Factors influencing zooplankton communities in small freshwater lochs

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Factors Influencing Zooplankton Communities in Small Freshwater Lochs

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Abstract

The work contained here examined the zooplankton of small freshwater lochs in Highland Scotland. Investigations focused on zooplankton community composition based upon environmental factors and the viability of these lochs as ‘canaries’ of climate change. Sampling occurred at 30 sites which were divided into three types based upon predicted allochthonous nutrients; altitude lochs, with no input, bird lochs with loafing and roosting sites, and stock lochs located in pastoral lands. Sites were further split by fish presence and absence, yielding six terminal subtypes. Physical and chemical analysis confirmed differences between subtypes, notably salinity, conductivity, pH, area, and altitude. Of these sites, 26 were analysed for community composition; 675 Rotifers, Cladocerans and Copepods, of 68 species were identified. Zooplankton community structure and diversity were examined to determine relationships with nutrient status and to establish baseline data from which future monitoring may derive. Findings supported previous research indicating a complex fish community produces a richer assemblage than a non-complex or fishless community. The stock lochs were the only ‘type’ supported by analytical data, though it was insufficient to support the hypothesis that nutrient chemistry alone was a significant predictor of zooplankton community composition. Owing to issues with sequencing, metabarcoding techniques were unable to support morphological data, and total N was missing from the final analyses. This work recorded zooplankton communities previously uncharacterised including several indicators of warming temperature, meso- and eutrophy which may be indicative of a shift in community in response to climate change. Further investigation is necessary to establish ‘canary’ status including more thorough chemical analysis and temporal biomonitoring.

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Contents

Chapter 1: Introduction

Plankton

Plankton, from the Greek planktos, meaning ‘wandering’, is the name given to aquatic organisms that are unable to completely control their movement against a current. Most often associated with oceanic waters, plankton can be found in a variety of freshwater environments including lakes, streams, puddles, and tyre tracks (De Bie, 2008). Plankton function as a ubiquitous basis for aquatic food webs, fuelling not only the carbon cycle but also the wider nutrient cycles of their environments (Brierley, 2017).

Plankton can be split into two main groups: phytoplankton and zooplankton. Phytoplankton are either mixotrophs or obligate photoautotrophs with little to no self-mediated movement. These organisms are at constant risk of sinking out of the photic zone and reproduce quickly to compensate (Huisman et al., 2002). Zooplankton are heterotrophs that prey on phytoplankton or other unicellular plankton. Whilst larger movement occurs via currents, Zooplankton can move towards prey or away from danger (Suthers et al., 2019).

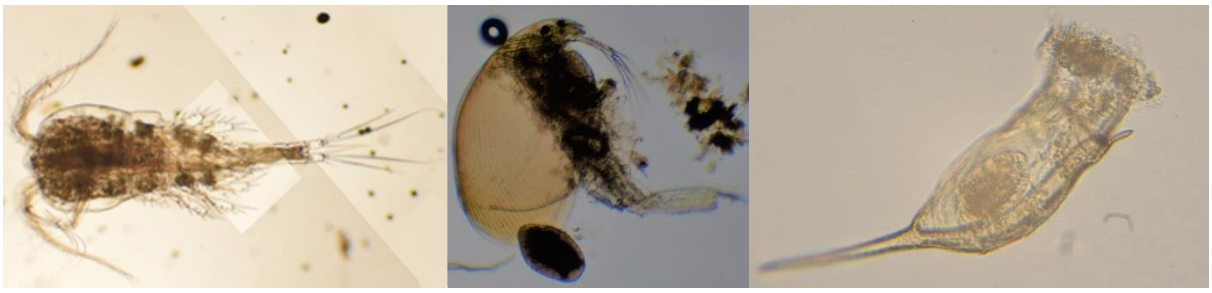


Figure 1.1, From left to right: Copepod, *Ectocyclops phalereatus* (composite), Cladoceran, *Alonopsis elongata*, Rotifer, *Keratella cochlearis.*
The above images were taken over the course of this project. *E. phalereatus* was isolated from Loch Feur on 19/08/21, *A. elongata* was isolated from Loch na Fiedil on 20/08/21 whilst *K. cochlearis* was isolated from ‘Lilyloch’ on 22/08/21.

Plankton are also categorised by size. At the largest end of the spectrum, ‘megaplankton’ include organisms such as Cnidaria and range from 2 cm – 36 m. This study will focus upon mesoplanktonic (0.2 – 20 mm) Crustacea; Cladocerans (Branchiopoda) and Copepods (Hexanauplia), and Rotifera (Suthers *et al.,* 2019). Copepods (Figure 1.1a) are teardrop shaped planktonic crustaceans with two sets of antennae, a two-pronged ‘tail’ (furcal ramus) and a segmented armoured exoskeleton, typically 0.5 - 2mm in length (Williamson and Reid, 2001). Most free-living Copepods feed directly on phytoplankton, although larger species may be

predators. Cladocerans (Figure 1.1b) are generally spherical to rectangular in shape likewise, possessing two pairs of antennae and a folded carapace which covers most of the body. Typically, 0.2 - 3 mm in length (Rizo *et al.,* 2019), some can reach 5 mm. Cladoceran reproduction is dominated by cyclical parthenogenesis augmented by annual sexual reproduction. Except for a few taxa, Cladocerans feed on phytoplankton and are an important food source for planktivorous fish. Rotifers (Figure 1.1c) are bilaterally symmetrical microorganisms ranging from 0.2 – 1 mm (Pontin, 1978). They may or may not have a rigid cuticle (Kobayashi *et al.,* 2019). Typically, they have a corona of cilia on the head which aids in swimming and ingestion (Melone, 1998). They feed on phytoplankton and bacterioplankton.

Copepods and Cladocerans evolved in the late Cretaceous and Paleogene periods respectively (Rigby and Milson, 2000). Earliest fossil records of rotifers indicate that they were present at the Eocene-Oligocene boundary (Waggoner and Poinar Jr, 1993), although their soft-bodied nature makes a true estimation of their origination difficult. Today's planktonic life likely echoes and fills similar niches to the first organisms to have evolved on Earth. In modern times, zooplankton function as both primary and secondary trophic links, functioning as grazers, nutrient cyclers, and food sources for larger animals and detritovores (Funk, 2013).

Aquatic food webs

Illustrated in Fig 1.2 is the freshwater aquatic food cycle, apexed by piscivorous fish, which predate upon planktivorous fish, which in turn feed upon large zooplankton (10 – 20 mm) and planktivorous invertebrates. Large zooplankton and planktivorous invertebrates feed upon small zooplankton (<10 mm), which predate upon phytoplankton and bacterioplankton (Carpenter and Kitchell, 1985). Phytoplankton photosynthesise and take in nutrients from the surrounding water. Phytoplanktonic mixotrophs may feed upon bacterioplankton when they are unable to photosynthesise (Wilken *et al.*, 2018). *In situ* nutrients such as dissolved organic carbon (DOC) are metabolised by bacterioplankton as they consume detritus from higher trophic levels. Those nutrients are then returned as bacterioplankton are consumed, thus creating the 'microbial loop' (Brönmark and Hansson, 2018). In this way nutrients are consistently cycled through the food chain.

Plankton communities are often diverse, perhaps up to 30 different species within a water body with multiple organisms realising the same niche. The co-existence of plankton communities in equilibrium has long been a question; the so-called 'paradox of the plankton' (Hutchinson, 1961), i.e., how can so many different competing species coexist? Initial answers to this paradox postulated that equilibrium could not be achieved due to consistent changes in climactic conditions (Schindler *et al.,* 2011). However, even in stable, closed laboratory conditions over a time scale of up to 10 years, equilibrium was unachievable. Scheffer *et al.* (2003) postulated that the cause for this is fluctuations from a high number of interactions by a large ensemble of species with sensitive dependence on initial conditions. In her thesis, Benincà (2010) displayed that oscillations of chaos and synchronicity are evident within the complex planktonic food web. Despite this apparent disarray, she also showed that the food web can support fluctuations in species composition over time.

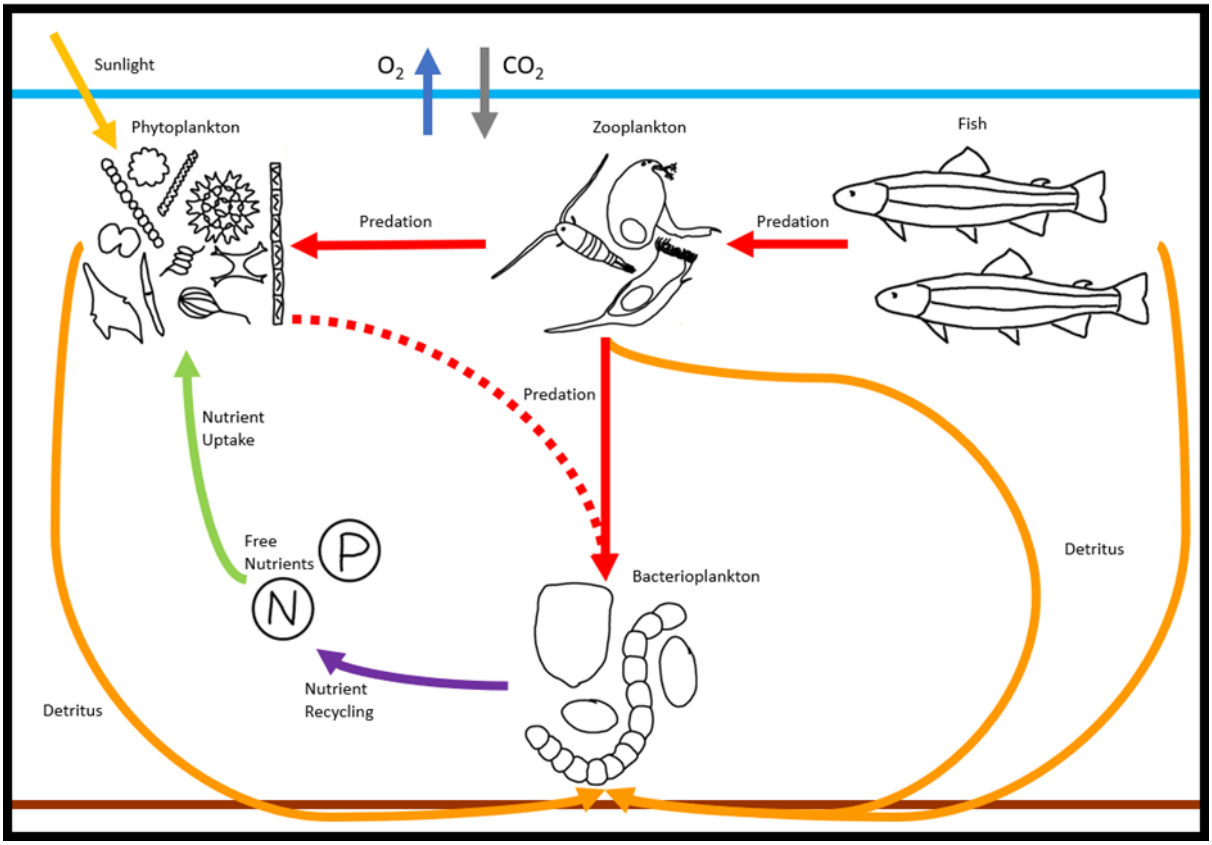


Figure 1.2, Aquatic Food Cycle

Whole lines indicate consistent interaction, dashed lines indicate occasional interaction, e.g., in the case of mixotrophic phytoplankton. This diagram shows the food chain from planktivorous fish to bacterioplankton and the microbial loop from bacterioplankton to phytoplankton and back again.
Image adapted from multiple sources.

The Trophic Cascade

Located between primary producing phytoplankton and consuming zooplanktivores, zooplankton are the link between these two trophic levels. The food web is in places circular (Fig. 5.1), and changes can have profound impact upon community structure. Influences are characterised as ‘top-down’ (TD) or ‘bottom-up’ (BU) controls (Hunter and Price, 1992). BU processes are typified by nutrient flow to primary producers and the effects of increased primary production on higher trophic levels. TD processes centre around predation effects upon lower trophic levels. Research has yet to clearly establish the principal effect, and it is likely that both are equally important factors.

Hairston, Smith and Slobodkin (1960) were the first to offer a model (the ‘HSS Model’) for these processes. It states that all trophic levels are limited by their respective resources, with interspecific competition found on each level. This simple model was the basis for the Trophic Cascade theory which states that the dominant factors in a chain will change dependent upon the neighbouring factors. In an aquatic system, for example, if the predator population (A) expands and reduces the population of predator population (B), the population of herbivores (C) will expand, which reduces the photosynthetic population (D). Thereby population D has been controlled by population A. Likewise, if there is an influx of allochthonous nutrients, for example leaf windfall, this will increase the population of bacterioplankton (E), which will cause an algal bloom (D), and the effect will cascade up the food chain (Scheffer, 1998). BU controls can also come in the form of inhibition, where the release of toxins by some phytoplankton reduces zooplankton volume allowing the phytoplankton to bloom (Banerjee and Venturino, 2011).

Trophic Status

Trophic status is the classification of a water body based upon its productivity. Measurement of water body status is made using several scales, the most famous of which is the Trophic State Index (TSI), which numerically classifies lake trophicity (Carlson, 1977), and even after 50 years is still widely used for temperate lakes. Four possible states are available, each one illustrating a different level of biomass and production. Moving from low to high production, these four states are; oligotrophic, mesotrophic, eutrophic and hypereutrophic. In order to classify, measurements are taken of total phosphorous (TP), clarity (measured as visibility

with a Secchi disk) and chlorophyll-α (phytoplankton biomass). A set of formulae is then followed to yield a numerical value which is assigned a classification.

As classification is a measure of biomass and primary production, there is continuous feedback between internal ecology and state of the water body. The release of toxins by phytoplankton is thought to be an annual response to over-grazing by zooplankton in the spring, in a phenomenon known as the 'clear water phase' (Banjeree and Venturino, 2011; Roy and Chattopadhyay, 2007; Lampert *et al.,* 1986). This well documented phenomenon is typified by a spring algal bloom, followed by a rise in zooplankton biomass and a clearing of the water, leading into a second algal bloom (of toxin-producing phytoplankton) which lasts until autumn. From this, and the above discussion of food webs we can conclude that zooplankton are often the trophic level upon which water body status depends (Carpenter and Kitchell, 1985). Further to this, it is possible to measure trophic status using the extant biota as a measure.

Zooplankton as Indicators

The use of zooplankton as indicators of trophic status and water quality is so well documented and verified that the exclusion of these organisms from government advice (European Parliament, 2000) has sparked outrage and petition in the scientific community (Jeppesen *et al.,* 2011; Cid *et al.*, 2014; Padovesi-Fonseca, 2020). Their use as indicators of trophic status and water quality has precedent as far back as 1978 (Gannon and Stemberger). Ejsmont-Karabin (2012) devised formulae to measure trophic status in north-east Poland by using rotifers as indicators. This functions because of BU control of rotifers by phytoplankton biomass, and the relief of pressure from the TD control of competing organisms. Likewise, in South America, cyclopoid Copepods were used to indicate water quality in reservoirs, with certain species being highly associated with chl-α and TP (Perbiche-Neves *et al.,* 2021). Finally, Cladocerans are often used in paleolimnology to identify historical trends in trophic status and environment (Leoni *et al.,* 2021).

Plankton communities are abundant and diverse and can reflect environmental productivity (phosphorus) (Simões *et al.,* 2015), fish community structure (Hessen *et al.,* 2006), water chemistry, water body size (De Bie, 2008), anthropogenic effect and climate change. Skala (2015) showed that community composition depends

upon species' requirements regarding total organic carbon (TOC), chlorophyll-α (chl-α), pH and depth. As chemical factors adjust in response to the changing climate, zooplankton communities fluctuate in phenology, abundance, distribution, size spectra and community structure (Vadadi-Fülöp *et al.,* 2012).

Climate Change

Climate change has been shown to erode demographic resilience and reduce habitat suitability for zooplankton (Pinceel *et al.,* 2018). Stenotherms are likely to find themselves with reduced habitat and an invasion of eurythermic taxa (Krajick, 2004). Insular environments such as lakes or alpine regions exacerbate this phenomenon, as movement between like habitats can be difficult, if not impossible. Foremost in the global consciousness when considering climate change is a rise in temperature. For many taxa, temperature is a governing factor in survivability, affecting among other things, speed of metabolism, viscosity of water, availability of nutrients and changes in biome size. The increase in temperature since the mid-1900s is seemingly exponential. A global surface temperature rise of ~ 0.8°C has occurred since 1940, with the last 10 years (2013 – 2023) all ranking in the top 10 warmest on record (NOAA National Centers for Environmental Information, 2023).

Evidence of increasing influence of climate change is observable; Northern hemisphere ice duration has decreased by 28 days (average) in the last 150 years (Woolway *et al.*, 2020). If the increase in temperature is sustained, effects on lake chemistry and biota will persist, moving further away from established baselines. As these baselines become more historical the case can be made for ongoing observation of sites to ensure sites are being meaningfully monitored. Lakes have long been known as 'sentinels' of change, responding quickly to stimuli (Adrian, 2009). It is suspected that the quick responses will be even more apparent in smaller water bodies with higher water to land interference ratios. There is some evidence for this suspicion (Vinnå *et al.,* 2021) however it requires greater investigation.

Water Body Size

When assessing climate change and environmental effects on water bodies and the communities within them it is important to distinguish between large (> 10 ha), small (< 10 ha) and tiny (< 1 ha), as size plays a significant role in the robustness of their internal ecologies. Macrophyte abundance and richness, for example, change with lake size and depth (Søndergaard, Jeppesen and Jensen, 2005), an important factor for zooplankton as macrophytes provide a refuge from planktivores. Additionally, waterbody size, shape and depth has major implications for pelagic (algae) (Tessier and Woodruff, 2002) or benthic (periphyton) (Vadeboncoeur *et al.,* 2008) phytoplankton, and thus for the zooplankton that graze upon them. De Bie, (2008) examined waterbodies varying in size from wheel tracks to lakes and showed significant relationship ($p < 0.001$) of composition and richness of zooplankton and water body size. Reasons posited are; susceptibility to water loss, degree of connectivity between water bodies, possibility of local stress events and size of existing population as a buffer to loss. A previous study of afforestation effect on small (0.3 – 3 ha) water bodies in Tayside, Scotland, showed that type of water body (lotic, lentic, size) is a key factor in determining zooplankton community composition (Jones, 1986).

Lake area has been shown to influence a variety of factors, including supporting knock-on effects such as variation in fish presence (Søndergaard *et al.,* 2005), amount of periphyton (Vadeboncoeur *et al.,* 2008) or the importance of terrestrial carbon (Wilkinson *et al.,* 2014). However, lake depth and the presence of stratification are arguably bigger contributors to the differences between ‘large’ and ‘small’ lakes. For example, submerged macrophytes are more plentiful in some shallow lakes owing to greater space-light ratios. Where submerged macrophytes are plentiful they fundamentally change the communities around them (Wu *et al.*, 2007), and influence zooplankton, planktivore volume and community composition of fish (Jeppesen *et al.*, 1997). Additionally, internal phosphorous loading is more prevalent in shallow lakes owing to the increased chance of aeration and subsequent resuspension of sediment (Scheffer, 1998). This has the knock-on effect of increasing algal blooms in summer, with consequences for lake management. As a result of this, shallow lakes are more prone to eutrophication than deep lakes (Qin *et al.,* 2006).

Sensitivity of Small Lakes

Lacustrine responses to changes in climate are heterogenous and complex, and span water clarity, temperature, stratification, and biotic responses (McCullough *et al.,* 2019). Despite this, Adrien *et al.* (2009) illustrated that these complicating factors can be accounted for, making lake habitats important 'sentinels' of climate change. Measurable responses such as plankton composition and dissolved organic carbon (DOC) indicate the influence of climate change not only for the specific lake but also the catchment and can highlight regional reactions to change.

Small lakes are especially sensitive to changes in climate associated with heating such as reduction in mixing and ice cover (Crossman, 2016; Vinnå *et al.,* 2021). This response is different to large lakes; even when wind is considered as a mixing factor in small lakes, the low levels of turbulent mixing reduce the climate change signal in stratified waters. In shallow lakes, where stratification is limited, small alterations in temperature can fundamentally alter chemical balance having a profound impact on biota and trophicity (Scheffer, 1998). Likewise, changes in temperature can alter DOC levels and thus have an impact not only on internal ecology, but also on the global carbon budget (Read and Rose, 2013). Downing *et al.* (2010) extrapolated global carbon capture capacity of small lakes as 4-fold higher than that of the oceans, wildly outweighing previously understood contributions. The sensitivity of these environments to change may make them key 'canaries' in investigations into climate change, highlighting the importance of acquiring a 'baseline' prior to further change.

When temperatures increase lakes tend towards eutrophy (Trolle *et al.,* 2011), an effect which upsets both carbon capture and green-house gas emissions. When mixed with climate change these factors (among others) are likely to be confounding. If we are to accept the premise that small lakes are 'canaries' to the larger lake 'sentinels', this must be taken into consideration by researchers. One caveat to this is that climate change is not always a singular effecter upon lakes of any size. Contaminants from farming and industry have unbalanced many aquatic ecosystems, with one review identifying trophic cascades in 60% of analysed cases (Fleeger, Carman and Nisbet, 2003).

Scottish Highland Lochs

With this caveat in mind, it is necessary to look for potential ‘canaries’ that are relatively untouched by anthropogenic practices. Virtually all regions of Britain have been affected by human activity. 91.1%, 92.5% and 95% of England, Scotland and Wales respectively has an agricultural or forestry land use (The Scottish Parliament, 2021; Department for Levelling Up, Housing and Communities, 2022; Natural Resources Wales, 2023), with most of the remaining portion used for urban or industrial purposes. In much of the UK, farming can be intensive and urban areas are distributed throughout agricultural lands. Highland Scotland is comparatively remote with little industry or urbanisation, and much land is considered ‘less favoured’ - soils are largely peat with acidic ground waters, making them nutrient and base-poor (Langan and Soulsby, 2001). This means that the major agricultural land uses in Highland Scotland are crofting and sheep grazing (The Scottish Parliament, 2021), which have a lesser impact on soil and removes the need for fertilisers, pesticides and other chemicals that can negatively impact the environment.

Within a warming climate lake trophy trends towards eutrophication, and for the ultra-oligotrophic lochs in Highland Scotland that is the only probable direction. The anthropogenic impact on these remote lochs is as reduced as possible within the context of Britain, making these lochs ideally located as potential ‘canaries’ for climate change.

Aims

This study will provide a preliminary examination of small freshwater lochs in Wester Ross, Scotland, with an aim of investigating the effect of environmental factors upon zooplankton community composition. The term ‘small’ in the context of lake size will hereafter refer to lakes which are <10 ha in size, drawing from work by Meyer *et al.* (2020), who created the Global Lake Area, Climate and Population (GLCP) dataset excluding, for the present, lakes <10 ha in size. Study sites were divided into three types based upon predicted nutrient enrichment by animals. Site type 1, ‘Stock Lochs’ are generally located within low altitude pastoral farmland likely to receive mammalian faeces. Site type 2, ‘Bird Lochs’ are low altitude, maritime, sea bird loafing and roosting sites, likely to receive avian faeces. Site type 3, ‘Altitude Lochs’

are at greater altitude with negligible faecal input. Each ‘type’ was further subdivided into fish present (‘fish lochs’) or fish absent (‘fishless lochs’) yielding 6 terminal subtypes.

This thesis focuses upon ‘small’ lochs which remain relatively under investigated and aims to answer the question of how much environmental factors, fish presence/absence and allochthonous nutrients influence small loch zooplankton community composition. Chemical and physical factors are used to ascertain that separation of sites into categories is a workable premise (Chapter 2). Zooplankton community assemblages are then investigated, using metabarcoding and traditional morphological analyses respectively (Chapters 3 and 4), and examined through the lens of chemical and physical variables. Finally, Chapter 5 critically evaluates the findings of this study within the context of the wider literature, and makes suggestions for future research.

The working hypotheses for this project are as follows;

H_{A1} – Significant chemical differences will be found between the loch types; those with greater allochthonous (avian and stock) inputs will be higher in nutrient value than those with lower inputs. H_{01} – No significance will be found in the nutrient value between the loch types regardless of input.

H_{A2} – Zooplankton community composition will show association with nutrient status suggesting significant influence from allochthonous inputs and the nutrient values found within the loch types, with those of higher inputs having a larger community (either of a dominating organism or a more diverse composition) than those of lower inputs. H_{02} – No significance will be found within the differences of community composition relating to allochthonous inputs and nutrient values.

H_{A3} – Fish presence or absence will have the greater impact on community composition than allochthonous inputs in this region. H_{03} – Fish presence or absence and allochthonous inputs will have an equal impact on community composition in this region.

Chapter 2: Study sites

2.1 Introduction

Scotland

Scottish lochs are relatively understudied, which is remarkable considering that water covers over 2% of Scotland, 5% of the Highlands and Islands, and 10% of the Western Isles, forming approximately 31,000 lochs and lochans, most of which are <1 ha (Warren, 2009).

This is not to say that several investigations in Scotland related to zooplankton interactions have not occurred; recent research on North Uist studied zooplankton in relation to *Gasterosteus aculeatus*, presence (Chitheer, 2018). Romo (1990), showed that the dominant zooplankton of 'small' (<24 ha) oligotrophic Loch Rusky was controlled by season and continuous fish stocking. A large survey by [1]Kernan *et* al. (2009) of over 350 high altitude lakes (>400 m.a.s.l) all >1 ha across Europe included 30 from Scotland. They showed that the high-altitude loch's biological assemblages could be split into two groups; low alkalinity low vegetation and high vegetation with peaty soils ([2]Kernan *et al.,* 2009), highlighting the effect of environmental factors upon communities. Another study into artificial enrichment within a three-loch series (Loch of the Lowes, Balgavies Loch and Forfar Loch; Stirling) showed zooplankton biomass increased with phosphorus concentration (Harper, 1986), a trend echoing that of industrial eutrophication of large Loch Leven.

Thus, primarily the lower altitude lochs, especially those that are 'tiny' (<1 ha), have received the least research focus. Yet, they comprise most Scottish waters, are morphologically and hydrologically heterogeneous. They provide a range of waterbodies which, by virtue of their 'smallness' are likely to experience rapid change due to environmental fluctuations. As such, they may, if adequately researched and monitored, offer a more sensitive means by which to measure climate change in this region of the world. If, however, such monitoring is to occur, baseline studies are required now.

Wester Ross

This study was located within the Wester Ross region of the Scottish Highlands. Sample sites (Fig. 2.1) are located both on the Gairloch peninsula and to the southeast; specifically, west of the Moine Thrust and south of the Loch Maree Fault. The geology of northwest Scotland is complex, consisting of overlain folded strata. Wester Ross itself is dominated by two bedrock types: Archean to Paleoproterozoic Lewisian Gneiss Complex (LGC) in the south and east, and fluvial Torridonian sandstone (TS) on the peninsula. The LGC in this region can further be broken down into undifferentiated LGC, hereafter referred to as LGC, and igneous Loch Maree Group (LMG). A fourth type of bedrock, Cambro-Ordovician Quartz (COQ), can be found running along the western side of the Moine Thrust where it overlays LGC and TS, however this is only identified as bedrock for one sample site (Trewin, 2002). Whilst bedrock is not the primary influence on lake characteristics, it is a contributory factor and should be noted (Kamenik *et al.,* 2001). All lochs in this area are bordered by peaty gleys soil, although this is relationship is not explored.

Sample Sites

Thirty lochs were sampled from the 19th – 29th August 2021. The notation for the six subtypes is as follows: A- and A+, B- and B+, and S- and S+ for fishless and fish altitude, bird and stock lochs respectively. A singular 'Large Loch' was included for comparison (Table 2.1). Location of lochs can be seen in Figure 2.1, with further information on each sample site available on Table 2.2.

Table 2.1, Sampled Lochs from Wester Ross, Scotland; Loch Types, Area and Altitude

Fish +/- is shorthand for fish presence/absence, respectively.

Subtype	Fish	Notation	#	Area Range (ha)	Altitude Range (masl)
Altitude	-	A-	6	0.012 – 0.41	205 - 332
Altitude	+	A+	3	0.28 – 0.48 (with outlier of 11.1)	205 - 333
Bird	-	B-	2	0.1 – 3.99	124 - 184
Bird	+	B+	8	1.1 – 27.18 (with outlier of 81.7)	75 - 128
Stock	-	S-	6	0.32 – 3.48	65 - 94
Stock	+	S+	4	4.10 – 13.37 (with outlier of 68.94)	21 - 74
Large	+	L+	1	3242	10

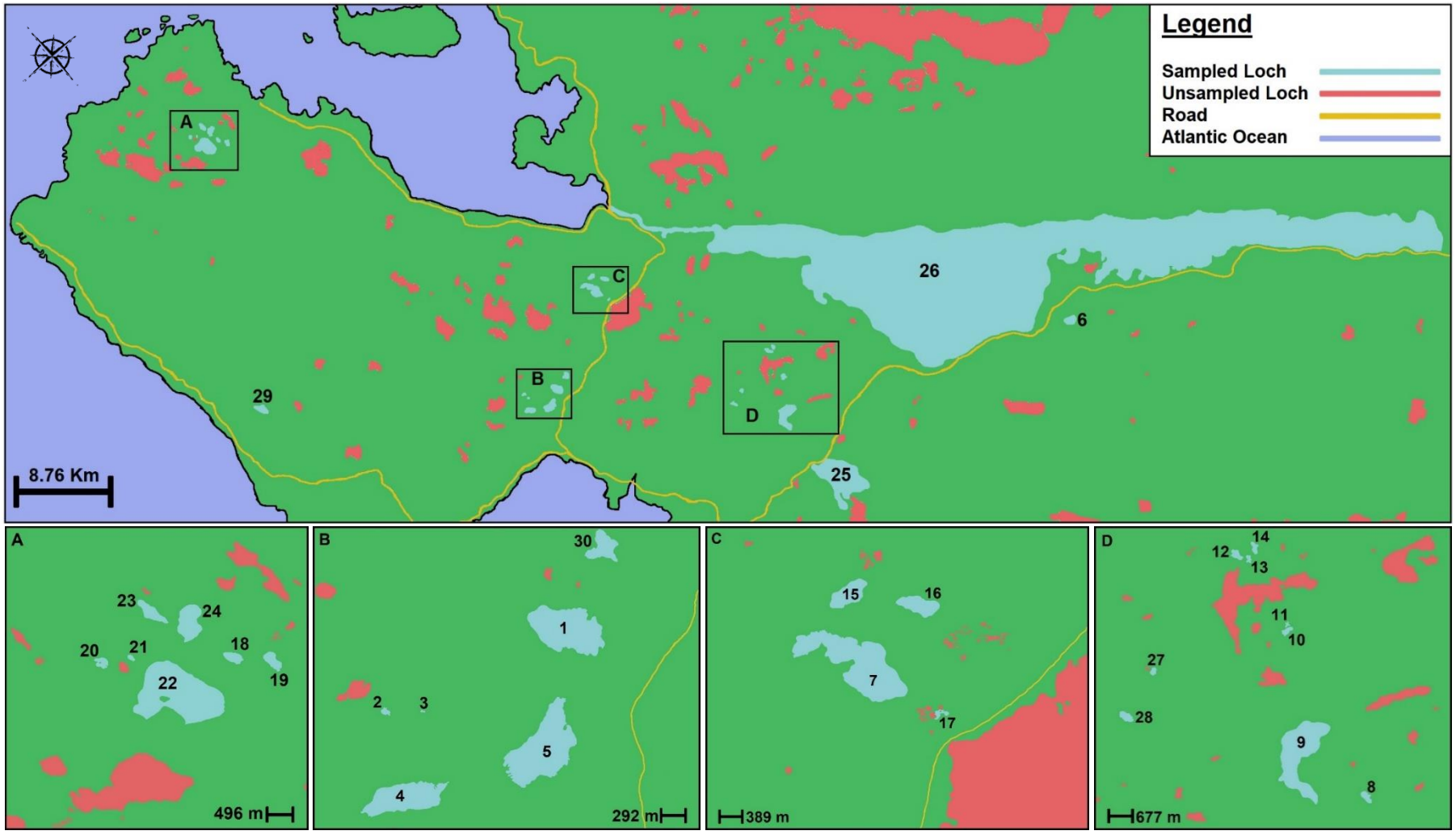


Figure 2.1, Map of Wester Ross, Scotland

The above map shows the sample area, including sampled (light blue) and unsampled (red) lochs. Sample sites are numbered, with four smaller areas included with exploded view (A, B, C and D). Hereafter samples are referred to as Loch #, or L# for short. For example, Loch 26 may be noted L26.

Table 2.2, Physical Characteristics of the 'Altitude Lochs'

For Tables 2.2 - 2.5; site names in parentheses have been given by the sampling team for ease of reference. These lochs are otherwise untitled. Geology data from Ballantyne and Bradwell, 2021.
Fish +/- is shorthand for fish presence/absence, respectively.

Loch ID	Site Name	Fish +/-	Long/Lat	Date Sampled	Area (ha)	Altitude (masl)	Geology
L8	'Lily Loch'	-	N57:41:40 W05:36:09	22/08/2021	0.34	205	LGC
L9	Loch nam Buainichean	+	N57:42:00 W05:36:16	22/08/2021	11.1	205	LGC
L10	Loch Dubh Dughaill	+	N57:42:26 W05:35:41	23/08/2021	0.28	333	LGC
L11	'Little Dughaill'	-	N57:42:29 W05:35:36	23/08/2021	0.012	331	LGC
L12	'Hot Parritch' (Goldilochs)	-	N57:42:53 W05:35:32	24/08/2021	0.41	324	LGC
L13	'Perfect Parritch' (Goldilochs)	-	N57:42:49 W05:35:28	24/08/2021	0.16	331	LGC
L14	'Cold Parritch' (Goldilochs)	-	N57:42:50 W05:35:22	24/08/2021	0.28	332	LGC
L27	'Lochan of the Great Diver'	-	N57:42:38 W05:37:19	27/08/2021	0.17	308	LGC
L28	'Cassius Loch'	+	N57:42:39 W05:37:16	28/08/2021	0.48	298	LGC

Table 2.3, Physical Characteristics of the 'Bird Lochs'

Loch ID	Site Name	Fish +/-	Long/Lat	Date Sampled	Area (ha)	Altitude (masl)	Geology
L1	Loch nam Breac	+	N57:44:27 W05:40:30	19/08/2021	4.93	81	TS, LMG, LGC
L4	Loch Coire na h-Airigh	+	N57:44:31 W05:41:20	19/08/2021	13.27	81	LMG
L5	Loch Feur	+	N57:44:21 W05:40:57	19/08/2021	27.18	75	TS, LMG
L7	Loch Boor	+	N57:45:10 W05:37:58	21/08/2021	9.23	120	LGC
L15	Lochan Feoir	+	N57:45:21 W05:37:47	25/08/2021	1.58	122	LGC
L16	Loch na Cloiche	+	N57:45:12 W05:37:33	25/08/2021	1.3	127	LGC
L17	'Lochran'	-	N57:44:57 W05:37:51	25/08/2021	0.1	124	LGC
L25	Loch Bad an Sgalaig	+	N57:40:47 W05:36:14	27/08/2021	81.7	112	TS, LMG
L29	Loch Meall a'Bhainne	-	N57:47:07 W05:46:46	29/08/2021	3.99	184	TS
L30	Loch Cregan Doire na Suaine	+	N57:44:34 W05:40:03	29/08/2021	1.1	128	LGC

Table 2.4, Physical Characteristics of the Large Loch, Loch Maree

Loch ID	Site Name	Fish +/-	Long/Lat	Date Sampled	Area (ha)	Altitude (masl)	Geology
L26	Loch Maree	+	N57:39:44 W05:24:20	20/08/2021 & 27/08/2021	3242	10	TS, LMG, LGC, CO

Table 2.5, Physical Characteristics of the 'Stock Lochs'

Loch ID	Site Name	Fish +/-	Long/Lat	Date Sampled	Area (ha)	Altitude (masl)	Geology
L2	'Matchless'	-	N57:44:45 W05:41:14	19/08/2021	3.48	94	TS
L3	'Tiny'	-	N57:44:40 W05:41:14	19/08/2021	0.32	93	TS
L6	Loch na Fiedil	+	N57:40:17 W05:28:47	20/08/2021	4.10	21	TS
L18	'Loch Caora'	-	N57:50:32 W05:43:02	26/08/2021	2.47	73	TS
L19	'Loch Reithe'	-	N57:50:25 W05:42:50	26/08/2021	3.48	68	TS
L20	'Loch Uan'	-	N57:50:47 W05:43:29	26/08/2021	1.75	66	TS
L21	'Loch Earball an Uain'	-	N57:50:43 W05:43:23	26/08/2021	0.35	65	TS
L22	Loch Airigh an Eilein	+	N57:50:34 W05:43:25	26/08/2021	68.94	59	TS
L23	'Loch Caoraich'	+	N57:50:47 W05:43:07	26/08/2021	5.44	74	TS
L24	'Loch Caoir-chaorach'	+	N57:50:38 W05:42:59	26/08/2021	13.37	71	TS

2.2 Methodology

Water sampling

Water samples were taken by filtering 500 mL surface water through 1 µm G/F Whatman filters using a hand pump. Filters were folded, wrapped in aluminium foil, kept chilled in a cool box and, within 6 hours, were stored at -20°C for Chl-α analysis. Filtered water was stored at 4°C for organic and inorganic compound analysis and dissolved organic carbon analysis. pH was measured in-field using an APERA PH20 pH Tester.

Chlorophyll-α

Prior to extraction filters were brought to room temperature. Extraction was performed by submersing torn filters in 10 mL 100% Acetone and storing at -20°C for >24 hours. Extract was centrifuged at 1000 g for 20 minutes to remove filter particulates. Samples were analysed fluorometrically on a FLUOstar Omega (BMG Labtech, Ortenberg) plate reader at an excitation of 430 nm and emission of 675 nm.

Organic Chemistry

Respectively, NO_3^-, NO_2^-, NH_4 and TP were measured using LCK339, LCK342, LCK304 and LCK348 Hach cuvette tests on a Hach DR3900 (Hach, Manchester). All field testing was done within 5 hours of sampling. Nutrient ratios could not be calculated as total nitrogen (TN) could not be measured and NO_2 was often too low to be measured. Meaningful Secchi data could only be obtained from a few lochs owing to sampling water clarity and bankside sampling loci and were thus unsuitable for analysis. Because TP values were all below the minimum calculable value of 0.75 mg/L, only Chl-α data was used to calculate TSI using the following formula (Carlson, 1977);

$$TSI(Chl-\alpha) = 9.81 \ln(Chl-\alpha) + 30.6$$

If lochs had negative TSI values they were corrected to 0 for statistical analysis.

Inorganic Chemistry

Cl^- was measured using the LCK311 Hach cuvette test on a Hach DR3900 (Hach, Manchester) within 5 hours of sampling. Na^+, K^+, Mg^{2+}, Ca^{2+}, Si^{2+}, Al^{3+}, Cu^{2+}, Zn^{2+} and Fe^{3+} were measured via ICP-OES on an X Series II (Thermo Fisher Scientific, Loughborough). Cd^{2+}, Cr^{2+} and Pb^{2+} were measured using ICP-MS on an iCAP RQ (Thermo Fisher Scientific, Loughborough). Performance report for the iCAP RQ can be seen in Appendix A.

Salinity was calculated using the following formula;

$$Salinity\ (ppt) = 0.0018066\ x\ Cl^-\ (^{mg}/_{L})$$

Where possible, Cu^{2+} and Zn^{2+} data was transformed from dissolved to bioavailable using the UK Technical Advisory Group's Metal Bioavailability Assessment Tool (MBAT). Hardness was calculated using the following formula;

$$Hardness = (2.497\ x\ Ca^{2+}) + (4.11\ x\ Mg^{2+})$$

Dissolved Organic Carbon

Total Carbon and Total Inorganic Carbon were measured using a Shimadzu TOC-VCPN analyser (Shimadzu, Milton Keynes). Dissolved Organic Carbon (DOC) was then calculated using the following formula:

$$DOC = TC - IC$$

Where TC = Total Carbon and IC = Inorganic Carbon. DOC was used in preference of TOC owing to filtration of water upon sampling.

Statistical Analysis

Raw chemical data for all variables can be seen in Appendix B, whilst variance and normality tests can be seen in Appendix C. Analysis of Variance (ANOVA), Kruskall-Wallace, and 2-sample T-tests were used as appropriate on the software Minitab 20.2. Tests were performed looking for differences and similarities between loch subtype (A+, A-, B+, B-, S+, S-) type (Altitude, Bird, Stock), and fish presence/absence (all samples) to investigate support for the groupings postulated in the aims. Loch Maree was excluded from all physical characteristic analysis; its inclusion in this study is as a reference point and its size is such that it skews physical data. Area was further examined using Levene's test to examine variance. Principal component analysis (PCA) was employed to illustrate and support the findings of other statistical tests such as ANOVA. Fish and invertebrate ('Inverts') presence was converted to a binary for the PCA, where 1 indicates presence and 0 indicates absence.

