STUDY ON THE DENSITY, HYDROSTSTIC SETTING VEOCITY AND LOCOMOTION OF *BIOMPHALARIA STRAMINEA*

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ABSTRACT: Schistosomiasis mansoni is one of the most prevalent neglected tropical diseases, affecting millions of people in developing countries. The freshwater snail *Biomphalaria straminea*, as an intermediate host of *S. mansoni*, is expanding its geographic range in Gonuangdong Province, China. It has caused potential risk of an epidemic of *S. mansoni* in southern China. This study explored its primary parameters of the hydraulics characteristics including density, hydrostatic setting velocity and locomotion in different water depth to evaluate its potential risk on transmission of *schistosomiasis mansoni*. The density and hydrostatic setting velocity of *B. straminea* were measured using the drainage volume and setting tube method, respectively. The behavior and distribution characteristics of *B. straminea* in different water depths were observed by the Plexiglas tube method. The effects of hydrostatic pressure on the climbing speed of *B. straminea* were also analyzed. The results show that the average density of the *B. straminea* is 1.08 g/cm^3. The hydrostatic setting velocity of *B. straminea* is between 2.32 cm/s and 12.92 cm/s in the water, there is no significant difference between the settling velocity and their shape type. In the Plexiglas tubes with different depths, we observed that the locomotion can occur in six manners. The *B. straminea* distributed mainly along the surface and at the bottom layer of the tubes and the proportion of the *B. straminea* on the surface water generally raised as time increased. Also, we noted a piecewise linear relationship between the climbing speed of the *B. straminea* and hydrostatic pressure in different water depth. It revealed that the climbing speed of *B. straminea* increases first and then decreases with the water depth increase, and reaches the maximum when the water depth at 120cm. The findings of this study indicate that there are significant differences between the density, the hydrostatic setting velocity and climbing speed of *B. straminea* compared with *Oncomelania hupensis*. Future studies should be conducted more deeply on hydraulics characteristics of *B. straminea* and thus establish adequate water conservancy measures to control its dispersion.

1. BACKGROUND

Schistosomiasis, a parasitic disease of considerable public health and economic significance, is caused by the trematodes of the genus *Schistosoma*. It is epidemic in 76 countries around the world with approximately 240 million people affected, which is considered by the World Health Organization as the second most prevalent parasitic disease after malaria(Gryseels, 2016). Among the pathogenic schistosomes, *Schistosoma mansoni* is the most widespread species .It is found in large parts of sub-Saharan Africa, Middle East, South America, and Caribbean, with more than 80 million people infected (Crompton, 1999). The emergence and transmission of *S. mansoni* is closely tied to freshwater snails of the genus *Biomphalaria*. It has been proved that 18 species of *Biomphalaria* serve as intermediate hosts of *S. mansoni* so far (Chitsulo *et al*, 2000). The *B. straminea* belongs to Mollusca, Pulmonata, and Planorbidae, is an important intermediate host for *S. mansoni* transmission in South America especially Brazil (Morgan *et al*, 2001). It has been expanding its geographic for its long-distance dispersal and colonization capabilities (Pointier, *et al*, 2005). In 1974, it is first reported near the Lam Tsuen valley in Hong Kong and then begins to colonize it (Meier-Brook, 1974).

In mainland China, *Oncomelania hupensis* is the obligatory intermediate host of *Schistosoma japonicum* and an essential link for schistosomiasis transmission (Hu *et al*, 2020). In 1982, the introduced *B. straminea* was first found in several watercourses in Shenzhen River region (Wang and Zhang, 1985). This phenomenon was neglected until the results of a 2012 and 2013 survey directed by the Guangdong Provincial Center for Disease Control and Prevention. It reported that the snail species was widely distributed in Guanlan River, Dasha River, Yantian River, and Kui Chung River in Shenzhen. Additionally, *B. straminea* were captured in Shima River and Danshui River in the adjacent area (Doangguan and Huizhou), which are connected to the Shenzhen water system (Huang *et al*, 2014), indicating the presence of the intermediate host snails and their survival, reproduction, spread, and formation of new habitats in natural environments of southern China. The transmission of human schistosomiasis coincides with the existence and geographic distribution of the intermediate host snails. With the promotion of the Belt and Road Initiative and rapid development of foreign economics and trade, the increasing number of migrant workers, foreign aid projects, and global trade has been contributing to a sharp increase in the numbers of imported *S. mansoni* cases in China since the 1990s (Lu *et al*, 2014; Wang *et al*, 2013). Thus, close attention should be paid to the potential risk of the transmission of *S. mansoni* in mainland China (Wang *et al*, 2013; Zeng *et al*, 2017)

