NATURAL PARASITIC INFECTION OF THE GOLDEN APPLE SNAIL, *POMACEA CANALICULATA*

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Abstract. Golden apple snails, *Pomacea canaliculata,* were collected once a month during a year to search for their natural parasites. The collections were made at two localities having different ecological environments. Of 576 collected snails from a canal, 176 individuals (30.6%) were infected by three groups of metacercariae. These parasites were amphistome, distome and echinostome metacercariae, which had prevalences of 23.5, 19.5 and 0.5%, respectively. The incidence of infection was highest (68.4% in October) when the snail population was composed of the old, juvenile and young *Pomacea.* Amphistome metacercariae were found most frequently and echinostome metacercariae the least frequently; both parasites were localized in the foot muscle of the snails and had a Shannon index of zero. The range of amphistomes was 1 to 115 with the mean ± SD of 1 ± 2 and 95% CL of 1, 2. Distome metacercariae were found primarily in the heart (range : 1-13), and also in the foot muscle (range : 1-5) and kidney (range : 1-14), with a Shannon index of 0.4. The means ± SD (with 95% CL) were 3 ± 4 (95% CL = 1, 5), 3 ± 4 (95% CL = 2, 4) and 2 ± 1 (95% CL = 1, 2) for the foot muscle, heart and kidney, respectively.

The snails from a pond, another locality, had a low proportion of infected individuals. Of 605 snails, only 24 individuals (4.0%) were infected, with the prevalence of amphistomes, distomes and echinostomes being 0.8, 1.8 and 2.1%, respectively. The incidence of infection for each month was zero or less than 10%, except in May when it was 30.2%.

INTRODUCTION

The golden apple snails, *Pomacea canaliculata* (Orbigny), have been found in large numbers in central and southern Thailand since they were introduced into the country less than ten years ago. They can be consumed because they have a palatable foot muscle. Thai farmers like to cook the snails rare, which is insufficient to destroy the parasites inside the snails. *Angiostrongylus cantonensis* is one of the parasites detected in *P. canaliculata.* Chao *et al* (1987) reported susceptibility of the golden apple snails to this nematode in the laboratory. They supplied the first stage larvae of *A. cantonensis* to the snails. All snails were susceptible, and the larvae developed into the infective third stage inside their hosts. The larvae were found in almost all snail organs, *ie* head-foot, mantle, digestive tract, heart and ovary. The head-foot was the region where the larvae were localized most frequently, while the liver (digestive gland), ovary and heart had fewer parasites.

Man is not the natural host of *Angiostrongylus cantonensis,* but the parasites can survive in man and migrate to the brain. The third-stage larval parasites can cause eosinophilic meningoencephalitis, resulting in neurological disorders or death to infected man (Harinasuta *et al,* 1965).

Saxena *et al* (1987) reported that *Pomacea* snails were distributed heavily in the ricefields of the Philippines and preferentially destroyed young stems of rice much more than old ones. At the same time, Duangsawasdi (1987) reported concerning the feeding behavior of *Pomacea canaliculata* in Thailand that the snails were not very selective for their food; they might be used for controlling aquatic weeds if they were not pests themselves. When Lauhachinda *et al* (1988) fed algae, water hyacinths and rice seedlings to *P. canaliculata,* they found that the snails preferred algae the most. The snails did not reproduce if they ate the water hyacinth.

There are three species of *Pomacea* in Thai-

land. They can be identified using shell morphology in conjunction with the male reproductive organs and genetic patterns (Keawjam and Upatham, 1990). At present, these snails are considered important pests of rice and aquatic vegetables by the Thai Ministry of Agriculture and Cooperatives.

Most reports of the golden apple snails have dealt with their distribution, feeding behavior and susceptibility to parasitic infection in captivity. There is no study of natural parasites of these snails in the field. It is our interest to determine whether Thai *Pomacea* are infected naturally, what kinds of parasites they have, and which organs of the snails are likely inhabited by the parasites. Furthermore, their environments as factors of the host-parasite relationship have been investigated.

