The Functional Ecology of *Potamogeton rutilus* Wolfg.

by

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DECLARATION

I declare that this thesis is composed of work carried out by myself unless otherwise acknowledged. This thesis, in whole or part, has not been submitted for any other degree.

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ABSTRACT

Potamogeton rutilus Wolfg. is a rare threatened macrophyte which, within the British Isles, is confined to oligo-mesotrophic Scottish lochs. The species has been lost from some of its former recorded sites, thought due to eutrophication and other environmental habitat changes. The plants limited distribution may be partly related to its lack of seed production that may limit long distance bird seed dispersal. *P. rutilus* appears to be reliant on clonal growth for reproduction, so may have a reduced genetic diversity that can reduce fecundity. To assist the development of strategies for the future conservation of *P. rutilus*, a series of investigations was carried out to reveal the autecological factors that influence the plant's distribution and survival.

TWINSPAN analysis was used to determine the floristic community types of *P. rutilus* from both Scotland and Finland. The analysis revealed that *P. rutilus* in Scotland had an oligo-mesotrophic community type, whilst Finnish *P. rutilus* was associated with a more eutrophic habitats. There appeared to be some distinct floristic differences between current and former Scottish *P. rutilus* loch habitats. More than half of the former *P. rutilus* sites formed a distinctive machair, marine influenced, TWINSPAN floristic group dominated by *Myriophyllum spicatum* and *Potamogeton pectinatus*.

The standing water classification of Palmer *et al* (1992) produced broadly similar oligo-mesotrophic floristic groups to the *P. rutilus* TWINSPAN analysis. The National Vegetation Classification (NVC) match analysis (Malloch 1986) of Scottish *P. rutilus* lochs produced one main A13, *Potamogeton perfoliatus-Myriophyllum alterniflorum* community with two A13 sub-communities. The only Scottish *P. rutilus* lochs that had a different NVC community type were the Outer Hebridean former *P. rutilus* lochs which supported an A11 *Potamogeton pectinatus – Myriophyllum spicatum* community with two A11 sub-communities. Finnish *P. rutilus* sites supported a range of eutrophic NVC community types, which further confirms their eutrophic floristic affinities. However, the NVC classification of Finnish *P. rutilus* sites has its limitations as the NVC classification system was formulated using British plant data.

The water chemistry, sediment chemistry and light extinction coefficients of lochs supporting *P. rutilus* were analysed to reveal the range of ecological conditions that support the plant and its macrophyte community type. Many of the current and former Scottish *P. rutilus* lochs appear to have a strong coastal influence, such as salinity which could have contributed to the extinction of *P. rutilus* in the most saline trophic group 4 lochs. The high alkalinity levels of Scottish *P. rutilus* lochs, may contribute to the success of the species in such alkaline environments, if it is able to utilise bicarbonate as a carbon source for photosynthesis, like some other members of its community type. Finnish *P. rutilus* trophic groups were found to have significantly higher aquatic total phosphate levels than Scottish trophic loch groups, confirming that Finnish *P. rutilus* tends to inhabit more eutrophic sites, compared to the more mesotrophic Scottish lochs supporting the plant. *P. rutilus* loch abundance and macrophyte diversity significantly declined with decreasing light availability.

The present study, based on two years of experimental growth investigations, showed that *P. rutilus* has a erect canopy forming growth habit with significant stem elongation growth under reduced light. This increased stem growth with reduced light availability did not produce any significant differences in plant biomass with different phosphorus availability, suggesting the different phosphate nutrient levels were not a limiting factor in plant biomass growth. However, turion development and turion size did appear to be significantly affected by light and phosphate treatments, with increased turion size being observed with increased phosphate levels, with optimum turion growth being achieved with eutrophic conditions under a half light regime. The significant differences in *P. rutilus* turion size with treatments seemed to be a good measure of plant fitness, whilst turion production per plant was not a good measure of fitness, as they were not significantly different under different treatments. Turion size and light availability appeared to influence *P. rutilus* germination rates and germination success. The growth experiments also revealed *P. rutilus* is a long day plant for turion development, with turion formation being initiated by a photoperiod of high summer long days and temperatures above 15 ^{o} C.

Genetic analyses using Randomly Amplified Polymorphic DNA (RAPDS) were undertaken on seven populations of *Potamogeton rutilus* (six from Scotland and one from Finland). Five RAPD primers produced a total of 54 bands, 18 (33.3 %) of which are polymorphic for the seven sampled populations. Thirtysix different genotypes were found in the total of 96 sampled *P. rutilus* plants, with the most common genotype being found in all sampled populations apart from the one Finnish site. Some identical RAPD genotypes are likely to have arisen via clonal growth. However, in some cases, sexual reproduction amongst similar genotypes could not be excluded as the source of identical genotypes. There was higher genotypic variation in small populations, which may indicate increasing sexual reproduction in more marginal habitats.

In summing up the main findings of the investigation, it was found that Scottish and Finnish *P. rutilus* have different trophic plant community types, with Scottish *P. rutilus* inhabiting less nutrient rich oligo-mesotrophic lochs than the more nutrient rich, eutrophic, Finnish *P. rutilus* habitats. Salinity may have caused *P. rutilus* to be lost from some of its former machair lochs. *P. rutilus* abundance and macrophyte diversity significantly declined with reduced light availability in lochs. The growth experiments revealed that *P. rutilus* turion size is a good measure of plant fitness and fecundity and eutrophic phosphate conditions, under a half-light regime, produced optimum *P. rutilus* turion growth. The genetic investigations suggest there is limited gene flow between *P. rutilus* populations and this could be due to the lack of seed production for inter-population seed dispersal. However, *P. rutilus* does not totally rely on clonal growth for reproduction, as genetic evidence suggested there was some degree of sexual reproduction in some populations.

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1.0 SUMMARY

Potamogeton rutilus Wolfg. is a rare threatened macrophyte that is confined to oligo-mesotrophic Scottish lochs within the British Isles. The species has been lost from some of its former recorded sites, thought due to eutrophication and other environmental habitat changes. The plant's limited distribution may be partly related to its lack of seed production, which may limit long distance bird seed dispersal. *P. rutilus* appears to be reliant on clonal growth for reproduction, so may have a reduced genetic diversity that can reduce fecundity. To ensure the future conservation of *P. rutilus,* a series of investigations was carried out to reveal the autecological factors that influence the plant's distribution and survival.

Keywords: *Potamogeton rutilus,* oligo-mesotrophic, eutrophication, macrophtye, sexual reproduction, clonal growth, bird dispersal, conservation, Red Data Book, Biodiversity Action Plan.

1.1 INTRODUCTION

Potamogeton rutilus Wolfg., Shetland Pondweed, is a rare fresh-water plant, which has been lost from some of its former Scottish loch sites. The extinctions in Scotland are thought partly due to the effects of eutrophication of its oligo-mesotrophic habitats (Preston & Croft 1997, Wallace & Murphy 2002), but other environmental and biotic factors may also be contributing to some extinctions, see Table 1.1.

P. rutilus is a northern European endemic with its British distribution confined to northern Scotland (Preston & Croft 1997). Due to the plant's limited British occurrence it has been listed as a near threatened species (Preston & Croft 1997, Palmer 2001). The species is also the subject of a Biodiversity Action Plan, to help maintain its known populations and possibly restore it to some of its former sites (Anon 1995). Aquatic plants form the largest category of plant biodiversity loss in Scotland, with aquatic plants being lost from the greatest number of 10 km squares compared to terrestrial habitats (Sydes 1997).

On a European scale, *P. rutilus* is considered a rare Red Data Book species, as some populations are declining from pollution and salinisation ((Kotiranta *et al.* 1998, Kazmierczakowa & Zarzycki 2001, Andrusaitis 2003). However, not all European *P. rutilus* populations have been in decline, as in Finland there has been an expansion of the plant's range with it colonising new sites (Barkman 2000, Virola *et al.* 2001).

1.2 TAXONOMY AND DESCRIPTION

P. rutilus belongs to the family *Potamogetonaceae.* This includes of the largest and most widespread of aquatic plant genera, *Potamogeton,* numbering some 80 to 90 species (Wiegleb 1988). The *Potamogeton* genus is divided into two major subgenera, *Potamogeton* and *Coleogeton,* with the subgenus *Potamogeton* consisting of three further recognised British sub-sections (Preston 1995). *Potamogeton* sub-sections

consist of *Potamogeton* that are rhizomatous and have broad submerged leaves, apart from *P. natans,* and do not produce turions; section *Graminifolii* are non-rhizomatous and have narrow, linear submerged leaves that usually produce turions and lack floating leaves; and, the remaining section *Batrachoseris* with only one species, *P. crispus,* is characterised by a compressed grooved stem with serrate leaves (Preston 1995). *P. rutilus* belongs to the narrow leafed *Graminifolii* sub-section and does not have any hybrids, see Plate 1.1 photo.

Plate 1.1 *Potamogeton rutilus* specimen from Loch Eilein, Tiree.

P. rutilus is a submerged macrophyte except for its small aerial inflorescence, which projects above the water surface. The plant is usually grass green in colour (Clark 1943), but some plants can have a red-brown tinge as in Loch Ussie (personal observation). The long, narrow leaf gradually tapers into an acute apex, whilst the leaf bases are surrounded by stiff membranous stipules, see Fig 1.1 (D), for *P. rutilus* leaf tip structure compared with other narrow leaved *Potamogetons.*

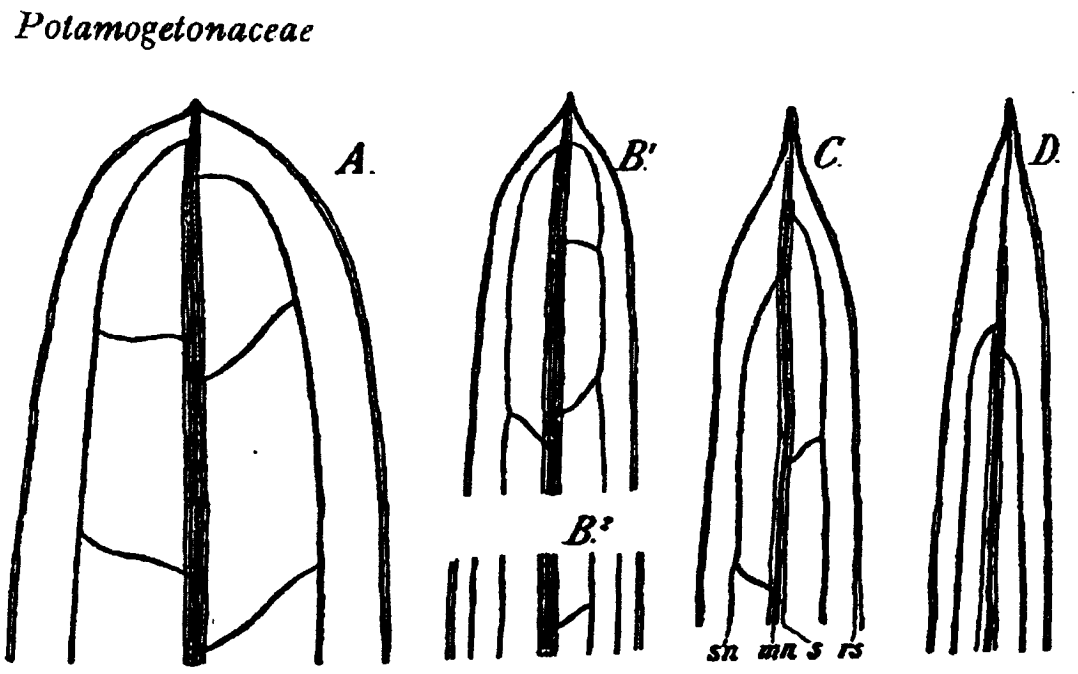


Figure 1.1 Comparing leaf structure of *P. rutilus* (D), with other fine leaved *Potamogetons*: *P. obtusifolius* (A), *P. Friesii* (B), and *P. pusillus* (C), from Suuri Kasvirkirja I, 1958.

The *P. rutilus*, 3 nerved, leaf tip has a much longer tapered leaf point than the other three narrow leafed *Potamogetons* shown in Fig 1.1. However, due to the variability and plasticity in leaf shape, this can often be an unreliable character to distinguish it from other narrow leafed *Potamogetons.* Comparing leaf stipules is a more reliable characteristic in separating *P. rutilus* from similar looking *P. berchtoldii* and *P.*

pusillus (Preston 1995). *P. rutilus* stipules are closed at their base with ribbed veins, a useful characteristic in distinguishing the plant from the other two similar fine-leafed *Potamogetons, P. berchtoldii* having more open based stipules, that are less prominently veined as in the similar *P. pusillus.* (Preston 1995). Dandy (1980) mentions that *P. rutilus*'s rigid leaves with an almost bristle like apex are another characteristic to separate it from the similar *P. pusillus.*

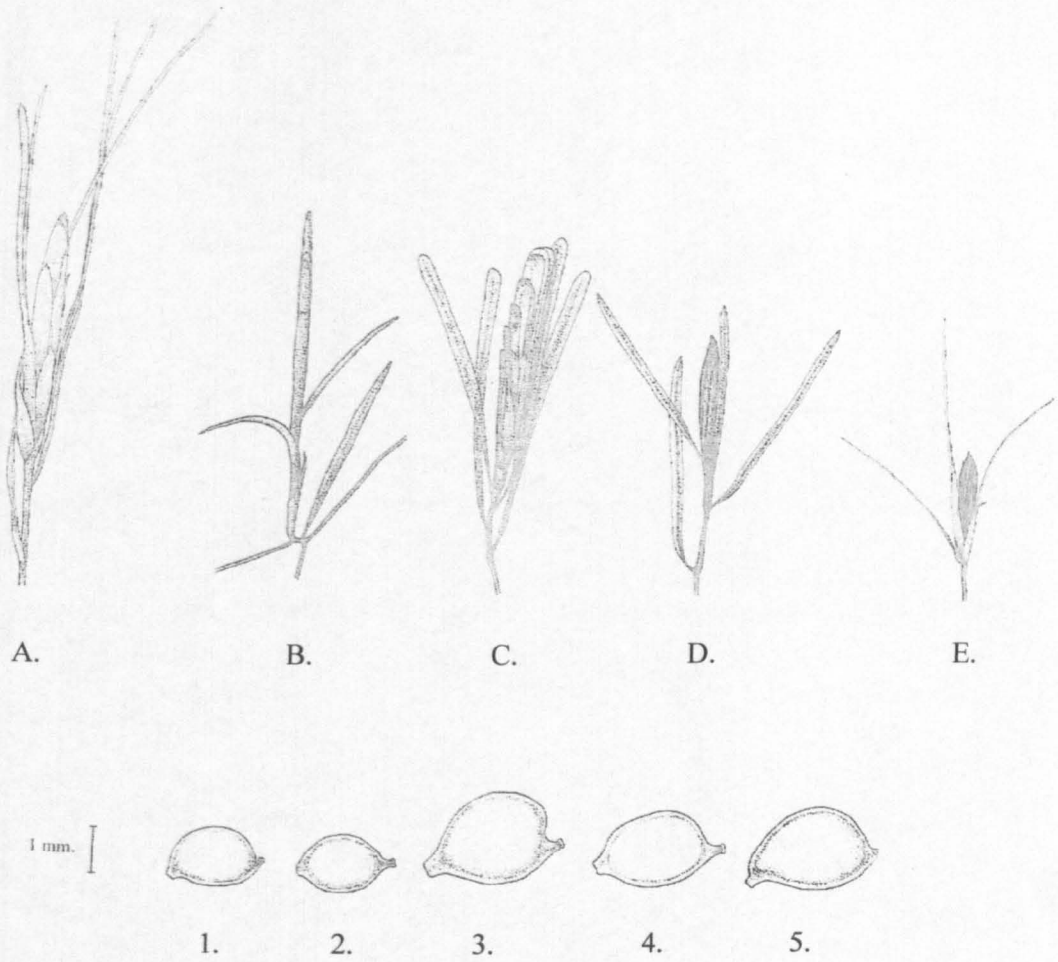


Figure 1.2 Comparing turion and seed type of *P. rutilus* (A.1), with turion and seed types of other fine leaved *Potamogetons*: *P. pusillus* (B.2), *P. obustifolius* (C.3), *P. Berchtoldii* (D.4), and *P. friesii* (E.5), turions not scale, modified diagrams from Preston 1995.

The very slender, compressed stems usually grow up to a length of 60 cm (Kotiranta *et al.* 1998), but in deeper water can grow over a metre long (personal observation). Inflorescences are borne on very slender, compressed peduncles and usually consist of 6 flowers (Preston 1995). Plants producing seeds are seldom recorded in Britain, apart from Clark's (1943) record of plants from Loch Scarie, North Uist, and my own personal observation of plants producing seeds in Loch an Eilein, Tiree, in 2002. If seeds are found it can be a helpful characteristic in distinguishing *P. rutilus* from other fine leaved *Potamogetons*. *P. rutilus* seeds are usually smaller than other similar *Potamogeton* species, apart from *P. pusillus* seeds. The similar sized seeds of *P. rutilus* and *P. pusillus* can be distinguished, as the *P. rutilus* seed has a curved beak (persistent remains of style and stigma), whilst *P. pusillus* has a straight seed beak, see Fig 1.2.

It appears that *P. rutilus* in Europe as Britain is mostly reliant on reproduction by bud like turions that form on the end of branches in late summer. (Preston 1995, Kotiranta *et al.* 1998). The asexually produced turions are usually 2 to 6 cm in length, green in colour and have a stiff, slightly resinous texture that may help protect the overwintering propagules. The long and stiff turions of *P. rutilus* are another useful characteristic for distinguishing *P. rutilus* from other fine leaved *Potamogetons*, especially from the similar *P. pusillus*, which has short and delicate turions (Preston 1995), see Fig 1.2. Vegetative clonal reproduction by macrophytes such as *P. rutilus*, appears to be a common method of reproduction in aquatic plants (Grace 1993).

P. rutilus is an annual species, as for all the other members of *Potamogeton* Section *Graminifolii* except for *P. epihydrus* (Preston 1995). The *P. rutilus* plants reach maturity in mid to late summer and then die down in autumn. Plants regrow in the spring from mostly turions as the plant rarely produces seed (Kotiranta *et al.* 1998). See life-cycle diagram Fig 1.3, for *P. rutilus* growth phases.

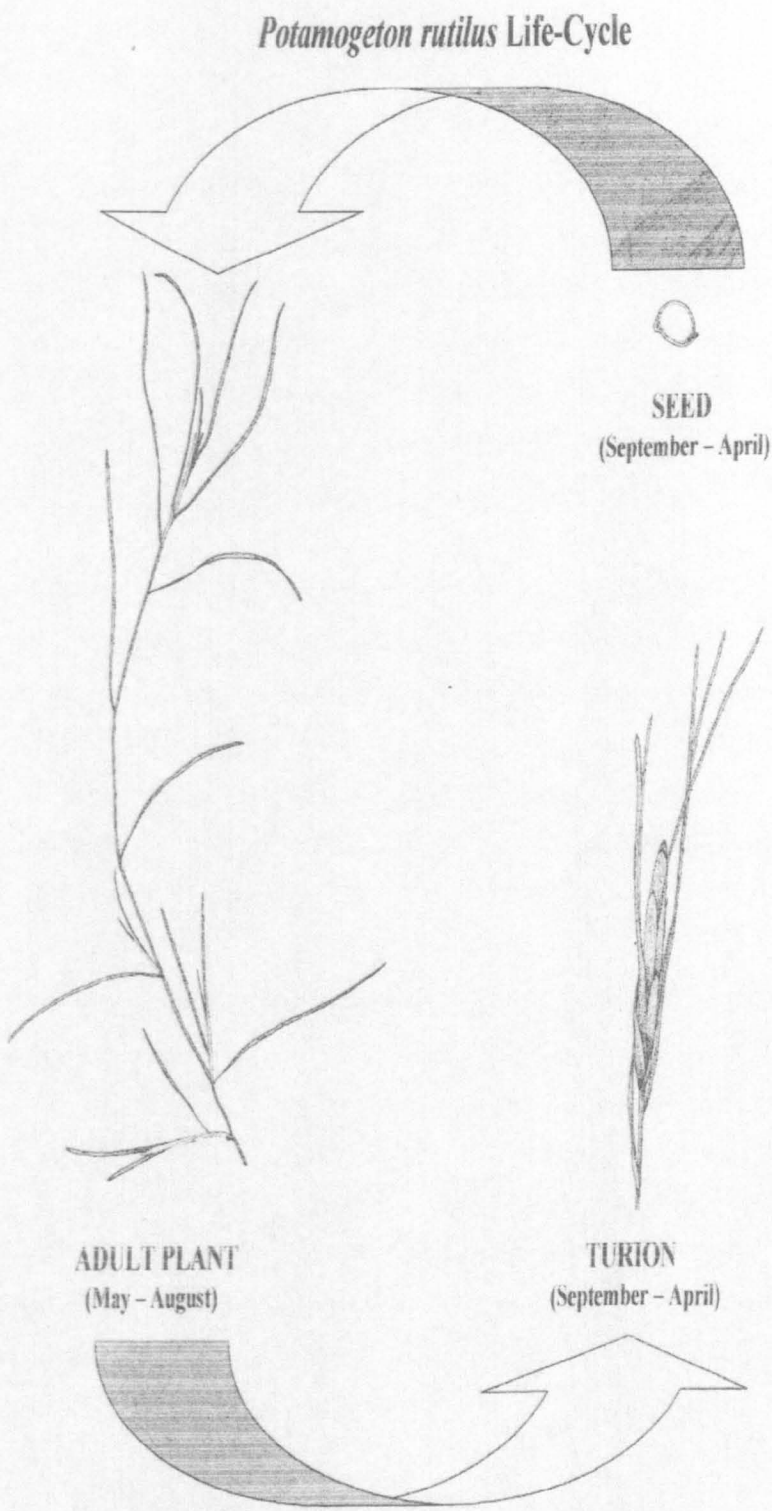


Figure 1.3 *Potamogeton rutilus* life-cycle stages and core growth periods

1.3 *P. RUTILUS* RECORDS IN BRITAIN

The first records of *P. rutilus* in Britain occurred in the late nineteenth century, but the occurrence of this plant and its identification was much misunderstood during this period (Dandy & Taylor 1938). The first published record for *P. rutilus* was in the Flora of West Yorkshire (1888) where the species was classified as a variety of the similar looking *P. pusillus*. However, this *P. rutilus* record was erroneous as Dandy & Taylor (1938) found the plant to be *P. berchtoldii.* Further records of *P. rutilus* occurrence in such places as Stafford, Coventry, East Sussex, Anglesey, and Orkney, all proved to be erroneous, being confused with *P. pusillus* (Dandy & Taylor 1938).

It was not until 1921 that Druce correctly identified and confirmed the presence of *P. rutilus* in Britain: specimens being found in lochs Tingwall and Bardister, Shetland (Dandy & Taylor 1938). However, Druce was not the first to discover *P. rutilus* in Britain as 31 years earlier, Beeby, in 1890 gathered specimens from Loch Bardister but misidentified them as *P. pusillus* (Beeby 1891). It was not until many years later after Beeby's death that it was discovered that unknowingly he was the first to add *P. rutilus* to the British flora (Scott *et al.*2002).

Clark (1943) discovered *P. rutilus* in the Outer Hebrides, occurring in the North Uist machair lochs, Grogary, Scarie and Mhor. The first genuine record for *P. rutilus* on the mainland was by Dandy in 1967 from Loch Eye, East-Ross-shire, (Duncan, 1980). More recently *P. rutilus* has been discovered in other Scottish mainland locations and also been located in the Inner Hebridean lochs of Tiree, Coll and Islay. The most recent discoveries for *P. rutilus* are in Perthshire, with the plant being first recorded in 1997 by N. Stewart in Loch Fingask (Nick Stewart personal communication). Another Perthshire loch has produced the most recent record of the plant, with I. Gunn finding a fragment of the plant in Loch Drumore, 2004.

1.4 EUROPEAN DISTRIBUTION AND PRESENT BRITISH DISTRBUTION STATUS

1.4.1 European Distribution

P. rutilus is an endemic northern European species (Preston & Croft 1997, Kottiranta *et al.* 1998), The northern Scottish distribution of this species are the western outliers of its distribution, with it being mainly concentrated in the Baltic region (Murphy 2002). The species primarily occurs in northern Germany, Scandinavian countries, Karelia, southwest Russia, Belorussia, Baltic countries and Poland (Hulten & Fries 1986, Mossberg *et al.* 1995, Kottiranta *et al.* 1998). *P. rutilus* was also recorded in Normandy, northern France (Lecointe 1995), but on a 2002 survey visit to the Pont l' Eveque lake the plant was not found, and so it may possibly be extinct in its only recorded French location.

P. rutilus has a scattered distributed throughout the Scandinavian countries with the plant being most rare in Norway, whilst still being rare, it is slightly more common in Sweden, Denmark and Finland (Kottiranta *et al.* 1998, Sand-Jensen *et al.* 2000, EUNIS 2005). In Russia *P. rutilus* is concentrated in the southwestern part of the country, with it being recorded in six sites that are located in the large lake regions of Karelia and northern Leningrad (Kottiranta *et al.* 1998).

P. rutilus is relatively frequent throughout Lithuania compared to the other Baltic countries, where it is more rare in Latvia and Estonia (Andusaitis 2003, Mossberg *et al.* 1995). Evidence for the long term presence of *P. rutilus* in this area of the Baltic has been found in late Pleistocene glacial pollen records of the plant, found in a small Lithuanian kettlehole lake (Satkunas *et al.* 2003). It has been stated that *P. rutilus* is most common in Belorussia and Poland (Kottiranta *et al.* 1998). However, many of the Polish distribution records for its 13 different localities are historical, being reported in the 19th century, so the true distribution of the plant is possibly reduced as it has been lost from some of its former Polish sites due to anthropogenic impacts (Kazmierczakowa & Zarzycki 2001).

1.4.2 British Distribution

Scotland has an estimated total of at least 31460 freshwater lochs (Lyle & Smith 1994). Since 1983, 2350 lochs have been surveyed for macrophytes, from a representative range of Scottish habitats, by Scottish Natural Heritage, SNH, and their predecessor Nature Conservancy Council, NCC (Lassiere & Duncan 1997). This SNH loch survey data and additional survey data from Glasgow University and other sources (Clark 1943), revealed that nineteen lochs have been previously recorded for *P. rutilus.* My recent surveys of these nineteen previously recorded *P. rutilus* sites have found a total of eleven loch sites still host the plant. In recent years two additional mainland sites in Perthshire have been discovered for *P. rutilus*, giving a total of thirteen sites.

Recorded *P. rutilus* is concentrated in five main geographical areas within Scotland: Outer Hebrides, Inner Hebrides, Shetland, mainland sites Inverness-shire and Perthshire, see Fig 1.4.

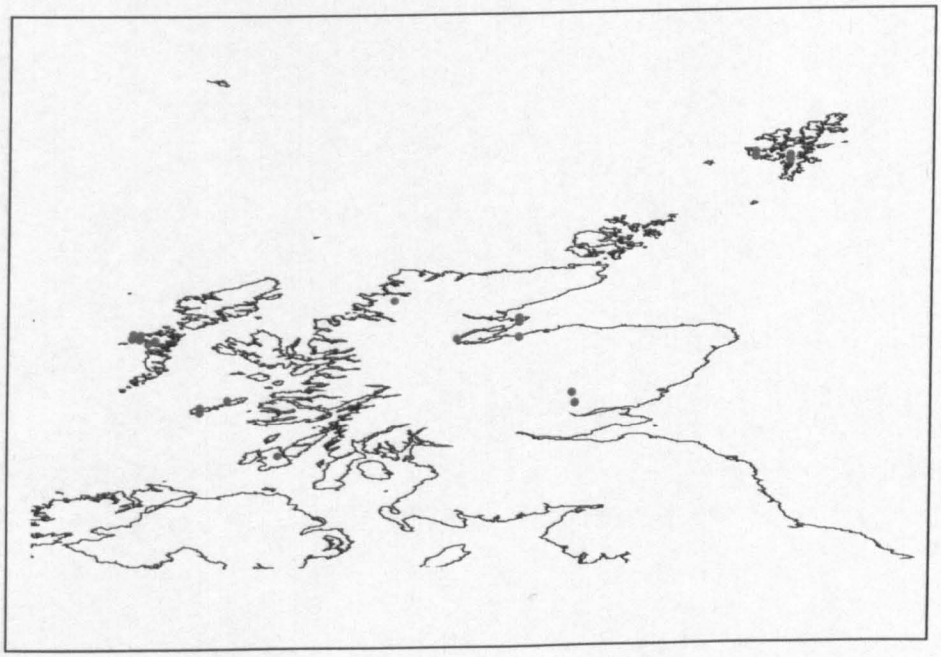


Figure 1.4 The current and former distribution of *P. rutilus* in Britain. Red = current sites, blue = former sites.

Table 1.1 below shows the changing status of *P. rutilus* for its known Scottish sites. In the case of the thirteen confirmed *P. rutilus* sites, seven seem to have stable populations whilst five sites have suffered declines in populations. These declining sites have small localised *P. rutilus* populations, suggesting they may be under threat from extinction.

Table 1.1 Surveys 2001/02/05 of previously recorded *P. rutilus* loch sites.

P. rutilus Lochs	Location	Grid ref	*P. rutilus* previous recorded presence	2001/2/5 *P. rutilus* surveys	Update of status	Current Threats
Eye	Mainland	NH832798	NCC Aug 85. O	23 Jul 02 R	Threatened	Enrichment
Flemington	Mainland	NH810520	Mc Callum , 1975	18 Jun 02 nil	Extinct	
Ussie	Mainland	NH505570	SNH Jun 94 LA	20 Jun 02 LA	Stable	
Bayfield	Mainland	NH821718	SNH Jul 94 LF	20 Jun 02 LF	Stable	Enrichment
Fingask	Mainland	NO165431	N. Stewart 1997 A	22 Jul 05 A	Stable	Enrichment
Drumore	Mainland	NO166608	I. Gunn 2004 fragment	22 Jul 05 nil	Not Found	
Awe	Mainland	NC246153	E.Charter 1988, not verified	Aug 01 nil	Not Found	
Lossit	Islay	NR409653	GU Aug 00 LO	29 July 02 LO	Stable	Enrichment
Ballyhaugh	Coll	NM176580	SNH Aug 94 O	2 Aug 02 R	Threatened	
an Eilein	Tiree	NL985436	SNH Aug 94 O	31July 02 LF	Stable	
a Chlair	Tiree	NL983445	SNH 26 Jun 93 O	1 Aug 02 LO	Threatened	Enrichment
Grogary	N Uist	NF716712	SNH Aug 95 F	6 Aug 02 R/LO	Threatened	Invasives
Scarie	N Uist	NF717708	SNH Aug 95 LF	6 Aug 02. LO	Threatened	Invasives
na Reivil	N Uist	NF708714	T.Roberts 1988, not verified	7 Aug 02 nil	Not Found	
Leodasay	N Uist	NF809632	J.W. Clark, 1975	8 Aug 02 nil	Not Found	
Mhor	N Uist	NF792622	W.A. Clark, 1942 Wash up	8 Aug 02 nil	Extinct	
nam Feithean	N Uist	NF713704	1998 not verified	6 Aug 02 nil	Not Found	
Asta	Shetland	HU413417	J.E.Lousley, 1980 O	20 Aug 02 nil	Extinct	
Tingwall	Shetland	HU417429	NCC Aug 86 O	20 Aug 02 nil	Extinct	
Kirkigarth	Shetland	HU238497	SNH Sep 97. W ash up	21 Aug 02 O	Stable	
Bardister	Shetland	HU237502	SNH Sep 97. F/LA	21 Aug 02 F	Stable	

DAFOR scale: D = dominant, A = abundant, F = frequent, O = occasional, R = rare, L = Locally. Record institutions: GU Glasgow University, NCC Nature Conservancy Council, SNH Scottish Natural Heritage. Stable = no decrease in DAFOR, Threatened = decline in DAFOR to LO/R

North Uist, Outer Hebrides

The two North Uist sites Grogary and Scarie appear to have suffered a decline since 1995, possibly due the competitive affects of invasive *Elodea nuttallii*, which has

greatly increased in abundance since1995. On surveying Loch Mhor in 2002, which has an old 1942 record of *P. rutilus* (Clark 1943), the plant was not found in 2002. The water chemistry for Loch Mhor shows a higher than normal salinity for a freshwater loch and high levels of algal growth, both factors which may have contributed to the demise of the plant. The 2002 survey of Loch Leodasay did not reveal *P. rutilus*, which was last recorded in 1975 by J.W. Clark. The apparent loss of the plant from Leodasay may also be due to the high salinity levels of the loch. *P. rutilus* was not found in 2002 in either lochs nam Feithean and na Reivil.

Islay, Tiree and Coll, Inner Hebrides

The Inner Hebridean *P rutilus* loch sites have increased from three to four sites with the Glasgow University 2000 discovery of the plant in Loch Lossit, Islay. Loch Lossit and Loch an Eilein appear to have stable *P. rutilus* populations, whilst Loch a Chlair and Ballyhaugh populations seem to be declining, see Table 1.1.

Scottish Mainland

Currently Loch Flemington is the only former *P. rutilus* site where the species appears to be extinct, as the plant was recently rediscovered in Loch Eye in 2002 (Wallace 2002 unpublished data), where it had not been recorded since 1985, despite subsequent surveys. Loch Eye, being situated in an intensive agricultural catchment, has always had a threat of eutrophication. In addition, Loch Eye may also be suffering from nutrient enrichment from the large numbers of protected waterfowl that overwinter on the loch (Lassiere & Duncan 1997).

In Loch Bayfield and Ussie, *P. rutilus* populations seem to be stable with small levels of enrichment in Loch Bayfield indicated by recent water chemistry monitoring. The previous mainland 1988 *P. rutilus* record for Loch Awe appears to be unverified and so possibly an erroneous record, with no trace of the plant being found in more recent intensive surveys.

P. rutilus records for Perthshire suggest that Loch Fingask has a stable plant population. In the case of the most recent Scottish record of *P. rutilus,* in Loch Drumore, it maybe a rare transient species for this site as only a fragment of the plant was found when first discovered in 2004. Previous to 2004, surveys of Loch Drumore have not found *P. rutilus* (N. Stewart, personal communication), nor has the most recent 2005 survey.

Shetland

Shetland has lost half of its former *P. rutilus* sites, with the loss of the plant from Lochs Asta and Tingwall, possibly due to previous eutrophication events (Pulford & Murphy 1996). The remaining Shetland lochs, Bardister and Kirkigarth seem to have good *P. rutilus* populations.

From the assessment of *P. rutilus*'s known current populations it would seem that three lochs, Flemington, Tingwall and Asta have suffered extinction's due to past eutrophication events, as these lochs are known to have suffered nutrient enrichment, (Pulford & Murphy 1996, Preston & Croft 1997, May *et al.* 2001). The two Outer Hebridean lochs, Mhor and Leodasay may have lost their *P. rutilus* populations due to the higher salinitys of these lochs, as the plant is known to be sensitive to salinity (Kazmierczakowa & Zarzycki 2001).

1.5 DISCUSSION

1.5.1 Rarity and limited distribution

Comparing recent *P. rutilus* loch surveys with past surveys it is clear that *P. rutilus* has been lost from some of its former sites, Table 1.1. In Scotland, *P. rutilus* inhabits mostly mesotrophic lochs that are sensitive to nutrient enrichment, which often causes a decline in macrophyte species diversity (Murphy 2002), and extinction of rare species such as *P. rutilus* and *Najas flexilis* (Preston & Croft 1997, Wingfield *et al.* 2004). However, *P. rutilus* appears to thrive in eutrophic conditions in Finland (Barkman 2000, Virola *et al.*

2001), suggesting that nutrient rich conditions may not be the main factor contributing to the plant's decline.

Even taking into account of some extinctions of *P. rutilus* from habitat changes, there is no clear explanation for the very localised distribution of the plant populations, being restricted to five main geographic areas in northern Scotland, see Fig 1.3. The restricted distribution of some rare plants such as *P. rutilus* is possibly not so much to do with habitat loss, but more connected with the plants biology (Henderson 2001). It has been found that rare or threatened plants have a tendency to produce fewer seeds (Pilgrim *et al.* 2004), and often rely on clonal reproduction that can reduce plant diversity and fecundity (Legg *et al.* 2003, Wilcock 2002). This may be the case for *P. rutilus.*

Not only is clonal reproduction common in some rare plants, but this mode of asexual reproduction seems to be much more common in aquatic than terrestrial plants (Hutchinson 1975). It has been suggested there exists a trade-off between sexual reproduction and clonal reproduction, and that clonal growth is favoured in circumstances where sexual reproduction is unsuccessful (Sculthorpe 1967).

Clonal plants may not only have limited populations through genetic factors, but also through reduced seed production which limits the opportunities for long range seed dispersal (Grace 1993, Figuerola & Green 2002). Water-birds feeding on aquatic plants appear to be an important agent of long distance seed dispersal (Rich & Fitzgerald 2002, Figuerola & Green 2002), with ingested seeds being deposited as viable seeds some considerable distance from where they originated (Agami & Waisel 1986). The limited geographic distributions of *P. rutilus* maybe partly due to its inability to produce seeds for long distance bird dispersal. *P. rutilus* limited distribution is even apparent at a more localised scale, where it is often found restricted to pairs of closely adjacent lochs, suggesting that short-distance dispersal is dependent on water-borne turions than pass through the stream channels that link these lochs.

It is known that more widespread species have a greater capacity to disperse and so are more able to colonise new areas and so maintain gene flow between these new populations (Bohonak 1999). If *P. rutilus*'s limited distribution is due to lack of seed production and reliance on clonal reproduction, it may reduce gene flow and so encourage genetic differentiation between more isolated populations (Wilcock 2002). However, recent studies have revealed that geographic distance is usually unrelated to genetic distance between aquatic plant populations (Hollingsworth *et al.*1996, Freeland *et al.* 2000), suggesting indirect evidence of long distance dispersal and gene flow between aquatic habitats, with waterbirds being a main vector (Figuerola & Green 2002). For example, genetic differentiation studies of Baltic populations of *Potamogeton pectinatus*, suggest that migratory swans may promote gene flow among populations (King *et al.* 2002).

Many species of migratory herbivorous waterfowl use macrophyte rich lakes as an important food source, with their grazing sometimes influencing macrophyte abundance (Santamarai & Rodriguez-girones 2002, Jalene *et al.* 2003). It has been suggested that the abundance of submerged macrophytes can be an important determinant of herbivorous bird numbers (Milberg *et al.* 2002, Nolet *et al.* 2002). This may be the case for macrophyte rich *P. rutilus* lakes in Finland, which have high numbers of waterfowl and for this reason are known in Finland as bird-lakes (Marja Koistinen personal communication).

1.5.2 Conservation of *P. rutilus*

P. rutilus inhabits mostly mesotrophic lakes, a rare habitat in the British Isles (Murphy 2002). Mesotrophic lakes have a high conservation value, as they host one of the most diverse macrophyte floras, which in addition to *P. rutilus,* include other rare protected species such as *Najas flexilis* (Wingfield 2004, Murphy 2002).

To ensure future protection of mesotrophic lake habitats from such threats as eutrophication, many have been designated as protected wildlife sites. Some mesotrophic lakes have been nationally designated as Sites of Special Scientific Interest

(SSSI) and internationally under the European Habitats Directive as Special Areas of Conservation (SAC), (NCC 1989, Preston 2000). In addition many of these lakes have been designated under the European Wild Birds Directive as Special Protection Areas (SPA) for their diverse birdlife (Pritchard *et al.*1992).

There have been no previous detailed research studies of *P. rutilus* and its Scottish mesotrophic loch habitats, partly due to the remoteness of many of the loch sites (Preston & Croft 1997). The lack of autecological knowledge has resulted in the urgent need to investigate the habitat requirements of this rare threatened plant species, which has become extinct from some of its former sites. The increased autecological knowledge of rare plants such as *P. rutilus* can provide a better assessment of the plants habitat requirements and reasons for their scarcity (Thompson & Hodgson 1996, Legg *et al.* 2003).

To fully assess the habitat needs and requirements of a rare species it is essential to fully understand the plant's life cycle and the factors that limit the regeneration and survival of the different life-cycle growth stages of the plant (Legg *et al.* 2003). Such life-cycle growth information for *P. rutilus* would provide a better understanding of the factors that regulate the population dynamics of the plant and possibly reveal the most vulnerable life-cycle growth stages.

The population dynamics of *P. rutilus* may operate over several scales, as plant metapopulation dynamics can range from the smaller habitat patch and loch scale to much larger geographic regions (Freckleton & Watkinson 2002). This may be the case in *P. rutilus* if long distance bird-mediated dispersal is contributing to the dispersal of the plant, as it can have a major influence on metapopulation dynamics (Figuerola & Green 2002). In addition, the degree of asexual versus sexual reproduction may also influence population dynamics and metapopulation structure as suggested by genetic analysis of plant population structures (Pandit & Babu 2003, Rossetto *et al.* 2004).

1.6 AIMS OF STUDY

P. rutilus is the subject of a Biodiversity Action Plan (BAP) to help maintain its known populations and possibly restore it to some of its former sites (Anon 1995).
The BAP for *P. rutilus* has the following main objectives:

1. the maintenance of existing known populations
2. the restoration of populations
3. the restoration of the species to suitable areas where it has been lost.

To help achieve some of the above BAP objectives this research investigated the autecology and habitat preferences of *P. rutilus* by fulfilling the following aims:

- To investigate the range of plant community types that *P. rutilus* is found in Scotland and how this may differ with European *P. rutilus* sites.

- To investigate whether the current plant community types are different from its former loch sites.

- To investigate the range of water chemistry and light regimes for *P. rutilus* community types for both former and present *P. rutilus* sites.

- To use plant trophic types and environmental data to formulate hypotheses to be tested by *P. rutilus* growth experiments, to reveal how some environmental variables effect the different growth stages of the plant's life cycle.

- To investigate the patterns of gene flow (as a measure of dispersal ability) and asexual versus sexual reproduction among populations of *P. rutilus.*

- To evaluate the current and possible future threats to *P. rutilus* and recommend future research and practical conservation management for the protection of the plant and its loch habitats.

CHAPTER 2: Floristic Community Types of *Potamogeton rutilus*

2.0 SUMMARY

TWINSPAN analysis was used to determine the floristic community types of the rare fresh water macrophyte *Potamogeton rutilus* Wolfg. from both Scotland and Finland. The analysis revealed that *P. rutilus* in Scotland had an oligo-mesotrophic community type, whilst Finnish *P. rutilus* was associated with more eutrophic habitats. There appeared to be some distinct floristic differences between current and former Scottish *P. rutilus* loch habitats. More than half of the former *P. rutilus* sites formed a distinctive machair, marine influenced, TWINSPAN floristic group dominated by *Myriophyllum spicatum* and *Potamogeton pectinatus*. The standing water classification of Palmer *et al* .1992) produced broadly similar oligo-mesotrophic floristic groups to the *P. rutilus* TWINSPAN analysis. The National Vegetation Classification (NVC) match analysis (Malloch 1986) of Scottish *P. rutilus* lochs produced one main A13, *Potamogeton perfoliatus-Myriophyllum alterniflorum* community with two A13 sub-communities. The only Scottish *P. rutilus* lochs that had a different NVC community type were the Outer Hebridean former *P. rutilus* lochs which supported an A11 *Potamogeton pectinatus – Myriophyllum spicatum* community with two A11 sub-communities. Finnish *P. rutilus* sites supported a range of eutrophic NVC community types, which further confirms their eutrophic floristic affinities. However, the NVC classification of Finnish *P. rutilus* sites has its limitations as the NVC classification system was formulated using British plant data.

Keywords: oligo-mesotrophic, eutrophic, trophic type, TWINSPAN floristic group, Standing Waters Classification, National Vegetation Classification (NVC), community type.

2.1 INTRODUCTION

To understand the ecological habitat requirements of rare plants such as *P rutilus* it is essential to evaluate the plants floristic community types and phytosociological relationships (Henderson 2001, Preston *et al.* 2000).

These plant community classifications can take several forms, ranging from the assemblage-based classification systems, such Rodwell's National Vegetation Classification (Rodwell 1995), to functional classifications such as Grime's Plant Growth Strategies approach, based on plant life forms. Grime (1974) identified particular environmental factors, competition, stress and disturbance to which all plants are subjected, in a greater or lesser degree. Depending on a plant's environment, it will have evolved a growth strategy that is best suited to surviving in its particular environment; these plant growth strategies have been classified into three groups by Grime, stress-tolerators, ruderals (disturbance-tolerators) and competitors. Grime (1979) was able to attribute certain morphological, life history and physiological characteristics that were associated with each of the three growth strategies.

Elements of Grime's plant growth form strategies are important in the phyto-sociological classifications of aquatic plant communities because different macrophyte community growth forms are good indicators of environmental stresses acting on aquatic systems, such as acidity and nutrient enrichment (Seddon 1972, Farmer & Spence 1986, Kautsky 1989, Palmer 1989, Murphy 2002). Several aquatic plant classification systems have been developed using floristic phyto-sociological analysis, one of the best known being Palmer's TWINSPAN botanical classification of British standing waters (Palmer 1992.) More recently Rodwell (1995) produced a phyto-sociological classification system for aquatic and emergent plants based on National Vegetation Classification (NVC), plant community types derived from TWINSPAN analysis of surveyed British plant communities.

These phyto-sociological systems, described above, have provided some information on the plant community types that are associated with *P. rutilus*. The NVC places *P. rutilus* in the A13, *Potamogeton perfoliatus – Myriophyllum alterniflorum* community type, which Rodwell (1995) describes as a diverse macrophyte community, confined mostly to the north and west of Britain, and having a wide range of *Potamogeton* species. Palmer's botanical classification of standing waters does not mention *P. rutilus* (Palmer 1989, Palmer *et al.* 1992), but a study by Preston *et al.* (2000) of Inner Hebridean lochs, placed *P. rutilus* in two TWINSPAN groups which they described to be similar to Palmer's standing water type 4. This standing water type is described as a community type with a range of trophic influences that consist of coastal lochs in northern Scotland, which include machair lochs in the Outer Hebrides (Palmer 1992).

