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# **Ecology of ponds: anthropogenic and environmental effects on biodiversity**



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## **Abstract**

Ponds are a vital component of the freshwater ecosystem but have been understudied worldwide. The paucity of research is especially disturbing due to increasing pressure on freshwater ecosystems. Pond ecosystems are vulnerable due to anthropogenic activities and changing environmental conditions. Ponds are the most ubiquitous freshwater ecosystems found in the Auckland Region of New Zealand. Despite their abundance (occurring in all landscapes) and significant ecological role, there is a lack of knowledge on the ecology of pond ecosystems in New Zealand. Further, literature on the impact of land use/ land cover (LULC) and human population density on pond water quality and biodiversity is lacking globally. I studied aspects of ponds (natural and man-made) ecology in the Auckland region by assessing both abiotic and biotic factors in the summer and winter seasons. Specifically, my study focused on six aspects of the ecology of pond ecosystems: i) the relationship between water quality and LULC at multiple spatial scales across the seasons, ii) the temporal community composition of macroinvertebrates and the relationship between the communities and the abiotic factors, iii) the influence of anthropogenic activities (measured as human population density and as pond types or function) on the macroinvertebrate communities, iv) the phytoplankton communities in ponds across the seasons, v) the limiting nutrient(s) in periphyton biomass in ponds using an *in-situ* experiment, and finally, vi) a single case study tracking a newly formed pond in a restoration area and development of its macroinvertebrate community over one year.

I sampled 50 ponds in the Auckland region across two seasons (summer and winter) to assess pond water quality (evaluated using seven physicochemical variables: pH, percentage dissolved oxygen '% DO', conductivity, temperature, total dissolved solids 'TDS', salinity, nitrate, phosphate and ammoniacal nitrogen). My aim was to understand the relationship between water quality and the landscape features (physical variables and LULC types: 'forest, grass, and impervious surface') at multiple spatial scales (10m, 100m, 500m and pond catchment) from the pond. I found a significant seasonal difference in the water quality of ponds. All the water quality variables measured apart from ammoniacal nitrogen were higher in summer, suggesting that the water was of lower quality at that time. Also, the effect of LULC on the physicochemical water quality parameters varied spatially and seasonally. LULC at the catchment and 500m scale

influenced the water quality in winter, while the LULC at 100m affected the water quality in summer. The results highlight the critical and complex role of environmental factors and LULC in determining the water quality in ponds.

I assessed the macroinvertebrate community compositions and water quality in 12 ponds across two seasons for two years. I found an average of 15 macroinvertebrate taxa in focal ponds. Insects were the most diverse group found, although Crustacea were most abundant. The community composition of the macroinvertebrates varied among ponds and varied across seasons and years. Macroinvertebrates were more abundant, and the community was more diverse in summer. The % DO in the ponds was negatively correlated to the macroinvertebrate abundance. My results suggest that macroinvertebrates and water quality in ponds are temporally variable.

I assessed the influence of anthropogenic activities on the macroinvertebrate communities of 11 ponds. Four categories of human population density were used (rural, small urban, large urban, and major urban; in order of increasing human population) to group ponds for analysis. By applying taxonomic and trait-based (functional feeding groups) approaches, I found that high human population density was negatively associated with the macroinvertebrate communities, especially in summer. Ponds in rural areas had the highest diversity of macroinvertebrates and the highest composition of functional foraging group relative to the other areas assessed. This finding suggests that ponds in rural areas had the lowest anthropogenic impact. Ornamental ponds were rich in macroinvertebrates, primarily due to a comparatively more heterogeneous pond habitat.

I sampled and analysed the phytoplankton community composition of 12 ponds in summer and winter. Overall, the communities were dominated by taxa in the phylum Chlorophyta (green algae) and class Bacillariophyceae (Diatoms). Although I did find seasonal differences in the phytoplankton communities, these were influenced by temperature and conductivity. In addition, ponds within areas of denser human populations had the most motile diatoms in summer, suggesting high siltation. Despite being moderately polluted, these results show that all ponds generally had healthy phytoplankton communities. Furthermore, by using an *in-situ* nutrient diffusion experiment, I found that nitrogen is likely to be the limiting nutrient for periphyton growth in ponds.

Finally, I sampled and monitored a newly created and a nearby established pond for a year to obtain insights into the progression of a pond from creation into a functional ecosystem using macroinvertebrates as indicators. I found that Crustaceans were the first to colonise the new pond. The macroinvertebrate community in the new pond was more taxonomically distinct than the established pond at the end of the first year of its creation. Shannon Weiner's diversity index was similar between the ponds, and environmental factors influenced the macroinvertebrate abundance. My results indicate that new ponds can create new habitats and boost local freshwater biodiversity.

By combining water quality analyses, and detailed biodiversity assessment, my thesis demonstrates that pond ecosystems support a high diversity of macroinvertebrates and phytoplankton. Environmental variables, LULC, and human population density influence the biodiversity in ponds, and the extent, relationship, and impact of these are complex and vary seasonally. My study provides new baseline information and valuable insights for future research on pond ecosystems in New Zealand.

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## Table of Content

Abstract.....	i
Acknowledgements .....	iv
Table of Content .....	vii
List of Appendices.....	xii
List of Figures.....	xiii
List of Tables .....	xiv
<b>Chapter 1 Introduction.....</b>	<b>1</b>
1.1 Introduction .....	2
1.1.1 Status of freshwater biodiversity in New Zealand .....	3
1.1.2 Pond ecosystems, significance, and diversity .....	4
1.1.3 Water quality; its drivers and importance for biodiversity.....	5
1.1.4 Anthropogenic influences on the pond ecosystems.....	8
1.1.5 Seasonality in pond ecosystems .....	9
1.1.6 History of research and status of ponds in New Zealand .....	10
1.1.7 How is the ecological status of a pond assessed, and what is the current knowledge gap? .....	11
1.1.8 Employing a holistic method for assessing ponds biodiversity across a varied landscape	13
1.1.9 Thesis aims .....	14
1.2 Thesis outline.....	15
1.3 References .....	17
<b>Chapter 2 Pond, their catchment, and spatio-temporal variations in water quality in an urbanised region.....</b>	<b>25</b>
2.1 Abstract.....	26
2.2 Introduction .....	27
2.2.1 Aims and hypotheses .....	30
2.3 Methods and analyses.....	31
2.3.1 Study Area .....	31
2.3.2 Data collection .....	33
2.2.3 Data Analysis .....	35
2.4 Results.....	36
2.4.1 The relationship between pond and catchment areas.....	36
2.4.2 Seasonal comparison of the physicochemical variables between the seasons.....	36
2.4.3 Seasonal variations in the physicochemical water quality variables (Multivariate approach) .....	37
2.4.4 Relationship between physicochemical water quality variables.....	39
2.4.5 Effect of LULC at multiple spatial scales on water quality variables.....	40
2.4.6 The relationships between the physical, LULC and physicochemical variables at multiple scales.....	42
2.4.7 The effect of LULC and physical parameters on pond water quality across the seasons..	44
2.4.8 The physicochemical water quality variables of ponds along the urban-rural gradient ...	47
2.4.9 Land use and land cover along the urban-rural gradient .....	49
2.5 Discussion .....	50
2.5.1 Relationship between pond area and catchment area.....	50
2.5.2 Seasonal and spatial variation in water quality .....	50

2.5.3 Land use/land cover and water quality .....	52
2.5.4 The effect of human population density on physicochemical water quality .....	54
<i>Implications for freshwater systems management</i> .....	55
2.6 Conclusion .....	56
2.7 References.....	57
<b>Chapter 3 Macroinvertebrate communities in ponds.....</b>	<b>63</b>
3.1 Abstract .....	64
3.2 Introduction .....	65
3.2.1 Macroinvertebrate community composition in ponds .....	65
3.2.2 Factors that influence macroinvertebrate compositions in ponds .....	66
3.2.3 State of macroinvertebrates in New Zealand freshwater ecosystems and the knowledge gap .....	66
3.2.4 Aims and hypotheses.....	67
3.3 Methods and analyses .....	68
3.3.1 Methods .....	68
3.3.1.1 Sorting and identification .....	69
3.3.2 Data analyses.....	70
3.3.2.1: Inventory of macroinvertebrates in ponds and temporal variations.....	70
3.3.2.2 Relationship between biotic (macroinvertebrate) and abiotic factors (physicochemical and physical parameters) .....	70
3.4 Results .....	71
3.4.1 Section 1: Inventory and temporal variation of macroinvertebrates in ponds.....	71
3.4.1.1 The abundance of macroinvertebrates in ponds .....	71
3.4.1.2 The abundance of dominant macroinvertebrates in ponds.....	72
3.4.1.3 Distribution of macroinvertebrates in ponds in Auckland .....	75
Biodiversity in individual ponds.....	76
3.4.1.4 Temporal trend in macroinvertebrate community .....	76
3.4.1.5 Annual variations in macroinvertebrates communities in summer.....	77
3.4.1.6 Annual variations in macroinvertebrates communities in winter.....	78
3.4.1.7 Biodiversity indices (Margalef's richness, Piloni's evenness and Shannon Weiner) .....	79
3.4.2 Section 2: Water quality in ponds .....	79
3.4.2.1 Multivariate analyses of physicochemical water variables .....	80
3.4.2.2 Drivers of physicochemical water quality in summer .....	80
3.4.2.3 Temporal drivers of physicochemical water quality .....	81
3.4.2.4 Relationship between macroinvertebrate community composition and physicochemical water quality.....	82
3.4.2.5 Relationship between water quality variables and macroinvertebrate biodiversity indices .....	83
3.4.2.6 Physical parameters around the ponds.....	84
3.4.2.7 Relationship between physical parameters of the ponds and biodiversity .....	85
3.4.2.8 Physicochemical, physical and land use, land cover (LULC) parameters and macroinvertebrate community composition .....	86
3.5 Discussion.....	88
3.5.1 Macroinvertebrate community composition and distribution .....	88
3.5.2 Temporal variation in macroinvertebrate composition .....	90
3.5.3 Relationship between macroinvertebrates and physicochemical water quality and physical parameters of the ponds.....	92
<i>Implications of the findings for macroinvertebrate conservation</i> .....	94
3.6 Conclusion .....	94
3.7 References.....	96
<b>Chapter 4 Anthropogenic influence on macroinvertebrate communities in ponds.....</b>	<b>103</b>

4.1 Abstract.....	104
4.2 Introduction.....	105
4.2.1 Macroinvertebrates as biological indicators.....	106
4.2.2 The use of the taxonomic approach.....	106
4.2.3 The use of traits approach.....	107
4.2.4 Functional feeding groups and food availability.....	107
4.2.5 Taxonomic versus trait-based approach.....	108
4.2.6 Aims and hypotheses.....	109
4.3 Methods and analyses.....	110
4.4 Results.....	112
4.4.1 Section 1: Impact of human population density on the macroinvertebrates in the ponds (Taxonomic approach).....	112
4.4.1.1 Macroinvertebrate communities along the urban-rural gradient.....	112
4.4.1.2 Distribution of macroinvertebrates along the urban-rural gradient.....	114
4.4.1.3 Macroinvertebrate heterogeneity along the urban-rural gradient in summer.....	116
4.4.1.4 Macroinvertebrate heterogeneity along the urban-rural gradient in winter.....	117
4.4.1.5 Biodiversity along the urban-rural gradient.....	118
4.4.1.6 Physicochemical water quality variables along the urban-rural gradient.....	119
4.4.1.7 Water quality variables along the urban-rural gradient.....	121
4.4.2 Impact of human population density on macroinvertebrates composition (functional feeding group approach).....	121
4.4.2.1 Functional feeding group richness in the ponds.....	122
4.4.3 Section 2: Macroinvertebrates community in different types of pond.....	123
4.4.3.1 Macroinvertebrate communities in three different artificial pond types.....	123
4.5 Discussion.....	127
4.5.1 The effect of human population density.....	127
4.5.2 Functional feeding group.....	129
4.5.3 The macroinvertebrate community in three types of artificial ponds.....	130
<i>Implications of the findings for freshwater management</i> .....	132
4.6 Conclusion.....	133
4.7 References.....	134
<b>Chapter 5 Phytoplankton community composition and environmental correlates in ponds.....</b>	<b>139</b>
5.1 Abstract.....	140
5.2 Introduction.....	140
5.2.1 Types of algae based on habitat types.....	141
5.2.2 Classification of algae.....	141
5.2.3 Ecological significance of phytoplankton.....	146
5.2.4 Factors that affect phytoplankton community structure.....	147
5.2.5 Phytoplankton indices and metrics.....	148
5.2.6 Phytoplankton community in New Zealand freshwater ecosystems.....	149
5.2.7 Knowledge gaps.....	149
5.2.8 Aims and hypotheses.....	150
5.3 Methods and data analyses.....	151
5.4 Results.....	154
5.4.1 The phytoplankton community in 12 ponds.....	154
5.4.2 Seasonal occurrence and distribution of phytoplankton in ponds.....	158
5.4.3 Seasonal taxa heterogeneity.....	159
5.4.4 Community composition across the urban-rural gradient.....	161
5.4.5 Biodiversity along the urban-rural gradient.....	163

5.4.6 Physicochemical properties of pond water .....	164
5.4.7 Relationship between environmental factors and phytoplankton biodiversity indices in summer.....	165
5.4.8 Relationship between environmental factors and phytoplankton biodiversity indices in winter .....	166
5.4.9 Correlation between the abundance of phytoplankton phyla and physicochemical water quality variables.....	166
5.4.9.1 Relationship between phytoplankton groups and physicochemical water quality variables in summer .....	166
5.4.9.2 Relationship between phytoplankton groups and physicochemical water quality variables in winter .....	167
5.4.10 The relationship between water quality and phytoplankton community .....	168
5.5 Discussion.....	169
5.5.1 Seasonal differences in phytoplankton community.....	171
5.5.1.1 Seasonal shifts in phytoplankton composition.....	172
5.5.2 Effect of human population density on phytoplankton community.....	173
5.5.3 Effect of human population density on biotic metrics .....	174
5.5.4 Relationship between phytoplankton and water quality.....	174
5.5.5 Phytoplankton as indicators .....	175
<i>Implication of the finding for freshwater management</i> .....	176
5.6 Conclusion .....	177
5.7 References.....	178
<b>Chapter 6 Identifying the limiting nutrient(s) in ponds.....</b>	<b>183</b>
6.1 Abstract .....	184
6.2 Introduction .....	185
6.2.1 Aim and hypotheses .....	188
6.3 Method and analyses .....	188
6.3.1 Experimental design .....	188
6.3.2 Statistical analysis.....	192
6.4 Results.....	192
6.4.1 Overall effects of nutrient treatment.....	192
6.4.2 Comparisons within respective nutrient treatments .....	193
6.4.2.1 Nitrogen treatment .....	193
6.4.2.2 Phosphorus treatment .....	193
6.4.2.3 Nitrogen and phosphorus treatment .....	193
6.4.2.4 Control treatment .....	194
6.4.3 Comparing different concentrations levels of nutrients between treatments.....	195
6.5 Discussion.....	197
<i>Implications of the findings for nutrient management</i> .....	199
6.6 Conclusion .....	199
6.7 References.....	200
<b>Chapter 7 Establishment of a pond: monitoring the succession and dynamics in macroinvertebrate diversity of the Matuku link pond.....</b>	<b>203</b>
7.1 Abstract .....	204
7.2 Introduction .....	205
7.2.1 Aims and hypotheses.....	207
7.3 Methods and analyses .....	208
7.3.1 Study area - History of the Matuku Link Reserve.....	208
7.3.1.1 Description of the ponds .....	209
7.3.2 Methods: data collection.....	211

7.3.2.1 Data preparation.....	212
7.3.3 Data analyses .....	212
7.4 Results.....	213
7.4.1 Physicochemical water quality parameters.....	213
7.4.2 Temporal trends in physicochemical water quality parameters .....	215
7.4.3 Temporal variation in taxa abundance .....	216
7.4.4 Temporal dynamics of macroinvertebrates in the ponds.....	218
7.4.5 Abundant taxa in the ponds.....	218
7.4.6 Macroinvertebrates community structure .....	219
7.4.7 Insect diversity in the ponds .....	220
7.4.8 The relative abundance of non-insect taxa .....	221
7.4.9 Biodiversity measures .....	222
7.5 Discussion .....	223
7.5.1 Water quality trends .....	223
7.5.2 Macroinvertebrate trends and dynamics in the ponds .....	224
7.5.3 Macroinvertebrate colonisation and succession in the new pond.....	226
<i>Implications of the findings</i> .....	227
7.6 Conclusion .....	228
7.7 References .....	229
<b>Chapter 8 Conclusions.....</b>	<b>253</b>
8.1 A holistic assessment of the pond ecosystem.....	234
8.1.1 The influence of abiotic factors on the pond ecosystems .....	235
8.1.2 The biotic communities of the pond ecosystems .....	236
8.1.3 Interaction between the biotic and abiotic parameters in pond ecosystems.....	238
8.1.4 Anthropogenic impacts on pond ecosystems.....	239
8.2 Recommendations for future research .....	241
8.3 References .....	243
APPENDICES.....	249

## List of Appendices

Appendix A: Section 1 Methods for nutrients test.....	249
Appendix A: Table 1 Spearman correlation between the physicochemical variables in summer and winter.....	250
Appendix A: Table 2 Results of PERMANOVA model testing for differences in water quality along the urban-rural gradient.....	250
Appendix A: Table 3 Range of nutrients concentrations in ponds (this study), rivers and lakes in New Zealand.....	251
Appendix B: Table 1 Physical and geographical features of the 12 ponds sampled.....	252
Appendix B: Table 2 Transformations carried out on physicochemical water quality variables in the ponds.....	253
Appendix B: Figure 1 Pictures of macroinvertebrates sampled from the ponds.....	253
Appendix B: Figure 2 Draftsman plot of the correlations between physicochemical variables in each sampling period.....	254
Appendix B: Figure 3 Linear regression plot of biodiversity indices and physicochemical water qualities in summer 2018. p-values are reported.....	254
Appendix B: Table 3 BIOENV results.....	255
Appendix C: Table 1 SIMPER results for summer showing the distinguishing taxa among different areas along the urban-rural gradient. ....	255
Appendix C: Table 2 SIMPER results for winter showing the distinguishing taxa among different areas along the urban-rural gradient.....	256
Appendix D: Table 1 Abundance of the major taxonomic groups in phytoplankton phylum...257	
Appendix D: Table 2 Spearman correlation between the ten most important genera and the physicochemical water quality parameters in summer and winter.....	258
Appendix D: Figure 1a A picture of <i>Pleurotaenium</i> (Chlorophyta) taken under magnification x1000.....	258
Appendix D: Figure 1b A photo of <i>Pinnularia</i> (Ochrophyta) taken under magnification x1000.....	259
Appendix D: Figure 1c A picture of <i>Oscillatoria</i> (Cyanophyta) taken under magnification x1000.....	259
Appendix D: Figure 1d A picture of <i>Euglena</i> (Euglenophyta) taken under magnification x1000.....	260
Appendix E: Section 1 Protocols for Preparation of Nutrient Diffusing Substrates (NDS).....	260
Appendix F: Figure 1 Pictures of the new pond on each sampling period.....	261
Appendix F: Figure 2 Pictures of the established pond on each sampling period.....	262
Appendix F: Figure 3 A scatter plot of spearman correlation of physicochemical water quality variables in both ponds.....	262
Appendix F: Table 1 Simper results between the established and new pond.....	263

## List of Figures

Figure 1.1 A diagram showing the environmental factors that affect water quality and pond biodiversity.....	6
Figure 1.2 A diagram of the structure of the thesis. ....	14
Figure 2.1 A map of Auckland showing the distribution of the 50 ponds sampled.....	33
Figure 2.2 Regression plot of the relationship between pond area (m <sup>2</sup> ) and catchment area (m <sup>2</sup> ) .....	36
Figure 2.3 An nMDS plot of the Euclidean distance based on normalised physicochemical water quality variables.....	38
Figure 2.4 Ordination diagrams of the physicochemical water quality variables and landscape features at four spatial scales in summer.....	45
Figure 2.5 Ordination diagrams of the physicochemical water quality variables and landscape features at four spatial scales in winter.....	46
Figure 2.6 Percentage of LULC (forest, grass, urban) type along the urban-rural gradient.....	49
Figure 3.1 Percentage composition of macroinvertebrate sub-phylum in the ponds.....	72
Figure 3.2 The frequency of the number of taxa occurring in the macroinvertebrate's samples.....	75
Figure 3.3 A stacked bar of the relative abundance of the taxonomic composition of macroinvertebrates in the 12 ponds. ....	76
Figure 3.4 Bar plots of the relative abundance of the macroinvertebrate groups.....	78
Figure 3.5 Linear regression plot of biodiversity indices.....	84
Figure 3.6 Variation in the macroinvertebrate community explained by abiotic factors at different scales in a: 2018 and b: 2019.....	87
Figure 4.1 A relative percentage abundance of the macroinvertebrate groups in ponds along the urban-rural gradient in a: summer and b: winter.....	114
Figure 4.2 Percentage relative abundance of the common macroinvertebrate functional feeding group along the urban-rural gradient in summer.....	122
Figure 4.3 Bar plots of a: relative percentage taxa richness of macroinvertebrate groups and b: number of families in insect orders in stormwater, ornamental, and golf course ponds.....	124
Figure 5.1 Percentage contribution of different phyla to the phytoplankton community in ponds.....	154
Figure 5.2 The relative percentage abundance of phytoplankton phylum .....	157
Figure 5.3 Percentage contribution of phyla to the phytoplankton community in 12 ponds in a) summer and b) winter.....	158
Figure 5.4 An nMDS plot of phytoplankton communities in the ponds showing a) seasonal and site variations.....	159
Figure 5.5 Percentage relative abundance of phytoplankton phylum along the urban-rural gradient. ....	162
Figure 5.6 Biodiversity indices along the urban-rural gradient.....	163
Figure 6.1 A picture of the NDS setup at different stages.....	190
Figure 6.2. A diagram of the linear mixed model of treatments.....	194
Figure 6.3. The effect of different nutrient levels between treatments.....	195
Figure 7.1 A picture of the established pond taken on 17 April 2019.....	210
Figure 7.2 A picture of the new pond taken on 17 April 2019.....	211
Figure 7.3 An nMDS plot of the established and new ponds' water quality.....	214
Figure 7.4 Temporal changes in physicochemical water quality variables.....	216

Figure 7.5 Temporal trend of macroinvertebrates abundances.....217  
Figure 7.6 Abundance  $\pm$  SE of the five most common taxa in the new and established pond.....218  
Figure 7.7 An nMDS plot of macroinvertebrates abundance.....219  
Figure 7.8 Relative abundance of macroinvertebrate community structure in (a) established pond and (b) new pond.....220  
Figure 7.9 Temporal trend of the relative abundance of five insect orders from the (a) established pond and (b) new pond.....221  
Figure 7.10 Temporal trend of the relative abundance of non-insect taxa.....222  
Figure 7.11 Temporal trend of biodiversity indices.....223

## List of Tables

Table 2.1 A summary of the physicochemical variables in summer and winter.....37  
Table 2.2 A summary table of PCA loadings of the physicochemical variables in summer and winter.....39  
Table 2.3 A summary table of the physical and landcover parameters of the ponds.....40  
Table 2.4 Spearman correlation's rank between physicochemical variables and LULC at the multiple spatial scales in summer and winter.....41  
Table 2.5 Predictive ability (adjusted  $R^2$ ) for the multiple linear regression (MLR) models at the four spatial scales for each water physicochemical variable in summer and winter.....43  
Table 2.6 A summary table of the physicochemical variables along the urban-rural gradient in summer and winter.....48  
Table 3.1 Results of permutational multivariate analysis of variance (PERMANOVA) of the macroinvertebrate community.....77  
Table 3.2 Temporal physicochemical variables of the ponds in 2018 and 2019 in summer and winter.....80  
Table 3.3 Loadings of physicochemical variables in principal component axes in summer (2018 and 2019) and winter (2018 and 2019) .....81  
Table 3.4 Results of DISTLM for summer (2018 and 2019) and winter (2018 and 2019).....82  
Table 3.5 Physical parameters of the ponds sampled.....85  
Table 4.1 Description of taxa numbers, similarities and differences in macroinvertebrate compositions and abundance along the urban-rural gradient in summer and winter.....113  
Table 4.2 Results of permutational multivariate analysis of variance (PERMANOVA) of the macroinvertebrate community along the urban-rural gradient.....115  
Table 4.3 An ANOSIM pairwise result of the macroinvertebrate community along the urban-rural gradient in summer and winter.....115  
Table 4.4 SIMPER result of taxa that contributed to 70% of the abundance of macroinvertebrates sampled along the urban-rural gradient in summer.....116  
Table 4.5 Result of SIMPER of taxa that contributed to 70% of the abundance of macroinvertebrates sampled along the urban-rural gradient in winter.....118  
Table 4.6 Alpha and Gamma diversity  $\pm$  standard deviation along the urban-rural gradient in summer and winter.....118  
Table 4.7 Physicochemical water quality variables along the urban-rural gradient in the summer and winter.....120

Table 4.8 Results of permutational multivariate analysis of variance (PERMANOVA) of the water quality along the urban-rural.....	121
Table 4.9 Taxa richness of collector gatherer and predators in the ponds along the urban-rural gradient.....	123
Table 4.10 Physicochemical water quality parameters of the ponds in summer and winter....	126
Table 4.11 Physical features of the ponds sampled. Average values are reported with '±' standard deviation. In addition, the result of the ANOVA test is reported.....	126
Table 5.1 Characteristics of algal groups .....	142
Table 5.2 SIMPER results showing the characteristic taxa for each season. The genera contribute to 70% of the community.....	160
Table 5.3 Seasonal phytoplankton biodiversity indices in the ponds.....	161
Table 5.4 Occurrence of phytoplankton metrics in the ponds along the urban-rural gradient in summer and winter.....	164
Table 5.5 Physicochemical water quality variables in summer and winter.....	164
Table 5.6 Relationships between summer and winter phytoplankton biodiversity indices and environmental variables.....	165
Table 5.7 The spearman correlation between phytoplankton phyla and physicochemical water quality in summer and winter.....	167
Table 5.8 Coefficients in the linear combinations of physicochemical variables making up the principal components in summer and winter.....	169
Table 6.1 A summary of the initial nutrient in the ponds and concentrations used in the NDS.....	189
Table 6.2 A grid showing how the NDS plates were arranged on the tray.....	190
Table 6.3 Pairwise comparison between different nutrient levels between treatments.....	195
Table 7.1 A summary of physicochemical water quality parameters of the new and established pond.....	214
Table 7.2 A comparison of the macroinvertebrate composition in the new and established ponds.....	217



# Chapter 1

## Introduction



## 1.1 Introduction

Freshwater environments provide valuable ecosystem services such as primary productivity, biodiversity, nutrient cycling, carbon sequestration and recreation (Dudgeon 2010). Globally, freshwater ecosystems are faced with the highest rate of loss resulting from impacts of human activities such as overexploitation, pollution, habitat destruction and degradation (Vörösmarty *et al.* 2010). Issues relating to freshwater quality that affect biodiversity are among the greatest environmental challenges currently facing New Zealand (Weeks *et al.* 2016). In New Zealand, nearly 65 freshwater invertebrates out of over 638 species are at risk of extinction, making New Zealand a lead contributor to the world's endangered and threatened freshwater species diversity (Weeks *et al.* 2016). Research and conservation of freshwater ecosystems in New Zealand are currently focused on streams, rivers, and lakes (Collier 2014; Collier *et al.* 2009; Downs *et al.* 2008; Galbraith and Burns 2010; Greenwood *et al.* 2016; Schowe and Harding 2014).

Like many countries, 85% of New Zealand's population reside in urban areas, although urban lands cover 0.8% of the total land area (Dudley *et al.* 2020; Ministry for the Environment and Stats NZ 2017; Ministry for the Environment and Stats NZ 2019). This high urban population density results in stress on the freshwater ecosystems. One of the primary challenges faced by freshwater managers is stopping or reversing water quality deterioration related to Land Use/Land Cover (LULC) and human population density (Larned *et al.* 2020; Maxted *et al.* 2005). Policies formulated to protect freshwater ecosystems mainly focus on the economic value of water quality, usually to the disadvantage of aspects of the biodiversity that inhabits these systems (Weeks *et al.* 2016). This neglect notwithstanding, lotic (flowing) systems are usually of higher water quality than lentic (still) systems (Downs *et al.* 2008). To maintain the integrity of freshwater ecosystems, it is vital to manage both the lotic and lentic ecosystems holistically. The regional councils in New Zealand monitor aquatic ecosystems, principally lotic systems, to evaluate their integrity. However, in monitoring lentic systems, the focus has mainly been on larger bodies of water such as lakes, with little attention given to ponds (defined as a water body either permanent or ephemeral and occupying an area between 1m<sup>2</sup> and 2ha (Biggs *et al.* 2005; Drake *et al.* 2011; Schallenberg *et al.* 2011).

Ponds are important freshwater habitats that in some regions of the world support more uncommon aquatic macroinvertebrates than other freshwater ecosystems (Jooste *et al.* 2020;

Oertli 2018). Yet, they are one of the most vulnerable ecosystems with respect to the impacts of climate change and anthropogenic activities due to their small size. In spite of this, research on the biodiversity of ponds and how different LULC types affect these ecosystems has received little attention either globally or in New Zealand (Greig and Galatowitsch 2016; Oertli 2018). This limited research makes the ecological basis for the conservation of ponds weak (but see Hill and Wood 2014; Thornhill *et al.* 2017, Wissinger *et al.* 2009). Therefore, it is imperative to understand the impact of LULC on pond ecosystems to protect and improve their water quality.

To develop conservation strategies for an ecosystem and achieve conservation goals, it is important to study and determine the biodiversity within the ecosystem and the relationship between the abiotic factors and the biotic community. My thesis i) investigates the water quality and biodiversity in ponds, ii) assesses the relationship between water quality and biodiversity, and iii) determines the spatiotemporal variability in pond ecosystems in an urbanised landscape. A clearer understanding of the pond ecosystems will inform management decisions to derive the maximum benefits (economic, conservation, recreational) from ponds and mitigate the challenges faced by freshwater ecosystems.

### **1.1.1 Status of freshwater biodiversity in New Zealand**

Freshwater ecosystems play a vital role in the economy, culture, and livelihood of indigenous people in many countries, including New Zealand (Noble *et al.* 2016; Quinn 2009; Weeks *et al.* 2016). Freshwater ecosystems also support a diversity of biota and have high recreational value (Ballantine & Davies-Colley 2014). The freshwater biodiversity of New Zealand includes 66 fish species, of which half are endemic, a minimum of 675 endemic invertebrate species (including about 447 insect species and 78 threatened species), about 34 bird species and 38 endemic plants species (Collier 1993; Drinan *et al.* 2020; Weeks *et al.* 2016). While habitat loss has severe impacts on all ecosystems, freshwater ecosystems in New Zealand have been affected more directly by anthropogenic activities than the marine and terrestrial ecosystems (Joy 2015; Robertson 2016).

The water quality and biodiversity of most freshwater ecosystems in New Zealand have rapidly declined, particularly over the past four decades (Elston *et al.* 2015; Gadd *et al.* 2020; Weeks *et al.* 2016). The decline in the quality of freshwater systems elicits complex responses from the biotic community within these ecosystems. Anthropogenic activities that cause this deterioration include agriculture intensification, forestry, and mining (Cullen *et al.* 2006; Weeks

*et al.* 2016). The others are urbanization (Ballantine and Davies-Colley 2014; Collier 1993; Elston *et al.* 2015; Gadd *et al.* 2020), the introduction of exotic and invasive species (Leathwick *et al.* 2016), and climate change (Mantyka-Pringle *et al.* 2016). The decline (the causes and how to mitigate them) in the quality of freshwater ecosystems has received much attention from many stakeholders in the environment sector (Salmond *et al.* 2014). Recently a policy document released by the New Zealand Ministry for the Environment seeks to make 90% of freshwater bodies (rivers and lakes) suitable for human contact (i.e., improving water quality and reducing *Escherichia coli* and cyanobacteria concentrations) by 2040 (Ministry for the Environment 2020).

Nonetheless, studies have shown that lotic systems usually have higher water quality than lentic systems (Downs *et al.* 2008). The regional councils in New Zealand monitor aquatic ecosystems, predominantly lotic systems, to evaluate their integrity. In monitoring lentic systems, the focus has mainly been on larger ones (lakes), with little attention to ponds (Schallenberg *et al.* 2011).

### **1.1.2 Pond ecosystems, significance, and diversity**

Ponds serve as habitats and sometimes act as refugia for rare species not found in other freshwater bodies (Drinan *et al.* 2020; Epele and Miserendino 2016; Maxted *et al.* 2005; Naselli-Flores and Padisak 2016; Oertli *et al.* 2005). Ponds in New Zealand contain invertebrates that are a major food source for water birds, such as rails (Sanders 2000). Ponds also serve as stepping stones for frogs, enhancing their movements across different ponds (Howell *et al.* 2020). Ponds are not only important as wildlife habitats; they also provide ecosystem services such as recreation, mineral extraction, nutrient cycling, agriculture, and water management. They have significant aesthetic, ecological, geochemical, cultural, and economic value (Hassall 2014; Maxted *et al.* 2005; Mueller *et al.*, 2016; Wood *et al.* 2003). Furthermore, microbial communities in ponds are useful in bioremediation (breaking down pollutants) (Hassall 2014).

Because they are responsive to environmental variation, ponds are ecosystems that give early warning signs about climate change when studied over time (Epele and Miserendino 2016; Oertli *et al.* 2008). As a consequence of their relatively small surface area, ponds are vulnerable to climate change and species loss. Therefore, quantifying the biodiversity in ponds is important for managers to develop a comprehensive approach to freshwater conservation (Naselli-Flores *et al.* 2016; Oertli *et al.* 2014). Ponds are considered discontinuous habitats, and due to their patchy nature, any land management activity undertaken close to them will likely impact the

pond ecosystem (Naselli-Flores *et al.* 2016). Any changes in land use activities may lead to considerable pressure on pond biodiversity, threatening their viability (Naselli-Flores *et al.* 2016; Kuczynska-Kippen and Joniak 2016).

### **1.1.3 Water quality; its drivers and importance for biodiversity**

Water quality is defined as "the measure of the suitability of water for supporting a range of water values, including habitat for aquatic life and human use, including recreation" (Davies-Colley 2013; Johnson *et al.* 1997). In turn, the water quality of a pond is influenced by its size (area and depth) and local landscape features (Blicharska *et al.* 2016; Chiandret and Xenopoulos 2016; Nobre *et al.* 2020; Novikmec *et al.* 2016). Therefore, species diversity (abundance and richness) and population structures in ponds are influenced by the interactions between prevailing physical, biological, and chemical constituents of the pond (Oertli *et al.* 2005). The physical conditions of a pond, such as altitude, geography, connectivity or proximity to other ponds or aquatic systems, surrounding land use type and size, affect the biological community by influencing biogeochemical and ecological processes (Figure 1.1) (Blicharska *et al.* 2016; Eden 2016; Hassall *et al.* 2011; Steele and Heffernan 2014). Different taxa may also respond differently to local conditions, further influencing biodiversity (Batzer *et al.* 2004). There are few studies that have combined the effects of physical, chemical, and biological parameters on a pond's biodiversity at a large (regional) scale (Briers 2014; Hill *et al.* 2016). Most studies have only considered the effect of individual parameters such as acidity or hydroperiod on species diversity (Biggs *et al.* 2005; Hassall *et al.* 2011). My study combines the quantification of water quality and landscape features (LULC and physical parameters) to understand their interactive effects on pond biodiversity.

In general, freshwater studies describe freshwater quality for ecological purposes (to support the biotic community) by measuring eight key variables (percentage dissolved oxygen, acidity, temperature, conductivity, TDS, salinity, phosphates, and nitrates) (Novikmec *et al.* 2016). The drivers of these water quality variables and their significance to biodiversity in ponds are explained below.

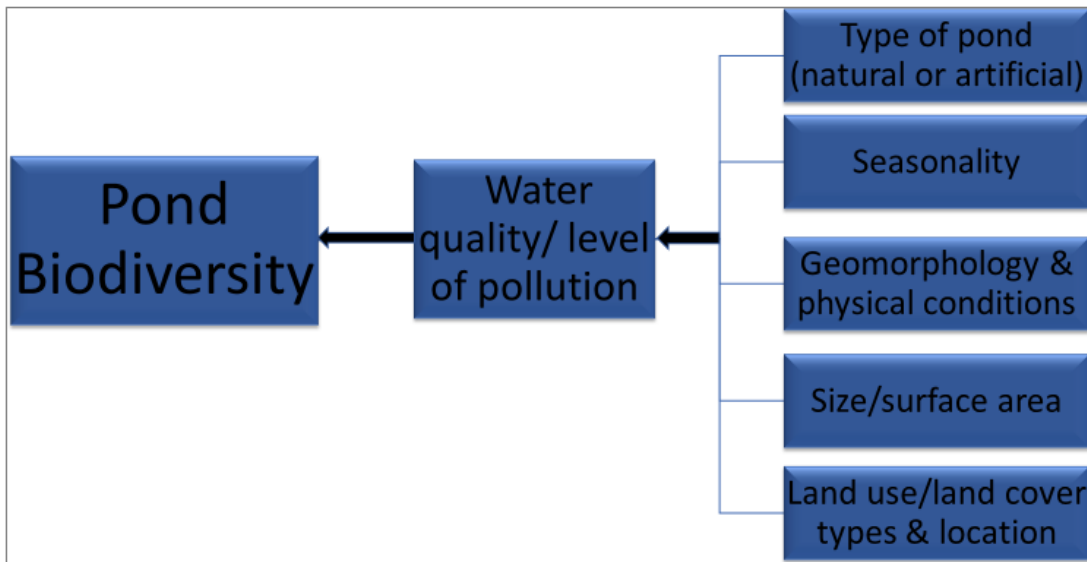


Figure 1.1 A diagram showing the environmental factors that affect water quality and pond biodiversity.

## pH

The pH of an aquatic ecosystem is partly determined by the underlying geology. For instance, ponds in areas of limestone have high pH values (Sharma *et al.* 2016). In contrast, increases in carbon dissolved dioxide, a by-product of cellular respiration, can reduce pH while photosynthesis uses up carbon dioxide, hence increasing pH. The pH of a pond determines which species (macroinvertebrates and algae) it can support (Hassall *et al.* 2011). For example, the algae, *Chlamydomonas*, *Cosmarium*, *Eunotia* survive in extremely acidic water (pH < 2) (Sabater *et al.* 2016). pH has a major influence on freshwater invertebrate community diversity in New Zealand (Suren *et al.* 2010). For instance, macroinvertebrate richness increases as pH increases (Hassall and Anderson 2015). Nonetheless, rare species have been recorded in acidic and alkaline ponds (Biggs *et al.* 2005). Therefore, the dominance of specific taxa can be indicative of the pH of the ecosystem.

## Temperature

Different organisms vary in their thermal tolerance. The temperature of the water is a key factor in determining the type of species that occur in a pond because temperature regulates the physiological processes (Smith *et al.* 2016). It also influences the distribution of macroinvertebrates and phytoplankton in the water column of the ponds (Barman and Gupta

2015; Orr and Buchwalter 2020; Saeiam *et al.* 2020; Yusuf 2020). High temperature increases photosynthesis and the diversity of primary producers in a pond, but temperatures over the tolerance level of species are lethal. Whilst Bacillariophyceae (diatoms) dominate in temperatures between 20°C – 25°C, Chlorophyta (green algae) usually abound at 30°C – 35°C (Smith *et al.* 2016). A sudden increase or decline in temperature can cause some species to undergo hibernation because it regulates physiological processes (Smith *et al.* 2016).

### **Conductivity**

Conductivity is a measure of the capacity of electric current to pass through water. It indicates the concentration of dissolved ions (e.g., nitrate, chloride, iron, sodium) in the water. Conductivity is affected by temperature, rain, and geology. Increase in temperature increases conductivity. Fresh rain has low ionic concentration and therefore decreases conductivity. High conductivity may be an indicator of pollution entering a pond. For example, runoff that contains high nutrients loads and sediments increases conductivity. Sea spray can increase conductivity in ponds close to the sea (Burns *et al.* 1984). An increase in conductivity reduces the diversity of some invertebrate taxa in ponds (Hassall and Anderson 2015).

### **Percentage dissolved oxygen**

Percentage dissolved oxygen is the percentage measure of oxygen present (oxygen saturation) in the water and a significant water quality parameter. The amount of oxygen accessible depends on oxygen inputs and outputs (Carey 2005). Aeration, photosynthesis, and anaerobic respiration are the sources of oxygen input in aquatic systems (Carey 2005; Tyagi *et al.* 2020; Ustaoglu *et al.* 2020). Oxygen is depleted through aerobic respiration and decomposition (Carey 2005; Swistock 2010). Warmer water tends to have lower % DO levels as higher temperatures reduce the solubility of oxygen (Carey 2005). Also, % DO levels are lowest in the early morning before sunrise and highest in the afternoon due to photosynthesis (Carey 2005). Additional factors that affect % DO levels in ponds are shade, flow, and organic matter input. Ponds in areas with closed vegetation are expected to have lower temperatures and have high % DO levels than ponds in areas of open or no vegetation (Carey 2005). Dissolved oxygen directly affects the species richness and species diversity in an aquatic ecosystem. Generally, there is a positive relationship between higher % DO levels and measures of species richness for both fish and macroinvertebrate communities (Carey 2005; Briers 2014).

## Nitrogen

Nitrogen is a naturally occurring chemical element in the environment that forms a critical component of the cells of fauna and flora but can also be a pollutant in high concentrations (Larned *et al.* 2016). Nitrogen is usually present in aquatic ecosystems in an inorganic state. Nitrogen occurs in the most stable state as nitrate ( $\text{NO}_3^-$ ) or in the unstable state as ammonia ( $\text{NH}_4$ ) and nitrite ( $\text{NO}_2^-$ ). An increase in nitrogen level can increase primary productivity, which can cause algal blooms and ammonia gas production, which can be toxic to biodiversity (Snelder *et al.* 2020; Sowa *et al.* 2020). High nitrogen concentration is an indication of contamination of the ecosystem (Larned *et al.* 2016). Sources of nitrogen in ponds include stormwater runoff as nitrates, organic matter as nitrogen and eroding sediments as ammonia (Carey 2005). Seasons associated with high precipitation have been associated with nitrogen levels (for example, during winter in New Zealand) due to leaching (Chaichana *et al.* 2011). Anthropogenic activities like fertiliser application, sewage production and release, commercial and industrial waste production, and vehicular emissions increase nitrogen concentration in aquatic systems in agricultural and urban areas (Chang 2005; Larned *et al.* 2016; Burns *et al.* 1984).

## Phosphorus

Phosphorus is an element that occurs naturally and is an essential nutrient for the cell development of aquatic organisms. Phosphorus occurs as either soluble organic phosphorus or phosphate. Sources of phosphorus include fertiliser, faecal matter, and waste from pets, especially dogs and waterfowls (Hobbie *et al.* 2017). Therefore, high phosphorus levels are typically recorded in aquatic systems in urban areas (Hobbie *et al.* 2017). Excess phosphorus causes increased growth in plants which leads to eutrophication. When the amount of light penetration to phosphorus ratio increases in the aquatic ecosystem, phosphorus becomes limited due to increased carbon, reducing algal growth (Sabater *et al.* 2016; Sterner *et al.* 1997).

### 1.1.4 Anthropogenic influences on the pond ecosystems

The physicochemical conditions and the biological community of a pond are mainly determined by Land Use/Land Cover (LULC) within the catchment area and the local climate (Novikmec *et al.* 2016). An increasingly dominant land use type in New Zealand and worldwide are urban and agricultural landscapes. As urbanization intensifies, the shape, size and connectivity of ponds are altered, affecting the biological community (Hill *et al.* 2016; Manoj and Padhy 2015; Oertli 2018; Steele and Heffernan 2014). Additionally, the composition and concentration of human-

derived nutrients, such as phosphorus and nitrogen, are also highest in urban and agricultural areas, which negatively impact macroinvertebrate communities (Adrian *et al.* 2016; Atique and An 2020; Bhat *et al.* 2020; Gadd *et al.* 2020; Mesa *et al.* 2013; Ministry for the Environment and Stats NZ 2019; Snelder *et al.* 2020; Song *et al.* 2015; Usio *et al.* 2017). Despite this concern, there has been limited effort to quantify ponds' nutrient levels or even assess pond water quality (Greig and Galatowitsch 2016; Snelder *et al.* 2020).

Further, although there is a high rate of freshwater species extinction, data on ponds' biodiversity are incomplete, especially in New Zealand (Weeks *et al.* 2016). Many ponds have recently been constructed in modified landscapes, predominantly urban areas, to prevent flooding and retain excessive nutrients from runoff. These artificial ponds also support biodiversity (Oertli 2018; Sun *et al.* 2019). Therefore, the lack of research on ponds represents a fundamental knowledge gap in freshwater systems, mainly due to the increase in freshwater habitat loss and degradation (Maxted *et al.* 2005). It is essential to assess the role of ponds in species conservation and how LULC and anthropogenic activities affect biodiversity. Such an assessment will be informative in developing appropriate conservation or restoration strategies.

### **1.1.5 Seasonality in pond ecosystems**

The New Zealand climate is characterised by four main seasons (winter, spring, summer, and autumn). However, the predictability of seasonality is low due to the high probability of monthly precipitation (Tonkin *et al.* 2017). Seasonality is an environmental feature that influences water quality and biodiversity in ponds by affecting the chemical composition and food availability (Desalegne 2018; García-Criado *et al.* 2017; Pratt and Chang 2012; Tonkin *et al.* 2017). Seasons characterised by high precipitation can increase individual chemical components in the water through runoff or soil percolation. In contrast, high precipitation can also reduce the concentration of nutrients due to dilution (Jeppesen *et al.* 2015). On the contrary, seasons characterised by low or no precipitation and high temperature lead to increased conductivity, nutrient load, and pH (Chowdhury and Husain 2020). Temperature, water volume, and photoperiod, which influence the hatching of macroinvertebrate eggs, are also seasonally variable (Boix *et al.* 2016). Hence seasons affect the composition and distribution of macroinvertebrate communities in ponds (Desalegne 2018; Jayawardana *et al.* 2017; Greig and Galatowitsch 2016; Kilroy *et al.* 2006).

Like macroinvertebrates, phytoplankton composition experiences high species turnover in many climates, usually associated with seasonality. For instance, a shift in dominant taxonomic groups such as Euglenophyta and Cyanophyta occurs with temperature increase (Saeiam *et al.* 2020; Yusuf 2020). Seasonal shifts in phytoplankton communities in New Zealand ponds has been rarely studied (Kuschel 2012), although shifts in phytoplankton community abundance in rivers have been reported (Lam 1981).

New Zealand macroinvertebrates show less seasonal variability in community structure compared to those in other temperate regions (Tonkin *et al.* 2017). The macroinvertebrate communities in New Zealand tend to be more nested (taxa present in a season is a subset of a more diversified community in a previous season) over time (Tonkin *et al.* 2017). The reduced seasonal variability is caused by the unpredictability of the climate and low organic input (due to the evergreen forest cover) into the freshwater ecosystems (Tonkin *et al.* 2017; Winterbourn *et al.* 1981). Additionally, biotic factors like predation pressure are influenced by seasonality (Baxter *et al.* 2004; Boix *et al.* 2016). Therefore, sampling in different seasons in an ecological study is vital to ascertain the importance of seasonal changes in environmental variables and the biological community (Zhang *et al.* 2019). This information is essential in developing management and conservation strategies suitable for all seasons.

### **1.1.6 History of research and status of ponds in New Zealand**

Published research work on pond ecosystems in New Zealand dates back to the 1960s (Stout 1964). Research on ponds has focused on the ecological patterns, such as the seasonal faunal diversity of single ponds and clusters of ponds adjacent to urban areas (Burns *et al.* 1984; Greig and Galatowitsch 2016). More recent studies, especially over the last two decades, have focused on biological controls of the biodiversity community structure (Greig *et al.* 2013; Kilroy *et al.* 2006; Suren *et al.* 2008; Wissinger *et al.* 2009). Others have assessed the evolution of life strategies that enable organisms to persist across disturbance gradients (Galatowitsch 2014). Also, the spread of invasive species in ponds has been studied (Leisnham *et al.* 2005; Parkes and Duggan 2012; Tanentzap *et al.* 2014). Specific aquatic community compositions, such as invertebrates (Blakely *et al.* 2005), ciliates and phytoplankton (Galbraith and Burns 2010), copepods (Jamieson 1986; 1988), fish and waterbirds (Garrett-Walker *et al.* 2020) and zooplankton have been assessed (Duggan *et al.* 2006; Le Quesne *et al.* 2021). Maxted *et al.* (2005) also carried out a study on ponds constructed on stream channels, but the study focused on the pond's effect on the stream biota. It was concluded that online ponds (ponds constructed

or located in the path of lotic systems that receive water inflow and release water in an outflow) negatively affect macroinvertebrate communities downstream due to the recorded low dissolved oxygen and high temperatures. However, none of these studies examined the effect of water quality on biodiversity in general.

Moreover, most New Zealand pond studies have been carried out in the South Island. For instance, a study on planktonic food web structure and how it is influenced by land use and water quality considered nine ponds and swamps in Otago (Burns and Galbraith 2007). In addition, a study on the effect of rotenone on macroinvertebrates was carried out by Blakely *et al.* (2005) in Motueka (South Island). However, the South Island has different climatic and environmental conditions, lentic nutrient concentrations, and biodiversity compared to the North Island (Cullen *et al.* 2006). In general, the scope and number of studies on ponds are limited relative to the importance and abundance of these ecosystems.

Finally, ponds have received little attention from environmental stakeholders such as freshwater ecologists, environmental managers, and policy makers (Galatowitsch and McIntosh 2016; Greig *et al.* 2013; Wissinger *et al.* 2009). A review of studies on ponds in New Zealand identified a gap in knowledge on the biodiversity of these specialised freshwater habitats (Greig and Galatowitsch 2016; Johnson and Rogers 2003). These gaps are largely related to the influence of LULC and other anthropogenic activities on ponds ecosystems (Greig and Galatowitsch 2016). This bias in research focus persists despite the increasing threats faced by pond ecosystems because ponds are often considered insignificant due to their small size and potential ephemeral nature (Hassall *et al.* 2012).

### **1.1.7 How is the ecological status of a pond assessed, and what is the current knowledge gap?**

Ponds support a network of organisms, including primary producers (phytoplankton, periphyton, and macrophytes), primary consumers (zooplankton, some invertebrates) and secondary consumers (fishes, amphibians, birds). All these organisms interact with the abiotic factors in transferring energy and nutrients (Hassall 2014). Until recently, only physical and chemical parameters were used to measure water quality and describe ponds' integrity (Özkundakci *et al.* 2014). These measures give a snapshot of the ecosystems current condition

without providing any information about previous conditions and how those conditions affect the biodiversity (Maxted *et al.* 2005).

Biological variables are often more efficient in assessing pond health because they are cost-effective and fast. They can also predict the pollution level and the ecological status over an extended period because they are sensitive to changes in the ecosystem (Lau *et al.* 2015; Menetrey *et al.* 2005; Sharma *et al.* 2008). The application of biological approaches to aquatic systems, including rivers and streams, is becoming more widespread (Menetrey *et al.* 2005). For instance, the Macroinvertebrate Community Index (MCI) is used to assess lotic systems' ecological integrity in New Zealand. The Stream Invertebrate Grade Number – Average Level (SIGNAL) and the Australian River Assessment System (AusRivAS) are used in Australia (Chessman 1995, Smith *et al.* 1999). The River Invertebrate Prediction and Classification System (RIVPACS) is used in the UK. The Empirical Biotic Index (EBI) is used in South Africa (Chutter 1972), and the Family-level Biotic Index (FBI) is used in the USA (Hilsenhoff 1988). Similarly, the trophic level index is also used to assess lakes in New Zealand (Clapcott *et al.* 2012; Özkundakci *et al.* 2014). Since ponds ecosystems are more responsive to environmental changes in contrast to lakes and streams, the lack of an index or metric to assess the ecological integrity of ponds is a challenge to their conservation and needs to be addressed.

Generally, differences in biotic indices across regions result from different responses of biota to degradation. In developing a biotic index, either a single taxa or groups of taxa are used as indicators of the system's health. However, indices that incorporate multi-taxonomic groups are widely accepted because they include several ecological dimensions of the ecosystem. They are also comparable among different sites in a similar biogeographical area (Menetrey *et al.* 2005). In the United Kingdom, The *Predictive System for Multimetrics* (PSYM) based on flora and fauna is used to assess ponds' ecological status (Biggs *et al.* 2005; Howard 2002). Also, Menetrey *et al.* (2005) developed a metric for assessing eutrophication in ponds by examining species richness and abundance of Coleoptera, Odonata, Gastropoda and Amphibia in Switzerland. Another method known as PLOCH (*Plans d'eau suisses*) has been developed and used to assess ponds' species richness and conservation value in Switzerland (Oertli *et al.* 2005). However, the use of these metrics is regionally specific in their application due to geographic variations in species composition.

Moreover, most pond metrics have failed to account for land use effect on the ecosystem biodiversity (Greig and Galatowitsch 2016; Oertli 2018). The LULC around a pond has

implications on the water quality and the biodiversity of the ecosystem. Land use, land cover may facilitate the improvement or degradation of the ecosystem. The lack of a metric for assessing the ecological status of pond ecosystems in New Zealand is a critical gap in understanding freshwater ecosystems. However, to develop a metric, knowledge of the ecology of the ecosystem is vital. My study seeks to fill this crucial knowledge gap.

### **1.1.8 Employing a holistic method for assessing ponds biodiversity across a varied landscape**

The use of macroinvertebrates and phytoplankton to assess the ecological integrity of ponds is widespread globally. These taxa are good indicators of ecological quality because they quickly respond to environmental stress (Das *et al.* 2018; Järvinen *et al.* 2013; McGowan *et al.* 2020). Moreover, the measures of physicochemical water quality variables, especially nutrient loads, are essential in energy flow and, hence, determine the ecosystem's biodiversity.

My thesis assesses the relationship between the biotic and abiotic components of the pond ecosystems in New Zealand. Specifically, it evaluates the association of human population density, LULC and other physical environmental variables on pond ecosystems. The effect of seasonality on water quality and biodiversity is also investigated. In addition, an *in-situ* experiment was conducted to determine the limiting nutrient(s) on periphyton biomass in pond ecosystems. Finally, I use a case study to explore how macroinvertebrates (as indicators) within a newly formed pond in a restoration area change as the pond becomes established over one year. By using various techniques and identifying the macroinvertebrates and phytoplankton communities, I provide holistic information on the ecology of pond ecosystems in the Auckland Region of New Zealand. Figure 1.2 shows the steps and the type of data used in each results chapter.

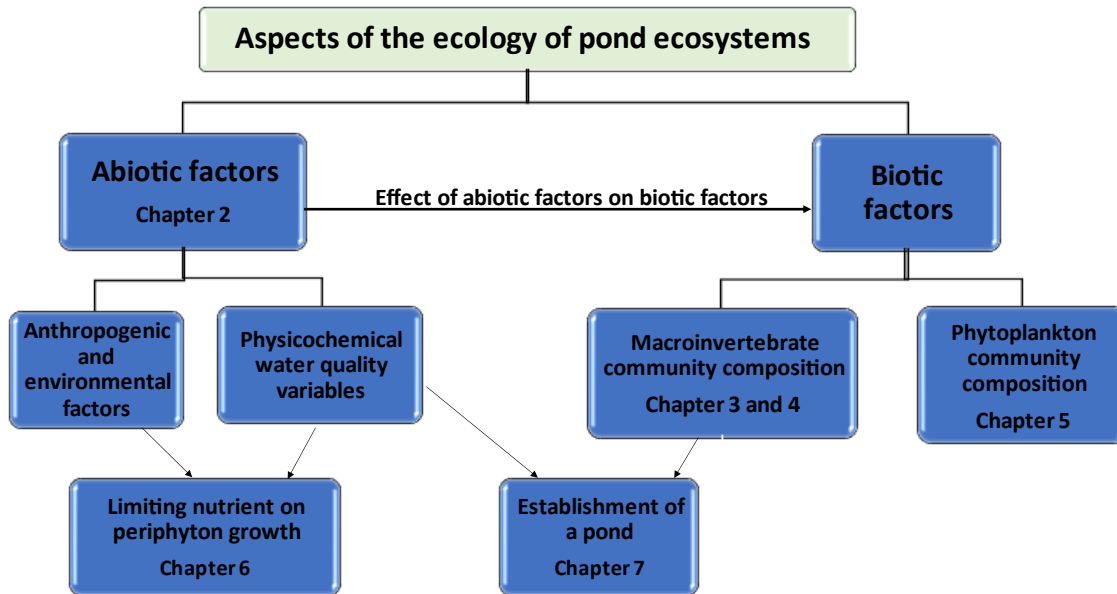


Figure 1.2 A diagram of the structure of the thesis. Chapters 3, 4 and 5 explore the impact of abiotic factors on the biotic factors. Chapter 6 investigates the limiting nutrient(s) in periphyton growth. Finally, Chapter 7 gives insight into the establishment of a pond using macroinvertebrates as indicators.

### 1.1.9 Thesis aims

This thesis seeks to address the gap in knowledge in New Zealand's pond ecosystems in six ways.

The aims of the thesis are to:

- i) assess the effect of LULC at multiple spatial scales on the water quality of 50 ponds and how this relationship varies temporally along a human population density gradient,
- ii) investigate the macroinvertebrate community in 12 ponds in two seasons (winter and summer) over two years and how the macroinvertebrates respond to environmental variables,
- iii) determine the effect of anthropogenic influence on ponds macroinvertebrate communities,
- iv) investigate the phytoplankton community composition in 12 ponds in two seasons (winter and summer) and determine how the community responds to environmental variables,
- v) determine the limiting nutrient(s) on periphyton growth in ponds,
- vi) assess the establishment of a functioning pond ecosystem using macroinvertebrates as a proxy.

## 1.2 Thesis outline

My thesis includes eight chapters. Chapter 1 provides the research background and justification for the study. It includes an outline of the research and summarises the objectives of each chapter.

**Chapter 2** describes the water quality of 50 ponds in the Auckland Region in two seasons. Here, water quality and associated landscape parameters are analysed for two seasons for 50 ponds to determine their relationship (Figure 1.2). Additionally, the water quality is related to the LULC at four different spatial scales. Finally, it determines the relationship between human population density and ponds' water quality. In **Chapter 3**, I provide an inventory of the macroinvertebrate community in ponds across seasons. I also assess the relationship between macroinvertebrate community diversity indices and environmental variables in summer and winter. Finally, I determine the abiotic parameters that are important in influencing macroinvertebrate communities.

**Chapter 4** addresses the effect of anthropogenic influence on ponds in two ways. Firstly, it investigates if the macroinvertebrate community composition varies along the urban-rural gradient (human population gradient). This assessment was done using taxonomic and trait-based methods. The significance of the relationship between the macroinvertebrate community and human population density is assessed for both summer and winter. The relationship between water quality and macroinvertebrates is determined. Secondly, I compare the macroinvertebrate community in three artificial pond types (stormwater, golf course, ornamental ponds) because they are common in Auckland and are influenced by different anthropogenic activities. In **Chapter 5**, I investigate the phytoplankton community composition in 12 ponds across seasons. The relationship between phytoplankton community composition and physicochemical water quality variables are examined. Also, the influence of human population density on the community is evaluated across seasons. In **Chapter 6**, I investigate the limiting nutrient(s) of periphyton biomass in ponds. I use a field experiment to determine how nutrient load (phosphates and nitrates) affect periphyton biomass in ponds. The concentration and the nutrients that result in high periphyton biomass is provided.

**Chapter 7** describes the succession of a new pond ecosystem from three months to a year after construction. I achieve this through a case study by monitoring the water quality and

macroinvertebrate composition in an established and a newly created pond. Finally, in **Chapter 8**, I present my key findings and highlight their significance in the broader context of freshwater conservation in New Zealand. My study's shortcomings are identified, and prospects for future work on pond ecosystems are explored. Overall, my thesis improves our understanding of pond ecosystems in an urbanised region in New Zealand and provides a foundation for future research on the role of ponds in freshwater conservation.

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# Chapter 2

## Ponds, their catchments and spatio-temporal variations in water quality in an urbanised region



## 2.1 Abstract

Understanding the spatial relationships between land use/land cover (LULC) and physicochemical water quality in pond ecosystems is vital to the conservation and management of ponds. This knowledge is especially critical to reconcile landscape planning and management with freshwater ecosystem conservation, particularly in urban regions with rapid population growth. In this study, I assessed 50 ponds in the Auckland region by i) determining the relationship between pond area and their catchment area, ii) seasonal differences in water quality, iii) the impact of the surrounding landscape at four spatial scales (10m, 100m, 500m and full catchment) on water quality, and iv) determining the impact of anthropogenic activities (based on an index relating to human population) on the water quality in the pond ecosystems. At each pond along the human population gradient, seven physicochemical water quality variables and nine landscape properties (LULC and physical features of the pond) were measured in winter and summer. There was a significantly positive relationship between pond area and catchment area. Also, there was a significant seasonal difference in water quality indicative of poorer water quality during summer. I found significantly higher measures of conductivity, total dissolved solids (TDS), percentage dissolved oxygen (% DO), pH, salinity, and the concentrations of phosphates, in summer. In contrast, ammoniacal nitrogen concentrations were higher in winter. A multiple linear regression model showed that LULC and physical landscape features had different influences on the physiochemical variables across different spatial scales and seasons. The landscape properties at all four spatial scales predicted the temperature and % DO of the ponds in summer only. Overall, variations in pond water quality were explained better by general landscape characteristics than by the LULC alone, at the catchment and 500m scale in winter and at the 100m scale in summer. Finally, the water quality of the ponds differed along the urban-rural gradient in summer. Nitrate concentrations were highest in ponds in rural areas, and TDS was highest in ponds in large urban areas. My study highlights the importance of including different spatial scales, seasons, landscape properties and anthropogenic activities when quantifying land-water interactions.

## 2.2 Introduction

The small size of pond ecosystems relative to other freshwater ecosystems, and the high biodiversity they contain, should make their protection more straightforward in comparison to other freshwater systems. Species richness in ponds is only weakly related to the pond's area, implying that there are small biodiversity differences in big and small ponds (Oertli *et al.* 2002; Sun *et al.* 2019). Blicharska *et al.* (2016), however, found that the size of a pond influenced its species richness. For instance, smaller ponds tend to dry out faster than larger ones, affecting the water quality and species diversity (Biggs *et al.* 2005). Furthermore, it is widely accepted that smaller ponds have smaller catchments, although this relationship has rarely been tested (Davies *et al.* 2008; Novikmec *et al.* 2016). The relationship between pond area and their catchments has implications for efficient management, particularly land use practices that affect water quality and biodiversity (Davies *et al.* 2008; Declerck *et al.* 2006; Novikmec *et al.* 2016). Water quality in ponds is affected by the catchment Land use/land cover (LULC); hence, determining the relationship between pond area and the catchment area is vital for landscape management.

Since the 1970s, researchers have explored the effects of LULC on freshwater quality (Rimer *et al.* 1978; Shen *et al.* 2015; Zhang *et al.* 2018). A strong negative relationship between anthropogenic LULC and water quality has largely been reported, and thus LULC is widely considered a primary stressor influencing freshwater ecosystem state. However, the effect of other stressors, including climate, geology and landscape features that contribute to altered conditions, cannot be ruled out (Bhat *et al.* 2020; Brainwood *et al.* 2004). Due to their small sizes and hence small water volume, ponds are sensitive to changes in LULC, mostly resulting from anthropogenic activities through the increased transfer of nutrients from the anthropogenic stressors. Stressors linked with anthropogenic LULC usually include decreased vegetation cover, increased impervious surfaces, increased soil erosion, increased contaminants, and nutrient fluxes that modify the integrity of pond ecosystems (Brabec *et al.* 2002; Vander Laan *et al.* 2013; Anim *et al.* 2019). These stressors result in increased transfer of nutrients from the surrounding catchment into the ponds (Declerck *et al.* 2006; Epele *et al.* 2018; Nielsen *et al.* 2012; Nobre *et al.* 2020). Anthropogenic activities occurring in catchments are associated directly or indirectly with major nonpoint pollutants such as nitrogen and phosphorus into ponds (Chaichana *et al.* 2011; Nielsen *et al.* 2012; Nobre *et al.* 2020; Paul *et*

*al.* 2012; Tu 2011). In addition, land use changes within the ponds' catchment area influence water quality through the exchange of materials, including chemicals between the ponds and the proximate LULC (Asare *et al.* 2018).

The extent of the effect of catchment LULC on the water quality depends on the LULC type, the size of the impacted area, and the closeness to the aquatic ecosystem (Declerck *et al.* 2006; Novikmec *et al.* 2016). For instance, urbanization in areas where wastewater treatment is not efficient (e.g., high amounts of impervious surfaces) results in low water quality due to runoff (Hobbie *et al.* 2017; Nobre *et al.* 2020). Additionally, ponds located in agricultural, industrial, or urban landscapes usually show higher nutrient levels (lower water quality) compared to ponds in pristine or less impacted areas (higher water quality) (Epele *et al.* 2018; Liu *et al.* 2018). Hence the relationship between water quality variables and different land use types are not consistent but vary geographically and at different spatial scales (Declerck *et al.* 2006; Nielsen *et al.* 2012; Novikmec *et al.* 2016).

Auckland is the most populated New Zealand region, with its population concentrated in urban areas (Stats NZ 2020). Auckland is characterised by a sub-tropical climate with warm summers and wet winters. Rapid urbanization resulting in LULC changes has been observed in the last decade (Larned *et al.* 2016; Gadd *et al.* 2020). These recent changes have resulted in increased amounts of impervious surfaces (e.g., roads, parking lots, and rooftops) and a reduced amount of forested and vegetated lands in these urban catchments. These significant changes affect freshwater quality and impact biodiversity due to the changes in the hydrological cycle, increased sedimentation and pollutant load (Council 2005; Baillie and Neary 2015; Chan *et al.* 2016; Gadd *et al.* 2020; Xu *et al.* 2020).

Impervious surfaces (primary landscape of cities) increase runoff and are among the largest uncontrolled pollution sources for freshwater ecosystems (Epele *et al.* 2018; Tang *et al.* 2020). While drinking water standards in Auckland are high, many urban freshwater systems are reported to be polluted and exhibit signs of ecological degradation (Larned *et al.* 2016; Larned *et al.* 2020). However, in non-urban or rural areas in Auckland, crop or animal farms and forests are the primary LULC types, increasing nitrogen and phosphorous concentration into freshwater ecosystems (Chan *et al.* 2016). When catchment land is covered by natural vegetation or forest, less nutrients are leached into freshwater ecosystems (Nobre *et al.* 2020). Many studies worldwide have shown that LULC substantially impacts water quality (Larned *et al.* 2020; Nobre *et al.* 2020; Tu 2011). Despite the relationship between LULC and water quality,

linkages between one specific LULC type and different water quality variables are still controversial due to other confounding differences between areas under study (Liu *et al.* 2018).

Furthermore, the influence of LULC on specific water quality measurements changes according to season (Buck *et al.* 2004; Pratt and Chang 2012). Pollutants and nutrients are transported from different landscapes, within the catchment areas, into aquatic systems through stormwater runoff from precipitation (Atique and An 2020). Hence, variability in precipitation between seasons influences surface runoff patterns, groundwater flow and outflows from aquatic and terrestrial ecosystems. The dynamics of this occurrence substantially impact pollutant concentrations, discharge, and storage (Chowdhury and Husain 2020).

Landscape-scale approaches are useful for exploring fundamental ecological patterns across a region and improving our knowledge about the influence of specific landscapes on ecosystems (Epele *et al.* 2018). By studying small, isolated water bodies (ponds), it is possible to recognise the spatial variations and the primary environmental controls across broad regional scales (Epele *et al.* 2018). This quantification is feasible because of the large number of ponds that can be sampled within local and regional scales. Moreover, because human activities differ in intensity in different areas, water quality degradation might be spatially heterogeneous and seasonally confounded (Buck *et al.* 2004; Chen *et al.* 2016). There is a limited understanding of the spatiotemporal variations in water quality, which imparts additional challenges in controlling water quality from degradation (Chan *et al.* 2016; Greig and Galatowitsch 2016; Epele *et al.* 2018). LULC and anthropogenic activities (measured in this study as human population) affect the water quality of ponds. However, it is still unclear at what hierarchical level these effects operate and predicting them can be challenging (Brainwood *et al.* 2004; Ramião *et al.* 2020).

Understanding the role of spatial scales in the relationship between landscape patterns and water quality in aquatic ecosystems is vital in protecting and improving water quality. This knowledge is required to plan, monitor, and manage landscape patterns (Zhang *et al.* 2019b; Mwaijengo *et al.* 2020; Xu *et al.* 2020). Therefore, it is vital to analyse the characteristics of pond LULC and their influence on pond water quality (Chan *et al.* 2016; Epele *et al.* 2018; Greig and Galatowitsch 2016). My study is particularly important due to the lack of knowledge about pond water quality and its relationships with the intensity of urban land use and human populations. In this study, I assessed the relationship between ponds and their catchment

areas, evaluated the water quality and analysed the impact of seasons on the relationship between water quality and LULC at multiple spatial scales and across an urban-rural gradient of sites.