MATERIALS AND METHODS

Sampling

Location : Two sites of a study area at Kasetsart University, Bangkok, with large populations of *Pomacea canaliculata* were chosen : one was a man-made canal, 800 m in length, and the other was a pond, having a surface area of 500 m^2. These two sampling stations were 1.5 km apart. The canal was shaded by a row of large trees along one bank and also covered by water lilies. This canal also receives effluent from office buildings. Moreover, there are grass and shrubs covering the canal bank (Fig 1). In contrast, the pond was in an open area through which sunlight could penetrate. The pond had a small area shaded by trees. There were bunches of water hyacinths and water lilies (Fig 2). Water buffalos were occasionally found in the pond. This pond has been periodically cleaned by getting rid of weeds.

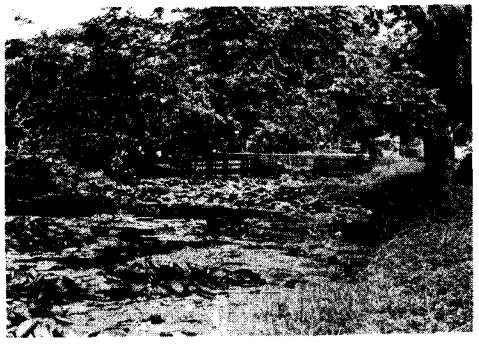

Fig 1—The canal shaded by a row of large trees along one bank and also covered by water lilies.

Fig 2—The pond, an open area through which sunlight can penetrate.

Sample processing

The snails were collected once a month and were randomly picked by a long-hand net, approximately 50 individuals per station. They were collected early in the morning, 6:30-8:00 am, transferred to plastic baskets and brought to the laboratory. The snails were anesthetized by placing them in a freezer at a temperature below 0°C; the animals were then removed without breaking the shell. After weighing the whole animal, the foot muscle was cut and weighed again. Each piece of foot muscle was chopped and put into a 50 ml beaker individually, to which sufficient digestive solution (5% pepsin) was added to cover the muscle tissue. To prepare the digestive solution, 5 g of pepsin A powder (BDH Chemicals, Ltd, England) was dissolved in 500 ml distilled water and 15 ml concentrated HCl; the solution was then brought to 1,000 ml by adding distilled water. The final pH of this solution was 1.7-1.8. The pepsin solution was refrigerated before use and then incubated to 37°C before digestion of the tissue. The mixture of 1 g muscle tissue/3 ml pepsin was incubated in an incubator at 37°C for 12 hours, and periodically agitated. Large pieces of tissue were removed using a sieve, while the remaining mixture was transferred to a 40 ml centrifuge tube and left until it sedimented. The sediment was cleaned by changing the above solution with dechlorinated

water until the liquid became transparent. The centrifuge tubes were then refrigerated (Poonswad, 1988).

To observe and count the parasites, Scott's chamber was used. Scott's chamber is a thick sheet of glass (4 × 7.5 cm) having a 0.3 cm wide × 0.5 cm deep × 7.5 cm long channel in the middle. This channel has open ends and a volume of 0.5 ml. The sediment along with water was added into the channel for examination and counting of the parasites within, using a dissecting microscope at 15 × magnification.

The snails were dissected to remove their heart and kidney. These two organs were placed between two pieces of glass (0.5 × 5 × 23 cm) and squashed to a thin film. Within this film the trematode parasites were examined. To identify the groups of trematodes present, a compound microscope equipped with camera was used at a magnification of 100 × or 400 ×.

Data evaluation

The percentage of infected snails for each month was calculated. For a whole year, the snails having the same group of parasites were pooled and also were divided according to which organs the parasites were within. The prevalence of each group of metacercariae found in the snails was recorded. The Shannon index, a quantitative measure of diversity for parasitic sites was calculated, using the equation (Zar, 1974):

$$H = \frac{n\log n - \sum_{i=1}^{k} fi \log fi}{n}$$

where H = Shannon index
n = sample size
k = number of categories
fi = number of observations in category i.

For a specific parasitic group, the snails having the same numbers of parasites were counted, and the mean ± SD and 95% confidence limits (CL) of that parasite were calculated.

