



# Radionuclide biokinetics in the Russian sturgeon and phylogenetic consistencies with cartilaginous and bony marine fishes



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## ABSTRACT

The biokinetics of eight radionuclides ( $^{241}\text{Am}$ ,  $^{109}\text{Cd}$ ,  $^{134}\text{Cs}$ ,  $^{75}\text{Se}$ ,  $^{54}\text{Mn}$ ,  $^{110\text{m}}\text{Ag}$ ,  $^{65}\text{Zn}$ ,  $^{60}\text{Co}$ ) absorbed from the aquatic medium by juvenile Russian sturgeon (*Acipenser gueldenstaedtii*) were experimentally determined in fresh (0.42‰) and brackish (9.0‰) waters, of a similar salinity range to the Caspian Sea, and in conjunction with chemical speciation modelling. Uptake and loss rate constants were determined for each radionuclide for a 14 day exposure at each salinity and during 28 days of exposure to radionuclide-free conditions. Whole body (wet): water concentration factors (CF) achieved over 14 days for these eight radionuclides were used in a comparison with the same radionuclide CFs previously determined experimentally for six species of marine teleosts and chondrichthyans, to further test a phylogeny-based model of multi-nuclide bioaccumulation based on marine chordates. Multivariate analyses (multidimensional scaling and hierarchical clustering) identified the relative affinities among these taxa and also those radionuclides which distinguished most between them, in their differing CFs. They consistently showed that sturgeon aggregated as a group, which was also slightly differentiated with salinity. Sturgeon were distinguished from all teleosts and chondrichthyans but were more dissimilar from chondrichthyans than teleosts, in accordance with sturgeon's different periods of divergence from them in evolutionary time. Variable salinity among experiments may also cause changes in radionuclide bioaccumulation due to variations in (i) bioavailability (ii) osmolarity, and (iii) competitive inhibition of a radionuclide's bioaccumulation by its stable analogue or metabolic model. Their potentially confounding effects on these patterns of radionuclide CFs among taxa were critically evaluated for those radionuclides which discriminated most between sturgeon and teleosts or chondrichthyans. Bioavailability, osmolarity and competitive inhibition effects were identified among salinity treatments, however they were not appreciable enough to override the phylogeny-based signal. The results of this study are thus consistent with a phylogeny-based model of radionuclide bioaccumulation by marine chordates being valid for a fish species living in lower salinity regimes.

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## 1. Introduction

There is increasing evidence for the value of employing phylogenetic information in various environmental sciences which are of relevance to the discipline of radioecology. These include the role of phylogeny in better resolving the comparative relationships among species in their patterns of trace element bioaccumulation (Hao

et al., 2015), bioaccumulation and sensitivity to environmental contaminants (Hammond et al., 2012; Poteat and Buchwalter, 2014) and more general physiological characteristics (Garland et al., 2005). Phylogeny has been used to more correctly define the slopes of allometric relationships for biota over a range of body size, which are also of current interest and application in radioecological extrapolation techniques (Garland et al., 2005; Beresford et al., 2016).

Phylogenetic investigations within radioecology have reported on the appreciable influence of evolutionary history on soil-to-plant transfers of radionuclides (Willey, 2010). Previous experimental studies on marine chordates have demonstrated, for aqueous exposure to multiples radiotracers, that differential

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relatedness among family representatives of bony and cartilaginous fishes and a cephalochordate, as defined by evolutionary divergences times, predicts the degrees of difference in their multi-elemental bioaccumulation patterns (Jeffree et al., 2006, 2010, 2013). These investigations showed differences among taxa could be discerned down to the family level for cartilaginous fishes and teleosts, to some degree. They also identified those elements which were most phylogenetically discerning among the marine taxa compared, by scales that could exceed several orders of magnitude as well as those elements which were phylogenetically less differentiating among the studied taxa.

This experimental study on multi-radionuclide biokinetics in the Russian sturgeon, *Acipenser gueldenstaedtii*, was undertaken to further test this phylogenetic bioaccumulation model. The Russian sturgeon represents a taxon of fish with a well-established point of divergence from a common ancestor with bony fish, which is more recent than its divergence time from cartilaginous fish. Moreover, declining catches in the Caspian Sea of this IUCN Red List Threatened Species ([www.iucnredlist.org](http://www.iucnredlist.org)) have been attributed to their contamination by metal pollutants (Khodorevskaya et al., 1997) as for other sturgeon species (Doering et al., 2015); some of which are investigated in this multi-element bioaccumulation study.

The objectives of this radiotracer study were three-fold; viz. (i) to characterise the whole body bioaccumulation kinetics of eight radionuclides ( $^{110m}\text{Ag}$ ,  $^{241}\text{Am}$ ,  $^{109}\text{Cd}$ ,  $^{60}\text{Co}$ ,  $^{134}\text{Cs}$ ,  $^{54}\text{Mn}$ ,  $^{75}\text{Se}$  and  $^{65}\text{Zn}$ ) in the Russian sturgeon, from water, at two contrasting salinities ((0.42‰ (fresh) and 9.0‰ (brackish)) that are typical of different regions of the Caspian Sea, and (ii) to use these multi-tracer experimental data for sturgeon for a comparison with several species of both teleosts and chondrichthyans exposed under comparable conditions, in order to further evaluate a hypothesis related to phylogenetically-based bioaccumulation patterns in fishes (Jeffree et al., 2006, 2010, 2013), and (iii) to evaluate the potential effect of changes in salinity in order to explain any differences between Russian sturgeon and these other taxa in their bioaccumulation of radionuclides.

## 2. Materials and methods

### 2.1. The Russian sturgeon (*Acipenser gueldenstaedtii*)

The Russian sturgeon, *A. gueldenstaedtii* J. F. Brandt & Ratzeburg, 1833 (Actinopterygii: Acipenseriformes: Acipenseridae), is an IUCN-listed critically endangered species (Gesner et al., 2010) which historically occurred naturally in the Caspian, Black and Azov Sea basins. Aquaculture activities have also resulted in intentional and accidental introductions throughout Europe. It is currently known from the Caspian Sea, where it spawns in the rivers Ural and Volga, and the Black Sea where spawning occurs in the lower Danube and Rioni rivers. It is an anadromous species that inhabits both the fresh riverine waters that empty into the northern regions of Caspian Sea as well as its brackish waters, at different phases of its life-cycle.

#### 2.1.1. Phylogenetic relationships of *A. gueldenstaedtii* with teleosts and chondrichthyans

This study further investigates a hypothesis about the effect of phylogeny on multi-element bioaccumulation in fishes. Thus it is relevant to summarise recent findings on the phylogenetic relationships between *A. gueldenstaedtii* and the chondrichthyan and teleost species which were previously investigated (Jeffree et al., 2006, 2010) and which will also be included in this investigation.

The fish species previously employed were three chondrichthyans: dogfish (*Scyliorhinus canicula* - (Order Carcharhiformes, Family Scyliorhinidae); undulate ray (*Raja undulata* - Order

Rajiformes) and spotted torpedo (*Torpedo marmorata* - Order Torpediniformes), and three teleosts: turbot (*Scophthalmus maximus* (previously *Psetta maxima*) - Order Pleuronectiformes)); sea bream (*Sparus aurata* - Order Perciformes), and; sea bass (*Dicentrarchus labrax* - Order Perciformes).

There are twenty one species of sturgeon of the genus *Acipenser* and two species of the genus *Huso* recognized by the Integrated Taxonomic Information System (<http://www.itis.gov>). The evolutionary divergence of sturgeon from teleosts took place at 344 million years before present (MYBP) and from chondrichthyans at 473 MYBP, based on genetic clock techniques [<http://www.timetree.net>, Evolutionary time tree of life (TTOL) current (June, 2017) estimates, Hedges et al., 2015]. Using the same methodology, the evolutionary divergence between the branches of chondrichthyans and bony fishes is currently placed at 473 MYBP (TTOL estimate, Hedges et al., 2015) which is somewhat later than the value used in a previous comparison of teleosts and chondrichthyans (>500MYBP) (Jeffree et al., 2010).

These times of divergence based on genetic clock techniques are taken as independent measures of differential relatedness among the fish taxa, which were compared in this experimental investigation of *A. gueldenstaedtii* with regard to their multi-element bioaccumulation patterns.

#### 2.1.2. Phylogenetic hypotheses tested

Based solely on these times of divergence, the *a priori* phylogenetic hypotheses which are tested in this experimental investigation of multi-elemental bioaccumulation patterns are;

- i) individual sturgeon are more similar to each other than to individual teleosts and chondrichthyans of all six species,
- ii) sturgeon are distinct from all teleost and chondrichthyan species, and
- iii) as a result of the sturgeon's longer period of divergence from chondrichthyans (473 MYBP) than teleosts (344 MYBP) sturgeon are more distant from chondrichthyans than teleosts.

Another independent variable in these comparisons between fish taxa is the different salinities of experimental media (fresh and brackish) used for radionuclide exposures of sturgeon compared to that of Mediterranean seawater used for bony and cartilaginous fishes. It is plausible that such salinity differences could confound the evaluation of the three phylogenetic hypotheses detailed above, by modifying the bioavailability of the selected radionuclides. As *A. gueldenstaedtii* is a euryhaline fish it was also possible to assess the effect of variable salinity on these three hypotheses. To address this potentially confounding effect of variable salinity, geochemical speciation modelling was employed (as described below in Section 2.5 and previously used in Jeffree et al., 2006) to assess the effect of salinity on the bioavailability of each radionuclide (using the stable element as an analogue) used among experiments. Potentially, the finding of a 'non-effect' of salinity on the phylogenetic affinities of sturgeon relative to its marine cousins in their bioaccumulation characteristics could indicate that the phylogenetic bioaccumulation model based on marine chordate taxa could be extended beyond marine chordates to fishes also living in lower salinities. As in previous studies, multiple radionuclides were employed to assess these phylogenetic hypotheses for Russian sturgeon based on the following rationale (Jeffree et al., 2010). Each element is likely to label or represent a distinctive physiological characteristic of sturgeon in its bioaccumulation, and thus provide individual axes in the multi-variate bioaccumulation space. Thus by using multiple radio-tracers, sturgeon could be better discerned in their relationships with bony and cartilaginous marine fishes.