### 2.2.1 Aims and hypotheses

The aims of this chapter are to:

- i) determine the relationship between pond area and catchment area,
- ii) determine the physicochemical variable(s) that influences the variations of the water quality,
- iii) assess and determine if there is any seasonal difference in the water quality of ponds,
- iv) determine the relationships between different LULC types and the water quality variables at multiple spatial scales and,
- v) identify the influence of season on the relationships between LULC and physical parameters and water quality,
- vi) determine if water quality and LULC vary across an urban-rural gradient.

My hypotheses are that:

- i) the catchment area of ponds will be positively correlated to the area of the ponds,
- ii) nutrients (nitrate, phosphate and ammoniacal nitrogen) will influence variations in water quality in ponds,
- iii) water quality will be different between seasons because of differences in the amount of precipitation,
- iv) water quality in summer will be poorer than in winter due to an increase in temperature and reduction in rainfall, leading to a high concentration of ions in summer,
- v) winter will have a greater influence on the relationship between water quality and LULC due to higher rainfall and runoff into ponds,
- vi) LULC at the catchment will be the most important influencer of water quality in ponds. The highest amount of runoff into the ponds comes from the catchment; hence any LULC type within the catchment area will have a greater influence on pond water quality, and
- vii) ponds in major urban areas will have poorer water quality due to greater stormwater runoff from impervious surfaces.

## 2.3 Methods and analyses

### 2.3.1 Study Area

The Auckland Region is in the upper North Island of New Zealand, lying within longitude 174.617218 and latitude -36.118271 in the north-eastern, 174.173732, -36.461183 in the north-western, 175.294622, -37.021974 in the south-eastern and 174.657926, -37.282979 in the southwestern point. The region is located 13° south of the Tropic of Capricorn. The Auckland region covers 5600km<sup>2</sup>, representing two percent of the total area of New Zealand. The landmass of Auckland spans from the Awhitu peninsula in the south to the Te Ara dunes in the North. From east to west, the Auckland Region extends from the Hunua Ranges, including the Hauraki Gulf Islands, to the Tepora Peninsula and Kaipara Harbour (Council 2013; McClure 2016).

The climate in Auckland is a mixture of temperate and subtropical conditions. The region has a high absolute humidity record and is relatively warm, with an average annual temperature of 14°C to 16°C. The eastern sections of the Auckland Region are slightly warmer than the western portions (Chappell 2013). Auckland has an average elevation of 42m and a maximum elevation of 459m above sea level (Gradwell 1971). The landform in Auckland consists of mountains, dunes, and rocks with volcanic cones. The rock in the region is dominated by sedimentary rocks made up of sandstone and mudstone. The soil is predominantly loamy, formed from volcanic ash and lava. However, this rich soil has been podsolised by acid litter from the Kauri and other plants (Gradwell 1971; McClure 2016).

Auckland is the most densely inhabited region in New Zealand, with a population of 1,717,500 as of 30 June 2020, representing a third (28.9%) of the total population of New Zealand (Stats NZ 2020). Hence, the region is experiencing a high rate of urbanization. Data on human populations (based on an urban-rural classification) associated with the location of the ponds were obtained from Statistics New Zealand/ Geographic Information Systems (GIS) maps on urban-rural distribution in New Zealand (Stats NZ 2019). The GIS tool provides information on rural and urban locations in New Zealand. The categories by Stats NZ are rural (at most 200 residents per square kilometre or 100 address points per square kilometre), small urban (1,000 to 9,999 residents), large urban (30,000 to 99,999 residents), and major urban (100,000 or more residents) (Stats NZ 2019). The categories were derived from the population based on

the 2013 census data and projections of the 2018 census (Stats NZ 2019) and the infrastructural development associated with the sites. The major urban areas may be associated with increased commercial/ economic activities, a large proportion of impervious surfaces, and greater municipal wastewater discharge (Liyanage and Yamada 2017). These factors increase the type and quantity of chemicals input through stormwater runoff into freshwater ecosystems (Juma *et al.* 2014). As the human population decreases, the impact of the stormwater runoff reduces. Freshwater ecosystems in rural areas usually have high nutrient levels from fertiliser application associated with agricultural activities (Larned *et al.* 2016).

Despite being the largest urban area in New Zealand, non-urban land use still covers 89% of the region (Stats NZ 2018). The dominant agricultural land use is sheep and cattle farming representing over 40% of agricultural land, followed by dairy farming (>30%) (Stats NZ 2018). New Zealand is rich in freshwater ecosystems, and Auckland is no different. It has a diversity of freshwater ecosystems, of which many are under threat due to anthropogenic activities (Council 2013). There are 72 natural and artificial lakes in the Auckland region greater than 1ha. The council continuously monitors seven of these lakes to determine their water quality ecological status and assess their microbiological content (Council 2013).

The study was carried out in Auckland, located in the upper North Island of New Zealand. Auckland has a temperate climate, with humid and warm summers and mild and damp winters (Council 2015). Fifty ponds of varying sizes located in various landscapes along the urban-rural gradient (14 in major urban, 6 in large urban, 16 in small urban and 14 in rural areas) within the region were sampled (Figure 2.1).

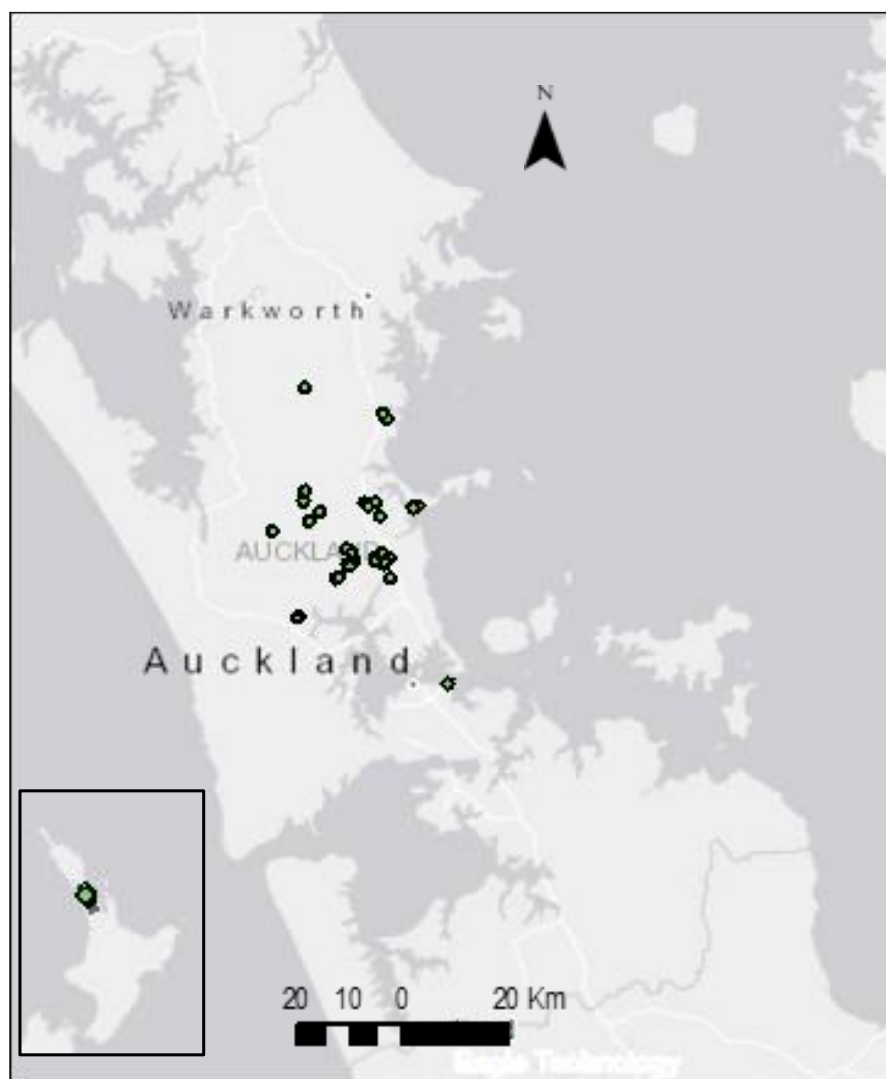


Figure 2.1 A map of Auckland showing the distribution of the 50 ponds sampled (map on the left); inset is the map of the North Island, New Zealand, with the dark green portion indicating the location of the Auckland Region. Basemap; New Zealand light grey canvas from Esri®. All rights reserved.

### 2.3.2 Data collection

The physicochemical, physical, and LULC data were collected from the ponds once during the summer (December to February) and winter (June to August) of 2017. The physicochemical water quality variables measured included pH, conductivity, temperature, TDS, %DO, salinity, phosphate ( $\text{PO}_4^{3-}$ ), ammoniacal nitrogen ( $\text{NH}_3\text{-H}$ ) and nitrate ( $\text{NO}_3\text{-N}$ ). The physical parameters measured were rainfall, altitude, percentage slope and percentage macrophyte cover.

At each pond site, physicochemical variables were measured, and nutrient tests were carried out simultaneously (11:00 am - 2:00 pm New Zealand Standard Time) and recorded. The pH,

conductivity, temperature, TDS, % DO, and salinity were measured with a calibrated Hanna multiparameter probe (Model H198194). The probe was gently lowered into the water column at each location without it touching the sediment. The probe was left in the water column for five minutes to adjust to the conditions in the pond before readings were recorded. The average of each of the variables measured at the three points was calculated and used for analysis. The margin of error or sensitivity for the physicochemical variables of the Hanna probe are temperature,  $\pm 0.15^{\circ}\text{C}$ , pH  $\pm 0.02$ , % DO  $\pm 1.5\%$ , conductivity  $\pm 1\mu\text{scm}^{-1}$ , TDS  $\pm 1\text{ppm}$  and salinity, PSU  $\pm 0.01$ . The concentrations of  $\text{PO}_4^{3-}$ ,  $\text{NH}_3\text{-H}$ , and  $\text{NO}_3\text{-N}$  were measured with the HACH nutrient test kits (DR 200) after the accuracy of the test kit was validated with standard nutrients in the lab. The limits of detection (LOD) of these nutrient tests were  $\text{PO}_4^{3-}$ :  $0.02\text{-}2.50\text{mg l}^{-1}$ ,  $\text{NH}_3\text{-H}$ :  $0.02\text{-}2.50\text{mg l}^{-1}$  and  $\text{NO}_3\text{-N}$ :  $0.01\text{-}0.50\text{mg l}^{-1}$  (see Appendix A: Section 1 for details of nutrient test).

Land Use/Land Cover (LULC) types (i.e., forest, grass and urban) was estimated at four spatial scales: i) 10m immediately surrounding the pond, ii) 100m radius from the centre of the pond, iii) 500m radius from the centre of the pond, and iv) the catchment area. The 10m, 100m, and catchment scales were selected following Novikmec *et al.* (2016) and Declerck *et al.* (2006), where the importance of these scales on pond water quality were highlighted. The 10m and 100m represent the buffer zones of the ponds.

The LULC data were analysed at 1:1000, at 30m resolution by measuring the area covered by each LULC type from aerial maps obtained from Geomaps from the Auckland Council (Council 2018). The LULC were categorised as grass, forest or urban (impervious surface). Table 1 summarises and provides a description of the landscape features and LULC parameters measured at multiple scales. The area of some of the ponds identified on the Auckland Council shapefiles was obtained from the attributes table in ArcGIS version 10.7.1 (ESRI 2016). The catchment area was evaluated using GIS tools on a Digital Elevation Model (DEM) with a resolution of 80m obtained from the Land Information New Zealand (LINZ) using the spatial analyst extension in ArcGIS 10.7.1 (ESRI 2019). The physical parameters measured were altitude, percentage slope, rainfall, and percentage macrophyte cover per sampling period. The altitude of the ponds was obtained from an aerial base map from the Auckland council Geomaps. The percentage slope was derived from the slope map calculated from the DEM. Percentage macrophyte cover of the ponds was visually estimated from the pond site relative to the total surface area of each pond at the time of sampling.

### 2.2.3 Data Analysis

I tested the relationship between pond catchment area and pond surface area using linear regression. The seasonal variation in the physicochemical water quality variables was compared using a student t-test. The principal physicochemical variables of the water quality were analysed using a Principal Component Analysis (PCA). A PERMAVOVA test was performed to test for differences in the water quality between the seasons. A PCA was used to determine the physicochemical variables that contribute most to water quality. The relationship between the percentage cover of each LULC type and water quality at the different spatial scales was analysed using Spearman rank correlation. The p-values were revised using Bonferroni corrections. The relationships between water quality variables, physical parameters and LULC patterns were explored with a multivariate approach using multiple linear regression (MLR) modelling and redundancy analysis (RDA). The water quality data were transformed as  $\text{Log}_e(x + 1)$  and normalised for the RDA and the MLR (Ding *et al.* 2016). The physical parameters were also normalised. The MLR model was used to evaluate the relationships between a response (i.e., a single water quality variable) and a scale of LULC in the presence of the physical parameters (rainfall, area, catchment area, altitude, % macrophyte cover, and slope).

The forecasting ability of the MLR model was measured by the adjusted coefficient (adjusted  $R^2$ ) (Xu *et al.* 2020). The best model for each physicochemical variable is the 'objective' MLR model and was chosen in a stepwise MLR analysis based on: (i) the adjusted  $R^2$  value of the model is the highest among all models, i.e., among all scales and (ii) the significance of the coefficients of the model and predictors are equal to or less than 0.05 (Ding *et al.* 2016).

Redundancy analysis (RDA) was used to explore the relationships between LULC, water quality and physical factors at each of the four spatial scales (Ding *et al.* 2016; Shi *et al.* 2017; Song *et al.* 2020). The full RDA simultaneously examined the impacts of multiple LULC and physical parameters on water quality (dependent variable) (Zhang *et al.* 2019a). Partial RDA (p-RDA) were performed to examine the effect of LULC alone on the water quality removing the effect of physical variables. The differences in physicochemical variables and water quality along the urban-rural gradient were compared using an Analysis of Variance (ANOVA) test and PERMANOVA. The analyses were done using PERMANOVA + (version 7.0.13) software extension (Clarke and Gorley 2006), R version 3.6. 1: (R Core Team 2019), and SPSS (version 25) software (IBM 2017).

## 2.4 Results

### 2.4.1 The relationship between pond and catchment areas

The area of the ponds varied from 22.7m<sup>2</sup> to 6957.2m<sup>2</sup>, and the catchment areas were between 428m<sup>2</sup> and 31762m<sup>2</sup>. The mean pond area and catchment area were 1698.5m<sup>2</sup> ± 1977.4m<sup>2</sup> and 3635.7 ± 5258.2m<sup>2</sup>. Figure 2.2 shows the relationship between the pond area and their catchment ( $p = 0.3$ ,  $p = 0.01$ ). A significant weak positive relationship was observed.

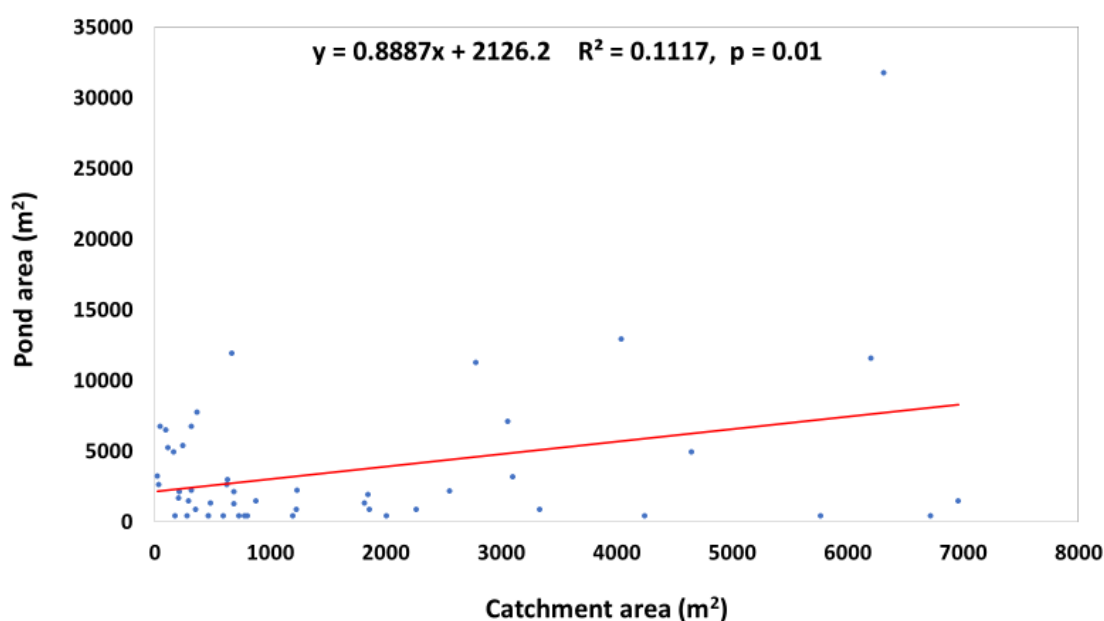


Figure 2.2 Regression plot of the relationship between pond area (m<sup>2</sup>) and catchment area (m<sup>2</sup>) of 50 ponds in the Auckland region. The linear regression equation, coefficient of determination (squared deviation of the mean), and p value are reported.

### 2.4.2 Seasonal comparison of the physicochemical variables between the seasons

There were seasonal variations in levels of the physicochemical variables measured. In particular, the pH ( $t = 2.36$ ,  $p = 0.02$ ) of the water and phosphate concentrations ( $t = 3.97$ ,  $p < 0.001$ ) were significantly higher in summer than in winter. The % DO in the ponds was significantly higher in summer but more variable in winter ( $t = 2.91$ ,  $p < 0.01$ ). Conductivity ( $t = 2.71$ ,  $p < 0.01$ ), TDS ( $t = 2.68$ ,  $p < 0.01$ ), salinity ( $t = 2.71$ ,  $p < 0.01$ ) and temperature ( $t = 23.27$ ,  $p < 0.001$ ) levels were all more variable and significantly higher in summer. In contrast, nitrate concentrations did not change significantly with seasons ( $t = 0.21$ ,  $p = 0.08$ ). and, finally,

ammoniacal nitrogen concentrations were significantly higher in winter ( $t = 2.76$ ,  $p < 0.01$ ) (Table 2.1).

Table 2.1 A summary of the physicochemical variables in summer and winter. Significance at  $p < 0.05$  are in bold. Con.: conductivity, Sal.: salinity, Tem.: Temperature,  $\text{NO}_3\text{-N}$ : Nitrate,  $\text{PO}_4^{3-}$ : Phosphate,  $\text{NH}_3\text{-H}$ : Ammoniacal nitrogen.

Variables	Summer		Winter		t	p value
	Range	Average	Range	Average		
pH	4.44 - 8.01	$6.63 \pm 0.67$	4.22 - 7.49	$6.33 \pm 0.59$	2.36	<b>0.02</b>
% DO	25.30 - 129.21	$73.49 \pm 31.53$	11.27 - 162.40	$56.59 \pm 26.25$	2.91	<b>&lt;0.01</b>
Con. ( $\mu\text{Scm}^{-1}$ )	21.6 - 920.3	$228.8 \pm 180.5$	14.4 - 434.7	$147.1 \pm 75.7$	2.79	<b>&lt;0.01</b>
TDS (ppm)	28.7 - 460.7	$117.7 \pm 98.7$	20.7 - 218.3	$80.4 \pm 43.4$	2.68	<b>&lt;0.01</b>
Sal. (psu)	0.01 - 0.50	$0.12 \pm 0.10$	0.01 - 0.52	$0.08 \pm 0.07$	2.71	<b>&lt;0.01</b>
Tem. ( $^{\circ}\text{C}$ )	19.37 - 32.34	$23.89 \pm 2.63$	8.39 - 16.83	$13.41 \pm 1.93$	23.27	<b>&lt;0.001</b>
$\text{NO}_3\text{-N}$ ( $\text{mg l}^{-1}$ )	0.01 - 0.92	$0.17 \pm 0.23$	0.01 - 0.52	$0.18 \pm 0.21$	0.21	0.8
$\text{PO}_4^{3-}$ ( $\text{mg l}^{-1}$ )	0.06 - 2.5	$1.03 \pm 0.85$	0.02 - 2.50	$0.47 \pm 0.65$	3.97	<b>&lt;0.001</b>
$\text{NH}_3\text{-H}$ ( $\text{mg l}^{-1}$ )	0.05 - 1.17	$0.20 \pm 0.17$	0.02 - 1.66	$0.20 \pm 0.27$	2.76	<b>&lt;0.01</b>

### 2.4.3 Seasonal variations in the physicochemical water quality variables (Multivariate approach)

Overall, I found I found a significant seasonal difference in the water quality (multivariate PERMANOVA analysis,  $F_{1,98} = 17.11$ ,  $p < 0.001$ ) as shown in the nMDS plot (Figure 2.3).

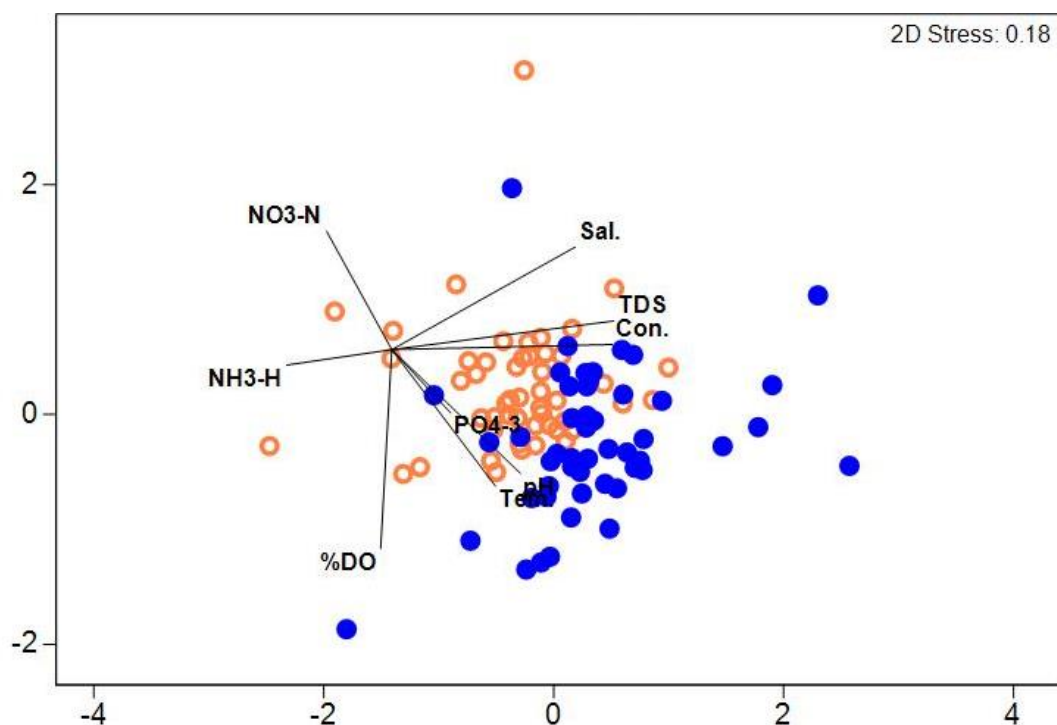


Figure 2.3 An nMDS plot of the Euclidean distance based on normalised physicochemical water quality variables in 50 ponds overlaid with vectors of variables. Blue circles represent summer water quality; orange open circles represent winter water quality. The distance between circles indicates the similarity in the water quality among the ponds. The closer the circles, the more similar the water quality and *vice versa*.

Variations in water were explained primarily by conductivity, salinity, and TDS and, to a lesser extent, by pH and % DO (PCA, Table 2.2). Overall, PC1, PC2 and PC3 explained 76% of the total variation in water quality. PC1 was negatively loaded by TDS (-0.56), salinity (-0.56) and conductivity (-0.52). PC2 was negatively loaded by ammoniacal nitrogen (-0.73) and positively loaded by pH (0.57) and % DO (0.47). Finally, PC3 was positively loaded by ammoniacal nitrogen concentration (0.48) (Table 4). Similarly, in winter, PC1, PC2 and PC3 explained 63.3% of the variation in the water quality. PC1 was negatively loaded by conductivity (-0.56) and TDS (-0.55). PC2 was negatively loaded by nitrate (-0.64) and % DO (-0.53), and PC3 was negatively loaded by phosphate concentration (-0.82) and % DO (-0.51) and positively by salinity (0.48) (Table 2.2).

Table 2.2 A summary table of PCA loadings of the physicochemical variables in summer and winter. Variables with loading > 0.4 are in bold. Con.: conductivity, Sal.: salinity, Tem.: Temperature, NO<sub>3</sub>-N: Nitrate, PO<sub>4</sub><sup>3-</sup>: Phosphate, NH<sub>3</sub>-H: Ammoniacal nitrogen.

Variable	Coefficients of the linear combinations of variables making up PC's					
	Summer			Winter		
	PC1	PC2	PC3	PC1	PC2	PC3
pH	-0.14	<b>0.57</b>	0.11	-0.35	-0.02	-0.26
% DO	0.2	<b>0.47</b>	-0.3	-0.2	<b>-0.53</b>	<b>-0.51</b>
Con. (μScm <sup>-1</sup> )	<b>-0.52</b>	0.09	-0.08	<b>-0.56</b>	-0.03	0.04
TDS (ppm)	<b>-0.56</b>	-0.03	-0.17	<b>-0.55</b>	0.16	0.27
Sal. (psu)	<b>-0.56</b>	-0.08	-0.19	-0.08	0.26	<b>0.48</b>
Tem. (°C)	0.03	0.07	-0.1	-0.01	0.01	-0.06
NO <sub>3</sub> -N (mg l <sup>-1</sup> )	0.39	-0.29	0.32	0.02	<b>-0.64</b>	0.02
PO <sub>4</sub> <sup>3-</sup> (mg l <sup>-1</sup> )	-0.21	0.01	0.17	0.18	-0.04	<b>-0.82</b>
NH <sub>3</sub> -H (mg l <sup>-1</sup> )	-0.14	<b>-0.73</b>	<b>0.48</b>	0.05	-0.15	-0.38
Eigenvalues	3.26	1.89	1.1	1.96	1.38	1.21
% Variation	39.6	23	13.4	27.3	19.2	16.8
Cumulative % Variation	39.6	62.6	76	27.3	46.5	63.3

#### 2.4.4 Relationship between physicochemical water quality variables

The relationships between the physicochemical variables measured varied across seasons. For example, in summer, conductivity was strongly positively correlated to TDS ( $\rho = 1$ ,  $p < 0.01$ ) and salinity ( $\rho = 0.9$ ,  $p < 0.01$ ). Salinity was also strongly positively correlated to TDS ( $\rho = 0.9$ ,  $p < 0.01$ ). Nitrate concentration was negatively correlated to pH ( $\rho = -0.5$ ,  $p < 0.01$ ) and % DO ( $\rho = 0.3$ ,  $p < 0.05$ ). Temperature was positively correlated to % DO ( $\rho = 0.6$ ,  $p < 0.01$ ).

In winter, conductivity was strongly positively correlated to TDS ( $\rho = 0.9$ ,  $p < 0.01$ ) and salinity ( $\rho = 0.8$ ,  $p < 0.01$ ). Salinity was also strongly positively correlated to TDS ( $\rho = 0.8$ ,  $p < 0.01$ ). Nitrate concentration was negatively correlated to conductivity ( $\rho = -0.3$ ,  $p < 0.05$ ) and TDS ( $\rho = -0.3$ ,  $p < 0.05$  Appendix A: Table 1).

### 2.4.5 Effect of LULC at multiple spatial scales on water quality variables

The percentage cover by the three LULC types (forest, grass, and urban) varied among the four spatial scales measured. For example, the highest mean urban cover ( $28.2 \pm 29.5\%$ ) was observed at the 500m scale, but for grass, the highest mean cover ( $54.9 \pm 35.6\%$ ) was at the catchment scale, and for forest, the highest mean cover ( $48.4 \pm 32.0\%$ ) was at the 10m scale (Table 2.3). Generally, there was a weak correlation between the physicochemical variables and the three LULC types. In summer, at the 500m scale, the percentage of grass cover was negatively correlated with temperature ( $\rho = -0.4$ ), and urban cover was positively correlated with % DO ( $\rho = 0.4$ ). However, in winter, urban cover at the 500m and catchment scales were significantly positively correlated with pH ( $\rho = 0.4$ ). Urban cover at the catchment scale was negatively correlated with  $\text{NH}_3\text{-H}$  ( $\rho = -0.5$ , Table 2.4).

Table 2.3 A summary table of the physical and landcover parameters of the ponds. Average values with standard deviations (SD) are reported. (n = 50)

Landscape parameters	Parameter	Minimum	Maximum	Average $\pm$ SD
Catchment physical structure	% Slope	11.1	39.3	$29.7 \pm 10.7$
	Catchment area (m <sup>2</sup> )	428	31762	$3635.7 \pm 5258.2$
	% Macrophyte cover	0	99	$32.2 \pm 33.01$
Pond topography	Altitude (m)	3	346	$37.7 \pm 48.6$
	Area (m <sup>2</sup> )	22.7	6957.2	$1698.5 \pm 1977.4$
Local climate	Rainfall mm (winter)	54	175	$88.9 \pm 32.4$
	Rainfall mm (summer)	12	31	$27.8 \pm 7.0$
LULC at 10m	Forest (%)	0	100	$48.4 \pm 32.0$
	Grass (%)	0	100	$44.1 \pm 30.4$
	Urban (%)	0	80	$6.2 \pm 15.0$
LULC at 100m	Forest (%)	0.8	83.0	$26.4 \pm 19.2$
	Grass (%)	0.7	93.8	$51.7 \pm 25.9$
	Urban (%)	0	71.0	$18.0 \pm 19.8$
LULC at 500m	Forest (%)	1	63	$18.4 \pm 15.2$
	Grass (%)	3	97	$48.8 \pm 25.8$
	Urban (%)	1	94	$28.2 \pm 29.5$
LULC at catchment area	Forest (%)	0	90	$28.8 \pm 31.8$
	Grass (%)	1	99	$54.9 \pm 35.6$
	Urban (%)	0	92	$12.4 \pm 22.5$

Table 2.4 Spearman correlation's rank between physicochemical variables and LULC at the multiple spatial scales in summer and winter. Significant correlations ( $p < 0.05$  with Bonferroni corrections) are in bold. Con.: conductivity, Sal.: salinity, Tem.: Temperature,  $\text{NO}_3\text{-N}$ : Nitrate,  $\text{PO}_4^{3-}$ : Phosphate,  $\text{NH}_3\text{-H}$ : Ammoniacal nitrogen.

Spatial scale	LULC types	Physicochemical variables								
		pH	% DO	Con. ( $\mu\text{Scm}^{-1}$ )	TDS (ppm)	Sal. (psu)	Tem. ( $^{\circ}\text{C}$ )	$\text{NO}_3\text{-N}$ ( $\text{mg l}^{-1}$ )	$\text{PO}_4^{3-}$ ( $\text{mg l}^{-1}$ )	$\text{NH}_3\text{-H}$ ( $\text{mg l}^{-1}$ )
<b>Summer</b>										
10m	Forest	0.2	0.2	0.2	0.3	0.3	0.1	-0.3	0.1	0.0
	Grass	-0.2	-0.2	-0.2	-0.2	-0.3	0.0	0.2	0.1	0.1
	Urban	0.1	-0.1	0.0	0.0	0.0	-0.1	0.1	-0.3	-0.2
100m	Forest	-0.1	0.1	-0.1	-0.1	-0.1	0.0	0.0	0.0	-0.1
	Grass	0.1	-0.1	0.0	0.0	0.0	0.0	0.0	-0.2	-0.1
	Urban	-0.1	-0.1	0.2	0.3	0.3	0.0	0.1	0.2	0.2
500m	Forest	-0.3	-0.3	0.0	0.0	0.1	-0.1	0.0	0.0	0.1
	Grass	-0.2	-0.2	-0.3	-0.3	-0.3	<b>-0.4</b>	0.1	-0.1	0.1
	Urban	0.3	<b>0.4</b>	0.1	0.1	0.0	0.3	-0.1	0.1	-0.3
Catchment	Forest	-0.2	0.0	-0.1	-0.1	-0.1	-0.2	0.0	0.1	0.0
	Grass	-0.1	-0.2	0.0	0.0	0.0	0.1	0.1	-0.2	0.2
	Urban	0.3	0.2	0.1	0.1	0.1	0.0	-0.2	0.0	-0.2
<b>Winter</b>										
10m	Forest	0.0	-0.1	0.1	0.2	0.1	-0.1	-0.1	0.0	0.1
	Grass	-0.1	0.1	-0.2	-0.2	-0.1	0.0	0.1	0.0	0.0
	Urban	0.2	0.0	0.1	0.0	0.1	0.0	0.1	0.2	-0.2
100m	Forest	0.0	0.2	-0.1	-0.1	-0.2	-0.1	0.1	-0.1	0.0
	Grass	0.0	-0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Urban	0.1	0.1	0.1	0.2	0.2	0.1	-0.1	0.2	-0.2
500m	Forest	-0.3	0.0	0.2	0.1	0.0	-0.3	0.1	0.1	0.2
	Grass	-0.3	0.1	-0.2	-0.3	-0.3	-0.1	0.2	-0.1	-0.1
	Urban	<b>0.4</b>	0.0	0.0	0.1	0.1	0.3	-0.1	0.0	-0.2
Catchment	Forest	-0.2	0.0	0.0	0.1	0.0	-0.3	0.1	0.0	0.1
	Grass	-0.1	0.0	0.1	-0.3	0.0	0.0	0.0	0.1	0.2
	Urban	<b>0.4</b>	0.0	-0.2	0.1	0.0	0.3	0.0	-0.1	<b>-0.5</b>

#### **2.4.6 The relationships between the physical, LULC and physicochemical variables at multiple scales**

There were variations in the relationship between the landscape features and the physicochemical parameters. In summer, the LULC and physical parameters at the 10m, 100m and catchment scale significantly explained the variation in pH levels of the ponds. The LULC and physical parameters at all four spatial scales (10m, 100m, 500m, and catchment) significantly explained the variation in % DO and temperature. LULC and physical parameters at 10m and 500m explained the variation in TDS. LULC and physical parameters at 10m and 500m explained the variation in salinity and conductivity, respectively (Table 2.5).

The predictability ( $\beta$ ) of water quality variables differed with scale and seasons. In summer pH, and % DO provided the best model at catchment scales. The key predictors for pH were pond area ( $\beta = 0.49$ ), catchment area ( $\beta = -0.30$ ) and urban cover ( $\beta = 0.28$ ). Key predictors of % DO were pond area ( $\beta = 0.35$ ), rainfall ( $\beta = 0.31$ ) and macrophyte cover ( $\beta = -0.34$ ). The best model for temperature, conductivity and TDS was at 500m. The key predictors of temperature were macrophyte cover ( $\beta = -0.43$ ), pond area ( $\beta = 0.39$ ), and forest cover ( $\beta = 0.31$ ). The key predictor for conductivity was urban cover ( $\beta = 0.32$ ) and for TDS were rainfall ( $\beta = -0.34$ ) and urban cover ( $\beta = 0.32$ ).

In winter, LULC and physical features at the 100m and 500m scale explained the variation in % DO. The LULC and physical parameters at 100m explained the variation in salinity levels. The best model for % DO was at 500m, and the key predictor was rainfall ( $\beta = 0.48$ ). The best model for salinity was at 100m, and the best predictor was rainfall ( $\beta = -0.34$ ).

Table 2.5 Predictive ability (adjusted R<sup>2</sup>) for the multiple linear regression (MLR) models at the four spatial scales for each water physicochemical variable in summer and winter. Objective models with p < 0.05 are in bold. Negative adjusted R<sup>2</sup> implies that the predictors' explanation power is extremely low or negligible in explaining variations in the response variable.

Spatial scale	Physicochemical (response) variables									
	Prediction	pH	%DO	Con. (µScm <sup>-1</sup> )	TDS (ppm)	Sal. (psu)	Tem. (°C)	NO <sub>3</sub> -N (mgL <sup>-1</sup> )	PO <sub>4</sub> <sup>3-</sup> (mgL <sup>-1</sup> )	NH <sub>3</sub> -H (mgL <sup>-1</sup> )
<b>Summer</b>										
10m	p value	<b>0.01</b>	< <b>0.001</b>	0.1	<b>0.03</b>	<b>0.04</b>	<b>0.002</b>	0.4	0.06	0.7
	Adj. R <sup>2</sup>	0.2	0.47	0.12	0.18	0.17	0.32	0.02	0.15	0.1
100m	p value	<b>0.02</b>	< <b>0.001</b>	0.2	0.2	0.3	< <b>0.001</b>	0.36	0.1	0.1
	Adj. R <sup>2</sup>	0.20	0.41	0.08	0.07	0.03	0.33	0.02	0.29	0.13
500m	p value	0.6	< <b>0.001</b>	<b>0.01</b>	<b>0.03</b>	0.2	< <b>0.001</b>	0.5	0.4	0.6
	Adj.R <sup>2</sup>	0.28	0.43	0.25	0.19	0.1	0.41	-0.004	0.04	-0.1
Catchment	p value	< <b>0.01</b>	< <b>0.001</b>	0.05	0.09	0.2	<b>0.001</b>	0.4	0.3	0.6
	Adj.R <sup>2</sup>	0.3	0.5	0.16	0.12	0.05	0.35	0.01	0.04	-0.04
<b>Winter</b>										
10m	p value	0.2	0.1	0.4	0.3	0.1	0.2	0.5	0.6	0.6
	Adj. R <sup>2</sup>	0.1	0.1	0.003	0.03	0.1	0.05	-0.02	-0.04	-0.04
100m	p value	0.1	<b>0.03</b>	0.4	0.4	<b>0.03</b>	0.2	0.3	0.3	0.6
	Adj. R <sup>2</sup>	0.1	0.2	0.01	0.01	0.2	0.04	-0.02	0.04	-0.03
500m	p value	0.1	<b>0.02</b>	0.3	0.1	0.1	0.1	0.3	0.5	0.9
	Adj. R <sup>2</sup>	0.11	0.2	0.04	0.09	0.1	0.1	0.05	-0.02	-0.11
Catchment	p value	0.1	0.6	0.5	0.4	0.1	0.2	0.8	0.4	0.6
	Adj. R <sup>2</sup>	0.1	0.15	-0.02	0.01	0.1	0.06	-0.07	0.01	-0.03

#### **2.4.7 The effect of LULC and physical parameters on pond water quality across the seasons**

In summer, the full RDA model considering the effect of landscape features on water quality was significant and explained 32% ( $p = 0.01$ ) at the 10m scale, 34% ( $p < 0.0001$ ) at the 100m scale, 29% ( $p = 0.004$ ) at 500m scale, and 32% at the catchment scale ( $p = 0.001$ ) of variations in water quality. In the p-RDA, after accounting for the effect of the physical features, LULC explained 7% at the 10m and catchment scales, and 4% at the 500m scale of the variations in water quality. However, LULC was significant ( $p = 0.01$ ) and explained 9.7% of the water quality variations at 100m spatial scale (Figure 2.4).

In winter, the full RDA model considering the effect of landscape features on water quality in ponds explained 16% ( $p = 0.5$ ) of variations in water quality at the 10m scale. The model explained 19% ( $p = 0.1$ ) at 100m scale, 18% ( $p = 0.01$ ) at 500m scale, and 26% ( $p = 0.003$ ) at the catchment scale. However, after accounting for the effect of physical parameters, LULC accounted for just 3% at 10m and 5% at 100m scale of variations in water quality. At the catchment and 500m scale, LULC explained 1% ( $p = 0.003$ ) and 9.8% ( $p = 0.01$ ) of variations in water quality respectively (Figure 2.5).

In the full models, phosphate levels were positively associated with rainfall in summer but negatively associated in winter. Percentage macrophyte cover was negatively associated with temperature in summer but positively associated in winter at all four spatial scales (Figure 2.4 and Figure 2.5)

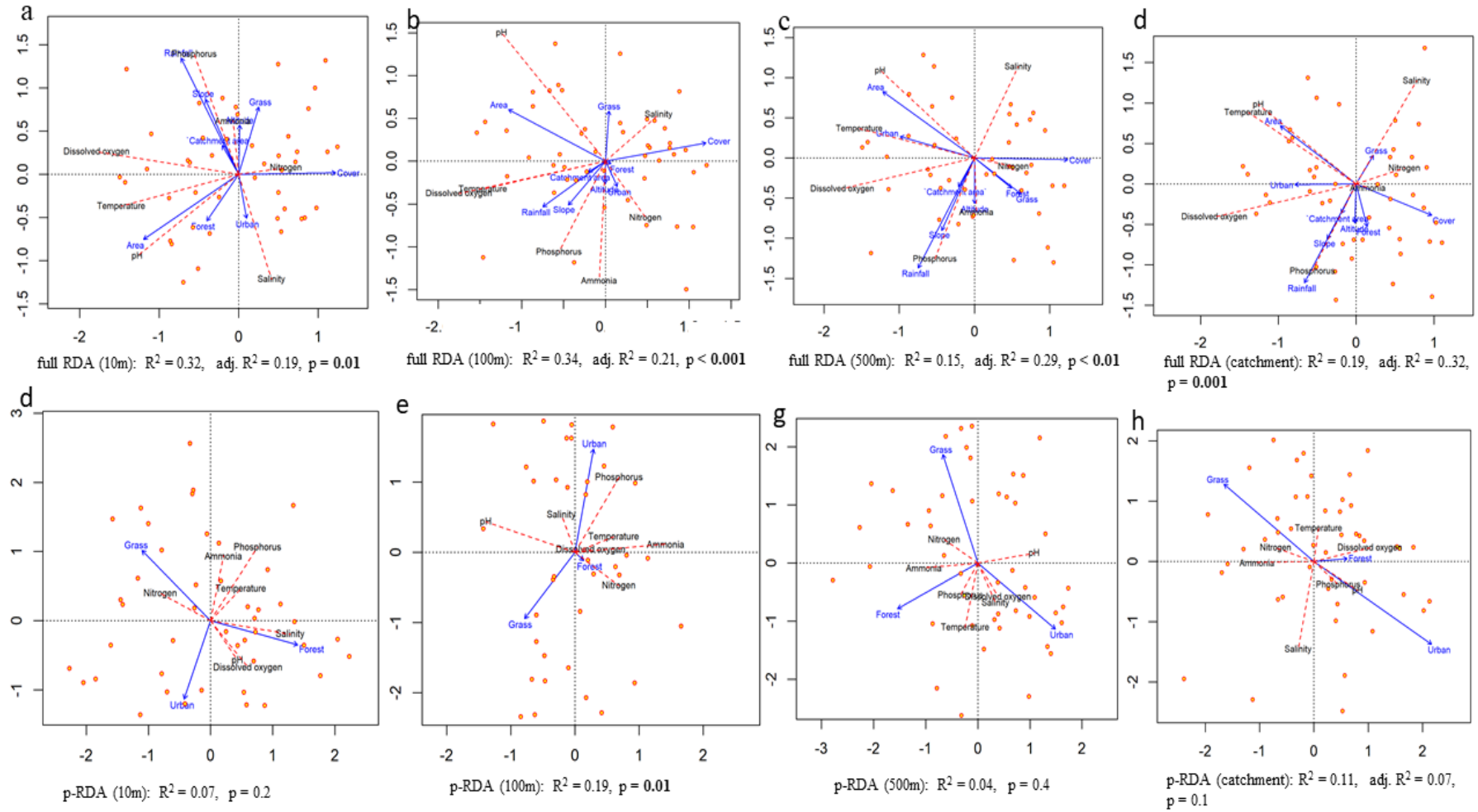


Figure 2.4 Ordination diagrams of the physicochemical water quality variables (red dotted lines) and landscape features (blue lines) at four spatial scales in summer according to the results of full RDA (a, b, c, and d) and p-RDA (e, f, g, and h). Cover represents percentage macrophyte cover.  $R^2$ , adjusted  $R^2$  and  $p$ -values for each model is reported. Significant  $p < 0.05$  are in bold. Non-objective models have no adjusted  $R^2$  value.

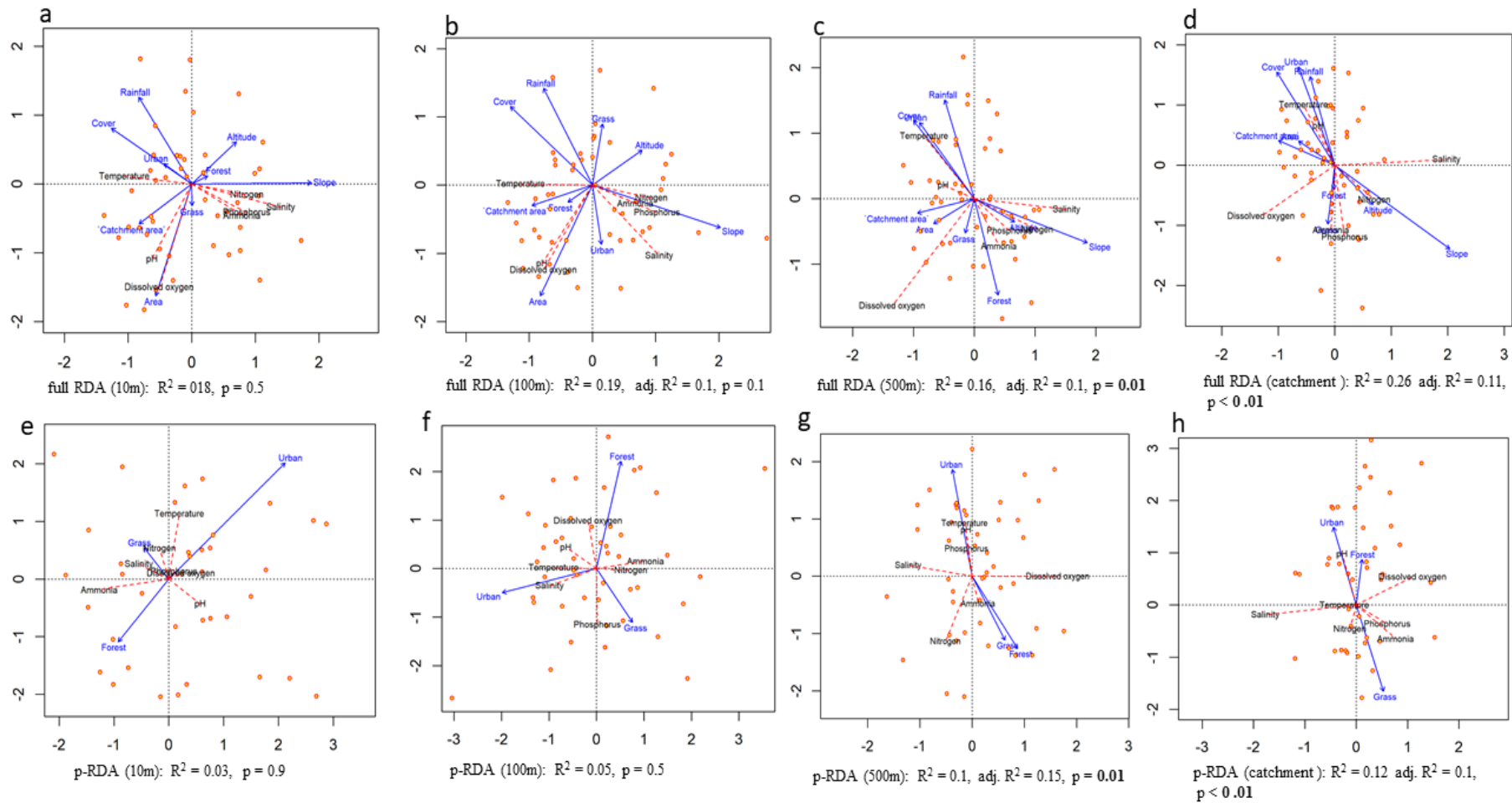


Figure 2.5 Ordination diagrams of the physicochemical water quality variables (red dotted lines) and landscape features (blue lines) at four spatial scales in winter according to the results of full RDA (a, b, c, and d) and p-RDA (e, f, g, and h). Cover represents percentage macrophyte cover.  $R^2$ , adjusted  $R^2$  and  $p$ -values for each model is reported. Significant  $p < 0.05$  are in bold. Non-objective models have no adjusted  $R^2$  value.

### 2.4.8 The physicochemical water quality variables of ponds along the urban-rural gradient

The physicochemical variables varied along the urban-rural gradient across the seasons. While some variables were significantly different along the gradient (e.g., TDS), others like % DO were not. Ponds in large urban areas had the highest TDS, whilst ponds in small urban areas had the least TDS (Table 2.6). In contrast to summer, the amount of % DO in winter was significantly different along the urban-rural gradient ( $F_{3,46} = 4.1$ ,  $p = 0.01$ ). TDS ( $p = 0.002$ ) also varied along the gradient (Table 2.6).

A PERMANOVA test showed a significant seasonal difference in pond water quality along the urban-rural gradient ( $F_{1,46} = 9.9$ ,  $p < 0.01$ ). There were also significant differences in water quality along the urban-rural gradient ( $F_{3,46} = 1.3$ ,  $p = 0.02$ ) and there was no significant interaction between season and urban-rural gradient ( $F_{1,3} = 1.3$ ,  $p = 0.02$ , Appendix A, Table 2). Within group, comparison showed significant difference in water quality in summer between i) major urban and large urban ( $t = 2.1$ ,  $p < 0.01$ ), ii) small urban and rural ( $t = 1.7$ ,  $p = 0.02$ ), and iii) small urban and large urban ( $t = 2.7$ ,  $p = 0.001$ ). In winter, there were significant differences in water quality between small urban and i) large urban ( $t = 1.8$ ,  $p = 0.1$ ) and ii) major urban ( $t = 1.6$ ,  $p = 0.3$ ).

Table 2.6 A summary table of the physicochemical variables along the urban-rural gradient in summer and winter. Average values,  $\pm$  standard deviation, and results of the ANOVA test and p-values are reported. Significant p-values are in bold.

Physicochemical variables	Urban-rural gradient								F	p value
	Major urban (n = 14)		Large urban (n = 6)		Small urban (n = 16)		Rural (n = 14)			
	Min-Max	Average	Min-Max	Average	Min-Max	Average	Min-Max	Average		
<b>Summer</b>										
pH	4.44 - 7.65	6.75 $\pm$ 0.78	6.06 - 7.71	7.11 $\pm$ 0.63	5.74 - 7.42	6.51 $\pm$ 0.54	5.4 - 8.01	6.42 $\pm$ 0.62	1.6	0.1
% DO	39.95 - 129.21	92.47 $\pm$ 28.35	54.83 - 119.4	75.59 $\pm$ 23.22	37.47 - 117.53	70.07 $\pm$ 28.79	25.3 - 126.13	57.52 $\pm$ 33.16	1.8	0.1
Con. ( $\mu\text{Scm}^{-1}$ )	88 - 305.67	196.38 $\pm$ 62.48	161 - 920.33	466 $\pm$ 302.84	57 - 234	141.94 $\pm$ 53.80	21.6 - 754.33	258.71 $\pm$ 207.14	1.1	0.3
TDS (ppm)	44 - 153	100.024 $\pm$ 32.47	80.5 - 460.67	233.14 $\pm$ 151.63	28.67 - 117	71.81 $\pm$ 27.18	30 - 460.04	144.19 $\pm$ 123.11	3.2	<b>0.03</b>
Sal. (psu)	0.04 - 0.15	0.09 $\pm$ 0.03	0.07 - 0.45	0.22 $\pm$ 0.15	0.02 - 0.11	0.07 $\pm$ 0.02	0.01 - 0.50	0.15 $\pm$ 0.13	1.5	0.1
Tem. ( $^{\circ}\text{C}$ )	21.99 - 32.34	24.90 $\pm$ 2.94	23.1 - 26.38	25.21 $\pm$ 1.21	19.51 - 26.96	23.18 $\pm$ 2.48	19.31 - 26.82	23.12 $\pm$ 2.06	1.4	0.2
NO <sub>3</sub> -N ( $\text{mg l}^{-1}$ )	0.01 - 0.46	0.12 $\pm$ 0.18	0.01 - 0.52	0.11 $\pm$ 0.20	0.01 - 0.52	0.16 $\pm$ 0.22	0.02 - 0.62	0.27 $\pm$ 0.27	3.2	<b>0.03</b>
PO <sub>4</sub> <sup>3-</sup> ( $\text{mg l}^{-1}$ )	0.12 - 2.5	1.41 $\pm$ 0.89	0.14 - 1.63	0.72 $\pm$ 0.65	0.06 - 2.17	0.76 $\pm$ 0.77	0.06 - 2.3	1.09 $\pm$ 0.89	0.01	0.9
NH <sub>3</sub> -H ( $\text{mg l}^{-1}$ )	0.08 - 1.17	0.23 $\pm$ 0.29	0.16 - 0.22	0.19 $\pm$ 0.02	0.05 - 0.38	0.16 $\pm$ 0.09	0.09 - 0.36	0.22 $\pm$ 0.08	0.2	0.8
<b>Winter</b>										
pH	5.79 - 7.27	6.58 $\pm$ 0.37	5.74 - 7.10	6.514 $\pm$ 0.47	4.22 - 7.49	6.19 $\pm$ 0.80	5.3 - 7.13	6.16 $\pm$ 0.47	1.6	0.1
% DO	38 - 79.65	56.07 $\pm$ 14.75	21.4 - 50.7	36.44 $\pm$ 11.63	28.5 - 162.4	63.91 $\pm$ 34.14	11.27 - 113.2	57.38 $\pm$ 27.01	4.1	<b>0.01</b>
Con. ( $\mu\text{Scm}^{-1}$ )	68 - 218.67	146.31 $\pm$ 51.78	69 - 434.67	201.61 $\pm$ 130.68	45.6 - 189.67	114.38 $\pm$ 42.95	14.44 - 277	161.96 $\pm$ 85.29	4.2	<b>&lt; 0.01</b>
TDS (ppm)	34 - 156.5	82.17 $\pm$ 33.63	56 - 218.33	129.17 $\pm$ 76.66	20.67 - 95	57.30 $\pm$ 21.54	21 - 137.33	84.61 $\pm$ 37.47	5.6	<b>0.002</b>
Sal. (psu)	0.03 - 0.01	0.07 $\pm$ 0.02	0.03 - 0.21	0.10 $\pm$ 0.06	0.01 - 0.09	0.05 $\pm$ 0.02	0.01 - 0.52	0.11 $\pm$ 0.12	5.9	<b>0.002</b>
Tem. ( $^{\circ}\text{C}$ )	9.09 - 15.63	14.08 $\pm$ 1.87	11.31 - 15.9	13.69 $\pm$ 1.88	8.39 - 16.83	13.50 $\pm$ 2.02	9.04 - 16.29	12.51 $\pm$ 1.75	2.4	0.07
NO <sub>3</sub> -N ( $\text{mg l}^{-1}$ )	0.01 - 0.48	0.1 $\pm$ 0.16	0.01 - 0.07	0.023 $\pm$ 0.02	0.01 - 0.52	0.27 $\pm$ 0.22	0.01 - 0.51	0.20 $\pm$ 0.23	1.3	0.3
PO <sub>4</sub> <sup>3-</sup> ( $\text{mg l}^{-1}$ )	0.1 - 2.5	0.49 $\pm$ 0.66	0.03 - 2.5	0.52 $\pm$ 0.97	0.02 - 1.68	0.41 $\pm$ 0.41	0.02 - 2.4	0.51 $\pm$ 0.79	1.6	0.1
NH <sub>3</sub> -H ( $\text{mg l}^{-1}$ )	0.04 - 0.45	0.28 $\pm$ 0.13	0.17 - 0.45	0.29 $\pm$ 0.11	0.02 - 1.66	0.37 $\pm$ 0.39	0.04 - 0.73	0.30 $\pm$ 0.15	0.4	0.7

### 2.4.9 Land use and land cover along the urban-rural gradient

There were significant differences in LULC scores around the ponds along the urban-rural gradient at 10m ( $F_{3,49} = 2.7, p = 0.03$ ), 500m ( $F_{3,49} = 28.1, p < 0.001$ ), and catchment ( $F_{3,49} = 4.9, p < 0.001$ ) scales (Figure 2.6). LULC at 100m scale was not significantly different ( $F_{3,49} = 1.2, p = 0.3$ ) (Figure 3.5) around the ponds. Additionally, the percentage of forest ( $F_{3,49} = 2.7, p < 0.01$ ), grass ( $F_{3,49} = 5.3, p = 0.0001$ ) and urban cover ( $F_{3,49} = 12.9, p < 0.0001$ ) differed along the urban-rural gradient.

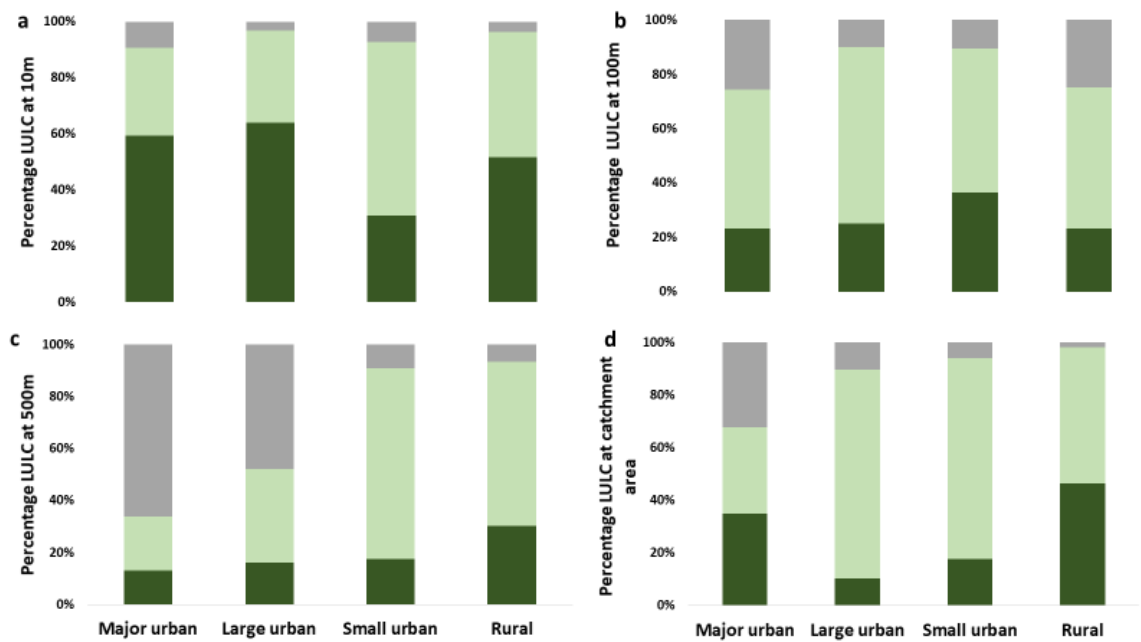


Figure 2.6 Percentage of LULC (forest, grass, urban) type along the urban-rural gradient at a) 10m, b) 100m, c) 500m and d) catchment scale. Olive bars: forest cover, green bar: grass, and grey: urban cover.

## 2.5 Discussion

The quality of water in a freshwater ecosystem is critical for the aquatic ecosystem's resilience by influencing trophic processes and niche availability. The quality of water governs the ecological health, particularly of the biotic community. I evaluated ponds, their catchments, and spatio-temporal variations in water quality. I also determined the relationships between water quality and LULC at multiple spatial scales in Auckland (an urbanised region).

### 2.5.1 Relationship between pond area and catchment area

This study's finding corroborated the widely recognised view of a positive relationship between the pond size and the catchments area (Davies *et al.* 2008; Gordon *et al.* 2004; Novikmec *et al.* 2016). However, the relationship was weak and similar to that reported by Novikmec *et al.* (2016) in Slovakia and Galbraith and Burns (2007) in New Zealand. The observed weak positive correlation indicates that there were some large ponds with a small catchment area and vice versa. The positive relationship has implications for the construction and management of ponds and adds to the discussion about the appropriate size of ponds to conserve (or create) to maintain biodiversity.

Nonetheless, it is easier and cost-effective to construct and manage ponds with small catchments (Davies *et al.* 2008; Novikmec *et al.* 2016; Sun *et al.* 2019). It must also be noted that pond area does not significantly affect biological diversity (Hamerlík *et al.* 2014; Novikmec *et al.* 2016; Williams *et al.* 2008). Therefore, it could be cost effective to manage smaller ponds with smaller catchments and still conserve biodiversity (Novikmec *et al.* 2016; Williams *et al.* 2008).

### 2.5.2 Seasonal and spatial variation in water quality

My findings showed significant seasonal variations in the physicochemical variables similar to previous studies by Zhang *et al.* (2019a), Ray *et al.* (2020), and Xu *et al.* (2020). Also, the spatial variations in variables can be related to temporal heterogeneity in catchment characteristics (Pratt and Chang 2012). These findings are indicative of the important seasonal and spatial characteristics in pond ecosystems.

The pH recorded in this study is similar to the range of 6.5 - 8.5 for freshwater ecosystems in New Zealand (ANZECC 2000). The % DO in the ponds was between 11.27% - 162.4%. The average % DO in the ponds was below the recommended saturation (80 - 90%) for freshwater ecosystems (ANZECC 2000). The % DO was higher in summer than winter, which was surprising because % DO levels typically decrease with increasing temperature (Tyagi *et al.* 2020). This higher % DO levels in summer than winter may be due to increased photosynthesis (Carey 2005), but further investigations will be required to confirm this hypothesis. The concentration of nitrates, phosphate, and ammoniacal nitrogen in the ponds was higher on average than in streams and rivers (see Appendix A: Table 3). However, the concentrations of these nutrients in some ponds were lower than those found in rivers (ANZECC 2000). The high concentration of nutrients in the ponds compared to rivers is not surprising as lotic systems have lower nutrient loads than small lentic systems (Galbraith and Burns 2007). The high concentrations of the nutrients in the ponds may be due to organic waste input in the ponds, especially for ponds in urban areas (Lake 2001; Novikmec *et al.* 2016; Ray *et al.* 2020; Sharma *et al.* 2016).

As expected, pond temperatures were higher in summer because of the photoperiod and the sun's intensity. The increase in temperature during the summer leads to increased photosynthesis and increased degradation of organic substances (e.g., microbial degradation) through photooxidation, photodegradation and the subsequent excessive release of ions into water bodies (Carey 2005; Ray *et al.* 2020). These processes contribute to higher pH levels in summer (Smith *et al.* 2016). In contrast, the lower pH in winter could be due to high precipitation diluting the water's alkaline materials' effects and the concentrations of chemicals (Ray *et al.* 2020; Tyagi *et al.* 2020). In addition, high evaporation in summer results in a reduction in the water volume, leading to an increment in the salt concentration of the water, possibly contributing to higher TDS, salinity, and conductivity levels of ponds.

Nevertheless, variables like conductivity and TDS are mostly governed by the LULC at broader spatial scales like the catchment scale (Jayawardana *et al.* 2017; Olson and Hawkins 2017). The concentration of TDS may also reflect anthropogenic impacts on the ecosystem (Olson and Hawkins 2017; Omoigberale *et al.* 2020; Pratt and Chang 2012). The correlations among the TDS, conductivity and salinity indicates that these variables are interdependent (Botwe *et al.* 2018; Tyagi *et al.* 2020).

The high concentrations of ammoniacal nitrogen in winter may be due to leaching (Larned *et al.* 2016; Malcolm *et al.* 2018; Shi *et al.* 2017). The high precipitation and low evaporation in winter

result in increased runoff and leaching of nutrients from the ground into soils and aquatic ecosystems (Malcolm *et al.* 2018). In contrast to ammoniacal nitrogen, phosphates levels were higher in summer. My results are similar to Carpenter *et al.* (2018), Zhang *et al.* (2019a), and Chaichana *et al.* (2011), who also reported high phosphorus levels in summer in watersheds in the United States of America, reservoirs in China and ponds in the UK, respectively. Carpenter *et al.* (2018) and Zhang *et al.* (2019a) found a positive association between agriculture and urban land cover with phosphate levels in summer. The concentration of nitrates did not differ between the seasons in my study. According to NIWA (2019), nitrate concentrations in New Zealand lentic ecosystems do not show seasonal differences, similar to our findings

Conductivity, TDS, and % DO were critical components of the water quality in the ponds across the seasons. Therefore, the hypothesis that nutrients (nitrate, phosphate and ammoniacal nitrogen) are the principal drivers of water quality is rejected. While ammoniacal nitrogen, salinity and pH were essential in summer, nitrate and phosphate were significant in winter. The differences in the importance of variables may be due to interactions between anthropogenic activities and geographical and climatic factors (Tyagi *et al.* 2020).

### **2.5.3 Land use/land cover and water quality**

The relationship between LULC pattern and water quality variables has been shown to vary at multiple spatial scales by many studies (Houlahan and Findlay 2004; Novikmec *et al.* 2016; Zhou *et al.* 2016; Nobre *et al.* 2020). This variation is because pollution sources and watershed characteristics vary spatially and differ in different regions (Nobre *et al.* 2020; Novikmec *et al.* 2016; Tu 2011; Zhou *et al.* 2016). After accounting for the effect of physical features, my results show that LULC at the catchment and 500m scales significantly determine the water quality in winter. This finding is similar to Nielsen *et al.* (2012) and Novikmec *et al.* (2016). Also, Nobre *et al.* (2020) and Houlahan and Findlay (2004) found that land use at the catchment scale was a key driver of water quality in lakes in Denmark and ponds in Slovakia. In the South Island of New Zealand, Galbraith, and Burns (2007) also reported that catchment LULC is a major driver of water quality of lentic ecosystems. In summer, however, LULC was significant at only the 100m scale. This result is similar to Declerck *et al.* (2006), who reported that adjoining land use of ponds in agricultural areas was significant in defining the water quality. However, direct comparison among freshwater ecosystems in different studies can be misleading and need to be done with caution considering differences in the environmental variables measured (Novikmec *et al.* 2016).

My models for summer, especially the full models ( $R^2$ : 29 - 34%), explained a higher proportion of variability in my dataset compared to the winter models, contrary to my hypothesis. The high variation in summer accounted for by the models is similar to the finding by Xu *et al.* (2020) and Zhang *et al.* (2019a). The model's high variation may be due to a higher concentration of pollutants input from runoff in summer compared to winter. During summer, nutrients are transported to aquatic systems through subsurface flows, meaning LULC and physical features significantly influence the physicochemical water quality variables (Zhang *et al.* 2019a). There is still, however, considerable unexplained variance. Other factors were beyond the scope of my study, for example, underlying soil type and the age of the pond, surface flow, trophic state, fish presence, and algal biomass. These unaccounted geomorphological variables and other biological and hydrological factors, such as surface flow, could account for some of the unexplained variations (Nobre *et al.* 2020; Novikmec *et al.* 2016).

Additionally, the relatively high variance explained in the full model compared to the partial model is indicative of the vital role of other physical features. In particular, the surrounding slope and altitude of ponds may influence water quality, especially in summer. Previous studies have found that the topographical characteristics (slope and altitude) of catchment areas are major factors affecting water quality (Ramião *et al.* 2020; Xu *et al.* 2020).

The RDA results revealed a seasonal difference in interactions between LULC and physicochemical water quality parameters. This seasonal difference may be due to increased overland runoff input to the ponds during winter. The RDA results also gave extensive descriptions of the effects of LULC on pond water quality at multi-spatial scales. My results showed that water quality was significantly influenced by LULC catchment and 500m scales in winter and all four spatial scales in summer. The impact of LULC has been linked to high levels of precipitation (Nobre *et al.* 2020). Therefore, it is not surprising that the influence of LULC was more consistent in summer. Despite the expected effects of precipitation on nutrient runoff, my results show an interactive effect of precipitation variability and LULC at different spatial scales on water quality, similar to findings by Nobre *et al.* (2020).

It is important to note that the variables we measured did not show similar trends and correlations in the two seasons studied. Nitrate concentration was weakly negatively associated with impervious surfaces but positively associated with grass and forest cover at both the catchment and 500m scales in summer. Ammoniacal nitrogen was negatively associated with catchment scale urban cover in both seasons. These associations indicate the complexity of

water quality determinants in anthropogenically influenced landscapes (Gadd *et al.* 2020). It is salient to note that the variables measured did not show similar trends and correlations in the two seasons studied. The negative correlations observed between slope and phosphorus, nitrate and ammoniacal nitrogen concentrations in winter are similar to those reported by Galbraith and Burns (2007). However, it is worth noting that their study sampled water quality only in the summer. The urban cover was positively related to phosphorus load at the catchment in summer but negatively correlated in winter. Most of the phosphorus input probably came from household and pet faeces exported through stormwater runoff into the ponds, as reported by Müller *et al.* (2020) and Hobbie *et al.* (2017). However, this seasonal disparity in the relationship may be because of precipitation. The nitrate concentrations were negatively correlated to urban cover at the catchment and 500m scale. This relationship may indicate the relatively lower importance of urban cover to the nitrate load in the ponds. The weak positive correlation between grass cover and nitrate concentrations may be due to the grass's absence or minimum grazing (Galbraith and Burns 2007). Also, the weak correlation between forest cover at both catchment and 500m scale and nitrate concentration is expected as nitrate influx is reported to be low in areas of high forest cover (Galbraith and Burns 2007).

This finding suggests that the overall variation of water quality variables is explained better by LULC at the catchment scale and 500m scale. However, in summer, some water quality variables such as pH, % DO, and TDS strongly correlated with LULC at a smaller scale (10m and 100m). These effects of scale imply that management protocols need to be applied on a large scale, but the impacts of management at a small scale should also be considered (Xu *et al.* 2020).

