

## EFFECT OF STARVATION ON THE GLYCOGEN RESERVES IN *CHICOREUS VIRGINEUS* AND *RAPANA RAPIFORMIS* (NEOGASTROPODA: PROSOBRANCHIA)

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### ABSTRACT

The utility of glycogen reserves during prolonged periods of starvation in muricid gastropods, *Chicoreus virgineus* and *Rapana rapiformis*, was studied in the laboratory in relation to weight and glycogen level of the foot, mantle, adductor muscle and digestive gland. Weight and glycogen levels decreased more in the mantle than in other organs of both species. *C. virgineus* survived up to 157 days, and *R. rapiformis* up to 150 days without consuming any feed. Females died a few days earlier than males.

### INTRODUCTION

Many molluscs, particularly bivalves and gastropods, appear to have remarkable tendencies to withstand a prolonged period without feeding. During unfavourable conditions the animals retreat into their shells and close the operculum. They can remain in dormant condition for several months to a year (Meenakshi 1957). Locomotion, growth, and reproduction are reduced, or completely arrested during starvation. Prolonged starvation results in drastic depletion of the glycogen content stored in body organs. Glycogen occurs in most gastropods and serves as an energy source (Livingstone & de Zwaan 1983). Carbohydrate depletion due to starvation has been shown in gastropods by von Brand *et al.* (1950, 1955), Meenakshi (1957), Emerson (1967), Christie *et al.* (1974), and Heeg (1977). The aim of the present study is to provide data on utilisation of glycogen by the muricid gastropods *C. virgineus* and *R. rapiformis* during a period of starvation. To our knowledge, there are no previous studies on the muricids

### MATERIALS AND METHODS

*C. virgineus* and *R. rapiformis* were collected from the littoral region of Parangipettai

(11°29'N; 79°47'E) coastal waters, southeastern coast of India. The snails were acclimatized 15 days prior to the experiment. They were fed with bivalves, *Meretrix meretrix*. A total of 150 animals of both species were selected: *C. virgineus*: 6.31-7.15 cm total length; 45.40-66.50 g total weight. *R. rapiformis*: 5.84-6.26 cm total length; 42.60-58.31 g total weight. Analysis was carried out on 10 specimens sampled at 15 days intervals. Foot, mantle, adductor muscle and digestive gland were dissected out and their weight and glycogen content were measured separately in males and females. Mortality during starvation was estimated in 25 *C. virgineus* and 25 *R. rapiformis* maintained separately in the laboratory.

### Glycogen extraction

Foot, mantle, adductor muscle, and digestive gland were quickly removed and placed in a Petri dish kept between ice cubes to avoid post-mortem breakdown of glycogen. Tissue weighing 100-150 mg was sliced and the glycogen extracted by boiling for 15 min in 1 ml 30% KOH. After cooling, 1.5 ml 95% ethanol was added to precipitate the glycogen. The extracts were left overnight at 0-4 °C for flocculation of the glycogen. The glycogen was precipitated using a centrifuge

at 3000 rpm for 15 min and the supernatant was slowly decanted without disturbing the glycogen sediment. The glycogen was redissolved in glass distilled water and reprecipitated with 95 % ethanol. The precipitated glycogen was finally dissolved in 5 ml glass distilled water and suitably diluted for estimation. The glycogen was estimated colorimetrically using anthrone reagent following the method of Carrol *et al.* (1956).

## RESULTS

A steep fall in the weight of the investigated organs were recorded in both the species during the period of starvation (Tables 1 and 2). There was no significant difference between males and females. At the beginning of the experiment (day 0), mantles showed

the highest amount of glycogen reserves, followed by foot, adductor muscle and digestive gland (Tables 3 and 4). A steep fall in the level of glycogen was noticed in all the tissues and the percentage of reduction was 65.68 %, 78.85 %, 69.52 % and 60.38 % in *C. virgineus* and 78.02 %, 84.84 %, 82.77 % and 67.79 % in *R. rapiformis* (foot, mantle, adductor muscle and digestive gland respectively; Tables 3 and 4).

Mortality as a result of starvation was observed after 141 days and onwards in *R. rapiformis* and after 146 days in *C. virgineus* (Table 5). Generally, females died somewhat earlier than males of both species. *C. virgineus* survived starvation up to 157 days and *R. rapiformis* up to 150 days.

