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Biological control of golden apple snail, *Pomacea canaliculata* by Chinese soft-shelled turtle, *Pelodiscus sinensis* in the wild rice, *Zizania latifolia* field

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ABSTRACT: The wild rice, *Zizania latifolia* Turcz, used to be one of the important aquatic vegetables cultivated in China. Recently, the golden apple snail - GAS (*Pomacea canaliculata* (Lamarck)) was found to be a major invasive pest attacking *Z. latifolia*. To control efficiently GAS, predation by the Chinese soft-shelled turtles (*Pelodiscus sinensis*) on GAS was evaluated in laboratory and field trials. *P. sinensis* had a strong predatory capacity and selectivity for GAS both in laboratory and field conditions. All the sizes of *P. sinensis* prefer to capture smaller snails. The optimum number of *P. sinensis* released in *Z. latifolia* field was dependent on the density of over-wintered GAS, and varied between 30 and 50 turtles per 666.7 m^2. The number of GAS declined in the fields with turtles as compared to turtle-free field. A pattern of releasing *P. sinensis* in *Z. latifolia* fields was developed and widely adopted by farmers because of much more benefit besides biologically controlling GAS.
Keywords: invasive pest, predatory capacity, turtles, snails

Introduction

The golden apple snail (GAS), *Pomacea canaliculata* (Lamarck), native to South America, now is a major rice exotic invasive pest in Asia (Mochida, 1991; Halwart, 1994). The yield loss caused by GAS in rice was estimated varying from 5 % to 100 % depending on locality and the level of infestation in most Asian countries (Halwart, 1994; Naylor, 1996). In China, GAS was first introduced into Guangdong province in 1981and became a serious pest of rice since 1984 (Halwart, 1994). The first record for GAS infestation on rice and *Zizania latifolia* in Yuyao City of Zhejiang province was found in 2002, and became a serious pest in rice and *Z. latifolia* fields in 2004 (Pan et al., 2008). *Z. latifolia*, is one of the most important aquatic and economic vegetable crops cultivated in the Southeast China since ancient time (Guo, 2007). Nowadays, around 100 thousands hectares of *Z. latifolia* were planted in more than ten provinces in China (Chen, 1991; Zhai et al., 2001).

Numerous measures have been taken to control GAS, in *Z. latifolia* fields. The molluscicide was usually used to kill GAS with a serious environmental and human health consequence, and the hand-picking was proved to be a time-consuming work (Yu et al., 2001; Chen et al., 2003). Thus, local farmers did not adopt these methods extensively for GAS control. Recently, the biological control was applied to suppress the occurrence of GAS by releasing biological agents such as fishes and ducks in crop fields. However, due to the low efficiency of fishes and ducks, especially for controlling the adult GAS (Yoshie and Yusa, 2008), the Chinese soft-shelled turtle (*Pelodiscus sinensis*), was tentatively selected as a new biological agent to control GAS in *Z. latifolia* field (Zheng et al., 2005). *P. sinensis* is widely distributed in Eastern Asia and usually take up 4 ~ 6 years to reach sexual maturity adulthood. *P. sinensis* consumes insect larvae, small fish, small aquatic animals and seeds of marsh plants (Nuangsaeng and Boonyaratapalin, 2001). Furthermore, *P. sinensis* is of high commercial value and is commonly cultured in Malaysia, Indonesia and China for food consumption (Jia et al., 2005). The objective of this study is to quantify the role of *P. sinensis* in biologically controlling GAS in *Z. latifolia* field.

Materials and Methods

All tested GAS were collected semimonthly from *Z. latifolia* fields located in Yuyao City (E 121°10′, N 30°02′), Zhejiang province, China. The laboratory population of GAS was mass-reared in greenhouse continuously at 25 ± 2°C. New-hatched snails were maintained on carp food, and adult snails were reared on the wild rice shoots. The tested Chinese soft-shelled turtles were provided by the local fish farmers in Yuyao City. In this study, the wild rice variety Zheda Jiaobai, was planted in each spring and harvested twice in the fall and the following summer.

