (1974) reported significant temperature effect, with higher temperature conferring a greater efficiency on the assimilation of *S.gairdneri* (Faecal calorie loss 28.2% at 5°C; falling to 15.2% at 20°C). Caulton (1978) also noticed higher efficiency of assimilation at high temperature (34°C) in the tropical cichlid, *Tilapia rendalli* (Boulenger) thus confirming temperature dependency of assimilation efficiency. Beenakkers et al. (1971) in locusts, Roe et al. (1980) in *Acheta domesticus* (Linnaeus) and Palanichamy et al. (1982) in *Eupterote mollifera* found a much higher assimilation efficiency and reduced feeding rate at lower temperature and attributed them to slower passage of food through the gut under such thermal conditions. Krishnan and Chockalingam (1988) stated that assimilation efficiency in larval *P.ricinii* was inversely related to the feeding rate of the larvae and level of temperature. Brett et al. (1969) found a reduction in assimilation efficiency as ration approaches the maximum for a given temperature in the sockeye salmon, *O.nerka*. Lower assimilation efficiency at higher rations has also been shown later in the brown trout, *S.trutta* (Elliott, 1976). These observations by different authors in different species could well be taken as a base and supporting evidence to explain changes in assimilation efficiency in *P.granulosa* at different temperature regimes vis-a-vis life history stages.

**Starvation**

Presence of significant differences between fed and starved leeches may be a reflection of the involvement of protein metabolism (Buttery and Annison, 1973). Records of N-excretion in starved animals provide a near approximation of the endogenous fraction, particularly poikilothermic vertebrates that normally endure prolonged periods of starvation (Stover, 1967). Significant decrease in endogenous excretion with prolonged starvation duration from 30 to 90 days at GP, IP and RP (Table 8) indicate that due to depleted protein resources leeches may reduce the utilization of the same in order to maintain integrity of the tissues. In different size groups of siberian sturgeon, *A.baeri* (60 g to 2 kg) endogenous ammonia excretion was found to decrease even after 8 days of starvation (Salin and Williot, 1991). This further shows that blood sucking leeches in which the frequency of feeding is low (Zebe et al. 1986) due to high storage ability although survive prolonged periods of starvation, exhibit utilization of body proteins following 30 day starvation. An increase in ammonia excretion, indicating increased energy costs,
following food ingestion has also been observed in several species (Brett and Zala, 1975; Caulton, 1978; Zebe et al. 1986; Davenport et al. 1990; Gonzalez et al. 1990). Stressful conditions in fish lead to an increase in nitrogen loss (Hunn, 1982) thus enhancing the loss of combustible matter. Davenport et al. (1990) found that ammonia output was approximately doubled 24 hr after a meal in the captive atlantic halibut, *H. hippoglossus* and lemon sole, *M. kitt*. Putter (1907) noticed excretion of water, ammonia and other compounds following uptake of blood in *H. medicinalis*. On the other hand significant increase in exogenous excretion and total energy lost in excretion with prolonged duration (Table 8) could be attributed to increased food ingestion (Table 2). This could be due to increased gut capacity with increase in the starvation duration. Zebe et al. (1986) observed that large proportion of ammonia in *H. medicinalis* after several days in the ambient water must be derived from the breakdown of components of the ingested blood or serum.

An increase in percent energy loss in excretion with increase in body size and starvation duration at each life history stage in *P. granulosa* suggest that the rate of metabolic processes increase with increase in food ingestion (Tables 1 & 2) which occurred with progress in body size or duration of starvation. But at reproductive phase 60 and 90 day starved *P. granulosa* showed similar percent energy losses (Fig. 10) indicating the amount of energy loss in excretion remains as a constant factor of ingested food beyond certain level.

Results presented in table 10 showing an increase in assimilated energy with increase in starvation duration from 30 to 60 days and insignificant change between 60 and 90 day starvation during GP indicate that while short-time starvation although leads to higher utilization of ingested energy along with increased rate of ingestion (Table 2), prolonged starvation do not offer any additional ability of either ingestion or absorption especially in leeches of growth phase. This effect was found to be existing even beyond 30 day of starvation during inflection point. Significant increase in assimilated energy with increase in starvation duration during reproductive phase however indicate that due to higher energy demands of gonadal growth the animal perhaps maximizes the utilization of available resources.

*P. granulosa* at each life history stage fed following 30, 60 or 90 day starvation was found to exhibit more than 85% assimilation efficiency indicating greater amounts of energy available for metabolism (R) and growth (G). Although assimilation efficiency in the fresh water snail, *Stagnicola elodes* (Say) was found to be higher in nutritionally poor habitats, no direct effect of starvation duration on assimilation efficiency has been reported among invertebrates (Hunter, 1975). Although starvation is expected to result in increased assimilation efficiency, no such response has been noticed in *P. granulosa* during GP, IP and RP (30 and 60 day starvation) (Fig. 12). However only 90 day starved *P. granulosa* during RP showed significant elevation in assimilation efficiency
indicating that combined effects of prolonged starvation and additional energy requirements of gonadal growth could elevate the efficiency component to higher levels.

**Resting and Active Oxygen consumption and Aerobic scope**

Measurement of oxygen consumption is the most frequently used method to measure metabolism (Lampert, 1984; Wrona and Davies, 1984; Davies et al. 1992). Understanding the oxygen requirements of aquatic animals is of great importance for aquaculture. Respiratory data provide a reliable basis for the calculation of carrying capacities, particularly of semi-intensive culture systems. The major factors affecting the oxygen requirements of aquatic organism are biomass, environmental temperature, salinity and diet. Apparently maintenance metabolism (measured as oxygen consumption) is the most important route of daily energy expenditure in most animals (Studier et al. 1975). Evaluation of various factors which might significantly affect oxygen consumption provides a means of assessing the importance of these intrinsic and extrinsic factors to the daily energy budget of the species. Respiration rate is modelled as a function of biomass, activity, temperature and specific dynamic action (Post, 1990). Minimum rate of oxygen consumption of an organism at rest in the postabsorptive state is known as 'standard metabolism'. The rate of oxygen consumption during maximum sustained activity is known as 'active metabolism'. The relationship between activity and resting metabolism (standard metabolism) in fish has most often been studied in terms of the 'scope' for activity as introduced by Fry (1947). The difference in the rate of oxygen consumption between the active state (maximum aerobic activity) and the resting state is called 'aerobic scope' for activity and indicates the maximum amount of oxygen available at a particular temperature for covering the energy costs of all kinds of biological activity. The difference between the maximum and the routine level of aerobic metabolism is considered to represent a measure of the amount of aerobic energy available under more or less natural conditions. Since this difference will always be a fraction of the scope for activity as defined by Fry (1947, 1971) and Brett (1964), the term relative scope for activity is popularly used.

