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CONSIDERING THE ROLE OF THE ASSASSIN SNAIL *Anentome helena* AS A BIOLOGICAL CONTROL OF *Bithynia siamensis goniomphalos*, THE FIRST INTERMEDIATE HOST OF *Opisthorchis viverrini*

Surat HARUAY[1], Supawadee PIRATAE[2]

[1] Community Health Program, Faculty of Public Health, Ubon Ratchathani Rajabhat University, Ubon Ratchathani 34000, Thailand.
[2] One Health Research Unit, Faculty of Veterinary Sciences, Mahasarakham University, Maha Sarakham 44000, Thailand.

Corresponding author:
Supawadee Piratae
supawadee.p@msu.ac.th

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Abstract

This study aims to investigate whether the assassin snail *Anentome helena* may serve as a biological control agent of *Bithynia siamensis goniomphalos,* the 1st intermediate host of *Opisthorchis viverrini*. Experiments were carried out in the laboratory, and the results found that *A. helena* showed the variation and selection of mollusc prey. *A. helena* can consume *B. siamensis goniomphalos,* which is remarkable because this snail can compete with other snails and could be used as a biological control. The consumption rate of the predator was compared, and it was found that *A. helena* prefers to consume *Indoplanorbis exustus,* followed by *Pomacea canaliculata*, *Melanoides tuberculata*, *Filopaludina sumatrensis speciosa, Lymnaea* sp., and *B. siamensis goniomphalos*. This is the first report of an experimental study controlling *B. siamensis goniomphalos* using *A. helena.* Our data imply that *A. helena* can control the *B. siamensis goniomphalos* population with good results, especially in the absence of other snail species.

Keywords: Biocontrol. *Clea helena*. Freshwater snail. Opisthorchiasis. Predator.

1. Introduction

Freshwater snails play a role as intermediate hosts of many trematode parasites, including blood flukes, gastrointestinal flukes, lung flukes and liver flukes (Bayne 2009; Keiser and Utzinger 2009). Infections with these flukes are reported to be universal and persistent public health problems worldwide. One of the most important freshwater snails is *Bithynia siamensis goniomphalos*, the 1st intermediate host of *Opisthorchis viverrini,* which causes opisthorchiasis, a disease endemic to Southeast Asia, especially Thailand (Piratae 2015). Infection with *O. viverrini* remains an important public health problem in Southeast Asia because it is a risk factor for the development of cholangiocarcinoma (Sithithaworn and Haswell-Elkins 2003). In the parasite life cycle, *Bithynia* snail development consists of miracidium, sporocyst, redia and cercariae stages. After cercariae are released from infected snails, they invade cyprinoid fish and transform into metacercariae, which is the infective stage for definitive hosts, including humans. Prevention of opisthorchiasis is one of multiple strategies, including consuming cooked food, changing behaviour, or keeping sanitary conditions. In addition, an important preventative process is to control the population of the 1st intermediate snail host.

Many methods have been reported for snail control, such as physical methods by water resource management (Laamrani et al. 2000), chemical methods by using molluscicides or pesticides (Wang et al.

2018) and biological control by using challenger species. The advantage of using a biological control is reducing the use of pesticides or other chemicals that have undesired environmental effects, including pest residuals, progression of pesticide resistance and the destruction of other aquatic animals. The biological control of snails by using competitor snails is an attractive strategy for parasitic infection control (Pointier et al. 2011).

In previous reports, several ways have been described for controlling the *B. siamensis goniomphalos* population, including using molluscicide (Tesana et al. 2012) and plant extracts (Aukkanimart et al. 2013). However, no study reported using a competitor snail as a biological control of this snail. Interestingly, *Anentome helena* snail has been proposed as a biological control for *Melanoides tuberculata* and *Tarebia granifera* (Oleh et al. 2018; Yakovenko et al. 2018). Thus, we were interested in whether *A. helena* can be used as a biological control for *B. siamensis goniomphalos*.

2. Material and Methods

Experimental snails

The experiments were performed in the Parasitology Laboratory of Public Health, Faculty of Ubon Ratchathani Rajabhat University. The snails used in this experiment were collected from three locations: 15°14'35.53"N 104°52'38.76"E; 15°12'24.24"N 104°51'47.35"E and 15°16'41.65"N 104°50'0.50"E in Ubon Ratchathani Province (Figure 1). All collected snails were examined for trematode infection by cercarial shedding. Snails that were free from trematode infection were then used in the experiment.

