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Effect of Crowding, Food quality and Body Size on Food Utilization of the Exotic Snail, *Pomacea canaliculata* (Lamarck), a Potential Pest of Rice in Sri Lanka.

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Abstract

Pomacea canaliculata (Family: Ampullaridae) is an exotic freshwater snail introduced to Sri Lanka as an ornamental commodity. Although *P. canaliculata* is a potential rice pest, its food preference and energy budget under Sri Lankan conditions are not known. In order to assess the above, the snails were fed with three commonly found fresh water weed species, i.e. *Salvinia molesta, Azolla* sp. and *Hydrilla verticellata*, and their energy budget (i.e. feeding rate, absorption rate, food conversion rate, metabolic rate, absorption efficiency and conversion efficiency) was investigated in relation to the body size and crowding.

The snails preferred to consume *H. verticellata*, the rooted aquatic weed, than the other two species which were floating. Young snails showed the highest energy budget while the old snails showed the lowest. Similarly, the energy budget of the snails in less crowded aquaria was higher than in the highly crowded aquaria.

Considering the morphology of *H. vericellata* which is the highly preferred food by *Pomacea*, the present preliminary study urges the importance of conducting further investigations to assess the potential of this snail to infest rice cultivation in Sri Lanka as it has become a major pest of rice in many countries in the south-east Asian region.

Introduction

Pomacea canaliculata (Lamarck) (Order: Prosobranchia; Fam.: Ampullaridae), also known as golden apple snail living in stagnant freshwaters, is reported to have originated in the Amazon river basin in South America (Edra 1991) and therefore is also known as Argentine apple snails (Cazzaniga 1990) (hereafter referred to as *Pomacea*). From Argentina and Brazil they have become established in Florida, from where they have been extensively propagated and exported to other states in the USA and to other countries in Europe, Africa and Asia (Purchon 1968; Edra 1991; Halwart 1992). *Pomacea* was initially introduced to Southeast Asian countries in the early 1980's for human consumption and also as an export commodity (Halwart 1992; Halwart *et al.* 1998).

It is generally accepted that *Pomacea* has been introduced to Sri Lanka through aquarium fish trade. They soon became popular in Sri Lanka because of their beauty as well as their effectiveness in scavenging on uneaten fish food, rotting plant matter and algae in the aquaria. However, the aquaculturists have discovered that *Pomacea* not only feed on the eggs of fishes but also produce their own eggs which adhere to the sides of the tanks and ponds, which makes them visually unpleasant. This has led to the snails being discarded live to outside waters such as drains and ponds by aquaculturists. They were first observed in the water-bodies in the Colombo area in early 1980's (Gunawardena 1996).

In the new habitats in Sri Lanka *Pomacea* has turned out into a voracious feeder of aquatic weeds. Presently it has spread into areas around Colombo and also into some areas in the districts of Gampaha, Galle, Kandy, Kalutara and Ratnapura.

In the recent past, *Pomacea* was recognized as a serious pest of rice in both irrigated and rainfed cultivations because of its resilience, fast growth, phenomenal reproductive capacity and voracious appetite for vegetation (Basilio 1991; Guzman & Enriquez 1991; Acosta & Pullin 1991; Halwart 1992). For example, *Pomacea* has developed to be a major pest of rice in the Philippines since 1986 and it had been reported that the rice yield lost due to *Pomacea* was about 75% and by 1988 it had invaded around 426,000 ha of rice cultivation (Escalanda 1989; Rondon & Sumangil 1991; Halwart 1992). Around 3 million US dollars were spent each year on molluscicides to destroy this pest which amounted to about 20% of farmer's income (Acosta & Pullin 1991). In Taiwan, *Pomacea* has invaded nearly 100,000 ha since 1982, and about 1 million US dollars were spent annually for its control. Vietnam is the latest country where *Pomacea* has become a rice pest and it invaded nearly 30,000 ha of rice fields since 1992. Moreover the Vietnam Government has banned the rearing of *Pomacea* (Gunawardena 1996). Surinam had a similar problem of *Pomacea* infestation 25-30 years ago where it was a major rice pest (Acosta & Pullin 1991).

Apart from rice, *Pomacea* attacks other ornamental and commercially important plants such as corn, citrus and ramie (Baldia & Pantastico 1991).

Because of the economic importance of *Pomacea* as a pest, several studies on the different aspects of its biology (Guerrero1991; Cazzaniga 1990), genetics (Thiengo *et al.* 1993), morphology (Fujio & Von Brand 1990) and control practices (Guzman & Enriquiz 1991; Cruz 1991; Dupo & Ferido 1991; Rodriguez & Lorenzana 1991; Rondon & Sumangil 1991; Halwart *et al.* 1998) have been carried out.