An increase in both Rm and Am of *P.granulosa* with increase in body size at 18, 28 and 38°C (Table 11) suggests an increase in biomass, temperature and physical activity considerably increases the total energy requirements of the animal. The effect of size on the standard metabolic rate of animals has been the focus of much research and conjecture (Zeuthen, 1953, 1970; Kleiber, 1961; Gordon, 1972). Several investigators reported increased oxygen consumption with increase in biomass in fish (Winberg, 1956; Paloheimo and Dickie, 1966; Brett and Groove, 1979; Hughes, 1984; Oikawa and itazawa, 1984; Itazawa and Oikawa, 1986). Diana (1983) found an increased respiration with increase in age (0 to 3 years old) in a northern pike, *E.lucius* while
Yamamoto (1991) observed increased oxygen consumption with increase in body weight in the carp, *Cyprinus carpio* (Linnaeus) under resting and normoxic condition. Kalarani et al. (1993), Davies and Kalarani (1993) and Davies et al. (1996) reported increased resting and active oxygen consumption with dry biomass in *N. obscura* and *E. montezuma*. Suarez and Xiques (1969) showed the relationship between body weight and oxygen consumption in *Penaeus schmitti* (Burkenroad). The relationship between metabolism and fish weight follows a power law, values of the weight exponent varying between 0.7 and 0.9, with an extreme range of 0.5 - 1.0 (Winberg, 1956; Beamish, 1964; Brett, 1964; Fry, 1971). Several authors observed a linear relationship between the logarithms of metabolism and fish weight (Brett, 1972; Brett and Grooves, 1979). Brett and Glass (1973) also observed decreased standard metabolic rate with increasing size at all levels of temperature in a sockeye salmon, *O. nerka* and different molluscan species (Ghiritti, 1966). Studies on the effect of size in the sockeye salmon (Brett, 1965) showed a continuous change in the weight exponent from 0.78 to 0.97 with increasing levels of activity (at 15°C). The mean value of slope for the active metabolic rate of this species at all temperatures (5-20°C) was 0.98 indicating an almost insignificant effect of weight for most temperature regimes (Brett and Glass, 1973).

Decreased As with increase in body size at each temperature could be attributed to greater increase in Rm (18°C-128.3%; 28°C-82.72%; 38°C-101.3%) compared to the that in Am (18°C-62.19%; 28°C-32.74%; 38°C-45.37%) over the size range considered (Table 11). A decrease in As with increase in biomass has been observed in the shrimp, *Heterosquilla tricarinata* by Innes (1985) and in the larvae of some small (<10 mg wet weight) cyprinids such as *Chalcalburnus chalcoides* (Gueldenstaedt) and *R. rutilus* (Kaufmann, 1990).

Decrease in aerobic scope with increase in body size observed in *N. obscura* (Kalarani et al. 1993) has been attributed to differences in the slopes between resting and active oxygen consumption over five life history stages (Davies and Kalarani, 1993). Smith and Davies (1996) also observed declined scope for activity (SFA) with increase in biomass in *N. obscura* maintained in groups with varied groups sizes (1, 5, 10, 25, 50 or 100 numbers in each group).

**Temperature**

Poikilotherms can respond to chronic temperature changes in a number of ways and it is important that these changes be interpreted in relation to the life history of the organism under consideration (Burky, 1983; Cossins and Bowler, 1987). The ability to acclimate physiological rates in the face of changes in environmental temperature has long been considered as an adaptation for organisms that inhabit thermally unstable environments. Temperature is known to have both
acute (short term) and chronic (long term) effects on the rate of oxygen consumption in poikilothermic animals (Hornbach, 1992). Short-term effects are usually expressed in terms of $Q_{10}$ (respiratory quotient) values while long-term influences are referred to as acclimation or acclimatization effects.

The effects of ecological factors such as temperature, salinity etc., on the metabolic rate can be related to a defined state of activity of the animals (Forstner and Wieser, 1990). The temperature relationship of metabolism in spontaneously swimming fish consists of two elements: one is the level of metabolic intensity due to the rate of chemical reactions and the other is a change in the patterns of activity due to the effects of temperature on the central control of activity (Wieser, 1973). The effects of temperature on standard (resting) metabolism and active metabolism have been reviewed first by Fry (1957, 1971) As observed by Fry (1971), such temperature related changes of locomotor activity are probably responsible for most of the so called “plateau effects” and other non-linear relations between metabolic rate and temperature which have often been interpreted as indicating the existence of temperature compensation or homeostatic control of metabolism in these poikilothermic animals.