At the start of each experiment, all snails were free from trematode infection, and their approximate sizes were measured. We used 270 *A. helena* (shell length 1.3-1.8 cm), 360 *B. siamensis goniomphalos* (shell length 0.7-0.9 cm), 30 *Pomacea canaliculata* (shell length 1.00-1.50 cm), 30 *Filopaludina sumatrensis speciosa* (shell length 1.00-1.50 cm), 30 *Melanoides tuberculata* (shell length 1.00-1.50 cm), 30 *Sulcospira housei* (shell length 1.00-1.50 cm), 30 *Indoplanorbis exustus* (shell width 0.6-1.00 cm) and 30 *Lymnaea* sp. (shell length 1.00-1.50 cm) (Figure 2). Snails were maintained in a tank with a diameter of 40 cm, filled with 15 L of dechlorinated tap water, covered bedding with 2 mm thick sand, and oxygen was added by an oxygen pump. Experimental snails were provided with synthetic snail food *ad libitum*. Water quality was measured at pH = 6.35±0.34, DO = 5.2±0.9 mg/L, salt = 0.02%, conductivity = 0.307±0.021 mS, and temperature = 25±2 °C (Lutron WA-2017SD).

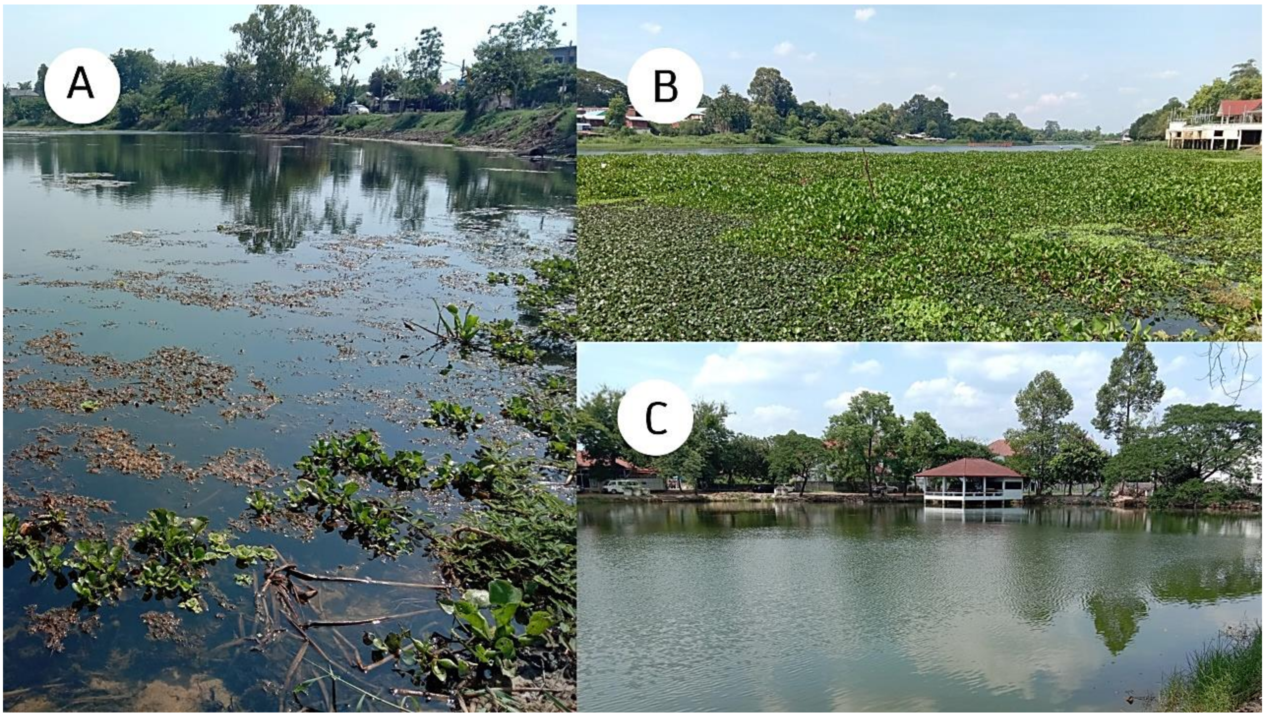

Figure 1. Photographs of sampling locations: A - 15°14'35.53"N 104°52'38.76"E; B - 15°12'24.24"N 104°51'47.35"E and C - 15°16'41.65"N 104°50'0.50"E.