Experimental determination of feeding rate, absorption rate, food conversion rate, metabolic rate, absorption efficiency and conversion efficiency which are commonly known as food utilization parameters or energy budget, are known to offer important clues in assessing the relative importance of different species and populations contributing to the structure, productivity and functioning of communities (Odum 1971; Haniffa 1980a; Lei & Armitage 1980). In view of the above, considerable work on the effects of various factors on food utilization of molluscs have been conducted in the recent past. These include the studies on food quality and quantity (Carefoot 1970; Haniffa 1980a), crowding (Cameron & Carter 1979; Haniffa 1980b; O'Keeffe 1985), aestivation (Haniffa 1978a, 1989), starvation (Haniffa 1989) and body size (Haniffa & Pandian 1974).

A few studies have been conducted on molluscs inhabiting the freshwaters of Sri Lanka. Most of them are, however, confined to taxonomic studies (e.g. Ellepola &

Fernando 1962; Starmuhlner 1974; 1977) and only a few have been carried out on biological aspects (Costa & Indrasena, 1984). As *Pomacea* can become a serious rice pest, various aspects of the biology and feeding ecology of this snail under Sri Lankan conditions will be of great importance.

The present preliminary study investigates the food utilization parameters of *Pomacea* in relation to their crowding and body size when fed on three species of aquatic macrophytes, namely *Salvinia molesta* (Mitchel) (hereinafter referred to as *Salvinia*), *Hydrilla verticillata* (Linnaeus) (hereinafter referred to as *Hydrilla*) and *Azolla* sp (hereinafter referred to as *Azolla*), that are commonly found in Sri Lankan water bodies. This study is the first of a series of studies planned to be conducted on the biological and ecological aspects of *Pomacea* in Sri Lanka.

Materials and Methods

Live specimens of *Pomacea* were obtained from a local aquarium and transported in polythene containers to the Zoology laboratory of the University of Kelaniya. They were acclimated for one week in glass aquaria with aged-tap water under static conditions. During the acclimatization period, the snails were regularly fed on natural food plants *Ad libitum*. After every 24 hours, water was changed and unconsumed plants were removed to prevent contamination of water.

The acclimated snail were exposed to air and left undisturbed for four hours to release the excess water contained in the palial chamber. They were then weighed and divided into three size classes namely young (3±0.5g), intermediate (7±0.5g) and old (11±0.5g).

Snails belonging to each size class were stocked at three stocking densities viz. 5, 10 and 15 individuals in 8 l capacity glass aquaria with with aged tap water. Three replicates of each stocking density in each size class were used and the snails in each aquarium were fed daily on *Hydrilla* of 10% of their body weight for a period of one week. Before feeding, the wet weight of *Hydrilla* was measured using an electronic balance.

Unconsumed *Hydrilla* parts in each aquarium were weighed and the food consumed by the snails was estimated on a daily basis. Appropriate corrections were made for the amount of plant substances produced due to photosynthesis during the experimental period following Vivekanandan *et al.* (1974) and Haniffa & Pandian (1974). The temperature and dissolved oxygen concentrations of the water in each aquarium were determined daily using a standard glass-mercury thermometer and Winkler's method respectively. After the seven days experimental period, the wet weight of each snail in each aquarium was measured using an electronic balance.

Fecal pellets of *Pomacea* accumulated in the bottom of each aquarium were collected by filtering the entire water content through a 125µm diameter sieve with a minimum disturbance to the test individuals. Feces were dried at 105^0C to a constant weight and the dry weight was measured using an electronic balance.

The above experimental procedure was repeated by feeding a fresh batch of test snails using *Salvinia* and *Azolla* as food.

Fifteen randomly selected individuals inclusive of shells of a wide weight range (0.8-12g) were taken from the original snail stock and their wet weights were measured. They were dried at 105^0C for 24 hours and the corresponding dry weights were also measured. Similarly, the wet weights and corresponding dry weights of *Hydrilla, Salvinia* and *Azolla* were also determined. When the wet weight of a test snail or a test plant is known its corresponding dry weight could be graphically calculated using these relationships.

The calculated dry weight loss of food plants and the dry weight gain of the test snails were used to determine the food utilization parameters such as the feeding rate (FR), absorption rate (AR), food conversion rate (CR), metabolic rate (MR), absorption efficiency (AE) and conversion efficiency (CE) following Haniffa (1978a), Sampath & Ramasunder (1992) and Jayalakshmi & Natarajan (1994).

