Brown trout in the Falkland Islands: invasion ecology, population structure and genetic diversity

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Summary

Biological invasions are important causes of biodiversity loss, particularly in remote islands. Non-native salmonids, such as brown trout (*Salmo trutta*), have been widely introduced throughout the Southern Hemisphere, impacting endangered native fauna, particularly galaxiid fishes, through predation and competition. However, due to their importance for sport fishing and aquaculture, they are often protected, and any attempts to curtail their impacts are generally met with limited support, which poses a conservation conundrum. The best prospect of protecting native galaxiids is to predict where and how salmonids might disperse. This thesis aims to answer three main questions about brown trout in the Falkland Islands in order to provide resource managers with information to facilitate conservation planning to minimize the impacts of brown trout on native galaxiids. (1) determine the distribution of invasive brown trout and native zebra trout, *Aplochiton zebra* and *Aplochiton taeniatus*. (2) estimate patterns of movement and (3) assess the population structuring and estimate levels of gene flow between different rivers and populations of brown trout in the Falklands. To meet these aims, I used state-of-the-art methods, including SNP genotyping, stable isotope analysis, acoustic tagging, and environmental DNA (eDNA) analysis. The results of this thesis suggest that establishment success (calculated as the proportion of historical introductions where brown trout became established) was ~88% and that brown trout are continuing to spread from their original sites of introduction. The native *Aplochiton* species have disappeared from most rivers invaded by brown trout. Four genetically distinct clusters of brown trout were identified, with high levels of gene flow indicating widespread dispersal of brown trout across the Falkland Islands. Without strong containment, brown trout are predicted to invaded nearly all suitable freshwater habitats in the Falklands within the next ~70 years, which might put native galaxiids at a high risk of extinction.

Declaration and Statements

I, **Jessica Minett**, certify that this work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

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STATEMENT 1

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Authorship Declaration

The following people and institutions contributed to the publication of work undertaken as part of this thesis:

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List of Abbreviations

Abbreviation	Definition
A	Angling
AIC	Akaike Information Criterion
AUC	Area Under the Curve
BIC	Bayesian Information Criterion
bp	Base pair
CI	Confidence Intervals
DAPC	Discriminant Analysis of Principle Components
df	Degrees of freedom
DNA	Deoxyribonucleic acid
E	Estimate
EC	Electrical conductivity
eDNA	Environmental DNA
eDNA-HRM	Environmental DNA-high-resolution melt
EF	Electrofishing
F	F-statistic
FIG	Falkland Islands Government
F_{IS}	Inbreeding coefficient
F_{ST}	Proportion of the total genetic variance
g	Grams
GB	Great Britain
Ho	Heterozygosity
HRM	High-resolution melt
hrs	Hours
Hs	Gene diversity
Hz	Hertz
IBD	Isolation By Distance
k	Thousand
K	Number of centroids
Km	Kilometres
Km^2	Kilometres squared
L	Litre
LOD	Limit of detection
LOOCV	Leave One Out Cross Validation
LOQ	Limit of quantification
LRT	Likelihood ratio test
MAF	Minor Allele Frequency
min	Minutes
ml	Millilitre
mg	Milligrams
MH	Middleton Hatchery
mm	Millimetre
mt	Mitochondrial
N	Number
N_e	Effective population size
Nm	Gene flow parameter – product of effective population number and rate of migration

ng	Nanograms
No.	Number
p	P-value
PCR	Polymerase chain reaction
PES	Polyethersulfone
r	Correlation coefficient
SE	Standard error
Sec	Seconds
SIA	Stable isotope analysis
SN	Seine netting
SNP	Single Nucleotide Polymorphism
Spp.	Species
t	Test statistic
TDS	Total dissolved solids
TL	Trophic level
tm	Melt temperature
µl	Microliter
µm	Micrometre
µM	Micromolar
USGS	United States Geological Survey
UV	Ultraviolet
qPCR	Quantitative PCR
v	volt
VIF	Variance Inflation Factor
χ^2	Chi-squared statistic
‰	Parts per thousand
°C	Degrees Celsius
%	Percentage
~	Approximately
δ	Delta
‰	Parts per thousand

Chapter 1 Introduction

1.1 Freshwater Ecosystems and Invasive Species

Freshwater ecosystems contain only 0.01% of the world's water and cover 5-7% of the Earth's surface. Yet, they are home to one-third of all vertebrate species, including almost 50% of global fish diversity, over 14,736 species (Lundberg *et al.*, 2000; Lehner and Do, 2004; Balian *et al.*, 2008; Vega and Wiens, 2012). Despite such species richness freshwater ecosystems are experiencing declines in biodiversity at a far greater rate than any terrestrial ecosystems. In 2016 the Living Planet Index reported that freshwater vertebrates declined by 81% between 1970 and 2012, representing an annual decline of 3.9%. In contrast, land and sea vertebrates declined by 38% and 36% respectively (Collen *et al.*, 2009; World Wildlife Foundation, 2016). Therefore, freshwater ecosystems could be classified as the most endangered ecosystem in the world (Sala *et al.*, 2000; Dudgeon *et al.*, 2006). Previous work by Dudgeon et al. (2006) identified five main challenges to freshwater ecosystems; however, a more recent study by Reid et al. (2019) named 12 emerging threats to freshwater biodiversity; amongst both was the introduction of invasive species, **Table 1.1** (Dudgeon *et al.*, 2006; Reid *et al.*, 2019). Without appropriate measures, freshwater ecosystems which provide critical ecosystem services, essential to humanity and freshwater biodiversity will be endangered (Sala *et al.*, 2000; Rockström and Karlberg, 2010).

Strong human dependence on freshwaters has resulted in widespread water pollution, habitat degradation and biodiversity loss due to changes in land use, the introduction of invasive species and flow modification (Malmqvist and Rundle, 2002; Dudgeon *et al.*, 2006; Reid *et al.*, 2019). Due to the expanding human population requiring more urban and agricultural zones, the demand for freshwater resources is increasing (Martinuzzi *et al.*, 2014). Additional agricultural zones are required to meet a growing need for food production. However, this can lead to increased levels of sediment, pesticides and nutrients entering freshwaters (Schaller *et al.*, 2004), consequently resulting in changes to the water chemistry, causing eutrophication and harmful algal blooms (Heisler *et al.*, 2008; Moss, 2008). Endocrine-disrupting chemicals and antimicrobials have also been found entering freshwater from urban wastewater. Synthetic hormones can result in the development of intersex in male fishes which can have transgenerational effects and reduced species fitness and abundance (Jobling *et al.*, 2002; Harris *et al.*, 2011; Schwindt *et al.*, 2014). Changes in land use can also alter stream flows and diminish flood pulses through flow modification in order to support

agricultural systems, consequently decreasing stream-channel and riparian habitats, biodiversity, and reducing native fish movement (Schlosser, 1995; Poff *et al.*, 1997; Roy *et al.*, 2003; Scanlon *et al.*, 2007; King *et al.*, 2011).

The flow of freshwater systems can be modified through instream infrastructure and impact many aspects of an ecosystem, for example, dams, weirs, and culverts can affect species movement, alter water chemistry and sediment dynamics, and create discontinuities in temperature (Andersson *et al.*, 2000; Stanley and Doyle, 2002; Mueller *et al.*, 2011; Pépino *et al.*, 2012; McIntyre *et al.*, 2015; Oele *et al.*, 2019). The development of instream infrastructure is increasing at unprecedented rates with 11 small hydropower plants for every large dam globally (Couto and Olden, 2018). However, low head structures (<1m) are often overlooked and missing from records, with field surveys indicating that barrier density is underestimated by over 60% in current databases (Jones *et al.*, 2019; Belletti *et al.*, 2020). Thus <1% of catchments are free of artificial barriers in the UK (Jones *et al.*, 2019), and only 37% of rivers >1000 km remain free-flowing globally (Grill *et al.*, 2019).

Overexploitation is another major cause of freshwater biodiversity loss, although it predominantly impacts fishes, some reptiles and amphibians, certain aquatic invertebrates and mammals can also be affected. Despite primarily only being considered a problem with marine fisheries, overexploitation also widely affects freshwater ecosystems (Raby *et al.*, 2011), especially in poorer and remote countries where freshwater species provide a critical source of protein (Allan *et al.*, 2005; Welcomme *et al.*, 2010; He *et al.*, 2017). Overexploitation can contribute to species declines through targeted harvest, for example, sturgeon and paddlefish are being pushed to the brink of extinction due to intense fishing pressure for caviar, resulting in many fisheries crashing 7-20 years after launching and decreasing harvests, putting the survival of these species in the wild at risk (Pikitch *et al.*, 2005), or through by-catch, as observed in the Yangtze River dolphin (*Lipotes vexillifer*), which was the first recorded extinction of a cetacean species due to human activity. Declines in the Yangtze River dolphin populations were mainly attributed to by-catch in local fisheries where electrofishing, gill and fyke nets, and rolling hooks were used, with 40% of Yangtze River dolphin mortality recorded during the 1990s attributed to electrofishing alone (Turvey *et al.*, 2007).

Climate change is likely to exacerbate and magnify many of the other threats and challenges faced by freshwater ecosystems, as well as increasing water temperatures, creating changes in

flow and water discharge, and shifts in species distribution. Extreme events (i.e., storm events, floods, and droughts) are likely to become more prevalent, with rising temperatures modifying species distributions and disease outbreaks. For example, cold-water species may experience a reduction in range (Meisner, 1990; Rahel, 2002; Xenopoulos *et al.*, 2005), whereas temperate- and warm-water species may experience range expansions (Chu *et al.*, 2005; Buisson *et al.*, 2008). However, such range expansions may severely impact fish communities already inhabiting those watersheds, especially when invading species are top predators (Chu *et al.*, 2005; Rahel and Olden, 2008). Climate change can impact population dynamics and community composition. For brook trout (*Salvelinus fontinalis*) increases in mean stream temperature have been shown to reduce the survival of the youngest age class, thus, decreasing overall population size (Bassar *et al.*, 2016). Changes in population dynamics can have knock-on ecological impacts for communities, as observed in Rio Grande, New Mexico, where the onset of spawning advanced by 4-28 days between 2008-2010 compared to 1995. Therefore, decreasing the resource partitioning between species and altering the species composition (Krabbenhoft *et al.*, 2014), similar effects have also been observed in aquatic insect communities (Thompson, 2016). In addition to warming temperatures, cold shock events, such as the rapid decrease in water temperature in Bolivia in 2010 that caused the mass mortality of fishes in the Amazon, are likely to become more widespread (Szekeres *et al.*, 2016). Species in the tropics are more susceptible due to little seasonal variation, ensuing species are less adapted to fluctuations in temperature (Szekeres *et al.*, 2014; Gutiérrez-Pesquara *et al.*, 2016). Global temperature rises have resulted in a shift in the oviposition date of the Chinese alligator (*Alligator sinensis*) by 10 days between 1987-2005 (Zhang *et al.*, 2009). Indicating the potential and substantial effects climate change can have on species phenology. Furthermore, rising temperatures can also impact species demographics by altering sex ratios (Thompson, 2016), in fishes the sex ratio can be significantly changed from 1:1 (males: females) to 3:1 (males: females) by an increase of only 1-2°C (Piferrer, 2008).

A wide range of species have been introduced to freshwaters around the world through accidental introductions, whereby species hitchhike on ships/planes transporting people and/or goods, or intentional introductions, where species are the commodity and, therefore, deliberately transported/released (Hulme, 2009). Many freshwater fish have been introduced for recreational purposes (e.g., angling) or as a result of aquaculture activities (Savini *et al.*, 2010). For example, in Chile and Argentina it is commonplace to stock salmonids, mainly

rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) (Pascual *et al.*, 2007). In addition, Chile became the second-largest producer of salmon in the 1990s and now generates 25% of global salmon production (Asche *et al.*, 2013; Iversen *et al.*, 2020). Yet, as salmon production in Chile increased, so did escapes from aquaculture net pens, leading to fish straying into rivers in Chile and Argentina and forming self-sustaining populations (Pascual *et al.*, 2007; Arismendi *et al.*, 2009; Di Prinzio and Pascual, 2009). As global trade and e-commerce has grown so has the intentional introduction of species to novel environments through exotic pet trade (Padilla and Williams, 2004; Keller and Lodge, 2007). Exotic plants and animals are now sold internationally via the internet and online auctions (Kay and Hoyle, 2001; Humair *et al.*, 2015), resulting in species becoming established and invasive through accidental escapes and intentional releases (Lockwood *et al.*, 2019). Seven to eight percent of pet owners have admitted to intentionally releasing pets into the wild due to excessive growth, aggressive behaviour, lack of space or rapid reproduction (Gertzen *et al.*, 2014; Banha *et al.*, 2019). Such introductions have resulted in 84% of the non-native amphibians and reptiles introduced to Florida arriving as a result of pet trade (Krysko *et al.*, 2011), and over 10,000 fishes estimated to be released every year in Montréal, Canada (Gertzen *et al.*, 2014). The trade of live bait has also contributed to the introduction of invasive species as anglers accidently or intentionally release unused bait, which can contain multiple non-target, and possibly invasive, species into waterbodies (Mahon *et al.*, 2014; Nathan *et al.*, 2014). Over 50% of anglers have released live unused bait in the United States (Litvak and Mandrak, 1993; Kilian *et al.*, 2012) with approximately 5% of bait samples containing at least one target invasive fish species (Nathan *et al.*, 2014), such releases have resulted in the known introduction of 47 freshwater species to the Mid-Atlantic slope region of the United States (Kilian *et al.*, 2012).

Biological invasions are frequently thought of as a staged process (Richardson et al., 2000; Colautti and MacIsaac, 2004). Although often not linear, they are seen to be divided by barriers or ecological filters that species must pass before progressing to the next stage (Richardson et al., 2000; Colautti and MacIsaac, 2004; Mitchell et al., 2006). With the number of species reaching each stage diminishing due to the process (Williamson, 1993; Williamson and Fitter, 1996; Levine et al., 2004). Viewing invasion in such a way allows ecologists to conceptualise factors that may enable species to move from one stage to the next, guiding research and management actions. For example, Williamson's rule of ten states that 1 in 10 will pass from one stage to the next, with the stages being importation,

introduction, establishment, and pest (Williamson and Fitter, 1996). However, biological invasions are a complicated process composed of many steps and drivers (e.g., propagule pressure and various biotic and abiotic factors), and the contribution of these different mechanisms to invasion success is unknown and likely varies depending on the invading species, time, and space (Pyšek and Richardson, 2006; Catford et al., 2009).

Although not all introduced species become invasive, those that do have been observed to have dramatic impacts on native species and ecosystems (Gallardo *et al.*, 2016). Aquatic invasive species directly impact native populations through predation (McIntosh *et al.*, 1994; Kats and Ferrer, 2003; Habit *et al.*, 2010), competition (Jackson, 2002; Martin *et al.*, 2010; Richter-Boix *et al.*, 2013), and hybridization (Perry *et al.*, 2002; Dufresnes *et al.*, 2016), or indirectly through habitat modification (Jackson, 2002; Matsuzaki *et al.*, 2009; Emery-Butcher *et al.*, 2020) and the spread on of novel diseases (Miaud *et al.*, 2016; Martín-Torrijos *et al.*, 2019). In addition, the introduction of invasive species can have impacts of local communities as observed in Guangdong Province, China where the introduction of Nile tilapia (*Oreochromis niloticus*) reduced the growth, density, and abundance of native mud carp leading to socio-economic impacts due to a reduction in income for local fishermen (Gu *et al.*, 2015).

The transport of species around the world has resulted in biotic homogenization, the widespread introduction of species outside of their native range and the loss of native and endemic species, reducing regional biodiversity and distinctiveness (Mckinney and Lockwood, 1999; Rahel, 2000). More than 600 freshwater fish species had been introduced outside of their native range for fishing, aquaculture, and ornamental reasons by the end of the 20th century, many of which tend to dominate the communities and ecosystems they invade (Rahel, 2007; Gozlan, 2008; Gozlan *et al.*, 2010). However, only a few introduced species contribute to worldwide homogenization (Toussaint *et al.*, 2016). Increasing homogenization is of critical importance as it can result in the extinction of endemic species, and the loss of species diversity and geographic uniqueness as species become common, and communities become more uniform in geographically distinct and distant areas (Taylor, 2004). For instance, widespread introductions to enhance food and sport fisheries across the United States resulted in an average similarity increase of 7.2% (Rahel, 2000). The introduction of invasive species has direct effects on native ecosystems, by reducing the abundance and distribution of native fauna and flora, but can also indirectly affect ecosystems, for example, decreasing the availability of host species for obligate parasitic

freshwater mussels which could potentially result in evolutionary and demographic consequences, particularly if host specificity is critical (Douda *et al.*, 2013; Huber and Geist, 2019).

Table 1.1. Main threats faced by Freshwater ecosystems.

Threat	Impact	Reference
Water pollution	Change water quality (eutrophication, sedimentation, and harmful algal blooms); cause developmental and reproductive abnormalities affecting species fitness and abundance through endocrine disruption	(Guillette *et al.*, 1994; Heisler *et al.*, 2008; Moss, 2008; Schwindt *et al.*, 2014)
Instream infrastructure	Alter movement of species; reduce access to upstream spawning ground; fragment populations; alter the natural flow, diminish flood pulses, and create thermal discontinuities; decrease channel and riparian habitats; modify water chemistry and sediment dynamics; increase the prevalence of water-related diseases	(Andersson *et al.*, 2000; Stanley and Doyle, 2002; Roy *et al.*, 2003; Steinmann *et al.*, 2006; Pépino *et al.*, 2012; Ong *et al.*, 2016; Oele *et al.*, 2019)
Overexploitation	Declines in the abundance of species and local populations; increased mortality and risk of extinction due to targeted harvest and by-catch.	(Pikitch *et al.*, 2005; Turvey *et al.*, 2007; Raby *et al.*, 2011)
Climate change	Changes in species distribution and survival; disease outbreaks; rising temperatures; the increased prevalence of extreme events (storm events, drought, floods, cold shock); alterations in annual precipitation potential impact on population demographics in reptiles; interactions with other stressors/threats	(Chu *et al.*, 2005; Piferrer, 2008; Zhang *et al.*, 2009; Krabbenhoft *et al.*, 2014; Bassar *et al.*, 2016; Szekeres *et al.*, 2016; Thompson, 2016)
Invasive species	Changes in species abundance and distribution through predation, competitive exclusion and hybridization; introduction of diseases; habitat modification though altering food web structure, water chemistry and sedimentation	(McIntosh *et al.*, 1994; Jackson, 2002; Richter-Boix *et al.*, 2013; Gallardo *et al.*, 2016; David *et al.*, 2017; Martín-Torrijos *et al.*, 2019)

1.2 The Falkland Islands

The Falkland Islands are an archipelago in the South Atlantic located 500km off the mainland of South America. The islands cover an area of 12,200 km^2 and are composed of two large main islands (East and West Falkland) surrounded by 780 smaller islands (McDowall *et al.*, 2001; Broughton and McAdam, 2005; Fowler, 2013). The Falklands have a cool temperate oceanic climate with mean summer and winter temperatures of 9.4°C in January and 2.2°C in July respectively, low levels of precipitation (mean annual rainfall of 640mm for Stanley between 1944-1978) and an average wind speed of approximately 16 knots (Poncet *et al.*, 2011). The landscape is generally hilly, with Mt. Usborne on East Falkland being the tallest mountain at 705m high (Broughton and McAdam, 2005). Although the islands were uninhabited until 1764, they were likely exposed to invasive rats (*Rattus norvegicus* and *Rattus rattus*) and mice (*Mus musculus*) through passing whaling and sealing vessels (Poncet *et al.*, 2011). There are now many introduced species on the islands including 192 non-native plants (Broughton and McAdam, 2005; Lewis and Gardens, 2014), a number of invertebrates, and many vertebrates including feral cats (*Felis catus*), the domestic goose (*Anser anser*) and brown trout (*Salmo trutta*) (Rendell, 2011).

The Falkland Islands are home to three species of native freshwater fish, two species of zebra trout, *Aplochiton zebra* and *Aplochiton taeniatus*, and the Falklands minnow, *Galaxias maculatus* (McDowall *et al.*, 2001; Vanhaecke *et al.*, 2012b). Other species, such as *Galaxias platei* and the pouched lamprey, *Geotria australis*, have also been reported although these reports have never been substantiated, due to only one specimen of *G. platei* ever recorded in 1905, and there is debate over the origin of the specimen (McDowall, 2005). Although the pouched lamprey has been recorded sporadically in the islands, these occurrences are believed to result from individuals migrating from South America and South Georgia, with the last known occurrence in 2016. The family Galaxiidae are one of the most threatened and endangered families of fish (Helfman, 2007; McIntosh *et al.*, 2010), with declines of native galaxiid fishes in New Zealand (Townsend and Crowl, 1991; Townsend, 1996; McDowall, 2003; McDowall, 2006) and South America (Arismendi *et al.*, 2009; Young *et al.*, 2009; Habit *et al.*, 2010; Young *et al.*, 2010; Elgueta *et al.*, 2013) associated with the introduction of salmonids (Garcia de Leaniz *et al.*, 2010).

1.1 Introduction of Salmonids to the Falkland Islands

Brown trout were introduced to the Falkland Islands between 1947-1962 from Chile and the UK. Over this 18-year period, approximately 113,000 brown trout ova were imported and introduced to 29 watersheds across the Falklands (Stewart, 1973; Stewart, 1980; Fowler, 2013; Minett *et al.*, 2021a). Subsequently, the once abundant *Aplochiton* spp. is now limited to uninvaded refugia in the south if the islands and classified as threatened in the Falkland Islands (McDowall *et al.*, 2001; Ross, 2009). For more information on the introduction of brown trout, see Chapter 2.

Several other salmonid species have been introduced to the Falklands. In 1944 small quantities of rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) were shipped from Chile (Crawford and Muir, 2008; MacCrimmon, 2011; Monzón-Argüello *et al.*, 2014b). Atlantic salmon (*Salmo salar*) was introduced from the UK in 1960-1964; yet only brown trout have survived and formed self-sustaining populations (Arrowsmith and Pentelow, 1965). Rainbow and brook trout were introduced in small quantities; therefore, their lack of survival and establishment is likely due to their low propagule pressure (number and frequency of introductions) (Lockwood *et al.*, 2005; García-Díaz *et al.*, 2015). However, Atlantic salmon were introduced in similar quantities to those of brown trout (approximately 104,000 and 113,000 respectively). Although Atlantic salmon were reported to survive well in rivers, they have never been recorded returning from sea and spawning in the islands. Similar disappearances of introduced Atlantic salmon have also been observed in other Southern Hemisphere locations. Atlantic salmon were introduced to the Kerguelen Islands, a French sub-Antarctic archipelago in the Southern Ocean, in the 1970s, however, they failed to establish anadromous populations (Lecomte *et al.*, 2013).

Two small scale fish farms have been set up in the Falklands, one in 1986 farming Atlantic salmon in Fox Bay, West Falkland, the second in 2013 farming brown trout in Fitzroy sound, East Falkland (Fowler, 2013; Bridson, 2018). The Atlantic salmon farm was a small-scale project which imported 28,500 eggs to the Falklands, from which at least 2000 salmon were transported to sea cages. However, this project was deemed unsuccessful and shut down in 1990 (Fowler, 2013). Escapes from sea cages are not uncommon in salmon farming and are the main source of introduction for invasive salmonids in the Southern Hemisphere (Arismendi *et al.*, 2009; Consuegra *et al.*, 2011). Although no salmon were reported to escape it is possible that some may have, though Atlantic salmon have never established breeding populations in the Falklands. The farming of brown trout started with the transfer of 10,000

sea trout smolts from local broodstock into sea cages in Fitzroy Harbour, today brown trout are still farmed in the islands at a freshwater hatchery at Moody Brook, and sea cages in Fitzroy Harbour. All farmed brown trout originated from local broodstock, except for fish from 2014/2015 where eggs were imported to the islands from Howietoun hatchery, UK. The level of escape from the sea cages and the possible impacts on the wild brown trout populations is unknown.

Despite Atlantic salmon never returning from sea and forming self-sustaining populations in the Falklands local fishermen have occasionally reported catching a 'salmon'. These reports are, however, likely to be unusual sea trout, due to their highly plastic phenotype. In 2011 and 2019 a chinook salmon (*Oncorhynchus tshawytscha*) (Fowler, 2013) and a coho salmon (*Oncorhynchus kisutch*) were caught, respectively, these salmon possibly originated from escaped farmed fish from Patagonia (Ciancio *et al.*, 2005; Correa and Gross, 2008), demonstrating that the Falklands are not completely isolated and there is the potential for future invasions by other salmonid species.

1.2 Brown Trout as an Invasive Species

The native range of brown trout extends from Iceland and the northern coasts of Europe to North Africa and towards the northern slopes of the Himalayas (MacCrimmon and Marshall, 1968). Brown trout were first introduced beyond their native range in 1864, when 300 ova from the Wey and river Itchen, UK were introduced to the Plenty River, Tasmania (MacCrimmon and Marshall, 1968). Subsequently, brown trout have been introduced to rivers and lakes on every continent except Antarctica (**Figure 1.1**). Despite little information on the state of local fauna in the Southern Hemisphere before their introduction (Morgan *et al.*, 2004; Pascual *et al.*, 2007), brown trout have been linked to declines in native species and are now classified as one of the 100 world's worst invasive species (MacCrimmon and Marshall, 1968; Lowe *et al.*, 2000; Cambray, 2003). The invasion biology and impacts of brown trout on native fauna and ecosystems has been studied around the world, but extensively in New Zealand and South America where brown trout have been associated with the decline in native galaxiid fishes, one of the most endangered fish families in the world (McIntosh *et al.*, 2010). Negative relationships have been observed between the abundance of native fishes and salmonids in Chile (Soto *et al.*, 2006), Patagonia (Arismendi *et al.*, 2009) and New Zealand (Townsend, 1996) rivers, with predation and competition considered the main cause for these declines.

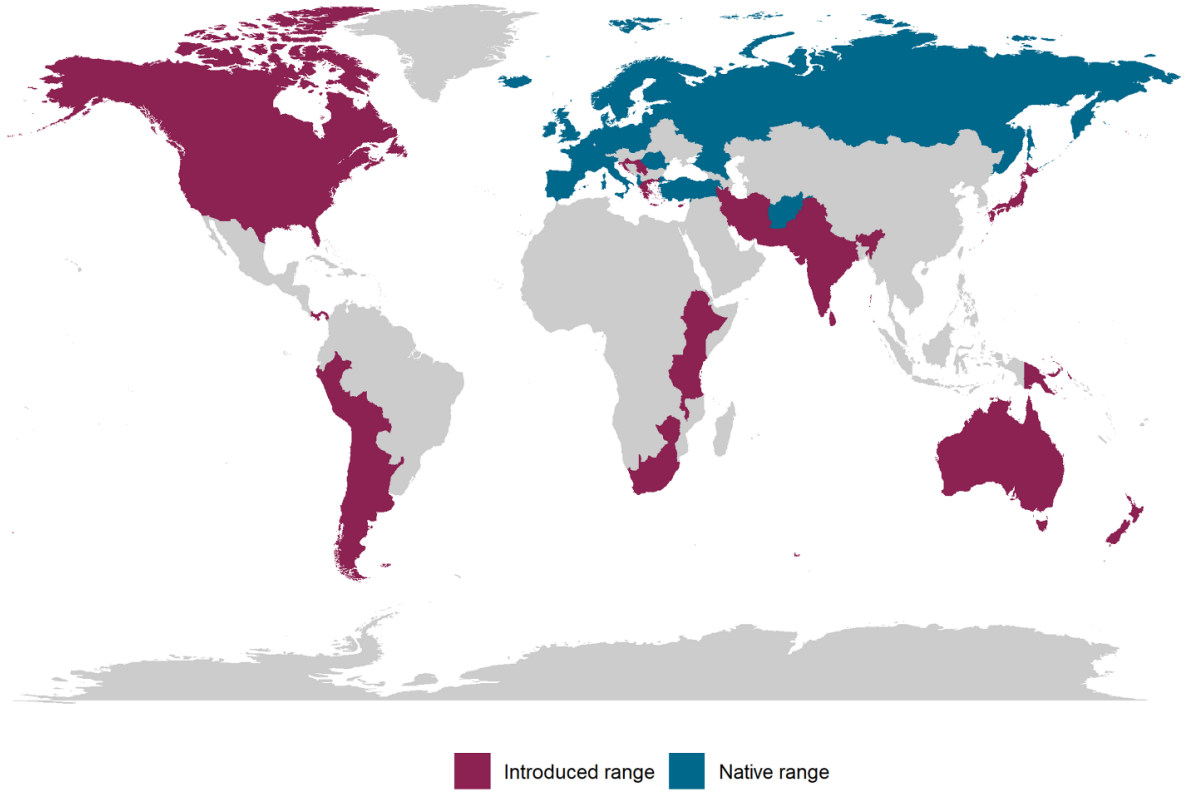

Figure 1.1. Global distribution of brown trout (*Salmo trutta*), data source: FishBase. This map does not indicate the countrywide presence, but that brown trout is categorized as an introduced species within that country.

Brown trout are a highly plastic species that possess a wide range of life history strategies and traits. The life history strategies utilised by brown trout can vary between individuals and populations, and are influenced by environmental, genetic, and physiological processes (Birnie-Gauvin et al., 2021). Brown trout exploit both freshwater and marine environments, while some fish will migrate to brackish, or saltwater's others will remain resident (**Figure 1.2 and Figure 1.3**). In their native range brown trout reproduce in Autumn/Winter on gravel bottom streams where they dig their nest and bury their eggs, in the Falkland Islands brown trout have been observed to start breeding in late May (Autumn/Winter in the Southern hemisphere), coinciding with native spawning periods (pers comms., Nick Bonner). Depending on water temperature, eggs are incubated for one to several months in the gravel before hatching in Spring (Wild Trout Trust, 2021), with longer incubation periods required at lower temperatures (Klemetsen et al., 2003). Fry and parr are territorial and will intensively compete for resources, with the dominance hierarchy determining foraging status, for example, dominant fish will feed at dusk, the most beneficial feeding time (Alanärä et al., 2001; Lahti et al., 2001). Habitat use by brown trout is flexible and typically dependant on

habitat availability and time of day (Greenberg et al., 2001; Heggnes et al., 2002). During their first-year young brown trout tend to inhabit shallow, fast flowing areas along the riverbank, whereas they typically prefer deeper and slow flowing areas as adults (Roussel and Bardonnet, 1999; Heggenes, 2002; Wild Trout Trust, 2021). Where brown trout have access to the sea, they typically form anadromous populations (Klemetsen et al., 2003). Often individuals migrating to the marine environment are found in shallow coastal areas close to the mouth of their home river, however, some individuals have been shown to migrate further out to sea (Bendall et al., 2005; Birnie-Gauvin et al., 2019; Davidsen et al., 2021). The time spent in the marine environment can also vary between individuals, with some fish only spending a summer and others remaining in the sea for many years (Jonsson and Jonsson, 2002; Birnie-Gauvin et al., 2019).

Although, brown trout are among the most studied fish species, a lot is still unknown about the variation in their migration and life history strategies. The lifecycle of brown trout was assumed to be relatively fixed, however, research has shown that many aspects are flexible and have been overlooked (**Figure 1.2**) (Limburg et al., 2001; Birnie-Gauvin et al., 2019). For example, migrating parr have been shown to enter the marine environment, resident fish may migrate and undergo late smoltification, autumn migrants may represent up to 40% of the spring smolt class, and migrating smolts may assume residency through desmoltification (Taal et al., 2014; Winter et al., 2016; Aarestrup et al., 2018; Birnie-Gauvin et al., 2019; Birnie-Gauvin and Aarestrup, 2019).

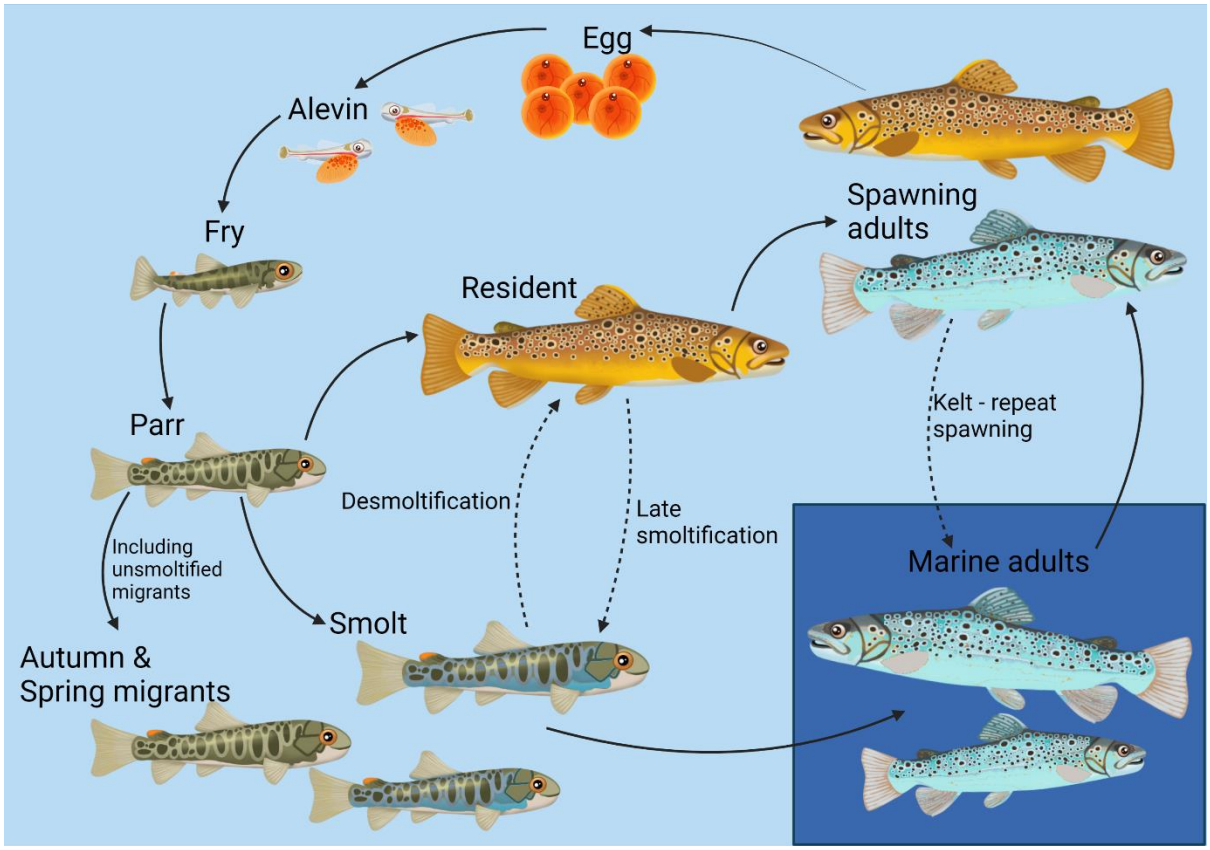

Figure 1.2. Brown trout life cycle, images obtained from RiverLife and produced in BioRender.

The wide-ranging life history tactics possessed by brown trout can also contribute to and assist with invasion success, thus, allow them to thrive in many ecological settings (Arismendi et al., 2014; Sloat et al., 2014). For example, the ability to migrate into the marine environment is likely to contribute to successful establishment and rapid spread. Straying, visiting, or inhabiting a watershed other than the one the fish was born in, is not uncommon in brown trout (Euzenat et al., 1999; Birnie-Gauvin et al., 2019). Studies around Norway and Scotland have recaptured individuals in non-native rivers (Pratten and Shearer, 1983; Berg and Berg, 1987), with one study in the Baltic Sea finding 16% of recaptures were a result of fish being caught in non-natal rivers (Degerman et al., 2012). In the Falkland Islands migratory ecotypes of brown trout have been present since 1956 and have, therefore, likely aided in the spread of brown trout throughout the Islands (Salmon and Trout Association, 2012).

In comparison very little is known about the life history strategies of *Aplochiton* spp., and their ecology and conservation status remain poorly understood (Young et al., 2010; Alò et al., 2013). Morphological similarities between the *A. zebra* and *A. taeniatus* have led to high levels of misidentification, further complicating their conservation (**Figure 1.3**). In addition,

further confusion surrounds their life history strategies as zebra trout were believed to have a marine living larval stage (McDowall et al., 2001; McDowall, 2006), although, research by Alò et al (2013) found no evidence of diadromy in either *A. zebra* or *A. taeniatus*. In Chile, both *Aplochiton* spp. are considered in danger of extinction due to the impacts of introduced salmonids, while in the Falklands the species are considered seriously threatened and have been protected since 1999 (Falkland Islands Government, 1999; McDowall et al., 2001; Alò et al., 2013). Neither species has been evaluated for the IUCN red list of threatened species as little information is available regarding their abundance and distribution (World Conservation Monitoring Centre, 1996).

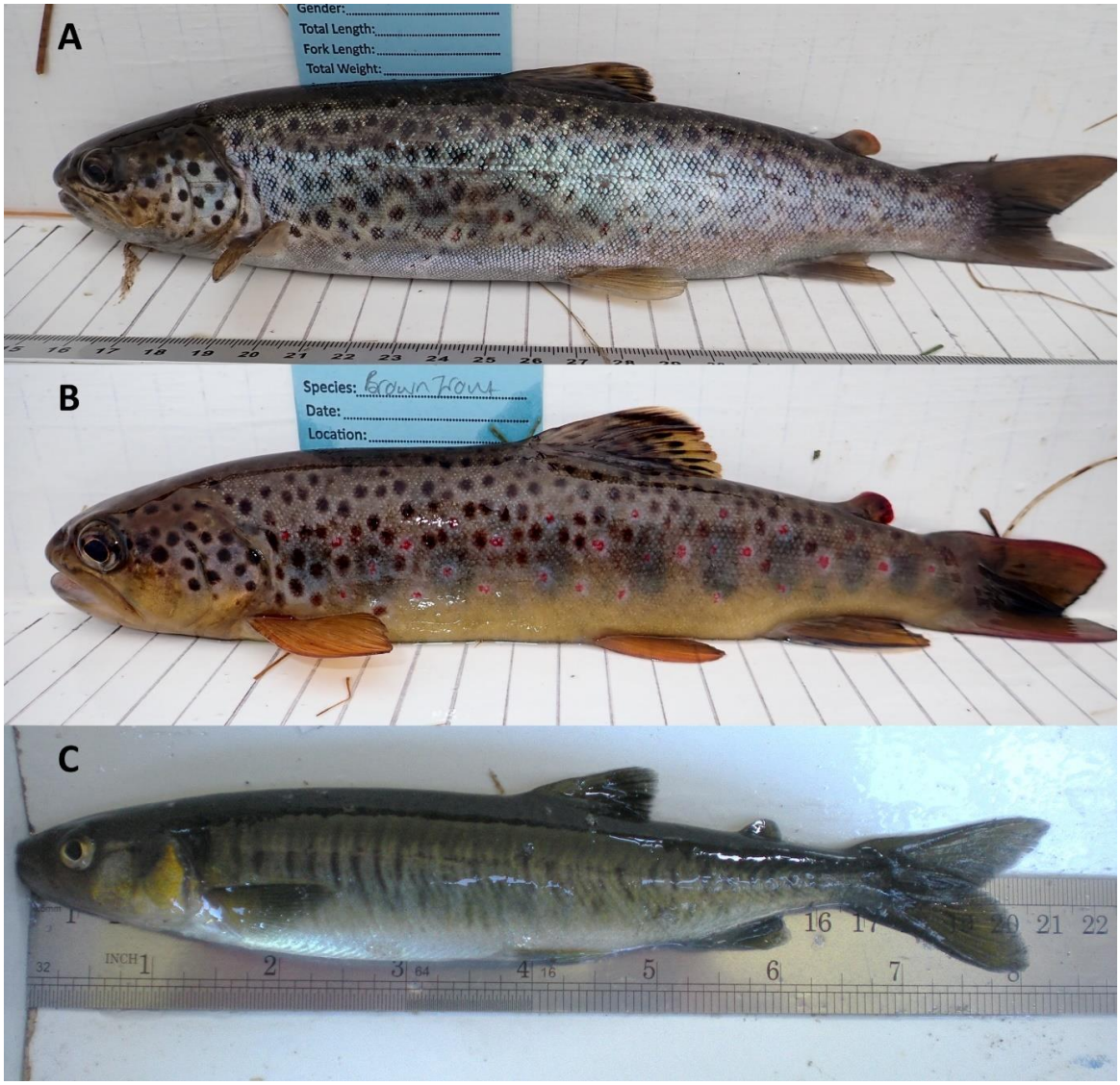

Figure 1.3. Examples of brown trout (A and B) and zebra trout (C) caught in the Falkland Islands, image (C) kindly provided by Sonia Consuegra and Carlos Garcia de Leaniz.

Size-specific predation is evident in New Zealand where medium-sized brown trout (100-120mm) prey heavily on small galaxiids (McIntosh *et al.*, 1994; Macchi *et al.*, 2007; Habit *et al.*, 2010), resulting in reduced abundance, or disappearance of galaxiid species and/or size classes (McIntosh, 2000; Glova, 2003; McIntosh *et al.*, 2010). Both *Aplochiton* spp. (*A. zebra* and *A. taeniatus*) present in the Falkland Islands commonly reach sizes of 110-170mm and are, therefore, vulnerable to predation from brown trout at all life stages (McDowall, 1971; Pascual *et al.*, 2007; McIntosh *et al.*, 2010). Galaxiids are particularly susceptible to brown trout predation as fry. Although galaxiids and brown trout generally spawn at the same time of year, brown trout tend to hatch earlier and, therefore, have a size advantage allowing even trout fry to consume galaxiid fry (Crowl *et al.*, 1992). Hence, in areas where brown trout and galaxiids are present trout can have major impacts on galaxiid fry survival and recruitment (McIntosh *et al.*, 2010).

Brown trout can impact native species not only through direct predation, but also by interference competition, altering their behaviour, and by feeding on the same prey items. Brown trout can feed on very diverse prey within a stream, including smaller fish and terrestrial prey (Huryn, 1996). Therefore, they can strongly influence stream invertebrate communities by altering species composition (Nyström and McIntosh, 2003), total biomass (Flecker and Townsend, 1994), and invertebrate behaviour (Flecker, 1992; McIntosh and Townsend, 1995; McIntosh and Townsend, 1996) leading to changes in nutrient dynamics (Simon *et al.*, 2004). For example, in the presence of trout, invertebrates evolved an adaptive response to heavier predation and altered their behaviour to become more cryptic and spend more time beneath cobblestones (McIntosh and Townsend, 1998; McIntosh, 2000) or switch to a nocturnal feeding pattern to avoid predation (Flecker, 1992; Cowan and Peckarsky, 1994). These changes in behaviour can have knock-on effects and result in a reduction in the number of insects and other invertebrates on exposed surfaces, decreasing grazing pressure on periphyton and leading to its increased abundance (Flecker and Townsend, 1994; McIntosh and Townsend, 1996; Biggs *et al.*, 2000; McDowall, 2003).

The feeding strategies of brown trout and galaxiids are similar (Glova *et al.*, 1992; Glova and Sagar, 1993), which can result in habitat overlap. Trout can displace native galaxiids from optimal foraging locations or prevent foraging through competition, termed competitive exclusion/displacement (McIntosh *et al.*, 1992; Edge *et al.*, 1993; McDowall, 2003). In Chile, *A. zebra* were observed to shift their foraging behaviour to consume less winged Diptera (Brown *et al.*, 2006) in order to avoid predation from brown trout, causing a reduction in

trophic position which significantly reduced their condition factor. Although this shift in diet reduced their predation risk, it may have also increased competition between these two species, as the diet of *A. zebra* and brown trout become more similar (Elgueta *et al.*, 2013). Furthermore, significant changes in condition factor have previously been inversely correlated with population abundance (Arismendi *et al.*, 2011); thus, the presence of brown trout may result in a reduction in population size for *A. zebra*. Therefore, these substantial impacts exerted on the trophic ecology of native galaxiids may have disastrous effects on their abundance (Elgueta *et al.*, 2013).

Although brown trout and invasive salmonids are known to impact native ecosystems and cause declines in native species, they are classified as naturalised in some areas of the Southern Hemisphere due to where, when, and why they were introduced, and the economic benefit they provide (Rowe, 2001; Iriarte *et al.*, 2005; Valiente *et al.*, 2010b). Therefore, conservation managers are tasked with a conservation oxymoron, to protect native species and habitats, as well as the invasive species that have caused their decline (Garcia de Leaniz *et al.*, 2010). For example, in Patagonia, National Parks were set up to increase conservation and forestry management resources; however, they have restrictions on economic activities and do not protect native species but instead protect invasive trout species and sport fishing (Pascual *et al.*, 2007). Due to the cultural and economic value of brown trout in the Falkland Islands they have been conferred a protected status since 1999 despite the declines in abundance and distribution observed in the native *Aplochiton* species following their introduction (McDowall *et al.*, 2001; Ross, 2009). This protected status prevents brown trout from being captured outside the fishing season, while during the fishing season (1st September – 30th April) fishermen are restricted to a daily limit of six trout per day per person on most rivers (catch limit on the Murrell River is reduced to three) (Falkland Islands Government, 1999; Otley *et al.*, 2008).

Three presence and absence surveys have been conducted following the introduction of brown trout to the Falklands: in 1999, 2008-09, and 2011-12 by McDowall et al. (2001), Ross (2009) and Fowler (2013), respectively. Although no surveys were conducted before their introduction, locals have reported the disappearance of the native zebra trout following the arrival of brown trout (McDowall *et al.*, 2001). The first survey in 1999, 37 years after introductions had ceased, revealed that brown trout had formed self-sustaining populations and were now widespread throughout East and West Falkland. With the first sea-run brown trout reported in 1956 (Salmon and Trout Association, 2012), their spread throughout the

Falklands was presumably aided by their anadromous lifestyle allowing individuals to migrate to new rivers and streams through the sea. However, some secondary translocations have occurred as brown trout are also found in landlocked areas not believed to be initial introduction sites (McDowall *et al.*, 2001). Due to the reduction in the abundance and distribution of zebra trout, they were regarded as 'severely threatened' following McDowall's 1999 survey and were included as a protected species under the 1999 Conservation of Wildlife and Nature Ordinance (Falkland Islands Government, 1999). However, before 2012 only one species of zebra trout was thought to be present in the Falklands with *A. taeniatus* misidentified as *A. zebra* due to their morphological and ecological similarities which may have confounded their identification (Vanhaecke *et al.*, 2012b). The study by Vanhaecke et al. (2012) determined that *A. zebra* was less widespread than previously thought, further complicating its conservation (McDowall, 2006; Vanhaecke *et al.*, 2012b).

1.3 Environmental DNA

The distribution and impacts of invasive species are traditionally monitored through visual detection and counting, such sampling relies on practical and taxonomic expertise and often requires the physical capture (i.e., through trapping and netting) of individuals which can cause stress, injury, and even mortality (Hopkins and Freckleton, 2002; Miranda and Kidwell, 2010; Panek and Densmore, 2011). Analysing DNA obtained from environmental samples (environmental DNA), i.e., water, soil, or air, offers a non-invasive alternative approach to physical capture of individuals (Ficetola *et al.*, 2008; Goldberg *et al.*, 2011; Taberlet *et al.*, 2012a; Wilson and Wright, 2016), and can increase the data available regarding the occurrence of rare or endangered species, the detection of invasive species, alongside estimating biodiversity (Goldberg *et al.*, 2016).

DNA is released into the environment through faeces, urine, skin, mucus, and blood. Detection of environmental DNA (eDNA) provides a method to assess and monitor biodiversity in variety of settings including sediments, ice cores, lakes, and rivers, from both present-day and ancient samples (Willerslev *et al.*, 2007; Haile *et al.*, 2009; Jerde *et al.*, 2011; Jørgensen *et al.*, 2012; Thomsen *et al.*, 2012; Xie *et al.*, 2018; Duyke *et al.*, 2019; Hellström *et al.*, 2019; Holman *et al.*, 2019). With evidence of a correlation between eDNA concentration and species abundance, there is now the potential to assess and estimate species abundance without the need for traditional methods such as mark and recapture techniques or netting/electrofishing surveys which can be costly and time-consuming (Lacoursière-Roussel

et al., 2016b; Lacoursière-Roussel *et al.*, 2016a). Therefore, sampling eDNA can further reduce field costs and time, allowing more sites to be sampled (Goldberg *et al.*, 2011; Biggs *et al.*, 2015; Evans *et al.*, 2017).

To protect and conserve rare and threatened species and habitats, it is of utmost importance to detect and monitor species of direct interest, including invasive species. Riverine habitats are often difficult to sample due to their topography, poor access, and flow rate. In addition, many riverine species display cryptic colouration and are often found at low densities (Bayley and Peterson, 2001; Mehta *et al.*, 2006). Sampling in such systems becomes increasingly complicated when sample sites are in remote areas. Traditional monitoring tools, such as netting and/or electrofishing, are only reliable indicators of species presence when target organisms are at moderate-to-high abundance due to their low capture and detection probabilities (Magnuson *et al.*, 1994). Therefore, rare species can often be presumed absent when they are in fact present (Gu and Swihart, 2004), and the only possible solution is to increase sampling effort, which is often unfeasible (Mcdonald, 2004). Environmental DNA analysis provides a way of assessing the distribution of rare and cryptic species or where sampling efforts could harm protected species (Beja-Pereira *et al.*, 2009; Biggs *et al.*, 2015; Doi *et al.*, 2015; Doi *et al.*, 2017; Robinson *et al.*, 2019a), which is particularly useful in conservation and invasive species monitoring programs.

Analysis of eDNA can be completed using a targeted or general approach. A targeted approach, whereby the presence/absence of a single species is determined using species-specific primers and conventional PCR (PCR), quantitative PCR (qPCR) or digital droplet PCR (ddPCR), is typically used when detecting endangered or invasive species (Ficetola *et al.*, 2008; Jerde *et al.*, 2011; Thomsen *et al.*, 2012; Doi *et al.*, 2015; Capo *et al.*, 2019). However, targeted approaches are limited to the detection of a single species and are, therefore, not efficient when whole communities need to be identified. In contrast, a general approach uses conserved primers, i.e., primers with binding sites that are shared across multiple taxa and flank a highly variable region that allows discrimination between taxa and species, and high-throughput sequencing (metabarcoding), enabling whole communities to be sequenced (Taberlet *et al.*, 2012b; Valentini *et al.*, 2016; Deiner *et al.*, 2017). Metabarcoding is an attractive approach to analyse eDNA as it allows the simultaneous assessment of species diversity and distributions (Blackman *et al.*, 2017; Elbrecht *et al.*, 2017; Harper *et al.*, 2019), although this can come with a reduction in accuracy and sensitivity as the more prevalent species are more likely to amplify and may mask the detection of rarer species (Kelly *et al.*,

2014; Brandon-Mong *et al.*, 2015). This makes metabarcoding less suitable than targeted eDNA for the detections of rare species or when distribution data are required (Evans *et al.*, 2016; Bylemans *et al.*, 2019). In such cases, a targeted approach may be more appropriate to map species distribution accurately and quantify eDNA abundance to provide an indicator of species abundance (Takahara *et al.*, 2012; Takahara *et al.*, 2013; Sigsgaard *et al.*, 2015; Bylemans *et al.*, 2016; Lacoursière-Roussel *et al.*, 2016a; Doi *et al.*, 2017).

Although eDNA analysis has been shown to be as reliable as traditional sampling methods (Seymour *et al.*, 2020; Boivin-Delisle *et al.*, 2021; Seymour *et al.*, 2021), species detection using eDNA is affected by extraction efficiency, assay sensitivity, sample interference, and the ecology of the target organism (Goldberg *et al.*, 2016). Each step in the methodology (**Figure 1.4**) needs to be optimized and requires clean and consistent field and laboratory protocols, which are essential to minimize the risk of contamination. Negative controls in the form of clean water processed using the same equipment/protocols as the field samples should be included at all stages, including sample collection, extraction, and amplification to detect potential sources of contamination. To avoid cross-contamination between sites and samples, it is also necessary to decontaminate all equipment and use single-use disposable supplies. Where equipment is to be reused, it must be thoroughly cleaned and decontaminated beforehand. Samples should be stored and handled in dedicated areas/rooms separate from areas where high-quality DNA and PCR products are processed (Taberlet *et al.*, 1999; Goldberg *et al.*, 2016). Immediately after shedding, eDNA starts to decay due to microbial activity, chemical reactions, and mechanical forces (Lindahl, 1993; Nielsen *et al.*, 2007; Thomsen *et al.*, 2012) and hence samples should be preserved as soon as possible.

Environmental DNA can be concentrated in water samples through precipitation or filtration. Precipitation involves preserving small volumes of water (e.g., 15ml) with salt (e.g., sodium acetate) and absolute ethanol, then storing the sample at -20°C (Ficetola *et al.*, 2008; Turner *et al.*, 2015). Greater volumes of water (200ml to >100L) can be processed with filtration (Hinlo *et al.*, 2017; Sepulveda *et al.*, 2019; Schabacker *et al.*, 2020). Water can be filtered on-site, which preserves samples immediately and may be critical when working in remote locations, or they can be filtered in a laboratory, allowing for multiple samples to be filtered simultaneously, reducing field and processing time (Goldberg *et al.*, 2016). DNA from filtered samples can be preserved through freezing, immersion in ethanol or cell lysis buffer, or drying filters (Hinlo *et al.*, 2017; Spens *et al.*, 2017). Final detection rates can vary with eDNA concentration process (filtration or precipitation), filter volume, filter material and

pore size, and DNA extraction method (Deiner *et al.*, 2015; Renshaw *et al.*, 2015; Goldberg *et al.*, 2016). Sensitivity and specificity of single species detection can be further improved using a species-specific probe (Kutyavin *et al.*, 2000; Pilliod *et al.*, 2013; Wilcox *et al.*, 2013; Amberg *et al.*, 2015). Without probes, positive samples will require subsequent confirmation through sequencing to rule out false positives due to cross-contamination.