#### **2.5.4 The effect of human population density on physicochemical water quality**

In this study, the urban-rural gradient based on human population density was more influential in the physicochemical water quality in summer than winter. This result is not surprising because the high precipitation during winter is likely to dilute and override the effect of anthropogenic activities on the chemicals in the pond. Nitrate levels increased along the urban-rural gradient, where the lowest concentrations occurred in major and large urban ponds and the highest in rural ponds in summer. The results suggest that the nitrate concentration in the ponds may either be influenced directly by human activities or indirectly by organic matter mineralisation associated with nitrification (Ramião *et al.* 2020). Phosphate and ammoniacal nitrogen concentration did not vary significantly along the gradients. This finding supports the

supposition that natural processes rather than human activities determine the concentrations of these nutrients in the ponds (Ramião *et al.* 2020). Nitrates are prone to leach and are transported through subsurface flow, while ammonia and phosphate tend to be absorbed into the sediments (Carey 2005; Ramião *et al.* 2020). My findings suggest that anthropogenic activities impact nitrates more than ammoniacal nitrogen and phosphates concentrations.

Although the multivariate analysis showed no difference in water quality along the gradient in winter, individual physicochemical variables (conductivity, TDS, salinity, and % DO) differed. The relative high forest cover explains the relatively low temperatures recorded in ponds in rural areas (Orr and Buchwalter 2020). The high macrophyte cover in ponds in small urban areas could also be associated with the high % DO, low conductivity, TDS, and salinity observed in this study. The high salinity, conductivity and TDS levels recorded in ponds in the large urban area could be due to sea spray (Ray *et al.* 2020), as most of the ponds in these areas were closer to the sea than the ponds in the other areas. The ponds in the major urban areas were expected to be the most anthropologically impacted and have the poorest water quality. However, this was not so because of the relative high forest cover within these ponds' catchment areas, which likely provided a buffer for nutrient and sediment transport into the ponds (Nobre *et al.* 2020).

### ***Implications for freshwater systems management***

The protection and restoration of freshwater systems require an improved understanding of catchment LULC changes in urban areas and the response of the ecosystems to these LULC changes. This study provides an important step in improving the current understanding of such interactions. The findings in my study highlighted that the water quality of freshwater systems is strongly linked to LULC changes at larger scales. This relationship suggests that as catchments become increasingly urbanised, management measures should be instituted to minimise overland runoff flux that carries pollutants into the ponds. It is widely recognised that stormwater runoff is a primary source of stress to freshwater ecosystems (Walsh *et al.* 2012; Anim *et al.* 2019). Therefore catchment-scale stormwater control measures that have retention, detention, infiltration and harvesting objectives (e.g., bioretention systems) can be incorporated in urban development to capture and treat polluted stormwater before it reaches the ponds. Studies have shown that such an approach helps reduce water quality impairment of freshwater systems even in urban areas (Hatt *et al.* 2009; Bell *et al.* 2017). This intervention will particularly be critical in summer to minimize the worsening of water quality.

## 2.6 Conclusion

In this chapter, I investigated the relationship between ponds areas and catchment areas and assessed the water quality of ponds. I measured and compared a range of variables of 50 ponds along the human population gradient in Auckland (an urbanised region) in summer and winter. I also analysed the seasonal influence on the relationship between water quality and landscape properties at four different spatial scales from the ponds. Overall, there was a positive relationship between pond area and catchment area. Smaller ponds are expected to have smaller catchments, although this relationship was only weak. Nonetheless, based on my findings, the construction of many small ponds could be a better choice for conserving high biological diversity and reducing management costs. Water quality in the ponds was significantly poorer in summer than winter. Conductivity, TDS, and % DO were the main variables controlling the water quality in the ponds in both seasons. Additionally, LULC marginally influenced pond water quality in the Auckland region. The effects of LULC and physical features on water quality varied at multiple spatial scales and were seasonally dependent. LULC had a slightly stronger correlation with water quality variables in summer than winter. Moreover, the influence of LULC on overall water quality in the ponds were higher at the catchment scale than at 10m, 100m and 500m scales in winter but was prominent at the 100m scale in summer. The effects of the LULC types on water quality also varied at different spatial scales. These results indicate that to safeguard water quality, the protection and conservation of adjacent areas of the aquatic systems are essential. Therefore, it is fundamental to consider the LULC characteristics of the entire catchment area. The results demonstrate that land and water management decisions need to be spatially specific and account for seasonal variations, even when objectives are spatially uniform. The need for spatial specificity arises because ecological responses to nitrate and phosphate enrichment vary spatially and temporally. Water quality varied along the urban-rural gradient in summer, indicating the complexity of LULC, climate, and anthropogenic activities in determining water quality. Finally, my study provides vital insights into the relationship and the effects of LULC at multiple scales on pond water quality. These findings have implications for effective water quality management, contributing appreciably to the sustainable development of aquatic ecosystems.

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# Chapter 3

## Macroinvertebrate communities in ponds



### 3.1 Abstract

Ponds are habitats for unique macroinvertebrates communities and contribute to the biodiversity of freshwater ecosystems. However, the macroinvertebrate community structure and diversity in ponds have received little attention despite being one of the most widespread freshwater habitats in New Zealand landscapes. Therefore, understanding the diversity and the role of the environment in structuring macroinvertebrate communities in pond ecosystems is vital in predicting the impact of environmental changes on the community. In this chapter, I document the macroinvertebrate taxa composition in 12 ponds sampled across two different seasons (summer and winter) in Auckland, the most densely populated region in New Zealand. I also investigated the relationship between macroinvertebrate community diversity indices and water quality. I found a total of 68 taxa in the ponds, ranging between 15 and 47 per pond. Crustacea and insects were the dominant macroinvertebrates groups in the ponds. Hydrachnidae, Cladocera, Oligochaeta, Chironominae, *Sigara*, Notonectidae and Dugesidae occurred in all the ponds. The community composition of the macroinvertebrate varied among ponds, seasons and years. The taxa richness in the ponds were 55 and 52 in summer and winter, respectively. Water quality and other environmental factors influenced the macroinvertebrate community. The key water quality variables structuring the macroinvertebrate communities were the pH, salinity, and percentage dissolved oxygen (% DO). pH was negatively correlated with the number of taxa, and % DO was negatively correlated with macroinvertebrate abundance. Additionally, the percentage macrophyte cover and altitude of the ponds were the important physical features shaping the macroinvertebrate communities. Macroinvertebrate abundance and richness was positively correlated with percentage macrophyte cover. The number of taxa was positively correlated with the altitude of the ponds in winter. My findings show that ponds are rich in macroinvertebrates, but the communities are temporally variable. The potential ways for pond management to enhance their suitability for macroinvertebrates are discussed.

## 3.2 Introduction

Aquatic macroinvertebrates are animals greater than one millimetre long, without vertebrae and belong to the phyla Nematoda, Mollusca, Annelida, Turbellaria, Platyhelminthes, Cnidaria, Bryozoa, Nematoda, Porifera and Arthropoda (Batzer and Boix 1999; Chessman 2003). Macroinvertebrates can be as big as crayfish (approximately 400mm) or as small as a water flea (6mm) and can be seen with the eye in good light (Johnson *et al.* 1993; Mandaville 2002; Rosenberg and Resh 1993).

### 3.2.1 Macroinvertebrate community composition in ponds

Most research on pond macroinvertebrates has reported the dominance of insects, Crustacea, and sometimes annelids and gastropods (Baxter *et al.* 2004; Cottenie *et al.* 2003; Hill *et al.* 2016; Meland *et al.* 2020). For instance, in Spain, the macroinvertebrates were dominated by 27 insect taxa, especially Coleoptera and Hemiptera (Perez-Bilbao *et al.* 2015). Similarly, insects dominated in 31 ponds sampled in Norway (Meland *et al.* 2020). Insects (dominant taxa), mollusc, Crustacea, Oligochaeta, Collembola (springtails) and Hirudinea (leeches) has been reported in ponds in the United Kingdom (UK) (Briers and Biggs 2003; Cottenie *et al.* 2003; Hassall *et al.* 2012; Hill *et al.* 2015; Hill *et al.* 2016). Insects, mites, and Oligochaeta have been reported in stormwater ponds in Denmark (Stephansen *et al.* 2016). In Minnesota (USA), 45 taxa of macroinvertebrates sampled from 66 ponds consisted of insects, molluscs, and Crustacea (Baxter *et al.* 2004). Macroinvertebrate communities in New Zealand ponds tend to comprise ostracods, insects (mainly *Anisops*, *Sigara* and beetles), molluscs, and annelids (Barclay 1966; Kuschel 2012; Wissinger *et al.* 2009).

Different macroinvertebrates adapt to different environmental conditions (Batzer and Boix 1999; Chessman 2018). Most successful macroinvertebrates in ponds are habitat generalists with high tolerance range (Baxter *et al.* 2004; Lewis-Phillips *et al.* 2020). Although the macroinvertebrate community compositions at the higher taxonomic level are similar, they differ at the lower taxonomic level (Lewis-Phillips *et al.* 2020; Meland *et al.* 2020). Different community composition occurs between ponds within the same or different geographical areas (Lewis-Phillips *et al.* 2020). These disparities are because of differences in the pond's water quality, size, and hydroperiod (Batzer and Boix 1999; Boix *et al.* 2016; Wissinger *et al.* 2006; Wissinger *et al.* 2009).

### 3.2.2 Factors that influence macroinvertebrate compositions in ponds

Apart from the importance of water quality (see in Chapter 1, section 1.1.3), the type(s) of mesohabitat present in pond ecosystems are essential in structuring macroinvertebrate communities. Ponds with diverse mesohabitats have a higher diversity of macroinvertebrates than ponds with homogenous outlay. The different mesohabitats and riparian vegetation provide different food types to diverse taxa than a pond with identical mesohabitats and no riparian vegetation (Bazzanti *et al.* 2010; Blicharska *et al.* 2016; Milesi *et al.* 2016).

Furthermore, a positive correlation exists between the taxonomic richness of macroinvertebrates and macrophyte cover (Declerck *et al.* 2006; Johansson *et al.* 2019). Macrophytes provide abundant food and mesohabitats (Blicharska *et al.* 2016). Macrophytes play vital roles in shaping community diversity in ponds through nutrient availability, increased oxygen level and sediment stabilization (Blicharska *et al.* 2016; Declerck *et al.* 2006). Macrophytes also protect macroinvertebrates from predation (Bazzanti *et al.* 2010). Notwithstanding the benefits, higher densities of macrophytes in ponds may negatively affect the taxonomic composition of macroinvertebrates (Blicharska *et al.* 2016).

The amount of nutrients in a pond also, to a large extent, influences the type of macroinvertebrate community. For example, ponds with less organic pollutants have more sensitive taxa than those that are more polluted. This trend has been observed in many New Zealand freshwater systems (Biggs 2000). Furthermore, macroinvertebrate communities are affected by physical habitat conditions relating to topography and catchment position. For instance, muddy or weedy and less shaded ponds tend to support low macroinvertebrate richness (Moore 2003).

### 3.2.3 State of macroinvertebrates in New Zealand freshwater ecosystems and the knowledge gap

There is a minimum of 638 endemic freshwater macroinvertebrate species in New Zealand freshwater ecosystems. As of 2013, about 52% of these were classified as either at risk, threatened or data deficient (Weeks *et al.* 2016). Also, 82 taxa were at risk of extinction (Weeks *et al.* 2016). Recently, Drinan *et al.* (2020) reported that there is deficient data on 26% of the 675 freshwater macroinvertebrates, further emphasising the limited knowledge of freshwater macroinvertebrates (Weeks *et al.* 2016). In lentic ecosystems, 13 taxa are threatened whilst 15

taxa are at risk (Drinan *et al.* 2020). These latest findings have stressed the need for urgent attention, especially research into the cause of decline to prevent more losses and inventories to provide data on freshwater macroinvertebrate distribution. Worldwide, ponds are known to have taxa not found in other freshwater systems, which is a possibility for ponds in New Zealand (Oertli 2018). Therefore, the lack of data on macroinvertebrates in ponds represents a fundamental gap in freshwater conservation in New Zealand.

Research on the macroinvertebrates community in New Zealand has led to the development of biotic tools to assess the ecological status and level of impairment of some freshwater ecosystems. For example, the Macroinvertebrate Community Index (MCI) developed to assess rivers and streams is based on sensitivity scores assigned to 140 taxa in line with global trends (Greig and Galatowitsch 2016; Stark and Maxted 2007). These scores are assigned mainly to insects and Crustacea, which are the dominant taxa in streams and rivers (Shearer *et al.* 2015; Townsend *et al.* 1997). Apart from the MCI, the Wetland Community Index (WCI) and the Index of Ecological Integrity (IEI) has been developed for assessing wetlands (Maxted *et al.* 1999; Suren *et al.* 2011). However, no index has been developed for assessments of ponds. The lack of an index can be due to the lack of research on pond ecosystems. Since ponds are distinct from other aquatic ecosystems, there is a need to develop appropriate tools to evaluate their ecological integrity (Oertli *et al.* 2005). To develop an index, information on the community structure and diversity of macroinvertebrates is needed. In contrast to rivers, streams and lakes, knowledge of macroinvertebrate communities in ponds in New Zealand is still poorly developed despite an increase in research on freshwater ecosystems over the last two decades (Greig and Galatowitsch 2016; Drinan *et al.* 2020). Thus, a better understanding of temporal biodiversity patterns and the community structure of pond ecosystems is needed to provide baseline information. In this chapter, I assessed the temporal variation in macroinvertebrate community compositions in ponds and determined the relationship between the communities and environmental factors.

### **3.2.4 Aims and hypotheses**

The aims are to:

- i) enumerate and describe the macroinvertebrate community composition and distribution in ponds,
- ii) determine the temporal variations in the macroinvertebrate communities,

- iii) evaluate the influence and relationship of physicochemical water quality variables and physical features on the macroinvertebrate communities,
- iv) determine the abiotic factors that structure the macroinvertebrate communities.

I tested the following hypotheses:

- i) the macroinvertebrate community compositions will vary among ponds,
- ii) macroinvertebrate diversity indices will be higher in summer than in winter,
- iii) macroinvertebrate community will be positively correlated with macrophyte cover,
- iv) the difference in the seasonal community composition is associated with changes in physicochemical variables in the ponds.

### 3.3 Methods and analyses

#### 3.3.1 Methods

Twelve ponds in different landscapes (a subset of 50 ponds sampled in Chapter 2) were sampled once during the winter and summer seasons from 2018 - 2020. For details of the physical features of the sampled ponds, see Appendix B: Table 1. Details of the methods used in sampling physicochemical variables and other environmental factors are in Chapter 2, section 2.3.2.

Macroinvertebrate sampling was done between 11:00 am and 2:00 pm NZT from the ponds during summer and winter 2017 and 2018. Sampling was done after water quality (physicochemical variables) was measured. In many ponds, the locations where macroinvertebrates were sampled were similar to where the physicochemical variables were tested. Macroinvertebrates were sampled using a D-frame net (0.5mm). The number of mesohabitats for each pond was used to determine the number of samples to be taken and the duration of each sweep to obtain a total of 3 minutes of sweeping for the pond. Mesohabitats are distinct sections in the pond characterised by vegetation, rocky surfaces, open water, or shaded by trees that overhang the ponds (e.g., pond margin). On average, each mesohabitat was swept for  $\leq 1$  minute. During sampling, the net was vigorously swept vertically and horizontally from the bottom of the water column to the surface in each mesohabitat to collect macroinvertebrates (Epele and Miserendino 2016). A timer was started when sweeping began. After each sweep, the nets were turned inside out into a bucket to pour out the contents. Some of the pond water was used to wash out the net contents to empty the net of the

macroinvertebrates. Buckets containing the samples were labelled and transported to the laboratory for sorting and identification. The D-frame net was thoroughly washed and dried after sampling for each pond to prevent the spread or transfer of organisms between ponds.

### 3.3.1.1 Sorting and identification

The buckets' content was poured into a white plastic tray at the lab, a little at a time to pick out all macroinvertebrates without missing any. All unwanted debris and materials were carefully washed with water to dislodge any macroinvertebrate attached to them before they were discarded. Next, macroinvertebrates were sorted using forceps, pipettes, and a hand lens from the tray. The plastic pipette was used to pick out fragile organisms like oligochaetes and other worms like flatworms. The picked macroinvertebrates were placed in labelled (pond name, date of collection, and name of collector) 100ml plastic containers filled to a third with 70% ethanol for identification and preservation. Macroinvertebrates were identified as soon as possible after sorting by using standard keys. Macroinvertebrates were placed on a slide and positioned under a dissecting microscope (Olympus SZX7) (up to 40 x magnification) (Brown *et al.* 2016). The microscope was fitted with a camera (Infinity1-1M) connected to a monitor using the infinity capture software programme. All the macroinvertebrates in a sample were observed under the microscope. The photographs of the macroinvertebrates were captured, labelled, and used in identification.

Macroinvertebrate's identification guides used in the identification included NIWA and Landcare freshwater invertebrates guide (Manaaki Whenua 2017; NIWA 2016). Identification was done to the family level for about 70% of the macroinvertebrates collected. Organisms belonging to the taxonomic group Nematoda, Oligochaeta, Ostracoda and Cladocera were left at the higher taxonomic levels (Biggs *et al.* 1998; Vermond *et al.* 2009). These taxonomic levels were used because i) they are standard methods used in freshwater ecological studies (see Botwe 2017; Fierro *et al.* 2018; Hill *et al.* 2016), ii) there were limited experts who could identify to the species level, and ii) I was constrained by time to identify all individuals to the species level. Seven photographs of unidentified macroinvertebrates were sent to entomologist and freshwater taxonomist; Professor Russell Death to identify. Numbers of macroinvertebrates belonging to the same taxa were summed up as their abundance in the sample.

### 3.3.2 Data analyses

#### 3.3.2.1: Inventory of macroinvertebrates in ponds and temporal variations

The first section of the results documents the taxa that make up the macroinvertebrate communities in ponds and describes their temporal variation. Data used in this section were pooled across all 12 ponds and all seasons sampled. The inventory of macroinvertebrates in ponds consists of:

- i) a description of macroinvertebrates distribution in the 12 ponds sampled
- ii) abundances and relative abundances of different taxonomic groups
- iii) a description of the distribution of the five most abundant groups (Insects, Crustacea, Annelida and Chelicerata and Gastropoda).

I describe the macroinvertebrate community in each pond and the population of each taxa in all the ponds. The temporal variation (seasonal and annual) in the ponds macroinvertebrate community was assessed using PERMANOVA. A Bray-Curtis dissimilarity matrix was calculated on a 4<sup>th</sup> root transformed macroinvertebrate abundance data before PERMANOVA test was conducted (Bray and Curtis 1975). I tested for seasonal differences in the abundance of the dominant groups, and a SIMPER test was done to identify taxa contributing to the observed seasonal differences (Clarke & Ainsworth, 1993; Clarke and Gorley 2015). The PERMANOVA design was used to test for the temporal variations in the macroinvertebrate communities. Pond site was treated as a random effect ( $n = 12$ ), and season ( $n = 2$ ) and year ( $n = 2$ ) were treated as fixed factors. Sixty-eight variables were included in the analysis. The results highlight; i) the seasonal and annual differences in macroinvertebrate communities and diversity indices (Margalef's richness, Pilon's evenness, Shannon Weiner index) in the ponds, and ii) the taxa that contribute to the seasonal and annual differences in the communities. A general linear model was used to analyse the temporal variance in the biodiversity indices.

#### 3.3.2.2 Relationship between biotic (macroinvertebrate) and abiotic factors (physicochemical and physical parameters)

This section determined the relationship between the macroinvertebrate community composition, biodiversity indices, and the physical and chemical water quality variables. Also, I determined the environmental variables that were best predictors and explained the

macroinvertebrate community. Variance in the macroinvertebrate community explained by the physicochemical variables was analysed using a distance-based linear model (DISTLM) (Anderson *et al.* 2008). The physicochemical variables data were transformed (see Appendix B: Table 2 for details of transformation), normalised and standardised. Also, a Bray-Curtis dissimilarity was carried on a 4<sup>th</sup> root transformed data of the macroinvertebrate abundance data. The DISTLM analysis was carried out for each of the four sampling periods due to the annual and seasonal variation in the macroinvertebrate community. Spearman correlation was used to test the correlation between macroinvertebrate diversity indices and the physical parameters of the pond. Additionally, the effect of abiotic (Land use/land cover, physical and physicochemical variables) parameters on the macroinvertebrate community was analysed using DISTLM. Finally, the BIOENV routine in PRIMER was used to determine the best physical and physicochemical predictors of macroinvertebrates (Clarke and Warwick 2001; Anderson *et al.* 2008). All analyses were carried out using the PRIMER v 7 and R version 3.6. 1 software (Clarke and Gorley 2005; R Core Team 2019).

## 3.4 Results

### 3.4.1 Section 1: Inventory and temporal variation of macroinvertebrates in ponds

#### 3.4.1.1 The abundance of macroinvertebrates in ponds

A total of 26,801 macroinvertebrates belonging to six phyla, eight subphyla, and 68 taxa groups (Family, genus) were sampled and identified from the 12 ponds (Appendix B: Figure 1 shows photos of some of the macroinvertebrates sampled). Each of the eight subphyla was made up of different classes and orders. The most numerically abundant subphylum was Crustacea (38% of all macroinvertebrates sampled, followed by Hexapoda (26%). Hydrozoa (>1%) was the least abundant (Figure 3.1). The subphylum Hexapoda consisted of the class Insecta and Enthognatha, and the subphylum Crustacea consisted of the classes Branchiopoda, Ostracoda and Malacostraca. The subphylum Platyhelminthes consisted of classes Neophora and Rhabditophora. However, the three most abundant taxonomic classes were Insecta > Branchiopoda > and Rhabditophora. The least abundant classes were Hydrozoa, Enthognatha and Neophora.

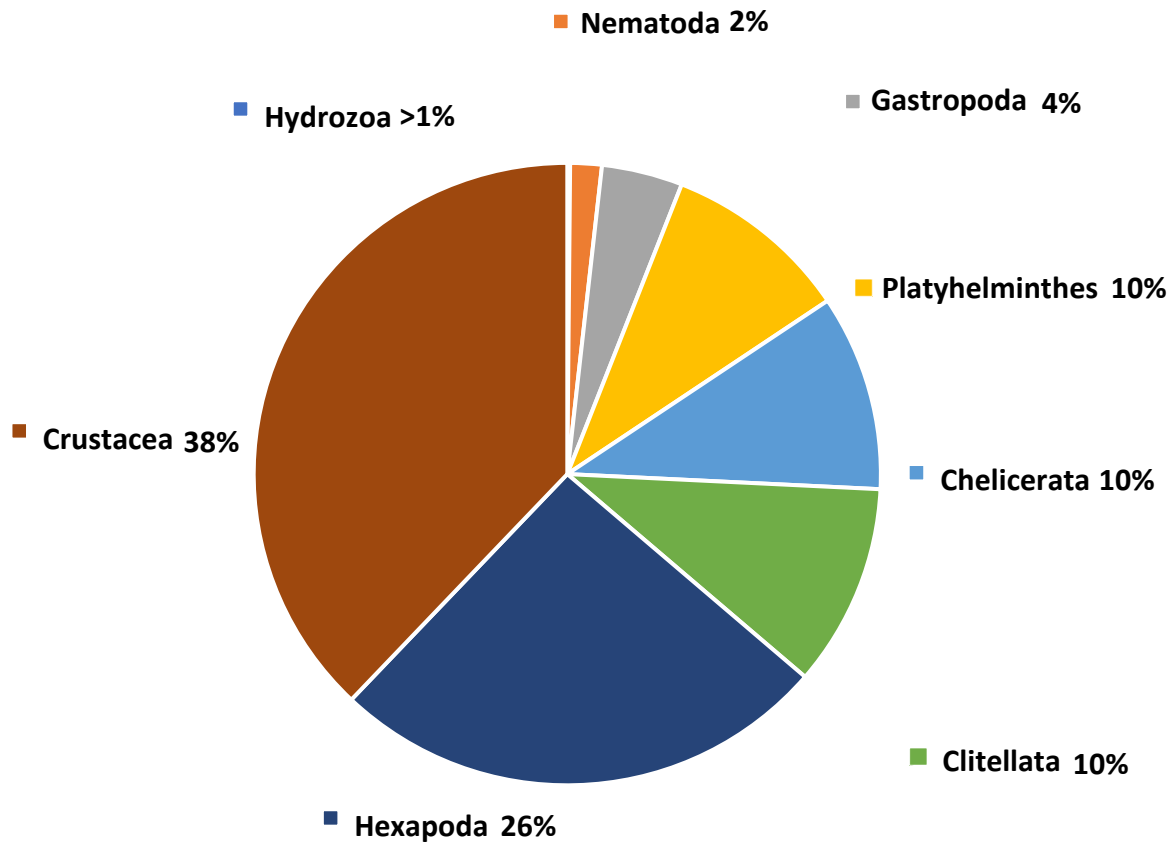


Figure 3.1 Percentage composition of macroinvertebrate sub-phylum in the ponds (n = 12).

#### 3.4.1.2 The abundance of dominant macroinvertebrates in ponds

##### Phylum Annelida

The phylum Annelida consisted of Oligochaeta (constituted 94% of the annelids sampled), Glossophoniidae (4%) and Salifidae (2%). The annelids constituted 9.5% of the macroinvertebrate's community. A total of 66% of the annelids occurred in winter. The community composition was not significantly different between seasons ( $F_{1,11} = 0.8, p = 0.4$ ) or among ponds ( $F_{1,11} = 0.8, p = 0.6$ ).

### **Subphylum Crustacea**

The subphylum Crustacea was most abundant in winter, constituting 43% and 33% in summer of the total macroinvertebrate sampled. Crustacea in the ponds consisted of seven taxonomic orders (Cladocera, Decapoda, Calanoida, Cyclopoida, Amphipoda, Isopoda and Ostracoda). Out of the 11 taxa, ten occurred in winter, and eight taxa occurred in summer. They consisted of 55.5% Cladocera, 22% Copepods and 21% Ostracods (which were the dominant groups), 2% Amphipods and < 1% Isopods. A PERMANOVA test showed that the community composition of Crustacea in the ponds was significantly different between seasons ( $F_{1,11} = 2.8$ ,  $p < 0.01$ ) and among sites ( $F_{1,11} = 2.2$ ,  $p < 0.0002$ ).

### **Subphylum Chelicerata**

The subphylum Chelicerata was represented by two families (Hydrachinidae: 99% of total abundance, and Pisauridae: 1%). They constituted 10% of the total macroinvertebrates sampled. Sixty-nine percent of all the Chelicerata occurred in winter. The community composition of this group was not significantly different between seasons ( $F_{1,11} = 1.8$ ,  $p = 0.1$ ) but different among sites ( $F_{1,11} = 2.1$ ,  $p = 0.04$ ).

### **Class Insecta**

The class Insecta consists of 37 families and seven taxonomic orders: Hemiptera (76.5% of the total insect abundance), Diptera (16%), Odonata (5.4%), Coleoptera (0.8%), Ephemeroptera (0.004%), Plecoptera (0.001%), and Trichoptera (1.2%). Hemiptera, Diptera, Odonata, and Trichoptera's were the common taxa and contributed to 97% and 96% of the insects' abundance in summer and winter. The taxonomic orders Coleoptera, Plecoptera and Ephemeroptera were rare. Coleoptera occurred in both seasons. Plecoptera was present only in summer but Ephemeroptera only in winter samples. Six taxonomic orders were recorded in winter and summer, respectively.

The most abundant insects were the Order Hemiptera, which constituted 80% and 62% of all insects in summer and winter. All the freshwater taxonomic families of Hemiptera in New Zealand were recorded. They included Corixidae (*Sigara*, *Diaprepocoris*), Mesovillidae, Vellidae, Notonectidae, and Hydrometridae. The families Corixidae and Notonectidae were the most dominant taxa in both seasons. In contrast, Hydrometridae was rare and was recorded only in summer.

Diptera made up 13.3% and 25.9% of the total insects recorded in summer and winter. Diptera was the most diverse order of insects in the ponds consisting of eleven taxonomic families. Three families (Chironomidae, Dixidae, Culicidae) dominated and occurred in both winter and summer. The rare families included Tabanidae, Muscidae, Tipulidae.

Odonata had a relative abundance of 4.1% and 9.0% in summer and winter, respectively, and included three common families (Lestidae, Coenagrionidae, Libellulidae). The families Corduliidae and Aeshnidae were rare and occurred in summer at only two sites (a farm and an ornamental pond).

The order Trichoptera contributed 1.4% and 0.8% of the insects' abundance in summer and winter. Eight families of Trichoptera were recorded in the ponds. However, the families Helicophidae, Leptoceridae and Hydroptilidae were common in both seasons. The rare families were Conoesucidae, Hydrobiosidae, Hydropsychidae, and only recorded in winter. In contrast, the families, Oeconesidae and Polycentropidae, were recorded only in summer.

Insects consisted of 36.7% ( $5390 \pm 49.61$ ) of the macroinvertebrates sampled in summer. The total abundance of insects was 500% more in summer than in winter ( $1498 \pm 20.34$ ), where this group was 12.3% of all macroinvertebrates. One pond recorded all seven taxonomic orders of insects. Insects were more evenly distributed in the ponds in winter ( $J = 0.7$ ) than in summer ( $J = 0.6$ ). Also, Shannon Weiner diversity was higher in summer (1.23) than in winter (1.06).

Multivariate analysis showed that, the insect community composition was significantly different among ponds ( $F_{1,11} = 2.4$ ,  $p < 0.0001$ ), between seasons ( $F_{1,11} = 4.5$ ,  $p < 0.0001$ ) and between years ( $F_{1,11} = 2.5$ ,  $p = 0.01$ ). The insect communities in the ponds were more similar (44%) in summer than in winter (37%). Drivers of seasonal dissimilarity based on SIMPER analysis were *Sigara* (12.5%), which was abundant in summer and Chironomidae (11%), Notonectidae (10%) and *Diaprepocoris* (8.6%), which were more abundant in winter.

### **Class Gastropoda**

The Class Gastropoda constituted 4% of the macroinvertebrates sampled and consisted of four families: Physidae (49% of the total Gastropod), Tateidae (23%), Planorbidae (27%) and Lymnaeidae (> 1%). Eighty-two (82%) of the gastropods occurred in summer. The community

composition of the gastropods was not significantly different between seasons ( $F_{1,11} = 1.1$ ,  $p = 0.4$ ) but different among ponds ( $F_{1,11} = 4.1$ ,  $p < 0.01$ ).

### 3.4.1.3 Distribution of macroinvertebrates in ponds in Auckland

Overall, 48 samples (4 samples from each pond) of macroinvertebrates were collected, identified, and analysed. The number of taxa in a sample ranged from 3 to 29, with an average of 15 taxa for the ponds. Forty percent of the samples had between 11 to 15 taxa present (Figure 3.2). The least number of taxa occurred in the pond in a water storage pond ('Supe'), and the highest occurred in an ornamental pond ('Bric'). The number of individuals for a sample ranged from 9 to 1746, with an average of  $558 \pm 456$ . The macroinvertebrate communities varied significantly among the ponds (Table 3.1). The distribution of macroinvertebrates in the ponds is shown in Figure 3.3. Crustacea and insects were dominant in all the ponds apart from a stormwater and golf course pond ('Hugh' and 'Helle'), where Platyhelminthes and Chelicerata were the dominant groups. Seven taxa (Hydrachinidae, Cladocera, Oligochaeta, Chironominae, *Sigara*, Notonectidae and Dugesiidae) out of the 68 taxa occurred in all 12 ponds; 25 taxa were common in half (6) of the ponds. The taxa Hydrozoa and Enthognatha occurred in 10 and six ponds, respectively (Figure 3.3).

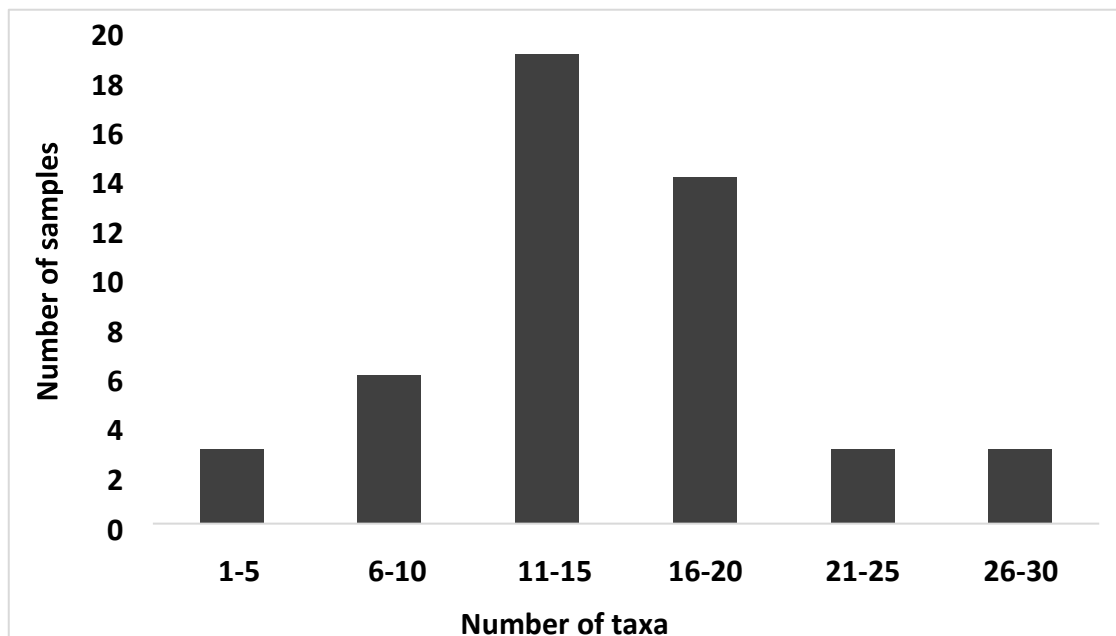


Figure 3.2 The frequency of the number of taxa occurring in the macroinvertebrate's samples. A total of 48 samples from 12 ponds.

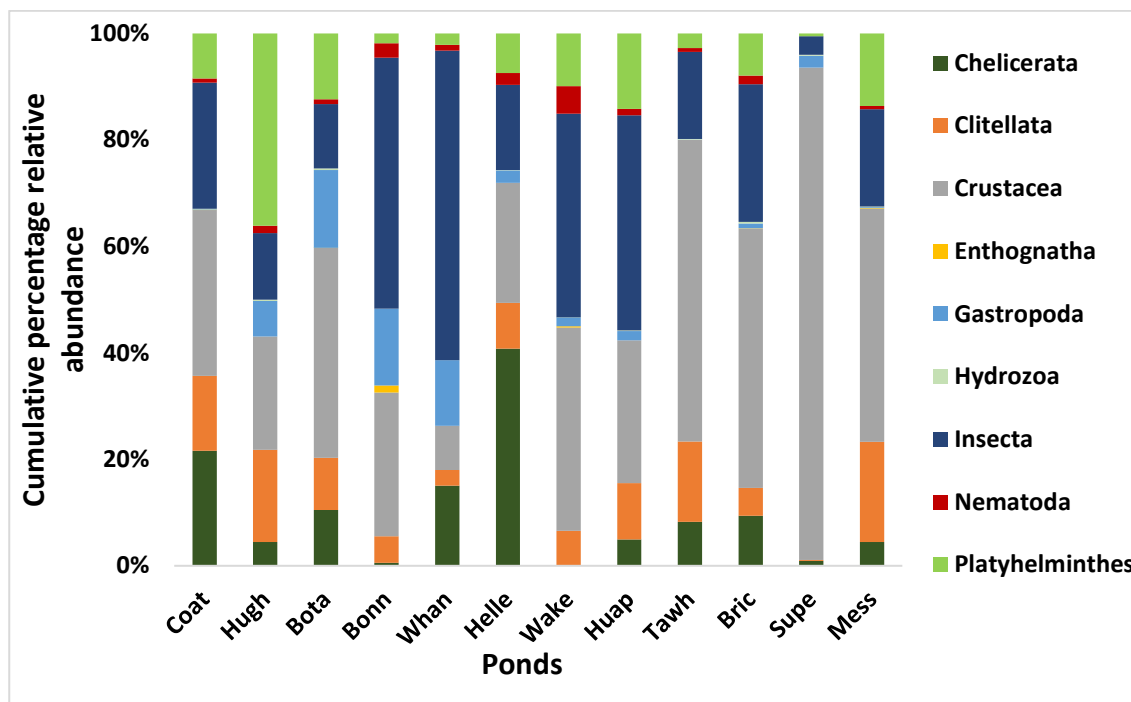


Figure 3.3 A stacked bar of the relative abundance of the taxonomic composition of macroinvertebrates in the 12 ponds.

### Biodiversity in individual ponds

The number of taxa in ponds ranged from 15 to 47, with an average of  $32 \pm 8$ . The highest Margalef's richness occurred in 'Bric' (3.7), and the least occurred in 'Supe' (1.4). The abundance of macroinvertebrates for a pond ranged from 548 to 3926, with an average of  $2234 \pm 1147$ . The highest macroinvertebrate abundance for a pond was 1313 ('Tawp'; a water storage pond), and the least was 246 ('Supe'). The highest and lowest Shannon Weiner diversity occurred at 'Bric' ( $2.15 \pm 0.30$ ) and 'Supe' ( $1.01 \pm 0.6$ ).

#### 3.4.1.4 Temporal trend in macroinvertebrate community

From the 48 samples, 14538 individuals were recorded in summer, and 12263 were recorded in winter. The macroinvertebrate abundance across the seasons were not significantly different ( $t = 0.7$ ,  $p = 0.5$ ). Fifty-two taxa were recorded in winter, and 55 taxa were recorded in summer. The number of taxa was not significantly different between seasons ( $t = 1.6$ ,  $p = 0.06$ ). Forty taxa were common in both seasons; 12 taxa occurred only in winter, and 15 taxa occurred only in summer. However, there was a significant difference ( $t = 1.6$ ,  $p = 0.0004$ ) in the community composition between the seasons.

The PERMANOVA analyses showed that macroinvertebrate community composition differed temporally (Table 3.1). The communities in 2019 were more similar than in 2018. On average, in 2018, the community composition between summer and winter was 42.6% similar, but in 2019, the communities were 45.7% similar. The highest variation (EMS: 483) in the macroinvertebrate communities was related to the ponds, followed by season (EMS: 140) (Table 3.1).

Table 3.1 Results of permutational multivariate analysis of variance (PERMANOVA) of the macroinvertebrate community. Df represents degrees of freedom; EMS represents estimated mean squares. Bold numbers indicate significant p-values.

	<b>Df</b>	<b>EMS</b>	<b>F</b>	<b>p value</b>
Ponds	11	483	3.1	<b>&lt; 0.001</b>
Season	1	140	4.6	<b>&lt; 0.001</b>
Year	1	121	4.1	<b>&lt; 0.001</b>
Season x Year	1	297	4.8	<b>&lt; 0.001</b>
Residual	33			

## Temporal macroinvertebrate heterogeneity

### 3.4.1.5 Annual variations in macroinvertebrates communities in summer

The macroinvertebrates communities differed between the two years sampled. Clitellata constituted 10% and 5% of macroinvertebrate abundance in 2018 and 2019. Gastropoda constituted 4% and 8% of the total abundance in 2018 and 2019. Also, there were more insects' than nematodes in 2018 than in 2019 (Figure 3.4a). A SIMPER analysis showed eight taxa (Notonectidae, DugesIIDae, *Sigara*, Oligochaeta, Hydrachinidae, Chironomidae, Cladocera, Ostracod) contributed to 71.6% and 71.9% of total abundance in 2018 and 2019, respectively. Although the major contributing taxa were similar, their abundances were different between the years. The taxa driving the annual differences and were also more abundant in 2018 were Hydrachinidae (Diss/SD = 1.24), Oligochaeta (Diss/SD = 1.31), and Notonectidae (Diss/SD = 1.23). On the other hand, Cladocera, which contributed to the annual difference (Diss/SD = 1.44), was more abundant in 2019.

### 3.4.1.6 Annual variations in macroinvertebrates communities in winter

The macroinvertebrates communities differed between the two years sampled. While insects constituted 20% of the total abundance in 2018, only 7% were insects in 2019. On the contrary, the relative abundance of Arachnida in 2018 was 11% and 17% in 2019 (Figure 3.4b). A SIMPER analysis showed that ten taxa contributed to 71.6% of the abundance in 2018, while eight taxa contributed to 71% of the macroinvertebrate abundance recorded in 2019. The taxa Hydrachnidae, Cladocera, and Ostracod, were more abundant in 2019 compared to 2018. Five taxa (Chironomidae, Oligochaeta, Cyclopoida, Dugesidae and Physidae) contributed to 30% and 39% of the total abundances in 2018 and 2019, respectively. The taxa that distinguished between the communities in 2018 and 2019 were nematodes (Diss/SD = 1.44), Glossiphonidae (Diss/SD = 1.19) and *Sigara* (Diss/SD = 0.97), which were all more abundant in 2018 and Hydrachnidae (Diss/SD = 1.21) which was more abundant in 2019.

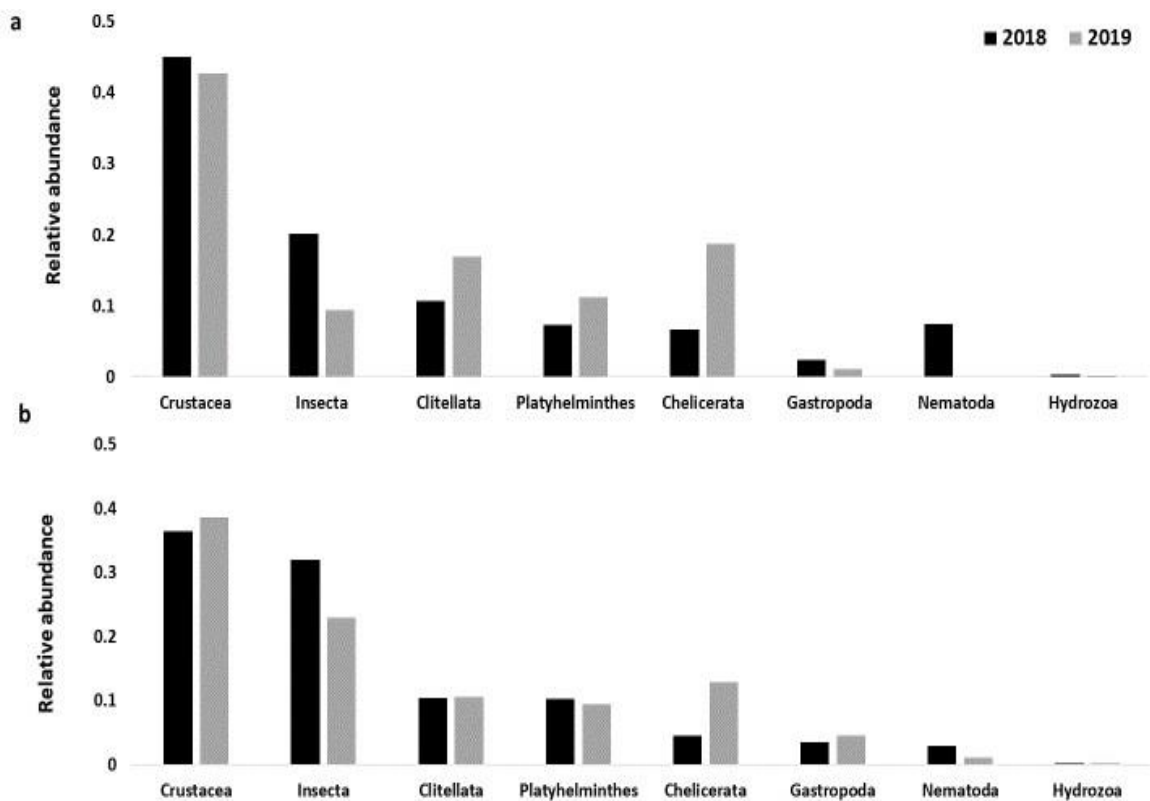


Figure 3.4 Bar plots of the relative abundance of the macroinvertebrate groups in a: summer and b: winter. Black bars represent 2018, and grey bars represent 2019.

### 3.4.1.7 Biodiversity indices (Margalef's richness, Pilon's evenness and Shannon Weiner)

Margalef's richness varied significantly between seasons ( $F = 4.5$ ,  $p < 0.01$ ), years ( $F = 18.4$ ,  $p < 0.01$ ) and among the ponds ( $F = 5.3$ ,  $p < 0.01$ ). Evenness did not differ across ponds ( $F = 0.1$ ,  $p = 0.9$ ), and between seasons ( $F = 2.1$ ,  $p = 0.1$ ); however, it differed between years ( $F = 5.8$ ,  $p < 0.01$ ). Evenness was higher in 2019 ( $0.74 \pm 0.10$ ) than in 2018 ( $0.64 \pm 0.14$ ). Shannon Weiner diversity differed among ponds ( $F = 3.6$ ,  $p < 0.01$ ), between year ( $F = 13.7$ ,  $p < 0.01$ ) where, it was significantly higher in 2018 ( $1.96 \pm 0.26$ ) than in 2019 ( $1.64 \pm 0.46$ ). However, Shannon Weiner did not differ between seasons ( $F = 0.1$ ,  $p = 0.8$ ). The generalised linear models (GLMs) explained more of the variation in macroinvertebrate richness ( $R^2 = 0.41$ : AIC = 99.4) than Shannon Weiner diversity ( $R^2 = 0.269$ : AIC = 42.3) or evenness ( $R^2 = 0.18$ : AIC = 43.7).

### 3.4.2 Section 2: Water quality in ponds

Spearman correlation between physicochemical variables in each sampling season is shown in a draftsman plot in Appendix B: Figure 2. There were some seasonal and annual variations in the physicochemical water quality variables in the ponds. For example, the pond water was more acidic in winter than in summer. Water pH was significantly higher in the summer of 2018 compared to 2019. Water temperature was significantly ( $p < 0.001$ ) higher in summer than in winter. There was more variability in the temperature in summer (Table 3.2). The percentage of dissolved oxygen (% DO) in the ponds was significantly higher ( $p < 0.01$ ) and more variable in winter ( $53.04\% \pm 2.74\%$ ) than in summer. The conductivity levels were not significantly different ( $p = 0.3$ ) between seasons. The salinity was not significantly different between seasons ( $p = 0.7$ ). Total dissolved solids did not differ between years in summer but differed in winter. Nitrate concentration was not significantly different between seasons. Similarly, the concentration of ammoniacal nitrogen was not significantly different between seasons ( $p = 0.7$ ). However, within seasons, ammoniacal nitrogen concentration in winter was significantly higher in 2018 ( $0.26\text{mg l}^{-1} \pm 0.26\text{mg l}^{-1}$ ) than in 2019 ( $0.11\text{mg l}^{-1} \pm 0.08\text{mg l}^{-1}$ ). Table 3.2 shows the temporal variations in physicochemical variables in the ponds.

Table 3.2 Temporal physicochemical variables of the ponds in 2018 and 2019 in summer and winter. Significant p-values (5%) between years are in bold.

Physicochemical variable	Summer			Winter		
	2018	2019	p value	2018	2019	p value
pH	6.94 ± 0.64	6.64 ± 0.75	<b>&lt; 0.01</b>	6.49 ± 0.56	6.60 ± 0.65	0.4
% DO	39.7 ± 19.7	36.4 ± 14.6	0.3	41.9 ± 23.7	64.9 ± 16	<b>&lt; 0.01</b>
Con. ( $\mu\text{Scm}^{-1}$ )	193.4 ± 143.8	188.1 ± 118	0.6	178.4 ± 114.8	228.6 ± 156.3	<b>0.03</b>
TDS (ppm)	94.9 ± 67.3	94.2 ± 59.5	0.8	89.3 ± 55.2	131.3 ± 106.6	<b>0.03</b>
Sal. (psu)	0.09 ± 0.07	0.09 ± 0.05	0.5	0.08 ± 0.05	0.13 ± 0.10	<b>0.03</b>
Tem. ( $^{\circ}\text{C}$ )	21.21 ± 2.81	20.15 ± 2.11	<b>0.01</b>	13.05 ± 1.04	14.36 ± 1.07	<b>&lt; 0.001</b>
$\text{NO}_3\text{-N}$ ( $\text{mg l}^{-1}$ )	0.19 ± 0.20	0.23 ± 0.22	0.6	0.20 ± 0.21	0.35 ± 0.15	0.3
$\text{PO}_4^{3-}$ ( $\text{mg l}^{-1}$ )	1.16 ± 0.80	1.31 ± 0.07	0.6	1.08 ± 0.90	1.64 ± 0.73	0.2
$\text{NH}_3\text{-H}$ ( $\text{mg l}^{-1}$ )	0.23 ± 0.14	0.16 ± 0.15	0.3	0.26 ± 0.26	0.11 ± 0.08	<b>&lt; 0.01</b>

### 3.4.2.1 Multivariate analyses of physicochemical water variables

PERMANOVA test showed that the water quality varied among ponds ( $F_{1,11} = 5.51$ ,  $p < 0.001$ ), between seasons ( $F_{1,22} = 12.11$ ,  $p < 0.001$ ), and years ( $F_{1,22} = 5.04$ ,  $p < 0.001$ ). There was also significant interaction between season and years ( $F_{1,11} = 3.10$ ,  $p = 0.01$ ). The water quality in summer did not vary between the years ( $F_{1,22} = 0.56$ ,  $p = 0.6$ ) but did for winter ( $F_{1,22} = 3.3$ ,  $p = 0.01$ ). There was more variability in the water quality parameters measured in winter compared to summer.

### 3.4.2.2 Drivers of physicochemical water quality in summer

A PCA for the physicochemical water quality variables was constructed for each year and season. This individual construction was because of the significant differences in the macroinvertebrate community assemblage in the years and seasons. This partitioning enabled the accurate determination of relationships between each sampling season. Conductivity, salinity, and TDS explained more variation in the physicochemical water quality variables in both seasons. In addition, ammoniacal nitrogen and pH were essential variables in summer. Phosphate and % DO were important in winter irrespective of the year (Table 3.3).

### 3.4.2.3 Temporal drivers of physicochemical water quality

In summer, PC1, PC2 and PC3 explained 86.4% of the total variation in the physicochemical water quality variables in 2018, while PC1 and PC2 explained 78% in 2019. Conductivity, TDS, and salinity were negatively loaded on PC1 in both years. PC2 in 2018 was heavily negatively loaded by phosphate and ammoniacal nitrogen. The PC3 was heavily loaded negatively by phosphate and positively by pH. The PC2 in 2019 was negatively loaded by nitrate and pH. Table 3.3 summarises the variations explained by each PC and the loading of each variable.

In winter, the first three and two PCA in 2018 and 2019 explained 80.2% and 76% of the total variation in the physicochemical water quality variables. Conductivity, TDS, and salinity were negatively loaded on PC1 in both years. Whilst phosphate was positively loaded on all three PCs in 2018, and nitrate was positively loaded on PC2 and PC3. However, PC2 was negatively loaded by pH in 2019 (Table 3.3).

Table 3.3 Loadings of physicochemical variables in principal component axes in summer (2018 and 2019) and winter (2018 and 2019). Loading  $\leq 0.4$  are in bold.

Eigenvectors of variables	Coefficients of linear combinations of variables making up PC's									
	Summer					Winter				
	2018			2019		2018			2019	
Variable	PC1	PC2	PC3	PC1	PC2	PC1	PC2	PC3	PC1	PC2
pH	0.3	-0.17	0.57	0.36	<b>-0.59</b>	0.01	-0.26	0.31	0.12	<b>-0.73</b>
% DO	0.32	0.05	0.11	0.21	0.04	0.32	-0.17	-0.32	0.26	-0.38
Con. ( $\mu\text{Scm}^{-1}$ )	<b>-0.47</b>	0.02	0.3	<b>-0.4</b>	-0.08	<b>-0.48</b>	0.10	0.16	<b>-0.5</b>	0.02
TDS (ppm)	<b>-0.45</b>	0.03	0.23	<b>-0.4</b>	-0.08	<b>-0.44</b>	0.17	0.13	<b>-0.57</b>	-0.12
Sal. (psu)	<b>-0.46</b>	0.03	0.25	<b>-0.41</b>	-0.04	<b>-0.44</b>	0.19	0.12	<b>-0.55</b>	-0.12
Tem. ( $^{\circ}\text{C}$ )	0.26	-0.2	0.23	0.23	-0.01	0.02	0.02	0.02	0.03	-0.14
$\text{NO}_3\text{-N}$ ( $\text{mg l}^{-1}$ )	0.32	0.2	0.37	-0.11	<b>-0.74</b>	-0.01	<b>0.63</b>	<b>-0.64</b>	0.13	-0.03
$\text{PO}_4^{3-}$ ( $\text{mg l}^{-1}$ )	0.0	<b>-0.65</b>	<b>-0.41</b>	0.03	0.29	<b>0.51</b>	<b>0.60</b>	<b>0.57</b>	-0.11	-0.35
$\text{NH}_3\text{-H}$ ( $\text{mg l}^{-1}$ )	-0.09	<b>-0.68</b>	0.32	<b>-0.53</b>	-0.07	-0.14	0.28	0.10	-0.08	-0.39
Eigenvalues	4.93	1.2	1.01	4.67	1.51	3.97	1.57	1.06	4.03	1.43
% Variation	59.6	14.5	12.3	59	19	48.2	19	12.9	56.1	19.9
Cumulative % variation	59.6	74.1	86.4	59	78	48.2	67.3	80.2	56.1	76

### 3.4.2.4 Relationship between macroinvertebrate community composition and physicochemical water quality

In summer 2018, the distance-based linear model (DISTLM) showed that PC1 - PC3 of the physicochemical water quality variables explained just 35% of the variation in the macroinvertebrate communities (AIC = 89.8) in the ponds. The first PC axis of the model was significant ( $p = 0.03$ ) in explaining the macroinvertebrate community composition but only explained 16.5% of the variation. In summer 2019, PC1 and PC2 of the model explained 15.5% of the macroinvertebrate communities (AIC = 89.7) but did not explain the variation in the community composition (Table 3.4). In winter, PC1 - PC3 in 2018 explained 30.1%, and PC1 and PC2 in 2019 explained 35% of the variations in the macroinvertebrate communities. All PCs except PC2 ( $p = 0.004$ ) in 2019 were insignificant in explaining the variation in the communities (Table 3.4).

Table 3.4 Results of DISTLM for summer (2018 and 2019) and winter (2018 and 2019). Significant p values (5%) are in bold.

PC	F	p value	Variation explained	Cumulative variation
Summer 2018 (AIC = 89.8 $R^2 = 0.36$ )				
PC1	1.99	<b>0.04</b>	0.17	0.17
PC2	0.84	0.5	0.07	0.24
PC3	1.51	0.2	0.12	0.36
Summer 2019 (AIC = 89.8 $R^2 = 0.36$ )				
PC1	1.28	0.2	0.11	0.11
PC2	0.42	0.9	0.04	0.15
Winter 2018 (AIC = 88.6 $R^2 = 0.30$ )				
PC1	1.36	0.2	0.12	0.12
PC2	1.69	0.1	0.14	0.26
PC3	0.49	0.9	0.04	0.3
Winter 2019 (AIC = 87.2 $R^2 = 0.35$ )				
PC1	1.04	0.4	0.09	0.09
PC2	3.54	<b>0.004</b>	0.26	0.35

#### 3.4.2.5 Relationship between water quality variables and macroinvertebrate biodiversity indices

In summer 2018, the water's pH was significantly and strongly negatively correlated with the total number of taxa ( $\rho = -0.7, p < 0.01$ ). The pH was positively correlated with Pilou's evenness ( $\rho = 0.7, p = 0.01$ ). Total dissolved solids were also strongly positively correlated with macroinvertebrate abundance ( $\rho = 0.7, p = 0.01$ ). Margalef's richness was significantly and negatively correlated with nitrate concentration ( $\rho = 0.7, p = 0.01$ ). Additionally, % DO was significantly and positively correlated with evenness ( $\rho = 0.7, p = 0.02$ ) but negatively correlated with the macroinvertebrate abundance ( $\rho = -0.7, p = 0.01$ ) (see Appendix B: Figure 3). In 2019, the physicochemical water quality variables were not significantly correlated with the biodiversity indices. Evenness was negatively correlated with pH ( $\rho = -0.6$ ) and the Shannon Wiener index was negatively correlated with pH ( $\rho = -0.6$ ) and temperature ( $\rho = -0.5$ ).

In contrast, in winter 2018, the physicochemical water quality variables were not significantly correlated with the biodiversity indices. The number of taxa was negatively correlated with % DO ( $\rho = -0.5$ ). Macroinvertebrate abundance was negatively correlated with temperature ( $\rho = -0.4$ ) and ammoniacal nitrate ( $\rho = 0.4$ ). Margalef's richness was also negatively correlated with % DO ( $\rho = -0.5$ ) and orthophosphate ( $\rho = -0.5$ ). Shannon Wiener diversity was negatively correlated with % DO ( $\rho = -0.5$ ) but positively correlated with nitrate ( $\rho = 0.5$ ). In winter 2019, pH was significantly and negatively correlated with number of taxa ( $\rho = -0.7, p = 0.01$ ) but positively with Pilou's evenness ( $\rho = -0.7, p < 0.01$ ). Macroinvertebrate abundance was significantly and negatively correlated with % DO ( $\rho = -0.8, p = 0.001$ ) and temperature ( $\rho = -0.7, p < 0.01$ ) (Figure 3.5).

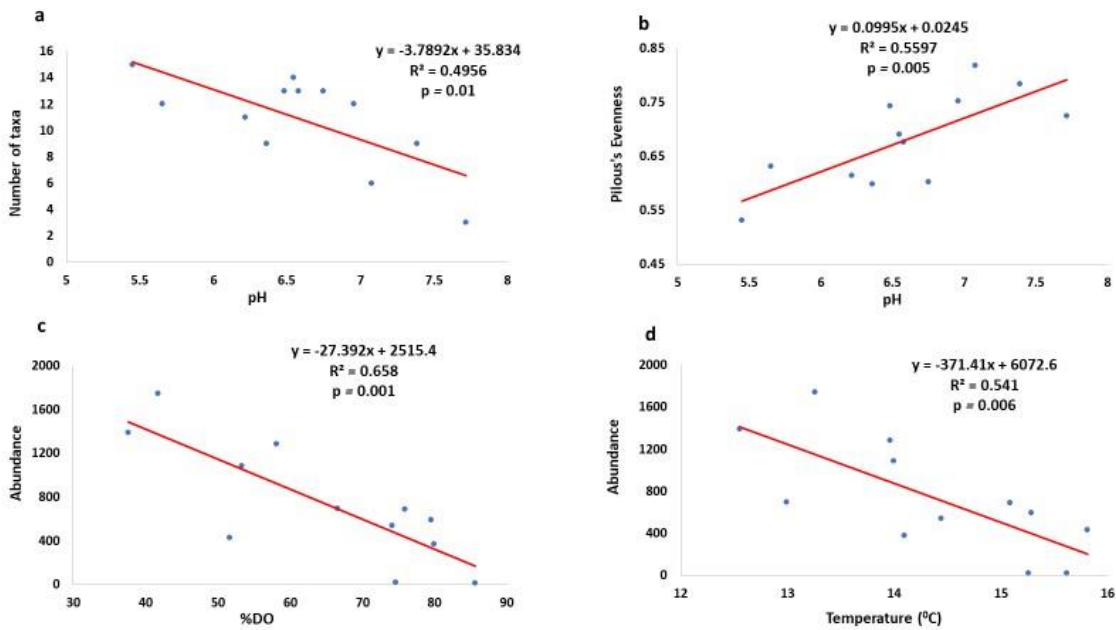


Figure 3.5 Linear regression plot of biodiversity indices (a: number of taxa and temperature, b: pilous' evenness and pH, c: abundance and % DO and d: abundance and temperature) and physicochemical water qualities in winter 2019. P-values and the regression equation is reported.

#### 3.4.2.6 Physical parameters around the ponds

The physical parameters of the ponds assessed were catchment area, area, depth, altitude, percentage cover by macrophyte, slope, and distance to road and water. Most of the pond's physical properties stayed the same throughout the sampling period apart from the depth and percentage macrophyte cover. The area of the pond ranged from 51m<sup>2</sup> to 7202.8m<sup>2</sup>. The distance between the closest pond to a road was 4.6m, and the farthest was 354.6m. The shortest distance between a pond and a nearby aquatic system was 14.7m, and the farthest was 317m. The average pond depth was 1.08m ± 0.14m. The % macrophyte cover in the ponds was 0 - 95% (Table 3.5). The ponds catchment areas were significantly negatively correlated with the pond's depth ( $\rho = -0.7$ ,  $p = 0.01$ ). Additionally, the pond's altitude was significantly positively correlated with the % macrophyte cover ( $\rho = 0.7$ ,  $p < 0.01$ ). The pond area was positively but weakly correlated ( $\rho = 0.1$ ,  $p = 0.6$ ) to the catchment area (Table 3.5).

Table 3.5 Physical parameters of the ponds sampled. Average values are reported with '±' standard deviation

Physical parameters	Min-Max	Average ± SD
Area (m <sup>2</sup> )	51.0 - 7202.7	3084.0 ± 2468.9
Catchment area (m <sup>2</sup> )	428 - 31762	5130.2 ± 8715.9
Altitude (m)	4.5 - 55.5	28.3 ± 16.1
% Cover	0 - 95	43.5 ± 38.7
% Slope	11.3 - 39.3	30.2 ± 11.5
Distance to road (m)	4.6 - 354.6	99.1 ± 116.5
Distance to waterbody (m)	14.7 - 317	68.4 ± 85.1
Depth(m)	0.8 - 1.4	1.1 ± 0.1
Land Cover/Land Use (LULC)		
% Forest (500m)	2 - 46	16 ± 14
% Grass (500m)	1 - 69	26 ± 26
% Urban (500m)	17 - 83	52 ± 20
% Forest (catchment)	1 - 83	23 ± 29
% Grass (catchment)	5 - 99	68 ± 35
% Urban (catchment)	0 - 65	9 ± 2

#### 3.4.2.7 Relationship between physical parameters of the ponds and biodiversity

The relationship between the physical parameters of the ponds and the macroinvertebrate diversity indices varied temporally. In summer 2018, the % macrophyte cover was significant and strongly positively correlated with the number of taxa ( $\rho = 0.7$ ,  $p < 0.01$ ) and macroinvertebrate abundance ( $\rho = 0.8$ ,  $p = 0.04$ ). The forest cover at the 500m distance away from the pond was positively correlated with the number of taxa ( $\rho = 0.7$ ,  $p = 0.01$ ) and Margalef's richness ( $\rho = 0.7$ ,  $p = 0.02$ ). In summer 2019, the physical parameters were weakly correlated with the biodiversity parameters. However, the forest cover at the 500m distance away from the pond was strongly positively correlated to Margalef's richness ( $\rho = 0.8$ ,  $p = 0.001$ ) and the number of taxa ( $\rho = 0.8$ ,  $p < 0.01$ ).

In winter 2018, the abundance of macroinvertebrate was strongly correlated to altitude ( $\rho = 0.8$ ,  $p = 0.002$ ). The amount of forest cover ( $\rho = -0.8$ ,  $p = 0.001$ ) and grass cover ( $\rho = 0.8$ ,  $p < 0.01$ ) at the catchment area was significantly and correlated to Shannon Weiner diversity. Similarly, % forest cover at the 500m was positively correlated to Margalef's richness ( $\rho = 0.7$ ,  $p = 0.02$ ). In winter 2019. The number of taxa was positively correlated with altitude ( $\rho = 0.8$ ,  $p < 0.01$ ) and

% macrophyte cover ( $\rho = 0.7$ ,  $p < 0.01$ ). Margalef's richness was also positively correlated with altitude ( $\rho = 0.7$ ,  $p = 0.01$ ). Macroinvertebrate abundance was strongly negatively correlated with the slope ( $\rho = -0.7$ ,  $p < 0.01$ ) and % forest cover at the 500m distance but positively correlated with % macrophyte cover ( $\rho = 0.7$ ,  $p = 0.02$ ) and % forest cover at the 500m distance ( $\rho = 0.8$ ,  $p < 0.001$ ) away from the ponds.

#### **3.4.2.8 Physicochemical, physical and land use, land cover (LULC) parameters and macroinvertebrate community composition**

The land use, land cover (LULC) types combined with the physical parameters and the water quality influenced the macroinvertebrate community differently at the 500m and catchment scales than water quality alone. These variables with the LULC at the catchment scale better structured the community than at the 500m scale (Figure 3.6). Other important abiotic factors were ammoniacal nitrogen (500m scale), conductivity, and salinity (catchment scale). A DISTLM and dbRDA analysis showed that in summer 2018, altitude was an important contributor to the variation in the macroinvertebrate community at the 500m and catchment scales. In summer 2019, % grass cover and the catchment areas were essential at both LULC scales. Additionally, the percentage of urban cover and macrophyte covers were important factors in the catchment and 500m scales. In winter, the pH and altitude were the essential abiotic factors at the catchment scale in 2018 and 2019 and the 500m scale in 2019. At the 500m scale, in 2018, conductivity and phosphate were significant in structuring the macroinvertebrate community.

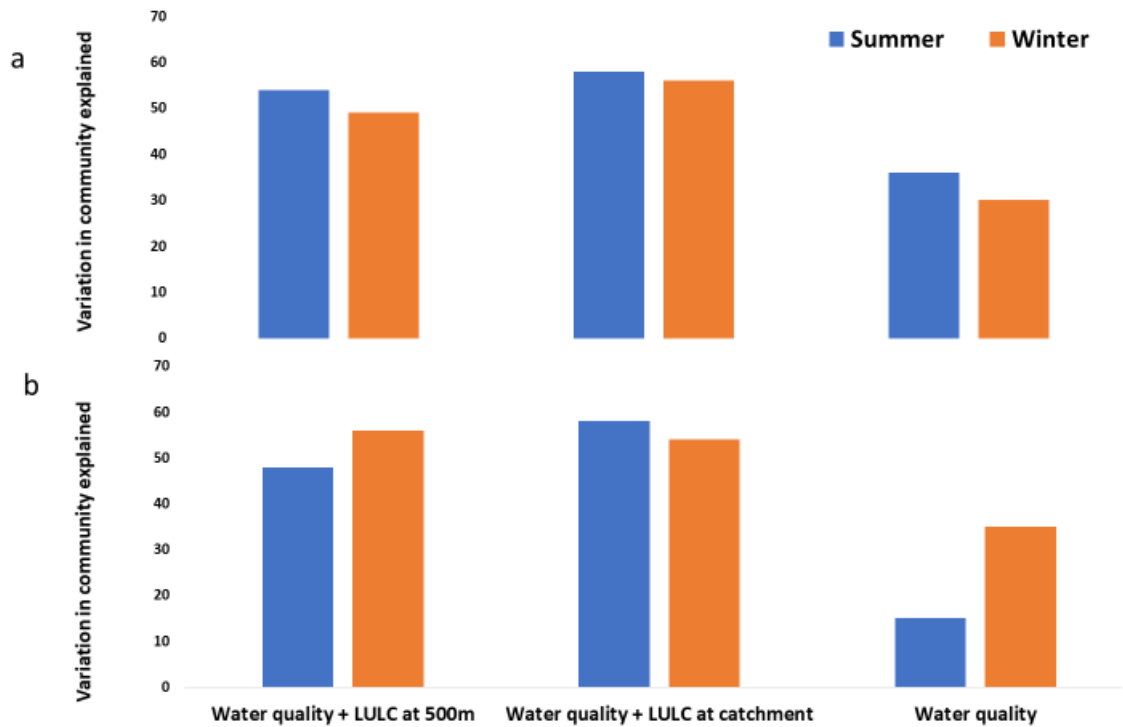


Figure 3.6 Variation in the macroinvertebrate community explained by abiotic factors at different scales in a: 2018 and b: 2019. Blue bars represent variations explained in summer, and the orange bar represents variations explained in winter.

Finally, I used the BIOENV routine to predict the environmental parameters that best predicted the macroinvertebrate composition irrespective of the season. Out of the 18 parameters, pH, salinity, altitude, distance to road, and pond depth were significant ( $p = 0.003$ ) and the best predictors of the macroinvertebrate community. These five parameters together yielded a weighted Spearman correlation of 0.29. See Appendix B: Table 3 for details.

## 3.5 Discussion

### 3.5.1 Macroinvertebrate community composition and distribution

In this chapter, I investigated the temporal macroinvertebrate communities in ponds and their relationship with environmental factors. The results show that ponds support a rich macroinvertebrate faunal community. The macroinvertebrate richness for a sample was highly variable (3 to 29 taxa). Most samples had 11 to 20 taxa, similar to those in rivers in the Auckland region (Moore and Neale 2008). The least taxa richness occurred in an artificial water storage pond which was lined with polyethylene. This richness was exceptionally low as the next taxa richness was 18. Similar to sample richness, the highest taxa richness in the ponds is comparable to taxa richness in rivers and streams in this region. In the rivers in Auckland, Tablada (2018) reported the highest taxa richness to be 45, with an average of 10 to 24 taxa. The lowest taxa richness in the ponds from my study was 100% higher than the lowest taxa richness of 7, recorded in Oakley stream (Auckland) (Moore and Neale 2008; Tablada 2018). Similar findings have been recorded in the UK, where ponds supported relatively higher taxa richness than rivers (Hassall and Anderson 2015; Hill *et al.* 2016).