The data were tested for homogeneity of variance using the Bartlett test ($\alpha = 0.05$) prior to parametric analysis. Differences of the food utilization parameters between the size classes and stocking densities at each feed type were initially tested using three-way ANOVA ($\alpha = 0.05$). When a significant main effect due to feed type was noted, the data were analyzed separately using two-way or one-way ANOVA ($\alpha = 0.05$) as appropriate. When the ANOVA revealed a significant main effect or interaction, Tukey's multiple comparison tests ($\alpha = 0.05$) (Zar 1996) were carried out to determine significant differences between the densities and size classes. The dry weights of test animals and plants were regressed against their respective wet weights to determine the dry weight-wet weight relationship. Data were analyzed using Minitab for Windows (version 10.2) statistical package.

Results

The temperature and dissolved oxygen content of test aquaria varied within a range of 26-30°C and 3.14-3.26 mg l^{-1} respectively during the experimental period.

The relationships between the wet and dry weights of *Pomacea, Hydrilla, Salvinia* and *Azolla* are shown in Table 1. These relationships were found to be highly significant (regression analysis; $p<0.05$).

Table 1. The relationships between the dry (y) and wet (x) weights of *Pomacea, Hydrilla, Salvinia* and *Azolla*. R^2 = coefficient of determination, R = correlation coefficient, P = probability level.

Type	Number of samples	Regression equation	R^2	R	P
Pomacea	15	y = 0.4506x + 0.0073	0.9805	0.9902	0.0001
Hydrilla	15	y = 0.0565x + 0.0013	0.9664	0.9830	0.0001
Salvinia	15	y = 0.0144x + 0.0131	0.9456	0.9724	0.0001
Azolla	15	y = 0.0526x + 0.0132	0.9843	0.9921	0.0001

The summary of the 3-way ANOVA of each of the six food utilization parameters (i.e. FR, AR, CR, MR, AE and CE) of different size classes of *Pomacea* at the three stocking densities when fed with the three feed types (i.e. *Hydrilla, Salvinia* and *Azolla*) are shown in tables 2-7 respectively.

The feeding rate of *Pomacea* on *Hydrilla* was significantly higher than those on *Azolla* and *Salvinia* (Tukey's pairwise tests after one-way ANOVA; $p<0.05$). This showed that *Pomacea* showed a preference for the consumption on *Hydrilla* over other feed types. *Pomacea* did not show a preference to feed on *Salvinia* to *Azolla* (Tukey's pairwise tests after one-way ANOVA; $p>0.05$) (Fig. 1).

Table 2. Summary of the 3-way ANOVA showing the effects of feed type, body size and stocking density on the feeding rates (FR) of *Pomacea*. DF= degrees of freedom; SS= sum of squares; MS = Mean squares; F= Calculated F-value; P= probability level.

Source	DF	SS	MS	F	P
Main factors					
Feed Type (FT)	2	76.72	38.36	314.71	0.0001
Body Size (BS)	2	58.34	29.17	239.31	0.0001
Stocking Density (SD)	2	34.84	17.42	142.9	0.0001
Interactions					
FT x BS	4	16.17	4.04	33.16	0.0001
FT x SD	4	10.76	2.69	22.08	0.0001
BS x SD	4	10.78	2.70	22.12	0.0001
FT x SD x BS	8	11.17	1.39	11.45	0.0001
Error	54	6.58	0.12		
Total	80	225.36			

Table 3. Summary of the 3-way ANOVA showing the effects of feed type, body size and stocking density on the absorption rate (AR) of *Pomacea*. Abbreviations are as in Table 2.

Source	DF	SS	MS	F	P
Main factors					
Feed Type (FT)	2	16.78	8.39	194.40	0.0001
Body Size (BS)	2	15.55	7.78	180.24	0.0001
Stocking Density (SD)	2	11.91	5.95	137.99	0.0001
Interactions					
FT x BS	4	6.41	1.60	37.16	0.0001
FT x SD	4	2.26	0.56	13.08	0.0001
BS x SD	4	4.42	1.10	25.59	0.0001
FT x SD x BS	8	1.84	0.23	5.34	0.0001
Error	54	2.33	0.04		
Total	80	61.49			

Food utilization parameters of young *Pomacea* (size range 0-3.0g) were significantly higher than those of intermediate and old size classes (Tukey's pair-wise tests after 3-way ANOVA; $p<0.05$) (Tables 2-7 and Figs. 2, 4 and 6). This showed that the energy budget of young *Pomacea* is higher than those of the other two size classes.