2.3 Results

Table 2.6, Water Chemistry for the 'A+ Lochs'

Variable	Unit	N	Min	Max	Mean	Median	StDev
Cond	µS	2	34.00	34.00	34.00	34.00	0.00
pH		2	6.85	7.00	6.93	6.925	0.11
Temp	°C	2	17.20	19.30	18.25	18.25	1.48
NO_3^-	µg/L	2	214.00	319.00	266.50	266.50	74.20
TP	µg/L	2	7.00	42.00	24.50	24.50	24.70
NH_4	µg/L	2	16.00	510.00	263.00	263.00	349.00
Cl-	µg/L	2	9.85	13.60	11.72	11.72	2.65
Chl-α	µg/L	2	16.30	127.20	71.70	71.70	78.40
TSI		2	0.00	10.37	5.18	5.18	7.33
DOC	µg/L	2	8.64	8.66	8.65	8.65	0.01

Table 3.7, Water Chemistry for the 'A- Lochs'

Variable	Unit	N	Min	Max	Mean	Median	StDev
Cond	µS	7	19.00	32.00	25.14	25.00	1.71
pH		7	5.50	6.85	6.26	6.44	0.21
Temp	°C	7	17.00	20.10	19.43	19.90	0.42
NO_3^-	µg/L	7	164.00	633.00	381.30	335.00	62.60
TP	µg/L	7	6.00	23.00	13.43	14.00	2.15
NH_4	µg/L	6	0.00	616.00	193.00	32.00	113.00
Cl-	µg/L	7	6.70	10.20	8.47	8.63	0.51
Chl-α	µg/L	7	55.40	208.70	102.00	78.30	20.60
TSI		7	2.22	15.23	7.22	5.61	1.70
DOC	µg/L	4	6.80	40.80	18.17	12.55	7.74

Table 2.8, Ion Concentrations for the 'A+ Lochs'

*For ion concentration tables, * Denotes total dissolved concentration, † denotes bioavailable concentration as calculated using the UK Technical Advisory Group's Metal Bioavailability Assessment Tool.*

Variable	Unit	N	Min	Max	Mean	Median	StDev
Al^{3+}	µg/L	2	20.70	44.10	32.40	32.40	16.50
Ca^{2+}	mg/L	2	1.15	2.94	2.05	2.05	1.27
Cu^{2+*}	µg/L	1	283.00	283.00	283.00	283.00	
$Cu^{2+†}$	µg/L	1	0.19	0.19	0.19	0.19	
Cr^{2+}	µg/L	2	0.22	1.15	0.68	0.68	0.66
Cd^{2+}	µg/L	2	0.14	0.36	0.25	0.25	0.15
Fe^{3+}	µg/L	2	12.29	22.64	17.46	17.46	7.31
K^{+}	mg/L	2	0.27	0.29	0.28	0.28	0.01
Mg^{2+}	mg/L	2	0.75	0.80	0.77	0.77	0.03
Na^{+}	mg/L	2	5.23	5.26	5.25	5.25	0.02
Pb^{2+}	mg/L	2	0.51	7.69	4.10	4.10	5.08
Si^{2+}	mg/L	2	0.15	0.40	0.27	0.27	0.18
Zn^{2+*}	µg/L	1	8.28	8.28	8.28	8.28	
$Zn^{2+†}$	µg/L	1	2.45	2.45	2.45	2.45	

Table 2.9, Ion Concentrations for the 'A- Lochs'

Variable	Unit	N	Min	Max	Mean	Median	StDev
Al^{3+}	µg/L	6	23.19	62.97	36.23	32.35	13.99
Ca^{2+}	mg/L	6	0.05	1.36	0.55	0.40	0.53
Cu^{2+*}	µg/L	2	1.00	755.00	378.00	378.00	534.00
$Cu^{2+†}$	µg/L	3	0.02	0.29	0.11	0.03	0.15
Cr^{2+}	µg/L	5	0.22	0.38	0.31	0.33	0.06
Cd^{2+}	µg/L	5	0.10	0.15	0.12	0.11	0.02
Fe^{3+}	µg/L	6	0.00	783.00	179.00	80.00	298.00
K^{+}	mg/L	6	0.17	0.46	0.27	0.22	0.12
Mg^{2+}	mg/L	6	0.53	0.86	0.64	0.58	0.13
Na^{+}	mg/L	6	3.45	5.93	4.59	4.53	0.80
Pb^{2+}	mg/L	5	0.63	4.46	1.71	1.36	1.59
Si^{2+}	mg/L	6	0.07	0.35	0.17	0.16	0.10
Zn^{2+*}	µg/L	2	6.85	25.54	16.19	16.19	13.22
$Zn^{2+†}$	µg/L	3	0.62	5.64	2.53	1.32	2.72

Table 2.10, Water Chemistry for the ‘B+ Lochs’

Variable	Unit	N	Min	Max	Mean	Median	StDev
Cond	µS	8	16.40	110.00	52.17	44.00	27.26
pH		4	5.90	6.90	6.30	6.20	0.43
Temp	°C	8	16.50	20.90	17.84	17.30	1.39
NO_3^-	µg/L	8	164.00	722.00	385.90	286.00	205.60
TP	µg/L	8	6.00	43.00	15.25	12.00	12.16
NH_4	µg/L	8	40.00	586.00	264.40	198.50	238.80
Cl-	µg/L	8	10.60	21.80	15.09	14.00	4.54
Chl-α	µg/L	8	75.00	471.20	182.00	164.70	125.90
TSI		8	5.19	23.22	12.31	12.86	5.66
DOC	µg/L	8	7.20	21.06	11.84	10.20	5.01

Table 2.11, Water Chemistry for the ‘B- Lochs’

Variable	Unit	N	Min	Max	Mean	Median	StDev
Cond	µS	2	48.00	59.00	53.50	53.50	7.78
pH		2	4.70	6.00	5.35	5.35	0.92
Temp	°C	2	16.10	18.30	17.20	17.20	1.56
NO_3^-	µg/L	2	172.00	699.00	436.00	436.00	373.00
TP	µg/L	2	8.00	20.00	14.00	14.00	8.49
NH_4	µg/L	2	439.00	538.00	488.50	488.50	70.00
Cl-	µg/L	2	14.10	22.40	18.25	18.25	5.87
Chl-α	µg/L	2	14.70	58.70	36.70	36.70	31.10
TSI		2	0.00	2.78	1.39	1.39	1.97
DOC	µg/L	2	22.47	29.41	25.94	25.94	4.91

Table 2.12, Ion concentrations for the 'B+ Lochs'

Variable	Unit	N	Min	Max	Mean	Median	StDev
Al^{3+}	µg/L	8	3.55	41.90	21.41	16.47	14.13
Ca^{2+}	mg/L	8	0.34	6.50	2.86	1.87	2.29
Cu^{2+*}	µg/L	5	0.45	8.61	2.94	1.21	3.45
$Cu^{2+\dagger}$	µg/L	3	0.10	0.26	0.16	0.13	0.09
Cr^{2+}	µg/L	8	0.25	0.37	0.29	0.27	0.04
Cd^{2+}	µg/L	8	0.08	0.25	0.16	0.16	0.05
Fe^{3+}	µg/L	8	8.10	423.60	134.00	87.70	146.70
K^{+}	mg/L	8	0.25	0.66	0.40	0.33	0.17
Mg^{2+}	mg/L	8	0.93	2.26	1.35	1.10	0.51
Na^{+}	mg/L	8	5.74	11.85	8.13	7.04	2.19
Pb^{2+}	mg/L	8	0.40	1.51	0.95	0.93	0.34
Si^{2+}	mg/L	8	0.09	0.94	0.58	0.57	0.27
Zn^{2+*}	µg/L	4	4.93	11.53	8.38	8.52	3.58
$Zn^{2+\dagger}$	µg/L	3	1.41	2.44	1.86	1.72	0.53

Table 2.13, Ion concentrations for the 'B- Lochs'

Variable	Unit	N	Min	Max	Mean	Median	StDev
Al^{3+}	µg/L	2	26.45	34.33	30.39	30.39	5.57
Ca^{2+}	mg/L	1	0.24	0.24	0.24	0.24	
Cu^{2+*}	µg/L	1	1.99	1.99	1.99	1.99	
$Cu^{2+\dagger}$	µg/L	1	0.35	0.35	0.35	0.35	
Cr^{2+}	µg/L	2	0.26	0.34	0.30	0.30	0.06
Cd^{2+}	µg/L	2	0.14	0.21	0.17	0.17	0.05
Fe^{3+}	µg/L	2	128.00	450.00	289.00	289.00	228.00
K^{+}	mg/L	2	0.32	0.60	0.46	0.46	0.20
Mg^{2+}	mg/L	2	0.85	1.24	1.04	1.04	0.27
Na^{+}	mg/L	2	7.31	11.14	9.23	9.23	2.71
Pb^{2+}	mg/L	2	1.57	7.34	4.46	4.46	4.08
Si^{2+}	mg/L	2	0.09	0.31	0.20	0.20	0.16
Zn^{2+*}	µg/L	1	9.73	9.73	9.73	9.73	
$Zn^{2+\dagger}$	µg/L	1	2.44	2.44	2.44	2.44	

Table 2.14, Water Chemistry for the 'S+ Lochs'

Variable	Unit	N	Min	Max	Mean	Median	StDev
Cond	µS	5	46.00	62.00	55.20	55.00	6.98
pH		5	4.90	8.30	6.54	6.60	1.21
Temp	°C	5	16.30	22.30	20.10	20.50	2.26
NO_3^-	µg/L	5	152.00	532.00	286.00	256.00	150.80
TP	µg/L	5	8.00	15.00	11.20	12.00	3.11
NH_4	µg/L	4	273.00	444.00	354.80	351.00	70.00
Cl-	µg/L	5	9.05	18.10	15.63	17.10	3.75
Chl-α	µg/L	5	66.85	102.72	77.94	73.37	14.87
TSI		5	4.06	8.27	5.43	4.97	1.75
DOC	µg/L	4	7.01	31.99	16.87	14.25	10.84

Table 2.15, Water Chemistry for the 'S- Lochs'

Variable	Unit	N	Min	Max	Mean	Median	StDev
Cond	µS	5	61.00	136.00	98.20	81.00	34.10
pH		3	4.60	5.70	5.00	4.70	0.61
Temp	°C	5	19.00	22.90	20.78	21.30	1.65
NO_3^-	µg/L	5	532.00	1020.00	713.20	707.00	187.40
TP	µg/L	5	16.00	25.00	18.20	16.00	3.90
NH_4	µg/L	5	9.00	493.00	289.60	388.00	201.10
Cl-	µg/L	5	16.30	22.60	19.88	19.50	2.40
Chl-α	µg/L	5	35.90	154.90	86.70	76.60	44.10
TSI		5	0.00	12.30	5.96	5.40	4.53
DOC	µg/L	5	16.13	33.63	25.01	26.93	8.31

Table 2.16, Ion concentrations for the 'S+ Lochs'

Variable	Unit	N	Min	Max	Mean	Median	StDev
Al^{3+}	µg/L	5	8.25	30.09	16.78	14.43	8.60
Ca^{2+}	mg/L	5	0.35	4.57	1.51	1.02	1.74
Cu^{2+*}	µg/L						
$Cu^{2+\dagger}$	µg/L	5	0.04	0.81	0.21	0.06	0.34
Cr^{2+}	µg/L	5	0.19	0.32	0.24	0.21	0.06
Cd^{2+}	µg/L	5	0.09	0.13	0.11	0.12	0.02
Fe^{3+}	µg/L	4	6.50	181.40	57.60	21.30	83.40
K^{+}	mg/L	5	0.34	0.43	0.40	0.40	0.04
Mg^{2+}	mg/L	5	0.92	1.41	1.08	1.04	0.19
Na^{+}	mg/L	5	6.34	9.45	8.39	8.49	1.24
Pb^{2+}	mg/L	5	0.14	0.69	0.46	0.45	0.22
Si^{2+}	mg/L	5	0.08	1.11	0.37	0.19	0.42
Zn^{2+*}	µg/L						
$Zn^{2+\dagger}$	µg/L	5	0.03	2.11	0.82	0.22	0.96

Table 2.17, Ion concentrations for the 'S- Lochs'

Variable	Unit	N	Min	Max	Mean	Median	StDev
Al^{3+}	µg/L	5	20.43	59.08	35.84	28.93	17.18
Ca^{2+}	mg/L	5	0.21	15.05	6.53	2.42	7.04
Cu^{2+*}	µg/L	2	0.59	2.08	1.34	1.34	1.06
$Cu^{2+\dagger}$	µg/L	3	0.07	0.08	0.08	0.08	0.01
Cr^{2+}	µg/L	5	0.22	0.45	0.31	0.31	0.08
Cd^{2+}	µg/L	5	0.13	0.18	0.16	0.16	0.02
Fe^{3+}	µg/L	5	47.40	323.70	114.50	72.40	117.80
K^{+}	mg/L	5	0.28	0.95	0.53	0.52	0.26
Mg^{2+}	mg/L	5	0.96	2.24	1.64	1.52	0.53
Na^{+}	mg/L	5	9.01	12.21	11.28	11.80	1.29
Pb^{2+}	mg/L	5	0.79	1.52	1.19	1.30	0.35
Si^{2+}	mg/L	5	0.06	1.28	0.46	0.26	0.51
Zn^{2+*}	µg/L	2	5.08	20.58	12.83	12.83	10.96
$Zn^{2+\dagger}$	µg/L	3	1.34	3.24	2.29	2.29	0.95

Geology

Stock lochs are exclusively based on Torridonian Sandstone (TS), whilst the altitude lochs are exclusively based on Lewisian Gneiss Complex (LGC). The bird lochs are unevenly split between exclusive and combinations of TS, LGC and Loch Maree Group (LMG). Loch Maree has a combination of all previously stated geological types, and a fourth type, Cambro-Ordovician Quartz on the eastern edge (Table 2.18).

Table 2.18, Subtypes of Lochs and Their Geological Types

Geological Type	# Stock Lochs	# Altitude Lochs	# Bird Lochs	# Large Lochs
LGC	-	9	5	-
LMG	-	-	1	-
TS	10	-	1	-
TS, LMG	-	-	2	-
TS, LMG, LGC	-	-	1	-
TS, LMG, LGC, COQ	-	-	-	1

Physical Characteristics

When analysed, altitude data supported ($p = 0.000$) the classification of lochs into subtypes, which is illustrated in Fig 2.2A and 2.2B. Clear separation of loch type (altitude, bird, stock) can be seen, whereby altitude lochs are the highest and stock lochs are the lowest. When all samples were divided into fishless/fish lochs and compared it was clear that fishless lochs had a higher altitude ($p = 0.001$) and smaller area ($p = 0.012$) than fish lochs, with fishless lochs consistently measuring <10 ha. These findings are echoed by loch subtype statistics (Table 2.1), which show that B- and S- lochs are smaller with greater altitude than B+ and S+ lochs, respectively. The altitude lochs support the size findings in that A- lochs are smaller than A+, however the altitude range is almost the same, and differences are statistically insignificant. Overall, the altitude lochs were the smallest, the largest loch being 11.1 ha, whilst the bird and stock lochs had three and two lochs > 10 ha respectively, although this variable cannot be used to justify the classifications.

Pictured in Fig 2.2B, distance from the coast (DisCoast) isolates ($p = 0.012$) the altitude lochs from bird and stock lochs, with all altitude lochs being a minimum 3.4 Km DisCoast. Bird and stock lochs are statistically the same DisCoast, even with their outliers, which includes Loch na Fiedil (12 Km DisCoast), not pictured.

Organic Chemistry – DOC, NO_3^- and TSI

DOC data was unobtainable for samples ‘Lilyloch’ (L8), ‘Perfect Parritch’ (13), ‘Loch Caoraich’ (L23) and Loch Maree (L26). Concerning DOC, a difference (p = 0.009) in concentration could only be observed within the stock lochs, where S- and S+

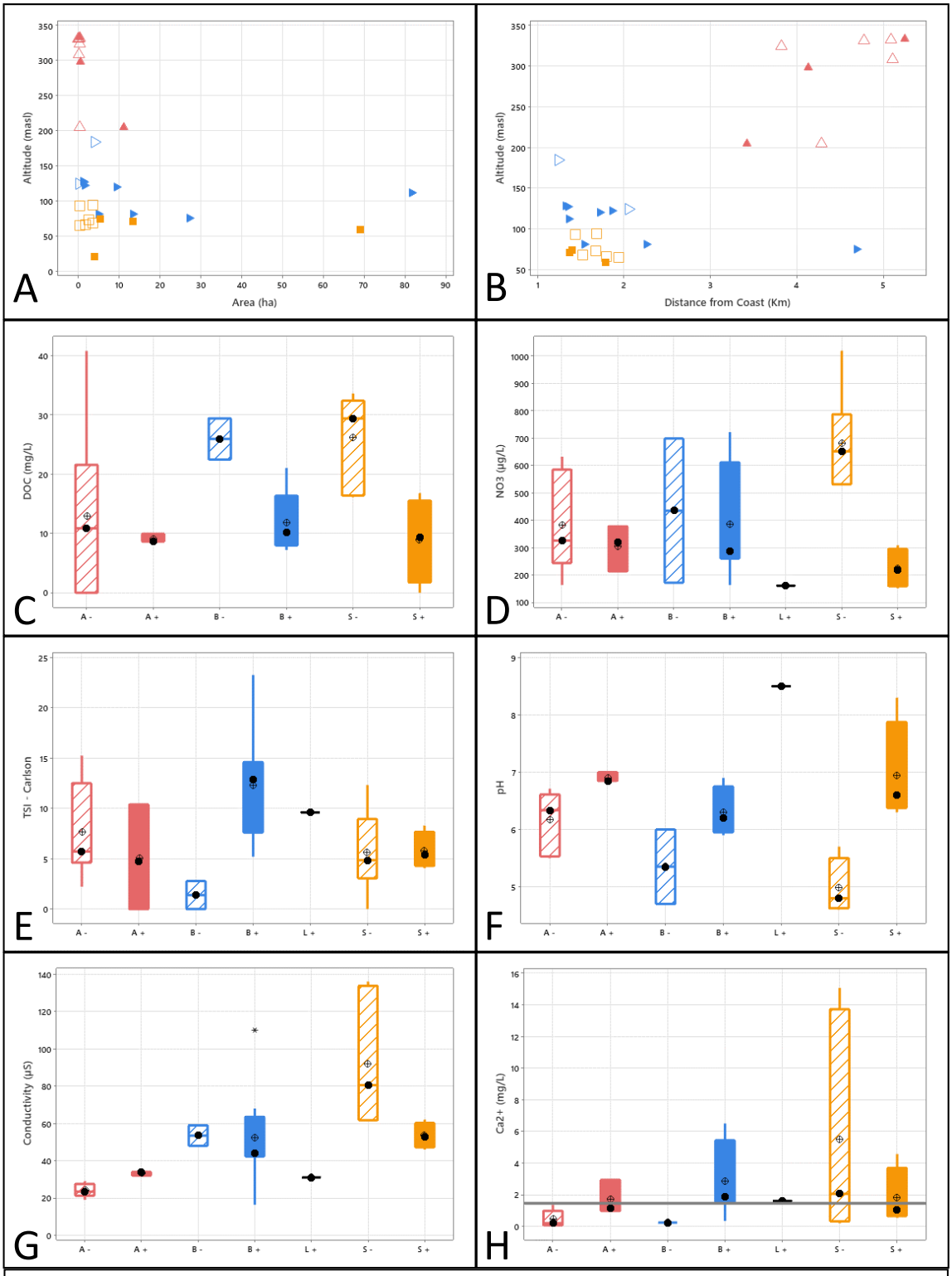


Figure 2.3, Physical and Chemical ranges of Lochs in Wester Ross

Graphs A – F are histograms, indicating subtype statistics - indicator colours are , and, for altitude, bird, and stock, respectively. Fish presence and absence is indicated by a filled and cross-hatched image, respectively. ● indicates median and the sun-cross symbol indicates mean. The grey line in plot H indicates Weyenmeyer’s minimum critical survival threshold (2019).

had mean concentrations of 26.18 and 8.88 mg/L respectively. Illustrated in Fig 2.2C, the remaining groups (A-, A+, B-, and B+) displayed insignificant differences in this variable. NO_3^- concentration showed the biggest difference ($p = 0.001$) in S- (Fig 2.3D), which had a mean concentration of 683 µg/L. When analysed without S-, differences in NO_3^- concentrations were not found either between the remaining subtypes (A-, A+, B-, B+, and S+), within remaining loch types (A- and A+, B- and B+) or using stand-alone fish presence and absence data. Analysis of TSI (Fig 2.3E) shows that the bird lochs had a wide disparity in trophy; some B+ lochs were almost mesotrophic, whilst B- lochs had the lowest trophy of all.

Inorganic Chemistry – pH and Conductivity

pH data is unavailable for L1 – 5 and Loch Boor (L7) due to technical errors in-field. Lochs had a markedly acidic pH, with only three lochs (Loch Maree, Loch na Fiedil, and 'Cassius Loch') measuring at pH 7 or higher. Illustrated in Fig 2.3F, fish were consistently ($p = 0.001$) observed in lochs with a higher pH and were not observed where pH was <5.9. Differences were observed within the stock and altitude subtypes, with the stock lochs having the most (S-) and least (S+) acidic lochs of all. Likewise, with conductivity, the altitude and stock loch subtypes exhibited differences, but this is not linked to fish absence. Conductivity (Fig 2.2G) is consistently <140 µS, with S- having the highest mean and the biggest range.

Heavy Metal Ions

'Lilyloch' (L8) was removed from heavy metal analysis owing to technical difficulties. Figure 2.3H shows Ca^{2+} concentration across subtypes, with a grey line to indicate Weyhenmeyer's 'minimum critical survival threshold' (2019) of 1.5 mg/L. 16/28 lochs have values below this threshold, including all the lochs in A- and B-.

Most ion concentrations were well below safe levels for a healthy ecosystem (Table 2.6). Cd^{2+} levels were all below the maximum allowable concentration highlighted by the Scottish Environment Protection Agency (SEPA) and had no statistical changes between samples. Of those values able to be transformed, only one (Loch Maree) showed Cu^{2+} values above recommended levels. K^+ concentrations were found to be within drinking standards, as little information on ecological safety was

available, there was no statistical differences between type or subtype. Concentrations of Pb^{2+} were all below the maximum allowed concentration, with 11 samples showing concentrations above the annual average, although it is unclear if these values are static. Al^{3+} displayed no statistical variation and was below safe drinking levels. Further guidance for safe levels of dissolved Al is unavailable as the danger with this element comes with speciation, especially in acidic waters such as the ones under study. Safe levels are available for what is considered 'reactive' Al, however neither this nor speciation were analysed.

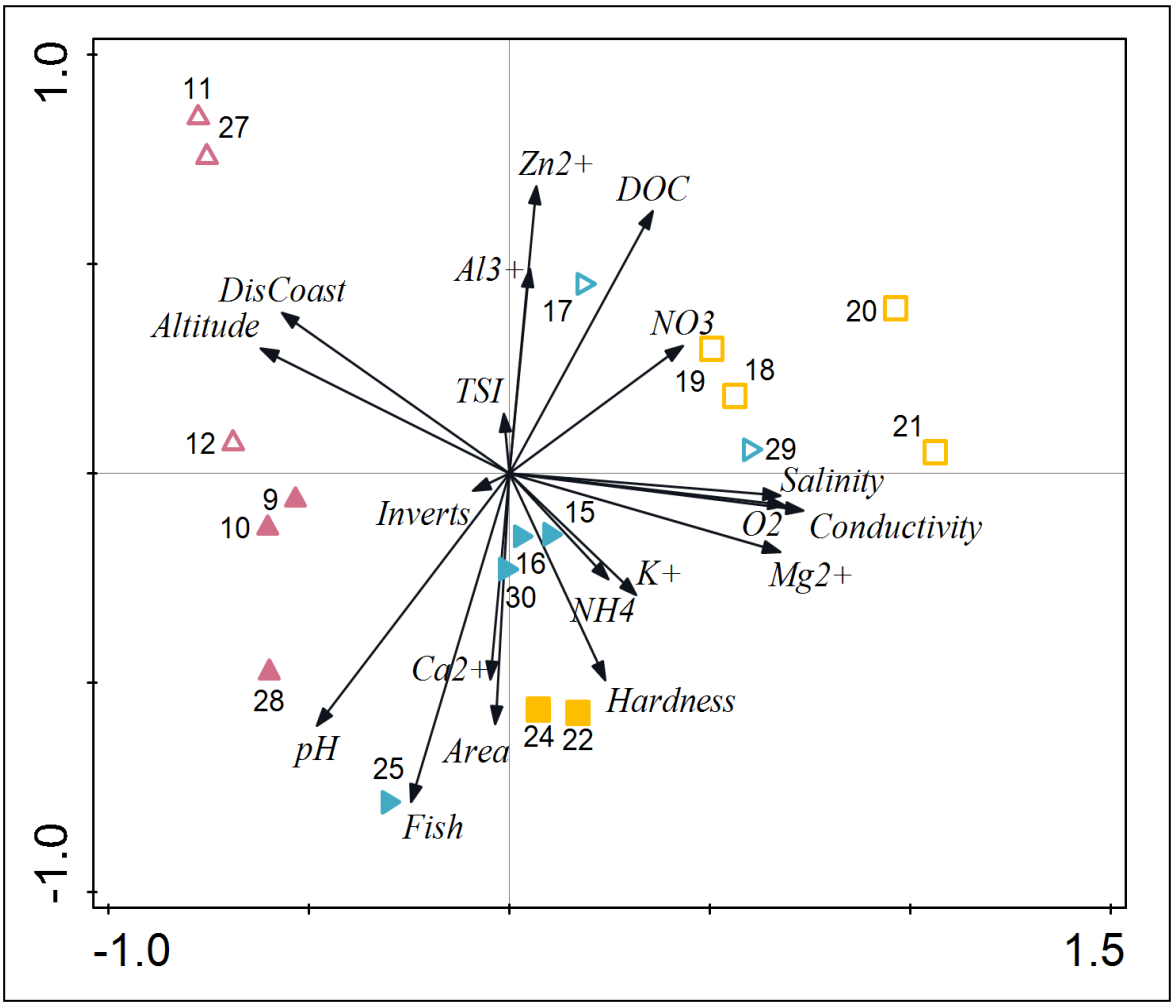
Table 2.19, Guidance on Safe Ion Levels in Water

Element	Advised maximum (µg/L)	Advisor	Sample max (µg/L)	Lochs above advised limit
Al^{3+}	100 (drinking level)	WHO (2003)	62.000	0
Cd^{2+}	0.45 (max allowable)	SEPA (2020)	0.361	0
Cr^{2+}	4.7 (III) 3.4 (VI)	SEPA (2020)	1.152	0
Cu^{2+}	1 (bioavailable)	SEPA (2020)	5.570	1
Fe^{3+}	1000	SEPA	0.782	0
K^{+}	1 (drinking level)	WHO (2009)	0.952	0
Mg^{2+}	3	Mooney *et al.* (2020)	2.250	0
Pb^{2+}	14 (max allowable)	SEPA (2020)	7.691	0
Si^{2+}	30	Book and Backhaus (2022)	1.270	0
Zn^{2+}	10.9 (bioavailable)	SEPA (2020)	5.640	0

PCA for Environmental Factors

The environmental variation of lochs can be seen in Fig 2.3 as a PCA plot, with Eigenvalues of 0.3234 and 0.1885 for Axis 1 and 2, accounting for 32.34 and 18.85% of the variation respectively. Axis 1 is correlated with salinity, which in turn correlates with conductivity and O_2. Axis 2 is associated with TSI, which correlates with Zn^{2+} and Al^{3+}, which are negatively correlated to area and Ca^{2+}. Sites with fish plot to the lower half of the PCA biplot, in contrast to fishless plots which appear exclusively north of Axis 1. Ca^{2+}, area and fish plot closely together, as does pH, supporting earlier findings that fish are not found at lower pH, and are associated with higher Ca^{2+} concentrations.

The altitude lochs plot exclusively on the left-hand side of the biplot but are extended along the area-Zn^{2+} gradient of Axis 2. Fish is correlated with pH, which negatively correlates with DOC. DOC behaves like a weak acid and partially regulates acidity (Bishop, Laudon, and Köhler, 2000). The bird lochs plot along the fish-DOC gradient sitting closest to Axis 2, excepting Loch Meall a'Bhainne (L29), which has a greater association with Axis 1 and salinity. The stock lochs plot to the right of Axis 2, with the fishless lochs showing a strong association with salinity and NO_3^-, whilst the fish lochs have a greater association with Axis 2 and Hardness, which correlates with NH_4 and K^+.



Sampled from Wester Figure 2.5, PCA plot for Lochs Sampled from Wester Ross

Where ▲ represents altitude lochs, ▶ represents bird lochs, ■ represents stock lochs, with an empty and filled symbol indicating a fishless and fish loch, respectively.

2.4 Discussion

This section examines the initial separation of lochs into subtypes based on environmental context and fish presence/absence with an emphasis upon chemical and physical data to determine whether these subtypes are justified. Soil data was not used for justification as Wester Ross lochs subsist mostly on peaty gleys soil (Soil Survey of Scotland Staff, 1981). Likewise, hydrogeology was not considered a factor as the region is dominated by the Precambrian North groundwater type which is weakly mineralised (Dochartaigh *et al.,* 2015). Seven lochs (Loch Coire na h-Airigh, Loch Feur, Loch nam Buainichean, Loch Airigh an Eilein, 'Loch Caoir-chaorach' and Loch Bad an Sgalaig) are larger than the prescribed 10 ha designated at the beginning of this work, their inclusion offers important insights into variation within the subtypes so were analysed as part of the dataset. Statistical analysis indicates differences in subtypes based on altitude, DOC, NO_3^-, TSI, pH, conductivity and Ca^{2+}, with altitude a known factor in the initial partitioning of sites, and a defining characteristic of the Altitude loch type.

Geological Type

All stock lochs have a base geology of 'Torridonian' Sandstone, and all altitude lochs have a base geology of Lewisian Gneiss Complex. Bird lochs were unevenly spread between the geological types, with 50% having a base of Lewisian Gneiss Complex, 20% have a base of 'Torridonian' sandstone with Loch Maree Group, and the remaining 30% having bases of Torridonian Sandstone or Loch Maree Group or Torridonian sandstone with Loch Maree Group and Lewisian Gneiss Complex. The large loch, Loch Maree, had all three types with a small section of additional Cambro-Ordovician Quartz.

Conductivity varied though it was always low, and the water is soft. While significance could be found relating to geological type, and TS is known to have the highest transmissivity of all bedrock in the study area (Dochartaigh *et al.,* 2015), it is unlikely that this solely reflects causation. The causes for differences in conductivity are likely complex and beyond the scope of this research project, although the data will be used to analyse the relationship between zooplankton

community composition and conductivity in future chapters. Salinity data followed trends that might be expected from loch location; those located closer to the coast possessed the highest salinities. Salinity was, however, well within ranges expected of freshwater bodies and none could be classified as brackish.

Loch Type

Excluding Loch Maree, Loch na Fiedil and 'Cassius Loch', all lochs were acidic, likely due to the local dominance of *Sphagnum* and peat (Clymo, Kramer and Hammerton, 1984). pH was significantly related to fish presence/absence, and factors that affect pH could only be explained through the lens of fish presence and absence. NO_3^- was higher in fishless lochs than in fish lochs, this may be because the absence of fish allows for greater grazing by zooplankton leading to higher levels of NH_4 which is oxidised into NO_3^- (Christoffersen *et al*., 1993). Secondly, DOC was markedly higher in fishless lochs than in fish lochs. In all instances the fishless subtypes (A-, B-, S-) had higher DOC and lower pH, although these values were only statistically relevant for stock and altitude lochs.

Salmo trutta, which are widely found in Scotland, can survive a pH of 4.8 provided there is an absence of inorganic Al (Al_i) (Buffam, Bishop, and Laudon, 2021). Where Al_i is present, increased acidification exacerbates speciation of toxic Al, which has been reported from Scotland (Harriman *et al.,* 1987). Despite its role as a weak acid and subsequent contribution to acidity, DOC complexes with labile Al and reduces its toxicity. This phenomenon however does not seem to contribute to extant fish populations within the sample pool. It is possible that the absence of fish in the high DOC low pH environments is due to low recruitment or vectors, however chemical interactions are just as suspect. The toxicity of Al-species at low pH cannot be understated; toxic Al, inhibits respiration (Lacroix, 1989) and suppresses mineral uptake of Ca^{2+}, K^+, and Na^+ reducing skeletal calcification (Reader *et al.,* 1989). In environments where Ca^{2+} is already low (A- and B-) it is possible that Ca^{2+} was a limiting factor of fish persistence. In the S- lochs, where Ca^{2+} is often above Weyhenmeyer's critical survival threshold (2019), pH is lowest and DOC highest – it is likely that more complex interactions are occurring than can be deduced from the current data.

A possible cause for the higher DOC is the reduction of light quality due to browning (dystrophy) which results in a reduction of primary production and clarity therefore negatively affecting fish abundance (Symons *et al.,* 2018). However, the findings imply that a reduction in chl-α would be expected, which was not supported by present data, and clarity from bankside sampling loci was ubiquitously very high. It is possible that increased primary production was coming from periphyton (Seekell *et al.,* 2015), however this was not measured. For aquatic chl-α concentrations, types and subtypes were functionally the same until conversion to TSI. TSI in this case is a proxy for chl-α, as Secchi data was unobtainable and TP concentration was too low to be used in the calculation. The low concentration of TP, and conferring literature from existing Scottish research (Smith, 1990; Jones *et al.,* 1996; [1]Kernan *et al.,* 2009) indicate that it is a limiting factor in the region. TSI analysis showed that all lochs are oligotrophic, and only the bird lochs showed any disparity in trophy. Some B+ lochs (Loch Boor, TSI 23.22) were almost mesotrophic whilst the B- lochs were the most oligotrophic (avg TSI 1.39). It is unclear why this disparity exists - it may not have anything to do with bird presence at all. The B- lochs are low pH, high DOC with Ca^{2+} levels below the critical survival threshold. Ca^{2+} concentration is not affected by guano (Leentvaar, 1966), thus it may be that these lochs are just inhospitable based on water chemistry.

The increased levels of NO_3^- and DOC go some way to explaining why pH is lower in fishless lochs, although without Al-speciation the data is incomplete.

To summarise, simplify and give context to the differences between subtypes, statistically relevant metrics have been assessed and combined; the final metrics to be used are altitude, ‘dissolved elements’ (DE) made up of conductivity, hardness, and salinity, and ‘acidic components’ (AC) made up of pH, NO_3^- and DOC. Thus: A- is high altitude, low dissolved elements, medium acidic components, A+ is high altitude, medium DE, low AC, B- is medium altitude, high DE, low AC, B+ is medium altitude, medium DE, medium AC, S- is low altitude, high DE, high AC, and S+ is low altitude, medium DE, and low AC.

Conclusion

The findings of the physical characteristic analysis support the separation of sites by subtype. Further, although differences cannot always be explained, chemical aspects such as salinity, conductivity, pH, and TSI also supported the split. Although the lack of TN data prevents true indication of nutritional values within the lochs and thus the subtypes the first half of H_{A1} is satisfied, in that significant chemical differences were found between the lochs. The lack of TN is a limitation, and its measurement is recommended for future studies. Clearly not all factors can be investigated in this small aspect of the overall study however categorisation has been supported and will be used to inform the analysis of zooplankton community composition.

Chapter 3: Metabarcoding

3.1 Introduction

The development of standardised methods for species identification based on DNA sequences, bioinformatic methods and public databases has streamlined the characterisation of biological samples. DNA metabarcoding is the technique of amplifying and sequencing specific short highly conserved genomic regions, then comparing those fragments with existing ones. The mitochondrial gene cytochrome *c* oxidase subunit I (COI) is a standardised molecular marker for animal species (Rodrigues, Morelli, and Jansen, 2017). COI has high resolution to species but can be difficult to amplify across highly heterogenous groups such as zooplankton. Although amplification has historically been a difficult process, protocols are continuously being improved (Elías-Gutiérrez *et al.,* 2018).

To mitigate amplification issues a second genetic marker is often used, usually one that compliments the limitations of COI. The small subunit ribosomal gene (18S rRNA) is the most widely used marker for Eukarya (Gong and Marchetti, 2019), with conserved primer sites and wide amplification across taxonomic groups but has issues with species resolution and sequence alignments. COI and 18S have been used together to successfully amplify zooplankton across a variety of different environments (Zhang *et al.,* 2018; Questel *et al.,* 2021), and will be likewise employed here.

Metabarcoding is best used in conjunction with morphological identification as all techniques come with their own set of challenges and biases that must be accounted for. For metabarcoding there are difficulties amplifying DNA, primer bias and the potential loss of taxa from the final result (Elbrecht and Leese, 2015). When identifying taxa through a microscope as is required for zooplankton, species resolution can be difficult to attain. As such best practice is to combine the techniques and compare the final results (Groendahl, Kahlert and Fink, 2017). Morphological techniques can be seen in Chapter 4, whilst this chapter focuses on metabarcoding of species from 27 Wester Ross lochs.

3.2 Methodology

Sampling

Zooplankton samples were collected by throwing a 40µm plankton net for ~5m and drawing it back to the bank. Net draw occurred 3 times so average total draw distance was 15m. 3x 45 mls of each sample were collected and transported back to the field lab where they were euthanised within 6 hours of sampling. Euthanised samples were allowed to settle overnight and then the supernatant was removed and replaced with ethanol to a final concentration of 75% in 50 ml. Owing to their shallow nature, 'Matchless' (L2) and 'Tiny' (L3) were excluded from zooplankton sample collection.

DNA Extraction and PCR

Samples were condensed from 50 ml by centrifuging at 4000 rpm for 15 minutes and removing the supernatant. Condensed samples were aliquoted to 2ml centrifuge tubes and centrifuged for 3 minutes at >12 g and the remaining supernatant was replaced with 99% ethanol. DNA was extracted from the samples using the DNeasy Blood and Tissue Kit (Qiagen, Manchester) with slight modifications. 500 µl of the condensed sample was aliquoted and centrifuged at >12 g for 3 minutes, after which the maximum amount of supernatant was removed without disturbing the pellet. Samples were heated to 50°C for 3 hours until dry. 100 µl Buffer ATL was added and samples were macerated for 2 minutes, with an additional 80 µl Buffer ATL being added and macerated for a further 1 minute. 20 µl Proteinase K was added, solution mixed by inversion once and incubated at 56°C overnight. The remaining protocol followed kit instructions.

Extracted DNA was quantified using a NanoDrop™ 2000/2000c Spectrophotometer (Thermo Scientific™, Waltham). PCR reactions were set up with the following primers; Leray *et al.* (2013) (forward primer, mlCOIintF: GGWACWGGWTGAACWGTWTAYCCYCC, reverse primer, mlCOIintR: GGRGGRTASACSGTTCASCCSGTSCC) and Nolte *et al.* (2010) (forward primer: ATTAGGGTTCGATTCCGGAGAGG, reverse primer: CTGGAATTACCGCGGSTGCTG) primers for COI and 18S RNA regions

respectively. COI and 18S primers included the Illumina adapters required for downstream processes. 2.5 µl of 5 ng/µl DNA was combined with 5 µl of both forward and reverse primers (10 µM), and 12.5 µl Taq Mix Red (PCRBIO, London). For thermocycler programmes see Table 3.1. Amplification was checked using gel electrophoresis on 1% Agarose, where Leray primers produced 1 band and Nolte primers produced 2. Samples that failed to amplify both primers were excluded from downstream processing.

Table 3.1, Thermocycler Programmes for Leray (2013), Nolte (2010) and Nextera Primers

Primers	Initial	Denature	Annealing	Extension	End
Leray	95°	94°C	62°C, -1°C every cyc	68°C	
	10 min	10 sec	30 sec	60 sec	
	1 cycles	16 cycles ----------→			
		94°C	46°C	68°C	72°C
		10 sec	60 sec	60 sec	10 min
		25 cycles ----------→			1 cycle
Nolte	95°C	95°C	50°C	72°C	72°C
	3 min	45 sec	90 sec	45 sec	10 min
	1 cycles	35 cycles ----------→			1 cycle
Nextera XT Index Primers	95°C	95°C	55°C	72°C	72°C
	3 min	30 sec	30 sec	30 sec	5 min
	1 cycles	8 cycles ----------→			1 cycle

Products were pooled by sample, cleaned using room temperature AMPure XP beads (Beckman-Coulter™, Brea) and quantified using a Qubit™ dsDNA Assay Kit (Invitrogen™, Carlsbad). Nextera XT Index Primers (Illumina, San Diego) were added with a final solution of 5 µl PCR product, 5 µl of both forward and reverse primers, 25 µl 2x KAPA HiFi HotStart Readymix (Sigma-Aldrich Co., St. Louis) and 10 µl nuclease free water, programme detailed in Table 3.1. PCR products were cleaned using room temperature AMPure XP beads before quantification via 4200 TapeStation (Agilent, Santa Clara).

Sequencing

Individual libraries were quantified using the following formula with an average library fragment size of 370 bp (average library size was calculated from the two Nolte amplicons of 150 and 300 bp, and the Leray amplicon of 450 bp), formula as follows;

$$\frac{Concentration\ in\ {}^{ng}/_{\mu l}}{(660\ {}^{g}/_{mol}\ x\ average\ library\ size)}\ x\ 10^{6} = Concentration\ in\ nM$$

Individual libraries were normalised to 4 nM buffered by Tris pH 8.5 before being pooled together. PhiX was used as internal standard (spike-in control) and was diluted to 4 nM/µl with Tris pH 8.5 to a final volume of 5 µl.

Cartridge was thawed in-bag in a 21°C water bath for 6 hours. Pooled library was diluted to 1 nM with room temperature Tris-HCl, pH 8.5 to a final volume of 100 µl. PhiX was similarly diluted to 1 nM and added to the pooled library as a 15% spike-in. Library with PhiX spike-in was vortexed briefly, then centrifuged at 280 g for 1 minute. Cartridge was inverted five times to mix reagents, and then tapped (label up) five times to ensure aspiration. 20 µl diluted library with PhiX spike-in was loaded into the reservoir of an iSeq 100 (Illumina, San Diego). iSeq Control Software v.2.0.0.661 was set to Paired Ends with 150 cycles for Read 1 and ran for ~20 hours. Demultiplexing was performed by the iSeq 100 at the end of the run.

Analysis

Quality of data output was analysed via FastQC (Andrews, 2010). FastQ data files were processed through Geneious Prime v. 1.2, Build 2023-04-27 14:16, Java Version 11.0.18+10 (64 bit) drawing from Geneious Academy Amplicon Metagenomics Tutorial (Geneious, 2023). Forward and reverse reads were paired and BBDuk ([1]Joint Genome Institute, 2023) was used to trim, with minimum quality of 30 and discard of reads <60 bp long. Paired reads were merged using BBMerge ([2]Joint Genome Institute, 2023) with a rate of high. De novo assembly occurred with custom sensitivity. Gap size was set to 1 with a 1% maximum per read. Minimum overlap was set to 100 with a minimum 98% overlap identity. Word length was 24, Index word length of 14 and word repeats >200 were ignored. The re-analyse threshold was 16, with 2% maximum mismatches per read and a maximum

ambiguity of 4. Consensus sequences and unused reads lists were run through the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) both for individual samples and full assembly for each fragment. BLAST is performed to associate fragments from known species on the database with fragments from the samples under study. The Nucleotide collection (nr/nt) database was searched via Megablast with hit table and maximum hit of 1. Low Complexity Filter was active and the Max E-value was set to 1e-1. Scoring was set at 1 -2 with a word size of 28. BLAST hits were extracted and classified using the Geneious Sequence Classifier (GSC). GSC settings had a high/medium sensitivity with a minimum overlap of 40 bp. Minimum overlap identity to classify was 75% with a minimum % identity as 0.2%. Minimum overlap identity to classify at the lowest, second lowest and third lowest taxonomic levels were set at 95%, 90% and 85% respectively.

