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ANALYSIS OF BACTERIOPLANKTON COMMUNITIES IN TENGIZ-KORGALZHYN LAKES SYSTEM USING FULL-LENGTH 16S NANOPORE SEQUENCING DATA

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DECLARATION

I hereby declare that the thesis is my original work, and it has been written by me in its entirety. I have duly acknowledged all the sources of information which have been used in the thesis. This thesis has also not been submitted for any degree in any university previously.

Polina Len

(student's name)

28 April 2023

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SUMMARY

Tengiz-Korgalzhyn Lakes system, designated under the Ramsar Convention and UNESCO World Heritage Sites, is a unique ecosystem of wetlands inhabited by more than a hundred protected and endemic species. In the context of the constant ecological pressure in the area, it is critical to investigate the lake bacterioplankton species and their relationship with abiotic factors, especially since microbiome studies of the region are practically absent in the literature.

This study aims to investigate the role of salinity gradient in shaping bacterial communities in lake ecosystems, as well as the extent to which the overall abiotic factor explains the heterogeneity of microbiome composition across the region.

Data on microbial communities is based on the full-length 16S amplicons obtained with the MinION mk1c. Species-level classification and analysis are performed in Emu, R , and R Studio, using packages phyloseq and vegan.

Our research has confirmed the importance of the salinity gradient in shaping the microbiome composition in limnetic and oligohaline lakes. We have shown that out of all abiotic factors, salinity exerts the most influence on the composition of microbial communities. The abundance of *Beta-* and *Gammaproteobacteria* classes changed in parallel with raising salinity levels across all sampling sites: decreasing and increasing, respectively. Moreover, salinity negatively correlated with the community evenness index across distinct small lakes, implying the presence of dominant species. The high degree of variability between isolated water bodies was mainly attributed to the geographical separation.

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ABBREVIATIONS

NGS	Next Generation Sequencing
ONT	Oxford Nanopore Technologies
TDS	Total Dissolved Solids
DNA	Deoxyribonucleic Acid
PCR	Polymerase Chain Reaction
ANOSIM	ANalysis Of Similarities

1 INTRODUCTION

1.1 Background and Literature Review

Active exploration of microbial biogeography started only in the beginning of the 21st century, soon after the emergence of new sequencing techniques that allowed handling large amounts of genomic data (Martiny *et al*, 2006). It was yet to be determined whether bacterial biogeography actually exists and, furthermore, if reflects current or/and historical environmental events; however, it was clear that understanding of microbial communities and their dynamics is crucial on the ecosystem level because it affects the most fundamental biogeochemical processes, such as respiration, production, decomposition, nitrogen cycling, etc. (McGrady-Steed *et al*, 1997; Naeem & Li, 1997; Bell *et al*, 2005). The issue of microbial communities becomes even more pressing when put into the context of unstable ecosystems or endangered inhabiting species that require protection and constant monitoring.

Preservation of natural habitats ranks highly among many pressing environmental concerns. It is especially relevant to Qazaqstan – with its diverse biomes and ecosystems. 'Zooming' into the Qazaq steppe, there is a special area designated under the UNESCO World Heritage Sites and Ramsar Convention, "Saryarka: Steppe and Lakes of Northern Qazaqstan." As a part of the protected territory, the Tengiz-Korgalzhyn Lakes system in the Korgalzhyn State Nature Reserve is represented by the wetlands and surrounding grounds inhabited by numerous protected and endemic species of plants and animals (Burlibayev *et al*, 2007), that have been extensively studied and described in literature (Кошкин, 2017; Березовиков *et al*, 2014; Ерохов & Березовиков, 2001). Despite numerous efforts, the ecological and hydrological state of lakes located in this semi-arid is still under great pressure of salinization and desiccation, as it is largely dependent on the abundance of annual spring floods, works on the nearby buttress dams, as well as other anthropogenic factors coming from adjacent rural and urban areas. We believe that understanding microbial communities is crucial because of their strong relationship with system stability and productivity, especially since microbiome studies of the region are practically absent in the literature.

Studies of the similar ecosystems, represented by lakes under variable haline stress, have identified a set of relationships between microbial composition and abiotic factors that can be used in characterization purposes. Research shows that four main *Proteobacteria* classes strongly correlate with the salinity gradient in limnetic to mesohaline environments: *Betaproteobacteria* dominating in freshwater bodies, while *Alpha-* and *Gammaproteobacteria* classes dominating in saline environments (Wu *et al*, 2006; Lew *et al*, 2022; Laas *et al*, 2022). Laas et al (2022) also highlighted that, with salinity being the only environmental variable with significant effect on microbiome composition, spatial and ecological classification accounted for a larger percentage of variability in given models. The effect of such geographical factors is highlighted in other works as well (Wang *et al*, 2015).

In this study, we are focusing on the water lands of the Tengiz-Korgalzhyn Lakes system. Our aim is to describe the microbiome composition of the lakes and establish the correlations between a diversity of the bacterioplankton (alpha- and beta-diversity) and a set of environmental characteristics. It will allow us to identify the patterns in microbial composition and the specific response of organisms to changes in the environment.

1.2. Research question

What is the composition of bacterioplankton communities in the Tengiz-Korgalzhyn lakes, and how do abiotic factors such as salinity, conductivity, TDS and pH affect their structure?

1.3. Hypothesis

We hypothesize that the structure of the bacterial community of the lakes is shaped by the salinity gradient, in terms of its quantitative and qualitative composition. We also anticipate that geographical location of the lakes, apart from the salinity gradient, also exerts influence in the composition of microbiome.

2 MATERIAL AND METHODS

2.1. Sampling sites, collection and storage

Water samples were collected at different locations across the Tengiz-Korgalzhyn Lakes system as a part of a previous expedition to the Korgalzhyn Nature Reserve in July - August 2021: collection depth, volume, and sample code were registered. The following physicochemical parameters were recorded: temperature, conductivity, pH, oxygen saturation, total dissolved solids (TDS), and salinity. Samples were filtered in the lab using a Vacuum pump with the 0.22 μm (pore size) membrane filter, which are further stored in collection tubes at -80C.

