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Effect of Ecological Factors on Snails Infection by *Schistosoma japonicum* in Central Sulawesi, Indonesia

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Abstract: In Central Sulawesi, Napu valley and Lindu Lake (Indonesia), *Schistosoma* are spread in the host snails (*Oncomelania hupensis lindoensis)*. In the present work, the influence of ecological factors on the spreading of *Schistosoma japonicum cercariae* infection in the snails were analyzed. The *Oncomelania hupensis lindoensis* snails were collected and analyzed by ring method in the form of graphs and matrices. The results showed that, the ecological environment and such as temperature, pH, humidity, light intensity, dissolved oxygen, and salinity, as well as food, have significant effect on the increase of cercariae infected snails. These results also reflect that Schistosomiasis is still a health problem that could potentially be re-emerging diseases, especially in those areas and therefore much interesting must considered to break their cycle in nature.

Keywords: Ecology, *O.h. lindoensis*, Cercariae *S. japonicum*

Introduction

Schistosomiasis is an infectious disease caused by several species of *Schistosoma* worms. It still being health problem in some certain endemic areas in Africa, as well as in America and Asia (Richards *et al.*, 2006; Gryseels, 2012; Wu *et al*., 2005). The recorded infected persons were over 200 million around the world (Steinmann *et al.*, 2006; Vidal *et al*., 2010).

Schistosoma japonicum is the most infection species around the Lindu Lake, Napu and Bada within the Valley of Central Sulawesi in Indonesia (Hadidjaja and Sudomo, 1976; Rosmini *et al.*, 2014). Humans are the main or definitive host, while other mammals such as dog, pig and buffalo are serve as reservoir hosts as well (Sudomo and Pretty, 2007). Fresh water snail namely *Oncomelania hupensis lindoensis* is the intermediate host, where the worms continue their life cycle, starting from hatching of eggs in water then continued by penetration of miracidium into the body of snails until the production of cercariae (Hadidjaja and Sudomo, 1976). However, both humans and mammals are infected via penetration of the free swimming cercariae that come from the infected snails (Zhou *et al.*, 2009).

Trials of the controlling of Schistosomiasis in Central Sulawesi has been done since 1974, through multisectoral approach, including the treatment of patients, snails eradication using moluscicide and agro-engineering (Rosmini *et al.*, 2014). The main target of those programs were to decrease the prevalence of Schistosomiasis to be less than 1% (Rosmini *et al*., 2010) but up to date the prevalence of it is still fluctuative. The survey of snails during 2016 showed that, from 3560 collected snails in Lindu district, ~53 (1,50%) of snails were positive infected by cercariae of *S. japonicum* (Sutrisnawati *et al*., 2016). This ratio is higher than the former recorded ratio at 1,46% (Sudomo and Pretty, 2007). The former work in the Napu region showed that, from ~6874 collected snails, ~67 (0.97%) snails were infected by cercariae (Sudomo and Pretty, 2007).

The persistence of cercariae *S. japonicum* infection in snails may be due to ecological conditions. Therefore, the present study is conducted to identify the predominant ecological factors or the effect of environment on the occurrence of cercariae infection of *S. japonicum* worms in snails in Lindu and Napu endemic areas. Such data can give idea for further work and try how we can break this cycle.

In the present work, we focus on the ecological factors effect in the cercariae infection of *S. japonicum* worms in snails. These factors include mainly air temperature, water medium and its pH value, salinity, humidity and sunlight. We used advanced techniques in collecting the snails and statistical methods, Xlstat version 5.0. PCA and Statistical Product and Service Solutions (SPSS) version 2.0, in clarify the results. In the fact, these factors can give us an idea about how we can break this cycle in situ of its growing.

Methods

Data

In the present study, determination of the ecology of the habitats condition of *O.h. lindoensis*, comprise three analysis there were physical, chemical and biological factors in Central Sulawesi (include four villages in Lindu and another in Napu) (Fig. 1).

For ecologic determinations, collection of the samples, biologic condition, Principle Component Analysis (PCA) and the statistical analysis were our detailed studies.