In contrast to these mostly mesotrophic communities that Scottish *P. rutilus* appears to be found in (Preston 1995), continental *P. rutilus* plant communities are described eutrophic in character (Sinkeviciene 1998, Barkman 2000, Virola *et al.* 2001). For example, Lithuanian *P. rutilus* is associated with eutrophic plant communities dominated by *Zannichellia palustris* (Sinkeviciene 1998), a species usually associated with eutrophic or brackish conditions (Preston & Croft 1997, Palmer 1992). In Finland, *P. rutilus* is considered a coloniser of lakes that have become eutrophic in character (Barkman 2000, Virola *et al.* 2001).

The continental eutrophic *P. rutilus* plant community associations, raises the question how significant are these trophic and botanical differences between Finnish *P. rutilus*, described as a eutrophic specie, and Scottish *P. rutilus*, which is associated with mesotrophic conditions ? Further, Finnish *P. rutilus* appears to thrive in highly eutrophic lake conditions (Barkman 2000), whilst Scottish *P. rutilus* is said to decline and some cases become extinct when their loch sites become too eutrophic (Preston & Croft 1997). To fully understand these different trophic changes that may affect *P. rutilus* and to possibly determine the reasons for its loss from some of its former Scottish sites, this present study aims to:

- To determine if there are distinct differences between *P. rutilus* community types in both Scotland and Finland.

- To determine if there are any significant differences in macrophyte communities of present and former Scottish *P. rutilus* sites, which would possibly indicate site trophic changes.

- To evaluate the different macrophtye trophic classifications for assessing *P. rutilus* communities and their trophic habitats.

- To determine what changes in plant trophic groups may indicate possible threats to *P. rutilus.*

2.2 METHODS

In order to investigate the floristic characteristics of *P. rutilus* communities and their habitat types, recent macrophyte survey data was used in a TWINSPAN analysis to categorise current and former *P. rutilus* aquatic communities, to assess if there is any differences in their floristic communities. Included in this analysis were potential *P. rutilus* - colonised lochs, comprising similar nearby habitats, which were surveyed but not found to have *P. rutilus.* Both Scottish and Finnish *P. rutilus* plant surveys were also analysed by TWINSPAN to determine how large geographical differences may affect *P. rutilus* floristic community types. Finally, to evaluate how the floristic TWINSPAN analysis related to other macrophyte classification systems, the survey data were also analysed using the standing water types approach of Palmer *et al.* (1992), and by NVC Match computer analysis (Malloch 1986).

2.2.1 Surveyed Scottish and Finnish Loch Sites

Plate 2.1 One of the surveyed study sites, Loch Ballyhaugh, Coll, Inner Hebrides

There was a total of 27 sites surveyed for *P. rutilus*. This consisted of 19 Scottish sites that had been chosen for survey as they had been previously recorded for the presence of *P. rutilus.* In addition, 3 other Scottish loch sites were also surveyed as potential *P. rutilus* sites, as they where in the vicinity of lochs that are currently occupied by *P. rutilus* and appeared to have similar plant community types. Five Finnish *P. rutilus* sites were also chosen for survey to evaluate if floristic and trophic differences exist between Scottish and European *P. rutilus.* The majority of the Scottish sites, 22 in total, were surveyed and sampled in 2002, whilst the 5 Finnish *P. rutilus* sites were surveyed and sampled in 2003. See Table 2.1.

Table 2.1 Lakes surveyed for *P. rutilus* and associated macrophyte species

LOCH SITE	UK GRID REFERENCE or LAT/LONG	*P. rutilus* Status		
Scottish Mainland		**Current**	**Former**	**Potential**
Loch Ussie	NH 505570	√		
Loch Bayfield	NH 821718	√		
Loch Eye	NH 832798	√		
Loch Flemington	NH 810520		√	
Loch Awe	NC 246153		√	
Inner Hebrides				
Loch an Eilein	NL 985436	√		
Loch a Chlair	NL 983445	√		
Loch Ballyhaugh	NM 176580	√		
Loch Lossit	NR 409653	√		
Loch Phuill	NL 955419			√
Loch Bhasapoll	NL 972470			√
Outer Hebrides				
Loch Grogary	NF 716712	√		
Loch Scarie	NF 717708	√		
Loch na Reivil	NF 708714		√	
Loch Leodasay	NF 809632		√	
Loch Mhor	NF 792622		√	
Loch nam Feithean	NF 713704		√	
Shetland				
Loch Bardister	HU 237502	√		
Loch Kirkigarth	HU 238497	√		
Loch Asta	HU 413417		√	
Loch Tingwall	HU 417429		√	
Loch Benston	HU 457540			√

Table 2.1 continued

LOCH SITE	UK GRID REFERENCE or LAT/LONG	*P. rutilus* Status		
Finland		**Current**	**Former**	**Potential**
Lake Simpele (Siikalahti)	27° E : 6831 6361	√		
Lake Simpele (K. Hovi)	27° E : 6803 6302	√		
Lake Grundtrask	27° E : 6683 3722	√		
Lake Rytilampitrask	27° E : 7335 6119	√		
Lake Lampistrask	27° E : 6699 3234	√		

Current:sites currently hosting *P. rutilus*, Former: sites no longer hosting *P. rutilus*, Potential: nearby similar sites that could be possibly colonised by *P. rutilus*.

2.2.2 Field Survey Techniques

To ensure my survey data were comparable with previous survey data all lake surveys were carried out using a standardised Nature Conservancy Council (NCC) lake survey technique (Palmer 1989). This entailed walking around the perimeter of the loch and recording aquatic species in the shallows. During the loch perimeter walk a hand thrown grapnel was used at regular intervals to sample macrophytes in deeper water. Grapnel sampling intervals were approximately every 10 metres, but this may increase or decrease, as it was dependent on the presence of macrophyte vegetation and safe access. Boat surveys were also carried out on the larger inaccessible lochs, by boat trailed grapnel transects. Macrophyte abundance's were evaluated used a standard DAFOR abundance scale: D: Dominant, A: Abundant, F: Frequent, O: Occasional, R: rare. The term DAFOR abundance has no precise definition (Kirby 1992), but in these surveys it was determined by the frequency of a plant species and their estimated total cover for a site.

Macrophyte taxonomic keys used in the surveys, included: Pondweeds of Great Britain and Ireland, (Preston 1995), British Water Plants (Haslam *et al.* 1982), Charophytes of

Great Britain and Ireland (Moore 1986) and New Flora of the British Isles (Stace 1991). Collected voucher specimens were pressed and dried.

2.2.3 Palmer's Trophic Analysis of *P. rutilus* Communities

Palmer's trophic classification has been used to establish a range of trophic types that host *P. rutilus* and its associated plant community. The trophic assessment also involves evaluating loch plant communities that no longer host *P. rutilus.* In addition, some potential *P. rutilus* sites were assessed. The potential *P. rutilus* sites are those lochs that do not host *P. rutilus* but have a similar plant community and occur in the same geographic vicinity of current *P. rutilus* lochs.

The trophic classification of Palmers *et al.* (1992), defined UK lakes as occurring one of 10 possible types. The classification scheme was developed on the basis of TWINSPAN analysis of submerged and floating aquatic flora recorded at 1124 lake sites in Great Britain. The lowest trophic status was classified as lake type one (dystrophic), with increasing trophic status being described by increase in lake type number to reach lake type ten, broadly the most nutrient rich eutrophic lake type: see Appendix 1 for Palmers lake type categories.

2.2.4 TWINSPAN Data Analysis

In addition to Palmer's trophic classification, a separate TWINSPAN analysis of *P. rutilus* plant community data was carried out (Hill 1979). Only true aquatic macrophyte species were used in the analysis, because the inclusion of emergents does not always truly reflect the aquatic trophic character of a site. DAFOR abundance scores were replaced by presence or absence of species as some site surveys did not have comparable comprehensive species abundance scores: see Appendix 2 for the macrophyte species data set used in the TWINSPAN analysis.

In this TWINSPAN analysis three different data sets were assessed for plant community groupings. These included current Scottish and Finnish *P. rutilus* lakes to distinguish if there are any trophic floristic similarities or differences between Scottish and European sites.

The current and former *P. rutilus* lochs were also analysed by TWINSPAN to reveal any floristic differences, that may indicate trophic or other environmental changes between the two groups. Finally, another TWINSPAN analysis was carried out on current and former *P. rutilus* lochs with the addition of potential *P. rutilus* lochs, sites that were surveyed as they seemed potentially colonisable by *P. rutilus,* being nearby and similar in character to lochs hosting *P. rutilus.* The analysed data sets were as follows:

Current Scottish and Finnish *P. rutilus* lochs	16 sites
Current and previous Scottish *P. rutilus* lochs	19 sites
Current, previous, and potential Scottish *P. rutilus* lochs	23 sites

The TWINSPAN program divides plant species data into floristically similar groups, known as end-groups. These end-groups are sites with similar vegetational characteristics that are defined in a key by indicator species for each group.

2.2.5 National Vegetation Classification (NVC) Match Data Analysis

NVC analysis was used to fully evaluate the range of *P. rutilus* phytosociological relationships and how this categorisation may contradict or relate to both TWINSPAN and Palmer's trophic classifications

The plant community data was analysed using the NVC Match computer program, which gives a coefficient of goodness of fit (Malloch 1996). A match coefficient of 100

is a perfect match and zero coefficient shows no relationship. Species data were analysed using species presence or absence as for previous classification methods.

2.3 RESULTS

2.3.1 TWINSPAN Analysis of Current Scottish and Finnish *P. rutilus* sites

The current 11 Scottish and 5 Finnish *P. rutilus* sites analysed by TWINSPAN produced four main macrophyte community end groups, see Table 2.2 for loch site groupings. The divisions divided the lochs into geographical groupings with Group 1 and Group 2 representing Finnish Lakes. Group 3 consisted of the Inner and Outer Hebridean sites, whilst the remaining Scottish *P rutilus* sites, mainland and Shetland lochs, formed end Group 4.

Table 2.2 Scottish and Finnish *P. rutilus* TWINSPAN Loch Groups

Group 1	**Group 2**	**Group 3**	**Group 4**
Lake Grundtrask (F)	Lake Simpele (F)	Loch Grogary (O)	Loch Ussie (M)
Lake Rytilampitrask (F)	Lake K. Hovi (F)	Loch Scarie (O)	Loch Bayfield (M)
Lake Lampistrask (F)		Loch an Eilein (I)	Loch Eye (M)
		Loch a Chlair (I)	Loch Kirkigarth (S)
		Loch Ballyhaugh (I)	Loch Bardister (S)
		Loch Lossit (I)	

F = Finland, O = Outer Hebrides, I = Inner Hebrides, M = Mainland Scotland, S = Shetland

Table 2.3 was drawn up from the Scottish and Finnish sites and species matrix from the TWINSPAN output, to show the range of macrophyte species and their constancy of occurrence within each of the four loch groups. The macrophyte species constancy table

shows the groupings of distinctive (indicator) species, which have been used by TWINSPAN to separate site into groups.

Table 2.3 Floristic Constancies of Scottish and Finnish *P. rutilus* Loch Types

Macrophyte species	**Group 1 (n = 3)**	**Group 2 (n = 2)**	**Group 3 (n = 6)**	**Group 4 (n = 5)**
Elodea canadensis		V		IV
Isoetes lacustris		III		III
Nitella walhbergiana		III		
Potamogeton friesii		III		
Potamogeton obtusifolius		III		II
Potamogeton pusillus		V	I	II
Subularia aquatica		III		II
Sparganium emersum		III		
Hydrocharis morsus-ranae		III		
Chara sp.	III	III		
Nitella flexilis		III	I	II
Sparganium angustifolium		V	II	II
Apium inundatum			III	II
Baldellia ranunculoides			II	
Chara aspera			IV	II
Elodea nuttallii			II	
Fontinalis antipyretica			IV	IV
Littorella uniflora			V	V
Myriophyllum alterniflorum		III	V	V
Myriophyllum spicatum			II	
Najas flexilis			II	
Nymphaea alba			I	II
Potamogeton gramineus		III	V	IV

Frequency score :V = 81-100%, IV = 61-80%, III = 41-60%, II = 21-40%, I =up to 20%

Table 2.3 continued.

Macrophyte species	Group 1 (n = 3)	Group 2 (n = 2)	Group 3 (n = 6)	Group 4 (n = 5)
Potamogeton natans		III	V	V
Potamogeton pectinatus			III	II
Potamogeton polygonifolius			II	II
Persicaria amphibia	III			
Filamentous algae			IV	III
Callitriche hamulata				III
Callitriche stagnalis				II
Chara curta				II
Chara virgata				IV
Elatine hexandra				II
Isoetes echinospora				II
Juncus bulbosus			II	III
Lemna minor				II
Lobelia dortmanna				III
Nitella sp.				II
Potamogeton alpinus				II
Potamogeton crispus			I	III
Potamogeton praelongus				II
Potamogeton x *nitens*			II	III
Ranunculus trichophyllus				II
Utricularia vulgaris			I	
Zannichellia palustris				II
Potamogeton filiformis	II		IV	III
Potamogeton perfoliatus	II		V	V
Nitella opaca	II			II
Callitriche hermaphroditica	II		II	I

Frequency score :V = 81-100%, IV = 61-80%, III = 41-60%, II = 21-40%, I =up to 20%

Table 2.3 continued.

Macrophyte species	**Group 1 (n = 3)**	**Group 2 (n = 2)**	**Group 3 (n = 6)**	**Group 4 (n = 5)**
Potamogeton rutilus	V	V	V	V
Potamogeton berchtoldii	IV	V	II	II
Stratiotes aloides	II	II		
Chara globularia	II			
Chara strigosa	II			
Myriophyllum sibiricum	II			
Potamogeton compressus	II			
Ceratophyllum demersum	II			
Nuphar lutea	IV			
Nuphar pumila	II			
Sagittaria sagittifolia	II			
Najas tenuissima	II			
Lemna trisulca	V			
Lemna spirodela	II			
Sparganium gramineus	II			

Frequency score :V = 81-100%, IV = 61-80%, III = 41-60%, II = 21-40%, I =up to 20%

2.3.2 Descriptions of Scottish and Finnish Floristic Loch Types

Group 1: Finnish Nuphar lutea - Lemna trisulca lakes

The Finnish *P. rutilus* lakes grouped together as group1 sites share the key indicator species *Lemna trisulca* with the high constancy presence of *Nuphar lutea*. In addition to these indicator species, there is a high constancy of *Perscaria amphibia*, which is not present in any of the other *P. rutilus* groups. Three species, *Myriophyllum alterniflorum, Potamogeton gramineus* and *Potamogeton natans,* occur at high constancy frequencies in the other *P. rutilus* groups but do not feature in this type 1 group.

Group 2: Finnish Elodea canadensis –Stratoites/Hydrocharis lakes

The Finnish lakes in group 2 consist of an *Elodea canadensis* community type with the presence of floating macrophyte *Hydrocharis morsus-ranae* and *Stratiotes aloides.* The shared presence of *Stratiotes aloides* is found in both Finnish loch groups 1 and 2, with this species not being found in any of the Scottish *P. rutilus* lochs. *Potamogeton berchtoldii* has a high constancy frequency presence in both Finnish groups, whilst having a much lower frequency in the Scottish *P. rutilus* groups.

Group 3: Hebridean Machair Chara aspera –Potamogeton filiformis Lochs

This grouping of *P. rutilus* sites consists of the Inner and Outer Hebridean lochs, which have a high frequency of *Chara aspera* and *Potamogeton filiformis* compared to the other groups. In addition, two other species, *Apium inundatum* and *Potamogeton pectinatus*, are also found at a reasonably high constancy frequency in this group 3. Three species found in this group and not found in any other *P. rutilus* group are *Myriophyllum spicatum, Baldellia ranunculoides* and the rare *Najas flexilis* all at low frequency. The invasive *Elodea nuttallii* is also found in this group in contrast to the other invasive, *Elodea canadensis,* which is found in Scottish mainland *P. rutilus* lochs belonging to group 4 and in Finnish group 2 lochs.

Group 4: Elodea canadensis - Chara virgata Lochs

The mainland and Shetland lochs that host *P. rutilus* are confined to group 4. *Callitriche hamulata* and *Chara virgata* at high frequency are key indicator species for this group, not found in the previous group 3. The isoetids *Lobelia dortmanna, Isoetes lacustris* and *Subularia aquatica* feature strongly in this group compared to their absence in the other Scottish loch type, group 3. *Juncus bulbosus* also occurs at a reasonably high constancy frequency and does not occur in either of the Finnish loch types

2.3.3 TWINSPAN Analysis of Current and Former Scottish *P. rutilus* sites

The 11 current and 8 former Scottish *P. rutilus* sites analysed by TWINSPAN produced 4 main macrophyte community end groups, see Table 2.4 for loch site groupings.

The lochs were divided into mainly geographical groupings, with Group 1 being mainly Shetland lochs plus the northern mainland site Loch Eye. Group 2 consists of a mixture of mainland and outer Hebridean Lochs. Hebridean lochs made up Group 3 with one mainland former *P. rutilus* site, Loch Flemington. In the case of Group 4, all sites consisted of Outer Hebridean sites, with four of these being former *P. rutilus* lochs.

Table 2.4 Scottish Current and Former *P. rutilus* TWINSPAN Loch Groups

Group 1	**Group 2**	**Group 3**	**Group 4**
Eye (M)	Bayfield (M)	Grogary (O)	Scarie (O)
Kirkigarth (S)	Ussie (M)	Flemington (M) *	na Reivil (O) *
Bardister (S)	Awe (M) *	an Eilein (I)	Mhor (O) *
Asta (S) *	Ballyhaugh(I)	a Chlair (I)	Leodosay (O)*
Tingwall (S) *	Lossit (I)		nam Feithean (O) *

O = Outer Hebrides, I = Inner Hebrides, M = Mainland Scotland (* Former *P. rutilus* sites)

Table 2.5 was drawn up from these sites and species from the TWINSPAN output, to show the range of macrophyte species and their constancy of occurrence within each of the four loch groups.

Table 2.5 Floristic Constancies of Scottish Current and Former *P rutilus* Lochs

Macrophyte species	**Group 1 (n = 5)**	**Group 2 (n = 5)**	**Group 3 (n = 4)**	**Group 4 (n = 5)**
Callitriche stagnalis		I		
Lemna minor		I		
Nitella opaca		II		
Juncus bulbosus	I	V		
Najas flexilis		II		
Nymphaea alba		II		
Potamogeton alpinus		II		
Sparganium angustifolium		V		
Utricularia intermedia		I		
Utricularia minor		I		
Callitriche hamulata	I	II		
Potamogeton praelongus	III			
Chara curta	III			
Elatine hexandra	I			
Isoetes echinospora	II			
Nitella translucens	III			
Zannichellia palustris	III	I		
Chara virgata	III	II		
Isoetes lacustris	III	II		
Lobelia dortmanna	II	II		
Ranunculus trichophyllus	I	I		
Subularia aquatica	I	I		
Utricularia vulgaris	I	I		
Callitriche hermaphroditica	III	I	II	
Potamogeton berchtoldii	III	III		I

Frequency score :V = 81-100%, IV = 61-80%, III = 41-60%, II = 21-40%, I =up to 20%

Table 2.5 continued

Macrophyte species	Group 1 (n = 5)	Group 2 (n = 5)	Group 3 (n = 4)	Group 4 (n = 5)
Nitella flexilis	II		II	
Potamogeton x nitens	III	I	III	
Fontinalis antipyretica	IV	IV	III	I
Potamogeton natans	IV	V	III	IV
Potamogeton perfoliatus	V	V	III	V
Potamogeton rutilus	III	IV	III	I
Elodea canadensis	I	II	II	
Potamogeton crispus	I	III		II
Potamogeton polygonifolius		III	II	II
Apium inudatum		II	II	
Baldellia ranunculoides		I	II	
Potamogeton obtusifolius		I	II	
Potamogeton gramineus	III	V	V	II
Potamogeton filiformis	III	II	IV	II
Potamogeton pusillus	II		II	I
Littorella uniflora	V	V	V	IV
Myriophyllum alterniflorum	V	IV	V	IV
Filamentous algae	V	I	III	V
Chara sp.		II	II	III
Potamogeton pectinatus	I	I	I	V
Chara hispida				II
Elodea nuttallii			II	I
Myriophyllum spicatum			I	V
Potamogeton friesii				III
Ranunculus baudotii				II
Persicaria amphibia		I	III	III

Frequency score :V = 81-100%, IV = 61-80%, III = 41-60%, II = 21-40%, I =up to 20%

Table 2.5 continued.

Macrophyte species	Group 1 (n = 5)	Group 2 (n = 5)	Group 3 (n = 4)	Group 4 (n = 5)
Chara aspera	I	I	III	

Frequency score :V = 81-100%, IV = 61-80%, III = 41-60%, II = 21-40%, I =up to 20%

2.3.4 Descriptions of Current and Former Scottish *P rutilus* Floristic Loch Types

Group1: Meso-eutrophic Lochs (Potamogeton praelongus-Callitriche hermaphroditica)

All the current and former Shetland *P. rutilus* lochs are confined to this group, apart from Loch Eye, a northern mainland *P. rutilus* loch. High frequency of *Callitriche hermaphroditica, Chara virgata* and *Potamogeton praelongus* are key indicators for this group.

Group 2: Oligo-mesotrophic Lochs *(Sparganium angustifolium-Juncus bulbosus)*

The main group 2 indicator species are *Sparganium angustifolium* and *Juncus bulbosus,* which are only found in this group, apart from the low-frequency occurrence of *J. bulbosus* in group 1. Three species, with up to a 40% constancy, only present in this group, are *Nymphaea alba, Potamogeton alpinus* and the rare *Najas flexilis.* Group 2 has the lowest constancy presence of filamentous algae.

Group 3: Shallow Hebridean Lochs (Chara aspera-Potamogeton filiformis)

This grouping of *P rutilus* sites consists mostly of the Inner and Outer Hebridean lochs which have a high frequency of *Chara aspera, Potamogeton filiformis* and which all still host *P. rutilus.* This group also contains one former *P. rutilus* mainland site, Loch Flemington. *Potamogeton berchtoldii* and *Potamogeton crispus* are two species found in all other groups, but absent from this group.

Group 4: Shallow Machair Lochs (Myriophyllum spicatum-Potamogeton pectinatus)

All the Outer Hebridean lochs that previously hosted *P. rutilus* are confined to group 4, together with one site, Loch Scarie, that still hosts the plant. *Myriophyllum spicatum* and

Potamogeton pectinatus at high frequencies are key indicator species for this group, Table 2.5. There is no presence of *Chara aspera* in this group, being replaced by *Chara hispida*, with *Ranunculus baudotii* being only present in this group.

2.3.5 TWINSPAN Analysis of Current, Former and Potential Scottish *P. rutilus* lochs

The current eleven, eight former and three possible Scottish *P. rutilus* sites were analysed by TWINSPAN to produced four main macrophyte community types; see Table 2.6 for loch site groupings. These four groupings are very similar to Table 2.4 TWINSPAN analysis of current and former *P. rutilus* lochs with the potential *P. rutilus* sites being grouped in Type 1 and 3 loch groups. The potential *P. rutilus* sites are those that do not host *P. rutilus* but are in the geographical vicinity of current *P. rutilus* sites and appear to have similar macrophyte communities with similar trophic/sediment conditions.

The potential *P. rutilus* lochs in floristic groups 1 and 2 coincide with their geographical distributions. Loch Benston is a Shetland site, being categorised in Group 1 Shetland dominated lochs, whilst the two potential *P. rutilus* Inner Hebridean sites are grouped in the Hebridean dominated group 3 loch type.

Table 2.6 Scottish Current, Former and Potential *P. rutilus* TWINSPAN Loch Groups

Group 1	**Group 2**	**Group 3**	**Group 4**
Eye (M)	Bayfield (M)	Grogary (O)	na Reivil (O) *
Kirkigarth (S)	Ussie (M)	Ballyhaugh (O)	Mhor (O) *
Bardister (S)	Awe (M) *	Scarie (O)	Leodosay (O) *
Asta (S) *	Lossit (I)	an Eilein (I)	nam Feithean (O) *
Tingwall (S) *		a Chlair (I)	
Benston (S) ^		Phuill (I) ^	
		Bhasopoll (I) ^	
		Flemington (M) *	

O = Outer Hebrides, I = Inner Hebrides, M = Mainland Scotland, S = Shetland

(* Former *P. rutilus* sites, ^ Potential *P. rutilus* sites)

The Floristic Constancy Table 2.7 below was drawn up from current, former and potential Scottish loch sites and floristic species matrix from the TWINSPAN output. This shows the range of macrophyte species and their constancy of occurrence within each of the four loch groups.

Table 2.7 Floristic Constancies of Scottish Current, Former and Potential *P. rutilus* Lochs.

Macrophyte species	**Group 1 (n = 6)**	**Group 2 (n = 4)**	**Group 3 (n = 8)**	**Group 4 (n = 4)**
Callitriche stagnalis		II		
Lemna minor		II		
Nitella opaca		III		
Nymphaea alba		III		
Potamogeton alpinus		III		
Utricularia intermedia		II		
Utricularia minor		II		
Callitriche hamulata	1	III		
Elodea canadensis	I	III	I	
Juncus bulbosus	II	V	I	
Sparganium angustifolium		V	1	
Isoetes lacustris	III	III		
Lobelia dortmanna	III	III		
Ranunculus trichophyllus	I	II		
Subularia aquatica	I	II		
Utricularia vulgaris	I	II		
Chara virgata	III	III	I	
Chara curta	III			
Elatine hexandra	I			
Isoetes echinospra	II			
Nitella translucens	III			
Potamogeton praelongus	III			
Zannichellia palustris	IIII	II		
Callitriche hermaphroditica	IV	II	I	

Frequency score :V = 81-100%, IV = 61-80%, III = 41-60%, II = 21-40%, I = up to 20%

Table 2.7 continued

Macrophyte species	**Group 1 (n = 6)**	**Group 2 (n = 4)**	**Group 3 (n = 8)**	**Group 4 (n = 4)**
Nitella flexilis	II		I	
Potamogeton berchtoldii	IV	III	II	
Potamogeton crispus	I	III	I	
Fontinalis antipyretica	IV	IV	III	II
Littorella uniflora	V	V	V	IV
Myriophyllum alterniflorum	V	V	V	IV
Potamogeton natans	V	V	IV	IV
Potamogeton perfoliatus	V	V	IV	V
Potamogeton rutilus	IV	III	IV	
Potamogeton x nitens	IV	II	III	
Najas flexilis		II	I	
Potamogeton obtusifolius		II	I	
Potamogeton polygonifolius		III	II	II
Potamogeton filiformis	IV	III	IV	II
Potamogeton gramineus	IV	V	V	II
Potamogeton pusillus	II		II	II
Filamentous algae	V		III	V
Chara sp.	I	III	III	III
Apium inundatum		II	III	
Baldellia ranunculoides			II	
Chara aspera			III	
Elodea nuttallii			II	
Potamogeton pectinatus			II	V
Myriophyllum spicatum			III	V
Persicaria amphibia			IV	III

Frequency score :V = 81-100%, IV = 61-80%, III = 41-60%, II = 21-40%, I = up to 20%

Table 2.7 continued.

Chara hispida				III
Potamogeton friesii				IV
Ranunculus baudotii				III

Frequency score :V = 81-100%, IV = 61-80%, III = 41-60%, II = 21-40%, I = up to 20%

2.3.6 FloristicType Groupings of Scottish Lochs which appear to be Potential Sites for *P. rutilus*

Group1: Meso-eutrophic Lochs (Potamogeton praelongus-Callitriche hermaphroditica)

The potential *P. rutilus* site Loch Benston is placed in TWINSPAN group 1. All the current and former Shetland *P. rutilus* lochs also form part of this TWINSPAN group, apart from mainland Loch Eye.

Group 3: Shallow Hebridean Lochs (Potamogeton gramineus-Potamogeton filiformis)

The potential *P. rutilus* lochs, Phuill and Bhasopoll are grouped into the Hebridean dominated TWINSPAN groups 3. The high floristic frequency of *P. gramineus* in group 3, with the absence of key group 4 indicator species *Potamogeton friesii,* separates these two Hebridean dominated TWINSPAN groups 1 and 3.

2.3.7 Palmer's Floristic Trophic Ranking Analysis of *P. rutilus* Lochs

Palmer's trophic analysis of the known current *P. rutilus* lochs produces 4 different loch trophic types which host *P. rutilus,* Table 2.8. The trophic types range from the most nutrient poor of the four, oligotrophic Type 3, to the most nutrient rich, eutrophic Type 7. Loch Type 4, defined as oligotrophic with eutrophic influences, appears to be the most prevalent loch type that currently hosts *P. rutilus.* Note that the two Perthshire *P. rutilus* lochs, Fingask and Drumore, that were not known about until the very late stages of this study, belong to trophic type 5A.

Table 2.8 Palmer's Loch Types for Current Scottish *P. rutilus* Lochs

Loch Type 3	**Loch Type 4**	**Loch Type 5A**	**Loch Type 7**
Loch Eye (M)	Loch Grogary (O)	Loch Bayfield (M)	Loch Scarie (O)
Loch Ussie (M)	Loch an Eilein (I)	Loch Kirkigarth (S)	
Loch Ballyhaugh (I)	Loch Lossit (I)	Loch Bardister (S)	
	Loch an Chlair (I)		

O = Outer Hebrides, I = Inner Hebrides, M = Mainland Scotland, S = Shetland

The Table 2.9 shows the loch type groupings of current, former and possible *P. rutilus* lochs.

Table 2.9 Palmer's Loch Types for Scottish Current, Former and Potential *P. rutilus* Lochs

Loch Type 3	**Loch Type 4**	**Loch Type 5A**	**Loch Type 7**
Eye (M)	Grogary (O)	Bayfield (M)	Scarie (O)
Ussie (M)	an Eilein (I)	Flemington (M) *	Mhor (O) *
Ballyhaugh (I)	Lossit (I)	Kirkigarth (S)	Leodasay (O) *
Awe (M)	a Chlair (I)	Bardister (S)	na Reivil (O) *
	Asta (S) *		nam Feithean (O) *
	Tingwall (S) *		Bhasapoll (I) ^
	Benston (S) ^		
	Phuill (I) ^		

O = Outer Hebrides, I = Inner Hebrides, M = Mainland Scotland, S = Shetland

(* Former *P. rutilus* sites, ^ Potential *P. rutilus* sites)

The Table 2.10 gives the lake type standing water classification for Finnish *P. rutilus* sites.

Table 2.10 Palmer's Lake Types for Finnish *P. rutilus* Lakes

Lake Type 3	**Lake Type 9**	**Lake Type 10**
Lake K. Hovi	Lake Grundtrask	Lake Simpele (10A)
	Lake Rytilamptrask	Lake Lampistrask (10B)

2.3.8 National Vegetation Classification of *P. rutilus* Lochs

The National Vegetation Analysis (NVC) Match coefficient analysis, Table 2.11 shows that current *P. rutilus* lochs consist of one main NVC aquatic community type A13, *Potamogeton perfoliatus – Myriophyllum alterniflorum* community, see below definitions of NVC community types:

Table 2.11 Scottish Current, Former and Potential *P. rutilus* NVC Match Loch Groups

A13a Community		**A13b Community**		**A11/A11b Community**		**A11c Community**	
Lochs	**% Match**	**Lochs**	**% Match**	**Lochs**	**% Match**	**Lochs**	**% Match**
Eye (M)	78	Grogary (O)	77	Leodosay (O) *	85	na Reivil (O) *	74
Bayfield (M)	76	Scarie(O)	84	Mhor (O) *	71	Nam Feithean (O) *	88
Ussie (M)	56	Kirkigarth (S)	68				
Awe (M) *	59	Bardister (S)	82				
Flemington (M) *	80	Asta (S) *	88				
Ballyhaugh (I)	56	Tingwall (S) *	69				
		an Eilein (I)	89				
		Lossit (I)	76				
		a Chlair (I)	67				
		Benston (S) ^	86				
		Bhasopoll (I) ^	84				
		Phuill (I) ^	70				

O = Outer Hebrides, I = Inner Hebrides, M = Mainland Scotland, S = Shetland. (* Former *P. rutilus* sites, ^ Potential *P. rutilus* sites)

A13, *Potamogeton perfoliatus – Myriophyllum alterniflorum* community

A13b *Potamogeton perfoliatus – Myriophyllum altereniflorum,* sub-community *Potamogeton filiformis*

A11 *Potamogeton pectinatus-Myriophyllum spicatum* community

A11b *Potamogeton pectinatus-Myriophyllum spicatum,* sub-community *Elodea canadensis*

A11c *Potamogeton pectinatus-Myriophyllum spicatum,* sub-community *Potamogeton filiformis* (Rodwell 1995).

The A13 *P. rutilus* lochs consist of two *P. perfoliatus – M. alterniflorum* sub-community types. This consists of A13a *Potamogeton berchtoldii* sub-community which includes all the current and former *P. rutilus* mainland lochs, and A13b *Potamogeton filiformis* sub-community which includes the majority of Hebridean and Shetland lochs that still host *P. rutilus.* The former Shetland *P. rutilus* lochs and possible *P. rutilus* lochs are also classified as A13b sub-communities. All of the former Outer Hebridean *P. rutilus* lochs are classified within the A11 *Potamogeton pectinatus-Myriophyllum spicatum* community type. These former *P. rutilus* lochs consist of several A11 sub-communities, which include A11b *Elodea canadensis*, and A11c *P. filiformis* sub-communities, see comparisons Table 2.12.

Table 2.12 Comparison of NVC Categories/Palmer's Trophic Types with Scottish TWINSPAN Groups (current, former and potential *P. rutilus* lochs)

Lochs	**NVC Category**	**Palmer's Trophic Type**	**TWINSPAN Group**
Eye (M)	A13a	3	1
Bayfield (M)	A13a	5A	2
Ussie (M)	A13a	3	2
Awe (M) *	A13a	3	2
Flemington (M) *	A13a	5A	3
Ballyhaugh (I)	A13a	3	3
Grogary (O)	A13b	4	3
Scarie (O)	A13b	7	3
Kirkigarth (S)	A13b	5A	1
Bardister (S)	A13b	5A	1

Table 2.12 continued

Lochs	NVC Category	Palmer's Trophic Type	TWINSPAN Group
Asta (S) *	A13b	4	1
Tingwall (S) *	A13b	4	1
an Eilein (I)	A13b	4	3
Lossit (I)	A13b	4	2
a Chlair (I)	A13b	4	3
Benston (S) ^	A13b	4	1
Bhasapoll (I) ^	A13b	7	3
Phuill (I) ^	A13b	4	3
Leodosay (O) *	A11/A11b	7	4
Mhor (O) *	A11/A11b	7	4
na Reivil (O) *	A11c	7	4
nam Feithean (O) *	A11c	7	4

O = Outer Hebrides, I = Inner Hebrides, M = Mainland Scotland, S = Shetland.

(* Former *P. rutilus* sites, ^ Potential *P. rutilus* sites)

The Table 2.13 gives the NVC match groupings for Finnish *P. rutilus* lakes. The NVC aquatic community types are defined as follows:

A2b *Lemna minor,* sub-community *Lemna trisulca*

A8 *Nuphar lutea* community

A11b *Potamogeton pectinatus-Myriophyllum spicatum,* sub-community *Elodea canadensis*

A15 *Elodea canadensis* community (Rodwell 1995)

Table 2.13 Finnish *P. rutilus* NVC Match Loch Groupings

A 2b Community		A 8 Community		A 11b Community		A 15 Community	
Lakes	% Match	Lakes	% Match	Lakes	% Match	Lakes	% Match
Lampistrask	29	Grundtrask	27	Simpele	32	K. Hovi	30
		Rytilampitrask	39				

2.4 DISCUSSION

2.4.1 TWINSPAN floristic evaluation of *P. rutilus* community types

Finnish P. rutilus Lakes

The floristic TWINSPAN analysis *P. rutilus* of sites clearly revealed that Scottish and Finnish sites were quite different, being divided into distinctive floristic groups: Table 2.3. The Finnish lake group 1 appears to have higher trophic influences than the other groups, with the presence of indicator species *Lemna trisulca* and high constancy presence of *Nuphar lutea,* both species being usually associated with meso-eutrophic conditions (Palmer *et al.* 1992, Rodwell 1995, Preston & Croft 1997). In addition, certain species only present in this group, namely *Persicaria amphibia, Ceratophyllum demersum* and *Sagittaria sagittifolia,* have strong eutrophic affinities (Rodwell 1995).

The absence from group 1 of *Myriophyllum alterniflorum* and *Potamogeton gramineus* that occur at high constancies in all the other groups, further suggests this Finnish lake type is more eutrophic in character, as these absent species have strong oligo-mesotrophic floristic affinities (Palmer *et al.* 1992, Heegard *et al.* 2001, Murphy 2002).

Both Finnish floristic groups share a high constancy presence of *Potamogeton berchtoldii,* with a low frequency occurrence of *Stratiotes aloides,* which is not found in the Scottish *P. rutilus* groups. These two meso-eutrophic species with *Chara* are the only constancy species that the two Finnish *P. rutilus* groups share. *Chara* species in Finland are generally regarded to occur in fairly eutrophic lakes (Rintanen 1996). However, some floristic mesotrophic influences are reflected in this eutrophic type group with the low constancy presence of *Potamogeton compressus, Callitriche hermaphroditica, Nuphar pumila* and the rare *Najas tenuissima* (Palmer *et al.*1992, Kotiranata *et al.*1998).

The constant species, which distinguish Finnish *P. rutilus* group 2, are the high constancies of *Elodea canadensis, Potamogeton pusillus* and *Potamogeton berchtoldii*;

species mostly associated with meso-eutrophic lakes (Palmer 1992). The high constancy presence of oligotrophic associated *Sparganium angustifolium* with the presence of *Myriophyllum alterniflorum* and *Potamogeton gramineus* suggest some oligo-mesotrophic floristic influences in this Finnish *P. rutilus* group.

Scottish P. rutilus Lochs

In this Finnish and Scottish TWINSPAN analysis the Scottish *P. rutilus* lochs are divided into two main groups, Hebridean group 3 lochs, whilst mainland and Shetland lochs are combined to form a group 4 loch type. The Hebridean lochs group type have a high constancy of indicator species *Chara aspera* with *Potamogeton filiformis*, suggesting some mesotrophic base rich floristic characteristics, as found in many machair lochs (Preston *et al.* 2000, Palmer 1992). TWINSPAN floristic analysis of Inner Hebridean lochs by Preston *et al.* 2000 placed *P. rutilus* lochs in their E and F groupings that they described as having machair base-rich influences, which have similar floristics to Hebridean *P. rutilus* group 4, in this analysis. The high constancy *Potamogeton pectinatus* and the only presence of *Myriophyllum spicatum* in this mesotrophic group further confirm floristic, maritime, base-rich influences (Palmer *et al.*1992, Murphy 2002).

Finally, group 4 lochs that include mainland and Shetland *P. rutilus* lochs have characteristic indicator species *Callitriche hamulata* and *Chara virgata* which are not found in any other groups. These indicator species with the high constancy presence of isoetids, *Lobelia dortmanna, Isoetes lacustris* and the only presence of *Subularia aquatica*, indicate there is greater oligotrophic floristic influence in this *P. rutilus* group (Farmer & Spence 1986, Palmer 1992, Gacia *et al.* 1994). The greater abundance of *Juncus bulbosus,* a calcifuge species (Preston & Croft 1997) in this group further confirms the lower trophic influences in this floristic loch group.

2.4.2 Floristic loch type evaluation of changes in Scottish *P. rutilus* distribution

Scottish current and former P. rutilus sites

This TWINSPAN analysis was carried out on current and former Scottish *P. rutilus* sites and produced four distinct floristic loch groups, see Table 2.4. Group 2 had the highest constancy presence of *P. rutilus* with group 4 having the lowest constancy presence, see Table 2.5.

Group 2 appeared to be the most oligotrophic floristically of all these group categories, having the high constancy indicator species *Sparganium angustifolium* with the highest constancies of *Juncus bulbosus* and *Potamogeton polygonifolius.* These three high constancy species are regarded as having strong oligotrophic floristic affinities (Palmer *et* al.1992, Sand-Jensen *et al.* 2000, Murphy 2002). However, the presence at high floristic constancy of oligo-mesotrophic species, *Potamogeton gramineus, Myriophyllum alterniflorum, Littorella uniflora* and the moss *Fontinalis antipyretica* suggests strong mesotrophic influences (Palmer *et al.* 1992, Preston *et al.* 2000). This group has the lowest constancy presence of filamentous algae, which are usually abundant in more nutrient rich eutrophic sites (Haslam 1990).

These above core mesotrophic species are also found at a high floristic constancies in the other *P. rutilus* groups, apart from Group 4 lochs, see Table 2.5. The least mesotrophic group 4 machair type lochs have the highest number of former *P. rutilus* sites. *Myriophyllum spicatum* and *Potamogeton pectinatus* are key indicator species of group 4 lochs, which considering their machair coastal locations, suggesting base rich sites with strong maritime influences as found in many Outer Hebridean machair lochs (Palmer *et al.* 1992, Rodwell 1995). This base rich maritime floristic influence is further emphasized by *Potamogeton friesii, Ranunculus baudotii* and *Chara hispida*, species found only in this group, that have been described as having maritime and/or calcareous affinities (Moore 1986, Preston 1995, Preston & Croft 1997).

The other loch groupings 1 and 2 have similar constancy presences of *P. rutilus.* Group 1 sites consist of all the Shetland current and former *P. rutilus* lochs and one mainland site, Loch Eye. All these group 1 lochs appear to have floristic meso-eutrophic influences, having high constancy indicator species *Callitriche hermaphroditica, Potamogeton praelongus* with *Potamogeton berchtoldii* and *Zannichellia palustris*, which have meso-eutrophic affinities (Palmer *et al.* 1992, Preston 1995, Preston & Croft 1997). This Shetland dominated loch group with the presence of isoetids, *Isoetes lacustris, I. echinospora* and *Lobelia dortmanna* indicate some oligotrophic influences on this loch group. (Palmer *et al.* 1992, Farmer & Spence 1986).

Finally, group 3 sites dominated by Hebridean machair lochs have more mesotrophic base rich influences, compared to the Shetland lochs, having as indicator species *Potamogeton filiformis* and *Chara aspera* (Preston & Stewart 2000). The inclusion of the former mainland *P. rutilus*, Loch Flemington in this Hebridean loch grouping, reflects the arbitrary nature of TWINSPAN when classifying species poor sites such as Loch Flemington. A macrophyte species poor site, such as Loch Flemington with only 7 species, appear to have too few species to produce an adequate TWINSPAN grouping of the site, compared to more species rich sites.

In evaluating *P. rutilus* Hebridean loch distribution, it appears to favour the mesotrophic floristic machair group 3, compared to the more maritime/base rich machair group 4 lochs which has a much lower *P. rutilus* frequency.

2.4.3 How *P. rutilus* TWINSPAN evaluation fits with other loch floristic classification systems

Palmer's Botanical Standing Waters Classification

Classification of current Scottish *P. rutilus* lochs using the method of Palmer *et al.* 1992 produced four different trophic types, Table 2.7. These four loch types are defined as follows:

Loch Type 3: Oligotrophic, usually base poor waters with a range of isoetids.
Loch Type 4: Waters with a mixture of oligo-mesotrophic influences, which are mostly in northern Scotland coastal lochs, including many machair lochs.
Loch Type 5A: Meso-eutrophic species rich sites.
Loch Type 7: Eutrophic or base rich waters, similar to type 4, but with strong marine influences, found predominantly in northern Britain.

The Table 2.8 results clearly showed that *P. rutilus* was restricted to three main loch trophic types, ranging from oligotrophic to meso-eutrophic floristic types, with oligo-mesotrophic type 4 having the most *P. rutilus* loch sites.

Of the four different TWINSPAN groupings, floristic groups 3 and 4 appeared to fit best the *P. rutilus* standing water classifications, see TWINSPAN Tables 2.5, 2.6 and standing water Tables 2.8 & 2.9. TWINSPAN group 3 with its mainly Hebridean *P. rutilus* lochs was classified in the oligo-mesotrophic standing water type 4. It is no surprise that not only floristically but geographically this equivalent grouping fits, being geographically distinct machair lochs with a mixture of acid peat and calcareous sand trophic influences (Palmer 1989, Preston 1995, Preston *et al.* 2000).

The TWINSPAN group 4, dominated by Outer Hebridean former *P. rutilus* lochs were classified as a standing water type 7. This loch type 7 geographical classification, Outer Hebridean lochs with strong marine influences (Palmer 1989), fits with the TWINSPAN group 4 which all consists of machair lochs. The brackish marine influences on this Hebridean loch type 7 can be clearly seen in the floristics of the former *P. rutilus* lochs that dominate this group 4, Table 2.9. This machair coastal group is dominated by indicator species *Myriophyllum spicatum* and *Potamogeton pectinatus* accompanied by species such as *Ranunculcus baudotii*, suggests a saline influence (Palmer *et al.* 1992, Rodwell 1995). Interestingly, this loch type 7 hosts the greatest number of former *P. rutilus* sites with only one current *P. rutilus* site, Loch Scarie, being included here. This

would suggest that saline influences might be also contributing to the decline of *P. rutilus* in Loch Scarie, as well as the possible competitive impacts of invasive *Elodea nuttallii*.

The trophic floristic TWINSPAN group 2 has two lochs, Ussie and Ballyhaugh that are classified in the standing waters, oligotrophic type 3. However, there is some classification discrepancies, for example Loch Eye from group 1 was also classified as type 3. Loch Eye has a mixture of floristic trophic influences, having both mesotrophic *Potamogeton* species and more oligotrophic isoetids, which can explain how it could be classified in several trophic floristic groups.