Knowledge of the distribution and dispersion of *B. straminea* can provide helpful information to set-up effective snail control programmers. Previous studies have focused on the biotic and abiotic factors such as temperature, precipitation, elevation, vegetation, water quality, velocity, substrate type (Wang and Zhang, 1985; WHO, 1957; Kloos H *et al*, 2001; Rollinson, 2011; Yang *et al*, 2018). As an aquatic snail, the *B. straminea* spread with water flow is an important way to expand its range for its weak athletic ability (Barbosa and Barbosa, 1994; Sarquis *et al*, 1998). Thus, its hydraulic characteristics contribute greatly to its dispersal in water column, because the features of setting, threshold, and transport directly affect its migration and diffusion in the water, which in turn affects its spread in different regions, but the current research is relatively limited. In this paper, we analyzed and studied the snails' density, hydrostatic setting velocity and locomotion of *B.straminea* to promote the understanding of its hydraulic characteristics that can provide the basis for taking water conservancy measures to prevent and control the diffusion of *B. straminea* in the near future.

2. MATERIALS AND METHODS

2.1 Materials

The *B. straminea* were sampled from the wetland at Shenzhen East Lake Park in Apr, 2019, it was undertaken by two experienced field collectors using scoops and forceps. The Sampling time was fixed to 2 hours and performed 8:30 h and 10:30 h. Sampling area was along streams shoreline with the lengths of 50 m. Collected snails were transferred in perforated plastic containers to the laboratory of Hubei Provincial Center for Disease Control and Prevention where they were processed. Snails were identified to species level based on shell morphological and reproductive system characteristics, following the published identification guidelines and descriptions from previous reports on snail sightings in the region (Pan American Health Organization, 1968; Liu *et al*, 1982; Habib *et al*, 2018).Thus, approximately 1000 alive *B. straminea* were collected. The snails were maintained at several aquariums with the dechlorinated water (temperature 23 ± 0.5 ℃) and fed with lettuces every day. These containers were cleaned every two days and water was drained and refilled to maintain water quality.

2.2 Methods

2.2.1 Determination of B. straminea's density

The density of *B. straminea* was determined by volumetric drainage method (i.e., Archimedes' principle) at the laboratory. Before the measurement, we carefully took out the living snails using tweezers and put them on water-absorbent paper to make the surface fully dry. The test steps were as follows: (1) took a dry clean cylinder (size 25 ml in 20 ℃) and individually weighed the quality (M_1, the precision is 0.0001 g); (2) randomly selected 20~30 dry *B. straminea* and put them into the cylinder, and weighed the quality of the snails and the cylinder (M_2); (3) measured the distilled water temperature, then carefully add distilled water into the cylinder near 25 ml and lightly shaking the cylinder to make the bubble discharge. (4) kept the cylinder static for 1~2 h to make the water fill the body of *B. straminea*, fixed the capacity to 25 ml, and obtained the weight of the cylinder, snails, and water (M_3); (4) poured the water (including *B. straminea*) from the cylinder, cleaned and wiped the cylinder surface, added water to 25 ml, and obtained the quality of the water and cylinder (M_4). The density (ρ_s) was obtained as follows:

$$\rho_s = \frac{m_2 - m_1}{m_2 - m_1 + m_4 - m_3} \bullet \rho_w \tag{1}$$

where ρ_w refers to the density of distilled water at measured temperature. The experiment was repeated 10 parallel times, and took the arithmetic mean of the 10 analytical results as average density.

