THE POPULATION STRUCTURE OF WILD SCOTTISH BROWN TROUT (*SALMO TRUTTA* L.1758) OF LOCH MAREE, WESTER ROSS: SPATIAL GENETIC STRUCTURE AFTER POPULATION DECLINE

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ABSTRACT

The wild trout of Loch Maree is an understudied population that recently experienced a population decline after once having a world status for its sea trout fisheries. Tissues and specimens sampled over the last decade exist in archives and have yet to undergo population genetic analysis. This study aims firstly to determine the best approach to characterising the genetic structure of the Salmo trutta *population of Wester Ross with the available archived tissues/specimens. The population structure observed will then be analysed so as to examine the effects of the Wester Ross geography and topography upon the trout population's genetic diversity. Nine markers from the Beaufort Trout MicroPlex microsatellite panel were used to genotype 192* Salmo trutta *samples sampled across 35 sites within Loch Maree and neighbouring catchments, split into four major resident regions: NW-, CW- and SE Loch Maree, and Gairloch, as well as sea trout from the Ewe and Flowerdale systems. Results suggest the population genetics of brown trout in Wester Ross are structured at regional and river scales, and suggests a genetic bottleneck caused by the population decline is still detectable. "Within-population" genetic diversity seems similar between regional populations sampled. Differences observed in population allele frequencies suggest the Gairloch and NW Loch Maree populations are the most similar regional populations analysed, with significant departures from Hardy-Weinberg equilibrium in almost all their loci, suggesting they may be under significant environmental pressure. The Wester Ross sea trout seem to be an admixture of the resident trout populations, with overlapping coastal ranges. Headwater populations have reduced genetic diversity compared downstream which may be influenced by genetic drift in isolation caused by barriers such as waterfalls and dams. A positive correlation was also observed between geo-hydrological distance and genetic divergence within regional resident trout populations, with a degree of introgression between all populations that are hydrologically connected. The analysis also suggest Loch Maree was likely colonised initially at the NW point by ancient migrant sea trout rather than a freshwater-radiation from an inland glacial refuge since the retreat of the last glacial maximum.*

A C K N O W L E D G E M E N T S

This project owes its existence to the following individuals, without any one of them you would not be reading this report.

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C O N T E N T S **Page**

GLOSSARY OF ABBREVIATIONS

Acronym /Symbol	Terminology Definition	Formula (if applicable)	Reference
GENERAL			
WR	Wester Ross A western area of the Northwest Highlands of Scotland.		WRFT (no date)
WRFT	Wester Ross Fisheries Trust Established in 1966 in response to the need for solutions to fisheries problems and to improve the management of wild fisheries in Wester Ross.		WRFT (no date)
BTMP	Beaufort Trout MicroPlex A microsatellite panel made up of 38 loci, tailored and tested for use in *Salmo trutta* population genetic studies.		Keenan *et al*. (2013)
STUDY SAMPLE GROUPS			
NWLM	North West Loch Maree Brown trout sampled in the NW catchment region around Loch Maree and neighbouring catchments that drain out to Poolewe.		*This study*
CWLM	Central-West Loch Maree Brown trout sampled in the Central West catchment region around Loch Maree.		*This study*
SELM	South East Loch Maree Brown trout sampled in the SE catchment region around Loch Maree.		*This study*
WCG	West Coast Gairloch Brown trout sampled in catchments that drain out to Gairloch on the West Coast of Scotland.		*This study*
ECLL	East Coast Loch Leven Brown trout sampled in Loch Leven on the east Coast of Scotland.		*This study*

EST	Ewe Sea Trout Sea trout sampled returning up the River Ewe.	*This study*
FST	Flowerdale Sea Trout Sea trout sampled around the Flowerdale estuarine outflow.	*This study*
CST	Canaird Sea Trout Sea trout sampled in Canaird estuary and its outflowing river.	*This study*

POPULATION GENETICS

A_R	Allelic Richness The number of alleles present at a locus or the mean number across multiple loci in a population.	Crow and Kimura (1970)
	Heterozygosities How likely an individual in a population will carry two different alleles at a locus or across loci, based on the allele frequencies of a population sample - expressed as a decimal value.	Crow and Kimura (1970)
H_E	Expected heterozygosities What the level of heterozygosity would be expected to be if the number of alleles observed in a population sample were under HWE.	Crow and Kimura (1970)
H_O	Observed heterozygosities The actual level of heterozygosity observed in a population sample based on the samples number of alleles.	Crow and Kimura (1970)
A_f	Allele Frequencies How often a particular allele appears in a population expressed as a decimal.	Crow and Kimura (1970)
HWE	Hardy-Weinberg equilibrium $p^2 + 2pq + q^2 = 1$	

	Hardy-Weinberg equilibrium describes an equal distribution of all possible allelic combinations present in a population, which is expected to be present in populations that have lasted many generations without significant selective forces. The classical expression for a biallelic marker under HWE is shown.		Crow and Kimura (1970)
F_{ST}	*F*-statistics Fixation indices measure population substructure using the heterozygosities of the total and subpopulations.	$F_{ST} = (H_T - H_S) / H_T$	Wright (1965)
D_{ST}	Nei's *D* Nei's standard genetic distance (with or without sample size bias correction) calculates the genetic differentiation between any pair of populations. It is calculated as shown, where I is the normalised identity of the markers between two population samples.	$D_{ST} = -\log_e I$	Nei (1972)
G_{ST}	Nei's G_{ST} A method of analysing gene diversity (heterozygosity) of a subdivided population, within and between subpopulations - calculated by dividing D_{ST} by the total population heterozygosity (H_T).	$G_{ST} = D_{ST} / H_T$	Nei (1973)
MDE	Mutation-drift equilibrium Mutation-drift equilibrium occurs when a population's size has remained stable for a sufficient number of generations, leading to an equal probability that a locus shows an heterozygosity excess or deficit.		(Cornuet and Luikart, 1996)

S T A T I S T I C A L

p	P-value Statistical value of probability that describes how likely the observational data represents the nature of what has been measured.		(Heumann and Schomaker, 2017)
p_s	Multiple P-values		(Heumann and

	Multiple statistical probability values calculated separately but presented together as "greater or lower than" the alpha value or significance level.		Schomaker, 2017)
SD	Standard Deviation Typical range of deviation from the mean for each data point within a dataset.	$\sigma = \sqrt{\Sigma(x-\bar{x})^2/n}$	(Heumann and Schomaker, 2017)
SE	Standard Error Error range of the population sample mean.	$\sigma_{\bar{x}} = \sigma/\sqrt{n}$	(Heumann and Schomaker, 2017)
r	Correlation coefficient Indicates the type and strength of the relationship observed between two variables.		(Heumann and Schomaker, 2017)

LIST OF TABLES & FIGURES

Page

TABLES

Appendices

F I G U R E S

Introduction

Chapter 1: Study methodology development - DNA quality assessment and optimisation of microsatellite markers
1.1: Methodology

1.2: Results

Chapter 2: Population genetic structure analyses of Wester Ross brown trout (*Salmo trutta*)

2.1: Methodology

2.2: Results

Appendices

Statistical appendices figures and results are numbered in their sections but are not listed here due to sheer volume. Please refer to contents page to locate statistical appendices.

INTRODUCTION

SALMO TRUTTA (L.1758)

Salmo trutta is one of the most widespread freshwater species in the northern hemisphere (Leitwein *et al.,* 2016). Its native range includes Eurasia and North Africa (Bernatchez, 2007; García-Marín *et al*., 1999), and its adaptability has permitted successful introduction into North and South America, South and East Africa, and Australasia (Watson, 1999). The incentive for introduction around the world reflects the species' socioeconomic value due to its worth in commercial fisheries, recreational fishing, and other local industries associated with sport fishing.

Figure 1 Mature brown trout (~ 35 cm fork length) from "Elf's Loch", July 2014.

LIFE STRATEGIES OF *SALMO TRUTTA* – BROWN, SEA & FEROX TROUT

S. trutta exhibits considerable phenotypic plasticity with morphs adopting distinct life strategies, often occupying the same watercourse (Watson, 1999; Elliot, 1994). Ecologically, there are two major morphs: freshwater residents, known as 'brown trout', and anadromous 'sea trout'; once argued to be taxonomically separate, research into their genetics and development has demonstrated they are one species (Watson, 1999; Hindar *et al*., 1990).

Brown trout (*Figure 1*) spend their entire lives in freshwater, never making a sea-run despite having access to the sea (Watson, 1999; Elliot, 1994; Hindar *et al*., 1990; Campbell, 1977). These freshwater residents then enter various niches, becoming predominantly riverine (*Salmo trutta* m. *fario*), known as river trout, or lacustrine (*Salmo trutta* m. *lacustris*), as lake trout. There are also resident headwater populations that are 'landlocked', where access to the sea is either no-longer present or one-way, preventing return (e.g. above impassable waterfalls) (Hindar *et al*., 1990).

Instead of staying in freshwater, some individuals smoltify (turn silvery) and migrate to sea where they spend most of their time, before returning to freshwater to spawn as sea trout (*Figure 2*) (Watson, 1999; Elliot, 1994). Individuals that adopt anadromous lifestyles are usually female (Kinnison *et al*., 2001; Jonsson and Jonsson, 1993) and is believed to be triggered by a combination of genetics and

the environment (most likely a lack of food or territory) (Watson, 1999). There is a higher risk of mortality associated with the marine environment, but the risks are rewarded with richer feeding (Sahashi and Morita, 2013; Wysujack *et al*., 2009), allowing sea trout to grow up to 3kg in weight, dwarfing their resident siblings upon return which rarely exceed 300g (Watson, 1999; Elliot, 1994).

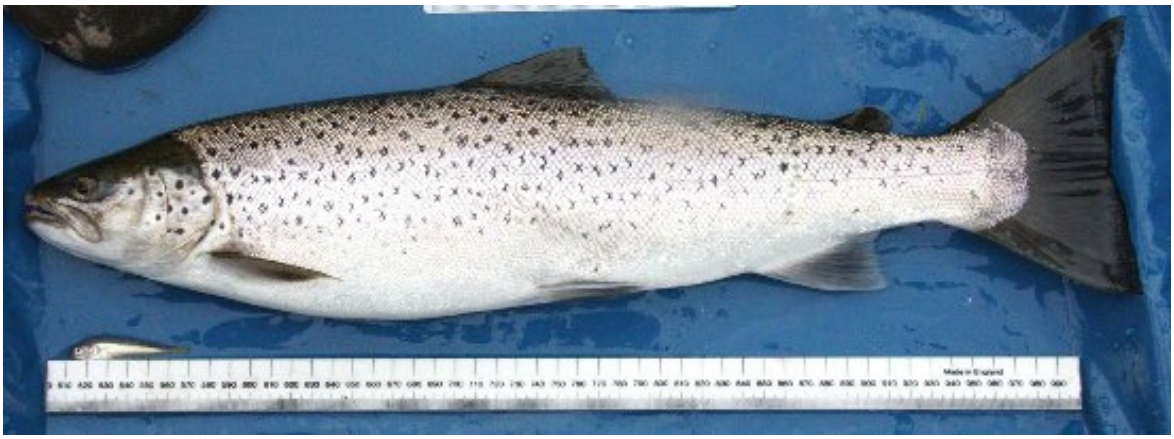

Figure 2 Mature sea trout (52 cm fork length, 1.6 kg weight) caught and released in Loch Ewe, June 2014.

Sea trout have many different names depending on location, gender, life stage, and duration at sea (e.g. Finnock are young sea trout that have spent up to a year in coastal waters) (Watson, 1999). As sea trout life stages are not the focus of this study, any *S. trutta* specimens that have adopted anadromy in their life history will simply be referred to as 'sea trout' from hereon.

Ferox trout are a third, somewhat enigmatic morph and are thought to be resident lacustrine forms of trout that have adopted a piscivorous diet, preying primarily on arctic charr (*Salvelinus alpinus* L.1758) (Watson, 1999; Elliot, 1994). Piscivory seems to trigger once a 'ferox-destined' trout has reached a threshold length of 30 – 35 cm (Watson, 1999; Campbell, 1979), and is otherwise indistinguishable from brown trout prior. The fish-based diet allows ferox trout to become typically larger than other sympatric resident trout, resembling sea trout in terms of their size and shape, while resembling brown trout in colouration (*Figure 3*). Ferox also have increased longevity, late-maturation, and lower reproductive rates which make them more vulnerable to habitat loss (Quesnelle *et al*., 2014). Again, whether ferox trout are a separate species from brown trout has been debated for some time (Watson, 1999), though there is some evidence to suggest that ferox may be genetically distinct (Duguid *et al.* 2006; Ferguson and Taggart, 1991; Campbell, 1979). The International Union for Conservation of Nature (IUCN) has in fact listed *Salmo ferox* as a separate species to *Salmo trutta*, however its status is classified as '*data deficient*' with an '*unknown*' population trend (Freyhof and Kottelat, 2008); however, Scottish authorities do not consider Scottish ferox to be taxonomically distinct from *S. trutta* (Scottish Government, 2017).

Figure 3 Mature (male) ferox trout (47.5 cm fork length) caught and released in Loch Kernsary, October 2013.

Despite their various life strategies, all *S. trutta* morphs instinctually *home* in on their natal streams to breed and can migrate to and from to do so several times in their lives (Watson, 1999; Jonsson, 1985; Campbell, 1977). This homing instinct creates a level of reproductive isolation, promoting divergence as they genetically drift away from each other over isolated generations, with migrants between populations providing some potential for gene flow (Quinn, 2005; Watson, 1999).

EUROPEAN BROWN TROUT

The evolutionary history that has led to European brown trout is complex (Leitwein *et al.,* 2016). Europe was colonised by *Salmo trutta* after the last glacial period ~ 15 000 years ago (Bernatchez, 2007; García-Marín, 1999; Hamilton *et al*., 1989). As the glaciers melted and retreated northwards, they carved out many of the highland freshwater courses in Europe (Sisson, 2016; Finnergan *et al*., 2013) which allowed at least five major lineages, inferred by mitochondrial DNA (mtDNA), originating from the Atlantic, Mediterranean, Adriatic, Danubian and Marmoratus to establish populations across the continent (Bernatchez, 2007).

Since its European radiation, many populations have become geographically isolated at catchment and sub-catchment levels, especially where populations are restricted to highland waters (Elliot, 1994). The typical geography and topography of highland landscapes, coupled with the trout's tendency to 'home in' on their natal streams to breed has led to distinct genetic structuring within and between catchments, with local subpopulations being genetically differentiated over a relatively small geographic range (Stelkens *et al*., 2012; Ferguson, 1989).

Due to its cosmopolitan range, the global population of *S. trutta* is classified as *'least concern'* by the IUCN (Freyhof, 2011); however there are many endemic populations under threat (WTT, 2019), such as the wild trout population of Loch Maree (WRFT, no date).

LOCH MAREE WILD TROUT

The wild population being studied is native to Loch Maree catchment, Wester Ross, in the northwest Highlands of Scotland (coordinates: 57° 41' 23" N, 5° 27' 27" W, OS grid reference: NG 960 686). The catchment area covers Beinn Eighe, Slioch, Fisherfiefld, Glen Docherty, Coulin, Slattadale, and Talladale, most of which flow into Loch Maree before draining out to sea through the River Ewe at Poolewe (Loch Ewe). At 20 km long, with a maximum width of 4 km, a maximum depth of 38 m and a total surface area of 28.6 km^2, Loch Maree holds approximately 1 billion tonnes (1 090 000 000 kL) of water, making it the fourth largest freshwater loch in Scotland. Loch Maree's trout population includes resident brown trout, anadromous sea trout, and piscivorous ferox trout thought to be rare and isolated to a few watercourses where arctic charr also exist (WRFT, no date). Until the late 1980s Loch Maree was the most productive sea trout fishery in Scotland, since then the sea trout population has collapsed, indicated by a 68 % decline in the five year mean catch, falling from 2 500 in 1982 to 800 in 2000 (WRFT, no date) and has yet to recover (Walker. 2016; Cunningham, 2014; WRFT, no date). This negatively impacted nutrient dynamics within local ecosystems, as well as the local communities who depended on industries associated with sport fishing for their livelihoods (WRFT, no date).

POPULATION GENETICS OF *SALMO TRUTTA*

Over recent decades, genomic techniques have advanced considerably, increasing the quantity and quality of genomic data that can be obtained from samples (Morin *et al*., 2004). Single-Nucleotide Polymorphism (SNP) marker data sets have become popular due to their flexibility and relatively low-costs (Shirasawa *et al*., 2016; Leitwein *et al*., 2016). SNPs also provide data across broader genome coverage, creating a Reduced Representation Library (RRL) of a genome, whilst maintaining equivalent statistical power compared to microsatellites or mtDNA markers (Morin *et al*., 2004). Advances in Next-Generation Sequencing (NGS) technologies have led to more cost-effective techniques such as Restriction enzyme Associated DNA sequencing (RADseq), making it possible for genome-wide SNP marker sets to now be routinely produced (Shirasawa *et al*., 2016; Leitwein *et al*., 2016; Morin *et al*., 2004). This has contributed to the development of conservation management strategies for several salmonid populations (Bradbury *et al*., 2015; Abadía-Cardoso *et al*., 2011; Morin *et al*., 2004, Meier *et al.,* 2011); however there is currently a limited number of SNP markers characterised for *S. trutta* (SušnikBajec *et al*., 2015; Pustovrh *et al*., 2012) and no genetic data available for the Loch Maree catchment area (WRFT, no date).

Population genomic data has been used to characterise the population structure, local adaptations, ecology, evolution, and genetic diversity in many salmonid populations (Hecht *et al*., 2015; Hohenlohe *et al*., 2013; Hansen *et al*., 2000). The focus of many studies have been to investigate its evolutionary history (Finnergan *et al*., 2013), monitoring the effects of restocking on genetic diversity (Berrebi, 2015; Hansen *et al*., 2000), genetic comparisons of wild and domestic stocks (Aho *et al*., 2006), and local phylogeographies (Bernatchez, 2007). Much of this has been done using traditional genetic markers such as allozymes (Sanz *et al*., 2006; Kreig & Guyomard, 1985), microsatellite loci (Thaulow *et al*., 2014; Aho *et al*., 2006; Hansen *et al*., 2000; Poteaux *et al*., 1999), and mitochondrial

markers (Sanz *et al*., 2006; Bernatchez, 2007). Advancements in NGS techniques, such as RADseq, are now capable of producing thousands of SNP markers per individual to produce genome-wide scale RRLs which contrasts the few genetic markers (often < 20) that are generally used in many previous studies which may limit their potential in establishing a baseline to monitor the effects on genetic diversity from management policies or environmental changes (Leitwein *et al*., 2016; Keenan *et al*., 2013) - though there are exceptions (Keenan *et al*., 2013; Hansen & Mensberg, 2009).

The "Beaufort trout MicroPlex: a high throughput multiplex platform comprising of 38 informative microsatellite loci for use in resident and anadromous (sea trout) brown trout *Salmo trutta* genetic studies" provides 38 microsatellite markers informative for *Salmo trutta* for population genetic research, this offers a cheaper, faster alternative approach to NGS methods, and benefits from already being tailored for brown trout population genetic studies (Keenan *et al*., 2013).

The choice of approach to any population genetic study is influenced by a number of factors which ultimately limits the choice of methods which result in reliable data (Graham *et al*., 2015; Shiozawa *et al*., 1992). Studies using traditional markers such as microsatellites or mtDNA usually have less concerns, while studies using an NGS approach such as single-nucleotide polymorphisms (SNPs) often face implications that can result in data loss (Graham *et al*., 2015).

The aims of this project are therefore to determine the best approach and to characterise the genetic structure of the *Salmo trutta* population in Loch Maree and neighbouring catchments based on available archived tissues/specimens; and to then map the spatial distribution of that structure to the Wester Ross geography and topography, to examine its effects upon the trout's genetic diversity, in hopes of informing conservation management of this once world-renowned population of *Salmo trutta*.

CHAPTER 1: STUDY METHODOLOGY DEVELOPMENT: DNA QUALITY ASSESSMENT AND OPTIMISATION OF MICROSATELLITE MARKERS

1.1: METHODOLOGY

STANDARD LAB PROTOCOLS

DNA EXTRACTIONS

All manual DNA extractions in this study were conducted using the Qiagen DNeasy® Blood & Tissue DNA kit (© 2006 Qiagen) following the protocol: Purification of Total DNA from Animal Tissues Spin Column Protocol (Qiagen, 2006). The protocol was altered to use chilled isopropanol instead of ethanol during 'step 3'. If an RNAse treatment was included, 1 µL of RNAse was added to the sample for 1 minute at room temperature immediately after cell lysing (addition of AL buffer). When extracting DNA from frozen-muscle tissue, a centrifuge step was introduced at this point (during 'step 3'), immediately after cell lysing by AL buffer, before the use of chilled isopropanol (or ethanol).

Automated DNA extractions at the Natural History Museum were conducted by NHM staff (Stephen Russell, Senior Laboratory Manager) on a Qiagen Biosprint 96®.

MEASURING DNA INTEGRITY, QUANTITY & PURITY

The following assays were used to assess DNA quantity, purity, and integrity.

DNA Quantity -Qubit

DNA quantity was calculated using Qubit 1.0 and 3.0 fluorometers (© 2014 Thermo Fisher Scientific Inc.), following the kit guidelines and protocol for 'dsDNA broad spectrum analysis'. Both machines were tested on identical samples to ensure they both had reliable and consistent measurements between them.

DNA Purity - Nanodrop

DNA purity was measured using a Nanodrop 2000c spectrophotometer (© 2009 Thermo Fisher Scientific Inc.) following the standard protocol to assess 'Nucleic Acid 260/230 and 260/280 ratios' (Thermo Fisher Scientific, 2009, pp. 26). Each sample was analysed in 1 µL volumes.

DNA Integrity - Agarose gel

DNA integrity was assessed using 0.8 % and 1.5 % agarose gels, all gels in this project were made using the following protocols:

Gel preparation for every 100 mL of agarose gel required had 0.8 % or 1.5 % w/v (800 mg or 1500 mg) added to every 100 mL of TAE buffer, which was then microwaved until the solution was clear. After letting the solution cool to ~ 60-70°C, 5 µL of SafeView nucleic acid stain (© 2018 NBS Biologicals) was added and swirled evenly before casting the gel.

Sample preparation utilised 2 µL of DNA loading dye mixed with 5 µL of each specimen DNA before being added to the gel in separate wells.

If required, 3 µL of 100 bp or 1000 bp DNA ladder (Bioline HyperLadder™) was also added to a well per lane in the gel.

Choice of agarose concentration and DNA ladder size depended on the expected size range of DNA fragments. Raw gDNA is relatively larger requiring 0.8 % agarose and the use of a 1000 bp ladder; microsatellite amplicons are relatively small, requiring 1.5 % agarose and 100 bp ladder for quality assessment. Electrophoresis was run between 90 - 110 V until the bands separated sufficiently before being imaged on a UV Trans-illuminator.

Evaluating agarose gels

DNA integrity was assessed with considerations based on the work by Graham *et al.* (2015), which investigated raw DNA integrity from blood and muscle tissue of another salmonid (Lake Whitefish - *Coregonus clupeaformis* M.1818) after varying durations at room temperature. Analysis showed that that DNA degradation did not cause significant data loss in muscle tissue until after 96 hours (*Figure 4, E*).

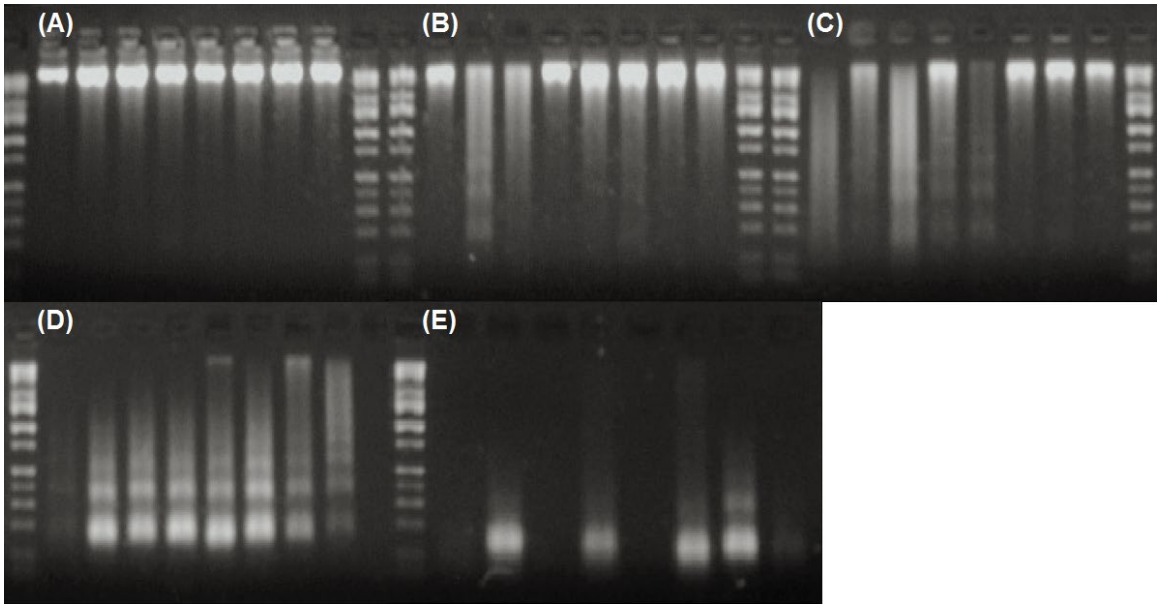

Figure 4 Agarose gel image showing the size distribution of untreated DNA extracted from Lake Whitefish tissue at the following time points at room temperature post euthanasia: (A) 0 h blood, (B) 0 h muscle (C) 12 h muscle, (D) 48 h muscle and (E) 96 h muscle. Each lane of the gel represents an individual lake whitefish. The size standards shown are DNA ladders ranging from 50 to 10 000 bp (Graham et al., 2015).

Based on the results found by Graham *et al*. (2015) (*Figure 4*), any gel lanes that appear as 'bottom-up smears' (D & E) were classified as having 'low' integrity (due to > 50 % data loss observed by Graham *et al*. [2015]), 'top-down smears' (B, C) as having 'moderate' (< 10% data loss), and 'distinct bands' at the top of the lane (A) as 'high' integrity (< 5 % data loss).

DNA QUALITY ASSESSMENT

A subsample of *S. trutta* fin clips (*n* 96) in the available archive had DNA extracted to assess its raw integrity (*Figure 8*). DNA was also extracted from a specimen of each tissue type and mode of storage available to investigate which best maintained DNA integrity. Sample types available differed by tissue type: internal muscle, pectoral fin, and adipose fin; and mode of storage: fins were stored either dry or in ethanol, internal muscle was frozen within archived specimens with a varying number of freeze-thaw cycles (*Figure 9*).

EXTRACTION OF DNA FOR POPULATION GENETIC ANALYSIS: PLATE 1 & 2

Two 96 deep-well plates were prepared for automated DNA extraction at the Natural History Museum (NHM). Plate 1 contained frozen-internal muscle tissue (*Figure 5*). Plate 2 contained dried pectoral and ethanol-stored adipose fins.

PLATE 1: CRYO–TISSUE EXTRACTION

Frozen specimens had internal muscle tissue extracted in their frozen states under aseptic conditions. Before the tissue was extracted, a 96 deep-well plate was placed in a small polystyrene box with liquid nitrogen (N_2) at a depth of ~ 1 - 2 cm. The plate was placed in the centre of the box directly in the nitrogen. A polystyrene board with a cut to fit the plate in its centre was placed around the plate floating on the nitrogen which decreased the rate of evaporation, improving visibility (*Figure 5*).

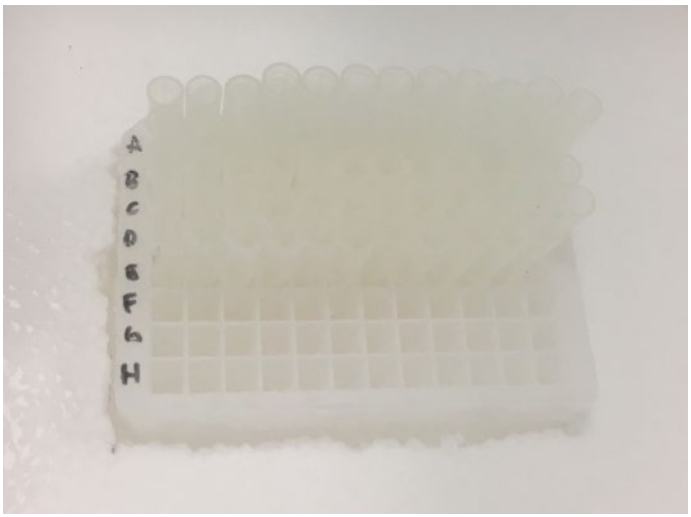

Figure 5 Cryo-tissue extraction: frozen 96 deep-well plate in liquid nitrogen, pipette tips with extracted frozen tissue inside can be seen in the top half of the plate.

Specimens were removed from the freezer in groups of five by site. A sterile disposable scalpel blade was used for each specimen to remove the scales and skin from the site of extraction (to minimise field sampling cross contamination), which was dorsal above the gills on either lateral side (*Figure 6*).

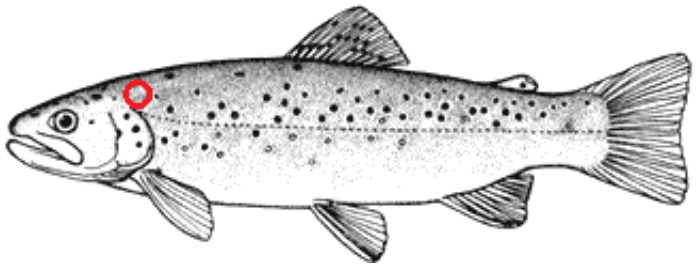

Figure 6 Left-lateral view of brown trout (*Salmo trutta* L.1758) indicating the area of dissection (circled in red).

The apex of a sterile 1000 µl pipette tip was cut with a sterile pair of scissors to create a wider aperture, which was used to bore into the frozen specimen (*Figure 7*). The pipette tip with the frozen tissue extract was then placed in its plate position (*Figure 5*). Once the specimens had their tissue extracted, they were immediately returned to the freezer.

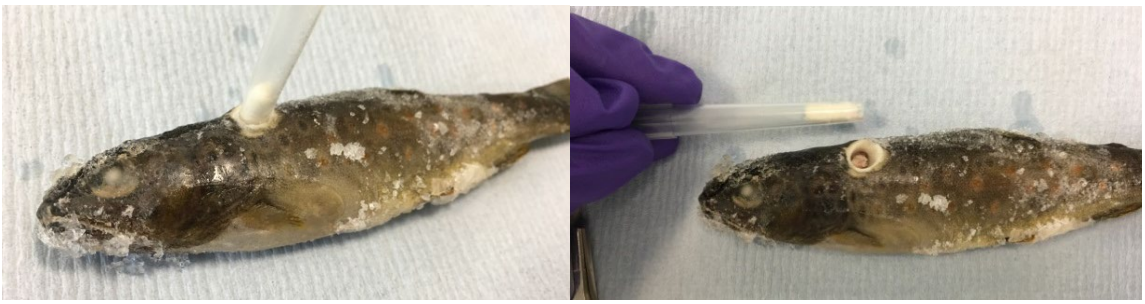

Figure 7 Cryo-tissue extraction using a cut pipette tip: frozen-tissue being extraction (left); frozen-tissue extracted in pipette tip (right).

The pipette tips were carefully inverted in their plate positions and the tissue was prodded free using the sterile ends cut off the pipette tips to drop the tissue into the well. The plate was then sealed with sticky foil and kept frozen at -80°C before being transported to the NHM on ice packs for automated DNA extraction on a Qiagen Biosprint 96® (© 2013–2018 Qiagen).

PLATE 2: PECTORAL AND ADIPOSE FIN CLIPS

Fin clips had the 'cut-ends' of the clip removed with a sterile scalpel to minimise any residual contamination from field sampling. Residual ethanol was left to evaporate off any fin clips before being added to the plate. The plate was sealed and kept frozen at -80°C before being transported to the NHM on ice packs for automated DNA extraction

DNA QUALITY COMPARISON POSTONE CRYO–EXTRACTION

Additional extractions were required to increase DNA quantity for low yield extractions, this provided an opportunity to investigate DNA degradation in frozen-muscle tissue following one cryo-tissue extraction, as well as to compare the DNA quality obtained between automated and manual DNA extraction methods.

Muscle tissue of aforementioned samples were cryo-extracted again and their DNA extracted manually. During the extraction process, a centrifuge step was added *during* 'step 3' - after the addition and AL (lysing) buffer (to suspend the DNA) and before the use of ethanol (or chilled isopropanol in this study, which precipitates the DNA) (Qiagen, 2006). This removed solid impurities

which were abundant after digestion and lysing to prevent the spin column mesh becoming clogged which significantly decreased DNA yield and purity in initial attempts. The successful extractions were pooled to bring DNA yield to optimum levels. Comparisons were made with the same specimens auto-extracted previously at the NHM (*Table 7*).

RNASE TREATMENT

Some of the samples were not RNAse treated during initial extractions, obscuring agarose gel results. To degrade the RNA, 1 µL of RNAse was added to these samples and left at room temperature for at least 1 minute.

DNA PURIFICATION AND CONCENTRATION

Phenol-chloroform extraction was used to purify DNA samples of any lipid and protein contaminants such as RNAse. Carried out inside a fume hood, a solution of phenol-chloroform-isoamyl alcohol (at 25:24:1 ratio) was made by mixing equal volumes of 100 % phenol with a mixture of chloroform-isoamyl alcohol (24:1). The mixture was then added to each DNA sample in equal volumes, and was then gently shaken by hand for approximately 20 seconds. Each sample was then centrifuged at 16,000 x g for 5 minutes at room temperature. The upper aqueous phases were carefully removed and transferred to sterile tubes without disturbing or pipetting any of the lower organic phases containing the contaminant proteins and lipids. For low yield samples where more DNA could no longer be obtained, 100 µL nuclease-free water was added to their tubes again, the tubes were shaken, and the aqueous layer extracted once more and pooled.

Ethanol precipitation was undertaken immediately after each phenol-chloroform extraction to purify samples further from residual contaminants following the extraction process, as well as to standardise DNA concentrations. To do this, 1 µL of glycogen (20 µg/µL) was added to each sample, followed by adding 0.5 x sample volume of ammonium acetate-NH_4OAc (7.5M), then adding 2.5 x 'the sample volume + the added ammonium acetate volume' of 100 % ethanol. The tubes were stored at -80°C for at least 1 hour, the samples were then centrifuged at ≤ 4°C for 30 minutes at 16,000 x g to pellet the DNA. The supernatant was removed being careful not to disturb the DNA pellet adhered to the tube. 150 µL of 70 % ethanol was added to wash the pellet of any remaining contaminants. The supernatant was carefully removed and the samples left open in a fume hood to dry out before adding AE buffer at varying volumes depending on the initial DNA yield and target concentration.

Comparisons were made between samples prior and post Phenol-Chloroform-Isoamyl alcohol – Ethanol (PCI-E) purification (*Table 8*).

COMPLETE DNA QUALITY ASSESSMENT

Over the course of this study 384 specimens had their DNA extracted. Though many were not suitable for the study due to low DNA qualities, compiled, they provide information on the raw DNA integrity, quantity, and purity observed, and were collated and compared by tissue type and storage (*Table 5*).

MICROSATELLITE MARKERS: BTMP–CUSTOM–PANEL-4

Microsatellites markers were chosen to characterise the population genetics of the Loch Maree trout using the Beaufort Trout MicroPlex (BTMP) Panel-4 by Keenan *et al*. (2013) (*Table 1*). BTMP-Panel-4 was modified to ensure no overlapping size ranges within each fluorophore group.

Table 1 Information on primer sequences, size ranges. Red boxes outline where markers have overlapping size ranges and where fluorophore groups have either too many or only one marker in the original panel; and subsequently indicate which marker was substituted in, as well as which marker had moved fluorophore groups to remove outlined size overlaps in the custom panel.

	Panel/locus	Forward primer	Reverse primer	Size range (bp)
Original BTMP-Panel-4	*Ssa*D170	NED-GGAGGCAGTTAAGAGAACAAAAG	gttTCACCTACCCTTCTCATTCAAG	148–217
	Sasa-UBA	NED-GGAGAGCTGCCCAGATGACTT	gtttCAATTACCACAAGCCCGCTC	268–523
	*Ssa*413UoS	PET-GTAGACGCCATCGGTATTGTG	gtttCGTGATGCCGCTGTAGACTTG	225–282
	*Ssa*407UoS	FAM-TGTGTAGGCAGGTGTGGAC	gtttCACTGCTGTTACTTTGGTGATTC	204–320
	*Ssa*D48	FAM-GAGCCTGTTCAGAGAAATGAG	gtttCAGAGGTGTTGAGTCAGAGAAG	304–558
	CA054565a	VIC-TCTGTGGTTCCCGATCTTTC	gtttCAACATTTGCCTAGCCCAGA	101–120
	CA054565b	–	–	125–163
	m*One*101	VIC-TGCTAAATGACTGAAATGTTGAGA	gtttGAGAATGAATGGCTGAATGGA	155–194
	CA060177	VIC-CGCTTCCTGGACAAAAATTA	gtttGAGCACACCCATTCTCA	234–315
	m*One*108	VIC-GTCATACTACTCATTCCACATTA	gtttACACAGTCACCTCAGTCTATTC	371–518
Custom BTMP-Panel-4	*Ssa*D170	NED-GGAGGCAGTTAAGAGAACAAAAG	gttTCACCTACCCTTCTCATTCAAG	148–217
	Sasa-UBA	NED-GGAGAGCTGCCCAGATGACTT	gtttCAATTACCACAAGCCCGCTC	268–523
	m*One*101	PET-TGCTAAATGACTGAAATGTTGAGA	gtttGAGAATGAATGGCTGAATGGA	155–194
	*Ssa*413UoS	PET-GTAGACGCCATCGGTATTGTG	gtttCGTGATGCCGCTGTAGACTTG	225–282
	*Ssa*407UoS	FAM-TGTGTAGGCAGGTGTGGAC	gtttCACTGCTGTTACTTTGGTGATTC	204–320
	*Str3*QUB	FAM-CTGACCGCTGCACACTAA	gtttGGCTCTAATCGACTGGCAGA	115–175
	CA054565a	VIC-TCTGTGGTTCCCGATCTTTC	gtttCAACATTTGCCTAGCCCAGA	101–120
	CA054565b	–	–	125–163
	CA060177	VIC-CGCTTCCTGGACAAAAATTA	gtttGAGCACACCCATTCTCA	234–315
	m*One*108	VIC-GTCATACTACTCATTCCACATTA	gtttACACAGTCACCTCAGTCTATTC	371–518

Information on the original BTMP-Panel-4 primer sequences (*Table 1*) outline the fluorophore labelled forward primers, expected amplicon size range in base pair length (bp). 'm' prefixed loci names had been modified in Keenan *et al.,* (2013) study from their original sources for use in *Salmo trutta*. Unlabelled primers are prefixed with 'gttt' (or 'pigtailed') (Brownstein *et al.,* 1996).

To remove overlapping size ranges with *SSa*407UoS, *Ssa*D48 was replaced with *Str3*QUB from BTMP-Panel-2 (Keenan *et al*., 2013). m*One*101's fluorescent dye was changed from VIC to PET as there was only one loci using the PET fluorophore while VIC was being used by five loci - including CA054565b which overlapped with the expected size-range of m*One*101. This ensured no overlapping size ranges. The red boxes outline these changes and why they were made, resulting in the Custom BTMP-Panel-4 (*Table 1*).

Evaluation and optimisation of primers

The Custom BTMP-Panel-4 was tested for compatibility with Wester Ross *S. trutta* samples. Forward primers for the BTMP customised panel were ordered modified with assigned fluorescent labels from Thermo Fisher (© 2016 Thermo Fisher Scientific Inc) and the reverse primers from Sigma Aldrich, now Merck (© 2018 Merck KGaA). 100 µM stock solutions were prepared and all loci were tested against a small subsample from separate catchments (*n* 3).

Multiplex preparation & evaluation

The panel was tested to ensure successful multiplexed amplification, and whether 10 µL reaction volumes could amplify successfully. The primer multiplexed stock solution and 10 µL PCR reaction volumes were prepared as follows:

Table 2 Multiplex preparation of BTMP Custom Panel, volumes of primers and solvent used.

Reagent	Volume (µL)
Volume/primer (100 µM/primer)	10
Nuclease-free H_2O	320
Total volume (2 µM/primer):	**500**

For all 9 forward and reverse primers, 10 µL of each starting at 100 µM molarity were mixed together with 320 µL of nuclease-free water, making an end volume of 500 µL, with each primer now at 2 µM molarity (*Table 2*).

Table 3 PCR multiplex working solution & reaction mix preparation.

Reagent	Volume/specimen (µL)
PCR Master mix	5
Primer multiplex (2 µM each)	1
Nuclease free H_2O	3
Multiplex working solution volume/specimen:	9
Specimen DNA:	1
Total PCR reaction volume/specimen:	**10**

PCR reaction volumes (10 µL) was prepared by mixing 9 µL of working solution with 1 µL of each specimen's DNA sample (*Table 3*). PCR conditions consisted of four cycle sets (*Table 4*).

Table 4 PCR cyclic conditions for amplification of the BTMP microsatellites (Keenan *et al.,* 2013).

	1 Temp - 1 Cycle	3 Temp – 5 Cycle			3 Temp – 22 Cycle			1 Temp – 1 Cycle	∞
Temperature (°C)	95	95	55	72	95	57	72	60	4
Time (min:seconds)	15:00	00:45	01:30	01:00	00:45	01:30	01:00	30:00	∞
Number of cycles	1	5			22			1	∞

AMPLIFICATION OF BTMP-CUSTOM-PANEL–4: RUNS 1-4

All 192 specimens were amplified successfully across 4 separate runs (each run *n* 48) using the multiplexed PCR protocols described in *Tables 2, 3* & *4*.

DNA INTEGRITY INDEX RATING

In an attempt to objectively quantify and compare the DNA integrity of sample groups, the DNA Integrity Index Rating (IIR) is proposed: each specimen's integrity is given the following the values based on its integrity assessed by agarose gel lane pattern, as mentioned previously: Low integrity = 0; Moderate integrity = 0.5, High integrity = 1. The datasets produced can use descriptive statistics to allow basic, quantitative descriptions within, as well as comparisons between sample groups (*Table 6*).

1.2: RESULTS

DNA QUALITY ASSESSMENT

The DNA purity (molecular and chemical, defined by 260/230 and 260/280 wavelength (nm) absorption ratios) and quantity (or yield, defined by molecular mass), was optimal for many of the initial 96 specimens subsampled from the archive with mean purity ratios: *260/230* 2.31 ± 2.31 SD, *260/280* 2.06 ± 0.06 SD; and mean yield: 14.21 µg/µL ± 11.88 SD.

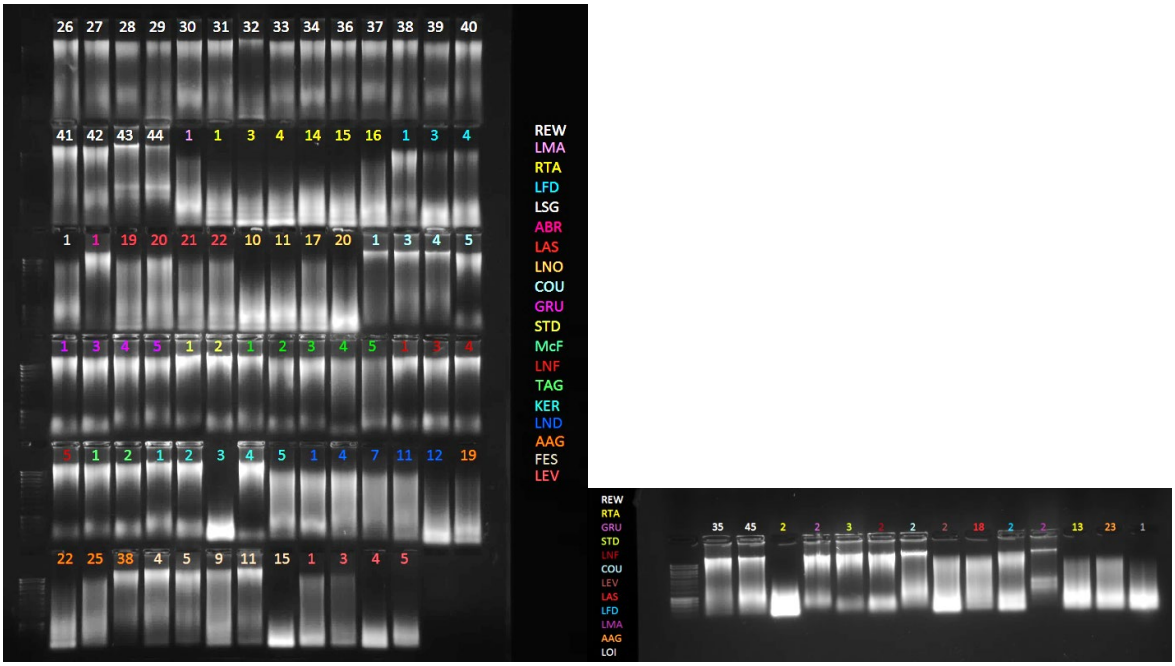

Figure 8 Agarose gel images of 96 DNA extractions from *S. trutta* fin clips. Each lane represents a specimen. 1 kb ladders were used as size standards.

Many samples had compromised DNA integrity (defined as the 'intactness' of the DNA, which decreases by degradation). Of the 96 samples, 49 (51.04 %) maintained moderate DNA integrity, 38 had low integrity (39.58 %), and only 9 yielded high integrity DNA (9.38 %) (*Figure 8*).

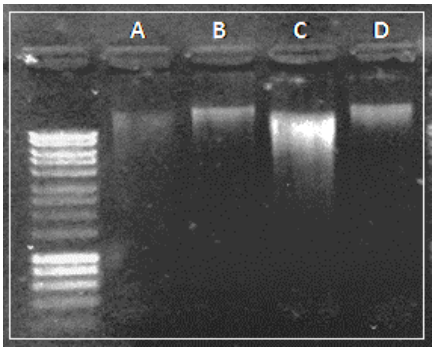

Figure 9 Agarose results for tissue type and storage DNA quality, which tested frozen muscle that has been through at least one freeze-thaw cycle (A), frozen muscle that has never been defrosted (B), pectoral fin stored dry (C), and adipose fin stored in ethanol (D). 1 kb ladders were used as size standards.

DNA extracts from tissue types and modes of storage available in the archive indicated muscle tissue that had never been defrosted maintained the highest DNA integrity DNA (B), followed by adipose

fins stored in ethanol (D), while muscle tissue with at least one freeze-thaw cycle (A) and ethanol-stored adipose (C) suffered moderate degradation (*Figure 9*).

COMPLETE DNA QUALITY ASSESSMENT

DNA quantity was highly variable between all tissue and storage types and ranged between 0.68 – 26.53 µg (*Table 5*).

Table 5 DNA quality, quantity, purity for all extractions of *S. trutta* for available tissue types and their storage mode.

Tissue type & storage	% DNA Integrity (*n*)				Mean DNA Quantity			Mean DNA Purity		
	n	High	Moderate	Low	*n*	Yield (ng)	SD	*n*	260/280	260/230
Dried-Adipose clips	46	23.91 (*11*)	56.52 (*26*)	19.57 (*9*)	46	19699.37	18187.75	29	2.08	2.24
Ethanol-Adipose clips	97	31.96 (*31*)	49.48 (*48*)	18.56 (*18*)	97	6548.03	7423.28	70	2.05	2.42
Frozen-Adipose clips	3	100.00 (*3*)	0.00 (*0*)	0.00 (*0*)	3	679.09	268.09	-	-	-
Dried-Pectoral fins	95	6.32 (*6*)	34.74 (*33*)	58.95 (*56*)	100	26531.9	21798.11	38	2.06	2.21
Ethanol-Pectoral fins	13	0.00 (*0*)	0.00 (*0*)	100.00 (*13*)	13	20594.74	11695.73	13	2.07	1.95
Frozen-Muscle tissue	98	71.43 (*70*)	19.38 (*19*)	9.18 (*9*)	99	947.31	559.1	-	-	-
Defrosted-Muscle tissue	26	0.00 (*0*)	7.69 (*2*)	92.31 (*24*)	26	14425.6	7068.22	26	2.08	2.22

Man-Whitney tests showed the median yields in dried-adipose fins yielded significantly more than the ethanol-stored ($p < 0.0001$, 95% Confidence Interval 6567-11 869 [ng]) but this was not observed for pectoral fins (p 0.7021). Dried-adipose fin-clips also provided significantly less yield than dried-pectoral fins (p 0.0109, 95% CI 1314-9376). Yield was lowest in all frozen tissues compared to non-frozen storage methods ($p_s < 0.005$). Yield difference between defrosted-muscle and dried-adipose fins was not significant (p 0.4781), but defrosted-muscle was significantly lower than dried-pectoral fins (p 0.0024, 95% CI 2496-11 625). Mean purity was consistent between all samples (~ 2) with some variability (SD < 0.3) (*Table 5*). The proportions of DNA integrity observed are illustrated below (*Figure 10*).

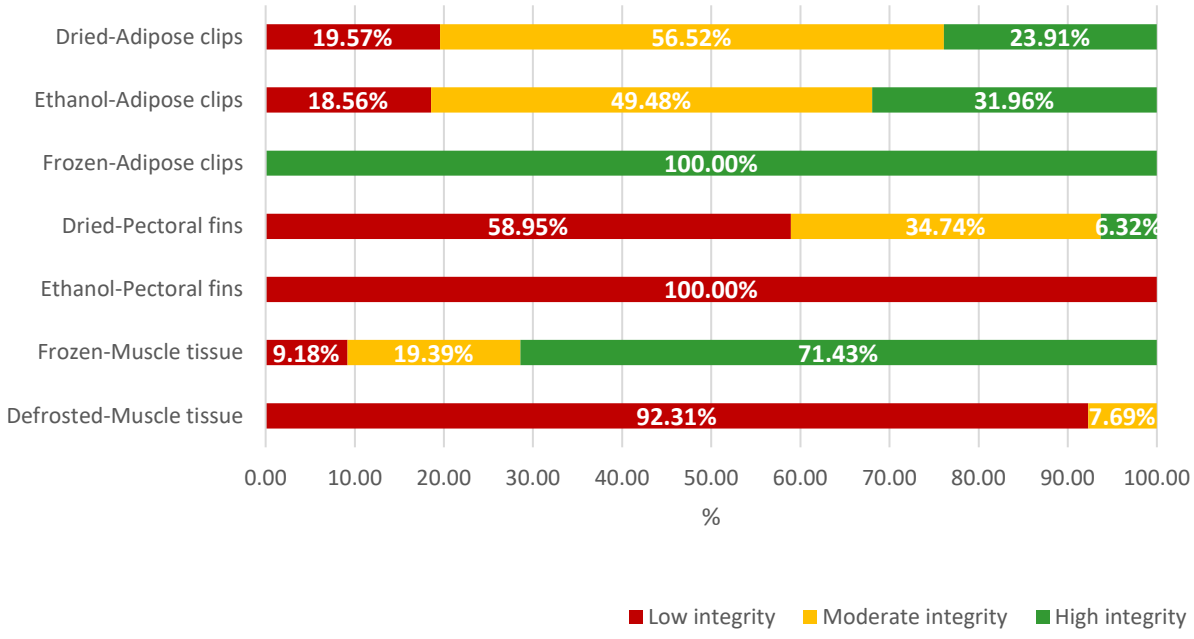

Figure 10 Stacked bar chart showing percentages of extractions that yielded DNA of high, moderate, and low integrity from *S. trutta* tissue types and their modes of storage, data derived from *Table 5*.

DNA integrity was highly variable between the samples. Dried- and ethanol-stored adipose fins yielded similar proportions of low integrity DNA (19.57 % and 18.56 %), with ethanol-storage yielding 8.05 % more high integrity DNA, the remainder of both containing moderate integrity (*Figure 10*). Frozen-adipose fins provided very promising results, but with a sample size of three, this result is precarious. Dried-pectoral fins yielded 58.95 % low integrity DNA and only 6.32 % of high integrity. Ethanol-stored pectoral fins provided only low integrity but this result is also cautionary (*n* 13) (*Table 5*). Frozen-muscle tissue yielded the lowest 9.18 % of low integrity, and the most 71.43 % of high integrity DNA. Defrosted-muscle tissue yielded 92.31 % of highly degraded DNA, and only 7.69 % DNA of moderate integrity. Frozen adipose consistently provided DNA of high integrity (100%) followed by frozen-muscle tissue (71.43%) which also provided the next lowest proportion of low integrity DNA (9.18 %). Ethanol-stored pectoral fins provided the most low integrity DNA (100%), followed by defrosted-muscle tissue (92.31 %) which also failed to provide any of high integrity (*Figure 10*).

DNA INTEGRITY INDEX RATING

Descriptive statistics of the IIR describe the typical DNA integrity found across all the extractions undertaken in this study, separated by tissue and storage type are displayed (*Table 6*).

Table 6 Descriptive statistics of Integrity Index Rating of DNA extracted from every *S. trutta* in this study by tissue and storage type.

Tissue/storage	*n*	Mean	SD	Minimum	Q1	Median	Q3	Maximum
Dried-adipose	46	0.52	0.33	0.00	0.50	0.50	0.63	1.00
Ethanol-adipose	97	0.57	0.35	0.00	0.50	0.50	1.00	1.00
Frozen-adipose	3	1.00	0.00	1.00	1.00	1.00	1.00	1.00
Dried-pectoral	95	0.23	0.30	0.00	0.00	0.00	0.50	1.00
Ethanol-pectoral	13	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Frozen-muscle	98	0.81	0.33	0.00	0.50	1.00	1.00	1.00
Defrosted-muscle	26	0.04	0.14	0.00	0.00	0.00	0.00	0.50

Abbreviations: *n*, number of specimens in sample; SD, standard deviation; Q1, first interquartile range; Q3, third interquartile range.

IIR data is likely non-parametric so interpretations should include considerations of the median, mean, SD, and interquartile ranges (*Table 6*). Defrosted-muscle DNA extraction was considered a positive control for DNA degradation and had an index of 0.04 ± 0.14 SD, which was significantly less than the mean IIR for frozen-muscle tissue (0.81 ± 0.33) ($p < 0.0001$). No significant difference was seen between dried and ethanol-stored adipose fins (p 0.4866) and adipose fins yielded significantly greater proportions of high integrity DNA compared to pectoral fins of the same storage mode ($p_s < 0.0001$). These results coincide with the results observed earlier (*Table 5*), including the interquartile ranges that reflect *Figure 10*.

EXTRACTION OF DNA FOR POPULATION GENETIC ANALYSIS: PLATE 1 & 2

The following gel images are of Plate 1 & 2's DNA auto-extracted at the NHM.

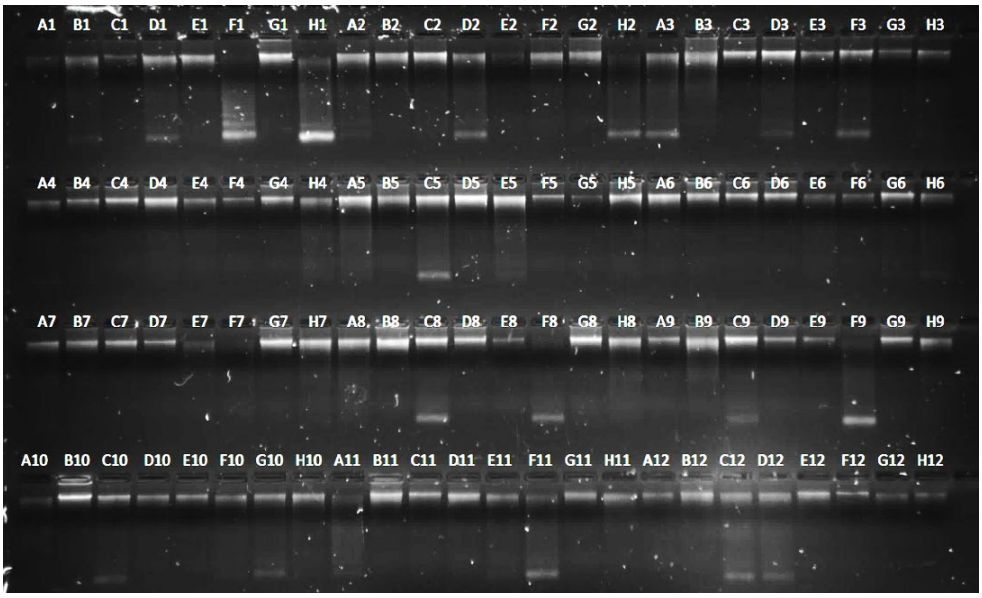

Figure 11 Agarose gel image of plate 1 - 96 DNA extractions from *S. trutta* frozen muscle tissue mostly, and a few fin clips. Each lane represents an individual trout.

DNA integrity for 76.04 % (*n* 73) of plate 1 extractions were optimal (*Figure 11*). Mean IIR across the plate was 0.83 ± 0.32 SD; mean yield was 1.02 µg/µL ± 0.60 SD.

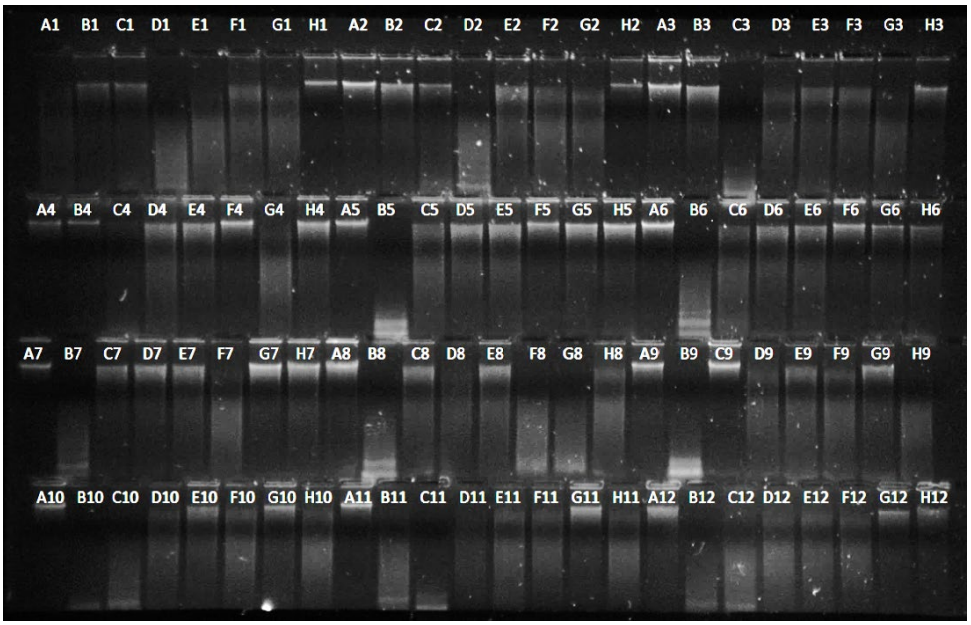

Figure 12 Agarose gel image of plate 2 - 96 DNA extractions from *S. trutta* dry-stored pectoral and ethanol-stored adipose fin clips. Each lane represents an individual.

