Using stable isotopes to assess the distribution of reproduction by migratory and resident *Salmo trutta* within river systems: some complicating factors.

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ABSTRACT

Understanding the distribution of spawning resident and migratory trout in a river system is important for fishery management. In most cases this information is lacking or only partial, and mostly derived from surveys of breeding adults. Stable isotope analysis of fry provides an alternative method by which to determine the relative importance of different areas for migratory and resident fish breeding. The ratios of carbon and nitrogen stable isotopes in fish tissue vary predictably between offspring from different forms; sea trout typically show higher abundance of the heavier stable isotopes of both elements. These are passed to their offspring, allowing distinction at this phase. Here we show the results from applying this technique to two different river systems; the Gala Water tributary of the River Tweed and tributaries of the River Deveron. Although it is generally possible to distinguish migratory and resident offspring, interpretation is complicated by considerable isotopic variation within river systems. This may be linked to the influence of freshwater feeding, catchment-scale land-use patterns and isotopic variation in adults. In order to maximise the utility of stable isotope-based techniques in fisheries management, these factors need to be more fully understood.

Keywords: *Salmo trutta* ; stable isotope analysis; migration, catchment; population composition; reproduction.

INTRODUCTION

Migratory and resident forms of trout *Salmo trutta* L. are commonly found within the same river system and, with complex and overlapping distributions, there is also commonly extensive interbreeding between the two forms (Charles *et al*., 2005). The distribution and abundance of reproduction by migratory and resident forms of trout and other salmonids is thought to be influenced by physical conditions within rivers and the difficulty of migrating distances upriver (Bohlin *et al*., 2001; Kristensen *et al*., 2010; Berejikian *et al*., 2013) but patterns of distribution are often complex. Understanding the distribution of reproduction of the two forms is important for fishery management: for example to ensure that migratory access is maintained and that important breeding areas are protected. A number of different techniques have been employed to help distinguish between migratory and resident fish, given that phenotypic characteristics are not always reliable. These include the assessment of strontium levels in scales, eggs or otoliths (Rieman *et al*., 1994; Howland *et al*., 2001; Koksvik & Steinnes 2005; Kristensen *et al*., 2010), carotenoid profiles (Youngson *et al*., 1997; Briers *et al*., 2013) and stable isotope ratios (McCarthy & Waldron, 2000; Curry, 2005; Briers *et al*., 2013). These techniques can be used on both adult fish and eggs/offspring. The presence of adults is not necessarily indicative of the reproductive contribution to any particular area unless spawning is observed. Ideally each assessment should be based on the characteristics of the population of offspring that are contributing to the next generation. Stable isotope ratios are increasingly widely used in a variety of contexts within ecology and environmental management, including tracking migration of species (Brattström *et al*., 2010) and distinguishing trophic characteristics (Eloranta *et al*., 2014). Studies of both trout and other salmonids have used stable isotope ratios to distinguish the offspring from migratory and resident fish

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and to determine the relative proportions of the different forms within populations (Doucett *et al*., 1999; Charles *et al*., 2004; Curry, 2005; Charles *et al*., 2006; Jardine *et al*., 2008). The use of the stable isotope ratios can provide valuable information in the context of fisheries management, particularly when combined with other information such as surveys of juvenile abundance and assessment of adult populations. The aim here, through work undertaken within the River Deveron and River Tweed (specifically the Gala Water tributary) in Scotland, is to highlight some significant issues in the interpretation of stable isotope information that need to be given careful consideration to avoid potential mis-interpretation of the data provided.

BASIS OF THE METHOD AND SAMPLING

The ability to distinguish between the offspring of migratory and resident individuals is based on relatively predictable differences in the relative amounts of the heavier stable isotopes of carbon and nitrogen, namely ^{13}C and ^{15}N. Stable isotope ratios are generally expressed in terms of delta values (δ) in units of parts per thousand (‰). These represent the ratio of the heavier to lighter isotope within the sample relative to the ratio of a standard. In the case of ^{13}C this is a marine fossil, the Pee Dee Belemnite, and for ^{15}N it is atmospheric N. In both cases, migratory trout have a greater abundance of the heavier isotope than resident fish within their tissues. This is linked to the greater abundance of both forms in marine systems in comparison to freshwaters (Peterson & Fry, 1987; McCarthy & Waldron, 2000). The stable isotope ratios of the eggs deposited reflect the characteristics of the spawning females and thus have similar stable isotope ratios to the adult tissues. This difference is retained by young fry, although the distinction gradually reduces due to freshwater feeding within a matter of months (Doucett *et al*., 1999; Briers *et al*., 2013).