A significant decrease in $R_m$ and $A_m$ of $P$. granulosa with decrease in temperature from 28 (control) to 18°C reveals lower metabolic demands of maintenance and physical activity respectively at low temperatures. On the other hand a significant increase in $R_m$ and $A_m$ with increase in temperature from 28 to 38°C reflects the influence of higher temperature on the maintenance metabolism and activity costs respectively. Cech Jr. et al. (1979) stated that the depression in respiratory metabolism in the large mouth bass, $M$. salmoides at various experimental temperatures (20, 25 and 30°C) appears to be caused by insufficient oxygen to meet the ‘resting routine’ metabolic needs of the fish. However, increased oxygen consumption rates with increase in temperature from 20 to 30°C have been noticed in the same species by Cech et al. (1979). Soofiani and Hawkins (1982) also observed that the rate of oxygen consumption in the juvenile cod, $G$. morhua increased with temperature, reaching a maximum at 15°C and tend to decrease above this temperature. Martinez et al. (1996) stated that the resting respiration rate, expressed as weight specific rate, was linearly related to temperature in the shrimp, $P$. vannamei (Boone). Increased metabolic rate in fish under forced swimming varied with species and temperature (Fry, 1957). Scholander et al. (1953) and Wohlschlag (1960, 1964) conducted studies comparing the temperature effect on standard metabolic rates of fish from different climates. Brett (1964) and Beamish (1978) observed that the maximum rate of aerobic energy metabolism in fish increases more steeply with temperature than any other physical factor. Hornback (1992) also found lower rates of oxygen consumption at 5°C than at 10, 15 or 20°C in the fresh water clam, $M$. partumeium (Say). Kalarani and Davies (1994) observed a significant increase in both
resting and active respiration of unfed *N. obscura* from 5 to 20°C indicating higher metabolic demands with increase in temperature. But a decrease in both these parameters at 25°C has been attributed to either oxygen becoming a limiting factor at high temperatures or *N. obscura* reaching its upper limit of temperature tolerance. Similar observations have been made in some other species like sockeye salmon and cray fish (Brett, 1964; Fry 1971; Rutledge and Pritchard, 1981). There is also provisional evidence that oxygen becomes a respiratory limiting factor at high temperatures for some salmonids as the oxygen content of air-saturated water decreases with increase in temperature (Brett, 1964; Fry, 1971). An increase in environmental temperature from 1.5 to 10°C was found to result in a linear increase in the rates of oxygen consumption in the yellowfin sole, *L. aspera* (Paul et al. 1990). The routine metabolism of *Tilapia rendalli* (Boulenger) nearly reaching a plateau at high temperatures, is considered as an important energy saving function in fish foraging at the margins of warm water lakes (Caulton, 1977, 1978, a, b).

Decrease in *As* at 18°C and increase at 38°C at each life-history stage compared to control *P. granulosa* suggest higher potential energy available for activity at higher temperatures than at lower temperatures (Table 11). In fact in all the cases so far studied (Brett, 1964; Beamish, 1978) the maximum rate of aerobic energy metabolism in fish increases more steeply with temperature than the standard rate, causing the scope for activity to increase with temperature to a maximum level which is reached at approximately the preferred temperature of the species or population in question. At each temperature As decreased with increase in body size indicating differential changes in the rates of increases in *Rm* and *Am* at 18 and 38°C similar to the controls. Wieser (1985) observed no change in scope for activity in the rainbow trout, *S. gairdneri* with increase in biomass (0.1 to 10 gm) at 4°C which increased with body size at 12°C. Koch and Wieser (1983) earlier observed lower swimming activity in fish during synthesis of new gonadal tissues, the reduction ranging from approximately 40% at 18°C to less than 8% at 6°C. Greater decrease in As at each temperature during reproductive phase may be attributed to increased energy demands of gonadal development. In any organism different energy consuming processes compete with each other for the same source of energy. That the reduction of locomotor activity may compensate for costs of production was shown in some homeotherms (Koch and Wieser, 1983; Prosser, 1990). The data of the present study show that, even a poikilothermic animal may well be able to defray at least a part of the costs of reproduction by saving on the energy demands of locomotion – a similar response exhibited by higher organisms.

A significant increase in the percentage of consumed energy allocated to *Rm* from GP to RP at 18, 28 (control) and 38°C (Fig. 13) indicates that maintenance energy demands increase in *P. granulosa* with increase in body size irrespective of temperature. But decreased percent energy allocation at 18°C and increased percent energy allocation at 38°C compared to the control at each
life-history stage reflect the depressive effects of lower temperature and higher energy demands of higher temperature respectively.

A significant decrease in the percentage of consumed energy allocated to As from GP to RP at 18, 28 and 38°C (Fig. 14) suggests that due to gradual increase in maintenance energy demands, P.granulosa exhibits compensatory mechanism by reducing the potential energy required for physical activity. While low percent energy allocation to As at 18°C during each life history stage could be attributed to reduced proportion of energy allocation to both Am and Rm, high percent energy allocation to As at 38°C could be attributed to the elevation of both Rm and Am and, perhaps, to higher rate of increase in Am than Rm (Fig. 14).

Starvation

Organisms when subjected to starvation undergo a number of physiological and biochemical changes. Starvation induces rapid breakdown of food reserves for maintenance and survival. There have been a number of studies on the influence of starvation on metabolism of invertebrates (Lee and Campbell, 1965; Ghiretti, 1966; Djangmah, 1970; Ramamurthi and Subramanyam, 1976; Lane and Lawrence, 1979; Barclay et al. 1983; Prasad Rao and Jayasree, 1983; Hunter et al. 1983; Reddy et al. 1985; Dall and Smith, 1987; Duncan et al. 1987 a, b; Maya and Kuruppaswamy, 1987; Surendranath et al. 1987, 1988; Fried et al. 1989; Helen and Maria, 1991). In leeches (Man, 1958), snails (Calow, 1975), bivalves (Bayne, 1973) and crabs (Wallace, 1973) the most common response to food deprivation is a decrease in the rate of oxygen uptake. Decline in the rate of oxygen uptake has usually been attributed either to reduction in spontaneous activity (Hagerman, 1970) or to loss of respiring tissue (Hubbell, 1971). However the effect of refeeding following starvation on oxygen consumption has received cursory attention.

An increase in Rm and Am with increase in body size in P.granulosa fed following 30 (control), 60 and 90 day starvation (Table 12) indicated that duration of starvation do not affect the relationship between body size and oxygen consumption. Higher Rm upon refeeding following 60 and 90 day starvation compared to the control group at each life history stage suggests that additional energy costs of maintenance are associated with tissue repair a consequence of prolonged starvation (Table 12). Earlier higher resting respiration rates of N.obscura following winter stresses (low temperature; low dissolved oxygen; reduced feeding) were attributed to the costs of maintaining, mobilizing and transportation of materials for tissue repair (Davies and Kalarani, 1993). Hence it is clear that following stress (single or multiple), animals try to compensate the basic metabolic requirements such as oxygen.
No change in As during growth phase and inflection point in *P. granulosa* fed following 60 day starvation compared to the control indicates that despite higher costs of Rm (caused by tissue repair), *P. granulosa* may maintain normal levels of energy for physical activity by increasing the level of Am to its maximum cut off level as has been seen in some higher animals under different stress conditions. Post (1990) reported that higher metabolic rates of young fish accelerate the utilization of body energy stores and, therefore, reduce starvation time in situations of inadequate food supply. But this was found to be not the case during reproductive phase which is associated with both costs of repair and costs of gametogenesis which in turn leads to further enhancement in Rm leaving very little scope for other activities. In 90 day starved *P. granulosa*, a decrease in As right from growth phase further provide evidence that prolonged starvation which results in greater tissue damage and thus higher energy requirements for repair leads to lower aerobic scope even during non-reproductive stages of the life cycle.