### 2.1.3. Choice of the aqueous exposure pathway for hypothesis testing

Previous experimental comparisons of bony and cartilaginous fish species with respect to their bioaccumulation of multiple radionuclides (Jeffree et al., 2010) justified the use of the seawater exposure pathway as less potentially confounded compared to the dietary pathway in the testing of a hypothesis of a phylogenetic signal. This rationale was based on the previously demonstrated greater discriminating capacity of the seawater compared to the dietary pathway for the bioaccumulation of radionuclides (Jeffree et al., 2006; Mathews et al., 2008; Mathews and Fisher, 2009). Greater differences in feeding habits and trophic levels among the variety of species employed (Stergiou and Karpouzi, 2002), and distinct contrasts in the ingestion, food transport and digestion rates between and among bony and cartilaginous fishes (Holmgren and Nilsson, 1999), could effectively vary their exposure to radio-labelled food over the duration of a relatively short-term radio-tracer experiment. The use of the seawater exposure pathway for these experiments with marine fish was also fortuitous in that it facilitated the subsequent comparison with the cephalochordate amphioxus (Jeffree et al., 2013), which has a filter-feeding technique and dietary composition distinctly different to these fishes (Barrington, 1970).

Such a rationale detailed above also holds for the choice of the aqueous exposure pathway to evaluate a 'phylogenetic signal' hypothesis for the sturgeon's bioaccumulation of radionuclides. Moreover, the other independent variable being assessed, in addition to period of phylogenetic divergence of sturgeon from bony and cartilaginous marine fishes, is that of salinity, a characteristic inherent to the experimental aquatic medium, rather than that necessarily associated with a dietary exposure pathway.

### 2.2. Acclimation and experimental exposure of sturgeon to radio-labelled waters

Russian sturgeon of about 10–12 month's age, within a range of 9–27 g wet weight and 12.5–24.5 cm total length (snout to caudal fin) were sourced from aquaculture production. Individuals were chosen to be comparable as practicable in their ranges of age and size to the teleosts and chondrichthyans used in the previous experiments, which were compared with sturgeon in this study (see Table 1 in Jeffree et al., 2010). Individuals were transported overnight in freshwater within plastic bags which were inflated with oxygen and that were held within insulated boxes. Following their arrival at the IAEA Monaco laboratory, they were divided into two groups and were maintained with no mortality for one month of acclimation in either freshwater (FW) or diluted (carbon-filtered) Mediterranean seawater (BW) at  $19 \pm 1$  °C (Table 1). Acclimation conditions were virtually identical to those used during the subsequent long-term exposure experiment (70 L tanks with regular changes of water every 1–2 days), and with a similar feeding regime. All sturgeon were fed during their acclimation period with chironomids (*Chironomus* sp.), in accordance with their benthic feeding habits (Karpinski et al., 2005), at a daily average rate of 0.65 g (wet weight) per fish.

Fish were exposed to eight radionuclides ( $^{110m}\text{Ag}$ ,  $^{241}\text{Am}$ ,  $^{109}\text{Cd}$ ,  $^{60}\text{Co}$ ,  $^{134}\text{Cs}$ ,  $^{54}\text{Mn}$ ,  $^{75}\text{Se}$  and  $^{65}\text{Zn}$ ) in both fresh and brackish experimental waters (0.5 Bq/mL for  $^{110m}\text{Ag}$ ,  $^{241}\text{Am}$ ,  $^{60}\text{Co}$ ,  $^{54}\text{Mn}$ ,  $^{75}\text{Se}$  and  $^{65}\text{Zn}$ , 1.0 Bq/mL for  $^{134}\text{Cs}$  and 1.5 Bq/mL for  $^{109}\text{Cd}$ ; Table 1) in order to reflect both the salinity range of the Caspian Sea and also the high spatial and multi-annual variability, especially near river mouths that represents one of its most distinguishing hydrochemical features (Kosarev and Yablonskaya, 1994; Tuzhilkin et al., 2005).

Two glass aquaria (70 L) were prepared with ten fish in each –

**Table 1**  
Physico-chemistry of the experimental waters.<sup>a</sup>

	Fresh water	Brackish water
pH	8.10 $\pm$ 0.5	8.05 $\pm$ 0.5
Temperature (°C)	19 $\pm$ 1	19 $\pm$ 1
Salinity (‰)	0.42 (0.02)	9.0 <sup>c</sup> (0.3)
Organic carbon (mg/L) <sup>b</sup>	<0.1	<0.1
Na (mg/L)	38.7 (1.7)	2680 (68)
K (mg/L)	1.84 (0.10)	99.2 (2.9)
Ca (mg/L)	69.1 (3.3)	102 (4.9)
Mg (mg/L)	15.5 (0.7)	318 (8.9)
HCO <sub>3</sub> (mg/L)	144 (7.2)	52 (2.8)
Cl (mg/L)	70.2 (3.4)	4800 (144)
SO <sub>4</sub> (mg/L)	99.1 (4.7)	673 (20.2)
NO <sub>3</sub> (µg/L)	1380 (72)	568 (23)
PO <sub>4</sub> (µg/L)	80 (4.5)	60 (3.2)
Fe (µg/L)	25.2 (1.3)	19.1 (1.0)
Al (µg/L)	54 (2.7)	40.8 (2.4)
Mn (µg/L)	1.89 (0.10)	1.52 (0.07)
Ag (µg/L)	0.047 (0.0025)	0.042 (0.0021)
Cd (µg/L)	0.039 (0.0022)	0.036 (0.0018)
Co (µg/L)	0.066 (0.0036)	0.047 (0.0024)
Se (µg/L)	0.25 (0.014)	0.23 (0.013)
Zn (µg/L)	0.47 (0.025)	0.44 (0.023)

<sup>a</sup> Mean (and standard error) values (n = 8).

<sup>b</sup> Filtered through a 0.2 µm polysulfone membrane.

<sup>c</sup> Salinity can be as high as 13‰ (Peeters et al., 2000).

one with FW and the other with BW. Fish were exposed for 14 days to the eight radionuclides in solution. There was no measured change in pH after addition of the radionuclides to each aquaria. The water temperature was maintained at  $19 \pm 1$  °C, with dissolved oxygen levels of 90–100% saturation (via aeration) for both salinities. During the experimental exposures, each sturgeon was fed with chironomids (unlabelled) for 45 min each day, and ingested a daily average of 0.65 g/fish. Experimental waters and radiotracer spikes were renewed daily for 5 days, and then every second day in order to maintain constant activities. Activities of radionuclides in fresh and brackish experimental waters were measured daily, both before and after each water renewal to determine their time-integrated activities.

For the 28 day depuration phase of the experiment, seven sturgeon in each aquaria were exposed to a radionuclide-free experimental water under the same physico-chemical conditions and feeding regime as the uptake phase of the experiment.

During the full experimental period of acclimation (30 days), uptake (14 days) and depuration (28 days) there was an average increase of 25% in fish masses (c. 0.3% per day), indicating the adequacy of the laboratory conditions and feeding regime for the health of the experimental fish. All animals used in this experimental study were treated in accordance with the animal care and ethics standards required by the Principality of Monaco and the International Atomic Energy Agency (IAEA).

### 2.3. Radionuclide analysis in sturgeon and experimental waters

Radionuclides were purchased from Amersham (UK) and the Isotope Product Laboratory (USA). The chemical forms for each isotope were as follows;  $\text{AmCl}_3$  in HCl 1.0 M for  $^{241}\text{Am}$ ,  $\text{CdCl}_2$  in HCl 0.1 M for  $^{109}\text{Cd}$ ,  $\text{CsCl}$  in HCl 0.1 M for  $^{134}\text{Cs}$ ,  $\text{MnCl}_2$  in HCl 0.1 M for  $^{54}\text{Mn}$ ,  $\text{ZnCl}_2$  in HCl 0.5 M for  $^{65}\text{Zn}$ ,  $\text{CoCl}_2$  in HCl 0.1 M for  $^{60}\text{Co}$ ,  $\text{Na}_2\text{SeO}_3$  in saline solution for  $^{75}\text{Se}$  and  $\text{AgNO}_3$  in  $\text{HNO}_3$  1.0 M for  $^{110m}\text{Ag}$ . The isotopes used to label fish were measured with a high-resolution gamma-spectrometry system consisting of four coaxial Germanium (N- or P-type) detectors (EGNC 33–195-R, Inter-technique, Ortec and Canberra; 40–50% efficiency) that were connected to a multi-channel analyzer and a personal computer

employing spectral analysis software (Interwinner 6, Intertechnique).

The Germanium detectors were calibrated with a standard sturgeon phantom in order to define the specific efficiency detection curves for each radionuclide. Individual fish were repeatedly live-counted during both the uptake and depuration phases of the experiment in clean FW or BW at  $19 \pm 1$  °C for a maximum of ten minutes to minimise stress and asphyxia. During repeated counting, the specimens were situated consistently relative to the counter, and their movements were minimised by prior sedation in an MS-222 bath and light restraint within the counting tube. Counting times for water samples were adapted to obtain count rates with relative propagated errors of less than 5%, viz. 1–12 h. The radio-analytical techniques used in this study were identical to those used in previous experiments on teleosts and chondrichthyans (Jeffree et al., 2006, 2010).

#### 2.4. Chemical analyses of experimental waters

The pH and temperature of the experimental waters was measured using a Hanna (211/Checktemp) hand held instrument. Salinity was measured using a TPS (WP-84) hand-held conductivity/salinity meter, which was calibrated against both temperature and a series of conductivity standards which encompassed the range of the two experimental waters used with sturgeon. The concentrations of Ag, Cd, Co, Mn, Se and Zn in the experimental waters (diluted 4-fold for the brackish waters prior to analyses) were measured using inductively coupled plasma mass spectrometry (ICPMS, HP Agilent 4500), whereas Ca, Mg, Na, K, Fe and Al were measured using inductively coupled plasma atomic emission spectrometry (Varian Vista AX). A multi-element calibration standard and a reagent blank were analysed with every ten samples to monitor signal drift. For all elements, the signals changed by less than 8%, but typically 3–5%. Where ICPMS was used, gallium and indium were employed as internal standards to correct for any non-spectral interferences.

Chloride (Cl), sulfate (SO<sub>4</sub>), nitrate (NO<sub>3</sub>) and phosphate (PO<sub>4</sub>) were measured using ion chromatography (Dionex DX600). Bicarbonate concentrations were determined via measurement of dissolved inorganic carbon using flow injection gas diffusion, coupled with photometric detection. Dissolved (0.2 µm polysulphone filter) organic carbon was measured by catalytic combustion (Tekmar Dohrmann Apollo 8000 TOC analyzer) following standard method 5310B.