Samples can be obtained from young fry either by taking a sample of tissue, typically the white muscle, or by fin clippings. Only a small amount of tissue is required for analysis (typically 0.6 - 0.8 mg dry weight depending on the equipment used) so fin clippings, particularly from the adipose fin, can provide a non-destructive method of characterisation of fish (Hanisch *et al*., 2010). For small fry however, the mass of the adipose fin may be insufficient to allow analysis (Thornton *et al*., 2015), thus requiring destructive sampling. There are also differences between isotope ratios of fin clippings and those of muscle, particularly for the adipose fin in terms of carbon isotope ratios, mostly due to its high lipid content (Graham *et al*., 2013). Recent studies have also found variation in stable isotope ratios even within individual fins (Hayden *et al.*, 2015). Therefore, to reduce the influence of inter-tissue variability in isotope ratios, it is generally preferable to use a consistent tissue type for characterisation. As indicated above, samples are dried and then prepared for analysis of stable isotope ratios in an Isotope Ratio Mass Spectrometer (IRMS). Characterisation can be undertaken from fresh, frozen or preserved samples, although prolonged preservation, and the preservative used, may influence stable isotope ratios to some extent (Kelly *et al*., 2006; Vizza *et al*., 2013).

EXAMPLES OF USE IN POPULATION CHARACTERISATION

RIVER DEVERON

The first example is based on analysis of fry from ten sites within the River Deveron system which flow directly into the Moray Firth, North-East Scotland. The freshwater sampling sites were chosen to represent a range of different potential population characteristics. Some sites were chosen as 'reference' sites with known characteristics based on previous fishery information and others, notably a number of coastal streams, were chosen specifically to provide information on the characteristics of the population to aid fishery management. Details of each of the sampling sites are given in Table 1 and the location of the sites in Figure 1.

At each site, ten fry (standard length 5-8cm) were sampled in August 2013 and analysed for stable isotope ratios of both carbon and nitrogen. Following analysis, values were plotted on a dual isotope biplot to visualise the differences in stable isotope ratios between fry from different sites (Figure 2).

The patterns shown by the fish at the Deveron sites demonstrate clearly the distinction between migratory and resident fish in terms of stable isotope ratios. The most obvious difference is shown between the reference resident population (Upper Bogie, above an impassable waterfall) and the fish at the Burn of Boyne and Water of Philorth sites which represent offspring from entirely migratory reproduction. The results for the Tore Burn are a good example of where the technique can be used to support or reject other information about the population and inform management. This site is another coastal stream, but one which has a large gravel bar situated across the mouth of the river which was thought could act as an impediment to migration. The stable isotope results would support this interpretation, as the values for all fish sampled at this site would tend to indicate that the reproduction is solely derived from resident fish. The results for the two sites on the Isla tributary (Crooksmill and Auchindachy) are also informative. The lower site at Crooksmill would appear to be dominated by the progeny of migratory fish whereas the site at Auchindachy, which is above a series of in-stream barriers, has a distinctly different isotope composition, one that is intermediate between the two extremes.

Interpretation of 'intermediate' isotope ratio values such as these is not straightforward. For some sites that fall into this 'intermediate' zone, a wide spread of values that spans the range of values those which are characteristic of both forms would tend to suggest a mixed reproduction. Often values within a site will fall into two separate clusters, representing migratory and resident offspring and allowing proportions of the different forms within the site to be estimated (Charles *et al*., 2004; Curry, 2005). Separation may not always be so marked however, leading to some potential ambiguity in interpretation (such as those observed in fish obtained at King Edward Burn).