The prey–predator relationship between snails and turtle was studied in the aquarium with an arena of L 75 cm × W 60 cm × H 75 cm. The aquarium was filled with tap water to a depth of 30 cm, and one turtle was placed in each aquarium. The tested GAS was divided into four classes in this study according to their body weight: Class I, < 0.3 g; Class II, 0.3 ~ 1.5 g; Class III, 1.5 ~ 6.5 g; Class IV, 6.5 ~ 70 g. Class I to Class III correspond to young snails, and Class IV corresponds to adult snails. The tested turtles were also divided into three classes: juvenile (50 ~ 250 g), developing turtle (250 ~ 500 g) and developed turtles (500 ~ 750 g). To balance the encounter probability of turtle to snails with various sizes in the tested arena, the combination of different classes of snails was arranged in each treatment. In choice trials, a mixture of 60 Class I, 40 Class II, 20

Class III and 10 Class IV snails were placed in the same aquarium. However, in no-choice trials, 120 Class I, 60 Class II, 20 Class III or 10 Class IV snails were placed in each aquarium. In each aquarium, one turtle was released for the feeding test, and all treatments were replicated 4 times. After 5 days, all the snails were collected from the aquaria and the number of snails consumed by the turtle was calculated by subtracting the number of the remaining snails from the initial number placed in the aquarium. The snails were replenished to the initial quantity after recording. The procedure was repeated until four replicates were obtained.

Four randomized experimental plots (3 m wide and 3 m long) were set in *Z. latifolia*field, and 18 seedlings of *Z. latifolia* were planted in each plot. The plots were enclosed with one-meter-high color-coated steel-tile fence. Two individuals of *P. sinensis* with a weight of about 450 g were released in each plot. A mixture of 360 Class I, 240 Class II, 120 Class III and 60 Class IV snails were released in each plot in Jun. 2006. The number of GAS consumed by *P. sinensis* was counted by subtracting the remaining GAS from the initial number placed in the plots 15 days later.

Experimental trials were conducted in *Z. latifolia* field located in GAS heavy infested areas in Yuyao Cityin 2006. The wild rice variety 'Zheda Jiaobai' was planted in the density of 50 cm × 100 cm in field on Mar.2006. Two or four ditches of about 80 cm width and 50 cm depth along the field were dug to offer turtles a refuge area during farming and high temperature in summer. Besides, 6 ~ 8 mounds of soil per 667 m^2 were constructed for turtle rest in the middle of experimental fields, accounting for 5 ~ 10 % area of the field. The fields were enclosed with 1 m-high color-coated steel-tile fence to prevent the escape of turtles (Figure 1).

A field trial was conducted in four fields (field I, II, III and IV), and each field was about 1067 m^2. Fields I and II were two adjacent areas with a high density (about 1,100 Class IV overwintered snails and 2,000 egg masses) of GAS, and moderate densities (about 330 Class IV over-wintered snails and 600 egg masses) of GAS were recorded in both fields III and IV. *P. sinensis* was released into the Fields II and IV in June 4, 2006, and Field I and Field III were turtle-free as control. Fifty-five and sixty-five turtles were respectively released into Fields II and IV, according to the number of GAS in both fields, corresponding to 30 to 50 turtles per 667 m^2, respectively.

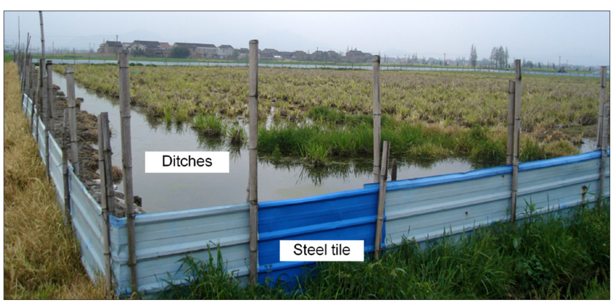

Figure 1 – The fences designed to enclose Chinese soft-shelled turtle *Pelodiscus sinensis* in the field with wild rice *Zizania latifolia*.

