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Starvation as a stress factor influencing the metabolism of a tropical intertidal gastropod *Morula granulata* (Duclos)

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Abstract. The snail *Morula granulata* (Duclos) survived the starvation stress over a period of 30 days but 50% mortality occurred on the 70th day. The changes in the level and content of different biochemical constituents viz carbohydrates, glycogen, free sugars, protein, total ninhydrin positive substances and lipids were investigated in foot, gonad digestive gland complex and viscera of the animal at intervals of 10 days up to 70 days. All these biochemical constituents (both level and content) were found to decrease with the period of starvation showing their utilisation except total ninhydrin positive substances which exhibited an increase. Of all the 3 tissues, the gonad digestive gland complex was affected most during starvation. 'Lipid-oriented' metabolism was observed in this animal showing maximum utilisation of lipids during starvation. Respiratory studies revealed a decreased pattern of oxygen consumption with increasing period of starvation when calculated per snail or per tissue weight. The recalculated intercept values (a) also showed a decreasing trend when exposed to starvation.

Keywords. Starvation stress; biochemical constituents; tropical intertidal gastropod; lipid oriented metabolism; oxygen consumption

1. Introduction

Lack of food constitutes a stress for the animal and the ability of the animal to withstand this nutritive stress for prolonged periods depends on its ability to utilise the stored nutrients economically. A good quantity of information is available on the effect of starvation stress in molluscs (Martin and Goddard 1966; Giese 1966; Emerson 1967; Emerson and Duerr 1967; Stickle and Duerr 1970). Interestingly, some of the gastropods show polysaccharide-oriented metabolism (Barry and Munday 1959) whereas in other gastropods, it is lipid-oriented (Stickle and Duerr 1970).

Several investigations on the starvation metabolism of gastropod molluscs revealed that, though there is a preferential utilisation of one particular body reserve during the course of starvation typical for a particular species (Emerson 1967; Stickle and Duerr 1970; Ricardo and Brennes 1981), the other products also are being utilised equally or to a lesser extent. Thus work was done on several types of snails where specifications exist among the various types of gastropods.

Although the gastropods were studied extensively, there appears to be little information on the nutrient stores and respiration in relation to starvation of either carnivorous prosobranch snails or opisthobranch snails. Therefore, in the present investigation, an attempt has been made to study the level and content of organic constituents, and respiration in a carnivorous snail *Morula granulata* (Duclos) during starvation.

2. Materials and methods

Animals of uniform size (dry weight of soft parts ranged from 80–150 mg) were collected from the rocks of Palm beach of Visakhapatnam on east coast of India. They were equilibrated in the laboratory in an aquarium containing sea water (32‰) at 25° ± 0·5°C for 24 h.

Preliminary investigations were carried out to study the mortality rate of animals exposed to starvation stress. A batch of 100 animals were taken in an aquarium filled with Whatman 42 filtered sea water. The filtered sea water was aerated continuously and the water was changed daily. Observations were made for intervals of 10 days up to 70 days where 50% mortality occurred.

2.1 *Sampling technique*

For biochemical studies, a group of 300 animals were taken in an aquarium and 20 animals from this group were sacrificed which served as initial (0 days). The rest of the animals were subjected to starvation stress as explained above. Sampling intervals were fixed for every 10 days up to 70 days depending on the above observations on mortality rate. At each interval, 20 animals were sacrificed for biochemical analysis.

Immediately after sacrificing the animals, the different body components viz foot, gonad digestive gland complex (GDG complex) and viscera were excised and pooled up separately. As the gonad and digestive gland are inseparable they were considered as GDG complex hereafter. Wet weights of the pooled body components were taken before they were dried in an oven at 90°C for 48 h to a constant weight and dry weights of all the samples were also taken. The dried samples were powdered and were kept in glass vials stored in a desiccator. The biochemical composition of each component in *M. granulata* was estimated separately at various stages of starvation.

2.2 *Biochemical analysis*

Anthrone method was followed for the estimation of total carbohydrates and glycogen (Carrol *et al* 1956). The difference between the amount of total carbohydrates and glycogen was taken as free sugars. Protein content was estimated by the method of Lowry *et al* (1951). Total ninhydrin positive substances (TNPS) were estimated using the method of Moore and Stein (1954). Total lipids were determined by applying the chloroform: methanol (2:1) extraction procedure described by Folch *et al* (1957).