Most previous studies have sampled fry more or less immediately after emergence. Sampling of very early stages may not always be practical due to field conditions; most standard fisheries surveys are undertaken later in the year, once the fish have been active in freshwater for several months. In this case the fry were sampled in August, by which time the influence of freshwater feeding on stable isotope ratios is likely to be evident (Jardine *et al*., 2008; Briers *et al*., 2013), leading to less marked distinction between offspring of different sources and the consequent ambiguity evident at some of the sites. The composition of the food consumed is likely to influence the rate and extent of change in isotope values of the young fish (Thornton *et al*., 2015), along with other factors such as individual variation in metabolism (Heady & Moore 2013) and prevailing environmental conditions such as temperature.

For offspring of migratory fish, although the isotopic variation between marine and freshwater ecosystems is the basis of the technique employed, there is also evidence, at least for Atlantic salmon (*Salmo salar* L.), that migration to different sea feeding areas also leads to variation in stable isotope values (Dempson *et al*., 2009; MacKenzie *et al*., 2012). It is a reasonable assumption that similar patterns may be evident in migratory trout, if fish returning to breed in a particular river may have spent time at sea feeding in different areas and thus have distinct stable isotope values, could further complicate interpretation. Given the known wide variation in stable isotope ratios of adult trout, even within a single waterbody (Etheridge *et al*., 2008), a better understanding of the extent and nature of variation in ratios in adult migratory trout returning to breed would be highly beneficial.

GALA WATER, RIVER TWEED.

The second example is drawn from sites on the Gala Water tributary of the River Tweed in the Scottish Borders and forms part of an ongoing larger, catchment-wide characterisation. Sampling was similar to that undertaken in the Deveron; ten fry were sampled from each site in August 2013. The first useful comparison to draw is between isotope values for both river systems overall (Figure 3). From this it is clear that the overall pattern of variation in stable isotope ratios is similar between the two river systems. This is not always the case however, as comparison of the values given here with values obtained from other rivers and lakes shows wider ranges of variation in young stages of trout in both isotopes considered (McCarthy & Waldron, 2000; Charles *et al*., 2006). Therefore it is not possible to give stable isotope ratios that are 'characteristic' of resident or migratory fish; the values vary between waterbodies and can only be interpreted relative to each other in the context of the system under study.

For the Gala Water, there are much more strongly overlapping ranges of values between sites (Figure 4) compared to that shown in the Deveron sites, even if the overall range of variation is similar to the Deveron. It is still possible to distinguish populations where reproduction is predominantly or entirely derived from either resident or migratory forms (e.g. Site 17 for migratory fish), but the majority appear to be mixed populations where both forms appear to be contributing to recruitment. Previous work on the Gala Water (Holmes, 2015), showed an inconsistent relationship between the relative contribution of sea trout spawning activity with either distance upstream from the mouth of the river; or altitude. This would suggest that other, more local, factors may also be important in determining the location of sea trout spawning sites.

What is evident from Figure 4 is that there is clustering of sites into two fairly distinct groups in the opposite axis of variation to that typical of the overall resident-migratory distinction. When these sites are mapped (Figure 5), it is clear that the groups relate to areas of the river catchment with contrasting land-use patterns, suggesting a potentially strong influence of catchment on overall variation in both isotopes. Most notable is the strong distinction between the cluster of sites at the top left of Figure 4, which are in an area which drains mostly pasture and those towards the bottom right, which drain areas of mostly moorland and natural grassland landuse. There were significant differences in both isotope ratio values between these two groups (Welch's t-test, $\delta^{15}N$: $t = 14.75$, $df = 131.76$, $p<0.0001$, $\delta^{13}C$: $t = 7.06$, $df = 111.32$, $p<0.0001$), with pasture sites having lower $\delta^{13}C$ and higher $\delta^{15}N$ values than moorland/grassland sites.