The food utilization parameters, when the stocking density was 5 individuals per aquarium, were significantly higher than those when the stocking densities were 10 and 15 individuals per aquarium (Tukey's pairwise tests after 3-way ANOVA; $p<0.05$) (Tables 2-7 and Figs. 3, 5 and 7). This showed that in less crowded conditions, the energy budget of *Pomacea* is high.

Table 4. Summary of the 3-way ANOVA showing the effects of feed type, body size and stocking density on the food conversion rate (CR) of *Pomacea*. Abbreviations are as in Table 2.

Source	DF	SS	MS	F	P
Main factors					
Feed Type (FT)	2	0.84	0.42	496.67	0.0001
Body Size (BS)	2	0.52	0.26	308.29	0.0001
Stocking Density (SD)	2	0.39	0.19	223.51	0.0001
Interactions					
FT x BS	4	0.35	0.09	104.24	0.0001
FT x SD	4	0.18	0.04	51.71	0.0001
BS x SD	4	0.14	0.03	41.06	0.0001
FT x SD x BS	8	0.08	0.01	12.59	0.0001
Error	54	0.05	0.0008		
Total	80	2.54			

Table 5. Summary of the 3-way ANOVA showing the effects of feed type, body size and stocking density on the metabolic rate (MR) of *Pomacea*. Abbreviations are as in Table 2.

Source	DF	SS	MS	F	P
Main factors					
Feed Type (FT)	2	10.13	5.06	116.39	0.0001
Body Size (BS)	2	10.30	5.15	118.34	0.0001
Stocking Density (SD)	2	7.89	3.95	90.75	0.0001
Interactions					
FT x BS	4	7.89	0.94	21.53	0.0001
FT x SD	4	3.75	0.29	6.63	0.0001
BS x SD	4	1.15	0.74	16.96	0.0001
FT x SD x BS	8	2.95	0.14	3.31	0.0001
Error	54	1.15	0.04		
Total	80	2.95			

Table 6. Summary of the 3-way ANOVA showing the effects of feed type, body size and stocking density on the absorption efficiency (AE) of *Pomacea*. Abbreviations are as in Table 2.

Source	DF	SS	MS	F	P
Main factors					
Feed type (FT)	*2*	*10008.68*	*5004.3*	*136.14*	*0.0001*
Body size (BS)	2	1214.39	607.19	16.52	0.0001
Stocking density (SD)	2	2606.40	1303.2	35.45	0.0001
Interactions					
FT x BS	4	536.93	134.23	3.65	0.0011
FT x SD	4	941.04	235.26	6.40	0.0001
BS x SD	4	175.03	43.76	1.19	0.326
FT x SD x BS	8	456.80	57.10	1.55	0.161
Error	54	1985.03	36.76		
Total	80	17924.23			

Table 7. Summary of the 3-way ANOVA showing the effects of feed type, body size and stocking density on the conversion efficiency (CE) of *Pomacea*. Abbreviations are as in Table 2.

Source	DF	SS	MS	F	P
Main factors					
Feed type (FT)	2	1355.22	677.61	34.38	0.0001
Body size (BS)	2	384.02	192.01	9.74	0.0001
Stocking density (SD)	2	105.14	52.57	2.67	0.079
Interactions					
FT x BS	4	276.29	69.07	3.50	0.013
FT x SD	4	30.66	7.67	0.39	0.816
BS x SD	4	42.19	10.55	0.54	0.710
FT x SD x BS	8	13.33	2.04	0.10	0.999
Error	54	1064.36	19.71		
Total	80	3274.22			

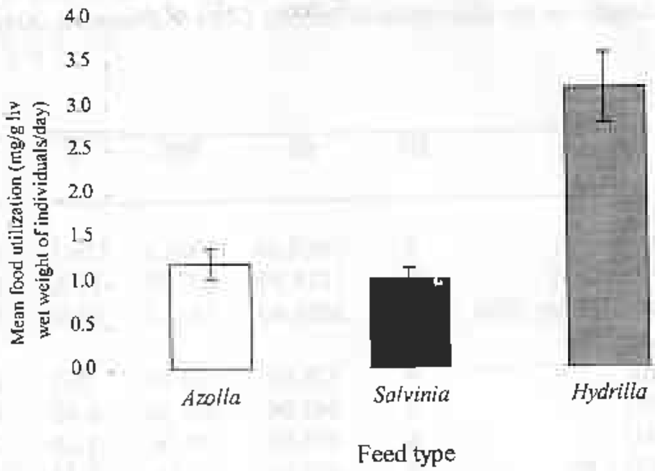

Fig. 1. Food preference (feeding rate) of *Pomacea* (n = 90) fed with *Azolla, Salvinia* and *Hydrilla*. Columns denote mean food utilization and vertical bars denote standard error (n=90).

