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MOLECULAR PHYLOGENY STUDY OF *SCHISMATOGLOTTIS* FROM DIFFERENT REGIONS IN SABAH USING INTERNAL TRANSCRIBED SPACER REGION

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ABSTRACT. *The Schismatoglottideae tribe, primarily in Borneo, is notably diverse within the Araceae family. It encompasses various rainforest herbs, adapting to terrestrial, lithophytic, and rheophytic habitats. The Schismatoglottis genus, with over 175 species, mostly exclusive to Borneo due to geological preferences, stands out. A study aimed to understand genetic relationships among Schismatoglottis species from Kadamaian-Kinabalu Park, Kionsom Recreational Area, Imbak Canyon Conservation Area, Mensalong Forest Reserve, Danum Valley Conservation Area, Tawau Hills Park, and Mantailang Crocker Range Park. The Internal Transcribed Spacer (ITS) regions of twelve Schismatoglottis species were sequenced. Genomic DNA was extracted from dried-silica-gel leaf tissue using a commercial kit (Promega, USA). ITS region lengths ranged from 528 bp to 708 bp. BLAST analysis accurately identified species within the Schismatoglottis genus (95% to 98% similarity). Phylogenetic analysis revealed a highly supported sister clade, uniting Schismatoglottis porpax species from Mensalong and Kionsom, despite differing elevational habitats. This pioneering study delves into Schismatoglottis phylogenetics, particularly in Sabah, with seven of the twelve species showing potential for new species classification, pending further research.*

KEYWORDS. Araceae, *Schismatoglottis*, phylogenetic analysis, Malaysia, Sabah.

INTRODUCTION

The Araceae family consists of about seven subfamilies, 32 tribes, and 144 genera with 6,000 estimated species (Mayo *et al.*, 1997). Currently, 4,000 species of Araceae have been formally described and updated in an ongoing list (Boyce & Croat, 2011). The family is defined by having minute sessile either unisexual or bisexual flowers located on the spadix and covered by a modified leaf called a spathe.

The tribe Schismatoglottideae Nakai has more than 120 species, more than 95% of which are endemic, making it the most varied and speciose aroid taxon in Borneo (Low *et al.*, 2018). The tribe includes *Schismatoglottis* Zoll. & Moritz, the biggest genus, in addition, includes four minor genera, or "satellites": *Bucephalandra* Schott, *Aridarum* Ridl, *Piptospatha* N.E. Br. and *Phymatarum* M. Hotta. Although the Schismatoglottideae tribe is prevalent in southeast Asia, it poses many challenges to the current definition of the genus boundaries.

Genus *Schismatoglottis* (included within the Schismatoglottideae tribe) has more than 175 species with a majority endemicity in Borneo with strictly geological obligated (Kartini *et al.*, 2017; Kartini *et al.*, 2020; Low *et al.*, 2018; Wong *et al.*, 2010). Fewer research was done based on aroids especially the genus of *Schismatoglottis* in Sabah resulting in poor understanding of the taxonomy and phylogeny.

The Araceae of Borneo currently stands at 670 species, of which 40% from them is new species and have not been formally described (Wong, 2016). In Sabah, field trips that were conducted often resulted in numerous undeterminable species of aroids which on subsequent flowering in cultivation have proved to be taxonomic novelties (Kartini *et al.*, 2017). In previous molecular phylogenetic studies, mainly examining the Araceae family as a whole, only two or three taxa from the Schismatoglottideae were included. The molecular study indicated that the Schismatoglottideae are not monophyletic (Barabé *et al.*, 2004).

The rapid advancements in molecular techniques, as demonstrated by the polymerase chain reaction (PCR), have had a profound impact on the

application of DNA sequences in molecular phylogenetic studies (Topi & Adi, 2010). DNA-based molecular markers provide a more efficient and reliable method for characterizing germplasm and conducting phylogenetic analysis. An additional advantage of these markers lies in their ability to withstand various environmental variables (Barnajee *et al*., 2016).

The chosen molecular markers must be conservative to facilitate PCR amplification and alignment of sequences among distant species, but they must alsobe sufficiently variable to permit the identification of the nearest species. Internal

Transcribed Spacer (ITS) DNA markers are the potential universal marker for plants and animals (Chen *et al.*, 2010). It contains enough variation to identify a wider range of plant taxa and even distinguish between closely related species (Yao *et al.*, 2010), and can be used in conjunction with morphological features analysis (Zhang *et al.*, 2015).

To the best of our knowledge, few molecular investigations on the evolutionary relationships of the tribe Schismatoglottideae were recently investigated utilizing a variety of sequences in Borneo (Ting *et al.*, 2012; Low *et al.*, 2011; Wong *et al.*, 2010; Wong & Boyce, 2007). To date, the vast diversity of *Schismatoglottis* species in Sabah has not been adequately researched, particularly in terms of taxonomy and biosystematics, genetics, and molecular biology. Therefore, this research was done to investigate the evolutionary relationships and provide a basic analysis of a short fragment of the ITS region.

MATERIALS AND METHODS

Plant Materials

In this study, a total of 12 *Schismatoglottis* species were collected from seven different localities in Sabah, Malaysia as presented in Figure 1 and Table 1.

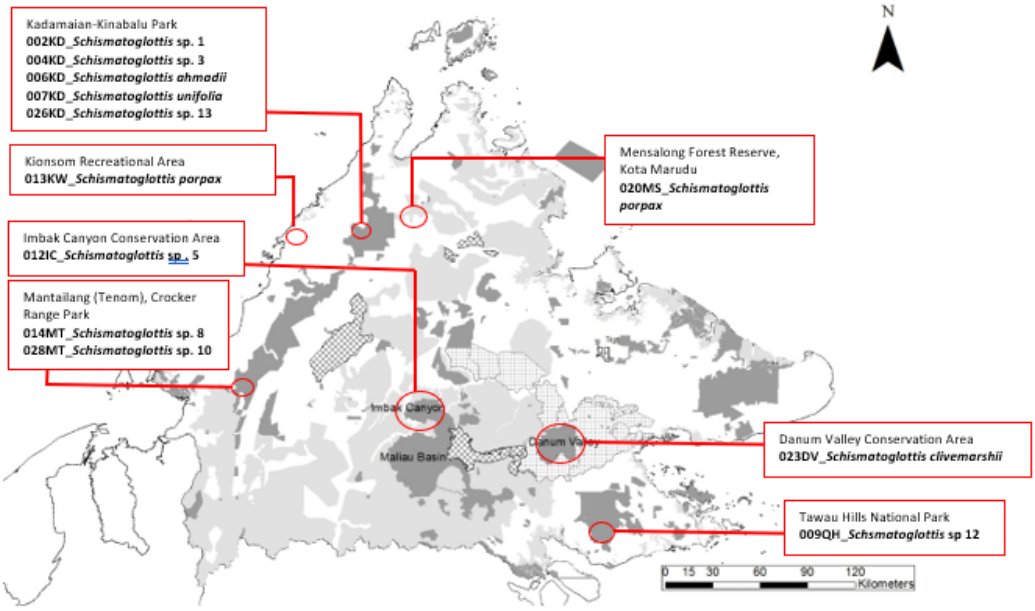

Figure 1. The location of the sampling area.

Table 1: Tabulation of sampling locations and their elevation

No.	Location	Abbreviation	GPS coordinate	Elevation (m a.s.l)
1	*Kadamaian-Kinabalu Park (Kota Belud)*	*K-KP*	*6.105º N, 116.303º E*	*500-1400*
2	*Kionsom Recreational Area*	*KRA*	*5.973° N, 116.206° E*	*200-300*
3	*Imbak Canyon Conservation Area*	*ICCA*	*5.936° N, 116.627° E*	*150-1500*
4	*Mensalong Forest Reserve (Kota Marudu)*	*MFR*	*6.170° N, 116.450º E*	*250-1100*
5	*Danum Valley Conservation Area*	*DVCA*	*4.961° N, 117.689° E*	*500-1100*
6	*Tawau Hills Park*	*THP*	*4.503° N, 117.935° E*	*400-900*
7	*Mantailang (Tenom), Crocker Range Park*	*MCRP*	*5.286° N, 115.978° E*	*500*

Identification of the species is referred to previous references such as Mayo *et al.*, 1997 and Hay & Yuzammi, 2000. In addition, photographs and morphological descriptions of these species were also sent to one of the Araceae experts, Associate Professor Madya Dr. Kartini Saibeh for validation of the species (Table 2). Each species was cut approximately 10 cm with a sterile blade and stored in a tea bag filled with 2 g silica gel (Sigma Aldrich, Germany) to reduce humidity. Once arrived at the laboratory, the samples were then stored at -80 ^{o}C before further use.

Table 2. List of twelve samples collected from different localities

No .	Samples ID	Locality
1	002KD_*Schismatoglottis* sp. 1	Kadamaian
2	004KD_*Schismatoglottis* sp. 3	Kadamaian
3	006KD_*Schismatoglottis ahmadii*	Kadamaian
4	007KD_*Schismatoglotttis unifolia*	Kadamaian
5	026KD_*Schismatoglottis* sp. 13	Kadamaian
6	012IC_*Schismatoglottis* sp. 5	Imbak Canyon
7	009QH_*Schismatoglottis* sp. 12	Imbak Canyon
8	013KW_*Schismatoglottis porpax*	Kionsom
9	020MS_*Schismatoglottis porpax*	Mensalong
10	014MT_*Schismatoglottis* sp. 8	Mentailang
11	028MT_*Schismatoglottis* sp. 10	Mentailang
12	023DV_*Schismatoglottis clivemarshii*	Danum valley

Total genomic extraction

Total genomic DNA was extracted from silica-gel-dried leaf tissue by using the Wizard® Genomic DNA extraction kit (Promega, USA) following the manufacturer's instructions. The quality of DNA was measured using a NanoDrop^{TM} One Microvolume UV-Vis Spectrophotometer, (Thermo Fisher Scientific, USA). The extracted DNA was labeled and stored at -20^{o}C until further use.

PCR amplification and sequence analysis

The PCR fragments were amplified using primer pairs of the Internal Transcribe Spacer (ITS) regions 1 (5' TCCGTAGGTGAACCTGCGG 3') and 4 (5' GCTGCGTTCATCGATGC 3') (White *et al.*, 1990). All PCR reactions were carried out in a 20 µl volume containing 1× GoTaq Flexi PCR buffer (Promega, USA), 3.0 mM $MgCl_2$, 0.2 mM dNTP, 0.2 µM of each primer, 2.0 units of Taq DNA polymerase, GoTaq G2 Flexi (Promega, USA), 0.2 mg/ml of BSA (bovine serum albumin, New England Biolabs), 1.5 µl of DNA template (100 ng/µl to 200 ng/µl) and sterile double deionized water.

The expected amplicon size for the Internal Transcribed Spacer is 500-1000 base pairs (bp). The amplification of ITS region was carried out using Bio-Rad T100^{TM} thermal cycler with a thermal profile of primary denaturation for 2 minutes at 95^{o}C, followed by 35 cycles of 30 seconds at 95^{o}C, 1 minute for 57^{o}C, 1 minute and 30 seconds at 72^{o}C and followed by final extension of 72^{o}C at 5 minutes. The PCR was amplified based on Wong *et al.*, (2010) with slight modification.

PCR purification and sequencing

PCR products were checked on 1% TBE agarose gel with 1 Kb DNA ladders. The PCR products are stained using GelRed and visualized under Vilber Lourmat UV scanner (UK) to observe the existing band. The positive PCR products were sent to Apical Scientific Sdn. Bhd. (Selangor, Malaysia) for purification and sequencing.

Sequence search and phylogenetic construction

The sequence of twelve samples was searched for homolog sequence using BLAST analysis against the NCBI database (http://www.ncbi.nlm.nih.gov/blast.cgi). The multiple sequence alignments were performed using the ClustalW program followed by visual adjustment and and then edited using Bioedit version 7.2.5 before constructing the phylogenetic tree. The construction of maximum likelihood (ML) was performed using CIPRES (https://www.phylo.org/portal2/login!input.action) which is an online software. The IQ TREE on XSEDE (Nguyen *et al*., 2015)

was chosen to construct the maximum likelihood phylogenetic tree, whereas, Bayesian phylogenetic analyses were performed using CIPRES (https://www.phylo.org/portal2/login!input.action) which is an online tool and the MrByes 3.2.7a on XSEDE (Ronquist *et al.*, 2011) was chosen to construct the bayesian inference phylogenetic tree.

RESULTS AND DISCUSSION

PCR amplification

The sequence alignments obtained from Apical Scientific Sdn. Bhd. (Selangor, Malaysia) were successfully generated a total of 12 DNA sequences of *Schismatoglottis* in this study. Figure 2 shows the successful PCR amplification of *Schismatoglottis*.

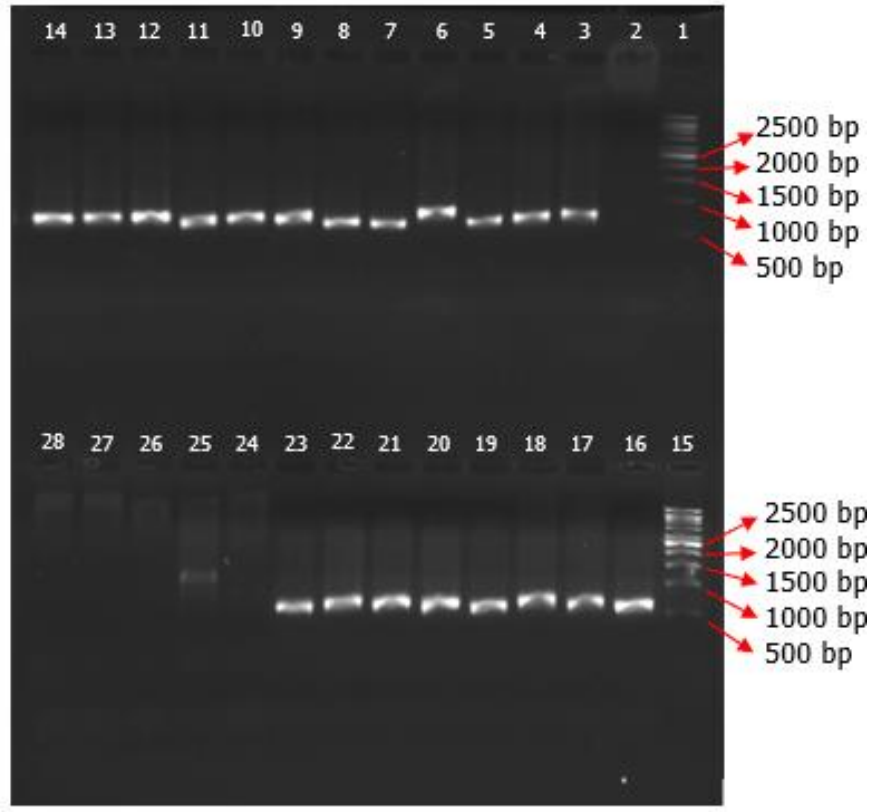

Figure 2. PCR amplification of Schismatoglottis. 1: 1kb ladder, 2: negative control, 3: positive control, 4-5: 002KD_*Schismatoglottis* sp. 1, 6-7: 004KD_*Schismatoglottis* sp. 3, 8-9: 006KD_*Schismatoglottis ahmadii*, 10-11: 007KD_*Schismatoglotttis unifolia* , 12-13: 026KD_*Schismatoglottis* sp. 13. 14-15: 012IC_*Schismatoglottis* sp. 5, 16-17: 009QH_*Schismatoglottis* sp. 12, 18-19: 013KW_*Schismatoglottis porpax*, 20: 020MS_*Schismatoglottis porpax*, 21: 014MT_*Schismatoglottis* sp. 8. 22: 028MT_*Schismatoglottis* sp. 10, 23: 023DV_*Schismatoglottis clivemarshii*, 24-28: unsucessful band.

BLAST analysis

The DNA sequences were verified by comparing them with the sequences of other species by BLAST search in the NCBI. The BLAST analysis was shown in Table 3. The length of the Internal Transcribed Region (ITS) region ranged from 528 bp to 708 (bp).

ITS successfully identified 95% to 98% of specimens, respectively at the species level belonging to the genus *Schismatoglottis*. This information could potentially be used in future studies on the phylogeny of *Schismatoglottis* in Sabah

Table 3. Analysis of BLAST X result by using ITS region.

Species	Accession number	E value	Identity	Query cover
Schismatoglottis calyptrata	KU748782.1	0	98.84	99%
***Schismatoglottis* sp. SLL-2016**	KT732169.1	0	98.84	99%
Schismatoglottis ahmadii	KU748781.1	0	98.41	99%
***Schismatoglottis* sp. AR3956**	KP748510.1	0	95.66	99%
***Schismatoglottis* sp. AR3586**	KP748505.1	0	95.64	99%
***Schismatoglottis* sp. AR4331**	KP748501.1	0	95.64	99%
***Schismatoglottis* sp. AR4270**	KP748512.1	0	95.25	99%
***Schismatoglottis* sp. AR4666**	KP748516.1	0	95.36	99%
***Schismatoglottis* sp. AR4096**	KP748511.1	0	95.36	99%
***Schismatoglottis* sp. AR3679**	KP748509.1	0	95.36	99%
***Schismatoglottis* sp. AR3615**	KP748506.1	0	95.24	99%
***Schismatoglottis* sp. AR2549**	KP748503.1	0	95.36	99%
***Schismatoglottis* sp. AR1632**	KP748498.1	0	95.36	99%
***Schismatoglottis* sp. AR1941**	KP748502.1	0	95.11	99%
***Schismatoglottis* sp. AR382**	KP748496.1	0	95.07	99%
***Schismatoglottis* sp. AR1240**	KP748497.1	0	94.95	99%
Schismatoglottis ranchanensis	KP744728.1	0	94.95	99%
***Schismatoglottis* sp. AR4651**	KP748515.1	0	95.18	98%
***Schismatoglottis* sp. AR2588**	KP748504.1	0	94.93	99%
***Schismatoglottis* sp. AR1638**	KP748500.1	0	95.18	98%

Phylogenetic datasets

One dataset was generated, which comprised 12 ITS regions with 22 Bornean samples of *Schismatoglottis* species (Table 4) from GenBank (https://www.ncbi.nlm.nih.gov/genbank/). The outgroup species had relatively relevant sequences, with ITS sequences ranging from 640 bp (*Apoballis mutata*).

Table 4. List of species taken from GenBank with accession number

Name	Locality	GenBank (ITS)	Publication
Schismatoglottis nervosa	Sarawak	JX857111	Low *et al.*, 2018
Schismatoglottis nervosa	Sarawak	JX857125	Low *et al.*, 2018
Schismatoglottis sp. AR-2078	Sarawak	JX857121	Low *et al.*, 2018
Schismatoglottis sp. AR-1105	Sarawak	JX857113	Low *et al.*, 2018
Schismatoglottis sp. AR-1930	Sarawak	JX857119	Low *et al.*, 2018
Schismatoglottis asperata	Sarawak	KP744717	Low *et al.*, 2018
Schismatoglottis asperata	Sarawak	KP744712	Low *et al.*, 2018
Schismatoglottis asperata	Sarawak	KP744727	Low *et al.*, 2018
Schismatoglottis asperata	Sarawak	KP744716	Low *et al.*, 2018
Schismatoglottis asperata	Sarawak	KP744715	Low *et al.*, 2018
Schismatoglottis sp. AR1638	Sarawak	KP748500	Low *et al.*, 2018
Schismatoglottis sp. AR3662	Sarawak	KP748507	Low *et al.*, 2018
Schismatoglottis sp. AR3673	Sarawak	KP748508	Low *et al.*, 2018
Schismatoglottis sp. AR382	Sarawak	KP748496	Low *et al.*, 2018
Schismatoglottis sp. AR3679	Sarawak	KP748509	Low *et al.*, 2018
Schismatoglottis sp. AR4096	Sarawak	KP748511	Low *et al.*, 2018
Schismatoglottis sp. AR4023	Sarawak	KP748499	Low *et al.*, 2018
Schismatoglottis sp. AR4651	Sarawak	KP748515	Low *et al.*, 2018
Schismatoglottis sp. AR3615	Sarawak	KP748506	Low *et al.*, 2018
Schismatoglottis patentinervia	Sarawak	KT732134	Low *et al.*, 2018
Schismatoglottis nervosa	Sarawak	JX857116	Low *et al.*, 2018
Schismatoglottis sp. AR3956	Kalimantan	KP748510	Low *et al.*, 2018
Apoballis mutata AR3595	Sarawak	KM433710	Low *et al.*, 2018

Analysis of phylogenetic tree

A combined analysis based on maximum likelihood and Bayesian inference tree based on nuclear Internal Transcribed Spacer (ITS) region sequence data is shown in Figure 3.