**Table 1.** Mean weight (g) of foot, mantle, adductor muscle, and digestive gland of male and female *Chicoreus virgineus*. A total of 5 male and 5 female snails were sacrificed at 15 days intervals during the period of starvation. Day 0 = the beginning of the experiment.

Days	Foot		Mantle		Adductor muscle		Digestive gland	
	male	female	male	female	male	female	male	female
0	5.9	6.3	2.8	3.2	4.6	4.9	1.10	1.30
15	5.2	5.8	2.7	3.0	4.4	4.6	1.06	1.00
30	4.8	5.4	2.5	2.8	4.0	4.2	1.01	0.98
45	4.4	4.0	2.3	2.5	3.8	4.0	0.90	0.95
60	3.8	3.8	2.1	2.3	3.7	3.7	0.84	0.88
75	3.2	3.4	2.0	2.0	3.5	3.6	0.80	0.81
90	3.0	3.2	1.8	1.8	3.3	3.4	0.71	0.70
105	2.8	3.0	1.7	1.6	3.0	3.1	0.64	0.62
120	2.6	2.8	1.5	1.5	2.8	2.7	0.59	0.55
135	2.5	2.6	1.4	1.2	2.6	2.5	0.51	0.49
150	2.4	2.8	1.2	1.1	2.5	2.3	0.47	0.45

**Table 2.** Mean weight (g) of foot, mantle, adductor muscle, and digestive gland of male and female *Rapana rapiformis*. A total of 5 male and 5 female snails were sacrificed at 15 days intervals during the period of starvation. Day 0 = the beginning of the experiment.

Days	Foot		Mantle		Adductor muscle		Digestive gland	
	male	female	male	female	male	female	male	female
0	6.3	7.1	2.5	2.8	3.8	4.2	1.05	1.10
15	6.0	6.8	2.3	2.4	3.5	4.0	1.03	1.00
30	5.7	6.4	2.1	2.1	3.2	3.8	1.01	0.91
45	5.3	6.0	2.0	2.0	3.0	3.6	0.93	0.82
60	5.0	5.8	1.8	1.9	2.8	3.5	0.81	0.76
75	4.7	5.4	1.7	1.8	2.6	3.1	0.76	0.68
90	4.4	4.9	1.7	1.7	2.5	2.7	0.52	0.50
105	4.1	4.5	1.5	1.6	2.2	2.4	0.48	0.41
120	3.9	4.0	1.3	1.4	2.1	2.0	0.31	0.33
135	3.8	3.5	1.1	1.0	0.9	0.8	0.28	0.26
150	3.7	3.3	0.8	0.7	0.7	0.5	0.22	0.19

**Table 3.** Mean glycogen levels (mg glucose per gram wet weight) of foot, mantle, adductor muscle, and digestive gland of male and female *Chicoreus virgineus*. A total of 5 male and 5 female snails were sacrificed at 15 days intervals during the period of starvation. Day 0 = the beginning of the experiment.

Days	Foot		Mantle		Adductor muscle		Digestive gland	
	male	female	male	female	male	female	male	female
0	93.18	98.21	123.27	131.38	98.57	103.28	66.24	70.31
15	85.56	87.54	110.58	120.16	80.11	92.10	58.16	62.79
30	71.89	70.86	101.35	102.17	72.57	85.38	47.35	57.15
45	60.67	59.45	90.56	91.53	63.61	70.15	40.07	50.13
60	52.38	48.38	81.35	78.17	54.56	61.68	36.34	46.35
75	40.83	33.41	66.59	61.56	45.63	50.53	28.55	38.97
90	28.57	25.06	50.51	53.80	31.12	42.58	21.33	30.10
105	19.78	16.14	42.53	41.54	25.50	33.99	12.17	22.18
120	14.33	10.25	28.75	23.56	18.19	20.17	9.63	13.76
135	9.56	8.36	19.19	18.12	11.31	11.06	7.12	9.27
150	6.12	5.07	12.56	10.36	8.51	7.18	4.00	4.31

**Table 4.** Mean glycogen levels (mg glucose per gram wet weight) of foot, mantle, adductor muscle, and digestive gland of male and female *Rapana rapiformis*. A total of 5 male and 5 female snails were sacrificed at 15 days intervals during the period of starvation. Day 0 = the beginning of the experiment.

