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Marine radionuclide transfer factors in chordates and a phylogenetic hypothesis

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Previous radiotracer experiments that compared multi-elemental whole organism: water transfer factors among chondrichthyan and teleost fishes, including an ICRP reference flatfish *Psetta maxima*, demonstrated distinctive contrasts in their bioaccumulation characteristics, with generally elevated bioaccumulation in chondrichthyans. These results supported a hypothesis that phylogenetic divergence may influence marine radionuclide transfer factors. This notion has been further evaluated in an amphioxus species *Branchiostoma lanceolatum*, sub-phylum Cephalochordata. This taxon diverged about 800 MYBP from a common ancestor of the teleosts and the chondrichthyans, which in turn diverged from each other around 500 MYBP. Our experimental results indicate that amphioxus is indeed more divergent in its multi-elemental bioaccumulation patterns from teleosts and chondrichthyans than they are from each other, consistent with our hypothesis. The experimental comparisons with the ICRP reference flatfish *P. maxima* also revealed an unexpectedly enhanced capacity in amphioxus to accumulate all eight tested trace elements from seawater, and for some by more than two orders of magnitude. These results have practical applications for the strategic selection of marine biota for further radioecological investigations to better guarantee the radiological protection of marine biodiversity. Such seemingly anomalous results for understudied biota like amphioxus and chondrichthyans suggest that more effort in marine radioecology be directed to assessing the bioaccumulatory capacities of other phylogenetic groups that have received less attention so far, particularly those that are phylogenetically more remote from commonly investigated taxa and those nominated as ICRP marine reference organisms.

1. Introduction

It is generally held that more closely related organisms will have similar environmental transfer factors (TFs) for radionuclides (ICRP, 2009; Howard et al., 2013), but would the converse also hold? To what extent could more distantly related organisms be expected to show dissimilarities in their TFs? This question of the influence of phylogenetic relatedness on bioaccumulation of environmental contaminants has received some attention to date. Rainbow (1998) interpreted differences between marine crustacean taxa in their metal accumulation patterns, in the context of differences in their phylogeny as well as their biology and ecology, and the contaminant patterns of PCBs among species of Arctic seabirds have also suggested a phylogenetic association (Borga et al., 2005). Buchwalter et al. (2008) have also shown variations in Cd bioaccumulation and susceptibility in three orders of aquatic insects that are taxon-related. More generally, in other areas of biological science that are also relevant to radioecology a phylogenetic perspective is providing fruitful research agendas. In various physiological and biochemical studies a phylogenetic approach has been helpful or even crucial in the design and interpretation of results from comparative investigations between taxa (Garland et al., 2005). Studies in population and community ecology can also be better informed by accurate knowledge of the evolutionary relationships between co-existing species (Webb et al., 2002; Westoby, 2006).

In previous experiments we have investigated a phylogenetic question in a comparison of multi-elemental whole-body: water concentration factors (CFs) between distantly related cartilaginous and bony fishes (including a flatfish). These two major phylogenetic groups had diverged from a common ancestor over more than 500 million years before the present (MYBP) (Blair, 2009). These studies using nine radioactive trace elements showed very distinctive differences between these two taxa in the rankings of their CFs for the nine trace elements and also in the absolute values of the CFs for single trace elements, in both their whole bodies and also

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among dissected body components (Jeffree et al., 2006, 2010). The results for the three teleosts investigated showed that they have similar bioaccumulation patterns and the CFs for individual trace elements were also generally comparable between the three tested species, being within one order of magnitude of each other. Hence the results were consistent with the teleost flatfish being representative of other teleost fishes in its bioaccumulatory behaviours. The results for chondrichthyans did not accord with this interpretation and, depending on the radionuclide responsible for the radiation dose, they may be more exposed to many of the radionuclides examined in this previous study, but less exposed to radiocaesium.

The experimental study reported here was designed to further critically evaluate two linked components of a phylogenetic hypothesis, *viz*; i) that multi-elemental bioaccumulation characteristics differ between taxa and ii) that the degree of difference increases with their period of evolutionary divergence. To this end we chose a representative species of the subphylum Cephalochordata, whose members are modern survivors of this ancient chordate lineage. This taxon was chosen solely on the basis of its much greater period of divergence time from a common ancestor compared to that for teleost and chondrichthyan fishes; however it is acknowledged that the rates of evolution of each these taxa following divergence may well be different. Species of the subphylum Cephalochordata are essentially sedentary and usually found within sand in shallow coastal regions of temperate and tropical seas where they filter particles from the water with their ciliated pharynx (Barrington, 1970; Romer, 1970). In Asia they are also harvested commercially as food for both humans and for domesticated animals. To our knowledge there are no published transfer factors for radionuclides for any representatives of this subphylum.