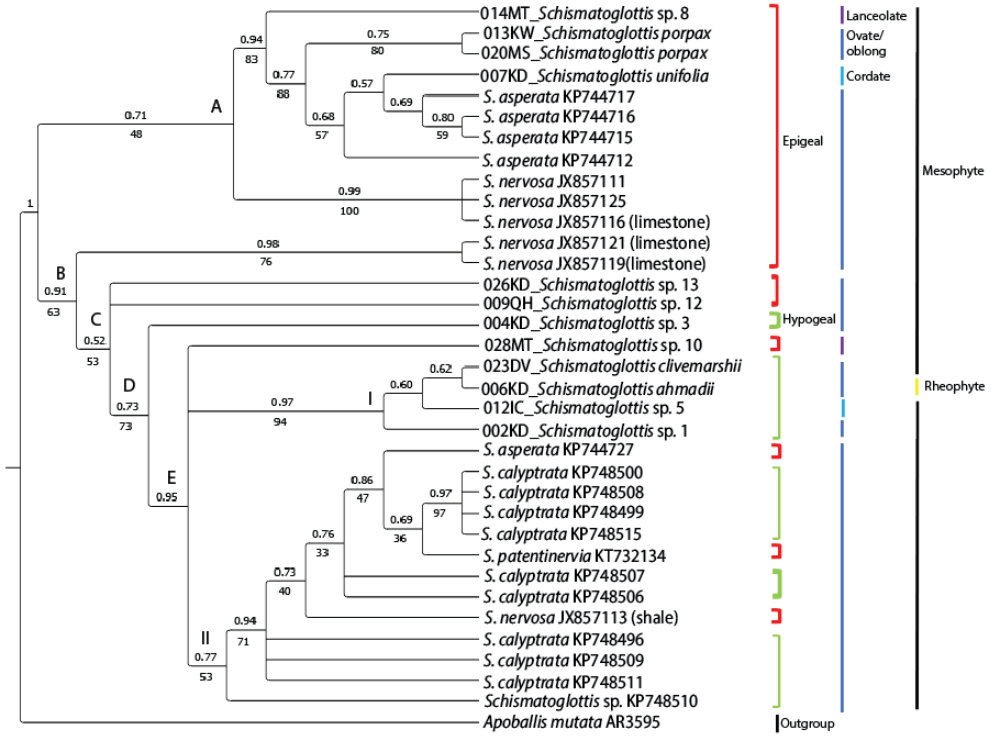

Figure 3. The combined consensus tree results from Maximum Likelihood analysis (lower branch) and optimal tree results from Bayesian analysis (upper branch) generated from the Internal Transcribed Spacer (ITS) region.

Based on the consensus tree, five major clades were formed. Clade A moderately supported with 0.71 posterior probability and 48% bootstrap. *Schismatoglottis porpax* taken from Mensalong Forest Reserve and Kionsom Recreational Area was grouped together with moderately high support value (PP: 0.75, BS: 80%). *Schismatoglottis unifolia* formed a basal root to *Schismatoglottis asperata* (KP744717, KP744716, and KP744715) with a moderately bootstrap support value of 57%. *Schismatoglottis unifolia* is a unique single leaf of the *Schismatoglottis* genus. This species is endemic to Sabah and usually found on steep slopes (Figure 4).

Figure 4. A colony of *Schismatoglottis unifolia* in its habitat at Kadamaian-Kinabalu Park.

In Clade B, *Schismatoglottis nervosa* from the limestone area formed a sister clade with a strongly supported posterior probability of 0.98 and bootstrap of 76%. *S. nervosa* JX857121 and JX857119 belong to clade B, form a separate clade from *S. nervosa* JX857116, which belongs to clade A. These species are thought to have originated in the limestone areas of Sarawak but have not been identified yet.

The presence of aromatic vegetative tissues (terpenoids), longitudinally ribbed petioles, and leaf blades with tessellate tertiary venation led to the placement of these species in the Nervosa Grade.

Schismatoglottis nervosa is an intriguing plant species that exemplifies the complex process of evolutionary divergence among populations. Schismatoglottis nervosa, which has a common ancestor, has endured significant changes over time. The species initially flourished as a single population, but environmental changes led to the separation of the population into two distinct groups. In isolation, these communities may have **Molecular**

accumulated genetic variations through mutations, a hallmark of evolution. Over the course of generations, these genetic differences manifested as unique traits and adaptations in each group, such as variations in leaf morphology and growth patterns. As a result, the two groups progressively evolved into distinct clades within the larger *Schismatoglottis nervosa* species, each with a unique evolutionary history. Phylogenetic analysis, which entails the study of the genetic material of individuals from each clade, reveals the complex branching pattern of their evolution. This analysis provides not only insights into the intriguing history of *Schismatoglottis nervosa*, but also a broader comprehension of how species can diversify in response to their environments.

In Clade C, *Schismatoglottis* sp. 13 from Kadamaian-Kinabalu Park and *Schismatoglottis* sp. 12 form sister taxa with a posterior probability of 0.52 and bootstrap of 53%. Both of these species share similar morphology traits which they have an ovate to oblong shape of leaf (Figure 5).

Figure 5. A) *Schismatoglottis* sp. 12; B) *Schismatoglottis* sp. 13, both of these species have an ovate to oblong shape of leaf (red circles).

For Clade D, this clade is supported with posterior probability of 0.73 and bootstrap of 73%. Moving to Clade E, this clade is divided into two clades which are Clade E-I and Clade E-II. In Clade E-I, *Schismatoglottis* sp. 1 formed basal root to *Schismatoglottis* sp. 5, *Schismatoglottis ahmadii* and

Schismatoglottis clivemarshii with posterior probability of 0.97 and bootstrap of 94%.

Schismatoglottis genus had a lack of identification based on ITS rDNA sequence data however they have a significantly moderate level of nucleotide variation between species that are closely related species.

CONCLUSION

To conclude, the phylogenetic tree of combined analysis for ITS gene in this study produced five clades. For Clade A, there are 11 species were included that is *Schismatoglottis* sp. 8, *Schismatoglottis porpax*, *Schismatoglottis porpax*, *Schismatoglottis unifolia*, *Schismatoglottis asperata* (KP744717, KP744716, KP744715, KP744712) and *Schismatoglottis nervosa* (JX857111, JX857125, JX857116). In Clade B, there are two species included that is *Schismatoglottis nervosa* (JX857121 and JX857119). In Clade C, there are two species also that is *Schismatoglottis* sp. 13 and *Schismatoglottis* sp. 12. Whereas in Clade E, is separated into two subclades: Clade E-I includes four species that is *Schismatoglottis clivemarshii, Schismatoglottis ahmadii, Schismatoglottis* sp.5 and *Schismatoglottis* sp.1. Lastly, in Clade E-II, 13 species included that is *Schismtoglottis asperata* (KP744727), *Schismatoglottis calyptrata* (KP748500, KP748508, KP748999, KP748515, KP748507, KP748506, KP748496, KP748509, KP748511), *Schismatoglottis patentinervia* (KT732134), *Schismatoglottis nervosa* (JX857113) and *Schismatoglottis* sp. (KP748510). The conclusions drawn from the phylogenetic tree lead to the formation of one highly supported sister clade. The sister clades are the *Schismatolgottis porpax* (from Mensalong) and *Schismatolgottis porpax* (from Kionsom). Even though they lived at different elevations, the two species of *Schismatoglottis porpax* were closely related to one another. The results of this study are the pioneers of the phylogenetic study of *Schismatoglottis* focusing on species in Sabah. A total of seven (7) out of 12 species would have a high potential to be described as a new species, however, further confirmation needs to be made in future research.

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ZERO-INFLATED POISSON TRANSMUTED WEIGHTED EXPONENTIAL DISTRIBUTION: PROPERTIES AND APPLICATIONS

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ABSTRACT. *Count observations with high frequencies of zero counts abound in diverse fields. In actuary science, for example, insurance claims are often underreported, leading to a higher frequency of zero counts. This invariably reduces the mean and leads to over-dispersion. Different techniques to model such occurrences exist. This study uses the cubic rank transmutation map to compound the weighted exponential distribution and obtain a new count distribution in the mixed Poisson paradigm. The zero-inflated form of the proposition is obtained along with some mathematical properties. Simulated skewness, kurtosis, and dispersion index for the new distributions reveal they are suitable for model dispersed observation with positive skewness. Five datasets with high frequencies of zero counts are used to assess the performance of the new distribution along with some popular count distributions by using the maximum likelihood for parameter estimation. Results show that the natural form of the new proposition performs creditably better than its zero-inflated forms, even when there is a higher proportion of zero counts. The classical negative binomial distribution is also observed to outperform its zero-inflated form in most cases. In contrast, the zero-inflated Poisson distribution better fits the datasets than the classical Poisson distribution.*

KEYWORDS: Mixed Poisson distribution, mixing distribution, rank transmutation map, weighted exponential distribution, excess zero counts.

INTRODUCTION

Results obtained from count data modelling may be misleading if there are too many or too few zero counts. Zero-deflation arises when the zero frequency in a dataset is lower than the expected frequency, while zero inflation occurs when there are too many zero counts. The former is rare, while the latter is more observed (Conceição *et al*., 2017). The Poisson distribution is often considered for modelling count data (Tajuddin & Ismail, 2022; Wagh & Kamalja, 2017). The distribution assumes equality of mean and variance for observations (Ong *et al*., 2021). Observations with a relatively higher frequency of zero counts are usually dispersed (Sellers & Raim, 2016). Since most count data are dispersed (Adetunji &

Sabri, 2021), there is model misspecification when the classical Poisson distribution is assumed for dispersed observation (Asamoah, 2016). In order to overcome challenges that characterize the Poisson distribution, several methods that can handle dispersed count observations with excess zero have been proposed (Das *et al*., 2018; Ong *et al*., 2021). One of the techniques often utilized for modelling such observation is the mixed Poisson, first proposed in the early 20th century (Greenwood & Yule, 1920) when the gamma distribution is assumed for the parameter of the classical Poisson distribution resulting in the negative binomial distribution. The process of obtaining a mixed Poisson distribution involves assuming a continuous distribution (called the mixing distribution) with positive supports for the parameter of the Poisson distribution.

Several choices of mixing distributions have been proposed to improve the flexibility and general applicability of the mixed Poisson paradigm. A detailed survey on the choice of the mixing distribution is provided in Ong *et al*. (2021), while Karlis & Xekalaki (2005) gave several properties of the mixed Poisson distribution.

In this study, the cubic rank transmutation map (Rahman *et al*., 2019) is used to extend the weighted exponential distribution (Gupta & Kundu, 2009) and obtain a new mixing distribution assumed for the parameter of the Poisson distribution. The mixing distribution is used to obtain a new mixed Poisson distribution and its zero-inflated form.

Zero-inflated distribution is often used to model observations with several zeros by assigning an extra probability to zero occurrences (Lambert, 1992). The distribution is applied when the frequency of excess zeros is assumed to have come from two processes; the first, where zero counts are obtained by chance like the ones, twos, etc., and the second are obtained when some data are constrained to be zeros (Lambert, 1992; Shahmandi *et al*., 2020). The claim frequency in actuary science is an example of such modelling since observed zeros could have been from two scenarios. A policyholder may have no claim (no case of an accident) reflecting a true zero. Also, there are situations when the policyholder may be involved in a minor accident and hence, may have no urge to report such for a claim, mainly due to the usually cumbersome processes and procedures involved in getting the claim.

MATERIALS AND METHOD

Weighted Exponential Distribution

The weighted exponential distribution (Gupta & Kundu, 2009) with the CDF defined in equation (1) as:

$$G(t) = 1 - \frac{1}{a}e^{-\theta t}\left(a + 1 - e^{-a\theta t}\right) \qquad (1)$$

Gupta and Kundu (2009) showed that the shape of equation (1) is identical to that of other two-parameter continuous distributions like generalized exponential, Weibull, and gamma, hence can be used as their alternative. The 3-parameter form of the distribution was proposed by Altun (2019). Zamani *et al*. (2014) used the mixing distribution to obtain a new mixed Poisson distribution with applications in claim frequency.

Cubic Rank Transmutation Map

Several extensions of different baseline distributions have been proposed to improve flexibility and general applicability. Among many recently proposed techniques are: the Quadratic Transmutation (QT) map (Shaw & Buckley, 2007); and Cubic Rank Transmutations (CRT) map (Al-kadim, 2018; Aslam *et al*., 2018; Rahman *et al*., 2019). Given a baseline distribution with distribution function $G(t)$, the cubic rank transmutation map (Rahman *et al*., 2019) has the CDF given in equation (2) as:

$$F(t) = (1-p)G(t) + 3p\big(G(t)\big)^2 - 2p\big(G(t)\big)^3 \quad (2)$$

Inserting equation (1) into equation (2) gives the distribution function for the Cubic Transmuted Weighted Exponential Distribution (CTWED) in equation (3) as:

$$F(t) = \left(\frac{(a+1)e^{-\theta t} - e^{-(1+a)\theta t} - a}{a^3}\right)\left(pae^{-(1+a)\theta t} - 4p(a+1)e^{-(2+a)\theta t} + 2pe^{-2(1+a)\theta t} - pa(a+1)e^{-\theta t} + 2p(a+1)^2 e^{-2\theta t} - a^2\right) \quad (3)$$

The corresponding PDF is obtained in equation (4) as:

$$f(t) = \frac{\theta(a+1)}{a^3}\left[-6p(2a+3)e^{-(2a+3)\theta t} + 6p(a+1)(a+3)e^{-(a+3)\theta t} + a^2(p-1)e^{-(a+1)\theta t} - 6pa(a+2)e^{-(a+2)\theta t} + 6pe^{-3(a+1)\theta t} + 6pae^{-2(a+1)\theta t} - 6p(a+1)^2 e^{-3\theta t} + 6pa(a+1)e^{-2\theta t} - a^2(p-1)e^{-\theta t}\right] \quad (4)$$

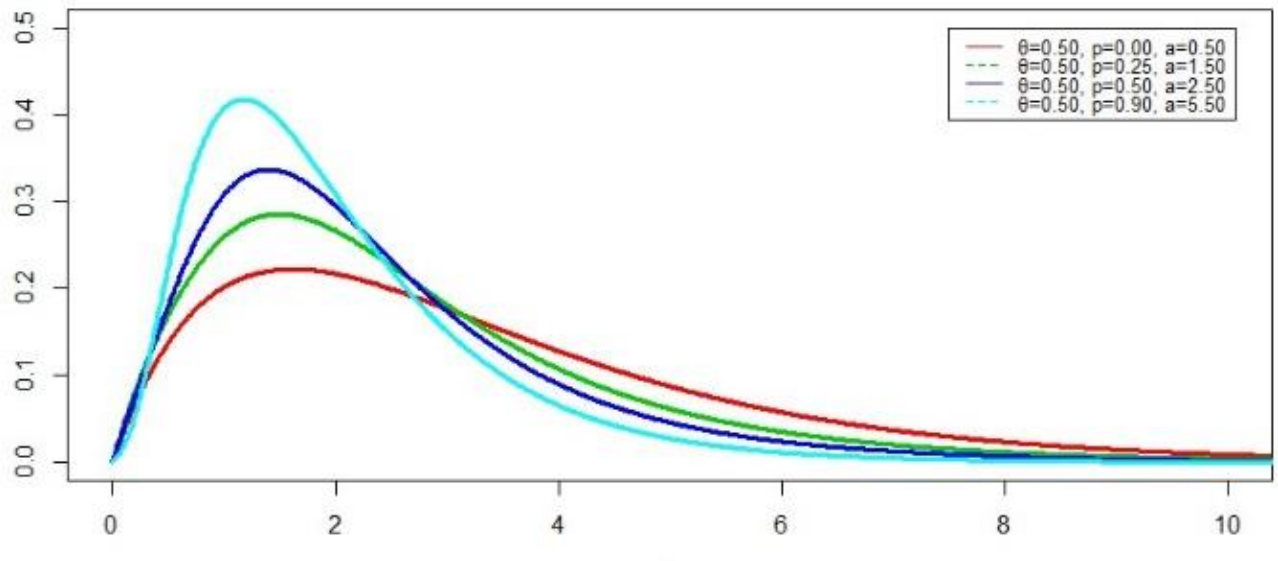

Figure 1. Shapes of the PDF of the CTWED

Figure 1 shows that the PDF of the CTWED is positively skewed and unimodal.

Moment and Moment Generating Function

Proposition 1: If a random variable *T* has a CTWED, the rth moment is obtained in equation (5) as:

$$E(t^r) = \left(\frac{(a+1)r!}{a^3\theta^r}\right)\left(\frac{-6p}{(2a+3)^r} + \frac{6p(a+1)}{(a+3)^r} + \frac{a^2(p-1)}{(a+1)^{r+1}} - \frac{6pa}{(a+2)^r} + \frac{2p}{3^r(a+1)^{r+1}} + \frac{3pa}{2^r(a+1)^{r+1}} - \frac{2p(a+1)^2}{3^r} + \frac{3pa(a+1)}{2^r} - a^2(p-1)\right) \quad (5)$$

Proof

$$E(t^r) = \int_0^\infty t^r f(t)\,dt$$

$$= \int_0^\infty t^r \frac{\theta(a+1)}{a^3}\left[-6p(2a+3)e^{-(2a+3)\theta t} + 6p(a+1)(a+3)e^{-(a+3)\theta t} + a^2(p-1)e^{-(a+1)\theta t} - 6pa(a+2)e^{-(a+2)\theta t} + 6pe^{-3(a+1)\theta t} + 6pae^{-2(a+1)\theta t} - 6p(a+1)^2e^{-3\theta t} + 6pa(a+1)e^{-2\theta t} - a^2(p-1)e^{-\theta t}\right]dt$$

$$= \frac{\theta(a+1)}{a^3}\left[-6p(2a+3)\int_0^\infty t^r e^{-(2a+3)\theta t}dt + 6p(a+1)(a+3)\int_0^\infty t^r e^{-(a+3)\theta t}dt + a^2(p-1)\int_0^\infty t^r e^{-(a+1)\theta t}dt - 6pa(a+2)\int_0^\infty t^r e^{-(a+2)\theta t}dt + 6p\int_0^\infty t^r e^{-3(a+1)\theta t}dt + 6pa\int_0^\infty t^r e^{-2(a+1)\theta t}dt - 6p(a+1)^2\int_0^\infty t^r\, e^{-3\theta t}dt + 6pa(a+1)\int_0^\infty t^r e^{-2\theta t}dt - a^2(p-1)\int_0^\infty t^r e^{-\theta t}dt\right]$$

$$= \left(\frac{\theta(a+1)r!}{a^3\theta^{r+1}}\right)\left(\frac{-6p(2a+3)}{(2a+3)^{r+1}} + \frac{6p(a+1)(a+3)}{(a+3)^{r+1}} + \frac{a^2(p-1)}{(a+1)^{r+1}} - \frac{6pa(a+2)}{(a+2)^{r+1}} + \frac{6p}{3^{r+1}(a+1)^{r+1}} + \frac{6pa}{2^{r+1}(a+1)^{r+1}} - \frac{6p(a+1)^2}{3^{r+1}} + \frac{6pa(a+1)}{2^{r+1}} - a^2(p-1)\right)$$

$$= \left(\frac{(a+1)r!}{a^3\theta^r}\right)\left(\frac{-6p}{(2a+3)^r} + \frac{6p(a+1)}{(a+3)^r} + \frac{a^2(p-1)}{(a+1)^{r+1}} - \frac{6pa}{(a+2)^r} + \frac{2p}{3^r(a+1)^{r+1}} + \frac{3pa}{2^r(a+1)^{r+1}} - \frac{2p(a+1)^2}{3^r} + \frac{3pa(a+1)}{2^r} - a^2(p-1)\right)$$

Proposition 2: If a random variable *T* has a CTWED, the MGF is obtained in equation (6) as:

$$E(e^{zt}) = \frac{\theta(a+1)}{a^3}\left(\frac{-6p(2a+3)}{2a\theta+3\theta-z} + \frac{6p(a+1)(a+3)}{a\theta+3\theta-z} + \frac{a^2(p-1)}{a\theta+\theta-z} - \frac{6pa(a+2)}{a\theta+2\theta-z} + \frac{6p}{3a\theta+3\theta-z} + \frac{6pa}{2a\theta+2\theta-z} - \frac{6p(a+1)^2}{3\theta-z} + \frac{6pa(a+1)}{2\theta-z} - \frac{a^2(p-1)}{\theta-z}\right) \quad (6)$$

Proof

$$E(e^{zt}) = \int_0^\infty e^{zt} f(t)\ dt$$

$$= \int_0^\infty e^{zt}\frac{\theta(a+1)}{a}\left[-6p(2a+3)e^{-(2a+3)\theta t} + 6p(a+1)(a+3)e^{-(a+3)\theta t} + a^2(p-1)e^{-(a+1)\theta t} - 6pa(a+2)e^{-(a+2)\theta t} + 6pe^{-3(a+1)\theta t} + 6pae^{-2(a+1)\theta t} - 6p(a+1)^2e^{-3\theta t} + 6pa(a+1)e^{-2\theta t} - a^2(p-1)e^{-\theta t}\right]\ dt$$

$$= \frac{\theta(a+1)}{a^3}\int_0^{\infty}\big(-6p(2a+3)e^{-(2a\theta+3\theta-z)t} + 6p(a+1)(a+3)e^{-(a\theta+3\theta-z)t} + a^2(p-1)e^{-(a\theta+\theta-z)t} - 6pa(a+2)e^{-(a\theta+2\theta-z)t} + 6pe^{-(3a\theta+3\theta-z)t} + 6pae^{-(2a\theta+2\theta-z)t} - 6p(a+1)^2\, e^{-(3\theta-z)t} + 6pa(a+1)e^{-(2\theta-z)t} - a^2(p-1)e^{-(\theta-z)t}\big)dt$$

$$= \frac{\theta(a+1)}{a^3}\left(\frac{-6p(2a+3)}{2a\theta+3\theta-z} + \frac{6p(a+1)(a+3)}{a\theta+3\theta-z} + \frac{a^2(p-1)}{a\theta+\theta-z} - \frac{6pa(a+2)}{a\theta+2\theta-z} + \frac{6p}{3a\theta+3\theta-z} + \frac{6pa}{2a\theta+2\theta-z} - \frac{6p(a+1)^2}{3\theta-z} + \frac{6pa(a+1)}{2\theta-z} - \frac{a^2(p-1)}{\theta-z}\right)$$

