

Environmental factors influencing overwintering success of the golden apple snail, *Pomacea canaliculata* (Gastropoda: Ampullariidae), in the northernmost population of Japan

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Abstract

The effect of environmental factors on the overwintering success of *Pomacea canaliculata* in the northernmost population of Japan was examined to determine the location of overwintering habitats in the agricultural water system. The snail overwintered only in a portion of the water canals, and did not overwinter in dried paddy fields. In the canal, the density of the snail was not necessarily high at the overwintering site before winter. Overwintering success in the water canal was not explained by temperature and water velocity, but by pH, dissolved oxygen (DO) and depth of water among the sampling sites. To determine the location of a proper overwintering site, it is important to investigate various habitats including places where the snail density is low, and measure various environmental variables including pH, DO, and depth of water.

Key words: Golden apple snail, overwintering habitat, water canal, paddy field, northernmost population

INTRODUCTION

The golden apple snail, *Pomacea canaliculata* (Lamarck), is a freshwater snail introduced to Southeast Asian countries, including Taiwan, the Philippines, and Japan from South America around 1980 as an edible snail for aquaculture and farming (Mochida, 1991; Halwart, 1994). Because consumers did not like the taste of the snail, the snail lost commercial value and was discarded. Through waterways and canals, escaping and abandoned snails were dispersed to diverse freshwater habitats such as paddies, ponds, and canals. This snail is well known as a serious rice pest in most Southeast Asian countries because it damages the young seedlings of rice (Halwart, 1994; Naylor, 1996; Yusa and Wada, 1999).

Damage by *P. canaliculata* only occurs in the rice-planting season, when the snail eats young rice seedlings (Chang, 1985; Miyahara et al., 1986; Litsinger and Estano, 1993). In most regions of Japan, rice transplanting is done in late spring, and *P. canaliculata* reproduces mainly from summer to autumn (Suzuki and Fukuda, 1988; Ozawa and Makino, 1989; Tanaka et al., 1999). Hence, most of

the damage is caused by snails that have overwintered. The damage depends on snail density and size (Oya et al., 1986; Yano and Nakatani, 1989; Litsinger and Estano, 1993). In order to reduce snail damage, it is important to forecast and increase the overwintering mortality of the snail.

Previous studies show that *P. canaliculata* can overwinter in various habitats (Kiyota and Okuhara, 1987; Ozawa and Makino, 1988). In dried paddies, low temperature in winter causes high mortalities depending on shell height (Ozawa and Makino, 1988; Hirota and Ohki, 1989; Kondo and Tanaka, 1991; Syobu et al., 2001). Although various environmental factors influence the freshwater benthic organisms (Giller and Malmqvist, 1998), the effect of environmental variables except temperature on the overwintering success of *P. canaliculata* has not yet been investigated.

In this paper, the relationship between environmental variables and overwintering success of *P. canaliculata* was examined in a Japanese agricultural water system, including paddies and water canals.

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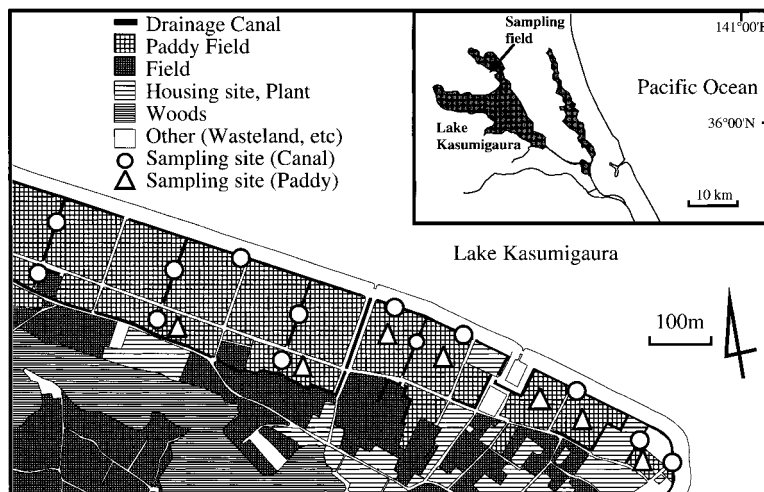


Fig. 1. Map showing the study site.