2.2. DNA extraction and amplification

Filtered samples that include bacterioplankton and other particles greater than 0.22 um in diameter are used as a source of genetic material. DNA is extracted with the PowerWater DNA Isolation Kit (Qiagen, USA) according to the manufacturer's instructions. This kit utilizes the Inhibitor Removal Technology to eliminate both solid and dissolved PCR inhibiting compounds and extract high-quality DNA. The final concentration and purity of the DNA are assessed with Nanodrop (Thermo Fisher Scientific). A concentration of 6 ng/ul and above is considered satisfactory. The 260/280 ratio of about 1.8 is considered satisfactory. Purified DNA products are stored at -20C until further use.

The ONT 16S Barcoding Kit SQK-16S024 (Oxford Nanopore Technologies, UK) and the relevant manufacturer's protocol are used for library preparation and PCR. Provided primers include 27F and 1492R gene regions for full-length 16S sequencing, as well as 24 different barcodes. PCR is performed under standard conditions with Dream Taq Hot Start PCR Master Mix 2X (Thermo Fisher Scientific, USA). Purification step is performed with AMPure XP (Beckman Coulter, USA) according to the protocols provided by the Nanopore Community. In addition to the Barcoding Kit, the Flow Cell Priming Kit (EXP-FLP002) is used for priming and loading (Oxford Nanopore Technologies, UK).

Basecalling and demultiplexing is done with the Guppy Basecalling Software (version 6.4.6+ae70e8f) by the Oxford Nanopore Technologies (UK). Reads are filtered based on the length (1400 - 1650 bp) and quality (Qscore of 7 and higher).

2.3. Taxonomic classification and downstream analysis

Taxonomic assignment and relative abundance estimation is performed using the Emu algorithm (Curry *et al*, 2022). Further downstream analysis is performed with R (R Core Team, 2023), R Studio (Posit team, 2023), and R packages *phyloseq* (McMurdie & Holmes, 2013) and *vegan* (Oksanen J *et al*, 2022). Single and multivariable linear regression was used to estimate the relationship between environmental parameters and diversity indices. Mantel (Mantel, 1967) and ANOSIM (Clarke, 1993) tests are used for the statistical assessment of sample separation and correlation. The required significance level is a p-value of less than 0.05.

3 AIMS OF THE THESIS PROJECT

I. Collect water samples from different locations across the Tengiz-Korgalzhyn Lakes system along with physicochemical parameters of water
II. Obtain data on taxonomic units of bacterial communities
III. Conduct computational analysis of microbial communities
    A. relative abundance of taxonomic units
    B. alpha-diversity (richness)
    C. beta-diversity

4 RESULTS

4.1. Sample collection

Overall, 43 probes across the Tengiz-Korgalzhyn Lakes system were collected and filtered: 21 from Tengiz Lake (S) and 22 from Korgalzhyn lakes (T). Locations (Figure 1) and physicochemical parameters (Supplementary Table 1, Table 2) were recorded for all of the locations. Probes were collected into the 1.5 liter bottles and brought to the lab for further filtration and storage.

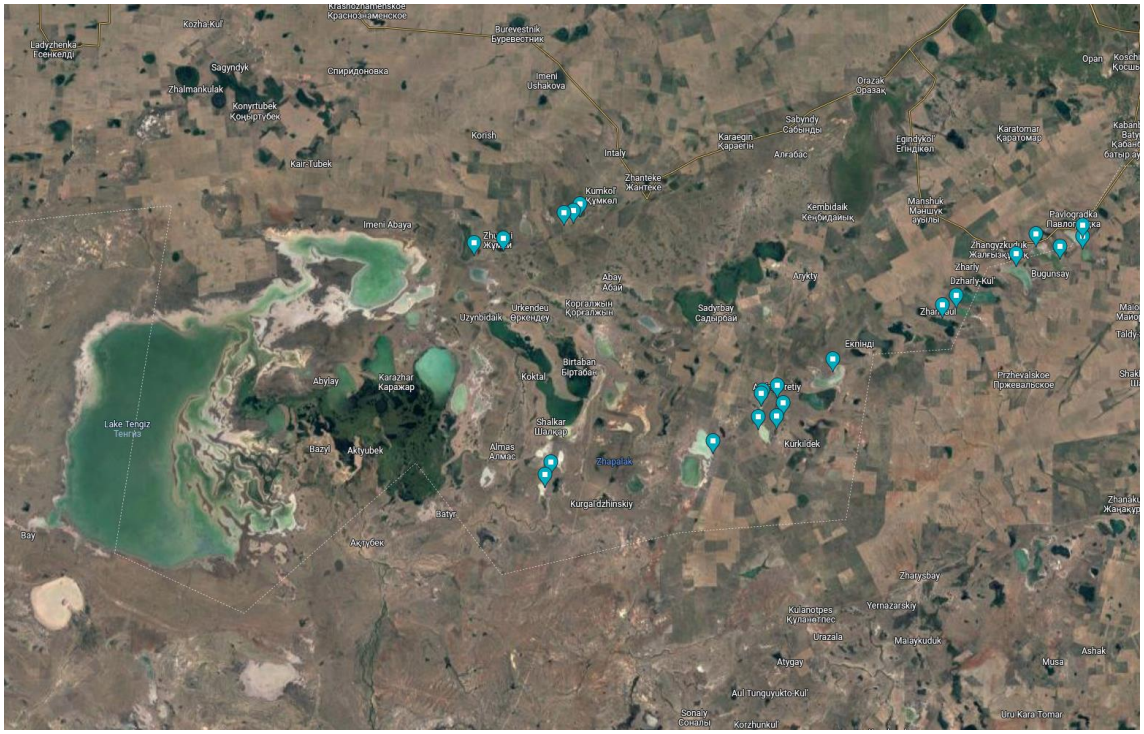

Figure 1. Geolocations of the sites of water sample collection across the region

Most variability was observed in salinity and oxygenation levels (%). While temperature and pH measurements remained relatively constant across the whole dataset, the other two parameters fluctuated between sites. Tengiz can be generally characterized as a β-oligohaline lake, with the exception of one point shifting more toward the α-oligohaline range (Venice System, 1958). Salinity in small lakes varied greatly, with some being in the range of freshwater and others reaching the top of the β-oligohaline group.