2.2.2 Determination of the B. straminea's hydrostatic setting velocity

The setting tube method was applied to determine the hydrostatic velocity. Maximum shell diameter and apertural height of the *B. straminea* were recorded by a vernier caliper (made by Shanghai Tool Works Co., Ltd with accuracy of 0.02mm) before the experiment. The setting tube was 160 cm long and the inner diameter was 10 cm, with an effective observation length of 130 cm. The settling time was measured by an electronic stopwatch with a precision of 0.01 s. The *B. straminea* was put into the setting tube which filled with water (temperature 22.0 ± 0.5 ℃, pH = 6.8) and allowed it to freely set in the water individually. We observed and recorded the setting state and settling time of the *B. straminea* during the settlement process. Thus, the hydrostatic setting velocity ($v = L/T$) of each snail could be calculated by the setting distance (L) and time (T). Three delivery methods are used when the snails put into the tube: aperture upward, aperture downward, and flatwise, and the setting velocity was calculated respectively.

2.2.3 Locomotion of B. straminea

The locomotion experiment was conducted in several Plexiglas tubes with a height of 160 cm and an inner diameter of 10 cm. The plexiglass tube was divided into 15 groups according to the water depths of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, and 150 cm (24.0 ± 0.8 ℃, pH = 6.9). Twenty live snails were put into the bottom of each tube, and then we observed the climb height and recorded the motion direction of *B. straminea* in each tube every 10 minutes. The experiment was repeated three times for each group and obtained the average value of the motion speed of *B. straminea* for each group.

2.2.4 Data analysis

All data were set up by Microsoft Excel 2003 (Microsoft Corporation; Redmond, WA, USA), and statistical analysis were carried out by SPSS 13 (SPSS, Inc.; Chicago, IL, USA) statistical software. The hydrostatic settling velocity and spatial distribution of *B. straminea* in tubes were fitted by frequency distribution; and the water pressure level and climbing speed was fitted by the linear regression method. The differences in the hydrostatic setting velocity in different types of snails and the climbing speed among the *B. straminea* under different water pressure levels was analyzed by one-way Analysis of Variance, and a pairwise comparison was made by the Student-Newman-Keuls method. The differences in the percent of *B. straminea* at top 5cm layer (or bottom 5cm layer) in different time was also analyzed by one-way ANOVA , P-values less than 0.05 were considered statistically significant.

3. RESULTS

3.1 The density of *B. straminea*

The density of B. straminea ranged from 1.04 to 1.15 g/cm^3, and the mean average density was 1.08± 0.03 g/cm^3.

3.2 The hydrostatic setting velocity of *B. straminea*

The *B. straminea* curled up its visceral mass to the shell when we placed it into the water. The setting state of *B. straminea* in water could be divided into two types: (1) circling down in a horizontal state (the number of snail occupied by this state accounted for 95% of the total number of snail); and (2) swaying down (the number of snail occupied by this state accounted for 5% of the total number of snail). In general, the setting velocities of the snails ranged from 2.32 to 12.92 cm/s, but mainly were focused between 7.00 and 11.0 cm/s. The results of one-way ANOVA showed that there was no significant difference between the hydrostatic velocity of snails with different group (Ⅰ~Ⅴ) (F = 0.701, P = 0.592)（Tab.1）.

Table 1 Hydrostatic settling velocity of *B. straminea*

Group	Number of *B. straminea*	*B. straminea shape*		Settling velocity		
		Diameter (mm)	Apertural height (mm)	Max. value (cm/s)	Min. value (cm/s)	Average value (cm/s)
Ⅰ	36	8.00~9.28	3.24~3.80	12.36	7.32	9.15
Ⅱ	100	7.00~7.88	3.02~3.22	12.61	4.94	9.15
Ⅲ	134	6.00~6.98	2.60~3.00	12.92	2.32	8.79
Ⅳ	36	5.02~5.96	2.40~2.58	11.31	4.65	8.66
Ⅴ	20	3.44~4.92	2.18~2.32	10.16	6.77	8.61
Average		6.75	5.51	11.87	5.2	8.87

3.3 The motion way of *B. straminea*

Different experimental groups of *B. straminea* present different motion features after we put them into the bottom of Plexiglas tubes. The following behavioral patterns were observed: climbing up along the pipe, climbing down along the pipe, climbing up to the surface water and hanging still, climbing up to a certain height and hanging still, crawling at the bottom of the tube, and keeping still at the bottom of the tube.