To estimate the actual density of GAS in *Z. latifolia* field, snails were sampled in five 15 m^2 quadrats in each filed. The number of adult and young snails was recorded every 15 days until the end of experiment. The number of egg masses on each plant was also counted every 15 days, and ten plants were selected randomly in each field.

All statistical analyses were done using the DPS© package (version 8.01 for windows) (Tang and Feng, 2007). To analyze the predatory capacity of different Class of *P. sinensis* on GAS in both choice and no-choice trials, data were subjected to one-way analyses of variance (ANOVA) with repeated measures and Tukey's multiple comparison methods. The number of GAS consumed by *P. sinensis* in aquarium and field was compared using two-way ANOVA. Data for snail egg masses in turtle-released fields and control fields at the same stage were compared by Student-*t* test. All tests were considered at $p \leq 0.05$.

Results

When the turtles were provided with only one Class of snails, the predatory capacity in a decreasing order was developed turtles > developing turtles > juvenile turtles (Table 1). However, there was no difference of predatory capacity on GAS between developing and developed turtles, except for that on Class IV snails.

When provided four Classes of GAS in the same arena of aquarium, the juvenile turtles preferred to eat young snails (Class I and Class II), and only 3.4 % Class III snails in total snails were attacked and Class IV was not consumed. For adult turtles, all classes of GAS can be consumed when provided with a mixture of four Classes of snails. The developing turtles had a higher predatory capacity of Class I and II snails than developed turtles, while the developed turtles showed a higher predatory capacity of Class III and IV snails, which occupied 11.8 % in total snails. For Class IV snails, there was no difference of predatory capacity between developing and developed turtles. Therefore, it was concluded that the turtle's predatory selectivity for GAS in a decreasing order was juvenile turtles > developing turtles > developed turtles, which was opposite to the order of predatory capacity. To balance the predatory capacity and selectivity of *P. sinensis* on GAS, the developing turtles were recommended for the field trials.

P. sinensis also preferred to prey young GAS in field trials. Almost all GAS in Class I and II were consumed by *P. sinensis* after 15 days, but the numbers of GAS in Class III and Class IV consumed by *P. sinensis* were10.89 ± 1.39 and 3.28 ± 0.57 per 5 days. It indicated more GAS in field trials were consumed by *P. sinensis* than those in the arena of aquaria (F = 4.8530, df = 1, p = 0.0311, for Class III; F = 3,423, df = 1, p = 0.002, for Class IV).

Table 1 – Golden apple snails (GAS) consumed by turtles *Pelodiscus sinensis* in no-choice and choice trials, under laboratory conditions.

Treatment		No. of GAS consumed by different classes of *P. sinensis*		
		Juvenile turtles	Developing turtles	Developed turtles
No-choice trial (one class of GAS in one arena)	I	47.6 ± 3.28 b	107.29 ± 9.25 a	115.5 ± 10.85 a
	II	45.3 ± 4.16 b	91.82 ± 8.55 a	114.35 ± 11.42 a
	III	7.5 ± 0.74 b	12.88 ± 1.01 a	16.65 ± 1.26 a
	IV	1.50 ± 0.50 c	3.24 ± 0.59 b	5.65 ± 1.18 a
Choice trial (four different classes of GAS in one arena)	I	26.12 ± 2.16 b	32.6 ± 2.87 a	28.6 ± 2.45 b
	II	30.2 ± 2.89 a	33.5 ± 3.21 a	26.8 ± 2.62 a
	III	1.98 ± 0.28 c	6.8 ± 0.28 b	9.34 ± 0.83 a
	IV	0	2.1 ± 0.21 a	2.84 ± 0.56 a

Note: Snails classes: I = 0 ~ 0.3 g; II = 0.3 ~ 1.5 g; III = 1.5~ 6.5 g; IV = 6.5 ~ 70 g. Turtle classes: Juvenile turtles = 50 ~ 250 g; Developing turtles = 250 ~ 500 g; Developed turtles=500 ~ 750 g. Different letters in the same row indicate difference among means (one-factor ANOVA and Tukey's multiple comparisons, $p \leq 0.05$).

