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# 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 ($^{241}$Am, $^{109}$Cd, $^{134}$Cs, $^{75}$Se, $^{54}$Mn, $^{110m}$Ag, $^{65}$Zn, $^{60}$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.



## 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 cartilagenous fish. Moreover, declining catches in the Caspian Sea of this IUCN Red List Threatened Species (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 ($^{110m}$Ag, $^{241}$Am $^{109}$Cd, $^{60}$Co, $^{134}$Cs, $^{54}$Mn, $^{75}$Se and $^{65}$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 Carcharhiniformes, 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.