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Characteristics of Feeding Preference and Nutrients Utilization of Golden Apple Snail (*Pomacea canaliculata*) on Macrophytes in Paddy Fields

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Abstract: Golden apple snail was a harmful invasive gastropod in Asian wetlands. In order to clarify the effect of *Pomacea canaliculata* on macrophytes in paddy fields, feeding preference and nutrients utilization of snail were studied. Feeding preference of snail was *Alternanthera philoxenoides*>*Monochoria vaginalis*>*Oryza sativa* L. Snail showed a higher approximate digestion coefficient on *Oryza sativa* L. than that on *Monochoria vaginalis*. Nitrogen utilization coefficient of snail on *Alternanthera philoxenoides* was significantly higher than that on *Oryza sativa* L. Snail exhibited a higher calcium utilization coefficient on *Alternanthera philoxenoides* and *Monochoria vaginalis* than that on *Oryza sativa* L. *Oryza sativa* L. was not preferred among three plants under the same available and exposure condition.

Keywords: Herbivorous, macrophyte, *Pomacea canaliculata*, utilization coefficient

INTRODUCTION

Wetlands have been regarded as an important component in aquatic ecosystem on earth. In wetland ecosystem, macrophyte plays a vital role, which is helpful to keep the water body in the clear state by uptaking the nutrients, stabilizing the sediment in roots growth and also controlling the phytoplankton development (Newman *et al*., 1996; Fang *et al*., 2010). Meanwhile, they could provide an appropriate micro-habitat for the diverse aquatic organisms to settle and reproduce to some degree (Tanner *et al*., 2004).

In a natural wetland ecosystem, the biomass of macrophyte is generally influenced by the balanced result controlled by the plants growth and herbivore behaviors of grazers (Jones and Sayer, 2003; Li *et al*., 2009). However, such balance in the wetlands is easily interrupted by the entrance of some non-indigenous species such as fish (*Cyprinus carpio*, *Oreochromis mossambicus*), crayfish (*Orconectes rusticus*, *Orconectes neglectus*) in some areas (Dudgeon *et al*., 2006; Matsuzaki *et al*., 2009). Compared to the indigenous grazers, wetland invaders are often voracious in feeding a large variety of macrophytes. In about 1980, a kind of mollusc called golden apple snail (*Pomacea canaliculata*) native from South America was introduced into Asian countries for commercial purposes. However, these snails escaped away from the aquaculture environment and invaded into the various natural and semi-artificial wetlands such as small rivers, lakes and paddy fields in China for lacking of local efficient predators (Estebenet and Martín, 2002; Carlsson and Lacoursière, 2005).

Recent studies reported that *Pomacea canaliculata* exhibited complex appetites and flexibility dietary on macrophytes in natural wetlands (Burlakova *et al*., 2009; Qiu and Kwong, 2009). In paddy field *Pomacea canaliculata* tended to ravage seedlings and resulted in the mass loss of rice production (Sanico *et al*., 2002). At the same time, many paddy weeds have also suffered from the greedy feeding behaviors of *Pomacea canaliculata*. Even some researchers tried to apply such harmful snails in paddy fields as weeds control tools (Okuma *et al*., 1994). However, it was a pity that no appetite comparison experiments between rice (*Oryza sativa* L.) and other macrophytes from paddy fields were performed up to date. It was also unclear that the relationship between the appetites of *Pomacea canaliculata* and the chemical properties of above plants. This study aimed to compare the herbivorous preference of *Pomacea canaliculata* on rice (*Oryza sativa* L.) and other two macrophytes from paddy fields and also to analyze the element composition and utilization coefficient. The study results would be helpful to clarify the effect of *Pomacea canaliculata* on macrophytes in paddy fields.

