Beresford, N.A.; Yankovich, T.L.; Wood, M.D.; Fesenko, S.; Andersson, P.; Muikku, M.; Willey, N.J. 2013. **A new approach to predicting environmental transfer of radionuclides to wildlife: a demonstration for freshwater fish and caesium**

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3   ►An alternative approach to estimating radionuclide transfer to wildlife is presented
4   ►Analysed a dataset comprising 53 freshwater fish species collected from 67 sites
5   ►Residual Maximum Likelihood mixed model regression used
6   ►Model output takes account of the effect of inter-site variation
7   ► Successfully predicted ^{137}Cs concentrations in different fish species from 27 lakes

A new approach to predicting environmental transfer of radionuclides to wildlife: a demonstration for freshwater fish and caesium

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Abstract

The application of the concentration ratio (CR) to predict radionuclide activity concentrations in wildlife from those in soil or water has become the widely accepted approach for environmental assessments. Recently both the ICRP and IAEA have produced compilations of CR values for application in environmental assessment. However, the CR approach has many limitations most notably that transfer of most radionuclides is largely determined by site-specific factors (e.g. water or soil chemistry). Furthermore, there are few, if any, values for many radionuclide-organism combinations. In this paper we propose an alternative approach and, as an example, demonstrate and test this for caesium and freshwater fish. Using a Residual Maximum Likelihood (REML) mixed-model regression we analysed a dataset comprising 597 entries for 53 freshwater fish species from 67 sites. The REML analysis generated a mean value for each species on a common scale after REML adjustment taking account of the effect of the inter-site variation. Using an independent dataset, we subsequently test the hypothesis that the REML model outputs can be used to predict radionuclide, in this case radiocaesium, activity concentrations in unknown species from the results of a species which has been sampled at a specific site. The outputs of the REML analysis accurately predicted ^{137}Cs activity concentrations in different species of fish from 27 Finnish lakes; these data had not been used in our initial analyses. We recommend that this alternative approach be further investigated for other radionuclides and ecosystems.

Keywords

Concentration ratio, residual maximum likelihood model, caesium, freshwater fish

1. Introduction

Over recent years a number of approaches and associated tools have been developed to assess the exposure of wildlife to ionising radiation (e.g. Copplestone et al., 2001; USDOE, 2002; Brown et al., 2008; ICRP, 2008; ICRP, 2009; Beresford et al., 2008a). These tools use models to predict radionuclide activity concentrations in wildlife to enable internal absorbed dose rates to be estimated. Most commonly this is achieved by using a simple concentration ratio ($CR_{wo-media}$) which relates the whole organism activity concentration to the activity concentration in the appropriate medium for a given environment (e.g. soil or air for the terrestrial environment, water for aquatic environments) (Beresford et al., 2008a).

Given the large number of organisms and radionuclides that may need to be considered to allow assessment of the many source terms and different environments, it is perhaps not surprising that there are many cases where empirical data to derive $CR_{wo-media}$ are lacking. Where this is the case a variety of extrapolation approaches have been used to enable the estimation of whole organism activity concentrations (Copplestone et al., 2003; Beresford et al., 2008b; Higley et al., 2003; ICRP, 2009). Although recent attempts to collate CR values for wildlife have led to improved databases, there are still many gaps in our knowledge (Howard et al., 2013; Copplestone et al., in-press; ICRP 2009). Consequently, there is still a need to develop robust extrapolation approaches most especially: (i) to enable initial screening tier assessments for which site-specific data are not available (Brown et al., in-press); (ii) for protected species for which it may be impossible to acquire sufficient data (e.g. Copplestone et al., 2003); and (iii) for the International Commission on Radiological Protection's Reference Animals and Plants (RAPs) which are defined specifically at the taxonomic family level but for which there are relatively few specific data (ICRP, 2009; Copplestone et al., in-press).

Soil-to-plant transfer of elements of radiological interest has been related to plant evolutionary history, or phylogeny, for Cs (Broadley et al., 1999; Willey et al., 2005), Sr (Willey and Fawcett, 2005a), Ru (Willey and Fawcett, 2006), Cl (Willey and Fawcett, 2005b), Co (Willey and Wilkins, 2008) and U (Willey, 2010). Such phylogenetic relationships present a potential approach to enable predictions of transfer, with some scientific justification, for taxonomic groups for which there are no data either at the generic or site-specific level (Willey, 2010). The potential to derive phylogenetic relationships for

organisms other than plants has also been demonstrated by Jeffree et al. (2010; in-press) who suggested that the transfer of a number of radionuclides to marine teleost and chondrichthyan fishes and the amphioxus (fish like chordate) species *Branchiostoma lanceolatumis* is influenced by phylogeny. However, the work of Jeffree et al. was based upon the results of laboratory studies. Whilst this usefully removes the influences of many confounding factors it is not directly applicable to environmental conditions as foodchain transfer was excluded.

The objective of the work described in this paper was to explore if phylogeny could be used to explain variation in the transfer of radiocaesium to freshwater fish species based on field observations analysed using Residual Maximum Likelihood (REML) mixed-model regression (Willey, 2010) (see section 2.2).

2. Materials and Methods

2.1 Data sources

The primary source of data for the analyses was the database on radionuclide transfer to freshwater organisms as described by Yankovich et al., (in-press) (see also Copplestone et al., in-press). This contains concentration ratios relating the fresh weight (FW) whole organism activity concentration to the activity concentration in water. Where:

$$CR_{wo\text{-}water} = \frac{\text{Activity concentration in whole organism (Bq kg}^{-1}\text{ FW)}}{\text{Activity concentration in (filtered) water (Bq l}^{-1}\text{)}}$$

This database contains 535 $CR_{wo\text{-}water}$ entries describing the transfer of caesium to freshwater fish; some entries are mean values and other single data points. The data set includes $CR_{wo\text{-}water}$ based on both radiocaesium and stable caesium values. The $CR_{wo\text{-}water}$ values are categorised by species, feeding strategy (benthic, predatory or forage) and freshwater ecosystems type ('lake' or 'flowing water'). Some of these data were excluded from this analysis as no species information was recorded (e.g. the source reference specified 'freshwater fish' only). For each study site, the REML analysis (see section 2.2) requires that data are available for more than one species and that at least one of these species must occur at other sites. Excluding data which did not meet these criteria left a total of 248 entries. As we were using the REML model it was possible to supplement the $CR_{wo\text{-}water}$ values with data from studies reporting Cs concentrations in fish; these additional data had not been used by Yankovich et al. (in press) as corresponding water concentrations were not available and hence $CR_{wo\text{-}water}$ values could not be calculated. Concentration data had to adhere to the same requirements as the $CR_{wo\text{-}water}$ values to be included in this analysis. An additional 349 data

entries reporting activity concentrations which met these criteria were identified (Copeland and Ayers 1972; Copeland et al., 1973; Smith et al., 2003 Andersson pers. comm.[1]). In total 597 entries were available for 53 freshwater fish species from 67 sites; note whilst in most instances sites were identified in the source references, in a few cases it was necessary to assume that all the data in a given reference came from one site (these represented <10 % of the total dataset). Table 1 presents a summary of the available data; data were also available for one species in the order Cyprinodontiformes but could not be used as this species occurred at only one site within the database and data for other species were not available for this site.