The high taxonomic richness reported in ponds is associated with diverse food resources and better environmental conditions, such as the presence of macrophytes and algae. High taxa richness in the ponds is indicative of a healthy community (Dube *et al.* 2020; Mandaville 2002; Tamiru 2018) and explains why sensitive taxa (Ephemeroptera, Trichoptera, Plecoptera) were observed in ponds with high richness. Compared to ponds in the UK and Spain, the ponds I sampled had low taxa richness (Hassall and Anderson 2015; Hill *et al.* 2016; Perez-Bilbao *et al.* 2015). This low richness is not surprising due to the geographical isolation and the generally low biodiversity richness in New Zealand, a Southern Hemisphere temperate country (Garrett-Walker *et al.* 2020).

Overall, the community composition of macroinvertebrates differed among the ponds. Ponds exhibit higher variability in taxa richness than other freshwater ecosystems (Angélibert *et al.* 2004; Hassall and Anderson 2015). These differences can be due to the macroinvertebrates adaptability to survive in each pond (Botwe 2017). In addition, there were differences in the habitat structure and the substrata of individual ponds. For example, some of the ponds sampled had a combination of rocky and soft bottom substrate, while others had only soft bottom

substrate. Also, while some ponds had a high macrophyte cover, others had a low cover. These habitat heterogeneities provided diverse opportunities for different macroinvertebrate taxa to colonize and thrive, resulting in high community variability (Hill *et al.* 2016).

Crustacea and insects were the most abundant and most taxa rich groups in the ponds, similar to findings by Perez-Bilbao *et al.* (2015) in Spain and Boix *et al.* (2016) in the Mediterranean. Insects are ectothermic organisms; therefore, temperature greatly influences their physiology. The temperature changes related to their physiology causes a seasonal cycle in insect abundance, especially in temperate regions (Haggag *et al.* 2018; Hawkins *et al.* 1997). Temperatures below 15°C slow down development in some insects and cause others to undergo diapause (suspend development) (Gilbert and Raworth 1996; Hawkins *et al.* 1997). This strategy can lead to a low abundance and richness of insects in winter, as most taxa will likely hibernate.

Few taxa of the insect Order Diptera dominated by Chironomidae, Dixidae, and Culicidae, were recorded compared to the high richness of this order in rivers and streams (Moore 2003). On the contrary, all the freshwater taxonomic families of the Order Hemiptera recorded in New Zealand were present in the ponds. This high richness suggests that ponds are suitable for Hemiptera, confirmed by the occurrence of Corixidae and Notonectidae in all the ponds. The dominance of Hemiptera (Corixidae, Notonectidae) also suggests a high level of organic pollution and confirms their large temperature tolerance levels (Haggag *et al.*, 2018). The taxa *Sigara* and Notonectidae are common in pond ecosystems (Perez-Bilbao *et al.* 2015). Additionally, the insect taxa composition was expected as Ephemeroptera, Trichoptera, and Plecoptera were recorded in low abundance and low diversity. These taxa are sensitive to water quality and are more abundant in rivers and streams with low organic pollution and high dissolved oxygen levels in New Zealand (Haggag *et al.* 2018; Moore 2003).

Generally, the dominant taxa in the ponds included Chironomidae, Oligochaeta, Hydrachinidae, Cladocera, and Ostracod. Similar results have been reported in lakes in Canterbury (New Zealand) (Timms 1983). Again, Oligochaeta, Chironomidae, and Crustacea are well adapted and dominant groups in pond ecosystems (Brooks 2000; Martins *et al.* 2020). Chironomidae has a broad pollution tolerance level and can survive even in severely polluted (organic) and harsh environmental conditions (Haggag *et al.* 2018; Martins *et al.* 2020; Scheibler and Ciocco 2011). Therefore, their abundance may indicate pollution in the ponds. Similarly, the abundance of Oligochaeta in the ponds is expected due to their ability to survive in ecosystems with high conductivity and pollution levels (Medupin 2020; Scheibler and Ciocco 2011). High abundance

and distribution of Oligochaeta have been reported in rivers in the UK and urban streams in the Auckland region (Allibone *et al.* 2001; Medupin 2020). In this study, the abundance of Oligochaeta was positively related to the increase in macrophyte cover. Macrophytes provide refuge to reduce predation risk and enhance oligochaetes' ability to obtain food from periphyton (Bazzanti *et al.* 2010).

The Shannon Wiener diversity reported from the ponds in this study (0.2 to 2.3) is similar to diversity values in Alpine ponds and lakes in Europe, where the diversity varied between 1.3 to 1.9 (Martins *et al.* 2020). However, the taxa richness and the variability of community composition compensate for the low Shannon Wiener diversity. The pond with the lowest diversity is not surprising because that pond (water storage pond) was lined with polyethylene to preserve the clarity of the water. The polythene substrate prevented macrophytes growth; hence, few mesohabitats, low or no oviposition sites and limited food for macroinvertebrates.

### 3.5.2 Temporal variation in macroinvertebrate composition

Several studies have reported temporal variation in the macroinvertebrate communities in aquatic ecosystems (Briers 2014; Hassall *et al.* 2012; Hill *et al.* 2016). Macroinvertebrate communities in ponds show the most temporal variations (Brooks 2000). These variations are influenced mainly by seasonality (Martins *et al.* 2020; Yanygina *et al.* 2018). It is therefore not surprising that the macroinvertebrate communities varied seasonally and annually.

Although the abundance of the taxa varied annually, the composition was reasonably stable, similar to findings by Brooks (2000) in the USA. The annual difference recorded was likely a function of variation in abundance than changes in composition. Macroinvertebrate community compositions also varied among individual ponds and across the years. The inter-annual disparities explained these variations in community compositions in the pond's physicochemical variables, similar to the finding by Hassall and Anderson (2015). Similar variations and relationships have been reported in ponds in France (Angélibert *et al.* 2004). These differences may also have been observed because of differences in the taxa's survival abilities and the taxa's niche preference in the ponds (Botwe 2017; Sanderson *et al.* 2005). These findings support the assertion that no two ponds have similar macroinvertebrate compositions (Meland *et al.* 2020; Perez-Bilbao *et al.* 2015).

Macroinvertebrate community compositions also varied seasonally within ponds. Ponds were more similar in their community compositions in winter than in summer. Similar seasonal variations have been reported in streams in Australia (Botwe 2017). The differences in community compositions between seasons may be because different taxa show differential success between seasons according to their particular resilience (Blanchette and Pearson 2012; Botwe 2017). Particularly, the high abundance of Hemiptera in summer is expected because they tolerate high temperatures (Haggag *et al.* 2018). In summer, the relative higher abundance of insects was also expected because insects thrive and develop faster at high temperatures between 15°C to 27°C (Gilbert and Raworth 1996; Hawkins *et al.* 1997). Additionally, the contribution of Notonectidae and Corixidae (*Sigara*) to the community abundance in summer indicates that summer conditions in the ponds are more suitable for these taxa. The findings suggest that sampling in more than one season will provide better information on the taxa diversity in the pond, but for a rapid study, sampling in summer is recommended.

There was more variability in the biodiversity indices (Margalef's richness, Piloni's evenness and Shannon Weiner diversity) in winter compared to summer. The higher taxa richness recorded in summer than winter may be due to a more favourable summer condition (favourable physicochemical variables and food availability). Additionally, higher rainfall (characteristic in winter) is associated with lower taxa richness (Barquín and Death 2004). Williams (1996) and Brooks (2000) reported seasonal differences in taxa richness in ponds.

Although SIMPER selected similar taxa to contribute to the community composition in summer, their abundance was different between the years. This similarity in the summer compositions correlates with the physicochemical water quality conditions and may indicate that the community compositions in summer are more stable. Nematodes, Glossiphoniidae, *Ischnura* (Odonata), Cyclopoida, and Physidae, were more abundant in winter. Nematodes have been documented to colonise and increase in abundance in aquatic systems in winter (Michiels and Traunspurger 2005). The high richness of Odonata in winter in New Zealand is associated with high humidity, which is more favourable for Odonata (Manwar *et al.* 2016). The high abundance of Physidae recorded in winter was not surprising because they prefer lower temperatures. This preference notwithstanding, Physidae have high-temperature tolerance ability (Zukowski and Walker 2009). The abundance of eurythermal Cyclopoida in winter has been reported by Gaponova (2014) in ponds in Ukraine.

### 3.5.3 Relationship between macroinvertebrates and physicochemical water quality and physical parameters of the ponds

The physical and chemical variables in the ponds also showed seasonal variations. There were seasonal differences in variables like conductivity, temperature, percentage dissolved oxygen concentration, and pH. Similar findings have been reported in Alpine ponds in Switzerland (Martins *et al.* 2020). The physicochemical variables like conductivity, salinity and TDS were essential in the water quality of the ponds. Conductivity was negatively correlated to macroinvertebrate diversity in ponds. This correlation is contrary to Meland *et al.* (2020) findings but similar to Johnson *et al.* (2013). The negative correlation is likely due to the intolerance of the taxa to the conductivity levels. An increase in conductivity can cause a shift in the macroinvertebrate compositions from sensitive taxa to pollution tolerant taxa (Cianciolo *et al.* 2020; Dube *et al.* 2020). Conductivity is significant in discriminating among the community composition in different gradients and drive macroinvertebrate composition differences (Botwe 2017; Meland *et al.* 2020). The positive correlation between TDS and macroinvertebrate abundance is expected, and similar results have been reported by Barman and Gupta (2015).

Dissolved oxygen is considered the most important abiotic factor that influences the aquatic systems (Haggag *et al.* 2018), and its depletion is an indication of pollution. The low % DO levels in summer are expected due to the high temperatures, as reported by Haggag *et al.* (2018). Also, the low % DO concentration in summer could be due to the high decomposition rate of macrophytes and suspended bacteria facilitated by high temperatures (Martins *et al.* 2020). The significant negative correlation between macroinvertebrate abundance and % DO suggests pollution in the ponds (Barman and Gupta 2015). This poor water quality is evident in the high abundance of the pollution tolerant taxa recorded. The negative correlation between % DO and nitrate richness and abundance has also been reported in shallow lakes in China, Alpine ponds, and Karst ponds in Slovenia (Ji *et al.* 2020; Martins *et al.* 2020; Zelnik *et al.* 2018).

Acidity is a significant predictor of macroinvertebrate communities (Briers 2014; Nicolet *et al.* 2004). Since the temperature was positively correlated with pH, the negative correlation between pH with taxa number and abundance is expected. The abundant taxa in the ponds (Cladocera, Chironomidae, Hydrachinidae) are associated with low pH (<5.6) in New Zealand (Suren *et al.* 2011). The concentration of nitrate and phosphate were not different between the seasons. Although this was unexpected, nitrate and phosphate concentration has been reported

to be stable all year round in ponds and rivers in the UK and France (Angélibert *et al.* 2004; Medupin 2020). Nitrate levels were negatively correlated with Magalef's richness, which is expected. High nutrient levels indicate low water quality and hence only suitable for a few pollution tolerant taxa. The positive relationship between macrophyte cover and taxa richness is expected and has been reported in several studies (Declerck *et al.* 2011; Martins *et al.* 2020; Wissinger *et al.* 2016).

Macroinvertebrate abundance and Margalef's richness was positively correlated with the altitude of the ponds. Altitude influences the amount of runoff that drains into the ponds and impacts the water quality and then the macroinvertebrate community compositions (Barquín and Death 2004; Collier 2008; García-Criado *et al.* 2017; Townsend *et al.* 1997; Wissinger *et al.* 2006; Zelnik *et al.* 2018). This result is similar to Medupin (2020), which reports that the altitude of rivers is positively correlated with invertebrate density in the UK.

The positive correlation between pond surface area, connectivity, taxa richness has been reported in other studies in Asia, Europe, and the USA (Brooks 2000; Johansson *et al.* 2019; Kadoya *et al.* 2004; Meland *et al.* 2020; Sanderson *et al.* 2005). The positive correlation between the ponds surface area and the richness and diversity of macroinvertebrates is in accordance with the positive species-area relationship (Biggs *et al.* 2005; Wilson and MacArthur 1967; Zelnik *et al.* 2018). Surface area is directly related to the amount of available space that can be occupied (Zelnik *et al.* 2018). However, this relationship contradicts Hamerlík *et al.* (2014) and Blicharska *et al.* (2016), who reported no association between pond area and macroinvertebrate richness. The type of land use/ land cover (LULC) at the catchment scales seemed more relevant in shaping the macroinvertebrate communities than the other spatial scales. This finding is similar to Novikmec *et al.* (2016), Nobre *et al.* (2020) and Asare *et al.* (2018), who reported that catchment land use significantly affects water quality in ponds. For instance, high forest cover in the catchment area reduces the water temperature, leading to higher richness and abundance, especially in summer.

In summer, the importance of conductivity and salinity in structuring the macroinvertebrate communities in aquatic ecosystems have been widely accepted (Cañedo-Argüelles *et al.* 2013; Muresan *et al.* 2020). In winter 2018, nutrients, especially phosphates, played a vital role in the macroinvertebrate community structure. Although the physicochemical water quality variables explained higher variations in the communities in summer than winter, 64% to 70% of the variations were not explained. More variations in the communities were explained by including

physical and LULC, suggesting that physical parameters and the LULC are essential in structuring the macroinvertebrate community in ponds (Zelnik *et al.* 2012).

### ***Implications of the findings for macroinvertebrate conservation***

The use of bioindicators to determine the conservation value of ecosystems is widely accepted (Parmar *et al.* 2016). Additionally, the conservation of freshwater biodiversity requires an improved understanding of the temporal dynamics in the community structure of the bioindicators. This chapter provides a vital step in advancing our understanding of the temporal macroinvertebrate community structure in ponds and the niche preference of different taxa. The findings highlight that the environment, particularly water quality, primarily influences the temporal dynamics and diversity in macroinvertebrate communities. Hence, as the environment becomes degraded, more pollution tolerant taxa will dominate the macroinvertebrate communities and reduce diversity (Botwe 2017). Therefore, management measures should be instituted to minimise pollution from overland runoff, particularly conductivity levels in freshwater systems, by planting riparian vegetation. Studies have emphasised the importance of riparian vegetation in improving water quality and biodiversity in freshwater ecosystems (Dosskey *et al.* 2010, Dunea *et al.* 2021, Sargac *et al.* 2021, Swanson and Bohlman 2021). This intervention will enhance the macroinvertebrate community diversity even in winter.

## **3.6 Conclusion**

In this chapter, I investigated the macroinvertebrate community compositions, their temporal variations, and their relationship with environmental parameters. The findings indicate that Crustacea is the most abundant group of macroinvertebrates, whilst insects are the most diverse group in the ponds. Macroinvertebrate compositions in the ponds varied seasonally and annually, but the abundance and taxa richness were similar in both seasons. My findings show that in addition to the variations in taxa richness between ponds, each pond supported different macroinvertebrate compositions. The differences in community compositions are related to variations in physicochemical and environmental parameters within and around the ponds. Therefore, it is imperative to conserve all ponds, regardless of location or size. Similar to the macroinvertebrates, the water quality of ponds was also seasonally variable. Percentage macrophyte cover and altitude were important physical parameters that influenced

macroinvertebrate composition. However, the critical role of physicochemical variables like salinity (or conductivity) and pH in structuring the communities cannot be overlooked. These findings contribute to increasing knowledge of the drivers of macroinvertebrates in ponds in the Auckland region of New Zealand. Ponds are a vital component of freshwater ecosystems and contribute substantially to macroinvertebrates regional diversity. Moreover, ponds are resilient in providing diverse mesohabitats for diverse taxa. Despite the low water quality, some sensitive taxa were present in ponds. Therefore, it is essential to maintain good chemical and physical parameters of ponds and construct new ones to maximize their benefits.

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# Chapter 4

## Anthropogenic influence on macroinvertebrate communities in ponds



## 4.1 Abstract

Pond ecosystems are affected by the anthropogenic activities around them. Assessing the influence of these activities using macroinvertebrates as indicators is advantageous in determining the ecological status of ponds. I evaluated the macroinvertebrate composition in 11 ponds along the human population gradient (major, large, small urban and rural areas) in two years in summer and winter using taxonomic and trait (functional feeding group 'FFG') based approaches. I also assessed the physicochemical water quality variables of the ponds along the gradient. Additionally, I compared the macroinvertebrates community compositions among three artificial ponds (stormwater, ornamental and golf course). Finally, the relationship between the environmental parameters and the macroinvertebrate communities was determined. The taxonomic approach showed that the macroinvertebrate communities varied along the human population gradient. Further, ponds in the major urban and small urban areas had similar macroinvertebrate compositions. However, ponds in rural areas had the highest taxonomic and FFG richness. Sixty percent of the macroinvertebrates were predators, while shredders and detritivores were less than 1% each in the ponds. The FFG in the ponds differed between the seasons, but the composition varied along the urban-rural gradient in summer only. There were more predators in summer and more collector gatherers in winter. Furthermore, the macroinvertebrate community and Shannon Weiner diversity index differed among the three types of artificial ponds in summer. Ornamental ponds had the highest taxonomic richness and diversity compared to golf course ponds. The macroinvertebrate community compositions in the stormwater ponds were similar to the ornamental and golf course ponds. However, the community compositions in the golf course and ornamental ponds were different. The percentage of macrophyte cover contributed to the differences observed. Hence the environment in and around the pond influences the macroinvertebrate composition, diversity, and richness. My findings indicate that; i) increase in anthropogenic activities (measured by human populations and pond function) negatively affect the macroinvertebrate communities in ponds, especially in summer, and ii) the taxonomic approach is a better predictor of the impact of anthropogenic activities on the macroinvertebrate communities compared to the FFG approach.

## 4.2 Introduction

Anthropogenic influence on biodiversity arises primarily through land use/land cover (LULC) changes, including those associated with urbanization and agriculture. Over the years, conservation planning and management have focused on protecting natural habitats (e.g., primary forests and pristine habitats) (Ausseil *et al.* 2011; Robertson 2016). However, this paradigm is shifting towards including a more comprehensive range of land uses, especially those within urban areas (Clements *et al.* 2006; Rosenzweig 2003).

The macroinvertebrate community compositions in an ecosystem are significantly influenced by the prevailing environmental and historical precedence (Barquín and Death 2004; García-Criado *et al.* 2017). Some of the historical factors are metapopulation dynamics and biogeographical factors such as dispersal and speciation (García-Criado *et al.* 2017). The environmental conditions arise from natural and anthropogenic sources and spatial patterns (García-Criado *et al.* 2017; Moreno *et al.* 2010). Additionally, macroinvertebrates' spatial and temporal distribution depends on their resilience or resistance to different abiotic and biotic stresses and on their dispersal ability (Sanderson *et al.* 2005; Townsend *et al.* 1997). These factors operate at the local (individual pond) and the regional (ponds in the landscape) scales. As a result, different types of LULC affect water quality and the macroinvertebrates communities (distribution and abundance). The effect of land use on macroinvertebrate communities in ponds has been reported in many countries. For instance, in the United Kingdom (UK), ponds in floodplains and urban areas support more taxa than those on arable land (Hill *et al.* 2016), and stormwater ponds have higher taxa richness than natural ponds in Norway (Meland *et al.* 2020). Additionally, farm ponds in Japan with higher turbidity and phosphorus levels are poorer in macroinvertebrate communities having lower diversity (Usio *et al.* 2017). Further, biodiversity in golf course ponds contributes to regional diversity, although the low water quality may negatively impact biodiversity (Bock and Easton 2020; Colding *et al.* 2009; Müller *et al.* 2020). Understanding how anthropogenic activities drive the functional and structural compositions of macroinvertebrate communities at different spatial and temporal scales can predict the impact of climate change and LULC changes on the ecosystem (García-Criado *et al.* 2017; Greig and Galatowitsch 2016).

#### 4.2.1 Macroinvertebrates as biological indicators

Macroinvertebrates are widely accepted tools for water quality and biomonitoring (Tamiru 2018). They are good indicators because the tolerance levels of species and their taxonomy, ecology, evolution, and phenology have been extensively studied (Bravo-Utrera 2010; Pilière *et al.* 2016; Tamiru 2018). Macroinvertebrates are easily sampled and identified and a cost-effective tool (Dube *et al.* 2020; Pilière *et al.* 2016; Tamiru 2018). They also provide information on the aquatic ecosystems' evolutionary, biogeographical, and ecological state (Tamiru 2018). Macroinvertebrates are ubiquitous and sensitive to environmental changes and stress. Macroinvertebrates have been used to monitor heavy metals accumulation and pollution and human disturbance (organic pollution, habitat destruction and alteration, acid–mine drainage) in freshwater ecosystems (Wallace and Webster 1996).

Macroinvertebrates are primary consumers and are vital in the food chain of freshwater ecosystems by controlling primary productivity and maintaining energy flow (Ramírez and Gutiérrez-Fonseca 2014; Tamiru 2018). They are also involved in nutrient cycling through mineralization and detritus breakdown (Ramírez and Gutiérrez-Fonseca 2014). Macroinvertebrates are used in biomonitoring by assessing either their taxonomic or functional composition (Culp *et al.* 2011). The taxonomic measure is appropriate for determining the diversity and richness, whilst the functional measure is used to classify the ecosystem condition (Cummins *et al.* 2005). However, the functional methods require relatively little taxonomic effort compared to the taxonomic method.

#### 4.2.2 The use of the taxonomic approach

The basic description of the macroinvertebrate community is obtained by quantifying the diversity of the taxonomic composition. The quantitative measures used include relative abundance, species diversity, richness, and evenness. Additionally, qualitative methods such as the presence of sensitive taxa as applied mostly for lotic systems have been developed for ecological assessment (Botwe 2017). This method is suitable for assessing the impacts of the environment on aquatic ecosystems because the presence, abundance and distribution of macroinvertebrates are affected by environmental variability and anthropogenic activities (Moreno *et al.* 2010). Therefore, an ecologically healthy system will likely support a more diverse macroinvertebrate community than an impacted system (Clapcott *et al.* 2017; Tamiru 2018).

However, there are exceptions to this assertion as diversity can be reduced by high and low disturbance levels (Clapcott *et al.* 2017).

#### **4.2.3 The use of traits approach**

Macroinvertebrates possess traits that enable them to adapt to their environment. These properties are related to mobility, feeding, life history and reproduction (Boix *et al.* 2016; Botwe 2017; Céréghino *et al.* 2012). Measuring functional traits requires less taxonomic expertise and time but gives more information about changes in habitat conditions (Cummins *et al.* 2005; Céréghino *et al.* 2012; Pilière *et al.* 2016; Yadamsuren *et al.* 2020). Hence the application of trait-based biomonitoring has gained much attention, especially over the past decade (Pilière *et al.* 2016; Tamiru 2018).

Macroinvertebrates are classified into functional groups based on their traits. Functional groups are groups of species that respond in the same way to the environmental conditions and exert similar effects on ecological processing (Ruhí *et al.* 2012). The functional group either affects (effect groups) ecological processes or respond (response groups) to the changes in ecological processes (Yoshimura *et al.*, 2006). For example, a functional feeding group is based on a feeding trait. Feeding strategies (functional effect groups) are the primary determinant of species functional role in the ecosystem (Cummins *et al.* 2005; Merritt and Cummins 1996; Ramírez and Gutiérrez-Fonseca 2014; Zilli *et al.* 2008). The functional feeding groups (FFG) present in aquatic ecosystems provide valuable information about available food resources and, therefore, the ecosystem's ecological status (Nesemann *et al.* 2017; Ramírez and Gutiérrez-Fonseca 2014). The relative abundance of FFG is one of the measures used to indicate the ecological condition and is beneficial when assessing land use impacts on the ecosystem (Menetrey *et al.* 2005).

#### **4.2.4 Functional feeding groups and food availability**

Macroinvertebrates exhibit different behavioural traits (food-acquisition system) in obtaining food from the environment based on their physical characteristics, such as the morphology of mouthparts (Wallace and Webster 1996). Macroinvertebrates are classified as predators, collector gatherers, scrapers, detritivores, shredders, piercers, or filter feeders based on their feeding behaviour (Nesemann *et al.* 2017). Predators either have specialized mouthparts (e.g., Odonata and Hemiptera) or modified legs (for example, Nepidae) to capture their prey which they either engulf and eat wholly or partly (Ramírez and Gutiérrez-Fonseca 2014). Families which

employ this mechanism include Libellulidae, Dytiscidae, Tabanidae, Coenagrionidae, and Corduliidae. Other predators engulf and pierce through the body wall and suck off the tissue fluids of their prey. Taxa involved in this are Notonectidae, Hydrometritidae Veliidae, Mesovilidae and Glossiphoniidae (Nesemann *et al.* 2017).

Collector gatherers are dominant FFG in aquatic systems with abundant fine particles. Collector gatherers consume pieces of leaves less than one millimetre in size (Ramírez and Gutiérrez-Fonseca 2014). The feeding of collector gatherers often leads to a suspension of fine particles in the water column (Ramírez and Gutiérrez-Fonseca 2014). Scrapers graze on algae, macrophytes and other microorganisms such as fungi and bacteria that grow on substrates in the water (Nesemann *et al.* 2017). Shredders feed by cutting or chewing on decaying plant tissues and microflora attached to decaying plants. This feeding mechanism increases the release of dissolved organic matter into the aquatic system (Nesemann *et al.* 2017). Detritivores also feed on decaying plant material and vascular plant tissue (Ramírez and Gutiérrez-Fonseca 2014). The families Pyralidae, adult Hydrophillidae, and some Chironomidae are examples of detritivores (Nesemann *et al.* 2017). Finally, shredders and scrapers facilitate leaf litter breakdown by chewing plants' leaves into coarse particulate organic matter (Cummins *et al.* 2005). Shredders convert about 30% of the coarse particulate organic matter (CPOM) into fine particulate organic matter (FPOM) on which filter feeders depend. Therefore, the abundance of shredders is likely to affect the abundance of filter feeders in ponds (Cummins *et al.* 2005; Milesi *et al.* 2016). However, some macroinvertebrates exhibit plasticity in feeding behaviour depending on the stage in their life cycle and the type of food available. Piercers are macroinvertebrates that cut and perforate plant tissue to suck up the plant fluids. Some species of the family Trichoptera and Hydroptilidae are piercers (Ramírez and Gutiérrez-Fonseca 2014).

#### 4.2.5 Taxonomic versus trait-based approach

Despite its usefulness, the use of the taxonomic approach is geographically restrictive. The transfer of information among different countries and regions is not feasible due to the differences in taxonomic compositions across geographic barriers. However, findings based on the trait approach are transferrable across different geographic locations (Clapcott *et al.* 2017; Culp *et al.* 2011). Moreover, functional traits appear to be more stable among seasons than taxonomic composition. Traits are more sensitive to changes in environmental variables (especially changes resulting from anthropogenic influences such as LULC changes) and directly associated with ecosystem processes and function compared to the taxonomic compositions

(Coccia 2021; Clapcott *et al.* 2017; Culp *et al.* 2011; Hill *et al.* 2021; Thornhill *et al.* 2018). Furthermore, functional richness has been shown to correlate positively with taxonomic richness (Schriever and Lytle 2016). Finally, functional richness (or composition) explains more of the biological community variance associated with environmental variables than taxonomic composition (Botwe 2017; Coccia 2021; Hill *et al.* 2021).

The recent deterioration of the quality of freshwater ecosystems and the decline in macroinvertebrate taxa in New Zealand has been of concern. This situation is worse in places with intensive land use resulting from population growth (Larned *et al.* 2016). This challenge notwithstanding, ponds have been constructed in these landscapes to serve diverse purposes. However, the impact of anthropogenic activities on ponds has barely been studied in New Zealand (Greig and Galatowitsch 2016). Particularly, the role of artificial ponds in macroinvertebrate diversity has not been assessed (Greig and Galatowitsch 2016). Therefore, evaluating the impact of anthropogenic activities on macroinvertebrate communities is vital, especially in a rapidly urbanizing region like Auckland. Also, it is essential to account for the role of artificial ponds in macroinvertebrate conservation. The findings will fill the knowledge gap and predict the impact of anthropogenic influence on pond biodiversity. In this chapter, I evaluate the impact of anthropogenic influence on macroinvertebrate community compositions in ponds in two ways. First, I determine the effect of the human population (as a measure of anthropogenic activities) on the macroinvertebrate community. Here, both taxonomic and trait based (FFG) groups were used to evaluate the impact. Secondly, I assessed and compared the macroinvertebrate composition in three artificial pond types (stormwater, ornamental and golf course).

#### **4.2.6 Aims and hypotheses**

The aims of the chapter are to:

- i) determine the macroinvertebrate community compositions in ponds along the human population gradient,
- ii) determine the FFG richness in the ponds along the human population gradient,
- iii) investigate the macroinvertebrate community compositions among three artificial pond types (stormwater, ornamental, and golf course ponds),
- iv) investigate the relationship between environmental factors and the macroinvertebrate communities along the urban-rural gradient and the three pond types.

The hypotheses being tested are:

- i) human population influences macroinvertebrate communities. Densely populated areas will have lower diversity,
- ii) the FFG will discriminate among the macroinvertebrate communities along the urban-rural gradient. The diversity of the FFG in high populated areas will be low due to an increase in pollution,
- iii) the FFG is better at predicting the impact of the human population on the macroinvertebrate communities than the taxonomic measures,
- iv) different types of artificial ponds will have different macroinvertebrate compositions. The differences will correlate with the environment in and around the ponds.

### 4.3 Methods and analyses

To determine the macroinvertebrate compositions along the urban-rural gradient, 11 ponds (major urban: 3, large urban: 2, small urban: 3, and rural: 3) were sampled twice during the winter and summer seasons from 2018 to 2020 (these ponds are a subset of the 12 ponds sampled in Chapter 3. The pond with polyethylene lining was excluded from this analysis). First, the macroinvertebrates were identified taxonomically and functionally based on their foraging groups. Secondly, a focused analysis was carried out on nine of these 11 ponds (three stormwater, three ornamental, and three golf course ponds) to determine the effect of their use or type on the macroinvertebrate's composition. Pond type is described as the purpose or the function for which the pond was constructed. Further, the physicochemical water quality and the environmental parameters were measured from these ponds. Chapter 3 provide details of the sampling methods and macroinvertebrate identification.

I present the data analyses and results in two sections. In section one, I determined the impact of the human population on the macroinvertebrates in the ponds. These were based on the first three aims. Using the taxonomic approach, I initially describe the macroinvertebrate assemblages along the urban-rural (human population) gradient. The macroinvertebrate assemblages along the urban-rural gradient are described based on the major taxonomic groups' percentage abundances. The differences in macroinvertebrate communities along the urban-rural gradient was tested using a Bray-Curtis dissimilarity resemblance matrix of pooled data of all the ponds in each gradient. A PERMANOVA test was carried out with sites nested in urban-

rural gradient to determine any interaction between temporal (year and season) and spatial (urban-rural gradient) factors. Analysis of similarity (ANOSIM) test was used to determine the assemblages along the urban-rural gradient (Anderson and Walsh 2013; Clarke and Gorley 2015). Taxa that contribute to the differences in the macroinvertebrate communities along the gradient was analysed using the SIMPER on the Bray Curtis similarity matrix. Finally, the biodiversity indices (Taxa richness, Alpha and Gamma diversity) along the gradient were assessed.

Secondly, the effect of the human population on the macroinvertebrate communities based on the FFG (trait-based approach) was determined. Classification of FFG was based on different taxonomic resolution as outlined by Mesa *et al.* (2013) and Ramírez and Gutiérrez-Fonseca (2014). The family level taxonomic resolution was used for insects. The Class or Order taxonomic resolutions were used for the other taxa. The relative abundance, diversity (alpha and gamma) and richness of the FFG along the urban-rural gradient were compared. The difference in FFG along the urban-rural gradient was tested using PERMANOVA based on log (X+1) transformed and Bray-Curtis dissimilarity FFG data matrix. ANOVA test was used to analyse the variance in the richness of predators and collector gatherers.

## Section 2: The macroinvertebrate community in different types of ponds

In this section, I assessed and compared the macroinvertebrate community in three artificial ponds. I determined the differences in macroinvertebrate communities among the pond types using PERMANOVA based on a Jaccard dissimilarity index. Also, the biodiversity indices (taxa richness, Shannon Weiner, Alpha and Gamma diversity) among the pond types were determined. All analyses were carried out using the PRIMER e software (Anderson *et al.* 2008)

## 4.4 Results

### 4.4.1 Section 1: Impact of human population density on the macroinvertebrates in the ponds (Taxonomic approach)

#### 4.4.1.1 Macroinvertebrate communities along the urban-rural gradient

There were variations in the macroinvertebrate communities' structure in the ponds along the urban-rural gradient. For example, there were 500% more Crustacea in the major urban ponds than in the large urban ponds. Forty-seven percent of the gastropods sampled were from the ponds in the major urban areas, and the lowest abundance of gastropods occurred in ponds in the rural areas.

The community compositions were significantly different between the seasons in the major urban areas and rural ponds only (Table 4.1). For example, in the major urban areas, Crustacea were more abundant in winter, but there were 20% more insects and 98% more gastropods in summer than winter. On the other hand, although 28 taxa were common across seasons in the rural areas, Crustacea and Clitellata constituted 55% and 21% of the communities in winter (Figure 4.1).

There were 17 taxa common to both seasons in the large urban areas. However, the taxa composition varied across seasons. For example, there were more than 300% insects and over 100% Crustacea in summer than in winter. On the contrary, there was 100% more nematodes and platyhelminths in winter (Figure 4.1). For ponds in the small urban areas, 20 taxa occurred in both seasons. However, whereas the communities in summer consisted of 50% insects, insects were only 13% in winter. On the contrary, the abundance of Crustacea was 100% more in winter than in summer (Figure 4.1).

Table 4.1 Description of taxa numbers, similarities and differences in macroinvertebrate compositions and abundance along the urban-rural gradient in summer and winter. The total number of taxa recorded in each category and the results of the ANOSIM test between the compositions across seasons are in brackets. Significant p values (5%) are in bold.

Community compositions	Summer	Winter
	Major urban (42, t = 1.7, p = <b>0.01</b> )	
Total taxa	34	32
% similarity	58	62
Exclusive taxa	10 (Tateidae, Anisopodidae, Hydrophilidae, Tanypodinae, Helicophidae, Dytiscidae, Veliidae, Mesovilidae, Dixidae)	8 (Atydae, Pelecorhynhidae, Hydraenidae, Elmidae, Tipulidae, Polypedulum, Dalyellidae and Lymnaeidae)
Abundance (t = 1.22, p = 0.2)	56% of total abundance	44% of total abundance
	Large urban (35, t = 1.1, p = 0.1)	
Total taxa	32	22
% similarity	36	39
Exclusive taxa	17 (Oeconesidae, Stytonicidae, Elmidae, Tipulidae, Hydrophilidae, Nematoda, Notonectidae, Calanoida, Salifidae, Culicidae, Sigara, Dixidae and Dugesiiidae)	5 (Pelecorhynchidae, Helicophidae, Tabinidae, Corduliidae and Conoesucidae)
Abundance (t = 1.7, p = 0.08)	69% of total abundance	31% of total abundance
	Small urban (37, t = 1.1, p = 0.3)	
Total taxa	32	25
% similarity	57	64
Exclusive taxa	12 (Aeshnidae, Elmidae, Hydrophilidae, Tanypodinae, Orthocladinae, Paucispinigera, Lestidae, Dixidae, Hydridae, Salifidae, Mesovilidae, and Leptoceridae)	5 (Ceratopogonidae, Dolichopodidae, Dalyellidae, Muscidae and Arrhopalitidae)
Abundance (t = 0.9, p = 0.3)	57% of total abundance	53% of total abundance
	Rural (58, t = 1.7, p = <b>0.01</b> )	
Total taxa	49	37
% similarity	53	52
Exclusive taxa	21	9
Abundance (t = 0.9, p = 0.3)	44% of total abundance	56% of total abundance

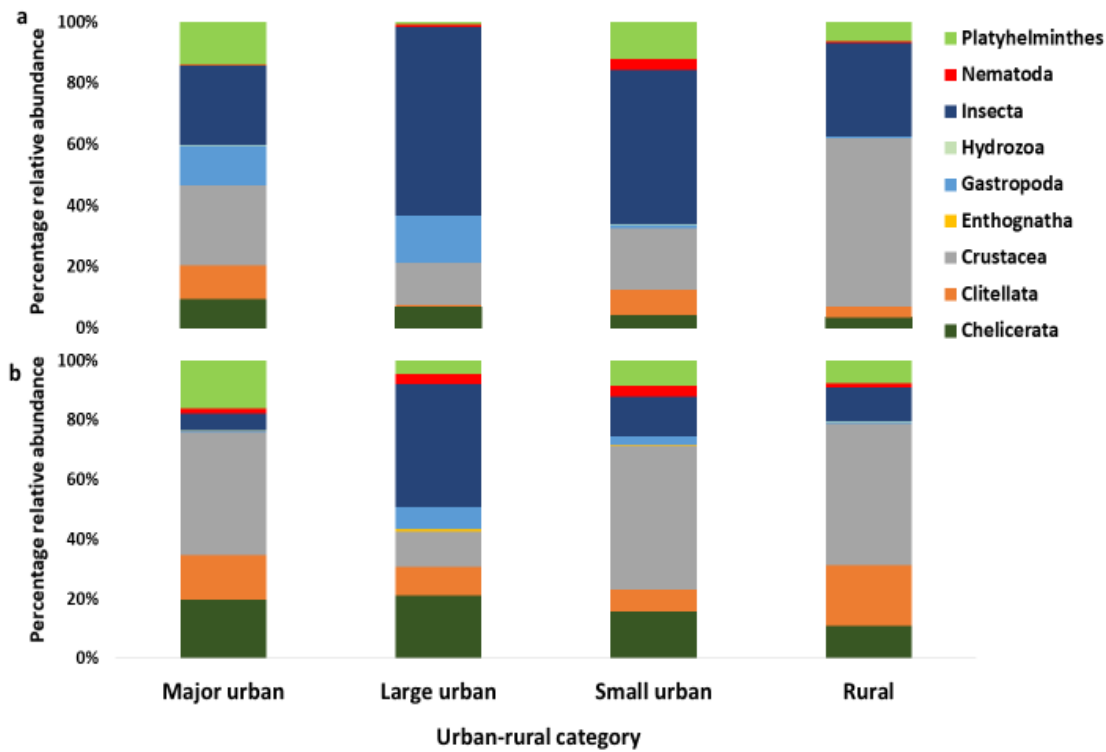


Figure 4.1 A relative percentage abundance of the macroinvertebrate groups in ponds along the urban-rural gradient in a: summer and b: winter.

#### 4.4.1.2 Distribution of macroinvertebrates along the urban-rural gradient

A PERMANOVA test showed a significant difference in taxa composition along the urban-rural gradient ( $F_{3,7} = 2.1, p < 0.01$ ) and no significant interaction between season and urban-rural gradient ( $F_{1,3} = 1.4, p = 0.09$ ) (Table 4.2). A further pairwise test indicated that while the communities in the major urban and rural areas differed significantly seasonally, those in large and small urban areas were not significantly different. Table 4.1 shows the number of taxa and exclusive taxa in the ponds along the gradients across the seasons.

Table 4.2 Results of permutational multivariate analysis of variance (PERMANOVA) of the macroinvertebrate community along the urban-rural gradient. Df represents degrees of freedom; MS represents mean squares. Bold numbers indicate significant p-values (5%).

Terms	df	MS	F	p
Season	1	4162.5	5.9	<b>&lt; 0.001</b>
Year	1	5309.6	4.5	<b>&lt; 0.001</b>
Urban-rural	3	3254.4	1.9	<b>&lt; 0.01</b>
Pond (Urban-rural)	7	1711.0	2.1	<b>&lt; 0.01</b>
Season x Year	1	4082.9	5.2	<b>&lt; 0.001</b>
Season x Urban-rural	3	1004.0	1.4	0.09
Year x Urban-rural	3	677.93	0.8	0.6
Pond (Urban-rural) x Season	7	698.3	0.9	0.7
Pond (Urban-rural) x Year	7	771.1	1.0	0.5
Season x Year x Urban rural	3	859.2	1.1	0.3
Residual	7	1324.1		

An analysis of similarity (ANOSIM) showed that the macroinvertebrate communities were significantly different along the human population gradient in summer (Global statistic  $R = 0.3$ ,  $p < 0.001$ ) and winter (Global statistic  $R = 0.2$ ,  $p < 0.001$ ). A pairwise t-test indicated a significant difference between ponds in, for example, i) major urban and large urban areas, ii) large urban and small urban areas, and iii) small urban and rural areas in both seasons. However, the taxa compositions in the major and small urban areas were similar in both seasons. Table 4.3 gives details of the pairwise comparison of the macroinvertebrate communities along the urban-rural gradient.

Table 4.3 An ANOSIM pairwise result of the macroinvertebrate community along the urban-rural gradient in summer and winter. Significant p values (5%) are in bold.

Pairwise test	Summer		Winter	
	R	p value	R	p value
Major urban and large urban	0.5	<b>&lt; 0.01</b>	0.5	<b>&lt; 0.01</b>
Major urban and small urban	0.05	0.3	0.2	0.06
Major urban and rural	0.2	<b>0.04</b>	0.02	0.5
Large urban and small urban	0.4	<b>&lt; 0.01</b>	0.3	<b>0.02</b>
Large urban and rural	0.3	0.05	0.3	<b>0.02</b>
Small urban and rural	0.3	<b>0.01</b>	0.2	<b>0.04</b>

#### 4.4.1.3 Macroinvertebrate heterogeneity along the urban-rural gradient in summer

SIMPER identified 11 taxa that contribute to 70% of the macroinvertebrate abundance observed along the human population gradient. Different taxa contributed highly to the communities in each category. For example, Dugesiidae and Notonectidae were important in all four categories along the gradient (Table 4.4).

The taxa identified to contribute to the difference between the communities in the major and large urban ponds were Cladocera (Diss/SD = 2.24), Physidae (Diss/SD = 2.06), and Orthoclad (Diss/SD = 1.98), which were more abundant in major urban areas. On the other hand, *Sigara* (Diss/SD = 1.72) was more abundant in ponds in large urban areas (see Appendix C: Table 1).

The taxa identified as driving the differences between the major urban and rural areas were Veliidae (Diss/SD = 2.13) and Notonectidae (Diss/SD = 1.45), which were more abundant in rural areas. The others were Physidae (Diss/SD = 1.69) and Hydrachinidae (Diss/SD = 1.43), which were more abundant in ponds in major urban areas (see Appendix C: Table 1).

The taxa identified to drive the differences between the communities in the small urban and rural areas were Ostracod (Diss/SD = 1.59), Veliidae (Diss/SD = 1.42), and Ischunura (Diss/SD = 1.5), which were more abundant in ponds in small urban areas. In contrast, Physidae (Diss/SD = 1.46) was more abundant in rural ponds (see Appendix C: Table 1).

Table 4.4 SIMPER result of taxa that contributed to 70% of the abundance of macroinvertebrates sampled along the urban-rural gradient in summer. The average similarity among ponds within a gradient is in brackets. The highest percentage contribution of a taxon in each category is in bold.

Taxa	Major urban (57.3%)	Large urban (36.91%)	Small urban (59.35%)	Rural (52.11%)
Dugesiidae	<b>13.08</b>	7.54	11.79	5.9
Cladocera	11.7		8.62	11.67
Oligochaeta	11.17		10.41	
Chironominae	9.42		6.94	9.11
Notonectidae	8.2	24.43	<b>14.89</b>	<b>13.02</b>
Physidae	8.19		6.87	
Ostracod	8.02		7.76	10.87
Hydrachinidae	5.42			4.76
<i>Sigara</i>		<b>25.71</b>	9.1	6.84
<i>Diaprepocoris</i>		9.02		
Veliidae		7.15		8.02
Total % contribution	75.2	73.85	76.38	70.19

#### 4.4.1.4 Macroinvertebrate heterogeneity along the urban-rural gradient in winter

The macroinvertebrate communities differed along the human population gradient. A pairwise test indicated significant difference between ponds in i) major and large urban areas ( $R = 0.5$ ,  $p < 0.01$ ), ii) large and small urban areas ( $R = 0.3$ ,  $p = 0.02$ ), iii) large and small urban areas ( $R = 0.3$ ,  $p = 0.02$ ), iv) large urban and rural areas ( $R = 0.3$ ,  $p = 0.02$ ), and v) small urban and rural areas ( $R = 0.2$ ,  $p = 0.04$ ). Macroinvertebrate community compositions in the major and large urban ponds were the most distinct ( $R = 0.5$ ). In contrast, the communities in the major urban and rural area ponds were most similar (Table 4.5). An nMDS plot indicated that the community composition in large urban areas showed the most variation (32.2%). Communities in the small urban areas were the least variable (54.6% similar). Community compositions in major urban and rural areas were 49.7% and 48%, similar, respectively.

The common taxa identified by SIMPER to contribute to 70% of the total abundance of the macroinvertebrate community compositions along all the four human population gradients were Cladocera, Oligochaeta (Table 4.5). The differences between major urban and large urban communities were driven by Ostracod, Cyclopoida Glossiphonidae, and Dugesiidae, which were all more abundant in the major urban areas (see Appendix C: Table 2). Differences between large and small urban communities were driven by Ostracod, Dugesiidae, Diaprepocoris, and Cyclopoida, which were all more abundant in small urban areas (see Appendix C: Table 2).

The taxa that contributed to the differences between communities in the major and rural area ponds were Glossiphonidae and Oligochaeta, which were more abundant in major urban areas. Ostracod and *Sigara* were more abundant in ponds in rural areas (see Appendix C: Table 2). Finally, the taxa that contributed to the differences in the small urban and rural area ponds were Physidae and *Diaprepocoris*, which were more abundant in the small urban areas. The others were Ostracod and *Sigara*, which were more abundant in ponds in rural areas (see Appendix C: Table 2).

Table 4.5 Result of SIMPER of taxa that contributed to 70% of the abundance of macroinvertebrates sampled along the urban-rural gradient in winter. The average similarity among ponds within a gradient is in brackets. The highest percentage contribution of a taxon in each category is in bold.

Taxa	Major urban 61.09%	Large urban (39.53%)	Small urban (63.77%)	Rural (56.60%)
Cladocera	<b>13.96</b>	20.7	<b>15.7</b>	10.9
Oligochaeta	13.85	11.87	10.33	<b>13.94</b>
Dugesiidae	13.57		11.92	12.57
Cyclopoida	11.62		8.21	
Hydrachinidae	11.55	11.76		7.92
Ostracod	9.26		9.86	11.67
Notonectidae		<b>24.33</b>	11.05	
Chironominae		7.88		7.89
Physidae			9.48	
<i>Sigara</i>				9
Total % contribution	73.81	76.54	76.55	73.89

#### 4.4.1.5 Biodiversity along the urban-rural gradient

The alpha and gamma diversities were highest in ponds in rural areas across the seasons. Conversely, the lowest alpha diversity occurred in large urban ponds, and the lowest gamma diversity occurred in ponds in major urban areas. A summary result is shown in Table 4.6.

Table 4.6 Alpha and Gamma diversity  $\pm$  standard deviation along the urban-rural gradient in summer and winter.

Gradient	Summer		Winter	
	Alpha	Gamma $\pm$ SD	Alpha	Gamma $\pm$ SD
Major urban	34	46 $\pm$ 9	32	48 $\pm$ 16
Large urban	30	48 $\pm$ 5	22	55 $\pm$ 15
Small urban	32	54 $\pm$ 6	25	51 $\pm$ 9
Rural	49	62 $\pm$ 5	37	71 $\pm$ 13

#### 4.4.1.6 Physicochemical water quality variables along the urban-rural gradient

Conductivity, TDS, and salinity were significantly different along the urban-rural gradient across the seasons. The temperature was significantly different along the gradient ( $F_{3,7} = 9.52$ ,  $p < 0.001$ ) in summer but not in winter ( $F_{3,7} = 0.57$ ,  $p = 0.6$ ). The concentrations of nitrates, phosphate, and ammoniacal nitrogen were similar along the gradient in summer. In winter, pH ( $F_{3,7} = 4.04$ ,  $p = 0.02$ ) and nitrate concentrations ( $F_{3,7} = 4.04$ ,  $p = 0.02$ ) were different along the gradient. The highest and lowest pH was recorded in ponds in the small ( $7.06 \pm 0.29$ ) and large urban areas ( $6.16 \pm 0.60$ ). Also, nitrate concentrations were highest in ponds in small urban areas ( $0.44 \pm 0.06$ ) and lowest in ponds in the major urban areas ( $0.12 \pm 0.15$ ). Table 4.7 provides details of the physicochemical variables along the urban-rural gradient in both seasons.

Chapter 4 | Anthropogenic influence on macroinvertebrate communities in ponds

Table 4.7 Physicochemical water quality variables along the urban-rural gradient in the summer and winter. Results of the ANOVA test is reported, and a significant p value(5%) is in bold.

Physicochemical variables	Summer				F	p value	Winter				F	p value
	Major urban	Large urban	Small urban	Rural			Major urban	Large urban	Small urban	Rural		
pH	6.95 ± 0.43	7.02 ± 0.36	6.82 ± 0.62	6.16 ± 0.64	2.67	0.07	6.59 ± 0.26	7.06 ± 0.29	6.16 ± 0.60	6.24 ± 0.49	4.04	<b>0.02</b>
% DO	33.87 ± 18.53	38.775 ± 2.69	45.27± 19.57	31.35 ± 15.12	1.19	0.3	47.47 ± 19.19	59.54 ±17.86	62.00 ± 21.85	36.66 ±23.71	1.74	0.1
Con. (µScm <sup>-1</sup> )	169.0 ± 18.5	249.7 ± 115.6	102.1 ±31.8	306.9 ± 171.6	4.22	<b>0.01</b>	187.8 ± 62.5	263.7 ± 84.1	100.2 ± 35.2	325.4 ± 178.5	4.83	<b>0.01</b>
TDS (ppm)	84.6 ± 39.6	119.4 ± 36.9	51.1 ± 15.9	153.8 ± 85.5	4.72	<b>0.01</b>	93.7 ± 31.3	183.2 ± 127.9	52.8 ± 15.7	160.2 ± 90.4	3.5	<b>0.03</b>
Sal. (psu)	0.08 ± 0.02	0.12 ± 0.05	0.05 ± 0.02	0.15 ± 0.08	4.39	<b>0.01</b>	0.09 ± 0.03	0.18 ± 0.13	0.05 ± 0.01	0.16 ± 0.09	3.6	<b>0.03</b>
Tem. (°C)	20.83 ± 2.28	21.17 ± 1.29	22.45 ± 1.76	17.59 ± 0.39	9.52	<b>&lt; 0.001</b>	13.95 ± 1.69	14.19 ± 0.89	13.55 ± 1.26	13.25± 0.84	0.57	0.6
NO <sub>3</sub> -N (mg l <sup>-1</sup> )	0.15 ± 0.19	0.24 ± 0.26	0.17±0.85	0.18 ± 0.24	0.09	0.9	0.12 ± 0.15	0.25 ± 0.22	0.44 ± 0.06	0.26 ± 0.19	4.04	<b>0.02</b>
PO <sub>4</sub> <sup>3-</sup> (mg l <sup>-1</sup> )	0.91 ± 0.70	1.26 ± 0.85	1.48 ± 0.85	1.25 ± 0.81	0.23	0.8	1.67 ± 0.87	1.57 ± 0.78	1.48 ± 0.75	0.81 ± 0.96	1.21	0.3
NO <sub>3</sub> -N (mg l <sup>-1</sup> )	0.23 ± 0.19	0.24 ± 0.14	0.12 ± 0.15	0.20 ± 0.08	1.16	0.3	0.17 ± 0.14	0.27 ± 0.10	0.21 ± 0.16	0.14 ± 0.13	0.77	0.5

#### 4.4.1.7 Water quality variables along the urban-rural gradient

A PERMANOVA test showed no significant difference in water quality along the urban-rural gradient ( $F_{3,7} = 2.2$ ,  $p = 0.06$ ) but a significant interaction between season and urban-rural gradient ( $F_{1,3} = 2.2$ ,  $p = 0.03$ ) (Table 4.8). A further pairwise test indicated that the water quality in the major urban ( $t = 1.9$ ,  $p < 0.001$ ), large urban ( $t = 1.7$ ,  $p = 0.01$ ), small urban ( $t = 3.05$ ,  $p < 0.001$ ) and rural areas ( $t = 1.7$ ,  $p = 0.02$ ) differed significantly seasonally.

Table 4.8 Results of permutational multivariate analysis of variance (PERMANOVA) of the water quality along the urban-rural gradient. Df represents degrees of freedom; MS represents mean squares. Bold numbers indicate significant p-values (5%).

Terms	df	MS	F	P value
Season	1	38.45	12.7	<b>&lt; 0.001</b>
Year	1	16.91	5.3	<b>&lt; 0.004</b>
Urban-rural	3	27.23	2.2	0.06
Pond (Urban-rural)	7	11.93	3.5	<b>&lt; 0.001</b>
Season x Year	1	11.21	3.3	<b>0.01</b>
Season x Urban-rural	3	6.78	2.2	<b>0.03</b>
Year x Urban-rural	3	4.63	1.4	0.2
Pond (Urban-rural) x Season	7	3	0.9	0.6
Pond (Urban-rural) x Year	7	3.16	0.9	0.5
Season x Year x Urban rural	3	4.34	1.3	0.2
Residual	7	3.32		

#### 4.4.2 Impact of human population density on macroinvertebrates composition (functional feeding group approach)

The FFG of the macroinvertebrates across the ponds consisted of 60% predators, 29% collector gatherers, 10% scrapers, 1% piercers, and < 1% each of shredders and detritivores. The taxa *Sigara* and Notonectidae contributed highly to the predator numbers. The FFG compositions were significantly different ( $F_{1,3} = 21.5$ ,  $p < 0.001$ ) across the seasons. Feeding group composition in summer was relatively more diverse than in winter. There were 55% and 68% of predators in summer and winter. Detritivores were present in summer only. Additionally, there were 4% more collector gatherers in winter than in summer.

In summer, the ponds in small urban areas had the highest relative abundance of predators, and the rural ponds had the highest abundance of collector gatherers. The relative abundance of scrapers was higher in ponds in the major and large urban areas (Figure 4.2). The rural ponds

had the most diverse functional groups present, recording all six FFG. Predators, collector gatherers, scrapers, and piercers were observed in ponds in the major, large, and small urban areas. The FFG compositions were significantly different along the urban-rural gradient ( $F_{3,7} = 2.28$ ,  $p = 0.02$ ). A pairwise t-test showed that FFG in ponds in i) small urban and rural areas ( $t = 1.55$ ,  $p = 0.04$ ) and ii) rural and large urban areas ( $t = 1.67$ ,  $p = 0.03$ ) were different.

In winter, the FFG in the ponds did not differ along the urban-rural gradient ( $F_{3,7} = 1.80$ ,  $p = 0.08$ ). Piercers were only present in small urban areas where predators were relatively more abundant than the other gradients. The FFG in ponds in the large urban areas were the least diverse, consisting of predators, collector gatherers, and scrapers. Finally, shredders were present only in ponds in the major urban and rural areas.

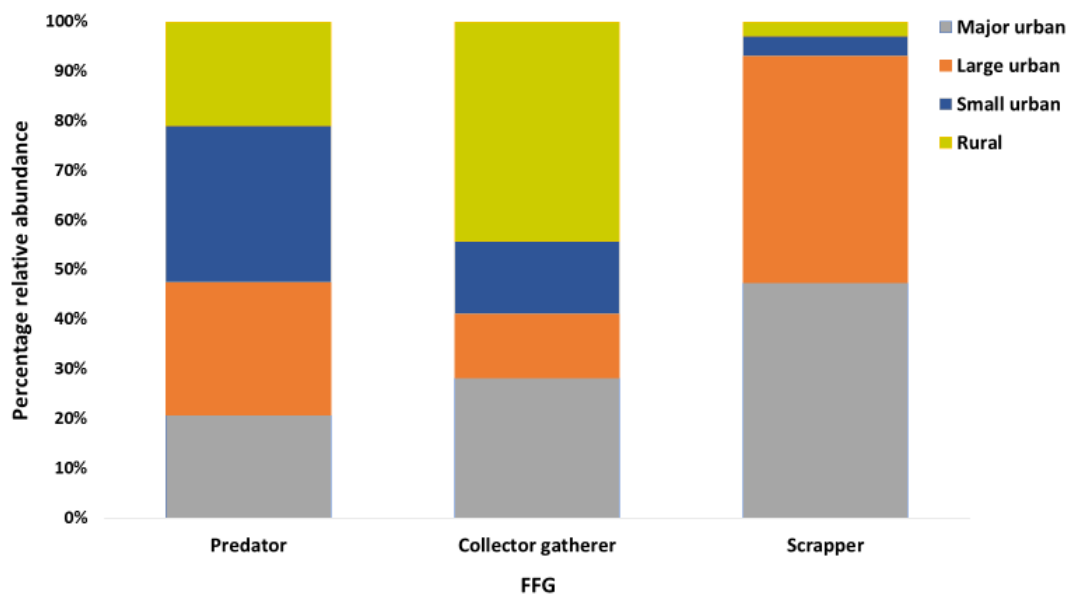


Figure 4.2 Percentage relative abundance of the common macroinvertebrate functional feeding group along the urban-rural gradient in summer.

#### 4.4.2.1 Functional feeding group richness in the ponds

FFG richness was similar along the urban-rural gradient across the seasons. Ponds in rural areas were the richest in predator and collector gatherers taxa, especially in summer (Table 4.9).

Table 4.9 Taxa richness of collector gatherer and predators in the ponds along the urban-rural gradient. Results of the ANOVA test is reported.

Taxa richness	Urban-rural gradient					F	p value
	Season	Major urban	Large urban	Small urban	Rural		
Predator	Summer	13	13	15	21	1.43	0.2
	Winter	10	10	12	12	1.46	0.1
Collector gatherer	Summer	5	5	7	9	3.06	0.05
	Winter	7	3	3	4	0.67	0.8

#### 4.4.3 Section 2: Macroinvertebrates community in different types of pond

##### 4.4.3.1 Macroinvertebrate communities in three different artificial pond types

A total of 54 taxa were recorded from 36 samples in all three artificial pond types (stormwater, ornamental and golf course). The highest taxonomic richness for a sample was recorded from an ornamental pond (29 taxa), and the least was recorded in a stormwater pond (6 taxa). Three taxa (Anisopodidae, Dolichopodidae, Pelecorhynchidae) were exclusive to stormwater ponds. Also, 15 taxa (Conoesucidae, Corduliidae, Corophiidae, Gripopterygidae, Helicophidae, Hydrometridae, Hydrophilidae, Hydropsychidae, Lymnaeidae, Paraleptamphopidae, Phreatogammaridae, Polycentropodidae, Scirtidae, Talitridae, and Tipulidae) were found only in ornamental ponds. About 20% of macroinvertebrate taxa in ornamental ponds were Crustacea compared to 16% in golf courses and 14% in stormwater ponds (Figure 4.3a). On average, insects constituted a larger proportion of taxonomic richness recorded in ornamental (58%) compared to stormwater (56%) and golf course (50%) ponds. However, the family richness of insects varied among the ponds (Figure 4.3b).

There was a significant seasonal difference ( $F_{1,2} = 2.8, p < 0.001$ ) in macroinvertebrates' compositions and abundance. Macroinvertebrate abundance was higher in summer in the stormwater and golf course ponds but lower in the ornamental ponds. Additionally, the communities in ornamental ponds were more similar in winter (Bray Curtis Similarity, 51.16%) than in summer (48.86%).

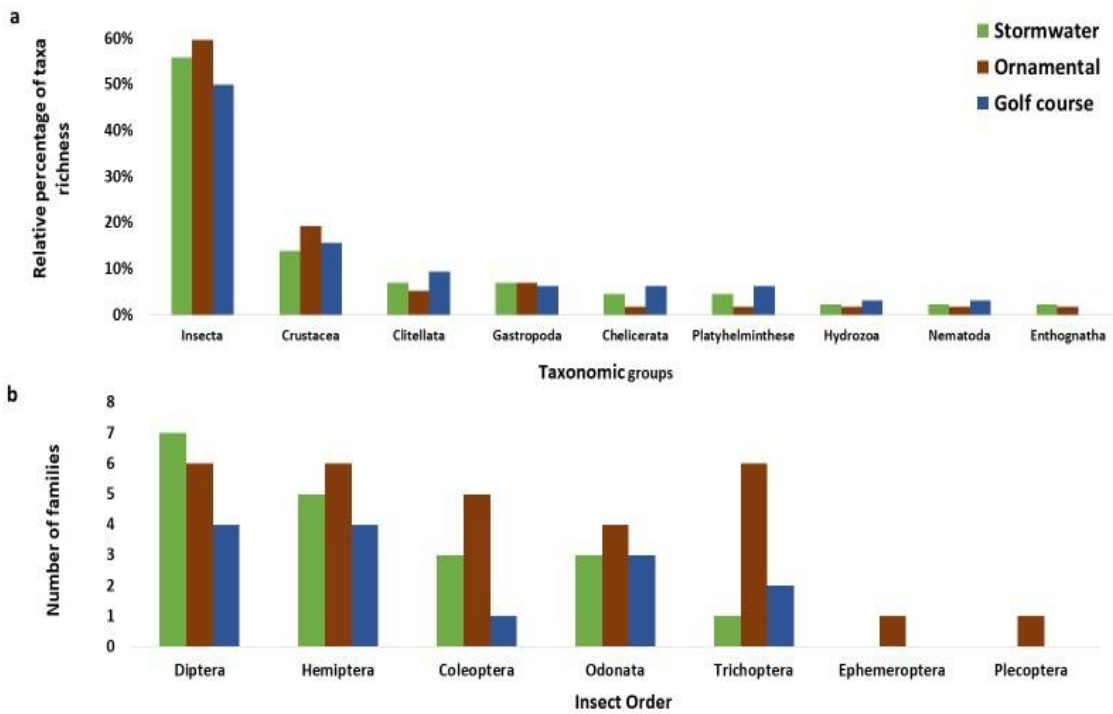


Figure 4.3 Bar plots of a: relative percentage taxa richness of macroinvertebrate groups and b: number of families in insect orders in stormwater (green bars), ornamental (brown bars) and golf course (blue bars) ponds.

#### 4.4.3.2 Seasonal macroinvertebrate composition in the three pond types

In summer, 36 taxa occurred in stormwater ponds, 29 in golf course ponds and 47 in ornamental ponds. There was a significant difference in the total number of taxa in the three artificial ponds in summer ( $F = 6.6, p < 0.01$ ). In addition, the Shannon Weiner diversity was significantly different ( $F = 14.4, p < 0.001$ ) among the ponds. The highest Shannon Weiner diversity was recorded in ornamental ponds ( $2.20 \pm 0.13$ ) compared to stormwater ( $1.75 \pm 0.07$ ) and golf course ( $1.68 \pm 0.09$ ) ponds.

In winter, 30 taxa were recorded in stormwater ponds, 24 in the golf course ponds, and 40 in ornamental ponds. The number of taxa ( $F = 1.9, p = 0.1$ ) and abundance were not significantly different among these ponds. The Shannon Weiner diversity was highest in golf course ponds ( $1.90 \pm 0.14$ ) compared to stormwater ( $1.79 \pm 0.11$ ) and ornamental ponds ( $1.80 \pm 0.13$ ); however, they were not significantly different ( $F = 0.2, p = 0.7$ ).

A PERMANOVA test indicated that after accounting for seasonal differences, there was no significant difference among the macroinvertebrate community compositions in the three pond types ( $F_{1,2} = 1.4, p = 0.1$ ).

#### **4.4.4.3.3 Physicochemical water parameters of the different pond use types**

I observed significant differences in the % DO, conductivity, TDS, salinity and ammoniacal nitrogen across the pond in summer. For example, golf course ponds had the highest % DO (50.67), and stormwater ponds had the least (25.97). However, only conductivity was different ( $F = 4.57$ ,  $p = 0.03$ ) among the ponds, with the highest observed in stormwater ponds in winter. Table 4.10 provides details of the physicochemical variables in the ponds across the seasons.

A PERMANOVA test indicated that there was no significant difference among the water quality in the three pond use types ( $F_{1,2} = 2.5$ ,  $p = 0.05$ ).

#### **Physical features of the ponds**

Golf course ponds were farthest away from roads ( $279.3\text{m} \pm 58.3\text{m}$ ), and stormwater ponds were closest to roads ( $28.3\text{m} \pm 8.0\text{m}$ ). Also, golf course ponds were the least covered by macrophytes ( $2.3\% \pm 2.1\%$ ) and showed the least variability, while stormwater ponds showed higher variability (Table 4.11). The environmental parameters of the ponds were also different in the three pond types. The water quality and the physical features in and around the pond were significantly different among the three pond types in summer ( $F_{2,8} = 3.38$ ,  $p < 0.001$ ) and winter ( $F_{2,8} = 3.07$ ,  $p < 0.001$ ). In summer, a pairwise test showed that i) stormwater and golf course ponds ( $t = 2.1$ ,  $p < 0.01$ ) and ii) ornamental and golf course ponds ( $t = 2.0$ ,  $p < 0.01$ ) were significantly different. Similarly, in winter, i) stormwater and golf course ponds ( $t = 1.9$ ,  $p < 0.01$ ) and ii) ornamental and golf course ponds ( $t = 2.0$ ,  $p < 0.01$ ) were significantly different.

Table 4.10 Physicochemical water quality parameters of the ponds in summer and winter. ANOVA test that showed significant difference ( $p < 0.05$ ) among the ponds have bold p values.

Physicochemical variable	Summer			ANOVA Test		Winter			ANOVA Test	
	Stormwater	Ornamental	Golf course	F	p value	Stormwater	Ornamental	Golf course	F	p value
pH	6.81 ± 0.44	6.37 ± 1.27	7.05 ± 0.32	3.68	0.1	6.69 ± 0.37	6.33 ± 0.56	6.54 ± 0.66	0.66	0.5
% DO	25.97 ± 13.83	42.20 ± 14.34	50.7 ± 12.23	5.78	<b>0.01</b>	45.87 ± 17.90	48.99 ± 23.94	61.12 ± 22.90	0.82	0.4
Con. ( $\mu\text{Scm}^{-1}$ )	244.4 ± 82.2	172.8 ± 58.1	111.2 ± 42.59	7.08	<b>0.007</b>	249.0 ± 213.36	194.4 ± 58.6	125.3 ± 79.51	4.6	<b>0.03</b>
TDS (ppm)	115.4 ± 28.3	86.8 ± 29.1	58.83 ± 24.5	6.89	<b>0.008</b>	158.7 ± 106.73	94.3 ± 25.1	65.3 ± 37.9	3.0	0.08
Sal. (psu)	0.12 ± 0.04	0.08 ± 0.03	0.05 ± 0.02	7.21	<b>0.006</b>	0.15 ± 0.10	0.09 ± 0.02	0.06 ± 0.04	3.02	0.08
Tem. (°C)	19.86 ± 1.68	19.31 ± 4.05	22.62 ± 1.30	4.49	<b>0.03</b>	14.34 ± 1.26	13.27 ± 1.44	14.06 ± 0.04	1.29	0.3
NO <sub>3</sub> -N ( $\text{mg l}^{-1}$ )	0.14 ± 0.19	0.17 ± 0.19	0.32 ± 0.24	1.31	0.3	0.15 ± 0.18	0.17 ± 0.16	0.39 ± 0.19	3.34	0.06
PO <sub>4</sub> <sup>3-</sup> ( $\text{mg l}^{-1}$ )	1.01 ± 0.73	1.38 ± 0.72	1.49 ± 0.86	0.63	0.5	1.77 ± 0.71	0.85 ± 1.05	1.61 ± 0.44	2.34	0.1
NH <sub>3</sub> -H ( $\text{mg l}^{-1}$ )	0.33 ± 0.17	0.16 ± 0.10	0.12 ± 0.11	3.86	<b>0.04</b>	0.21 ± 0.14	0.11 ± 0.11	0.17 ± 0.08	1.09	0.3

Table 4.11 Physical features of the ponds sampled. Average values are reported with '±' standard deviation. In addition, the result of the ANOVA test is reported. Significant p values (5%) are in bold.

Physical variable	Stormwater			Ornamental			Golf course			ANOVA Test	
	Min	Max	Average ± SD	Min	Max	Average ± SD	Min	Max	Average ± SD	F	p value
Distance to a road (m)	20.3	39.2	28.7 ± 8.0	4.6	121.5	59.3 ± 48.0	212.6	354.6	279.3 ± 58.3	19.47	<b>0.002</b>
Connectivity (m)	16.7	317	123.4 ± 137.2	21.6	101.5	61.4 ± 32.6	14.7	110.1	46.6 ± 44.9	0.45	0.6
Area (m <sup>2</sup> )	684	6312	3184 ± 2340	50.99	4648	2600 ± 1910	2265	6957	4093 ± 2050	0.25	0.7
Catchment area (m <sup>2</sup> )	2106	31762	12004 ± 13971	3155	6734	4942 ± 1461	856	7101	3146 ± 2808	0.64	0.5
% Cover by macrophyte	0	80	43.3 ± 33.0	30	60	46.7 ± 12.5	0	5	2.33 ± 2.05	2.92	0.1

## 4.5 Discussion

### 4.5.1 The effect of human population density

I assessed anthropogenic influence on macroinvertebrate communities in ponds using two approaches (based on human population density and artificial ponds). In terms of the human populations, based on the taxonomic approach, I found an association between the human population density and the macroinvertebrate compositions. This finding was expected as anthropogenic activities affect land use and are evidently a key determinant of variation in macroinvertebrate abundance and taxonomic diversity (Hill *et al.* 2016; Olson and Hawkins 2017). Ponds in the rural areas were most diverse both at the local and regional levels. This diversity could be due to the variety of mesohabitats in the ponds. Also, the land use and management practices around these ponds involving the introduction of macrophytes (both floating and emergent) and the minimum impact of anthropogenic activities are likely to contribute to the observed trend. The ponds in the rural areas had a high percentage cover of macrophytes hence the high taxa richness. Although most farming activities that negatively impact macroinvertebrate composition occur in rural areas, the ponds I sampled in the rural areas did not show this trend. Though one of the ponds had an organically managed farm adjacent to it, the macroinvertebrate community was remarkably diverse compared to the other ponds. On the contrary, ponds in large urban areas recorded lower taxa richness due to the relatively low macrophyte cover. The ponds in the rural areas were also closely connected to other ponds or other aquatic ecosystems, which is likely to facilitate the dispersal of taxa across the different aquatic systems leading to high taxa richness (Hassall and Anderson 2015; Thornhill *et al.* 2017).

