Effects of predation on the exotic freshwater snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae) by the indigenous turtle *Chinemys reevesii* (Testudines: Geoemydidae)

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(Received 6 December 2007; Accepted 2 May 2008)

Abstract

We studied the predatory potential of the turtle *Chinemys reevesii* on the apple snail *Pomacea canaliculata* using two series of experiments. First, we investigated the relationship between turtle body size and the maximum size of snails consumed over a period of 3 days within 0.37 m² containers. The maximum snail size consumed was positively related with turtle size. Secondly, we investigated the predation of snails by turtles over a period of 8 weeks. We released 200 snails (10–30 mm shell height) and an adult turtle (155–183 mm carapace length) into each of two 0.82 m² plots with soil and rice plants. Subsequently, snail density was monitored every week and 200 snails were added to low density plots up to twice a week. Two control plots with the same initial density of snails but without turtles were also monitored. The density and survival rate of snails were lower in plots with a turtle than in control plots. We estimated that a single turtle consumed >2,000 snails in 8 weeks. In addition, the biomass of duckweed (given as food for snails) was greater in turtle plots than in control plots, suggesting that the presence of turtles had an indirect effect on weed.

Key words: Alien species; apple snail; biological control; Mollusca; natural enemy

INTRODUCTION

The apple snail, *Pomacea canaliculata* is a freshwater snail which originates from South America. It was introduced into many Asian countries, including Japan, for human consumption in the 1980s. Subsequently, wild populations were established and it is now a critical rice pest in these countries (Halwart, 1995; Naylor, 1996; Wada, 1997).

One reason why this snail has become widely established appears to be the scarcity of its natural enemies in the introduced areas. Currently, biodiversity in rice fields and adjacent areas is generally low, especially in Japan, due to the heavy use of pesticides (Hidaka, 1998) and concrete-lined fields and canals (Fujio and Lane, 1997; Lane and Fujio, 1998). A series of laboratory experiments have demonstrated that 26 out of 46 aquatic animal species living in Japan prey on *P. canaliculata* (Yusa et al., 2006). However, most of these inhabit rivers, ponds and creeks; only a few predators, such as the leech *Whitmania pigra* (Ozawa et al., 1989) or larvae of the dragonfly *Pantala flavescens* (Suzuki et al., 1999), commonly inhabit rice fields (Yusa, 2006; Yusa et al., 2006); therefore, releasing an efficient predator may be an effective control measure for apple snails in rice fields and artificial canals.

The effectiveness of predators, such as the common carp *Cyprinus carpio* (Halwart, 1995; Yusa et al., 2001) and the mallard duck *Anas platyrhynchos* (Furuno, 1992; Teo, 2001), has been quantified in the field. However, both predators are impractical for use in non-organic rice fields in Japan: carp require water deeper than 10 cm (Yusa et al., 2001), which is too deep for a normal rice field, and keeping ducks requires electric fences to prevent escape as well as a lot of care, including feeding every day (Furuno, 1992). Furthermore, even in canals, the potential negative impact of these species on native organisms is a concern (see Lowe et al., 2000 for

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DOI: 10.1303/ac.2008.475
carp as one of the world’s worst invasive alien species). Among other potentially effective predators, indigenous turtles, such as *Chinemys reevesii* and *Pelodiscus sinensis*, may be suitable for release into canals and rice fields since they do not require special care. Unlike *P. sinensis*, *C. reevesii* is commonly found in these habitats and is easy to rear. A preliminary study has shown that adult *C. reevesii*, although omnivorous, does not attack rice seedlings once the plants reach the 3-leaf stage (Yoshie, personal observations). However, the effectiveness of *C. reevesii* as a snail predator has not been fully investigated, except for the approximate size and amount of snails eaten by adult turtles in the laboratory (Yusa et al., 2006).

In this study, we conducted two series of experiments to investigate the effectiveness of *C. reevesii* as a predator of *P. canaliculata*. First, we investigated the relationship between turtle body size and the maximum size of snails consumed in small containers. Secondly, we investigated the number of snails eaten by adult turtles over an 8-week period in larger containers and under simulated natural conditions (with soil and rice plants) to examine the long-term effects of the turtles.