3.3 Results

The metabarcoding process started with DNA extraction, which was successful for all but Loch nam Breac (L1). Loch nam Breac was consistently poor; no taxa could be identified morphologically either. After DNA extraction PCR was performed using two primer pairs, COI (Leray) and 18S (Nolte). Table 3.2 indicates the success of PCR across all lochs excepting 'Matchless' (L2) and 'Tiny' (L3) which were too shallow to sample from. Nolte primers produced two products at 150 and 300 bp, where one product of 180 – 200 bp was expected (Nolte *et al.,* 2010). This is non-specific amplification, where the primers work on regions that were not the target. COI primers were less successful than 18S, although where 18S failed to amplify COI failed also.

Table 3.2, Amplification of DNA from Wester Ross Lochs by COI and 18S Primers

Loch Name	Loch ID	Leray (COI)	Nolte (18S)
Loch nam Breac	L1	No	No
Loch Coire na h-Airigh	L4	Yes	Yes
Loch Feur	L5	No	Yes
Loch na Fiedil	L6	No	Yes
Loch Boor	L7	No	Yes
'Lilyloch'	L8	No	Yes
Loch nam Buainichean	L9	No	No
Loch Dubh Dughaill	L10	Yes	Yes
'Little Dughaill'	L11	No	No
'Hot Parritch' (Goldilochs)	L12	Yes	Yes
'Perfect Parritch' (Goldilochs)	L13	Yes	Yes
'Cold Parritch' (Goldilochs)	L14	Yes	Yes
Lochan Feoir	L15	Yes	Yes
Loch na Cloiche	L16	Yes	Yes
'Lochran'	L17	No	No
'Loch Caora'	L18	Yes	Yes
'Loch Reithe'	L19	Yes	Yes
'Loch Uan'	L20	Yes	Yes
'Loch Earball an Uain'	L21	No	Yes
Loch Airigh an Eilein	L22	Yes	Yes
'Loch Caoraich'	L23	Yes	Yes
'Loch Caoir-chaorach'	L24	Yes	Yes
Loch Bad an Sgalaig	L25	No	No
Loch Maree	L26	No	No
'Lochan of the Great Diver'	L27	Yes	Yes
'Cassius Loch'	L28	Yes	Yes
Loch Meall a'Bhainne	L29	No	No
Loch Cregan Doire na Suaine	L30	No	Yes

FastQC

Pictured in Figure 3.1 is FastQC data from Loch Dubh Dughaill (L10), which is a typical representation of the sample pool. Quality scores are logarithmically representative of the probability that that a nucleotide or sequence is incorrect based on signal strength from that nucleotide or sequence (Piper and Khetani, 2023). Graph A shows a distribution of quality scores on a base-by-base scale, and indicates sequencing output is of poor quality. Quality loss towards the end of the sequence is expected from Illumina sequencing however, the quality pictured here is extremely poor, and indicative of a failed run despite overall sequence quality being high (>30) (Figure 3.1B). Figure 3.1C shows three peaks in sequence length at ~60bp, ~80bp and ~150bp, likely the three fragments produced by PCR. Sequence duplication (Figure 3.1D) is low although there is a high volume of overrepresented sequences, in the case of L10 they are 30% of sequences. The proportion of overrepresented sequences varies by sample but is always indicative of poor quality. The overrepresented sequences could not be identified – they are not matching primer-dimer, nor can they be recognised via BLAST.

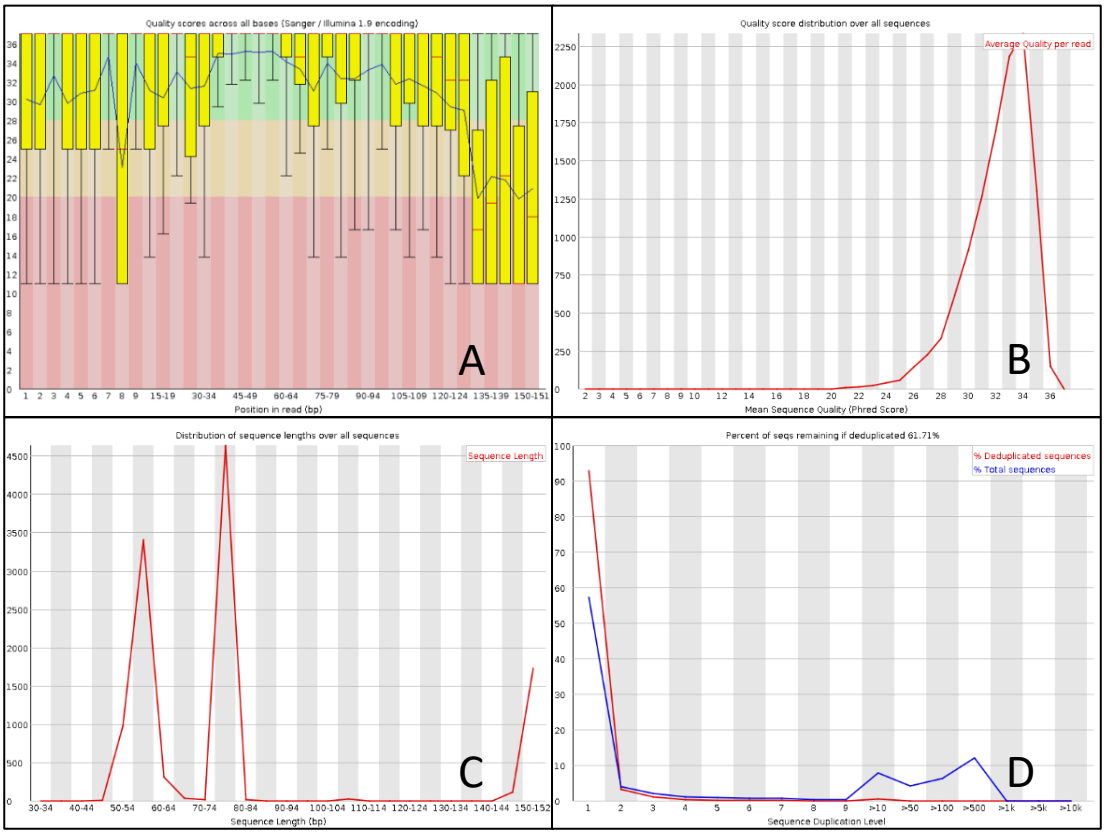


Figure 3.1, FastQC Graphs for Sample L10

The above graphs show quality data for sample L10 generated by the FastQC analysis software. Graph A shows Per Base Sequence Quality, with base position on the x-axis and quality scores on the y-axis. Graph B shows Per Sequence Quality, with average quality score on the x-axis and the number of sequences with that average on the y-axis. Graph C shows distribution of sequence lengths, with sequence length (bp) on the x-axis and the number of sequences with that length on the y-axis. Graph D shows sequence duplication, with duplication level on the x-axis and percentage on the y-axis.

Geneious Processing

The Geneious metabarcoding pipeline starts with a quality control step in which poor data is removed. In agreement with the FastQC results presented above, only a limited amount of data was retained after this step, which is detailed in Table 3.3. Initial output from the iSeq indicated that there were 3,142,814 sequencing reads, which is in line with existing literature. After processing, expected average output per sample could be ~60k and ~25k for 18S and COI respectively (Zhao *et al.,* 2021), however this run only produced 140 final sequences of acceptable quality, with 24 that could not be attributed to any loch. Analysis of pre-processed products could not confirm the presence of the control (PhiX spike-in), despite multiple researchers being confident of its inclusion, further illustrating the failure of this run.

Table 3.3, Processing results from Geneious

Detailed here is the results of the Geneious processing of sequenced data.
#Paired Reads shows the number of sequences that were paired by forward and reverse primers.
#Trims indicates the number of sequences remaining after trimming, which removed all sequences with quality <30 and length <60 bp.
#Merges shows the number of paired reads that contained a sufficient overlap by which it is indicative that they are opposite ends of the same sequence and are thus mergeable into a single sequence.
Undetermined reads indicate sequences that could not be attributed to a sample (had no Nextera primers).

Loch Name	Loch ID	# Paired Reads	# Trims	# Merges
Loch Coire na h-Airigh	L 4	50,549	10	3
Loch Feur	L 5	74,256	6	2
Loch na Fiedil	L 6	48,782	12	4
Loch Boor	L 7	123,268	20	9
‘Lilyloch’	L 8	64,199	28	3
Loch Dubh Dughaill	L 10	11,334	20	4
‘Hot Parritch’ (Goldilochs)	L 12	10,417	36	6
‘Perfect Parritch’ (Goldilochs)	L 13	24,371	38	5
‘Cold Parritch’ (Goldilochs)	L 14	29,722	16	4
Lochan Feoir	L 15	56,994	12	3
Loch na Cloiche	L 16	46,720	14	5
‘Loch Caora’	L 18	16,531	72	9
‘Loch Reithe’	L 19	25,491	32	5
‘Loch Uan’	L 20	60,762	54	6
‘Loch Earball an Uain’	L 21	70,736	32	12
Loch Airigh an Eilein	L 22	38,094	44	9
‘Loch Caoraich’	L 23	51,704	24	6
‘Loch Caoir-chaorach’	L 24	55,502	16	4
‘Lochan of the Great Diver’	L 27	19,197	34	8
‘Cassius Loch’	L 28	57,060	12	4
Loch Cregan Doire na Suaine	L 30	55,417	18	5
Undetermined reads		580,301	952	24

BLAST

BLAST was performed to compare the 140 final sequences of acceptable quality to existing databases to identify which taxa are present.

The first thing to note with Table 3.4, is that no COI fragments have been identified within the final sequence pool. One problem that can arise from using COI with the iSeq is that the iSeq only outputs sequences that are 150 bp, and the COI fragment is 450 bp – therefore there cannot be sufficient overlap in the middle of the sequences for them to be merged. However, this does not appear to be the issue here as the COI fragments could not be identified anywhere in the data.

Whilst the E-values detailed in Table 3.4 are very small, they are actually too big to be considered a good match for confident species identification. An E-value of 10^{-50} constitutes a 90% match identity, which is appropriate for analysis to family rather than species (Fernández *et al.,* 2018). This is well illustrated in the ecological and locational data for several taxa, which have not been recorded from Britain (*Vorticella sp., Eudiaptomus padanus, Stygocyclopia sp., Allonais pectinata, Bosminopsis zernowi, Cyclestheria hislopi*). Interestingly, one non-zooplankton taxon (*Odontocerum albicorne*) has possibly been sighted in this study (Chapter 4), although it was not identified to species. *O. albicorne* was the only taxa to be identified from Chromosome 15, a genomic region which was not a target amplicon of PCR, likely the result of the non-specific amplification from Nolte primers. Although no conclusions can be drawn about community composition from these findings, the presence of *O. albicorne* indicates that sequencing is still a useful tool for identification of taxa from this region.

Table 3.4, Taxa Identified from BLAST

This table shows the taxa that were identifiable via BLAST. The table shows the species name, the loch it could be found in and the subtype of that loch as determined at the start of this project.
Gene refers to the gene fragment that was used to identify the species, which is ssrRNA in all but one case.
#BLAST Hits refers to the number of fragments that could be identified from the database. This will always be 2 per fragment, as per the complementary nature of DNA. Please note, the ssrRNA gene (aka 18S) is the dsDNA gene that codes for ssrRNA. In brackets is the size of each fragment in base pairs.
E-values denote the likeliness that an alignment occurs by chance, smaller numbers mean a smaller chance.
UK Sighting data is taken from the National Biodiversity Network database (NBN, 2023)

Taxa	Loch ID	Subtype	Gene	# BLAST Hits	E-value
Vorticella sp.	L8 L4 L6	A- B+ S+	ssrRNA	2 (163 bp) 2 (159 bp)	1.70^{-77} 5.96^{-72}
Ecology	Ciliate epibiont, bacterioplanktivore. May use other organisms as a substrate, prey for some rotifers (Gilbert, 2022; Wałach and Blagden, 2023)				
UK Sightings	*Vorticella sp.* are as yet unrecorded in the UK, although *Peridinium cinctum* which has also been named *Vorticella cincta* has confirmed presence on South Uist, Scotland				
Testudinella reflexa	L10	A+	ssrRNA	2 (133 bp)	6.18^{-61}
Ecology	Periphytic Rotifer, cosmopolitan in freshwater (Pontin, 1978)				
UK Sightings	No recorded presence in the UK				
Eudiaptomus padanus	L12 L13 L14 L16 L30 L20 L21 L22 L23 L24	A- A- A- B+ B+ S- S- S+ S+ S+	ssrRNA	2 (133 bp) 2 (163 bp) 2 (167 bp) 2 (168 bp) 2 (171 bp) 2 (172 bp)	6.18^{-61} 3.68^{-74} 4.87^{-78} 2.93^{-80} 6.87^{-82} 5.06^{-78}
Ecology	Calanoid Copepod exclusively recorded from Italy and Croatia. Dominant with highly variable trophy, although likely to be eliminated by *E. gracilis* upon eutrophication (Błędzki and Rybak, 2016)				

UK Sightings	*E. padanus* has no recorded presence in the UK. E. gracilis has a few records in Scotland although none further north than Perth, whilst E. vulgaris has recordings from South East England only				
Stygocyclopia sp. DZMB587	L12 L13 L14 L16 L30 L20 L21 L22 L23 L24	A- A- A- B+ B+ S- S- S+ S+ S+	ssrRNA	2 (177 bp)	3.52-85
Ecology	Cyclopoid Copepod with four recorded organisms in the genus, ubiquitously stygobionts (Jaume and Boxshall, 1995; Jaume, Fosshagen and Illiffe 1999; Jaume, Boxshall and Humphreys, 2001; Belmonte, 2022)				
UK Sightings	No recorded presence in the UK				
Allonais pectinata	L15	B+	ssrRNA	2 (178 bp)	4.05-84
Ecology	Segmented sludge worm, detritovore, previously not identified in Europe (Lee and Jung, 2014; Vargas and Zardoya, 2014).				
UK Sightings	No recorded presence in the UK				
Odontocerum albicorne	L18, S- Chromosome 2 (168 bp) 3.32-80 L19 S- 15				
Ecology	Mortarjoint case-building caddisfly, commonly found in Scotland (Knowler, Flint, and Flint, 2016).				
UK Sightings	Confirmed presence nationally				
Galleria mellonella	L18, L19	S- S-	ssrRNA	2 (149 bp)	1.04-69
Ecology	Greater wax moth, commonly found in the British Isles (Kwadha *et al.*, 2017)				

UK Sightings	Some sightings as far north as Aberdeen, but mostly located in southern England				
Daphnia pulicaria	L18, L19	S- S-	ssrRNA	2 (178 bp)	9.86-86
Ecology	Daphnid Cladocera native to Britain and oligotrophic humic lakes such as those found in Highland Scotland (Błędzki and Rybak, 2016).				
UK Sightings	No recorded presence in the UK				
Acroperus harpae	L18, L19	S- S-	ssrRNA	2 (168 bp)	3.32-80
Ecology	Chydorid Cladocera native to Britain. Cold tolerant, acid tolerant, classified 'Arctic' species with low tolerance for eutrophication (Błędzki and Rybak, 2016).				
UK Sightings	Recorded on South Uist				
Bosminopsis zernowi	L18, L19	S- S-	ssrRNA	2 (149 bp)	1.04-69
Ecology	Bosmoinid Cladocera also known as *B. deitersi,* although genetic differences across distance may indicate sibling species. Not previously recorded in Britian but present in central Europe (Błędzki and Rybak, 2016)				
UK Sightings	No recorded presence in the UK				
Moina sp. 1 JRdW-2005	L18, L19	S- S-	ssrRNA	2 (172 bp)	4.02-69
Ecology	Moinidae Cladocera with two species (*Moina brachiate* and *Moina (Exomoina) macrocopa*) previously identified in Britain (Błędzki and Rybak, 2016).				
UK Sightings	*Moina macrocopa* has 1 unconfirmed sighting near Inverness, all other *Moina* spp. are identified from mid-southern England				
Cyclestheria hislopi	L18, L19	S- S-	ssrRNA	2 (151 bp)	8.17-71
Ecology	Eurytopic, 'clam shrimp', sister group of Cladocera with a wide geographic distribution (Sonia, Ramanibai and Kanniga, 2012)				
UK Sightings	No recorded presence in the UK				

3.4 Discussion

It is clear from the results that the sequencing run failed, however there is still knowledge gained within this work. Specifically, condensation of crude samples, DNA extraction and PCR protocols were optimised. Although the actual sequencing results are not reliable, of the 12 taxa detailed in Table 3.3 half have been recorded from Britain either by the NBN or existing literature. *D. pulicaria* and *A. harpae* are within scope for this project, and have ecological traits in line with the Highland Scotland environment. Caddisfly larvae (potentially *O. albicorne*) were observed during morphological identification, although they were not identified to species. Unfortunately, none of the sequenced taxa were identified during morphological ID (Chapter 4), however if the sequencing data is only reliable to family it is likely close relations to sequenced taxa are extant within Highland Scotland.

Metabarcoding challenges are not new, and genomics in general can be a tricky discipline (Coissac, Riaz and Puillandre, 2012; Piper *et al.,* 2019; Keck, Couton and Altermatt, 2022). Among other issues, problems can arise from primer specificity among zooplankton populations (Bucklin *et al.,* 2016) as observed here. The presence of non-zooplankton within the sequencing data (*G. mellonella, A. pectinate, C. hislopi, O. albicorne*) is likely due to the use of Nolte primers; the forward primer is known to have broad specificity in Eukarya (Nolte *et al.,* 2010). It is unclear why the sequencing run failed, however, with time and continued effort these challenges will be overcome and zooplankton metabarcoding will become a less problematic standard in ecological analysis (Huggett *et al.,* 2022). The work detailed here will provide a good basis for future metabarcoding research in freshwater Highland Scotland.

Conclusion

In summary, the sequencing run failed, but useful information and context can still be harvested from the methodology and data. This experiment is the first attempt to sequence zooplankton from the region and provides a good foundation for future metabarcoding based research.

Chapter 4: Morphological Identification

4.1 Introduction

The identification of organisms by their morphology is manual, and as such restricted by human skill, experience, error, and technology. Advanced imaging techniques like scanning electron microscopes (SEM) allow greater resolution than binocular high-power microscopes, but they are not always available, and identification of diagnostic characteristics is still a difficult human process. This is especially true when observing microfauna such as zooplankton, which can only be seen in detail using microscopes; zooplankton can be complex in structure and difficult to manipulate to see defining characteristics. Even so, the body of research surrounding species morphology, ecology, distribution, and phenotype is well developed, wide ranging and built on centuries of observation. Modern technologies such as deep learning models and flowcell techniques like ZOOSCAN and FlowCAM are still in their infancy (Xiong *et al.,* 2020). There is still no gold standard substitution for the human eye, with the caveat of course that morphological identification is best supported my modern techniques such as metabarcoding.

This chapter will focus on the morphological identification of species from 27 Wester Ross Lochs. Statistical methods will be used to analyse the assemblages and test the loch type, categorisation and hypotheses postulated earlier in this thesis.

4.2 Methodology

Sampling

Zooplankton samples were collected by throwing a 200 mm diameter, 40µm mesh plankton net ~5m and drawing it back to the bank. Net draw occurred 3 times at 45º, 90º and 135º to bank edge respectively. At time of sampling fish, invertebrate and floral presence were recorded. Zooplankton were transported back to the field lab where they were euthanised and stored in 75% ethanol.

Microscopy

Samples were (except for L1) diluted to 250 ml in a Stempel pipette and homogenised for 5 minutes before being decanted into a 27.5 mL Bogorov cell. Organisms were then identified, measured, and counted under a microscope at 40x, 100x and 400x magnifications. Planktonic crustacea were identified according to Błędzki and Rybak (2016) whilst Rotifera were identified using Pontin (1978).

One full 250 mL Stempel pipette represented 471.3 L of loch water. Each sample was investigated for 12 hours, with total volume observed ranging from 20 mL to 250 mL dependent upon the richness of each sample. Species numbers were adjusted to be representative of the total volume sampled.

Statistical Analysis

Principal Component Analysis (PCA) was performed on taxonomic groups to discern their relationships to each other. At a species level, Two-Way Indicator Species Analysis (TWINSPAN) via WinTWINS v2.3 2005 was used to establish loch groupings based upon species composition and provide a comparison for the loch types used in this study. Rare species (<2 occurrences) and *K. cochlearis* were excluded as indicators; new research indicates that *K. cochlearis* is a complex (Cieplinski, Obertegger and Weisse, 2018) with a diverse range of ecological requirements. As in-depth analysis of morphotype was beyond the scope of this project the taxa is considered 'cosmopolitan' and not representative of environmental factors. Community composition of TWINSPAN groups and loch types was calculated by using indi/L to ascertain the % contribution of each taxon to each loch, then averaging the values for each group to avoid skew from dominant organisms.

Normality and variance tests (ANOVA) (visible in Appendix C) were performed on Minitab 20.2, which analysed the differences between Cladocera, Copepod and Rotifer populations within the TWINSPAN groups, Kruskall-Wallace for the differences between loch and subtypes, and Mann-Whitney for fish presence and absence. Simpson's Diversity Index (SDI) was performed to identify diversity within each sample and within loch groupings. The formula is as follows;

$$D = \frac{\sum n\,(n-1)}{N(N-1)}$$

Where n was equal to the number of individuals of a single species and N was equal to the number of individuals in the population. Calculation of SDI was followed by analysis with Minitab, where ANOVA, Kruskall Wallace and a 2-Sample T-Test were performed to establish if there were differences in diversity between loch type and subtype, TWINSPAN groupings and fish presence/absence respectively.

Finally, Canonical Correspondence Analysis (CCA) and Redundancy Analysis (RDA) were performed on Canoco 5 v.5.15 to illustrate the relationships between samples, species, and environmental factors. Fish and invertebrate (Inverts) presence/absence was converted to nominal values where 1 equalled presence and 0 equalled absence. Loch Maree (L26) was excluded from statistical analyses owing to its position as a reference loch within the sample set. The entire sample pool was

examined using CCA, however analysis of ecological and environmental factors was either CCA or RDA dependent upon the variables under study. Determination of test (RDA or CCA) was based upon response data gradient, whereby a gradient <2 Standard Deviation units (SD) determined RDA and a gradient >2 SD determined CCA. Variables (Table 5.1) were split into three groups. 'Physical', including area, DisCoast and altitude. 'Organic', including fish presence and absence, #Taxa (total number of individuals in each sample) NH_4, DOC, NO_3^- and O_2. 'Inorganic', including conductivity, hardness, salinity, pH, Zn^{2+}, Mg^{2+}, Al^{3+} and K^+. Community composition was analysed through subtype, type and fish presence and absence, with additional analysis occurring where zooplankton were classified by known ecological preferences: body size, biome, depth, lake size, pH, trophy, macrophyte coverage and zone. Taxa with no known preference (NkP) were grouped.

Table 5.1, Zooplankton Ecological Preferences and Ranges

*Classifications denoted with * indicate that species may be counted in multiple sub-classifications as per their ecology.*

Classifications denoted with † have sub-classification definitions that deviate from those previously stated, or entirely unstated within this writing. In this instance definitions of those sub-classifications are deferred to the references noted.

Classification	Sub-classification	# Taxons	References
Biome*	New/Temporary	20	Pontin, 1978; Bērziņš and Pejler, 1989; Błędzki and Rybak, 2016; Novichkova *et al.*, 2020
	Palearctic	11	
	Shaded	1	
	Swamp	21	
	NkP	18	
Body size	<0.5 mm	9	Pontin, 1978; Błędzki and Rybak, 2016
	0.5 – <1.5 mm	29	
	1.5 – <2.5 mm	15	
	>2.5mm	6	
Depth†	Deep	5	Pejler and Bērziņš, 1989; Adamczuk, 2014; Błędzki and Rybak, 2016; Świdnicki et al., 2016
	Shallow	21	
	Variable	14	
	NkP	19	
Lake size†	Large	6	Pontin, 1978; Błędzki and Rybak, 2016
	Small	20	
	Variable	17	
	NkP	16	
Macrophytes†	Mid	19	Pontin, 1978; Dole-Olivier et al., 2000; Kuckzyńska-Kippen, 2001; Ratushnyak and Trushin, 2007; Adamczuk, 2014; Błędzki and Rybak, 2016; Karpowicz and Ejsmont-Karabin, 2021;
	Poor	4	
	Rich	5	
	Variable	9	
	NkP	26	

pH	Minimum <6	30	Locke, 1991; Maier, 1990; Hessen, Faafeng and Andersen, 1995; Walseng and Schartau, 2001; Jersabek, 2013; Anufriieva, Hołyńska and Shadrin, 2014; Jersabek, 2015; Jersabek, 2016; Błędzki and Rybak, 2016; [1]Jersabek, 2017; [2]Jersabek, 2017; Jersabek, 2018; Jersabek, 2020
	Minimum 6-7.5	12	
	NkP minimum	17	
	Maximum <7.5	12	
	Maximum >8	22	
	NkP maximum	26	
Trophy*	Eutrophic	16	Gannon and Stemberger, 1978; Esjmont-Karabin, 2012; Xue *et al.,* 2014; Błędzki and Rybak, 2016; Mnatsakanova, 2016; Kuckzyńska-Kippen, Klimaszyk and Piotrowics, 2017; Jaturapruek, Fontaneto and Maiphae, 2021
	Mesotrophic	19	
	Oligotrophic	21	
	NkP	29	
Zone*	Benthic	11	Matveeva, 1986; Maier, 1992; Korovchinsky, 2000; Adamczuk, 2014; Shumka, 2014; Kattel *et al.*, 2015; Skála, 2015; Błędzki and Rybak, 2016; Wærvågen and Andersen, 2017; Gaponova, 2019; Karpowicz and Ejsmont-Karabin, 2021
	Littoral	34	
	Pelagic	2	
	NkP	13	

4.3 Results

'Matchless' (L2) and 'Tiny' (L3) were excluded as they were too shallow to collect a zooplankton sample from. Loch nam Breac (L1) was similarly excluded as no zooplankton were found.

From the 26 sites in the final analysis 675 individual zooplankters from 68 taxa were identified. Following adjustment for equivalent sample volume, taxa concentration was 0.8 indi/L. The most common taxa were *B. longiros*tris, *P. pediculus*, *B. longispina*, *S. crystallina*, and *A. elongata* (Cladocerans), *L. macrurus* (Copepod), *K. longispina,* and *K. cochlearis* (Rotifers).

PCA of Taxonomic Groups

A PCA plot (Fig. 4.1) of total taxa indicate that there is a positive correlation between rotifers and Copepods, and Copepods and Cladocerans, whilst rotifers and Cladocerans are negatively correlated. Axes 1 and 2 are shown, with Eigenvalues of 0.4055 and 0.3960 respectively. Further analyses with loch type, subtype and fish presence/absence show that these metrics explain <21% of variation within the sample and are statistically insignificant.

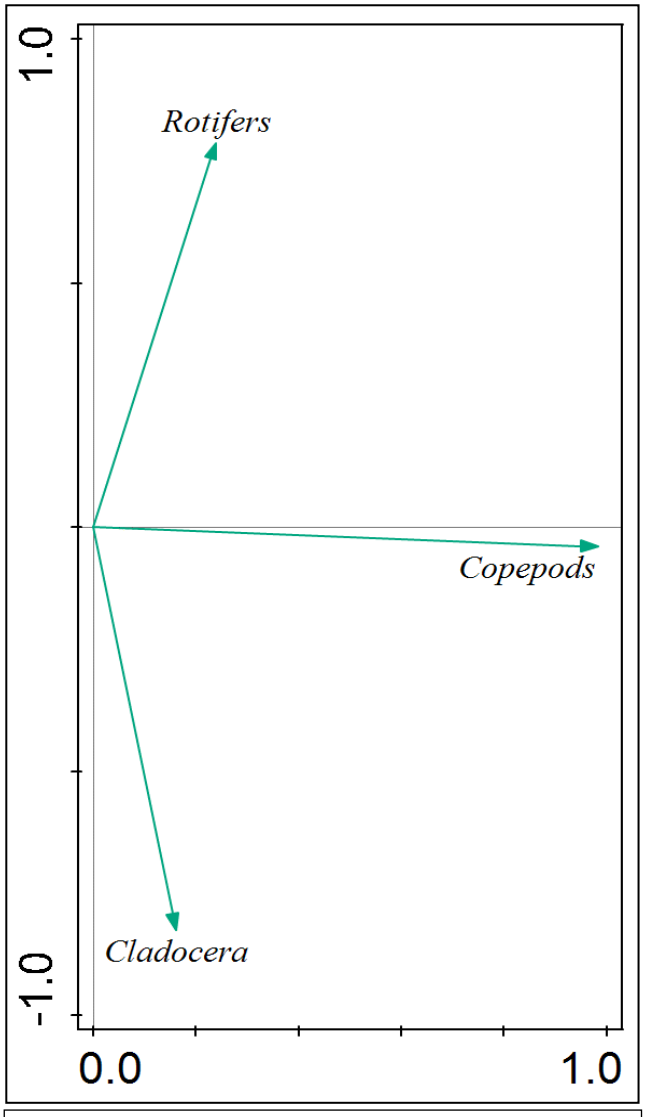


Figure 4.1, PCA of Total Cladoceran, Copepod and Rotifer Populations

Twinspan

TWINSPAN analysis is shown as a dendrogram in Fig 4.2, and as venn diagrams in Fig 4.3. Fig 4.2 demonstrates three levels of division of the 26 sample sites yielding four terminal groups with *A. ecaudis, P. pediculus, O. tenicaudis, B. longispina, S. crystallina, Bdelloid spp., B. longicornis* and *K. longispina* as indicators. The first of the four terminal groups, Group 3 (G3) is separated with *A. ecaudis* as the only indicator. Loch Dubh Dughaill (L10) and 'Little Dughaill' (L11) are close together and geographically isolated from the other altitude lochs, as can be seen in Figure 2.1. The second terminal group, Group 5 (G5) is predicated on the presence of *Bdelloid spp.* All except 'Lilyloch' (L8), 'Cold Parritch' (L14) and 'Lochan of the Great Diver' (L27) have fish presence. L8 and L27 have known planktivorous invertebrates (caddisfly larvae and newts). Excepting Loch na Fiedil (L6, stock), all lochs in this sample are from the Bird or Altitude types. Group 6 (G6) exclusively contains Stock Lochs, three with fish absence (L19 – L21) and two with fish presence (Loch Airigh an Eilein [L22] and 'Loch Caoir-chaorach' [L24]). The final

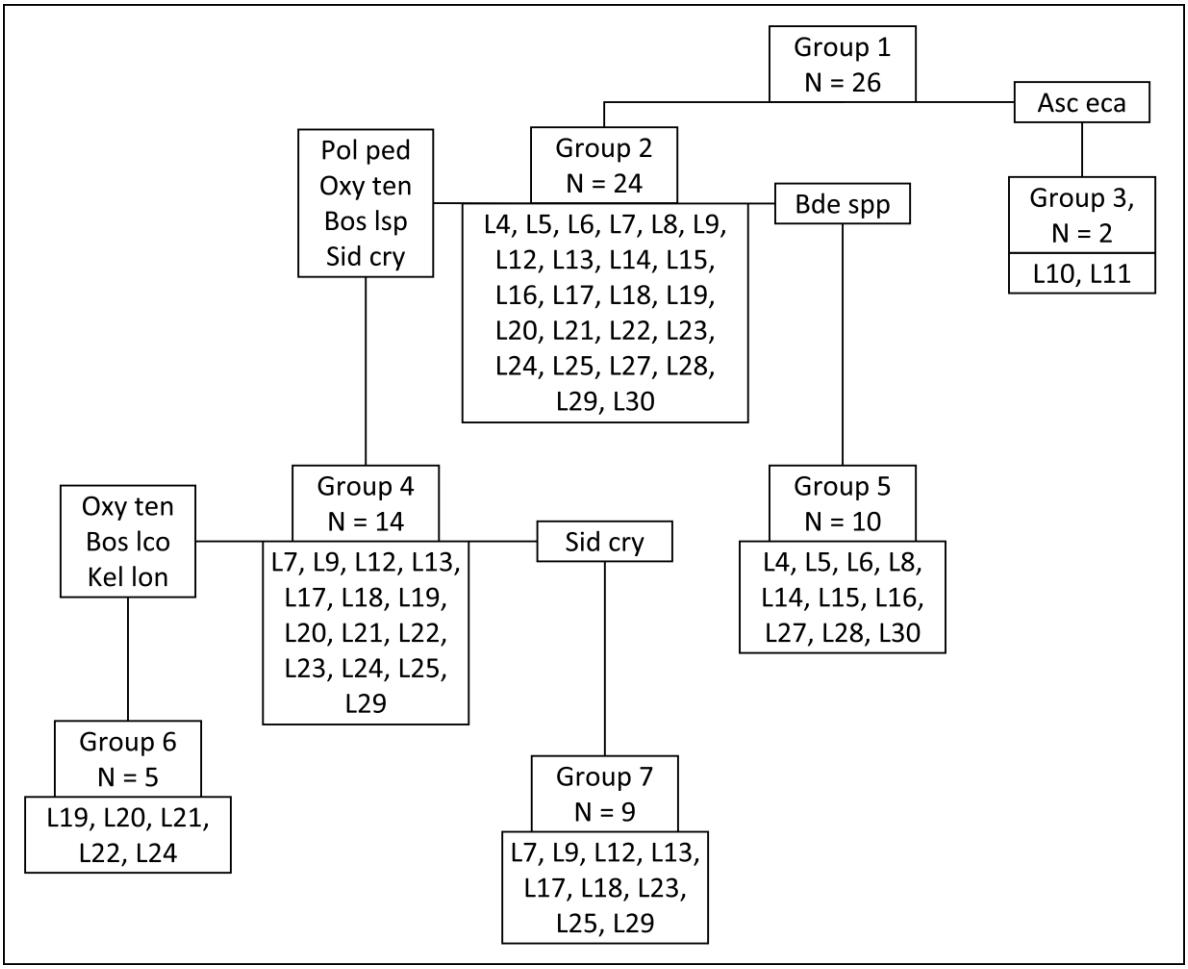


Figure 4.2, TWINSPAN dendrogram showing site classification and indicator species for the total zooplankton assemblage. Figure 2.5, PCA plot for Lochs

group, Group 7 (G7), contains four lochs with fish presence from all types: Loch Boor (L7) and Loch Bad an Sgalaig (L25) (Bird), Loch nam Buainichean (L9)(Altitude), and 'Loch Caoraich' (L23) (Stock). The remaining lochs are fishless and similarly from all types; 'Hot Parritch' (L12) and 'Perfect Parritch' (L13) (Altitude), 'Lochran' (L17) and Loch Meall a'Bhainne (L29) (Bird), and 'Loch Caora' (L18) (Stock). Species abbreviations can be seen in Appendix B.

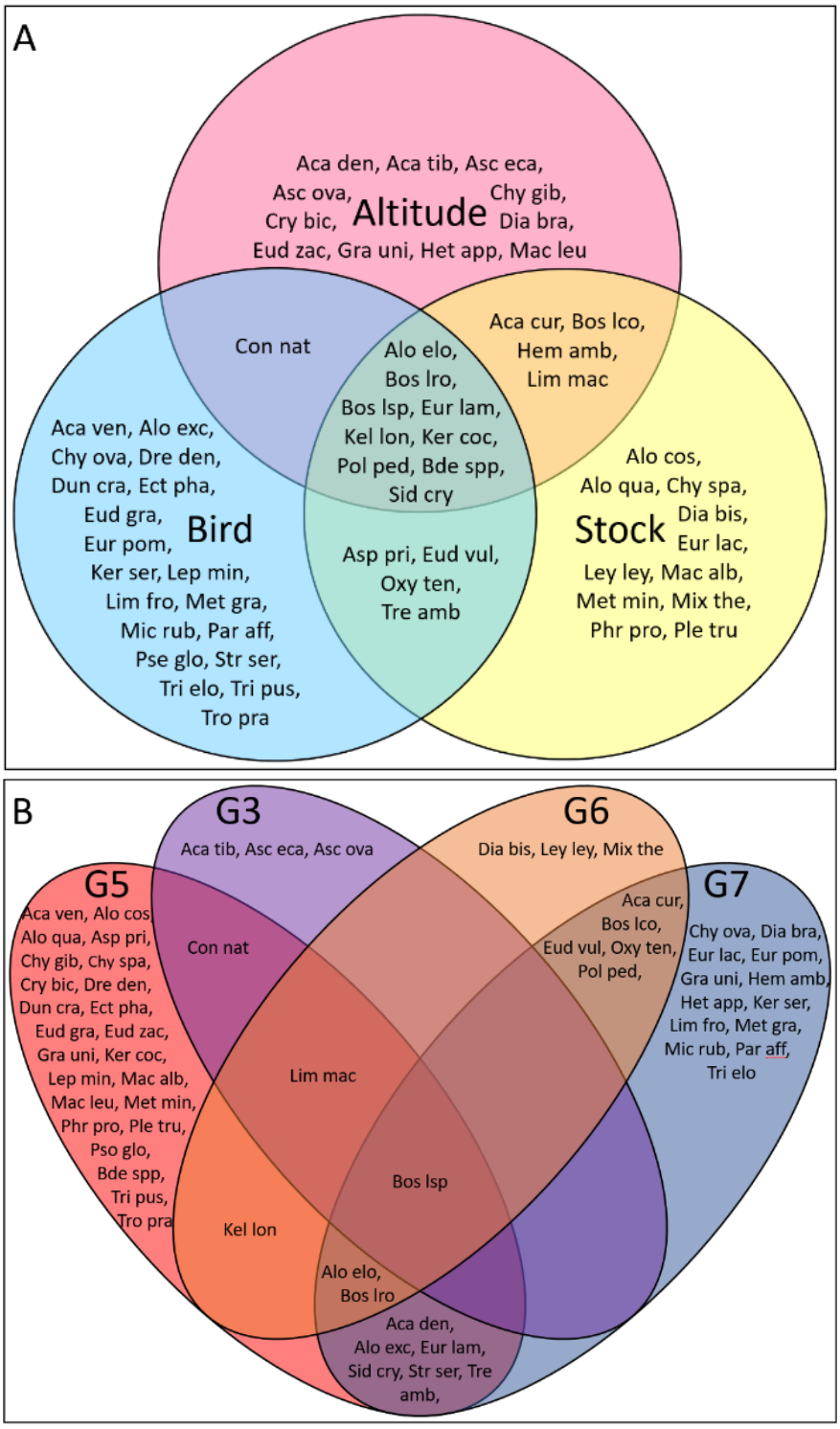


Figure 4.3, Venn Diagrams Highlighting Taxa Associations Across Different Groupings

Colour representations are as follows; altitude, bird, stock, G3, G5, G6 and G7. For species abbreviations please see Appendix B.

Group Composition

Figure 4.4 shows composition of the zooplankton assemblage across loch type, fish presence/absence and TWINSPAN terminal groups. Cladoceran, Copepod and Rotifer richness made up 44.43, 44.10 and 11.47% of the total assemblage respectively. Fish only had a significant impact (*P = 0.016)* on the Rotifer population, whose contribution to the assemblage changes from 2.11 to 16.02% in their presence. Cladocera alone were impacted by loch type (*P = 0.038)*. No statistically relevant findings could be observed for subtype. TWINSPAN groups show differences in the Cladoceran (*P = 0.014*) and Rotifer (*P = 0.041*) volumes.