The relative lower gamma diversity recorded in urban ponds can be due to heavy metal pollution, as reported in streams in the Auckland region (Stephansen *et al.* 2016; Tablada 2018). Pollution is associated with low biodiversity in aquatic ecosystems (Clapcott *et al.* 2017; Tamiru 2018). The relatively high abundance of pollution tolerant taxa like Platyhelminthes (*Cura*) and gastropods and the low diversity observed in the urban ponds suggest that the urban ponds may be polluted. Despite the marginal differences observed in the water quality along the gradient, the macroinvertebrate communities are better indicators of the state of freshwater ecosystems (Parmar *et al.* 2016).

The variability in macroinvertebrate compositions aligns with the differences in the environmental parameters. The similarity in the community compositions in the ponds in small and major urban areas was due to the similar habitat conditions (such as salinity, conductivity, and TDS levels). The differences between the ponds in the major and large urban areas were driven by a high abundance of insects (*Sigara*, *Diaprepocoris*, and Veliidae) in the large urban areas. Also, a high abundance of Crustacea (Ostracod, Cladodera, Cycloipoida) in the major urban areas contributed to the differences. The variations in insect' abundance can be due to differences in the altitude of the ponds (Barquín 2004). Ponds in the large urban areas occurred on a lower elevation (ponds in large urban areas were 2x lower than major urban ponds), which is likely to facilitate immigration and colonization of the ponds by insects. This result is similar to findings by Barquín and Death (2004) and Hinden *et al.* (2005). They reported insects' richness and biodiversity, in general, to be negatively correlated with altitude in streams in New Zealand and ponds in Switzerland, respectively. Due to little macrophyte cover, the ponds in large urban areas had a relatively higher open water surface area. The open water is likely to be attractive for *Sigara*, *Diaprepocoris* and Veliidae that prefer open water for skating. Additionally, the differences in physicochemical variables like % DO, conductivity, and TDS could contribute to the differences in macroinvertebrate compositions observed between the major and large urban ponds.

Environmental factors, especially water quality, varied between the ponds in small urban and rural areas across the seasons, resulting in significant species composition and abundance changes. The ponds in the rural areas were more silted (high TDS) and therefore had a relatively lower abundance of insects and Chelicerata. Nevertheless, the high percentage of macrophyte cover and the heterogeneous habitat structure led to high macroinvertebrate diversity in these rural ponds, similar to findings by Dalu and Chauke (2020) and Milesi *et al.* (2016).

Although the human population influenced the macroinvertebrate communities, taxa richness and the community compositions of the densely (major urban areas) and sparsely (rural areas) populated areas were not distinct in winter. The high precipitation during the winter season in New Zealand is likely to enhance the dispersal of taxa among aquatic ecosystems. Therefore, it is expected that the communities will be less spatially structured in winter despite the differences in environmental variables (Zhang *et al.* 2019). This similarity in richness could also result from factors other than the environmental parameters assessed, such as geology. Differences in geology have influenced macroinvertebrate community assemblage in New Zealand by affecting sedimentation and water chemistry (Shearer and Young 2011). Since ponds

in the rural area had the highest diversity, the hypothesis that the human population influences macroinvertebrate compositions in ponds is confirmed though this influence is seasonally variable.

#### **4.5.2 Functional feeding group**

The high abundance of Notonectidae and *Sigara* contributed to predator dominance in the ponds irrespective of the season. Predators have been reported to show high abundance in diverse landscapes in China (Fu *et al.* 2016) and Argentina (Mesa *et al.* 2013; Neesemann *et al.* 2017), similar to my findings. The dominance of predators signifies a heterotrophic dominant food web and pollution in the ponds (Neesemann *et al.* 2017). Predators are secondary consumers, and their abundance indicates a higher abundance of primary consumers (mainly other macroinvertebrates) and primary producers in the ponds.

The relative abundance of collector gatherers in rural ponds compared to other gradients may be due to the high availability of food resources in the ponds related to the observed pond's habitat complexity and also reported by Barquín and Death (2004), Bazzanti *et al.* (2010) and (Mesa *et al.* 2013). Collector gatherers are sensitive to water quality and anthropogenic activities; hence, their dominance indicates a relatively higher water quality and the importance of seston in the water column (Mesa *et al.* 2013; Neesemann *et al.* 2017). According to Solimini *et al.* (2008), the low diversity of FFG in ponds indicates stressful conditions. Therefore, it can be deduced that the rural ponds were less anthropogenically impacted compared to the ponds in the other gradients. Further, the relative higher richness of FFG in the ponds in the rural area suggests higher resilience of the community against the absence or loss of any taxa belonging to any FFG (Thornhill *et al.* 2018).

The scarcity of shredders is expected as they have a long life cycle and are slow in colonizing ecosystems (Mesa *et al.* 2013; Thornhill *et al.* 2021). Shredders are indicators of good water quality but are usually uncommon in freshwater ecosystems, especially those in urban landscapes (Shearer and Young 2011). Consequently, the rarity of shredders in the ponds affirms the relatively low water quality compared to rivers and streams. Thus, the FFG provides evidence of widespread anthropogenic influence on the pond ecosystems. However, it could not differentiate among the communities in winter.

Compared to the taxonomic measures, the FFG was weak in differentiating among levels of anthropogenic impact, similar to Thornhill *et al.* (2018), who found a weak relationship between functional diversity and land use gradient (urban and agriculture) in ponds. However, FFG discriminates among levels of anthropogenic impact in lotic systems (Botwe 2017; Mesa *et al.* 2013; Neesemann *et al.* 2017 but see Palmer *et al.* 1996). Based on my findings, functional and structural approaches should be combined to assess anthropogenic impacts on ponds. Further, increasing the number of replicates will increase the sensitivity of these approaches to differentiate among levels of anthropogenic impacts (Estevez *et al.* 2017).

#### **4.5.3 The macroinvertebrate community in three types of artificial ponds**

There were high taxa richness and a high variation in taxa richness at the local level (alpha diversity: 6-29) in the three types of artificial ponds. The high variability indicates that the macroinvertebrate composition in each pond is different. Hill *et al.* (2016) reported similar high variability in alpha diversity in ponds in the UK. Due to this high variation in alpha diversity, ponds supported higher gamma diversity at the landscape level. The distinct catchment conditions and the habitat heterogeneity within and among ponds created habitats for different taxa resulting in high diversity observed at the regional scale (Hill *et al.* 2016; Williams *et al.* 2004).

There was a difference in the macroinvertebrate taxa among the three pond types. The high abundance of Odonata and Diptera in ornamental ponds is expected. These taxa prefer aquatic systems with macrophytes to provide refuge for larvae against predators. The relative high cover by macrophytes in the ornamental ponds also provides oviposition sites for more taxa (Zelnik *et al.* 2018). The high richness of Coleoptera, Hemiptera, Trichoptera, Plecoptera and Ephemeroptera in the ornamental ponds indicate suitable habitat conditions, including good water quality (Moore 2003; Tablada 2018). The dominance of Diptera (with broad tolerance for environmental conditions) in stormwater ponds are indicative of the presence of macrophyte, isolation (lacking close connectivity) and pollution of these ponds (Hill *et al.* 2016; Sun *et al.* 2019). This finding supports the assertion that ponds connected to other aquatic ecosystems tend to have a more heterogeneous macroinvertebrate community than unconnected ponds (Hill *et al.* 2016; Thornhill *et al.* 2017). Further, the relatively higher conductivity, salinity and TDS, and low % DO levels observed in the stormwater ponds compared to the others signifies pollution.

Ornamental ponds supported significantly higher taxa richness than stormwater and golf course ponds. The ornamental ponds high diversity is not surprising because they had a complex layout consisting of different macrophyte types (submerged, floating, emergent, and rocky surfaces) (Declerck *et al.* 2006; Dube *et al.* 2020; Johansson *et al.* 2019; Martins *et al.* 2020). These provided different mesohabitats and additional habitats for the macroinvertebrates (Harabiš 2016). The difference in the macroinvertebrate richness may also be due to the location of the ponds. The ornamental ponds were either located in reserve or rural areas where anthropogenic influence is minimal compared to the stormwater ponds which were located in urban areas. The proximity of the stormwater ponds to major highway roads exposes them to high pollution and runoff, evidenced by high conductivity and the lower taxa richness (Greenway 2010; Meland *et al.* 2020). The golf course ponds were located in a managed landscape where the courses were fertilized to enhance grass growth. This management activity increases the nutrient load causing these ponds to have high nitrate levels, negatively affecting taxa richness (Boone *et al.* 2008; Meland *et al.* 2020). Again, the golf course ponds were managed to reduce macrophyte cover. Therefore, it is not surprising that the taxa richness of the ponds was low despite occupying comparatively more extensive areas.

Connectivity to other aquatic systems increases macroinvertebrate richness (Hill *et al.* 2016; Meland *et al.* 2020; Noble and Hassall 2015). The golf course ponds were most closely connected to other aquatic bodies (connectivity enhance dispersal of macroinvertebrates) within the courses. However, they were affected by the same management practices, reducing the potential of close connectivity in increasing taxa richness.

The similarity in community composition between stormwater and ornamental ponds iterates the vital role of macrophytes in increasing macroinvertebrates richness and diversity. It also affirms the importance of stormwater ponds in maintaining regional macroinvertebrate diversity (Briers 2014; Greenway 2010; Meland *et al.* 2020; Sinclair *et al.* 2020; Stephansen *et al.* 2016). Colding *et al.* (2009) reported no difference in species composition between the ponds in golf courses and urban parklands. Similarly, stormwater and golf course ponds had similar macroinvertebrate compositions in this study. This result reflects similar anthropogenic effects on these ponds. The differences in the environmental variables contributed mainly to the different macroinvertebrate compositions recorded in the different ponds. However, these differences were not evident in the community composition in winter.

Ornamental ponds in this study supported the highest taxa richness and diversity of macroinvertebrates. Despite this, stormwater and golf course ponds also harboured taxa exclusive to them and contributed to the high regional diversity. Therefore, stormwater and golf course ponds must be seen as habitats for aquatic fauna and not only for the aesthetic value (golf course ponds) or water retention (stormwater ponds). So, it is important to enhance the habitat suitability of golf courses and stormwater ponds through reconciliation ecology. Reconciliation can be achieved by modifying these habitats to support biodiversity while maintaining the efficiency of their primary purpose to achieve the conservation of freshwater macroinvertebrates (Rosenzweig 2003).

### ***Implications of the findings for freshwater management***

As human population increases, the water quality and the macroinvertebrate community of ponds are likely to degrade due to increased pollutants resulting in low diversity, including dominance by pollution tolerant taxa and an increase in non-native and invasive species (Sinclair *et al.* 2020; Thornhill *et al.* 2017). Ponds in urban areas, particularly those in public spaces, are more predisposed to these impacts. From my results, ponds in densely populated areas had a higher abundance of pollution tolerant taxa; therefore, enhanced management action is necessary. For example, macrophytes should be incorporated in ponds to improve their quality (Declerck *et al.* 2006). Also, minimising nutrient input, especially from wrong household drainage connections. Furthermore, natural filtration (for example, the use of porous materials for pavement construction) of surface runoff into freshwater ecosystems in urban areas should be prioritised in managing these ecosystems (Sinclair *et al.* 2020; Thornhill *et al.* 2017). Additionally, the construction of ponds should be such that they form a network to enhance the dispersal of taxa among ponds. Finally, mesohabitat within ponds should be heterogeneous to improve taxa diversity (Bilton *et al.* 2001; Thornhill *et al.* 2017).

## 4.6 Conclusion

In this chapter, I present the effect of anthropogenic activities on macroinvertebrate community compositions in ponds from highly populated areas and low populated areas. The impact was assessed using both the taxonomic and functional approaches. Additionally, I compared the macroinvertebrate community in three types of artificial ponds. The macroinvertebrate taxonomic composition in the most populated area (major urban areas) and least populated areas (rural) were similar. However, the macroinvertebrate richness was significantly different. Ponds in rural areas were the most diverse and, therefore, the least impacted site. There were variations in the FFG composition in the ponds. Predators were the most dominant group, which generally signifies low water quality. The FFG compositions in the ponds differed along the urban-rural gradient in summer. The FFG approach also indicated that ponds in the rural areas were of higher water quality. In comparing the two approaches, the taxonomic composition was better at discriminating among the ponds along the human population gradient irrespective of the season. The macroinvertebrate communities varied among the three types of artificial ponds. Ornamental ponds were the most taxa rich, followed by stormwater ponds and golf course ponds. Although stormwater and ornamental ponds had similar macroinvertebrate compositions, golf courses and ornamental ponds have different community compositions. Ornamental ponds were also rich in sensitive insect taxa such as Ephemeroptera, Plecoptera, and Trichoptera. The significant differences in the environmental conditions, including anthropogenic activities around the ponds, contributed to the variation observed. Despite the limitation of generalization of the results due to small sample sizes, the findings of this chapter show that human population density is associated with macroinvertebrate communities in ponds. In addition, macroinvertebrates sensitive to pollution (habitat degradation) were observed in ponds in areas with less anthropogenic influence. Nonetheless, the anthropogenic impact was seasonally dependent. Finally, all ponds contributed to the high regional macroinvertebrate diversity observed irrespective of the function or location.

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#### Chapter 4 | Anthropogenic influence on macroinvertebrate communities in ponds

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#### Chapter 4 | Anthropogenic influence on macroinvertebrate communities in ponds

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# Chapter 5

## Phytoplankton community composition and environmental correlates in ponds



## 5.1 Abstract

Phytoplankton is one of the most widely distributed taxa globally and has been widely used as an indicator of aquatic ecosystem quality. Ponds are important freshwater ecosystems and common features in anthropogenic landscapes. This chapter documents the phytoplankton community composition in ponds and how they vary seasonally and across a human population density gradient. It also explores the relationship between the phytoplankton community and physicochemical water quality variables. Phytoplankton was sampled from 12 ponds in two different seasons (summer and winter) in 2018. I discovered that the phytoplankton community showed significant seasonal differences. Phytoplankton abundance, richness, and the number of taxa were significantly higher in summer than winter. Pielou's evenness was, however, significantly higher in winter. Chlorophyta was the dominant phylum in both seasons. Bacillariophyceae was the dominant class and showed a significant difference in abundance between the seasons. Overall, 126 genera of phytoplankton ( $\gamma$  diversity) were recorded, and the number of taxa ( $\alpha$  diversity) for a pond varied from 44 and 76. The physicochemical water quality variables varied seasonally. Temperature and conductivity played a significant role in the phytoplankton communities; human population density also influenced the summer's phytoplankton communities. Ponds in the major urban area were more silted. Although the ponds in the Auckland region had low to moderate organic pollution levels, they contained healthy phytoplankton communities.

## 5.2 Introduction

Algae are one of the most widely distributed taxa globally and occur in all types of ecosystems and habitats (Musa 2015). They are present in all surface water, including rivers, lakes, canals, ponds, estuaries, and canals (Flint 1966). Their size ranges from single microscopic cells to larger kelps in oceans (Hoek *et al.* 1995). Algae are eukaryotes (cells having a membrane-bound nucleus) (Hoek *et al.* 1995). Some prokaryotes (cells lacking a membrane-bound nucleus) known as cyanobacteria can also be classified as algae.

Algae are autotrophic and primary producers forming the food chain base in aquatic ecosystems (Lévesque *et al.* 2020). They have chlorophyll molecules that are used to attract energy from the sunlight and obtain nutrients (nitrogen and phosphorus) from the surroundings to produce organic carbon, releasing oxygen as a by-product (Biggs 2000a). Other organisms rely upon algae's oxygen for their metabolism (Dung *et al.* 2017).

### 5.2.1 Types of algae based on habitat types

Algae live in aquatic ecosystems either as suspended (floating) in the water column (phytoplankton) or attached to various substrates (periphyton) (Naselli-Flores and Padiak 2016). Periphyton can be epipelagic, epilithic, epipsammic, epizoic, or epiphytic, growing on silt, rocks, sand, animals, or plants, respectively (John *et al.* 2011). When algae occur in the bottom part of the aquatic system, they are referred to as benthic (John *et al.* 2011). Although benthic algae are appropriate for determining the effect of pollution on specific locations in an ecosystem, phytoplankton reflects the impacts of organic pollution on the whole ecosystem (McCormick and Cairns 1994). Though phytoplankton contributes to about 50% of the global primary productivity (Field *et al.* 1998, Branco *et al.* 2020), phytoplankton in freshwater contributes approximately 29% to the global freshwater primary productivity (Likens 1973). This chapter focuses on the phytoplankton community in ponds within a human-modified landscape.

### 5.2.2 Classification of algae

Generally, algae are classified into taxonomic groups based on the predominant pigment they possess, how they move, and their cell formation (Biggs 2000a). The major phylum of algae has been classified into various groups by different authors, but the main groups are Chlorophyta, Ochrophyta, Cyanophyta, Euglenophyta, Cryptophyta, which are dominant in freshwater ecosystems and Dinophyta. Also, the phylum Rhodophyta which are prevalent in the marine environment (Necchi 2016b; van Vuuren *et al.* 2006).

Each group comprises diverse algae communities, which are further classified based on their shape, number of cells, number of chloroplasts, and colour (Table 5.1). Although some major groups can be distinguished with the unaided eye, identification, especially of microalgae to genera and species taxonomic level, is only possible using a high-power magnification on a microscope to distinguish the patterns of branching or reproductive structures or cell morphology (Biggs 2000a).

Table 5.1 Characteristics of algal groups (adapted from Biggs 2000a; van Vuuren *et al.* 2006). Note there are exceptions from the table below.

Group	Colour	Cell formation	Movement	Other features
Ochrophyta	Golden brown or yellow, yellowish-brown	Single or colonial, mostly unicellular	Motile, immotile, or gliding	Cells consist of two halves, raphe present, siliceous cell wall, two unequal flagella, pseudopods present
Chlorophyta	Grass green	Single, colonies or filaments (branched and unbranched)	Immotile, motile, or gliding	Two to four flagella
Cryptophyta	Red, blue-green, olive-green, olive-brown	Unicellular	Motile	Slightly unequal flagella
Cyanophyta	Blue-green or olive-green	Single, colonial, or filamentous	Motile or gliding	Sheath present
Dinophyta	Brown to yellow brown	unicellular	Motile	Has two equal flagella arising from a groove
Euglenophyta	Bright green, sometimes with a red eyespot	Unicellular	Swimming	Ability to change shape
Rhodophyta	Grey-green	Mostly filamentous		

### Phylum Chlorophyta (Green algae)

Chlorophyta is one of the most diverse algal groups in terms of abundance, occurrence, and richness (van Vuuren *et al.* 2006). They are characterized by the presence of pyrenoids that store starch and possess both chlorophyll 'a' and 'b' in their chloroplast (Sherwood 2016). Some members possess a stigma (red eyespot), whilst others possess two to four equal anterior flagella, which they use for swimming (Table 5.1, van Vuuren *et al.* 2006). Green algae generally thrive in aquatic systems with moderate to high nitrogen to phosphate concentration (van Vuuren *et al.* 2006). When lentic ecosystems, especially ponds, have their phytoplankton community dominated by Chlorophyta, the water often appears dark green.

## Phylum Ochrophyta

Ochrophyta is a major group composed of about 15 classes, but the three dominant classes are Bacillariophyceae, Chrysophyceae, Xanthophyceae.

### Class Bacillariophyceae (Diatoms)

Bacillariophyceae are unicellular algae distinguished from other algae by their cells (frustule) consisting of two halves (valves). The cell wall is made of silica and has a slit through it known as the raphe (Burliga and Kociolek 2016; Musa 2015). Bacillariophyceae store their food in a form known as chrysolaminarin and oil droplets, which keeps them buoyant (van Vuuren *et al.* 2006). Bacillariophyceae produce approximately 20% of the oxygen on earth annually and are responsible for about 45% of the global gross primary production (Musa 2015). They are used in the production of biofuels, cosmetics, food and paints, and their frustules are used in filtration (Musa 2015; van Vuuren *et al.* 2006).

Many studies have widely used Bacillariophyceae to determine aquatic ecosystems' ecological status (Castillejo *et al.* 2018; Lobo *et al.* 2016; Rimet *et al.* 2015; Temizel *et al.* 2017). Their biotic indicator quality is based on their capacity to respond to changes in the water quality and their sensitivity to organic and inorganic pollution (Temizel *et al.* 2017). Several indices have been developed based on diatom community composition to assess the ecological quality of aquatic ecosystems. These include the Trophic Diatom Index (TDI) (Kelly and Whitton 1995), Pollution Tolerance Index (PTI), Specific Pollution Sensitivity Index (SPI), and Biological Diatom Index (BDI) (Musa 2015; Temizel *et al.* 2017).

### Class Chrysophyceae (golden-brown algae) and Xanthophyceae (yellow-green algae)

These classes are common but are not very diverse and are the least studied (Ashrafi 1999). They occur mostly in oligotrophic freshwater systems (Izaguirre *et al.* 1998; Necchi 2016a). Chrysophyceae are autotrophs and heterotrophs and have an accessory pigment (fucoxanthin) which masks the colour of chlorophyll 'a', 'c<sub>1</sub>' and 'c<sub>2</sub>' (van Vuuren *et al.* 2006). Some species of Chrysophyceae may be good bio-indicators because they have a minimum tolerance range for temperature, conductivity, pH, and nutrient levels (Ashrafi 1999; Necchi 2016a). Xanthophyceae and Chrysophyceae, when present, occur in low abundance in freshwater ecosystems (Padisák

*et al.* 1998). Chrysophyceae dominate the algae community in lakes in the Antarctic peninsula (Izaguirre *et al.* 1998).

### **Phylum Cyanobacteria (Blue-green algae)**

Cyanophyta are true bacteria and lack membrane-bound organelles. Cyanophyta are photo oxygenic bacteria found in all aquatic systems irrespective of their water quality and may be singular, filamentous, or colonial (Table 5.1). Cyanophyta possess three photosynthetic pigments: chlorophyll 'a' (a green pigment), phycocyanin (blue pigment) and phycoerythrin (red pigment). A combination of these pigments gives Cyanophyta either a blue-green or a purple appearance (van Vuuren *et al.* 2006).

Cyanophyta are usually small in size and have limited morphological features (Casamatta and Hašler 2016). Some species can fix inorganic nitrogen from the atmosphere and produce about 30% of the global oxygen annually (Casamatta and Hašler 2016; Sabater *et al.* 2016). Cyanophyta, especially some filamentous and colonial forms, thrive and dominate algae communities in eutrophic (nutrient-rich) aquatic ecosystems (Flint 1966; Järvinen *et al.* 2013). Anthropogenic factors mostly cause Cyanophyta blooms primarily related to land use, leading to increased nitrogen and phosphorus concentrations, although warm weather conditions can serve as a catalyst (van Vuuren *et al.* 2006). Low nitrogen to phosphate concentrations increases Cyanophyta pollutions or bloom likelihood in aquatic ecosystems (Amengual-Morro *et al.* 2012). Cyanophyta blooms are mostly an indication of degradation (Casamatta and Hašler 2016).

Cyanotoxins can be neurotoxic, derma-toxic or hepatotoxic, posing a health risk to humans and animals that may come into contact with contaminated water (Wood *et al.* 2017). The genera *Dolichospermum* (previously *Anabaena*) and *Microcystis* produce toxins linked to animals' death (Amengual-Morro *et al.* 2012). For example, *Microcystis* has been linked to farm animals' death in Waipukurau in New Zealand (Flint 1966).

Not all taxa of Cyanophyta are associated with nutrient-rich ecosystems. For example, some genera such as *Calothrix* and *Tolypothrix* prefer unenriched aquatic systems and have been found to dominate the algal biomass in oligotrophic streams in New Zealand (Biggs 2000a; Casamatta and Hašler 2016; Wood *et al.* 2017). Therefore, although Cyanophyta is a good predictor of aquatic ecosystem health, they have been rarely used as indicators due to uncertainty surrounding their systematics and difficulty in identification.

### **Phylum Euglenophyta**

Taxa belonging to the phylum Euglenophyta are unicellular with bright green chloroplast and a red eyespot on the front end (Table 5.1; (van Vuuren *et al.* 2006)). Phylum Euglenophyta flourish in aquatic systems with high nutrient levels, rich in decaying organic matter (van Vuuren *et al.* 2006), and they are sensitive to grazing (Aboim *et al.* 2020).

### **Phylum Cryptophyta**

Members of the phylum Cryptophyta are few but widespread (van Vuuren *et al.* 2006). They are dorsoventrally flattened; usually leaf or heart-shaped, possess a gullet and are motile (Table 5.1). Cryptomonads are an excellent food source for zooplankton and are widely used in fish farming (van Vuuren *et al.* 2006).

### **Phylum Dinophyta**

Members of this phylum may be autotrophic, heterotrophic or mixotrophic (engulf other organisms) (van Vuuren *et al.* 2006). They have flagella for swimming and have cell walls, and may possess chlorophyll for photosynthesis (Table 5.1) (van Vuuren *et al.* 2006). The distinguishing feature of phytoplankton belonging to the phylum Dinophyta is the possession of thecal (armour-like) plates, which covers them (van Vuuren *et al.* 2006). Dinoflagellates can cause blooms known as “red tide” and release neurotoxins that are harmful and sometimes lethal to humans and animals (van Vuuren *et al.* 2006).

### **Phylum Rhodophyta**

Rhodophyta is usually filamentous and typically occur in marine environments but may occur in lotic systems and rarely in lentic ecosystems (Table 5.1) (Necchi 2016b). In New Zealand, they are rare and restricted to bedrock habitats (Biggs 2000a). The occurrence and distribution of Rhodophyta are primarily controlled by light quality and intensity and photoperiod (Necchi 2016b), and they are considered indicators of good water quality (Necchi 2016b). For example, the *Audouinella* is known to dominate oligotrophic streams in New Zealand (Biggs 2000a).

### 5.2.3 Ecological significance of phytoplankton

Phytoplankton are significant primary producer in lentic freshwater ecosystems. They often respond to nutrient concentration changes in ponds and are good indicators of water quality (Carvalho *et al.* 2013). However, phytoplankton are present in polluted and unpolluted aquatic systems with different taxa adapted to surviving in different pollution levels (Jafari and Gunale 2006). Phytoplankton provide vital information on the relationships in energy flow in aquatic ecosystems because of their integral role in ecosystem energetics (primary productivity) and biogeochemical cycling (Galbraith and Burns 2010; McCormick and Cairns 1994). Phytoplankton contributes significantly to primary productivity, and as phototrophs, they produce most of the oxygen on earth. (Amengual-Morro *et al.* 2012). Phytoplankton also have a secondary role in absorbing nutrients such as nitrogen and phosphorus (Amengual-Morro *et al.* 2012) from ecosystems, thereby improving water quality (Biggs 2000a).

Despite the importance of phytoplankton, their excessive growth has been associated with odour production and biodiversity loss, such as fish and macroinvertebrates (Biggs 2000a; Peretyatko *et al.* 2007; Novis *et al.* 2020). Some taxa in the phylum Cyanophyta can also produce toxic compounds, while other taxa form mats capable of clogging pipes and reducing the aesthetic value of the aquatic system (Hamill 2001; Novis *et al.* 2020; Ryan *et al.* 2003; Wood *et al.* 2017). These negative impacts of phytoplankton reduce the economic, social, cultural and biodiversity value of the aquatic ecosystem, which usually requires a lot of investment for restoration (Biggs 2000a).

Phytoplankton are sensitive to a broad range of environmental stressors, reproduce quickly, are ubiquitous, have high species richness and are easy to sample (Aboim *et al.* 2020; McCormick and Cairns 1994). Finally, phytoplankton provides a cost-effective monitoring tool in terms of information gained per unit effort (McCormick and Cairns 1994). The changes in water quality and hence phytoplankton compositions are associated with seasonal changes or land use/ land cover factors such as an increase in the input of nutrients (Aboim *et al.* 2020; Lévesque *et al.* 2020; Saeiam *et al.* 2020). Sometimes, water quality change leads to a change in the dominant phytoplankton group (Hu *et al.* 2020; Yusuf 2020). Therefore, identifying the phytoplankton taxa present and relating it to the water quality parameters can help understand the ecosystem's ecological dynamics (Amengual-Morro *et al.* 2012).

In Europe, phytoplankton is the only biotic component of the biodiversity approved by the Water Framework Directive (Directive 2000/60/CE) to classify lentic ecosystems (Martins *et al.* 2020). Similarly, in New Zealand, the trophic level index used in classifying lentic systems considers chlorophyll 'a' as a proxy for phytoplankton biomass (Biggs 2000b).

#### 5.2.4 Factors that affect phytoplankton community structure

Phytoplankton communities in aquatic ecosystems are influenced by abiotic and biotic factors (Mosisch *et al.* 2001; Santana *et al.* 2017). The abiotic factors include geographic (altitude, latitude), climatic (length of daylight, precipitation, temperature), geological (soil type, hydrological regimes), and land use/ land cover (LULC) (nutrient availability) types. The biotic factors include invertebrate grazer community composition and intensity of predation or grazing (Mosisch *et al.* 2001).

In chapter 1, I highlighted some of the effects of water quality on phytoplankton. Further, some studies have reported that changes in pH (reduction in pH) increase the number of specific phytoplankton taxa of green algae (Zygnemataceae) and Bacillariophyceae (*Pinnularia*, *Eunotia*) (Hill *et al.* 2000; van Vuuren *et al.* 2006). Apart from the water quality, phytoplankton richness increases with increasing altitude (Barinova and Chekryzheva 2014). Taxa such as Cyclotella, Dinoflagellates, Cryptophyta and Chrysophyta dominate freshwater ecosystems in high latitude and high altitudes (Järvinen *et al.* 2013).

Decreases in the abundance of decomposers and invertebrate grazers increase phytoplankton abundance and change their population structure (Yan *et al.* 2020). Despite the effect exerted by individual factors, there are usually interactions between multiple biotic and abiotic factors on phytoplankton community composition. For instance, Fairchild *et al.* (1989) showed that algae increase with increasing nutrient load even if more grazers are present in the ecosystem. Furthermore, differences in phytoplankton community composition occur due to the demand of specific taxa for specific water quality conditions and geomorphological characteristics (Sabater *et al.* 2016). At the regional level, landscape parameters such as land use/ land cover and slope and anthropogenic activities such as habitat alteration determine the phytoplankton community composition. At the local level, the phytoplankton community is influenced by the temperature, nutrient availability, and trophic structure (Chang *et al.* 2021; Fonseca and Bicudo 2008).

### 5.2.5 Phytoplankton indices and metrics

Both structural and functional parameters of phytoplankton are used in water quality monitoring. Measures of phytoplankton function include gross primary productivity, growth rates, the chemistry of mats and nutrient limitation experiments. These measures are time consuming and are not directly related to anthropogenic disturbances (Biggs 2000a). On the contrary, phytoplankton structural parameters measures which include community composition, percentage sensitive or tolerant species, diversity indices, and biomass (Martins *et al.* 2020; McCormick and Cairns 1994), provide a more reliable estimate of ecosystem condition than measuring biomass (McCormick and Cairns 1994). Measures of phytoplankton community structure efficiently provide accurate ecosystem conditions (Biggs 2000a; McCormick and Cairns 1994). The taxonomic grouping has been widely used to indirectly include important environmental drivers due to the possession of important adaptive features that affect the phytoplankton composition and maintain taxonomic separations (Paul *et al.* 2012). These factors make structural measures a preferred choice (Biggs 2000a). The structural metrics include:

- i) percentage of Bacillariophyceae present: Studies have shown that diatoms dominate phytoplankton community composition in less polluted sites. Sites with communities dominated by other taxa (like green algae and cyanobacteria) indicate more pollutants (Hill *et al.* 2000; Palmer 1969),
- ii) dominant Bacillariophyceae taxa present: Different diatom taxa are adapted to specific pollution levels. More sensitive diatom genera dominate the diatom community in habitats with good water quality. Polluted sites will be dominated by species that can tolerate pollution (Hill *et al.* 2000). Hence the genera of diatom that dominate the community indicate the aquatic system's pollution status (Hill *et al.* 2000),
- iii) acidophilic Bacillariophyceae: This metric is based on the pH tolerance or sensitivity levels of diatoms. It is expected that a change in pH will favour specific diatom taxa and thus cause these taxa to dominate under these conditions (Hill *et al.* 2000; Palmer 1969). Acidophilic diatom taxa include *Eunotia*, *Pinnularia* and *Tabellaria* (Hill *et al.* 2000),
- iv) eutraphentic Bacillariophyceae are taxa that tolerate nutrient enrichment (pollution) and provide a metric that increases with increasing pollution levels (Hill *et al.* 2000). Eutraphentic diatom includes *Gomphonema*, *Nitzschia* and *Melosira* (Hill *et al.* 2000),
- v) motile Bacillariophyceae: Studies have shown that the percentage of motile diatoms increases with increasing siltation, caused mainly by human activities. This metric is

used to classify sites according to siltation level (Hill *et al.* 2000). Examples of motile diatoms include *Gyrosigma*, *Surirella* and *Navicula* (Hill *et al.* 2000),

- vi) percentage Cyanobacteria: This metric accounts for the percentage of cyanobacteria in a phytoplankton community; and increases with increasing nutrient and toxic inputs into the aquatic ecosystem (Hill *et al.* 2000; Palmer 1969).

### 5.2.6 Phytoplankton community in New Zealand freshwater ecosystems

The phytoplankton community in aquatic ecosystems in New Zealand differs among different freshwater ecosystems and between similar ecosystems but is often dominated by Bacillariophyceae and Chlorophyta, with desmids occasionally common (Kuschel 2012). The taxa Cyanophyta, Chlorophyta and Bacillariophyceae have been reported as the dominant groups in lakes in Rotorua (Paul *et al.* 2012), in the Waikato River (Lam 1981), and in reservoirs and lakes in Otago (Downs *et al.* 2008). However, Biggs (2000b) found that Xanthophyceae (Ochrophyta) was more common than Chlorophyta and Bacillariophyceae in some rivers in the Otago. Cyanophyta is common in warm, shallow, and nutrient-rich ponds and lakes (Flint, 1966).

Occasionally, blooms of Cyanophyta and toxic algae have occurred in several farm ponds (Flint, 1966). The latitudinal gradient influences the ponds' temperatures and hence the phytoplankton community such that the most northerly ponds have the highest minimum temperature, and the southerly ponds have the lowest maximum temperature (Kuschel 2012).

### 5.2.7 Knowledge gaps

Although the phytoplankton communities in lentic freshwater ecosystems have been studied in New Zealand, these studies have focused on lakes (Cassie 1969; Cassie 1974; Cassie and Freeman 1980; Duthie and Stout 1986; Flint 1938; Flint 1966; Galbraith and Burns 2010; Paul *et al.* 2012). These studies have found seasonal variation in phytoplankton communities (Cassie 1969; Duthie and Stout 1986; Flint 1938). Galbraith and Burns (2010) reported a significant influence of land use on water quality that, in turn, is a driver of the phytoplankton community in the South Island's freshwater ecosystems. Further, Paul *et al.* (2012) studied the influence of catchment land use and trophic state on phytoplankton composition in lakes. They found that the trophic state is correlated negatively with native forest but positively with pasture and urban area.

Studies on the phytoplankton community in ponds have received little attention in New Zealand. The few studies on phytoplankton in ponds that have provided preliminary findings were also undertaken several decades ago (Flint 1966). This gap makes the knowledge base on phytoplankton community composition in lentic systems, especially ponds, incomplete, and the phytoplankton community in the ponds is poorly understood. With the growing pressure on freshwater ecosystems and the deteriorating state of these ecosystems in New Zealand (Weeks *et al.* 2016), understanding phytoplankton's community structure in the most abundant lentic ecosystem in the most populous region is an emerging priority.

In this chapter, I describe the phytoplankton compositions of 12 ponds in the Auckland region. I explore and evaluate the effect of the water quality and seasonal variability on phytoplankton groups in ponds and determine the relationship between phytoplankton community compositions and pond water quality. Elucidation of specific phytoplankton groups' responses to environmental changes in different seasons can improve our understanding of annual phytoplankton succession (Deng *et al.* 2019). Furthermore, my findings provide a foundation for future studies to monitor and assess changes (and improvements) in the pond ecosystems.

### 5.2.8 Aims and hypotheses

The aims of this chapter are to:

- i) characterize phytoplankton compositions in ponds in the Auckland region,
- ii) assess if there are seasonal differences in phytoplankton composition,
- iii) determine if phytoplankton composition varies with the degree of urbanization, and
- iv) determine the relationships between key environmental variables and the phytoplankton communities.

My hypotheses are:

- i) phytoplankton compositions will differ among ponds because of varying environmental conditions,
- ii) the abundance of phytoplankton will be higher in summer than winter because of higher temperatures,
- iii) the human populations will influence the abundance and composition of the phytoplankton community. I predict that Cyanophyta will dominate in the major urban areas due to high pollution levels,
- iv) phosphates and nitrates concentrations are the main drivers of the phytoplankton compositions regardless of the season.

## 5.3 Methods and data analyses

Phytoplankton was sampled from 12 ponds once in the winter and summer of 2018. These ponds differed in location and were of varying sizes. Details of each pond's location, size, and depth are provided in Appendix B: Table 1.

Phytoplankton sampling was carried out once in the winter and summer of 2018. Sampling was carried out between the hours of 10:00 am – 3:00 pm NZT. At the ponds, one litre of water was collected from three different locations (same as where the physicochemical variables were measured) around the pond and poured into a bucket. The bucket was swirled, and a subsample poured into two labelled 100ml opaque sterilised bottles. The label had information on the pond's name, the collector's name, the time, and the date of sample collection. One percent Lugols' iodine solution (three drops) was added to one of the bottles containing the algae for preservation. The samples were stored in the dark between 2-6°C and then transported to the laboratory for identification. The sample without the Lugols' iodine solution was analysed within 48 hours after collection. The fast analysis prevented photo-oxidation and the phytoplankton from dying or losing their colour (Hall and Karol 2016). The sample containing the 1% Lugols' iodine was put into the fridge at a temperature of 2° to 4°C as a reference.

### Phytoplankton identification

#### *Preparation of slides*

Standard microscopic slides to be used for identifying algae under the microscope were thoroughly cleaned with 70% ethanol to wipe off any dust particles that might be on the slide. Dust particles can obstruct algae's identification by interfering with the colour and sometimes the algal cell's shape. The bottle containing the phytoplankton was swirled, and a pipette was used to take a 10µm subsample which was dropped on the cleaned slide. A clean coverslip was then used to spread the sample evenly on the slide. The slide was placed under the microscope (Leica DMRBE Research microscope), which had a camera attached to it and connected to a monitor. The camera made it easier to view algal cells under the microscope on the monitor and not from the eyepiece. Contents on the slide were first viewed under a magnification of 400×. Next, observations were done by moving the slide gently from left to right until the entire slide

contents had been captured. This step provided a general overview of the cells present in the sample.

After observing under 400×, a drop of oil was added to the coverslip. Phytoplankton cells were then observed under a magnification of 1000×. At this magnification, the algal cells became clearer to aid in identification. The slide was moved again gently from left to right in a grid pattern to ensure the entire slide was inspected. Photographs of each phytoplankton were taken each time they were brought into focus until all the cells present on the slide were captured. The captured pictures of the phytoplankton were saved in folders with exact details as the label on the sample and a slide number. This process of taking 10µm of the sample, preparation of slides, observations, and photographs was repeated ten times for each sample collected from each pond. A total of 240 slides (120 slides per season) were prepared and observed under the microscope.

#### *Phytoplankton identification*

All phytoplankton were identified from the photos that were taken from the slides and recorded to the genus level. Identification guides and resources used included NIWA and Landcare websites (Manaaki Whenua 2017; NIWA 2016). The 'Easy identification to most common freshwater algae' by van Vuuren *et al.* (2006) was also used in identification. Counts were made based on the presence of a genus in each slides' sample. The number of times a genus was present in all the ten slides per pond sample was used as a surrogate for the genera's abundance in the pond (Adon *et al.* 2019). For example, a genus scored 10 if the taxa occurred in all ten samples.

#### **Data analyses**

I related structural phytoplankton measures to the pond's habitat quality (pollution levels). The structural metrics I used included the percentage of diatom present, percentage cyanobacteria and acidophilic metric. The others are eutrphentic, and motile Bacillariophyceae present and species diversity indices. These metrics for a pond sample were calculated as follows:

- i) Bacillariophyceae metric: number of Bacillariophyceae cells/total number of algal cells,
- ii) dominant Bacillariophyceae metric:  $1 - (\text{number of dominant Bacillariophyceae} / \text{total of number Bacillariophyceae})$ ,

- iii) acidophilic Bacillariophyceae metric:  $1 - (\text{number of acidophilic Bacillariophyceae} / \text{total number of Bacillariophyceae})$ ,
- iv) eutrphentic Bacillariophyceae metric:  $1 - (\text{number of eutrphentic Bacillariophyceae} / \text{total number of Bacillariophyceae})$ ,
- v) motile Bacillariophyceae metric:  $1 - (\text{number motile Bacillariophyceae} / \text{total number of Bacillariophyceae})$ , and
- vi) Cyanobacteria metric:  $1 - (\text{number of Cyanobacteria cells} / \text{total number of algal cells})$  (Hill *et al.* 2000).

Differences in the phytoplankton communities between seasons and the urban-rural gradient were analysed using PERMANOVA on square-root transformed and Bray-Curtis dissimilarity matrix of the abundance data. A SIMPER test was used to determine the genera that contributed to the seasonal differences in the communities. The correlation between phytoplankton community diversity structure and water quality parameters was analysed using the Spearman correlation coefficient. Finally, the amount of variation in the phytoplankton communities explained by the water quality was explored using DISTLM. The analyses were done using PERMANOVA + (version 7.0.13) software extension (Clarke and Gorley 2006).

## 5.4 Results

### 5.4.1 The phytoplankton community in 12 ponds

The distribution and composition of phytoplankton varied significantly among the ponds. Overall, a total of 126 phytoplankton genera were identified based on their morphology. These genera belonged to seven phyla: for example, Chlorophyta (38% of total abundance), Ochrophyta (34%), Cyanophyta (16%), Euglenophyta (6%), Dinophyta (4%), and 17 classes (Figure 5.1) (See Appendix D: Figure 1a -1d for photos of some four genera of phytoplankton). The number of genera in a pond ranged from 44 to 76. However, only 12 genera were common in all the ponds. *Nitzschia* and *Navicula* were the most widespread genera occurring in all the samples. Numerically, the most abundant genera were *Navicula*. Class Bacillariophyceae (diatoms) were the most widespread, speciose, and abundant class accounting for 30% of the total abundance of phytoplankton identified.

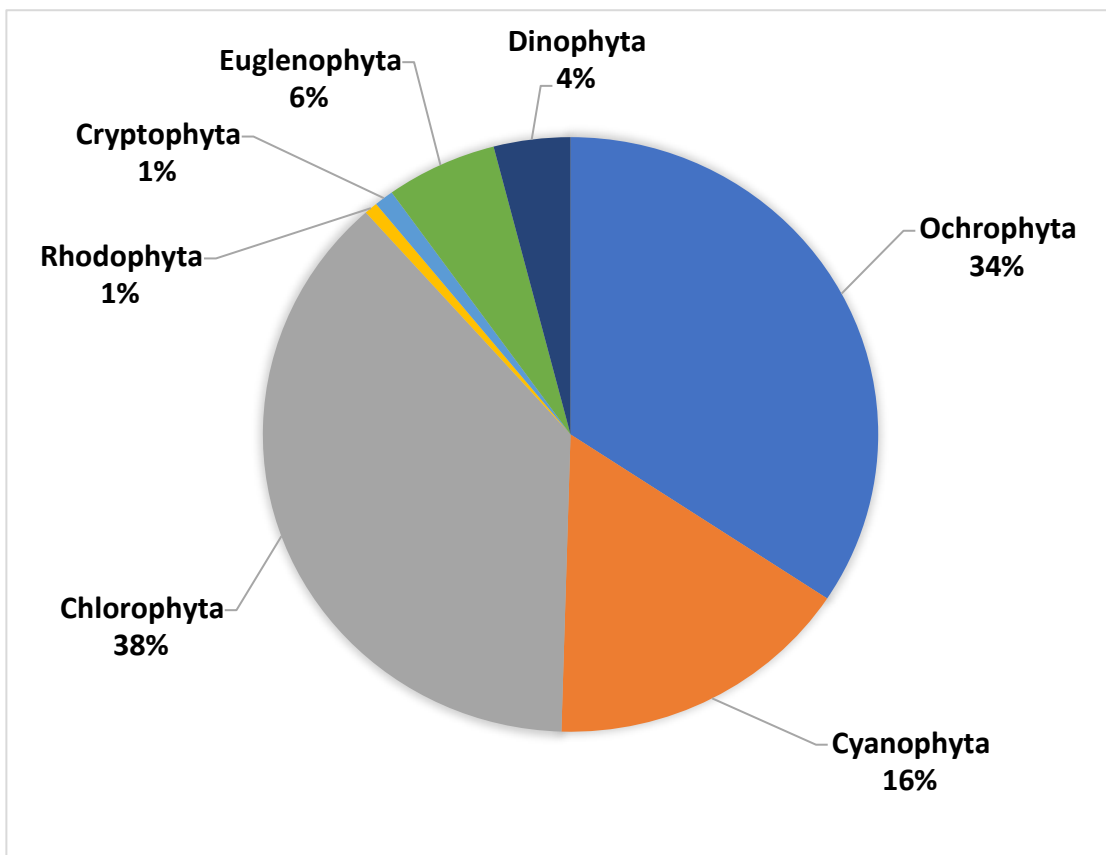


Figure 5.1 Percentage contribution of different phyla to the phytoplankton community in ponds.

### Phylum Chlorophyta

A total of 53 genera belonging to six classes (Chlorophyceae, Coleochaetophyceae, Klebsormidiophyceae, Trebouxiophyceae, Ulvophyceae, and Zygnematophyceae) were identified, making it the richest phylum found in the ponds sampled. The relative abundance (%) of Chlorophyta for a pond ranged between 25% and 54%. Forty-nine percent of the total Chlorophyta occurred in four (33%) ponds (Helle (golf course): 12%, Huap (golf course): 13% Wake (farm): 13%, and Bric (ornamental): 11%, Figure 5.2a). The overall four most dominant classes were Chlorophyceae (52% of all Chlorophyta), Zygnematophyceae (23%), Trebouxiophyceae (20%), and Ulvophyceae (2%) (see Appendix D: Table 1). The five most widespread and abundant genera were *Chlorella* (8.8% of all Chlorophyta), *Oocystis* (6.7%), *Cosmarium* (6.6%), *Golenkinia* (5.4%), and *Oedogonium* (5.1%).

Chlorophyta was the most abundant group in the ponds in both seasons (35% in summer and 41% in winter, Figure 5.3). It did not show a significant seasonal difference in abundance ( $t = 1.6$ ,  $p = 0.1$ ) (Figure 5.3). The relative abundance of Chlorophyta per pond ranged from 20% to 51% in summer and from 19% to 58% in winter. There was significant seasonal difference ( $F_{1,11} = 2.5$ ,  $p = 0.001$ ) in the Chlorophyta community composition. The genera that were driving these differences in communities between the seasons (identified by SIMPER analyses) were *Spirogyra* (Diss/SD = 1.22), *Tetraedron* (Diss/SD = 1.52), which were more abundant in summer, and *Antikistrodesmus* (Diss/SD = 1.23), and *Dictyosphaerum* (Diss/SD = 1.52) which were most abundant in winter. The genera *Chlorella*, *Cosmarium*, and *Golenkinia*, were the most widespread in summer, occurring in 92% of the ponds. In winter, the genera *Oocystis* and *Spirogyra* were widespread, occurring in 83% of the ponds.

### Phylum Ochrophyta

A total of 36 genera (second richest phylum) belonging to four classes (Bacillariophyceae, Chrysophyceae, Mediophyceae, Xanthophyceae) were identified. Ochrophyta abundance in individual ponds ranged from 22% - 49%. The highest (49%) was in Bota, an ornamental pond, and the least (22%) was in Huap (Figure 5.2b). The three most abundant classes were Bacillariophyceae (91% of all Ochrophyta), Mediophyceae (5%), and Chrysophyceae (4%). Within the Class Bacillariophyceae, the dominant taxonomic Orders were Navicules (33% of all Bacillariophyceae), Cymbelles (25%), Fragilariales (13%), and Baccillariales (11%) (see Appendix D: Table 1).

Phytoplankton belonging to Ochrophyta constituted 35% and 30% of the phytoplankton community's total abundance in summer and winter, respectively (Figure 5.3). The abundance within ponds differed significantly between the seasons ( $t = 2.6$ ,  $p = 0.02$ ). There was significant seasonal difference in Ochrophyta community composition in the ponds ( $F_{1,11} = 6.2$ ,  $p = 0.0001$ ) with 44.5% similarity and the community composition differed significantly across ponds ( $F_{1,11} = 1.4$ ,  $p = 0.02$ ). The genera that were driving the differences in communities between the seasons (identified by SIMPER analyses) were *Navicula* (Diss/SD = 1.93), *Nitzschia* (Diss/SD = 1.93), *Gomphoneis* (Diss/SD = 1.52), which were more abundant in summer and *Cyclotella* (Diss/SD = 1.52) which was most abundant in winter.

### Phylum Cyanophyta

There were 25 genera recorded belonging to six taxonomic Orders (Chroococcales, Nostocales, Oscillatoriales, Pleurocapsales, Stigonematales, and Synechococcales). The four most abundant taxonomic orders were Oscillatoriales (34% of all Cyanophyta), Nostocales (23%), Chroococcales (21%), and Synechococcales (19%). The relative abundance (%) in ponds ranged from 6% - 21%. The pond with the highest relative abundance of Cyanophyta (21%) was Wake (21%), and the lowest was in Hugh (6%), a stormwater pond (Figure 5.2c).

Cyanophyta made up 16.4% and 15.8% of the total phytoplankton abundance in summer and winter, respectively. Cyanophyta abundance in ponds did not differ significantly seasonally ( $t = 2.13$ ,  $p = 0.05$ ). Cyanophyta community communities differed significantly between seasons ( $F_{1,11} = 2.2$ ,  $p = 0.02$ ). The abundance in summer was nine percent more than in winter (Figure 3c). Cyanophyta community communities were 34.2% similar between seasons. The genera that were driving the differences in communities between the seasons (identified by SIMPER analyses) were *Dolichospermum* (Diss/SD = 1.14), *Leptolyngbya* (Diss/SD = 1.13), *Lyngbya* (Diss/SD = 1.52), which were more abundant in winter and *Aphanocapsa* (Diss/SD = 1.09) which was most abundant in summer. The genus *Oscillatoriales* was widespread in summer, occurring in all ponds and constituting 25% of the Cyanophyta sampled. In winter, the genera *Lyngbya* and *Oscillatoriles* were common, occurring in 75% of the ponds (see Appendix D: Table 1).

### Phylum Euglenophyta

Four taxonomic genera; *Trachelomonas* (34% of the total Euglenophyta), *Phacus* (30%), *Euglena* (23%), and *Strombomonas* (13%) belonging to the Class Euglenoidea were present in the ponds. They constituted 6.5% and 4.8% of the phytoplankton abundance in summer and winter. Sixty-five percent of total Euglenophyta were found in summer, but the relative abundance of Euglenophyta for individual ponds ranged from 1% - 12%.

The contribution of individual ponds to the total Euglenophyta abundance ranged from 2% - 20%. The highest abundance of Euglenophyta occurred in the pond Bric (12%) and the lowest (1%) in Huap (Figure 5.2d). *Phacus* was the most widespread in winter, occurring in 66.6% of the ponds. *Trachelomonas* was most dominant, occurring in 83% of the ponds in summer (see Appendix D: Table 1).

The community of Euglenophyta between ponds was similar between seasons ( $F_{1,11} = 1.8$ ,  $p = 0.1$ ), however abundance differed significantly between seasons ( $t = 2.4$ ,  $p = 0.02$ ). There was no record of Euglenophyta in two ponds (Hugh and Supe) in winter. Similarly, in summer, Euglenophyta was present in all ponds except one (Huap).

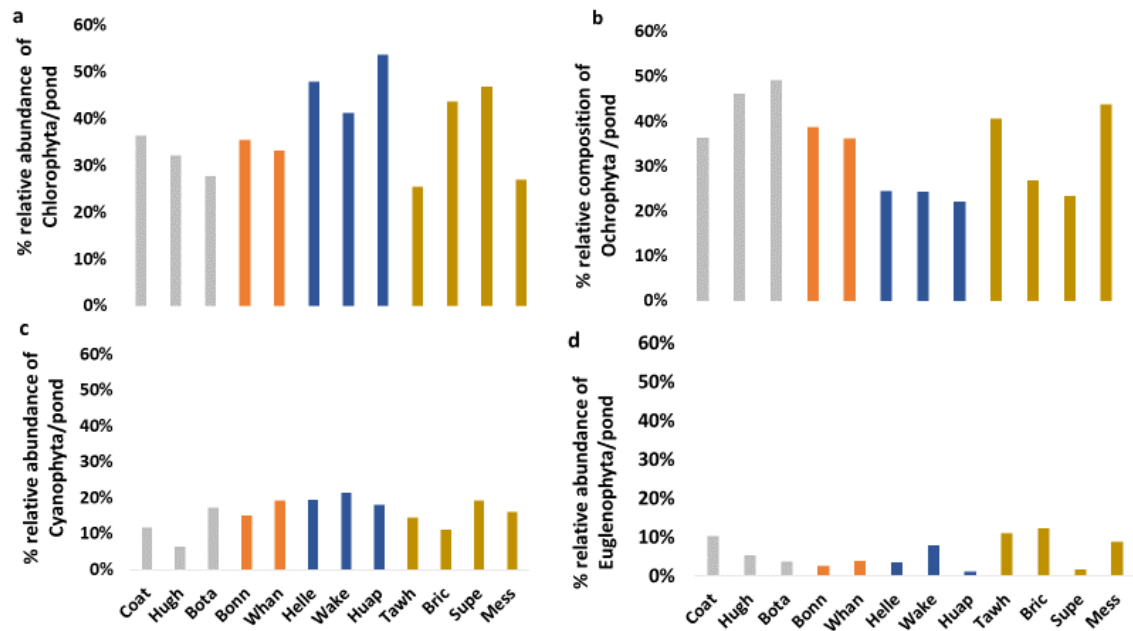


Figure 5.2 The relative percentage abundance of phytoplankton phylum a) Chlorophyta, b) Ochrophyta, c) Cyanophyta, and d) Euglenophyta in 12 ponds. Black bars = major urban area, orange bars = large urban areas, blue = small urban area, and gold = rural areas.

### 5.4.2 Seasonal occurrence and distribution of phytoplankton in ponds

Phytoplankton abundance was slightly higher in summer compared to winter (58% vs 42%). Out of the 126 genera, 97 genera (77%) were common to both seasons, 90.4% of the total phytoplankton genera occurred in summer, and 86.5% of the total phytoplankton genera occurred in winter. Less than 10% of the total genera: 12 genera (Chlorophyta: *Chlorogonium*, *Crucigeniella*, *Cylindrocapsa*, *Eremosphaera*, *Hyalotheca*, *Oocystella*, *Selanastrum*, *Sirogonium*; Rhodophyta: *Audouinella Compsopogon*; Cyanophyta: *Stigonema* and Ochrophyta: *Tabellaria*) were exclusive to winter. Thirteen percent (17 genera) of the total genera (Chlorophyta: *Ankyra*, *Chordatella*, *Elliptochloris*, *Euastrum*, *Pseudokirchieniella*, *Quandrigula*, *Xanthidium*; Cyanophyta: *Aphanothese*, *Dichothrix*, *Microcoleus*, *Rhabdoderma*, *Rhodomonas*, *Scytonema*, *Wilmottia*, *Xenococcus*; Ochrophyta: *Coconeis*, *Pleurosigma*,) were exclusive to summer. Different phyla contributed different percentages to the phytoplankton community in the two seasons. Chlorophyta constituted the highest abundance in summer (36%) and winter (41%), respectively. Rhodophyta was the least abundant in summer (< 1%) (Figure 5.3).

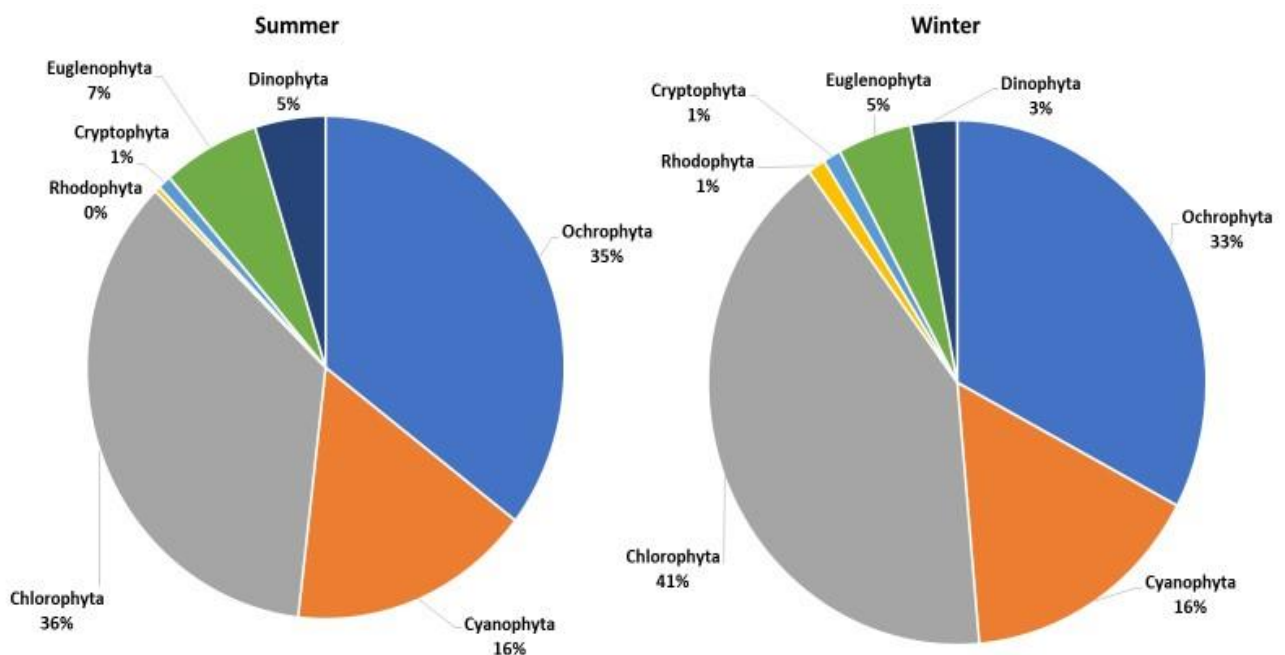


Figure 5.3 Percentage contribution of phyla to the phytoplankton community in 12 ponds in a) summer and b) winter.

Multivariate analyses showed that phytoplankton abundance and composition differed significantly among ponds ( $F_{1,11} = 1.5$ ,  $p < 0.001$ ) and between the seasons ( $F_{1,11} = 3.7$ ,  $p < 0.001$ ). Generally, there was more variability in phytoplankton communities among ponds. Variation in phytoplankton communities in individual ponds explained more (mean square: 325.34) of the difference in the data than seasons (mean square: 291.12).

### 5.4.3 Seasonal taxa heterogeneity

A SIMPER analysis showed that 20 (out of 114 genera) and 19 genera (out of 109 genera) contributed to 70% of the total abundance in summer and winter, respectively. These genera were characteristic of the phytoplankton community in the seasons.

The genera that were driving the differences in communities between the seasons (identified by SIMPER analyses) were *Peridium* (Diss/SD: 1.73), *Cymbella* (Diss/SD: 1.73), *Gomphonema* (Diss/SD 1.42), which occurred in high abundance in summer and *Spirogyra* (Diss/SD:1.41) which occurred in high abundance in winter (Table 5.2, Figure 5.4).

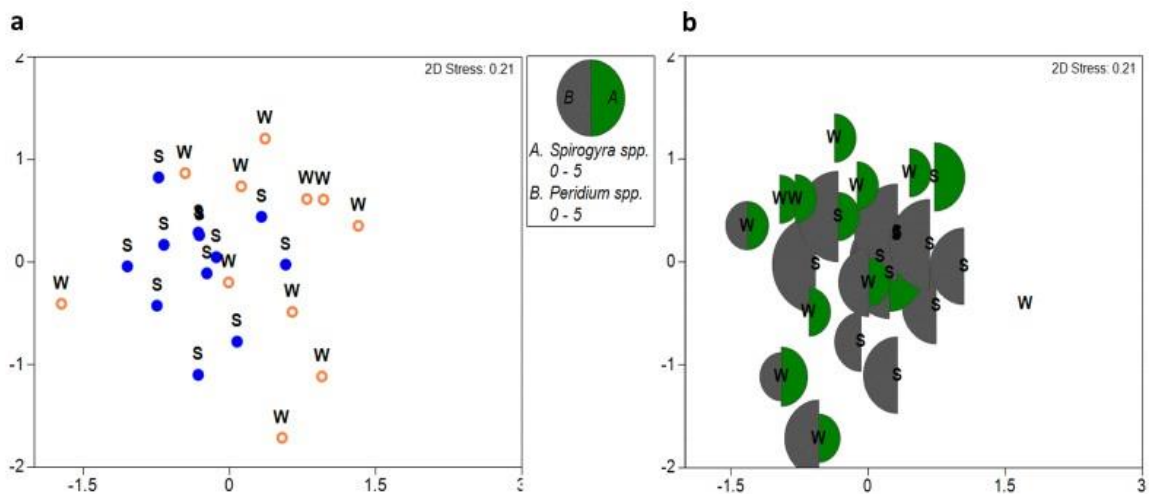


Figure 5.4 An nMDS plot of phytoplankton communities in the ponds showing a) seasonal and site variations and b) seasonal distribution of phytoplankton overlaid with bubbles of the abundance of *Spirogyra* (green bubble) and *Peridium* (grey bubble). The plots are based on Bray-Curtis similarity on square-root transformed phytoplankton abundance data. Orange circles represent winter, and blue circles represent summer. The distance between circles indicates the level of similarity of the phytoplankton communities. The closer the circles, the more similar the communities and *vice versa*. In plot 'b', the size of the bubble indicates the abundance of the taxa. S = summer, W = winter.

Table 5.2 SIMPER results showing the characteristic taxa for each season. The genera contribute to 70% of the community in each season. The phylum to which each genus belongs is in brackets. Ochrophyta (OC), Chlorophyta (CL), Dinophyta (DN), Cyanophyta (CN), Euglenophyta (EG). Genera in 'bold' were important in summer only, and genera in 'italics' were only in winter.

Characteristic taxa Genera	% Contribution to total abundance	
	Summer	Winter
<i>Navicula</i> (OC)	6.1	5.8
<i>Synedra</i> (OC)	5.8	4.1
<i>Nitzschia</i> (OC)	5.3	5.5
<i>Chlorella</i> (CL)	4.9	3.7
<i>Cymbella</i> (OC)	4.9	2.5
<b><i>Peridium</i> (DN)</b>	4.8	
<i>Pinnularia</i> (OC)	4.7	5.5
<i>Lyngbya</i> (CN)	4.0	3.0
<i>Oscillatoria</i> (CN)	3.6	3.3
<i>Cosmarium</i> (CL)	3.5	3.3
<i>Oocystis</i> (CL)	3.3	4.2
<i>Golenkinia</i> (CL)	3.0	3.3
<i>Trachelomonas</i> (EU)	3.0	2.2
<i>Oedogonium</i> (CL)	2.5	2.2
<b><i>Ankistrodesmus</i> (CL)</b>	2.2	
<b><i>Gomphonema</i> (OC)</b>	2.0	
<b><i>Eunotia</i> (OC)</b>	1.72	
<b><i>Anabaena</i> (CN)</b>	1.62	
<i>Phacus</i> (EG)	1.54	2.7
<b><i>Tetraedron</i> (CL)</b>	1.51	
<b><i>Euglena</i> (EG)</b>	1.50	
<i>Cyclotella</i> (OC)		5.2
<i>Spirogyra</i> (CL)		4.0
<i>Achnanthes</i> (OC)		3.1
<i>Tribonema</i> (OC)		2.6
<i>Leptolyngbya</i> (CN)		2.5
<i>Microcystis</i> (CN)		2.0

There was a significant ( $t = 3.06$ ,  $p = 0.01$ ) seasonal difference in the number of phytoplankton genera present in ponds. The average number of taxa per pond ( $t = 3.06$ ,  $p = 0.01$ ) and phytoplankton abundance ( $t = 3.11$ ,  $p < 0.01$ ) were significantly higher in summer than winter. Margalef's richness was significantly ( $t = 2.9$ ,  $p = 0.01$ ) higher in summer than in winter. Phytoplankton evenness was significantly ( $t = 6.6$ ,  $p < 0.001$ ) higher in winter than in summer. However, Shannon Wiener diversity did not differ significantly ( $t = 1.52$ ,  $p = 0.1$ ) between season (Table 5.3). Alpha diversities of 114 and 109 were recorded in summer and winter, respectively. The regional diversity (Chao 1 index estimate) for summer was  $136 \pm 13$  and  $110 \pm 2$  for winter.

Table 5.3 Seasonal phytoplankton biodiversity indices in the ponds. Average values  $\pm$  standard deviations are reported, and significant p values at a 5% significant level are in bold.

<b>Biodiversity Index</b>	<b>Summer <math>\pm</math> SD</b>	<b>Winter <math>\pm</math> SD</b>	<b>t values</b>	<b>p value</b>
Number of taxa (S)	45.1 $\pm$ 10.9	35 $\pm$ 12.4	3.06	<b>0.01</b>
Abundance (N)	137.9 $\pm$ 41.7	100.16 $\pm$ 40.68	3.11	<b>&lt; 0.01</b>
Richness (d)	8.9 $\pm$ 1.8	7.33 $\pm$ 2.14	2.9	<b>0.01</b>
Evenness (j)	0.9 $\pm$ 0.0	0.95 $\pm$ 0.0	6.6	<b>&lt; 0.001</b>
Shannon Weiner diversity (H)	3.5 $\pm$ 0.3	3.3 $\pm$ 0.5	1.52	0.1
Simpson index (1- $\lambda$ )	1.0 $\pm$ 0.0	1.0 $\pm$ 0.0	0.82	0.4

#### 5.4.4 Community composition across the urban-rural gradient

The composition of phytoplankton varied along the urban-rural gradient. The phylum Ochrophyta were dominant in the major (44%) and large urban areas (37%). In contrast, the phylum Chlorophyta was dominant in ponds in the small urban (47%) and rural areas (38%). Euglenophyta was the fourth most dominant phylum in all the areas apart from the large urban area where Cryptophyta was more common. Rhodophyta occurred during summer in large and small urban ponds, but it was present in major urban and rural ponds in winter.

There were 92 genera in the ponds in the major urban area (79 occurred in summer and 67 in winter) (Figure 5.5a). The most abundant phylum was Ochrophyta. The abundance of phytoplankton in summer was 63.7% of the total recorded. Phytoplankton communities in the ponds in the major urban areas were significantly different ( $t = 1.65$ ,  $p < 0.001$ ) (Figure 5.5a) across the seasons.

There were 78 genera of phytoplankton recorded in ponds in the large urban area; 58 occurred in summer and 50 in winter. The most abundant phylum was Ochrophyta (37%), and the least was  $< 1\%$  Rhodophyta (occurred only in summer) (Figure 5.5b). The abundance of phytoplankton in summer was 57.1% of the total recorded. Phytoplankton community in these ponds did not differ significantly between the seasons ( $t = 1.46$ ,  $p = 0.1$ ) (Figure 5.5b).

There were 98 phytoplankton genera in ponds in the small urban area (82 occurred in summer and 68 in winter). The most abundant phylum was Chlorophyta (47%) and had comparatively

the highest Cyanophyta abundance (Figure 6.5c). A total of 58% of the phytoplankton occurred in summer. Phytoplankton communities in the ponds were not significantly different between the seasons ( $t = 1.88$ ,  $p = 0.06$ ) (Figure 5.5c).

There were 97 genera of phytoplankton recorded in ponds in the rural area (78 occurred in summer and 73 in winter). Ponds in the rural areas recorded the highest percentage (9%) abundance of the phylum Euglenophyta than the other gradient. The most abundant phylum was Chlorophyta (38%), and the least was Rhodophyta (occurred only in winter) and Cryptophyta. The community composition was not significantly different between the seasons ( $t = 0.78$ ,  $p = 0.4$ ) (Figure 5.5d).