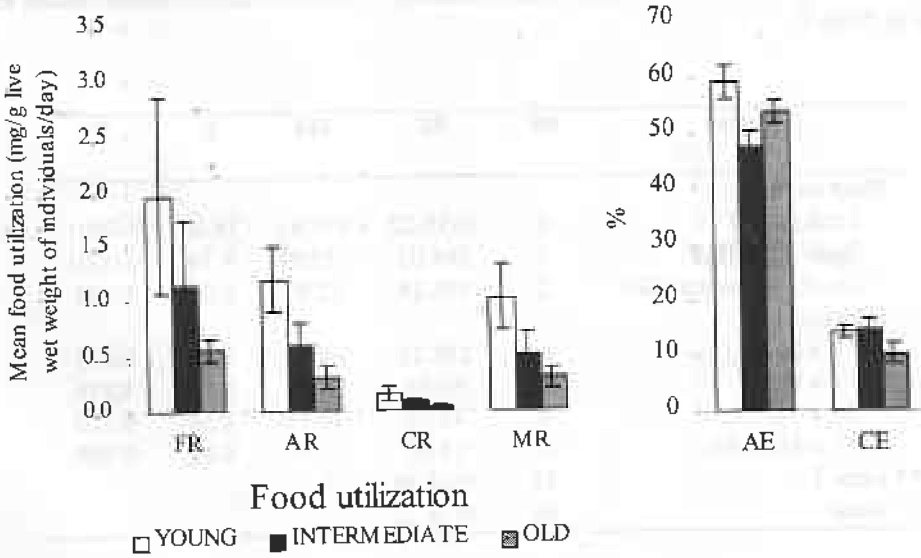

Fig. 2. Effect of size class on food utilization of *Pomacea* fed with *Azolla* for 7 days. FR=feeding rate, AR= absorption rate, CR= conversion rate, MR= metabolic rate, AE= absorption efficiency and CE= conversion efficiency. Columns denote mean food utilization and vertical bars denote standard error (n=9).

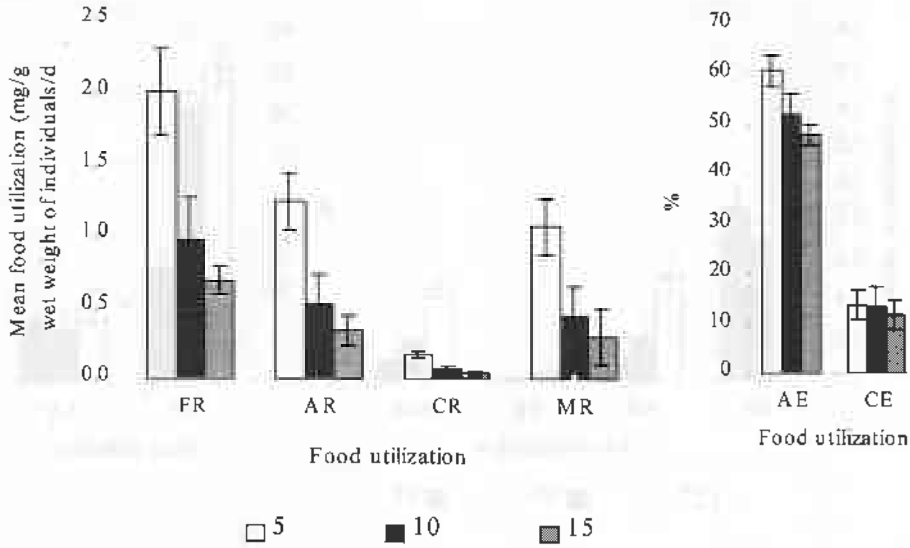

Fig. 3. Effect of stocking densities (5, 10 and 15 individuals per aquarium) on food utilization of *Pomacea* fed with *Azolla* for 7 days. FR=feeding rate, AR= absorption rate, CR= conversion rate, MR= metabolic rate, AE= absorption efficiency and CE= conversion efficiency. Columns denote mean food utilization and vertical bars denote standard error (n=9).