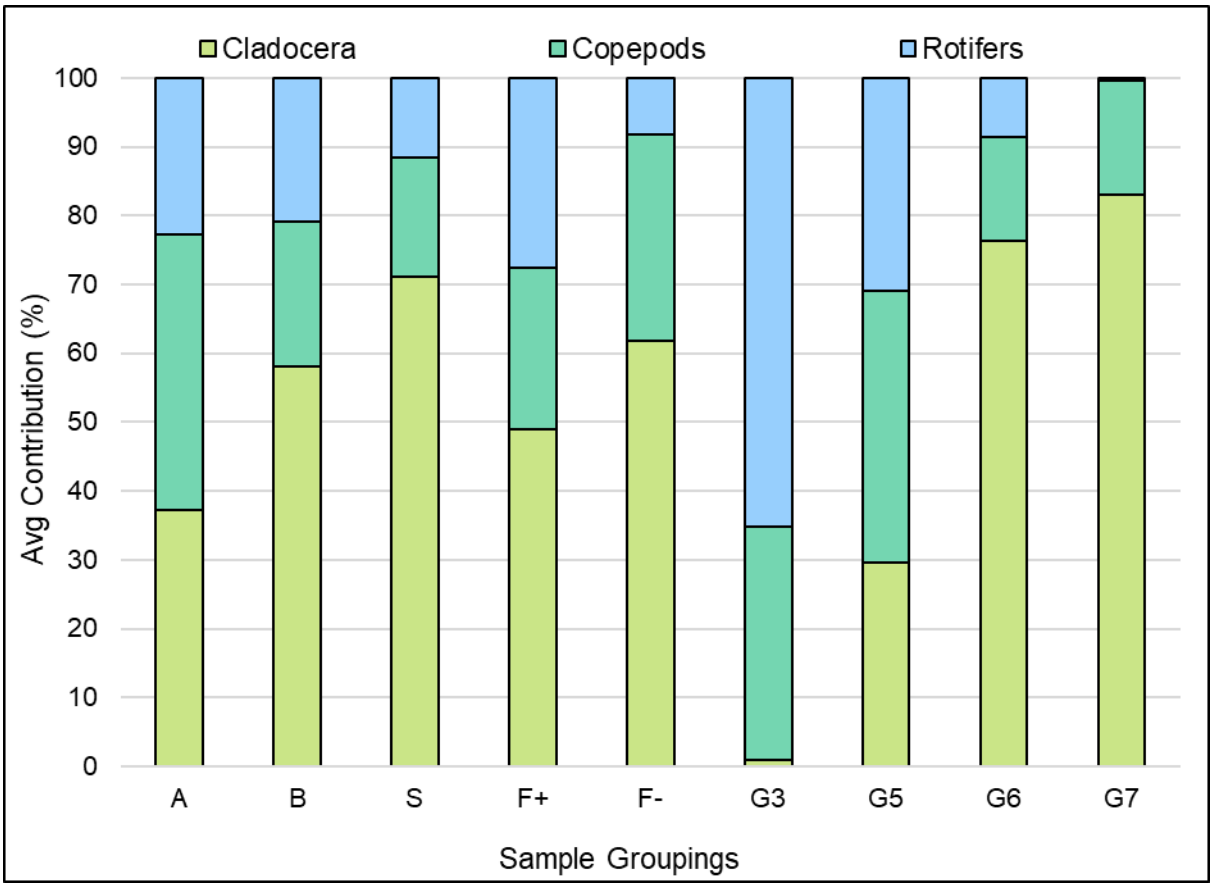


Figure 4.4, Zooplankton Assemblage Across Loch Types, Fish Presence/Absence and TWINSPAN Terminal Groups

Simpson's D

Simpson's Diversity Index (SDI) shows that almost half (44%) of all sampled lochs have high diversity with SDI scores >0.66. Low diversity was observed in Loch nam Buainichean (L9), Loch Dubh Dughaill (L10) and 'Cassius Loch' (L28) (A+), 'Lochan of the Great Diver' (L27) (A-), Loch Boor (L7), Loch na Cloiche (L16) and Loch Cregan Doire na Suaine (L30) (B+) and Loch Airigh an Eilein (L22) (S+). A+ displayed (0.024 SDI), significantly lower ($p = 0.047$) diversity than the other subtypes. Statistically, TWINSPAN groupings had the same diversity. G3 (Loch Dubh Dughaill [L10] and 'Little Dughaill' [L11]) had the lowest (0.226 SDI).The reference loch, Loch Maree had an SDI of 0.76. Dominance of a species can be determined using Simpson's D and proportions, and is shown in Table 4.1.

Table 4.1, Dominance of Taxa and their Ecology

Loch ID	Loch Type	Dominant Species	Proportion of Sample	SDI
L7	B+	*Sida crystallina*	96%	0.071
L9	A+	*Diaphanosoma brachyurum*	98%	0.033
L10	A+	*Conochilus natans*	99%	0.007
L16	B+	*Keratella cochlearis*	93%	0.117
L22	S+	*Polyphemus pediculus*	85%	0.261
L27	A-	*Acanthodiaptomus denticornis*	99%	0.004
L28	A+	*Cryptocyclops bicolor*	98%	0.033
L29	B-	*Acanthocyclops venustus*	99%	0.018

Canonical Correlation Analysis

Figure 4.5 shows the relationship between species, samples and environmental factors as analysed via CCA, with only the 20 best fitting species displayed. Total variation is 8.254 with explanatory values accounting for 83.46% total variation. Axis 1 - 4 Eigenvalues were 0.8196, 0.7833, 0.7405 and 0.7189 respectively. Axis 1 and 2 are shown, where axis 1 is associated with conductivity, Mg^{2+}, salinity and O_2, which are negatively correlated with #Taxa and NH_4. Axis 2 is associated with fish and invertebrates, which are negatively correlated with DOC. Axis 3 (not shown) is most associated with DOC and salinity, which are negatively correlated with altitude. Axis 4 (not shown) is most associated with Zn^+ and negatively correlated with NH_4. The TWINSPAN indicators are visible as best fitting species in Figure 4.5, except *K. longispina* (*Kel lon*), which had strongest associations with axis 3, and is not shown.

Loch type has been illustrated in (Fig. 4.5a). pH, fish, and invertebrates trend together along Axis 2 and have a negative trend with DOC. The stock lochs are clustered on the left-hand side of Axis 2, and trend along the fish-DOC gradient, showing mostly strong-medium associations with salinity, conductivity, and O^2, and Mg^{2+} which trend together. All stock lochs show a weak negative association with altitude and DisCoast.

The bird lochs are mostly spread in relation to fish, with varying associations to area, #Taxa and Zn^+. #Taxa correlates with fish supporting the abundance findings (Fig. 4.4). ‘Lochran’ (L17) and Loch Meall a-Bhainne (L29) (B-) show strong association with the stock lochs, whilst Loch na Fiedil (L6, S+) shows a strong association with the bird lochs.

66% of the A- lochs have a weak association to altitude and sit along the Fish-DOC gradient, with varying associations to #Taxa. Those lochs that have a stronger association to altitude and DisCoast are additionally pulled by other metrics such as #Taxa, DOC, NH_4 and Zn^+.

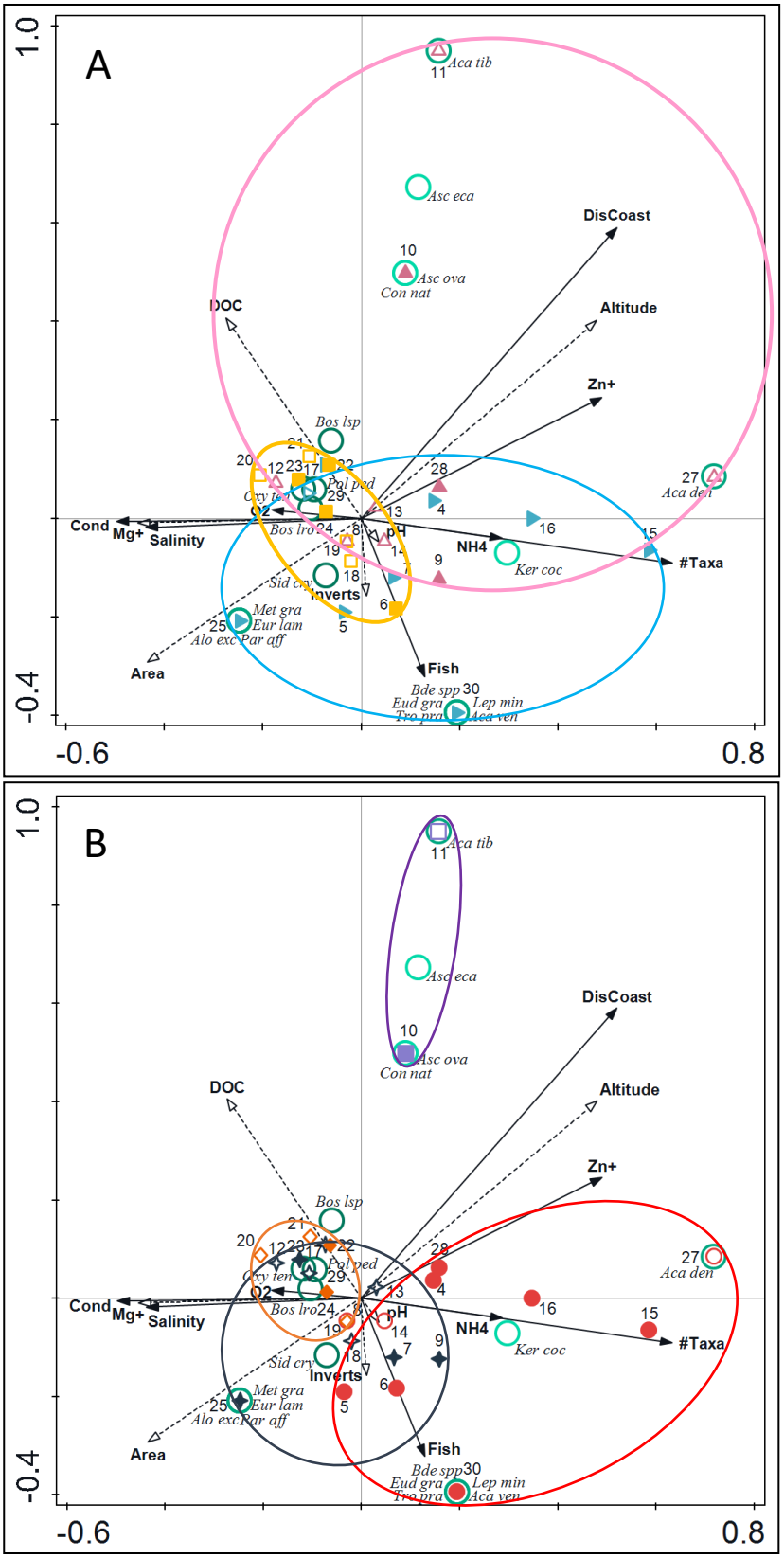


Figure 4.5, CCA for Total Zooplankton Assemblage with Type, Category and TWINSPAN Groupings

These graphs illustrate the relationship between species, samples, and environmental factors. Solid lines with filled arrows and dashed lines with hollow arrows indicate a statistically significant and insignificant variable respectively. Filled and hollow symbols represent fish presence and absence respectively. ○ indicates a species, where the lightest, medium, and darkest hue represents Rotifers, Copepods and Cladocerans respectively. Graph A highlights loch category whereby ▲, ▶, and ■ represent altitude, bird, and stock lochs respectively. Graph B shows TWINSPAN groupings, where ■, ●, ♦, and ✦ represents Groups 3, 5, 6 and 7, respectively.

Graph B shows clear clustering for Groups 3 and 6. G6 exclusively contains the stock lochs that are strongly pulled by Axis 4 and DOC, whilst G3 contains the two altitude lochs that are affected by DOC and DisCoast. G5 is mostly affected by fish presence and #Taxa with varying degrees of association to altitude, area, invertebrates, Zn^{+}, and DisCoast. conductivity/Mg^{2+}/O_2 which trend together. G7 sits mostly along the pH gradient with varying associations to fish, #Taxa, area, invertebrates, DOC and the variables along axis 4.

'Lilyloch' (L8), 'Cold Parritch' (L14), 'Loch Caora' (L18) and 'Loch Reithe' (L19) appear weakly affected by fish, despite being classified fishless. Loch Airigh an Eilein (L22), 'Loch Caoraich' (L23) and 'Loch Caoir-chaorach' (L24) are affected by fish absence whilst being fish lochs. Taxa appear mostly affected by area, and the fish-DOC correlation, with a few other associations. Positively affected by fish presence and localised around Loch Cregan Doire na Suaine (L30) are the unknown Bdelloid spp. (*Bde spp.*), *E. gracilis* (*Eud gra*), *L. minutus* (*Lep min*), *T. prasinus* (*Tro pra*), and *A. venustus* (*Aca ven*). With strongest associations to area and localised around Loch Bad an Sgalaig (L25) are *E. lamellatus* (*Eur lam*), *M. gracilis* (*Met gra*), *A. excisa* (*Alo exc*) and *P. affinis* (*Par aff*). Most associated with the DOC-axis 4 correlation and the stock lochs/G6 and G7 are *O. tenuicaudis* (*Oxy ten*), *P. pediculus* (*Pol ped*), *B. longirostris* (*Bos lro*), and *B. longispina* (*Bos lsp*). Correlating tightly with G3 are *C. natans* (*Con nat*), *A. ecaudis* (*Asc eca*), *A. ovalis* (*Asc ova*), and *A. tibetinus* (*Aca tib*). *K. cochlearis* (*Ker coc*) was strongly associated with #Taxa and NH_4, whilst *A. denticornis* (*Aca den*) was positioned between #Taxa and the Zn^{+}-DisCoast correlation with 'Lochan of the Great Diver' (L27). *S. crystallina* (*Sid cry*) was mostly related to area and invertebrates.

Table 4.2, Physical variables affecting ecological preferences of zooplankton

For Tables 4.2 – 4.4, ‡ indicates variables which are significant only as conditional term effects.

Variable	#Sig	Metric	Parameter	P-value	Conditional Term Effects
Area	1	Body Size	<0.5 mm	0.0221	
Altitude	5	Biome*	Swamp*	0.0019	
		Macrophytes†	Rich†	0.0087	
		pH	Minimum NkP	0.0377	
			Maximum NkP	0.0329	
		Trophy*	NkP*	0.0306‡	DisCoast
DisCoast	6	Biome	NkP	0.0039	
		Lake Size†	NkP†	0.0108	
		Macrophytes†	Mid†	0.0023	
		Trophy*	NkP*	0.0003	
		Zone*	Littoral*	0.0496	

		Total Assemblage	0.0070	

Table 4.3, Organic variables affecting ecological preferences of zooplankton.

Variable	#Sig	Metric	Parameter	P-value	Conditional Term Effects
DOC	7	Biome*	NkP*	0.0198‡	O_2
		Depth†	NkP†	0.0319	
		Lake Size†	Small†	0.0052	
		Macrophytes†	Mid†	0.0086	
		pH	Minimum <6	0.0319	
			Maximum NkP	0.0209	
		Zone*	Littoral*	0.0417	
Fish	10	Biome*	Palearctic	0.0269	
		Depth†	Variable†	0.0494	
			NkP†	0.0387	
		Macrophytes†	NkP†	0.0288	
		pH	Maximum NkP	0.0308	
		Trophy*	Eutrophy*	0.0324	
			Mesotrophy*	0.0459	
			Oligotrophy*	0.0381	
		Zone*	Pelagic*	0.0185	
		Total Assemblage		0.0432	
NH_4	3	Biome*	New/Temporary*	0.0450	
		Body Size	1.5 – 2.4 mm	0.0443	
		Total Assemblage*		0.0107‡	Conductivity, DisCoast, O_2, Salinity, #Taxa, Zn^+
NO_3^-	1	Macrophytes	Rich	0.0350‡	O_2
O_2	8	Biome*	New/Temporary*	0.0063	
			NkP	0.0350	
		Depth†	Deep†	0.0323‡	Fish, NH_4
		Lake Size†	NkP†	0.0260	
		Macrophytes†	Mid†	0.0320	
			Rich†	0.0361‡	NO_3^-
		Trophy*	NkP*	0.0298	
		Total Assemblage		0.0185	
#Taxa	9	Biome*	New/Temporary*	0.0208	
		Body Size	0.5 – 1.4 mm	0.0301	
			1.5 – 2.4	0.0329	
		Depth†	Shallow†	0.0380	
		Lake Size†	Small†	0.0382	
		Macrophytes†	NkP†	0.0133	
		pH	Maximum NkP	0.0335	
		Zone*	Littoral*	0.0274‡	DOC
		Total Assemblage		0.0081	

Table 4.4, Inorganic variables affecting ecological preferences of zooplankton.

Variable	#Sig	Metric	Parameter	P-value	Conditional Term Effects
Conductivity	4	Biome*	Swamp*	0.0305	
		pH	Minimum NkP	0.0094	
			Maximum NkP	0.0266	
		Total Assemblage*		0.0348‡	DisCoast, NH_4, O_2, Salinity, #Taxa, Zn^+
Hardness	2	Biome*	New/Temporary*	0.0208	
		Trophy*	NkP*	0.0354	
K^+	2	Body Size*	<0.5 mm	0.0316‡	Ca^{2+}
		Macrophytes†	Variable†	0.0052	
		pH	Minimum NkP	0.0331‡	Cond, Zn^+
Mg^{2+}	3	Biome*	Swamp*	0.0391	
		Macrophytes†	Mid†	0.0159‡	Salinity
		pH	Minimum NkP	0.0247	
Salinity	11	Biome*	New/Temporary*	0.0155	
			Swamp	0.0093	
		Lake Size†	Small†	0.0311	
		Macrophytes†	Mid†	0.0130	
		pH	Minimum <6	0.0436	
			Minimum NkP	0.0261	
			Maximum NkP	0.0031	
		Trophy*	Mesotrophy*	0.0341	
			Oligotrophy*	0.0184	
		Zone*	Pelagic*	0.0181	
		Total Assemblage		0.0215	
Zn^{2+}	4	Body size	>2.5 mm	0.0398	
		Macrophytes†	Poor†	0.0453	
		pH	Minimum NkP	0.0485‡	Cond, K^+
		Total Assemblage*		0.0374‡	Conductivity, DisCoast, NH_4, O_2, Salinity, #Taxa

The variables affecting different ecological preferences are visible in Tables 4.2, 4.3 and 4.4. Of the physical variables, altitude and DisCoast had the strongest effects, affecting 5 and 6 ecological metrics, although only DisCoast affected the total assemblage. Fish, O_2 and #Taxa were strong organic effectors, showing relationships with >7 different metrics and the total assemblage. Salinity was the biggest inorganic effector, with significant relationship to 11 metrics and the total assemblage. Organisms with NkP for pH were affected by multiple parameters. Those with a minimum NkP were strongly affected by inorganic factors (salinity, conductivity, K^+, Mg^{2+} and Zn^{2+}) and altitude, whilst those with maximum NkP were affected by factors from all three classes of variable (altitude, DOC, #Taxa, conductivity, and salinity). Organisms with a known preference (kP) for new/temporary biomes showed significant relationships to organic (fish, O_2, NH_4 and #Taxa) and inorganic variables (hardness and salinity). Organisms with a kP for swamp biomes exhibited a significant relationship with inorganic variables (conductivity, Mg^{2+} and salinity) and altitude. Organisms with a kP for small$^+$ lakes showed a relationship with DOC, salinity and #Taxa, whilst those with NkP were correlated with DisCoast and O_2. Organisms with a kP for variable and large lakes exhibited no statistical relationships.

4.4 Discussion

The final section of Chapter 4 investigates zooplankton community composition and diversity of 27 sampled sites and challenges the initial categorisation and type allocation of sites at the start of this project with statistical analysis.

Fish, Diversity, and the Zooplankton Assemblage

The effect of fish on the zooplankton assemblage is long documented in the discussion of bottom up (BU) vs top down (TD) controls. The G5 lochs have the most equally weighted assemblage of all the groups, with the Cladocera, Copepods and Rotifers making up 29.63, 39.50 and 30.87% of the assemblage respectively. Predation seems to be a major factor for this group, with the fishless lochs, 'Lilyloch' (L8), 'Cold Parritch' (L14) and 'Lochan of the Great Diver' (L27) having noted Pleurodelinae or *Trichoptera* larvae, and *Utricularia* is present in Lochan Feoir (L15) and Loch na Cloiche (L16). Findings indicate that zooplankton species richness is enhanced in the presence of fish, as evidenced by the change in rotifer volume. Further, zooplankton densities in fish present waters averaged 1.00 indi/L, whilst densities in fishless waters averaged 0.57. Increased richness is expected in lakes with complex fish communities, where larger predatory zooplankton are suppressed by multiple feeding strategies and small zooplankton can become established (Donald *et al.,* 2001; Tiberti, Hardenberg and Bogliani, 2014).

'Lochan of the Great Diver' (L27, A-) and Loch Meall a'Bhainne (L29, B-) are the only fish negative lochs to host a dominant taxon. In both cases taxa are large (>2.5 mm) predators (*A. denticornis* and *A. venustus* respectively), an expected norm for a fishless lake (O'Brien, 1979). It is possible that the low pH (5.5 and 6 respectively) restricts invasion of small zooplankton (Arnott and Vanni, 1993). Where fish are present and diversity is low, three of the taxa are small (<1 mm), indicating a non-complex fish community (possibly the result of independent fish stocking). The large predatory *P. pediculus* is likely able to survive owing to the presence of plant refuges in much the same way as *S. crystallina* (Timms and Moss, 1984).

Loch Maree has a long history of fishing and management, and undoubtedly contains a complex fish community, which is reflected in the SDI.

Loch Groupings – Allochthonous Type and TWINSPAN.

Initial loch groupings into type and subtype were postulated and supported by physical and chemical data detailed in Chapter 2. Observation of the assemblage indicates that only Cladocera volumes were affected by loch type, having a reduced volume in the altitude lochs. Subtype had no statistical bearing on the assemblage. TWINSPAN analysis created four terminal groups, which indicate that a higher proportion of Cladocera does not occur with a high proportion of Rotifers and vice versa. This pattern typifies the ability of Cladocera, especially large-bodied organisms to suppress Rotifer populations via mechanical interference competition and exploitative competition (MacIsaac and Gilbert, 1991; Gilbert, 1988). Of these, G6 alone supports the grouping of lochs into types postulated at the start of this thesis.

Stock lochs are the only robust loch typing, supported by the G6 TWINSPAN calculation, and mostly condensed positioning within the CCA (Fig. 5.1) showing associations with salinity and the fish-DOC anticorrelation. The literature shows some limited support for the correlation of salinity and DOC, although most studies focus on brackish or marine environments rather than ‘mildly saline’ freshwaters. The hard cap for classification as brackish is 0.05% salinity, and as the highest salinity noted in these samples is 0.041% (L20, ‘Loch Uan’) none qualify. Regardless, studies show that DOC and dissolved inorganic carbon (DIC) concentrations tend to be elevated in more saline environments, and it is possible those trends are mirrored here, albeit at a reduced level (Harvey, Kratzer and Andersson, 2015; Song *et al.,* 2019; Lee, Kim and Kim, 2020). The presence of stock lochs clustered around the salinity gradient is unsurprising as they are (excepting L6, Loch na Fiedil) located at a low elevation and a short DisCoast. The presence of L6 outside of the stock loch ‘cluster’ (Fig. 4.5), G4 (Fig. 4.2), and the presence of non-stock, non-G6 lochs within the ‘cluster’ indicates that community composition for this loch type more likely stems from localised physiochemical factors than mammalian allochthonous inputs.

These findings indicate rejection of H_{A2}, which stated that “zooplankton community composition will be significantly influenced by allochthonous inputs, and the nutrient values found within the loch types, with those of higher inputs having a larger community (either of a dominating organism or a more diverse composition) than those of lower inputs”. Cladoceran reduction in relation to loch type is not enough to

satisfy this hypothesis, especially when it cannot be established whether nutrient levels are different between loch subtypes owing to the inability to measure total nitrogen (TN) and the low levels of TP detailed in Chapter 2.

Community Composition and Environmental Factors

Previous investigations into Scottish taxa indicate that the most important explanatory variable for crustacean zooplankton was the percentage of organic carbon in surface sediments ([1]Kernan *et al.,* 2009). Whilst surface sediment was not measured, Cladocera in this study show a strong association with DOC and the lochs with macrophyte presence (G4) that are likely to have higher sedimentary carbon. G4 macrophyte populations include emergent grasses, *Meyanthes trifoliata*, Nymphaeaceae and *Lobelia,* environments that *S. crystallina* and *O. tenicaudis* have known preference for (Błędzki and Rybak, 2016). The separation of G6 from G4 was predicated on the absence of the *S. crystallina* and the presence of *O. tenicaudis, B. longicornis* and *K. longispina.* 'Loch Reithe' (L19) and Loch Airigh an Eilein (L22) of G6 both had emergent grass presence, with the other three sites having *Meyanthes trifoliata* ('Loch Uan' [L20], 'Loch Earball an Uain' [L21]), Nymphaeaceae (L21, 'Loch Caoir-chaorach' [L24]) and *Lobelia* (L24). *S. crystallina* has a strong association to *Phragmites australis* and *Paspalum distichum* with little regard for fish predation (Choi *et al.,* 2016), which may affect the difference in assemblage between these groups.

The lochs with a negative association to DOC exist mostly within G5 and have varying associations to fish and #Taxa with a higher pH. Limiting the study, the indicator for this group is unfortunately not identifiable to species; identification of Bdelloids is difficult upon death (Turner, 1999) and all organisms were euthanised prior to identification. Body size for this group was made up of organisms mostly 0.5 – 1.4 mm in length, supporting the impact of fish upon this cohort. Whilst the overall richness of taxa can be increased in combination with fish presence, and some assemblages are impacted, the presence of taxa who ignore fish presence in the samples under study indicate a rejection of H_{A3}. *S. crystallina, B. longirostris, E. gracilis* and *M. albidus* are known to have little regard for fish presence, either by the ability of subitaneous eggs to pass through fish digestive systems (Bartholmeé *et al.,* 2005), or their epiphytic ecology (Choi *et al.,* 2016). This hypothesis is a

localised version of the persisting top-down bottom-up debate, which seems to be resolving as scientists iron out the complexities of aquatic ecologies. In this instance, as with wider findings, the hypothesis must be rejected; the debate cannot be simplified down to a binary equation. Bottom-up and top-down processes that are prevalent in one location may have no bearing on another (Skala, 2003, quoted in [1]Kernan, 2009), their affects completely bypassing taxa dependent upon ecologies, preferences, and other environmental pressures (Bhele *et al.,* 2022). As such these controls are somewhat site and taxa specific, and each habitat should be examined with this in mind.

Conclusion

The zooplankton assemblage supports existing literature that indicate a complex fish community produces a richer assemblage than a non-complex or absent fish community. The hypothesis (HA_2) that lochs with higher nutrient inputs would have a larger community (either of a dominant organism or a more diverse composition) has been rejected based upon the data gathered. Further, the final hypothesis (HA_3) that states that fish presence or absence will have a greater impact on community composition than allochthonous inputs must also be rejected. Despite ascertained differences between loch types detailed in Chapter 1, community composition is not governed by these findings alone. Stock lochs are the only type supported by statistical data with a bearing on community composition, but the supported grouping (G6) does not contain all designated stock lochs. It is likely that community composition for the stock lochs is based upon physio-chemical and locational factors rather than allochthonous nutrients from the catchment. Bird and altitude typing has small bearing on the overall assemblage, only affecting Cladocera. TWINSPAN groupings have a robust support from ecological data and statistical grounding in comparison.

Chapter 5: Critical Evaluation of Methods and Zooplankton Composition

Introduction

The three hypotheses proposed at the start of this thesis are as follows; H_{A1} – Significant chemical differences will be found between the loch types; those with greater allochthonous (avian and stock) inputs will be higher in nutrient value than those with lower inputs. H_{A2} – Zooplankton community composition will be significantly influenced by allochthonous inputs, and the nutrient values found within the loch types, with those of higher inputs having a larger community (either of a dominating organism or a more diverse composition) than those of lower inputs. H_{A3} – Fish presence or absence will have the greater impact on community composition than allochthonous inputs in this region.

The inability to measure total nitrogen (TN) means that all three have been rejected, although H_{A1} was partially satisfied. This final chapter will critically evaluate the findings and methodologies used, discuss limitations, and make recommendations for future investigations.

Loch Types

The lack of differentiation between loch type may be down to a few factors. Previous studies have indicated that pastoral land use has a marked impact on lake chemistry. A generalised expectation is an increase in N resulting from urine and faecal deposits in the lake catchment (Abell *et al.,* 2011), leading to eutrophication. The primary grazers for the stock lochs were sheep, and compared to deer and cattle the effect of sheep is much reduced. Sheep have lower urination frequency and thus their contribution of N is less than would be expected for cattle or deer (McDowell and Wilcock, 2011). Sediment erosion resulting in a loss of P is also a factor for cattle and deer, however this effect is again reduced for sheep as they prefer not to loiter near water (Drewry, Littlejohn and Paton, 2000). As P is ubiquitously low across the samples and there is no differentiation between the loch

types it is likely that this is a feature of the region and not effected by pastoral land use.

Avian impact on water quality is more complex, partly due to the three forms of nutrient interaction. The phenomenon of Guanotrophication is largely predicated on taxa-specific feeding and roosting strategies, detailed in Fig. 5.1 (Adhurya, Das and Ray, 2020). *Larus argentatus* (European Herring Gull) was the most prevalent species found at the bird lochs, with additional observations of *Larus marinus, Larus fuscus,* and herons. Seabirds such as gulls are nutrient importers, and previous studies have found that even small colonies can have eutrophic implications, especially where local landfill is a nutrient source as is the case here (Gould and Fletcher, 1978; Signa, Mazzola and Vizzini, 2012; Winton and River, 2017). Obviously, eutrophication is not a factor in the lochs under study, and it is likely therefore that the measurement of *in situ* P does not provide a complete picture. In low pH, sedimentary iron is a precipitating agent for P (Scheffer, 1998), and it is possible that much bird-imported P is being restricted from entering the water column by this factor. Future investigations would benefit from analysis of sedimentary P and Fe^{3+}.

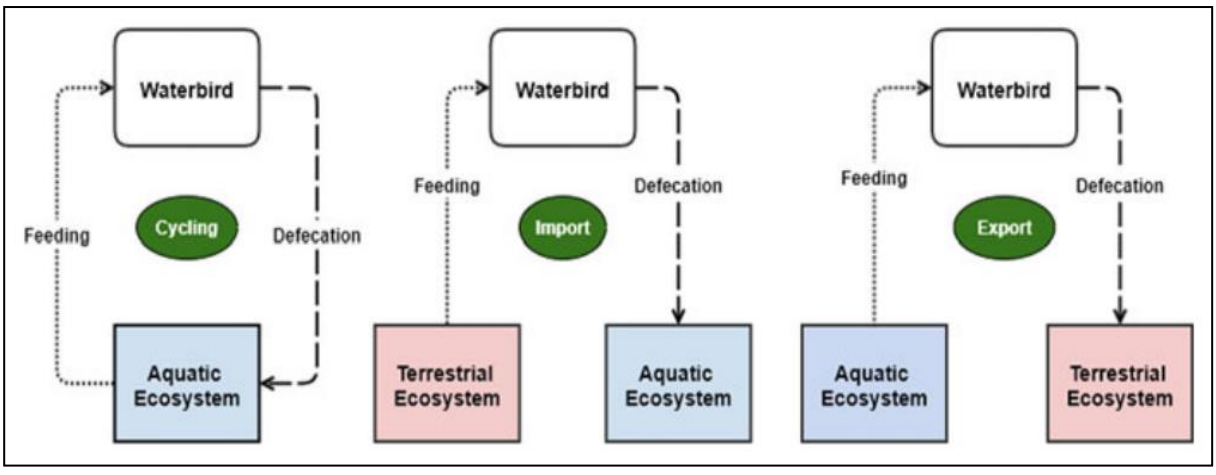


Figure 5.1, Waterbirds as Nutrient Vectors (Adhurya, Das and Ray, 2020)
The three forms of nutrient interaction are as follows; (i) Nutrient cycling, where waterbirds (moorhens, coots, pygmy geese, grebes) feed from and defecate into the same water body thereby converting nutrients into a different form. (ii) Import, where waterbirds (ducks, geese, swans) roost in aquatic habitats but feed in terrestrial or marine ones, therefore importing terrestrial and marine nutrients into freshwater ecosystems. (iii)

Chemistry

Undoubtedly the biggest barrier to the chemical examination was the inability to measure TN owing to methodological issues in-field. This meant that true nutrient ratios could not be calculated or compared between type, subtype or later, TWINSPAN groupings. The measurement of TN is recommended for all future analyses, especially where nutrition is a vital component of the hypotheses. Alkalinity is vital for discovering the buffering capabilities of lakes against pollution and climate changes and thus the status of small lochs as ‘canaries’. Previous investigation has found that alpine Scottish lochs have low alkalinity due to poorly buffered bedrock and peat soils ([1]Kernan *et al.,* 2009). It is likely that these factors indicate reduced alkalinity in Gairloch although this is not analytically confirmed. The absence of Al speciation also limits context on fish presence and absence.

This study would benefit from a more thorough testing of chemical factors and identification of allochthonous inputs in the catchment. Methods detailed here looked at wide-ranging factors and applied them as exclusives; a stock loch only has pastoral usage, and a bird loch only has roosting and loafing usage. The amount of usage by birds or stock was not measured despite observational differences and some lochs had usage of both, which was not considered. Moving forward, each loch should be looked at each loch as a unique microcosm rather than an arbitrary type. It is recommended that researchers perform more robust observation upon sampling, and include the number of roosting and loafing sites, visiting animals over time and number of animal droppings within a reasonable distance of the loch, to build a more precise picture of external site ecology.

Metagenomic and Manual Identification of Taxa

A better way to measure faecal inputs and thus allochthonous nutrient ingress could be to examine the lochs for eDNA from local faunal populations, which may be an argument for the use of eDNA over traditional metabarcoding methods. Issues with metagenomic methodologies have been examined in Chapter 3 and will not be repeated, other than to say that genomic analyses can provide a secondary identification, support and validation for projects where manual morphological identification is the chosen method. These two techniques complement each other in their limitations (Matthews, Goetze and Oham, 2021) and should still be used together in future work.

Issues with the manual identification of taxa within this study are subtler. Due to the difficulties of transporting boats across rough terrain and the lack of an anchor, vertical hauls were unobtainable and so data is qualitative rather than quantitative. Previous researchers have alleviated some of the risk factors by transporting a rigid inflatable boat and spreading the load across a larger team (Kernan, 2021, personal communication). At an identification level, whilst resources are wide ranging within science, up to date official taxonomic keys are not always available. Pontin's (1978) key, as used here, is still the most recommended key for use in the British Isles, but there is no escaping that it is almost 50 years old. Species ecology is somewhat sparse and needs updating, and it is possible for organisms to be misidentified based upon the limitations of the key. *Rotaria,* for example, has only one species noted whereas several have been identified within the British Isles. The issue of identification is, of course the remit of the researcher to investigate and use as many resources as possible to solve, but it must be noted that the lack of easy-access resources is symptomatic of the taxonomist shortage (Engel *et al.,* 2021), and creates a barrier to this work and student entry.

Scottish Lochs as ‘Canaries’

The chemistry alone does not have enough data to ascertain whether small Scottish lochs are suitable ‘canaries’ however the taxa may tell a different story. Several taxa normally associated with eutrophy are present (*C. spaericus, M. leuckarti* and *T. pusilla*) (Ochocka and Karpowicz, 2022). Whilst abundance for these taxa is currently low (Proportionally 1.8% of Loch na Fiedil [L6], 21% of ‘Lilyloch’ [L8] and 21% of Loch Coire na h’Airigh [L4]), presence alone could indicate an impending shift. Interestingly all three of these lochs are rich in well-developed *Nymphaea,* which can act as a buffer against eutrophication by removing excess nutrients (Wang *et al.,* 2022) and suppressing phytoplankton (Kurashov, Krylova and Protopopova, 2022, pp. 75). *B. longirostris,* indicative of mesotrophy (Muñoz-Colmenares, Soria and Vicente, 2021) had a much wider distribution and was found in 9/27 sites. *D. brachyurum* (dominant and present in Loch nam Buainichean [L9] only) is indicative of eutrophy, but perhaps more concerning is its status as an indicator for rising water temperature (Hamil *et al.,* 2020). When combined with meteorological data (Fig 5.2), we can see that Wester Ross, just like the rest of the planet, is warming.

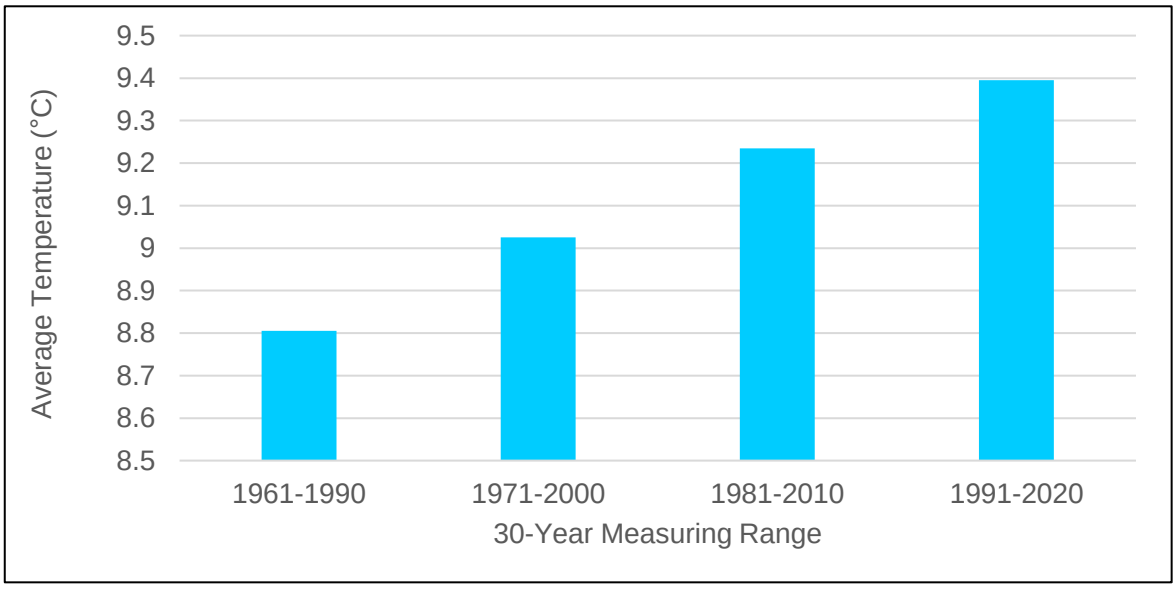


Figure 5.2, 30-Year Average Temperature in Altbea, North Scotland (Meteorological Office, 2023)

The temperature fluctuations recorded in 2023 highlight that climate change is not a far-off eventuality, it is here now, and affecting the biosphere in drastic ways (Copernicus Climate Change Service, 2023). Without historical data to compare, community composition cannot tell the ‘canary’ story, however the presence of meso- and eutrophic indicators underlines the need for further monitoring of these sites. As the baseline drifts from earlier (and cooler) reference conditions it is vital that we continue monitoring freshwater ecosystems so that we can recognise and report on change as it occurs (Woodward *et al.*, 2010).

Conclusion

In conclusion, differences in chemistry between loch type and subtype was established, although this did not extend to a true evaluation of nutrition owing to missing TN data. Recommendations for further chemical analysis have been made, namely TN, Al species, alkalinity, and sedimentary data. Whilst TP is the likely limiting factor in the region true nutrient ratios must be determined to draw reliable conclusions. Initial groupings were somewhat supported in the data, but not to the extent that community compositional hypotheses could be supported. The stock loch grouping was the most robust, with grounding in multiple statistical tests, but composition is more likely due to physico-chemical and locational factors, rather than allochthonous inputs. Supporting genetic work did not bear fruit owing to issues with sequencing that have been discussed. Despite the issues highlighted in this text, genetic data is still advised as a good counterpoint to manual identification of taxa. Even though this study did not confirm any hypotheses, it does provide information regarding hitherto almost disregarded freshwater habitats. The species data gathered here is the first recording of zooplankton in the region and hints at the possible ‘canary’ status of these lochs. The continued sampling of these (and all) environments is needed to monitor ecosystems throughout climate change and mitigate damage where possible.