RESULTS

The golden apple snails, *Pomacea canaliculata* (Fig 3), in the canal were distributed throughout the water body, but in the pond the snails were found only in the small areas of shade, such as underneath trees, bunches of water hyacinth and water lilies. The snails collected from both sampling locations were found to have trematode metacercariae as their parasites, but their occurrence was different for the two localities.

Fig 3—*Pomacea canaliculata* is laying eggs out of the female opening which is located at the right side.

Table 1 shows the number and percentage of infected snails for a one year period in the canal compared with those in the pond. Of 575 snails collected from the canal, 176 individuals (30.6%) were infected by three groups of metacercariae. For four months (January, February, July and December), the percentage of infection was less than 10%; the infection was highest in October (68.4%). The parasitic metacercariae were identified as amphistome, having an oral sucker at one end and another at the other end (Fig 4), distome, having an oral sucker and another elsewhere on the ventral surface, and echinostome, having spines around the oral sucker and another sucker elsewhere on the ventral surface. (Pictures of distome and echinostome were not available). Amphistomes

Table 1

Comparison of percentage of infected snails from two different localities according to times of the year.

Month	Snails collected from canal			Snails collected from pond		
	No. examined	No. infected	% infected	No. examined	No infected	% infected
January	15	0	0	15	0	0
February	47	2	4.3	56	1	1.8
March	51	9	17.7	55	0	0
April	56	34	60.7	61	1	1.6
May	45	15	33.3	43	13	30.2
June	49	20	40.8	52	5	9.6
July	50	3	6.0	48	1	2.1
August	54	17	31.5	52	0	0
September	49	13	26.5	54	0	0
October	57	39	68.4	64	0	0
November	52	20	38.5	52	0	0
December	50	4	8.0	53	3	5.7
Total	575	176	30.6	605	24	4.0

were the major parasites specifically localized in the foot muscle of the 135 snails, with a prevalence of 23.5% (Tables 2, 3). Distomes were fewer in number and found in the foot muscle, heart and kidney of 21, 64 and 27 snails, respectively, with a Shannon index (index of diversity) of 0.4. The prevalence of distomes was 19.5%. Echinostomes were found the least, only in three snails, and specifically in the foot muscle of the hosts. The prevalence of echinostomes was 0.5%.

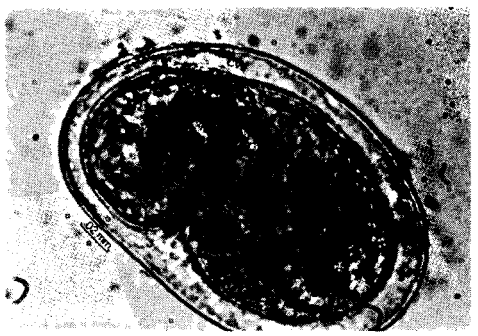

Fig 4—Amphistome metacercaria of *Pomacea canaliculata*.
CW = cyst wall, AS = anterior sucker, PS = posterior sucker

The total number of host snails listed in Table 2 is higher than that in Table 1, even though both are from the same samples, because some of the snails harbored more than one group of metacercariae or were infected in two or three organs, as follows:

Amphistomes were found in concurrence with distomes in the foot muscle of 22 host snails. In addition, amphistomes were in the foot muscle, while distomes were also in the heart and kidney (of nine snails) and in the heart (of seven snails). In a rare case (one snail), amphistomes were in the foot muscle in concurrence with distomes, and distomes were also in the heart and kidney.

Amphistomes were found in concurrence with echinostomes in the foot muscle of three snails, two of which had distomes in the heart and kidney or only heart.

In nine snails, only distomes were found, but they were localized in both foot muscle and heart. Four other snails had distomes in both heart and kidney. There was only one snail that had distomes in all three organs.

The number and percentage of infected snails in the pond are shown in Table 1. Of 605 snails, 24 individuals (4.0%) were infected. No parasites

Table 2

Comparison of distribution of trematode metacercariae in three organs of infected *Pomacea* snails collected from two different localities.