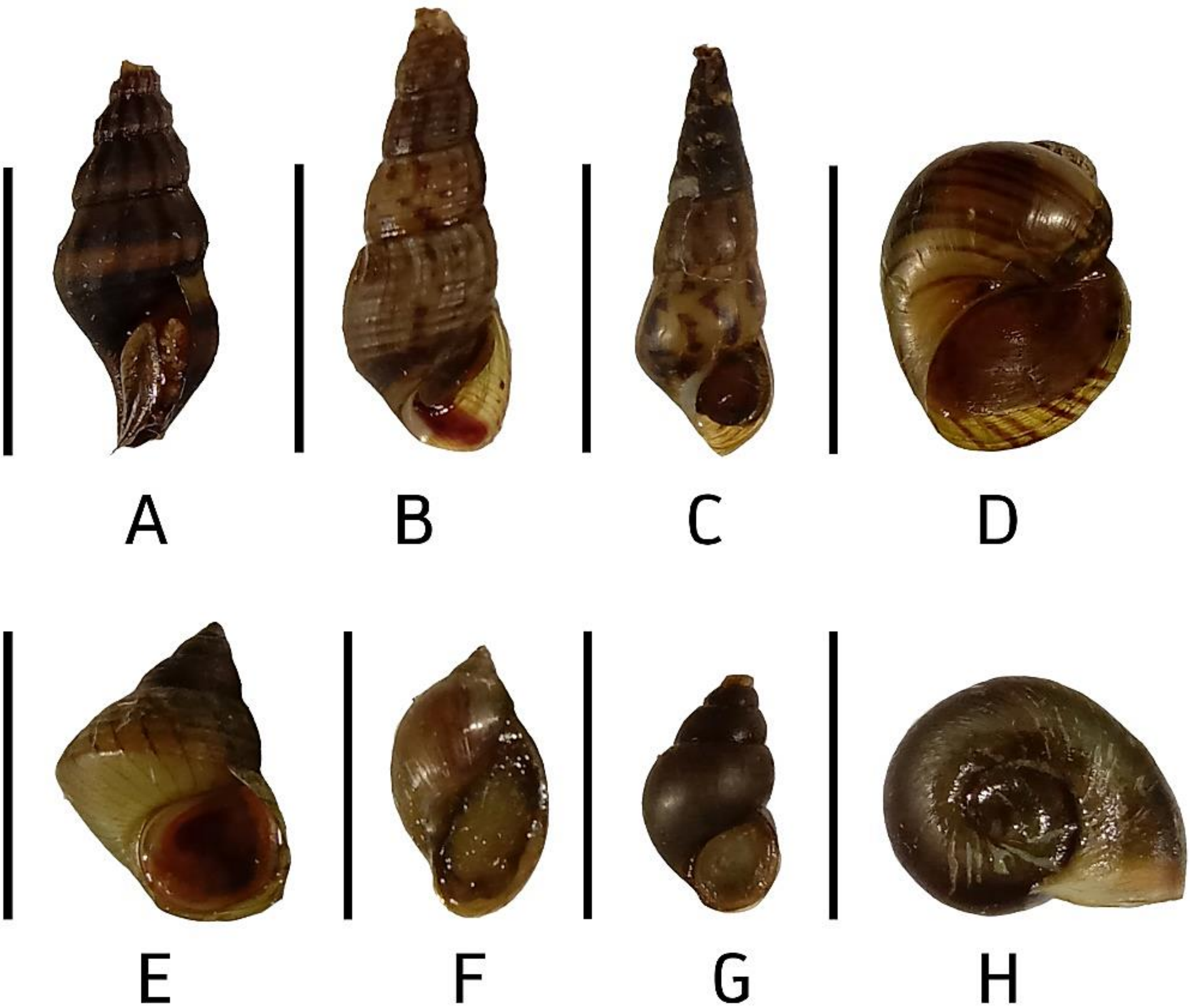

Figure 2. Shell morphology of eight snail species: A - *Anentome helena*; B - *Melanoides tuberculata*; C - *Sulcospira housei*; D - *Pomacea canaliculata*; E - *Filopaludina sumatrensis speciosa*; F - *Lymnaea* sp.; G - *Bithynia siamensis goniomphalos*; H - *Indoplanorbis exustus.* Scale bar: A - H = 1 cm.