Previous work has shown that $\delta^{15}N$ values in particular within entire aquatic food webs are influenced by the nature of run-off from the catchment (Anderson & Cabana, 2005; Moore *et al.*, 2014). ^{13}C isotope ratios in terrestrial vegetation also vary in relation to land-use e.g. Hobson (2007) and therefore the isotopic characteristics of detritus entering and being incorporated into the aquatic food web are also likely to vary. This may influence both the isotope values of resident adult fish, which are the passed on to the offspring, and also the rate and extent of temporal change in isotope characteristics of fry from either source (Briers *et al.*, 2013) through variation in isotopic characteristics of their food and differences in growth rates driven by variation in the overall productivity of the rivers (Jonsson *et al.*, 2011).

KEY ISSUES

The aim of this study was to explores some of the issues that need to be taken into consideration when using stable isotope techniques to provide information on the composition of offspring populations. Previous work on relatively small numbers of sites and using very early stages have generally found fairly clear-cut differences between resident and migratory offspring of trout and other salmonids (Doucett *et al.*, 1999; Charles *et al.*, 2004; Curry, 2005; Charles *et al.*, 2006; Jardine *et al.*, 2008; Briers *et al.*, 2013). This study highlights that there may commonly be a considerable amount of variation between the stable isotope ratios of fish found at different sites, even sites that are consistently derived from either migratory or resident reproduction. Drawing a distinction between offspring from resident and migratory females using stable isotope analysis is therefore not always straightforward without an understanding of the extent and pattern of variation in stable isotope ratios within the river under consideration. The most significant influences are summarised below:-

Influence of Sampling Time and Freshwater Feeding

There are quite rapid temporal changes in isotope values following emergence (Curry, 2005) as maternal resources become used up and offspring stable isotope ratios begin to reflect those of local freshwater food sources (Briers *et al.*, 2013). The small size and rapid growth of fry at this stage mean that stable isotope ratios change more rapidly than in larger fish. Sampling during the very early stages of post-emergence is therefore best for enabling maximum distinction, but may be subject to practical limitations. Interpreting the stable isotope ratios of larger fry requires consideration of the effects of freshwater feeding, which may also vary between sites depending on diet, individual variation in physiology and environmental influences such as temperature and catchment productivity (see below).

Effect of Catchment Characteristics

The characteristics of the river catchment in terms of land-use can have an important influence on both the extent and nature of temporal change in stable isotope ratios, through influences on productivity, growth and also the overall carbon and nitrogen stable isotopic characteristics of fish populations at different sites, due to effects at the level of the entire food web. Given that the extent of difference, particularly in nitrogen stable isotopes (Cole *et al.*, 2004; Anderson & Cabana, 2005; Diebel & Vander-Zanden, 2009) can be linked to land-use characteristics, most notably agricultural impacts, it may prove possible to 'correct' stable isotope values for land-use influences, although this awaits further development.

Variation in Adult Stable Isotope Ratios

This has a potentially strong influence on the stable isotope ratios of progeny from both resident and migratory fish. For migratory trout, few data exist on variation in stable isotope ratios of adults in relation to marine feeding areas but it is reasonable to assume that similar patterns will be shown as have been found for Atlantic salmon *Salmo salar* L. (Dempson *et al*., 2009; MacKenzie *et al*., 2012). The stable isotope ratios of adult trout are known to vary along a continuum between typical resident and migratory values, even within a single waterbody (Etheridge *et al*., 2008) and this may reflect both marine feeding and variation relating to catchment influences.

CONCLUSION

In summary, while stable isotope analysis of offspring provides a potentially powerful technique for assigning fry to the progeny of resident or migratory females, in order to make most effective use of the technique for fishery management or other purposes, the potential role of the sources of variation highlighted above need to be considered and accounted for when drawing interpretation from surveys and analysis of young stages.

ACKNOWLEDGEMENTS

A number of people assisted in the fieldwork, preparation and analysis of the samples discussed here; in no particular order: Iain Reid, Viora Weber, Ian Martin and Nora Washbourne. The analysis was supported by funding and donations of time from the Atlantic Salmon Trust, the Wild Trout Trust, the Deveron, Bogie and Isla River Trust, the Deveron Salmon Fishery Board, NERC and Edinburgh Napier University. Stable isotope analysis was undertaken at the NERC Stable Isotope Facility in East Kilbride; thanks are due to Rona McGill and Jason Newton for assistance and advice.

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Table 1. Characteristics of the sites sampled in the River Deveron system.