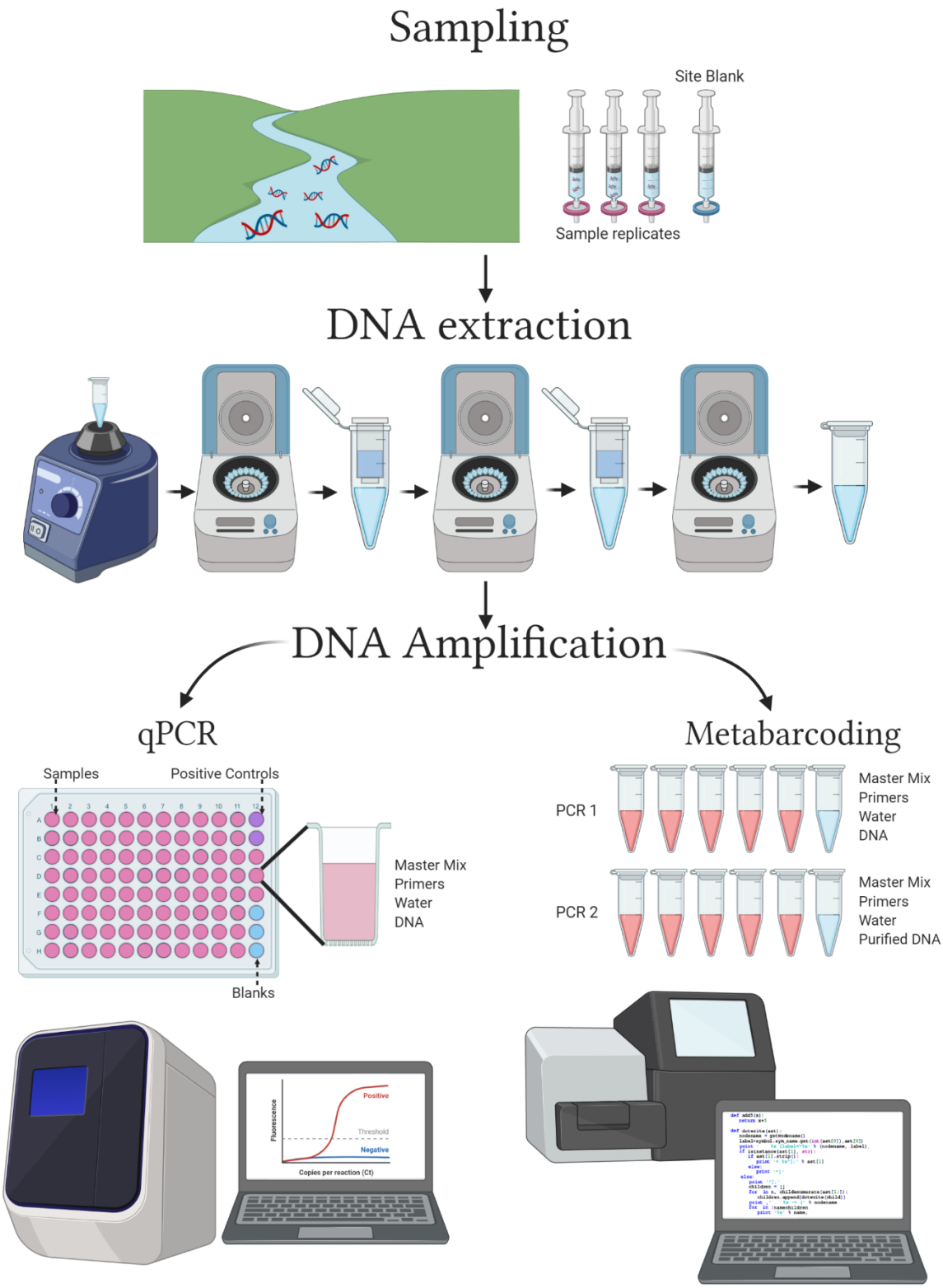

Figure 1.4. Environmental DNA sample process flow chart, created in BioRender.

1.4 Population Genetics of Invasive Species

Population genetics focuses on the distribution and amount of genetic variation within and between populations. Genetic variation arises through mutations, i.e., base pair changes (single nucleotide polymorphisms), insertions/deletions, and rearrangement of DNA fragments. Over time these mutations accumulate, and subsequently different occurrences of each mutation will be present in the population, thus characterising their genetic variation (Le Roux and Wieczorek, 2009). Such genetic variation can be monitored through a variety of molecular markers, including Single Nucleotide Polymorphisms (SNPs).

SNPs are single base changes in the genome that differ across species and/or populations under study, they occur as a result of mutations which create base-pair differences among sequences. Due to their high abundance and widespread distribution throughout the genome they are a useful source of information regarding genetic variation and have been used to study population structure, adaption, and evolution (Brumfield *et al.*, 2003; Morin *et al.*, 2004; Leaché and Oaks, 2017). Previously, microsatellite markers were commonly used, however, the use of SNPs is becoming more commonplace as they are typically more efficient, do not require standardization across detection platforms and can be replicated between laboratories, enabling results to be directly compared (Coates *et al.*, 2009; Seeb *et al.*, 2011).

Molecular markers can be applied in a variety of ways to gain a better understanding of invasive species populations facilitating the implementation of management and control measures (Sakai *et al.*, 2001; Resh *et al.*, 2018; Du *et al.*, 2021; Resh *et al.*, 2021). To effectively manage invasive populations, it is essential to correctly identify species. However, this can be difficult for various reasons such as cryptic taxa or high diversity (Stepien and Tumeo, 2006; Le Roux and Wieczorek, 2009). Taxonomic misidentification can prevent early detection of cryptic invasive taxa or result in ineffective management strategies, particularly when utilizing biological controls, as they may only be efficient against a particular species or variant (May and Marsden, 1992; Stepien and Tumeo, 2006). Molecular markers can also be used to detect hybridisation between populations and species (Vanhaecke *et al.*, 2012b; Deines *et al.*, 2014; Sušnik Bajec *et al.*, 2015). For example, the implementation of species diagnostic SNP markers in southern Africa determined the distribution of invasive largemouth bass (*Micropterus salmoides*), Florida bass (*Micropterus floridanus*) and their hybrids, revealing widespread introgression between these two invasive species, resulting in populations dominated by hybrids (Hargrove *et al.*, 2019). Hybridisation

can strongly influence invasive and native species fitness, resulting in the masking of deleterious alleles, increasing the fitness of invasive species (Abbott, 1992; Le Roux and Wieczorek, 2009). However, hybridisation can also severely threaten native populations by impacting their genetic integrity. Hybridisation between introduced sheepshead minnow (*Cyprinodon variegatus*) and native pupfish (*Cyprinodon bovinus*) in Dexter, New Mexico has resulted genetic introgression of the entire wild population.

Spread of invasive species can be limited through the implementation of targeted management strategies, however, such strategies require knowledge of invasive potential, movement pathways and sources of introduction (Sakai *et al.*, 2001; Le Roux and Wieczorek, 2009; Resh *et al.*, 2018; Du *et al.*, 2021; Resh *et al.*, 2021). This information can be obtained through SNP genotyping and assessing the population structure of introduced species. Previous studies examining invasive populations of Northern snakehead in the United States revealed the presence of more genetic populations than previously estimated, and by assessing population sizes, researchers were able to demonstrate their invasive potential (Resh *et al.*, 2018). In addition, analysis of SNPs enabled these invasive populations to be traced back to their original sources in the Yangtse river, China, thus providing valuable information to managers which can be used to prevent future introductions and further spread of the species in the United States (Resh *et al.*, 2018; Resh *et al.*, 2021). SNPs also provide an opportunity to estimate migration rates and gene flow between sites and populations, therefore, enabling possible dispersal pathways to be assessed (Mamoozadeh *et al.*, 2020).

1.5 Stable Isotope Analysis

Naturally occurring stable isotopes can be used to study and detect animal movement. Although movement is typically studied through marking and recapturing individuals with visible or electronic markers, stable isotope analysis can provide information regarding individual movements due to assimilated site-specific signatures (Rubenstein and Hobson, 2004; Cunjak *et al.*, 2005). Many elements including carbon and nitrogen possess multiple stable isotopes, these isotopes are present in different proportions and their composition can change predictably through natural geochemical and biochemical processes. Variations in these proportions can be measured as isotopic differences compared to international standards and are reported in delta (δ) values as parts per thousand (‰) (Peterson and Fry, 1987). Isotopic signatures can vary spatially between food webs or environments and these signatures can be assimilated and passed on through feeding where information is retained in

tissues (Grey, 2001; Fry *et al.*, 2003). Spatial and temporal movement patterns can then be inferred from these signatures when individuals move between isotopically distinct resources (Rubenstein and Hobson, 2004; Durbec *et al.*, 2010). However, retention of isotopic signatures will vary depending on tissue and elemental turnover rates (Tieszen *et al.*, 1983; Heady and Moore, 2013).

Different tissue can be analysed to determine isotopic signatures depending on the research question and the timeframe studied. Metabolically inert tissues such as bones and otoliths in fish reflect the isotopic record at the location where the tissue was formed, whereas metabolically active tissues reflect the isotopic signature over time depending on the turnover rate of the tissue selected (Tieszen *et al.*, 1983; Campana and Neilson, 1985; Tzadik *et al.*, 2017). Liver and blood plasma can provide information in days, while muscle tissue typically reflects changes in weeks to months, and longer-term information can be obtained from otoliths and scales (Perga and Gerdeaux, 2005; Ramsay *et al.*, 2011). Obtaining muscle tissue and otoliths requires lethal sampling which is often not appropriate when working with threatened, endanger, or protected species (Barnett *et al.*, 2010; Jardine *et al.*, 2011). However, studies are increasingly using non-lethally sampled tissues, including scales, fins (both adipose and rayed), and mucus (Fincel *et al.*, 2012; Graham *et al.*, 2013; Heady and Moore, 2013; Winter *et al.*, 2019). Carbon and nitrogen stable isotope values obtained from fins and muscle tissue are strongly correlated, therefore, fin tissue presents a non-lethal alternative (Kelly *et al.*, 2006; Sanderson *et al.*, 2009; Hanisch *et al.*, 2010; Jardine *et al.*, 2011; Graham *et al.*, 2013).

Stable isotopes have been successfully used to investigate movement in a wide range of taxa and ecosystems (Fry *et al.*, 2003; Rubenstein and Hobson, 2004; Hobson, 2008; Durbec *et al.*, 2010; Steenweg *et al.*, 2017). Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes have been analysed in a variety of metabolically inert and active tissue to estimate carbon flow in food webs and trophic levels (Rounick and Winterbourn, 1986; Post, 2002). Typically, $\delta^{15}N$ values are enriched by 3-4‰ as nitrogen moves through the food web, thus enabling consumer trophic levels to be estimated. In comparison, $\delta^{13}C$ tends to be unaffected as carbon moves through the food web, however, $\delta^{13}C$ provides information regarding the initial source of carbon and facilitates differentiation of isotopic signatures when sources change (Deniro and Epstein, 1981; Minagawa and Wada, 1984; Rounick and Winterbourn, 1986; Peterson and Fry, 1987; France and Peters, 1997). Individuals reflect the stable isotope signatures of a

particular site as they feed, assimilate isotopic signatures, and assume equilibrium with their diet, enabling movement patterns to be discerned when individuals move to or from a new feeding site (Fry *et al.*, 2003). However, turnover time of tissues needs to be accounted for when assessing this movement (Tieszen *et al.*, 1983; Heady and Moore, 2013). Analysis of $\delta^{13}C$ enabled feeding habitats for juvenile fish to be distinguished in Chwaka Bay, Zanzibar, as carbon values differed between mangroves, mud and sand flats and seagrass habitats, thus revealing the importance of feeding areas for different species, while intermediate values between feeding habitats established movement and connectivity between sites (Lugendo *et al.*, 2006).

Movement patterns have been described at a range of spatial scales using stable isotopes (Rubenstein and Hobson, 2004; Hobson, 2008). Large scale migrations such as those conducted by monarch butterflies have been tracked back to their natal origins (Hobson *et al.*, 1999). In comparison, relatively fine scales have also been studied, for example where movement of fish species has been estimated within a river or estuary (Haas *et al.*, 2009; Rasmussen *et al.*, 2009).

1.6 Aims and Objectives

This thesis explores the invasion ecology of brown trout in the Falkland Islands with the aim to facilitate conservation planning and minimise the impacts of brown trout on native galaxiids. SNP genotyping, stable isotope analysis, acoustic tracking, and environmental DNA analysis were used to determine the distribution of invasive brown trout and its potential impacts on native endangered *Aplochiton* spp. in the Falklands. The following main objectives were addressed in four data chapters:

- Chapter 2. To determine the distribution of brown trout and native galaxiids in the Falkland Islands using environmental DNA. This chapter was published as Minett, J.F., Garcia de Leaniz, D., Brickle, P. & Consuegra, S. (2020) A new high-resolution melt curve eDNA assay to monitor the simultaneous presence of invasive brown trout (Salmo trutta) and endangered galaxiids. *Environmental DNA,* 3, 561-572. https://doi.org/10.1002/edn3.151

- Chapter 3. To reconstruct the introduction and colonization of brown trout in the Falklands and model their dispersal. This chapter is under review in Biological Invasions.

- Chapter 4. To assess the population structure and likely origin of brown trout in the Falklands and evaluate the level gene flow between populations using SNPs. This chapter is published in Evolutionary Applications as Minett, J.F., Garcia de Leaniz, D., Sobolewska, H., Brickle, P., Crossin, G. T. & Consuegra, S. (2021) SNP analysis and acoustic tagging reveal multiple origins and widespread dispersal of invasive brown trout in the Falkland Islands. *Evolutionary Applications*, 11-1. https://doi.org/10.1111/eva.13274

- Chapter 5. To examine the life history strategies of brown trout in the Falklands and determine the incidence of anadromy using SIA. This chapter is in preparation as Stable isotope analysis reveals multiple life history strategies and the extent of migratory brown trout in the Falkland Islands.

Chapter 2 A new high-resolution melt curve eDNA assay to monitor the simultaneous presence of invasive brown trout (*Salmo trutta*) and endangered galaxiids

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2.1 Introduction

Understanding species' niche characteristics is essential to predict the consequences of biological invasions (Korsu *et al.*, 2007), but requires being able to accurately identify particular species and their distributions (Darling and Blum, 2007). Species identification can be difficult if they are threatened, at low densities (Jerde *et al.*, 2011) and/or morphologically cryptic (Bickford *et al.*, 2006). This is important because the establishment and dispersal of non-native species often impact native fauna through increased predation, competition for resources and disease transmission (Gozlan *et al.*, 2010; Ellender and Weyl, 2014). Competition for resources and/or predation can result in the displacement of native species and introgression/hybridisation with introduced species, potentially leading to their decline, extirpation or extinction (Huxel, 1999). These negative impacts can be particularly severe for endemic species, especially those found in low abundance and having limited geographic range (Burlakova *et al.*, 2011; Hobbs *et al.*, 2011), and particularly in freshwater ecosystems where invasive species are one of the main drivers of biodiversity loss (Dudgeon *et al.*, 2006; Reid *et al.*, 2019).

The introduction and spread of non-native fishes in freshwater ecosystems has often been attributed to aquaculture and recreational fishing, particularly in the case of salmonid fishes (Garcia de Leaniz *et al.*, 2010), one of the most widespread groups of introduced fishes (Rahel, 2007). Although, few species are known to have become extinct due to the effects of introduced salmonids, declines in abundance and distribution of native and endemic fishes are evident in many countries (Woodford and Impson, 2004; Habit *et al.*, 2010; McIntosh *et al.*, 2010; Young *et al.*, 2010; Kadye *et al.*, 2013). In New Zealand for example, the extinction of the native grayling *Prototroctes oxyrhynchus* has been attributed in part to the introduction of brown trout *Salmo trutta* (McDowall, 2006). Galaxiid fishes, endemic of the Southern Hemisphere, constitute one of the freshwater fish families most seriously threatened by salmonid expansions (Garcia de Leaniz *et al.*, 2010; Habit *et al.*, 2010). Invasive salmonids exert strong selection pressure upon native galaxiids across their ranges, including New Zealand (McIntosh *et al.*, 2010), Chile (Habit *et al.*, 2010), and Australia (Hardie *et al.*, 2006), mainly through predation and competition (Soto *et al.*, 2006; Macchi *et al.*, 2007; Arismendi *et al.*, 2009; Penaluna *et al.*, 2009).

In Chile and the Falkland Islands, the distribution of galaxiids (*Aplochiton* spp.) is determined by historical colonisation but also shows strong population structuring, isolation,

and reduced genetic diversity in areas affected by salmonids (Vanhaecke *et al.*, 2015). In particular, brown trout have caused widespread ecological damage to areas they have been introduced, and as a result, they have been classified as one of the '100 of the world's worst invasive species' (Lowe *et al.*, 2000). In the Falkland Islands, since its introduction in 1947-1962, brown trout has spread around East and West Falkland (Arrowsmith and Pentelow, 1965; Stewart, 1973), resulting in the once-common native galaxiid, zebra trout (*Aplochiton zebra*) to be classed as threatened, and limited to refuges uninvaded by brown trout south of the islands (McDowall *et al.*, 2001; Ross, 2009). Conservation of *Aplochiton* spp. is complicated because the two known species (*A. zebra* and *A. taeniatus*) are ecologically and morphologically similar and include resident and migratory ecotypes that may confound identification (McDowall, 2006). In fact, until recently both species had been misidentified as *A. zebra* in the Falklands (Vanhaecke *et al.*, 2012b). The small sizes of *A. zebra* and *A. taeniatus* juveniles makes them particularly susceptible to salmonid predation and displacement (Macchi *et al.*, 2007; Arismendi *et al.*, 2009), which also potentially increases inbreeding and hybridisation as a result of population reductions and limited suitable habitat uninvaded by brown trout (Wolf *et al.*, 2001; Vanhaecke *et al.*, 2012b). In contrast, the abundance of salmonids seems to be related to propagule pressure (Consuegra *et al.*, 2011) and habitat connectivity (Habit *et al.*, 2012). Previous studies conducted 10 and 20 years ago to assess the distribution of brown trout and native galaxiids in the Falklands (McDowall *et al.*, 2001; Ross, 2009; Fowler, 2013) showed marked reduction in the abundance and distribution of zebra trout since the introduction of brown trout. However, traditional monitoring exercises based on electrofishing are limited by their cost and by the protected and rare nature of *Aplochiton* spp. Electrofishing of rare species often requires increased effort, possibly reducing the number of reaches that can be sampled (Reynolds *et al.*, 2003) and increasing the cost of sampling each reach (Evans *et al.*, 2017). In addition, electrofishing can reduce survival in embryos (Bohl *et al.*, 2009) as well as cause stress, injury and mortality (Miranda and Kidwell, 2010; Panek and Densmore, 2011), which could impact rare and threatened populations.

Environmental DNA (eDNA) released from organisms through blood, urine, skin, mucus and faeces, increasingly is used to detect aquatic species that are difficult to locate, identify and/or are in low abundance, and is particularly useful for conservation programs (Biggs *et al.*, 2015; Robinson *et al.*, 2019a). Whilst eDNA metabarcoding is used to target multiple species and often to assess the biodiversity of a system (Deiner *et al.*, 2015; Lacoursière-Roussel *et*

al., 2018), quantitative PCR (qPCR) targets single species and constitutes a reliable method for detecting endangered and invasive species when combined with in vitro controls and amplicon sequencing (Díaz-Ferguson *et al.*, 2014; Carlsson *et al.*, 2017). qPCR in combination with high-resolution melt (HRM) curve analysis allows single-base variations in DNA sequences to be detected based on the DNA product melt temperature in water samples (Wittwer, 2009; Ramón-Laca *et al.*, 2014; Robinson *et al.*, 2018), and has been used with environmental DNA as a sensitive method to detect individual or multiple species, including fishes (Behrens-Chapuis *et al.*, 2018; Robinson *et al.*, 2019b), invertebrates (Robinson *et al.*, 2018; Robinson *et al.*, 2019a), sea turtles (Harper *et al.*, 2020), and plants (Emenyeonu *et al.*, 2018). Here, I developed eDNA-HRM curve analysis assays to map the current distribution of brown trout and both *Aplochiton* species in the Falkland Islands in a non-destructive way, to identify refuges for zebra trout, which then can be prioritised for conservation.

Here, the main aims were to develop an eDNA-HRM curve analysis assay to detect the presence of both *Aplochiton* species. Develop and eDNA-HRM curve analysis assay to detect the presence of brown trout and map the current distribution of brown trout and both *Aplochiton* species and identify refuges for zebra trout.

2.2 Methods

qPCR primer design and optimisation

Aplochiton zebra and *A. taeniatus* qPCR primers (AzebAtaeCytbF: 5'-ATGAAATTTTGGCTCTCT-3' and AzebAtaeCytbR: 5'-GAAATATCGGAGGTGTAG-3') were designed to amplify an 89 bp fragment of the cytochrome *b* region of the mitochondrial (mt) genome (product melt temperature 77.8°C and 79.2°C for *A. zebra* and *A. taeniatus* respectively). Species-specific qPCR primers (StruttaCytbF: 5'-TATCCTCCATACCTCTAA-3' and StruttaCytbR: 5'-GACCGATGATAATGAATG-3') were designed for *Salmo trutta* to amplify a 139 bp fragment of the mitochondrial cytochrome *b* region. Both sets of primers were designed using OligoArchitect Primer and Probe Design online software and checked in silico for cross-amplification using NCBI Primer-BLAST (Ye *et al.*, 2012). Both AzebAtaeCytb- and StruttaCytb-qPCR primers were tested in vitro for non-specific amplification against all freshwater fishes present in the Falklands (*A. zebra*, *A. taeniatus*, *Galaxias maculatus* and *S. trutta*, except *Geotria australis* that may occur intermittently) (McDowall *et al.*, 2001; Vanhaecke *et al.*, 2012b).

Primers were assessed using positive tissue controls (fin clips and muscle tissue) from 12 different *A. zebra* and *A. taeniatus* individuals. DNA was extracted using Qiagen DNeasy Blood and Tissue Kit (Qiagen, UK). A 10-fold dilution series using pools of DNA from each species (consisting of DNA from six *A. zebra* and six *A. taeniatus*) ranging from 19.7ng/µl to 1.97×10^{-4}ng/µl and 14.8ng/µl to 1.48×10^{-4}ng/µl respectively was conducted in order to determine the limit of detection (LOD) and the limit of quantification (LOQ) as in Robinson et al., (2018). Amplification efficiency, also estimated from the dilution curve, was 79.5% for *A. zebra* and 84.6% for *A. taeniatus* (Bio-Rad, 2013). The annealing temperature for AzebAtaeCytb primers was optimised at 61.5°C. The AzebAtaeCytb-qPCR protocol began with a two min denaturation step at 95°C, followed by 45 cycles of 95°C for 10 s and 61.5°C for 30 s. A HRM step was applied at the end of the real-time PCR reaction, ranging from 65°C to 95°C in 0.1°C increments to test the consistency of amplicon melt temperatures (tm) for each species. To account for any potential intraspecific variation in qPCR product tm, six individuals from five *A. zebra* populations and six from three *A. taeniatus* populations were used for HRM analysis. To assess the ability to detect *A. zebra* and *A. taeniatus* in the same reaction, equal volumes of both species' DNA were pooled from six different individuals of

both species at various concentration ratios ranging from 10:90 to 50:50 (e.g. 30:70 dilutions represented in **Figure 2.1**).

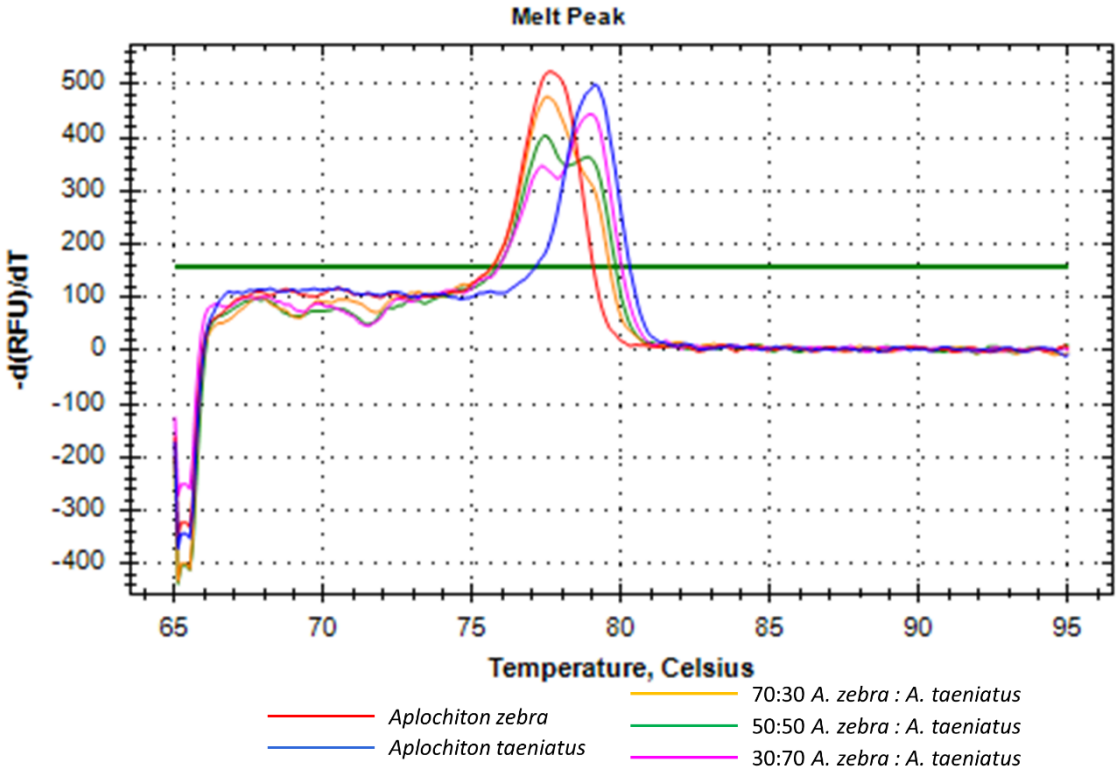

Figure 2.1. qPCR product melt curve profile for ratios of *A. zebra: A. taeniatus DNA*, red and blue peaks corresponds to positive *A. zebra* and *A. taeniatus* tissue samples respectively, whilst green peak is from a 50:50 *A. zebra: A. taeniatus* mix and orange and pink peaks correspond to 70:30 and 30:70 *A. zebra* and *A. taeniatus* mixes respectively.

StruttaCytb-qPCR primers were assessed in vitro using positive tissue controls (fin clips) from nine individual brown trout from a range of populations. DNA was extracted using the Qiagen Blood and Tissue Kit (Qiagen, UK), and amplified in real-time PCR-HRM analysis using the following StruttaCytb protocol: 95°C for 3 min, followed by 40 cycles of 95°C for 10 sec and 60°C for 30 sec, a HRM step was applied to the end of the real-time PCR reaction, ranging from 65°C to 95°C in 0.1°C increments. The annealing temperature for the StruttaCytb primers was optimised at 60°C resulting in an efficiency of 89.4%. A 10-fold dilution series was also carried out ranging from 35.4 ng/µl to 3.54×10^{-4}ng/µl to determine the LOD and LOQ.

AzebAtaeCytb and StruttaCytb primers also were tested using positive eDNA controls (sites where species had been seen during the sampling period) to ensure that the primers would amplify environmental DNA (**Figure 2.2**). eDNA samples (nine samples from three different sites × three technical PCR replicates) were spiked with positive control DNA (1µl of *A. zebra* DNA from six individuals, 9.85ng/µl) to test for possible inhibition in separate reactions.

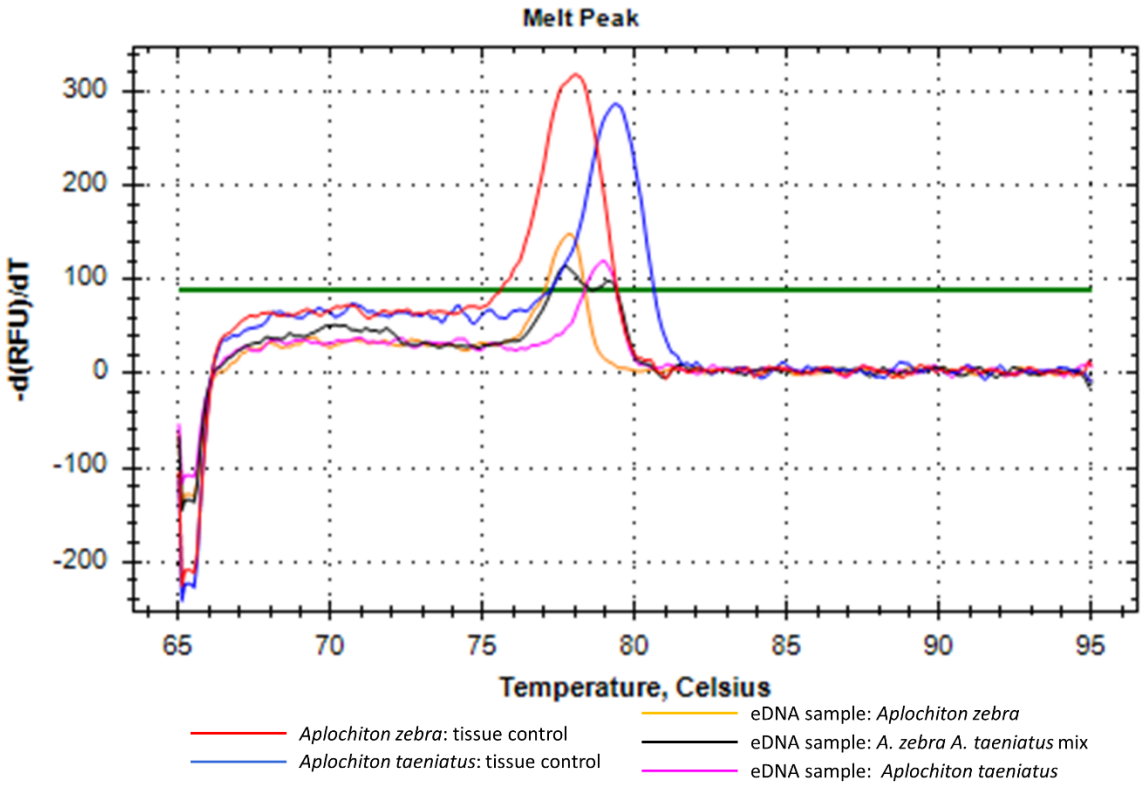

Figure 2.2. qPCR product melt curve profile for positive tissue controls for *Aplochiton zebra* and *Aplochiton taeniatus* and eDNA sample amplifications. Red and blue peaks corresponds to positive A. *zebra* and *A. taeniatus* tissue samples respectively, the black peak is from an eDNA sample amplifying both *A. zebra* and *A. taeniatus* simultaneously, and orange and pink peaks correspond to eDNA samples amplifying *A. zebra* and *A. taeniatus* respectively.

Study populations and eDNA sample collection

Nineteen rivers and ponds were sampled across the Falkland Islands (**Figure 2.3**), eight on East Falkland (five in the North and three in the South), and 11 on West Falkland (five in the North and six in the South). Locations were chosen based on information from monitoring studies conducted 10 and 20 years ago (McDowall *et al.*, 2001; Ross, 2009; Fowler, 2013). Zebra trout had previously been detected at seven of the 19 locations, co-occurring with

brown trout at only two locations. Six locations solely supported brown trout populations. The remaining seven rivers had not been surveyed previously (N = 5) or were rivers that had been surveyed but where zebra trout or brown trout had not been recorded.

Two sites per river/pond were sampled except for R19 Neil Clark Nature Reserve where three sites were sampled; at each site, two water samples were collected from the surface of the water in areas of low flow near the bank of the river, taking precautions to avoid contamination following Robinson, Garcia de Leaniz, Rolla, et al. (2019). Three water replicates of 100-200ml (the final volume depending on the level of particulate organic matter present in the waterbody) were filtered at each site (**Table 2.1**). Water was pushed through a syringe filter containing a polyethersulfone (PES) filter membrane with a 0.45µm pore size using a sterile 50ml disposable syringe. Filters were then dried by pushing through air before being preserved in 95% ethanol and stored at -20°C until further analyses. To prevent contamination, water sampling bags, syringes and gloves were disposed of between sites. Negative controls consisting of autoclaved or ultrapure water were filtered instead of river/pond water before sampling at each site. River width, temperature, shade cover, pH, total dissolved solids, and electrical conductivity were measured at each sampling site where possible (**Table 2.1**). Due to time and weather constraints, sampling was conducted over two field seasons April-May (Autumn) and September-October (Spring) in 2018, three additional waterbodies were sampled by local citizens, two in May (Autumn) 2019 and a final site sampled in December 2019 (**Table 2.1**).

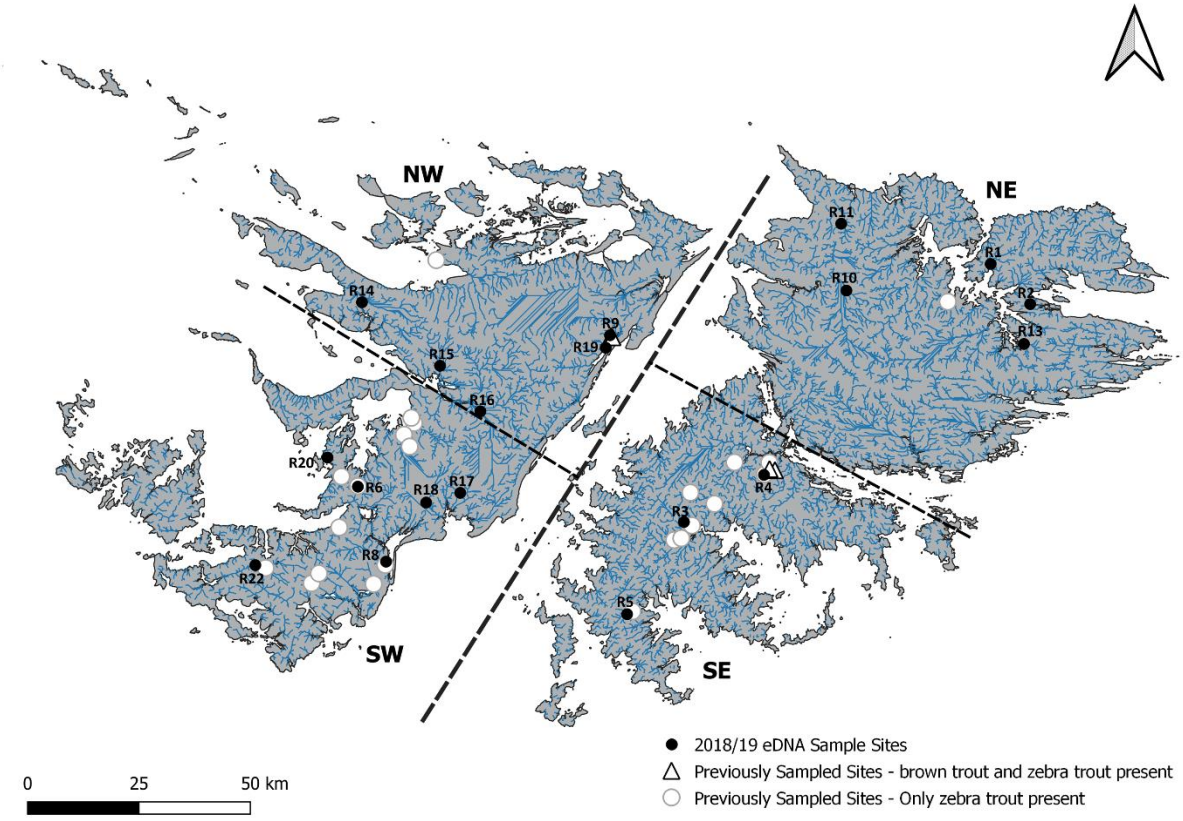

Figure 2.3. eDNA sampling locations in the Falkland Islands. Current eDNA sampling locations (black circles), previously sampled sites where only zebra trout were present (white circles) and previously sampled sites with zebra trout and brown trout present (white triangle), previous sampled data obtained from McDowall et al (2001), Ross (2009) and Fowler (2012).

eDNA extraction and amplification

eDNA was extracted from 273 field samples (19 waterbodies, 39 sites × two water samples × three replicates and one blank per site, **Table 2.1**) using the Qiagen DNeasy PowerSoil Kit (Qiagen, UK), following the manufacturer's instructions. DNA extractions took place in a dedicated eDNA area within an extraction cabinet equipped with a flow-through air system and UV light to minimise the risk of contamination. Extracted DNA was quantified with a Qubit 3.0 fluorometer. Six technical PCR replicates of each sample were amplified in a Bio-Rad CFX96 Touch Real-Time PCR Detection System (Bio-Rad, UK), in 10µl reaction consisting of 5µl of iTaq Universal SYBR Green Supermix (Bio-Rad, UK), 0.25µl (10µM) of each AzebAtaeCytbF and AzebAtaeCytbR, 2.5µl of ultrapure water and 2µl of extracted DNA. Amplifications were carried out using the standard AzebAtaeCytb-qPCR protocol as described above, only samples which consistently amplified in at least two technical PCR replicates per site at the target DNA product tm (either 77.8°C ± 0.2 or 79.2°C ± 0.2) were

considered to be a positive result. Reactions of 10µl also were carried out using the StruttaCytb primers consisting of 5µl of iTaq Universal SYBR Green Supermix (Bio-Rad, UK), 0.25µl (10µM) of each forward and reverse primer, 1.5µl of ultrapure water, and 3µl of DNA. Amplification was carried out using the standard StruttaCytb-qPCR protocol (described above) and only samples that amplified consistently in at least two technical PCR replicates per site at the target DNA product tm (78.7°C ± 0.1) were considered a positive result. qPCR reactions were carried out in a dedicated eDNA area; reaction mix was loaded in a DNA free PCR hood with a flow-through air system and UV light before being transferred to a separate PCR hood to load DNA. Once all eDNA samples had been loaded and sealed two positive controls (one for each species) and a negative control consisting of brown trout or *Galaxias maculatus* DNA also were loaded to control for false positives. Negative filter and extraction controls were run throughout the process. Three additional negative amplification controls consisting of ultrapure water were also added to test for contamination during the entire process (both with eDNA and positive control samples). To confirm primer specificity, a subset of eDNA samples (N = 4 brown trout and N = 9 *Aplochiton* spp.) was amplified with the qPCR primers using end-point PCR and cloned into a pCR 4-TOPO plasmid cloning vector (TOPO TA Cloning Kit for Sequencing, Invitrogen). In total, 10-25 clones were sequenced per sample using T3 and T7 primers. All samples were cleaned using a sodium acetate/EtOH solution, resuspended in 10µl HiDi Formamide (Applied Biosystems) and analysed using Sanger Sequencing on an ABI 3730 DNA Analyser (Applied Biosystems). Resulting sequences were aligned in BioEdit (v 7.2.5) (Hall, 1999), and input to BLAST (Ye *et al.*, 2006) to confirm species identity.

To determine if sampling conditions (volume filtered, season, temperature, and shade, **Table 2.1**) affected amplifications, a generalised linear model using binomial error family was performed in R3.5.3. Using the *drop1* function, individual predictors were dropped from the model until the optimal model based on AIC was obtained.

Table 2.1. Locations and environmental data for eDNA sampling sites, including latitude and longitude, temperature (°C), shade cover (0-3), river width, pH, total dissolved solids (TDS), electrical conductivity (EC) and total volume filtered.

Sampling Date	Site No.	Waterbody	Previously Sampled	Fish Status	Latitude	Longitude	Temp (°C)	Shade Cover	Width (m)	pH	TDS (ppm)	EC (S/m)	Total Volume Filtered (ml)
04/04/18	R1a	Johns Brook	NA	Unknown	-51.48339	-58.29203	5.6	0	1.5	4.5	92	46	872
04/04/18	R1b		NA		-51.48137	-58.29257	6.0	0	5	4.9	102	51	823
04/04/18	R2a	Monty Deans Creek	1999		-51.56585	-58.16645	5.0	0	2	6.1	240	120	850
04/04/18	R2b		1999		-51.56715	-58.15749	5.0	0	2	6.7	240	120	650
09/04/18	R3a	Spots Arroyo	2009	Zebra trout	-51.9902	-59.30946	5.0	0	3	6.9	364	182	900
09/04/18	R3b		2009		-51.9896	-59.28561	6.8	0	3	7.3	370	185	1200
18/04/18	R4a	Findley Creek Stream	2011	Brown trout & zebra trout	-51.89972	-59.04361	6.8	0	<1	7.3	240	120	1200
18/04/18	R4b		2011		-51.93139	-59.06011	7.4	0	<1	7.5	288	144	1200
19/04/18	R5a	Northwest Arm House Stream	2012	Zebra trout	-52.17283	-59.50553	9.4	0	2	6.8	482	234	1200
19/04/18	R5b		2012		-52.16641	-59.49236	11.6	0	3	7.1	479	239	1108
01/05/18	R6a	Fish Creek (2)	2012	Zebra trout	-51.89306	-60.36861	4.0	1	1	5.5	508	254	1200
01/05/18	R6b		2012		-51.89306	-60.36861	4.0	0	3	6.7	382	191	1200
02/05/18	R8a	Fish Creek (1)	2012	Zebra trout	-52.05583	-60.29111	4.2	1	2	4.5	240	120	635
02/05/18	R8b		2012		-52.04722	-60.28778	4.2	0	5	4.6	242	121	650
03/05/18	R9a	House Creek	1999	Brown trout & zebra trout	-51.6075	-59.52972	4.2	0	3	4.8	56	28	1100
03/05/18	R9b		1999		-51.61111	-59.52333	4.2	0	3	4.9	58	29	950
22/09/18	R10a	San Carlos	1999	Brown trout	-51.5095	-58.822	1.6	0	20	3.9	70	35	1200
30/09/18	R10b		1999		-51.531111	-58.760278	NA	0	15	NA	NA	NA	1200

03/10/18	R11a	Elephant Beach Pond Stream	1999	Brown trout	-51.395556	-58.771944	2.6	0	5	4.5	92	46	1200
03/10/18	R11b		1999		-51.434444	-58.773611	5.8	1	2	4.7	94	47	1200
03/10/18	R13a	Estancia Creek	2008	Brown trout	-51.6475	-58.195833	5.4	2	<1	5.3	92	46	1200
03/10/18	R13b		2008		-51.646389	-58.188611	5.4	0	15	5.6	704	4352	1200
08/10/18	R14a	Herbert Stream	1999	Brown trout	-51.5208333	-60.3277778	5.8	0	10	NA	288	148	900
08/10/18	R14b		1999		-51.5308333	-60.2427778	6.2	1	5	NA	226	110	1200
08/10/18	R15a	Teal House River	NA	Unknown	-51.6194444	-60.1102778	5.2	1	3	NA	72	36	1200
08/10/18	R15b		NA		-51.6561111	-60.0841667	6.8	2	4	NA	90	45	1200
08/10/18	R16a	Chartres River	1999	Brown trout	-51.7516667	-59.9594444	7.8	1	25	NA	92	46	1192
09/10/18	R16b		1999		-51.8366667	-59.9611111	4.6	1	3	NA	304	152	600
09/10/18	R17a	Doctors Creek	2012	Brown trout	-51.9411111	-60.0522222	4.0	1	3	NA	364	182	1200
09/10/18	R17b		2012		-51.9147222	-60.0358333	3.6	2	<1	NA	246	123	1200
09/10/18	R18a	Malo Arroyo	NA	Unknown	-51.9313889	-60.1483333	4.0	0	4	NA	364	182	614
11/10/18	R18b		NA		-51.9597222	-60.1569444	1.4	0	7	NA	328	164	600
29/10/18	R19a	Neil Clark Nature Reserve	NA	Unknown	-51.632444	-59.54519	NA	NA	NA	NA	NA	NA	1200
09/10/18	R19b		NA		-51.9411111	-60.0522222	3.0	0	50	NA	1660	830	1200
14/12/19	R19c		NA		-51.632222	-59.545556	NA	2	<1	NA	NA	NA	1150
06/05/19	R20a	Spring Point	NA	Unknown	-51.8314	-60.4628	NA	2	1	NA	NA	NA	900
06/05/19	R20b		NA		-51.823	-60.4454	NA	2	2	NA	NA	NA	1000
06/05/19	R22a	Whiskey Creek	2009	Zebra trout	-52.0542	-60.7891	NA	2	2	NA	NA	NA	900
06/05/19	R22b		2009		-52.0416	-60.7155	NA	2	3	NA	NA	NA	900

2.3 Results

AzebAtaeCytb and StruttaCytb assays were tested in silico for cross-amplification using NCBI Primer-BLAST (Ye *et al.*, 2012) (**Table 2.2**), I found no cross amplifications with any species present in the Falkland Islands. Primers were also tested in vitro against *S. trutta* and *G. maculatus*, and both species of zebra trout and *G. maculatus* respectively; no cross amplifications were detected. A 10-fold dilution series of positive control *A. taeniatus* and *A. zebra* DNA (from six individuals respectively) revealed that for *A. taeniatus* the limit of detection (LOD) was 1.97×10^{-4}ng/µl and for *A. zebra* the LOD was 1.48×10^{-4}ng/µl. The detection threshold for both species of zebra trout at the lowest LOD was 42 cycles and the product melting temperatures (tm) were consistent throughout the dilution series. qPCR product tm showed no overlap between the two species of zebra trout (77.8°C and 79.2°C ± 0.2 for *A. zebra* and *A. taeniatus* respectively; these might vary in zebra trout from different regions, if there were polymorphisms in the amplified region). Using the diagnostic melt curve produced it was possible to detect the presence of both species when combining varying ratios of pooled DNA (**Figure 2.2**). Results from a 10-fold dilution series revealed that the LOD for brown trout was 3.54×10^{-4}ng/µl for the *S. trutta* qPCR assay with a detection threshold of 37 cycles. The nine eDNA samples spiked with positive control *A. zebra* DNA amplified with qPCR product tm at 77.8°C, indicating no signs of inhibition.

Table 2.2. Species that could be cross amplified with AzebAtaeCytb and StruttaCytb primers, in silico cross amplification checks conducted using Primer-BLAST. None of the species are present in the Falkland Islands and in all cases the region sequenced has at least one base pair difference with the target species.

AzebAtaeCytb Primers		**StruttaCytb Primers**
Astatotilapia burtoni	*Kryptolebias marmoratus*	*Atherinomorus endrachtensis*
Acantopsis dialuzona	*Lobocheilos melanotaenia*	*Barilius bendelisis*
Acrossocheilus yunnanensis	*Lobocheilos* spp.	*Salmo akairos*
Allodontichthys tamazulae	*Luciogobius pallidus*	*Salmo marmoratus*
Argyrops spp.	*Lutjanus adetii*	*Salmo munzuricus*
Barbus balcanicus	*Mastacembelus ansorgii*	*Salmo obtusirostris*
Barbus barbus	*Micropoecilia bifurca*	*Salmo ohridanus*
Barbus biharicus	*Mystus singaringan*	*Salmo platycephalus*
Barbus caninus	*Neoheterandria cana*	*Salmo salar*
Barbus peloponnesius	*Olyra longicaudatus*	*Salmo tigridis*
Barbus petenyi	*Orthodon microlepidotus*	
Barbus plebejus	*Paracanthobrama guichenoti*	
Barbus plebejus spp.	*Pethia longicauda*	
Betta simplex	*Pethia sahit*	
Brachyrhaphis hartwegi	*Pimelodus pictus*	
Brachyrhaphis roseni	*Plotosus canius*	
Channa quinquefasciata	*Poeciliopsis retropinna*	
Chiloglanis brevibarbis	*Pseudomystus siamensis*	
Crenuchus spilurus	*Pterolebias peruensis*	
Distoechodon hupeinensis	*Rhodeus amurensis*	
Galaxias zebratus	*Rhodeus atremius*	
Galaxiella pusilla	*Rhodeus sinensis*	
Galaxiella toourtkoourt	*Rhodeus uyekii*	
Gambusia eurystoma	*Risor ruber*	
Gambusia sexradiata	*Rivulus marmoratus*	
Gymnothorax margaritophorus	*Rivulus santensis*	
Gymnothorax niphostigmus	*Scarus flavipectoralis*	
Haplochromis burtoni	*Tanakia limbate*	
Hara jerdoni	*Thamnaconus modestus*	
Henicorhynchus lineatus	*Trigonectes cf.*	
Ichthyoelephas longirostris	*Xenocypris hupeinensis*	

273 eDNA samples were extracted from 19 rivers and ponds in the Falklands retrieving DNA concentrations between 0 and 15 ng/ µl across all sites (57 samples had no detectable DNA). Zebra trout DNA was successfully detected in three of the 19 rivers sampled (**Table 2.3** and **Table 2.4**), *Aplochiton zebra* in two rivers and *Aplochiton taeniatus* in three, whereas brown trout DNA was detected in six out of 19 rivers (**Table 2.3** and **Table 2.4**), three of being the first time. Previously, brown trout and zebra trout had been found together in two of the rivers, R4 (Findley Creek Stream) and R9 (House Creek); however, no indication of either species was found in those rivers. Brown trout and zebra trout DNA was detected at sites

where they had been previously found (N = 3 in each case) and also at sites where there was visual confirmation eDNA collection (**Table 2.3**), supporting the effectiveness of these assays in the field. All negative controls (sampling blanks, extraction blanks and PCR blanks) failed to amplify for both zebra trout species and brown trout.

Table 2.3. Previous and current presence/absence data for the three study species at all sampling sites based on previous sampling using electrofishing and on current sampling using eDNA. Y = species present, N = Species not present/detected. * zebra trout seen during eDNA sampling; ** brown trout caught/seen during eDNA sampling period.

Waterbody	**Site No.**	**Previously sampled**	**Zebra trout previously present**	**Zebra trout current presence**		***Salmo trutta* previously present**	***Salmo trutta* current presence**
				A. zebra	***A. taeniatus***		
Johns Brook	R1	NA	NA	N	N	NA	Y
Monty Deans Creek	R2	1999	N	N	N	N	N
Spots Arroyo	R3	2009	Y	N	Y	N	N
Findley Creek Stream	R4	2011	Y	N	N	Y	N
North West Arm House Stream *	R5	2012	Y	Y	Y	N	N
Fish Creek (2)	R6	2012	Y	Y	Y	N	N
Fish Creek (1)	R8	2012	Y	N	N	N	N
House Creek	R9	1999	Y	N	N	Y	N
San Carlos **	R10	1999	N	N	N	Y	Y
Elephant Beach Pond Stream	R11	1999	N	N	N	Y	Y
Estancia Creek	R13	2008	N	N	N	Y	N
Herbert Stream **	R14	1999	N	N	N	Y	N
Teal House River	R15	NA	NA	N	N	NA	Y
Chartres River	R16	1999	N	N	N	Y	N
Doctors Creek**	R17	2012	N	N	N	Y	Y
Malo Arroyo **	R18	NA	NA	N	N	NA	N
Neil Clark Nature Reserve	R19	NA	NA	N	N	NA	Y
Spring Point	R20	NA	NA	N	N	NA	N
Whiskey Creek Stream	R22	2009	Y	N	N	N	N

Table 2.4. Total number of amplifications in waterbodies where brown trout or zebra trout were detected.

Waterbody	Site No.	Total Volume Sampled	No. Zebra Trout Amplifications		No. Brown Trout Amplifications
			A. zebra	A. taeniatus	
Johns Brook	R1.1	872	0/36	0/36	4/36
	R1.3	823	0/36	0/36	2/36
Spots Arroyo	R3.1	900	0/36	2/36	0/36
	R3.3	1200	0/36	4/36	0/36
North West Arm House Stream	R5.1	1200	6/36	5/36	0/36
	R5.3	1108	34/36	0/36	0/36
Fish Creek (2)	R6.1	1200	0/36	0/36	0/36
	R6.3	1200	9/36	5/36	0/36
San Carlos	R10.1	1200	0/36	0/36	12/36
	R10.2	1200	0/36	0/36	21/36
Elephant Beach Pond Stream	R11.1	1200	0/36	0/36	19/36
	R11.3	1200	0/36	0/36	6/36
Teal House River	R15.1	1200	0/36	0/36	13/36
	R15.2	1200	0/36	0/36	12/36
Doctors Creek	R17.1	1200	0/36	0/36	19/36
	R17.2	1200	0/36	0/36	1/36
Neil Clark Nature Reserve	R19.1	1200	0/36	0/36	0/36
	R19.3	1200	0/36	0/36	5/36
	R19.5	1150	0/36	0/36	0/36

Cloning of four brown trout samples resulted in 58 successfully transformed clones whose sequences matched 97.89 – 100% *S. trutta* sequences in BLAST (Ye *et al.*, 2006). *Aplochiton* spp. cloning resulted in the successful transformation of 84 clones from nine eDNA samples (N = 2 *A. zebra*, N = 3 *A. taeniatus*, and N = 2 mixed samples), 78 matching 89.66 – 100% *A. zebra*, and six matching 91.67 – 100% *A. taeniatus* in BLAST, confirming the species identity of the peaks at each of the melting temperatures. Only *A. zebra* sequences were identified in the mixed samples and non-specific amplification was observed in the remaining clones.

To determine if amplifications were affected by sampling conditions (volume filtered, season, and shade, **Table 2.1**) a generalised linear model using binomial error family was performed. Individual predictors were dropped from the model until the optimal model based on AIC was obtained. Starting model contained volume (estimate = 0.005, SE = 0.002, t = 2.384, p = 0.017), season (estimate = 0.485, SE = 0.853, t = 0.569, p = 0.570), and shade (estimate = -0.894, SE = 0.549, t = -1.628, p = 0.106) as predictor variables. Following the *drop1* function season was removed first, followed by shade (**Table 2.5**). In the final model total water volume sampled was the sole significant predictor (estimate = 0.005, SE = 0.002, t = 2.293, p

= 0.022, AIC = 47.773), indicating that larger volumes of water were more likely to yield successful amplifications.

Table 2.5. Model output and AIC for all possible models used to determine if amplifications were affected by sampling conditions. Predictor variables included volume, shade, and season.

Model	Volume	Shade	Season	AIC
Starting model	E = 0.005	E = -0.894	E = 0.485	48.622
	SE = 0.002	SE = 0.549	SE = 0.853	
	t = 2.384	t = -1.628	t = 0.569	
	p = 0.017	p = 0.106	p = 0.570	
Intermediate model	E = 0.005	E = -0.793		46.946
	SE = 0.002	SE = 0.500		
	t = 2.530	t = -1.588		
	p = 0.011	p = 0.112		
Final Model	E = 0.005			47.773
	SE = 0.002			
	t = 2.293			
	p = 0.022			

2.4 Discussion

The application of the novel AzebAtaeCytb assay allowed the detection of two threatened galaxiids, which coexisted in some of the sampling locations, and confirmed their presence at three rivers where they had previously been detected with conventional sampling. In addition, using the StruttaCytb assay, brown trout DNA was detected in six rivers, including three where they had not previously been sampled. The assays were validated by sequencing and visual identification.