DNA integrity for 50 % (*n* 48) of plate 2 extractions were low, and 25 % (*n* 24) of both moderate and high (*Figure 12*). Mean IIR was 0.38 ± 0.42 SD; mean yield was 24.36 µg/µL ± 23.77 SD and was significantly lower than the DNA IIR of plate 1 ($p < 0.0001$).

DNA QUALITY COMPARISON POSTONE CRYO–EXTRACTION

DNA quality and quantity of the manual DNA extractions of frozen muscle tissue post 1 cryo-extraction are compared with that of the same specimens prior to the last cryo-extraction (*Table 7*).

Table 7 DNA quality and quantity measurements for additional extractions - Comparison between automated (Qiagen Biosprint 96®) and manual extraction (Qiagen DNeasy® Blood & Tissue) post 1 cryo-extraction.

	Automated			**Manual**		
Sample ID	**Yield (ng)**	**Quality**	**IIR**	**Yield (ng)**	**Quality**	**IIR**
LFD5	987.45	High	1	455.04	High	1
RTA5	684.97	Moderate	0.5	476.71	Moderate	0.5
LMG2	665.28	High	1	773.93	High	1
LMG3	488.65	High	1	744.98	High	1
LMG5	566.95	High	1	399.51	Moderate	0.5
LMG6*	-	-	-	891.66	Moderate	-
LMG6*	-	-	-	648.48	High	-
LFM34	506.05	High	-	-	-	-
LFM35	658.30	High	1	582.86	Moderate	0.5
LFM36	774.30	High	1	972.72	High	1
LFM37**	930.90	High	1	3956.50	High	1
LAP8	951.84	High	1	492.15	Moderate	0.5
LAP19	723.55	Moderate	0.5	638.83	Moderate	0.5
LAP29	997.60	High	1	660.06	High	1
LAP30	961.35	Moderate	0.5	824.11	Moderate	0.5
RCA1	458.20	High	1	611.81	High	1
Mean:	**739.67**		**0.88**	**655.20****		**0.77**
SD:	**189.34**		**0.21**	**164.48****		**0.26**

*LMG6 had twice as much tissue cryo-extracted and therefore was extracted in two separate tubes, as the specimen had not been extracted before and was used to replace another specimen for the same site.

**Mean and SD values calculated with the removal of sample LFM37 which heavily skewed the averages.

Results from the additional extractions show that DNA integrity was maintained at the same IIR post one cryo-extraction for all extractions except LMG5, LFM35, and LAP8 which deteriorated from high to moderate integrity. The mean IIR of the DNA extracted subsequently fell from 0.88 ± 0.21 SD (for the automated), to 0.77 ± 0.26 SD after one cryo-extraction cycle (for the manual), an insignificant decrease of 0.12 (p 0.2565, n 13) (*Table 7*).

DNA PURIFICATION AND CONCENTRATION

Phenol-Chloroform and ethanol purification successfully removed contaminant proteins following RNAse treatments, increasing the mean IIR from 0.56 ± 0.27 SD to 0.63 ± 0.36 SD (*n* 32) (*Table 8*).

Table 8 DNA yield post phenol-chloroform isoamyl alcohol and ethanol precipitation.

	Sample ID		PRE-PCI-E	POST-PCI-E	Loss %	PRE-PCI-E		POST-PCI-E		PRE-	POST-
#	Label	ID	Yield (ng)	Yield (ng)	Yield (ng)	260/280	260/230	260/280	260/230	IIR	IIR
1	REW	43	29336.00	13578.00	53.72	2.1	2.3	1.8	2.2	1	1
2	REW	44	23160.00	4138.50	82.13	2.1	2.2	1.8	2.0	1	1
3	REW	45	84148.00	21948.00	73.92	2.1	2.3	1.8	2.0	1	0.5
4	LMA	2	6851.50	1506.60	78.01	2.1	2.1	1.6	1.1	1	1
5	GRU	1	3270.00	1460.10	55.35	2.0	2.4	1.7	1.2	0.5	0.5
6	GRU	2	2550.00	1274.10	50.04	2.0	2.5	1.5	1.1	0.5	0.5
7	GRU	3	5312.00	1506.60	71.64	2.0	2.4	1.6	1.1	0.5	0.5
8	GRU	4	6851.00	1720.50	74.89	2.1	2.5	1.7	1.1	0.5	0.5
9	GRU	5	8143.00	1043.40	87.19	2.0	2.5	1.5	1.2	0.5	1
10	STD	1	11424.00	3966.80	65.28	2.0	2.3	1.6	1.1	0.5	1
11	STD	2	10200.00	3064.40	69.96	2.0	2.6	1.5	1.1	0.5	1
12	STD	3	20400.00	-	100.00	2.0	2.6	1.5	1.6	0.5	0
13	McF	1	3184.00	-	100.00	2.0	2.7	1.5	1.0	0.5	0.5
14	McF	2	6749.00	676.80	89.97	2.1	2.5	1.6	1.0	0.5	1
15	McF	3	5152.00	755.76	85.33	2.0	2.6	1.6	1.4	0.5	0
16	McF	4	4432.00	500.08	88.72	2.0	2.7	1.5	0.9	0.5	0
17	McF	5	1416.00	355.32	74.91	2.0	2.9	1.5	1.5	0	0
18	LNF	1	1102.40	1193.80	0.00	2.0	2.8	1.6	2.7	0.5	1
19	LNF	2	4256.00	1156.20	72.83	2.0	2.5	1.5	1.4	0.5	0.5
20	LNF	3	2960.00	742.60	74.91	2.0	2.5	1.5	1.7	0.5	0.5
21	LNF	4	5040.00	396.68	92.13	2.0	2.5	1.6	1.8	0.5	1
22	LNF	5	1872.00	708.76	62.14	2.0	2.7	1.5	1.9	0.5	0.5
23	TAG	1	1728.00	738.84	57.24	2.0	2.8	1.6	1.4	0.5	0.5
24	TAG	2	11594.00	7238.00	37.57	2.0	2.5	1.7	1.7	0.5	1
25	KER	1	3136.00	1090.40	65.23	2.1	2.5	1.6	1.8	0.5	0.5
26	KER	2	2816.00	849.76	69.82	2.1	2.5	1.6	1.6	0.5	1
27	KER	3	3344.00	919.32	72.51	2.1	2.5	1.5	1.8	0	0.5
28	KER	4	4512.00	321.48	92.88	2.0	2.5	1.5	2.4	0.5	0
29	KER	5	6562.00	2801.20	57.31	2.0	2.5	1.9	2.5	0	0.5
30	COU	1	1251.84	1193.80	4.64	2.2	2.1	1.6	1.7	1	1
31	COU	2	5760.00	6956.00	0.00	2.2	2.1	1.9	2.4	1	1
32	COU	3	2419.20	4361.60	0.00	2.1	2.2	1.9	2.3	1	0.5
		Mean:	**9091.62**	**2938.78**	**64.38**	**2.1**	**2.5**	**1.6**	**1.6**	**0.56**	**0.63**
		SD:	**15034.75**	**4507.75**	**28.02**	**0.1**	**0.2**	**0.1**	**0.5**	**0.27**	**0.36**

DNA yield prior and post PCI-E purification indicated substantial losses of DNA had occurred. Mean loss of DNA yield was 64.38 % ± 28.02 SD after PCI-E purification (*Table 8*). Purity was also a consistent issue across all samples after PCI-E with mean 260/280 and 260/230 ratios of 1.61 ± 0.13 and 0.49 SD respectively. This made many samples unsuitable for a RADseq approach and so a microsatellite marker approach using the BTMP (Keenan *et al*., 2013) was adopted to carry out the study from hereon.

BTMP-CUSTOM-PANEL–4 OPTIMISATION & MULTIPLEXING

Fluorescent labelled primers successfully amplified target regions individually across three representative samples (*Figure 13*) and when multiplexed at 20 and 10 µL reaction volumes (*Figures 15 & 16*). Which allowed all samples to be amplified following the 10 µL multiplexed protocols outlined in *Tables 3-5* (*Figure 16*).

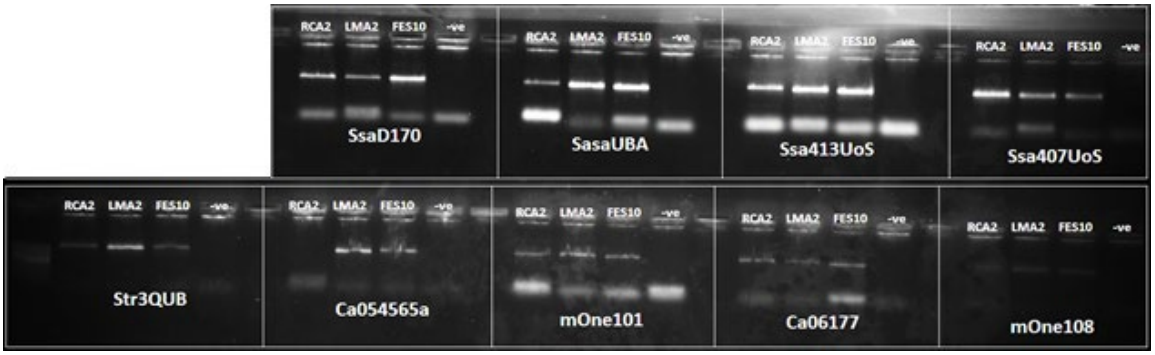

Figure 13 Agarose gel (1.5%) image of labelled BTMP test 2. 100bp ladder was used as size standards.

All modified primers amplified successfully in all except RCA2 for loci Ca054565a (*Figure 13*).

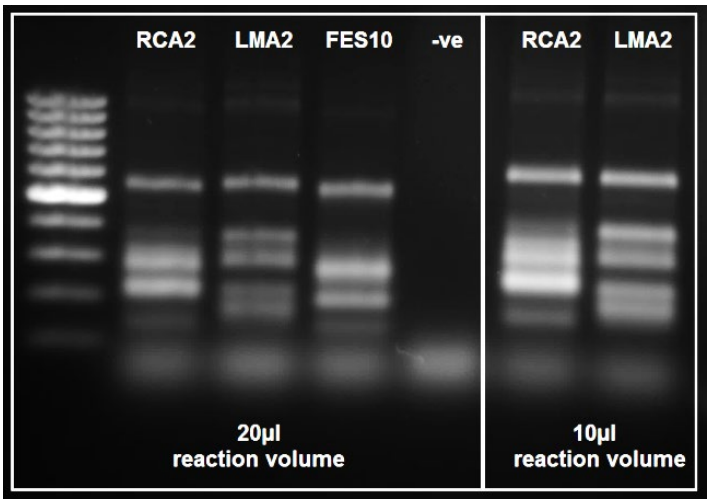

Figure 14 Agarose gel (1.5%) image of labelled BTMP multiplex test 1 with reaction volumes 20 µL and 10 µL for RCA2 and LMA2 . 100 bp ladder was used as size standards.

Gel results showed that both reaction volumes amplified successfully, with the 10 µL reaction volume also giving a stronger signal (due to doubled starting concentration). Identical banding patterns for RCA2 and LMA2 was observed between both reaction volumes (*Figure 14*).

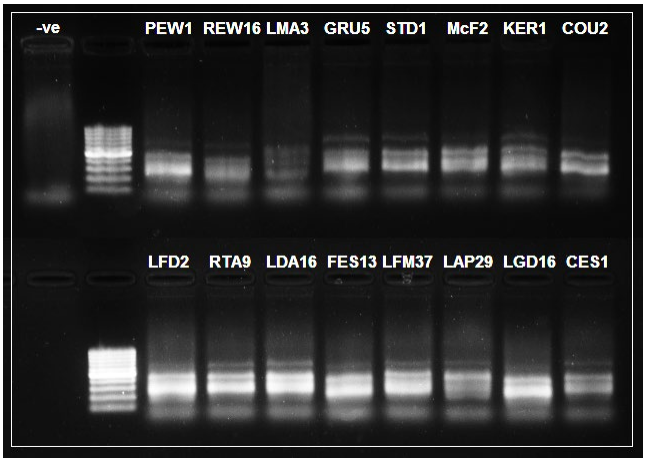

Figure 15 Agarose gel (1.5%) image of labelled BTMP multiplex test 2 with 10 µL reaction volumes. 100 bp ladder was used as size standards.

Multiplexed 10 µL reaction volumes amplified successfully in a representative sample (*n* 16) (*Figure 15*).

BTMP-CUSTOM-PANEL-4 PCR RUNS 1–4

All 192 samples of trout DNA were successfully amplified across four separate runs (*n* 48) (*Figure 16*). Despite low fluorescence in a few samples, their bands are visible (e.g. LLm16). Gel images of all runs can be found in *Appendix 1*.

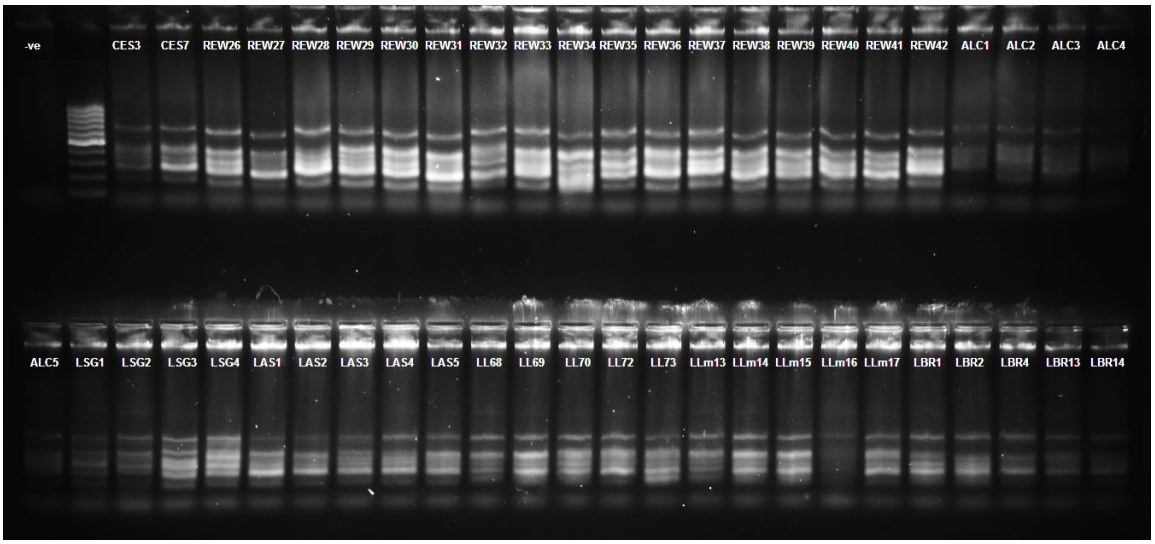

Figure 16 Agarose gel (1.5%) image of BTMP-custom-panel-4 amplification run 3 using 10 µL reaction volumes. 100bp ladder was used as size standards.

1.3: DISCUSSION

DNA QUALITY ASSESSMENT

The trout tissue archive contained samples of varying storage age and previous research use. Consequently, it was necessary to undertake an analysis of DNA integrity, quantity, and purity. These parameters influence choice of methodology when conducting population genetic studies, and requirements for successful NGS methods are very strict (Muhammad *et al*., 2016; Bonin *et al.,* 2004; Ross *et al*., 1990; Crow and Kimura, 1970). Results from the initial 96 fin-clips subsampled from the archive indicated the specimens had suffered varied levels of DNA degradation (*Figure 8*), with 39.58 % creating a 'bottom-up smear pattern' indicating DNA of 'low integrity' (e.g. RTA group). 'Top-down smears' are visible across 51.04 % lanes, indicating DNA of 'moderate integrity', which may still be sufficient for NGS analysis and are certainly more than adequate for extracting information on traditional markers such as microsatellites (Graham *et al*., 2015). 'Distinct bands at the top of the lanes' indicating high integrity DNA was observed in only 9.38 % of the samples (e.g. REW 43-44). These extractions were not RNAse treated before the gel, meaning some lanes showing moderate degradation may in fact be high quality obscured by fluorescing RNA which smears the lane. Taken together, these results suggest the archived fin-clips are not optimal for genotyping by NGS. When DNA integrity was investigated for each sample type (*Figure 9*), frozen muscle tissue that had never been defrosted, and ethanol-stored adipose fins yielded high integrity DNA, with frozen muscle showing slightly less degradation. Muscle that been through a freeze-thaw cycle and dried-pectoral fins provided DNA of, at best moderate, to low integrity. This suggested frozen-muscle tissue should be prioritised for extracting high integrity DNA.

COMPLETE DNA QUALITY ASSESSMENT

When raw quality data for all extractions in this study were pooled, mean DNA yield was variable between tissue types and was consistently highest in dried-storage for fin clips compared to other storage modes of the same fin-type (*Table 5*). Pectoral fins yielded the greatest quantities of DNA out of all tissue types, but the high proportions of low integrity DNA made pectoral fins suboptimal tissues for an NGS approach (*Figure 10*). However this was not the case with adipose-fins, therefore, if dry storage is all that is available, adipose fins are the recommended tissue type for sampling and archiving, as the large proportion of samples with moderate DNA integrity may still be suitable for NGS techniques (Graham *et al*., 2015). Frozen-storage was optimal over dried- and ethanol-storage for maintaining high DNA integrity in muscle and adipose fin tissue (*Figure 10*), but suffered from significantly lower DNA yields (*Table 5*) – this reduction in yield was observed in another study (Ross *et al*., 1990), suggesting as much tissue as sustainably possible should be sampled if freezing is the storage method of choice. High levels of DNA degradation made dried-pectoral fins and defrosted-muscle suboptimal for population genetic studies archiving and should be avoided (*Figure 10*). Mean purity ratios were optimal across all groups and did not show any clear trends (*Table 5*).

DNA INTEGRITY INDEX RATING

The mean IIR (proposed in this study) provides an index value where reaching 1 indicates all DNA samples have high integrity, as seen in the frozen-adipose tissue; 0 indicates all to be highly degraded as in ethanol-stored pectoral fins. Around 0.5 indicates possibilities: either most samples are of moderate integrity, or the sample has similar proportions of high and low integrity DNA, or a combination of the two, as seen in the dried and ethanol-stored adipose fins (*Figure 10*). Dried-pectoral fins and frozen-muscle tissue show contrasting percentages with both having either mostly high or low quality with a small amount of the opposite, and an intermediate amount of moderate quality DNA - the mean index of each reflects this contrasting difference at 0.23 and 0.81. IIR descriptive statistics show their interquartile ranges also reflect the stacked bar chart (*Figure 10*), and also allows the use of standard deviations to get an idea of typical sample variability, furthermore, the statistical results also reinforce earlier observations (*Table 5 & Figure 10*). Based on this reasoning, rather than describing multiple percentages when comparing groups of extractions, DNA Integrity Index Rating or IIR will be used to describe and compare DNA integrity of sample groups from hereon. Frozen-adipose and ethanol-stored pectoral fins showed extreme results but were not statistically analysed due to insufficient sample sizes.

EXTRACTION OF DNA FOR POPULATION GENETIC ANALYSIS

The gel results from plate 1 and 2 indicated frozen muscle tissue consistently provided higher quality DNA than fin clips (*Figures 12 & 13*). Mean IIR was 0.82 ± 0.34 SD for the frozen-muscle tissue on Plate 1, and was significantly greater than the DNA IIR obtained from the adipose and pectoral fins on Plate 2 (IIR 0.38 ± 0.42 SD), reinforcing frozen-muscle as the tissue and storage of choice for acquiring high integrity DNA. Mean yield for Plate 1-frozen muscle was below optimal limits (1.02 µg/µL ± 0.60 SD) and required additional extractions. Plate 2-fin clips provided high yields except for one or two small adipose-clips (mean yield: 24.36 µg/µL ± 23.77 SD), coinciding with earlier results.

DNA QUALITY COMPARISON POST ONE CRYO–EXTRACTION

The cryo-extraction method may have minimised degradation for all but three samples where DNA integrity decreased from high to moderate. A controlled test is needed to further validate this hypothesis; if true, removing and returning the fish to the freezer one at a time would minimise degradation further. Sufficient yield was extracted in all additional manual extractions and is comparable to the automated extractions (*Table 7*). DNA was pooled so quantity and quality for most samples were then optimal for NGS.

DNA PURIFICATION AND CONCENTRATION

RNAse treatment was successful for the required extractions, increasing mean IIR from 0.56 ± 0.27 SD to 0.63 ± 0.36 SD (*n 32*) (*Table 8*). Subsequent purification by PCI-E extraction resulted in intolerable losses of DNA yield (mean recovery 41.64 % ± 37.79 SD), which made multiple samples, some highly informative and irreplaceable, suboptimal for a RADseq approach

which was initially intended for this study. Tests were conducted, measuring DNA yield at each step in the PCI-E protocols (results not shown), but the results could not show where the loss occurs as the loss was minimal during tests. When purifying the study's actual samples, the unexpected losses occurred.

The yield loss coupled with low integrity in many samples made a microsatellite approach more appealing, as it is more robust with samples of lower quality, as well as allowing the inclusion of the highly informative, irreplaceable samples that were no longer suitable for NGS and would have been excluded otherwise. Ultimately, the BTMP microsatellite marker array became the project approach hereon.

MICROSATELLITE MARKERS: BTMP–CUSTOM–PANEL-4

The panel of microsatellite markers used in this study initially developed by Keenan *et al*., (2013) – The Beaufort Trout MicroPlex, comprised of 38 loci over 4 panels. BTMP-Panel-4, comprised of nine loci, had two overlapping bp size ranges for the microsatellite amplicons on fluorophore groups FAM and VIC (*Table 1*), which could result in erroneous allele scoring for peaks within the overlap in their traces. *Ssa*D48, labelled with the FAM fluorescent dye, has an overlapping size range with *SSa*407UoS; m*One*101 overlaps with CA054565b which is co-amplified (from CA054565a) using the same VIC label. This led to the reconfiguration for the Custom BTMP-Panel-4 (*Table 1*) which removed overlapping allele size ranges which would have occurred between 304-320 bp on the FAM fluorophore group, as alleles 304, 312, and 320 did appear on *SSa*407Uos (Locus 2), and could have been obscured by potential alleles in *Ssa*D48. Relabelling *m*One101 with the PET fluorophore was also successful at reducing overlapping size-ranges that was present from the co-amplification of CA054565b (which was not scored due to noise-filled erratic traces).

SUGGESTIONS FOR FUTURE SAMPLING

Based on results, freezing whole trout specimens for population genetic studies could be prioritised where and when it is sustainably possible. Despite the results, adipose-fins are not recommended to be taken from catch-and-release specimens as recent literature has shown that it is a sensory organ that affects swimming efficiency in brown trout (Buckland-Nicks, 2016; Buckland-Nicks *et al*., 2011), however, results suggest it does maintain DNA integrity better than pectoral-fins and could be used when extracting tissue from frozen specimens - though yield is expected be the relatively low due to freezing conditions (Ross *et al*., 1990) coupled with the relatively small mass of adipose fins. Pectoral-fins should be further tested with alternative storage modes (such as frozen, or perhaps ethanol-frozen) to examine the extent of their usefulness for population genetic studies, particularly with regards to NGS techniques. Progress has been made in DNA extraction techniques from fish scales which may provide another, non-invasive alternative (Kumar *et al*., 2007), and could be tested for their efficacy on salmonid scales.

CHAPTER 2: POPULATION GENETIC ANALYSES OF WESTER ROSS BROWN TROUT (*SALMO TRUTTA*)

2.1: METHODOLOGY

SAMPLING

Sampling within Wester Ross included 35 different sites, all but one were within 15 km of Loch Maree, with Loch Canaird (which is just over 30 km north-east) as the exception. The sample contained 192 Scottish trout, of which 187 were sampled from Wester Ross. Of these, 36 brown trout were from sites around North-West Loch Maree that outflow directly into Poolewe estuary, or the River Ewe which then flows directly into Poolewe. Central-West Loch Maree had 32 brown trout sampled from its tributaries, while 26 trout were sampled from rivers in South-East Loch Maree – both of which drain directly into their respective cardinal points of Loch Maree before outflowing to Poolewe via the River Ewe. Another 35 originate nearby on the West-Coast, coming from sites which drain out to Gairloch estuary. The last 5 brown trout came from Loch Leven (~ 200 km away) which is next to the East-Coast in Kinross, ~ 27 km north of Edinburgh. These groupings constitute the regional populations of brown trout sampled, and are considered to represent reproductive groups caught in their natal watercourses. The last 58 specimens are sea trout, sampled outside their natal streams; 35 were caught heading up the River Ewe, 12 in Flowerdale bay and its outflow, 3 in Poolewe, and 6 from Canaird estuary and its outflow, and another 2 caught in Loch Maree (one sea and one lake trout) (*Table 9 & Figure 17*). Specimens were sampled using a combination of electrofishing, seine netting, sweep netting, fyke netting, and fly fishing techniques.

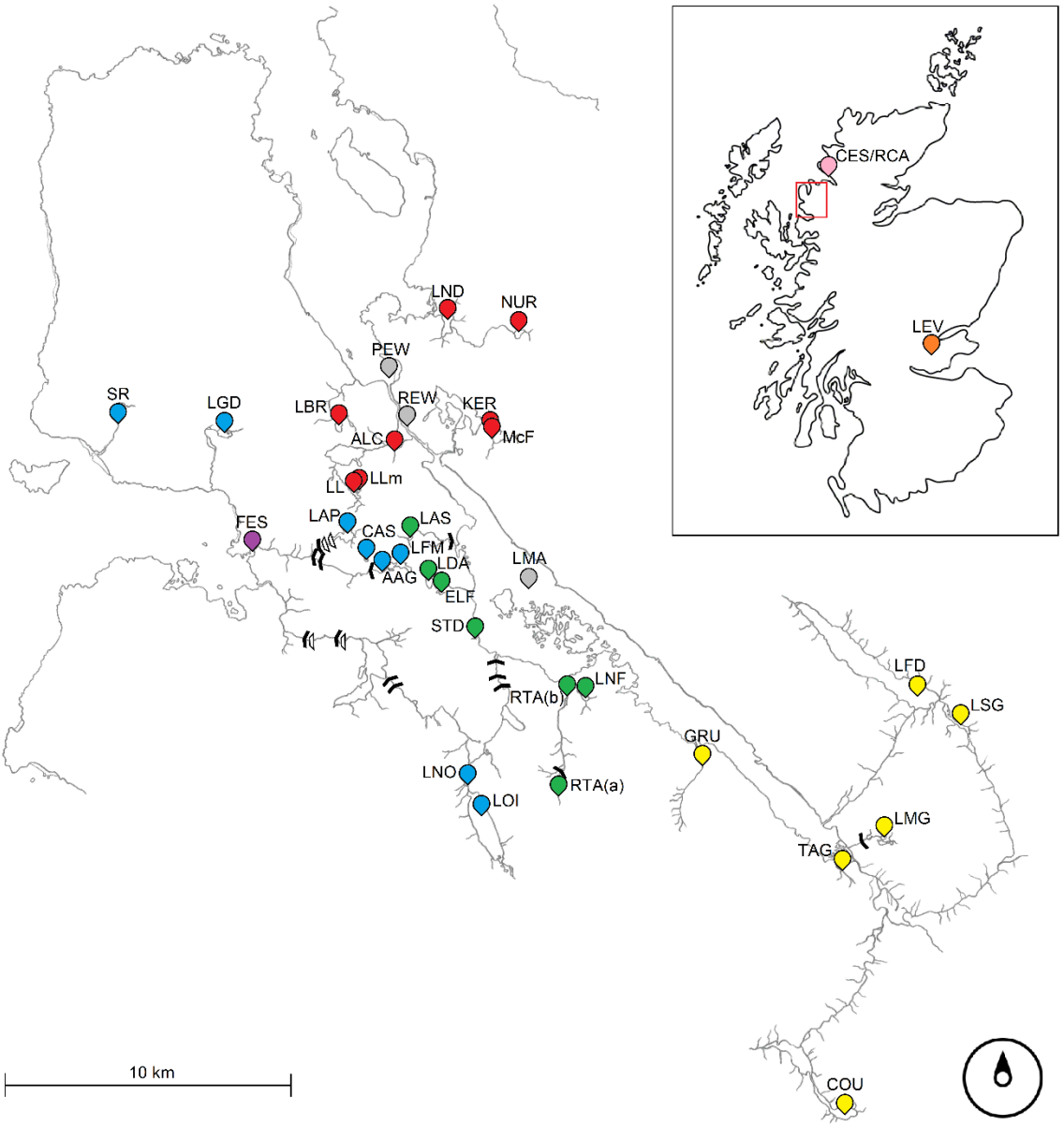

Figure 17 Hand traced line-drawing of Wester Ross study area showing Loch Maree and neighbouring catchments that were sampled. Red icons represent sample sites that drain into NW Loch Maree and Poolewe. Green represents sites that drain into CW Loch Maree. Yellow mark sites draining into SE Loch Maree. Blue sites drain into Gairloch on the West Coast. Purple marks where sea trout were caught in Flowerdale estuary. Grey shows were sea trout were sampled within Poolewe, the Ewe river, and Loch Maree. In the satellite map: Pink marks estuarine Loch Canaird and its outflow. Orange marks Loch Leven on the east coast of Scotland. Significant barriers to fish movement are indicated as: Black chevrons for impassable waterfalls. Black and white trapezia for dams. (Source map: Ordnance Survey acquired from Digimap, Edina).

Table 9 Summary of sites sampled in Loch Maree, its neighbouring catchments, and Loch Leven, site labels, number of specimens genotyped (*n*), morphs present, sampling dates (with *n* sampled in which year if samples were obtained across multiple years, the tissue types used to extract specimen DNA, and sample site GPS coordinates.

#	Sample sites	Label	*n*	Morphs	Sampling dates				Tissue type used	GPS Coordinates	
					M	Years (*n* sampled if across multiple years)				Latitude	Longitude
(1)	**NORTH-WEST LOCH MAREE**		**(36)**								
	Boor catchment										
1	Loch Boor	LBR	5	Brown	7	2012 (1)	2013 (2)	2014 (2)	Frozen muscle	57° 45' 11.40" N	5° 37' 56.22" W
	Tournaig catchment		***7***								
2	Loch nan Dailthean	LND	5	Brown	-	2010			Dried pectoral fins	57° 47' 20.10" N	5° 34' 17.85" W
3	Nursery lochan	NUR	2	Brown	7	2016			Frozen muscle	57° 47' 13.55" N	5° 30' 54.50" W
	Loch Maree catchment		***82***								
	Ewe system		**24**								
4	Burn Allt an Leth-chreige	ALC	5	Brown	7	2014			Frozen muscle	57° 44' 44.53" N	5° 35' 56.90" W
5	Loch Laraig	LL	5	Brown	7	2012			Frozen muscle	57° 43' 58.17" N	5° 37' 20.45" W
6	Loch Laraig "micro"	LLm	4	Brown	7	2012			Frozen muscle	57° 43' 59.30" N	5° 37' 7.03" W
7	Kernsary mainstream	KER	5	Ferox	10	2013			Ethanol adipose fins	57° 45' 13.33" N	5° 32' 37.29" W
8	McFarlane burn	McF	5	Ferox	10	2013			Ethanol adipose fins	57° 45' 5.93" N	5° 32' 31.39" W
(2)	**CENTRAL-WEST LOCH MAREE**		**(32)**								
9	Loch an Aird-sheilg	LAS	5	Brown	9	2009			Ethanol adipose fins	57° 43' 9.14" N	5° 35' 14.18" W
10	Loch Doire na h-Airighe	LDA	5	Brown	7	2012 (4)	2014 (1)		Frozen muscle	57° 42' 17.23" N	5° 34' 29.50" W
11	Elf's Loch	ELF	5	Brown	7	2012 (1)	2014 (4)		Frozen muscle	57° 42' 7.77" N	5° 34' 3.11" W
12	Slattadale burn	STD	3	Brown	10	2013			Ethanol adipose fins	57° 41' 26.22" N	5° 32' 37.62" W
13	River Talladale (above WF)	RTA(a)	5	Brown	7	2015			Frozen muscle	57° 38' 32.51" N	5° 29' 34.82" W
14	River Talladale (below WF)	RTA(b)	4	Brown	7	2015			Dried pectoral fins	57° 40' 15.40" N	5° 29' 25.86" W
15	Loch na Fideil burn	LNF	5	Brown	10	2013			Ethanol adipose fins	57° 40' 28.65" N	5° 29' 8.59" W
	Loch na h-Oidhche outflow	LNO	4	Brown	7	2015			Dried pectoral fins	57° 38' 25.29" N	5° 32' 47.16" W
	Loch na h-Oidhche	LOI	1	Brown	7	2015			Dried pectoral fins	57° 38' 21.31" N	5°32'34.68" W
(3)	**SOUTH-EAST LOCH MAREE**		**(26)**								
16	River Grudie	GRU	5	Brown	10	2013			Ethanol adipose fins	57° 39' 4.53" N	5° 24' 30.88" W
17	Loch Coulin	COU	5	Brown	11	2009 (2)	2011 (3)		Ethanol adipose fins	57° 32' 37.10" N	5° 18' 58.89" W
18	Taagan burn	TAG	2	Brown	10	2013			Ethanol adipose fins	57° 37' 17.01" N	5° 19' 34.02" W
19	Loch Meallan Ghobhar	LMG	5	Brown	7	2015			Frozen muscle	57° 37' 51.87" N	5° 18' 1.84" W
20	Lochan Fada	LFD	5	Brown	7	2014 (2)	2016 (3)		DPF (2), FM (3)	57° 40' 23.41" N	5° 17' 00.72" W
21	Loch an Sgeireach	LSG	4	Brown	7	2014			Frozen muscle	57° 40' 23.37" N	5° 16' 1.67" W

#	Sample sites	Label	*n*	Morphs	Sampling dates						Tissue type used	GPS Coordinates	
					M	Years (*n* sampled if across multiple years)						Latitude	Longitude
(4)	**WEST-COAST, GAIRLOCH**		**(35)**										
	Sands catchment												
22	Sands River	SR	5	Brown	7	2012					Frozen muscle	57° 44' 57.74" N	5° 45' 40.27" W
	Smithstown catchment												
23	Loch a' Gharbh-Doire	LGD	5	Brown	7	2015					DAF (1), DPF (4)	57° 44' 52.34" N	5° 42' 2.64" W
	Flowerdale catchment		***20***										
24	Loch na Feithe Mugaig	LFM	5	Brown	7	2016					Frozen muscle	57° 42' 40.98" N	5° 35' 41.57" W
25	Burn Allt a' Ghlinne	AAG	5	Brown	7	2015					Dried pectoral fins	57° 42' 28.61" N	5° 36' 12.46" W
26	Cassius' Lochan	CAS	5	Brown	7	2010 (3)	2012 (2)				Dried adipose fins	57° 42' 36.59" N	5° 37' 9.39" W
27	Loch Airigh a' Phuil	LAP	5	Brown	7	2012 (1)	2014 (4)				Frozen muscle	57° 43' 10.51" N	5° 37' 28.11" W
	Kerrysdale catchment		***5***										
28	Loch na h-Oidhche outflow	LNO	4	Brown	7	2015					Dried pectoral fins	57° 38' 25.29" N	5° 32' 47.16" W
29	Loch na h-Oidhche	LOI	1	Brown	7	2015					Dried pectoral fins	57° 38' 21.31" N	5° 32' 34.68" W
(5)	**EAST-COAST, LOCH LEVEN**		**(5)**										
	Leven catchment												
30	Loch Leven	LEV	5	Brown	8	2011					Ethanol adipose fins	56° 11' 57.53" N	3° 22' 47.39" W
(6)	**NON-RESIDENT GROUPS**		**(58)**										
	Canaird estuary sea trout		***6***										
31	River Canaird (estuary outflow)	RCA	2	Sea (finnock)	7	2014					Frozen muscle	57° 57' 18.81" N	5° 10' 40.75" W
32	Loch Canaird (estuary)	CES	4	Sea (3)/brown (1)	7	2011 (2)	2013 (1)	2014 (1)			Ethanol adipose fins	57° 56' 39.51" N	5° 10' 59.33" W
	Flowerdale estuary sea trout												
33	Flowerdale estuary	FES	12	Sea	7	2014 (3)	2015 (2)	2016 (7)			DAF (4), EAF (3), FM (5)	57° 42' 43.56" N	5° 40' 43.34" W
	River Ewe sea trout												
34	Poolewe (estuary)	PEW	3	Sea (finnock)	7	2014					Ethanol adipose fins	57° 46' 7.42" N	5° 36' 13.27" W
35	River Ewe (estuary outflow)	REW	35	Sea (finnock)	7	2006 (2)	2012 (1)	2014 (10)	2015 (21)	2016 (2)	DPF (1), EAF (10), DAF (24)	57° 45' 44.15" N	5° 36' 02.23" W
36	Loch Maree	LMA	2	Lake/sea-hybrid?	7	2016	2017				DAF (1), EPF (1)	57° 41' 23" N	5° 27' 27" W

Abbreviations: *n*, number of specimens, parenthesised and **bold** counts measure subsequent totals; M, month of the year/s sampled; within "Tissue type used" column: DAF, dried adipose fins; DPF, dried pectoral fins; EAF, ethanol adipose fins; EPF, ethanol pectoral fins; FM, frozen muscle.

A summary of site information sampled in this study (*Table 9*), which includes Loch Maree, its neighbouring catchments, and Loch Leven. The information displays sample site labels, the number of specimens genotyped (*n*) – also grouped by in bold by catchment (in italic) and region (in parenthesis), the trout morphs present, sampling dates

(with *n* sampled in which year if samples were obtained across multiple years, the tissue types used to extract specimen DNA, and sample site GPS coordinates. Regional and sea trout cohorts have been colour matched with the same groups in *Figures 17, 28 & 29.*

Sample sites were selected based on locality, hydro-connectivity and the presence of specific trout morphs. McFarlane burn and the Kernsary mainstream represent known Ferox habitats (Hughes *et al*., 2016). Neighbouring catchments were selected to investigate any levels of introgression between catchments. Loch Leven trout was once used to stock Loch Maree (WRFT, no date) and was added as an 'outgroup', and to investigate possible prevailing genetic contributions. Tissue type and mode of storage was also considered when selecting specimens per site, prioritising frozen-muscle tissue and ethanol-stored fin clips where available.

MICROSATELLITE ALLELE SCORING

All microsatellite amplicons were sent to the Natural History Museum, London, for analysis on an Applied Biosystems Capillary Array 3130 Series System (© 2004 Applied Biosystems). The raw data was calibrated and alleles scored using Genenious 8.0.5 (© 2005 - 2014 Biomatters Ltd.) and its associated microsatellite plugin, following its tutorial (Biomatters, 2018) and guided by the BTMP allele scorin g notes (Keenan *et al*., 2013) resulting in the alleles table found in *Appendix 3*; additional details pertinent to allele scoring are provided in *Appendix 2*.

F–STATISTICS

Intra-population statistics such as allelic richness (A_R), expected (H_E) and observed heterozygosities (H_O), and inter-population stats such as G_{ST} and D_{ST} was calculated using POPTREEW - a web-based software package for calculating population statistics, genetic distances, and to construct population dichotomous trees (Takezaki, Nei, and Tamura, 2014, 2010). Allele frequencies were calculated using Arlequin v3.5.2.2 (© 2015 L. Excoffier).

UNIQUE AND SHARED ALLELES

Unique alleles found within and shared between populations were identified using Venny 2.1 (Oliveros, 2007-2015).

DEPARTURES FROM HARDY–WEINBERG EQUILIBRIUM

Significant departures from Hardy-Weinberg equilibrium were tested for using Arlequin v3.5.2.2 (© 2015 L. Excoffier).

INTER–POPULATION GENETIC DIVERGENCE BY DISTANCE

Mantel tests were performed between inter-population pairwise genetic distance, and geo-hydrological distances between populations using GenAlEx v6.5 (Peakall and Smouse, 2012, 2006) (a plugin for Excel © 2010 Microsoft). Observed D_{ST} is reported for comparisons between samples of similar sizes, while corrected results are cautiously reported for biased comparisons.

HEADWATER POPULATIONS & BARRIERS TO MOVEMENT

In order to assess whether physical barriers had a significant effect on the population structure of the wild trout around Loch Maree, sample sites above and below barriers had their genetic diversity (allelic richness and expected heterozygosities) statistically compared. Sample sizes were balanced by random selection of specimens from larger samples to match group size of available data from smaller samples (*Figures 22-26*).

MUTATION–DRIFT EQUILIBRIUM

Mutation-drift equilibrium was statistically tested for using software Bottleneck v1.2.02 (Cornuet and Luikart, 1999).

POPULATION TREE

Neighbour joining trees were constructed from D_{ST} values using POPTREEW (Takezaki, Nei, and Tamura, 2014, 2010).

STATISTICS

Statistical analyses (e.g. correlational, differential analysis) of data was performed using Minitab® 17.2.1 (© 2013, 2015 Minitab Inc.). Pearson's correlation, one-way ANOVA, 2-sample t-tests were used to analyse parametric data, and Spearman's rank correlation and Mann-Whitney tests were used to analyse non-parametric data. All data sets were tested for normality and equal variances before any tests for difference or association were conducted.

All statistical tests on all platforms were conducted with a significance α 0.05. Precise P-values are presented for possible interpretations (Greenland *et al*., 2016); unless describing multiple *p,* then p_s; or if p 0.0000, then $p < 0.0001$. 95% Confidence Interval (CI) ranges are also presented with significant results for differences. Test statistics will also be presented for significant results (e.g. *f* stats).

Bonferroni Corrections

Bonferroni correction was not initially applied to the study's statistical analysis. The routine use of bonferroni corrections has been criticised and it was deemed not necessary as this study's data analysis was exploratory, not confirmative; it was not imperative to avoid type 1 errors; and there was no single test of a universal null hypothesis that all tests are not significant (Armstrong, 2014).

Nonetheless the corrections were made and explored post-hoc.

GEOGRAPHIC ANALYSIS

Geographic information such as geo-hydrological distances and the presence of hydrological barriers, were gathered using Google Earth Pro v7.3.1.4507 (© 2018 Google Inc.) and Ordnance Survey Maps, provided by Digimap, Edina (© The University of Edinburgh) (© Crown Copyright and Database Right 2018. Ordnance Survey Digimap Licence).

ANALYTICAL SCOPE AND SAMPLE POOLING STRATEGY

Regional groups were defined by the location of their natal river outflows to either the sea, or into cardinal regions of Loch Maree (for North-West, Central-West, and South-East Loch Maree). All sites which outflow in close proximity were considered a region with < 10 km of hydrological distance between the two furthest outflows in all regional groups (*Figure 17*). All sample sites within each region where then pooled into the defined regional sample groups.

PRINCIPLE COMPONENT ANALYSIS (POST–HOC)

A Principle Component Analysis (PCA) was conducted post hoc using the software R v3.4.1 (© 2017 The R Foundation for Statistical Computing) to investigate individual sample relationships in relation to their geographic origin, as well as the discriminatory power of the microsatellite markers.

To do so, the “hierfstat” package (Goudet, 2004) was downloaded and installed from secure CRAN mirror: UK (London 1) [https]. Once the hierfstat package was loaded onto a workspace, the following commands were used in the following order to execute the analysis:

1. test<-read.fstat("***File name***", na.s = c("0", "00", "000", "0000", "00000", "000000", "NA"))
2. x<-indpca(test)
3. plot(x)

The distribution of individuals of each sample group population were plotted (*Figures 28 & 29*).

2.2: RESULTS

ALLELE SCORING

Out of the 3 456 scoring attempts 3 432 (99.3 %) were scored successfully. Twelve specimens were homozygotes for a null allele at one locus, nine of these were found at Locus 5 (*m*One108), one on Locus 6 (*SSa*D170) and two on Locus 7 (*Sasa*-UBA); no other ambiguous traces were found (*Table 10*).

Table 10 Number of alleles found in and outside of expected size ranges, and number null alleles found across the trout 192 specimens genotyped.

#	Locus	*n* genotyped	A_R	Null *A*	*n* outside range	*A* outside	Expected SR (bp)	Expanded SR (bp)
1	*Str3*QUB	192	9	0	4	1	115–175	115-185
2	*Ssa*407UoS	192	38	0	-	-	204–320	-
3	CA054565a	192	7	0	2	1	101–120	101-121
4	CA060177	192	16	0	5	4	234–315	234-340
5	m*One*108	183	36	9	-	-	371–518	-
6	*Ssa*D170	191	25	1	45	9	148–217	123-258
7	*Sasa*-UBA	190	29	2	-	-	268–523	-
8	m*One*101	192	7	0	-	-	155–194	-
9	*Ssa*413UoS	192	12	0	2	2	225–282	220-282
	Total:	**1716**	**179**	**12**	**58**	**17**		
	Mean:	**190.67**	**19.89**	**1.33**	**11.60**	**3.40**		

Abbreviations: *n*, number of specimens successfully genotyped; A_R found, number of different alleles found; Null *A*, number of alleles that failed to amplify; *n* outside range; number of specimens with an allele outside the expected size range; *A* outside range, number of alleles found outside expected size range; Expected SR (bp), the size range observed by Keenan *et al*. (2015) in base-pair length; Expanded SR (bp), the expanded size ranges made in this study.

A few samples with weak signals could still be scored when their traces were viewed at maximum scale/resolution (e.g. LLm16, *Figure 16*). The expected size range was expanded for five out of nine loci to allow scoring of novel alleles, determined if a locus in question would otherwise be null and if there was at least a second allele beyond the same range. Alleles outside the expected size range were most common on Locus 6 - *Ssa*D170 with 11.7% (45/384) found outside; at the other four loci < 2 % were found outside expected ranges (*Table 10*).

INTRA–POPULATION GENETIC DIVERSITY

Allelic richness was similar between Loch Maree cardinal populations (NWLM, CWLM, and SELM) with an average mean allelic richness of 10.0 ± 0.4 SD, and is similar to Gairloch resident trout (10.7 ± 1.8 SE) (*Table 11*).

Table 11 Mean allelic richness, multi-locus H_E & H_O, and mean error, for regional populations of *S. trutta* in Wester Ross & Loch Leven, and at varying coalesced geographic ranges.

Population	*n*	A_R		H_E		H_O	
		Mean	SE	Mean	SE	Mean	SE
NW Loch Maree	36	10.0	2.3	0.650	0.106	0.641	0.104
CW Loch Maree	32	10.4	2.4	0.622	0.105	0.612	0.104
SE Loch Maree	26	9.6	2.3	0.642	0.098	0.629	0.096
W Coast Gairloch	35	10.7	1.8	0.710	0.079	0.699	0.078
Ewe ST*	40	15.0	2.8	0.770	0.069	0.761	0.069
Flowerdale ST	12	6.7	1.2	0.672	0.095	0.644	0.091
Canaird ST**	6	5.2	1.1	0.655	0.116	0.600	0.107
E Coast Leven	5	4.7	0.6	0.734	0.094	0.658	0.084
Loch Maree residents*	**94**	**14.1**	**3.5**	**0.669**	**0.103**	**0.666**	**0.103**
Wester Ross residents**	**129**	**16.6**	**3.7**	**0.696**	**0.098**	**0.693**	**0.097**
Wester Ross sea trout***	**58**	**16.0**	**3.2**	**0.752**	**0.076**	**0.745**	**0.075**
All of Wester Ross****	**187**	**19.4**	**4.2**	**0.717**	**0.090**	**0.715**	**0.090**
All residents*****	**134**	**17.1**	**3.7**	**0.701**	**0.097**	**0.698**	**0.097**
All specimens	**192**	**19.8**	**4.1**	**0.720**	**0.090**	**0.718**	**0.090**

Abbreviations: A_R, allelic richness; heterozygosities: H_E, expected; H_O, observed; *n*, sample size; SE, standard error.

*Ewe ST (EST) combines River Ewe (REW) & Poolewe (PEW) sea trout, and the two lake trout caught in Loch Maree (LMA).

** Canaird ST (CST) include the sea trout caught in Loch Canaird estuary (CES) and its outflowing river (RCA).

***Loch Maree residents** are all the resident trout caught in NW, CW, and SE Loch Maree (NWLM, CWLM, SELM).

****Wester Ross residents** describe all the residents sampled in Wester Ross (all of Loch Maree and Gairloch),

*****WR sea trout** group the sea trout from Flowerdale (FES), Ewe (REW, PEW, and LMA) and Canaird (CES, RCA).

******All of Wester Ross** collate all of its resident and sea trout.

*******All residents** coalesce Wester Ross and Leven residents.

Means and SE were calculated across all nine loci for each population (*Tables 12-14*).

No significant difference was observed between the Loch Maree cardinal populations (One-way ANOVA, *p* 0.965, *f* 0.04), or with Gairloch resident populations (*p* 0.985, *f* 0.05). The highest richness seen at regional level was in the Ewe sea trout at 15.0 ± 2.8 SE, and was still greater than the Loch Maree regional populations when coalesced despite differences in sample sizes (14.1 ± 3.5 SE) (*Table 11*). This was also observed at higher ranges; Wester Ross sea trout A_R was 16.0 ± 3.2 SE (*n* 58), which was greater than the WR residents when sample bias was removed (13.0 ± 2.9 SE) (*n* 58). Though this difference was not found statistically significant (*p* 0.486), suggesting genetic diversity at the allelic level is similar between WR residents and sea trout. When all the WR trout (resident and anadromous) are grouped as one population (*n* 187) the combined richness reaches 19.4 ± 4.2 SE, suggesting the presence of, on average, three alleles per loci between the resident and anadromous populations that are also not shared – suggesting the sample range and/or sample size may have been insufficient in locating the origins of these sea trout alleles. Despite the small sample, when Leven trout (*n* 5) was grouped with all specimens (*n* 192), allelic richness reaches 19.8 ± 4.1 SE, brought by the addition of a Leven-unique allele at loci 5, 8, and 9 (*Table 12*).

Mean A_R across all loci across all populations was 9.0 ± 1.8 SE and ranged between 2.5 ± 0.7 SE (Locus 3 – Ca054565a) and 15.6 ± 2.2 SE (Locus 2 - *Ssa*407UoS) tied with 15.6 ± 2.6 SE (Locus 5 - *m*One108). At population level it ranged between 4.7 ± 0.6 SE (Loch Leven) and 15.0 ± 2.8 SE (Ewe sea trout). Mean A_R at locus level across all specimens in the study was 19.8 ± 4.1 SE, and ranged between 7 (Locus 3 CA054565a tied with Locus 8 *m*One101) and 36 (Locus 5 *m*One108) (*Table 12*).

Normality tests showed that allelic richness was normally distributed (or Gaussian distributed) across all nine loci in all but one population – the Gairloch residents, suggesting brown trout around Gairloch may have influences on their allele distribution (*Appendix 6.9*).

Table 12 Locus allelic richness (A_R) for populations of *S. trutta* of Wester Ross & Loch Leven, and at varying geographic ranges.

Population	*n*	Locus 1 *Str3*QUB	Locus 2 *Ssa*407UoS	Locus 3 CA054565a	Locus 4 CA060177	Locus 5 m*One*108	Locus 6 *Ssa*D170	Locus 7 *Sasa*-UBA	Locus 8 m*One*101	Locus 9 *Ssa*413UoS
NW Loch Maree	36	4	18	1	7	19	16	15	4	6
CW Loch Maree	32	6	24	3	8	18	15	12	4	4
SE Loch Maree	26	4	17	1	8	20	14	14	4	4
W Coast Gairloch	35	5	15	5	12	16	16	16	5	6
Ewe ST*	40	9	23	6	14	28	22	20	5	8
Flowerdale ST	12	4	10	2	5	11	10	10	5	3
Canaird ST**	6	3	11	1	5	8	9	4	4	2
E Coast Loch Leven	5	3	7	1	4	5	5	6	6	5
Mean:		4.8	15.6	2.5	7.9	15.6	13.4	12.1	4.6	4.8
SE:		0.7	2.2	0.7	1.2	2.6	1.9	1.9	0.3	0.7
LM residents*	**94**	**7**	**31**	**3**	**9**	**27**	**19**	**20**	**5**	**6**
WR residents**	**129**	**8**	**34**	**6**	**13**	**30**	**21**	**24**	**5**	**8**
WR sea trout***	**58**	**9**	**26**	**6**	**14**	**31**	**24**	**20**	**6**	**8**
All of WR****	**187**	**9**	**38**	**7**	**15**	**35**	**25**	**29**	**6**	**11**
All residents*****	**134**	**8**	**34**	**6**	**13**	**31**	**22**	**25**	**6**	**9**
All specimens	**192**	**9**	**38**	**7**	**15**	**36**	**25**	**29**	**7**	**12**

*Footnotes match *Table 11.*

Mean H_E ranged from 0.622 ± 0.105 SE (CW Loch Maree) to 0.770 ± 0.069 SE (Ewe sea trout) and showed no significant difference between resident populations of Loch Maree (*p* 0.981, *f* 0.02), or with the Gairloch residents (*p* 0.929, *f* 0.15), indicating the level of intra-population genetic diversity is similar between the Wester Ross brown trout populations (*Table 13*). The same is true when the Ewe and Flowerdale sea trout are also compared together with the resident trout (*p* 0.885, *f* 0.34).

Mean H_E across all nine loci within all regional populations is 0.682 ± 0.095 SE, with a range between 0.100 ± 0.050 SE (Locus 3 – CA054565a) and 0.924 ± 0.009 SE (Locus 5 – *m*One108) (*Table 13*).

Table 13 Locus expected heterozygosities (H_E) for populations of *S. trutta* of Wester Ross & Loch Leven, and at varying geographic ranges.

Population	*n*	Locus 1 *Str3*QUB	Locus 2 *Ssa*407UoS	Locus 3 CA054565a	Locus 4 CA060177	Locus 5 m*One*108	Locus 6 *Ssa*D170	Locus 7 *Sasa*-UBA	Locus 8 m*One*101	Locus 9 *Ssa*413UoS
NW Loch Maree	36	0.597	0.910	0.000	0.793	0.945	0.879	0.842	0.320	0.563
CW Loch Maree	32	0.490	0.952	0.092	0.774	0.915	0.835	0.837	0.205	0.496
SE Loch Maree	26	0.544	0.874	0.000	0.574	0.936	0.914	0.826	0.575	0.533
W Coast Gairloch	35	0.633	0.863	0.238	0.864	0.913	0.907	0.875	0.475	0.619
Ewe ST*	40	0.694	0.949	0.388	0.865	0.959	0.945	0.922	0.534	0.675
Flowerdale ST	12	0.562	0.906	0.083	0.627	0.888	0.924	0.906	0.736	0.420
Canaird ST**	6	0.591	0.985	0.000	0.727	0.939	0.939	0.773	0.773	0.167
E Coast Loch Leven	5	0.711	0.911	0.000	0.778	0.893	0.822	0.889	0.778	0.822
Mean:		0.603	0.919	0.100	0.750	0.924	0.896	0.859	0.550	0.537
SE:		0.026	0.015	0.050	0.037	0.009	0.016	0.017	0.075	0.068
LM residents*	**94**	**0.568**	**0.938**	**0.032**	**0.770**	**0.947**	**0.914**	**0.878**	**0.389**	**0.587**
WR residents**	**129**	**0.612**	**0.938**	**0.091**	**0.815**	**0.954**	**0.917**	**0.901**	**0.412**	**0.625**
WR sea trout***	**58**	**0.658**	**0.953**	**0.299**	**0.823**	**0.956**	**0.943**	**0.929**	**0.610**	**0.595**
All of WR****	**187**	**0.632**	**0.945**	**0.157**	**0.814**	**0.955**	**0.926**	**0.919**	**0.480**	**0.626**
All residents*****	**134**	**0.618**	**0.939**	**0.088**	**0.815**	**0.954**	**0.921**	**0.902**	**0.440**	**0.633**
All specimens	**192**	**0.636**	**0.945**	**0.154**	**0.814**	**0.954**	**0.928**	**0.919**	**0.497**	**0.632**

*Footnotes match *Table 11*.

Mean H_O across all nine loci across within regional resident populations was 0.645 ± 0.018 SE, ranging from 0.612 ± 0.104 SE (CW Loch Maree) to 0.699 ± 0.078 SE (Gairloch) (*Table 14*).

Table 14 Loci observed heterozygosities (H_O) for populations of *S. trutta* of Wester Ross & Loch Leven, and at varying geographic ranges.

Population	*n*	Locus 1 *Str3*QUB	Locus 2 *Ssa*407UoS	Locus 3 CA054565a	Locus 4 CA060177	Locus 5 m*One*108	Locus 6 *Ssa*D170	Locus 7 *Sasa*-UBA	Locus 8 m*One*101	Locus 9 *Ssa*413UoS
NW Loch Maree	36	0.589	0.898	0.000	0.782	0.932	0.867	0.830	0.316	0.555
CW Loch Maree	32	0.482	0.938	0.090	0.762	0.901	0.822	0.823	0.202	0.488
SE Loch Maree	26	0.533	0.857	0.000	0.563	0.918	0.896	0.810	0.564	0.523
W Coast Gairloch	35	0.624	0.850	0.234	0.852	0.897	0.894	0.863	0.468	0.610
Ewe ST*	40	0.685	0.937	0.383	0.854	0.947	0.933	0.911	0.528	0.667
Flowerdale ST	12	0.538	0.868	0.080	0.601	0.851	0.885	0.868	0.705	0.403
Canaird ST**	6	0.542	0.903	0.000	0.667	0.861	0.861	0.708	0.708	0.153
E Coast Loch Leven	5	0.640	0.820	0.000	0.700	0.781	0.740	0.800	0.700	0.740
Mean:		0.579	0.884	0.098	0.723	0.886	0.862	0.827	0.524	0.517
SE:		0.024	0.015	0.050	0.038	0.019	0.021	0.021	0.067	0.064
LM residents*	**94**	**0.565**	**0.933**	**0.032**	**0.766**	**0.942**	**0.909**	**0.873**	**0.387**	**0.583**
WR residents**	**129**	**0.610**	**0.934**	**0.090**	**0.812**	**0.950**	**0.914**	**0.898**	**0.410**	**0.622**
WR sea trout***	**58**	**0.652**	**0.945**	**0.296**	**0.816**	**0.948**	**0.934**	**0.920**	**0.605**	**0.590**
All of WR****	**187**	**0.630**	**0.943**	**0.157**	**0.812**	**0.952**	**0.924**	**0.916**	**0.479**	**0.624**
All residents*****	**134**	**0.616**	**0.935**	**0.088**	**0.812**	**0.950**	**0.917**	**0.898**	**0.438**	**0.631**
All specimens	**192**	**0.634**	**0.943**	**0.154**	**0.812**	**0.952**	**0.925**	**0.917**	**0.496**	**0.630**

*Footnotes match *Table 11*.

Differences between H_E and H_O are negligible and is consistently similar at all population ranges (max difference estimated within WR residents was 2.02% or 0.013 for SE Loch Maree), with larger differences attributed to smaller sample sizes (e.g. Loch Leven and Canaird ST). The differences also decreased to < 1.0 % when populations were grouped at higher ranges (*Table 11*).

UNIQUE/SHARED ALLELES AND ALLELIC FREQUENCIES

The proportions of unique alleles within and shared between the WR brown trout populations are illustrated (*Figure 18*).