Snail Collection

Based on a standard method of APHA (1998), snails were collected, from 12 stations (Fig. 2A), using the iron bracelet method (sized 1/70 m^2). The iron bracelet was thrown in the snail's habitat and all the snails inside the bracelet were collected. After accumulation the samples, the snails were broken to collect snail-containing and the snail-free of cercariae *S. japonicum* inside the snail's body (Fig. 2B and 2C).

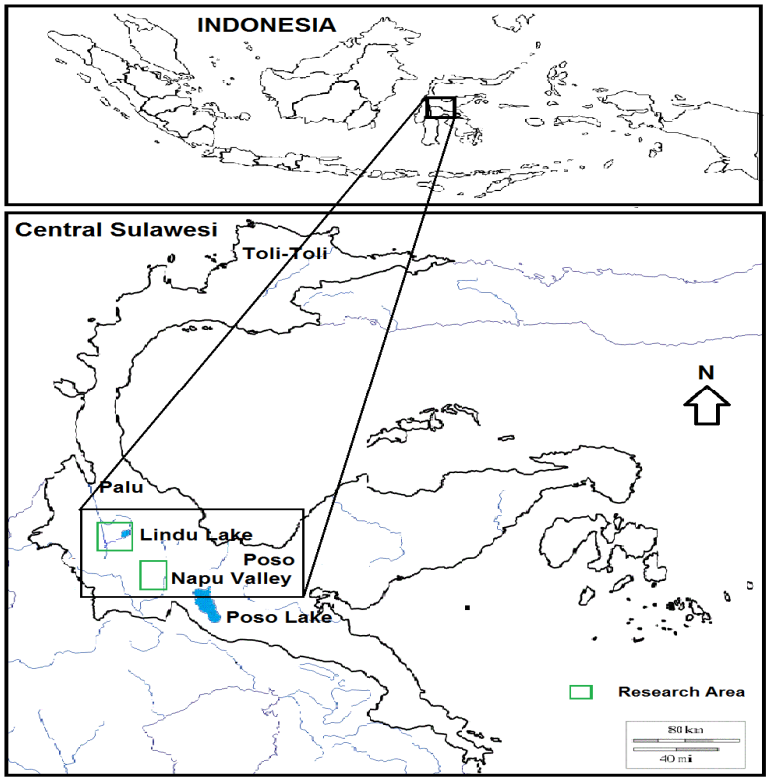


Fig. 1: The area of *O.h. lindoensis* snail collection in the region of Lindu and Napu, Central Sulawesi Province

Fig. 2: Habitat and Snail Collection. A. One of *O.h. lindoensis* snail's habitat, B. Collection of *O.h. lindoensis* snail, C. Some of the *O.h. lindoensis* snails which were found

Data Analysis

In the data analysis, Principal Components Analysis (PCA) was used for each factor using Xlstat version 5.0. PCA procedure. The later program is important in reducing the original independent variables that are correlated by using factor analysis. However, each factor of analysis represents the variables analyzed by the amount of variance called **eigenvalue**. Therefore, the first step was to determine the number of factors and the eigenvalue will show the variance of each of the main components.

According to the analysis of factors, criteria of eigenvalue greater than one was considered valid and being the number of factors formed (Umar, 2009). However if the total eigenvalue value was less than one, then the factor stated could not explain the variable well, therefore, it could not be included in the formation of variables (Abdi *et al*., 2013). We also used Statistical Product and Service Solutions (SPSS) version 2.0 (Campbell and Campbell, 2008) to know the effect of the ecology on the positive infected snails to know its prevalence.

Results

Prevalence of S. japonicum cercariae in O.h. lindoensis Snails in the Lindu and Napu

In this research, the number of *O.h. lindoensis* snail that infected with cercariae of *S. japonicum* found in the area Lindu was 133 from a total number of 7783 collected snails. The data showed that the average prevalence of snail infected by cercariae were 1.58%. In Napu region the total number of snail that successfully collected were 3630 and the average prevalence of infected snail were 6.23% (Table 1).

In Lindu area; include villages of Tomado, Palili, Anca and Paku; the ratio of infected snails/ non-infected ones was 133/7783 whereas in Napu that were, include village of Sedoa, Mekarsari, Alitupu and Dololo, the ratio was 178/3630 (Table 1). Therefore, the average percentage of the infected snails in Lindu and Napu were 3.90% and both Anca and (Lindu) Mekarsari (Napu) villages have the highest infected snails, by *S. japonicum*, of 2.34 and 18.61% respectively (Table 1) and Fig. 3.