Zebra trout were not detected in three rivers where they had previously been identified, including two where the species previously were found to coexist with brown trout. This failure to detect coexistence could be due to brown trout outcompeting native zebra trout, as seen in other streams throughout the Falklands and other counties (Garcia de Leaniz *et al.*, 2010; Valiente *et al.*, 2010a). It is possible that the trout caught in Findley Creek Stream and House Creek were new invaders into these areas during the first sampling and, therefore, co-existence between these species may have been short-lived. However, failure to detect brown trout and zebra trout at rivers where they had previously been found using traditional methods also could be due to low filtration volume, as filtering larger volumes of water increases eDNA capture (Deiner *et al.*, 2015; Muha *et al.*, 2019) and may facilitate detection of rare species and populations (Turner *et al.*, 2014). Although all target species were detected using relatively small volumes of water (100-200ml per replicate), which were previously shown to be sensitive enough to detect rare species (Robinson *et al.*, 2019b), the analysis indicated that amplifications were affected by the total volume filtered, with detections being more likely with higher volumes (Turner *et al.*, 2014; Schultz and Lance, 2015; Egeter *et al.*, 2018). Therefore, I suggest filtering larger water volumes, at least 1L per replicate, to maximise detection of rarer target species (Mächler *et al.*, 2016; Capo *et al.*, 2019).

Weather conditions might also have played a role in the detection rates, as sampling was carried out across two field seasons, the first April-May 2018 (Autumn) and the second September-October 2018 (Spring), coinciding with high volume of rain and snowmelt, resulting in more water and faster flowing rivers than in the first sampling season. These high/fast flowing conditions could have led to DNA being flushed out/downstream more quickly, potentially reducing the probability of detecting target species' DNA (Pilliod *et al.*, 2014; Laramie *et al.*, 2015). In addition, seasonal changes in eDNA concentration can occur with breeding, whereby DNA is released into the environment with gametes (Buxton *et al.*, 2017; Doi *et al.*, 2017). Environmental factors such as temperature also can have seasonal

impacts, with temperature not only influencing the release of DNA through increased activity, but also impacting its degradation rates (Lacoursière-Roussel *et al.*, 2016b; Buxton *et al.*, 2017). However, statistical analyses indicated that season had no effect on amplification, so sampling in two different seasons did not seem to have affected the detection probability in this case. In addition, the spatial distribution, and densities of individuals in a river could affect the detection of target DNA, if animals congregated in a specific area and water movement resulted in the clumping of DNA (Furlan *et al.*, 2016). Finally, it is possible that the presence of brown trout and zebra trout in some streams could not be detected as they no longer inhabited those areas.

The analyses distinguished between the morphologically similar *A. zebra* and *A. taeniatus*, enabling the determination of species assemblages when either or both species are present, highlighting the sensitivity of qPCR-based methods over traditional approaches (Wilcox *et al.*, 2013; Evans *et al.*, 2017). Previously, morphological identification was mainly based on stomach size and length, and dorsal spots; however, individuals can lack colour patterns especially when small and this colouration should be interpreted with caution (Alò *et al.*, 2013). In addition, identifying species through stomach size and length (Mcdowall and Nakaya, 1988) requires destructive sampling, which is not ideal when working with a threatened species (Barnett *et al.*, 2010; Jardine *et al.*, 2011). Although it is possible to identify *Aplochiton* spp. though DNA barcoding of tissue samples (e.g., fin clips and muscle), this type of sampling could increase mortality as it requires capturing and handling individuals (Vanhaecke *et al.*, 2012b), it is more time consuming than collecting water, particularly for rare species such as zebra trout (Reynolds *et al.*, 2003), and is not appropriate endangered species (Falkland Islands Government, 1999; Sanderson *et al.*, 2009).

The introduction of brown trout to the Falkland Islands has posed many risks to the native galaxiids, and the impacts can be seen in all three native species (*Galaxias maculatus* and both *Aplochiton* species) (McDowall *et al.*, 2001; Ross, 2009). Since the introduction of brown trout, zebra trout abundance and distribution has shown a marked decline that resulted in the species being considered threatened in the Falklands (Falkland Islands Government, 1999; McDowall *et al.*, 2001; Ross, 2009). Although, I did not detect any coexistence of brown trout and zebra trout in this study, their co-occurrence had been previously observed in the Falkland Islands (McDowall *et al.*, 2001) and in Patagonia, where brown trout has caused dietary changes and decreased body condition in both species of zebra trout (Elgueta *et al.*, 2013).

eDNA from both *Aplochiton* species was also found in two locations where their coexistence had not been previously observed (Vanhaecke *et al.*, 2012b). Such species mixing could lead to increased hybridisation, known to occur at very low frequencies (Vanhaecke *et al.*, 2012b), potentially resulting in outbreeding depression, demographic swamping, and/or genetic assimilation (Esa *et al.*, 2000; Wolf *et al.*, 2001). Hybridisation effects of invasions have been observed in pupfish (*Cyprinodon bovinus*) in Texas and Mozambique tilapia (*Oreochromis mossambicus*) in southern Africa where native and invasive species are hybridizing (Echelle and Echelle, 1997; Firmat *et al.*, 2013), and also in New Zealand where introgression between two species of native galaxiid (*Galaxias depressiceps* and *Galaxias sp D*) has been human induced (Esa *et al.*, 2000). It is unknown whether hybrids between *A. zebra* and *A. taeniatus* would be viable, but further research on the potential risks is needed.

To protect the native galaxiids in the Southern Hemisphere it is important to determine their current distribution and that of invasive salmonids, for which eDNA provides an efficient and cost-effective non-invasive tool, as in many recent conservation and monitoring programs (Jerde *et al.*, 2011; Rees *et al.*, 2014). This is particularly valuable in remote/inaccessible areas (Lacoursière-Roussel *et al.*, 2018), such as the Falklands, where it can be very difficult and costly to access and sample using traditional methods due to the limited road network. Information on remaining refugia for galaxiids can be used to prioritise sites for conservation (McGeoch *et al.*, 2016), for example in designating nature reserves and/or Ramsar sites, implementing semi-permeable fish barriers that allow movement of only small native fishes or physically removing brown trout from galaxiid refuges (Chadderton, 2001).

In summary, using newly developed non-destructive eDNA assays, I identified brown trout in locations where it had previously been undetected, suggesting potential expansion of the species in the Falklands, and also detected the coexistence of both *Aplochiton* species. With further optimisation, such as using synthetic genes at known concentrations (Wilcox *et al.*, 2013), it may be possible to gain relative estimates of species abundance using qPCR (Lodge *et al.*, 2012; Lacoursière-Roussel *et al.*, 2016a), although the results indicate that water volume is critical for the detection sensitivity. These tools can be used to monitor both threatened galaxiids and invasive brown trout and have the potential to inform conservation managers on their range expansion or contraction to better target areas for intervention (Rees *et al.*, 2014).

Chapter 3 Conservation of endangered galaxiid fishes in the Falkland Islands required urgent action on invasive brown trout

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3.1 Introduction

Invasive species represent one of the major threats to freshwater biodiversity, and yet their introduction has in many cases been intentional. For example, salmonids have been deliberated translocated all over the world to provide fishing and aquaculture opportunities since the 19th century (McDowall, 2006), despite being responsible for the demise of native fish fauna (Garcia de Leaniz *et al.*, 2010; Young *et al.*, 2010).

Human activities have not only been responsible for the introduction of invasive species but have also helped in many cases with their expansion (Hulme, 2015). Yet, the importance of human assisted dispersal of non-native species is often difficult to assess due to lack of accurate introduction records and confounding environmental factors (Tabak *et al.*, 2017). Islands provide ideal scenarios to examine the dispersal of invasive species as the date and location of introductions are typically well known, and there is often baseline information on the status of native species before the invasion (Ewel and Högberg, 1995).

Brown trout (*Salmo trutta*) is one of the most successful freshwater invaders and has been included as one of the '100 of the world's worst invasive alien species' (Lowe *et al.*, 2000) due to its widespread ecological damage. The species has been implicated in the decline of native galaxiid fishes in many parts of the Southern Hemisphere (McDowall, 2006), most notably in South America (Young *et al.*, 2010; Elgueta *et al.*, 2013), New Zealand (McDowall, 2003), and the Falkland Islands, where it has benefitted from protected status (Falkland Islands Government, 1964; Falkland Islands Government, 1999; McDowall *et al.*, 2001). This has created a conservation conundrum as protecting non-native salmonids to boost sport fishing may have put native fish at risk (Garcia de Leaniz *et al.*, 2010).

Three surveys, conducted 10-20 years ago, concluded that brown trout had severely impacted two of the three native galaxiids, *Aplochiton zebra* and *Aplochiton taeniatus* (McDowall *et al.*, 2001; Ross, 2009; Fowler, 2013), which appear to have contracted their range and are threatened by secondary releases (i.e., invasions following the initial introductions). However, little is known about the current distribution of the endangered galaxiids, or the roles that natural and human-mediated dispersal may have played in the dispersal of brown trout following the initial introductions.

Here, the main aims were to reconstruct the introduction and establishment of brown trout in the Falkland Islands using historical records. Model the dispersal of brown trout using anthropogenic and bioclimatic variables to derive risk maps which could be used to prioritise

conservation of native galaxiid populations. In addition to predicting the invasion of brown trout in the Falklands under different management scenarios.

3.2 Methods

Reconstructing the introductions of brown trout

Historical records on the introduction of brown trout in the Falkland Islands were obtained from Arrowsmith and Pentelow (1962), Stewart (1973, 1980) and Chilean sources, Basulto (2003) and Faundez et al. (1997). This information was supplemented with angler accounts, newspaper, magazine, and blog articles (Arrowsmith and Pentelow, 1965; Salmon and Trout Association, 2012), Master's thesis (Fowler, 2013) and grey literature (Stewart, 1973) to reconstruct the origin of brown trout introductions, due to a paucity of published literature.

A database of presence/absence records of the four species of freshwater fish present in the Falklands (three native galaxiids, *A. zebra, A. taeniatus* and *G. maculatus*) was compiled using records from McDowall et al. (2001), Ross (2009) and Fowler (2013). McDowall's (McDowall *et al.*, 2001) first survey of the Falkland Islands (2001) employed seine, gill and fyke netting, spotlighting at night and electrofishing. Electrofishing was mostly conducted on 50 m stretches of river and survey sites were primarily located around the road network to optimize the number of sites that could be sampled. Ross (Ross, 2009) also utilized electrofishing, seine netting and visual checks, primarily focused on *Aplochiton* spp. but also to expand the presence/absence database of freshwater fish across the Falklands. Fowler (Fowler, 2013) used single-pass electrofishing (Smith-Root ELBP2), seine netting and visual surveys in 2011/2012 sampling seasons. Active fishing effort varied between 159 and 1800 seconds depending on location.

Species distribution modelling

The Falklands were divided into 8,813 1×1km^2 grid cells, excluding those with less than 70% land and those which contained no rivers (Rodríguez-Rey *et al.*, 2019). Brown trout presence was modelled using a generalised linear model and presence/absence records from 134 sites obtained from McDowall et al. (2001), Ross (2009) and Fowler (2013) were used to train and test the model (**Figure 3.1**). The model employed 12 anthropogenic and 9 bioclimatic predictors (**Table 3.1**) for which mean values or values from the centre of the grid cells were extracted using zonal statistics and sample raster value tools in QGIS 3.4 (QGIS Development Team, 2020). To examine human-mediated range expansion, Euclidean distance to closest settlement and road as indicators of human pressure and accessibility was included. Euclidean distance to the nearest river-road crossing and number of river-road crossings in the watershed was calculated to account for factors which may have contained

the spread of brown trout, as well barrier free length (fragment length between consecutive barriers) and barrier-free length share (proportion of total river length free of culverts) (Jones *et al.*, 2019). Bioclimatic predictors included slope, altitude, minimum winter temperature, annual rainfall, and land cover type, in addition to river density (total river length per watershed) and flow accumulation (accumulation of flow downstream from the grid cell). To examine marine dispersal, flow accumulation was included as a proxy for distance to the river mouth as sites with low flow accumulation are further from the mouth of the river and, therefore, less likely to become invaded by anadromous dispersal. *Aplochiton* spp. could act as a potential food source for brown trout, therefore, their presence/absence was included as a variable. East/West was also included as a variable to determine if there were any differences in the invasion of brown trout between the two main islands. In addition, introduction site (whether the grid cell contained an introduction site), and introduction basin (does the drainage basin contained an introduction site) were included as if the drainage basin contained an introduction site other sites within the same drainage basin would be more likely to become invaded. Euclidean distance to the nearest invaded and introduction sites was included to account for human translocation of fish, whereas; the distance to the nearest invaded and introduction sites around the coast was included to account for natural marine-mediated colonization. Euclidean distances were calculated using the distance matrix tool from the centroid of the grid to the point of interest (e.g., introduction sites) in QGIS. Distances around the coast were calculated using a purpose-built function (pers comms., William P. Kay) in R3.5.3 (R Core Team, 2019) using *rgdal*, *sp*, *raster*, *gdistance*, *dplyr* and *tidyverse* packages.

The Variance Inflation Factor (VIF) was calculated using the corvif function in R (Zuur *et al.*, 2009) and predictors with a VIF <3 were retained to reduce bias due to collinearity (Kock and Lynn, 2012). On this basis, three variables (basin introduction, river density and distance to the nearest culvert, **Table 3.1**) were excluded, resulting in 10 anthropogenic and eight bioclimatic variables being retained. Equal numbers of presence and absence records were randomly divided into training and testing datasets with an 80:20 split. Species distribution was predicted using a generalised linear model and Leave One Out Cross Validation (LOOCV), see Appendix 2 for details (Rennie *et al.*, 2005; Hooten and Hobbs, 2015). The *drop1* function from the lme4 package (Bates *et al.*, 2015) was used to test the significance of individual predictors and arrive at the best model based on the lowest AIC value (see **Table S1** for a breakdown of models). Model performance was assessed using the *evaluate* function

in *dismo* to examine the area under the curve (AUC) criterion (Fielding and Bell, 1997), and compared against a null model of all variables built using the same testing and training datasets as used for the real model (Rodríguez-Rey *et al.*, 2019). The final and null models were compared using parametric bootstrapping (1000 simulations) methods in R3.5.3 (R Core Team, 2019) using *PBmodcomp* in *pbkrtest* (Halekoh and Højsgaard, 2014). Risk maps were generated in QGIS 3.10.3 using the predicted probability of invasion calculated using LOOCV for all 8,813 grid cells.

Establishment success

To calculate establishment success, the proportion of introduction sites that still had brown trout ~50 years later were compared against the random 50% expectation using a binomial test. Presence/absence data for brown trout and the three native galaxiids was used to assess how the presence of brown trout influenced the presence of native galaxiids by calculating relative risks.

Predictive modelling of brown trout invasions under different management scenarios

The future dispersal of brown trout over a 130-year period from 1947 onwards was modelled and predicted considering three different management scenarios: (1) No containment, (2) moderate containment (a 10% reduction in the probability of invasion at each cell), and (3) strong containment (a 30% reduction in the probability of invasion at each cell). All scenarios were modelled using the invasion probabilities calculated with the brown trout occurrence model and LOOCV. For scenarios 2 and 3 the probability of invasion was reduced by 10% and 30%, respectively. Grid cells with an original probability of ≥0.8 remained the same as they would not be targeted for management due to their high invasion risk. Using these probabilities, the invasion status, invaded or not-invaded, was estimated using a random binomial distribution generator with the *rbinom* function in R version 3.5.3 (R Core Team, 2019). As not every instance of invasion is successful (Sax and Brown, 2000), sites that became invaded were randomly selected using *rbinom* to remain invaded or return to a not-invaded status. As grid cells were found to be more likely to become invaded if they were close to invaded sites (see results) Euclidean distance to the nearest invaded site was calculated using the *sf* version 0.9-6 (Pebesma, 2018) and *geodist* version 0.0.6 packages (Padgham and Sumner, 2020). Invasion probabilities were then updated at each iteration under the three scenarios outlined above. To obtain a mean percentage occupancy and 97.5% confidence intervals each scenario was run for over 300 iterations (Vose, 2008). The

observed rate of expansion (0.9% increase in occupancy/year since 1950) was used to calibrate the model and convert the number of model iterations into calendar years (one iteration = ~24 years or ~4 generations), see supplementary material for details.

Table 3.1. Predictor variables used to generate species distribution model. Variables in bold had a VIF scores <3 (Kock and Lynn, 2012) and were included in the species distribution model.

Predictor	Description	Source
Anthropogenic predictors		
Eucl_dist_inv	Euclidean distance to the nearest invaded site in a straight line (km)	Own creation
Coast_dist_inv	Distance to the nearest invaded site around the coast (km)	Own creation using modified R script from William P. Kay and processed using the sunbird cluster from Supercomputing Wales
Eucl_dist_intro	Euclidean distance to the nearest introduction site in a straight line (km)	Own Creation
Coast_dist_intro	Distance to the nearest introduction site around the coast (km)	Own creation using modified R script from William P. Kay and processed using the sunbird cluster from Supercomputing Wales
Intro_site	Introduction site (y/n)	**Table 3.2**
Intro_basin	Introduction basin (y/n)	**Table 3.2**
Settle_dist	Distance to the nearest settlement (km)	Own creation, FIG IMS-GIS Centre
Road_dist	Distance to the nearest road (km)	Own creation, FIG IMS-GIS Centre
Road_cross_No	Number of river-road crossings in the river basin	Own creation, FIG IMS-GIS Centre
Road_cross_dist	Distance to nearest river-road crossing (km)	Own creation
BFL	Barrier Free Length, length of river between consecutive river-road crossings (km)	Own creation
BFL_share	Proportion of total river length free from river-road crossings	Own creation
Bioclimatic predictors		
Ap	Presence of *Aplochiton* spp. (y/n); coded no if unknown	**Table 3.3**
EW	East or West Island	Own creation
Slope	Mean slope of each grid cell	USGS
Alt	Mean altitude of each grid cell	USGS
River_dens	River network in the basin (km)	Own creation, SAERI/FIG IMS-GIS Centre
Flow_accum	Mean flow accumulation scaled by max flow accumulation in basin	Own creation, SAERI/FIG IMS-GIS Centre
Min_winter_temp	Minimum winter temperature (°C)	SAERI/FIG IMS-GIS Centre
Rain	Annual precipitation	SAERI/FIG IMS-GIS Centre
LC	Land cover/substrate type	SAERI/FIG IMS-GIS Centre, DPLUS065 Project

3.3 Results

Introduction and dispersal of brown trout

Approximately 113,000 brown trout eggs were dispatched to the Falkland Islands on eight separate occasions over an 18-year period (1944-1962, **Table 3.2**; **Figure 3.1**) (Arrowsmith and Pentelow, 1965). Although original records are missing, many consignments were described as arriving in 'excellent condition' (Stewart, 1973). The first introductions took place in Moody Brook during 1944, but due to missing records, their origin is unclear. However, these first introductions likely came from Chile as 30,000 eggs from the Lautaro hatchery (River Cautín, Chile) were sent to the Falklands three years later in 1947 (Arrowsmith and Pentelow, 1965; MacCrimmon and Marshall, 1968). Eggs from the Lautaro hatchery were primarily sourced from Germany from non-anadromous parents (Faundez *et al.*, 1997; Basulto, 2003). Subsequent eggs came from three sources in the United Kingdom: Surrey, Pentlands and Lancashire. The Surrey and Pentlands fish were from non-anadromous parents, while the Lancashire trout were from sea-trout caught in the River Lune (Arrowsmith and Pentelow, 1965; Stewart, 1973). Thus, both anadromous and non-anadromous brown trout were introduced to the Falklands. The provenance of the Pentlands stock is unclear, but they may have originated from Cobbinshaw Loch (Arrowsmith and Pentelow, 1965; Stewart, 1973), Loch Leven (Fish Loch Leven, 2019), or the Howietoun Hatchery (Ross Gardiner, pers. comm.). The Howietoun hatchery had reared trout from Loch Leven and many other sources, however, no records were found of fish having ever been sent to the Falkland Islands.

In total 28 sites were stocked, but three rivers within a 25km radius of the capital Stanley (Moody Brook, Murrell River and Malo River) received most of the introductions. Fish were transported around the islands in sea-planes, milk churns, on horseback in panniers and in Bren-gun carriers (Arrowsmith and Pentelow, 1965; Stewart, 1973; Salmon and Trout Association, 2012). Trout were reported to become quickly established and colonized new areas aided by marine dispersal, with the first sea-run trout detected in 1956-57 (Arrowsmith and Pentelow, 1965; Stewart, 1973; Salmon and Trout Association, 2012), four years before any anadromous trout were introduced (**Table 3.2**).

Of the 17 stocked sites for which there are fish survey data, 15 sites still had brown trout ~50 years later. Establishment success can therefore be estimated as 88% (95CI = 62-98%), which is significantly better than chance ($\chi^2 = 8.47$, df = 1, $p = 0.004$).

Table 3.2. Sites of introductions of brown trout in the Falkland Islands

Stock origin	Year	Quantity	Introduction Site	Latitude	Longitude
Unknown (likely German stock via Chile; non-anadromous)					
	1944	'Small quantities'	Unknown	Unknown	Unknown
			Moody Brook	-51.6857	-57.9222
			Moody Brook	-51.6857	-57.9222
Lautaro Hatchery (Chile, German Stock; non-anadromous)					
	1947	30,000	Moody Brook	-51.6857	-57.9222
			Malo River	-51.6171	-58.3018
			Murrell River	-51.6535	-57.9951
Surrey Trout Farm (UK; non-anadromous)					
	1948	10,000	Malo River	-51.6171	-58.3018
			Murrell River	-51.6535	-57.9951
			Hill Cove	-51.4736	-59.9764
			Chartres River	-51.6428	-59.9283
			Port Howard/Warrah River	-51.4554	-59.6245
Surrey Trout Farm or Pentlands (UK; non-anadromous)					
	1949	15,000	Port San Carlos	-51.5095	-58.8220
			Elephant Beach Pond/Stream	-51.3807	-58.7690
			Head of the Bay	-51.6061	-59.0142
			Lorenzo Pond	-51.3593	-58.6730
			Swan Inlet	-51.8239	-58.6161
			Fitzroy River	-51.7546	-58.3068
			Kidney Pond	-51.6251	-57.7739
			Pebbly Pond	-51.7270	-57.8740
			Johnsons Harbour	-51.4995	-58.0044
			Fox Bay East	-51.9421	-60.0500
			Fox Bay West	-51.9510	-60.0897
			Hill Cove	-51.4736	-59.9764
Surrey Trout Farm or Pentlands (UK; non-anadromous)					
	1950	10,000	Darwin (Camilla Creek)	-51.7711	-58.9457
			Malo River	-51.6171	-58.3018
			Port San Carlos	-51.5095	-58.8220
			Fitzroy River	-51.7546	-58.3068
			Pebble Island	-51.3199	-59.5741
			Chartres River	-51.6428	-59.9283
			Hill Cove	-51.4736	-59.9764
			Port Howard/Warrah River	-51.4554	-59.6245
			Port Stephens	-52.0980	-60.8321
Surrey Trout Farm or Pentlands (UK; non-anadromous)					
	1951	10,000	Malo River	-51.6171	-58.3018
			Swan Inlet	-51.8239	-58.6161
			Darwin (Camilla Creek)	-51.7711	-58.9457
			North Arm	-52.1291	-59.3709
			Port San Carlos	-51.5095	-58.8220
			Murrell River	-51.6535	-57.9951
Surrey Trout Farm or Pentlands (UK; non-anadromous)					
	1952	10,000	Murrell River	-51.6535	-57.9951
			Malo River	-51.6171	-58.3018
			Johns Brook	-51.4865	-58.2932
			Lorenzo Pond	-51.3593	-58.6730
			Fitzroy River	-51.7546	-58.3068
			Swan Inlet	-51.8239	-58.6161
			North Arm	-52.1291	-59.3709
			Pebbly Pond	-51.7270	-57.8740
			Kidney Pond	-51.6251	-57.7739
Middleton Hatchery – Lancashire Fisheries Board (UK; anadromous)					
	1961	20,000	Chartres River	-51.6428	-59.9283

		Mac's Paddock Brook	-51.4849	-58.2927
		Pasa Maneas	-51.6338	-58.3261
		Malo River	-51.6171	-58.3018
		Port San Carlos	-51.5095	-58.8220
		Port Howard/Warrah River	-51.4554	-59.6245
Middleton Hatchery – Lancashire Fisheries Board (UK; anadromous)				
1962	8,000	Felton's Stream	-51.6894	-57.9033
		Mile Pond	-51.7214	-57.8835
		Round Pond	-51.7268	-57.8835
		Pebbly Pond	-51.7270	-57.8740
		Salvador Camp	-51.4020	-58.3954

Farming of brown trout in the Falklands began in 2013 with the transfer of 10,000 sea trout smolts from local broodstock into sea cages at Fitzroy Sound, although ova were imported from Howietoun Hatchery UK in 2014 and 2015. Small quantities of fish have been translocated between river/ponds to form populations in new uncolonised areas or landlocked locations that would never be naturally colonized (McDowall *et al.*, 2001). These rivers/ponds are not believed to be part of the initial introduction sites (**Table 3.2**), for example, trout found in the landlocked pond at Mary Hill Quarry are believed to be a result of such movements (Jay Moffatt, pers. comms.)

The three surveys conducted in 1999, 2009 and 2012 by McDowall et al. (2001), Ross (2009) and Fowler (2012) (see **Table 3.3** for more information on sample sites) revealed a marked decline in the once abundant *Aplochiton* spp*.,* which local previously described as widespread and found throughout East and West Falkland (McDowall *et al.*, 2001). These native galaxiids now confined to the South of the Islands (**Figure 3.1**). At the time of the last survey (2012), brown trout occupied 54% of all sampled sites, with *Aplochiton* spp*.* only occupying 18%.

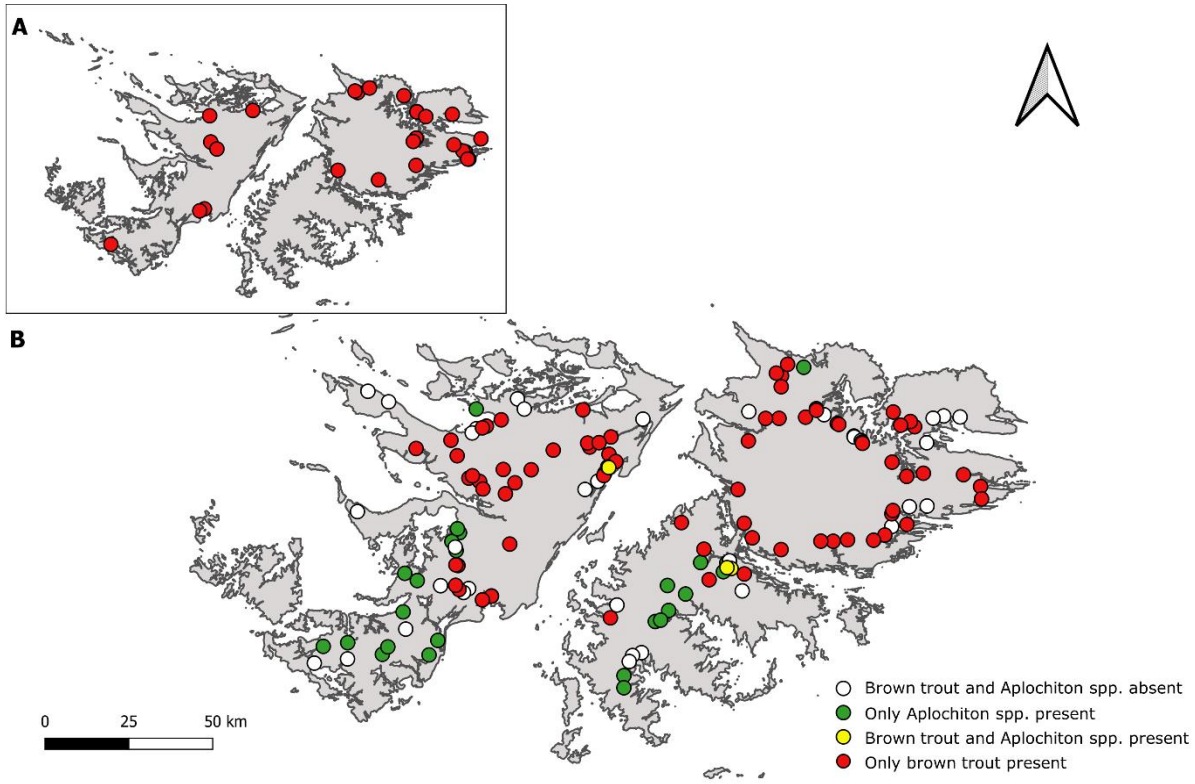

Figure 3.1. Map of the Falkland Islands showing (A) sites of the historical introductions of brown trout during 1944-1962 (details given in **Table 3.2**) and (B) presence/absence of brown trout and native *Aplochiton* species based on 1999-2012 surveys (detailed in **Table 3.3**) with six additional sites sampled in 2018-2019.

Table 3.3. Presence and absence of brown trout (*St*), *Aplochiton* spp. (*Ap*) and *Galaxias maculatus* (*Gm*) in the Falkland Islands. Sites marked with an asterisk denote brown trout introduction sites (see **Table 3.2**).

Sample Site	Island	Date Sampled	Latitude	Longitude	*St*	*Ap*	*Gm*	Reference
Arrow Harbour Arroyo	East	2011	-51.9062	-58.9508	+	-	+	Fowler (2013)
Arroyo Pedro	East	1999	-51.5111	-58.5346	+	-	-	McDowall et al. (2001)
Big Pond	East	2018	-51.8433	-51.7522	+	-	+	Minett (unpubl.)
Bodie Creek	East	2011	-51.9191	-59.1042	+	-	+	Fowler (2013)
Bull Pass Stream	East	2011	-51.8909	-59.0074	+	+	+	Fowler (2013)
Clay Ditch	East	1999	-51.4925	-58.6700	+	-	-	McDowall et al. (2001)
Colorado Pond	East	2018	-51.7136	-58.4717	+	-	-	Minett et al. (2021)
Comoda Ditch	East	1999	-51.8219	-58.5003	+	-	-	McDowall et al. (2001)
Congo Ponds Area	East	2009	-51.9787	-59.5072	-	-	-	Ross (2009)
Dan's Shanty Stream	East	1999	-51.5236	-58.2020	+	-	+	McDowall et al. (2001)
Darwin (Camilla Creek) *	East	1999	-51.7711	-58.9457	+	-	-	McDowall et al. (2001)
Deep Arroyo	East	2011	-51.9553	-59.2080	-	+	+	Fowler (2013)
Ditches into New Haven	East	2009	-51.7646	-59.2166	+	-	+	Ross (2009)
Duffins Bridge Stream	East	2009	-52.1078	-59.4073	-	-	+	Ross (2009)
Elephant Beach Pond*	East	1999	-51.3807	-58.7690	+	-	+	McDowall et al. (2001)
Elmer's Ditch Tributary	East	1999	-51.4939	-58.7840	+	-	-	McDowall et al. (2001)
Estancia Brook	East	2008	-51.6480	-58.1678	+	-	-	Fowler (2013)
Felton Stream*	East	NA	-51.6894	-57.9033	NA	NA	NA	Not sampled
Findley Creek Stream	East	2011	-51.8882	-59.0250	+	+	+	Fowler (2013)
Findlay Harbour (Wreck House)	East	2009	-52.0122	-59.5375	+	-	-	Fowler (2013)
Fitzroy River*	East	2012	-51.7546	-58.3068	+	NA	NA	Angler
Frying Pan	East	2011	-51.8111	-58.3387	+	-	-	Fowler (2013)
Gonzales Arroyo	East	2009	-51.9513	-58.9621	-	-	+	Ross (2009)
Green Pass Brook Tributary	East	1999	-51.4098	-58.7715	+	-	-	McDowall et al. (2001)
Head of the Bay*	East	2012	-51.6061	-59.9764	+	NA	NA	Angler
Head of the Creek Stream	East	1999	-51.4961	-58.0763	-	-	+	McDowall et al. (2001)
Hunter's Arroyo	East	1999	-52.1147	-59.4470	-	-	+	McDowall et al. (2001)
John's Brook*	East	2018	-51.4834	-58.2930	+	-	NA	Minett et al. (2020)
Johnsons Harbour*	East	NA	-51.4995	-58.0044	NA	NA	NA	Not sampled
Kidney Pond*	East	NA	-51.6251	-57.7739	NA	NA	NA	Not sampled
Laguna Isla	East	2011	-51.8439	-58.7890	+	-	-	Fowler (2013)
L'Antioja Stream	East	2012	-51.8249	-58.5627	+	-	-	Fowler (2013)
Little Creek Stream	East	1999	-51.3504	-58.7418	+	-	-	McDowall et al. (2001)
Lorenzo Pond*	East	1973	-51.3593	-58.6730	-	+	+	Stewart (1973)
Mac's Paddock Brook*	East	NA	-51.4849	-58.2927	NA	NA	NA	Not sampled
Magellan Pond	East	1999	-51.4995	-58.0062	-	-	+	McDowall et al. (2001)
Malo River*	East	1999	-51.6171	-58.3018	+	-	+	McDowall et al. (2001)
Mary Hill Quarry	East	2018	-51.6844	-57.7894	+	NA	NA	Minett (unpubl.)
Mile Pond*	East	NA	-51.7214	-57.8835	NA	NA	NA	Not sampled
Moody Brook*	East	2012	-51.6857	-57.9222	+	-	-	Fowler (2013)
Mullet Creek	East	1999	-51.7187	-57.9185	+	-	+	McDowall et al. (2001)
Murrell River*	East	2013	-51.6535	-57.9951	+	-	-	Fowler (2013)
North Arm*	East	2011	-52.1291	-29.3709	-	-	+	Fowler (2013)
Northwest Arm House	East	2012/18	-52.1674	-59.4874	-	+	+	Fowler (2013),

Stream								Minett et al. (2020)
Northern Stream	East	1999	-51.5018	-58.1223	-	-	+	McDowall et al. (2001)
Orequta Arroyo	East	2011	-51.8373	-59.1229	+	-	-	Fowler (2013)
Pasa Maneas*	East	NA	-51.6338	-58.3261	NA	NA	NA	Not sampled
Pebbly Pond*	East	NA	-51.7270	-57.8740	NA	NA	NA	Not sampled
Round Pond*	East	NA	-51.7268	-57.8835	NA	NA	NA	Not sampled
Rumford Brook	East	2011	-51.6557	-58.2399	+	-	-	Fowler (2013)
Salvador Camp*	East	NA	-51.4020	-58.3954	NA	NA	NA	Not sampled
San Carlos River at Ford*	East	1999/ 2018	-51.5095	-58.220	+	-	-	McDowall et al. (2001), Minett et al. (2020)
Shepherds Brook	East	1999	-51.6808	-58.9688	+	-	+	McDowall et al. (2001)
Spots Arroyo	East	2009/18	-52.0260	-59.3432	-	+	+	Ross (2009), Minett et al. (2020)
Stream at Colorado Pass	East	2009	-51.8702	-59.0137	-	-	+	Ross (2009)
Stream at Gibraltar Gate	East	1999	-51.4933	-58.8417	+	-	-	McDowall et al. (2001)
Stream near Hunter Arroyo	East	1999	-52.1303	-59.4622	-	-	+	McDowall et al. (2001)
Stream, Caneja Creek	East	1999	-51.5190	-58.2621	+	-	-	McDowall et al. (2001)
Stream, at Fitzroy	East	1999	-51.7838	-58.2425	+	-	+	McDowall et al. (2001)
Stream, Douglas Creek	East	1999	-51.4704	-58.6229	+	-	-	McDowall et al. (2001)
Stream, Elephant Beach Pond*	East	1999/ 2018	-51.3731	-58.7911	+	-	+	McDowall et al. (2001), Minett et al. (2020)
Stream, Fitzroy	East	1999	-51.7887	-58.3085	-	-	-	McDowall et al. (2001)
Stream, Monty Dean's Creek	East	1999	-51.5669	-58.1515	-	-	+	McDowall et al. (2001)
Stream, Mount Pleasant	East	1999	-51.8243	-58.3878	+	-	-	McDowall et al. (2001)
Stream, NW of Teal Inlet	East	1999	-51.5456	-58.4638	-	-	+	McDowall et al. (2001)
Stream NW of Teal Inlet	East	1999	-51.5481	-58.4629	-	-	+	McDowall et al. (2001)
Stream, Salt House Creek	East	2009	-52.1997	-59.4894	-	+	+	Ross (2009)
Stream, SE of Teal Inlet	East	1999	-51.5580	-58.4330	-	-	-	McDowall et al. (2001)
Stream, Smylie's Brook	East	1999	-51.4738	-58.9129	-	-	-	McDowall et al. (2001)
Stream, Teal Inlet	East	1999	-51.5654	-58.4278	+	-	-	McDowall et al. (2001)
Stream, SE of Teal Inlet	East	1999	-51.5622	-58.4294	-	-	+	McDowall et al. (2001)
Swan Inlet*	East	2012	-51.8239	-58.6161	+	NA	NA	Angler
Teal Creek Arroyo	East	2009	-51.8106	-58.9121	+	-	+	Ross (2009)
Third Corral Brook	East	1999	-51.5520	-58.9182	+	-	-	McDowall et al. (2001))
Trib., Halfway House Arroyo	East	2011	-51.9977	-59.2836	-	+	+	Fowler (2013)
Turners Stream	East	1999	-51.5137	-58.5277	+	-	-	McDowall et al. (2001)
Unnamed Stream	East	1999	-51.8726	-59.1386	-	+	+	McDowall et al. (2001)
Unnamed Stream	East	1999	-51.9311	-59.2855	-	+	+	McDowall et al. (2001)
Unnamed Stream	East	1999	-51.4869	-58.5905	-	-	-	McDowall et al. (2001)
Unnamed Stream	East	1999	-52.0228	-59.3197	-	+	+	McDowall et al. (2001)
Unnamed Stream	East	1999	-51.8987	-59.0414	-	+	+	McDowall et al. (2001)

Unnamed Stream	East	1999	-51.8753	-59.0254	-	+	+	McDowall et al. (2001)
Unnamed Stream	East	1999	-51.7461	-58.3020	+	-	-	McDowall et al. (2001)
Unnamed Stream	East	1999	-51.7366	-58.2306	-	-	+	McDowall et al. (2001)
Unnamed Stream	East	1999	-51.7357	-58.1542	-	-	-	McDowall et al. (2001)
Unnamed Stream	East	1999	-51.4747	-58.6230	+	-	-	McDowall et al. (2001)
Pebble Island*	Pebble	NA	-51.3199	-59-5741	NA	NA	NA	Not sampled
1st Arroyo	West	2009	-52.0838	-60.5346	-	+	-	Ross (2009)
2nd Pass Stream	West	1999	-51.6253	-60.1260	+	-	-	McDowall et al. (2001)
2nd Arroyo	West	2009	-52.0647	-60.5092	-	+	+	Ross (2009)
Arroyo Chico	West	1999	-51.9208	-60.1891	+	-	-	McDowall et al. (2001)
Arroyo Malo	West	1999	-51.9194	-60.1471	-	-	-	McDowall et al. (2001)
Ballan Stream	West	2009	-51.6471	-59.5714	-	-	+	Ross (2009)
Beach Stream	West	1999	-51.6258	-59.5402	+	-	+	McDowall et al. (2001)
Bull Hill Stream	West	1999	-51.5764	-59.5194	+	-	-	McDowall et al. (2001)
Bull Stream	West	1999	-51.4888	-60.0413	+	-	-	McDowall et al. (2001)
Campbell Creek Stream	West	2009	-52.0197	-60.4278	-	-	+	Ross (2009)
Cemetery Creek Estuary	West	1999	-51.5961	-59.4913	+	-	+	McDowall et al. (2001)
Chartres River*	West	1999	-51.6428	-59.9283	+	-	-	McDowall et al. (2001)
Daddy's Ditch	West	2009	-51.4195	-59.9033	-	-	+	Ross (2009)
Dean's River	West	2009	-52.0914	-60.6869	-	-	+	Ross (2009)
Dirty Ditch at the High Tide	West	1999	-51.5401	-60.3470	+	-	+	McDowall et al. (2001)
Double Stream	West	2009	-51.6677	-59.6277	-	-	+	Ross (2009)
Doyle River Tributary	West	1999	-51.7695	-60.1764	-	+	+	McDowall et al. (2001)
Dunbar Creek	West	2009	-51.4124	-60.4556	-	-	+	Ross (2009)
Edye Creek	West	2012	-51.8711	-60.4206	-	+	+	Fowler (2013)
Fish Creek 1	West	2012	-52.0538	-60.2908	-	+	+	Fowler (2013)
Fish Creek 2	West	2012/ 2018	-51.8918	-60.3681	-	+	+	Fowler (2013), Minett et al. (2020)
Fox Bay East* (Doctors Creek)	West	2012/18	-51.9421	-60.0500	+	-	NA	Minett et al. (2020)
Fox Bay West*	West	2012	-51.9510	-60.0897	+	NA	NA	Angler
Gibraltar Stream	West	2009	-52.0913	-60.3318	-	+	+	Ross (2009)
Green Hill Stream	West	2009	-51.5552	-59.6012	+	-	-	Ross (2009)
Herbert Stream	West	1999	-51.5222	-60.1959	+	-	-	McDowall et al. (2001)
Hawk's Nest Stream	West	1999	-51.8055	-59.9614	+	-	-	McDowall et al. (2001)
Hill Cove*	West	2008	-51.4736	-59.9764	+	-	-	Fowler (2013)
House Creek	West	1999	-51.6097	-59.5267	+	+	+	McDowall et al. (2001)
House Stream	West	2012	-51.6116	-59.5221	+	+	+	Fowler (2013)
Lake Sulivan, Outflow	West	2009	-51.7922	-60.2111	-	+	+	Ross (2009)
Lake Sulivan North	West	2011	-51.8167	-60.1941	-	+	+	Fowler (2013)
Lake Sulivan North Outflow	West	1999	-51.8073	-60.1976	-	-	+	McDowall et al. (2001)
Lake Sulivan South	West	2011	-51.8567	-60.1891	+	-	+	Fowler (2013)
Larger Stream, Hill Cove	West	1999	-51.4945	-60.0843	-	-	+	McDowall et al. (2001)
Leicester Stream	West	2009	-51.9084	-60.2682	-	-	+	Ross (2009)
Main Point Creek	West	2009	-51.4474	-59.8757	-	-	+	Ross (2009)
Many Branches Stream	West	1999	-51.5303	-59.5083	+	-	+	McDowall et al. (2001)

Mickey Doolan's Ditch	West	1999	-51.9292	-60.1703	-	-	+	McDowall et al. (2001)
Mt Adam Stream	West	1999	-51.6355	-60.0802	+	-	-	McDowall et al. (2001)
Mt Donald Pond Inflow	West	1999	-51.5643	-60.1720	+	-	-	McDowall et al. (2001)
Neil Clark Nature Reserve	West	2018	-51.6324	-59.5452	+	-	NA	Minett et al. (2020)
Poncho Valley Stream	West	2012	-51.9736	-60.4357	-	+	+	Fowler (2013)
Port Howard/Warrah*	West	1999	-51.4554	-59.6245	+	-	-	McDowall et al. (2001)
Port Stephens*	West	2009	-52.0980	-60.8321	-	-	+	Ross (2009)
River Doyle	West	2009	-51.7588	-60.1845	-	+	+	Ross (2009)
Rous Creek Stream	West	2009	-51.7006	-60.6122	-	-	+	Ross (2009)
Skull Pass Stream	West	1999	-51.5444	-59.6095	+	-	-	McDowall et al. (2001)
Stewart's Brook	West	2009	-52.0482	-60.6826	-	+	+	Ross (2009)
Stream by Mt Rosalie House	West	2009	-51.4856	-59.3685	-	-	+	Ross (2009)
Stud Paddock Stream	West	1999	-51.9416	-60.0497	-	-	+	McDowall et al. (2001)
Teal House River	West	2018	-51.6194	-60.1103	+	-	NA	Minett et al. (2020)
Teal Ponds and Waterfall Stream	West	1999	-51.6557	-60.0672	+	-	+	McDowall et al. (2001)
Top Hog Ground Stream	West	1999	-51.6711	-59.9714	+	-	-	McDowall et al. (2001)
Unnamed Stream	West	1999	-51.5443	-59.5602	+	-	-	McDowall et al. (2001)
Unnamed Stream	West	1999	-51.9092	-60.2030	+	-	-	McDowall et al. (2001)
Unnamed Stream	West	2012	-51.6098	-59.8563	+	-	-	Fowler (2013)
Unnamed Stream, Hill Cove	West	1999	-51.5053	-60.1034	-	-	+	McDowall et al. (2001)
Unnamed Stream, Hill Cove	West	1999	-51.4928	-60.0587	+	-	-	McDowall et al. (2001)
Warrah River	West	1999	-51.5598	-59.7581	+	-	-	McDowall et al. (2001)
Waterfall Stream	West	2009	-51.3817	-60.5421	-	-	+	Ross (2009)
Weedy Outlet	West	1999	-51.8548	-60.1980	+	-	+	McDowall et al. (2001)
West Lagoons	West	2013	-51.4421	-60.0817	-	+	+	Fowler (2013)
Whisky Creek	West	2009	-52.0546	-60.7896	-	+	-	Ross (2009)

Modelling of brown trout occurrence

The most plausible model of brown trout occurrence contained only three predictors, Euclidean distance to nearest invaded site (estimate = -0.238, SE = 0.067, t = -3.56, p = 0.0004), presence of *Aplochiton* spp. (estimate = -1.57, SE = 0.769, t = -2.04, p = 0.041) and number of river-road crossings in the drainage basin (estimate = 0.156, SE = 0.066, t = 2.37, p = 0.018). This model explained the occurrence of brown trout significantly better than chance (LRT (3) = 52.17, p = <0.0001, AUC = 0.85).

Impact of brown trout on native galaxiids

Native galaxiids were less likely to occur in streams invaded by brown trout than in uninvaded ones (**Figure 3.2**), but the impact of invasive brown trout was more pronounced in the case of *Aplochiton* spp. Calculation of relative risk indicated that *Aplochiton* spp. was 4.5 times less likely to persist in streams invaded by brown trout than in uninvaded streams (95CI = 1.8-11.2, $p<0.001$). For *Galaxias maculatus*, the presence of brown trout decreased the probability of occurrence 2.9-fold (95CI = 2.0-4.2, $p<0.001$).

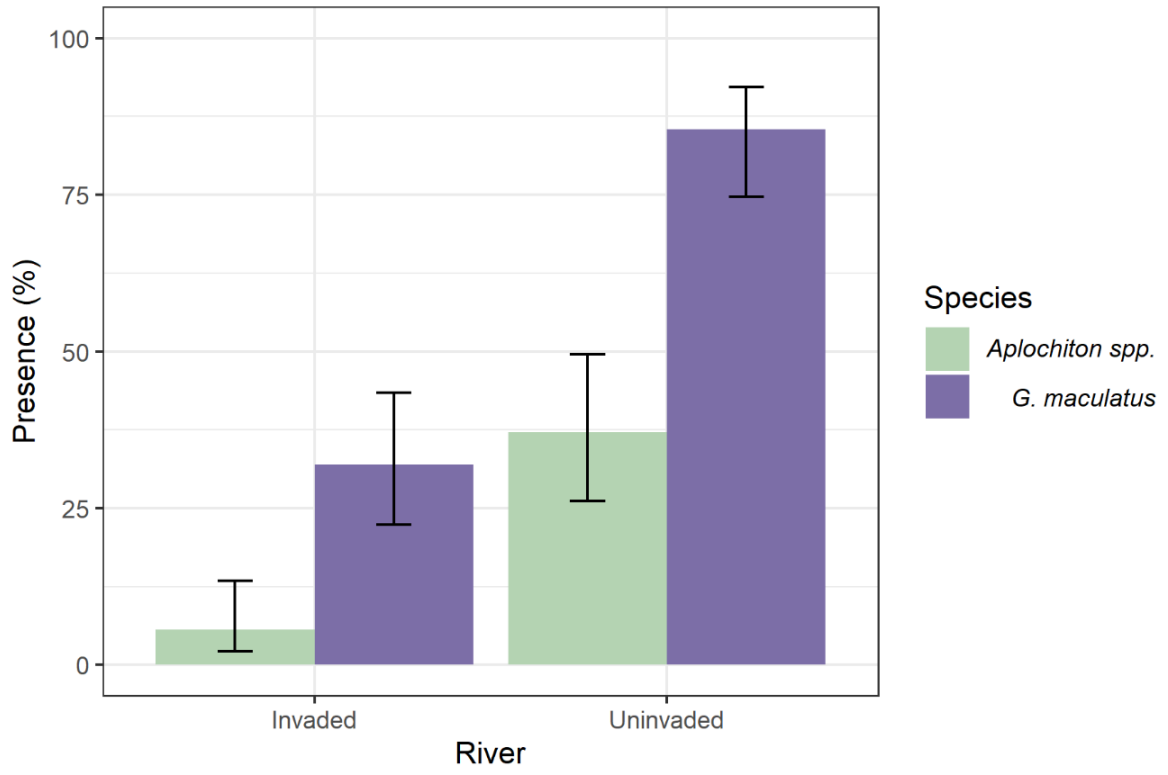

Figure 3.2. Frequency of occurrence (% and binomial upper 95CI) of native galaxiids (*Aplochiton* spp. and *Galaxias maculatus*) in streams invaded (N = 62) and in those uninvaded (N =72) by brown trout.

Risk of invasions

A risk map generated from the LOOCV probabilities identified 21% of cells with a very high risk of invasion (≥0.75), with a further 24% at high risk of invasion (≥0.5 to <0.75), and the remaining 17% and 40% being at medium (≥0.25 to <0.5) and low risk (<0.25) respectively (**Figure 3.3**). Overlaying *Aplochiton* spp. presence identified 10 high or very high-risk areas for native species where preventative measures should be prioritised to exclude brown trout and protect native freshwater fish (**Figure 3.3**).

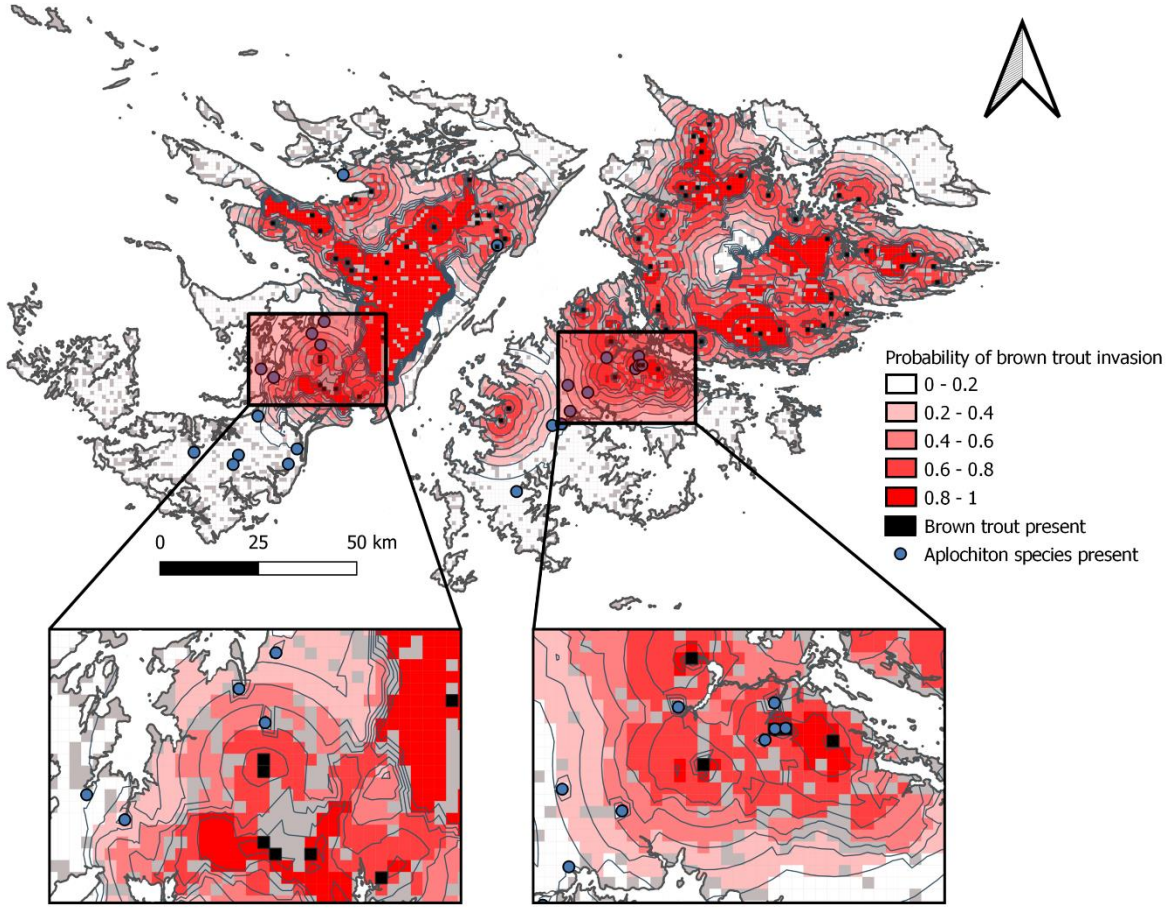

Figure 3.3. Risk map showing probabilities of brown trout invasion based on species distribution modelling. *Aplochiton* refugia at high risk of brown trout invasion are shown in zoomed insets.

Management scenarios

The simulation of brown trout invasion probabilities under three management scenarios indicated that if nothing is done (scenario 1: no containment) brown trout will likely increase their occupancy from 54% to 93% within the next 70 years (97.5CI = 70-99%). Under scenario 2 (moderate containment) occupancy is predicted to increase to 86% (97.5CI = 59-94%) and to 69% (97.5CI = 47-81%) with strong containment (scenario 3, **Figure 3.4**). Thus, occupancy is predicted to increase under all three scenarios, but only with strong containment can current *Aplochiton* refugia likely be protected from trout invasions.

