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Snails (Mollusca) diversity and its distribution in various type of microhabitats

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Abstract

Snails have some preferences in the way they choose to inhabit and multiply in certain areas. Therefore, a study was conducted to study the microhabitats that influence snail diversity. A total of nine species of 581 snails were collected and examined comprising both terrestrial and freshwater species. The number of snails found in each habitat was significantly different across all locations except for the habitat of the living plant. The water that exists in the sampling areas was examined and only water pH was found to have a positive relationship with the number of snails, ($r=0.573$, $P<0.05$). The dissolved oxygen has a significantly negative relationship with the total number of snails collected ($r=-0.776$, $P<0.01$). Besides, the relationship between vegetation species present in the area with several snails was also studied. The *Macrochlamys* sp. have a significant positive relationship with vegetation species *Mikania micrantha*, *Vigna* sp. and *Panicum repens*, ($r=0.710$, $P<0.01$), ($r=0.714$, $P<0.01$) and ($r=0.710$, $P<0.01$), respectively. These grasses are preferred by the *Macrochlamys* sp. snails probably due to the high ability of the grasses to absorb nutrients from the soil and their high tolerance to herbicides. Besides, these grasses could survive throughout the year despite extreme conditions and for that reason, they can serve the nutrients that snails needed.

1. INTRODUCTION

Snails belong to the phylum Mollusca which is a classification of invertebrate animals with soft unsegmented bodies and some of the members may have exoskeletons on or shells. The phylum may consist of over 100,000 living species and thousands of fossil species making it the second-largest animal phylum (WHO, 2015). Snails can be found everywhere despite of wide different range of habitats. They can be found in terrestrial, coastal, freshwater, and as many as in marine ("Snail World," 2014). The snail herbivory on green plants may damage plant crops as well as gardens. However, the pest status of snails varies greatly on location and time. In some countries, a snail is considered as delicious delicacy. From the street market to high-class restaurant, snail shares the same status as fish, chicken, swine and cow.

Freshwater snails and some terrestrial snails have received so much attention for their role as intermediate hosts for several types of helminths causing parasitic diseases as well as food-borne diseases in people and animals. Most digenetic trematode species which are generally termed flukes seem to require a molluscan host to complete their life cycles. They need at least one or as many as three additional hosts apart from the principal host (Sri-aroon et al., 2005). A study has shown that many species of molluscs in Thailand serve as intermediate hosts of parasites (Tesana, 2003). Parasitic disease infection caused by parasites that harbour snails as intermediate host is widespread and more prevalent in areas providing the snail favourable environmental conditions with a wide range of food source availability. Water resource development schemes have profound consequences on the introduction and spread of schistosomiasis.

WHO in 2014 estimated more than 350 snail species are possible for medical or veterinary importance with all people susceptible, and children have a higher rate of re-infection after treatment than adults. The intermediate host snail can be divided into two main groups: (1) aquatic snail and (2) amphibious snail. Aquatic snails live underwater and could not survive outside water, while amphibious snails are characterized by their ability to adapt to living in and out of water. In 2015, WHO estimated at least 56 million people were reported to be infected by pathogenic flukes other than schistosomes. Trematode infection is not only associated with human and livestock farms; it is also important in fish disease. Fish-borne zoonotic trematodes, such as Clonorchis sinensis, heterophyids, and others constitute a public health concern especially in some parts of northern Vietnam (Madsen and Hung, 2015). This trematode is often believed to have a link with fishpond culture (Thien et al., 2015). The transmission pattern of the trematode is determined by the distribution and density of the first intermediate host, which is a freshwater snail because each parasite develops in a specific snail species.

Horgan et al. (2014) studied the impact of invasive apple snails on the function and services of natural

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and managed wetlands. Their study showed that two species of snail, *Pomacea canaliculata* and *Pomacea maculata* have become major pests of aquatic crops such as rice in Southeast Asia and these species of apple snail have a remarkable preference for most classes of macrophyte. Other studies by Li et al. (2009) on snail herbivory on submerged macrophyte show that *Physella cubensis* had a high preference for grazing. They conducted a study in Lake Taihu, China and reported that *Physella cubensis* preferred to graze on the aquatic plant such as *Vallisneria spiralis*, *Potamogeton malaianus, Elodea nuttallii,* and *Hydrylla verticillata*.