The Result of Ecology Determinant and Principal Component Analysis (PCA)

The measurement of a biotic and biotic in the Lindu and Napu showed varying condition which can be seen in Table 2 and 3.

The Principle Component Analysis (PCA) in the Lindu area showed three factors have variance in total values (or Eigenvalue) greater than one, that include: 1. factors with eigenvalue 3.269 there were air temperature, humidity, water pH and dissolved oxygen (DO), 2. factors with eigenvalue 2.085 comprise water temperature, soil temperature and soil pH and 3. factors with eigenvalue 1.508 was salinity (Fig. 4A). In the other side, the PCA in Napu region, also showed three variance factors include: 1. factors with eigenvalue of 4.856 comprise water temperature, soil temperature, air temperature, DO, light intensity, 2. factors with eigenvalue value of 2.106 cover water pH, soil pH and 3. factor with eigenvalue of 0.941 only involved the salinity(Fig. 4B).

The aforementioned results of Principal Component Analysis method (PCA) showed that in Lindu region variability on first and second axes was 59.49% (Fig. 4A). This means that 59.49% of the analytical data can be explained up to the second major axis. The first and second component respectively describes 36.32% and 23.17, respectively (Fig. 4A and 4B). In the Napu region the variability of F1, F2 was 77.35%. Thus the result data analysis of 77.35% can be explained up to the second major axis (Fig. 5A and 5B). The first and second components explain 53.95%, 23.40% respectively (Fig. 5A and 5B).

Figure 5A showed a correlation of physical and chemical environment parameter in the Lindu regional.

The results of multiple regression analysis were done to know the influence of the environmental ecology determinant (X) on the increased of cercariae infected snail (Y) using software SPSS the results as follows (Table 4).

Table 1: The number of *S. japonicum* cercariae positive snails in several villages of Napu and Lindu

		Number of snail		
Region	Village	Total	Positive	Prevalence (%)
Lindu	Tomado	322.000	3.0	0.93
	Palili	3.345	52.0	1.55
	Anca	1.922	45.0	2.34
	Paku	2.194	33.0	1.50
	Number	7.783	133.0	6.33
	Average	1.945	33.0	1.58
Napu	Sedoa	300.000	2.0	0.67
	Mekarsari	360.000	67.0	18.61
	Alitupu	550.000	8.0	1.45
	Dodolo	2.420	101.0	4.17
	Number	3.630	178.0	24.90
	Average	907.500	45.5	6.22

Table 2: The characteristic of physical, chemical and biological environment in Lindu area

		Lindu area village				Average and standard deviation			
No	Ecology determinants	Tomado	Palili	Anca	Paku	Minimum	Maximum	Mean	SD
1	Water temp (°C)	27.67	28.33	27.33	25.33	25	29	27.16	1.26
2	Soil temp (°C)	25	25	27	24	23	28	25.25	1.42
3	Air temp (°C)	28.33	27.67	24.67	24.33	23	29	26.25	2.09
4	Humidity (%)	82.33	82.67	91	86.67	80	92	85.66	3.93
5	Water pH	7.3	7.53	6.9	7.23	6.7	7.8	7.24	0.31
6	Soil pH	6.4	6,20	6,27	6.37	6	6.6	6.3	0.16
7	Salinity (‰)	0.53	0.57	0.5	0.53	0.4	0.7	0.53	0.08
8	DO (Mg/L)	2.34	2.35	2.51	2.26	2.22	2.6	2.36	0.11
9	Light intensity (Cd)	89	87	81.67	86.67	80	90	86.08	4.03
	Biological Factor								
	a. Chlorophycea	+	++	+++	+++				
	b. Diatomeae	++	+	++	+++				
	c. Cyanophyceae	+	-	+	+				

Table 3: The characteristic of physical, chemical and biological environment in Napu Area