The TWINSPAN group 1 appears to be more equivalent to standing water type 5A with all current Shetland *P. rutilus* lochs being classified in this mesotrophic trophic type. This mesotrophic standing water type 5A did not include all the group 1 sites, as former *P. rutilus* Shetland lochs were classified as mesotrophic type 4. As mentioned for Loch Eye, many of these former and current *P. rutilus* lochs have a range of trophic floristics, which can result in them being classified in several related trophic types. These type 4 and type 5A lochs can have a wide range of oligo-mesotrophic influences (Palmer 1989, Preston *et al.* 2002). Note, the two Perthshire *P. rutilus* lochs, Fingask and Drumore, that were not known about until the very late stages of this study, also belong to trophic type 5A.

The TWINSPAN classification of both, current, former and potential *P. rutilus* lochs showed a strong geographical bias, with Shetland sites dominating group 1, mainland sites mostly in group 2, mixture of Inner and Outer Hebridean sites in group 3, and group 4 consisting of Outer Hebridean sites (Tables 2.4 and 2.6).

The more eutrophic nature of the Finnish floristic TWINSPAN groups is clearly seen in their standing water classification, producing lake types 9 and 10 (Table 2.10), which are defined as being mainly eutrophic lochs (Palmer 1989, Palmer *et al.* 1992).

However, one of the Finnish lakes, K. Hovi, appeared to show less eutrophic floristic affinities, being classified as oligotrophic standing water type 3, similar to some Scottish *P. rutilus* lochs. Floristic definitions of the standing water eutrophic lake types are as follows:

Loch Type 9: Mainly eutrophic sites, usually dominated by the water lilies *Nuphar lutea* and *Nymphaea alba,* a rare lake type in Scotland.
Loch Type 10: Mainly lowland eutrophic waters, with two variant types; one, type10A, characterized by *Elodea canadensis* and *Lemna minor* and the other, type 10b, by *Chara* species.

Two of the three Finnish lakes from TWINSPAN floristic group 1 were classified as standing water type 9, having a high constancy of eutrophic indicator species *Nuphar lutea* which is used in both classification systems. The remaining group 1 Finnish lake, Lampistrask was not classified as lake type 9 but the more eutrophic lake type 10B, characterised by *Chara* species (Palmer *et al.* 1992). The other eutrophic standing water type 10A, characterised by *Elodea* and *Lemna*, seemed to have its eutrophic floristic equivalent in Finnish TWINSPAN group 2 with its high constancy of *Elodea canadensis.* However, there was one classification discrepancy, with Lake K. Hovi of eutrophic TWINSPAN group 2 being classified as oligotrophic standing water type 3.

See Appendix 1 for Palmer's standing waters floristic descriptions of the above loch types.

National Vegetation Classification (NVC) of P. rutilus Lochs

The NVC match classification of Scottish current, former and possible *P. rutilus* lochs, produced two main NVC aquatic community types, A13, *Potamogeton perfoliatus – Myriophyllum alterniflorum* community and A11, *Potamogeton pectinatus – Myriophyllum spicatum* community. These two NVC community types consisted of several sub-communities, which showed a strong geographical bias. The mainland sites

were grouped in A13a *Potamogeton berchtoldii* sub-community, Hebridean and Shetland sites grouped in A13b *Potamogeton filiformis* sub-community, whilst all former *P. rutilus* Outer Hebridean sites were classified in A11, *P. pectinatus-M. spicatum* community and two sub-communities, A11b, *Elodea canadensis* and A11c *P. filiformis* sub-communities (Rodwell 1995).

This NVC community type match analysis of *P. rutilus* sites fits reasonably well with some of the *P. rutilus* floristic groups produced from TWINSPAN analysis. The floristic TWINSPAN group 4, former Outer Hebridean *P. rutilus* sites, dominated by a *M. spicatum* and *P. pectinatus* community type was classified as NVC A11 community and sub-communities, see Table 2.11. Most of the mainland current *P. rutilus* sites in TWINSPAN group 2 are classified in the NVC sub-community type13a, *P. perfoliatus – M. alterniflorum*/sub-community *P. berchtoldii.* The bulk of the current and potential *P. rutilus* sites from TWINSPAN floristic groups 1 and 3 were classified as sub-community A13b *P. perfoliatus – M. alterniflorum*/sub-community *P. filiformis.* The NVC, A13b classification of the two floristic TWINSPAN groups 1 and 3 has resulted from their high constancy presence of *P. filiformis* which forms this NVC sub-community (floristic Tables 2.5 and 2.7).

The high constancy presence *P. perfoliatus – M. alterniflorum* in all the *P. rutilus* TWINSPAN floristic groups demonstrates why most of these lochs are classified in the A13 community types, see floristic table 2.3. This high constancy presence of *P. perfoliatus – M. alterniflorum* also occurs in former *P. rutilus* sites that form TWINSPAN group 4, but with the high constancy of *M. spicatum* and *P. pectinatus* this TWINSPAN floristic group is classified as NVC, A11 community types, see Table 2.5. The NVC classification of Finnish *P. rutilus* lakes clearly demonstrates their eutrophic floristic affinities, with the four community types, A2b *Lemna minor,* sub-community *Lemna trisulca,* A8 *Nuphar lutea community,* A11b *P. pectinatus-M. spicatum,* sub-community *E. canadensis* and A15 *E. canadensis* community, all communities usually characteristic of eutrophic conditions (Rodwell 1995). However, the NVC match groupings of Finnish *P. rutilus* lakes appear to have a low % match coefficient, all less

than 40%, compared to Scottish *P. rutilus* sites with most % match coefficients in the 70 to 80 % range (Tables 2.11 and 2.12). The NVC classification is a much less accurate descriptor of Finnish *P. rutilus* communities as Rodwell's NVC community types have been based on phyto-sociological data from Britain.

2.4.4 The Distribution of *P. rutilus* Floristic Communities

The standing water trophic typing revealed that the greatest proportion of *P. rutilus* lochs were from loch types 4 and 5A, which forms only 6 % of the surveyed lochs found in Scotland, see below Table 2.14. In fact this figure would be lower than 6 % as loch type 5 proportion of lochs, see Table 2.14, includes both type 5 species variants, species rich type 5A and species poor type 5B. In addition, the mesotrophic loch resource would be a lot less than the calculated 6 % of total Scottish lochs, as mesotrophic lochs were very much over represented in the Scottish loch surveys, with the surveys not being random but biased towards botanically rich sites, such as mesotrophic lochs (Palmer *et al.* 1992). If these proportions of loch types found in surveyed lochs are reflected in the total number of 31460 estimated Scottish lochs (Lyle & Smith 1994), it would suggest a figure of a lot less than the 6 % (1888) of mesotrophic loch types capable of hosting *P. rutilus* constitute a limited habitat resource.

Table 2.14 Percentage frequency of loch types in Scotland
(from Lassiere & Duncan 1997)

	Percentage frequency by loch Type										
	U	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Lochs											
%	4.2	10.3	31.3	40.8	2.9	3.1	0.8	4.3	1.4	0.2	0.6
No	93	230	696	909	65	70	18	95	32	5	14

U = sites which could not be classified using the Palmer *et al.* (1992) scheme.

Three *P. rutilus* lochs had the standing water oligotrophic type 3, but are not totally oligotrophic as they have oligo-mesotrophic affinities as revealed in the TWINSPAN floristic groupings. These *P. rutilus* oligotrophic type 3 sites, with oligo-mesotrophic floristics, would not be equivalent to the many type 3 lochs in Table 2.14, as many would undoubtedly be strongly oligotrophic lochs that dominate many of the upland areas surveyed in north-western Scotland.

Only one *P. rutilus* site, Loch Scarie, was classified as type 7 (base rich type with strong coastal influences), suggesting the trophic conditions of this loch type is the least suitable of all the trophic types that support *P. rutilus,* and may be contributing to its decline in this loch. The majority of former *P. rutilus* lochs, all Outer Hebridean coastal lochs, being in this standing water type 7 further supports this unsuitability. These former (type 7) *P. rutilus* lochs also form a distinctive floristic TWINSPAN group 4 and NVC A11 community and sub-communities (see Table 2.12). This Outer Hebridean floristic community is dominated by *Myriophyllum spicatum* and *Potamogeton pectinatus* with strong maritime influences suggesting a high degree of salinity compared to other *P. rutilus* floristic groups, which is confirmed by water chemistry data (in chapter 3). This group's higher ionic levels/salinity, may possibly explain why the majority of former *P. rutilus* sites are in this group, as *P. rutilus* is particularly sensitive to salinisation (Kazmierczakowa & Zarzycki 2001)

Three of the former *P. rutilus* lochs, one mainland site, Loch Flemington and two Shetland sites, Loch Asta and Loch Flemington, have believed to have lost *P. rutilus* through nutrient enrichment (Murphy 1989, Preston & Croft 1997, May *et al.* 2001). However, these former *P. rutilus* sites macrophyte floristic communities, being classed as mesotrophic in loch type 4 and meso-eutrophic type 5A, currently do not reflect eutrophic conditions. In the case of eutrophic Loch Flemington, with so few macrophytes it can be easily misclassified by TWINSPAN and also hardly fits its species rich type 5A standing water classification (Table 2.12).

The loss of nutrient sensitive macrophytes due to eutrophication of soft water lakes (oligo-meso-eutrophic) is a European wide problem (Rintanen 1996, Sand-Jensen *et al.* 2000, Murphy 2002). Eutrophication has been implicated in the decline of *P. rutilus* in Poland (Kasmierczakowa & Zazycki 2001) and Russia (Kotiranta *et al.* 1998). In the case of Finland, eutrophication appears to have not caused a decline in *P. rutilus,* but seems to have increased its distribution (Barkman 2000, Virola *et al.* 2001). A recent study of macrophyte surveys of southern Finland lakes in the 1980s (Virola *et al.* 2001) revealed six new species including *P. rutilus*, not found in surveys during the 1930s. As well as *P. rutilus* these other new species are, *Sparganium glomeratum, P. pusillus, P. pectinatus, Zannichellia palustris* and *Najas marina,* which are said to favour more eutrophic alkaline conditions. These new species appear to be new colonizers of southern Finnish lakes, many of which have suffered eutrophication in the last 50 years (Virola *et al.* 2001).

The recent *P. rutilus* colonisation of more eutrophied southern Finnish lakes is clearly seen with it been recently found as a new species for the southern Finnish province of Nyland, where it has colonised Lake Grundtrask, a lake which has suffered a decline of the very rare elodeid *Najas tenuisssima* due to eutrophication (Barkman 2000). This Finnish *P. rutilus* preference for more eutrophic conditions than the more mesotrophic Scottish *P. rutilus* plant communities can be clearly seen in the more eutrophic floristic character of the Finnish TWINSPAN groups, dominated by species that favour more eutrophic conditions, such as *Nuphar lutea, Stratoites aloides,* and *Lemna* species (Table 2.3). The eutrophic nature of the Finnish *P. rutilus* communities is further revealed by their standing waters classification that produced mainly type 9 and type 10 communities, which are associated with eutrophic conditions (Palmer *et al.* 1992).

The Finnish *P. rutilus* ability to grow in eutrophic conditions compared to the less trophic mesotrophic Scottish conditions, suggest the possibility of locally adapted forms of the plant. There has been some evidence suggesting *Potamogeton* populations can show local adaptations to environmental conditions, such as found in *Potamogeton*

pectinatus populations from both fresh and brackish waters that showed a difference in their tolerance to salinity in controlled growth experiments (Van Wijk *et al.* 1988). This may suggest that some variation within a species may be genetically based. However, other growth experiments have found that widely distributed *P. pectinatus* populations show a range of phenotypically plasticity, rather than being locally specialised to differences in irradiance and photoperiod (Pilon & Santamaria 2002). Phenotypic plasticity to environmental changes has also been demonstrated in *P. pectinatus* response to temperature changes, with the plants overall pattern of thermal response being similar for clones originating from distant localities (Pilon & Santamaria 2001). These findings show *P. pectinatus* may be locally specialised for some abiotic factors such as salinity, but also has a high capacity for acclimation to other environmental changes such as light and temperature. In the case of *P. rutilus*, the Scottish plants from Loch Ussie did seem to show some degree of phenotypic plasticity with the ability to grow in a range of trophic and light conditions as demonstrated in the growth experiments chapter 4. However, it cannot be ruled out that the more geographical isolated Scottish plants may have developed some ecotypic adaptations for mesotrophic growth conditions compared to the Finnish and other European *P. rutilus* populations, which predominantly inhabit eutrophic lakes.

2.4.5 Conservation value of *P. rutilus* Floristic Communities

The Scottish lochs that host the rare *P. rutilus* have a high conservation value for not only having this Near Threatened plant, but also for hosting a range of other nationally scarce and rare macrophyte species (Preston & Croft 1997). Several *P. rutilus* lochs host the nationally and internationally protected *Najas flexilis,* both Biodiversity Action Plan species. Other macrophytes with a limited distribution found in *P. rutilus* lochs include *Elatine hexandra* – regionally uncommon in SEPA areas SE and HIG, *Isoetes echinospora* – regionally uncommon in SEPA area SE, *Potamogeton filiformis* and charophyte *Chara aspera* nationally scarce (Palmer, in press, Preston & Croft 1997).
P. rutilus lochs not only host a range of rare species but also many of these oligo-mesotrophic loch types have the greatest diversity of macrophytes (Palmer *et al.* 1992,

Murphy 2002). The high conservation value of many these sites not only results from their botanical interest but also from other factors such as their high degree of unspoiled naturalness, as found in many of the *P. rutilus* machair lochs (Preston *et al.* 2000).

2.5 CONCLUSIONS

It is clear that *P. rutilus* can be associated with a broad range of trophic floristic community types. Scottish *P. rutilus* appears to favour oligo-mesotrophic floristic communities, whilst in Finland the plant tends to have more eutrophic floristic affinities. The floristic analysis suggests that *P. rutilus* is more vulnerable to extinction in high base rich, marine influenced, *M. spicatum – P. pectinatus* communities, as more than half of the former *P. rutilus* sites form this community type. This *M. spicatum - P. pectinatus* community group has the highest salinity levels of all the *P. rutilus* floristic types (see water chemistry, chapter 3) and this may explain why it is now absent from these saline machair lochs, as *P. rutilus* is particularly sensitive to salinity (Kazmierczakowa & Zarzycki 2001).

The other former Scottish *P. rutilus* sites did not appear to show clear floristic community differences from the current *P. rutilus* oligo-mesotrophic floristic groups. However, Loch Flemington is eutrophic (May *et al.* 2001), and this former *P. rutilus* site has been clearly misclassified as oligo-mesotrophic. TWINSPAN analysis can be arbitrary when classifying species poor sites such as Loch Flemington.

TWINSPAN analysis of nearby lochs, potentially suitable *P. rutilus* colonising sites, revealed most had floristically similar habitats to current *P. rutilus* sites, but were uncolonised by the plant. The very localised distributions of *P. rutilus* in northeast Scotland, Hebrides and Shetland cannot clearly be explained by limitation of suitable oligo-mesotrophic loch habitats. Although oligo-mesotrophic lochs are one of the less common loch types (Lassiere & Duncan 1997), they are still much more wide spread

than the distribution of *P. rutilus*. Factors other than suitable habitat appear to be limiting the distribution of *P. rutilus* in Scotland.

One critical factor that has been said to limit the distribution of rare plants such as *P. rutilus* is the lack of seed production and reliance on clonal growth (Henderson 2001, Rich & Fitzgerald 2002, Wilcox 2002). However, this does not seem to be the case for *P. rutilus* in Finland, with the plant colonising new provinces in southern Finland (Virola *et al.* 2001, Barkman 2000), even though it fruits infrequently (Kotiranta *et al.* 1998). Virola *et al.* (2001) cites the nutrient enrichment of Finnish southern lakes for encouraging the spread of *P. rutilus* into southern Finland. However, other environmental and biotic factors need to operate to facilitate the long distance colonisation of new sites. Migrating waterbirds are known to be key long-distance dispersal agents of aquatic propagules (Figuerola & Green 2002). In Karelia and adjacent southern Finland there has been an increase in migratory breeding Whooper Swans in the last 25 years (Hokhlova & Artemjev 2002), a factor which could possibly explain the long distance dispersal and recent colonisation of southern Finnish lakes, by new macrophyte species such as *P. rutilus*. Swans are thought to have promoted the dispersal and gene flow of *Potamogeton pectinatus* in areas of the Baltic (King *et al.* 2002).

It is clear that floristic descriptions alone can only explain some aspects of *P. rutilus* distribution. The vegetation analysis has provided *P. rutilus* floristic descriptions but cannot take account of other habitat variables, such as water chemistry, light regime, substrate, and plant competition, which can determine macrophyte distribution (Spence 1967, Seddon 1972, Denny 1972, Denny 1980, Day *et al.* 1988, Rørslett 1991, Palmer *et al.* 1992). These issues are covered in Chapter 3.

CHAPTER 3 : *Potamogeton rutilus* Water/Sediment Chemistry and Light Conditions

3.0 SUMMARY

The water chemistry, sediment chemistry and light extinction coefficients of lochs supporting *P. rutilus* were analysed to reveal the range of ecological conditions that support the plant and its macrophyte community type. Many of the current and former Scottish *P. rutilus* lochs appear to have a strong coastal influence, such as salinity which could have contributed to *P. rutilus* extinctions in the most saline trophic group 4 lochs. The high alkalinity levels of *P. rutilus* lochs may contribute to its success in such alkaline environments if it is able to utilise bicarbonate as a carbon source for photosynthesis, like some other members of its community type. Finnish *P. rutilus* trophic groups were found to have significantly higher aquatic total phosphate levels than Scottish trophic loch groups, confirming that Finnish *P. rutilus* tends to inhabit more eutrophic sites, compared to the more mesotrophic Scottish lochs supporting the plant. *P. rutilus* loch abundance and macrophyte diversity significantly declined with decreasing light availability.

Keywords: mesotrophic, eutrophic, water chemistry, sediment chemistry, salinity, alkalinity, total phosphate, light extinction coefficient, nutrient use efficiency, macrophyte diversity, TWINSPAN, trophic group, ecological monitoring.

3.1 INTRODUCTION

Water chemistry is a major factor in determining the aquatic flora found in a particular site (Seddon 1972, Preston, 1975, Palmer *et al.* 1992, Gacia *et al.* 1994). In particular aquatic nutrient levels, which will determine trophic status, will have a strong influence on the presence or absence of species (Arts *et al.* 1990), especially highly nutrient sensitive macrophyte species (Jupp & Spence 1977, Farmer & Spence 1986). Two of the most significant plant nutrients that appear to influence macrophyte growth are nitrogen and phosphorus, with phosphorus being often the nutrient that limits growth (Preston 1995). Over the past 100 years, many nutrient sensitive macrophyte species have been lost from numerous lowland water bodies, which have suffered eutrophication from intensive agriculture and development (Balls *et al.* 1989, Arts *et al.* 1990, Sand-Jensen *et al.* 2000, Murphy 2002).

The decline of some macrophyte species with increased nutrient enrichment is thought to be brought about by several factors, such as change in competitive balances (Farmer & Spence 1986, Spink *et al.* 1995). It is thought that competitive balances between plants are partly determine by their growth form and their ability to utilise nutrients (Farmer & Spence 1986, Garbey *et al.* 2004). However, some researchers believe that macrophyte competition has little effect on macrophytes (Wilson & Keddy 1991), and that the main eutrophic influence on macrophyte growth is reduced light conditions due to increased algal growth (Jupp & Spence 1977, Balls *et al.* 1989). For example, it has been suggested that eutrophication can cause increased epiphyton and periphyton growth that can competitively reduce nutrient and light availability for macrophyte growth (Phillips *et al.* 1978, Preston 1995, Jones *et al.* 2002). However, reduced light availability by itself can be one of the key limiting factors in macrophyte growth, as found in turbid growth conditions (Blindow 1992).Blindow (1992) suggested that eutrophication caused the decline of charophytes due to reduced light conditions.

In addition to trophic conditions, alkalinity has been found to be an important factor in determining macrophyte distribution in lakes (Vestergaard & Sand-Jensen 2000, Kahara

& Vermaat 2003). Alkalinity is known to be a close descriptor of bicarbonate concentration, which is an important source of inorganic carbon in the photosynthesis of many aquatic plants (Sand-Jensen 1987, Vestergard & Sand-Jensen 2000). The ability of macrophytes to utilise bicarbonate as a carbon source appeared to be one of the main factors influencing species distribution in Danish Lakes (Vestergaard & Sand-Jensen 2000).

In the case of Scottish *P. rutilus* its distribution has been associated with unpolluted mesotrophic loch sites that have some base-enrichment (Preston & Croft 1997). For example, in Shetland the plant grows near limestone outcrops, whilst its Hebridean sites are usually situated at the junction of calcareous machair and acid rocks (Preston 1995). Mainland *P. rutilus* sites, such as Loch Eye, appear also to show a range of catchment nutrient influences, that has resulted in the establishment in a diverse range of plant communities, characteristic of both nutrient-rich and nutrient poor conditions (Lassiere & Duncan 1997). Highly eutrophic conditions have been associated with the loss of the plant, in sites such as Loch Flemington (Preston & Croft 1997). However, in comparison, Finnish *P. rutilus* appears to inhabit eutrophic lakes (Virola *et al.* 2001) and in some cases thrive in sites suffering from eutrophication (Barkman 2000).

Clearly there is a need to evaluate what environmental factors, such as some of those described above, that may determine the distribution and survival of *P. rutilus*.
To determine these environmental factors, this present study will evaluate how the relationships between water chemistry, sediment chemistry and light environments may influence the distribution and abundance of *P. rutilus*, by the following aims.

- To determine the environmental factors that may influence the abundance and distribution of *P. rutilus*.

- To evaluate how seasonal environmental changes may affect *P. rutilus* loch sites.

- To determine if there are any significant environmental differences between present and former Scottish *P. rutilus* trophic loch groups.

- To evaluate if there are any significant environmental differences between Scottish and Finnish *P. rutilus* trophic groups.

3.2 METHODS

The aquatic chemistry and light data gathered during *P. rutilus* surveys of Scottish and Finnish loch surveys, were used to further evaluate the TWINSPAN vegetation groups, produced in the previous chapter. Seasonal changes in water chemistry and light levels will be investigated, using one former and three present *P. rutilus* mainland sites that were monitored on a monthly basis, as a single water analysis may only give a limited snap shot analysis (Preston 1995).

3.2.1 Field Survey

In the summer months of late June, July and August 2002, one field visit was made to each of the 19 Scottish lochs where *P. rutilus* had been previously recorded, see Fig 3.1. In addition, 3 other Scottish sites that may potentially host *P. rutilus* (potential sites) were also surveyed, 1 in August 2002 and 2 in July 2003. The five Finnish *P. rutilus* lakes were visited in September 2003.

The field visits were used for a macrophyte survey of each loch to confirm the presence or absence of *P. rutilus* and to collect herbarium specimens. The field visits also provided the opportunity to collect water chemistry, light measurements and sediment samples for laboratory analysis.

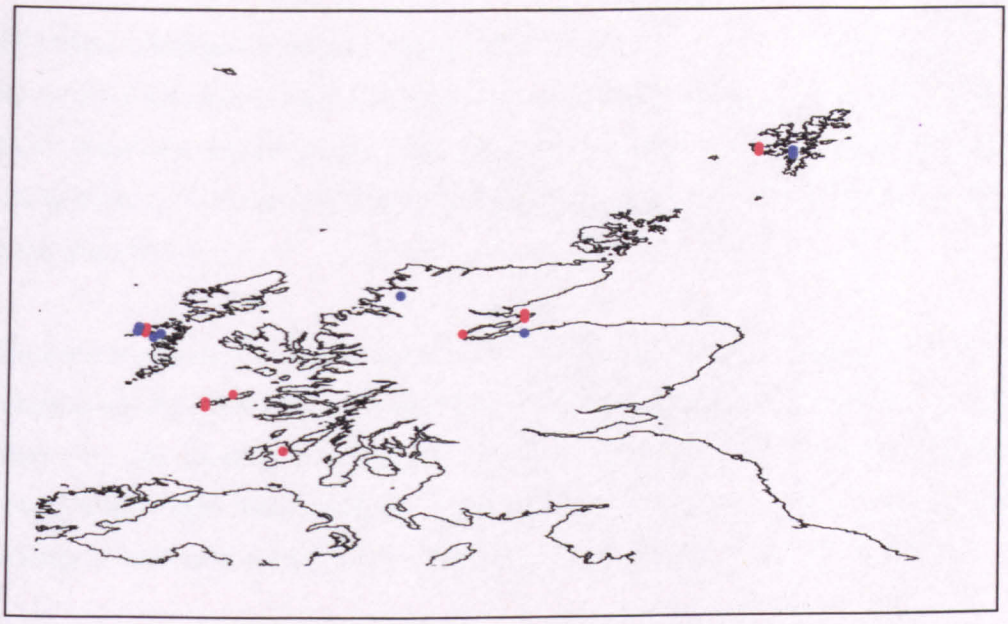


Figure 3.1 The Scottish distribution of *P. rutilus* sample sites 2001/2, lochs still hosting *P. rutilus,* indicated in red, lochs no longer hosting the plant, indicated in blue.

3.2.2 Sediment Sampling and Laboratory Analysis

For each loch site, *P. rutilus* vegetation was located by using a boat-trailed grapnel. On locating *P. rutilus* an Ekman grab was used to take one vegetation and sediment sample at each loch site. The sediment samples were placed in labelled plastic sample bags and kept in a cool box, until returned to the laboratory for analysis.

P. rutilus sediment samples were prepared for analysis by drying at an oven temperature of 80 ° C. The dried sediment samples were ground with a pestle and mortar and then sieved to remove solid particulates. Sediment samples were analysed by the Scottish Agricultural College for extractable phosphate, nitrogen, potassium, magnesium, calcium, iron and % organic content. The extractable analyte units are on a weight/volume basis (i.e. mg analyte /l of air-dried soil). Organic matter content units (by loss on ignition) are on a % (w/w) basis. Total kjeldahl nitrogen units are on a weight / weight basis (i.e. mg N/kg air-dried soil).

3.2.3 Water Temperature and Physico/Chemistry

For each of the once visited loch sites, one water sample was taken and the following water chemistry measurements were taken in the field. Water temperature, pH and conductivity were measured in the field using a portable CPH conductivity/temperature meter and pH meter.

The three mainland *P. rutilus* loch sites, Ussie, Bayfield, Eye plus Flemington which no longer hosts the plant, were also monitored monthly for seasonal changes in loch water chemistry. One monthly water sample was taken from each monitored loch. Monthly water samples and water chemistry measurements were always taken from the same location in each loch, usually from a boat jetty, as a boat was not always available.

Water samples were taken from each loch in order to undertake analysis of nutrient levels. Samples were sent to a Scottish Environment Protection Agency (SEPA) laboratory for analysis. The water samples were collected in glass sample bottles (acid washed to ensure no traces of phosphorus contamination). On collection, the water samples were kept cool in a cool-box until they were returned to the laboratory within a few days where they were kept refrigerated at 4 $^{\circ}$ C before being sent within a day to a SEPA laboratory for analysis.

Water samples were analysed for the following, total phosphate, dissolved reactive phosphate, nitrate, nitrite, total oxidised nitrogen, ammonia, sodium, chlorine, potassium, calcium, magnesium, iron, alkalinity, conductivity and pH, see Appendix 3. All these measurements were made after filtration so as to exclude particulates.

3.2.4 Light Measurements

To evaluate the available light energy for plant photosynthesis it is essential to measure Photosynthetically Active Radiation (PAR) (Holmes & Klein 1987), which is defined as being the energy flux within the 400 – 700 nm waveband (McCree 1972). The aquatic light environment can be greatly influenced by the reflective and scattering properties of the water and any dissolved substances and particulate matter (Holmes & Klein 1987).

Measuring the aquatic attenuation of PAR with depth can give an indication of the light available for macrophyte photosynthesis.

PAR was measured using SKYE SKP210 light sensors linked to a SKYE-SDL 2540 logger. Loss of PAR with depth was measured by placing one light sensor at the water subsurface and the other just above the sediment bed, so as to measure light attenuation with depth in each water body. From the PAR data the average extinction coefficient *k* measurements for each site was calculated (Moss 1988).

$$k = \frac{1}{(Z_2 - Z_1)} \log_e \frac{(Lo)}{(L)}$$

Where: Z_1 = sub-surface (0m)

Z_2 = depth (0.5m)

Lo = PAR measured at sub-surface (Z_1)

L = PAR measured at depth (Z_2)

3.2.5 Data Analysis

The water chemistry, light and sediment data for each individual site was analysed for relationships between these environmental variables and the presence and absence of *P. rutilus*. See Appendix 3 for the water chemistry/light data set used in the analysis and Appendix 4 for the sediment chemistry data set. This analysis included Spearmans Rank correlation coefficient and Canonical Correspondence Analysis (CCA) of the environmental and plant data. One-Way Anova with Tukeys mean separation test analysis determined significant differences between means. The TWINSPAN *P. rutilus* trophic groups formed from the plant community data analysis (see chapter 2) were also used to determine if mean environmental data for these trophic groups were significantly different. Results from the environmental and plant data analysis were also used to form hypothesis that were further investigated and tested by *P. rutilus* growth experiments.

3.3 RESULTS

3.3.1 Water Chemistry

The effects of maritime influence on water chemistry can be seen in the scatter plot Fig 3.2. The maritime influence on loch aquatic sodium, calcium and magnesium, can be seen with a gradual trend of decreasing concentration of ions with increased loch distance from the sea (Fig 3.2).

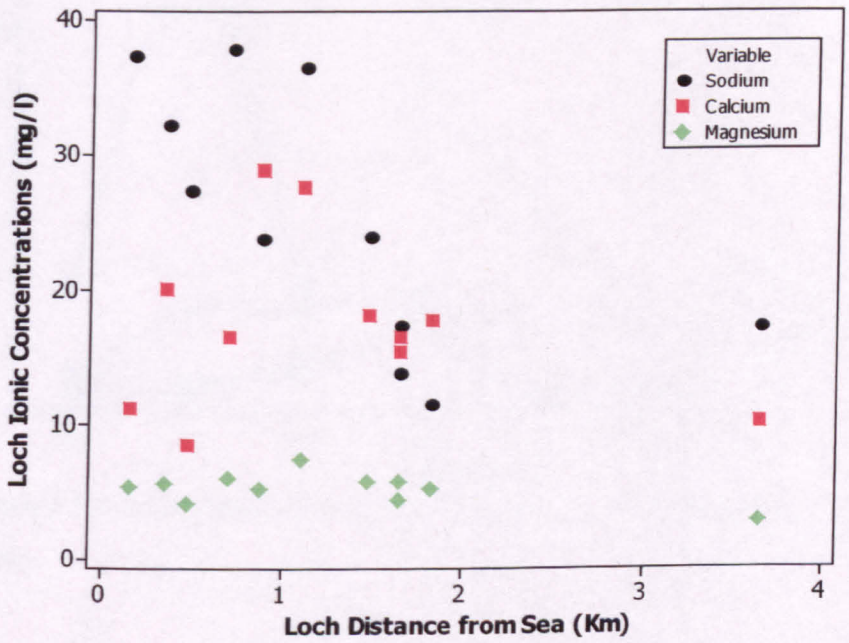


Figure 3.2 The decline in ionic concentrations with increased loch distance from the sea (sodium significant correlation r = – 0.68, P <0.05)

The decrease in sodium ion concentration for loch distance from the sea is significant r = –0.68, P < 0.05, and though not significant, there appeared to be a trend of increasing concentrations of calcium and magnesium with lochs situated closer to the sea.

In the case of changing potassium ion concentrations with increased loch distance from the sea there was no significant correlation r = -0.26, P > 0.05

3.3.2 Water Chemistry : Seasonal Changes and Mean Values

The monthly monitoring of loch total phosphorus levels Fig 3.3, show that Loch Flemington is much more nutrient enriched compared to the other three mainland lochs which still host *P. rutilus*.

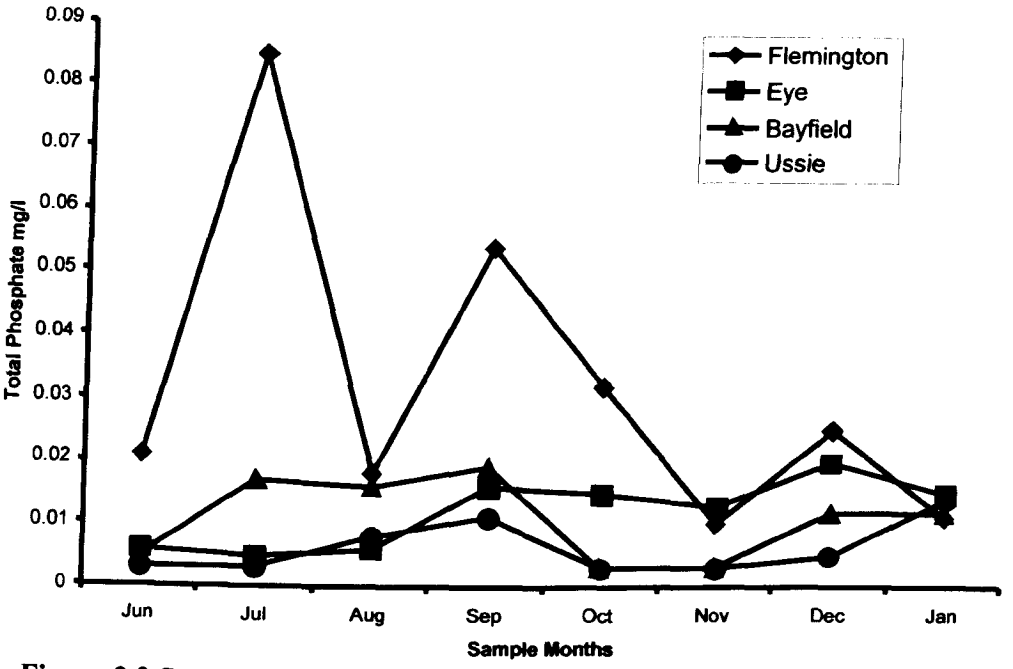


Figure 3.3 Seasonal changes in total phosphate levels for mainland lochs supporting *P. rutilus*..

It can be seen from graph Fig 3.3 that total phosphate levels in Loch Flemington peak during to two periods, with the highest phosphate level occurring in July with another lower peak occurring in late summer in the month of September. In contrast the other three mainland loch sites that still host *P. rutilus* show more constant seasonal changes in total phosphate levels, Fig 3.3. The seasonal conductivity changes for the four loch sites, reveal that Loch Flemington had the highest conductivity values with levels of around 300 μs/cm and above, whilst the other lochs had lower seasonal conductivities of around 150 to 250 μs/cm. Conductivities appeared to decline from June to August and increase to a peak in November and then decrease appreciably in December, Fig 3.4.

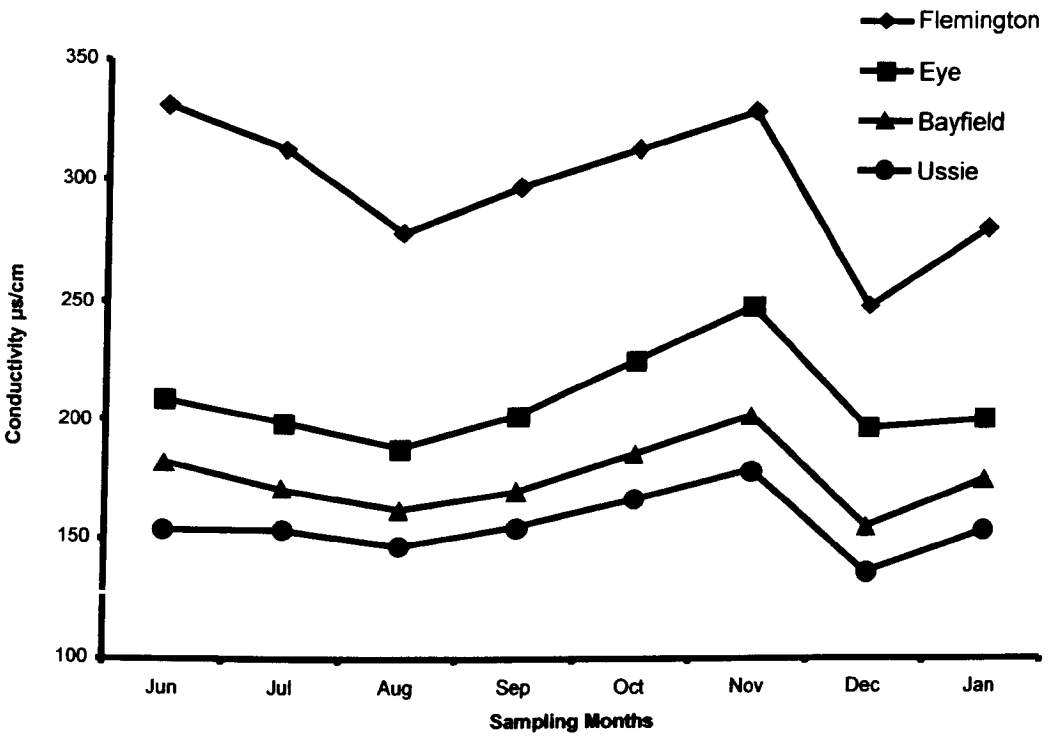


Figure 3.4 Seasonal changes in conductivity of mainland lochs supporting *P. rutilus*.

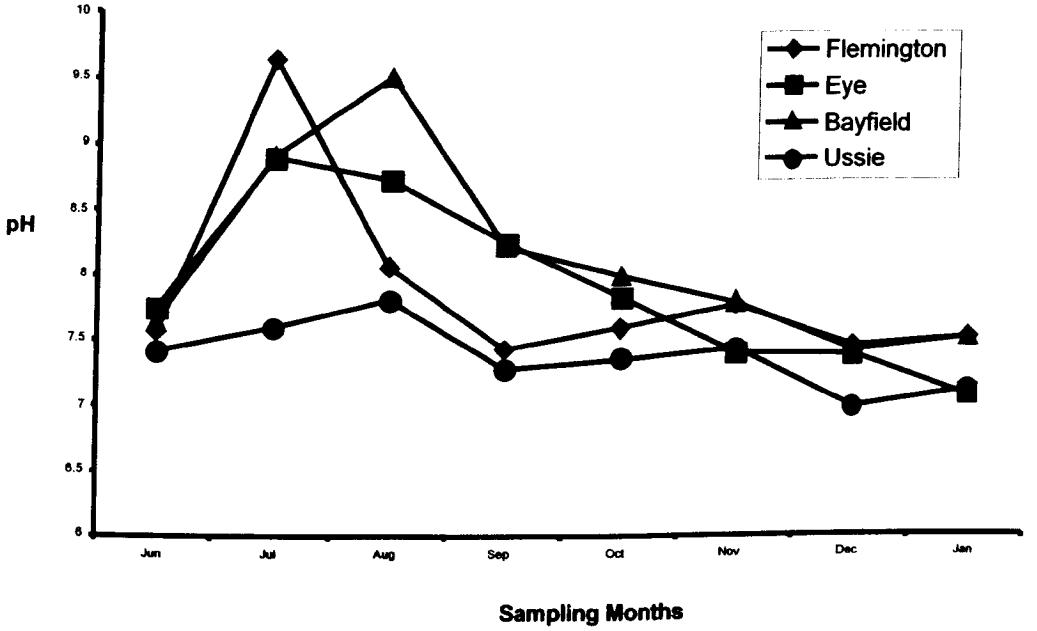


Figure 3.5 The seasonal changes of pH for *P. rutilus* mainland lochs.

The monitored seasonal changes of pH show a pH rise through the month of June, peaking in July, for Loch Flemington and Eye, with peaks occurring in Bayfield and Ussie in August. After the rise in pH for all the lochs there was a gradual decline in pH through the remaining summer and into the winter season, Fig 3.5.

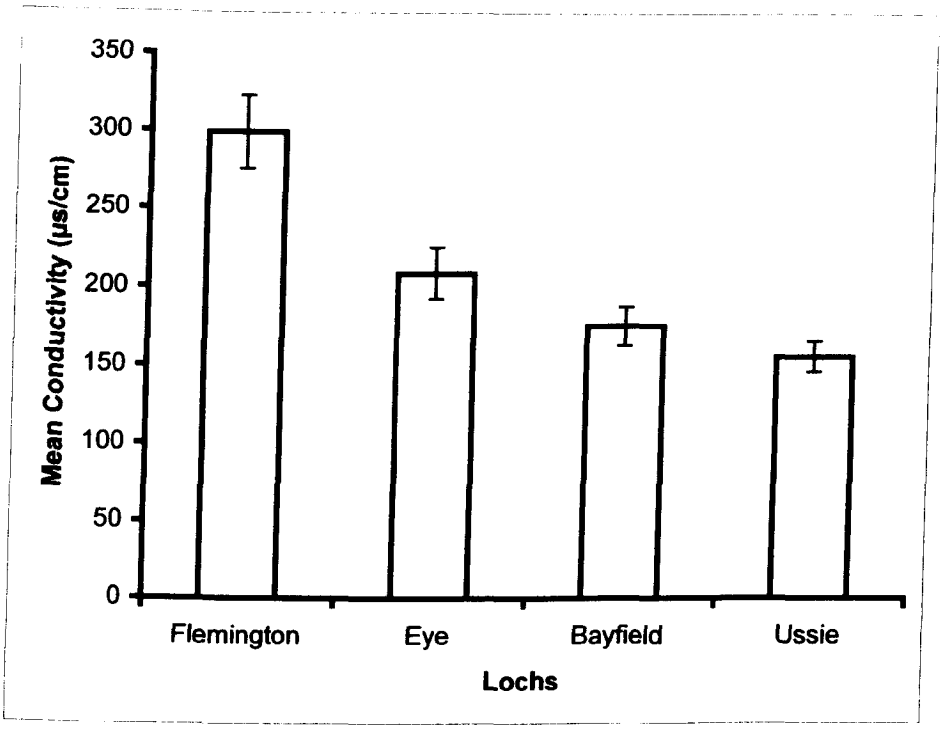


Figure 3.6 Mean conductivity of seasonally monitored *P. rutilus* loch sites.

The seasonal conductivity monitoring clearly shows that Loch Flemington, the former *P. rutilus* loch, has a mean seasonal conductivity significantly greater than the other three mainland lochs that still host *P. rutilus,* Fig 3.6. Loch Eye has the highest mean seasonal conductivity of the three mainland *P. rutilus* lochs, whilst Loch Ussie has the lowest mean seasonal conductivity, Fig 3.6.

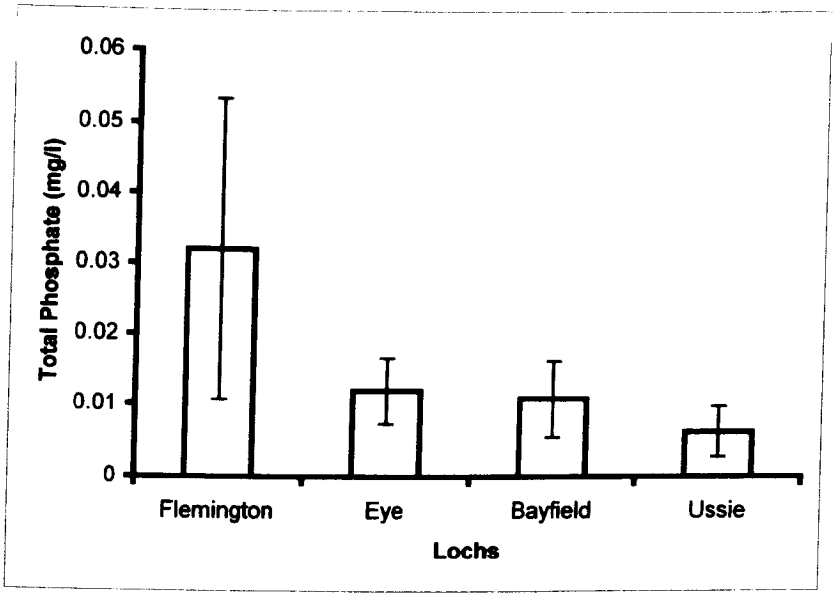


Figure 3.7 Mean total phosphate levels of seasonally monitored *P. rutilus* loch sites

The seasonal total phosphate of the four monitored mainland loch sites revealed, that former *P. rutilus* site Loch Flemington had two large seasonal peaks in total phosphate levels, Fig 3.3. However, when comparing the overall means of the seasonal phosphate levels, it was clear that Loch Flemington's higher seasonal peaks of phosphate level did not produce a significantly greater overall mean, 0.032 ± 0.0213, $P > 0.5$, due the large mean standard error, Fig 3.7.

3.3.3 PAR Light Related to P rutilus Abundance and Macrophyte Diversity

The scatter plot results in Fig 3.8, show there is a significant negative correlation ($r = -0.65$, $P < 0.05$) between increasing light extinction coefficient (LEC) and macrophyte diversity for the *P rutilus* lochs.

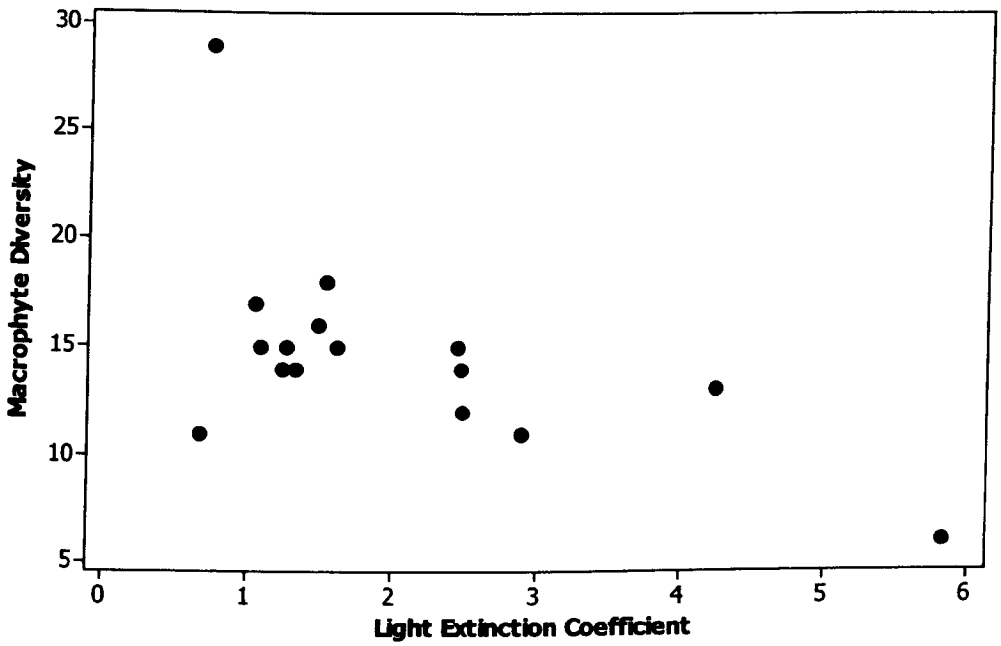


Figure 3.8 The decline in macrophyte diversity (number of different macrophyte species per loch) with increasing aquatic light extinction coefficients in current and former Scottish *P. rutilus* lochs (significant correlation $r = -0.603$, $P<0.05$).