Experiment 1: *A. helena* may serve as a biological control agent of *B. siamensis goniomphalos*

A. helena snails were used as predators to observe their ability to be competitor snails and were used as biological controls for *B. siamensis goniomphalos*. We cultured *A. helena* with different ratios of *B. siamensis goniomphalos* and divided them into 3 groups: Group I, 30 *A. helena* + 30 *B. siamensis goniomphalos*; Group II, 30 *A. helena* + 60 *B. siamensis goniomphalos;* and Group III, 30 *A. helena* + 90 *B. siamensis goniomphalos*. The experiments were performed for one week for five replicates in the laboratory. At the end of each week, the number of dead snails was recorded and analysed to qualify the daily consumption rates.

Experiment 2: Which snail species were preferred for hunting by *A. helena?*

A. helena snails were used as predators to observe their ability to be competitor snails and were used as biological controls for *B. siamensis goniomphalos* with other snail species. There were 6 groups comprising *A. helena* and various other snails as food in each group, which were raised together. *B. siamensis goniomphalos* and other snail species acted as prey. Each group contained 30 *A. helena,* 30 *B. siamensis goniomphalos* and another 30 snails, which were *Pomacea canaliculata*, *Filopaludina sumatrensis speciosa*, *Melanoides tuberculata*, *Sulcospira housei*, *Indoplanorbis exustus* and *Lymnaea* sp. in Groups I, II, III, IV, V and VI, respectively. The experiments were performed for 1 week in the laboratory (Figure 3). At the end of each day, the shells of prey were removed and replaced with another snail every day to maintain an equal snail ratio.

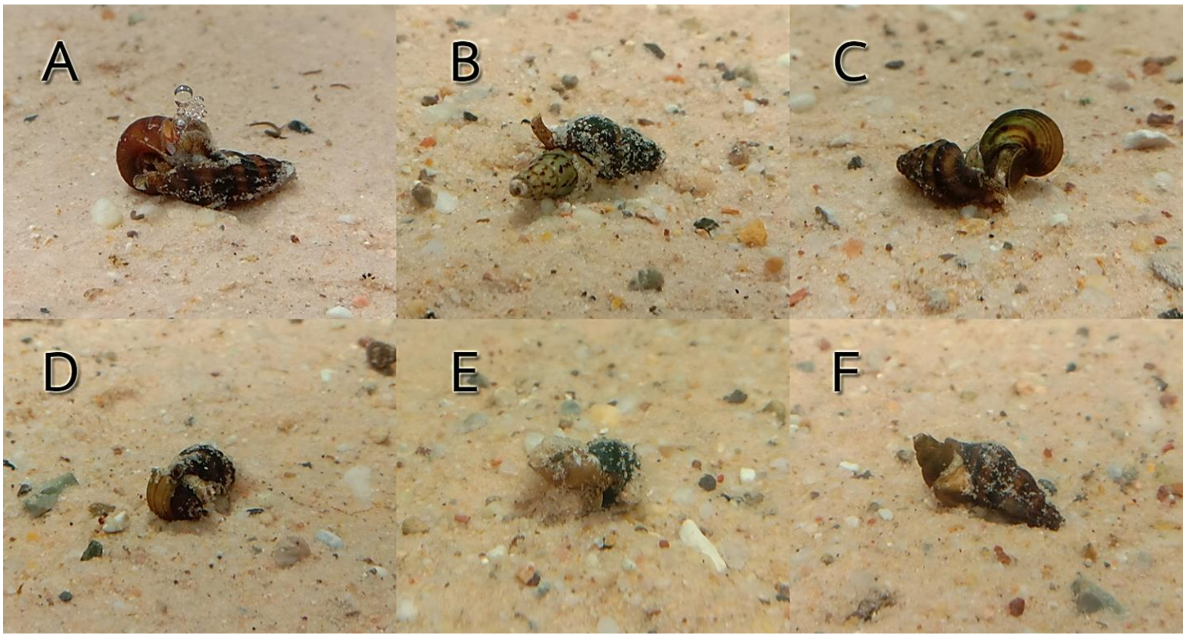

Figure 3. *A. helena's* consumption of other species: A - *Indoplanorbis exustus*; B - *Melanoides tuberculata*; C - *Pomacea canaliculata*; D - *Filopaludina sumatrensis speciosa*; E - *Lymnaea* sp.; F - *Bithynia siamensis goniomphalos.*

Statistical analysis

Descriptive statistics were calculated as the sum, average, and standard deviation. The differentiation of consumption was compared by independent samples t test with The R Project for Statistical Computing; https://www.r-project.org/. Statistical differences were considered when the *p* value was less than 0.05.