Burn / River	*Location*	*Easting*	*Northing*	*Notes*
Burn of Boyne	Bridge of Tilly Naught	359887	860962	Coastal burn
Tore Burn	U/S Nethermill	383857	865520	Coastal burn (gravel bar at mouth)
Water of Philorth	Rathen	399586	860759	Coastal burn
King Edward		370741	857789	Thought to be a sea trout burn
Upper Blackwater	Allt Daimh	333369	828008	Well studied brown trout population
Upper Deveron	Charach Water	336079	831183	Trout burn off upper Deveron
Isla	Crooksmill	342492	851422	Downstream of Keith
Isla	Auchindachy	340685	847509	Upstream of Keith (above various barriers)
Upper Bogie	Burn of Craig	344659	825208	Above impassable waterfall - brown trout reference
Bogie	Kirkney Water	358850	833579	Below barrier

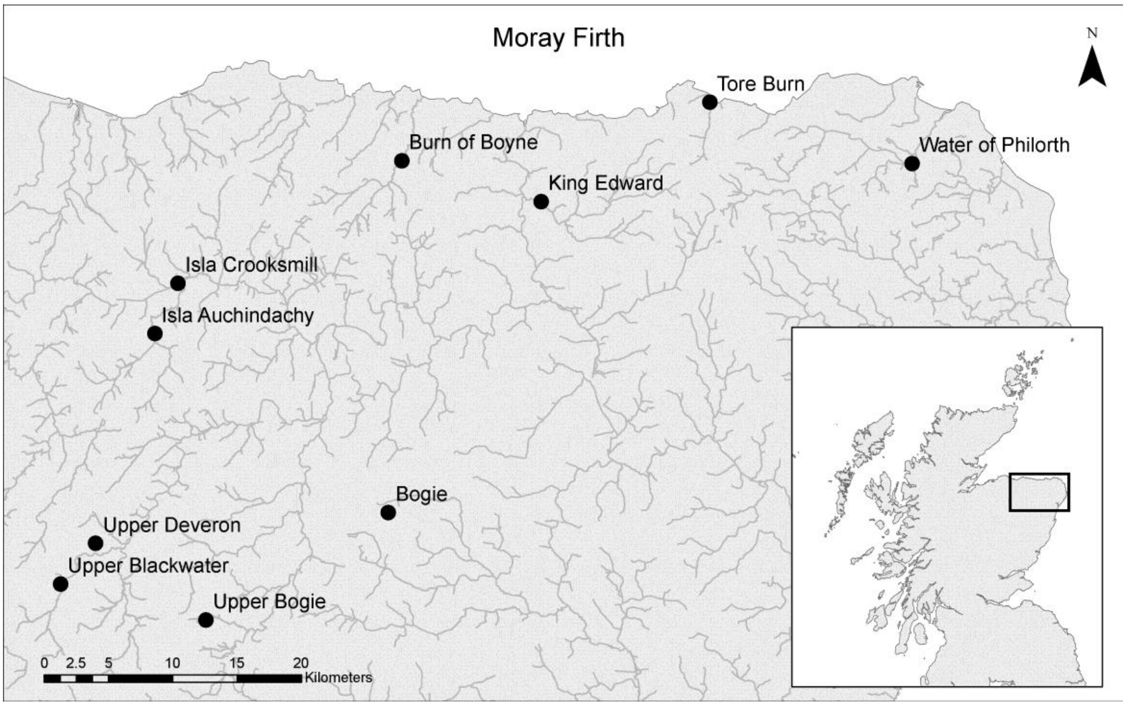

Figure 1. Map of sites sampled within the River Deveron system. Inset map shows location of study area within Scotland. © Crown Copyright and Database Right 2016, Ordnance Survey (Digimap licence).

