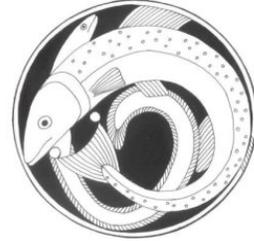




Lochaber Fisheries Trust Ltd



The effects of freshwater cage culture on the diet, growth and condition of brown trout (*Salmo trutta* L.) in Loch Shiel, Scotland

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Abstract:

This study compared diet, length-weight relationship, condition factor, and growth of brown trout in Loch Shiel, an oligotrophic loch in the west of Scotland. This was carried out with the objective of assessing the possible impacts of escaped feed waste from salmonid cage culture on the migratory behaviour of brown trout in light of diminishing anadromous sea runs. Comparisons were made between fish captured at two sites: (1) a site adjacent to Atlantic salmon smolt cages (farm site); and (2) a site 8 km from cage farming activity (control site). Farm site trout were composed of larger, older, fatter fish. The length weight relationship showed significant differences between sites with farm site trout becoming comparatively more rotund as they grew. Stomach contents identified that feed waste represented 33% of the immediate diet, whilst a carbon (C^{13}) and nitrogen (N^{15}) isotope multi-source mixing model estimated the contribution to be 48.5% of the assimilated diet (over previous weeks to months), supporting the hypothesis that feed waste forms part of the diet of farm site trout. Comparisons of growth through back-calculations from scales revealed higher rates in each of the first three years for the farm site trout, with the second and third years being significantly so. The condition factor (K) of farm site trout ($K=1.02$) was also significantly greater compared to that of the control ($K=0.89$). In addition isotopic analysis of organisms collected through benthic and pelagic sampling showed elevated signatures at the farm site consistent with feed waste entering the local food web. It is thought that the increased growth and improved condition identified in inhabiting fish farms may either cause a plastic response in otherwise anadromous trout to remain in freshwater, and/or cause migrant trout that return to their natal waters to switch to resident behaviour.

TABLE OF CONTENTS

	Page No.
1.0 INTRODUCTION _____	5
1.1 Nomenclature _____	5
1.2 Salmonid cage culture _____	6
1.3 Salmonid cage culture: The impact on wild fish _____	7
1.4 Trout reproduction and growth _____	8
1.5 Migrate or stay? _____	9
1.6 Loch Shiel _____	10
1.7 Techniques _____	11
1.8 Aims and Objectives _____	14
2.0 MATERIALS AND METHODS _____	15
2.1 Sampling site and collection _____	15
2.2 Laboratory methods _____	17
2.3 Statistical analyses _____	22
3.0 RESULTS _____	25
3.1 Population structure _____	25
3.2 Length-weight and length-girth relationships _____	32
3.3 Condition factor _____	32
3.4 Growth _____	35
3.5 Stomach contents analysis _____	38
3.6 Stable isotope analysis _____	41
4.0 DISCUSSION _____	51
4.1 Diet source and composition _____	51
4.2 Benthic and pelagic sampling _____	55
4.3 Growth and condition _____	57
4.4 Does cage waste influence trout migration? _____	58
4.5 Conclusion _____	59
4.6 Limitations of the study and future work _____	60
5.0 ACKNOWLEDGEMENTS _____	61
6.0 REFERENCES _____	62
7.0 APPENDICES _____	66
7.1 CD ROM. Raw data _____	66

LIST OF FIGURES

Figure 1 Map of Loch Shiel and its position in relation to Scotland..	16
Figure 2 Digital photograph of a trout captured at the farm site.....	18
Figure 3 Scale from a 4 year old trout (length 34 cm) captured at the farm site..	21
Figure 4 Relationship between standard length (cm) and scale radius for all trout captured at Loch Shiel.	21
Figure 5 Length frequency percentage distribution of brown trout..	27
Figure 6 Weight frequency percentage distribution of brown trout.....	27
Figure 7 Age frequency percentage distribution of brown trout.....	28
Figure 8 Linear regressions with log transformed data of standard length (SL) and weight (W)	29
Figure 9 Linear regressions with log transformed data of girth (G) and standard length (SL) between farm (separated into identified and non-identified pellet feeders) and control sites.....	30
Figure 10 Linear regressions with log transformed data of girth (G) and standard length (SL) between farm and control sites. Photographs of trout that fit the relationship are shown along with fish with both positive and negative residual values.	31
Figure 11 Relationships between condition factor (K) and standard length	33
Figure 12 Condition factor estimation based on mean (\pm SD) for separated groups...	34
Figure 13 Condition factor estimation between age groups	35
Figure 14 Mean (\pm SD) of back calculated growth at age for farm and control sites..	36
Figure 15 The relationship between trout age and length, (a) calculated from scale annuli, and (B) calculated from whole fish measurements (cm) and age at capture.	37
Figure 16 Overall diet composition of trout	38
Figure 17 Feed pellets in the stomach of a farm site trout.	39
Figure 18 Diet composition of trout per size class (cm)	40
Figure 19 Liver and muscle N ¹⁵ isotope ratios of trout.....	42
Figure 20 Muscle and liver C ¹³ isotope ratios of trout.....	42
Figure 21 Combined δ^{13} C and δ^{15} N signatures of liver (A) and muscle (B).....	45

Figure 22 Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}\%$ signatures of prey items and other organisms (fish liver).....	48
Figure 23 Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}\%$ signatures of prey items and other organisms (fish muscle).....	48
Figure 24 Comparison of average percentage contribution of food items to the diet of farm site trout.	50
Figure 25 Histograms of C^{13} and N^{15} of liver and muscle tissue.....	54

LIST OF TABLES

Table 1 Summary of fish caught.....	26
Table 2 Number; mean standard length, weight, and girth of brown trout caught	26
Table 3 Linear relationship of log transformed data of length and weight	29
Table 4 Linear relationship of log transformed data of length and girth	31
Table 5 Relationship of condition factor and standard length for brown trout from the two sites.	33
Table 6 Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope signatures from trout with the results of statistical analyses.	43
Table 7 Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope signatures from trout with the results of statistical analyses.	44
Table 8 Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}\%$ signatures of organisms collected in the plankton and benthic sampling, and stomach of trout.....	47
Table 9 Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}\%$ signatures of liver and muscle	47
Table 10 Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of sources used in the Isosource mixing model along with target mixture	50

1.0 INTRODUCTION

The trout species, *Salmo trutta* L. is a highly polytypic salmonid that often exhibits both anadromous, marine migratory (sea trout), and non-anadromous, freshwater resident (brown trout) individuals from within a population. Loch Shiel in the north west of Scotland has shown a decline in sea trout which has been accompanied by an apparent increase in large brown trout (Mackay 2008). These changes have coincided with the implementation of salmonid cage culture fish farming on the loch, which has led to suggestions of a causal link (Elberizon & Kelly 1998, Welch 2002, Baum & Smith 2008). One effect of salmonid fisheries is the potential impact of farmed fish feed material escaping the smolt cages and entering the local food web. This may create an artificial food source for the local fish population (Kilambi et al. 1978, Cornel & Whoriskey 1992). Freshwater loch or lake, cage culture of salmonids is recognised as a significant source of organic waste, which increases levels of nutrients (Cornel & Whoriskey 1992, Elberizon & Kelly 1998), enriching lake sediments and altering both the community composition and population structure of organisms (Phillips et al. 1985, Carss 1990). Uneaten pellets, faeces, scales and mucus may be taken up by trout through either direct consumption or by consuming lower trophic prey that have utilised the waste material themselves. This artificial resource may benefit the local trout population by increasing growth rate, possibly causing otherwise anadromous trout to remain resident, and not the natural expectation of a seaward migration. Trout, in particular sea trout support important and valuable sport fisheries that represent significant conservation value (Harris 2001). As such the need to address their decline is of major importance. This report sets out to identify the effect of cage culture fish farms on the diet, growth and condition of brown trout in Loch Shiel. By quantifying these effects it is hoped that more will be understood about the influence of fish farms on migratory behaviour in this species.

1.1 Nomenclature

Due to the lack of universal terminology in describing different forms of *Salmo trutta* (L), interpretations of the various names are defined here to avoid confusion to the reader: Within this review the term trout has been adopted to broadly represent all forms of this species as a whole; brown trout as representing all variations of the non-migratory form; and sea trout as representing the fully anadromous (migratory, sea going) form. Other terms have been used, but as more specific variations to those above. This is purely for the convenience of the writer, as conflicting terms exist in the literature.

1.2 Salmonid cage culture

The farming of salmonids in Scottish freshwater lochs expanded rapidly since the 1970's, and is now of considerable importance to parts of Scotland, bringing much needed employment and economic revenue to remote, rural areas (Harris 2001). However, this has been at a cost: Increases in salmonid production along the west coast of Scotland has coincided in a marked reduction in the abundance of wild *Salmo salar* (Atlantic salmon), and sea trout (MacLean & Walker 2002). These reductions have mainly been attributed to increased sea lice burdens due to intensive stocking practices of farmed salmon, and genetic integration and competition from escaped fish (Costello 2009). Cage culture is a method of rearing fish within an enclosed netting or mesh structure held within a large body of water, *i.e.* the coast or a lake. This system has several advantages over tank-based culture, including lower running costs, and relatively simple management (Phillips et al. 1985). Cage farms producing Atlantic salmon (hereafter' salmon) smolts are now found in over fifty lakes (Hennessy et al. 1996). Salmon smolts are produced for the purpose of supplying marine sites with new fish to be fed on to a marketable size (Hennessy et al. 1996). The rearing of salmon in freshwater requires high water quality, and a preference to sites in close proximity to marine facilities. This has led to a large concentration of freshwater salmon farms in the west coast of Scotland. The requisite for high water quality however creates a possible conflict, as the usage of fish farms, through many factors could reduce the water quality, at least within its locality (Hennessy et al. 1996). The

greatest concern in terms of waste output from cage fish farms relate to the feed pellets fed to the fish. Many studies have shown that the losses of feed from cages are considerable and reflect a high proportion of the total waste (Munro 1990). Estimates of feed passing through salmonid cages uneaten ranges from 5 - 20% (Hennessy et al. 1996) to 30% (Phillips et al. 1985). It has been calculated (Phillips et al. 1985) that 150-300 kg of waste feed and around 250-300 kg of faeces (dry weight) are introduced into the surrounding environment for every tonne of fish (inferred from rainbow trout). Reasons for this wastage include supply in excess of need, poor ingestion rates due to feeding practices and inefficient stocking densities (Munro 1990). As such feed wastage is an important contributor of effluent water and unintentional fertilisation of the lakebed. In addition, antibiotics and other therapeutants can enter the local environment through bath treatments, being uneaten in the feed or by passing through the fish in the faeces (Brown et al. 1987, Baum & Smith 2008). Plasmids with resistance to specific antibiotics used in fish farming have been shown to be more common in bacteria found in sediment beneath cages (Munro 1990). Salmonid farms are reliant on the use of compacted pellet forms of feed that are highly nutritious, increasing growth rate and reducing the age at which the fish smolt (Hennessy et al. 1996, Dempster et al. 2004, Costello 2009). Within marine sites, increasing effluent nutrient output is to some extent mitigated by the high water volume and flushing rates. This replenishes oxygen levels and dilutes the wastage (Hennessy et al. 1996). In contrast, lake systems used for aquaculture have smaller water volumes and much lower flushing rates. As such, freshwater sites are far more susceptible to the effects of cage wastage than marine sites (Cornel & Whoriskey 1992). Studies have identified that increased nutrient content in the vicinity of cages can change the population structure of benthic organisms (Kilambi et al. 1978). Different species of organisms do not respond in the same way to nutrient input (Brown et al. 1987), and as such, it can be expected that the composition of organisms within an affected area will alter (Cornel & Whoriskey 1992).

1.3 Salmonid cage culture; the impact on wild fish

There has been very little information published on the effect of aquaculture farming waste on fish populations, and even less specifically on freshwater systems. Also most

of the published research that has been carried out was produced more than a decade ago. Nonetheless, there is much evidence from this research that cage culture can enhance growth of indigenous fish (Rocha & Mills 1984, Phillips et al. 1985, Cornel & Whoriskey 1992). Growth enhancement of trout and particularly charr has occurred in other lochs where fish rearing cages are present (e.g. Arkaig, Garry, Earn) (MacLean & Walker 2002). The increased growth has been attributed to either the consumption of escaped feed pellets, and/or consuming a higher rate of prey organisms that increase in number through fertilisation of the water body (Kilambi et al. 1978, Phillips et al. 1985).

There can be little doubt that introduced feed waste can alter the behaviour of wild fish. A reliable and nutritious food supply, with minimal expenditure needed to acquire it will certainly offer advantages over 'natural' food or prey.

1.4 Trout reproduction and growth

Salmo trutta is part of the family Salmonidae, which is subdivided into three subfamilies of which seven genera exist. Within the genera *Salmo*, *Salmo trutta* is one of 29 species (Harris 2001). *Salmo trutta* (hereafter 'trout') spawn in well oxygenated open systems, usually their natal stream, from autumn to winter. Fertilised eggs are left buried under substrate to develop for a period of time (one to three months, depending on temperature), hatching in the subsequent spring as alevins. The alevin are initially fuelled by a yolk sac and move up from the substrate once this is absorbed. This is followed by a period of residency in freshwater, where young trout known as parr, establish territories and compete aggressively for resources (Campbell 1977, Harris 2001). Those that successfully feed and survive, disperse as they grow with increasing need for space and more plentiful food. The brown trout will from this point onwards remain in freshwater for its whole life cycle, whereas the sea trout will at some point, depending on its growth rate, undergo biochemical, physiological and morphological changes known as smoltification (Klemetsen et al. 2003). During this period sea trout alter their appearance, and develop a tolerance for seawater, before migrating downstream to enter the sea. They will remain at sea for a variable number of years depending on their growth before returning to freshwater to breed, which they may do several times. Trout growth and size between individuals and

populations can vary considerably (Harris 2001, Klemetsen et al. 2003). Resident brown trout tend to be smaller, slender and brownish, whilst anadromous sea trout tend to be larger, deeper bodied and silver in colouration. Fish at four years of age can vary in weight from 20 g in brook resident brown trout to 1 kg in anadromous sea trout, depending largely on food consumption and temperature.

1.5 Migrate or stay?

Spatial and temporal differences in survival and growth between habitats are major contributors to the development of migratory behaviour (Olsson et al. 2006). The ability for genotypes to react to cues in the environment has been viewed as an adaptation to changing conditions (Jonsson 1982, Skaala & Naevdal 1989, Pakkasmaa & Piironen 2001). Within the salmonids, anadromy (migration to the sea) is linked to better feeding and reproductive success (Elliott 1975, Harris 2001). However, the major costs include increased predation from a suite of marine predators (including man), and a highly energetic migration (Etheridge et al. 2008). Anadromous sea trout and resident forms often live in sympatry within early life stages and breeding periods. Despite clear differences in their life cycle, the two forms produce morphologically identical progeny, that can interbreed producing fertile, hybrid offspring (Charles et al. 2005), thus can be viewed as fractions of the same stock (Klemetsen et al. 2003, Etheridge et al. 2008). Due to this, trout are thought to be highly polymorphic, plastic species that exist in many different forms in response to different habitats (Harris, 2001). It is thought that migratory behaviour is dictated by a combination of genetic and environmental controls, such as food availability and temperature (Nordeng & Bratland 2006). Morphological differences usually occur when habitats favour specific abilities, and the degrees in which differences exist are expected to be reflected this disparity between environmental conditions (Harris, 2001). However, genetic differentiation between anadromous and resident trout has as yet not been identified (Charles et al. 2005, Olsson et al. 2006). In other salmonid species clear genetic differences have been found between migratory and resident forms, but research to date on trout has shown no such clear divergence (Charles et al. 2005). Despite this, much of the recent literature seems to accept that there must be an important measure of genetic control over the extent to which different forms exist in

different or changing environments (Skaala & Naevdal 1989, Harris 2001, Pakkasmaa & Piironen 2001). However, behavioural studies have shown clear results of environmental factors influencing decisions on migration. In an experiment from Olsson et al. (2006), trout were reciprocally transplanted between two sections of the same river; migratory behaviour developed in a river section where trout density was high and growth rates low, whereas non-migratory, resident behaviour developed where trout density was low and growth rate was high. This was followed up with experiments in the lab, where the availability of food was tested to see if it influenced development into either migrant or resident forms. Trout were found to be more likely to become migrant when food levels were low (87%), compared to when food levels were medium or high (56% and 57%). These results showed that, high food availability (subsequent growth) influences anadromous behaviour, and that migration is an adaptive response to environmental conditions.