The taxonomy of each species for which data were available was determined with reference to Nelson (2006) and Froese and Pauly (2012). The 53 species for which there were data all belonged to the class Actinopterygii (ray-finned fishes) with 10 orders, 14 families and 33 genera being represented in the dataset (Table 1). Prior to analyses, orders, families and genera were numerically coded based on the phylogenetic tree presented by Nelson (2006) (Figure 1) with approximate timescales for the evolutionary divergence for each order being identified from http://www.timetree.net. The 'oldest' order was defined as '1' and the most recent as '10' (data being available for a total of ten orders) (Figure 1). In some cases groups of orders diverged at the same time (e.g. Osmeriformes, Salmoniformes and Esociformes) in which case the order numbering was simply from left to right on Figure 1 and does not reflect differences in evolutionary age. To put some context to the order numbers, the clade containing Lepisosteiformes and Amiiformes diverged from the other orders considered here >300 million years ago whereas the clade containing Perciformes diverged from that containing Cyprinodontiformes around 100 millon years ago (see Figure 1). Each species was given a 'taxon number' starting with species in the oldest orders, so for the available dataset *Lepisosteus osseus* was defined as *taxon 1* (see Table 1).

2.2 Data analyses

The Residual Maximum Likelihood (REML) fitting of a mixed-model regression as described by Willey (2010) and originally developed by Broadley et al. (1999;, 2001) was used to analyse the data for any phylogenetic influence on Cs transfer. This technique enables the collation of data from different sources and the prediction of values that might be gained

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[1] Swedish Radiation Safety Authority see http://www.stralsakerhetsmyndigheten.se/Yrkesverksam/Miljoovervakning/Sokbara-miljodata/ for information on monitoring programme from which data obtained.

if they were all generated under an average set of conditions. The output consists of a mean value for each species on a common scale after REML adjustment (the fixed factor) taking account of the effect of the random factor (i.e. inter-site variation). This provides a method for statistically accounting for as much of the effect of site as possible within the collated data. The mean value output for each species provides a relative scaling value which can subsequently be used to infer $CR_{wo-water}$ values, or concentrations, from a known value for a given species or a group mean (Willey, 2010) (or indeed site specific activity concentrations if data are available for one species (see section 3.3 below)).

The REML procedure fits the model such that values for each species are made as nearly identical as possible across the studies. Consequently, we were able to include both $CR_{wo-water}$ values from Yankovich et al. (in-press) and concentration data where the crietia specified within section 2.1 were met. Here we are making the assumption that the relative difference between Cs concentrations between species at a site will be the same as the relative difference between Cs $CR_{wo-water}$ values at a site. The REML procedure minimises, as far as possible, variation due to factors such as water chemistry or study methodology (e.g. $CR_{wo-water}$ values may in some references be related to unfiltered water) by treating the 'site' as a random factor.

The REML analysis and associated analysis of variance was conducted on log-transformed data by adapting the Genstat (http://www.vsni.co.uk) code as presented in Willey (2010) (see Appendix 1 which presents the Genstat code for the overall REML analysis and hierarchical ANOVA). In addition to outputting REML-adjusted means by species (i.e. using the Genstat routine as presented in Appendix 1), REML-adjusted means were also estimated at the level of order, family and genus. To determine significant differences between specific taxonomic groupings the standard error of difference was estimated in a pair wise manner for all REML-adjusted means. The *t*-statistic was then calculated as the ratio of the difference between mean pairs and the associated standard error of difference. All other analyses were conducted using the General Linear Model option from the Minitab statistical package (http://www.minitab.com) or linear regression from Microsoft Excel.

3. Results and discussion

3.1 REML analysis

When all data were considered at the species level, the REML variance component analysis gave a significant (p<0.001) Wald statistic of 116 with significant variation in REML estimated mean values being explained by hierarchical ANOVA at the order level (ANOVA; p<0.001) but little additional variation explained by the effects of family within order or genus within family.

The Wald statistic for the analysis at the levels of order, family and genus were 51 (p<0.001), 54 (p<0.001) and 107 (p<0.001) respectively, also indicating significant data fits (Thompson and Welham 2001. Significant variation in REML estimated mean values was, however, explained by hierarchical ANOVA at the order level (ANOVA; p<0.001) with little additional variation explained by the effects of family within order or genus within family. REML-adjusted mean values are presented in Table 2 for the four different taxonomic levels considered. For each taxonomic level these values should be regarded as relative numbers and not actual estimates of $CR_{wo\text{-water}}$ (see 3.3 for examples of application).

From the estimated *t*-statistics Perciformes had a significantly higher REML-adjusted mean value than Anguilliformes, Clupeiformes, Osmeriformes, Salmoniformes and Cypriniformes (p<0.05). Esociformes also had a significantly higher adjusted mean value than Anguilliformes, Clupeiformes, Salmoniformes and Cypriniformes (p<0.05). Anguilliformes had a significantly lower (p<0.05) adjusted mean than all other orders for which comparisons were justified. Lepisosteiformes, Arniiformes and Siluriformes were not considered in statistical tests as they were present at three or fewer sites only. Significant differences, when tested at more refined taxonomic levels, were generally in agreement with those observed at the order level. For instance at the level of family Anguillidae had a significantly lower REML-adjusted mean than Osmeridae, Salmonidae, Esocidae, Centrarchidae, Moronidae, Clupeidae, Cypinidae and Percidae (p<0.05). Similarly, both Escocidae and Percidae had a significantly higher REML-adjusted means than Salmonidae, Clupeidae and Cypinidae (p<0.05). Comparatively few of the potential comparison at genus and species level could be shown to be significant due to low data availability for some species. Where significant differences were observed (p<0.05), these generally involved comparisons which included *Anguilla*, *Esox*, *Perca* or *Sander* species.