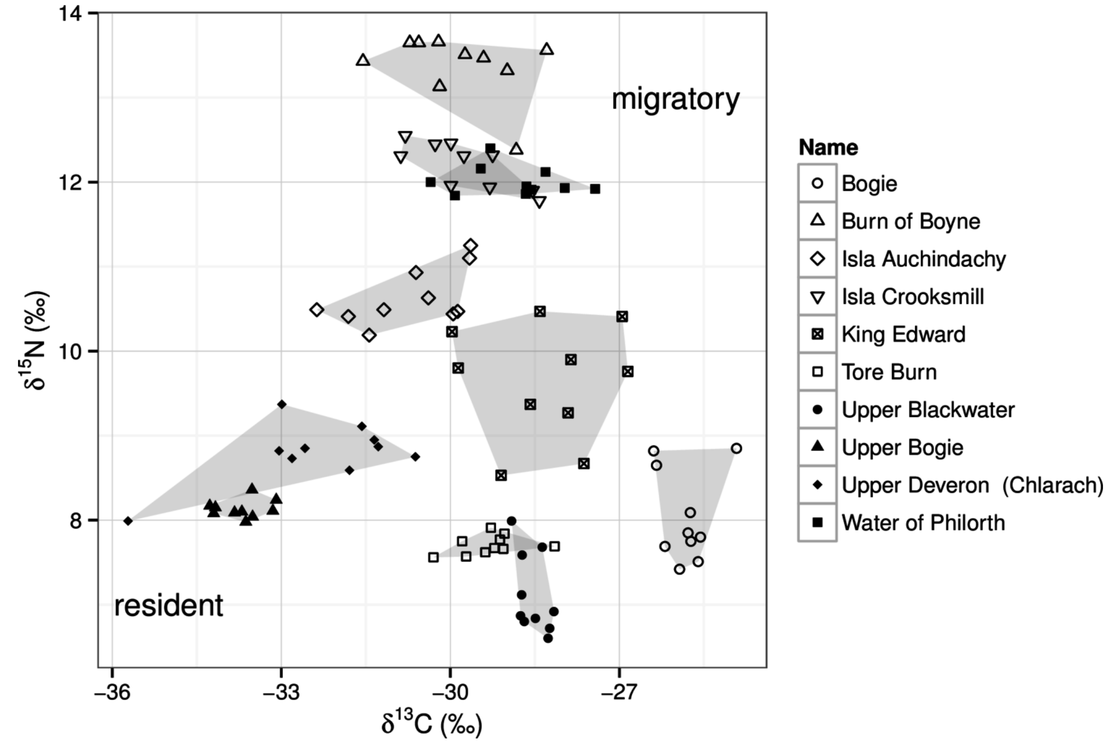

Figure 2. Dual isotope biplot of the trout fry sampled from sites within the River Deveron.