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MATERIALS AND METHODS

Tested *Pomacea canaliculata* and plant materials. Experiment was performed at labs located at South China Agricultural University from May to September, 2011. *Pomacea canaliculata* collected from paddy fields in the Research and Teaching Farms of South China Agricultural University (23°14′N, 113°38′E) were kept in lab to obtain eggs. After snails hatched and grew up to about 10 mm shell height, they were transferred into an aquarium (50 L) equipped with filtration pump, air pump and automatic thermostat (25±1°C) and fed with Chinese cabbage. Before starting the experiment, snails of 25±1 mm height were selected and put into a basin with the quantity ratio of male and female snails set to 1:1 (Seuffert *et al*., 2010). Only the active snails which climbed everywhere or attached to the wall of aquarium were used. The snails were starved in purified water for 24 h and then dried on the filter paper for 2 h. Rice species used was *Oryza sativa* L. (Huanghuazhan, *O. sativa* L.) in seedling stage (28 days). *Alternanthera philoxenoides* (*A. philoxenoides*) and *Monochoria vaginalis* (*M. vaginalis*) in vegetative stage collected from the paddy fields in farms of South China Agricultural University were cultivated in lab for 2 months. After being rinsed in purified water for 24 h and removed the surface water, leaves of three plants were weighed and used in herbivorous test and Dry Matter Content (DMC) determination (Elger and Willby, 2003).

Determination of feeding and excretion of *Pomacea canaliculata*: The test was performed in a device which could separate the excretions of snails and the plant residues in the process (Fig. 1).

The device was composed of a beaker, an iron mesh cage and a plastic cup. The four tiny holes on the edge were used to pull the plastic cup out from the beaker. Firstly, the plastic cup was placed into the beaker. Secondly, a snail and fresh leaves were simultaneously placed into the cage and then the cage was hooped on the beaker. At last, a 1 L of purified water was added into the beaker. Each plant was repeated in quadruplicate. The plastic cup was pulled out carefully after taking out the iron mesh cage. The excretions of snails were collected daily in 6 days to obtain the dry weight. Meanwhile, the plant residues were weighed in 5 days after absorbing the water on the surface. From 1 to 4 days, the plant residues were placed again into the cage after weighing. At 5 day, the plant residues were not put back in order to obtain the amount of excretion remained in the body of snails. Nutrients including C, N and Ca in plants and excretions were determined by conventional method (Bao, 2000).

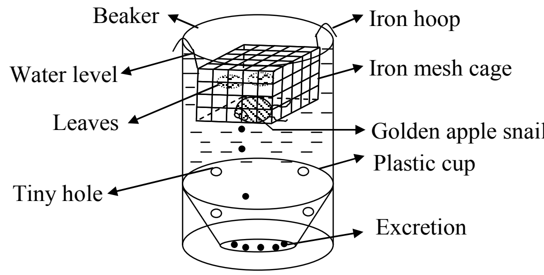

Fig. 1: Schema of the device of feeding and excrement collection from *Pomacea canaliculata*

Calculation of herbivorous amount and excretion of *Pomacea canaliculata*: In this experiment, the initial fresh weight was recorded as P_0 (1.00±0.10 g). The weight of plant residues in 5 days was recorded as P_t (t = 1-5 days). The weight of excrement was recorded as E_t (t = 1-6 days). The dry matter ratio of plants was calculated according to DMC and recorded as R. The nutrient content of nitrogen, carbon and calcium of plant and excrement were represented as N_1 and N_2, respectively. As a result, the accumulative herbivorous amount (H_t, g) was calculated as $(P_0\text{-}P_t)*R$; the accumulative excrement amount (E_t, g) was calculated as $\sum_{i=1}^{t} Et$ (t = 1-6 days); The approximate digestion coefficient was calculated as $1\text{-}E_6/(H_5*R)$; The nutrient utilization coefficient was represented as $1\text{-}(E_6*N_2)$ / (H_5*R*N_1) (Qin, 1987; Han, 1997).

Statistical analyses: Data was analyzed using the Excel and SPSS 13.0 software. ANOVA was applied directly to analyze the difference among different macrophytes treatments.