Standard reference materials (National Research Council of Canada riverine (SLRS-4) and estuarine (SLEW-3) water for trace metals) sample replicates, and reagent blanks were used to evaluate analytical accuracy, precision and limits, respectively. The mean concentrations of elements in the SRM were within their certified ranges. For replicate samples and SRM, the percentage coefficient of variation was <10% for all elements.

#### 2.5. Speciation modelling of radionuclides in fresh and brackish waters

A metal (or radionuclide) may exist in a range of physico-chemical forms, or species, (ie. dissolved, complexed with inorganic or organic ligands, or associated with colloids/particles) in an aquatic medium (Ure and Davidson, 2001). The distribution of a metal between the various physico-chemical forms, or speciation, plays an important role in determining the fraction that is taken up or bioaccumulated (Markich et al., 2001). Bioavailability, as applied here, represents the fraction of a metal (or radionuclide) that is taken up by a sturgeon fish from its aquatic medium. A convincing body of experimental evidence has concluded that the bioavailable

metal fraction is best predicted by the concentration of the dissolved “free” metal ion (e.g. for Zn it would be the Zn<sup>2+</sup> ion) and that metals complexed with inorganic ions (e.g. carbonate, chloride or sulfate) or natural dissolved organic matter (eg. fulvic or humic acid) typically have a low bioavailability (Brown and Markich, 2000). Speciation modelling was used in this study to calculate the physico-chemical distribution of a metal, including the bioavailable free metal ion fraction.

Any differences in the bioavailability of radionuclides in experimental waters that varied in salinity, both between experimental exposures of sturgeon (this experiment) and previous studies on teleosts and chondrichthyans (Jeffree et al., 2006, 2010), could potentially confound the use of the bioaccumulation data for sturgeon to evaluate the phylogenetic hypotheses central to this study (Section 2.1.2). Accordingly, speciation modelling of the experimental waters was undertaken for comparison with results of previous speciation modelling on marine experimental waters (Jeffree et al., 2006).

The speciation of <sup>110m</sup>Ag, <sup>241</sup>Am, <sup>109</sup>Cd, <sup>60</sup>Co, <sup>134</sup>Cs, <sup>54</sup>Mn, <sup>75</sup>Se and <sup>65</sup>Zn (as stable elements), in both the fresh and brackish experimental waters, was calculated using Visual MINTEQ (version 3.1; Gustafsson, 2016). The equilibrium constants used in the speciation calculations were derived from Markich (2017) and the input parameters were based on measured water chemistry data (Table 1). Given that the measured concentrations of dissolved organic carbon (DOC) in the test waters were negligible (<0.1 mg L<sup>-1</sup>), dissolved organic matter was not included in the modelling calculations. If the 95% confidence limits of the mean values of the bioavailable metal species overlapped, then they were deemed to be not significantly ( $p > 0.05$ ) different.

#### 2.6. Data analysis

##### 2.6.1. Biokinetic analysis

The uptake of radionuclides over 14 days of exposure to radio-labelled fresh or brackish water was expressed as the change in concentration factors (CF = Bq. g<sup>-1</sup> whole organism (wet) divided by the time-integrated Bq. g<sup>-1</sup> sea water) over time. Uptake kinetics in whole individuals was described by either using a single-component first-order kinetic model

$$CF_t = CF_{ss} (1 - e^{-k_e t}),$$

where:

CF<sub>t</sub> and CF<sub>ss</sub> represent the concentration factors at time  $t$  (d) and at steady state respectively, and  $k_e$  is the biological depuration rate constant (d<sup>-1</sup>) (Whicker and Schultz, 1982), or, if individuals did not tend towards a steady state during the exposure time course, by a simple linear regression model

$$CF_t = k_u t,$$

where  $k_u$  (uptake rate) is the regression slope (i.e. rate of increase in CF. d<sup>-1</sup>).

Linearity of the uptake kinetics expressed as CF<sub>ss</sub> was evaluated by a linearity test (one-way analysis of variance) for regression with replication (Zar, 1996).

Loss kinetics were expressed in terms of percentage of remaining radioactivity, i.e. radioactivity at time  $t$  divided by initial radioactivity (times 100) measured in the organisms at the beginning of the depuration (loss) period. Visual assessment of the



patterns of loss indicated that there was more than one rate of loss, indicating there could be two exchangeable pools for each radionuclide and salinity exposure. Consequently, the kinetics were described by a double-component exponential model

$$A_t = A_{0s} e^{-k_{es} t} + A_{0l} e^{-k_{el} t},$$

where:

$A_t$  and  $A_0$  are the remaining activities (%) at time  $t$  (d) and 0, respectively, and

$k_e$  is the biological depuration rate constant ( $d^{-1}$ ).

The determination of  $k_e$  allows the calculation of the radionuclide biological half-life ( $T_{b1/2} = \ln 2/k_e$ ). The “s” subscript refers to a short-lived component (loss of the fraction of radionuclide pool that is weakly associated to the organism) while the “l” subscript refers to a long-lived component (loss of the fraction of the radionuclide pool that is tightly bound in the organism) (Whicker and Schultz, 1982).

The uptake and loss constants (and their statistics) were estimated by iterative adjustment of the models and Hessian matrix computation, respectively, using the nonlinear curve-fitting routines in the STATISTICA<sup>®</sup>5.1 software (StatSoft Inc.; Tulsa, Oklahoma, USA).

### 2.6.2. MDS and hierarchical cluster analysis

The raw dataset used for these analyses consisted of 15 sturgeon (eight from FW and seven from BW) and forty other teleost and chondrichthyan fishes of six species (Jeffree et al., 2010) and was based on their individual whole body (wet): water CFs for the eight radionuclides used in this experiment undertaken with sturgeon. These data were  $\log(n+1)$  transformed, so that those radionuclides showing generally higher CF<sub>ss</sub> values (such as <sup>65</sup>Zn and <sup>110m</sup>Ag (Table 3 and Jeffree et al., 2010) and some right skewness in their frequency distributions, did not dominate the statistical comparisons. These transformed data were also normalised for mean and standard deviation to reduce the effect of differences in scale among radionuclides in their bioaccumulation capacity, so as to derive more meaningful measures of distances between samples. The PRIMER 7 program was used to calculate a resemblance matrix using Euclidean distance as the resemblance measure followed by both multi-dimensional scaling - non-metricMDS (nMDS) and metricMDS (mMDS) - and hierarchical cluster analyses. Analysis of similarity (ANOSIM) was used to test for significant ( $p \leq 0.05$ ) differences between groupings identified by MDS. SIMPER analysis identified the percentage contributions of individual radiotracers to the average dissimilarity measures between each grouping of bony and cartilaginous fish with sturgeon exposed at two salinities, as well as between sturgeon exposed in fresh and brackish water.

## 3. Results

### 3.1. Speciation modelling of radioisotopes in fresh and brackish waters

Table 2 provides the results of the chemical speciation modelling, detailing the species distribution of each radionuclide for both fresh and brackish experimental waters (Table 1), and for seawater used in previous experiments on marine cartilaginous and bony fishes (Jeffree et al., 2006, 2010).

The free metal ion ( $M^{n+}$ ), generally considered to be a good estimate of the bioavailable metal fraction (see Section 2.5), was calculated to be the major proportion of dissolved Cs (~98–99%), Co (71–83%), Mn (77–85%) and Zn (72–81%), with no significant

( $p > 0.05$ ) differences in the percentages of the free metal ion between fresh, brackish and sea water, for each metal. In the case of Cd, the free ion ( $Cd^{2+}$ ) formed a major proportion of dissolved Cd in fresh water, but declined markedly as salinity increased (i.e., 16% in brackish water and ~4% in sea water). The decline in  $Cd^{2+}$  was largely offset by increases in the proportions of Cd chloride complexes ( $CdCl^+$ ,  $CdCl_2$  and  $CdCl_3^-$ ; Table 2). In contrast to Cs, Co, Mn and Zn, the free Am ion ( $Am^{3+}$ ) was calculated to form only a very small proportion ((1–7% and not significantly ( $p > 0.05$ ) different amongst all three waters of differing salinity)) of dissolved Am, with carbonate ( $AmCO_3^+$ ) and hydroxy-carbonate ( $AmOHCO_3$ ) complexes being predominant. For dissolved Ag, the free ion ( $Ag^+$ ) was predicted to be a minor species (~20%) in fresh water and

**Table 2**

Predicted speciation of Am, Cd, Cs, Co, Mn, Se, Ag and Zn in experimental freshwater, brackish water and sea water.

Metal species <sup>a</sup>	Fresh water (0.42‰)	Brackish water (9‰)	Sea water (38‰)
<b>Americium</b>			
$Am^{3+}$	1.0 (5.2) <sup>b</sup>	4.1 (5.2)	7.4 (5.2)
$Am(OH)^{2+}$	1.0	7.0	14.6
$Am(OH)_2^+$	1.0	4.2	9.9
$AmOHCO_3$	34.6	28.8	21.3
$AmCO_3^+$	51.0	50.7	37.3
$Am(CO_3)_2^-$	10.9	2.2	0.7
$AmSO_4^+$	<1.0	1.3	6.3
<b>Cadmium</b>			
$Cd^{2+}$	73.9 (3.8)	16.1 (3.8)	3.6 (3.8)
$CdCl^+$	9.3	61.9	42.8
$CdCl_2$	<1.0	18.0	39.9
$CdCl_3^-$	<1.0	1.3	11.8
$CdSO_4$	6.2	1.4	<1.0
$CdCO_3$	8.7	<1.0	<1.0
<b>Cesium</b>			
$Cs^+$	99.3 (3.4)	98.6 (3.4)	97.6 (3.4)
$CsSO_4$	<1.0	1.3	2.1
<b>Cobalt</b>			
$Co^{2+}$	71.0 (6.4)	82.5 (6.4)	71.5 (6.4)
$CoCO_3$	23.4	3.3	<1.0
$CoSO_4$	5.1	6.3	8.1
$CoCl^+$	<1.0	5.5	17.4
<b>Manganese</b>			
$Mn^{2+}$	77.4 (4.8)	84.5 (4.8)	79.0 (4.8)
$MnCO_3$	16.3	2.3	<1.0
$MnSO_4$	5.2	6.2	5.5
$MnCl^+$	<1.0	6.0	14.7
<b>Selenium</b>			
$SeO_4^{2-}$	92.5 (4.8)	80.5 (4.8)	66.1 (4.8)
$CaSeO_4$	3.9	2.1	2.6
$MgSeO_4$	3.1	8.8	13.6
$NaSeO_4^-$	<1.0	8.6	17.7
<b>Silver</b>			
$Ag^+$	19.9 (4.4) <sup>b</sup>	<1 (4.4)	<1 (4.4)
$AgCl$	67.7	6.5	1.1
$AgCl_2^-$	12.0	75.5	53.5
$AgCl_3^{2-}$	<1.0	17.8	45.2
<b>Zinc</b>			
$Zn^{2+}$	71.6 (4.7)	80.5 (4.7)	75.3 (4.7)
$ZnOH^+$	4.3	3.1	2.0
$ZnCO_3$	16.8	3.0	<1.0
$ZnSO_4$	5.4	6.6	5.8
$ZnCl^+$	<1.0	5.2	14.5

<sup>a</sup> Each metal species is shown as a percentage of its measured dissolved concentration. Species comprising <1% of the total distribution are excluded for clarity. The bioavailable fraction is highlighted.

