



Influence of physico-chemical parameters on the occurrence of parasites in the freshwater snails in the endemic areas of Lanao del Norte

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Abstract

The correlation between the level of infection of the freshwater snails (infected with cercariae, infected with other parasites and non-infected) and physico-chemical parameters (pH, temperature, total dissolved solids, altitude, number of plant species and water depth) were studied at 30 areas composing four sites in Lanao del Norte from April 2017 – August 2018. *In situ* determinations of the parameters were done prior to the explorative investigative malacological sampling. The snails were cleaned and brought to the laboratory for microscopy to identify the parasites they harbored. ANOVA showed that pH, number of plant species and temperature significantly varied among the sampling sites. Consequently, the different levels of infection also had significant variation. Correlation analysis between pH and infected snails showed a negative correlation and a positive correlation to non-infected. Temperature showed a positive correlation to the infected and negative correlation to the non-infected. Number of plant species showed negative correlation to all levels of infection. Regression analysis showed a strong positive correlation between the pH and the non-infected snails. This finding can be in agreement to the previous studies conducted that the pH had a significant role in the metabolic activities of the snails further influencing their physiological response to parasite infection. Nevertheless, the importance of the other physico-chemical parameters used in this study cannot be discounted. They were part and parcel of the complex interplay of the habitat factors that may or may not affect the level of infection in the freshwater snails.

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Introduction

The interaction between the host and the parasites can be influenced by the environmental conditions including the physico-chemical factors of a given area because they regulate the physiological condition, reproduction and survival of both parasites and their host (Lenihan, *et al.*, 1999).

Physico-chemical factors, on the other hand, namely, pH, temperature, and depth can influence the diversity of the intermediate host of schistosomes (Alhassan, *et al.*, 2016). Several studies had been conducted on this field like the study by Alhassan, *et al.* (2017) which cited a significant influence of the physico-chemical parameters on schistosome intermediate host composition. Casavechia, *et al.* (2017) cited evidences in their study that pointed out the influence of environmental conditions on the occurrence of parasitic infection. Furthermore, a similar study conducted in Nigeria, cited the significant influence of pH, temperature and dissolved oxygen to the proliferation of the intermediate hosts of trematode parasites (Abe, *et al.*, 2017). Moreover, fluctuations in population density of planorbid host of *S. mansoni* were pointed to be influenced by climatic factors (Camargo, *et al.* 2015).

The knowledge on the factors that affected the level of infection of the parasites to the intermediate host can be very crucial especially if the main aim will be on the eradication of the debilitating zoonotic diseases. Through this information, localized strategies will be tailored to the local level to better guide and support control and management interventions of zoonosis. This study was conducted to provide information on the physico-chemical parameters that played an important role in the infection with parasites. Geared on the data that will be provided herein, strategic information can be provided to come up with a plan for the control and management of zoonotic diseases.

Materials and methods

Study area

Three endemic municipalities in the Province of Lanao del Norte (Figure 1) were purposely sampled for this study, namely: Municipality of Lala (07°58'N 123°45'E), Municipality of Kapatagan (07°54'N 123°46'E), and Municipality of Salvador (07°54'N 123°51'E). The selected municipalities were among the one hundred ninety (190) municipalities in the Philippines that are endemic for schistosomiasis. The areas consist wide plain mostly dedicated to rice farming.



Fig. 1. Geographical location of the study sites.

There were a total of 30 sampling areas (Table 1) which were divided into four sampling sites according to their land-use. The first site were all irrigation canals, including water served by the National Irrigation Authority (NIA) or private irrigation canals to rice fields and other agricultural lands. Second Site included ricefields – covering those which were recently plowed, harvested, idle and planted. The third site were flowing bodies of water including, river, streams, and creeks. And the last site composed of waterfalls and seepages.

Determination of physico-chemical parameters

In situ determination of the pH, temperature, total dissolved solid, altitude, number of plant species and water level were done prior to the actual collection of samples. pH, temperature and total dissolved solids were measured using portable probe instrument while the water level was measured using a steel tape. Coordinates and altitudes were also taken using a hand-held GPS.