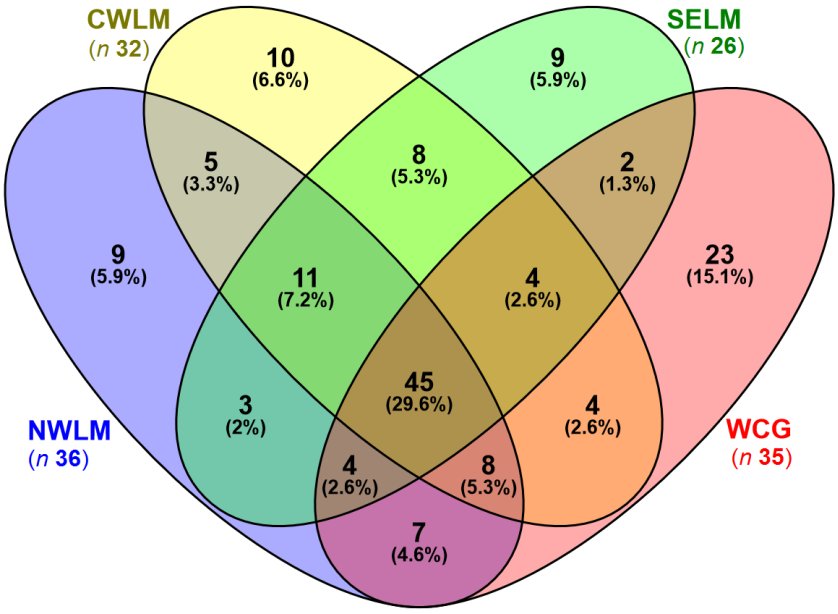

Figure 18 The proportions of alleles uniquely found and shared between the Wester Ross resident populations.

Allele frequencies for the 45 alleles shared between all populations are presented below (*Table 15*). Frequencies are calculated out of 384 possible positions per locus in 192 specimens. Full list of allele frequencies found unique and shared between resident populations are displayed in *Appendix 4.*

Table 15 Alleles and their frequencies found shared between the WR resident populations of brown trout. Frequencies ≥ 0.1 are shaded the same colour every 0.1 increment higher, frequencies < 0.1 are not shaded.

A(Locus)	NWLM	CWLM	SELM	WCG	*A*(Locus)	NWLM	CWLM	SELM	WCG
132(1)	0.319	0.031	0.038	0.443	471(5)	0.069	0.063	0.019	0.043
160(1)	0.542	0.688	0.635	0.414	174(6)	0.042	0.016	0.096	0.057
172(1)	0.125	0.203	0.231	0.071	178(6)	0.125	0.031	0.019	0.029
242(2)	0.097	0.047	0.077	0.086	182(6)	0.056	0.266	0.038	0.043
246(2)	0.028	0.094	0.115	0.071	186(6)	0.278	0.125	0.038	0.186
254(2)	0.014	0.016	0.038	0.014	190(6)	0.097	0.031	0.154	0.071
262(2)	0.042	0.063	0.115	0.057	194(6)	0.056	0.281	0.077	0.100
270(2)	0.083	0.031	0.019	0.071	198(6)	0.042	0.016	0.019	0.043
274(2)	0.194	0.016	0.019	0.029	202(6)	0.042	0.016	0.058	0.143
278(2)	0.111	0.016	0.019	0.014	206(6)	0.028	0.031	0.173	0.029
113(3)	1.000	0.953	1.000	0.871	210(6)	0.014	0.016	0.096	0.014
262(4)	0.292	0.391	0.154	0.143	292(7)	0.014	0.016	0.038	0.014
266(4)	0.125	0.078	0.058	0.171	296(7)	0.333	0.328	0.096	0.100
270(4)	0.306	0.188	0.635	0.129	306(7)	0.069	0.094	0.038	0.029
274(4)	0.125	0.016	0.077	0.243	307(7)	0.028	0.156	0.038	0.014
278(4)	0.028	0.188	0.019	0.086	309(7)	0.028	0.078	0.385	0.071
286(4)	0.056	0.016	0.019	0.029	169(8)	0.069	0.016	0.038	0.071
423(5)	0.069	0.016	0.077	0.043	177(8)	0.083	0.063	0.058	0.057
427(5)	0.042	0.094	0.019	0.014	181(8)	0.819	0.891	0.558	0.714
431(5)	0.056	0.016	0.019	0.014	230(9)	0.319	0.656	0.654	0.200
443(5)	0.111	0.063	0.077	0.100	233(9)	0.583	0.281	0.173	0.571
463(5)	0.042	0.016	0.038	0.014	260(9)	0.014	0.047	0.135	0.143
467(5)	0.014	0.031	0.077	0.014					

DEPARTURES FROM HARDY–WEINBERG EQUILIBRIUM

Significant deviations from Hardy-Weinberg equilibrium were observed in 39 out of 68 tests over all nine loci for all eight populations, minus a monomorphic Locus 3 (*CA054565a*) in four populations (*Table 16*).

Table 16 Proportions of loci genotyped under Hardy-Weinberg equilibrium in resident and anadromous populations of *S. trutta* in Wester Ross (and Loch Leven, east coast) residents.

Population	*n*	HWE	HWE %
NW Loch Maree	36	2/8*	25.00%
CW Loch Maree	32	5/9	55.60%
SE Loch Maree	26	4/8*	50.00%
Gairloch	35	1/9	11.10%
Ewe sea trout**	40	5/9	55.60%
Flowerdale sea trout	12	7/9	77.80%
Canaird sea trout***	6	7/8*	87.50%
Loch Leven	5	8/8*	100.00%

*Locus 3 is monomorphic and is discounted.

**Ewe sea trout (EST) collate River Ewe (REW) & Poolewe (PEW) sea trout, and the lake trout caught in Loch Maree (LMA).

*** Canaird sea trout (CST) include the sea trout caught in Loch Canaird estuary (CES) and its outflowing river (RCA).

The number of loci under HWE and their proportions vary between resident and anadromous populations. P-values for each test are tabulated in *Appendix 5.1*.

POPULATION DIFFERENTIATION

Mean G_{ST} values (based on H_O across all nine loci and populations) for WR trout at varying geographic ranges was analysed to assess how levels of genetic differentiation changed with increasing range and the inclusion of anadromous populations (*Table 17*). This shows the highest amount of differentiation was observed between the WR sea trout (total G_{ST} 0.285 ± 0.007 SE) and was almost equal to the level of differentiation seen within the Loch Maree resident populations (0.284 ± 0.006 SE) (*p* 0.001).

Table 17 Mean G_{ST} values across 9 microsatellite loci at various geographic and population ranges.

Populations	*n* pop'	*n*	*k*	G_{ST}	SE
Loch Maree residents*	3	94	9	0.284	0.006
Wester Ross residents**	4	129	9	0.246	0.006
Wester Ross sea trout***	3	58	9	0.285	0.007
All of Wester Ross****	7	187	9	0.190	0.011
All residents*****	5	134	9	0.242	0.012
All specimens	8	192	9	0.192	0.013

Abbreviations: *n* pop', number of subpopulations; *n*, number of specimens, *k*, number of loci; SE, standard error.

*Loch Maree residents are all the resident trout caught in NW, CW, and SE Loch Maree (NWLM, CWLM, SELM).

**Wester Ross residents are all the residents sampled in Wester Ross (all of Loch Maree and Gairloch).

***WR sea trout group the sea trout from Flowerdale (FES), Ewe (REW, PEW, and LMA) and Canaird (CES, RCA).

****All of Wester Ross collate all of its resident and sea trout.

*****All residents include Wester Ross and Leven residents.

When Gairloch was grouped with LM residents (in WR residents), total G_{ST} decreased significantly to 0.246 ± 0.006 SE (*p* 0.002), which suggests the Gairloch residents share genetic similarities with LM populations. When the WR residents and sea trout were analysed together (all of Wester Ross), the total G_{ST} found within Wester Ross decreased further to 0.190 ± 0.011 SE (*p* 0.0047), which makes sense if a significant proportion of the sea trout originated from the same resident populations. Wester Ross sea trout were also significantly more differentiated within than the WR resident trout (*p* 0.0023), reinforcing earlier suggestions that the sea trout are a more genetically diverse group than the resident trout in Wester Ross. When Loch Leven resident trout (*n* 5) are included with other residents (all residents), G_{ST} significantly decreases from 0.246 ± 0.006 SE (WR residents) to 0.242 ± 0.012 SE (All residents) (*p* 0.0134), however when Leven trout were included with all of Wester Ross (0.190 ± 0.011 SE), no significant difference was found (*p* 0.8253) (0.192 ± 0.013 SE for all specimens) suggesting Loch Leven may have a closer resemblance to the residents of Wester Ross, and not so much the sea trout.

INTER-POPULATION GENETIC DIVERGENCE BY DISTANCE

Mantel tests showed a significant positive correlation between population pairwise genetic distances (measured in D_{ST}) and between population geo-hydro distances (km) for WR resident population ranges (*Table 18*).

Table 18 Mantel tests between inter-population geo-hydro distances (km) and population pairwise genetic distances (D_{ST} – corrected and observed).

Populations included	*n*	matrix *n*	D_{ST}	*p*	*r*
Loch Maree* & Gairloch	4	6	Observed	***0.101***	0.867
Loch Maree, Gairloch, & Canaird**	5	10	Observed	0.014	0.732
Loch Maree, Gairloch, & Loch Leven	5	10	Corrected	0.008	0.804
Loch Maree, Gairloch, Canaird, & Loch Leven	6	15	Corrected	0.002	0.713
Loch Maree, Gairloch, EST, & FST	6	15	Observed	***0.135***	0.324
Loch Maree, Gairloch, Canaird, EST, & FST	7	21	Corrected	***0.238***	0.208
Loch Maree, Gairloch, Loch Leven, EST, & FST	7	21	Corrected	0.019	0.772
Loch Maree, Gairloch, Canaird, Loch Leven, EST, & FST	8	28	Corrected	0.017	0.705

Abbreviations: *n,* number of populations included; matrix *n*, number of cells in matrices compared.

All mantel tests were run with 9999 permutations.

*Loch Maree includes the three NW, Central-W, and SE populations (NWLM, CWLM, SELM).

**Canaird includes the sea trout and brown trout caught in river Canaird (RCA) and its estuary Loch Canaird (CES).

Relationships were significant between all resident trout population ranges (*Ps* < 0.05*)*, with significance rising with increasing *n* (increasing matrix *n* or data points exponentially), and a consistent trend (mean *r* 0.779 ± 0.070 SD), except for when the range covered only Loch Maree and Gairloch populations (*p* 0.101, *r* 0.867, *n* population 4) (*Table 18*).

Table 19 Inter-population pairwise D_{ST} (corrected) values between regional populations of *S. trutta* in Wester Ross, based on a custom BTMP panel comprising 9 microsatellite loci.

Populations	*n*	NWLM	CWLM	SELM	WCG	ECLL	FST	EST	CST
NW Loch Maree	36	-	0.095	0.192	0.070	0.263	0.162	0.058	0.210
CW Loch Maree	32		-	0.141	0.212	0.379	0.132	0.045	0.133
SE Loch Maree	26			-	0.307	0.321	0.073	0.086	0.108
W Coast Gairloch	35				-	0.352	0.268	0.150	0.312
E Coast Loch Leven	5					-	0.253	0.309	0.228
Flowerdale ST	12						-	0.073	0.026
Ewe ST*	40							-	0.097
Canaird ST**	6								-

*Ewe ST (EST) include the River Ewe (REW) & Poolewe (PEW) sea trout, and the two lake trout caught in Loch Maree (LMA).

** Canaird ST (CST) contains the finnock caught in Loch Canaird estuary (CES) and its outflowing river (RCA).

A relatively low pairwise D_{ST} was observed between the Gairloch and NW Loch Maree residents for the distance between them (D_{ST} 0.070) (*Table 19*); however as data points increase this relationship has little effect on the overall trend (*Figure 19*).

Table 20 Geo-hydrological distances (km) between regional populations of *S. trutta* in Wester Ross.

Populations	*n*	NWLM	CWLM	SELM	WCG	ECLL	FST	EST	CST
NW Loch Maree	36	-	27.9	67.5	75.0	882.8	75.0	5.0	120.8
CW Loch Maree	32		-	60.4	102.9	910.7	102.9	22.9	143.7
SE Loch Maree	26			-	170.5	978.2	170.5	67.5	188.3
W Coast Gairloch	35				-	957.8	5.9	75.0	195.8
E Coast Loch Leven	5					-	957.8	882.8	762.0
Flowerdale ST	12						-	43.5	195.8
Ewe ST*	40							-	120.8
Canaird ST**	6								-

*Footnotes match *Table 19. M*easurements were made using Google Earth (© 2018 Google Inc.).

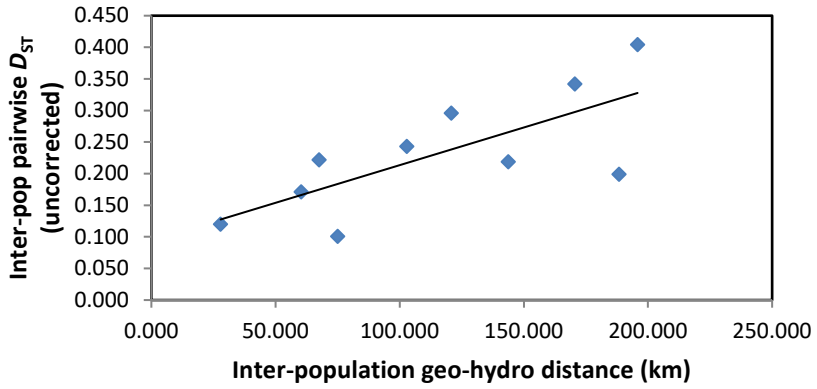

Figure 19 Scatterplot between geo-hydro distances and pairwise D_{ST} (observed) between the Loch Maree, Gairloch and Canaird residents, (n = 5; NWLM, CWLM, SELM, WCG, CST), p 0.014, r 0.732, at 9999 permutations.

When the sea trout populations are included in the tests with the WR residents, the results showed no significance or trend (p_s > 0.135, r_s < 0.325, e.g. *Figure 20*).

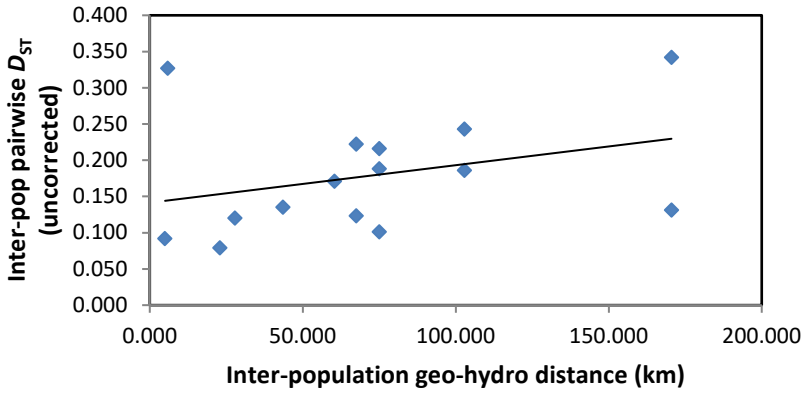

Figure 20 Mantel plot between geo-hydro distances and pairwise D_{ST} (observed) between the WR resident trout, and sea trout populations (n = 6; NWLM, CWLM, SELM, WCG, EST, FST), p 0.135, r 0.324, at 9999 permutations.

The trend is observed again when Loch Leven is added to the matrices (*Figure 21*).

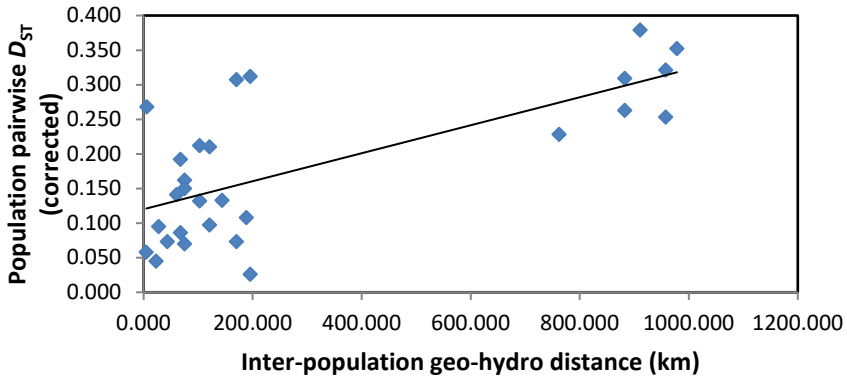

Figure 21 Mantel scatterplot between geo-hydro distances and pairwise D_{ST} (corrected) between all populations sampled (n = 8; NWLM, CWLM, SELM, WCG, CST, EST, FST, ECLL), p 0.017, r 0.705, at 9999 permutations.

HEADWATER POPULATIONS & BARRIERS TO MOVEMENT

When populations at sample sites above barriers (LAS, RTA(*a*), LMG, LFM, CAS, LAP, and LNO & LOI) were compared with sites downstream (*Figures 22-26*), a significant difference was observed between above and below barriers allelic richness and heterozygosity, with downstream populations having a greater genetic diversity than populations isolated above barriers (p_s 0.001).

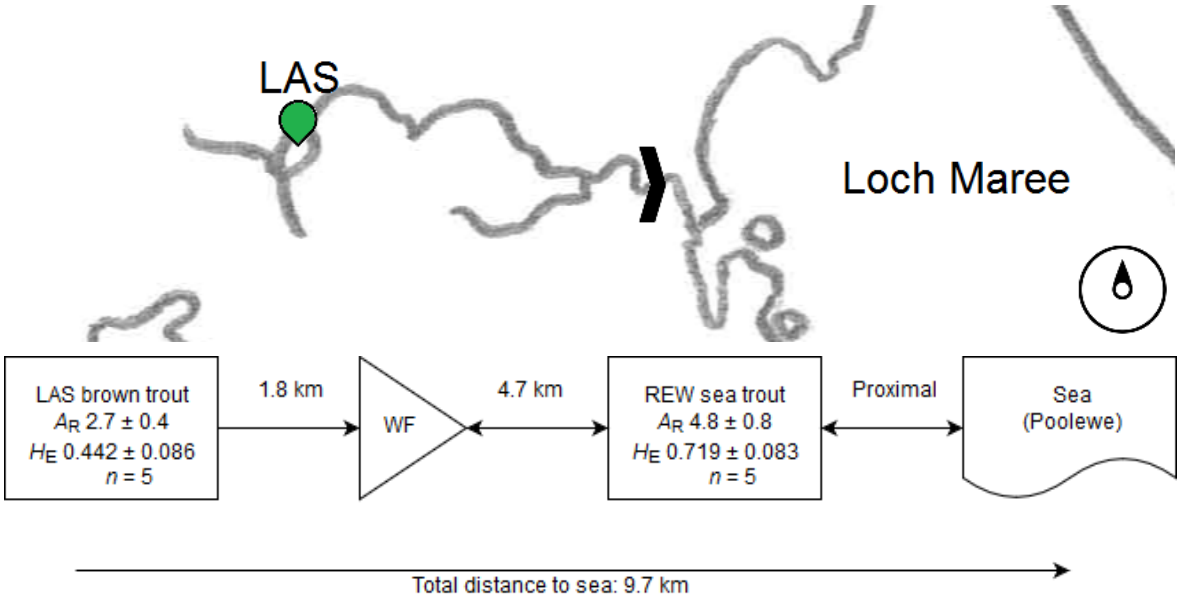

Figure 22 Line-map and flow diagram of Loch an Aird-sheilg (LAS) headwater population and downstream post-barrier comparison with sea trout caught in the River Ewe (REW), indicating the presence of barriers of distances between.

A highly negative correlation was observed between genetic diversity (A_R and H_E) and distance to sea (p_s < 0.002, r_s -0.753).

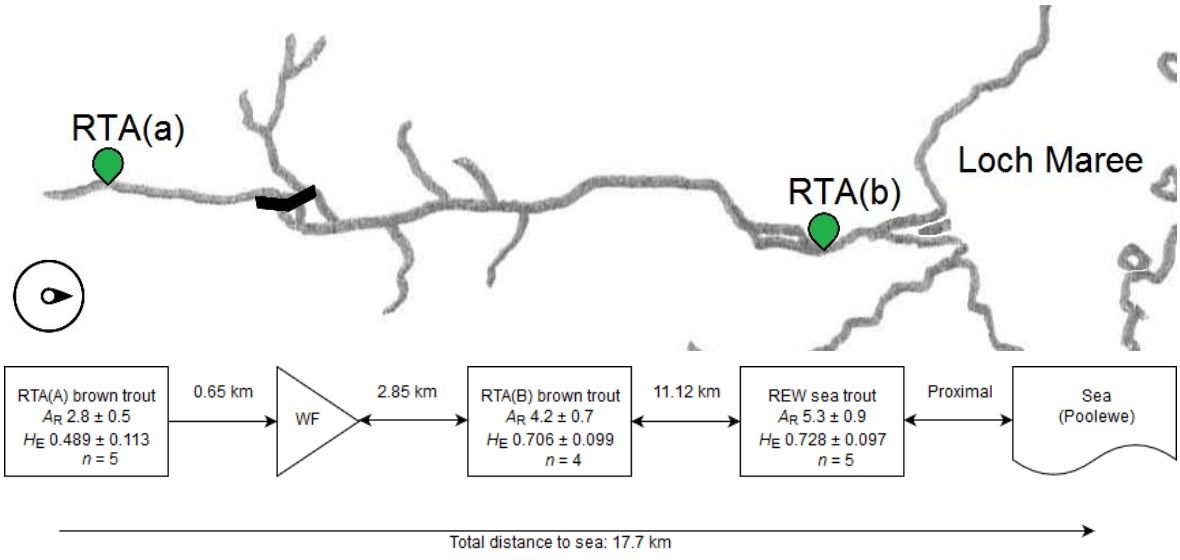

Figure 23 Line-map and flow diagram of River Talladale headwater population (RTA[a]) and downstream post-barrier comparison with another resident population (RTA[b]), as well as sea trout caught in the River Ewe (REW), indicating the presence of barriers of distances between.

The strongest relationships were observed between the groups when separated by life history (p_s < 0.001, r_s 0.862), and whether they were above or below barriers (p_s < 0.001, r_s 0.876), suggesting that populations with access to and life history at sea, are significantly more likely to have greater levels of allelic richness. Statistical tests are displayed in *Appendices 6.15-6.18*.

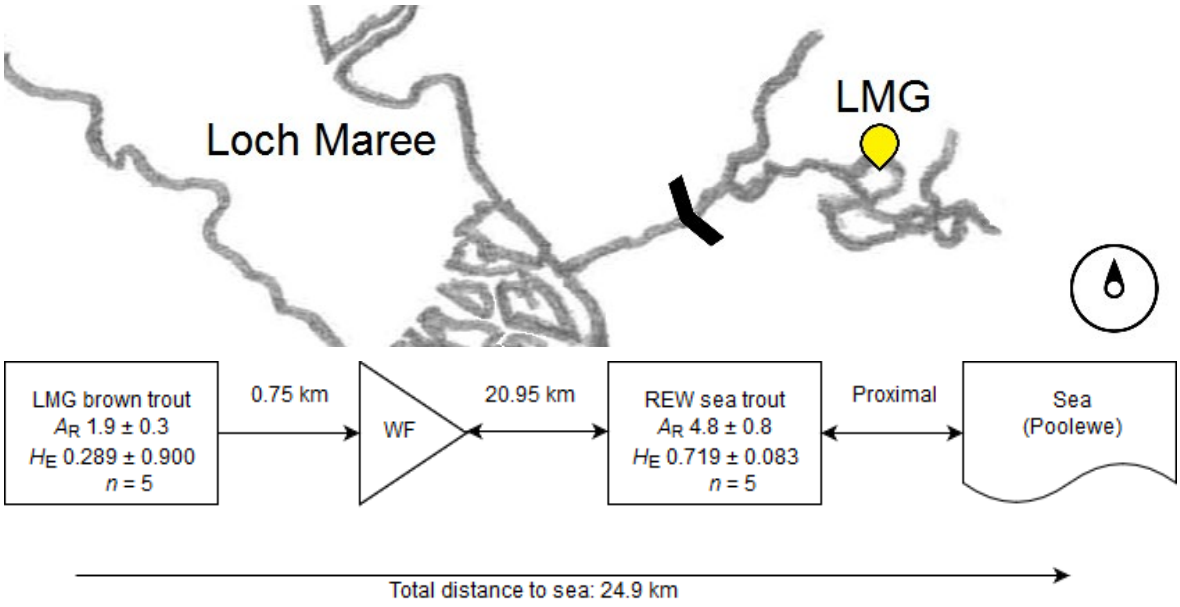

Figure 24 Line-map and flow diagram of Loch Meallan Ghobhar (LMG) headwater population and downstream post barrier comparison with sea trout caught in the River Ewe (REW), indicating the presence of barriers of distances between.

Further analysis was undertaken to assess if these differences were really based on the presence of barriers to movement, and not correlations due to distance from sea or life history. Populations with no barriers to prevent access to the sea (GRU, COU, LFD, SR, LGD, RTA(*b*), and LEV) were compared with the same randomly generated anadromous cohorts of equal size (*n* 5). Results showed no significant difference between genetic diversity and distance to sea (p_s > 0.249), and with life history (p_s > 0.160).

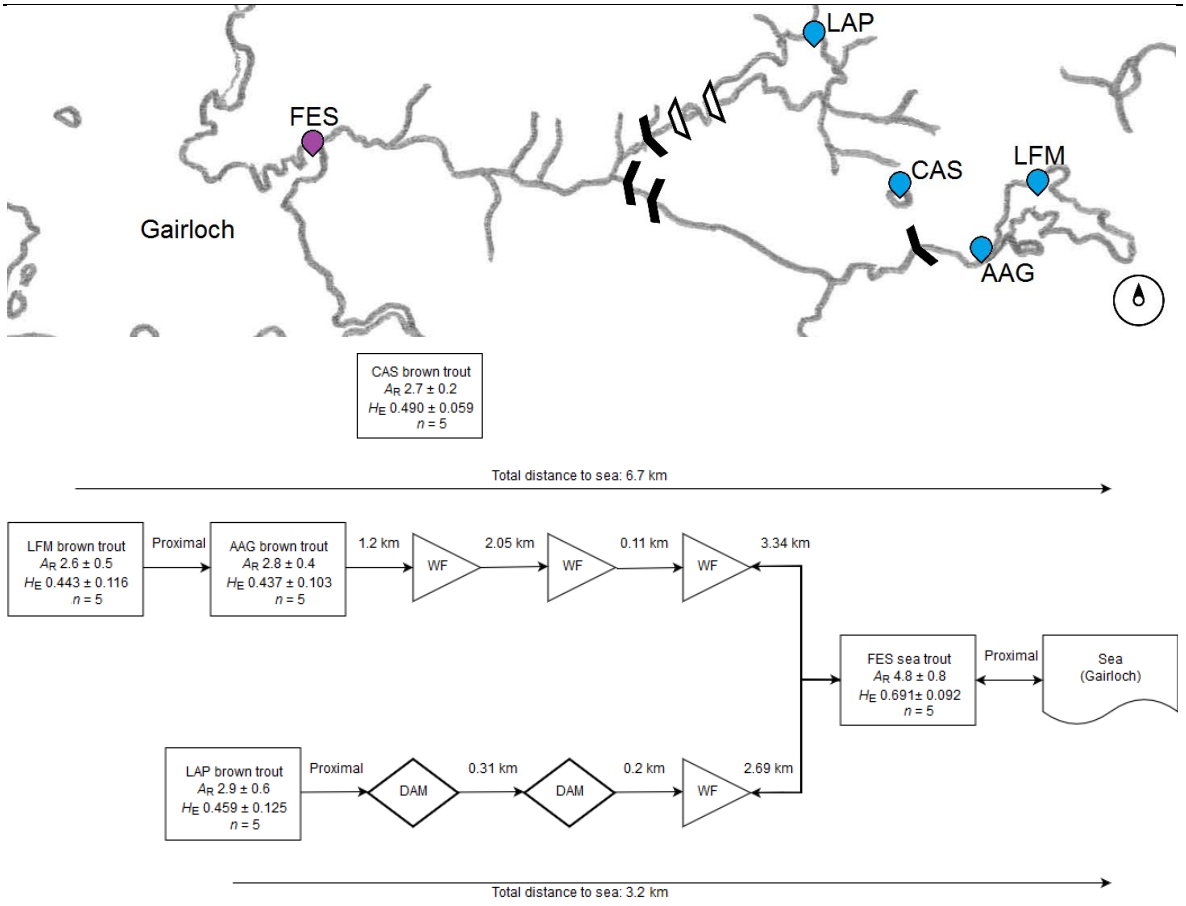

Figure 25 Line-map and flow diagram of Loch na Feithe Mugaig (LFM), its outflow river (AAG) and Loch Airigh a' Phuil (LAP) headwater population and downstream post barrier comparison with sea trout caught in Flowerdale bay (FES), as well as "Cassius lochan" (CAS), a small isolated lochan, indicating the presence of barriers of distances between.

Tests for difference between resident and anadromous genetic diversity in this group showed no significant difference ($p_s > 0.18$). Suggesting that populations not affected by barriers (to the sea) do not have significant influences on their genetic diversity as a result of distance to sea or their life history.

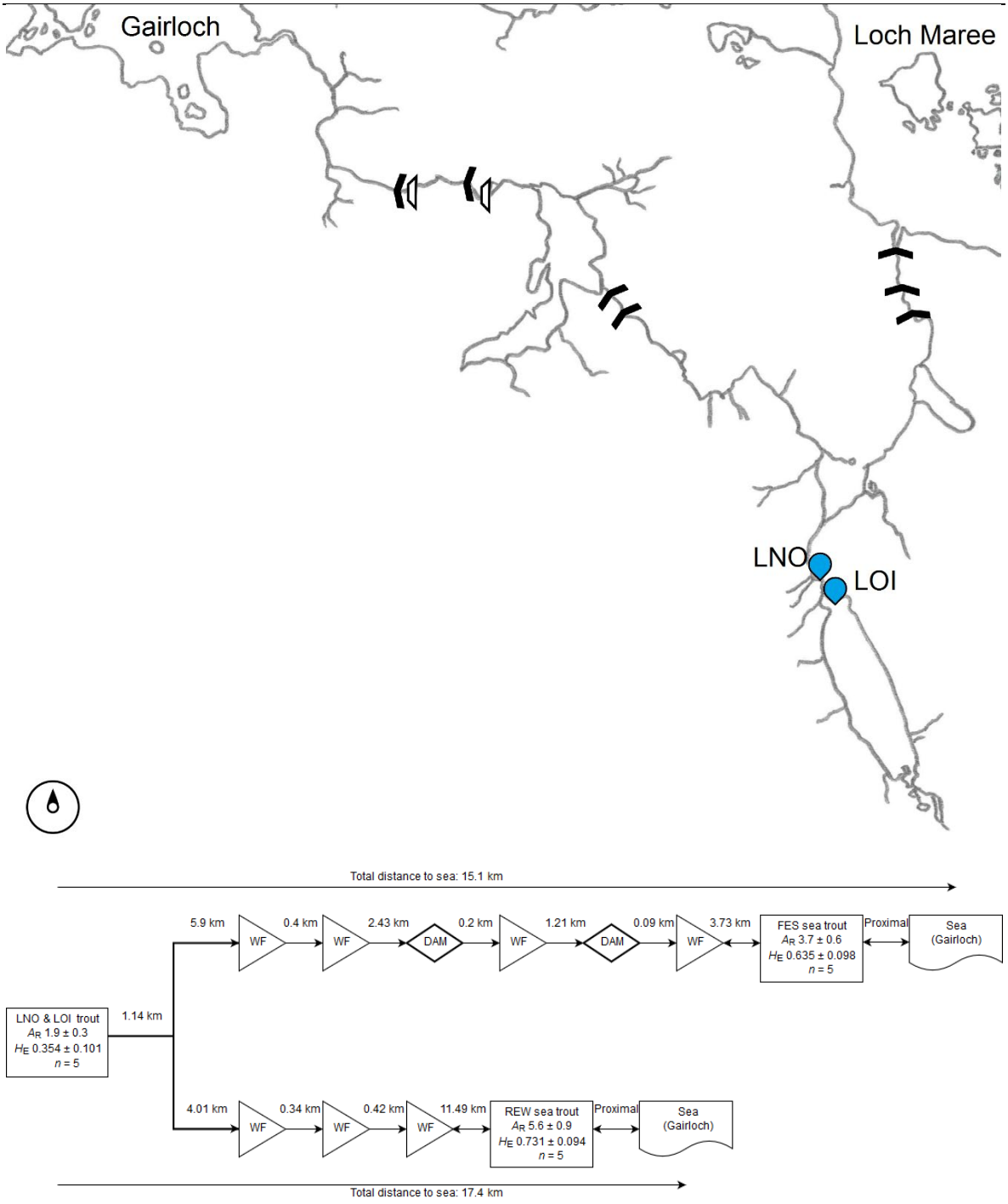

Figure 26 Line-map and flow diagram of Loch na h-Oidhche (LNO & LOI) headwater population and downstream post barrier comparison with sea trout caught in Flowerdale bay (FES) and the River Ewe (REW), indicating the presence of barriers of distances between.

When both populations with and without barriers were analysed as a whole data set, distance to sea showed no significant effect on trout populations' A_R and H_E ($p_s > 0.212$). Significant correlations were observed between site genetic diversity, and life history ($p_s < 0.001$, $r_s > 0.794$), and whether they were above or below barriers ($p_s < 0.001$, $r_s > 0.859$), with downstream populations being significantly more diverse genetically than the headwater populations (p_s 0.0001).

MUTATION–DRIFT EQUILIBRIUM

A mode shift analysis could not detect this in the allele frequencies distribution at regional level, showing an L-shaped distribution typical of equilibrium, however results from sign tests and standardised differences tests contradicts this and suggest the resident populations of brown trout sampled in this study are statistically likely to be in mutation-drift disequilibrium (p_s < 0.0002) (*Appendix 6*) (although the standardised differences results are less reliable than the sign test as only 8 polymorphic loci could be analysed, while 20 is recommended, nonetheless, these results do coincide), suggesting that the population may have experienced a detectable genetic bottleneck event. Sign tests suggest all resident populations are in MDE except NW Loch Maree, though the standardised differences test suggests it is. Furthermore, the mode shift analysis contradicts both sign and standardised differences test, suggesting the study's sample size and marker size were insufficient to produce reliable results. Therefore, these results are highly cautionary and require confirmation.

POPULATION STRUCTURE

A Neighbour-Joining (NJ) population tree was constructed using population pairwise D_{ST} values (*Figure 27*).

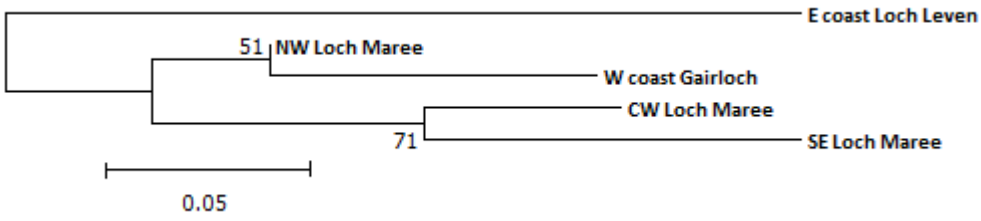

Figure 27 NJ tree illustrating the Wester Ross brown trout population genetic structure, based on D_{ST} (corrected) values generated using nine microsatellite loci.

Results suggest the population structure at regional level is split between two main branches within Loch Maree and Gairloch, with NW Loch Maree and Gairloch sharing a branch – though the bootstrap value is not significant. The other branch shows a relationship between the CW- and SE Loch Maree resident populations, perhaps suggesting the two populations are more similar to each other, but that they are also diverging. The population genetics of Wester Ross brown trout might be structured regionally in this way, though these results would preferably carry more statistical weight. As it is, this is a tentative result, which indicates the study's sample sizes were not optimal for discerning the true population structure. If the tree holds true however, the population structure observed at regional level suggest Loch Maree was initially colonised at the NW point from the sea at Poolewe, suggesting a sea-to-freshwater colonisation by ancient migrant sea trout is more likely than a freshwater-radiation from an inland glacial refuge since the retreat of the last glacial maximum.

PRINCIPLE COMPONENT ANALYSIS (POST–HOC)

The PCA results conducted at sample site and regional range revealed several outlier specimens that were identified as SR20 & 21 (33), REW 44 & 45 (30) and LMA 3 (20) (*Figure 28*).

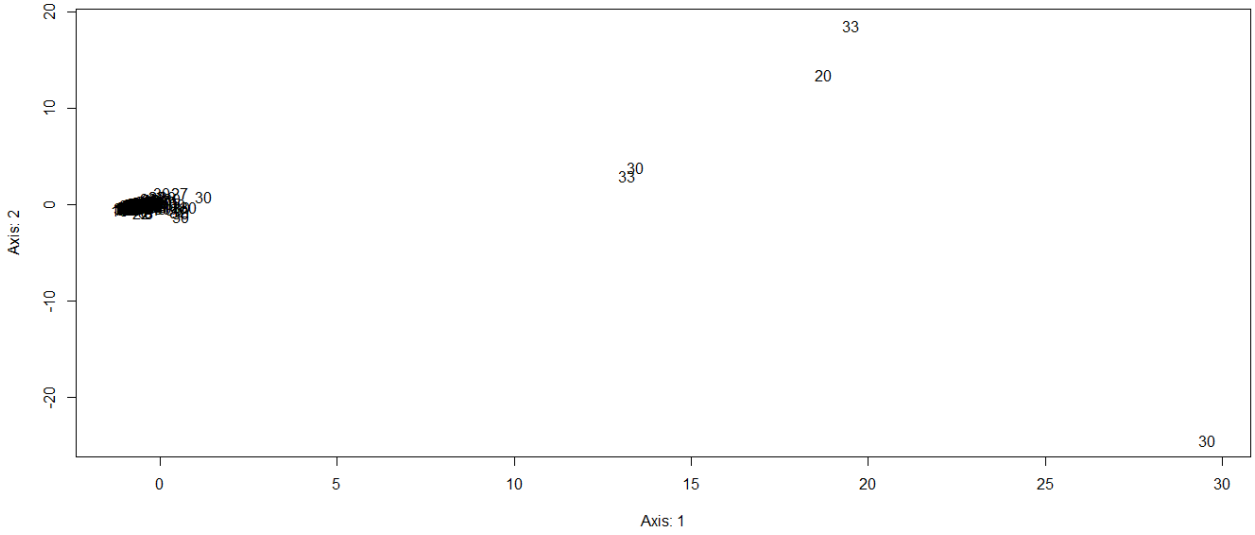

Figure 28 Initial PCA results plot showing five outliers outside the main cluster, these individuals have been identified as SR20 & 21 (33), REW 44 & 45 (30) and LMA 3 (20).

These specimens were subsequently removed to get a better understanding of the relationships among the other samples in the main cluster (*Figures 29 & 30*).

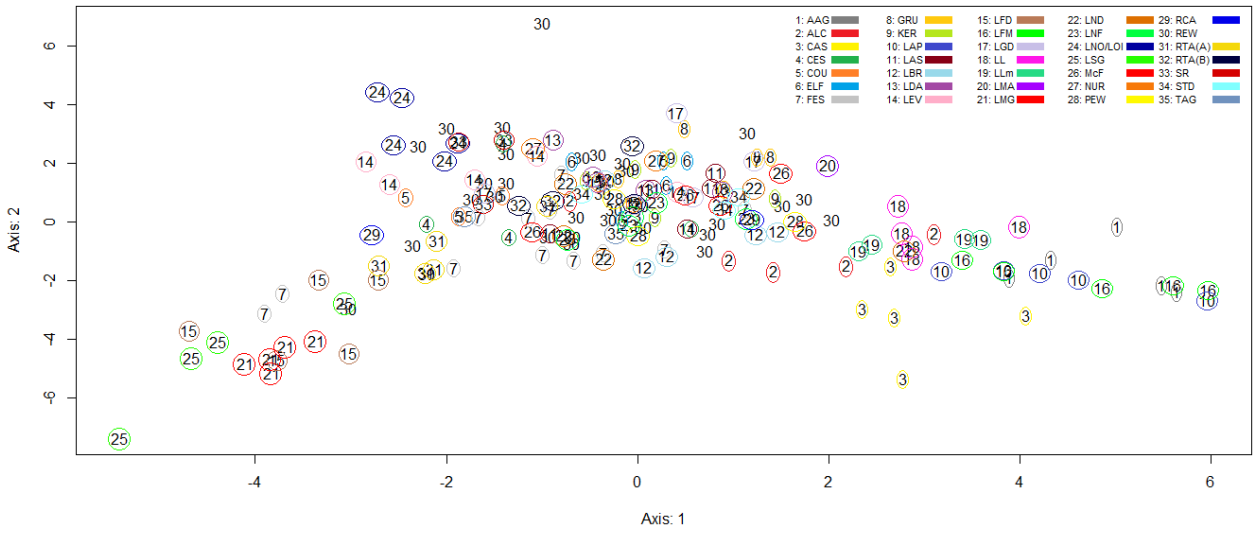

Figure 29 PCA two-axis plot of Wester Ross brown trout and sea trout at individual sample site range.

At sample site range, individuals sampled at the same sites were clustered together with varying degrees of compactness, as well as overlap with other sample site clusters (*Figure 29*).

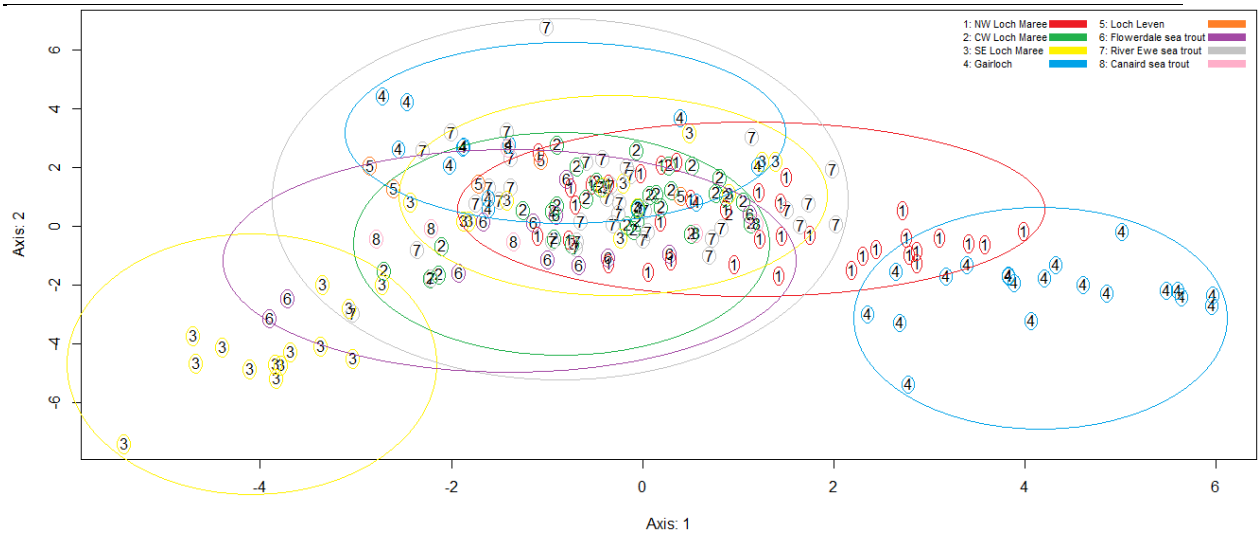

Figure 30 PCA two-axis plot of Wester Ross brown trout and sea trout in their regional groups.

At regional range, major clusters are clearly defined with two regions (SE Loch Maree-Group 3-Yellow and Gairloch-Group 4-Blue) having fragmented clusters. Considerable overlap between clusters is also visible in the centre of the plot (*Figure 30*).

BONFERRONI CORRECTIONS (POST–HOC)

The post-hoc bonferroni corrections made to the family-wise statistical P-values generated in this study are as follows:

- Man-Whitney tests (*n* 17): α = 0.05/17 = 0.0029
  Only **one** significant result would become null = Wester Ross Gst and Wester Ross resident Gst would have no significant difference in their median Gst (*Appendices 7.2, 7.4, 7.6, 7.8, **7.14** & 7.17*).

- Two sample t-tests (*n* 2): α = 0.05/2 = 0.025
  No significant results changed – both tests already null and well above alpha (*Appendices 7.10 & 7.17*).

- Spearman's ranked correlation (*n* 14): α = 0.05/14 = 0.0036
  No significant results changed – all tests still below corrected alpha value or are already well above alpha (*Appendix 7.18*).

- Analysis of Variance (*n* 5): α = 0.05/5 = 0.01
  No significant results changed – all tests already null and well above alpha (*Appendices 7.10 & 7.12*).

As only one significant result which was not essential in any conclusions became null, these results will not be discussed further.

2.3: DISCUSSION

Population genetic diversity determines a population's ability to adapt to environmental changes, and thus is of increasing concern due to climate-change (Lovejoy, 2005). The need to understand a population's genetic structure has become a priority for many in conservation management, particularly for *S. trutta* due to its socioeconomic value across its range (Keenan *et al*., 2013).

INTRA–POPULATION GENETIC DIVERISTY

Genetic diversity contributes to evolutionary potential, and can also be used to study evolutionary history, as well as to outline contemporary clades within populations (Hughes *et al*., 2008; Caballero and García-Dorado, 2013). There are various measures to estimate different aspects of a population's genetics, collectively known as *F*-statistics (where *F* stands for *fixation indices*)(Wright,1921) - heterozygosities and other measures based on allelic richness are fundamental interests, but these measures differ in their mathematics, interpretations, and what they represent (Caballero and García-Dorado, 2013; Crow and Kimura, 1970).

Allelic richness (A_R - the number of alleles present at any locus or the mean number across multiple loci) is the most basic measure of genetic diversity, and is the raw material for selection, making it fundamental to a populations adaptability to environmental changes (Greenbaum *et al*., 2014). Genetic diversity at the allelic level is similar between Wester Ross resident populations (NWLM, CWLM, SELM, & WCG) (*Table 12*). The sea trout cohort caught in the River Ewe heading upstream carried roughly 50 % (or 5) more alleles per loci than the resident populations in Wester Ross. When the WR residents are analysed as one population the combined allelic richness is still lower than that of the Ewe sea trout, despite the difference in sample size. These results indicate the Ewe sea trout of Loch Maree are more genetically diverse than their resident counter parts, which might suggest the sea trout are possibly admixtures originating from multiple resident populations, bringing together different alleles and increasing the cohort's diversity to higher than that of any of their resident counterparts. This seems plausible since the difference between Loch Maree's populations' mean A_R (10.0 ± 0.4 SD) and combined A_R within Loch Maree as a whole population (14.1 ± 3.5 SE) indicates alleles (on average 4 a locus) which are not being shared between the population samples. This suggests a degree of genetic distinction exists between freshwater populations found within Loch Maree catchment (*Table 12*) and contributes to their anadromous cohorts having a higher allelic diversity, since they then join sea trout from other resident populations. There is evidence to suggest allelic richness correlates positively with 'evolvability' - even when measured with neutral markers (Wagner, 2008), and negatively with inbreeding depression, and therefore has evolutionary consequences (Greenbaum *et al*., 2014). This emphasises the importance for conservation and management to maintain genetic diversity at the allelic level, particularly for populations with low effective sizes that are more at risk of allelic loss.

Comparisons with results of other studies is somewhat hindered by the study's sample range and sample sizes. Many studies with comparable data (i.e. microsatellite markers, similar number of loci, same species) have larger samples per site (often $n > 30$, here this study's mean n 5 per site) often spreading over a single catchment, if not, a river and its tributaries (Griffiths *et al*., 2009; Carlsson *et*

al., 1999), making WR regional groupings mean A_R tentatively comparable with other studies' results. Mean A_R at sample site level in this study was 3.3 with a mean *n* 5, and *k* 9 loci, which is similar to a study of Norwegian trout from the Nordre Finnvikelv, at 3.63 A_R, though the study had a mean sample size around *n* 62 ± 7 SD and *k* 6 (Carlsson *et al*., 1999). A study on the River Dart, south-east UK found a mean A_R per site of 5.52, with a mean *n* per site around 60 ± 30 SD over *k* 9 (Griffiths *et al*., 2009). These data suggest a similar level of allelic diversity between the three populations (at site level) if differences are considered between *n* and *k* - though this has not been statistically proven in this study, limiting any conclusions that can be drawn.

Heterozygosities (how likely a member of a diploid population will have different alleles at one or more loci) is the frequently chosen measure when presenting a genetic summary of a population (Greenbaum *et al*., 2014; Vonholdt *et al*., 2008), as a decrease in observed heterozygosity can reduce the average fitness of individuals (Andras *et al*., 2011; Szulkin *et al*., 2010, 2003; Toro *et al*., 2009; Reed and Frankham, 2003), and thus has observable ecological consequences. Expected heterozygosity (H_E) estimates what the heterozygosity would be if the number of alleles in the sample were under HWE and represents a population's genetic diversity, since it is calculated using allele frequencies, which take into account allelic richness and distribution. If a population remains in HWE, H_E positively correlates with A_R - as there are more alleles in the population, individuals are more likely to have two different copies, and less likely to have two copies of the same. For example, a fixed locus would equal 0.0 H_E, while a biallelic locus (containing only two alleles) will have a H_E of 0.5, since HWE = $p^2 + 2pq + q^2 = 0.5$ H_E - as there are 2/4 *pq* and only 1/4 p^2 and 1/4 q^2, therefore 50 % (or 0.5) are heterozygous for *pq* (Crow and Kimura, 1970). As allele richness increase by one, it brings one new homozygote state (e.g. x^2) and *n* new heterozygotes, where *n* = pre-existing A_R, which in this case is two (e.g. *xp, xq*). So with each new allele, the number of heterozygous states exponentially increases while homozygous states increase incrementally, causing a net increase in expected heterozygosity. This explains why expected heterozygosity represents genetic diversity better than observed and stresses the need to conserve allelic richness in smaller populations, particularly if inbreeding depression is a concern. Observed heterozygosity (H_O) estimates the population's actual heterozygosity based on the samples' allelic frequencies. A limitation with observed heterozygosities is null alleles. If an individual is heterozygous for one null allele at a locus, that sample will appear to be homozygous during allele scoring, which further contributes to why H_E is considered to better represent genetic diversity than H_O. Mean expected and observed heterozygosities was almost equal in all regional populations analysed separately (< 2.02 % $H_E - H_O$ difference) and as totalled populations (< 1.0 %) (*Table 11*). Expected heterozygosities suggest the Gairloch population is more genetically diverse than the Loch Maree resident populations, though no significant difference was found between the Wester Ross resident populations' H_E indicating the level of genetic diversity within and between them is similar. An interesting observation can be seen at regional ranges, where the heterozygosity increases with increasing *n* (and A_R) except for the Wester Ross sea trout, which displays the highest level of heterozygosity, despite the lowest population size (*n* 58) and second lowest A_R (16.0 ± 3.2) (*Table 11*), indicating the sea trout are more genetically diverse at the individual level than the resident population. This warrants a need for their protection, since sea trout survival and genetic contribution is therefore likely to be influential in

maintaining genetic diversity for both the resident and anadromous populations of *Salmo trutta* in Wester Ross.

The mean H_O observed across all populations and loci was 0.656 ± 0.086 SE, and varied from 0.600 (Canaird sea trout) to 0.761 (Ewe sea trout), which is similar to levels seen in the River Dart (mean H_O 0.6, range 0.55-0.74) (Griffiths *et al*., 2009). The study in Norway found an overall mean H_O of 0.52 which ranged from 0.44-0.6, and is only slightly less heterozygous (Carlsson *et al*., 1999) - though differences between study sample sizes and range require caution when comparing their data.

UNIQUE/SHARED ALLELES AND ALLELIC FREQUENCIES

The results show that out of the 182 alleles genotyped across the nine loci in this study, 152 (83.5 %) were found within Wester Ross resident trout populations (*Figure 18*). The cardinal regions of Loch Maree each have similar numbers of unique alleles, NW- and SE Loch Maree had 9 (5.9 %) while CW Loch Maree had 10 (6.6 %). Gairloch had 23 (15.1 %), more than twice as many unique alleles found in each Loch Maree population despite having similar allelic richness (*Table 11*). Allele frequencies (A_f) for almost all population unique alleles did not indicate population wide distribution with frequencies averaging < 4 % for all unique alleles (*Appendix 4.1*), suggesting the study's sampling may have been inadequate in discerning all of the populations' alleles. The only alleles with frequencies > 7 % were 459 (A_f 12.9 %) and a null allele that appeared in seven homozygotes (A_f 20 % - though this is an underestimation as the null allele may have appeared in heterozygotes but would not appear during allele scoring); both on Locus 5 (*m*One108) and only in the Gairloch resident population (except for one appearance in a Ewe sea trout). This suggests some degree of genetic distinction may exist between Gairloch and the Loch Maree populations of brown trout. Almost one third of the alleles genotyped in this study (45 or 29.6%) were shared amongst all WR resident populations, which indicate differences in allele commonality which might be more typical indicators of their populations (*Table 15*). The lack of population wide unique alleles might suggest a degree of introgression (or allele homoplasy) between all populations. A complete list of allele frequencies can be found shared and unique to populations are displayed in *Appendix 4*. Due to their low frequencies, Wester Ross brown trout population structure does not seem to be defined by population unique alleles, and suggests the study's sampling might have been insufficient. The data suggest it is the shared alleles and their varying frequencies between WR populations that identify population structure. One of the limitations with using microsatellite markers is the concept of allele homoplasy - due to their high polymorphism and the step-wise mutation model they're assumed to follow (Kimura and Ohta, 1978, Ohta and Kimura, 1973), it is possible that identical alleles in separate populations could arise by parallel evolution and not by descent. However, the chance that this has occurred for all 45 shared alleles observed in this study is less likely, therefore the presence of at least some of these shared alleles and their relative frequencies might indicate that there has been a degree of introgression between populations, such that population 'typical' alleles are shared between all – at least in low frequencies (*Table 15*). Maintaining sufficient hydrological-connectivity between populations may therefore be important in maintaining genetic diversity within the trout of Wester Ross.

DEPARTURES FROM HARDY–WEINBERG EQUILIBRIUM

Loci with significant deviations from Hardy-Weinberg equilibrium (HWE) were seen in all but one population, with variable proportions of loci in disequilibrium (*Table 16*). Locus 3 (CA054565a) was either in HWE or fixed in all populations, with the lowest mean A_R (2.5), and heterozygosities (H_E, 0.100; H_O, 0.098) (*Tables 12-14*), and is discounted where monomorphic. HWE describes an equal distribution of category states (or combinations) of two or more alleles for any locus in a population. Allelic distribution will remain under HWE from generation to generation if the following is true: mating is completely random, no mutations are occurring, no selection is occurring, natural or artificial, genetic drift is not occurring (less likely in sufficiently large populations), and no introgression (gene flow, or migration) (Crow and Kimura,1970). Significant differences between observed and expected measures suggest a population is out of HWE at that locus and suggests possible influences by one of the above (Chen *et al*., 2017). The resident trout population with the most loci in disequilibrium was the Gairloch population, where only Locus 3 was in HWE. The other eight loci might therefore be indicators of significant influences affecting the population's allele frequencies (e.g. non-random mating influenced by selection - whether it be natural or artificial) (Crow and Kimura, 1970), and may also explain the non-normal distribution observed in the Gairloch population's allele distribution across the nine loci genotyped. NW Loch Maree was similar to Gairloch with 25 % of the loci (2/8) in HWE. NW Loch Maree drains out to sea nearby at Loch Ewe (Poolewe), another sea loch that drains all of Loch Maree and neighbouring catchments (e.g. Boor and Tournaig, *Table 9, Figure 17*). Sea trout mortality is known to be influenced by marine predation, parasitisation (Walker, 2016; Sahashi and Morita, 2013; Wysujack *et al*., 2009) and feeding opportunities (Watson, 1999); both Gairloch and NW Loch Maree populations are in greater proximity to the sea (5.9 and 5 km) than CW and SE Loch Maree populations (12.6 and 26.6 km); therefore, their significant departures from HWE might suggest they are more reliant on sea trout returns for reproduction. Loch Gairloch and Loch Ewe are sea lochs (with estuarine bays). A harbour south of the village and North-East of Flowerdale bay in Loch Gairloch has a working port which supports local and east coast fishing boats, Royal Navy vessels, marine wildlife tourism, and a pier for in inshore fisherman to cast lines, and freshwater aquaculture (Scotland's Aquaculture, no date), amongst other local activities (The Highland Council, no date), all of which may be adding to the significant influences on the allele frequencies of the local trout population. Leven, Flowerdale and Canaird trout have small sample sizes which make their measures preliminary and less comparable.

POPULATION DIFFERENTIATION

Genetic distance is the amount of differentiation between the genetics of any two populations (Crow and Kimura, 1970). There are a number of formulated estimators and all take into account heterozygosities, which captures allelic richness and distribution. F_{ST} is widely presented as a measure of differentiation, but it was originally formulated to measure biallelic markers (a loci that only has two alleles present in a population) (Putman and Carbone, 2014; Meirmans and Hedrick, 2011; Wright, 1969, 1921), and was later reformulated to handle multi-allelic markers, referred to as G_{ST} (often F_{ST} and G_{ST} are used interchangeably) (Takezaki *et al*., 2014, 2010; Nei, 1973), which indicates the level of genetic differentiation between multiple subpopulations across one or more loci based on their observed heterozygosities. Though G_{ST} and related measures of differentiation have been criticised before for underestimating genetic differentiation between clearly divergent populations (Putman and Carbone, 2014; Carreras-Carbonel *et al*., 2006), more recent literature argues against this and that G_{ST} is still more appropriate than proposed replacements - Hedrick's G_{ST} (Hedrick, 2005) (which measures the maximum possible differentiation possible based on the subpopulations 'observed homozygosities') and Jost's *D* (Jost, 2008) (which measures allelic differentiation, based on number of effective alleles calculated from the sample), and argue they are also affected by the same underestimations imposed on G_{ST} (Wang, 2015; Ryman and Leimar, 2009). Therefore, any conclusions derived from these measures should be backed by other evidence and context. According to G_{ST} values (*Table 17*), WR sea trout was significantly more differentiation than the WR resident trout and to all residents as a whole. When the WR sea trout and residents were analysed together as 'all of Wester Ross', the amount of genetic differentiation significantly decreased, which would make sense if a significant proportion of the sea trout originated from the same resident populations. Interestingly, the WR sea trout had almost identical levels of differentiation to that found between the LM resident populations. The differentiation observed within LM decreases significantly when Gairloch and the LM populations were analysed together as 'WR residents', suggesting Gairloch shares some genetic similarities within the LM populations. The inclusion of Leven trout, though a small sample size (*n* 5) significantly decreased G_{ST} found in the WR residents, but not when Leven was included with all of Wester Ross, which might suggest Leven trout share more genetic similarities with WR resident trout and not so much the sea trout – though Leven's small sample size means this hypothesis requires greater testing to be certain.