Mixed Poisson CTWED

Proposition 3: Given that a random variable $X \sim Poisson(T)$ and $T \sim CTWED\ (\theta, p, a)$. A discrete random variable *X* has a mixed Poisson-CTWED (PCTWED) if its PMF is defined in equation (7) as:

$$P_x = \frac{\theta(a+1)}{a^3}\left(\frac{6p(a+1)(a+3)}{(1+a\theta+3\theta)^{x+1}} - \frac{6p(2a+3)}{(1+2a\theta+3\theta)^{x+1}} + \frac{a^2(p-1)}{(1+a\theta+\theta)^{a+1}} - \frac{6pa(a+2)}{(1+a\theta+2\theta)^{x+1}} + \frac{6p}{(1+3a\theta+3\theta)^{x+1}} + \frac{6pa}{(1+2a\theta+2\theta)^{x+1}} - \frac{6p(a+1)^2}{(1+3\theta)^{x+1}} + \frac{6pa(a+1)}{(1+2\theta)^{x+1}} - \frac{a^2(p-1)}{(1+\theta)^{x+1}}\right) \quad (7)$$

Proof

$$P_x = \int_0^{\infty}\frac{t^x e^{-t}}{x!}\frac{\theta(a+1)}{a^3}\big[-6p(2a+3)e^{-(2a+3)\theta t} + 6p(a+1)(a+3)e^{-(a+3)\theta t} + a^2(p-1)e^{-(a+1)\theta t} - 6pa(a+2)e^{-(a+2)\theta t} + 6pe^{-3(a+1)\theta t} + 6pae^{-2(a+1)\theta t} - 6p(a+1)^2\, e^{-3\theta t} + 6pa(a+1)e^{-2\theta t} - a^2(p-1)e^{-\theta t}\big]\,dt$$

$$= \frac{\theta(a+1)}{a^3 x!}\int_0^{\infty} t^x\big(6p(a+1)(a+3)e^{-(1+a\theta+3\theta)t} - 6p(2a+3)e^{-(1+2a\theta+3\theta)t} + a^2(p-1)e^{-(1+a\theta+\theta)t} - 6pa(a+2)e^{-(1+a\theta+2\theta)t} + 6pe^{-(1+3a\theta+3\theta)t} + 6pae^{-(1+2a\theta+2\theta)t} - 6p(a+1)^2\, e^{-(1+3\theta)t} + 6pa(a+1)e^{-(1+2\theta)t} - a^2(p-1)e^{-(1+\theta)t}\big)\,dt$$

$$= \frac{\theta(a+1)}{a^3}\left(\frac{6p(a+1)(a+3)}{(1+a\theta+3\theta)^{x+1}} - \frac{6p(2a+3)}{(1+2a\theta+3\theta)^{x+1}} + \frac{a^2(p-1)}{(1+a\theta+\theta)^{a+1}} - \frac{6pa(a+2)}{(1+a\theta+2\theta)^{x+1}} + \frac{6p}{(1+3a\theta+3\theta)^{x+1}} + \frac{6pa}{(1+2a\theta+2\theta)^{x+1}} - \frac{6p(a+1)^2}{(1+3\theta)^{x+1}} + \frac{6pa(a+1)}{(1+2\theta)^{x+1}} - \frac{a^2(p-1)}{(1+\theta)^{x+1}}\right)$$

Sub-Model: Equation (7) becomes the PMF of the mixed Poisson Weighted Exponential Distribution (Zamani *et al*., 2014) when the $p = 0$.

Figure 2 shows different shapes of the PCTWED for different parameter values. The figure reveals that the distribution is suitable for unimodal distribution and observations with excess zeros, resembling the shapes of the PDF of the CTWED in Figure 1.

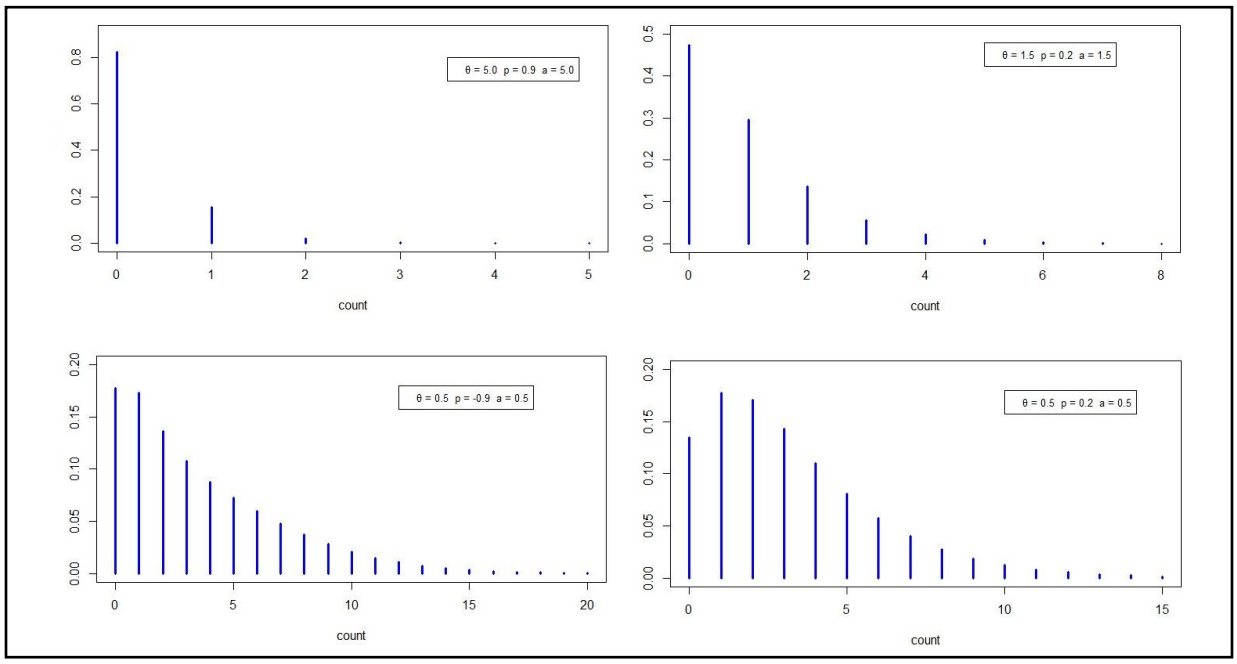

Figure 2. Shapes of the PMF of the PCTWED

The CDF of the PCTWED

Proposition 4: The CDF of a random variable *X* with the PCTWED is defined in equation (8) as:

$$F(x) = 1 - \left(\frac{6p(a+1)^2}{a^3(1+a\theta+3\theta)^{x+1}} - \frac{6p(a+1)}{a^3(1+2a\theta+3\theta)^{x+1}} + \frac{(p-1)}{a(1+a\theta+\theta)^{x+1}} - \frac{6p(a+1)}{a^2(1+a\theta+2\theta)^{x+1}} + \frac{2p}{a^3(1+3a\theta+3\theta)^{x+1}} + \frac{3p}{a^2(1+2a\theta+2\theta)^{x+1}} - \frac{2p(a+1)^3}{a^3(1+3\theta)^{x+1}} + \frac{3p(a+1)^2}{a^2(1+2\theta)^{x+1}} - \frac{(p-1)(a+1)}{a(1+\theta)^{x+1}}\right) \quad (8)$$

Proof

$$F(X) = P(X \leq x)$$

$$= 1 - P(X > x)$$

$$= 1 - \sum_{k=x+1}^{\infty} P_k$$

$$= 1 - \sum_{k=x+1}^{\infty} \frac{\theta(a+1)}{a^3}\left(\frac{6p(a+1)(a+3)}{(1+a\theta+3\theta)^{k+1}} - \frac{6p(2a+3)}{(1+2a\theta+3\theta)^{k+1}} + \frac{a^2(p-1)}{(1+a\theta+\theta)^{k+1}} - \frac{6pa(a+2)}{(1+a\theta+2\theta)^{k+1}} + \frac{6p}{(1+3a\theta+3\theta)^{k+1}} + \frac{6pa}{(1+2a\theta+2\theta)^{k+1}} - \frac{6p(a+1)^2}{(1+3\theta)^{k+1}} + \frac{6pa(a+1)}{(1+2\theta)^{k+1}} - \frac{a^2(p-1)}{(1+\theta)^{k+1}}\right)$$

$$= 1 - \frac{\theta(a+1)}{a^3}\left(\frac{6p(a+1)(a+3)}{\theta(a+3)(1+a\theta+3\theta)^{x+1}} - \frac{6p(2a+3)}{\theta(2a+3)(1+2a\theta+3\theta)^{x+1}} + \frac{a^2(p-1)}{\theta(a+1)(1+a\theta+\theta)^{x+1}} - \frac{6pa(a+2)}{\theta(a+2)(1+a\theta+2\theta)^{x+1}} + \frac{6p}{3\theta(a+1)(1+3a\theta+3\theta)^{x+1}} + \frac{6pa}{2\theta(a+1)(1+2a\theta+2\theta)^{x+1}} - \frac{6p(a+1)^2}{3\theta(1+3\theta)^{x+1}} + \frac{6pa(a+1)}{2\theta(1+2\theta)^{x+1}} - \frac{a^2(p-1)}{2\theta(1+\theta)^{x+1}}\right)$$ **Zero-Inflated Poisson**

$$= 1 - \left(\frac{6p(a+1)^2}{a^3(1+a\theta+3\theta)^{x+1}} - \frac{6p(a+1)}{a^3(1+2a\theta+3\theta)^{x+1}} + \frac{(p-1)}{a(1+a\theta+\theta)^{x+1}} - \frac{6p(a+1)}{a^2(1+a\theta+2\theta)^{x+1}} + \frac{2p}{a^3(1+3a\theta+3\theta)^{x+1}} + \frac{3p}{a^2(1+2a\theta+2\theta)^{x+1}} - \frac{2p(a+1)^3}{a^3(1+3\theta)^{x+1}} + \frac{3p(a+1)^2}{a^2(1+2\theta)^{x+1}} - \frac{(p-1)(a+1)}{a(1+\theta)^{x+1}}\right)$$

Mathematical Properties of the PCTWED

Proposition 5: If $f(t)$ is the PDF of the mixing distribution for a discrete random *X*, the Probability Generating Function (PGF) is obtained as:

$$P_x(z) = \int_0^\infty e^{t(z-1)} f(t)dt$$

$$= \int_0^\infty e^{t(z-1)} \frac{\theta(a+1)}{a^3}\left[6\mathrm{p(a+1)(a+3)e^{-(a+3)\theta t}} - 6\mathrm{p(2a+3)e^{-(2a+3)\theta t}} + \mathrm{a^2(p-1)e^{-(a+1)\theta t}} - 6\mathrm{pa(a+2)e^{-(a+2)\theta t}} + 6\mathrm{pe^{-3(a+1)\theta t}} + 6\mathrm{pae^{-2(a+1)\theta t}} - 6\mathrm{p(a+1)^2\, e^{-3\theta t}} + 6\mathrm{pa(a+1)e^{-2\theta t}} - \mathrm{a^2(p-1)e^{-\theta t}}\right]dt$$

$$= \frac{\theta(a+1)}{a^3}\int_0^\infty \left(6p(a+1)(a+3)e^{-(1+a\theta+3\theta-z)t} - 6p(2a+3)e^{-(1+2a\theta+3\theta-z)t} + a^2(p-1)e^{-(1+a\theta+\theta-z)t} - 6pa(a+2)e^{-(1+a\theta+2\theta-z)t} + 6pe^{-(1+3a\theta+3\theta-z)t} + 6pae^{-(1+2a\theta+\theta-z)t} - 6p(a+1)^2\, e^{-(1+3\theta-z)t} + 6pa(a+1)e^{-(1+2\theta-z)t} - a^2(p-1)e^{-(1+\theta-z)t}\right)dt$$

Therefore, the PGF is obtained in equation (9) as

$$P_x(z) = \frac{\theta(a+1)}{a^3}\left(\frac{6p(a+1)(a+3)}{(1+a\theta+3\theta-z)} - \frac{6p(2a+3)}{(1+2a\theta+3\theta-z)} + \frac{a^2(p-1)}{(1+a\theta+\theta-z)} - \frac{6pa(a+2)}{(1+a\theta+2\theta-z)} + \frac{6p}{(1+3a\theta+3\theta-z)} + \frac{6pa}{(1+2a\theta+\theta-z)} - \frac{6p(a+1)^2}{(1+3\theta-z)} + \frac{6pa(a+1)}{(1+2\theta-z)} - \frac{a^2(p-1)}{(1+\theta-z)}\right) \quad (9)$$

The Moment Generating Function (MGF) is obtained in equation (10) by replacing $\boldsymbol{z}$ with e^z in equation (9).

$$M_X(z) = \frac{\theta(a+1)}{a^3}\left(\frac{6p(a+1)(a+3)}{(1+a\theta+3\theta-e^z)} - \frac{6p(2a+3)}{(1+2a\theta+3\theta-e^z)} + \frac{a^2(p-1)}{(1+a\theta+\theta-e^z)} - \frac{6pa(a+2)}{(1+a\theta+2\theta-e^z)} + \frac{6p}{(1+3a\theta+3\theta-e^z)} + \frac{6pa}{(1+2a\theta+\theta-e^z)} - \frac{6p(a+1)^2}{(1+3\theta-e^z)} + \frac{6pa(a+1)}{(1+2\theta-e^z)} - \frac{a^2(p-1)}{(1+\theta-e^z)}\right) \quad (10)$$

The first four central moments are obtained in equations (11) - (14) as:

$$E(X) = \frac{(12-2p)a^4+(102-15p)a^3+(318-34p)a^2+(432-38p)a+216-19p}{6\theta(a+1)(a+2)(a+3)(2a+3)} \quad (11)$$

$$E(X^2)=\left(\frac{\theta(1+a)}{a^3}\right)\left(\frac{12p(a+1)(a+3)}{(a\theta+3\theta)^3}+\frac{6p(a+1)(a+3)}{(a\theta+3\theta)^2}-\frac{12p(2a+3)}{(2a\theta+3\theta)^3}-\frac{6p(2a+3)}{(2a\theta+3\theta)^2}+\frac{2a^2(p-1)}{(a\theta+\theta)^3}+\frac{a^2(p-1)}{(a\theta+\theta)^2}-\frac{12pa(a+2)}{(a\theta+2\theta)^3}-\frac{6pa(a+2)}{(a\theta+2\theta)^2}+\frac{12p}{(3a\theta+3\theta)^3}+\frac{6p}{(3a\theta+3\theta)^2}+\frac{12pa}{(2a\theta+2\theta)^3}+\frac{6pa}{(2a\theta+2\theta)^2}-\frac{8p(1+a)^2-27pa(1+a)+36a^2(p-1)}{18\theta^3}-\frac{4p(1+a)^2-9pa(1+a)+6a^2(p-1)}{6\theta^2}\right) \quad (12)$$

$$E(X^3)=\left(\frac{\theta(a+1)}{a^3}\right)\left(\frac{36p}{(3a\theta+3\theta)^4}+\frac{36p}{(3a\theta+3\theta)^3}+\frac{6p}{(3a\theta+3\theta)^2}-\frac{36p(2a+3)}{(2a\theta+3\theta)^4}-\frac{36p(2a+3)}{(2a\theta+3\theta)^3}-\frac{6p(2a+3)}{(2a\theta+3\theta)^2}+\frac{36pa}{(2a\theta+2\theta)^4}+\frac{36pa}{(2a\theta+2\theta)^3}+\frac{6pa}{(2a\theta+2\theta)^2}+\frac{36p(a+1)(a+3)}{(a\theta+3\theta)^4}+\frac{36p(a+1)(a+3)}{(a\theta+3\theta)^3}+\frac{6p(a+1)(a+3)}{(a\theta+3\theta)^2}-\frac{36pa(a+2)}{(a\theta+2\theta)^4}-\frac{36pa(a+2)}{(a\theta+2\theta)^3}-\frac{6pa(a+2)}{(a\theta+2\theta)^2}+\frac{6a^2(p-1)}{(a\theta+\theta)^4}+\frac{6a^2(p-1)}{(a\theta+\theta)^3}+\frac{a^2(p-1)}{(a\theta+\theta)^2}-\frac{216a^2(p-1)-81pa(a+1)+16p(a+1)^2}{36\theta^4}-\frac{36a^2(p-1)-27pa(a+1)+8p(a+1)^2}{6\theta^3}-\frac{4p(a+1)^2-9pa(a+1)+6a^2(p-1)}{6\theta^2}\right) \quad (13)$$

$$E(X^4)=\left(\frac{\theta(a+1)}{a^3}\right)\left(\frac{144p}{(3a\theta+3\theta)^5}+\frac{216p}{(3a\theta+3\theta)^4}+\frac{84p}{(3a\theta+3\theta)^3}+\frac{6p}{(3a\theta+3\theta)^2}-\frac{144p(2a+3)}{(2a\theta+3\theta)^5}-\frac{216p(2a+3)}{(2a\theta+3\theta)^4}-\frac{84p(2a+3)}{(2a\theta+3\theta)^3}-\frac{6p(2a+3)}{(2a\theta+3\theta)^2}+\frac{144pa}{(2a\theta+2\theta)^5}+\frac{216pa}{(2a\theta+2\theta)^4}+\frac{84pa}{(2a\theta+2\theta)^3}+\frac{6pa}{(2a\theta+2\theta)^2}+\frac{144p(a+1)(a+3)}{(a\theta+3\theta)^5}+\frac{216p(a+1)(a+3)}{(a\theta+3\theta)^4}+\frac{84p(a+1)(a+3)}{(a\theta+3\theta)^3}+\frac{6p(a+1)(a+3)}{(a\theta+3\theta)^2}-\frac{144pa(a+2)}{(a\theta+2\theta)^5}-\frac{216pa(a+2)}{(a\theta+2\theta)^4}-\frac{84pa(a+2)}{(a\theta+2\theta)^3}-\frac{6pa(a+2)}{(a\theta+2\theta)^2}+\frac{24a^2(p-1)}{(a\theta+\theta)^5}+\frac{36a^2(p-1)}{(a\theta+\theta)^4}+\frac{14a^2(p-1)}{(a\theta+\theta)^3}+\frac{a^2(p-1)}{(a\theta+\theta)^2}-\frac{32p(a+1)^2+1296a^2(p-1)-243pa(a+1)}{54\theta^5}-\frac{16p(a+1)^2+216a^2(p-1)-81pa(a+1)}{6\theta^4}-\frac{56p(a+1)^2+252a^2(p-1)-189pa(a+1)}{18\theta^3}-\frac{4p(a+1)^2+6a^2(a-1)-9pa(a+1)}{6\theta^2}\right) \quad (14)$$

Skewness and Kurtosis

The skewness and kurtosis for the PCTWED are obtained from the central moments (De Jong & Heller, 2008) as:

$$S_k=\frac{E(X^3)-3E(X^2)E(X)+2(E(X))^3}{(Var(X))^{\frac{3}{2}}}$$

$$Kurt=\frac{E(X^4)-4E(X^3)E(X)+6E(X^2)(E(X))^2-3(E(X))^4}{(Var(X))^2}$$

Tables 1 – 3 show simulated Skewness, Kurtosis, and Dispersion Index for some parameters distribution.

Table 1. Skewness for some parameters of the PCTWED

	$a = 0.5$			$a = 2.5$			$a = 7.5$		
	θ =0.1	*θ =2.0*	*θ =10.0*	*θ =0.1*	*θ =2.0*	*θ =10.0*	*θ =0.1*	*θ =2.0*	*θ =10.0*
p = -0.9	1.68	2.44	4.06	1.76	3.08	5.82	1.94	4.27	8.80
p = -0.5	1.82	2.48	4.15	1.89	3.13	5.97	2.03	4.36	9.04
p = 0.0	2.00	2.50	4.25	2.02	3.18	6.17	2.10	4.48	9.38
p = 0.5	2.10	2.46	4.35	2.05	3.20	6.38	2.06	4.59	9.76
p = 0.9	1.77	2.34	4.41	1.74	3.18	6.55	1.80	4.67	10.09

Table 2. Kurtosis for some parameters of the PCTWED

	$a = 0.5$			$a = 2.5$			$a = 7.5$		
	θ =0.1	θ =2.0	θ =10.0	θ =0.1	θ =2.0	θ =10.0	θ =0.1	θ =2.0	θ =10.0
p = -0.9	6.60	10.61	21.61	7.06	14.46	39.12	7.96	23.38	82.65
p = -0.5	7.56	10.98	22.30	7.90	14.83	40.80	8.62	24.15	86.98
p = 0.0	9.02	11.25	23.06	9.09	15.13	43.03	9.39	25.06	93.01
p = 0.5	10.40	10.97	23.59	9.93	15.01	45.33	9.59	25.76	99.84
p = 0.9	8.72	9.82	23.68	8.17	14.34	47.16	7.96	26.02	105.97

Table 3. Dispersion Index for some parameters of the PCTWED

	$a = 0.5$			$a = 2.5$			$a = 7.5$		
	θ =0.1	θ =2.0	θ =10.0	θ =0.1	θ =2.0	θ =10.0	θ =0.1	θ =2.0	θ =10.0
p = -0.9	9.86	1.44	1.09	4.80	1.19	1.04	2.56	1.08	1.02
p = -0.5	8.99	1.40	1.08	4.42	1.17	1.03	2.41	1.07	1.01
p = 0.0	7.67	1.33	1.07	3.86	1.14	1.03	2.18	1.06	1.01
p = 0.5	6.00	1.25	1.05	3.14	1.11	1.02	1.88	1.04	1.01
p = 0.9	4.35	1.17	1.03	2.44	1.07	1.01	1.59	1.03	1.01

Remarks:

For fixed p and a, skewness and kurtosis increase while the dispersion index decreases as θ increases. When p and θ are fixed, skewness and kurtosis increase while the dispersion index reduces as a increases. For fixed a and θ, skewness, kurtosis increase while the dispersion index decreases as p increases in most cases. Both skewness and kurtosis peak at $p = 0.5$.