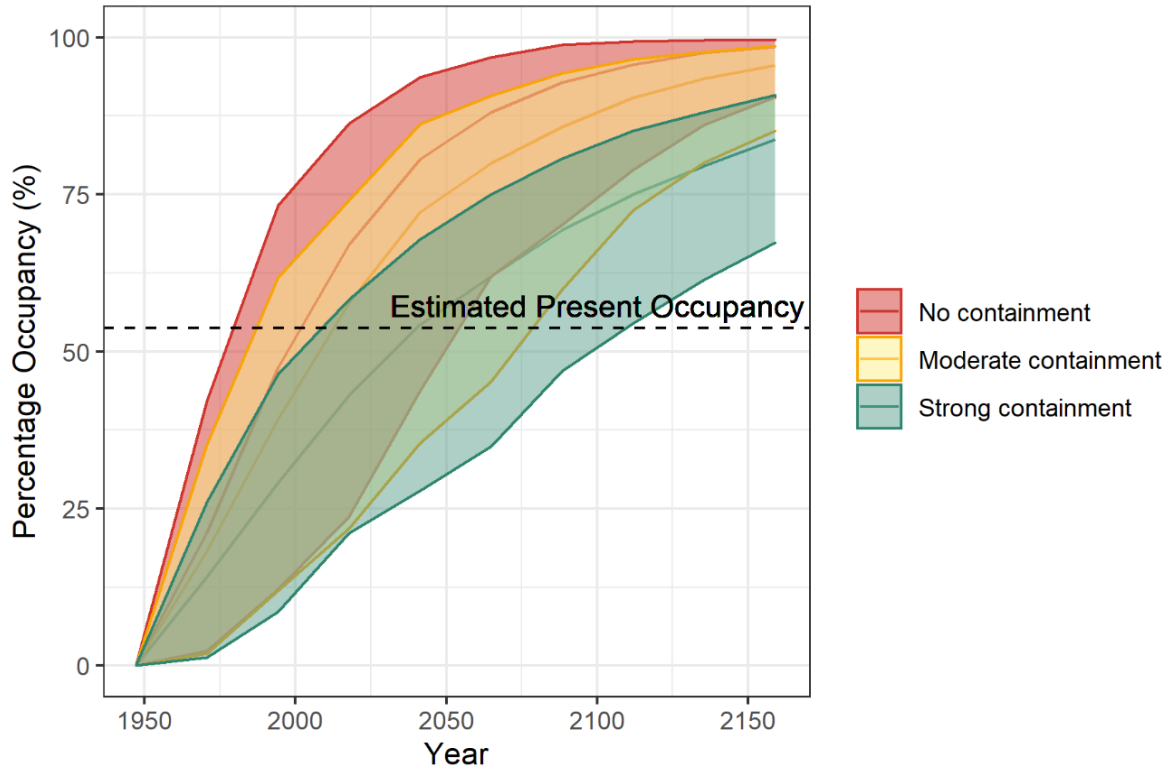

Figure 3.4. Modelled expansion of brown trout in the Falkland Islands under three different management scenarios.

3.4 Discussion

This study indicates that brown trout have already invaded 54% of the streams in the Falkland Islands since they were introduced in 1944-1962 and are impacting on native freshwater fish. Streams invaded by brown trout were significantly less likely to harbour native galaxiids than uninvaded streams, suggesting that the impacts are substantial. This finding is consistent with competitive exclusion of native galaxiids by invasive brown trout (Young *et al.*, 2009; Garcia de Leaniz *et al.*, 2010), exacerbated by predation and trophic interference (Elgueta *et al.*, 2013; Arismendi *et al.*, 2014). These simulations suggest that unless more stringent measures are put in place, brown trout will likely invade nearly all the suitable freshwater habitats in the Falklands within the next ~70 years. Given that endangered *Aplochiton* spp. only occupy ~18% of the area, mostly confined to southern part of the Islands, this could drive the species to extinction.

The establishment success of brown trout in the Falkland Islands was very high (88%), as seen elsewhere in the Southern Hemisphere (Young *et al.*, 2010; Lecomte *et al.*, 2013; Arismendi *et al.*, 2014; Davidsen *et al.*, 2021), and remarkably, in Argentina, no failed introduction of brown trout was ever reported (Baigún and Quirós, 1985). Several factors may help explain this. Firstly, this study shows that brown trout introduced into the Falkland Islands originated from at least four different origins with two life history strategies (anadromous, non-anadromous), which resulted in genetic admixture (Minett *et al.*, 2021b). Multiple origins and genetic admixture can increase genetic diversity and facilitate adaptation to novel conditions (Consuegra *et al.*, 2011), which along with repeated introductions may increase invasion success. Establishment success may have also been facilitated by phenotypic plasticity and marine dispersal, as demonstrated recently by acoustic tracking (Minett *et al.*, 2021b).

However, marine dispersal alone cannot explain the current distribution of brown trout in the Falklands; secondary translocations must have also taken place because the species is now found in land-locked sites, where it could not have reached without human intervention. Transporting brown trout has been illegal in the Falklands since 1999, but some translocations must have taken place (McDowall *et al.*, 2001). Indeed, these results indicate that brown trout presence was predicted by proximity to other invaded sites (overland, but not around the coast) and by the density of river-road crossings, which is consistent with secondary translocations facilitated by the road network, as seen in many other aquatic

invasive species. For example, roads facilitated the expansion of smallmouth bass (*Micropterus dolomieu*) in remote lakes in Canada (Kaufman *et al.*, 2009) and of bluegill (*Lepomis macrochirus*) in Japan (Kizuka *et al.*, 2014). The Falklands has ~800 km of roads that crisscross a dense river network, most of which were built over the last three decades (Fowler and Garcia de Leaniz 2012), and it is likely that this may have facilitated the expansion of brown trout. Recent eDNA analysis of water samples (Minett *et al.*, 2020) has revealed the presence of brown trout in additional streams since the last survey, suggesting that the species is expanding at a rate of ~0.9%/year. The data used to build the invasion risk model was derived from studies conducted 10-20 years ago (Fowler, 2013; McDowall et al, 2001; Ross, 2009). Thus, it is possible that if this model were to be rerun with new up to data information on the distribution of brown trout in the Falklands, which could be obtained from eDNA analysis, other predictor variables could have a larger influence.

Invasion risk was modelled using a GLM. GLMs are commonly used to model species distribution and are user friendly, however, they are not always the most appropriate method to use as they only implement linear (straight-line) functions (Austin, 2007; Breiner et al., 2015). Therefore, it is important to consider model accuracy and test model performance against null models (Rodríguez-Rey et al., 2019), as was done here (see methods and results).

Other invasive salmonids are also threatening the native fish fauna of the Falklands. For example, both chinook salmon (*Oncorhynchus tshawytscha*) (Fowler, 2013) and coho salmon (*Oncorhynchus kisutch*) are increasingly being caught off West Falkland, most likely originating from Chile or Argentina, highlighting the potential for further salmonid invasions. Similarly, the recent development of sea trout farming in open-net cages in the Falklands in 2013 poses a risk of escapees, which could further compromise the survival of native galaxiids, as seen in Patagonia (Consuegra *et al.*, 2011; Vanhaecke *et al.*, 2012a), particularly if sea cages are located close to *Aplochiton* refugia. Given the widespread ecological damage caused by invasive salmonids, being able to identify areas at high risk of invasion is critical for managing and curtailing their expansion. In this sense, these risk maps (**Figure 3.3**) may aid conservation officers tasked with the protection of native fish fauna to monitor high risk areas and develop an integrated management strategy for invasive salmonids in the Falkland Islands.

Conclusions & Recommendations

Galaxiids rank among the most severely threatened fish in the world due to the introduction of invasive salmonids (McDowall, 2006; Garcia de Leaniz *et al.*, 2010). This modelling suggests that without containment and strict measures brown trout will likely invade all remaining suitable freshwater bodies in the Falklands before the end of the century, putting the endangered native freshwater fish at high risk of extinction.

Existing legislation makes it illegal to transport or propagate brown trout in the Falkland Islands (Falkland Islands Government, 1964), but this seems insufficient as the species is also afforded a protected status (Falkland Islands Government, 1999), and fishing for trout is widely promoted (Falkland Islands Government, 2015), which may facilitate its spread. The road network appears to be a main route of human-assisted translocations and it is therefore essential that more stringent measures are put in place. This may involve making people more aware of the impacts of salmonid invasions and passing more stringent legislation. Exclusion barriers could also be deployed around galaxiid refugia to reduce the risk of salmonid invasions (Jones *et al.*, 2021b), but care must be taken to ensure this does not impact on native galaxiids, which may pose a challenge as even small barriers can have negative impacts on weak swimmers (Jones *et al.*, 2021a). Changes to angling regulations might also be useful. Currently, brown trout anglers are subject to a daily bag limit and a strict fishing season (Falkland Islands Government, 1999; Falkland Islands Government, 2015), lifting these restrictions may help slow down the invasion front in some places. Intensive fishing could be used to eradicate brown trout and establish buffer zones around *Aplochiton* spp. refugia although this may not be effective if there is compensatory density-dependent mortality (Saunders *et al.*, 2015). Analysis of eDNA from water samples could be used to delineate galaxiid refugia (Minett *et al.*, 2020), to serve as an early warning of brown trout invasions, and to establish whether containment or eradication measures have been successful.

Since McDowall's call for action 20 years ago (McDowall *et al.*, 2001), brown trout has continued to expand while native galaxiids have continued to decline. *Aplochiton* spp. feature on a Falklands postal stamp while *Galaxias maculatus* is called 'Falklands minnow', testifying to their importance for local islanders, and their place in the natural and cultural heritage of the Falkland Islands. Brown trout has brought wealth and recreation opportunities

to the Falklands but has also caused the demise of native freshwater fish. This study shows that urgent protection measures are needed to safeguard their survival.

Chapter 4 SNP analysis and acoustic tagging reveal multiple origins and widespread dispersal of invasive brown trout in the Falkland Islands

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4.1 Introduction

The spread of invasive species can occur via accidental introduction, deliberate release and/or through natural processes. Controlling biological invasions is increasingly important because they impact native species and communities leading to loss of biodiversity and ecosystem functionality (Mills *et al.*, 2003; Doherty *et al.*, 2016; Mollot *et al.*, 2017), particularly in remote islands with low native diversity (Moser *et al.*, 2018). However, control measures can face social opposition, for example if the costs are high (Sheremet *et al.*, 2017) or the introduced species has acquired socio-cultural importance (Lohr and Lepczyk, 2014; Roberts *et al.*, 2018). Thus, in some cases, managing the damage caused by invaders can be the best option (Hanley and Roberts, 2019). Management of invasive species requires an understanding of propagule pressure (introduction effort), number of different origins (Du *et al.*, 2021), and the pathways and patterns of dispersal (Sakai *et al.*, 2001; Resh *et al.*, 2018). However, unless introductions are deliberate and thorough records are kept, the number and routes of introductions are generally unknown. Molecular techniques, such as microsatellites and Single Nucleotide Polymorphisms (SNPs) can be used to assess the evolution and dispersal of invasive species and design targeted plans of containment or eradication (Le Roux and Wieczorek, 2009; Resh *et al.*, 2021).

Brown trout (*Salmo trutta*) is native to Europe, Western Asia and Northern Africa, however, since 1864 it has been widely introduced outside of their native range and are currently found on all continents except Antarctica (MacCrimmon and Marshall, 1968). Such introductions have resulted in extensive ecological damage making brown trout one of the 100 world's worst invasive species (Lowe *et al.*, 2000). Invasive brown trout have had strong negative impacts on native fishes in New Zealand (McIntosh *et al.*, 1994; McIntosh, 2000; McDowall, 2006; McIntosh *et al.*, 2010), Chile (Penaluna *et al.*, 2009; Habit *et al.*, 2010), North America (McHugh and Budy, 2006; Budy and Gaeta, 2018), and Japan (Kitano, 2004; Morita, 2018), causing severe decreases in native biodiversity and loss of ecosystem function though predation, competition and habitat modification (Macchi *et al.*, 2007; Penaluna *et al.*, 2009).

Brown trout from Great Britain (approximately 83,000) and Chile (approximately 30,000- with a potential bridgehead effect (Bertelsmeier *et al.*, 2018)) were introduced to the Falkland Islands nearly 80 years ago, over an 18-year period between 1944-1962, although much of the information regarding introduction sites and stocks has been lost. Chilean stocks from Lautaro hatchery were primarily sourced from Germany (Faundez *et al.*, 1997; Basulto,

2003), whereas trout from Great Britain originated from three sources: the Surrey trout farm, Pentlands (Scotland), and the Middleton hatchery in Lancashire (Arrowsmith and Pentelow, 1965; Stewart, 1973; Stewart, 1980), and included anadromous trout. The exact sources of the Pentlands stock are unknown but believed to originated from Cobbinshaw Loch or Loch Leven (Minett *et al.*, 2021a).

Since their introduction, brown trout have widely spread throughout East and West Falkland (McDowall *et al.*, 2001; Fowler, 2013; Minett *et al.*, 2021a). Their dispersal has been facilitated by marine dispersal, as in other places (Jonsson, 1985; Nevoux *et al.*, 2019), with anadromous brown trout (sea trout) having been documented in the Falklands since 1956 (Salmon and Trout Association, 2012). Additionally, brown trout have been moved intentionally amongst various locations (McDowall *et al.*, 2001). The native fish community, mainly zebra trout (*Aplochiton zebra* and *A. taeniatus*) and the Falklands minnow (*Galaxias maculatus*), has been severely impacted by brown trout (McDowall *et al.*, 2001), and zebra trout are currently regarded as seriously threatened and protected by law (Falkland Islands Government, 1999; McDowall *et al.*, 2001; Ross, 2009). However, brown trout can be difficult and costly to eradicate once established (Bosch *et al.*, 2019; Healy *et al.*, 2020) and have become an important source of income through angling tourism in the Falklands (Ross, 2009). Therefore, to maintain a balance between trout fishing and the protection of native galaxiids, targeted management plans should be implemented to limit trout dispersal and prevent further invasion. These need geographical information (e.g., concave, and complex coastlines seem to favour brown trout invasion (Labonne *et al.*, 2013)), as well as information on dispersal routes and population connectivity. Here I analysed the movement and genetic status of brown trout populations across the Falkland Islands to provide information that can be used for future management plans aimed at preventing further dispersal of the species.

Here, the main aims were to examine the population structure of brown trout in the Falkland Islands. Analyse levels of migration and gene flow between populations in order to establish their patterns of dispersal and to assess potential origins of current brown trout populations.

4.2 Methods

Sampling

A total of 290 brown trout were non-lethally sampled from 14 rivers and ponds across the Falkland Islands, nine on East Falkland and five on West Falkland (**Figure 4.1**; **Table 4.1**), during two field seasons (April-May 2018 [autumn] and September-October 2018 [spring]). Sampling locations were chosen based on brown trout presence information from previous work conducted by McDowall et al (2001), Ross (2009) and Fowler (2013). Fish were captured using either seine netting, angling or backpack electrofishing (Model: Smith-Root LR-24, 160-280v and 50Hz). Adipose fin clips and scale samples were obtained from all fish greater than 50mm, and weight (g) and fork length (mm) were recorded. Fish were then returned alive to their location of capture. Adipose fin clips were stored in 90% ethanol at -20°C for subsequent genetic analysis.

DNA extraction, SNP calling and filtering

DNA from adipose fins was extracted using Qiagen DNeasy 96 plate tissue kits (Qiagen, UK) following the manufacturer's protocol. Concentration and QC parameters were determined using a NanoDrop 8000 spectrophotometer (ThermoFisher Scientific, UK) and samples normalised to 50ng/µl. SNP array analysis was completed by Neogen Europe (Ayr, UK), using a custom design 24k *Salmo salar* / *Oncorhynchus mykiss* Illumina Infinium beadchip array, under permission of Hendrix Genetics (Hendrix Genetics BV., Boxmeer, Netherlands). Data analysis was completed by Noahgene Ltd (Alloa, UK). Raw data was imported into the software package Genome Studio 2.0.4 (Illumina Inc., San Diego, Ca., USA) for cluster analysis and SNP calling. SNP calls were exported as a Genome Studio FR.txt files and imported into SNP Variation Suite 8.9.0 (Golden Helix Inc., Bozeman, Mt., USA). QC parameters threshold call rate >0.85 and MAF >0.025 were applied and filtered SNP genotypes (N = 14, 195) exported to Microsoft Excel for downstream analysis. Invariant loci (N = 12,233), SNPs with more than 3% missing data overall, and any individuals with more than 10% missing data were removed.

Genetic differentiation, isolation by distance and effective population size

Heterozygosity (Ho), gene diversity (Hs) and F_{IS} were calculated for each sample site using the *basic.stats* function in R 3.5.3 (Goudet, 2005; R Core Team, 2019; Goudet and Jombart, 2020). Deviations from Hardy-Weinberg equilibrium were estimated using the *hw.test* (Guo

and Thompson, 1992) function from the *adegenet* package. Genetic differentiation, between rivers and genetic clusters was calculated using the *hierfstat* 0.5-8 package. Weir and Cockerham pairwise F_{ST} values were calculated using the *pairwise.WCfst* function and 97.5% confidence intervals were obtained by bootstrapping using the *boot.ppfst* function (1,000 permutations). Overall F_{ST} estimates were calculated using the *betas* function (Weir and Cockerham, 1984). Nei's distance between populations (Nei, 1987) was calculated using the *genet.dist* function and used to produce a dendrogram of the population relationships. Effective population size (N_e) was estimated using linkage disequilibrium method implemented in *NeEstimator* v2 (Hill, 1981; Do *et al.*, 2014a).

To examine the extent of isolation by distance (IBD) a Mantel test between genetic distance (pairwise Weir and Cockerham F_{ST} values) and geographic distance matrix was conducted using 999 randomisations in the *ade4* package in R (Mantel, 1967; Thioulouse *et al.*, 2018). Two measures of geographic distance were used: pairwise distance between river mouths around the coast (to reflect marine dispersal), and shortest Euclidean distances between sampling sites (to reflect potential human-mediated translocation of fish). River mouth distances around the coast were calculated using *rgdal* (Bivand *et al.*, 2019), *sp* (Pebesma and Bivand, 2005; Bivand *et al.*, 2013), *raster* (Hijmans, 2020), and *gdistance* (van Etten, 2017) packages using a purpose-built function. Euclidean distances were calculated in QGIS v3.10.14 (QGIS Development Team, 2020). IBD was calculated for all sampling sites across the Falklands, as well as for East and West Falklands separately.

Genetic cluster identification, admixture, and gene flow

Genetic clusters of related individuals were identified through analysis of SNP genotypes using Discriminant Analysis of Principal Components (DAPC) using the *adegenet* 2.1.3 R package (Jombart, 2008; Jombart and Ahmed, 2011). The optimal number of genetically distinct clusters were determined by K-means cluster analysis based on the lowest associated Bayesian Information Criterion (BIC) value, with a maximum K of 14 (the total number of sites sampled in the Falklands), using the *find.clusters* function. To examine the genetic structure and describe diversity between clusters I preformed DAPC using the *dapc* function and the clusters defined by K-means. The number of principal components retained in DAPC was determined based on their alpha-scores using the *optim.a.score* function, resulting in the retention of 5 principle components (Jombart *et al.*, 2010). The level of admixture was assessed through individual assignment to different clusters; assuming that an individual was

admixed if it had less than 90% probability of belonging to a single cluster (Noble *et al.*, 2010).

Admixture between clusters was also examined using the *snapclust* function in the *adegenet* R package. Using the clusters defined by K-means I simulated F1 and F2 backcrosses between pairs of clusters and *snapclust* was run to reassign individuals to one of six possible classes: parental 1, parental 2, F1 hybrid, or F2 backcross with either parental population.

Directional migration rates were calculated as a proxy for gene flow between sample sites using the *divMigrate* function in the *diveRsity* v1.9.90 R package using genetic diversity and differentiation statistics (Keenan *et al.*, 2013; Sundqvist *et al.*, 2016).

Origin of brown trout introduced into the Falkland Islands

The stocking history of brown trout in the Falkland Islands during 1948-1962 was reconstructed and the putative sources from the literature and historical records were identified (Minett *et al.*, 2021a). Fresh or archived tissue samples were also obtained for genetic analysis from three of the putative sources in Great Britain (**Table 4.1**), the River Wey for the Surrey trout farm, N = 12; and Howietoun hatchery (N=6) and Loch Leven (N=14) representing the Pentlands. It was not possible to obtain samples from Cobbinshaw Loch (it was not a natural brown trout population and trout are no longer stocked in the loch) or Germany (the original stock is no longer cultured). DNA extraction and genotyping was carried out as above except for 10 samples obtained from Loch Leven whose DNA had already been extracted. SNP data from putative origins and the Falklands' samples were combined into a single database and analysed as above to examine genetic clustering and differentiation, using a maximum K of 17.

Acoustic tracking

To gain additional insight into brown trout movements and costal dispersal around the Falkland Islands, 25 putative sea trout with a size range between 175mm and 545mm were captured (by angling) and tagged from San Carlos River (see supplementary material **Table S2** for a breakdown of the fish tagged). San Carlos was chosen as it was accessible and surrounded by East and West Falkland rivers with brown trout presence, allowing the movement between the two islands to be detected. All fish were tagged with 9mm ID-2LP9 acoustic transmitters (Thelma Biotel) inserted in the abdominal cavity, following standard surgical tagging procedures (Lacroix *et al.*, 2005). Tags were programmed to transmit every

180s for approximately 2.5 years, had an acoustic range of ~450m and a transmitter failure rate reported by manufacturers <2% (Newton *et al.*, 2016). Ten acoustic receivers (VR2W; Vemco Ltd) were deployed in five rivers (two receivers per river; **Figure 4.1**) configured to record directional movements for a maximum of 23 months, although two receivers were recovered after 11 months. To assess movement within and between islands, acoustic receivers were deployed in three sites on East Falkland and two sites on West Falkland, no range testing was conducted. Detection data was downloaded from all acoustic receivers, exported to Microsoft Excel, and visually examined to determine if any tagged fish had moved between sites, no statistical analysis was conducted.

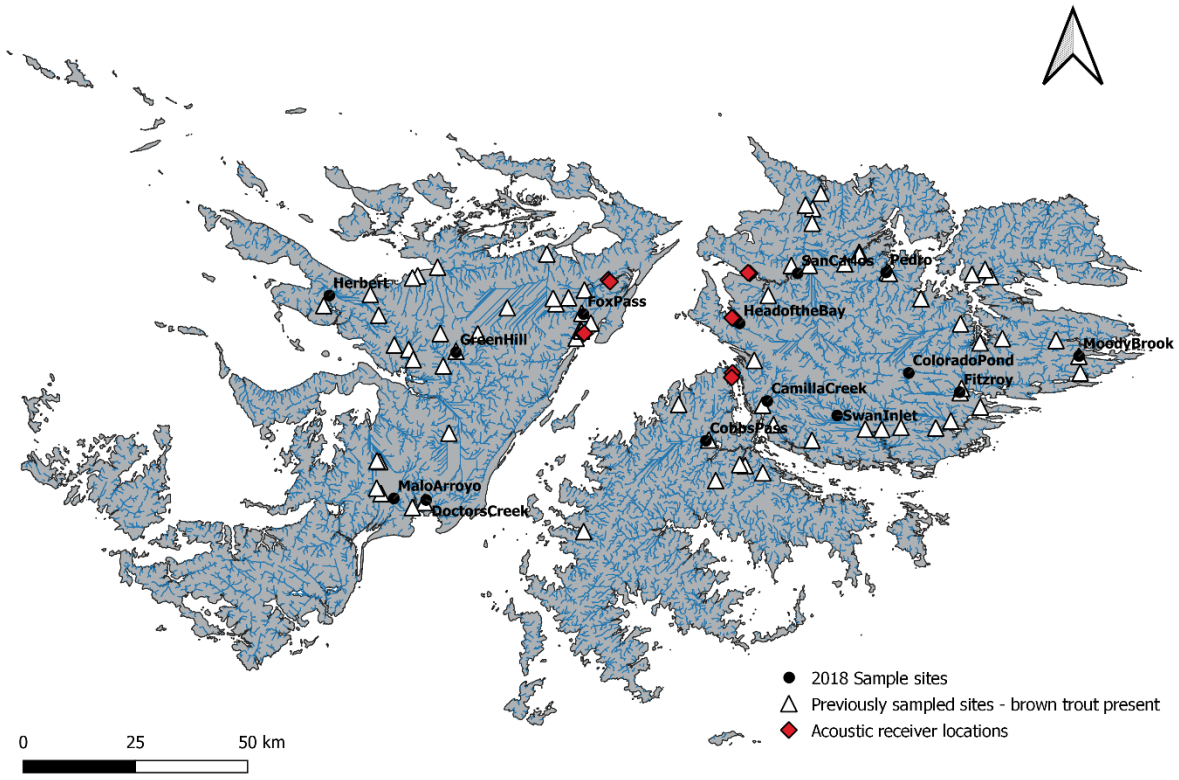

Figure 4.1. Sampling locations for this study in the Falkland Islands (black circles) and sites where brown trout had been previously detected (white triangles) from McDowall et al. (2001), Ross (2009), and Fowler (2013). Sites where acoustic receivers deployed (red diamonds).

Table 4.1. Details of sampling sites in the Falkland Islands and likely origin of the introduced stocks. Number in brackets corresponds to number of samples for Falklands-GB comparisons when number of samples differed from Falklands only analysis. Seine netting (SN), angling (A), and electrofishing (EF). Surrey trout farm (STF), Pentlands (P), German origin from Chile (G), and Middleton Hatchery (MH).

River	No. brown trout	No. samples analysed	Sampling Method	Island	Introduction Stock
Camilla Creek *	25	12	EF	East Falkland	STF/P
Cobbs Pass	22	22	SN	East Falkland	NA
Colorado Pond	25	25	SN & A	East Falkland	NA
Doctors Creek *	24	24	EF	West Falkland	STF/P
Fitzroy *	16	16	EF	East Falkland	STF/P
Fox Pass	17	16	EF	West Falkland	NA
Green Hill *	23	21 (23)	EF	West Falkland	STF/P/MH
Head of the Bay *	25	25	EF	East Falkland	STF/P
Herbert	14	13	EF	West Falkland	NA
Malo Arroyo	9	9	EF	West Falkland	NA
Moody Brook *	11	10	EF	East Falkland	G
Pedro	25	25	EF	East Falkland	NA
San Carlos *	29	14	A	East Falkland	STF/P/MH
Swan Inlet *	25	25	EF & A	East Falkland	STF/P
River Lune	2	(0)		UK	MH
River Wey	12	(12)		UK	STF
Loch Leven	14	(12)		UK	P
Howietoun	6	(6)		UK	P

4.3 Results

Genomic data

Two hundred and sixty-five fish were successfully genotyped from 14 sites (**Table 4.1**) and 32 fish from three putative origins. After removing invariant SNPs and samples with missing data, 477 SNPs were available for the genetic analysis of 257 trout in the Falkland Islands and 592 SNPs for comparisons with three of the putative origins (N = 289 trout). the relatively low number of variable SNPs is likely the result of using an array designed for other salmonid species, possibly combined with the history of the introduced populations, that originated from limited number of stocks of hatchery/farm origin, as reflected in the current population structuring. Low intra-population genetic diversity and high structuring had also been observed in the Falklands brown trout analysed using microsatellites (Monzón-Argüello *et al.*, 2014a). Eight SNPs (Ax-87899852, AX-87986668, AX-880117788, AX-88166365, omy19_28513692, omy22_31997564, omy22_39402264, omy_28375016) deviated significantly from Hardy-Weinberg equilibrium in four or more sites in the Falkland Islands but were retained as their exclusion did not change the genetic clustering of individuals (**Appendix Figure S1a**). For Falklands/GB comparisons, nine SNPs (the same as above in addition to AX-88095436) deviated significantly from Hardy-Weinberg equilibrium but were also retained as their exclusion did not affect clustering (**Appendix Figure S1b**).

Genetic differentiation, isolation by distance and effective population size

The inbreeding coefficient (F_{IS}) was negative for all sampling sites, indicating a small excess of heterozygotes (**Table 4.2** and **Table 4.3**). Overall F_{ST} for all Falklands sites was 0.090. The smallest pairwise genetic distance (F_{ST} = 0.011) was observed between Green Hill and Herbert, in contrast the largest pairwise genetic distance (F_{ST} = 0.215) observed between Cobbs Pass and Colorado Pond (**Table 4.3** and **Figure 4.2**).

Estimates of N_e based on linkage disequilibrium, ranged from 11.5 (95% CI = 3-965) at Moody Brook to 489 (95% CI = 167-infinity) at Doctors Creek (**Table 4.2**).

No significant isolation by distance was found, using either geographic distance around the coast (r = 0.085; p = 0.272) or Euclidean distance (r = -0.042; p = 0.593), for the Falklands together or for West Falkland (coastal distance r = -0.084; p = 0.601; Euclidean distance r = 0.144; p = 0.343). However, a significant correlation was observed for rivers in East Falkland

between genetic and coastal distance (r = 0.391; p = 0.004) but not for Euclidean distance (r = 0.282; p = 0.116), suggesting a role for marine dispersal.

Table 4.2. Estimates of genetic diversity (observed heterozygosity, Ho; observed gene diversity, Hs; F_{IS}, overall F_{ST}) and effective population size (N_e) calculated according to linkage disequilibrium.

Sample Site	Ho	Hs	F_{Is}	F_{ST}	Sample size	N_e
Camilla Creek	0.110	0.105	-0.051	0.109	12	inf (inf-inf)
Cobbs Pass	0.106	0.095	-0.126	0.254	22	20.0 (10.3-55.9)
Colorado Pond	0.095	0.088	-0.080	0.195	25	45.6 (27.4-105.3)
Doctors Creek	0.125	0.116	-0.078	0.009	24	489.0 (167.4-inf)
Fitzroy	0.115	0.104	-0.106	0.116	16	221.5 (47.2-inf)
Fox pass	0.121	0.109	-0.116	0.073	16	41.1 (10.6-inf)
Green Hill	0.116	0.110	-0.055	0.064	21	149.0 (52.8-inf)
Head of the Bay	0.115	0.105	-0.098	0.108	25	70.6 (36.5-357.7)
Herbert	0.114	0.114	-0.002	0.029	13	inf (112.6-inf)
Malo Arroyo	0.109	0.104	-0.046	0.114	9	inf (265.9-inf)
Moody Brook	0.108	0.104	-0.035	0.116	10	11.5 (3.0-965.3)
Pedro	0.120	0.114	-0.050	0.028	25	130.7 (69.4-698.0)
San Carlos	0.129	0.121	-0.061	-0.032	14	16.3 (7.1-80.5)
Swan Inlet	0.112	0.109	-0.033	0.077	25	111.9 (60.3-521.4)

Table 4.3. Pairwise F_{ST} values for Falkland Islands sample sites, calculated according to Weir and Cockerham.

	Camilla Creek	Cobbs Pass	Colorado Pond	Doctors Creek	Fitzroy	Fox Pass	Green Hill	Head of the Bay	Herbert	Malo Arroyo	Moody Brook	Pedro	San Carlos
Cobbs Pass	0.133												
Colorado Pond	0.172	0.215											
Doctors Creek	0.083	0.100	0.121										
Fitzroy	0.146	0.181	0.027	0.083									
Fox pass	0.074	0.143	0.170	0.065	0.127								
Green Hill	0.069	0.103	0.126	0.025	0.095	0.072							
Head of the Bay	0.101	0.136	0.172	0.075	0.137	0.055	0.083						
Herbert	0.049	0.105	0.115	0.020	0.080	0.066	0.011	0.069					
Malo Arroyo	0.122	0.168	0.177	0.089	0.146	0.130	0.088	0.149	0.093				
Moody Brook	0.093	0.169	0.153	0.071	0.106	0.091	0.064	0.096	0.062	0.109			
Pedro	0.067	0.109	0.132	0.050	0.092	0.078	0.031	0.088	0.027	0.086	0.044		
San Carlos	0.063	0.110	0.158	0.052	0.122	0.024	0.045	0.023	0.042	0.103	0.073	0.054	
Swan Inlet	0.068	0.061	0.141	0.037	0.111	0.074	0.036	0.081	0.039	0.096	0.077	0.052	0.049

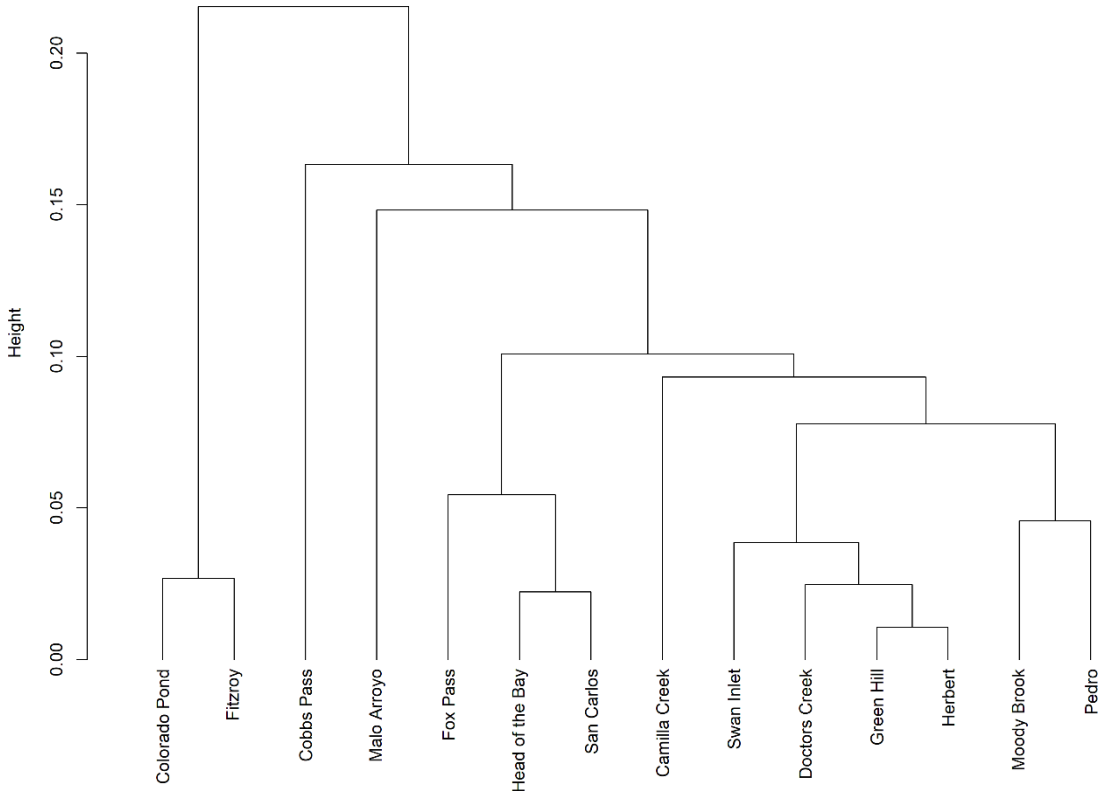

Figure 4.2. Cluster dendrogram of Falkland Islands sites, based on Nei's distance.

Genetic cluster identification, admixture, and gene flow

Results of the DAPC analysis support four genetically distinct genetic clusters of brown trout in the Falkland Islands (K = 4 BIC = 1983.383; **Figure 4.3** and **Figure 4.4a**). Cobbs Pass largely formed its own cluster, cluster 1, which contained 25 individuals including a few from Swan Inlet. One cluster (cluster 2) contained 41 individuals from Colorado Pond and Fitzroy and was well differentiated from the rest of the sampling sites (**Table 4.4**). Another cluster (cluster 3) consisted of 61 individuals primarily from Fox Pass, Head of the Bay and San Carlos. The remaining fish formed cluster 4, which contained 130 individuals from all sampling sites except Colorado Pond, Fox Pass and Head of the Bay. All clusters were clearly distinct (**Figure 4.5a**). The lowest pairwise distance was observed between cluster 1 and cluster 4 (F_{ST} of 0.081), and the largest between cluster 1 and cluster 2 (F_{ST} of 0.202, **Table 4.4**).

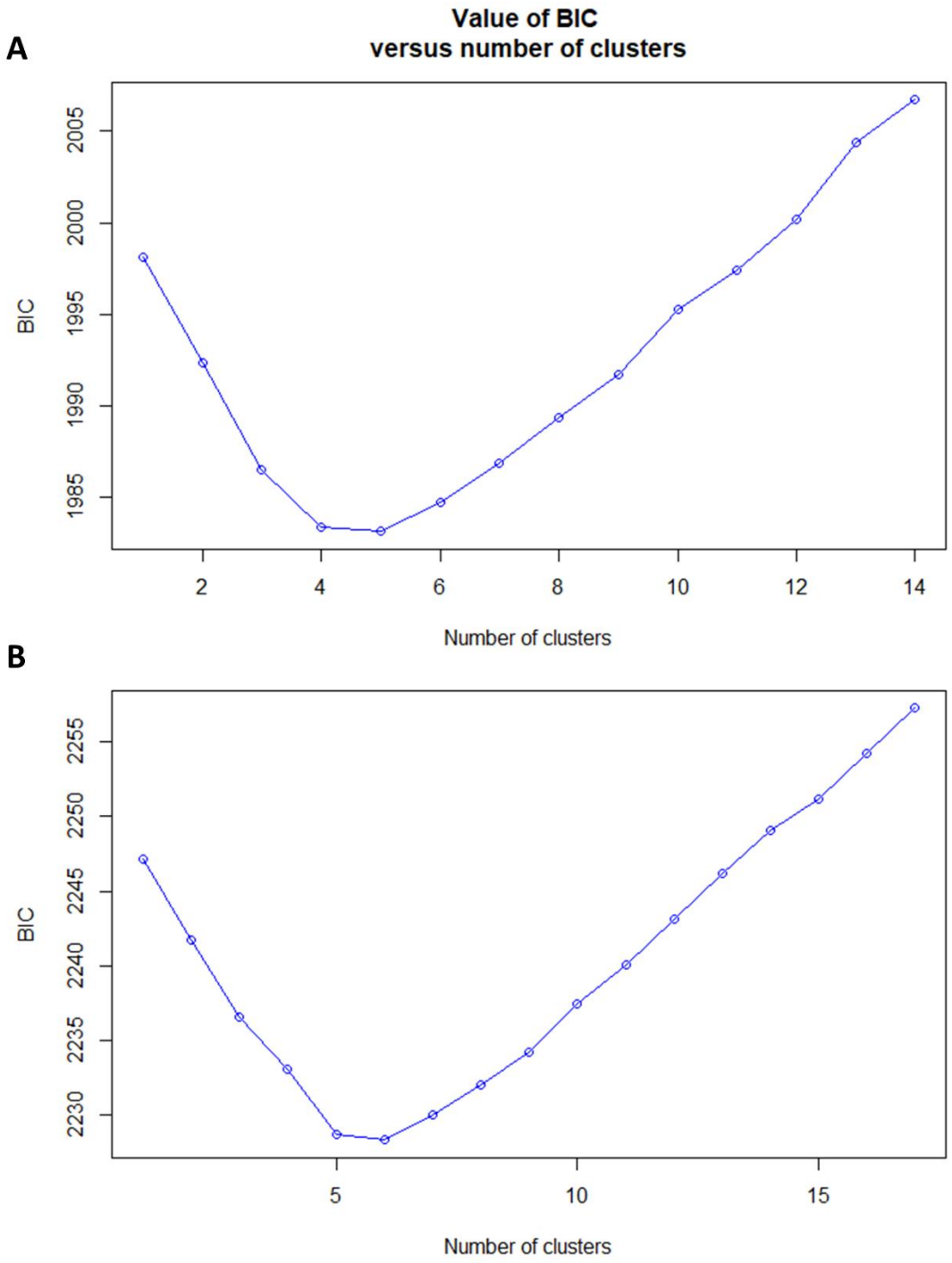

Figure 4.3. Bayesian Information Criterion (BIC) values for the different number of clusters in (A) Falkland Islands only data (optimal K = 4) and (B) Falkland Islands GB comparisons (optimal K = 5).

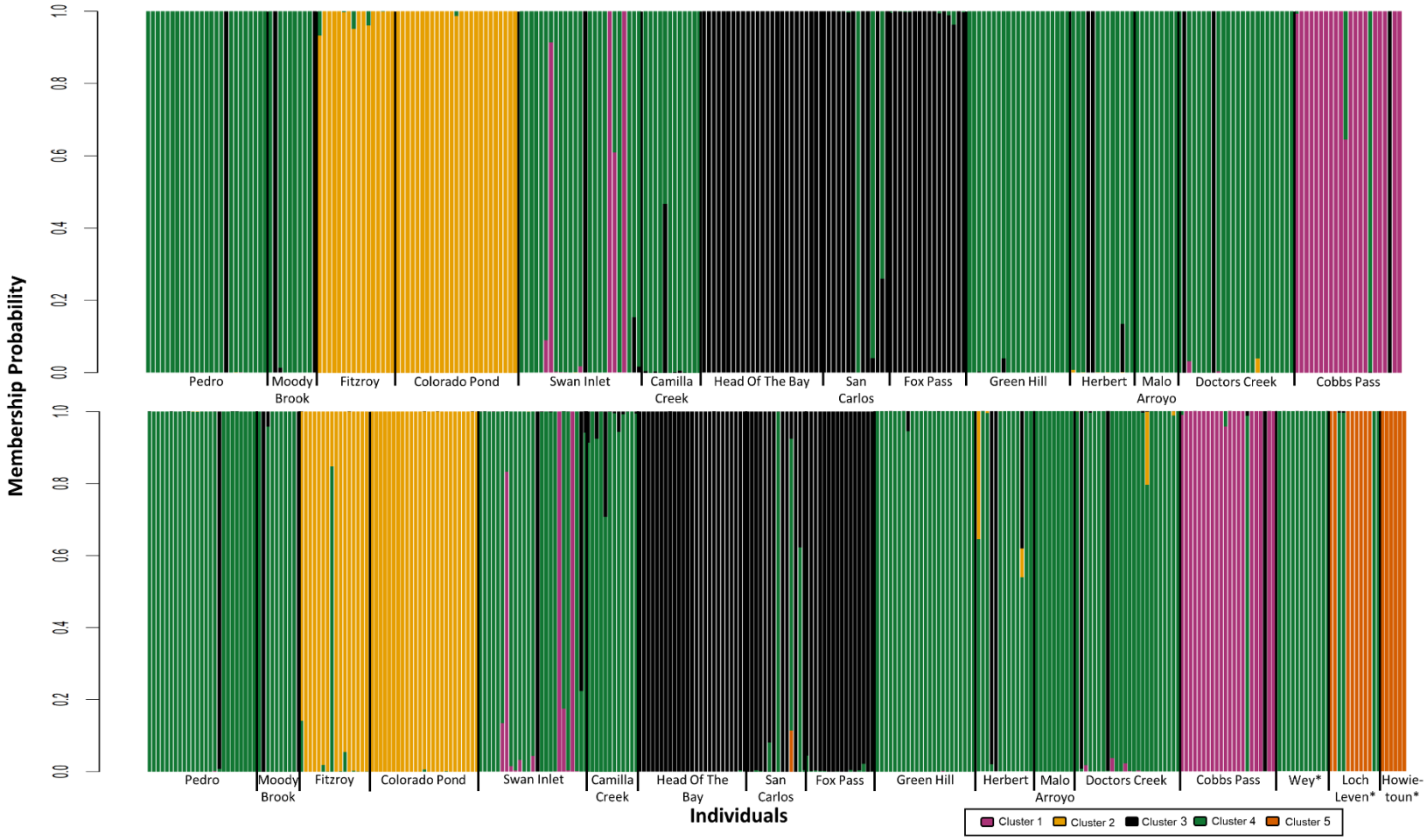

Figure 4.4. Discriminant Analysis of Principle Components (DAPC) analysis of population structure for (A) Falkland Islands brown trout based on 477 SNPs and K = 4 and (B) Falkland Islands and GB brown trout based on 592 SNPs and K = 5. Each bar corresponds to an individual and colours represent genetic clusters. (*) Indicate GB sites.

Only six individuals from five sites displayed evidence of admixture between the distinct genetic backgrounds (clusters), mostly between clusters 3 and 4 and between clusters 1 and 4, with the greatest number of admixed individuals being from Swan Inlet (**Figure 4.4**). Admixed individuals included 9% F1 hybrids and 0.6% - 28% backcrosses (**Figure 4.6**).

Estimates of migration rates were consistent with the DAPC analysis (**Table 4.5** and **Figure 4.7**). The greatest inferred gene flow was between Head of the Bay and San Carlos (Nm = 1.00), while Malo Arroyo was the only clearly isolated site (Nm ≤0.26).

Table 4.4. Pairwise F_{ST} values for cluster (K = 4) of Falkland Islands samples, calculated according to Weir and Cockerham.

	Cluster 1	**Cluster 2**	**Cluster 3**
Cluster 2	0.202		
Cluster 3	0.122	0.136	
Cluster 4	0.081	0.088	0.046

Table 4.5. Relative migration rates for Falkland Islands sample sites, migration rates calculated using Nm.

	Camilla Creek	Cobbs Pass	Colorado Pond	Doctors Creek	Fitzroy	Fox Pass	Green Hill	Head of the Bay	Herbert	Malo Arroyo	Moody Brook	Pedro	San Carlos
Cobbs Pass		0.19	0.12	0.29	0.14	0.27	0.28	0.24	0.38	0.16	0.18	0.34	0.32
Colorado Pond	0.22		0.12	0.30	0.14	0.17	0.25	0.20	0.25	0.13	0.14	0.29	0.23
Doctors Creek	0.16	0.13		0.25	0.81	0.15	0.27	0.17	0.27	0.14	0.16	0.24	0.17
Fitzroy	0.29	0.26	0.22		0.26	0.36	0.71	0.38	0.71	0.23	0.29	0.50	0.44
Fox pass	0.18	0.15	0.74	0.32		0.19	0.32	0.20	0.32	0.15	0.22	0.30	0.20
Green Hill	0.32	0.21	0.15	0.40	0.17		0.35	0.46	0.35	0.16	0.25	0.37	0.78
Head of the Bay	0.35	0.27	0.21	0.89	0.25	0.33		0.33	0.89	0.25	0.30	0.67	0.49
Herbert	0.28	0.22	0.16	0.40	0.15	0.56	0.36		0.37	0.14	0.28	0.36	1.00
Malo Arroyo	0.43	0.28	0.23	0.84	0.26	0.36	0.95	0.40		0.22	0.32	0.64	0.50
Moody Brook	0.16	0.14	0.13	0.24	0.14	0.16	0.24	0.16	0.21		0.17	0.26	0.20
Pedro	0.22	0.15	0.17	0.25	0.19	0.22	0.31	0.23	0.28	0.17		0.44	0.26
San Carlos	0.36	0.25	0.20	0.54	0.26	0.31	0.68	0.32	0.62	0.24	0.36		0.43
Swan Inlet	0.25	0.23	0.15	0.35	0.17	0.48	0.34	0.49	0.35	0.16	0.25	0.37	

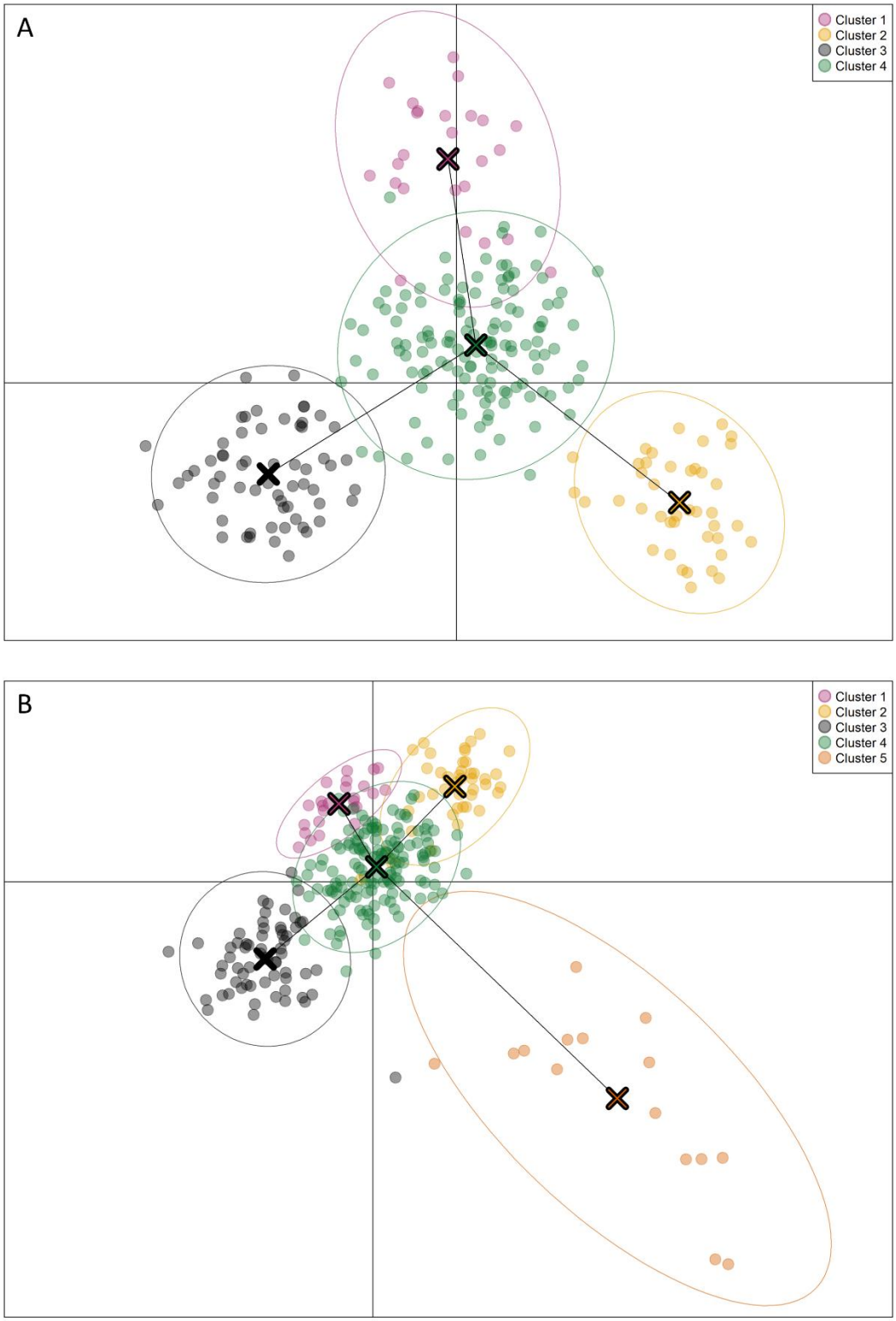

Figure 4.5. Discriminant Analysis of Principle Components (DAPC) for (A) Falkland Islands brown trout based on 477 SNPs and K = 4 and (B) Falkland Islands and GB brown trout based on 592 SNPs and K = 5. Dots represent individuals and colours represent genetic clusters.

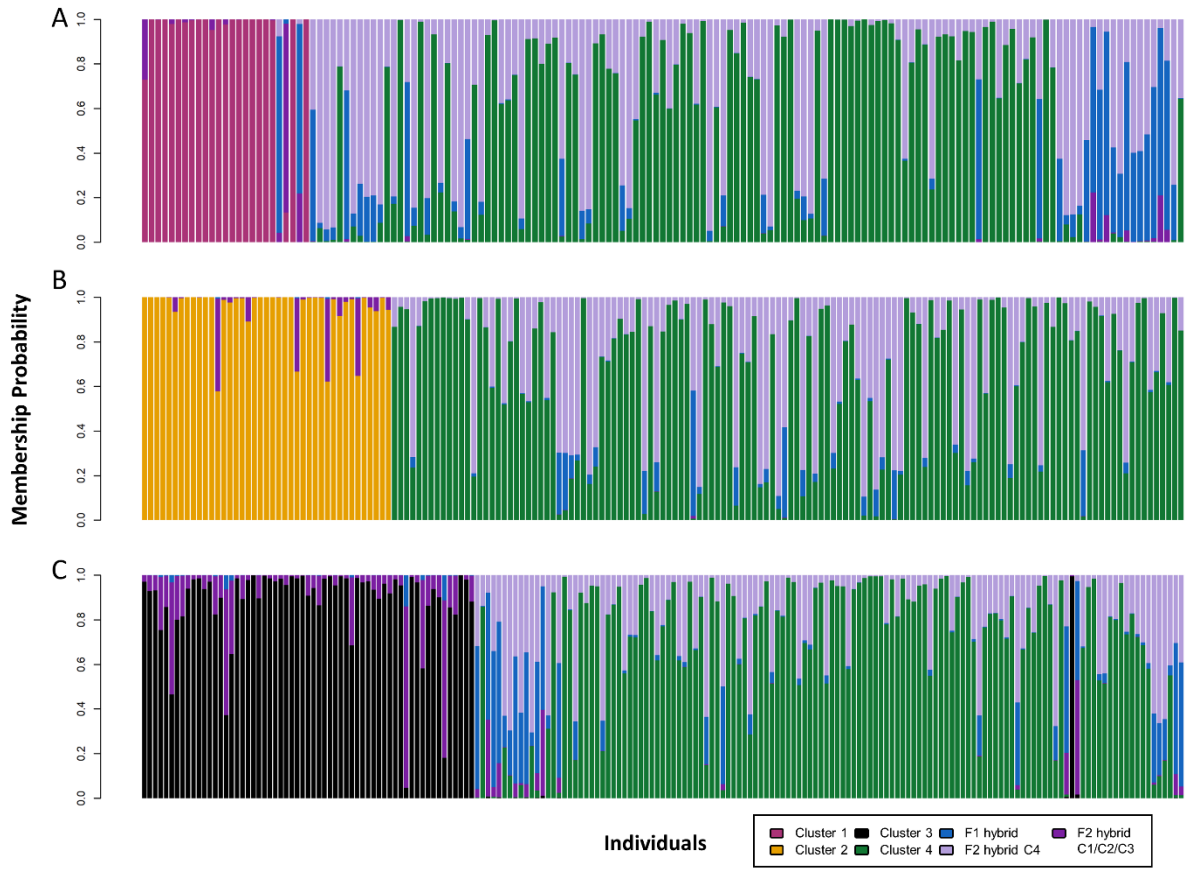

Figure 4.6. Population structure of Falkland Islands brown trout when F1 hybrids and F2 backcrosses are accounted for. Hybridisation analysed between (A) cluster 1 and 4, (B) cluster 2 and 4, and (C) cluster 3 and 4. Bars represent individuals and colours represent cluster and hybrid group.