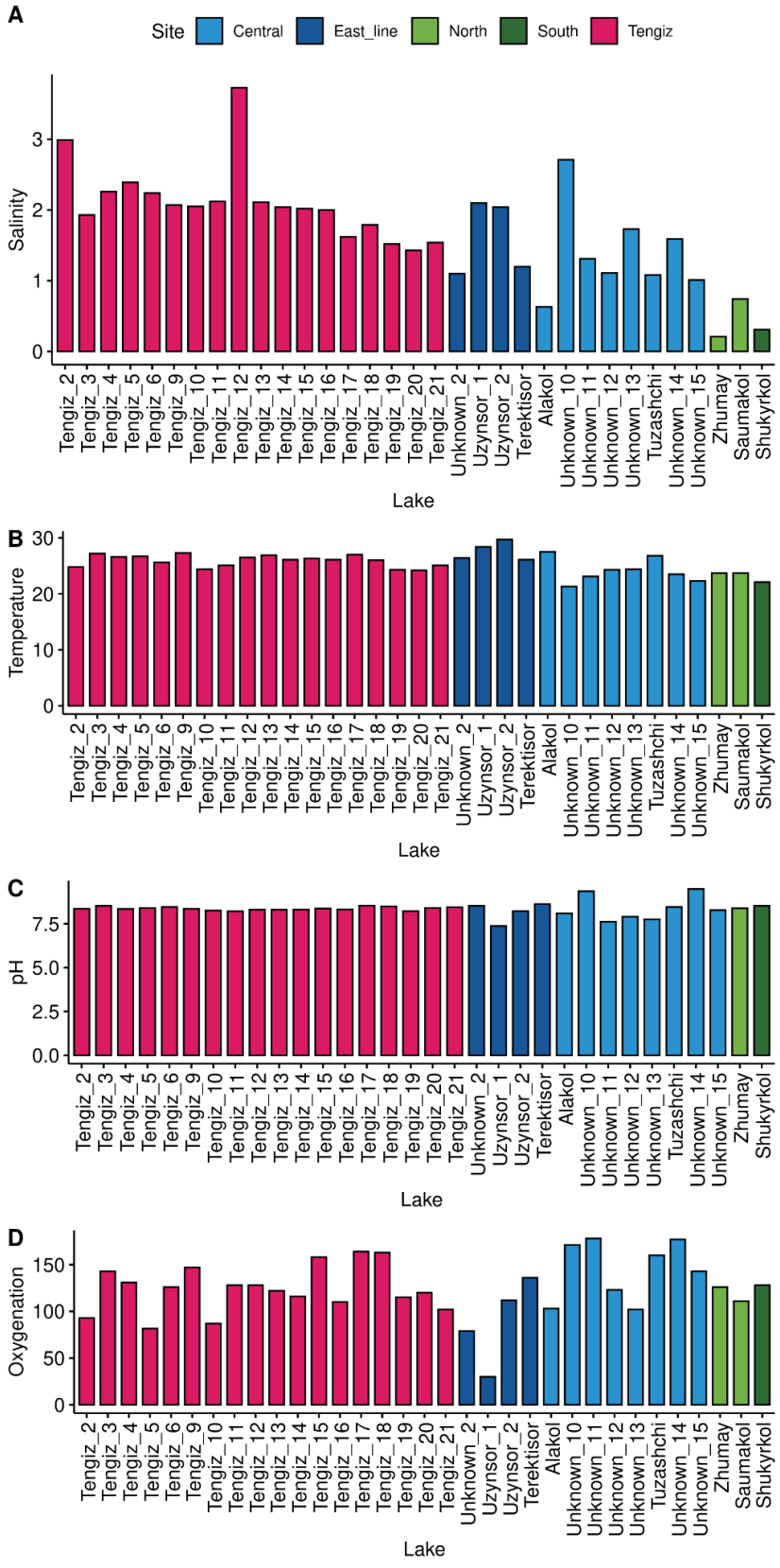

Figure 2. Distribution of physicochemical parameters across Tengiz-Korgalzhyn lakes system

4.2. DNA extraction and purification

After extraction and purification, impure samples with DNA concentration less than 6 ng/ul (Tengiz 1, Tengiz 8, Tengiz 22, Tuz, Karasor, Uzynkol, Ashchikol,
Unknown small lake 18, Temirastau, Unknown small lake 23) were discarded from further analysis. Figure 2 shows the metadata summary of remaining samples.

4.3. Library preparation and Nanopore Sequencing

In total, two successful sequencing rounds of sequencing were performed. Table 1 provides an overview of the duration and output of both runs. After approximately 38 hours, the runs were terminated due to the exhaustion of available sequencing units (pores), yielding 4-5 million reads each. The estimated average read length was consistent with the expected 16S rRNA gene length (~1550 bp) and average read quality of about Q11 (92% accuracy).

Table 1. Overview of sequencing results and output quality estimates

Parameter	**Run 1 (S)**	**Run 2 (T)**
Flow Cell	FLO-MIN106D	FLO-MIN106D
Kit	SQK-16S024	SQK-16S024
Run Length	1d 13h 30m	1d 15h 44m
Reads Generated	4.12 M	5.72 M
Estimated Bases	8.33 Gb	10.53 Gb
Average length	1,561.1 bp	1,562.6 bp
Average q-score	11.0	10.9

4.4. Taxonomic classification and downstream analysis

Species-level classification performed with the Emu algorithm identified a total of 2790 unique taxa across 33 available samples. Based on the resulting rarefaction curve (Figure 3), reads were sampled without replacement with the cutoff value of 50,000 reads per sample. As a result of rarefaction, seven more samples were excluded from further analysis: Tengiz 16, Tengiz 8, Tengiz 19, Tengiz 20, Unknown small Lake 2, Terektisor,

and Unknown small Lake 14. Bacterial phyla distribution across the normalized dataset is shown in Figure 4.