1.6 Loch Shiel

Loch Shiel (Figure 1) is a 20.2 km² oligotrophic glacial freshwater loch in south Lochaber, Highland Scotland (Bennion et al. 2004). It is 28 km long with a mean breadth of only 0.7 km. It lies in a North-east/south-west direction; with a westerly bend at the lower end that discharge out to the sea into Loch Moidart via the only outflow, the 4 km River Shiel. Along its length are two basins connected by a narrow channel. The overall mean depth is 40.5 m, with its deepest point being 128 m within the upper basin, a flooded glacial trough. The lower basin is smaller and shallower with many sheltered bays and sand banks. Its primary inflows are the rivers Finnan, Callop and Polloch. The naturally nutrient poor status of the loch is key to its characteristic flora and fauna. Emergent vegetation is sparse, but there are submerged communities of macrophytes, typical of oligotrophic waters (Baum & Smith 2008). The loch supports Atlantic salmon as well as brown and sea trout, eels (*Anguilla anguilla*), three-spined sticklebacks (*Gasterosteus aculeatus* L.) and minnow (*Phoxinus phoxinus*). The loch is designated as a SSSI for its oligotrophic loch interest and a Special Protection Area (SPA) for its breeding population of black-throated diver (*Gavia arctica*). The loch was well known for its sport fishing (coarse and fly) for salmon, and sea trout, with the River Shiel being once regarded as one of

the finest rivers on the west coast for such activities (Mackay 2008). Unfortunately, there has been increasing concern in recent years over the general decrease in both salmon and sea trout over the past 30 years in the west coast of Scotland (MacLean & Walker 2002, Mackay 2008). Data from rod catches (MacLean and Walker 2002) has shown the decline in sea trout to be mirrored on Loch Shiel. This decline coincides with the implementation of cage culture fish farming on the loch which is linked to increases in nutrients through organic waste (Hennessy et al. 1996). Oligotrophic lakes are by definition low in nutrient content and primary productivity. The major artificial source of nutrients within the loch is from fish farming. The nutrient cycling of large, deep lochs is little understood but phosphorous is thought to be an important nutrient determining productivity (Welch 2002). There are currently two sets of salmon smolt cages on the loch found at the northern and southern sites. Anecdotal evidence from various sources, such as anglers and the Sea Trout Group (STG), relating specifically to Loch Shiel, suggests that the presence of smolt rearing cages is significantly altering the lochs ecology. Also trout caught close to the cage complexes grow larger and fatter than normal (Mackay 2008). A major concern is that cage wastage could create a reliable source of high nutrient food for trout that may stimulate increased growth over 'natural' conditions, and alter the natural selection process by influencing trout towards non-migratory behaviour (Mackay 2008). Salmonid fish stocks are one of the major resources for recreational and commercial fishing, generating substantial economic benefits to rural and urban communities (Harris 2001). Anadromous species are also seen as bio-indicators of environmental water quality. Thus a need to understand the effects of smolt cage salmonid farming on the migratory behaviour of trout is paramount.

1.7 Techniques

The use of stomach contents analysis (SCA) has in the past been the sole determinant of fish diet in relation to the effects of freshwater smolt cages. This method is a very useful approach, and has proved successful in helping to determine the recent diet of fish prior to capture. However, the diet of trout is particularly seasonal (Grey 2001), and the 'snap shot' approach of SCA is unable to tell if captured fish are consuming waste products over a longer period of time, and to what extent. There are other

weaknesses to this approach on its own, such as variations in food assimilation rates and misidentification through partial digestion. Other laboratory procedures have been employed to identify a marine from freshwater diet in fishes, such as the analysis of strontium content of scales and carotenoid pigment profiling (Choubert et al. 2008, Waite et al. 2008), but the study of naturally occurring stable isotopes has become the most robust and practical method. Stable isotope analysis (SIA) of carbon and nitrogen has been for some time used as a tool to help answer questions in ecology. Stable isotopes are atoms of the same element that are identical in chemical and physical properties except those determined by mass. For example carbon: C^{12} has a mass of 12 (6 protons and 6 neutrons), whilst Isotope: C^{13} has a mass of 13 (6 protons and 7 neutrons). The abundance of naturally occurring stable isotopes of $C^{13} = 1.11\%$ ($C^{12} = 98.89\%$), and $N^{15} = 0.336\%$ ($N^{14} = 99.633\%$) (Cherel 1999). Their use in ecology lies in the fact that stable isotope ratios in proteins of consumers reflect those of their diet in a predictable, systematic manner (Bearhop et al. 1999, Hobson 1999, Bearhop 2004). Expressed as $\delta^{15}N$ (‰) the (δ) “delta notation” is used to express that the obtained values were determined by a comparison against a recognised standard. The ratio of ^{15}N to N^{14} exhibits a stepwise enrichment (increased in the value of $\delta^{15}N$ at each trophic level (Post 2002). This stepwise enrichment occurs because of isotopic fractionation, the process of differential partitioning of isotopes between two compounds, (such as from prey to consumer), due to preferential excretion of the lighter isotope. Consequently the $\delta^{15}N$ values in the tissues of consumers increase by $\delta^{15}N = 3.4\text{‰} \pm 0.98\text{‰}$ (Hobson 1994, Post 2002). As such, nitrogen is an excellent indicator of trophic level, and is frequently used as the primary method for understanding trophic relationships. Carbon also increases with each trophic level, but to a lesser degree, enriching by $\delta^{13}C = 1.0\text{‰} \pm 1.30\text{‰}$, with each trophic level (Hobson 1994, Post 2002), and as such is less useful in determining trophic level due to its sensitivity to other factors. However, because of this relatively small $^{12}C/^{13}C$ alteration, carbon isotope ratios can be used to identify the source of food (Rounick & Hicks 1985). This is particularly useful in understanding animals that migrate between marine and terrestrial environments. C^{13} values are distinctly different between freshwater and marine environments, with a gradient existing between the two habitats (Bearhop et al. 1999, McCarthy & Waldron 2000, Etheridge et al. 2008), which correlate with salinity (Etheridge, 2008). This baseline difference in $C^{13}\text{‰}$ is due to the difference between bicarbonate (fixed carbon in marine sources) and

carbon dioxide (fixed carbon in terrestrial/freshwater sources) (Bearhop et al. 1999). This allows for the diets of animals to be easily distinguished between environments, and has been carried in numerous studies to infer feeding location (Rounick & Hicks 1985, Bearhop et al. 1999, McCarthy & Waldron 2000, Etheridge et al. 2008, Moreno-Rojas et al. 2008). The sources of feed material in salmon feed pellets are of marine origin (fish meal, oil) and therefore show a distinct difference in C^{13} compared to freshwater sources. Values of C^{13} are conserved up the food chain, but vary at the base of the food chain, thus the δC^{13} of aquatic consumers can provide information about the sources of energy to higher consumers (Vander Zanden & Rasmussen 1999). Different tissues can integrate dietary information over different time scales (e.g. weeks, months, years) as different tissue types represent different metabolic pathways (Hobson 1998, Pinnegar & Polunin 1999, Thompson et al. 2005). Liver changes rapidly and thus responds quickly to changes in diet (days to weeks), but is also sensitive to other factors, such as the condition of fish at the time of sampling (Trueman et al. 2005). White muscle turnover (growth, dilution and repair) equates to months on a temporal scale, giving a longer-term perspective (Sweeting et al. 2005). By combining the techniques of SCA and SIA, the stomach contents can provide information on recently (within hours) consumed food items of fish which may be identified with high taxonomic resolution, whilst the stable isotope signatures of liver and muscle tissue may infer the diet source of fish from days to months.

However, to avoid misinterpretation of SIA, many considerations are required, such as spatial, temporal and ontogenetic variability for both predator and prey feeding behaviours (Christensen & Moore 2009). Also the productivity of the system and any potential sources of carbon that may influence isotopic values should also be taken into account (Vander Zanden & Rasmussen 2001, Vadeboncoeur et al. 2002). In determining diet, the more possible food sources identified and isotopically tested, the greater the confidence in the subsequent interpretation.

Length-weight relationships (LWR) in fisheries biology are important because they are able to describe mathematically their relationship, allowing for an estimation of weight from a given length (Ayoade & Ikulala 2007). They are also useful for assessing the relative physical condition of fish populations, and for comparing populations of a particular species between different environments. Measuring the expected weight for length of fish can have indications of fatness and general well

being. This measure of condition, referred as “K factor” is usually influenced by age, season, maturity and sex, but also the environmental conditions that the fish are exposed to such as temperature and food resources. The growth and condition of trout has been described as variable with habitat, and quantity and type of food (Forseth & Jonsson 1994, Gabrielsen 1999). Thus the condition factors of fish living in a food rich environment should be expected to be higher than fishing living in a relatively food poor environment, if other parameters are equal.

An important factor that may determine migratory decisions is early growth and condition. Sea trout typically smolt after one to four years residency in freshwater (Labeelund et al. 1989). However, if food availability is high (equating to a potential increase in growth and enhanced conditioning), the advantages for trout to remain resident over seaward migration are increased (Olsson et al. 2006). This may reduce the proportion of anadromous trout within the population. Thus growth rates and condition of trout are perhaps good measures of the likelihood of a seaward migration.

1.8 Aims and objectives

Using condition and growth as measures of suitability for remaining resident in their locality. This report aims to provide data on the LWR, condition factor, and diet (SCA and SIA) of trout in Loch Shiel that may aid in understanding effects of smolt cages on wild trout.

All parameters mentioned were examined and compared from catches of trout between two separate sites on the loch. The first site located in close proximity to smolt rearing cages (farm site), and a second site located at the furthest point from cages on the Loch (control site). In addition, organisms collected from benthic and pelagic sampling were tested isotopically, giving an estimate of cage waste effects on the base of the food web. Specifically selected food items found within the stomachs of trout were tested isotopically, along with specimens of prey collected in the field through sampling procedures. This allowed for the evaluation of the diet of trout using a multiple source mixing model, Isosource (Phillips & Gregg 2003). Data collected from sampling at Loch Shiel were utilised to answer the following research objectives:

Objective 1: Determine the composition of trout diet from both farm and control sites through (a) stomach contents, and (b) stable isotope analysis in conjunction with mixing models.

Hypothesis 1: Feed waste will be identified as a diet component in farm site trout from (a) the stomach contents, and (b) significantly enriched (marine signal) C¹³ signatures in trout liver and muscle tissue, including the output from mixing models.

Objective 2: Determine if identified trout prey species are consumers of feed waste.

Hypothesis 2: Sampled organisms (from the farm site) identified through the stomach contents of farm site trout will exhibit enriched (marine signal) C¹³ signatures indicative of assimilation of feed waste.

Objective 3: Determine if farm site trout exhibit (a) higher growth rates (0-3 years), and (b) better condition factor values over the control site trout.

Hypothesis 3: Trout collected from the farm site will show significantly higher (a) growth rates, and (b) condition factors than the control site trout.

The results of these objectives are discussed in the context of the influence of fish farms on the migratory decision on Loch Shiel trout.

2.0 MATERIALS AND METHODS

2.1 Sampling site and collection

The study was conducted between the 14th and the 20th of June 2009 at Loch Shiel, South Lochaber, Highland, Scotland. 56° 50'N, 5° 28'W.

2.1.1 Wild Fish

Two sites were chosen for the study (Figure 1), one at the northern cages at Carn Mhic Labhraich (56° 51'36. 84"N, 5 ° 27'30. 03"W) and a second at a control site at the midpoint of the loch (56° 48'24. 90"N, 5 ° 32'29. 10"W), the furthest point from the two cage systems. Salmonids were sampled using two multi-mesh gillnets with 1.8 m

vertical panels and varying mesh size, which catch a range of fish, 5-50 cm, so as to be representative of the population. Gill nets were set perpendicular to the shore and extended to depths around 15-20 m and were left for approximately 16 hours overnight. European eels were sampled using two fyke nets that were sunk to depths around 15 m. Attempts to capture minnow and three-spined stickleback using electro-fishing from the shoreline of each site was unsuccessful, however further eels and juvenile salmonids were recovered.

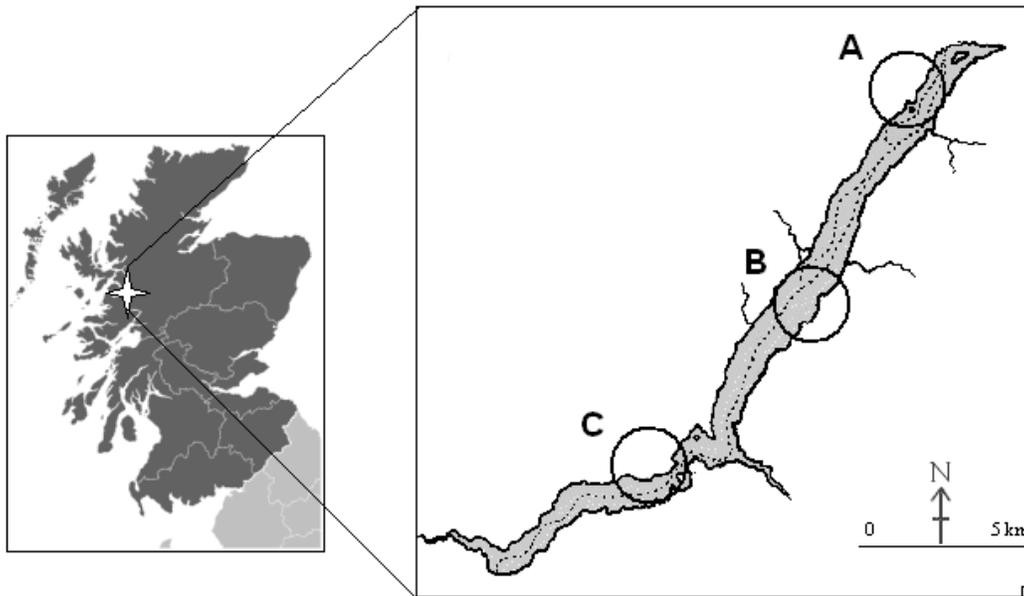


Figure 1 Map of Loch Shiel and its position in relation to Scotland. Including the position of the (A) farm and (B) control sampling sites, and (C) the position of southern smolt cages on the loch.

Farmed Salmon

A total of 15 farmed salmon were also collected from cages at Carn Mhic Labhraich so as to gain a reflection of isotopic values of a closely related species being fed solely on feed pellets. The salmon were given an overdose of anaesthetic, as were fish that were still alive upon capture. Freezing within 4 hrs of collection preserved all fish.

Feed pellets

Feed pellets used at the farm were collected for later analysis of isotopic values, allowing for an accurate measure of fractionation from comparisons in the farmed fish and an estimate of feed diet expressed in the signatures of wild fish.

Food web sampling

Plankton was sampled from areas of open water at both sites towing a plankton net (mesh size 110 μm) from an outboard powered dinghy travelling at approximately five km per/hr. Plankton was then emptied into plastic screw top sampling bottles. Benthic sampling was carried out at the farm site by dropping an Eckman grab sampler to the loch bed from a smolt cage platform to depths of approximately 15-20 m. Grab samples at the control site were taken from a dinghy at the same approximate depth. The samples were then emptied out into buckets before being washed through a series of sieves, separating the invertebrate fauna from the sediment, before being placed into screw top sampling bottles. All sampled organisms were frozen within 3 hrs of collection for preservation.

2.2 Laboratory methods

Fish samples

All fish collected were rinsed clean in deionised water, and identified to which species by visual observation. Removing the gonads and visually comparing them with photographs of male/female salmonid gonads at various stages of maturity identified gender. This was followed by measurements in cm from the tip of the snout to the tip of the caudal peduncle (SL) using a measuring board, and weight in g using an electric balance. A digital photograph was then taken of each individual fish with its unique identification according to the site and method of capture for later determination of fish girth (Figure 2), through digital analysis using ImageJ free software. Following this approximately 15 scales were removed from an area midway between the posterior of the dorsal fin and the lateral line (trout only). Scales were

removed by either scraping the skin with a knife or by plucking individual scales using tweezers, depending on their size. The scales were then placed in brown paper scale envelopes and stored for later analysis to determine growth (see age and growth, laboratory methods).

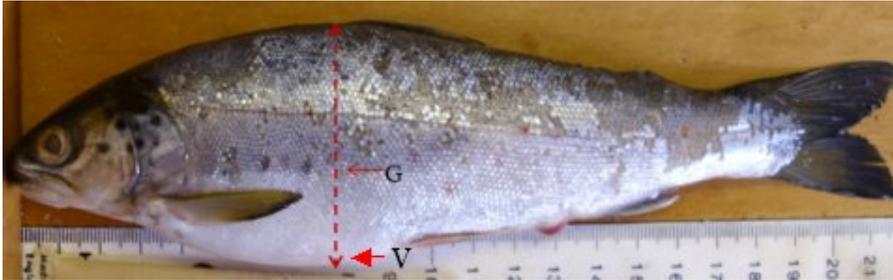


Figure 2 Digital photograph of a trout captured at the farm site. Girth (G) measurements were taken of each trout using ImageJ free software. Fatty visceral area (V) also highlighted.