Not only did aquatic light availability (Light Extinction Coefficient, LEC) appear to be a good predictor of aquatic macrophyte diversity, Fig 3.8, but was also a good predictor of *P rutilus* abundance, Fig 3.9. The scatter plot shows there is a significant inverse correlation between LEC and *P. rutilus* loch abundance, when the highly skewed LEC value of 4.23 for Loch Eilein is removed from the analysis Fig 3.9.

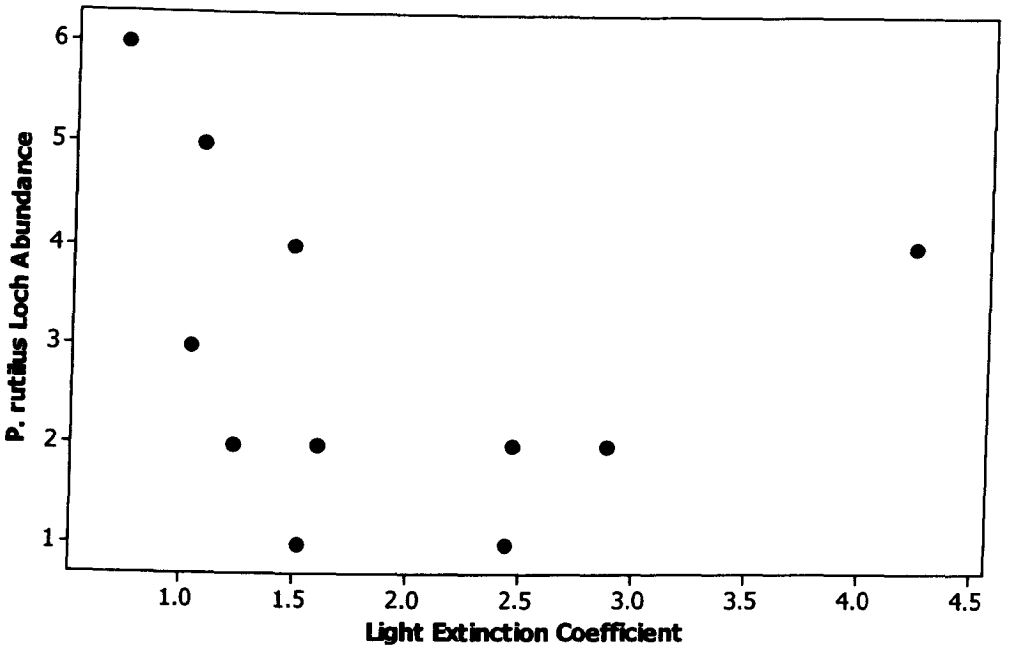


Figure 3.9 The decline in *P. rutilus* abundance with increasing light extinction coefficients in current Scottish *P. rutilus* lochs (significant correlation r = -0.64, P = 0.04 <0.05, when highly skewed 4.23 LEC value is removed from analysis). (DAFOR *P. rutilus* abundance per loch converted to numerical values using pseudospecies method, Hill *et al.* 1975): A = 7, LA = 6, F = 5, LF = 4, O = 3, LO = 2, R = 1)

3.3.4 *P. rutilus* Finnish and Scottish Trophic Groups

Table 3.1 shows the water chemistry and light extinction coefficients for trophic groups formed from the TWINSPAN classification of *P. rutilus* vegetation community's (see chapter 2 for TWINSPAN vegetation groups). It can be seen that the aquatic total phosphate levels were highest in the Finnish trophic group 1 sites. Phosphate levels in Finnish group 1 being nearly 4 times greater than Scottish *P. rutilus* trophic groups 3 and 4, with these mean differences being significant, P< 0.5, Tukeys, One Way Anova, Table 3.1 and Fig 3.10. Finnish group 2 trophic sites also had near twice the total phosphate levels than Scottish trophic groups, but were not significantly different. There appeared to be very little difference in total phosphate levels between Scottish trophic

groups 3 and 4, with both phosphate levels just less than 0.01 mg/l, see Table 3.1 and Fig 3.10.

Table 3.1 Loch environmental conditions for different *P. rutilus* TWINSPAN loch groups, Finnish groups 1 & 2, Scottish groups 3 & 4. One-Way Anova, Tukeys significant differences between means P<0.5, denoted by superscript a, b.

Means ± SE	**Group 1**	**Group 2**	**Group 3**	**Group 4**
pH	7.6 ± 0.14	7.5 ± 0.1	7.8 ± 0.13	8.4 ± 0.26
Conductivity (µs/cm)	193.3 ± 47.9 [a b]	112 ± 2 [a]	258.8 ± 25.6 [b]	199.6 ± 18.5 [a b]
Alkalinity (mg/l)	34.14 ± 6.2	35.25 ± 0.15	60.07 ± 8.1	38.54± 4.7
N-NH_3 (mg/l)	0.049 ± 0.05	0.1 ± 0.06	0.087 ± 0.05	0.112 ± 0.07
P-TP (mg/l)	0.038 ± 0.012 [a]	0.02 ± 0.01 [a b]	0.0082 ± 0.004 [b]	0.008 ± 0.005 [b]
Cl (mg/l)	22.4 ± 19.4	3.75 ± 1.05	42.5 ± 5.75	40.5 ± 8.55
Na (mg/l)	13.8 ± 11.6	4.35 ± 0.55	25.9 ± 3.5	24.8 ± 5.24
K (mg/l)	2.3 ± 0.81	2.6 ± 0.0	0.91 ± 0.06	2.01 ± 0.62
Ca (mg/l)	11.33 ± 0.88	10.3 ± 0.3	20.22 ± 3.06	13.98 ± 1.39
Mg (mg/l)	4.13 ± 1.14	4.3 ± 0.9	5.55 ± 0.43	4.74 ± 0.63
Light Extinction Coefficient	1.24 ± 0.1	1.7 ± 0.7	2.47 ± 0.4	1.17 ± 0.15

One-Way Anova, Tukeys significant differences between means, denoted by superscript a, b.

The high alkalinity levels of Scottish group 3, 60.07 ± 8.1 mg/l, compared to the other trophic groups, reflects the alkaline coastal influences of this machair dominated loch group, Table 3.1.

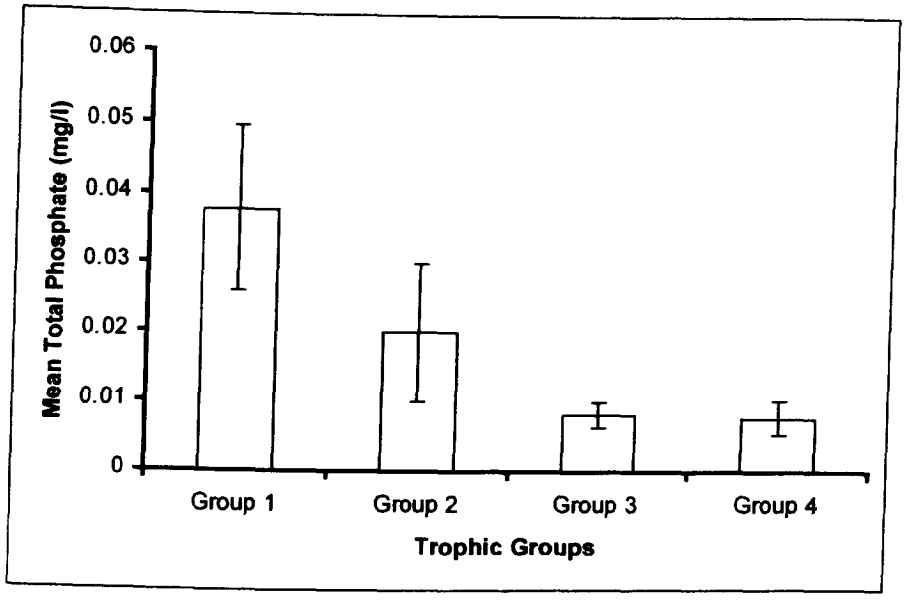


Figure 3.10 Mean total phosphate levels for different *P. rutilus* trophic groups, Finnish Groups 1 & 2 and Scottish Groups 3 & 4.

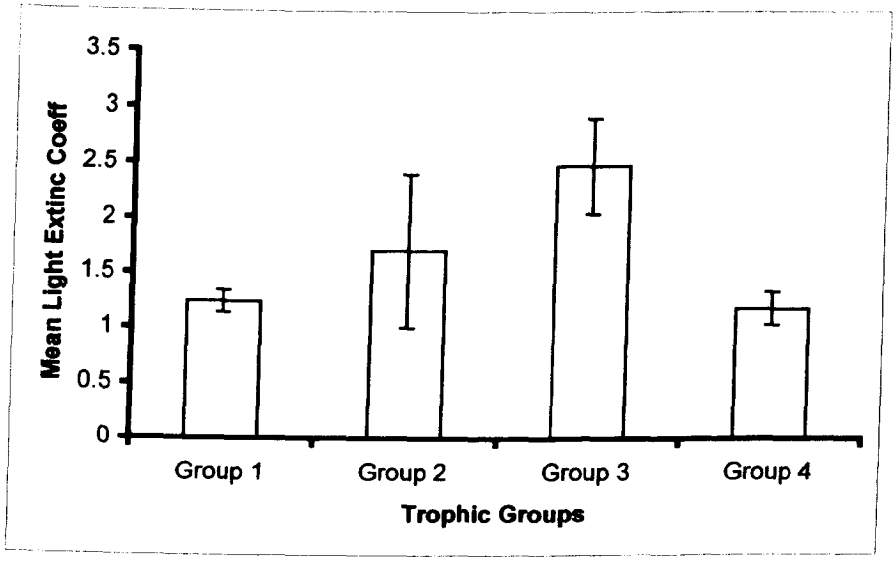


Figure 3.11 Mean light extinction coefficients for different *P. rutilus* trophic groups, Finnish Groups 1 & 2 and Scottish Groups 3 & 4.

The mean light extinction coefficients (LEC) for different Scottish and Finnish trophic groups revealed that Scottish group 3 has a higher mean LEC than Finnish group 1 and Scottish group 4 sites, see Table 3.1 and Fig 3.11.

3.3.5 *P. rutilus* Scottish Trophic Groups

In analysis of current and former *P. rutilus* TWINSPAN water chemistry groups, it was found that mean alkalinity of group 4 is significantly greater than group 2, see Table 3.2 and Fig 3.12. The trophic group 4 lochs consists of Outer Hebridean sites, many with strong coastal influences which would explain their significantly greater mean alkalinity than trophic group 2 which is dominated by mainland lochs, having more inland, less alkaline coastal influences.

Table 3.2 Loch environmental conditions for different, present and former, Scottish *P. rutilus* TWINSPAN loch groups. One-Way Anova, Tukeys significant differences between means $P<0.5$, denoted by superscript a, b.

Means $\pm$ SE	**Group 1**	**Group 2**	**Group 3**	**Group 4**
pH	8.3 $\pm$ 0.16	7.8 $\pm$ 0.3	8.4 $\pm$ 0.4	8.7 $\pm$ 0.2
Conductivity(µs/cm)	231.2 $\pm$ 12.4	157.4 $\pm$ 23.8	278 $\pm$ 15.3	2604 $\pm$ 1833
Alkalinity (mg/l)	47.5 $\pm$ 6.85 $^{a\,b}$	33.48 $\pm$ 8.4 ^{a}	64.20 $\pm$ 6.6 $^{a\,b}$	67.1 $\pm$ 7.5 ^{b}
N-NH_3 (mg/l)	0.112 $\pm$ 0.07	0.096 $\pm$ 0.06	0.04 $\pm$ 0.0	0.044 $\pm$ 0.004
P-TP (mg/l)	0.006 $\pm$ 0.0007	0.01 $\pm$ 0.003	0.026 $\pm$ 0.02	0.01 $\pm$ 0.002
Cl (mg/l)	48.6 $\pm$ 5.85	26.8 $\pm$ 4.87	48.7 $\pm$ 5.94	760.9 $\pm$ 568
Na (mg/l)	30.1 $\pm$ 3.6	16.6 $\pm$ 2.8	29.3 $\pm$ 3.2	464 $\pm$ 347
K (mg/l)	1.9 $\pm$ 0.6	1.2 $\pm$ 0.4	1.83 $\pm$ 0.8	18.3 $\pm$ 13.9
Ca (mg/l)	18.5 $\pm$ 3.3	11.3 $\pm$ 2.6	22.9 $\pm$ 2.4	36.5 $\pm$ 9.9
Mg (mg/l)	4.5 $\pm$ 0.3	3.5 $\pm$ 0.7	5.9 $\pm$ 0.39	56.9 $\pm$ 44.3
Light Extinction Coefficient	1.1 $\pm$ 0.14 ^{a}	1.35 $\pm$ 0.35 ^{a}	3.85 $\pm$ 0.76 ^{b}	1.77 $\pm$ 0.26 ^{a}

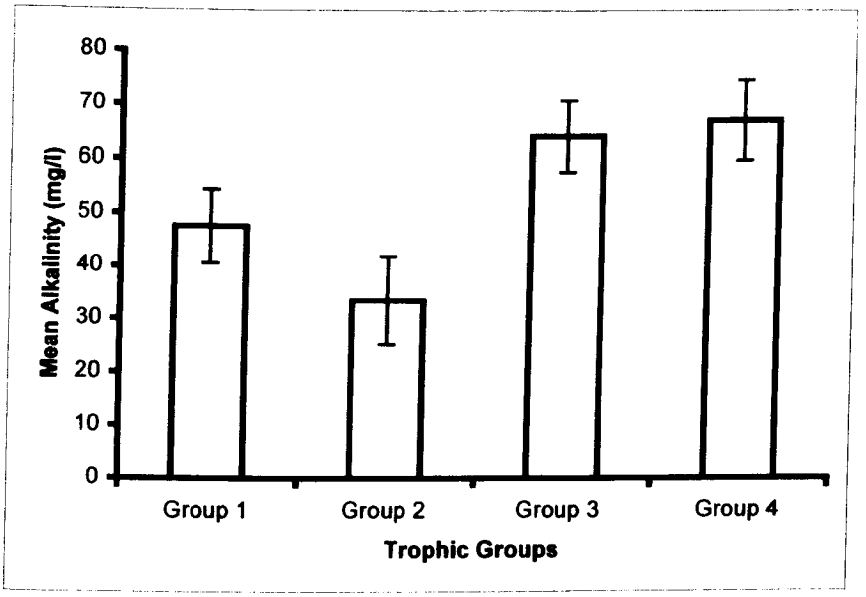


Figure 3.12 The mean alkalinity of *P. rutilus* trophic groups for present and former Scottish loch sites.

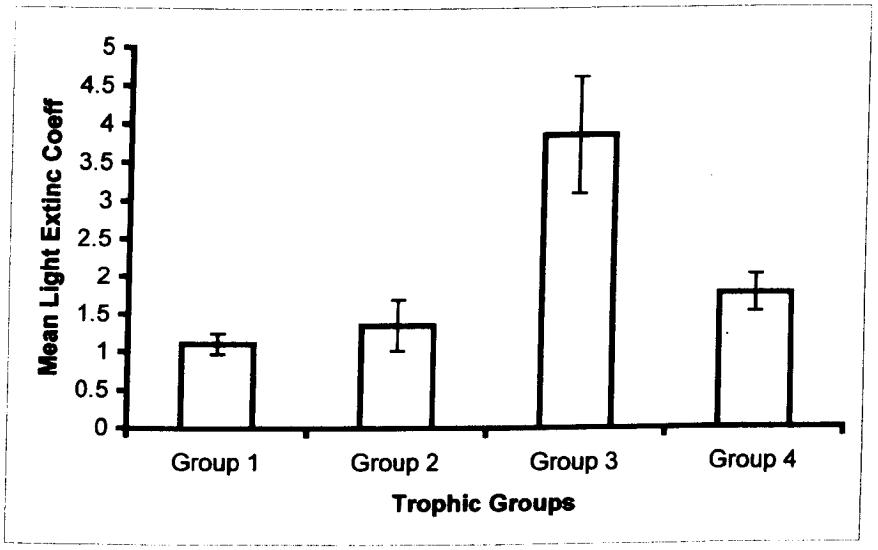


Figure 3.13 The mean light extinction coefficients of *P. rutilus* trophic group for present and former Scottish loch sites.

The mean light extinction coefficient (LEC) for group 3, is significantly greater than the other Scottish trophic groups, whilst trophic group 1, dominated by Shetland current and former *P. rutilus* lochs, has the lowest LEC of all the loch groups, Table 3.2 and Fig 3.13.

3.3.6 Sediment Chemistry

The analysis of mean sediment chemistry values of TWINSPAN trophic groups for current Scottish *P. rutilus* sites revealed some differences, Table 3.3.

The sediment organic content of *P. rutilus* trophic groups ranges from the lowest 12.03 ±7.9 % in the Shetland dominated group 1 sites to the highest of 29.23 % for the sole trophic group 4 Outer Hebridean site, Loch Scarie, see Table 3.3. The other two *P. rutilus* trophic groups 2 (mainland dominated) and 3 (Hebridean) have % organic contents level of 19.15 ± 8.7 and 20.87 ± 8.49, respectively. Sediment phosphate levels, 27.8 ±13.3 mg/l, in trophic group 1 were much greater than in trophic groups 2, 10.03 ± 5.08 mg/l and 9.07 ±1.87 mg/l for trophic group 3, whilst the sole loch in group 4, Loch Scarie, has a 29.23 mg/l of sediment phosphate, Table 3.3.

Table 3.3 Sediment chemistry for different Scottish *P. rutilus* trophic groups (Tukeys mean differences between trophic groups are not significant $P < 0.05$).

Means ±SE	**Group 1**	**Group 2**	**Group 3**	**Group 4**
% Organic content	12.03 ±7.9	19.15 ±8.7	20.87 ±8.49	29.23
Phosphate (mg/l)	27.8 ±13.3	10.03 ±5.08	9.07 ±1.87	18.66
K (mg/l)	82.9 ±30.1	51.5 ±13.1	91.7 ±34	136.7
Mg (mg/l)	104.2 ±35.5	132.8 ±32.2	123.3 ±34.8	136.7
Ca (mg/l)	3428 ± 3022	1295 ±195	14720± 12550	25500
Fe (mg/l)	364 ±119	632 ±191	558 ±398	181
N (mg/kg)	4049 ±2702	6046 ±2221	6873 ±3618	10630

Extractable analyte units are on a weight/volume basis (i.e. mg analyte /l of air dried soil). Organic matter content units (by Loss on Ignition) are on a % (w/w) basis. Total Kjeldahl N units are on a weight / weight basis (i.e. mg N/kg air-dried soil).

The sediment calcium levels are clearly much higher in the machair loch dominated groups 3 and 4, compared to trophic groups 1 and 2, Table 3.3. This association of machair trophic groups 3 and 4 with high calcium sediment levels can be clearly seen in the CCA ordination, where they are clustered along the calcium environmental axis, Fig 3.14.

The CCA ordination of Scottish *P. rutilus* lochs (sites that still host the plant), constrained on sediment variables show that the axis for sediment calcium are dominated by the Outer Hebridean machair lochs group 3 and group 4, Fig 3.14. The cumulative variance of species-sediment environmental relation is 29.3 % for axis 1; 26.1 % for axis 2; 90.6 % for all four axes combined. All variables shown are significant ($p < 0.05$), Monte-Carlo permutation.

These sediment environmental gradients also separated some of the lochs into their defined trophic groups. Fig 3.14. The lochs belonging to the trophic group 3 (open circles) were clearly associated with the relatively alkaline influence of sediment calcium, reflecting the coastal machair distribution of this loch trophic group. There also appeared to be some grouping of Shetland loch sites, Bardister and Kirkigarth along the sediment phosphate gradient, but this was not the case for the mainland site, Loch Eye, belonging to the same loch trophic group 1 (square symbol), Fig 3.14.

The trophic group 2 (triangle symbol) lochs appear not to show any clustering, being more dispersed over the different environmental axis, with only Loch Lossit showing a strong association along the total sediment nitrogen and organic matter, Fig 3.14.

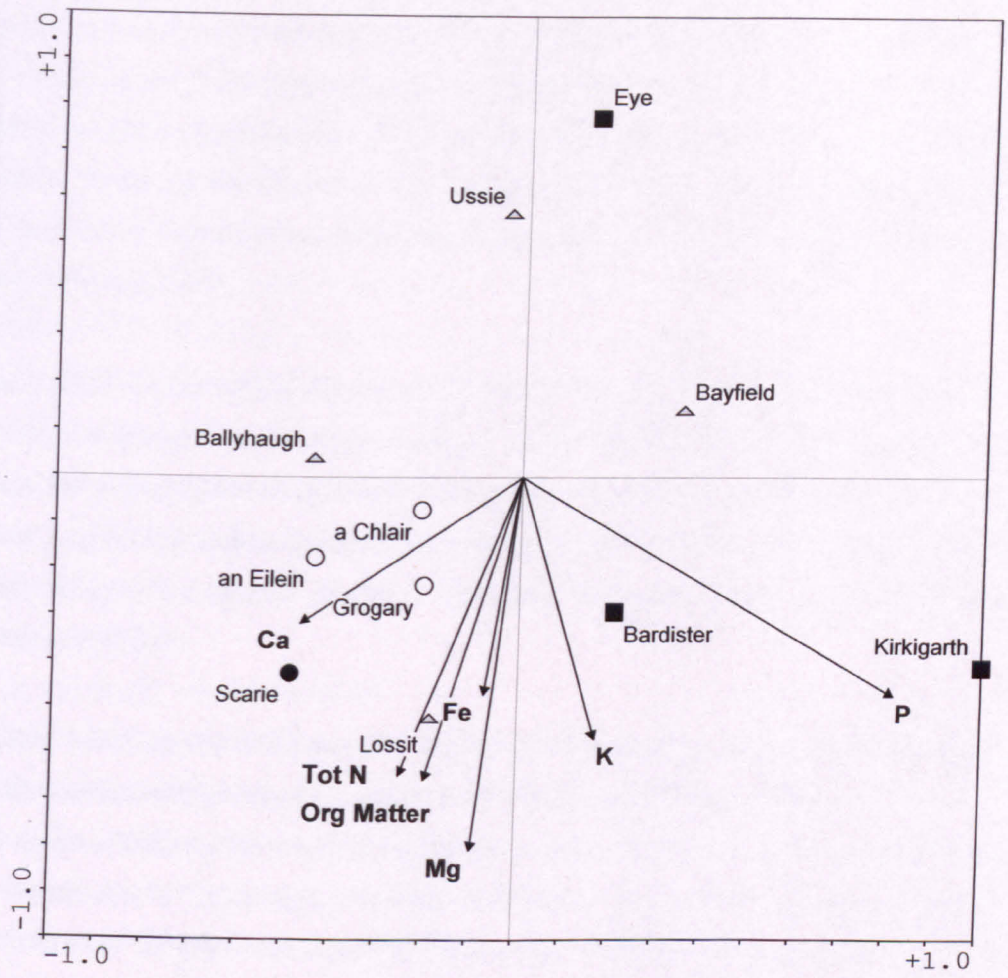


Figure 3.14. CCA ordination of *P. rutilus* trophic groups and species data constrained on sediment environmental variables (lochs belonging to a particular trophic group denoted by same symbol; square = trophic group 1, triangle = trophic group 2, open circle = trophic group 3, filled circle = trophic group 4).

3.4 DISCUSSION

3.4.1 Coastal Influences - Salinity

The geographical distribution of the *P. rutilus* lochs appears to influence their trophic status and water chemistry, with most of the lochs being situated close to the sea having a strong coastal influences, Fig 3.2. Maritime effects on loch water chemistry were found to influence sodium ion levels, with a significant trend of increasing of sodium ions in lochs situated closer to the coast, Fig 3.2. These higher salinities of lochs situated closer to the sea, are usually due to their greater exposure to wind and salt water spray (Preston 1995).

The TWINSPAN analysis of current and former Scottish *P. rutilus* lochs revealed that the trophic group 4 has the highest salinity, Table 3.2. This trophic group 4 consists of Outer Hebridean lochs situated very close to the sea, that have NaCl mean salinities 30 times greater than trophic group 2 (mainland and inner Hebridean lochs) and 15 to 16 times greater than trophic groups 1 (Shetland dominated lochs) and 3 (Hebridean dominated lochs).

Salinity levels above 1000 mg/l are known to be damaging to the growth phases of plants, such as seed germination, (Hart *et al.* 1981, Nielsen *et al.* 2003a).
The upper salinity levels tolerated by freshwater aquatic plants is said to be 4000 mg/l, but levels above 1000 mg/l, although sub-lethal, can reduce macrophyte growth (Neilsen *et al.* 2003b). Sub-lethal salinity has been found to affect macrophyte growth in several ways, which include reduced fertility, plant growth and shoot elongation (Blindow *et al.* 2003, James & Hart 1993). Macrophyte fertility, both sexual and asexual reproduction has found to be suppressed by salinity levels above 1000 mg/l, such as reducing the production of asexual propagules and flower growth necessary for sexual seed reproduction (James & Hart 1993, Warwick & Bailey 1996). As *P. rutilus* is mainly dependent on asexual turion growth for reproduction, any reduction in turion growth through salinity could reduce the plant's ability to survive in saline influenced sites. This would suggest that group 4 lochs having a mean salinity of 1225 mg/l, may

have a detrimental effect on the growth and reproduction of *P. rutilus* level, as has been found in some other macrophyte species, (Nielsen *et al.* 2003b).

The possible adverse effects of salinity on macrophyte growth, may explain why *P. rutilus* exhibits the greatest number of extinctions in loch group 4, this being the most saline influenced trophic type. Lake salinity has been implicated for the decline of *P. rutilus* in some Polish water-bodies (Kazmierczakowa & Zarzycki 2001). The possible salinity threat to *P. rutilus* and its freshwater habitats, situated near the coast, may only be increasing with global warming. Coastal freshwater habitats may suffer increases in salinity, as global warming is predicted to cause a rise in sea levels, which will result in more flooding and saline intrusion of some coastal freshwater habitats (Galbraith *et al.* 2002), such as *P. rutilus* machair lochs. Global warming is predicted to cause more frequent storms and a resulting increase in wind speeds (Watt *et al.* 1997), which will expose freshwater coastal habitats to an increase in wind borne salts from more sea spray.

3.4.2 Alkalinity and Bicarbonate

In the case of alkalinity there appears to be a trend, though not significant, of increasing levels of magnesium and calcium ions with *P. rutilus* lochs that are situated closer to the coast, Fig 3.2. These high concentrations of magnesium and calcium ions in *P. rutilus* Scottish lochs situated close to the sea brings about the high alkalinities of many of these sites. The water chemistry of loch groups, formed from TWINSPAN vegetation analysis, revealed that the Scottish lochs are much more alkaline than Finnish trophic groups, Table 3.1. This can be especially seen in Scottish group 3, which has nearly double the calcium and alkalinity levels of Finnish trophic groups, Table 3.1. Scottish trophic group 3 sites are dominated by Hebridean machair type lochs, which are known for their alkaline influenced waters (Palmer *et al.*1992, Preston *et al.* 2000). The higher alkalinity in machair lochs can also be seen in the TWINSPAN groups formed from current and former *P. rutilus* Scottish sites. The Hebridean loch groups 3 and 4 having an alkalinity twice that of group 2 sites with lowest alkalinity, mainland dominated

sites. The Shetland lochs forming group 1 also had a lower alkalinity than the Hebridean groups 3 and 4, Fig 3.12.

In terms of water chemistry, alkalinity has been found to be an important factor in determining aquatic flora (Spence 1967, Seddon 1972, Sand-Jensen 1987, Vastergaard & Sand-Jensen 2000). Vastergaard and Sand-Jensen 2000, found that alkalinity was the main determining factor for the macrophyte species distribution of Danish lakes. Alkalinity is a good descriptor of bicarbonate concentration (Vestergaard & Sand-Jensen 2000), which is an important source of inorganic carbon for the photosynthesis of many submerged macrophytes. Plants with the ability to use bicarbonate as a photosynthetic carbon source, as an alternative to carbon dioxide, would have a competitive growth advantage when carbon dioxide as a source of carbon is limiting photosynthesis (Boston 1986, Carr *et al.*1997).

The affinity for bicarbonate appears to be high in macrophyte species in mineral rich, hard waters and in marine species (Sand-Jensen 1987). Many of the *Potamogeton* species found in the *P. rutilus* macrophyte communities appear to be bicarbonate users, such as *P. pectinatus, P. perfoliatus* and *P. crispus* (Vestergaard & Sand-Jensen 2000, Van den Berg *et al.* 2002). Other macrophyte bicarbonate users found in *P. rutilus* communities include *Chara* species, (Van den Berg *et al.* 2002, *Elodea canadensis* (Vadstrup & Madsen 1995) and *Callitriche hermaphroditica* (Maberly & Madsen 2002).

The bicarbonate utilisation system provides direct access to bicarbonate ions as a source of dissolved inorganic carbon for photosynthesis, in addition to free carbon dioxide, which would be highly advantageous in high pH waters (Bowles 1987, Carr *et al.* 1997). This may partly explain why *P. rutilus* and its associated community types are mostly found in Scottish lochs with an alkaline influences, where they may have a competitive advantage of utilising bicarbonate as a photosynthetic carbon source. *P. rutilus* is also known to inhabit Finnish sites with an alkaline influence (Kotiranta *et al.* 1998, Vestergaard & Sand-Jensen 2000), but they appear to be at lower alkalinities

than most of the Scottish trophic groups, Tables 3.1 & 3.2. The more oligotrophic isoetid species that are CO_2 users (Boston 1986, Carr *et al.*1997), are also present in some of the *P. rutilus* communities, but are not so prevalent and dominant as the elodeid bicarbonate user species (see vegetation community types, chapter 2).

In some macrophytes bicarbonate affinity varies with environmental conditions (Sand-Jensen & Gordon 1986). Experiments have revealed that *Elodea canadensis* bicarbonate affinity is plastic and CO_2 is preferred when available (Sand-Jensen 1987). *E. canadensis* use of bicarbonate appears to increase when light, nutrients and bicarbonate are high, but is suppressed when CO_2 is high and light is low (Sand-Jensen 1987). It may be the case if Scottish *P. rutilus* is dependant on bicarbonate as a nutrient source, any great reductions in its light environment, which may occur with eutrophication, will suppress the growth of the plant and reduce the plants competitive ability. In contrast, Finnish *P. rutilus* living in a less bicarbonate rich environment may have a lesser affinity for bicarbonate and so be less dependant on this dissolved inorganic carbon (DIC) nutrient source, which can be greatly light limited in eutrophic conditions.

If Scottish *P. rutilus* is dependant on bicarbonate, it may not only be environmental differences that determine its affinity for this DIC, but ecotypic differences may also exist between Scottish and Finnish populations. See section 3.4.3 below for further discussion on nutrient use and ecotypes.

3.4.3 Total Phosphate Levels

The TWINSPAN groups formed from Finnish and Scottish *P. rutilus* groups revealed that total phosphate levels were much greater in Finnish trophic groups than Scottish trophic groups, with Finnish group 1 having more than four times the level of total phosphate, Table 3.1. Though not significantly different, Finnish trophic group 2 had nearly twice the total phosphate levels than Scottish *P. rutilus* trophic groups, Fig 3.10.

These phosphate chemistry results confirm that *P. rutilus* appears to inhabit more eutrophic water-bodies in Finland, compared to the oligo-mesotrophic loch types which it inhabits in Scotland. This is further confirmed by the previous chapter's TWINSPAN vegetation classifications that found Finnish *P. rutilus* community types much more eutrophic than the Scottish *P. rutilus* communities, which are more mesotrophic in character (Wallace & Murphy 2002). The presence of *P. rutilus* in more trophic rich Finnish lakes is also confirmed by other investigators where it has been found to be a coloniser of Finnish lakes that have become more eutrophic (Barkman 2000, Virola *et al.* 2001).

It would appear that *P. rutilus* is able to inhabit a wide range of trophic conditions; suggesting that this species may have a high phosphate nutrient use efficiency (NUE) and storage capacity enabling it to occur over a broader ecological range (Garbey *et al.* 2004). Nutrient-use efficiencies depend on a plant's physiological capacity for nutrient uptake and its ability to integrate phosphate in its vegetative structure (Garbey *et al.* 2004). This would appear to be the case in *P. rutilus,* as growth experiments revealed that the plant can thrive in a range of phosphate levels, with both mesotrophic and eutrophic phosphate conditions producing significantly larger reproductive turions (see chapter 4).

However, even if *P. rutilus* appears to have a high phosphate NUE, it cannot be ruled out that the plants ability to live in different Scottish and Finnish trophic conditions may be partly due to ecotypic differences, such as in their possible affinity for bicarbonate as a DIC source. Ecotypic differences can develop in plant populations living in different environmental conditions, as for example, Belgium populations of *Silene nutans* are differentiated into two parapatric ecotypes that are associated with calcareous or siliceous substrates (Van Rossum *et al.* 1997, 1999).

3.4.4 Seasonal Changes in Water Chemistry and Light Availability

The high pH levels in July and August for the mainland *P. rutilus* lochs, followed by a gradual decline in pH with approaching winter, may reflect peak summer macrophyte

photosynthetic activity. Increased macrophyte photosynthesis in the summer can be one of the main factors for increasing loch pH by elevating photosynthetic bicarbonate uptake from the water, which enhances alkalinity, and so pH (Van den Berg *et al.* 2002).

The monthly water chemistry monitoring of the four mainland *P. rutilus* lochs revealed that total phosphate levels of Loch Flemington peaked during the months of July and September and were much higher than in the other three lochs that still host *P. rutilus* (Fig 3.3). These high summer peak levels of total phosphate in Loch Flemington would explain the prolific algal blooms (May *et al.* 2001), which greatly reduce water transparency and so light availability to submerged macrophytes.

Loch Flemington's high levels of total phosphate is further reflected in its significantly greater levels of mean seasonal conductivity compared to the other three mainland *P. rutilus* lochs, Fig 3.6. Loch Flemington's reduction in light availability, through algal blooms, would likely to be one of the major factors contributing to the demise of submergent macrophytes such as *P. rutilus*, as has been found in other nutrient enriched lochs (Balls *et al.*1989, Blindow 1992).

Aquatic light availability through effects on photosynthetic ability has found to be an important factor in determining distribution and abundance of aquatic plants (Spence 1967, Preston 1995). This maybe the case for current and former *P. rutilus* lochs, with a significant inverse relationship between light availability and macrophyte diversity, (Fig 3.8). This significant trend of decreasing water transparency, with a reduction in loch macrophyte diversity, resulted in a macrophyte diversity of only five species for the loch with the highest light extinction coefficient, Fig 3.8.

The abundance of *P. rutilus* in Scottish lochs also appears to be influenced by light availability, with *P. rutilus* loch abundance significantly declining with an increase in light extinction coefficient, Fig 3.9. Light availability is known to limit macrophyte distribution and growth, through different stages in the plant's life-cycle, with one of the

most vulnerable plant growth stages being turion germination and growth (Holmes & Klein 1987, Jian *et al.* 2003, as found in *P. rutilus* growth experiments (see chapter 4).

3.4.5 Loch Sediment Chemistry

The availability of suitable rooting habitat for *P rutilus* turions, such as sand and/or soft sediments, will also influence abundance and distribution of *P. rutilus*. *P. rutilus* is known to be found rooted in fine textured mineral sediments (Kotiranta *et al.*1998), and it can also be found growing on sandy substrates as in the machair loch sites (Preston & Croft 1997). *P. rutilus* can also be found growing on shallow stony and silt type substrates, but the plant is much more dispersed than when growing in fine textured substrates (personal observation), which may be partly due the greater loch exposure to wave action associated with many stony substrates (Spence 1972).

In addition to substrate type and structure, nutrient content of substrates can have a strong influence on the growth of rooted macrophytes (Barko & Smart 1981, Barko *et al.*1991). Sediment organic content has been found to have an effect on macrophyte growth and abundance (Pearsall 1920, Macan 1977), with increasing sediment organic content reducing macrophyte growth (Carpenter 1981, Barko & Smart 1986). Sediment organic contents above 10 % have found to greatly reduce the growth of *Myriophyllum spicatum* and *Hydrilla verticillata* (Barko & Smart 1986).

However, organic substrate levels above 10 % are found in Scottish *P. rutilus* substrates, with organic content, ranging from the lowest level of 12.03 $\pm$ 7.9 % in Shetland dominated group 1 sites, to the highest level of 29.23 % for the sole trophic group 4 Outer Hebridean Loch Scarie, see Table 3.3. The other two *P. rutilus* trophic groups 2 (mainland dominated) and 3 (Hebridean) have near twice the 10 % organic contents level of 19.15 $\pm$ 8.7 and 20.87 $\pm$ 8.49, respectively. This suggests that *Potamogeton* species such as *P. rutilus* are able to tolerate and grow in substrates with higher organic content levels than that of some other submerged macrophyte species, such as *Myriophyllum spicatum*.

Important plant growth nutrients, nitrogen and phosphate are said to regulate macrophyte production (Carr *et al.* 1997), with these nutrients being incorporated from both the open water and sediments (Chambers *et al.*1989, Barko *et al.*1991). In the case of *P. rutilus* trophic groups, sediment phosphate levels ranged from a low 10.03 $\pm$ 5.08 mg/l in group 2 mainland dominated sites, to the highest phosphate level of 27.8 $\pm$ 13.3 mg/l for the group 1 Shetland dominated sites, Table 3.3. These trophic groupings along phosphate sediment gradients can be seen in the CCA ordination of sites, with group 1 Shetland sites such as Bardister and Kirkigarth grouped along the higher sediment phosphate axis compared to group 2 mainland sites Ussie and Bayfield in the opposite lower axis, Fig 3.14.

It is unclear how much these sediment phosphate levels may influence *P. rutilus* growth and distribution, as some macrophytes can satisfy their demand for mineral nutrients by leaf uptake alone (Madsen & Cedergreen 2002). Plant micronutrients such as calcium, magnesium and potassium are known to be mainly taken up from water and not sediments (Barker *et al.* 1991). This may also be the case for nitrogen, with its uptake being by one or both nutrient pathways. In some cases the range of substrate nutrient levels, such as nitrogen, gives no measure of a plant's ability to utlilise these nutrients, as has been found by investigators comparing plant tissue nutrient levels with environmental levels and finding in many cases no significant relationship (Wong & Clark 1979, Madsen & Cedergreen 2002, Garbey *et al.* 2004).

3.5 CONCLUSIONS

The coastal distribution of many of the Scottish *P. rutilus* lochs is reflected in their maritime influenced water chemistry . The alkalinity of *P. rutilus* lochs may be a factor in determining *P. rutilus* occurrence, as the plant may be able to utilize bicarbonate ions as an alternative carbon source for photosynthesis. In comparing the environmental variables there appears to be more significant differences in trophic group water chemistry than for sediment chemistry. In comparing *P. rutilus* water total phosphate

levels, there was clearly a significant trophic difference between the more eutrophic Finnish lakes and the more oligo-mesotrophic Scottish loch types. These trophic differences were further confirmed by the previous chapter's TWINSPAN vegetation trophic classification. The significant inverse relationships of macrophyte diversity and *P. rutilus* abundance with increasing light extinction coefficients revealed that light availability may have a strong influence on *P. rutilus* and its community type.

The seasonal changes in water chemistry clearly showed that highly eutrophic sites, such as Loch Flemington, produce high summer peaks of conductivity and total phosphate levels, compared the less trophic mainland sites that still retain *P. rutilus*. These high summer peaks of nutrient levels in Loch Flemington would be undoubtedly contributing to the lochs large summer algal blooms, which greatly reduce the loch's light availability for macrophyte growth. Clearly there is a need to investigate how *P. rutilus* growth and survival are affected by high nutrient levels and reduced light environments.

CHAPTER 4 :Experimental Investigation of the Effects of Phosphate and Reduced Light Regime on the Growth of *Potamogeton rutilus*

4.0 SUMMARY

The present study, based on two years of experimental growth investigations showed that *P. rutilus* has a erect canopy forming growth habit with significant stem elongation growth under reduced light. This increased stem growth with reduced light availability did not produce any significant differences in plant biomass with different phosphate levels, suggesting the different phosphate nutrient levels were not a limiting factor in plant biomass growth. However, turion development and turion size did appear to be significantly affected by light and phosphate treatments with increased turion size being observed with increased phosphate levels, with optimum turion growth being achieved at half light eutrophic conditions. The significant differences in *P. rutilus* turion size with treatments seemed to be a good measure of plant fitness as turion numbers produced per plant were not significantly different under different treatments. Turion size and light availability appeared to influence turion germination rates and germination success. The growth experiments also revealed *P. rutilus* is a long day plant for turion development, with turion formation being initiated by a photoperiod of high summer long days and temperatures above 15 ^{o} C .

Keywords: *Potamogeton. rutilus*, life-cycle, turion growth, trophic levels, photoperiod, phosphate levels, nutrient-use efficiency (NUE), ecotypes.

4.1 INTRODUCTION

The species rich low-nutrient oligo-mesotrophic lochs that host the rare Red Data Book *Potamogeton rutilus* Wolfg. are vulnerable to eutrophication. Diffuse agricultural pollution, domestic sewage waste and other sources has been implicated as the main factors causing nutrient enrichment and the loss of oligo-mesotrophic macrophyte species, such as *P rutilus* (Lassiere & Duncan 1997, Murphy & Pulford 1996, Preston & Croft 1997, May *et al.* 2001). Elevated levels of aquatic phosphorus resulting from eutrophication has been found to reduce macrophyte species diversity (Balls *et al.* 1989, Jupp & Spence 1977, van Groenendael *et al.* 1996). High phosphate levels encourage greater macrophyte productivity, which reduces species diversity as the most competitive and productive macrophytes thrive at the expense of less competitive species (Jupp *et al.* 1974).

Competitive exclusion brought about nutrient enrichment is not always responsible for the decline in macrophyte diversity, as increased phytoplankton growth can cause such a decline through reduced water transparencies. (Blindow 1992, Hough *et al.* 1989, Wilhelm & Solander 1988). This appears to be the case in *P. rutilus* lochs, as the plant's abundance and associated macrophyte species diversity significantly declines with reduced water transparencies that accompany phytoplankton blooms (Wallace 2003, unpublished data).

Scottish *P. rutilus* is found in oligo-mesotrophic lochs and it has been suggested that its disappearance from some lochs be due to eutrophication (Preston & Croft 1997). However, in the case of Finnish *P. rutilus* there appears to be a difference in trophic preferences as it seems to inhabit more eutrophic environments than Scottish *P. rutilus* sites, see chapter 3 water chemistry results. *P. rutilus* has been classified as a more eutrophic species in Scandinavian trophic plant classifications (Fremstad 1997, Swedish EPA, 2002), and the spread of *P. rutilus* in southern Finland has been thought due to southern Finnish lakes becoming more eutrophic in the past 20 to 30 years (Virola *et al.* 2001, Barkman 2000). Clearly there is a need to evaluate the above contradictions of which trophic conditions best favour the growth of *P. rutilus*.

4.1.1 Plant Growth Experiments and Life-Cycle

Many plant growth investigations only provide limited inferences from results, as many are short term, lasting a year or less, often measuring only vegetative growth rather than reproductive success (Gibson *et al.* 1999). To ensure predictive power from plant growth experiments it is necessary to assess the critically important plant growth phases, which can be a good measure of fitness (Goldberg & Fleetwood 1987). In addition to vegetative biomass, turion production would be a good measure of plant fitness for *P. rutilus*, as it is an essential reproductive and dispersal life-cycle phase for a plant that does not often produce seeds (Kotiranta *et al.* 1998). Turions serve as a multi-functional organ for carbohydrate storage, propagation and dispersal, so understanding their germination behaviour under simulated field conditions is essential to understanding plant population dynamics (Jian *et al.* 2003).

The two growth condition variables used in the experiments were phosphate nutrient levels and light levels. Phosphate nutrient levels were chosen as an experimental variable as it is usually the most limiting aquatic growth nutrient (Moss 1988, Mason 1981), and the nutrient most implicated in lake eutrophication (Lee 1973, Jupp & Spence 1977, Marsden *et al.* 1995). Light levels were chosen as the second growth variable as different light levels appeared to influence *P. rutilus* abundance and macrophyte diversity (see chapter 3), with light variability known to be a key factor in determining macrophyte growth and distribution (Holmes & Klein 1987, Moss 1988, Blindow 1992).

To ascertain the growth and fitness of *P. rutilus* under different nutrient and light conditions, a series of laboratory experiments were set up. *P. rutilus* life-cycle phases were grown under different concentrations of phosphate with varying levels of light transparency to evaluate the plant's growth and fecundity. Experiments were carried out over a period of two years, to include two life-cycle growth generations of the plant so as to include turion production and germination phases of the plant.