2.3 *Biochemical level and content*

The different biochemical constituents are presented as mg/g dry weight of the tissue (biochemical level). The biochemical content (total present per component of a 100 g standard animal) was estimated by the method of Stickle (1975). The body component indices of *M. granulata* subjected to starvation stress have been reported earlier by Uma Devi *et al* (1985).

2.4 *Respiration*

For respiratory studies, 10 animals of equal size (as described earlier) were selected and labelled serially from 1–10. Their initial oxygen consumption was measured and then they were subjected to lack of food by keeping them in Whatman 42 filtered sea water (salinity 32%, pH 8). The animals were kept in a glass trough at a temperature of 25°±0·5°C with continuous aeration. Oxygen consumption was studied for all the 10 animals individually at 10 days intervals (10, 20, 30, 40, 50, 60 and 70) over a period of 70 days under starvation at the same temperature (25°C). Respiratory measurements were made according to the method used by Prasada Rao and Uma Devi (1981). After measuring the oxygen consumption, they were transferred back to the experimental troughs.

2.5 *Statistical evaluation*

All the data are presented as mean ± standard deviation. One way analysis of variance (ANOVA) (Snedecor and Cochran 1967) was used to determine the significance of variation in biochemical level and content during starvation. Duncan's Multiple Range test (Snedecor and Cochran 1967) was used to study the above data further. Respiratory rates were also studied by using the above test.

3. Results

Mortality data (cumulative) with reference to starvation are given in table 1. 50% mortality was recorded after 70 days of starvation. Based on this observation, the present investigation has been planned.

3.1 *Biochemical level*

The changes in the level of biochemical constituents of foot, GDG complex and viscera are presented in figures 1–3. From ANOVA, it is clear that there are significant variations ($P<0·05$) in all the biochemical constituents of different body components. Carbohydrates decreased significantly ($P<0·05$) from 30, 40 and 50

Table 1. Effect of starvation on the respiration of *Morula granulata* (see text for details of oxygen consumption and log *a* values).

No. of Days	Mortality (%)	Oxygen consumption $\mu l\ O_2 \cdot h^{-1} \pm SD$	Decrease (%)	Oxygen consumption $\mu l\ O_2\ g^{-1} \cdot h^{-1} \pm SD$	Decrease (%)	log *a*
0	0	58·93±8·18	—	0·3982±0·0553	—	0·8193
10	0	49·10±10·07	16·68	0·3672±0·0753	7·79	0·7594
20	0	44·19±9·88	25·01	0·3710±0·0830	6·83	0·7356
30	0	39·57±9·04	32·85	0·3365±0·0769	15·50	0·6901
40	10	34·15±7·73*	42·05	0·3012±0·0682*	24·36	0·6331
50	20	27·90±5·50*	52·66	0·2527±0·0498*	36·54	0·5504
60	35	18·92±3·37*	67·89	0·1775±0·0316*	55·42	0·3883
70	50	18·50±3·64*	68·81	0·1823±0·0359*	54·22	0·3880