Collection and Identification of Freshwater Snails

An explorative-investigative sampling technique was employed in the malacological sampling that was done on the months of April 2017 – August 2018. A standardized 15-minute period was applied to all sites to ensure uniformity in the collection of data. The samples were cleaned, stored in a poly-ethylene bag and transported to the laboratory for identification, grouping and cercarial examination.

Examination and Detection of Parasite infection

There were two steps for the determination of the

parasite infection used in this study, the Isolation Method/Monitoring of cercariae shedding, for larger sized snails, following the steps used by Frandsen, *et al.* (1984) and Squash technique, for the smaller sizes, using the procedures by Caron, *et al.* (2008). Consequently, the identification of the cercariae and parasites were made possible by the identification manuals of Frandsen, *et al.* (1984), Farahnak, *et al.* (2006), Mohammed, *et al.* (2015), and Chontanarath, *et al.* (2017).

Data analysis

Variation in the physicochemical characteristics were determined using the Analysis of Variance (ANOVA) at $p \leq 0.05$. Furthermore, the determination of the correlation between the significant physico-chemical characteristics and the level of infection were computed using the correlation coefficient and were further subjected to regression analysis to test for further significant relationship of the correlation.

Results and discussions

Physico-chemical parameters and level of infection

Table 2 shows the mean, standard error and significance of the physicochemical parameters and level of infection of the collected snails.

It can be observed from the table that among the six physicochemical parameters that were tested, only pH, number of plant species and temperature showed significant difference in one or more sampling sites. The other three parameters, namely, total dissolved solids, altitude and water level did not manifest any significant differences among the sites.

Table 1. Complete Details of the Sampling Sites.

Site	Coordinates		Vegetation
Irrigation Canal (Site 1)			
Pinuyak 1, Lala, LDN	7°55.580"N	123°43.583"E	<i>Oryza sativa</i> , <i>Ipomoea aquatic</i> , <i>Paspalum conjugatum</i> Brgius, <i>Musa acuminata</i> , <i>Bambuseae</i> , <i>Cocos nucifera</i>
Tenazas, Lala, LDN	7°56.100"N	123°46.783"E	<i>Musa acuminata</i> , <i>Paspalum conjugatum</i> Brgius, <i>Colocasia esculenta</i>
Sto. Thomas 1, Kapatagan, LDN	7°54.770"N	123°47.384"E	<i>Musa acuminata</i> , <i>Colocasia esculenta</i> , <i>Paspalum conjugatum</i> Brgius, <i>Cocos nucifera</i> , <i>Terminalia catappa</i>
Sto. Thomas 2, Kapatagan, LDN	7°54.758"N	123°47.377"E	<i>Paspalum conjugatum</i> Brgius
Curva-Miagao, Salvador, LDN	7°54.052"N	123°49.271"E	<i>Colocasia esculenta</i> , <i>Paspalum conjugatum</i> Brgius
Inagasan IC, Salvador, LDN	7°55.025"N	123°50.827"E	<i>Paspalum conjugatum</i> Brgius
Cumpra IC, Salvador, LDN	7°54.106"N	123°49.583"E	<i>Musa acuminata</i> , <i>Colocasia esculenta</i>