MATERIALS AND METHODS

Study site. Sampling and observations of *P. canaliculata* were conducted on drainage canals and paddy fields along the coast of Kasumigaura Lake (140°22–23'E, 36°06–07'N), in the southern part of Ibaraki Prefecture, Japan (Fig. 1). This area is thought to be the northern boundary of this species (Wada, 1997). In this area, the snail is distributed in a range of only about 1.5 km×0.3 km, and the study sites were set in the center of the distribution. Preliminary observations indicated that *P. canaliculata* occurred in both paddies and canals in the summer. Paddies in this area were dried up from September to April, though there was water in the canals year round. The water in the canals was mainly derived from ground water and the drainage from houses and food factories throughout the year, and from paddies from May to August. The water level of this canal was roughly under human control. This canal was a U-shaped concrete structure with a great deal of sediment (mud, sand, and dead plants) deposited on the bottom (Fig. 2). Vegetation, such as grass (e.g. *Paspalum distichum*, *Persicaria hydropiper*, etc.) and reeds (*Phragmites australis*), was mainly observed in shallow sites of the canal.

Examination of paddy fields. To examine the overwintering process in dried paddy fields, snail sampling was conducted from February to March in 2001. Seven paddies where many *P. canaliculata* were found in summer and which had not (or partially) been rototilled since autumn were selected

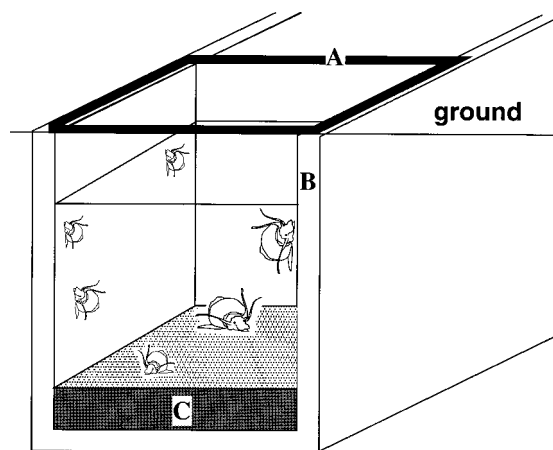


Fig. 2. A sketch of sampling procedures in drainage canals. A: quadrat (0.36 m²), B: drainage canal, C: sediment.

as sampling sites (Fig. 1). Most of the snails were found less than 10-cm deep in the paddy soil (see Results), so snails distributed under 10-cm in the soil were ignored. One to three clods of soil (80×45×10 cm deep) were randomly taken from the non-rototilled part of the paddies. All soil samples were sieved *in situ* using 5-mm mesh to collect the snails. Collected snails were put into water in the laboratory to check whether they were dead or alive. Temperatures in the dried paddies (surface and 5-cm under ground) were recorded using a data logger (Testo GDP-1710) from 14 February to 24 March. Water temperatures in the two canal sites were also measured to compare the temperatures between paddies and canals.

Examination in canals. In November 1999 and

May 2000, snails were collected from 13 sampling sites in the drainage canals (Fig. 1). Snails were sampled using a hand net with a mesh size of 3×3 mm. Eight 0.36 m² quadrates were set on the canal at each sampling site and all snails were sampled from these quadrates (Fig. 2). When the water was turbid and/or deep, snails were sampled by scraping the top of the sediment (about 5 cm depth) and the wall, and sieving the sample *in situ* with a 4-mm mesh. The shell height of collected snails was measured with calipers to the nearest 0.1 mm. In this study, small snails whose shell height was under 10-mm shell were excluded from the analysis because the sampling efficiency of smaller snails seemed to vary depending on site conditions such as water depth and turbidity. When the water deepened, the area of the wall underwater became large. Therefore, the value of the snail density in the deep portion of the canal was presumably overestimated by this method. This error is considered to have little influence on the result of this research because no positive relationship was observed between water depth and snail density in this canal (Ito, unpublished data).