Maximum Likelihood Estimation of the PCTWED

Given random samples of size *n* drawn from the PCTWED with (θ, p, a) as defined in equation (7), the log-likelihood function for the distribution is obtained in equation (15) as:

$$\ell = \sum_{i=1}^{n} log\left(\frac{\theta(a+1)}{a^3}\left(\frac{6p(a+1)(a+3)}{(1+a\theta+3\theta)^{x+1}} - \frac{6p(2a+3)}{(1+2a\theta+3\theta)^{x+1}} + \frac{a^2(p-1)}{(1+a\theta+\theta)^{x+1}} - \frac{6pa(a+2)}{(1+a\theta+2\theta)^{x+1}} + \frac{6p}{(1+3a\theta+3\theta)^{x+1}} + \frac{6pa}{(1+2a\theta+2\theta)^{x+1}} - \frac{6p(a+1)^2}{(1+3\theta)^{x+1}} + \frac{6pa(a+1)}{(1+2\theta)^{x+1}} - \frac{a^2(p-1)}{(1+\theta)^{x+1}}\right)\right) \quad (15)$$

Estimators for (θ, p, a) denoted with $(\hat{\theta}, \hat{p}, \hat{a})$ are the solutions for the log-likelihood function. The equations form a non-linear equation system that can only be solved numerically. This research uses the optimr function (Nash *et al.*, 2019) in the **R language** (R-Core Team, 2020) is used.

Zero-Inflated PCTWED

Proposition 6. If a discrete random variable *X* has a PCTWED with PMF P_x and its realization at $x = 0$ as P_0. If the zero-inflation parameter is denoted with π, then a discrete random variable X_Z has a zero-inflated PCTWED if its PMF is defined in equation (16) as:

$$P_x^Z = \begin{cases} \pi + (1-\pi)P_0, & x = 0 \\ (1-\pi)P_x, & x = 1,2,3,\ldots \end{cases} \quad (16)$$

where

$$P_x = \frac{\theta(a+1)}{a^3}\left(\frac{6p(a+1)(a+3)}{(1+a\theta+3\theta)^{x+1}} - \frac{6p(2a+3)}{(1+2a\theta+3\theta)^{x+1}} + \frac{a^2(p-1)}{(1+a\theta+\theta)^{a+1}} - \frac{6pa(a+2)}{(1+a\theta+2\theta)^{x+1}} + \frac{6p}{(1+3a\theta+3\theta)^{x+1}} + \frac{6pa}{(1+2a\theta+2\theta)^{x+1}} - \frac{6p(a+1)^2}{(1+3\theta)^{x+1}} + \frac{6pa(a+1)}{(1+2\theta)^{x+1}} - \frac{a^2(p-1)}{(1+\theta)^{x+1}}\right)$$

and

$$P_0 = \frac{\theta(a+1)}{a^3}\left(\frac{6p(a+1)(a+3)}{(1+a\theta+3\theta)} - \frac{6p(2a+3)}{(1+2a\theta+3\theta)} + \frac{a^2(p-1)}{(1+a\theta+\theta)} - \frac{6pa(a+2)}{(1+a\theta+2\theta)} + \frac{6p}{(1+3a\theta+3\theta)} + \frac{6pa}{(1+2a\theta+2\theta)} - \frac{6p(a+1)^2}{(1+3\theta)} + \frac{6pa(a+1)}{(1+2\theta)} - \frac{a^2(p-1)}{(1+\theta)}\right)$$

Proof

The proof is obtained by appropriate substitution. Hence the result.

Mathematical Properties of the Zero-Inflated PCTWED

If the PGF of the PCTWED is denoted with $P_x(z)$, then the PGF of the Zero-Inflated PCTWED denoted by $P_x^Z(z)$ is obtained using: $P_x^Z(z) = (1-\pi)P_x(z)$

Hence, the PGF of the Zero-Inflated PCTWED is given in equation (17) as:

$$P_x^Z(z) = (1-\pi)\left(\frac{\theta(a+1)}{a^3}\left(\frac{6p(a+1)(a+3)}{(1+a\theta+3\theta-z)} - \frac{6p(2a+3)}{(1+2a\theta+3\theta-z)} + \frac{a^2(p-1)}{(1+a\theta+\theta-z)} - \frac{6pa(a+2)}{(1+a\theta+2\theta-z)} + \frac{6p}{(1+3a\theta+3\theta-z)} + \frac{6pa}{(1+2a\theta+\theta-z)} - \frac{6p(a+1)^2}{(1+3\theta-z)} + \frac{6pa(a+1)}{(1+2\theta-z)} - \frac{a^2(p-1)}{(1+\theta-z)}\right)\right) \quad (17)$$

The corresponding MGF is therefore expressed in equation (18) as:

$$M_x^Z(z) = (1-\pi)\left(\frac{\theta(a+1)}{a^3}\left(\frac{6p(a+1)(a+3)}{(1+a\theta+3\theta-e^t)} - \frac{6p(2a+3)}{(1+2a\theta+3\theta-e^t)} + \frac{a^2(p-1)}{(1+a\theta+\theta-e^t)} - \frac{6pa(a+2)}{(1+a\theta+2\theta-e^t)} + \frac{6p}{(1+3a\theta+3\theta-e^t)} + \frac{6pa}{(1+2a\theta+\theta-e^t)} - \frac{6p(a+1)^2}{(1+3\theta-e^t)} + \frac{6pa(a+1)}{(1+2\theta-z)} - \frac{a^2(p-1)}{(1+\theta-e^t)}\right)\right) \quad (18)$$

If the r^{th} raw moment of the PCTWED is denoted by $E(X^r)$, then the r^{th} raw moment of the zero-inflated PCTWED is generally defined as:

$$m_r = E(X_Z^r) = (1-\pi)E(X^r)$$

Given the first four raw moments of the PCTWED as $E(X)$, $E(X^2)$, $E(X^3)$, and $E(X^4)$ as obtained in equations (11) to (14), the first four raw moments of the zero-inflated PCTWED are given in equations (19) to (22).

$$m_1 = (1-\pi)E(X) \quad (19)$$
$$m_2 = (1-\pi)E(X^2) \quad (20)$$
$$m_3 = (1-\pi)E(X^3) \quad (21)$$
$$m_4 = (1-\pi)E(X^4) \quad (22)$$

The variance, dispersion index, skewness, and kurtosis are obtained respectively from equations (19) to (22) as:

$$Var(X_Z) = m_2 - [m_1]^2$$

$$DI = \frac{Var(X_Z)}{m_1}$$

$$S_k = \frac{m_3 - 3m_2m_1 + 2(m_1)^3}{\left(Var(X_Z)\right)^{\frac{3}{2}}}$$

$$kurt = \frac{m_4 - 4m_3m_1 + 6m_2(m_1)^2 - 3(m_1)^4}{\left(Var(X_Z)\right)^2}$$

Maximum Likelihood Estimation of the Parameter of Zero-Inflated PCTWED

If a random variable *X* is assumed to follow the Zero-Inflated PCTWED with PMF P_x^Z indexed with parameter (θ, p, a, π), then the distribution parameters can be estimated using the MLE method. The likelihood function is defined as:

$$\mathcal{L}(\theta, p, a, \pi) = \prod_{n_0}(\pi + (1-\pi)P_0)\prod_{n_1}\left((1-\pi)P(x>0)\right)$$

where: n_0 is the frequency of zero in the observation; n_1 is the frequency of non-zero observations; the sample size $n = (n_0 + n_1)$, P_0 is the realization of P_x at $x = 0$. The log-likelihood function is obtained as:

$$\ell = n_0 \ln(\pi + (1-\pi)P_0) + n_1 \ln(1-\pi) + \left(\sum_{n_1} \ln(P_x)\right)$$

$$= n_0 \ln\left(\pi + (1-\pi)\left(\frac{\theta(a+1)}{a^3}\left(\frac{6p(a+1)(a+3)}{(1+a\theta+3\theta)} - \frac{6p(2a+3)}{(1+2a\theta+3\theta)} + \frac{a^2(p-1)}{(1+a\theta+\theta)} - \frac{6pa(a+2)}{(1+a\theta+2\theta)} + \frac{6p}{(1+3a\theta+3\theta)} + \right.\right.\right.$$

$$\left.\left.\left.\frac{6pa}{(1+2a\theta+2\theta)} - \frac{6p(a+1)^2}{(1+3\theta)} + \frac{6pa(a+1)}{(1+2\theta)} - \frac{a^2(p-1)}{(1+\theta)}\right)\right)\right) + n_1 \ln(1-\pi) + \sum_{n_1} \ln\left(\frac{\theta(a+1)}{a^3}\left(\frac{6p(a+1)(a+3)}{(1+a\theta+3\theta)^{x+1}} - \right.\right.$$

$$\left.\left.\frac{6p(2a+3)}{(1+2a\theta+3\theta)^{x+1}} + \frac{a^2(p-1)}{(1+a\theta+\theta)^{x+1}} - \frac{6pa(a+2)}{(1+a\theta+2\theta)^{x+1}} + \frac{6p}{(1+3a\theta+3\theta)^{x+1}} + \frac{6pa}{(1+2a\theta+2\theta)^{x+1}} - \frac{6p(a+1)^2}{(1+3\theta)^{x+1}} + \frac{6pa(a+1)}{(1+2\theta)^{x+1}} - \right.\right.$$

$$\left.\left.\frac{a^2(p-1)}{(1+\theta)^{x+1}}\right)\right)$$

$$\frac{\partial \ell}{\partial \pi} = \frac{n_0(1-P_0)}{\pi+(1-\pi)P_0} - \frac{n_1}{(1-\pi)}$$

$$\hat{\pi} = \frac{n_0}{n_1} - \frac{n_1}{n}\left(\frac{P_0}{1-P_0}\right)$$

The MLE for parameters space (θ, p, a, π) are obtained numerically by solving $\frac{\partial \ell}{\partial \pi} = 0$, $\frac{\partial \ell}{\partial \theta} = 0$, $\frac{\partial \ell}{\partial p} = 0$, and $\frac{\partial \ell}{\partial a} = 0$. This is done using different algorithms that come with the **optimr** package (Nash *et al*., 2019) in **R language** (R-Core Team, 2020).

APPLICATIONS

Data

Five count datasets characterized by many zeros are considered to assess the performance of the PCTWED and zero-inflated PCTWED. The new propositions are compared with the Poisson and negative binomial distributions (along with their respective zero-inflated forms). Dataset I consists of the number of claims on motorcycle insurance from WASA (a Swedish Insurance Firm) from 1994 – 1998. The data has been previously utilized (Omari *et al*., 2018) on count distributions. The second dataset is the number of automobile insurance policies in Australia between 2004 and 2005, as previously presented in De Jong and Heller (2008). Dataset III comprises of frequency of claim insurance in a Belgium firm in 1993. The data was used to assess claim distributions (Zamani *et al.*, 2014). The fourth dataset is the frequency of claims of 10, 814 policyholders of a Turkish insurance firm between 2012 and 2014. The data were assessed on Poisson-related distributions (Meytrianti *et al*., 2019). The fifth dataset is the yeast cell counts per square, as previously examined on Poison Lindley distribution (Shanker & Hagos, 2015). All five datasets are dispersed and positively skewed, with very high percentages of zero counts (table 4).

Table 4. Summary of Datasets

X	Dataset I	Dataset II	Dataset III	Dataset IV	Dataset V
0	63878	63232	57178	8544	128
1	643	4333	5617	1796	37
2	27	271	446	370	18
3		18	50	81	3
4		2	8	23	1
% of 0	98.96	93.19	90.33	79.01	68.45
Dispersion Index	1.07	1.08	1.08	1.26	1.32
Kurtosis	118.26	18.50	14.59	7.71	2.80
Skewness	10.46	4.07	3.52	2.56	1.75

RESULTS AND DISCUSSION

With the least values of the chi-square statistic and – LL, the proposed distribution (PCTWED) provide the best fit to the first dataset (table 5) while its zero-inflated form (ZI-PCTWED) performs worst. It is also observed that the negative binomial distribution (another mixed Poisson distribution) performs better than its zero-inflated form, while the ZIP performs better than the Poisson distribution.

For the second dataset (table 6), the PCTWED also gives the best fit with the lowest values of both – LL and chi-square statistic. Like the first data, the ZiNB also performs better than the classical negative binomial distribution, while the ZIP performs better than the Poisson distribution.

Table 7 shows that the ZiNB best fits the third dataset; the PCTWED follows this. The ZI-PCTWED gives the worst fit to the data with the highest values of both – LL and chi-square.

Tables 8 and 9 show that the new proposition (PCTWED) best fits both datasets IV and V. The negative binomial provides a better fit than its zero-inflated form, while the ZIP gives a better fit than

the Poisson distribution for both datasets. Dataset V has the lowest percentage of zero counts among the five datasets assessed. The ZI-PCTWED gives a relatively better fit in this dataset than the negative binomial distribution and its zero-inflated form.

Table 5. Data I (Swedish Claim Frequency)

X		Freq.	PCTWED	ZI-PCTWED	Poisson	ZIP	Neg. Bin.	ZiNB
0		63878	63878.13	63877.74	63854.63	63878.11	63877.73	63893.56
1		643	642.83	632.94	689.63	643.61	644.62	629.97
2		27	26.76	37.38	3.72	25.58	24.43	23.26
Estimates	θ		89.097	504.897	0.011	0.080	0.154	0.003
	p		-4.759	-0.761		0.864	0.934	0.926
	a		-4.926	-3.590				-40.710
	π			-54.232				
	$-LL$		3840.44	3853.79	3872.00	3840.88	3841.50	3857.32
	Chi-Square		0.00	3.04	148.64	0.08	0.27	0.88

Table 6. Data II (Australian Claim Frequency)

X		Freq.	PCTWED	ZI-PCTWED	Poisson	ZIP	Neg. Bin.	ZiNB
0		63232	63230.90	63239.55	63091.61	63230.49	63230.60	63317.89
1		4333	4332.84	4310.28	4593.07	4325.83	4330.57	4252.49
2		271	273.98	305.84	167.19	286.59	276.48	261.98
3		18	17.14	0.33	4.06	12.66	17.22	21.45
4		2	1.07	0.00	0.07	0.42	1.06	1.97
Estimates	θ		15.167	387.325	0.073	0.133	1.157	0.007
	p		-0.045	-5.445		0.451	0.941	0.878
	a		9.406	-4.253				-76.060
	π			-135.921				
	$-LL$		18049.64	18148.72	18101.50	18052.20	18049.68	18105.58
	Chi-Square		0.89	2585.95	177.66	9.07	0.98	48.15

Table 7. Data III (Belgium Claim Frequency)

X		Freq.	PCTWED	ZI-PCTWED	Poisson	ZIP	Neg. Bin.	ZiNB
0		57178	57178.64	57187.37	56949.763	57177.48	57188.34	57249.63
1		5617	5598.70	5587.71	6019.590	5584.80	5581.31	5558.90
2		446	477.08	523.00	318.135	504.87	485.28	438.37
3		50	40.67	0.91	11.209	30.43	40.47	45.91
4		8	3.57	0.01	0.296	1.38	3.30	5.40
Estimates	θ		9.573	271.157	0.106	0.181	1.279	0.008
	p		0.354	-6.343		0.415	0.924	0.843
	a		13.223	-4.312				-71.130
	π			-111.862				
	$-LL$		22063.30	22311.68	22150.54	22075.30	22064.31	22136.57
	Chi-Square		9.74	10122.64	413.84	51.55	12.33	2.44

Table 8. Data IV (Turkish Claim Frequency)

X	Freq.	PCTWED	ZI-PCTWED	Poisson	ZIP	Neg. Bin.	ZiNB
0	8544	8545.14	8544.04	8292.42	8544.19	8543.47	8561.78
1	1796	1789.69	1771.07	2201.64	1759.23	1795.62	1807.66
2	370	380.67	492.20	292.27	430.75	375.71	331.89
3	81	78.92	6.34	25.87	70.31	78.50	81.03
4	23	15.78	0.33	1.72	8.61	16.39	22.23
Estimates	θ	4.353	41.119	0.266	0.490	1.009	0.006
	p	-0.506	-4.526		0.458	0.792	0.635
	a	12.831	-4.408				-82.430
	π		-19.730				
	$-LL$	7029.56	7334.26	7153.16	7038.91	7029.71	7057.06
	Chi-Square	2.68	2452.35	484.41	35.02	2.84	4.52

Table 9. Data V (Yeast cell counts per square)

X	Freq.	PCTWED	ZI-PCTWED	Poisson	ZIP	Neg. Bin.	ZiNB
0	128	127.31	127.48	118.06	128.00	126.73	180.68
1	37	38.36	35.34	54.30	38.35	42.08	4.51
2	18	16.72	21.26	12.49	15.49	12.84	1.15
3	3	3.86	2.51	1.91	4.17	3.80	0.39
4	1	0.65	0.35	0.22	0.84	1.11	0.15
Estimates	θ	9.003	97.851	0.4609	0.808	1.195	0.004
	p	-12.457	-0.232		0.431	0.722	0.490
	a	0.013	-1.467				-11.720
	π		-31.216				
	$-LL$	168.60	170.31	173.83	168.80	170.02	172.05
	Chi-Square	0.53	1.92	12.16	0.81	2.88	517.31

CONCLUSION

The skewness, kurtosis, and dispersion index for some parameter combinations are presented to assess the behaviour of the distributions. The shapes of the PCTWED show that it can be effectively utilized to model observations with an unusual frequency of zeros.

Performances of the new propositions are assessed on five count observations with varying percentages of zero counts. Comparisons are made with the Poisson and negative binomial distributions (along with the irrespective zero-inflated forms). The maximum likelihood estimation using different algorithms that come with the optimr package in R-language is used to provide estimates for the parameters of the distributions. The chi-square goodness of fits and the – LL are used for model selection. Results show that the PCTWED outperforms its zero-inflated form in particular and all the competing

distributions in most cases. The classical negative binomial distribution provides a better fit for datasets with above 70% of zero counts than its zero-inflated form. In contrast, the zero-inflated Poisson outperforms the Poisson distribution in all cases.

The finding shows that mixed Poisson distributions and the negative binomial distribution (when the gamma distribution is used as the nixing distribution) are naturally suitable to model observation with higher than usual zero counts in count observation. Therefore, it is unnecessary to obtain their zero-inflated form to model observations with many zero counts.

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CURRENT PROBIOTICS APPLICATION FOR AQUACULTURE FEED: A REVIEW

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ABSTRACT. *Probiotics are a helpful alternative that can be used to increase aquaculture output in a sustainable way. For probiotics to have the desired effects when applied to a certain aquaculture species, first, the proper strain and dosage must be chosen. The second, probiotics can be given intravenously, orally, or as feed additives, the last of which is frequently employed in aquaculture. Probiotics application in aquaculture production provides several advantages, including enhanced stress tolerance, enhanced disease resistance, enhanced immunological defence against infections, enhanced disease performance, and enhanced feed utilization. To improve the economic performance of the aquaculture species, probiotics can be used at the farm level. This review seeks to assess the current apprehension of probiotics as fish-feed supplements in aquaculture, types of probiotics, administration methods, mechanism of action, benefits, and effects. It also constrains the approach of contribution probiotics application to the aquaculture activities which enhance the fish health and growth based on the results of experimental studies.*

KEYWORDS. Aquaculture, benefits, effects, mechanism of action, probiotics

INTRODUCTION

Aquaculture activities have experienced significant growth worldwide, emerging as a crucial contributor to the global food production sector. Aquaculture is the term used to describe the farming of aquatic organisms in coastal and inland settings employing alterations to the growing process to boost productivity. According to the United Nations Food and Agriculture Organisation (FAO) (2020), from 25.7% in 2000 to 46% in 2018, aquaculture's share of the world's fish production increased. From 1961 to 2017, fish consumption increased on average by 3.1% annually, outpacing increases in consumption of all other animal protein foods (meat, dairy, milk, etc.) and the global population growth rate of 1.6%. In 2017, consumption of fish accounted for 7% of all proteins ingested and 17% of the animal protein intake of the entire world's population (FAO, 2020). All statement points to a huge increase in worldwide fish consumption over other sources of animal protein.