Tissue preparation prior to stable isotope analysis

Approximately 1-2 g (wet weight) of white muscle tissue was extracted from the left side of each fish below the dorsal fin and above the lateral line. A further sample of liver (the whole liver if less than 1 g) was extracted from each fish and weighed using an electric balance, and immediate freezing preserved both tissue samples.

Stomach contents analysis

Fish had their stomachs removed (Figure 17) and the contents recorded as frequency occurrence of each prey item in all non-empty stomachs. Each item was identified to the highest possible resolution by visual observation through a binocular microscope and the use of taxonomic keys, from either; Macan (1963) or Croft (1986). Items were then separated into categories: (1) Insects (Chironomidae, Gyrinidae, and Notonectidae), (2) feed pellets, (3) fish (partly digested, presumably salmonid), (4) other invertebrates (Oligochaetes and *Strigamia maritima*), (5) zooplankton (*Daphnia* spp. and *Canthocamptus* spp.), (6) plant matter, (7) fish scales, and (8) unknown. Invertebrates with their exoskeleton still intact and possible pellet material found within trout stomachs were washed in 10% hydrochloric acid (HCL), before being

rewashed in deionised water and preserved by immediate freezing for later preparation for isotopic analysis.

Food web sampling

Using taxonomic keys from either Macan (1964), or Croft (1986) organisms within the benthic and pelagic sampling were identified. Invertebrates that were identified in the stomachs of the fish and those at or close to the base of the food chain were washed in deionised water and preserved by freezing in preparation for SIA. Isotopic analysis of specimens allowed for comparisons of signatures of prey from both sites and to identify if species caught in close proximity to the farm site were utilising cage waste. In addition, SIA of primary (phytoplankton) and secondary producers (zooplankton) allowed for an estimate of base, or close to base values in δC and δN found in the loch at each site.

Final preparation for stable isotope analysis

All samples were thawed and rewashed with deionised water. Samples were then dried to a constant weight in a laboratory oven at 60° for approximately 72 hours, before being homogenized to a powder with a pestle and mortar. After which between 1.1 and 1.3 mg of mixed powdered tissue was weighed into tin capsules using an electro balance. Due to the small size of many invertebrate specimens, multiple individuals of the same species were pooled to obtain adequate dry weight.

Note: Tissues containing large amounts of lipid (such as liver) tend to be more C^{13} -depleted due to lipid synthesis (Deniro & Epstein 1981, Grey 2001). Thus it is often standard practice to extract lipid from the tissue before isotopic analysis. However the process of lipid extraction may alter nitrogen isotope ratios (Pinnegar & Polunin 1999). As this study was consistent in its procedures and not comparing results from data outside of this report, it was decided that lipid extraction would not be carried out.

Stable isotope analysis

Stable isotope analysis was carried out by UC Davis Stable Isotope Facility, USA using a PDZ Europa ANCA-GSL elemental analyzer in line with a PDZ Europa Hydra 20/20 continuous-flow isotope ratio mass spectrometer. All stable isotope ratios are reported in per mil (‰) using the δ notation according to the following equation:

$$X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

Where X is $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$, and R is the corresponding ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. R_{standard} for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ is the Pee Dee Belemnite and atmospheric nitrogen (AIR). Repeat analysis of an internal standard showed that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ can be measured with accuracy and precision of $\pm 0.2\%$.

Age and growth

Scales were removed from their envelopes, cleaned in water before being mounted between two microscope slides. Digital images of scales with clear, focused centres were taken using a digital camera mounted on a dissecting microscope. A graticule was taken next to each image in order to calculate its length. The age of the trout was determined by counting the number of annuli from each scale from the captured image. Size at age was estimated by back calculating trout length from the distance between annuli (Heidarsson et al. 2006) using ImageJ freeware (Figure 3). Whole scale radius was paired with measurements of standard length in a regression and showed a linear relationship (Figure 4), with a slope of 1.47 and y-intercept of 0.57. The R^2 value was 0.928. The slope of the relationship (1.47) was multiplied to the scale growth values (arbitrary units), so that growth was represented in standard length (cm).

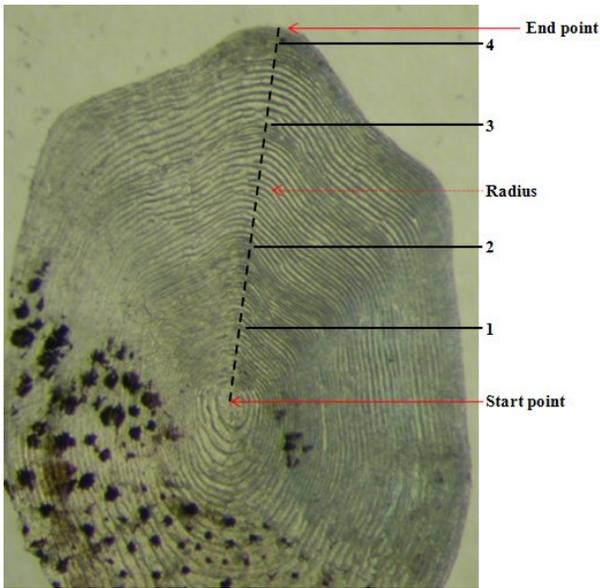


Figure 3 Scale from a four-year-old trout (length 34 cm) captured at the farm site. The radius start (S) and end (E) points are shown, together with annuli (1-4).

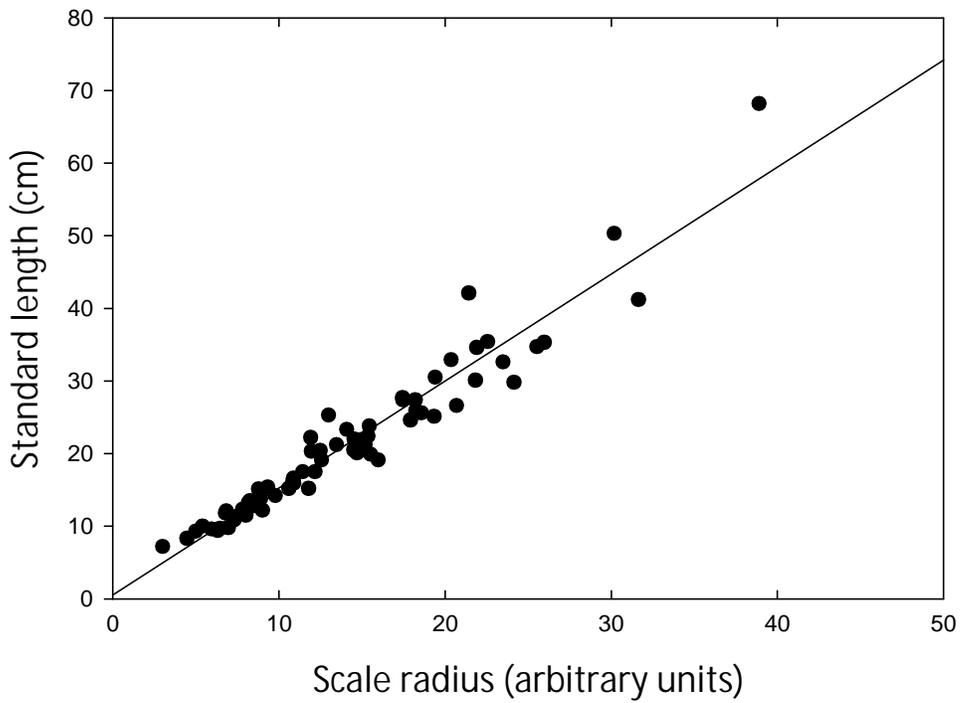


Figure 4 Relationship between standard length (cm) and scale radius (um) for all trout (farm and control sites) captured at Loch Shiel.

2.3 Statistical analysis

Catch data

Frequency distributions for age, length and weight were plotted for both sites. Catch per unit effort in hrs (CPUE (hours)) was calculated for the gill netting procedure, along with the number of fish caught and sex ratio of trout from both sites.

Length-weight, length girth relationship and condition factor

Standard length and girth measurements were used to estimate length-girth relationships for the two sites, using least squares regression. The slopes of the length-girth relationships were compared using analysis of covariance (ANCOVA).

The length-weight relationship (LWR) was estimated by using the equation: $W = aL^b$ where W = weight (g), L = standard length (cm), a = constant, b = growth exponent. A logarithmic transformation was used to make the relationship linear $\log W = \log a + bL$. For trout at each site, the slope of length-weight regression was compared to 3 using a student's t -test to ascertain whether growth was isometric. The slopes from the regression lines for each site were also compared against each other using ANCOVA. The values of the compiled growth exponent were used for the calculation of Fulton's condition factor K using the equation $K = 100 \times (W/L^b)$. Where K = condition factor, W = weight (g), L = standard length (cm), b = growth exponent (slope value taken from LWR.). A regression analysis using condition factor and standard length was used to determine if condition factor was independent of standard length for trout in Loch Shiel. Farm site trout containing pellets identified in the SCA were distinguished from the regression plot.

Condition factor was assessed for trout from the farm and control sites. Condition factor was then separated into age groups (age 1-3+) and tests for significant differences between sites were carried out using two independent t -test's once the variance was shown to be homogenous (Levene's test, $p > 0.05$). The overall condition factor for the farm site was then separated into three groups: (1) All trout at the farm site; (2) only trout containing pellets; and (3) all trout not containing pellets. These were then compared against each other and that of the control site using a non-

parametric Kruskal-Wallis test once the variances were shown not to be homogenous (Levene's test, $p < 0.05$), followed by Mann-Whitney U tests to determine from which groups significant differences existed.

Incremental growth

Incremental growth at age (years 0-3), and combined growth (years 0-3) from back calculations of scale analysis was compared for both sites using either two independent t-tests, if the variances were shown to be homogenous (Levene's test, $p > 0.05$), or Mann-Whitney U tests, if variances were shown not to be homogenous (Levene's test, $p < 0.05$).

Stomach contents analysis

A frequency of occurrence of each food/prey item category (insects; pellets; fish; invertebrates (other); zooplankton; plant matter; and scales) was plotted for both sites. In addition the composition of the diet of trout for different size classes were shown for each site. The stomach fullness of the trout was scored subjectively from 0 – 4, based on visual observation of the stomach (0 = empty, 1 = 0-25%, 2 = 25-50%, 3 = 50-75%, and 4 = 75-100%).

Stable isotope analysis

Linear regressions were used to determine relationships between C^{13} and N^{15} and length, and examine differences between the two trout populations

After performing a test for homogeneity of variance (Levene's test, $p > 0.05$), either a two independent sample t-test, or a Mann-Whitney U test was used to determine if differences existed in the signatures of C^{13} and N^{15} (liver and muscle tissue) for trout between sites.

Mean (\pm SD) isotopic signatures (δC^{13} and δN^{15}) of all organisms collected were plotted to represent a visual isotopic interpretation of the Loch Shiel food web at both sites.

Using a combination of SCA, SIA and a dual isotope, multi source-mixing model, estimates of the proportions of food items in the assimilated diet of trout were made. This was achieved by testing isotopically, food items captured in the field that matched those found in the stomachs of the fish. The level of fractionation used for the model, was identified through the observed difference in isotopic signatures between feed pellets, and farmed Salmon (fed solely on feed pellets). The Isosource model (Phillips & Gregg 2003) determines all possible solutions to the proportions of sources that make up a mixture (C^{13} and N^{15}). The programme achieves this by examining every possible combination by incremental adjustments of each source (that always total 100%). The resulting output identifies all viable solutions according to a set tolerance (mass balance tolerance, in this case set at 1%,) with the mean, maximum, and minimum proportion of each source in a viable mixture given. This was only possible for the farm site trout as an inadequate sample of food sources was collected from the control. A simpler single isotope, two source mixing model was also used to determine the relative contribution of carbon derived from freshwater consumers and marine signal feed waste (Phillips & Gregg 2001). This method assumes that only two sources exist, but allowed for a comparison between the two sites.

All statistical tests throughout this report were performed using SPSS v16. Graphs were produced using SigmaPlot v11.

3.0 RESULTS

3.1 Population Structure

A total of 84 fish were caught between both the farm and control sites, using gill nets, fyke traps and electro-fishing techniques. Identical gill netting procedures for both sites resulted in the capture of 38 trout (two were decapitated and excluded from the length/weight data) from the farm site, and 32 trout from the control site. The sex ratio of trout showed a disproportionate number of males at both sites, with a ratio of 61:39 at the farm site, and 63:37 at the control (Table 1). Eight age categories of trout were distinguished from the farm site (1+ to 7+, and one fish age 12+) and six from the control (0+ to 5+) by counting the number of annuli on each scale (Figure 7). Normally the age at which anadromous forms of salmonids migrate to sea can be told by the analysis of scales; since growth increases in these fish once they reach the marine environment (Treasurer, 1975). However, a large number of farm site trout that appeared to be of the resident form from their morphology and colouration, showed patterns of increased growth in their second, third, and fourth years, similar to what would be expected in anadromous fish. It was decided therefore that the observed increased growth might in fact be the result of a sudden utilisation of feed waste, not the result of a marine diet. Thus the analysis of scales was used for ageing and determining the growth of trout, and not to distinguish between forms. As such, all trout (resident or migratory) were grouped together.

The standard length for the farm site trout ranged from 9.3-68.2 cm, while the weight ranged from 12g – 7.38 kg (Figure 5 & 6). The control site trout were smaller by comparison with a standard length from 7.2-42.1 cm, and weight from 5-937 g (Figure 5 & 6). The mean length, weight, girth and age of trout were significantly higher at the farm site (Table 2). The weight frequency distribution highlights the disparity between sites. Farm site trout are far more evenly spread out across the weight categories, with only three categories not being represented across the size range. In contrast the control site trout show a majority peak (57%) in the weight class 0-49 g, with a reduction in the proportion of heavier categories, and no representatives from 250 – 649 g. However, a small percentage of the total were found in the two heaviest classes. The maximum weight attained was 7.3 kg, and 557 g for the farm

and control sites respectively. Fuller stomachs in the farm site trout may to some degree affect this (see stomach contents). The length, weight and age frequency distributions showed that the farm site trout population were older, larger and heavier than those of the control site.

Table 1 Summary of fish caught, including CPUE (fish per hour), for gillnetting procedure at both the fish captured in close proximity to the smolt rearing cages (Farm site) and those caught at the loch mid-point (Control site). ‘Other’ refers to fish captured using electro-fishing or fyke trapping techniques. ‘Combined’ refers to the total fish captured at each site.

Farm site					
Species	<i>N</i>	CPUE	Other	Combined	M: F% ratio
			<i>n</i>	<i>n</i>	
<i>S. trutta</i>	36	1.5	1	37	61:39
<i>S. salar</i>	2	0.1	2	4	
<i>A. anguilla</i>	1	0.05	6	7	

Control site					
Species	<i>N</i>	CPUE	Other	Combined	M: F% ratio
			<i>N</i>	<i>n</i>	
<i>S. trutta</i>	32	1.3	1	3	66:34
<i>S. salar</i>	0	0	2	0	
<i>A. anguilla</i>	0	0	6	1	

Table 2 Number; mean standard length, weight, and girth of brown trout caught from the farm and control sites.

	<i>n</i>	Mean SL (cm)	Mean weight (g)	Mean girth (cm)	Mean age (years +)
Farm site	36	25.21	556.9	6.78	3.56
Control site	32	16.8	114.28	3.71	2.31

All parameters were significantly different between sites: Length (values double log transformed, two sample *t*-test, $t = -3.693$), $p = 0.001$; weight (values log10 transformed, two sample *t*-test, $t = -4.163$), $p = 0.001$; girth (values double log transformed, two sample *t*-test, $t = 4.951$), $p = 0.001$; age (Mann-Whitney U test) $p = 0.005$.

