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The Relationship between *Odorrana hosii* Skin Histology and Habitat Water Quality in Different Locations of Sarawak

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ABSTRACT

Recent study has revealed that the poison gland in *Odorrana hosii* skin act as defensive mechanism and can help in wound healing management of a frog species. Since skin is the first organ that come into contact with the surrounding, the habitat of the species should be related to the properties of the frogs' skin. Thus, we investigated the *O. hosii* skin histology in relation to water quality parameters at different localities in Sarawak. The goal of study is to uncover a link between *O. hosii* habitat and its skin histology at four different locations: Ranchan Recreational Park (RRP), Matang Wildlife Centre (MWC), Borneo Highlands (BH), and Santubong National Park (SNP). The water quality criteria for each area were pH, turbidity, temperature, and dissolved oxygen (DO). The results showed that DO concentration of the habitat is the most essential element in affecting the health of the amphibian skin, as well as the forested areas (MWC, SNP, and BH) that protect the skin from direct sunlight. The DO of the habitat differed significantly for the mean number of mucous and seromucous glands at 0.01 ($\alpha = 0.05$) and 0.03 ($\alpha = 0.05$), respectively. The number of mucous glands and DO were found to have a strong positive correlation at 0.701. However, the number of seromucous glands showed a strong negative correlation with DO at -0.623. *O. hosii* skin from the four different localities was found to have cutaneous gland variations that reflect the environment in the number of glands. Mucous glands play an important role in indicating the localities' water quality, which reflects adaptation of *O. hosii* to its habitat. This highlights the importance of preserving natural habitats for this sensitive species.

Keynote: Bornean frog, histology, *Odorrana hosii*, skin, water quality

INTRODUCTION

Anurans, such as *Odorrana hosii*, have integument instead of scales, enabling them to live in a wide range of terrestrial settings. However, their "bare" skin makes them prone to illness and injury. To defend themselves from harmful germs, anurans employ integument glands to respond to their environment (Seki *et al*., 1995; Zainudin *et al*., 2018) which are classified as mucosal or serous depending on their secretion type (Barrionuevo, 2017). Patterned or evenly positioned glandular glands are found on the dorsal skin, and the concentration of these glands may affect the rate of wound epithelialisation (Rasit *et al*., 2018). *Odorrana hosii* has a green dorsum from snout to anus with brown sides and black crossbars (Inger *et al*., 2005; Inger *et al*., 2017). The International Union for Conservation of Nature (IUCN) Red List of Threatened Species considers *O. hosii* to be of least concern (IUCN). Water-contaminated woodlands, rivers, and rocky streams are typical habitats for *O. hosii* (Inger *et al*., 2005; Inger *et al*., 2017). It prefers to rest on rocks, branches, or plants near water bodies for its foraging activities (Inger *et al*., 2017; Zainudin *et al*., 2017). A study by Shahabuddin *et al*. (2018) demonstrated that antimicrobial skin peptides (AMP) extracted from the skin of *O. hosii* are bacteriostatic and bactericidal. *O. hosii* peptides may be exploited to create new antibiotics. AMP is a source of possible antibiotics that kill microorganisms and prevent sepsis (Raghavan *et al*., 2010; Shahabuddin *et al*., 2018).

Water quality is critical for biological life, and the utility of its characteristics are often overlooked (Chaplin, 2001). Excessive mineral pollution reduces the quality of water, which can cause mortality or restrict biological life on land and in the sea. The Sarawak Natural Resource and Environment Board has been monitoring river water quality since 1998, taking into account sedimentation and wastewater discharge, which place additional strain on aquatic systems.

The Malaysian Department of Environment (DOE) employs Water Quality Index (WQI) and National Water Quality Standards (NWQS) to assess water quality. In addition to pH, turbidity, temperature, and electrical conductivity, the water quality indexes also assess DO and mineral or ion concentration. Water bodies' physical, chemical, and biological characteristics determine their long-term sustainability (Meybeck, 1996). Organic pollution depletes oxygen levels in aquatic settings, suffocating aquatic animals when dangerous levels are exceeded (Mansano *et al*., 2018). The biochemical oxygen demand (BOD) quantifies microbial oxygen consumption during organic matter degradation, which is produced by the decomposition of organic waste and harms both terrestrial and aquatic creatures. Moreover, a mineral or element concentration influences another water quality measure; in water, DO content and temperature are inversely proportional. According to Mansano *et al*. (2018), DO is 9.08 mgL^{-1} at 20 °C and 10.07 mgL^{-1} at 15 °C. High BOD levels indicate water contamination, as DO declines.