A gradual increase in the percentage of consumed energy allocated to Rm from GP to RP following 30, 60 and 90 day starvation suggest that energetic demands of recovery increase with increase in starvation duration. This in turn was found to influence the energy available for aerobic activities causing a decrease in percent of consumed energy allocated to As from GP to RP in *P. granulosa* fed following 30, 60 or 90 day starvation (Fig. 16). Since energy is usually limiting which should be partitioned among there will be a conflict among the different consuming processes for its use (Koch and Wieser, 1983). Higher percent energy allocation during GP in *P. granulosa* at 30 and 60 day starvation reveals increased energetic demands for growth at short duration of starvation. Higher percent energy allocation to As during IP and RP implies that the energetic demands of metabolic activities are much higher following 60 day starvation.

Refeeding following starvation was found to increase energy allocation to Rm which is directly proportional to the duration of starvation (Table 12). On the other hand increased Rm was found to result in reduced As (Tables 11 and 12). While 30 day starved group stand as a good example of this situation (Fig. 16), highest percentage energy allocation to As along with increased Rm at GP, IP and RP by 60 day starvation in *P. granulosa* indicate that upon refeeding following starvation for moderate periods leeches may even show over compensation in some life history traits by increasing food ingestion and allocation of energy to some life history traits. However, decreased percent energy allocation to As following 90 day starvation (Table 12) despite increased food ingestion (Table 1) indicate that leeches following prolonged starvation may not maintain even normal levels of As due to higher costs of maintenance (including tissue repair) and/or costs of gonadal growth. *N. obscura* was found to show compensation in growth due to adjustments in the bioenergetic budget when food ration is reduced to two meals from three meals per week.
However, one meal per week was found to result in increased percent energy allocation to active respiration rather than to reproductive growth (Smith and Davies, 1995).

This study shows that during recovery following prolonged stress, animals tend to give priority to increase their fitness and to produce the progeny. Thus compensation occurs when individuals respond to a stress such as reduced prey availability, by making adjustments in energy allocation pattern (Bayne, 1985) to help maintain fitness and can either be partial or, if fitness is fully maintained, complete.

Resting oxygen consumption of fed (Rm fed) and unfed (Rm unfed) *P. granulosa* and apparent specific dynamic action (ASDA)

The length of time for which consumption of food exerts its influence upon heat production depends upon many factors. Chief amongst them are the quantity and quality of food and water temperature (Cho et al. 1982). The impact of starvation is felt sooner in active fish than in sluggish ones. Brody (1945) reported that apparent heat increment in domestic animals depends on the balance of energy-yielding dietary nutrients. The energy loss associated with feeding can be attributed to several causes including excited locomotor and other incidental activities, the mastication, digestion and absorption of food and biochemical transformation of absorbed material. The latter is especially associated with the metabolism of protein and amino acids (Nelson and Cox, 2000) but also includes the release of energy accompanying lipid and carbohydrate metabolism. Kleiber (1961) preferred to use the term ‘heat increment’ to these energy changes instead of ‘specific dynamic action’ (SDA) formerly applied to denote the changes accompanying protein deamination. SDA is only one component of apparent SDA, a term coined by Beamish (1974) to describe the total postprandial rise in metabolic rate. Hence apparent SDA includes energy costs associated with the behavioural responses to the presence of food, the movement of food residues through the gut, enhancement of digestive secretions and finally the biochemical processes attributed to SDA. Energy expenditure for apparent SDA is found to be influenced by meal size (Muir and Niimi, 1972), environmental temperatures (Brett, 1976), fish size (Beamish, 1974) and diet (Cho et al. 1976; Smith et al. 1978; Tandler, 1978; Tandler and Beamish, 1980).

A significant increase in resting oxygen consumption of fed (Rm fed) and unfed (Rm unfed) *P. granulosa* and ASDA with increase in body size in both control and experimental groups (Tables 13 and 14) suggest that the metabolic costs of maintenance and/or mobilization of materials for tissue growth increased with increase in body size. The energy requirements for maintenance and growth in large mouth bass are positively related to body weight when meal size
is expressed relative to fish biomass (Niimi and Beamish, 1974). On the basis of a given meal relative to biomass ASDA of largemouth bass rose directly with biomass. Positive relationship between SDA and both biomass and amount eaten would be predicted from comparable studies in fish (Beamish, 1974; Tandler and Beamish, 1979). Apparent heat increment increases with meal size and biomass but, on the basis of a fixed intake of food it declines with biomass in carnivorous fish (Beamish and Trippel, 1990). Carefoot (1990) reported that SDA asymptotically increased with ration and had significant positive effect on biomass in the supra littoral isopod, L. pallasii. In contrast Tandler and Beamish (1981) stated that ASDA in largemouth bass decreased with increase in biomass for a given intake of the experimental diet.

**Temperature**

The relationship between environmental temperature and oxidative metabolic rate in aquatic ectotherms is well documented (Schmidt – Nielsen, 1975). Fry (1971) studied the effect of temperature on standard metabolism within species. Relation between duration of elevated metabolic rate and water temperature was described by Saunders (1963). Acclimation of metabolic rates in response to variable temperature has classically been viewed as an important mechanism of compensation for animals (Segal, 1961; Kinne, 1970; Vernberg and Vernberg, 1972; Precht et al. 1973; Prosser, 1973; Newell, 1979).