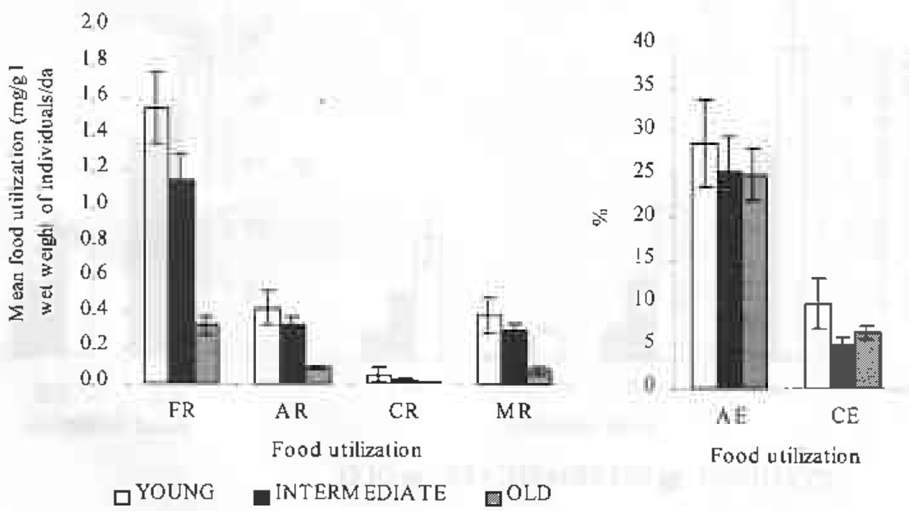

Fig. 4. Effect of size class on food utilization of *Pomacea* fed with *Salvinia* for 7 days. FR=feeding rate, AR= absorption rate, CR= conversion rate, MR= metabolic rate, AE= absorption efficiency and CE= conversion efficiency. Columns denote mean food utilization and vertical bars denote standard error (n=9).

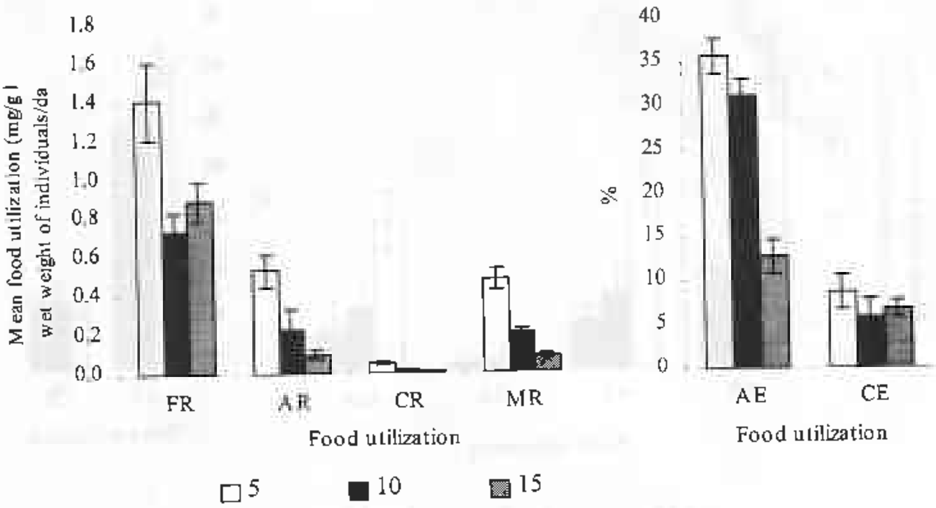

Fig. 5. Effect of stocking densities (5, 10 and 15 individuals per aquarium) on food utilization of *Pomacea* fed with *Salvinia* for 7 days. FR=feeding rate, AR= absorption rate, CR= conversion rate, MR= metabolic rate, AE= absorption efficiency and CE= conversion efficiency. Columns denote mean food utilization and vertical bars denote standard error (n=9).