INTER-POPULATION GENETIC DIVERGENCE BY DISTANCE

Results from the mantel tests suggests Wester Ross resident trout populations that are further apart are more likely to be genetically differentiated, and the degree of differentiation is positively correlated with distance between the populations in Wester Ross. However this trend did not apply between NW Loch Maree and Gairloch, indicating less genetic variation between the two groups than would be predicted based on geographic-hydrological distances. This may suggest a greater degree of introgression between the two populations historically, than compared to other population pairs found in this study. Compared to CW- and SE Loch Maree residents, the Gairloch and NWLM trout are much closer to sea (mean site distances to sea approximately 5 km NWLM and 5.9 km Gairloch; 12.6 km CWLM, and 26.6 km SELM). The frequencies of four, maybe five alleles shared between all WR resident groups are similarly more common within NWLM and Gairloch, and similarly less common in CWLM and SELM, and vice versa (*Table 15*), suggesting a degree of separation between the two population pairs. The overall trend lost its significance when the Wester Ross sea trout populations were included in the analysis. This shows that genetic variation and geo-hydro distance between the sea trout cohorts where they were sampled did not follow the trend seen in the resident samples, suggesting some of the sea trout caught in the River Ewe and Flowerdale bay/Gairloch did not originate from the outflows nearby in this study, suggesting their natal streams were elsewhere, and that the sea trout cohort of Wester Ross are possibly an admixture of the resident trout with overlapping coastal ranges. If this is the case, resident populations may be affected by the large overlapping coastal ranges of their anadromous members, as point source coastal activities may result in widely diffused consequences in terms of anadromous members' return (or not) to natal streams). If the presence of shared alleles found in this study is the result of historical gene flow, then a coastal range large enough to allow the Gairloch and Loch Maree sea trout to introgress makes sense, and would at least partially explain the genetic similarities between the two groups. The trend is observed again when Loch Leven is added to the matrices. Despite *n* 5, Leven trout show sufficient genetic variation that they also extrapolate the trend (*Figure 21*) - whether or not the sea trout cohorts are included as well - suggesting the sea trout cohort may have a range that covers Wester Ross but not the country (which would have been striking considering the coastal distance is ~ 750 km between Leven and Canaird). These results resonate with literature sources that demonstrate significant genetic divergence between populations of *Salmo trutta* over relatively small geographic distances (Stelkens *et al*., 2012; Ferguson, 1989).

These data demonstrate the analytical power of the BTMP Panel, despite only using one quarter of the total panel (9/38 loci), the markers were able to distinguish genetic differences between Scottish brown trout residing on opposite coasts. Though the statistical power of one BTMP panel on Scottish trout had not been tested before and did lack analytical power required (at sample sizes available for this study) to produce reliable results at lower geographic (site) ranges, these results should be taken with caution, with many hypotheses rather than conclusions being drawn for further research. Re-examination including the rest of the BTMP panel is recommended and would provide broader coverage per specimen (Keenan *et al.,* 2013) and would increase confidence and reliability in the data, as well as greater phylogeographic resolution.

HEADWATER POPULATIONS & BARRIERS TO MOVEMENT

Seven populations sampled at LAS, RTA(*a*), LMG, LFM, CAS, LAP and LNO (& LOI) are isolated above dams or waterfalls, some with multiples of each (*Figures 22-26*), creating unidirectional barriers preventing upstream return. Sample bias was randomly balanced (*n* 5) to compare allelic richness between the headwater populations with other trout populations downstream. Allelic richness is consistently lower in the isolated headwaters and is significantly greater in populations below barriers with sea accessibility. Isolated populations of the salmonid cutthroat trout (*Orncorhynchus clarkii*) have demonstrated a consistent loss of allelic richness over time due to genetic drift (Carim *et al*., 2016), and may echo the low allelic richness observed in the isolated populations found in Wester Ross. A negative trend indicated that populations closer to sea were more genetically diverse, though this is ruled out as it was not present in populations without barriers. The statistical evidence therefore suggests a strong indication that position above or below barriers was the most likely cause for the significant differences seen, with the smallest distance between two divergent populations over a single barrier in a river being < 4 km (RTA(a) above and RTA(b) below, River Talladale, *Figure 23*). This echoes results seen in other populations of trout with barriers between populations (Palmé *et al.,* 2013; Stelkens *et al*., 2012; Griffiths *et al*., 2009; Duguid *et al*., 2006; Ferguson, 1989). An example of two sites that are proximal with no barrier between them show that genetic diversity is similar in the absence of a barrier (LFM and AAG, *Figure 25*). A small isolated ("Cassius Lochan") site also exists in proximity to LFM and AAG and there is anecdotal evidence (unnamed angler, personal communication, 2016) that it receives unofficial stocking with trout originating from them (*Figure 25*). Results indicate CAS trout genetic diversity was similar to LFM and AAG, despite it being isolated from them. This makes sense since there is thought to be a lack of reproductively suitable gravel beds, and that all stocked trout here do not reproduce. These results suggest the presence of barriers created significant influences on the observed genetic diversity between populations above and below barriers in Wester Ross trout populations. This stresses an importance in conserving headwater populations that likely have lower effective sizes and may be under the effects of genetic drift, creating highly divergent populations between above barriers and below. Literature has shown that such headwater populations in other salmonids around the globe, are much more affected by local environmental selection, specialising and adapting these populations to catchment environmental parameters, increasing their local fitness and survival rates, and thus could be sources of local adaptions useful to populations downstream (Hetch, 2015; Watson, 1999).

MUTATION–DRIFT EQUILIBRIUM

Genetic drift has a tendency to reduce variation in a population, while mutation introduces it (Crow and Kimura, 1970). Since finite populations in nature experience both forces, which have opposing effects on genetic variation, the combined effects of these is of much interest. Mutation-drift equilibrium occurs if the effective size of a population has remained stable for many generations, resulting in an almost equal probability that a locus shows an heterozygosity excess or deficit (i.e. whether the heterozygosity observed in a sample is larger or smaller than the heterozygosity expected based on the number of alleles found in the sample if the population were at mutation-drift

equilibrium) (Cornuet and Luikart, 1996). A population that experiences significant reduction of its effective size generally develops heterozygosity excess at selectively neutral loci (Nei *et al*., 1975). Results from sign tests, and standardised differences tests indicate all Wester Ross resident populations are not under mutation-drift equilibrium, suggesting all regional populations included within this study, may have undergone a recent depression in effective population size. There is a suggestion that such a depression event occurred in the late 1980's (Walker, 2016; WRFT, no date). Walker's (2016) report posted on Salmon and Trout Conservation website, investigated it's cause and offers evidence based on Loch Maree annual angler catch returns that marine salmonid aquaculture (one still active in Loch Ewe [Scotland's aquaculture, no date]) is likely to be responsible for Loch Maree's sea trout population collapse, with the year of sudden decline coinciding with the installation of the coasts first two salmon farms. The high density of domestic stocks create high density blooms of salmonid marine parasites ("sea-lice", *Lepeophtheirus salmonis* K.1837), that persist in sea trout coastal territories. The report cites various literature that also reports the same population decline in response to salmon farming installations in Ireland, Norway, and other parts of Scotland, as well as the first reports of sea-lice blooms following in those countries (Walker, 2016). Unfortunately, no genetic baseline pre-collapse exist for the Loch Maree trout population, and so the true magnitude of effects this has had since, or whether the trout are recovering or not has not been studied before. This study hopes to provide a starting point towards creating a baseline for future and possibly historical comparisons. If fin clips sampled before 1987 still exist in archives, they might provide information that could help illuminate the Loch Maree wild trout pre-collapse population's ecological and evolutionary history.

By way of a contrast, mode shift analysis (Luikart *et al*., 1997) of allele frequencies distribution did not suggest any of the Wester Ross resident regional populations had undergone any recent genetic bottleneck events (such as an extirpation [local extinction] or founder event), showing an L-shaped distribution for all resident groups as expected under mutation-drift equilibrium, which is thought to be detectable for only the last few dozen generations (Luikart *et al*., 1997). One limitation such a study faces is that extirpation in highland waters is more likely at waterbody/tributary scales than at regional or catchment scales. This apparent disparity between the results of different tests may simply emphasise the difficulties of such analyses based on small samples acquired over a large geographic range. When such data are analysed, the definition they offer may be insufficient for the detection of any influential bottleneck events at this scale, and may be detectable at site level with a larger sample per site and or a greater genomic coverage per specimen (Cornuet and Luikart, 1996).

TEMPORAL GENETIC VARIATION

A limitation of this study is possible temporal genetic variation. Of the 192 samples genotyped, *n* 2 were sampled in year 2006, *n* 7 in 2009, *n* 8 in 2010, *n* 10 in 2011, *n* 24 in 2012, *n* 28 in 2013, *n* 41 in 2014, *n* 52 in 2015, *n* 19 in 2016, and *n* 1 in 2017 (*Table 9*). A total range of 11 years, however the bulk of the samples (85.4 %) were obtained over 4 years between 2012 and 2016. There is evidence in literature to suggest that genetic variance is temporally stable for at least up to 20 years in *Salmo trutta* if effective sizes remain stable (Palm *et al*., 2003), and studies compared earlier also did not find significant temporal genetic differences, albeit their samples only covered two years (Griffiths *et al*., 2009; Carlsson *et al.,* 1999). Therefore, there is the chance that some temporal

variation may be present in this study, but it was not statistically addressed as there was not enough sample sizes from the same sites (mean site *n* 5) split over different sample years to make data sets of any significant weight (which is was due to sampling strategy focusing on high DNA integrity rather than temporal differences).

REGIONAL POPULATION STRUCTURING OF WESTER ROSS BROWN TROUT

A constructed population (neighbour-joining) tree reflects pairwise D_{ST} values, based on population allele frequencies (*Figure 27*). Results suggest the population structure at regional level is split between two main branches, with NW Loch Maree and Gairloch sharing a branch, the other branch shows a relationship between the CW- and SE Loch Maree resident populations, suggesting the two populations are more similar to each other. Loch Maree and Gairloch have two geo-hydrological routes between them, one along the west coast and the other via two rivers that drain from Loch na h-Oidhche (LNO & LOI), one into Gairloch just south of Flowerdale estuary, the other into CW Loch Maree between Slattadale burn (STD) and River Talladale (RTA) (*Figure 17*). Both routes downstream contain multiple unidirectional barriers (waterfalls and dams); trout that make it past the barriers and successfully reproduce downstream may contribute a loss of variation between these two populations. If this was the case however, it would bring into question why resident trout from CW Loch Maree are not also genetically similar to Gairloch residents. This may suggest gene flow between the two populations is more coastal or LNOs genetic contribution to the two is negligible. The relationship between CW- and SE Loch Maree and their relative positions to NW Loch Maree suggest that Loch Maree was colonised first at the NW Loch Maree end, suggesting a sea trout colonisation following the last glacial retreat, and not a freshwater radiation from a glacial refuge in Loch Maree. The population genetics of Wester Ross brown trout might be structured regionally in this way, though these results would preferably carry more statistical weight with higher bootstrap values. As it is, this is a tentative result, which indicates the study's sample sizes were not optimal at discerning the true population structure. Nonetheless, the data obtained in this study suggests population genetic structure is present in the wild brown trout of Wester Ross, and that its current structure has been largely affected by a population crash, leading to a bottleneck event that is still statistically detectable. Significant marine pressure and relative proximity to sea is hypothesised to be influencing NW Loch Maree and Gairloch's divergence from CW- and SE Loch Maree inland populations, if the NJ tree holds true (*Figure 27*). Significant genetic differentiation between barriers at site level was observed, however site sample sizes were insufficient at producing a tree with any confidence at that population and geographic scale – a major limitation of this study.

PRINCIPLE COMPONENT ANALYSIS (POST–HOC)

The principle component analysis (PCA) that was conducted post hoc was to assess the validity of the study's sample groups at sample site and at pooled regional range.

Five specimens appeared initially as outliers and were subsequently removed from the analysis (*Figure 28*). Two of these specimens (REW44 & 45) were sampled in 2006 and could represent some temporal differences in the microsatellite profiles of the sea trout in Loch Maree. LMA 3 was also one of the outliers and interestingly is thought to be a possible trout/salmon hybrid based on morphological observations made on the specimen (observations not made in this study or by the study's author). The last two specimens came from the SR sample site and were sampled on the same date as the rest of the SR samples and are believed to be resident brown trout as well (*Table 9*). All five outliers uniquely carry allele 119 at Locus 3 (CA054565*a*) (*Appendix 3*) which may represent a salmon allele, though this conjecture is based on a single specimen (LMA 3).

With these five outliers removed, results show that trout sampled at the same sample sites had similar microsatellite profiles, as individuals sampled at the same site were clustered together when results were plotted (*Figure 29*). This suggests that the microsatellite allele scoring was successful as an *a priori* assumption was that trout from the same resident sample sites would share similar microsatellite profiles due to the trout's reproductive homing nature. Considerable overlap was observed between many sites in the centre of the plot, suggesting a degree of genetic exchange between these sites and their populations is likely to have occurred.

Headwater sample sites from small isolated lochs such as CAS (3-Yellow) and LMG (21-Red) appear divergent from the central overlapping clusters, suggesting the microsatellite profiles found in trout from these sites had more unique microsatellite profiles than trout from connected sites. Furthermore, sites that were closer geographically, such as AAG (1-Grey), LFM (16-Light green), LAP (10-Navy), tended to overlap or cluster beside each other (*Figures 17 & 28*).

At pooled regional range, results demonstrate that two out of four of the Wester Ross resident regional groups are supported genetically, as NW- (Group 1-Red) and CW Loch Maree (Group 2-Green) regional groups showed a distinct cluster. Considerable overlap was also seen between all sites in both regions' clusters (except RTA(a) isolated above a waterfall and the Gairloch hill lochs of NW Loch Maree [ALC, LL, LLm]), suggesting gene flow between the other sites in these neighbouring regions (*Figures 17, 28 & 29*).

SE Loch Maree (Group 3-Yellow) and Gairloch (Group 4-Blue) were both fragmented into two sub-clusters (*Figure 30*) with sample sites within each fragment having no overlap with each other within their own region. These fragmentations for both SE Loch Maree and Gairloch correlate with sites that are in separate catchments or are geographically isolated above barriers to fish movement (*Figures 17 & 28*).

Within SE Loch Maree clusters, sample sites LMG, LSG and LFD are clustered together away from sites GRU, TAG and COU. Although both clusters of sites are within the same region geographically

and are proximal in the results, the sites exist in separate Loch Maree tributaries and without overlap in the results (*Figures 17, 28 & 29*).

The fragmented sub-clusters within the Gairloch cluster demonstrate the same separation but on a larger scale, both geographically (*Figure 17*) and genetically according to results (*Figure 30*). SR, LGD and LNO/LOI are clustered away from LFM, AAG, CAS and LAP which makes sense since SR and LMG are part of their own small isolated catchments but neighbour each other west from the Flowerdale catchment which holds LFM, AAG, CAS and LAP (*Figure 17*). Interestingly, LNO is geographically separate from SR and LMG existing above many significant barriers to gene flow in a separate catchment (Kerrysdale catchment) south of the Flowerdale catchment (*Figure 17*).

Two sample sites in NW Loch Maree, LL and LLm are clustered in proximity to the Flowerdale cluster of Gairloch (*Figures 28 & 29*). These sites are also in relatively close proximity geographically (*Figure 17*) and suggests a degree of genetic similarity exist between these sites. However, there was an *a priori* assumption that these sites, did not have gene flow due to a presumed lack of hydrological connectivity between these sites. These results might contradict either one or both of these assumptions (or perhaps suggest anthropogenic activity) and would at least partially explain the high degree of genetic similarity seen between the two regions (NW Loch Maree and Gairloch) in earlier results.

The sea trout clusters (River Ewe sea trout [Group 7-Grey] and the Flowerdale sea trout [Group 6-Purple]) overlap primarily with NW Loch Maree (Group 1-Red), CW Loch Maree (Group 2-Green), one of SE Loch Maree clusters (Group 3-Yellow-southern cluster) and one of the Gairloch clusters (Group 4-Blue). But have a clear separation from the other Gairloch cluster, the Flowerdale catchment (Group 4-Blue-bottom right) (*Figure 30*). If these results correspond to the sea trout's resident origins, it suggests almost all that were sampled and genotyped, at least in the study, have no origins from the Flowerdale catchment and the north side of SE Loch Maree (Group 3-Yellow-bottom left cluster), except for two sea trout caught in the Flowerdale (*Figures 28 & 29*).

Re-analysis of the data with population or reproductive units based on these results might be insightful, with the Gairloch hill lochs (LFM, AAG, CAS and LAP [Flowerdale catchment] and LL and LLm [from NW Loch Maree]) as a distinct region. SE Loch Maree could be separated into its north and southern regions. SR and LGD could be better grouped with NW Loch Maree. LNO/LOI should be grouped with CW Loch Maree rather than with Gairloch (Figures 17 & 28). The rest of the sample sites could remain as currently grouped; however, larger sample sizes and greater genomic coverage would be required to further validate these suggested groupings.

The hypothesis drawn is that the fragmented clusters may alter the conclusions drawn if the data were to be re-analysed in the suggested groupings, however any changes to the conclusions are not hypothesised to be substantial at regional range (except for one), and that these results would be better used to further inform earlier results at sample site range. The conclusion expected to change at regional level is the amount of genetic similarity seen between NW Loch Maree and Gairloch, if the Gairloch hill lochs become one total region, they are hypothesised to reduce the genetic similarity seen between NW Loch Maree and Gairloch observed earlier in this study.

2.4: CONCLUSIONS

The data in this study suggest population genetic structure is present in the wild brown trout of Wester Ross suggesting a non-panmictic population exists and that its current structure may still show some evidence for having been influenced by a population crash, which led to a genetic bottleneck event that may still be detectable. Intra-population genetic diversity appears similar between regional populations sampled, however, significant differences were seen in population allele frequencies which suggest the Gairloch and NW Loch Maree populations had significant departures from Hardy-Weinberg equilibrium in almost all their loci, suggesting they may be under the most environmental pressure. The data indicates this may be related to their proximity to the sea, which suggests they may also be more reliant on sea trout returns for reproduction. The sea trout sampled in this study, seem to be a large admixture of the resident trout populations, with overlapping coastal ranges, that appear to mostly originate from Loch Maree. Headwater populations have reduced genetic diversity compared to populations downstream which may have been influenced by genetic drift in isolation, causing significant genetic variation over distances as low as ~ 4 km over a barrier. A trend was observed where the greater the geo-hydrological distances between any two resident trout populations the more divergent they were genetically (except between NW Loch Maree & Gairloch), though population allele frequencies suggest a degree of introgression may exist between all populations that are hydrologically connected. The population structure observed at regional level suggest Loch Maree was initially colonised at the NW point from the sea at Poolewe, suggesting a sea-to-freshwater colonisation by ancient migrant sea trout is more likely than a freshwater-radiation from an inland glacial refuge since the retreat of the last glacial maximum.

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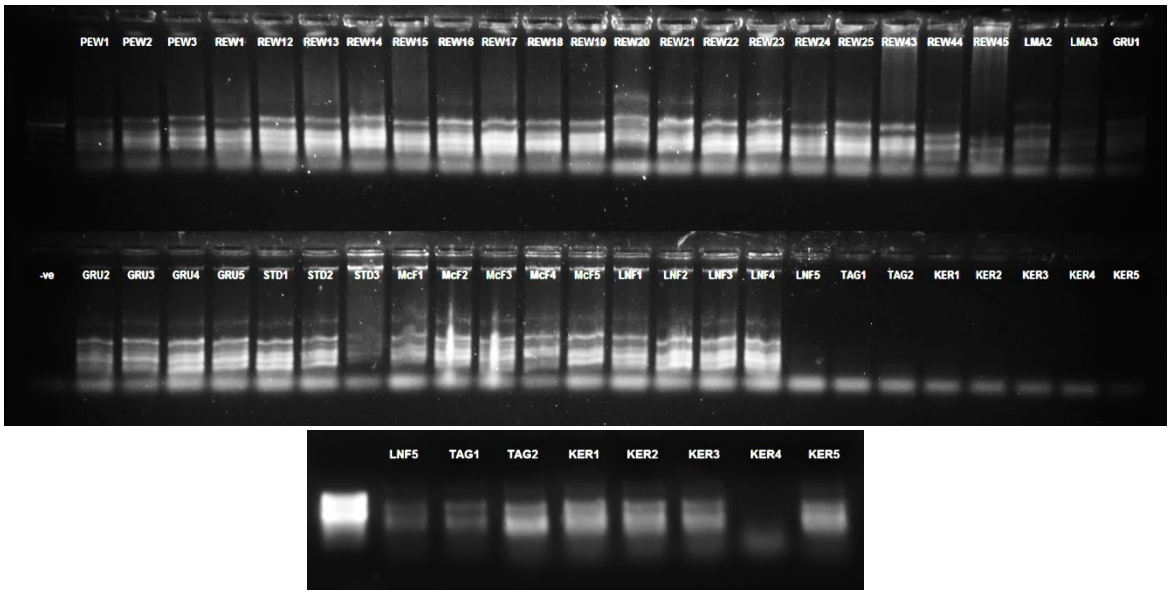
APPENDICES

Any results and analyses, notes or comments that contributed to the study's results are displayed in chronological order as described in the thesis. Some of these may not have been referred to in the writing but are displayed nonetheless. Any additional information on tests or results not shown here are available on request.

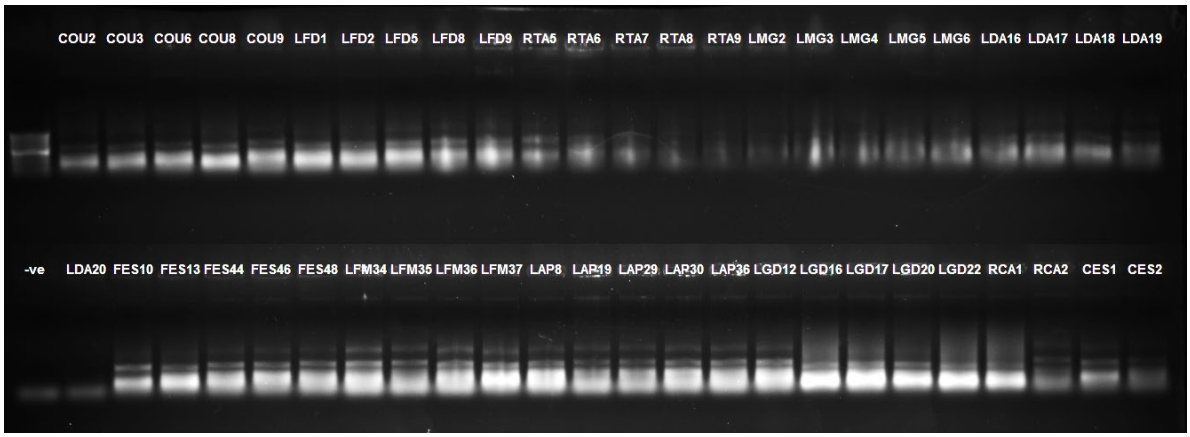
APPENDIX 1 AMPLIFICATION OF BTMP–CUSTOM–PANEL–4: RUNS 1–4



Appendix 1.1 Agarose gel (1.5%) image of BTMP run 1 in 10 µL reaction volumes. 100 bp ladder was used as size standards.

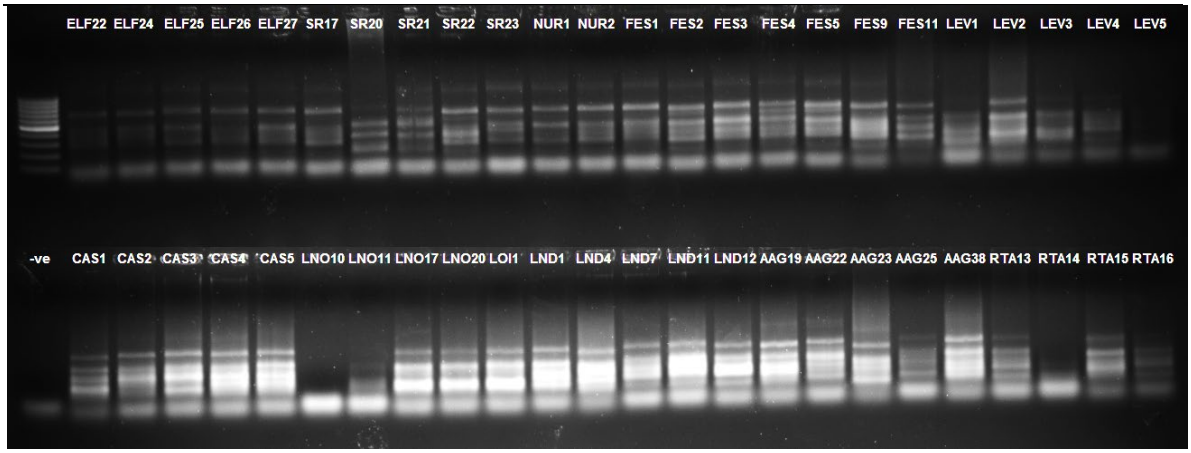
All samples amplified successfully except for the last 8 (LNF5, TAG, and KER samples). These samples were using a different loading dye to the rest which seemed to have failed. A rerun of these failed samples with a new loading dye was successful (*Appendix 1.1*).

The next 48 specimens were amplified using the same multiplex protocols as run 1.



Appendix 1.2 Agarose gel (1.5%) image of BTMP run 2 in 10 µL reaction volumes. 100 bp ladder was used as size standards.

All samples amplified successfully in run 2, except for specimen LDA20 (*Appendix 1.2*). The last 48 specimens were amplified in run 4.



Appendix 1.3 *Agarose gel (1.5%) image of BTMP run 4 in 10 µL reaction volumes. 100bp ladder was used as size standards.*

All specimens amplified successfully except LNO10 and RTA14 (*Appendix 1.3*), but were amplified successfully in reruns (results not shown).

APPENDIX 2 NOTES ON ALLELE SCORING

The following notes outline defining qualities that led to allele scoring of all 9 loci.

LOCUS 1 *Str*3QUB

Allele signals, or peaks, have a small merged artefact pattern downstream (left of peak), and are of the similar height or intensity. Artefacts peaks are small and present across trance range, but did not impede scoring. Locus range was extended from 115-175 to 115-185 bp to incorporate unique alleles found in REW37, and RTA6, 8, and 9.

LOCUS 2 *Ssa*407UoS

Peaks have one or two artefacts downstream 4 bp apart. Artefacts are present across trace range and may overlap with allele calls. This locus has some noise across the range.

LOCUS 3 CA054565a (CA054565b)

Peaks are stuttered (have repeating small artefact peaks evenly spaced between them) with 3-6 peaks 1 bp apart downstream, often with a small artefact 2 bp upstream. Artefacts at 104 and 114 bp (undescribed previously by Keenan *et al.* 2013), often seen simultaneously and can are present even where there are two true peaks. Locus range increased from 101-120 to 101-121 bp to incorporate unique alleles found in REW44 and SR20. All samples in Keenan *et al*. (2013) were monomorphic for this locus - in the WR samples, monomorphism was only seen in this in locus in 4 out of the 8 populations.

CA054565*a* is co-amplified by the CA054565 primer set, and the other co-amplified region CA054565*b* had large noise (artefacts of variable sizes in concentration) signals across traces which made scoring of CA054565*b* unreliable.

LOCUS 4 CA060177

Peaks stuttered by at least one artefact 4 bp downstream. Noise may appear across entire trace. Locus size range increased from 234-315 to 234-340 bp to include novel alleles in REW44/45.

LOCUS 5 m*One*108

Peaks have 2 artefact stuttering 4 bp downstream. A frequent artefact was found at 458 bp which may overlap with allele signals at 459.

LOCUS 6 *Ssa*D170

Alleles have a normal peak pattern with a slight merger at the base and one smaller artefact 4 bp downstream. Locus range expanded from 148–217 to 123-258 bp to accommodate peaks outside ranges in LFD8; LSG1, 3, 4; REW43, 44, 45; SR20, 21; PEW3; COU3; LNO, and LOI.

LOCUS 7 *Sasa*–UBA

A common artefact peak appears at 456 bp and noise between ranges 342-397 bp appeared in some samples. Locus range increased to cover novel alleles found in REW44 and 45.

LOCUS 8 m*One*101

Peaks have a small merger on the peaks base, and are off similar height to one another. Some common noise present within bin ranges, but are much smaller than allele peaks on the same trace. A significant ‘free dye’ peak is described at 181 bp in Keenan *et al*. (2013), however the peak at this location appears to behave as an allele in the present study.

LOCUS 9 *Ssa*413UoS

Allele peaks have a smaller artefact peak 3 bp downstream, Artefact at 236 bp has the same peak stutter as allele peaks, is present in every sample and is often the largest peak on the trace, and can overlap true peaks, particularly at 232 bp. Locus range increased at the lower range from 225-282 to 220-282 bp to incorporate unique alleles in REW45 and SR21.

APPENDIX 3 ALLELES TABLE

	Sample ID			LOCUS 1 *Str3*QUB		LOCUS 2 *Ssa*407UoS		LOCUS 3 CA054565*a*		LOCUS 4 CA060177		LOCUS 5 m*One*108		LOCUS 6 *Ssa*D170		LOCUS 7 *Sasa*-UBA		LOCUS 8 m*One*101		LOCUS 9 *Ssa*413UoS	
#	Label	ID	Population	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
1	AAG	19	Gairloch	132	160	238	242	113	113	266	274	443	447	186	186	315	315	181	181	233	233
2	AAG	22	Gairloch	132	132	282	282	111	113	274	274	443	443	186	194	296	315	181	181	233	233
3	AAG	23	Gairloch	132	132	282	282	113	113	266	274	459	459	194	194	296	319	181	181	233	233
4	AAG	25	Gairloch	132	132	282	282	113	113	266	274	455	455	186	194	315	319	181	181	230	233
5	AAG	38	Gairloch	132	132	282	282	113	113	270	274	447	455	182	182	307	309	181	181	233	233
6	ALC	1	NW Loch Maree	160	172	268	278	113	113	262	286	423	423	186	206	**NPIL**	**NPIL**	177	181	230	233
7	ALC	2	NW Loch Maree	132	132	238	242	113	113	262	270	451	455	190	218	296	315	181	181	230	230
8	ALC	3	NW Loch Maree	132	160	242	282	113	113	262	266	431	455	174	186	315	315	177	181	230	233
9	ALC	4	NW Loch Maree	132	160	238	266	113	113	262	274	451	451	198	218	296	319	177	181	230	230
10	ALC	5	NW Loch Maree	160	160	238	266	113	113	266	266	451	455	198	198	296	296	181	181	230	233
11	CAS	1	Gairloch	132	160	234	234	111	113	270	270	443	459	158	158	315	315	181	185	233	233
12	CAS	2	Gairloch	132	132	234	234	105	113	246	266	**NPIL**	**NPIL**	190	202	275	296	173	185	230	233
13	CAS	3	Gairloch	132	132	234	234	113	113	262	266	459	459	202	202	296	315	185	185	230	233
14	CAS	4	Gairloch	132	160	234	234	113	113	262	266	459	459	158	190	296	315	185	185	233	233
15	CAS	5	Gairloch	132	160	282	282	113	113	266	270	459	459	158	202	315	315	181	185	233	233
16	CES	1	Canaird ST	160	172	230	230	113	113	270	274	435	463	182	186	292	292	173	181	230	230
17	CES	2	Canaird ST	160	160	228	278	113	113	278	278	443	471	162	174	306	306	169	181	230	230
18	CES	3	Canaird ST	160	172	250	258	113	113	266	270	427	427	166	210	291	306	181	185	230	230
19	CES	7	Canaird ST	160	160	274	320	113	113	270	270	443	455	182	182	292	306	169	181	230	233
20	COU	2	SE Loch Maree	172	172	246	250	113	113	270	290	419	467	178	182	291	302	181	181	230	233
21	COU	3	SE Loch Maree	160	160	246	254	113	113	270	294	423	483	182	222	304	304	181	181	233	233
22	COU	6	SE Loch Maree	160	160	220	286	113	113	270	278	431	467	190	202	296	296	181	181	230	230
23	COU	8	SE Loch Maree	160	160	250	266	113	113	262	270	383	463	202	218	304	476	177	177	230	230

	Sample ID			LOCUS 1 *Str3*QUB		LOCUS 2 *Ssa*407UoS		LOCUS 3 CA054565*a*		LOCUS 4 CA060177		LOCUS 5 m*One*108		LOCUS 6 *Ssa*D170		LOCUS 7 *Sasa*-UBA		LOCUS 8 m*One*101		LOCUS 9 *Ssa*413UoS	
#	Label	ID	Population	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
24	COU	9	SE Loch Maree	160	160	246	274	113	113	262	262	451	467	194	218	303	309	181	185	230	230
25	ELF	22	CW Loch Maree	160	160	288	288	111	113	262	262	431	439	190	194	304	304	181	181	233	233
26	ELF	24	CW Loch Maree	160	160	220	258	113	113	262	278	435	435	194	226	296	304	181	181	230	233
27	ELF	25	CW Loch Maree	160	172	286	290	113	113	262	278	503	503	174	182	296	306	181	181	230	230
28	ELF	26	CW Loch Maree	160	160	286	312	113	113	270	270	439	503	194	194	**NPIL**	**NPIL**	181	181	230	233
29	ELF	27	CW Loch Maree	160	160	258	286	113	113	262	262	451	483	186	194	296	296	181	181	230	230
30	FES	1	Flowerdale ST	132	172	258	286	113	113	270	270	439	451	158	186	305	305	181	185	230	230
31	FES	2	Flowerdale ST	160	160	238	284	113	113	262	274	391	415	182	190	296	305	169	169	230	230
32	FES	3	Flowerdale ST	160	160	262	284	113	113	270	282	431	443	174	202	304	304	181	185	230	230
33	FES	4	Flowerdale ST	144	160	278	284	113	113	262	282	379	383	182	186	303	315	173	181	230	230
34	FES	5	Flowerdale ST	160	172	242	286	111	113	270	270	399	399	182	186	298	298	185	185	230	230
35	FES	9	Flowerdale ST	160	172	238	274	113	113	262	274	495	495	186	190	291	315	169	181	230	233
36	FES	10	Flowerdale ST	160	160	238	262	113	113	270	286	427	439	202	210	476	476	169	177	230	230
37	FES	11	Flowerdale ST	160	160	238	242	113	113	262	270	439	439	158	210	315	315	173	181	230	233
38	FES	13	Flowerdale ST	160	172	234	284	113	113	270	270	399	399	162	206	292	309	173	181	230	260
39	FES	44	Flowerdale ST	160	172	234	262	113	113	270	270	439	443	158	162	292	303	173	181	230	233
40	FES	46	Flowerdale ST	160	160	238	260	113	113	262	270	391	439	190	206	292	292	181	181	230	260
41	FES	48	Flowerdale ST	132	172	234	286	113	113	270	270	439	451	162	214	305	305	181	181	230	233
42	GRU	1	SE Loch Maree	160	160	254	272	113	113	270	286	455	471	170	174	296	296	181	181	230	233
43	GRU	2	SE Loch Maree	132	144	266	286	113	113	262	270	443	487	174	194	304	477	181	181	233	239
44	GRU	3	SE Loch Maree	144	144	266	286	113	113	262	262	443	487	174	194	304	476	169	181	233	239
45	GRU	4	SE Loch Maree	144	144	286	304	113	113	262	270	427	443	170	174	296	476	181	181	233	233
46	GRU	5	SE Loch Maree	160	160	246	270	113	113	262	270	439	487	174	194	307	307	181	181	230	233
47	KER	1	NW Loch Maree	160	160	220	242	113	113	262	262	411	431	142	186	303	319	181	181	230	233
48	KER	2	NW Loch Maree	160	160	238	242	113	113	262	266	399	439	142	142	303	303	181	181	233	233

	Sample ID			LOCUS 1 *Str3*QUB		LOCUS 2 *Ssa*407UoS		LOCUS 3 CA054565*a*		LOCUS 4 CA060177		LOCUS 5 m*One*108		LOCUS 6 *Ssa*D170		LOCUS 7 *Sasa*-UBA		LOCUS 8 m*One*101		LOCUS 9 *Ssa*413UoS	
#	Label	ID	Population	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
49	KER	3	NW Loch Maree	160	160	242	242	113	113	262	262	411	439	142	174	296	296	177	181	233	248
50	KER	4	NW Loch Maree	132	160	278	278	113	113	262	266	427	435	142	170	303	303	173	173	230	233
51	KER	5	NW Loch Maree	160	172	270	278	113	113	262	278	411	427	142	190	303	303	177	181	233	233
52	LAP	8	Gairloch	132	132	238	282	113	113	270	274	**NPIL**	**NPIL**	158	194	315	315	181	181	233	233
53	LAP	19	Gairloch	132	160	274	282	113	113	266	266	411	411	202	206	296	305	181	181	233	233
54	LAP	29	Gairloch	132	132	282	282	113	113	270	274	**NPIL**	**NPIL**	186	190	291	315	181	181	233	233
55	LAP	30	Gairloch	132	132	262	282	113	113	266	274	455	455	186	202	315	319	181	181	233	233
56	LAP	36	Gairloch	132	160	238	282	113	113	274	274	431	455	186	206	296	319	181	181	233	233
57	LAS	1	CW Loch Maree	160	160	282	286	113	113	262	262	427	479	182	182	307	307	181	181	230	230
58	LAS	2	CW Loch Maree	160	172	266	282	113	113	262	278	403	479	182	182	296	296	181	181	230	233
59	LAS	3	CW Loch Maree	160	160	266	266	113	117	262	266	403	427	182	182	307	307	181	181	230	230
60	LAS	4	CW Loch Maree	160	160	266	282	113	117	262	262	427	427	182	186	306	306	181	181	230	233
61	LAS	5	CW Loch Maree	160	160	270	278	113	113	262	262	427	479	182	194	296	296	181	181	230	233
62	LBR	1	NW Loch Maree	132	172	230	278	113	113	278	282	423	471	174	178	306	306	181	181	230	230
63	LBR	2	NW Loch Maree	132	160	262	266	113	113	270	282	435	491	186	194	476	476	181	181	233	233
64	LBR	4	NW Loch Maree	160	172	282	282	113	113	270	270	419	423	186	186	476	480	181	181	230	230
65	LBR	13	NW Loch Maree	160	172	230	262	113	113	270	270	471	471	178	186	296	296	181	181	233	233
66	LBR	14	NW Loch Maree	160	172	238	266	113	113	270	282	419	419	186	186	306	476	181	181	233	233
67	LDA	16	CW Loch Maree	172	172	220	262	113	113	278	278	443	443	194	226	291	304	181	181	230	230
68	LDA	17	CW Loch Maree	160	160	262	282	113	113	262	278	435	483	182	226	296	306	181	181	230	230
69	LDA	18	CW Loch Maree	160	172	246	282	113	113	262	278	443	503	190	194	291	296	177	181	230	230
70	LDA	19	CW Loch Maree	160	160	246	246	113	113	262	262	483	483	194	226	304	304	181	181	230	230
71	LDA	20	CW Loch Maree	160	160	282	282	113	113	262	278	479	479	194	194	291	296	181	181	230	230
72	LEV	1	Loch Leven	132	132	226	270	113	113	270	290	**NPIL**	**NPIL**	166	190	296	296	173	177	233	239
73	LEV	2	Loch Leven	132	172	250	256	113	113	270	282	427	451	166	170	302	302	169	173	230	266

	Sample ID			LOCUS 1 *Str3*QUB		LOCUS 2 *Ssa*407UoS		LOCUS 3 CA054565*a*		LOCUS 4 CA060177		LOCUS 5 m*One*108		LOCUS 6 *Ssa*D170		LOCUS 7 *Sasa*-UBA		LOCUS 8 m*One*101		LOCUS 9 *Ssa*413UoS	
#	Label	ID	Population	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
74	LEV	3	Loch Leven	132	160	242	242	113	113	278	278	371	371	142	166	300	303	173	181	233	260
75	LEV	4	Loch Leven	160	172	270	270	113	113	270	270	439	439	166	174	301	303	173	189	230	239
76	LEV	5	Loch Leven	160	160	244	274	113	113	290	290	435	435	170	190	291	296	173	185	239	239
77	LFD	1	SE Loch Maree	172	172	262	266	113	113	266	270	399	403	206	210	309	309	181	185	230	260
78	LFD	2	SE Loch Maree	160	172	262	266	113	113	266	270	403	411	198	206	309	309	185	185	260	260
79	LFD	5	SE Loch Maree	160	172	242	246	113	113	270	274	395	467	206	210	292	309	181	185	230	260
80	LFD	8	SE Loch Maree	160	172	266	294	113	113	270	270	399	439	214	218	300	478	181	185	230	260
81	LFD	9	SE Loch Maree	160	160	262	288	113	113	270	274	423	483	210	210	300	328	181	185	230	230
82	LFM	34	Gairloch	132	160	270	282	113	113	270	270	447	471	182	198	315	315	181	181	233	233
83	LFM	35	Gairloch	132	132	238	282	113	113	274	274	447	447	186	198	315	319	181	181	233	233
84	LFM	36	Gairloch	132	160	238	262	113	113	262	274	447	447	190	202	315	315	181	181	233	233
85	LFM	37	Gairloch	160	160	282	282	113	113	266	274	455	455	186	202	319	319	181	181	233	233
86	LFM	38	Gairloch	132	132	262	282	113	113	266	274	**NPIL**	**NPIL**	186	202	315	319	181	181	233	233
87	LGD	12	Gairloch	172	172	248	274	111	113	262	282	443	443	150	186	306	309	177	181	233	248
88	LGD	16	Gairloch	160	172	248	270	113	113	274	286	415	415	198	202	303	303	181	181	230	230
89	LGD	17	Gairloch	156	160	234	234	113	113	282	286	427	447	178	178	291	306	169	181	233	248
90	LGD	20	Gairloch	160	160	234	244	113	113	270	290	415	423	194	194	303	303	177	181	230	230
91	LGD	22	Gairloch	132	172	262	270	113	113	262	262	443	471	186	186	292	309	177	181	248	275
92	LL	68	NW Loch Maree	132	132	270	274	113	113	270	274	443	443	178	186	296	296	181	181	233	233
93	LL	69	NW Loch Maree	132	160	274	282	113	113	270	270	471	483	182	186	296	296	181	181	233	233
94	LL	70	NW Loch Maree	132	160	274	274	113	113	270	270	443	447	178	210	296	315	181	181	233	233
95	LL	72	NW Loch Maree	160	160	270	274	113	113	262	270	443	443	178	186	296	315	181	181	233	233
96	LL	73	NW Loch Maree	132	132	266	274	113	113	270	270	439	443	178	182	296	315	181	181	233	233
97	LLm	13	NW Loch Maree	132	132	274	274	113	113	270	270	427	447	178	186	296	296	181	181	233	233
98	LLm	14	NW Loch Maree	132	160	266	270	113	113	274	274	443	447	182	186	296	296	181	181	233	233

	Sample ID			LOCUS 1 *Str3*QUB		LOCUS 2 *Ssa*407UoS		LOCUS 3 CA054565*a*		LOCUS 4 CA060177		LOCUS 5 m*One*108		LOCUS 6 *Ssa*D170		LOCUS 7 *Sasa*-UBA		LOCUS 8 m*One*101		LOCUS 9 *Ssa*413UoS	
#	Label	ID	Population	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
99	LLm	15	NW Loch Maree	132	160	266	270	113	113	270	274	439	451	178	186	297	297	181	181	233	233
100	LLm	17	NW Loch Maree	160	160	274	274	113	113	270	274	419	471	182	186	296	315	181	181	233	233
101	LMA	2	River Ewe ST	132	132	238	238	113	113	262	286	439	443	174	174	296	296	169	169	233	263
102	LMA	3	River Ewe ST	128	128	266	298	113	119	306	338	359	479	126	214	273	273	165	165	227	230
103	LMG	2	SE Loch Maree	160	172	266	266	113	113	270	270	403	419	190	190	309	309	185	185	230	230
104	LMG	3	SE Loch Maree	160	160	266	266	113	113	270	270	403	423	206	206	309	309	181	185	230	230
105	LMG	4	SE Loch Maree	160	160	266	266	113	113	270	270	403	419	190	190	309	309	181	185	230	230
106	LMG	5	SE Loch Maree	160	172	262	266	113	113	270	270	399	403	190	206	309	309	181	185	230	230
107	LMG	6	SE Loch Maree	160	172	266	266	113	113	266	270	419	419	190	206	309	309	185	185	230	230
108	LND	1	NW Loch Maree	132	132	274	282	113	113	262	266	447	447	178	190	296	306	181	181	230	230
109	LND	4	NW Loch Maree	132	160	258	274	113	113	262	262	415	435	194	230	296	303	181	181	230	233
110	LND	7	NW Loch Maree	160	172	226	246	113	113	270	282	439	479	158	190	307	307	169	181	233	239
111	LND	11	NW Loch Maree	148	160	230	238	113	113	262	274	419	463	194	230	298	309	181	181	230	230
112	LND	12	NW Loch Maree	160	172	238	298	113	113	262	274	447	451	190	194	306	309	181	181	230	230
113	LNF	1	CW Loch Maree	144	172	238	242	113	113	270	270	427	439	142	186	307	307	181	181	230	233
114	LNF	2	CW Loch Maree	160	160	242	290	113	113	270	282	443	471	186	186	292	480	181	181	233	260
115	LNF	3	CW Loch Maree	160	160	246	272	113	113	266	278	403	447	178	186	296	296	181	181	230	233
116	LNF	4	CW Loch Maree	132	160	230	266	113	113	262	278	403	451	182	202	300	300	181	181	230	233
117	LNF	5	CW Loch Maree	160	160	236	240	113	113	262	278	439	471	194	206	296	305	181	185	230	233
118	LNO	10	Gairloch	160	160	242	242	113	113	278	278	**NPIL**	**NPIL**	142	142	300	303	173	181	260	260
119	LNO	11	Gairloch	160	160	242	246	113	113	282	282	**NPIL**	**NPIL**	142	142	300	300	173	181	260	260
120	LNO	17	Gairloch	160	160	242	246	111	113	282	282	471	499	142	142	300	300	181	181	260	260
121	LNO	20	Gairloch	160	160	246	254	111	113	278	282	495	495	142	142	300	300	173	181	260	260
122	LOI	1	Gairloch	160	160	246	246	113	113	278	282	**NPIL**	**NPIL**	142	142	300	303	181	181	260	260
123	LSG	1	SE Loch Maree	160	160	242	242	113	113	270	270	403	419	214	218	309	309	181	181	230	230

	Sample ID			LOCUS 1 *Str3*QUB		LOCUS 2 *Ssa*407UoS		LOCUS 3 CA054565*a*		LOCUS 4 CA060177		LOCUS 5 m*One*108		LOCUS 6 *Ssa*D170		LOCUS 7 *Sasa*-UBA		LOCUS 8 m*One*101		LOCUS 9 *Ssa*413UoS	
#	Label	ID	Population	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
124	LSG	2	SE Loch Maree	172	172	278	292	113	113	270	270	399	435	206	214	309	309	181	185	230	230
125	LSG	3	SE Loch Maree	160	160	250	266	113	113	270	270	403	439	206	218	328	328	181	185	230	260
126	LSG	4	SE Loch Maree	160	160	292	296	113	113	274	274	399	419	210	218	292	328	185	185	230	260
127	McF	1	NW Loch Maree	160	160	242	270	113	113	262	262	431	435	202	206	303	303	177	181	233	233
128	McF	2	NW Loch Maree	160	160	240	246	113	113	270	274	423	483	202	214	292	303	181	181	230	230
129	McF	3	NW Loch Maree	132	172	238	238	113	113	266	270	431	439	142	202	305	305	181	181	233	263
130	McF	4	NW Loch Maree	132	132	234	274	113	113	262	282	435	463	**NPIL**	**NPIL**	296	304	181	181	239	263
131	McF	5	NW Loch Maree	160	160	254	262	113	113	266	266	443	467	186	186	296	476	181	181	230	239
132	NUR	1	NW Loch Maree	160	160	278	278	113	113	286	286	479	479	186	190	302	302	169	169	233	260
133	NUR	2	NW Loch Maree	160	160	274	278	113	113	262	286	451	463	186	190	304	304	169	169	233	233
134	PEW	1	River Ewe ST	160	160	274	274	111	113	270	286	439	507	186	214	306	309	181	181	230	233
135	PEW	2	River Ewe ST	156	160	220	282	113	113	270	270	391	443	186	194	298	309	177	181	230	233
136	PEW	3	River Ewe ST	160	172	234	234	113	117	266	282	447	455	170	230	303	315	181	181	230	233
137	RCA	1	Canaird ST	132	160	238	282	113	113	262	278	447	447	158	170	291	306	173	181	230	230
138	RCA	2	Canaird ST	172	172	242	254	113	113	270	270	439	463	186	190	302	302	169	185	230	230
139	REW	1	River Ewe ST	144	172	268	270	111	113	262	274	455	475	186	226	304	305	169	181	230	233
140	REW	12	River Ewe ST	160	172	276	298	113	113	270	294	419	439	142	190	298	305	181	185	230	233
141	REW	13	River Ewe ST	132	160	220	230	113	113	266	278	403	427	194	214	292	301	181	181	230	230
142	REW	14	River Ewe ST	160	172	230	286	113	113	262	266	415	447	186	206	296	306	181	181	230	233
143	REW	15	River Ewe ST	160	172	250	272	111	113	262	286	451	451	206	222	291	298	181	181	230	233
144	REW	16	River Ewe ST	132	160	238	274	113	113	274	274	383	475	170	202	304	476	181	181	230	233
145	REW	17	River Ewe ST	144	160	258	262	113	113	266	270	423	475	182	202	296	305	169	181	230	260
146	REW	18	River Ewe ST	160	172	246	250	113	113	270	294	471	475	194	202	302	306	181	181	230	230
147	REW	19	River Ewe ST	144	172	258	266	113	115	270	282	423	431	178	194	296	296	181	181	230	230
148	REW	20	River Ewe ST	160	172	262	270	113	113	270	270	439	447	210	222	298	306	181	185	230	230

	Sample ID			LOCUS 1 *Str3*QUB		LOCUS 2 *Ssa*407UoS		LOCUS 3 CA054565*a*		LOCUS 4 CA060177		LOCUS 5 m*One*108		LOCUS 6 *Ssa*D170		LOCUS 7 *Sasa*-UBA		LOCUS 8 m*One*101		LOCUS 9 *Ssa*413UoS	
#	Label	ID	Population	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
149	REW	21	River Ewe ST	160	172	246	254	113	113	270	270	407	439	178	186	303	476	177	181	230	230
150	REW	22	River Ewe ST	160	172	250	278	111	113	262	270	475	479	174	210	305	476	181	181	233	233
151	REW	23	River Ewe ST	160	172	254	270	111	113	262	274	463	467	174	218	304	476	177	177	230	233
152	REW	24	River Ewe ST	160	160	246	250	111	113	262	270	447	467	178	182	296	309	177	181	260	260
153	REW	25	River Ewe ST	160	160	242	270	113	113	262	266	403	487	182	190	307	476	181	185	233	260
154	REW	26	River Ewe ST	160	160	230	270	113	113	262	278	411	471	198	202	296	476	181	181	233	233
155	REW	27	River Ewe ST	160	160	250	274	113	113	270	274	443	467	174	174	304	476	177	181	230	233
156	REW	28	River Ewe ST	160	172	262	282	113	117	262	270	423	475	182	190	306	476	181	181	230	230
157	REW	29	River Ewe ST	160	160	270	270	113	113	266	266	439	491	190	194	304	305	181	181	230	233
158	REW	30	River Ewe ST	160	172	254	268	113	113	274	278	431	455	182	214	301	305	181	181	233	233
159	REW	31	River Ewe ST	148	160	258	266	113	115	262	278	419	427	178	186	296	306	169	181	239	260
160	REW	32	River Ewe ST	144	160	234	270	111	113	274	290	467	467	158	234	305	325	169	177	230	263
161	REW	33	River Ewe ST	148	160	246	270	113	113	262	270	391	475	202	202	296	476	177	181	230	263
162	REW	34	River Ewe ST	144	172	266	290	113	113	282	286	443	467	142	214	292	305	169	177	230	239
163	REW	35	River Ewe ST	160	160	234	258	113	113	266	286	403	487	202	214	304	480	169	181	230	233
164	REW	36	River Ewe ST	160	172	266	282	113	113	266	270	435	467	186	194	296	307	181	181	260	260
165	REW	37	River Ewe ST	172	184	242	242	113	113	270	286	403	435	170	194	296	476	181	181	230	233
166	REW	38	River Ewe ST	148	160	240	240	113	113	270	270	443	459	186	198	301	303	181	181	230	269
167	REW	39	River Ewe ST	160	172	250	288	113	113	262	294	395	419	178	214	304	305	181	185	230	233
168	REW	40	River Ewe ST	160	172	266	278	111	113	262	290	427	443	190	214	307	307	181	181	230	230
169	REW	41	River Ewe ST	148	160	238	246	113	113	262	298	435	447	174	218	298	476	181	181	230	233
170	REW	42	River Ewe ST	160	172	238	250	113	113	270	290	459	479	186	198	303	303	181	181	230	263
171	REW	43	River Ewe ST	160	172	246	250	111	113	270	298	451	467	210	230	304	476	181	181	230	263
172	REW	44	River Ewe ST	128	144	258	266	119	121	330	334	351	491	246	258	321	327	165	169	227	230
173	REW	45	River Ewe ST	140	144	262	266	117	119	330	338	**NPIL**	**NPIL**	226	226	315	323	165	169	224	230

	Sample ID			LOCUS 1 *Str3*QUB		LOCUS 2 *Ssa*407UoS		LOCUS 3 CA054565*a*		LOCUS 4 CA060177		LOCUS 5 m*One*108		LOCUS 6 *Ssa*D170		LOCUS 7 *Sasa*-UBA		LOCUS 8 m*One*101		LOCUS 9 *Ssa*413UoS	
#	Label	ID	Population	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
174	RTA	5	CW Loch Maree	160	172	256	256	113	113	274	282	439	471	182	194	476	476	181	181	230	230
175	RTA	6	CW Loch Maree	172	184	238	256	113	113	282	290	415	439	170	194	309	309	181	181	230	230
176	RTA	7	CW Loch Maree	160	172	260	260	113	113	270	270	415	439	182	206	476	476	181	181	230	230
177	RTA	8	CW Loch Maree	160	184	260	262	113	113	270	270	439	439	170	170	309	476	181	181	230	230
178	RTA	9	CW Loch Maree	160	184	242	262	113	113	282	290	439	439	194	194	309	309	181	185	230	230
179	RTA	13	CW Loch Maree	160	160	274	304	113	113	262	290	439	439	182	210	296	307	181	181	230	233
180	RTA	14	CW Loch Maree	160	172	246	286	113	113	262	270	467	483	194	194	296	477	169	177	233	233
181	RTA	15	CW Loch Maree	132	172	220	270	113	113	270	286	403	499	162	170	296	296	177	181	230	260
182	RTA	16	CW Loch Maree	160	172	256	304	113	113	266	282	379	439	182	198	296	307	177	181	230	230
183	SR	17	Gairloch	160	172	234	270	113	113	262	278	467	499	174	174	302	302	181	181	230	230
184	SR	20	Gairloch	128	128	242	250	119	121	306	326	499	499	126	222	277	321	169	169	230	230
185	SR	21	Gairloch	128	128	208	278	113	119	306	338	475	491	126	226	282	321	169	169	221	230
186	SR	22	Gairloch	160	160	230	250	113	113	262	278	463	499	174	210	302	302	177	181	230	233
187	SR	23	Gairloch	160	160	250	270	113	113	262	262	423	423	174	190	309	309	181	181	230	275
188	STD	1	CW Loch Maree	160	160	254	294	113	113	262	266	463	479	178	182	296	306	181	181	233	233
189	STD	2	CW Loch Maree	160	172	266	282	113	113	266	278	467	479	182	186	296	306	181	181	233	260
190	STD	3	CW Loch Maree	156	160	240	246	113	113	262	270	423	471	186	214	307	307	181	181	230	239
191	TAG	1	SE Loch Maree	132	160	242	262	113	113	270	270	423	443	186	202	304	476	177	181	230	230
192	TAG	2	SE Loch Maree	160	160	246	262	113	113	270	270	387	463	186	190	306	306	169	181	230	230

Abbreviations: NPIL, No peaks in locus.

APPENDIX 4 ALLELE FREQUENCIES

Appendix 4.1 Population unique alleles and their frequencies found within Wester Ross brown trout populations.

NWLM	A_f	CWLM	A_f	SELM	A_f	WCG	A_f
148(1)	0.014	184(1)	0.047	292(2)	0.038	128(1)	0.057
226(2)	0.014	236(2)	0.016	296(2)	0.019	208(2)	0.014
268(2)	0.014	256(2)	0.063	294(4)	0.019	248(2)	0.029
298(2)	0.014	260(2)	0.047	383(5)	0.019	244(2)	0.014
230(6)	0.028	290(2)	0.031	387(5)	0.019	105(3)	0.014
?(6)	0.028	312(2)	0.016	395(5)	0.019	119(3)	0.029
298(7)	0.014	117(3)	0.031	487(5)	0.058	121(3)	0.014
297(7)	0.028	379(5)	0.016	328(7)	0.077	246(4)	0.014
263(9)	0.028	503(5)	0.063	478(7)	0.019	306(4)	0.029
Mean:	**0.020**	162(6)	0.016	**Mean:**	**0.032**	326(4)	0.014
SE:	**0.002**	**Mean:**	**0.034**	**SE:**	**0.007**	338(4)	0.014
SD:	**0.007**	**SE:**	**0.006**	**SD:**	**0.022**	459(5)	0.129
		SD:	**0.019**			475(5)	0.014
						495(5)	0.029
						?(5)	0.200
						126(6)	0.029
						150(6)	0.014
						275(7)	0.014
						277(7)	0.014
						282(7)	0.014
						321(7)	0.029
						221(9)	0.014
						275(9)	0.029
						Mean:	**0.034**
						SE:	**0.009**
						SD:	**0.044**

Almost population specific alleles had a frequency < 7 % except for alleles 459(5) and ?(5) which had frequencies of 12.9 and 20 % (*Appendix 4.1*).

Appendix 4.2 Population unique alleles and their frequencies found shared pairwise between Wester Ross resident trout populations.

	CWLM	*Y f*	*X f*	SELM	*Y f*	*X f*	WCG	*Y f*	*X f*
NWLM	240(2)	0.014	0.031	399(5)	0.014	0.096	234(2)	0.014	0.171
	258(2)	0.014	0.031	419(5)	0.069	0.135	491(5)	0.014	0.014
	479(5)	0.042	0.109	218(6)	0.028	0.115	158(6)	0.014	0.071
	480(7)	0.014	0.016	**Mean:**	**0.037**	**0.115**	315(7)	0.097	0.286
	?(7)	0.028	0.031	**SE:**	**0.017**	**0.011**	319(7)	0.028	0.114
	Mean:	**0.022**	**0.044**	**SD:**	**0.029**	**0.019**	173(8)	0.028	0.057
	SE:	**0.006**	**0.017**				248(9)	0.014	0.043
	SD:	**0.012**	**0.037**				**Mean:**	**0.030**	**0.108**
							SE:	**0.012**	**0.035**
							SD:	**0.030**	**0.094**
CWLM	-	-	-	144(1)	0.016	0.096	156(1)	0.016	0.014
				272(2)	0.016	0.019	111(3)	0.016	0.071
				286(2)	0.078	0.077	499(5)	0.016	0.071
				288(2)	0.031	0.019	226(6)	0.063	0.014
				294(2)	0.016	0.019	**Mean:**	**0.027**	**0.043**
				304(2)	0.031	0.019	**SE:**	**0.012**	**0.016**
				403(5)	0.078	0.154	**SD:**	**0.023**	**0.033**
				477(7)	0.016	0.019			
				Mean:	**0.035**	**0.053**			
				SE:	**0.010**	**0.018**			
				SD:	**0.027**	**0.051**			
SELM	-	-	-	-	-	-	250(2)	0.058	0.043
							222(6)	0.019	0.014
							Mean:	**0.038**	**0.029**
							SE:	**0.019**	**0.014**
							SD:	**0.027**	**0.020**

Alleles and their frequencies that were found shared pairwise within WR resident populations.