The results of these analyses, therefore, demonstrate differences in Cs transfer to freshwater fish based upon phylogenetically derived taxonomic groupings. Does this then mean that we have demonstrated an evolutionary, or phylogenetic, relationship for the Cs transfer to different freshwater species? On the basis of the data included in our analyses presented here

we cannot establish this. For instance, evolutionarily Lepisosteiformes are most closely related to Amiiformes yet the REML-adjusted means for the two orders differ by a factor of >2 which is more than the difference between Lepisosteiformes and Perciformes, the most distantly related orders (REML-adjusted means being within *c*. 20% of each other). Similarly, whilst the REML-adjusted means for Salmoniformes (560) and Osmeriformes (550) are similar they are considerably lower than that for the order Esociformes (810) which is in the same clade. Our inability to conclude a 'phylogenetic effect' on Cs transfer to freshwater fish is likely due, in part, to the relatively few species and taxonomic groups for which we had data. Whilst we had a relatively large dataset to consider, data were only available for 53 of the total 11952 freshwater species (Nelson, 2006), representing only 10 orders and one class.

Fish within a given taxa are likely to share many characteristics such as feeding strategy. The $CR_{wo-water}$ data presented by Yankovich et al. (in-press) are categorised by feeding type: piscivorous (feeding primarily on smaller fish but also amphibians, mammals and birds), forage (feeding on primary producers and invertebrates and zooplankton) feeding, or benthic (feeding on benthic-dwelling organisms) feeding. When analysed by feeding group the Cs $CR_{wo-water}$ value for piscivorous fish is significantly higher ($p<0.05$) than those for both benthic and forage feeding fish (Figure 2). This is in agreement with the findings of a number of previous authors who have reported higher Cs concentrations in piscivorous, or predatory, fish compared to fish feeding on benthos, invertebrates and primary producers (e.g. Kryshev 1995, Kryshev et al., 1993; Rowan et al., 1998; Rowan and Rasmussen, 1994; Saxén and Ilus, 2008; Smith et al., 2000). We should acknowledge that the data from some of these papers were included within our analyses, although they comprise a relatively small proportion of the total data available to us via the database described by Yankovich et al. (in-press).

3.2 Effect of other variables on $CR_{wo-water}$

This paper represents the first analysis, for any radionuclide, of the $CR_{wo-water}$ dataset presented by Yankovich et al. (in-press). In addition to categorising data by species and feeding group, data in the underlying database are also categorised as coming from either 'flowing' (i.e. rivers and streams) or 'lake' (i.e. lakes, reservoirs and ponds) freshwater ecosystems. There is a significant difference between the mean $CR_{wo-water}$ values estimated for the two ecosystem types with values from lakes being higher than those for flowing waters (see Figure 2). Figure 2 also presents a comparison of $CR_{wo-water}$ values for freshwater

fish derived from measurements of ^{137}Cs (n=506) compared to those derived from stable Cs measurements (n=214). The mean stable Cs $CR_{wo-water}$ value is significantly higher than that derived from ^{137}Cs data ($p<0.05$). Although this may initially be interpreted as a need to question the increasing usage of stable element data to provide CR values for radiological assessments (Beresford 2010, Howard et al., 2013, Copplestone et al., in-press, ICRP, 2009) further consideration of the underlying database is required. All of the stable Cs data are derived from North America (Yankovich, 2010; Rowan and Rasmussen, 1994; Vanderploeg et al., 1975), with the majority being from Canada (n=197). Whereas the majority of ^{137}Cs data originated from studies in Europe with observations from Russian language publications (see Fesenko et al., 2010) contributing *c.* 45% of the ^{137}Cs $CR_{wo-water}$ values. Furthermore, and likely of more importance than data source, piscivorous species (171 of 214 values) dominated the stable Cs data, which tend to have comparatively high $CR_{wo-water}$ values (Figure 2); *c.* 50% of the $CR_{wo-water}$ values for ^{137}Cs were for forage and benthic feeding species. Rowan (2013), observed that ^{137}Cs $CR_{wo-water}$ values in piscivorous fish exposed to repeated short releases into a river were higher than those for ^{133}Cs determined in the same fish. Further consideration of the application of stable element data to predict radioisotope transfer in wildlife assessment models can be found in Wood et al. (submitted).

We should also acknowledge that although $CR_{wo-water}$ is defined on the basis of filtered water activity concentrations, in some instances, source references are unclear as to if water has been filtered or not. This could contribute to the observed uncertainty in $CR_{wo-water}$ values, however, as noted above the effect of methodological differences on $CR_{wo-water}$ should be minimised by the application of REML analyses.

Typically $CR_{wo-water}$ values for freshwater fish and other aquatic organisms vary over orders of magnitude, as do $CR_{wo-media}$ values for organisms in other ecosystem types. This is demonstrated for freshwater fish in Table 3 which presents a summary of Cs $CR_{wo-water}$ values from the compilation of Yankovich et al. (in-press) as will be used in a forthcoming IAEA handbook of transfer parameters for wildlife (see Howard et al., 2013). In large part this variability is due to site specific factors which influence radionuclide transfer. In the case of Cs and freshwater fish a key site specific factor is the K concentration in water (e.g. Smith et al. (2000) demonstrate approximately two-orders of magnitude variation in $CR_{wo-water}$ explained by water K concentration) with water pH and Ca concentration also being suggested to influence Cs transfer (Smith et al., 2002). Consequently, there is often large variation between the outputs of models using CR_{wo} values to predict activity concentrations

in wildlife (Beresford et al., 2008c; Yankovich et al., 2010; Johansen et al., 2012) and the approach is open to criticism as being too simplistic (ICRP 2009). However, pragmatically the CR_{wo} approach is easy to apply and has the most comprehensive datasets available, and hence it continues to be recommended in international compilations (Howard et al., 2013; ICRP, 2009). The REML-adjusted means presented in Table 2 potentially provide a more refined approach. By taking into account inter-site variation, they in effect provide a mechanism of accounting for site specific variables such as, the K concentrations in water in the case of Cs transfer to fish being considered here. Comparison of Tables 2 and 3 suggests that the variation in the transfer of Cs to fishes between studies/sites (two to three orders of magnitude) is considerably greater than the likely variation between taxonomic groups at a given site (*circa* one order of magnitude or less). This further demonstrates the crude nature of generic CR values if trying to make site specific predictions.

3.3 Testing the REML outputs

We propose the hypothesis that the REML model outputs can be used to predict the radionuclide, in this case radiocaesium, activity concentrations in unknown species from the results of a species which has been sampled at a specific site. To test this hypothesis we require data which had not been included in the already comprehensive compilation needed to conduct the analysis described above. A large monitoring programme of fish from 590 Finnish lakes has been conducted since deposition from the 1986 Chernobyl accident and data from this programme have recently been made available on request via the EURATOM network of excellence in radioecology (www.star-radioecology.org). These data were not used within the analysis we have conducted above and so provide an opportunity to independently test our hypothesis. The monitoring programme is in part described by Saxén and Koskelainen (2005), Saxén (2007), Saxén and Ilus (2008), Vetikko and Saxén (2010), with a meta data record available on-line (STUK, 2012).

