REGENERATION OF SIPHONAL CANALS IN THE MURICIDS *CHICOREUS VIRGINEUS* AND *RAPANA RAPIFORMIS* (PROSOBRANCHIA: NEOGASTROPODA)

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ABSTRACT

Males and females of *Chicoreus virgineus* (6.2-7.3 cm length) and *Rapana rapiformis* (6.0-6.7 cm length) were selected. In the first experiment, 5 mm of the siphonal canal was amputated in 125 specimens of both species. *C. virgineus* males regenerated in 183 days; females in 207 days; *R. rapiformis* males regenerated in 195 days; females in 223 days. The mantle, foot, and adductor muscle lost weight during the regenerative process. *C. virgineus* had minimal concentrations of protein, carbohydrate and lipid after 120 days. A similar pattern was found in *R. rapiformis*, except for tissue of the foot where all relative concentrations were minimal after 140 days in both sexes. In the second experiment, the feeding rate was highly reduced in labial tooth regenerating animals. It took 90 days for male *C. virgineus*, and 100 days for females, to regenerate amputated labial teeth. During the first 50 days of regeneration, the consumption of bivalves, *Meretrix meretrix* was 9-11 bivalves 10 d^{-1} ind^{-1} against a consumption of 18-20 bivalves 10 d^{-1} ind^{-1} in snails with untreated labial teeth.

INTRODUCTION

Molluscs are capable of repairing damage inflicted to the shell. The repair, often called shell regeneration, is accomplished by a deposition of new shell material in the damaged area. The process of repair may or may not be similar to that of normal shell formation and the characteristics of the repaired shell vary with the organism and the region of the shell damage. Recent physiological and ultrastructural investigations have revealed that the mechanisms are intimately associated with neurosecretory or hormonal action of the organisms.

For a number of reasons, shells of molluscs are often broken in nature: rough weather, missed attacks by predators, and the day to day life activities (Hylleberg & Khokiattiwong 1992). Hence energy must be derived from the soft body for repair of the damaged area. The present study was designed to investigate the regenerative processes of the edible muricid gastropods *Chicoreus virgineus* and *Rapana rapiformis*. The metabolic costs related to regeneration was examined in terms of soft body changes, specifically the wet weight of mantle, foot, adductor muscle, and the amounts of carbohydrate, protein, and lipid in these tissues. Since the labial tooth of *Chicoreus virgineus* is important in feeding (Patterson *et al.* 1992, Fig 1B), a laboratory study was designed to investigate the effect of losing the tooth on the feeding rate of this species.

MATERIALS AND METHODS

The specimens were collected from Parangipettai (11°29'N; 79°47'E) coastal waters of Bay of Bengal, southeastern coast of India. They were fed with bivalves, *Meretrix meretrix* for one month during acclimatization in the laboratory.

Both sexes of *C. virgineus* (total length 6.21-7.28 cm; total width 4.02-4.41 cm) and *R. rapiformis* (total length of 5.96-6.65 cm; total width 4.23-4.91 cm) were selected for the regeneration study. Exactly 5 mm of siphonal canal was amputated in 125 specimens of both species. Addition of new shell material was measured every 20 days using Vernier callipers. Weight and biochemical composition: protein (according to Raymont *et al.* 1964), carbohydrate (Dubois *et al.* 1956), and lipid (Folch *et al.* 1966), of foot, mantle and adductor muscle were measured every 20 days in 5 specimens sacrificed of both sexes and both species. The labial tooth was amputated in 10 *C. virgineus*. The rate of regeneration was measured every 10 days. Regenerating snails, and 10 snails maintained as control (same size group), were fed *ad libitum* with the clam *Meretrix meretrix*.

RESULTS

The regenerative capacity was greater in males than females. It took 183 and 195 days for males of *C. virgineus* and *R. rapiformis*, respectively, to regenerate 5 mm length of siphonal canal, while it was 207 days for female *C. virgineus* and 223 days for female *R. rapiformis* (Table 1).