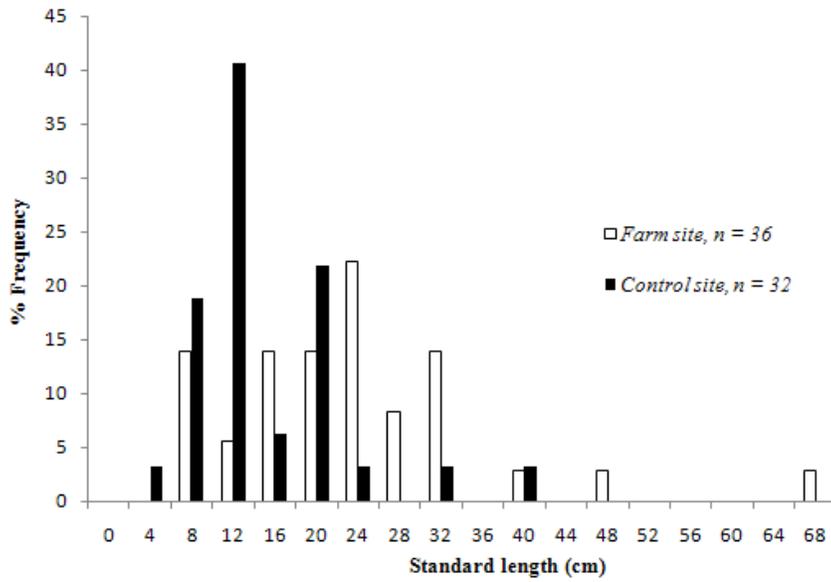


Figure 5 Length frequency percentage distribution of brown trout. n = number of fish.

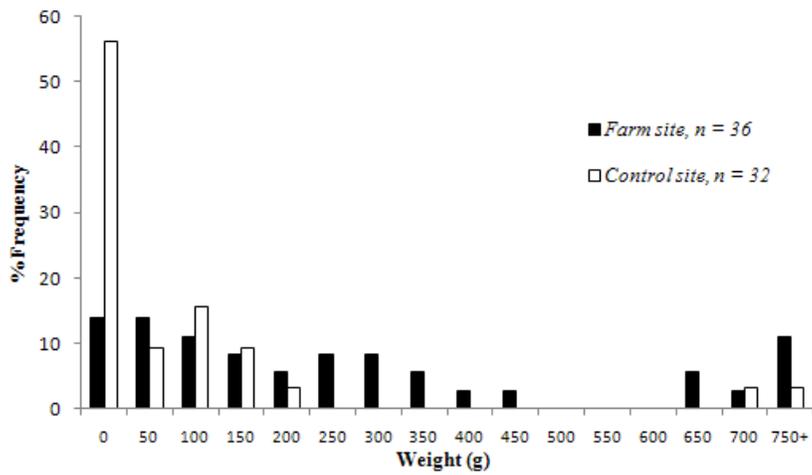


Figure 6 Weight frequency percentage distribution of brown trout. n = number of fish.

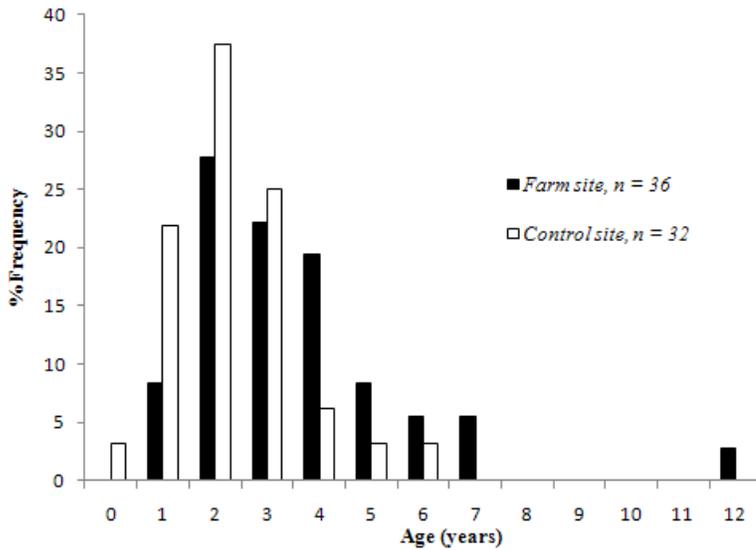


Figure 7 Age frequency percentage distribution of brown trout. n = number of fish.

3.2 Length-weight and length-girth relationships

The results of length-weight analysis are presented in Table 3, and Figure 8. Both farm and control site trout length-weight relationships were highly significant ($p = 0.0001$). The length-weight relationships obtained for farm and control site trout were significantly different (ANCOVA, $F = 14.96$, $p = 0.005$) $\log(W) = 3.11^* \log(SL) - 1.9303$; $R^2 = 0.9862$, $n = 36$, for the farm site; $\log(W) = 3.061^* \log(SL) - 1.9377$; $R^2 = 0.9889$, $n = 32$, for the control site). Despite significant differences between sites, both exhibited allometric growth with ‘ b ’ values not significantly different from 3. Farm site trout had a higher b value, with trout becoming more rotund as they grow. This is supported up by the significantly different length girth relationship between trout at each site, (ANCOVA, $F = 24.77$, $p = 0.001$) $\log(G) = 1.15^* \log(SL) - 1.9303$; $R^2 = 0.914$, $n = 36$, for the farm site; $\log(G) = 1.039^* \log(SL) - 1.9377$; $R^2 = 0.939$, $n = 32$, for the control site). This shows that the girth of trout at the farm site increases to a higher degree with length (Figure 9, and Table 4). However, the fuller

stomachs of the farm trout may to some degree affect this (see stomach contents). A visual representation of the differences in girth is displayed in Figure 10.

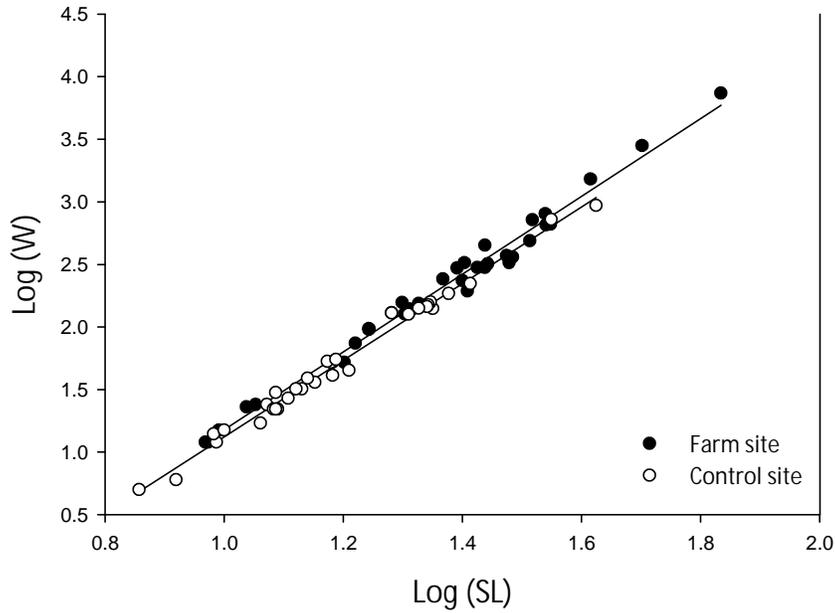


Figure 8 Linear regressions with log transformed data of standard length (SL) and weight (W) between farm and control sites.

Table 3 Linear relationship of log transformed data of length and weight for brown trout from the two sites.

	<i>n</i>	Slope (S.E)	Intercept	R ²
Farm site	36	3.11 (0.063)	-6.437	0.989
Control site	32	3.061 (0.059)	-6.412	0.986
Combined	66	3.16 (0.044)	-2.026	0.987

$\text{Log}(W) = b * \text{log}(SL) = a$, where *W* is the weight, *SL* is the standard length, *b* is the slope and *a* is the intercept. *n* is the number of individuals used, R² is the explained variation and standard length range.

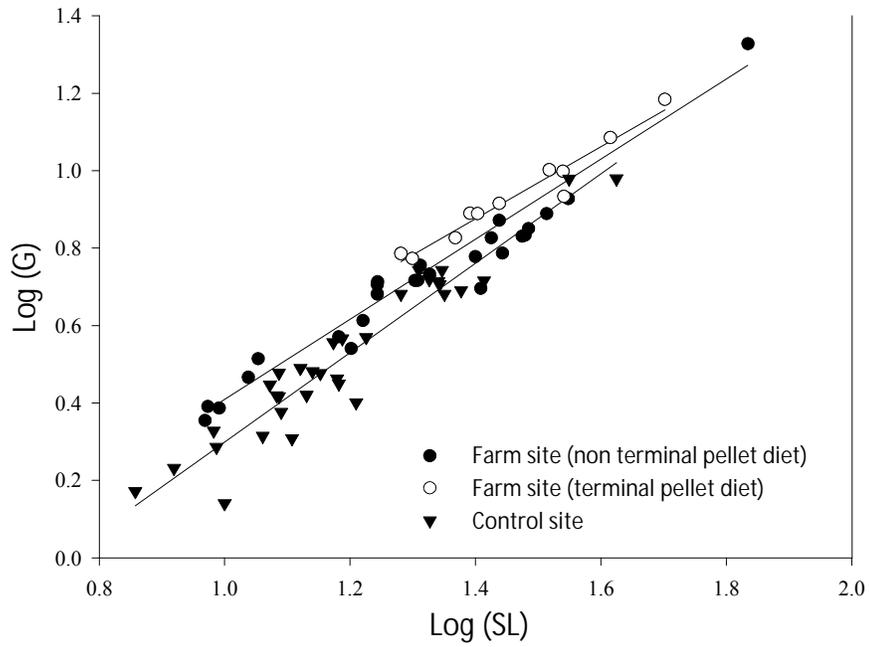


Figure 9 Linear regressions with log transformed data of girth (G) and standard length (SL) between farm (separated into identified and non-identified pellet feeders) and control sites.

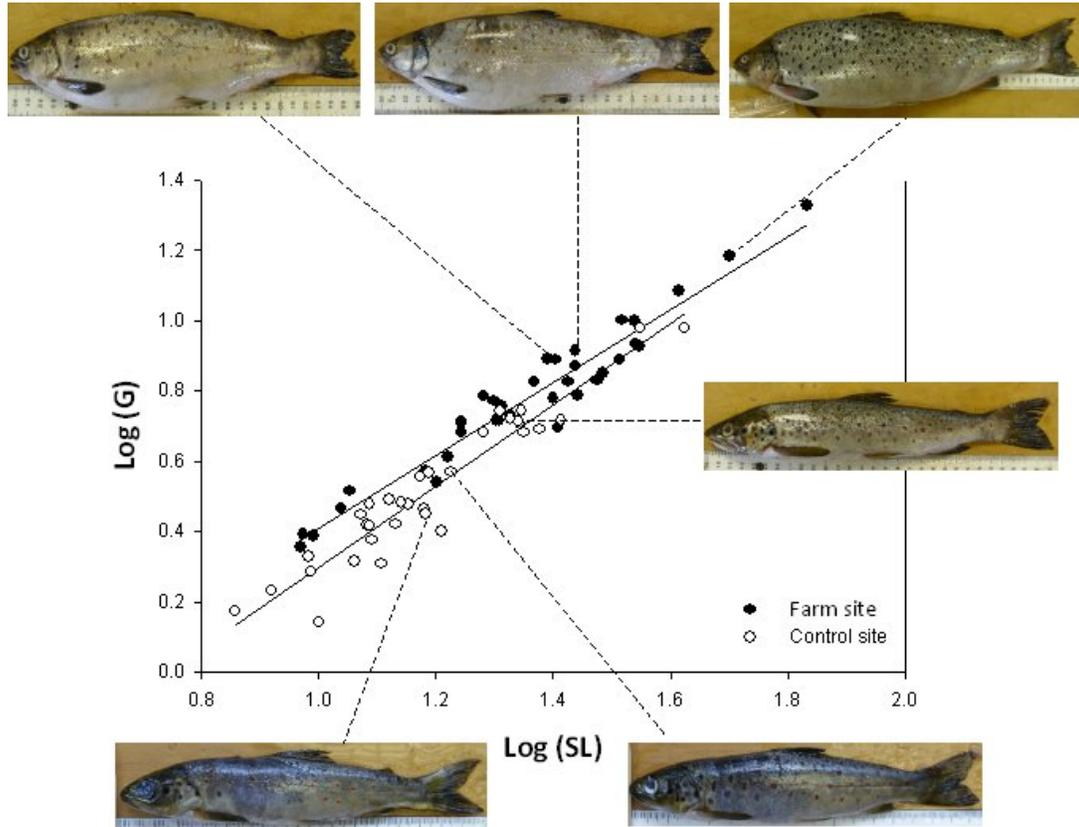


Figure 10 Linear regressions with log transformed data of girth (G) and standard length (SL) between farm and control sites. Photographs of trout that fit the relationship are shown along with fish with both positive and negative residual values.

Table 4 Linear relationship of log transformed data of length and girth for brown trout from the two sites.

	<i>n</i>	<i>b</i> (S.E)	<i>a</i>	R ²
Farm site	36	1.154 (0.065)	-0.855	0.914
Control site	32	1.039 (0.045)	-0.632	0.939
Farm site (pellet feeders)	36	0.930 (0.025)	-0.426	0.937
Farm site (non pellet feeders)	32	1.035 (0.060)	-0.625	0.949
All trout total	68	1.185	-0.850	0.926

$\text{Log}(G) = b * \text{log}(\text{SL}) + a$, where *G* is the girth, *SL* is the standard length, *b* is the slope and *a* is the intercept. *n* is the number of individuals used, *R*² is the explained variation and standard length range.

3.3 Condition factor

Overall

The condition factor (K) for both the farm and control site trout were found to be independent of standard length using a regression analysis (ANOVA, $p = 0.973$), for farm site trout; (ANOVA, $p = 0.090$), the control site trout; and (ANOVA, $p = 0.512$), for the combined sites (Table 5, Figure 11). These analyses permitted overall comparisons of the condition factor between the two sites, irrespective of the differences in length (SL) frequencies. Condition factor of trout was assessed and compared between both farm and control sites. The condition factor for the farm site trout ranged from 0.685-1.285 with a mean value of 1.018, whilst for the control site ranged from 0.677-1.154, and a mean value of 0.889. In addition the trout from the farm site were separated into groups: Group A; farm site trout, B; trout containing feed pellets in their terminal diet (from SCA), and group C; trout not containing feed pellets; presented in Figure 12. There were significant differences between the farm site, control site and farm sub groups (A and B) (Kruskal-Wallis, $p = 0.0001$). Farm site trout had significantly higher mean K factor than control site trout (Mann-Whitney U, $p = 0.001$). Sub group A had significantly higher mean K factor than the control site (Mann-Whitney U, $p = 0.0001$), and sub group B (Mann-Whitney U, $p = 0.0001$). Sub group B was not significantly different from the control site trout (Mann-Whitney U, $p = 0.083$). The overall condition of farm site trout is better than those at the control site, with known cage feed waste consumers being the best conditioned.

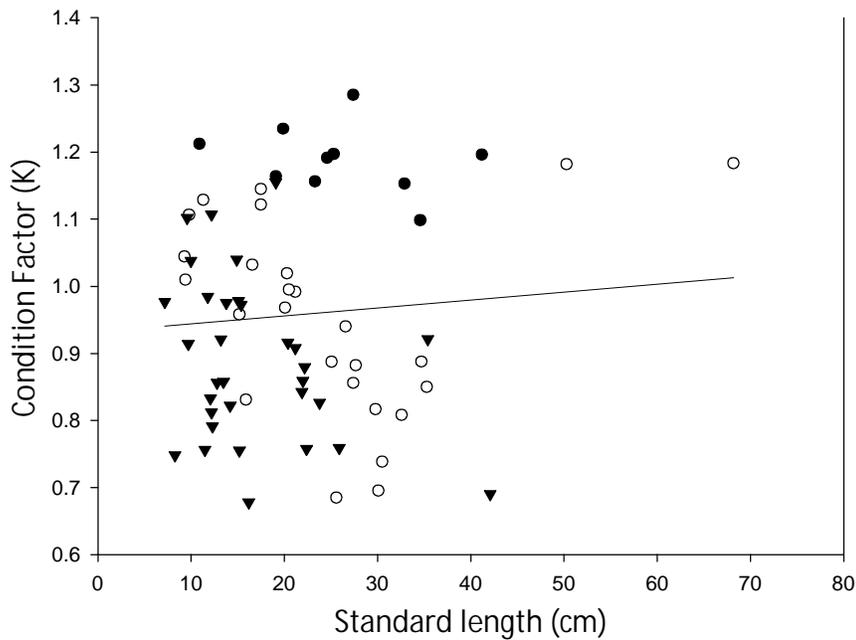


Figure 11 Relationships between condition factor (K) and standard length for farm (circles) and control (triangles) sites. Trout with a terminal pellet diet from the farm site (closed) are distinguished from other farm site trout (open). Slopes were not statistically significant; farm site ($p > 0.973$), control site ($p > 0.090$), and combined ($p > 0.512$).

Table 5 Relationship of condition factor and standard length for brown trout from the two sites.