We have selected data from 1988, which were collected from 27 Finnish lakes for which ^{137}Cs activity concentrations were available for four or more fish species. In total data were available for 11 fish species: *A. brama, E. lucius, P. fluviatilis, R. rutilus, S. trutta, S. lucioperca, Coregonus albula, Coregonus lavaretus, Blicca bjoerkna, Leuciscus idus, Abramis ballerus* and *Lota lota*. As *P. fluviatilis* was present at all 27 sites and was also well represented within the dataset used for the REML analysis, we selected this as the species

from which to calculate activity concentrations for the other species which were treated as unknowns. To calculate the ^{137}Cs activity concentrations, the ratios of the REML-adjusted mean (Table 2) for each unknown species to that of *P. fluviatilis* was estimated (e.g. for *R. rutilus* the ratio was 0.48). For each lake the ^{137}Cs activity concentration in different species were then estimated as the product of this ratio and the geometric mean ^{137}Cs activity concentration in *P. fluviatilis* at that site. This approach was possible for *A. brama, E. lucius, R. rutilus, S. trutta* and *S. lucioperca* all of which were in the dataset used for the REML analysis. Although other *Coregonus* spp. were present within our initial dataset, the two species sampled in Finnish lakes were not. Therefore, the ratio of the REML-adjusted mean for the genus *Coregonus* to that for *Perca* was used to estimate ^{137}Cs activity concentrations in both species; a similar genus based approach was used for *A. ballerus*. No data for the genus *Blicca* or *Leuciscus* were available for our REML analysis. Therefore, as both species are Cypinidae the ratio of the REML-adjusted mean for this family to that of Percidae was estimated and used to predict ^{137}Cs activity concentrations in both *B. bjoerkna* and *L. idus*. No predictions could be made for *L. lota* as it is a Gadiforme and no representatives of this order were present in the database used to populate the REML analysis (values for *L. lota* are within the CR$_{wo-water}$ dataset described by Yankovich et al. (in-press) but they did not meet the selection criteria required for the REML analysis). In total this allowed predictions in 100 fish samples across the 27 lakes.

A comparison of predicted ^{137}Cs activity concentrations with measured values is presented in Figure 3. There was relatively good agreement between predicted and measured values with a linear regression fit to all 100 data points yielding an R^2 of 0.83 (p<0.001) and a slope (±standard error) of 0.98±0.04 (p<0.001). The intercept was not significantly different to zero for this or any of the subsequent regressions discussed and hence a zero intercept was used. Linear regressions were also fitted individually for *A. brama, E. lucius*, *and R. rutilus,* with these three Salmonidae being considered together given there were few observations for them. All regressions yielded R^2 values close to 0.8 (0.76 – 0.84) (p<0.001). Slopes (±standard error) were: *A. brama* (0.96±0.12)*, E. lucius* (0.81±0.06)*, R. rutilus* (1.32±0.12)*, S. lucioperca* (0.69±0.07) and Salmonidae species (0.87±0.08) thus suggesting a tendency to under-predict for some species (notably *E. lucius* and *S. lucioperca*) and over-predict for *R. rutilus*.

The results of this comparison therefore look promising. There is obviously some scope for the results obtained to be influenced by the selection of *P. fluviatilis* as our 'known' species.

For instance, *E. lucius* was present in the sample from 26 of the lakes if we select this as our known species for comparison with the results using *P. fluviatilis* then we can make predictions for 96 fish samples. Whilst all predicted values were within a factor of *c.* 5 of the measured data there was a tendency towards over prediction (Figure 4).

For comparison with our results, if the appropriate feeding group geometric mean $CR_{wo-water}$ values from Yankovich et al. (in-press) are used to predict the ^{137}Cs activity concentrations in fish from the Finnish lakes there is a general under-prediction with a regression of predicted to measured activity concentrations yielding a slope of only 0.31.

The approach tested here should account for site specific factors, and as demonstrated appears to work relatively well. However, we should acknowledge some limitations in the available data which may influence the resultant REML adjusted means. As noted above, there is geographical bias in available data and, therefore, site-specific variability may not be adequately compensated for in some species. For instance, whilst *A. anguilla* data are available from 5 sites (Table 1) these were all within Sweden and hence the sites may be relatively similar. Similarly, limitations in sample size for the Finnish lakes dataset, which ranged from 1 to >400 fish, may have impacted upon some comparisons of predicted and observed activity concentrations.

Given the variation in biological half-lives across different organisms, the REML approach is unlikely to be applicable in situations where activity concentrations in the environment are rapidly changing. By 1988 water activity concentrations in Finnish lakes as a consequence of deposition from the Chernobyl accident were not changing rapidly; Saxén (2007) reports ecological half-lives for ^{137}Cs in Finnish lake waters of *circa* 1 to 5 years at this time. However, we acknowledge that a lack of equilibrium may have influenced the comparison of our predicted activity concentrations in fish with measured values. For example, increases in ^{137}Cs activity concentrations have been observed with increasing body mass for some species of predatory fish, potentially as a consequence of changing diets as fish age (Smith and Beresford, 2005).

4. Conclusions

Whilst we have demonstrated differences between the Cs transfer to different taxa of freshwater fish, based upon the data available to us (all species originating from just 10 orders in one class) we cannot describe detailed phylogenetic relationships. Earlier analyses which have suggested phylogentic relationships for the transfer of radionuclides to plants (see

Willey, 2010) and marine fish (Jeffree et al., 2010; in-press) have included species encompassing much wider evolutionary time scales (e.g. >500 million years in the case of marine fish (Jeffree et al., 2010; in-press)).

The commonly used CR approach to estimating the radionuclide activity concentrations in wildlife is open to criticism, as CR values can be highly variable, largely due to site-specific factors. The analyses of available data using the REML analysis, as demonstrated here, should compensate for inter-site variation, assuming sufficient data are available for the analysis. For freshwater fish the outputs of the REML analysis predicted ^{137}Cs activity concentrations in an independent dataset well. Caesium was selected for this paper as an example and because there was a relatively large amount of data available. We recommend that this approach of producing relative values be further investigated and developed for other radionuclides and across a wider range of organisms. A disadvantage of the approach is that it requires relatively large datasets which much meet specific criteria. However, recent data compilations (Howard et al., 2013; Yankovich et al., in-press; Copplestone et al., in-press) should enable similar analysis to be conducted for a number of elements for terrestrial, marine and freshwater species. The ICRP (2009) suggested identifying a series of terrestrial, freshwater and marine sites from which samples of their Reference Animals and Plants (RAPs) could be sampled and analysed to serve as 'points of reference'. Such studies have been initiated and results are starting to be published (Barnett et al., 2013). However, such data are highly site specific, potentially limiting their wider value. However, applying the approach as conducted in this paper to data such as that presented by Barnett et al. (2013) to derive relative values for different organisms should provide a more generic set of 'reference data'. In taking the REML approach forward if will be beneficial to target studies to provide data that will fill gaps in the input data reducing uncertainties and biases in the REML outputs.