**MATERIALS AND METHODS**

**Animals.** All individuals of *P. canaliculata* used in this study were collected from rice fields in Kashiwagi Town, Nara City, Japan (34°67’N; 135°79’E). The collected snails were stored dry at 25°C. Before use, snails were put into water and confirmed to be alive.

Very small individuals (less than 40 mm carapace length) of *C. reevesii* were purchased from a pet shop, and larger juveniles and adults were caught in Saho River and the adjacent rice fields in Nara City. The turtles were reared in outdoor round aquaria (65 cm diameter × 33 cm height) and fed live earthworms and artificial food pellets (“kame-no-gohan”, Itosui Co. Ltd., Tokyo) for at least one month prior to use, to acclimate them to the experimental conditions.

In each experiment, sufficient artificial food pellets were given to the turtles at the start, but no further food was given except for apple snails during the experiment.

**Maximum size of snails consumed by turtles.** A series of experiments was conducted in outdoor plastic containers (0.37 m²; 49×75×20 cm height) in Nara Women’s University, Nara City, from July 29 to September 13, 2005. In each experiment, a turtle and ten snails of various sizes (Table 1) were introduced into a container. The water was kept at 4 cm deep and at 25–35°C, and the containers were covered with 6-mm mesh nets to prevent snails and turtles from escaping and being preyed upon by other animals. The shell heights of snails and the carapace lengths of the turtles were measured with calipers at the start of the experiment. Three days later, snails which had not been eaten by the turtles were collected and identified by their shell heights. Throughout the course of the experiment, dead snails with unbroken shells were immediately replaced with living ones of a similar size. Snails showed no apparent growth during the experimental period. Ten turtles of various sizes were tested (Table 1).

**Long-term experiment.** We conducted an 8-week experiment from August 17 to October 11, 2005 using four plastic containers (2.08 m²; 119×175×61 cm height), placed outside at the university. At the bottom of each container was a layer of soil 4–5 cm deep and water was kept 10 cm deep above the soil. A plastic box (13×21×13 cm height) was placed in the center of each container to provide a platform for the turtle to rest on. This was also included in the control plots without a turtle. To prevent snails from escaping, copper tape (≈1 cm wide) was glued above the water level inside the containers. Eight hills of rice plants of approximately equal numbers at the early vegetative stage were transplanted into each container, 40–50 cm apart. The water was not changed in the first week; however, after the second week, the water in all experimental containers was changed once or twice a week because it became polluted by turtle feces in the containers with turtles. Water temperature was maintained at 14–31°C throughout the experiment, with the use of 200 W water heaters from September 30 (seventh week) onwards.

We introduced a male turtle (carapace length: 155 mm, weight: 500 g, measured at the beginning of the experiment) into one of the containers and a female turtle (carapace length: 183 mm, weight: 779 g) into another. The remaining two (control plots) were without a turtle. The plots with a turtle (turtle plots) were randomly chosen.

We released 200 snails into each plot at the be-
beginning of the experiment on August 17. We then monitored the number of snails by weekly census (see below), supplemented by occasional observations. When the estimated number decreased to less than half within 1 week, we added 200 snails up to twice a week until September 27 (sixth week) to supplement naturally dead and killed snails. Snails of 10–30 mm shell height were used throughout the experiment, which were within the normal size range of overwintering snails in rice fields (Watanabe et al., 2000) and mostly below the size at maturity (25 mm; Estoy et al., 2002). The average of the total blot-dry weight of 200 snails was 172 g, and at each release we kept the difference in the total weight at less than 2% among plots.

To evaluate the impact of predation by the turtle on the next generation of snails, egg masses laid during the experiment were measured using the longest axis and allowed to hatch. We estimated the hatching rate to the nearest 5%; however, we added 200 hatchlings to each plot on September 14 (fourth week), because the snails rarely laid eggs during the experiment.

As food for snails, ~100 g of duckweed (Spirodela punctata and Lemna paucicostata) was placed in each control plot twice a week from August 25 (second week) to October 5 (seventh week; total 11 times); however, we added duckweed only on August 25 and 31 (second week) to the male turtle plot (twice) and up to September 13 (fourth week) to the female turtle plot (6 times) because weed density at the water surface in the turtle plots tended to remain high.