	<i>n</i>	<i>b</i> (S.E)	<i>a</i>	R^2
Farm site	36	0.005 (.002)	1.020	0.000
Control site	32	-0.005 (.003)	0.003	0.093
Combined	68	0.001 (.002)	0.932	0.007

$K = b * (SL) + a$, where K is the condition factor, SL is the standard length, *b* is the slope and *a* is the intercept. *n* is the number of individuals used, R^2 is the explained variation and standard length range.

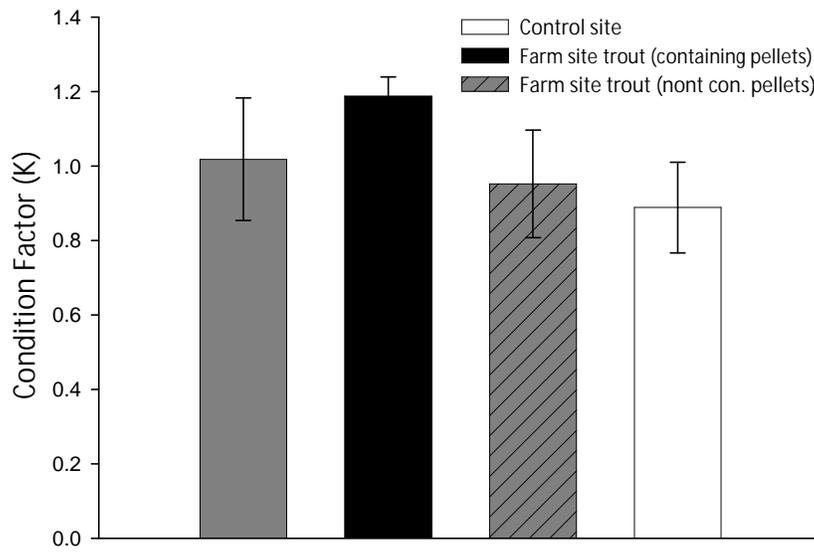


Figure 12 Condition factor estimation based on mean (\pm SD) for separated groups. Farm site ($n = 36$), control site ($n = 32$), Farm site (containing pellets) ($n = 11$), farm site trout (not containing pellets) ($n = 25$).

Age groups

The condition factor for the farm and the control site trout were grouped into age groups and were statistically analysed for years 1-3+ (Figure 13). The condition factor for farm site trout increased annually, with three years + fish having the highest values. In contrast the control site trout decreased in condition annually for the first three years of growth. For age 1+ trout, farm site (mean = 1.053, Standard deviation (SD) = 0.048) was not significantly higher than control site trout (mean = 0.925, SD = 0.124), (two sample t -test, degrees of freedom (DF) = 8, $t = 1.688$, $p = 0.130$). For age 2+ trout, farm site (mean = 1.074, SD = 0.123) was significantly higher than control site trout (mean = 0.892, SD = 0.126), (two sample t -test, DF = 20, $t = 3.395$, $p = 0.003$). For age 3+ trout, farm site (mean = 1.096, SD = 0.142) was significantly higher than control site trout (mean = 0.884, SD = 0.125), (two sample t -test, DF = 14, $t = 1.60$, $p = 0.007$). The condition factor of all trout between 0+ and 3 + age were compared between sites and were found to be significantly higher in the farm site trout, farm site (mean = 1.080, SD = 0.119), control site (mean = 0.9012, SD = 0.120), (Mann-Whitney U, $p = 0.0001$). These results show that the condition of the

farm site trout increases annually for the first three years, whilst the control trout showed a decrease in their condition over the same age range.

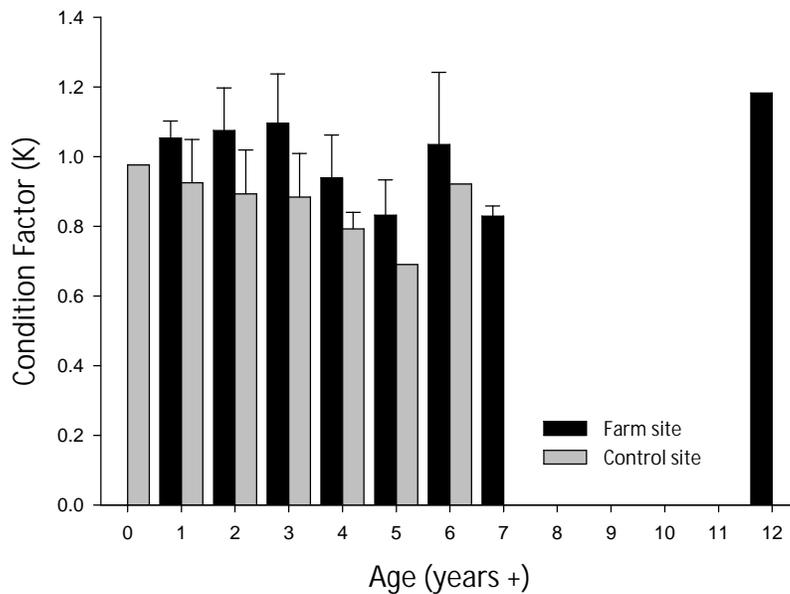


Figure 13 Condition factor estimation between age groups for trout from farm ($n = 36$) and control sites ($n = 32$), based on mean (\pm SD).

3.4 Growth

Back-calculated growth for the first three years of growth

The back calculated growth for the farm site and the control site trout were grouped into the first to seventh year's of growth (Figure 14), but with only the first, second and third years analysed. The growth rate (cm/year) of farm trout increased annually for the first three years. However, control site trout reached their maximum growth in year two, before a decrease in growth rate. For the first year of growth from trout, farm site (mean = 4.22, SD = 1.22) was not significantly different from the control

site trout (mean = 3.88, SD = 1.05), (two sample *t*-test, DF = 66, $t = 1.214$, $p = 0.229$). For the second year of growth, farm site (mean = 5.01, SD = 1.628) was not significantly different from the control site (mean = 4.39, SD = 1.255), (two sample *t*-test, DF = 54, $t = 1.523$, $p = 0.133$). The third year of growth did show significant differences, farm site trout (mean = 5.77, SD = 2.079), control site (mean = 3.70, SD = 0.652), (Mann-Whitney U test, $p = 0.0001$). The relationship between age and length (Figure 15) clearly shows farm site trout experiencing increased growth rates over the control.

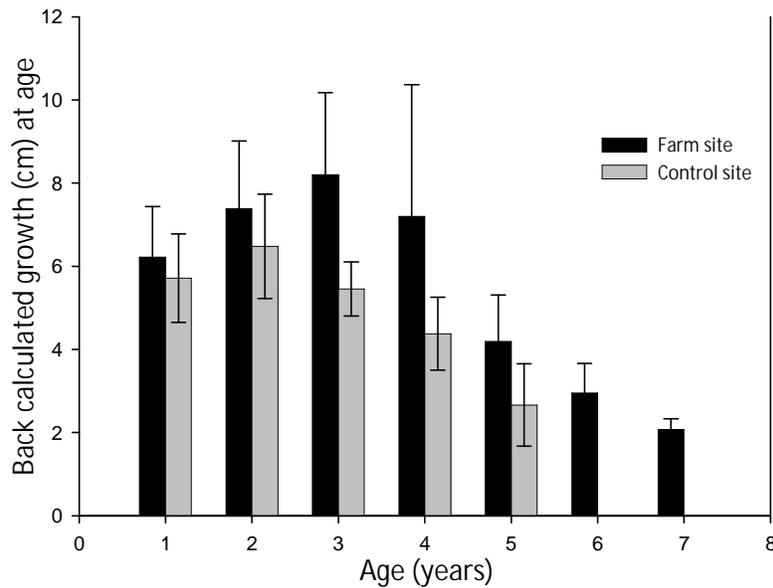


Figure 14 Mean (\pm SD) of back calculated growth at age for farm and control sites. Classes divided into ages: 1 (First year growth); 2 (second year growth); 3 (third year growth); 4 (fourth year growth); 5 (fifth year growth); 6 (six year growth); and 7 (seventh year of growth).

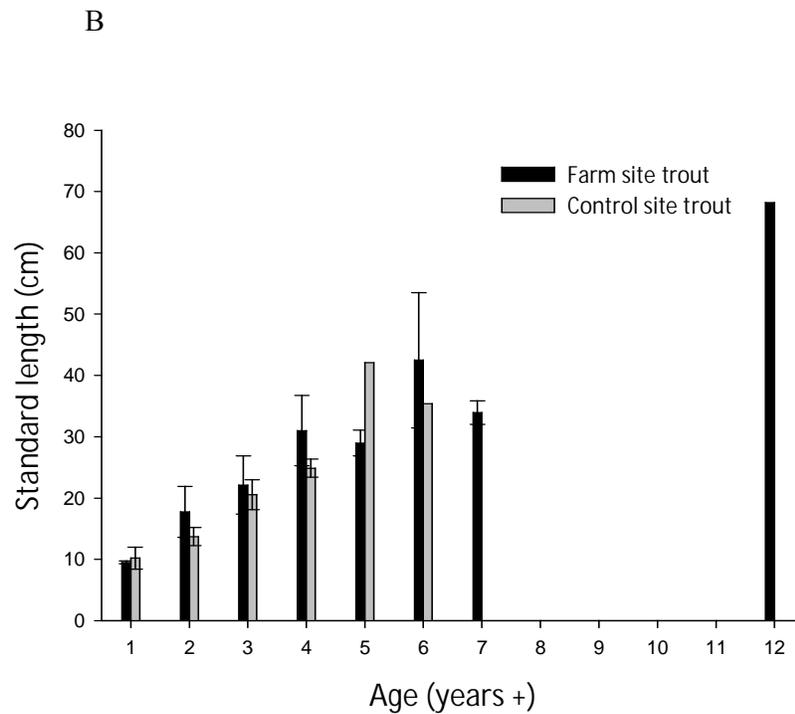
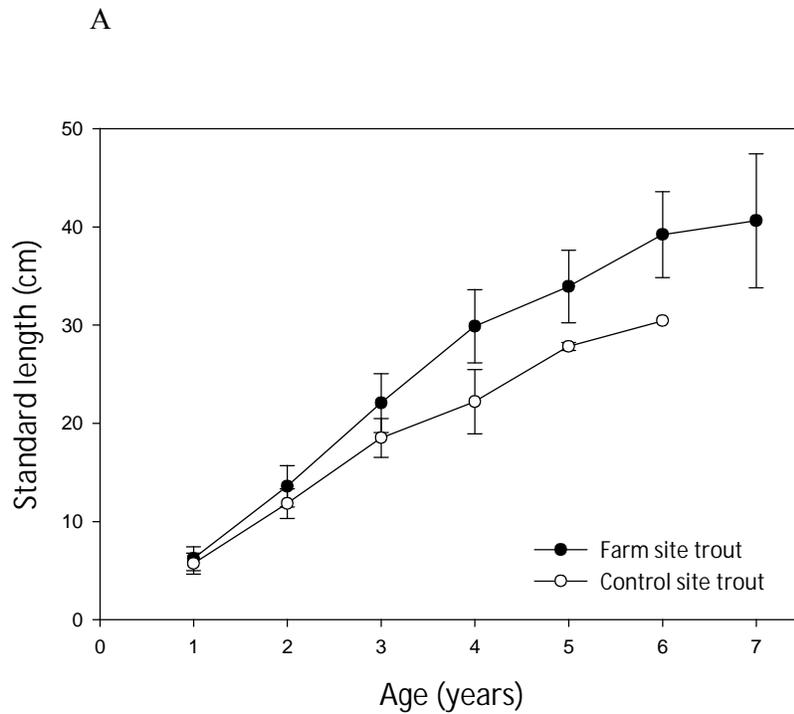


Figure 15 The relationship between trout age and length, (a) calculated from scale annuli, and (B) calculated from whole fish measurements (cm) and age at capture. Re: Graph (a) One 12+-year-old farm site trout was excluded from the data due to scarring from spawning marks on the scales, making measurements between annuli difficult.

3.5 Stomach contents analysis

Diet composition

A total of 68 trout (36 from the farm site and 32 from the control site) had their stomach removed and analysed for contents. Of these 56 were found to contain food items (farm site trout, $n = 33$ (91.6 %)), and control site, $n = 23$ (71.9 %). Insects were the most frequently observed source of food at both sites, with 39.5 % in stomachs of trout in the farm site, and 34.6 % at the control site (Figure 16). However, the frequencies of food items differ markedly from here on. 32.6 % of farm site trout contained feed pellets (Figure 17). Six trout stomachs were completely full of feed pellets and nothing else. Followed by fish (9 %), (unidentifiable due to partial digestion, but thought to be salmonid), plant matter (7 %), fish scales and zooplankton (each 4.7 %). In contrast, without the resource of feed pellets, other food items were of higher frequency for trout at the control site. Plant matter was found in 30.8 % of trout, followed by zooplankton (19 %).

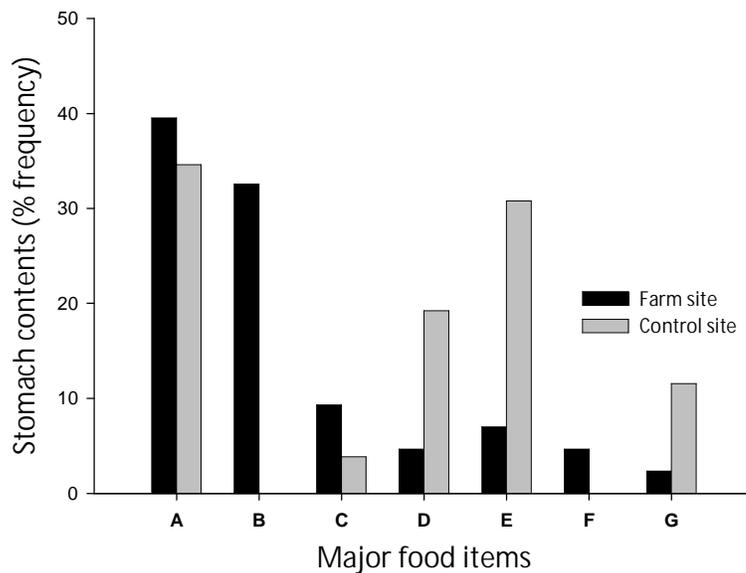


Figure 16 Overall diet composition of trout for farm site ($n = 33$), and control site ($n = 23$). (A) Insects; (B) pellets; (C) fish; (D) zooplankton; (E) plant matter; (F) fish scales; and (G) unknown. Insects consisted of Chironomidae, Gyrinidae, and Notonectidae. Pellets (escaped fish feed pellets). Fish consisted of unidentified fish (presumed salmonids). Other invertebrates consisted of Oligochaetes and *Strigamia maritima*. Zooplankton consisted of *Daphnia* spp. and *Canthocamptus* spp.



Figure 17 Feed pellets in the stomach of a farm site trout.

By attributing the frequency observed food items into trout size classes (Figure 18), farm site trout from each size class were found to consume feed pellets, but larger fish consisted of higher frequencies. Fish found in the stomachs of farm site trout (piscivorous diet) were only found in larger trout (+24 cm (SL)). A piscivorous diet was also only observed in the larger control site trout also (+40 cm (SL)), (=24 cm, being not represented).