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Appendix A

Performance Report for ICP-MS Analysis on iCAP RQ

System

Start time:	3/4/2022 11:02:59 AM		
Instrument:	iCAP RQ		
Operator:	OPTIPLE-KTE4I1D\Administrator		
Template:	2. KED Middlesex Uni		
Instrument Serial Number:	RQ02977		
Last Autotune:	Autotune-!CaliTune	KEDS	Line1-20220304-110127928.imatdat
Solution:	No solution specified		

Sensitivity & Stability Test

Result	Runs	Sweeps
Passed	5	30

Sensitivity

Analyte	Result	Value	Condition	Limit
Bkg4.5	Passed	0.022 CPS	Less than	0.5 CPS
Bkg220.7	Passed	0.0 CPS	Less than	2.0 CPS
77Se	Passed	8.1 CPS	Less than	50.0 CPS
59Co	Passed	49,632.0 CPS	Greater than	30,000.0 CPS
238U	Passed	677,887.0 CPS	Greater than	85,000.0 CPS
209Bi	Passed	461,095.0 CPS	Greater than	42,500.0 CPS
140Ce.16O/140Ce	Passed	0.0094	Less than	0.01
115In	Passed	81,884.0 CPS	Greater than	35,000.0 CPS

Stability

Analyte	Value	Limit
59Co	0.4%	2
238U	0.6%	2
209Bi	0.7%	2
115In	0.4%	2

Mass Calibration Test

Result	Channels	Dwell	MeasureWidth	PointSpacing	Sweeps
Passed	75	0.04	1.5	0.02	5

Analyte	Result	Centroid Mass [u]	Offset	Peak width [u]	Peak width min [u]	Peak width max [u]
59Co	Passed	58.9512	0.0180	0.700	0.650	0.850
115In	Passed	114.9135	0.0096	0.715	0.650	0.850
209Bi	Passed	208.9875	0.0072	0.699	0.650	0.850

3/4/2022

Vacuum Check

Parameter	Result	Value
Analyzer Pressure		5.050e-7
Interface Pressure		1.654e+0

Detector Voltages

Analog	Counting
-1737.50	1787.50

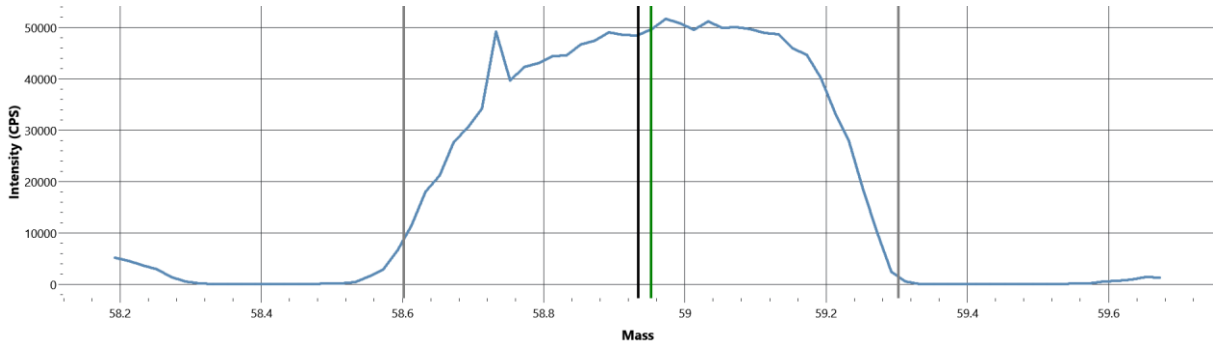
3/4/2022

Tune Settings

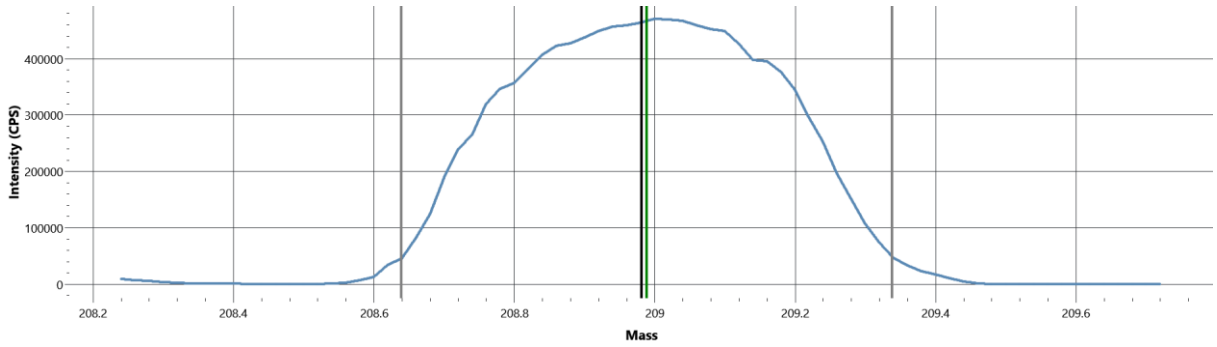
Parameter	Value
Additional Gas Flow 1	0.00
Additional Gas Flow 2	0.00
Additional Gas Flow 3	0.00
Angular Deflection	-382.40
Auxilliary Flow	0.80
CCT Bias	-21.00
CCT Entry Lens	-105.98
CCT Exit Lens	-40.00
CCT Focus Lens	-3.60
CCT1 Flow	4.59
CCT1 Shut-Off Valve	1.00
CCT2 Flow	0.00
CCT2 Shut-Off Valve	0.00
Cool Flow	14.00
D1 Lens	-355.00
D2 Lens	-158.00
Deflection Entry Lens	-35.01
Dry Pump Speed	100.00
Extraction Lens 1 Negative	0.00
Extraction Lens 1 Polarity	0.00
Extraction Lens 1 Positive	0.00
Extraction Lens 2	-177.00
Focus Lens	-7.50
Nebulizer Flow	1.03
Peristaltic Pump Speed	30.00
Plasma Power	1550.00
Pole Bias	-18.00
Quad Entry Lens	-54.00
Sampling Depth	5.00
Spray Chamber Temperature	2.70
Torch Horizontal Position	-1.22
Torch Vertical Position	-0.50
Virtual CCT Mass Maximum Dac Limit Set	4095.00
Virtual CCT Mass parameter b	1.00
Virtual CCT Mass to Dac Factor	60.00
Virtual CCT Mass to Dac Offset	40.00

Mass Calibration Peaks

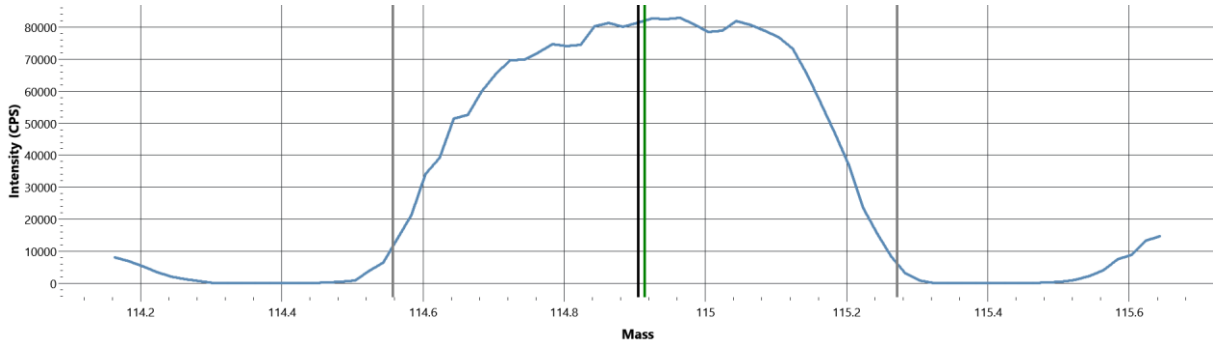
Analyte: 59Co



Analyte: 209Bi



Analyte: 115In



3/4/2022 11:08:28 AM

Appendix B

Table B.1, Inorganic Chemistry of Water from the 'Altitude Lochs' – Full data

For all tables, * Denotes total dissolved concentration, † denotes bioavailable concentration as calculated using the UK Technical Advisory Group's Metal Bioavailability Assessment Tool.

Loch ID	Subtype	Conductivity (µS)	pH	Temperature °C
L8	Altitude -	29	6.71	20.0
L9	Altitude +	34	6.85	19.3
L10	Altitude	32	6.85	19.3
L11	Altitude -	19	6.23	20.1
L12	Altitude -	27	6.58	19.9
L13	Altitude -	22	6.44	20.1
L14	Altitude -	22	5.54	19.6
L27	Altitude -	25	5.50	17.0
L28	Altitude +	34	7.00	17.2

Table B.2, Organic Chemistry of Water from the 'Altitude Lochs' – Full data

Loch ID	Subtype	NO_3^- µg/L	TP µg/L	NH_4 µg/L	Chl-α µg/L	TSI	DOC mg/L
L8	Altitude -	633	14	35	78.26	5.61	-
L9	Altitude +	319	7	16	127.17	10.37	8.66
L10	Altitude	378	23	616	71.74	4.75	9.93
L11	Altitude -	570	7	28	79.89	5.81	40.80
L12	Altitude -	271	14	4	55.43	2.22	15.17
L13	Altitude -	335	6	0	143.48	11.55	-
L14	Altitude -	318	15	-	76.63	5.40	6.80
L27	Altitude -	164	15	472	208.70	15.23	-
L28	Altitude +	214	42	510	16.30	0.00	8.64

Table B.3, Ion concentrations a for the 'Altitude (and Large) Lochs' – Full data

Loch ID	Subtype	Al^{3+} µg/L	Ca^{2+} mg/L	Cd^{2+} µg/L	Cl^- mg/L	Cu^{2+} µg/L	Cr^{2+} µg/L
L8	Altitude -	-	-	-	8.63	-	-
L9	Altitude +	44.128	1.150	0.144	13.60	0.190†	0.217
L10	Altitude -	34.392	0.981	-	9.74	-	-
L11	Altitude -	62.966	0.593	0.098	6.70	0.020†	0.347
L12	Altitude -	23.186	1.361	0.110	10.20	0.030†	0.376
L13	Altitude -	28.940	0.209	0.098	6.81	0.290†	0.323
L14	Altitude -	37.564	0.053	0.120	8.21	0.936*	0.327
L27	Altitude -	30.312	0.101	0.150	9.02	755.480*	0.218
L28	Altitude +	20.736	2.941	0.361	9.85	283.000*	1.152

Table B.4, Ion concentrations b for the 'Altitude (and Large) Lochs' – Full data

Loch ID	Subtype	Fe^{3+} µg/L	K^{+} mg/L	Mg^{2+} mg/L	Na^{+} mg/L	Pb^{2+} µg/L	Si^{2+} mg/L	Zn^{2+} µg/L
L8	Altitude -	-	-	-	-	-	-	-
L9	Altitude +	22.635	0.290	0.748	5.231	0.506	0.403	2.450†
L10	Altitude -	29.726	0.463	0.858	5.931	-	0.171	6.848*
L11	Altitude -	0.190	0.165	0.573	3.451	0.634	0.347	1.320†
L12	Altitude -	93.962	0.357	0.716	4.555	0.656	0.230	0.620†
L13	Altitude -	782.908	0.229	0.589	4.370	1.359	0.144	5.640*†
L14	Altitude -	65.908	0.193	0.547	4.512	1.450	0.082	-
L27	Altitude -	102.820	0.217	0.528	4.744	4.464	0.071	25.540*
L28	Altitude +	12.292	0.272	0.796	5.263	7.691	0.145	8.280*

Table B.5, Inorganic Chemistry of Water from the 'Bird Lochs' – Full data

Loch ID	Subtype	Conductivity (µS)	pH	Temperature °C
L1	Bird +	16	-	16.5
L4	Bird +	68	-	17.2
L5	Bird +	110	-	16.9
L7	Bird +	50	-	17.2
L15	Bird +	45	6.10	18.5
L16	Bird +	42	6.30	18.1
L17	Bird -	48	4.70	18.3
L25	Bird +	43	6.90	20.9
L29	Bird -	59	6.00	16.1
L30	Bird +	43	5.90	17.4

Table B.6, Organic Chemistry of Water from the 'Bird Lochs' – Full data

Loch ID	Subtype	NO_3^- µg/L	TP µg/L	NH_4 µg/L	Chl-α µg/L	TSI	DOC mg/L
L1	Bird +	285	16	57	148.37	11.88	10.73
L4	Bird +	164	10	54	99.46	7.96	7.20
L5	Bird +	280	7	49	197.28	14.68	12.16
L7	Bird +	254	6	40	471.20	23.22	8.24
L15	Bird +	676	19	493	189.13	14.26	21.06
L16	Bird +	722	14	496	180.98	13.83	17.75
L17	Bird -	699	20	439	58.70	2.78	22.47
L25	Bird +	287	7	340	75.00	5.19	9.66
L29	Bird -	172	8	538	14.67	0.00	29.41
L30	Bird +	419	43	586	94.57	7.46	7.91

Table B.7, Ion concentrations for the 'Bird Lochs' – Full data

Loch ID	Subtype	Al^{3+} µg/L	Ca^{2+} mg/L	Cd^{2+} µg/L	Cl^{-} mg/L	Cu^{2+} µg/L	Cr^{2+} µg/L
L1	Bird +	13.600	2.779	0.130	16.50	3.796*	0.263
L4	Bird +	3.552	6.318	0.144	21.80	0.608*	0.368
L5	Bird +	8.689	6.500	0.119	21.60	0.450*	0.256
L7	Bird +	16.663	1.436	0.081	13.80	8.612*	0.286
L15	Bird +	41.895	1.859	0.250	10.60	0.130†	0.311
L16	Bird +	36.553	1.735	0.178	10.80	0.100†	0.289
L17	Bird -	34.332	-	0.136	14.10	1.990*	0.263
L25	Bird +	34.008	1.872	0.173	11.40	1.213*	0.255
L29	Bird -	26.455	0.236	0.211	22.40	0.350†	0.343
L30	Bird +	16.280	0.344	0.185	14.20	0.260†	0.254

Table B.8, Ion concentrations for the 'Bird Lochs' – Full data

Loch ID	Subtype	Fe^{3+} µg/L	K^{+} mg/L	Mg^{2+} mg/L	Na^{+} mg/L	Pb^{2+} µg/L	Si^{2+} mg/L	Zn^{2+} µg/L
L1	Bird +	20.500	0.493	1.492	9.153	0.712	0.370	11.400*
L4	Bird +	8.145	0.595	1.970	10.726	1.248	0.614	5.644*
L5	Bird +	33.153	0.664	2.255	11.849	1.016	0.944	4.933*
L7	Bird +	97.093	0.249	1.007	7.078	0.845	0.519	11.530*
L15	Bird +	423.630	0.309	1.149	7.009	1.042	0.824	1.410†
L16	Bird +	286.575	0.256	1.052	6.600	1.513	0.782	1.720†
L17	Bird -	128.345	0.323	0.849	7.307	1.573	0.088	9.725*
L25	Bird +	78.208	0.355	0.926	5.736	0.400	0.534	-
L29	Bird -	450.235	0.604	1.236	11.143	7.339	0.307	2.440†
L30	Bird +	124.912	0.252	0.956	6.874	0.843	0.087	2.440†

Table B.9, Inorganic Chemistry of Water from the 'Stock Lochs' – Full data

Loch ID	Subtype	Conductivity (µS)	pH	Temperature °C
L2	Stock -	136	-	19.2
L3	Stock -	133	-	19.0
L6	Stock +	46	8.30	16.3
L18	Stock -	61	4.70	21.5
L19	Stock +	62	4.90	20.3
L20	Stock -	80	4.60	21.3
L21	Stock -	81	5.70	22.9
L22	Stock +	55	6.30	22.3
L23	Stock +	62	6.60	20.5
L24	Stock +	51	6.60	21.1

Table B.10, Water Chemistry for the 'Stock Lochs' – Full data

Loch ID	Subtype	NO_3^- µg/L	TP µg/L	NH_4 µg/L	Chl-α µg/L	TSI	DOC mg/L
L2	Stock -	532	16	9	68.48	4.30	16.13
L3	Stock -	710	16	153	35.87	0.00	26.93
L6	Stock +	256	15	-	79.89	5.81	7.01
L18	Stock -	597	25	493	154.89	12.30	16.50
L19	Stock +	532	13	273	66.85	4.06	31.99
L20	Stock -	707	16	388	76.63	5.40	33.63
L21	Stock -	1020	18	405	97.83	7.80	31.88
L22	Stock +	309	8	444	102.72	8.27	16.80
L23	Stock +	152	12	347	66.85	4.06	-
L24	Stock +	181	8	355	73.37	4.97	11.69

Table B.11, Ion concentrations for the 'Stock Lochs' – Full data

Loch ID	Subtype	Al^{3+} µg/L	Ca^{2+} mg/L	Cd^{2+} µg/L	Cl^- mg/L	Cu^{2+} µg/L	Cr^{2+} µg/L
L2	Stock -	20.428	15.053	0.172	19.50	2.083*	0.313
L3	Stock -	22.081	13.266	0.164	19.50	0.591*	0.446
L6	Stock +	19.896	4.572	0.085	9.05	0.810†	0.288
L18	Stock -	48.702	1.697	0.139	16.30	0.080†	0.265
L19	Stock +	30.093	0.348	0.109	17.70	0.060†	0.324
L20	Stock -	28.928	0.209	0.130	22.60	0.080†	0.221
L21	Stock -	59.076	2.421	0.175	21.50	0.070†	0.312
L22	Stock +	14.425	0.535	0.118	17.10	0.070†	0.212
L23	Stock +	8.251	1.017	0.122	18.10	0.060†	0.194
L24	Stock +	11.217	1.054	0.130	16.20	0.040†	0.187

Table B.12, Ion concentrations for the 'Stock Lochs' – Full data

Loch ID	Subtype	Fe^{3+} µg/L	K^+ mg/L	Mg^{2+} mg/L	Na^+ mg/L	Pb^{2+} µg/L	Si^{2+} mg/L	Zn^{2+} µg/L
L2	Stock -	47.385	0.518	2.238	11.848	0.787	0.624	20.58*
L3	Stock -	72.359	0.952	2.098	11.798	0.870	1.279	5.082*
L6	Stock +	33.470	0.337	1.409	6.338	0.689	1.110	2.11†
L18	Stock -	79.650	0.521	0.956	9.008	1.523	0.060	3.24†
L19	Stock +	181.429	0.394	1.049	9.262	0.635	0.083	1.59†
L20	Stock -	49.615	0.281	1.391	11.556	1.299	0.058	2.29†
L21	Stock -	323.693	0.353	1.518	12.207	1.489	0.261	1.34†
L22	Stock +	9.118	0.404	0.950	8.405	0.399	0.188	0.22†
L23	Stock +	6.535	0.425	1.043	9.450	0.446	0.267	0.17†
L24	Stock +	-	0.422	0.924	8.487	0.142	0.177	0.03†

Table B.13, Inorganic Chemistry of Water from Loch Maree – Full data

Loch ID	Subtype	Conductivity (µS)	pH	Temperature °C
L26	Large +	31	8.50	-

Table B.14, Organic Chemistry of Water from Loch Maree – Full data

Loch ID	Subtype	NO_3^- µg/L	TP µg/L	NH_4 µg/L	Chl-α µg/L	TSI	DOC mg/L
L26	Large +	161	6	5	117.39	9.58	-

Table B.15, Ion concentrations from Loch Maree – Full data

Loch ID	Subtype	Al^{3+} µg/L	Ca^{2+} mg/L	Cd^{2+} µg/L	Cl^- mg/L	Cu^{2+} µg/L	Cr^{2+} µg/L
L26	Large +	16.792	1.601	0.085	9.07	5.570†	0.278

Table B.16, Ion concentrations b from Loch Maree – Full data

Loch ID	Subtype	Fe^{3+} µg/L	K^+ mg/L	Mg^{2+} mg/L	Na^+ mg/L	Pb^{2+} µg/L	Si^{2+} mg/L	Zn^{2+} µg/L
L26	Large +	-	0.268	0.664	4.504	0.692	0.399	4.24*†

Table B.17, Zooplankton Species Names and Abbreviations

Species name	Abbrev.	Species name	Abbrev.
Acanthocyclops venustus	Aca ven	*Graeteriella unisetigera*	Gra uni
Acanthodiaptomus denticornis	Aca den	*Hemidiaptomus amblyodon*	Hem amb
Acanthodiaptomus tibetanus	Aca tib	*Heterocope appendiculata*	Het app
Acantholeberis curvirostris	Aca cur	*Kellicottia longispina*	Kel lon
Alona costata	Alo cos	*Keratella cochlearis*	Ker coc
Alona quadrangularis	Alo qua	*Keratella serrulata*	Ker ser
Alonella excisa	Alo exc	*Leptodioptamus minutus*	Lep min
Alonopsis elongata	Alo elo	*Leydigia leydigia*	Ley ley
Ascomorpha ecaudis	Asc eca	*Limnocalanus macrurus*	Lim mac
Ascomorpha ovalis	Asc ova	*Limnosida frontosa*	Lim fro
Asplanchna priodonta	Asp pri	*Macrocyclops albidus*	Mac alb
Bosmina longicornis	Bos lco	*Mesocyclops leukarti*	Mes leu
Bosmina longirostris	Bps lro	*Metacyclops gracilis*	Met gra
Bosmina longispina	Bos lsp	*Metacyclops minutus*	Met min
Chydorus gibbis	Chy gib	*Microcyclops rubellus*	Mic rub
Chydorus ovalis	Chy ova	*Mixodiaptomus theeli*	Mix the
Chydorus spahericus	Chy spa	*Oxyurella tenuicaudis*	Oxy ten
Conochilus natans	Con nat	*Paracyclops affinis*	Par aff
Cryptocyclops bicolour	Cry bic	*Phreatalona protzi*	Phr pro
Diacyclops bisetosus	Dia bis	*Pleuroxus truncatus*	Ple tru
Diaphanosoma brachyurum	Dia bra	*Polyphemus pediculus*	Pol ped
Drepanothrix dentata	Dre den	*Pseudochydorus globosus*	Pse glo
Dunhevedia crassa	Dun cra	*Bdelloid spp.*	Bde spp
Ectocyclops phaleratus	Ect pha	*Sida crystallina*	Sid cry
Eudiaoptamus gracilis	Eud gra	*Streblocerus serricaudatus*	Str ser
Eudiaptomus vulgaris	Eud vul	*Tretocephala ambigua*	Tre amb
Eudiaptomus zachariasi	Eud zac	*Trichocerca elongata*	Tri elo
Eurycercus lamellatus	Eur lam	*Trichocerca pusilla*	Tri pus
Eurycercus pompholygodes	Eur pom	*Tropocyclops prasinus*	Tro pra
Eurytemora lacustris	Eur lac		

Table B.18, Zooplankton Composition in Altitude Lochs

Loch ID	L8	L9	L10	L11	L12	L13	L14	L27	L28
Fish ± ve	-	+	+	-	-	-	-	-	+
A. elongata	*								
K. longispina							*		
E. lamellatus	*								
K. cochlearis							*	*	**
Bdelloid spp.							*		
M. leukarti	*								
S. crystallina	*	*				*			
L. macrurus	*		*				*		*
C. bicolour	*								***
G. unisetigera	*						*		
C. gibus	*								
D. brachyurum		***							
B. longicornis		**							
C. natans			***						
B. longispina			*			*	*		
A. ecaudis			*						
A. ovalis			*						
A. tibetanus				*					
H. appendiculata					*				
S. kieferi					*	*	*		
B. longirostris					**		*		*
H. amblyodon					*				
P. pediculus					*	*			
A. denticornis						*		***	
A. curvirostris						*			
E. zachariasi							*		
S. pallidus							*		

Table B.19, Zooplankton Composition in Bird Lochs

Loch ID	L4	L5	L7	L15	L16	L17	L25	L29	L30
Fish ± ve	+	+	+	+	+	-	+	-	+
A. priodonta		*							
A. elongata	*								
K. longispina		*							*
E. lamellatus							*		
K. cochlearis	*				*				*
A. costata			*						
Bdelloid spp.		*							*
S. crystallina			***			*	*	*	*
C. natans	*								
P. pediculus						*		**	
S. serricaudatus			*	*					
T. ambigua	*	*		*		*			
L. frontosa						*			
O. tenuicaudis								*	
E. vulgaris								*	
M. gracilis							*		
P. affinis							*		
A. excisa		*					*		
E. phaleratus		*							
K. serrulata								*	
A. venustus									***
L. minutus									*
T. prasinus									*
E. gracilis									*
D. dentata	*								
P. globosus	*								
T. pusilla	*								
D. crassa	*								
M. rubellus			*						
C. ovalis			*						
E. pompholygodes			*						
T. elongata			*						
B. longispina			*			***			
B. longirostris						***	*	*	

Table B.20, Zooplankton Composition in Stock Lochs

Loch ID	L6	L18	L19	L20	L21	L22	L23	L24
Fish ± ve	+	-	-	-	-	+	+	+
M. albidus	*							
M. minutus	*							
A. priodonta	*							
A. elongata	*	*	*				*	
P. protzi	*							
K. longispina	*					*		**
A. quadrangularis	*							
C. spahericus	*							
P. truncatus	*							
E. lamellatus	*							
K. cochlearis	*							
A. costata	*							
Bdelloid spp.	*							
S. crystallina		*						
L. macrurus			*					*
H. amblyodon							**	
P. pediculus		*	*	*	**	**	*	**
A. curvirostris			*					
T. ambigua		*						
L. leydigia			*					
O. tenuicaudis			*	*	*			*
M. theeli				**				
E. vulgaris					**			
E. lacustris							**	
D. bisetosus								*
B. longicornis			*					**
B. longispina					**	*	**	
Bongirostris		*		**			**	

Appendix C

Table B.7 Na^+ Concentration (mg/L) in Categorised Lochs

Loch Subtype	Number	Mean	Standard Deviation	99% CI
Altitude Loch -	6	4.5940	0.7970	(3.232, 5.956)
Altitude Loch +	2	5.2474	0.0225	(2.8878, 7.6070)
Bird Loch -	2	9.2300	2.710	(6.87, 11.58)
Bird Loch +	8	8.1280	2.192	(6.948, 9.308)
Stock Loch -	5	1.2930	1.293	(6.896, 9.881)
Stock Loch +	5	1.2360	1.236	(9.791, 12.776)
Large Loch +	1	4.5040	-	(1.167, 7.841)

Table B.8 Cl^- Concentration (mg/L) in Categorised Lochs

Loch Subtype	Number	Mean	StDev	99% CI
Altitude Loch -	7	8.473	1.347	(5.766, 11.180)
Altitude Loch +	2	11.72	2.655	(6.66, 16.79)
Bird Loch -	2	18.25	5.870	(13.19, 23.31)
Bird Loch +	8	15.09	4.540	(12.6, 17.62)
Stock Loch -	5	19.88	2.400	(16.68, 23.08)
Stock Loch +	5	15.63	3.750	(12.43, 18.83)
Large Loch +	1	9.070	-	(1.908, 16.232)

Table B.9 Area (ha) of Categorised Lochs

Items in bold display median.

Loch Subtype	Number	Mean	StDev	99% CI
Altitude Loch -	7	0.236	0.1325	(-20.148, 20.6205)
Altitude Loch +	2	5.790	7.5100	(-32.35, 43.93)
Bird Loch -	2	2.040	2.7500	(-36.09, 40.18)
Bird Loch +	8	7.080	-	-
Stock Loch -	5	1.675	1.3700	(-22.444, 25.79)
Stock Loch +	5	5.400	-	-
Large Loch +	1	3242	-	(3188, 3296)

Table B.10, Conductivity of Categorised Lochs

Loch Type Subtype	Number	Mean	StDev	99% CI
Altitude Loch -	7	25.14	4.53	(2.76, 47.52)
Altitude Loch +	2	34.00	0.00	(-7.87, 75.87)
Bird Loch -	2	53.50	7.78	(11.63, 95.37)
Bird Loch +	8	52.17	27.26	(31.24, 73.11)
Stock Loch -	5	98.20	34.10	(71.7, 124.7)
Stock Loch +	5	55.20	6.98	(28.72, 81.68)
Large Loch +	1	31.00	-	(28.21, 90.21)

Table B.11, Conductivity in Lochs by Geological Type

Geological Type	Number	Mean (µS)	StDev	99% CI
LGC	14	33.71	10.34	(16.06, 51.37)
LMG	1	68.00	-	(1.94, 134.06)
TS	11	75.09	31.23	(55.17, 95.01)
TS, LMG	2	76.50	47.40	(29.8, 123.2)
TS, LMG, LGC	1	16.40	-	(-49.66, 97.06)
TS, LMG, LGC, CO	1	31.00	-	(-35.06, 97.06)

Table B.12 Altitude (masl) of Categorised Lochs

Loch Subtype	Number	Mean	StDev	99% CI
Altitude Loch -	7	309.1	46.80	(273.8, 344.5)
Altitude Loch +	2	251.5	65.80	(158.3, 317.7)
Bird Loch -	2	154.0	42.40	(87.8, 220.2)
Bird Loch +	8	105.8	22.70	(72.65, 138.85)
Stock Loch -	5	78.2	14.31	(36.33, 120.07)
Stock Loch +	5	58.6	21.76	(16.73, 100.47)
Large Loch +	1	10.0	-	(-83.61, 103.61)

Table B.13, Zooplankton Assemblage Statistics for Cladocerans Across Type, Subtype, Fish P/A and TWINSPAN Group

	Num	Test	Mean	Median	StDev	P-Value	99% CI
Fish Presence	14	Mann-Whitney		21.0		0.699	(-48, 114)
Fish Absence	12			23.5			
Altitude	9	Kruskall-Wallace		12.0		0.038	
Bird	9			22.0			
Stock	8			85.0			
A+	6	ANOVA	69.5		145.00	0.947	(-94.9, 233.9)
A-	3		98.0		168.00		(-134.5, 330.5)
B+	2		5.5		7.78		(-279.28, 290.28)
B-	7		102.3		192.40		(-49.9, 254.5)
S+	4		55.8		33.60		(-145.6, 257.1)
S-	4		112.0		67.30		(-89.4, 313.4)
G3	2	ANOVA	1.5		2.12	0.014	(-151.44, 154.44)
G5	10		11.4		8.97		(-57.00, 79.80)
G6	5		88.6		46.90		(-8.1, 185.3)
G7	9		131.0		122.50		(58.9, 203.1)

Table B.14, Zooplankton Assemblage Statistics for Copepods Across Type, Subtype, Fish P/A and TWINSPAN Group

	Num	Test	Mean	Median	StDev	P-Value	99% CI
Fish Presence	14	Mann-Whitney		8.5		1.000	(-22, 170)
Fish Absence	12			5.0			
Altitude	9	Kruskall-Wallace		4.0		0.634	
Bird	9			6.0			
Stock	8			5.5			
A+	6	ANOVA	9.67		9.35	0.075	(-85.11, 104.44)
A-	3		43.7		58.7		(-90.4, 177.7)
B+	2		231		233		(67, 395)
B-	7		59.6		86.9		(-28.2, 147.3)
S+	4		20.5		26.9		(-95.6, 136.6)
S-	4		51.5		89.2		(-64.6, 167.6)
G3	2	ANOVA	2.000		0.000	0.366	(-260.246, 264.246)
G5	10		124.1		191.8		(6.8, 241.4)
G6	5		19.4		23.4		(-146.5, 185.3)
G7	9		42.7		77.2		(-81.0, 166.3)

Table B.15, Zooplankton Assemblage Statistics for Rotifers Across Type, Subtype, Fish P/A and TWINSPAN Group

	Num	Test	Mean	Median	StDev	P-Value	99% CI
Fish Presence	14	Mann-Whitney		8		0.016	(0.00, 37)
Fish Absence	12			0			
Altitude	9	Kruskall-Wallace		1		0.720	
Bird	9			2			
Stock	8			0			
A+	6	ANOVA	4.170		7.910	0.142	(-36.74, 45.08)
A-	3		67.000		86.000		(9.1, 124.9)
B+	2		1.000		1.410		(-69.86, 71.86)
B-	7		11.430		14.220		(-26.45, 49.30)
S+	4		0.000		0.000		(-50.103, 50.103)
S-	4		35.300		53.200		(-14.9, 85.4)
G3	2	ANOVA	82.500		115.300	0.041	(14.9, 150.1)
G5	10		16.500		14.390		(-13.72, 46.72)
G6	5		23.000		50.300		(-19.7, 65.7)
G7	9		0.444		0.882		(-31.407, 32.296)

Table B.16, ANOVA Data for Simpson's Diversity Index for Lochs Separated by Subtype

Subtype	Num	Mean	StDev	99% CI
A+	3	0.02443	0.0148	(-0.42150, 0.47036)
A-	6	0.606	0.3290	(0.291, 0.921)
B+	7	0.416	0.3360	(0.124, 0.708)
B-	2	0.5527	0.0533	(0.0066, 1.0989)
S+	4	0.579	0.2400	(0.192, 0.965)
	4	0.7184	0.1620	(0.3322, 1.1046)

Table B.14, Altitude (m.a.s.l.) Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	14	Mann-Whitney	65	332	185.6	154.0	115.8	0.228	(-29, 209)
Fish +	15		21	333	127.1	96.5	87.3		
Altitude	9	ANOVA	205	333	296.3	324.0	53.2	0.000	(262.1, 330.5)
Bird	9		75	184	115.4	121.0	31.9		(83.0, 147.8)
Stock	8		21	94	68.4	69.5	20.2		(35.96, 100.84)
A -	6	ANOVA	205	332	305.2	327.5	49.9	0.000	(264.2, 346.1)
A +	3		205	333	278.7	298.0	66.2		(220.8, 336.5)
B -	2		124	184	154.0	154.0	42.4		(83.1, 224.9)
B +	8		75	128	105.8	116.0	22.8		(70.32, 141.18)
S -	6		65	94	76.5	70.5	13.5		(35.58, 117.42)
S +	4		21	74	56.3	65.0	24.4		(6.1, 106.4)
G3	2	ANOVA	331	333	332.0		1.4	0.018	(148.89, 515.11)
G5	10		21	332	169.7	300.5	109.5		(87.8, 251.6)
G6	5		59	71	65.8	69.5	4.4		(-50.01, 181.61)
G7	9		73	331	171.9	264.5	98.5		(85.6, 258.2)

Table B.14, Al^{3+} Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	14	2-Sample T-Test	0.00	62.97	32.36	29.52	16.16	0.051	(-3.96, 26.05)
Fish +	16		3.55	44.13	21.32	16.73	12.73		
Altitude	9	ANOVA	0.00	62.97	31.36	30.31	17.25	0.640	(16.91, 45.80)
Bird	9		3.55	41.90	23.20	21.56	13.16		(9.50, 36.91)
Stock	8		8.25	59.08	26.31	21.25	16.28		(12.61, 40.01)
Large	1		-	-	16.79	-	-		(-26.54, 60.13) (-24.92, 58.50)
A -	6	ANOVA	0.00	62.97	30.50	29.63	20.45	0.316	(13.47, 47.52)
A +	3		20.74	44.13	33.09	34.39	11.75		(9.00, 57.17)
B -	2		26.46	34.33	30.39	30.39	5.57		(0.90, 59.89)
B +	8		3.55	41.90	21.40	16.47	14.13		(6.66, 36.15)
S -	6		20.43	59.08	34.88	29.51	15.54		(17.86, 51.91)
S +	4		8.25	19.90	13.45	12.82	4.98		(-7.41, 34.30)
G3	2	ANOVA	34.40	63.00	48.70	48.70	20.20	0.166	(18.4, 79.0)
G5	10		0.00	41.90	21.55	20.32	14.75		(7.99, 35.11)
G6	5		11.22	59.08	28.75	28.93	18.93		(9.57, 47.92)
G7	9		8.25	48.70	29.41	28.95	12.71		(15.11, 43.70)

Table B.14, Area Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	14	Mann-Whitney	0.012	3.99	1.24	0.34	1.483	0.001	(-13.208, -0.6159)
Fish +	15		0.280	81.70	16.27	5.44	25.120		
Altitude	9	Kruskall-Wallace	0.010	11.10	1.47	0.28	3.610	0.012	
Bird	9		0.100	81.70	14.44	4.46	25.020		
Stock	8		0.320	68.94	10.37	3.48	20.910		
A -	6	ANOVA	0.012	0.41	0.23	0.23	0.144	0.304	(-21.422, 21.880)
A +	3		0.280	11.10	3.95	0.48	6.190		(-26.67, 34.57)
B -	2		0.100	3.99	2.04	2.04	2.750		(-35.46, 39.55)
B +	8		1.100	81.70	17.54	7.08	27.370		(-1.21, 36.29)
S -	6		0.321	3.48	1.98	2.11	1.431		(-19.674, 23.628)
S +	4		4.100	68.90	23.00	9.40	30.900		(-3.6, 49.5)
G3	2	Kruskall-Wallace	0.012	0.28	0.15	0.15	0.190	0.165	
G5	10		0.170	27.18	4.98	1.20	8.750		
G6	5		0.300	68.90	17.60	3.50	29.200		
G7	9		0.100	81.70	12.73	3.99	26.160		

Table B.14, Ca^{2+} Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	12	Mann-Whitney	0.050	15.050	2.960	0.470	5.300	0.099	(-2.4308, 1.0168)
Fish +	16		0.344	6.500	2.293	1.668	1.910		
Altitude	8	ANOVA	0.053	2.941	0.924	0.787	0.954	0.376	(-2.702, 4.550)
Bird	9		0.236	6.500	2.564	1.859	2.316		(-0.854, 5.983)
Stock	10		0.210	15.050	4.020	1.380	5.510		(0.77, 7.26)
Large	1		-	-	1.601	-	-		(-8.655, 11.857) (-8.753, 11.956)
A -	5	ANOVA	0.053	1.361	0.463	0.209	0.545	0.414	(-4.167, 5.094)
A +	3		0.981	2.941	1.691	1.150	1.086		(-4.287, 7.669)
B -	1		0.236	0.236	0.236	0.236	*		(-10.118, 10.591)
B +	8		0.344	6.500	2.855	1.866	2.293		(-0.805, 6.516)
S -	6		0.210	15.05	5.500	2.060	6.780		(1.27, 9.73)
S +	4		0.535	4.572	1.794	1.035	1.867		(-3.383, 6.972)
G3	2	ANOVA	0.593	0.981	0.787	0.787	0.275	0.161	(-2.640, 4.215)
G5	10		0.053	6.500	2.714	1.859	2.550		(1.098, 4.330)
G6	5		0.209	2.421	0.913	0.535	0.902		(-1.254, 3.081)
G7	9		0.209	1.872	1.122	1.255	0.619		(-0.592, 2.836)

Table B.14, Cd^{2+} Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	13	2-Sample T-Test	98	211	139.38	136.0	34.0	0.834	(-67.2, 57.9)
Fish +	16		0	361	144.10	130.0	79.9		
Altitude	8	ANOVA	0	361	135.10	115.0	102.3	0.604	(72.1, 198.1)
Bird	10		81	250	160.70	158.5	48.8		(104.3, 217.1)
Stock	10		85	175	134.40	130.0	28.9		(78.05, 190.75)
Large	1		-	-	85.00	-	-		(-93.19, 263.19) (-98.88, 268.88)
A -	5	ANOVA	98	150	115.20	110.0	21.5	0.700	(32.97, 197.43)
A +	3		0	361	168.00	144.0	182.0		(62, 274)
B -	2		136	211	173.50	173.5	53.0		(43.5, 303.5)
B +	8		81	250	157.50	158.5	51.1		(92.5, 222.5)
S -	6		109	175	148.20	151.5	26.4		(73.1, 223.2)
S +	4		85	130	113.75	120.0	19.8		(21.81, 205.69)
G3	2	ANOVA	0	98	49.00	49.0	69.3	0.073	(-71.4, 169.4)
G5	9		85	361	176.90	150.0	83.8		(120.1, 233.6)
G6	5		109	175	132.40	130.0	25.4		(56.3, 208.5)
G7	9		81	211	134.90	136.0	39.4		(78.1, 191.6)

Table B.14, Chl-α Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	14	Mann-Whitney	14.70	208.70	86.90	76.60	50.7	0.100	(-92.935, 17.935)
Fish +	16		16.30	471.20	132.00	101.10	103.1		
Altitude	9	ANOVA	16.30	208.70	95.30	78.30	56.4	0.153	(18.2, 172.4)
Bird	10		14.70	471.20	152.90	123.90	127.2		(79.8, 226.1)
Stock	10		35.87	154.89	82.34	75.00	31.4		(9.22, 155.46)
Large	1		-	-	117.39	-	-		(-113.8, 348.6) (-104.5, 339.3)
A -	6	ANOVA	55.40	208.70	107.10	79.10	58.0	0.162	(16.5, 197.7)
A +	3		16.30	127.20	71.70	71.70	55.4		(-56.4, 199.9)
B -	2		14.70	58.70	36.70	36.70	31.1		(-120.2, 193.6)
B +	8		75.00	471.20	182.00	164.70	125.9		(103.5, 260.5)
S -	6		35.90	154.90	83.40	72.60	40.3		(-7.2, 174.0)
S +	4		66.85	102.72	80.71	76.63	15.6		(-30.26, 191.67)
G3	2	ANOVA	71.74	79.89	75.82	75.82	5.8	0.750	(-108.71, 260.34)
G5	10		16.30	208.70	122.10	97.00	66.1		(39.6, 204.6)
G6	5		66.85	102.72	83.48	76.63	15.8		(-33.23, 200.18)
G7	9		14.70	471.20	129.70	75.00	136.1		(42.7, 216.7)

Table B.14, Conductivity Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	14	Mann-Whitney	19.0	136.0	57.40	53.50	39.10	0.835	(-22, 36)
Fish +	16		16.4	110.0	47.65	44.00	20.87		
Altitude	9	ANOVA	19.0	34.0	27.11	27.00	5.53	0.001	(4.88, 49.34)
Bird	10		16.4	110.0	52.44	46.50	24.19		(31.35, 73.53)
Stock	10		46.0	136.0	76.70	62.00	32.40		(55.6, 97.8)
Large	1		-	-	31.00	-	-		(-35.69, 97.69) (-30.99, 92.99)
A -	6	ANOVA	19.0	29.0	24.00	23.50	3.69	0.001	(-1.31, 49.31)
A +	3		32.0	34.0	33.33	34.00	1.16		(-2.454, 69.121)
B -	2		48.0	59.0	53.50	53.50	7.78		(9.67, 97.33)
B +	8		16.4	110.0	52.17	44.00	27.26		(30.26, 74.09)
S -	6		61.0	136.0	92.20	80.50	33.90		(66.9, 117.5)
S +	4		46.0	62.0	53.50	53.00	6.76		(22.51, 84.49)
G3	2	ANOVA	19.0	32.0	25.50	25.50	9.19	0.107	(-14.08, 65.08)
G5	10		22.0	110.0	46.40	42.50	25.94		(28.70, 64.10)
G6	5		51.0	81.0	65.80	62.00	13.99		(40.77, 90.83)
G7	9		22.0	62.0	45.11	48.00	14.80		(26.45, 63.77)

Table B.14, Cr^{2+} Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	13	Mann-Whitney	218	446	313.7	323.0	62.2	0.025	(-25, 126)
Fish +	16		0	1152	300.6	259.5	240.5		
Altitude	8	ANOVA	0	1152	370.0	325.0	338.0	0.730	(186, 554)
Bird	10		254	368	288.8	274.5	40.1		(124.4, 453.2)
Stock	10		187	446	276.2	276.5	78.8		(111.8, 440.6)
Large	1		-	-	278.0	-	-		(-242.0, 798.0) (-263.8, 819.8)
A -	5	ANOVA	218	376	318.2	327.0	59.8	0.828	(75.9, 560.5)
A +	3		0	1152	456.0	217.0	612.0		(144, 769)
B -	2		263	343	303.0	303.0	56.6		(-80.1, 686.1)
B +	8		254	368	285.3	274.5	39.2		(93.7, 476.8)
S -	6		221	446	313.5	312.5	75.6		(92.3, 534.7)
S +	4		187	288	220.3	203.0	46.4		(-50.6, 491.1)
G3	2	ANOVA	0	347	174.0	174.0	245.0	0.411	(-213, 560)
G5	9		218	1152	384.8	289.0	291.1		(202.8, 566.8)
G6	5		187	324	251.2	221.0	62.4		(7.0, 495.4)
G7	9		194	376	280.2	265.0	58.7		(98.2, 462.2)

Table B.14, Cu^{2+} Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	12	Mann-Whitney	0.59	8.78	2.06	1.62	2.19	0.105	(-6.5071, 0.4957)
Fish +	14		0.45	15.31	4.53	2.84	4.71		
Altitude	5	Kruskall-Wallace	0.60	5.72	1.92	0.98	2.14	0.174	
Bird	10		0.450	8.78	3.45	2.84	2.98		
Stock	10		0.59	13.47	2.88	1.94	3.75		
Large	1		-	-	15.31	-	-		(6.60, 24.01)
A -	4	ANOVA	0.60	1.35	0.97	0.96	0.30	0.013	(-3.384, 5.319)
A +	1		-	-	5.72	-	-		(-2.981, 14.426)
B -	2		1.99	8.78	5.39	5.39	4.80		(-0.77, 11.54)
B +	8		0.45	8.61	2.96	2.84	2.61		(-0.116, 6.038)
S -	6		0.59	2.11	1.68	1.94	0.59		(-1.878, 5.229)
S +	4		1.53	13.47	4.68	1.87	5.86		(0.33, 9.04)
G3	1	ANOVA	-	-	0.60	-	-	0.679	(-9.181, 10.390)
G5	7		0.45	13.47	3.50	2.78	4.56		(-0.20, 7.19)
G6	5		1.40	2.01	1.80	1.85	0.26		(-2.575, 6.177)
G7	9		0.98	8.78	3.59	1.99	3.22		(0.33, 6.85)