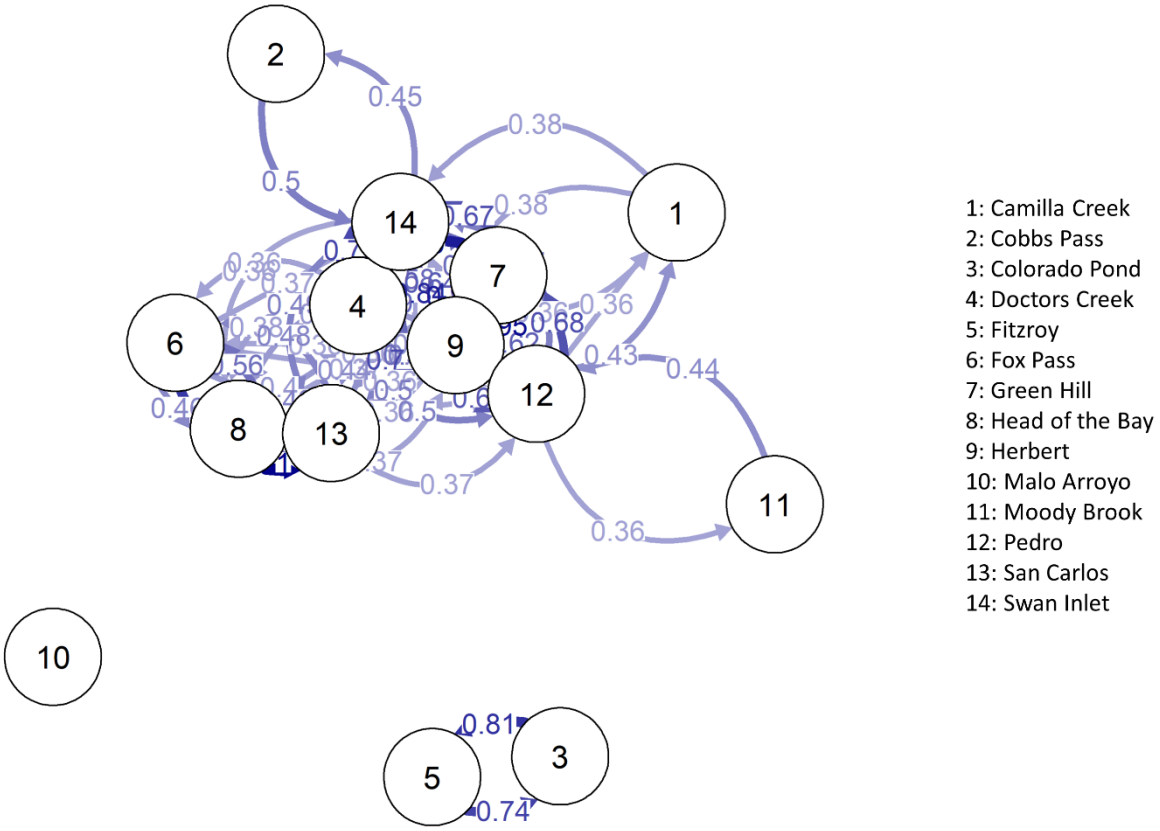

Figure 4.7. Relative migration network among Falkland Islands sampled rivers/lakes, migration rates calculated using Nm, threshold set to 0.35.

Genetic assignment to putative populations of origin

The results of the DAPC analysis of samples from both the Falkland Islands and Great Britain indicated the existence of five genetically distinct clusters (**Figure 4.4b** and **Figure 4.5b**), the 4 clusters identified in Falklands-only analysis and one additional cluster (5), which consisted of individuals from Loch Leven and the Howietoun hatchery (**Figure 4.4**). Trout from the River Wey, representative of the Surrey trout farm, were included in cluster 4, suggesting that they could be the origin of the trout populations in Camilla Creek, Doctors Creek, Green Hill, Herbert, Malo Arroyo, Moody Brook, Pedro, and Swan Inlet. Despite Loch Leven samples forming part of cluster 5, four of the 12 individuals belonged to cluster 4, suggesting Loch Leven as another possible source of these populations. The smallest genetic distance was between cluster 2 and 4 (F_{ST} = 0.047) and the greatest between cluster 1 (Falklands) and 5 (Great Britain) (F_{ST} = 0.205, **Table 4.6**).

Table 4.6. Pairwise F_{ST} values for cluster (K = 5) of Falkland Islands-GB comparisons, calculated according to Weir and Cockerham.

	Cluster 1	**Cluster 2**	**Cluster 3**	**Cluster 4**
Cluster 2	0.117			
Cluster 3	0.193	0.131		
Cluster 4	0.079	0.047	0.082	
Cluster 5	0.205	0.167	0.185	0.129

Acoustic tracking

Movements of 12 fish (mean length 365mm) in San Carlos were detected (**Table 4.7**), three of which also moved to Head of the Bay (separated by 26km around the coast), confirming the migration between different rivers through marine dispersal. The remaining 13 fish (mean length 367mm) were not detected on any of the acoustic receivers. Of the three fish that were detected in both San Carlos and Head of the Bay, two fish moved from San Carlos to Head of the Bay and back. A third fish moved between the two sites twice and was detected around Head of the Bay initially for 22 days before being detected in San Carlos 26 days later.

Table 4.7. Breakdown of acoustic receiver detection data in the Falklands Islands at three sites.

Receiver	River	Date range	Transmitter ID	No. of detections	Total No. of detections	Latitude	Longitude
VR2W-132982	San Carlos	18/02/19 – 12/11/20	A69-1303-4439	4	2567	-51.5069	-58.9757
		20/01/19 – 16/11/19	A69-1303-4441	209			
		20/02/19 – 25/02/19	A69-1303-4448	635			
		28/11/19 – 02/01/20	A69-1303-4450	8			
		29/12/19 – 03/01/20	A69-1303-4453	115			
		24/02/19 – 18/10/20	A69-1303-4454	3			
		20/01/19 – 17/02/19	A69-1303-4457	97			
		20/01/19 – 16/02/19	A69-1303-4458	1488			
		08/03/19 – 12/12/19	A69-1303-4459	6			
		23/01/20	A69-1303-4460	1			
		12/11/19	A69-1303-4461	1			
VR2W-132983	San Carlos	18/02/19	A69-1303-4439	1	986	-51.5057	-58.9811
		20/01/19 – 17/11/19	A69-1303-4441	51			
		21/01/19 – 22/02/19	A69-1303-4448	14			
		28/11/19 – 03/01/20	A69-1303-4450	6			
		29/12/19 – 03/01/20	A69-1303-4453	39			
		27/01/19 – 01/02/19	A69-1303-4457	29			
		20/01/19 – 15/02/19	A69-1303-4458	836			
		01/02/19 – 12/12/19	A69-1303-4459	4			
		05/11/19 – 23/01/20	A69-1303-4460	4			
		12/11/19	A69-1303-4461	1			
		12/12/19	A69-1303-4466	1			
VR2W-132983	Head of the Bay	10/11/19	A69-1303-4439	2	28	-51.5947	-59.0370
		09/11/19	A69-1303-4454	1			
		04/12/19 – 09/11/20	A69-1303-4460	25			

4.4 Discussion

This analysis revealed the presence of four genetically distinct clusters of invasive brown trout in the Falkland Islands, likely reflecting their different origins, although the effects of founder effects on the structuring cannot be discarded. The four clusters had high levels of genetic diversity and low levels of admixture, although high levels of gene flow were detected between rivers within each cluster. Relatively variable effective population sizes (N_e ranging from 12 to 489) were also observed, with high 95% confidence intervals in several cases, potentially due to low sample sizes (Do *et al.*, 2014b). These estimates were greater than those estimated by a previous study 10 years ago, for which N_e ranged between 16-46 (Monzón-Argüello *et al.*, 2014a). The difference in N_e could reflect the expansion of brown trout in the Falklands, potentially aided by marine dispersal as evidenced from the estimates of the number of migrants, supported by acoustic tagging. However, this comparison must be treated with caution as there were differences in the molecular markers and methods used in both studies (microsatellites and maximum likelihood in 2014, SNPs and linkage disequilibrium method here), as well as in the target populations. Yet, both the current and previous analysis identified strong population structuring, lack of isolation by distance as well as the presence of anadromous trout (Monzón-Argüello *et al.*, 2014a).

Putative F1 and backcrossed individuals were identified between two genetic clusters (cluster 2 and 4) that could have resulted from marine dispersal, but also from admixture with farmed escapees. One of the rivers in cluster 2 (Fitzroy) is close to the location where sea trout are being farmed in open net sea cages since 2013, initially with locally captured brood stock from Camilla Creek (cluster 4), and then with ova imported from Howietoun Hatchery UK in 2014 and 2015. Although I found no direct evidence of mixing between Fitzroy and Howietoun fish, escapes from sea pens are not uncommon in salmonid farming and are the main route of introduction of invasive salmonids in the Southern Hemisphere (Arismendi *et al.*, 2009; Consuegra *et al.*, 2011; Monzón-Argüello *et al.*, 2014a). Thus, the presence of farmed fish in close proximity to naturalised populations could have resulted in admixture, as for rainbow trout in Chile (Consuegra *et al.*, 2011; Monzón-Argüello *et al.*, 2013), potentially increasing dispersal. Alterations in dispersal patterns can be expected by genetic admixture between wild (naturalised is this case) and captive-bred trout and could apply to this case (Saint-Pé *et al.*, 2018), considering the anadromous nature of the farmed stock.

The highest level of genetic diversity was observed in trout from the rivers San Carlos and Green Hill. These are the sites with the greatest number of documented introductions and the most diverse origins, including three sources from Great Britain (Arrowsmith and Pentelow, 1965; Stewart, 1973; Fowler, 2013). In comparison, lower genetic diversity was observed in Cobbs Pass, where no fish were stocked and where the population likely represents the result of secondary invasions and natural dispersal, as suggested by earlier studies in the Falkland Islands (Monzón-Argüello *et al.*, 2014b; Monzón-Argüello *et al.*, 2014a) and the Kerguelen Islands, where current genetic diversity largely reflects the pattern of historical introductions (Launey *et al.*, 2010).

The analysis of some of the donor populations from Great Britain suggests that many trout in the Falklands must have originated from the River Wey (representative of the Surrey trout farm) as reported in the early literature (Arrowsmith and Pentelow, 1965; Stewart, 1973) and, to a lesser extent possibly from Loch Leven. Although I did not have samples from two other potential sources (Middleton hatchery or Cobbinshaw Loch), the results indicate that trout in the Falklands likely originate from four distinct sources, that can be used to trace new colonisation events. Migration between sampling sites could have been the result of human mediated translocations, as reported by McDowall et al (2001). However, strong sea runs of sea trout have been observed since 1956 (Salmon and Trout Association, 2012) indicating the ability of brown trout to colonize new rivers through marine dispersal (Launey *et al.*, 2010; Westley and Fleming, 2011), a fact also supported by these results from acoustically tagged fish, which were detected moving between two rivers ~26km apart (San Carlos and Head of the Bay). A recent study using environmental DNA (eDNA) has detected brown trout in sites where the species had not previously been recorded, suggesting it is continuing to spread across the Falklands (Minett *et al.*, 2020). Without containment measures in place, there is a risk that brown trout may invade the last remaining refuges for the native endangered *Aplochiton* spp.

Further examination of the movements from acoustically tagged fish in the Falklands is required and ongoing (acoustic receivers have been redeployed and additional fish tagged). Only three for the 25 individuals tagged were detected, this could be due to a variety of reasons, all the fish were tagged in the river and classified as putative sea trout based on their coloration, however, this is not a reliable method to determine if a fish is going to migrate to sea (Youngson *et al.*, 1997; Birnie-Gauvin *et al.*, 2019). In addition, it is possible that some of these fish could have been returning to the river and may have not re-entered the sea after

tagging (Birnie-Gauvin *et al.*, 2019). Due to time and weather constraints, there was also a delay between tagging the fish (September-October) and deploying the receivers (December-February), therefore, some individuals may have travelled downstream and into the marine environment before receivers were deployed and, hence, were not detected.

The popularity of brown trout as a sport fish is common to other countries where it is also highly invasive, such as US or New Zealand (Jones and Closs, 2018), however, awareness of the negative impacts on the native ecosystems is increasing and plans for containment or eradication are starting to be implemented (Saunders *et al.*, 2015; Budy and Gaeta, 2018). Mechanical removal of trout tends to increase the density of small fish (Meyer *et al.*, 2006; Saunders *et al.*, 2015) and is not appropriate management strategy, although they seem to be contained by beaver dams and natural competition at high densities of native fish (Budy and Gaeta, 2018). A combination of molecular markers, eDNA and acoustic tagging, as used here, can be employed to monitor the expansion of brown trout, and put measures in place to limit its dispersal, for example through the installation of selective barriers (Jones *et al.*, 2021b).

Chapter 5 Stable isotope analysis reveals multiple life history strategies and the extent of migratory brown trout in the Falkland Islands

This work is in preparation for publication.

5.1 Introduction

Brown trout possess flexible life history strategies owing to their facultative anadromous nature thus allowing individuals to choose to either complete their whole life cycle in freshwater (resident) or migrate to the marine environment (anadromous), termed partial migration (Ferguson *et al.*, 2019; Marco-Rius et al., 2013). This enables individuals to benefit from better food and reach larger size and fecundity (Ferguson *et al.*, 2016). This plasticity contributes to their resilience to environmental variability and facilitates their adaptability to new environments (Guiry *et al.*, 2020), enabling brown trout to rapidly succeed in colonizing areas where they have been introduced outside of their native range. Hence, brown trout are now found on all continents except Antarctica (MacCrimmon and Marshall, 1968). Introductions of brown trout have caused extensive ecological damage, particularly in the Southern Hemisphere where they have exerted strong negative impacts on native fish and freshwater communities. In New Zealand, brown trout invasions have caused widespread reductions in the abundance and distribution of native galaxiids, mainly due to size-specific predation (McIntosh *et al.*, 1994; McIntosh *et al.*, 2010). In addition, strong predation pressure on invertebrates has caused shifts in community structure resulting in ecosystems dominated by algae (McIntosh and Townsend, 1996; Simon and Townsend, 2003; Townsend, 2003). Thus, due to their impacts on native communities' brown trout have been classified as one of the 100 world's worst invasive species (Lowe *et al.*, 2000).

Brown trout were introduced to 29 watersheds in the Falkland Islands over an 18-year period between 1944-1962 (Arrowsmith and Pentelow, 1965; Stewart, 1973; Fowler, 2013; Minett *et al.*, 2021a). Despite the reduced opportunity for growth in freshwaters in the Falklands due to poor abundance and diversity of invertebrates and low biological productivity brown trout are found in both resident and migratory ecotypes (Stewart, 1973; McDowall *et al.*, 2001; Flower, 2005; Fowler, 2013). Trout have spread throughout East and West Falklands (McDowall *et al.*, 2001; Fowler, 2013; Minett *et al.*, 2021a), with their dispersal aided by marine migration, which has been documented in the Falklands since 1956, even though anadromous stock were only introduced in 1961 (Stewart, 1973; Salmon and Trout Association, 2012; Minett *et al.*, 2021a). Severe impacts have been observed on the native fish community, composed of two *Aplochiton* spp. (*Aplochiton zebra* and *Aplochiton taeniatus*) and the Falklands minnow (*Galaxias maculatus*), following the introduction of brown trout (McDowall *et al.*, 2001; Vanhaecke *et al.*, 2012b). *Aplochiton* spp. were previously found in great abundance all over the islands, but they are now limited to a few

refugia mainly in the south of the East and West Falkland and are now classified as seriously threatened and protected by law (Falkland Islands Government, 1999; McDowall *et al.*, 2001; Ross, 2009). In order to protect these native species, it is imperative to manage brown trout populations in the Falklands, limit any future spread and prevent further invasions, particularly in areas that could be refuges for native *Aplochiton* spp.

To effectively manage populations, it is important to understand their life history strategies. Salmonids have previously been tracked using a variety of methods including genetics (Chapter 4), tagging and scale reading (Jonsson and Jonsson, 2002; Cunjak *et al.*, 2005; Ferguson *et al.*, 2016; Wollebaek *et al.*, 2018; Ferguson *et al.*, 2019). Although resident and migratory ecotypes can typically be distinguished based on colour, size and body form (i.e., resident fish are usually smaller and brownish whilst migratory fish tend to be silvery in colour and larger in size) identification can be problematic due to considerable overlap in phenotypes, especially as a result of unsmoltified migrants and desmoltification (Eek and Bohlin, 1997; Youngson *et al.*, 1997; Birnie-Gauvin *et al.*, 2019). However, differences between marine and freshwater environments can be detected through analysis of naturally occurring carbon and nitrogen stable isotopes (McCarthy and Waldron, 2000; Charles *et al.*, 2004). Stable isotope analysis (SIA) is normally conducted using muscle tissue, otoliths, or scales, with the tissue chosen depending on the timeframe being analysed and sampling strategy. Whereas muscle tissue reflects shorter term changes, typically weeks to months, depending on the growth and turnover of the tissue, longer-term information can be obtained from otoliths and scales (Perga and Gerdeaux, 2005; Ramsay *et al.*, 2011). However, obtaining muscle tissue and otoliths requires lethal sampling (Graham *et al.*, 2013). Sampling a small fin clip of the adipose fin presents a non-lethal alternative to muscle tissue and has previously been shown to be provide comparable $\delta^{13}C$ and $\delta^{15}N$ values at similar turnover rates (McCarthy and Waldron, 2000; Suzuki *et al.*, 2005; Hanisch *et al.*, 2010; Graham *et al.*, 2013).

Here, the aims were to distinguish between resident and anadromous brown trout in the Falklands using carbon and nitrogen stable isotopes values obtained non-lethally from adipose fins clips, and to use this information to assess the incidence of different migratory ecotypes across the Falkland Islands.

5.2 Methods

Sampling

A total of 156 brown trout were sampled across 14 rivers and ponds in the Falkland Islands, nine on East Falkland and five on West Falkland (**Table 5.1**; **Figure 5.1**). Sampling was conducted throughout two field seasons, April-May 2018 [Autumn] and September-October 2018 [Spring] and coincided with the start and the end of the fishing season (Falkland Islands Government, 1999). Fish were captured using seine netting, angling, or backpack electrofishing (Smith-Root LR-24, 160-280v and 50Hz). Fish were weighted (g) and the fork length (mm) recorded, adipose fin clips and scales were obtained from all fish >50mm before releasing them alive at point of capture. Fin clips were stored in 90% ethanol and at -20°C for subsequent analysis. In addition, the level of silvery (0-3) was recorded as a proxy for resident/anadromous trout, where 0 = brown/yellowish colour typical of resident brown trout with no silvering and 3 = almost completely silvery (Dannewitz and Petersson, 2001; Thomson and Lyndon, 2018). Invertebrate samples were obtained for assessment of prey consumption (N = 11 sampling sites including Mary Hill Quarry where no trout were included sampled, **Table 5.1**) and prey items identified to class level (family level where possible) for processing and analysis.

Table 5.1. Summary of samples collected from each site and sample size (N). Electrofishing (EF); seine netting (SN); angling (A). * Sites where invertebrate samples were also collected, $^{+}$sites where Falklands minnows (*Galaxias maculatus*) were also found.

Sample site	Date of collection	N	Sampling method
Camilla Creek*	September	12	EF
Cobbs Pass	April/May	11	SN
Colorado Pond*	April	25	SN & A
Doctors Creek*	October	17^{+}	EF
Fitzroy*	May	2^{+}	EF
Fox Pass	October	2	EF
Green Hill*	October	7	EF
Head of the Bay*	September	17	EF
Herbert*	October	6	EF
Malo Arroyo*	October	6^{+}	EF
Moody Brook*	October	6	EF
Pedro	May	4	EF
San Carlos*	September/October	20	A
Swan Inlet	May/September	20	EF & A

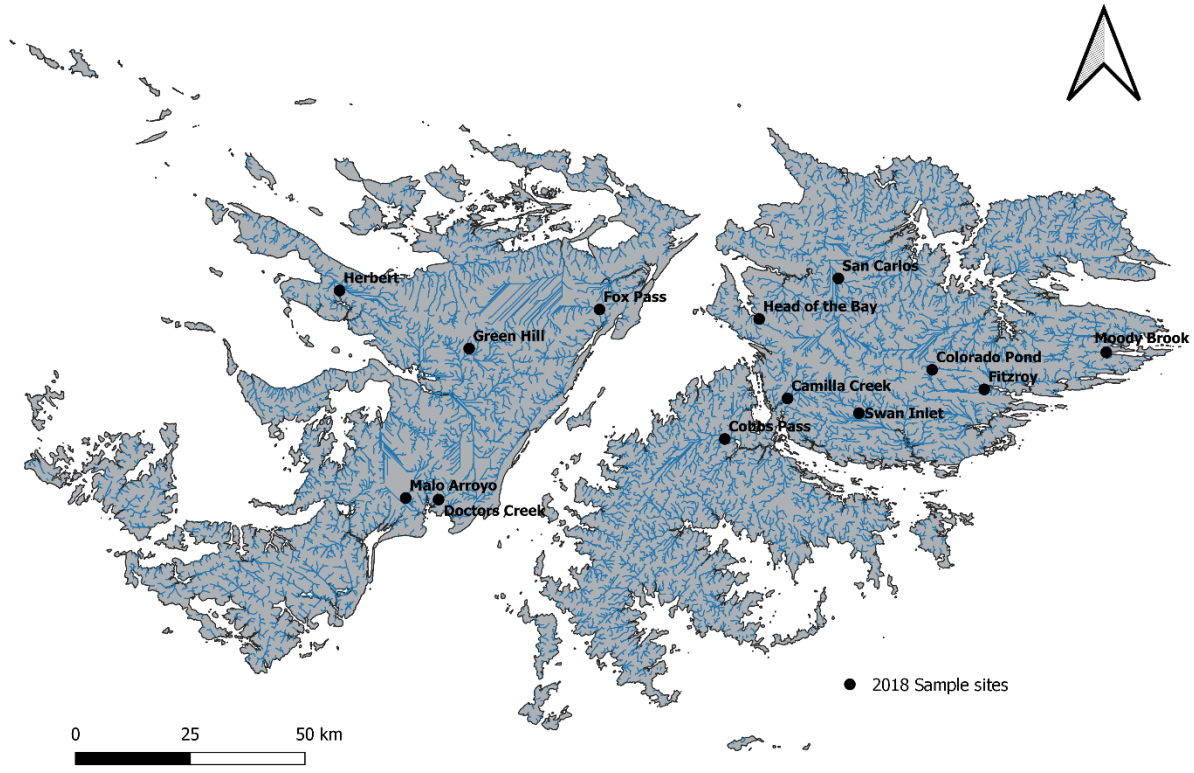

Figure 5.1. Map of sampling locations in the Falkland Islands

Laboratory Analysis

All samples (adipose fin clips and invertebrates) were oven dried at 50°C for 48hrs or until samples reached a constant weight. Dry samples were cut into fine pieces using scissors and ground using a pestle and mortar before weighing 0.5mg (±0.05mg) into tin capsules. A mix of invertebrate's present was produced for each sample site as well as a mix of individual invertebrate groups across all sample sites. Samples were combusted in excess oxygen over chrome oxide at 1000°C in an ANCA GSL Elemental Analyser interfaced with a Sercon 20/20 Mass Spectrometer (Swansea University) as per (McCarroll and Loader, 2004). Oxides of nitrogen were reduced over hot copper (600°C) and any traces of water removed using a chemical water trap. Carbon and nitrogen isotope ratios, expressed in delta notation (δ) as parts per thousand (‰), were calculated relative to the international standard for ^{13}C, the Vienna Pee Dee Belemnite (VPDB) standard (Coplen, 1995), and the standard N_2 of the atmosphere (Mariotti, 1983). Acetanilide references (Sigma-Aldrich Co., UK) were analysed between tissue samples to calibrate the machine and compensate for any machine drift. C:N ratios ranged from 2.82 to 3.79 in all brown trout samples. Adipose fin clip samples were not

lipid normalised as C:N ratios were only greater than 3.5 in four out of 156 samples. All C:N ratios for invertebrate samples were greater than 3.5, therefore the following lipid normalisation equation was used to derive lipid corrected $\delta^{13}C$ values as recommended by (Post *et al.*, 2007) for aquatic organisms:

$$\delta^{13}C_{corr.} = \delta^{13}C - 3.32 + 0.99 \times C{:}N$$

All $\delta^{13}C$ values reported for invertebrates were lipid-corrected in this way. Recommendations for stable isotope terminology from (Fry, 2006) and (Coplen, 2011) were followed; the term 'enriched' refers to samples with higher δ values and 'depleted' to samples with lower δ values.

Data Analysis

All data analysis was conducted in R 3.5.3 (R Core Team, 2019). Separate linear models were run to compare carbon and nitrogen content, $\delta^{13}C$ and $\delta^{15}N$, in brown trout adipose fin clips using sample site, fork length and level of silvery as predictors, due to the small sample sizes samples from Fitzroy and Fox Pass were excluded from analysis. Correlations between length and $\delta^{13}C$ or $\delta^{15}N$ were also calculated using the *cor.test* function and spearman method (Best and Roberts, 1975).

To determine if there was any clustering based on the carbon and nitrogen SIA signatures a K-means cluster approach was undertaken using the *factoextra* and *stats* packages in R (Kassambara and Mindt, 2020). The optimal number of clusters (K) was calculated using the *fviz_nbclust* function using the total within sum of squares (WSS) method, and the clusters were determined with the *kmeans* function.

Trophic level was calculated for each cluster from $\delta^{13}C$ and $\delta^{15}N$ values, using a one baseline model in the jagsBayesianModel and TPmodel functions in the tRophicPosition R package. These functions use a Markov Chain Monte Carlo Bayesian modelling framework through JAGS (Quezada-Romegialli et al., 2018) and utilise invertebrate samples as baseline values and trophic discrimination factors (Post, 2002). Kruskal-Wallis tests were conducted to compare length, $\delta^{13}C$ and $\delta^{15}N$ values for all clusters using the Kruskal.test function. Where significant differences were found, a pairwise Wilcox test was performed to compare pairs of clusters using the *pairwise.wilcox.test* function and the *BH* method to adjust p-values for false discovery (Benjamini and Hochberg, 2007).

5.3 Results

Stable isotope data were successfully obtained for all brown trout samples; however, one sample was removed from analysis due to extremely low $\delta^{13}C$ and $\delta^{15}N$ values. The size of brown trout ranged between 142 and 770mm in fork length and stable isotope values ranged from -32 to -10 for $\delta^{13}C$ and 7 to 20 for $\delta^{15}N$.

Twenty-three invertebrate samples were removed from analysis due to errors in analysis or strange $\delta^{13}C$ and $\delta^{15}N$ values. All invertebrates identified had previously been found in the Falkland Islands (Flower, 2001). Invertebrates were identified from Amphipoda, Oligochaeta, Hirudinea, Diptera, Coleoptera, Gastropoda, Plecoptera and Tricoptera (**Table S3** for breakdown of invertebrates found at each site).

Sampling site ($\delta^{13}C$: df = 11, F=39.634, p<0.001; $\delta^{15}N$: df = 11, F=31.300, p<0.001), length ($\delta^{13}C$: F=36.634, p=<0.001; $\delta^{15}N$: F=98.895, p<0.001), and level of silvery ($\delta^{13}C$: df = 3, F=13.150, p<0.001; $\delta^{15}N$: df = 3, F=13.554, p=0.001) all influenced brown trout $\delta^{13}C$ and $\delta^{15}N$ signatures (**Table 5.2** and **Table 5.3**). A positive correlation was observed between both carbon and nitrogen isotopic signatures and length ($\delta^{13}C$: *rho* = 0.63, p<0.001; $\delta^{15}N$: *rho* = 0.70, p<0.001, **Figure 5.2a/b**), and enriched $\delta^{13}C$ and $\delta^{15}N$ signatures with and increasing level of silvery.

Table 5.2. Output for $\delta^{13}C$ model

Variable	Estimate	Standard error	*t* value	*p* value
Intercept	-25.343	1.052	-24.087	<0.001
Cobbs Pass	1.812	1.095	1.660	0.099
Colorado Pond	-2.542	0.905	-2.810	0.006
Doctors Creek	-4.371	0.965	-4.527	<0.001
Green Hill	-2.818	1.224	-2.302	0.023
Head of the Bay	2.635	1.004	2.625	0.010
Herbert	-1.183	1.280	-0.924	0.357
Malo Arroyo	-1.838	1.253	-1.467	0.145
Moody Brook	-1.313	1.263	-1.039	0.301
Pedro	1.368	1.460	0.936	0.351
San Carlos	0.816	1.076	0.759	0.449
Swan Inlet	2.840	1.008	2.818	0.006
Length	0.010	0.003	3.704	<0.001
Silvery level 1	1.083	0.811	1.335	0.184
Silvery level 2	4.278	0.791	5.410	<0.001
Silvery level 3	4.512	0.808	5.586	<0.001

Table 5.3. Output for $\delta^{15}N$ model

Variable	Estimate	Standard error	*t* value	*p* value
Intercept	6.705	0.736	9.111	<0.001
Cobbs Pass	1.347	0.766	1.771	0.079
Colorado Pond	-0.592	0.633	-0.935	0.352
Doctors Creek	0.528	0.675	0.782	0.435
Green Hill	1.311	0.856	1.531	0.128
Head of the Bay	2.464	0.702	3.510	<0.001
Herbert	1.902	0.895	2.124	0.036
Malo Arroyo	-1.841	0.876	-2.101	0.038
Moody Brook	2.229	0.883	2.524	0.013
Pedro	-0.400	1.021	-0.392	0.696
San Carlos	1.365	0.752	1.815	0.072
Swan Inlet	1.314	0.705	1.864	0.065
Length	0.013	0.002	7.364	<0.001
Silvery level 1	1.179	0.567	2.077	0.040
Silvery level 2	3.036	0.553	5.489	<0.001
Silvery level 3	3.234	0.565	5.725	<0.001

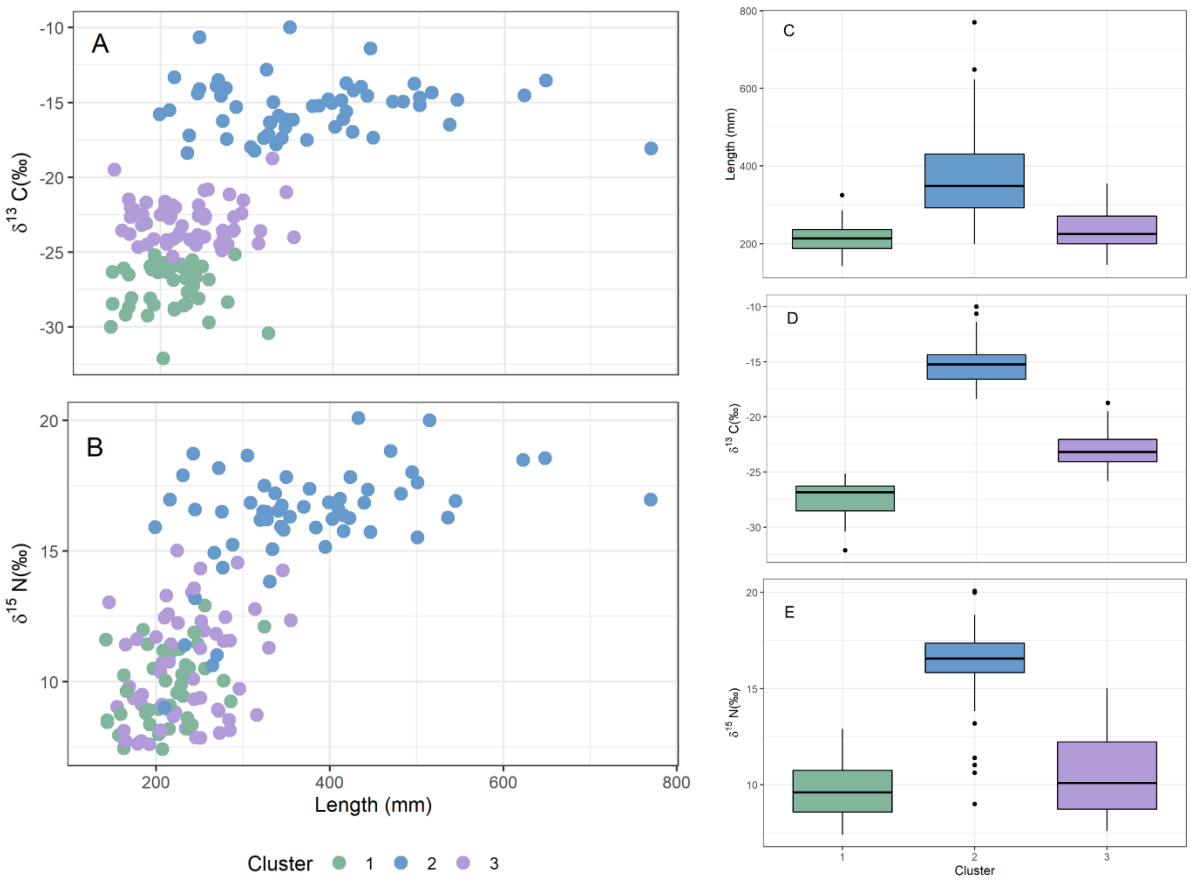

Figure 5.2. Relationship between length and $\delta^{13}C$ (A) and $\delta^{15}N$ (B), grouped by clusters determined from K-means clustering analysis. Mean and 95% confidence intervals (whiskers) of length (C), $\delta^{13}C$ (D) and $\delta^{15}N$ (E) for each cluster. Stable isotope values obtained from adipose fin clips collected from brown trout in the Falkland Islands.

Results of the K-means cluster analysis from adipose fin clips resulted in an optimal K of three (**Table 5.4**; **Figure 5.3**, K = 3 WSS = 963.654). Cluster 1 contained a total of 40 individuals from six sites, with 78% of fish in cluster 1 originating from Colorado Pond and Doctors Creek, 43% and 35%, respectively. Cluster 2 contained a total of 58 individuals from eight sites, including all individuals from Fitzroy, and San Carlos, with the majority of fish, 66%, derived from Swan Inlet and Head of the Bay, 34% and 31% respectively. Cluster 3 contained 57 individuals from 12 sites and contained all individuals from Fox Pass, Malo Arroyo (**Table 5.4**).

All clusters were clearly distinct (**Figure 5.3**), with clusters 1 ($\delta^{13}C$ = -27; $\delta^{15}N$ = 10) and 2 ($\delta^{13}C$ = -15; $\delta^{15}N$ = 16) exhibiting isotopic signatures typical of freshwater and marine environments, respectively (McCarthy and Waldron, 2000; Ciancio *et al.*, 2008a). Cluster 1 displayed depleted $\delta^{13}C$ and $\delta^{15}N$ values compared to cluster 2, representative of a freshwater background, with cluster 3 exhibiting intermediate values ($\delta^{13}C$ = -23; $\delta^{15}N$ = 10), therefore,

likely reflecting intermediate fish (estuarine/slob trout) (Appendix **Table S4**). Based on stable isotope values, 26% and 37% of the fish sampled were classified as cluster 1 (freshwater resident) and cluster 2 (anadromous), respectively, with the remaining 37% belonging to intermediate cluster (3) (**Table 5.4**). In addition, 96% of the individuals in cluster 2 were classified as a silvery level 2 or 3, while cluster 1 and 3 contained 37% and 53% of individuals classified as a level 0 or 1, respectively (**Figure 5.4**). The highest trophic level, 4.9, was detected in cluster 2, whereas cluster 1 exhibited the lowest at 3.1 (**Table 5.5**). Therefore, the different isotopic signatures likely reveal different life history strategies.

Significant variation was observed in length, $\delta^{13}C$ and $\delta^{15}N$ between all clusters (length: χ^2 = 75.62, p = <0.001; $\delta^{13}C$: χ^2 =135.34, p = <0.001; $\delta^{15}N$: χ^2 = 95.90, p = <0.001). Pairwise comparisons revealed significant variation between all cluster for length (clusters 1 and 2 p = <0.001; clusters 1 and 3 p = 0.041; clusters 2 and 3 p = <0.001, **Figure 5.2c**). In addition, significant variation was observed between clusters 1 and 2 and clusters 2 and 3 when comparing $\delta^{13}C$ and $\delta^{15}N$ (pairwise Wilcox test all <0.001, **Figure 5.2d/e**), whereas cluster 1 and 3 only varied significantly for carbon and not nitrogen (<0.001 and 0.14, respectively, **Figure 5.2**).

Table 5.4. Life history strategies of Falkland Islands brown trout predicted through K-means cluster analysis based on $\delta^{13}C$ and $\delta^{15}N$ values from adipose fin tissue from 14 sampling sites.

Site	N	Cluster 1 (resident) %	Cluster 2 (anadromous) %	Cluster 3 (intermediate) %
Camilla Creek	12	0	17	83
Cobbs Pass	11	0	27	73
Colorado Pond	25	68	0	32
Doctors Creek	17	82	6	12
Fitzroy	2	0	100	0
Fox Pass	2	0	0	100
Green Hill	7	57	0	43
Head of the Bay	17	6	65	29
Herbert	6	17	17	67
Malo Arroyo	6	0	0	100
Moody Brook	6	50	0	50
Pedro	4	0	0	100
San Carlos	20	0	100	0
Swan Inlet	20	0	90	10
Overall	155	26	37	37

Table 5.5. Summary of brown trout stable isotope signatures for cluster 1, 2 and 3. Sample size (N), mean Trophic level (TL)

Cluster	Isotope	Mean δ‰	Min, max (δ‰)	N	TL (SD)	Mean length (range)
Cluster 1 (freshwater)	$\delta^{13}C$	-27.46	-32.11, -25.14	40	3.07 (0.50)	211.8 (142-325)
	$\delta^{15}N$	9.75	7.41, 12.90			
Cluster 2 (anadromous)	$\delta^{13}C$	-15.34	-18.37, -10.66	58	4.95 (0.53)	374.8 (199-770)
	$\delta^{15}N$	16.32	9.00, 20.08			
Cluster 3 (intermediate)	$\delta^{13}C$	-23.01	-25.81, -18.75	57	3.23 (0.49)	233.5 (146-355)
	$\delta^{15}N$	10.47	7.61, 15.01			

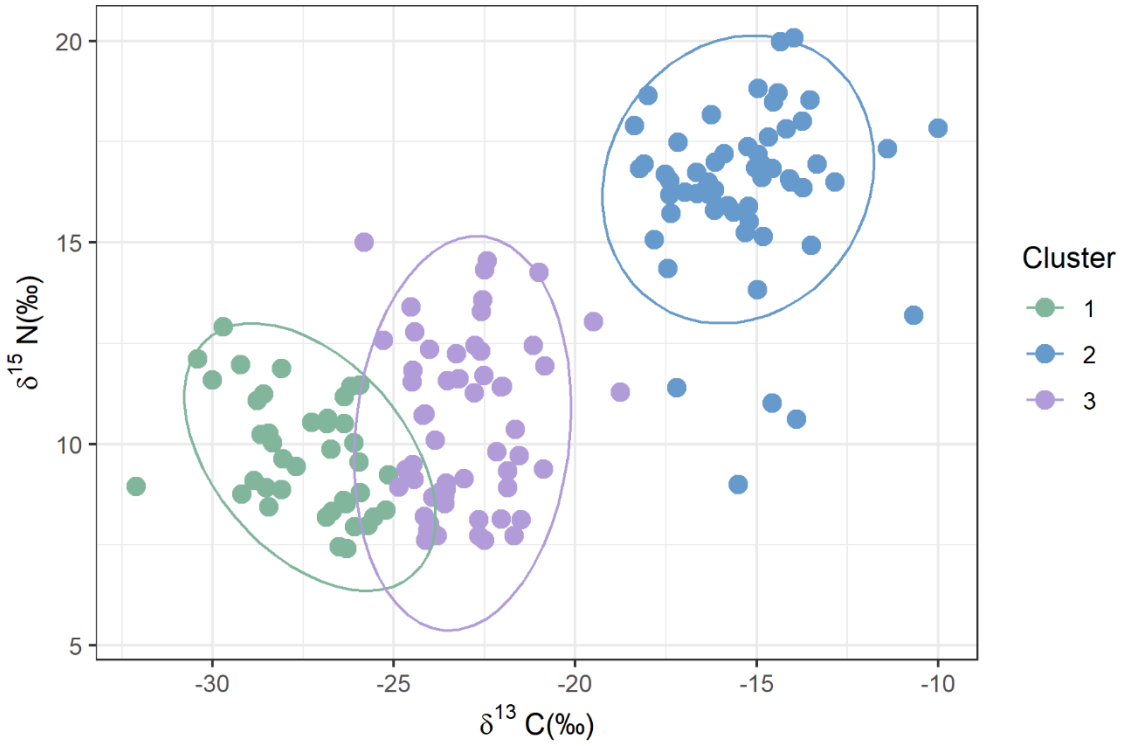

Figure 5.3. Scatter plot of results of K-means cluster analysis using $\delta^{13}C$ and $\delta^{15}N$ stable isotopes found in adipose fin clips of brown trout in the Falkland Islands.

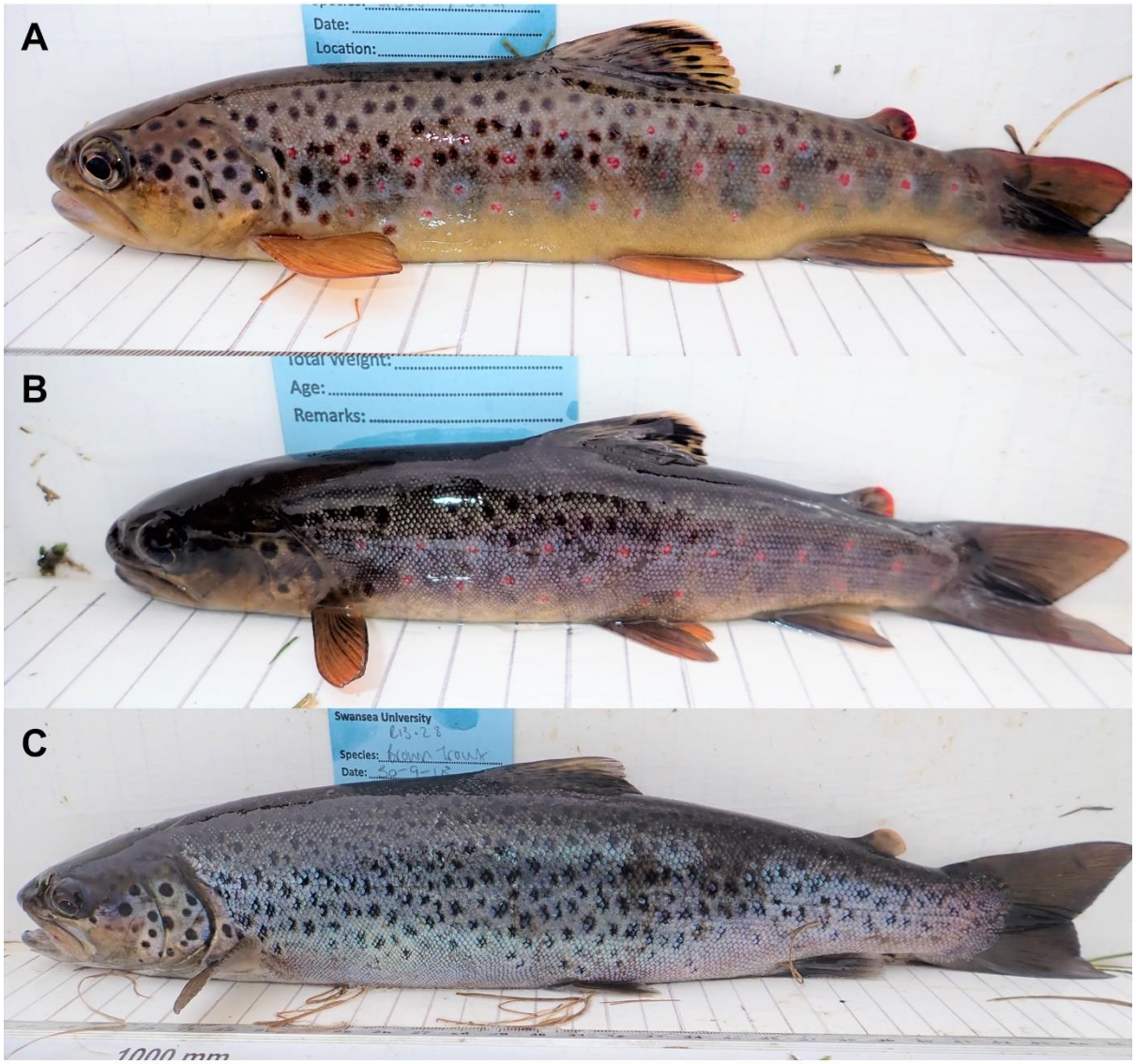

Figure 5.4. Examples of brown trout from cluster 1 (A), cluster 3 (B), and cluster 2 (C). Brown trout from Head of the Bay, Camilla Creek, and San Carlos, respectively.

5.4 Discussion

The analysis of stable isotopes revealed the presence of three distinct clusters of brown trout in the Falkland Islands, based on $\delta^{13}C$ and $\delta^{15}N$ values from adipose fin clips, likely reflecting their different ecotypes/life history strategies: resident (freshwater), intermediate (estuarine/slob trout), and anadromous (marine). Strong relationships were observed with $\delta^{13}C$ and $\delta^{15}N$ values and length likely reflecting the faster growth of brown trout in the marine environment compared to the relatively poor freshwater environments typical of the Falkland Islands (McDowall *et al.*, 2001; Flower, 2005; Ferguson *et al.*, 2019). Furthermore, migratory (estuarine and marine) brown trout were detected in all sample sites, indicating the potential for brown trout to spread beyond their current distribution in the Falklands, which could possibly cause additional impacts to the already restricted and threatened native galaxiid populations of *Aplochiton zebra* and *A. taeniatus*.

A wide range of both carbon and nitrogen stable isotope values were observed, similar to those observed in other studies for both freshwater resident and sea migrating anadromous fish in both native and invasive brown trout populations (McCarthy and Waldron, 2000; Ciancio *et al.*, 2008a; Ciancio *et al.*, 2008b). In addition, an intermediate cluster (cluster 3) was identified, which exhibited enriched $\delta^{13}C$, mean increase of 4‰, yet similar $\delta^{15}N$ values compared to freshwater fish, and depleted $\delta^{13}C$ and $\delta^{15}N$ values compared to marine fish, approximately 4‰ and 6‰ lower, respectively. Thus, these intermediate isotopic values in fish identified as cluster 3 could be due to estuarine feeding at a similar tropic level to freshwater fish. Estuarine/slob trout have previously been observed in native (McCarthy and Waldron, 2000) and invasive (Ciancio *et al.*, 2008a) populations of brown trout and are thought to arise due to competition for resources. Previous work examining freshwater ecosystems in the Falklands has indicated low biological productivity due to low invertebrate abundance and diversity (McDowall *et al.*, 2001; Flower, 2005), consequently, individuals may adopt an estuarine feeding strategy due to inter- and intraspecific competition for food. All intermediate (cluster 3) fish except one were classified as a silvery level 0 or 1, resident trout have been known to adapt to brackish conditions without undergoing smoltification (McCarthy and Waldron, 2000).

Carbon and nitrogen stable isotope values allow ecotype/life history strategies to be assumed, through information on the source of carbon in the food web (δ13C) and the trophic level at which individuals feed (δ15N) (McCarthy and Waldron, 2000; Charles et al., 2004).

However, other more conclusive methods could have been used to determine migratory status with more certainty. For example, examining strontium stable isotope ratios in otoliths, scales or eggs has previously been shown to be able to distinguish between freshwater-resident and marine migrating individuals (Eek and Bohlin, 1997; Zimmerman, 2005; Gibson-Reinemer et al., 2009; Kristensen et al., 2011). It was not possible to examine strontium in these samples due to limited tissue availability.

Strong relationships between length and $\delta^{13}C$ and $\delta^{15}N$ reflect the use and importance of feeding habitats and strategies. Smaller individuals are more likely to feed in freshwaters and at lower trophic levels while fish that migrated to the marine environment obtained larger sizes. While migration to the marine environment can incur costs from increased risk of predation and physiological changes, it can also provide access to better and more abundant food sources (Klemetsen *et al.*, 2003; Ferguson *et al.*, 2019). The relationship between $\delta^{15}N$ enrichment and increasing size was also reflected in the trophic level calculated for each cluster. In a biologically poor freshwater system, dispersal to the marine environment increases the abundance and sources of possible prey (Klemetsen *et al.*, 2003), which would otherwise be limited in freshwaters such as the Falkland Islands due to their limited invertebrate community and presence of few native fish species (McDowall *et al.*, 2001; Flower, 2005). Thus, migrating to the marine environment enables individuals to grow to larger sizes and exhibit increased fecundity (Jensen *et al.*, 2019). Anadromy was first observed in Falklands brown trout 20-years after initial introductions and five years before any stock from anadromous parents was introduced (Arrowsmith and Pentelow, 1965; Stewart, 1973; Minett *et al.*, 2021a). This switch to a migratory ecotype is likely due to changes in freshwater prey, as observed in Patagonia, where brown trout considerably reduced the abundance of native galaxiids and depleted invertebrate biomass through direct predation, consequently, driving fish to migrate to sea in order to obtain sufficient food resources (O'Neal and Stanford, 2011). Similar reductions in both abundance and distribution of native galaxiids (two *Aplochiton* species) have also been observed in the Falklands following the introduction brown trout. Thus, the migratory life history strategy in the Falklands likely developed as a result of a limited food supply, perhaps due to a reduction in the abundance of native fish, which brown trout have been known to predate on (Arismendi *et al.*, 2009; Ross, 2009), and competition for already limited invertebrate food resources (Flecker and Townsend, 1994; McDowall *et al.*, 2001; Townsend, 2003; Flower, 2005).

Migratory ecotypes, either estuarine or marine migrants, were detected in all sample sites, with 74% of fish sampled belonging to a migratory life history strategy. This is consistent with previous work estimating the level of migration and gene flow between the same sites, calculated using single nucleotide polymorphisms (SNPs). Previous SNP analysis (Chapter 4) identified high levels of migration between all sites, indicating the potential of widespread migration of brown trout across the Falklands via anadromous dispersal (Minett *et al.*, 2021b). Migratory brown trout are not restricted to certain rivers or introduction sites, therefore, suggesting that all rivers containing brown trout can act a source for future spread, thus increasing the potential for brown trout to invade new areas. Further spread could lead to the invasion of the last known refuges for threatened *Aplochiton* species, possibly resulting in the extinction of these native fishes in the Falklands (McDowall *et al.*, 2001).

In summary, the presence of three distinct clusters, which correspond to three life history strategies/ecotypes were detected through the analysis of $\delta^{13}C$ and $\delta^{15}N$ stable isotopes from adipose fin clips in brown trout. Although anadromy likely occurred in the Falklands due to limited freshwater productivity, it is now widespread across the Falklands, thus enabling further migration and spread (Launey *et al.*, 2010; Westley and Fleming, 2011). Previous work on invasive brown trout populations in Chile and New Zealand has revealed impacts on invertebrate communities (Townsend, 2003; O'Neal and Stanford, 2011), further work is required in the Falklands to determine the diet of resident, estuarine and anadromous brown trout in the Falklands, and how their presence impacts native fauna.

Chapter 6 General discussion

Freshwater ecosystems are home to ~50% of global fish diversity (Lundberg *et al.*, 2000; Lehner and Do, 2004; Balian *et al.*, 2008; Vega and Wiens, 2012), yet they are experiencing declines in biodiversity at a far greater rate than any terrestrial ecosystem (Collen *et al.*, 2009; World Wildlife Foundation, 2016) and are classified as the most endangered ecosystem in the world (Sala *et al.*, 2000; Dudgeon *et al.*, 2006). Such declines are attributed to many reasons including the introduction of invasive species (Dudgeon *et al.*, 2006; Reid *et al.*, 2019). Despite the impacts brown trout have had on native communities they have been introduced around the world and are now classified as one of the 100 world's worst invasive species (Lowe *et al.*, 2000). Islands, such as the Falklands, provide ideal scenarios to study invasive species, particularly when the introduction history is well known and there are few native species (Ewel and Högberg, 1995). Thus, the Falklands provide an ideal setting to study the invasion ecology of brown trout, with data collected here not only contributing to the conservation of native species in the Falklands but being also relevant to invasive brown trout populations elsewhere.

This thesis aimed to explore the invasion ecology of brown trout in the Falkland Islands in order to assess their current distribution and dispersal to inform management and facilitate the conservation of native galaxiids. First, I developed eDNA assays to detect the presence of brown trout and native *Aplochiton* species (*A. zebra* and *A. taeniatus*), enabling the distribution of invasive brown trout and native *Aplochiton* spp. populations to be monitored without physical capture, which can be difficult when species are rare or present in low densities (Jerde *et al.*, 2011). Subsequently, I developed a species distribution model to predict the invasion risk of brown trout across the Falklands, demonstrating the importance of human-mediated translocations, and determined the possible effects that varying levels of containment would have on their spread, revealing that brown trout are likely to continue spreading and invade all sites within ~70 years unless strong containment and strict measures are implemented. Thirdly, I assessed the population structure of brown trout populations in the Falklands and the results indicated high levels of gene flow suggesting widespread migration of brown trout, likely due to anadromous dispersal, as supported by data from acoustically tagged fish. Finally, this thesis examined the life history strategies of brown trout, thus allowing the incidence of anadromy to be uncovered, exposing the extensive

presence of migratory ecotypes, which likely enable the migration and spread of invasive brown trout in the Falkland Islands.

Work presented in this thesis shows two possible methods that have been employed to enable brown trout to spread from their site of introduction and across East and West Falklands. Species distribution modelling (Chapter 3) revealed the importance of Euclidean distance to the nearest invaded site, representing human-mediated translocations, over distance around the coast, representing natural dispersal through the marine environment. In addition, population genetics (Chapter 4) and stable isotope analysis (Chapter 5) uncovered the importance of anadromy in the Falklands as demonstrated by estimates of gene flow between all sites, the correlation between genetic and geographic distance (distance around the coast), and the prevalence of migratory ecotypes in all sample sites. Intentional releases of brown trout into uninvaded and landlocked sites have previously been reported in the Falkland Islands (McDowall *et al.*, 2001). However, their occurrence is believed to have decreased following the conservation of wildlife and nature ordinance in 1999, making it an offence to transport any protected animal, including brown trout (Falkland Islands Government, 1999). Therefore, it is likely that the most recent spread is due to natural dispersal, as indicated by SNPs and stable isotope analysis.