Appendix 4.3 Population unique alleles and their frequencies found shared between Loch Maree resident trout populations.

A(Locus)	NWLM	CWLM	SELM
220(2)	0.014	0.047	0.019
266(2)	0.097	0.094	0.308
435(5)	0.069	0.047	0.019
439(5)	0.083	0.219	0.058
451(5)	0.097	0.031	0.019
483(5)	0.028	0.078	0.038
170(6)	0.014	0.063	0.038
214(6)	0.014	0.016	0.058
304(7)	0.042	0.094	0.115
476(7)	0.069	0.078	0.077
239(9)	0.042	0.016	0.038
Mean:	**0.052**	**0.071**	**0.072**
SE:	**0.010**	**0.017**	**0.025**
SD:	**0.033**	**0.056**	**0.083**

Alleles and their frequencies found shared within all Loch Maree Populations.

APPENDIX 5 DEPARTURES FROM HARDY–WEINBERG EQUILIBRIUM

Test parameters
Guo, S. and Thompson, E. (1992) and Levene H. (1949)
Exact test using a Markov chain (for all Loci):
Forecasted chain length:1000000 Dememorization steps:100000

Appendix 5.1 Full list of test results for departures from HWE, calculated using Arlequin 3.5.2.2

Population	Locus	*n*	H_E	H_O	P-value	SD	HWE	*n* Permutations
NWLM	1	36	0.52778	0.59703	0.41649	0.00050	HWE	1001000
NWLM	2	36	0.77778	0.91041	0.01257	0.00009	DE	1001000
NWLM	3	This locus is monomorphic						
NWLM	4	36	0.61111	0.79264	0.01823	0.00011	DE	1001000
NWLM	5	36	0.77778	0.94484	0.00849	0.00008	DE	1001000
NWLM	6	35	0.85714	0.87909	0.11586	0.00016	HWE	1001000
NWLM	7	35	0.45714	0.84224	0.00000	0.00000	DE	1001000
NWLM	8	36	0.19444	0.32042	0.00040	0.00002	DE	1001000
NWLM	9	36	0.33333	0.56260	0.00640	0.00008	DE	1001000
		Mean:	0.56706	0.73116	0.07231	0.00013	**2/8**	
		SD:	0.23360	0.21742	0.14426	0.00016	-	
		SE:	0.08259	0.07687	0.05100	0.00006	-	
CWLM	1	32	0.46875	0.49008	0.57309	0.00056	HWE	1001000
CWLM	2	32	0.81250	0.95238	0.00672	0.00003	DE	1001000
CWLM	3	32	0.09375	0.09177	1.00000	0.00000	HWE	1001000
CWLM	4	32	0.65625	0.77431	0.00156	0.00004	DE	1001000
CWLM	5	32	0.71875	0.91518	0.00732	0.00005	DE	1001000
CWLM	6	32	0.71875	0.83532	0.46241	0.00041	HWE	1001000
CWLM	7	31	0.45161	0.83659	0.00000	0.00000	DE	1001000
CWLM	8	32	0.18750	0.20486	0.11702	0.00032	HWE	1001000
CWLM	9	32	0.43750	0.49554	0.47490	0.00050	HWE	1001000
		Mean:	0.50504	0.62178	0.29367	0.00021	**5/9**	
		SD:	0.24685	0.31591	0.35451	0.00023	-	
		SE:	0.08228	0.10530	0.11817	0.00008	-	
SELM	1	26	0.30769	0.54374	0.00069	0.00002	DE	1001000
SELM	2	26	0.80769	0.87406	0.05252	0.00013	HWE	1001000
SELM	3	This locus is monomorphic						
SELM	4	26	0.50000	0.57391	0.64795	0.00051	HWE	1001000
SELM	5	26	0.96154	0.93590	0.07573	0.00008	HWE	1001000
SELM	6	26	0.84615	0.91403	0.01123	0.00009	DE	1001000
SELM	7	26	0.42308	0.82579	0.00000	0.00000	DE	1001000
SELM	8	26	0.50000	0.57541	0.16715	0.00035	HWE	1001000
SELM	9	26	0.38462	0.53318	0.01373	0.00013	DE	1001000
		Mean:	0.59135	0.72200	0.12113	0.00016	**4/8**	
		SD:	0.24406	0.18023	0.22014	0.00018	-	
		SE:	0.08629	0.06372	0.07783	0.00006	-	
WCG	1	35	0.34286	0.63271	0.00012	0.00001	DE	1001000
WCG	2	35	0.60000	0.86253	0.00000	0.00000	DE	1001000
WCG	3	35	0.22857	0.23768	0.13863	0.00035	HWE	1001000
WCG	4	35	0.68571	0.86418	0.00055	0.00002	DE	1001000
WCG	5	28	0.42857	0.91299	0.00000	0.00000	DE	1001000
WCG	6	35	0.60000	0.90725	0.00000	0.00000	DE	1001000
WCG	7	35	0.57143	0.87536	0.00000	0.00000	DE	1001000
WCG	8	35	0.31429	0.47495	0.00499	0.00007	DE	1001000
WCG	9	35	0.25714	0.61905	0.00000	0.00000	DE	1001000
		Mean:	0.44762	0.70963	0.01603	0.00005	**1/9**	
		SD:	0.17023	0.23640	0.04600	0.00011	-	
		SE:	0.05674	0.07880	0.01533	0.00004	-	

Appendix 5.1 Continued

Population	Locus	*n*	H_E	H_O	P-value	SD	HWE	*n* Permutations
ECLL	1	5	0.60000	0.71111	1.00000	0.00000	HWE	1001000
ECLL	2	5	0.60000	0.91111	0.05278	0.00020	HWE	1001000
ECLL	3	This locus is monomorphic						
ECLL	4	5	0.40000	0.77778	0.18880	0.00043	HWE	1001000
ECLL	5	4	0.25000	0.89286	0.00894	0.00010	DE	1001000
ECLL	6	5	1.00000	0.82222	1.00000	0.00000	HWE	1001000
ECLL	7	5	0.60000	0.88889	0.15022	0.00030	HWE	1001000
ECLL	8	5	1.00000	0.77778	1.00000	0.00000	HWE	1001000
ECLL	9	5	0.80000	0.82222	0.89836	0.00028	HWE	1001000
		Mean:	0.65625	0.82550	0.53739	0.00016	**7/8**	
		SD:	0.26651	0.06923	0.47175	0.00016	-	
		SE:	0.09422	0.02448	0.16679	0.00006	-	
FES	1	12	0.58333	0.56159	0.24980	0.00048	HWE	1001000
FES	2	12	1.00000	0.90580	1.00000	0.00000	HWE	1001000
FES	3	12	0.08333	0.08333	1.00000	0.00000	HWE	1001000
FES	4	12	0.58333	0.62681	0.29470	0.00038	HWE	1001000
FES	5	12	0.66667	0.88768	0.01334	0.00008	DE	1001000
FES	6	12	1.00000	0.92391	0.85073	0.00025	HWE	1001000
FES	7	12	0.41667	0.90580	0.00043	0.00002	DE	1001000
FES	8	12	0.66667	0.73551	0.16314	0.00035	HWE	1001000
FES	9	12	0.50000	0.42029	1.00000	0.00000	HWE	1001000
		Mean:	0.61111	0.67230	0.50802	0.00017	**7/9**	
		SD:	0.28260	0.28449	0.44400	0.00019	-	
		SE:	0.09420	0.09483	0.14800	0.00006	-	
EST	1	40	0.77500	0.69399	0.00428	0.00006	DE	1001000
EST	2	40	0.85000	0.94873	0.00726	0.00006	DE	1001000
EST	3	40	0.40000	0.38797	0.12704	0.00031	HWE	1001000
EST	4	40	0.85000	0.86519	0.68112	0.00032	HWE	1001000
EST	5	39	0.94872	0.95937	0.28465	0.00025	HWE	1001000
EST	6	40	0.90000	0.94494	0.06411	0.00012	HWE	1001000
EST	7	40	0.87500	0.92215	0.02970	0.00011	DE	1001000
EST	8	40	0.42500	0.53449	0.01785	0.00011	DE	1001000
EST	9	40	0.70000	0.67500	0.23764	0.00032	HWE	1001000
		Mean:	0.74708	0.77020	0.16152	0.00018	**5/9**	
		SD:	0.20273	0.20822	0.22027	0.00011	-	
		SE:	0.06758	0.06941	0.07342	0.00004	-	
CST	1	6	0.50000	0.59091	1.00000	0.00000	HWE	1001000
CST	2	6	0.83333	0.98485	0.09100	0.00012	HWE	1001000
CST	3	This locus is monomorphic						
CST	4	6	0.50000	0.72727	0.25383	0.00038	HWE	1001000
CST	5	6	0.66667	0.93939	0.08165	0.00016	HWE	1001000
CST	6	6	0.83333	0.93939	0.40193	0.00041	HWE	1001000
CST	7	6	0.50000	0.77273	0.19175	0.00039	HWE	1001000
CST	8	6	1.00000	0.77273	0.86020	0.00033	HWE	1001000
CST	9	6	0.16667	0.16667	1.00000	0.00000	HWE	1001000
		Mean:	0.62500	0.73674	0.48505	0.00022	**8/8**	
		SD:	0.26352	0.26528	0.40276	0.00017	-	
		SE:	0.09317	0.09379	0.14240	0.00006	-	

APPENDIX 6 MUTATION–DRIFT EQUILIBRIUM

Results calculated using online software Bottleneck v1.2.02 (Cornuet and Luikart, 1999).

Sign tests suggest all resident populations are in MDE except NW Loch Maree, though the standardised differences test suggests it is (*Appendix 6.1*). Only 8 polymorphic loci were used for the standardised differences making it less reliable (Cornuet and Luikart, 1999).

Appendix 6.1 Results from sign and standardised differences tests

		Sign test					Standardised differences test			
	n	***n*** k_E H_{exc}	***n k*** H_{def}	***n k*** H_{exc}	**P-value**	**MDE**	***n poly. k***	**T2**	**P-value**	**MDE**
NWLM	36	4.7	5	3	0.19325	E	8	-3.514	0.00022	D
CWLM	32	5.33	9	0	0.00031	D	9	-6.745	0.00000	D
SELM	26	4.71	7	1	0.01021	D	8	-5.812	0.00000	D
WCG	35	5.25	9	0	0.00038	D	9	-5.801	0.00000	D
LEV	5	4.47	2	6	0.22644	E	8	-0.145	0.44225	E
EST	40	5.46	6	3	0.09150	E	9	-4.99	0.00000	D
FST	12	5.17	5	4	0.32149	E	9	-1.102	0.13516	E
CST	6	4.03	4	4	0.63627	E	8	NAN	NAN	NAN?

Abbreviations: *n*, number of samples; *n* k_E H_{exc}, number of loci expected to be in heterozygosity excess; *n k* H_{def}, number of loci in heterozygosity deficit; *n k* H_{exc}, number of loci in heterozygosity excess; MDE, mutation-drift equilibrium; *n poly. K*, number of polymorphic loci; T2, test statistic.

Furthermore, the mode shift analysis disagrees with both sign and standardised differences test (*Appendix 6.2*), suggesting the study's sample size and marker size was insufficient in producing reliable results. Therefore these results are cautionary, with the sign test results holding the most validity.

Appendix 6.2 Results from the mode shift test

		Mode shift	
	n	**Distribution**	**MDE**
NWLM	36	L	E
CWLM	32	L	E
SELM	26	L	E
WCG	35	L	E
LEV	5	Shifted	D
EST	40	L	E
FST	12	L	E
CST	6	L	E

Furthermore, the mode shift analysis contradicts both sign and standardised differences test, suggesting the study's sample size and marker size was insufficient in producing reliable results. Therefore these results are cautionary.

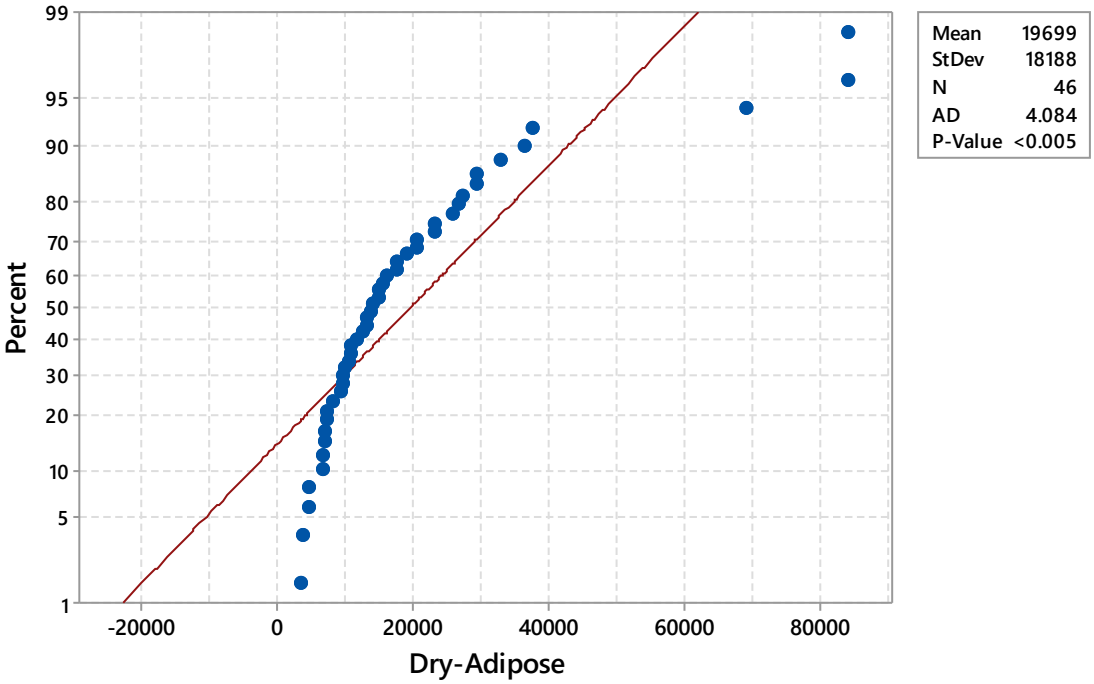
APPENDIX 7 STATISTICAL ANALYSIS

The following are all the intermediate and output results from conducting all the statistical analyses in this project using Minitab®. Presented in the same chronological order as in the thesis.

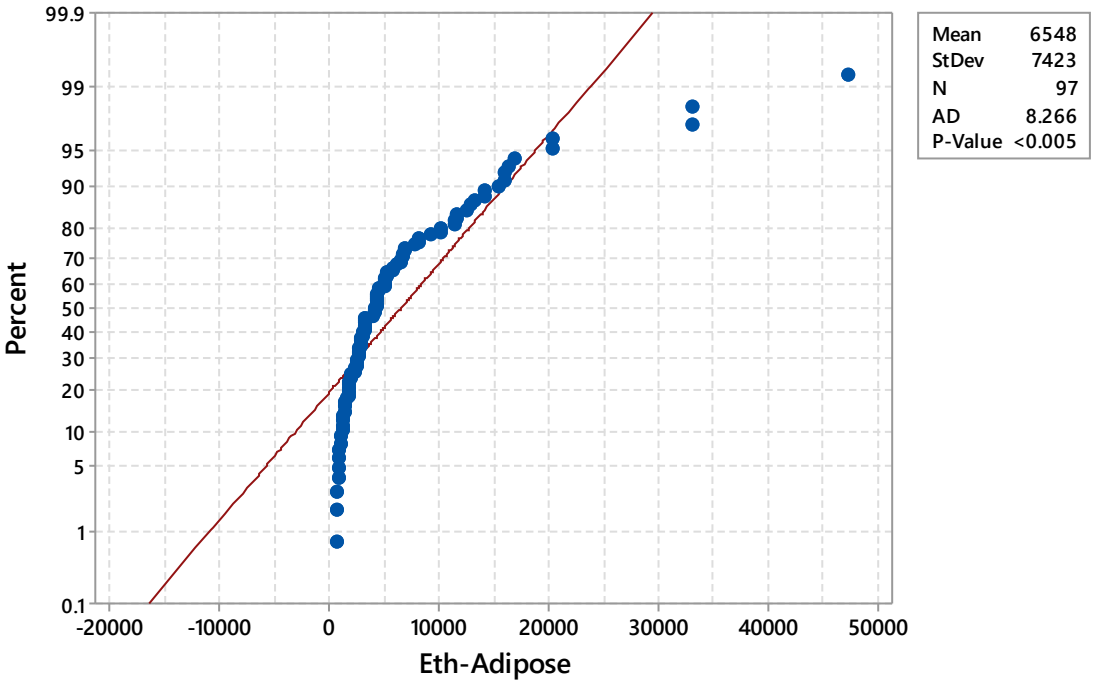
COMPLETE DNA QUALITY ASSESSMENT

APPENDIX 7.1 DNA QUANTITY - NORMALITY TESTS

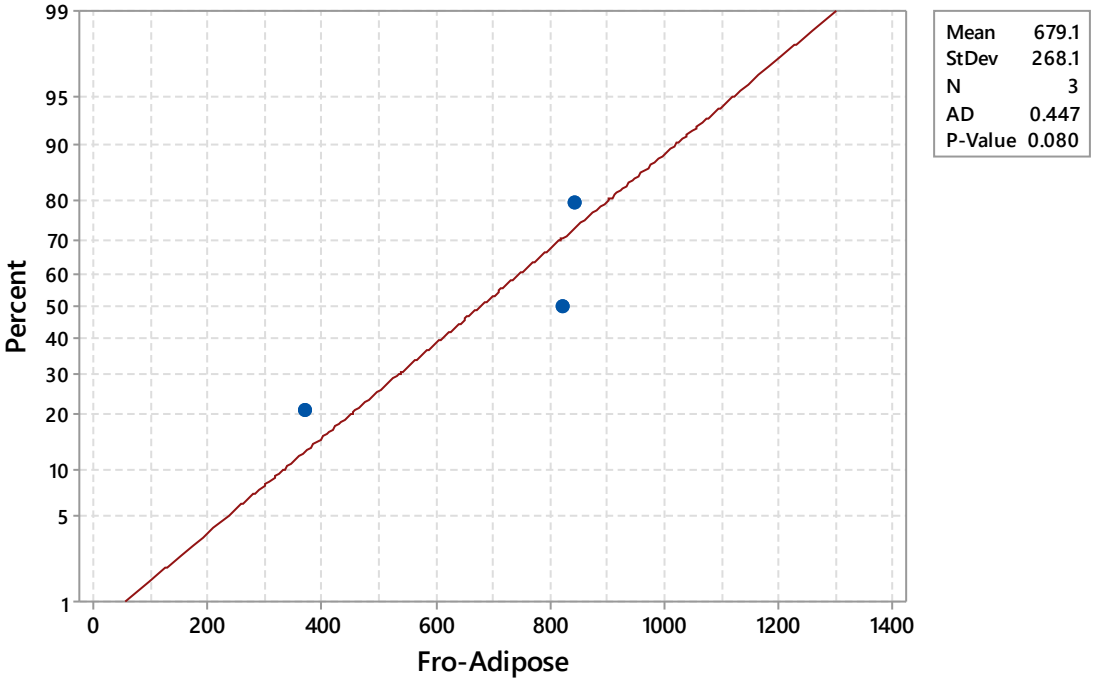
- Only ethanol-pectoral and frozen-adipose fins had normally distributed DNA yields (ng).



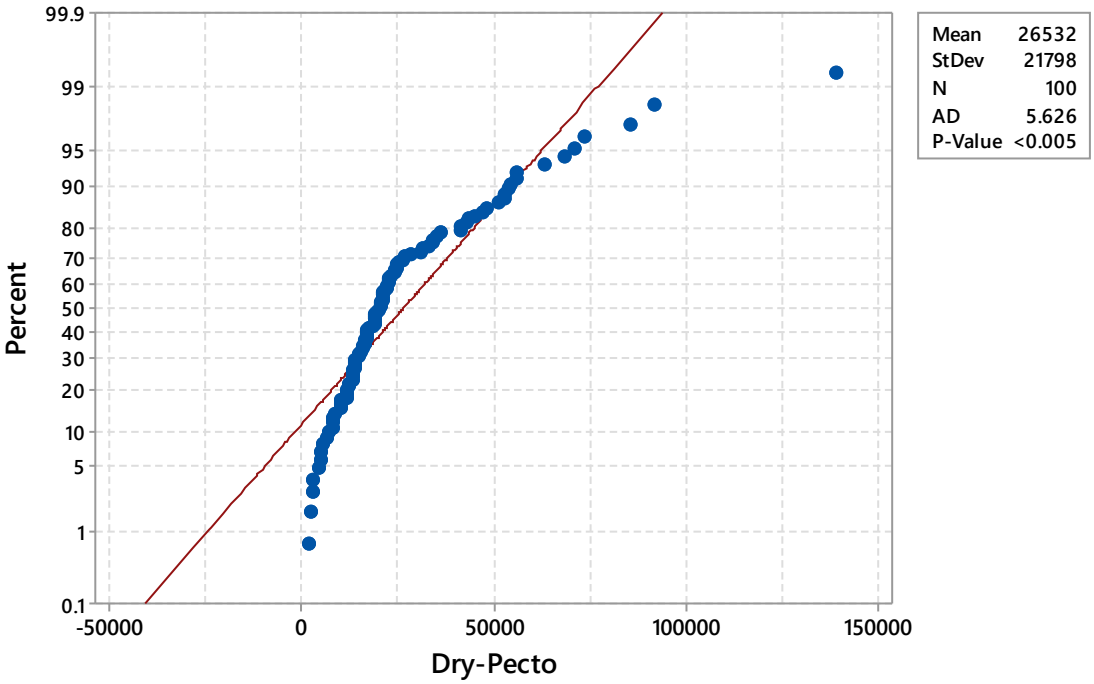
Appendix 7.1.1 Normality test result for Dry-adipose fins DNA yield (ng) = Non-normal distribution



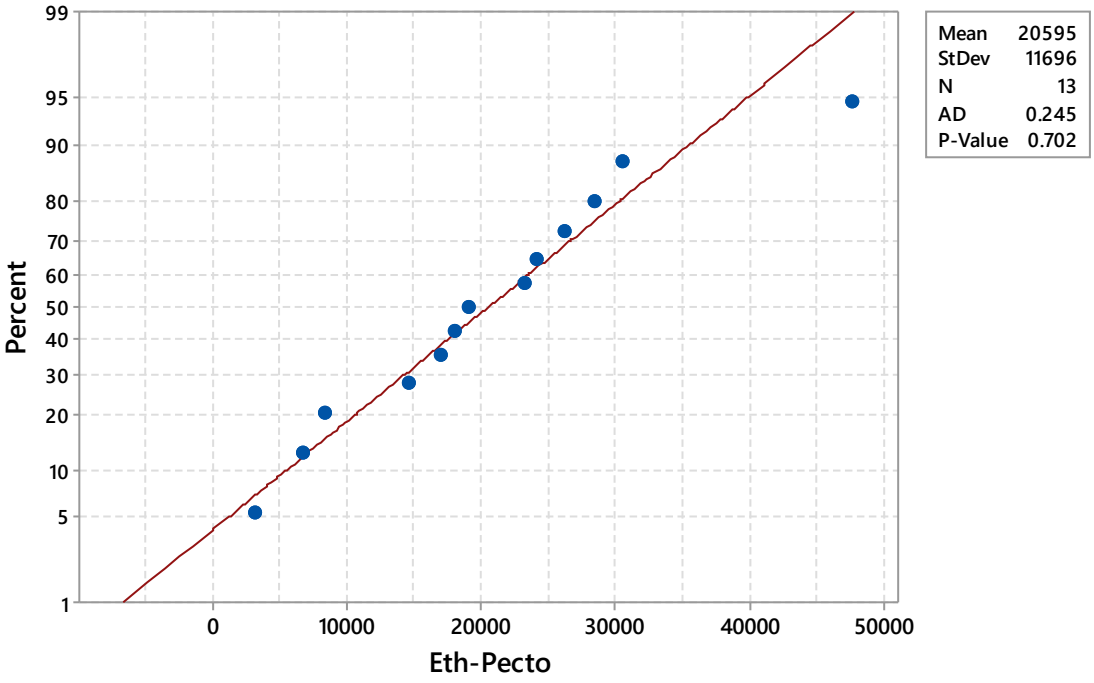
Appendix 7.1.2 Normality test result for Ethanol-adipose fins DNA yield (ng) = Non-normal distribution



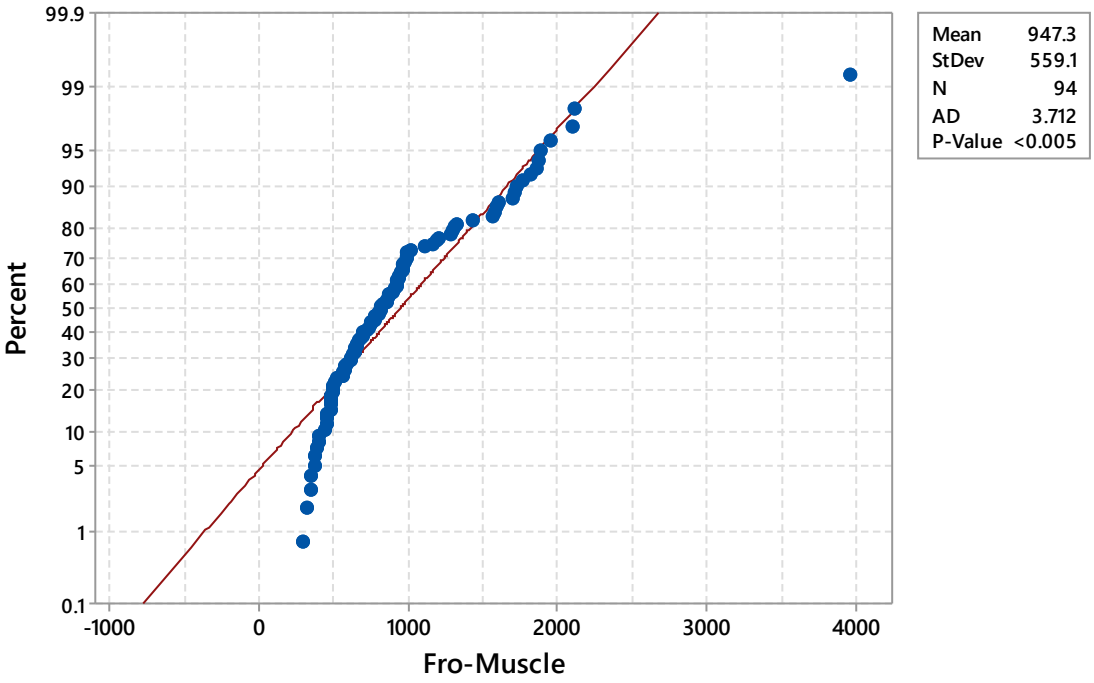
Appendix 7.1.3 Normality test result for Frozen-adipose fins DNA yield (ng) = Normal distribution



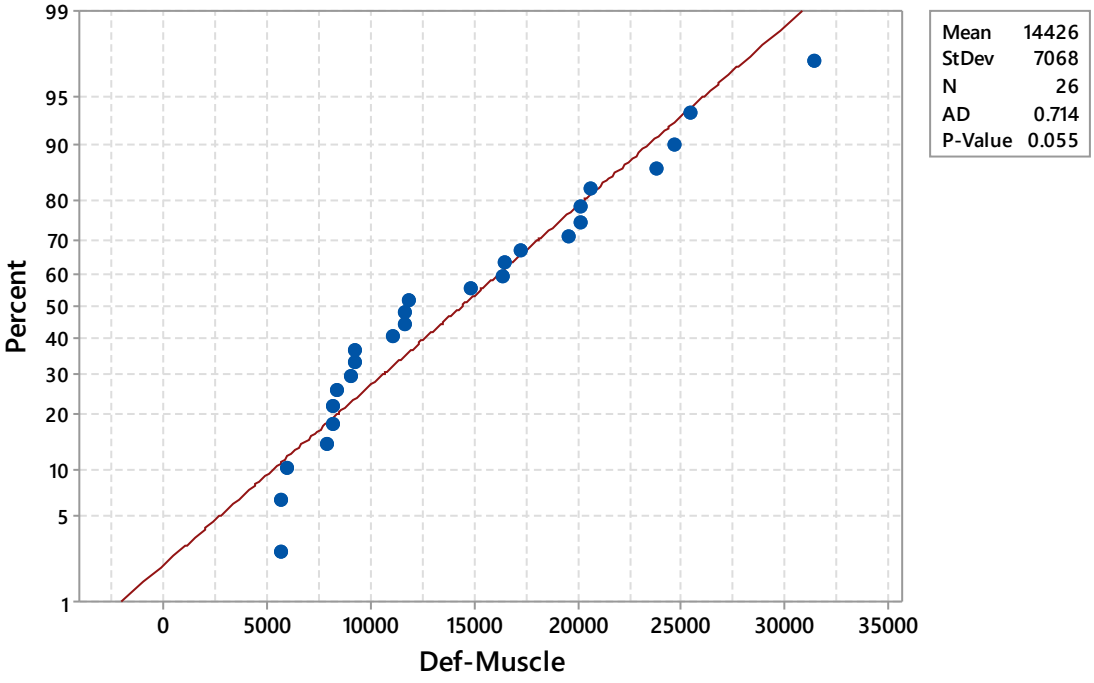
Appendix 7.1.4 Normality test result for Dry-pectoral fins DNA yield (ng) = Non-normal distribution



Appendix 7.1.5 Normality test result for Ethanol-pectoral fins DNA yield (ng) = Normal distribution



Appendix 7.1.6 Normality test result for Frozen-muscle tissue DNA yield (ng) = Non-normal distribution



Appendix 7.1.7 Normality test result for Defrosted-muscle tissue DNA yield (ng) = Normal distribution

APPENDIX 7.2 DNA QUANTITY - MANN–WHITNEY TESTS

Mann-Whitney Test and CI: Dry-Adipose, Eth-Adipose

```
              N  Median
Dry-Adipose  46   14064
Eth-Adipose  97    4256


Point estimate for η1 - η2 is 9026
95.0 Percent CI for η1 - η2 is (6567,11869)
W = 4890.0
Test of η1 = η2 vs η1 ≠ η2 is significant at 0.0000
The test is significant at 0.0000 (adjusted for ties)
```

Mann-Whitney Test and CI: Dry-Pecto, Eth-Pecto

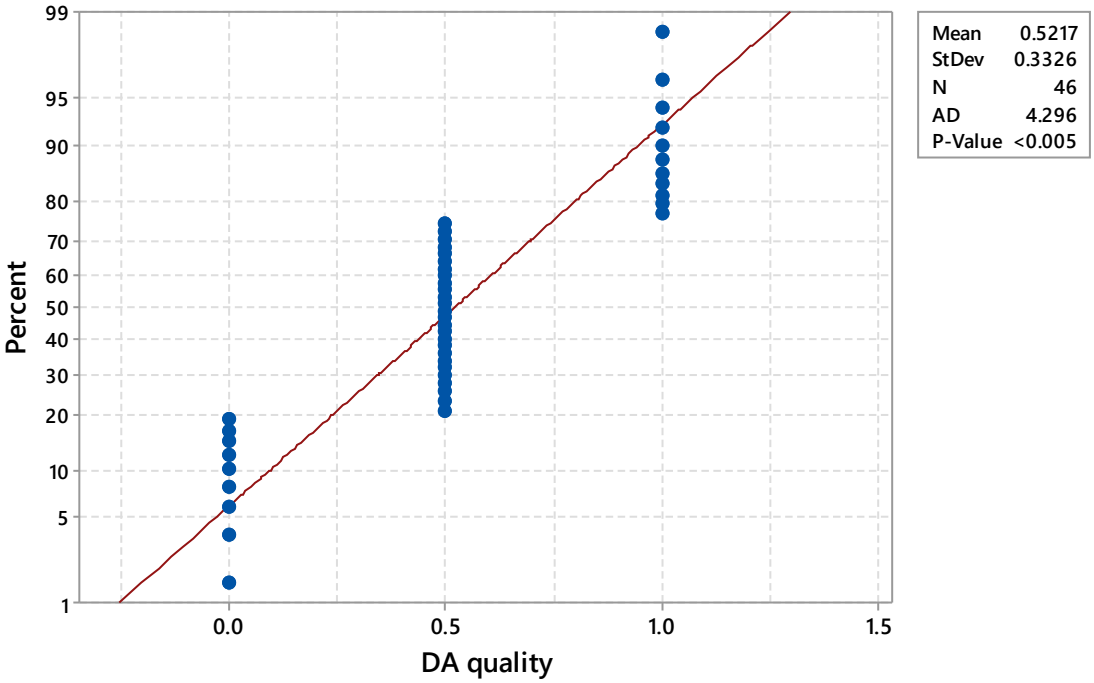
```
              N  Median
Dry-Pecto   100   20199
Eth-Pecto    13   19188


Point estimate for η1 - η2 is 1552
95.1 Percent CI for η1 - η2 is (-5383,10059)
W = 5743.0
Test of η1 = η2 vs η1 ≠ η2 is significant at 0.7021
The test is significant at 0.7021 (adjusted for ties)
```

Mann-Whitney Test and CI: Fro-Muscle, Def-Muscle

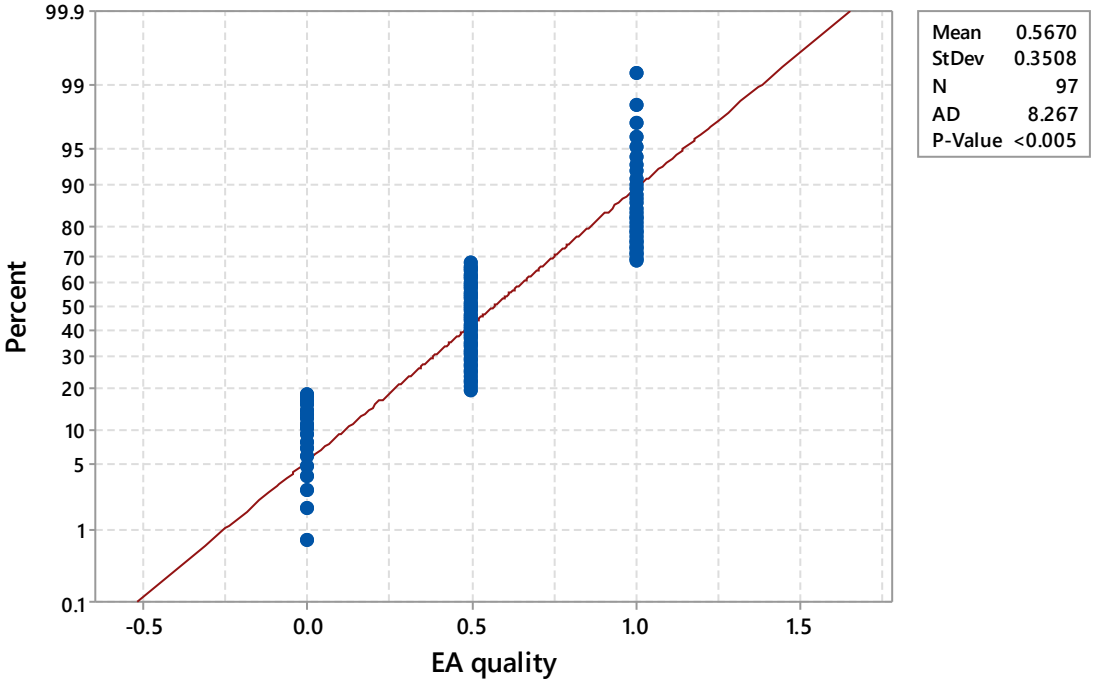
```
              N  Median
Fro-Muscle   94     822
Def-Muscle   26   11756


Point estimate for η1 - η2 is -11060
95.1 Percent CI for η1 - η2 is (-15671,-8794)
W = 4465.0
Test of η1 = η2 vs η1 ≠ η2 is significant at 0.0000
The test is significant at 0.0000 (adjusted for ties)
```

APPENDIX 7.3 DNA INTEGRITY: INTEGRITY INDEX RATINGS - NORMALITY TESTS

- All IIR data sets not normally distributed.

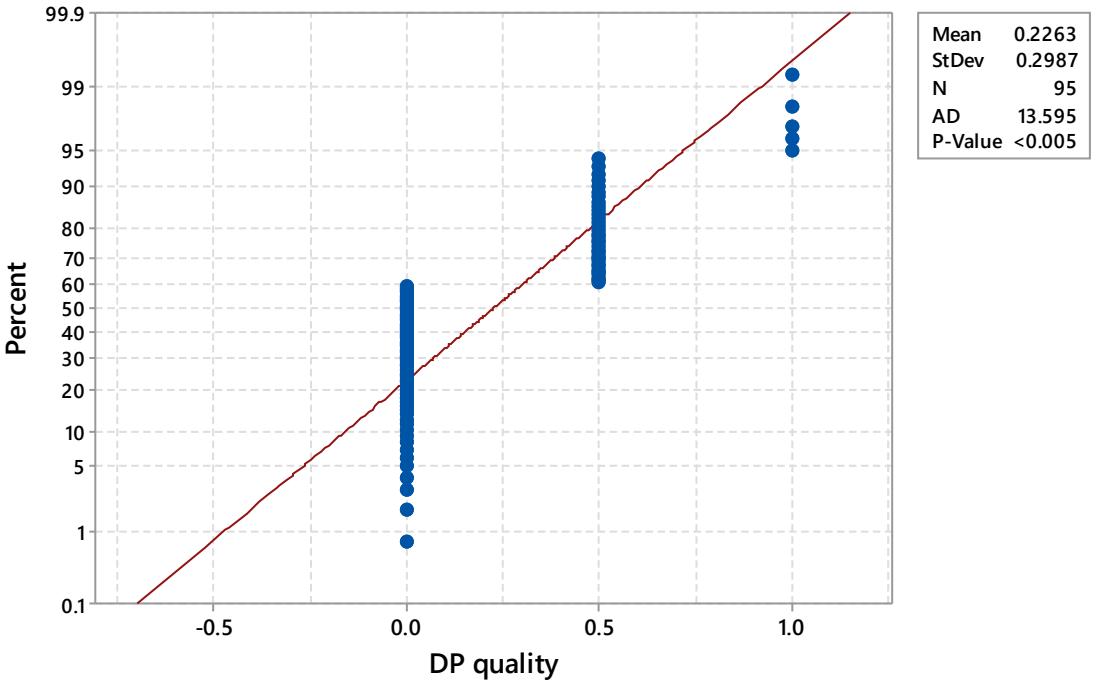


Appendix 7.3.1 Normality test result for Dry-adipose fins DNA IIR = Non-normal distribution



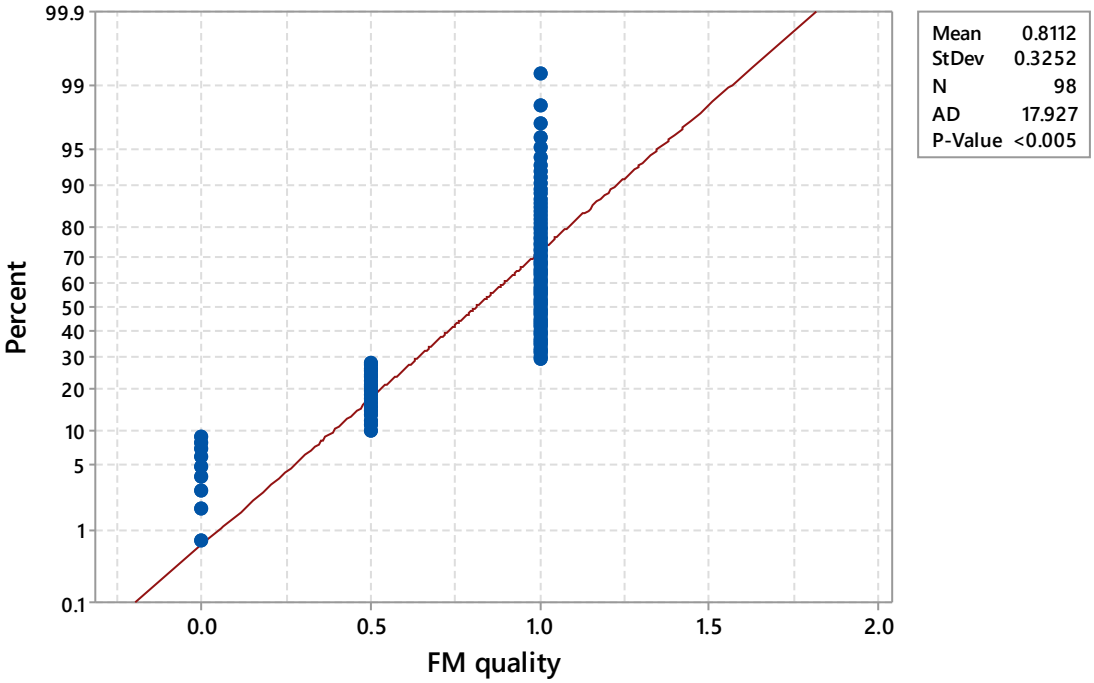
Appendix 7.3.2 Normality test result for Ethanol-adipose fins DNA IIR = Non-normal distribution

Unable to compute normality test for Frozen-adipose fins DNA IIR data – data homogenous.

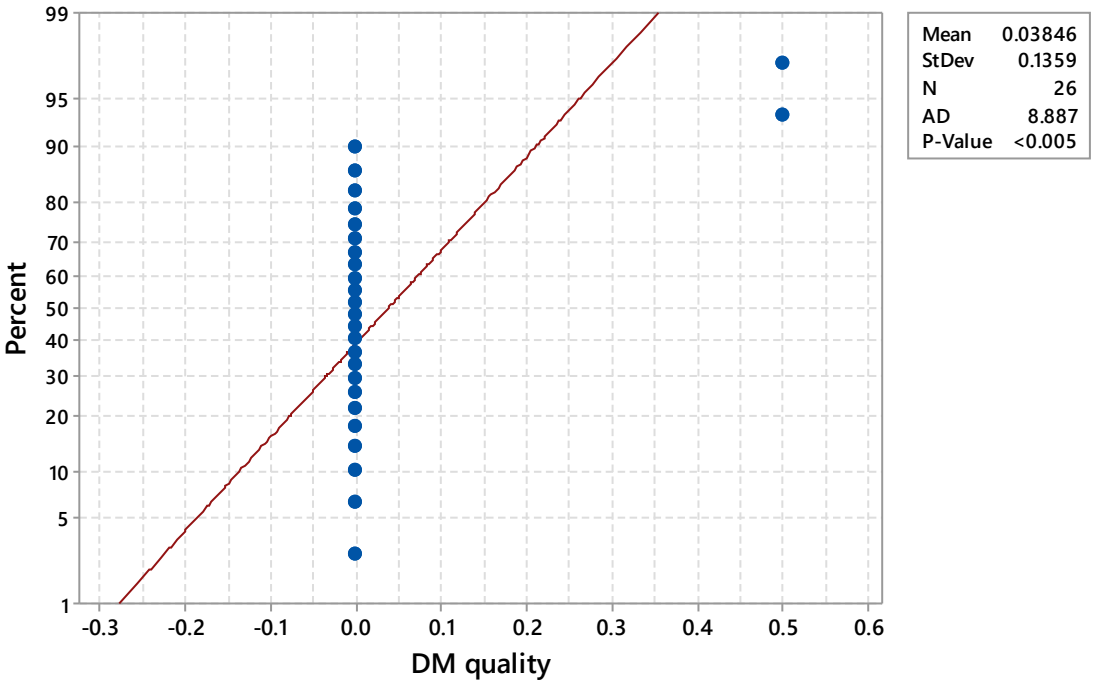


Appendix 7.3.3 Normality test result for Dry-pectoral fins DNA IIR = Non-normal distribution

Unable to compute normality test for Ethanol-pectoral fins DNA IIR data – data homogenous.



Appendix 7.3.4 Normality test result for Frozen-muscle tissue DNA IIR = Non-normal distribution



Appendix 7.3.5 Normality test result for Defrosted-muscle tissue DNA IIR = Non-normal distribution

APPENDIX 7.4 DNA INTEGRITY (IIR) - MANN–WHITNEY TESTS

Mann-Whitney Test and CI: FM quality, DM quality

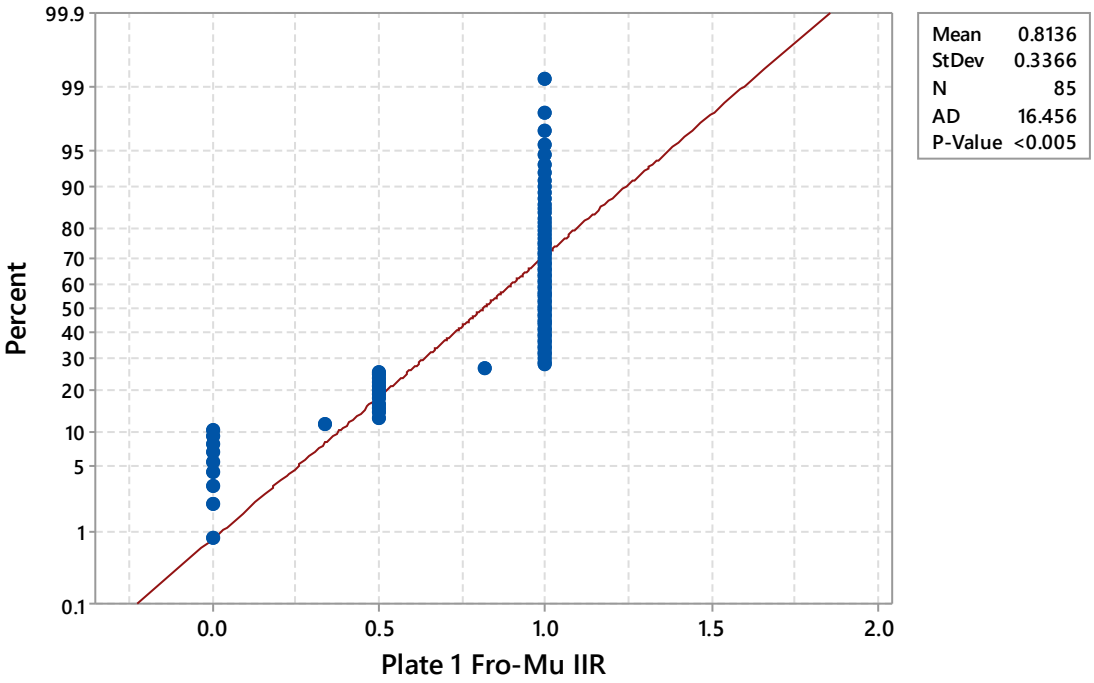
```
              N  Median
FM quality   98  1.0000
DM quality   26  0.0000


Point estimate for η1 - η2 is 1.0000
95.0 Percent CI for η1 - η2 is (1.0000,1.0000)
W = 7254.0
Test of η1 = η2 vs η1 ≠ η2 is significant at 0.0000
The test is significant at 0.0000 (adjusted for ties)
```

Mann-Whitney Test and CI: DA quality, DP quality

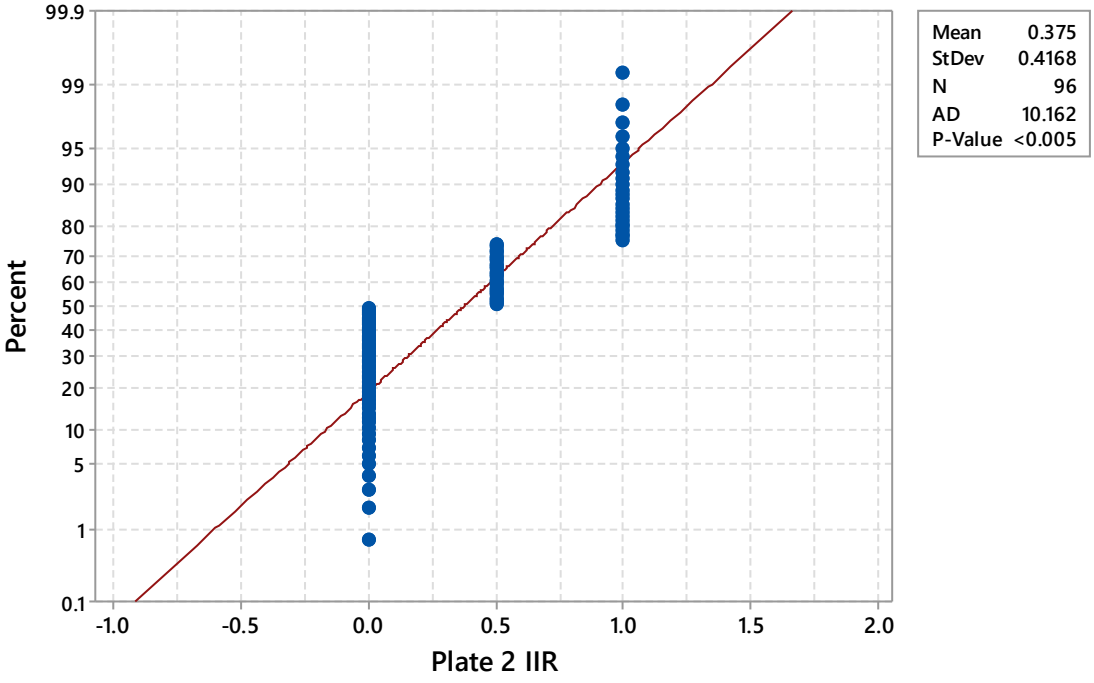
```
              N  Median
DA quality   46  0.5000
DP quality   95  0.0000


Point estimate for η1 - η2 is 0.5000
95.0 Percent CI for η1 - η2 is (-0.0000,0.4999)
W = 4266.0
Test of η1 = η2 vs η1 ≠ η2 is significant at 0.0000
The test is significant at 0.0000 (adjusted for ties)
```

APPENDIX 7.5 PLATE 1 & 2: DNA IIR - NORMALITY TESTS

- IIR data is non-parametric



Appendix 6.5.1 Normality test result for Plate 1 Frozen-muscle tissue DNA IIR = Non-normal distribution



Appendix 6.5.2 Normality test result for Plate 2 adipose and pectoral fin tissue DNA IIR = Non-normal distribution

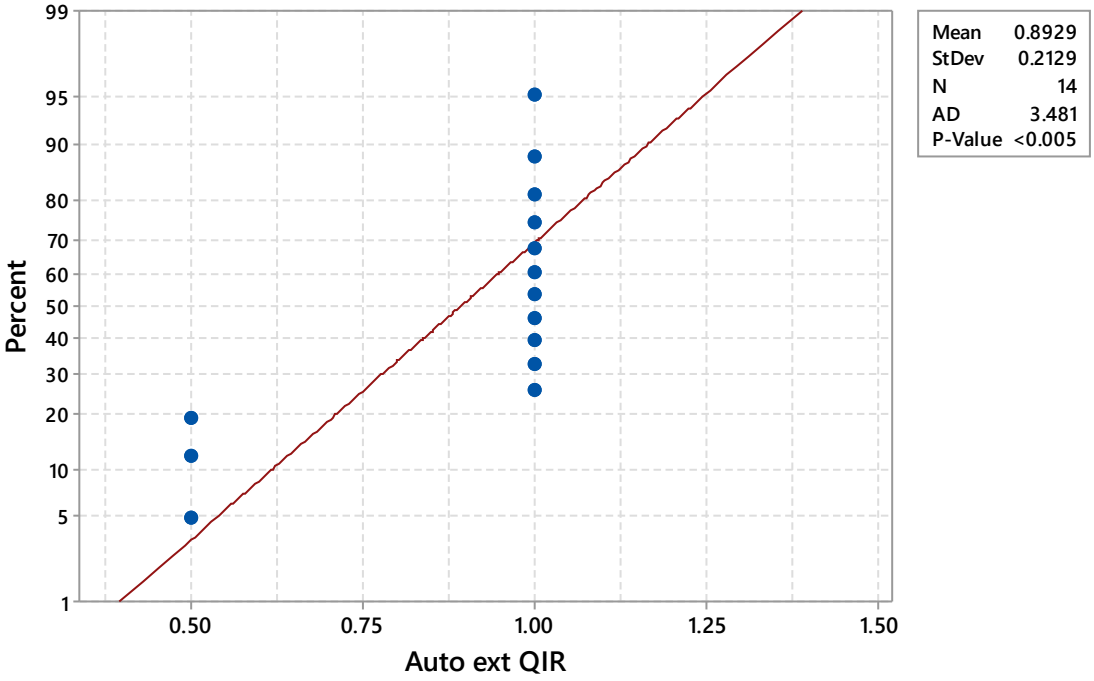
APPENDIX 7.6 PLATE 1 & 2: DNA IIR - MANN–WHITNEY TEST

Mann-Whitney Test and CI: Plate 1 Fro-Mu IIR, Plate 2 IIR

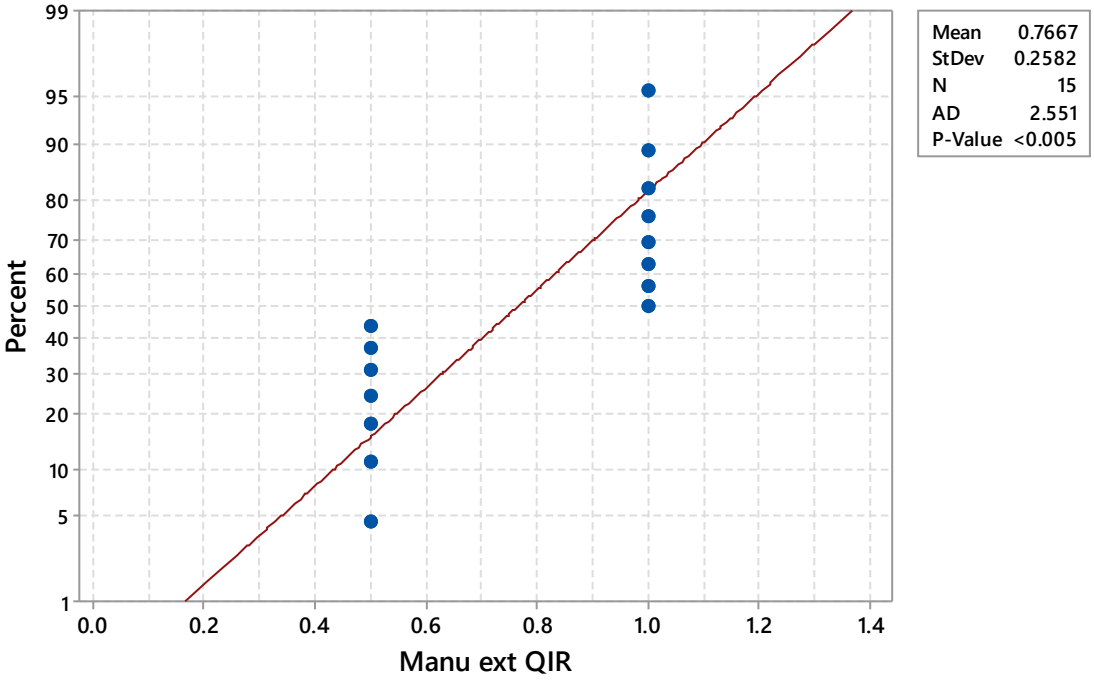
```
                     N  Median
Plate 1 Fro-Mu IIR  85  1.0000
Plate 2 IIR         96  0.2500

Point estimate for η1 - η2 is 0.5000
95.0 Percent CI for η1 - η2 is (0.5000,0.5000)
W = 9919.0
Test of η1 = η2 vs η1 ≠ η2 is significant at 0.0000
The test is significant at 0.0000 (adjusted for ties)
```

APPENDIX 7.7 DNA QUALITY COMPARISON POSTONE CRYO–EXTRACTION: NORMALITY TESTS

- Both IIR data sets not normally distributed.