Table B.14, DisCoast Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	14	Mann-Whitney	1.230	5.114	2.941	1.994	1.576	0.445	(-1.431, 2.528)
Fish +	16		1.319	12.872	3.649	1.823	3.677		
Altitude	9	Kruskall-Wallace	3.421	5.250	4.520	4.778	0.637	0.002	
Bird	10		1.230	4.699	1.937	1.625	1.029		
Stock	10		1.370	12.060	2.660	1.670	3.310		
A -	6	ANOVA	3.819	5.114	4.645	4.779	0.502	0.075	(2.306, 6.985)
A +	3		3.421	5.250	4.270	4.139	0.922		(0.961, 7.579)
B -	2		1.230	2.052	1.641	1.641	0.581		(-2.411, 5.693)
B +	8		1.319	4.699	2.011	1.625	1.132		(-0.015, 4.037)
S -	6		1.430	1.936	1.670	1.672	0.183		(-0.6700, 4.0090)
S +	4		1.370	12.060	4.150	1.590	5.270		(1.29, 7.02)
G3	2	ANOVA	4.778	5.250	5.014	5.014	0.334	0.090	(0.703, 9.325)
G5	10		1.319	12.056	4.217	4.215	3.150		(2.289, 6.145)
G6	5		1.370	1.936	1.679	1.782	0.230		(-1.048, 4.406)
G7	9		1.230	4.781	2.383	1.717	1.289		(0.351, 4.415)

Table B.14, Fe^{3+} Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	13	Mann-Whitney	47.40	782.90	197.60	102.80	211.8	0.004	(16.15, 190.39)
Fish +	16		0.00	423.60	74.10	26.20	118.1		
Altitude	8	Kruskall-Wallace	12.30	782.90	162.60	79.90	257.2	0.208	
Bird	10		8.10	450.20	165.10	111.00	163.6		
Stock	10		0.00	323.70	80.30	48.50	100.4		
Large	1		-	-	0.00	-	-		
A -	5	Kruskall-Wallace	66.00	783.00	247.00	103.00	303.0	0.013	
A +	3		12.29	29.73	21.55	22.63	8.8		
B -	2		128.00	450.00	289.00	289.00	228.0		
B +	8		8.10	423.60	134.00	87.70	146.7		
S -	6		47.40	323.70	125.70	76.00	108.8		
S +	4		0.00	33.47	12.28	7.83	14.6		
G3	2	ANOVA	29.70	190.40	110.10	110.10	113.6	0.823	(-275.6, 495.7)
G5	9		8.10	423.60	121.20	65.90	142.4		(-60.6, 303.0)
G6	5		0.00	323.70	112.80	49.60	138.4		(-131.1, 356.7)
G7	9		6.50	782.90	193.30	94.00	256.7		(11.5, 375.1)

Table B.14, Hardness Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	13	Mann-Whitney	2.38	46.79	11.35	5.67	14.89	0.076	(-6.9427, 2.9188)
Fish +	16		4.79	25.50	10.40	8.10	6.42		
Altitude	8	ANOVA	2.38	10.62	5.06	4.89	2.78	0.229	(-5.322, 15.436)
Bird	10		3.49	25.50	11.06	8.57	7.67		(1.78, 20.34)
Stock	10		5.18	46.79	15.61	7.49	15.60		(6.33, 24.89)
Large	1		-	-	6.73	-	-		(-22.629, 36.082)
A -	5	Kruskall-Wallace	2.38	6.34	3.58	2.94	1.65	0.034	
A +	3		5.94	10.62	7.51	5.98	2.69		
B -	2		3.49	5.67	4.58	4.58	1.54		
B +	8		4.79	25.50	12.68	9.01	7.76		
S -	6		5.18	46.79	20.07	10.22	18.97		
S +	4		5.24	17.21	8.93	6.63	5.56		
G3	2	ANOVA	3.83	5.98	4.90	4.90	1.51	0.181	(-6.43, 16.24)
G5	9		2.38	25.50	11.65	9.37	8.70		(6.30, 16.99)
G6	5		5.18	12.28	7.08	6.25	2.97		(-0.09, 14.24)
G7	9		2.94	8.48	6.18	6.34	1.94		(0.832, 11.518)

Table B.14, K^+ Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	13	2-Sample T-Test	165.2	951.6	392.7	352.7	215.7	0.834	(-180.4, 209.2)
Fish +	16		248.8	664.1	378.3	345.8	126.4		
Altitude	8	ANOVA	165.2	462.6	273.1	250.7	97.4	0.095	(117.6, 428.7)
Bird	10		248.8	664.1	409.9	339.0	162.9		(270.8, 549.0)
Stock	10		281.5	951.6	460.5	413.2	187.8		(321.4, 599.7)
Large	1		-	-	268.3	-	-		(-171.7, 708.2) (-183.9, 720.4)
A -	5	ANOVA	165.2	356.7	232.1	217.1	73.8	0.220	(29.9, 434.3)
A +	3		272.4	462.6	341.5	289.6	105.2		(80.4, 602.6)
B -	2		323.0	604.0	463.0	463.0	198.0		(144, 783)
B +	8		248.8	664.1	396.5	331.9	165.7		(236.6, 556.4)
S -	6		281.5	951.6	503.0	455.8	238.9		(318.4, 687.6)
S +	4		336.6	424.5	396.9	413.2	41.2		(170.8, 623.0)
G3	2	ANOVA	165.0	463.0	314.0	314.0	210.0	0.933	(35, 593)
G5	9		192.5	664.1	343.8	272.4	168.5		(212.2, 475.3)
G6	5		281.5	422.4	370.8	393.5	56.1		(194.4, 547.3)
G7	9		229.0	603.8	372.3	354.9	124.4		(240.8, 503.9)

Table B.14, Mg^{2+} Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	13	2-Sample T-Test	0.528	2.238	1.099	0.956	0.576	0.845	(-0.585, 0.508)
Fish +	16		0.664	2.255	1.137	0.982	0.440		
Altitude	8	ANOVA	0.528	0.858	0.669	0.652	0.126		(0.2650, 1.0735)
Bird	10		0.849	2.255	1.289	1.100	0.475		(0.928, 1.651)
Stock	10		0.924	2.238	1.358	1.221	0.479		(0.996, 1.719)
Large	1		-	-	0.664	-	-		(-0.4795, 1.8072) (-0.4544, 1.7821)
A -	5	ANOVA	0.528	0.716	0.590	0.573	0.074	0.012	(0.0903, 1.0905)
A +	3		0.748	0.858	0.801	0.796	0.055		(0.1550, 1.4462)
B -	2		0.849	1.236	1.042	1.042	0.274		(0.251, 1.833)
B +	8		0.926	2.255	1.351	1.100	0.508		(0.956, 1.746)
S -	6		0.956	2.238	1.542	1.455	0.530		(1.086, 1.999)
S +	4		0.924	1.409	1.082	0.997	0.224		(0.522, 1.641)
G3	2	ANOVA	0.573	0.858	0.715	0.715	0.202	0.292	(-0.103, 1.534)
G5	9		0.528	2.255	1.185	1.052	0.599		(0.799, 1.571)
G6	5		0.924	1.518	1.167	1.049	0.271		(0.649, 1.685)
G7	9		0.589	1.236	0.896	0.926	0.195		(0.5105, 1.2824)

Table B.14, Na^+ Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	13	2-Sample T-Test	3.451	12.207	8.135	9.008	3.425	0.514	(-2.40, 3.84)
Fish +	16		4.504	11.849	7.415	6.942	2.085		
Altitude	8	ANOVA	3.451	5.931	4.757	4.649	0.738	0.000	(2.989, 6.525)
Bird	10		5.736	11.849	8.348	7.193	2.184		(6.766, 9.929)
Stock	10		6.338	12.207	9.836	9.356	1.936		(8.254, 11.417)
Large	1		-	-	4.504	-	-		(-0.498, 9.505)
		ANOVA						0.000	(-0.056, 9.064)
A -	5		3.451	4.744	4.326	4.512	0.507		(2.287, 6.366)
A +	3		5.231	5.931	5.475	5.263	0.395		(2.843, 8.108)
B -	2		7.310	11.140	9.230	9.230	2.710		(6.00, 12.45)
B +	8		5.736	11.849	8.128	7.044	2.192		(6.516, 9.741)
S -	6		9.008	12.207	10.946	11.677	1.421		(9.085, 12.808)
S +	4		6.338	9.450	8.170	8.446	1.311		(5.890, 10.450)
G3	2	ANOVA	3.450	5.930	4.690	4.690	1.750	0.050	(0.05, 9.33)
G5	9		4.512	11.849	7.102	6.600	2.555		(4.914, 9.289)
G6	5		8.405	12.207	9.983	9.262	1.780		(7.049, 12.918)
G7	9		4.371	11.143	7.098	7.078	2.367		(4.910, 9.285)

Table B.14, NH_4 Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	13	Mann-Whitney	0.0	538.0	249.0	273.0	215.4	0.369	(-340, 216)
Fish +	15		5.0	616.0	293.9	347.0	230.9		
Altitude	8	ANOVA	0.0	616.0	210.1	31.5	270.3	0.448	(-9.3, 429.5)
Bird	10		40.0	586.0	309.2	389.5	232.0		(112.9, 505.5)
Stock	9		9.0	493.0	318.6	355.0	152.4		(111.7, 525.4)
Large	1		-	-	5.0	-	-		(-615.62, 625.62)
		ANOVA						0.267	(-595.17, 605.17)
A -	5		0.0	472.0	107.8	28.0	204.1		(-160.6, 376.2)
A +	3		16.0	616.0	381.0	510.0	320.0		(34, 727)
B -	2		439.0	538.0	488.5	488.5	70.0		(64.1, 912.9)
B +	8		40.0	586.0	264.4	198.5	238.8		(52.2, 476.6)
S -	6		9.0	493.0	286.8	330.5	180.0		(41.8, 531.8)
S +	3		347.0	444.0	382.0	355.0	53.8		(35.5, 728.5)
G3	2	ANOVA	28.0	616.0	322.0	322.0	416.0	0.749	(-132, 776)
G5	8		35.0	586.0	336.9	482.5	243.2		(109.6, 564.1)
G6	5		273.0	444.0	373.0	388.0	64.4		(85.6, 660.4)
G7	9		0.0	538.0	246.3	340.0	228.3		(32.1, 460.6)

Table B.14, NO_3^- Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	14	2-Sample T-Test	164	1020	518.6	551.0	241.7	0.015	(-14.6, 419.6)
Fish +	16		152	722	316.1	282.5	167.8		
Altitude	9	ANOVA	164	633	355.8	319.0	154.3	0.358	(147.3, 564.2)
Bird	10		164	722	395.8	286.0	220.8		(198.0, 593.6)
Stock	10		152	1020	499.6	532.0	276.4		(301.8, 697.4)
Large	1		-	-	161.0	-	-		(-464.4, 786.4) (-361.4, 683.4)
A -	6	ANOVA	164	633	381.8	326.5	181.4	0.017	(168.6, 595.1)
A +	3		214	378	303.7	319.0	83.1		(2.1, 605.3)
B -	2		172	699	436.0	436.0	373.0		(66, 805)
B +	8		164	722	385.9	286.0	205.6		(201.2, 570.6)
S -	6		532	1020	683.0	652.0	183.2		(469.7, 896.3)
S +	4		152	309	224.5	218.5	71.4		(-36.7, 485.7)
G3	2	ANOVA	378	570	474.0	474.0	135.8	0.423	(17.8, 930.2)
G5	10		164	722	384.6	299.0	216.0		(180.6, 588.6)
G6	5		181	1020	550.0	532.0	332.0		(261, 838)
G7	9		152	699	342.9	287.0	185.0		(127.8, 557.9)

Table B.14, O_2 Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	14	2-Sample T-Test	28.0	76.0	43.56	41.10	14.28	0.739	(-14.87, 18.97)
Fish +	16		0.7	88.0	45.62	46.15	19.07		
Altitude	9	ANOVA	28.0	39.8	32.63	33.00	3.74	0.052	(18.47, 46.79)
Bird	10		0.7	88.0	46.63	42.75	23.98		(33.20, 60.06)
Stock	10		42.0	76.0	52.98	49.50	9.37		(39.55, 66.41)
Large	1		-	-	50.00	-	-		(7.52, 92.48) (4.86, 95.14)
A -	6	ANOVA	28.0	33.8	30.72	30.40	2.30	0.253	(12.287, 49.147)
A +	3		33.6	39.8	36.47	36.00	3.13		(10.40, 62.53)
B -	2		40.2	55.7	47.95	47.95	10.96		(16.03, 79.87)
B +	8		0.7	88.0	46.30	42.75	26.86		(30.34, 62.26)
S -	6		42.0	76.0	54.95	53.30	11.80		(36.52, 73.38)
S +	4		47.3	55.0	50.03	48.90	3.40		(27.45, 72.60)
G3	2	ANOVA	28.0	33.6	30.80	30.80	3.96	0.237	(2.96, 58.64)
G5	10		30.4	88.0	48.16	40.30	19.33		(35.71, 60.61)
G6	5		47.7	57.4	51.90	49.00	4.69		(34.29, 69.51)
G7	9		28.7	55.7	40.93	40.20	10.16		(27.81, 54.06)

Table B.14, Pb^{2+} Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	13	Mann-Whitney	634	7339	1852	1359	1925	0.030	(-58, 1127)
Fish +	16		0	7691	1137	702	1791		
Altitude	8	ANOVA	0	7691	2095	1008	2646	0.519	(245, 3945)
Bird	10		400	7339	1653	1029	2029		(-1, 3308)
Stock	10		142	1523	828	738	472		(-827, 2482)
Large	1		-	-	692.00	-	-		(-4539.8, 5923.8) (-4166.7, 5550.7)
A -	5	ANOVA	634	4464	1713	1359	1585	0.160	(-460, 3885)
A +	3		0	7691	2732	506	4302		(-73, 5538)
B -	2		1573	7339	4456	4456	4077		(1020, 7892)
B +	8		400	1513	952	931	338		(-765, 2670)
S -	6		635	1523	1101	1085	384		(-883, 3084)
S +	4		142	689	419	423	224		(-2010, 2848)
G3	2	ANOVA	0	634	317	317	448	0.492	(-3688, 4322)
G5	9		689	7691	2217	1248	2347		(329, 4105)
G6	5		142	1489	793	635	580		(-1740, 3326)
G7	9		400	7339	1627	845	2192		(-261, 3516)

Table B.14, pH Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	12	2-Sample T-Test	4.60	6.71	5.633	5.620	0.773	0.001	(-2.126, -0.307)
Fish +	12		5.90	8.50	6.850	6.725	0.800		
Altitude	9	ANOVA	5.50	7.00	6.411	6.580	0.556	0.078	(5.560, 7.263)
Bird	6		4.70	6.90	5.983	6.050	0.722		(4.940, 7.026)
Stock	8		4.60	8.30	5.963	6.000	1.256		(5.059, 6.866)
Large	1		-	-	8.500	-	-		(5.946, 11.054) (6.773, 10.227)
A -	6	ANOVA	5.50	6.71	6.167	6.335	0.526	0.000	(5.461, 6.872)
A +	3		6.85	7.00	6.900	6.850	0.087		(5.9027, 7.8973)
B -	2		4.70	6.00	5.350	5.350	0.919		(4.129, 6.571)
B +	4		5.90	6.90	6.300	6.200	0.432		(5.436, 7.164)
S -	4		4.60	5.70	4.975	4.800	0.499		(4.111, 5.839)
S +	4		6.30	8.30	6.950	6.600	0.911		(6.086, 7.814)
G3	2	ANOVA	6.23	6.85	6.540	6.540	0.438	0.422	(4.750, 8.330)
G5	8		5.50	8.30	6.419	6.200	0.923		(5.524, 7.314)
G6	5		4.60	6.60	5.620	5.700	0.864		(4.488, 6.752)
G7	8		4.70	6.90	6.096	6.510	0.905		(5.201, 6.991)

Table B.14, Salinity Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	13	2-Sample T-Test	12.10	40.83	27.04	29.45	11.01	0.621	(-8.45, 12.06)
Fish +	16		16.35	39.38	25.23	24.75	7.65		
Altitude	8	ANOVA	12.10	24.57	16.74	16.95	3.98	0.000	(10.05, 23.43)
Bird	10		19.15	40.47	28.40	25.56	8.40		(22.42, 34.38)
Stock	10		16.35	40.83	32.08	32.34	6.72		(26.09, 38.06)
Large	1		-	-	16.39	-	-		(-2.54, 35.31) (-1.61, 34.39)
A -	5	ANOVA	12.10	18.43	14.79	14.83	2.69	0.001	(6.74, 22.84)
A +	3		17.60	24.57	19.99	17.80	3.97		(9.59, 30.38)
B -	2		25.47	40.47	32.97	32.97	10.60		(20.24, 45.70)
B +	8		19.15	39.38	27.26	25.29	8.19		(20.89, 33.62)
S -	6		29.45	40.83	35.26	35.23	4.21		(27.91, 42.61)
S +	4		16.35	32.70	27.30	30.08	7.44		(18.30, 36.30)
G3	2	ANOVA	12.10	17.60	14.85	14.85	3.88	0.039	(-1.52, 31.22)
G5	9		14.83	39.38	23.11	19.15	9.63		(15.39, 30.83)
G6	5		29.27	40.83	34.36	31.98	5.14		(24.01, 44.71)
G7	9		12.30	40.47	25.43	24.93	8.22		(17.72, 33.15)

Table B.14, Si^{2+} Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	13	2-Sample T-Test	58.5	1279.1	279.6	143.7	341.5	0.132	(-534, 152)
Fish +	16		87.5	1110.1	470.8	401.2	311.2		
Altitude	8	ANOVA	70.5	403.3	199.1	158.0	120.4	0.283	(-123.7, 521.9)
Bird	10		87.5	944.2	506.9	526.2	296.2		(218.2, 795.6)
Stock	10		58.5	1279.0	411.0	224.0	446.0		(122, 699)
Large	1		-	-	399.1	-	-		(-513.9, 1312.1) (-537.9, 1336.1)
A -	5	ANOVA	70.5	347.1	174.7	143.7	115.3	0.433	(-244.3, 593.8)
A +	3		144.8	403.3	239.8	171.3	142.2		(-301.2, 780.8)
B -	2		88.0	307.0	198.0	198.0	155.0		(-465, 860)
B +	8		87.5	944.2	584.2	574.0	274.3		(252.9, 915.5)
S -	6		58.0	1279.0	394.0	172.0	485.0		(12, 777)
S +	4		177.0	1110.0	435.0	227.0	452.0		(-33, 904)
G3	2	ANOVA	171.3	347.1	259.2	259.2	124.3	0.137	(-311.4, 829.8)
G5	9		71.0	1110.0	518.0	614.0	421.0		(249, 787)
G6	5		58.5	260.7	153.2	176.6	82.5		(-207.6, 514.1)
G7	9		60.3	533.7	283.5	266.5	174.5		(14.6, 552.5)

Table B.14, DOC Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	14	2-Sample T-Test	0.00	40.80	20.47	19.48	12.69	0.014	(-0.64, 20.60)
Fish +	15		0.00	21.06	10.50	9.66	5.08		
Altitude	9	ANOVA	0.00	40.80	11.66	8.66	12.19	0.301	(1.88, 21.43)
Bird	10		7.20	29.41	14.66	11.45	7.59		(5.39, 23.93)
Stock	10		0.00	33.63	19.26	16.65	11.49		(9.98, 28.53)
A -	6	ANOVA	0.00	40.80	12.95	10.87	15.22	0.019	(2.73, 23.17)
A +	3		8.64	9.929	9.076	8.660	0.739		(-5.377, 23.529)
B -	2		22.47	29.41	25.94	25.94	4.91		(8.24, 43.64)
B +	8		7.20	21.06	11.84	10.19	5.01		(2.99, 20.69)
S -	6		16.13	33.63	26.18	29.41	7.96		(15.96, 36.40)
S +	4		0.00	16.80	8.88	9.35	7.14		(-3.64, 21.39)
G3	2	ANOVA	9.90	40.80	25.40	25.40	21.80	0.026	(6.4, 44.3)
G5	10		0.00	21.06	10.35	8.27	6.18		(1.86, 18.84)
G6	5		11.69	33.63	25.20	31.88	10.18		(13.19, 37.21)
G7	9		0.00	29.41	12.23	9.66	9.76		(3.28, 21.18)

Table B.14, TP Statistics

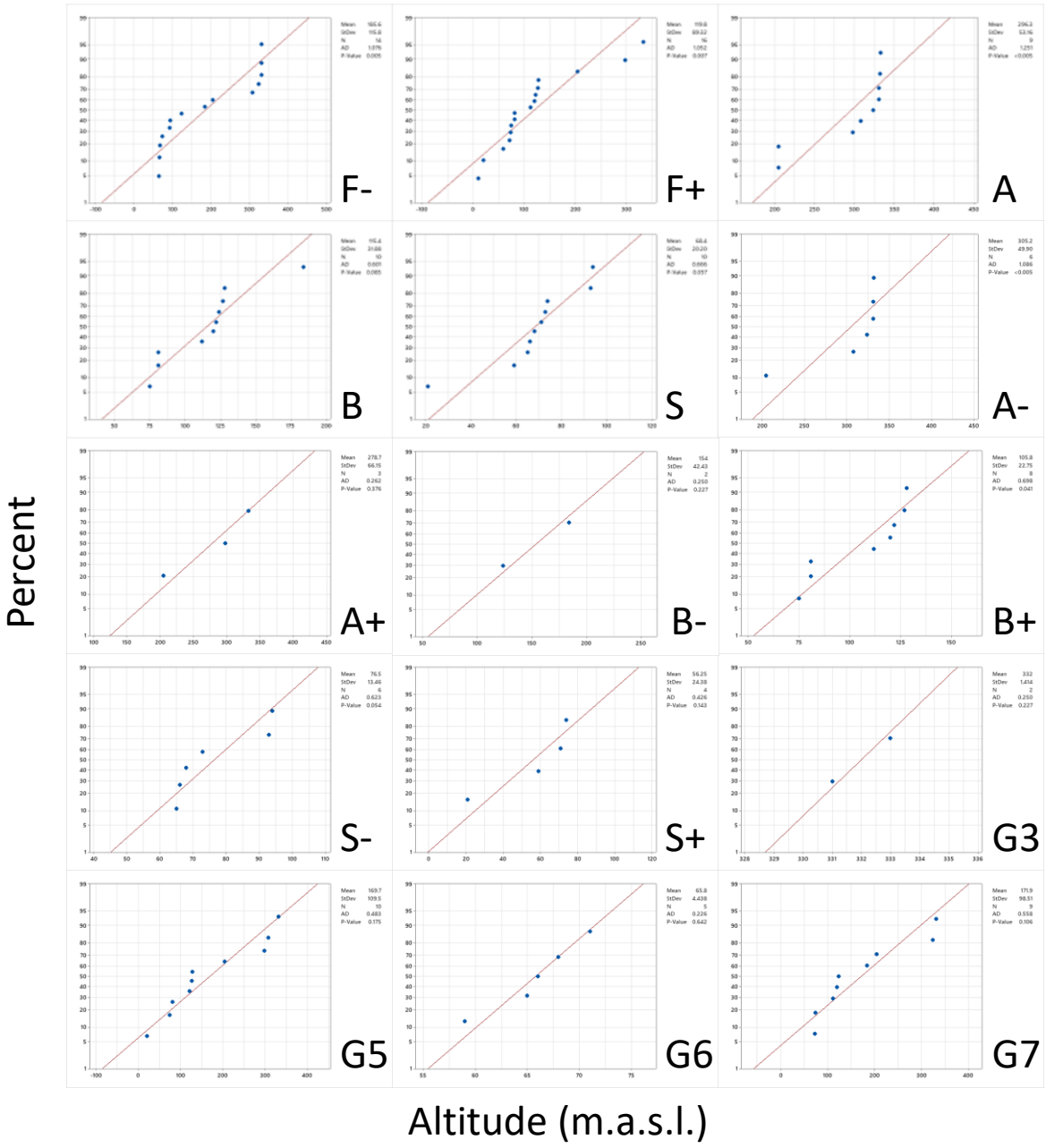
Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	14	2-Sample T-Test	6	25	14.50	15.00	5.06	0.834	(-9.91, 8.53)
Fish +	16		6	43	15.19	11.00	11.78		
Altitude	9	ANOVA	6	42	3.72	14.00	11.16	0.804	(7.12, 24.66)
Bird	10		6	43	3.51	12.00	11.11		(6.68, 23.32)
Stock	10		8	25	1.57	15.50	4.97		(6.38, 23.02)
Large	1		-	-	6.00	-	-		(-20.299, 32.299) (-19.692, 31.692)
A -	6	ANOVA	6	15	11.83	14.00	4.17	0.463	(1.34, 22.32)
A +	3		7	42	24.00	23.00	17.50		(9.2, 38.8)
B -	2		8	20	14.00	14.00	8.49		(-4.17, 32.17)
B +	8		6	43	15.25	12.00	12.16		(6.17, 24.33)
S -	6		13	25	17.33	16.00	4.08		(6.84, 27.82)
S +	4		8	15	10.75	10.00	3.40		(-2.10, 23.60)
G3	2	ANOVA	7	23	15.00	15.00	11.31	0.342	(-4.06, 34.06)
G5	10		7	43	19.40	15.00	12.59		(10.88, 27.92)
G6	5		8	18	12.60	13.00	4.56		(0.55, 24.65)
G7	9		6	25	11.67	8.00	6.84		(2.68, 20.65)

Table B.14, TSI - Carlson Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	14	2-Sample T-Test	0.00	15.230	5.89	5.40	4.50	0.104	(-8.54, 2.09)
Fish +	16		0.00	23.220	9.14	8.12	5.56		
Altitude	9	ANOVA	0.00	15.230	6.77	5.61	4.77	0.273	(1.98, 11.56)
Bird	10		0.00	23.220	10.13	9.92	6.82		(5.58, 14.67)
Stock	10		0.00	12.300	5.70	5.19	3.25		(1.15, 10.24)
Large	1		-	-	9.58	-	-		(-4.790, 23.960)
A -	6	ANOVA	2.22	15.230	7.64	5.71	4.79	0.056	(2.34, 12.94)
A +	3		0.00	10.370	5.04	4.75	5.19		(-2.45, 12.53)
B -	2		0.00	2.780	1.39	1.39	1.97		(-7.78, 10.57)
B +	8		5.19	23.220	12.31	12.86	5.66		(7.72, 16.90)
S -	6		0.00	12.300	5.64	4.85	4.13		(0.34, 10.94)
S +	4		4.06	8.275	5.78	5.39	1.81		(-0.710, 12.269)
G3	2	ANOVA	4.75	5.809	5.28	5.28	0.75	0.711	(-5.731, 16.294)
G5	10		0.00	15.230	9.02	7.71	5.18		(4.10, 13.95)
G6	5		4.06	8.275	6.10	5.40	1.84		(-0.864, 13.066)
G7	9		0.00	23.22	7.97	5.19	7.21		(2.78, 13.16)

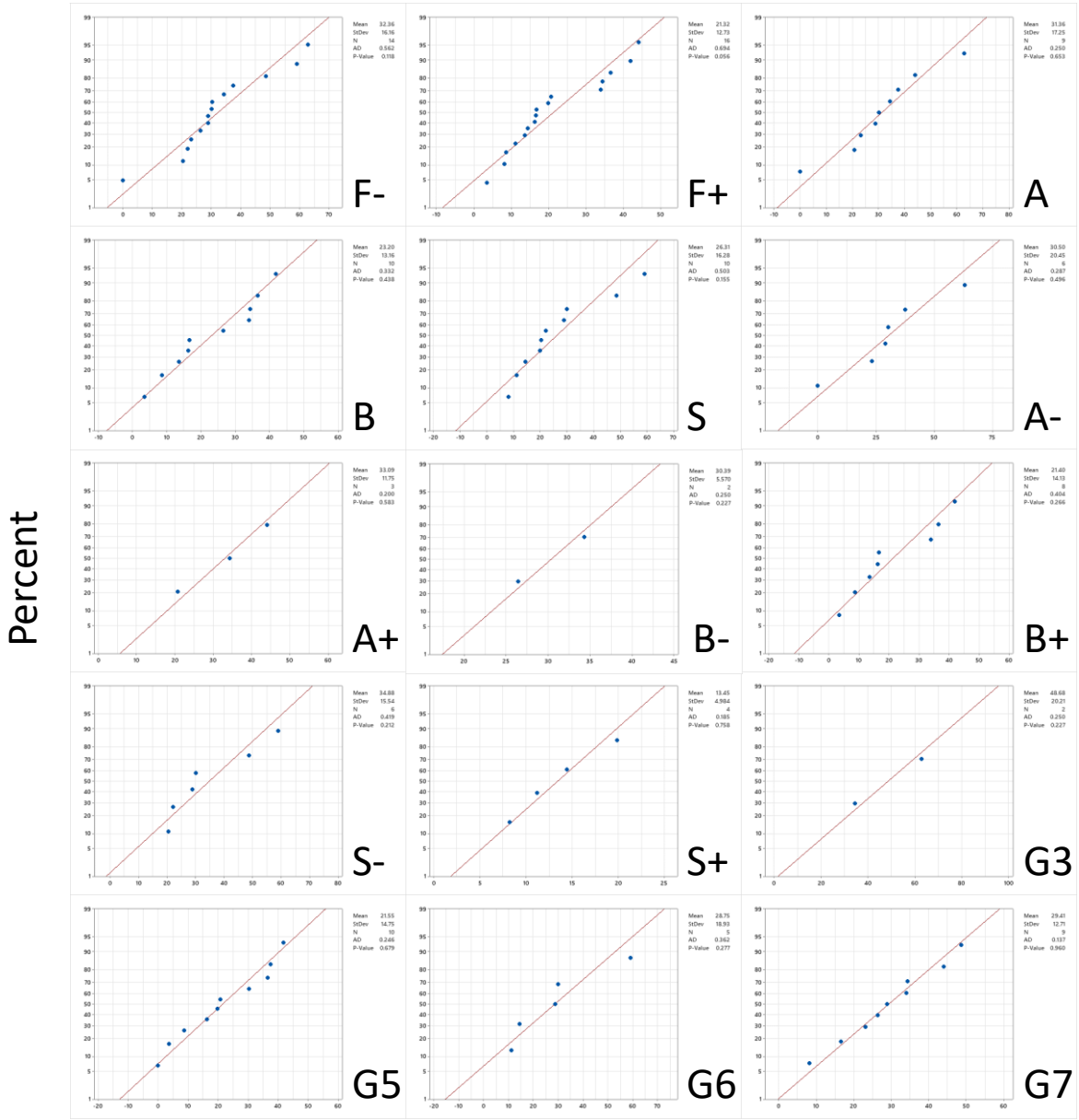
Table B.14, Zn^{2+} Statistics

Group	#	Test	Min	Max	Mean	Median	StDev	P-Value	99% CI
Fish -	14	2-Sample T-Test	0.00	25.54	9.89	8.65	7.43	0.087	(-2.33, 10.25)
Fish +	16		0.000	11.526	5.932	6.246	3.661		
Altitude	9	ANOVA	0.00	25.54	7.29	6.85	7.59	0.927	(1.49, 13.08)
Bird	10		0.00	15.47	8.22	9.00	4.35		(2.73, 13.72)
Stock	10		0.59	20.58	8.13	7.43	6.56		(2.64, 13.63)
Large	1				4.2403				(-13.146, 21.627)
A -	6	ANOVA	0.00	25.54	7.10	4.44	9.58	0.285	(0.47, 13.73)
A +	3		6.848	8.280	7.666	7.869	0.737		(-1.709, 17.041)
B -	2		9.72	15.47	12.60	12.60	4.06		(1.11, 24.08)
B +	8		0.00	11.53	7.13	7.01	3.89		(1.39, 12.87)
S -	6		5.08	20.58	11.79	11.12	5.64		(5.16, 18.41)
S +	4		0.59	7.18	2.66	1.43	3.05		(-5.46, 10.77)
G3	2	ANOVA	6.848	8.183	7.516	7.516	0.944	0.989	(-4.733, 19.764)
G5	10		0.00	25.54	7.51	6.41	7.13		(2.04, 12.99)
G6	5		1.17	13.11	6.55	7.68	5.09		(-1.19, 14.30)
G7	9		0.00	15.47	7.69	7.87	5.79		(1.91, 13.46)



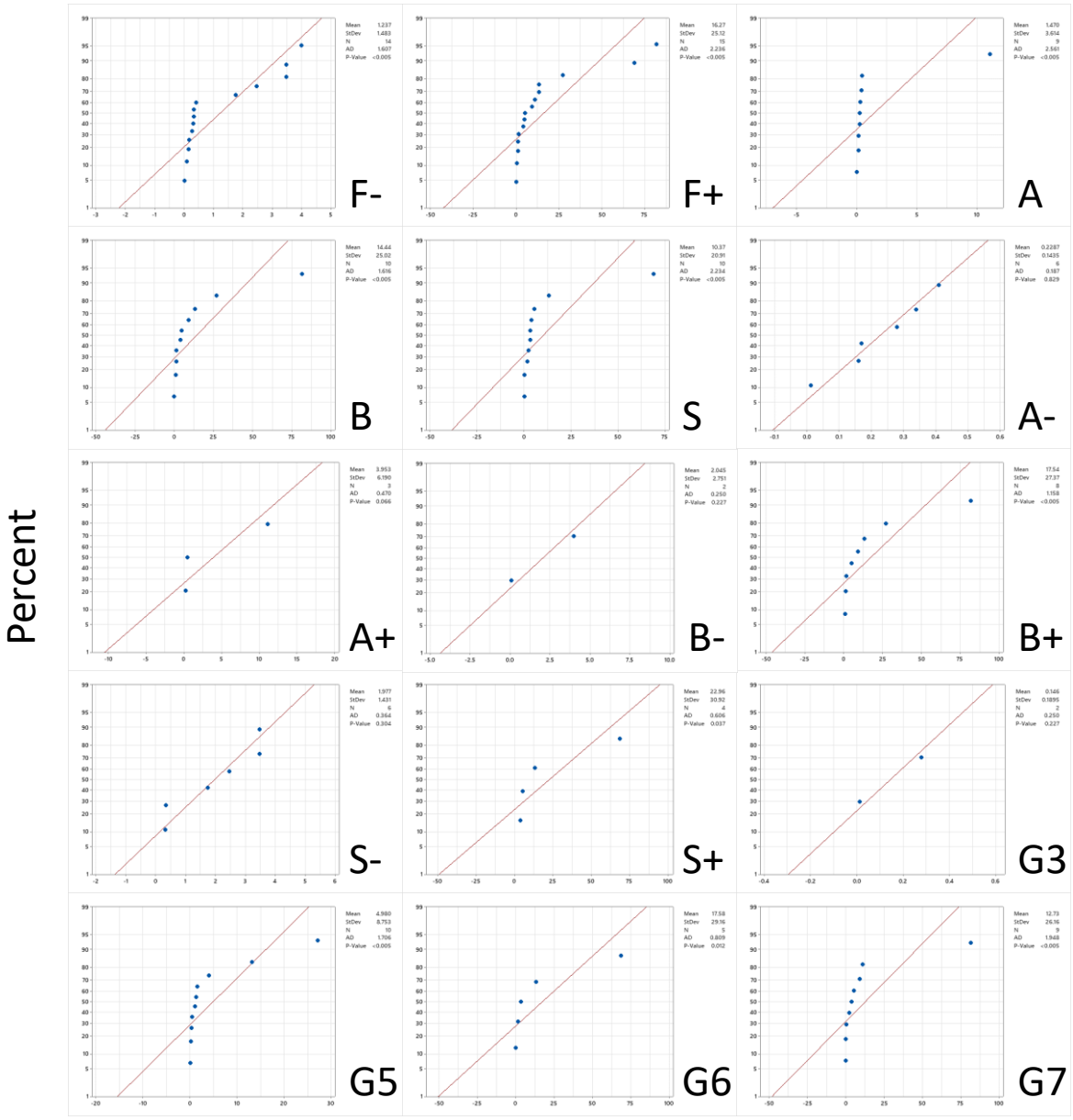
Normality tests for Altitude by Loch Groupings

For all tables in the appendix abbreviations are as follows; F- is fish absence, F+ is fish presence, A, B, and S for altitude, bird and stock loch types respectively, A-, B-, and S- represent fish absent altitude, bird and stock lochs respectively, and A+, B+, and S+ indicate fish present altitude, bird and stock lochs respectively.



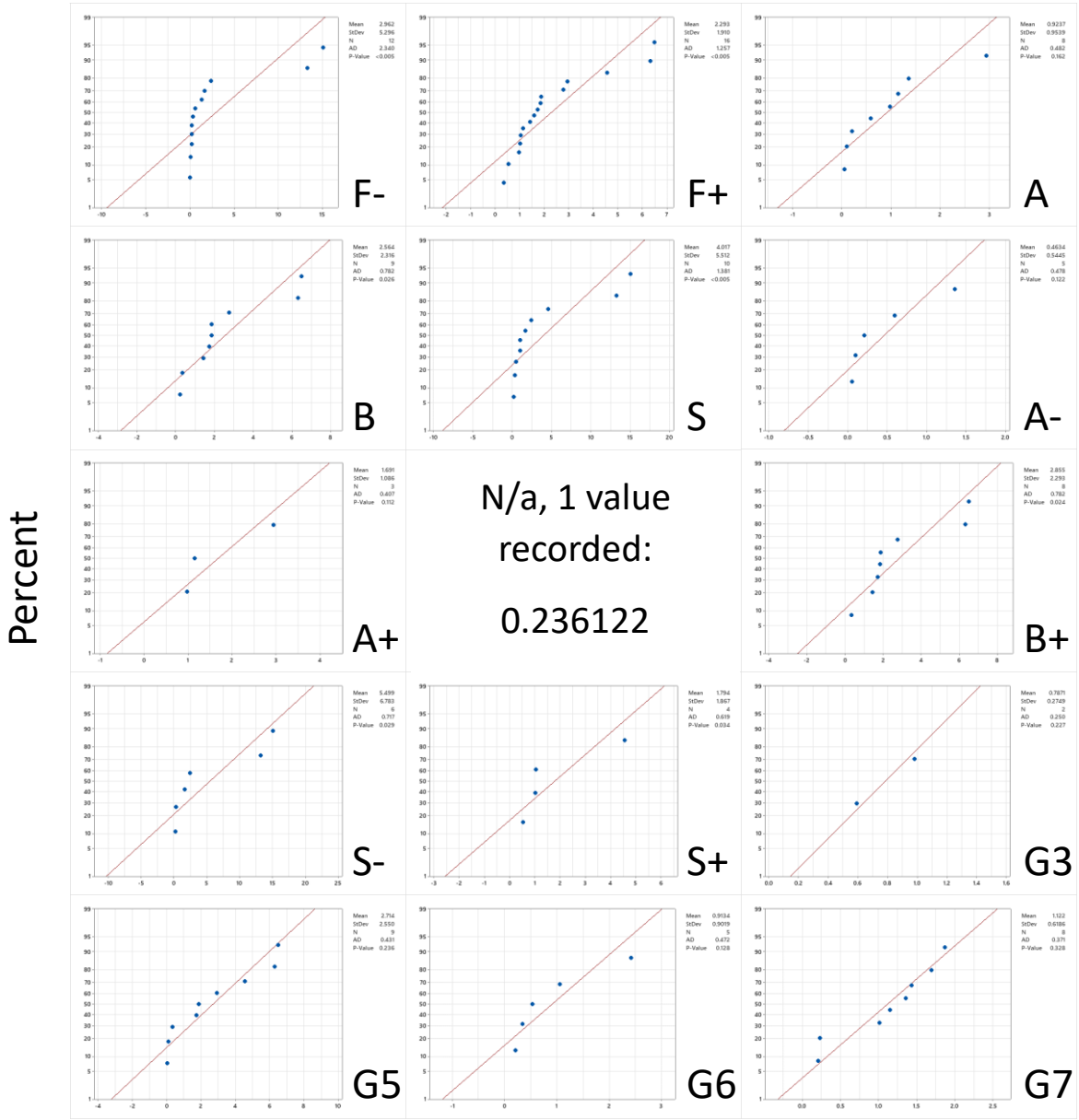
Normality tests for Al^{3+} by Loch Groupings

For all tables in the appendix abbreviations are as follows; F- is fish absence, F+ is fish presence, A, B, and S for altitude, bird and stock loch types respectively, A-, B-, and S- represent fish absent altitude, bird and stock lochs respectively, and A+, B+, and S+ indicate fish present altitude, bird and stock lochs respectively.