4.2 METHODS

4.2.1 Experimental Approach

The *P. rutilus* growth experiments were conducted in a two factorial random block design under different trophic and light conditions. Varied trophic conditions were simulated by different phosphate concentrations from low phosphate (oligotrophic) to high phosphate levels (hyper-eutrophic). The experimental design also incorporated three different light levels, natural light, half natural light and quarter natural light, to evaluate how both varied nutrient levels in conjunction with different light levels may affect *P. rutilus* growth.

4.2.2 Experimental Design

The plants were grown in 32 litre plastic growth tanks situated in a protected outside growth environment to maintain natural temperature conditions for growth as a warm greenhouse provides unsuitable growth conditions for this northern temperate plant.
Each growth treatment tank was replicated in triplicate giving a total of 36 growth tanks for the experiment. Water from Loch Ussie was used as the oligotrophic water source for experiments as Glasgow University tap water was too high in phosphates for the experiments. The mesotrophic to hyper-eutrophic phosphate concentrations were produced by adding NaH_2PO_4 to oligotrophic water from Loch Ussie, see Table 4.1 below for the experimental variables:

Table 4.1 *P rutilus* phosphate and light growth treatments.

Trophic Level	Phosphate Conc (mg/l)	Full light	0.5 light	0.25 light
Oligotrophic	0.016	3 (replicates)	3	3
Mesotrophic	0.032	3	3	3
Eutrophic	0.064	3	3	3
Hyper-eutrophic	0.128	3	3	3

4.2.3 Light regimes

The experimental light regime covers the range of light attenuation levels sampled from *P. rutilus* lochs. The two different shade light levels were achieved by covering the growth tanks with lids constructed from two different types of low and high shade geotextile material. The photosynthetically-active radiation (PAR) produced by the shade levels were measured with a twin sensor Skye SKP210 PAR linked to a SKYE Datalog SDL 2540 logger.

4.2.4 Plant Growth and Turion Formation - Experiment 1

P. rutilus plants were obtained as germinating turions that had been freshly washed up along the shores of Loch Ussie, in early June. The plants were sorted for standardized size, approximately 6 to 8 cm, and washed and then planted in pots of a well mixed sand substrate from Loch Ussie and observed for growth and uniformity before being selected as stock plants for growth experiments. For each growth tank, three plants were planted into a plastic growth pot containing well mixed sand, which was then placed in the growth tank. The three growth tank replicates for each treatment, each containing three plants, give a total of nine plants for each treatment.

The first stage of the experiments was conducted in 2003 over the main *P. rutilus* growing season months, June and July. Plant growth measurements were taken approximately every ten days, which involved the measurement of the length of each plant and recording the dates when the first signs of shoot turion formation took place for each plant. During the period of the experiment water chemistry measurements, pH, conductivity and temperature were monitored. The growth tanks water depths were maintained constant throughout the experiment by addition of Loch Ussie water.

Plant growth measurements ceased when it was found that no further shoot elongation took place. The plants were then weighed and harvested for their turions. The turions were buried in growth pots just below the surface of well mixed sand and returned to their same, refilled, growth treatment tanks for the following years second stage experiments.

4.2.5 Turion Germination and Plant Growth - Experiment 2

The second stage of experiments was conducted during spring and early summer 2004, when *P. rutilus* turions started to germinate in early spring, in the unseasonally mild spring temperatures, and so completed growth by by the end of 2004.

This involved monitoring the buried turions from the previous year for their first signs of germination and then measuring their shoot growth as in the previous year's experiment. *P. rutilus* germination times, growth rate and final turion size/weight were used as a measure of plant fitness and fecundity for the different growth treatments. The *P. rutilus* turion germination and growth experiments lasted for a period of approximately eight weeks, beginning in early March 2004 and ending in the first week of May 2004.

4.3 RESULTS

4.3.1 Effects of variable light levels on plant vegetative growth under different trophic conditions

The *P. rutilus* vegetative (stem length) growth for the 2003 and 2004 growth experiments revealed the following results in Tables 4.2 and 4.3. It can be seen from both tables there is a trend of greater mean vegetative growth length with reduced light levels for the different trophic levels, with the trend more apparent in 2004 growth data, see Tables 4.2 and 4.3.

In the 2003 experiment the greatest vegetative elongation under full light was under eutrophic conditions. The greatest vegetative elongation under half light regime was produced with poly-eutrophic conditions, with it being significantly greater than for full light treatment. Hyper-eutrophic conditions also produced the second largest vegetative stem growth, for quarter light treatment, see Table 4.2.

Table 4.2 *P. rutilus* mean final vegetative growth lengths (cm) for different light and trophic conditions, 2003. Treatments with different superscript letters are significantly different. Tukeys mean differences $P < 0.05$

Trophic Level	Phosphate conc (mg/l)	Full light	0.5 light	0.25 light
Oligotrophic	0.016	9.96 ± 1.23	11.38 ± 3.57	14.52 ± 1.77
Mesotrophic	0.032	10.39 ± 0.96	12.30 ± 2.07	10.13 ± 0.82
Eutrophic	0.064	12.06 ± 1.63	11.50 ± 1.68	11.37 ± 1.50
Hyper-eutrophic	0.128 $^{a\,b}$	9.56 ± 0.71 ^{a}	16.71 ± 1.97 ^{b}	13.70 ± 1.61 $^{a\,b}$

This trend of greater vegetative stem elongation with increased trophic conditions under reduced light levels can be clearly seen in the 2004 experiments, Table 4.3. In half light there was a significantly greater stem length growth under eutrophic condition 9.37 ± 1.04 cm compared to the less trophic oligotrophic, 5.09 ± 0.54 cm, and mesotrophic, 5.78 ± 0.53 conditions. Hyper-eutrophic conditions under half light also produced significantly greater plant stem growth than oligotrophic, but not mesotrophic conditions, Fig 4.1.

Table 4.3 *P. rutilus* mean final vegetative growth lengths (cm) for different light and trophic conditions, 2004. Treatments with different superscript letters are significantly different, lower case compare different light regimes, upper case compares different trophic regimes. Tukeys mean differences $P < 0.05$

Trophic Level	Phosphate conc (mg/l)	Full light	0.5 light	0.25 light
Oligotrophic	0.016	5.71 ± 0.46 ^{a}	5.09 ± 0.54 ^{aA}	9.96 ± 0.65 ^{b}
Mesotrophic	0.032	7.19 ± 0.57 $^{a\,b}$	5.78 ± 0.53 ^{aA}	8.30 ± 0.87 ^{b}
Eutrophic	0.064	6.67 ± 0.39	9.37 ± 1.04 ^{B}	9.45 ± 1.00
Hyper-eutrophic	0.128	6.66 ± 0.62	8.54 ± 0.81 ^{B}	9.37 ± 0.90

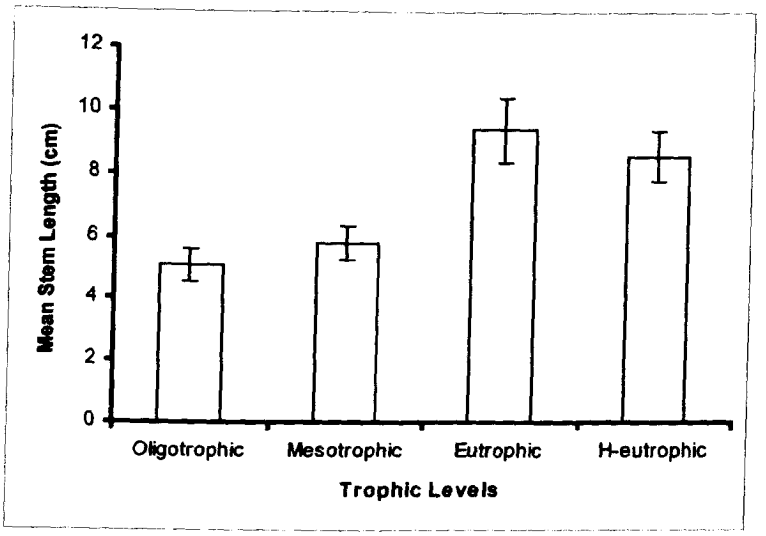


Figure 4.1 The effects of different trophic regimes on the final *P. rutilus* mean stem length under half light conditions, 2004.

This greater eutrophic growth under half light was also reflected in total above ground production, being greater for eutrophic conditions compared to lesser trophic levels, see Table 4.4. It can also be seen that the above ground *P. rutilus* biomass growth was larger, though not significantly, under full light conditions for all trophic levels apart for mesotrophic, which has a slightly less final biomass than for the quarter light treatment, Table 4.4.

Table 4.4 *P. rutilus* mean final vegetative growth dry weights (mg) for different light and trophic conditions, 2004. No significant differences, Tukeys mean differences $P < 0.05$

Trophic Level	Phosphate conc (mg/l)	Full light	0.5 light	0.25 light
Oligotrophic	0.016	11.03 $\pm$ 4.4	3.77 $\pm$ 0.98	6.70 $\pm$ 3.52
Mesotrophic	0.032	8.83 $\pm$ 0.89	3.60 $\pm$ 1.15	8.93 $\pm$ 3.34
Eutrophic	0.064	9.13 $\pm$ 1.19	7.23 $\pm$ 2.60	8.50 $\pm$ 0.67
Hyper-eutrophic	0.128	9.60 $\pm$ 2.40	4.47 $\pm$ 1.01	7.53 $\pm$ 4.20

4.3.2 Effects of different light and trophic levels on plant turion production

The effects on *P. rutilus* mean turion production of different light regimes and trophic conditions are revealed in Table 4.5 below. Mean turion production per plant under natural light appears slightly greater under eutrophic conditions, 2.67 ± 0.50, than with oligotrophic and mesotrophic conditions producing similar number of turions, 2.56. There also appears to be a trend, though not significant, of reduced turion production with reduced light levels for each trophic treatment, but this is not the case for hyper-eutrophic light treatments. Hyper-eutrophic conditions under different light regimes produced the least mean number of turions per plant, than the three lower trophic treatments.

Table 4.5 Mean number of *P. rutilus* turions per plant for different light and trophic conditions, 2003. No significant differences found, Tukeys mean differences $P < 0.05$

Trophic Level	Phosphate conc (mg/l	Full light	0.5 light	0.25 light
Oligotrophic	0.016	2.56 ± 0.65	2.11 ± 0.31	1.89 ± 0.35
Mesotrophic	0.032	2.56 ± 0.50	2.44 ± 0.24	2.11 ± 0.46
Eutrophic	0.064	2.67 ± 0.50	2.33 ± 0.44	1.89 ± 0.35
Hyper-eutrophic	0.128	1.78 ± 0.32	1.89 ± 0.31	1.78 ± 0.55

The greatest range of turions produced per plant was in the full light oligotrophic treatment, 1 to 7 turions per plant, Table 4.6. Under full light conditions the smallest range number of 1 to 3 turions per plant was produced under hyper-eutrophic conditions, with a similar range of turions per plant in mesotrophic and hyper-eutrophic under a half light treatment. Hyper-eutrophic treatments under quarter light produced the minimum range of zero turions per plant, range 0 to 5.

Table 4.6 Minimum and maximum range of *P. rutilus* turions produced per plant for different light and trophic conditions, 2003.

Trophic Level	Phosphate conc (mg/l	Full light	0.5 light	0.25 light
Oligotrophic	0.016	1 – 7	1 – 4	1 – 5
Mesotrophic	0.032	1 – 5	1 – 3	1 – 5
Eutrophic	0.064	1 – 5	1 – 5	1 – 4
Hyper-eutrophic	0.128	1 – 3	1 – 3	0 – 5

4.3.3 Effects of different light and trophic levels on turion growth

The measurements of turion length was used as a measure of size/fitness as it was not possible to measure turion dry weight for the 2003 experiments, as these turions were to be used in the 2004 turion germination experiments. However, length appears to be a good measure of size, as the 2004 turion length measurements correlate significantly with turion dry biomasses ($r = 0.604$ $P < 0.004$), see Fig 4.2.

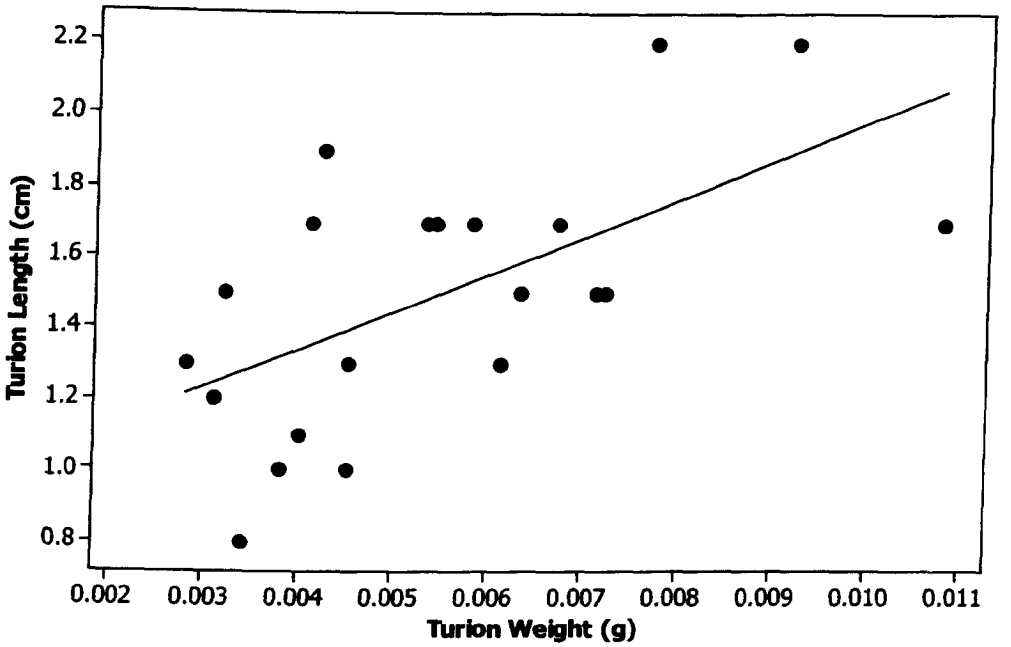


Figure 4.2 The relationship between turion length and turion dry weight, r = 0.604 P < 0.004.

The *P. rutilus* mean final turion lengths for the 2003 growth experiments revealed the following results in Table 4.7. It shows a trend of reduced turion growth size with decreasing light availability under oligotrophic and mesotrophic conditions.
However, under eutrophic conditions with a reduced light regime there was a increase in turion size, with a significantly greater turion size produced under half light compared to full light conditions.

Table 4.7 *P. rutilus* mean ± s.e. final turion growth lengths (cm) for different light and trophic conditions, 2003. Treatments with different superscript letters are significantly different, lower case compare different light regimes, upper case compare different trophic regimes. Tukeys mean differences $P < 0.05$

Trophic Level	Phosphate Conc (mg/l)	Full light	0.5 light	0.25 light
Oligotrophic	0.016	1.44 ± 0.06 ^{A}	1.27 ± 0.06	1.30 ± 0.07
Mesotrophic	0.032	1.67 ± 0.07 ^{AB}	1.43 ± 0.08	1.55 ± 0.82
Eutrophic	0.064	1.60 ± 0.05 ^{ABa}	2.02 ± 0.09 ^{b}	1.82 ± 0.16 ^{ab}
Hyper-eutrophic	0.128	1.89 ± 0.09 ^{B}	1.62 ± 0.10	1.83 ± 0.14

The Table 4.7 reveals that under a full light regime *P. rutilus* turion growth appears to be significantly greatest under hyper-eutrophic conditions, compared to more lower trophic conditions. This trend of greater turion growth size with increasing trophic conditions under full light, is also found in 2004 experimental results. The exception being, eutrophic conditions which has significantly smaller turion size than the less trophic mesotrophic full light treatments, Table 4. 8.

Table 4.8 *P. rutilus* mean final turion growth lengths (cm) for different light and trophic conditions, 2004. Treatments with different superscript letters are significantly different, lower case compare different light regimes, upper case compares different trophic regimes. Tukeys mean differences $P < 0.05$

Trophic Level	Phosphate conc (mg/l)	Full light	0.5 light	0.25 light
Oligotrophic	0.016	1.42 ± 0.08 ^{aA}	0.94 ± 0.16 ^{bA}	1.98 ± 0.11 ^{cA}
Mesotrophic	0.032	1.48 ± 0.08 ^{aA}	0.86 ± 0.06 ^{bA}	1.73 ± 0.13 ^{aB}
Eutrophic	0.064	1.14 ± 0.06 ^{aB}	1.82 ± 0.11 ^{bB}	1.33 ± 0.09 ^{aB}
Hyper-eutrophic	0.128	1.51 ± 0.13 ^{A}	1.27 ± 0.06 ^{A}	1.55 ± 0.20 ^{B}

The reduction in eutrophic turion length was further confirmed with eutrophic conditions, under full light, producing the smallest biomass production of all the trophic treatments, with biomass being significantly smaller than the mesotrophic full light treatment. Eutrophic turions size also significantly reduced in length and biomass for full light compared to eutrophic half light conditions for both year's experiments, Tables 4.7, 4.8 and 4.9. Quarter light, eutrophic regime, also produced smaller turion length and biomass than the half light eutrophic treatment, for both years experiments, with it being significantly different for 2004, Table 4. 8.

However, half light regimes tended to produce significantly greater eutrophic turion growth than the other trophic half light treatments. This significantly greater eutrophic turion length under half light conditions, compared to the other trophic half light conditions can be seen in Fig 4.3.

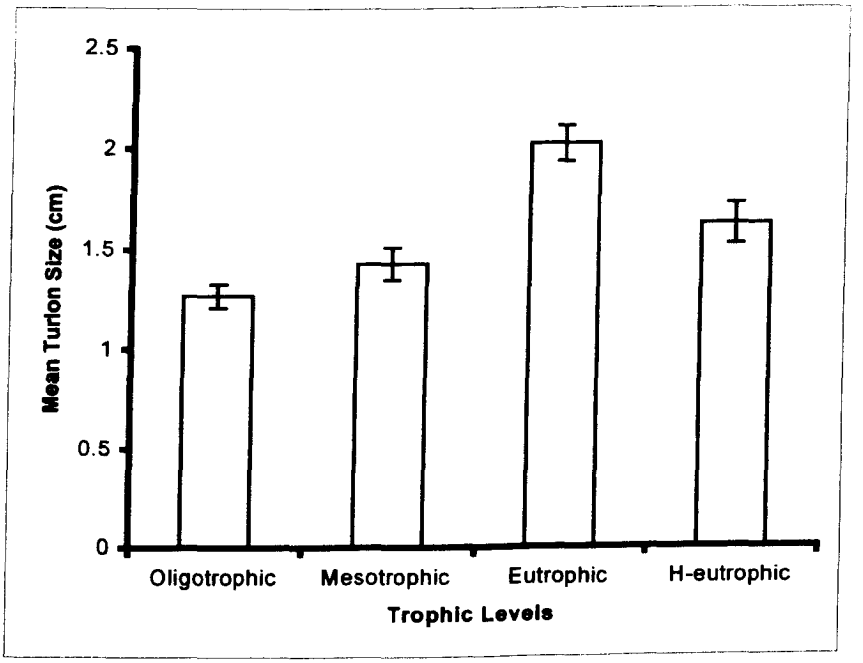


Figure 4.3 The effects of different trophic regimes on the final length of *P. rutilus* turions under half light conditions, 2003.

The significantly greater eutrophic turion length under half light conditions compared to the other trophic half light trophic levels was also repeated for turion length and biomass 2004 experimental results, Tables 4.8, 4.9 and graph Fig 4.4.

Table 4.9 *P. rutilus* mean final turion weights (mg) for different light and trophic conditions, 2004. Treatments with different superscript letters are significantly different, lower case compare different light regimes, upper case compares different trophic regimes. Tukeys mean differences $P < 0.05$

Trophic Level	Phosphate conc (mg/l)	Full light	0.5 light	0.25 light
Oligotrophic	0.016	4.96 ± 0.57^{aAB}	2.27 ± 0.54^{bA}	5.78 ± 0.71^{a}
Mesotrophic	0.032	5.36 ± 0.45^{aA}	2.21 ± 0.39^{bA}	5.61 ± 0.71^{a}
Eutrophic	0.064	3.21 ± 0.41^{aB}	6.07 ± 0.40^{bB}	4.40 ± 0.64
Hyper-eutrophic	0.128	4.70 ± 0.73^{AB}	2.59 ± 0.21^{A}	5.47 ± 1.11

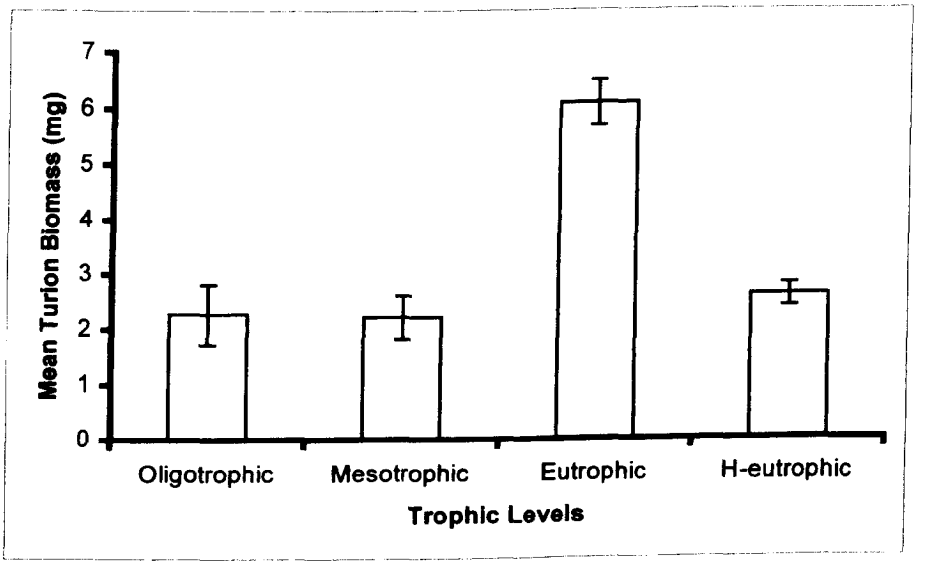


Figure 4.4 The effects of different trophic regimes on the final dry biomass production of *P. rutilus* turions under half light conditions, 2004

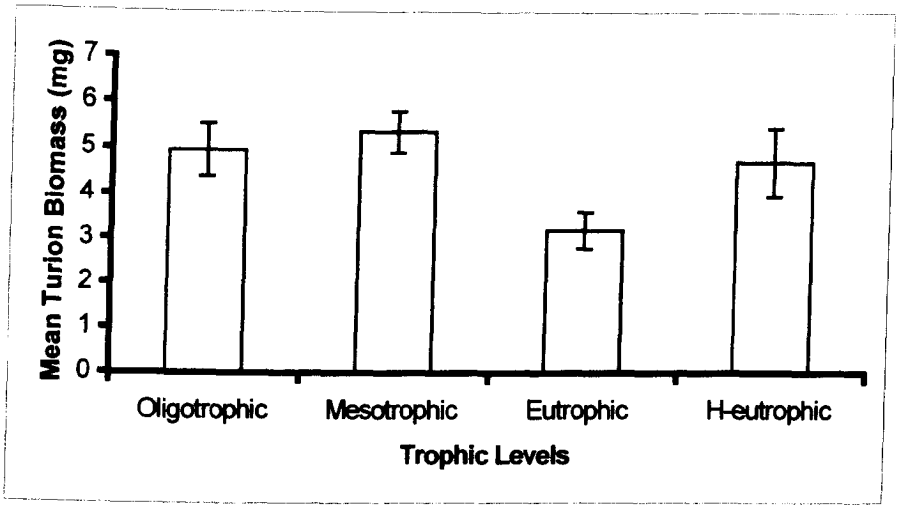


Figure 4.5 The effects of different trophic regimes on the final dry biomass production of *P. rutilus* turions under full light conditions, 2004.

It can be seen under natural light conditions that the mesotrophic treatment produces the largest turion size, with it being significantly larger than full light eutrophic treatment (Fig 4.5).

4.3.4 Effects of different light and trophic levels on turion germination

The experimental results, Table 4.10, show both mesotrophic and eutrophic treatments produced the largest and similar, 16.7 %, germination rates for a full light regime, whilst the lower oligotrophic and poly-eutrophic germination rates are similar for full light treatments, Table 4.10. Eutrophic half light treatment regime produced the highest germination rate of 25 %, with a germination rate twice that of the other trophic levels under a half light regime, Table 4.10. However, these germination rates for different treatments appear they may also be partly influenced by turion size, with germination rate increasing with turion size, producing a near significant relationship, Fig 4.6.

Table 4.10 The % turion germination rate per week for different light and trophic conditions, 2004 (% turion germination rate per week is the 100 % for complete germination, divided by the total number of weeks that took for completion of germination for each growth treatment).

Trophic Level	Phosphate conc (mg/l)	Full light	0.5 light	0.25 light
Oligotrophic	0.016	12.5	11.1	12.9
Mesotrophic	0.032	16.7	12.5	18.4
Eutrophic	0.064	16.7	25	12.5
Hyper-eutrophic	0.128	12.5	12.5	14.3

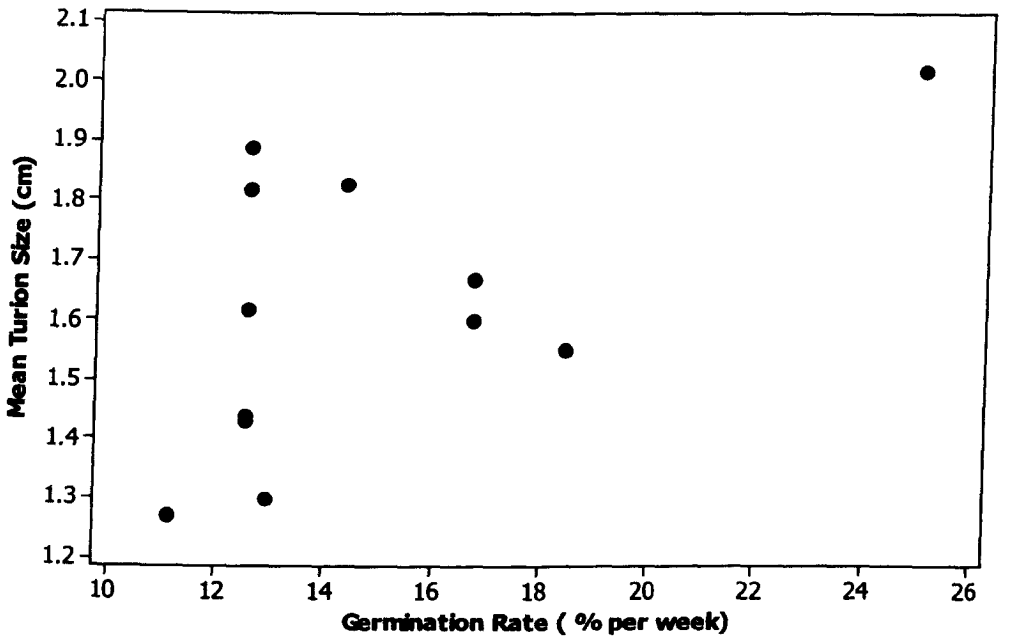


Figure 4.6 The relationship between *P. rutilus* turion mean size (for each treatment) and germination rate, 2004, $r = 0.53$ $P < 0.07$.

Both full light and half light treatments produced 100% *P. rutilus* turion germination, apart from the oligotrophic half light treatment, which resulted in a reduced germination of 89.5 %, see Table 4.11. The quarter light treatment also produced less than a 100 % turion germination, with oligotrophic conditions produced the lowest, 64.7 %, turion germination. Eutrophic treatment produced the largest turion germination, 94.4 % of all the quarter light treatments, graph Fig 4.7.

Table 4.11 The % total turion germination for different light and trophic conditions, 2004

Trophic Level	Phosphate conc (mg/l)	Full light	0.5 light	0.25 light
Oligotrophic	0.016	100	89.5	64.7
Mesotrophic	0.032	100	100	73.7
Eutrophic	0.064	100	100	94.4
Hyper-eutrophic	0.128	100	100	71.4

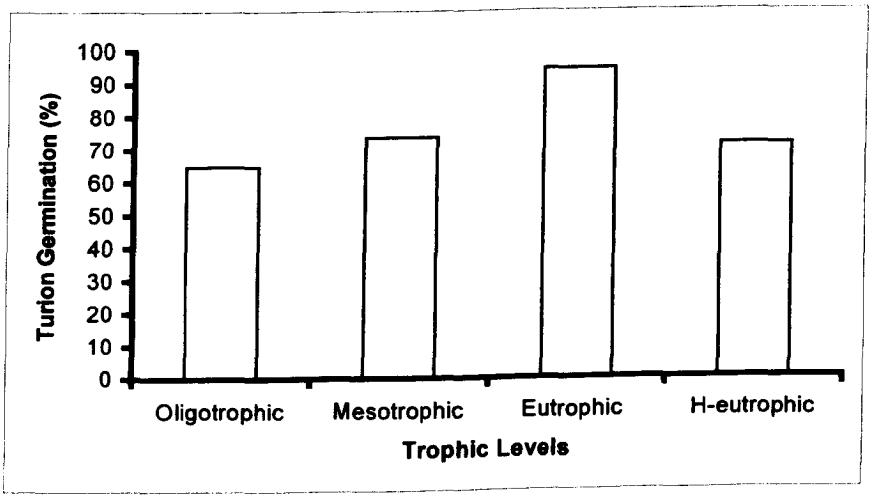


Figure 4.7 The effects of a quarter light regime on the % germination of *P. rutilus* turions under different trophic conditions, 2004.

Smaller turion length also seemed to be linked to reduced germination, as the smallest mean turion lengths of all the treatments had the least turion germination under oligotrophic reduced light regimes, Tables 4.7 & 4.11. The average day length graph, Fig 4.8, shows *P. rutilus* turion germination began in early March when average day length was 11 + hours, with germination being completed by late April when average day length was 14 + hours.

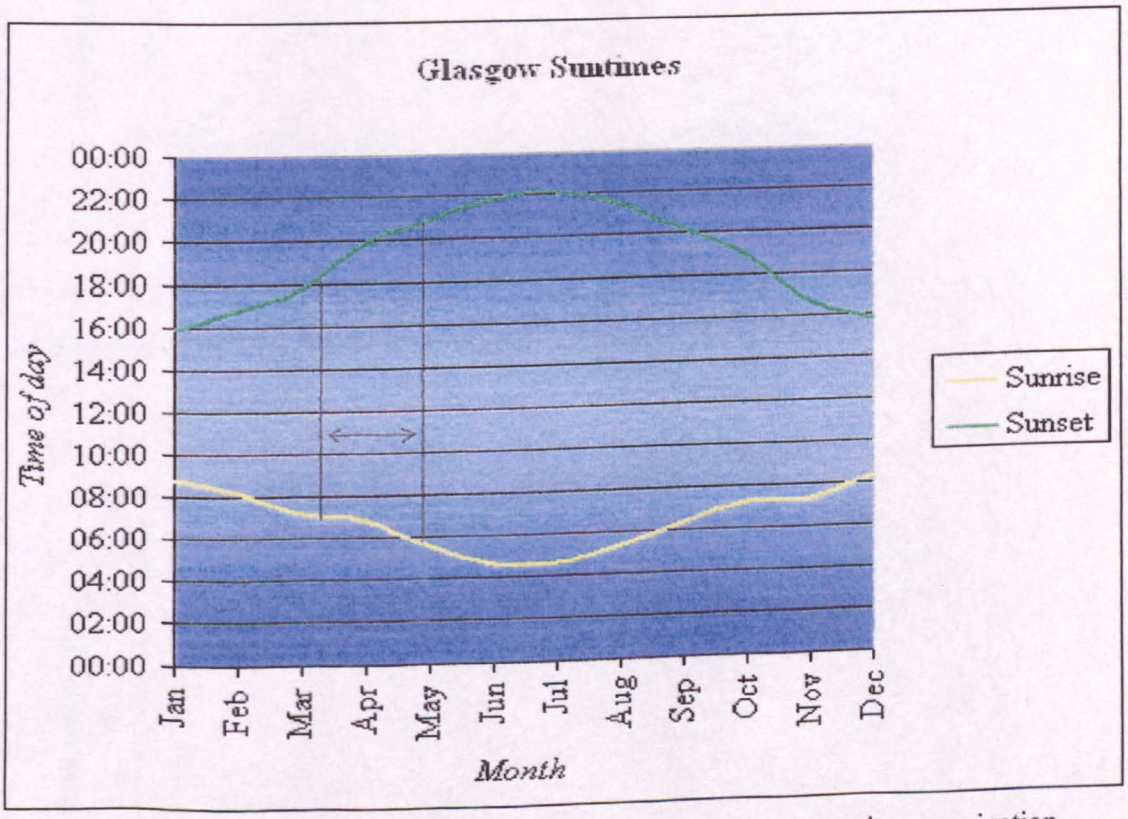


Figure 4.8 The average day length photoperiod during *P. rutilus* turion germination, from early March to late May 2004

The germination for *P. rutilus* turions appears to take place when water temperature starts increasing from a low 6 ° C to a higher 13 ° C , where cumulative germination greatly increases, see Figs 4.10 & 4.11. Both full and half light mesotrophic treatments achieved 100 % turion germination, whilst under a quarter light treatment only 73.7 % of the turions germinated, with no more germinations occuring after 5 weeks, Fig 4.9.

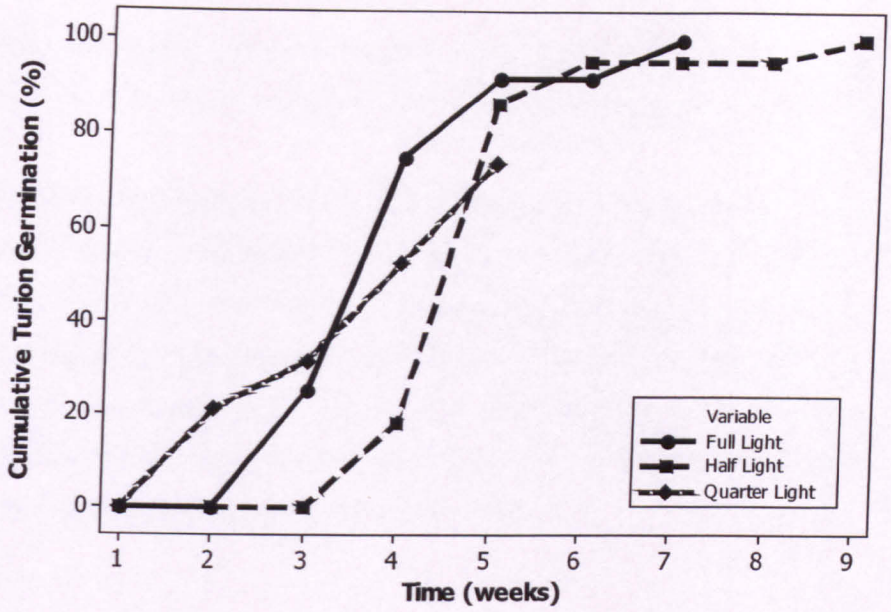


Figure 4.9 The % germination of *P. rutilus* turions under different light regimes in mesotrophic conditions, 2004.

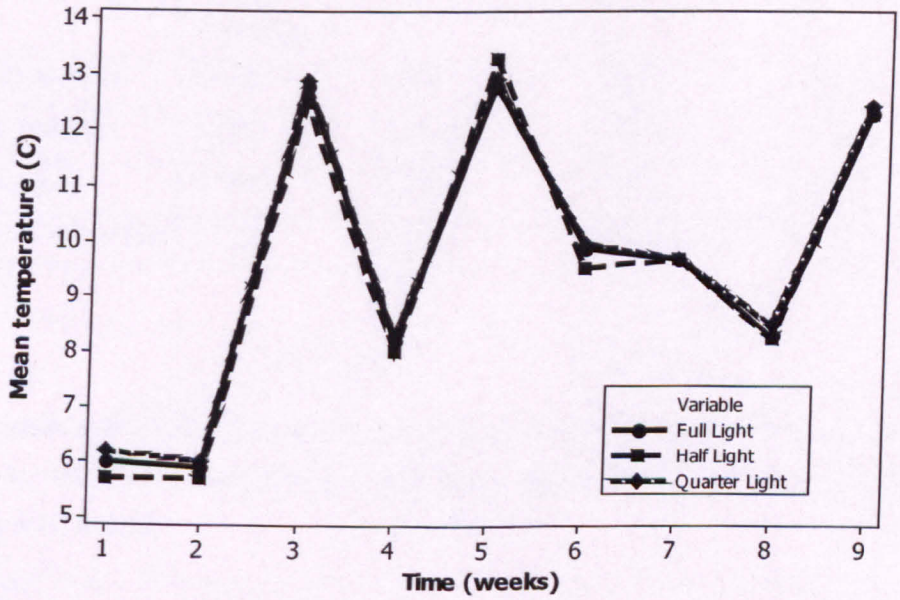


Figure 4.10 *P. rutilus* growth experiment water temperatures for different light regimes under mesotrophic conditions, 2004.

Similar turion germinations coinciding with a rise in water temperatures also occurs in other growth treatments, such as for eutrophic conditions (see Appendix 5).

4.3.5 Effects of different light and trophic levels on turion formation rate

Turion formation rate appears to be similar for all the different trophic levels under a full light treatment. The full light treatment produces higher turion formation rates than half and quarter light treatments under oligotrophic and hyper-eutrophic conditions. Eutrophic conditions have the highest turion formation rate for half light regimes, whilst both mesotrophic and eutrophic conditions produce the highest formation rates for quarter light treatments, see Table 4.12, and Fig 4.11.

Table 4.12 The % turion formation (% of total turions formed for each treatment) rate per week for different light and trophic conditions, 2004.

Trophic Level	Phosphate conc (mg/l)	Full light	0.5 light	0.25 light
Oligotrophic	0.016	16.7	13.5	13.5
Mesotrophic	0.032	16.7	13.5	16.7
Eutrophic	0.064	16.7	16.7	16.7
Hyper-eutrophic	0.128	16.7	13.5	13.5

P. rutilus turion formation appeared to take place when water temperatures reached 15 ^{o} C +, with turion formation rate rising very quickly above this 15 ^{o} C + temperature, Figs 4.11 & 4.12.

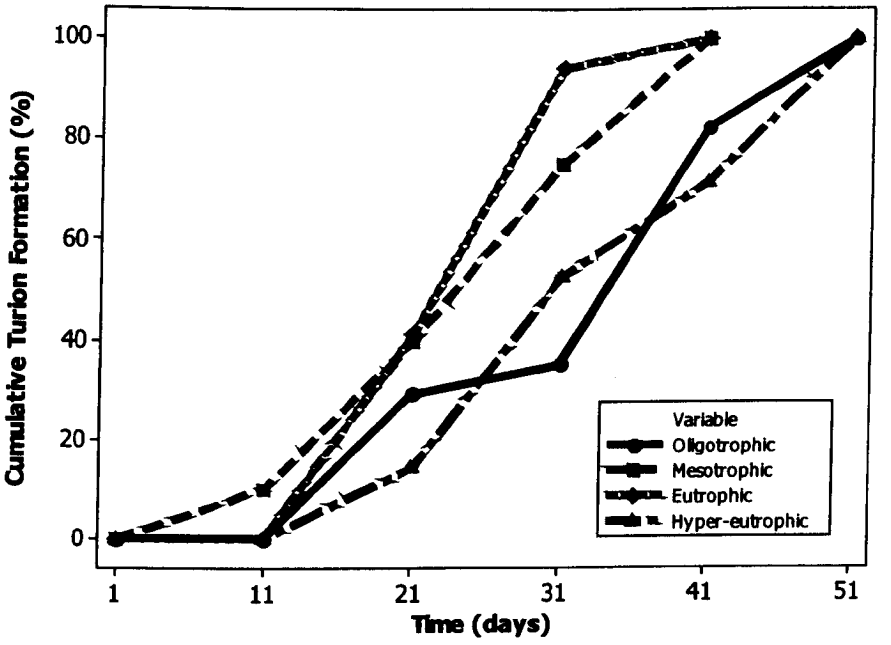


Figure 4.11 The effects of quarter light treatments on % *P. rutilus* turion formation under different trophic conditions, 2004.

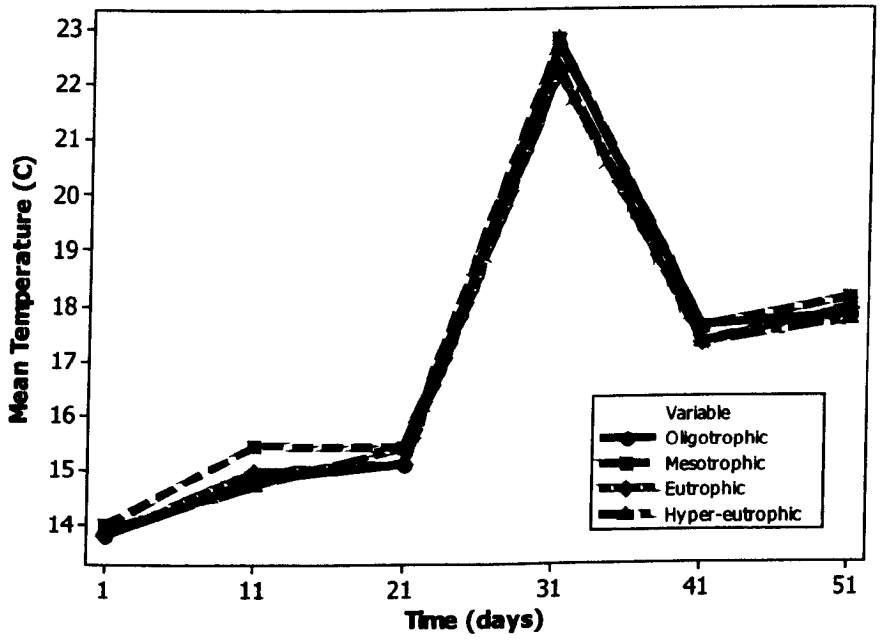


Figure 4.12 *P. rutilus* growth water temperatures for quarter light regimes under different trophic conditions, 2004.

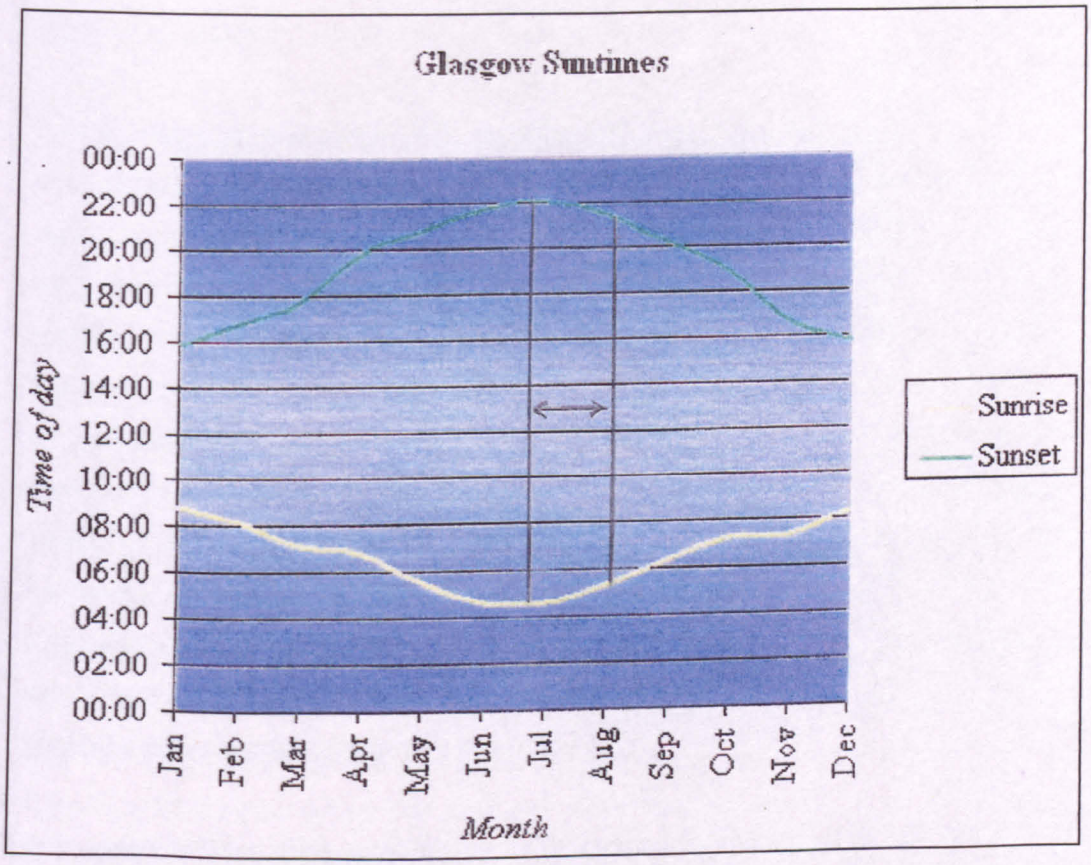


Figure 4.13 The average day length photoperiod during *P. rutilus* turion formation, from late June to early August 2003.

The graph Fig 4.13 shows that *P. rutilus* turion development phase for turion formation started in late June and was completed by early August. *P. rutilus* turion development first started during high summer with a maximum photoperiod of 17 hours + and was completed by early August with day length decreasing to 16 hours, Fig 4.13.

4.4 DISCUSSION

4.4.1 *P. rutilus* growth under different light and trophic conditions

The vegetative growth experiments revealed that there was a trend of increasing *P. rutilus* stem growth with reduced light levels for the different trophic treatments. The more nutrient rich conditions, eutrophic and hyper-eutrophic, produced the largest heights, with hyper-eutrophic conditions, under a half light regime producing significantly greater vegetative growth than for full light growth conditions. This trend of increasing stem growth with reduced light and richer nutrient conditions was also apparent in 2004 results, but was not significant. However, there was a significantly greater stem elongation under a half light regime, with eutrophic and hyper-eutrophic treatments producing significantly greater stem growth than oligotrophic and mesotrophic treatments. Greater stem elongation in higher phosphate conditions may be also caused by indirect shading of plants, by increased periphyton growth which is known to effect macrophyte growth (Jones *et al.* 2002).