RESULTS AND DISCUSSION

The accumulative feeding amount of *Pomacea canaliculata*: The accumulative feeding amounts of *Pomacea canaliculata* on *A. philoxeroides*, *M. vaginalis* and rice were observed in this test (Fig. 2). It was obvious that the feeding amount of snails on three macrrophytes were different significantly. The accumulative feeding amounts of snails on *A. philoxeroides* were significant higher than those of snails on *M. vaginalis* and *O. sativa* L. ($p<0.01$). From 1 to 3 days, the difference of feeding amounts of snails on between *M. vaginalis* and *O. sativa* L. was not significant. At 4 and 5 days, the accumulative feeding amounts of snails on *M. vaginalis* were significantly higher than those on *O. sativa* L. ($p<0.01$). As a result, the feeding amount of snails on three macrophytes was *A. philoxeroides*>*M. vaginalis*>*O. sativa* L. However, it was reported that *A. philoxeroides* was unpalatable for *Pomacea canaliculata*, which was possibly related with some unidentified chemical substances

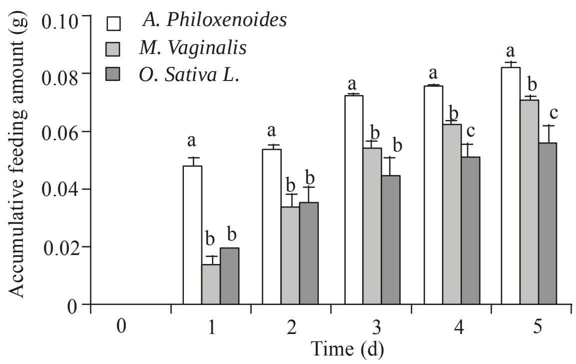

Fig. 2: The accumulative feeding amount of *Pomacea canaliculata* on *Alternanthera philoxenoides*, *Monochoria vaginalis* and *Oryza sativa* L.

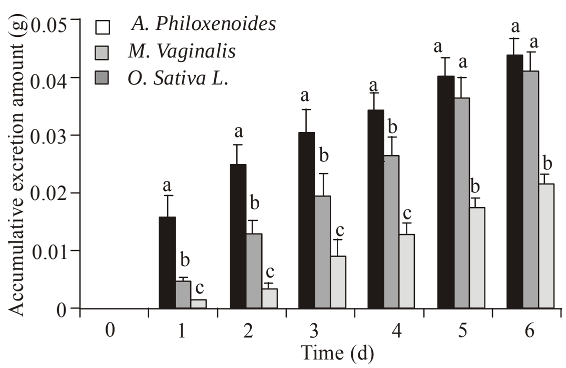

Fig. 3: The accumulative excretion amount of *Pomacea canaliculata* feeding on *Alternanthera philoxenoides*, *Monochoria vaginalis* and *Oryza sativa* L.

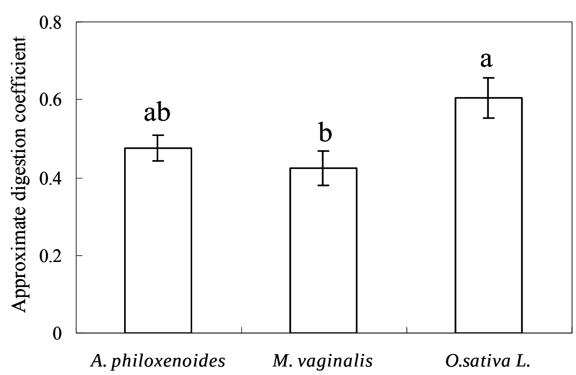

Fig. 4: The digestion coefficient of *Pomacea canaliculata* feeding on the *Alternanthera philoxenoides*, *Monochoria vaginalis* and *Oryza sativa* L.

contained in leaves (Wong *et al*., 2010). So, our experimental results indicated that *M. vaginalis* and *O. sativa* L. would be more unpalatable to *Pomacea canaliculata* than *A. philoxeroides* did. And this experiment showed that although *Pomacea canaliculata* always feed on the rice seedlings in paddy fields, rice plant was not a preferred food actually among the three tested plant materials under the same available and exposure condition, The reason may be that rice seedlings are usually fresh, tender and more massive and exposed than other weeds in paddy field, so they would be easy to be found and eaten by *Pomacea canaliculata*.

The accumulative amount of excretion of *Pomacea canaliculata*: With the feeding amount increased, the accumulative excretion of *Pomacea canaliculata* improved accordingly in the experiment (Fig. 3).