Acknowledgements

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Table 1. Summary of data available for REML analysis to investigate any phylogentic influence on the transfer of Cs to freshwater fish.

Order	Family	Genus	Species	Sites species present	Taxon number
Lepisosteiforme	Lepisosteida	*Lepisosteus*	*osseus*	1	1
Amiiformes	Amiidae	*Amia*	*calva*	1	2
Anguilliformes	Anguillidae	*Anguilla*	*anguilla*	5	3
Clupeiformes	Clupeidae	*Dorosoma*	*cepedianum*	1	4
Clupeiformes	Clupeidae	*Alosa*	*pseudoharengus*	10	5
Osmeriformes	Osmeridae	*Osmerus*	*mordax*	10	6
Salmoniformes	Salmonidae	*Coregonus*	*clupeaformis*	2	7
Salmoniformes	Salmonidae	*Coregonus*	*hoyi*	2	8
Salmoniformes	Salmonidae	*Coregonus*	*artedi*	2	9
Salmoniformes	Salmonidae	*Coregonus*	spp.	6	10
Salmoniformes	Salmonidae	*Oncorhynchus*	*kisutch*	5	11
Salmoniformes	Salmonidae	*Oncorhynchus*	*mykiss*	4	12
Salmoniformes	Salmonidae	*Oncorhynchus*	*tschawytscha*	3	13
Salmoniformes	Salmonidae	*Salmo*	*trutta*	11	14
Salmoniformes	Salmonidae	*Salvelinus*	*alpinus*	1	15
Salmoniformes	Salmonidae	*Salvelinus*	*fontinalis x namaycush*	1	16
Salmoniformes	Salmonidae	*Salvelinus*	*namaycush*	8	17
Salmoniformes	Salmonidae	*Salvelinus*	*siscowet*	1	18
Salmoniformes	Salmonidae	*Stenodus*	*leucichthys*	1	19
Esociformes	Esocidae	*Esox*	*lucius*	38	20
Esociformes	Esocidae	*Esox*	*niger*	1	21
Cypriniformes	Catostomida	*Catostomus*	*catostomus*	2	22
Cypriniformes	Catostomida	*Catostomus*	*commersoni*	1	23
Cypriniformes	Catostomida	*Moxostoma*	*aureolum*	1	24
Cypriniformes	Cyprinidae	*Abramis*	*brama*	7	25
Cypriniformes	Cyprinidae	*Carassius*	*auratus*	3	26
Cypriniformes	Cyprinidae	*Carassius*	*carassius*	2	27
Cypriniformes	Cyprinidae	*Cyprinus*	*carpio*	3	28
Cypriniformes	Cyprinidae	*Notemigonus*	*crysoleucas*	1	29
Cypriniformes	Cyprinidae	*Rutilus*	*rutilus*	5	30
Cypriniformes	Cyprinidae	*Scardinius*	*erythrophthalmus*	3	31
Cypriniformes	Cyprinidae	*Notropis*	*hudsonius*	7	32
Cypriniformes	Cyprinidae	*Gobio*	*gobio*	1	33
Cypriniformes	Cyprinidae	*Tinca*	*tinca*	7	34
Siluriformes	Ictaluridae	*Ictalurus*	*punctatus*	2	35
Siluriformes	Ictaluridae	*Ictalurus*	spp.	1	36

Perciformes	Centrarchida	*Ambloplites*	*rupestris*	2	37
Perciformes	Centrarchida	*Lepomis*	*gulosus*	1	38
Perciformes	Centrarchida	*Lepomis*	*macrochirus*	3	39
Perciformes	Centrarchida	*Lepomis*	*gibbosus*	1	40
Perciformes	Centrarchida	*Lepomis*	*microlophus*	1	41
Perciformes	Centrarchida	*Micropterus*	*dolomieu*	6	42
Perciformes	Centrarchida	*Micropterus*	*salmoides*	5	43
Perciformes	Centrarchida	*Pomoxis*	*annularis*	1	44
Perciformes	Centrarchida	*Pomoxis*	*nigromaculatus*	1	45
Perciformes	Moronidae	*Morone*	*chrysops*	4	46
Perciformes	Percidae	*Perca*	*flavescens*	8	47
Perciformes	Percidae	*Perca*	*fluviatilis*	28	48
Perciformes	Percidae	*Sander*	*lucioperca*	3	49
Perciformes	Percidae	*Sander*	*canadensis*	1	50
Perciformes	Percidae	*Sander*	*vitreus*	9	51
Perciformes	Percidae	*Gymnocephalus*	*cernuus*	1	52
Perciformes	Sciaenidae	*Aplodintus*	*grunniens*	1	53

Table 2. REML-adjusted means for different taxonomic groups. Note these are relative values and not absolute values of $CR_{wo\text{-}water}$.

Order		Family		Genus		Species	
Lepisosteiformes	6.8	Lepisosteidae	6.9	*Lepisosteus*	7.6	*osseus*	7.4
Amiiformes	3.2	Amiidae	2.9	*Amia*	3.6	*calva*	3.0
Anguilliformes	1.8	Anguillidae	1.9	*Anguilla*	1.9	*anguilla*	2.1
Clupeiformes	5.0	Clupeidae	4.9	*Dorosoma*	4.7	*cepedianum*	4.3
				Alosa	4.0	*pseudoharengus*	3.7
Osmeriformes	5.6	Osmeridae	5.5	*Osmerus*	4.5	*mordax*	4.2
Salmoniformes	5.5	Salmonidae*	5.5	*Coregonus*	3.8	*clupeaformis*	3.9
						hoyi	5.0
						artedi	3.5
						spp.	3.2
				Oncorhynchus	8.1	*kisutch*	9.3
						mykiss	6.5
						tschawytscha	8.9
				Salmo	6.0	*trutta*	5.8
				Salvelinus	7.8	*alpinus*	7.9
						fontinalis x namaycush	5.6
						namaycush	8.0
						siscowet	10.8
				Stenodus	5.4	*leucichthys*	5.6
Esociformes	8.1	Esocidae	8.3	*Esox*	8.5	*lucius*	8.8
						niger	3.0
Cypriniformes	4.6	Catostomidae	5.3	*Catostomus*	5.0	*catostomus*	4.5
						commersoni	4.4
				Moxostoma	4.7	*aureolum*	4.2
		Cyprinidae	4.5	*Abramis*	4.5	*brama*	4.8
				Carassius	4.4	*auratus*	4.3
						carassius	4.9
				Cyprinus	1.2	*carpio*	1.2
					6.2	*crysoleucas*	5.7
				Rutilus	4.8	*rutilus*	5.0
				Scardinius	4.4	*erythrophthaimus*	4.7
				Notropis	3.9	*hudsonius*	3.6
				Gobio	6.2	*gobio*	6.5
				Tinca	3.1	*tinca*	3.2
Siluriformes	7.6	Ictaluridae	7.6	*Ictalurus*	6.2	*punctatus*	5.7
						spp.	5.1
Perciformes	8.6	Centrarchidae	7.0	*Ambloplites*	14.2	*rupestris*	13.8
				Lepomis	4.1	*gulosus*	5.3