A significant decrease in ASDA and resting oxygen consumption of fed (Rm fed) *P. granulosa* at 18°C compared to the control suggest decreased energy demands of food processing associated with decreased food ingestion (Table 1). Cech Jr. et al. (1979) found that the rate of oxygen consumption decreased with decrease in temperature from 30 to 20°C in fed *M. salmoides*. Soofiani and Hawkins (1982) also observed a decrease in the rate of oxygen consumption with decrease in temperature from 20°C to 5°C in *G. morhua*. In contrast a significant increase in ASDA and resting oxygen consumption of fed (Rm fed) *P. granulosa* at 38°C (Table 13) compared to the control could be attributed to increased energy costs of food processing usually associated with increased food ingestion (Table 1). An increase in apparent heat increment with temperature was earlier reported for coho salmon and atlantic cod (Averett, 1969; Soofiani and Hawkins, 1982). A pattern of increase in apparent heat increment was also found in rainbow trout between 8 and 15°C for each of the two formulated diets (Beamish et al. 1986). The suspension of feeding in the gastropod, *Crepidula fornicata* (Linnaeus) caused an increase in metabolic rate and filtration rate with exposure to elevated temperatures (Newell and Kofoed, 1977). Ansell and Sividas (1973) found elevation in both metabolic and filtration rates with elevated temperature in the bivalve, *Donax vittatus* (Dacosta). But apparent heat increment for an equivalent intake of
food did not change with temperature in either bluegills (Pierce and Wissing, 1974) or plaice (Jobling and Davies, 1980).

No particular pattern was noticed in the percentage of energy allocation to ASDA with increase in body size (Fig. 17). This implies that although the absolute amount of energy utilized for ASDA depends on the amount food ingested which in turn is influenced by body size of the animal percentage of energy consumed may not be either a constant proportion of ingested food or influenced by body size. However, as in the present study (Fig. 17), temperature was found to influence the proportion of energy allocated to ASDA. While low temperature (18°C) was found to result in lesser proportion of energy utilization, high temperature (38°C) was found to cause higher proportion of energy utilization for ASDA. Kalarani and Davies (1994) in *N. obscura* also observed that the proportion of absorbed energy utilized for ASDA decreased from 10.3% at 5°C to 6.1% at 15°C and increased to 10.3% at 25°C.

**Starvation**

Apparent heat increment depends on the quantity, quality and balance of the dietary energy components (Krebs 1964; Harper, 1971; Buttery and Annison, 1973) and on the nutritional status of the animal (Smith, 1981; Lied et al. 1982). Apparent heat increment varies directly with meal size for a wide variety of fish species fed with a broad spectrum of natural and formulated diets (Averett, 1969; Hamada and Ida, 1973; Beamish, 1974; Vahl and Davenport 1979).

A significant increase in ASDA and resting oxygen consumption of fed *P.granulosa* with increase in starvation duration from 30 to either 60 or 90 days at all the three life-history stages (Table 14) suggest that increased food ingestion which is influenced by the duration of starvation causes higher utilization of energy for food processing. Active species which normally have high metabolic rates cannot withstand the starvation stress for too long as they do not accumulate food reserves, whereas sluggish animals with low metabolic rates can survive longer periods of starvation (Dratnal et al. 1992; Kalarani et al. 1996). Nutritional state was found to influence oxygen consumption in several species (Hart, 1980; Vernberg, 1987). Ansell (1973) and Dall and Smith (1986) found starvation to decrease daily oxygen consumption in the crustacean, *Cancer pagurus* (Edwards, E) and in the prawn, *Penaeus esculentus* (Haswell) respectively. The medicinal leech, *H.medicalis* is extremely resistant to starvation and, having a very low metabolic rate can survive many months without food. When there is an opportunity a leech was found to ingest huge quantities of blood increasing its biomass five fold or even more (Zebe et al. 1986). But this study indicates that sluggish animals like leeches which are well known to survive longer periods without food also suffer from the stress of starvation.
Du Preez et al. (1992) found a significant increase in oxygen consumption associated with excited locomotion in the shrimp, *P. monodon* provided with food. Similarly *L. pallasii* during meal time was found to exhibit an elevation in oxygen consumption in the presence of chemical diet even though it did not consume food (Carefoot, 1990). Hence it is clear that not only the level of appetite but also other behavioural responses like excitement could lead to elevated metabolic costs following starvation.

Increased percentage of consumed energy allocated to ASDA from growth phase to inflection point and inflection point to reproductive phase following 60 and 90 day starvation (Fig. 18) show that the animal spends higher amounts of energy for digestion, absorption and assimilation inorder to maximize energy utilization of obtained food perhaps to meet higher demands which prevail following stress. This effect is found to be more predominant following 90 day starvation at each life-history stage (Fig. 18) indicating longer the duration of stress higher the energy spent on ASDA.
Mean (±SD; n=5) values of faeces and mucus (Fe+Mu) produced (mg DWT/30 days) at three different life history stages of *Poecilobdella granulosa* reared at 18, 28 (control group) and 38°C and fed *ad libitum* once in 30 days.

(Values in parentheses represent energy values in Kilo joules)

<table>
<thead>
<tr>
<th>Temperature regimen/Variable</th>
<th>18°C</th>
<th>28°C</th>
<th>38°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Life history stage</td>
<td>Biomass (gm wet wt.)</td>
<td>Fe+Mu</td>
<td>Biomass (gm wet wt.)</td>
</tr>
<tr>
<td>Growth phase</td>
<td>2.82 ±0.21</td>
<td>265.2±15.13</td>
<td>3.62 ±0.23</td>
</tr>
<tr>
<td>Inflection point</td>
<td>6.95 ±0.37</td>
<td>400.0** ±22.32</td>
<td>7.35 ±0.47</td>
</tr>
<tr>
<td>Reproductive phase</td>
<td>12.63 ±0.41</td>
<td>669.5@ ±27.61</td>
<td>13.85 ±0.77</td>
</tr>
</tbody>
</table>

Values similarly marked are not significantly different (P<0.05) from each other.
Table 4

Mean (±SD; n=5) values of faeces and mucus (Fe+Mu) produced (mg DWT/30 days) at three different life history stages of Poecilobdella granulosa reared at 28°C and fed ad libitum following 30 (control group), 60 and 90 day starvation.

(Values in parentheses represent energy values in Kilo joules)

<table>
<thead>
<tr>
<th>Life history stage</th>
<th>Feeding schedule/Variable</th>
<th>30 d</th>
<th>60 d</th>
<th>90 d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biomass (gm wet wt.)</td>
<td>Fe+Mu</td>
<td>Biomass (gm wet wt.)</td>
<td>Fe+Mu</td>
</tr>
<tr>
<td>Growth phase</td>
<td>3.62 ±0.23</td>
<td>273.9±12.13 (6.35)</td>
<td>2.67 ±0.18 (7.83)</td>
<td>339.1*±21.3 (7.83)</td>
</tr>
<tr>
<td>Inflection point</td>
<td>7.35 ±0.47</td>
<td>400.0±21.71 (9.24)</td>
<td>6.11 ±0.47 (10.54)</td>
<td>456.5**±26.2 (10.54)</td>
</tr>
<tr>
<td>Reproductive phase</td>
<td>13.85 ±0.77</td>
<td>530.4±26.12 (12.25)</td>
<td>11.23 ±0.93 (13.95)</td>
<td>604.3±35.6 (13.95)</td>
</tr>
</tbody>
</table>

Values similarly marked are not significantly different (P<0.05) from each other.
TABLE – 5

Mean (±SD; n=5) amount of absorbed energy (Ab) [kilo Joules] at three different life history stages of *Poecilobdella granulosa* reared at 18, 28 (control group) and 38°C and fed *ad libitum* once in 30 days.

