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Experimental comparison of the bioaccumulation of anthropogenic radionuclides by egg and juvenile life stages of a small shark

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ABSTRACT

This study compared the bioaccumulation of anthropogenic nuclides (^{65}Zn, ^{134}Cs, ^{60}Co and ^{241}Am) between the egg and juvenile life stages of a small shark (*Scyliorhinus canicula*), based on previously published experimental data. Rates of accumulation over 15 days were derived and summed for the transfer pathways which were specific to these two life stages. Radionuclide transfers to the egg and its embryo & yolk were quantified for i) the maternal pathway following her uptake of radionuclides via food and seawater and ii) from seawater following its oviposition. For the juvenile, the transfer of radionuclides were measured for aqueous & dietary pathways. The results show that, compared to juveniles, eggs have equivalent rates of accumulation of ^{65}Zn and ^{134}Cs but enhanced accumulation of ^{241}Am by a factor of five and of ^{60}Co by two orders of magnitude. The radiological exposure of the embryo due to radionuclides maternally transferred to the embryo & yolk is also enhanced for the alpha-emitting ^{241}Am. This enhanced accumulation of ^{241}Am and ^{60}Co, as well as the equivalent accumulation of ^{65}Zn and ^{134}Cs, suggest greater likely vulnerability to radiation damage in eggs as compared to juveniles. Radiological dose assessment confirmed highest doses to the egg which is predominantly due to accumulated ^{241}Am.

1. Introduction

Studies of the bioaccumulation of radionuclides in marine biota have been predominantly focussed on the adult phase of their life cycles. This has been a consequence of the initial interest in radiological exposure of humans via consumption of seafood, based on the capacities of these organisms to accumulate various radionuclides from their marine environment, as defined by their organism-to-seawater concentration factors (CFs) (IAEA, 2004). More recent concern with the radiological effects of accumulated radionuclides on marine biota themselves has led to dose assessments and methodologies which have been mainly based (by necessity) on these organism-to-seawater CFs determined for the adult phases of their life cycles (Beresford, 2010; Howard et al., 2013).

Whereas these CFs based on the adult phase of the life cycle for marine biota are appropriate for assessment of radiological impact on humans through their consumption, such radioecological data may not be adequate to assess potential radiological impacts on marine biota themselves. This follows from the likelihood of radiological sensitivities and responses that vary throughout stages of the life-cycle, similar to those well established for humans (ICRP, 1990). The dose-effect variation with life stage is relatively poorly defined for most other organisms. However in the teleost *Danio rerio* (zebrafish), which is an established radiobiological model organism, radiation-induced lethality and morphological perturbations are more pronounced earlier in embryogenesis, supporting the hypothesis that events occurring at or before gastrulation are particularly radiosensitive (Geiger et al., 2006; Hu et al., 2016; Walker and Streisinger, 1983). Moreover, the long-term effects on gene expression of the irradiation of embryonic zebrafish differs strikingly from that seen in adults (Jaafar et al., 2013).

These results contrast irradiation effects on different life stages which have been equally exposed under experimental conditions. However, under natural conditions separate life stages could well vary in their irradiations due to their different capacities to bioaccumulate radionuclides, particularly when their combinations of transfer pathways for radionuclides are not the same and also

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their morphologies and physiologies are different [e.g. age-dependent doses to humans (ICRP, 1990)].

The objective of this study was to evaluate the hypothesis of different capacities for the bioaccumulation of four anthropogenic radionuclides (^{65}Zn, ^{134}Cs, ^{60}Co and ^{241}Am) between two contrasting life-stages of the lesser spotted dogfish *Scyliorhinus canicula*. We tested a null hypothesis of no appreciable differences between the two life stages in their capacities to accumulate these four radionuclides. Its egg-laying mode of reproduction is representative of about 40% of sharks and all skates. In contrast to bony fishes it has reduced fecundity with enhanced maternal investment in a small number of slowly developing progeny (Wourms and Demski, 1993). We compared the bioaccumulation of these radionuclides under experimental conditions between the encased embryonic and free-swimming juvenile life stage of *S. canicula*, and for exposure pathways that were specific to these two life stages.