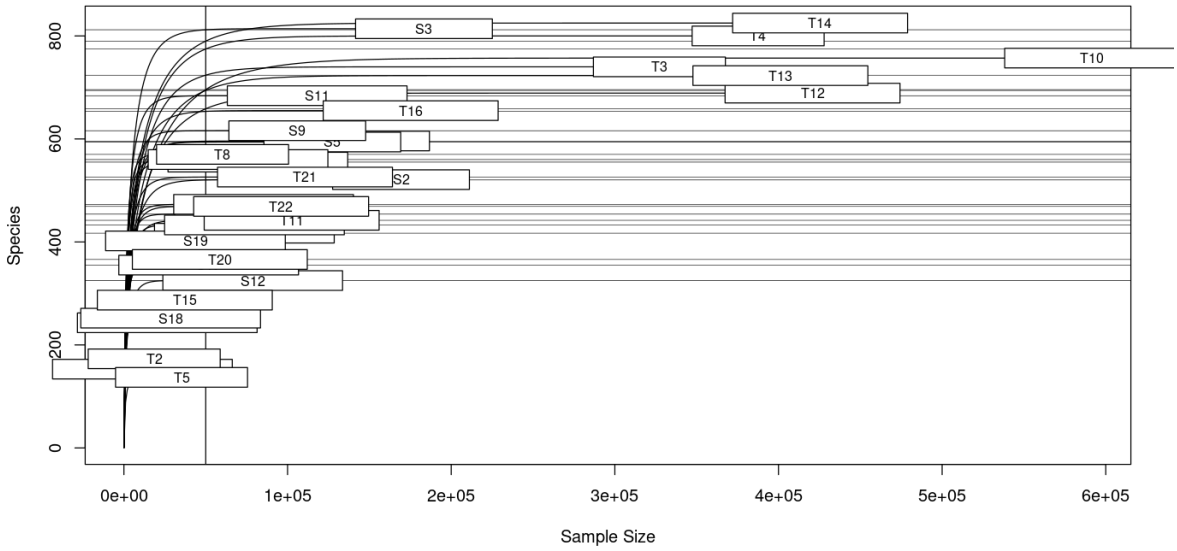

Figure 3. Rarefaction curves based on Emu classification outputs; the vertical line indicates a 50,000 cutoff threshold.

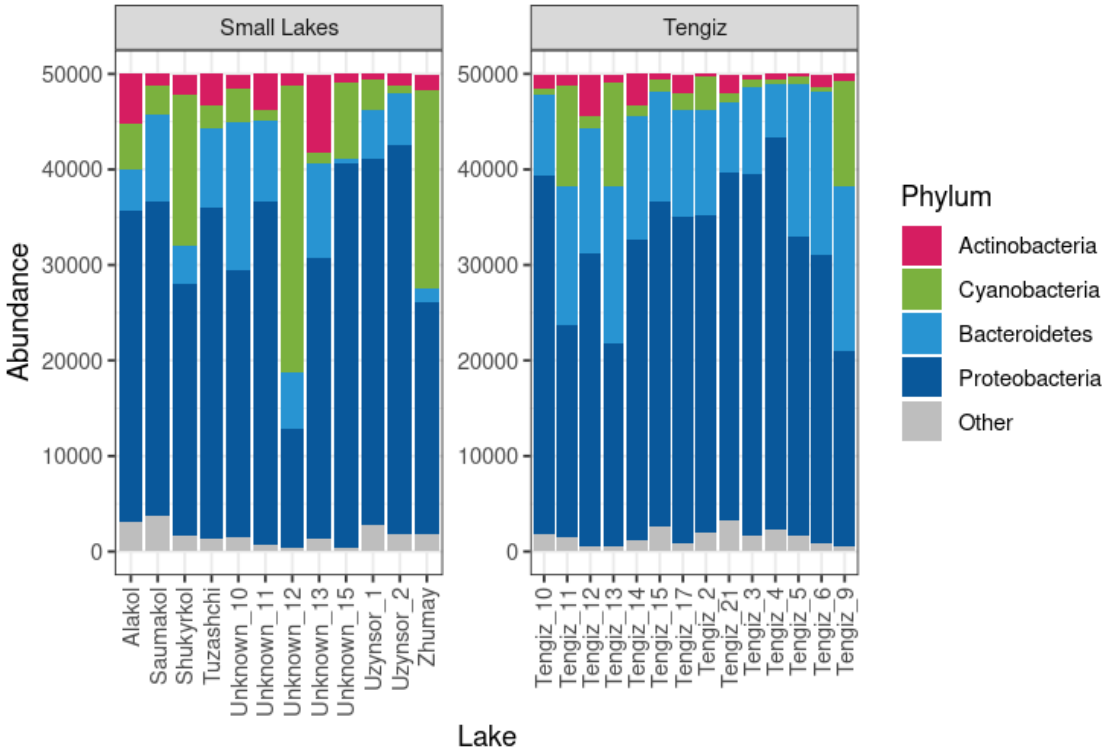

Figure 4. Relative distribution of bacterial phyla across 26 samples from the Tengiz-Korgalzhyn Lakes system.