<table>
<thead>
<tr>
<th>Temperature regimen/Variable</th>
<th>Life history stage</th>
<th>18°C</th>
<th>28°C</th>
<th>38°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biomass (gm wet wt.)</td>
<td>Ab</td>
<td>Biomass (gm wet wt.)</td>
<td>Ab</td>
</tr>
<tr>
<td>Growth phase</td>
<td>2.82 ±0.21</td>
<td>32.51±3.21</td>
<td>3.62 ±0.23</td>
<td>49.60*±4.01</td>
</tr>
<tr>
<td>Inflection point</td>
<td>6.91 ±0.37</td>
<td>42.72±3.81</td>
<td>7.35 ±0.47</td>
<td>67.63**±4.11</td>
</tr>
<tr>
<td>Reproductive phase</td>
<td>12.63 ±0.41</td>
<td>57.34±4.21</td>
<td>13.85 ±0.77</td>
<td>80.24@±6.81</td>
</tr>
</tbody>
</table>

Values similarly marked are not significantly different (P<0.05) from each other.
TABLE – 6

Mean (±SD; n=5) amount of absorbed energy (Ab) [kilo Joules] at three different life history stages of *Poecilobdella granulosa* reared at 28°C and fed *ad libitum* following 30 day (control group), 60 and 90 day starvation.

<table>
<thead>
<tr>
<th>Life history stage</th>
<th>Feeding schedule/ Variable</th>
<th>30 d</th>
<th></th>
<th>60 d</th>
<th></th>
<th>90 d</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biomass (gm wet wt.)</td>
<td>Ab</td>
<td>Biomass</td>
<td>Ab</td>
<td>Biomass</td>
<td>Ab</td>
<td></td>
</tr>
<tr>
<td>Growth phase</td>
<td>3.62 ±0.23</td>
<td>49.60 ±4.01</td>
<td>2.67 ±0.18</td>
<td>59.45* ±4.72</td>
<td>2.17 ±0.13</td>
<td>65.44* ±5.82</td>
<td></td>
</tr>
<tr>
<td>Inflection point</td>
<td>7.35 ±0.47</td>
<td>67.63 ±4.11</td>
<td>6.11 ±0.47</td>
<td>78.56** ±5.33</td>
<td>5.75 ±0.42</td>
<td>84.86** ±7.63</td>
<td></td>
</tr>
<tr>
<td>Reproductive phase</td>
<td>13.85 ±0.77</td>
<td>80.24 ±6.81</td>
<td>11.23 ±0.93</td>
<td>101.45[</td>
<td>10.66 ±0.89</td>
<td>109.64[</td>
<td></td>
</tr>
</tbody>
</table>

Values similarly marked are not significantly different (P<0.05) from each other.
TABLE – 7

Mean (±SD; n=5) values of endogenous (endo) and exogenous (exo) ammonia excretion (mg. NH₃/30 days) at three different life-history stages of *Poecilobdella granulosa* reared at 18, 28 (control group) and 38°C and fed *ad libitum* once in 30 days (values in parentheses represent energy values in Kilo joules)

<table>
<thead>
<tr>
<th>Life history stage</th>
<th>18°C</th>
<th>28°C</th>
<th>38°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biomass (gm wet wt.)</td>
<td>Endo</td>
<td>Exo</td>
</tr>
<tr>
<td>Growth phase</td>
<td>2.82</td>
<td>±0.21</td>
<td>(2.93)</td>
</tr>
<tr>
<td></td>
<td>±0.37</td>
<td>±3.21</td>
<td>61.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.95</td>
</tr>
<tr>
<td>Inflection point</td>
<td>143.7</td>
<td>±3.72</td>
<td>61.62</td>
</tr>
<tr>
<td></td>
<td>173.7</td>
<td>±4.63</td>
<td>173.7</td>
</tr>
<tr>
<td>Reproductive phase</td>
<td>12.63</td>
<td>±0.41</td>
<td>(7.14)</td>
</tr>
<tr>
<td></td>
<td>±4.63</td>
<td>±3.71</td>
<td>61.62</td>
</tr>
</tbody>
</table>

All Values are significant at P<0.05
TABLE – 8

Mean (±SD; n=5) values of endogenous (endo) and exogenous (exo) ammonia excretion (mg. NH₃/30 days) at three different life-history stages of *Poecilobdella granulosa* reared at 28°C and fed *ad libitum* following 30 (control group), 60 and 90 day starvation. (values in parentheses represent energy values in Kilo joules)

<table>
<thead>
<tr>
<th>Life history stage</th>
<th>30 d</th>
<th>60 d</th>
<th>90 d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biomass (gm wet wt.)</td>
<td>Endo</td>
<td>Exo</td>
</tr>
<tr>
<td>Growth phase</td>
<td>3.62 ±0.23</td>
<td>166.3 ±13.91</td>
<td>83.16 ±5.91</td>
</tr>
<tr>
<td>Inflection point</td>
<td>7.35 ±0.47</td>
<td>224.2 ±14.25</td>
<td>142.7 ±7.42</td>
</tr>
<tr>
<td>Reproductive phase</td>
<td>13.85 ±0.77</td>
<td>272.7 ±14.84</td>
<td>192.0 ±10.93</td>
</tr>
</tbody>
</table>

All Values are significant at P<0.05
TABLE – 9

Mean (±SD; n=5) amount of assimilated energy (Ass[kilo Joules]) at three different life history stages of *Poecilobdella granulosa* reared at 18, 28 (control group) and 38°C and fed *ad libitum* once in 30 days.