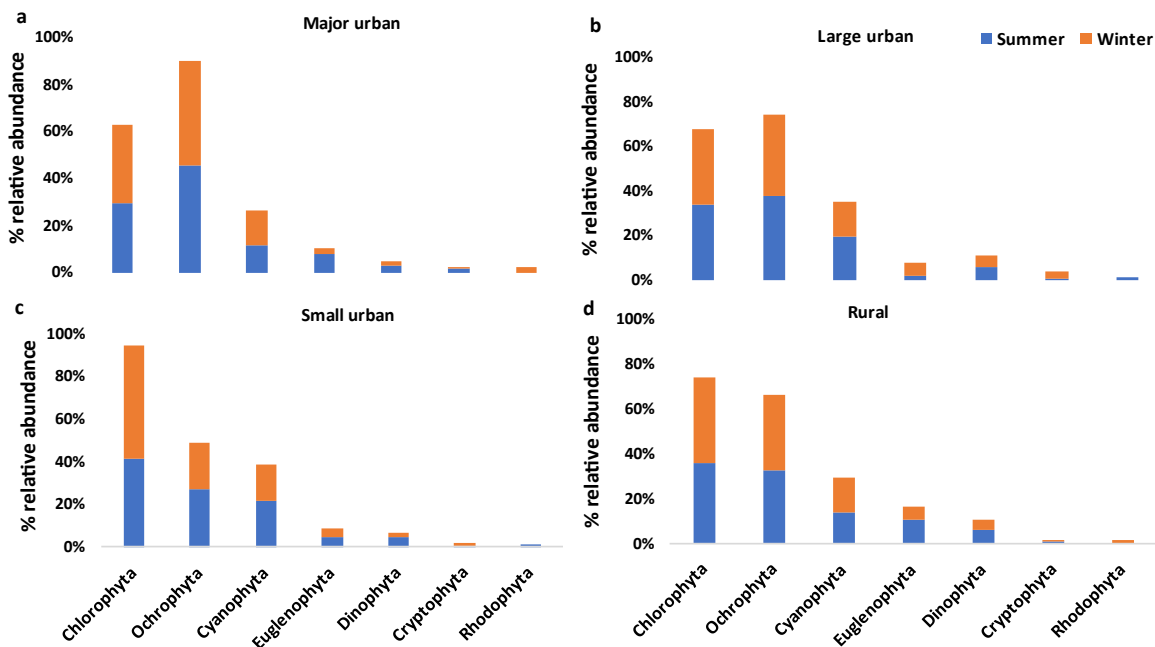


Figure 5.5 Percentage relative abundance of phytoplankton phylum along the urban-rural gradient. A result of the seasonal test is shown at the top left corner of each graph. a: ponds in the major urban areas with a significant seasonal difference (reported in bold), b: ponds in the large urban areas, c: ponds in small urban areas, and d: ponds in the rural areas.

Due to the significant seasonal differences in the phytoplankton communities, each season was analysed separately to determine if the communities varied along the urban-rural gradient. A PERMANOVA test showed a significant difference ( $F_{3,11} = 1.3$ ,  $p = 0.04$ ) in the phytoplankton communities along the urban-rural gradient in summer. However, in winter, the phytoplankton communities were not significantly different ( $F_{3,11} = 1.1$ ,  $p = 0.1$ ) along the urban-rural gradient.

### 5.4.5 Biodiversity along the urban-rural gradient

In summer, the highest phytoplankton abundance occurred in small urban ponds ( $160 \pm 38$ ) and the least in rural ponds ( $106 \pm 8$ ) (Figure 5.6a). Similarly, the highest number of taxa was recorded in the small urban areas ( $54 \pm 7$ ), and the least occurred in the rural ponds ( $37 \pm 5$ ) (Figure 5.6b). The highest Shannon Weiner diversity occurred in small urban ponds ( $3.7 \pm 0.1$ ) and the least in large urban area ponds ( $3.5 \pm 0.4$ ) (Figure 5.6c). The phytoplankton communities in the small urban areas were the richest ( $10.3 \pm 1.2$ ) (Figure 5.6d). There were no significant differences in the biodiversity indices along the urban-rural gradient.

Similar to the trend in summer, phytoplankton abundance in winter was highest in the small urban ponds ( $54 \pm 1$ ) and least in large urban ponds ( $31 \pm 4$ ) (Figure 5.6a). The highest number of taxa occurred in small urban ponds ( $127 \pm 21$ ) and the least in large urban ponds ( $88 \pm 57$ ) (Figure 5.6b). The highest Shannon Weiner diversity occurred in ponds in small urban areas ( $3.6 \pm 0.0$ ) and the least in ponds in major urban areas ( $3.0 \pm 0.9$ ) (Figure 5.6c). The phytoplankton communities in the small urban area were the richest ( $8.8 \pm 0.2$ ), while ponds in the major urban areas were the least rich ( $6.6 \pm 3.6$ ) (Figure 5.6d). There was no significant difference in the biodiversity indices along the urban-rural gradient.

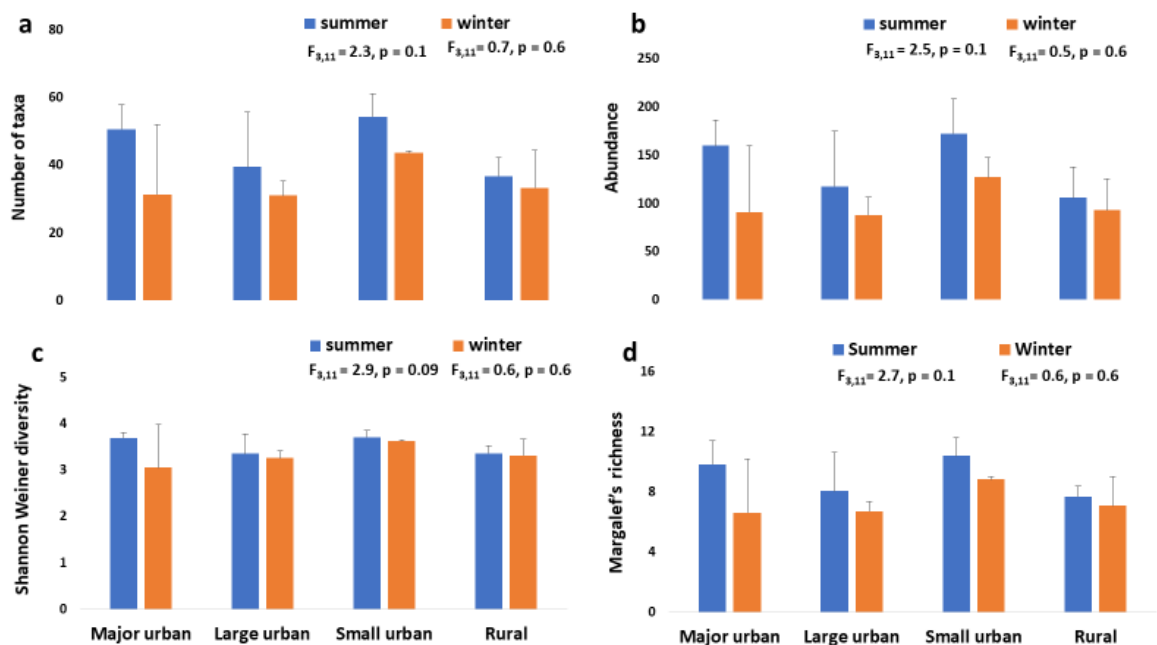


Figure 5.6 Biodiversity indices along the urban-rural gradient, a) Number of taxa, b) Abundance, c) Shannon Weiner, and d) Margalef's richness. The blue bars represent summer, and the orange bars represent winter with standard error bars. The F value and p value of the PERMANOVA test along the urban-rural gradient are shown on the index.

### Indicator metrics

Motile metrics in the ponds in the major urban area was significantly different and higher in summer (Table 5.4).

Table 5.4 Occurrence of phytoplankton metrics in the ponds along the urban-rural gradient in summer and winter. Significant p values at 5% significant level are in bold.

Season	Metric	Urban-rural gradient			ANOVA test		
		Major urban	Large urban	Small urban	Rural	f	p value
Summer	Acidophilic	0.8 ± 0.1	0.9 ± 0.0	0.8 ± 0.1	0.8 ± 0.1	0.6	0.6
	Eutraphentic	0.7 ± 0.1	0.5 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.8	0.5
	Motile	0.7 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.1	4.6	<b>0.03</b>
	Cyanophyta	19.0 ± 12.5	23.0 ± 21.2	37.0 ± 9.6	14.5 ± 6.2	2.3	0.1
	Bacillariophyceae	64.7 ± 19.6	43.5 ± 9.2	44.0 ± 13.5	29.2 ± 8.3	4.0	0.05
Winter	Acidophilic	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.0	0.8 ± 0.1	0.8	0.5
	Eutraphentic	0.7 ± 0.1	0.7 ± 0.0	0.81 ± 0.1	0.8 ± 0.1	2.4	0.1
	Motile	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	1.1	0.3
	Cyanophyta	13.3 ± 11.5	13.5 ± 2.1	21.7 ± 5.5	14.5 ± 5.9	0.8	0.5
	Bacillariophyceae	31.7 ± 25.1	28.5 ± 12.0	21.3 ± 3.5	23.7 ± 8.4	0.3	0.8

### 5.4.6 Physicochemical properties of pond water

Conductivity, pH, total dissolved solids, salinity, and temperature were more variable in summer. Overall, pH, conductivity, and water temperature were significantly higher in summer compared to winter (Table 5.5). There were overall significant seasonal ( $F_{1,11} = 5.6$ ,  $p < 0.001$ ) and site differences ( $F_{1,11} = 2.9$ ,  $p < 0.0001$ ) in the physicochemical water quality variables.

Table 5.5 Physicochemical water quality variables in summer and winter. The table reports the average ± standard deviation, the minimum (min) and maximum (max) values, and the result of a 't' test between the seasons. Significant p values at 5% significant level are in bold.

Variable	Summer (n = 12)			Winter (n = 12)			t	p value
	Average ± SD	Min	Max	Average ± SD	Min	Max		
pH	6.94 ± 0.64	5.82	8.24	6.48 ± 0.53	5.43	7.14	2.7	<b>0.01</b>
% DO	39.83 ± 19.62	12.47	66.3	41.18 ± 22.76	6.97	72.23	0.2	0.8
Con. ( $\mu\text{Scm}^{-1}$ )	193.4 ± 143.8	50.3	545.3	175.4 ± 109.9	52	413	3.8	<b>&lt; 0.01</b>
TDS (ppm)	94.9 ± 67.3	25	272.7	87.8 ± 52.8	26	208.3	1.1	0.2
Sal. (psu)	0.09 ± 0.07	0.02	0.26	0.08 ± 0.05	0.02	0.2	1.2	0.2
Tem. ( $^{\circ}\text{C}$ )	21.21 ± 2.81	17.19	24.53	13.19 ± 1.12	11.54	14.81	8.8	<b>&lt; 0.001</b>
$\text{NO}_3\text{-N}$ ( $\text{mg l}^{-1}$ )	0.19 ± 0.20	0.01	0.5	0.18 ± 0.21	0.01	0.5	0.05	0.9
$\text{PO}_4^{3-}$ ( $\text{mg l}^{-1}$ )	1.16 ± 0.80	0.25	2.45	1.15 ± 0.90	0.02	2.39	0.01	0.9
$\text{NH}_3\text{-H}$ ( $\text{mg l}^{-1}$ )	0.23 ± 0.14	0.02	0.46	0.26 ± 0.13	0.06	0.51	0.7	0.4

### 5.4.7 Relationship between environmental factors and phytoplankton biodiversity indices in summer

Overall, conductivity negatively correlated with total phytoplankton taxa number ( $\rho = -0.5$ ,  $p = 0.1$ ), phytoplankton abundance ( $\rho = -0.5$ ,  $p = 0.09$ ), phytoplankton Margalef's richness ( $\rho = -0.5$ ,  $p = 0.1$ ), and with Shannon Weiner species diversity ( $\rho = -0.4$ ,  $p = 0.1$ ). The altitude of the ponds ranged from 4.5m to 55.5m and was strongly positively relationship with phytoplankton abundance ( $\rho = 0.6$ ,  $p = 0.03$ ) and Shannon Wiener diversity ( $\rho = 0.5$ ,  $p = 0.1$ ). Proximity to other aquatic systems (distance to water body) ranged from 14.7m to 317.0m and was positively correlated with Margalef's richness ( $\rho = 0.1$ ,  $p = 0.7$ ) (Table 5.6).

Table 5.6 Relationships between summer and winter phytoplankton biodiversity indices and environmental variables. S = number of taxa, N = abundance, d = Margalef's richness, J' = Pilon's evenness, H' = Shannon Weiner diversity,  $1 - \lambda$  = Simpson's index. Correlations with "\*" are significant at  $p < 0.05$ .

Physicochemical water quality variables	Biodiversity indices											
	Summer						Winter					
	S	N	d	J'	H'	1- $\lambda$	S	N	d	J'	H'	1- $\lambda$
pH	0.0	0.0	0.0	-0.2	0.0	0.0	-0.3	-0.3	-0.3	-0.0	-0.2	-0.2
% DO	0.0	0.1	0.0	-0.2	0.0	0.0	0.1	0.2	0.0	0.0	0.1	0.1
Con. ( $\mu\text{Scm}^{-1}$ )	-0.5	-0.5	-0.5	0.3	-0.4	-0.4	-0.6*	-0.5	-0.6*	-0.5	-0.6*	-0.6*
Sal. (psu)	-0.5	-0.5	-0.5	0.2	-0.4	-0.3	-0.6*	-0.5	-0.6*	-0.5	-0.6*	-0.6*
TDS (ppm)	-0.5	-0.5	-0.4	0.2	-0.4	-0.4	-0.5	-0.5	-0.6*	-0.5	-0.6*	-0.6*
Tem. ( $^{\circ}\text{C}$ )	0.2	0.3	0.2	-0.4	0.2	0.2	0.1	0.0	0.1	-0.2	0.1	0.1
$\text{NO}_3\text{-N}$ ( $\text{mg l}^{-1}$ )	0.4	0.1	0.4	0.2	0.4	0.4	0.1	0.0	0.0	-0.1	0.0	0.0
$\text{PO}_4^{3-}$ ( $\text{mg l}^{-1}$ )	0.1	0.4	0.1	-0.4	0.1	0.1	0.0	0.1	0.0	-0.1	0.0	0.0
$\text{NH}_3\text{-H}$ ( $\text{mg l}^{-1}$ )	0.0	0.3	0.0	0.0	0.0	0.0	-0.3	-0.1	-0.4	-0.3	-0.4	-0.4
Area ( $\text{m}^2$ )	0.0	0.3	0.0	0.3	0.0	0.0	0.2	0.0	0.2	0.6*	0.2	0.3
Altitude (m)	0.5	0.6*	0.4	0.1	0.5	0.4	0.2	0.4	0.1	0.2	0.2	0.0
Distance to road (m)	0.3	0.0	0.3	0.0	0.2	0.2	0.3	0.2	0.4	0.2	0.3	0.3
Distance to waterbody (m)	0.2	0.0	0.1	-0.1	0.2	0.1	0.5	0.6*	0.3	0.1	0.5	0.3
% Macrophyte Cover	0.1	0.2	0.0	0.3	0.1	0.1	-0.2	-0.0	-0.3	-0.1	-0.2	-0.3
Depth (m)	0.0	-0.4	0.0	0.0	0.1	0.0	0.0	-0.1	0.2	-0.2	0.1	0.2

#### **5.4.8 Relationship between environmental factors and phytoplankton biodiversity indices in winter**

Some of the environmental variables measured correlated with phytoplankton community indices. Conductivity was negatively correlated and significant to phytoplankton number of taxa ( $\rho = -0.6$ ,  $p = 0.04$ ), Margalef's richness ( $\rho = -0.6$ ,  $p = 0.03$ ), Shannon Weiner diversity ( $\rho = -0.6$ ,  $p = 0.03$ ), and Simpson's index ( $\rho = -0.6$ ,  $p = 0.02$ ) (Table 5.6).

Macrophyte cover ranged 0% to 80% cover and was weakly negatively correlated with number of phytoplankton taxa ( $\rho = -0.2$ ,  $p = 0.5$ ), Margalef's richness ( $\rho = -0.3$ ,  $p = 0.3$ ), Shannon Weiner diversity ( $\rho = -0.2$ ,  $p = 0.5$ ) and Simpson's diversity ( $\rho = -0.3$ ,  $p = 0.3$ ). The depth of the ponds was weakly positively correlated with Margalef's richness ( $\rho = 0.2$ ,  $p = 0.5$ ) and Simpson diversity ( $\rho = 0.2$ ,  $p = 0.5$ ) and Pielou's evenness was significantly and positively correlated to pond area  $\rho = 0.6$ ,  $p = 0.03$ ) (Table 5.6).

#### **5.4.9 Correlation between the abundance of phytoplankton phyla and physiochemical water quality variables**

##### **5.4.9.1 Relationship between phytoplankton groups and physicochemical water quality variables in summer**

There were variations in the relationship between the relative abundance of the phyla and the physicochemical variables. For example, the relative abundance of phylum Chlorophyta in summer was strongly negatively correlated to TDS ( $\rho = -0.8$ ,  $p < 0.01$ ), conductivity ( $\rho = -0.7$ ,  $p < 0.01$ ), and salinity ( $\rho = -0.7$ ,  $p = 0.01$ ). Also, the relative abundance of phylum Ochrophyta was significant and positively correlated with conductivity ( $\rho = 0.7$ ,  $p = 0.01$ ), and salinity ( $\rho = 0.6$ ,  $p = 0.02$ ). The relative abundance of phylum Cyanophyta was strongly negatively correlated and significant to conductivity ( $\rho = -0.6$ ,  $p = 0.02$ ) and salinity ( $\rho = -0.6$ ,  $p = 0.04$ ). Finally, the relative abundance of phytoplankton in the phylum Euglenophyta was significantly strongly negatively correlated to temperature ( $\rho = -0.8$ ,  $p < 0.01$ ), pH ( $\rho = -0.8$ ,  $p < 0.01$ ), and % DO ( $\rho = -0.7$ ,  $p = 0.01$ ). Euglenophyta was negatively correlated to nitrate concentration ( $\rho = -0.5$ ,  $p = 0.06$ ) (Table 5.7).

In terms, of the genera composition, in summer, *Bulbochaete* was significantly negatively correlated with conductivity ( $\rho = -0.8$ ) and positively correlated with temperature ( $\rho = 0.6$ ).

*Calothrix* was significantly negatively correlated with conductivity ( $\rho = -0.7$ ) and positively correlated with temperature ( $\rho = 0.6$ ). *Cymbella* was significantly positively correlated with ammonia ( $\rho = 0.8$ ). *Navicula* was positively correlated to temperature ( $\rho = -0.7$ ), and *Pinnularia* was negatively correlated with % DO ( $\rho = -0.6$ ) (Appendix D: Table 2).

Table 5.7 The spearman correlation between phytoplankton phyla and physicochemical water quality in summer and winter. Correlations coefficients with ‘\*’ are significant at  $p < 0.05$ .

Phylum	Physicochemical water quality variables								
	pH	% DO	Con. ( $\mu\text{Scm}^{-1}$ )	TDS (ppm)	Sal(psu)	Tem. ( $^{\circ}\text{C}$ )	$\text{NO}_3\text{-N}$ ( $\text{mg}^{\text{l}^{-1}}$ )	$\text{PO}_4^{3-}$ ( $\text{mg}^{\text{l}^{-1}}$ )	$\text{NH}_3\text{-H}$ ( $\text{mg}^{\text{l}^{-1}}$ )
<b>Summer</b>									
Chlorophyta	0.4	0.3	-0.7*	-0.8*	-0.7*	0.4	0.3	0.1	-0.2
Ochrophyta	-0.2	-0.4	0.7*	0.7*	0.6*	-0.3	-0.3	-0.0	0.5
Cyanophyta	0.2	0.5	-0.6*	-0.5	-0.6*	0.4	0.4	0.3	-0.1
Euglenophyta	-0.8*	-0.7*	0.4	0.4	0.4	-0.8*	-0.5	0.0	-0.2
Rhodophyta	0.0	0.3	-0.3	-0.3	-0.3	0.2	0.2	0.5	0.2
Cryptophyta	-0.5	-0.2	0.4	0.4	0.4	-0.6	-0.1	-0.2	0.1
Dinophyta	0.0	0.1	0.1	0.1	0.1	-0.1	0.1	-0.2	0.0
<b>Winter</b>									
Chlorophyta	-0.3	0.3	-0.8*	-0.8*	-0.8*	0.3	0.5	0.4	-0.4
Ochrophyta	0.2	-0.1	0.6*	0.5	0.6*	-0.2	-0.5	-0.4	0.1
Cyanophyta	0.0	-0.1	-0.1	-0.1	-0.1	-0.1	0.4	0.0	0.3
Euglenophyta	-0.4	-0.2	0.4	0.4	0.4	-0.1	-0.1	0.0	0.1
Rhodophyta	-0.2	-0.6*	0.2	0.4	0.3	0.6	-0.1	-0.1	-0.1
Cryptophyta	0.3	-0.1	0.3	0.3	0.3	-0.1	0.1	0.1	-0.5
Dinophyta	0.4	0.0	0.1	0.2	0.2	0.2	0.1	-0.2	-0.2

#### 5.4.9.2 Relationship between phytoplankton groups and physicochemical water quality variables in winter

Similar to summer, there were variations in the relationship between the relative abundance of the phyla and the physicochemical variables. For example, the relative abundance of phylum Chlorophyta was weakly correlated with pH ( $\rho = -0.3$ ,  $p = 0.5$ ) but strongly correlated with conductivity ( $\rho = 0.8$ ,  $p = 0.002$ ), TDS ( $\rho = 0.8$ ,  $p = 0.003$ ) and salinity ( $\rho = 0.8$ ,  $p = 0.002$ ). Also, the relative abundance of phylum Ochrophyta was positively and significantly correlated with conductivity ( $\rho = 0.6$ ,  $p = 0.04$ ), and salinity ( $\rho = 0.6$ ,  $p = 0.04$ ) (Table 5.7).

In terms of the genera composition, *Achnanthes* was significantly negatively correlated with pH ( $\rho = -0.6$ ) but positively to nitrate ( $\rho = 0.6$ ). *Synedra* was negatively correlated to nitrate ( $\rho = -0.7$ ). *Chlorogonium* was significantly negatively correlated to % DO. *Cyclotella* ( $\rho = 0.6$ ) and *Navicula* ( $\rho = 0.7$ ) were positively correlated to temperature (Appendix D: Table 2).

In addition to the taxonomic assessment, the phytoplankton biomass for the ponds was analysed for summer and winter 2019. Like the taxonomic composition, the chlorophyll 'a' biomass varied among the ponds, and the average biomass was higher in summer ( $0.09\text{mgm}^{-2} \pm 0.19\text{mgm}^{-2}$ ) than in winter ( $0.01 \pm 0.02\text{mgm}^{-2}$ ). Also, the biomass related differently to the physicochemical water quality variables. For example, phytoplankton biomass was significantly negatively correlated to pH ( $\rho = 0.8$ ,  $p < 0.01$ ) in summer. Similarly, in winter, phytoplankton biomass was significantly negatively correlated to % DO and temperature (Appendix D: Table 3).

#### 5.4.10 The relationship between water quality and phytoplankton community

The first two principal component axes (PC1 = 59.6, PC2 = 18.9) explained 78.5% of the variation in the physicochemical water quality variables. The first principal components were highly loaded by salinity (0.47), TDS (0.46), and conductivity (0.45). The second principal component was heavily loaded by ammoniacal nitrogen (-0.64) and pH (-0.5). Using a DISTLM, the first two PCA explained a total of 13% of the variation in the phytoplankton community assemblage. Both PCA's were not significant in explaining the variations. PC1 explained 8.2% of the variation ( $p = 0.8$ ) and PC2 explained 5.1% of the variation ( $p = 0.7$ ) (Table 5.8).

In winter, the first three principal component axes (PC1 = 44.1, PC2 = 20.1, PC3 = 12.9) cumulatively explained 77.1% of the variations in the physicochemical water quality variables. The first principal components were highly loaded by TDS (0.48), conductivity (0.47), salinity (0.46), and phosphate (-0.43). The second principal component was heavily loaded by nitrate (-0.67), phosphate (-0.56), and ammoniacal nitrogen (-0.42). The third principal component was heavily loaded by pH (-0.62) and ammoniacal nitrogen (-0.56) (Table 5.8). From DISTLM, the first three PCA explained 23% of the variations in the phytoplankton community assemblage. All three PCs were not significant in explaining the variations. PC1 explained 9.8% of the variations ( $p = 0.4$ ), PC2 explained 7.2% of the variations ( $p = 0.7$ ), and PC3 explained 6.3% of the variations ( $p = 0.7$ ).

Table 5.8 Coefficients in the linear combinations of physicochemical variables making up the principal components in summer and winter. Physicochemical variables significant in the principal axes are in bold.

Physicochemical variable	Season				
	Summer		Winter		
	PC1	PC2	PC1	PC2	PC3
pH	0.30	<b>-0.50</b>	0.00	0.20	<b>-0.62</b>
% DO	0.37	-0.35	-0.31	-0.04	-0.27
Con. ( $\mu\text{Scm}^{-1}$ )	<b>-0.46</b>	-0.26	<b>0.47</b>	-0.04	-0.10
TDS (ppm)	<b>-0.46</b>	-0.19	<b>0.48</b>	-0.09	-0.05
Sal. (psu)	<b>-0.47</b>	-0.24	<b>0.46</b>	-0.09	-0.04
Tem. ( $^{\circ}\text{C}$ )	0.19	-0.20	-0.02	0.00	0.08
$\text{NO}_3^-$ -N ( $\text{mg l}^{-1}$ )	0.31	-0.14	0.08	<b>-0.67</b>	0.39
$\text{PO}_4^{3-}$ ( $\text{mg l}^{-1}$ )	0.02	-0.01	<b>-0.43</b>	<b>-0.56</b>	-0.23
$\text{NH}_3$ -H ( $\text{mg l}^{-1}$ )	-0.07	<b>-0.64</b>	0.22	<b>-0.62</b>	<b>-0.56</b>
PC Eigenvalues	5.08	1.61	3.59	1.63	1.05
% Variation	59.6	18.9	44.1	20.1	12.9
Cumulative % Variation	59.6	78.5	44.1	64.2	77.1
% variation explained in phytoplankton community by DISTLM	8.2	5.1	9.8	7.2	6.3
Total variation explained		13.2		23.3	

## 5.5 Discussion

My study provides one of the first reports of the phytoplankton community compositions in ponds in the Auckland region of New Zealand. This study aimed to characterize the phytoplankton community composition, determine their seasonal composition, and assess the human population's influence on the abundance and composition of phytoplankton. I also investigated phytoplankton's response community to the environmental factors, particularly the potential physiochemical water quality variables that affect the community structure.

The number of genera of phytoplankton identified in the ponds suggests that ponds host a taxonomically diverse community compared to 69 genera identified in the Waikato River (Lam 1981). Different taxonomic compositions have been reported in different aquatic systems globally (Lévesque *et al.* 2020; Yusuf 2020), including New Zealand (Paul *et al.* 2012). The

taxonomic groups Cyanophyta, Chlorophyta, Cryptophyta, Ochrophyta, Euglenophyta, and Dinophyta have been recorded in 11 lakes in Rotorua, New Zealand, with different taxonomic groups dominating in different lakes (Paul *et al.* 2012). The group Rhodophyta recorded in ponds in this study was absent in lakes in Rotorua.

The phytoplankton assemblage (composition and distribution) was different among the ponds. Similar differences were recorded in lakes in Rotorua (Paul *et al.* 2012). These differences in assemblages among the ponds could be due to differences in the ponds' environmental conditions, such as the conductivity, TDS, and pH levels (Saeiam *et al.* 2020). In addition, the group Cryptophyta are sporadic both in occurrence and abundance (Martins *et al.* 2020). The most diverse phylum was Chlorophyta, similar to British Isle and the Waikato River (John *et al.* 2011; Lam 1981). The dominance of Chlorophyta has also been documented in fishponds in the USA (Tucker and Lloyd 1984), China, Nigeria, and Canada (Hu *et al.* 2020; Lévesque *et al.* 2020; Li *et al.* 2019; Yan *et al.* 2020; Yusuf 2020). Phylum Chlorophyta is adapted to living in areas of optimal light intensity (photophilic), making ponds suitable for them due to their shallowness (Adon *et al.* 2019; Yan *et al.* 2020; Yusuf 2020) and hence the dominance of Chlorophyta in the phytoplankton community. Aquatic ecosystems with Chlorophyta dominating the algal flora are considered to have a healthy algae community assemblage (Amengual-Morro *et al.* 2012).

The phylum Ochrophyta was the second most abundant group, including the common and most abundant and diverse class (Bacillariophyceae). The class Bacillariophyceae's dominance and widespread nature are expected as taxa belonging to this class are common in all aquatic ecosystems (Yan *et al.* 2020). The dominance of Bacillariophyceae is due to their resilience and ability to withstand a broad range of environmental conditions, as observed in the ponds (Yusuf 2020). The class Bacillariophyceae has been reported to dominate aquatic ecosystems with various nutrient concentrations in New Zealand (Biggs 2000b). Although Bacillariophyceae are the most abundant algae, they prefer benthic environments (Adon *et al.* 2019; Yan *et al.* 2020). This preference notwithstanding, they are comparatively more diversified in the ecosystem's pelagic, benthic habitat than the benthos (Adon *et al.* 2019).

Phylum Cyanophyta was the third dominant group in the ponds. The high light intensity and the pond's high transparency could be why the low dominance of Cyanophyta in the ponds. D'Alessandro *et al.* (2020) report that Cyanophyta dominates in turbid water because it is superior to low light intensity.

Although Euglenophyta prefers aquatic systems rich in organic pollution (Saeiam *et al.* 2020), they were uncommon, not diverse. Contrary to expectation, Euglenophyta occurred in lower abundance than Chlorophyta, Ochrophyta, and Cyanophyta in the ponds. Euglenophyta taxa such as *Trachelomonas*, which are known to prefer eutrophic waters with high oxygen concentration and high temperatures (Grabowska and Wołowski 2014), were recorded in the ponds and were prevalent in summer. The lower abundance recorded in the ponds could be due to unfavourable conditions in the ponds to support them, including low temperature and high grazing (Aboim *et al.* 2020).

### 5.5.1 Seasonal differences in phytoplankton community

I found significant differences in the community compositions across seasons, including significantly higher phytoplankton abundances in summer. Furthermore, there were significant seasonal variations in the assemblages of the phyla Chlorophyta, Ochrophyta, and Cyanophyta. Euglenophyta did not show any significant difference in composition between the seasons, but the abundance in summer was higher than in winter. Seasonal differences have been recorded in phytoplankton's community structure and composition in many freshwater ecosystems worldwide, with summers characterized by high abundance and phytoplankton diversity (Hall and Karol 2016; Tucker and Lloyd 1984).

The groups that contributed most to the observed seasonal differences belonged mainly to the phyla Chlorophyta and Ochrophyta, most likely because they are diverse groups with taxa adapted to a wide range of environmental conditions (Hoek *et al.* 1995; Ray *et al.* 2020). The seasonal differences in compositions are due to changing environmental variables, especially the physicochemical water quality variables. Phytoplankton assemblages change rapidly in response to changes in water quality. This property of phytoplankton makes a change in seasons long enough to alter the composition (Martins *et al.* 2020). In my study, I found temperature and salinity (conductivity) to be the key factors affecting the seasonal abundance of phytoplankton. Besides, I found that temperature was positively correlated, and conductivity was negatively correlated to phytoplankton abundance irrespective of the season, similar to findings by Saeiam *et al.* (2020). Temperature influences all metabolic functions and is a major independent factor in structuring phytoplankton communities (Saeiam *et al.* 2020; Taş 2016). The increase in temperature during summer is also associated with a relatively low percentage of dissolved oxygen levels, leading to high phytoplankton abundance (Amengual-Morro *et al.* 2012). Additionally, an increase in pH leads to an increase in phytoplankton abundance by

increasing photosynthesis (Carvalho *et al.* 2013). This temperature and pH increase can lead to algal blooms in summer.

Generally, I found that the total abundance of phytoplankton negatively correlated with conductivity, salinity, and total dissolved solids. I also found that conductivity was significant in predicting the biodiversity indices in both winter and summer. Conductivity is known to influence phytoplankton assemblage variance in Brazil's rainy season (Aboim *et al.* 2020). The increase in conductivity led to a decrease in biodiversity indices apart from evenness. An increase in total dissolved solids decreases solar radiation intensity that can be captured by phytoplankton for photosynthesis (Yan *et al.* 2020; Yusuf 2020). This relationship may account for the negative association observed between total dissolved solids and phytoplankton biodiversity indices (number of taxa, richness, Pielou's evenness, Shannon Weiner diversity, and Simpson's index).

There was a lower abundance of phytoplankton in the ponds in winter. Winter in New Zealand is associated with rain and decreased temperature, shorter sunlight (daylight), and environmental activities. These factors most likely contributed to the lower abundance of phytoplankton abundance in winter (Saeiam *et al.* 2020). Additionally, the lower abundance, richness, and taxa number recorded during winter could best be attributed to the dilution of essential growth nutrients (lower nitrogen and phosphorus concentrations) in the ponds. The dilution could arise from increased erosion and water volumes (Martins *et al.* 2020; Yusuf 2020).

#### **5.5.1.1 Seasonal shifts in phytoplankton composition**

Although there were significant seasonal differences in the phytoplankton assemblages, there was no shift in the dominant group (see also Aboim *et al.* 2020; Li *et al.* 2019). Cyanophyta has been reported as the dominant taxa in aquatic ecosystems in summer in many regions of the world (Poland, Grabowska & Wołowski, 2014, Nigeria, Yusuf, 2020, China, Yan *et al.* 2020). In Brazil, Chlorophyta and Bacillariophyceae were dominant in the dry season, while Cyanophyta dominated the wet seasons. A similar shift in dominance has been reported in reservoirs by Li *et al.* (2019). The lack of a dominant taxa shift is likely because the ponds were not enriched in nutrients enough to support other taxa. Although nutrients (phosphate and nitrate) play an important role in phytoplankton community compositions, there was no significant seasonal change in the nutrient levels, which may be due to the type of land use/land cover. Also,

Bacillariophyceae are dominant in all nutrient levels in New Zealand and may not necessarily be displaced by larger, more competitive taxa in ponds (Biggs 2000a).

### 5.5.2 Effect of human population density on phytoplankton community

The phytoplankton community differed among ponds along the human population gradient in summer but not winter. The community structure differences among ponds along the gradient indicate the phytoplankton community's sensitivity to environmental factors (Yan *et al.* 2020). Chlorophyta and Ochrophyta were the two dominant groups in all ponds; however, Chlorophyta was dominant in ponds in the small urban area while Ochrophyta was dominant in the major urban area. The spatial variations in abundances are reflective of changes in environmental variables. The relatively high abundance of Chlorophyta and Cyanophyta in the small urban area indicates a higher level of pollution in these ponds (Su *et al.* 2017). Of the three ponds in the small urban areas, one pond had sheep around it, and another had about 30 ducks. The excreta of the animals are likely to increase the nutrients load in the ponds. Thus, these ponds had high levels of some physicochemical variables (e.g., temperature, nitrate, and phosphate levels).

Similarly, the relatively high abundance of Ochrophyta and low abundance of Cyanophyta in the major urban area suggests comparatively lower pollution levels (lower nitrate levels) to the small urban area. This finding contradicts expectations of higher nutrient levels in densely populated areas and related higher abundance of Cyanophyta. The high variability in the phytoplankton composition in the major urban areas may indicate that these ponds may experience higher taxa replacement between winter and summer due to high runoff into the ponds. The dominance of Chlorophyta in ponds in rural areas is expected due to low anthropogenic impact.

A study by Paul *et al.* (2012) reveals an influence of land use types on lakes in New Zealand. They report that forest cover is negatively correlated, but urban land is positively correlated with trophic index in lakes. Similarly, Galbraith and Burns (2007) and Burns and Galbraith (2007) found that nutrient concentrations in the open water of 45 freshwater systems (both lotic and lentic) in the Otago region are positively related to land use in the catchment and increased with farming and urbanization. However, these findings are inconsistent with mine because the ponds' phytoplankton communities are affected more by other environmental factors apart from nutrients. Also, the land use intensity in the Auckland region is relatively lower than in dairy farms that experience high runoff.

### 5.5.3 Effect of human population density on biotic metrics

The phytoplankton community did not show a significant difference in pollution level along the urban-rural gradient in winter. On the contrary, the motile diatom metrics did show a difference along the gradient. Based on this metric, the ponds in major urban areas were more silted in summer (Hill *et al.* 2000). This result implies that the motile diatom metrics may be more sensitive to anthropogenic activities than the other metrics or even the whole community composition. The rest of the biotic indices' inability to differentiate among pollution levels along the urban-rural gradient, although surprising, indicates that anthropogenic activities had no significant influence on the phytoplankton metrics. The biodiversity indices were also similar along the gradient irrespective of the season.

### 5.5.4 Relationship between phytoplankton and water quality

Different phytoplankton groups responded differently to environmental conditions changes despite the significantly higher abundance of phytoplankton in summer. Chlorophyta was more abundant in winter. The relatively lower abundance of Chlorophyta in summer is likely due to the higher conductivity. Since Chlorophyta are photophilic, an increase in TDS can decrease their abundance (Yusuf 2020). Higher TDS reduces transparency, minimizing the amount of light permeating the water (Yusuf 2020); hence the negative relationship between conductivity, TDS, salinity, and Chlorophyta. In contrast to Chlorophyta and Cyanophyta, Ochrophyta was positively related to conductivity TDS and salinity. Ochrophyta is usually more abundant in the middle and lower section of the water column and has diverse taxa adapted to different nutrient levels (Biggs 2000a; Yan *et al.* 2020; Yusuf 2020). The observed positive correlation between dissolved oxygen and the abundance of Cyanophyta in summer may be because photosynthesis results in oxygen production; therefore, an increase in phytoplankton abundance comes with a resultant increase in dissolved oxygen concentration (Yusuf 2020).

In this study, the ponds' nutrient (phosphates and nitrates) concentrations were not significantly different between the seasons. Hence, they are likely not to be the reason for the difference in assemblages and abundance recorded. A study by Saeiam *et al.* (2020) on phytoplankton community dynamics in ponds in Thailand showed that although nitrogen and phosphorus affect the types of autotrophic organisms, they did not affect the amount of phytoplankton in ponds. This relationship may explain the weak correlation between phytoplankton abundance and the nitrate and phosphorus concentrations in the ponds in both seasons. My results showed that

phosphates and nitrates were not the main drivers of the phytoplankton communities in the ponds as hypothesized, contrary to the report by Jin *et al.* (2020).

Different phytoplankton groups, however, exhibited different responses. For instance, Ochrophyta, Chlorophyta, and Euglenophyta were negatively correlated with nitrate concentration, while Cyanophyta was positively correlated. Although nutrients, especially nitrate concentrations, are important in predicting phytoplankton community composition, temperature and hours of sunshine are more vital in phytoplankton community compositions (Li *et al.* 2019). My research results reveal that water temperature played a role in accounting for the differences in phytoplankton communities, similar to studies by Li *et al.* (2019). The temperature was also positively correlated with phytoplankton abundance, as hypothesized.

### 5.5.5 Phytoplankton as indicators

Phytoplankton diversity is associated with the availability of niches and resources such as photoperiod and sporadic disturbance (Paul *et al.* 2012). From the results of this study, it is possible that more niches and resources were available in summer than winter; however, intermediate disturbances caused by high precipitation during winter could have resulted in new niches and hence the similar Shannon Wiener diversity value recorded. These findings indicate that phytoplankton diversity in the pond does not vary irrespective of the season.

My findings suggest that the ponds were moderately polluted because of the Shannon Wiener diversity values of  $\approx 3$  recorded in both seasons (Rasal *et al.* 2019; Taş 2016; Yusuf 2020). Within aquatic ecosystems with phytoplankton, a Shannon Wiener diversity index  $< 1$  indicates heavy pollution, 1-3 moderately polluted, and  $> 3$  clean water (Wilm and Dorris 1966).

The Ochrophyta taxa, *Navicula* and *Nitzschia*, are characteristic of shallow aquatic ecosystems and good indicators of pollution because they are sensitive to nutrient depletion (Aboim *et al.* 2020; Palmer 1969; Reynolds *et al.* 2002; van Vuuren *et al.* 2006). Hence their dominance in the ponds in both seasons indicates pollution (Palmer 1969; Yusuf 2020). *Cyclotella* is also found in mesotrophic lentic systems and is sensitive to a rise in pH (Aboim *et al.* 2020), which explains why they were more abundant in winter, where a lower pH value was recorded.

The presence of taxa such as i) *Peridium* and *Ankistrodesmus*, which are associated with a lower level of organic pollution, ii) *Eunotia* (associated with acidic environments), *Dolichospermum*

(associated with warm environments and high light saturation), and iii) *Tetraedron*, *Euglena* and *Gomphonema* (associated with high pollution and temperature) are indicative of the environmental conditions associated with the ponds in summer (Aboim *et al.* 2020; van Vuuren *et al.* 2006).

Similarly, in winter, the presence and abundance of the i) *Cyclotella* (adapted to low nutrient concentrations) (Järvinen *et al.* 2013), ii) *Spirogyra* (sensitive to pH increase) (Aboim *et al.* 2020; van Vuuren *et al.* 2006) iii) *Achnanthes* and *Tribonema* (associated with well-mixed systems), and iv) *Leptolyngbya*, and *Microcystis* (associated with nutrient-rich systems) (Van Vuuren *et al.* 2006; Aboim *et al.* 2020) are indicative of the environmental variables that prevailed in the ponds.

### ***Implication of the finding for freshwater management***

Ponds are sensitive to stochastic events in and around them, either due to anthropogenic activities or seasonal variations, mostly leading to increased nutrient concentration and increased phytoplankton abundance (Kallscheuer *et al.* 2021). This phenomenon is especially prevalent in summer when there is a longer photoperiod and high temperature. This study provides a piece of important information in improving our understanding of the phytoplankton compositions in ponds. The findings showed that the phytoplankton communities in ponds are mainly influenced by water quality, particularly temperature. Thus, as temperature increases, management interventions should be instituted to minimize their levels in the freshwater ecosystems. For instance, trees should be planted around freshwater ecosystems, including those by roadsides, to provide shade, reduce temperature, and reduce the temperature of runoff into the aquatic systems (Peretyatko *et al.* 2007). Within ponds, the introduction and maintenance of submerged macrophytes will also control phytoplankton proliferation (Peretyatko *et al.* 2007). This intervention is especially vital during summer to reduce nutrient concentration and reduce the proliferation of cyanobacteria to maintain healthy phytoplankton communities in freshwater ecosystems, especially in the lentic systems.

## 5.6 Conclusion

To my knowledge, this research is one of the first studies on phytoplankton in ponds in the Auckland region. This study investigated the phytoplankton community composition in ponds and how they vary seasonally and across the human population gradient. Also, the relationship between the communities and the physicochemical water quality variables was determined. The phytoplankton communities in the ponds are diverse, and the dominance by Chlorophyta in both seasons is indicative of a healthy phytoplankton community. The phytoplankton community compositions showed seasonal changes in the ponds. Phytoplankton abundance in the ponds was higher in summer, and the community compositions differed between seasons. Similarly, the physicochemical water quality variables in the ponds varied across the seasons. Conductivity and temperature were important in structuring the phytoplankton communities seasonally. The nutrient concentrations in the ponds did not appear to determine the abundance of the phytoplankton. However, the Bacillariophyceae metric used indicated that ponds were more silted and organically polluted in summer; this was confirmed by the dominance of taxa such as *Navicula*, *Nitzschia*, *Cyclotella*, and *Synedra*. The phytoplankton communities showed a significant influence of anthropogenic activities in summer.

## 5.7 References

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# Chapter 6

## Identifying the limiting nutrients in ponds



## 6.1 Abstract

Anthropogenic activities, including a high rate of urbanization, are associated with increased nutrient levels in freshwater ecosystems. In New Zealand, increased nutrients (nitrogen and phosphorus) levels are considered the primary cause of freshwater deterioration. These nutrients are essential for primary productivity, but higher levels can easily lead to the eutrophication of many lakes and ponds. Eutrophication increases algal biomass, making ponds and other freshwater ecosystems unsuitable for biodiversity. Ponds generally have a higher nutrient load, but research on the limiting nutrient on algal growth has not been given much attention. This information is increasingly important in managing ponds, the most abundant freshwater ecosystem globally and in New Zealand. In this chapter, I assessed the effect of different nutrient concentrations on periphyton growth in ponds. Chlorophyll 'a' (a measure of algal biomass) yield of periphyton grown in different nutrient concentrations was used to assess the limiting nutrients in ponds. I investigated this by experimentally depositing nutrient diffusion substrates (NDS) in ponds and measuring periphyton biomass. Three-level concentrations of (low, medium, high) nitrogen, phosphorus, and a combination of nitrogen and phosphorus were used in the experiment. The results show a variable response of periphyton to the addition of different nutrient concentrations. Overall, moderate nutrient additions yielded the highest chlorophyll 'a' biomass. Nitrogen at moderate concentrations ( $0.15\text{mg l}^{-1}$ ) yielded the highest chlorophyll 'a' biomass. The chlorophyll 'a' biomass yielded by the low concentrations of the nutrient treatments were not significantly different regardless of the ambient nutrient concentrations. The control treatment yielded the least chlorophyll 'a' biomass regardless of the ponds and treatment level they were placed. The results suggest that nitrogen is the main limiting nutrient for chlorophyll 'a' biomass in the ponds.

## 6.2 Introduction

Anthropogenic activities, especially those relating to urbanization, have increased nutrient loads in freshwater ecosystems (Jani *et al.* 2020). This phenomenon has arisen due to the sensitivity of freshwater ecosystems to land use activities around them. Typically, the increase in nutrient concentrations results in the ecosystem's degradation and sometimes leads to cultural eutrophication (Schindler 2012; Tsoi *et al.* 2020; Weeks *et al.* 2016), impacting not only biodiversity but its aesthetic and recreational values (Elliott *et al.* 2006; Maberly *et al.* 2020; Sutherland *et al.* 2020).

Globally, nitrogen and phosphorous concentrations are the primary nutrients responsible for freshwater deterioration (Tsoi *et al.* 2020). High concentrations of these nutrients affect the structure and function of aquatic ecosystems by increasing primary productivity because they are essential for the growth of algal cells (Trochine *et al.* 2014; Tsoi *et al.* 2020). High nutrient concentrations also adversely affect other freshwater flora and fauna by altering the water chemistry (Abell *et al.* 2010; Biggs 2000; Death *et al.* 2007; Maberly *et al.* 2020). Approximately 30% (n > 1000) of lentic ecosystems in New Zealand have very poor water quality and may be suffering from cultural eutrophication and algal proliferation (Abell *et al.* 2010; Ministry for the Environment 2007). Therefore, the management of nutrient inputs into New Zealand freshwater ecosystems is crucial for environmental agencies responsible for maintaining or improving the health of the freshwater ecosystems (Death *et al.* 2007).

Algae is the principal primary producer in freshwater ecosystems (Tsoi *et al.* 2020; Vadeboncoeur and Steinman 2002), making algal biomass a key indicator of the biological water quality in aquatic ecosystems (Song *et al.* 2020). The term "algae" is a useful ecological grouping of chlorophyll 'a' containing organisms that occur in mixed communities in aquatic habitats (Biggs and Kilroy 2000). Therefore chlorophyll 'a' is a quantitative proxy measure of algae concentration and, thus, primary productivity.

Chlorophyll 'a' is an especially useful measure because it indicates the total amount of (predominantly) autotrophic organisms in a sample (Biggs and Kilroy 2000). Additionally, measuring chlorophyll 'a' is cheaper and more efficient than identifying and enumerating algae under the microscope (DeNicola and Kelly 2014). It is also less predisposed to errors related to

allochthonous organic matter (Biggs and Kilroy 2000). An increase in algae biomass (chlorophyll 'a' concentration) has been traced to elevated levels of phosphorus and nitrogen, although other contributory factors, including geological, land use, and temperature, also contribute to biomass changes (Death *et al.* 2007; Kilroy *et al.* 2020; Song *et al.* 2020).

Nitrate and hence nitrogen have been frequently reported to limit algal biomass in freshwater ecosystems because algae is an important consumer of nitrogen (Abell *et al.* 2010; Maznah and Makhrough 2015). Chu (1943) hypothesised that the nitrogen concentration in freshwater determines, to a large extent, the amount of chlorophyll 'a'. In comparison, phosphorus availability appears to have no significant effect on algal growth in reservoirs (Maznah and Makhrough 2015), suggesting that for these ecosystems, optimum algal growth rates are mainly affected by high nitrogen concentration. On the contrary, phosphorus is considered a limiting nutrient in algal growth in lentic ecosystems (Trochine *et al.* 2014). Downing and McCauley (1992) showed that phosphorus is more important than nitrogen in regulating algal biomass in lakes. Additionally, Atique and An (2020) report that phosphorus is the important nutrient limiting primary productivity in lentic systems. The differences in limiting nutrients are due to geology, climate, and hydrology, which regulate nutrient flux through soil erosion and runoff into freshwater ecosystems (Abell *et al.* 2010; Death *et al.* 2007; Downing and McCauley 1992). For example, streams on alluvial soils are likely to be limited by phosphorus, while streams draining volcanic areas are likely to have nitrogen limitations (Death *et al.* 2007)

Many studies on nutrient limitations in lentic ecosystems have focused on phytoplankton with little focus on periphyton (but see Fairchild *et al.* (1985) and Rodusky *et al.* (2001). However, periphyton plays a significant role in primary productivity (Lepori and Robin 2014; Oleksy *et al.* 2021; Vadeboncoeur and Steinman 2002). The proliferation of phytoplankton in aquatic ecosystems is usually preceded by an increase in periphyton (Jones *et al.* 2002; Lepori and Robin 2014). Besides, periphyton is a key part of the food web and a vital food source for many grazers such as macroinvertebrates and zooplankton (Lepori and Robin 2014; Vadeboncoeur and Steinman 2002). Further, periphyton is a useful indicator of point source nutrient inputs in lentic ecosystems (Fairchild *et al.* 1985).

Due to their relatively small size and lentic nature, ponds are highly susceptible to algal blooms, especially during summer when evaporation and temperatures levels are highest. However, research on the limiting nutrient in ponds has received little attention globally, and in New Zealand, such information is scarce. There is scant information on how nutrient concentrations

impact pond ecosystems and how it affects primary productivity (Trochine *et al.* 2014). Abell *et al.* (2010) recommend that the limiting nutrient in primary productivity in pond ecosystems in New Zealand needs to be investigated. However, this has not been done though there are more ponds than other freshwater ecosystems. A pond's nutrient limitation status may be inferred from experimental (laboratory cultivation) and observational data through field simulations or direct field samplings (Abell *et al.* 2010; Elliott *et al.* 2006; Hogan *et al.* 2014).

In this study, chlorophyll 'a' biomass of periphyton grown in different nutrient concentrations was elucidated by experimentally depositing nutrient diffusion substrates in ponds. The stimulation of periphyton biomass by adding nitrogen, phosphorus, or both nutrients experimentally using nutrient diffusion experiments is common in determining the limiting nutrients in aquatic ecosystems (Oleksy *et al.* 2021). Nutrient diffusing substrates (NDS) is one of the methods of manipulating nutrient concentrations *in-situ* to determine the type and concentration of nutrients that limit primary productivity in freshwater ecosystems (Hogan *et al.* 2014; Tsoi *et al.* 2020). In this method, agar is impregnated with different nutrient treatments and covered with a substrate that becomes the settlement material. Algae attach, grow, and sometimes form a mat on the settlement material (Khatoon *et al.* 2007; Larned 2010).

After a defined time, usually between 1-30 days, the substrates are processed for chlorophyll 'a' to evaluate and compare algal growth in response to the different nutrient treatments. The treatment that yields the highest chlorophyll 'a' indicates the limiting nutrient for algae growth (Biggs and Kilroy 2000; Death *et al.* 2007; Larned 2010; Tsoi *et al.* 2020). In other words, an individual nutrient is defined as limiting if its addition results in a significant positive algal growth response measured in chlorophyll 'a' concentration (Abell *et al.* 2010). Such experiments give baseline information on the nutrient responsible for limiting algal growth (Death *et al.* 2007). These are necessary for the subsequent development of management strategies to minimise and monitor these nutrient levels in the aquatic systems (Cooper *et al.* 2016).

### 6.2.1 Aim and hypotheses

In this chapter, I aimed to identify the limiting nutrient(s) of periphyton growth in an *in-situ* experiment using NDS. In addition, I assessed the response of periphyton to different nutrient concentrations in ponds. The questions I addressed were:

- i) do different phosphorus and nitrate concentrations affect periphyton growth and hence chlorophyll 'a' concentrations in ponds?
- ii) which nutrient has a greater effect on chlorophyll 'a' biomass in ponds?

Based on these questions, I formulated two null and alternative hypotheses. These are:

- i) algae biomass does not change with increasing nutrient concentrations,
- ii) both phosphorus and nitrogen have equal effects on periphyton growth.

The alternate hypotheses are:

- i) algae biomass (chlorophyll 'a' concentration) varies with different nutrient concentrations,
- ii) both phosphorus and nitrates are the limiting nutrients in the periphyton growth in ponds.

## 6.3 Method and analyses

### 6.3.1 Experimental design

Agar solutions were impregnated with phosphorus in the form of ( $\text{KH}_2\text{PO}_4$ ) and nitrogen in the form of ( $\text{NaNO}_3$ ). These compounds are commonly used in NDS (Beck *et al.* 2017; Oleksy *et al.* 2021). Three different nutrient concentrations were used in this experiment. The nutrient concentrations and ponds used were based on water quality data collected from earlier surveys (Tsoi *et al.* 2020). The lowest, moderate, and highest nutrient concentrations recorded for each nutrient were multiplied by a factor of 3 (see table 6.1) for the nutrients used.

The NDS was prepared by adding different concentrations of nutrients to agar. Distilled water was the solvent used in the nutrient diffusing substrate. There were four treatments: i) control, ii) nitrogen (N), iii) phosphorus (P), and iv) a combination of nitrogen and phosphorus addition

(NP) (Table 6.1). The control treatment did not have any nutrients added (agar only). Therefore, its algal biomass was from the ambient water quality of the ponds. Settlement nylon mesh was attached to each treatment (see Appendix E, section 1 for details of the protocol). Each pot consisted of agar solutions with one each of the four nutrient treatments. The four treatment pots were placed 10–30cm apart within each experimental set, with four replicates per treatment (Figure 6.1a). There were 16 NDS in each setup.

A total of six ponds were used for this study; two ponds received the same concentration of nutrients of low, moderate, and high nutrient treatments. The two ponds were to minimise the bias that environmental factors may cause. Each pond received three experimental sets. The ponds were divided into low, moderate, and high nutrient contents (Table 6.1). The setup consisted of a tray with the treatment's pots. Before deployments, the trays were cable tied to a basket with weights to prevent them from floating in the pond. Each experimental setup was enclosed in a wire mesh to prevent birds (Pukeko and Ducks) from accessing it (Figure 6.1a). The setups were deployed between November and December 2018. The experimental setup was placed in designated ponds for four weeks (28 days) because the diffusion rate during this period is likely to be constant (Tsoi *et al.* 2020). Each set up was placed at least three metres into the pond. They were placed so that the distances between the setups were approximately equal. The pottles were submerged entirely at approximately 5cm below the pond surface at the beginning of the experiment (Figure 6.1b). After four weeks, the experimental setups were removed from ponds, and the algal biomass of the pottles was quantified for each treatment.

Table 6.1 A summary of the initial nutrient in the ponds and concentrations used in the NDS.

Ponds	Nutrient concentration (mg l <sup>-1</sup> ) in the pond			Concentrations of nutrients used (mg l <sup>-1</sup> )		
	N	P	Treatment level	N	P	NP
Pond 1	0.01	0.12	Low	0.03	0.03	0.0015 (NO <sub>3</sub> ) / 0.0015 (P)
Pond 2	0.01	0.18	Low	0.03	0.03	0.0015 (NO <sub>3</sub> ) / 0.0015 (P)
Pond 3	0.04	1.03	Medium	0.15	1.5	0.0075 (NO <sub>3</sub> ) / 0.075 (P)
Pond 4	0.02	1.05	Medium	0.15	1.5	0.0075 (NO <sub>3</sub> ) / 0.075 (P)
Pond 5	0.16	1.89	High	1.5	7.5	0.075 (NO <sub>3</sub> ) / 0.375 (P)
Pond 6	0.32	2.39	High	1.5	7.5	0.075 (NO <sub>3</sub> ) / 0.375 (P)

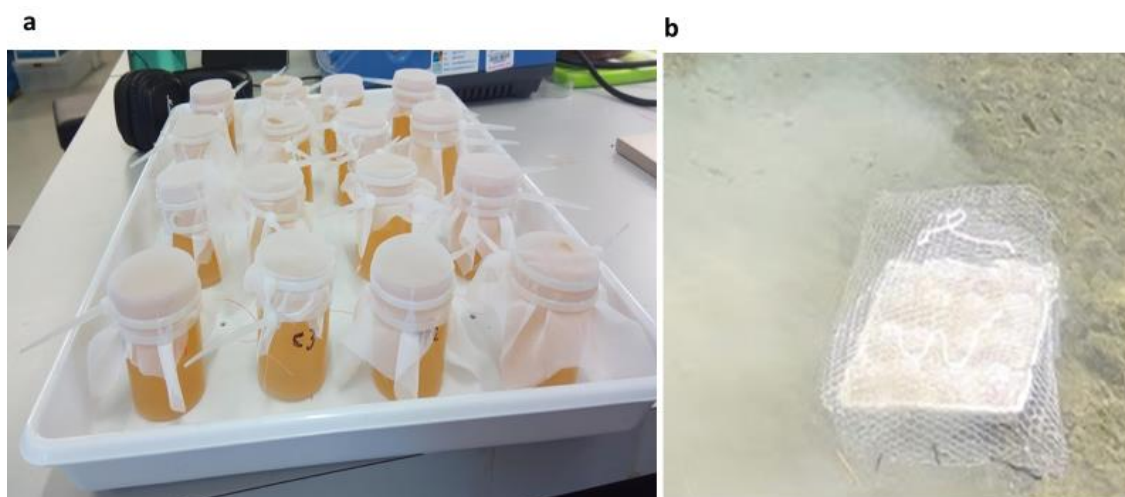


Figure 6.1 A picture of the NDS set up at different stages. a) show the random placement of treatment pots on a tray, and b) shows the NDS setup in the ponds.

Table 6.2 A grid showing how the NDS plates were arranged on the tray. The numbers indicate the replicate. N = nitrogen, P = phosphorus, NP = nitrogen + phosphorus, and C = control treatment.

NP1	N2	P3	C4
C3	P4	N1	NP2
N4	NP3	C2	P1
P2	C1	NP4	N3

### Collection of periphyton biomass

At the end of 28 days in the ponds, periphyton was sampled from the fine mesh screen over each jar. Each mesh screen was trimmed to the standard diffusible area directly in direct contact with the agar ( $28.66\text{cm}^2$ ) by cutting off the excess mesh. Then, a known water volume (70-100ml) was used to wash the mesh and added to the scraped periphyton. The mesh content was transferred into a labelled sample tube and immediately stored on ice in a portable freezer for algal biomass analyses. The methods for the chlorophyll 'a' extraction is outlined below

### Chlorophyll 'a' extraction and algal biomass

Chlorophyll 'a' extraction was done based on the methods by Biggs and Kilroy (2000). The samples were kept in a labelled dark container and exported on ice to the lab. These samples were kept frozen ( $-20^\circ\text{C}$ ), removed a day before chlorophyll 'a' extraction, and kept in the dark

to defrost and thaw. After thawing, the samples were shaken thoroughly to mix the contents. Then, a known volume (2-7ml) of each sample was filtered through a Whatman GF/C (47mm) glass fibre filter. The samples were filtered until a green colouration was seen on the filter paper. The filter papers were then folded and placed in a labelled polythene centrifuge tube containing 10ml of 90% ethanol to extract the chlorophyll 'a'. The centrifuge tubes were loosely closed and placed in a water bath in a fume hood to boil at 78°C for five minutes. After boiling, the tubes were removed and placed in the refrigerator (2°C - 4°C) overnight for chlorophyll extraction to continue. The tubes were then removed from the refrigerator the following day.

The centrifuge tubes were opened, and the glass fibre filters were pushed to the bottom of the tubes before closing them. The tubes were placed into the centrifuge rotor such that their weights were evenly distributed. The tubes were centrifuged at 40449g for 15 minutes, after which they were removed. The chlorophyll 'a' concentrations of three replicates of each sample were measured using their absorbance in a spectrophotometer, and the average was used for analysis. The chlorophyll 'a' in the samples were measured against a standard laboratory chlorophyll 'a'. In its preparation, the standard chlorophyll 'a' was added to 10ml of ethanol in a centrifuge tube and boiled at 78°C for 5 minutes. The content was left to cool to room temperature, and then the absorbance was read on a spectrophotometer, 'Shimadzu-UV-1601'.

A pair of 3.5ml plastic cuvettes were used for taking the absorbance readings. Each cuvette was initially filled with 3ml of 90% ethanol. These were placed in the spectrophotometer and used as the blank solution. Next, one of the cuvettes was taken out and filled with 3ml of the standard chlorophyll solution, and the reading was taken. The peak absorbance was recorded at wavelength 665nm. This wavelength was used as the benchmark for all the other extracted chlorophyll samples. For each sample, 3ml of the chlorophyll solution was pipetted into the same cuvette. The absorbances of the samples at the wavelength 750nm and 665nm were recorded (Venter *et al.* 2013).

After the first readings were recorded, the cuvette with the chlorophyll was acidified to correct for phaeopigment. This correction was done by removing the cuvette from the spectrophotometer and adding 0.75ul of three molar hydrochloric acid ( $3\text{kgmol}^{-1}$  of HCL) to the chlorophyll, and the second reading was taken. After taking each sample's initial and after acidification readings, the cuvette was rinsed with 90% ethanol before the next chlorophyll sample was poured into the cuvette to avoid contamination between samples. The chlorophyll

'a' concentration was calculated with the equation: chlorophyll 'a' ( $\text{mgm}^{-2}$ ) =  $\frac{\{(A_{665} - A_{750}) - (A_{665a} - A_{750a}) \times 28.66 \times \text{volume of extractant} \times \text{volume of sample} / (\text{filtered sub-sample volume})\}}{\text{area of sample}}$ , where 28.66 is the absorption coefficient for chlorophyll a as defined by Sartory and Grobbelaar (1984),  $A_{665}$  and  $A_{665a}$  (and  $A_{750}$ ,  $A_{750a}$ ) represent absorbance at 665nm (and 750nm) before and after acidification, (Biggs and Kilroy 2000; Venter *et al.* 2013).

### 6.3.2 Statistical analysis

The average of all chlorophyll 'a' concentrations was estimated ( $n = 4$ ) for each treatment. The effect of nutrient application on chlorophyll 'a' biomass was determined using a linear mixed model (LMM) after checking for normality with the Shapiro-Wilk test. Using the package "*lmerTest*", nutrient type (control, nitrogen, phosphorus, and NP) with nutrient level (low, moderate, and high) were selected as fixed effects with pond location as a random effect, followed by ANOVA. After LMM, a set of contrasts between treatments were performed using the "*lsmeans*" package with Bonferroni adjustment. Before performing analysis, the data were transformed by  $\log_e(x + 1)$  to satisfy the assumptions of ANOVA (homogeneous variances and approximate normality) (Death *et al.* 2007; Tsoi *et al.* 2020), and a  $p$ -value  $< 0.05$  was always considered significant. The analyses were done using R version 3.6. 1: (R Core Team 2019).

## 6.4 Results

### 6.4.1 Overall effects of nutrient treatment

Results of the linear mixed model showed that both nutrient type ( $F_{3, 60} = 8.43$ ,  $p < 0.001$ ) and levels of concentration ( $F_{8, 60} = 8.43$ ,  $p < 0.001$ ) were significantly associated with high chlorophyll 'a' yield. In contrast, the pond location explained only a fraction of variance ( $\sigma^2 = 0.33$ ).

With the control group as a reference, the addition of nitrogen ( $t = 4.26$ ,  $p < 0.001$ ) and phosphorus ( $t = 3.51$ ,  $p = 0.001$ ) significantly increased chlorophyll 'a' biomass, while nitrogen and phosphorus combination (NP) only had a minor positive impact ( $t = 0.84$ ,  $p = 0.4$ , Figure 6.2).

The set of contrast between nutrient treatment also revealed a significantly higher chlorophyll 'a' biomass in response to nitrogen addition than phosphorus ( $t = 3.01$ ,  $p < 0.01$ ) and NP additions ( $t = 4.70$ ,  $p < 0.001$ ), while mean yield was similar for phosphorus compared to NP addition ( $t = 0.62$ ,  $p = 0.6$ ).

## 6.4.2 Comparisons within respective nutrient treatments

### 6.4.2.1 Nitrogen treatment

Overall nitrogen (N) addition positively increased chlorophyll 'a' biomass ( $\beta = 0.42$ ,  $t = 3.53$ ,  $p = 0.002$ ). The moderate N concentration treatments yielded the highest chlorophyll 'a' concentrations ( $10.20\text{mgm}^{-2} \pm 6.9\text{mgm}^{-2}$ ), then low ( $1.35\text{mgm}^{-2} \pm 0.73\text{mgm}^{-2}$ ) and high N concentration treatments ( $4.40\text{mgm}^{-2} \pm 1.44\text{mgm}^{-2}$ ). The pairwise analysis showed a significant difference between the mean yields of low and high ( $t = 4.47$ ,  $p < 0.001$ ) and low and moderate N additions ( $t = 3.98$ ,  $p = 0.001$ ), while that of high and moderate N additions was not significant ( $t = 0.32$ ,  $p = 0.7$ ) (Figure 6.2).

### 6.4.2.2 Phosphorus treatment

Phosphorus (P) addition significantly increased chlorophyll 'a' biomass in the ponds ( $t = 2.31$ ,  $p = 0.03$ ). The moderate P concentration treatments yielded the highest mean chlorophyll 'a' concentration ( $4.17\text{mgm}^{-2} \pm 2.38\text{mgm}^{-2}$ ), compared to low ( $2.27\text{mgm}^{-2} \pm 1.81\text{mgm}^{-2}$ ) and high ( $3.56\text{mgm}^{-2} \pm 1.11\text{mgm}^{-2}$ ). The differences were significant between low and moderate ( $t = 2.97$ ,  $p = 0.01$ ) and low and high ( $t = 3.38$ ,  $p < 0.01$ ) P additions. The difference was not significant between the biomass of high and moderate P treatments ( $t = 0.40$ ,  $p = 0.7$ ) (Figure 6.2).

### 6.4.2.3 Nitrogen and phosphorus treatment

On the other hand, the addition of nitrogen and phosphorus (NP) treatment had a minor negative effect on chlorophyll 'a' biomass ( $t = -1.30$ ,  $p = 0.2$ ). The high concentration treatments of NP yielded the highest chlorophyll 'a' ( $3.76\text{mgm}^{-2} \pm 1.05\text{mgm}^{-2}$ ), followed by the moderate ( $2.60\text{mgm}^{-2} \pm 1.63\text{mgm}^{-2}$ ) and low ( $0.94\text{mgm}^{-2} \pm 0.25\text{mgm}^{-2}$ ) concentrations (Figure 6.3).

6.4.2.4 Control treatment

The control treatments were also generally associated with a negative effect on the chlorophyll 'a' yield ( $t = -4.55, p < 0.001$ ), with mean yields relatively lower than other treatments. The chlorophyll 'a' biomass recorded in this group were ( $0.89\text{mgm}^{-2} \pm 1.05\text{mgm}^{-2}$ ), ( $2.14\text{mgm}^{-2} \pm 1.04\text{mgm}^{-2}$ ) and ( $1.6\text{mgm}^{-2} \pm 0.70\text{mgm}^{-2}$ ) for low, moderate, and high respectively. Differences were significant for low and high ( $t = 3.39, p < 0.01$ ) and low and moderate ( $t = 2.60, p = 0.02$ ) treatments (Figure 6.2).