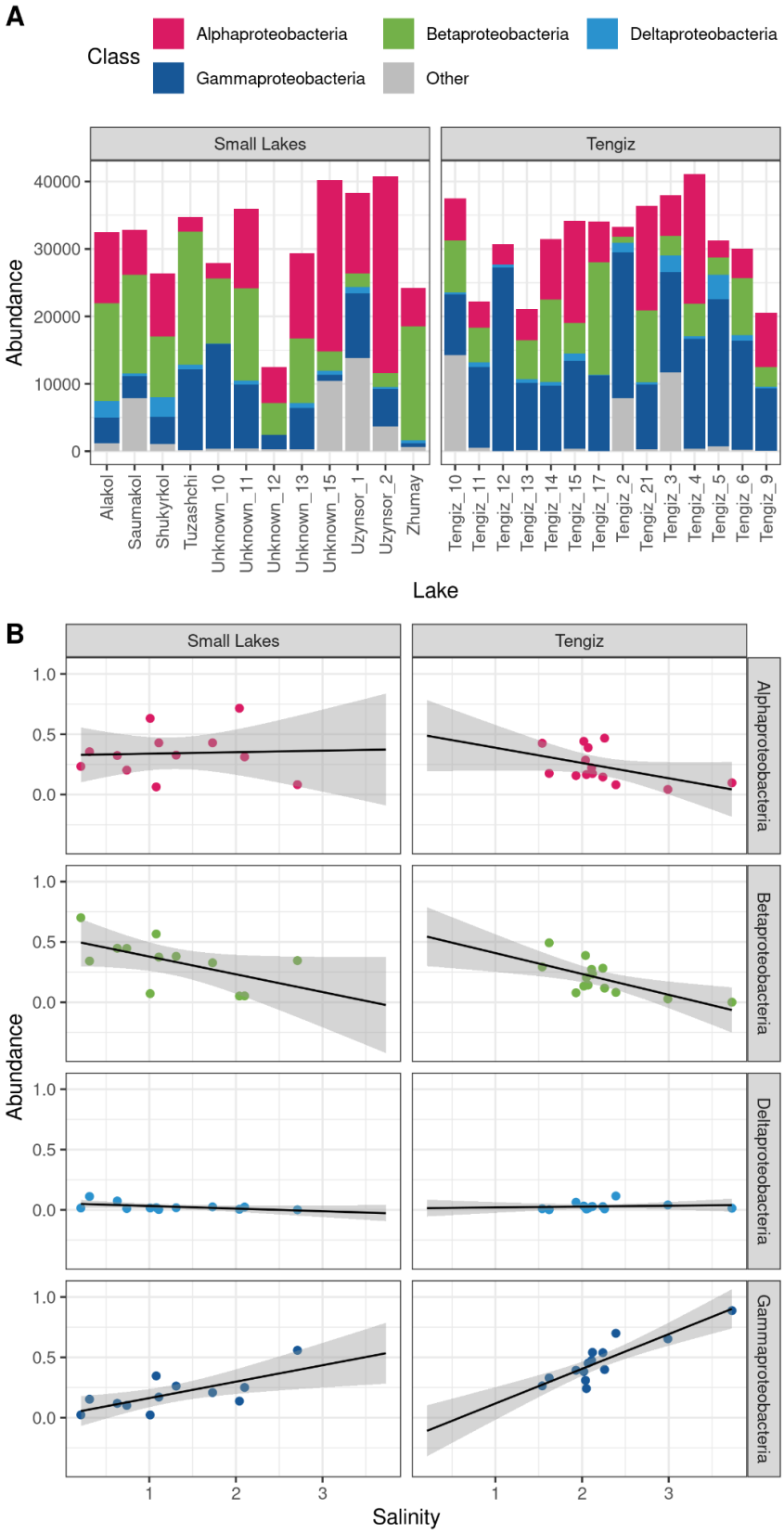

Figure 5. Relationship between the relative abundance of *Proteobacteria* classes and lake salinity.

Classification has identified that the four most abundant bacterial phyla present in the samples are *Proteobacteria*, *Bacteroidetes*, *Cyanobacteria*, and *Actinobacteria* (Figure 4). In order to assess the relationship between relative abundance of these taxa and water salinity, linear regression models were estimated for two groups of samples (Figure 5). No significant correlation was found between salinity percentage and the abundance of *Alphaproteobacteria* and *Deltaproteobacteria*. Yet, we have identified strong negative correlation between *Betaproteobacteria* (p-value of 0.059 and 0.0074 for Small Lakes and Tengiz, respectively.) In contrast, *Gammaproteobacteria* showed a significant positive correlation with lake salinity (p-value of 0.011 and <0.001, for Small Lakes and Tengiz, respectively).

4.4.1. Alpha-diversity (richness) of the samples

Four main indexes were chosen to estimate the alpha-diversity of lake bacterioplankton: Observed richness, represented by the number of unique OTUs, Chao1, Shannon and Gini-Simpson (1 – □) indexes (Figure 6). On average, samples collected from the small lakes are represented by a slightly larger number of unique species, as can be seen from Observed and Chao1 plots. At the same time, richness (Shannon) and evenness (Simpson) indexes indicate that communities from small lakes have, though insignificantly, lower evenness compared to Tengiz.

Moreover, variability in the richness of communities from small lakes seems to be strongly dependent on salinity levels (Figure 7B, D, F). Interestingly, the total number of unique species is strongly correlated with increasing salinity (R-squared: 0.4231, p-value: 0.01309). Yet, as it is indicated by Shannon and Simpson estimators (Figure 7D, F), the evenness of the communities is inversely proportional to salinity levels (R-squared: 0.4323, p-value: 0.012, for Shannon; R-squared: 0.4731, p-value: 0.008, for Simpson). No significant trends between richness and salinity were observed for Tengiz samples.