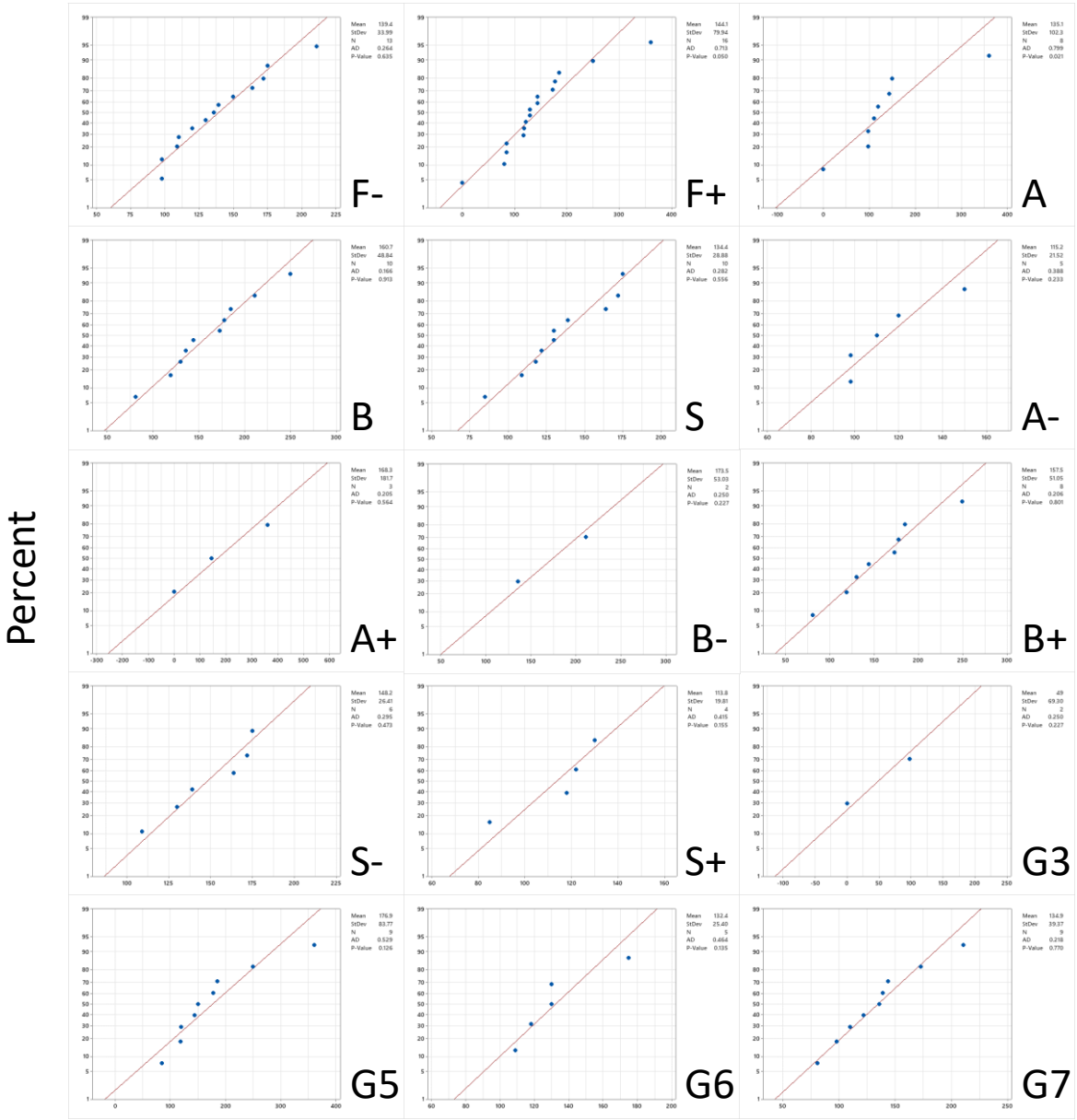
Normality tests for Area by Loch Groupings

For all tables in the appendix abbreviations are as follows; F- is fish absence, F+ is fish presence, A, B, and S for altitude, bird and stock loch types respectively, A-, B-, and S- represent fish absent altitude, bird and stock lochs respectively, and A+, B+, and S+ indicate fish present altitude, bird and stock lochs respectively.



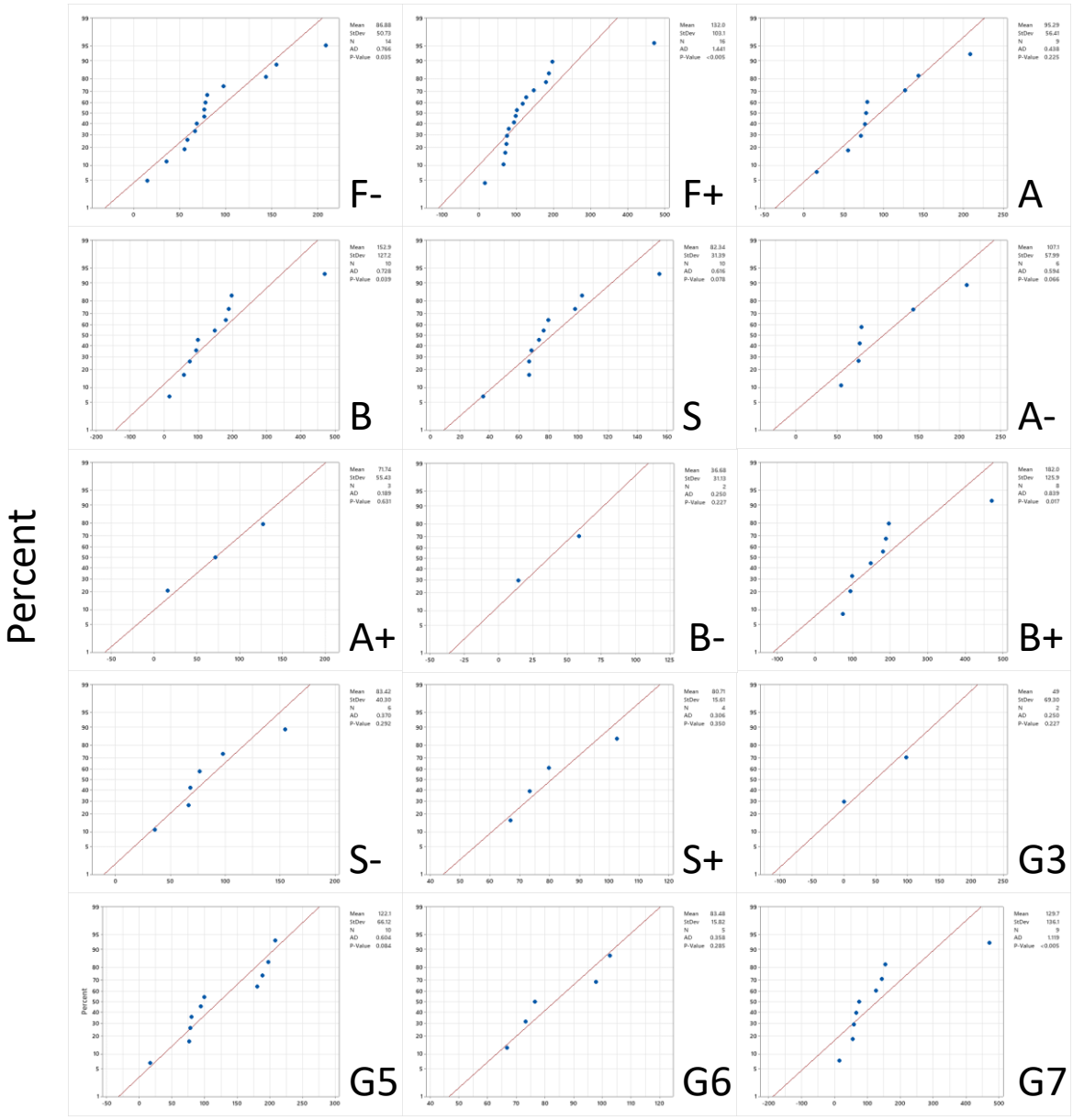
Normality tests for Ca^{2+} by Loch Groupings

For all tables in the appendix abbreviations are as follows; F- is fish absence, F+ is fish presence, A, B, and S for altitude, bird and stock loch types respectively, A-, B-, and S- represent fish absent altitude, bird and stock lochs respectively, and A+, B+, and S+ indicate fish present altitude, bird and stock lochs respectively.



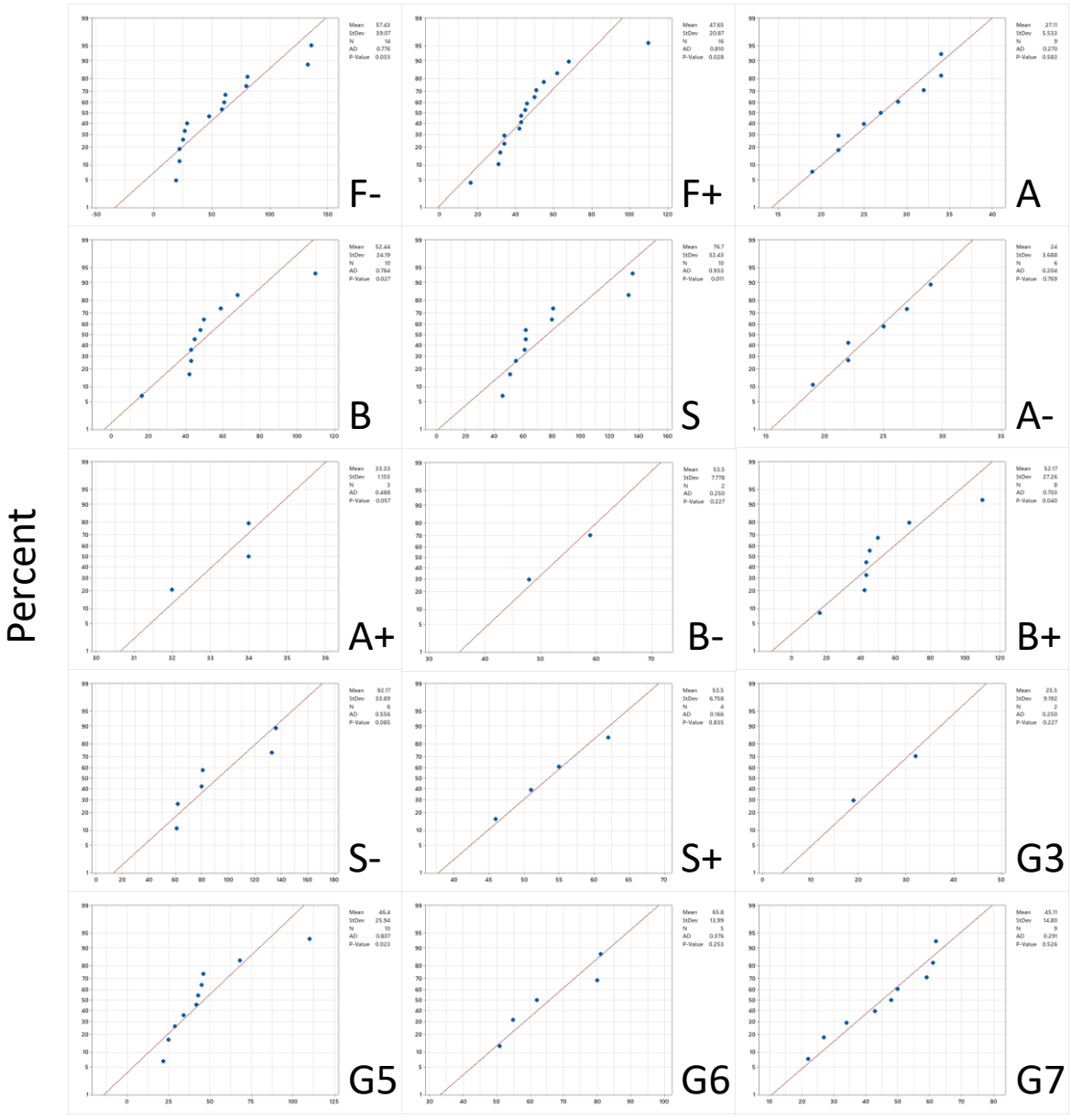
Normality tests for Cd^{2+} by Loch Groupings

For all tables in the appendix abbreviations are as follows; F- is fish absence, F+ is fish presence, A, B, and S for altitude, bird and stock loch types respectively, A-, B-, and S- represent fish absent altitude, bird and stock lochs respectively, and A+, B+, and S+ indicate fish present altitude, bird and stock lochs respectively.



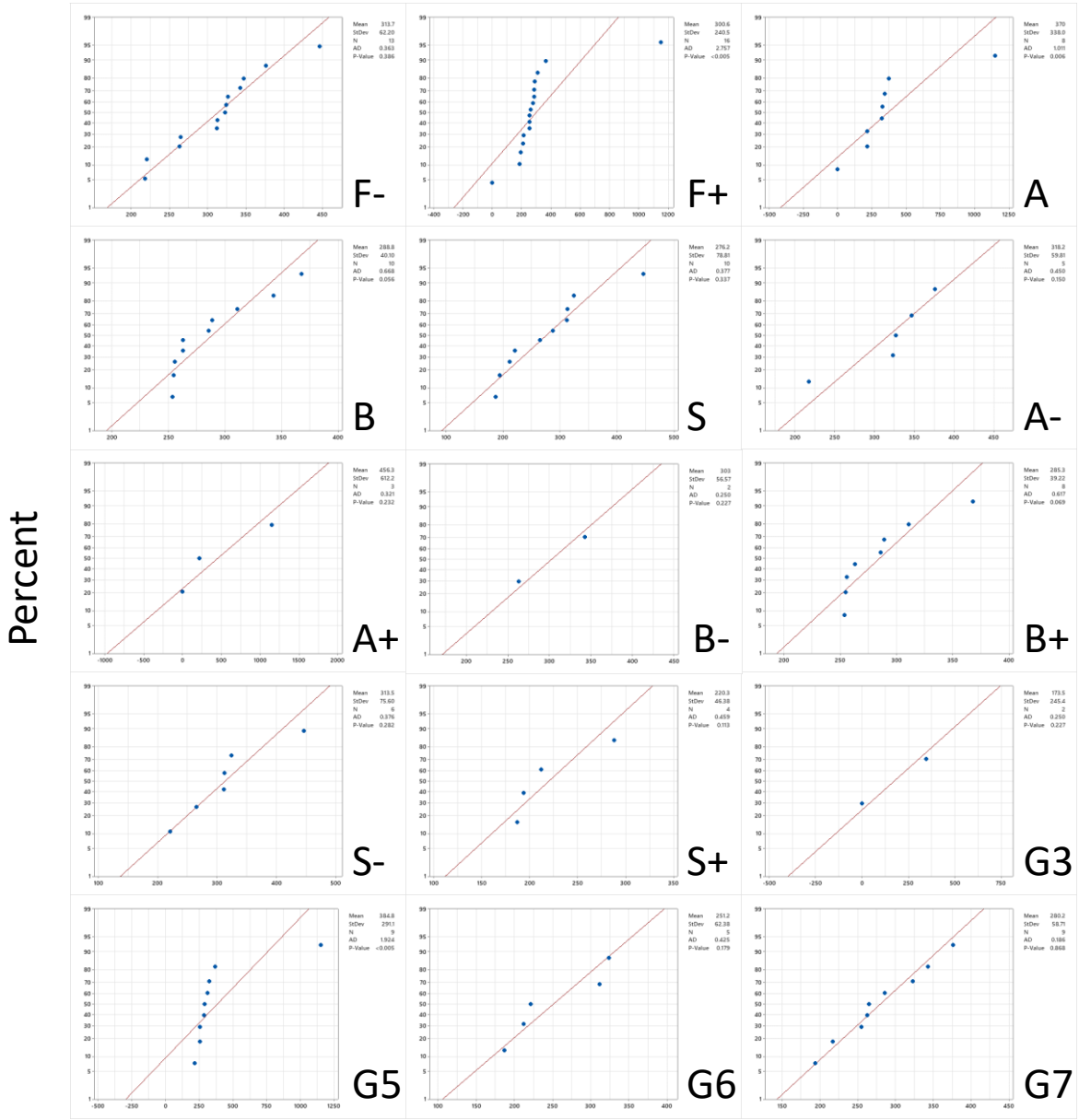
Normality tests for Chl-α by Loch Groupings

For all tables in the appendix abbreviations are as follows; F- is fish absence, F+ is fish presence, A, B, and S for altitude, bird and stock loch types respectively, A-, B-, and S- represent fish absent altitude, bird and stock lochs respectively, and A+, B+, and S+ indicate fish present altitude, bird and stock lochs respectively.



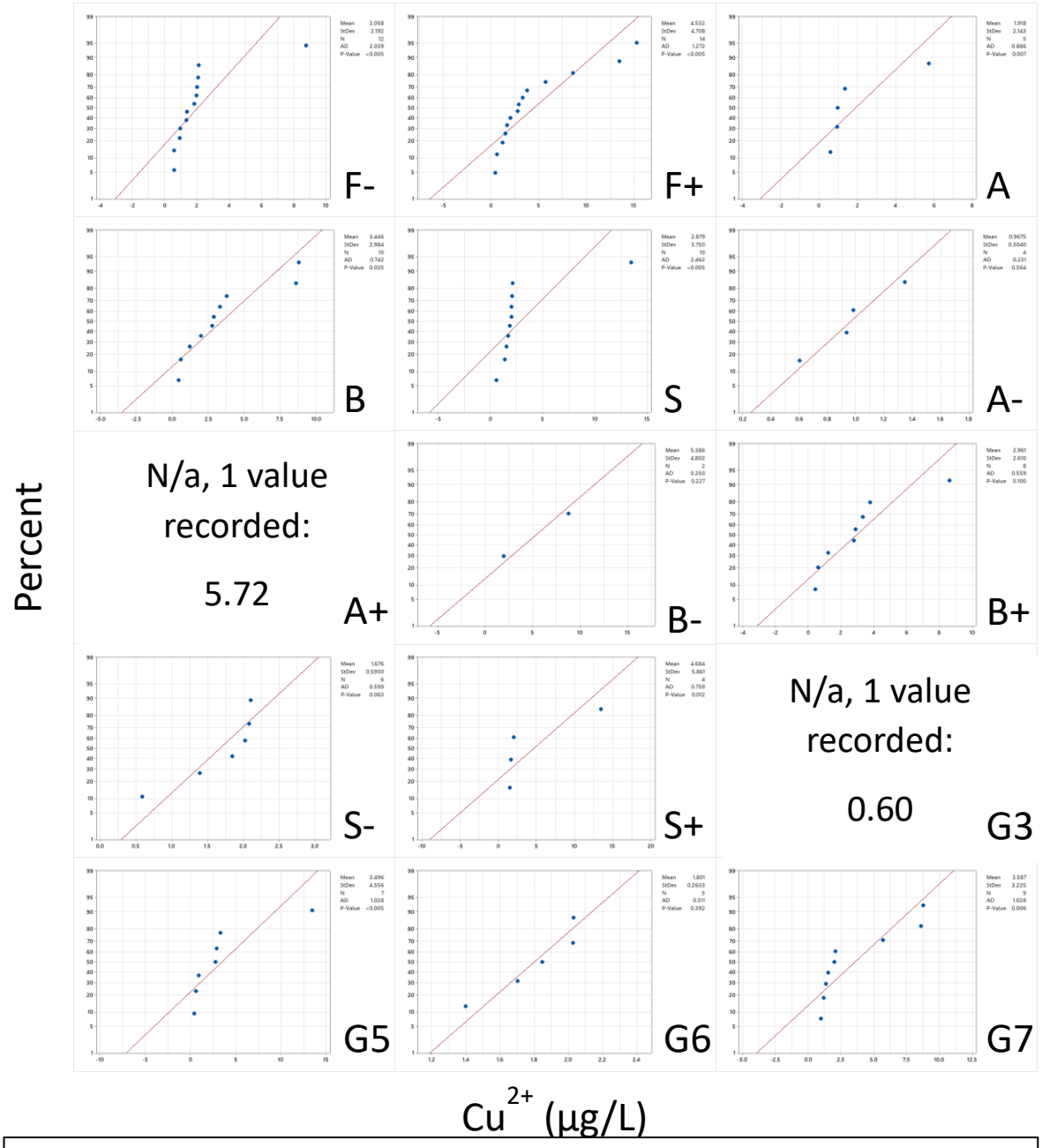
Normality tests for Conductivity by Loch Groupings

For all tables in the appendix abbreviations are as follows; F- is fish absence, F+ is fish presence, A, B, and S for altitude, bird and stock loch types respectively, A-, B-, and S- represent fish absent altitude, bird and stock lochs respectively, and A+, B+, and S+ indicate fish present altitude, bird and stock lochs respectively.



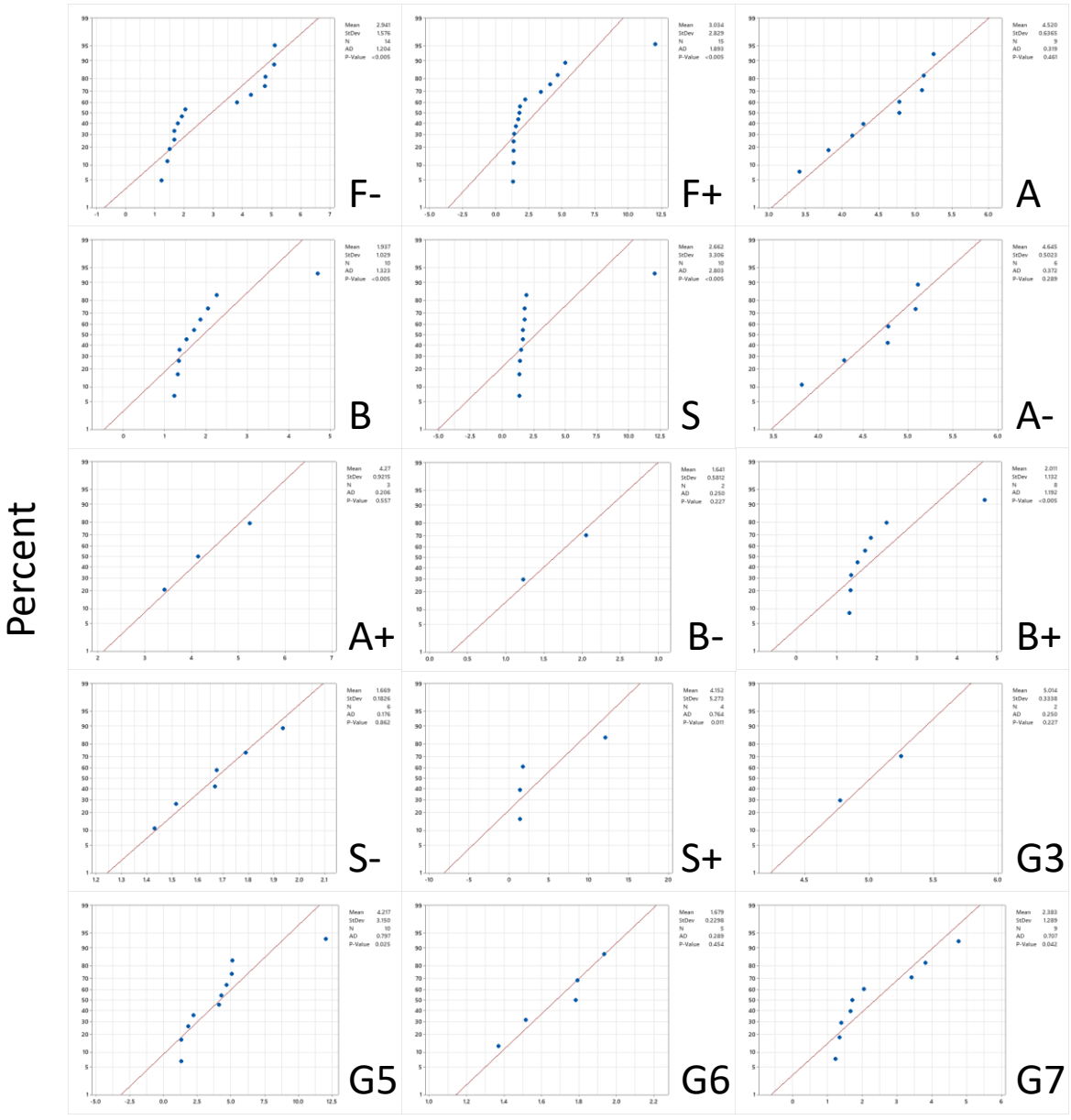
Normality tests for Cr^{2+} by Loch Groupings

For all tables in the appendix abbreviations are as follows; F- is fish absence, F+ is fish presence, A, B, and S for altitude, bird and stock loch types respectively, A-, B-, and S- represent fish absent altitude, bird and stock lochs respectively, and A+, B+, and S+ indicate fish present altitude, bird and stock lochs respectively.



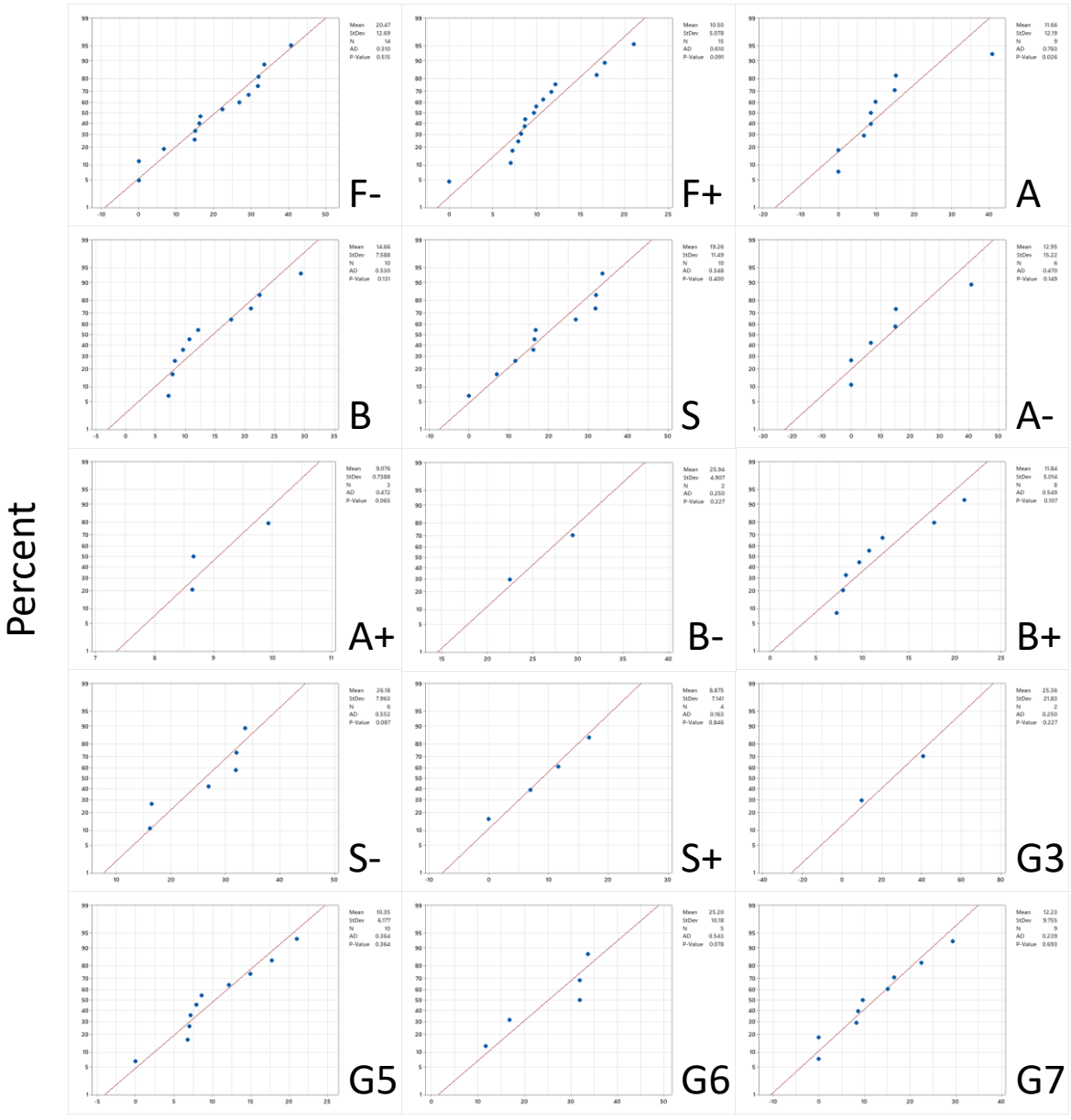
Normality tests for Cu^{2+} by Loch Groupings

For all tables in the appendix abbreviations are as follows; F- is fish absence, F+ is fish presence, A, B, and S for altitude, bird and stock loch types respectively, A-, B-, and S- represent fish absent altitude, bird and stock lochs respectively, and A+, B+, and S+ indicate fish present altitude, bird and stock lochs respectively.



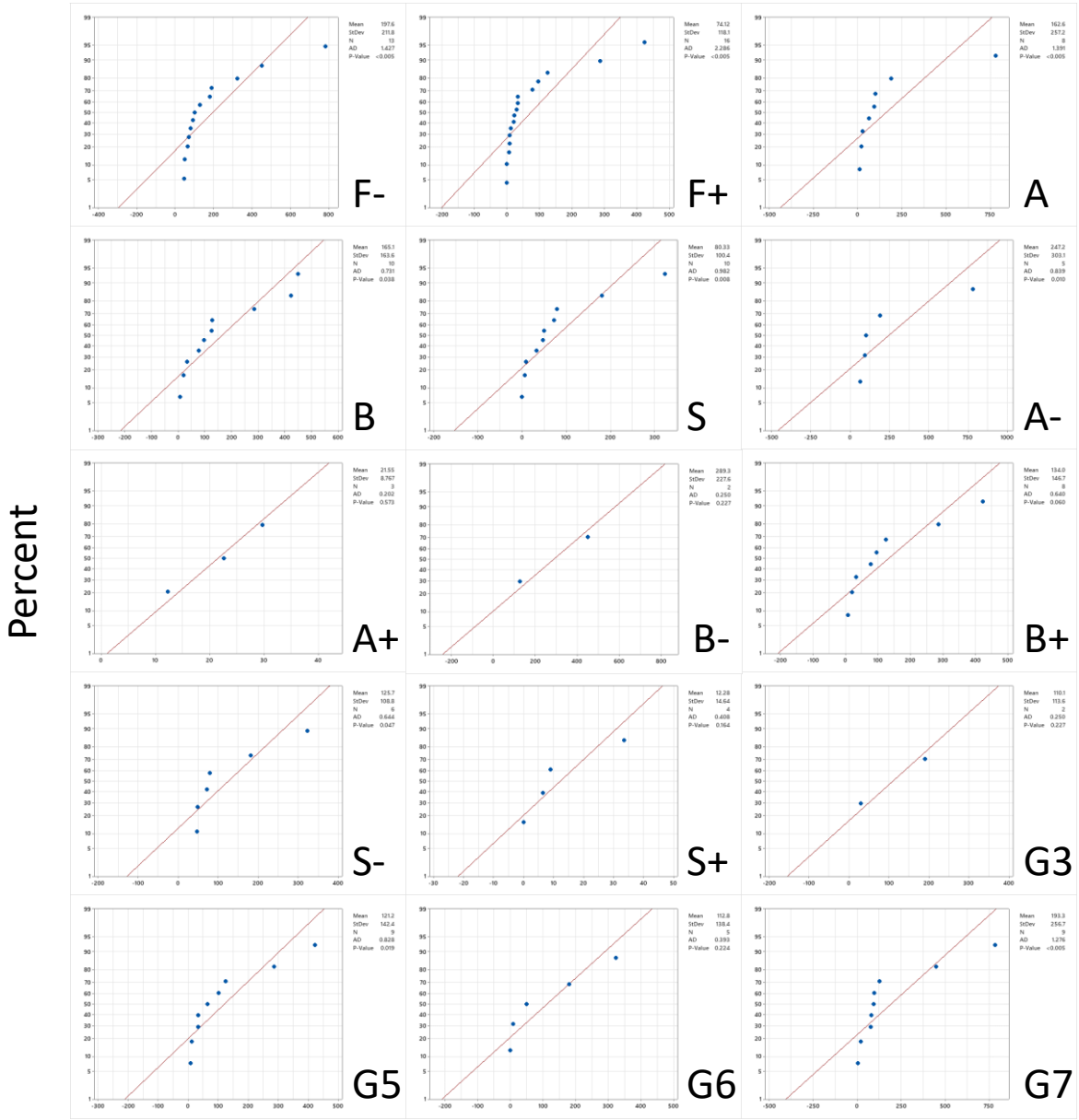
Normality tests for DisCoast by Loch Groupings

For all tables in the appendix abbreviations are as follows; F- is fish absence, F+ is fish presence, A, B, and S for altitude, bird and stock loch types respectively, A-, B-, and S- represent fish absent altitude, bird and stock lochs respectively, and A+, B+, and S+ indicate fish present altitude, bird and stock lochs respectively.



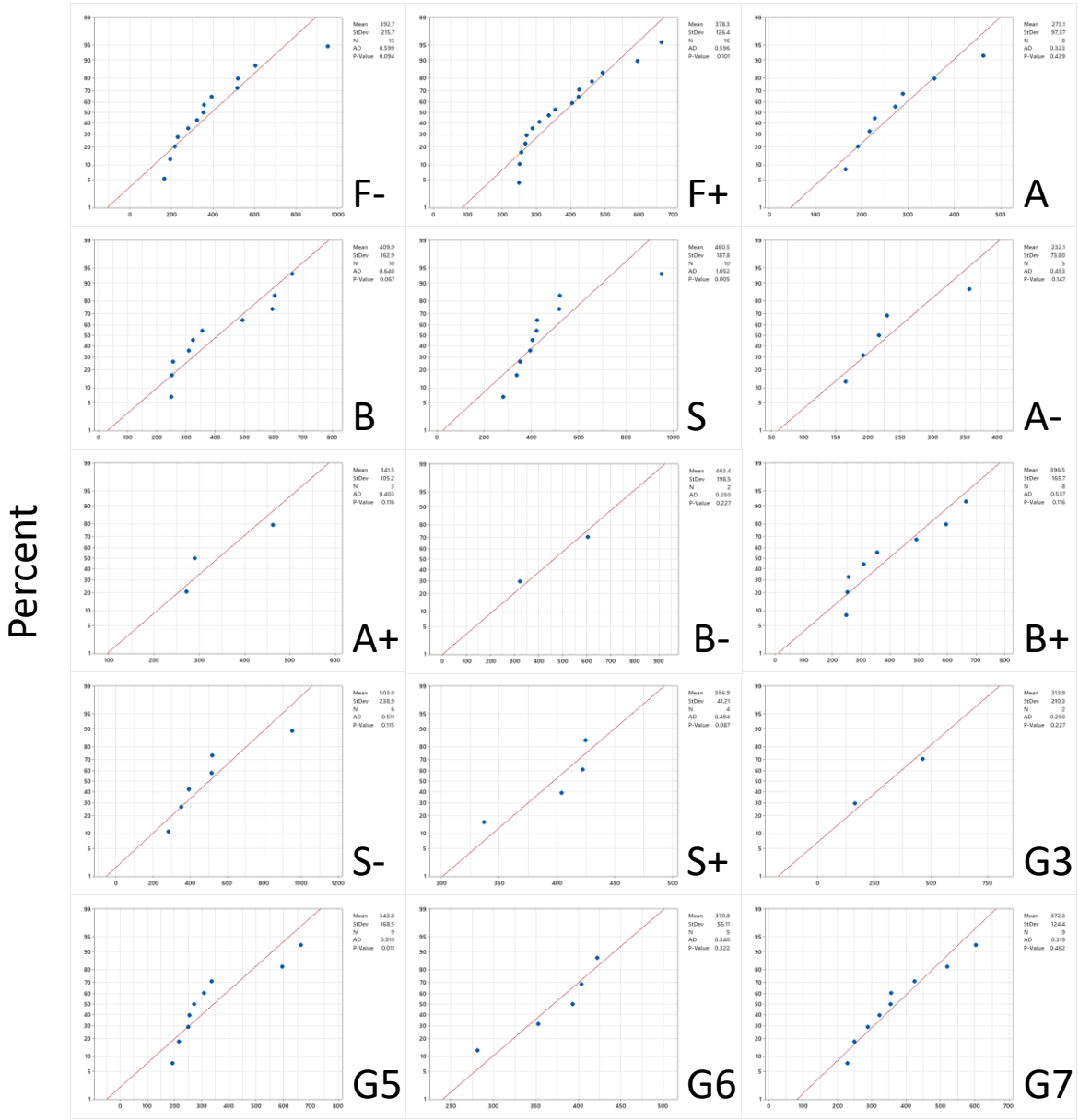
Normality tests for DOC by Loch Groupings

For all tables in the appendix abbreviations are as follows; F- is fish absence, F+ is fish presence, A, B, and S for altitude, bird and stock loch types respectively, A-, B-, and S- represent fish absent altitude, bird and stock lochs respectively, and A+, B+, and S+ indicate fish present altitude, bird and stock lochs respectively.



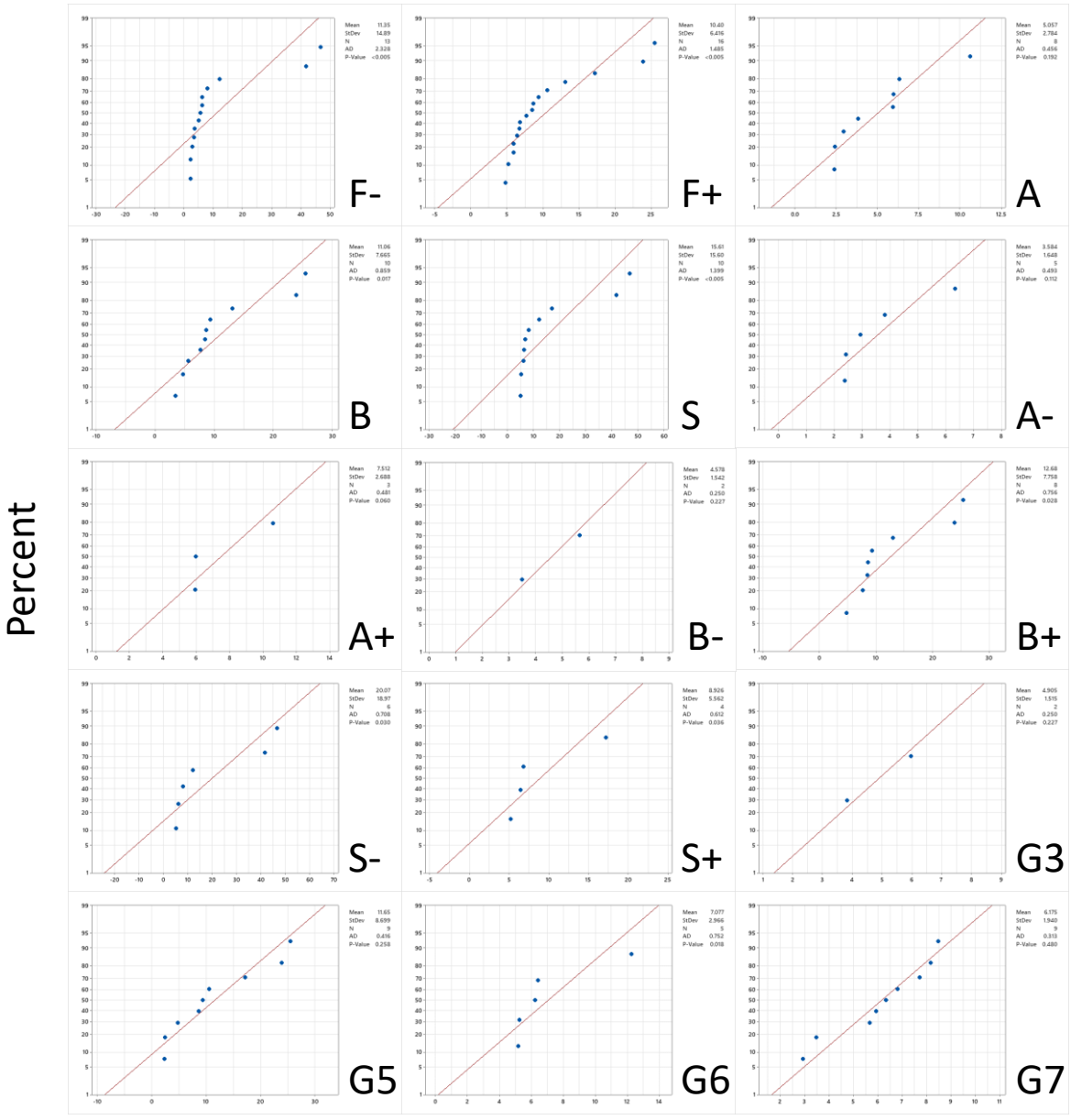
Normality tests for Fe^{2+} by Loch Groupings

For all tables in the appendix abbreviations are as follows; F- is fish absence, F+ is fish presence, A, B, and S for altitude, bird and stock loch types respectively, A-, B-, and S- represent fish absent altitude, bird and stock lochs respectively, and A+, B+, and S+ indicate fish present altitude, bird and stock lochs respectively.



Normality tests for K^+ by Loch Groupings

For all tables in the appendix abbreviations are as follows; F- is fish absence, F+ is fish presence, A, B, and S for altitude, bird and stock loch types respectively, A-, B-, and S- represent fish absent altitude, bird and stock lochs respectively, and A+, B+, and S+ indicate fish present altitude, bird and stock lochs respectively.