Many *B. straminea* crept up along the pipe wall within 30 min (Fig. 1), especially in the experimental groups with water depths greater than 40 cm. In the water depth of 130 cm, the proportion of the snails that climbed up along the pipe was the largest. When the water depth was less than 40 cm, the proportion of the snails that climbed up along the pipe was less than 20%. At the end of our observation (120 min), numerous *B. straminea* had climbed to the surface water and remained motionless, which was a significant increase when compared with the results after 30 min.

Some snails also climbed down along the pipe wall (Fig. 1). Within 30 min, this case accounted for a small proportion of the total snails with an average value of 7.4% in the experimental groups, whereas the maximum value of 36.8% appeared at a depth of 40 cm. After 120 min, the number of snails that climbed down along the pipe wall increased significantly compared with the other times, and the average value was 13.1%. The maximum value appeared at a water depth of 140 cm, up to 35.5%. At the same time, some *B. straminea* remained at the bottom or crawled at the bottom. As time increased, this proportion of *B. straminea* at bottom gradually decreased and remained stable below 15% when the water depth was more than 80 cm.

The motion pattern also showed that most *B. straminea* tended to move toward the water surface in different experimental groups. As time increased, the proportion of the snails' distribution in the surface water became larger, whereas the snails at the bottom of the water constantly decreased. After 120 min, most of the *B. straminea* had distributed in the water surface and at the bottom, and both could be accounted for the largest proportion (94.4%) of the total snails in the 30 cm experimental group, whereas the smallest proportion (50.8%) appeared in the 80 cm experimental group, and the average value was 69.8%. The results of one-way ANOVA revealed that there was significant differences in the percent of *B. straminea* at top 5cm layer or bottom 5cm layer in different time (F=13.219, P = 0.000).

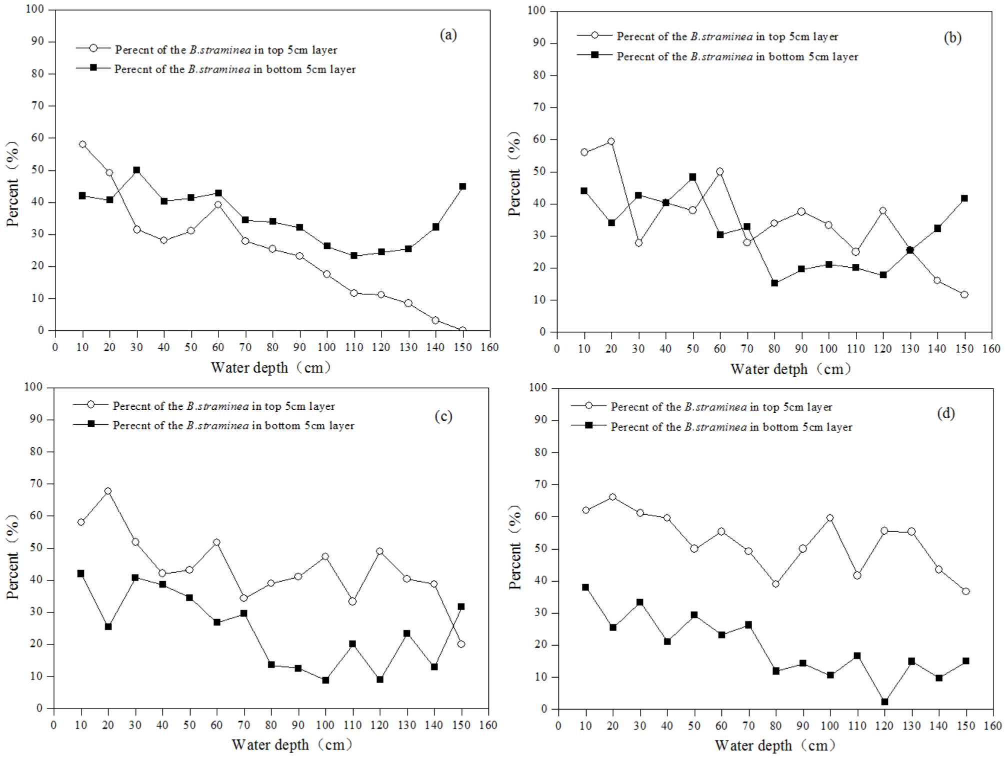

Figure 1 Percent of different motion types of B. straminea account for total snails, in different water depths at different time after the experiment beginning (a: 30min; b: 60min; c: 90 min; d: 120min).