<sup>b</sup> Mean (and 95% confidence limit).

**Table 3**

Radionuclide biokinetic rates for sturgeon based on a) a first order kinetics or b) a simple linear regression model.

Radionuclide	Fresh water			Brackish water		
a) First order kinetics model						
	CF <sub>ss</sub>	k <sub>e</sub> <sup>ua</sup>	r <sup>2</sup>	CF <sub>ss</sub>	k <sub>e</sub> <sup>ub</sup>	r <sup>2</sup>
<sup>54</sup> Mn	1.17	0.31	0.59	1.08	0.09 <sup>c</sup>	0.7
<sup>60</sup> Co	1.12	0.51	0.58	1.01	0.17	0.66
<sup>65</sup> Zn	4.66	0.22	0.62	3.41	0.18	0.69
<sup>75</sup> Se	19.17	0.07	0.84	16.48	0.07 <sup>c</sup>	0.83
<sup>109</sup> Cd	8.22	0.15	0.76	3.42	0.16	0.55
<sup>134</sup> Cs	2.61	0.18	0.44	3.82	0.05 <sup>c</sup>	0.77
<sup>241</sup> Am	0.98	0.44 <sup>c</sup>	0.22	3.23	0.17 <sup>c</sup>	0.24
b) Simple linear regression model						
	k <sub>u</sub> <sup>a</sup>		r <sup>2</sup>	k <sub>u</sub> <sup>a</sup>		r <sup>2</sup>
<sup>110m</sup> Ag	2.33		0.57	4.74		0.44

<sup>a</sup> p ≤ 0.01.

<sup>b</sup> p ≤ 0.05.

<sup>c</sup> p > 0.05.

negligible (<1%) in brackish and sea water. Moreover, Ag forms strong complexes with chloride (AgCl, AgCl<sub>2</sub><sup>-</sup> and AgCl<sub>3</sub><sup>2-</sup>) in all three waters, with the species distributions shifting as salinity increases (see Table 2). In contrast to all the above-mentioned elements (which occur as cations), Se exists as an anion in the form of selenate (SeO<sub>4</sub><sup>2-</sup>) in oxic waters (+6 oxidation state). The calculated proportion of SeO<sub>4</sub><sup>2-</sup> decreased (p ≤ 0.05) with increasing salinity (i.e. ~93% in fresh water decreasing to 66% in sea water), which was largely offset by an increase in NaSeO<sub>4</sub>, also believed to be bioavailable (Griffith, 2017).

### 3.2. Experimental exposure of sturgeon

#### 3.2.1. Uptake

The results for the modes of uptake of each radioisotope from water by sturgeon at both salinities are shown in Fig. 1, where the whole body (wet): water CF is plotted as a function of period of exposure to radio-labelled water during 14 days. The derived CF<sub>ss</sub> values for each radionuclide and loss rate constants (k<sub>e</sub><sup>u</sup>) are given in Table 3. The fitted regression lines (Fig. 1) are based on a one compartment model with first order kinetics, and a simple linear analysis for <sup>110m</sup>Ag which had no significant k<sub>e</sub><sup>u</sup> in either salinity (Table 3). Based on these fitted regression lines (Fig. 1) the patterns of bioaccumulation at either salinity show that this species has a minimal capacity to accumulate the radiotracers <sup>54</sup>Mn, <sup>60</sup>Co and <sup>134</sup>Cs which approximate to equilibrium whole body (wet): water CF's within a period of 4–7 days. Their CFs range from ≤1 to 2, by the end of the period of experimental exposure. There is a greater capacity to accumulate <sup>65</sup>Zn, <sup>75</sup>Se, <sup>241</sup>Am and <sup>109</sup>Cd which is reflected in whole body (wet): water CF's attained by the end of the experimental exposure that range from about 3 to 60 in both salinities. Whereas <sup>65</sup>Zn approximates to an equilibrium whole body (wet): water CF <sup>75</sup>Se, <sup>241</sup>Am and particularly <sup>109</sup>Cd do not attain equilibrium CFs during the period of experimental exposure. Sturgeon do show a heightened capacity to accumulate <sup>110m</sup>Ag which attains CF's of between c. 30–60 in both salinities over the experimental exposure and it has the least indicated tendency to reach equilibrium among all the radioisotopes used, as supported by the linear regression analysis (Table 3). The effect of the salinity of the experimental waters on the patterns of accumulation of these radioisotopes in sturgeon is also evident in Fig. 1. In order to provide more quantitative comparisons among these uptake data for statistical assessment of salinity effects the average whole body (wet): water CFs (and their 95% confidence limits) for each radionuclide were determined for data at the end of the 14 day exposure, (Table 4). These mean CFs varied from 0.8 for <sup>54</sup>Mn in brackish

water to 61 for <sup>110m</sup>Ag in brackish water, i.e. a factor of c. 75. Between salinities the CFs varied by only factors of 1.4 (<sup>60</sup>Co and <sup>65</sup>Zn) and 2.2 for <sup>75</sup>Se. Statistical comparisons (*t*-test) between mean CFs showed that <sup>109</sup>Cd was accumulated to a significantly (p ≤ 0.05) greater extent in fresh than brackish water, whereas <sup>75</sup>Se accumulation was significantly (p ≤ 0.05) enhanced in brackish compared to fresh water. For the other six radionuclides there were no significant (p > 0.05) differences in bioaccumulation between fresh and brackish water exposures, based on these data.

The results of biokinetic modelling of the empirical data shown in Fig. 1, based on a first order kinetics model for seven radionuclides (Table 3), provide predictions of steady state whole body: water CFs (CF<sub>ss</sub>), many of which would not have been achieved within 14 days of experimental exposure to radio-labelled water. These CF<sub>ss</sub> data indicate the following: i) they are highest for <sup>75</sup>Se in both salinities, ii) <sup>109</sup>Cd is more than a factor of two higher in fresh than brackish water (8.2 vs. 3.4) and <sup>241</sup>Am is more than 3 times higher in brackish than freshwater, and iii) for the other four radionuclides their values, ranging only from 1.0 to 4.7, are similar between fresh and brackish exposures.

#### 3.2.2. Loss

The patterns of depuration over 28 days for sturgeon exposed to each radionuclide in fresh and brackish waters, expressed in terms of percentage of remaining radioactivity and also their fitted loss curves based on a double-component exponential model (Section 2.6.1), are shown in Fig. 2. Table 5 reports the calculated loss rate constants for the long-lived compartment (k<sub>el</sub>) and its size as a percentage of the combined short and long-lived fitted compartments. The long-lived compartment is ≥52% of both compartments for all radionuclides, irrespective of salinity, and as high as 96% for <sup>109</sup>Cd in sturgeon exposed to freshwater. It is appreciably greater in sturgeon from freshwater compared to brackish water for <sup>54</sup>Mn and <sup>75</sup>Se and only <sup>60</sup>Co shows a larger compartment in brackish than freshwater. For <sup>241</sup>Am, sturgeon have consistently reduced compartment sizes in both salinities (61 and 52%). The calculated loss rate constants (Fig. 2) show that for <sup>134</sup>Cs the k<sub>el</sub> is greater by a factor of two for sturgeon held in brackish compared to fresh water.