It is apparent from these results that a combination of light and nutrient levels are affecting *P. rutilus* stem growth characteristics. These two factors, light and phosphorus levels are known to significantly influence macrophyte growth form and macrophyte distribution (Garbey *et al.* 2004, Carr & Chambers 1998, Spink *et al.* 1995, Chambers 1987). The shoot elongation in *P. rutilus* appears to increase with increased shade, especially in nutrient rich conditions, which is known to be one of the adaptive mechanisms for angiosperms to survive in poor light, turbid conditions (Blindow 1992). Shoot elongation is known to be common among angiosperms in poor light availability, Spence *et al.* 1987, Abernethy *et al.* 1996, Barko & Smart 1981).

This shoot elongation in macrophytes in reduced light conditions is common among canopy forming erect species (Blindow 1992), enabling them to concentrate there biomass in the upper water layer where photosynthesis is at its maximum (Cenzato & Ganf 2001, Boston *et al.* 1989, Barko *et al.* 1981). This canopy forming erect habit was

reflected in there being no significant differences in biomass production between significantly taller or smaller *P. rutilus* growth forms, as biomass production was similar but distributed differently according to the different light regimes. Even in low nutrient, oligotrophic, treatment conditions, where low phosphorus availability may limit macrophyte production (Solander 1978, Solander 1983), there was significantly larger *P. rutilus* stem elongation under reduced light compared to full light regimes. The growth response of macrophytes has shown to be phenotypically plastic, with pronounced vertical stem extension in low irradiance (Pilon & Santamaria 2002).

It appears that light availability is a significant controlling factor in *P. rutilus* stem and canopy growth. Light is known to be one of the main limiting factors in the growth and distribution of macrophytes, especially in turbid conditions (Jupp & Spence 1977, Chambers 1987, Blindow 1992, Abernethy *et al.* 1996). The erect canopy growth habit of *Potamogeton* species such as *P. rutilus* gives such plants a competitive advantage in reduced light conditions over smaller rosette macrophyte growth forms, such as isoetid and some charophyte species (Farmer & Spence 1986, Blindow 1992). Competition light experiments have shown that tall plant growth forms have a competitive advantage over shorter plant forms, as they are able to intercept more light (Wilson & Keddy 1991, Gaudet & Keddy 1988, Grime 1973).

The loss of smaller growth form macrophyte species from nutrient enriched sites is thought more to be due to reduced light levels from both phytoplankton (Balls *et al.* 1989, Graneli & Solander 1988), and more competitive, canopy forming plants (Farmer & Spence 1986, Jupp & Spence 1977). However, light availability may be one of the most important factors influencing the growth of *P. rutilus,* but seasonal studies of rooted macrophytes have found in many cases there is no single dominant environmental factor limiting plant growth *in situ* (Carr *et al.* 1997, Madsen & Adams 1988).

4.4.2 *P. rutilus* turion formation and growth under different light and trophic conditions

The light and trophic experimental treatments revealed that mean turion production was greatest under natural light for all trophic regimes, apart for the most nutrient rich, poly-eutrophic, treatments where a half light treatment produced slightly a greater mean turion production per plant. Eutrophic full light treatment produced the slightly larger mean number of turions, 2.67 $\pm$ 0.5, whilst full light oligotrophic and mesotrophic treatments, 2.56 $\pm$ 0.65/0.50 produced the second largest turion production for all the different light treatments. The greatest range in the number of turions produced per plant was with full light oligotrophic treatment, with a maximum of 7 and minimum of 1 per plant.

It is known that turion formation and production is a light and temperature mediated process (Spence 1972, Chambers *et al.*1985). In the case of the experimental treatments there was no significant temperature differences in treatments, which suggests that light difference was a key factor in determining plant turion production. This was further indicated by the trend of reduced turion production with reduction in light availability, with the exception of hyper-eutrophic half light treatment. As for *P. rutilus*, it is known that reduced light regimes can reduce the number of turions, as observed in *P. crispus* where production of turions was confined to the upper 1.5m of the water column were light was higher (Spence *et al.* 1987).

The light sensitive plant development of turions, is thought due to light sensitive plant phytochromes which control plant photomorphogenic growth development, independently of photosynthesis (Smith 1975). The two main functions of phytochrome photomorphogenesis are first to produce morphological and physiological states which adapt photosynthesizing plants to local light climates and secondly to synchronize plant development phases with changing seasons (Spence *et al.* 1987).

The synchronized development of turions with season is thought to be controlled by a photomorphogenic phytochrome process (Sastroutomo 1981, Weber & Nooden 1976).

For example, *Potamogeton crispus* is known to be a long day plant in turion formation, producing in mid summer with long days (16h +) and high temperatures (16 ° C +), with formed turions germinating in autumn time. The reverse process would be expected with *P. rutilus*, to form turions through cues of shorter photoperiod days and lower late summer temperatures, being a temperate zone species that produces overwintering turions. (Frankland *et al.* 1987). However, the experimental results indicated this is not the case and turion long day formation cues, as in *P. crispus*, appear to initiate turion formation with high summer long days (17h +) and high temperatures above 15 ° C +, Figs 4.12 & 4.13.

This turion development initiated by high summer cues, long day photoperiod and high temperatures, resulted in *P. rutilus* turion formation beginning in late June and being completed by the beginning of August. Similar turion development phases occur in other graminoid *Potamogetons*, such as *Potamogeton pusillus* with numerous turions being formed from July through to August (Kadono 1984). These turion development phases may vary by a few weeks, as light and temperature environmental cues will be influenced by variable seasonal weather conditions, whilst latitudinal effects will delay turion development in colder northern latitudes (Virola *et al.* 2001).

4.4.3 Turion growth and biomass production under different light and trophic conditions

There were much more significant differences in turion size and biomass for the different light and trophic treatments, than for differences in turion production per plant. Not only is propagule production a good measure of plant fecundity and fitness (Rodriguez-Girones *et al.* 2003), but propagule size is also an important measure of plant fitness (Rodriguez-Girones *et al.* 2003, Blindow 1992). The 2003 results revealed that there was a trend of mean reduction in turion size with reduction in light regime under oligotrophic and mesotrophic conditions, Table 4.7. However, this trend was not so clear in the 2004 turion size and biomass results, with oligotrophic and mesotrophic

treatments producing significantly smaller turion size/biomass in only half light treatments but not quarter light treatments Tables 4.8 & 4.9.

Aquatic plants are known not only to take up phosphorus by roots but also through leaves (Madsen & Cedergreen 2002, Garbey *et al.* 2004), and these experimental results show that low nutrient, oligotrophic and mesotrophic, phosphate levels appear to limit turion growth when combined with half reduced light levels. Half light levels appear to significantly affect *P. rutilus* plant turion phosphate assimilation at lower nutrient levels compared to normal light conditions, Tables 4.8 & 4.9. However, reduced light may not be the key overall limiting factor in plant phosphate assimilation and turion growth in low nutrient conditions, as lower, quarter light conditions produced greater turion sizes than for half light treatments under oligotrophic and mesotrophic conditions, Tables 4.8 & 4.9.

In contrast the more phosphate rich eutrophic conditions under half-light, appeared to produce optimum conditions for turion growth. Eutrophic conditions under half light conditions produced the largest overall turion size of all the treatments, significantly larger than those produced under eutrophic conditions with full light and with lower phosphate half light treatments, Tables 4.7, 4.8 & 4.9. Increasing turion size with higher nutrient levels was further seen in greater turion size under full light hyper-eutrophic treatments when compared with the lower trophic full light treatments.

P. rutilus turion final biomass can be considered a measure of plant fitness and fecundity, since fecundity and survival normally increase with propagule size, (Rodriguez-Girones *et al.* 2003, Geritz 1995, Blindow 1992, Westoby *et al.*1992). It can be seen that overall optimum turion growth was achieved under half light eutrophic conditions, whilst mesotrophic conditions provided optimum turion growth under natural light conditions, Figs 4.4 & 4.5. The least favourable conditions for turion growth were half light treatments for lowest and highest trophic conditions, apart from eutrophic half light conditions that produced the overall largest turion biomass size for all treatments, Table 4.8.

The increase in phosphate appears to be increasing turion growth, which suggests *P. rutilus* plants has the ability to take up phosphate from the aquatic environment and assimilate it in its turion growth. This ability suggests a high nutrient-use efficiency (NUE) and ability to integrate P rapidly which enables such species to occur over broader ecological range than species with lower storage capacities and NUE (Garbey *et al.* 2004).

This wide ecological range of light and trophic conditions that *P. rutilus* can inhabit can be seen not only from these growth experiments, but is also reflected in the range of aquatic conditions which this rare plant can be found. For example, Scottish *P. rutilus* is found in oligo-mesotrophic lochs, some with eutrophic influences (Preston 1995, Preston & Croft 1997, Wallace 2002,) whilst in its Scandinavian range it tends to inhabit more eutrophic habitats (Swedish EPA 2002, Kotiranta *et al.* 1998, Fremstad 1997). However, if *P. rutilus* is able to inhabit a range of trophic conditions it raises the question why the Scottish plant is only found growing in mesotrophic lochs and not also in eutrophic lochs as found in Finland and other Scandinavian lakes ?

The Scottish *P. rutilus* growth experiments demonstrated that turions grew best in mesotrophic conditions when under full light conditions. It may be the case that the mesotrophic growth habit of Scottish *P. rutilus* represents some degree of ecotypic differentiation from Scandinavian plants that appear to thrive in eutrophic lake habitats. Ecotypic variation has been found in aquatic species such as *Ranunculus flammula* that has shown ecotypic differences in tolerances to dessication (Cook & Johnson 1968), whilst ecotypic differences in the tolerance to salinity have been found in *Potamogeton pectinatus* (Van Wijk *et al.* 1988).

However, it may not be just ecotypic differences, but also environmental differences between Finnish and Scottish habitats that determine the ability of *P. rutilus* to live in different trophic conditions. For example, latitudinal differences of lower temperatures and reduced light levels of the higher Finnish latitudes (Virola *et al.* 2001), may favour the survival of *P. rutilus* in eutrophic lakes when under these habitat conditions. This

was partly supported by the growth experiments, that showed eutrophic phosphate growth conditions, under a half light regime, produced the largest *P. rutilus* turion growth, which was shown to be a good measure of plant fitness.

4.4.4 Effects of different light and trophic regimes on turion germination

The *P. rutilus* germination rates under different treatments appeared to be the highest under mesotrophic and eutrophic full light conditions, whilst eutrophic half-light conditions produced the largest overall germination rate, Table 4.9. However, the largest overall germination rate for the half-light eutrophic treatment, was produced by the largest mean turion size of all the treatments, suggesting turion size may also be influencing germination rate. This was further confirmed by a near significant relationship between increasing germination rate and increasing mean turion size, Fig 4.6.

In the case of macrophytes, large winter buds, tubers or turions offer these plants the carbohydrate stores which are necessary for a high initial growth rate during spring, independently of light availability in the water column (Blindow 1992). It is known that the most critical period in a plant's life-cycle is when carbohydrate depletion weakens overwintering organs, such as turions (Crawford & Palin 1981, Stewart & Bannister 1973). This would appear to be the case for the overwintering *P. rutilus* turions. Larger turion size promotes early germination and a high germination rate compared to smaller turions, which in some cases faileded to germinate in reduced light conditions.

Turion germination is known to be a light-demanding process, with successful germination and germination rate decreasing with the reduced light availability of increasing water depth (Jian *et al.* 2003). In the case of *P. rutilus*, the greater turion size appears to increase the fitness of the plant, enabling it to germinate successfully at reduced light regimes, which suggests it could survive larger reductions in light so colonising deeper water depths than when reliant on smaller turions.

If a plant has a limited amount of resources that it can invest in propagules, it can produce either many small propagules, or fewer larger ones (Rodriguez-Girones *et al.* 2003). This trade-off between propagule size and propagule number did not seem apparent in *P. rutilus*, as although there was some difference in turion number for different growth treatments, they were not significant compared to the more significant size and biomass differences. This suggests that turion size would be a good correlate of *P. rutilus* fitness, as yearly recruitment seems to depend almost exclusively on turion production, as seed recruitment is rare (Kotiranta *et al.*1998).

Turion germination is not only influenced by turion size and light intensity, but also by seasonal changes of temperature and photoperiod, which appear to break turion winter dormancy and stimulate germination (Jian *et al.* 2003, Frankland *et al.* 1987, Weber & Nooden 1976). For example, Chambers *et al.* (1985) revealed that short days and low temperatures mostly likely cause the breaking of *P. crispus* turion dormancy. In the case of *P. rutilus*, winter dormancy is possibly broken by winter chilling and then a rise in spring water temperature and increase in photoperiod, as in other macrophytes with overwintering turions (Sibasaka & Oda 1979, Winston & Gorman 1979).

The germination of *P. rutilus* turions appeared to take place when spring water temperatures doubled, increasing from a low 6 °C to 13 °C, which resulted in a greatly increasing cumulative turion germination, Figs 4.9 & 4.10. The changes in photoperiod that took place when *P. rutilus* turion germination occurred, began in early March when average day length increased above 11 + hours, with germination being completed by late April when average day length was 14 + hours, Fig 4.8.

4.5 CONCLUSIONS

The two years of growth experiments revealed that *P. rutilus* plant vegetative growth was not adversely affected by different phosphate nutrient levels. The reduced light levels appeared to increase significantly stem elongation but these differences were not significant in terms of plant growth. However, different phosphate and light levels

appear to effect *P. rutilus* growth and development in the turion development phase of the plant's life cycle.

The fecundity of the plant appeared to be determined by the effects of light and phosphate on turion size development, which in turn was a good measure of plant fitness, as it partly determined turion germination rate and germination success. Turion development conditions were optimum for eutrophic conditions under half light, revealing that reduced light levels were sufficient for optimum *P. rutilus* reproductive growth, under appropriate nutrient conditions. This maximum *P. rutilus* growth under reduced light is typical of most submerged aquatic macrophytes, categorized as shade plants, as leaf photosynthesis is saturated at an irradiance of less than half full sunlight below the water surface (Bowes 1987, Bowes *et al.* 1977, Van *et al.* 1976).

The reduced light and eutrophic trophic preferences for optimum turion reproductive growth suggests that the Scandinavian eutrophic conditions that *P. rutilus* inhabits are ideal for reproductive growth, where higher eutrophic nutrient levels would reduce aquatic light levels in conjunction with the more northern latitudes. In contrast *P. rutilus* turion biomass production, under full light regime, was largest under mesotrophic conditions, which possibly explains the success of *P. rutilus* in Scottish mesotrophic lochs, where such higher light mesotrophic conditions would also favour turion reproductive growth.

Chapter 5: Investigating Patterns of Gene Flow and Asexual Versus Sexual Reproduction among Populations of *Potamogeton rutilus*

5.0 Summary

Genetic analyses using Randomly Amplified Polymorphic DNA (RAPDS) were undertaken on seven populations of *Potamogeton rutilus* (six from Scotland and one from Finland). Five RAPD primers produced a total of 54 bands, 18 (33.3 %) of which are polymorphic for the seven sampled populations. Thirtysix different genotypes were found in the total of 96 sampled *P. rutilus* plants, with the most common genotype being found in all sampled populations apart from the one Finnish site. Some identical RAPD genotypes are likely to have arisen via clonal growth. However, in some cases, sexual reproduction amongst similar genotypes could not be excluded as the source of identical genotypes. There was higher genotypic variation in small populations, which may indicate increasing sexual reproduction in more marginal habitats.

An analysis of molecular variance (AMOVA) found genetic variation to be greatest between individuals within beds with less variation occurring between sample beds and between populations. Nei's genetic distances revealed that increasing genetic distance between *P. rutilus* populations significantly correlated with increasing spatial geographic distance when including the distant Finnish *P. rutilus* population. There was also a trend of increasing genetic distance with spatial distance between Scottish *P. rutilus* populations, but this was not significant. However, over smaller spatial scales significant differentiation of populations was detected indicating limited gene flow. This limited gene flow may be the result of sporadic seed production that can greatly reduce the chance inter-population dispersal.

Keywords: analysis of molecular variance, clonal diversity, genotypes, genetic distance, metapopulations, polymorphic bands, *P. rutilus,* spatial structure, RAPDs, waterfowl dispersal.

5.1 INTRODUCTION

Potamogeton rutilus has a very limited distribution within the British Isles being currently known from thirteen different Scottish lochs, with apparent extinctions in several previously recorded sites (Preston 1997, Wallace 2002 unpublished data). The reasons for this rare plant's limited distribution could be several fold, but one important factor maybe the lack of long distance dispersal ability, through limited seed set and a reliance on asexual clonal reproduction, a factor that appears to be limiting the distribution of other clonally reproducing rare Scottish plants (Henderson 2001, Wilcock 2002).

Seed production can facilitate long distance dispersal by via vectors, such as waterfowl feeding on aquatic plants (Agami & Waisel 1986, Cavino-Canella 2002, Jordi & Green 2002, Jordi *et al.* 2002, Proctor 1968). In comparison, clonally reproducing plants have limited methods of long distance dispersal from the parent plants (Grace 1993, Wilcock 2002). Secondly, populations of clonally reproducing plants are found to suffer from reduced genetic variation (Wilcock 2002, Hutchinson 1975), although this is not always the case (Torimaru *et al.* 2003). Reduced genetic variation in clonally reproducing plants can result in inbreeding depression and reduced plant fecundity, further reducing seed producing ability (Aigner 2004). The reduced ability of clonal plant populations to reproduce by seeds can lead to extinction's due to habitat loss and environmental change, as clonal plants have neither the ability to escape unfavourable conditions by long distance seed dispersal or survive in situ as dormant seeds (Wilcock 2002).

In the case of *P. rutilus* there have been no records in the literature of the plant producing seeds in Scotland (Preston 1995), apart from Clark (1943) finding fruiting specimens in Loch Scarie, North Uist. In more recent times, my surveys of Scottish *P. rutilus* lochs revealed seed producing plants in only one loch, Loch Eilein, Tiree (Wallace, 2002 unpublished data). Thus although seed production in the UK is certainly uncommon, it can occur, but its relative importance in the population biology of *P. rutilus* in Britain is unknown.

To clarify the levels of genetic diversity in *P. rutilus* populations and the possible influences of asexual vegetative reproduction and gene flow (extent of population differentiation), genetic variation will be investigated over several ecological spatial scales as follows:

1. mesoscale (genetic variation within small discrete patches of plants)

2. macroscale (genetic variation between spatially separate plant patches within a loch)

3. geographic scale (genetic variation between different plant populations from different loch sites over a north-south and east-west latitudinal scale).

The mesoscale and macroscale genetic investigations aim to reveal how much clonal diversity exists within plant populations of each loch and give an indication of the degree of sexual or asexual reproduction involved in maintaining these populations.
The geographic investigation of *P. rutilus* genetic diversity covers a range of spatial distances, from short distances between adjacent lochs to much greater distances, between Scottish islands/mainland and with Finland. The geographic scale investigation will give a measure of population differentiation (a surrogate of quantification of the movement of genes among populations) which can be correlated to the dispersal ability of a population (Bohonak 1999), whilst also possibly indicating sources of colonisation and their directional pathways(Abbott & Brochmann 2003).

It is usually the case that increasing geographical distance from a colonising source will lead to increasing genetic isolation of a population (Bockelmann *et al.* 2003). However, recent evidence suggests that geographic distance is not always related to the genetic distance between populations (Hollingsworth *et al.* 1996, Mader *et al.* 1998) and that the long distance dispersal by migratory waterbirds may negate genetic isolation brought about by geographic distance (Figuerola & Green 2002, Freeland *et al.* 2000). In the case of *P. rutilus* populations, if genetic distance is not related to geographic

distance, it would support the case for migratory bird propagule dispersal in this plant species.

5.2 MATERIALS AND METHODS

5.2.1 Study Sites

This study focused on four main regions (map Fig 5.1)

1. The most northern sites in Scotland are two lochs in Shetland, Bardister and Kirkigarth.

2. The two mainland lochs, Ussie and Bayfield, are the most eastern Scottish sites

3. Loch Eilein and Loch a Chlair, two inner Hebridean sites, are the most western loch sites included in this study.

4. One Finnish loch site was also included in the study to compare for genetic differences between Finnish and Scottish *P. rutilus* populations.

Figure 5.1 The distribution of the *P. rutilus* genetic analysis sample sites

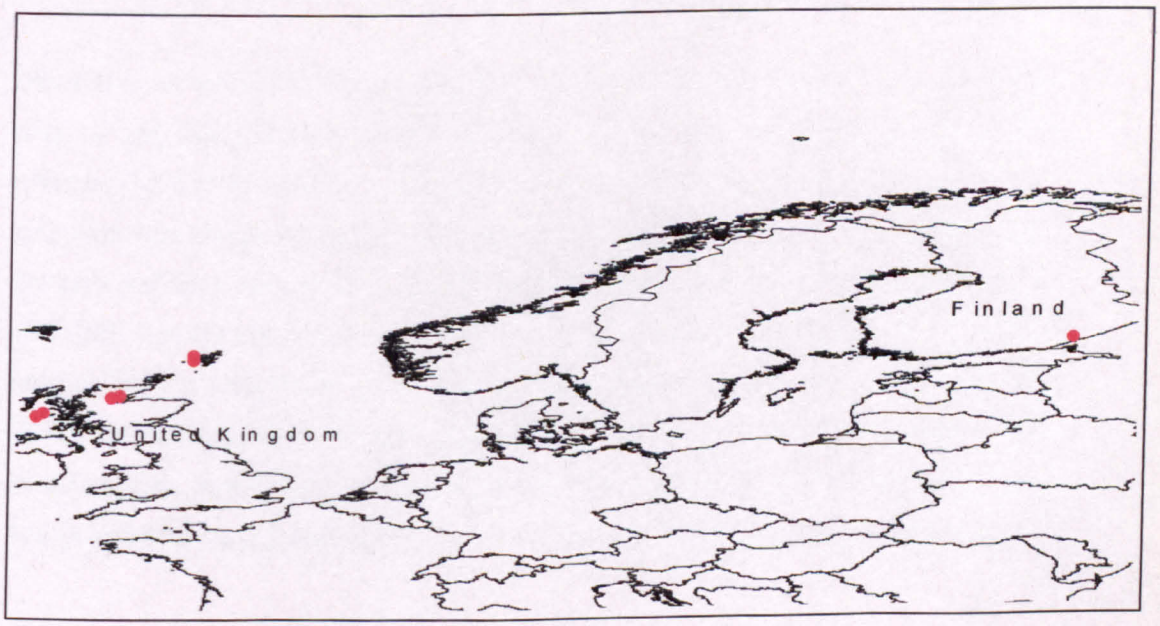


Table 5.1 Loch sites which *P. rutilus* was collected from RAPD genetic analysis.

Loch site	Grid reference	Loch area (ha)	Plant Abundance
Scottish Mainland			
Loch Ussie	NH 505570	84	LA
Loch Bayfield	NH 821718	9	F
Inner Hebrides			
Loch Eilein	NL 985436	12	LF
Loch a Chlair	NL 983445	5.4	LO/R
Shetland			
Loch Bardister	HU 237502	9.2	F
Loch Kirkigarth	HU 238497	7.4	O
Finland			
Lake Simpele (Siikalahti)	27 °E: 6830:636	147	LF

DAFOR scale: D=dominant, A=abundant, F= frequent, O=occasional, R=rare (L = Locally)

5.2.2 Field Sampling Methods

For each loch a hierarchical nested plant-sampling regime was used over several scales, within distinct patches of *P. rutilus* and at macroscale, three spatially separate patches situated around each loch.

Depending on the area of a plant bed, leaf samples were removed from the centre to the edge of each bed, with more samples taken for larger beds to ensure that sample size reflected the area of each bed. Minimum distances of 1 m between sample points were used to ensure ranges of spatial scales were covered to detect clonal growth and genetic diversity within a sample bed. Individual plants were sampled at each sample point, with leaf samples approximating to 1cm x 1cm equivalent leaf tissue. Leaf samples were collected in duplicate, for backup testing, and preserved in vials of concentrated CTAB (hexadecyltrimethylammonium bromide) until DNA extraction. Environmental measurements, water depth, and light regime were also recorded for each sample site. The position of each sampled bed were recorded by Global Positioning System (GPS)

for analysing spatial distances between plant sample beds on a Geographical Information System (GIS), 1.10 000 ordnance survey mapping system. Plate 5.1 shows a sample bed of *P. rutilus* in Loch Eilein, Tiree, Inner Hebrides.

Plate 5.1 *P. rutilus* sample bed situated in Loch Eilein, Tiree, Inner Hebrides

5.3 LABORATORY METHODS

5.3.1 DNA Extraction

Total genomic DNA was extracted from ground up leaf material using hexadecyltrimethylammonium bromide (CTAB) extraction buffer method (Doyle & Doyle 1990). Approximately 1cm ^{2} of *P. rutilus* leaf material was removed from each CTAB preserved leaf sample and washed in distilled water to remove CTAB and any possible contaminants such as algae, and then shock frozen in liquid nitrogen (-197 $^\circ$C). The frozen leaf material was then macerated with a small amount of sand using a plastic pestle and then had 500 µL of pre-heated CTAB extraction buffer (2 % CTAB, 20mM EDTA, 100 mM Tris-HCI pH 8.0, 1.4M NaCl, + 0.2 % mercaptoethanol) added and

mixed to a green homogenate. Another 500 μL of CTAB extraction buffer and a pinch of polyvinylpyrolidone (PVP) were added and the sample was incubated at 65 °C in a heated block for 30 minutes and then cooled to ambient temperature before being mixed with 500 μL of chloroform isoamyl alcohol (24:1). The resulting solution was mixed for at least 10 minutes using an orbital shaker and then centrifuged for 10 minutes at 13,000 rpm to produce a supernatant phase containing DNA.

The supernatant was removed and subjected to a second chloroform: isoamyl alcohol extraction. The supernatant was placed in a clean eppendorf and mixed with freezer cold isopropanol to precipitate DNA, which was pelleted by centrifuging for 10 minutes at 13,000 rpm. On removing the supernatant from the pelleted DNA, 1mL of wash buffer was added to the DNA, which was vigorously agitated to release the pellet from bottom of tube. After leaving the DNA pellet sample for at least 30 minutes it was centrifuged for 5 minutes at 13,000 rpm before removing the supernatant and vacuum drying. The resulting DNA pellet was then resuspended in 75 μl of TE buffer (10 mM Tris-HCI, 0.1mM EDTA, pH 7.5).

5.3.2 DNA Quality Check

Agarose gel electrophoresis was carried out on the extracted plant DNA to first check for the quantity and quality of DNA extract. On establishing sufficient quality and quantity of DNA extract, DNA samples for each population were amplified using the RAPD PCR technique.

5.3.3 Screening of Primers

A total of 34 primers were tested for producing clear and repeatable polymorphic bands, from which five of the best primers were chosen for the analysis. The DNA primer samples were run with negative controls (samples without plant DNA), in every PCR run to ensure accurate and consistent genotyping. Duplicating or some cases triplicating PCR runs assessed reproducibility. Some DNA re-extractions were also undertaken to check for repeatability of results with the chosen primers and to reveal any anomalies with original extractions. The chosen five primers A10, P9, P10, P14 and P17 produced

a total of 54 repeatable polymorphic bands. See Table 5.2 below for the list of tested primers and those chosen for the RAPD work.

Table 5.2 List of primers tested for the RAPD *P. rutilus* plant population analysis.

Tested Operon Primer	Sequence of selected primers	Repeatable Bands	Selected Primers
A4		4	
A5		6	
A6		1	
A7		5	
A10	OPA-10 5'-GTGATCGCAG-3'	8	√
A14		0	
C6		4	
F2		4	
F3		5	
F6		3	
F10		7	
F12		4	
F14		3	
F15		3	
F16		2	
F18		2	
F19		1	
G5		6	
G6		4	
G12		1	
P2		4	
P4		7	

Table 5.2 continued.

Tested Operon Primer	Sequence of selected primers	Repeatable Bands	Selected Primers
P6		1	
P7		5	
P9	OPP-9 5'-GTGGTCCGCA-3'	16	√
P10	OPP-10 5'-TCCCGCCTAC-3'	11	√
P11		7	
P12		3	
P13		4	
P14	OPP-14 5'-CCAGCCGAAC-3'	10	√
P16		7	
P17	OPP-17 5'-TGACCCGCCT-3'	9	√
P18		3	
P20		0	

5.3.4 Random Amplified Polymorphic DNA (RAPD) Analysis

The 5 selected primers were tested on extracted plant DNA using Random Amplified Polymorphic DNA (RAPD) technique (Hadrys *et al.* 1992, Newbury & Ford-Lloyd 1993). For RAPD, 25 µL PCR reaction contains: 14.8 µL H_2O, 2.5 µL dNTPs, 2.5 µL Bioline Buffer, 1.25 µL $MgCl_2$, 0.5 µL formamide, 0.2 µl Bio *Taq* (*Thermus aquaticus*) Bioline), 1.25 µL primer and 2 µL extracted plant DNA. The PCR RAPD reactions were carried out in a GeneAmp PCR system 9700 DNA thermocycler (Perkin-Elmer). The programme began with an initial denaturation at 94° C for 4 min, followed by 35 cycles of 1 min at 94° C, 1 min at 36° C and 2 min at 72 ° C, and ended with 4 min at 72 ° C.

5.3.5 RAPD Agarose Gel Electrophoresis

The entire volume of RAPD product were run alongside a HyperLadder 1 molecular weight marker (Bioline) and run on 2% 1 X TBE (Tris-borate-EDTA) agarose gels with added ethidium bromide to reveal the DNA banding in the gel when observed under UV light.

5.3.6 RAPD Marker Band Scoring

Bands were first checked for scoring by placing the electrophoresis gels in a UV light box to confirm first that distinct clear bands were present. The gel was then transferred to the gel analysis computer to produce positive and negative photographic print outs of the gel band patterns. These photographic band patterns for each primer were scored for each primer by aligning banding patterns for all plant populations, and individual bands scored as present (1) or absent (0).

Only bands of high intensity, which were reproducibly scorable, were considered. The RAPD marker bands were then scored if they displayed a clear polymorphism (i.e. presence or absence) with no faint amplification (Andrea & Stocklin 2004). If there was a doubt about the repeatability of the band, the PCR reaction was repeated. If there was still any doubt about the banding scoring, plant DNA was re-extracted and re-amplified. Inconsistent bands were excluded from the analysis. Of the total of 105 plant individuals tested, 96 of these, from a total of seven loch populations (six from Scotland and one from Finland) were scored for polymorphic bands for the chosen five primers.

5.4 DATA ANALYSIS

5.4.1 Clonal diversity

The RAPD bands, were used to form a matrix of all the individual plant samples. Identity of all RAPD band positions was considered consistent with two samples being of the same genotype (= clone, = genet). However, identical genotypes can potentially

arise via sexual reproduction among similar genotypes. To test the likelihood of identical genotypes being sexually derived, the simulation software MLGsim was employed (Stenberg *et al.* 2003). This MLGsim method uses a simulation approach, to calculate the significance values of the likelihood that a multilocus genotype observed more than once in a population is the result of sexual reproduction.

5.4.2 Population Genetic Structure

Genetic variation within each population was evaluated by the percentage of loci polymorphic (P) at the 5 % level and average heterozygosity, (He = gene diversity) calculated using TFPGA version 1.3. This program was also used for calculating the allele frequency of the 54 band loci and an estimate of population differentiation (F_{ST}). An analysis of molecular variance (AMOVA) was carried out using, Arlequin, Version 1.1; to compare genetic variation between *P. rutilus* loch populations, compared with variation between sample beds within lochs, and variation between individuals within a bed.

5.4.3 Genetic Structure and Spatial Distance

Genetic distances between the seven different *P. rutilus* loch populations were measured using the Nei's (1978) genetic distance statistic in TFPGA programme, Version 1.3, software. The spatial distribution of the 96 *P. rutilus* samples were plotted an ARC View Geographical Information System (GIS), using plant sample GPS positions. The spatial distance between each of the sampled genets within a *P. rutilus* loch population were measured in metres using GIS measuring software.

5. 5 RESULTS

5.5.1 RAPD Clonal Genotypes

The 7 populations of *Potamogeton rutilus* produced 36 different genotypes based on 18 polymorphic bands from the 96 sampled individual plants, with eleven of these genotypes being potentially clonal (e.g. found in more than one plant), see Table 5.3.

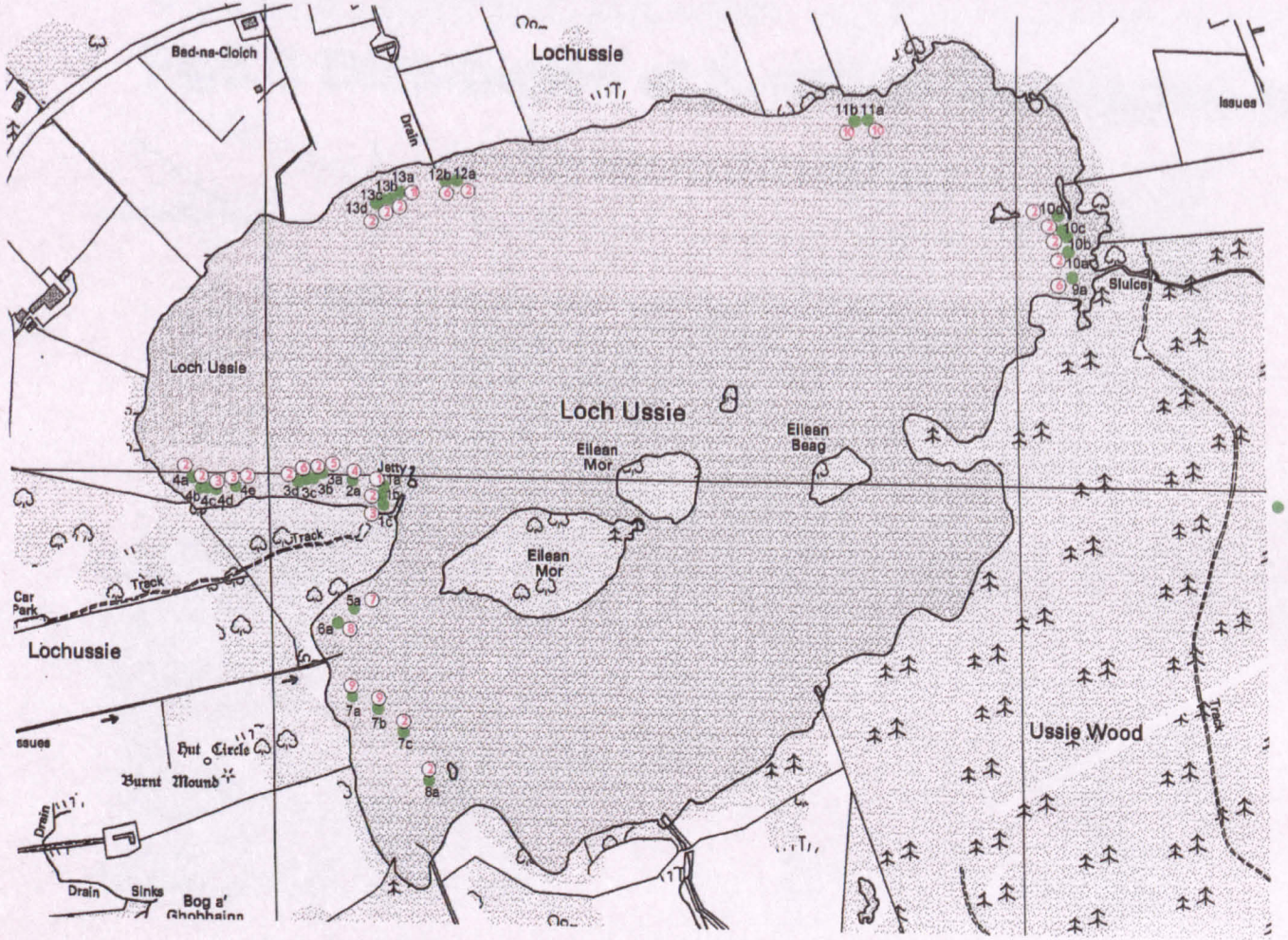
Genotype 2 was the most widespread, found in all the Scottish populations but not in the Finnish plant population. Genotype 2 represents 40 of the total 96 individual *P. rutilus* plants, which are most common in the mainland lochs, Ussie, Bayfield, and island lochs, Eilein and Bardister. In the other two Scottish Lochs, the common genotype 2 only occurred once in a Chlair and Kirkigarth sampled populations. Only one genotype 30 was found both in Scottish and Finnish populations. Twenty-five of the total 36 genotypes were found in only one sample (Table 5.3). The maps Figs 2 to 7 show the spatial distribution of the sampled plant genotypes for the five *P. rutilus* loch populations.

Table 5.3 *P. rutilus* genotypes for the six Scottish and one Finnish loch populations.

		Genotypes																																						
Pop No	**Pop.Name**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**Pop Total**
1	Ussie	1	16	3	1	1	3	1	1	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32
2	Bayfield	0	7	0	0	0	3	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14
3	Eilein	0	10	0	0	0	0	0	0	0	0	0	0	0	0	4	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18
4	A Chlair	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	8
5	Bardister	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	8
6	Kirkigarth	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1	1	1	0	0	0	9
7	Simpele	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	3	1	1	7
Total Genotypes		**1**	**40**	**3**	**1**	**1**	**6**	**1**	**1**	**2**	**3**	**1**	**1**	**1**	**1**	**4**	**2**	**1**	**1**	**1**	**2**	**1**	**1**	**1**	**1**	**3**	**1**	**1**	**1**	**1**	**3**	**1**	**1**	**1**	**3**	**1**	**1**	**96**

The maps Figs 5.2 to 5.7 show the spatial distribution of plant genotypes for the six Scottish *P. rutilus* loch populations.

Fig 5.2 Distribution of P. rutilus Genotypes in Loch Ussie

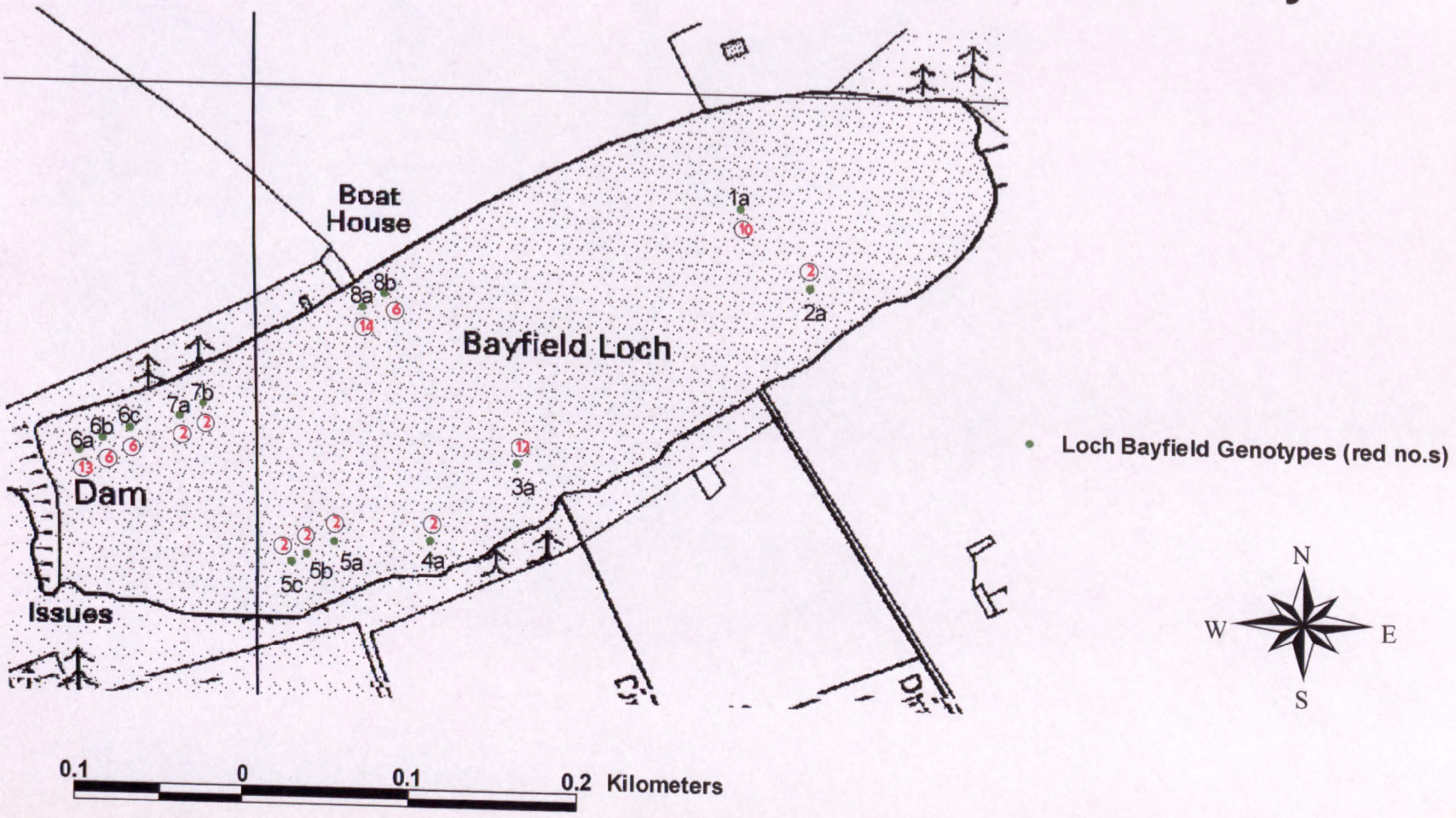


0.4 0 0.4 0.8 Kilometers

Loch Ussie Genotypes (red no.s)