2. Materials & methods

2.1. Collection, acclimation and experimental exposure of *Branchiostoma lanceolatum*

Individuals of *B. lanceolatum*, were collected from the English Channel by staff of the Roscoff Marine Biological Station, France, and transported to the IAEA Marine Radioecology Laboratory in Monaco. They were maintained on a diet of *Isochrysis galbana* at 5×10^4 cells/mL and were acclimated for three weeks to laboratory experimental conditions. The amphioxus used in this 14 day experiment were regularly removed from the experimental bath and allowed to feed for about 60 min on unlabelled *Isochrysis* algal cells in suspension at 5×10^4 cells/mL in a 3 L close-system aquarium, before their return to the radio-labelled experimental medium. Individuals maintained their weights during this period, with there being no significant ($P < 0.05$) regression between wet weight and the duration of experimental exposure.

For the experimental exposure 20 individuals that ranged in total length between 3.5 and 6.0 cm were exposed to eight radioisotopes in seawater. Their experimental conditions were chosen to be very similar to those of two previous experiments with fishes (Jeffree et al., 2006, 2010) to enhance the validity of the comparisons of results between these experiments. Amphioxus were exposed at 16° ± 1 °C, salinity 38 and pH 8.05 in 280 L volumes with a light/dark cycle of 10 h/14 h for two weeks. The counts for each individual determined the total body burdens and their concentration factors (CF = Bq g^{-1} wet organism divided by the time-integrated Bq g^{-1} seawater) attained after 14 days of experimental exposure. As in a previous experiment with the teleost *Psetta maxima* and chondrichthyan *Scyliorhinus canicula* (Jeffree et al., 2006) each individual was measured repeatedly at intervals over the 14 day exposure for its radionuclide contents to assess patterns of accumulation during experimental exposure. The radioactivity levels in individuals were determined by comparison with known standards of appropriate geometry and were corrected for background and isotopic decay. Counting times were adapted to obtain count rates with relative propagated errors of less than 5% that were typically 10–30 min.

Data plots indicated that for most radio-isotopes amphioxus did not attain equilibrium concentration factors in 14 days. These results for amphioxus contrasted with those for *P. maxima* which reached or approached equilibrium for radiotracers of Co, Cd, Zn, Am and Cr. Similarly *S. canicula* attained or approached equilibrium for radiotracers of Co, Cd, Mn, Cs, Am and Cr in 14 days of uptake (Jeffree et al., 2006).

The water exposure pathway was used in previous experiments because it was less potentially confounded when comparing between fish species with different natural diets and assimilation efficiencies for trace elements (Jeffree et al., 2010), ie. the fractions of trace elements ingested in food that are absorbed. The seawater exposure pathway used for previous experimental comparisons between teleosts and chondrichthyans had yielded differences of more than 2 orders of magnitude among trace elements in whole body and tissue CFs between these two major taxa (Jeffree et al., 2006, 2010). In contrast, an experimental comparison between dogfish *S. canicula* and sea bream *Sparus aurata* of the trophic transfer of seven of the radiotracers used in this experiment had shown no significant differences ($P > 0.05$) between these two species in their assimilation efficiencies of Am, Cd, Cs or Zn (Mathews et al., 2008) when they ingested identical diets. However, among various trace elements the seawater exposure pathway is not necessarily the major exposure pathway for their accumulation in marine fishes (Mathews and Fisher, 2009).

Fortuitously, prior employment of the seawater exposure pathway to compare between fish taxa was also the most appropriate one to use in a comparison of these previous results with those produced in this study for amphioxus, which has a completely different feeding technique (Barrington, 1970) and dietary composition compared to these marine fishes.

The eight radioactive elements used in this experimental exposure were Mn-54, Co-60, Zn-65, Cs-134, Se-75, Am-241, Ag-110m and Cd-109. Multiple elements were again employed to evaluate our phylogenetic hypothesis because each element is likely to be metabolised uniquely. Each trace element thus represents a different physiological trait and provides an individual axis in a 'multi-dimensional bioaccumulation space' where the location of each species in lower dimension ordinations could better discern any differences that were taxon-specific in their overall patterns of bioaccumulation. For ease of reference we have used the collective term 'trace elements' in the text for the eight radioactive elements. During the experimental exposure radio-isotopic activities were measured before and after each daily seawater renewal in order to keep exposure activities constant and to determine their time-integrated activities in the experimental water (Mn-54 0.5 KBq/L, Co-60 0.5 KBq/L, Zn-65 0.5 KBq/L, Cd-109 1.3 KBq/L, Ag-110m 0.5 KBq/L, Se-75 0.5 KBq/L, Cs-134 1.0 KBq/L, Am-241 0.2 KBq/L). No change of pH was detectable after isotope solution addition in 280 L batches.

To ensure a consistently adequate quality all seawater was carbon-filtered prior to delivery into the tanks that were used during the acclimation period and the experimental exposure. Accordingly the calculations with geochemical modelling codes that we used to predict the various chemical species in the experimental water were determined for DOC levels at <0.1 mg L^{-1}. The details of the modelling procedures employed and speciation results are given in Jeffree et al. (2006) for most of the trace elements that were also used in this experiment. The free aquo ions