*$P<0·05$

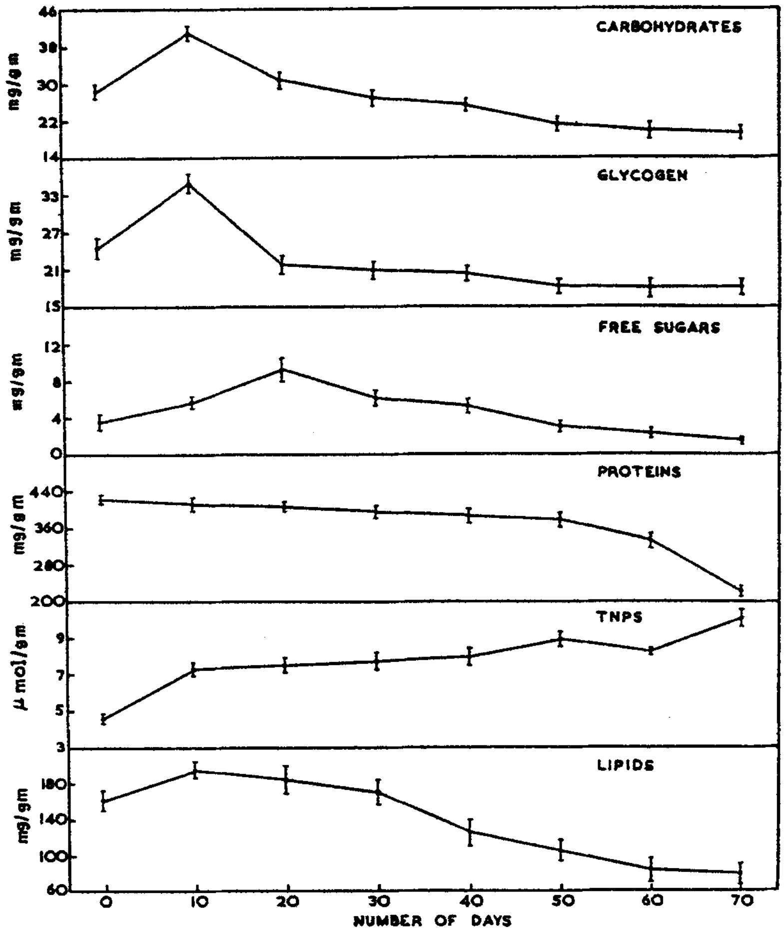

Figure 1. Effect of starvation on the biochemical composition (level) of foot in *M. granulata* (vertical bars represent the standard deviation).

days in foot, GDG complex and viscera, respectively. Significant decrease ($P < 0.05$) in glycogen levels of foot was observed from the same period as for carbohydrates but in GDG complex, the decrease in glycogen level occurred at 20 days and it continued up to 70 days. In viscera, the same trend was noticed as in foot. The free sugars of foot and viscera decreased ($P < 0.05$) from 50 days onwards. The protein levels of GDG complex and viscera showed gradual decrease ($P < 0.05$) immediately after 10 days. In foot, significant decrease of protein levels was observed from 40 days. The TNPS levels were found to increase gradually in foot, GDG complex and viscera and this increase was significant from 40 days. In all the body components studied, the lipid levels decreased significantly ($P < 0.05$) from 50 days onwards.

3.2 *Biochemical content*

ANOVA of different body biochemical contents reveals significant variations ($P < 0.05$) in their composition during different intervals of starvation. However, it is