		Napu area village				Average and standard deviation			
No	Ecology determinants	Sedoa	Mekarsari	Alitupu	Dodolo	Minimum	Maximum	Mean	SD
1	Water temp (°C)	29	27	29	27.33	26	30	28.08	1.31
2	Soil temp (°C)	26.67	23	27.33	23.33	22	28	25.08	2.1
3	Air temp (°C)	29.67	25.67	30.33	23.67	23	31	27,33	2.93
4	Humidity (%)	80.67	81.67	85.33	88.67	80	89	84.08	3.37
5	Water pH	6.9	7.27	7.2	6.57	6	7.4	6.98	0.41
6	Soil pH	6.57	6.47	6.43	6.67	6.4	6.7	6.53	0.1
7	Salinity (‰)	0.67	0.53	0.43 *	0.73	0.4	0.8	0.59	0.13
8	DO (Mg/L)	3.05	3.68	2.57	3.82	2.52	4.2	3.28	0.6
9	Light intensity (Cd)	82.33	91.33	81.67	86.67	80	92	85.5	5.03
	Biological Factor								
	a. Chlorophycea	++	+++	++	+++				
	b. Diatomeae	+	+++	++	+++				
	c. Cyanophyceae	-	-	+	+				

*At high sea level (>1500 m) and in heavy rainfall the water salinity may be lower and have un-normal value as in Alitupu village (0.43 ppt)

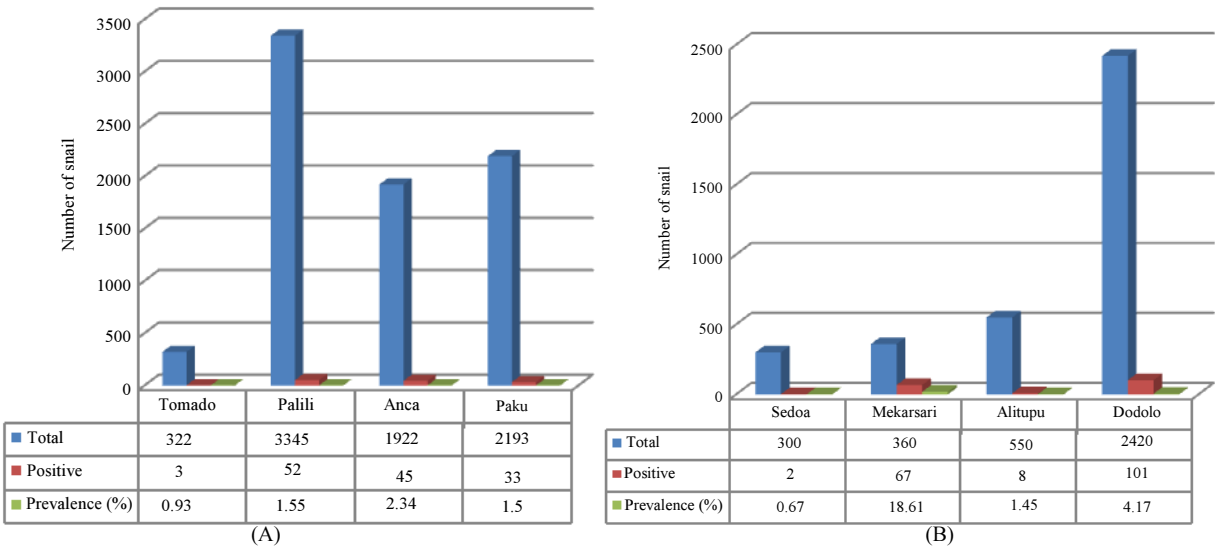


Fig. 3: The number of *S. japonicum cercariae* positive and prevalence snails in several villages of Lindu (A) and Napu (B)