Without the implementation of sufficient management actions, brown trout will continue to spread and invade all possible sites. Native species have already been severely impacted by the introduction of brown trout, whereas *Aplochiton* spp. were previously found all over the islands, they now are mainly located in refuges in the South of the Islands (McDowall *et al.*, 2001; Ross, 2009). The continued spread of brown trout is likely to cause additional impacts and further limit the distribution of native species, possibly leading to their extinction on the islands, as was similarly observed with the native grayling *Prototroctes oxyrhynchus* in New Zealand (McDowall, 2006). Although zebra trout are protected from being deliberately captured and killed, little is known about their current distribution or abundance. Only one species (*Aplochiton zebra*) is referred to under the current legislation. Distinguishing between *A. zebra* and *A. taeniatus* is challenging due to their similar ecology and morphology (McDowall, 2006). *A. taeniatus* was only identified to be found in the Falklands in 2012, further complicating their conservation as *A. taeniatus* was found to be more widespread, and therefore *A. zebra* populations were more at risk than previously believed (Vanhaecke *et al.*, 2012b). In addition, the introduction of brown trout seems to be increasing the occurrence of

sympatry between both *Aplochiton* spp., potentially leading to hybridisation, which could cause further declines and their extirpation in the Falklands (Huxel, 1999; Vanhaecke *et al.*, 2012b). Therefore, to conserve native freshwater fauna in the Falklands, it is essential to limit the spread of brown trout and protect native habitats and refugia, which could be achieved through strong and stringent management; see Chapter 2 conclusions and recommendations for possible ways brown trout distribution and spread could be limited.

Furthermore, freshwater ecosystems in the Falklands need to be monitored for future invasions from salmonids from Chile and Patagonia. Chinook (*Oncorhynchus tshawytscha*, (Fowler, 2013)) and coho salmon (*Oncorhynchus kisutch*) have already been captured in/around the islands, demonstrating that the Falklands are not completely isolated and have the potential to be invaded by other species.

Without substantial and urgent management, brown trout will continue to spread throughout the Falkland Islands, impacting native freshwater fauna. Despite the recreational and economic opportunities, the introduction of brown trout has brought to the Falklands (Ross, 2009), they have also had major impacts on the native freshwater fish (McDowall *et al.*, 2001), as has also been observed around the world, particularly in the Southern Hemisphere (McDowall, 2003; McDowall, 2006; Young *et al.*, 2010; Elgueta *et al.*, 2013). Although existing legislation makes it illegal to propagate and transport brown trout (Falkland Islands Government, 1964; Falkland Islands Government, 1999), more needs to be done to limit the spread and conserve habitats and refugia for native *Aplochiton* spp., without which their survival in the Falklands is at risk.

- Brown trout are continuing to spread throughout the Falklands and will likely be distributed throughout the Falklands if management is not put in place. Similar invasion risk models could be conducted in other areas/countries with invasive brown trout populations using similar anthropogenic and bio-climatic variables, enabling information regarding the area's most at risk of invasion and those areas that would likely benefit from management to be determined.
- Using a combination of markers (both SNPs and stable isotopes) it was possible to reveal the importance of anadromy in the Falklands, uncovering that all occupied sites could act as a potential source for future invasions. Such methods could enable brown trout in newly invaded sites to be traced back to source populations, thus, allowing

management measures, such as the use of selective barriers, to be implemented to limit their dispersal.

- The use of environmental DNA assays, such as those developed here, allows for the detection of invasive and rare species without the need for visual confirmation which can be difficult when species are at low densities. These markers could be used to determine the distribution of brown trout and native zebra trout both in the Falklands and in Chile. Such information is essential for monitoring these threatened and protected native species and could be used and built upon to evaluate the extinction risk of zebra trout.

Suggested management actions

- Increase public awareness of the impacts from salmonid invasions and the importance of conserving native galaxiid populations.

- Implement more stringent legislation to prevent the deliberate movement and release of brown trout around the islands, and to prevent further importation of brown trout.

- Deploy exclusion barriers around *Aplochiton* spp. refugia to prevent and reduce the risk of brown trout invading these sites. However, the impacts of installing such barriers need to be carefully monitored to ensure that they do not negatively impact native galaxiids.

- Angling regulations: remove/increase bag limit and fishing season in areas around *Aplochiton* spp. refugia/invasion front to slow down the spread of brown trout. Intensive fishing could help eradicate brown trout from these regions and establish a buffer zone between brown trout and *Aplochiton* spp. populations.

- Set up an eDNA monitoring program to establish the locations of *Aplochiton* spp. refugia, act as an early warning system for brown trout invasions and invasions of any other salmonid species (e.g., chinook/coho salmon), and to determine whether containment measures are successful.

- Develop a species action plan to aid in the conservation of native *Aplochiton* spp. and consider designating sites containing *Aplochiton* spp. as Nature Reserves to protect populations.

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Appendix 1: Supplementary Table and Figures

Table S1. Model output and AIC for all possible models used to determine species distribution model. For information on predictor variables used see **Table 3.1**.

Model	Eucl_dist _inv	Road_cross _No	Ap	Coast_dist _intro	Coast_dist _inv	Min_winter _temp	Slope	Settle_dist	Road_dist	LC
Starting model	E = -0.169	E = 0.093	E = -2.092	E = 0.029	E = -0.038	E = 0.242	E = -0.343	E = 0.121	E = -0.173	E = 0.121
	SE = 0.100	SE = 0.087	SE = 1.088	SE = 0.024	SE = 0.022	SE = 0.178	SE = 0.244	SE = 0.102	SE = 0.133	SE = 0.120
	t = -1.695	*t* = 1.075	*t* = -1.922	*t* = 1.223	*t* = -1.740	*t* = 1.362	*t* = -1.406	*t* = 1.178	*t* = -1.302	*t* = 1.001
	p = 0.090	*p* = 0.282	*p* = 0.055	*p* = 0.221	*p* = 0.082	*p* =0.173	*p* = 0.160	*p* =0.239	*p* = 0.193	*p* =0.317
Intermediate	E = -0.168	E = 0.094	E = -2.095	E = 0.029	E = -0.038	E = 0.242	E = -0.334	E = 0.121	E = -0.172	E = 0.121
model 1	SE = 0.097	SE = 0.086	SE = 1.088	SE = 0.024	SE = 0.022	SE = 0.177	SE = 0.205	SE = 0.102	SE = 0.132	SE = 0.120
	t = -1.731	*t* = 1.097	*t* = -1.925	*t* = 1.229	*t* = -1.746	*t* = 1.362	*t* = -1.633	*t* = 1.195	*t* = -1.303	*t* = 1.005
	p = 0.083	*p* = 0.272	*p* = 0.054	*p* = 0.219	*p* =0.081	*p* =0.173	*p* = 0.103	*p* =0.232	*p* =0.193	*p* =0.315
Intermediate	E = -0.171	E = 0.095	E = -2.113	E = 0.029	E = -0.038	E = 0.244	E = -0.335	E = 0.120	E = -0.173	E = 0.120
model 2	SE = 0.089	SE = 0.085	SE = 1.063	SE = 0.022	SE = 0.022	SE = 0.174	SE = 0.204	SE = 0.099	SE = 0.131	SE = 0.120
	t = -1.922	*t* = 1.114	*t* = -1.988	*t* = 1.294	*t* = -1.743	*t* = 1.399	*t* = -1.643	*t* = 1.209	*t* = -1.319	*t* = 1.004
	p = 0.055	*p* = 0.266	*p* = 0.047	*p* = 0.196	*p* = 0.081	*p* = 0.162	*p* = 0.100	*p* =0.227	*p* =0.187	*p* =0.316
Intermediate	E = -0.172	E = 0.096	E = -2.094	E = 0.028	E = -0.037	E = 0.241	E = -0.341	E = 0.116	E = -0.173	E = 0.118
model 3	SE = 0.089	SE = 0.084	SE = 1.057	SE = 0.022	SE = 0.021	SE = 0.174	SE = 0.202	SE = 0.097	SE = 0.131	SE = 0.118
	t = -1.924	*t* = 1.144	*t* = -1.981	*t* = 1.291	*t* = -1.744	*t* = 1.386	*t* = -1.692	*t* = 1.192	*t* = -1.321	*t* = 0.995
	p =0.054	*p* = 0.253	*p* = 0.048	*p* = 0.197	*p* =0.081	*p* =0.166	*p* = 0.091	*p* =0.233	*p* =0.186	*p* =0.320
Intermediate	E = -0.176	E = 0.094	E = -2.099	E = 0.029	E = -0.037	E = 0.255	E = -0.340	E = 0.125	E = -0.176	E = 0.120
model 4	SE = 0.087	SE = 0.084	SE = 1.058	SE = 0.021	SE = 0.021	SE = 0.165	SE = 0.201	SE = 0.092	SE = 0.130	SE = 0.118
	t = -2.008	*t* = 1.114	*t* = -1.983	*t* = 1.361	*t* = -1.732	*t* = 1.364	*t* = -1.695	*t* = 1.1347	*t* = -1.348	*t* = 1.019
	p =0.045	*p* = 0.265	*p* = 0.047	*p* = 0.174	*p* = 0.083	*p* = 0.173	*p* = 0.090	*p* =0.178	*p* =0.178	*p* =0.308
Intermediate	E = -0.171	E = 0.095	E = -2.161	E = 0.030	E = -0.038	E = 0.239	E = -0.320	E = 0.103	E = -0.081	E = 0.113
model 5	SE = 0.086	SE = 0.085	SE = 1.044	SE = 0.021	SE = 0.021	SE = 0.163	SE = 0.196	SE = 0.083	SE = 0.129	SE = 0.118
	t = -1.988	*t* = 1.125	*t* = -2.070	*t* = 1.438	*t* = -1.833	*t* = 1.468	*t* = -1.631	*t* = 1.240	*t* = -1.405	*t* = 0.960
	p =0.047	*p* =0.261	*p* =0.039	*p* =0.151	*p* =0.067	*p* =0.142	*p* =0.130	*p* =0.215	*p* =0.160	*p* =0.337
Intermediate	E = -0.175	E = 0.132	E = -2.361	E = 0.031	E = -0.037	E = 0.217	E = -0.318	E = 0.112	E = -0.205	E = 0.117
model 6	SE = 0.085	SE = 0.078	SE = 1.030	SE = 0.021	SE = 0.021	SE = 0.157	SE = 0.195	SE = 0.084	SE = 0.127	SE = 0.118
	t = -2.061	*t* = 1.706	*t* = -2.292	*t* = 1.456	*t* = -1.779	*t* = 1.382	*t* = -1.635	*t* = 1.333	*t* = -1.611	*t* = 0.992
	p = 0.039	*p* =0.088	*p* =0.022	*p* =0.145	*p* =0.075	*p* =0.167	*p* =0.102	*p* =0.182	*p* =0.107	*p* =0.321
Intermediate	E = -0.185	E = 0.125	E = -2.287	E = 0.026	E = -0.035	E = 0.167	E = -0.287	E = 0.117	E = -0.193	E = 0.126
model 7	SE = 0.083	SE = 0.075	SE = 1.014	SE = 0.020	SE = 0.020	SE = 0.133	SE = 0.188	SE = 0.083	SE = 0.122	SE = 0.117
	t = -2.243	*t* = 1.663	*t* = -2.255	*t* = 1.332	*t* = -1.729	*t* = 1.254	*t* = -1.523	*t* = 1.404	*t* = -1.582	*t* = 1.074
	p =0.025	*p* =0.096	*p* =0.024	*p* =0.183	*p* =0.084	*p* =0.210	*p* =0.128	*p* =0.160	*p* =0.144	*p* =0.283
Intermediate	E = -2.05	E = 0.149	E = -2.326	E = 0.028	E = -0.035	E = 0.179	E = -0.263	E = 0.120	E = -0.152	E = 0.128
model 8	SE = 0.081	SE = 0.078	SE = 1.008	SE = 0.019	SE = 0.020	SE = 0.128	SE = 0.182	SE = 0.083	SE = 0.109	SE = 0.114
	t = -2.528	*t* = 1.918	*t* = -2.308	*t* = 1.461	*t* = -1.729	*t* = 1.401	*t* = -1.443	*t* = 1.457	*t* = -1.392	*t* = 1.131
	p =0.012	*p* =0.055	*p* =0.021	*p* =0.144	*p* =0.123	*p* =0.161	*p* =0.149	*p* =0.145	*p* =0.164	*p* =0.258

Appendix 1: Supplementary Table and Figures

Model	BFL_share	EW	BFL	Rain	Intro_site	Flow_accum	Eucl_dist _intro	Alt	AIC
Starting model	E = 2×10^{-5}	E = -0.869	E = -0.965	E = 0.002	E = -0.291	E = -0.219	E = -0.005	E = 0.002	112.76
	SE = 2×10^{-5}	SE = 0.926	SE = 1.137	SE = 0.004	SE = 0.866	SE = 1.401	SE = 0.079	SE = 0.025	
	t = 1.215	*t* = -0.939	*t* = -0.849	*t* = 0.462	*t* = -0.336	*t* = -0.156	*t* = -0.067	*t* = 0.063	
	p = 0.224	*p* = 0.348	*p* = 0.396	*p* = 0.644	*p* = 0.737	*p* = 0.876	*p* = 0.947	*p* = 0.950	
Intermediate model 1	E = 2×10^{-5}	E = -0.872	E = -0.957	E = 0.002	E = -0.285	E = -0.228	E = -0.006		110.77
	SE = 2×10^{-5}	SE = 0.925	SE = 1.128	SE = 0.004	SE = 0.861	SE = 1.392	SE = 0.078		
	t = 1.221	*t* = -0.943	*t* = -0.848	*t* = 0.535	*t* = -0.331	*t* = -0.164	*t* = -0.075		
	p = 0.222	*p* = 0.346	*p* = 0.396	*p* = 0.593	*p* = 0.740	*p* = 0.870	*p* = 0.940		
Intermediate model 2	E = 2×10^{-5}	E = 09.885	E = -0.936	E = 0.002	E = -0.279	E = -0.264			108.77
	SE = 2×10^{-5}	SE = 0.908	SE = 1.092	SE = 0.004	SE = 0.857	SE = 1.307			
	t = 1.227	*t* = -0.975	*t* = -0.857	*t* = 0.535	*t* = -0.325	*t* = -0.202			
	p = 0.220	*p* = 0.330	*p* = 0.392	*p* = 0.592	*p* = 0.745	*p* = 0.840			
Intermediate model 3	E = 2×10^{-5}	E = -0.895	E = -0.956	E = 0.002	E = -0.274				106.82
	SE = 2×10^{-5}	SE = 0.907	SE = 1.092	SE = 0.004	SE = 0.856				
	t = 1.241	*t* = -0.986	*t* = -0.876	*t* = 0.499	*t* = -0.321				
	p = 0.125	*p* = 0.324	*p* = 0.381	*p* = 0.618	*p* = 0.749				
Intermediate model 4	E = 2×10^{-5}	E = -0.814	E = -0.905	E = 0.002					104.92
	SE = 2×10^{-5}	SE = 0.864	SE = 1.018	SE = 0.004					
	t = 1.212	*t* = -0.942	*t* = -0.837	*t* = 0.556					
	p = 0.226	*p* = 0.346	*p* = 0.403	*p* = 0.579					
Intermediate model 5	E = 2×10^{-5}	E = -0.717	E = -0.871						103.23
	SE = 2×10^{-5}	SE = 0.855	SE = 1.084						
	t = 1.260	*t* = -0.838	*t* = -0.804						
	p = 0.208	*p* = 0.402	*p* = 0.422						
Intermediate model 6	E = 2×10^{-5}	E = -0.524							101.89
	SE = 1×10^{-5}	SE = 0.817							
	t = 1.072	*t* = -0.641							
	p = 0.284	*p* = 0.521							
Intermediate model 7	E = 1×10^{-5}								100.29
	SE = 1×10^{-5}								
	t = 0.985								
	p = 0.325								
Intermediate model 8									99.489

Appendix 1: Supplementary Table and Figures

Model	Eucl_dist _inv	Road_cross _No	Ap	Coast_dist _intro	Coast_dist _inv	Min_winter _temp	Slope	Settle_dist	Road_dist	AIC
Intermediate	E = -0.195	E = 0.141	E = -2.480	E = 0.031	E = -0.028	E = 0.192	E = -0.254	E = 0.120	E = -0.154	98.84
model 9	SE = 0.079	SE = 0.076	SE = 1.003	SE = 0.018	SE = 0.018	SE = 0.126	SE = 0.176	SE = 0.083	SE = 0.110	
	t = -2.475	*t* = 1.864	*t* = -2.471	*t* = 1.705	*t* = -1.577	*t* = 1.526	*t* = -1.441	*t* = 1.452	*t* = -1.401	
	p = 0.013	*p* = 0.062	*p* = 0.014	*p* = 0.088	*p* = 0.115	*p* = 0.127	*p* = 0.150	*p* = 0.147	*p* = 0.161	
Intermediate	E = -0.204	E = 0.167	E = -2.074	E = 0.020	E = -0.026	E = 0.110	E = -0.187	E = 0.064		99.14
model 10	SE = 0.077	SE = 0.077	SE = 0.920	SE = 0.016	SE = 0.017	SE = 0.099	SE = 0.167	SE = 0.073		
	t = -2.661	*t* = 2.176	*t* = -2.254	*t* = 1.237	*t* = -1.466	*t* = 1.108	*t* = -1.123	*t* = 0.879		
	p = 0.008	*p* = 0.030	*p* = 0.024	*p* = 0.216	*p* = 0.143	*p* = 0.028	*p* = 0.262	*p* = 0.379		
Intermediate	E = -0.199	E = 0.168	E = -1.996	E = 0.022	E = -0.026	E = 1.112	E = -0.146			97.94
model 11	SE = 0.074	SE = 0.078	SE = 0.929	SE = 0.016	SE = 0.018	SE = 0.098	SE = 0.160			
	t = -2.690	*t* = 2.143	*t* = -2.148	*t* = 1.403	*t* = -1.495	*t* = 1.147	*t* = -0.913			
	p = 0.007	*p* = 0.032	*p* = 0.032	*p* = 0.161	*p* = 0.135	*p* = 0.251	*p* = 0.361			
Intermediate	E = -0.215	E = 0.151	E = -2.059	E = 0.022	E = -0.022	E = 0.093				96.78
model 12	SE = 0.073	SE = 0.073	SE = 0.918	SE = 0.016	SE = 0.017	SE = 0.096				
	t = -2.928	*t* = 2.065	*t* = -2.242	*t* = 1.382	*t* = -1.319	*t* = 0.965				
	p = 0.003	*p* = 0.039	*p* = 0.025	*p* = 0.167	*p* = 0.187	*p* = 0.335				
Intermediate	E = -0.210	E = 0.149	E = -2.110	E = 0.022	E = -0.022					95.72
model 13	SE = 0.071	SE = 0.074	SE = 0.902	SE = 0.016	SE = 0.016					
	t = -2.976	*t* = 2.007	*t* = -2.338	*t* = 1.404	*t* = -1.357					
	p = 0.003	*p* = 0.045	*p* = 0.019	*p* = 0.160	*p* = 0.175					
Intermediate	E = -0.246	E = 0.153	E = -1.883	E = 0.010						95.77
model 14	SE = 0.069	SE = 0.071	SE = 0.868	SE = 0.012						
	t = -3.573	*t* = 2.155	*t* = -2.171	*t* = 0.808						
	p = <0.001	*p* = 0.031	*p* = 0.030	*p* = 0.419						
Final model	E = -0.238	E = 0.156	E = -1.572							94.46
	SE = 0.067	SE = 0.006	SE = 0.769							
	t = -3.559	*t* = 2.370	*t* = -2.044							
	p = <0.001	*p* = 0.018	*p* = 0.041							

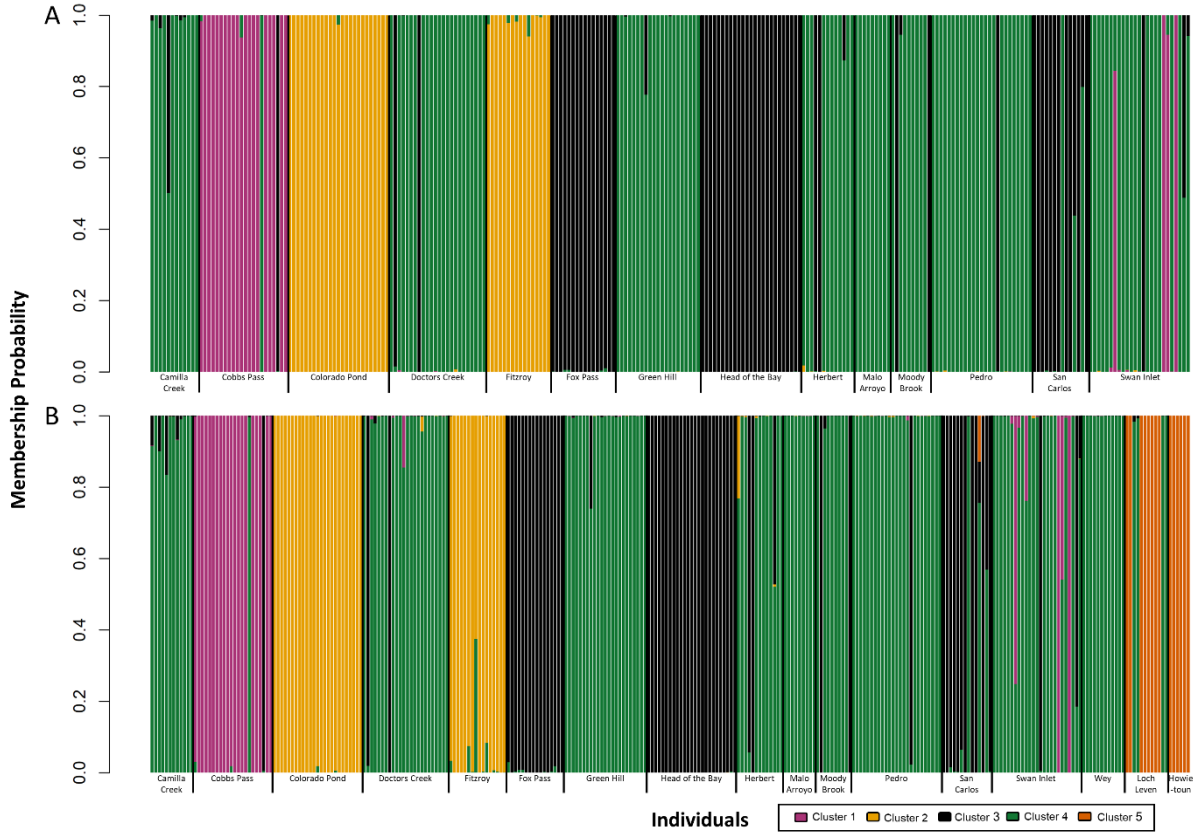

Figure S1. Population structure for Falkland Islands (A) and Falkland Islands GB comparison (B) when SNPs that significantly deviate from Hardy-Weinberg equilibrium are removed. A total of eight and nine SNPs were removed from Falkland Islands only and Falklands-GB comparisons, respectively.

Table S2. Breakdown of fish acoustically tagged in the San Carlos River, Falkland Islands, including latitude and longitude, acoustic tag number, Length (mm), and date tagged.

Fish ID	Acoustic tag	Length (mm)	Latitude	Longitude	Date
R13.1	4451	536	-51.5311	-58.7603	30/09/2018
R13.6	4449	345	-51.5311	-58.7603	30/09/2018
R13.10	4452	447	-51.5311	-58.7603	30/09/2018
R13.11	4450	397	-51.5311	-58.7603	30/09/2018
R13.14	4446	334	-51.5311	-58.7603	30/09/2018
R13.16	4448	347	-51.5311	-58.7603	30/09/2018
R13.23	4441	309	-51.5311	-58.7603	30/09/2018
R13.26	4443	412	-51.5311	-58.7603	30/09/2018
R13.27	4445	501	-51.5311	-58.7603	30/09/2018
R13.28	4439	331	-51.5311	-58.7603	30/09/2018
R13.30	4444	328	-51.5311	-58.7603	30/09/2018
R19.1	4453	423	-51.5311	-58.7606	14/10/2018
R19.3	4455	341	-51.5311	-58.7606	14/10/2018
R19.4	4464	325	-51.5311	-58.7606	14/10/2018
R19.5	4454	545	-51.5311	-58.7606	14/10/2018
R19.8	4466	403	-51.5311	-58.7606	14/10/2018
R19.9	4458	320	-51.5311	-58.7606	14/10/2018
R19.12	4459	305	-51.5311	-58.7606	14/10/2018
R19.14	4462	277	-51.5231	-58.7056	14/10/2018
R19.18	4465	188	-51.5231	-58.7056	14/10/2018
R19.21	4461	495	-51.5231	-58.7056	14/10/2018
R19.22	4463	198	-51.5231	-58.7056	14/10/2018
R19.24	4460	329	-51.5231	-58.7056	14/10/2018
R19.25	4457	175	-51.5231	-58.7056	14/10/2018
R19.27	4456	544	-51.5081	-58.7769	14/10/2018

Appendix 1: Supplementary Table and Figures

Table S3. Breakdown of invertebrate's present (1) and absent (0) at each sampling site, number of samples for sample isotope analysis (N) and mean and standard error for Carbon and Nitrogen stable isotopes.

Site	Amphipoda	Oligochaeta	Hirudinea	Diptera	Coleoptera	Gastropoda	Plecoptera	Tricoptera	Unknown	N	$\delta^{13}C$		$\delta^{15}N$	
											Mean	SE	Mean	SE
Camilla Creek	1	0	0	1	0	0	0	1	0	2	-27.3	0.3	68.3	3.1
Colorado Pond	1	1	1	1	1	1	0	1	0	3	-29.3	0.2	37.5	3.2
Doctors Creek	1	1	0	1	0	1	0	1	1	3	-28.6	1.3	8.1	2.6
Fitzroy	1	1	0	1	0	0	1	1	1	1	-26.7	NA	24.0	NA
Green Hill	1	1	0	1	1	1	1	1	1	3	-30.4	0.5	4.0	2.5
Head of the Bay	1	0	0	1	0	0	0	1	1	0	NA	NA	NA	NA
Herbert	1	1	1	1	0	1	1	1	1	3	-28.7	1.5	7.1	2.5
Malo Arroyo	1	1	1	1	0	1	0	1	0	2	-26.7	2.1	11.5	12.6
Mary Hill Quarry	1	1	1	0	1	1	0	0	1	3	-21.4	0.1	57.3	3.9
Moody Brook	1	1	0	1	0	0	1	1	0	3	-30.4	0.3	1.6	1.3
San Carlos	1	1	1	1	0	1	1	1	1	3	-28.6	0.3	0.1	0.1

Table S4. Stable isotope and sample data for fish from each sample site assigned to each cluster.

Cluster	Site	$\delta^{13}C$	$\delta^{15}N$	Length	Silvery
Freshwater (1)	Colorado Pond	-27.3	10.5	238	1
	Colorado Pond	-25.1	9.2	286	0
	Colorado Pond	-26.3	7.4	207	0
	Colorado Pond	-26.7	9.9	229	1
	Colorado Pond	-25.7	8.0	203	0
	Colorado Pond	-26.0	11.5	248	0
	Colorado Pond	-26.4	10.5	197	2
	Colorado Pond	-26.2	11.4	190	0
	Colorado Pond	-25.2	8.4	193	1
	Colorado Pond	-26.1	7.9	157	0
	Colorado Pond	-26.5	7.5	163	0
	Colorado Pond	-26.4	8.6	236	0
	Colorado Pond	-26.9	8.2	215	0
	Colorado Pond	-27.7	9.4	231	0
	Colorado Pond	-26.8	10.5	256	0
	Colorado Pond	-28.8	11.1	216	1
	Colorado Pond	-26.1	10.0	211	0
	Doctors Creek	-30.4	12.1	325	0
	Doctors Creek	-28.4	10.3	230	0
	Doctors Creek	-28.9	9.1	216	0
	Doctors Creek	-32.1	8.9	203	0
	Doctors Creek	-29.7	12.9	256	0
	Doctors Creek	-29.2	12.0	185	0
	Doctors Creek	-28.3	10.0	278	0
	Doctors Creek	-26.7	8.3	241	0
	Doctors Creek	-28.1	8.9	188	0
	Doctors Creek	-28.7	10.2	163	0
	Doctors Creek	-29.2	8.8	159	0
	Doctors Creek	-26.0	9.6	224	0
	Doctors Creek	-28.6	11.2	227	0
	Doctors Creek	-28.5	8.9	192	0
	Green Hill	-28.5	8.4	144	0
	Green Hill	-28.1	9.6	166	0
	Green Hill	-26.3	8.5	144	0
	Green Hill	-30.0	11.6	142	0
	Head of the Bay	-25.6	8.2	237	0
	Herbert	-28.1	11.9	244	0
	Moody Brook	-26.8	10.6	234	0
	Moody Brook	-25.9	8.8	188	0
	Moody Brook	-26.4	11.2	208	0
Marine (2)	Camilla Creek	-13.5	18.5	648	2
	Camilla Creek	-18.1	17.0	770	3
	Cobbs Pass	-15.8	15.9	199	1
	Cobbs Pass	-12.8	16.5	323	2
	Cobbs Pass	-10.7	13.2	254	0
	Doctors Creek	-14.0	16.5	276	3
	Fitzroy	-14.0	20.1	433	0
	Fitzroy	-15.5	9.0	210	0
	Head of the Bay	-11.4	17.3	444	2
	Head of the Bay	-13.5	14.9	267	3
	Head of the Bay	-13.3	17.0	216	3
	Head of the Bay	-14.3	20.0	515	2
	Head of the Bay	-10.0	17.8	350	2
	Head of the Bay	-13.7	16.4	416	2
	Head of the Bay	-14.9	16.6	410	3
	Head of the Bay	-14.1	16.6	245	3

	Head of the Bay	-15.0	16.9	399	2
	Head of the Bay	-18.4	17.9	231	3
	Head of the Bay	-16.2	18.2	272	2
	Herbert	-14.4	18.7	243	3
	San Carlos	-16.5	16.3	536	3
	San Carlos	-17.4	15.7	447	3
	San Carlos	-14.5	18.5	623	0
	San Carlos	-17.8	15.1	334	3
	San Carlos	-16.2	15.8	347	3
	San Carlos	-18.2	16.8	309	3
	San Carlos	-16.1	17.0	412	3
	San Carlos	-15.2	15.5	501	3
	San Carlos	-15.0	13.8	331	3
	San Carlos	-16.4	16.2	328	3
	San Carlos	-16.7	16.7	345	3
	San Carlos	-17.0	16.3	423	3
	San Carlos	-18.0	18.7	305	3
	San Carlos	-17.4	14.4	277	2
	San Carlos	-13.7	18.0	495	2
	San Carlos	-17.4	16.5	341	3
	San Carlos	-17.2	17.5	325	2
	San Carlos	-14.8	16.9	545	3
	San Carlos	-16.6	16.2	403	2
	San Carlos	-17.4	16.2	320	3
	Swan Inlet	-14.8	15.2	395	3
	Swan Inlet	-15.6	15.8	416	3
	Swan Inlet	-15.9	17.2	337	3
	Swan Inlet	-14.2	17.8	424	1
	Swan Inlet	-15.2	17.4	377	1
	Swan Inlet	-14.6	11.0	270	1
	Swan Inlet	-14.9	18,8	470	2
	Swan Inlet	-15.2	15.9	384	2
	Swan Inlet	-16.3	16.5	327	2
	Swan Inlet	-15.0	17.2	482	3
	Swan Inlet	-13.9	10.6	265	2
	Swan Inlet	-15.3	15.2	288	2
	Swan Inlet	-17.2	11.4	233	1
	Swan Inlet	-17.5	16.7	370	2
	Swan Inlet	-14.7	17.6	501	1
	Swan Inlet	-16.2	16.3	354	2
	Swan Inlet	-16.1	15.9	344	3
	Swan Inlet	-14.5	16.8	440	3
Intermediate (3)	Camilla Creek	-24.5	11.5	278	0
	Camilla Creek	-22.6	12.3	252	0
	Camilla Creek	-21.0	14.3	364	0
	Camilla Creek	-22.0	8.1	205	0
	Camilla Creek	-22.7	7.7	165	0
	Camilla Creek	-23.9	10.1	243	0
	Camilla Creek	-21.9	8.9	214	0
	Camilla Creek	-23.1	9.1	183	0
	Camilla Creek	-22.5	7.6	179	0
	Camilla Creek	-24.2	10.7	207	0
	Cobbs Pass	-23.8	7.7	164	0
	Cobbs Pass	-22.0	11.4	217	1
	Cobbs Pass	-23.2	11.6	178	0
	Cobbs Pass	-23.3	12.2	225	1
	Cobbs Pass	-22.5	14.3	251	0
	Cobbs Pass	-23.5	11.6	285	0
	Cobbs Pass	-23.6	8.9	272	0
	Cobbs Pass	-24.0	7.8	251	1

Colorado Pond	-20.8	11.9	255	0
Colorado Pond	-24.5	11.8	269	0
Colorado Pond	-24.9	8.9	271	0
Colorado Pond	-24.0	8.8	273	0
Colorado Pond	-24.2	8.2	234	0
Colorado Pond	-20.9	9.4	251	0
Colorado Pond	-21.6	10.4	205	0
Colorado Pond	-22.7	8.1	285	0
Doctors Creek	-23.6	9.0	155	0
Doctors Creek	-24.4	9.1	207	0
Fox Pass	-22.8	12.4	210	0
Fox Pass	-24.5	13.4	241	0
Green Hill	-24.4	12.8	314	0
Green Hill	-25.3	12.6	214	0
Green Hill	-22.2	9.8	169	0
Head of the Bay	-21.2	12.5	280	0
Head of the Bay	-19.5	13.0	146	0
Head of the Bay	-24.1	10.8	215	0
Head of the Bay	-22.5	11.7	200	0
Head of the Bay	-22.0	11.4	165	0
Herbert	-24.5	9.5	184	0
Herbert	-24.7	9.4	174	1
Herbert	-23.7	8.8	222	0
Herbert	-25.8	15.0	224	0
Malo Arroyo	-24.1	7.9	246	0
Malo Arroyo	-23.9	8.7	220	0
Malo Arroyo	-23.6	8.5	284	0
Malo Arroyo	-23.6	8.7	316	0
Malo Arroyo	-24.0	12.4	355	0
Malo Arroyo	-24.1	7.6	192	2
Moody Brook	-22.6	13.6	244	0
Moody Brook	-22.4	14.5	294	0
Moody Brook	-22.6	13.3	212	0
Pedro	-22.8	11.3	251	0
Pedro	-21.9	9.3	244	0
Pedro	-21.5	8.1	163	0
Pedro	-21.7	7.7	183	0
Swan Inlet	-18.8	11.3	330	0
Swan Inlet	-21.5	9.7	296	0