3. Results

A. helena* may serve as a biological control agent of *B. siamensis goniomphalos

This experiment observed the influence of a predator snail on the biological control of *B. siamensis goniomphalos* in the laboratory. All snails were maintained in the laboratory for 3 months before performing the experiment for laboratory adaptation. The experiments divided into 3 groups differed in the number ratio of *A. helena*: *B. siamensis goniomphalos,* with 1:1, 1:2 and 1:3 in Groups I, II and III, respectively.

The average consumption rates of the 30 *A. helena* were 16.4 ± 3.51, 25.6 ± 5.03 and 22.4 ± 3.21 snails per week in Groups I, II and III, respectively. The approximate consumption rates of *A. helena* were 0.55 ± 0.12, 0.85 ± 0.17, and 0.75 ± 0.11 snails per week in Groups I, II and III, respectively. We also found a significantly different consumption rate between Groups I and II ($t = -3.355$; $p = 0.010$) and Groups I and III ($t = -2.822$; $p = 0.022$). However, no relationship was observed between Groups II and III ($t = 1.199$; $p = 0.265$) (Table 1).

Which snail species were preferred for hunting by *A. helena?*

We kept *B. siamensis goniomphalos* with other interspecies snails to study which snails were preferred for hunting by *A. helena*. Thirty *A. helena* were reared in each group together with 30 *B. siamensis goniomphalos* and another 30 snails, which were *Pomacea canaliculata*, *Filopaludina sumatrensis speciosa*, *Melanoides tuberculata*, *Sulcospira housei*, *Indoplanorbis exustus* and *Lymnaea* sp. in Groups I, II, III, IV, V and VI, respectively. This study was conducted for 1 week and found that *A. helena* favoured snails other than *B. siamensis goniomphalos,* except for *S. housei*. We found that *A. helena* preferred *I. exustus,* followed by *P. canaliculata*, *M. tuberculata*, *F. sumatrensis speciosa* and *Lymnaea* sp.

with consumption rates of 2.67, 1.9, 1.63, 0.83 and 0.53 snails/week, respectively. No *A. helena* consumed *S. housei* (Table 2). Remarkably, *A. helena* killed *Lymnaea* sp. and ate part of them except the footpad.

Table 1. The assassin snail *A. helena* consumption of *B. siamensis goniomphalos.*

Ratio of predator: prey	Number of killed *B. siamensis goniomphalos*					Average consumption rate of 30 *A. helena* per week (mean ± S.D.)	Average consumption rate of *A. helena* per week (mean ± S.D.)
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
1:1	21	18	17	12	14	16.4 ± 3.51	0.55 ± 0.12
1:2	33	23	28	20	24	25.6 ± 5.03	0.85 ± 0.17
1:3	24	19	20	27	22	22.4 ± 3.21	0.75 ± 0.11
Total							0.72 ± 0.09

Rep = Replication.

Table 2. *A. helena* consumption of other snails that were raised together with *B. siamensis goniomphalos.*

Group	Number of freshwater snails raised together	Number of *B. siamensis goniomphalos* killed	Number of other snails killed	*A. helena* consumption rate of other snails per week (mean)
I	30 *A. helena,* 30 *B. siamensis goniomphalos,* 30 *Pomacea canaliculata*	0	57	1.9
II	30 *A. helena,* 30 *B. siamensis goniomphalos,* 30 *Filopaludina sumatrensis speciosa*	6	25	0.83
III	30 *A. helena,* 30 *B. siamensis goniomphalos,* 30 *Melanoides tuberculata*	1	49	1.63
IV	30 *A. helena,* 30 *B. siamensis goniomphalos,* 30 *Sulcospira housei*	20	0	0
V	30 *A. helena,* 30 *B. siamensis goniomphalos,* 30 *Indoplanorbis exustus*	0	80	2.67
VI	30 *A. helena,* 30 *B. siamensis goniomphalos,* 30 *Lymnaea* sp.	4	16	0.53