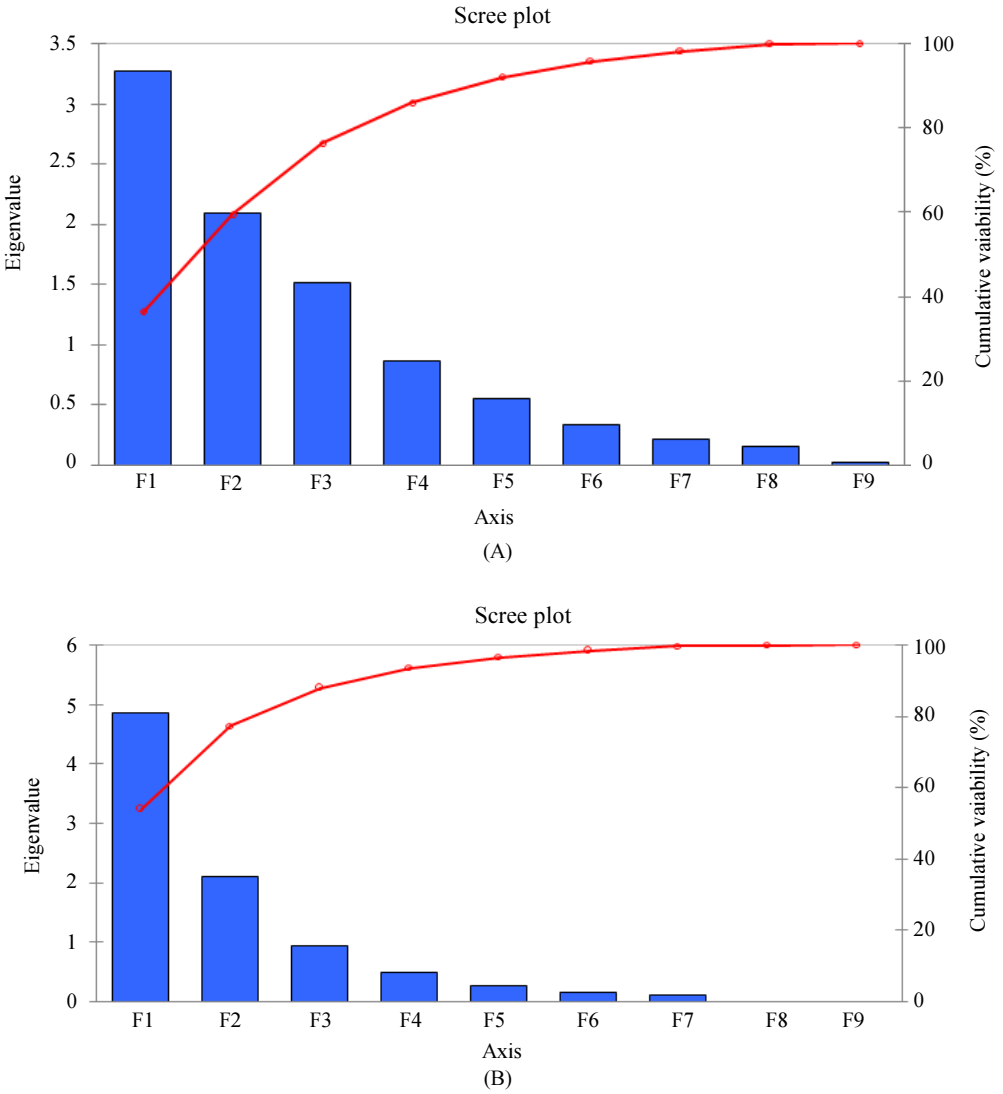
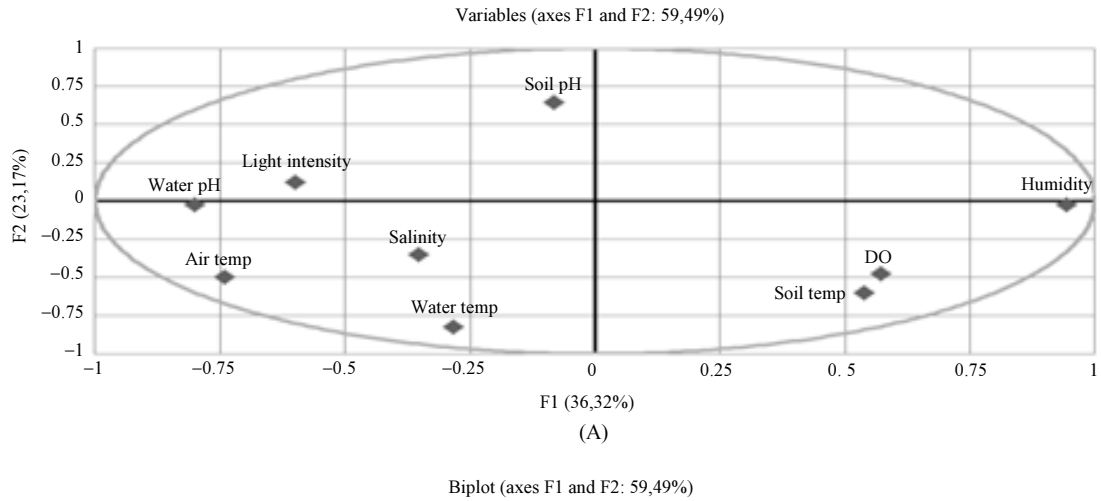