Cumpra IC 2, Salvador, LDN	7°54.108"N	123°49.581"E	<i>Musa acuminata, Colocasia esculenta</i>
Ricefield (Site 2)			
Lala Proper Schoolside RF, Lala, LDN	7°57.855"N	123°44.508"E	<i>Oryza sativa, Paspalum conjugatum Brgius, Colocasia esculenta</i>
Butadon, Kapatagan, LDN	7°53.220"N	123°44.701"E	<i>Colocasia esculenta, Paspalum conjugatum Brgius, Cocos nucifera</i>
CumpraMiagao RF, Salvador, LDN	7°53.572"N	123°49.561"E	<i>Paspalum conjugatum Brgius, Musa acuminata</i>
Flowing Waterbodies (Site 3)			
Lala Proper Schoolside Creek	7°57.343"N	123°48.082"E	<i>Bambuseae, Oryza sativa, Colocasia esculenta, Citrus, Cocos nucifera, Mangifera indica, Musa acuminata, Pandanus amaryllifolius</i>
Suba 5, Tenazas, Lala, LDN	7°56.395"N	123°47.535"E	<i>Gliricidia sepium, Chrysophyllum cainito, Cocos nucifera, Durio, Sandoricum koetjapa, Leucaena leucocephala, Terminalia catappa, Colocasia esculenta, Mangifera indica</i>
Abaga Bridge, Lala, LDN	7°56.232"N	123°46.038"E	<i>Colocasia esculenta, Pteridophytes, Bambuseae, Paspalum conjugatum Brgius, Musa acuminata, Cocos nucifera</i>
Simpak Creek, Lala, LDN	7°56.170"N	123°44.918"E	<i>Colocasia esculenta, Cocos nucifera, Terminalia catappa, Musa acuminata, Bambuseae, Pteridophytes</i>
Tunaan Creek, Lala, LDN	7°58.742"N	123°45.806"E	<i>Paspalum conjugatum Brgius, Gliricidia sepium, Musa acuminata, Cocos nucifera</i>
Rebe 1, Lala, LDN	7°54.822"N	123°47.818"E	<i>Nymphaea spp, Bambuseae, Ipomoea aquatica, lemon, Swietenia macrophylla, Annona squamosa, Gliricidia sepium</i>
Rebe 2, Lala, LDN	7°54.262"N	123°47.840"E	<i>Bambuseae, Musa acuminata, Artocarpus odoratissimus, Colocasia esculenta</i>
Cabasagan Stream, Lala, LDN	7°58.007"N	123°48.068"E	<i>Musa acuminata, Mangifera indica, Colocasia esculenta, Bambuseae, Cocos nucifera, Imperata cylindrica, Tamarindus indica, Saccharum, Gliricidia sepium, kaimito, Terminalia catappa, Artocarpus odoratissimus, citrus</i>
Cabasagan Creek, Lala, LDN	7°57.343"N	123°48.082"E	<i>Bambuseae, Oryza sativa, Colocasia esculenta, citrus, Cocos nucifera, Mangifera indica, Musa acuminata, Pandanus amaryllifolius</i>
Maguindanao Creek, Lala, LDN	7°56.805"N	123°48.084"E	<i>Gliricidia sepium, Cocos nucifera, Bambuseae, Leucaena leucocephala, Moringa oleifera, Paspalum conjugatum Brgius, Colocasia esculenta</i>
Cabasagan Tributary 2, Lala, LDN	7°57.602"N	123°47.054"E	<i>Paspalum conjugatum Brgius, Colocasia esculenta, Moringa oleifera, Cocos nucifera</i>
Cabasagan Tributary 1	7°57.701"N	123°46.984"E	<i>Paspalum conjugatum Brgius, Colocasia esculenta, Moringa oleifera, Cocos nucifera</i>
Adjacent to Municipal Hall, Lala, LDN	7°57.896"N	123°46.640"E	<i>Paspalum conjugatum Brgius, Leucaena leucocephala</i>
Near Lanipaobrgy. Hall, Lala, LDN	7°57.613"N	123°46.603"E	<i>Paspalum conjugatum Brgius, Colocasia esculenta,</i>
Cumpra Miagao River, Salvador, LDN	7°53.552"N	123°49.625"E	<i>Colocasia esculenta, Paspalum conjugatum Brgius, Bambuseae</i>
Pinuyak 2, Lala, LDN	7°55.588"N	123°43.689"E	<i>Leucaena leucocephala, Musa acuminata, Paspalum conjugatum Brgius, Colocasia esculenta, Ipomoea aquatica, Musa acuminata, Cocos nucifera, Swietenia macrophylla, Nymphaea spp, Crescentia cujete</i>
San Vicente Creek, Kapatagan, LDN	7°53.141"N	123°46.724"E	<i>Paspalum conjugatum Brgius, Colocasia esculenta, Cocos nucifera, Terminalia catappa</i>
Waterfalls/Seepages (Site 4)			
Cathedral Hills seepages, Kapatagan, LDN	7°52.266"N	123°46.315"E	
Waterfalls, Kapatagan, LDN	7°52.233"N	123°46.639"E	<i>Oryza sativa, Colocasia esculenta, Cocos nucifera, Leucaena leucocephala, Gmelina arborea</i>