Order		Family		Genus		Species	
						macrochirus	3.7
						gibbosus	3.7
						microlophus	2.9
				Micropterus	9.6	*dolomieui*	8.7
						salmoides	8.5
				Pomoxis	8.3	*annularis*	7.3
						nigromaculatus	9.3
		Moronidae	7.5	*Morone*	9.1	*chrysops*	8.8
		Percidae	9.0	*Perca*	9.4	*flavescens*	7.3
						fluviatilis	10.5
				Sander	10.0	*lucioperca*	7.8
						canadensis	12.3
						vitreus	11.8
				Gymnocephalus	1.9	*cernuus*	2.0
		Sciaenidae	15.9	*Aplodinotus*	11.2	*grunniens*	10.3

*The ICRP Reference Trout (the freshwaster fish RAP) is defined as the Salmonidae family.

Table 3. Summary of Cs $CR_{wo-water}$ values for fish from Yankovich et al. (in-press).

Feeding group	Arithmetic mean ±SD	Geometric mean(GSD)	Minimum	Maximum	N
Benthic feeding	(1.0±2.0)E+3	4.6E+2(3.5)	1.8E+1	2.0E+4	156
Forage feeding	(9.2±16)E+2	4.7E+2(3.2)	1.7E+1	8.6E+3	125
Piscivorous	(4.5±6)E+3	2.7E+3(2.8)	1.3E+1	8.2E+4	439
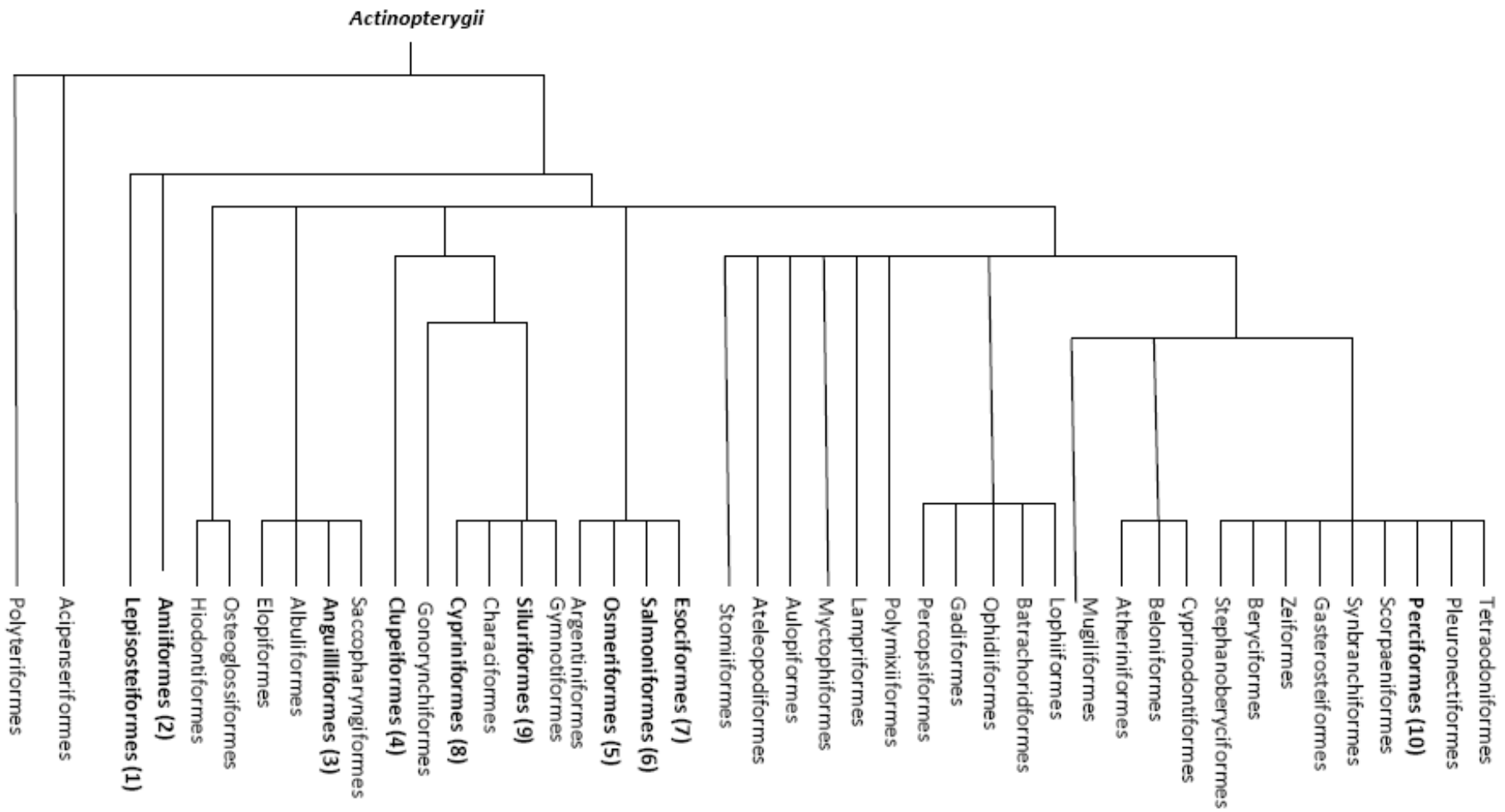
Figure 1. Sequence of orders within the class Actinopterygii (ray-finned fishes) adapted from Nelson (2006). Orders for which data are available are identified in **bold**; the number in parenthesis denotes that given to the order based upon evolutionary time for statistical analyses.