Normality tests for Hardness by Loch Groupings

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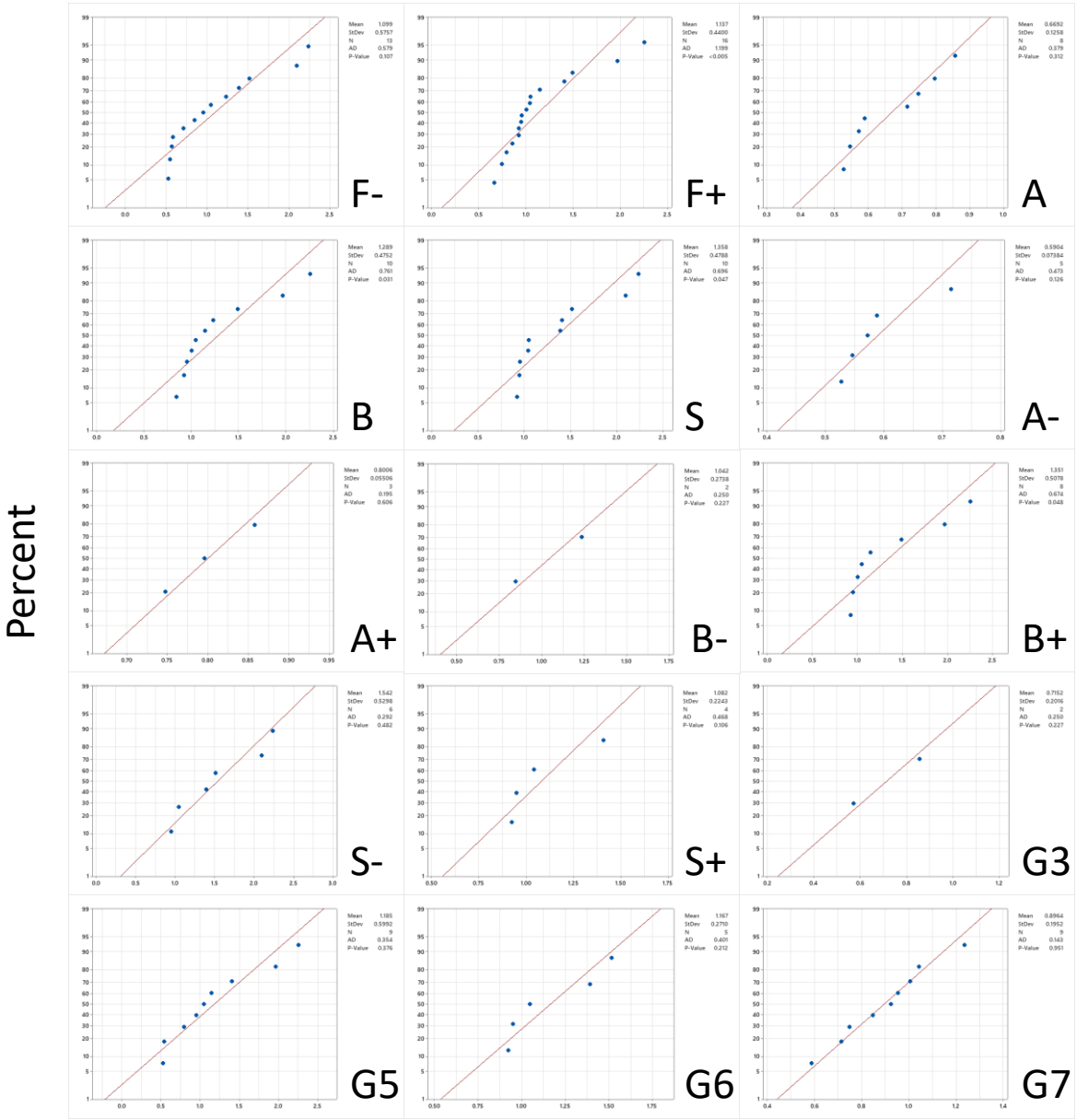
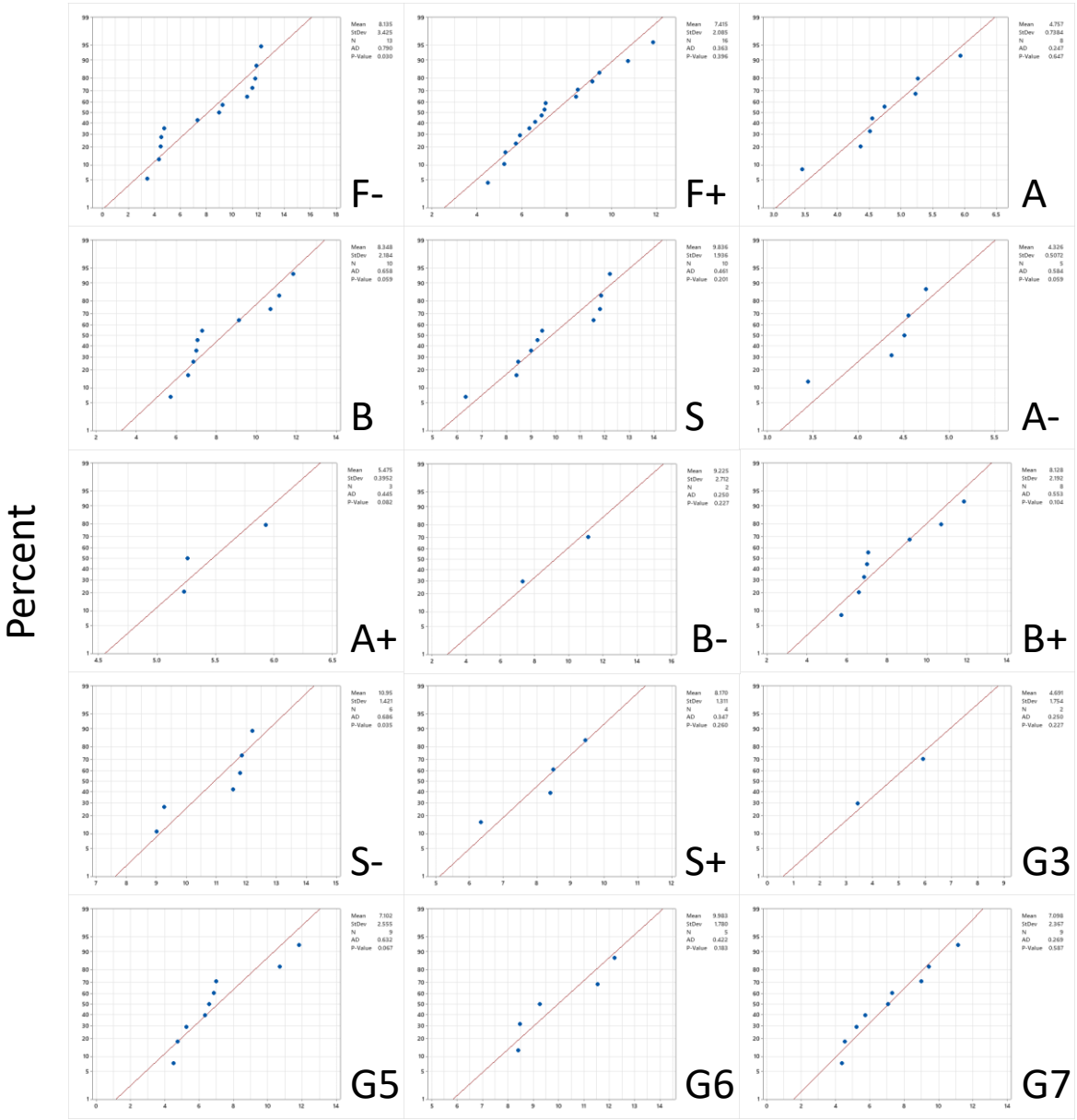


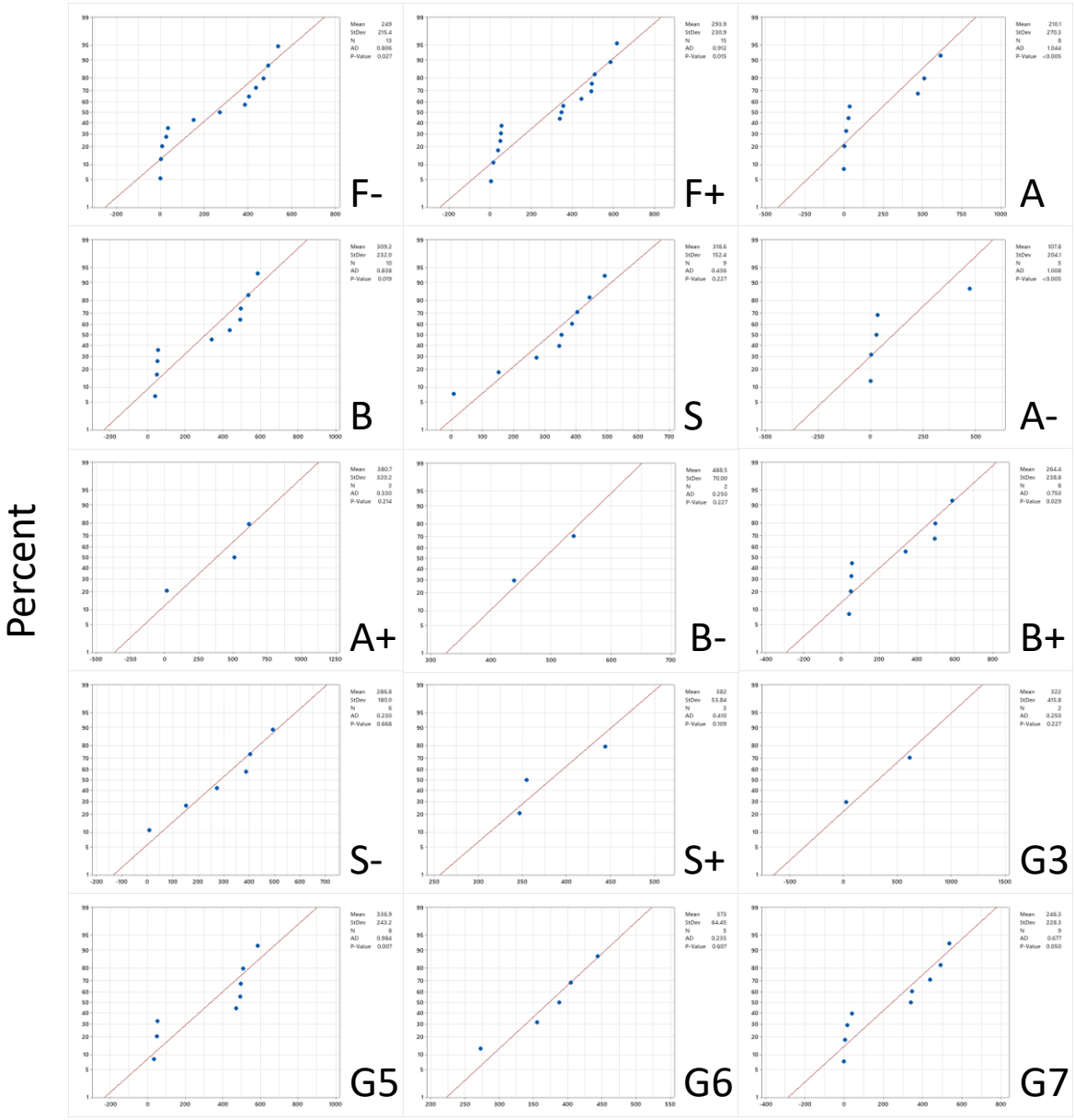
Normality tests for Mg^{2+} by Loch Groupings

For all tables in the appendix abbreviations are as follows; F- is fish absence, F+ is fish presence, A, B, and S for altitude, bird and stock loch types respectively, A-, B-, and S- represent fish absent altitude, bird and stock lochs respectively, and A+, B+, and S+ indicate fish present altitude, bird and stock lochs respectively.



Normality tests for Na^+ by Loch Groupings

For all tables in the appendix abbreviations are as follows; F- is fish absence, F+ is fish presence, A, B, and S for altitude, bird and stock loch types respectively, A-, B-, and S- represent fish absent altitude, bird and stock lochs respectively, and A+, B+, and S+ indicate fish present altitude, bird and stock lochs respectively.



Normality tests for NH_4 by Loch Groupings

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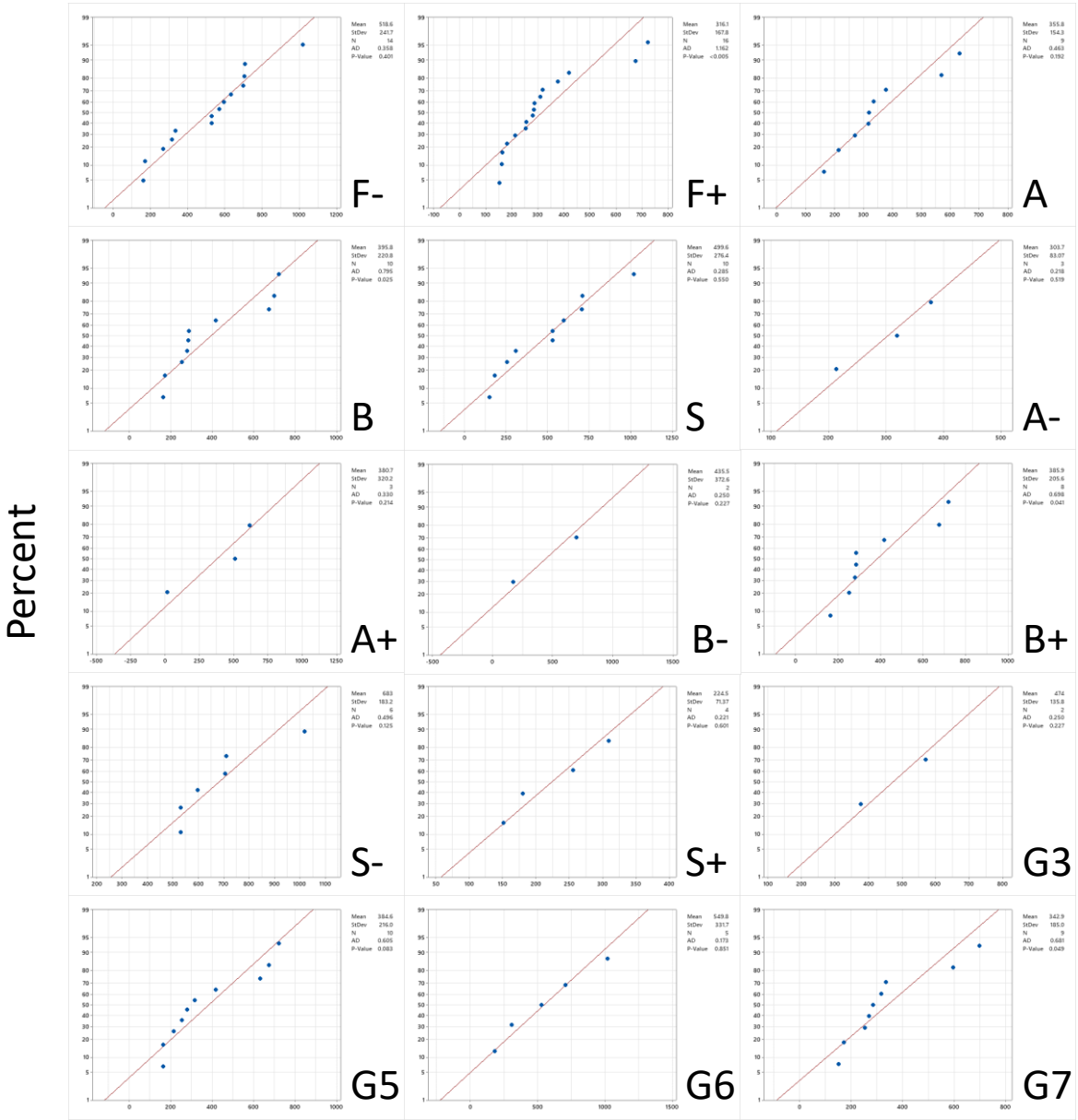
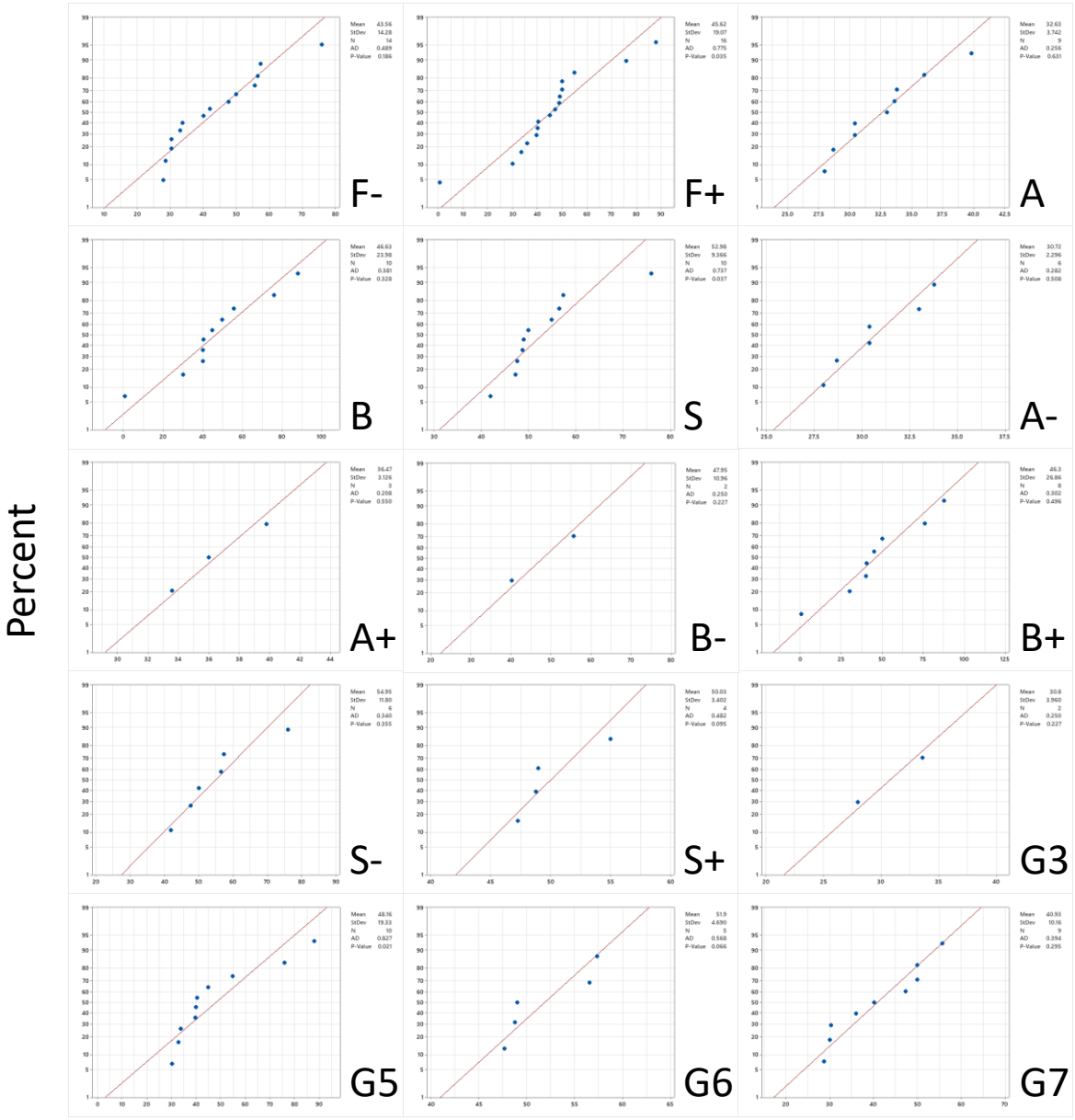


Normality tests for NO_3^- by Loch Groupings

For all tables in the appendix abbreviations are as follows; F- is fish absence, F+ is fish presence, A, B, and S for altitude, bird and stock loch types respectively, A-, B-, and S- represent fish absent altitude, bird and stock lochs respectively, and A+, B+, and S+ indicate fish present altitude, bird and stock lochs respectively.



Normality tests for O_2 by Loch Groupings

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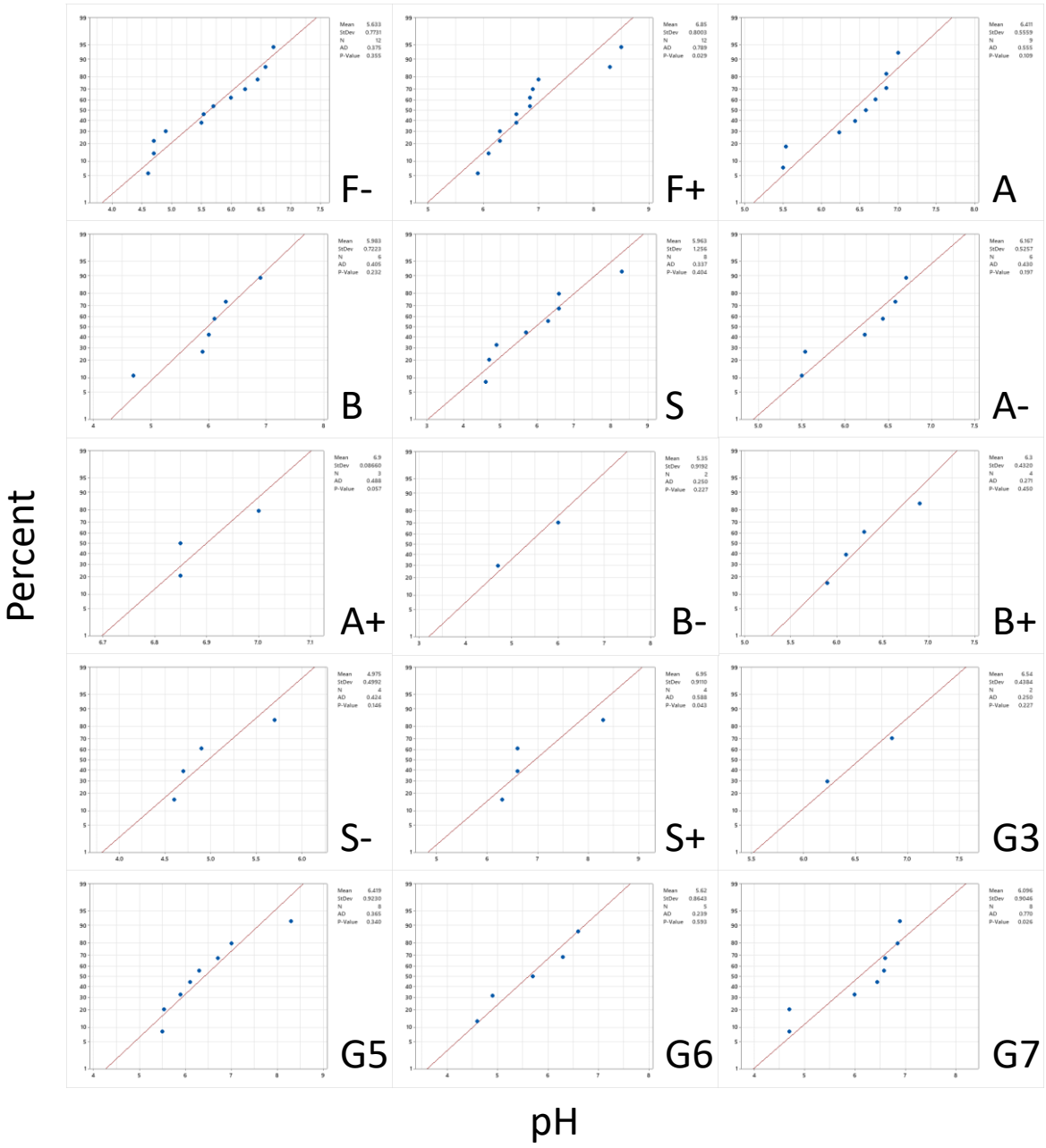
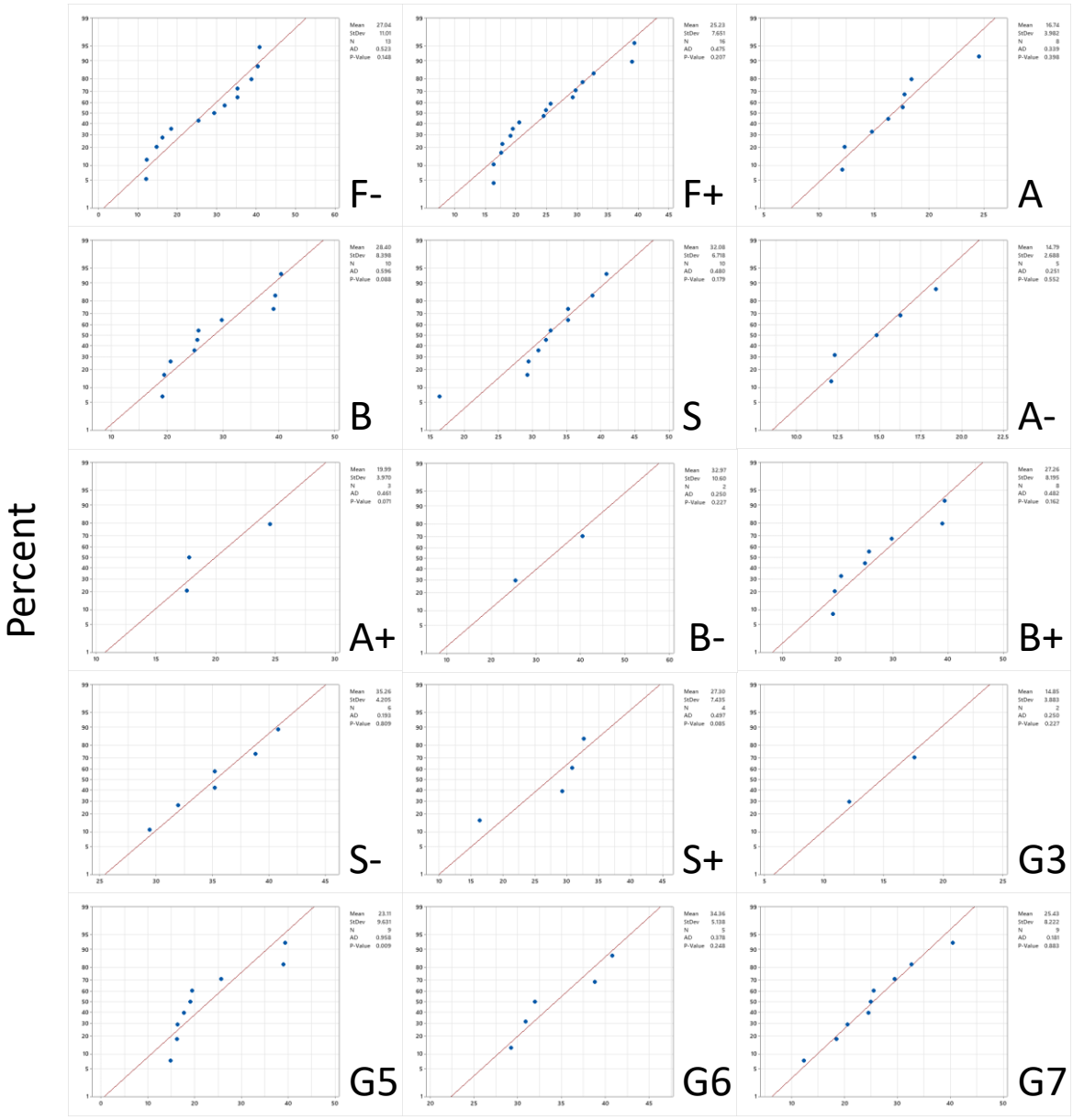


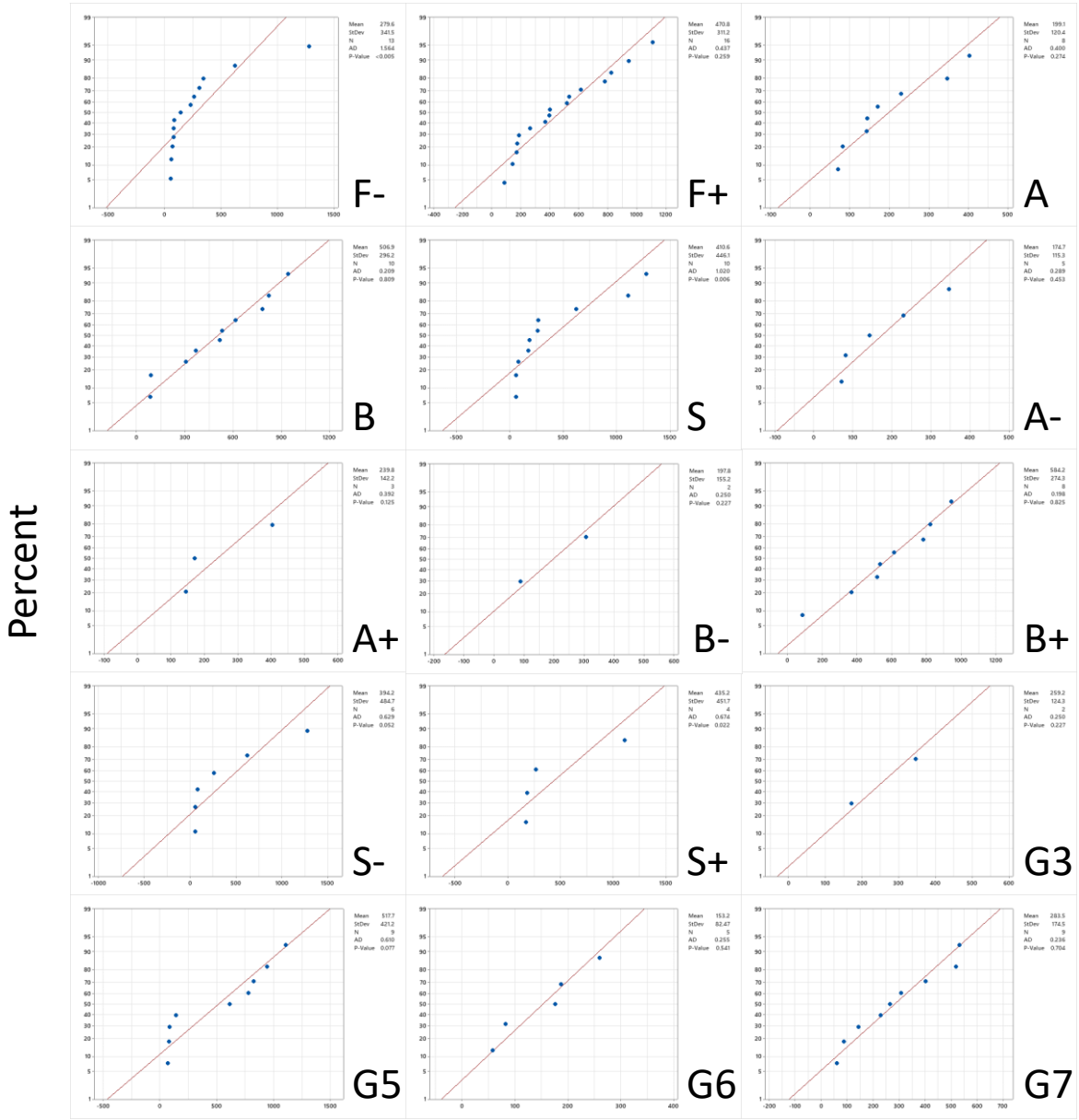
Normality tests for pH by Loch Groupings

For all tables in the appendix abbreviations are as follows; F- is fish absence, F+ is fish presence, A, B, and S for altitude, bird and stock loch types respectively, A-, B-, and S- represent fish absent altitude, bird and stock lochs respectively, and A+, B+, and S+ indicate fish present altitude, bird and stock lochs respectively.



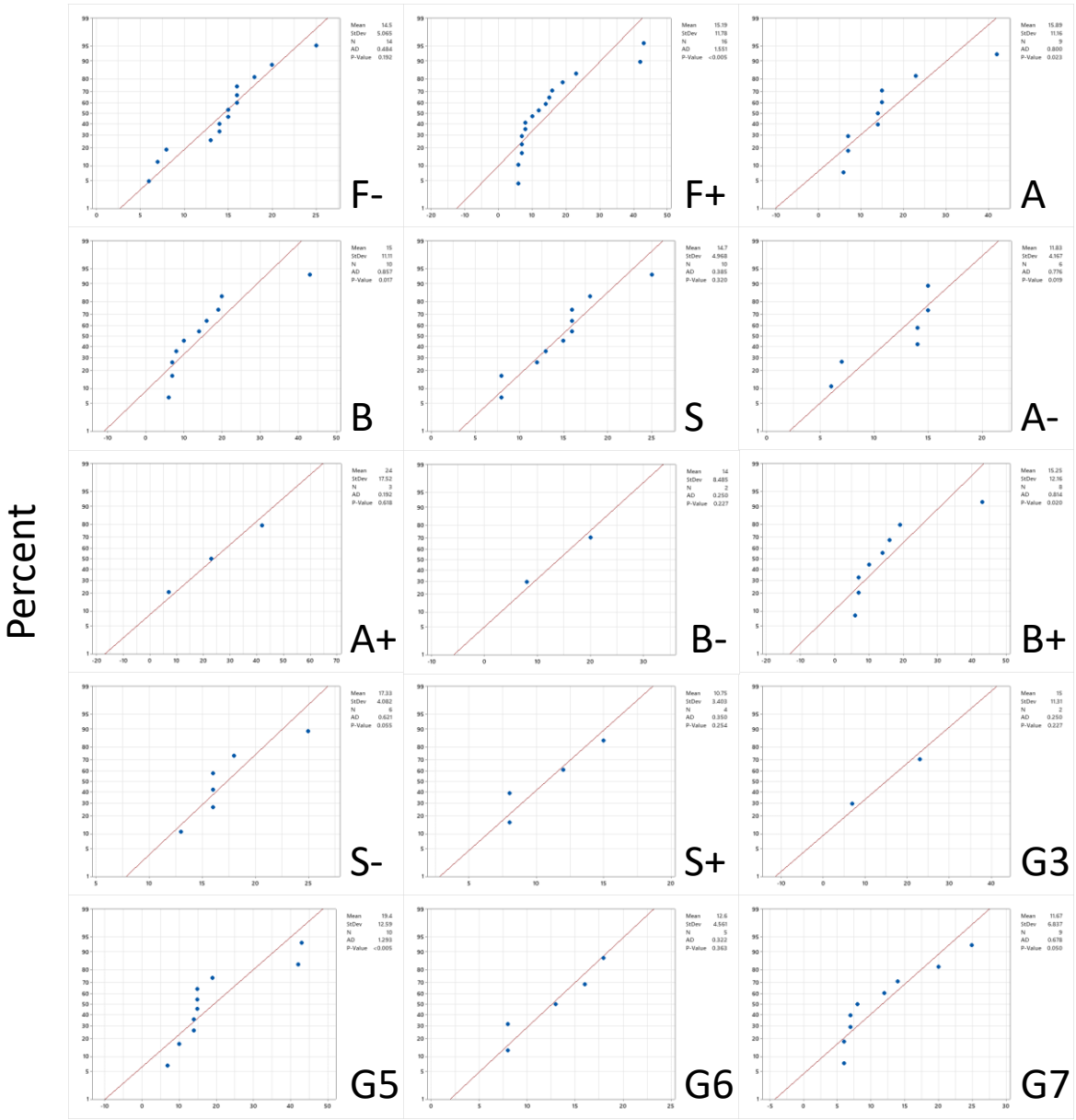
Normality tests for Salinity by Loch Groupings

For all tables in the appendix abbreviations are as follows; F- is fish absence, F+ is fish presence, A, B, and S for altitude, bird and stock loch types respectively, A-, B-, and S- represent fish absent altitude, bird and stock lochs respectively, and A+, B+, and S+ indicate fish present altitude, bird and stock lochs respectively.



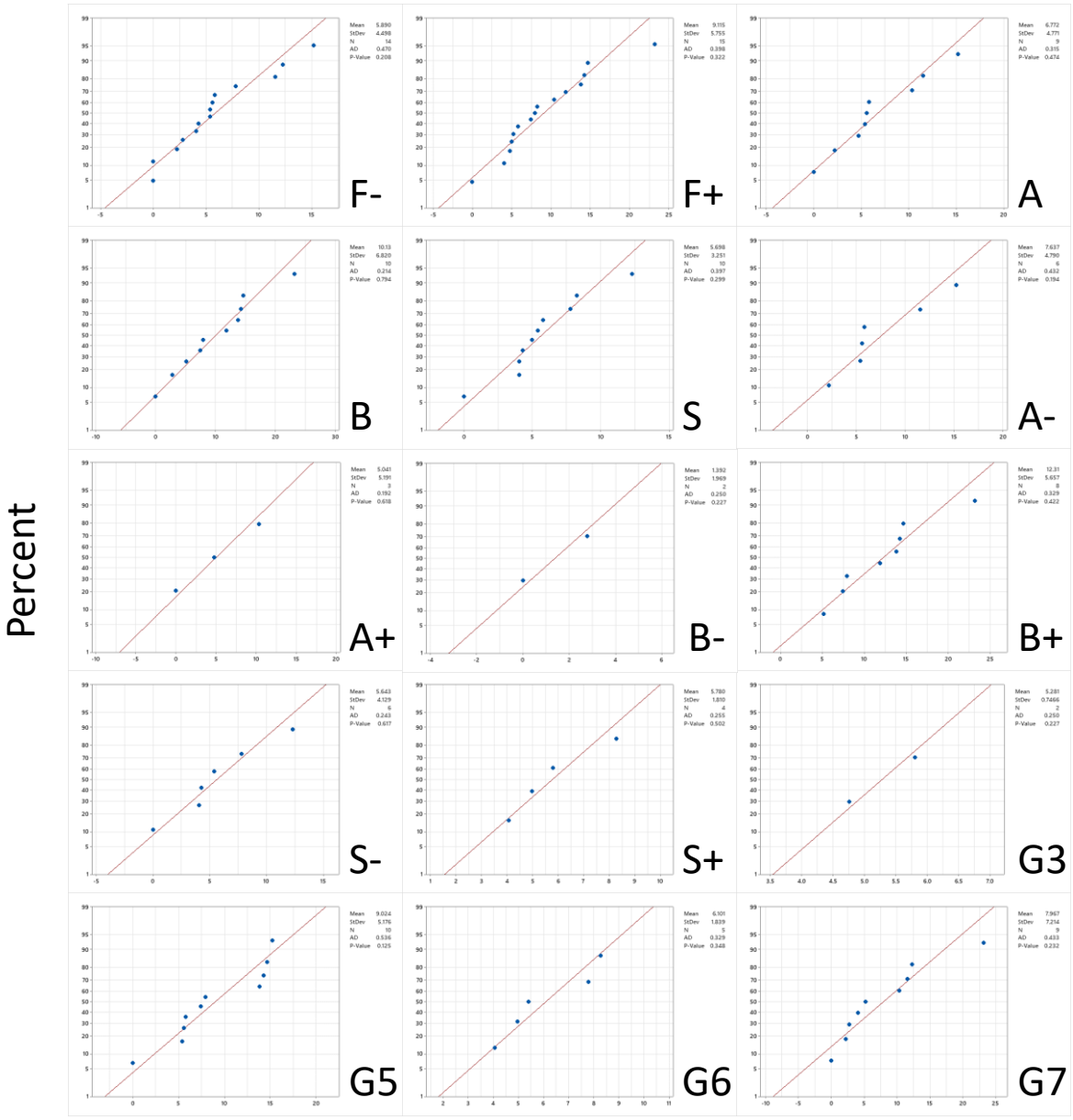
Normality tests for Si^{2+} by Loch Groupings

For all tables in the appendix abbreviations are as follows; F- is fish absence, F+ is fish presence, A, B, and S for altitude, bird and stock loch types respectively, A-, B-, and S- represent fish absent altitude, bird and stock lochs respectively, and A+, B+, and S+ indicate fish present altitude, bird and stock lochs respectively.



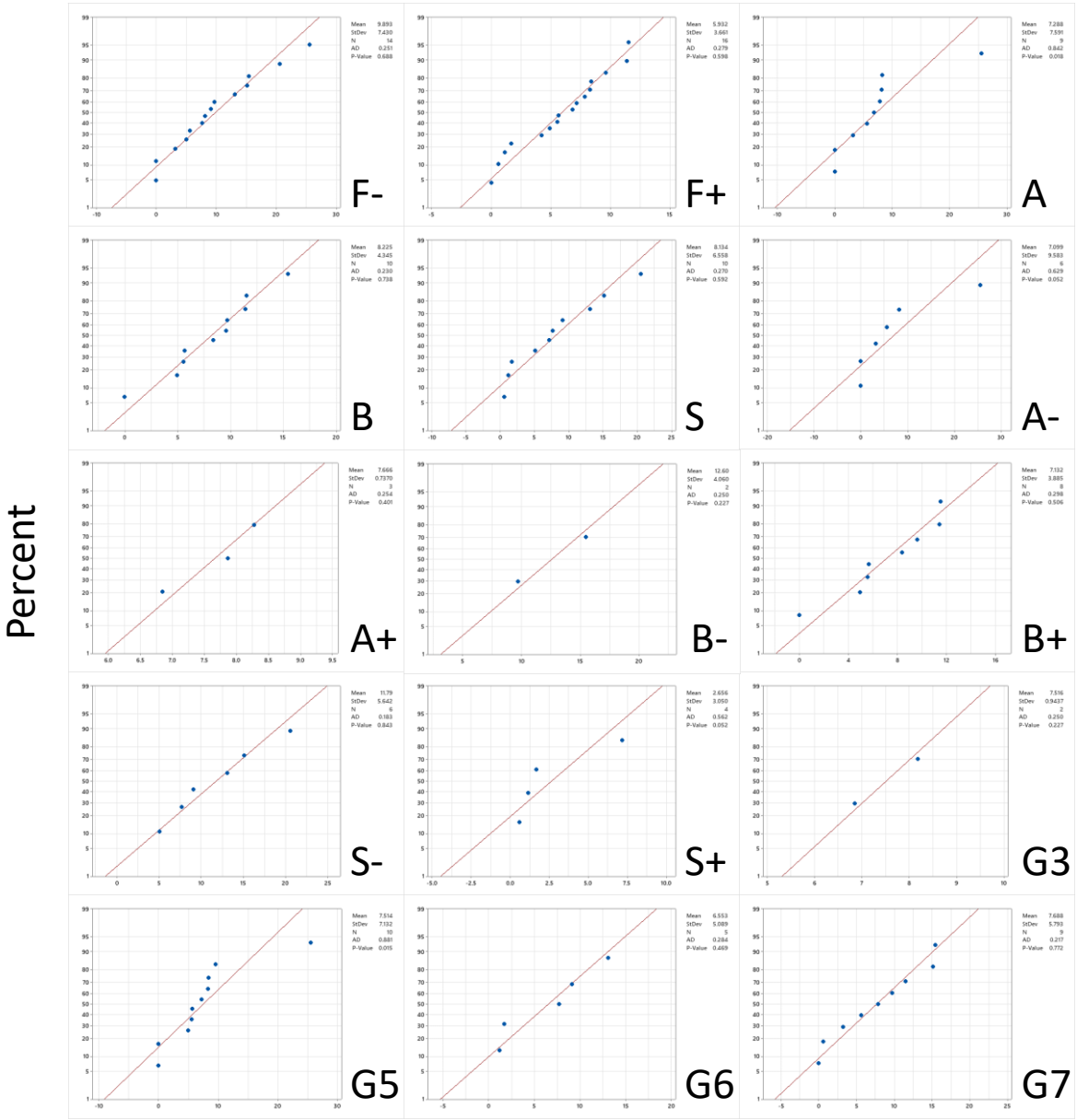
Normality tests for TP by Loch Groupings

For all tables in the appendix abbreviations are as follows; F- is fish absence, F+ is fish presence, A, B, and S for altitude, bird and stock loch types respectively, A-, B-, and S- represent fish absent altitude, bird and stock lochs respectively, and A+, B+, and S+ indicate fish present altitude, bird and stock lochs respectively.



Normality tests for TSI by Loch Groupings

For all tables in the appendix abbreviations are as follows; F- is fish absence, F+ is fish presence, A, B, and S for altitude, bird and stock loch types respectively, A-, B-, and S- represent fish absent altitude, bird and stock lochs respectively, and A+, B+, and S+ indicate fish present altitude, bird and stock lochs respectively.



Normality tests for Zn^{2+} by Loch Groupings

For all tables in the appendix abbreviations are as follows; F- is fish absence, F+ is fish presence, A, B, and S for altitude, bird and stock loch types respectively, A-, B-, and S- represent fish absent altitude, bird and stock lochs respectively, and A+, B+, and S+ indicate fish present altitude, bird and stock lochs respectively.