Snails perhaps have some preference in the way they choose to inhabit and multiply in that area. This study was conducted to determine the habitat preference of different species of snails and tried to understand the relationship of snail abundance with the environmental parameters present in that specific area such as the vegetation species, and the chemical properties of the water including the pH, dissolved oxygen, and others. The outcome of this study can be used in control and management strategies to reduce the number of snails that is the intermediate host and eventually reduce the economic damage and control disease outbreak.

2. MATERIALS AND METHODS

2.1 The study area

A total of four sampling sites with different habitats were sampled: livestock farm, river, paddy field and terrestrial (Table 1). The first site was a livestock farm located in Kg. Valdor, Sg. Bakap, Penang. Sampling site in Kg. Valdor has long grasses and weeds that cover the area. The field is used as a grazing area for the non-commercial farmers that live nearby. There was a chicken poultry located beside the field which was poorly managed and not clean. The chicken feces and other organic waste were washed off into the drainage that separated the poultry from the grass field. The soil in the area was humic and moist.

The next location is Kg Titi Tok Aris located near an oil palm plantation. The palm trees were tall in which sunlight can barely penetrate the farm floor. The grasses and the weeds present there were medium-sized tall. There was a small cowshed beside the sampling area where all the waste material was washed off to the small drainage that separate these two areas. The owner of the cowshed let his cow forage in the palm tree area, thus the cow feces can easily be seen everywhere.

Another sampling site was in Sg. Kob, Kulim, Kedah and Gua Kerbau, Kodiang, Kedah. The river in Sg. Kob was wide, deep, and had a relatively strong current with many large rocks in the middle of the river. There were houses near one side of the riverbank and the other side was covered with many types of vegetation. The residents threw their domestic waste into the river as many non-degradable materials can be seen in the river.

Table 1: Sampling site with coordinates.

Sampling site	Coordinate
Kg Titi Tok Aris	5°26'35.2"N100°33'16.9"E
Kg Valdor	5°14'07.0"N100°29'20.9"E
Sg. Kob	5º42'35.25"N100º64'24.12"E
Gua kerbau	6°23'40.36"N100°18'55.09"E
Kg. Bendahara	6°21'55.2"N100°20'21.3"E
Kg. Kodiang Lama	6°22'53.2"N100°19'09.2"E
USM lake area	5°35’37.5”N100°30’06.3”E
USM lecture hall	5°35’70.2”N100°30’14.5”E

The sampling site in Gua Kerbau is in the river at the recreational park. The river was wide and shallow with many pebbles. There were boulders and rocks in the middle of the river which slowed down the flowing of water to the shallower part of the river. The river was surrounded by paddy fields. Another sampling site was at a paddy field, Kg. Bendaha and Kg. Kodiang Lama. Both paddy fields have relatively the same environmental condition. The sampling was conducted during the pre-harvesting season when the paddy plant was tall but not yet ripening. Fern from the genus *Azola* covered the exposed water surfaces. The last sampling site was studied within USM main campus located in an area near the lake as well as in the lecture hall. The area near the lake was shaded from sunlight as there were many tall trees all around. The area was covered with grasses and some weeds in small size.

2.2 Snail and habitat parameters samplings

At each sampling site, a 1-meter square quadrate was chosen randomly. Three quadrates were applied randomly. All snails within this area were observed and collected. Visible snail present within the parameter was collected by hand and/or forceps for minute snail. The presence of submerged snails was observed and collected from the water using the aquatic net. All collected snails were kept in a container with all the resources (water, soil, vegetation). Collected snails were kept alive. All containers used were labelled according to date, location, and replicates. Collected snails were observed for their characteristics and were identified to family, genera, and species level where possible. The shell of the snail gave distinguishable characteristics for identification. The identification was based on various key identification from a combination of multiple literature materials authored by Wright (1957), Kathryn et al. (2004), and Somsak and Burch (2004). After identification, the snails were released back to their habitat. Meanwhile, the chemical properties of water such as water pH, dissolved oxygen (DO) and water temperature were carefully checked using the YSI multiprobe meters.