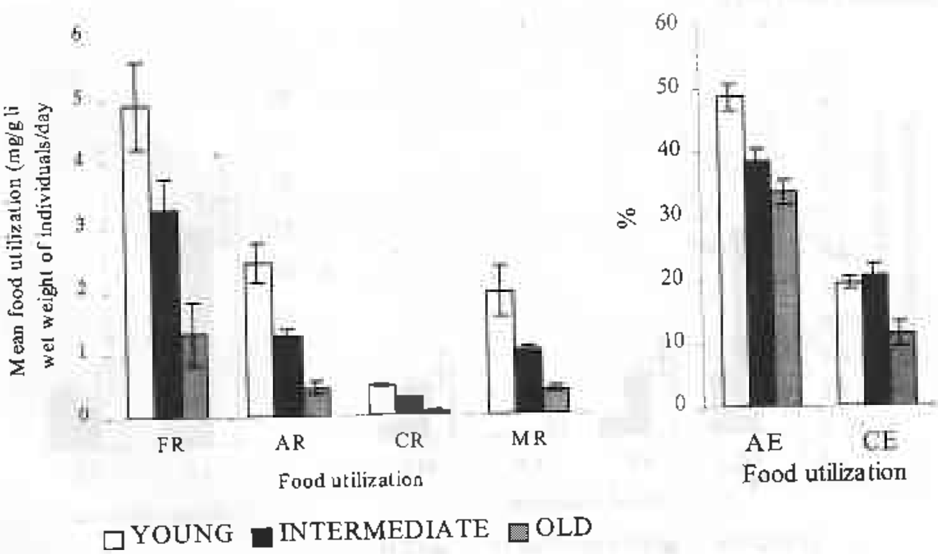

Fig. 6. Effect of size class on food utilization of *Pomacea* fed with *Hydrilla* for 7 days. FR=feeding rate, AR= absorption rate, CR= conversion rate, MR= metabolic rate, AE= absorption efficiency and CE= conversion efficiency. Columns denote mean food utilization and vertical bars denote standard error (n=9).

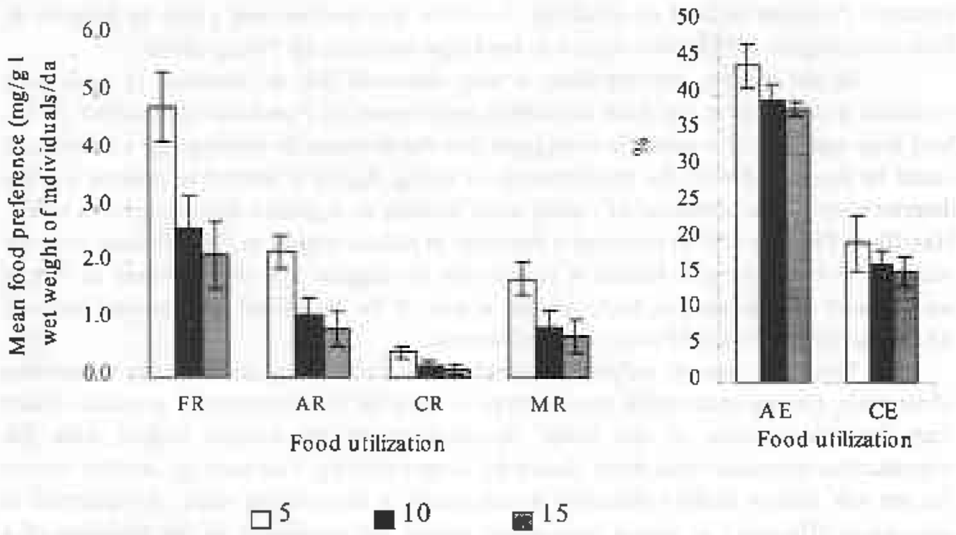

Fig. 7. Effect of stocking densities (5, 10 and 15 individuals per aquarium) on food utilization of *Pomacea* fed with *Hydrilla* for 7 days. FR=feeding rate, AR= absorption rate, CR= conversion rate, MR= metabolic rate, AE= absorption efficiency and CE= conversion efficiency. Columns denote mean food utilization and vertical bars denote standard error (n=9).

Discussion

Pomacea is already well known to be a voracious feeder on aquatic plants in other countries (Purchon 1968; Guerrero 1991) and, has been manipulated under laboratory conditions to obtain information on its food utilization patterns in relation to crowding, body size and feed types. In the present study, three species of aquatic plants commonly found in ponds and streams in Sri Lanka were tested as different feed types of *Pomacea* under laboratory conditions with a view to investigate food preference and energy budget of the snails. Feeding rate of *Pomacea* was selected to compare the food preference because it is a sensitive parameter for assessing the energy budget (Haniffa 1982).

In the present experiment, *Pomacea* showed the highest preference for *Hydrilla* irrespective to its density and body size while the lowest preference was shown for *Salvinia*. These differences could be attributed to the morphology and composition of the plant types tested and, to the condition of radula of *Pomacea*.

Hydrilla is a rooted macrophyte and has a simple stem to provide a good support for the snails to hang on while feeding. Nevertheless, both *Azolla* and *Salvinia* occur on the water surface and do not produce this facility. As such, the snails have to spend more energy to come to the water surface for feeding than in feeding in the water column. This may possibly be the reason for preference for *Hydrilla*.