The aquaculture industry grapples with various challenges due to the expansion and commercialization of production in response to rising demand. These challenges encompass maintaining water quality, controlling diseases and epizootics, enhancing broodstock domestication and improvement, devising suitable feeding systems, and advancing hatchery and grow-out technologies. (FAO, 2022). Out of these, disease outbursts are presently the foremost obstacles to the aquaculture production for many species, which isolating both financial and social growth in many nations (Mugimba *et al*., 2021). Diseases outbreaks caused significant financial losses to global finfish aquaculture, which are projected to range from US$ 1.05 to US$ 9.58 billion annually (Tavares-Dias & Martins, 2017). The aquaculture industry's expansion is hindered by very high mortality rates caused by harmful pathogens. In intensive aquaculture, bacterial infections have been found to represent biological production bottlenecks, necessitating the use of medications and antibiotics in health management techniques (Okocha *et al*., 2018). Antibiotics have been used traditionally in aquaculture for a number of reasons, including the improvement of growth and feed conversion efficiency as well as the management and prevention of fish infections (Leong *et al*., 2022). Probiotics play a vital role in the aquaculture industry, preserving overall fishes' health. Numerous research has been carried out to investigate the presence of bacteria and their features, including antibiotic resistance, in a natural water environment, such as a river, because the problem of antibiotic-resistant bacteria has grown more serious in aquatic habitats (Leong *et al*., 2018). The unrestricted use of antibiotics causes an imbalance in the gut flora and the predominance of antibiotic-resistant microbes, which impairs the health of fish and leads to residue building up in the muscle, posing a possible health risk to consumers (Lihan *et al*., 2021). Probiotics are an alternate strategy to control fish health in the aquaculture business because of the uncertainty surrounding the use of antibiotics in aquaculture. Probiotics can be utilised in aquaculture to increase growth, feed utilisation, disease resistance, immunological response, and water quality (Subedi & Shrestha, 2020). Therefore, probiotic is a health-promoting alternative used in fish feed to help reduce the negative impact of antibiotics and enhance aquaculture production in a sustainable way (Hai, 2015). This review seeks to assess the current understanding of probiotics as fish-feed supplements in aquaculture, summarize the benefits and the effects.

PROBIOTIC USED IN FISH FEED

The probiotic term means "for life," and it comes from the Greek words "pro" and "bios" (Pradhan *et al*., 2020). Hill *et al*. (2014) explain a probiotic as living microorganisms that, when given to a host in sufficient quantities, improve their health. Hoseinifar *et al*. (2018) described probiotics as microbial feed additives that modify the gut microbiota to confer host organism. Due to technological advancements, probiotics have become readily accessible in our everyday food products, and they are even integrated into animal feed as functional additives. (Manik *et al*., 2021). El-Saadony *et al*. (2021) claim that probiotics used in aquaculture contain bacteria that improve the quality of the water as well as the water itself or prevent diseases from growing in the water. The World Health Organisation (WHO) and the Food and Agriculture Organisation of the United Nations (FAO) both stated that living microbes are beneficial to the host's health when administered in the right quantities. Probiotics have recently been interpreted as "living or dead, or even a component of the microorganisms that work under multiple modes of action in delivering good benefits to the host or its environment" under an aquaculture understanding by Lazado & Caipang (2014). Probiotics must undergo a proper selection as the appropriate strains can have detrimental consequences on the host (Lingoh *et al*., 2020; Schuurman *et al*., 2022).

When choosing potential probiotics, it is ideal to look for strains with the following qualities: (i) enhanced growth or resistance to disease in the host animal; (ii) non-pathogenic and non-toxic; (iii) present as live cells, preferably in large numbers; and (iv) capacity to survive and metabolise in the gut environment; and (v) stability and ability to remain viable for longer periods under sterile conditions. Probiotics may be given orally, intravenously, or directly submerged in water (Anjana & Tiwari, 2022) which can be applied singly or in combination (Hai, 2015). By controlling intestinal microbial stability, secreting antibacterial compounds such as bacteriocins and carboxylic acids, fighting with pathogens to stop their attachment to the gut, pathogen survival, and producing an antitoxin impact, probiotics have an antimicrobial effect (Jahangiri & Esteban, 2018). In terrestrial animals, lactic acid-producing bacteria are frequently used, whereas a wide variety of microorganisms are used in aquaculture, where effective supply of both Gram-positive and Gram-negative bacteria (Hai, 2015). The concept of probiotic therapy has provided fresh perspectives on the importance of gut flora in disease prevention (Abd AL-Khaliq, 2019). Aquaculture frequently utilizes non-bacterial probiotics such as bacteriophages, microalgae, and yeasts. Basically, there are five groups of probiotics listed in Table 1. In aquaculture, probiotics can be provided in a variety of ways, including through feeding, injection, or direct submersion in water (Hai, 2015; Anjana & Tiwari, 2022). The methods of probiotic preparation are illustrated in Figure 1.

Table 1. Methods of probiotic preparation.

Types of probiotics	Descriptions
Freeze dried	Lyophilization process to remove moisture from the probiotics while preserving their viability and functionality.
Fermentation	Produced through fermentation process.
Viable	This is a live system that contains a certain number of organisms, has a routine for counting, and is extremely reliable and effective.
Non-viable	Dead organism

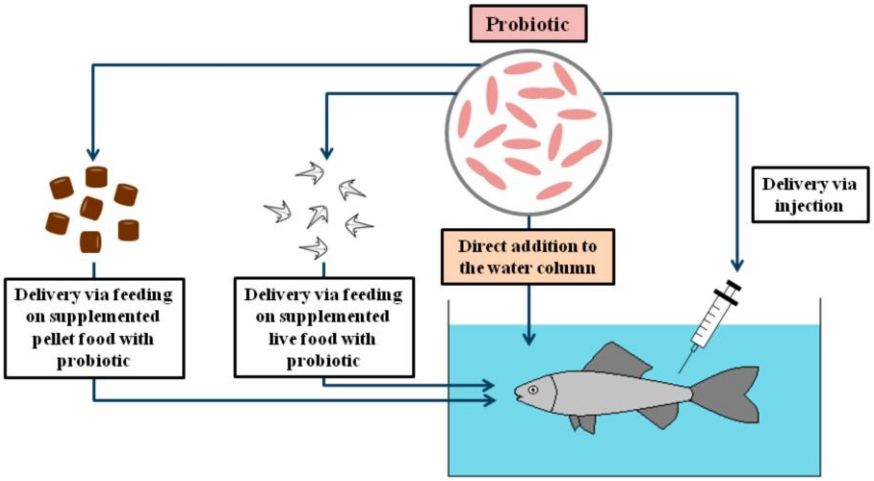

Figure 1. Different methods of probiotic administration (Jahangiri & Esteban, 2018).

Mechanism of action

Hancz (2022) described that there has been a lot of discussion about using probiotics in the creation of microbial management strategies and reducing the usage of medicinal drugs and antibiotics to achieve a more ecologically friendly and sustainable aquaculture. The probiotics' mechanisms of action are well defined and include creating pathogen-unfriendly surroundings by producing an inhibitor (hydrogen peroxide, lysozymes, proteases, and bacteriocins), competing with adherence sites for essential nutrients, enhancing host immune responses, supplying extra essential nutrients, vitamins, and enzymes, and dissolving direct absorption (Sankar *et al.*, 2016; Plaza-Diaz *et al*., 2019; Kouhounde *et al*., 2022; Tegegne & Kebede, 2022; Zhang *et al*., 2023). Multiple screening steps are required to confirm the viability of probiotic bacteria for field application. Stringent screening steps are usually followed by molecular identification to confirm the identity of the good bacteria. world.

The conflict between microbial species over food sources and a territory is known as microbial antagonism. Thus, one organism outcompetes another, the organism that didn't succeed in the environment is inhibited. *In vitro* antagonistic screening against several strains of pathogenic bacteria can be used to assess the competitive exclusion by putative probiotic bacteria. Environmental factors that encourage the growth of bacterial species, such as culture water and abiotic or biotic factors, have a significant effect on the composition of microbial communities. The metabolic and physiological roles of the gut microbiota have been the subject of several investigations. According to Ray *et al.* (2012), attribution of the precise contribution of the gastrointestinal microbiota in exothermic animals is challenging due to the complexity and variable ecologies of different fish species' digestive tracts. This is true even though numerous studies have suggested that microbial activity in the gastrointestinal system may be an essential nutrient and enzyme for the host. The non-specific immune system has been demonstrated to benefit from probiotics in several investigations using rats and in vitro models (Shi *et al*., 2016). Besides, the similar immune response system is stimulated by phagocytosis and antibacterial activity as shown in the Chinese mitten crab (*Eriocheir sinensis*), which is largely responsible for this protection (Wang *et al*., 2019). Abdel-Latif *et al*. (2023) described that supplementing fish with probiotics, either mono-species or multi species mixtures, can improve their immune response. Probiotics could be an alternative used to control the occurrence of viral infection prevention in aquaculture. According to Abomughaid (2020) and Dauda *et al*. (2013),

probiotics with antiviral action against infectious IHNV (Infectious Hematopoietic Necrosis Virus) includes *Pseudomonas s*p., *Vibrio* sp., *Aeromonas* sp., and *Coryneform* bacteria isolated from salmonid hatcheries, with a plaque reduction of more than 50%. One of the variables is water quality which is linked to farm-based fish disease outbreaks. The jumble of organic and nitrogenous wastes such as ammonia and even nitrite, is a great concern especially in fish breeding. Probiotics, including *Bacillus* sp., can enhance pond water quality by breaking down organic matter and releasing CO_2, which is especially advantageous during intensive production (Hlordzi *et al*., 2020). In commercial shrimp farms in Malaysia, photosynthetic bacteria and *Bacillus* sp. can enhance water quality, juvenile shrimp survival rates, and growth rates.

Advantages and disadvantages of using probiotics

Systems for growing aquatic life in fresh, brackish, and marine waters are all examples of aquaculture systems. However, the disease's occurrence is utmost impediment to its long-term viability. Probiotics are frequently used in aquaculture systems to control bacterial pathogens. *Streptococcus thermophilus, Lactobacillus, Bifidobacterium, Pediococcus* sp., *Carnobacterium* sp., *Flavobacterium* sp., *Bacillus* sp., *Cytophaga, Pseudomonas* sp., *Alteromonas* sp., *Aeromonas* sp., *Enterococcus* sp., *Nitrobacter* sp., *Nitrosomonas* sp., and *Vibrio* sp. are examples of marketable probiotics. The detrimental impacts of probiotics on aquaculture systems and the environment, however, have largely gone unstudied. Due to temperature-related stress, the benefits of utilising probiotics include *Lactobacillus sporogenes*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, and *Lactococcus lactis* could increase the production of red blood cells and immunology system in fish (Mohapatra *et al*., 2014). Besides, *Lactobacillus delbrueckii* has positive effects on European sea bass growth and body weight (Pérez-Pascual *et al*., 2020). Resistance to *Aeromonas* sp. and *Salmonicida* sp. has improved by using *Lactobacillus rhamnosus* (Nikoskelainen *et al*., 2001). Because nutrients and other microbes are also involved, probiotics have drawbacks of their own. The mode of action is still unknown; further research into the processes of bacterial competition is required; and it is also necessary to determine the ecological significance of the various in-situ processes. Aquatic species' intestinal contents contain substantially higher microbial populations than the surrounding water when those populations are cultured. As a result, there is a chance that aquaculture conditions could spread resistant germs to people. According to article published in International Magazine For Animal Feed & Additives Industry (2023), there is a possibility of the introduction of non-native microbes into the aquatic environment, which might change the ecology and have an impact on native species; a risk that pathogenic microbes which has been used as probiotic may mutate and contaminate aquaculture products; probiotic overuse; lastly the high production cost which will be the burden for small scale aquaculture farm.

Effects of probiotics on fish

According to Rahman *et al.* (2021), “For life” was the phrase introduced by Parker from the Greek word. Probiotics are chemicals and living organisms that support intestine and microbial balance. A probiotic organism needs to be resistant to the stomach's acidic condition, bile, and pancreatic enzymes, access to the intestinal mucosa layers, and the ability to colonise for an extended period in order to function well inside the host. Probiotic effects on fish performance include growth, feed conversion ratio, protein efficiency ratio, digestibility, body composition, immune system response, and pathogenicity and challenge capacities, according to Allameh *et al.* (2017). According to the article, how probiotic bacteria affect the growth performance as it depends on what type of probiotic

bacteria was used to feed in fish. Some have an effect on reducing the stress factors in fish. Some have effect inside the digestive tract. The probiotics that were provided to the fish diets could improve their protein efficiency ratio. Next, it also has a part in increasing the functions of the immune feedback in fish. One of the instances is the utilization of lactic acid bacteria (LAB) to boost immunity and control populations of possible infections. Fish immune systems, both specialised and generalised, can be stimulated by probiotics. Probiotic bacteria can also improve fish's immune systems by increasing their acidophilic granulocytes and immunoglobulin cells. Probiotics can also lower the risk of disease outbreaks or lower the frequency of illness.

Improve feed utilization

Most of the research has proved that probiotic has the ability to alter enzymes production and hence improve the fish's feed utilization. *Lactobacillus pentosus* supplementation increased the feed utilisation in white prawns (*Litopenaeus vannamei*), according to research by Zheng & Wang (2017). The addition of heat-killed *Lactobacillus plantarum* at 50, 100, or 1000 mg/kg over 12 weeks significantly improved the amylase, lipase, and protease activities of Nile tilapia (Dawood *et al*., 2019) and thin clawed crayfish (Valipour *et al*., 2019). Besides, feed efficiency increased with increasing probiotic dosage as shown by most researchers. Some of the significant results were discussed as Muchlisin *et al.* (2017) stated that the optimal probiotic dosage for keureling fish (*Tor tambra*) fry was 10 ml kg -1 with feed conversion rate of 2.37. Compared to prebiotic supplementation, probiotic-based diets have a greater favourable impact on the feed conversion rate (1.43-1.8), protein efficiency rate (1.33-1.71) and survival (100%) of *Channa striata* fingerlings (Munir *et al*., 2016). Application of multi-strain probiotics for 12 weeks in cage cultured striped catfish (*Pangasianodon hypophthalmus*) in Bangladesh has proved that feed efficiency increased (from 2.69 to 3.03 protein efficiency) (Chowdhury & Roy, 2020). Probiotic such as *Saccharomyces cerevisiae* obtained the best performance at concentration of 4 g kg^{-1} while *Bacillus subtilis* required higher dose of 10 g kg^{-1} in order to present with better feed conversion ratio (1.61) and significantly higher protein level (86-89%) (Opiyo *et al.*, 2019).

Growth stimulator

The direct impact of bacterial probiotics on fish development performance is one of the anticipated outcomes of their use, either by increasing nutrition intake directly or by delivering nutrients (Roque Joel *et al*., 2020). Feeding with probiotics will enhance gut microflora health, increase in the release of digestive enzymes, stimulation of hunger, creation of vitamins, degradation of indigestible components, and increase the release of digestive enzymes (Lingoh *et al*., 2020). Supplementation of *Bacillus subtilis* in feed at 10^7 and 10^9 CFU/kg food for five weeks significantly increased the growth of Litopenaeus vannamei, a Pacific white shrimp (Kewcharoen & Srisapoome, 2019). The mud crab, *Scylla paramamosain*, gained weight and had a much faster rate of specific growth after receiving dietary supplements of *Enterococcus faecalis* and *Pediococcus acidilacti* (Ray *et al*., 2012). Ahmadifar *et al.* (2020) shown that feeding *Pediococcus acidilactici* to zebra fish (*Danio rerio*) improved their growth performance. Probiotic supplementation of 0.2% in striped catfish in Bangladesh resulted in impressive growth (29.66%) and economic performance (66.37%) as showed by (Chowdhury & Roy, 2020). Different probiotic are shown with different level of performance, for example *Saccharomyces cerevisiae* and *Bacillus subtilis* required different concentration in order to present with higher final weight (255.31g) and specific growth rates (SGR) (0.77%) (Opiyo *et al.*, 2019). Furthermore, application of probiotics in fin fishes which significantly increased their growth

rate were proven as *Bacillus pumilus* in juvenile golden pompano, *Paenibacillus ehimensis* (Lin *et al*., 2022); *Bacillus circulans* PB7 in South Asian carp, Catla (Bandyopadhyay & Das Mohapatra, 2009), *Bacillus megaterium* and *Pediococcus pentosaceus* in catfish (*Clarias* sp.) (Hamka & Widanarni, 2020); *Pediococcus pentosaceus* in grass carp (Gong *et al*., 2019); *Lactobacillus plantarum* used in Nile tilapia (*Oerochromis niloticus*) (Gewaily *et al*., 2021) are well documented.

Disease resistance

The ability of probiotic microorganisms to secrete substances like bacitracin and polymyxin, which have a bactericidal or bacteriostatic effect on pathogenic bacteria in the intestine of the host, boosts the host's resistance to disease. Disease resistance is shown by supplementation of *Enterobacter* sp. in rainbow trout which enhanced the disease protection against *Flavobacterium psychrophilum* (LaPatra *et al*., 2014); The majority of people are focusing on *Aeromonas* sp. either as pretreated pathogen probiotic or as fish pathogen because different species of *Aeromonas* bacteria can inhibit the growth of each other. Previously Irianto and Austin (2002) have observed that consuming *Aeromonas hydrophila* as a probiotic reduces the mortality caused by other *Aeromonas* pathogen such as *Aeromonas salmonicida* in rainbow trout. Besides, Irianto *et al.* (2003) showed that feeding goldfish (*Carassius auratus*) with formalin-inactivated *A. hydrophila* A3-51 boosted resistance against *A. salmonicida*. Additionally, Pieters *et al*. (2008) showed that feeding rainbow trout with 10^8 cells/ g of *A. sobria* GC2 and 10^{10} cells/ g of *Brochothrix thermosphacta* BA211 increased their resistance to the causative agent of fin rot, *A. bestiarum*. However, *A. hydrophila* is causing disease in most carp fish, thus application of other Aeromonas sp. may be able to stop the disease. This is shown by Chi *et al*. (2021) proved that common carp's disease resistance to *Aeromonas hydrophila* was improved by applying *A. veronii* and *Flavobacterium sasangense* in farming; It has been demonstrated that *Lactococcus garvieae*, which is present in raw cow's milk, increases Nile tilapia's resistance to *Staphylococcus aureus*. (Abdelfatah & Mahboub, 2018); *Cromileptes altivelis* resistance to *Vibrio harveyi* was improved by *Lactococcus lactis* (Sun *et al*., 2019); Disease resistance of rainbow trout against *Lactococcus garvieae* was enhanced by *Enterococcus faecalis* present in commercial probiotics (Baños et al., 2019); Numerous significant fish infections, such as *Aeromonas hydrophila*, *Aeromonas sobria*, *Aeromonas veroni*, *Eelwardsiella tarda*, *Plesiomonas shigelloide*, and *Lactococcus garviae* are effectively combatted by *Pediococcus pentosaceus* (Raheem *et al*., 2021).

Immune response

The pathogens can be fought by producing inhibitory substances. Probiotics can prevent pathogen infection by boosting the host immune system and cellular and non-specific immunity in the body (Raheem *et al*., 2021). According to Munirasu *et al.* (2017) and Avella *et al*. (2010), the effects of probiotics towards fish is to increase survivorship, increase growth and development and reduce stress in fish. Based on Avella *et al*. (2010), for the probiotic to be most effective is when the fish is in its early stage of development. This can increase the survivorship by reducing the chances of getting infected by disease of the fish in future. Probiotics can also help alleviate fish stress and foster growth. Munirasu *et al*. (2017) proved that when the fish were fed with probiotics as supplements, the fish's feed value, enzymatic contribution to digestion, inhibition of pathogenic microbes, antimutagenic and anticarcinogenic activity, growth-promoting factors, and elevated biochemical activities all improved. Most lactic acid bacteria, including *Leuconostoc mesenteroides*, *Lactococcus lactis*, and *Lactobacillus sakei* increased the fraction of phagocytically active head kidney cells and activated

the complement receptor expression in rainbow trout, improving both cellular and hormonal immune capabilities (Balcázar *et al*., 2007). In nile tilapia, *Lactobacillus plantarum* and even *Bacillus velezensis* increased innate immune markers such as lysozyme and peroxidase activity in skin mucus, serum lysozyme and peroxidase, alternative complement, phagocytosis, and respiratory burst activities (Doan *et al*., 2018). Complement activity was greatly improved by *Lactobacillus plantarum* at 10^8 and 10^9 CFU/g supplementation. Lysozyme and respiratory burst activity were also significantly increased in black eared catfish, *Pangasius larnaudii* (Silarudee *et al*., 2019). The application of *Pediococcus pentosaceus* to common carp causes a significant increase in the number of erythrocytes, phagocytes, hematocrit, total serum antibody level, alternative complement protein, protease and lysozyme activities, and antibacterial activity (Ahmadifar *et al*., 2019).