Amphibians are well-known environmental indicators, and embryo mortality and abnormalities may be linked to eutrophic conditions such as pH and water temperature (Boyer & Grue, 1995). *O. hosii* is an omnivore, and water quality affects its metabolism (Mansano *et al*., 2018). *O. hosii* spends much of its life near flowing water, and the aquatic environment it dwells in may cause skin histological changes due to dermal absorption of contaminants (Boyer & Grue, 1995).

O. hosii is a stream-dwelling frog, and dermal absorption of pollutants or toxicants influences its growth and development (Boyer & Grue, 1995). Shahabuddin *et al*. (2018) and Tennessen (2009) found selection pressure in frog skin peptides correlated with prior microbe contacts.

In addition, Md. Sungif *et al*. (2015) revealed that the poison gland in *O. hosii*'s skin act as defensive mechanism and can help in wound healing management of a species. Therefore, the frog's environment influences its adaption, and water quality is critical in investigating *O. hosii*'s histology. Hence, this study focuses on investigating the correlation between the water quality and histology of *O. hosii* skin.

MATERIALS AND METHODS

The frogs, *Odorrana hosii* were obtained at four sites: Ranchan Recreational Park (RRP) (1°, 110.583°), Matang Wildlife Center (MWC) (1.6°, 110.1591°), Borneo Highland (BH) (1.5°, 110.15°), and Santubong National Park (SNP) (1.7°, 110.3325°) in Kuching Division (Figure 1). On-site water samples were collected and analysed using the Eutech pH 150 pH metre and the Eutech ECTN 100 IR waterproof portable turbidimeter. The water quality parameters were measured at each field site, including pH, turbidity, DO, and temperature. The sampling of water quality parameters was conducted at the natural habitat shelters, ensuring that readings were taken at three different times to minimise the impact of immediate rainfall or weather conditions.

Animal Model

The Universiti Malaysia Sarawak Animal Ethics Committee (UNIMAS-AEC) authorised and monitored the experimentation. It was conducted on 12 non-breeding adult *O. hosii*, with three individuals from four site samplings. An adult *O. hosii* was euthanised with 99% ethanol and a short incision was made from the lower thighs to the dorsal end for skin harvesting (Heyer, 1994). Skin was taken from both the dorsal and ventral specimens (Figure 2). Skin grossing and mounting of the skin onto filter sheets were also conducted.