Appendix 2: Script Chapter 2

```
Invasion model
####################################
####     Brown Trout Invasion Model    ####
####      Jess Minett and Josh Jones      ####
####################################
####Load Libraries ####
library(tidyverse)
library(caret)
library(moments)
library(mgcv)
library(dismo)
library(ggplot2)
library(readr)
library(lme4)
library(broom)
library(gridExtra)
# library(pbkrtest)
library(effects)
library(lmerTest)
library(scales)
library(MuMIn)
library(dismo)
library(pbkrtest)
#library(tidyverse); theme_set(theme_classic())

#### Load Data ####
FIsampled<-read.csv("data/New.FI.Model.SmallDF.csv", header=TRUE)
#DF contains - gridRef, lon, lat, st, eastWest, intro, zebra, basinInv, basinIntro, d2set, d2r, d2culvert, d2introW,
d2introL
#d2invW, d2invL, slope, height, nCulverts, density, fraglen, bfl, FA_scales, winterTmin, annualRain, landType
#changing some of the variables to factors
FIsampled$gridRef<-as.factor(FIsampled$gridRef)
invad<-FIsampled[which(FIsampled$st==1),]
absent<-FIsampled[which(FIsampled$st==0),]
#randomly sampling invaded data = so same number of points as absent data
invad<-invad[sample(nrow(invad), 62),]
#spliting data into testing and training
#arbitarily assign group 1 as the testing data group
testing.group <- 1
#creat a vector of group memberships
group.presence <- kfold(x = invad, k =5)
head(group.presence)
table(group.presence) # should see even representation in each group
#output table shows how many points have been assigned to each of the 5 groups
#separate observations into training and testing groups
presence.train <- invad[group.presence !=testing.group,]
presence.test <- invad[group.presence == testing.group,]
#repeat process for pseudo-asence points
group.background <- kfold (x = absent, k = 5)
background.train<-absent[group.background != testing.group,]
background.test <- absent[group.background == testing.group,]
#combine presence and absence traing and testing data
dframe1<-rbind(presence.train, background.train)
dframe.test<-rbind(presence.test, background.test)

#### Checking Coliniarity of Covariates ####
#Corvif Function https://github.com/biometry/muledeer/blob/master/HighstatLibV6_correlation_functions.R
```

```
## put correlations on the panels,
## with size proportional to the correlations.
panel.cor <- function(x, y, digits=1, prefix="", cex.cor)
{
  usr <- par("usr"); on.exit(par(usr))
  par(usr = c(0, 1, 0, 1))
  r1=cor(x,y,use="pairwise.complete.obs")
  r <- abs(cor(x, y,use="pairwise.complete.obs"))
  txt <- format(c(r1, 0.123456789), digits=digits)[1]
  txt <- paste(prefix, txt, sep="")
  if(missing(cex.cor)) cex <- 0.9/strwidth(txt)
  text(0.5, 0.5, txt, cex = cex * r)
}
panel.smooth2=function (x, y, col = par("col"), bg = NA, pch = par("pch"),
                        cex = 1, col.smooth = "red", span = 2/3, iter = 3, ...)
{
  points(x, y, pch = pch, col = col, bg = bg, cex = cex)
  ok <- is.finite(x) & is.finite(y)
  if (any(ok))
    lines(stats::lowess(x[ok], y[ok], f = span, iter = iter),
          col = 1, ...)
}
panel.lines2=function (x, y, col = par("col"), bg = NA, pch = par("pch"),
                       cex = 1, ...)
{
  points(x, y, pch = pch, col = col, bg = bg, cex = cex)
  ok <- is.finite(x) & is.finite(y)
  if (any(ok)){
    tmp=lm(y[ok]~x[ok])
    abline(tmp)}
}
panel.hist <- function(x, ...)
{
  usr <- par("usr"); on.exit(par(usr))
  par(usr = c(usr[1:2], 0, 1.5) )
  h <- hist(x, plot = FALSE)
  breaks <- h$breaks; nB <- length(breaks)
  y <- h$counts; y <- y/max(y)
  rect(breaks[-nB], 0, breaks[-1], y, col="white", ...)
}
#VIF
myvif <- function(mod) {
  v <- vcov(mod)
  assign <- attributes(model.matrix(mod))$assign
  if (names(coefficients(mod)[1]) == "(Intercept)") {
    v <- v[-1, -1]
    assign <- assign[-1]
  } else warning("No intercept: vifs may not be sensible.")
  terms <- labels(terms(mod))
  n.terms <- length(terms)
  if (n.terms < 2) stop("The model contains fewer than 2 terms")
  if (length(assign) > dim(v)[1] ) {
    diag(tmp_cor)<-0
    if (any(tmp_cor==1.0)){
      return("Sample size is too small, 100% collinearity is present")
    } else {
      return("Sample size is too small")
    }
  }
  R <- cov2cor(v)
```

```
  detR <- det(R)
  result <- matrix(0, n.terms, 3)
  rownames(result) <- terms
  colnames(result) <- c("GVIF", "Df", "GVIF^(1/2Df)")
  for (term in 1:n.terms) {
    subs <- which(assign == term)
    result[term, 1] <- det(as.matrix(R[subs, subs])) * det(as.matrix(R[-subs, -subs])) / detR
    result[term, 2] <- length(subs)
  }
  if (all(result[, 2] == 1)) {
    result <- data.frame(GVIF=result[, 1])
  } else {
    result[, 3] <- result[, 1]^(1/(2 * result[, 2]))
  }
  invisible(result)
}
corvif <- function(dataz) {
  dataz <- as.data.frame(dataz)
  #correlation part
  cat("Correlations of the variables\n\n")
  tmp_cor <- cor(dataz,use="complete.obs")
  print(tmp_cor)
  #vif part
  form    <- formula(paste("fooy ~ ",paste(strsplit(names(dataz)," "),collapse=" + ")))
  dataz   <- data.frame(fooy=1,dataz)
  lm_mod  <- lm(form,dataz)
  cat("\n\nVariance inflation factors\n\n")
  print(myvif(lm_mod))
}
myvif <- function(mod) {
  v <- vcov(mod)
  assign <- attributes(model.matrix(mod))$assign
  if (names(coefficients(mod)[1]) == "(Intercept)") {
    v <- v[-1, -1]
    assign <- assign[-1]
  } else warning("No intercept: vifs may not be sensible.")
  terms <- labels(terms(mod))
  n.terms <- length(terms)
  if (n.terms < 2) stop("The model contains fewer than 2 terms")
  if (length(assign) > dim(v)[1] ) {
    diag(tmp_cor)<-0
    if (any(tmp_cor==1.0)){
      return("Sample size is too small, 100% collinearity is present")
    } else {
      return("Sample size is too small")
    }
  }
  R <- cov2cor(v)
  detR <- det(R)
  result <- matrix(0, n.terms, 3)
  rownames(result) <- terms
  colnames(result) <- c("GVIF", "Df", "GVIF^(1/2Df)")
  for (term in 1:n.terms) {
    subs <- which(assign == term)
    result[term, 1] <- det(as.matrix(R[subs, subs])) * det(as.matrix(R[-subs, -subs])) / detR
    result[term, 2] <- length(subs)
  }
  if (all(result[, 2] == 1)) {
    result <- data.frame(GVIF=result[, 1])
  } else {
```

```
    result[, 3] <- result[, 1]^(1/(2 * result[, 2]))
  }
  invisible(result)
}
corvif <- function(dataz) {
  dataz <- as.data.frame(dataz)
  #correlation part
  cat("Correlations of the variables\n\n")
  tmp_cor <- cor(dataz,use="complete.obs")
  print(tmp_cor)
  #vif part
  form    <- formula(paste("fooy ~ ",paste(strsplit(names(dataz)," "),collapse=" + ")))
  dataz   <- data.frame(fooy=1,dataz)
  lm_mod  <- lm(form,dataz)
  cat("\n\nVariance inflation factors\n\n")
  print(myvif(lm_mod))
}
#need a df that only contains predictors - need to remove gridRef, eastWest, lon, lat and st
#checking coliniarity - removing d2uninvaded also as dont think should be included in model- as it is distance to
nearest known
#(sampled) uninvaded site not true uninvaded site- dont know what means in practice
vifdf <- FIsampled[, c(6:16, 18:27)]
summary(vifdf)
str(vifdf)
# VIF < 3 is good
#https://www.researchgate.net/post/Multicollinearity_issues_is_a_value_less_than_10_acceptable_for_VIF
#https://www.researchgate.net/post/How_high_of_VIF_value_in_regression_can_be_accepted
corvif(vifdf)
#remove d2culvert = 86.2
vifdf <- FIsampled[, c(6:11, 13:16, 18:27)]
corvif(vifdf)
#density = 5.0
vifdf <- FIsampled[, c(6:11, 13:16, 18:20, 22:27)]
corvif(vifdf)
#basinIntro = 3.5
vifdf <- FIsampled[, c(6:8,10,11, 13:16, 18:20, 22:27)]
corvif(vifdf)
#all below 3

#### Building Models ####
#need to change 2 variable numbers as have the wrong way around
#dframe1[55,7]=1
#dframe1[71,7]=0
#now fixed in csv file loaded in r
#try and build a model with everything in it.
#only basinInv not included as model freaks out
model4 <- glm(st ~ d2invL.km. + d2r.km. + zebra + d2invW..km. +  d2introW.km. + d2introL.km. + intro +
eastWest +
              slope + nCulverts + fraglen + FA_scaled + landType + bfl + winterTmin + annualRain + d2set.km. +
height,
            family = binomial(link = "logit"), maxit = 100,
            data = dframe1)  #only basinInv not included
drop1(model4, test = "Chi")
#remove height
model4 <- glm(st ~ d2invL.km. + d2r.km. + zebra + d2invW..km. +  d2introW.km. + d2introL.km. + intro +
eastWest +
              slope + nCulverts + fraglen + FA_scaled + landType + bfl + winterTmin + annualRain + d2set.km.,
            family = binomial(link = "logit"), maxit = 100,
            data = dframe1)
drop1(model4, test = "Chi")
```

```
# remove d2introL.km.
model4 <- glm(st ~ d2invL.km. + d2r.km. + zebra + d2invW..km. +  d2introW.km. + intro + eastWest +
            slope + nCulverts + fraglen + FA_scaled + landType + bfl + winterTmin + annualRain + d2set.km.,
          family = binomial(link = "logit"), maxit = 100,
          data = dframe1)
drop1(model4, test = "Chi")
#remove FA_scaled
model4 <- glm(st ~ d2invL.km. + d2r.km. + zebra + d2invW..km. +  d2introW.km. + intro + eastWest +
            slope + nCulverts + fraglen + landType + bfl + winterTmin + annualRain + d2set.km.,
          family = binomial(link = "logit"), maxit = 100,
          data = dframe1)
drop1(model4, test = "Chi")
# remove intro
model4 <- glm(st ~ d2invL.km. + d2r.km. + zebra + d2invW..km. +  d2introW.km. + eastWest +
            slope + nCulverts + fraglen + landType + bfl + winterTmin + annualRain + d2set.km.,
          family = binomial(link = "logit"), maxit = 100,
          data = dframe1)
drop1(model4, test = "Chi")
#remove annualRain
model4 <- glm(st ~ d2invL.km. + d2r.km. + zebra + d2invW..km. +  d2introW.km. + eastWest +
            slope + nCulverts + fraglen + landType + bfl + winterTmin + d2set.km.,
          family = binomial(link = "logit"), maxit = 100,
          data = dframe1)
drop1(model4, test = "Chi")
#remove bfl
model4 <- glm(st ~ d2invL.km. + d2r.km. + zebra + d2invW..km. +  d2introW.km. + eastWest +
            slope + nCulverts + fraglen + landType + winterTmin + d2set.km.,
          family = binomial(link = "logit"), maxit = 100,
          data = dframe1)
drop1(model4, test = "Chi")
#remove eastWest
model4 <- glm(st ~ d2invL.km. + d2r.km. + zebra + d2invW..km. +  d2introW.km. +
            slope + nCulverts + fraglen + landType + winterTmin + d2set.km.,
          family = binomial(link = "logit"), maxit = 100,
          data = dframe1)
drop1(model4, test = "Chi")
#remove fraglen
model4 <- glm(st ~ d2invL.km. + d2r.km. + zebra + d2invW..km. +  d2introW.km. +
            slope + nCulverts + landType + winterTmin + d2set.km.,
          family = binomial(link = "logit"), maxit = 100,
          data = dframe1)
drop1(model4, test = "Chi")
#remove landType
model4 <- glm(st ~ d2invL.km. + d2r.km. + zebra + d2invW..km. +  d2introW.km. +
            slope + nCulverts + winterTmin + d2set.km.,
          family = binomial(link = "logit"), maxit = 100,
          data = dframe1)
drop1(model4, test = "Chi")
#remove d2r.km.
model4 <- glm(st ~ d2invL.km. + zebra + d2invW..km. +  d2introW.km. +
            slope + nCulverts + winterTmin + d2set.km.,
          family = binomial(link = "logit"), maxit = 100,
          data = dframe1)
drop1(model4, test = "Chi")
#remove d2set.km.
model4 <- glm(st ~ d2invL.km. + zebra + d2invW..km. +  d2introW.km. +
            slope + nCulverts + winterTmin,
          family = binomial(link = "logit"), maxit = 100,
          data = dframe1)
drop1(model4, test = "Chi")
```

```
#remove slope
model4 <- glm(st ~ d2invL.km. + zebra + d2invW..km. +  d2introW.km. +
              nCulverts + winterTmin,
            family = binomial(link = "logit"), maxit = 100,
            data = dframe1)
drop1(model4, test = "Chi")
#remove winterTmin
model4 <- glm(st ~ d2invL.km. + zebra + d2invW..km. +  d2introW.km. +
              nCulverts,
            family = binomial(link = "logit"), maxit = 100,
            data = dframe1)
drop1(model4, test = "Chi")
#remove d2invW..km.
model4 <- glm(st ~ d2invL.km. + zebra +  d2introW.km. +
              nCulverts,
            family = binomial(link = "logit"), maxit = 100,
            data = dframe1)
drop1(model4, test = "Chi")
#remove d2introW.km.
model4 <- glm(st ~ d2invL.km. + zebra +
              nCulverts,
            family = binomial(link = "logit"), maxit = 100,
            data = dframe1)
drop1(model4, test = "Chi")
#all variables significant
inv.mod <- glm(st ~ d2invL.km. + zebra + nCulverts,
             family = binomial(link = "logit"),
             data = dframe1)
summary(inv.mod)
plot(inv.mod)
AIC(inv.mod) #94.343

#### Comparing models for Null Model ####
nullMod <- glm(st ~ 1,
             family = binomial(link = "logit"),
             data = dframe1)
#compare models with anova
#comparing models to Null
anova(nullMod, inv.mod, test = "LRT") # chi p-value = 2.602x10-11
#seem to be significantly different from Null
#LRT = likihood ratio test statistic - assuming LRT has a chi-squared distribution
#PBtest = fraction of simulated LRT values that are greater or equal to the observed LRT value
PBmodcomp(inv.mod, nullMod, nsim = 1000) #LRT = 2.602x10-11     PBtest = 0.01961
#get the same results when you compare with anova or PBmodcomp
#think this means that model is significantly different from random (null model)
#will now try predicting some values using model and seeing how they compare to actual results
#can also do some SDM evaluation - as done with SDM with R

#### SDM - Chapter 7 Model Evaluation ####
#there are a number of different measure that can help access how good a model fits the data
#AUC is a measure of rank correlation -
#high AUC indicates sites with high predicted suitability values are areas of known presence
#locations with model prediction tend to be areas where species are known to be absent
#AUC score of 0.5 = model is as good as ransom guesses
#need to have the presence and absence data split into testing and training datasets
bc.inv <- glm(st ~ d2invL.km. + zebra + nCulverts,
            family = binomial(link = "logit"),
            data = dframe1)
e.inv <- evaluate(dframe.test[dframe.test$st == 1,], dframe.test[dframe.test$st == 0,], bc.inv)
e.inv
```

```
#get 12 presences and 12 absences which is correct
#AUC = 0.847
#cor = 0.600
plot(e.inv, 'ROC')
#now we will try with k-fold instead of a single random sample
#already have df of presence and df of absence =
pres <- invad
back <- absent #background data will only be used for model testing and doesnt need to be partioned
#now need to split data into 5 groups (k=5)
k <- 5
group <- kfold(pres, k)
group[1:10]
unique(group)
#now we can fit and test the model 5 times
e <- list()
for (i in 1:k) {
  train <- pres[group !=i,]
  test <- pres[group == i,]
  bc <- glm(st ~ d2invL.km. + zebra + nCulverts,
            family = binomial(link = "logit"),
            data = dframe1)
  e[[i]] <- evaluate(p=test, a=back, bc)
}
#we can extract ment things from onjects in e - just looking at AUC, max sum of sensitivity (true positive rate),
and
#specificity (true negative rate) - sometimes used as threshold for setting presence/absence
auc <- sapply(e, function (x){slot(x, 'auc')})
auc
#0.864  0.855 0.895 0.916 0.828
mean(auc) #0.872
sapply(e, function(x){x@t[which.max(x@TPR + x@TNR)]})
#-0.463 -0.4638  -0.589 0.232  -1.122

#### LOOCV - Prediciting Results ####
#going to try and predict results for model
#combining dframe1 and dframe.test so i have a larger df n=124 containing the same number of presnce and
absence records
dframe.all <- rbind(dframe1, dframe.test)
#write.csv(file = "output/EqualPresAbsData.csv", dframe.all)
#Try prediciting for inv.mod
#first have to define training control
train.control <- trainControl(method = "LOOCV")

model.inv <- train(st ~ d2invL.km. + zebra + nCulverts,
                   data = dframe1, method = "glm",
                   family = binomial(link = "logit"),
                   trControl = train.control)
#summarise results
print(model.2)
#RMSE = 0.383   R2 = 0.415    MAE = 0.286
predict.inv <- predict(model.inv, dframe.all)
summary(predict.inv) # ranges from 0.000 to 0.995
inv.predicitions <- cbind(predict.inv, dframe.all)
write.csv(file = "output/model_inv_Predictions.csv", inv.predicitions)
# Now have predicted results for dframe.all for inv.mod - now need to compare predictions

#### Predicting Invasion for All Points ####
invasionData_All <- read.csv("data/InvasionModel_DataNeeded.csv", header = TRUE)
#this is a df containing data for all ~8800 points for gridRef, lon, lat, st, zebra, d2invL, nculverts (what needed
for model)
```

```
#zebra = 1 or 0 (o = sites know dont inhabit or sites we dont know if present or not (sites that havent been
sampled))
summary(invasionData_All)
#Invasion Model
#First define training control
train.control <- trainControl(method = "LOOCV")
#Training Model
invasion.model <- train (st ~ d2invL.km. + zebra + nCulverts,
                         data = dframe1, method = "glm",
                         family = binomial (link = "logit"),
                         trControl = train.control)
#Summarise Results
print(invasion.model)
invasion.predict <- predict(invasion.model, invasionData_All)
summary(invasion.predict)
#ranges from 0.000 to 0.998
InvasionPredictions <- cbind(invasion.predict, invasionData_All)
write.csv(file = "output/InvasionPredictions.csv", InvasionPredictions)



Invasion scenarios
##########################################################
####      Invasion Scenario Predictions with Standard Error      ####
####                    Jess Minett and Josh Jones                    ####
##########################################################
#### Packages ####
library(tidyverse)
library(sf)
library(caret)
#install.packages("geodist")
library(geodist)
set.seed(321)
#### Data ####
testdata <- read.csv("data/Scenario1_SEdata.csv", header = TRUE) #scenario 1 with upper and lower SE
#testdata <- read.csv("data/Scenario2_SEdata.csv", header = TRUE) #scenario 2 (-0.1) data with upper and
lower SE
#testdata <- read.csv("data/Scenario3_SEdata.csv", header = TRUE) #scenario 3 (-0.3) data with upper and
lower SE
testdata <- testdata %>%
  mutate(st = if_else(is.na(st), 0 , as.numeric(st))) %>%
  rename(invaded = st)
dframe1 <- read.csv("data/dframe1 data for loocv.csv", header = TRUE) #data for training model
#rename st to invaded and distance to invaded to nn_dist
names(dframe1)[names(dframe1)=="st"] <- "invaded"
names(dframe1)[names(dframe1)=="d2invL.km."] <- "nn_dist"

#### Quick distance calculations ####
# special projection for quick distance calculation
(wkt <- 'GEOGCRS["spherical",
  DATUM["MySphere",
  ELLIPSOID["WGS 84Sphere", 6378137, 0,
  LENGTHUNIT["metre",1]]],
  PRIMEM["Greenwich",0,
  ANGLEUNIT["degree",0.0174532925199433]],
  CS[ellipsoidal,2],
  AXIS["geodetic latitude (Lat)",north,
  ORDER[1],
  ANGLEUNIT["degree",0.0174532925199433]],
  AXIS["geodetic longitude (Lon)",east,
```

```
  ORDER[2],
  ANGLEUNIT["degree",0.0174532925199433]],
  USAGE[
  SCOPE["unknown"],
  AREA["World"],
  BBOX[-90,-180,90,180]]
  ]')
sph <- st_crs(wkt)

#### Invasion Model ####
# no need to retrain the model each time
#First define training control
train.control <- trainControl(method = "LOOCV")
#Training Model
suppressWarnings(invasion.model <- train (invaded ~ nn_dist + zebra + nCulverts,
                                  data = dframe1, method = "glm",
                                  family = binomial (link = "logit"),
                                  trControl = train.control))

#### Scenario 1 - no containment ####
invade <- function(x) {
  x1 <- x #making new df of test data = x1
  n <- 0 #number of runs
  invaded_vec <- c(0) #invaded vector
  n_vec <- c(0)
  n_uninvaded <- x1 %>%
    filter(invaded != 1) %>%
    nrow() #filtering out only uninvaded sites
  n_invaded <- x1 %>%
    filter(invaded == 1) %>%
    nrow() #filtering out only invaded sites
  message("run number ", n) #prints message for run number
  message("n invaded ", n_invaded) #prints message for number of invaded sites - start with 72 (sites sampled)
  message("n uninvaded = ", n_uninvaded) #prints message for number of uninvaded sites - start with 62 (sites
sampled)
  # keep invading until all cells are invaded
  # or n threshold is reached
  #this tells function to run until reach number of runs want or all sites become invaded
  while (nrow(x1) > 1) { #n_uninvaded > 1 - would get results output if less than 50
    #at the start of each run it will reprint number of runs, number invaded, and number uninvaded (should
change between runs)
    message("run number ", n)
    message("n invaded ", n_invaded)
    message("n uninvaded = ", n_uninvaded)
    invaded <- x1 %>%
      filter(invaded == 1) #Filter only invaded sites
    uninvaded <- x1 %>%
      filter(invaded != 1) #Filter only uninvaded sites
    #roll dice for only uninvaded sites - this determines
    # which uninvaded sites could become invaded based on invasion prob
    uninvaded$invaded <- rbinom(length(uninvaded$invasion.predict),
                                size = 1,
                                prob = uninvaded$invasion.predict)
    uninvaded_invaded <- uninvaded %>%
      filter(invaded == 1)
    uninvaded_uninvaded <- uninvaded %>%
      filter(invaded != 1)
    # Then sample those sites and set that random sample to 0
    # use runif() to get a random number between 1 and nrow(uninvaded)
    uninvaded_sample <- uninvaded_invaded[sample(nrow(uninvaded_invaded),
```

```
                                    runif(1, 1, nrow(uninvaded_invaded))), ]
  # set the sample to be uninvaded
  uninvaded_sample$invaded <- 0
  # get a list of gridRefs that have had the invasion revered
  not_invaded <- unique(uninvaded_sample$gridRef)
  # get the rest of the uninvaded_invaded that weren't reversed
  uninvaded_invaded <- uninvaded_invaded %>%
    filter(!gridRef %in% not_invaded)
  # remove those gridRefs from uninvaded_invaded
  # and add the other uninvaded sites
  # this is now all uninvaded sites
  uninvaded_with_reversed <- uninvaded_uninvaded %>%
    add_row(uninvaded_sample)
  # rejoin all the data
  x1 <- invaded %>%
    add_row(uninvaded_with_reversed) %>%
    add_row(uninvaded_invaded)
  x1$n <- n #number of run
  sum_invaded <- sum(x1$invaded) #number become invaded
  sum_prob <- sum(x1$invasion.predict) #sum of probabilities from all invaded sites
  #sum_invaded and sum_prob should increase with each run (as long as a site has become invaded)
  # update the prob ability if a site becomes invaded
  #where a site has become invaded we update the invasion probability to 1
  x1 <- x1 %>%
    mutate(invasion.predict = if_else(invaded == 1, 1, invasion.predict))
  message(sum_invaded, " cells invaded in total.") #prints sum of invaded sites (how many sites invaded)
  message(sum_prob, " = sum of prob. Should increase.") #prints of of invasion probability (sum of prob for all
sites invaded)
  #now need to re speaparate uninvaded and invaded sites as site have become invaded through rolling dice on
probability
  # get uninvaded sites
  # e.g. uninvaded <- x1 %>% filter(invaded != 1)
  uninvaded <- x1 %>% filter(invaded != 1) #filtering to look at only uninvaded sites
  message("nrow(uninvaded ", nrow(uninvaded)) #prints number of uninvaded sites
  # get invaded sites
  # e.g. invaded <- x1 %>% filter(invaded == 1)
  invaded <- x1 %>% filter(invaded == 1) #filtering to get only invaded sites
  message("nrow(invaded ", nrow(invaded)) # prints number of invaded sites
  #Need to get sf object of all uninvaded and invaded sites to recalcualte distatnce to nearest invaded site
  # all invaded sites with lat lon in sf object
  # MAKE it an sf object
  invaded_sf <- st_as_sf(x = invaded,
                         coords = c("lon", "lat"),
                         crs = sph) %>%
    rename(id = gridRef) #renaming gridref as id as called id in distance function above
  # all uninvaded sites with lat lon in sf object
  # MAKE it an sf object
  uninvaded_sf <- st_as_sf(x = uninvaded,
                           coords = c("lon", "lat"),
                           crs = sph) %>%
    rename(id = gridRef) #renaming gridref as id as called id in distance function above
  #make matrix of invaded and uninvaded sites - need this for the nn_geo function
  # recalculate distance to nearest invaded site
  a1 <- st_coordinates(uninvaded_sf)
  message(nrow(a1)) #prints number of invaded sites
  b1 <- st_coordinates(invaded_sf)
  # nearest neighbor function
  # Ignore the >100 km warning
  # as these will have a low prop of
  # being invaded anyway
```

```
  nn_geo <- function(i) {
    from <- a1[i, ]
    to <- b1
    # Great Circle distance since in lat/lon
    dist <- geodist(from, to)
    min_dist <- min(dist)/1000 #converts m to km
    # message("distance to nearest neighbour, excluding self and repeat pairs = ", min_dist)
    return(c(i, min_dist))
  }
  #nn_geo measures distance from all uninvaded sites in circles to all invaded sites - then select for the smallest distance
  # this is all of the uninvaded sites with a distance to nearest invaded site column
  out <- as.data.frame(do.call(rbind,
                               lapply(seq(1:nrow(a1)),
                                      nn_geo))) %>%
    rename(gridRef = V1,
           nn_dist = V2)
  #for every uninvaded site we get the site id and the distance to the nearest invaded site in km
  #we then need to recover gridref id which we can get from uninvaded df
  # recover id
  out$gridRef <- uninvaded_sf$id
  # add the other variables back to this df
  # e.g.
  # outModel <- out %>%
  #  left_join(siteVariables, by = "gridRef")
  outModel <- out %>%
    full_join(uninvaded, by = "gridRef") #added all varaibles and new distances back together
  # message("colnames(outModel) ", colnames(outModel))
  # re run glm to get new probabilities using new distances
  #Summarise Results
  invasion.predict <- predict(invasion.model, outModel)
  #adding new probailities to uninvaded
  uninvaded$invasion.predict <- invasion.predict
  #uninvaded doesnt have new distances nn_dist but dont think this matters as is recalculated in outmodel each time
  #and outmodel is used to calculate the new invasion.predict
  #now need to reduce any probabilities <0.8 by 0.1 for scenario 2 and 0.3 for scenario 3 and set min probability to
  n_uninvaded <- x1 %>%
    filter(invaded != 1) %>%
    nrow() #get new number of uninvaded sites
  ####
  ## add new probabilities
  #uninvaded$invasion.predict <- invasion.predict
  message("uninvaded nrow ", n_uninvaded) #prints number of uninvaded sites
  # x1 needs to be all sites, invaded and uninvaded
  # this is take back to the top of the while statement
  # and processed again
  x1 <- invaded %>% add_row(uninvaded) #add uninvaded sites back into x1 with new invasion probabilities for uninvaded sites
  message("all sites (x1) nrow ", nrow(x1)) #prints number of sites in x1 - should be 8813
  n <- n + 1 # addes a 1 to the number of runs - will increase each time and stop running when reach number of cycles wanted
  message("n cycles = ", n)
  # outputs
  n_vec <- append(n_vec, n) #vector of number of runns
  n_invaded <- nrow(x) - n_uninvaded # number of invaded sites
  invaded_vec <- append(invaded_vec, n_invaded) #number of uninvaded sites
  #this will then cycle back upto the while function until we reach a specified number of runs (50)
  #or all sites become invaded
```

```
    # message("n_vec ", n_vec)
    # message("invaded_vec ", invaded_vec)
    if (nrow(uninvaded_invaded) <3) { # you might want to play with thresholds
      message("All cells invaded, well nearly. Stopping….")
      return(c(x1, data.frame(n_vec, invaded_vec)))
      stop()
      if (n == 50) {
        message("1000 runs complete. Stopping")
        return(c(x1, data.frame(n_vec, invaded_vec)))
        stop()
      }
    }
  }
}

#Data
testdata <- read.csv("data/Scenario1_SEdata.csv", header = TRUE) #scenario 1 with upper and lower SE
testdata <- testdata %>%
  mutate(st = if_else(is.na(st), 0 , as.numeric(st))) %>%
  rename(invaded = st)
#use the function to run as many repeats/iterations that would want one after another
#Runnign Results
b <- 1
df1 <- data.frame(n_vec = as.numeric(),
                  invaded_vec = as.numeric())
while (b<300){
  output <- invade(testdata)
  temp <- data.frame(matrix(unlist(output[12:13]), nrow = length(output[12:13]), byrow = TRUE))
  b <- b + 1
  temp <- as.data.frame(t(temp))
  names(temp)[names(temp) == "V1"] <- "n_vec"
  names(temp)[names(temp) == "V2"] <- "invaded_vec"
  df1 <- bind_rows(df1, temp)
}
#df to store all repeats data in
df_scen1 <- data.frame(n_vec = as.numeric(),
                       invaded_vec = as.numeric())
df_scen1 <- bind_rows(df_scen1, df1) #bind data = add new rows for each run (1-50)
write.csv(df_scen1, "output/df_scen1_raw.csv")

#### Secenario 2 - moderate containment ####
#Update function to account for -0.1 in invasion probability
invade2 <- function(x) {
  x1 <- x #making new df of test data = x1
  n <- 0 #number of runs
  invaded_vec <- c(0) #invaded vector
  n_vec <- c(0)
  n_uninvaded <- x1 %>%
    filter(invaded != 1) %>%
    nrow() #filtering out only uninvaded sites
  n_invaded <- x1 %>%
    filter(invaded == 1) %>%
    nrow() #filtering out only invaded sites
  message("run number ", n) #prints message for run number
  message("n invaded ", n_invaded) #prints message for number of invaded sites - start with 72 (sites sampled)
  message("n uninvaded = ", n_uninvaded) #prints message for number of uninvaded sites - start with 62 (sites
sampled)
  # keep invading until all cells are invaded
  # or n threshold is reached
  #this tells function to run until reach number of runs want or all sites become invaded
```

```
  while (nrow(x1) > 1) { #n_uninvaded > 1 - would get results output if less than 50
    #at the start of each run it will reprint number of runs, number invaded, and number uninvaded (should
change between runs)
    message("run number ", n)
    message("n invaded ", n_invaded)
    message("n uninvaded = ", n_uninvaded)
    invaded <- x1 %>%
      filter(invaded == 1) #Filter only invaded sites
    uninvaded <- x1 %>%
      filter(invaded != 1) #Filter only uninvaded sites
    #roll dice for only uninvaded sites - this determines
    # which uninvaded sites could become invaded based on invasion prob
    uninvaded$invaded <- rbinom(length(uninvaded$invasion.predict),
                                size = 1,
                                prob = uninvaded$invasion.predict)
    uninvaded_invaded <- uninvaded %>%
      filter(invaded == 1)
    uninvaded_uninvaded <- uninvaded %>%
      filter(invaded != 1)
    # Then sample those sites and set that random sample to 0
    # use runif() to get a random number between 1 and nrow(uninvaded)
    uninvaded_sample <- uninvaded_invaded[sample(nrow(uninvaded_invaded),
                                                 runif(1, 1, nrow(uninvaded_invaded))), ]
    # set the sample to be uninvaded
    uninvaded_sample$invaded <- 0
    # get a list of gridRefs that have had the invasion revered
    not_invaded <- unique(uninvaded_sample$gridRef)
    # get the rest of the uninvaded_invaded that weren't reversed
    uninvaded_invaded <- uninvaded_invaded %>%
      filter(!gridRef %in% not_invaded)
    # remove those gridRefs from uninvaded_invaded
    # and add the other uninvaded sites
    # this is now all uninvaded sites
    uninvaded_with_reversed <- uninvaded_uninvaded %>%
      add_row(uninvaded_sample)
    # rejoin all the data
    x1 <- invaded %>%
      add_row(uninvaded_with_reversed) %>%
      add_row(uninvaded_invaded)
    x1$n <- n #number of run
    sum_invaded <- sum(x1$invaded) #number become invaded
    sum_prob <- sum(x1$invasion.predict) #sum of probabilities from all invaded sites
    #sum_invaded and sum_prob should increase with each run (as long as a site has become invaded)
    # update the prob ability if a site becomes invaded
    #where a site has become invaded we update the invasion probability to 1
    x1 <- x1 %>%
      mutate(invasion.predict = if_else(invaded == 1, 1, invasion.predict))
    message(sum_invaded, " cells invaded in total.") #prints sum of invaded sites (how many sites invaded)
    message(sum_prob, " = sum of prob. Should increase.") #prints of of invasion probability (sum of prob for all
sites invaded)
    #now need to re speaparate uninvaded and invaded sites as site have become invaded through rolling dice on
probability
    # get uninvaded sites
    # e.g. uninvaded <- x1 %>% filter(invaded != 1)
    uninvaded <- x1 %>% filter(invaded != 1) #filtering to look at only uninvaded sites
    message("nrow(uninvaded ", nrow(uninvaded)) #prints number of uninvaded sites
    # get invaded sites
    # e.g. invaded <- x1 %>% filter(invaded == 1)
    invaded <- x1 %>% filter(invaded == 1) #filtering to get only invaded sites
    message("nrow(invaded ", nrow(invaded)) # prints number of invaded sites
```

```
  #Need to get sf object of all uninvaded and invaded sites to recalcualte distatnce to nearest invaded site
  # all invaded sites with lat lon in sf object
  # MAKE it an sf object
  invaded_sf <- st_as_sf(x = invaded,
                         coords = c("lon", "lat"),
                         crs = sph) %>%
    rename(id = gridRef) #renaming gridref as id as called id in distance function above
  # all uninvaded sites with lat lon in sf object
  # MAKE it an sf object
  uninvaded_sf <- st_as_sf(x = uninvaded,
                           coords = c("lon", "lat"),
                           crs = sph) %>%
    rename(id = gridRef) #renaming gridref as id as called id in distance function above
  #make matrix of invaded and uninvaded sites - need this for the nn_geo function
  # recalculate distance to nearest invaded site
  a1 <- st_coordinates(uninvaded_sf)
  message(nrow(a1)) #prints number of invaded sites
  b1 <- st_coordinates(invaded_sf)
  # nearest neighbor function
  # Ignore the >100 km warning
  # as these will have a low prop of
  # being invaded anyway
  nn_geo <- function(i) {
    from <- a1[i, ]
    to <- b1
    # Great Circle distance since in lat/lon
    dist <- geodist(from, to)
    min_dist <- min(dist)/1000 #converts m to km
    # message("distance to nearest neighbour, excluding self and repeat pairs = ", min_dist)
    return(c(i, min_dist))
  }
  #nn_geo measures distance from all uninvaded sites in circles to all invaded sites - then select for the smallest
distance
  # this is all of the uninvaded sites with a distance to nearest invaded site column
  out <- as.data.frame(do.call(rbind,
                               lapply(seq(1:nrow(a1)),
                                      nn_geo))) %>%
    rename(gridRef = V1,
           nn_dist = V2)
  #for every uninvaded site we get the site id and the distance to the nearest invaded site in km
  #we then need to recover gridref id which we can get from uninvaded df
  # recover id
  out$gridRef <- uninvaded_sf$id
  # add the other variables back to this df
  # e.g.
  # outModel <- out %>%
  #  left_join(siteVariables, by = "gridRef")
  outModel <- out %>%
    full_join(uninvaded, by = "gridRef") #added all varaibles and new distances back together
  # message("colnames(outModel) ", colnames(outModel))
  # re run glm to get new probabilities using new distances
  #Summarise Results
  invasion.predict <- predict(invasion.model, outModel)
  #adding new probailities to uninvaded
  uninvaded$invasion.predict <- invasion.predict
  #uninvaded doesnt have new distances nn_dist but dont think this matters as is recalculated in outmodel each
time
  #and outmodel is used to calculate the new invasion.predict
  #now need to reduce any probabilities <0.8 by 0.1 for scenario 2 and 0.3 for scenario 3 and set min
probability to
```

```
    uninvaded <- uninvaded %>%
      mutate(invasion.predict = if_else(invasion.predict <0.8, invasion.predict-0.1, invasion.predict)) %>%
      mutate(invasion.predict = if_else(invasion.predict <0, 0, invasion.predict))
    n_uninvaded <- x1 %>%
      filter(invaded != 1) %>%
      nrow() #get new number of uninvaded sites
    ####
    ## add new probabilities
    #uninvaded$invasion.predict <- invasion.predict
    message("uninvaded nrow ", n_uninvaded) #prints number of uninvaded sites
    # x1 needs to be all sites, invaded and uninvaded
    # this is take back to the top of the while statement
    # and processed again
    x1 <- invaded %>% add_row(uninvaded) #add uninvaded sites back into x1 with new invasion probabilities
for uninvaded sites
    message("all sites (x1) nrow ", nrow(x1)) #prints number of sites in x1 - should be 8813
    n <- n + 1 # addes a 1 to the number of runs - will increase each time and stop running when reach number of
cycles wanted
    message("n cycles = ", n)
    # outputs
    n_vec <- append(n_vec, n) #vector of number of runns
    n_invaded <- nrow(x) - n_uninvaded # number of invaded sites
    invaded_vec <- append(invaded_vec, n_invaded) #number of uninvaded sites
    #this will then cycle back upto the while function until we reach a specified number of runs (50)
    #or all sites become invaded
    # message("n_vec ", n_vec)
    # message("invaded_vec ", invaded_vec)
    if (nrow(uninvaded_invaded) <3) { # you might want to play with thresholds
      message("All cells invaded, well nearly. Stopping….")
      return(c(x1, data.frame(n_vec, invaded_vec)))
      stop()
      if (n == 50) {
        message("1000 runs complete. Stopping")
        return(c(x1, data.frame(n_vec, invaded_vec)))
        stop()
      }
    }
  }
}

#Running 300 times = 300 repeats to build CI
# Data ##
testdata <- read.csv("data/Scenario2_SEdata.csv", header = TRUE) #scenario 1 with upper and lower SE
testdata <- testdata %>%
  mutate(st = if_else(is.na(st), 0 , as.numeric(st))) %>%
  rename(invaded = st)

#repeating 300 times
b <- 0
df2 <- data.frame(n_vec = as.numeric(),
                  invaded_vec = as.numeric()) #clear anything previously stored in df1
while (b<300){
  output <- invade2(testdata)
  temp <- data.frame(matrix(unlist(output[12:13]), nrow = length(output[12:13]), byrow = TRUE))
  b <- b + 1
  temp <- as.data.frame(t(temp))
  names(temp)[names(temp) == "V1"] <- "n_vec"
  names(temp)[names(temp) == "V2"] <- "invaded_vec"
  df2 <- bind_rows(df2, temp)
}
```

```
#df to store all repeats data in
df_scen2 <- data.frame(n_vec = as.numeric(),
                       invaded_vec = as.numeric())
df_scen2 <- bind_rows(df_scen2, df2) #bind data = add new rows for each run (1-50)
write.csv(df_scen2, "output/df_scen2_raw.csv")

#### Scenario 3 - strong containment ####
invade3 <- function(x) {
  x1 <- x #making new df of test data = x1
  n <- 0 #number of runs
  invaded_vec <- c(0) #invaded vector
  n_vec <- c(0)
  n_uninvaded <- x1 %>%
    filter(invaded != 1) %>%
    nrow() #filtering out only uninvaded sites
  n_invaded <- x1 %>%
    filter(invaded == 1) %>%
    nrow() #filtering out only invaded sites
  message("run number ", n) #prints message for run number
  message("n invaded ", n_invaded) #prints message for number of invaded sites - start with 72 (sites sampled)
  message("n uninvaded = ", n_uninvaded) #prints message for number of uninvaded sites - start with 62 (sites
sampled)
  # keep invading until all cells are invaded
  # or n threshold is reached
  #this tells function to run until reach number of runs want or all sites become invaded
  while (nrow(x1) > 1) { #n_uninvaded > 1 - would get results output if less than 50
    #at the start of each run it will reprint number of runs, number invaded, and number uninvaded (should
change between runs)
    message("run number ", n)
    message("n invaded ", n_invaded)
    message("n uninvaded = ", n_uninvaded)
    invaded <- x1 %>%
      filter(invaded == 1) #Filter only invaded sites
    uninvaded <- x1 %>%
      filter(invaded != 1) #Filter only uninvaded sites
    #roll dice for only uninvaded sites - this determines
    # which uninvaded sites could become invaded based on invasion prob
    uninvaded$invaded <- rbinom(length(uninvaded$invasion.predict),
                                size = 1,
                                prob = uninvaded$invasion.predict)
    uninvaded_invaded <- uninvaded %>%
      filter(invaded == 1)
    uninvaded_uninvaded <- uninvaded %>%
      filter(invaded != 1)
    # Then sample those sites and set that random sample to 0
    # use runif() to get a random number between 1 and nrow(uninvaded)
    uninvaded_sample <- uninvaded_invaded[sample(nrow(uninvaded_invaded),
                                                 runif(1, 1, nrow(uninvaded_invaded))), ]
    # set the sample to be uninvaded
    uninvaded_sample$invaded <- 0
    # get a list of gridRefs that have had the invasion revered
    not_invaded <- unique(uninvaded_sample$gridRef)
    # get the rest of the uninvaded_invaded that weren't reversed
    uninvaded_invaded <- uninvaded_invaded %>%
      filter(!gridRef %in% not_invaded)
    # remove those gridRefs from uninvaded_invaded
    # and add the other uninvaded sites
    # this is now all uninvaded sites
    uninvaded_with_reversed <- uninvaded_uninvaded %>%
      add_row(uninvaded_sample)
```

```
  # rejoin all the data
  x1 <- invaded %>%
    add_row(uninvaded_with_reversed) %>%
    add_row(uninvaded_invaded)
  x1$n <- n #number of run
  sum_invaded <- sum(x1$invaded) #number become invaded
  sum_prob <- sum(x1$invasion.predict) #sum of probabilities from all invaded sites
  #sum_invaded and sum_prob should increase with each run (as long as a site has become invaded)
  # update the prob ability if a site becomes invaded
  #where a site has become invaded we update the invasion probability to 1
  x1 <- x1 %>%
    mutate(invasion.predict = if_else(invaded == 1, 1, invasion.predict))
  message(sum_invaded, " cells invaded in total.") #prints sum of invaded sites (how many sites invaded)
  message(sum_prob, " = sum of prob. Should increase.") #prints of of invasion probability (sum of prob for all
sites invaded)
  #now need to re speaparate uninvaded and invaded sites as site have become invaded through rolling dice on
probability
  # get uninvaded sites
  # e.g. uninvaded <- x1 %>% filter(invaded != 1)
  uninvaded <- x1 %>% filter(invaded != 1) #filtering to look at only uninvaded sites
  message("nrow(uninvaded ", nrow(uninvaded)) #prints number of uninvaded sites
  # get invaded sites
  # e.g. invaded <- x1 %>% filter(invaded == 1)
  invaded <- x1 %>% filter(invaded == 1) #filtering to get only invaded sites
  message("nrow(invaded ", nrow(invaded)) # prints number of invaded sites
  #Need to get sf object of all uninvaded and invaded sites to recalcualte distatnce to nearest invaded site
  # all invaded sites with lat lon in sf object
  # MAKE it an sf object
  invaded_sf <- st_as_sf(x = invaded,
                         coords = c("lon", "lat"),
                         crs = sph) %>%
    rename(id = gridRef) #renaming gridref as id as called id in distance function above
  # all uninvaded sites with lat lon in sf object
  # MAKE it an sf object
  uninvaded_sf <- st_as_sf(x = uninvaded,
                           coords = c("lon", "lat"),
                           crs = sph) %>%
    rename(id = gridRef) #renaming gridref as id as called id in distance function above
  #make matrix of invaded and uninvaded sites - need this for the nn_geo function
  # recalculate distance to nearest invaded site
  a1 <- st_coordinates(uninvaded_sf)
  message(nrow(a1)) #prints number of invaded sites
  b1 <- st_coordinates(invaded_sf)
  # nearest neighbor function
  # Ignore the >100 km warning
  # as these will have a low prop of
  # being invaded anyway
  nn_geo <- function(i) {
    from <- a1[i, ]
    to <- b1
    # Great Circle distance since in lat/lon
    dist <- geodist(from, to)
    min_dist <- min(dist)/1000 #converts m to km
    # message("distance to nearest neighbour, excluding self and repeat pairs = ", min_dist)
    return(c(i, min_dist))
  }
  #nn_geo measures distance from all uninvaded sites in circles to all invaded sites - then select for the smallest
distance
  # this is all of the uninvaded sites with a distance to nearest invaded site column
  out <- as.data.frame(do.call(rbind,
```

```
                        lapply(seq(1:nrow(a1)),
                               nn_geo))) %>%
    rename(gridRef = V1,
           nn_dist = V2)
  #for every uninvaded site we get the site id and the distance to the nearest invaded site in km
  #we then need to recover gridref id which we can get from uninvaded df
  # recover id
  out$gridRef <- uninvaded_sf$id
  # add the other variables back to this df
  # e.g.
  # outModel <- out %>%
  #  left_join(siteVariables, by = "gridRef")
  outModel <- out %>%
    full_join(uninvaded, by = "gridRef") #added all varaibles and new distances back together
  # message("colnames(outModel) ", colnames(outModel))
  # re run glm to get new probabilities using new distances
  #Summarise Results
  invasion.predict <- predict(invasion.model, outModel)
  #adding new probailities to uninvaded
  uninvaded$invasion.predict <- invasion.predict
  #uninvaded doesnt have new distances nn_dist but dont think this matters as is recalculated in outmodel each
time
  #and outmodel is used to calculate the new invasion.predict
  #now need to reduce any probabilities <0.8 by 0.1 for scenario 2 and 0.3 for scenario 3 and set min
probability to
  uninvaded <- uninvaded %>%
    mutate(invasion.predict = if_else(invasion.predict <0.8, invasion.predict-0.3, invasion.predict)) %>%
    mutate(invasion.predict = if_else(invasion.predict <0, 0, invasion.predict))
  n_uninvaded <- x1 %>%
    filter(invaded != 1) %>%
    nrow() #get new number of uninvaded sites
  ####
  ## add new probabilities
  #uninvaded$invasion.predict <- invasion.predict
  message("uninvaded nrow ", n_uninvaded) #prints number of uninvaded sites
  # x1 needs to be all sites, invaded and uninvaded
  # this is take back to the top of the while statement
  # and processed again
  x1 <- invaded %>% add_row(uninvaded) #add uninvaded sites back into x1 with new invasion probabilities
for uninvaded sites
  message("all sites (x1) nrow ", nrow(x1)) #prints number of sites in x1 - should be 8813
  n <- n + 1 # addes a 1 to the number of runs - will increase each time and stop running when reach number of
cycles wanted
  message("n cycles = ", n)
  # outputs
  n_vec <- append(n_vec, n) #vector of number of runns
  n_invaded <- nrow(x) - n_uninvaded # number of invaded sites
  invaded_vec <- append(invaded_vec, n_invaded) #number of uninvaded sites
  #this will then cycle back upto the while function until we reach a specified number of runs (50)
  #or all sites become invaded
  # message("n_vec ", n_vec)
  # message("invaded_vec ", invaded_vec)
  if (nrow(uninvaded_invaded) <3) { # you might want to play with thresholds
    message("All cells invaded, well nearly. Stopping….")
    return(c(x1, data.frame(n_vec, invaded_vec)))
    stop()
    if (n == 50) {
      message("1000 runs complete. Stopping")
      return(c(x1, data.frame(n_vec, invaded_vec)))
      stop()
```

```
    }
   }
  }
}
# Data ##
testdata <- read.csv("data/Scenario3_SEdata.csv", header = TRUE) #scenario 1 with upper and lower SE
testdata <- testdata %>%
  mutate(st = if_else(is.na(st), 0 , as.numeric(st))) %>%
  rename(invaded = st)
#Runnign Results
#repeating 200-500 times
b <- 0
df3 <- data.frame(n_vec = as.numeric(),
                  invaded_vec = as.numeric()) #clear anything previously stored in df1
while (b<300){
  output <- invade3(testdata)
  temp <- data.frame(matrix(unlist(output[12:13]), nrow = length(output[12:13]), byrow = TRUE))
  b <- b + 1
  temp <- as.data.frame(t(temp))
  names(temp)[names(temp) == "V1"] <- "n_vec"
  names(temp)[names(temp) == "V2"] <- "invaded_vec"
  df3 <- bind_rows(df3, temp)
}
df_scen3 <- data.frame(n_vec = as.numeric(),
                       invaded_vec = as.numeric())
df_scen3 <- bind_rows(df_scen3, df3) #bind data = add new rows for each run (1-50)
write.csv(df_scen3, "output/df_scen3_raw.csv")
```

Appendix 3: Ethics and health and safety

Approval No: SU-Ethics-Student-160118/307

Reference No: STU_BIOL_82474_261017111138_2

Approval No: SU-Ethics-Student-090118/299

1. Title of research project/teaching activity:

Brown trout in the Falklands: Invasive ecology, population structure and genetic diversity

2. College:

Science

3. Staff contact:

Carlos Garcia De Leaniz - [redacted]

4. Summary of project/activity:

Acoustic/ combined acoustic-radio tagging: Up to 80 brown trout (>17.5 cm) caught from electrofishing will be anesthetized (2 phenoxy-ethanol) one at a time, fish will then be placed into a V-trough board covered with wetted absorbent towel, ventral side up. Acoustic or combined radio-acoustic tags (VEMCO model V13 13 mm x 36 mm, weight in water 6 g) will be sterilized for a minimum of 15 minutes in a iodine solution before use and all hands and work surfaces will be washed with the same solution. A small mid-ventral incision will be made starting 1cm anterior to the pelvic girdle. The disinfected tag will then be rinsed in sterile saline and positioned to lie directly under the incision in the pectoral cavity. The incision will be closed with three simple interrupted stitches tied with surgeon's knots. The closed incision will then be cleaned with iodine and a small abound of liquid tissue adhesive. After surgery, fish will be placed in a recovery tank and allowed to recover from anesthesia before being released. The operation will be carried under veterinary supervision or by trained member of the team signed off by the local veterinary. Please note this is NOT a regulated procedure in the Falkland Islands and does not require a project licence there.

The number of fish to be tagged (60-80 over 2 years) has been calculated based on the advice of the local sponsor, the need to sample 6 watersheds (10-12 fish per watershed) and the results from other studies (Crossin et al al,.2016; Cons Physiology 4: 2-12) The local sponsors (SAERI Falkland Islands, Falklands Conservation, Env Dept) are handling the local ethical application and will also issue the necessary sampling permits.

Environmental DNA will allow us to detect the presence of brown trout and native galaxiids and predict the number of individuals present in an area. However, to gain information on the health and reproductive status of the population we will need to catch (via electrofishing) and examine individuals. Electrofishing will also give us the opportunity to tag individuals, allowing us to track their movement, and take samples for genotyping and isotope analysis which will allow us to determine population structure and how brown trout and native species compete and interact.

Brown trout and native galaxiids caught during electrofishing we will measured for length and body mass, and fin clips will be taken to do genotype and isotope analysis. Samples of possible brown trout and native galaxiid prey will also be obtained for isotope analysis. Water samples will also be taken from different catchments for environmental DNA.

I will also obtain fin clips from brown trout and native galaxiids, a minimum of 15 native galaxiids and 15 brown trout will be sampled per catchment (preferably 30 fish per catchment) in order to preform genotype analysis to determine their population structure, and isotope analysis to determine what fish are feeding from and how invasive brown trout and native species are interacting and competing for resources.

5. Location(s) at which the proposed project/activity is to be undertaken:

Falklands Islands

6. External approvals required for the proposed project/activity:

Yes, these are being processed by the Local sponsor (SAERI, Falklands)

7. Does the proposed project/activity involve schedule 1 method (as defined by ASPA 1986) being carried out by members of this University's staff or by its student? If yes, please list the individuals involved:

Yes, for stomach content and elemental composition analysis.
Jessica Minett - I have been trained in Schedule I

The AWERB committee cannot approve this application but welcomes a re-submission once the following comments have been addressed (30/11/2017)

R1 – My only comments are regarding the use of 2-phenoxy for this. Would MS222 be more appropriate for released fish if there is a possibility of the fish ending up in the human food chain? Also, can the student comment on how welfare of the fish during recovery will be monitored and what will happen if a fish is showing adverse effects from the procedure? Can the student also obtain the relevant ethical permissions from colleagues in the Falklands for this work and provide them to AWERB?

R2 – No ethical concerns regarding the implant of tags within the fish peritoneal cavity as is happening here.

R1 & 3's point about anaesthetic choice would also fall into this category (MS-222 still has a 70-degree-day meat withdrawal after use, there is also a synthetic clove oil product called Aqui-S licenced in nearby Chile that has a 0-day meat withdrawal but it is not licenced in UK, and I presume Falklands) - if their veterinary governing body has approved the use of phenoxyethanol for this work then I don't have ethical concerns.

R3 – I was wondering which fin(s) the samples will be taken from? The applicant mentioned using fin clips for genotype and isotope analysis. To obtain enough material for both types of analysis could there be a risk of removing too much material, that could then affect the performance of the subject when released back into the natural environment? I had a brief look online and found a few articles that describe the pros and cons of fin clipping. For captive fish this is not such a problem but for fish released back into their natural habitat it might not only affect their ability to swim against strong currents but might also affect their breeding performance.

Could an alternative method be considered for obtaining samples for genotype analysis, such as body swabs? This could reduce the amount of fin material required.

Applicants comments to the issues/concerns raised for the previous submission

Following the advice of the AWERB committee we have considered alternatives to the use of 2-phenoxy-ethanol as an anaesthetic. Of these, clove oil (isoeugenol, marketed as AQUI-S) is licenced for use in the Falklands, does not enter the food chain and has also antiseptic properties, so we propose to use this at a concentration of 40-60 p.p.m, as this was found to induce rapid anaesthesia and a relatively short recovery time in juvenile trout (Keene et al. 1998). Fish will be assessed over three stages of recovery to monitor individual welfare. During stage one individuals remain immobilised and start to regain opercula movements, stage two individuals begin to regain body

movements, and stage three individuals regain equilibrium and their pre-anaesthetic appearance. Any fish that show adverse effects to the anaesthetic will be monitored and if they do not recover fully they will be killed using schedule 1 procedures. A small clip of the adipose fin (c. 2mm) and a sample of 2-3 scales will be taken for genetic identification and isotope analysis. These are standard procedures in salmonid field studies and do not compromise the welfare of the fish, provided these are larger than c. 50 mm (Vander Haegen et al. 2005; Petersson et al. 2014; Andrews et al. 2014). A project proposal is being submitted for ethical review in the Falklands and this will be submitted to AWERB when approval is granted.

Andrews, M., Stormoen, M., Schmidt-Posthaus, H., Wahli, T. and Midtlyng, P. J. (2015), Rapid temperature-dependent wound closure following adipose fin clipping of Atlantic salmon Salmo salar L. J Fish Dis, 38: 523–531. doi:10.1111/jfd.12261

Keene, J. L., Noakes, D. L. G., Moccia, R. D. and Soto, C. G. (1998), The efficacy of clove oil as an anaesthetic for rainbow trout, Oncorhynchus mykiss (Walbaum). Aquaculture Research, 29: 89–101. doi:10.1046/j.1365-2109.1998.00927.x

Petersson, E., Rask, J., Ragnarsson, B., Karlsson, L. and Persson, J., 2014. Effects of fin-clipping regarding adult return rates in hatchery-reared brown trout. Aquaculture, 422, pp.249-252.

Vander Haegen, G.E., Blankenship, H.L., Hoffmann, A. and Thompson, D.A., 2005. The effects of adipose fin clipping and coded wire tagging on the survival and growth of spring Chinook salmon. North American Journal of Fisheries Management, 25(3), pp.1161-1170.

College Ethics Committee/AWERB Group DECISION on Ethical Review

Having examined the information included in the above application with Reference No. STU_BIOL_82474_241017115552_1, this Committee has decided to:

☑Approve this application

With the following reputation risk to the university

☑Low risk ☐Moderate Risk ☐High Risk

Approval No: SU-Ethics-Student-081217/307

1. Title of Research Project

Brown trout in the Falklands: Invasive ecology, population structure and genetic diversity

2. Staff/students undertaking research:

Jessica Minett

3. Primary staff contact detail (Name, E-mail, Phone):

Carlos Garcia de Leaniz - [redacted]

4. Location where the study will take place:

Falkland Islands

5. If the proposal involves working with a partner body or organisation, please provide the following information
    a. Full official title of the partner(s);
    b. Details of the work to be carried out (a) at the partner(s) and (b) at the university;
    c. Details of the relevant ethical approval processes at the partner(s).

South Atlantic Environmental Research Institute (SAERI), assistance with all aspects of the project Fishermen in the Falklands, help with tagging and collecting fin clips for genotyping and isotope analysis Dr Glenn Crossin, Assistance with tagging brown trout in the Falklands

6. Please state or tick, as appropriate, the following questions relating to your project: (tick any that apply during the progression of an experiment)
    a. Species and taxon:

Brown trout (Salmo trutta)

    b. Approximate number:

Tag 60-80 individuals with acoustic tags/ combined acoustic-radio tags. Fin clips from a minimum of 15 brown trout and 15 native galaxiids per catchment for genotype and isotope analysis (preferably 30 per catchment)

c. Life stages:

Juvenile/Adults ☑

Mammal, bird or reptile embryo beyond halfway through incubation/gestation period ☐

Amphibian, cephalopod or fish larvae capable of independent feeding ☐

Strictly only gametes/very early developmental stages of embryos ☐

7. Provide a **brief** scientific background for the work, and describe any pilot work undertaken:

Brown trout (Salmo trutta) were introduced to the Falkland Islands several times in the 1940's and 1950's, mostly to support recreational fishing opportunities. Since introduction, there has been a marked decline in the native fish fauna, which consists of only two species of galaxiid fishes (zebra trout Aplochiton zebra and the Falklands minnow Galaxias maculatus). Given the threats to the long-term sustainability of the native galaxiids, fundamental knowledge about the movement ecology of brown trout, and their overlap and interactions with galaxiids, is critically needed. However, at present virtually nothing is known about the extent and seasonality of brown trout movements throughout the Falkland Islands and their impacts on native galaxiids, and no studies have documented the distribution and abundance of native galaxiids prior to brown trout introductions.

8. Please provide a clear methodology for the work to be undertaken:

Acoustic/ combined acoustic-radio tagging: Up to 80 brown trout (>17.5 cm) caught from electrofishing will be anesthetized (2 phenoxy-ethanol) one at a time, fish will then be placed into a V-trough board covered with wetted absorbent towel, ventral side up. Acoustic or combined radio-acoustic tags (VEMCO model V13 13 mm x 36 mm, weight in water 6 g) will be sterilized for a minimum of 15 minutes in a iodine solution before use and all hands and work surfaces will be washed with the same solution. A small mid-ventral incision will be made starting 1cm anterior to the pelvic girdle. The disinfected tag will then be rinsed in sterile saline and positioned to lie directly under the incision in the pectoral cavity. The incision will be closed with three simple interrupted stitches tied with surgeon's knots. The closed incision will then be cleaned with iodine and a small abound of liquid tissue adhesive. After surgery, fish will be placed in a recovery tank and allowed to recover from anaesthesia before being released. The operation will be carried under veterinary supervision or by trained member of the team signed off by the local veterinary.

Brown trout and native galaxiids caught during electrofishing we will measured for length and body mass, and fin clips will be taken to do genotype and isotope analysis. Samples of possible brown trout and native galaxiid prey will also be obtained for isotope analysis. Water samples will also be taken from different catchments for environmental DNA.

9. Provide a **brief** statement of how science will advance or people or animals will benefit from this project:

This project will provide information on how invasive brown trout are effecting native species in the falklands, we will be able to determine the abundance of brown trout and native galaxiids in different catchments, how they are interacting and what effect brown trout have on native species. We will also be able to determine if there are any refugia for native species and provide information on possible ways to prevent the spread on invasive brown trout.

10. Why do animals have to be used in this study? Explain your choice of species, and justify the number of subjects to be used with a power analysis where appropriate.

The number of fish to be tagged (60-80 over 2 yers) has been calculated based on the advice of the local sponsor, the need to sample 6 watersheds (10-12 fish per watershed) and the results from other studies (Crossin et al al,.2016; Cons Physiology 4: 2-12) The local sponsors (SAERI Falkland Islands, Falklands Conservation, Env Dept) are handling the local ethical application and will also issue the necessary sampling permits.

Environmental DNA will allow us to detect the presence of brown trout and native galaxiids and predict the number of individuals present in an area. However, to gain information on the health and reproductive status of the population we will need to catch (via electrofishing) and examine individuals. Electrofishing will also give us the opportunity to tag individuals, allowing us to track their movement, and take samples for genotyping and isotope analysis which will allow us to determine population structure and how brown trout and native species compete and interact.

11. What effects will your research have on the study organisms, and how will suffering be kept to a minimum?

Fish will be anesthetized during tagging and all other samples required (a c 2 mm clip of the adipose fin) is minimally invasive, nonlethal, and routinely carried out on salmonids.

12. How will you dispose of carcasses/animals (tick any that apply):

Landfill ☐

Sampled/analysis/other destruction of biomass ☐

Released ☑

Sent live to external organisation ☐

DECLARATION

I certify that the answers to the questions given above are true and accurate to the best of my knowledge and belief and I take full responsibility for it. I also conform that I have read the University's Policy Framework on Research Ethics & Governance and will abide by its ethical guidelines, as well as the ethical principles underlying good practice appropriate to my discipline ☑

College Ethics Committee/AWERB Group DECISION on Ethical Review

Having examined the information included in the above application with Reference No. STU_BIOL_82472_2610171111382, this Committee has decided to:

☑Approve this application

With the following reputation risk to the university

☑Low risk ☐Moderate Risk ☐High Risk

Comments:

The CoS Ethics Committee approves this application but recommends that the following points are considered

R1 - given that the invasive procedures will be carried out by non-Swansea researchers, under non-UK licences, I understand we will not require an AWERB submission. Conditional on this being correct, the procedures appear to me to be fairly standard and acceptable and the supervisor involved certainly has the necessary knowledge and experience to train the student. Overall the question addressed is important, especially from a conservation/management point, hence the data that will be collected justify the procedures involved. Green light from my part.

R2 - I agree with R1 that this is important work and the supervisor is clearly experienced. My understanding is that we still need institutional oversight, however, and that this still needs to be reviewed through AWERB

Appendix 3: Ethics and health and safety

Falkland Islands Government

Policy Unit The Secretariat Stanley Falkland Islands

Telephone: (+500) 28427

E-mail: environmental.officer@sec.gov.fk

Research Licence No: R18/2018

CONSERVATION OF WILDLIFE AND NATURE ORDINANCE 1999

SECTION 9

LICENCE TO CARRY OUT SCIENTIFIC RESEARCH

1. Licensee:

Name of the person leading the research	Jessica Minett
Affiliation	Swansea University
Position	PhD Student
Postal Address	[redacted] Singleton Park, Swansea, SA2 8PP, Wales, UK
Phone number	[redacted]
Email	[redacted]

2. Nature of licence:

This licence is issued to Jessica Minett under Section 9 of the Conservation of Wildlife and Nature Ordinance 1999. It is granted to Jessica Minett to permit her staff and bona fide field assistants or researchers employed on their behalf or under their overall jurisdiction. It is granted only for the following activities using methods specified in the research licence application on research on **Brown trout in the Falklands: invasion ecology, population structure and genetic diversity** submitted to the Environmental Committee on 12th April 2018 and amendment submitted to the Environmental Officer on the 12 April 2021:

Electrofishing – to assess health and reproductive status of the population and examine individuals as well as to tag individuals and take samples for genotyping and isotope analysis. The length and body mass of brown trout and native galaxiids caught during electrofishing will be measured, and any additional samples required can be obtained. In locations where electrofishing is not possible seine and fyke nets will be used.

Genotype and isotope analysis –to determine the population structure and what brown trout and native species are feeding on and how they are interacting and competing. Individuals will be anesthetized (clove oil at a proposed concentration of 40-80 p.p.m) one at a time and a 2mm fin clip and a sample of 2-3 scales will be obtained from brown trout and native galaxiids. Samples of possible brown trout and native galaxiid

prey will be obtained along with stomach contents and muscle samples from dead individuals for isotope analysis. A minimum of 15 native galaxiids and 30 brown trout will be sampled per catchment (preferably 30 fish pre catchment).

Tagging fish – to tag a maximum of 80 individuals from six different watersheds with acoustic tags/ combined acoustic radio tags to gain an understanding into how brown trout move in and between watersheds. Place pairs of acoustic receivers (VR2Ws) at the mouth of six watersheds (N=12 receivers), which will allow us to detect the movement of individuals in and out of the rivers/estuaries. We will also place additional receivers (N=8) up into a single watershed that is populated by brown trout to track finer-scale movements. Up to 80 brown trout (>20 cm) caught from electrofishing or rod and line will be anesthetized (clove oil at a proposed concentration of 40-80 p.p.m) one at a time, fish will then be placed into a V-trough board covered with wetted absorbent towel, ventral side up. Acoustic or combined radio-acoustic tags (Thelma Biotel model ID-2LP9 9 mm x 28 mm, weight in water 2.4 g) will be sterilized for a minimum of 15 minutes in ethanol or an iodine solution before use and all hands and work surfaces will be washed with the same solution. A small mid-ventral incision will be made starting 1cm anterior to the pelvic girdle. The disinfected tag will then be rinsed in sterile saline and positioned to lie directly under the incision in the pectoral cavity. The incision will be closed with three simple interrupted stitches tied with surgeon's knots. The small amount of liquid tissue adhesive will then be placed on the closed incision. The operation will be carried under veterinary supervision or by trained member of the team signed off by the local veterinary.

Catch and release of brown trout – Will provide us with information on their abundance around the Falklands, as well as providing insights into their movements between rivers and estuaries. A few experienced anglers will be trained by the local vet and me to PIT tag, they will be provided with PIT tags (12 x 2 mm) so that any brown trout (>20cm) the capture can be tagged and recorded when recaptured. All fish to be PIT tagged will be anaesthetised with clove oil (40-80 ppm).

2.2 This licence shall not be construed as authorising the licensee to enter upon the land of another without the owner's permission or consent.

2.3 Anaesthesia and tagging of fish will be undertaken in the presence of a FIG vet.

2.4 This licence does not constitute a permit to remove biological items from protected species from the Falkland Islands. An export licence should be sought from the Customs and Immigration Department to allow for the removal of any biological material or protected species from the Falkland Islands.

3. Period of licence

3.1 This licence is valid for the period commencing on 17th April 2018 and terminating on the 31st October 2021.

3.2 This licence may be revoked at any time by the Governor, but otherwise shall be valid for the period stated in paragraph 3.1.

4. Conditions of licence

4.1 This licence is issued on condition that the licensee shall:

a) Submit to the Environmental Officer, Policy Unit, Secretariat, Stanley, Falkland Islands, not later than 1st June 2021, a report detailing the research work carried out and the methods used in that research; and

b) Deposit with the Environmental Officer copies of all subsequent reports on the research work carried out.

c) Deposit with the Environmental Officer copies of any data collected as part of this study. Data will be stored and will not be published or circulated without researcher approval for a period of 2 years.

5. Purpose of Research

The purpose of the research work carried out by the licensee is set out in the research proposal submitted to the Environmental Committee on 12th April 2018 and amendment submitted to the Environmental Officer on the 12 April 2021.

Signed:

Denise Blake
Environmental Officer

Dated: 13 April 2021

Bioscience and Geography Protocol Risk Assessment Form

Protocol #1	**Title: eDNA Extraction, Stabilization, and Purification Using a Commercial Kit.**
Associated Protocols #.....	**Description:** The protocol comprises the extraction, collection, stabilization, and purification of eDNA using a commercial kit, for example, DNeasy PowerSoil Kit (QIAGEN):

Location: Wallace 130/130a and 131/131a

Circle which Bioscience and Geography Local Rules apply –

~~**Boat Field Genetic Manipulation**~~ **Laboratory** ~~**Office/Facility Radioisotope**~~

Identify here risks and control measures for work in this environment, <u>additional</u> to Local Rules

Protocol protection – wear lab coat and latex gloves

Storage – store chemicals in labeled bottles in the chemical cabinets when not in use.

Chemicals	**Quantity**	**Hazards**	**Category (A,B,C,D)***	**Exp.Score**
Kits may contain: DNase or DNase, RNase and/or Protease which may cause sensitization by inhalation and skin contact.	1	Harmful	C	1
Ethanol	1	Highly Flammable Causes Eye Irritation	C	1
Guanidine hydrochloride	1	Flammable Skin Irritant	C	1
Isopropanol	1	Flammable Skin and Eye Irritant	C	1

Hazard Category (known or potential) ~~**A** (e.g. carcinogen/teratogen/mutagen)~~ ~~**B** (e.g. v.toxic/toxic/explosive/pyrophoric)~~ **C** (e.g. harmful/irritant/corrosive/high flammable/oxidising) ~~**D** (e.g. non classified)~~	**Exposure Potential** Circle the **highest Exposure Score** above. Use this to calculate the exposure potential for the entire protocol (see handbook). Indicate this value below. ~~**Low**~~ **Medium** ~~**High**~~
Primary containment (of product): sealed bottle(s) **Storage conditions and maximum duration** :- All components should be stored dry and at room temperature. When stored under the recommended conditions and handled correctly, full efficacy of reagents is retained until the expiry date indicated on the outer box label. Each bottle will be labelled with date opened and initials.	
Secondary containment (of protocol) Fume-hood and open bench	
Disposal Autoclaving of biohazardous material and sent to Swansea University chemical disposal	
Identify other control measures Latex/Nitrile gloves and Laboratory Coat	
Justification and controls for any work outside normal hours N/A	
Emergency procedures Wipe up any spillages **After Inhalation** If unconscious place in recovery position and seek medical advice. **After Swallowing** If accidentally swallowed obtain immediate medical attention. Rinse mouth with water. Never give anything by mouth to an unconscious person. **After Contact with Eyes** Immediately flush eye(s) with plenty of water. Remove any contact lenses. Protect unharmed eye. Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician. **After Contact with Skin** Wash off immediately with soap and plenty of water while removing all contaminated clothes and shoes	
Supervision/training for worker Already trained	
Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures.	

Name & signature of worker. [redacted]*Jessica Minett* *Name & counter-signature of supervisor* [redacted] *Date 10/01/18*
Date of first reassessment Frequency of reassessments

Bioscience and Geography Protocol Risk Assessment Form

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol #3	**Title: Nucleic acid Quantification**
Associated Protocols #.....	**Description:** Nucleic acid Quantification using Nanodrop or Qubit. Both Absorbance based nucleic acid quantification methods. Qubit quantification imply sample preparation using fluorometer and sensitive specific Qubit™ quantitation assays.

Location: N 130-Lab Qubit // N131-Nanodrop

circle which Bioscience and Geography Local Rules apply –

~~**Boat Field Genetic Manipulation**~~ **Laboratory** ~~**Office/Facility Radioisotope**~~

Identify here risks and control measures for work in this environment, <u>additional</u> to Local Rules

Protocol protection – wear lab coat and latex gloves

Storage – store chemicals in labeled bottles in the chemical cabinets when not in use.

Chemicals	**Quantity**	**Hazards**	**Category (A,B,C,D) ***	**Exp.Score**
Qubit® dsDNA BR Reagent *200X concentrate in DMSO*	1	No Hazards	D	1
Qubit® dsDNA BR Buffer	1	No Hazards	D	1
Qubit® dsDNA BR Standards	1	No Hazards	D	1
Qubit® dsDNA HS Reagent *200X concentrate in DMSO*	2	No Hazards	D	1
Qubit® dsDNA HS Buffer	1	No Hazards	D	1
Qubit® dsDNA HS Standards	1	No Hazards	D	1
Qubit® dsRNA BR Reagent *200X concentrate in DMSO*	1	No Hazards	D	1
Qubit® dsRNA BR Buffer	1	No Hazards	D	1
Qubit® dsRNA BR Standards	2	No Hazards	D	1
Qubit® dsRNA HS				

Reagent *200X concentrate in DMSO* Qubit® dsRNA HSBuffer Qubit® dsRNA BR Standards				

Hazard Category (known or potential) ~~**A** (e.g. carcinogen/teratogen/mutagen)~~ ~~**B** (e.g. v.toxic/toxic/explosive/pyrophoric)~~ ~~**C** (e.g. harmful/irritant/corrosive/high flammable/oxidising)~~ **D** (e.g. non classified)	**Exposure Potential** Circle the **highest Exposure Score** above. Use this to calculate the exposure potential for the entire protocol (see handbook). Indicate this value below. **Low** ~~-~~ **Medium** ~~**High**~~

Primary containment (of product) sealed flask/bottle/glass/plastic/other (state) :- sealed bottle

Storage conditions and maximum duration :- Room temperature

Secondary containment (of protocol) open bench/fume hood/special (state) :- OB

Disposal e.g. autoclaving of biohazard, SU chemical disposal: UWS chemical disposal

Identify other control measures (circle or delete) - latex/nitrile/~~heavy gloves; screens; full face mask; dust mask; protective shoes; spillage tray; ear defenders; other (state)~~

Justification and controls for any work outside normal hours N/A

Emergency procedures (e.g. spillage clearance; communication methods): Absorb spillage with liquid binding material

SECTION 4: First aid measures

Description of first aid measures

Skin contact	Rinse with plenty of water. Immediate medical attention is not required.
Eye contact	Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.
INGESTION	Not expected to present a significant ingestion hazard under anticipated conditions of normal use. If you feel unwell, seek medical advice.
Inhalation	Not expected to be an inhalation hazard under anticipated conditions of normal use of this material. Consult a physician if necessary.
Notes to Physician	Treat symptomatically.