The composition of *Hydrilla*, especially with reference to the content of ash, water, protein etc. may differ from those of other two feed types and this may have attracted *Pomacea* to feed on *Hydrilla*. A similar explanation was given by Haniffa & Sethuramalingam (1985) with regard to feed type selection by *Pila globosa*.

In the present investigation, it was observed that an increase in body size produces a decrease in the food utilization parameters of *Pomacea* irrespective of the feed type supplied. It is possible to suggest that the decrease in feeding rate of *Pomacea* could be associated with the phenomenon of aging. Aging is known to operate via the deterioration in the condition of radula teeth leading to digestive disturbances in snails. Haniffa & Pandian (1974) reported a decrease in radula weight in *Pila globosa* with the increase in body weight. Hence it is possible to suggest that the decrease in radula weight with the increase in body weight is one of the important mechanisms through which the feeding rate of *Pomacea* is influenced.

Similarly, growth progresses slowly or completely ceases with the maturation of animals. During maturation more energy is used for development of gonadal tissues than for maintenance of the body. A variation of the energy budget with the reproductive demands have been shown by Jaeger (1974). This may be another reason for the low energy budget observed in old snails in the present study. An increase in absorption efficiency in young individuals reflect the possibility of the presence of a spectrum of digestive enzymes, which are more active than in older individuals.

Experimental evidence of the present investigation showed that an increase in the number of snails per aquarium resulted in a decrease in food utilization parameters. Growth experiments carried out with other snails such as *Cypraea* sp. and *Pila globosa* have shown that increase in density has depressed growth (Cameron & Carter 1979 ; Haniffa 1980a). Such density effects as shown in this study are well known from studies conducted on natural populations of both land snails and freshwater snails (Eisenberg 1966) and as well as on snails under laboratory conditions (Thomas *et al.* 1974). Increase in crowding is usually related to decrease in growth or activity, but it has been shown that in the case of the freshwater snail *Biomphalaria glabrata*, there is an optimum density or level of conditioning above and below which the growth rate declines (Thomas & Benjamin 1974; Thomas *et al.* 1974).

The inverse relationship between stocking density and food utilization parameters could be related to the oxygen consumption and reproduction of the snails. When the aquaria become crowded the dissolved oxygen becomes a limiting factor hence snails have to come to the water surface to acquire oxygen. The intra-specific competition for oxygen between the individuals in highly crowded aquaria may ultimately result in taking lesser food, and much energy will be spent on acquiring oxygen (Chen & Prowse 1964). This may result in low values for food utilization under high stocking densities.

Similarly, more chances to spend time in copulation at high densities result in a decrease in food utilization in adult *Pomacea*. Previous work (Cameron & Carter 1979) has also shown that a decrease in fecundity occurs as a result of crowding.

Chemical substances and some physical factors play a significant role in the feeding rate of *Pomacea*. For example, growth inhibiting pheromones in snail mucous and many chemicals conditioning the water may result in lowering the feeding rates of adult individuals (Thomas *et al.* 1974; Aram 1975). Similarly, the feeding rate may also be lowered due to the accumulation of metabolites between successive water changes.

During the experiment, the snails were fed daily with plant food of 10% of their body weight. However, it was seen that this daily food ration was not completly eaten by the snails suggesting that the preference for the food types tested in the present experiment is not maximal. Given opportunities the snails may go for other feed types. One such food type is the rice plant. When the feeding ecology and feeding behaviour of *Pomacea* are considered, rice plants in aquatic habitats appear to be very good candidates for its feeding, thus *Pomacea* could become a potential pest of rice in agricultural lands all over the country.

The only redeeming feature of the spread of *Pomacea* in Sri Lanka so far is that its spread is still restricted to in and around major cities and towns and has not spread to the farming areas and rice growing areas in the country. However, this situation may not remain the same in the near future. The irrigation canals in the farming areas support a luxuriant growth of the weeds tested in the present experiment and this may sustain populations of *Pomacea* in such areas. When the suitable conditions arise, *Pomacea* could at any time, invade the rice cultivation. Direct observations in the field have shown that already there is a growing threat of *Pomacea* becoming a pest on the rice plant in areas surrounding the Bellanwila-Attidiya sanctuary, Boralasgamuwa, Kotte and Rajagiriya, the areas close to Colombo.

Although the present study was carried out under laboratory conditions, it provides some valuable information on the food utilization patterns of *Pomacea*. It also highlights the potential of *Pomacea* to affect commercially important crops such as rice. It is therefore necessary to carry out studies on the food preferences of *Pomacea* under natural conditions in Sri Lanka to investigate their potential to become pests of commercially important crops.

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