No of snails harboring parasites in their organs	Metacercarial group in snails in canal*			Metacercarial group in snails in pond**		
	A***	D	E	A	D	E
Foot muscle	135	21	3	5	2	13
Heart	none	64	none	none	8	none
Kidney	none	27	none	none	1	none
Total (n)	135	112	3	5	11	13
Shannon index	0	0.4	0	0	0.3	0

* The snails collected from the canal were 575
** The snails collected from the pond were 605
*** A : Amphistome; D : Distome; E : Echinostome

Table 3

Comparison of prevalence of three groups of metacercariae found in *Pomacea* snails collected from two different localities.

Metacercariae	Snails collected from canal			Snails collected from pond		
	No. examined	No. infected	Prevalence %	No. examined	No. infected	Prevalence %
Amphistome	575	135	23.5	605	5	0.8
Distome	575	112	19.5	605	11	1.8
Echinostome	575	3	0.5	605	13	2.1

were found during six of the monthly intervals: January, March, August, September, October and November, and in the other six months the parasites inside the snails were generally few in number, except in May. The highest percentage of infection was 30.2% in this month.

Groups and distribution of metacercariae in snails inhabiting the pond were similar to those of the canal. However, the echinostomes were the most numerous, being found in 13 snails, while amphistomes were the least common, inhabiting five snails (Table 2). Distomes were found in the foot muscle, heart and kidney with a Shannon index of 0.3. The total number of host snails was higher in Table 2 than in Table 1 because one individual had both amphistomes and distomes in the foot muscle, two individuals had distomes in the heart and also echinostomes in the foot muscle and one individual had both distomes and echinostomes in the foot muscle. The prevalence of amphistomes, distomes and echinostomes was 0.8, 1.8 and 2.1%, respectively (Table 3).

In our observations, we saw the adult snails were active in copulating and laying eggs in the rainy season (June-September). A tremendous number of egg clusters were attached to any objects above the water level. The eggs hatched to be young snails in about 14-18 days. We found many snails in October as juveniles and small-sized snails. The snails were growing and might have been preparing themselves to estivate to escape from drought during winter and summer (December-April) (Keawjam and Upatham, 1990).

Table 4

Intensity of amphistome metacercaria infection in foot muscle associated with *Pomacea* snails collected from canal.

No. of metacercariae	No. of host snails	No. of metacercariae	No. of host snails
1	47	19	1
2	9	20	2
3	17	22	1
4	16	31	1
5	4	35	1
6	6	40	1
7	3	46	1
9	3	47	1
10	2	48	1
11	4	54	1
12	3	58	1
13	2	76	1
14	1	93	1
17	1	103	1
18	1	115	1

No. of snails examined = 575; total no. of infected snails = 135
Range of amphistomes = 1-115
Mean ± SD of no. of amphistomes = 1 ± 2
95% confidence limits of amphistomes = 1, 2

Tables 4 and 5 show the number of host snails collected from the canal and harboring various numbers of metacercariae. In Table 4, 47 out of 135 snails had the lowest number of parasites, one amphistome. As the number of amphistomes increased, the number of host snails was generally one individual; the highest number was 115 amphistomes in a little snail (W × H : 22 × 17 mm). The number of amphistomes in the foot muscle varied from 1 to 115 with the mean ± SD of 1 ± 2, and 95% CL of 1, 2.

Distome metacercariae were found in three organs of the snails collected from the canal (Table 5); they occurred most frequently in the heart (64 snails), next, in the kidney (27 snails) and in the foot muscle (21 snails). The most common number of distomes in these three organs was one (in 15, 30 and 10 snails relative to the foot muscle, heart and kidney, respectively). The highest number were 13 distomes in the heart and 14 in the kidney. The number of distomes varied from 1 to 5 in the foot muscle, 1 to 13 in the heart and 1 to 14 in the kidney. Means ± SD were 3 ± 4 (95% CL = 1, 5), 3 ± 4 (95% CL = 2, 4) and 2 ± 1 (95% CL = 1, 2) for the foot muscle, heart and kidney, respectively.