From 1 to 4 days, the accumulative excretion of snails feeding on *A. philoxeroides* was significantly higher than those feeding on *M. vaginalis* and *O. sativa* L. ($p<0.01$). Meanwhile, the accumulative excretion of snails feeding on *M. vaginalis* was also higher than that on *O. sativa* L. ($p<0.01$). At 5 and 6 days, the difference of accumulative excretion of snails feeding on between *A. philoxeroides* and *M. vaginalis* was not significant. The accumulative excretion of snails feeding on *O. sativa* L. was the lowest. All in all, the more macrophytes were fed by *Pomacea canaliculata*, the more excretion was produced. This result implied that the food storing capacity of *Pomacea canaliculata* was not very large and the food stayed in its stomach only for a short time. As a result, *Pomacea canaliculata* always excreted as they fed on plants. It was known that *Pomacea canaliculata* had imposed strong negative effect on many macrophytes (Carlsson and Lacoursière, 2005), this model of growth of *Pomacea canaliculata* may provide a good understandings for its harmfulness.

Approximate digestion coefficient of *Pomacea canaliculata*: The approximate digestion coefficients of *Pomacea canaliculata* on three macrophytes were shown in Fig. 4. *Pomacea canaliculata* showed a higher digestion coefficient on *O. sativa* L. than that on *M. vaginalis*. Meanwhile, the digestion coefficient of snails on *O. sativa* L. was similar to that on *A. philoxeroides*. It should be noted that the order of approximate digestion coefficient was not consistent with that of the feeding amount. The approximate digestion coefficients were influenced by feeding amount and excretion amount simultaneously. It was actually a comprehensive result from less feeding amount on *O. sativa* L. and less excretion of *Pomacea canaliculata*. As a result, this behavior of snail made the digestibility of *O. sativa* L. stayed in stomach of *Pomacea canaliculata* was higher than that of other two plant species. Furthermore, *Pomacea canaliculata* contained many enzymes related to decompose organic substances in its tissues (Hirata *et al*., 1996; Imjongjirak *et al*., 2008), it was also possible that *Pomacea*

Table 1: Traits of *Alternanthera philoxenoides*, *Monochoria vaginalis* and *Oryza sativa* L.

Plant species	DMC (g)	N (mg/g)	C (mg/g)	C:N (mg/g)	Ca (mg/g)
A. philoxenoides	0.08±0.01b	41.62±0.27a	418.03±4.21b	10.04±0.08c	66.62±0.11a
M. vaginalis	0.09±0.01ab	20.57±0.24b	383.37±3.46c	18.66±0.05b	50.86±0.14b
O. sativa L.	0.11±0.02a	18.33±0.09c	439.03±1.29a	24.21±0.07a	28.73±0.16c

Data were expressed as mean values±standard errors; The significant level was at 0.05

canaliculata improved the digestion ability of stomach as the *O. sativa* L. was unpalatable. *Pomacea canaliculata* attacked plenty of seedlings in paddy fields and high digestion coefficient on *O. sativa* L. was helpful to its growth partly.

DMC, nitrogen content, C:N and calcium of tested plants: In fact, it was reported that the feeding amount of gold apple snails was closely related to many traits of macrophytes such as DMC, nitrogen content and C:N (Wong *et al*., 2010). In this study, these traits of *A. philoxeroides*, *M. vaginalis* and *O. sativa* L. were also analyzed (Table 1). Results showed that the nitrogen and calcium content of three macrophytes were *A. philoxeroides*>*M. vaginalis*>*O. sativa* L. ($p<0.05$); the carbon content of three macrophytes were *O. sativa* L.>*A. philoxeroides*>*M. vaginalis* ($p<0.05$); the C:N values of three macrophytes were in the order of *O. sativa* L.>*M. vaginalis*>*A. philoxeroides* ($p<0.05$).