N
W E
S

Fig5.3 Distribution of P. rutilus Genotypes in Loch Bayfield



Fi g5.4 Distribution of P. rutilus Genotypes in Loch an Eilein

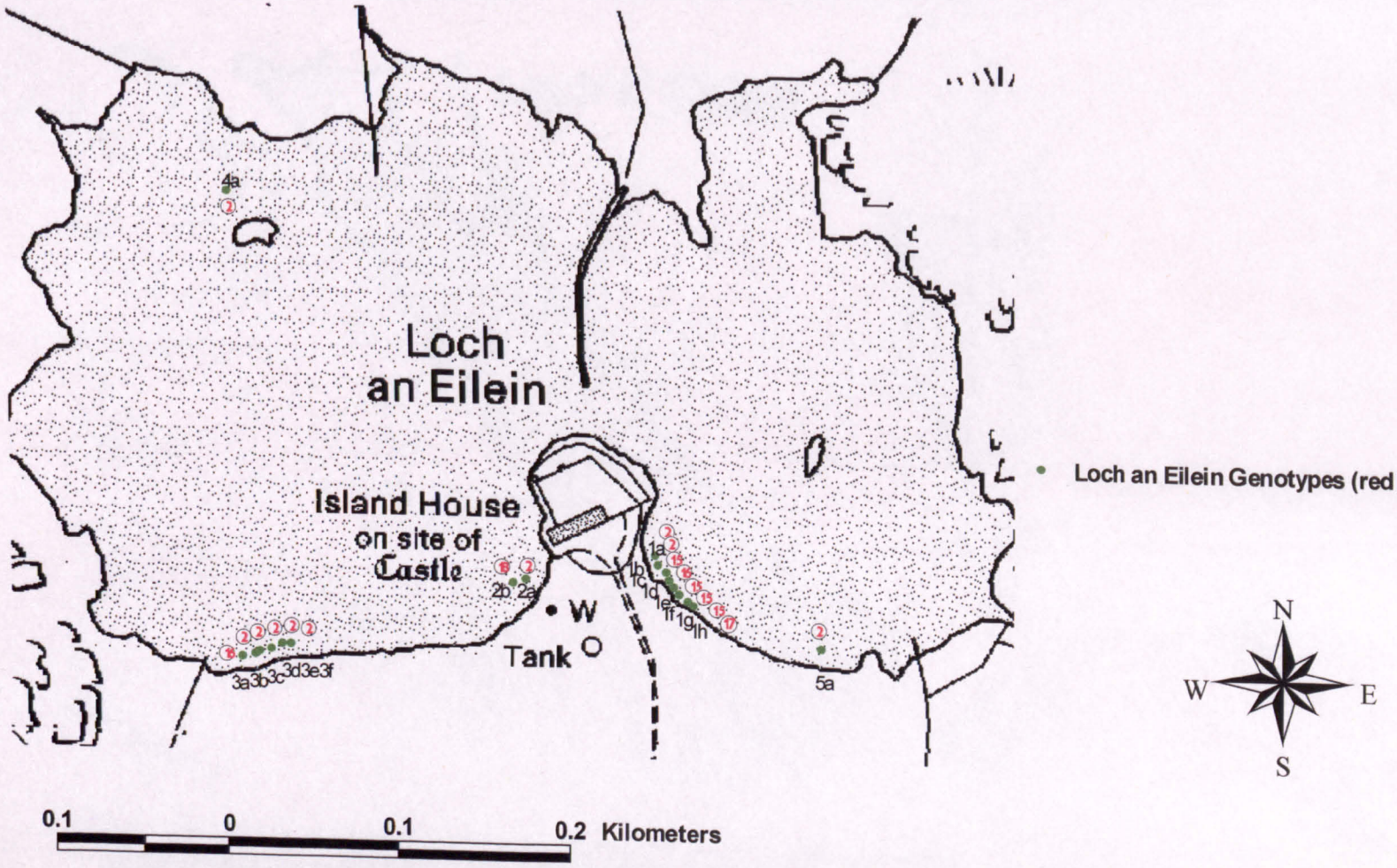


Fig5.5 Distribution of P. rutilus Genotypes in Loch a Chlair

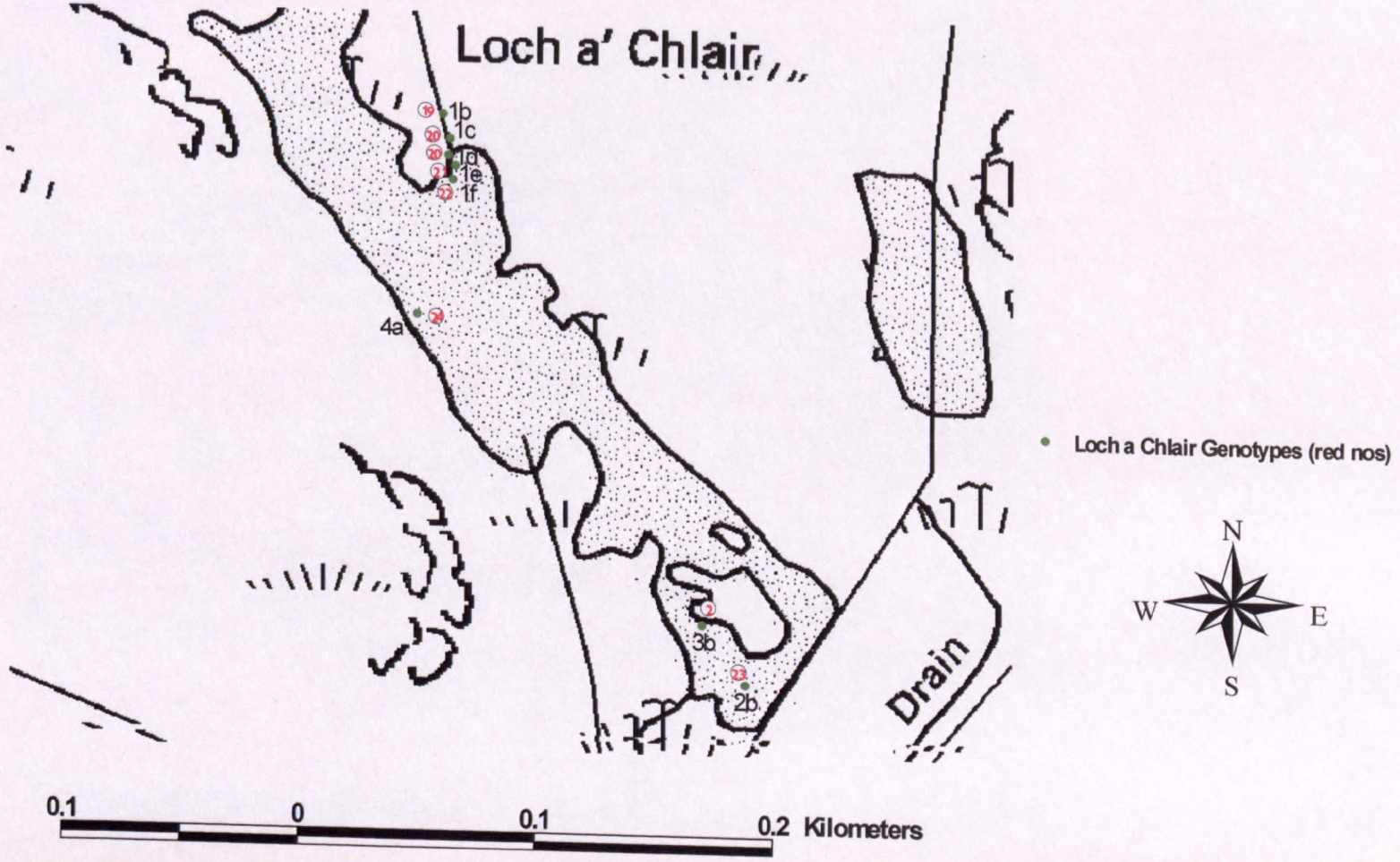


Fig5.6 Distribution of P. rutilus Genotypes in Loch Bardister

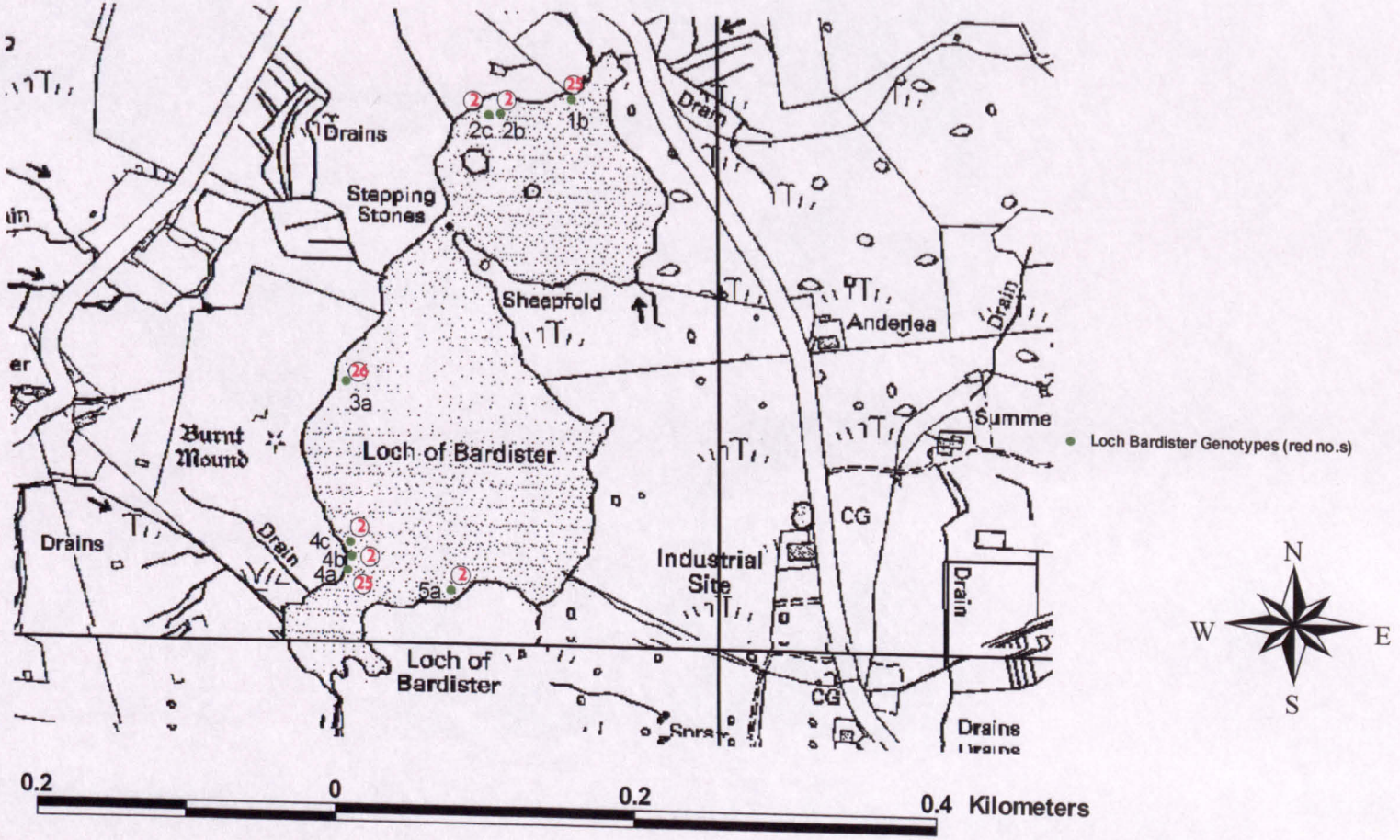
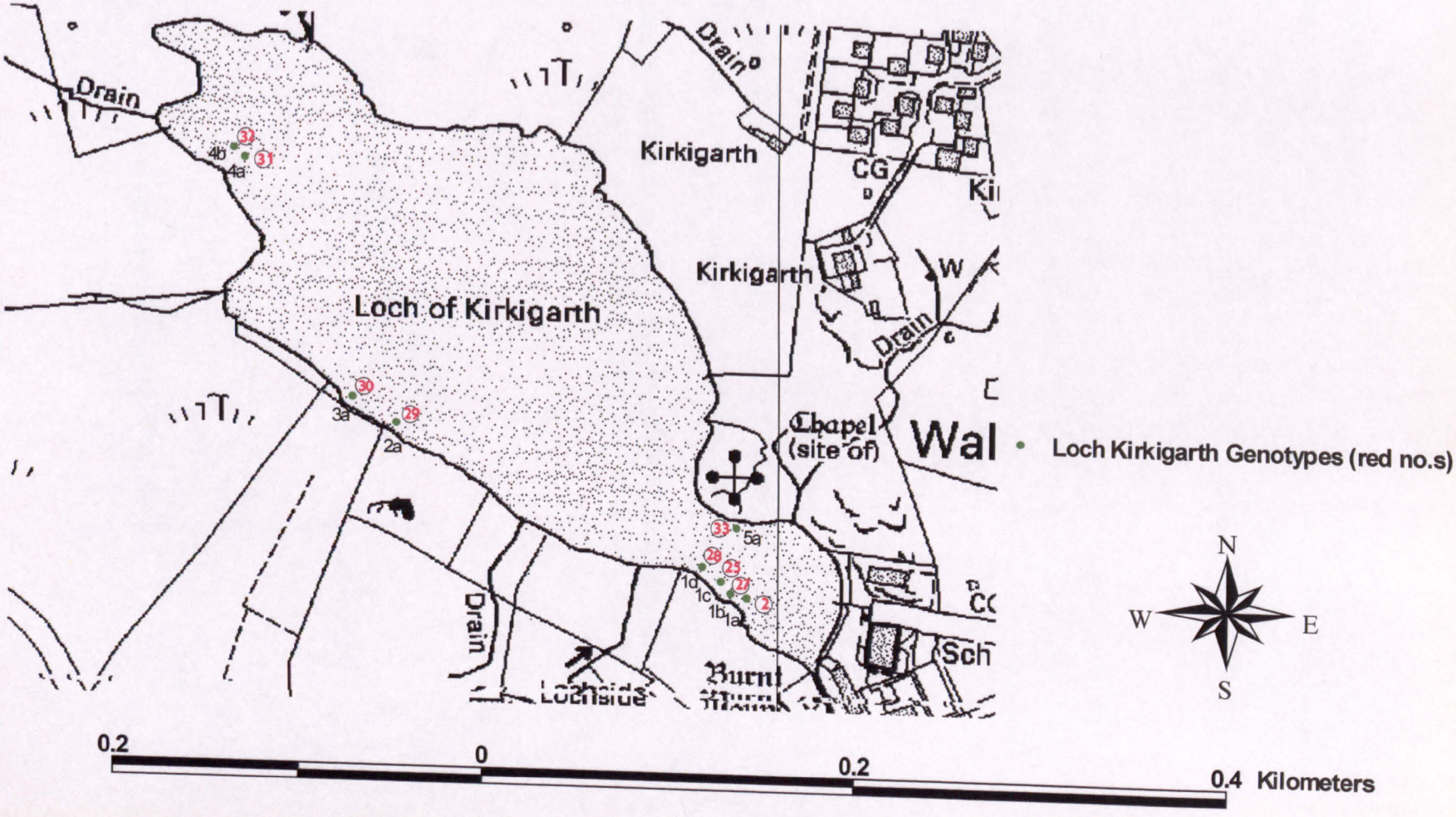


Fig5.7 Distribution of P. rutilus Genotypes in Loch Kirkigarth

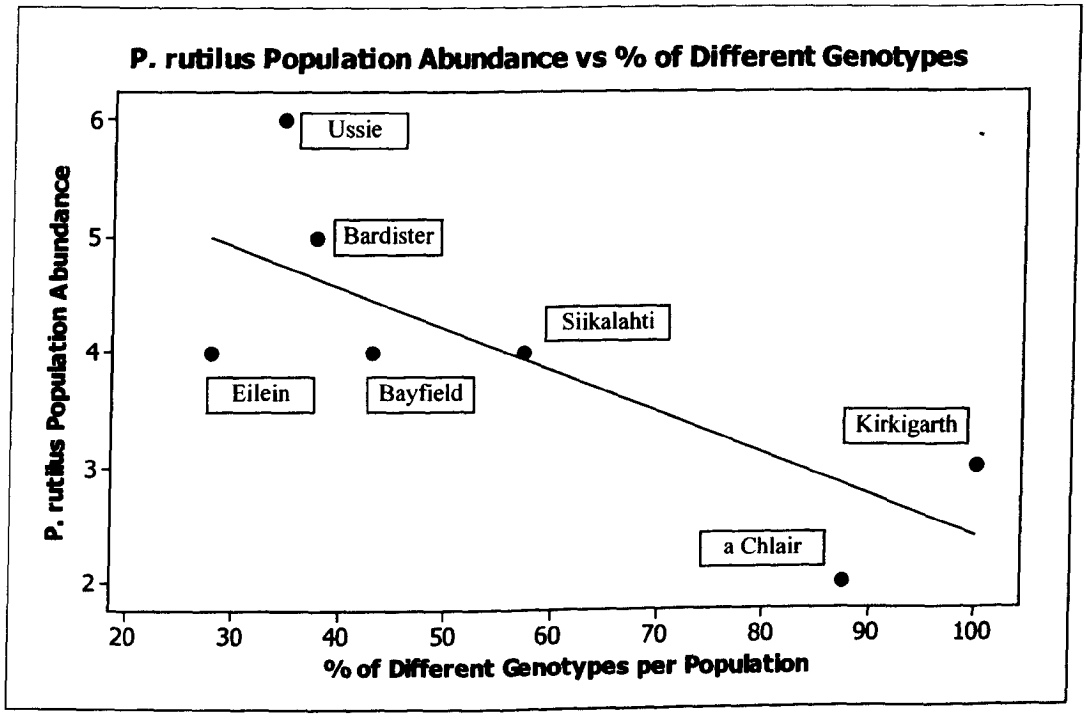


5.5.2 RAPD Clonal Genotypes

The genotype distribution maps show clustering of genotypes (red circles), such as genotypes 3, 9 and 10 in Loch Ussie (Fig 5.2 Map), and genotype 15 in Loch an Eilein (Fig 5.4 Map). However, it can be seen that some shared genotypes, such as genotype 2, are well dispersed and intermingled with other genotypes.

The genotypic diversity results indicate there is a significant trend of increasing *P. rutilus* diversity with decreasing *P. rutilus* population size for sampled sites, see Figure 5.8. Large *P. rutilus* populations such as Loch Ussie and Loch Bardister, have much lower genotypic variation compared to the smaller populations of Loch a Chlair and Kirkigarth.

Figure 5.8 *P. rutilus* population size versus % of different genotypes (genotypic diversity) for each sampled loch population. Pearson correlation = -0.778, P-Value < 0.039. *P. rutilus* numerical y-axis values derived from DAFOR abundance scores



A binomial simulation program (MLGsim) can be used to detect the likelihood that identical multilocus genotypes (MLGs) in a population could be the result of sexual reproduction, Psex value being greater than critical *p* value (Stenberg *et al.* 2003). For example, the Loch Ussie *P. rutilus* genotype 2, of which there are 16 of this genotype (Table 5.4), was analysed with MLGsim and found that the critical *p* value = 0.026 was greater than Psex value = 0.023, so there is a significant likelihood that the identical genotype 2 in the Loch Ussie *P. rutilus* population is not being produced by sexual reproduction. MLGsim results indicate that there is a significant likelihood of identical *P. rutilus* genotypes in Loch Ussie and Loch Bardister not being produced by sexual reproduction, see Table 5.4 .

Table 5.4 MLGSim analysis for detecting the likelihood of identical *P. rutilus* genotypes being sexually derived

Loch Site	Genotype code	Number of Genotype	Psex:Critical *p*[1]
Ussie	2	16	0.023 < 0.026*
Bayfield	2	7	0.326 > 0.026
Eilein	2	10	0.341 > 0.047
a Chlair	20	2	0.032 > 0.001
Bardister	2	5	0.039 < 0.056 *
Kirkigarth	-	All *different*	-
Siikalahti	34	3	0.039 > 0.007

[1]Psex value is the probability of the genotype occurring in the given number of samples due to sexual reproduction, based on the allele frequencies of RAPD markers in that population and the total number of individuals sampled from the site. The critical *p* value is derived from simulations, and is the threshold below which Psex values are considered significant at the 5 % level. * = Psex values that are statistically significant (e.g. the given number of samples sharing the named genotypes are unlikely to have

arisen due to sexual reproduction). In cases where the Psex value is greater than the critical *p* value, sexual reproduction among similar genotypes cannot be excluded as the source of identical genotypes.

5.5.3 Gene Diversity and Polymorphism

The 5 RAPD primers produced a total of 54 bands, 18 (33.3 %) of which are polymorphic for the total of 36 genotypes found in the sampled *P. rutilus* populations, see Table 5.5.

Table 5.5 Number of *P. rutilus* genotypes for the different sampled plant populations

Loch site	**Number of plants sampled**	**% bands polymorphic**	**Gene diversity (He)**	**Number of population genotypes**	**% of different genotypes for each population**
Scottish Mainland					
Loch Ussie	32	18.5	0.018	11	34.4
Loch Bayfield	14	9.3	0.014	6	42.9
Inner Hebrides					
Loch Eilein	18	7.4	0.011	5	27.8
Loch a Chlair	8	14.8	0.06	7	87.5
Shetland					
Loch Bardister	8	3.7	0.009	3	37.5
Loch Kirkigarth	9	16.7	0.055	9	100
Finland					
Siikalahti, L.Simpele	7	13	0.026	4	57.1
Entire populations	96	33.3	0.028	36	37.5

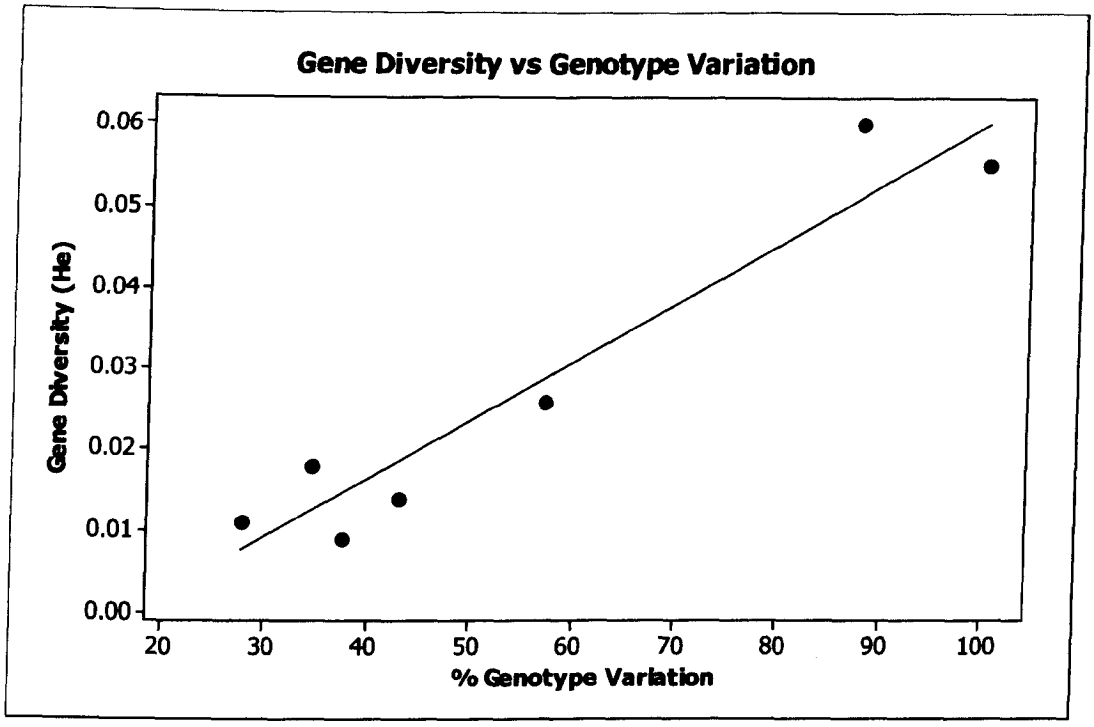


Figure 5.9 The relationship between gene diversity and genotype variation, Pearson correlation $r = 0.96$, $P < 0.001$.

Figure 5.9 shows a significant trend of increasing gene diversity with increasing genotype variation (% of different genotypes in a sampled population, see Table 5.5). *P. rutilus* populations with a high gene diversity, such as Loch Kirkigarth and a Chlair, have also a high genotype variation. Conversely, *P. rutilus* populations with lower gene diversity such as Loch Ussie and Bardister show lower genotype variation, see Table 5.5.

5.5.4 Genetic Structure

According to the AMOVA test, most of the variance was found between individuals within sample beds, 53.46 %, with less than half this variation, 20.68%, between beds within lochs and a slightly greater variation, 25.86%, between loch populations. The significance tests of variation, for 1000 permutations, showed the variations were highly

significant, see Table 5.6. The global estimate of population differentiation from TFPGA was $F_{ST} = 0.247$ ($p < 0.05$).

Table 5.6 Analysis of molecular variation (AMOVA) for seven *P. rutilus* loch populations. $P < 0.001$, significance levels are based on 1000 permutations.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	F Statistics	P-value
Between Lochs	6	33.644	0.32576 Va	25.86	CT= 0.259	<0.001
Between population beds within lochs	40	46.647	0.26048 Vb	20.68	SC= 0.279	<0.001
Between Individuals within beds	49	33.000	0.67347 Vc	53.46	ST= 0.465	<0.001
Total	95	113.292	1.25971			

5.5.5 Genetic Structure and Spatial Distance

Nei's (1978) genetic distance analysis reveals that Loch Ussie and Bayfield, the two mainland sites are most genetically similar, with Loch an Eilein, Tiree, and Bardister, Shetland showing some genetic similarity to the two mainland sites. The two remaining Scottish *P. rutilus* loch, Loch Kirkigarth, Shetland and a Chlair, Tiree showed a much greater genetic distance from the other Scottish loch sites. The Finnish site, Lake Siikalahti, exhibits the largest genetic distance, being much greater than Scottish *P. rutilus* genetic distances, see Figure 5.10.

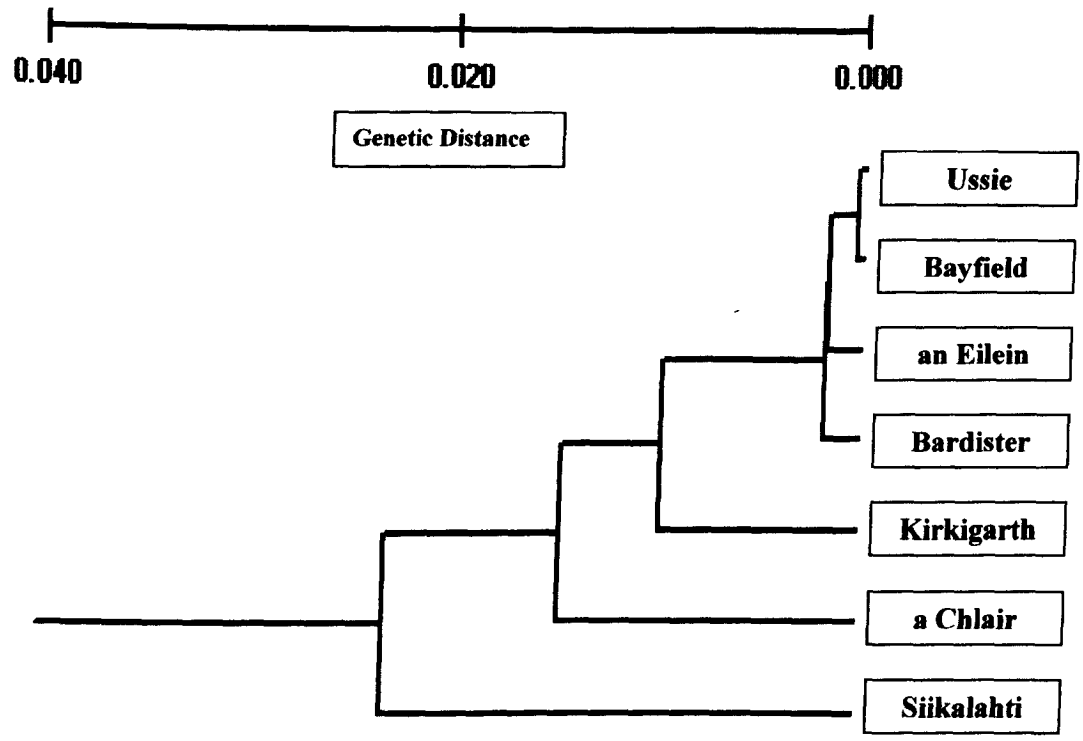


Figure 5.10 Genetic distance between six Scottish loch populations and one Finnish (Siikalahti) lake population of *P. rutilus*

The spatial and genetic distances for the seven sampled *P. rutilus* sites are compared below in Table 5.7. The genetic/spatial distances in graph Figure 5.11, indicates there is a trend of increasing genetic distance with increasing spatial distance between *P. rutilus* loch populations, although this relationship is less clear among UK populations.

Table 5.7. Genetic and Spatial distances for Scottish and Finnish loch populations of *P. rutilus* (Loch Ussie being the most southerly located sample site was chosen as the starting point distance of zero for comparing spatial distances with more northerly located sample sites)

Populations Compared	Genetic Distance	Spatial Distance (Km)
Ussie vs. Bayfield	0.0003	34
Ussie vs. Eilein	0.0013	184
Ussie vs. a Chlair	0.0144	184.25
Ussie vs. Bardister	0.0018	343
Ussie vs. Kirkigarth	0.0089	342.6
Ussie vs. Siikalahti	0.0221	1902.22

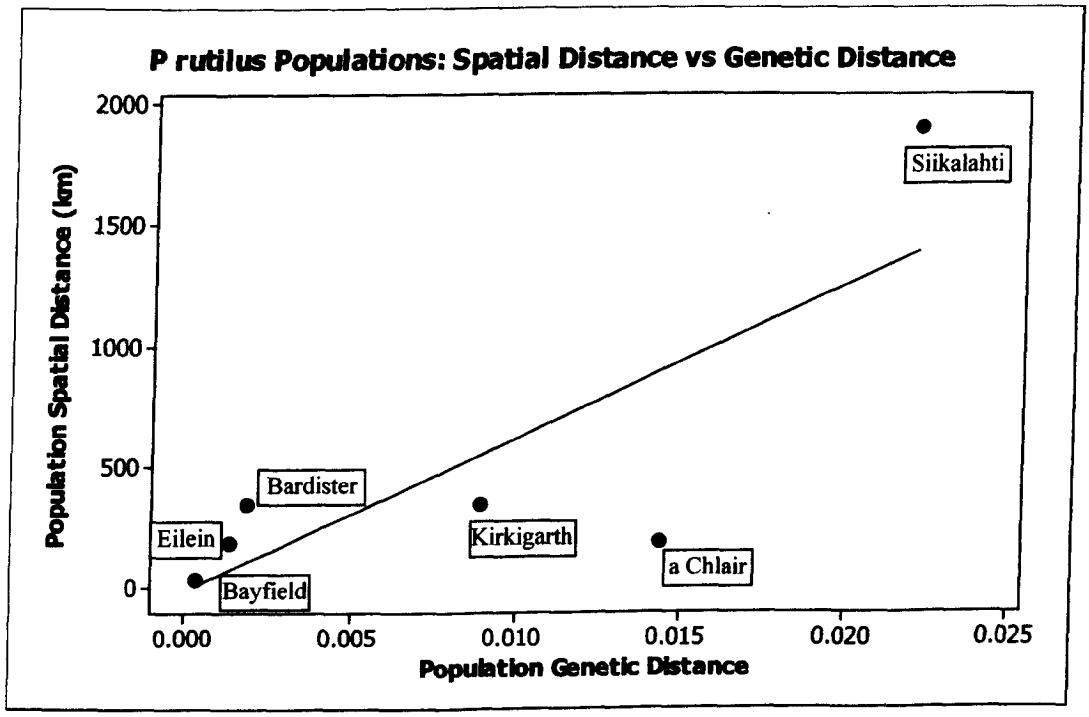


Figure 5.11 Genetic distance versus spatial distance for *P. rutilus* populations compared with Loch Ussie as zero distance, Pearson correlation $r = 0.796$, $P < 0.058$ (if skewed Loch a Chlair value is removed) $r = 0.956$, $P < 0.011$

5.6 DISCUSSION

RAPD analysis of populations has detected genetic and genotypic variation within and among populations of *P. rutilus*. However, only a limited number of polymorphic markers were detected (18) in the total dataset, which to some extent reduces the biological inferences that can be made. Nevertheless, while interpretations of the data are made with the proviso in mind, the data offers some insights into the population biology of *P. rutilus*.

5.6.1 Clonal Growth and Genotype Variation

RAPD genetic analysis of the seven different *P. rutilus* populations revealed that many plants shared the same genotype, with genotype 2 being the most prevalent, occurring in 41.7 % of the 96 sampled plants. At the level of individual lochs, the Loch Eilein *P. rutilus* population showed the greatest genotypic similarity between individuals, with only 27.8 % of the samples having unique genotypes, whilst neighbouring Loch a Chlair had very few similar genotypes resulting in a high level of genotypic diversity (87.5 % of individuals had unique genotypes). Likewise, 100 % of the Loch Kirkigarth, Shetland, samples had unique genotypes, compared to 37.5% at the neighbouring Loch Bardister.

There was some clumping in the distribution of similar genotypes, but some were more widespread. The aggregated distributions of some similar genotypes may have resulted from clonal growth, but sexual reproduction could not be ruled out in the similar genotypes found in *P. rutilus* populations of Bayfield, Eilein, a Chlair and Siikalahti. As the MLGsim analysis of similar *P. rutilus* population genotypes revealed that only at Loch Ussie and Bardister could sexual reproduction be rejected as a possible source of identical genotypes and hence, genotypic identity be more confidently equated with clonality, Table 5.4. Elsewhere in the dataset, the low level of genetic variation detected means that identical genotypes do not necessarily equate to clonal growth. Thus in summary, the data contain some evidence for sexual reproduction (different genotypes within and between lochs), some evidence for clonality, and some cases where it is not

possible to distinguish clonality and sexual reproduction as the source of identical RAPD genotypes.

In terms of *P. rutilus* population size and clonality, there is a significant trend of decreasing genotype variation with increasing population size. The increase in genotypic variation in smaller populations, such as Loch a Chlair and Loch Kirkigarth, may reflect the decrease in success of asexual reproduction in potentially unfavourable environments, with sexual reproduction being favoured in more stressed environmental circumstances (Sculthorpe 1967). In the case of Loch a Chlair and Loch Bardister the small plant populations are confined to more shallow loch edges habitats, which can suffer more changeable environmental conditions. For example, Loch a Chlair populations of *P. rutilus* are found mostly in shallow marginal areas of the loch that have greater transparency, as many of the deeper areas have reduced light availability due to turbidity. Plants in these more marginal habitats are known to suffer environmental stress, such as periodical drying out which would favour desiccation-resistant propagules over less tolerant vegetative growth (Hollingsworth *et al.* 1996). Shallow habitats may also encourage sexual reproduction through successful *P. rutilus* flowering, as often deeper water populations, growing to depths of 3 m are usually sterile as flowering has to take place above the waters surface (Kotiranta *et al.* 1998).

It has been found that sexual reproduction increases in clonal plants in unfavourable habitats that increase the mortality rate of the clone (Sakai 1995, Van Zandt *et al.* 2003). The larger more clonal *P. rutilus* populations, found in lochs with good growth conditions such as Loch Ussie, can possibly be related to the effective success of clonal asexual reproduction in favorable aquatic environments (Grace 1993).

These results reveal there can be a great variation in different *P. rutilus* loch populations indicating that not only reproduction methods, but also other factors may be operating. Other factors that are known to influence patterns of genetic diversity are number of founding individuals, population size, population age and ecological factors such as habitat niches (Hangelbroek *et al.* 2002, Rossetto *et al.* 2004, Kingston *et al.* 2004).

5.6.2 Genetic Structure and Spatial Distance

The AMOVA analysis of *P. rutilus* genetic structure revealed that the greatest genetic variation, 53. 5 %, occurred between individuals within *P. rutilus* sample beds, whilst less than half of this variation, 20.7 %, was found between beds within lochs. Population variation between lochs, 25.9 %, was just slightly greater than the 25.9 % variation between beds. Similar results, have been found for other *Potamogeton* clonal studies, where genetic variation was greatest between individuals within beds, than between beds (Hangelbroek *et al.* 2002). Hangelbroek *et al.* (2002) suggests that the apparent high genetic variation between individuals within beds could indicate that seedling recruitment in addition to clonal growth might be occurring in *Potamogeton* beds. Even though genetic variation between beds within lochs was less than half that for individuals within beds, it was still significant, indicating a small scale spatial population differentiation for *P. rutilus* beds.

The genetic variation between *P. rutilus* loch populations was also small but significant indicating that genetic variation is occurring at a greater geographic scale. This large geographic scale genetic structure between *P. rutilus* loch populations was clearly revealed by Nei's (1978) genetic distance analysis, with genetic distance increasing with spatial distance. However, when excluding the Finnish site, and just considering Scottish *P. rutilus* populations there would not appear to be a significant relationship between genetic and spatial distance, see Figure 5.11. The Scottish *P. rutilus* mainland sites did show more genetic similarity than further removed Scottish island sites such as Loch a Chlair and Loch Kirkigarth. However, other Scottish *P. rutilus* loch populations, such as the Inner Hebridean sites are genetically grouped with more distant populations, such as those located in mainland and Shetland sites. Finally, the Finnish site with its much greater spatial distance from Scottish sites, had also the greatest genetic distance from Scottish *P. rutilus* populations, see Figure 5.10.

This large-scale spatial geographic genetic structure of *P. rutilus* populations would suggest that gene flow over large distances is restricted by limited propagule gene dispersal (Bocklelmann *et al.* 2003, Bohonak 1999). This limited long distance gene

flow may be partly due to *P. rutilus* reliance on clonal growth and/or its limited seed production. Limited seed production can greatly reduce the chance of long distance seed dispersal by feeding migratory waterfowl, which can enhance gene flow and reduce genetic population isolation brought about by large geographic distances (Hollingsworth *et al.* 1995, Hollingsworth *et al.* 1996, Mader *et al.* 1998). However, in practice migratory bird seed dispersal may not always reduce variation between populations, as other factors such as frequency/direction of flight, genetic constitution of source populations and episodic seed dispersal events, can all lead to enhanced differentiation between populations (Hollingsworth *et al.* 1996).

5.6.3 Colonisations and Extinctions

Some of the above described effects that can enhance genetic differentiation in populations may have brought about the greater genetic differentiation of Loch a Chlair and Loch Kirkigarth compared to their neighbouring loch sites Eilein and Bardister, see Table 5.5. Loch Eilein appears to have been recently recolonised by *P. rutilus* (P. Hollingsworth, personal communication) and so has a relatively young population, compared to upstream Loch a Chlair, a possible turion colonising source for Eilein. The age of a plant population, determined through colonisation and extinction events can promote local genetic differentiation (McCauley 1991, Ibrahim *et al.* 1996). Older populations, such as Loch a Chlair, can be more genetically diverse as founder events usually reduce the genetic diversity of newly established populations (Gaggiotti *et al.* 2004), as may be the case in Loch Eilein.

The large genetic difference between the two adjacent *P. rutilus* Shetland sites, Loch Bardister and Loch Kirkigarth may be also be explained by colonisation and extinction events. Loch Kirkigarth maybe more genetically diverse than Bardister as it is an older population and/or may have had an originally more diverse colonising source. If only Loch Kirkigarth received a diverse colonising seed source, it would be less likely to colonise upstream Loch Bardister as the turion colonisation from Kirkigarth to Bardister would have to take place against the directional flow of the connecting stream.

Directional water flow is known to limit propagule dispersion, gene flow, from moving upstream against the water flow (Gornall *et al.* 1998). The limited seed production of *P. rutilus* and reliance on clonal vegetative turion growth appears not only to limit long distance dispersal, but also seems to limit more local, water mediated, turion dispersal between adjacent stream connected lochs.

5.6.4 Metapopulations

The population dynamics of colonisation and extinctions may partly explain *P. rutilus* clumped regional distributions, with Scottish *P. rutilus* lochs forming four distinct geographic groups, Mainland, Inner Hebrides, Outer Hebrides and Shetland. These regional distributions would partly appear to be the consequence of both limited dispersal and the availability of suitable *P. rutilus* loch habitats within this limited dispersal range. Hanski (1997) states the persistence and dynamics of metapopulations are critically dependent on the amount and regional configuration of suitable habitats. The population dynamics and habitat distribution of *P. rutilus* appears to fit a metapopulation model, which is based on the assumption that suitable habitats occur as discrete patches within a matrix of unsuitable habitat (Freckleton & Watkinson 2002). In the case of Scottish *P. rutilus* the suitable habitats are the oligo-mesotrophic lochs, which the species inhabits, in the matrix of unsuitable terrestrial and aquatic habitats.

The genetic structure of *P. rutilus* populations seems to be partially influenced by spatial distance that is more significant over larger geographic scales. This reflects the isolation by distance theory, where colonies are more likely to be founded by individuals drawn from nearby populations and not by random individuals from the entire metapopulation (Wade & McCauley 1988). *P. rutilus* clonal habit with its limited dispersal may reinforce genetic differentiation, as long distance waterfowl-mediated dispersal between clonally reproducing metapopulations is highly unlikely (King *et al.* 2002). In the case of *Potamogeton* that have a greater seed production such as *P. pectinatus* it has been found that genetic distance is lower for populations visited by

swans than populations not visited, suggesting that waterfowl can promote gene flow among visited plant populations (Mader *et al.* 1998).

5.6.5 Conservation Genetics

The RAPD genetic analysis of *P. rutilus* revealed that despite a high degree of clonality in most of the *P. rutilus* populations most of the genetic variation appeared to be between individuals within beds. This genetic variation in *P. rutilus* offers some chance of adapting to changing environments, as genetic diversity is associated with population viability in changing environments (Pandit & Babu 2003, Aigner 2004).

The *P. rutilus* genetic variation seems to have been partly produced by sexual seed reproduction, and even if occasional seed establishment takes place within a population it can be a powerful mechanism of generating genetic diversity (Soane & Watkinson 1979). However, a high level of diversity among individuals may also be partially maintained through clonal persistence of genets from the founding population (Hangelbroek *et al.* 2002).

Recent studies of rarity in the native British flora reveal that species that produce fewer seeds are more threatened (Henderson 2001, Wilcock 2002, Pilgrim *et al.* 2004). The lack of seed fecundity is known to reduce a species ability to buffer extinction events as seed banks (Kunin & Gaston, 1993) This limited seed production of *P. rutilus* would contribute to the localised meta-population dynamics of colonisations and extinctions. This would partially explain the observed clumped distributions of *P. rutilus* metapopulations that result from the limited short distance dispersal of turions.

5.6.6 Translocation

The genetic data combined with *P. rutilus* distribution and field observations suggests that limited seed production may contribute to the plant's rarity as it reduces the plants ability to colonise new sites, or in some cases recolonise former suitable sites. Conservation of *P. rutilus* would appear to best facilitated by translocation of vegetative

offspring or seeds, if available. Translocation has been successfully used as a method in helping rare clonal plants colonise and establish in suitably chosen habitats (Rossetto *et al.* 2004, Kingston *et al.* 2004). In re-establishing populations of rare clonal plants, one of the main aims is to re-initiate sexual reproduction to produce viable seeds for dispersal and re-colonisations of new areas (Rossetto *et al.* 2004). However, due to the clonal habit of many aquatic plants (Grace 1993), re-establishment of *P. rutilus* populations may not greatly increase seed production but would still help sustain metapopulation dynamics (Frekleton & Watkinson 2002).

Translocations within *P. rutilus* metapopulations would be especially targeted at recolonisation of suitable former sites and also colonisations of new suitable neighbouring sites. Supplementing *P. rutilus* colonising abilities with translocations between loch populations would help stabilize and possibly enhance metapopulation dynamics of a region (Freckleton & Watkinson 2002). *P. rutilus* genetic data combined with trophic classifications of present and former *P. rutilus* sites would help develop a plant conservation strategy, for choosing the most suitable loch sites for translocations (Pandit & Babu 2003). Translocations are discussed in more detail in chapter 6.

5.7 CONCLUSIONS

In conclusion, *P. rutilus* exhibits both asexual clonal and sexual seed reproduction. Genetic variation appeared to be greatest between individuals within sample beds with less variation between beds and populations. There appears to be limited gene flow between *P. rutilus* loch populations, which was indicated by genetic distance increasing between populations at large geographic scales and significant population differentiation over local scales. This limited gene flow may be the result of seed production being only sporadic and so greatly reducing the chance of waterfowl mediated, inter-population seed dispersal. The limited propagule dispersal seems to be contributing to the clustered *P. rutilus* loch distributions and potential metapopulation dynamics of local colonisations and extinctions.

CHAPTER 6: General Discussion and Conservation Implications

6.0 GENERAL DISCUSSION SUMMARY

- Scottish and Finnish *P. rutilus* occur in different trophic plant community types.

- Scottish *P. rutilus* inhabits less nutrient rich oligo-mesotrophic lochs compared to the more nutrient rich, eutrophic lakes, that Finnish *P. rutilus* inhabits.

- The effects of salinity may have caused *P. rutilus* to be lost from some of its former machair lochs in Scotland.

- *P. rutilus* abundance and macrophyte diversity both significantly decline with reduced light availability.

- *P. rutilus* turion size is a good measure of plant fitness and fecundity.

- Eutrophic (high phosphate) conditions, under a half-light regime, produced optimum *P. rutilus* turion growth under experimental conditions.

- Genetic evidence suggests there is limited gene flow between *P. rutilus* populations and this could be due to the lack of, or infrequent, seed production for inter-population seed dispersal.

- Genetic analysis revealed that *P. rutilus* does not totally rely on clonal growth for reproduction as genetic evidence suggested there was some degree of sexual reproduction in the populations.

- The conservation implications of the research were assessed to produce a series of practical management recommendations for the conservation management of *P. rutilus*.

6.1 INTRODUCTION

The plant community TWINSPAN analysis and water chemistry analyses discussed in chapters 2 and 3, revealed the range of aquatic conditions of *P. rutilus* habitats. Plant community data showed that Scottish *P. rutilus* inhabited lochs of a mainly oligo-mesotrophic trophic type. The chemistry data revealed that many of the *P. rutilus* lochs, especially the machair lochs, had a strong coastal influence with high alkalinities. If *P. rutilus*, like some other species of its community type (e.g.Vestergaard & Sand-Jensen 2000, Van den Berg *et al.* 2002), is able to utilise the high availability of bicarbonate for photosynthesis in such alkaline environments, it may partly explain its success in such lakes.

One of the community types, TWINSPAN group 4, dominated by machair former *P. rutilus* lochs, showed a high degree of salinity, which was reflected in a distinctive plant community type. The high levels of salinity in these former *P. rutilus* lochs suggested that the plant may have been lost from these sites due to salinity increase, as *P. rutilus* is known to be vulnerable to high salinity (Kazmierczakowa & Zarzycki 2001).

Plant community and water chemistry analysis data showed significant differences between Scottish and Finnish *P. rutilus* lake habitats. Scottish *P. rutilus* and its community type inhabited less nutrient rich oligo-mesotrophic loch conditions compared to Finnish *P. rutilus* lake groups, which had significantly higher levels of total phosphate and a eutrophic plant community type.

These results show that *P. rutilus* can thrive in a range of trophic conditions, even in more eutrophic conditions, as found in Finnish *P. rutilus* lakes. However, in Scotland there is evidence that *P. rutilus* has been lost from some of its sites due to eutrophication (Preston 1997). The environmental and plant data revealed that this may be due to reduced availability of light in eutrophic conditions, as reduced light seems to be significantly related to the reduced abundance of both *P. rutilus* and macrophyte diversity. However, as mentioned in previous chapters, the trophic tolerances between

Scottish and Finnish *P. rutilus* populations may partly be the result of ecotypic habitat specialisation. Possible ecotypic population differences will be discussed further in section 6.3.

The limiting effects of reduced light on *P. rutilus* growth and reproduction were revealed by the growth experiments, with the lowest light levels producing the greatest reduction in successful turion germination. Turion size seemed to be a good measure of plant fitness and a good predictor of turion germination rate, with germination rates increasing with turion size. Development conditions for *P. rutilus* turions were optimum in eutrophic conditions under half-natural light, whilst under full light conditions the greatest mean turion size was achieved under mesotrophic phosphate conditions. These preferred eutrophic and mesotrophic experimental growth conditions, tended to reflect the range of habitat conditions that *P. rutilus* successfully grows in, from mesotrophic Scottish loch types to the more eutrophic Finnish lake types.

The maximum *P. rutilus* turion growth achieved under half light conditions, seems to reflect the ability of some macrophytes, as shade plants, to grow in reduced light as leaf photosynthesis of such aquatic plants is saturated at irradiance of less than half full sunlight (Bowes 1987, Van *et al.* 1976). However, when *P. rutilus* light levels are greatly limited, as seen in the quarter light experimental growth levels, this reduces both turion size and germination rate.

The above information has revealed a range of variables and habitat conditions that affect the success of *P. rutilus* growth, highlighting how some growth conditions, such as salinity and eutrophication that can reduce the survival of the plant. However, other possible threats to *P. rutilus* that have yet to be considered, are the impacts of invasive plants and the possible impacts of global warming, discussed in the following section 6.2. Aspects of *P. rutilus* genetics, distribution, and conservation further discussed in section 6.3.

6.2 POSSIBLE THREATS

6.2.1 Invasive Plants

There are no records of any British endemic plant being made extinct by the spread of invasive alien plants (Dickson 2001). However, some rare plants in mesotrophic lakes may be suffering decline from more vigorous growing invasive *Elodea* species (Rørslett *et al.* 1986, Wallace & Murphy 2002, Wingfield *et al.* 2004). For example, Rørslett *et al* (1986) found the rare mesotrophic macrophyte *Najas flexilis* had its distribution greatly reduced by strong competition from *Elodea canadensis.* Lake observations by Rørslett (*et al.* 1986) revealed that in the presence of this invasive species, *N. flexilis* had its habitat narrowed to the 5-6 m depth zone at the very fringe of its realised niche in Lake Steinsfjord, where light-induced mortality rates would be expected to rise sharply.