Subsequently, the level of parasite infection varied among the sites based from the computed p value of 0.04, 0.02 and 0.00 for snails infected with cercariae, infected with other parasites and non-infected snails, respectively. These differences in the level of infection can be attributed to the different predisposing factors, like anthropogenic practices (Karesh, *et al.*, 2012), environmental changes (McMichael, *et al.*, 2018) and other abiotic and biotic factors, which can be suitable and conducive for the parasites and host snails to survive. The fact that most of the water running in these sites primarily came from the Municipality of Salvador, the different level of infection would

support the claim that the current levels of human-ecosystem interaction were driven by increased environmental encroachment and land-use change as cited by Hassell, *et al.* (2016).

As presented in Figure 2, it can be observed that most (58.31%) of the non-infected snails were sampled from Site 3, followed by those snails sampled from the Site 4 (25.64%), Site 1 (10.64%) and from the Site 2 (5.41%). This data could show that the bodies of different freshwater in the areas provided a conducive environment for the gastropods to grow and develop the necessary immunity against parasite infection,

hence, resulting to a highest level of non-infected snails from these sites. On the other hand, the low number of non-infected snails in rice fields could show that the different intervention done by the

farmers to control the freshwater gastropods infestation in their farm altered the physiology of the snails making them prone to infection.

Table 2. The mean \pm standard error and level of significance of each physicochemical parameter and data on the level of infection.

		Site 1	Site 2	Site 3	Site 4	Significance
Physico-chemical Parameters	pH	6.88 \pm 0.14	7.13 \pm 0.34	7.30 \pm 0.05	7.37 \pm 0.18	0.03*
	Temperature	30.33 \pm 0.48	33.58 \pm 1.78	30.29 \pm 0.41	30.30 \pm 1.17	0.04*
	TDS	92 \pm 10.03	152.11 \pm 90.2	97.35 \pm 5.69	152 \pm 85	0.24
	Water level	21.25 \pm 5.32	6.87 \pm 2.05	21.12 \pm 2.74	22.50 \pm 14.50	0.33
	Number of plant species	2.5 \pm 1.41	2.67 \pm 0.58	6 \pm 2.90	2.5 \pm 3.53	.01*
	Altitude	29.4 \pm 14.8	23.67 \pm 11.93	25.56 \pm 7.78	35.5 \pm 6.36	0.51
Level of Infection	Number of snails Infected with cercariae	5.00 \pm 0.63	2.33 \pm 2.33	0.06 \pm 0.06	3 \pm 3	0.04*
	Number of snails infected with other parasites	51.25 \pm 19.38	47.67 \pm 28.64	9.88 \pm 2.51	0.00 \pm 0.00	0.02*
	Non-infected snails	22.88 \pm 10.99	31 \pm 16.52	59 \pm 18.15	220.50 \pm 47.50	0.00*

*significant at $p \leq 0.05$.

Consequently, the high level of snails infected with other parasites were observed to be highest on Site 1 (56.87%), followed by Site 3 (23.30%) and on Site 2 (19.83%). Site 4 did not have snails infected with other forms of parasites. The irrigation canals had been a suitable place for many parasite because it had been a depository place of many organic waste from residential houses, excretion from the grazing

animals and the out flow of organic minerals from the rice fields which had been a conducive resource that can support the proliferation of parasites.