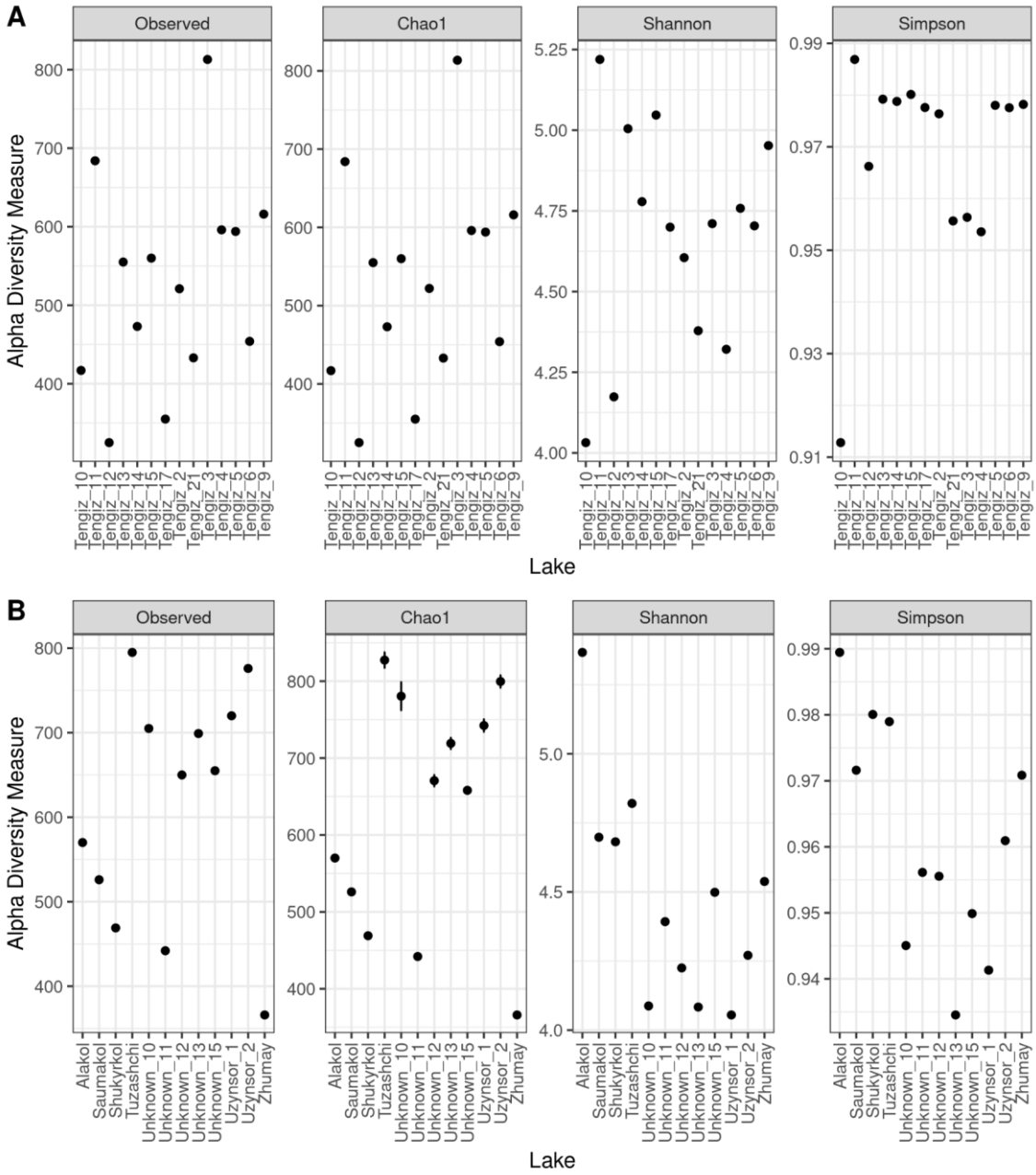

Figure 6. Richness estimates for (A) Tengiz Lake and (B) Small Lakes samples.

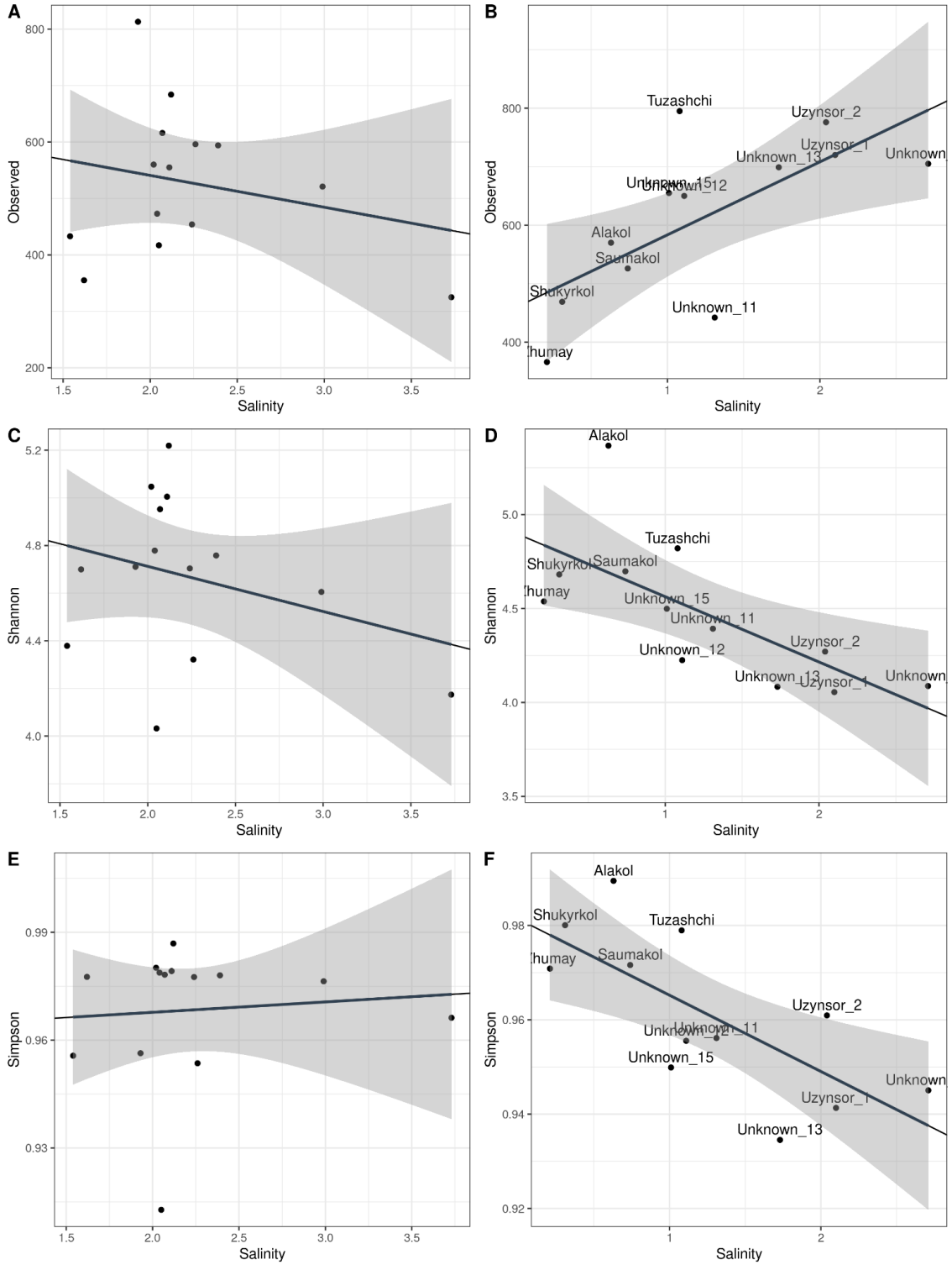

Figure 7. Relationship between alpha-diversity estimators and lake salinity. Three main indexes are shown: (A-B) Observed OTUs, (C-D) Shannon index, and (E-F) Simpson index. Graphs A, C, and E include samples from Tengiz Lake, and graphs B, D, and F include Small Lakes.