Fig. 4: Scree plot of the eigenvalue in the Lindu (A) and Napu (B)



Biplot (axes F1 and F2: 59,49%)

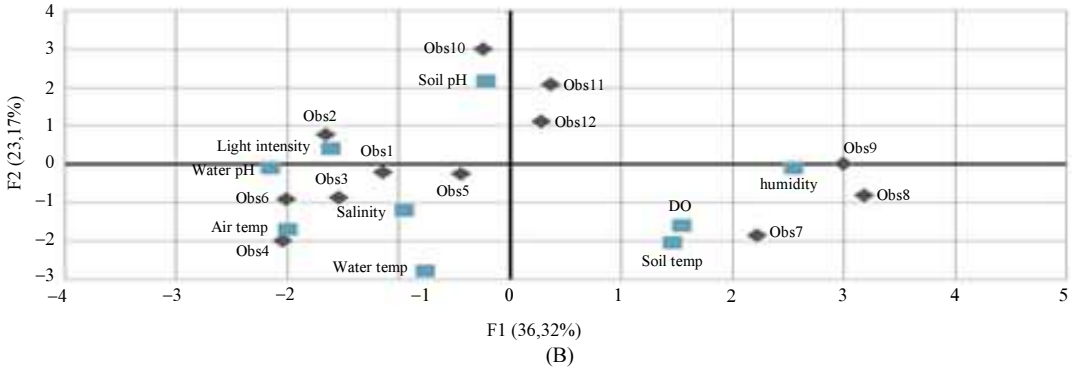


Fig. 5: A. Circle charts showed a correlation of physical and chemical environment parameter on an axis 1 and 2 based. B. Correlation between physical and chemical factors of the environment with observation stations in the Lindu regional.

Table 4: The influence of environmental ecology determinant on the increased of cercariae infected snail *O.h. lindoensis* in Lindu and Napu

				Change statistics		
Region	R	R square	Adjusted R square	R square change	F change	Sig. F change
Lindu	0.953	0.909	0.827	0.909	11.085	0.000
Napu	0.995	0.989	0.980	0.989	102.945	0.000

The results of regression analysis revealed that environmental the ecology determinant was significantly influenced the increasing of cercariae infection on *O.h. lindoensis* snails in the Lindu and in the regions Napu ($p = 0.00$). The correlation between the determinants of environmental ecology and the increasing of cercariae infection in both regions is very strong, with looking to the correlation coefficient (R) of 0.95 and 0.99 (Table 4).

Discussion

The high rate of snail infection in both areas (Lindu and Napu) was caused by the ecology determinant. Both areas are in the height >1500 meter above the sea level and they always moist watery that allows a great distribution of water biota components as macro-fauna and micro-fauna which are suitable habitats condition for life of *O.h. lindoensis* snail. Habitats that highly favored by *O.h. lindoensis* snail is always muddy places throughout the year and are under shadow of the direct sunlight. Also, the traces of crops that has been left for a long time, the ruins of water channel that surrounding by thick and muddy grass-grown and the outskirts of the forest protected from sunlight that is always moist with water are highly favored for *O.h. lindoensis* snail (Sudomo and Pretty, 2007; Garjito *et al*., 2008). *O.h. lindoensis* snails were found scattered and improved in a great variety of habitats, such as rice fields, irrigation channels, river, a ditch and lake/pool (Rosmini *et al*., 2014). A kind of fresh-water gastropod that are shady and shielded more preferred by snails than habitats that are open this is due to many freshwater snails of the nature have negative phototaxis (Dillon, 2004). Actually the destroy of the snail regions was hard because it spread under mountains of hard topography habitation in the forest area.