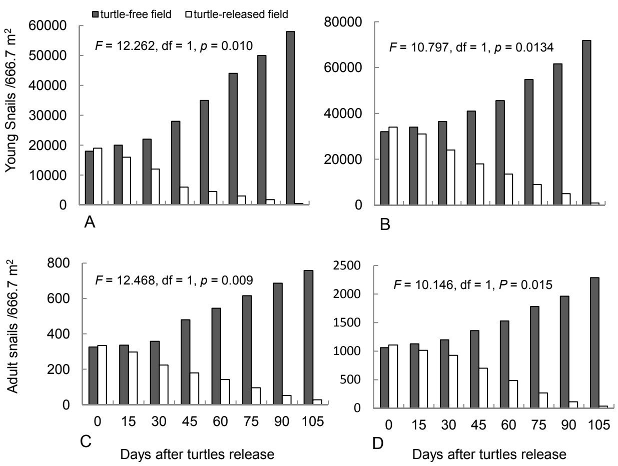

Figure 2 – Golden apple snails (GAS) consumed by Chinese soft-shelled turtle in moderately snail-infested fields (Figure 2A for young snails and Figure 2C for adult snails) and heavy snail-infested fields (Figure 2B for young snails and Figure 2D for adult snails). The turtles were released on 04 Jun. 2006. The number of snails in turtle-released field and turtle-free field were compared using two-way analyses of variance (ANOVA) without replications. The tests were considered at $p \leq 0.05$.

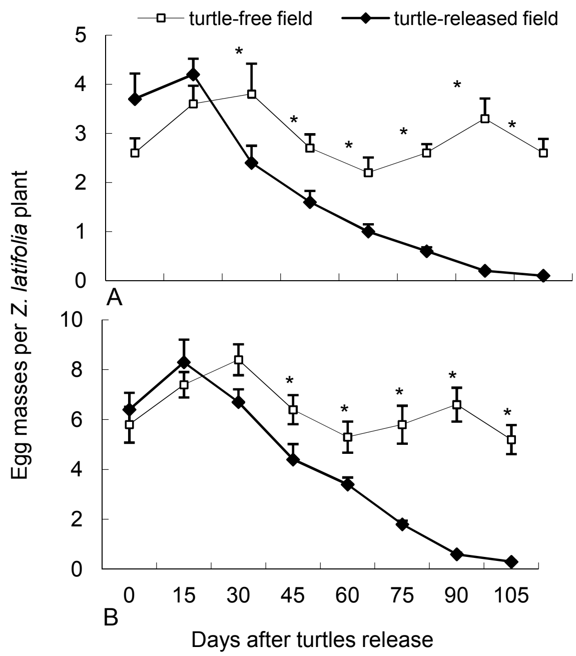

Figure 3 – Egg masses on each *Zizania latifolia* plant in moderately snail-infested fields (upper) and heavy snail-infested fields (below). The Chinese soft-shelled turtles *Pelodiscus sinensis* were released on 04 Jun. 2006. The data expressed as Means ± standard error (n = 10). At the same stage, the data with asterisks are different (*$p < 0.05$) by Student's *t*-test.

In the turtle-free field, the number of GAS increased rapidly, especially the young snails. In the turtle-released field, the number of both young and adult snails declined as compared to the turtle-free field (Figure 2). During first 15 days, after turtles were released, the number of both young and adult GAS hardly decreased, while the number of GAS in both tested snail-infested fields was effectively suppressed within 90 days after turtles were released. Furthermore, the number of egg masses laid by GAS was also declined 30 days day after turtles released as compared to turtle-free field (Figure 3). By the end of experiment, the new egg masses were hardly found in the turtle-released fields.