4.4.2. Beta-diversity (ordination) of the samples

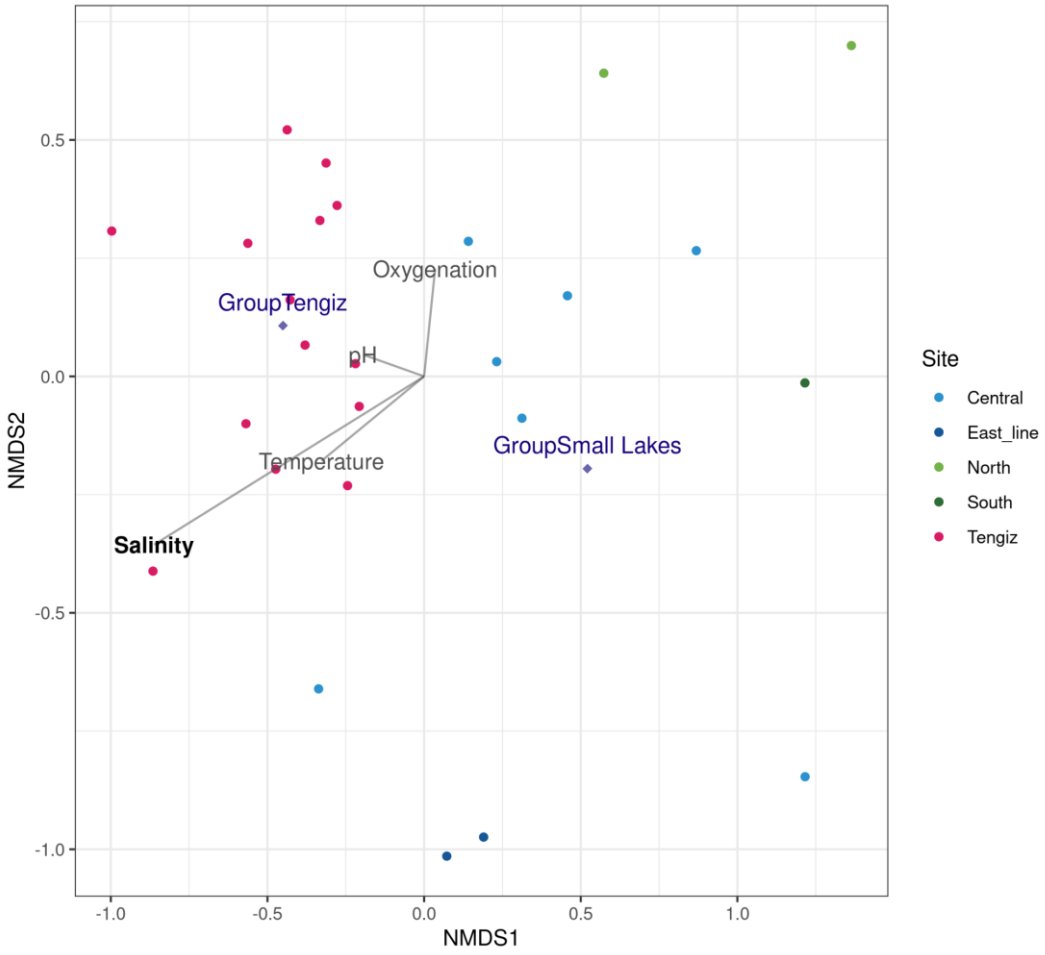

Figure 8. Non-parametric ordination of samples microbiome composition based on Bray-Curtis distance. Colors indicate lake location. Significant correlation with continuous physicochemical parameters is shown with bold dark labels. Purple diamonds indicate correlation with categorical parameters.

Analysis of the beta-diversity has shown significant differences in microbial communities among collected samples (Figure 8). Fitting of environmental parameters has identified salinity as being the major contributor to differences in bacterioplankton composition (Mantel test: r of 0.5469, p-value < 0.001), other factors, i.e., temperature, pH, and oxygenation, on the other hand, did not significantly affect the community composition. Interestingly, while contributing to variability in microbial communities, salinity does not explain the major differences observed between samples from Tengiz and small lakes. ANOSIM test has shown that geographical classification itself had a significant influence over bacterioplankton composition (ANOSIM statistic R 0.492, and p-value of < 0.001).

5 DISCUSSION

The Tengiz-Korgalzhyn Lakes system of the Korgalzhyn Nature Reserve is an area of international importance. Lakes, represented by a wide range of mesohaline, oligohaline, and freshwater water bodies, and adjacent territories are inhabited by more than 100 protected and endemic species of animals and plants. Yet, despite its fundamental role in maintaining the ecological balance of the system, the composition of the bacterioplankton and its relationship with abiotic factors remains unrecounted in today's literature. The results of our study shed light on the biodiversity of bacterial species across different lakes and the role of salinity gradient in shaping the composition of these communities.

We have identified four main bacterial phyla to be present in all of the samples: *Proteobacteria*, *Bacteroidetes*, *Cyanobacteria*, and *Actinobacteria* (Figure 4). Based on the results of correlation analysis, several classes of the *Proteobacteria*, as the most abundant phylum, were shown to have a strong correlation with salinity gradient in both lake groups. As indicated in the literature (Lew *et al*, 2022; Laas *et al*, 2022; Wu *et al*, 2006), species of the *Betaproteobacteria* were found to be dominating in a limnetic (freshwater) and lower ranges of β-oligohaline environment. The abundance of the *Gammaproteobacteria* species, on the other hand, increased with salinity, which shows their preference towards oligohaline environments. Data regarding *Alphaproteobacteria* and *Deltaproteobacteria* were inconclusive, potentially due to the narrow salinity range of the lakes.