CONCLUSION

Probiotics used as fish feed supplement comes in two forms; bacterial or nonbacterial microorganisms that represent the alternative additive for sustainable aquaculture. The most essential step in increasing the efficacy of probiotics is to choose the right strain from the favourable characteristics. Only the most effective probiotic at the right dose has the most beneficial effect on a certain species. There are pros and cons to using these probiotics upon the fish feed. One of the benefits is that fish growth has improved a lot. Meanwhile, long-term human exposure to bacteria from aquaculture still poses potential risk. Besides, the application of probiotics in either way is still costly to most small-scale farmers. Overall, the probiotic application shows positive advancement as it influences growth, reduces stress in fish, and enhances immunisation in fish by increasing their immune system.

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GEOCHEMICAL CHARACTERISTICS OF TROPICAL SALT LICKS IN SEGALIUD LOKAN FOREST RESERVE, SANDAKAN, SABAH MALAYSIA

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***ABSTRACT*:** Mineral licks are important for animals, especially wildlife, to nourish their diets, not only in terms of supporting their mineral intake deficiencies but also in regulating toxins in their bodies. This study characterised the geochemical properties of salt licks located in Segaliud Lokan Forest Reserve (SLFR). Soil samples were collected from five selected salt licks in the study area. The physico-chemical results show that the pH of the salt-lick soil varied from slightly acidic to slightly alkaline. The percentage of moisture content and organic matter ranges from 25.22% to 44.78% and 0.95% to 7.83, respectively. The electrical conductivity reading ranges from 48.59 µS/cm to 260.88 µS/cm. The soil samples were digested using aqua regia and analysed using inductively coupled plasma-optical emission spectrometry (ICP-OES). The concentrations of Ca (1101.92 mg/kg–11551.64 mg/kg), K (910.27 mg/kg–2355.41 mg/kg), Na (106.36 mg/kg–727.34 mg/kg), and Mg (1442.14 mg/kg–5305.13 mg/kg) in the five salt licks varied considerably and were higher than in the control soil samples. High chemical concentrations in salt licks are due to the pH of soils, which ranges from slightly acidic to slightly alkaline.

KEYWORDS: Salt licks, soil, physico-chemical, geochemical, tropical forest

INTRODUCTION

Salt licks are a location that is frequently visited by animals. It is a place rich in minerals animals seek to supplement their diets and regulate their body function (Parker et al., 2004; Blake et al., 2011; Lazarus et al., 2019). Salt licks can be identified from traces of animals, such as footprints on soil or bite marks on rock walls (Lameed & Adetola, 2012; Molina et al., 2014). There are two types of salt licks that exist

naturally, known as hydromorphic licks and lithomorphic licks (Panichev et al., 2013). According to Panichev et al. (2013), hydromorphic licks are formed by running water springs where clay rocks saturated with chemical concentrations become licks in water discharge areas, whereas lithomorphic licks are an exposure of rocks searched for and consumed by animals. Chong et al. (2005) divide the natural salt licks found in Peninsular Malaysia into two groups known as spring salt licks and dry land salt licks, whose local names vary according to location.

Salt licks can provide resources for animal nutrient deficiency that are difficult to obtain (Hon & Shibata, 2013; King et al., 2016; He et al., 2022); thus, they have been classified as important places within landscapes (Montenegro, 2004; Tawa et al., 2021). Salt licks are an important source of minerals for many species of mammals and birds in the lowland forests of Malaysia (Magintan et al., 2015).

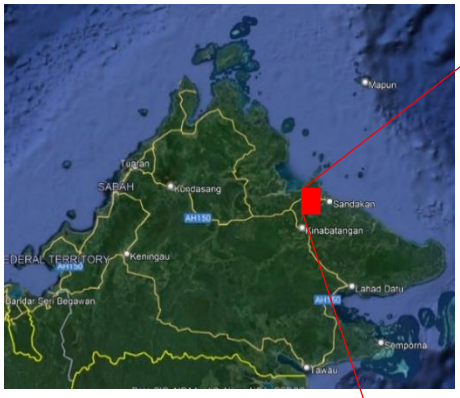
Previous studies of salt licks have mostly focused on the chemical properties, patterns of use, and interpretations of why the salt licks are frequently visited (Matsubayashi et al., 2007; Tobler et al., 2009; Elyau et al., 2012; Matsuda et al., 2015; Razali et al., 2020). Typical results for geochemical analysis studies found in salt licks content is abundant in essential elements such as calcium (Ca), potassium (K), sodium (Na), and magnesium (Mg) (Brightsmith & Muñoz-Najar, 2004; Lizcano & Cavalier, 2004; Lameed & Jenyo-Oni, 2012; Wahab et al., 2020; Sitienei et al., 2012; Griffiths, 2022).

There hasn't been much research done on the physico-chemical and chemical composition of the important elements in salt lick soil in SLFR. Thus, the study was conducted to determine the geochemical composition of salt lick soil from Segaliud Lokan Forest Reserve (SLFR), Sandakan Sabah.

MATERIALS AND METHODS

Study Area

Segaliud Lokan Forest Reserve is located in Sandakan, on the eastern side of Sabah. The study area lies within a latitude of 05026’N to 05036’N and a longitude of 117030’E to 117036’E. The sampling stations for the different forest zones and compartments are given in Figure 1. This forest reserve is a logging area managed by KTS Plantation Sdn. Bhd. and covers approximately 57,247 hectares of land. It was zoned into a Natural Forest Management and Industrial Tree Plantation area. The SLFR was gazetted as a timber production forest in 1955 and has been managed by a few private companies to date (Wilting & Mohamed, 2010). The SLFR is divided by Sungai Lokan in the north, Sungai Tabalion in the south, and Sungai Rawog in the center, whereas the eastern area of the forest consists of part of the basin formed by three rivers: Sungai Lokan to the north, Sungai Rawog Besar, and Sungai Tabalion Besar to the south (Hasmat et al., 2020).



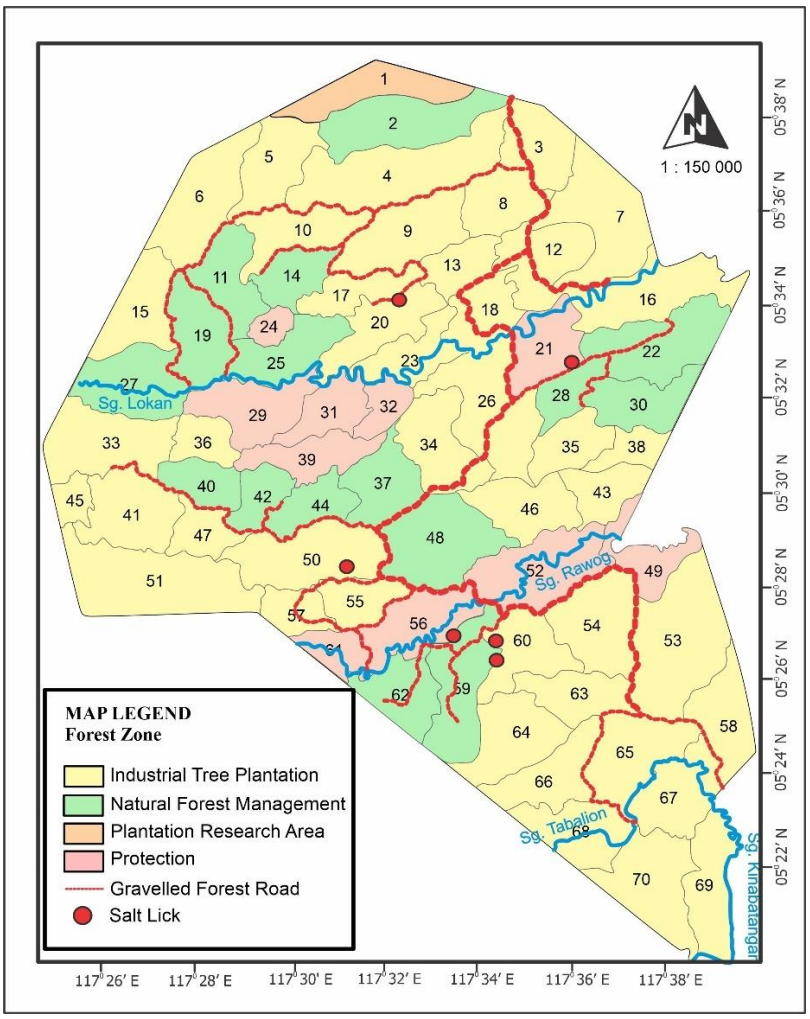


Figure 1 Map of Segaliud Lokan Forest Reserve, Sabah showing the forest zones and numbers of compartments.

(Source: https://www.segaliudlokan.com/)

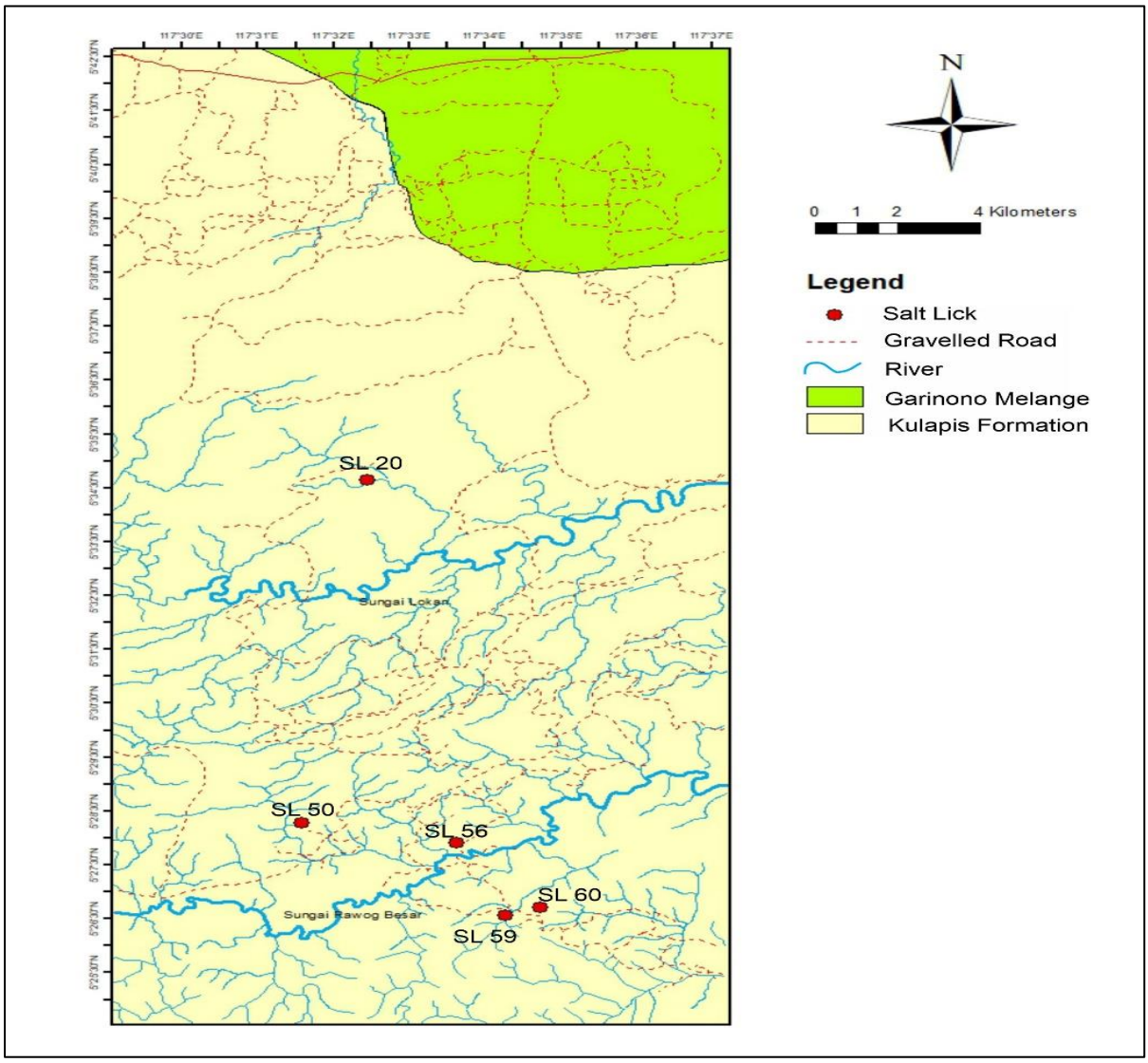


Figure 2 Geological map of Segaliud Lokan Forest Reserve
(Mineral and Geoscience Department of Malaysia, 2015)

This forest reserve is made up of the Kulapis Formation, as shown in Figure 2 (Clennell, 1991; Hutchison, 2005). According to Noad (1998), Kulapis Formation lithofacies consist of thick massive pink coarse-grained sandstone grading up to fine-grained, thick beds of featureless red mudstone, thin dark-red sandstone, and thin interbeds of very fine-grained red sandstone and thicker mudstone. Kulapis Formation is a distinctive wholly red bed formation where all the rocks have a red colour varying from pink sandstones to chocolate-brown shales, probably indicating an iron-rich source, perhaps from the nearby uplifted ophiolite (Hutchison, 2005).

The location of the salt licks is approximately 500 metres from the nearest gravel road. Researchers went by foot through the forest to reach the salt licks area, as no open access was provided. Each salt lick is named after the forest's compartment where they are located. The salt lick's site description is shown in Table 1.

Table 1: Licks, GPS coordinates, and site description.

Licks	Coordinate	Site Description
SL20 (n=11)	N 05^{O} 34’ E 117^{0} 32’	It is the farthest from other licks and located at the north of the research area. This salt lick is situated right next to an unpaved road. The slope height is approximately 2 to 3 metres from the road surface. Mud puddles can be seen on the surface of lick soils, as well as boulders scattered around the lick. The soil is dark in colour with no vegetation. Animal footprints were found in the lick. 
SL50 (n=5)	N 05^{O} 28’ E 117^{0} 31’	The salt licks are situated in the western part of the study area. This lick shows a flat and rocky area. Shrubs and low-canopy plants grow in the vicinity and are covered with vegetation. A tiny stream flows through seasonally, depending on the total rainfall. Gravel-sized rocks and shells are also present in the soil, as are weathered leaves. The soil is dark in colour. Animal footprints were found nearby, and leeches were also present in the area. 

SL56 (n=3)	N 05^{O} 27’ E 117^{0} 33’	The salt lick area is small compared to other licks and rocky. The size of the rocks in the locality varies with each other. Water puddles exist on the soil surface as well as animal waste products. A small pool can also be seen forming in one place. Soil is dark in colour and available in small amounts with no vegetation. 
SL59 (n=14)	N 05^{O} 26’ E 117^{0} 34’	The small river stream flows through, dissecting the licks into two parts. The flow rate of the stream depends on the rainfall. During the summer season, the stream dries up, and water puddles form on the lick soil surface. This lick is situated near the Rawog River branch. The depth of the salt lick is approximately 2–3 m from the slope. Soil colours range from yellowish to darker brown. Greyish-black soil is also present within the lick, indicating the presence of carbon. Flocks of butterflies are observed, as are vegetation and animal footprints. 

SL60 (n=11)	N 05^{O} 26’ E 117^{0} 34’	It takes a hike to get to the salt lick spot. Outcrops were spotted before being crushed by animal activities. Large boulders made up the lick vicinity, and puddles of water were spotted on the soil surface with no vegetation. The lick is approximately 2–3 m deep and darker. 

Sampling

A total of five (5) natural mud from salt licks were found scattered in the forest. Soil sampling was collected using the horizontal (Photo 1) and vertical (Photo 2). Horizontal sampling is carried out by taking out a thin layer of the topsoil. Vertical sampling was collected using PVC pipes with a diameter of 10 cm and a length of 100 cm. The pipes were closed tightly using styrofoam cork to avoid contamination. Once the samples arrived in the laboratory, the core samples were sliced and divided 10 cm thick accordingly. Controlled soil samples were also collected approximately 500 m away from the salt lick location for comparison. The control site is soil taken underneath shades of trees with zero influence of water. A total of forty-four (44) salt lick soil and eight (8) control soil samples were obtained.

Photo 1 (a) Horizontal sampling was carried out by clearing the top layer of the soil surface before taking soil samples. (b) Vertical soil sampling was carried out using PVC pipe hammered into the soil profile.

Physico-chemical analysis

The physico-chemical parameters were pH, moisture content (MC), organic matter (OM), and electrochemical conductivity. The pH analysis was conducted shortly after the samples arrived in the laboratory (BS 1377-3: 1990). The samples were air dried at 1050 for 8 hours in an oven (BS 1377-2: 1990). The sample preparation for organic matter was conducted according to BS 1377-3:1990, where the samples were left overnight in a furnace at 4000 °C with the dry combustion method. Electrical conductivity analysis for the soil samples was determined using a 1:5 suspension of soil in water (BS 7755-3.4:1995).

Geochemical analysis

Geochemical analysis for calcium (Ca), potassium (K), sodium (Na), and magnesium (Mg) in soil was determined by using inductively coupled plasma-optical emission spectrometry (ICP-OES) with the Perkin Elmer Optima model 5300DV. For the sample preparation, the soil was air dried, ground until powdered, and sieved through a 0.063-micrometre sieve for easier metal digestion. About 1 gramme of soil is weighed, added to 14 ml of aqua regia solution, and left overnight. The aqua regia were produced by mixing hydrochloric and nitric acid in a 3:1 ratio. The solution is then heated for 90 minutes and cooled down before adding 4 ml of aqua regia and heating again for 30 minutes (USEPA, 1996). The solution is then left to cool to room temperature, filtered through a 0.45-micrometre membrane filter, and diluted to 50 mL with deionized water.

RESULTS AND DISCUSSIONS

Results

Physico-chemical properties

Table 2 shows the soil physico-chemical properties of soils in five salt licks from the study area. The result for pH is slightly acidic to slightly alkaline, with an average of 7.30 ± 0.08. Muddy environment licks (SL20 and SL60) are slightly alkaline, whereas gravelled licks are slightly acidic (SL56 and SL59). The SL50 lick shows an almost neutral soil pH. The moisture content, organic matter, and electrical conductivity were (33.88 ± 1.67%), (4.12 ± 0.30%), and (177.46 ± 4.76 uS/cm), respectively. Generally, silt percentage (44.79 ± 21.15%) is the main component that makes up the salt lick soil, followed by sand (31.66 ± 22.97%) and clay (23.45 ± 11.95%), whereas sand percentage (47.69 ± 10.28) is the highest particle detected in the control soil.

Table 2 Physico-chemical properties (average values) of licks and control soil.

Sampel	SL20 (n=11)	SL50 (n=5)	SL56 (n=3)	SL59 (n=14)	SL60 (n=11)	Salt Lick Mean ± SD (44)	Range	Control (n=8)	Control SD (8)
pH	8.58	7.13	6.04	6.06	8.03	7.30 ± 0.08	4.69-9.49	4.90	± 0.07
MC (%)	26.04	38.36	36.00	44.78	25.22	33.88 ± 1.67	4.84-61.65	13.99	± 0.74
OM (%)	0.95	3.80	5.27	7.83	2.41	4.12 ± 0.30	0.08-12.11	3.05	± 0.23
EC (uS/cm)	260.88	243.32	224.39	48.59	100.69	177.46 ± 4.76	14.12-375.04	38.05	± 1.53
Sand (%)	33.56	42.79	66.79	30.73	16.29	31.66 ± 22.97	4.41-80.08	47.69	± 10.28
Silt (%)	40.88	34.83	14.69	46.43	59.36	44.79 ± 21.15	2.89-82.94	28.81	± 6.90
Clay (%)	25.56	21.48	18.53	22.84	24.35	23.45 ± 11.95	4.98-78.48	23.50	± 7.29
Texture	Loam	Sandy Loam	Sandy Loam	Silty Clay Loam	Silt Loam			Sandy Loam	

Essential element concentration

The geochemical concentration of essential elements in each lick's soil varies with each other (Table 3). Generally, lick soils contain a higher nutrient composition compared to control soil. Calcium (Ca) has

the highest concentration in all salt-lick soils, followed by potassium (K), magnesium (Mg), and sodium (Na). The SL20 and SL60 show significantly higher concentrations of Na and Mg compared to other licks. SL20 and SL50 are rich in Ca.

Table 3 Chemical concentration (average values) of soil from salt licks.

Sample	Concentration (mg/kg)			
	Ca	**K**	**Na**	**Mg**
SL20 (n=11)	7966.03	2355.41	615.09	5305.13
SL50 (n=5)	11551.64	1456.29	199.60	2501.74
SL56 (n=3)	1101.92	980.54	154.56	1442.14
SL59 (n=14)	1914.28	910.27	106.36	1446.85
SL60 (n=11)	3288.95	2053.73	727.34	5208.01
Controls (n=8)	1470.31	1102.05	186.73	1692.65

Discussions

Physico-chemical properties

The slightly alkaline soil observed in muddy salt licks is associated with high-soluble salts. According to Ardahanlioglu et al. (2003), the high sodium concentration affects the soil's overall pH value, which otherwise explains the lower sodium concentration in slightly acidic licks.