The waterfalls, on the other hand, did not have any parasites sampled from the snails because of the less vegetational cover, fast water flow, complete exposure to sunlight and rocky substrate.

Table 3. Correlation coefficient between the significant physico-chemical parameter (pH) and the level of infection.

	pH	cercariae infection	infection with non-cercariae	not infected
pH	1.00000			
cercariae infection	-0.21196	1.00000		
infection with non-cercariae	-0.55731	0.20216	1.00000	
not infected	0.04170	0.27114	-0.29220	1.00000

Table 4. Regression Analysis between pH and level of infection.

	Coefficients	Standard Error	t Stat	P-value
Intercept	7.34	0.09	84.21	0.00
cercaria infection	-0.01	0.03	-0.38	0.71
infection with non-cercaria	-0.01	0.00	-3.27	0.00
not infected	0.00	0.00	-0.61	0.55

Cercariae infected snails, on the other hand, were highest on the samples from Site 2 (36.84%), followed by those from Site 4 (31.58%), Site 1 (26.32%) and from Site 3 (5.26%). It can be observed that the

cercariae sampled from this study favored areas which were less turbulent, stable and had a direct sun exposure. The snails shedding these cercariae were sampled from the areas which were shallow, less

disturbed and more stable. It was generally observed from the study that those areas with fast flow were less likely to have snails infected with parasites or cercariae. However, those samples from Sites 1, 3 and 4 that harbored parasites or cercariae were sampled from more stable areas with less water current disturbance.

Correlation and regression analysis between the pH and levels of infection

Although all of the physico-chemical parameters that were tested in this study were relevant in the

abundance and distribution of both the snails and parasites, only the significant parameters were further tested to other statistical tools.

The significant parameters were then subjected to coefficient correlation to verify for the significant relationship of each parameter to the level of infection in the different sites. This test was done to further elucidate the contribution of these parameters to the infection of parasites in the different freshwater gastropods sampled in the area. The first significant physico-chemical parameter tested was the pH.

Table 5. Correlation coefficient between the significant physico-chemical parameter (Temperature) and the level of infection

	<i>Temp</i>	<i>cercariae infection</i>	<i>infection with non-cercariae</i>	<i>not infected</i>
<i>Temp</i>	1.00000			
<i>cercariae infection</i>	0.06664	1.00000		
<i>infection with non-cercariae</i>	0.00941	0.20216	1.00000	
<i>not infected</i>	-0.26463	0.27114	-0.29220	1.00000

The hydrogen ion concentration of the water had been an important factor for the metabolic activities of the aquatic organism (Wang, *et al.*, 2002; Puinyabati, *et al.*, 2013). Based from the data obtained (Table 3), it can be observed that pH was negatively correlated to the number of snails with cercariae infection and to the snails infected with other parasites. This result means that as the pH of the water in the sites increased, the level of infection

would decrease. Parasite may favor slightly acidic environment over basic.

This result was in contrast to the findings of the study of McDevitt-Galles, *et al.*, (2018) where pH, among other factors, had a significant positive effect on the infection status of the parasite to their host. They further added that any changes in the pH can alter the survival of free-living infectious stage of the parasite.

Table 6. Regression Analysis between Temperature and level of infection.

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>
<i>Intercept</i>	31.19	0.55	56.26	0.00
<i>cercariae infection</i>	0.20	0.21	0.94	0.36
<i>infection with non-cercariae</i>	-0.01	0.01	-0.65	0.52
<i>not infected</i>	-0.01	0.01	-1.71	0.10

Subsequently, pH was positively correlated to the number of non-infected snails. These findings implied that as the pH of the water in the area would increase, there would also be an increased in the number of uninfected snails. The correlation between the significant parameters and the level of infection were further subjected to another statistical test to additionally explore the significant connection between these correlations. Tables 4, showed the

regression analysis between pH and the level of infection.