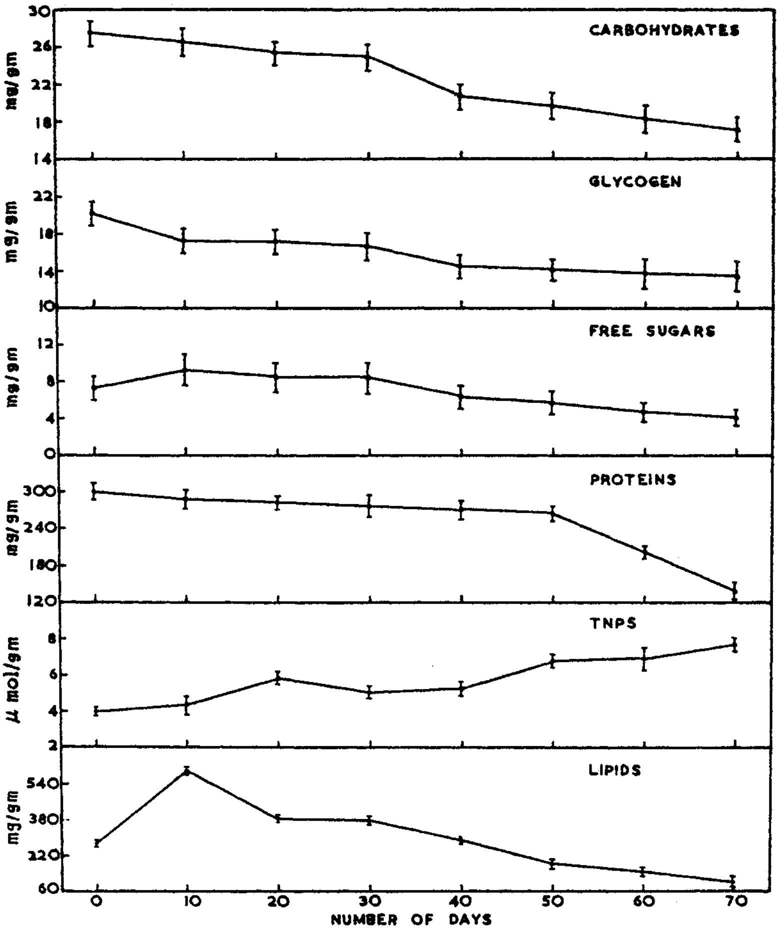

Figure 2. Effect of starvation on the biochemical composition (level) of GDG complex in *M. granulata* (vertical bars represent the standard deviation).

evident from Duncan's analysis that carbohydrates, glycogen, free sugars, proteins and lipids decreased with increasing period of starvation in foot, GDG complex and viscera as was observed earlier for biochemical levels. The significant increase ($P < 0.05$) in the content of TNPS is similar to that of level in all the body components during starvation.

It is clear from the results that the lipid quantities exhibited significant decrease ($P < 0.05$) in all the body components of *Morula granulata*. Therefore, it can be concluded that this snail belongs to the category of molluscs showing lipid-oriented metabolism when exposed to starvation stress.

3.3 *Respiration*

The details of oxygen consumption of *M. granulata* during different intervals of starvation are presented in table 1. It is clear from the percentage calculation that the decrease in oxygen consumption, when considered on per animal basis, was gradual

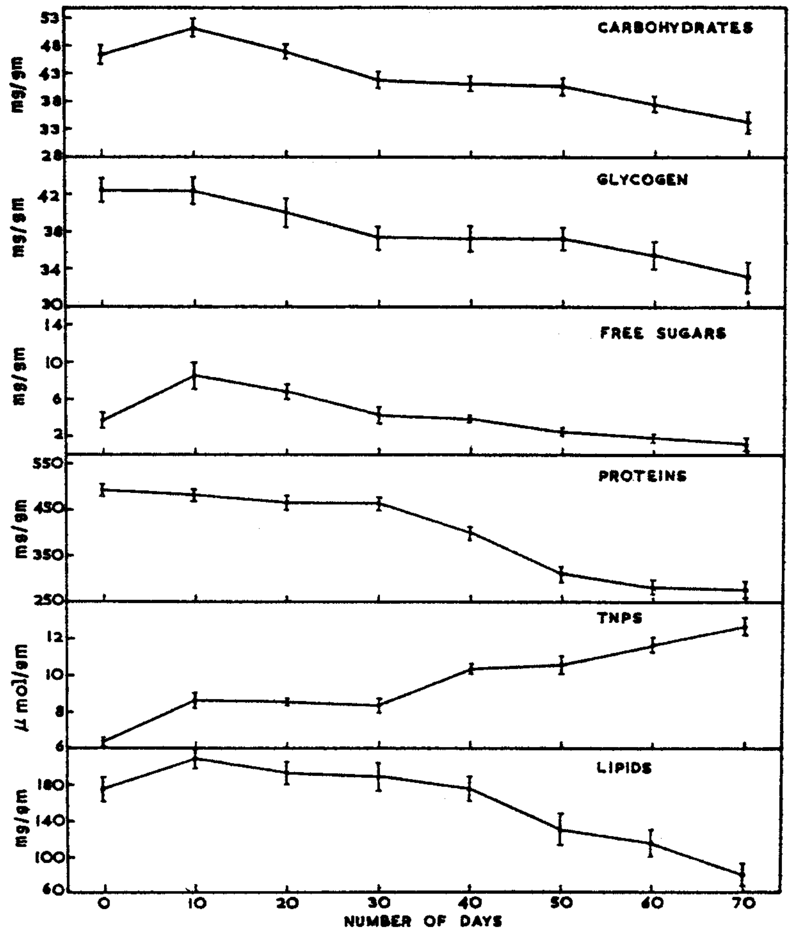

Figure 3. Effect of starvation on the biochemical composition (level) of viscera in *M. granulata* (vertical bars represent the standard deviation).

with the period of starvation. From Duncan's multiple range test, it is found that the decrease is significantly different ($P < 0{\cdot}05$) from 40 days of starvation. The oxygen consumption values when calculated basing on tissue weight (this was estimated from percentage decrease in dry tissue weights during starvation), also showed a decreasing trend. But this percentage decrease was less when compared to per animal basis (table 1). The log a values of exponential equation $Y = aW^b$ are calculated by using the 'b' value of 0·4382 reported earlier for this animal (Prasada Rao and Uma Devi 1981). These log a values are also found to decrease with increasing period of starvation.