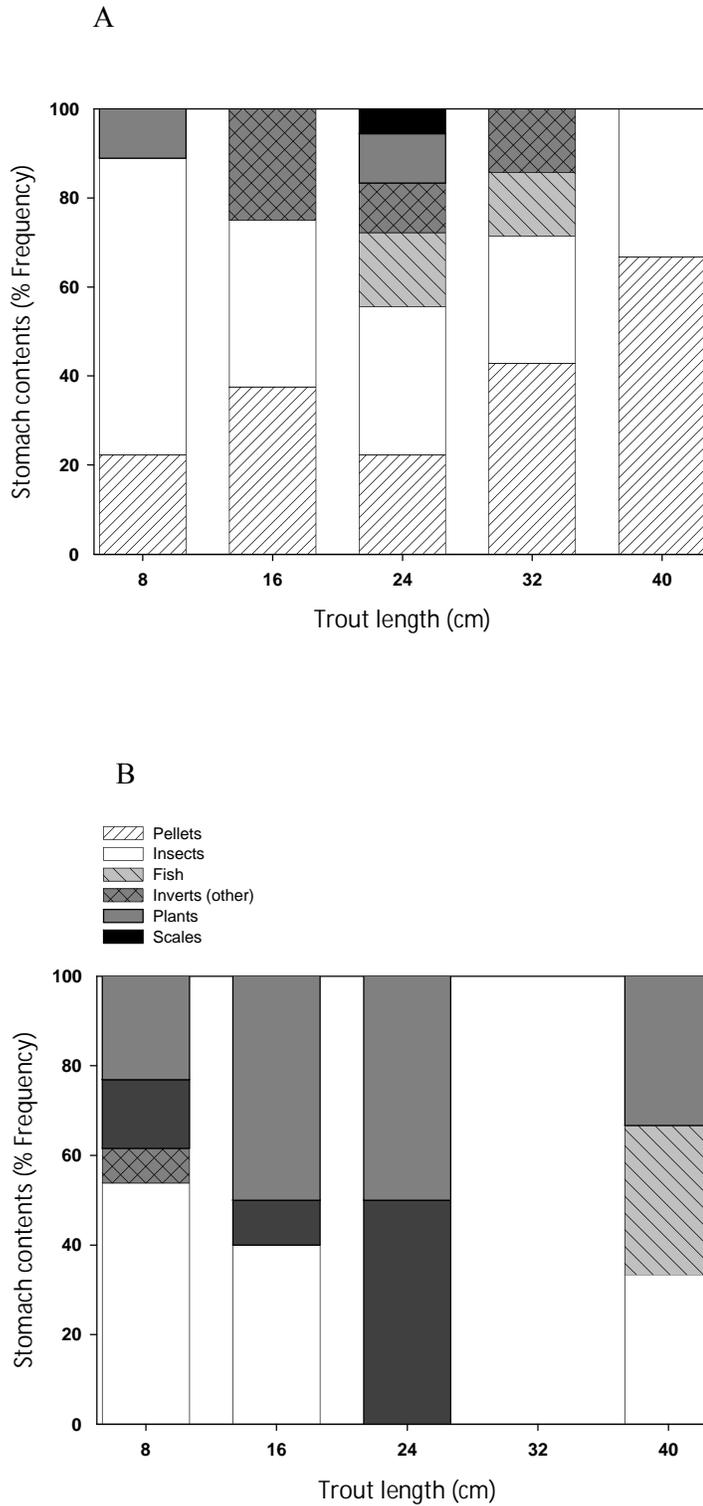


Figure 18 Diet composition of trout per size class (cm) for (A) farm site ($n = 33$), and (B) control site ($n = 23$). Classes divided into sizes: 8 (8.0-15.9); 16 (16.0-23.9); 24 (24.0-31.9); 32 (32.0-39.9); and 40 (40.0+). Insects consisted of Chironomidae, Gyrinidae, and Notonectidae. Pellets (escaped fish feed pellets). Fish consisted of trout or minnow. Other invertebrates consisted of Oligochaetes and *Strigamia maritima*. Zooplankton consisted of *Daphnia pulex* and *Canthocamptus staphylinus*.

Stomach fullness

Stomach fullness scores were compared between sites, and shown to be significantly different (Mann-Whitney U test, $p = 0.01$), with a mean of 2.23 in the farm site trout and 1.35 in the control site, suggesting that farm site trout are feeding more regularly.

3.6 Stable isotope analysis

A total of 74 trout, 15 farmed Salmon parr, eight eels, and four wild Salmon were tested isotopically for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of liver and muscle. In addition other organisms collected during benthic and pelagic sampling were also tested (Table 8 & 9).

Length - $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ relationship in Loch Shiel trout

Regression analyses were performed using length as a variable to be plotted against isotopic signatures for both liver and muscle signatures from trout.

Control site trout

The nitrogen isotopic ratios of trout liver ranged from 7.66‰ to 12.89‰ (Figure 19). The smallest parr were generally more ^{15}N -depleted than larger trout, though several smaller trout exhibited relatively higher ^{15}N signatures. However, trout signatures became progressively more enriched in ^{15}N with increasing length ($y = 0.106x + 7.72$, $R^2 = 0.56$, $p = 0.001$ (ANOVA)). For muscle tissue the ratios ranged from 8.29‰ to 13.06‰ (Figure 19), with again signatures becoming progressively more enriched with increasing length ($y = 0.11x + 8.44$, $R^2 = 0.69$, $p = 0.001$ (ANOVA)). The carbon isotopic ratios of trout for both liver and muscle showed a large degree of variability (Figure 20), ranging from -30.10‰ to -23.93‰ (liver), and -26.92‰ to -21.84‰ (muscle). In contrast to the nitrogen ratios, there was no relationship ($R^2 = 0.05$, $p = 0.59$ (liver); $R^2 = 0.02$, $p = 0.44$ (muscle)).

Farm site

The nitrogen isotopic ratios of trout liver ranged from 9.84‰ to 14.01, and 10.15 to 14.82 for muscle tissue ‰ (Figure 19). However, in contrast to the control site trout, no relationship between length and ^{15}N were detected ($R^2=0.0^{13}0$, $p=0.057$ (liver); $R^2=0.103$, $p=0.052$). The carbon ratios ranged from -21.01 to -28.50 in liver, and -19.92 to -26.75 for muscle ‰ (Figure 20). As with the control trout, no discernable relationship could be found ($R^2=0.045$, $p=0.217$ (liver); $R^2=0.046$, $p=0.207$).

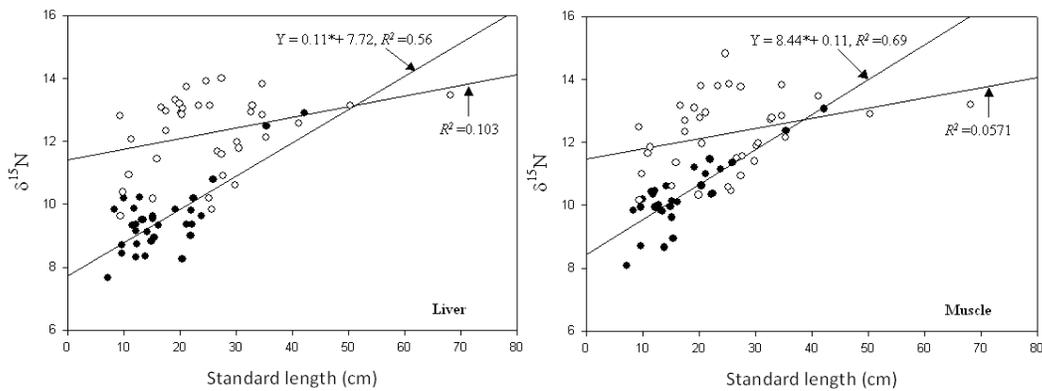


Figure 19 Liver and muscle N^{15} isotope ratios of trout collected from the farm (open circles), and control (closed circles) sites. Equation of linear regression is only shown when R^2 values are significant.

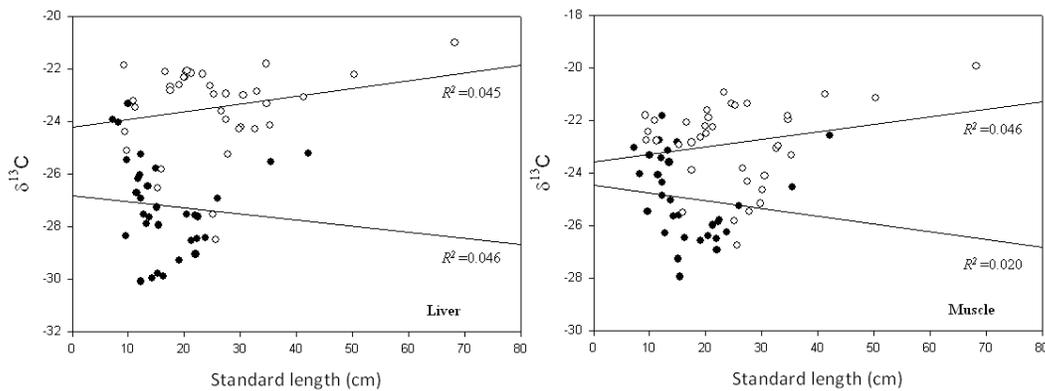


Figure 20 Muscle and liver C^{13} isotope ratios of trout collected from the farm (open circles), and control (closed circles) sites. Equation of linear regression is only shown when R^2 values are significant.

Control and farm site $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures

There was a large degree of variation in the sizes of individuals collected from each site, but the lack of relationship between isotopes signatures and length allowed for direct comparisons of the overall means.

The stable carbon and nitrogen signatures for trout were significantly different between sites for each tissue type; with farm site trout enriched in C^{13} and N^{15} compared to trout at the control site (Table 6, and Figure 21). Though all comparisons were significant, liver showed a notably higher degree of difference in C^{13} signatures between the sites.

Table 6 Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope signatures from trout with the results of statistical analyses.

Carbon ¹³					
Tissue	Farm site	Control site	Difference	Test	Sig.
Liver	-23.51 (1.64)	-27.22 (1.77)	3.71%	Two sample t-test	p =0.001
Muscle	-22.90 (1.56)	-24.98 (1.53)	2.08%	Two sample t-test	p =0.001
Nitrogen 15					
Tissue	Farm site	Control site	Difference	Test	Sig.
Liver	12.07 (1.21)	9.50 (1.05)	2.57%	Two sample t-test	p =0.001
Muscle	12.25 (1.43)	10.16 (0.99)	2.09%	Mann-Whitney U test	p =0.001

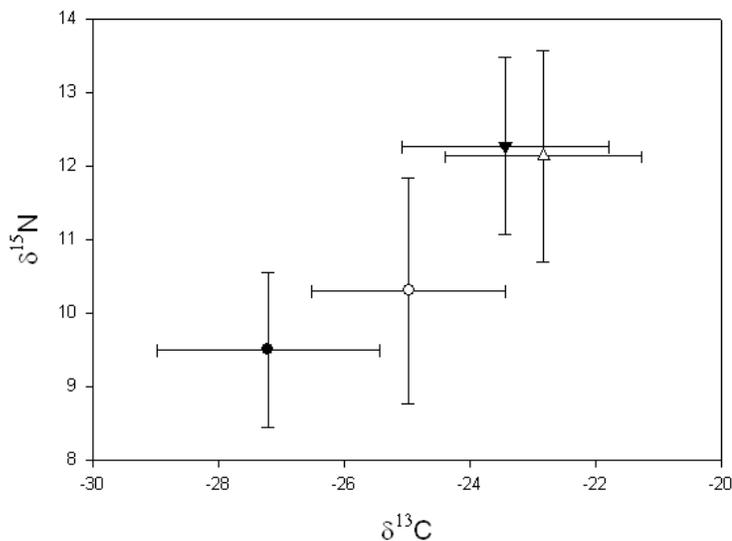


Figure 21 Dual isotope plot of mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures. Farm site (triangles); control site (circles); liver (closed); and muscle (open).

Effect of trout containing pellets

By separating farm site trout by either containing or not containing feed pellets in their stomachs it was possible to identify the effect of these trout on the overall signature for the group. In all but the C¹³ signatures from liver tissue, significant differences were shown between the sub groups. In every comparison, identified pellet feeders were more enriched in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Table 7 and Figure 22a-b). By subtracting the identified pellet feeders from the farm site, mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures were again compared between the two sites. Despite removing the known pellet feeders from the data, farm site trout were still significantly more enriched (Table 7, Figure 22a-b).

Table 7 Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope signatures from trout with the results of statistical analyses.

Carbon ¹³					
Tissue	Pellet trout (SCA)	Farm site - Pellet trout	Difference	Test	Sig.
Liver	-22.64 (0.45)	-23.78 (1.85)	1.11%	Mann-Whitney U test	p =0.093
Muscle	-21.71 (0.67)	-23.27 (1.62)	1.56%	Mann-Whitney U test	p =0.002
Nitrogen 15					
Tissue	Pellet trout (SCA)	Farm site - Pellet trout	Difference	Test	Sig.
Liver	13.29 (0.45)	11.84 (1.19)	1.45%	Two sample t-test	p =0.001
Muscle	13.22 (1.13)	11.71 (1.35)	1.51%	Mann-Whitney U test	p =0.001
Carbon ¹³					
Tissue	Control site	Farm site - Pellet trout	Difference	Test	Sig.
Liver	-27.22 (1.77)	-23.78 (1.85)	3.44%	Mann-Whitney U test	p =0.001
Muscle	-24.98 (1.53)	-23.27 (1.62)	1.71%	Two sample t-test	p =0.001
Nitrogen 15					
Tissue	Control site	Farm site - Pellet trout	Difference	Test	Sig.
Liver	9.50 (1.05)	11.84 (1.19)	2.34%	Two sample t-test	p =0.001
Muscle	10.16 (0.99)	11.71 (1.35)	1.55%	Mann-Whitney U test	p =0.001
Carbon ¹³					
Tissue	Control site	Pellet trout (SCA)	Difference	Test	Sig.
Liver	-27.22 (1.77)	-22.64 (0.45)	4.58%	Two sample t-test	p =0.001
Muscle	-24.98 (1.53)	-21.71 (0.67)	3.27%	Two sample t-test	p =0.001
Nitrogen 15					
Tissue	Control site	Pellet trout (SCA)	Difference	Test	Sig.
Liver	9.50 (1.05)	13.29 (0.45)	3.79%	Two sample t-test	p =0.001
Muscle	10.16 (0.99)	13.22 (1.13)	3.06%	Two sample t-test	p =0.001

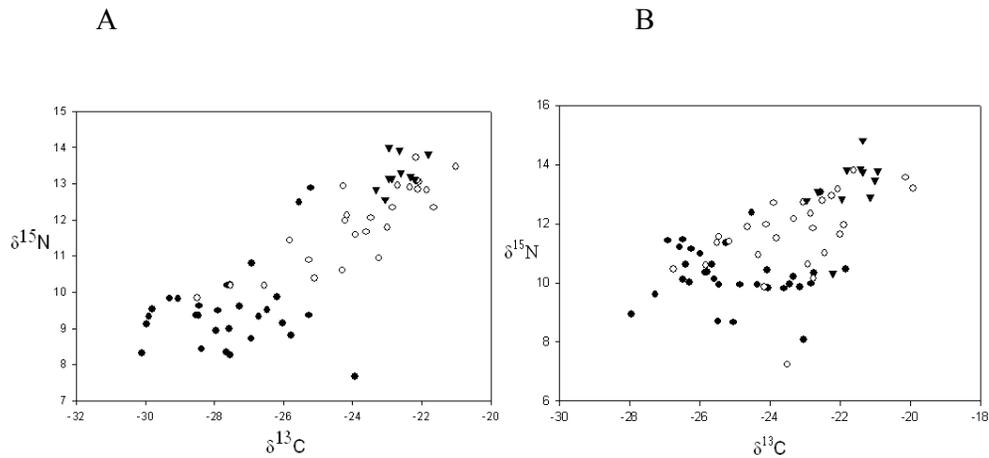


Figure 22 Combined $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of liver (A) and muscle (B) from farm site trout (open circles), identified pellet feeding farm site trout (closed triangles); and control site trout (closed circles).

Reconstructed food web

Fish and feed pellets

The signatures of the analysed feed pellets ranged from $\delta^{13}\text{C} = -22.14$ to -22.40 , and $\delta^{15}\text{N} = 9.71$ to 10.59 (Figures 23 & 24). However, the isotopic signature of one feed pellet was subtracted from the data as it was deemed erroneous (^{13}C , -25.86‰ ; ^{15}N , 9.93).

The farmed Salmon showed a predictable narrow range. The results of a consistent controlled diet. The liver ^{13}C signatures ranged from -21.96‰ to -22.59‰ , whilst muscle showed a relatively higher enrichment with signatures ranging from -19.97‰ to -20.31‰ . Nitrogen values ranged from 11.89‰ to 12.83‰ in liver, and 13.29‰ to 13.82‰ , relatively more enriched (Figures 3 & 4). Wild Salmon were captured in either gill nets or through Electrofishing at the farm site. These fish showed almost identical $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures as farmed Salmon. The liver ^{13}C signatures ranged from -21.79‰ to -21.89‰ , whilst muscle ranged from -19.94‰ to -20.32‰ . Nitrogen values ranged from 12.45‰ to 12.56‰ in liver, and 13.44‰ to 13.82‰ (Figures 3 & 4). Eels collected at the farm site ranged in their ^{13}C signatures from -26.37‰ to -23.13‰ (liver), and -26.05‰ to -21.25‰ (muscle). The ^{15}N signatures

ranged from 10.98‰ to 14.90‰ (liver), and 10.1‰ to 11.90‰ (muscle). Only one eel was caught at the control site (Table 9).