Days	Foot		Mantle		Adductor muscle		Digestive gland	
	male	female	male	female	male	female	male	female
0	89.48	93.54	118.32	124.47	96.77	103.53	54.58	61.31
15	81.35	81.80	110.00	107.13	88.91	92.18	49.85	56.57
30	76.56	74.48	102.07	96.11	73.15	81.31	43.11	51.35
45	68.53	66.53	90.55	83.57	66.51	73.17	38.67	47.31
60	60.17	59.61	82.53	70.50	52.31	67.46	32.21	40.07
75	52.59	41.37	71.99	61.27	40.59	53.53	28.28	36.28
90	40.18	33.55	58.69	53.87	31.68	42.41	23.14	22.18
105	21.96	25.17	41.78	40.63	26.21	36.56	17.31	17.56
120	16.27	15.91	28.67	21.38	18.36	28.31	10.38	9.15
135	10.23	10.78	17.13	16.13	11.15	16.15	7.67	5.30
150	7.37	6.91	11.26	19.34	8.01	8.23	3.70	3.91

## DISCUSSION

**Table 5.** The percentage dead *Chicoreus virgineus* and *Rapana rapiformis*. A total of 25 individuals of each species were exposed to starvation until the last individual died after 157 days. (-) = all animals dead.

Days	<i>Chicoreus virgineus</i>			<i>Rapana rapiformis</i>		
	male	female	total	male	female	total
0 to						
140	0	0	0	0	0	0
141	0	0	0	8	4	12
142	0	0	0	0	8	8
143	0	0	0	12	0	12
144	0	0	0	12	4	16
145	0	0	0	0	4	4
146	12	0	12	16	8	24
147	4	8	12	4	4	8
148	4	4	8	4	-	4
149	4	8	12	4	-	4
150	0	4	4	8	-	8
151	8	8	16	-	-	-
152	12	4	16	-	-	-
153	8	-	8	-	-	-
154	4	-	4	-	-	-
156	4	-	4	-	-	-
157	4	-	4	-	-	-
Total	64	36	100	68	32	100

Glycogen occurs in the tissues of almost all gastropods along with galactogen forming the main storage of polysaccharide. Glycogen is distributed throughout the body with high levels in foot, mantle, adductor muscle, and digestive gland. The average concentration of glycogen in gastropods is approximately 20 to 30 % of the dry weight (Livingstone & de Zwaan 1983).

In the present investigation the glycogen level was highest in the mantle tissue of both *C. virgineus* and *R. rapiformis*. This agrees with the general finding that molluscs have special cells for glycogen storage, and these cells occur in large numbers in the mantle edge (Sminia 1972). In the freshwater snail *Lymnaea stagnalis*, the vesicular connective tissue cells (glycogen cells) are the major storage cells for glycogen, and these cells are concentrated in the mantle. It was also found that the glycogen accumulation coincided with a two or three fold increase in the activity of glycogen synthetase in the mantle (Sminia 1972).

Glycogen levels of the muricids were quickly reduced from the initial day of starvation to

the death of the animals. In *C. virgineus*, the glycogen level was decreased up to 60.4-78.9 %; in *R. rapiformis* 67.8-84.8 % in different tissues after 150 days of starvation. Barry & Munday (1959) found that the glycogen concentrations of all the tissues were extremely low in the prosobranch *Patella* during prolonged starvation (120 days). Similarly, Emerson (1967) reported that the pulmonate *Planorbis corneus* lost 95 % of the carbohydrate during 58 days of starvation. However, Barry & Munday (1959) found that before maturation, starvation had no immediate effect on the glycogen levels of *Patella*. The steep fall of glycogen concentrations in the present muricids indicated that they depended on glycogen as an energy source during starvation.

The last *R. rapiformis* died a few days prior to *C. virgineus*. This might be due to the quantity of glycogen stored in tissues in agreement with a higher glycogen level of *C. virgineus* than in *R. rapiformis*. However, in both species, the females had slightly higher concentrations of glycogen. Nevertheless, the females died a few days earlier than males, indicating that the relationship between glycogen and tolerance to starvation may not be straightforward. The cycle of reproduction seems to be an important factor explaining the early death of females. Barry & Munday (1959) reported that the glycogen level decreased during gonad development, maturation and spawning.

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