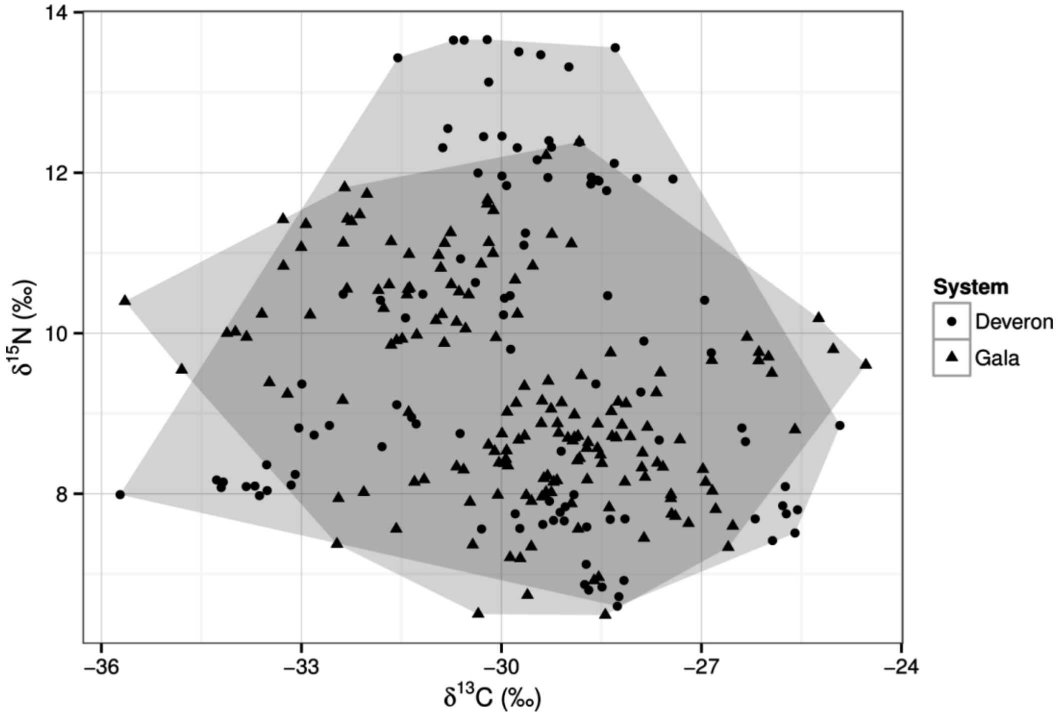

Figure 3. Dual isotope biplot of all fish sampled from the River Deveron and Gala Water.

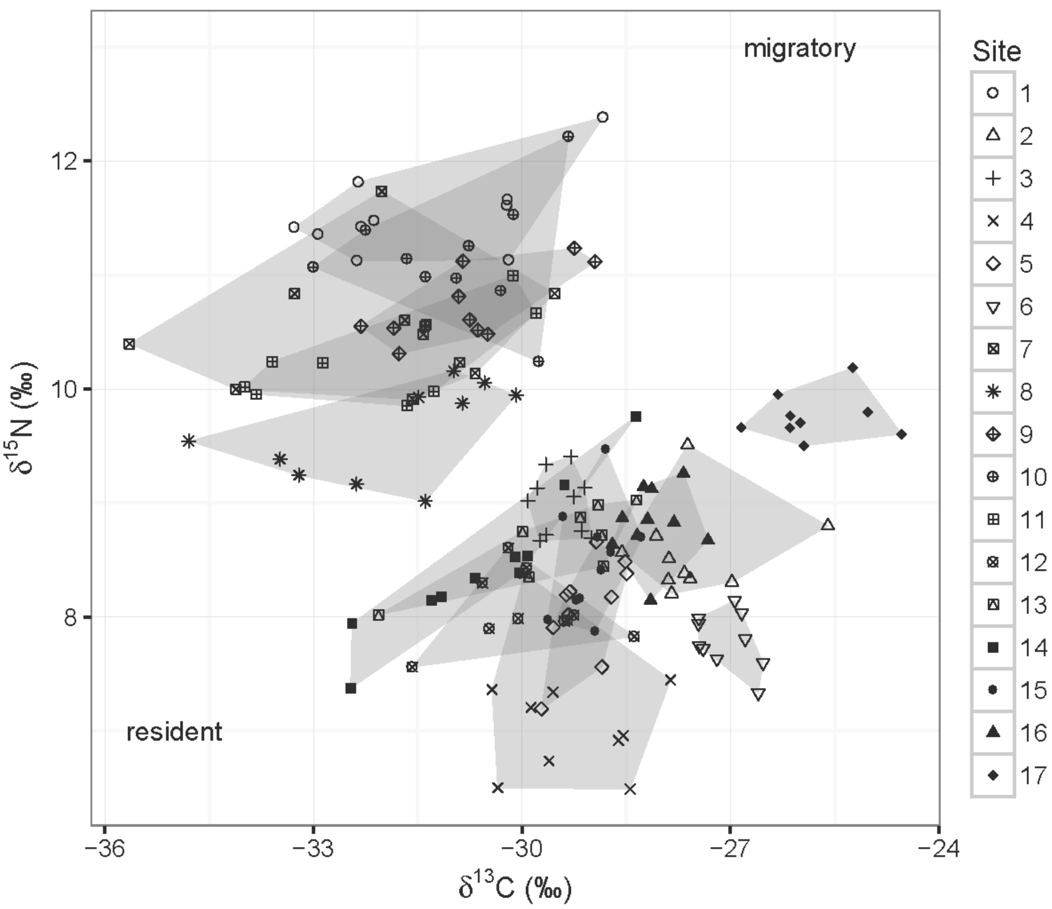

Figure 4. Dual isotope biplot of the trout fry sampled from sites within the Gala Water.