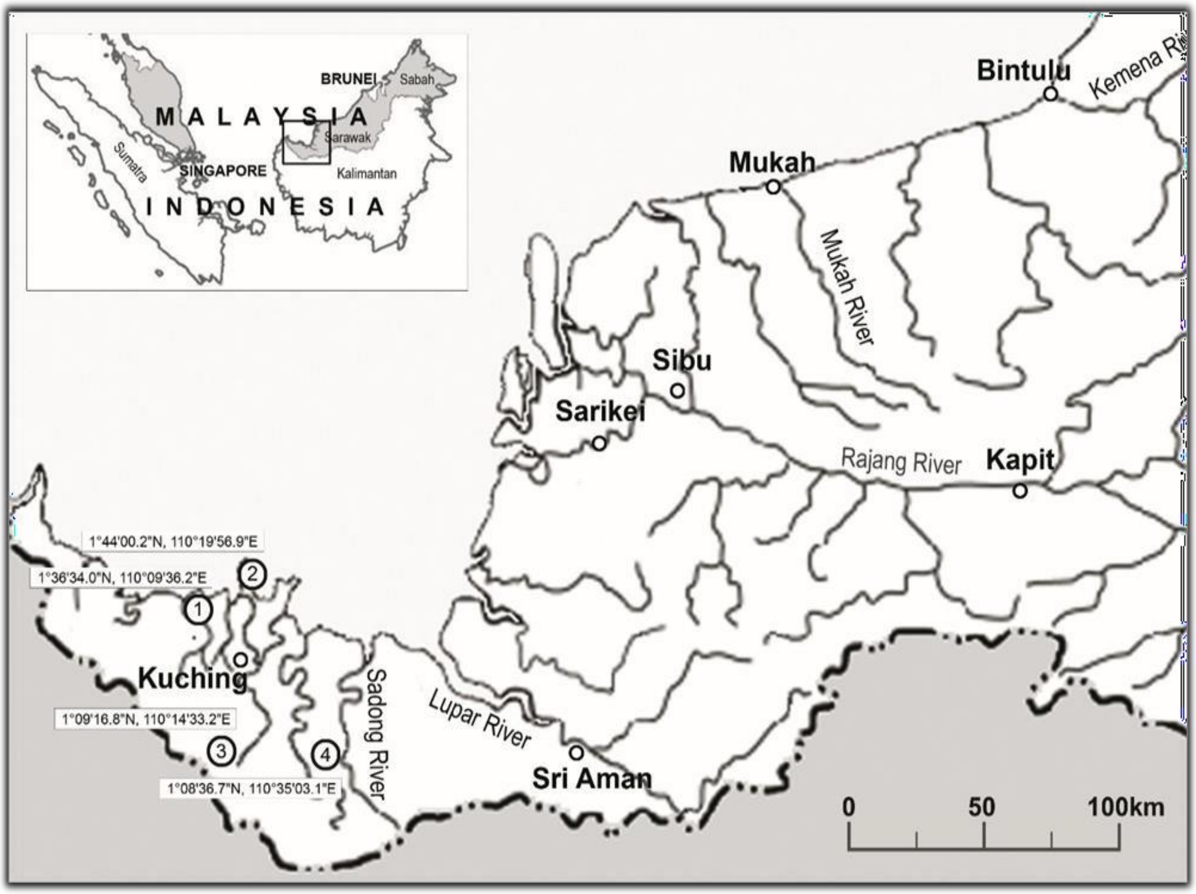

Figure 1. Sampling site at Matang Wildlife Center (1), Santubong National Park (2), Borneo Highlands (3) and Ranchan Recreational Park (4)