3.4 The climbing speed of *B. straminea*

According to the experimental water depth of 10~150 cm of the different tubes, we divided the water pressure in the Plexiglas tube into 1~15 corresponding levels (a 10 cm depth of water corresponds to one level, which was approximately 980 Pa). The one-way ANOVA results showed a significant difference (F = 55.596, P = 0.000) in the average climbing speed of snails under different pressure levels in still water. Additionally, we used the Student-Newman-Keuls method to compare the climbing speed of different pressure grades with a pairwise comparison; the classification of each grade is shown in Tab. 2. From Tab. 2, the climbing speed of snails can be classified into six groups in 1–15 water pressure levels (corresponding to the depths of 10~150 cm), indicating that *B. straminea* is sensitive to different water pressure.

Table 2 Category of snail climbing speeds under different water pressure levels

Grade of water pressure (980Pa)	Category of snails climbing speeds (cm/s)					
	1	2	3	4	5	6
1	0.018431					
2		0.029458				
3		0.030375				
4			0.042601			
5			0.044156			
6				0.053251		
7				0.054362		
8				0.054769		
9					0.065233	
10					0.065458	
11					0.066233	
15						0.075181
14						0.078500
13						0.081578
12						0.084378

The relationship between the hydrostatic pressure level and the average climbing speed of the B. straminea is presented in Fig. 2. The average climbing speed of the snails increased as the water pressure increased when the water depth was below 120 cm. When the water depth was 120 cm, the average climbing speed reached the maximum 0.084 cm/s. The average climbing speed, however, decreased gradually as the water pressure increased when the water depth was more than 120 cm.

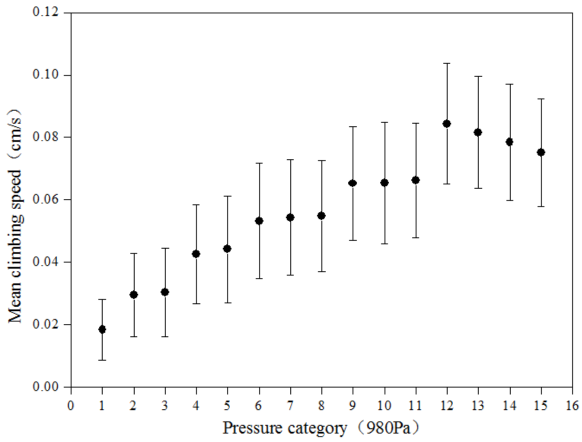

Figure 2 Mean climbing speed of *B. straminea* under different water pressures

Taking the average climbing speed of *B. straminea* as the dependent variable, and the static water pressure as the independent variable, thus, the regression equation of the average climbing speed and the hydrostatic pressure of the *B. straminea* in static water were able to obtained by the piecewise linear fitting method in equation (2)and (3).

$$v = 5\times10^{-6}P + 0.0178(10 \le h \le 120cm) \quad (2)$$

$$v = -3\times10^{-6}P + 0.1213(120 < h \le 150cm) \quad (3)$$

$$P = \rho g h_w \quad (4)$$

where v is the average climbing speed of the *B. straminea* in static water; P is the pressure of the location of the *B. straminea* in water; the unit is *Pa*, which can be obtained from equation (4); h_w is the height from the bottom of the water; ρ is the density of water; and g is the acceleration of gravity.