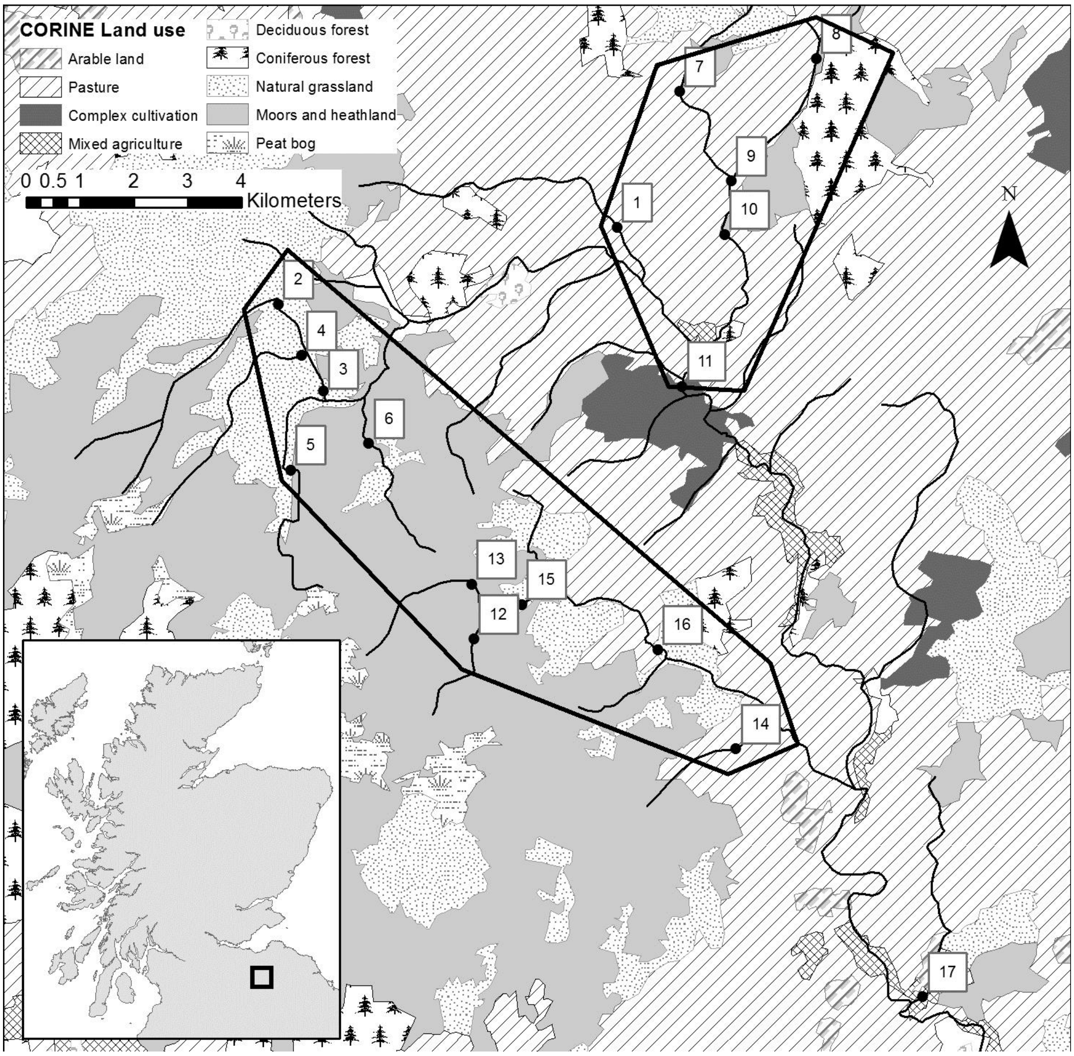

Figure 5. Map of the sites sampled within the Gala Water and the predominant land-use in different parts of the catchment. Inset map shows location of study area within Scotland © Crown Copyright and Database Right 2016, Ordnance Survey (Digimap Licence). Land-use data derived from CLC 2012 Corine Land Cover Classification © European Environment Agency, Copenhagen, 2013.

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