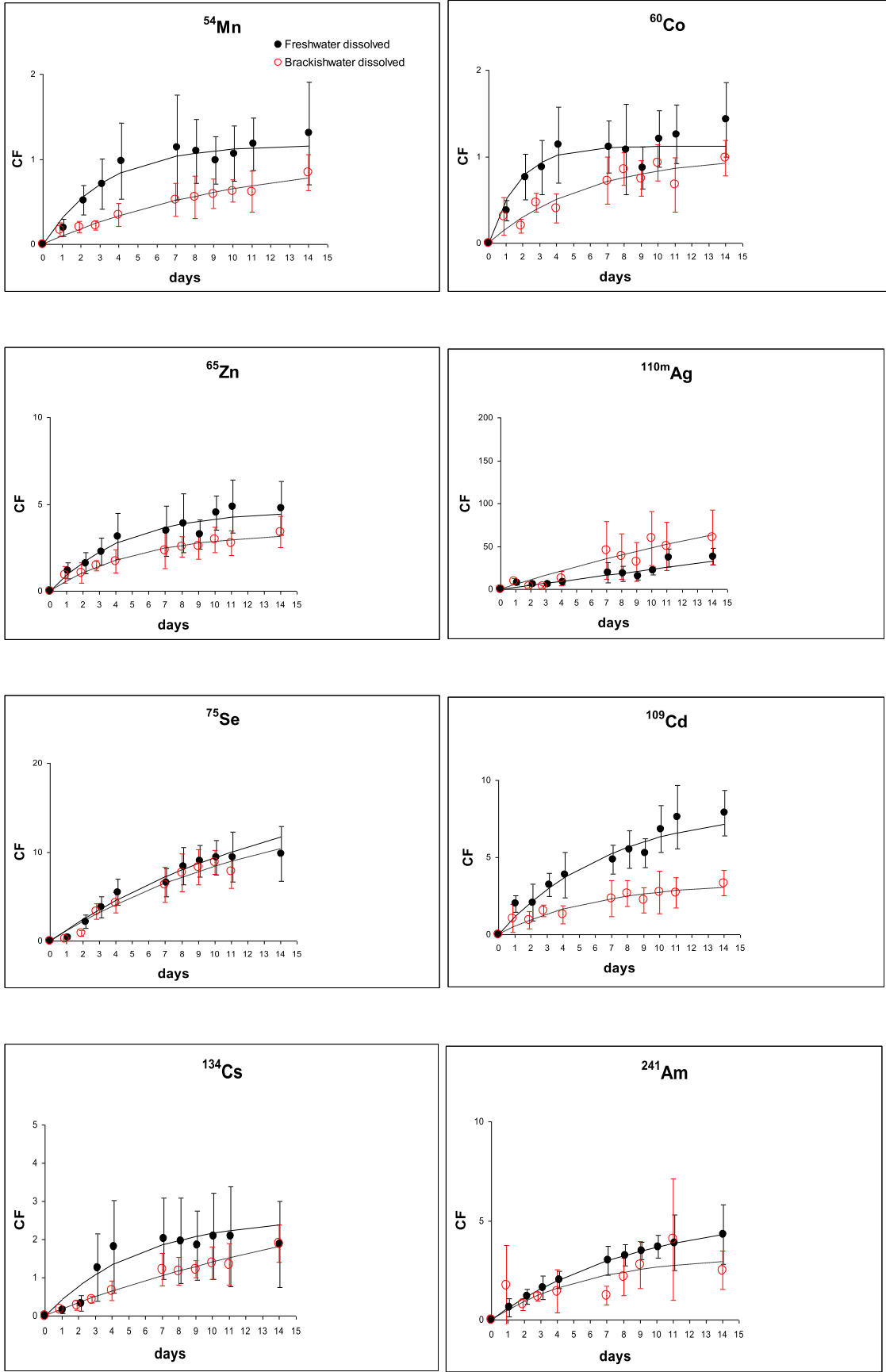
The very small percentage increases in average fish mass may have diluted the activity concentrations of radionuclides by c. 0.3% per day (Section 2.2). This percentage was insignificant in relation to the variation among individuals shown in Fig. 2 for the majority of radionuclides. Hence no adjustments in these loss data were deemed necessary for slight increases in fish size.

### 3.3. Comparison of sturgeon with marine teleost and chondrichthyan fish species

#### 3.3.1. Mean radioisotope CFs in sturgeon, turbot and dogfish

The whole body (wet): water CFs of eight radionuclides for sturgeon, juvenile turbot *S. maximus* and dogfish *S. canicula*, based on their mean whole body activity concentrations at the end of their 14 day experimental exposures, are given in Table 4. The whole body (wet): water CFs for *S. maximus* and *S. canicula* are also based on their 14 day exposures at 16.5 ± 1.0 °C to these eight radionuclides in seawater for individuals of ages (3–4 months) and mean wet weights (6.1 g for *S. canicula* and 6.7 g for *S. maximus*) (Jeffree et al., 2006), which are lower than these mean values for sturgeon (Section 2.2) but still provide a reasonable basis for comparison.

Compared to sturgeon exposed in either salinity the whole body (wet): water CFs for turbot are lower for six out of the eight radionuclides and by factors of up to 11–12 for <sup>75</sup>Se and <sup>110m</sup>Ag; only <sup>54</sup>Mn is slightly higher in turbot and <sup>60</sup>Co is similar between the two species. In contrast, the comparison of sturgeon with dogfish



**Fig. 1.** Mean whole body (wet): water CFs in sturgeon as a function of period of exposure to fresh and brackish water. Fitted non-linear and linear regressions (Table 3). Error bars represent 95% confidence limits from mean values.

**Table 4**

Mean (and 95% confidence limits, CL) radioisotope concentration factors (CF) of sturgeon, turbot and dogfish.

Water salinity	Whole body (wet): water CF <sup>a</sup>															
	Mn-54		Co-60		Zn-65		Se-75 <sup>b</sup>		Ag-110 m		Cd-109 <sup>b</sup>		Cs-134		Am-241	
	Mean	95% CL	Mean	95% CL	Mean	95% CL	Mean	95% CL	Mean	95% CL	Mean	95% CL	Mean	95% CL	Mean	95% CL
Fresh	<i>Acipenser gueldenstaedtii</i>															
	1.3	0.5	1.4	0.4	4.8	1.3	9.8	2.6	38.2	7.9	7.9	1.2	1.9	1.0	1.7	1.3
Brackish	<i>Scophthalmus maximus</i>															
	0.8	0.2	0.98	0.2	3.4	0.8	21.8	3.2	60.9	26.7	3.3	0.7	1.9	0.4	2.5	0.8
Marine	<i>Scyllorhinus canicula</i>															
	1.7	0.3	1.2	0.1	1.9	0.2	2.0	0.5	5.0	0.9	1.4	0.1	1.8	0.1	1.0	0.1
Marine	1.6	1.9	2.3	1.4	84	76	3.1	2.0	88	65	2.0	2.8	0.3	0.2	58	31

<sup>a</sup> Based on 14 day exposures to radio-labelled waters; *A. gueldenstaedtii* (n = 8), *S. maximus* (n = 12), *S. canicula* (n = 4).<sup>b</sup> Mean values are significantly different ( $p \leq 0.05$ ) between sturgeon exposed in freshwater and brackish water.

gives five radionuclides which are elevated in dogfish (<sup>54</sup>Mn, <sup>60</sup>Co, <sup>65</sup>Zn, <sup>110m</sup>Ag and <sup>241</sup>Am) and by factors as high as 25 for <sup>65</sup>Zn and 54 for <sup>241</sup>Am. Only <sup>75</sup>Se and <sup>109</sup>Cd are appreciably lower in their CFs in dogfish compared with sturgeon, by factors of up to 7 and 4, respectively.

### 3.3.2. MDS and hierarchical cluster analyses

The nMDS and mMDS analyses gave similar patterns of relationships among species. However the nMDS (Fig. 3a) showed a lower stress level (0.12) than did the mMDS (0.17) (Supplementary Fig. 1) and was therefore chosen as the better two-dimensional picture of the relationships among the phylogenetic groups, according to their multi-nuclide bioaccumulation behaviours. The stress level of 0.12 for the nMDS indicates a reasonably good representation of the degree of similarities among individual fishes in their multi-nuclide bioaccumulation characteristics (Clark and Gorley, 2015). This analysis shows that sturgeon form a cluster which is distinct from each species of chondrichthyan and teleost. Sturgeon are also more distant from chondrichthyans than teleosts. The nMDS analysis also indicated that although sturgeon exposed to fresh or brackish experimental water were similar in their bioaccumulation patterns they were still significantly ( $p = 0.02$ ) different, as confirmed by an ANOSIM.

The results of the hierarchical cluster analysis, that compares the multi-elemental CFs among sturgeon exposed at two salinities with individuals of the three teleost and three chondrichthyan species, are provided in Fig. 3b. This cluster analysis shows that all sturgeon are aggregated together at a Euclidean distance of c. 1.9, independent of all individuals of all six species of bony and cartilaginous fish. They also form a discrete grouping lower in the dendrogram for all individuals exposed to brackish water whereas those exposed to freshwater are more diverse among themselves. Sturgeon are clearly separated from both teleosts and chondrichthyans, but are more similar to the teleosts than to the chondrichthyans, aggregating with all teleosts at a lower Euclidean distance (c. 2.05) in the dendrogram, compared with the point of aggregation with two chondrichthyan species (ray and torpedo) at a distance of c. 2.4.

The results of the SIMPER analyses (Table 6) give information on which radionuclides discern best between sturgeon and the other tested phylogenetic groups, as well as between sturgeon exposed in brackish and fresh experimental waters. Among the four accumulated radionuclides which discriminate most between sturgeon in fresh or brackish water <sup>109</sup>Cd and <sup>60</sup>Co are higher in the fresh water exposure, whereas <sup>75</sup>Se and <sup>134</sup>Cs are higher in the brackish water exposure. The comparison between teleosts and sturgeon exposed in freshwater shows that, in descending order of importance, <sup>109</sup>Cd, <sup>110m</sup>Ag and <sup>75</sup>Se are elevated in sturgeon and <sup>54</sup>Mn is higher in teleosts. The same comparison with sturgeon from brackish water

shows similar discriminators, with <sup>75</sup>Se and <sup>110m</sup>Ag being elevated in sturgeon and <sup>54</sup>Mn being higher in teleosts; however <sup>109</sup>Cd is not a major discriminator. The radiotracers that discriminate between chondrichthyans and sturgeon exposed in either salinity show a common set of discriminators, with <sup>134</sup>Cs being elevated in sturgeon but <sup>241</sup>Am and <sup>65</sup>Zn being higher in chondrichthyans; however <sup>109</sup>Cd and <sup>75</sup>Se are elevated in sturgeon exposed in fresh and brackish waters, respectively.