The mantle, foot, and adductor muscle lost weight during the regenerative process (Table 2). The highest weight loss was noticed in the mantle of both sexes of the two species. In *C. virgineus*., the weight of mantle tissue was reduced to 1.0 g in males and 0.5 g in females after 100 days. In snails sacrificed at the beginning of the experiment, the mantles weighed 2.1-2.3 g, and 2.2-2.6 g respectively. In *R. rapiformis*, the mantle weighed 0.5 g in males and 0.6 g in females after 120-140 days of regeneration. (to be compared with 2.3-2.5 g and 2.4-2.6 g respectively, at the beginning of the experiment). The percentage protein, carbohydrate and lipid was analysed in the foot (Table 3), mantle (Table 4), and adductor muscle (Table 5). The relative concentrations of carbohydrate and lipid changed conspicuously, while the concentration of protein was less affected by regeneration (Tables 3, 4, 5) The males and females of *C. virgineus* had minimal concentrations of both protein, carbohydrate and lipid after 120 days. Occasionally the lowest content was measured after 100 days in males, or 140 days in females. (Tables 3, 4, 5). A similar pattern was found in *R. rapiformis*, except for tissue of the foot where all relative concentrations were minimal after 140 days in both males and females (Table 3). Then the concentrations gradually increased. After 200-220 days, the initial values had been attained, that is, at the time when regeneration of the amputated siphonal canal was completed.

It took 90 days for male *C. virgineus*, and 100 days for females, to regenerate the amputated labial tooth to its original length (Table 6).

Table 1. *Chicoreus virgineus* and *Rapana rapiformis*. Regeneration of the siphonal canal of 10 males and 10 females. The mean increment of the canal length (mm) is shown at 20 days intervals. (-) = no measurement. Day 0 = start of experiment.

	Regeneration (mm)			
	Chicoreus virgineus		*Rapana rapiformis*	
Days	Male	Female	Male	Female
0	0.0	0.0	0.0	0.0
20	0.9	0.8	0.8	0.8
40	1.6	1.3	1.3	1.2
60	2.1	1.9	1.9	1.8
80	2.9	2.5	2.6	2.2
100	3.3	3.1	3.1	2.8
120	3.9	3.7	3.7	3.1
140	4.3	4.1	4.0	3.9
160	4.8	4.5	4.6	4.1
180	5.0	4.8	4.8	4.5
200	-	5.0	5.0	4.8
220	-	-	-	5.0

Table 2. *Chicoreus virgineus* and *Rapana rapiformis*. Mean weight (g) of mantle, foot, and adductor muscle of male and female. A total of 5 male and 5 female snails of each species were sacrificed at 20 days intervals during the period when siphonal regeneration was measured. The range of soft body weights at the beginning of the experiment (day 0) is shown. (-) = no measurement.

	Chicoreus virgineus						*Rapana rapiformis*					
Days	Mantle		Foot		Add. Muscle		Mantle		Foot		Add. Muscle	
	male	female	male	female	male	female	male	female	male	female	male	female
0	2.1-2.3	2.2-2.6	4.7-4.9	5.0-5.3	3.2-3.4	3.5-3.7	2.3-2.5	2.4-2.6	5.6-5.8	5.9-6.1	3.8-4.3	4.0-5.2
20	1.8	2.0	4.8	5.0	3.3	3.6	2.4	2.6	5.7	6.0	3.2	3.4
40	1.6	1.8	4.3	4.7	3.0	3.5	2.0	2.3	5.5	5.7	3.0	3.1
60	1.2	1.0	4.0	4.3	2.8	3.3	1.5	2.0	5.1	5.1	2.7	3.0
80	0.8	0.7	3.7	4.0	2.7	3.0	1.0	1.8	4.7	4.8	2.7	2.8
100	1.0	0.5	3.5	3.8	2.5	2.7	0.7	1.5	4.0	4.2	2.6	2.6
120	1.3	1.0	3.4	3.6	2.5	2.5	0.5	1.0	3.8	3.9	2.2	2.0
140	1.6	1.6	3.8	4.0	2.8	2.8	1.2	0.6	4.1	4.2	2.5	2.5
160	1.8	2.0	4.5	4.3	3.0	3.0	1.8	1.2	4.7	4.7	2.8	2.7
180	2.1	2.1	5.0	4.8	3.4	3.2	2.1	1.8	5.1	5.2	3.1	3.0
200	-	2.3	-	5.3	-	3.7	2.5	2.0	5.8	5.8	3.3	3.2
220	-	-	-	-	-	-	-	2.6	-	6.1	-	3.5

Table 3. *Chicoreus virgineus* and *Rapana rapiformis*. Mean percentage of protein, carbohydrate, and lipid of the foot of male and female. A total of 5 male and 5 female snails of each species were sacrificed at 20 days intervals during the period when siphonal regeneration was measured. The range of soft body weights at the beginning of the experiment (day 0) is shown. (-) = no measurement.