Appendix 7.7.1 Normality test result for Automated DNA extractions IIR = Non-normal distribution



Appendix 7.7.2 Normality test result for Additional manual DNA extractions IIR = Non-normal distribution

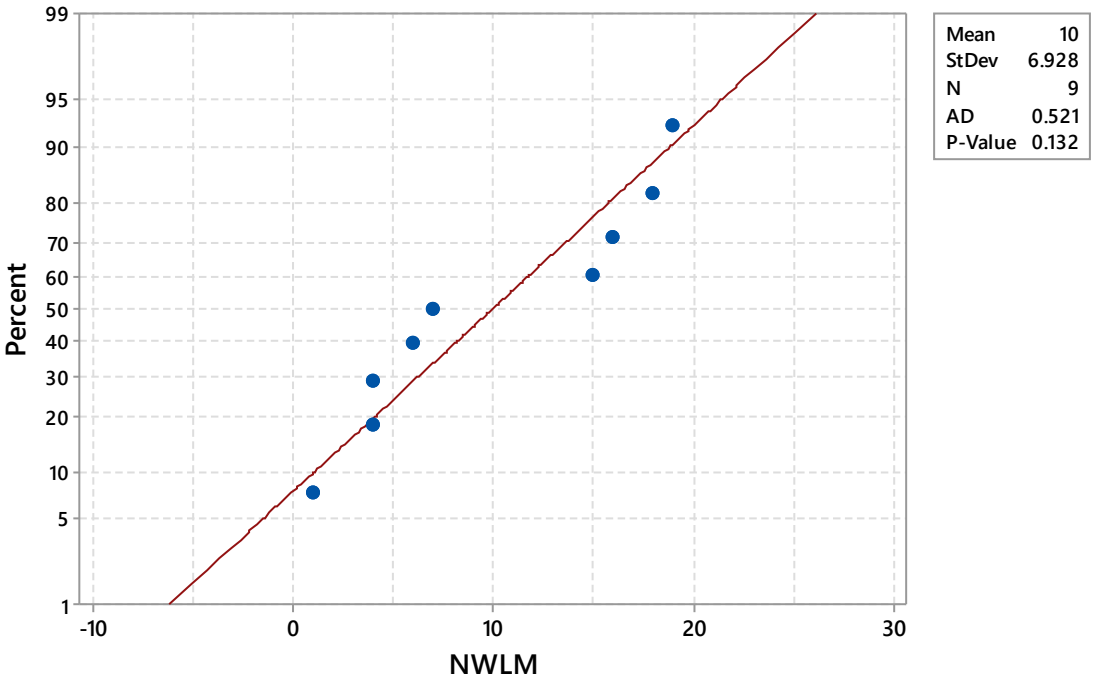
APPENDIX 7.8 DNA QUALITY COMPARISON POSTONE CRYO–EXTRACTION: MANN–WHITNEY TESTS

Mann-Whitney Test and CI: Auto ext IIR, Manu ext IIR

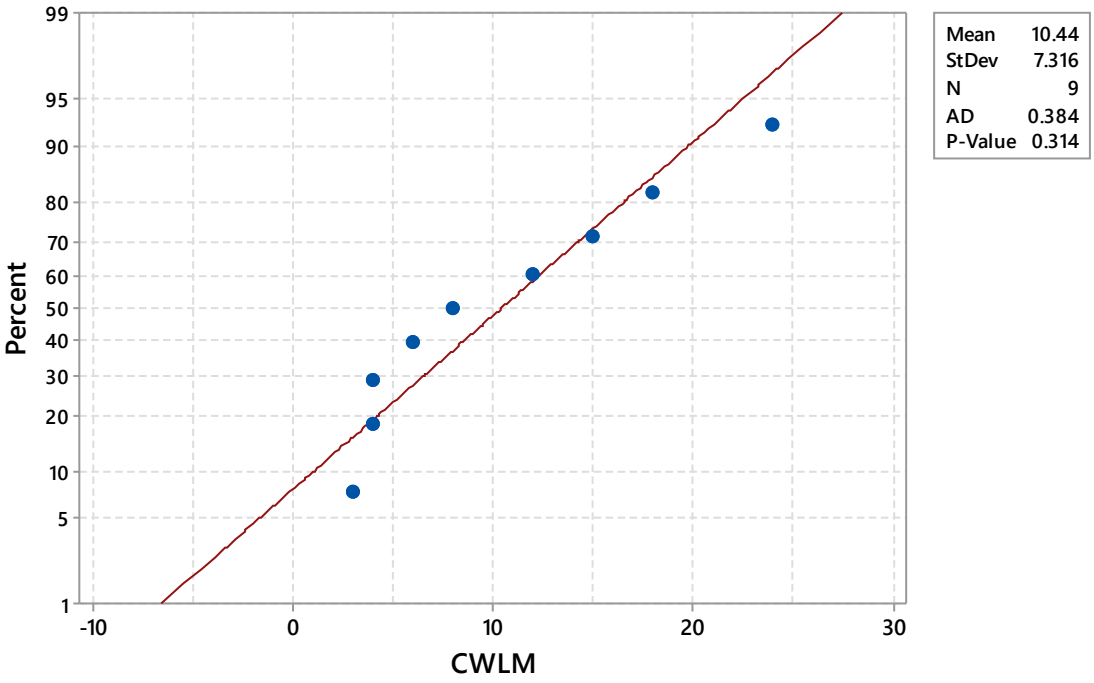
```
               N  Median
Auto ext IIR  14  1.0000
Manu ext IIR  15  1.0000

Point estimate for η1 - η2 is 0.0000
95.3 Percent CI for η1 - η2 is (-0.0001,0.5000)
W = 236.5
Test of η1 = η2 vs η1 ≠ η2 is significant at 0.2565
The test is significant at 0.1684 (adjusted for ties)
```

APPENDIX 7.9 ALLELIC RICHNESS - NORMALITY TESTS

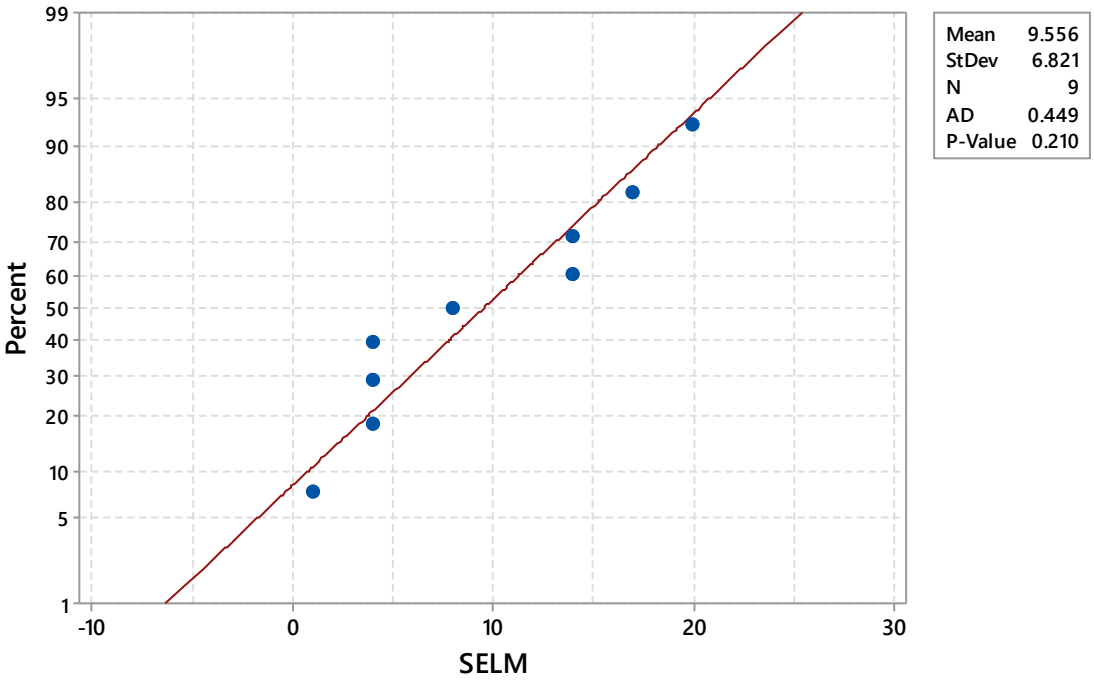
- Allelic richness normally distributed in all data sets except for Gairloch.



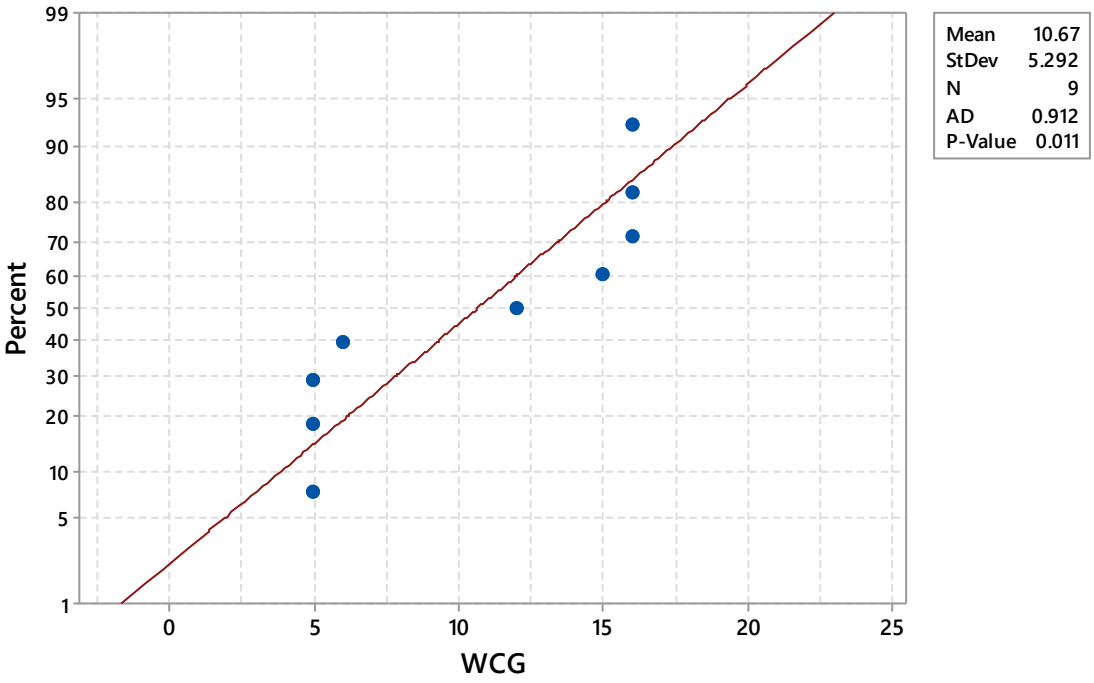
Appendix 7.9.1 Normality test result for NW Loch Maree A_R = Normal distribution



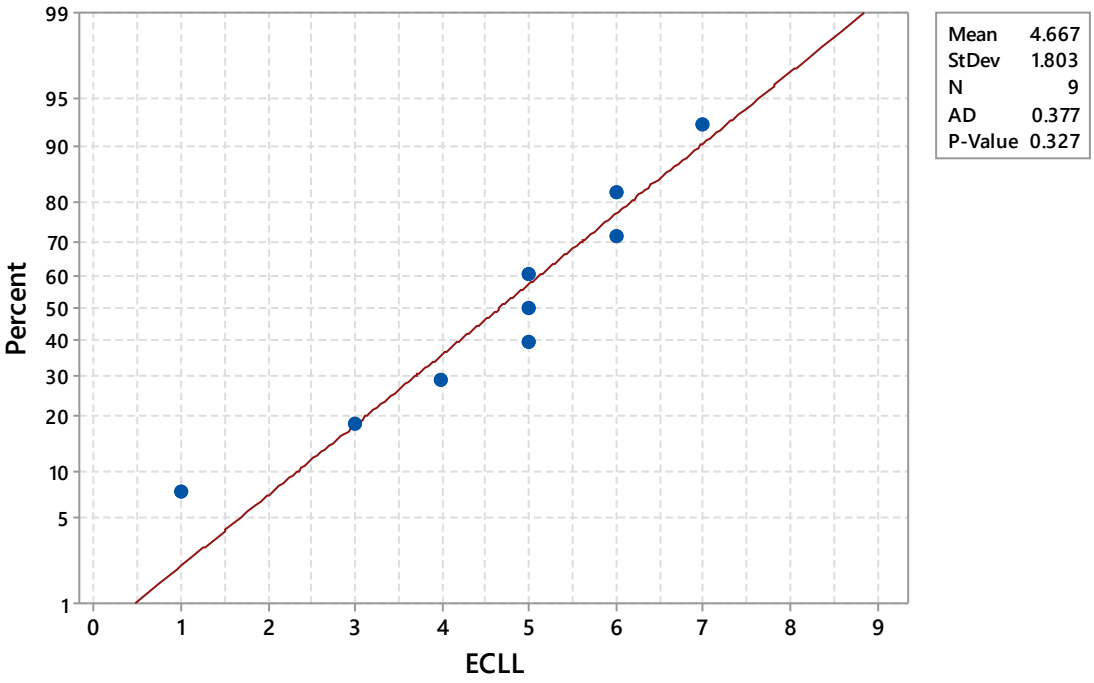
Appendix 7.9.2 Normality test result for CW Loch Maree A_R = Normal distribution



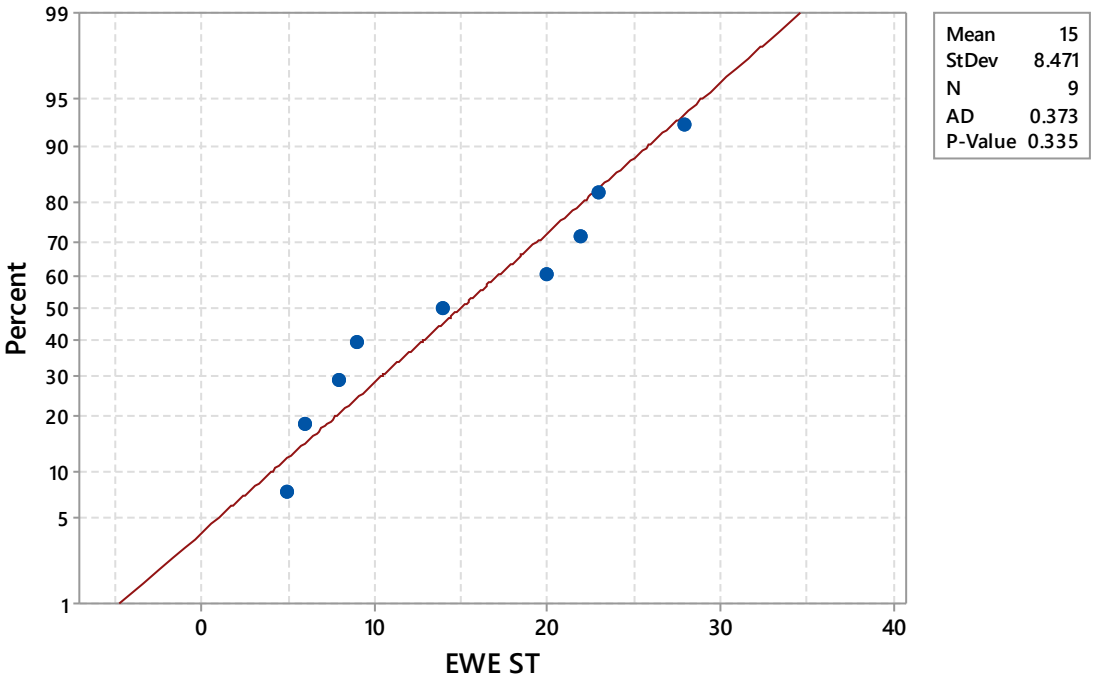
Appendix 7.9.3 Normality test result for SE Loch Maree A_R = Normal distribution



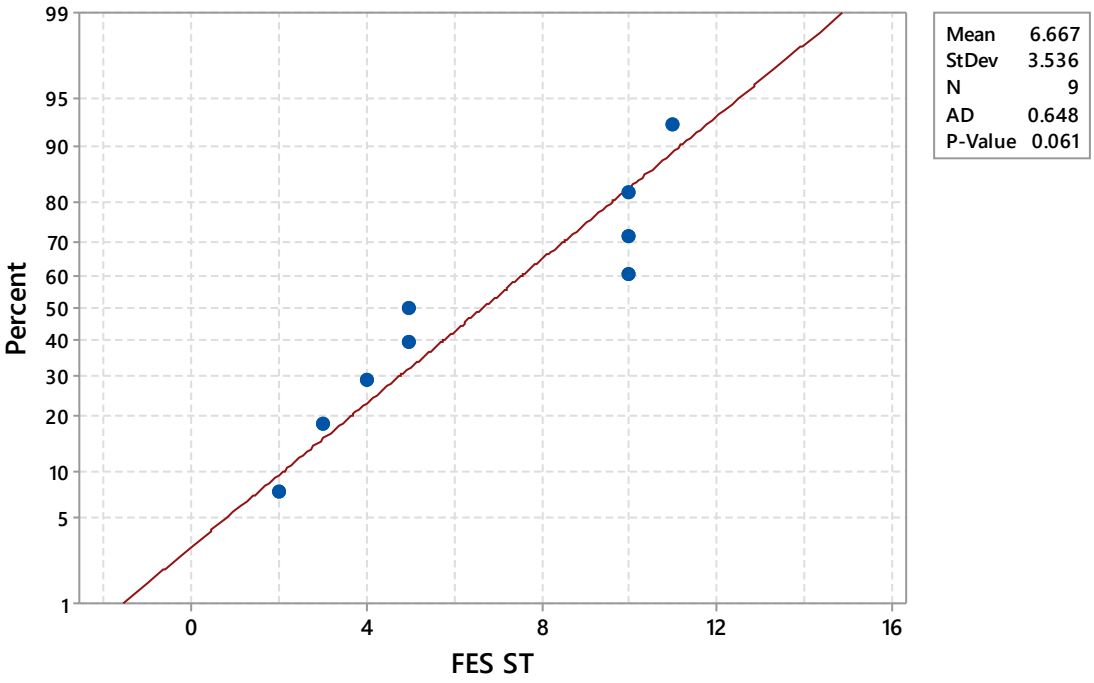
Appendix 7.9.4 Normality test result for Gairloch A_R = Non-normal distribution



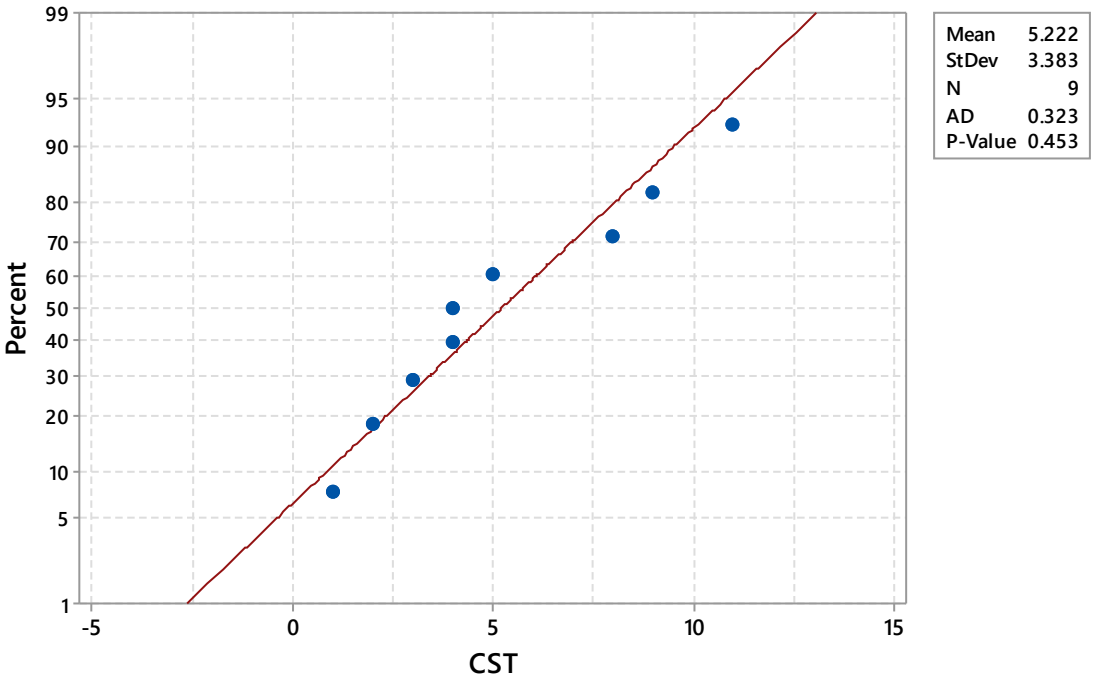
Appendix 7.9.5 Normality test result for Loch Leven A_R = Normal distribution



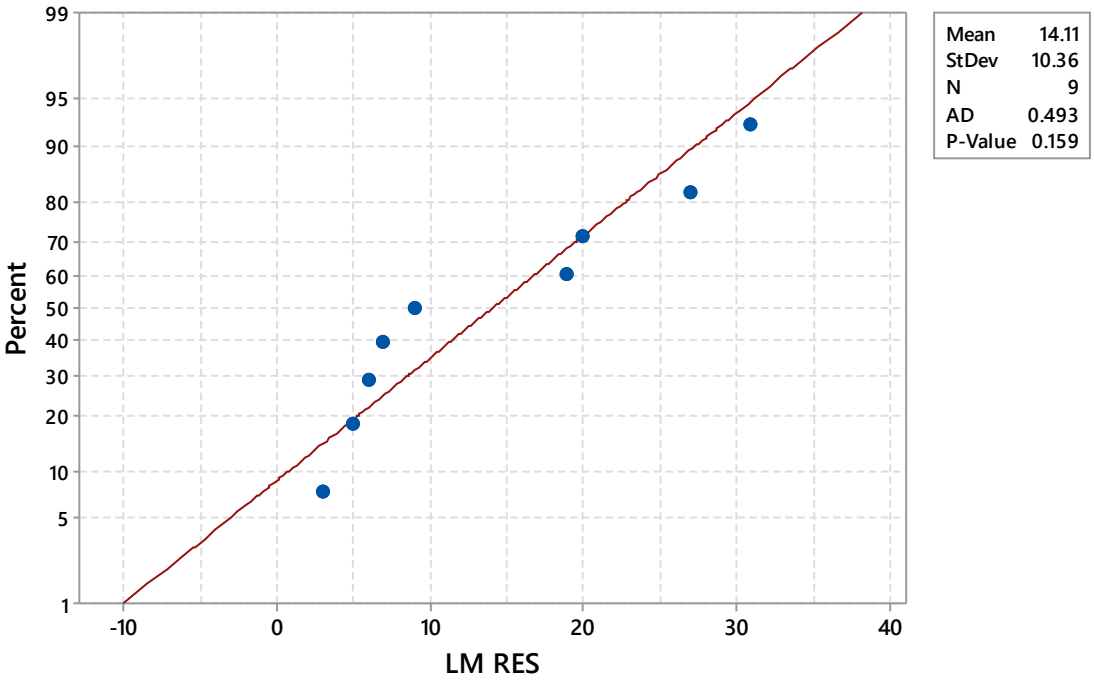
Appendix 7.9.6 Normality test result for Ewe sea trout A_R = Normal distribution



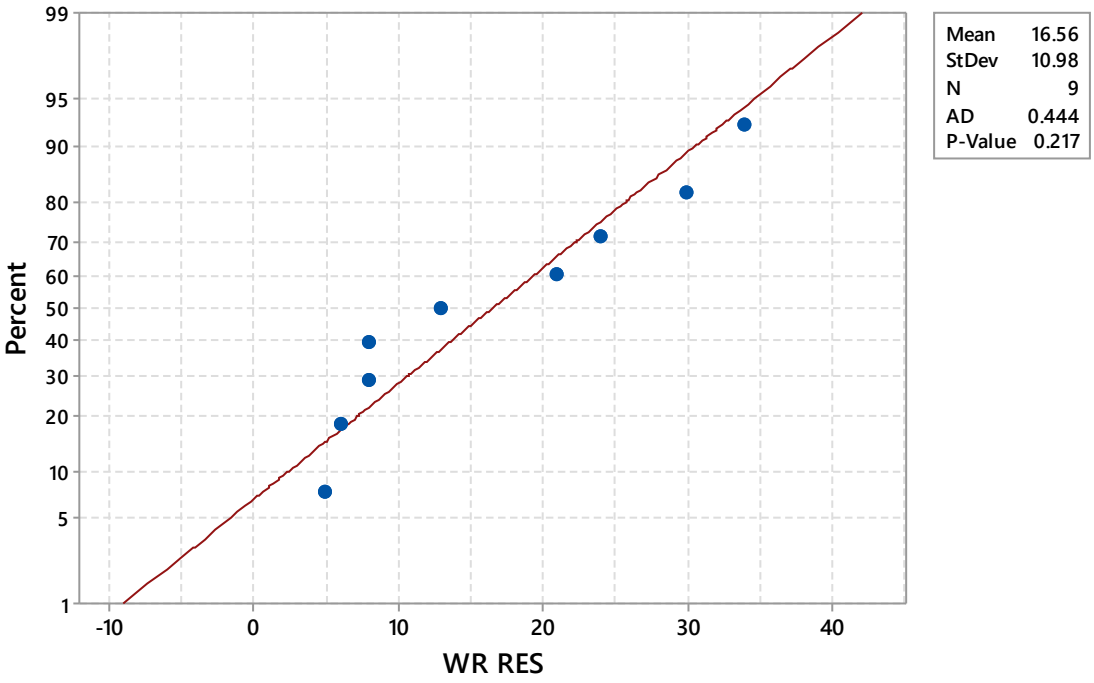
Appendix 7.9.7 Normality test result for Flowerdale sea trout A_R = Normal distribution



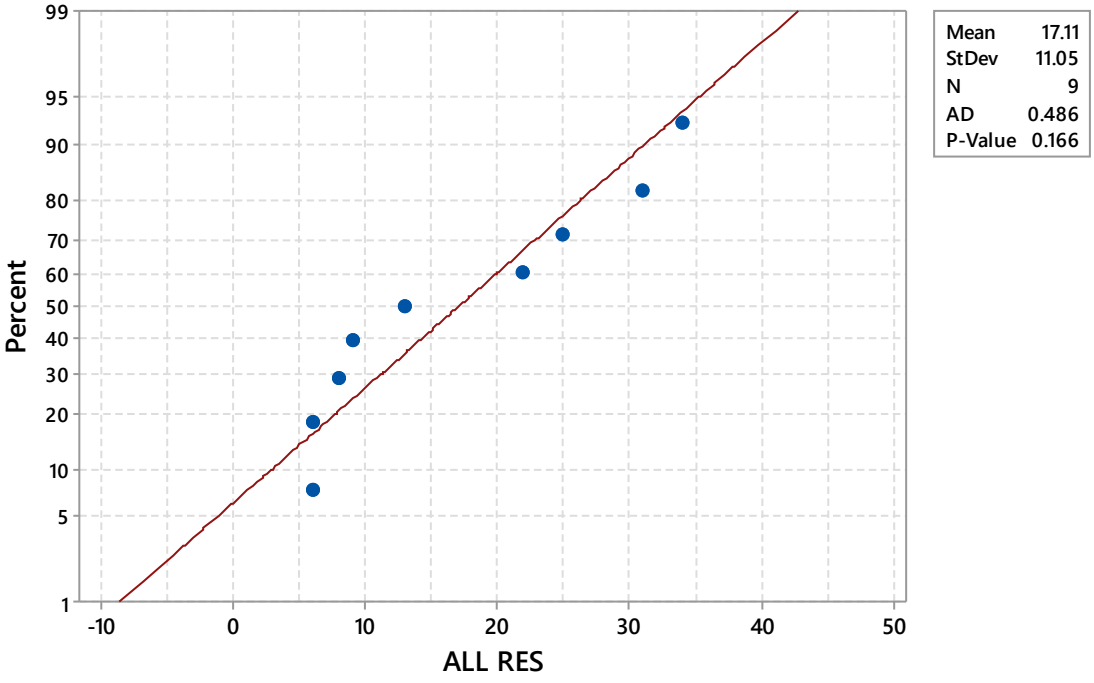
Appendix 7.9.8 Normality test result for Canaird sea trout A_R = Normal distribution



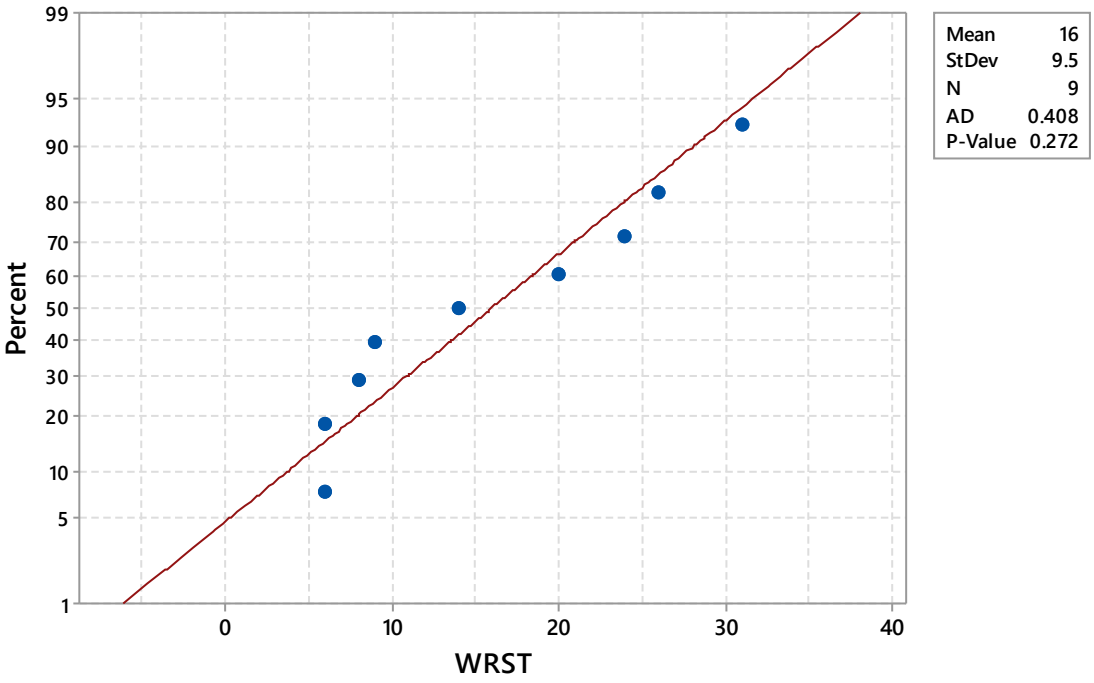
Appendix 7.9.9 Normality test result for coalesced Loch Maree resident trout A_R = Normal distribution



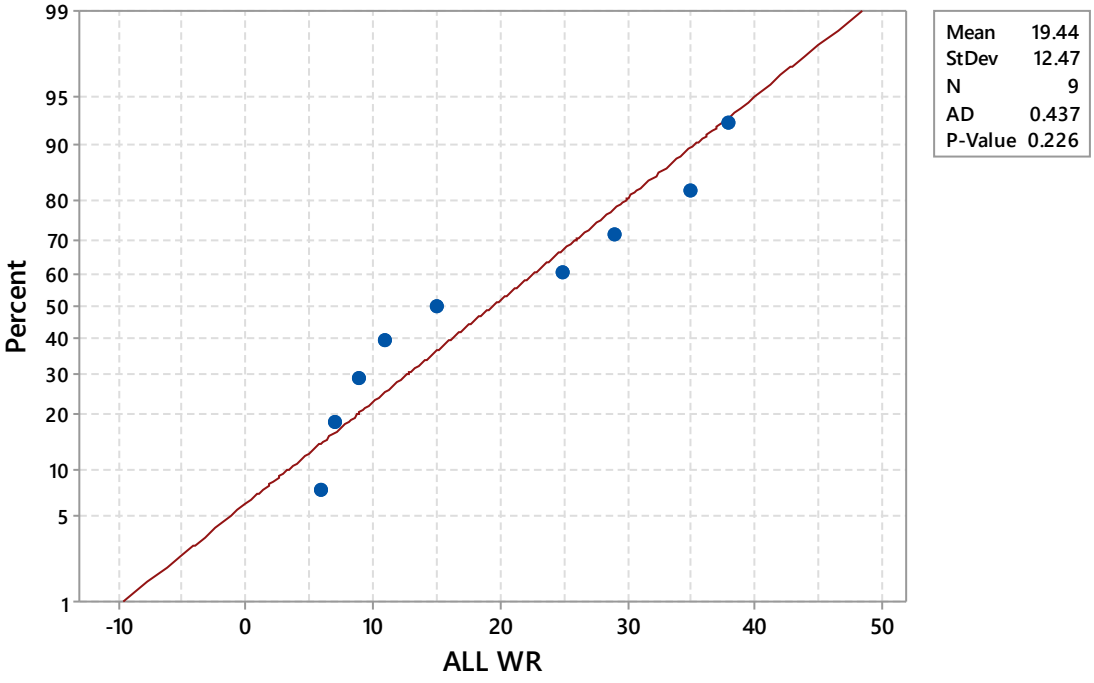
Appendix 7.9.10 Normality test result for coalesced Wester Ross resident trout A_R = Normal distribution



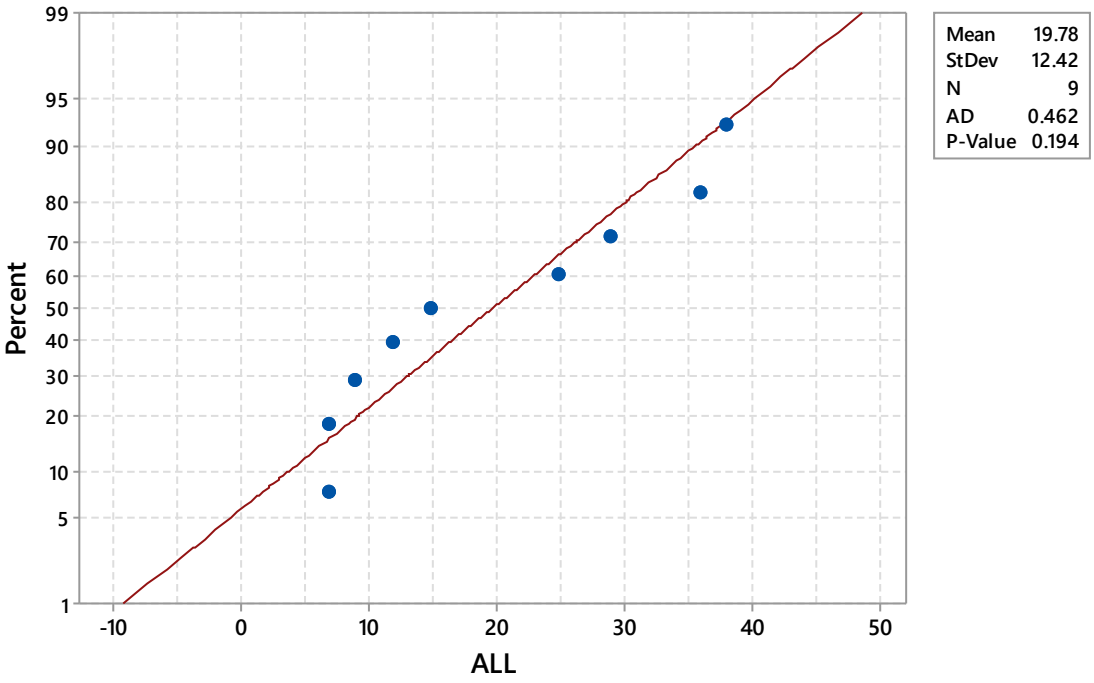
Appendix 7.9.11 Normality test result for coalesced WR and Loch Leven resident trout A_R = Normal distribution



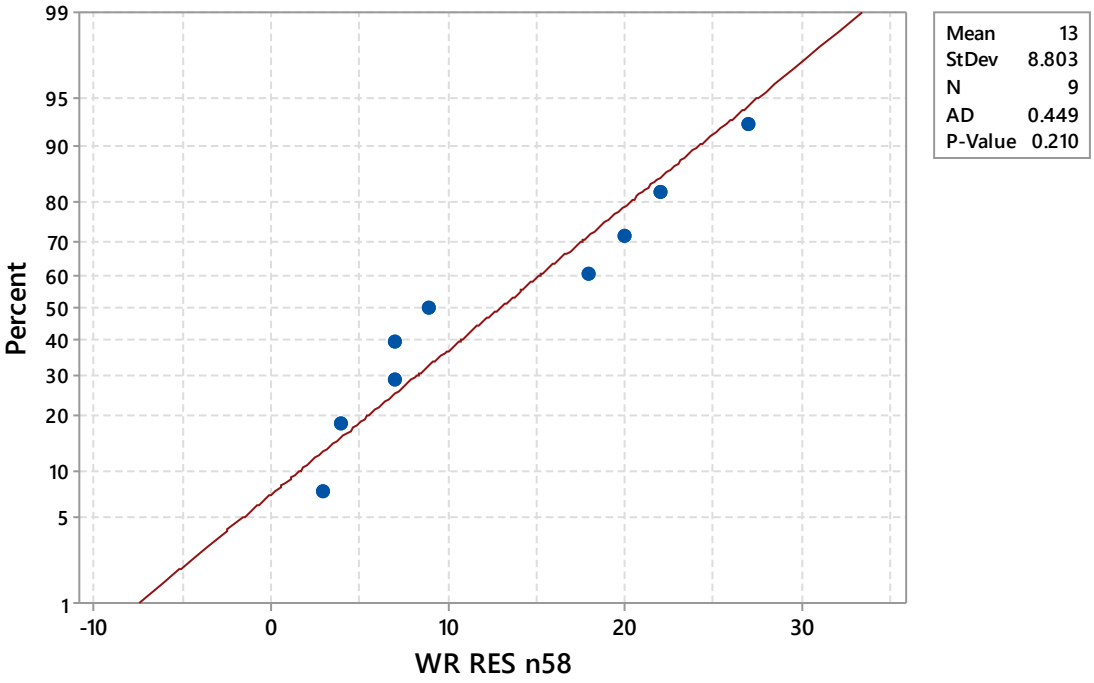
Appendix 7.9.12 Normality test result for coalesced Wester Ross sea trout A_R = Normal distribution



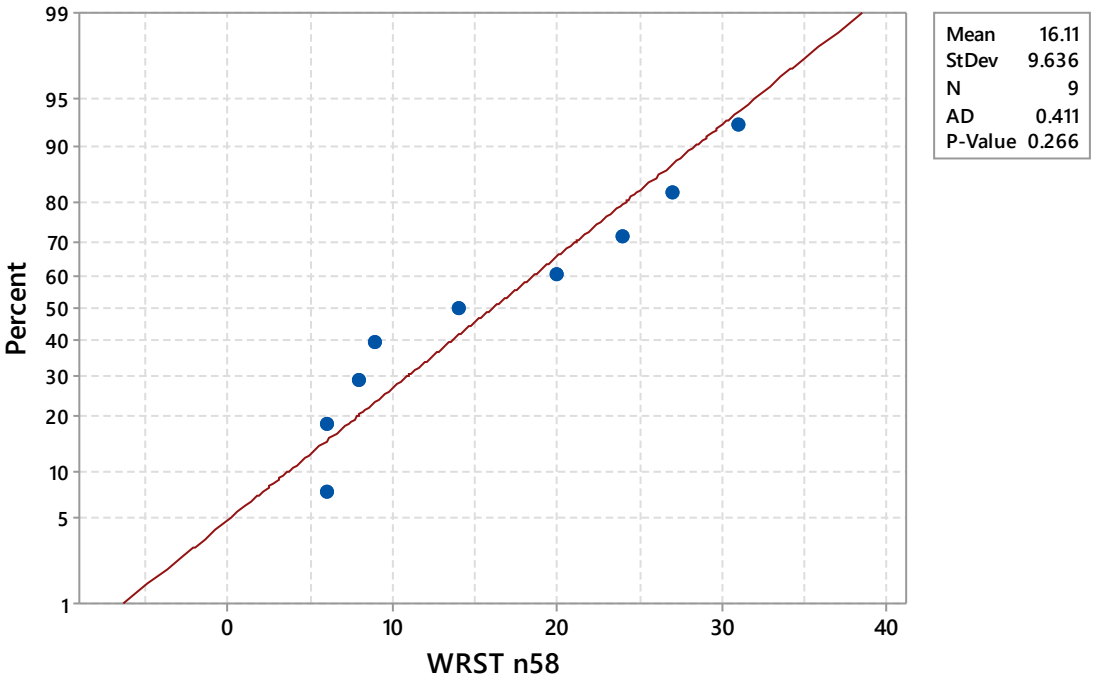
Appendix 7.9.13 Normality test result for coalesced Wester Ross resident and sea trout A_R = Normal distribution



Appendix 7.9.14 Normality test result for coalesced all trout specimens A_R = Normal distribution



Appendix 7.9.15 Normality test result for *n* 58 WR resident trout A_R = Normal distribution



Appendix 7.9.16 Normality test result for *n* 58 WR sea trout A_R = Normal distribution

APPENDIX 7.10 ALLELIC RICHNESS - TESTS FOR DIFFERENCES

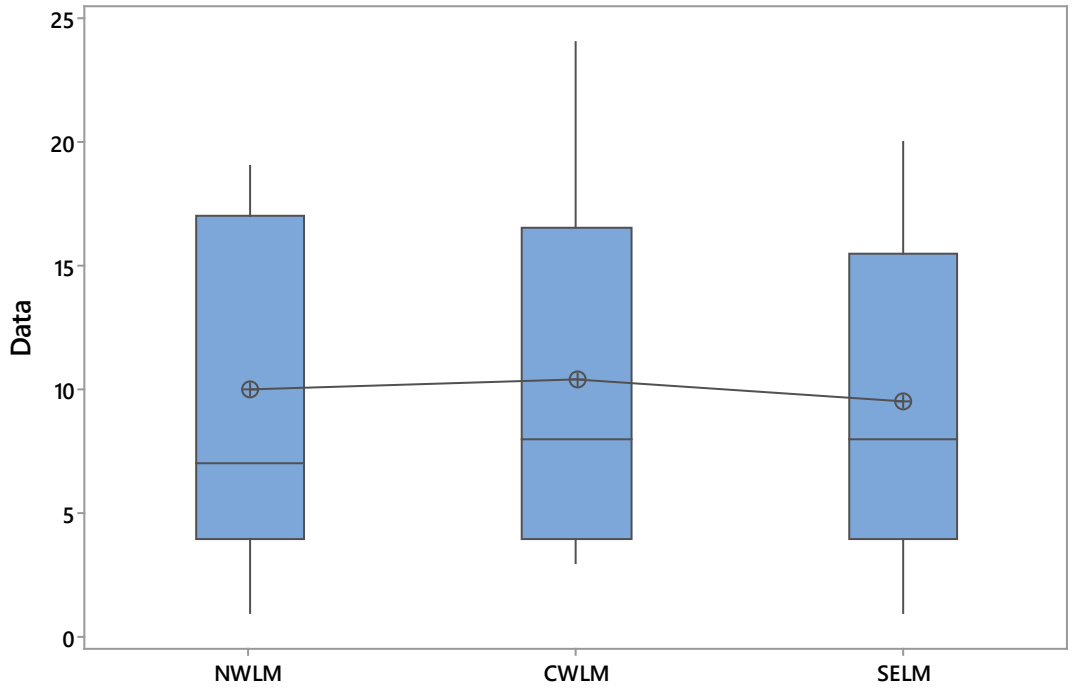
One-way ANOVA: NWLM, CWLM, SELM

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor  Levels  Values
Factor       3  NWLM, CWLM, SELM

Analysis of Variance

Source  DF   Adj SS  Adj MS  F-Value  P-Value
Factor   2     3.56   1.778     0.04    0.965
Error   24  1184.44  49.352
Total   26  1188.00

Model Summary

      S   R-sq  R-sq(adj)  R-sq(pred)
7.02509  0.30%      0.00%       0.00%
```

Means

Factor	N	Mean	StDev	95% CI
NWLM	9	10.00	6.93	(5.17, 14.83)
CWLM	9	10.44	7.32	(5.61, 15.28)
SELM	9	9.56	6.82	(4.72, 14.39)

Pooled StDev = 7.02509



Appendix 7.10.1 Boxplot of for NW, CW, and SE Loch Maree populations A_R

One-way ANOVA: NWLM, CWLM, SELM, WCG

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

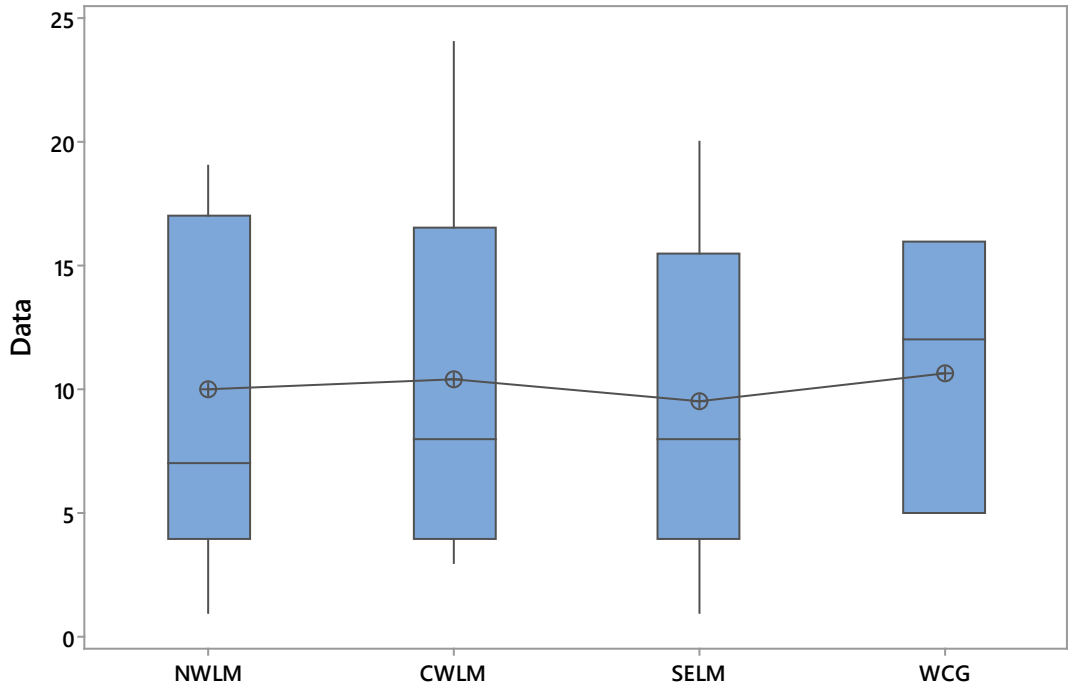
Factor	Levels	Values
Factor	4	NWLM, CWLM, SELM, WCG

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	3	6.56	2.185	0.05	0.985
Error	32	1408.44	44.014		
Total	35	1415.00			

Model Summary

```
      S   R-sq  R-sq(adj)  R-sq(pred)
6.63430  0.46%      0.00%       0.00%

Means

Factor  N   Mean  StDev      95% CI
NWLM    9  10.00   6.93  (5.50, 14.50)
CWLM    9  10.44   7.32  (5.94, 14.95)
SELM    9   9.56   6.82  (5.05, 14.06)
WCG     9  10.67   5.29  (6.16, 15.17)

Pooled StDev = 6.63430
```

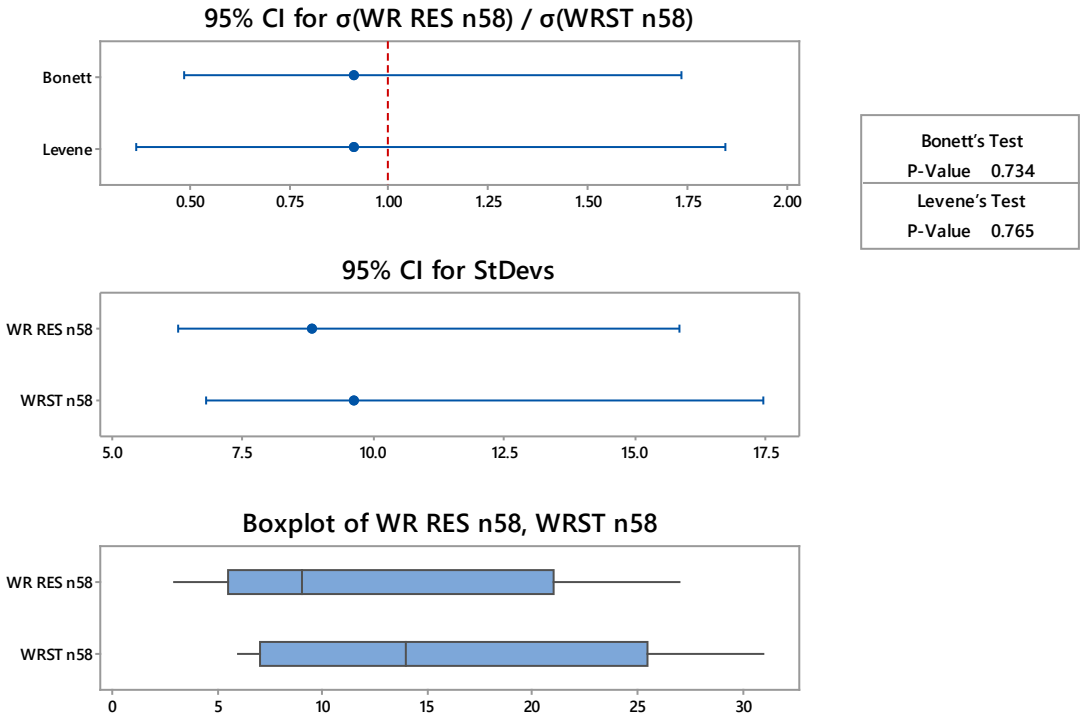


Appendix 7.10.2 Boxplot of for NW, CW, and SE Loch Maree, and Gairloch populations A_R

Test and CI for Two Variances: WR RES n58, WRST n58 BALANCED

```
Method

Null hypothesis         σ(WR RES n58) / σ(WRST n58) = 1
Alternative hypothesis  σ(WR RES n58) / σ(WRST n58) ≠ 1
Significance level      α = 0.05

Statistics
                                        95% CI for
Variable    N  StDev  Variance          StDevs
WR RES n58  9  8.803    77.500  (6.258, 15.833)
WRST n58    9  9.636    92.861  (6.799, 17.459)

Ratio of standard deviations = 0.914
Ratio of variances = 0.835

95% Confidence Intervals

                          CI for
         CI for StDev     Variance
```

```
Method       Ratio           Ratio
Bonett   (0.487, 1.732)  (0.237, 2.999)
Levene   (0.366, 1.842)  (0.134, 3.394)

Tests

                        Test
Method  DF1  DF2  Statistic  P-Value
Bonett    1    —       0.12    0.734
Levene    1   16       0.09    0.765
```



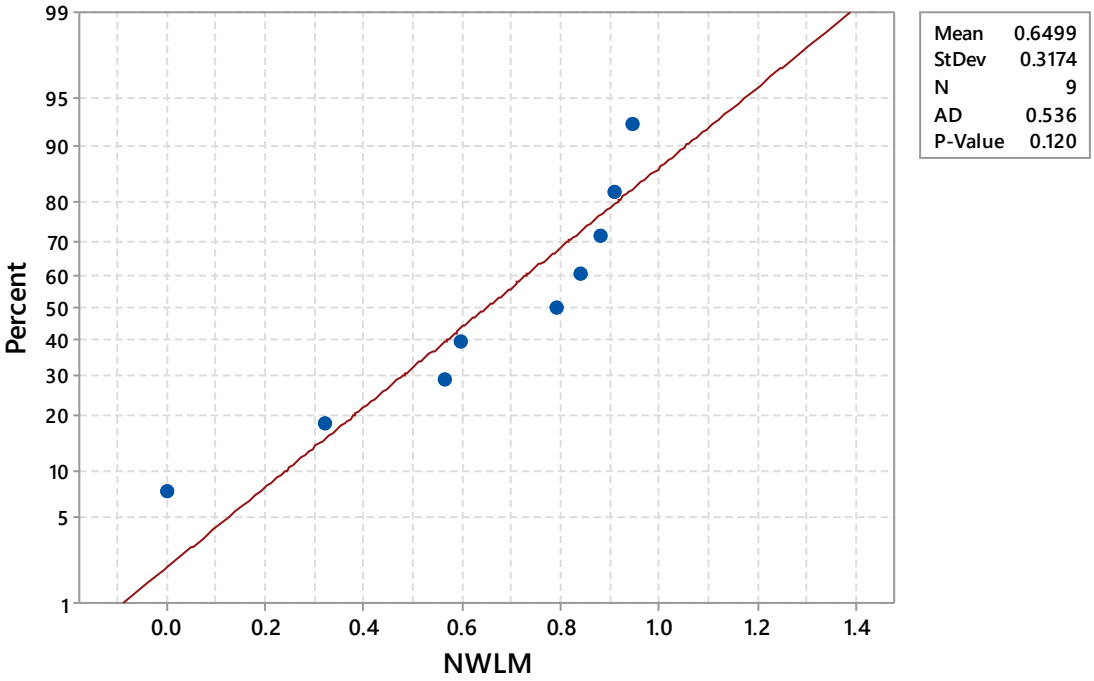
Appendix 7.10.3 Equal variance tests results between WR resident and sea trout with balanced sample sizes

Two-Sample T-Test and CI: WR RES n58, WRST n58 BALANCED

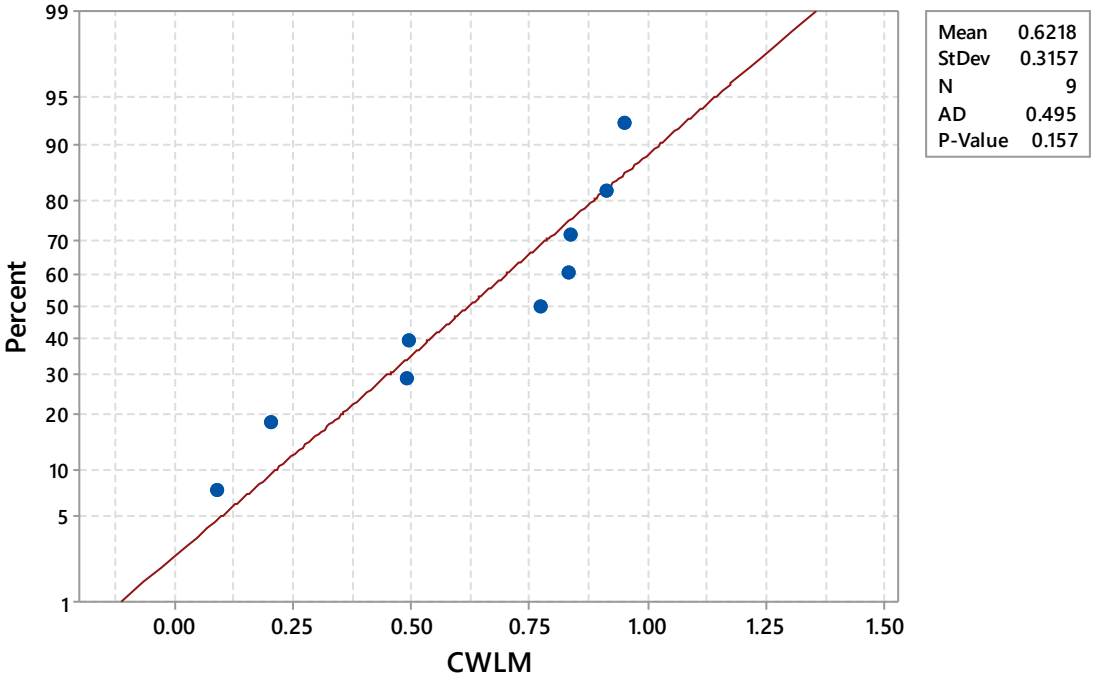
```
Two-sample T for WR RES n58 vs WRST n58

            N   Mean  StDev  SE Mean
WR RES n58  9  13.00   8.80      2.9
WRST n58    9  16.11   9.64      3.2