Regarding the snails collected from the pond, the most common number of metacercariae found in the snails was one. The highest number of amphistome metacercariae was ten, while the highest number of distomes and echinostomes were both four in the heart and foot muscle, respectively.

DISCUSSION

In the study of natural parasites of the golden apple snails, *Pomacea canaliculata* (Orbigny), useful information has been obtained. Firstly, the ecological conditions of the habitat can influence the susceptibility of the snails. The canal was under the shade of trees and also covered by water lilies, providing a good place for the snails to live. The effluent of the office buildings supplied nutrients which could promote growth of the snail population. Intermediate hosts of trematodes

Table 5

Intensity of distome metacercaria infection in foot muscle, heart and kidney associated with *Pomacea* snails collected from canal.

No. of metacercariae	No. of host snails of which organs are infected		
	Foot muscle	Heart	Kidney
1	15	30	10
2	2	11	3
3		9	2
4	3	4	1
5	1	2	4
6		3	
7		2	3
8			1
9		1	1
10		1	1
13		1	
14			1
Total no. of infected snails*	= 21	64	27
Range of distomes	= 1-5	1-13	1-14
Mean ± SD of no. of distomes	= 3 ± 4	3 ± 4	2 ± 1
95% confidence limits of distomes	= 1, 5	2, 4	1, 2

* of 575 examined snails

such as mice and rats may hide inside the effluent pipes and bushes. Due to the crowded snail population in the canal, the snails have much chance to contact each other, giving rise to the high percentage of infection of 30.6% for one year (Table 1). The prevalence of amphistomes (23.5%) and distomes (19.5%) (Table 3) are also noticeable. In contrast, the pond is a large open place, through which sunlight can penetrate. Therefore, the snails are found only in the small areas of the shade. Thus the environment in some areas may be too difficult for the snails to live. It is possible that there is less chance of two snails meeting resulting in a lower infection rate. The snails in the pond showed a low parasitic infection rate of 24 of 605 individuals (4.0%). Moreover, this pond has been periodically cleaned by getting rid of weeds; the snails are also thrown away together with the plants.

The snails collected from the canal had high infection rates of 60.7 and 68.4% in April and October (Table 1). It is possible that in April the snails leave estivation and come into the water body; some of them already have the parasites. In October, 68.4% of the snails were parasitized; almost all young (W : 21-29 mm; L : 17-24 mm) were infected. Thus the infection is due to some old snails and newly hatched ones.

In the pond, high infection has occurred in May with 13 of 43 snails (30.2%) being infected. The snails were leaving estivation and coming up to the ground, and they were already harboring parasites. After hatching out of the eggs in October - November, no infection was found. There are three possibilities to support this occurrence : the pond had just been cleaned, the infection is usually low with sometimes no parasitized snails being found, and the young snails have not enough safe places to live, with some of them dying.

There are three groups of metacercariae found in the snails. Amphistome metacercariae were localized specifically in the foot muscle with the range of 1 to 115 individuals in one snail and a

mean ± SD of 1 ± 2 individuals with 95% of all amphistomes obtainable lying between the limits of 1 and 2 individuals (Table 4). Distome metacercariae were localized mostly in the heart (range : 1-13), then in the kidney (range : 1-14) and foot muscle (range : 1-5) and have means ± SD (with 95% CL) as 3 ± 4 (95% CL = 2, 4), 2 ± 1 (95% CL = 1, 2) and 3 ± 4 (95% CL = 1, 5), respectively (Table 5). It can also be predicted to distribute over three organs with a Shannon index of 0.4, while no diversity occurred in amphistomes and echinostomes. Echinostome metacercariae were localized specifically in the foot muscle and are not common.

The natural parasites are all flukes. There were no nematodes found in 1,180 collected snails in one year. To identify the flukes, we use position of suckers in conjunction with other morphology. We cannot classify them into genera and species due to lack of miracidia, cercariae and adults which are in other hosts.

Finally, all snails were measured and weighed during the study, which could inform relationships between size of snail and susceptibility. For example, the small-and medium-sized snails from the canal tend to have amphistomes, but the number of amphistomes cannot be predicted by size of the snails.

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