As for DMC, the DMC of *A. philoxeroides* was significantly lower than that of *O. sativa* L. ($p<0.05$). In previous studies, DMC and C:N of macrophytes were found to be negatively related with the feeding amount of *Pomacea canaliculata*, but the nitrogen content had positive effect on feeding of *Pomacea canaliculata* (Wong *et al*., 2010). In this study, similar phenomenon was also observed. Feeding amounts of *Pomacea canaliculata* on *A. philoxenoides, M. vaginalis* were higher than those on *O. sativa* L. , which was contrary to the DMC, C:N ratio and consistent with the nitrogen content of three macrophytes. The relationship indicated that the nitrogen, DMC and C:N ratio of macrophytes were generally important for the herbivorous amount of snails. Interestingly, it was also discovered that the calcium content was significantly different in three macrophytes in this study ($p<0.05$). Calcium was an important element for snail growth and low calcium restricted it's disperse in some natural water bodies (Okland, 1992). The order of calcium content in three macrophytes was consistent with the feeding amount of *Pomacea canaliculata*. It was concluded that calcium content of macrophytes would be a nether possible reason that regulated the feeding behaviors of *Pomacea canaliculata*. The feeding amount of *Pomacea canaliculata* on *O. sativa* L. was the lowest at the end of experiment. However, carbon element content of *O. sativa* L. was the highest among three macrophytes ($p<0.05$), which indicated that carbon element of plants maybe imposed negative effect on the feeding behavior of *Pomacea canaliculata.*

Carbon, nitrogen and calcium utilization coefficient of *Pomacea canaliculata*: The nitrogen, carbon and calcium utilization coefficients of *Pomacea canaliculata* feeding on *A. philoxenoides*, *M. vaginalis* and *O. sativa* L. were shown in Fig. 5. The nitrogen utilization coefficient of snails on *A. philoxenoides* was significantly higher than that on *M. vaginalis and O. sativa* L. Meanwhile, calcium utilization coefficient of snails on *A. philoxenoides* and *M. vaginalis* were significantly higher than that of snails on *O. sativa* L. According to Table 1, content of nitrogen and calcium elements were found to be highest in *A. philoxenoides*.

In this study, high content of element resulted in the high utilization coefficient because it was more likely to be absorbed by *Pomacea canaliculata* in the digestion process. As for the carbon element, its utilization coefficient of snails on *O. sativa* L. was significantly lower than those on *A. philoxenoides* and *M. vaginalis*. Although the approximate coefficient of snails on *O. sativa* L. and carbon content in *O. sativa* L. was both very high in three macrophytes, it did not result in a high carbon utilization coefficient. This phenomenon indicated that a portion of carbon element was excreted in the form of excretion by *Pomacea canaliculata*. Furthermore, calcium utilizations of *Pomacea canaliculata* on three macrophytes were all significantly higher than carbon and nitrogen utilization ($p<0.05$).

Calcium was very important in forming the shells of snails in its growth (Kwong *et al*., 2008), it was easy to understand that high calcium utilization of *Pomacea canaliculata* on three macrophytes. However, considering the feeding amount of snail, it implied that a strong calcium demand existed in the feeding behavior of *Pomacea canaliculata*. As a result, *Pomacea canaliculata* maybe tends to choose the macrophytes rich in calcium element as its favorite food. As for three elements tested, low nutrient utilization coefficient of *Pomacea canaliculata* on *O. sativa* L. was observed. Although snails always attack rice seedlings in paddy fields, they in fact are in the status of low nutrients absorption and utilization. Hence they need to eat more rice seedlings to meet demands of their growth and activities for nutrients and energy. This is why snails can often result in a great loss to rice production. So trying to increase the