Isotopic analyses of stomach contents, grab and plankton net samples

The benthic and pelagic sampling was carried out to (1) identify and isotopically analyse organisms that matched those found in the stomachs of the fish; and (2) make comparisons in the isotopic signatures of matching organisms between the two sites. Two very different outcomes were found between sites. From the benthic grab samples taken from below the smolt cages, four different groups of organisms were discovered (table 8 and Figures 23-24). No faunal life was identified in the control site, and thus no direct statistical comparisons could be made. From the pelagic samples, algal phytoplankton was discovered in the cage site, and daphnia pulex from the control loch mid-point (Table 8 and 23-24). Three species of invertebrate found in the stomach contents that were not collected in the field were also isotopically analysed, including two species of water beetle, Notonectidae spp., and Gyrinidae Spp. (Whirligig), and *Strigamia maritima* (aquatic centipede). The stable isotope data suggest that the food webs of each site may be supported by different sources of carbon. It is noted that C¹³ signatures from liver tissue in the control site trout are depleted compared to all the collected animal taxa from the farm site.

Table 8 Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}\%$ signatures of organisms collected in the plankton and benthic sampling, and stomach of trout. Also feed pellets and farmed salmon collected from smolt cages on site. * Denotes animals collected from SCA. Underlined organisms are those identified as prey in the SCA.

		Farm site		
		Number	Mean $\delta^{13}\text{C}$ (\pm SD)	Mean $\delta^{15}\text{N}$ (\pm SD.)
Insects				
<u>Midge larvae</u>	chironomidae spp	3	-26.20 (1.67)	8.09 (1. ¹³)
<u>Midge adults</u>	chironomidae spp.	1	-26.19	7.82
<u>Water beetle**</u>	Notonectidae spp.	1	-24.01	0.39
<u>Whirligig*</u>	Gyrinidae spp.	1	-26.77	3.91
Other invertebrates				
Annelid worm	Oligochaete spp.	1	-23.47	9.47
Leech	Glossiphoniidae spp.	1	-25.57	8.30
Orb cockle	<i>Pissidium</i> spp.	1	-20.08	2.34
<u>Aquatic centipede*</u>	<i>Strigamia maritima</i>	1	-21.93	12.37
Plants				
<u>Algae</u>		3	-29.02 (0.32)	4.20 (0.29)
Salmon Farm				
<u>Feed pellets</u>		5	-22.25 (0.18)	10.14 (0.35)
Control site				
Plankton				
<u>Water flea</u>	<i>Daphnia</i> spp.	1	-31.48	3.30

Table 9 Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}\%$ signatures of liver and muscle for fish collected at each site.

		Farm site			
Common name		Liver		Muscle	
		Mean $\delta^{13}\text{C}$ (\pm SD)	Mean $\delta^{15}\text{N}$	Mean $\delta^{13}\text{C}$ (\pm SD)	Mean $\delta^{15}\text{N}$ (\pm SD)
Trout	38	23.51 (1.64)	12.07 (1.21)	-22.90 (1.56)	12.25 (1.43)
Wild salmon	5	-22.45 (0. ¹³)	11.84 (0.23)	-20.99 (0.85)	12.63 (0.58)
Eel	7	-23.78 (1.27)	12.76 (1.68)	-23.54 (1.78)	11.02 (0.62)
Smolt cage					
Farmed salmon	15	-22.33 (0.21)	12.49	-20. ¹³ (0. ¹³)	¹³ .57 (0.22)
Control site					
Trout	34	-27.22 (1.77)	-24.98 (1.53)	-24.98 (1.53)	10.15 (0.99)
Eel	1	-26.87	10.93	-23.97	10.65

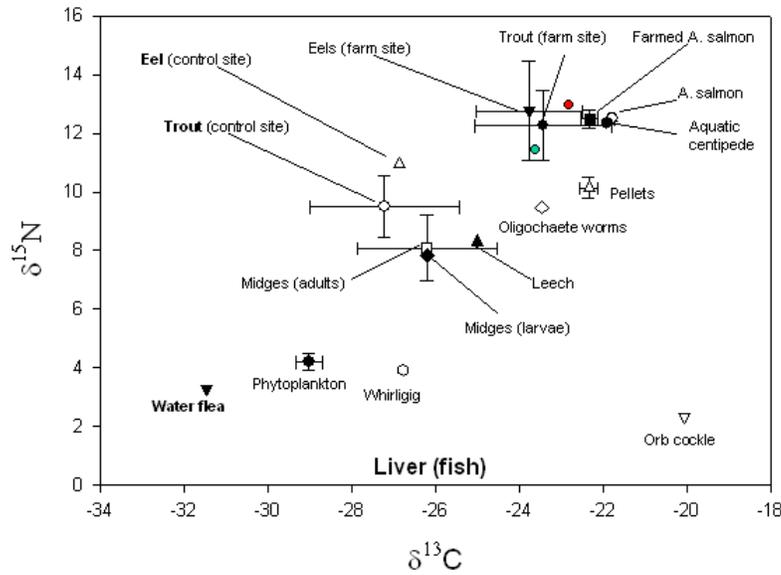


Figure 23 Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ‰ signatures of prey items and other organisms within the food web taken from the farm and control (bold) sites. Represents liver tissue in all sampled fish. The mean isotopic signatures of farm site trout pellet feeders (red closed circles), and non-pellet feeders (closed green circles) from SCA are included as well as the combined Mean (\pm SD) from the farm site trout. Full species names in table 2.

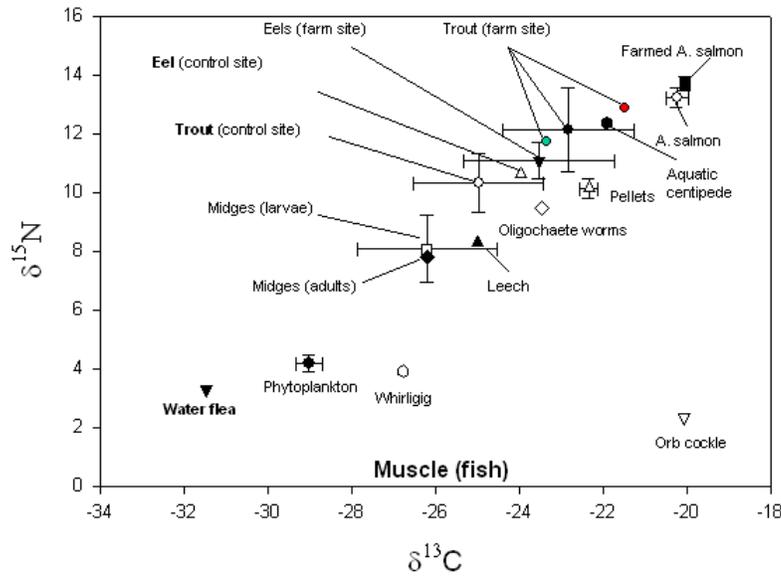


Figure 24 Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ‰ signatures of prey items and other organisms within the food web taken from the farm and control (bold) sites. Represents muscle tissue in all sampled fish. The mean isotopic signatures of farm site trout pellet feeders (red closed circles), and non-pellet feeders (closed green circles) from SCA are included as well as the combined Mean (\pm SD) from the farm site trout. Full species names in table 2.

Mixing models-composition of diets between sites

Isosource

The diets of the farm site trout were assessed using stable isotope analysis of muscle (months) and compared against that of the stomach contents analysis (immediate diet, 24 hrs). The mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures for the four most frequent observed diet items from the SCA (insects (38%), pellets (33%), and fish (9%), plant matter (algae) (4%) were used in the Isosource mixing model (Table 10). The mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures from the muscle of trout were used as the target mixture to represent the trout diet over a period of months (Figure below). From the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signature of trout muscle, the most important dietary source appeared to be escaped feed pellets, which contributed 48.5%. Fish, which contributed 22.3%, followed this; insects contributed 17.4%, and plant matter, 11.7 (based on the signature of algae). The isotopic signature used to represent the fish in the diet of farm site trout were taken from trout that were from 0-15.9 cm, the smallest size category. These were chosen because fish recorded in the stomach contents, though were partially digested, appeared to be salmonids within this size range (Table 10). The level of fractionation used in the model is the mean level identified from (Vander Zanden, 2005), where three different populations of salmonids were tested for trophic enrichment. The levels of fractionation found between feed pellets and farmed Salmon in this report were deemed outside a plausible range for fractionation in salmonids. The pellets fed to the farmed fish are made up of varying sources and thus different batches of feed may contain slightly different isotopic signatures. Only one source of feed was available to be tested at the time and it is thought that this may have contributed to the observed disparity. Also fast growing artificially fed fish have been shown to exhibit unpredictable levels of enrichment (Sweeting et al. 2005). The value used for insects is simply the mean signature of the insects analysed shown in Table 9. Unfortunately, due to the lack of prey diet analysed from the control site an Isosource mixing model could not be carried out.

Table 10 Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of sources used in the Isosource mixing model along with target mixture (mean isotopic signature of farm site trout) and fractionation level used.

Diet Source	Mean $\delta^{13}\text{C}$	Mean $\delta^{15}\text{N}$	<i>Muscle</i>	
			Target mixture $\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Feed pellets	-22.26	10.20	-22.90	12.25
Insects	-26.08	8.08		
Fish	-22.89	11.3		
Plant matter (algae)	-29.02	4.2		
Fractionation		=	0.36	3.49

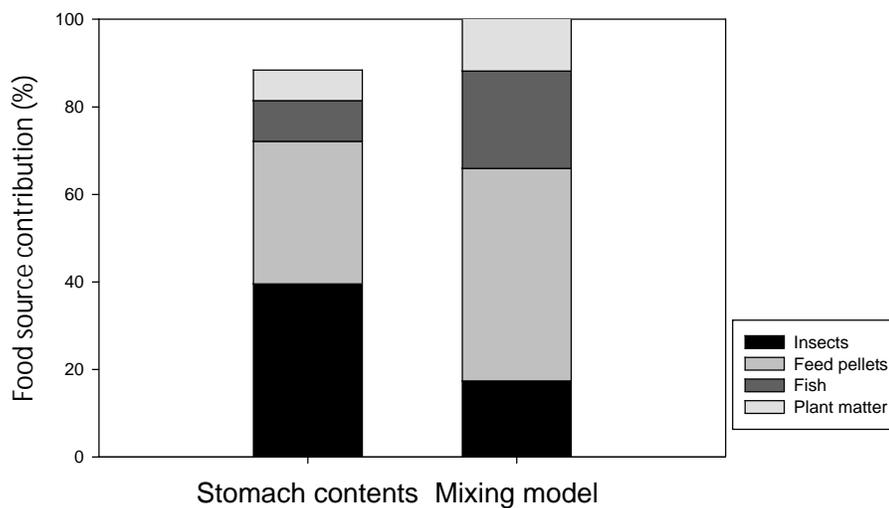


Figure 25 Comparison of average percentage contribution of food items to the diet of farm site trout.

Two source, single isotope linear model

The results of the model show the contribution of carbon assimilated into trout muscle from only two sources; feed waste (pellets, ^{13}C -22.26) and freshwater consumers (insects, ^{13}C -26.08). The mean source contribution of feed waste to farm site muscle tissue was 83.2% (lower quartile = 72.6%, upper = 93.8), with the mean contribution to control site muscle being 28.8% (lower quartile = 19.3%, upper = 38.3%). Showing a higher feed waste signal in the farm site trout. The same model was used again,

however this time running the mean ^{13}C values from trout at each site against individuals with the most enriched and depleted from the combined sites. For muscle the most depleted ^{13}C values (-27.95) represented 100% freshwater sourced carbon, and the most enriched ^{13}C values (-19.32) represented 100% feed waste sourced carbon. This time the farm site trout showed that 58.5% (standard error (SE) 0.30), and the control site 34.4% (SE 0.25) of carbon derived from feed waste representing the sampled muscle tissue in the fish. For liver the most depleted ^{13}C values were -30.10, and the most enriched were -21.01. The farm site trout showed 72.5% (SE 0.20), and the control 31.6% (SE 0.25) of carbon derived from feed waste representing the sampled liver tissue.

4.0 DISCUSSION

4.1 Diet source and composition

The combination of stomach contents, and stable isotope analysis of both liver and muscle has enabled this report to elucidate the diet of trout over different temporal scales. This study shows that trout caught in the two habitats of Loch Shiel differed considerably in their diet. The stomach contents data (immediate diet) shows that cage dwelling trout had high numbers of observed frequencies of insects and feed pellets. The method used however is biased towards smaller organisms, and does not take into account the total mass or potential degree of assimilation. With this in mind feed pellets are probably the most important (immediate) dietary item. Six from ten of the identified pellet feeders had full stomachs containing nothing else. For the same reasons, fish probably constitute a larger proportion of assimilated food than the results belie. In contrast to the farm site, the control site trout unsurprisingly showed no evidence of feeding on pellets. Though this group also showed a highest frequency of observed prey being insects, the proportions of all other food types were very different with both zooplankton and plant matter being of higher importance, though trout do not deliberately consume plant matter.

The higher proportion of stomachs containing food, and the higher average stomach fullness in the farm site trout, suggest that this site is a more productive ecosystem.

This disparity between the two trout groups diet is highlighted in the stable carbon and nitrogen signatures. Both the C^{13} and N^{15} signatures from each tissue of the farm site trout are enriched compared to the control site. Firstly looking at each isotope in isolation, the C^{13} signature of the farm site trout shows a distinctly more marine source. This was highlighted by the linear mixing model, which showed the contribution of marine or feed waste carbon to the muscle, and liver of farm site trout to be far higher than the control. Baring in mind that the location of the farm site is further (8 km) away from the sea than the mid-point control site, and that cage waste has a distinctly enriched C^{13} signature (Mazzola & Sara 2001), it seems likely that the source of carbon within the tissue of farm site trout is from feed waste, and less likely a true marine diet.

In isolation the relatively high nitrogen signatures of farm site trout could be the result of a longer food chain, and not necessarily identify cage waste as a reason for the observed level of enrichment. However, using the combined signatures of both isotopes brings more resolution to the results. Vander Zanden & Rasmussen (2001) showed a mean enrichment for N^{15} equalling 3.49‰, and C^{13} 0.36‰ from three populations of lake trout. Based on these levels, the difference in C^{13} signatures between the control and farm sites (2.08‰) is the equivalent of approximately five and a half instances of trophic fractionation. The level of difference between sites for N^{15} (2.10‰) is the equivalent of less than one instance of trophic enrichment. This shows that the farm site trout have a diet richer in C^{13} relative to N^{15} .

The results from the dual isotope, multi source mixing model is in close agreement with that of the stomach contents analysis, but with higher proportions of feed pellets and fish. Baring in mind that the isotopic signatures represent assimilated diet, this may be considered to be a close match. Also only four of the food item groups were included in the model, whereas seven were identified in the stomachs contents, which may alter the overall result. With the exception of feed pellets, the diets of brown trout found in this report are fairly typical of oligotrophic lakes (Carss 1990, Gabrielsen 1999, Grey 2001). However, there are conflicting reports regarding the feeding of brown trout on feed waste in freshwater lakes that contain cage culture. Phillips (1982) reported with the aid of underwater camera equipment that brown trout were observed feeding on pellets. However, reports by both Carrs (1990) and Gabrielsen (1999) showed that brown trout did not feed on escaped cage waste, and avoided fish cages altogether. In both these studies other species of salmonids were

found to have stomachs containing pellets, such as rainbow trout in Loch Awe, and Arctic charr in a lake in Western Norway. The reasons given for these differences in feeding strategies between salmonids was partly explained in habitat preferences, and inter-specific competition.

Despite significant differences in the means of both C^{13} and N^{15} between the two trout groups, a large degree of overlap exists, especially in the muscle tissue. Bearing in mind that muscle represents a longer-term view, it may be that some farm site trout are recent settlers, thus the slower metabolic turnover of muscle has not yet fully expressed a pellet-feeding signature.

A strong positive relationship between length and N^{15} observed in the control group is indicative of larger fish feeding at higher trophic levels than smaller individuals (Badalamenti et al. 2002, Jennings et al. 2002), due to alterations in their dietary intake through changes in growth and foraging tactics (Grey 2001). This however, was not the case in the farm site trout. Feed pellets are a constant and reliable food resource that are easily eaten and digested by salmonids. Thus, if feed pellets are the food item of choice, than N^{15} signatures are likely to be more uniform, and less reflective of size. That said, larger fish were shown to have a higher degree of fish in their stomachs, but relatively large proportions of feed pellets in the trout diet may have an equalising effect and reduce the relationship between size and N^{15} . Further evidence from farm site trout of a more consistent diet across size and temporal scales are the relatively similar means in C^{13} and N^{15} signatures from the two tissue types. Farm site trout showed a higher consistency in the isotopic signatures between the two tissue types compared to the control site trout. If the two tissue types represent different temporal scales (liver represents days to weeks, and muscle months), than these trout have remained relatively consistent in their signatures. This pattern has been shown in other species of salmonid. McCarthy et al. (2004) showed Arctic charr to exhibit consistent isotopic values (N^{15}) across both liver and muscle tissue, a result of long-term stability in their dietary choice.