	Chicoreus virgineus						*Rapana rapiformis*					
Days	Protein (%)		Carbohyd. (%)		Lipid (%)		Protein (%)		Carbohyd. (%)		Lipid (%)	
	male	female	male	female	male	female	male	female	male	female	male	female
0	37.0-38.0	34.31-35.26	7.0-8.0	9.0-9.7	1.2-1.38	1.50-1.69	29.0-30.0	33.0-34.5	9.0-10.5	11.0-12.40	1.10-1.28	1.40-1.53
20	34.12	37.32	7.01	9.01	1.30	1.38	30.02	33.21	10.12	11.38	1.25	1.49
40	30.20	35.11	6.78	8.85	1.12	1.12	28.21	32.53	9.80	10.21	1.15	1.28
60	28.31	33.23	6.12	8.21	1.00	1.03	27.98	30.26	8.53	9.58	1.02	1.20
80	23.12	30.12	5.90	7.78	0.98	0.97	27.31	29.81	7.78	8.31	0.92	1.01
100	20.83	28.50	5.25	7.50	0.81	0.80	26.81	28.53	7.07	8.08	0.80	0.91
120	23.51	24.12	4.50	6.07	0.61	0.71	26.06	26.21	6.31	7.30	0.61	0.78
140	28.52	26.34	5.31	7.31	0.83	0.62	25.31	25.38	6.01	6.75	0.50	0.61
160	32.71	30.22	6.78	7.99	1.20	1.07	26.53	27.32	7.98	7.81	0.83	0.98
180	35.00	34.91	7.31	8.38	1.38	1.34	28.24	29.28	9.02	8.38	1.07	1.16
200	-	38.01	-	9.58	-	1.69	30.21	31.83	10.21	10.85	1.28	1.28
220	-	-	-	-	-	-	-	34.62	-	12.38	-	1.53

Table 4. *Chicoreus virgineus* and *Rapana rapiformis*. Mean percentage of protein, carbohydrate, and lipid of the mantle of male and female. A total of 5 male and 5 female snails of each species were sacrificed at 20 days intervals during the period when siphonal regeneration was measured. The range of soft body weights at the beginning of the experiment (day 0) was lost. (-) = no measurement.

Days	*Chicoreus virgineus*						*Rapana rapiformis*					
	Protein (%)		Carbohyd. (%)		Lipid (%)		Protein (%)		Carbohyd. (%)		Lipid (%)	
	male	female	male	female	male	female	male	female	male	female	male	female
0	-	-	-	-	-	-	-	-	-	-	-	-
20	35.36	36.38	6.28	7.56	1.20	1.44	33.02	34.14	7.08	7.81	1.09	1.27
40	34.21	34.54	5.12	6.38	1.00	1.21	32.38	33.28	6.34	6.27	0.96	1.02
60	32.97	33.14	4.58	5.12	0.93	1.06	30.97	31.08	5.87	5.33	0.83	0.94
80	30.57	32.62	3.25	4.38	0.78	0.97	29.21	29.34	4.28	4.51	0.61	0.81
100	29.33	30.44	2.20	2.31	0.60	0.80	28.07	28.57	3.51	3.73	0.54	0.73
120	38.46	27.29	1.77	1.97	0.48	0.62	26.38	26.41	2.78	2.18	0.48	0.62
140	31.28	30.78	3.71	2.33	0.95	1.01	29.28	28.58	4.58	3.84	0.66	0.50
160	33.54	32.55	5.27	4.51	1.07	1.27	30.34	31.36	5.38	5.66	0.85	0.73
180	35.78	34.83	7.31	6.67	1.26	1.38	31.67	33.28	6.62	6.39	0.98	0.92
200	-	37.21	-	8.21	-	1.53	33.56	34.51	7.20	7.56	1.18	1.21
220	-	-	-	-	-	-	-	35.31	-	8.05	-	1.46

Table 5. *Chicoreus virgineus* and *Rapana rapiformis*. Mean percentage of protein, carbohydrate, and lipid of the adductor muscle of male and female. A total of 5 male and 5 female snails of each species were sacrificed at 20 days intervals during the period when siphonal regeneration was measured. The range of soft body weights at the beginning of the experiment (day 0) was lost. (-) = no measurement.