4. Discussion

Mortality rate of *M. granulata* is very slow (table 1) when subjected to prolonged starvation of 70 days. This clearly indicates that this animal has good capacity to withstand starvation stress. This is in agreement with previous work of Stickle and Duerr (1970) on *Thais lamellosa.*

The organic constituents of soft parts of *M. granulata* during starvation stress show trends of diminution with increase in the duration of the stress conditions. The decrease of these materials is somewhat slow during the earlier period of starvation, after which the decline is at a rapid rate (figures 1–3). Of all the principal biochemical constituents, the highest decrease was found for lipids (73·85% for content and 63·35% for level) in GDG complex (figure 2). However, this decrease was comparatively less in foot (70·97% for content and 52·03% for level) (figure 1) and viscera (61·71% for content and 54·62% for level) (figure 3). A remarkable decrease was observed for proteins but not as distinct as lipids (67·11% for content and 53·89% for level in GDG complex; 68·50% for content and 47·44% for level in foot). In viscera, proteins exhibited a decrease of 52·77% for content and 44·02% for level. The decrease in the percentage of total carbohydrates was: 58·70 for content and 31·86 for level in foot, 55·35 for content and 37·52 for level in GDG complex, 37·32 for content and 25·77 for level in viscera. However, glycogen and free sugars followed the same trend as explained above in different tissues (figures 1–3). Thus it is interesting to observe that during starvation, the percentage decrease of different nutrient classes is more when considered on basis of content than on the level. It may also be said that the effect of starvation is high on the GDG complex when compared to the other tissues.

A close examination of the data reveals that it is the lipid that is much reduced by starvation stress. Next to lipids, protein utilisation is more in *M. granulata.* In this study, total carbohydrates and glycogen occupy the last place in their role as a source of food when compared to other body constituents during starvation. The results of this investigation strongly suggest that the metabolism of the carnivorous prosobranch *M. granulata* is 'lipid-oriented'. This is in agreement with the previous work on a carnivorous prosobranch *Thais lamellosa* (Stickle and Duerr 1970) and on the intertidal prosobranch *Littorina planaxis* (Emerson and Duerr 1967).

Pulmonates appear to possess a polysaccharide-oriented metabolism (Emerson 1967) while prosobranchs have a lipid-oriented metabolism (Emerson and Duerr 1967). Pulmonate and prosobranch snails differ from each other not only morphologically but also in an equally fundamental metabolic way (Stickle and Duerr 1970).

Starvation results in an elevation of free amino acid (TNPS) level of the soft parts of *M. granulata.* This increase in free amino acids, as a result of proteolysis, would benefit the animal in two ways, one way that might be utilised as precursor for gluconeogenesis under stress conditions to meet the energy demands; secondly, since starvation is a stress condition imposed on the animal, the inherent ability of the molluscs to withstand the adverse condition in their nourishment depends on the capacity to regulate the ionic and osmotic concentrations of both blood and cells (Schoffeniels and Gilles 1972), so that the proteolysis should help to maintain the osmoregulatory balance. Free amino acids could also be utilized for the purpose of energy needs.

There is a continuous reduction in the total metabolism with progressive starvation up to 70 days. A decreased trend in the oxygen consumption in relation to starvation was observed by Berg *et al* (1958) in freshwater limpet *Ancylus fluviatilis*, Duerr (1965) in *Lymnaea palustris*, Lumbye and Lumbye (1965) in *Littorina planaxis.*

Although, a similar decrease in oxygen consumption during long term starvation has been reported in *Thais lapillus* (Stickle and Bayne 1982), no change was observed in the recalculated intercept (a). However, in the present investigation on *M. granulata*, the recalculated intercept values were found to decrease with increasing period of starvation which corroborates with the results of Bayne and Scullard (1978) on *T. lapillus*. Stickle and Duerr (1970) and Stickle (1971) have observed constant or increased rates of weight specific oxygen consumption in *T. lamellosa* during starvation. However, *M. granulata* exhibited a decreased pattern of weight specific oxygen consumption when exposed to starvation stress. It is possible that the observed decrease in oxygen consumption of *M. granulata* may be an adaptation enabling the snail to conserve the food stored.