Environmental variables were also measured in all 13 sampling sites during the winter. From November to May, maximum and minimum water temperatures in the bottom of the water were recorded every two weeks using a maximum-minimum thermometer. Water velocity on the surface was also measured using a float, and water depth in the center of the canal was measured to the nearest 1 cm at two-week intervals from November to May. From February to May, dissolved oxygen (DO) was measured at a depth of 10 cm using a handy DO meter (TOKO OX90) at two-week intervals. When the water depth was shallower than 10 cm, DO was measured at a shallower depth. pH was also measured at a depth of <5 cm in February and April 2000 using a digital pH meter (Hanna PIC-COLO2). All environmental variables were measured between 10:00 and 15:00. For statistical analysis (below), the mean values of six environment variables (maximum water temperature, minimum water temperature, water velocity, depth, DO, pH) were calculated for every sampling site.

Statistical analysis. Live snails were not found from seven out of 13 sampling sites in May 2000 (see Results). Univariate logistic regression analysis was used to examine the effect of the six envi-

Table 1. Temperature (°C) of water canals and dried paddies from February 14 to March 24 in 2001^a

Sampling site	Mean±SD	Maximum	Minimum
Canal 1	8.27±2.56	16.6	4.0
Canal 2	9.59±2.07	16.6	5.1
Dried paddy (surface)	5.79±3.07	16.7	0.0
Dried paddy (under 5 cm)	7.15±3.67	18.7	0.9

^a All data were measured with a data logger at 1-h intervals.

ronmental variables on overwintering success in those sampling sites. Likelihood-ratio analysis was used to test the logistic regression models. To examine the effect of snail size and density variation among the sampling sites on overwintering success of the snails, mean shell height and density in November 1999 were also investigated in this logistic regression analysis. These analyses were carried out using procedure LOGISTIC of SAS (SAS Institute, 1994). In this analysis, individual migration among sampling sites was ignored because *P. canaliculata* was inactive during the winter and water velocity was very low (Table 5).

RESULTS

Overwintering success in paddies

In order to determine the depth of the ground for investigation in a dry paddy, snails were sampled from the surface of the paddy, at 0 to 10 cm depth, and at 10 to 20 cm depth using a 0.36 m² quadrate on 27 February 2001. Of the 99 snails found, 58 snails were taken from the soil surface, 37 from a 0 to 10 cm depth, and only four from a 10 to 20 cm depth in the soil. This result implies that most of the snails were distributed from the surface of the ground to 10 cm in depth before winter. Hence, field collection in dried paddies was limited to a depth of 10 cm.

A total of 384 snails were sampled from seven paddies (100.07±100.35/m²: mean density±SD). In this survey, no live snails were found in these dried paddies. Table 1 shows the temperatures of both the paddies and the canals from 14 February to 24 March 2001. During this period, mean and minimum temperatures in the paddies were lower than those in the water canals.

Overwintering success and environmental variables in the canal

In the drainage canals, live snails were found at only six out of 13 sampling sites in May 2000 (Fig. 3). Table 2 shows the summary of snail sampling data in the drainage canals in November 1999 and May 2000. In November, there were significant variations in density and shell height among the sampling sites (Kruskal-Wallis test, $p < 0.0001$). Table 3 shows the effects of snail density and shell height in November 1999 on overwintering success at each sampling site. There was no statistically significant evidence that overwintering success was dependent on snail density and size in November.

Table 3 shows the correlation coefficients of six environmental variables in the water canals. A significant negative correlation was found between

water depth and DO, and a positive correlation was found between depth and pH.