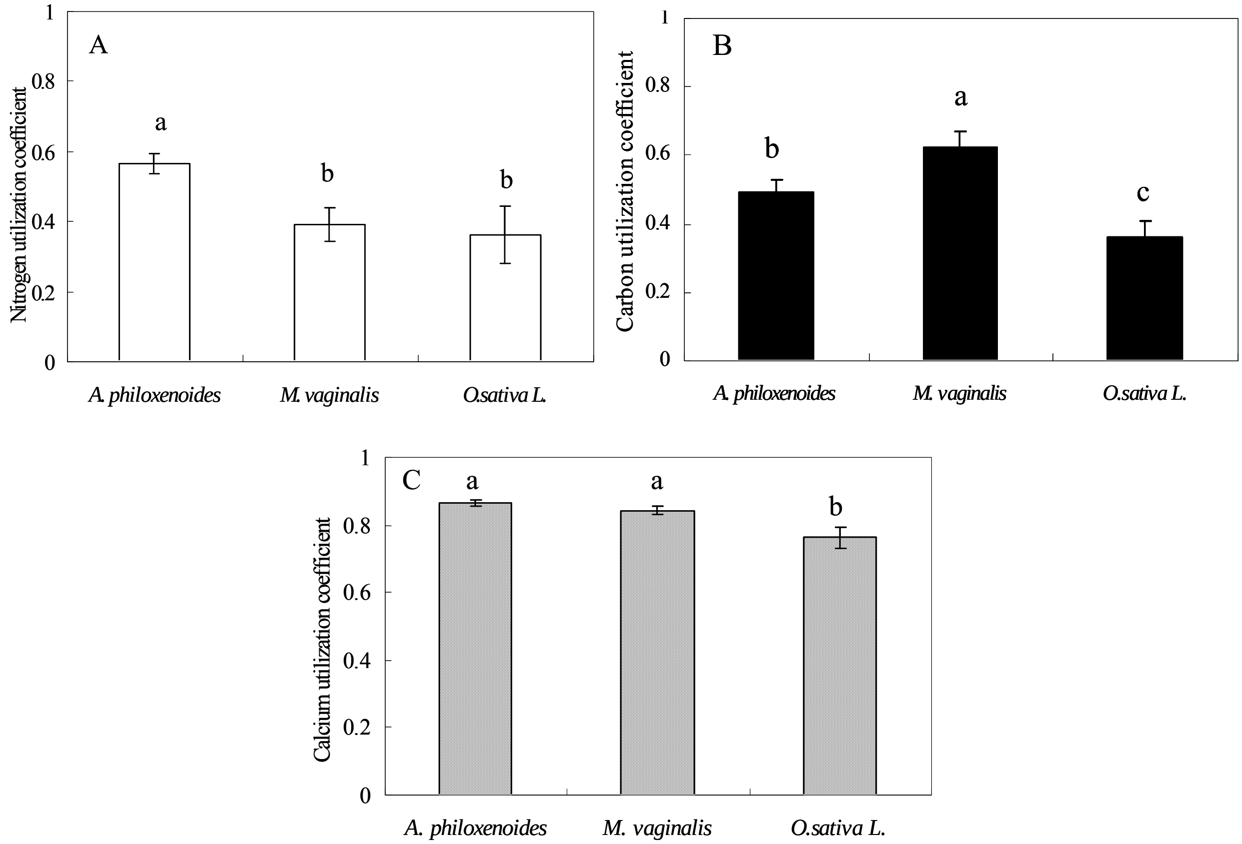

Fig. 5: Nitrogen (A), carbon (B) and calcium (C) utilization coefficients of *Pomacea canaliculata* on *Alternanthera philoxenoides*, *Monochoria vaginalis* and *Oryza sativa* L
The significance comparison was performed among different macrophytes at level of 0.05

macrophytes biodiversity was possibly another way to reduce the damage of *Pomacea canaliculata* on rice seedlings.

CONCLUSION

In this study, the feeding amount of *Pomacea canaliculata* on three macrophytes was *A. philoxeroides*>*M. vaginalis*>*O. sativa* L. The accumulative excretion of *Pomacea canaliculata* feeding on *O. sativa* L. was the lowest. *Pomacea canaliculata* showed a higher digestion coefficient on *O. sativa* L. than that on *M. vaginalis*. The calcium content was much more different in three macrophytes. Nitrogen utilization coefficient of *Pomacea canaliculata* on *A. philoxenoides* was significantly higher than that on *O. sativa* L. Calcium utilization coefficient of *Pomacea canaliculata* on *A. philoxenoides* and M. *vaginalis* was significantly higher than that on *O. sativa* L. Furthermore, calcium utilization of snails was very high in elements utilization tested. This study discovered that rice was not the favorite plant for *Pomacea canaliculata* among the three tested plant materials under the same available and exposure condition. Also, we confirmed that importance of DMC, nitrogen and C:N for snail grazing selection and discovered that calcium was another important factor in regulating feeding behavior of *Pomacea canaliculata*. This study was helpful to understand the negative influences of *Pomacea canaliculata* on macrophytes in paddy fields. To control the harmful effect of *Pomacea canaliculata* on rice seedlings, it was meaningful to increase the macrophytes biodiversity in paddy fields according to its feeding preferences.

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