In definitely, the results of physical and chemical parameters of the ecology in the Lindu region showed that humidity, air temperature, water pH, were the most contributing factors in the increasing of cercariae *S. japonicum* infections on *O.h. lindoensis* snails. The results of this study in accordance with the results of previous research explains that humidity, temperature and climate, is a limiting factor on the presence of snails *O.h. lindoensis* (Zhou *et al*., 2012). Water temperature is considered to be one of the important aquatic environmental factors, especially in areas with slightly different seasons. The high temperatures concomitant with water can affect the development of the snail population (Fakult and Yang, 2006; David *et al*., 1987). It is known that *O.h. lindoensis* snails is an amphibian snail and its larvae need water for its life (Körner, 2007). When *O.h. lindoensis* snails grown into adulthood, slugs tend to live in moist areas (Fakult and Yang, 2006). The optimal temperature for the life of *O.h. lindoensis* snail between 16-28°C with a pH value of water between 6-8 (Rosmini *et al*., 2014). The later temperature is in accordance with the measurement results in our research. Previous studies have shown that the development of *Schistosoma* worms in snails as intermediate hosts is closely related to environmental temperature. The snails

kept at 15.3°C, *S. japonicum* arrested their development, while their fast development occurred at 30°C (Yang *et al*., 2007). High temperatures accelerate the development of the population of miracidia into cercariae in the body of a snails (Bavia *et al*., 1999). In the Lindu region (Anca village) the main factors causing increased cercariae infections in snails are temperature (comprise temperature of air, soil and water) and disolved oxygen. The second factor causing an increase in cercariae infection in snails is pH (water and soil) and salinity. Donnelly *et al*. (1984) suggests cercariae *Schistosoma* live in low salinity i.e., 0.52 in fresh water. At higher salinity gradually cercariae will die. In the Napu region (Mekarsari village) the temperature conditions (comprise temperature of air, soil and water) in addition to the disolved oxygen that support the breeding of snails. The ideal temperature conditions are caused by high rainfall that can adjust the suitable condition for development. Temperature and humidity are the fundamental environmental factors to encourage or limit the distribution and abundance of a biological organism (Bavia *et al*., 1999; Sulieman *et al*., 2013). The sun light is also the principal stimulus that causes emergence of both *S. haematobium* and *S. mansoni* cercariae, thus, shedding of cercariae may take place at water temperatures between 10 to 30°C, or even at higher (Mouritsen, 2002).

The parameter of water biology that was observed was phytoplankton. Based on the identification of known phytoplankton, there were three classes of phytoplankton found in the waters of the research sites (Lindu and Napu Region) that comprise *Chlorophycea*, *Diatomae* and *Cyanophycea*. In our work, abundance of phytoplankton on the entire regions of collected snails showed that the class *Chlorophycea* is more dominant compared with other classes. However, other added that *Diatomae* class are the main food of *O.h. lindoensis* snails (Hadidjaja and Sudomo, 1976).

Based on R square value, the contribution of ecology determinant to infection of cercariae larvae at snail *O.h. lindoensis* at Lindu area was 90.9% and at Napu region was 98.9% (Table 4). The present study are in accordance with the previous research which explains that humidity, temperature and climate, are the limiting factors of the presence of *O.h. lindoensis* snails (Zhou *et al*., 2012). Really the environmental factors in any place not only include one factor, but usually various factors that mutually interact (David *et al*., 1987).

Conclusion

The results PCA and of regression analysis indicate that the determinant of the environmental ecology such as temperature, pH, humidity, light intensity, dissolved oxygen, salinity, food, significantly affect the increase of cercariae infections in snails of *O.h. lindoensis* in the region of Lindu and Napu. These findings provide information that the determinant of environmental ecology was conducive to the survival of the snails and will also support the development of miracidium to be cercariae stage in the body of snails.

Conflict of Interests

All authors declare that they have no conflict of interests.

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Author's Contributions

Sutrisnawati Mardin: Participate in all research, conduct data analysis and contribute to the writing of the manuscript.

Teguh Wahju Sardjono, Loeki Enggar Fitri and Aulanni'am: Supervisor (Contribute to the preparation and editing of articles).

Achmad Ramadhan: Assisting in field research, taking ecological data and analyzing data.

Ethics

This article is original and independency of this research was highly ensured. There will be no issues of ethics following the publication of this article.

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