Table 4 shows the relationships between environmental variables and overwintering success in the drainage canals. Overwintering success in the water canals was influenced by pH, DO, and depth variation among the sampling sites ($p < 0.05$). *P. canaliculata* could overwinter at shallow, high DO and at low pH sites in these canals. The sampling sites at which the snails could pass the winter were completely discriminated by the pH; the range of pH values at overwintering sites was from 6.29 to 6.63, and that at non-overwintering sites was from 6.79 to 7.46. In these analyses, there was no statistically significant evidence that overwintering success was influenced by water temperature or velocity.

Table 2. Summary of the golden apple snail data in the drainage canals based on 13 sampling sites

Sampling date	Density ^a (m ⁻²)			Shell height ^a (mm)		
	Mean \pm SD (<i>n</i>)	Max.	Min.	Mean \pm SD (<i>n</i>)	Max.	Min.
Nov. 1999	12.3 \pm 17.9 (13)	64.4	0.3	23.0 \pm 11.9 (459)	52.8	10.0
May 2000	1.8 \pm 3.2 (13)	11.5	0	27.0 \pm 10.1 (68)	57.0	12.1

^a Live snails.

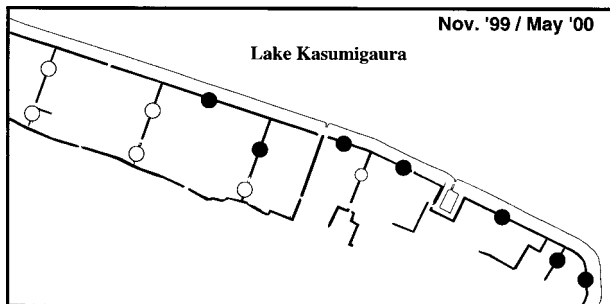


Fig. 3. Overwintering success of *Pomacea canaliculata* in water canals. ○: overwintered, ●: fail to overwinter.

Table 3. Likelihood-ratio analysis of univariate logistic regression models for overwintering success of the golden apple snail in water canals

Independent variables	χ^2	df	<i>p</i> -value
Snail density	1.466	1	0.2260
Mean shell height	0.024	1	0.8757

Table 4. Correlation coefficients of environmental variables in drainage canals

	Min. water temperature	Water velocity	Depth	DO	pH
Max. water temperature	-0.294	0.393	-0.371	0.500	-0.342
Min. water temperature		0.022	0.239	-0.254	-0.263
Water velocity			0.088	0.379	0.007
Depth				-0.648*	0.588*
DO					-0.397

* Significant at 5% level.

DISCUSSION

Although the snails were distributed in both paddies and canals in November, overwintered live snails could be found only in a portion of the drainage canals in May (Fig. 3). During the rice-growing season (May to August), the paddies were filled with water supplied by the pipeline, and much water flowed out of the paddy fields into the drainage canals. In addition, the water of the canals sometimes overflowed and flowed backward into the paddies depending on weather conditions. The migration of *P. canaliculata* was sometimes observed with the flow of water between the paddy and the canal. These results and observations imply that the snail population in this area was supported by recruitment of overwintered snails derived from the overwintering habitat in a limited area of the canal. For effective management of the golden apple snail in this area, it is quite important to detect this overwintering habitat.

Snail density in November could not explain wintering success in the water canals (Table 3). In addition, no overwintered snails could be found in the paddies although snail density in the paddy was about 100 individuals/m² in November. These re-

sults indicate that the snail density before hibernation was not helpful in the search of the overwintering habitat in this area. In previous studies, the overwintering mortality of *P. canaliculata* in the canals tended to be focused in the populations whose density was high before the winter season (Oya et al., 1987; Ozawa and Makino, 1988). In order to determine the overwintering place of this snail, investigation of various places, including those of low-density, should be undertaken.