The vegetation present within the 1-meter square quadrate was observed. The percentage abundance of each vegetation species present in the quadrate was observed and recorded. Ten samples for each vegetation species presence there were collected and identified. All the vegetation samples were observed for their characteristics and were identified to species level where possible, based on various key identification from literature materials authored by Mohamad et al. (1987), Helena (2005) and Barnes and Luz (1990).

2.3 Statistical analysis

The statistical analysis was used to determine the significant relationship between abundance of snails with the water parameter and type of vegetation present across the locations. All statistical analysis in this study were performed using IBM SPSS Statistics 22 software. The significant different between both water parameters and type of vegetation among locations were determined using Kruskal Wallis test as their values deviated the normality and homogeneity assumptions.

3. RESULTS

3.1 Snail composition at all selected locations

There was a total of 581 snails from eight locations sampled in this study (Table 2). Among them, Kg. Titi Tok Aris has the highest number of snails collected which were 265 individuals from all three quadrates with three different species. *Physella acuta* were found in the greatest number out of all the three species which were 126 individuals. The species with second highest number of individuals was *Physella cubensis* in which 33 individuals were collected in the location. The least number of snails found there was *Macrochlamys* sp. in which only seven snails were collected.

Table 2: Percentage of snail at each sampling sites.

Location	Snail species	Number of snail (%)
Kg. Titi Tok Aris	*Physella cubensis*	5.7
	Physella acuta	21.7
	Macrochlamys sp.	2.2
Kg. Valdor	*Macrochlamys* sp.	17.1
Sg. Kob	*Filopaludina* sp.	2.2
Gua Kerbau	*Pomacea maculata*	6.5
Kg. Bendahara	*Pomacea canaliculata*	2.4
	Pomacea maculata	5.2
Kg. Kodiang Lama	*Pomacea canaliculata*	2.6
	Pomacea maculata	3.4
USM lake area	*Subulina octona*	25.7
USM lecture hall	*Achatina fulica*	4.1
	Macrochlamys sp.	1.0

Only three locations recorded greatest snail species which were Kg. Titi Tok Aris, Kg. Bendahara and Kg. Kodiang Lama (Figure 1). In Kg. Titi Tok Aris, collected snails were from species *Physella cubensis*, *Physella acuta* and *Macrochlamys* sp. while in Kg. Bendahara and Kg. Kodiang Lama, the snail species found were *Pomacea canaliculata, Pomacea maculata* and *Pomacea* sp. The least number of species of snail were collected from Kg. Valdor, Sg. Kob and USM lake where only one species was found in each location. The total number of snails species collected in each location were significantly different at F=19.741, $P<0.05$. The median for the number of snails found in Kg. Titi Tok Aris was the highest among all locations (median = 58). The boxplot was comparatively tall suggesting that the number of snails was spread in a wide range (min = 39, max = 69). The shortest boxplot was in Sg. Kob that has a median of 2. It had the lowest value of Q1 and Q3 which were 1.5 and 3 respectively.

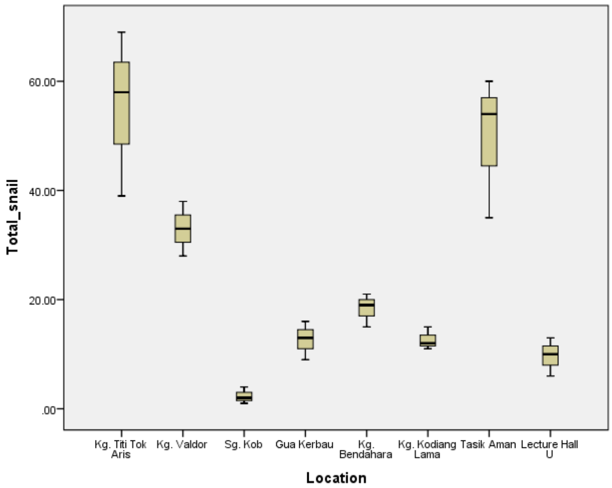

Figure 1: Boxplot of snail distribution in all locations.