Discussion

The Chinese soft-shelled turtle, *P. sinensis*, has a strong predatory capacity for controlling GAS both under laboratory and field conditions. By the use of *P. sinensis*, a method for biologically controlling GAS was developed in *Z. latifolia* field. As far as we know, it is the first report to use *P. sinensis* for biological control of GAS in wild rice fields or paddy fields. During controlling GAS, a pattern of stocking *P. sinensis* in *Z. latifolia* field was developed and was adopted extensively by local farmers because they can benefit from marketing both *Z. latifolia* and *P. sinensis*. Meanwhile, the cost of a large amount of chemical molluscicides was also saved. By releasing turtles, the yield of *Z. latifolia* shoot increased up to 1500 ~ 2000 kg per 667 m^2 as compared to the turtle-free field without use of synthetic molluscicides, and the income per 667 m^2 increased to 800 ~ 1000 US Dollars. Furthermore, although there was no outbreak of GAS both in 2008 and 2009, the local farmers continue to release *P. sinensis* in *Z. latifolia* fields for both economic and biological control reasons.

There are many animals known to feed on GAS or their eggs, including fish, birds, rats, lizards, frogs, toads, beetles and ants (Cowie, 2002). The turtles, including *Trachemysscripta*, *Chinemysreevesii*, *Mauremys japonica*, were first reported to have the ability to consume GAS in aquaria by Yusa et al. (2006). In the present study, predatory capacity of turtle, *P. sinensis*, was also conformed both in laboratory and field conditions, including preference to younger snails and strong predatory ability. The feeding behavior of *P. sinensis* was different depending on the body weight of GAS. The adult *P. sinensis* can swallow the whole apple snails of Class I and Class II because the unbroken snails were found in the gut of *P. sinensis* and a few empty shells remained in aquarium, and this feeding behavior was much like red tilapia *Anabastestudineus* (Teo, 2006). However, *P. sinensis* attacked and only ate the softy part of adult snail in Class III and Class IV when the snails sprawled up, and the whole empty shell of GAS remained after turtle attacking in test arena. This feeding behavior was also found in common carp *Cyprinuscarpio* (Teo, 2006). It is concluded that *P. sinensis* is one of the most hopeful biological agents for GAS because they not only prey the young snails, but also attack the adult ones efficiently.

To control GAS, several effective predators have been introduced into agricultural ecosystem, including various ducks and fishes (Teo, 2001; 2006). Domestic ducks were proven effective for biological control of young GAS in paddy field and could prey equally well under low and high pest population densities (Cowie, 2002). Common carp, *Cyprinuscarpio* Linnaeus, was also recommended for biological control of GAS in paddy fields after comparing five species of fish (Cowie, 2002). The black carp, *Mylopharyngodonpiceus*, was an efficient biological control agent of pest snails that bury underground (Ben-Ami and Heller, 2001). However, both fish and duck are impractical for use in rice fields because carp require water deeper than 10 cm, which is too deep for a normal rice field, and keeping ducks in fields requires a lot of care, including feeding every day (Yoshie and Yusa, 2008). In this study, *P. sinensis* showed the strong control capacity on GAS in *Z. latifolia* fields because of their strong predation on GAS and tolerance to starvation. Being different from rice seedlings, *Z. latifolia* can provide more shelter for turtles with a high and tense plant canopy, and *P. sinensis* can grow much well in *Z. latifolia* fields. Furthermore, the technique of stocking *P. sinensis* in *Z. latifolia* field was very easily to be mastered by farmers, rearing turtles required no more extra care as compared to keeping ducks (Yoshie and Yusa, 2008). In conclusion, both laboratory trials and field survey results showed that using *P. sinensis* biologically to control GAS was recommended as an optimal method in wild rice fields infected by GAS.

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