Figure 2. Comparisons of caesium $CR_{wo-water}$ values for freshwater fish summarised by feeding strategy (top), Cs isotope (middle) and broad habitat type (bottom). Values are geometric mean $CR_{wo-water}$ values; on each chart mean values with different letters are significantly different ($p<0.05$); statistical tests were performed on log-transformed data. Note: (i) these figues summarise $CR_{wo-water}$ values from Yanovich et al., in-press and not REML-adjusted means; (ii) to enable analyses of these data which include multiple entries of summarised data we have used the approach developed by Wood et al. (submitted) this results in some differences to the values of Yankovich et al. (in press) as presented in Table 3.
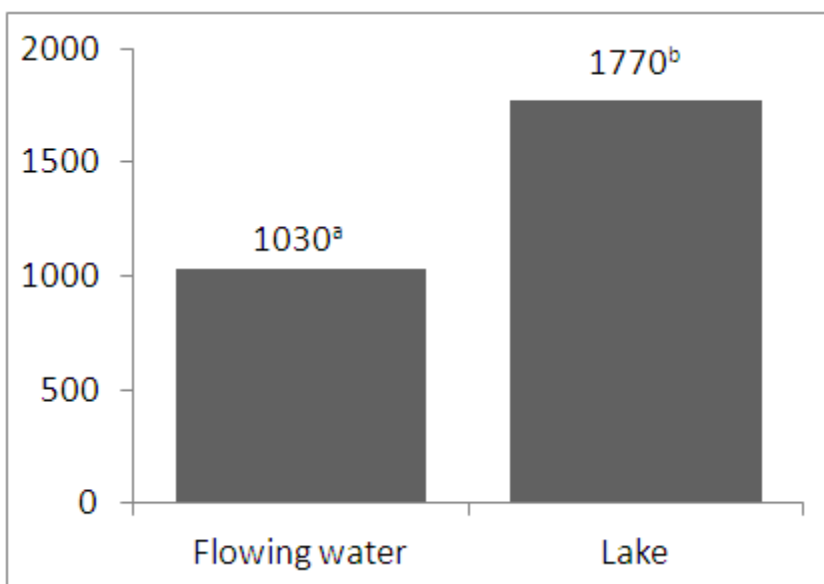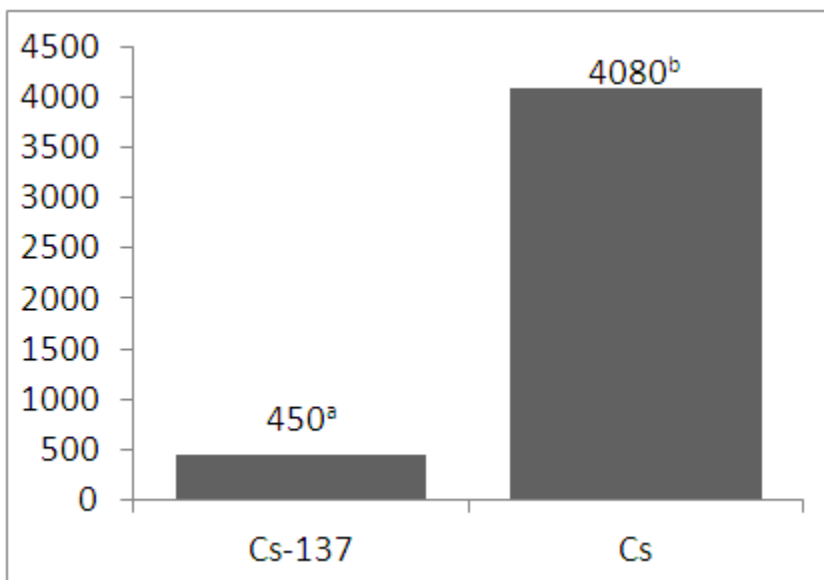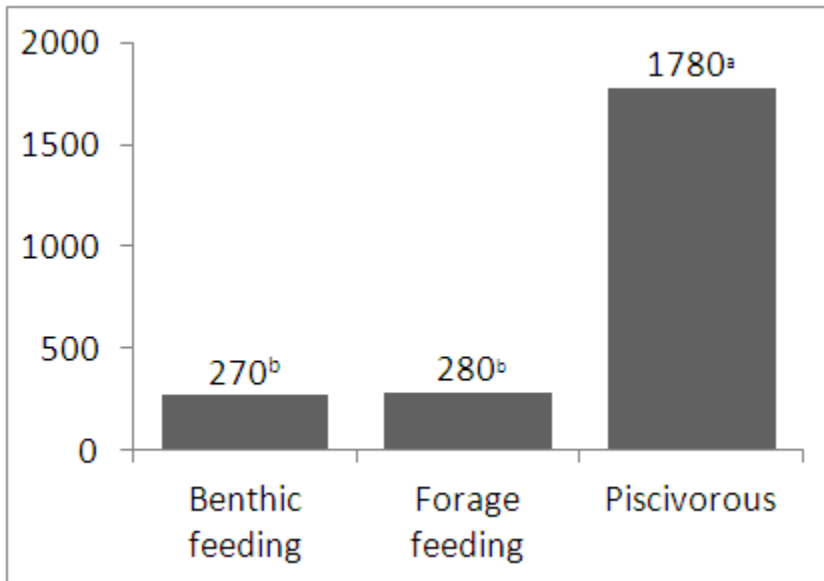
Figure 3. Comparison of measured ^{137}Cs activity concentrations in fish collected from 26 Finnish lakes in 1988 with predicted activity concentrations using the outputs of the REML analyses and data for *Perca fluviatilis* (line is 1:1 relationship). 'Other Cyprinidae' represents single values for *Blicca bjoerkna*, *Leuciscus idus* and *Abramis ballerus*.
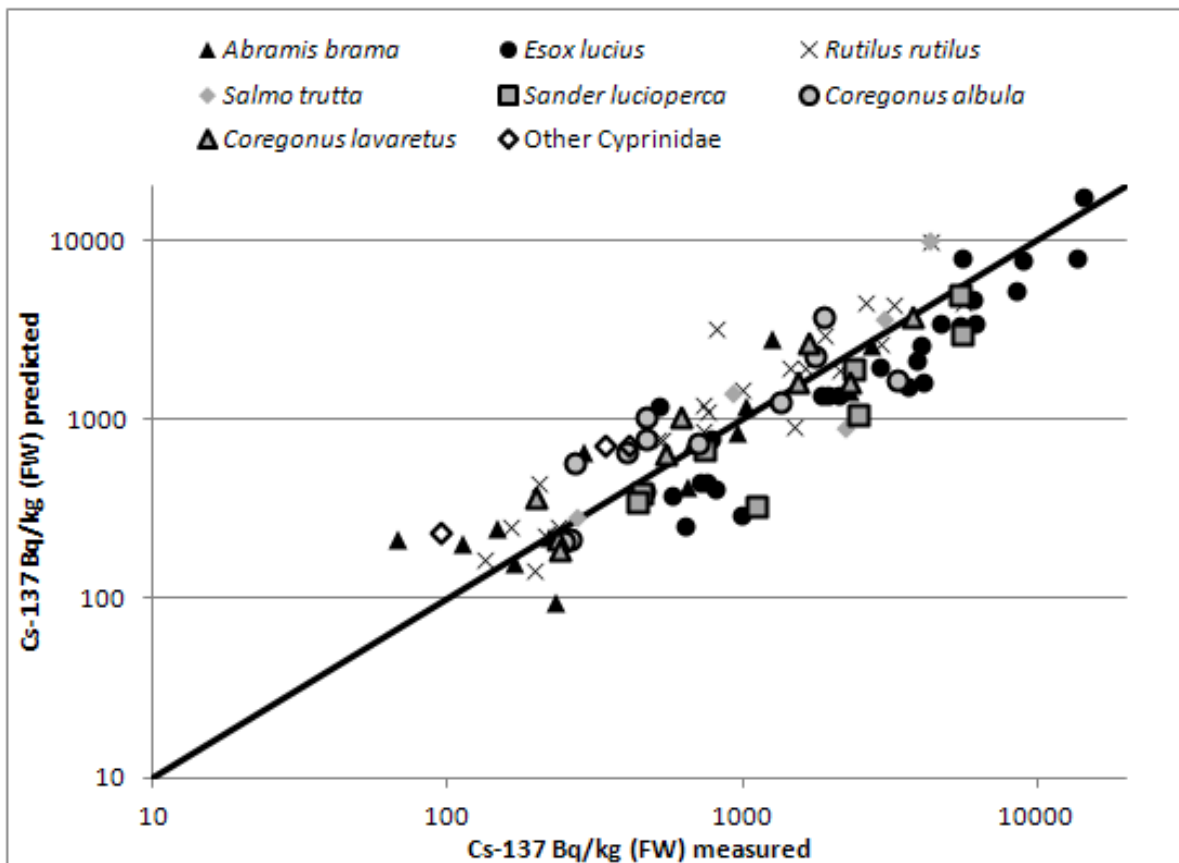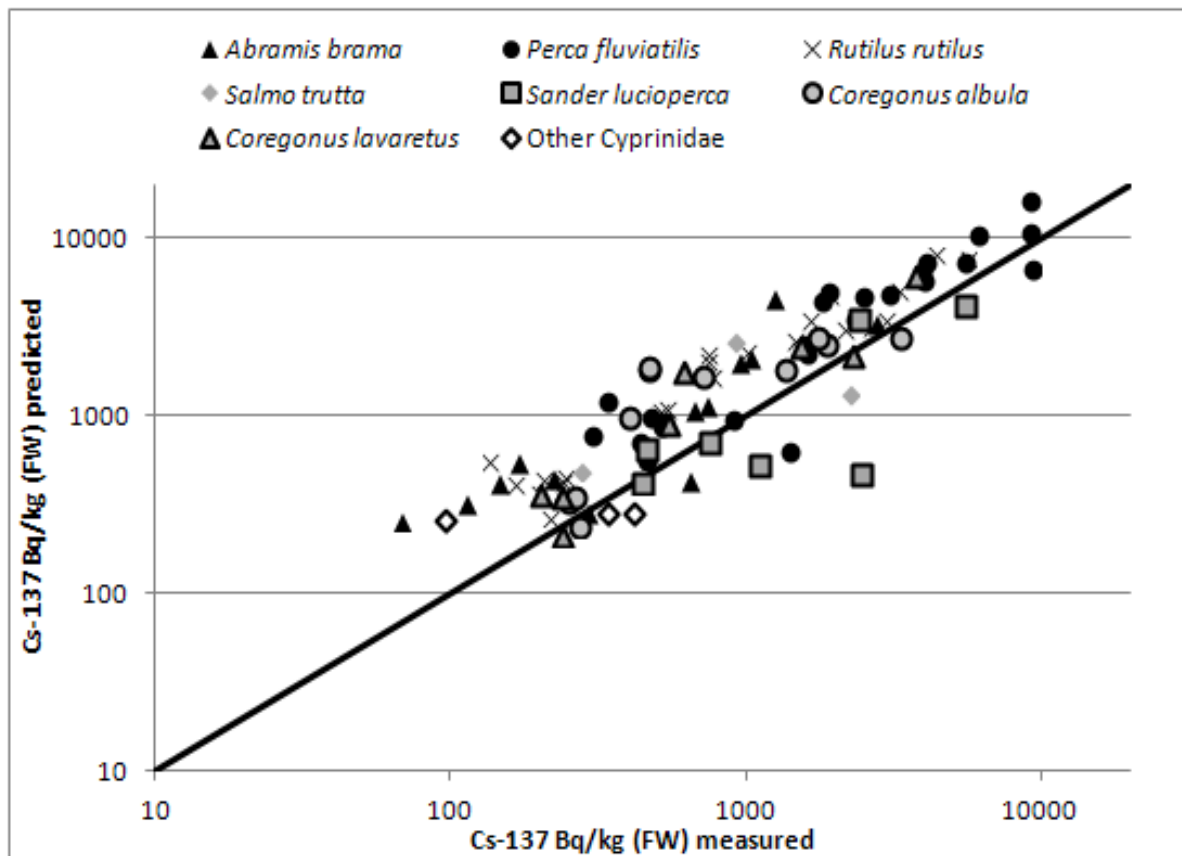
Figure 4. Comparison of measured ^{137}Cs activity concentrations in fish collected from 26 Finnish lakes in 1988 with predicted activity concentrations using the outputs of the REML analyses and data for *Esox lucius* (line is 1:1 relationship). 'Other Cyprinidae' represents single values for *Blicca bjoerkna, Leuciscus idus* and *Abramis ballerus*.