3.2 Snail habitats

There were several habitats examined during this study such as rocks, living plant, dead matter, intermittent puddle, soil, stream, and paddy field. Most of the snail species were found on rocks followed by intermittent puddle and soil. Among all locations sampled, there were four locations with the greatest number of snails found. In Kg. Titi Tok Aris, the habitats of snails *Physella acuta, Physella cubensis* and *Macrochlamys* sp. were the living plant, dead matter and intermittent puddle. Out of these three locations, snails *Physella acuta* and *Physella cubensis* were found in high number in the intermittent puddle (120 individuals). Only *Macrochlamys* sp. were found on living plants.

In Kg Bendahara, snails *Pomacea canaliculata, Pomacea maculata* and *Pomacea* sp. were found abundantly attaching themselves to different size of rocks. Snails were also found attaching themselves to dead matter and on a dead-decaying tree trunk, while the rest were collected in the river. The area near the USM lecture hall provided three different habitats for *Achatina fulica* and *Macrochlamys* sp. *Achatina fulica* was known as a garden pest and occurred in high numbers and always clumped together. Most of them were found attached to the wall of

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the building. As they moved, they defecated, thus leaving behind the mark of their existence. They are also commonly found near the edge of the building on the grass and on bare soil. Meanwhile, snails were found at USM Lake surrounding a terrestrial species. They are attracted to places with high humidity and constantly wet but not submerged. *Subulina octona* was the only species found in this location and commonly found attached to moist rocks that are shaded from sunlight. The highest number of snails was collected from intermittent puddles. The habitat that harbored the least number of snails was the stream which only 13 individuals were sampled.

The number of snails collected in each habitat was normally distributed but not homogenous, thus further analyses were done using the Kruskal-Wallis test. The number of snails collected in each habitat across all locations was significantly different except for the living plant (Table 3). Some snails were found in water, thus water body properties in those locations were studied. These locations were Kg. Titi Tok Aris, Sg. Kob, Gua Kerbau, Kg. Bendahara and K. Kodiang Lama (Table 4). The water temperatures of all five locations sampled were relatively the same. The location with the greater mean water temperature was Gua Kerbau (28.23±0.18℃) whilst the least mean water temperature was in Sg. Kob with 26.67±0.09℃. The mean water pH was recorded in Kg. Titi Tok Aris was slightly alkaline (8.59±0.09), while other locations recorded the same range of value (pH 6). Meanwhile, the dissolved oxygen in water (DO) for each location was different. The highest DO level was in Sg. Kob (79.5%). While the lowest DO level recorded in Kg. Bendahara (15.6%). The water in Sg. Kob also had the highest mean water velocity among all (1.07±0.03 m/s), whereas in other locations, the water was stagnant or relatively slow running. In every habitat observed, there were always biotic factors that took place, and this study was also interested in understanding the relationship between snails and vegetation.

USM Lake has the highest number of plant species collected. They were *Ischaemum inolicum, Paspalum orbiculare*, *Cyperus kyllingia*, *Ageratum conyzoides* and *Cyperus rotundus* (Table 5). Among these five vegetation species, *Ischaemum inolicum* reported the highest abundance (40%). The plant with the least percentage abundance was *Ageratum conyzoides* (5%).

The sampling location for both Kg. Bendahara and Kg. Kodiang Lama was a paddy field area, thus, 80% percent of the area was filled with *Oryza sativa*. Another 20% of the area was filled with *Azolla* sp. that covered the exposed water surface. The vegetation is present in Kg. Titi Tok Aris was *Ischaemum muticum* and *Alocasia* sp. The *Ischaemum muticum* covered most of the area with some of them submerged in the water puddle. The other 5% of the area was filled with *Alocasia* sp. where water was present. The *Macrochlamys* sp. were found in three different locations with different species of vegetation present. *Mikania micrantha, Vigna* sp. and *Panicum repens* have a significant positive relationship with the snail *Macrochlamys* sp, (r=0.710, P<0.01), (r=0.714, P<0.01) and (r=0.710, P<0.01), respectively.