During the experiment, the numbers of snails were monitored every week. Five quadrats (each 0.04 m²; 10×40 cm) were set at the same places in each plot, including the edge of the container and amongst the rice plants. During each census, snails on the surface were collected first, and then those under the soil were collected by hand, except for the first census in which only snails found on the surface of the substratum were collected. Snails were returned to their original plot after being counted.

At the end of the experiment, we collected duckweed from five quadrats, the two turtles and all of the snails found in the containers. We measured the wet weight of the weed. To collect all remaining snails from the containers, we continued collecting snails until November 28.

Data analyses and statistical methods. To determine the maximum size of snails consumed by each turtle, we conducted logistic regression analyses, with snail shell height as the independent variable and whether the snails were preyed on or not as the dependent variable for each replicate. When the regression was significant, the maximum size of snails eaten by the turtle was defined as the shell height at which the mortality of the snails was predicted to be 50% by regression. Subsequently, we regressed the maximum size of snails eaten against turtle carapace length using normal regression.

In the analyses of the long-term experiment, the number of snails in each plot \( N \) was estimated for each census as the number of snails in the quadrats divided by the proportion of surface areas covered by the quadrats (9.6%), although the final number was based on the collection of all snails found. Survival rate in each plot \( S \) was defined as the proportion of the estimated number at each census divided by the total number of snails \( T \) that had been released until the census \( S = N/T \). Cumulative dead snails \( D \) were defined as the total snails released until the census minus the estimated number of living snails at the census \( D = T - N \). Sometimes the value of cumulative dead snails was estimated to be below 0 in control plots; in such cases, it was corrected to 0. Naturally dead snails \( D_{\text{natural}} \) were defined as the average of the cumulative dead snails in the two control plots. Values for cumulative snails preyed upon by a turtle \( D_{\text{preyed}} \) were obtained by subtracting the numbers of naturally dead snails from the cumulative dead snails \( D_{\text{turtle}} \) in each turtle plot \( D_{\text{preyed}} = D_{\text{turtle}} - D_{\text{natural}} \).

Numbers of living or dead snails, survival rates and the amount of weed remaining were compared between treatments using \( t \)-tests. The number of living snails and the survival rate were tested in each census for descriptive purposes, but the definitive tests were those made in the last census. Although survival rates were proportions, they were not arcsine transformed because the estimated proportion sometimes exceeded 1. Cumulative dead snails were only tested using data from the last census because the data from each census were not independent.

We used a generalized linear model for the proportion of buried snails. Treatment (with or without a turtle), plot (within the treatment) and time of
observation were designated as independent variables and whether the snails buried themselves as a dependent variable. Interaction terms were not included because none had a significant effect.

All statistical analyses were conducted using JMP IN version 5.1 (SAS Institute, 2004).

RESULTS

Maximum size of snails consumed by turtles

We could determine the maximum size of P. canaliculata consumed by each individual of C. reevesii (size at 50% mortality) for six of 10 experiments, where the relationship between snail size and survival was statistically significant by logistic regression (Table 1). In the remaining four experiments, the turtle hardly preyed on the snails within the 3-day experimental period and hence these logistic regressions were not significant. In the six cases, the maximum size of snails consumed (y mm) was positively related with the carapace length (x mm) of the turtle (y=0.16x+3.7, \( R^2 = 0.99, p<0.001 \)).

Long-term experiment

We continued to add snails of 10–30 mm shell height for 8 weeks as necessary, based on the snail density at the previous census. As a result, the total number of snails released differed among plots, being 200 snails in each of the two control plots (165 and 167 g in total, respectively), 2,200 snails (1,952 g) in the plot with a female turtle and 2,600 snails (2,214 g) in the plot with a male turtle. Nonetheless, the number of living snails in turtle plots tended to be smaller than that in the control plots except for the second week, and this difference was significant from the fourth week until the end of the experiment (t-test, \( p<0.05 \); Fig. 1). The survival rate in the turtle plots was significantly lower than that in the control plots from the fourth week until the end of the experiment (t-test, \( p<0.05 \); Fig. 2).