The overall alpha-diversity of the microbial communities is strongly correlated with the salinity gradient, which we can observe across the Small Lakes system (Figure 7). Within the salinity range of 0.21 - 2.71%, higher salinity lakes are represented by more diverse yet disproportionate communities: the total number of unique species increases with salinity, while the evenness of their relative abundance goes down. We anticipate that no correlation was observed for the Tengiz Lake due to the narrow salinity range of the represented sampling sites. A similar conclusion is suggested by the beta-diversity analysis. The spatial distribution of samples represents dissimilarity in the composition of bacterioplankton communities across the lakes. Although the large portion of the dissimilarity in microbiome diversity across the whole dataset can be explained by

salinity, it does not account for the major differences between samples taken from Tengiz Lake and smaller water bodies. Geographical classification of lakes into two groups, as a categorical parameter, is by itself a factor significantly affecting similarity (or dissimilarity) in the composition of the lakes' microbiome (Laas *et al*, 2022; Wang *et al*, 2015). This apparent difference might be explained by more dynamic population genetics of the isolated water bodies on the one hand and the overall intermixing in Tengiz Lake on the other.

As originally suggested by our hypothesis, we have confirmed the importance of the salinity gradient in shaping the composition of the microbial community. More general salinity-associated trends, such as the prevalence of *Betaproteobacteria* in freshwater and *Gammaproteobacteria* in oligohaline lakes, were observed consistently in both groups of water bodies, Tengiz and Small Lakes. It has also been observed that increased salinity favors more diverse yet imbalanced microbial communities, as shown in the example of small lakes. Despite all these complex relationships between bacterial diversity and salinity, its effect could not resolve the dissimilarity between Tengiz Lake and other small lakes. Indeed, microbiome composition in samples collected from Tengiz appeared to be more similar, or homogenous, when compared to the rest of the dataset.

Further research would require differential and functional analysis in order to investigate the relationship and co-occurrence between various bacterial species, as well as a correlation between functional groups represented in the sample, and patterns in abiotic parameters.

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7 APPENDICES

Supplementary Table 1. Physicochemical parameters water probes collected at Tengiz-Korgalzhyn Lakes

Sample	Temperature	Conductivity	pH	Oxygen (%)	Oxygen (mg/L)	TDS	Salinity
S1	25.90	62.50	8.07	54.00	5.50	31.20	2.97
S2	24.80	61.60	8.36	93.00	7.40	30.80	2.99
S3	27.20	48.60	8.52	143.00	11.20	23.90	1.93
S4	26.60	47.50	8.34	131.00	10.20	23.80	2.26
S5	26.70	50.20	8.39	81.70	6.20	25.10	2.39
S6	25.60	47.10	8.46	126.00	9.80	23.40	2.24
S8	26.80	48.30	8.56	140.00	10.50	24.10	2.28
S9	27.30	45.70	8.35	147.00	21.40	22.80	2.07
S10	24.40	44.10	8.25	87.00	10.10	22.10	2.05
S11	25.10	44.20	8.21	128.00	10.10	22.20	2.12
S12	26.50	78.60	8.30	128.00	9.80	39.30	3.73
S13	26.90	44.90	8.30	122.00	9.20	22.40	2.11
S14	26.10	43.10	8.31	116.00	9.20	21.50	2.04
S15	26.30	43.10	8.37	158.00	11.90	21.50	2.02
S16	26.10	43.20	8.32	110.00	8.60	21.60	2.00
S17	27.00	41.20	8.53	164.00	12.90	20.50	1.62
S18	26.00	40.20	8.49	163.00	12.70	21.10	1.79
S19	24.30	39.40	8.22	115.00	9.30	19.60	1.52
S20	24.20	37.90	8.40	120.00	9.60	19.10	1.43
S21	25.10	37.60	8.45	102.00	8.10	18.80	1.54
S22	25.70	36.70	8.47	98.00	7.90	18.30	1.43
T1	28.90	154.00	6.73	70.00	7.00	67.50	6.70
T2	26.40	19.34	8.52	79.00	NA	9.67	1.10
T3	28.40	42.90	7.37	30.00	NA	21.60	2.10
T4	29.70	42.30	8.22	112.00	NA	21.10	2.04

T5	26.10	26.20	8.62	136.00	NA	13.10	1.20
T6	33.40	71.71	9.15	203.00	NA	35.50	3.45
T7	26.40	126.20	8.59	134.00	NA	0.63	6.90
T8	27.50	125.80	8.09	103.00	NA	6.29	0.63
T10	21.30	54.60	9.35	171.00	13.20	27.20	2.71
T11	23.10	28.30	7.62	178.00	14.10	14.10	1.31
T12	24.30	24.80	7.90	123.00	9.90	12.40	1.11
T13	24.40	35.90	7.75	102.00	8.10	17.90	1.73
T14	26.80	23.60	8.46	160.00	12.20	11.70	1.08
T15	23.50	32.90	9.48	177.00	14.10	16.50	1.59
T16	22.30	19.00	8.28	143.00	11.20	9.51	1.01
T17	21.60	13.60	8.01	106.00	9.00	6.80	0.74
T18	28.80	317.80	7.04	99.00	6.70	158.6	NA
T19	22.60	35.70	8.96	115.00	9.60	17.90	1.64
T20	23.70	4.70	8.38	126.00	10.10	2.34	0.21
T21	23.70	13.60	NA	111.00	9.90	6.80	0.74
T22	22.10	6.10	8.52	128.00	10.40	3.08	0.31
T23	22.40	100.00	8.86	98.00	7.70	49.90	5.00

This is to confirm I have read and approved this thesis for submission within BIOL490/491 course

supervisor's signature

5 May 2023