4. Discussion

Biological control is a method of controlling pests and pest effects using other organisms. This method can reduce pest populations by their natural enemies, including pathogens, parasitoids, predators and competitor species. The control of intermediate snail hosts of parasites is of interest in the control of snail-borne disease strategies (Sokolow et al. 2018). Many researchers have attempted to use predators to eliminate snail populations, such as carp (Ben-Ami and Heller 2001; Hung et al. 2013; Ip et al. 2014), water bugs (Younes et al. 2017, prawns (Sokolow et al. 2014), crayfish, cat fish (Monde et al. 2017), and soft shelled turtles (Dong et al. 2012). Moreover, biological control of snails using competitor snails, which have a higher ability to adapt for food, nutrients or habitat resources, has been previously reported (Gashaw et al. 2008). However, molluscivorous snails used as predators of snails are infrequently reviewed. Interestingly, we focused on *Anentome helena* (von dem Busch in Philippi 1847), which is known among aquarium enthusiasts as the "assassin snail" and is usually kept to prey on other snail species (Ng et al. 2016). *A. helena* is a freshwater snail in the family Nassariidae (Strong et al. 2017) that can be found in general water resources such as rivers, dams, ponds and ditches in a wide range of substrates, including sand, soil, mud and rock (Coelho et al. 2013). However, it is known as the assassin snail because of its ability to prey on other molluscs and it is usually kept to consume other snail species that are considered pests in home aquaria (Ng et al. 2016). Moreover, Coelho et al. (2013) reviewed the diets of *A. helena* and used *Melanoides tuberculata* as live prey for this snail. In addition, from our previous study, we found that *A. helena* consumed *Corbicula* sp., *B. siamensis goniomphalos* and *Filopaludina* spp. at the field site (Haruay and Piratae 2019).

In the present study, we evaluated the possibility of using *A. helena* as a biological control of *B. siamensis goniomphalos.* We also observed the ability of *A. helena* to be a predator of *Filopaludina sumatrensis speciosa, Indoplanorbis exustus, Melanoides tuberculata, Lymnaea* sp., *Pomacea canaliculata*, and *Sulcospira housei,* which generally live together with *A. helena* and *B. siamensis goniomphalos* in their habitats. The finding showed that *B. siamensis goniomphalos* and almost all prey species were consumed by *A. helena;* this correlates with a few studies that have focused on feeding behaviour between predator *A. helena* and other freshwater snails and concluded that *A. helena* can still be considered a generalist predator (Yakovenko et al. 2018; Berkhout and Morozov 2022) and is able to consume various snails in freshwater habitats that are known among aquarium enthusiasts as the assassin snail and is usually kept to prey on other snail species (Bogan and Hanneman 2013; Ng et al. 2016). Moreover, we found that *A. helena* preferred *I. exustus*, *P. canaliculata*, *M. tuberculata*, *F. sumatrensis speciosa,* and *Lymnaea* sp. over *B. siamensis goniomphalos*. The preference of *A. helena* for the consumption rate of their prey may occur due to various factors, such as the suitable size of target organisms or the characteristics of the shell, which affects snail aperture, speed of prey movement, and nutritional gains. In a previous study, *A. helena* feeding behaviour was observed on different prey species, including *Planorbella* sp., *Melanoides tuberculata, Lymnaea* sp. and *Tarebia granifera,* and the finding that this predator consumed all prey types was also supported. Moreover, the snail species *Planorbella* sp. was the most consumed overall, similar to our study because the most preferred snail, *I. exustus*, was classified as the same family as *Planorbella* sp. (Berkhout and Morozov 2022). Our data imply that *A. helena* can control *B. siamensis goniomphalos* and other snail populations and support forthcoming snail-control strategies by using assassin snails as a biological control agent.

5. Conclusions

In conclusion, we found that *A. helena* can consume *B. siamensis goniomphalos*; this finding is remarkable because it shows that this snail can be a competitor to snails and used as a biocontrol. Moreover, we found that *A. helena* preferred *I. exustus*, *P. canaliculata*, *M. tuberculata*, *F. sumatrensis speciosa,* and *Lymnaea* sp. which generally live together with *A. helena* and *B. siamensis goniomphalos* in their habitats. We conclude that *A. helena* may be an efficient biological control agent of *B. siamensis goniomphalos*, the first intermediate host of human liver fluke *O. viverrini*, an important result, especially in the absence of other snail species.

Authors' Contributions: HARUAY, S.: conception and design, acquisition of data, analysis of data; PIRATAE, S.: acquisition of data, drafting the article and critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: All experimental procedures using animals were approved by the Animal Care and Use Committee, Ubon Ratchathani Rajabhat University (AN63002).

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