Cumulative dead snails in the male turtle plot increased at an approximately constant rate until the seventh week, when we stopped releasing snails (Fig. 3). Cumulative dead snails in the female turtle plot did not increase in the second week; this is because the turtle did not eat many snails, probably due to heavy pollution of the water. Subsequently, we changed the water frequently and did not release snails in the third week. After this, the number of dead snails increased at an approximately constant rate up until the seventh week. In the control plots, the number of cumulative dead snails was small. The final cumulative dead snails were 41 and 28 snails in the two control plots, 2,188 in the female turtle plot and 2,597 in the male turtle plot, respectively. The final number was significantly larger in the turtle plots than in the control plots (t-test, \( p<0.01 \)). The total numbers of snails preyed upon by each turtle over 8 weeks were estimated to be 2,153 (1,897 g) in the female plot and 2,562 (2,162 g) in the male plot.

We found only two egg masses in a control plot.

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Table 1. Carapace length of C. reevesii, minimum and maximum shell heights of P. canaliculata used in the experiment, and shell height at 50% mortality calculated when logistic regression was significant

<table>
<thead>
<tr>
<th>Carapace length (mm)</th>
<th>Test snails Min (mm)</th>
<th>Max (mm)</th>
<th>( p )</th>
<th>Shell height at 50% mortality (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>4.4</td>
<td>13.3</td>
<td>&lt;0.001</td>
<td>8.7</td>
</tr>
<tr>
<td>34</td>
<td>4.2</td>
<td>13.4</td>
<td>&lt;0.001</td>
<td>9.1</td>
</tr>
<tr>
<td>36</td>
<td>4.4</td>
<td>13.4</td>
<td>&lt;0.001</td>
<td>10.7</td>
</tr>
<tr>
<td>97</td>
<td>15.4</td>
<td>30.4</td>
<td>&lt;0.01</td>
<td>19.8</td>
</tr>
<tr>
<td>158</td>
<td>25.4</td>
<td>39.3</td>
<td>&lt;0.05</td>
<td>31.8</td>
</tr>
<tr>
<td>183</td>
<td>30.7</td>
<td>55.1</td>
<td>&lt;0.01</td>
<td>33.7</td>
</tr>
<tr>
<td>76</td>
<td>17</td>
<td>26.3</td>
<td>--*</td>
<td>--</td>
</tr>
<tr>
<td>95</td>
<td>12.3</td>
<td>28.7</td>
<td>0.43</td>
<td>--</td>
</tr>
<tr>
<td>152</td>
<td>24.7</td>
<td>35.6</td>
<td>0.82</td>
<td>--</td>
</tr>
<tr>
<td>154</td>
<td>28.2</td>
<td>37.8</td>
<td>0.24</td>
<td>--</td>
</tr>
</tbody>
</table>

Dashes (---) indicate points where calculations were impossible because turtles consumed no (*) or few snails.
Predation on Pomacea Snails by a Turtle

Fig. 1. Temporal changes in the estimated number of *P. canaliculata*. Thick arrows indicate the time points when 200 snails were released into both turtle plots, and broken arrows those only into the male turtle plot. Asterisks indicate significant differences between treatments (*p<0.05, **p<0.01).

Fig. 2. Temporal changes in the survival rate of *P. canaliculata*. Asterisks indicate significant differences between treatments (*p<0.05, **p<0.01).

Fig. 3. Temporal changes in the cumulative dead number of *P. canaliculata*. Asterisks indicate significant differences between treatments (**p<0.01).

during the experiment. One was 22.4 mm long and the hatching rate was 25%. The other was 24.7 mm long, but the egg mass fell into the water before hatching. After adding 200 hatchlings per plot in the fifth week, the final number of hatchlings was 124 and 266 snails in the two control plots (the latter number includes hatchlings from the naturally-laid egg mass), 72 in the female turtle plot and 3 in the male turtle plot, respectively. Although the numbers appeared smaller in turtle plots, the difference between the treatments was not significant (*t*-test, *p*=0.18).