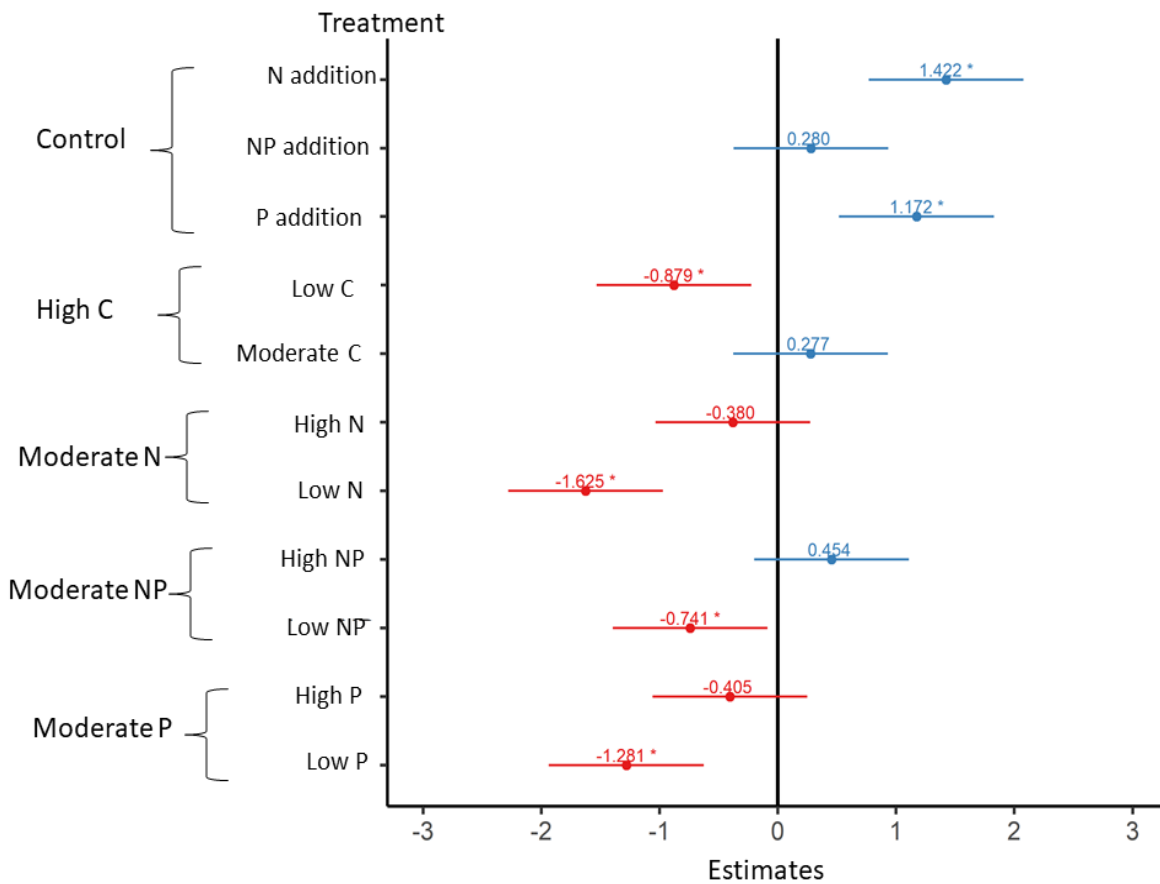


Figure 6.2. A diagram of the linear mixed model of treatments. The blue lines indicate positive effects of explanatory variables, while red lines represent negative effects, with 95% CI. The numbers on the lines show the coefficient of estimates, and asterisks (\*) indicate a significant impact ( $p < 0.05$ ). Treatments at the extreme left of the y-axis are used as references to which those in brackets are compared.

### 6.4.3 Comparing different concentrations levels of nutrients between treatments

A series of pairwise comparisons between the levels of nutrients were performed, following the linear mixed model. The results show that low concentration had no significant effect on chlorophyll 'a' biomass irrespective of the nutrient type added. In contrast, moderate and high nutrient concentrations were associated with variable effects. There was an interaction between the high and moderate nutrient levels of NP treatment (Figure 6.3, Table 6.3).

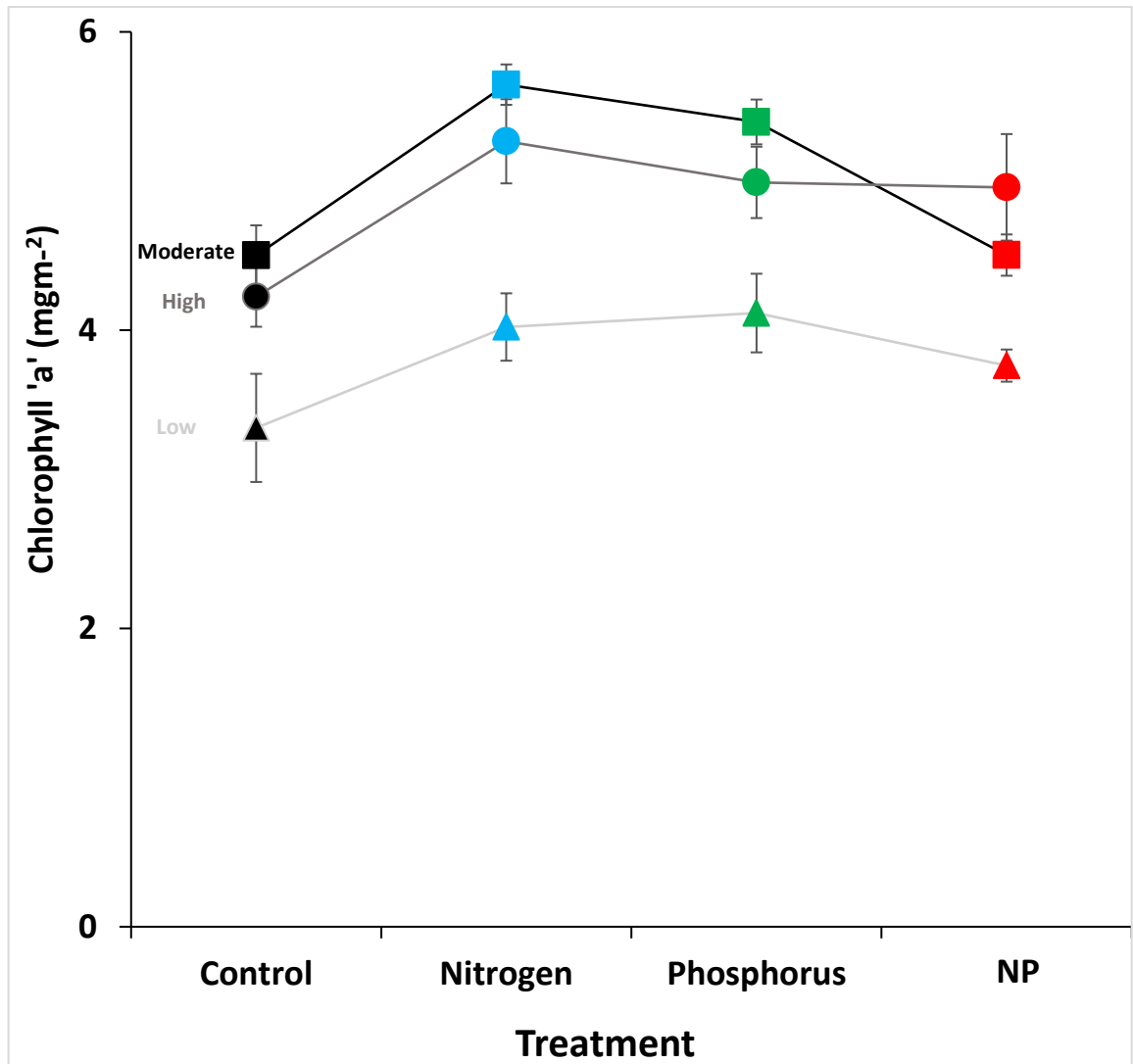


Figure 6.3. The effect of different nutrient levels between treatments. The points represent the mean chlorophyll 'a' biomass  $\pm$  standard error. The nutrient treatment is represented by different colours; black = control, blue = nitrogen, green = phosphorus and red = NP. The shapes represent different concentration levels; circle = high concentration, square = moderate concentration and triangle represent low concentration.

Table 6.3 Pairwise comparison between different nutrient levels between treatments, following a linear mixed model. Significant p-values (5%) from a paired t-test are in bold.

Nutrient level	Treatment	$\beta$	t	P
Low	C vs N	0.40	1.23	0.3
	C vs P	0.50	1.67	0.1
	C vs NP	0.15	0.46	0.7
	N vs P	0.18	0.57	0.6
	N vs NP	0.53	1.61	0.2
	P vs NP	0.08	0.24	0.8
Moderate	C vs N	0.87	2.65	<b>0.02</b>
	C vs P	0.62	2.09	0.07
	C vs NP	0.27	0.86	0.5
	N vs P	0.52	1.64	0.1
	N vs NP	1.42	4.29	<b>&lt;0.001</b>
	P vs NP	0.62	1.88	0.1
High	C vs N	0.77	2.34	<b>0.04</b>
	C vs P	0.49	1.66	0.1
	C vs NP	0.46	1.47	0.2
	N vs P	0.55	1.75	0.1
	N vs NP	0.58	1.76	0.1
	P vs NP	0.24	0.73	0.6

Abbreviations: control (C), nitrogen (N), phosphorus (P)

## 6.5 Discussion

Increasing nutrient loads within lentic ecosystems is a big challenge to freshwater ecosystems' quality in New Zealand, leading to eutrophication in some ecosystems, especially in summer. This experiment was conducted to determine the effect of different nutrient concentrations on periphyton growth and ascertain the limiting nutrient in ponds. The results showed that nutrient addition increased chlorophyll 'a' yield by a factor of one to six on average in the ponds. This outcome suggests that there are considerable amounts of nutrients available in ponds for primary productivity compared to the severe shortage reported by Hogan *et al.* (2014) in oligotrophic lakes in Greenland. Further, the chlorophyll 'a' yield is similar to the periphyton biomass in streams in New Zealand (Death *et al.* 2007), Lepori and Robin (2014) in alpine lakes in France and Arctic lakes (Hogan *et al.* 2014) but lower than lakes in the USA (Olesky *et al.* 2021; Roduski *et al.* 2001), and lakes in Sweden and Antarctica (Hansson 1992). The variations in the biomass are likely due to the impact of anthropogenically related nutrient concentrations in the study systems (Verburg *et al.* 2010)

The periphyton in the ponds responded differently to nitrogen and phosphorus addition. The highest chlorophyll 'a' biomass was below the 60mgm<sup>-2</sup> thresholds for oligotrophic-mesotrophic streams in New Zealand, favourable for conserving benthic flora and fauna (Biggs and Kilroy 2000; Biggs 2000). My finding suggests that ponds are generally less nutrient-rich. However, it is possible that heavy grazers (for example, snails) could have fed on the periphyton while in the ponds reducing the biomass.

The high chlorophyll 'a' biomass of nitrogen-only treatments across all concentrations demonstrate that primary productivity was limited by nitrogen in the ponds. Similar results are reported by Oleksy *et al.* (2021) on the Sky Pond in the USA. The nitrogen limitation may be because of usually high phosphorus concentrations in ponds (Biggs 2000; Death *et al.* 2007). Also, nitrogen limitation occurs when environmental factors impede nitrogen fixation (Trochine *et al.* 2014). For example, a low abundance of cyanobacteria can reduce nitrogen fixation (Trochine *et al.* 2014). Further, nitrogen and phosphorus's unequal removal from wetlands where denitrification leads to a higher rate of nitrogen loss than phosphorus can cause nitrogen limitation (Scott *et al.* 2005). Besides, since most of the freshwater in Auckland drains from volcanic geology, they are usually nitrogen-limited (Death *et al.* 2007). Moreso, nitrogen uptake

is high under warm or tropical conditions and may cause nitrogen limitation in the ponds (Maberly *et al.* 2020; Oleksy *et al.* 2021). Therefore, the nitrogen limitation in the ponds is not surprising.

My results support findings by Death *et al.* (2007) and Abell *et al.* (2010), who report nitrogen as the limiting nutrient in streams in the North Island and lakes in New Zealand, respectively. Similarly, Trochine *et al.* (2014) also report nitrogen limitation in an early summer experiment in Denmark. Further, Rodusky *et al.* (2001) also found nitrogen to be more limiting in periphyton growth than phosphorus in Lake Okeechobee (USA). The results contradict the finding that phosphorus is the limiting nutrients in lakes in other parts of the world (Abell *et al.* 2010). Although Cyanobacteria fix atmospheric nitrogen, thereby increasing nitrogen concentrations in freshwater ecosystems, they may not be abundant in the ponds (Hogan *et al.* 2014; Lepori and Robin 2014; Neumiller 2021). Moreso, the dominance of the phytoplankton by chlorophytes (non-nitrogen fixers) in the ponds (Chapter 5) could explain the nitrogen limitation. The nitrogen limitation suggests that nitrogen addition could lead to eutrophication in ponds (Lepori and Robin 2014; Scott *et al.* 2019). However, it should be noted that nutrient limitation can vary seasonally and spatially (Death *et al.* 2007; Rodusky *et al.* 2001; Trochine *et al.* 2014; Tsoi *et al.* 2020).

The moderate levels of the nitrogen and phosphorus treatments yielded higher chlorophyll 'a' biomass than the high and low concentrations, indicating that those concentrations may be the ambient for periphyton chlorophyll 'a' yield (Tsoi *et al.* 2020). However, the simultaneous addition of higher nitrogen and phosphorus concentrations led to a higher yield of chlorophyll 'a' similar to studies by Kniffin *et al.* (2009) and Trochine *et al.* (2014). The low yield of the low treatments indicates that when the nutrient concentration in ponds is low, chlorophyll 'a' biomass is likely to be low.

The low yield of the control treatments at all three levels is indicative of the importance of nutrients for periphyton proliferation. It also shows that the ambient nutrients in the water can sustain periphyton growth and the addition of nutrients stimulates the production of chlorophyll 'a' in ponds. Nitrogen limitation in ponds needs to be further investigated in more ponds and different seasons because my study was limited to six ponds and was carried out in summer due to time constraints. The inclusion of more ponds would have increased the statistical power of my results and allowed for comparison among ponds serving different functions. Furthermore, nutrient limitation in ponds may be variable and depend on prevailing environmental conditions.

Therefore, future environmental change scenarios carry a substantial risk of significantly altering nutrient limitation and severely altering pond structure and function (Hogan *et al.* 2014).

### ***Implications of the findings for nutrient management***

Lentic ecosystems are nutrient-rich, particularly when they occur in anthropogenically impacted landscapes (Howarth *et al.* 2021; Peretyatko *et al.* 2007). Nonetheless, evaluating the limiting nutrient is vital for management intervention to prevent eutrophication and, subsequently, algal blooms in these numerous lentic systems. My findings provide insights into the limiting nutrients in periphyton biomass in ponds upon nutrient addition. The findings suggest that nitrogen is the limiting nutrient in periphyton biomass. Based on the results, measures must be taken to prevent or reduce nitrogen inputs into these freshwater ecosystems. For example, input from point sources such as fertiliser use on lawns, parks, and golf courses should be minimised to reduce nitrogen input in aquatic ecosystems (Hobbie *et al.* 2017; Neumiller 2021).

Managing nutrient input, especially nitrogen in aquatic ecosystems, will improve the water quality of freshwater ecosystems (Death *et al.* 2007; Abell *et al.* 2010). This intervention will also reduce the cost of restoration of degraded freshwater ecosystems in New Zealand.

## **6.6 Conclusion**

I examined periphyton's response to the addition of nutrients (nitrogen and phosphorus) in ponds. The addition of the different nutrients yielded different chlorophyll 'a' concentrations. Experimental set up with moderate nutrient concentrations exhibited a strong response to periphyton growth. Further, the moderate nitrogen treatment yielded the highest amount of chlorophyll 'a' biomass. Therefore, increasing nitrogen concentration in these ponds will increase periphyton biomass and decrease species diversity. My study provides novel information on nutrient limitation on periphyton growth in ponds in Auckland. These findings add to the minimal research on periphyton biomass in ponds in this highly urbanised region. Further research is required to understand the sources of nutrients in the ponds and the effect of grazing and temperature on periphyton biomass yield.

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# Chapter 7

## **Establishment of a pond: monitoring the succession and dynamics in macroinvertebrate diversity of the Matuku link ponds**



## 7.1 Abstract

Recently, constructed ponds have become common in all landscapes and part of ecological restoration programmes. These ponds are constructed because of the ecosystem services they provide, including biodiversity conservation. Many ongoing freshwater ecological restoration programmes in New Zealand aim to restore biodiversity to a more ecologically sustainable and 'natural' state. Nonetheless, there is limited literature on the success of these restoration programs. Additionally, there is scant information on the temporal dynamics in biodiversity community structure, making the assessment of the success of restoration difficult. Newly constructed ponds provide an ideal system for studying succession as they can help track the community structure of biota from a known baseline. In this chapter, I expound on a case study of a pond's establishment, using macroinvertebrates as indicator taxa for biotic community establishment. I assessed, monitored, and compared the water quality and macroinvertebrate community compositions and diversity of a newly constructed and established pond bi-monthly over a year. I found that the water quality of the ponds was significantly different ( $F_{1,5} = 3.04$ ,  $p < 0.01$ ) from each other. The pairwise comparison revealed that conductivity, salinity, and total dissolved solids levels differed between the ponds. The highest macroinvertebrate abundance was recorded in August for both ponds, and the communities were dominated by Crustacea (Cladocera). The formation of the macroinvertebrate community in the new pond was rapid, resulting in a 75% resemblance to the established pond at the end of the first year of its existence. This result indicates a quick succession rate in the new pond. Furthermore, the Shannon Weiner diversity index was not significantly different ( $t = 1.4$ ,  $p = 0.2$ ) between the ponds at the end of the study. However, the ponds' macroinvertebrate community compositions were significantly different ( $F_{1,5} = 2.22$ ,  $p = 0.02$ ). The difference was due to the temporal taxonomic composition differences between the ponds. The high abundance of the taxa *Diplacodes*, *Physa*, and Ostracod in the new pond accounted for the difference between the two ponds. Differences in physicochemical variables contributed to the difference in the macroinvertebrate communities. Finally, the new pond provided additional and more diverse habitats for the macroinvertebrates by the end of the first year of construction.

## 7.2 Introduction

Ecological restoration is one of the vital tools used to reduce the impact and mitigate the extinction of the endemic fauna and flora of New Zealand (Norton 2009). Restoration seeks to enhance both the biotic and abiotic components of the environment. It also remodels the community structure to a functioning, sustainable, natural state (Caruso 2006). The goals of restoration programs are set to embrace the species, population, community ecology, and the ecological processes in the ecosystem (Lake 2001). These processes are both biotic (which include productivity, predation, nutrient cycling, and succession) and abiotic (such as water cycling) (Burbage 2005).

There are currently many ongoing restoration programmes in different ecosystems in New Zealand, including coastal forests, mainland islands, and freshwater (Atkinson 1994). More importantly, the construction of ponds and the restoration of freshwater ecosystems to compensate for these systems' loss have become necessary in recent times (Coccia *et al.* 2016). Assessing the role and value of these restored or new ecosystems to determine if they complement existing systems or replace the natural systems is vital for conservation and management. However, to achieve the restoration goal, knowledge of the temporal dynamics in community structure is essential. Monitoring provides essential benchmarks for restoration programs and planning, supporting projects to make realistic milestones for measuring success (Burbage 2005). The rate of progress of any restoration project will be determined in part by the initial state of the ecosystem and by the process of ecological succession (Burbage 2005). Succession is the process of change in community structure (species abundance and composition) over a period (Olmo *et al.* 2016). The succession process in an ecosystem is critical for understanding ecological processes as it is a vital indicator of a functioning ecosystem (Miguel Chinchilla *et al.* 2014).

Artificial ponds are useful for studying the successional process in freshwater ecosystems because they allow studying the species composition from the onset of creating the habitat until the community stabilises (Olmo *et al.* 2016). For organisms to colonise and be established in a newly established ecosystem, they must disperse actively or passively to the site. They must also be able to successfully establish in the presence of grazers, parasites, and competitors. Finally,

the habitat must be suitable for survival and reproduction, especially after a natural disaster has removed existing ecosystems (Olson *et al.* 2016; Wood *et al.* 2003).

Nonetheless, in situations where there is a relic of biotas like egg or seed banks, colonisation occurs when conducive environmental conditions occur (Bilton *et al.* 2001). Species composition changes throughout this succession process until a stable community is attained (Ruhí *et al.* 2013). Therefore, information on the temporal dynamics is vital in improving the knowledge of succession and providing information to construct similar ecosystems (Miguel Chinchilla *et al.* 2014).

Macroinvertebrates are one of the first organisms to colonise aquatic habitats. International studies on succession have found that macroinvertebrates communities become fully established, reaching high densities within the first year of pond creation (Cañedo-Argüelles and Rieradevall 2011; Ruhí *et al.* 2013; Solimini *et al.* 2003). Highly (active) dispersal taxa are early colonisers followed by passive dispersers or non-flying taxa (Cañedo-Argüelles and Rieradevall 2011). Although habitat conditions later shape macroinvertebrate community structure and compositions. Macroinvertebrates are good models to study succession and biological restoration as they are primary producers and play a vital role in the energy exchange in the ecosystem. Macroinvertebrates are also valuable for monitoring programs because they are sensitive to changes in ecological processes (Coccia *et al.* 2016).

Newly created ponds increase in macroinvertebrates diversity over time due to the availability of many unoccupied niches until they reach their carrying capacity (Coccia *et al.* 2016). Most newly created ponds lack macrophytes. However, after colonisation, macrophytes create additional habitats for macroinvertebrates (Coccia *et al.* 2016). Nevertheless, the associations between macrophytes and macroinvertebrates take time to become established. A cluster of ponds in a given area or landscape ('pondscape') is vital for the survival of some aquatic and semi-aquatic organisms such as frogs and insects that complete different life cycle stages in different ponds (Cañedo-Argüelles and Rieradevall 2011; Thornhill *et al.* 2017; Wood *et al.* 2003). These ponds act as networks playing valuable ecological roles at the metapopulation and metacommunity levels. They serve as corridors and provide refugee habitats for endemic and or endangered organisms (Cañedo-Argüelles and Rieradevall 2011). A cluster of ponds is also vital due to fluctuations in individual ponds resulting from stochastic environmental events (Hill *et al.* 2016; Thornhill *et al.* 2017). In a highly connected 'pondscape', colonisers are usually a subset of the populations in the established ponds (Ruhí *et al.* 2013).

Studies on the colonisation of wetlands have been carried out in the United States of America (Batzer and Resh 1992; Layton and Voshell Jr 1991), Europe (Boix *et al.* 2004; Cañedo-Argüelles and Rieradevall 2011; Culioli *et al.* 2006; Solimini *et al.* 2003), Africa (Guiral *et al.* 1994) and Australia (Bayly 2001). However, there is little information on the processes of primary succession of aquatic macroinvertebrates in artificial ponds. This information is significantly lacking in New Zealand, where there is limited information on freshwater macroinvertebrates in general. Newly created ponds provide an ideal system for studying succession because they are easy to monitor (Ruhí *et al.* 2011). The new pond's construction in the Matuku Link reserve provided a unique opportunity to study primary succession in a natural system. It also helped document the physical and chemical changes that occur as ponds undergo different successional changes. However, this study was an opportunistic one, and hence it lacked replicates; therefore, results relate to this system. In this chapter, I describe the evolution of a pond from formation to full ecological function compared to an established pond within the landscape.

### 7.2.1 Aims and hypotheses

This chapter aimed to:

- i) monitor the water quality trends of both the established and the new pond,
- ii) monitor the trends in macroinvertebrates' community structure, compositions, and diversity of both the new and established ponds,
- iii) determine the successional stages of a pond within a year using macroinvertebrates as the focus taxa in the new pond,
- iv) determine whether the macroinvertebrates community in the new pond resembles the community in the established pond,
- v) determine any difference in the macroinvertebrate communities between the two ponds.

Based on the results from other international studies on 'pondscape', I hypothesise that macroinvertebrate colonisation of the new pond will be fast and mainly linked to the different taxa's dispersal abilities. Secondly, there will be no difference in the species diversity between ponds by the end of the year.

## 7.3 Methods and analyses

### 7.3.1 Study area - History of the Matuku Link Reserve

The Matuku Link reserve is located in Te Henga (longitude 174.492592, latitude -36.861654), West Auckland. Matuku Link reserve is a 37-hectare bush and wetland bordering the Te Henga wetland (largest wetland area in the Auckland Region) and managed by a Trust. Prior to purchasing by the Trust and converting into a reserve in 2016, the site was privately owned by a family from the 1970s. Now a wetland undergoing restoration, a portion of the property used to be a horse farm in the 1970s. There are several trails between wetlands in the reserve created by a bulldozer. Another part of the reserve was a dumpsite for tyres and car wrecks. The location of the reserve was used as a film set for many episodes of the "Hercules", and the "Xena, Warrior Princess" TV series and the award-winning classic movie "The Piano" had some scenes shot there (John Sumich, pers comm).

The reserve is connected to other ecological reserves such as the Te Henga wetland (168ha), Matuku Reserve (120ha), Forest Ridge, and the Ark in the park (2000ha) hence its significance. Two native species (cabbage tree; *Cordyline australis* and Putaputaweta; *Carpodetus serratus*) dominate the trees in the reserve. The reserve has a rich diversity of native New Zealand birds, including Tui (*Prosthemadera novaeseelandiae*), Pukeko (*Porphyrio melanotus*), Grey warbler (*Geryone igata*), silver eye (*Zosteropidae lateralis*), and sacred kingfisher (*Todiramphus sanctus*). Other wetland birds such as Black shag (*Phalacrocorax carbo*), mallard (*Anas platyhynchos*), Brown teal (*Anas chlorotis*), and the bittern (*Ixobrychus novaeseelandiae*) also occur there. Additionally, one of the two native bats (*Mystacina* spp.) is present in the reserve (Matuku Link 2018). The reserve has a stream passing through it and three ponds (two established and a new one). The new pond was dug in November 2018 to improve the reserve's biodiversity status.

In December 2018, a preliminary survey of the two ponds' water quality on the Matuku Link Reserve indicated that the established pond had better water quality. However, physicochemical water quality variables only give a snapshot of the pond's condition at the time of measurement. Therefore, it was essential to assess the macroinvertebrates communities to understand the ecological status of both ponds. This assessment was vital because water quality influences the types of macroinvertebrates in a pond. Besides, macroinvertebrates serve as food for many native birds found in the reserve.

### 7.3.1.1 Description of the ponds

#### Established pond

The established pond, also known as Dr John's Pond (longitude: 174.489, latitude: -36.865), was shaped like a speech bubble located about 1km away from the nearest road (Bethells Road). The pond was surrounded by predominantly native vegetation and was connected to a marshland in the east. The established pond was artificial, covered 147m<sup>2</sup> and was about 0.5m deep when full. A bridle path and a paved road with gravel for vehicle access to the site are located less than two meters adjacent to the pond. The riparian vegetation consisted of new planting made of plants including flax (*Phormium tenax*), cabbage tree, Kowhai (*Sophora tetraptera*), Kahikatea (*Dacrycarpus decrydiodes*), hebe (*Hebe* spp.) and Manuka (*Leptospermum scoparium*). In addition, there were also established plants around the riparian zone, which included manuka, Totara (*Podocarpus totara*), buttercup (*Ranunculus* spp.), rushes (*Juncus* spp.), Kahikatea, and Kohuhu (*Pittosporum tenuifolium*). This riparian vegetation formed a shade of approximately 60% over the pond surface, but this may differ at different times.

There was a riparian vegetation replanting programme around the pond in November 2018. A large amount of submerged macrophytes in the pond was dominated by parrot feather (*Myriophyllum aquaticum*), an introduced aquatic plant (Figure 7.1). This macrophyte covered the non-shaded portion (about 50% of the pond's total pond area). Periodically, the parrot feather was removed from the pond. The macrophyte was removed twice during this research sampling period (in April and May 2019). The pond elevation was 13m above sea level, and the underlying soil was allophanic (perch gley) (Manaaki Whenua 2018). Although there was another pond in the reserve, the established Dr John's Pond was chosen because of its proximity to the new pond to allow for succession study. The water volume in this pond reduced during the warmer seasons, resulting in partial inundation between December and April at the pond's shaded portion. There was no major work around the riparian of the pond during the period of sampling.



Figure 7.1 A picture of the established pond taken on 17 April 2019, showing the domination of the parrot feather macrophyte. The darker portions of the pond represent the area shaded by overhanging trees.

### **New pond**

The new pond, also artificial (longitude: 174.489, latitude: -36.864), was shaped like a paramecium. This pond was constructed to serve as additional freshwater habitat to enhance the conservation function of the reserve. The new pond had an area of 180m<sup>2</sup> and was dug to 2m deep, but due to rapid siltation, the average depth during the sampling period was 1.2m. The pond was located about 1.2km from the nearest public road. Further from the bank (two metres), the vegetation comprised of reed (*Thamnochortus* spp.) and sedges (*Carex* spp.). There were also a few deciduous trees like kanuka, cabbage tree, totara, Kauri (*Agathis australis*), Kahikatea, bulrushes, Nikau (*Rhopalostylis sapida*), fern, and five fingers (*Pseudopanax colensoi*). The riparian vegetation was dominated by buttercups (Figure 7.2). Due to a steep slope on one side of the pond, it was expected to receive runoff from the adjacent walking path. The pond led to a swamp at the west end.



Figure 7.2 A picture of the new pond taken on 17 April 2019 showing the riparian vegetation's domination by buttercups.

### 7.3.2 Methods: data collection

Before this study was started, three months was allowed for the newly constructed pond's sediments to settle. Then, physicochemical water quality variables were measured (see Chapter 2 for details of physicochemical variable sampling), and macroinvertebrates were sampled from the ponds. Details of macroinvertebrate sampling and identification are in Chapter 3. Sampling was done bi-monthly from February 2019 to December 2019 to determine the temporal dynamics in the macroinvertebrate communities. Chlorophyll 'a' was extracted from water samples, and absorbance was read and recorded. Details of the methods used for chlorophyll 'a' are in Chapter 6. Additionally, a 5-minute bird count was conducted at each pond during each visit.

### 7.3.2.1 Data preparation

The three physicochemical variables data on all the sampling sessions for each pond were put together on an individual Microsoft excel spreadsheet. The data of the variables were analysed descriptively for each pond. Another spreadsheet was created for both ponds combined and analysed using a multivariate statistical approach. Similarly, each taxa's abundance in each sampling session for each pond was also recorded on a separate spreadsheet. These data were used to calculate the biodiversity measures. The individual data for each pond were then combined on a separate spreadsheet and used for multivariate statistical analysis.

### 7.3.3 Data analyses

Results of biological and physicochemical parameters were compared between the two ponds. A student t-test was used to check for a significant difference between the average value of the individual physicochemical parameters measured in the two ponds. Temporal variations of the water quality of both ponds were visualised on an nMDS plot. A PERMANOVA test was used to determine the amount of variability in the ponds water quality (Clarke and Warwick 2001). Differences in macroinvertebrate composition in the ponds were explored using PERMANOVA based on a Bray Curtis similarity calculated on the transformed (fourth root) and standardised data (Bray and Curtis 1957). The diversity indices (Margalef's richness, Piloni's evenness, Shannon Weiner, Simpson's, and the average taxa distinctness) were calculated for both ponds. The average taxa distinctness is the mean path length through the taxonomic tree connecting every pair of species in the list on the condition that they are both different species. It is calculated using the formula ' $\Delta^+ = [\sum_{i < j} \omega_{ij}] / s(s-1)/2$ '; where  $s$  is the number of taxa and for double summation,  $i$  and  $j$  range over these  $s$  taxa, and  $\omega_{ij}$  is the distinctness weight of the path linking taxa  $i$  to taxa  $j$  in a hierarchical classification of the taxa (Clarke and Warwick 1998). The average taxa distinctness was used to determine the similarity in the taxa in both ponds at each sampling period. The analyses were done using PERMANOVA + (version 7.0.13) software extension (Clarke and Gorley 2006).

## 7.4 Results

Fishes were absent from the newly created pond during the first visit (November 2018) and the subsequent sampling month (February 2019). However, mosquito fishes (*Gambusia* spp.) were present during the second sampling period (April 2019). Similarly, macrophyte was absent when the pond was constructed but was present at the second sampling session and was restricted to a small portion (10%) of the pond surface area. The macrophyte consisted mainly of emergent; common rush (*Juncus effusus* L.), broadleaved cattail (*Typha* spp.), redshank (*Persicaria maculosa*), and a submergent; parrot feather (see Appendix F: Figure 1). A management activity was done on 25 April 2019 to remove the emergent macrophyte; parrot feather. This management intervention was repeated on 25 May 2019. During the October survey, there was minimal vegetation on the riparian due to pond margin maintenance (see Appendix F: Figure 1 and 2). The grey warbler (*Gerygone igata*), mynah (*Gracula* spp.), Pukeko (*Porphyrio melanotus*), and Keruru (*Hemiphaga novaeseelandiae*) were some of the birds around the ponds. Others were the welcome swallow (*Hirundo neoxena*), goldfinch (*Carduelis carduelis*), Tui (*Prothemadera novaeseelandiae*), blackbirds (*Turdus merula*), and fantail (*Rhipidura* spp.). The distance between the established pond and the new pond is about 50m.

### 7.4.1 Physicochemical water quality parameters

The new pond recorded higher levels of some physicochemical water quality variables measured (conductivity, salinity, and total dissolved solids). Additionally, there was more variability in the temporal trend of the parameters measured in the new pond (Table 7.1). Also, the nMDS plot (Figure 7.3) showed more variability in the temporal trend of the water quality measured in the new pond. October and December were most similar in water quality in the established pond. However, water quality in February and June were most different in both ponds.

In the established pond, conductivity, salinity, and TDS levels were positively correlated ( $\rho = 8$ ). Also, temperature was negatively correlated to % DO ( $\rho = -7$ ) but positively correlated with ammoniacal nitrogen ( $\rho = 7$ ). In the new pond, temperature was negatively correlated to conductivity ( $\rho = -7$ ), salinity ( $\rho = -7$ ), and TDS ( $\rho = -7$ ). In addition, phosphorus was negatively correlated to pH ( $\rho = -7$ ) and nitrate ( $\rho = -7$ ), while conductivity was positively correlated to TDS and salinity ( $\rho = -7$ ) (Appendix F: Figure 3).

Table 7.1 A summary of physicochemical water quality parameters of the new and established pond showing means  $\pm$  standard deviations (SD) and range of the values. The parameters have their units in brackets. Significant p-values (5%) from a paired t-test are in bold.

Physicochemical variables	New pond		Established pond		t	p value
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range		
pH	6.77 $\pm$ 0.65	5.53 - 7.25	6.79 $\pm$ 0.55	6.13 - 7.80	-0.12	0.9
% DO	34.71 $\pm$ 32.41	2.70 - 92.90	30.84 $\pm$ 22.04	13.86 - 73.43	0.56	0.6
Con. ( $\mu\text{scm}^{-1}$ )	240.4 $\pm$ 32.2	207.0 - 285.7	214.7 $\pm$ 25.3	181 - 252	3.93	<b>0.01</b>
TDS (ppm)	120.3 $\pm$ 16.0	104 - 142.7	107.3 $\pm$ 12.9	90.67 - 127	3.90	<b>0.01</b>
Sal. (psu)	0.11 $\pm$ 0.01	0.1 - 0.14	0.10 $\pm$ 0.01	0.09 - 0.12	3.16	<b>0.02</b>
Tem. ( $^{\circ}\text{C}$ )	17.05 $\pm$ 5.77	10.62 - 24.65	15.67 $\pm$ 5.03	9.86 - 22.65	1.34	0.2
NO <sub>3</sub> -N ( $\text{mg l}^{-1}$ )	0.29 $\pm$ 0.21	0.02 - 0.49	0.32 $\pm$ 0.24	0.01 - 0.51	-0.30	0.1
PO <sub>4</sub> <sup>3-</sup> ( $\text{mg l}^{-1}$ )	1.25 $\pm$ 0.98	0.24 - 2.36	1.98 $\pm$ 0.94	0.09 - 2.68	-1.72	1
NH <sub>3</sub> -H ( $\text{mg l}^{-1}$ )	0.03 $\pm$ 0.04	0.01 - 0.12	0.03 $\pm$ 0.03	0.01 - 0.10	< -0.02	0.4
Chlo 'a' $\times 10^{-3}$ mgm <sup>-2</sup>	9.7 $\pm$ 17.0	4.0 - 43.7	38.2 $\pm$ 90.7	0.5 - 223.5	-0.94	0.4

\*Chlo 'a' = Chlorophyll 'a'

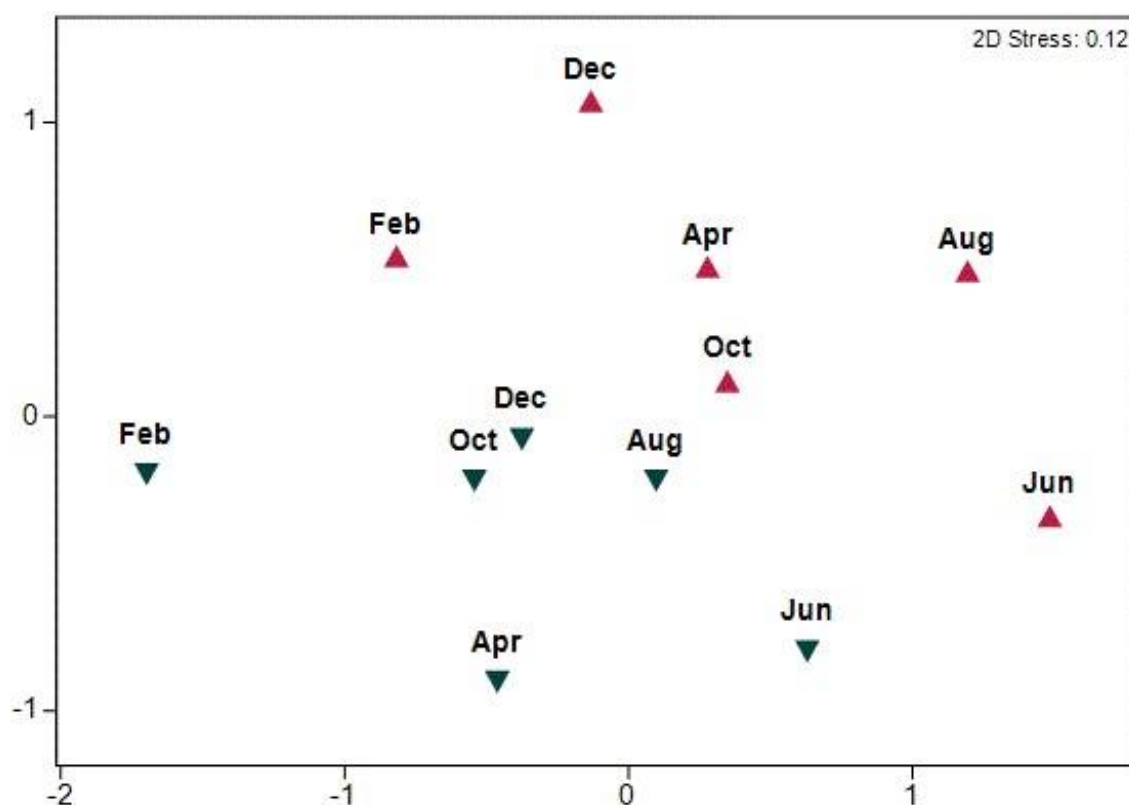


Figure 7.3 An nMDS plot of the established and new ponds' water quality based on Euclidian distance measure of transformed and normalised physicochemical water quality data. Red triangles indicate the water quality in the new pond, and the green triangle indicates water quality in the established pond. The physicochemical water parameters differed significantly between the ponds ( $F_{1,5} = 3.04$ ,  $p < 0.01$ ). The distance between triangles indicates the level of similarity of the macroinvertebrate communities. The closer the triangles, the more similar the communities and *vice versa*.

### 7.4.2 Temporal trends in physicochemical water quality parameters

The physicochemical water quality variables in the ponds varied temporally and between ponds. For example, conductivity was higher during the colder months than the warmer months for both ponds. Conductivity increased steadily from February to June, decreased from June to October, and then increased slightly in December in the established pond. The lowest conductivity levels were recorded in February (Figure 7.4b). The pH ranged from weak acidity to weak base (5.53 -7.8) for both ponds. The pH of the water varied between two units' points throughout the study period in both ponds. The highest pH was recorded in April and the lowest in December. In the new pond, pH ranged between 7.21 and 5.53 (Figure 7.4c). The % DO concentrations in both ponds increased marginally between February and April but increased by 300% between April and June (Figure 7.4d). The ammoniacal nitrogen concentrations in the established pond were marginally higher than in the new pond. The highest ammoniacal nitrogen concentration in the ponds was  $0.12\text{mg l}^{-1}$  and  $0.1\text{mg l}^{-1}$  for the new and established pond, respectively, for June (Figure 7.4e).

The Chlorophyll 'a' concentrations in the established pond were higher than in the new pond. Chlorophyll 'a' concentration in the new pond was highest in February ( $2 \times 10^{-2}\text{mgm}^{-2}$ ) and lowest in October ( $2 \times 10^{-5}\text{mgm}^{-2}$ ). The chlorophyll 'a' concentration in the established pond was highest in February ( $4.8 \times 10^{-2}\text{mgm}^{-2}$ ) and lowest ( $3 \times 10^{-5}\text{mgm}^{-2}$ ) in December (Figure 7.4f). In the established pond, chlorophyll 'a' concentration was strongly negatively correlated with conductivity ( $\rho = -0.6$ ) and nitrates ( $\rho = -0.6$ ) (Appendix F: Figure 3). In the new pond, chlorophyll 'a' was strongly negatively correlated with phosphate ( $\rho = -0.6$ ) but positively correlated ( $\rho = 0.5$ ) with temperature (Appendix F: Figure 3).

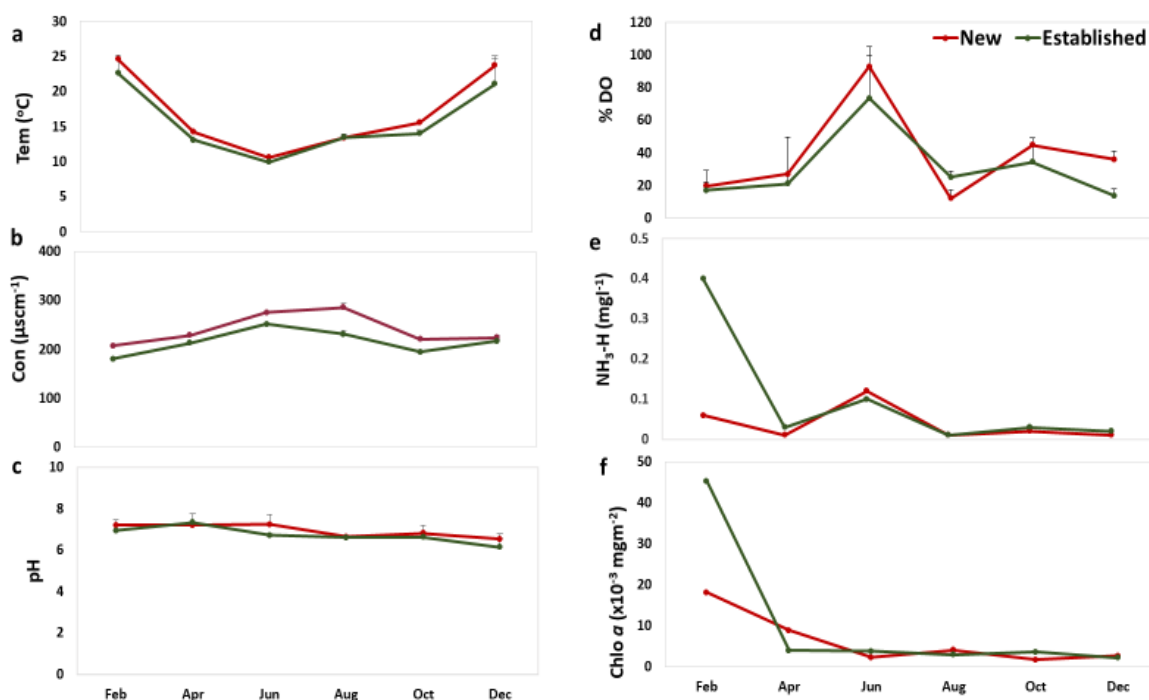


Figure 7.4 Temporal changes in physicochemical water quality variables (a: Temperature, b: conductivity, c: pH, d: % DO, e:  $\text{NH}_3\text{-H}$ , and f: Chlo 'a') in the ponds. The green line represents the established pond, and the red line represents the new pond. The plots 'a-d' show mean values of the variables  $\pm$  standard error.

### 7.4.3 Temporal variation in taxa abundance

A total of 7448 macroinvertebrates were sampled from the two ponds from February to December 2019. Forty-two taxa were recorded in the ponds, and 16 were consistently present in 50% of the samples. In addition, the taxa *Oligochaeta* and *Cura* were present in all the samples. Table 7.2 describes the occurrence of macroinvertebrates in the two ponds.

The minimum number of taxa occurred in June from both ponds (Figure 7.5a). The maximum number of taxa (23) per sampling period was recorded in August for the new pond. However, the maximum number of taxa (22) was recorded in October (Figure 7.5b) for the established pond. Thirteen taxa were recorded from both ponds during the first sampling period in February. Although the number of taxa increased marginally in the new pond during April and June, the established pond had 13 taxa recorded for April and June (Figure 7.5b).

Table 7.2 A comparison of the macroinvertebrate composition in the new and established ponds.

Descriptive measures	New pond	Established pond
Percentage of overall macroinvertebrates sampled	55%	45%
Average individuals per sample	688	554
The average number of taxa per sampling session	17	16
Total number of taxa present	36	35
Five common taxa	Cladocera, Ostracod, Orthocladinae, Cyclopoida and Hydrachinidae	Oligochaeta, <i>Cura</i> , Chironomus, Calanoida and Hydrachinidae
Rare and exclusive taxa	Stratiomyidae, Pelecorrhynchidae, and Stictocladus	Dolichopodid, Mesovilia, Shaeriidae and Paraleptamphopus
Percentage of exclusive taxa	14%	16%

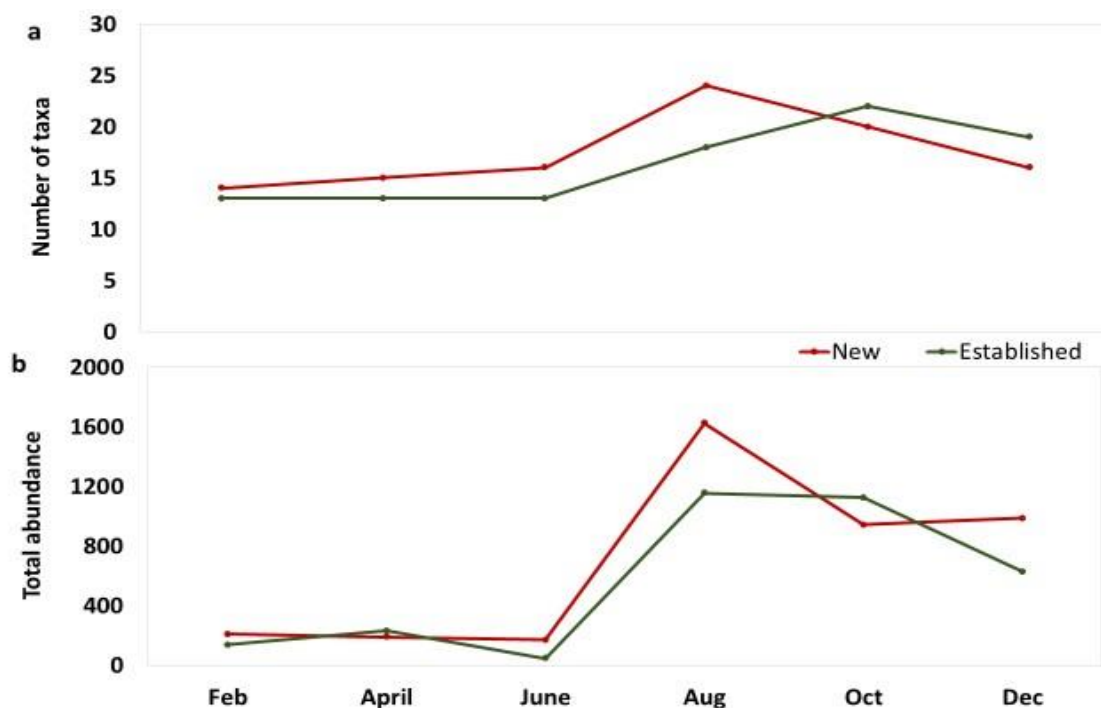


Figure 7.5 Temporal trend of macroinvertebrates abundances (a) and the number of taxa recorded from the ponds (b). The green line represents the values of the established pond, and the red line represents the values for the new pond.

#### 7.4.4 Temporal dynamics of macroinvertebrates in the ponds

The macroinvertebrate composition was most similar (Bray Curtis similarity: 75.2%) between the ponds in December. In June, the ponds were the least similar (Bray Curtis similarity: 31.9%) (Figure 7.7). There was a significant difference ( $F_{1,5} = 2.22$ ,  $p = 0.02$ ) between the macroinvertebrate community compositions in the two ponds. Additionally, there was more variability in the macroinvertebrate communities between sampling sessions in the established pond.

#### 7.4.5 Abundant taxa in the ponds

The five most abundant taxa were Chironomus, Orthocladinae, Hydrachinae, Oligochaeta, and Cladocera. Cladocera was the most abundant taxa in both ponds (Figure 7.6). Generally, Chironomus, Hydrachinae, and Oligochaeta were more abundant in the established pond (Figure 7.6). A SIMPER analysis showed that Cladocera, Oligochaeta, Ostracoda, *Cura*, *Physa*, *Xanthocnemis*, and *Diplacodes* cumulatively contributed to 73% of the total abundance of macroinvertebrates sampled from the new pond. Similarly, Oligochaeta, *Cura*, Cladocera, Hydrachinidae, *Sigara*, and Ostracoda cumulatively contributed to 72% of the total abundance of macroinvertebrates sampled from the established pond. Additionally, the taxa *Diplacodes* (Diss/SD 1.64), Ostracoda (Diss/SD 1.63), and *Physa* (Diss/SD 1.48), all of which were more abundant in the new pond, were the drivers of differences between the two ponds.

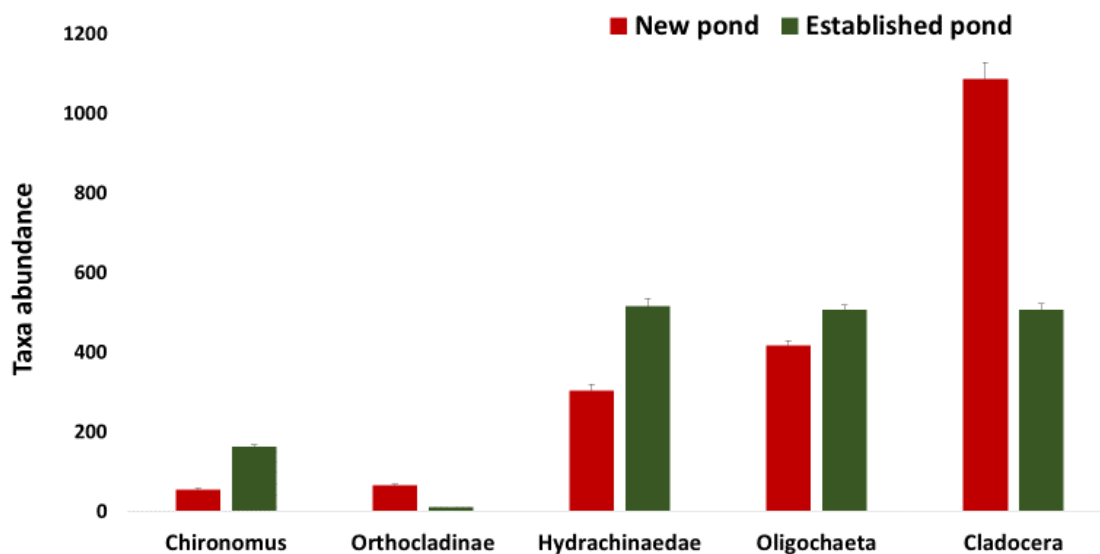


Figure 7.6 Abundance  $\pm$  SE of the five most common taxa in the new and established pond. Orange and blue bars represent abundance in the established and new pond, respectively.

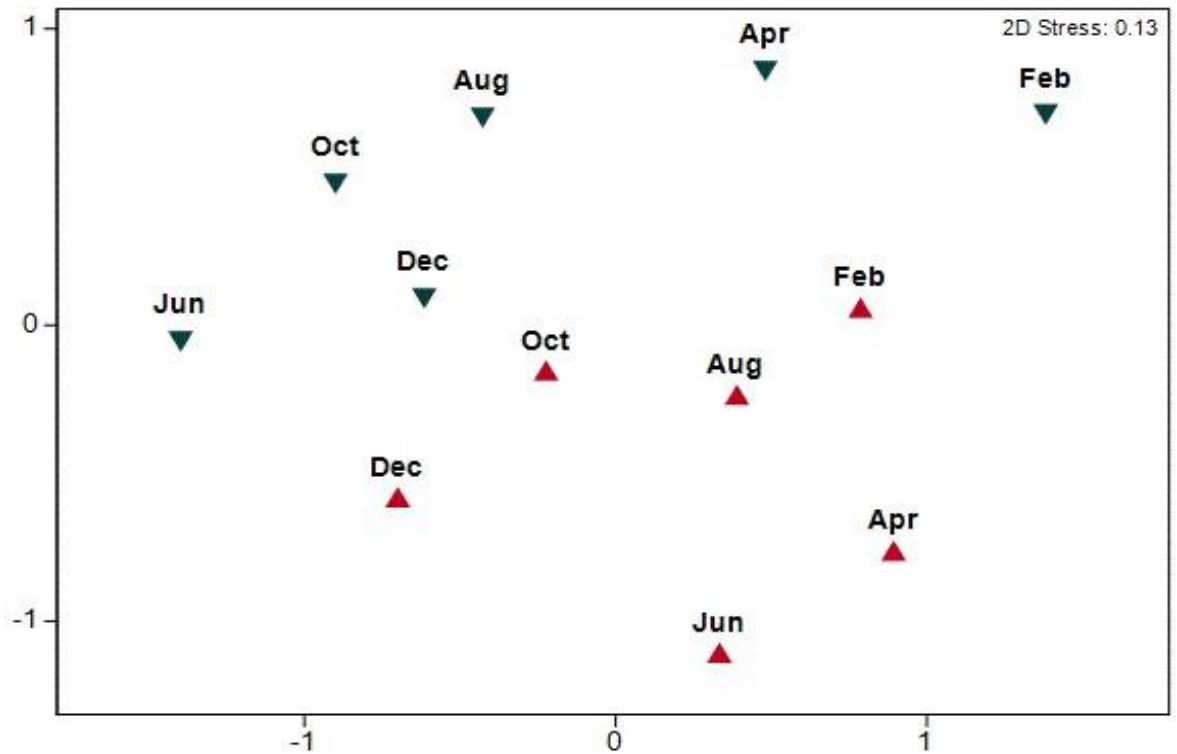


Figure 7.7 An nMDS plot of macroinvertebrates abundance (pond over time) based on Bray Curtis similarity measure a fourth root transformed and standardised by sample data. Red triangles indicate the water quality in the new pond, and the green triangle indicates water quality in the established pond. The distance between triangles indicates the level of similarity of the macroinvertebrate communities. The closer the triangles, the more similar the communities and *vice versa*.

#### 7.4.6 Macroinvertebrates community structure

The community structure in the ponds varied. For instance, non-insect taxa dominated the macroinvertebrate community in the established pond. The relative abundance of non-insect taxa was highest in April (96%) and lowest (54%) in December relative to the macroinvertebrate sampled (Figure 7.8a). In the new pond, non-insect taxa dominated the macroinvertebrate community structure for all months sampled except June. The highest abundance (1477) of macroinvertebrates occurred in August, of which 88% were non-insects. The lowest abundance (74; 40% of the total macroinvertebrates sampled) of non-insect taxa occurred in June. The high insect abundance was driven by the taxa Orthocladinae (Figure 7.8b).

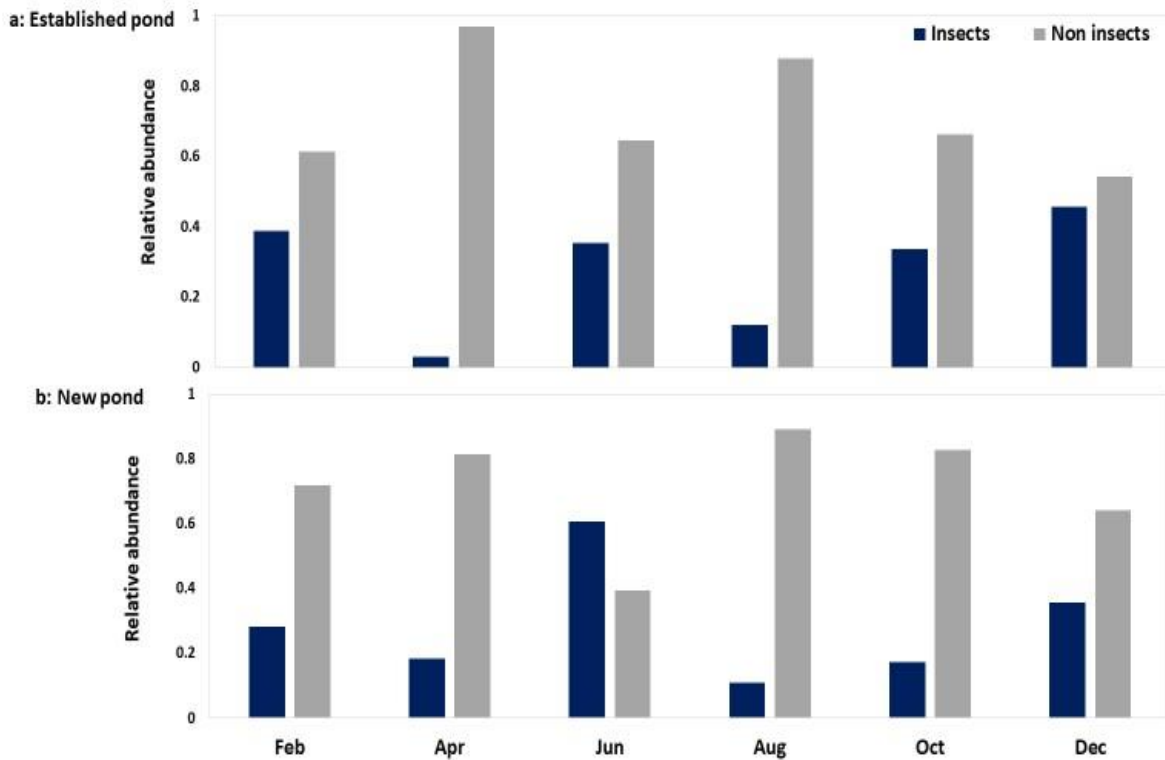


Figure 7.8 Relative abundance of macroinvertebrate community structure in (a) established pond and (b) new pond from February to December. Grey bars represent non-insect taxa, and blue bars represent insect taxa.

#### 7.4.7 Insect diversity in the ponds

Five insect orders: Coleoptera, Diptera, Hemiptera, Odonata, and Trichoptera, were recorded in both ponds showing varying abundances. Diptera dominated in February, April, and August in the established pond. Hemiptera dominated in June, October, and December. Insects sampled in April and October were less diverse in both ponds consisting of three taxa (Diptera, Hemiptera, and Odonata) (Figure 7.9a). In the new pond, Odonata dominated in February and April, Diptera dominated in June and August, and Hemiptera dominated in October and December (Figure 7.9b). Coleoptera was rare (present in 16.6% of the samples) in the new pond.

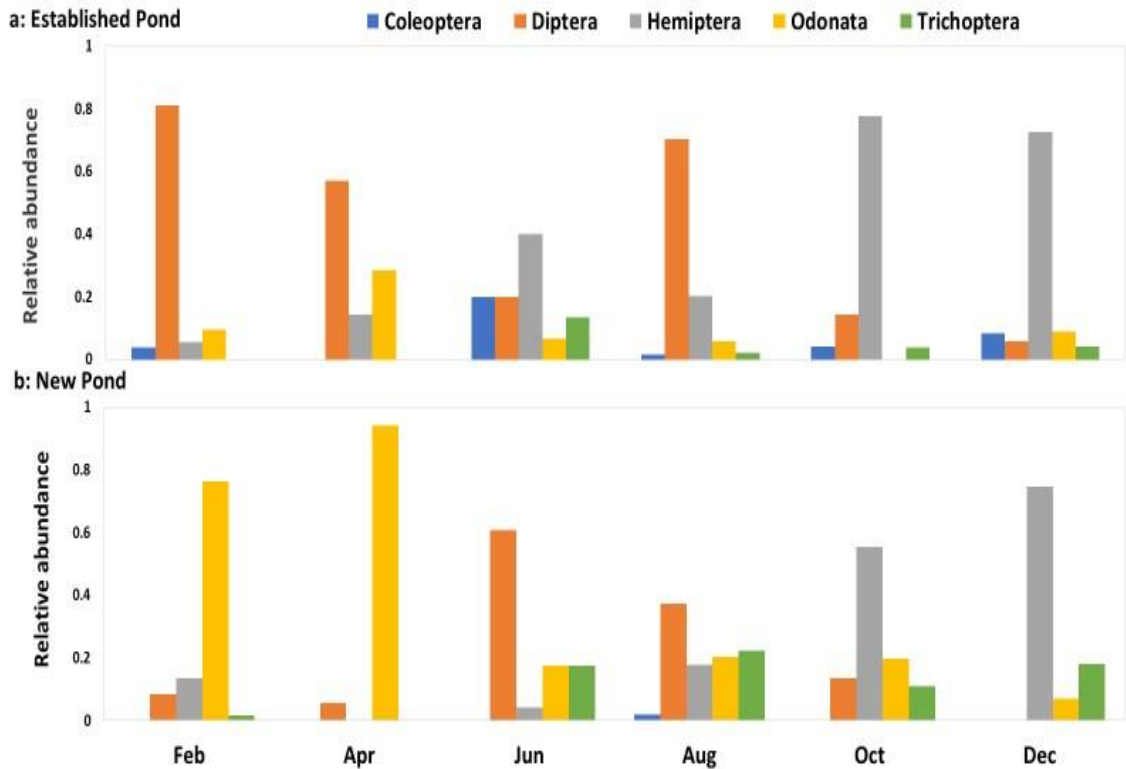


Figure 7.9 Temporal trend of the relative abundance of five insect orders from the (a) established pond and (b) new pond. Keys: blue (Coleoptera), orange (Diptera), grey (Hemiptera), yellow (Odonata), and green (Trichoptera).

#### 7.4.8 The relative abundance of non-insect taxa

The five most abundant non-insect taxa recorded were Oligochaete, Ostracod, Cladocera, *Cura*, and Hydrachinidae. Oligochaete and *Cura* were the most common (present in every sample) taxa in both ponds; however, the relative abundance of Oligochaete was higher than *Cura* in both ponds. Additionally, Hydrachinidae was rare (present in 33.3% of the sample) in the new pond but common in the established pond (present in 66.3%) (Figure 7.10).

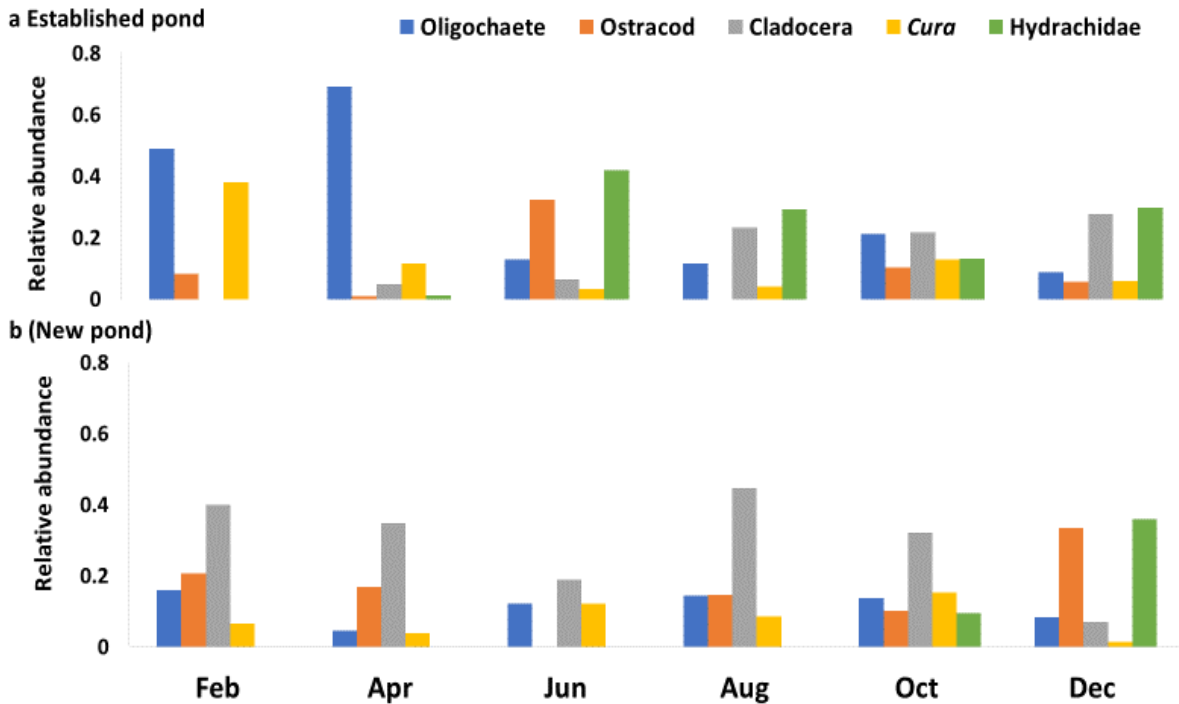


Figure 7.10 Temporal trend of the relative abundance of non-insect taxa from the (a) established and (b) new pond. Key: blue (Oligochaeta), orange (Ostracod), grey (Cladocera), yellow (*Cura*), and green (Hydrachinidae).

#### 7.4.9 Biodiversity measures

The biodiversity measures in the ponds showed variations between the ponds and varied temporally (Figure 7.11). For example, the average Shannon Weiner diversity indices were  $1.95 \pm 0.45$  and  $2.1 \pm 0.14$  for the established and new pond, respectively. The average Pielou's evenness was  $0.70 \pm 0.13$  and  $0.77 \pm 0.08$  for the established and new pond, respectively. Also, the average Margalef's richness for the established and new pond were  $2.66 \pm 0.30$  and  $2.62 \pm 0.36$ , respectively. There was no significant difference in Shannon Weiner diversity ( $t = 1.4$ ,  $p = 0.2$ ), macroinvertebrate evenness ( $t = 1.2$ ,  $p = 0.2$ ), and Margalef's richness ( $t = 0.02$ ,  $p = 0.9$ ) between the ponds.

Finally, macroinvertebrates sampled in April were the least taxonomically related, recording the highest  $\Delta^+$ . The lowest average taxonomic distinctness ( $\Delta^+$ ) for both ponds occurred in August. The macroinvertebrate communities in August recorded the lowest average taxonomic distinctness (Figure 7.11e).

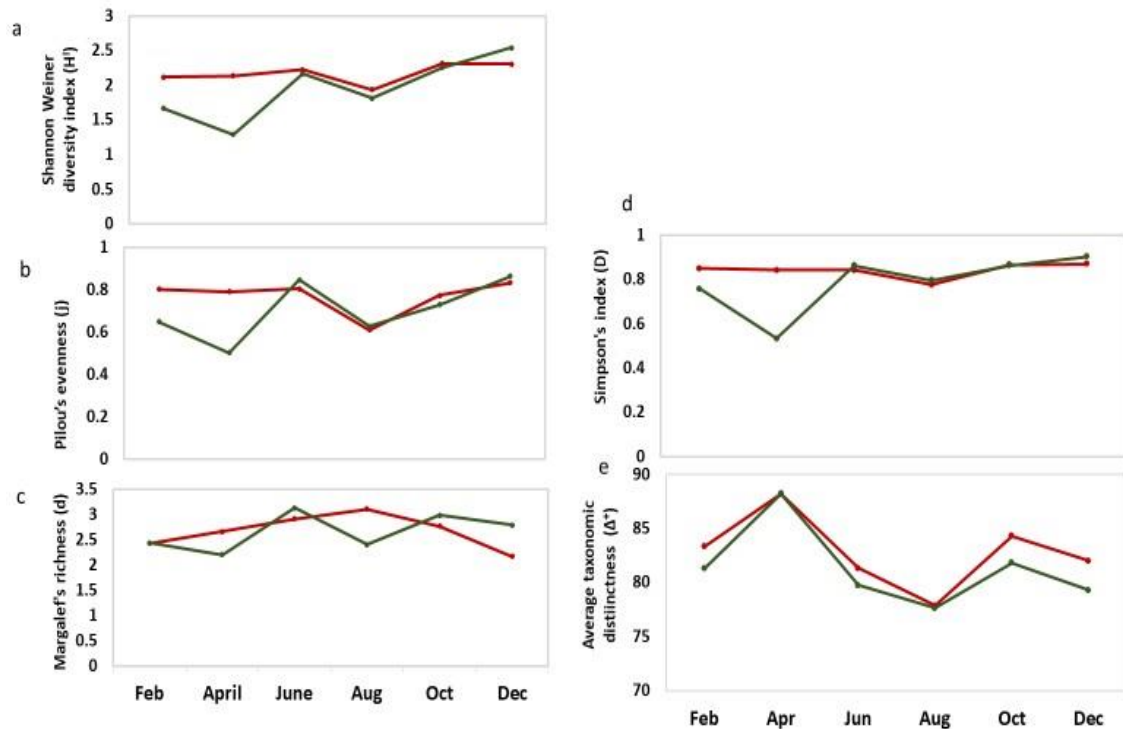


Figure 7.11 Temporal trend of biodiversity indices; a. Shannon Weiner, b. Pilou's evenness, c. Margalef's richness, d. Simpson's, and e, Average taxonomic distinctness between the established and new pond. The green and red lines are for the established and new pond, respectively.