Table 3: Statistical analysis of snails collected in each habitat across all locations using the Kruskal Wallis test.

Habitat	Chi-square value (x^2)	*P*-value
Rock	38.87	0.00
Living plant	12.29	0.10
Dead matter	30.62	0.00
Intermittent puddle	23.59	0.001
Soil	33.64	0.00
Stream	21.91	0.003
Paddy field	37.26	0.00

Table 4: Mean of physicochemical parameters of the water body for each location.

Location	Mean ± SE			
	temperature (℃)	pH	DO (%)	depth (cm)
Kg. Titi Tok Aris	27.7±0.75	8.59±0.09	16.43±0.45	22.33±1.76
Kg. Valdor	NA	NA	NA	NA
Sg. Kob	26.67±0.09	6.27±0.03	79.5±0.50	40.67±2.03
Gua Kerbau	28.23±0.18	6.33±0.03	34.73±0.07	3.93±0.97
Kg. Bendahara	27.03±0.15	6.10±0.10	15.57±0.03	10.33±0.60
Kg. Kodiang Lama	27.17±0.15	6.27±0.03	15.97±0.20	7.00±0.58
USM lake area	NA	NA	NA	NA
USM lecture hall	NA	NA	NA	NA

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Table 5: The abundance of vegetation species in all locations (%).

Location	Vegetation species	Percentage (%)
Kg. Titi Tok Aris	*Ischaemum muticum*	95
	Alocasia sp.	5
Sg Kob	NA	
Kg. Valdor	*Mikania micrantha*	25
	Vigna sp.	25
	Panicum repens	50
Kg. Bendahara	*Oryza sativa*	80
	Azolla sp.	20
Kg. Kodiang Lama	*Oryza sativa*	80
	Azolla sp.	20
USM lake area	*Ischaemum inolicum*	40
	Paspalum orbiculare	15
	Cyperus kyllingia	25
	Ageratum conyzoides	5
	Cyperus rotundus	15
USM Lecture Hall	*Axonopus compressus*	70
	Chrysopogon ocicularis	30

4. DISCUSSION

Snails collected in Gua Kerbau were from genus *Pomacea* that commonly found in paddy field (*Pomacea maculata* and *Pomacea canaliculata*). The river located near extensive paddy field area might be the reason for this snail's species to be recorded abundantly. Snail species from this genus were invasive species. Once they introduced to an area, they spread rapidly through bodies of water such as canals and rivers and during floods (Rama Rao et al,. 2018). Paddy fields provided a suitable breeding site for the parasite due to fertilizer used by farmers that acts as nutrient sources for the snails. Continual soil moisture throughout the year has helped snails to avoid desiccation thus remain in the paddy field for generations. *Pomacea canaliculata* or apple snail has become a serious pest of rice throughout many countries of south-east Asia. In the Philippines, it was considered the number one rice pest and has caused huge economic losses (Robert, 2004). In the paddy fields sampled, *Azolla* sp. was observed covering the water surface. This snail species was reported to have preference on this aquatic fern. This was supported by a previous study (Pouil et al, 2020) that used *Azolla* sp. as plant attractants served to divert the apple snail (*Pomacea canaliculata*) away from the rice field, hence facilitated the collection of the snails. This study concluded that the use of different *Azolla* species as biological attractants could control apple snail population.

Subulina octona that was found in USM lake area was a tropical snail and commonly found in ground litter and of moist surface. It fed mostly on plant materials and debris because its radula was not specialized (Lucie, 2006). This might be the reason a lot of them were found attached to rock and leaf litter. *Subulina octona* might become a minor pest in gardens or nurseries by making holes in cultivated plant leaves. This species was rarely found alone and mostly found in small groups. In the area of the lecture hall, two species were found: the *Achatina fulica* and *Macrochlamys* sp. The area was always moist and might be preferred by the snails. This species is also an invasive species that could inhabit a wide range of environments and the occurrence in high numbers is often regarded as a pest because it could damage plants and crops. *Achatina fulica* fed primarily on vascular plant matter, having no preference for whether it was alive or dead matter (Menno & Rutjes, 2001).