Though the two groups are significantly different, a high degree of overlap exists in each isotope (Figure 26). This may show that though the farm site trout have an overall signature indicative of feeding on waste, the variation that are observed may represent some individuals specialising in feed pellets, whilst others may have a more varied diet.

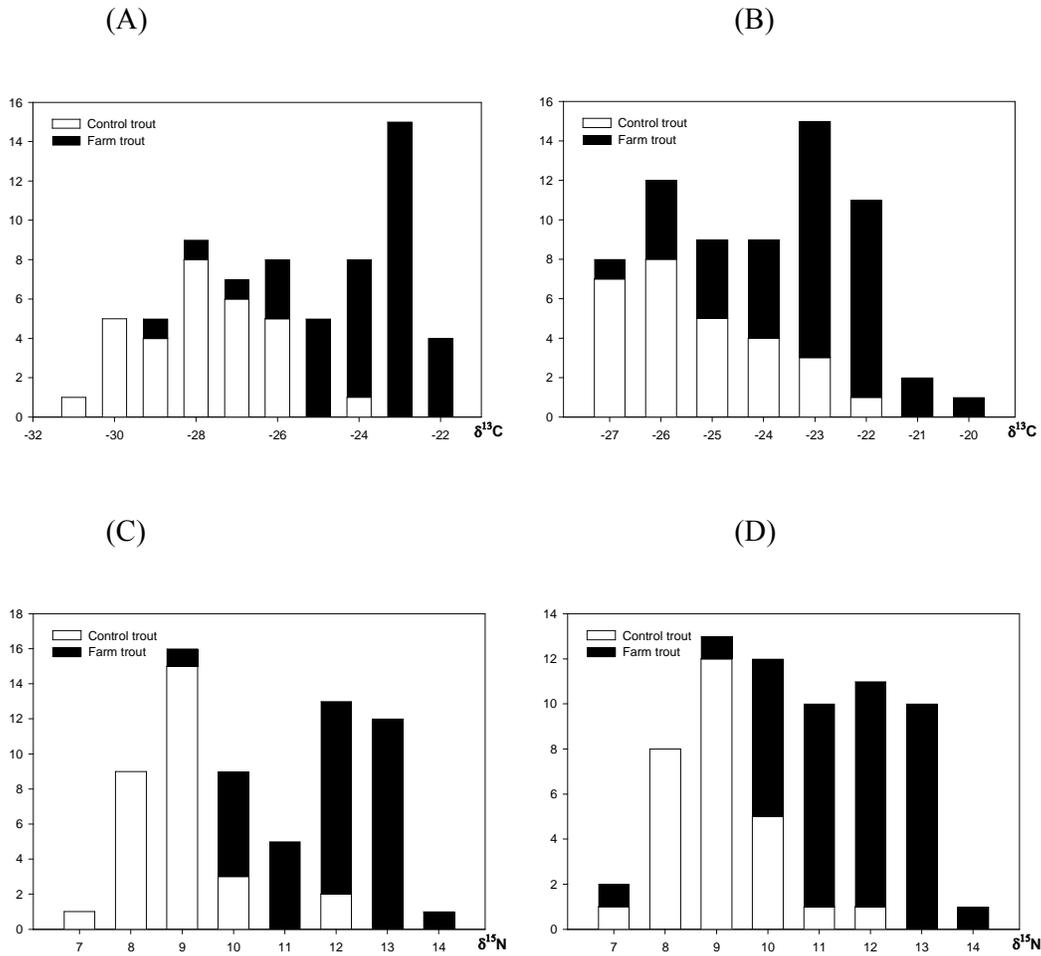


Figure 26 Histograms of C¹³ and N¹⁵ of liver and muscle tissue taken from trout at the farm and control sites. A and C liver, B and D muscle.

Indeed the identified pellet feeders when analysed separately as a group had significantly more enriched isotopic signatures than the remaining farm site trout. It is likely that many of these fish feed on waste to the exclusion of other food items. Whereas other individuals from the farm site had relatively depleted signatures, representing either a smaller proportion of feed waste in their diet or a reflection of being consumers from an enriched food web. The control site trout in contrast showed significantly different signatures between tissue types. It may be that trout not associated with smolt cages are adopting a more generalist or opportunistic strategy and dispersing further in search of food. Variation in the isotopic signatures of a species has been viewed as a measure of foraging niche width (Bearhop 2004). Thus farm site trout could be viewed as being the more specialists of the two trout groups, which fits with a consistent, reliable food source in feed pellets. Both the stable

isotope analysis and the stomach contents of brown trout from Loch Shiel revealed that fish captured in the vicinity of smolt cages had diets consistent with consuming a high proportion of cage waste. Therefore the evidence supports hypothesis 1.

The small number of Salmon parr caught close to the smolt cages are thought to be recent escapees. The stomach contents contained only insects, but the isotopic signature of the liver and muscle tissue was almost a match for the farmed Salmon. This would suggest their escape was so recent that changes in their diet have not yet been expressed in their isotopic signatures. The eels captured close to the smolt cages show a conflicting story between their stomach contents and their isotopic signatures. The eel's stomachs contained insect's parts, sediment and unidentifiable material, with no evidence of cage waste feeding. The isotopic signatures, in particular muscle, suggest a diet source more enriched in both C^{13} and N^{15} , suggesting they are feeding from a C^{13} enriched food web.

4.2 Benthic and pelagic sampling

The benthic and pelagic sampling did not involve recording the abundance or biomass of organisms, but rather it simply recorded their presence. Within the control site no benthic faunal life was discovered. The farm site sampling did however identify four different organisms, including oligochaetes and chironomid larvae. Doughty & McPhail (1995) reported that oligochaetes and chironomids were prevalent below fish farm cages in a Polish lake. Phillips (1985) found significant increases in chironomid abundances beneath cages in Loch Charn, while Shaw (1984 (noted in Doughty & McPhail 1995) found increased densities of oligochaetes below cages in Loch Earn. These organisms thus are directly linked to cage farming, and may benefit from fertilisation of the loch bed. The diets of these two very different organisms are somewhat similar, with algae, detritus, and silt being their primary food sources (Palmer et al. 1995, Yozzo & Smith 1995). In addition leaf, wood fragments, and animal remains have also been noted as a food source for chironomids (Palmer et al. 1995). The isotopic signatures of these two organisms do however imply very different diets. Oligochaetes are relatively enriched in C^{13} , suggesting that to some degree cage derived (C^{13} enriched) waste may be being assimilated by these worms as either faecal matter or broken down pellet material in a detritus state. The

chironomids have a relatively depleted C^{13} signature, which would suggest less assimilation of cage-derived material. However, compared against the signatures from liver tissue of the control site trout, these insects are more enriched in C^{13} . Freshwater or terrestrial environments are relatively depleted in C^{13} to that of estuarine or marine. Insect consumers from freshwater food webs would be expected to reflect this in their carbon signatures. Also the trout signatures are the result of higher accumulative trophic enrichment. Chironomids from 15 freshwater lakes were tested for their isotopic signatures (Vander Zanden & Rasmussen 1999) revealing mean signatures of $\delta^{15}N = 5.7$ (SD 1.1) and $\delta^{13}C = -30.8$, far less enriched than reported here. The only source of enriched C^{13} available to chironomids is through cage waste. Oligochaetes were not found in the stomach contents of any of the fish samples, and as such through this source there is no evidence of an indirect link to cage-derived material to the fish. Chironomid larvae and adults were numerous in the stomachs of trout, and may represent an indirect link of cage waste in the diet of farm site trout.

There is evidence that the presence of cage farms increases the density of benthic organisms (Kilambi et al. 1978). Grab samples from under the cages showed faunal life that was not present at the control site, which may contribute to the diet of fish. It was also found that completely different organisms dominated the composition of plankton at each site. Samples from the control site were dominated by zooplankton, whereas solely algae dominated the farm site. High water column algal production, driven by high nutrient loading from cage waste has been reported elsewhere (Kelly, 1995), and therefore it is not surprising that this is the case. The isotopic signatures of planktonic algae, (primary producers representing the base of the food web) collected at the farm site are enriched compared to daphnia (primary grazers) collected at the control site, giving further evidence to support that cage waste is entering the food web. Further evidence comes from the trout themselves. The farm site trout were still significantly enriched (from the control) in their C^{13} signature, even once the known pellet feeders were removed. This may imply that the isotopically enriched waste has influenced the food web. The very limited number of samples analysed makes any interpretation difficult to justify. However, these results support potential indirect sources of waste material reaching the trout. Therefore the evidence supports hypothesis 2.

4.3 Growth and condition

The evidence suggests that a large proportion of the farm site fish are utilising feed waste. With this in mind it would be expected that these fish would benefit from enhanced growth, as the feed itself is designed to do exactly that. The data suggest that this is the case. However, though growth in the farm site trout is higher than the control site for the first two years, it is only significantly so in the third year. These results are somewhat supported by the literature. An analysis of brown trout in Loch Charn showed an increased growth rate following the introduction of farm cages (Phillips et al. 1985). The same research also reported significant increases in the growth of roach *Rutilus rutilus* post the implementation of cage farming. These studies also report that growth was only significantly greater in the second and third years of fish compared to that of fish prior to cage farming.

The condition factor of farm site trout also increased annually for the first three years, whilst the control decreased in condition from the first year, with growth each year being higher in the farm site trout, and significantly for age three trout. The overall condition of all fish, irrespective of age, was also significantly higher in the farm site trout. However, by removing the known pellet feeders from the comparison reduced the mean condition factor, and were no longer significantly different from the control. These known pellet feeders, exhibited some of the highest condition values, and by this measure (K factor) were significantly better conditioned than the remaining farm site trout. Research on the effects of cage farms on Arctic charr (Gabrielsen, 1999), from an oligotrophic lake in Norway showed that individuals containing feed pellets had significantly higher condition factors than individuals that fed on natural food. The evidence shows that trout collected in the vicinity of smolt cages show increased growth and are better conditioned (with some individuals being excessively more rotund) than those collected from a control site. Thus the evidence supports hypothesis 3.

Population structure

The data show considerable differences between the two sites. Farm site trout are composed of larger, older, fatter fish. The length weight relationship showed significant differences between sites, though both exhibited allometric growth with

'*b*' values close to 3. Farm site trout had a higher *b* value, with trout becoming more rotund as they grow. This is supported up by the length girth relationship, showing that the girth of trout at the farm site increases to a higher degree with length. The presence of cage farms is likely to have dictated this disparity in size and age composition of trout. A possible scenario could be that with a reliable food resource filtering into the environment, trout may have been attracted to the site to utilise this resource. This would increase the density of trout around cages increasing the potential predation risk for smaller trout from larger piscivorous trout. As fish grow, the risk of predation decreases and thus the benefits of feeding around cages begin to outweigh the predation risk. This may also limit the number of new recruits to the site, as only larger fish would be able to feed here without the threat of being predated. This scenario fits with research by (Jonsson & Gravem 1985) where smaller younger trout occupy habitats where competition or predation risk is low, and larger, older fish are more likely to occupy habitats where feeding and growth are better. However, the same report shows that females are more abundant in these riskier habitats. This is in complete contrast to the observed data from farm site trout at Loch Shiel. The sex ratio of trout for the two sites were 61:39 (M/F), farm site, and 63:37 (M/F) for the control. However, the small sample size and potential misidentification of sex from younger trout could mean that this is not a true representation of the trout found at the farm site. Although a disproportionate number of males have also been observed at other loch systems that have under gone a severe decline in sea trout. Data from (Walker 1993) showed that the sex ratio of mature trout in the River Shiel was 74:26 (M/F).

4.4 Does cage waste influence migration?

These data show that the presence of smolt cages acts as an artificial food resource, significantly increasing the growth and condition of trout in the vicinity of cage farms on Loch Shiel. Olsson (2005) showed that the environment influences migratory behaviour in brown trout, indicating that migration is an adaptive response to habitat conditions, and a tendency to migrate correlated with low food availability. Migrants experience higher fitness related costs, but are able to attain increased growth rates and fecundity than non-migrants (Nordeng & Bratland 2006). With this in mind it seems plausible that the utilisation of high food availability in cage waste would

influence a plastic response in trout to remain resident in close proximity to smolt cages. However, from the relatively large size frequency of trout at the farm site, it may be that the risks of predation associated with smaller more vulnerable trout are too great. Possibly some of the farm site trout were previous marine migrants that have returned larger and more able to survive around large piscivorous trout and reap the benefits. Unfortunately this is hard to identify. Individuals that were thought to be anadromous by their appearance showed very similar patterns of growth (through scale analysis) to those identified as resident forms. As cage waste has the potential to accelerate growth, identifying trout type using scale analysis proved difficult. Thus identifying if an individual trout showed marine growth in the past or increased growth through cage wastage was unclear. Stable isotope analysis can differentiate between anadromous and non-anadromous populations with greater accuracy than that of visual appearance and morphology (McCarthy & Waldron 2000). However, identifying non-anadromous individuals feeding on waste that contain a marine signal from true marine consumers is a trickier proposition. From the farm site trout, three individuals were thought to be sea trout from the physical appearance and morphology, but their isotopic signatures revealed values similar to the mean of the group. It could be that these fish are previous migrants that have reverted back to freshwater residency. Regrettably the limitations of this study mean this can only be speculated.

This study focused on one set of cages located at the northern end of the loch. However, a second set is located at the southern end, close to the lochs only outflow, the River Shiel. Assuming the population structure of trout in the vicinity of the southern cages is similar to the northern site, than a high density of large piscivorous trout may cause a considerable predatory risk for migrating smolts in reaching the sea. Overall the evidence suggests that both the costs of anadromy and the benefits of resident behaviour have been increased on the loch. With this in mind it is likely that the presence of fish farms alter the balance between the two alternative strategies, increasing the selection pressure on remaining resident.

4.5 Conclusion

This study is the first to use stable isotope analysis to determine the contribution of feed waste in the population of wild fish. The study employed the use of two mixing

models. (1) Isosource that allowed the examination of four identified food sources in determining the diet of brown trout; and (2) a linear, two source mixing model that estimating the source contribution of carbon in the diet of trout at a farm and a control site. These techniques in conjunction with stomach contents identified that brown trout that aggregate in the vicinity of fish farms are utilising cage derived feed waste that represents a considerable proportion of their diet.

The back-calculated growth of trout through the analysis of scales identified significantly higher rates of growth in trout collected close to a fish farm compared to that of trout collected at a control site. In addition, these farm dwelling fish were in significantly better condition. It is thought these benefits associated with inhabiting fish farms may either cause a plastic response in otherwise anadromous trout to utilise the resources afforded and remain in freshwater, and/or cause migrant trout that return to their natal waters to switch to resident behaviour.

4.6 Limitations of the study and future work

Due to financial, time and manpower constraints only two sites could be afforded in this study. However, to assess the total area affected by feed waste, a more comprehensive design would include study sites with varying distances from the cages. For instance sampling could be conducted in; (1) the immediate vicinity of cages; (2) 500 metres from cages; and (3) 1000 metres from cages. In addition two sets of gill nets should be used in sampling trout. A limitation of this study was that the gill nets used targeted fish between 5 and 50 cm (fork length), excluding larger fish. This may have given a biased assessment of the size/age composition. Larger than 50 cm (FL) fish were caught at the farm site despite not being targeted, but these trout had entangled the net around their bodies and not through their gills. A second gill net to target larger fish may give an improved assessment of the size and age structure of trout from these varying distances, along with the other parameters. Food web sampling should also include methods to capture biota from the littoral fringe areas to complete a fuller picture of available food items within the environment. In order to assess the effects on a temporal scale (outside of isotopic range for liver and muscle tissue) sampling could be carried out both in the winter and summer (six month interval).

An idea worth considering in mitigating the effects of cage waste from fish farms being accessed by trout is to move cages into deeper parts of the loch. As stated earlier, two studies (Carss 1990, Gabrielsen 1999) showed that brown trout were not feeding on feed pellets despite the presence of cage fish farms, whilst other salmonid species were. Gabrielsen (1999) suggested that this was due at least in part to differences in habitat choice, with Arctic charr favouring more pelagic areas than the more littoral inhabitant brown trout. Presumably farm cages were found in deeper pelagic areas in this study, although not stated. Carrs (1990) showed rainbow trout and not brown trout to be consuming feed pellets. A similar explanation may be valid here too, as again rainbow trout are known to be a more pelagic feeding than brown trout (Giske & Salvanes 1995). The farm cages sampled from within this study were located on a trough at a depth of approximately 15-20 m (shoreline side) to approximately 60 m (lakeside). By simply moving these cages further out into deeper water may reduce the likelihood of trout to dwell around these cages. Clearly this strategy would require further investigation but maybe worth consideration.

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7.0 APPENDICES

7.1 CD ROM. Raw data