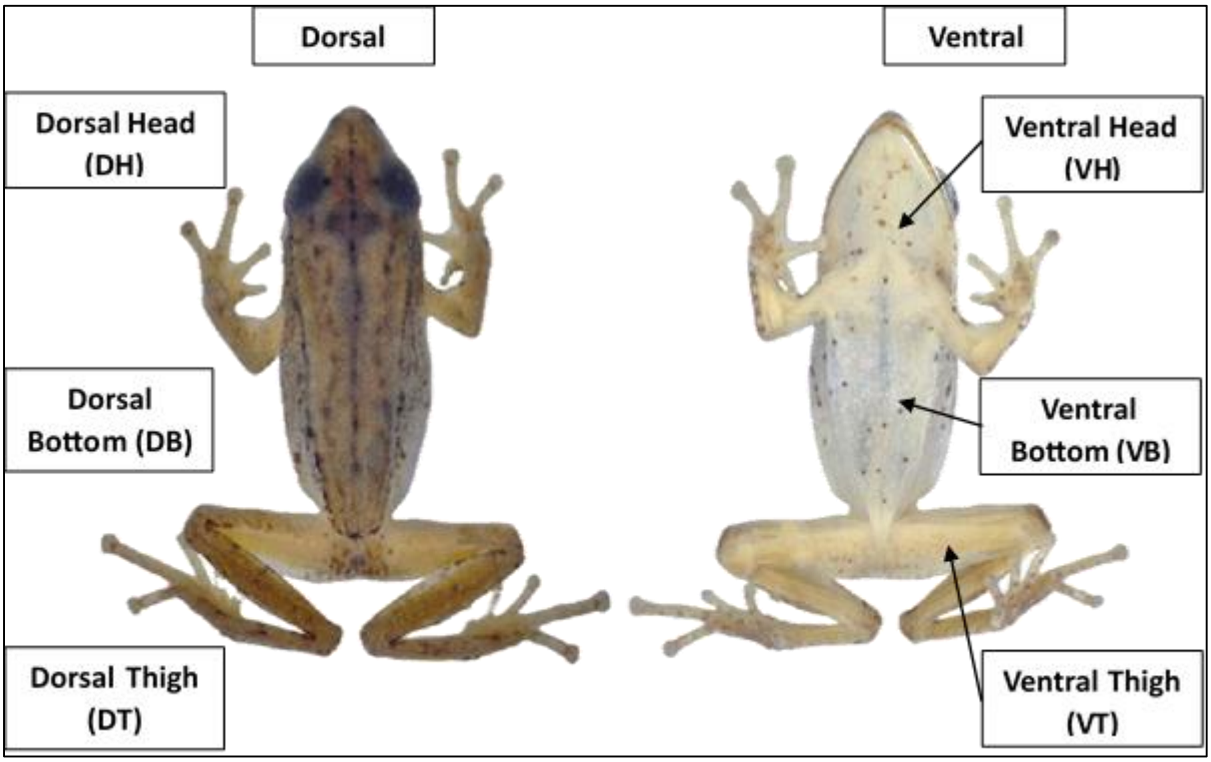

Figure 2. Six skin regions of *Odorrana hosii* used in histology assessment

Histological Technique

The frog skin was processed, fixed, and sectioned using a microtome machine (Rasit *et al*., 2018). The modified protocol of Hematoxylin and Eosin (H&E) was used for staining following Brown (2002). *O. hosii* skin slides were imaged using the Olympus Microscope BX51 and OlyVia software. The number of glands, average gland size, and average skin thickness were all investigated in *O. hosii* histology. The number of glands was counted in unit, while the area and length were measured in micrometres (µm).

Statistical Analysis

The statistical analysis using SPSS employed ANOVA and Post Hoc test were used to compare the four sites and their water quality parameters. The Pearson correlation test was performed to analyse the association between *O. hosii* histology finding and the quality of water parameter.

RESULTS

Water Quality Between Localities

The mean and standard deviation of water quality parameters, including pH, turbidity, DO, and temperature between locations were measured (Table 1). There was no significant difference between the four sites' pH ($p = 0.05$). However, the turbidity, DO, and temperature were found to vary across locations with $p = 0.000$ (Table 2).

There were two subsets for water turbidity, according to the post hoc test. RRP was grouped in subset two on its own, while MWC, SNP, and BH were grouped in subset one, which had moderate similarities at $p = 0.468$. RRP had the highest mean at 1.38 NTU, while MWC had the lowest mean at 0.357 NTU. The parameter of water quality for DO was divided into three subsets at the four sites. With a p-value of 1.00, RRP had the lowest mean concentration of 2.9 mg/L. MWC, with a mean concentration of 7.60 mg/L ($p = 1$), was revealed in subgroup two. The third subset group had 7.76 mg/L in BH and 7.83 mg/L in SNP ($p = 0.593$). The water quality parameter of water temperature was divided into two subgroups: RRP, SNP, and BH had a p-value of 0.813 and a mean temperature range of 25.2 °C to 25.37 °C, respectively. Another group, MWC, had a mean temperature of 26.73 °C.

The Thickness of *O. hosii* Skin in Different Locality

The mean thickness of the epidermis, stratum spongiosum, and stratum compactum in *O. hosii* skin varied across four different locations, ranging from 11.55 to 19.60 µm, 35.11 to 58.26 µm, and 37.50 µm to 75.36 µm, respectively (Table 3). The skin thickness of *O. hosii* showed a significant difference across all four localities ($p<0.05$) (Table 4). Post-hoc Tukey pairwise comparisons on the epidermis thickness revealed that there were three subsets formed. BH *O. hosii* had the lowest mean at 11.55 µm, while RRP had the highest at 19.60 µm. The epidermis thickness of *O. hosii* from BH, SNP, and RRP differed greatly, with no overlapping subgroups. *O. hosii* from MWC has similar epidermis thickness with *O. hosii* from BH and SNP. The Tukey post-hoc test on the stratum spongiosum thickness showed that BH, RRP, and SNP were clustered into one subgroup, with averages ranging from 35.11 µm to 44.65 µm. MWC's stratum spongiosum thickness differed from all other locations ($p = 1.00$). Three subgroups for stratum compactum thickness between localities were found. Within a subset, MWC and BH had $p = 0.687$ similarities. RRP and SNP were clustered into separate subgroups at $p = 1.0$. With MWC being the thinnest and SNP being the thickest, the stratum compactum was 37.5 µm thick.

Table 1. Mean of water quality parameter of localities

Locality	pH	Turbidity	Temperature	Dissolved Oxygen (DO)
Ranchan RP	6.84±0.25	1.38±0.15	25.20±0.00	2.91±0.10
Matang WC	6.19±0.91	0.36±0.02	26.73±0.35	7.60±0.01
Borneo Highlands	7.67±0.70	0.57±0.28	25.37±0.25	7.76±0.06
Santubong NP	6.50±0.40	0.44±0.13	25.20±0.17	7.83±0.06

Table 2. Summarised ANOVA of water parameters between localities

	df	F	Sig.	Mean±SE
pH	3	3.15	.087	6.8 ± 0.23
Turbidity	3	22.85	.000*	0.69 ± 0.13
Dissolved Oxygen	3	4362.86	.000*	6.52 ± 0.63
Temperature	3	31.06	.000*	25.63 ± 0.20