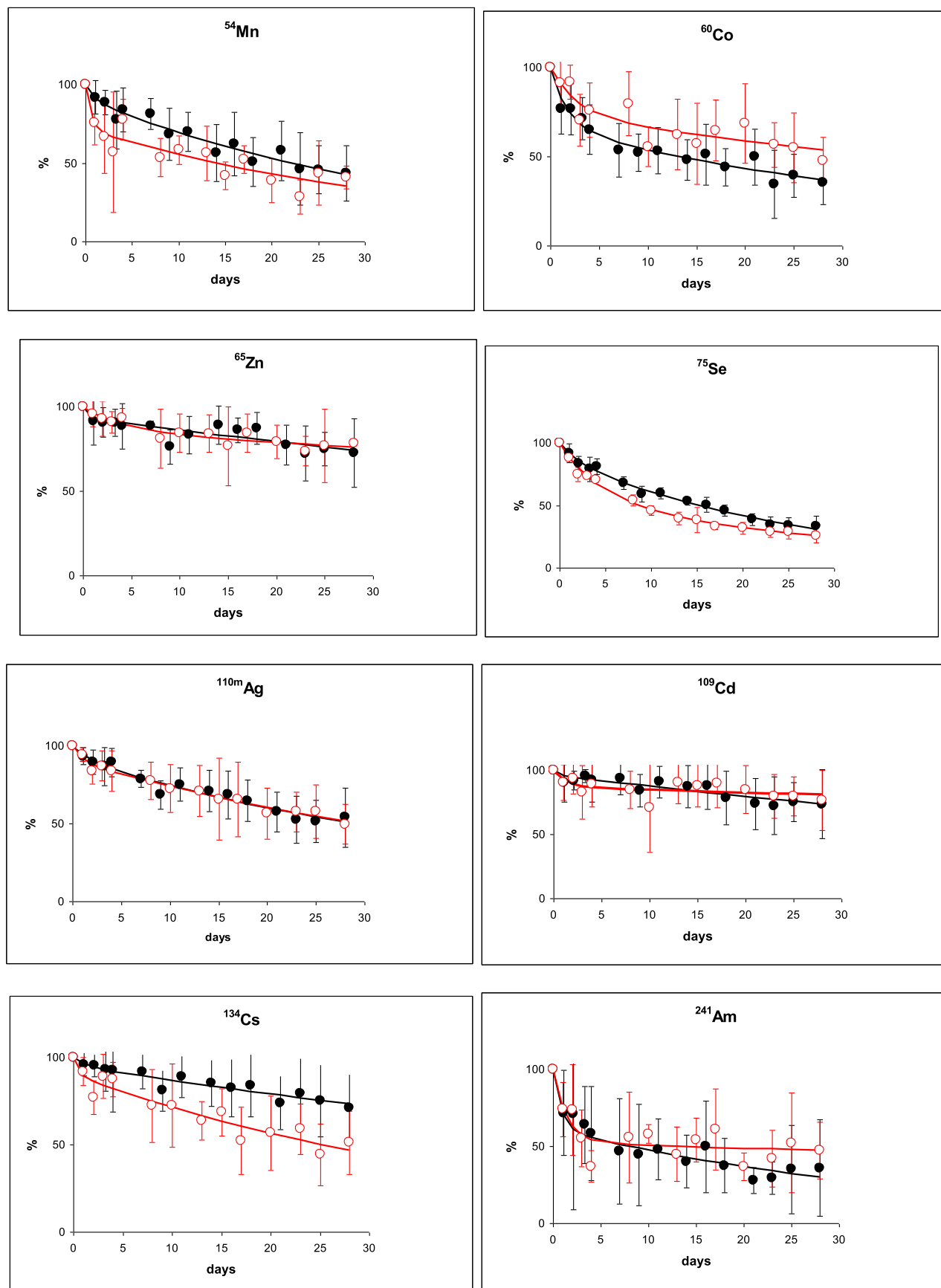
### 3.3.3. Evaluation of potentially confounding effects of metal speciation on the phylogenetic relationships between fish taxa

The results presented above (3.3.2; Fig. 3) are consistent with the phylogeny-based hypotheses which were a major component of this experimental investigation. The potential confounding role that different salinities among experimental treatments may have on the bioavailability, and hence the bioaccumulation of these eight radionuclides, is assessed below;

- Sturgeon exposed at different salinities*; the enhanced accumulation of <sup>109</sup>Cd from freshwater (Tables 4 and 6) where it contributes 26.4% to the average dissimilarity measure is also consistent with its predicted heightened bioavailability (Table 2) as Cd<sup>2+</sup> (74%) in freshwater compared to brackish (16%). Although <sup>75</sup>Se is a major contributor to this dissimilarity between salinity treatments (16.7%) it exhibits a similar bioavailability with varying salinity (Table 2; 92.5% v 89.1%). It bio-accumulates to a greater degree from brackish water (Tables 4 and 6). i.e. its bio-accumulatory behaviours cannot be explained by changes in its bioavailability between these two salinities. <sup>60</sup>Co and <sup>134</sup>Cs also make comparable contributions (14–16%) to the average dissimilarity between salinity exposures of sturgeon but do not vary significantly ( $p > 0.05$ ) in their bioavailability (Table 2). Among these four radiotracers which contribute most to the dissimilarity between salinity treatments (73.3%; Table 6) 46.9% is contributed by radiotracers whose bioavailability does not explain the separation between sturgeon (Fig. 3) and 26.4% is consistent with this interpretation.
- Sturgeon in fresh or brackish water and teleosts*: More than half the dissimilarity between sturgeon exposed in freshwater and marine teleosts (Table 6) is due to the elevated bioaccumulation by sturgeon of <sup>109</sup>Cd (33.4%) and to a lesser degree <sup>110m</sup>Ag (19%), which could be attributed to their appreciably enhanced bioavailability in freshwater compared to seawater (Table 2). Another 26.7% is explained by enhanced <sup>75</sup>Se and reduced <sup>54</sup>Mn in sturgeon, whose bioavailability is unaffected by salinity.

The dissimilarity between sturgeon exposed in brackish water and marine teleosts (Table 6) is mainly due to the elevations in





**Fig. 2.** Percentage radioactivity remaining in sturgeon as a function of days of exposure to tracer-free fresh and brackish water. Fitted two compartment loss equations (Table 5). Error bars represent 95% confidence limits for mean values.

**Table 5**Radionuclide loss rate constants<sup>b</sup> for the long-lived compartment in Russian sturgeon and marine fishes.

Radionuclide	<i>A. gueldenstaedtii</i>				<i>S. maximus</i>	<i>S. canicula</i>
	Fresh water		Brackish water		Marine	Marine
	Compartment size (%)	Loss rate constant (k <sub>el</sub> )	Compartment size (%)	Loss rate constant (k <sub>el</sub> )	Loss rate constant <sup>a</sup> (k <sub>el</sub> )	Loss rate constant <sup>d</sup> (k <sub>el</sub> )
<sup>54</sup> Mn	91	0.03	72	0.02	0.002	0.025
<sup>60</sup> Co	66	0.02	74	0.01 <sup>c</sup>	0.01 <sup>d</sup>	0.015 <sup>d</sup>
<sup>65</sup> Zn	83	0.01	85	0.004 <sup>c</sup>	0.007	0.01
<sup>75</sup> Se	88	0.04	53	0.03 <sup>c</sup>	—	—
<sup>110m</sup> Ag	92	0.02	90	0.02	—	—
<sup>109</sup> Cd	96	0.01	86	0.002 <sup>c</sup>	0.2	0.008
<sup>134</sup> Cs	95	0.01	90	0.02	0.027	0.014
<sup>241</sup> Am	61	0.03	52	0.003 <sup>c</sup>	0.007	0.001

<sup>a</sup> From Jeffree et al. (2006).<sup>b</sup> p < 0.02.<sup>c</sup> p > 0.05.<sup>d</sup> <sup>57</sup>Co.

sturgeon of <sup>75</sup>Se (40.1%) and <sup>110m</sup>Ag (27.1%), where bioavailability is not significantly ( $p > 0.05$ ) different between brackish and seawater (Table 2). <sup>54</sup>Mn also contributes to their separation (11.7%) by being elevated in teleosts, however there is no significant ( $p > 0.05$ ) difference in its bioavailability among brackish and marine waters (Table 2).

iii) *Sturgeon in fresh or brackish water and chondrichthyans*; the dissimilarity between chondrichthyans and sturgeon exposed in freshwater is predominantly due (49.4%; Table 6) to three radionuclides (<sup>134</sup>Cs, <sup>241</sup>Am and <sup>65</sup>Zn) whose bioavailability is not significantly different ( $p > 0.05$ ) between fresh and marine waters (Table 2). Another 23.6% of this dissimilarity is explained by elevated <sup>109</sup>Cd in sturgeon, and its bioavailability is appreciably enhanced in fresh compared to marine water (Table 2).

The divergence between sturgeon exposed in brackish water and chondrichthyans is predominantly (48.7%; Table 5) due to <sup>134</sup>Cs, <sup>241</sup>Am and <sup>65</sup>Zn, whose bioavailability is not significantly ( $p > 0.05$ ) affected in these two salinities. <sup>65</sup>Zn and <sup>241</sup>Am are elevated in chondrichthyans and <sup>134</sup>Cs is higher in sturgeon. Another 23.9% of the dissimilarity is due to elevated <sup>75</sup>Se in sturgeon (Table 6), where bioavailability is not significantly ( $p > 0.05$ ) different between brackish and seawater (Table 2).

Hence, <sup>109</sup>Cd contributes between 23.6 and 33.4% to the divergences between sturgeon exposed in freshwater from teleosts and chondrichthyans, as well as sturgeon exposed in brackish water (26.4%). In all comparisons <sup>109</sup>Cd is elevated in sturgeon exposed in freshwater which is consistent with its enhanced bioavailability in freshwater (73.9%) compared to both brackish (16.1%) and seawater (3.6%). To further evaluate the robustness of the hypothesised phylogenetic basis of the relationships between sturgeon with teleosts and chondrichthyans in their multi-nuclide bioaccumulatory behaviours (Fig. 3) both the nMDS and cluster analyses were rerun but with <sup>109</sup>Cd excluded from the dataset. The results of these analyses for the nMDS, based on the remaining seven radionuclides, are shown in Supplementary Fig. 2. Both analyses showed relationships between sturgeon with teleosts and chondrichthyans which conform to those previously determined (Fig. 3) when <sup>109</sup>Cd was included in the multi-tracer analyses, i.e. the relationships determined between these taxa are not dependent on, or particularly influenced by, their <sup>109</sup>Cd CFs among salinity treatments which may be influenced by differential bioavailability (Table 2). A comparison by ANOSIM between sturgeon from brackish and freshwater shows they remain significantly ( $p < 0.01$ ) different (Supplementary Fig. 2;  $R = 0.31$ ) after the exclusion of <sup>109</sup>Cd, even though there is no potentially confounding

phylogenetic signal in this comparison.