There is some evidence that *P. rutilus* can have its abundance reduced in the presence of abundant *E. canadensis,* as found in Loch Ussie. In some areas of Loch Ussie, *P. rutilus* biomass significantly declined with increasing biomass of *E. canadensis* (Wallace & Murphy 2002). The frequent occurrence of *E. canadensis* in Loch Ussie may not threaten the loch's relatively abundant populations of *P. rutilus.* However, this may change if *Elodea* becomes abundant or totally dominant, as it tends to extend from shallow water to depths of 3m (Preston & Croft 1997), precisely the depth growth zone that *P. rutilus* prefers (Preston & Croft 1997, Kotiranta *et al.* 1998). Competitive exclusion of *P. rutilus* by *E. canadensis* from its growth zone, may be further exacerbated by the fact that they favour similar substrate types. The optimum substrate type for *E. canadensis* growth is fine sediments with organic matter ranging from 10 % to 25 % (Nichols & Shaw 1986), which is similar to *P. rutilus* substrates that have mean organic contents ranging from 12 % to 29 %, see Table 3.3.

However, under highly nutrient rich conditions it may be difficult to distinguish the direct impacts of competition from *E. canadensis* and the environmental impacts of eutrophication on species such as *P. rutilus.* Populations of *E. canadensis* are able to

persist and thrive in nutrient rich, eutrophic conditions (Rørslett 1977, Spence 1964), whilst other macrophyte species have been eliminated by eutrophication (Spence 1964). *E. canadensis* exhibits phenotypically-expressed traits for tolerance of stress, disturbance, and competition from other species (Grime 1979), and so can survive in highly disturbed and low light environments, such as canals (Murphy & Eaton 1983). In contrast, *P. rutilus* turion growth and germination success is reduced in low light conditions (chapter 4), and its loss from hyper-eutrophic lochs, such as Loch Flemington, is possibly more to do with low light stress conditions than from direct competition from the abundant *E. canadensis.*

However, in more mesotrophic environments direct competition from invasive *Elodea nuttallii* on *P. rutilus* and other rare species such as *Najas flexilis* may be more of a problem (Wingfield 2004). The more recent invasive *Elodea nuttallii*, a more competitive species than *E. canadensis* (Simpson 1990), may be displacing *P. rutilus* and contributing to its decline in its two present Outer Hebridean locations. Evidence suggests that declining populations of *P. rutilus* in mesotrophic lochs, Grogary and Scarie, may partly be related to the increasing abundance of *E. nuttallii* that seems to be competitively excluding *P. rutilus.* In comparing old and recent macrophyte surveys of Loch Grogary and Scarie (SNH 1995/99 surveys with my more recent 2002 surveys), there appears to be a great increase in the abundance of *E. nuttallii* with a subsequent decline in frequency of *Potamogeton rutilus*, see Table 6.1

Table 6.1 The declining abundance of *P. rutilus* with increasing abundance of *E. nuttallii.*

Survey Year	Loch Grogary		Survey Year	Loch Scarie	
	P. rutilus	*E. nuttallii*		*P. rutilus*	*E. nuttallii*
1995	F	O/LF	1999	LF	F
2002	LO	D	2002	LO/R	LA

The 2002 boat surveys of the above Outer Hebridean lochs revealed an extensive spread of *Elodea nuttallii*, which formed a dense near-surface canopy over large parts of the loch. Small pockets of *P. rutilus* could only be found growing within small gaps in the *E. nuttallii* canopy. In a 1999 scuba survey of Loch Scarie it was found that *E. nuttallii* was most abundant in the 0.7-1.0 m depth zone, one of the depth zones most usually colonised by *P. rutilus* (Preston & Croft 1997). The increasing abundance of *E. nuttallii* may not only threatens *P. rutilus* and its habitats, but also possibly threaten the rare macrophyte *Najas flexilis* (Wingfield 2004), which has declined in both Loch Grogary and Scarie. This invasive ability of *E. nuttallii* is well demonstrated by the fact it has spread throughout much of the British Isles, since first being recorded near Oxford in 1969 (Preston & Croft, 1997).

6.2.2 Global Warming: Salinisation & Temperature Changes

One of the predicted effects of global warming is acceleration in the current rates of sea level rise, which will inundate many low-lying coastal areas (Galbraith *et al.* 2002). Even though sea levels are predicted to rise least in western Scotland (Cook & Harrison 2001), the low lying *P. rutilus* machair lochs will be more vulnerable to salinisation. *P. rutilus* has been found to be sensitive to salinity stress and has disappeared from some its former Polish locations due to increased salinity of its freshwater habitats (Kazmierczakowa & Zarzycki 2001).

Some of the former *P. rutilus* machair lochs have already higher than usual salinities for freshwaters, which may have contributed to their loss of *P. rutilus* (see chapter 3). The low-lying machair lochs, Grogary and Scarie, with already reduced *P. rutilus* populations, would particularly be vulnerable to environmental stress of increased salinities. Low levels of salinity stress may not be toxic enough to kill *P. rutilus,* but may be high enough to reduce the fertility and fecundity of the plant, as has been found in other saline-stressed macrophytes (Blindow *et al.* 2003, James & Hart 1993).

Another predicted consequence of global climate change is the rise in air and water temperatures (Mckee *et al.* 2002), with predictions of UK temperature rises being

between 0.7 ° C and 2.6 ° C by the 2050s (Cook & Harrison 2001). These increases in temperature will directly affect shallow lake macrophyte communities (Carpenter *et al.* 1992), as temperature influences macrophyte growth and reproduction (Holmes & Klein 1987, Santamaria & Van Vierssen 1997).

Variations in climatic factors are known to influence the population dynamics of plant communities (Bertness & Ewanchuk 2002), with individual growth responses of macrophyte species varying with plant growth strategy and adaptation to a particular climatic zone (Murphy 2002, Farmer & Spence 1986). For example, *Potamogeton filiformis*, a northwestern distributed species within the British Isles (Preston 1995), may have its climate space and distribution greatly reduced by predicted temperature rises for the 2050s (Cook & Harrison 2001). *P. rutilus* like *P. filiformis* may have its climate space affected by global temperature rises, as the distribution of the plant is confined to northern Scotland, which represents the most westerly location of this species, with an otherwise mainly Baltic distribution (Murphy 2002).

Temperature rises could affect *P. rutilus* seasonal growth as its development stages are sensitive to temperature cues that might cause earlier seasonal turion development and germination. These temperature changes may not adversely affect the life-cycle development of *P. rutilus*, as the plant may show some degree of thermal tolerance as has been found in different clonal populations of *P. pectinatus.* (Pilon & Santamaria 2002). However, the predicted increase in competition from invasive species (McKee *et al.* 2002), could have a detrimental effect on the survival of *P. rutilus*. These higher climatic temperatures may encourage the range expansion of macrophytes, such as invasive species, from warmer climates into cooler climatic zones (Mulholland *et al.* 1997), such as northern Scotland. At present there seems to be no invasive *Elodea* species in the Shetland Islands and their *P. rutilus* lochs (Murphy 2002), but this might change if these invasive species, are encouraged to move much further north with a warmer climate; as *Elodea canadensis* is already present as far north as the Orkney Islands (Preston & Croft 1997).

It is expected the most vulnerable plant communities to temperature increases would be in lake ecosystems that are already suffering from environmental stress such as eutrophication (McKee *et al.* 2002). For example, the two invasive *Elodea* species do not greatly increase their biomass growth with small temperature rises, but when combined with eutrophic nutrient levels, their growth rates greatly increase (McKee *et al.* 2002, Nichols & Shaw 1986).

Other effects of global climate change are the possible increases of aquatic dissolved inorganic carbon (DIC) resulting from elevated levels of atmospheric CO_2, which may benefit *P. rutilus* growth as a possible bicarbonate user, but non-bicarbonate users such softwater isoetid species may decline (Murphy 2002).

6.3 CONSERVATION OF P. RUTILUS

6.3.1 Genetics and Distribution

The genetic analysis of *P. rutilus* revealed that despite a high degree of clonality in most of the *P. rutilus* populations, there appeared to be some genetic variation between individuals and populations. This genetic variation in *P. rutilus* offers some chance of populations adapting to changing environments, as genetic diversity is associated with population viability in changing environments (Pandit & Babu 2003, Aigner 2004). The genetic variation in *P. rutilus* seems to have been partly produced by sexual seed reproduction, which would contribute to generating genetic diversity in populations (Soane & Watkinson 1979). However, a high level of diversity among individuals may also be partially maintained through clonal persistence of genets from the founding population (Hangelbroek *et al.* 2002).

The genetic results show that some degree of sexual seed production is taking place in *P. rutilus* populations, but in terms of seed production this is of limited occurrence (Preston 1995, Clark 1943). The limited seed occurrence may partly explain the distribution and rarity of *P. rutilus,* as other recent studies of rarity in the native British

flora reveal that species that produce fewer seeds are more threatened (Henderson 2001, Wilcock 2002, Pilgrim *et al.* 2004). Such plants with reduced seed fecundity are known to have a reduced ability to buffer extinction events via seed banks (Kunin & Gaston, 1993).

The limited seed production of *P. rutilus* may also be reducing the plant's ability to disperse and colonise new sites by long distance seed dispersal. This is further supported by genetic evidence, with genetic distance increasing with spatial distance between some *P. rutilus* populations, suggesting that there is limited gene flow between populations (Jordi *et al.* 2002, Ibrahim *et al.* 1996). This genetic isolation between plant populations and/or different environmental influences may in some cases contribute to a species differentiating into separate ecotypes, local races, (Crawford 1989, Van Rossum & Prentice 2004).

Ecotypes are locally adapted populations or races having distinctive characters, which resulted from the selective pressure of the local environment (Crawford 1989). For example, Belgian populations of *Silene nutans* have found to be differentiated into two parapatric ecotypes that are associated with calcareous or siliceous substrates (Van Rossum *et al.* 1997). In the case of *P. rutilus* the wide trophic tolerances the plant shows in inhabiting both Scottish and Scandinavian trophic habitats, may be partly due to ecotypic population differences. Scottish *P. rutilus* may have evolved ecotypic adaptations to the oligo-mesotrophic conditions it inhabits, whilst the Scandinavian plant that inhabits more nutrient rich conditions, may have evolved a eutrophic ecotype. This ecotypic habitat specialisation may partly explain the current oligo-mesotrophic distribution of Scottish *P. rutilus* and the extinctions in those lochs that have suffered eutrophication. In contrast, if a eutrophic ecotype exists for Finnish *P. rutilus*, it would partly explain why the plants are successfully expanding its range, by colonising some southern Finnish lakes that have become more eutrophic.

There may even be local ecotypic differences between Scottish *P. rutilus* populations, as has been found in other Scottish plant populations such as *Pinus sylvestris* (Perks &

Ennos 1999). The possible existence of local Scottish *P. rutilus* ecotypes would have genetic conservation implications for any population restoration methods, such as translocations. This will be considered in the following section on reintroductions.

In summary, the distribution of Scottish *P. rutilus* may be influenced by a combination of the factors described above, as plant distributions are known to be affected by the sum total of historical, geographical and environmental factors (Crawford 1989). In addition, biotic factors such as competition from invasive species may also determine plant survival and distribution in particular environments (Taylor 1949).

6.3.2 Reintroductions

One of the actions of the biodiversity action plan is to restore the species to suitable areas where it has been lost. This objective of reintroductions would seem best facilitated by translocation of vegetative offspring or seed if available to suitable sites. Translocation methods are known to be successful in helping rare clonal plants colonise and establish in chosen suitable habitats (Rossetto *et al.* 2004, Kingston *et al.* 2004). In re-establishing populations of rare clonal plants one of the main aims is to re-initiate sexual reproduction to produce viable seeds for dispersal and re-colonisations of new areas (Rossetto *et al.* 2004). However, due to the clonal habit of many aquatic plants (Grace 1993), re-establishment of *P. rutilus* populations may not greatly increase seed production, though it may help sustain metapopulation dynamics (Frekleton & Watkinson 2002).

In considering translocations of a rare species there are several conservation issues that need to be considered. For example, Hodder & Bullock (1997) listed ecological, genetic and anthropogenic factors that can affect successful translocations, whilst also highlighting the positive and negative effects that successful translocations may have on ecological interactions and genetic composition. Osborne (2004), identifies four important IUCN criteria that need to be met before a reintroduction of a species can take place:

- the factors that caused extinction must no longer operate
- the conditions in the proposed reintroduction area must still be suitable for the species
- there should be no adverse impact on the donor population
- there should be no adverse impact on the recipient ecosystem

The above ecological criteria for successful translocation would also include appropriate habitat conditions for the survival of the translocated species, with an adequate number of the translocated species to ensure the establishment of a viable, self-sustaining, population (Milton *et al.* 1999). As seed production by *P. rutilus* is unlikely the use of turions, which most plants produce, would be the best method for translocations. *P. rutilus* turions germinate readily in appropriate conditions as seen in the growth experiments in chapter 4. Selecting larger sized turions for translocations should increase germination success of any reintroductions, as turion size is a good measure of plant fitness as revealed in chapter 5. The appropriate habitat conditions for receiving translocated *P. rutilus* would be some of its former sites that have suitably recovered ecological conditions. For example, the two former *P. rutilus* Shetland lochs, Tingwall and Asta, appear to now have a stable mesotrophic trophic type which should be able to support the reintroduction of *P. rutilus* turions to appropriate loch habitats.

The success of any reintroductions will be dependent on the selection of suitable habitats within a loch for turion introduction. Suitable habitat conditions for turion planting would include soft sand/silt type substrates, with an adequate light environment with depths around 0.5 m to 1 m where conditions are not too exposed. The plant can grow in more shallow habitats (Preston & Croft 1997), but translocating turions to deeper depths can reduce the risk of waterfowl and grazing and disturbance, as found in *Potamogeton* species transplanted into shallow lakes (Lauridsen *et al.* 2003).

Other lochs such as Loch Flemington that still have very high nutrient levels and low light availability, due to algal blooms, would be unlikely to support a successful reintroduction of *P. rutilus* until there was much improvement in water quality.

Genetic considerations for evaluating the most appropriate translocations for a rare species include, the possible positive or negative affects a translocated species may have on the genetic structure of a host population. There is still ongoing debate on the restoration of sexual reproduction to rare threatened clonal plants by the deliberate introduction of cross-compatible mating partners (Wilcock 2002, Reinartz 1995). The genetic effects can be positive when they reduce inbreeding depression but negative when they cause outbreeding depression or result in the loss of ecotype or subspecies through hybridization (Milton *et al.* 1999, Warburton *et al.* 2000). Minimising the risk of losing ecotypes through translocations can be achieved by reintroducing a species to areas where they are extinct (Milton *et al.* 1999), or ensuring translocated species and receiving populations are of similar provenance (Perks & Ennos 1999).

Even within Scotland certain plant species are found to have adaptive genetic variation between geographical populations, as found in *Pinus sylvestris* that has distinctive ecotypes in different areas of Scotland (Perks & Ennos 1999). In the case of *P. rutilus,* supplementing small remnant populations of the plant by translocations, such as in Loch a Chlair, may be unwise as it could reduce the viability of any possibly adapted ecotypes. However, the reintroduction of the plant to extinct sites, such Tingwall and Asta, pose no problems of genetic loss, but there is the need to ensure that the translocated species between sites are of similar abiotic and biotic conditions (Perks & Ennos 1999), so ensuring translocated species have the best adaptive ability for survival. For example, the Shetland lochs, Kirkigarth and Bardister, would seem to be the most suitable local lochs to donate *P. rutilus* turions to the two extinct Shetland lochs, Tingwall and Asta, as they have similar climatic and trophic characteristics and so reduce the risk of ecotype incompatibility of habitats.

Local *P. rutilus* donor lochs could also be used to colonise new suitable neighbouring lochs that have been identified as potential lochs for colonisation. Translocations into new potential lochs would extend the *P. rutilus* population distribution and help safeguard the plant from localised extinctions. Some may argue that such artificial extension of rare clonal plant species distribution is a waste of time as the populations are not self-sustaining (Griffith *et al.* 1989). However, whether a translocation of a species to former or new sites, is a success or failure, it is essential to monitor in detail any experimental translocations to assess the successes or pitfalls of the technique for a particular species (Wilcock 2002, Osborne 2004).

P. rutilus genetic data, combined with trophic classifications and water chemistry data for present and former *P. rutilus* sites, should help determine the most suitable lochs for *P. rutilus* reintroductions. This ecological information would also be useful in developing a conservation strategy and monitoring program for the lochs chosen for reintroductions (Pandit & Babu 2003).

Supplementing *P. rutilus* colonising abilities with translocations between loch populations would help stabilise and possibly enhance metapopulation dynamics of a region (Freckleton & Watkinson 2002). These translocations would also help to achieve the biodiversity action plan objective of maintaining existing populations by enhancing metapopulation dynamics between lochs.

6.4 FUTURE RESEARCH

6.4.1 Invasive Competition Experiments

There is a need to investigate the current and future impacts of invasive macrophytes on the population dynamics of macrophyte communities, especially the impacts that these invasives will have on diverse mesotrophic macrophyte communities and their rare species. Investigations, such as field experiments, could evaluate the invasive threats to *P. rutilus* and *N. flexilis* by experimentally removing fixed quadrat areas of *E. nuttallii* from the two infested lochs, Grogary and Scarie, to monitor the recolonisation of these

cleared areas. The field experiments could evaluate whether controlled removal of *E nuttallii* from the main depth growth zones of *P. rutilus* and *N. flexilis* would encourage the plants to recolonise the cleared zones. In addition to the field experiments, laboratory experiments could also help evaluate the growth responses of *Elodea* to cutting and competition when under different controlled conditions (Abernethy *et al.* 1996), which may inform control measures for the plant (Nichols & Shaw 1986).

6.4.2 Investigating possible Global Warming Impacts

Scotland has a large variation in climatic zones, associated with altitudinal changes and distance from the sea, which combined with differences in geology, produces a wide range of habitats and vegetation types (Cannell *et al.* 1997). Each climatic zone with its distinctive habitat types does not have a great difference in mean January and July temperatures, with it being no more than 2 $^{\circ}$ C difference between climate zones (Cannell *et al.* 1997). Consequently even small predicted temperature rises of 1 $^{\circ}$ C might greatly affect the Scottish flora (Cannell *et al.* 1997).

The predicted rises in sea levels may not greatly affect Scotland as much as the rest of the UK. However, Scotland has some vulnerable coastal habitats such as the machair, which have a large number of macrophyte rich mesotrophic lochs that host *P. rutilus* and other rare plants such as *Najas flexilis.* It has already been predicted that *P. filiformis,* which forms an important part of the *P. rutilus* community type, may decline with climate change (Cook & Harrison 2001).

Other macrophyte species of this diverse mesotrophic community type may also be vulnerable to the predicted climatic changes. For example, *P. rutilus* is vulnerable to salinity (Kazmierczakowa & Zarzycki 2001), and is now absent from some of its former machair lochs, which have higher than usual salinities. This problem may only get worst with the predicted sea level rises that may cause further losses of *P. rutilus,* whilst also reducing macrophyte diversity, by affecting other macrophyte species that are vulnerable to salinity (Blindow *et al.* 2003, James & Hart 1993).

In this respect it would be prudent to investigate how such predicted temperature changes and rises in sea levels may affect the distinctive flora of Scottish mesotrophic lakes. Only with such research will we be able to take measures to conserve rare macrophytes and their habitats from the future impacts of climate change.

6.5 MANAGEMENT RECOMMENDATIONS

Defining appropriate management for habitats rather than each individual rare species is more likely possible with limited resources (Legg *et al.* 2003). In the case of *P. rutilus,* improved water quality is one of the key factors in the restoration and maintenance of *P. rutilus* populations and its associated mesotrophic community type. Reducing nutrient inputs from intensive agriculture and development would help protect mesotrophic lochs and their macrophyte habitats. For example, Loch Fingask, Perthshire, is surrounded by arable land with high fertiliser inputs that is causing nutrient enrichment of the loch by diffuse pollution. The loch at present has an abundant population of *P. rutilus* and a diverse macrophyte flora, but in recent years has being suffering from algal blooms and perturbation (N. Stewart, personal communication). Such lochs, threatened by eutrophication, need to be protected by reducing nutrient inputs through nutrient budgeting and by introducing vegetation buffer strips around lochs to reduce diffuse pollution from surrounding arable crops. To do this, we need to be informing local landowners about the adverse impacts of some of their farming methods and encourage them to join agri-environment schemes.

Bringing together the different issues discussed concerning the conservation of *P. rutilus* and its loch habitats, I have summarised a series of management recommendations to maintain and possibly increase the present population of *P. rutilus.*

- Maintain existing *P. rutilus* populations by increasing the protection of their mesotrophic loch habitats from nutrient enrichment, by methods described above.

- Restoration of *P. rutilus* to suitable former loch sites, such as Asta and Tingwall, by translocations of turions from local donor sites.

- If *P. rutilus* reintroductions are successful, consider extending the plants local distributions by introducing turions to suitable neighbouring lochs that have been identified as potential sites for colonisation.

- To evaluate the condition of current *P. rutilus* populations and success of any newly translocated populations it is essential to monitor the species and its loch habitats. Various criteria can be used to detect changes in *P. rutilus* and its community types such as monitoring trophic changes in lochs using macrophyte community types. The direct monitoring of *P. rutilus* may be necessary, especially where the populations are greatly reduced and aggregated in lochs, such as Loch a Chlair. For such populations, *P. rutilus* growth traits, turion length or turion biomass would be a good fitness indicator for monitoring these plant populations. Species trait and trophic monitoring could be combined with water chemistry sampling to give a comprehensive evaluation of any trophic changes in *P. rutilus* lochs.

- To further assess the current and future threats to *P. rutilus* there is an urgent need for more research to evaluate the impacts of invasive species such as *Elodea*, and the possible adverse habitat changes that may accompany global warming.

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APPENDIX 1 Palmer's standing water categories

(from Palmer *et al.* 1992)

LOCH TYPE Lochs are defined as one of 10 possible types. The classification scheme was developed on the basis of a TWINSPAN (Two way indicator species analysis) of submerged and floating aquatic flora recorded at 1124 sites in Great Britain. The 10 loch Types match up quite closely with water chemistry parameters and can be broadly described as follows:

Type 1 Dystrophic/ very acid: a species-poor group, characterised by submerged *Sphagnum* and *Juncus bulbosus*, often accompanied by *Potamogeton polygonifolius*. These include pools and small lochs on blanket bog.

Type 2 Oligotrophic/ base poor: sites are typified by *Juncus bulbosus and Potamogeton polygonifolius*, along with *Littorella uniflora*, *Lobelia dortmanna* and *Potamogeton natans*. Typical sites are peaty lochs in Northern Scotland.

Type 3 Oligotrophic/ base poor: Distinguished from Type 2 by the higher incidence of *Myriophyllum alterniflorum*, *Isoetes lacustris* and *Fontinalis antipyretica*. These lochs are similar to Type 2 but larger and rockier (e.g. Loch Lomond, LochAwe)

Type 4 Mixture of influences: contains elements of Type 3 with *Potamogeton f iliformis*, *Potamogeton praelongus*, *Myriophyllum spicatum* and *Chara* species commonly occurring.These sites are typified by coastal freshwater lochs of the Scottishislands, including machair lochs situated on calcareous sand, but with acid inflows from a peaty hinterland. These lochs often have very rich assemblages of water plants.

Type 5 Mesotrophic/ moderately rich: Type 5A consists of a species-rich group of sites characterised by *Littorella uniflora*, *Myriophyllum alterniflorum*, *Nitella* species, a wide range of *Potamogeton* species and *Elodea canadensis*. The variant 5B consists of species-poor sites dominated by *Potamogeton natans* and *Nymphaea alba*. e.g. Lake of Menteith

Type 6 Brackish: sites usually contain few species apart from *Potamogeton pectinatus*,
Ruppia species and seaweeds such as *Fucus ceranoides*. These sites are brackish sea lochs on islands off the north and west coasts of Scotland.

Type 7 Eutrophic/ base rich: similar to Type 4 sites but usually lacking *Juncus bulbosus* an *Myriophyllum alterniflorum*. These are lochs with a strong marine influence, on shell sand, limestone and Old Red Sandstone.

Type 8 Eutrophic/ base rich: These sites are poor in open water species, but rich in emergents, and are characterised by *Lemna minor*, *Callitriche stagnalis* and *Persicaria amphibia*.

Type 9 Eutrophic/ base rich: dominated by the water lilies *Nuphar lutea* and *Nymphaea alba*, frequently in combination. This is an uncommon loch type in Scotland.

Type 10 Eutrophic/ base rich: these sites often support *Myriophyllum spicatum* and *Potamogeton pectinatus*. This type has two variants; one characterised by *Elodea canadensis* and *Lemna minor* and the other by *Chara* species. Type 10 includes artificial sites such as gravel pits and little-used canals.The substrate of these water bodies is predominantly fine and most sites are situated in lowland areas on sedimentary rocks, often calcareous in nature

APPENDIX 2a Site code abbreviations

Loch Sites	**Loch Abbreviations**	***P. rutilus* Status**		
Scottish Mainland		**Current**	**Former**	**Potential**
Loch Ussie	Uss	√		
Loch Bayfield	Bay	√		
Loch Eye	Eye	√		
Loch Flemington	Fle		√	
Loch Awe	Awe		√	
Inner Hebrides				
Loch an Eilein	Eil	√		
Loch a Chlair	Chl	√		
Loch Ballyhaugh	Bal	√		
Loch Lossit	Los	√		
Loch Phuill	Phu			√
Loch Bhasapoll	Bha			√
Outer Hebrides				
Loch Grogary	Gro	√		
Loch Scarie	Sca	√		
Loch na Reivil	Rei		√	
Loch Leodasay	Leo		√	
Loch Mhor	Mho		√	
Loch nam Feithean	Fei		√	
Shetland				
Loch Bardister	Bar	√		
Loch Kirkigarth	Kir	√		
Loch Asta	Ast		√	
Loch Tingwall	Tin		√	
Loch Benston	Ben			√
Finland		**Current**	**Former**	**Potential**
Lake Simpele (Siikalahti)	Sii	√		
Lake Simpele (K. Hovi)	Kho	√		
Lake Grundtrask	Gru	√		
Lake Rytilampitrask	Ryt	√		
Lake Lampistrask	Lam	√		

APPENDIX 2b Species presence or absence data for sites

	Eye	Bay	Uss	Gro	Bal	Sca	Kir	Bar	Eil	Los	Chl	Ast	Tin	Rei
Apium inundatum	0	0	1	1	1	0	0	0	1	0	0	0	0	0
Baldellia ranunculoides	0	0	0	0	1	0	0	0	0	0	1	0	0	0
Callictriche hamulata	1	1	1	0	0	0	0	0	0	0	0	0	0	0
Callitriche hermaphroditica	0	0	0	1	0	0	1	0	0	1	0	1	1	0
Callitriche stagnalis	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Chara aspera	1	0	0	1	1	0	0	0	1	0	1	0	0	0
Chara globularia	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Chara curta	0	0	0	0	0	0	0	1	0	0	0	0	1	0
Chara virgata	0	1	1	0	0	0	1	1	0	0	0	0	0	1
Chara hispida	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chara strigosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chara sp.	0	0	0	0	0	1	0	0	0	1	1	0	0	0
Elatine hexandra	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Elodea canadensis	1	1	1	0	0	0	0	0	0	0	0	0	0	0
Elodea nuttallii	0	0	0	1	0	1	0	0	0	0	0	0	1	1
Fontinalis antipyretica	1	0	1	1	1	0	1	1	1	1	0	1	1	0
Isoetes lacustris	1	0	1	0	0	0	0	0	0	0	0	0	0	0
Isoetes echinospora	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Juncus bulbosus	0	1	1	0	1	0	1	0	0	1	0	0	0	0
Lemna minor	0	0	1	0	0	0	0	0	0	0	0	1	1	1
Littorella uniflora	1	1	1	1	1	1	1	1	1	1	1	0	0	0
Lobelia dortmanna	1	0	1	0	0	0	1	0	0	0	0	1	1	1
Myriophyllum alterniflorum	1	1	1	1	0	1	1	1	1	1	1	0	0	1
Myriophyllum spicatum	0	0	0	0	0	1	0	0	1	0	0	0	0	0
Myriophyllum sibiricum	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Najas flexilis	0	0	0	0	1	0	0	0	0	1	0	0	0	0
Nitella flexilis	1	0	0	0	0	0	0	0	1	0	0	0	0	0
Nitella opaca	0	1	1	0	0	0	0	0	0	0	0	1	0	0
Nitella walhbergiana	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitella translucens	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Nymphaea alba	0	0	1	0	0	0	0	0	0	1	0	1	1	0
Potamogeton alpinus	0	0	1	0	0	0	0	0	0	0	0	0	0	0

APPENDIX 2b continued

	Eye	Bay	Uss	Gro	Bal	Sca	Kir	Bar	Eil	Los	Chl	Ast	Tin	Rei
Potamogeton berchtoldii	1	1	0	0	1	1	0	0	0	0	0	0	0	0
Potamogeton compressus	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Potamogeton crispus	1	1	1	0	1	0	0	0	0	0	0	1	1	0
Potamogeton friesii	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Potamogeton filiformis	1	1	1	1	0	1	0	0	1	0	1	0	1	1
Potamogeton gramineus	0	1	1	1	1	1	1	1	1	1	1	0	0	0
Potamogeton natans	1	1	1	1	1	1	1	1	1	1	0	0	0	1
Potamogeton obtusifolius	0	0	1	0	0	0	0	0	0	0	0	1	1	1
Potamogeton pectinatus	1	0	0	1	0	1	0	0	0	1	0	0	0	1
Potamogeton perfoliatus	1	1	1	1	1	1	1	1	1	1	0	0	1	0
Potamogeton polygonifolius	0	0	1	0	1	0	0	0	0	0	1	0	1	0
Potamogeton praelongus	1	0	0	0	0	0	1	0	0	0	0	0	0	0
Potamogeton pusillus	0	0	0	1	0	0	0	1	0	0	0	0	1	0
Potamogeton rutilus	1	1	1	1	1	1	1	1	1	1	1	0	0	0
Potamogeton x nitens	1	0	1	1	0	0	0	1	0	0	1	0	0	0
Ranunculus baudotii	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ranunculus trichophyllus	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Sparganium angustifolium	0	1	1	0	1	0	0	0	0	1	0	0	0	0
Subularia aquatica	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Utricularia vulgaris	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Zannichellia palustris	0	1	0	0	0	0	0	1	0	0	0	1	1	0
Persicaria amphibia	0	0	0	0	1	1	0	0	1	0	0	0	0	1
Filamentous algae	1	0	0	0	1	1	1	1	1	0	1	1	1	1
Ceratophyllum demersum	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nuphar lutea	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nuphar pumila	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sagittaria sagittifolia	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Najas tenuissima	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lemna trisulca	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lemna spirodela	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stratiotes aloides	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sparganium gramineus	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sparganium emersum	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrocharis morsus-ranae	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX 2b continued

	Fle	Mho	Leo	Awe	Fei	Ben	Bha	Phu	Gru	Sii	Kho	Ryt	Lam
Apium inundatum	0	0	0	0	0	0	1	0	0	0	0	0	0
Baldellia ranunculoides	0	0	0	0	0	0	0	1	0	0	0	0	0
Callictriche hamulata	0	0	0	0	0	0	0	0	0	0	0	0	0
Callitriche hermaphroditica	0	0	0	0	0	1	0	0	1	0	0	0	0
Callitriche stagnalis	0	0	0	0	0	0	0	0	0	0	0	0	0
Chara aspera	0	0	0	0	0	0	0	0	0	0	0	0	0
Chara globularia	0	0	0	0	0	0	0	0	0	0	0	0	1
Chara curta	0	0	0	0	0	0	1	0	0	0	0	0	0
Chara virgata	0	0	0	0	1	0	0	0	0	0	0	0	0
Chara hispida	0	1	1	1	0	1	1	1	0	0	0	0	0
Chara strigosa	0	0	0	0	0	0	0	0	0	0	0	1	0
Chara sp.	0	1	0	0	0	0	0	0	0	1	0	0	0
Elatine hexandra	0	0	0	0	0	0	0	0	0	0	0	0	0
Elodea canadensis	1	0	0	0	0	0	0	0	0	1	1	0	0
Elodea nuttallii	0	0	0	1	0	0	0	1	0	0	0	0	0
Fontinalis antipyretica	0	0	0	1	0	0	0	0	0	0	0	0	0
Isoetes lacustris	0	0	0	0	0	0	0	0	0	0	1	0	0
Isoetes echinospora	0	0	0	1	0	1	0	0	0	0	0	0	0
Juncus bulbosus	0	0	0	0	0	0	0	0	0	0	0	0	0
Lemna minor	0	0	1	1	1	1	1	0	0	0	0	0	0
Littorella uniflora	1	0	0	1	0	1	0	0	0	0	0	0	0
Lobelia dortmanna	0	1	0	1	1	1	1	1	0	0	0	0	0
Myriophyllum alterniflorum	1	1	1	0	1	0	1	1	0	0	1	0	0
Myriophyllum spicatum	0	0	0	0	0	0	0	0	0	0	0	0	0
Myriophyllum sibiricum	0	0	0	0	0	0	0	0	0	0	0	0	1
Najas flexilis	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitella flexilis	0	0	0	0	0	0	0	0	0	1	0	0	0
Nitella opaca	0	0	0	0	0	0	0	0	0	0	0	0	1
Nitella walhbergiana	0	0	0	0	0	0	0	0	0	1	0	0	0
Nitella translucens	0	0	0	1	0	0	0	0	0	0	0	0	0
Nymphaea alba	0	0	0	1	0	1	0	1	0	0	0	0	0
Potamogeton alpinus	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX 2b continued.

	Fle	Mho	Leo	Awe	Fei	Ben	Bha	Phu	Gru	Sii	Kho	Ryt	Lam
Potamogeton berchtoldii	0	1	0	0	1	0	0	0	1	1	1	0	1
Potamogeton compressus	0	1	0	0	1	0	0	0	0	0	0	1	0
Potamogeton crispus	0	0	0	0	1	1	1	1	0	0	0	0	0
Potamogeton friesii	0	0	0	1	1	1	1	1	0	1	0	0	0
Potamogeton filiformis	0	1	0	1	1	1	1	0	0	0	0	1	0
Potamogeton gramineus	1	0	0	0	0	0	0	0	0	0	1	0	0
Potamogeton natans	0	1	1	0	1	0	1	0	0	1	0	0	0
Potamogeton obtusifolius	1	1	1	1	1	1	1	1	0	1	0	0	0
Potamogeton pectinatus	0	0	0	1	0	0	0	0	0	0	0	0	0
Potamogeton perfoliatus	0	0	0	0	0	0	0	0	1	0	0	0	0
Potamogeton polygonifolius	0	1	0	0	0	0	1	0	0	0	0	0	0
Potamogeton praelongus	0	0	0	0	0	0	0	0	0	0	0	0	0
Potamogeton pusillus	0	0	0	0	0	1	1	1	0	1	1	0	0
Potamogeton rutilus	0	0	0	0	0	0	0	0	1	1	1	1	1
Potamogeton x nitens	0	0	0	0	0	0	0	0	0	0	0	0	0
Ranunculus baudotii	0	0	0	1	0	0	0	0	0	0	0	0	0
Ranunculus trichophyllus	0	0	0	1	0	0	0	0	0	0	0	0	0
Sparganium angustifolium	0	0	0	1	0	0	0	0	0	1	1	0	0
Subularia aquatica	0	0	0	1	0	0	0	0	0	0	1	0	0
Utricularia vulgaris	0	0	0	1	0	0	0	0	0	0	0	0	0
Zannichellia palustris	0	0	0	0	0	0	0	0	0	0	0	0	0
Persicaria amphibia	1	0	0	0	1	0	1	0	0	0	0	0	0
Filamentous algae	0	1	1	0	1	1	0	0	0	0	0	0	0
Ceratophyllum demersum	0	0	0	0	0	0	0	0	1	0	0	0	0
Nuphar lutea	0	0	0	0	0	0	0	0	1	0	0	1	0
Nuphar pumila	0	0	0	0	0	0	0	0	1	0	0	0	0
Sagittaria sagittifolia	0	0	0	0	0	0	0	0	1	0	0	0	0
Najas tenuissima	0	0	0	0	0	0	0	0	1	0	0	0	0
Lemna trisulca	0	0	0	0	0	0	0	0	1	0	0	1	1
Lemna spirodela	0	0	0	0	0	0	0	0	1	0	0	0	0
Stratiotes aloides	0	0	0	0	0	0	0	0	0	1	0	1	0
Sparganium gramineus	0	0	0	0	0	0	0	0	0	0	0	0	1
Sparganium emersum	0	0	0	0	0	0	0	0	0	1	0	0	0
Hydrocharis morsus-ranae	0	0	0	0	0	0	0	0	0	1	0	0	0

APPENDIX 3 Site water chemistry data

Water Chemistry	Eye	Bay	Uss	Gro	Bal	Sca	Kir	Bar	Eil	Los	Chl	Ast	Tin	Rei
pH	8.9	8.9	7.6	8.2	7.4	8.2	8	8.5	7.9	7.6	7.7	8.1	8.2	8.7
Conductivity (µs/cm)	199	171	154	277	207	361	213	261	283	186	239	224	259	702
Alkalinity (mg/)	44.2	48	25.6	52.4	28.2	78.5	28.7	46.2	83	56.2	62.1	47	71.4	60.6
N-NH_3 (mg/l	0.4	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	0.32	<0.04	<0.04	<0.04	0.06
N TON (mg/l)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.17	<0.1	<0.1	<0.1	<0.1
NO_3-N (mg/l)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.17	<0.1	<0.1	<0.1	<0.1
NO_2-N (mg/l)	<0.01	<0.1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01
P-DRP(PO_4) (mg/l)	0.005	0.012	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	0.016	<0.003	<0.003	<0.003	<0.003
P-TP (mg/l)	0.005	0.017	0.008	0.007	0.006	0.008	0.004	0.005	0.009	0.016	0.003	0.006	0.008	0.013
Cl (mg/l)	30	21.1	29.3	52.9	44.9	59.8	62.3	59.9	39	18.9	39.4	46.3	44.4	168.4
Na (mg/l)	18.1	14	16.9	32.3	27.4	36.5	37.2	37.9	23.8	11.6	24	29.5	27.7	102.7
K (mg/l)	4.17	2.55	1.43	1.02	0.9	0.9	1.06	0.83	0.87	0.68	1.1	1.6	2.3	5.8
Ca (mg/l)	15.4	16.6	10.1	20.2	8.5	27.7	11.2	16.6	29	17.7	18.2	18.5	30.6	26.8
Mg (mg/l)	5.79	4.32	2.52	5.61	4.15	7.36	5.04	6.01	5.2	5.2	5.8	4.35	4.51	12.6
Fe (mg/l)	0.154	0.1	0.138			0.09	0.025	0.063		0.03		0.102	0.013	
Extinction Coefficient	1.52	1.48	0.73	2.46	2.44	1.23	1.04	1.08	4.23	1.6	2.88	0.67	1.25	2.48

APPENDIX 3 (continued)

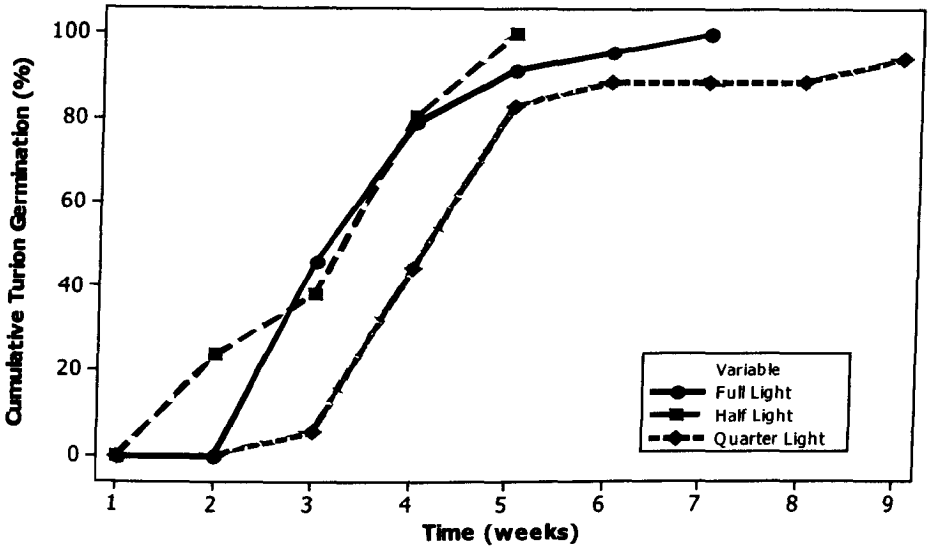
Water Chemistry	Fle	Mho	Leo	Awe	Fei	Ben	Bha	Phu	Gru	Sii	K ho	Ryt	Lam
pH	9.6	9.4	8.6	7.6	8.8	9.1	8.6	9.4	7.6	7.6	7.4	7.3	7.8
Conductivity (µs/cm)	313	1790	9860	69	308	226	339	376	289	114	110	142	149
Alkalinity (mg/)	59.3	82.4	41.1	9.4	72.9	47.4	61.7	62.3	44.4	35.4	35.1	0.7	46
N-NH_3 (mg/l	<0.04	<0.04	<0.04	<0.04	0.04	0.06	0.07	0.8	0.14	0.04	0.16	0.005	0.001
N TON (mg/l)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.42	0.91
NO_3-N (mg/l)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
NO_2-N (mg/l)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.1
P-DRP(PO_4) (mg/l)	0.082	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	0.026	0.005	0.027	<0.003	<0.003
P-TP (mg/l)	0.085	0.013	0.004	0.003	0.011	0.009	0.021	0.015	0.03	0.01	0.3	0.022	0.061
Cl (mg/l)	63.7	505.9	3009.8	20	60.4	53.1	63.2	77.1	61.1	2.7	4.8	1.6	4.4
Na (mg/l)	36.9	308.6	1836	13.3	36.9	33.8	39.2	47.8	36.9	4.9	3.8	1.1	3.3
K (mg/l)	4.33	11.5	73.3	0.28	0.2	1.4	1.4	1.5	3.8	2.6	2.6	1	2.1
Ca (mg/l)	24	28.1	75.9	3.6	24.1	18.9	18.5	18.7	13	10	10.6	11	10
Mg (mg/l)	4.2	35	232.8	1.1	7	4.4	5.6	5.6	6.4	5.2	3.4	2.8	3.2
Fe (mg/l)	0.231					0.026						0.19	
Extinction Coefficient	5.82	2.28	1.56	0.5	1.32	0.94	1.1	1.3	1.43	2.4	1	1.2	1.1

APPENDIX 4 Sediment chemistry of Scottish *P. rutilus* sites

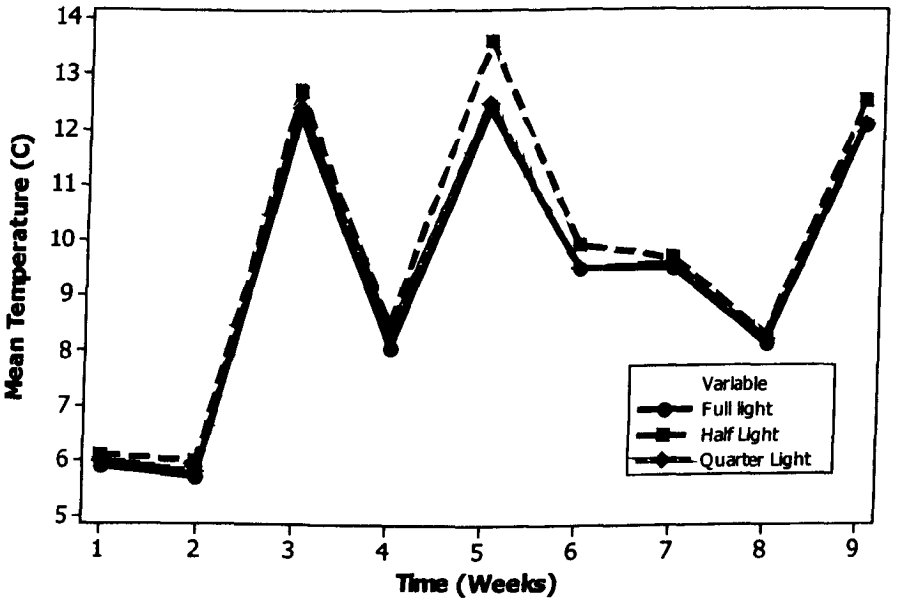
Sediment Chemistry	Eye	Bay	Uss	Gro	Bal	Sca	Kir	Bar	Eil	Los	Chl
% Organic Matter	1.5	16.2	5.75	37	-	29.23	7.1	27.5	8.2	35.5	17.4
Available P (mg/l)	6.2	25	5.05	9.6	2.7	18.66	52	25.3	12	7.35	5.6
Available K (mg/l)	24	79	61	159	16.9	170	123	101.75	50	49	66
Extractable Mg (mg/l)	35	115	60	180	141	136.7	125	152.5	60	215	130
Total Ca (mg/l)	406	1670	826	3050	1130	25500	-	6450	39800	1555	1310
Total Fe (mg/l)	131	496	223	1350	679	181	440	520	89.8	1130	234
Total N (mg/l)	410	6670	2140	13800	3320	10630	2410	9327.5	1600	12055	5220

Extractable analyte units are on a weight/volume basis (i.e. mg analyte /l of air dried soil). Organic matter content units (by Loss on Ignition) are on a % (w/w) basis. Total Kjeldahl N units are on a weight / weight basis (i.e. mg N/kg air-dried soil).

APPENDIX 5 Graphs of *P. rutilus* turion germination experiments and water temperature changes under eutrophic conditions



Appendix 5a *P. rutilus* % turion germination under eutrophic conditions for different light regimes



Appendix 5b *P. rutilus* growth experiment water temperatures