The moisture content in the lick soil is also higher than in the control soils. The presence of seasonal small streams and water seepage cutting through the lick promotes a high percentage of moisture content, apart from soil texture parameter influence. Fine-textured soils have higher water retention ability compared to coarse-textured soils (Tufaila et al., 2016) because fine-textured soils have more pore space (Zacharias & Wessolek, 2007; Eluozo, 2013; Mairghany et al., 2019) and an absorptive surface, thus having better water holding capacity (Nurhayati, 1986; Marakkala et al., 2018). The high percentage of moisture content in SL50, SL56, and SL59 is presumably affecting the high organic content in the licks. According to Bot and Benites (2005), soil conditions that are continuously saturated with water will cause poor aeration in the soil, thus causing low oxygen availability. This environment will result in low mineralization, where organisms become less active or dead. A prolonged water-saturated environment in the soil and a low decomposition rate could produce considerable organic matter. Low organic matter was observed in SL20 and SL60 compared to control soils. These may be due to the direct effect of the ungulates using the licks (Walters & Deluca, 2007), and active activities such as trampling in these areas cause soil compaction and further degrade soil physical properties (Greene et al., 1994).

Othaman et al. (2020) state that the soil's electrical conductivity values reflect the soil salinity (salt concentration), where the higher the electrical conductivity value, the higher the salt concentration in the soil, and vice versa. The SL59 shows lower electrical conductivity values, suggesting less soluble

salts are readily available in the soil. The low electrical conductivity value was perhaps due to good drainage conditions, which favoured the removal of bases by percolation (Rao et al., 2017). A small seasonal stream cutting through the soil affects the dilution process of mineral concentration in the soil (Anderson et al., 1997; Anderson & Dietrich, 2001).

Textural variances were also observed between salt lick soils and control soils. The differences were influenced by the nature of the source materials and some geomorphological events that occurred at these locations (Molina *et al.*, 2014). The mud salt licks show geochemical characteristics like weathering of rocks and other origins such as erosion of surface soil or sediments transported by water that are associated with the salt licks. The dissimilarities in grain size and particle content could be a result of the mixed structure of the mud salt licks, which continuously receive eroded materials from other components of the habitat both in and above the surface soil (Molina et al., 2014).

Essential element concentration

The salt lick SL50, which is almost neutral soil, shows a high concentration of calcium. This is due to the fact that the parent rock that made up the study area is calcareous lithic arenite sandstone (Hutchison, 2005), which is rich with calcium. According to Hutchison (2005), calcitic concretions are common in the thicker sandstone beds of Kulapis Formation. He also states that the sandstones are quartz-rich and contain plagioclase and chert grains. Thus, the co-existence of calcium elements and quartz in equilibrium may give rise to the neutral soil pH obtained in SL50. The existence of high calcium content in alkaline soils is influenced by the decomposition processes of animals, microorganisms, and plants. The decomposition process will cause calcium to be mineralized and released back into the soil (Attiwill & Adams, 1993). Mineralization rates are large and differ significantly among tree species, affecting the spatial pattern of soil acidity and calcium availability in a mixed-species forest stand (Dijkstra, 2003). A greater calcium concentration also indicates a higher clay content in the soil (Espinoza et al., 2012).

Potassium was found to be low in gravelled licks (SL 50 and SL 56) compared to muddy environments (SL 20 and SL 60). Mengel et al. (2001) state that the concentration of potassium in soil depends on the type of clay minerals present. In humid areas, illite was accompanied by vermiculite and smectite as the K+ (Shakeri & Abtahi, 2018). Potassium in acidic soil is low in concentration. Acidic soils are abundant with H+ and Al3+. During soil acidification, cations of calcium, magnesium, potassium, and sodium are leached out with rainwater and replaced by hydrogen and aluminium (Filipek, 2011).

According to Fang et al. (2021), high concentrations of salt and high pH often occur simultaneously in nature. Even though the soil contains a high concentration of soluble calcium, magnesium, and potassium salts, the predominant cause of salinity in soils is sodium salts (Rengasamy, 2006). Thus, the sodium concentration in SL20 and SL60 can be considered the predominant minerals in both licks, which are alkaline in nature. Acidic licks (SL56 and SL59) show almost similar nutrient concentrations to each other and are in the same range as control soils. Both of the salt licks are also influenced by water flows dissecting the licks. Continuous stream flows cause the elements to leach from

soils to the water body (Addiscott & Wagenet, 1985; Roos & Åström, 2005; Saarinen & Kløve, 2012) and carry away, thus the relatively low nutrient concentration compared to other licks.

Magnesium concentrations are higher in salt licks SL20, SL50, and SL60 compared to other salt licks and control soils. Generally, magnesium and calcium share the same element reaction for ion exchange (Mikkelsen, 2010). According to Wang et al. (2020), exchangeable magnesium concentration in acidic soil were low. This is because, relative to base ions, hydrogen ions and aluminium ions were more prevalent in acidic soil and trapped there by the clay colloid. Noticeable variation in element concentration from the five licks suggests that each lick serves different purposes in supplying nutrients for animal usage (Ayotte et al., 2006; Molina et al., 2014).

Table 4 compares the mineral concentrations reported in previous studies with the present study. Overall, salt licks located in Sabah (Deramakot Forest Reserve and Segaliud Lokan Forest Reserve) are higher in mineral concentration compared to salt licks found scattered in Sarawak. A comparison within local salt licks shows that the mineral concentration found in this study is higher than the salt lick found in Deramakot Forest Reserve, with the exception of potassium and magnesium concentrations, which are a bit lower. Interestingly, both of these forest reserves are located in the same formation, which is the Kulapis Formation. This proves that although the area is made up of the same rock formation, the soil formed from the weathering processes varies with mineral concentration.

Table 4 Chemical concentration (average values) of soil from salt licks.

Salt Lick Location	Concentration ranges (mg/kg)			
	Ca	**K**	**Na**	**Mg**
Deramakot Forest Reserve, Sabah Matsubayashi et. al (2007)	700 - 4400	1300 – 10060	200 – 600	1200 – 8600
Central to interior parts of Sarawak (Siong, 2020)	Nd – 1017	454 - 1834	Nd - 136	450 - 3627
Segaliud Lokan Forest Reserve (This Study)	1101 - 11551	910 - 2355	106 - 727	1442 - 5305

Nd – not detected

CONCLUSION

It was found that each salt lick in Segaliud Lokan Forest Reserve (SLFR) varies in physico-chemical composition and geochemical concentration. The salt lick soil's pH ranges from slightly acidic to slightly alkaline. The high moisture content in the salt lick's soil is the result of the occurrence of a seasonal stream with the support of a fine-grained texture that has high water retention abilities. The prolonged condition of water-saturated soil will cause anaerobic reactions and yield a considerable amount of

organic matter in the soil. The salt lick's soils also show a high electrical conductivity value, reflecting the abundance of salt found in the soil. The chemical concentration in Salt Lick's soils was higher compared to control soils. The abundance of calcium and magnesium, as well as elevated potassium and sodium concentrations in salt-licked soil, serve as sources of nutrients.

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ASSESSMENT OF THE CURRENT SPATIOTEMPORAL VARIATIONS OF TOTAL SUSPENDED SOLID ON THE SURFACE WATERS OF KUALA PERLIS, PERLIS

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ABSTRACT. *Rivers are vital water sources for human existence and environmental health. Due to freshwater scarcity, monitoring river water quality is crucial, especially concerning total suspended solids (TSS), which pose potential risks to health and the environment. Despite this importance, there is a lack of assessment of spatiotemporal TSS variation in Malaysia, particularly in Sungai Kuala Perlis. This study aims to evaluate spatial-temporal TSS variations in the surface water of Kuala Perlis, Perlis. Sampling points were GPS-recorded in December 2021. Five sites were established for morning, afternoon, and evening sampling. Water samples were collected and subjected to gravimetric analysis using the American Public Health Assessment (APHA) standard. ANOVA (p=0.05) in SPSS version 26 found TSS ranges of 110.67 mg/L -177.67 mg/L, 27.67 mg/L – 132 mg/L, and 78 mg/L – 304.67 mg/L for morning, afternoon, and evening, respectively. Surprisingly, no significant mean TSS differences were found for temporal (p>0.05) or spatial (p>0.05) variations. Factors influencing TSS variation such as water flow, salinity, and anthropogenic activities, were discussed. These findings inform researchers, governments, and NGOs for future planning in Kuala Perlis, promoting river health and eco-friendly management.*

KEYWORDS. Kuala Perlis, total suspended solids, water pollution, spatiotemporal study

INTRODUCTION

In recent decades, river water quality monitoring has been one of the major interesting research subjects as freshwater sources have become scarce and limited. Rivers are important sources of water for human existence and environmental health. Therefore, river water quality is a critical characteristic that must be conserved and closely monitored (Kamaruddin et al., 2018, 2021; Othman et al., 2012). Water quality, including hydrology, is a significant component of river health evaluations because it affects the spatial and temporal dynamics of various biological patterns and processes in major rivers (Kamaruddin et al., 2022; Mohd Rizal et al., 2022; Sheldon & Fellows, 2010). Studies have found that surface waters are the most polluted due to their easy availability for wastewater dumping (Kamaruddin et al., 2020; Samsudin et al., 2011). Malaysia's latest river water quality was examined in 2020 using 8,098 samples from 1,353 manual monitoring stations covering 672 rivers. Out of the 672 rivers monitored, 443 (66%) had good water quality, 195 (29%) were slightly polluted, and 34 (5%) were polluted (Department of Environment, 2020). The quality of water varies temporally and spatially because alterations in land cover surrounding rivers change over time and in different locations, influenced by both anthropogenic and natural factors (Hashim et al., 2023). This variability makes determining water conditions and pollution sources difficult and crucial for effective pollution control (Kamaruddin et al., 2021). Water quality monitoring should be conducted to properly understand rivers' current river health assessment, especially regarding the total solid content in the river ecosystem.

The total solid content in water bodies has adverse and detrimental effects on health and the ecosystem. Solids can degrade the quality of water or wastewater in various ways, and controlling biological and physical wastewater treatment processes requires solids analyses (Al-Badaii et al., 2013; Kamaruddin et al., 2020). Total solids include "total suspended solids," which a filter retains, and "total dissolved solids," which go through the filter (American Public Health Association, 1999). TSS is solid in water, including silt, decomposing materials, industrial waste, and sewage (Mohamed et al., 2015). TSS is a water quality indicator used to study sediment transport, aquatic ecosystem health, and engineering issues. Natural and anthropogenic processes such as runoff, coastal erosion, dredging activities, and waves account for most TSS in water bodies (Sa'ad et al., 2021). In short, a total suspended solid analysis should be carried out to understand the current river health in Malaysia.

Moreover, the total suspended solid is already an essential parameter in water quality monitoring in Malaysia. In the middle of the estuary and farther downstream, the TSS level increased due to wastewater dumping, an influx of runoff from the upper reaches, and fish feed for caged fish farming in Merbok Estuary (Fatema et al., 2014). The dry season has greater TSS levels than the wet season in Merbok Estuary (Fatema et al., 2014). However, this is contrary to a study conducted by Al-Badaii et al. (2013) in the Semenyih River, Selangor, as the rainy season had the highest TSS values because of the rainy season days, which caused substantial erosion on both sides of the riverbanks. According to the Malaysia Marine Water Quality and Criteria Standard, the allowable TSS amount for Class E (Interim) should be 30 mg/L (Department of Environment Malaysia, 2017). Therefore, the study of TSS in monitoring the river water quality assessment should be continually studied to acknowledge their adverse roles or impact on water bodies, especially in Sungai Kuala Perlis.

Previous studies showed that Sungai Kuala Perlis is designated as a Class III river, and it is currently undergoing severe erosion along its river banks and has become extremely shallow. Kuala Perlis has a landfill, directly impacting the river's water quality. Squatters near the river reserve area are also causing pollution. Shrimp livestock ponds, Kangar Wet Market, Sungai Perlis Esplanade, food vendors, and the Kuala Perlis Fisherman Jetty are further polluting sources (Samsudin et al., 2011). Previously, in 2006, Sungai Perlis had WQI scores of 68, which shows the status of slightly polluted and categorized as Class III (Department Of Environment, 2006). In 2019, Sungai Perlis' WQI scores ranged from 76 to 91, with only one river, Sungai Serai, being mildly contaminated (WQI score of 76, Class III); however, by 2020, the Sungai Perlis Basin's WQI had recovered to a range of 82 - 94 WQI scores, with all rivers classified as clean (Class I and Class II) (Department of Environment, 2020). Based on the current environmental report, Sungai Kuala Perlis's water quality has gradually been cleaned. The most recent study in Sungai Kuala Perlis revealed no significant variation in mean pH levels based on temporal variability. Despite the fact that the study discovered a considerable variance in pH readings due to spatial variations, the current spatiotemporal variation of TSS in Sungai Kuala Perlis is still not fully understood (Hashim et al., 2022). Prolonged and continued observation should be conducted to understand the current spatiotemporal variation of total solid content in Sungai Kuala Perlis.

Spatiotemporal variation of total suspended solids in Sungai Kuala Perlis should be properly conducted to assess the current condition and recent river health risk assessment. Temporally, water quality during high tide is Class II for upstream and downstream of the river. However, it is Class III during low tide. These results revealed that the river was less contaminated during high tide than during low tide. The volume and flow of water can

impact the river's water quality. However, there were no variations in WQI during high and low tides in the river's mainstream, classified as Class III (Che Ali et al., 2020). The TSS values at Sungai Perlis were higher during low than high tide (Che Ali et al., 2020). Spatially, according to Amneera et al. (2013), from Station 1 to Station 3, the TSS value was 50.25 mg/L, 30 mg/L, and 68.75 mg/L, respectively, from sample water taken in Sungai Kuala Perlis. Station 2 is classified as Class II, while Station 1 is classified as Class III, based on the NWQS parameter restrictions for Malaysia. Station 3 is Class III, with 50 to 150 mg/L concentrations. Previously, TSS profiles in Sungai Perlis exhibited fluctuations during high and low tides. In high tide, TSS levels ranged from 16.67 mg/L upstream, 30.25 mg/L in the middle stream, to 26.6 mg/L downstream. During low tide, TSS concentrations notably increased, with values of 42.67 mg/L upstream, 60.65 mg/L in the middle stream, and 57.34 mg/L downstream (Che Ali et al., 2020) . However, comprehensive and up-to-date data on the spatiotemporal variations of total suspended solids in Sungai Kuala Perlis remains limited. This study was initiated to provide preliminary data on the current assessment of the spatiotemporal variation of total suspended solids in Sungai Kuala Perlis and to understand the influence of the current anthropogenic landscape along with the river bodies that might be responsible for the pollution.

MATERIALS AND METHODS

This section briefly explained the sampling method, selection of sampling sites, and gravimetric analysis of total suspended solids. The observation during sampling activities was recorded to provide possible anthropogenic activities affecting the water quality and total suspended solid content.

Sampling method

Sampling was conducted in early December 2021, and five places were established, beginning with the first (SP1), Jetty Tok Kuning, and continuing until the river's mouth, the fifth location (SP5). Each sampling site was meticulously documented and recorded using the Global Positioning System (GPS). The sampling was done in the morning, afternoon, and evening. Water samples were obtained around one meter beneath the surface. At each sampling site, observations were made focusing on the natural landscape and potential anthropological components that could alter water quality and total solid content distribution. The water samples were collected in 1.5-litre plastic bottles, carefully labelled according to sampling sites, and transported to the laboratory for further investigation. Figure 1 illustrates the flow chart for TSS assessment in Sungai Kuala Perlis.

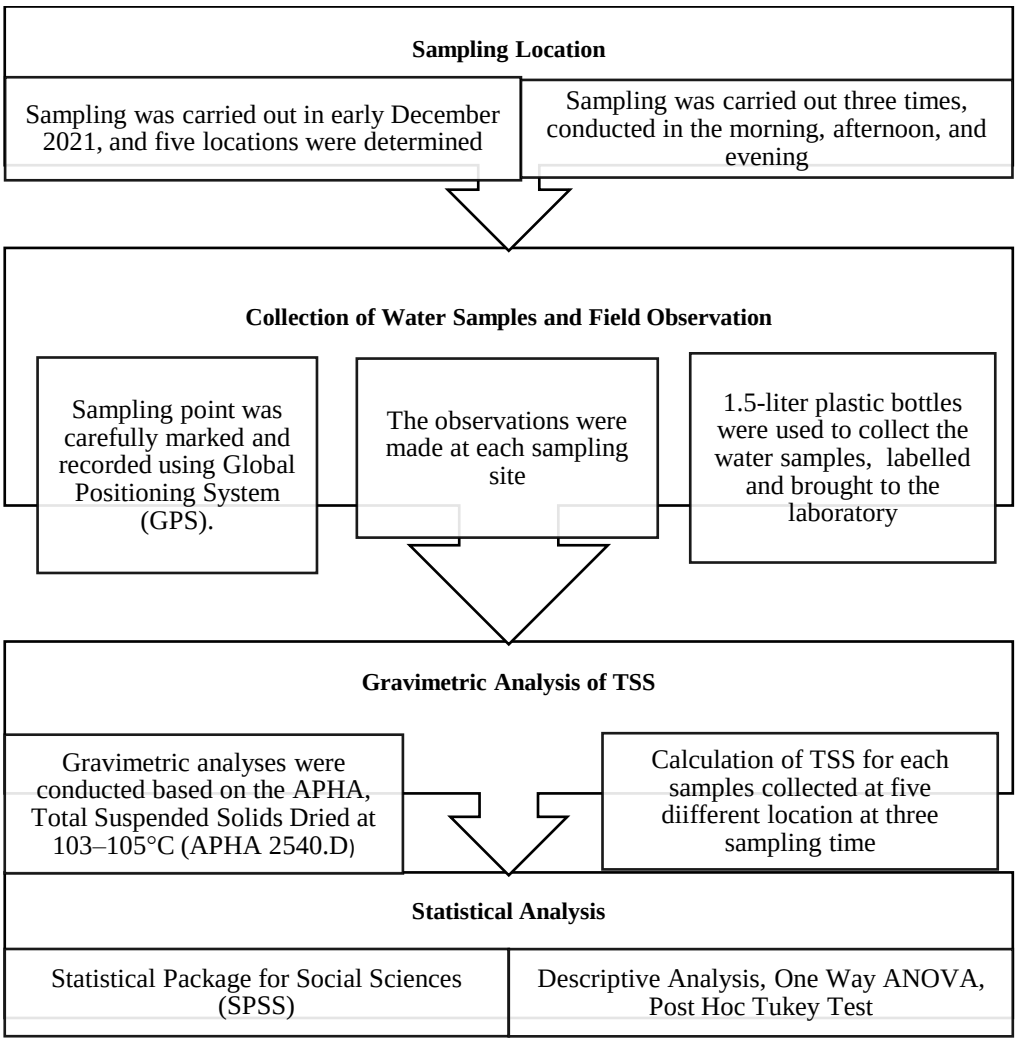

Figure 1. Flow chart for TSS assessment in Sungai Kuala Perlis

Sampling point location

The sampling sites were visited at three separate times: morning (AM), afternoon (AF), and evening (PM). During surface water sampling, each sampling point will be labelled (SP1-SP5), and the latitude and longitude will be recorded using a GPS. During the sample activities, probable anthropogenic activities were seen, and Table 1 indicates the sampling location and possible anthropogenic activities observed.

Table 1. Sampling location and possible anthropogenic activities

Sampling	Location	Coordinate	Possible Anthropogenic Activities
AM	SP1	6˚25'04.853"N 100˚09'01.853"E	Jetty Tok Kuning, Agriculture
	SP2	6˚25'19.128"N 100˚08'32.333"E	Agriculture, Aquaculture/ Fishing Pond
	SP3	6˚25'03.461"N 100˚08'22.434"E	Solar Power Plant
	SP4	6˚24'37.799"N 100˚08'23.903"E	Solar Power Plant, Roadside, Residential Area
	SP5	6˚24'28.511"N 100˚08'23.364"E	Restaurant, Floating Village
AF	SP1	6˚25'04.992"N 100˚09'00.774"E	Jetty Tok Kuning, Agriculture
	SP2	6˚25'17.364"N 100˚08'46.409"E	Agriculture, Aquaculture, Fishing Pond
	SP3	6˚25'20.808"N 100˚08'31.746"E	Fish Pond, Solar Power Plant, Roadside
	SP4	6˚24'57.449"N 100˚08'18.917"E	Roadside, Restaurant
	SP5	6˚24'27.263"N 100˚08'20.742"E	Floating Village
PM	SP1	6˚25'04.787"N 100˚09'01.290"E	Jetty Tok Kuning, Agriculture
	SP2	6˚25'22.188"N 100˚08'44.322"E	Agriculture, Aquaculture, Fishing Pond
	SP3	6˚25'00.653"N 100˚08'19.679"E	Aquaculture, Solar Power Plant
	SP4	6˚24'34.355"N 100˚08'29.033"E	Solar Power Plant, Roadside, Residential Area
	SP5	6˚24'27.479"N 100˚08'23.364"E	Restaurant, Floating Village

Furthermore, sampling locations in Sungai Kuala Perlis were plotted for surface water collection based on latitude and longitude for each sampling time. The map allows for precise sampling point positioning, and possible anthropogenic activities can be thoroughly

investigated. Figure 2 (Google Earth, 2022c) showed the sampling location for morning (AM) sampling; Figure 3 (Google Earth, 2022a) showed the sampling location for the afternoon (AF) sampling, and Figure 4 (Google Earth, 2022b) showed the sampling location for the evening (PM) sampling.