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References

Barry R J C and Munday D A 1959 Carbohydrate levels in Patella; *J. Mar. Biol. Assoc. UK* **38** 81–95

Bayne B L and Scullard C 1978 Rates of oxygen consumption by *Thais* (*Nucella*) *lapillus* (L.); *J. Exp. Mar. Biol. Ecol.* **32** 97–111

Berg K, Lumbye J and Ocklemann K W 1958 Seasonal and experimental variations of oxygen consumption of limpet *Ancylus fluviatilis*; *J. Exp. Biol.* **35** 43–73

Carrol N V, Longley R W and Roe J H 1956 The determination of glycogen in liver and muscle by use of anthrone reagent; *J. Biol. Chem.* **220** 583–593

Duerr F 1965 Some effects of diet on the respiratory rate of the freshwater pulmonate snail *Lymnaea palustris*; *Proc. S. D. Acad. Sci.* **44** 245

Emerson D N 1967 Carbohydrate oriented metabolism of *Planorbis corneus* (Mollusca, Planorbidae) during starvation; *Comp. Biochem. Physiol.* **22** 571–579

Emerson D N and Duerr F 1967 Some physiological effects of starvation in the intertidal prosobranch *Littorina planaxis* (Philippi 1847); *Comp. Biochem. Physiol.* **20** 45–53

Folch J, Lees H and Slone Stanley G H 1957 A simple method for the isolation and purification of total lipids from animal tissues; *J. Biol. Chem.* **266** 497–509

Giese A C 1966 Lipids in the economy of marine invertebrates; *Physiol. Rev.* **46** 244–298

Lowry O H, Rosenbrough N J, Farr A L and Randall R J 1951 Protein measurement with the Folin reagent; *J. Biol. Chem.* **193** 265–275

Lumbye J and Lumbye J 1965 The oxygen consumption of *Potamopyrgus jenkinsi* (Smith); *Hydrobiologia* **25** 489–500

Martin A and Goddard C 1966 Carbohydrate metabolism; in *Physiology of Mollusca* (ed) K M Wilbur and C M Yonge (New York: Academic Press) pp 275–308

Moore S and Stein W H 1954 A modified ninhydrin reagent for the photometric determination of amino acids and related compounds; *J. Biol. Chem.* **211** 907–913

Prasada Rao D G V and Uma Devi V 1981 Oxygen consumption in relation to body size in the intertidal gastropod *Morula granulata* (Duclos); *Geobios* **8** 249–253

Ricardo P J and Brennes R R 1981 Effect of environment and fasting on the lipids and fatty acid composition of *Diplodon patagonicus*; *Lipids* **16** 685–690

Schoffeniels E and Gilles R 1972 Ionoregulation and osmoregulation in mollusca; in *Chemical Zoology* (ed) M F Florkin and B T Scheer (New York: Academic Press) pp 301–342

Snedecor G W and Cochran W G 1967 *Statistical methods* (Iowa: The Iowa State University Press)

Stickle W B 1971 The metabolic effects of starving *Thais lamellosa* immediately after spawning; *Comp. Biochem. Physiol.* **A40** 627–634

Stickle W B 1975 The reproductive physiology of the intertidal prosobranch *Thais lamellosa* Gmelin. II Seasonal changes in the biochemical composition; *Biol. Bull.* **148** 448–460

Stickle W B and Bayne B L 1982 Effects of temperature and salinity on oxygen consumption and nitrogen excretion in *Thais lapillus*; *J. Exp. Mar. Biol. Ecol.* **58** 1–17

Stickle W B and Duerr F G 1970 Effects of starvation on the respiration and major nutrient stores of *Thais lamellosa*; *Comp. Biochem. Physiol.* **33** 689–695

Uma Devi V, Prabhakara Rao Y and Prasada Rao D G V 1985 Body component indices of an intertidal gastropod *Morula granulata* (Duclos) subjected to starvation; *Indian J. Mar. Sci.* **14** 44–45