Table 3. Mean of thickness of skin (µm) of *Odorrana hosii* between localities

Locality	Epidermis	Spongiosum	Compactum
Ranchan RP	19.60 ± 4.84	39.26 ± 1.79	63.29 ± 1.98
Matang WC	13.21 ± 2.37	58.26 ± 6.03	37.50 ± 4.72
Borneo Highlands	11.55 ± 0.81	35.11 ± 0.30	40.82 ± 15.53
Santubong NP	14.38 ± 0.32	44.65 ± 14.01	75.36 ± 17.41

Table 4. Summarised ANOVA of thickness of skin of *Odorrana hosii* between localities

	df	F	Sig.	Mean±SE
Epidermis	3	39.30	0.00*	14.675±0.55
Spongiosum	3	8.56	0.00*	44.652±3.44
Compactum	3	73.02	0.00*	54.244±2.12

The Number and Average Size of Glands on *Odorrana hosii* Skin Between Localities

The mean number of cutaneous glands (mucous, seromucous, and granular) in *O. hosii* skin varied across four different locations, ranging from 1.11 to 4.33, 4.89 to 8.05, and 3.00 to 10.44, respectively (Table 5). MANOVA was used to analyse the number of glands found on *O. hosii* between localities. The enumeration of all three cutaneous glands (mucous, seromucous, and granular) was significantly different for all four localities (p = 0.00; Table 6).

Post Hoc Tukey tests between number of glands and localities showed two subsets with no overlapping. The number of mucous glands found in RRP had the lowest mean (0.24) in subset one. Subset two consisted of three localities (SNP, MWC, and BH) with low similarities (p = 0.167). The BH had the highest mean for mucous glands (0.86). The number of seromucous glands between localities was grouped into two subsets. BH, SNP, and MWC had low similarities (p = 0.054) in subset one, with means ranging from 0.93 to 1.25. In subset two, seromucous glands in MWC overlapped with subset one, while RRP was unique with the highest mean (1.58). These two localities formed subset two with moderate similarities (p = 0.325). The number of granular glands between localities was grouped into two subsets. SNP had the lowest mean (0.91), while MWC had the highest mean (2.14). SNP, BH and RRP were grouped into subset one with high similarities (p = 0.910), while MWC formed its own subset (p = 1.00) with no overlap with the other localities tested.

Table 5. Mean number of glands of *Odorrana hosii skin* between localities

Locality	Mean of number of glands		
	Mucous	Seromucous	Granular
Ranchan RP	1.11±1.37	8.05±3.34	5.94±5.46
Matang WC	3.33±2.56	6.11±2.66	10.44±4.61
Borneo Highlands	4.33±2.26	4.89±2.10	4.33±2.26
Santubong NP	3.00±1.67	5.00±2.40	3.00±1.67

Table 6. Summarised ANOVA of number of gland of skin between localities

	df	F	Sig.	Mean±SE
Mucous	3	15.540	.000*	0.638±0.80
Seromucous	3	7.679	.000*	1.158±0.10
Granular	3	25.283	.000*	1.31±0.11

The mean average size of cutaneous glands (mucous, seromucous, and granular) in *O. hosii* skin varied across four different locations, ranging from 423.60 to 1395.38 µm², 988.97 to 1571.94 µm², and 3987.77 to 12725.84 µm², respectively (Table7).

The average size of mucous glands, and granular glands were found to be significantly different for all four localities, with p<0.001 (Table 8). Post Hoc tests showed that the average size of mucous glands between localities was grouped into two subsets, with MWC being in both subsets with a mean of 967.7 µm². RRP had the lowest mean for the average size of mucous glands at 423.6 µm² in subset one. Subset two consisted of three out of four localities: MWC, BH, and SNP, with a mean ranging from 967.7 µm² to 1395.4 µm². Subset one showed similarities at p = 0.127, while subset two showed moderately low similarities at p = 0.313.

Post Hoc tests showed that the average size of seromucous glands between localities showed high similarities between all four localities at p = 0.762 and formed only one subset. SNP had the lowest mean for the average size of seromucous glands at 989.0 µm², while MWC had the highest mean at 1571.9 µm². Two subsets were formed from the post hoc Tukey test of the average size of granular glands between localities. Three localities were grouped in subset one: RRP, BH, and SNP. These three localities showed high similarities at p = 0.740. The lowest average size mean was 3987.8 µm² for RRP. The average size of granular glands found in MWC was 12725.8 µm² with no overlapping with subset one.