4. DISCUSSION

As a part of integrated schistosomiasis control programmers, snail control strategy is considered a priority for the reduction of schistosomiasis transmission (Lardans and Dissous, 1998). In China, a large number of water conservancy projects shave been developed by engineers to prevent and control the spread of *O. hupensis* and block the transmission of *Schistosoma japonicum* for the intensive research on snail’s hydraulic characteristics(Rollinson *et al*, 2013). These measures have played an important role in schistosomiasis elimination agenda and attain great social and economic benefits in endemic areas(Lu *et al*, 2020; Yuan *et al*,2005) These experience could provide technical guidance for the *B. stramine* control, which should hopefully combating the infectious disease epidemics through China’s Belt and Road Initiative(Chen *et al*, 2019). However, as one of intermediate host snail of *S. mansoni*, the study on the hydraulics characteristics and dispersal laws of *B. stramine* is still deficient. Our study has been novel in revealing to elucidate the density, hydrostatic setting velocity, locomotion of *B. straminea*. The density and hydrostatic setting velocity of *B. straminea* are important parameters affecting its diffusion of in water column. As a basic physical parameter, the density directly influences its force condition and its drift distance in sluggish or torrent water. The hydrostatic setting velocity of *B. straminea* reflects how fast it sinks from the water surface to the bottom. Obviously, under the same flow conditions, the *B. straminea* with smaller setting velocity may drift further distance in water environment. The locomotion of *B. straminea* is a response to external biotic or abiotic stimuli, and they will move towards favorable places which suitable for them. These have a direct effect on its dispersion in water volume. This research represents several primary parameters of its hydraulic characteristics that can provide the information for taking water conservancy measures to control the diffusion of *B. straminea* and may facilitate the progress towards the elimination of *S. mansoni*.

Our results showed that the average density of the *B. straminea* is 1.08 g/cm^3, which is less than the average density of *O. hupensis* of 1.80 g/cm^3 (Xu *et al*, 1993), and slightly higher than the density of water, indicating that *B. straminea* do not easily sink in water. We also found that *B. straminea* could float on the water surface by stretching its body, and with the aid of the surface tension of water, could float for several hours without sinking, which was more obvious in smaller snails in the experiment. The study on the hydrostatic settling velocity of *O. hupensis* by Yang *et al.* (1994) showed that the settling velocity of the snail was between 0.94 and 16.25 cm/s and that the settling velocity of the *O. hupensis* of different ages was significantly different. With an increase in the age of the snail, the hydrostatic velocity also increased, and the Pan *et al.* (1998) experiment obtained similar results. In our experiment, we did not conduct the study on the *B. straminea*’s age; however, according to Ituarte’s (1989) research on the growth curves of *B. straminea* in Artigas, Uruguay, a significant exponential relationship was existed between size (diameter) and age for the snails, and the Von Bertalanffy's equation could be described it. Thus, the age increases with increasing shell diameter. There was no significant correlation between the hydrostatic setting velocity and the size of the *B. straminea*, indicating that there was no significant difference between the settling velocity and age, which is obviously different from *O. hupensis*. This suggests that if we adopt water conservancy measures to prevent and control the spread of *B. straminea*, we should fully consider the difference between it and *O. hupensis*.

Some experiments indicated that the intermediate host snail distributed inhomogeneous at different water depth. Corr *et al.* (1984) observed the distribution of *B. glabrata* in water by using several 15 cm long plastic tubes. He found that the *B. glabrata* spent 58% of the time in bottom, 35% in surface, and the snail in these two regions can accounted for 93% of the total number. Another study lead by Jurberg *et al.* (1987) on *B. glabrata*’s distribution was conducted in two 8.4 m long tubes. He notes that the frequency of *B. glabrata* distributed in surface area is 50% and 45%, while corresponding lower part is 16.6% and 29.2% individually. The proportion of the *B. glabrata* distributed in the surface and lower part of the water generally accounted for more than 65% of the total population in both two groups. Our study found that some *B. straminea* moved upward while some *B. straminea* move downward at the water column, but the proportion of *B. straminea* moved upward is larger than that moved downward (Fig. 2). The *B. straminea* distributed in the water surface and the bottom layers were more than 50% of the total population. Although the distribution of *Biomphalaria spp.* in different water layers is different, however, they had characteristics of tight ends and a thin middle in still water on the whole, indicting the middle part only a transition levels for the snails. Similar phenomenon also occurred on the distribution of *O. hupensis* in different water depth (Yuan *et al*, 2010).