The negative correlation between the pH and the infection of snails with cercariae was found to be statistically insignificant based on the p-value of 0.71. This means that the correlation was not significant and the increase in the pH did not necessarily imply a decrease in cercarial infection.

Table 7. Correlation coefficient between the number of plant species and the levels of infection.

	number of plant species	cercariae infection	infection with non-cercariae	not infected
Number of plant species	1.00			
cercariae infection	-0.32	1.00		
infection with non-cercariae	-0.31	0.20	1.00	
not infected	-0.25	0.27	-0.29	1.00

Moreover, the result of the regression analysis between the pH and the snails infected with other types of parasites including protozoan and microscopic organism or combination of both showed a significant negative correlation based on the p-value of 0.00. The result showed that the infection with other types of parasites in the freshwater gastropods sampled in the areas were affected by any changes in

the pH level, hence, any natural or artificial modification of the pH can be strategy to control or enhance parasite infection. This study found out that the increase in pH will decrease the infection rate of the snails, hence, interventions that can lessen the acidity of the water in the area should be implemented to control the population of the parasites.

Table 8. Regression Analysis between the number of plant species and the levels of infection.

	Coefficients	Standard Error	t Stat	P-value
Intercept	6.07	0.77	7.87	0.00
cercariae infection	-0.26	0.29	-0.87	0.39
infection with non-cercariae	-0.03	0.01	-1.98	0.06
not infected	-0.01	0.01	-1.66	0.11

Consequently, the positive correlation between the pH and the non-infected snails had a non-significant relationship based on a p-value of 0.55

Correlation and regression analysis between the temperature and levels of infection

Temperature was considered as one of the stress-inducing environmental factors that could interfere with the immune response of the snails to prevent from parasitic infection (Knight, *et al.* 2015; Coelho, *et al.*, 2006; Pila, *et al.* 2017). When temperature was tested with the level of infection (Table 7), it was observed that both the snails with cercarial infection and infected with other parasites were positively correlated with the temperature. However, the numbers of the non-infected snails were negatively correlated to this parameter. Positive correlation implied that when the temperature raises the infection of both the cercariae and other parasites would also increase, this is because accordingly, aside from impairing the immune response of the snails, the increase in the temperature could reduce the time

necessary for the parasites to develop or the cercarial development time (Camargo, *et al.*, 2015; Morley, *et al.*, 2010). Furthermore, the increase in temperature can also alter the distribution of the snails that are the intermediate host of the parasites, hence, increasing the potential number of parasite infection. The temperature rise can be an optimal condition for the host snails and parasites to breed, grow and survive. This outcome could also give agreement to the results of McCreech, *et al.*, (2014), Banhela, *et al.* (2017) that the infection rate of schistosomiasis can increase dramatically with small increase in the levels of temperature, thus, the onset of climate change can result to an alteration in the population size of the host snails, parasite density and disease epidemiology Kalinda, *et al.* (2017).

Consequently, the positive correlation between the temperature and the snails infected with cercariae and snails infected with other parasites had a non-significant relationship based in the p-value of 0.36 and 0.52, respectively. A non-significant relationship

was also evident on the negative correlation between the temperature and the non-infected snails based on a p-value of 0.10. This implied that there were no significant connections between the correlations. The temperature parameter as observed in this study cannot singly affect the infection rate of the snails, however, together with other environmental parameters they could also be one of the factors in the complex interplay of habitat components that either

be suitable or detrimental to the survival of the intermediate host snails and parasites. These findings can be in agreement to the observations made by Utzinger, *et al.* (1997) that none of the chemical parameters that they examined in their study was significant, hence, they concluded that there were interplay with complex interactions of habitat factors and pH among others was just only a part and parcel of that interaction.