Table 7. Mean average size of glands (µm²) of *Odorrana hosii* skin between localities

Locality	Mean of average size of glands(µm²)		
	Mucous	Seromucous	Granular
Ranchan RP	423.60±137.64	1490.68±44.25	3987.77±1598.68
Matang WC	967.71±283.41	1571.94±1245.46	12725.84±1066.80
Borneo Highlands	1172.36±306.51	1351.88±662.39	4795.76±3096.98
Santubong NP	1395.38±273.72	988.97±257.43	5334.66±3731.22

Table 8. Summarised ANOVA of average size of gland of skin between localities

	df	F	Sig.	Mean±SE
Mucous	3	5.624	.001*	989.77±175.37
Seromucous	3	.370	.774	1349.36±421.60
Granular	3	20.057	.000*	6693.72±905.84

The Correlation Between Histology of *Odorrana hosii* Skin and Water Quality Parameters

The Spearman correlation test showed significant differences for the number of mucous glands and the number of seromucous glands at 0.041 (α = 0.05). It showed a moderate negative correlation at -0.595 (Table 9). The water parameter, DO, showed significant differences for the number of both mucous and seromucous glands at 0.01 (α = 0.05) and 0.03 (α = 0.05) respectively. Mucous glands and DO were found to have a strong positive correlation at 0.701. Seromucous glands showed a strong negative correlation with DO at -0.623.

Table 10 showed a Spearman correlation between the area of glands (μm²) and water quality. Only one significant difference was noted; the correlation between the area of mucous glands and DO was 0.007 ($\alpha = 0.01$) with a strong positive correlation of 0.735.

DISCUSSION

Water Quality

The presence of pollution is indicated by the low pH levels observed in rivers and streams, which demonstrates acidification. It is generally recognized that rivers within the pH range of 6.5 to 8.5 are considered to be in a conserved state (WHO, 2017). The pH for all four localities measured was consistent with the Water Quality Index Classification of Malaysia (Department of Environment Quality Report, 2006). There were no significant differences for all the localities due to low anthropogenic activities, as the selected study sites offered some degree of protection by law and serve as habitats for various local frogs in Sarawak. The readings from the four sample sites showed turbidity with a reading of less than 5 NTU, which is within the standards of the National Water Quality Standards for Malaysia (NWQS). The turbidity value of the RRP sampled was the highest at 1.38 NTU compared to other localities, which is in accordance with the study done by Majit (2008). RRP as subset group two has high anthropogenic activities due to its easy accessibility to the waterfalls where *O. hosii* inhabits, hence, the silt or inorganic matters that were disturbed. MWC, SNP and BH were grouped in the same subset one with moderate similarities ($p = 0.468$). These three study sites sampled were more reserved compared to the RRP. The streams in subset one where *O. hosii* was found were inaccessible to the public. BH had the highest mean reading among the subsets one at 0.57 NTU due to the rainy weather. The water bodies in Sarawak were noted to have higher turbidity readings during the wet season (Majit, 2008).

Table 9. Spearman correlation between number of glands (unit) and water quality

		pH	Turbidity	Temperature	DO
Mucous	Correlation coefficient	.118	-.312	.107	.701*
	Sig. (2-tailed)	.715	.324	.740	.011
	N	12	12	12	12
Seromucous	Correlation coefficient	.121	.289	.105	-.623*
	Sig. (2-tailed)	.708	.361	.745	.030
	N	12	12	12	12
Granular	Correlation coefficient	-.260	-.411	.246	-.231
	Sig. (2-tailed)	.415	.185	.441	.471
	N	12	12	12	12

*Correlation is significant at the 0.05 level (2-tailed).

Table 10. Spearman correlation between average size of glands (μm²) and water quality

		pH	Turbidity	Temperature	DO
Mucous	Correlation coefficient	.025	-.312	-.142	.735**
	Sig.(2-tailed)	.940	.324	.659	.007
	N	12	12	12	12
Seromucous	Correlation coefficient	.028	.266	-.114	-.457
	Sig. (2-tailed)	.931	.403	.724	.135
	N	12	12	12	12
Granular	Correlation coefficient	-.445	-.473	.367	-.183
	Sig. (2-tailed)	.147	.121	.241	.570
	N	12	12	12	12

**Correlation is significant at the 0.01 level (2-tailed).

Water temperature is an important physical parameter that measures the water's heat content (Water Resources Management and Hydrology Division, Department of Irrigation and Drainage, 2009) and influences the mortality growth of aquatic organisms. It affects other parameters such as pH and DO concentrations. MWC had the highest average water temperature of 26.73 °C compared to the three study sites. The sample site was the shallowest compared to the three sites, which gave it a higher temperature (Yee *et al*., 2012). According to Haiyan *et al*. (2013), water temperature plays a role in affecting the pH and DO. The pH and DO decrease with an increase in temperature. Although no significant differences were found in pH values across the four study sites, significant differences were observed in water temperature and DO levels.