Biomphalaria spp. was considered to be perched in shallow bodies of water (Rollinson, 2011). Barbosa and Barbosa (1994) pointed out that the maximum depth of the life of the *B. straminea* generally does not exceed 1.52 m in nature environment. WHO (1957) also reported that it was difficult to find traces of *B. straminea* below water depths of 1.5~2.0 m. However, many investigators noticed the presence of *Biomphalaria spp.* in deeper water column. Mandahl-Barth (1954) demonstrated that the *Biomphalaria smithi* could be found at a depth of 4.3m in Lake Edward and *Biomphalaria choanomphala* at 12.2 m in Lake Victoria; Freitas (1976) noted *B. glabrata* at a depth of 4~5 m at the bottom at the Lagoa Santa in Brazil. Thus, the depth of survival of some *Biomphalaria spp* in water may exceed our usual cognition. Jurberg *et al.* (1988) built transparent chambers capable of withstanding pressures corresponding to 48.8 m depths, and found the *B. glabrata* can survival and later lay eggs freely during 48 hours. Our study revealed that under the water depth of 1.5m, the *B. straminea* can well survived and crawled at the bottom for a period of time. These implied that water pressure couldn't be the main reason for the distribution of the snail in water, but other factors, such as water temperature, light, food, dissolved oxygen, may play a more decisive role for the distribution of the snail in different water layers.

External stimulus would influence the movement of the *B. straminea* in the water. Several experiments had been conducted on the effect of light and molluscacide on the kinetic behavior of *B. straminea* (Sarquis *et al*, 1998; Schall *et al*, 1986), however, little is known about the locomotion speed of *B. straminea* in response to hydrostatic pressure. In our study, the measured mean climbing speed of *B. straminea* ranged from 0.018 to 0.084 cm/s at different hydrostatic pressure. This result is close to the locomotion rate of *B. straminea* in water with the illumination varying from 2.8 to 350 lux (Schall *et al*, 1986), but it is greater than the climbing speed of the *O. hupensis* under different water depth (Yuan *et al*, 2010). It indicated that the stimulation of light and water pressure on *B. straminea* was different, but the effects on kinetic behavior were similar. Also, the faster locomotion of *B. straminea* would be related to its more developed gastropod, and had greater ability to migrate from one location to another. Compared with the *O. hupensis* climbing speed decreases with the increase of water depth, there was a turning point in the climbing speed of *B. straminea* under the water depth of 120cm. It showed that at a depth less than 120 cm, with an increase in pressure, *B. straminea* was sensitive to stimulation and could crawl upward at a faster speed. When the water depth was more than 120 cm, however, *B. straminea*'s climbing speed gradually slowed, possibly because it may have exceeded its pressure bearing ability. According to equation (3), when the water depth exceeded 4.0 m, the speed of climbing up the pole snakehead was 0; this can be used as a reference for the reasonable establishment of snail control measures.

In Pearl River delta area, due to the abundant precipitation and numerous watercourses, the frequent floods would easily to facilitate *B. straminea* formation of new breeding sites. In addition, the anthropogenic activities, such as irrigation, fishing, aquaculture, may also enlarge its distribution in this area. Moreover, the ongoing global climate change, especially global warming, would certainly create an appropriate condition for its survival and reproduction in other places. It also have been predicted that *B. straminea* will spread to southern parts of Guangxi、Fujian and Yunnan provinces, as well as the North of Taiwan (Habib *et al*, 2016). Thus, the *B. straminea* dispersion should be closely monitored and effective snail control interventions should be implemented.

5. CONCLUSION

This study revealed the density, the hydrostatic setting velocity and locomotion of *B. stramine* in water column. We suggest that further studies should be undertaken on setting, threshold, transport and other hydraulic characteristics of *B. stramine* in natural water environment, especially in running water conditions. Also, the study on multiple habitat factors influence on the hydraulic characteristics should be conducted at the same time. All of these efforts will hopefully establish adequate water conservancy measures to control the spread of *B. stramine* and facilitate the progress towards the elimination of *S. mansoni*.

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