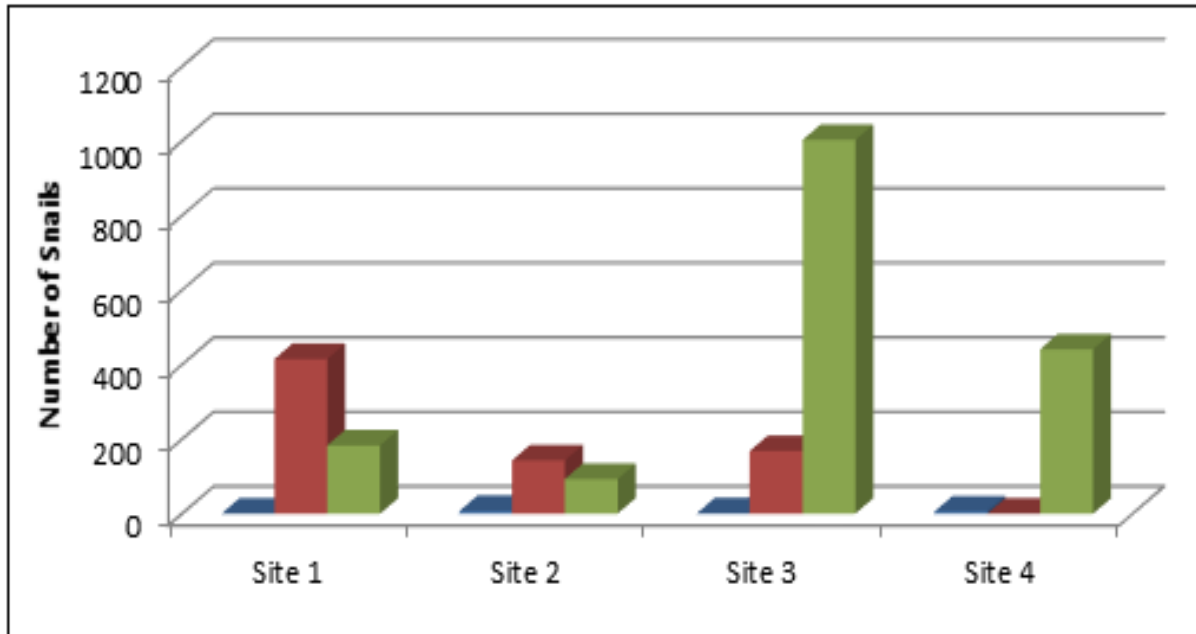


Fig. 2. The level of infection per site.

Correlation and regression analysis between the number of plant species and levels of infection

The number of the plant species had been a vital component for the survival and proliferation of the host snails of the parasites. They can support a high density of host snail population in the area (Usman, *et al.*, 2017). However, in the correlation between the number of plant species and the levels of snail infection, it was found out that the number of plant species had a negative correlation to all levels of infection, as shown in Table 7.

Table 8, further, showed the regression analysis between the negative correlation of the number of plants species and levels of infection. It can be observed that the infection with non-cercariae had a moderate negative correlation to the number of plant species while the other two levels of infection did not

have a significant role.

Conclusion

With the aim to elucidate the influence of the physico-chemical parameters to the level of parasite infection in the freshwater snails of Lanao del Norte, this study was carried out. Six physico-chemical parameters were tested namely: pH, temperature, total dissolved solids, altitude, number of plant species in the area and water level. Among these six parameters, only pH, number of plant species and temperature were found to be statistically significant.

The different levels of infection between the freshwater snails, namely infected with cercariae, infected with other parasites and non-infected, were all statistically distinctive in all sites. Correlation analysis showed either a negative or positive

correlation between the parameters and the level of infection, however, most of this correlation did not result to a significant relationship when further tested with regression analysis except for the analysis between the pH and the non-infected snails. This finding can be in agreement to the previous studies conducted that the pH had a significant role in the metabolic activities of the parasites further influencing their physiological response to enhance or decline infectivity.

Nevertheless, the importance of the other physico-chemical parameters used in this study cannot be also discounted. They were part and parcel of the complex interplay of the habitat factors that may or may not affect the level of infection in the freshwater snails as stated by Anderson, *et al.* (2010).

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