Physella acuta and *Macrochlamys* sp. snails were found in high abundance in Kg. Titi Tok Aris and Kg. Valdor, both locations were livestock farm areas. Grazing areas near the cowshed usually consist of green medium size grasses that may become suitable places for the snails to harbor their eggs and provide a source of food as well (Naomi et al., 2013). A well-rearing farm normally has only one grazing area that has been used continuously over a period of time. Too many livestock in one single grazing area will increase the risk of infection. A study conducted in Okinawa, Japan reported that *Macrochlamys* sp. was abundant in disturbed habitats and seems to be breeding in the wild.

Physella acuta in this study was found in both intermittent puddles and streams. This was confirmed by a study that reported that an important attribute to exploit both lotic and lentic water bodies was its relatively high tolerance of current velocity (Appleton, 2003). Besides, a study reported that the largest number of samples was reported from the habitat in which the water conditions were described as perennial, standing, and the high abundance of aquatic vegetation (Kock and Wolmarans, 2007). This supports the finding of this study in which *Physella acuta* was sampled in area covered with *Ischaemum muticum* and *Alocasia* sp.

Every collected snail species has a positive relationship with the vegetation present in the location. *Macrochlamys* sp. was found in three different locations with different species of vegetation per location. However, *Macrochlamys* sp. has a positive relationship with vegetation; *Mikania micrantha, Vigna* sp. and *Panicum repens.* Snails have food preferences that only feed on certain species of vegetation and are present abundantly in areas where the preferred vegetation was abundant. This result was in agreement with another study that found snail density is depending on the growth of macrophytes (Agnieszka, 2002). The snails probably preferred these

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grasses due to their high ability to absorb nutrients from the soil and high tolerance to herbicides. These grasses could survive throughout the year despite extreme conditions and served the snails the nutrients that they needed. *Panicum repens* were not sensitive to pH (Wilcut et al., 1988), had high tolerance to herbicides and its rhizomes absorbed more nutrients and water in a stressful environment (Peng, 2012).

Using this knowledge, a control strategy could be planned to control snails that have medical and economic importance. The number of snails could be reduced by managing their habitats. The preferred vegetation could be removed from the area, which in return reduced the number of snails present there. Farmers could also plant the preferred vegetation in small spots of the farm to attract snails, thus snails will not cause damage to the crop plant. The accumulated snails can easily be killed and disposed of without affecting other areas.

The number of total snails collected showed a negative relationship with the increasing dissolved oxygen level. Changes in dissolved-oxygen levels often impact freshwater invertebrates by causing their deaths. Each invertebrate can survive at different levels of oxygen, and so a change in oxygen level alters the varieties of invertebrates present in a body of water (David and Christopher,2020). The previous study found snails in the less oxygenated environment were more active, while snails in the oxygenated environment were mostly inactive (Taryn, 2005). One of the major characteristics of freshwater snails that affect their activity levels in the presence of low dissolved oxygen was their ability to self-regulate their oxygen intake. Some freshwater snails are capable of anaerobic metabolism, which allows them to survive in low-oxygen environments (David and Christopher,2020).

Snails have their optimum pH value to survive and reproduce, and in that case, the value may vary from one another. If the pH in the environment or surrounding was too low or too high, the aquatic organism living within it will move away or die. The water pH values were found higher in that area might be due to the high content of feces of cattle from the nearby cowshed.

5. CONCLUSION

Nine species of snails were collected and examined from all locations. They were *Physella cubensis*, *Physella acuta*, *Macrochlamys* sp., *Filopaludina* sp. *Pomacea maculata*, *Pomacea canaliculata*, *Subulina octana*, *Achatina fulica.* Different species of snails were recorded at different microhabitats in the sampling areas. This may be due to the different types of environmental resources and vegetation present in the area. The vegetation species: *Mikania micrantha*, *Vigna* sp., and *Panicum repens* have a significant positive relationship with the snail species. This study provides baseline information on the relationship between snail species and their habitat preferences.

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