The relationship between temperature and wintering mortality of *P. canaliculata* was previously examined to forecast the overwintering mortality (Ozawa and Makino, 1988; Hirota and Ohki, 1989; Kondo and Tanaka, 1991; Syobu et al., 2001). In dried paddies, yearly variation in overwintering mortality depended on the temperature (Syobu et al., 2001). However, water temperature variation among the sampling sites could not explain the overwintering success in the drainage canals (Table 5). This result may have been caused by the narrowness of temperature variation among the sampling sites of the water canals; the range of minimum water temperature in the canals was 2 to 8°C, and the water of the bottom was not frozen during the winter (Table 6).

Table 5. Likelihood-ratio analysis of univariate logistic regression models for overwintering success of the golden apple snail in water canals

Independent variables	χ^2	df	<i>p</i> -value
Mean value of maximum water temperature from November to May	1.284	1	0.2572
Mean value of minimum water temperature from November to May	0.725	1	0.3946
Water velocity	0.049	1	0.8243
Depth	5.461	1	0.0195
DO	6.059	1	0.0138
pH ^a	17.924	1	0.0001

^a Because the overwintering sites were completely discriminated with the value of pH (see Results), the maximum likelihood estimate did not exist (Tango et al., 1996). The results are based on the last maximum likelihood iteration.

Table 6. Summary of environmental variables in drainage canals

Environmental variables	Mean±SD	Maximum	Minimum
Mean value of maximum water temperature from November to May (°C)	15.0±1.1	17.3	13.7
Mean value of minimum water temperature from November to May (°C)	7.4±1.7	11.1	5.2
Minimum water temperature (°C)	4.1±1.8	8	2
Water velocity (cm/10 s)	18.6±27.5	101.2	0
Depth (cm)	28.6±13.0	59.2	11.4
DO (mg/l)	3.2±1.6	6.4	1.0
pH	6.79±0.34	7.46	6.29

In contrast, pH, DO, and depth variation explained the overwintering success in the water canals (Table 5). *P. canaliculata* could survive in the shallow portion of the canal that had high DO and low pH during the winter season. Therefore, hand picking of snails, dredging canals, and so on may be effective in such shallow canals during winter to reduce the overwintered snails in this area. Since there was significant correlation among the three environmental variables (Table 4), the effects of each of the three variables on overwintering success could not be distinguished (ter Braak and Looman, 1995). Before the winter, *P. canaliculata* was mainly distributed in the shallow water (Masuda, 1987; Ichinose et al., 2000), and sometimes breathed directly from the air using a siphon. In the winter season, however, most of the snails closed the operculum and sank to the bottom of the water (Kiyota and Okuhara, 1987; Estebenet and Cazzaniga, 1992). At low temperatures under 17.5°C, over 50% of the snails began to stop moving, and under 10°C, snails began to withdraw into their shells (Sugiura and Wada, 1999). In most periods of the winter season, water temperatures in the canals was under 17.5°C (Table 6). Inactive snails may not be able to escape from unsuitable environmental habitats, and as result, die during the winter.

Although the snails can pass the winter in dried paddies in the warmer southern regions (Oya et al., 1987; Ozawa and Makino, 1988; Hirota and Ohki, 1989; Watanabe et al., 2000), they can not overwinter in paddies in this northern area. In February and March, the lowest temperature on the ground was 0°C, and this was colder than the water canal (Table 1). In addition, yearly variations in overwintering mortality depended on temperature in dried paddies (Syobu et al., 2001). Therefore, such geographical variation in the wintering in paddies is mainly due to climatic differences among habitats.

In conclusion, *P. canaliculata* in the northernmost population of Japan can not overwinter in dried paddies, but can overwinter in limited areas of the water canals. Places of hibernation in these canals were characterized by shallowness, high DO, and low pH, not by water temperature variations. In addition, the snail density in an overwintering site was not necessarily high before winter. To determine the location of an overwintering habitat, it is important to investigate various habi-

tats, including those of low-density, and measure environmental variables other than temperature, such as pH, DO, and depth.

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