### 3.3.4. Evaluation of potentially confounding effects of osmolarity and competitive inhibition on the phylogenetic relationships between fish taxa

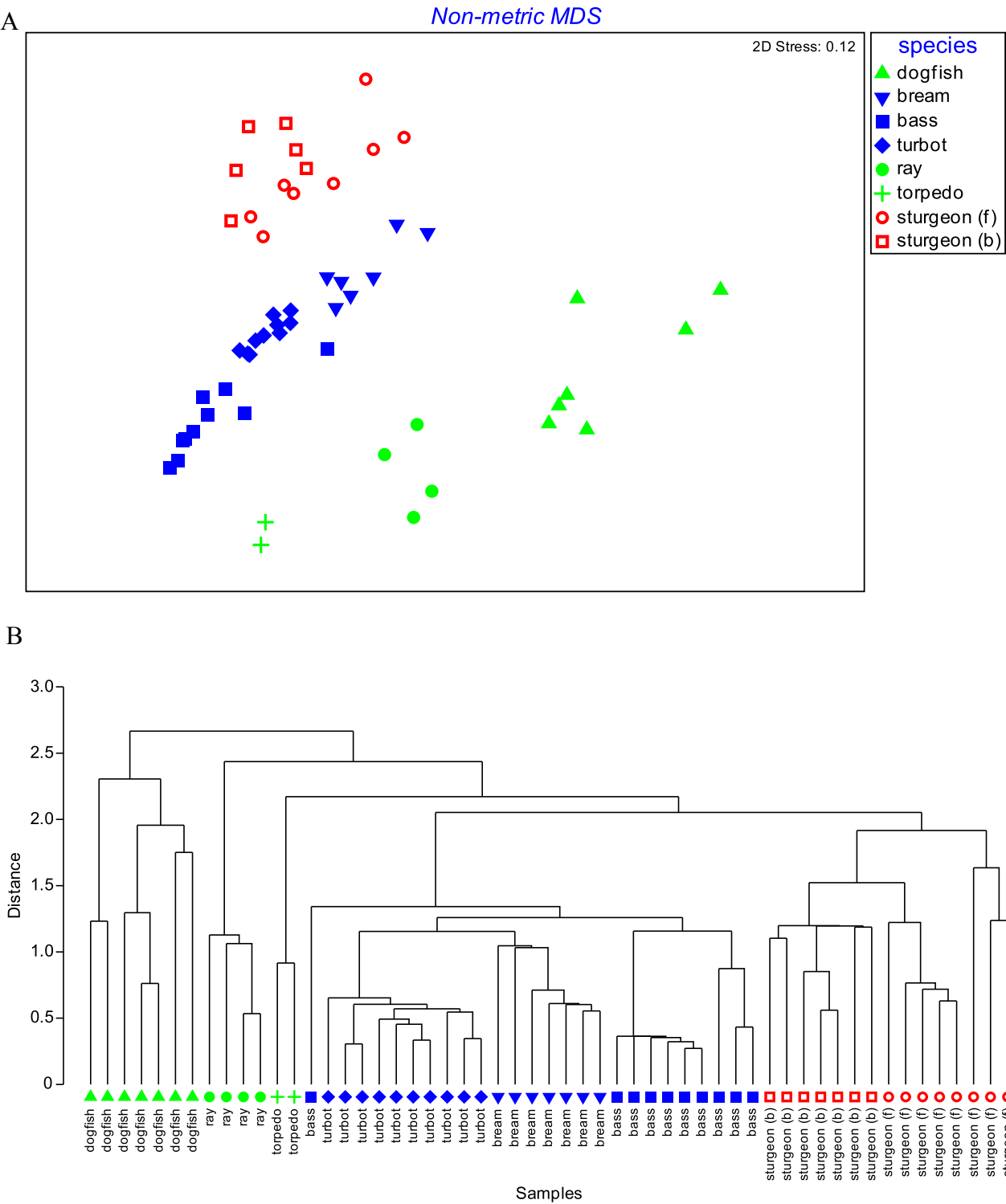
3.3.4.1. *Osmolarity effect*. The lowered osmolarity of brackish and fresh experimental waters compared to seawater could lead to the general enhancement in the uptake of ions and their radiotracer analogues across the gills of sturgeon to compensate for the loss of ions in large volumes of dilute urine, which is a general physiological characteristic of freshwater compared to saltwater fish (Kultz, 2015). More specifically to this study, the ionic and osmotic regulatory system of Russian sturgeon is known to differ from those of marine teleosts, where its kidney has tubules with a mechanism for intensive K secretion, although its ionic concentrations are similar in fresh and brackish Caspian Sea water (Natochin et al., 1985). A comparison of loss rate constants in Table 5 for <sup>134</sup>Cs, as an analogue of K, shows they are similar among sturgeon in both salinities and to *S. maximus*.

The comparison of mean whole body (wet): water CFs shown in Table 4 for sturgeon exposed at the two salinities with those for marine turbot and dogfish, where salinities have been reduced by factors of 4.2 for sturgeon in brackish water and a factor of 90.5 for their freshwater exposure compared to seawater, show the following;

- there are no systematic and appreciable factors of increase in CFs for sturgeon (in either fresh or brackish water) among all eight radionuclides, compared to turbot or dogfish,
- for <sup>109</sup>Cd there is such a trend of increasing CF with reducing salinity among the three species, and <sup>75</sup>Se shows enhanced CFs in sturgeon compared to marine fishes however its CF in sturgeon is higher in brackish (CF = 21.8) than fresh water (CF = 9.8.), thus not supporting an 'osmolarity effect' hypothesis.
- For <sup>54</sup>Mn, sturgeon have slightly lower CFs compared to turbot and dogfish.

The results of the SIMPER analysis (Table 6) also show that the dissimilarities identified between sturgeon and the other two phylogenetic groups (Fig. 3) are not due to consistently elevated CFs in sturgeon; the CF for <sup>54</sup>Mn is elevated in teleosts compared to sturgeon and both <sup>241</sup>Am and <sup>65</sup>Zn are elevated in chondrichthyans compared to sturgeon.

The multi-tracer comparison between species which employed the nMDS analysis (Fig. 3a) is based on the rankings of their whole



**Fig. 3.** a) Two dimensional nMDS plot and b) hierarchical cluster of experimentally exposed sturgeon, marine bony and cartilaginous fishes, based on their whole body (wet): water CFs for eight radiotracers [(sturgeon (f) - sturgeon exposed to fresh water; sturgeon (b) - sturgeon exposed to brackish water)].

body (wet): water CFs rather than their absolute values. Hence, such a method of multi-variate analysis would also actually circumvent the two potential problems described above of varying salinities between treatments confounding the phylogenetic comparison, if such an effect was actually present. It is also significant that the mMDS (Supplementary Fig. 1), which is based on the absolute values of the CFs rather than their rankings, gave a result

virtually identical to that for the nMDS (Fig. 3a)-consistent with the absence of a general salinity effect on the enhancement of absolute CFs in sturgeon. Additionally, the salinity variation in the sturgeon exposures (fresh or brackish) had little effect on their quantified dissimilarities with teleosts (15.5 and 15.8, respectively) and chondrichthyans (26.8 and 27.1, respectively) (Table 6).

**Table 6**Percentage contributions of the main radiotracers<sup>a</sup> to average dissimilarity measures between groupings of fishes.

Radiotracer	Average values for each group		% contribution to average dissimilarity measure	Average dissimilarity measure
<i>a) Sturgeon, fresh (SF) and Sturgeon, brackish (SB)</i>				
	<b>SF</b>	<b>SB</b>		6.5
Cd-109	1.8	0.5	26.4	
Se-75	1.0	1.9	16.7	
Co-60	0.3	−0.4	15.7	
Cs-134	0.5	0.7	14.5	
<i>b) Sturgeon, fresh (SF) and Teleosts (T)</i>				
	<b>SF</b>	<b>T</b>		15.5
Cd-109	1.8	−0.4	33.4	
Ag-110 m	0.8	−0.8	19.0	
Se-75	1.0	−0.5	16.3	
Mn-54	−0.1	0.4	10.4	
<i>c) Sturgeon, brackish (SB) and Teleosts (T)</i>				
	<b>SB</b>	<b>T</b>		15.8
Se-75	1.9	−0.5	40.0	
Ag-110 m	1.1	−0.8	27.1	
Mn-54	−0.6	0.4	11.7	
<i>d) Sturgeon, fresh (SF) and Chondrichthyans (C)</i>				
	<b>SF</b>	<b>C</b>		26.8
Cd-109	1.8	−0.6	23.6	
Cs-134	0.5	−1.5	17.3	
Am-241	−0.4	1.3	17.3	
Zn-65	−0.3	1.5	14.8	
<i>e) Sturgeon, brackish (SB) and Chondrichthyans (C)</i>				
	<b>SB</b>	<b>C</b>		27.1
Se-75	1.9	0.6	23.9	
Cs-134	0.7	−1.5	18.1	
Zn-65	−0.5	1.5	17.3	
Am-241	−0.2	1.3	13.3	

<sup>a</sup> ≥80% contribution.

3.3.4.2. *Competitive inhibition effect.* The lower salinities of the sturgeon exposures (Table 1) compared to teleosts and chondrichthyans exposed in seawater (Table 1, Jeffree et al., 2006) may lead to enhanced ratios in water of radiotracer to i) metabolic model (e.g.  $^{134}\text{Cs}$ : K;  $^{109}\text{Cd}$ : Ca), and ii) stable isotope (e.g.  $^{65}\text{Zn}$ : stable Zn) and thus could give rise to generally enhanced CFs in sturgeon. This competitive inhibition hypothesis can be tested for  $^{134}\text{Cs}$  as variable salinity does not affect its speciation (Table 2), and differences in K concentrations between fresh and brackish water (1.84 and 99.2 mg.L<sup>−1</sup>, respectively (Table 1)) with seawater (K = 445 mg.L<sup>−1</sup>) give ratios of 242 and 4.5, respectively.

However,  $^{134}\text{Cs}$  shows no enhancement in sturgeon exposed in fresh compared to brackish water (Table 4), even though their K water concentrations differ by more than a factor of 50 (Table 1). There is little difference in  $^{134}\text{Cs}$  CF between sturgeon with marine teleost (*S. maximus*) even though the factors of difference in K concentrations between fresh and brackish water with seawater (K = 445 mg.L<sup>−1</sup>) are 242 and 4.5, respectively. The comparison of sturgeon with the chondrichthyan *S. canicula* gives a factor of c.6 increase in sturgeon's CF for  $^{134}\text{Cs}$ , which is comparable to the factor of 4.5 reduction in K water concentration in brackish compared to seawater, i.e. a hypothesis of competitive inhibition, to explain elevated  $^{134}\text{Cs}$  in sturgeon in brackish (but not in fresh water) compared to chondrichthyan, is supported. Moreover,  $^{134}\text{Cs}$  contributes 18.1% in the dissimilarity between sturgeon in brackish water with chondrichthyans (Table 6), similar to that in the comparison of sturgeon in freshwater with chondrichthyans (17.3%). However, when the data subset of CFs for sturgeon in fresh and brackish waters and chondrichthyans were re-analysed, with the deletion of  $^{134}\text{Cs}$ , both nMDS and cluster analyses still distinctly separated the two phylogenetic groups. i.e. the hypothesised competitive inhibition effect of enhanced ratios of  $^{134}\text{Cs}$ : K with reduced salinity of brackish water was not appreciable enough to

remove the phylogenetic signal which separates sturgeon in brackish water from marine chondrichthyans.