Difference = μ (WR RES n58) - μ (WRST n58)
Estimate for difference:  -3.11
95% CI for difference:  (-12.38, 6.16)
T-Test of difference = 0 (vs ≠): T-Value = -0.72  P-Value = 0.486  DF =
15
```

APPENDIX 7.11 HETEROZYGOSITIES (H_E) - NORMALITY TESTS

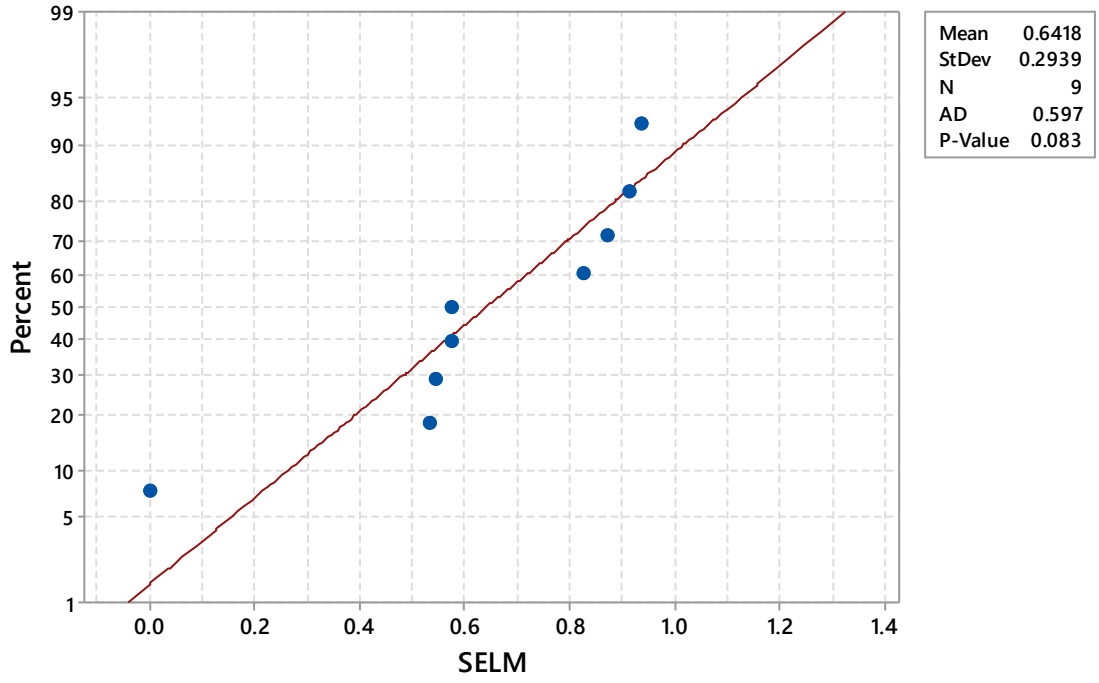
- All data sets normally distributed except for Loch Leven and Canaird sea trout.



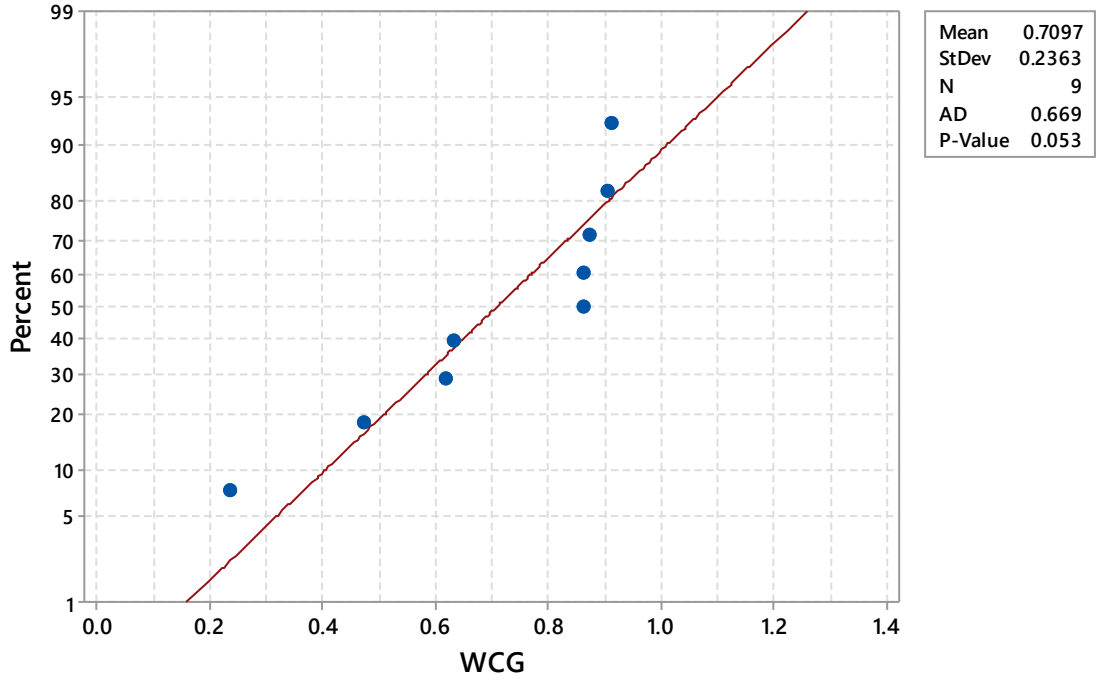
Appendix 7.11.1 Normality test result for NW Loch Maree H_E = Normal distribution



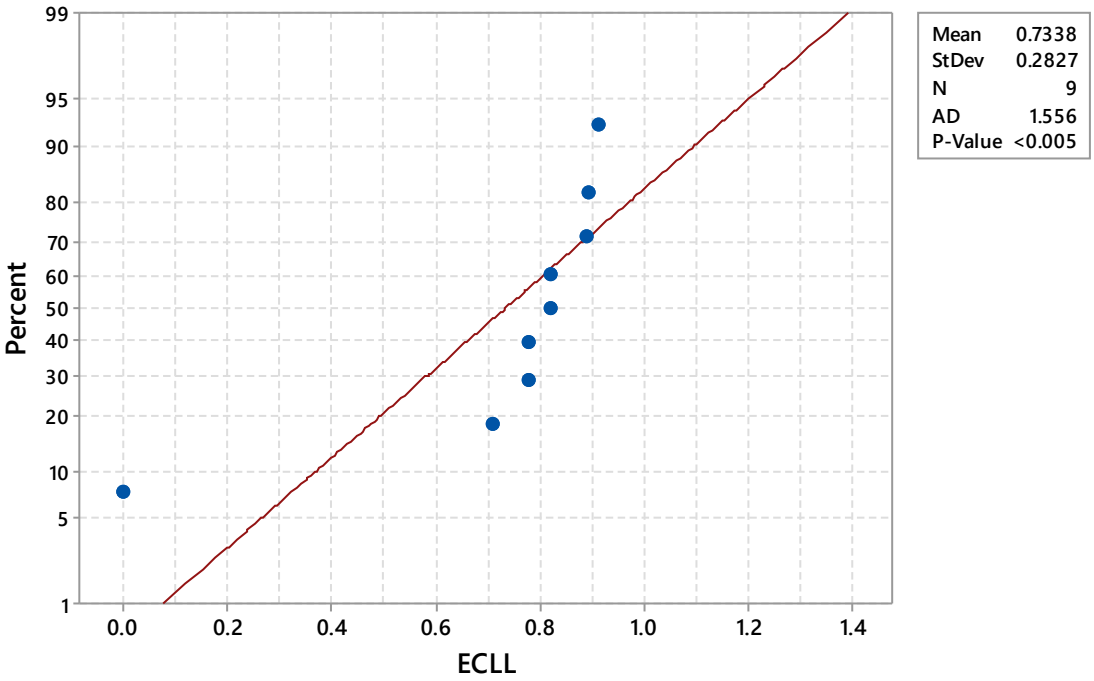
Appendix 7.11.2 Normality test result for CW Loch Maree H_E = Normal distribution



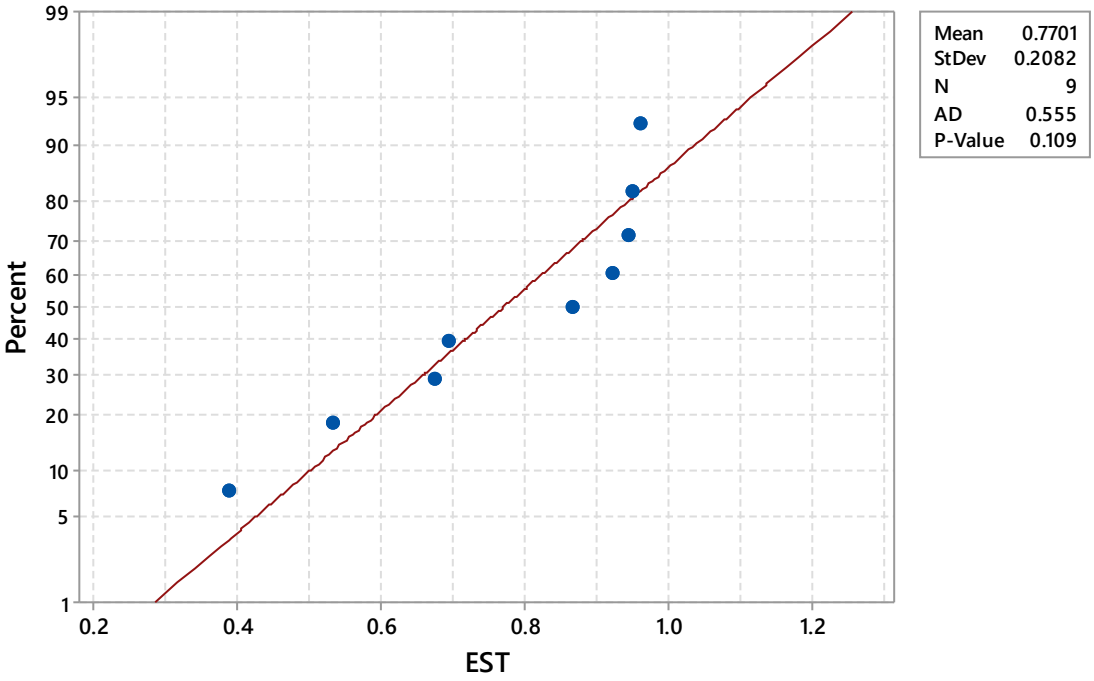
Appendix 7.11.3 Normality test result for SE Loch Maree H_E = Normal distribution



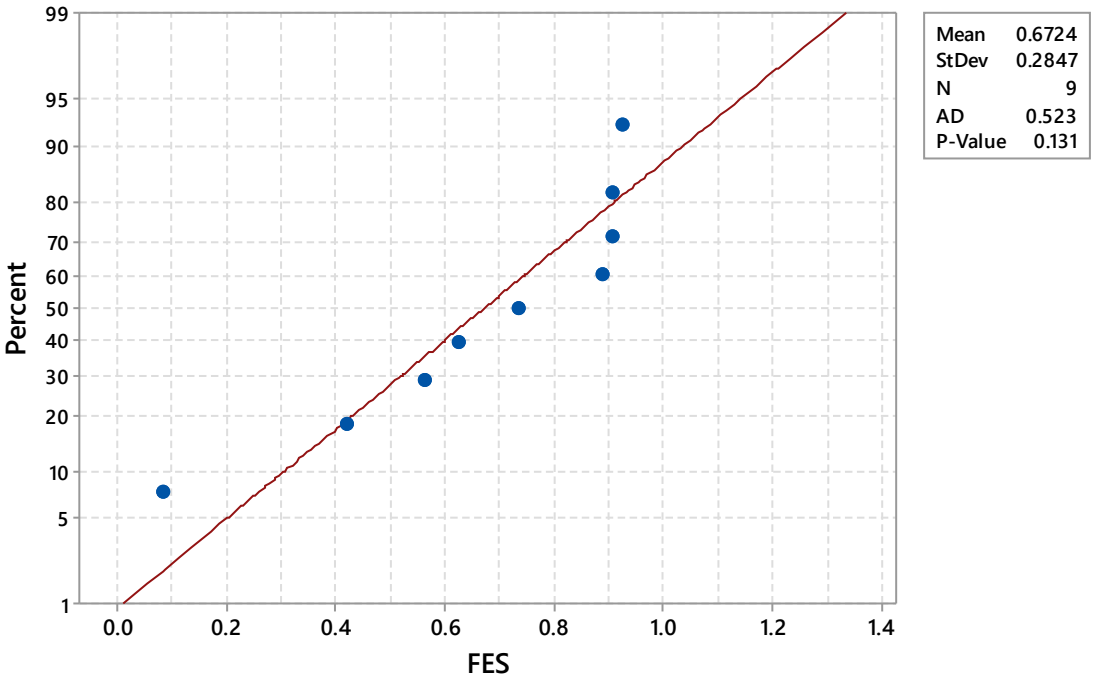
Appendix 7.11.4 Normality test result for Gairloch (West coast) H_E = Normal distribution



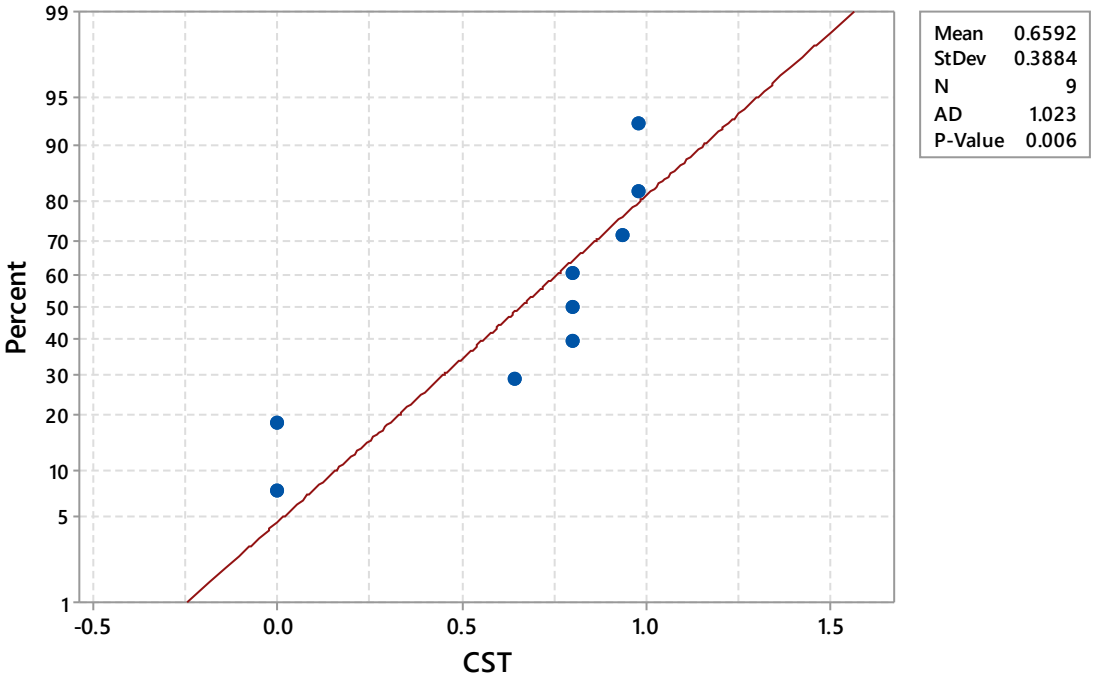
Appendix 7.11.5 Normality test result for Loch Leven (East coast) H_E = Non-normal distribution



Appendix 7.11.6 Normality test result for River Ewe sea trout H_E = Normal distribution



Appendix 7.11.7 Normality test result for Flowerdale sea trout H_E = Normal distribution



Appendix 7.11.8 Normality test result for Canaird sea trout H_E = Non-normal distribution

APPENDIX 7.12 EXPECTED HETEROZYGOSITIES (H_E) - ONE–WAY ANOVA

One-way ANOVA: NWLM, CWLM, SELM

Method

```
Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05
```

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	NWLM, CWLM, SELM

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.00377	0.001884	0.02	0.981
Error	24	2.29455	0.095606		
Total	26	2.29832			

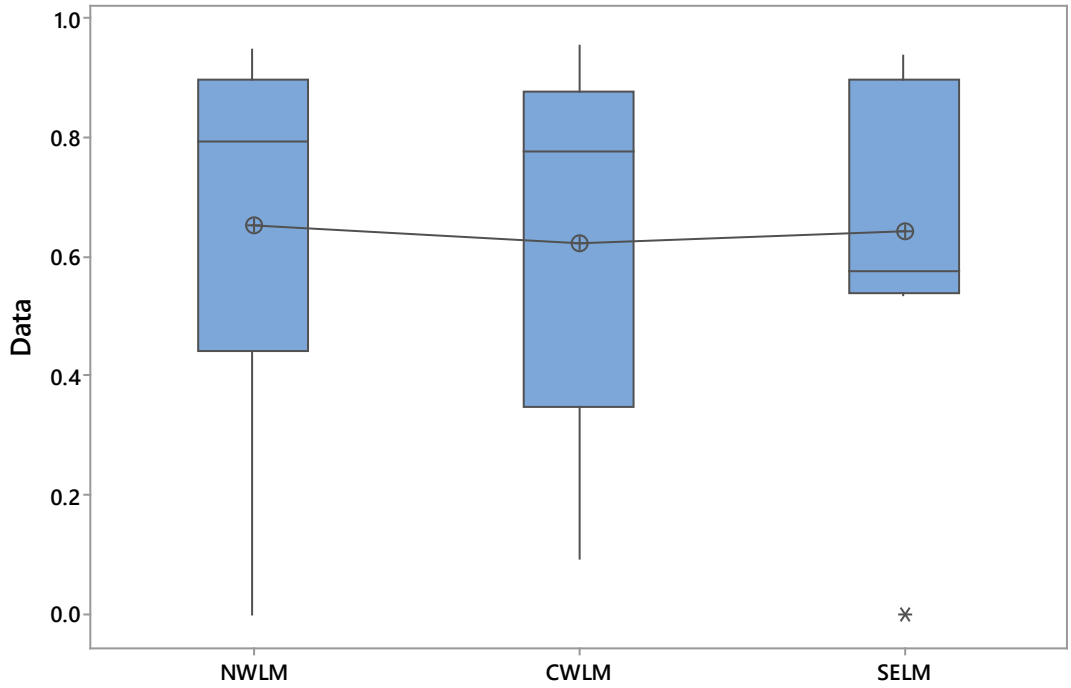
Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.309203	0.16%	0.00%	0.00%

Means

Factor	N	Mean	StDev	95% CI
NWLM	9	0.650	0.317	(0.437, 0.863)
CWLM	9	0.622	0.316	(0.409, 0.834)
SELM	9	0.6418	0.2939	(0.4291, 0.8545)

Pooled StDev = 0.309203

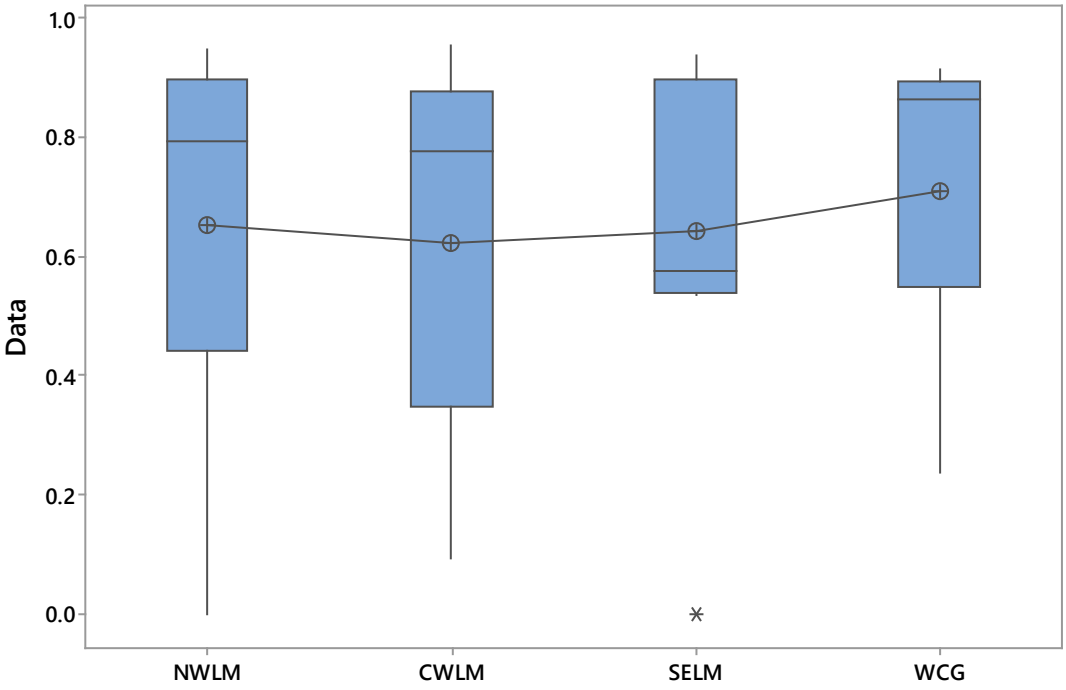


Appendix 7.12.1 Boxplot of for NW, CW, and SE Loch Maree populations H_E

One-way ANOVA: NWLM, CWLM, SELM, WCG

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor  Levels  Values
Factor       4  NWLM, CWLM, SELM, WCG

Analysis of Variance

Source  DF   Adj SS   Adj MS  F-Value  P-Value
Factor   3  0.03862  0.01287     0.15    0.929
Error   32  2.74114  0.08566
Total   35  2.77976

Model Summary

       S   R-sq  R-sq(adj)  R-sq(pred)
0.292678  1.39%      0.00%       0.00%



Means

Factor  N    Mean   StDev       95% CI
NWLM    9   0.650   0.317  ( 0.451,  0.849)
```

```
CWLM     9   0.622   0.316  ( 0.423,  0.820)
SELM     9  0.6418  0.2939  (0.4431, 0.8405)
WCG      9  0.7097  0.2363  (0.5109, 0.9084)

Pooled StDev = 0.292678
```



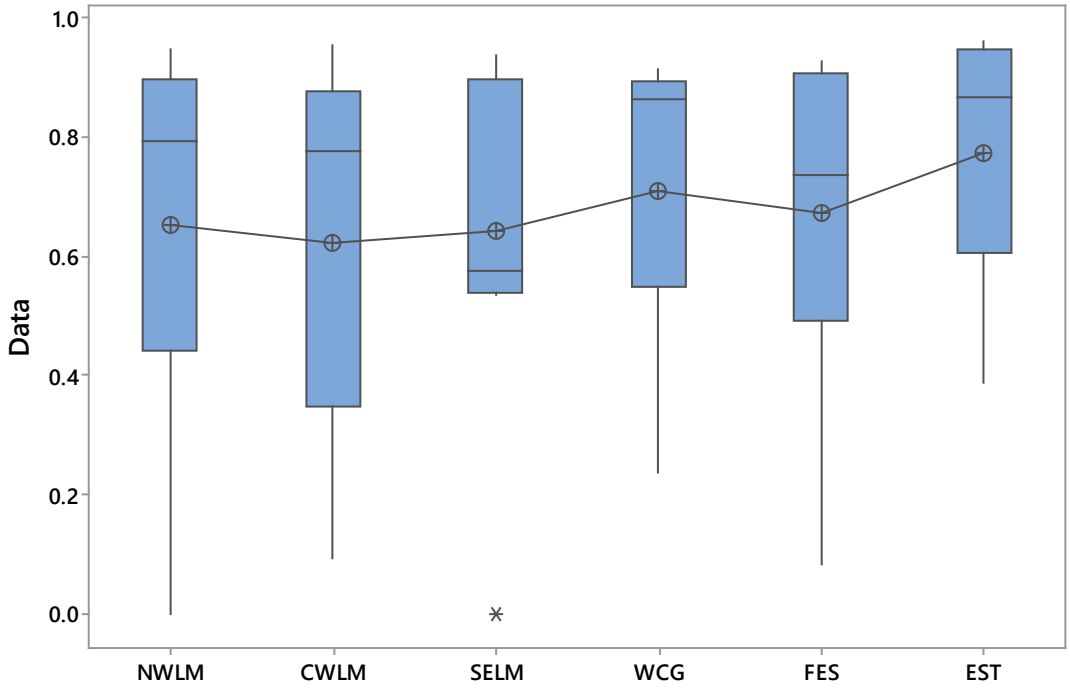
Appendix 7.12.2 Boxplot of for NW, CW, SE Loch Maree, and Gairloch populations H_E

One-way ANOVA: NWLM, CWLM, SELM, WCG, FES, EST

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor  Levels  Values
Factor       6  NWLM, CWLM, SELM, WCG, FES, EST

Analysis of Variance

Source  DF  Adj SS   Adj MS  F-Value  P-Value
Factor   5  0.1330  0.02660     0.34    0.885
Error   48  3.7364  0.07784
Total   53  3.8694

Model Summary

       S   R-sq  R-sq(adj)  R-sq(pred)
0.279001  3.44%      0.00%       0.00%

Means
```

Factor	N	Mean	StDev	95% CI
NWLM	9	0.650	0.317	(0.463, 0.837)
CWLM	9	0.622	0.316	(0.435, 0.809)
SELM	9	0.6418	0.2939	(0.4548, 0.8288)
WCG	9	0.7097	0.2363	(0.5227, 0.8967)
FES	9	0.6724	0.2847	(0.4855, 0.8594)
EST	9	0.7701	0.2082	(0.5831, 0.9571)

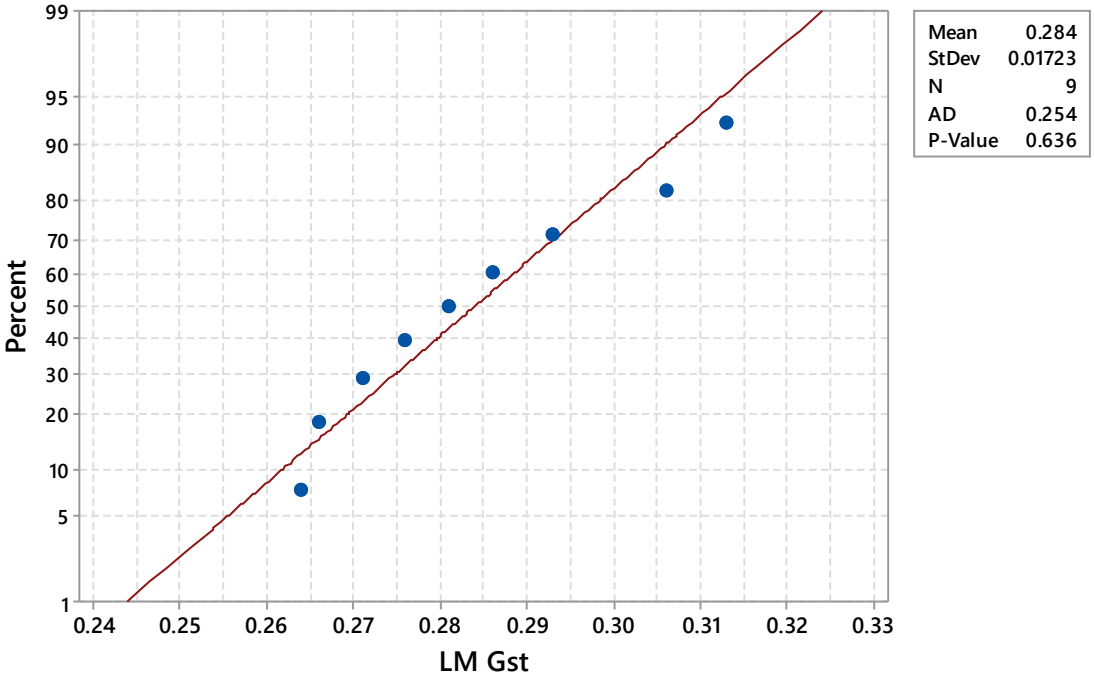
Pooled StDev = 0.279001



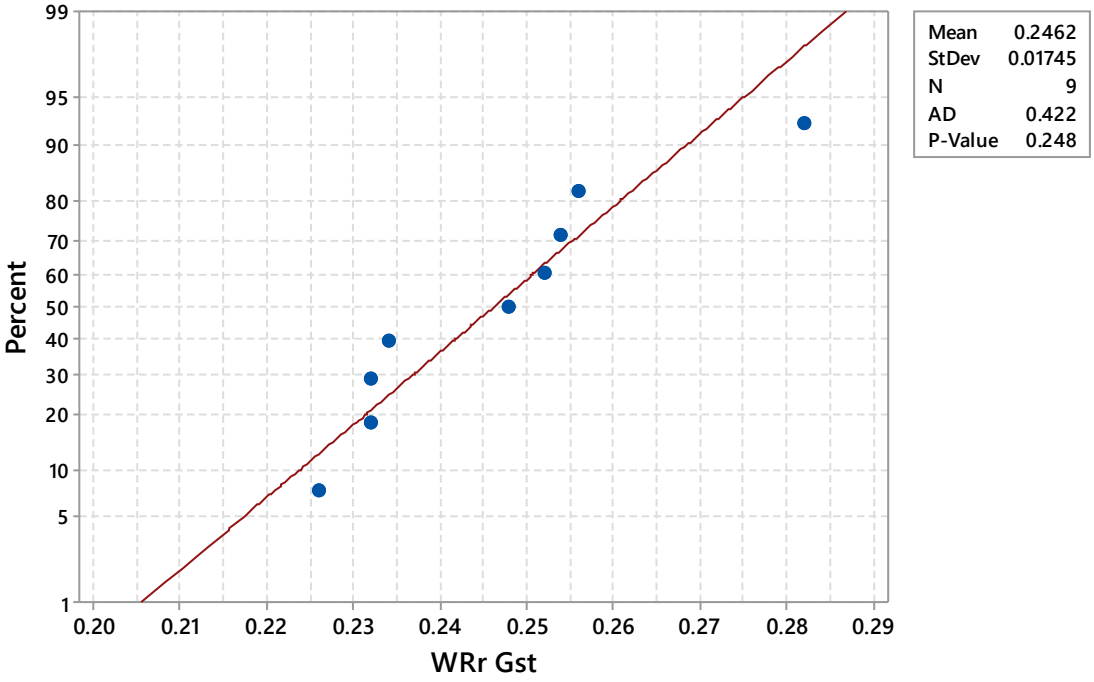
Appendix 7.12.3 Boxplot of for all parametric H_E data sets, including NW, CW, SE Loch Maree, Gairloch, and the Flowerdale and Ewe sea trout populations

APPENDIX 7.13 POPULATION DIFFERENTIATION - NORMALITY TESTS

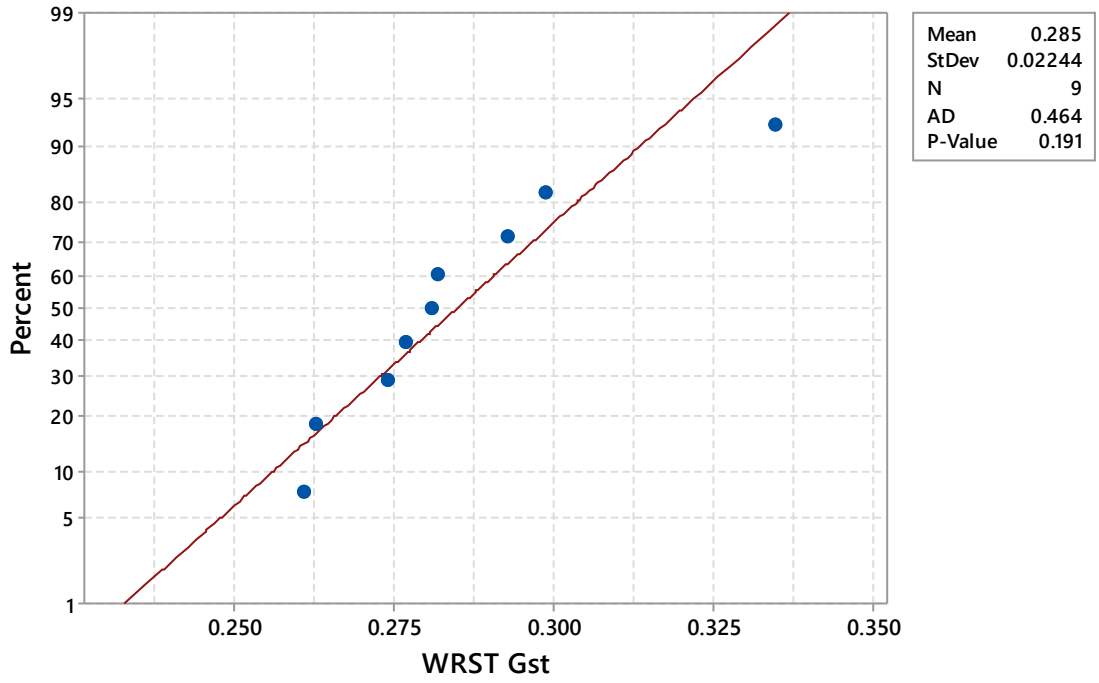
- Normal distributions seen in all G_{ST} measures except for groups All of Wester Ross, All residents, and all specimens.



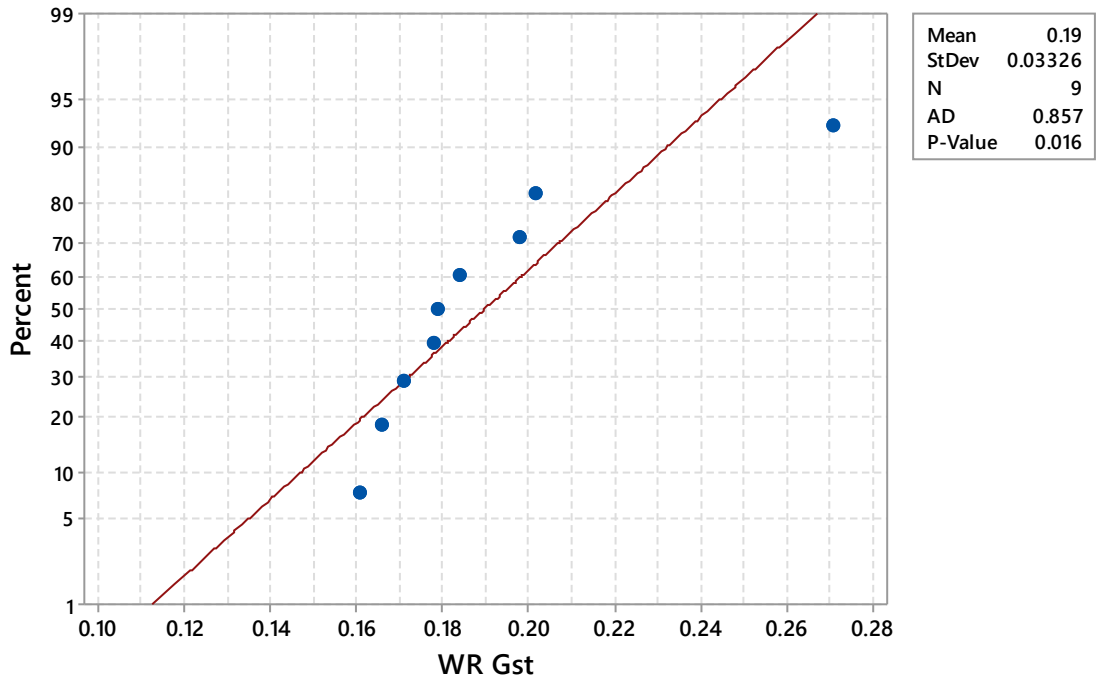
Appendix 7.13.1 Normality test result for Loch Maree populations G_{ST} = Normal distribution



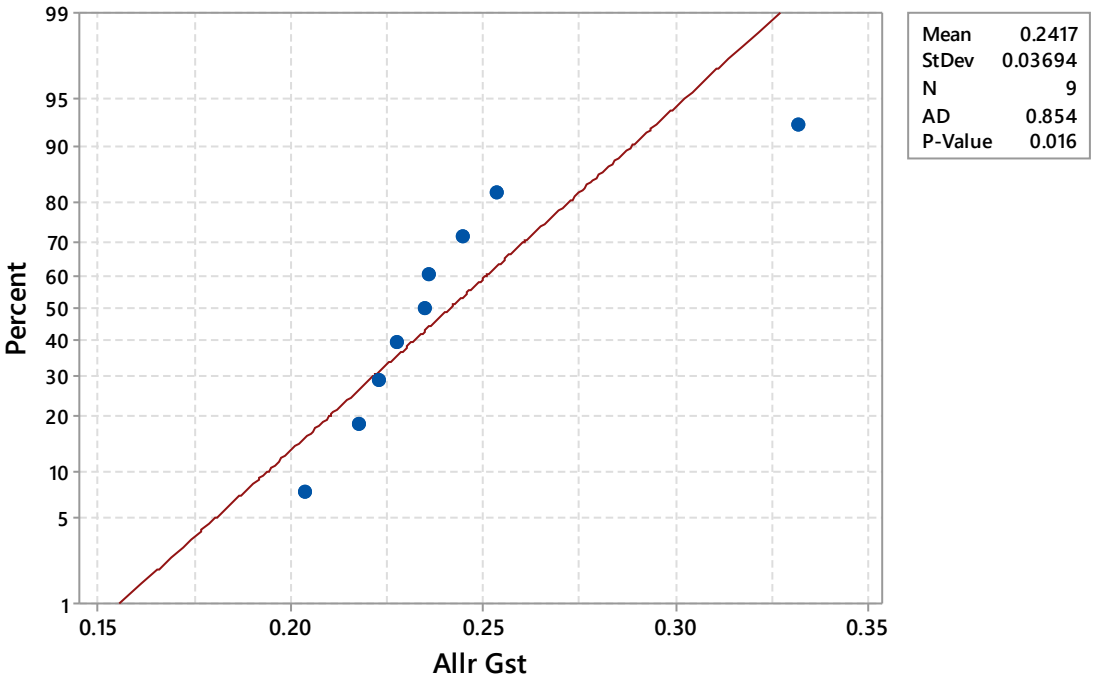
Appendix 7.13.2 Normality test result for Wester Ross resident populations G_{ST} = Normal distribution



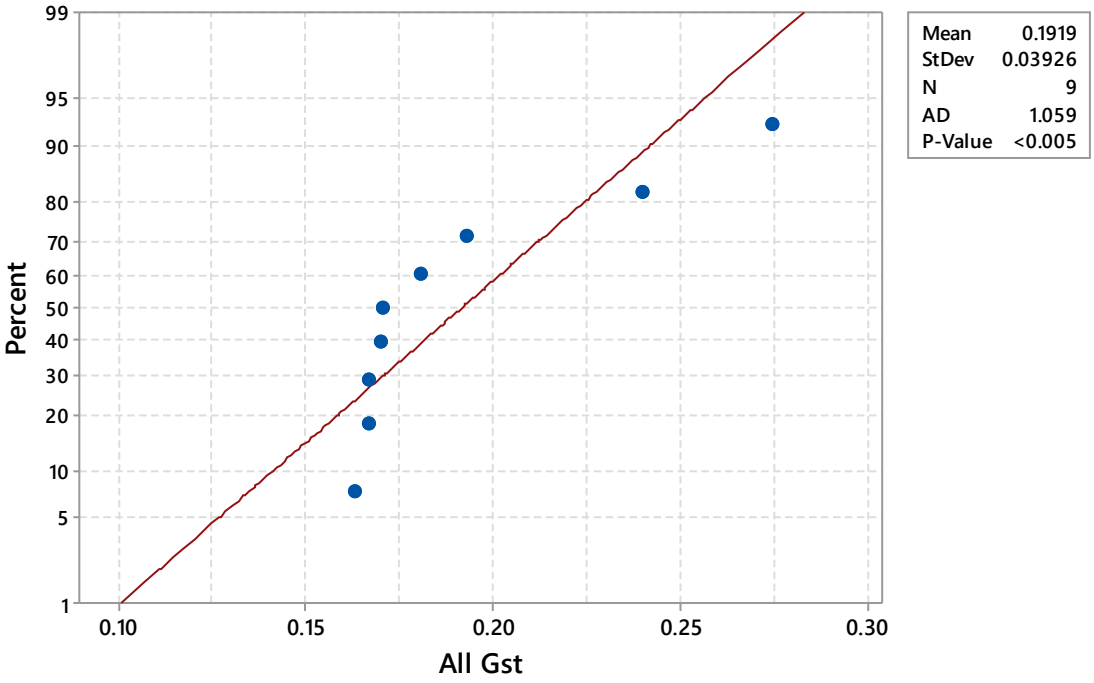
Appendix 7.13.3 Normality test result for Wester Ross sea trout populations G_{ST} = Normal distribution



Appendix 7.13.4 Normality test result for All of Wester Ross trout populations G_{ST} = Non-normal distribution

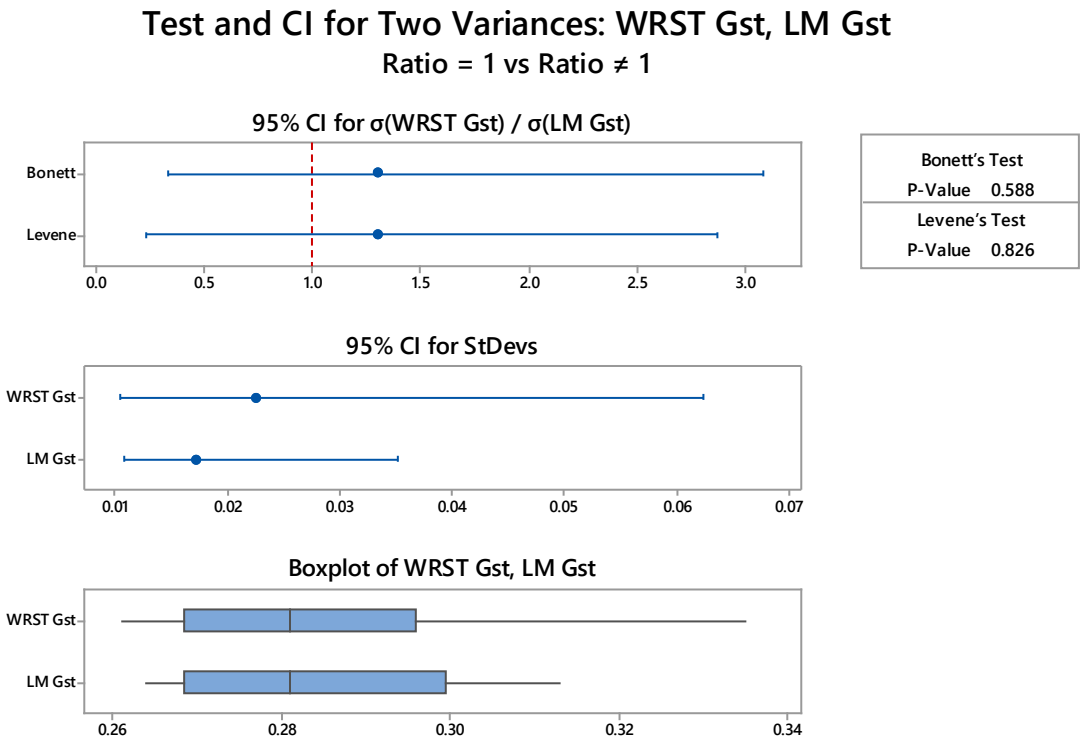


Appendix 7.13.5 Normality test result for All resident populations G_{ST} = Non-normal distribution



Appendix 7.13.6 Normality test result for All specimens G_{ST} = Non-normal distribution

APPENDIX 7.14 POPULATION DIFFERENTIATION – TESTS FOR DIFFERENCES



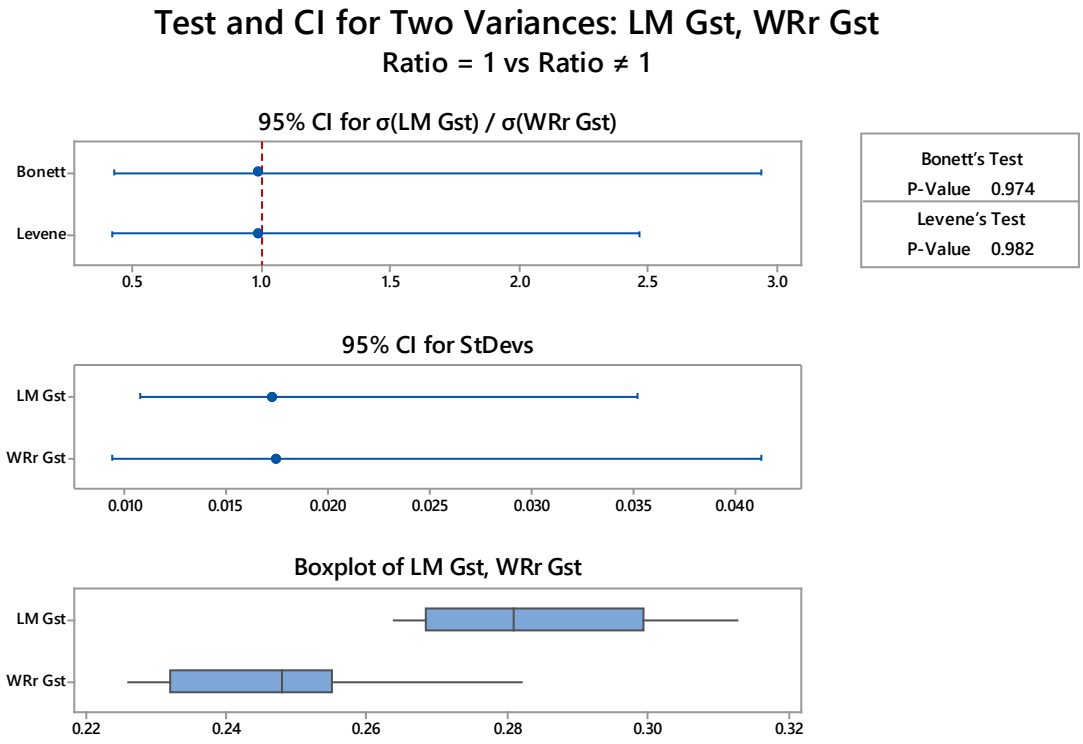
Appendix 7.14.1 Equal variance results between WR sea trout and Loch Maree resident G_{ST} = Equal

Two-Sample T-Test and CI: WRST Gst, LM Gst

```
Two-sample T for WRST Gst vs LM Gst

          N    Mean   StDev  SE Mean
WRST Gst  9  0.2850  0.0224   0.0075
LM Gst    9  0.2840  0.0172   0.0057


Difference = μ (WRST Gst) - μ (LM Gst)
Estimate for difference:  0.00100
```

Test and CI for Two Variances: LM Gst, WRr Gst

```
Method

Null hypothesis         σ(LM Gst) / σ(WRr Gst) = 1
Alternative hypothesis  σ(LM Gst) / σ(WRr Gst) ≠ 1
Significance level      α = 0.05


Statistics

                                  95% CI for
Variable  N  StDev  Variance       StDevs
LM Gst    9  0.017     0.000  (0.011, 0.035)
WRr Gst   9  0.017     0.000  (0.009, 0.041)

Ratio of standard deviations = 0.988
Ratio of variances = 0.976


95% Confidence Intervals

                          CI for
         CI for StDev     Variance
Method       Ratio         Ratio
Bonett  (0.422, 2.942)  (0.178, 8.654)
Levene  (0.421, 2.474)  (0.177, 6.121)


Tests

                        Test
Method  DF1  DF2  Statistic  P-Value
Bonett    1    —       0.00    0.974
Levene    1   16       0.00    0.982
```



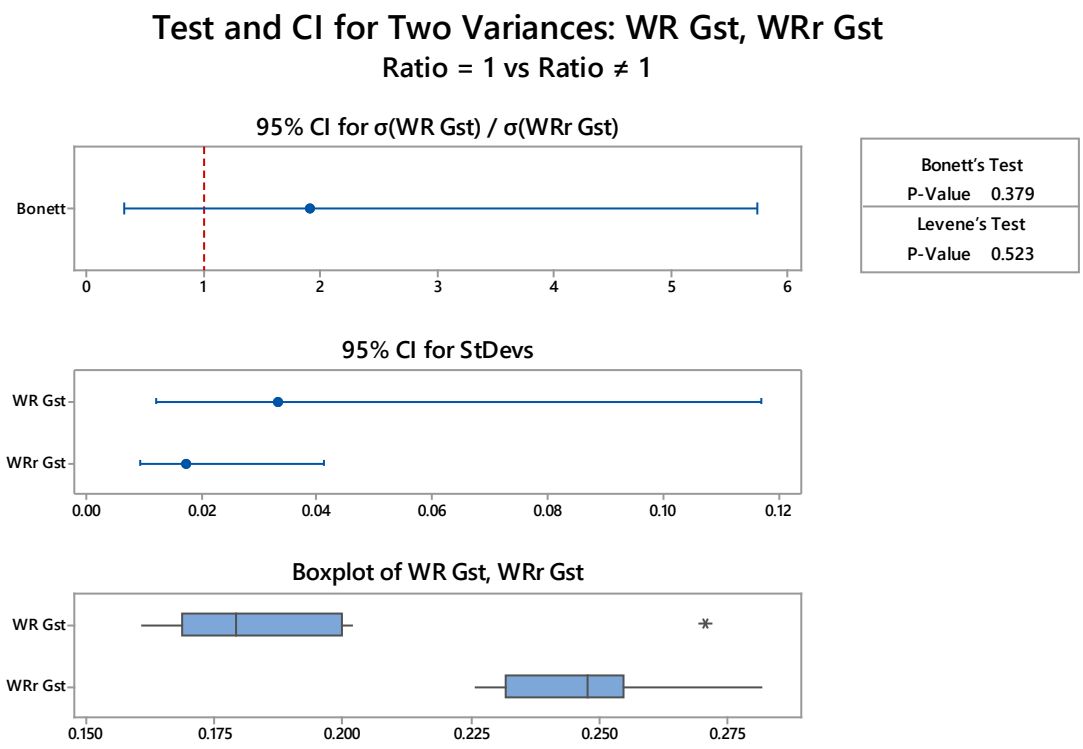
Appendix 7.14.2 Graphical results from equal variances test between Loch Maree and WR resident G_{ST} = Not-equal

Mann-Whitney Test and CI: LM Gst, WRr Gst

```
          N   Median
LM Gst    9  0.28100
WRr Gst   9  0.24800

Point estimate for η1 - η2 is 0.03800
95.8 Percent CI for η1 - η2 is (0.01901,0.05700)
W = 121.0
Test of η1 = η2 vs η1 ≠ η2 is significant at 0.0020
The test is significant at 0.0020 (adjusted for ties)
```

Test and CI for Two Variances: WR Gst, WRr Gst

```
Method

Null hypothesis         σ(WR Gst) / σ(WRr Gst) = 1
Alternative hypothesis  σ(WR Gst) / σ(WRr Gst) ≠ 1
Significance level      α = 0.05

Statistics
                                    95% CI for
Variable  N  StDev  Variance      StDevs
WR Gst    9  0.033     0.001  (0.012, 0.117)
WRr Gst   9  0.017     0.000  (0.009, 0.041)

Ratio of standard deviations = 1.906
Ratio of variances = 3.633

95% Confidence Intervals
                                CI for
          CI for StDev       Variance
Method       Ratio           Ratio
Bonett  (0.324, 5.753)  (0.105, 33.094)
Levene  (    *, 4.287)  (    *, 18.378)

Tests
                        Test
Method  DF1  DF2  Statistic  P-Value
Bonett    1    —       0.77    0.379
Levene    1   16       0.43    0.523
```



Appendix 7.14.3 Equal variance results between All of WR trout and WR residents G_{ST} = Not-equal

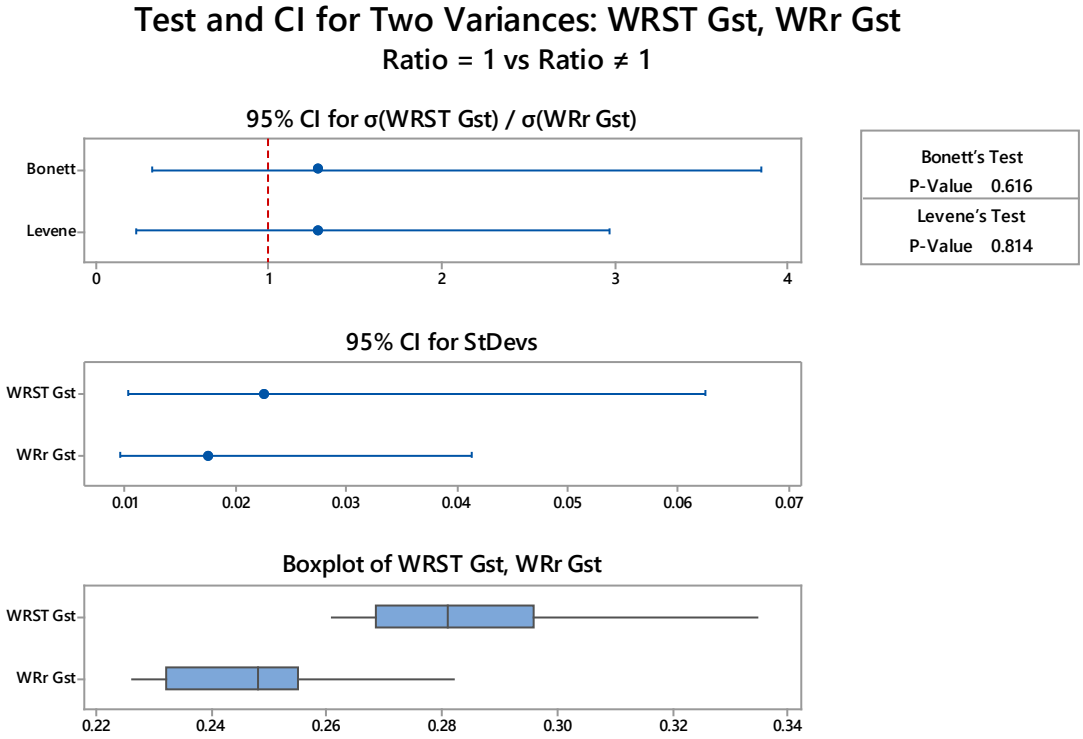
Mann-Whitney Test and CI: WR Gst, WRr Gst

```
          N   Median
WR Gst    9  0.17900
WRr Gst   9  0.24800


Point estimate for η1 - η2 is -0.06100
95.8 Percent CI for η1 - η2 is (-0.08100,-0.04201)
W = 53.0
Test of η1 = η2 vs η1 ≠ η2 is significant at 0.0047
The test is significant at 0.0047 (adjusted for ties)
```

Test and CI for Two Variances: WRST Gst, WRr Gst

```
Method

Null hypothesis         σ(WRST Gst) / σ(WRr Gst) = 1
Alternative hypothesis  σ(WRST Gst) / σ(WRr Gst) ≠ 1
Significance level      α = 0.05

Statistics
                                    95% CI for
Variable   N  StDev  Variance        StDevs
WRST Gst   9  0.022     0.001  (0.010, 0.062)
WRr Gst    9  0.017     0.000  (0.009, 0.041)

Ratio of standard deviations = 1.286
Ratio of variances = 1.655


95% Confidence Intervals
                                CI for
          CI for StDev        Variance
Method       Ratio             Ratio
Bonett  (0.326, 3.857)  (0.106, 14.875)
```

```
Levene   (0.233, 2.973)   (0.054,  8.839)

Tests
                        Test
Method   DF1   DF2   Statistic   P-Value
Bonett     1     —        0.25     0.616
Levene     1    16        0.06     0.814
```



Appendix 7.14.4 Equal variance test between WR sea trout and residents G_{ST} = Not-equal.

Mann-Whitney Test and CI: WRST Gst, WRr Gst

```
           N   Median
WRST Gst   9  0.28100
WRr Gst    9  0.24800

Point estimate for η1 - η2 is 0.03700
95.8 Percent CI for η1 - η2 is (0.01999,0.05500)
W = 120.5
Test of η1 = η2 vs η1 ≠ η2 is significant at 0.0023
The test is significant at 0.0023 (adjusted for ties)
```

Mann-Whitney Test and CI: All Gst, WRr Gst

```
          N   Median
All Gst   9  0.17100
WRr Gst   9  0.24800

Point estimate for η1 - η2 is -0.06400
95.8 Percent CI for η1 - η2 is (-0.08500,-0.03300)
W = 57.0
Test of η1 = η2 vs η1 ≠ η2 is significant at 0.0134
The test is significant at 0.0133 (adjusted for ties)
```

Mann-Whitney Test and CI: All Gst, WR Gst