(Values in parentheses represent energy values in Kilo joules)

<table>
<thead>
<tr>
<th>Temperature regimen/Variable</th>
<th>18°C</th>
<th>28°C</th>
<th>38°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Life history stage</td>
<td>Biomass (gm wet wt.)</td>
<td>Ass</td>
<td>Biomass (gm wet wt.)</td>
</tr>
<tr>
<td>Growth phase</td>
<td>2.82 ±0.21</td>
<td>29.62 ±2.73</td>
<td>3.62 ±0.23</td>
</tr>
<tr>
<td>Inflection point</td>
<td>6.91 ±0.37</td>
<td>38.51 ±3.53</td>
<td>7.35 ±0.47</td>
</tr>
<tr>
<td>Reproductive phase</td>
<td>12.63 ±0.41</td>
<td>50.23 ±3.95</td>
<td>13.85 ±0.77</td>
</tr>
</tbody>
</table>

Values similarly marked are not significantly different (P<0.05) from each other.
**TABLE – 10**

Mean (±SD; n=5) amount of assimilated energy (\textit{Ass} [kilo Joules]) at three different life history stages of \textit{Poecilobdella granulosa} reared at 28\(^0\)C and \textit{fed ad libitum} following 30 (control group), 60 and 90 day starvation.

<table>
<thead>
<tr>
<th>Life history stage</th>
<th>Feeding schedule / Variable</th>
<th>30 d</th>
<th>60 d</th>
<th>90 d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biomass (gm wet wt.)</td>
<td>Ass</td>
<td>Biomass (gm wet wt.)</td>
<td>Ass</td>
</tr>
<tr>
<td>Growth phase</td>
<td>3.62 ±0.23</td>
<td>44.51±2.90</td>
<td>2.67 ±0.18</td>
<td>51.63* ±3.24</td>
</tr>
<tr>
<td>Inflection point</td>
<td>7.35 ±0.47</td>
<td>60.16@±5.10</td>
<td>6.11 ±0.47</td>
<td>67.79@*±5.51</td>
</tr>
<tr>
<td>Reproductive phase</td>
<td>13.85 ±0.77</td>
<td>70.71±6.73</td>
<td>11.23 ±0.93</td>
<td>86.71 ±7.32</td>
</tr>
</tbody>
</table>

*Values similarly marked are not significantly different (P<0.05) from each other.*
Table 11

Mean (±SD; n=5) values of resting (Rm) and active (Am) oxygen consumption and aerobic scope (As) (mL O2/day) at three different life history stages of unfed (for 30 days) *Poecilobdella granulosa* reared at 18, 28 (control group) and 38°C.

(Values in parentheses represent energy values in Kilo joules)

<table>
<thead>
<tr>
<th>Temperature regimen / Variable</th>
<th>18°C</th>
<th>28°C</th>
<th>38°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biomass (gm wet wt.)</td>
<td>Biomass (gm wet wt.)</td>
<td>Biomass (gm wet wt.)</td>
</tr>
<tr>
<td>Life history stage</td>
<td>Rm</td>
<td>Am</td>
<td>As</td>
</tr>
<tr>
<td>Growth phase</td>
<td>2.82 ±0.21 (7.41)</td>
<td>12.21 ±1.10 (12.73)</td>
<td>20.95 ±1.31 (12.73)</td>
</tr>
<tr>
<td>Inflection point</td>
<td>6.95 ±0.37 (10.43)</td>
<td>17.16 ±1.21 (15.25)</td>
<td>25.08 ±1.63 (4.82)</td>
</tr>
<tr>
<td>Reproductive phase</td>
<td>12.63 ±0.41 (16.91)</td>
<td>27.88 ±1.82 (20.65)</td>
<td>33.98 ±2.07 (7.74)</td>
</tr>
</tbody>
</table>

Values similarly marked are not significantly different (P<0.05) from each other.
Table 12

Mean (±SD; n=5) values of resting (Rm) and active (Am) oxygen consumption and aerobic scope (As) (mlO₂/day) at three different life - history stages of *Poecilobdella granulosa* reared at 28°C and fed *ad libitum* following 30 (control group), 60 and 90 day starvation.

(Values in parentheses represent energy values in Kilo joules)

<table>
<thead>
<tr>
<th>Life history stage</th>
<th>Feeding schedule / Variable</th>
<th>30 d</th>
<th>60 c</th>
<th>90 d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biomass (gm wet wt.)</td>
<td>Rm</td>
<td>Am</td>
<td>As</td>
</tr>
<tr>
<td>Growth phase</td>
<td></td>
<td>3.62 ±0.23</td>
<td>20.95 ±1.62</td>
<td>35.30 ±2.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(12.73)</td>
<td>(21.48)</td>
<td>(8.75)</td>
</tr>
<tr>
<td>Inflection point</td>
<td></td>
<td>7.35 ±0.41</td>
<td>29.53 ±1.94</td>
<td>42.40 ±3.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(17.91)</td>
<td>(25.75)</td>
<td>(7.84)</td>
</tr>
<tr>
<td>Reproductive phase</td>
<td></td>
<td>13.85 ±0.43</td>
<td>38.28 ±2.63</td>
<td>46.86 ±3.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(23.25)</td>
<td>(28.48)</td>
<td>(5.23)</td>
</tr>
</tbody>
</table>

Values similarly marked are not significantly different (P<0.05) from each other.
### Table 13

Mean (±SD; n=5) values of resting oxygen consumption (mlO₂/30 days) of fed (Rm fed) and unfed (Rm unfed) *Poecilobdella granulosa* and apparent specific dynamic action (ASDA) (mlO₂/30 days) at three different life history stages and reared at 18, 28 (control group) and 38°C and fed *ad libitum* once in 30 days.