## 7.5 Discussion

### 7.5.1 Water quality trends

This study is one of the few in New Zealand to assess the rapid changes in a newly established pond using macroinvertebrate community measures and water quality variables. I monitored and compared the water quality and macroinvertebrate communities of a newly created pond and an adjacent established pond. I found that the physicochemical water quality parameters of the ponds were different. These differences between the ponds could be driven by conductivity, salinity, and TDS variations between the ponds. Also, the marginal differences in physical environmental parameters such as the percentage of trees that overhang the established pond, macrophyte cover, and depth could have contributed to this difference. Differences in water quality due to physical parameters have been reported by Angélibert *et al.* (2004) in a similar study. Macrophyte cover has been shown to have substantial water quality interactions in New

Zealand lakes (Ministry for the Environment 2006). Although the established pond was slightly shallower and expected to be warmer, the trees' shade appeared to have a greater effect on the water temperature than the small depth difference. The effect of shading likely contributed to the temperature difference in the ponds. The physicochemical water quality variables are similar to those in oxidation ponds (Council 1995), a coastal lake (Drake *et al.* 2011), and ponds in a native forest in the Kapiti region (Leisnham *et al.* 2005). Further, the trend in physicochemical parameters observed could be driven by seasonality (Angélibert *et al.* 2004; Xie *et al.* 2016).

### 7.5.2 Macroinvertebrate trends and dynamics in the ponds

The development of macroinvertebrate structure and composition in the new pond was rapid, resulting in a community that was over 75% similar to the established pond within one year. Similar results have been reported by (Olmo *et al.* 2016), where zooplankton community composition in a restored pond was similar to the nearby existing pond by the end of the first year of restoration. Rapid colonization of newly created ponds by macroinvertebrates has been reported by Scher and Thiery (2005). Although 42 macroinvertebrates taxa were recorded, few taxa dominated by abundance in both ponds. This trend suggests that less abundant (rare) taxa either use the ponds as a refuge habitat or are yet to establish thriving populations in the ponds (Botwe 2017). It might also be due to the life-history traits of the taxa, such as low mortality or high reproduction or rate. The low abundance of insects observed in the ponds may be because of their semi-aquatic life cycle and seasonality in colonising habitats (Lahr *et al.* 1999; Olmo *et al.* 2016; Suren and Lambert 2010).

In literature, different taxonomic groups, especially zooplankton, show variation in their peak abundance (Lahr *et al.* 1999), and this was observed in this study. Cladocera populations primarily drove the highest abundance of macroinvertebrates recorded in August for both ponds. Additionally, this high abundance of macroinvertebrates was recorded in August (the peak of the winter season in New Zealand). Winter is associated with higher water volume (due to high precipitation), supporting more diverse macroinvertebrates (Boix *et al.* 2000).

Measures of taxonomic distinctness reflect changes in community structure. The inconsistency in the taxonomic distinctness between the ponds over the study period can be attributed to variations in water quality, impacting intra-pond habitat quality (Jeffries 2005). This irregularity in taxonomic distinctness may also indicate seasonality in the macroinvertebrate compositions and community structures (Cañedo-Argüelles and Rieradevall 2011). The highest taxonomic

distinctness was recorded in April (Austral Autumn), which was neither the coldest nor warmest period. The taxa abundant during this period were the non-insect taxa (typically non-flying taxa).

The order Odonata are active dispersers and do not require vectors to carry them around (Coccia *et al.* 2016). This dispersing ability may have contributed to their high abundance in the new pond during the first two (February and April) sampling periods. Additionally, Odonata prefers larger ponds, and their larvae require relatively deeper ponds to develop (Kadoya *et al.* 2004; Oertli *et al.* 2002; Scher and Thiery 2005). Again, the high abundance of Odonata recorded in the new pond may also be why low Diptera abundance occurred during the same period. This relationship is because Odonata are predators of mosquito (Diptera) larvae (Leisnham *et al.* 2005; Lester and Pike 2003). The contrasting relative abundance of Diptera and Odonata in the ponds suggests that the new pond is more suitable for the Odonata than the established pond. It may also be due to high prey (Diptera) abundance or the high dispersal rate of adult Odonata from the ponds after emerging from larvae. This occurrence leads to increased prey since predators' limit prey numbers, explaining the high abundance of Odonata in the new pond. The high abundance of Chironomidae in the established pond may indicate instability of the habitat conditions in the pond (Boix *et al.* 2000). However, it is possible that the ponds may have stabilized because macroinvertebrates in New Zealand are considered generalist with the ability to withstand pressures in the habitat (Batzer *et al.* 2004; Galatowitsch and McIntosh 2016; Greig *et al.* 2013; Wissinger *et al.* 2009).

The low Crustacea (Cladocera, Ostracod) numbers could have accounted for the high chlorophyll 'a' concentration recorded in the established pond in February. These Crustacea feed and reduce the densities of phytoplankton in aquatic systems. Therefore, a decrease in the abundance of the Crustacea can be linked to the high phytoplankton biomass (Batzer and Boix 1999). The high chlorophyll 'a' concentration can also be due to the high temperatures recorded. The significant difference in the macroinvertebrate community compositions could be due to several factors, including taxa specific response to temporal changes in the environment. Also, habitat preferences and physicochemical conditions can lead to variations (Coccia *et al.* 2016; Ruhí *et al.* 2011). Different physiological needs by macroinvertebrates may also explain the difference in community composition (Jeffries 2005). Additionally, the differences in the age, type, and amount of macrophytes may contribute to the differences in macroinvertebrate compositions (Jeffries 2005; Oertli *et al.* 2002; Ruhí *et al.* 2011). Some studies show a positive relationship between faunal richness and pond age (Miguel Chinchilla *et al.* 2014; Olmo *et al.*

2016). However, the result of this study did not confirm that because both ponds, on average, had similar taxa richness comparable to findings by Sun *et al.* (2019).

### 7.5.3 Macroinvertebrate colonisation and succession in the new pond

The primary succession of the pond began with non-insect taxa such as Branchiopod, Clitellata, Gastropod and Ostracod. Insects were next and dominated the community in the 9<sup>th</sup> month of the ponds establishment (August) and then non-insects again in the proceeding months. This succession trend is similar to other reported studies where Crustaceans dominate in the early succession stages of ponds and wetlands (Coccia *et al.* 2016; Lahr *et al.* 1999). The colonization of the new pond was fast, as observed by the high diversity indices (evenness and richness) recorded for the first sampling period, which was the third month of the pond construction. This fast colonisation rate could be due to the presence of possible sources of colonisers and the absence of geographical barriers between the new and the established pond (Cañedo-Argüelles and Rieradevall 2011). Similar rapid colonisation has been reported in both experimental (Batzer and Boix 1999; Layton and Voshell Jr 1991) and non-experimental studies globally (Cañedo-Argüelles and Rieradevall 2011; Kim *et al.* 2014; Ruhí *et al.* 2011; Scher and Thiery 2005; Taylor and Duggan 2012).

Additionally, the high average taxonomic distinctness and macroinvertebrate richness recorded during the first sampling period (February) indicates a fast colonisation rate compared to the study by Ruhí *et al.* (2011) in Sweden. Initial colonisation sequence has been related to the dispersal abilities of the taxa Cladocera (Crustacea), Ostracod (Crustacea), and Odonata (Bilton *et al.* 2001; Cañedo-Argüelles and Rieradevall 2011; Olmo *et al.* 2016). There was a possibility of a Crustacean egg bank in the soil that probably hatched when the pond was constructed, contributing to the observed result. Reports suggest that Crustacean eggs survive prolonged periods of up to 20 years in dry conditions (Bilton *et al.* 2001; Lahr *et al.* 1999; Wissinger *et al.* 2009).

The relative high biodiversity indices recorded for the new pond during the first sampling period could be due to the availability of niches available for colonisation after the ponds' construction. Studies show that new lentic ecosystems located close to established ones without geographic barriers have high macroinvertebrate composition within the first year of creation (Cañedo-Argüelles and Rieradevall 2011). Thus, the proximity of the ecosystems facilitates the transfer of taxa. Additionally, in the first sample, the relatively high abundance of a passive disperser, *Physa*

(Gastropod), could be due to the eggs transfer by other vectors such as waterfowl or other individual dispersers (Cañedo-Argüelles and Rieradevall 2011; Taylor and Duggan 2012).

The new pond was constructed during late spring (November 2018), which coincided with increasing temperature and the beginning of high evaporation leading to lower water levels in the nearby established pond. Consequently, active fliers such as Odonata and obligate aquatic insects (Coleoptera and Hemiptera) might have found refuge in the new pond as the water level in the established pond reduced (Lahr *et al.* 1999; Olmo *et al.* 2016). Literature has touted that successful colonisation and consequent establishment depend on the colonisers' dispersal ability. However, the new habitat must be suitable for survival (Flory and Milner 2000; Olson *et al.* 2016).

Furthermore, the growth of macrophytes (emergent) by the second sampling period (April 2019) may have provided additional niches hence the increase in Shannon Weiner diversity and richness observed (Flory and Milner 2000; Olmo *et al.* 2016). However, the fluctuating trend in the Shannon Weiner diversity and Simpson's indices recorded in the new pond over the period suggest that the new pond may still have some unoccupied niches (Coccia *et al.* 2016). These fluctuations may also be due to environmental changes, including seasonal effects as observed in New Zealand lakes and ponds (in this chapter and Chapter 3) (Burns and Mitchell 1980; Christman and Voshell Jr 1993; Coccia *et al.* 2016; Ruhí *et al.* 2011).

### ***Implications of the findings***

As population decreases, coupled with the infilling of the freshwater ecosystem, the creation of ponds to provide various services has become a necessity (Oertli 2018). My findings suggest that a newly created pond can become a functioning ecosystem within a year, supporting a high diversity of macroinvertebrates. Therefore, managers of urban landscapes should include the creation of ponds in urban designs to enhance freshwater ecosystem conservation. Furthermore, studies show that diversity is enhanced when the ponds form a network with other ponds by enhancing the migration of biodiversity (Oertli 2018, Liao *et al.* 2021, Minot *et al.* 2021). Therefore, ponds should be sited such that they create a network.

## 7.6 Conclusion

In this chapter, I monitored the water quality and macroinvertebrate community succession in a newly created pond. I compared the water quality and macroinvertebrate communities between the new and the established pond. There was a difference in the water quality between the two ponds. The physicochemical water quality variables were unstable throughout the monitoring period, resulting in temporal variation in the ponds' water quality. Further, the new pond differed in environmental conditions by having less macrophyte cover and was deeper than the established pond. Similar to the water quality, the macroinvertebrate community compositions differed, but the biodiversity measures were similar. Non-insect taxa (Branchiopod, Clitella, Gastropod, and Ostracod) were the first to occur in high abundance in the new pond. The succession sequence observed in this study is similar to that reported in other studies and is determined by the taxa's dispersal ability. Oligochaeta and *Cura* were the common taxa observed in all the samples. The five most abundant taxa in the ponds were Chironomus, Orthocladinae, Hydrachinae, Oligochaeta, and Cladocera. The established pond had four exclusive taxa compared to three in the new pond. Changes in the community compositions and macroinvertebrate structure over the study period could be linked to environmental changes, including seasonal effects. The macroinvertebrate community compositions between the two ponds were 75% similar. The abundance of Ostracoda, *Physa*, and *Diplacodes* contributed to the differences in community between the ponds (see Appendix F: Table 1). Finally, the new pond i) provided additional and more diverse habitats for the macroinvertebrates and ii) contributed to the overall increase in biodiversity in the Matuku Link. My findings affirm that a cluster of ponds helps promote regional diversity.

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# Chapter 8

## Conclusions

Freshwater ecosystems play a pivotal role economically, ecologically, and culturally in New Zealand (Quinn 2009; Weeks *et al.* 2016). However, many of these ecosystems have been lost, and many of the remaining ones have degraded water quality and biodiversity due to anthropogenic activities (Ausseil *et al.* 2011; Elston *et al.* 2015; Joy 2014; Robertson 2016; Stewart-Harawira 2020; Weeks *et al.* 2016). In the midst of this, ponds, a critical component of the freshwater ecosystem, have become ubiquitous in most landscapes (Biggs *et al.* 2017). Ponds are small, highly diversified lentic systems that collectively play a critical role in the ecosystem (Garrett-Walker *et al.* 2020). Ponds serve as water purification systems and are highly species diverse, supporting rarer, endangered, and more aquatic macroinvertebrates than other freshwater systems (Hassall 2014; Williams *et al.* 2008). These lentic systems have been overlooked in many parts of the world, particularly in New Zealand, due to their small sizes and the perception of not being ecologically important (Craggs *et al.* 2012; Garrett-Walker *et al.* 2020; Hassall 2014). Research on ponds in New Zealand has focused on specific taxonomic groups (Blakely *et al.* 2005; Duggan *et al.* 2006; Galbraith and Burns 2010; Leisnham *et al.* 2005). Other studies have been concentrated in the South Island, with barely any study in the North Island. Additionally, there is little literature on the holistic research of pond ecosystems. This lack of research has resulted in a limited understanding of New Zealand pond ecosystems, which does not reflect their ecological significance.

Literature has mostly focused on the biodiversity of ponds in different landscapes (Hill *et al.* 2016; Meland *et al.* 2020). Further, studies on ponds have failed to account for the effect of anthropogenic activities such as land use/ land cover 'LULC' and human population on the pond ecosystem, especially in an urbanised landscape. This failure is a problem, especially now that ponds have become part of landscape planning in many countries, including New Zealand (Blicharska *et al.* 2016; Bowmar *et al.* 2016). Also, whatever happens in the pond ecosystem trickle into the other aquatic ecosystems (Maxted *et al.* 2005). It is, therefore, vital to assess the impact of anthropogenic activities on the abiotic and biotic factors of ponds. The results will inform the planning and management of these small but numerous ecosystems.

In my thesis, I studied some aspects of the pond ecosystems in an urbanised region. I assessed the abiotic (water quality and physical parameters) and biotic (macroinvertebrates and phytoplankton communities) components of pond ecosystems. I evaluated how these factors varied temporally and spatially, along with different levels of anthropogenic impacts. My research shows that ponds are highly variable ecosystems rich in biodiversity despite having poorer water quality than rivers and lakes. Also, the relationship between the ponds and the environment, seasons and anthropogenic activities are complex. In this chapter, I discuss my key findings, their significance, limitations, and future directions.

## 8.1 A holistic assessment of the pond ecosystem

Ponds support a network of organisms, including primary producers (phytoplankton, periphyton, and macrophyte), primary consumers (macroinvertebrates), and secondary consumers (fishes, amphibians). These organisms interact with the abiotic factors and are involved in energy and nutrient transfer (Stark and Maxted 2007). I assessed pond ecosystems holistically by determining various aspects of the biotic and abiotic communities and their relationships (Chapter 3, 4, 6 and 8) because water quality measures only give a snapshot of the current condition of ponds (Stark and Maxted 2007). However, the biological variables provide information about previous conditions and predict the nutrient state and the ecological status of ecosystems (Lau *et al.* 2015; Menetrey *et al.* 2005). Using both biotic and abiotic variables also help understand how the water quality measures affect the biological community (Stark

and Maxted 2007). The findings serve as a baseline to assess the complex relationship between the macroinvertebrates and phytoplankton.

### 8.1.1 The influence of abiotic factors on the pond ecosystems

In my thesis, I highlight the water quality in ponds (Chapter 2, 3, and 7). The quality of water in the ponds varied among ponds and between seasons. Seasonal differences in water quality have been reported to be mainly influenced by precipitation in many studies globally (Buck *et al.* 2004; Chowdhury and Husain 2020; Pratt and Chang 2012). Water quality variables (pH, conductivity, temperature, % DO, TDS, salinity, nitrate, phosphate) were higher in summer than in winter, indicating a poorer water quality in summer (Chapter 2, Table 2.1). The higher values in summer were due to higher evaporation and reduced dilution from precipitation (Chowdhury and Husain 2020; Ray *et al.* 2020). The conductivity, salinity, TDS were not only the important variables of the water quality but were also highly positively correlated with each other. An essential determinant of water quality and biodiversity is nutrient level. However, the nitrates concentrations in the ponds did not vary even when more ponds (50) were sampled. This result is likely due to the stochastic nature of ponds. On the other hand, phosphates and ammoniacal nitrogen concentrations (Chapter 2 and 3) differed seasonally irrespective of sample size. Consequently, an *in-situ* experiment showed that nitrates were the limiting nutrients in algal biomass in ponds (Chapter 6). This result is similar to a study by Death *et al.* (2007) in streams and by Abell *et al.* (2010) in lakes in New Zealand as well as Rodusky *et al.* (2001) in lakes in the USA. I found that the moderate nitrates concentration of  $0.15\text{mg l}^{-1}$  in the ponds yielded the highest periphyton biomass (Chapter 6). About 26% of the ponds had nitrate concentrations above this limit, and 58% of the ponds had concentrations below this limit. Though ponds generally have higher nutrient loads (i.e., poorer water quality) than other freshwater ecosystems in New Zealand (Galbraith and Burns 2007), the findings imply that most ponds may not experience algal blooms. This advantage may be due to the ability of ponds to regulate their nutrient concentrations quickly as a result of their relatively small volume.

Other abiotic factors like slope, rainfall, altitude and different LULC types influenced water quality in the ponds. However, their degree of influence was seasonally dependent (Chapter 2). For instance, grass cover was negatively correlated with % DO, temperature, and TDS in summer (Chapter 2, Table 2.4). The type of LULC, topographic features, and anthropogenic activities, especially those in the catchment areas of ponds, significantly impact the water quality of ponds (Galbraith and Burns 2007; Novikmec *et al.* 2016; Asare *et al.* 2018). The seasonal variability of

these abiotic factors' influence on water quality also occurs in lotic systems (Pratt and Chang 2012). My results highlight the importance of seasons and landscape features when quantifying land-water interactions. Therefore, as catchments become increasingly urbanised, management measures should be instituted to minimise overland stormwater runoff that carries pollutants into the ponds. My study could not account for the effect of other factors such as pond age, underlining soil, and type of macrophyte on water quality.

### 8.1.2 The biotic communities of the pond ecosystems

In contrast to rivers, streams and lakes, knowledge of ponds is still poorly developed in New Zealand. Therefore, to date, there is no biotic index to assess the ecological status of ponds. However, to develop a biotic index, an assessment of the biodiversity patterns, community structure, and pond ecosystems' function is needed (Oertli *et al.* 2005). In this thesis, I used multi-taxonomic groups and different biota (macroinvertebrates and phytoplankton) in ecosystem assessment. This approach is widely accepted because it incorporates several ecological dimensions, including energy flow, and enables comparison with other sites (Menetrey *et al.* 2005). Nationally, there is limited knowledge of freshwater macroinvertebrates, especially in ponds, which are macroinvertebrates' hotspots' (Céréghino *et al.* 2007; Weeks *et al.* 2016). Furthermore, studies on the phytoplankton community in ponds are either several decades old or were only carried out in the South Island (Cassie 1969; Cassie 1974; Cassie and Freeman 1980; Duthie and Stout 1986; Flint 1938; Flint 1966; Galbraith and Burns 2010).

Overall, I found 74 taxa of macroinvertebrates in the ponds, with each pond having between 15 - 47 taxa (Chapter 3 and 7) higher than what has been recorded in rivers in Auckland (Moore and Neale 2008; Tablada 2018) and floodplain ponds in Waikato (Garrett-Walker *et al.* 2020). The high macroinvertebrate richness affirms that ponds have higher taxa richness at the regional level than other freshwater ecosystems, as reported in the UK (Hassall and Anderson 2015; Hill *et al.* 2016). Crustaceans were the most abundant group in the ponds and seemed to be colonisers of ponds (Chapter 3 and 7). The class Insecta was the most diverse group in the ponds (Chapter 3 and 7). Hydrachinidae, Cladocera, Oligochaeta, Chironomidae, Notonectidae, DugesIIDae, Ostracod, Copepods and *Sigara* spp. were the most widespread and abundant macroinvertebrates (Chapter 3 and 7). The macroinvertebrate communities in the ponds were highly variable such that ponds that were about 50m apart had different communities (Chapter 7). The variability in the taxonomic composition of the ponds was expected. This variability can be attributed to the habitat heterogeneity of the ponds and the influence of varying landscape features on the ponds (Hill *et al.* 2016).

Further, I found 126 genera of phytoplankton in the ponds (Chapter 5). Like the macroinvertebrates, the phytoplankton composition in the ponds was highly variable. The phylum Chlorophyta was the most dominant group, contrary to other lotic systems in New Zealand, where the phylum Ochrophyta are most abundant (Biggs 2000). The results iterate the difference between the lotic systems and the lentic systems.

The high biodiversity indicates that ponds are highly biodiverse and contribute to overall freshwater biodiversity in New Zealand. Again, ponds can have rich biodiversity even three months after construction (Chapter 7). The fast rate of biodiversity colonization indicates that the construction of ponds and proper management can minimise the current trend in freshwater biodiversity loss in New Zealand. Further, the significant variation in the biodiversity among the individual ponds indicates that the conservation of each pond will enhance the overall conservation of freshwater biodiversity.

There were seasonal differences in the macroinvertebrates and phytoplankton community compositions (Chapter 3 and 5). Phytoplankton were more abundant in summer than winter, and there was a shift in the dominant genera between the seasons. The shift indicates that the phytoplankton community is responsive to seasonal changes (Grabowska & Wołowski, 2014; Yusuf, 2020; Yan *et al.* 2020). Further, the community compositions in the ponds were more similar in summer, indicating that the communities are more stable (Chapters 3 and 5). Biotic communities vary temporally, mostly as a response to seasonal changes in water quality, which affects the taxa's physiological response, leading to a change in their composition (Briers 2014; Hassall *et al.* 2012; Hill *et al.* 2016).

The dominance of pollution tolerant macroinvertebrate taxa in the ponds indicates that the ponds are polluted relative to rivers in New Zealand. In contrast, the phytoplankton community composition suggests that the ponds have a healthy phytoplankton community (Amengual-Morro *et al.* 2012). These results indicate that different biological communities respond differently to their environment. Based on my results, the development of an index for assessing the health of pond ecosystems should be multi-metric. The index should include water quality, macroinvertebrates, and phytoplankton communities similar to *Predictive System for Multimetrics* (PSYM) developed in the UK (Biggs *et al.* 2005; Howard 2002).

My study identified the biodiversity in the ponds to a relatively higher taxonomic level rather than focusing on the species level. This broader taxonomic approach enabled me to study the

macroinvertebrates and phytoplankton (good bioindicators of ecosystem health) compositions of many ponds in different landscapes within the limited time of my PhD. Moreover, using different levels of taxonomic classification for macroinvertebrates is common in freshwater ecological studies (Boix *et al.* 2016; Botwe *et al.* 2015; Garrett-Walker *et al.* 2020).

### 8.1.3 Interaction between the biotic and abiotic parameters in pond ecosystems

The decrease in water quality of varying degrees poses a threat to biodiversity and sometimes stresses some species to the point of being locally extinct (Stribling *et al.* 2013). In investigating the relationship between the biotic community and the abiotic factors, I found that water quality variations contributed to the differences in the macroinvertebrate and phytoplankton communities (Chapter 3, 5, and 7). Temperature, salinity, conductivity, pH, and nutrients were key influencers of phytoplankton and macroinvertebrate' abundance and diversity. The findings indicate that conductivity, salinity, TDS, and pH were negatively correlated with macroinvertebrates and phytoplankton abundance and diversity (Chapter 3 and 5), similar to findings by Saeiam *et al.* (2020) and Yusuf (2020). However, the relationship between the variables and i) phytoplankton differed across the different phyla, and ii) different macroinvertebrate diversity indices also differed. For example, the phylum Ochrophyta was positively correlated to salinity (Chapter 5, Table 5.7). The variability in the response of the communities to the physicochemical variables is expected because of differences in the physiological tolerance and resilience of individual taxa (Blanchette and Pearson 2012; Botwe 2017). Also, I found that the ponds' water quality in winter explained higher variations in both the phytoplankton and macroinvertebrate communities (Chapter 3 and 5). The higher precipitation and high stormwater flow into the ponds in winter created a more conducive environment for the biota (Shi *et al.* 2017).

Contrary to my prediction, the nutrients did not play a major role in the abundance of the phytoplankton and macroinvertebrate communities (Chapter 3 and 5) similar to results by Saeiam *et al.* (2020). Instead, the biotic community was influenced more by the temperature and conductivity (Li *et al.* 2019; Meland *et al.* 2020). Other important abiotic factors were altitude, pond size and distance to another waterbody. The size of the pond was positively correlated with Pielou's evenness in winter. This finding implies that the phytoplankton were more spread out in the bigger ponds than the smaller ones. Also, ponds closer to other aquatic bodies had more phytoplankton taxa, especially in winter (Chapter 5). Percentage macrophyte cover was positively correlated with macroinvertebrate abundance and richness (Chapter 3). It

was, however, negatively correlated with phytoplankton abundance in winter (Chapter 5). Similar results have been reported for macroinvertebrates (Declerck *et al.* 2006; Johansson *et al.* 2019) and phytoplankton (Iacarella *et al.* 2018; Vilas *et al.* 2018). Macrophytes provide food, oviposition sites, protection from predators, and increase oxygen levels in the water. These conditions are favourable for macroinvertebrates (Blicharska *et al.* 2016; Declerck *et al.* 2006). In contrast, macrophytes compete with phytoplankton for sunlight and nutrients (Declerck *et al.* 2006; Johansson *et al.* 2019). Finally, the altitude of the ponds was positively correlated to phytoplankton abundance and macroinvertebrate taxa richness (Chapter 3 and 5). However, the strength of the relationships was seasonally dependent, reflecting again the impact of seasonality structuring the biotic communities (Pratt and Chang 2012; White and Hastings 2020).

Based on the water quality and the dominant taxa of macroinvertebrates and phytoplankton, the ponds exhibited varying levels of pollution (Türkmen and Kazanci 2010). This study, however, could not find a clear relationship between nutrients and the biotic community, especially the phytoplankton.

#### **8.1.4 Anthropogenic impacts on pond ecosystems**

The physicochemical conditions and the biological community of a pond are determined mainly by the LULC type within the catchment area and the local climate (Novikmec *et al.* 2016). Anthropogenic activities, including urbanization, control these LULC types. Also, the relationship between water quality variables and different LULC types are not consistent but vary at different spatial scales (Declerck *et al.* 2006; Nielsen *et al.* 2012; Novikmec *et al.* 2016). In New Zealand, urbanization and other human-related activities have been identified as the primary cause of the decline in freshwater ecosystem quality (Ballantine and Davies-Colley 2014; Collier 1993; Elston *et al.* 2015; Gadd *et al.* 2020). However, most studies on ponds have failed to account for the effect of LULC on the ecosystems' biodiversity (Greig and Galatowitsch 2016). Additionally, the spatial effect is yet to be investigated.

My study has indicated that LULC differed at multiple spatial scales around the pond and along the urban-rural gradient. The LULC was different at 10m, 500m and the ponds' catchment area along the urban-rural gradient. Ponds in the major urban areas had the highest percentage of urban cover (impervious surface) at 10m, 500m and within the catchment area (Chapter 2, Figure 2.6). This high percentage of the impervious surface indicates a high concentration of anthropogenically related activities in the major urban area. However, forest cover was highest

in ponds in the rural and major urban areas within the catchment area. I also found that the effect of LULC types on water quality was spatially variable. The LULC at 500m and catchment area influenced the water quality in winter. The impact of LULC at the catchments has been reported to significantly influence water quality in New Zealand (Galbraith and Burns 2007) and elsewhere (Nielsen *et al.* 2012; Novikmec *et al.* 2016; Nobre *et al.* 2020). Nonetheless, in summer, LULC and physical features at all four spatial scales were critical influencers of the ponds' water quality (Chapter 2, Figure 2.4). The high concentration of pollution accumulated in the LULC during summer as opposed to the continuous flushing in winter makes the effect of LULC vital in summer. Similar seasonal influences have been recorded by Xu *et al.* (2020) and Zhang *et al.* (2019). Therefore, though the LULC occurring in the catchment areas are associated with water quality through the transfer of nutrients, the extent of influence is seasonally variable (Nielsen *et al.* 2012; Nobre *et al.* 2020; Tu 2011).

Additionally, the ponds' water quality differed along the urban-rural gradient in summer (Chapter 2). Ponds in rural areas had the highest nitrate concentration (Chapter 2, Table 2.6), indicating a negative influence of increasing human population density on nitrate concentration. Also, TDS levels were highest in ponds in the large urban areas because the ponds in these areas were comparatively closer to the seas and likely to experience sea spray. Overall, I also found that the effects of the same LULC type on water quality varied i) temporally, ii) at multiples scales, and iii) along the urban-rural gradient, similar to findings by Liu *et al.* (2018).

Further, there was an anthropogenic impact on the biotic community in the ponds. I found that ponds in rural areas had the highest taxonomic diversity, number of functional feeding groups (FFG), and richness of macroinvertebrates (Chapter 4). The results indicate that human activities least impacted the ponds in the rural area. On the contrary, ponds in small urban areas were the richest in phytoplankton genera (Chapter 5). The composition of the phytoplankton community (high abundance of Chlorophyta and Cyanophyta) in the small urban area indicates a relatively higher pollution level. I found that the ponds in the major urban areas were more silted in summer, highlighting the increasing impact of human activities on these ponds (Chapter 5) (Hill *et al.* 2000). My findings show that both macroinvertebrate FFG and phytoplankton composition indicated varying levels of anthropogenic impact only in summer (Chapter 4 and 5).

Ornamental ponds had the highest Shannon Weiner diversity index compared to stormwater and golf course ponds. The result was not surprising because the ornamental ponds had a heterogeneous layout composed of different macrophyte types (emergent, submerged, and

floating), providing habitat and resources for diverse macroinvertebrates. On the other hand, the macroinvertebrates' taxonomic composition differed along the urban-rural gradient in both seasons (Chapter 4). The abundance of pollution tolerant taxa (Oligochaeta, Dugesidae) in the ponds in the major urban areas suggests that they were most influenced by human activities (Chapter 4).

A major challenge I faced was the inability to sample ponds in pristine environments. It is critical in ecological assessment for data to be compared with reference conditions to assess the level of degradation (Jarvinen *et al.* 2013). However, the ponds available and accessible for this study did not meet the reference criteria given the rapid urbanization rate.

Understanding pond ecosystems, especially in the light of rapid urbanization, is vital to conserve them for maximum benefit, particularly for biodiversity. My study has indicated the ecological status of ponds and given insights into the general ecology of ponds. In my view, more information on the ecology of pond ecosystems will highlight the need for policies to protect and enhance the management and conservation of these vital but neglected ecosystems. My research has revealed the water quality and the rich diversity of fauna and flora in pond ecosystems. It has also identified the effect of human population density, LULC and environmental factors on ponds. My findings have filled a knowledge gap and have raised many questions about the pond ecosystem structure and function that need to be addressed in future studies.

## 8.2 Recommendations for future research

1. I recommend that future studies should consider the effect of pond age, underlining soil and the type of macrophyte on water quality. In addition, due to the abundance of pollution tolerant taxa in ponds, potential sources of pollution should be explored. Specifically, the relationship between specific phytoplankton taxa and nutrient concentrations in ponds on a large spatial scale should be investigated. Such studies can be done by assessing communities in pristine or less impacted ponds (based on selected physical and chemical parameters) as reference points for comparison.
2. Macroinvertebrates and phytoplankton should be identified to the species level using morphology or molecular methods. This level of precision will help determine if ponds

support any taxa not found in the other freshwater ecosystems. Also, the relationship between the macroinvertebrates and phytoplankton communities in ponds should be further investigated. Understanding how these two communities are linked may help predict the biodiversity and ecological status of ponds even when only one of these communities is sampled. Furthermore, the sampling of more ponds than used in my study is likely to find additional species because of the highly variable taxonomic composition I and others have found across ponds (Meland *et al.* 2020; Perez-Bilbao *et al.* 2015). Therefore, determining the number of ponds that need to be sampled to better estimate regional pond biodiversity should be a focus of further studies.

3. A specific area of recommended research is the expansion of investigations into the diatom community composition. Diatoms are promising taxa for the development of metrics and indices to assess ponds' ecological status because of the relationship of specific diatom taxa with different levels of siltation.
4. Finally, I recommend that the development of a robust index for assessing the health of the pond ecosystem should be multi-metric, including phytoplankton (especially diatoms), macroinvertebrates and water quality similar to *Predictive System for Multimetrics* (PSYM) developed in the UK (Biggs *et al.* 2005; Howard 2002). An index can be developed with a long-term and comprehensive study of ponds in different levels of anthropogenically impacted sites. I recommend that these future detailed ecological studies be done in the summer because the communities tend to be more stable more diverse, and anthropogenic activities are better observed in summer (Chapter 2, 3, 4, 5 and 5). Such an index would be a useful tool for managing pond health and biodiversity.

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# APPENDICES

## Appendix A: Section 1 Methods for nutrients test

### Phosphate's analysis

To determine the reactive phosphorus concentrations, the ascorbic acid powder pillow method 8048 was used. This method operates on the chemical reaction between the orthophosphates and molybdate in an acidic medium to produce an intense blue colour of molybdenum. The colourimeter then measures the blue colour's absorbance to provide the concentration of the reactive phosphorus in the water sample. The more intense the blue colour is, the higher the concentration of phosphate in the sample.

To determine the reactive phosphorus concentration, the program 490 on the colourimeter was started. A sample cell was filled with 10ml of water to be tested. The content of the PhosVer 3 phosphate reagent powder was added to the cell. The cell was closed and shaken vigorously for about 30 seconds to dissolve the powder. The 2-minute timer was started, and the solution was allowed to sit and react during this time. The second sample cell was then filled with 10ml of the sample water for the blank reading. After the expiration of the 2 minutes, the cells are cleaned. The blank sample was inserted into the cell holder, and the zero button was pushed. The reading 0.00mg/l  $\text{PO}_4^{3-}$  was displayed. The blank sample cell was replaced with the sample cell, which had the powder pillow. The read button was pushed, and the concentration of  $\text{PO}_4^{3-}$  in the sample was displayed, and the reading was recorded on the datasheet (Phosphorus, reactive PhosVer 3 method, Hach 2015).

### Ammoniacal nitrogen analysis

To measure the amount of nitrogen ammonia in the water, the salicylate (method 10023) test and tube method were used. In this technique, the ammonia in the water reacts initially with chlorine to produce monochloramine. The monochloramine later reacts with the salicylate to form 5-aminosalicylate, which is then oxidized in the presence of sodium nitroprusside (a catalyst) to produce a green colour resulting from a mixture of blue masked by yellow (Hach 2015). The program 342 was selected on the colourimeter. Two vials containing diluent reagent were used. The vials were labelled as blank and sample. To sample vial, 2ml of the pond water was added, and 2ml of distilled water was added to the second vial to prepare the blank. The content of one ammonia salicylate powder pillow was added to each vial. This was followed by the addition of the content of one powder pillow of ammonia cyanurate to each vial. The vials were closed and shaken thoroughly until the powders were fully dissolved. A 20-minute reaction timer was started on the colourimeter. After the 20 minutes expired, both vials were cleaned. The blank vial was inserted into the cell holder, and the zero button was pushed to display the 0.00mg/l. The blank vial was removed and replaced with a sample vial. The read button was pushed, and nitrogen ammonia concentration was recorded (Hach, 2015 Nitrogen-Ammonia, Salicylate method).

### Nitrate analysis

The cadmium reduction method, which measures the low range (0.01-0.5mg/l<sup>-1</sup>) nitrate concentration using powder pillows and the DR/ 900 colourimeter, was used. In this chemical reaction, cadmium metal reduces nitrate in the sample to nitrite. The nitrite in an acidic medium reacts with sulfanilic acid to form diazonium salt. The salt couples with chromotropic acid to produce a pink-coloured solution whose absorbance is measured by the colourimeter to provide the concentration of nitrate in the solution (Hach, Cadmium Reduction Method).

For this analysis, the program 351 was selected on the program file on the colorimeter. The mixing cylinder was filled to the 15ml mark of the mixing cylinder. The NitraVer 6 reagent powder pillow was emptied

## Appendices

into the cylinder and closed. The three-minute timer on the DR 900 was started. The content of the cylinder was mixed vigorously. When the three minutes expired, the sample was left to stand for two minutes. After two minutes, 10ml of the contents in the cylinder was poured into the sample cell. Care was taken not to transfer the undissolved cadmium particles in the cylinder. The content of the NutriVer 3 reagents powder pillow was then added to the sample cell. The sample cell was covered and shaken gently for 30 seconds, after which a 15min reaction time was started. The second sample cell was filled with 10ml of the sample water. After the 15minutes reaction time, the second sample cell (blank sample) was wiped with facial paper and inserted into the cell holder. The zero button was pushed, and this displays a reading of 0.00mg<sup>l</sup><sup>-1</sup> NO<sub>3</sub>-N. After this, the prepared sample cell was also cleaned and inserted into the sample holder. The read bottom is pushed to give the concentration of nitrates in the solution. The value was recorded on the datasheet.

Appendix A: Table 1 Spearman correlation between the physicochemical variables in summer and winter. ‘\*’ indicates significant relationships at p < 0.05, ‘\*\*’ indicates significance at p < 0.01. Cond.: conductivity, Sal.: salinity, Temp.: Temperature, Nit.: Nitrate, P.: phosphate, Amm.: Ammoniacal nitrogen.

Correlations of physicochemical water quality variables								
	pH	Con. % DO (μS cm <sup>-1</sup> )	TDS (ppm)	Sal. (psu)	Temp. (°C)	NO <sub>3</sub> -N (mg <sup>l</sup> <sup>-1</sup> )	PO <sub>4</sub> <sup>3-</sup> (mg <sup>l</sup> <sup>-1</sup> )	
<i>Summer</i>								
% DO	0.5**							
Cond. (μS cm <sup>-1</sup> )	0.2	-0.4*						
TDS (ppm)	0.1	-0.4**	1**					
Sal. (psu)	0.1	-0.4**	0.9**	0.9**				
Tem. (°C)	0.5**	0.6**	-0.1	-0.2	-0.2			
NO <sub>3</sub> -N (mg <sup>l</sup> <sup>-1</sup> )	-0.4**	-0.3*	0	0	0.1	-0.1		
PO <sub>4</sub> <sup>3-</sup> (mg <sup>l</sup> <sup>-1</sup> )	0	0.2	-0.1	-0.1	0	0.1	0.1	
NH <sub>3</sub> -H (mg <sup>l</sup> <sup>-1</sup> )	0	-0.1	0.1	0.1	0.1	0	0.1	0
<i>Winter</i>								
% DO	0.3*							
Con. (μS cm <sup>-1</sup> )	0.1	0.1						
TDS (ppm)	0.2	0	0.9**					
Sal. (psu)	0.2	-0.1	0.7**	0.8**				
Tem. (°C)	0.3*	0.1	0	0	0.1			
NO <sub>3</sub> -N (mg <sup>l</sup> <sup>-1</sup> )	-0.1	0	-0.3*	-0.3*	-0.1	0		
PO <sub>4</sub> <sup>3-</sup> (mg <sup>l</sup> <sup>-1</sup> )	0.2	0	0.1	0.1	0.1	-0.1	0	
NH <sub>3</sub> -H (mg <sup>l</sup> <sup>-1</sup> )	-0.1	0.1	0.2	0.2	0.2	-0.2	0.1	0.1

Appendix A: Table 2 Results of PERMANOVA model testing for differences in water quality along the urban-rural gradient. Ponds were nested in urban-rural gradient. Significant p-values are in bold.

Terms	df	MS	F	p value
Urban-rural gradient	3	18.683	3.3308	<b>&lt; 0.001</b>
Season	1	41.563	9.9477	<b>&lt; 0.001</b>
Pond (urban-rural)	46	5.6092	1.3425	<b>0.02</b>
Urban-rural *season	3	6.7725	1.6209	0.07

\*Interaction term

## Appendices

Appendix A: Table 3 Range of nutrients concentrations in ponds (this study), rivers and lakes in New Zealand. The source of the nutrient concentration levels is ANZECC 2000.

<b>Nutrient concentration</b>	<b>Ponds (This study)</b>	<b>Rivers</b>	<b>Lakes</b>
Nit. (nitrates) ( $\text{mg l}^{-1}$ )	0.01-0.92	0.17-0.44	Low band, 0.8-1.97, B 1.97-4.9
P. Filterable reactive phosphate FRP ( $\text{mg l}^{-1}$ )	0.02-2.50	0.009-0.01	Low median, 1.05-2.05, 4.88-5.99 (Auckland pond)
Amm. (ammonia) ( $\text{mg l}^{-1}$ )	0.02-1.66	0.01-0.21	$\leq 30$ band A (low level)

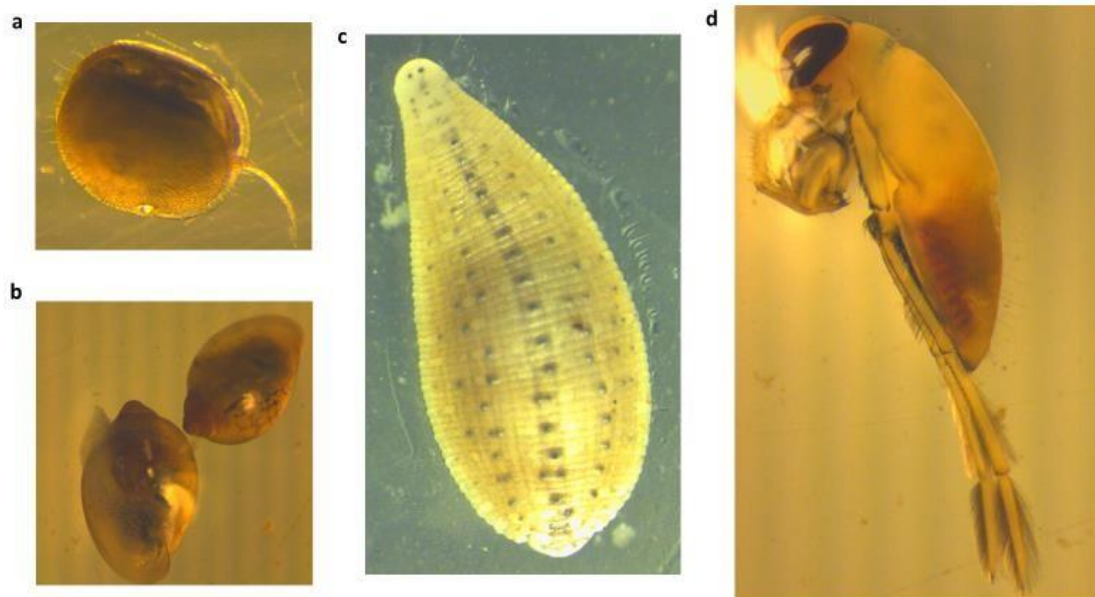
Appendix B: Table 1 Physical and geographical features of the 12 ponds sampled.

Pond	Distance to road (m)	Distance to waterbody (m)	Altitude	% macrophyte cover	Area (m <sup>2</sup> )	Depth (m)	Longitude	Latitude	Urban-rural gradient	Pond use
Coat	39.2	317	36.5	50	496.8	1.2	174.6418	36.71704	major urban	stormwater
Hugh	25.3	16.7	45	80	5981.0	0.8	174.7254	-36.7326	major urban	stormwater
Bota	4.6	101.5	50	50	3668.8	1.1	174.9074	-37.0108	major urban	ornamental
Bonn	20.3	36.4	5	0	2543.2	1.0	174.6986	-36.5965	large urban	stormwater
Whan	270.6	14.7	25.5	5	1974.2	1.2	174.7994	-36.6063	large urban	golf course
Helle	212.6	15.1	18.6	0	3009.5	1.1	174.5152	-36.6439	small urban	golf course
Wake	37	68.2	55.5	80	492.7	1.0	174.6307	-36.7448	small urban	farm
Huap	354.6	110.1	28	2	7202.8	1.1	174.5919	-36.7736	small urban	golf course
Tawp	18.6	39.4	25	80	780.6	1.1	174.8456	-36.3694	rural	water storage
Bric	51.7	61	20.5	30	4648.0	1.1	174.7234	-36.4055	rural	ornamental
Supe	33.1	19.1	4.5	0	6159.9	1.4	174.4154	-36.6671	rural	water storage
Mess	121.5	21.6	26	60	51.0	0.9	174.7094	-36.6288	rural	ornamental

## Appendices

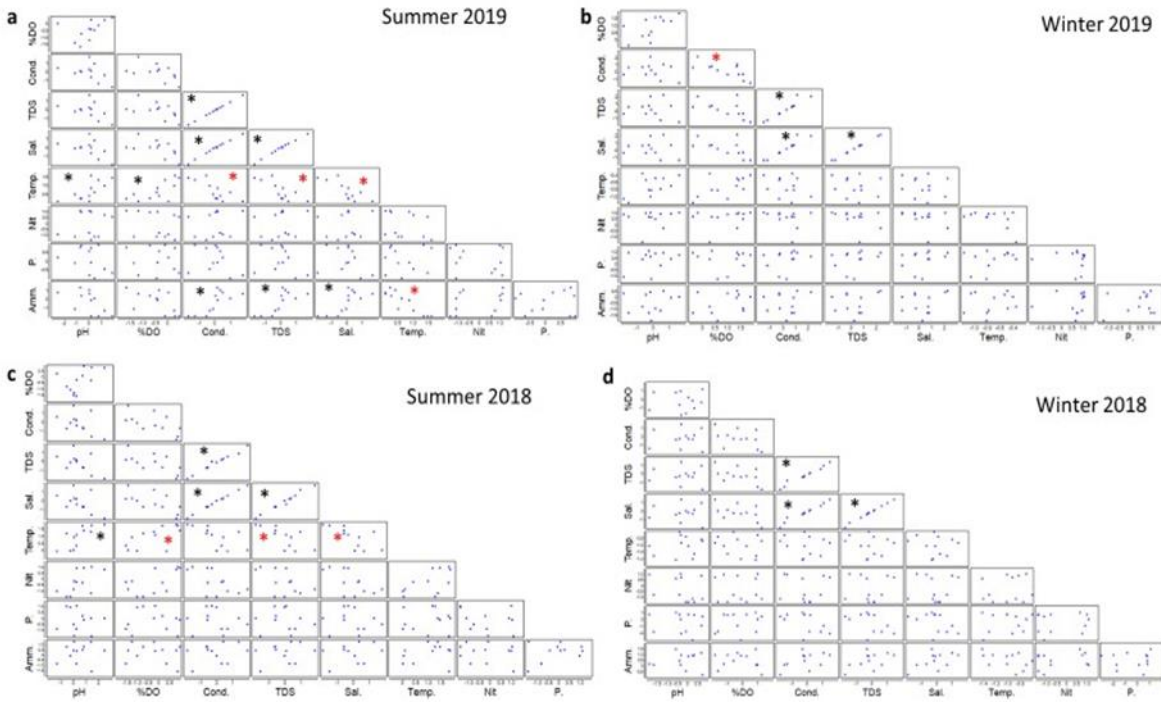
Appendix B: Table 2 Transformations carried out on physicochemical water quality variables in the ponds.

Variable	Chapter 3 & 4	Chapter 5	Chapter 6
pH	None	None	None
%DO	Square root	None	Square root
Con	Square root	Square root	Square root
TDS	Square root	Square root	Square root
Sal	Square root	Square root	Square root
Tem	None	$X*0.25$	None
NO <sub>3</sub> -N	None	$\log(X + 0.01)$	None
PO <sub>4</sub> <sup>3-</sup>	$\log(X + 0.01)$	$\log(X + 0.01)$	None
NH <sub>3</sub> -H	$\log(X + 0.01)$	None	Square root
Depth	$X*0.25$		

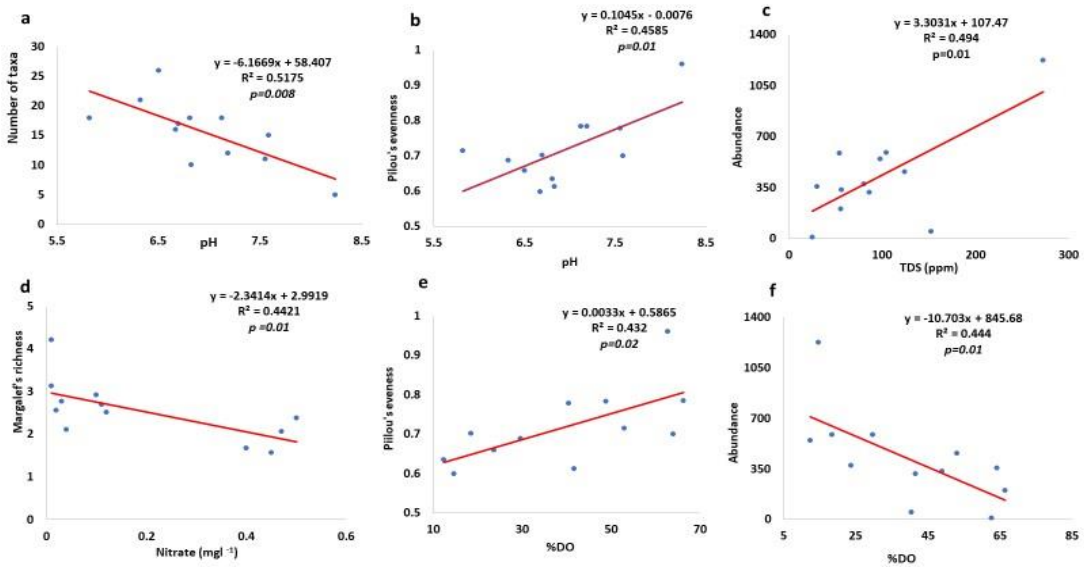


Appendix B: Figure 1 Pictures of macroinvertebrates sampled from the ponds. a) Ostracod, b) Physidae, c) Glossophonidae, and d) Notonectidae

## Appendices



Appendix B: Figure 2 Draftsman plot of the correlations between physicochemical variables in each sampling period. Red (\*) indicate significant negative correlation ( $p < 0.05$ ) and black (\*) indicate significant positive correlation. a: summer 2019, b: winter 2019, c: summer 2018, and d: winter 2018.



Appendix B: Figure 3 Linear regression plot of biodiversity indices and physicochemical water qualities in summer 2018.  $p$ -values are reported.

## Appendices

Appendix B: Table 3 BIOENV results

<b>BIOENV</b>	<b>Results</b>
Environmental factors used	pH, conductivity, TDS, salinity, temperature, NO <sub>3</sub> -N, PO <sub>4</sub> <sup>3-</sup> , NH <sub>3</sub> -H., pond area, catchment area, altitude, % macrophyte cover, % slope, distance to road, distance to water body, depth
Average weighted Spearman correlation coefficient (ρ)	0.289
Significance level of sample statistic (p)	0.003
Selected variables	pH, salinity, altitude, distance to road, depth

Appendix C: Table 1 SIMPER results for summer showing the distinguishing taxa among different areas along the urban-rural gradient. Values in bold represent shows where the taxa were most abundant.

<b>Taxa</b>	<b>Ave. Abundance</b>	<b>Ave. Abundance</b>	<b>Diss/SD</b>	<b>% Contribution</b>
	Group Major urban	Group Large urban		
Cladocera	<b>7.72</b>	2.36	2.24	5.27
Physidae	<b>6.54</b>	2.74	2.06	5
Orthocladinae	<b>4.42</b>	2.05	1.98	3.64
<i>Sigara</i>	4.49	<b>12.19</b>	1.72	6.7
	Group major urban	Group Rural		
Veliidae	0.66	<b>5.23</b>	2.13	4.72
Physidae	<b>6.54</b>	1.62	1.69	5.16
Notonectidae	6.51	<b>7.74</b>	1.45	2.78
Hydrachinidae	<b>5.09</b>	4.44	1.43	3.63
	Group Small urban	Group Rural		
Ostracod	5.63	<b>7.54</b>	1.59	3.36
Physidae	<b>5.46</b>	1.62	1.46	4.06
Veliidae	4.14	<b>5.23</b>	1.42	3.71
<i>Ischunura</i>	2.34	<b>2.88</b>	1.5	2.47

## Appendices

Appendix C: Table 2 SIMPER results for winter showing the distinguishing taxa among different areas along the urban-rural gradient. Values in bold represent shows where the taxa were most abundant.

Taxa	Ave. Abundance		Diss/SD	% Contribution
	Group Major urban	Group Large urban		
Ostracod	<b>7.11</b>	0	3.2	6.07
Cyclopoida	<b>8.68</b>	1.55	2.1	6.18
Glossiphoniidae	<b>4.7</b>	3.21	1.86	3.01
Dugesiiidae	<b>11.09</b>	2.71	1.79	7.84
	Group Large urban	Group Small urban		
Ostracod	0	<b>8.61</b>	2.36	8.12
Dugesiiidae	2.71	<b>9.16</b>	1.93	6.99
<i>Diaprepocoris</i>	3.95	<b>5.05</b>	1.71	6.11
Cyclopoida	1.55	<b>7.86</b>	1.6	6.44
	Group Major urban	Group Rural		
Glossiphoniidae	<b>4.7</b>	2.71	1.78	3.86
Ostracod	7.11	<b>9.33</b>	1.65	5.66
Oligochaeta	<b>10.58</b>	9.78	1.56	4.89
<i>Sigara</i>	2.2	<b>6.26</b>	1.53	5.15
	Group Small urban	Group Rural		
Ostracod	8.61	<b>9.33</b>	1.56	5.64
<i>Diaprepocoris</i>	<b>5.05</b>	0.78	1.58	5.18
<i>Sigara</i>	2.85	<b>6.26</b>	1.5	4.8
Physidae	<b>6.59</b>	1.47	3	5.82

## Appendices

Appendix D: Table 1 Abundance of the major taxonomic groups in phytoplankton phylum

<b>Taxonomic level</b>	<b>Abundance</b>	<b>Standard deviation</b>	<b>Standard error</b>	<b>Relative abundance</b>
<b>Cyanophyta Order</b>				
Oscillatoriales	160	31.8	1.3	0.35
Nostocales	109	23.1	1.0	0.24
Chroococcales	98	20.0	0.8	0.21
Synechococcales	87	17.6	0.7	0.19
Stigonematales	7	1.6	0.1	0.015
Pleurocapsales	1	0.3	0.0	0.002
<b>Chlorophyta Classes</b>				
Chlorophyceae	571	116.2	4.8	0.53
Zygnematophyceae	257	52.2	2.2	0.24
Trebouxiophyceae	219	44.5	1.8	0.20
Ulvophyceae	22	4.7	0.2	0.02
Coleochaetophyceae	11	2.5	0.1	0.01
Klebsormidiophyceae	4	0.8	0.0	0.004
<b>Ochrophyta</b>				
Bacillariophyceae	853	43.4	1.8	0.90
Mediophyceae	51	3.3	0.1	0.05
Chrysophyceae	41	3.9	0.2	0.04
<b>Euglenophyta Genera</b>				
<i>Euglena</i>	39	7.7	1.6	0.23
<i>Phacus</i>	50	9.8	2.0	0.30
<i>Strombonomas</i>	22	4.6	0.9	0.13
<i>Trachelomonas</i>	56	11.0	2.2	0.34

## Appendices

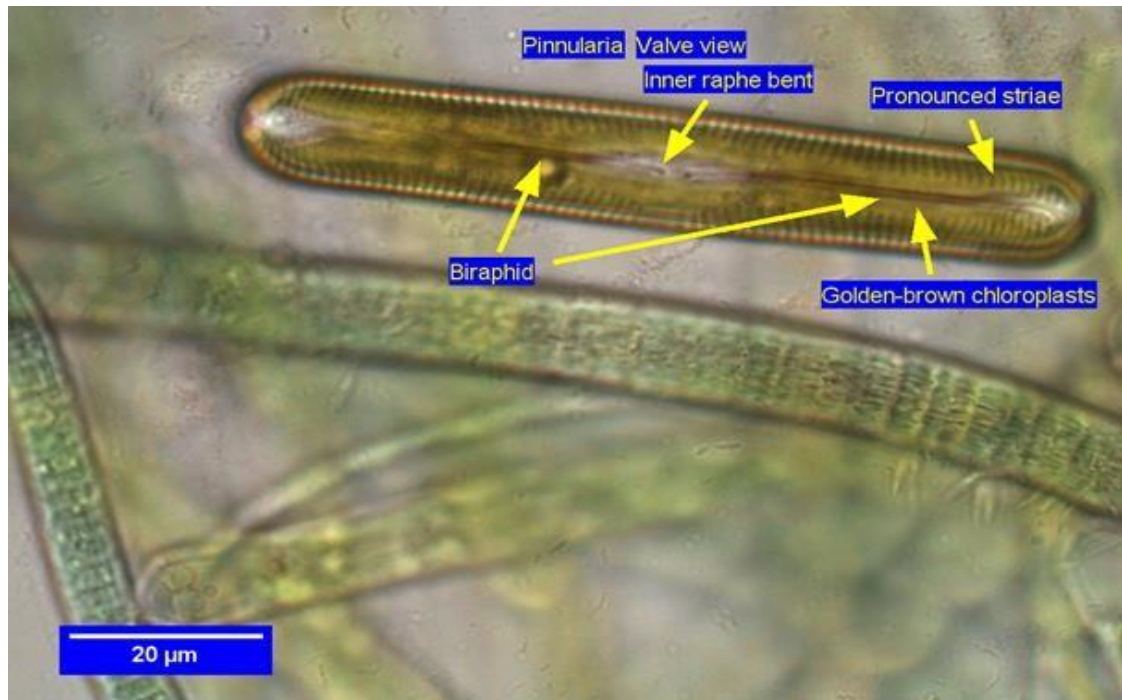
Appendix D: Table 2 Spearman correlation between the ten most important genera and the physicochemical water quality parameters in summer and winter. Correlations with '\*' and '\*\*' are significant at  $p < 0.05$  and  $p < 0.01$  respectively.

Genus	Physicochemical water quality variables							
	pH	% DO	Con	TDS	Tem	NO <sub>3</sub> -N	PO <sub>4</sub> <sup>3-</sup>	NH <sub>3</sub> -H
<b>Summer</b>								
<i>Ankistrodesmus</i>	-0.1	-0.4	0.1	0.0	-0.2	0.1	0.2	-0.3
<i>Bulbochaete</i>	0.5	0.4	-0.8**	-0.8**	0.6*	0.5	-0.2	-0.2
<i>Calothrix</i>	0.4	0.5	-0.7*	-0.7*	0.6*	0.4	0.1	-0.1
<i>Cymbella</i>	0.0	-0.2	0.0	0.1	0.2	-0.3	0.5	0.8**
<i>Chlorella</i>	0.1	0.0	-0.1	0.0	0.2	0.0	0.3	0.5
<i>Dichothrix</i>	-0.5	-0.3	0.4	0.4	-0.7*	-0.3	-0.4	-0.7*
<i>Navicula</i>	0.5	0.3	-0.4	-0.4	0.7**	0.1	0.5	0.5
<i>Nitzschia</i>	-0.3	-0.2	0.5	0.5	-0.3	-0.1	0.2	0.3
<i>Peridium</i>	-0.1	0.1	-0.5	-0.4	0.1	0.2	0.1	-0.1
<i>Pinnularia</i>	-0.3	-0.6*	0.3	0.3	-0.3	-0.2	-0.1	0.5
<b>Winter</b>								
<i>Achnanthes</i>	-0.6	0.3	-0.2	-0.2	0.0	.6*	-0.1	-0.1
<i>Anabaena</i>	0.1	0.3	-0.4	-0.4	-0.1	0.4	0.5	0.3
<i>Chlorogonium</i>	0.0	-0.7	0.5	0.5	0.2	-0.1	-0.5	-0.1
<i>Cladophora</i>	0.3	-0.2	0.2	0.2	-0.2	-0.4	0.1	0.3
<i>Cosmarium</i>	0.1	0.1	-0.2	-0.2	0.1	0.1	0.3	0.2
<i>Cyclotella</i>	0.2	0.0	-0.2	-0.2	0.6*	0.1	-0.4	-0.4
<i>Navicula</i>	0.4	-0.2	0.2	0.2	0.7*	0.1	-0.5	-0.2
<i>Nitzschia</i>	0.1	-0.3	0.2	0.3	0.4	0.2	-0.5	0.1
<i>Oedogonium</i>	-0.5	0.0	0.0	0.0	-0.3	0.4	-0.5	-0.2
<i>Synedra</i>	0.3	0.3	-0.4	-0.4	-0.2	-0.7	0.4	-0.3



Appendix D: Figure 1a A picture of *Pleurotaenium* (Chlorophyta) taken under magnification x1000

Appendices

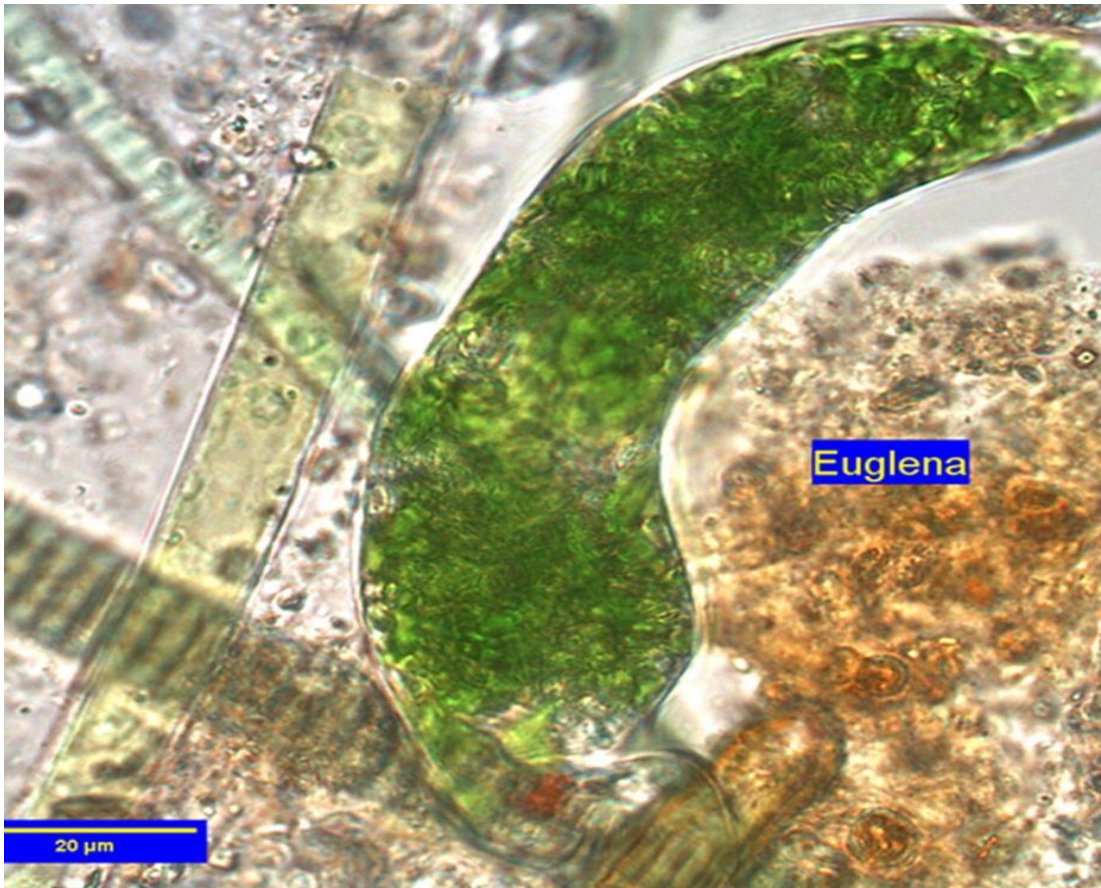


Appendix D: Figure 1b A photo of *Pinnularia* (Ochrophyta) taken under magnification x1000



Appendix D: Figure 1c A picture of *Oscillatoria* (Cyanophyta) taken under magnification x1000

## Appendices



Appendix D: Figure 1d A picture of *Euglena* (Euglenophyta) taken under magnification x1000

### Appendix E: Section 1 Protocols for Preparation of Nutrient Diffusing Substrates (NDS)

#### **Preparation of 2% agar solutions**

For each treatment, we prepared agar solutions in 280ml lots. 1120 ml in total for each setup. Agar and distilled milli-Q water (7g agar per 400ml water) were mixed by continually stirring over hotplates. A thermometer was placed in the beaker whilst continuing heating the solutions to 90°C to ensure agar is fully dissolved.

The solution was stirred for additional 20 minutes without heating to allow solutions to cool to 70°C.

#### **Preparation of Control- agar solutions**

An agar solution was added to four 70 ml screw bottom plastic pots. Care was taken to try not to overfill without making a mess. This is because the solution shrank a little when it cooled. The containers were clearly labelled with C for control.

#### **Preparation of Nutrient- agar solutions**

Nitrate (N) and/or phosphate (P) was added to agar solutions by gradually stirring in nutrients (see below for concentrations) for 5 minutes.

They were stirred for an additional 10 minutes to ensure nutrients were fully dissolved.

Care was taken not to contaminate different mixtures. The stirring rod was washed between each nutrient preparation.

Four pots of each nitrate concentration, four pots of each phosphate concentration, and four pots of each of phosphate and nitrate combined concentration were prepared.

Pots were labelled appropriately with P, N, N & P concentrations.

#### **Preparation of Nutrient diffusing substrates**

Nutrient-agar solutions were carefully poured into 70ml screw-top plastic pots.

The containers were covered with 100m mesh nylon netting and secured with a cable tie.

## Appendices

Sixteen pots (4 replicates of each treatment -see below) were screwed into rubber trays (400mm x 400mm). Pottles were arranged in the same, fully randomized order in each tray.

Pots were labelled appropriately with C, P, N or N&P.

Nutrient treatments:

C (Control) = no nutrients,

P (Phosphate) =  $\text{KH}_2\text{PO}_4$ ,

N (Nitrate) =  $\text{NaNO}_3$

N and P =  $\text{KH}_2\text{PO}_4$  &  $\text{NaNO}_3$

The C treatment pots were always placed in the most upstream position within each pot set further to reduce the risk of nutrient contamination within sets. The rest of the prepared pottles containing the nutrients were randomly screwed onto a tray.

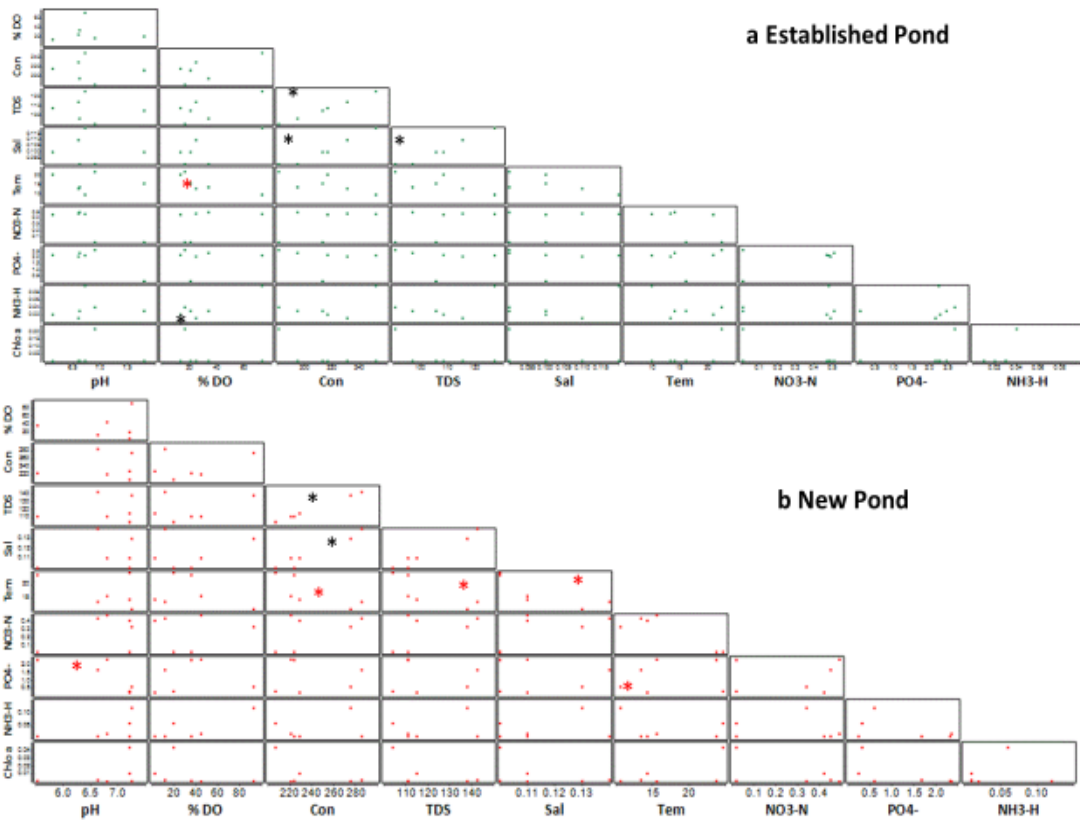


Appendix F: Figure 1 Pictures of the new pond on each sampling period.

Appendices



Appendix F: Figure 2 Pictures of the established pond on each sampling period.



Appendix F: Figure 3 A scatter plot of spearman correlation of physicochemical water quality variables in both ponds (a = established pond, green, b = new pond, red). Parameters with significant Spearman correlation have (\*): which indicates a strong positive correlation, and  $\rho \geq 7$ , and (\*) indicates a strong negative correlation.

## Appendices

Appendix F: Table 1 Simper results between the established and new pond

Taxa	Ave. Abundance	Ave. Abundance	Diss/SD	% Contribution
	New Pond	Established pond		
Ostracoda	9.26	6.31	1.63	5.51
<i>Physa</i>	6.2	2.91	1.48	3.88
Diplacodes	4.05	0.52	1.64	3.46
Hydrachinidae	3.57	9.45	1.43	7.66

Appendices