Days	*Chicoreus virgineus*						*Rapana rapiformis*					
	Protein (%)		Carbohyd. (%)		Lipid (%)		Protein (%)		Carbohyd. (%)		Lipid (%)	
	male	female	male	female	male	female	male	female	male	female	male	female
0	34.21-36.0	36.51-38.8	6.38-7.92	7.56-8.40	1.41-1.62	1.58-1.67	34.72-36.33	35.78-38.27	8.19-9.29	8.37-10.13	1.21-1.40	1.72-1.93
20	33.58	35.34	6.56	7.07	1.07	1.20	30.01	32.16	6.58	7.07	1.01	1.18
40	32.31	32.54	5.37	6.55	0.97	0.98	27.27	29.38	5.43	6.74	0.92	0.98
60	31.28	30.26	4.72	5.46	0.83	0.81	25.01	27.17	4.21	5.34	0.84	0.83
80	29.21	29.74	3.53	4.83	0.76	0.70	24.36	25.40	3.26	4.55	0.73	0.74
100	26.34	26.34	2.46	3.12	0.63	0.61	22.16	23.05	2.45	3.34	0.61	0.64
120	24.48	24.64	3.64	2.56	0.53	0.50	26.54	21.56	1.38	2.56	0.48	0.52
140	27.38	27.76	5.67	3.56	0.83	0.73	28.38	24.76	2.78	3.15	0.64	0.46
160	30.98	29.36	6.73	5.47	0.98	0.93	30.31	27.51	4.58	5.56	0.79	0.73
180	34.21	32.55	7.18	7.26	1.20	1.21	32.18	30.32	5.97	6.34	0.98	0.93
200	-	36.83	-	8.10	-	1.44	33.28	32.64	7.02	7.16	1.15	1.11
220	-	-	-	-	-	-	-	34.56	-	8.00	-	1.35

The consumption of bivalves, *Meretrix meretrix* was from 9-11 bivalves/10 days/snail against a consumption of 18-20 bivalves/10 days/snail in animals with untreated labial teeth (control). At the time the labial tooth had regenerated, the consumption of prey was equal in the two groups of animals (Table 6). There was no difference between males and females regarding consumption rates.

Table 6. *Chicoreus virgineus*. Regeneration of the labial tooth of 10 males and 10 females. Mean tooth length (mm) is shown at 10 days intervals. Feeding rate/10 days of the regenerating individuals is compared to 10 control specimens with complete labial teeth, and given the same amount of *M. meretrix*. (-) = no measurement. Day 0 = start of experiment.

	Growth of labial tooth (mm)		No. of *M. meretrix* consumed/individual		
Days	Male	Female	Control	Male	Female
0	0.0	0.0	0	0	0
10	0.2	0.2	20	11	10
20	0.6	0.5	18	9	10
30	0.9	0.8	19	10	9
40	1.2	1.1	20	10	11
50	1.8	1.7	20	11	11
60	2.2	2.1	19	13	12
70	2.5	2.4	18	14	14
80	2.7	2.6	19	16	17
90	3.0	2.7	18	19	20
100	-	3.1	20	-	20

DISCUSSION

Generally, the initiation and completion of shell repair is a slow process in aquatic molluscs (Beedham 1965). However, the repair is faster at the edges than in the central region where the mantle cannot retract (Timmerman 1973). In the present study, the rate of regeneration was 0.027 mm/d (males), and 0.025 mm/d (females) in *C. virgineus*. The corresponding values were 0.024 mm/d and 0.022 mm/d in *R. rapiformis*. These rates are higher than the regenerative rate of 0.006 mm/day in *Tegula funebralis* observed by Peppard (1964). But, comparisons are not simple since different parts of the shell regenerate at different rates. The amputated labial tooth of *C. virgineus* regenerated faster than the siphonal canal.

During the course of regeneration, the weight as well as biochemical constituents changed in the mantle, foot and adductor muscle. Major changes occurred in the mantle, both in terms of weight and biochemistry. Calcium cells are present in the connective tissues of mantle, foot, hepatopancreas. Epithelium of the mantle contains calcium carbonate or calcium phosphate spherules (Watabe *et al.* 1976; Simkiss 1976; Sminia *et al.* 1977). Calcium for shell repair is derived from the calcium cells of the mantle (Durning 1957; Guardebassi & Piacenza 1958; Watabe *et al.* 1976), and foot (Watabe *et al.* 1976). In addition to calcium, proteinaceous granules (Abolins-Krogis 1963, 1980) and protein components (Saleuddin *et al.* 1970), are released from the mantle and transported to the sites of shell repair. Those processes might be the reason for the changes in the mantle biochemistry during regeneration.

The feeding rate was highly reduced in the labial tooth regenerating animals. The tooth plays a prominent role in holding the valves of prey bivalves apart while feeding. If the valves could not be hold open by the tooth, the snails would feed by a drilling process which includes secretions from the accessory boring organ combined with radular action. In the present study labial tooth amputated animals made a hole on the prey's valve by drilling. Obviously, it affected the time needed for feeding since the rate of food consumption decreased during the initial period of regeneration.

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