Appendix 1: Genstat code for the overall REML analysis and hierarchical ANOVA.

```
%CD 'C:/Documents and Settings/NAB/My Documents'

job 'Analysis of Cs fish data July 2012 by taxon'

unit [597]

fact [lev=67] site

fact [lev=53] taxon

open 'C:/Documents and Settings/NAB/My Documents/Cs fish july 2012.prn'; ch=2; fi=in

skip [2] 1

read [ch=2] site,taxon,Cs

close ch=2

tabu [cl=site] Cs; mean=mtab; var=vtab; nobs=ntab

calc sdtab = sqrt(vtab)

calc cvtab = sdtab*100/mtab

print ntab,mtab,vtab,sdtab,cvtab; dec=0,4(4); fie=6,4(13)

calc lCs = log(Cs)

vcomponents [fixed=taxon] random=site

reml [print=model,components,Waldtests; method=fisher] lCs

vkeep terms=taxon; means=tmeans; SEDMEANS=sedtmeans

calc etmeans = exp(tmeans)

print tmeans,etmeans,sedtmeans

vtable table=tmeans; variate=mCs; class=!p(mtaxon)

unit [53]

fact species,genus,family,order

open 'C:/Documents and Settings/NAB/My Documents/Cs fish phylogeny july 2012.prn'; ch=2; fi=in

skip [2] 1

read [ch=2] taxoncode,species,genus,family,order

close ch=2

treat order/family/genus/species

anov [fact=3;fprob=yes] mCs

endjob
```