(Values in parentheses represent energy values in Kilo joules)

<table>
<thead>
<tr>
<th>Temperature regimen/Variable</th>
<th>18°C</th>
<th>28°C</th>
<th>38°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Life history stage</strong></td>
<td>Biomass (gm wet wt.)</td>
<td>Rm (fed)</td>
<td>Rm (unfed)</td>
</tr>
<tr>
<td><strong>Growth phase</strong></td>
<td>2.82 ±0.21</td>
<td>529.6 ±25.7 (10.75)</td>
<td>366.3 ±23.21 (7.41)</td>
</tr>
<tr>
<td><strong>Inflection point</strong></td>
<td>6.95 ±0.37</td>
<td>767.2 ±32.62 (15.57)</td>
<td>514.8 ±28.72 (10.43)</td>
</tr>
<tr>
<td><strong>Reproductive phase</strong></td>
<td>12.63 ±0.41</td>
<td>1163.3 ±43.93 (23.53)</td>
<td>836.6 ±30.23 (16.91)</td>
</tr>
</tbody>
</table>

Values similarly marked are not significantly different (P<0.05) from each other.
Table 14

Mean (±SD; n=5) values of resting oxygen consumption (mLO₂/30 days) of fed (Rm fed) and unfed (Rm unfed) *Poecilobdella granulosa* and apparent specific dynamic action (ASDA) (mLO₂/30 days) at three different life history stages and reared at 28°C and fed *ad libitum* following 30 (control group), 60 and 90 day starvation.

(Values in parentheses represent energy values in Kilo joules)

<table>
<thead>
<tr>
<th>Life history stage</th>
<th>Feeding schedule / Variable</th>
<th>30 d</th>
<th>60 d</th>
<th>90 d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biomass (gm wet wt.)</td>
<td>Rm (fed)</td>
<td>Rm (unfed)</td>
<td>ASDA</td>
</tr>
<tr>
<td>Growth phase</td>
<td>3.62 ±0.23</td>
<td>940.5 (19.05)</td>
<td>628.7 (12.73)</td>
<td>311.8 (6.32)</td>
</tr>
<tr>
<td>Inflection point</td>
<td>7.35 ±0.41</td>
<td>1242.5 (25.16)</td>
<td>886.1 (17.91)</td>
<td>356.4 (7.25)</td>
</tr>
<tr>
<td>Reproductive phase</td>
<td>13.85 ±0.43</td>
<td>1603.9 (32.48)</td>
<td>1148.5 (23.25)</td>
<td>455.4 (9.23)</td>
</tr>
</tbody>
</table>

All Values are significant at p<0.005
Fig. 5: Percentage of consumed energy lost in faeces and mucus (Fe+Mu) during growth phase (GP), inflection point (IP) and reproductive phase (RP) of *Poecilobdella granulosa* reared at $18 \pm 0.5^\circ C$, $28 \pm 0.5^\circ C$ (control) and $38 \pm 0.5^\circ C$ and fed *ad libitum* once in 30 days.

![Bar chart showing per cent loss of energy](image-url)
Fig. 6: Percentage of consumed energy lost in faeces and mucus (Fe+Mu) during growth phase (GP), inflection point (IP) and reproductive phase (RP) of *Poecilobdella granulosa* reared at 28 ± 0.5°C and fed *ad libitum* following 30 (control), 60 and 90 day starvation.
Fig. 7: Percent absorption efficiency during growth phase (GP), inflection point (IP) and reproductive phase (RP) of *Poecilobdella granulosa* reared at 18 ± 0.5°C, 28 ± 0.5°C (control) and 38 ± 0.5°C and fed *ad libitum* once in 30 days.
Fig. 8: Percent absorption efficiency during growth phase (GP), inflection point (IP) and reproductive phase (RP) of *Poecilobdella granulosa* reared at 28 ± 0.5°C and fed *ad libitum* once in 30 (control), 60 and 90 day starvation.
Fig. 9: Percentage of consumed energy lost in excretion during growth phase (GP), inflection point (IP) and reproductive phase (RP) of *Poecilobdella granulosa* reared at 18±0.5°C, 28±0.5°C (control) and 38±0.5°C and fed *ad libitum* once in 30 days.
Fig. 10: Percentage of consumed energy lost in excretion during growth phase (GP), inflection point (IP) and reproductive phase (RP) of *Poecilobdella granulosa* reared at $28 \pm 0.5^\circ$C, and fed *ad libitum* following 30 (control), 60 and 90 day starvation.
Fig. 11: Percent assimilation efficiency during growth phase (GP), inflection point (IP) and reproductive phase (RP) of *Poecilobdella granulosa* reared at $18 \pm 0.5^\circ C$, $28 \pm 0.5^\circ C$ (control) and $38 \pm 0.5^\circ C$ fed *ad libitum* once in 30 days.
Fig. 12: Percent assimilation efficiency during growth phase (GP), inflection point (IP) and reproductive phase (RP) of *Poecilobdella granulosa* reared at 28 ± 0.5°C and fed *ad libitum* following 30 (control), 60 and 90 day starvation.
Fig. 13: Percentage of consumed energy allocated to resting metabolism (Rm) during growth phase (GP), inflection point (IP) and reproductive phase (RP) of *Poecilobdella granulosa* reared at 18 ± 0.5°C, 28 ± 0.5°C (control) and 38 ± 0.5°C and fed *ad libitum* once in 30 days.
Fig. 14: Percentage of consumed energy allocated to aerobic scope ($A_s$) during growth phase (GP), inflection point (IP) and reproductive phase (RP) of Poecilobdella granulosa reared at $18 \pm 0.5^\circ C$, $28\pm0.5^\circ C$ (control) and $38\pm0.5^\circ C$ and fed ad libitum once in 30 days.
Fig. 15: Percentage of consumed energy allocated to resting metabolism (Rm) during growth phase (GP), inflection point (IP) and reproductive phase (RP) of *Poecilobdella granulosa* reared at 28 ± 0.5°C and fed *ad libitum* following 30 (control), 60 and 90 day starvation.
Fig. 16: Percentage of consumed energy allocated to aerobic scope (As) during growth phase (GP), inflection point (IP) and reproductive phase (RP) of *Poecilobdella granulosa* reared at 28 ± 0.5°C and fed *ad libitum* following 30 (control), 60 and 90 day starvation.
Fig. 17: Percentage of consumed energy allocated to apparent specific dynamic action (ASDA) during growth phase (GP), inflection point (IP) and reproductive phase (RP) of Poecilobdella granulosa reared at 18 ± 0.5°C, 28 ± 0.5°C (control) and 38 ± 0.5°C and fed ad libitum once in 30 days.
Fig. 18: Percentage of consumed energy allocated to apparent specific dynamic action (ASDA) during growth phase (GP), inflection point (IP) and reproductive phase (RP) of *Poecilobdella granulosa* reared at 28 ± 0.5°C and fed *ad libitum* following 30(control), 60 and 90 day starvation.