Figure 2. Morning sampling location (Google Earth, 2022c)

Figure 3. Afternoon sampling location (Google Earth, 2022a)

Figure 4. Evening sampling location (Google Earth, 2022b)

Gravimetric analysis of total suspended solids

Gravimetric analyses were conducted based on the APHA, Total Suspended Solids Dried at 103–105°C (APHA 2540.D) standard procedures in assessing solid analysis (American Public Health Association, 1999). Sample water collected for each sampling point (SP) will be tested for three batches of the sampling period, morning sampling (AM), afternoon sampling (AF), and evening sampling (PM).

Determination of total suspended solids

The filtering apparatus was assembled; 27 mm diameter, 0.45 µm-porous size was used in this analysis. The filter was seated first with a little reagent–grade or distilled water. 300mL of the sample was used for each sampling point. If possible, shear bigger particles with a magnetic stirrer to get a more uniform (ideally homogeneous) particle size. While stirred, 300 mL of sample were pipetted into the receiving flask, and suction was commenced. The filter was washed with three successive 10-mL volumes of reagent-grade water, complete drainage between washings was allowed, and suction was continued for about 3 min after filtration was complete. Samples with high dissolved solids may require additional washings. The filter was carefully removed from the filtration apparatus and transferred into an aluminium weighing dish as support. The filter was dried for at least 1 hour at 103 to 105°C in an oven, cooled in a desiccator to balance temperature, and weighed. The cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained or until the weight change is less than 4% of the previous weight or 0.5 mg, whichever is less. 10% of all samples were analyzed in duplicate. The weight of the filter, and the residue were

recorded, and the TSS value was calculated. Figure 5 shows the filter retaining the TSS before drying (a) and dried filter paper retaining TSS (b).

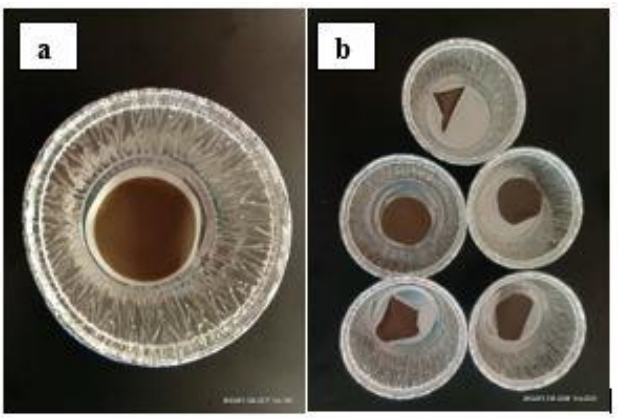

Figure 5. Filter retaining TSS before drying (a) and dried filter paper retaining TSS (b)

Calculation of TSS

The concentration of TSS was reported as mg/L or as mg/L TSS.

$$\text{TSS mg/L} = \frac{(A-B)\times 1000}{\text{sample volume, mL}} \quad (1)$$

Where:
A = weight of filter + dried residue, mg
B = weight of filter, mg

RESULTS AND DISCUSSION

All the results obtained from the spatiotemporal finding for Kuala Perlis's TSS variation were discussed distinctly for temporal and spatial variation of TSS, respectively, at Sungai Kuala Perlis. The data were tabulated and illustrated in tables and figures, respectively. The data analysis was conducted using the Statistical Package for the Social Sciences (SPSS) version 26. The current TSS spatiotemporal variation and possible factors affecting the spatiotemporal variation of TSS were further discussed.

Spatiotemporal variation of TSS in Sungai Kuala Perlis

The results showed three distinct ranges for TSS measurement along Sungai Kuala Perlis. For TSS, the ranges recorded for the morning, afternoon, and evening were 110.67 mg/L – 177.67 mg/L, 34.00 mg/L – 132.00 mg/L, and 78.00 mg/L – 304.67 mg/L, respectively, for the morning (AM), afternoon (AF), and evening (PM) sample times. The spatiotemporal variation of TSS will be discussed further below. Table 2 contains the distribution of the TSS for the five sampling points and the three sampling times.

Table 2. Data collection of TSS distribution at Sungai Kuala Perlis

Sampling	Location	TSS (mg/L)
AM	SP1	162.33
	SP2	145.33
	SP3	166.33
	SP4	177.67
	SP5	110.67
AF	SP1	132.00
	SP2	128.33
	SP3	50.00
	SP4	27.67
	SP5	34.00
PM	SP1	125.00
	SP2	304.67
	SP3	78.00
	SP4	111.33
	SP5	86.67

Spatiotemporal Variation of TSS

For the TSS spatiotemporal variation, it is apparent that the highest mean TSS was recorded during evening sampling at SP2 compared to the others. On the contrary, the lowest mean of TSS was recorded at the SP4 during the afternoon sampling. The ranges of the TSS distribution in the morning, afternoon, and evening were 110.67 mg/L – 177.67 mg/L, 27.67 mg/L – 132.00 mg/L, and 78.00 mg/L – 304.67 mg/L throughout five locations. Thus, it can be seen that TSS is particularly higher in the inshore part of the river compared to the region near the sea. The sampling point 2 (SP2) also showed the highest peak compared to the other location at a mean TSS of 192.78 ± 97.27 mg/L, while the lowest mean TSS was observed at SP5 with a mean TSS value of 77.11 ± 39.22 mg/L. Overall, from the plotted graph, it can also be seen that the TSS distribution is consistently higher during the morning sampling

(AM) exception at the SP2. The research predicted that the TSS value at the SP2 can be dramatically affected by the anthropogenic activities near the sample water collected; from the map and observation, SP2 can be seen near the agriculture site, aquaculture site, and fishing pond. The TSS might increase due to the scheduled water exchange of the aquaculture or fishing pond nearby. TSS spatiotemporal variation in Sungai Kuala Perlis is illustrated in Figure 6. Spatial and temporal variation of TSS along Sungai Kuala Perlis were recorded and analyzed in detail to understand the pattern of changes and to determine the current spatiotemporal variation of TSS on the surface waters of Sungai Kuala Perlis.

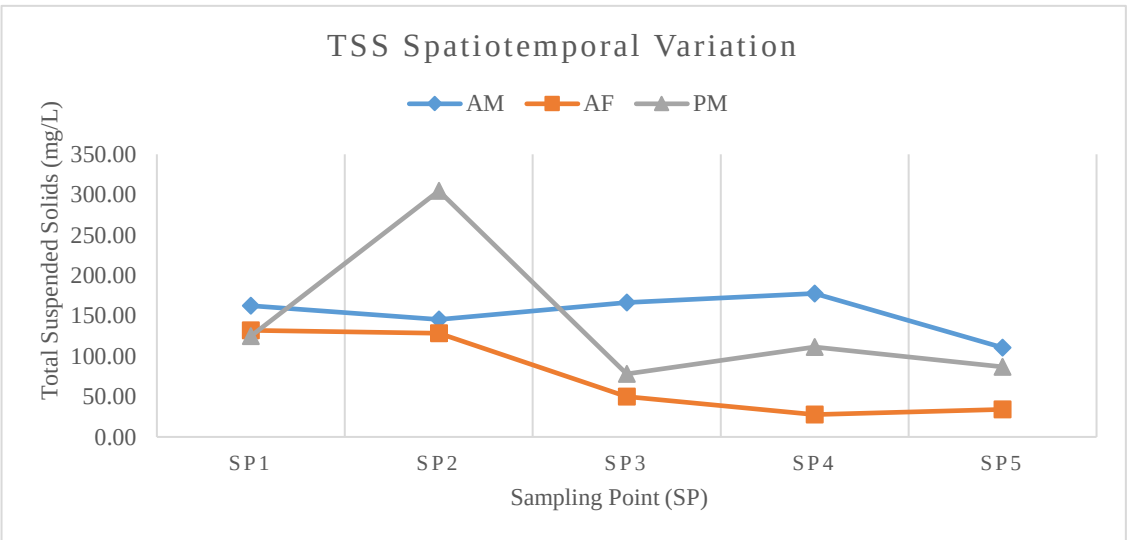

Figure 6. TSS spatiotemporal variation in Sungai Kuala Perlis

Spatial variation of TSS in Sungai Kuala Perlis

Spatially, the distribution of the total suspended solids was recorded. The finding revealed that the mean distribution of TSS at five different locations was 139.78 ± 19.84 mg/L, 192.78 ± 97.27 mg/L, 98.11 ± 60.72 mg/L, 105.56 ± 75.17 mg/L, 77.11 ± 39.22 mg/L, from SP1 till SP5. The highest mean TSS recorded for each sampling was at SP2. In contrast, the lowest mean of TSS was recorded at the fifth sampling point (SP5). A strong correlation, R2 = 0.5524, was found between the sampling point and the mean value of TSS at different sampling locations in Sungai Kuala Perlis. The mean TSS spatial distribution in Sungai Kuala Perlis is illustrated in Figure 7 below.

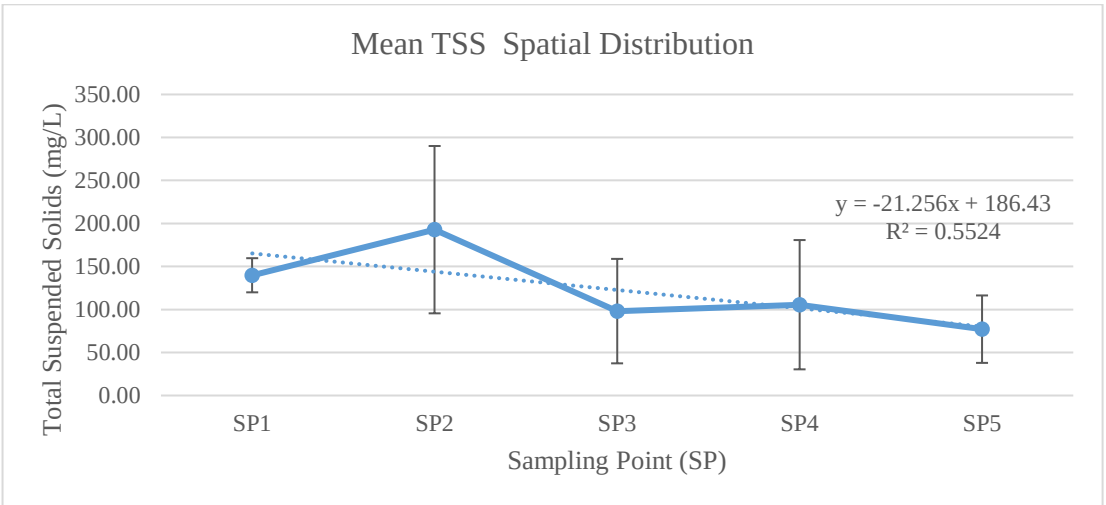

Figure 7. Mean of TSS spatial distribution

A one-way between-subject ANOVA was used to determine how the sampling point (SP1–SP5) affected the TSS value of the surface water in Sungai Kuala Perlis. At the $p < .05$ level for the five chosen sample points, there was no evidence of a substantial impact of the sampling location on the TSS value of surface water [$F(4,10) = 1.480$, $p = 0.280$], as can be seen in Table 3. These findings imply that the sampling site does not alter the TSS value of the water surface. In general, TSS assessment did not vary spatially in the study area of Sungai Kuala Perlis. As there was no significant difference in the spatial variation of surface water TSS measurement, post hoc Tukey HSD was not further performed.

Table 3. One-way ANOVA for spatial variation of TSS assessment

			Sum of Squares	df	Mean Square	F	Sig.
Between Groups	(Combined)		24538.296	4	6134.574	1.480	.280
	Linear Term	Contrast	13553.959	1	13553.959	3.269	.101
		Deviation	10984.337	3	3661.446	.883	.482
Within Groups			41460.148	10	4146.015		
Total			65998.444	14			

Temporal Variation of TSS in Sungai Kuala Perlis

For the temporal variation of TSS in Sungai Kuala Perlis, the temporal variation is observed based on the results obtained at three different sampling times conducted in the morning,

afternoon, and evening. The mean TSS for each sampling time was 152.47 ±26.09 mg/L for the morning sampling (AM), 74.40 ±51.57 mg/L for the afternoon sampling (AF), and 141.13 ±93.33 mg/L for evening sampling (PM), respectively. From the plotted graph for mean TSS for temporal distribution, a weak correlation, R2 = 0.018, was found between the sampling time and the mean value of TSS in Sungai Kuala Perlis. The lowest mean TSS for temporal distribution was during afternoon sampling compared to morning and evening sampling. Figure 7 shows the mean TSS temporal distribution.

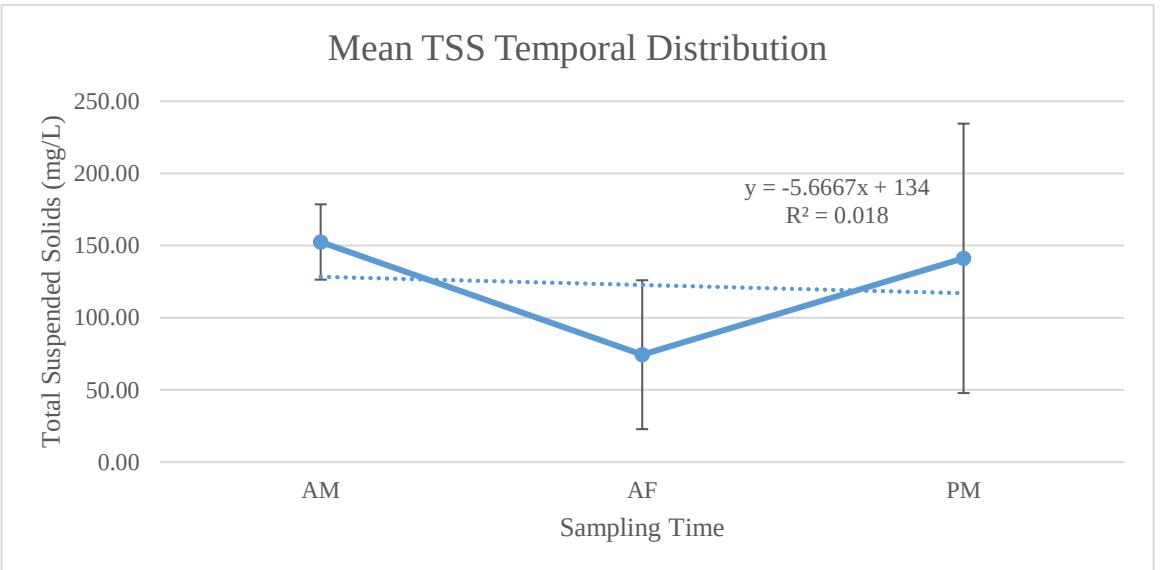

Figure 8. Mean of TSS temporal distribution

A one-way between-subject ANOVA was conducted to study the effect of the sampling time on surface water TSS value in the morning, afternoon, and evening. There was not a significant effect of the sampling time on surface water TSS value at the $p < .05$ level for the three conditions [$F(2,12) = 2.215$, $p = 0.152$], as can be seen in Table 4. These findings imply that sampling time in Kuala Perlis has no impact on TSS value. The TSS evaluation in the research region, Kuala Perlis River, generally did not vary over time. Since there was no significant difference in the time fluctuation of surface water TSS assessment, post hoc Tukey HSD was also not further performed.

Table 4. One-way ANOVA for temporal variation of TSS assessment.

			Sum of Squares	df	Mean Square	F	Sig.
Between Groups	(Combined)		17793.644	2	8896.822	2.215	.152
	Linear Term	Contrast	321.111	1	321.111	.080	.782
		Deviation	17472.533	1	17472.533	4.350	.059
Within Groups			48204.800	12	4017.067		
Total			65998.444	14			

Possible Factors Affecting Spatiotemporal Variation of TSS Distribution in Kuala Perlis River

In recent years, several studies have focused on the possible factors affecting the spatiotemporal variation of total suspended solids distribution in water bodies. Factors affecting the spatiotemporal distribution of TSS in river bodies are water flow rate, salinity, tidal movement and anthropogenic activities (Che Ali et al., 2020; Che Ngah et al., 2012; Dhungana & Wang, 2020; Håkanson, 2006; Rossi et al., 2006).

Researchers have studied the flow rate's effect on water bodies' total suspended solid distribution. Studies found that water flows positively influenced the TSS distribution in water bodies. A study by Dhungana & Wang (2020) confirmed that the flow rate increases the TSS concentration and eventually permits dam failure. TSS content in the water bodies, especially in lower drainage basins, is highly responsive to variations in flow rate (López et al., 2021). During frontal occurrences with extremely high peak flows in the examined estuaries, the average TSS values were higher than the other times. In short, spatially, the region with a high flow rate has higher TSS content. The collective finding indicates that water flow rate affects the spatiotemporal variation of TSS in river bodies.

Another factor that influences the distribution pattern of TSS concentration in river bodies is salinity. The dynamics of the inflow's salinity regime changes significantly impact the TSS concentration in estuaries and can assist in preserving nutrients (Paudel et al., 2019). It has been suggested that salinity could be a determining factor in the aggregation of the total suspended solids due to the presence of the salt ion (Håkanson, 2006). Therefore, the segmentation difference in freshwater and estuary in terms of salinity influences the spatiotemporal distribution of TSS in river bodies. The previous study found that salinity ranges along the Sungai Kuala Perlis ranged from 0.41 to 5.00 ppt, 0.79 to 24.74 ppt, and 0.33 to 14.00 ppt for the morning, afternoon, and evening periods (Hashim, Kamaruddin, Abd. Aziz, Tajam, Buyong, Abdullah, et al., 2023). The mean TSS for each sampling time was 152.47 ±26.09 mg/L for the morning sampling, 74.40 ±51.57 mg/L for the afternoon

sampling, and 141.13 ±93.33 mg/L for the evening sampling respectively. Consequently, research can highlight the connection between increased salinity and decreased TSS, especially during the afternoon sampling.

Moreover, the variation of TSS also being influenced by the daily tidal movement in Sungai Kuala Perlis. On Saturday, December 4th, 2021, in Kuala Perlis, the sunrise occurred precisely at 7:16:00 a.m., and the sunset took place at 7:02:50 p.m. The analysis of high and low tide data reveals specific timing, with the initial low tide recorded at 6:11 a.m., followed by another low tide at 6:02 p.m. The solitary high tide of the day was observed at 12:16 p.m. (tides4fishing.com, 2023). Consequently, the finding indicates that the TSS concentrations are significantly higher during low tide compared to high tide. This discovery aligns with the findings reported by Che Ali et al. (2020), which noted the elevation of TSS values during low tide conditions along Sungai Perlis compared to high tide conditions. It is postulated that the flow dynamics of the river during tidal events play a substantial role in regulating the concentration of both organic and inorganic materials within the river, driven by variations in river volume during high and low tides. During high tide episodes, TSS readings are reduced as dilution occurs due to the increased water volume.

In addition, the factor that influences the TSS spatiotemporal variation is anthropogenic activities. Typically, anthropogenic sources in rivers come from land-based sources, including mining, factories, and riverside development, which may influence the water quality, especially TSS (Azrina et al., 2006). In particular, sewage effluent and nutrient runoff from nearby residential areas can impact the TSS pollutants in river water (Bello & Haniffah, 2021; Tengku Ibrahim et al., 2021). Thus, anthropogenic activities are a major factor influencing the spatiotemporal variation of TSS content in river bodies.

To summarize, concerning Sungai Kuala Perlis, the water flow rate, salinity, and anthropogenic activities are possible factors affecting the TSS value in river bodies. The construction of a new residential area and other human activities close to Sungai Perlis have disturbed the soil surface, resulting in river pollution, erosion, increased runoff, and large-scale sediment movements (Che Ali et al., 2020). Sungai Kuala Perlis has become an alternative pathway for fishing vessels moving from the jetty to the sea, especially along the studied area. Thus, the water flow rate possibly increases from time to time, thus causing TSS suspension to rise on the surface. Different salinities of Sungai Kuala Perlis, from the jetty (SP1) moving forward approaching the sea, could also influence the TSS concentration. From the observation made during the sampling, there are rapid and dense human activities along Sungai Kuala Perlis, such as agriculture (paddy fields), aquaculture (aquaculture and fishing pond), floating residential areas, restaurants, and fresh markets. Floating trash and debris also can be seen free-floating along the stream flow. Figure 9(a) showed fishing

vessels along Sungai Kuala Perlis; Figure 9(b) (Residential Area/Fishers Village) and Figure 9(c) depicted evidence of free-floating trash and debris.

Figure 9. (a) fishing vessels along Sungai Kuala Perlis; (b) Residential area/fishers village and (c) evidence of free-floating trash and debris.

CONCLUSIONS

The findings effectively obtained preliminary data on the current spatiotemporal variance in TSS assessment on the Sungai Kuala Perlis surface water. The TSS results range was 110.67 mg/L -177.67 mg/L, 27.67 mg/L – 132 mg/L, and 78 mg/L – 304.67 mg/L, respectively, for the morning, afternoon, and evening sampling. However, the research found no significant difference in the mean TSS readings concerning temporal spatial variations. The study found no spatiotemporal variability in TSS measurements at any of the surface water locations and at any different time in Kuala Perlis. The preliminary information on TSS fluctuation can be used by the researcher, the government, and non-governmental groups to plan for future social and economic growth in the Kuala Perlis region by monitoring river health or assessing pollution. The data might also enhance environmentally friendly river management and safeguard the Sungai Kuala Perlis ecosystem.

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