DO measures the volume of oxygen contained in the water, which varies according to the rate of oxygen demand. RRP had the lowest DO at 2.9 mg/L. It showed a class IV reading, according to NWQS 2006, where high microbial activity occurs. MWC, BH, and SNP had DO > 7 mg/L, which makes them suitable to house sensitive aquatic species (Department of Environment Quality Report, 2006).

With reference to the above, the study agrees with Zainudin *et al*. (2019) in finding significant changes in species diversity, species assemblages, and physical tolerance (variance in physical elements that an organism population may resist and continue to thrive in an environment) to water quality. Hence, the number of species was found to be significantly influenced by water temperature, turbidity, salinity, and level of DO. These relates on how the species adapt to its environment as suggested by Zainudin *et al*. (2018) in which the distribution of serous and mucous glands on different regions of the skin might also reflect the habits of the horned frog, *Pelobatrachus nasutus* in its natural habitat.

The mean number and size of glands in both dorsal and ventral region of *O. hosii* skin were found to be significantly different in relation to DO. Only two glands, mucous glands and seromucous glands, were shown to be affected. The Kendal Correlation test showed that mucous glands and seromucous glands have an opposite correlation with one another. *O. hosii* has a high number of mucous glands but a low number of seromucous glands and vice versa.

RRP had the lowest DO, followed by MWC, BH, and SNP. A trend was noted where *O. hosii* that dwells in low DO waters had a high number of seromucous glands. *O. hosii*, found in RRP and BH, requires more adaptation mechanisms for higher AMP and homeostasis in low-DO environments. A low DO may indicate stagnant or still waters where a high amount of microbial activity may take place. Seromucous glands serve to function as *O. hosii*'s defense against the external environment and to facilitate oxygen diffusion into the skin.

The number of mucous glands was found to be the highest in *O. hosii* from BH and SNP, where DO is high. The frogs adapt to needing less secretion of AMP from seromucous glands that exhibit both mucous gland and granular gland properties. Hence, having a high number of mucous glands *O. hosii* from BH and SNP is enough to ensure its survivability in the environment.

The size of mucous glands has an above average correlation with DO when tested with the two correlation tests. Mucous glands play an important role in indicating the localities' water quality, which reflects on *O. hosii*'s adaptation to its habitat.

Frogs' Skin Histology

The granular glands present are dominant in the dorsal region, particularly the dorsal head. This corresponds to the behavior of predators that focus their attack on the anterior region of frogs before consuming them. Granular glands were observed to be the largest cutaneous glands in *O. hosii* (Moreno-Gomez *et al*., 2014; Rasit, 2018). The numerous granular glands on the dorsal head act as a defense mechanism for *O. hosii* as they secrete foul-smelling poison to deter predators and enable escape. This may also be the reason why the dorsal head has the thickest skin region from the epidermis layer to the dermis layer compared to the other skin regions studied. The stratum spongiosum, where the granular glands are located, shows a positive correlation to the thickness found in the dorsal head, which is in accordance with the study done by Rasit *et al*. (2018), and vice versa for the thigh skin region, where there were the least number of granular glands present. *O. hosii* is also commonly known as the Poisonous Rock frog, and from this study, it is reflecting its naming to have the greatest number of granular glands compared to the other cutaneous glands.

Frogs from MWC have the greatest number of granular glands, where there is a significant difference from the other three localities studied. This suggests the need for higher toxic secretion by *O. hosii* for defense adaptation. The undisturbed stream in MWC created an environment where fungus, bacteria, or predators thrived. In the post hoc test, RRP, BH, and SNP were found to have similarities in the number of granular glands. RRP has a diverse species of frogs found in small fragmented waterfalls and ponds. There is high competition within the population of frogs, hence there are also a high number of granular glands found for survival adaptation. *Odorrana hosii* found in BH and SNP streams was well hidden under canopies of trees and was solitary, thus needing fewer granular glands for its protection.