```
          N   Median
All Gst   9  0.17100
WR Gst    9  0.17900


Point estimate for η1 - η2 is -0.00300
95.8 Percent CI for η1 - η2 is (-0.02699,0.02702)
W = 82.5
Test of η1 = η2 vs η1 ≠ η2 is significant at 0.8253
The test is significant at 0.8251 (adjusted for ties)
```

APPENDIX 7.15 HEADWATER POPULATIONS & BARRIERS TO MOVEMENT

A summary of all P-values from the following tests are presented:

POPULATIONS ABOVE AND BELOW BARRIERS

Appendix 7.15.1 Spearman's Rho correlations based on ranked (RK) values for populations above and below barriers

Variable 1	V 2	P-Values	*r*
Distance to sea	A_R	0.001	-0.753
Distance to sea	H_E	0.001	-0.753
Life history	A_R	0.000	0.862
Life history	H_E	0.000	0.862
Above or below barrier	A_R	0.000	0.867
Above or below barrier	H_E	0.000	0.867

Appendix 7.15.2 Mann-Whitney tests for difference based on actual values for populations above and below barriers

Variable 1	V 2	P-Value	95% CI	
Landlocked A_R	Downstream A_R	0.001	1.8	2.9
Landlocked H_E	Downstream H_E	0.001	0.230	0.365

POPULATIONS WITH NO BARRIERS TO SEA

Appendix 7.15.3 Spearman's Rho correlations based on ranked (RK) values for populations without barriers to the sea

Variable 1	V 2	P-Value	*r*
Distance to sea	A_R	0.249	-0.380
Distance to sea	H_E	0.474	-0.242
Life history	A_R	0.160	0.454
Life history	H_E	1.000	0.000

Appendix 7.15.4 Tests for difference based on actual values for populations without barriers to the sea

Variable 1	V 2	P-Value	95% CI		Test
Resident A_R	Sea trout A_R	0.179	0.9	1.1	**MW test**
Resident H_E	Sea trout H_E	0.746	0.059	0.079	**T-test**

POPULATIONS WITH AND WITHOUT BARRIERS

Appendix 7.15.5 Spearman's Rho correlations based on ranked (RK) values for populations with and without barriers to sea

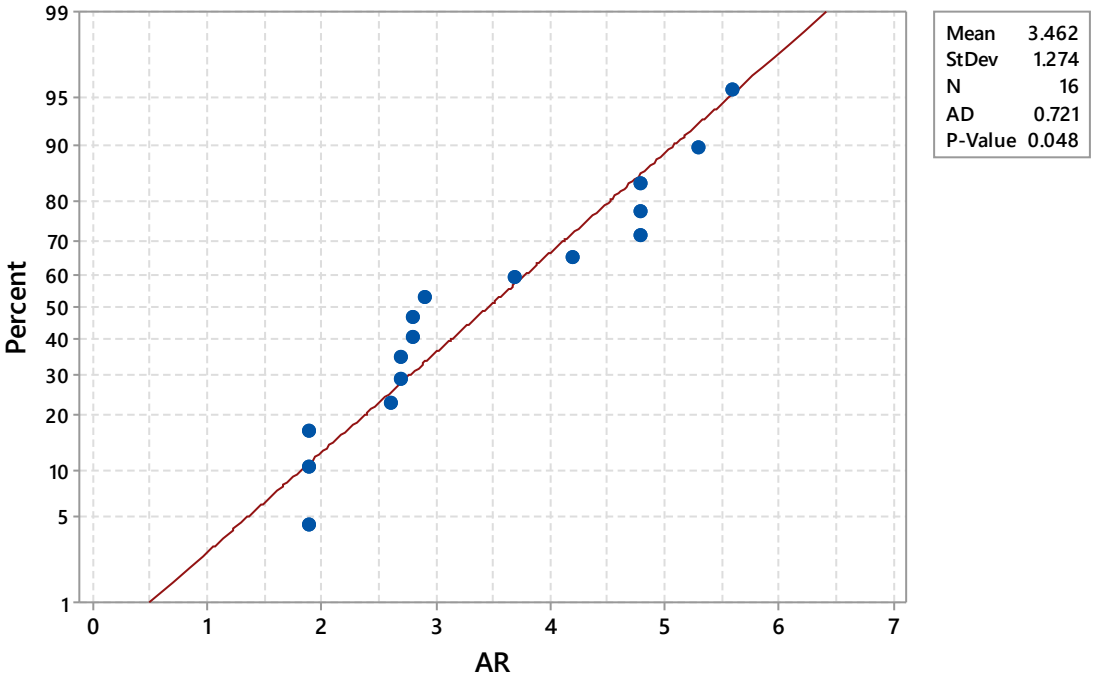
Variable 1	V 2	P-Value	*r*
Distance to sea	A_R	0.217	-0.281
Distance to sea	H_E	0.212	-0.284
Life history	A_R	0.000	0.869
Life history	H_E	0.000	0.794
Above or below barrier	A_R	0.000	0.862
Above or below barrier	H_E	0.000	0.859

Appendix 7.15.6 Mann-Whitney tests for difference based on actual values for populations with and without barriers

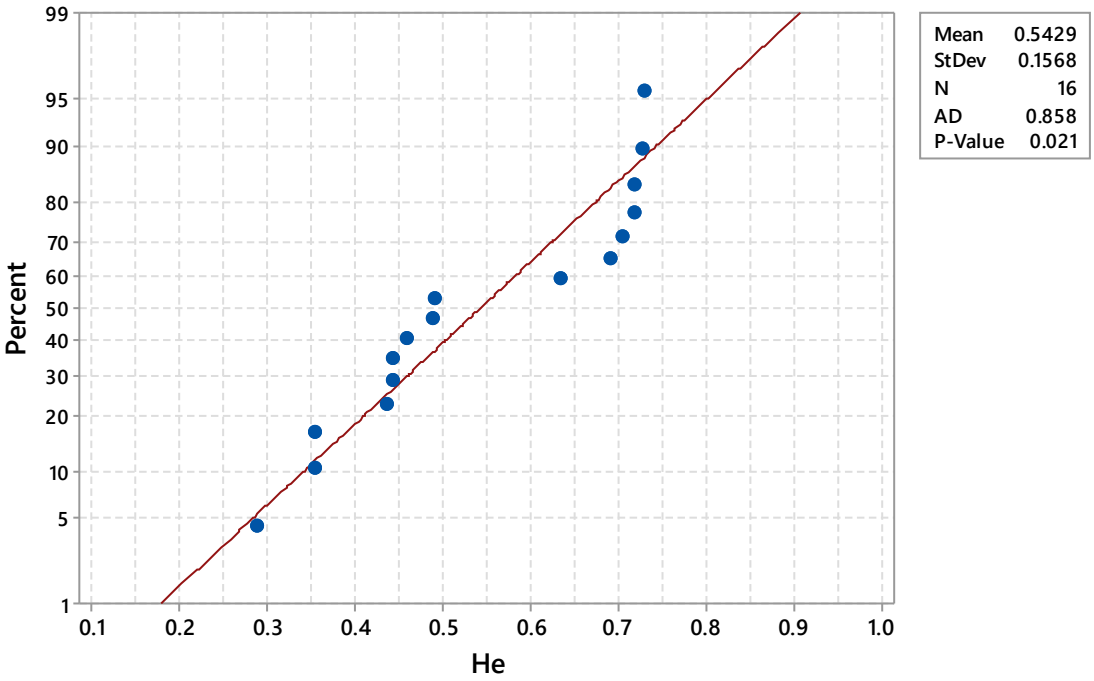
Variable 1	V 2	P-Value	95% CI	
Landlocked A_R	Downstream A_R	0.0001	1.49	2.50
Landlocked H_E	Downstream H_E	0.0001	0.210	0.337

APPENDIX 7.16 HEADWATER POPULATIONS & BARRIERS TO MOVEMENT - NORMALITY TESTS

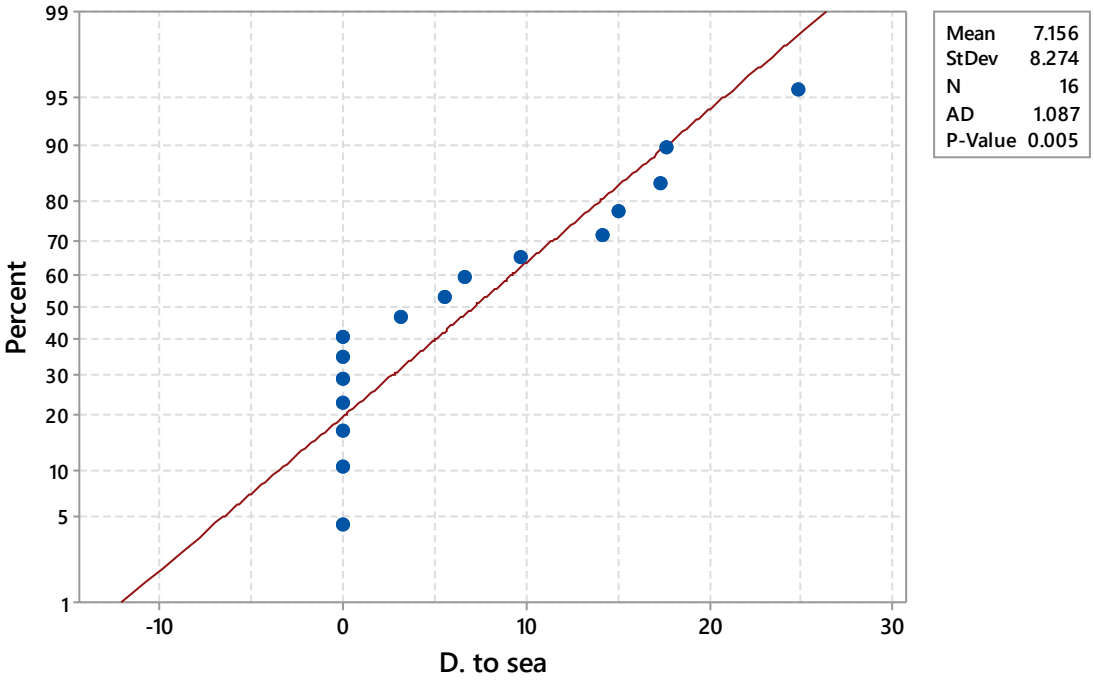
- Distances to sea was consistently non-normal, allelic richness and heterozygosity distribution is variable



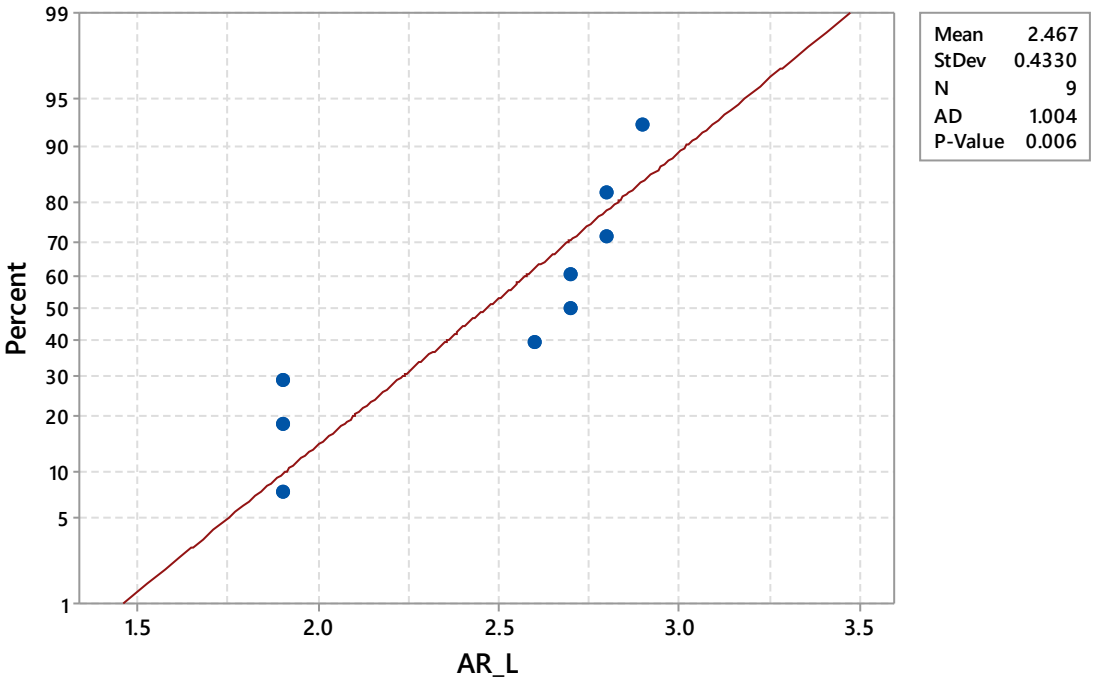
Appendix 7.16.1 Normality test of allelic richness for headwater populations and their downstream counterparts = Normal distribution



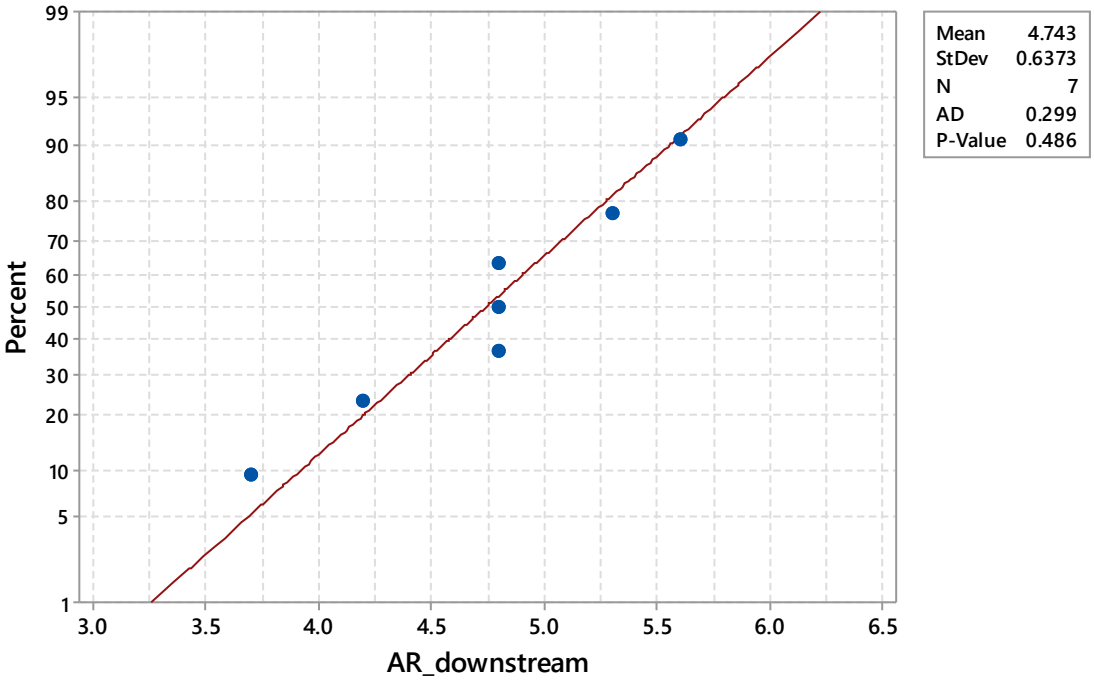
Appendix 7.16.2 Normality test of expected heterozygosities for headwater populations and their downstream counterparts = Normal distribution



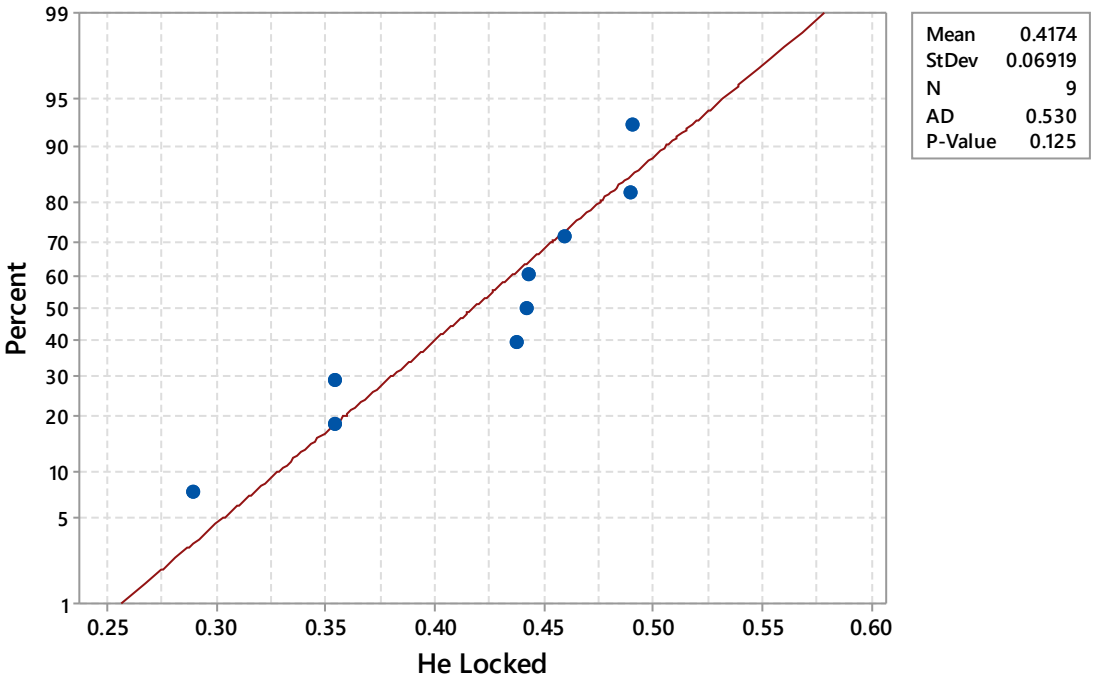
Appendix 7.16.3 Normality test of distances to sea (km) for headwater populations and their downstream counterparts = Non-normal distribution



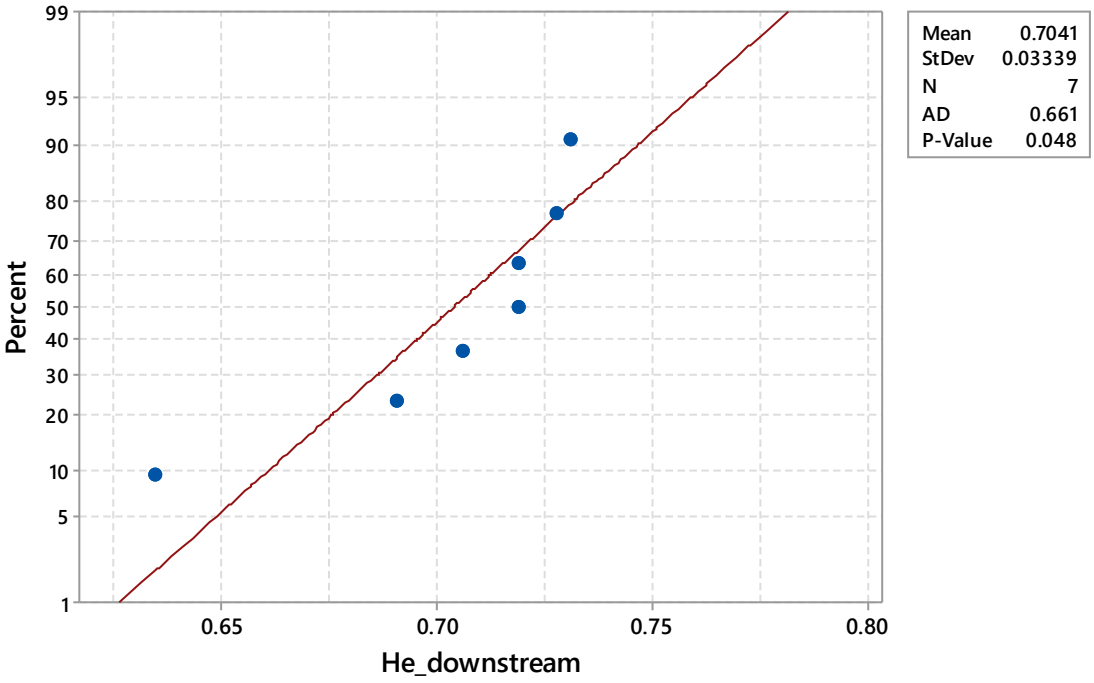
Appendix 7.16.4 Normality test of allelic richness for only headwater populations = Non-normal distribution



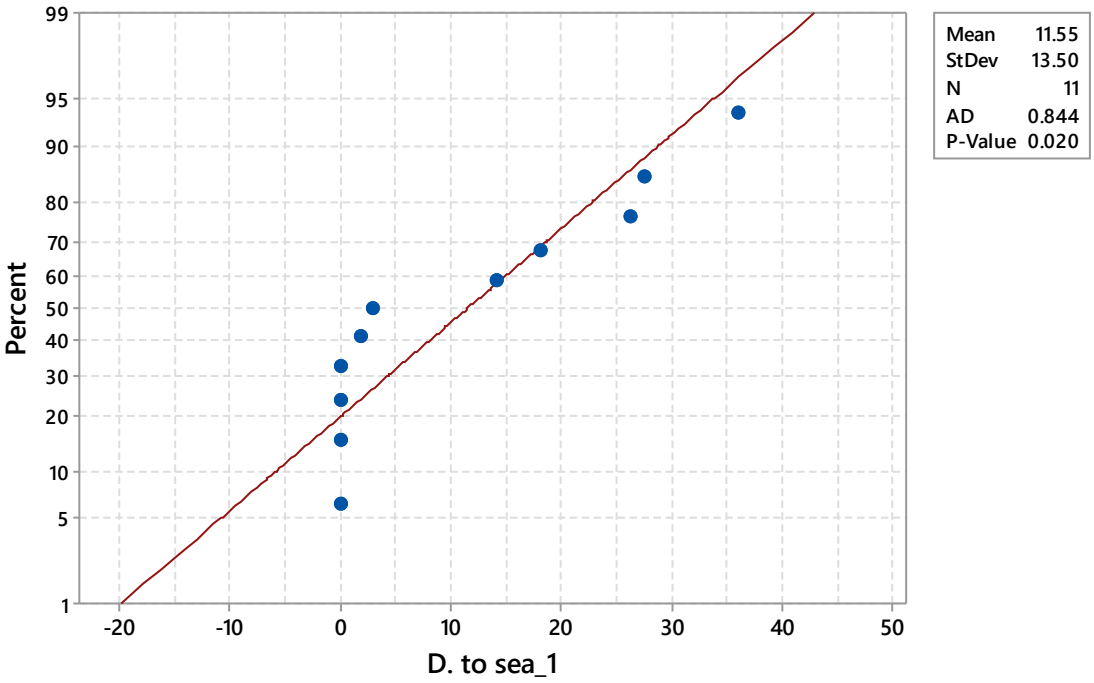
Appendix 7.16.5 Normality test of allelic richness for only the populations downstream of headwater populations = Normal distribution



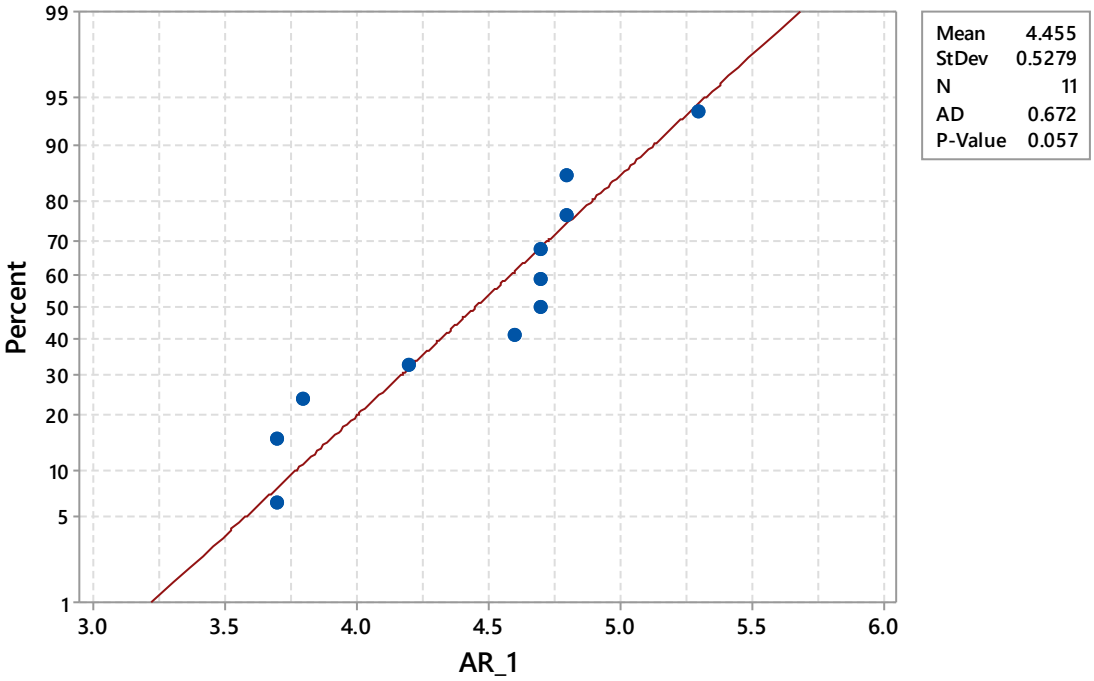
Appendix 7.16.6 Normality test of expected heterozygosities for only headwater populations = Normal distribution



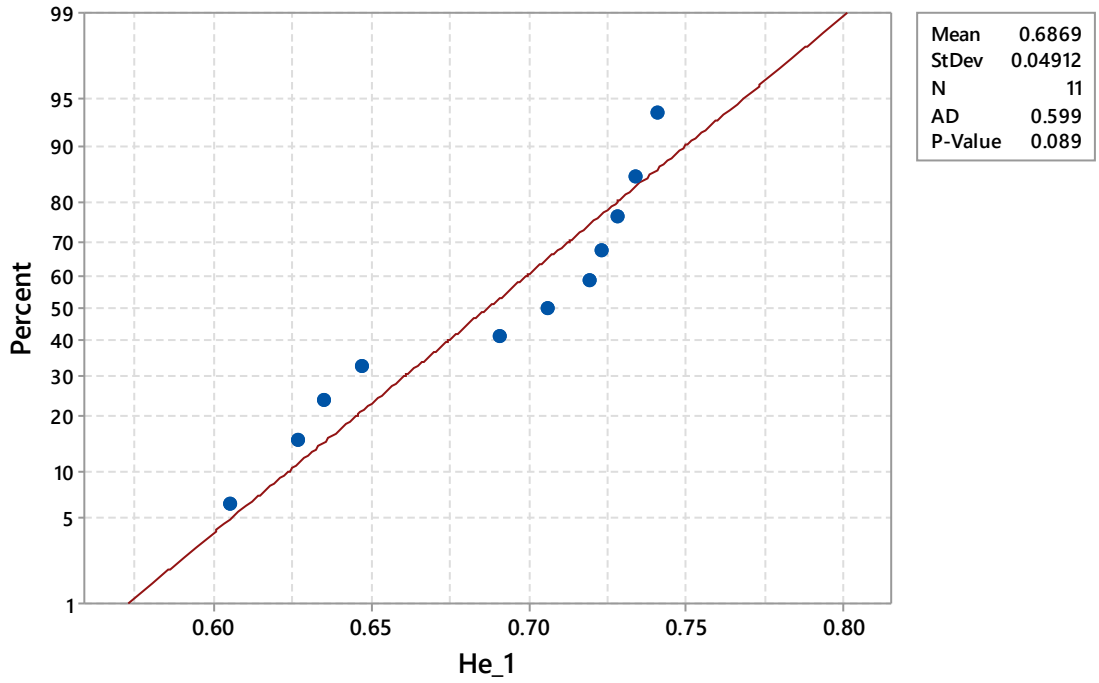
Appendix 7.16.7 Normality test of expected heterozygosities for only the populations downstream of headwater populations = Normal distribution



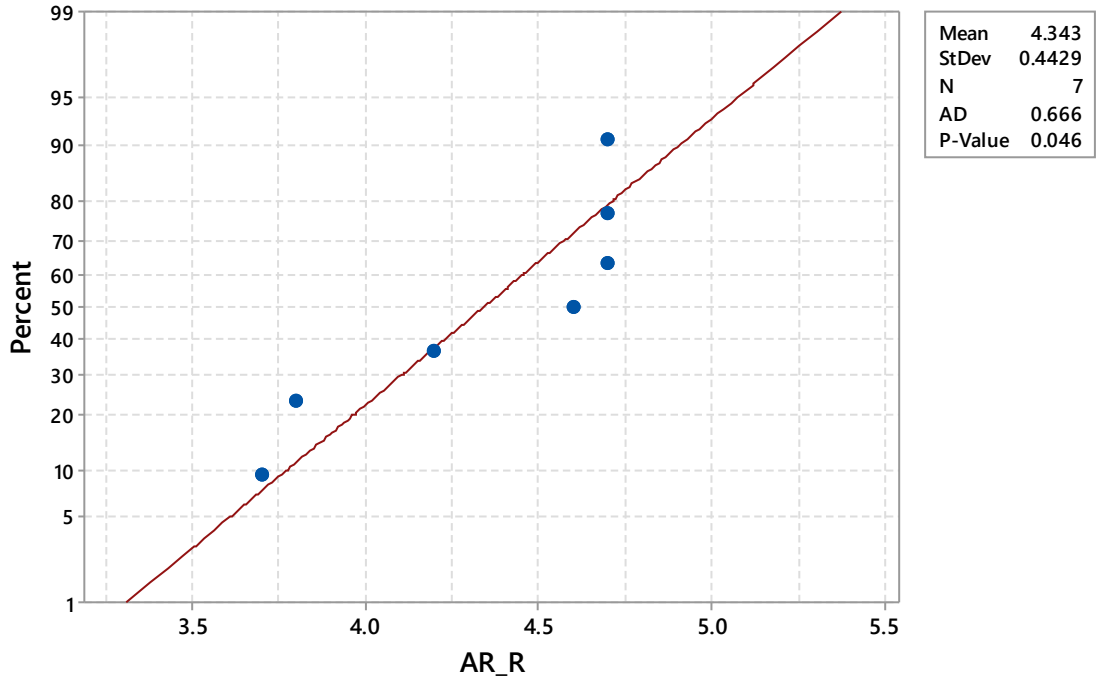
Appendix 7.16.8 Normality test of distances to sea (km) for populations without barriers to sea = Not-normally distributed



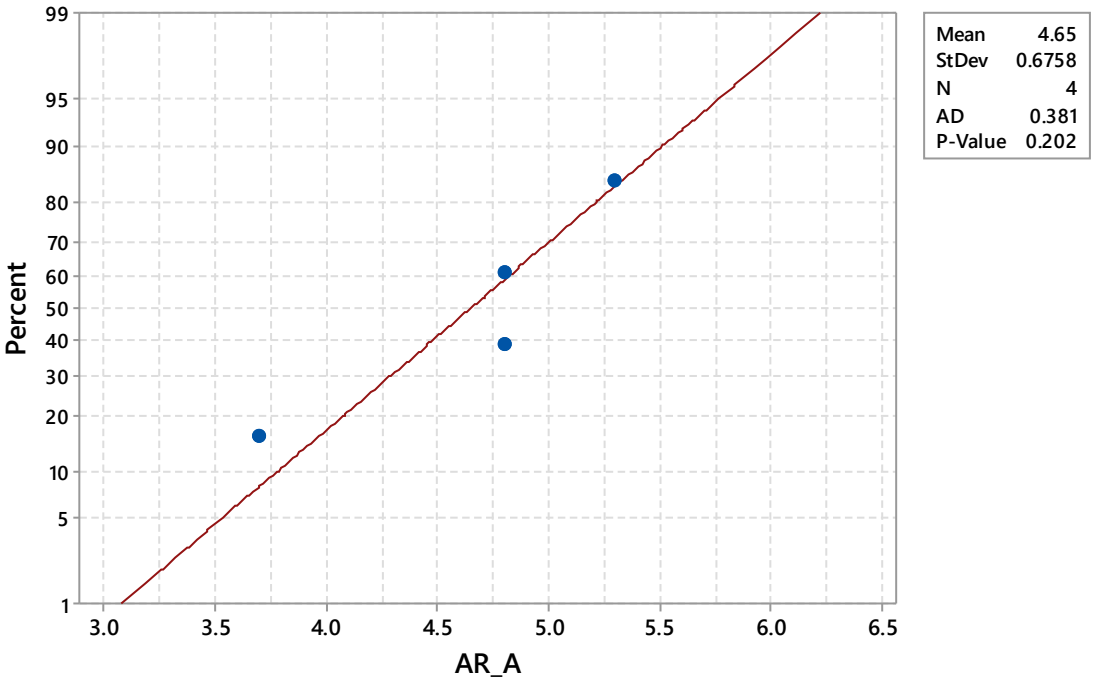
Appendix 7.16.9 Normality test of allelic richness found in populations without barriers to sea = Normally distributed



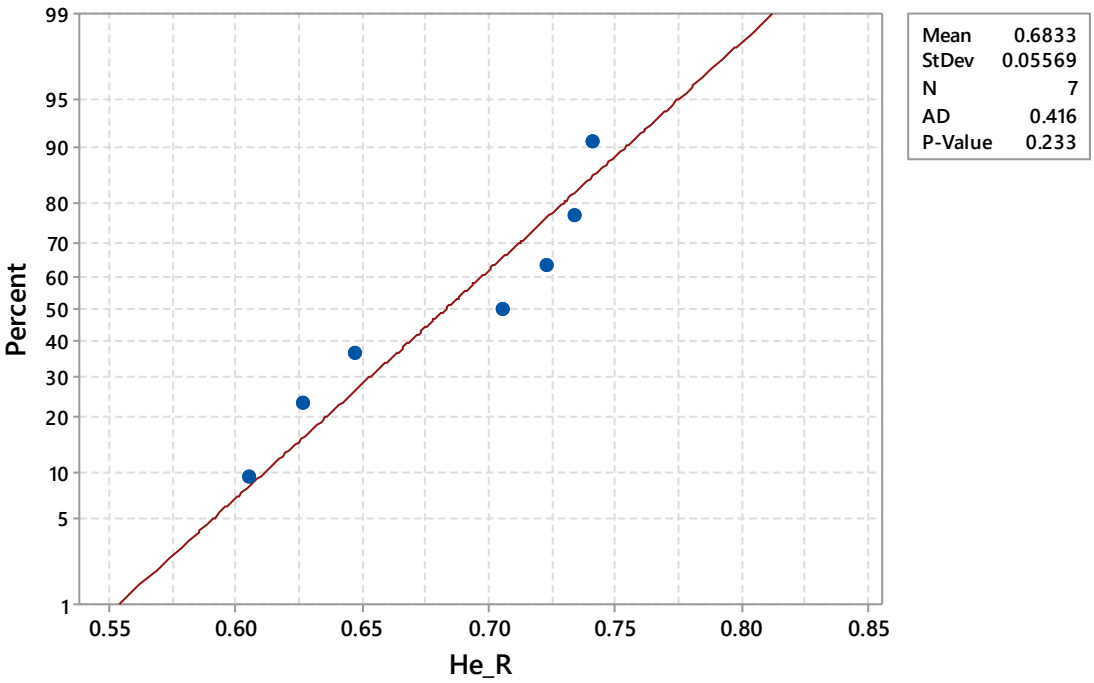
Appendix 7.16.10 Normality test of expected heterozygosities found in populations without barriers to sea = Normally distributed



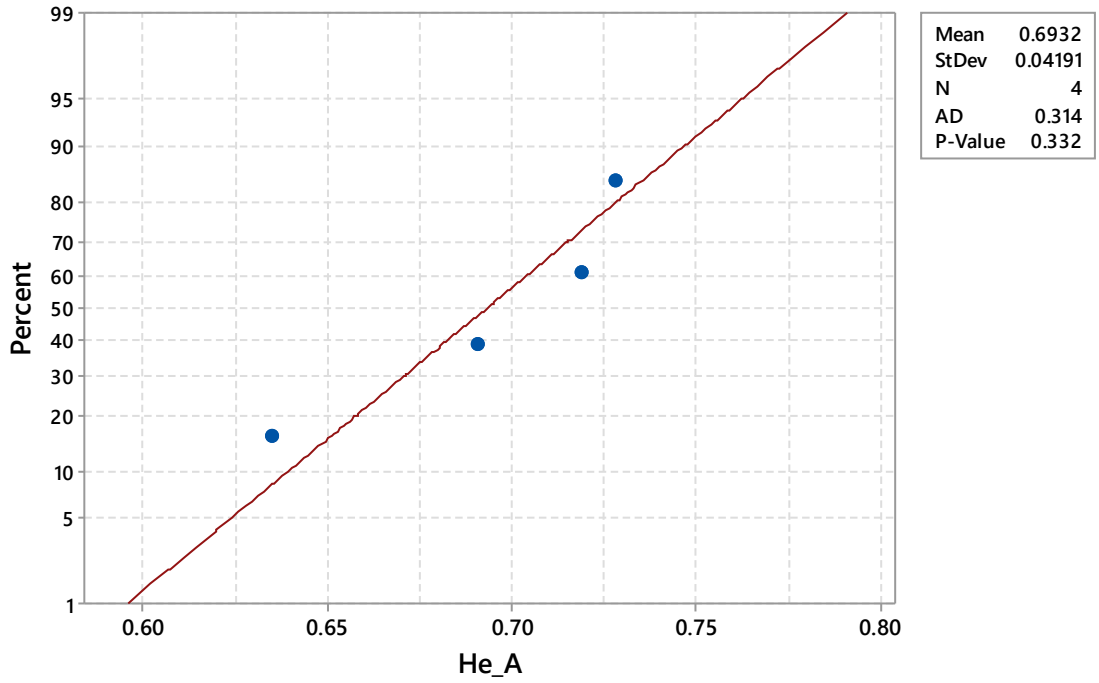
Appendix 7.16.11 Normality test of allelic richness found in resident populations without barriers to sea = Not-normally distributed



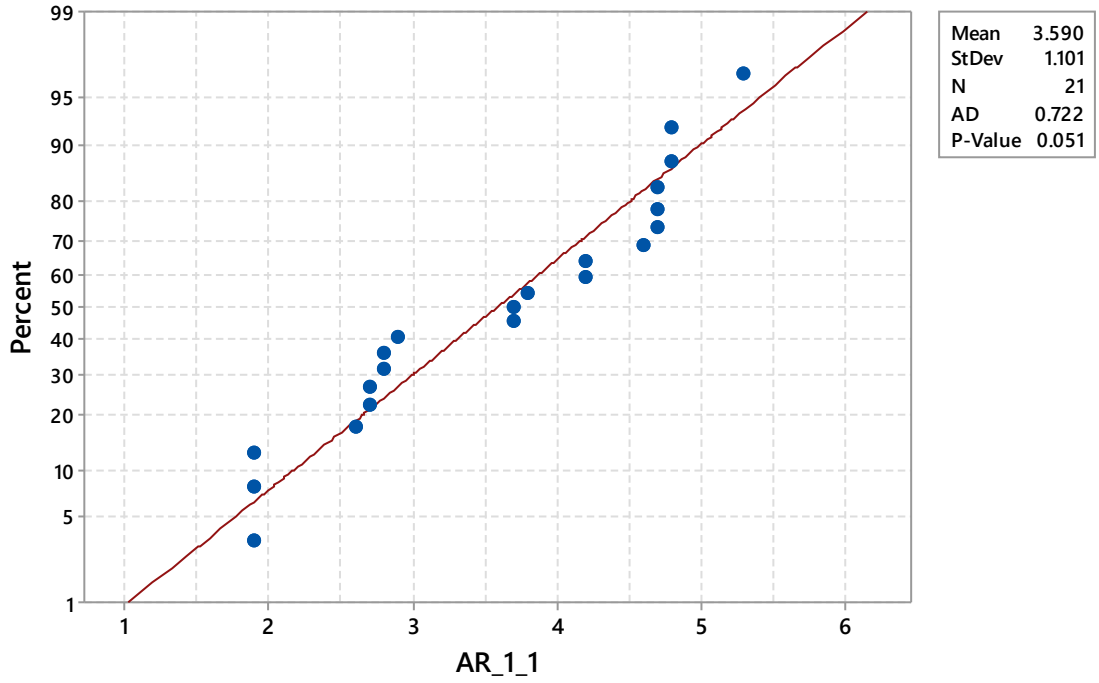
Appendix 7.16.12 Normality test of allelic richness found in anadromous populations without barriers to sea = Normally distributed



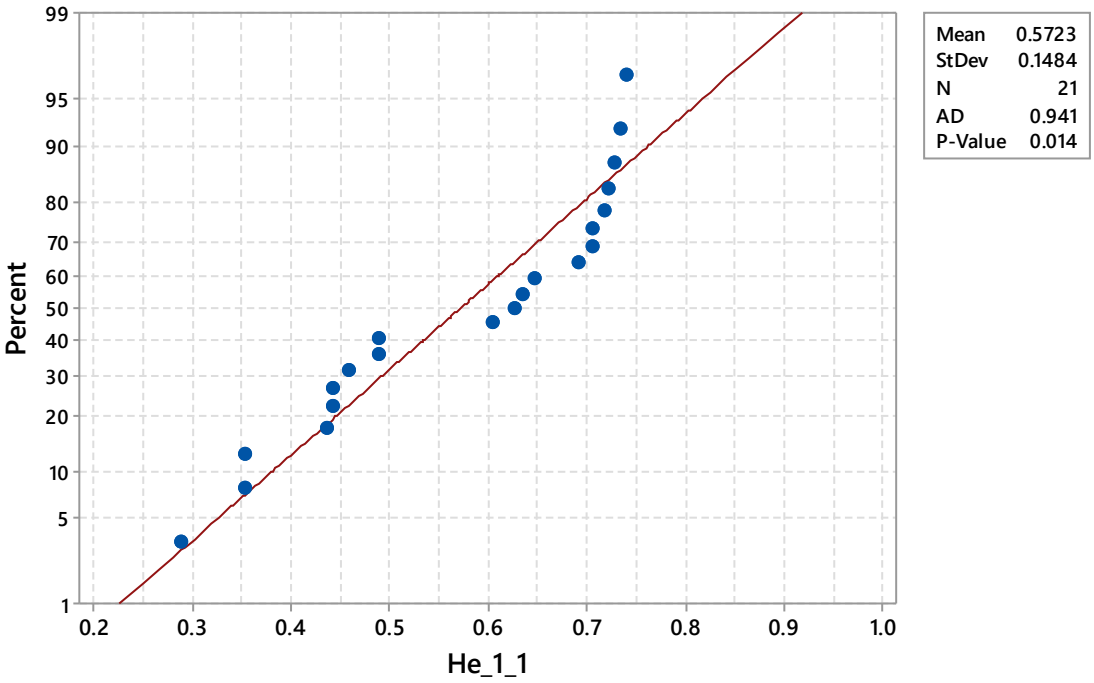
Appendix 7.16.13 Normality test of expected heterozygosities found in resident populations without barriers to sea = Normally distributed



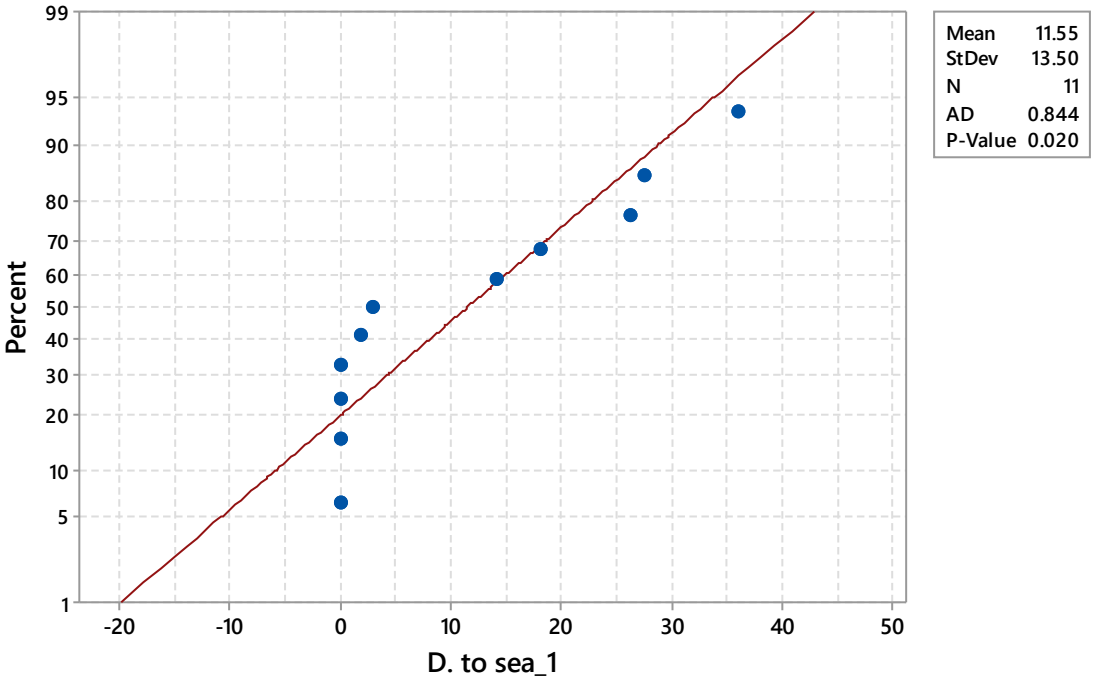
Appendix 7.16.14 Normality test of expected heterozygosities found in anadromous populations without barriers to sea = Normally distributed



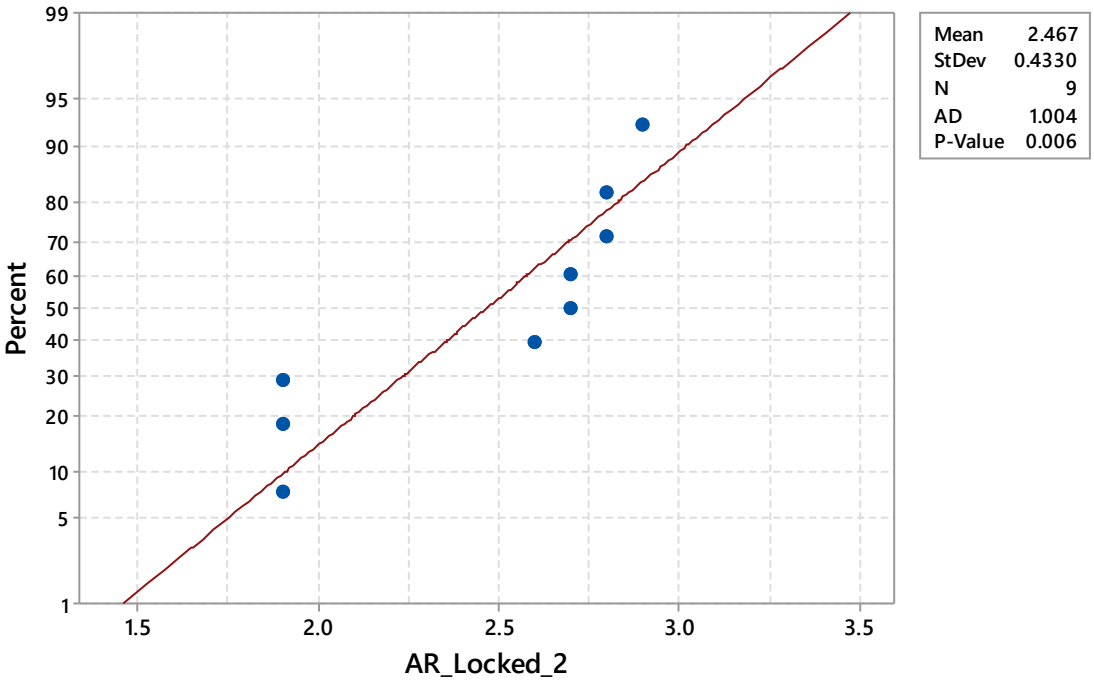
Appendix 7.16.15 Normality test of allelic richness found in populations with and without barriers to sea = Normally distributed



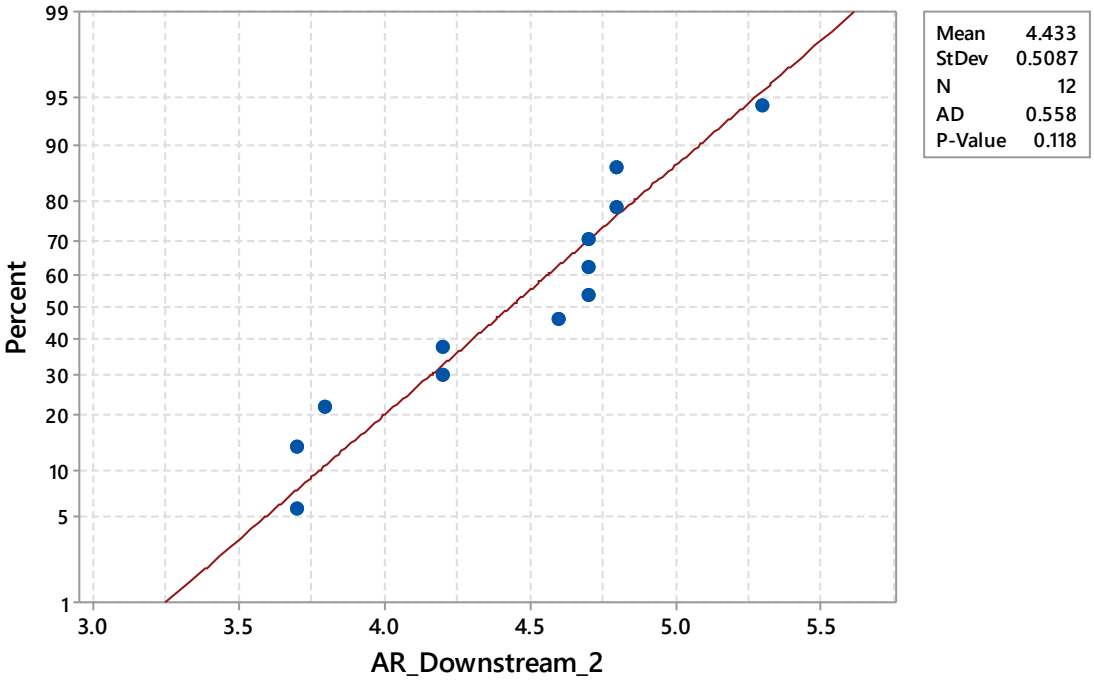
Appendix 7.16.16 Normality test of expected heterozygosities found in populations with and without barriers to sea = Non-normally distributed



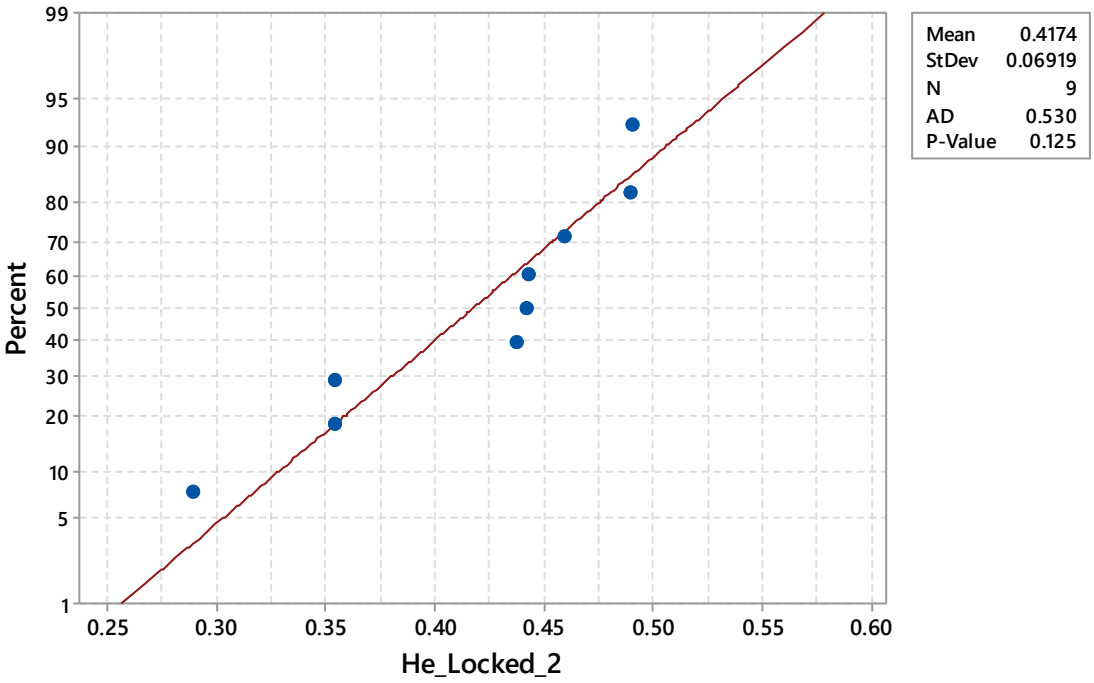
Appendix 7.16.17 Normality test of distances to sea (km) found in populations with and without barriers to sea = Non-normally distributed



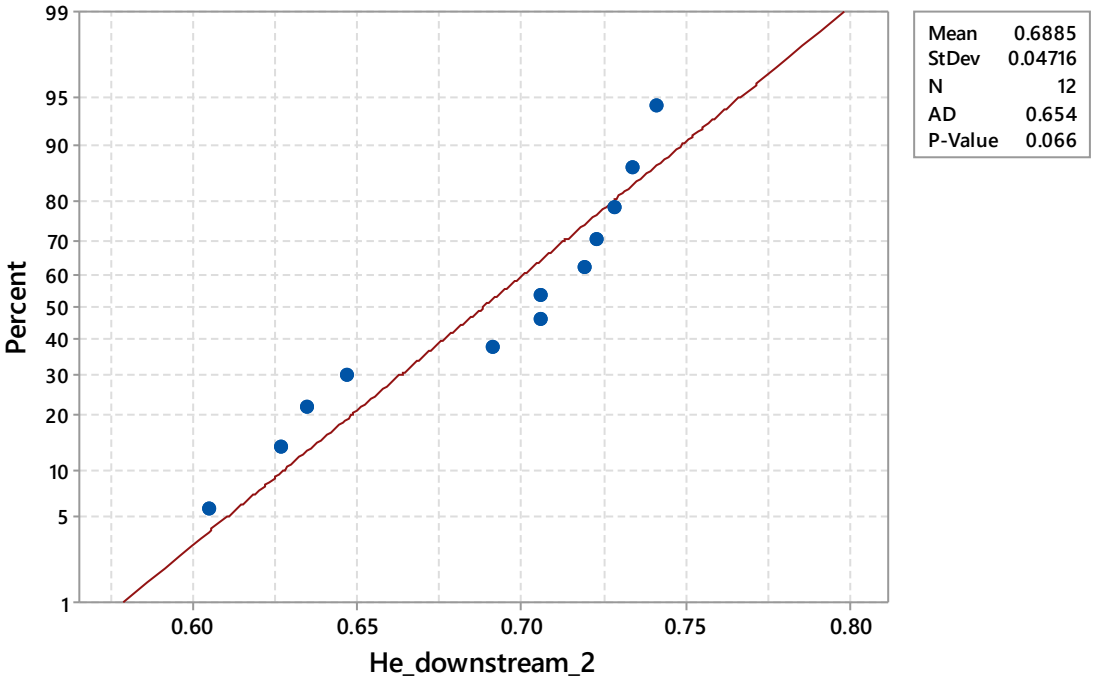
Appendix 7.16.18 Normality test of allelic richness found in headwater populations for analysis with and without barriers to sea = Non-normally distributed



Appendix 7.16.19 Normality test of allelic richness found in all populations below barriers for analysis with and without barriers to sea = Normally distributed



Appendix 7.16.20 Normality test of expected heterozygosity found in headwater populations for analysis with and without barriers to sea = Normally distributed



Appendix 7.16.21 Normality test of expected heterozygosity found in all populations below barriers for analysis with and without barriers to sea = Normally distributed

APPENDIX 7.17 HEADWATER POPULATIONS & BARRIERS TO MOVEMENT - TESTS FOR DIFFERENCES

Mann-Whitney Test and CI: AR_L, AR_downstream

```
               N  Median
AR_L           9   2.700
AR_downstream  7   4.800


Point estimate for η1 - η2 is -2.200
95.6 Percent CI for η1 - η2 is (-2.900,-1.800)
W = 45.0
Test of η1 = η2 vs η1 ≠ η2 is significant at 0.0010
The test is significant at 0.0009 (adjusted for ties)
```

Mann-Whitney Test and CI: He_locked, He_downstream

```
               N   Median
He_locked      9  0.44200
He_downstream  7  0.71900


Point estimate for η1 - η2 is -0.27600
95.6 Percent CI for η1 - η2 is (-0.36498,-0.23000)
W = 45.0
Test of η1 = η2 vs η1 ≠ η2 is significant at 0.0010
The test is significant at 0.0010 (adjusted for ties)
```

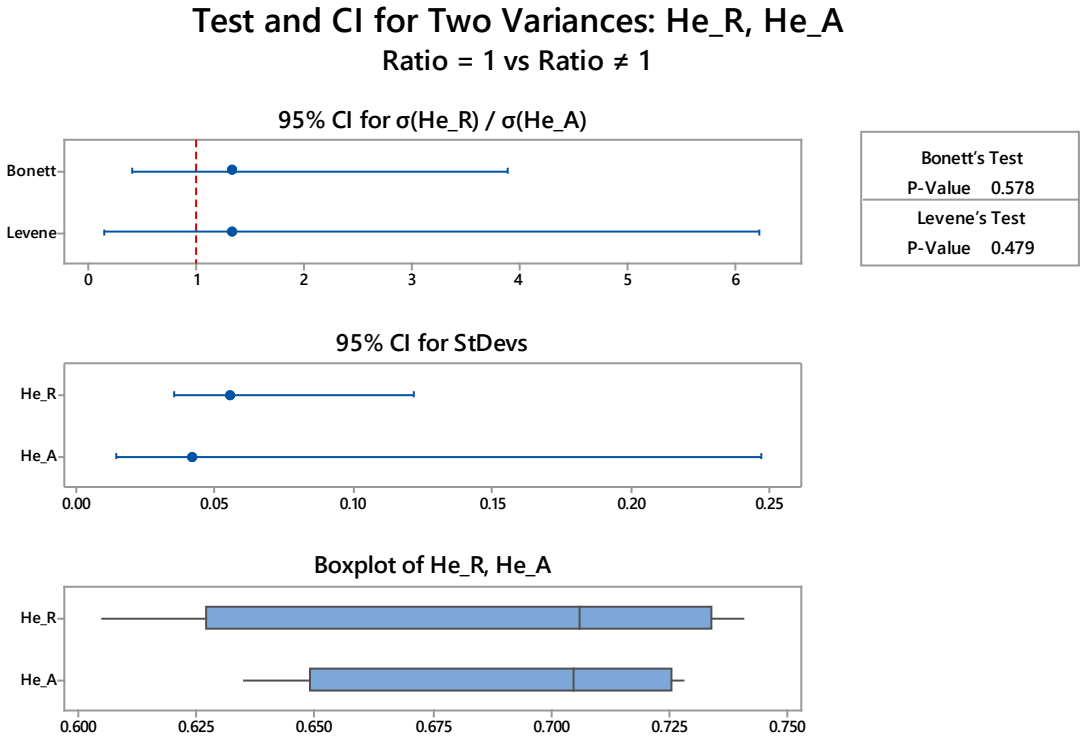
Mann-Whitney Test and CI: AR_R, AR_A

```
      N  Median
AR_R  7   4.600
AR_A  4   4.800


Point estimate for η1 - η2 is -0.200
95.3 Percent CI for η1 - η2 is (-1.100,0.900)
W = 34.5
Test of η1 = η2 vs η1 ≠ η2 is significant at 0.1859
The test is significant at 0.1798 (adjusted for ties)
```

Test and CI for Two Variances: He_R, He_A

```
Method

Null hypothesis         σ(He_R) / σ(He_A) = 1
Alternative hypothesis  σ(He_R) / σ(He_A) ≠ 1
Significance level      α = 0.05

Statistics
                                  95% CI for
Variable  N  StDev  Variance      StDevs
He_R      7  0.056     0.003  (0.035, 0.122)
He_A      4  0.042     0.002  (0.014, 0.247)

Ratio of standard deviations = 1.329
Ratio of variances = 1.766

95% Confidence Intervals
                              CI for
```

```
         CI for StDev       Variance
Method       Ratio           Ratio
Bonett   (0.395, 3.893)   (0.156, 15.154)
Levene   (0.134, 6.243)   (0.018, 38.971)

Tests
                        Test
Method  DF1  DF2  Statistic  P-Value
Bonett    —    —          —    0.578
Levene    1    9       0.55    0.479
```



Appendix 7.17.1 Equal variances test between expected heterozygosities for resident and anadromous trout without barriers to sea = Equal

Two-Sample T-Test and CI: He_R, He_A

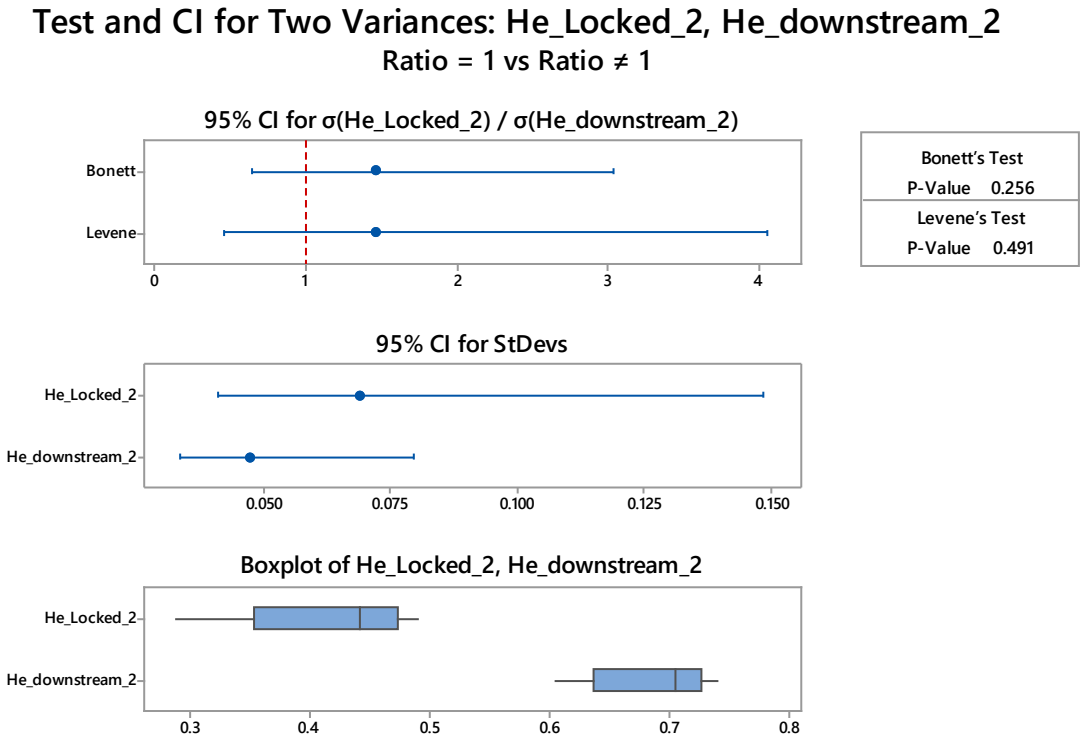
```
Two-sample T for He_R vs He_A

      N    Mean   StDev  SE Mean
He_R  7  0.6833  0.0557    0.021
He_A  4  0.6932  0.0419    0.021

Difference = μ (He_R) - μ (He_A)
Estimate for difference:  -0.0100
95% CI for difference:  (-0.0785, 0.0585)
T-Test of difference = 0 (vs ≠): T-Value = -0.34  P-Value = 0.746  DF = 8
```

Mann-Whitney Test and CI: AR_Locked_2, AR_Downstream_2

```
                  N  Median
AR_Locked_2       9  2.7000
AR_Downstream_2  12  4.6500

Point estimate for η1 - η2 is -1.9000
95.7 Percent CI for η1 - η2 is (-2.5002,-1.4999)
W = 45.0
Test of η1 = η2 vs η1 ≠ η2 is significant at 0.0001
The test is significant at 0.0001 (adjusted for ties)
```

Test and CI for Two Variances: He_Locked_2, He_downstream_2

```
Method

Null hypothesis         σ(He_Locked_2) / σ(He_downstream_2) = 1
Alternative hypothesis  σ(He_Locked_2) / σ(He_downstream_2) ≠ 1
Significance level      α = 0.05

Statistics
                                          95% CI for
Variable          N  StDev  Variance      StDevs
He_Locked_2       9  0.069     0.005  (0.041, 0.149)
He_downstream_2  12  0.047     0.002  (0.033, 0.080)

Ratio of standard deviations = 1.467
Ratio of variances = 2.152

95% Confidence Intervals
                                CI for
         CI for StDev       Variance
Method       Ratio            Ratio
Bonett   (0.647, 3.045)   (0.418,  9.275)
Levene   (0.459, 4.059)   (0.211, 16.476)

Tests
                       Test
Method  DF1  DF2  Statistic  P-Value
Bonett    —    —          —    0.256
Levene    1   19       0.49    0.491
```



Appendix 7.17.2 Equal variances test between expected heterozygosities for populations above and below barriers within all populations above and below = Not-equal

Mann-Whitney Test and CI: He_Locked_2, He_downstream_2

```
                  N  Median
He_Locked_2       9  0.4420
He_downstream_2  12  0.7060

Point estimate for η1 - η2 is -0.2665
95.7 Percent CI for η1 - η2 is (-0.3370,-0.2100)
W = 45.0
Test of η1 = η2 vs η1 ≠ η2 is significant at 0.0001
The test is significant at 0.0001 (adjusted for ties)
```

APPENDIX 7.18 HEADWATER POPULATIONS & BARRIERS TO MOVEMENT - CORRELATION ANALYSIS

Spearman Rho: D. to sea RK, AR RK

```
Spearman rho for D. to sea RK and AR RK = -0.753
P-Value = 0.001
```

Spearman Rho: D. to sea RK, He RK

```
Spearman rho for D. to sea RK and He RK = -0.753
P-Value = 0.001
```

Spearman Rho: Life H._1, AR RK

```
Spearman rho for Life H._1 and AR RK = 0.862
P-Value = 0.000
```

Spearman Rho: Life H._1, He RK

```
Spearman rho for Life H._1 and He RK = 0.862
```

Spearman Rho: Barrier RK, AR RK

```
Spearman rho for Barrier RK and AR RK = 0.867
P-Value = 0.000
```

Spearman Rho: Barrier RK, He RK

```
Spearman rho for Barrier RK and He RK = 0.867
```

Spearman Rho: D. to sea_1, AR_1

```
Spearman rho for D. to sea_1 and AR_1 = -0.380
P-Value = 0.249
```

Spearman Rho: D. to sea_1, He_1

```
Spearman rho for D. to sea_1 and He_1 = -0.242
P-Value = 0.474
```

Spearman Rho: Life H. RK_1, AR_1

```
Spearman rho for Life H. RK_1 and AR_1 = 0.454
P-Value = 0.160
```

Spearman Rho: Life H. RK_1, He_1

```
Spearman rho for Life H. RK_1 and He_1 = 0.000
P-Value = 1.000
```

Spearman Rho: D. to sea_1_1 RK, AR_1_1_1 RK

```
Spearman rho for D. to sea_1_1 RK and AR_1_1_1 RK = -0.281
P-Value = 0.217
```

Spearman Rho: D. to sea_1_1 RK, He_1_1 RK

```
Spearman rho for D. to sea_1_1 RK and He_1_1 RK = -0.284
P-Value = 0.212
```

Spearman Rho: Life H._1_1 RK, AR_1_1_1 RK

```
Spearman rho for Life H._1_1 RK and AR_1_1_1 RK = 0.869
P-Value = 0.000
```

Spearman Rho: Life H._1_1 RK, He_1_1 RK

```
Spearman rho for Life H._1_1 RK and He_1_1 RK = 0.794
P-Value = 0.000
```

Spearman Rho: Barrier_1 RK, AR_1_1_1 RK

```
Spearman rho for Barrier_1 RK and AR_1_1_1 RK = 0.862
P-Value = 0.000
```

Spearman Rho: Barrier_1 RK, He_1_1 RK

```
Spearman rho for Barrier_1 RK and He_1_1 RK = 0.859
P-Value = 0.000
```