The proportion of self-buried snails was 2% and 5% in the two control plots, 79% in the male turtle plot and 100% in the female plot, respectively (Fig. 4). The difference between treatments was significant (likelihood ratio test, *p*<0.001), whereas the differences were not significant between plots.
DISCUSSION

Size of snails consumed by turtles

The maximum size of *P. canaliculata* consumed by *C. reevesii* was positively related with the carapace length of the turtle. From direct observations, the turtles pinched the snails with their beaks, crushed the shells and then ate the flesh; therefore, the maximum size of snails eaten seems to be constrained by the beak size of turtles. Although less clearly shown, the maximum size of *P. canaliculata* consumed is also positively related to the body sizes of other predators, such as the turtle *Trachemys scripta* and several species of fish, including the common carp *Cyprinus carpio* (Halwart, 1995; Kai et al., 2001; Yusa et al., 2006).

This experiment demonstrated that a turtle of 183 mm carapace length, which is the standard size of an adult turtle (Okada et al., 1965), can prey on *P. canaliculata* of up to 34 mm shell height, which exceeds the size at maturity (about 25 mm shell height; Estoy et al., 2002); therefore, predation by *C. reevesii* is expected to be an effective means of snail control, especially since these turtles can reduce the number of reproducing adults as well as juveniles.

However, predation by adult turtles on the hatchlings of *P. canaliculata* may not be intense, judging from the long-term experiment where the effect of turtle predation on hatchlings was not significant.

Long-term effects on snail populations

We estimated that the female turtle preyed upon 2,153 snails and the male turtle preyed upon 2,562 snails in 8 weeks. Although the density of overwintering snails differs among rice fields and among regions, the average in common fields in Kyushu, where snail density tends to be high in Japan, is about 2 snails/m² (Watanabe et al., 2000; Wada et al., 2004). Consequently, the number of snails a single turtle could prey upon corresponds to the average number living in rice fields of 1,000 m² or more in this season, showing the very high predatory potential of the turtle. However, we cannot expect such high predatory activities in actual fields, as snail densities in the field are much lower than densities in the experimental plots. We might expect comparably intense predation in canals, where water flow ensures a steady supply of new snails. Therefore, the release of *C. reevesii* into canals may be a practical way to control populations of *P. canaliculata*; however, before releasing turtles, their effects on other indigenous organisms should be quantified.

In the turtle plots, the proportion of self-buried snails was higher than in the control plots. *P. canaliculata* bury themselves in response to the odors of predators or injured conspecifics in the laboratory (Ichinose et al., 2003; Carlsson et al., 2004). Therefore, our experimental results suggest that the snails exhibit self-burial behavior in response to predation, even under semi-natural conditions. We cannot completely exclude the possibility that the turtles preyed on snails on the soil surface and accordingly reduced the proportion of surface snails in this experiment. However, we think this unlikely as the sole explanation of the result because i) snails seem to self-bury and appear on the surface repeatedly rather than remaining self-buried (Wada and Yoshida, 2000) and ii) individuals of *P. canaliculata* self-bury in response to the odor of crushed conspecifics even in the field (Aizaki and Yusa, in press).

Duckweed in turtle plots increased greatly and the final weight was more than 400 g/m². On the other hand, no duckweed was found in control plots due to the high impact of snail feeding and despite restocking the weed. Although it is possible...
that the growth of duckweed was enhanced by turtle feces acting as a fertilizer in the turtle plots, the extreme decrease in control plots cannot be explained by this effect. Thus, the turtles not only affected the snail population but also vegetation through indirect effects. In general, indirect effects consist of density-mediated and trait-mediated interactions (Schmitz et al., 2004). In the present case, density-mediated indirect interaction is the reduction in the quantity of plant matter consumed by snails due to the decreased number of snails. A trait-mediated interaction is the reduction of plant damage through inducing self-burial and reducing the feeding behavior of each snail. Likewise, in paddy fields, damage to rice by \textit{P. canaliculata} might be controlled not only by reducing the absolute number of snails but also by changing their behavior. The effectiveness and relative importance of these indirect interactions in paddy fields should be investigated in detail.

ACKNOWLEDGEMENTS

We thank Drs. Keiji Wada and Hiroaki Sato, and two anonymous referees for valuable advice and comments. We also thank the members of the Laboratory of Animal Ecology, Nara Women’s University for discussion and assistance.

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