Mucous glands are responsible for the general homeostasis of the skin through saline and gas exchange, osmoregulation on land, and reduction in friction during swimming (Brizzi *et al*., 2001).

Odorrana hosii, a stream-dwelling frog, is consistently found either within the streams themselves or within a proximity of just a few meters from the streams (Inger *et al*., 2005). The mucous glands ranged the least among the cutaneous glands studied and were dominant in the dorsal region. Mucous glands were found to be the largest in the anterior region and least in the thigh region of the frog and were shown to have a positive correlation with the thickness of the stratum spongiosum. Exposure to surface desiccation is low and intake of water is limited due to *O. hosii*'s preference for wet environments, where mucous glands are responsible for ion intake and water regulation. Granular glands are the main defense strategy of *Odorrana hosii,* and while mucous glands are acidic in nature to cause unfavorable environments for its natural predators or parasites (Moreno-Gomez *et al*., 2014), the number of mucous glands needed for its survival is reduced.

Mucous glands found in SNP and BH were shown to have the largest size and highest enumeration, while the granular glands were found to be small and had low enumeration. This strongly suggests there is a need for protective secretion to protect *O. hosii* from its external environment, where its skin is vulnerable and susceptible to parasites or fungus. MWC, vice versa, has large granular glands and small mucous glands. RRP, however, has small-sized mucous glands and granular glands but a high enumeration of granular glands and a low enumeration of mucous glands.

Odorrana hosii seromucous glands are a mixture of granular glands (serous glands) and mucous glands that secrete fluid containing both synergic products of the two glands (Brizzi *et al*., 2001). The irritant products were released upon stress. However, at low dosage, the chemical is 'noxious' instead of being poisonous (Daly *et al*., 2005). According to Conlon *et al*. (2008), eight peptides with different antimicrobial activities were found in *O. hosii*'s secretion, which instead of causing mortality, serves as a protective film against pathogens. The enumeration of seromucous glands of *O. hosii* in this study was shown to be the second highest after granular glands and they were found to be dominant in the ventral region. The ventral region of the frog is constantly in contact with the substrate on the ground, where a high number of seromucous glands serve to reduce friction and ensure constant cellular regulation. The glands are also involved in the production of chemical signals and defensive secretions, as well as chemosignals which serve as individual recognition and territorial announcements (Brizzi *et al*., 2002).

In *O. hosii*, the highest number of seromucous glands was observed on the dorsal bottom and ventral head regions. These glands exhibit a positive linear relationship with granular glands and a negative linear relationship with mucous glands. It was noted that when a high number of granular glands were present, the seromucous glands also tended to be abundant. Among the study sites, RRP showed the highest number of seromucous glands, while SNP exhibited the lowest number. Additionally, the dorsal bottom of *O. hosii* displayed the largest size of seromucous glands.

All three cutaneous glands showed a positive correlation to the thickness of the stratum spongiosum found. The thickness of the epidermis only showed positive correlation to the two large-sized glands found (granular glands and seromucous glands), while the thickness of the stratum compactum only showed positive correlation to the seromucous glands, where the glands are minute but densely spread across. The thickness of stratum spongiosum accommodates the size and number of glands found, where the largest glands, the granular glands, found dominantly on the dorsal head, have the thickest epidermis, stratum spongiosum, and stratum compactum. The anterior of the frog has the thickest skin, while the posterior has the thinnest skin. This applies to both the dorsal and ventral regions of the frog. According to Prates *et al*. (2012), the granular glands coincide with the distribution of chromatophores in the dorsal regions, which serves to warn of potential predators.

CONCLUSION

The study identified two different water quality subset, with RRP (subset two) being more polluted than the subset one (MWC, SNP and BH). Forested areas (MWC, SNP and BH), where human activity is minimal, are the best habitats for this species due to their higher levels of DO with significant difference for both mucous and seromucous glands. Furthermore, a substantial positive association was discovered between mucous glands and DO, indicating the importance of mucous in assisting absorption of DO from air and while completely submerged in water especially during breeding season. In contrast, seromucous glands (a mixture of mucous and serous glands) showed a strong negative correlation with DO indicating its none or less function to the respiratory system of the amphibian.

Odorrana hosii skin from the four different localities was found to have cutaneous gland variations that reflect the environment in their number, size, and distribution of the glands. From the histology analysis, the size of mucous glands has an above average correlation with DO when tested with the two correlation tests. Mucous glands play an important role in showing the water quality of the habitat, which reflects on *O. hosii*'s adaptation to its natural surroundings.

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