Most important symptoms and effects, both acute and delayed
Not applicable.

Indication of any immediate medical attention and special treatment needed
None.

SECTION 5: Firefighting measures

Extinguishing media

Suitable Extinguishing Media	Water spray. Carbon dioxide (CO2). Foam. Dry chemical.
Unsuitable Extinguishing Media	No information available.

Special hazards arising from the substance or mixture Not Known.

Advice for fire-fighters Standard procedure for chemical fires.

SECTION 6: Accidental release measures

Personal precautions, protective equipment and emergency procedures
ELIMINATE all ignition sources (no smoking, flares, sparks or flames in immediate area). Use personal protection equipment. See Section 8 for more detail.

Environmental precautions
No special environmental precautions required. Avoid discharge into drains and waterways whenever possible.

Methods and material for containment and cleaning up
Soak up with inert absorbent material.

Reference to other sections
See section 8 for more information.

Supervision/training for worker (circle)

~~None required~~ Already trained ~~Training required~~ ~~Supervised always~~

Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures.

Name & signature of worker [redacted] *essica Minett*

Name & counter-signature of supervisor [redacted] *Date 10/01/18*

Bioscience and Geography Protocol Risk Assessment Form

Protocol #3	**Title: PCR of nucleic acid templates and DNA/RNA Agarose Gel Electrophoresis**
Associated Protocols ***#..........................***	**Description:** PCR of nucleic acid templates and DNA/RNA Agarose Gel Electrophoresis

Location: N 130-Lab // N131-Lab

~~Boat~~ ~~Field~~ ~~Genetic-manipulation~~ Laboratory **~~Office/Facility~~ ~~Radioisotope~~**

Identify here risks and control measures for work in this environment, <u>additional</u> to Local Rules

Protocol protection – wear lab coat and latex gloves

Storage – store chemicals in labeled bottles in the chemical cabinets when not in use.

Description of Risk	Hazard				Analyse / Evaluate Risk			Overall Risk Category (see explanation on last page)
	Source	Current Controls	Event	Category	Consequences	Exposure	Probability	
Burns from Heating Agarose in a Microwave Oven : Agarose is heated to boiling point in glassware using a microwave oven. There is a risk of sustaining burns from the hot flask and liquid (superheated and overflow) on being touched.	Thermal	PPE worn (gloves, labcoat, closed footwear) and safety glasses available. Prepare a small amount of gel in a large volume conical flask. Be careful not to overheat (watch while heating it up) and take care handling melted agarose - leave in microwave for 30secs to ensure it is safe to move. Wear heat resistant gloves.	Superheated liquid might bubble up and out of conical flask causing skin burns with contact. Microwave explosion causing body injury and burns from hot liquid. Dropping and breakage of flask containing hot liquid with spillage onto unprotected skin.	Contact or exposure to heat and cold	**Substantial :** Major burns to hand	**Frequent :** Agarose gels are usually made at least several times a week	**Remotely Possible :** Possible if control measures and care are not taken	Prob / Exposure: VR, R, U, O, F, C AC: Low, Mod, Subs, Subs, High, VH QP: Low, Low, Mod, Mod, Subs, High UP: Low, Low, Low, Mod, Mod, Subs RP: Low, Low, Low, Low, <u>Low</u>, Mod C: Low, Low, Low, Low, Low, Low PI: Low, Low, Low, Low, Low, Low
Exposure to UV Radiation : Agarose gels are examined with a UV light source in order to detect the presence of DNA and RNA. Nucleic acids bind SYBR Green in the gel and fluoresce under UV light. An image of the gel can be captured with a digital camera. Generally, this is done in an enclosed gel documentation system with no exposure of the operator to the UV light. Sometimes, it is necessary to use an older transilluminator to examine the gel and cut fragments from it for DNA purification while the light source is on.	Radiation	The gel documentation system has a safety mechanism by which the UV light is automatically switched off as soon as the door of the cabinet is opened but users are instructed to switch off the light themselves before opening the door so they will be protected in the unlikely event of the safety mechanism failing. When using the transilluminator it is necessary to wear a UV-resistant full face shield, nitrile gloves and lab coat with sleeves rolled down to protect the arms. Exposure time to the UV radiation should be minimised as much as possible.	Burns to skin and eyes	Exposure to radiation	**Substantial :** Burns due to UV radiation	**Occasional :** Not possible to avoid contact when cutting gel on UV light since bands are not visible without UV light	**Remotely Possible :** Wear all protective equipment minimise exposure time by cutting quickly therefore less likely to cause injury.	Prob / Exposure: VR, R, U, O, F, C AC: Low, Mod, Subs, Subs, High, VH QP: Low, Low, Mod, Mod, Subs, High UP: Low, Low, Low, Mod, Mod, Subs RP: Low, Low, Low, <u>Low</u>, Low, Mod C: Low, Low, Low, Low, Low, Low PI: Low, Low, Low, Low, Low, Low
General Public Exposure to Hazardous Chemicals – Trip to GelDoc : It is not unusual for personnel to transport gel to the GelDoc machine - sometimes located on a different floor.	Chemical	Place gel in dedicated transport box & affix lid securely. Remove lab coat before leaving lab but carry with you to the gel doc room. Remove old gloves and wash hands thoroughly. Put a clean glove on one hand and use this hand only to carry the gel box. Take extra gloves with you. Use bare hand only to open doors, push lift buttons etc. Once inside gel doc room, glove up, put on lab coat and process gel. When returning to lab, put gel back in transport box and affix lid securely. Put a fresh glove on one hand and remove labcoat. Use gloved hand to carry gel box.	Accidental SYBR Green contamination of passer by or public area.	Single contact with chemical or substance	**Substantial :** SYBR Green is an alternative to Ethidium Bromide. Its risks are reported to be low, however since its function is similar to Ethidium Bromide, appropriate care should be taken. At the concentrations left in the gel during transport the health effects are minimal	**Frequent :** Transporting gel to geldoc occurs often	**Conceivable :** PPE worn in PC2 facility	Prob / Exposure: VR, R, U, O, F, C AC: Low, Mod, Subs, Subs, High, VH QP: Low, Low, Mod, Mod, Subs, High UP: Low, Low, Low, Mod, Mod, Subs RP: Low, Low, Low, Low, Low, Mod C: Low, Low, Low, Low, <u>Low</u>, Low PI: Low, Low, Low, Low, Low, Low

Appendix 3: Ethics and health and safety

Description of Risk	Hazard				Analyse / Evaluate Risk			Overall Risk Category (see explanation on last page)
	Source	Current Controls	Event	Category	Consequences	Exposure	Probability	
Risk of Electrocution : Electrophoresis gel tanks are connected to a power source during operation. Bringing live equipment into contact with water can result in an electric shock. This can occur if operators touch equipment with wet hands or place live tank lids down on wet bench tops.	Electrical	Training in use of electrophoresis equipment and power packs. Caution signage. Operators are instructed to always switch off the power source while loading tanks and before opening tanks after running gels. All equipment is to be handled with dry gloved hands and benches are to be kept dry. Keep electrical appliances away from sink or water. Equipment should meet Australian Stds and be purchased from a reputable company. All mobile equipment is tested and tagged yearly. Power leads should be visually inspected before each use. Do not use double adaptors. If using multi outlet powerboards ensure they are individually switched and have an overload switch.	Forgetting to switch off power source and contacting live equipment with wet hands leading to electric shock or more serious electrocution	Contact with electricity	**Substantial :** Electric shock requiring medical treatment.	**Frequent :** Gel electrphoresis is a common procedure	**Practically Impossible :** If control measures are followed	Prob \ Exposure: VR / R / U / O / F / C AC: Low / Mod / Subs / Subs / High / VH QP: Low / Low / Mod / Mod / Subs / High UP: Low / Low / Low / Mod / Mod / Subs RP: Low / Low / Low / Low / Low / Mod C: Low / Low / Low / Low / Low / Low PI: Low / Low / Low / Low / Low / Low
Sharps Risk from a Scalpel Blade : A disposable scapel blade is used to excise DNA bands out of an agarose gel	Mechanical	Use scalpel blades on handles Dispose of scalpel blade via Qlicksmart device only. Dispose of broken glass & blades into Sharps bin or Glass recycling bins Care should be taken when handling sharp instruments. Training provided.	Cuts from needles, scalpel blade, razor blade, scissors	Being hit by moving object	**Substantial :** Cuts.	**Unusual :** Gel purification methods involving cutting bands out are infrequently used	**Remotely Possible :** Possible if control measures fail	Prob \ Exposure: VR / R / U / O / F / C AC: Low / Mod / Subs / Subs / High / VH QP: Low / Low / Mod / Mod / Subs / High UP: Low / Low / Low / Mod / Mod / Subs RP: Low / Low / Low / Low / Low / Mod C: Low / Low / Low / Low / Low / Low PI: Low / Low / Low / Low / Low / Low
Sharps Risk from Pipette Tips : Loading an agarose gel requires pipetting DNA into the wells. General hazards relate to the event of a sharps injury (i.e. piercing of skin).	Mechanical	PPE worn (blue nitrile gloves, labcoat and closed footwear). Disposal of tips into sharps container.	When ejector of the pipette fails the tip is removed manually or when ejected tip bounces back causing sharps injury (contact or exposure to chemical or biological substances).	Other contact with chemical or substance	**Minor :** A sharps injury from a pipette tip is unlikely to require more than first aid treatment.	**Frequent :** loading agarose gel is a common procedure.	**Conceivable :** Unlikely to occur - PPE is worn and pipette tip ejected straight into sharps container.	Prob \ Exposure: VR / R / U / O / F / C AC: Low / Low / Low / Low / Mod / Subs QP: Low / Low / Low / Low / Low / Mod UP: Low / Low / Low / Low / Low / Low RP: Low / Low / Low / Low / Low / Low C: Low / Low / Low / Low / Low / Low PI: Low / Low / Low / Low / Low / Low
Toxicity from SYBR Green Used to Stain the Agarose Gel : SYBR Green is used in SPARQed labs as an alternative to Ethidium Bromide. While its toxicity is reported as lower than Ethidium Bromide, the mode in which it works is similar. Therefore the use of SYBR Green in the laboratory mirrors that of Ethidium Bromide. Ethidium bromide is a chemical that can bind to DNA and cause mutations. It is commonly used in agarose gels with a concentrated solution of 10mg/mL. EtBr is added after the agarose has been melted in the microwave. Users are instructed to let the agarose cool to about 60C before adding the ethidium bromide - i.e. just before pouring the gel. An alternative approach is to stain the agarose gel with ethidium bromide after electrophoresis. In this case the gel is placed in a 0.5ug/ml solution of ethidium bromide for 15-20 minutes and then rinsed in water to remove excess ethidium bromide before visualising under UV light.	Chemical	Wearing gloves, safety glasses, labcoat and enclosed shoes. Have a dedicated area when working with SYBR Green to contain any spills.	Accident exposure to chemical through spill, splash, inhalation	Single contact with chemical or substance	**Substantial :** Exposure to ethidium bromide may result in mutation of DNA which could have long-term health consequences. SYBR Green is used instead.	**Frequent :** Agarose gel preparation and gel electrophesis is a common procedure.	**Remotely Possible :** Could possibly occur but appropriate PPE is worn (particularly use of nitrile gloves when handling SYBR Green). SYBR Green is used in place of ethidium bromide	Prob \ Exposure: VR / R / U / O / F / C AC: Low / Mod / Subs / Subs / High / VH QP: Low / Low / Mod / Mod / Subs / High UP: Low / Low / Low / Mod / Mod / Subs RP: Low / Low / Low / Low / Low / Mod C: Low / Low / Low / Low / Low / Low PI: Low / Low / Low / Low / Low / Low

Description of Risk	Hazard				Analyse / Evaluate Risk			Overall Risk Category (see explanation on last page)
	Source	Current Controls	Event	Category	Consequences	Exposure	Probability	
RSI – Repeated Pipetting : If a large number of reactions are performed (can be done as 96 well plate) then repetitive pipetting occurs. PCR and real-time PCR requires repeated pipetting into small tubes or wells.	Muscular (Strain)	Use of a "robot" for large scale projects	Long term repetitious pipetting results in injury to wrist and/or thumb	Repetitive movement with low muscular loading	**Substantial :** Long term injury to tendons in wrist and thumb.	**Unusual**	**Unusual But Possible**	Prob \ Exposure: VR / R / U / O / F / C AC: Low / Mod / Subs / Subs / High / VH QP: Low / Low / Mod / Mod / Subs / High UP: Low / Low / Low / Mod / Mod / Subs RP: Low / Low / Low / Low / Low / Mod C: Low / Low / Low / Low / Low / Low PI: Low / Low / Low / Low / Low / Low
Skin Burns from Hot Surfaces of PCR Cycler or Rotorgene Machine : Both thermocyclers and Rotorgene machines generate high heats (max ~98°C) during their cycling - thermal cyclers can also be used as heating blocks. Touching the thermoblock may result in burning injury; likewise, samples removed from a previous run inside the Rotorgene may still be hot.	Thermal	PPE (gown, gloves, and appropriate footwear). Protective lid/cover for Rotorgene machine and thermal cyclers during run. When a thermal cycling program is initiated in the Rotorgene, the door to machine is locked so it should not be possible to contact heating block during cycling. Use of forceps to remove tubes if hot. Temperature is held at 4°C at the end of the run after which the tubes are removed. Use of machine training provided to all staff Temperature is always displayed on the machine.	If tubes are unloaded prior to machine cool down period finger tips can suffer minor burns. Contact with the heating block when at high temperature during a thermal cycle (eg. adding polymerase to 'hotstart' PCR) . Ignoring heat warnings	Contact with or exposure to heat or cold	**Minor :** Minor burns to hand/fingers.	**Unusual :** In a standard thermal cycler, if lid/cover is in place. It is unlikely that this would be removed (thermal lid) and reactions are cooled to 4°C at end of cycle as part of standard cycling conditions.	**Conceivable :** Only conceivable in the thermal cycler if lid is lifted during cycling to add/remove something - care would be required. The lid is locked during the Rotorgene run but conceivable if tube removal is too soon after run is finished.	Prob \ Exposure: VR / R / U / O / F / C AC: Low / Low / Low / Low / Mod / Subs QP: Low / Low / Low / Low / Low / Mod UP: Low / Low / Low / Low / Low / Low RP: Low / Low / Low / Low / Low / Low C: Low / Low / Low / Low / Low / Low PI: Low / Low / Low / Low / Low / Low
SYBR Green Mix for Fluorescence : SYBR green binds to dsDNA and fluoresces	Chemical	Approriate PPE: gloves, gown and footwear.	Generally unspecified however ingestion and possible spillage may be harmful.	Single contact with chemical or substance	**Minor :** May bind to DNA.	**Occasional :** Perform as often as three times per day.	**Practically Impossible :** When handling SYBR green mix, gloves and gown must be worn.	Prob \ Exposure: VR / R / U / O / F / C AC: Low / Low / Low / Low / Mod / Subs QP: Low / Low / Low / Low / Low / Mod UP: Low / Low / Low / Low / Low / Low RP: Low / Low / Low / Low / Low / Low C: Low / Low / Low / Low / Low / Low PI: Low / Low / Low / Low / Low / Low

Explanation of Overall Risk Categories

Explanation of Overall Risk Categories

Exposure					
Very Rare (VR) – extremely rare (has not yet occurred	**Rare (R) –** hardly every occurs (but has been known to occur)	**Unusual (U) –** does not occur often (from once per month to once per year)	**Occasional (O) –** sometimes occurs (from once per week to once per month)	**Frequent (F) –** occurs often (approximately once daily)	**Continuous (C) –** occurs repeatedly (or many times daily)

Probability (Prob)					
Almost Certain (AC) – is the most likely and expected result if the hazard event takes place	**Quite Possible (QP) –** is quite possible, not unusual, has an even 50/50 chance	**Unlikely but Possible (UP) –** Would be an unusual sequence or coincidence	**Remotely Possible (RP) –** would be a remotely possible coincidence	**Conceivable (C) –** has never happened after many years of exposure, but is conceivably possible	**Practically Impossible (P) –** has never happened after many years of exposure and is virtually impossible

Overall Risk Category				
Low – risk is normally acceptable	**Moderate (Mod) –** should be dealt with as soon as possible but situation is not an emergency	**Substantial (Subs) –** should receive attention as soon as possible	**High –** immediate correction required	**Very High (VH) –** immediate correction required

The Overall Risk Category for each element is highlighted in green.

Chemicals	Quantity	Hazards	Category (A,B,C,D)*	Exp.Score
PCR				
Sterile Water	1-500ul	N/A	D	2
PCR Buffer	1-500ul	N/A	D	2
Polimerase	1-50ul	N/A	D	2
Primer mix	1-50ul	N/A	D	2
Nucleic acid template	1-50ul	N/A	D	2
Gel Electrophoresis				
Agarose	<10g	N/A	D	2
EDTA Buffer	1-100ml	N/A	D	2
Red Dye (GelRed)	<10ul	N/A	D	1
DNA loading dye	1-100ul	N/A	D	1
DNA	1-100ul	N/A	D	2
Sterile water	1-1000ul	N/A	D	2

Hazard Category (known or potential)

~~**A** (e.g. carcinogen/teratogen/mutagen)~~

~~**B** (e.g. v.toxic/toxic/explosive/pyrophoric)~~

~~**C** (e.g. harmful/irritant/corrosive/high flammable/oxidising)~~

D (e.g. non classified)

Exposure Potential Circle the **highest Exposure Score** above. Use this to calculate the exposure potential for the entire protocol (see handbook). Indicate this value below.

Low ~~**Medium**~~ ~~**High**~~

Primary containment (of product) sealed flask/bottle/glass/plastic/other (state) :- sealed bottle

Storage conditions and maximum duration :- Room temperature

Secondary containment (of protocol) open bench/fume hood/special (state) :- OB

Disposal e.g. autoclaving of biohazard, SU chemical disposal: UWS chemical disposal
Identify other control measures (circle or delete) - latex/nitrile/~~heavy gloves; screens; full face mask; dust mask; protective shoes; spillage tray; ear defenders; other (state)~~
Justification and controls for any work outside normal hours N/A
Emergency procedures (e.g. spillage clearance; communication methods): Absorb spillage with liquid binding material
Supervision/training for worker (circle) ~~None required~~ Already trained ~~Training required~~ ~~Supervised always~~
Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures. *Name & signature of worker.* [redacted] *Jessica Minett* *Name & counter-signature of supervisor* [redacted] *Date 10/01/18*
Date of first reassessment Frequency of reassessments

Bioscience and Geography Protocol Risk Assessment Form

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol #3	**Title: DNA/RNA extraction, stabilization, purification using commercial kits**
Associated Protocols #.....	**Description:** DNA/RNA extraction Collection, stabilization and purification using commercial kits: -DNeasy PowerLyzer PowerSoil Kit> Isolate DNA from tough soil microbes, optimized for use with bead-based homogenizers. - DNeasy kits (QIAGEN) - RNeasy kits (QIAGEN) - QIAmp kits (QIAGEN) - Allprep DNA/RNA/Protein Kits (QIAGEN) - ISOLATE II DNA kits (Bioline) - ISOLATE II RNA kits (Bioline) -Nexttec™ 1-step Isolation kits - Any other collection, stabilization and purification kit..

Location: N 130-Lab // N131-Lab

circle which Bioscience and Geography Local Rules apply –

~~**Boat Field Genetic Manipulation**~~ **Laboratory** ~~**Office/Facility Radioisotope**~~

Identify here risks and control measures for work in this environment, <u>additional</u> to Local Rules

Protocol protection – wear lab coat and latex gloves

Storage – store chemicals in labeled bottles in the chemical cabinets when not in use.

Chemicals	**Quantity**	**Hazards**	**Category (A,B,C,D)***	**Exp.Score**
Collection, stabilization, isolation or purification kit. Kits may contain: DNase or DNase, RNase and/or Protease which may cause sensitization by inhalation and skin contact.	1	Harmful	C	1

Hazard Category (known or potential)	**Exposure Potential** Circle the **highest Exposure Score** above. Use this to calculate the exposure potential for the <u>entire</u> protocol (see handbook). Indicate this value below.
~~**A** (e.g. carcinogen/teratogen/mutagen)~~	

~~**B** (e.g. v.toxic/toxic/explosive/pyrophoric)~~ **C** (e.g. harmful/irritant/corrosive/high flammable/oxidising) ~~**D** (e.g. non-classified)~~	~~**Low**~~ **Medium** ~~**High**~~

Primary containment (of product) sealed flask/bottle/glass/plastic/other (state) :- sealed bottle

Storage conditions and maximum duration :- All components should be stored dry and at room temperature.

When stored under the recommended conditions and handled correctly, full activity of reagents is retained until the expiry date indicated on the outer box label.

Secondary containment (of protocol) open bench/fume hood/special (state) :- OB

Disposal e.g. autoclaving of biohazard, SU chemical disposal: UWS chemical disposal

Identify other control measures (circle or delete) - latex/nitrile/~~heavy gloves; screens; full face mask; dust mask; protective shoes; spillage tray; ear defenders; other (state)~~

Justification and controls for any work outside normal hours N/A

Emergency procedures (e.g. spillage clearance; communication methods): Absorb spillage with liquid binding material

After Inhalation

Remove to fresh air. Keep airways free.

After Swallowing

Drink lots of water after ingestion.

After Contact with Eyes

Rinse eyes with running water with eyelids open. Tilt the head to prevent chemical transferring to uncontaminated eye.

After Contact with Skin

Remove contaminated clothing. Rinse the affected skin or mucous membrane thoroughly under running water. Use soap if possible.

Note to Physician

No additional recommendations

Supervision/training for worker (circle)

~~None required~~ Already trained ~~Training required~~ ~~Supervised always~~

Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures.

Name & signature of worker.  *Jessica Minett*

Name & counter-signature of supervisor  *Date 10/01/18*

Date of first reassessment	Frequency of reassessments

Bioscience and Geography Protocol Risk Assessment Form

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol #3	**Title: Real time quantitative PCR**
Associated Protocols #.....	**Description:** reverse transcription RNA. Quantitative PCR and high resolution melt curve analysis of cDNA/DNA with SYBR Green

Location: N 130-Lab // N131-Lab

circle which Bioscience and Geography Local Rules apply –

~~**Boat Field Genetic Manipulation**~~ **Laboratory** ~~**Office/Facility Radioisotope**~~

Identify here risks and control measures for work in this environment, additional to Local Rules

Protocol protection – wear lab coat and latex gloves

Storage – store chemicals in labeled bottles in the chemical cabinets when not in use.

Chemicals	Quantity	Hazards	Category (A,B,C,D)*	Exp.Score
DNAase	2ul	Not hazardous	D	Low
dNTP's	1ul	Not hazardous	D	Low
Random hexamers	1ul	Not hazardous	D	Low
Reverse transcriptase	1ul	Not hazardous	D	Low
SYBR Green	5ul	Not hazardous	D	Low

Hazard Category (known or potential)

~~**A** (e.g. carcinogen/teratogen/mutagen)~~

~~**B** (e.g. v.toxic/toxic/explosive/pyrophoric)~~

~~**C** (e.g. harmful/irritant/corrosive/high flammable/oxidising)~~

D (e.g. non classified)

Exposure Potential Circle the **highest Exposure Score** above. Use this to calculate the exposure potential for the entire protocol (see handbook). Indicate this value below.

~~**Low**~~ ~~**Medium**~~ ~~**High**~~

Primary containment (of product) sealed flask/bottle/glass/plastic/other (state) :- sealed vials

Storage conditions and maximum duration :- -20°C Freezer

Secondary containment (of protocol) open bench/fume hood/special (state) :- OB

Disposal e.g. autoclaving of biohazard, SU chemical disposal: UWS chemical disposal

Identify other control measures (circle or delete) - latex/nitrile/~~heavy gloves; screens; full face mask; dust mask; protective shoes; spillage tray; ear defenders; other (state)~~

Justification and controls for any work outside normal hours N/A

Emergency procedures (e.g. spillage clearance; communication methods): Absorb spillage with liquid binding material and dispose of safely

After Swallowing

Drink lots of water after ingestion.

After Contact with Eyes

Rinse eyes with running water with eyelids open. Tilt the head to prevent chemical

transferring to uncontaminated eye.

After Contact with Skin

Remove contaminated clothing. Rinse the affected skin or mucous membrane thoroughly

under running water. Use soap if possible.

Supervision/training for worker (circle)

~~None required~~ Already trained ~~Training required~~ ~~Supervised always~~

Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures.

Name & signature of worker. [redacted] *Jessica Minett*

Name & counter-signature of supervisor [redacted] *Date 10/01/18*

Date of first reassessment Frequency of reassessments

Bioscience and Geography Protocol Risk Assessment Form

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol #3	**Title: DNA extraction from animal tissue**
Associated Protocols #.....	**Description:** working in the laboratory extracting DNA from animal tissue

Location: N 130-Lab // N131-Lab

circle which Bioscience and Geography Local Rules apply –

~~**Boat**~~ ~~**Field**~~ ~~**Genetic Manipulation**~~ **Laboratory** ~~**Office/Facility**~~ ~~**Radioisotope**~~

Identify here risks and control measures for work in this environment, additional to Local Rules

Protocol protection – wear lab coat and latex gloves

Storage – store chemicals in labeled bottles in the chemical cabinets when not in use.

Chemicals	Quantity	Hazards	Category (A,B,C,D)*	Exp.Score
Nexttec TM 1-step DNA Isolation Kit	1	Harmful	C	1

Hazard Category (known or potential)

~~**A** (e.g. carcinogen/teratogen/mutagen)~~

~~**B** (e.g. v.toxic/toxic/explosive/pyrophoric)~~

C (e.g. harmful/irritant/corrosive/high flammable/oxidising)

~~**D** (e.g. non classified)~~

Exposure Potential Circle the **highest Exposure Score** above. Use this to calculate the exposure potential for the entire protocol (see handbook). Indicate this value below.

~~**Low**~~ **Medium** ~~**High**~~

Primary containment (of product) sealed flask/bottle/glass/plastic/other (state) :- sealed bottle

Storage conditions and maximum duration :- -room temperature

Secondary containment (of protocol) open bench/fume hood/special (state) :- OB

Disposal e.g. autoclaving of biohazard, SU chemical disposal: UWS chemical disposal

Identify other control measures (circle or delete) - latex/nitrile/~~heavy gloves; screens; full face mask; dust mask; protective shoes; spillage tray; ear defenders; other (state)~~

Justification and controls for any work outside normal hours N/A

Emergency procedures (e.g. spillage clearance; communication methods): Absorb spillage with liquid binding material and dispose of safely

Supervision/training for worker (circle)

~~None required~~ Already trained ~~Training required~~ ~~Supervised always~~
Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures. *Name & signature of worker.* [redacted] *Jessica Minett* *Name & counter-signature of supervisor* [redacted] *Date 10/01/18*
Date of first reassessment Frequency of reassessments

Bioscience and Geography Protocol Risk Assessment Form

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol #3	**Title: PCR of DNA templates**
Associated Protocols #.....	**Description:** PCR of DNA templates

Location: N 130-Lab // N131-Lab

circle which Bioscience and Geography Local Rules apply –

~~**Boat**~~ ~~**Field**~~ ~~**Genetic Manipulation**~~ **Laboratory** ~~**Office/Facility**~~ ~~**Radioisotope**~~

Identify here risks and control measures for work in this environment, additional to Local Rules

Protocol protection – wear lab coat and latex gloves

Storage – store chemicals in labeled bottles in the chemical cabinets when not in use.

Chemicals	Quantity	Hazards	Category (A,B,C,D)*	Exp.Score
Sterile water	1-500ul	N/A	D	2
PCR buffer	1-500ul	N/A	D	2
Primer mix	1-50ul	N/A	D	2
DNA	1-50ul	N/A	D	2

Hazard Category (known or potential)

~~**A** (e.g. carcinogen/teratogen/mutagen)~~

~~**B** (e.g. v.toxic/toxic/explosive/pyrophoric)~~

~~**C** (e.g. harmful/irritant/corrosive/high flammable/oxidising)~~

D (e.g. non classified)

Exposure Potential Circle the **highest Exposure Score** above. Use this to calculate the exposure potential for the entire protocol (see handbook). Indicate this value below.

~~**Low**~~ ~~**Medium**~~ ~~**High**~~

Primary containment (of product) sealed flask/bottle/glass/plastic/other (state) :- sealed bottle

Storage conditions and maximum duration :- room temperature

Secondary containment (of protocol) open bench/fume hood/special (state) :- OB

Disposal e.g. autoclaving of biohazard, SU chemical disposal: UWS chemical disposal

Identify other control measures (circle or delete) - latex/nitrile/~~heavy gloves; screens; full face mask; dust mask; protective shoes; spillage tray; ear defenders; other (state)~~

Justification and controls for any work outside normal hours N/A
Emergency procedures (e.g. spillage clearance; communication methods): Absorb spillage with liquid binding material and dispose of safely
Supervision/training for worker (circle) ~~None required~~ Already trained ~~Training required~~ ~~Supervised always~~
Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures. *Name & signature of worker.* [redacted] *Jessica Minett* *Name & counter-signature of supervisor* [redacted] *Date 10/01/18*
Date of first reassessment Frequency of reassessments

Bioscience and Geography Protocol Risk Assessment Form

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol #3	**Title: Working out of hours**
Associated Protocols #.....	**Description:** Working out of hours in the office/lab environment of N037, N130 and N131Wallace Building

Location: N 130-Lab // N131-Lab // N037-Office

circle which Bioscience and Geography Local Rules apply –

~~**Boat**~~ ~~**Field**~~ ~~**Genetic Manipulation**~~ **Laboratory** **Office/Facility** ~~**Radioisotope**~~

Identify here risks and control measures for work in this environment, <u>additional</u> to Local Rules

Fire safety – sign in and out of hours book in the foyer

Local security – do not let others into the building

Working prolonged periods – see VDU Safety

Laboratory work must not take place without supervision and prior assent from supervisor due to risks involved

Chemicals	**Quantity**	**Hazards**	**Category (A,B,C,D)***	**Exp.Score**
N/A				

Hazard Category (known or potential)

~~**A** (e.g. carcinogen/teratogen/mutagen)~~

~~**B** (e.g. v.toxic/toxic/explosive/pyrophoric)~~

~~**C** (e.g. harmful/irritant/corrosive/high flammable/oxidising)~~

~~**D** (e.g. non classified)~~

Exposure Potential Circle the **highest Exposure Score** above. Use this to calculate the exposure potential for the <u>entire</u> protocol (see handbook). Indicate this value below.

~~**Low**~~ ~~**Medium**~~ ~~**High**~~

Primary containment (of product) sealed flask/bottle/glass/plastic/other (state) :- N/A

Storage conditions and maximum duration :- N/A

Secondary containment (of protocol) open bench/fume hood/special (state) :- N/A

Disposal e.g. autoclaving of biohazard, SU chemical disposal: N/A

Identify other control measures (circle or delete) **-** none

Justification and controls for any work outside normal hours – access to building to access samples for analysis. Requirement to check welfare of livestock

Emergency procedures (e.g. spillage clearance; communication methods) – telephone communications
Supervision/training for worker (circle) ~~None required~~ Already trained ~~Training required~~ ~~Supervised always~~
Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures. *Name & signature of worker.* [redacted]*essica Minett* *Name & counter-signature of supervisor* [redacted] *Date 10/01/18*
Date of first reassessment Frequency of reassessments

Bioscience and Geography Protocol Risk Assessment Form

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol #3	**Title: Fieldwork**
Associated Protocols #.....	**Description:** Working out in the field in and around aquatic environments

Location:

Boat Field ~~Genetic Manipulation~~ ~~Laboratory~~ ~~Office/Facility~~ ~~Radioisotope~~

Identify here risks and control measures for work in this environment, additional to Local Rules

Slips, trips and falls – wear appropriate footwear and take care on slippery or uneven surfaces

Drowning – ensure you do not undertake fieldwork alone and never enter the water without someone else present

Hypothermia – appropriate warm clothing should be worn, any wet clothes should be removed and dry clothes put on and if any symptoms develop then the individual should be taken to a warm and dry area

Chemicals	Quantity	Hazards	Category (A,B,C,D)*	Exp.Score
N/A				

Hazard Category (known or potential)

~~**A** (e.g. carcinogen/teratogen/mutagen)~~

~~**B** (e.g. v.toxic/toxic/explosive/pyrophoric)~~

~~**C** (e.g. harmful/irritant/corrosive/high flammable/oxidising)~~

~~**D** (e.g. non classified)~~

Exposure Potential Circle the **highest Exposure Score** above. Use this to calculate the exposure potential for the entire protocol (see handbook). Indicate this value below.

~~**Low**~~ ~~**Medium**~~ ~~**High**~~

Primary containment (of product) sealed flask/bottle/glass/plastic/other (state) :- N/A

Storage conditions and maximum duration :- N/A

Secondary containment (of protocol) open bench/fume hood/special (state) :- N/A

Disposal e.g. autoclaving of biohazard, SU chemical disposal: N/A

Identify other control measures (circle or delete) **-** none

Justification and controls for any work outside normal hours – access to building to access samples for analysis.

Emergency procedures (e.g. spillage clearance; communication methods) – telephone communications

Supervision/training for worker (circle)

~~None required~~ Already trained ~~Training required~~ ~~Supervised always~~

Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures.

Name & signature of worker [redacted] *essica Minett* *Name & counter-signature of supervisor* [redacted] *Date 10/01/18*
Date of first reassessment Frequency of reassessments

Bioscience and Geography Protocol Risk Assessment Form

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol #3	**Title: VDU Safety**
Associated Protocols #.....	**Description:** working on computer at desk for prolonged periods of time

Location:

~~Boat~~ ~~Field~~ ~~Genetic Manipulation~~ ~~Laboratory~~ Office/Facility ~~Radioisotope~~

Identify here risks and control measures for work in this environment, additional to Local Rules

Take adequate breaks

Chemicals	Quantity	Hazards	Category (A,B,C,D)*	Exp.Score
N/A				

Hazard Category (known or potential)

~~**A** (e.g. carcinogen/teratogen/mutagen)~~

~~**B** (e.g. v.toxic/toxic/explosive/pyrophoric)~~

~~**C** (e.g. harmful/irritant/corrosive/high flammable/oxidising)~~

~~**D** (e.g. non classified)~~

Exposure Potential Circle the **highest Exposure Score** above. Use this to calculate the exposure potential for the entire protocol (see handbook). Indicate this value below.

~~**Low**~~ ~~**Medium**~~ ~~**High**~~

Primary containment (of product) sealed flask/bottle/glass/plastic/other (state) :- N/A

Storage conditions and maximum duration :- N/A

Secondary containment (of protocol) open bench/fume hood/special (state) :- N/A

Disposal e.g. autoclaving of biohazard, SU chemical disposal: N/A

Identify other control measures (circle or delete) – maximum of 90 minutes without taking a break. Look away regularly. Inform supervisor of any problems

Justification and controls for any work outside normal hours – access to building to access samples for analysis.

Emergency procedures (e.g. spillage clearance; communication methods) – telephone communications

Supervision/training for worker (circle)

None required ~~Already trained~~ ~~Training required~~ ~~Supervised always~~

Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures.

Name & signature of worker. [redacted] *ssica Minett* *Name & counter-signature of supervisor* [redacted] *Date 10/01/18*
Date of first reassessment Frequency of reassessments

PROJECT PROPOSAL AND RISK ASSESSMENT **FOR THE CONTAINED USE OF GENETICALLY MODIFIED ORGANISMS**

Section 1: General information

1.1 PROJECT SUPERVISOR	
Surname:	Consuegra
Forename:	Sonia
Email address:	[redacted]
College/Department/Other:	College of Science/Department of Biosciences

1.2 Title of Project
Brown trout in the Falkland Islands: invasion ecology, population structure and genetic diversity

1.3 GM PROJECT NUMBER (SCBHGM TO ALLOCATE)

1.4 IS THIS PROPOSAL IS AN EXTENSION OF A PREVIOUSLY-APPROVED PROJECT? *If so please tick box and enter previous reference number.*	
Previously-approved project	☐Yes ☒ No
Previous reference number	

Please complete the form, emphasising the connection between the original project and this application.

1.5 PROJECT LAY SUMMARY *Please describe the project, detailing aims and objectives, significance and outcomes, indicating how the GMMs will help to achieve the objectives of the project. This description should contain enough detail to help a non-specialist to understand the project.*
The project aims to determine the distribution of fish in the Falkland Islands using environmental

DNA. Water samples were collected from a number of streams across the Islands. From these samples an 89bp and 139bp fragment of the cytochrome b region of the mitochondrial genome were amplified. The DNA fragments amplified cannot be expressed. Several products were amplified; therefore, cloning and subsequent sequencing is necessary to validate the intended targets.

1.6 IS THIS GM ACTIVITY GOING TO FORM PART OF AN UNDERGRADUATE PRACTICAL CLASS?

If yes please provide details:

No

Section 2: Details of genetic modification

Please complete:

- Part A for Genetically modified microorganisms and/or
- Part B for Genetically modified higher organisms.

Part A: Projects involving the contained use of genetically modified microorganisms (GMMs).

2A.1 THE IDENTITY, SOURCE ORGANISM AND FUNCTION OF EACH SEQUENCE OF GENETIC MATERIAL TO BE INSERTED/MODIFIED.

Mitochondrial DNA from the fish…

2A.2 Is the donor organism pathogenic?

IF SO WHAT HARM DOES IT CAUSE.

No

2A.3 If the donor organisms has pathological or harmful characteristics, are the donated sequences implicated in them.

IF YES PLEASE GIVE DETAILS.

N/A

2A.4 Will the sequences cause harm if expressed in humans after accidental transfer?

IF YES, WHAT HARM WOULD OCCUR AND HOW SEVERE WOULD IT BE?
No
2A.5 Will the sequences cause harm if transferred to species in the environment *IF YES, WHAT HARM WOULD OCCUR AND HOW SEVERE WOULD IT BE?*
No
2A.6 Identity of the vector(s), and nature of any potential harmful properties(to humans and/or the environment). *INCLUDE IN YOUR DESCRIPTION THEIR ABILITY TO MOBILISE AND THE PRESENCE OF ACTIVE PROMOTERS OF EXPRESSION.* ***NOTE**: DISABLES VIRUSES USED AS A VECTOR SHOULD BE TREATED AS RECIPIENT ORGANISMS.*
Linearized plasmid for T-A cloning such as PGEM®T (Promega) or PCR™ 2.1. vector (Invitrogen). These vectors are not harmful to humans or the environment. These vectors are mobilization defective or non-mobilizable (i.e., the genomic information contained in the plasmid cannot be transferred from a bacterial cell to another).
2A.6 If using a disabled viral vector, state its origin and the mechanisms of attenuation.
N/A
2A.7 State identity [Species, strain(s)] and ACDP/SAPO hazard category of all recipient microorganisms.
E. coli (e.g., strain JM109, DH5-alpha)– lab adapted strains with a long history of safe use – equivalent to ACDP 1
2A.8 Are the intended recipient organisms pathogenic to humans? *IF YES WHAT HARM WILL THEY DO AND HOW SEVERE IS THE HARM?*
No
2A.9 Are the intended recipient organisms capable of independent survival in the environment, or will infect or transfer to other hosts?

IF YES PLEASE GIVE DETAILS
No. *E. coli* JM109 or DH5-alpha contains several disabling mutations, and is therefore considerably attenuated, and unlikely to be competitive in the environment.
2A.10 Natural host *(if any)* of recipient organism(s) and routes of transmission/infection (*if known)*.
N/A
2A.11 Characteristics of the genetically modified microorganisms. What effect will the modification have on the intended recipient organisms? *Include in your description any changes to pathogenicity or toxicity to humans*
None. *E. coli* is only used for cloning and plasmid replication and storage.
2A.12 Will the modification alter the recipient organisms ability to survive in the environment, compete with other organisms or transfer to them the inserted sequences? *IF YES PLEASE GIVE DETAILS.*
No. *E. coli* JM109 or DH5 -alpha contains several disabling mutations, and is therefore considerably attenuated, and unlikely to be competitive in the environment.

Part B: Projects involving the contained use of larger genetically modified organisms.

2B.1 LIST THE IDENTIFY OF ALL RECIPIENT ORGANISM(S) *Give common and scientific names and where relevant strain, cultivar or subspecies designations*
2B.2 Identity of the host/vector system or the method used for genetic modification
2B.3 Nature and identity of any toxic, allergenic or other potentially harmful effects attributed to the recipient organism, or its metabolic products

2B.4 Origin and intended function of inserted genetic material. Identify any harmful effects attributable to the inserted sequences

2B.5 Do these LGMO pose greater risk to humans than the unmodified parental organism			
Yes	☐	No	☐
Please summarise the justification for this statement			

Section 3: Risk Assessment

Please complete:

- Part A for Genetically modified microorganisms and/or
- Part B for Genetically modified higher organisms.

6.1 <u>Part A: Risk Assessment for Working with Genetically modified microorganisms</u>

3A.1 SUMMARISE ALL POTENTIALLY HAZARDOUS PROPERTIES OF EACH GMM IN RELATION TO HUMAN SAFETY. *Do not forget hazardous properties of the parental organism.* *Consider ALL properties of the host, vector, insert, and of the final GMM*
E. coli JM109 or DH5-alpha is classified as not hazardous to humans
E. coli DH5 alpha is classified as not hazardous to humans.

☒Laboratory workers, co-workers and other staff and students accessing laboratories

☐Other …

3A.2 Do any of these GMM pose a potential hazard to the environment? *CONSIDER ANIMALS, PLANTS ETC.*			
Yes	☐	No	☒

Please justify this statement.

The potential ecological risk due to exposure to *Escherichia coli* JM109 or DH5 alpha is likely to be minimal.

3A.3 What would be the consequence of these hazards being realised?

FOR THE PURPOSE OF THIS ASSESSMENT, ASSUME THAT THERE ARE NO BARRIERS TO PREVENT EXPOSURE. PLEASE GIVE DETAILS OF THE EXPECTED CONSEQUENCES AND USE THE TERMS "SEVERE, MEDIUM, LOW OR NEGLIGIBLE"

A. On human health

Negligible

B. On the environment

Negligible

3A.4 Is it possible to substitute these GMM with a safer alternative?

Yes	☐	**No**	☒

Please justify this statement.

Transformation of mitochondrial genes into *E. coli* are necessary for gene sequencing and storage of the clone sequences.

3A.5Likelihood of hazards associated with GMM being realised.

State the maximum culture volume to be used at any one time

60 ml (Three 90-mm Petri dishes)

20 ml of bacterial culture

Identify all types of operation with potential for dispersal (e.g. centrifugation, sonication, aspiration)

Plates and cultures will be inoculated on the bench close to a gas flame to protect samples from contamination.

Do any of these activities generate aerosols of splashes which could pose a risk to the worker? If so please provide details

No
If so is a Microbiological safety cabinet used to control these risks? If yes, please provide details of cabinet and location.
No
Are the GMM's to be centrifuged?
Yes
If so will sealed rotors and buckets be used for this and where will these buckets be opened?
Open bench
Please describe the culture conditions for the GMMs. E.g. shacking incubator, static shelves, rotary platforms etc.
Static Shelves and shaking incubators.

3A.6 PLEASE DESCRIBE THE TYPE OF WASTE GENERATED AND ITS DISINFECTION AND DISPOSAL ROUTE. *Remember to include liquid waste, sharps, solid waste.*
Solid media in Petri dishes, culture supernatant, tips, serological pipettes, and tubes.
What is the expected degree of kill
100%
How do you know that this degree of kill will be achieved
Autoclaving waste material for 40 minutes at 136 °C is more than sufficient to *E. coli*. Likewise, 2% biocleanse or 2% bleach for 12 h is enough to disinfect glassware and other labware. Waste will be autoclaved at 132 for 30 minutes. **A designated autoclave for GMO organisms is located in the Wallace Building room 001**. This autoclave is regularly (at least once a year) validated using a 12-point thermocouple technique. Staff technicians keep records of the validation. Once autoclaved the waste will be placed in tiger bags and disposed into the autoclave skip situated in the car park between Margam and ILS1.
3A.7 Please describe the emergency procedures for dealing with spills of GMMs

Microbial spill kits are available in lab 131A. This spill kit consists of paper towels, to absorb the bulk of the spill, concentrated biocleanse or suitable disinfectant, and disposable gloves, sharp container, and biohazard bags. In the event of a spill, the user will notify everybody in the lab. If any, the user will remove the sharps (broken glass) with tweezers or a spatula and dustpan and put them in the sharp container. Subsequently, the user will cover the spills with disinfectant to a final concentration of 2% and paper towels. The solution will be allowed to sit for 10 minutes before removing the paper towels, which will be disposed of in a biohazard bag. Surface will be cleaned down with a 2% disinfectant solution. Paper towels, and gloves will be placed in the biohazard bag, which will be sealed and disposed of appropriately. Utensils used to clean up the spill (e.g., tweezers, spatula, dustpan) will be disinfected with 2% biocleanse solution.

3A.8 Are animals to be infected with these GMOs?

IF YES PLEASE PROVIDE DETAILS.

No

3A.9 Are the GMM to be transported outside the laboratory to other areas of the university?

IF SO PLEASE PROVIDE DETAILS.

No

3A.10 Are the GMM to be transported outside Singleton campus?

IF SO PLEASE PROVIDE DETAILS.

No

3A.11 Are any microorganism or nucleic acid derived from a microorganism which is listed under Schedule 5 of the Anti-terrorism crime and security act 2001 as amended?

Yes	☐	No	☒

If yes – please provide details….

3A.12 Estimation of risk magnitude – to human health and safety

Based on the likelihood of exposure to GMM (following the procedures described above) and the

severity of the consequence of exposure, please select an estimation of risk magnitude from the matrix below.

	Likelihood			
Consequence	Probable	Possible	Unlikely	Highly improbable
Severe	☐High	☐High	☐Medium	☐Effectively 0
Moderate	☐High	☐Medium	☐Medium/low	☐Effectively 0
Minor	☐Medium/Low	☐Low	☐Low	☐Effectively 0
Negligible	☐Effectively 0	☐Effectively 0	☐Effectively 0	☒Effectively 0

IF NOT "EFFECTIVELY 0" PLEASE DESCRIBE THE ADDITIONAL MEASURES REQUIRED TO CONTROL THE RISK.

N/A

3A.13 Estimation of risk magnitude – to the environment

Based on the likelihood of release of GMM (following the procedures described above) and the severity of the consequence of release, please select an estimation of risk magnitude from the matrix below.

	Likelihood			
Consequence	Probable	Possible	Unlikely	Highly improbable
Severe	☐High	☐High	☐Medium	☐Effectively 0
Moderate	☐High	☐Medium	☐Medium/low	☐Effectively 0
Minor	☐Medium/Low	☐Low	☐Low	☐Effectively 0
Negligible	☐Effectively 0	☐Effectively 0	☐Effectively 0	☒Effectively 0

IF NOT "EFFECTIVELY 0" PLEASE DESCRIBE THE ADDITIONAL MEASURES REQUIRED TO CONTROL THE RISK.
N/A
3.A.14 Please state the proposed class of GM activity *(Class 1, 2 or 3)*
Class 1

Section 3B: Working with Larger Genetically modified organisms

3B.1 IDENTIFY ALL POTENTIALLY HAZARDOUS PROPERTIES OF THE LGMOS TO *HUMAN* HEALTH AND SAFETY *Take into account any toxic or allergenic effects, new reservoir for pathogens etc.*
3B.2 Identify persons who could be exposed to the hazard.
3B.3 What are the consequences of exposure of humans to these hazards?
3B.4 What are the measures put in place to prevent or control the risk?
3B.5 Identify all potentially hazardous properties of the lGMO's to the *environment* *ABILITY TO TRANSFER GENES TO OTHER ORGANISMS, COLONISE NEW ECOSYSTEMS, IMPROVED SURVIVAL ETC*
3B.6 What would be the consequence of release of these LHMO's on the local environment *PLEASE GIVE DETAILS OF THE EXPECTED CONSEQUENCES AND USE THE TERMS "SEVERE,*

MEDIUM, LOW OR NEGLIGIBLE"

3B.7 Describe the likely routes of release of the GMHO

3B.8 Describe the physical control measures that will be in place to minimise or prevent such release and identify control measures required to manage the risks.

3B.9 Describe the waste routes for GMHO (contaminated) material

3B.10 Estimation of risk magnitude – to human health and safety
Based on the likelihood of exposure to LGMO (following the procedures described above) and the severity of the consequence of exposure please select an estimation of risk magnitude from the matrix below.

	Likelihood			
Consequence	Probable	Possible	Unlikely	Highly improbable
Severe	☐High	☐High	☐Medium	☐Effectively 0
Moderate	☐High	☐Medium	☐Medium/low	☐Effectively 0
Minor	☐Medium/Low	☐Low	☐Low	☐Effectively 0
Negligible	☐Effectively 0	☐Effectively 0	☐Effectively 0	☐Effectively 0

If not "effectively 0" please describe the additional measures required to control the risk.

3B.11 Estimation of risk magnitude – to the environment
Based on the likelihood of release of LGMO (following the procedures described above) and the severity of the consequence of release please select an estimation of risk magnitude from the

matrix below.				
	Likelihood			
Consequence	Probable	Possible	Unlikely	Highly improbable
Severe	☐High	☐High	☐Medium	☐Effectively 0
Moderate	☐High	☐Medium	☐Medium/low	☐Effectively 0
Minor	☐Medium/Low	☐Low	☐Low	☐Effectively 0
Negligible	☐Effectively 0	☐Effectively 0	☐Effectively 0	☐Effectively 0
If not "effectively 0" please describe the additional measures required to control the risk.				

3B.12 Please state the proposed class of GM activity
☐ As safe as unmodified parental organism
☐ Harmful - pose additional risk to humans than the unmodified parental organism

Section 4: Administration

3.1 FACILITY DETAILS	
Building	Wallace Building
Laboratory number	[redacted]
Containment level	[redacted]
3.2 Local contacts ***please provide details of your*** college	
Health and Safety Coordinator	Dr Christopher Coates
GM /Biological safety Officer	Dr Almudena Ortiz-Urquiza

Lone Working Risk Assessment Form

Lone Workers Name: **Jessica Minett**	**Role of Lone Worker: PhD Student**	**Number of people exposed: 1**
School/College: College of Science	**Department: Bioscience**	
Risk assessment carried out by: Jessica Minett and Sofia Consuegra	**Date completed:03/03/20**	**Review date: 04/03/2020**
Description of work activity : Cloning DNA mitochondrial DNA fragments that cannot be expressed from fish for sequencing	**Assessor's signature: Sofia Consuegra** [redacted]	

Hazard:	Controls and precautions against the hazards:	Comments/ Action Required (including who and when):	Action Party:	By when:
Individual				
Medical fitness: Is the Lone Worker subject to any medical condition that may place them at increased risk when working alone. [Where they may be in doubt refer the Lone Worker to Human Resources or Student Support]	The Lone Worker must ensure that any medical conditions which might be relevant to their working alone are fully discussed with their line manager and, if necessary, Occupational Health and own GP. Individuals must not work alone if any such condition is assessed as placing them at increased risk. Any person who requires assistance to get out of the building in an emergency must not work alone.	No known medical conditions that will increase risk of working alone.		

Supervision: What arrangements are in place to maintain contact with the Lone Worker?	The Lone Worker must comply with the out of hours log in/out arrangements in operation within the building. Set up contact arrangements with family member. Examples include: • Regular contact by phone - identify who is responsible for maintaining contact with the Lone Worker and how it will be achieved. • End of shift contact • Periodic site visit to lone worker by supervisor • Identification of co-worker to be within visual or audible contact of the lone worker	Will not work out of hours (0800-1700) and will sign in the out of hours book when entering/leaving the building. Will email/text contact (Ryan Bevan Partner) outside building when starting and finishing work. Will contact supervisors (email, facetime) each evening and during work when needed.		
Training & Competency : Has necessary information instruction and training been given to the Lone Worker and is the Lone Worker competent to carry out the work alone?	Any person authorised to be in the building outside normal hours must be fully competent to carry out the work safely and be fully conversant with emergency procedures.	Have been trained for procedure and have performed the procedures before		
Location & Premises				
Building security: Is the building secure?	Access to the building is restricted to authorised personnel outside normal hours. In the event that the Lone worker has concerns about security or suspects there is an intruder in the building they must contact the Security.	Not after ours work requested		

Access: Is there a safe means of access/egress for the lone worker (consider lighting and personal security issues and means of escape in emergency)	Entrances in the vicinity of the building and car park are well lit. The Lone Worker should plan how to get to car/public transport after leaving, taking account of potential personal safety issues.	Will aces building during the day only		
Emergencies: Does the Lone Worker have access to emergency warning devices to raise the alarm in event of emergency e.g. fire alarm, motion sensors /manual device (panic alarms).	Lone Workers must know local arrangements on how respond in event of fire or other emergency.	Arranged		
First aid: Are there arrangements in place to deal with a situation where the Lone Worker becomes ill or has an accident? (Access to First aiders and facilities)	First aiders are unlikely to be present. First aid boxes are available and contents checked regularly. In the event of a Lone Worker feeling unwell they should if possible return home or contact the Security for assistance.	Arranged for emergencies with supervisors		
Welfare facilities: Is there adequate heating, lighting, access to drinking water and toilets.	The Lone Worker should be aware that heating/cooling in the area may be much reduced unless the business need for after hours working has been established.	Aware of lack of heating in the building		

Process/Work Activity				
Use of computers and general office equipment	Lone Worker should ensure their work station complies, and is set up in accordance with GSA's Display Screen Equipment Procedure and has undergone a DSE assessment. Lone Worker should take regular breaks from DSE work.			
Slip/trips/falls	Regular inspection of areas to ensure that any trip hazards [torn carpets, uneven flooring, trailing cables etc.] receive prompt attention. Individuals with temporarily impaired mobility must not work alone.			
Electrical equipment	Ensure all office equipment is electrically tested. Electrical cables and plugs visually inspected for damage. Do not interfere with plugs or power supply.			