The other identified tests of the competitive inhibition hypothesis were the effect of salinity on the ratios of  $^{65}\text{Zn}$ : stable Zn and  $^{109}\text{Cd}$ : Ca water concentrations. These were not valid due to i) little variation between fresh, brackish and marine waters in their stable Zn water concentrations (0.47, 0.44 and 0.25 μg.L<sup>−1</sup>; Table 1; Jeffree et al., 2006), and hence no competitive inhibition effect would be possible, and; ii) the confounding effect of salinity on the bioavailability of  $^{109}\text{Cd}$  (Table 2), although Ca water levels did vary across the increasing gradient (69.1, 102 and 458 mg.L<sup>−1</sup>; Table 1, Jeffree et al., 2006). Hence, differences between taxa where  $^{65}\text{Zn}$  makes an appreciable contribution (sturgeon in fresh and brackish water with chondrichthyans, Table 6) cannot be attributed to either varied bioavailability or competitive inhibition effects.

## 4. Discussion

### 4.1. Biokinetics of radionuclides in Russian sturgeon

The results of the speciation modelling undertaken for this study (Table 2) has clearly shown its importance for identifying those radionuclides whose bioavailability varies markedly with salinity (e.g.  $^{109}\text{Cd}$  and  $^{110m}\text{Ag}$ ), and the majority where their bioavailability does not vary significantly ( $p > 0.05$ ) with salinity. Such information was fundamental to enhancing the rigor of the assessment of the phylogenetic interpretation of the bioaccumulation patterns of sturgeon with other marine taxa, as well as further assessment of other potential confounders of this phylogenetic interpretation, i.e. competitive inhibition and osmolarity effects.

The characterisation of the radionuclide biokinetics in sturgeon under fresh and brackish exposures in combination with the



speciation modelling has indicated that enhanced bioavailability due to changed salinity does not necessarily lead to its enhanced bioaccumulation, in fact quite the opposite for  $^{110m}\text{Ag}$  (Tables 2 and 3, Fig. 1), i.e. modelled bioavailability, even when it predicts an appreciable effect of salinity, is not fully predictive for bioaccumulation patterns in sturgeon.

#### 4.2. Evaluation of the phylogeny-based model of multi-nuclide bioaccumulation with Russian sturgeon

A major objective of this experimental study was to further evaluate a hypothesis related to phylogenetically-influenced multi-radionuclide bioaccumulation patterns which was based on marine fishes and a marine cephalochordate (Jeffree et al., 2006, 2010; 2013), with the additional objective of evaluating whether it could be broadened to include species living in other salinity regimes. The Russian sturgeon had two qualities which were valuable for this investigation, viz., a) it naturally inhabits salinity regimes which are different from the taxa previously tested, and b) its ancestral relationships indicated that it would occupy a place distinctly between marine teleosts and chondrichthyans in their periods of divergence, thereby having an interpolative role in model assessment which balanced the more extrapolative nature of the salinity variations that laid outside the bounds of previous marine experimental tests of the phylogenetic model. The results of this study are assessed below against the set of *a priori* predictions (Section 2.1.2) which were defined to critically test this phylogenetic hypothesis, viz.

- a) *similarities between individual sturgeon compared to individual teleosts and chondrichthyans.* Clearly, individuals of the same species are closest in their phylogenetic relationships and if they did not show aggregation at the lowest level in their bioaccumulation characteristics then the phylogeny - based model would be falsified. In general accordance with the affinities of each other species represented in Fig. 3 (a and b), where individuals of a species are aggregated closest to each other rather than other species, sturgeon also aggregate independent of other species. However, sturgeon exposed in fresh and brackish water are separated and to a degree which is comparable to that between the teleosts, bass and turbot.
- b) *the distinction between sturgeon and all teleost and chondrichthyan species.* Given that separations exist between species of teleosts and between species of chondrichthyans, although their periods of divergence are less than those with sturgeon (Jeffree et al., 2010, Fig. 3), the phylogenetic model predicts that sturgeon are both distinct from and further separated from these taxa, given their longer periods of divergence. This model prediction is in agreement with the results shown in Fig. 3, where sturgeon exposed in either salinity are distinct from both marine taxa.
- c) *the greater divergence of sturgeon from chondrichthyans than teleosts.* The ancestral relationships between these two taxa with sturgeon is quite different, and thus should also be reflected in their bioaccumulation characteristics, if phylogeny plays a major role in their bioaccumulation patterns. Sturgeon's longer period of divergence from chondrichthyans (473 MYBP) than teleosts (344 MYBP) predicts their greater separation. This model prediction is also supported by the results in Fig. 3b, which shows sturgeon aggregating with teleosts lower in the dendrogram, before their collective aggregation with chondrichthyans; the nMDS (Fig. 3a) also shows sturgeon closer to teleosts than chondrichthyans.

In summary, the results for each of the tests which were

identified as ways to falsify the phylogenetic hypothesis for sturgeon were unequivocally consistent with this hypothesis.

Investigation of other salinity-influenced effects to explain these relationships between sturgeon and marine fish taxa (Fig. 3) did identify such potential effects due to changes primarily in bioavailability (Table 2), but also from changes in osmolarity and competitive inhibition. Assessment of the potential effects of such salinity-sensitive radionuclides by their i) exclusion from analysis (Supplementary Fig. 1), ii) evaluation of their relative contributions to measures of dissimilarity between taxa (Table 6), and iii) comparisons of CFs between salinity treatments (Table 4), indicated they had little or no appreciable effect on the underlying phylogeny-based relationships between taxa established in Fig. 3. Such salinity-influenced effects are also of a different investigative quality compared to the empirically-based patterns demonstrated in Fig. 3. They are notional, theoretically-based and wrought with underlying assumptions whose testing would require further experimentation. For example, tests of competitive inhibition of the accumulation of a metabolic analogue by its model, which varies in concentration between different waters, typically assume that the rates of model bioaccumulation are independent of their water concentrations. However this assumption may not always be true (Jeffree, 1991) and can lead to changes in the bioaccumulation of the metabolic analogue that vary from proportionality with the change in model water concentration. Nevertheless, consideration and assessment of their effects (albeit minimal) provide further confidence in the hypothesis that sturgeon have, in their bioaccumulation characteristics, the marks of their ancestral relationships with teleosts and chondrichthyans.

#### 4.3. Extension of the phylogenetic bioaccumulation model beyond marine biota

The results of this study have shown phylogenetic consistency in the patterns of accumulation of radionuclides in sturgeon experimentally exposed to fresh and brackish waters with those patterns of radionuclide accumulation by exclusively marine fishes. This consistency indicates that the phylogenetic bioaccumulation model, which has been initially established for marine chordates (Jeffree et al., 2006, 2010, 2013) may well incorporate biota which typically live in lower salinities. This indicated extension of the validity of the bioaccumulation model beyond marine taxa, albeit supported by the results of this one species of sturgeon tested at two salinities, would now need to be further tested for other fish species also living in lower salinities.

#### 4.4. Phylogenetically differentiating radionuclides

Prediction of the times of divergence of sturgeon from teleosts and chondrichthyans, which were used in this study, are based on a mechanism for genetic change which occurs between taxa over periods of evolutionary time. The process of speciation and consequent divergences between lineages is attributed to random genetic events, which nonetheless occur at a constant clock-like rate (Hedges et al., 2015), whereas adaptive change is largely a separate process from speciation. This mechanism of random genetic divergence between taxa over the periods of evolutionary time can also be drawn upon to derive interpretations about the roles of individual radionuclides in differentiating between taxa investigated in such multi-tracer bioaccumulation experiments.

Gene-mediated physiological traits which vary continuously (Garland et al., 2005) and influence the rates of accumulation of radionuclides may therefore be predicted to also vary randomly between diverging lineages. The suite of radionuclides which do discriminate between phylogenetic groups may thus represent a

Brownian motion, with any nuclide as likely as any other nuclide to be discerning between any two phylogenetic groups being compared in their bioaccumulation characteristics. Such physiological traits may also change the capacities for bioaccumulation under differing adaptive pressures between lineages. The following bioaccumulation characteristics determined in this study would suggest a degree of randomness in the way different radionuclides are discerning between diverging taxa;

- i) Among the radionuclides tested, all eight contribute to the highest four discriminating between marine taxa with sturgeon and also salinity treatments in sturgeon (Table 6), i.e. no element is phylogenetically indifferent and no element is consistently and overwhelmingly dominant in its capacity for discernment between compared taxa or salinity treatments.
- ii) For both salinity exposures of sturgeon  $^{75}\text{Se}$ ,  $^{110m}\text{Ag}$  and  $^{54}\text{Mn}$  collectively contribute 46–79% to their dissimilarity with teleosts, but the same comparisons of sturgeon with chondrichthyans shows a different set of discriminators, with  $^{134}\text{Cs}$ ,  $^{65}\text{Zn}$  and  $^{241}\text{Am}$  contributing 49% to their dissimilarity.

## 5. Conclusions

The results of this experimental study have met the criteria defined *a priori* to determine whether sturgeon's bioaccumulation characteristics were consistent in its ancestral relationships with teleosts and chondrichthyans, based on their previously determined multi-nuclide bioaccumulation patterns. These phylogeny-based signals were also resilient to confounding effects of variable bioavailability, osmotic and competitive inhibition effects which were identified for several elements and comparisons, and that were inherent in such comparisons among species accumulating radionuclides in different salinities.

This study represents another critical evaluation of a phylogeny-based bioaccumulation model initially derived for marine teleosts and chondrichthyans and also extended to the marine cephalochordate, amphioxus (*Branchiostoma lanceolatum*). It also represents successful extension of the model to much lower salinities and as such has broadened the scope of the model; although clearly more species living in lower salinities now need to be evaluated, employing the speciation modelling of radionuclide bioavailability and other methodologies identified and tested in this study.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jenvrad.2017.06.007>.

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