2. Materials and methods

2.1. Experimental exposures and radioanalysis

Below is summarised the exposure conditions for the set of previous experiments (Jeffree et al., 2006a, b; 2010, 2015; Mathews et al., 2008; Mathews and Fisher, 2009) from which data were drawn for the comparisons we have undertaken in this study. These experiments were comprised of aqueous, dietary and maternal transfers of four anthropogenic radionuclides (^{65}Zn, ^{134}Cs, $^{57/60}$Co and ^{241}Am) (Table 1). The four radionuclides used in these previous studies are typically associated with effluents entering the marine environment from coastal nuclear facilities for subsequent bioaccumulation (e.g. Alexander and Rowland, 1966; Whicker and Schultz, 1982). The types and combinations of exposure pathways are different between the two life stages, being aqueous and maternal for encased embryos but aqueous and dietary for free-swimming juveniles (Supplementary Fig. 1). Experimental data were available for each of these exposure pathway/life stage combinations so that the two life stages could be adequately compared in this synthesis study.

All comparisons of concentration factors (CFs) between exposure pathway/life stage combinations were based on the wet weight of the life stage and its components.

All acclimations and experiments were conducted in the IAEA Monaco Radioecology laboratory under very similar conditions of salinity 38 psμ, pH 8.05, a light/dark cycle of 10 h/14 h and temperature 16.0–17.0 °C. Prior to experimentation encased embryos, juveniles and adult egg-laying females were also acclimated for 3–6 weeks under these conditions.

Radioactivity concentrations were measured using a high resolution gamma spectrometry system consisting of four coaxial Germanium (N- or P-type) detectors (EGNC 33-195-R, Intertechnique; 40–70% efficiency) which were connected to a multi-channel analyser and a personal computer employing spectral analysis software (Interwinner 6, Intertechnique). The radioactivity concentrations of the samples were determined by comparison with known standards of appropriate geometry (phantoms of eggs and juveniles) and were corrected for background and isotope physical decay. Phantoms were made of radiolabelled tissue paper of comparable geometry, size and radionuclide content to the samples being analysed. Self-absorption of activity by samples was minimal for the gamma-emitting radionuclides used in these studies. Counting times were adapted to obtain count rates with relative propagated errors less than 5–10%, viz. typically 10–30 min for whole eggs or juveniles and 1–12 h for seawater and dissected components of eggs and juveniles.

2.1.1. Aqueous exposures of eggs and juveniles

During the experiments (Table 1) radio-isotopic activity concentrations were measured before and after each seawater renewal (24 or 48 h) in order to keep exposure activity concentrations constant. The time-integrated water activity concentrations of radio-isotopes were ^{65}Zn 0.5 kBq/L, ^{134}Cs 1 kBq/L, $^{57/60}$Co 0.5 kBq/L, ^{241}Am 0.2 kBq/L. No changes in pH were detectable after isotope solution additions. In this study, a rinsing protocol for organisms was not employed. Rather, consistent with natural conditions, they were allowed to absorb the radiotracers through external membranes, which for the egg has been shown to be significant. Dogfish eggs with developed embryos were exposed to radio-labelled seawater over 15 days and were regularly sampled from the experimental bath, transferred in a counting tube within clean seawater for radio-spectrometric analysis, and then returned to the experimental bath. During repeated counting, the specimens were situated consistently relative to the counter, and movement of the juveniles was minimized by light anesthesia and restraints. Whole egg-to-water concentration factors (CFs) were then calculated and plotted as a function of days of experimental exposure to determine the pattern and rate of bioaccumulation of each radio-isotope as a function of days of experimental exposure. The CFs were also determined at the end of the 15 day exposure for egg case, embryo, yolk, and glycosaminoglycan jelly (Jeffree et al., 2006b).

For the comparisons of eggs accumulating radionuclides directly from seawater with other combinations of exposure pathway and life-stage it was decided to employ the CFs associated with the egg case rather than the whole egg for the following reasons, in order to give a more valid comparison of potential radiation exposure of the embryo with the juvenile life-stage: a) data for radionuclide distributions among egg components showed ≥99% of ^{65}Zn, ^{57}Co and ^{241}Am were associated with the egg case, and their egg case-to-water CFs were two or more orders of magnitude greater than those for the other three egg components; b) the distribution of ^{134}Cs also showed a similar pattern of 69% being associated with the

Table 1
Pathways of experimental exposures of eggs and juveniles of *S. canicula* to ^{137}Cs, ^{65}Zn, $^{57/60}$Co and ^{241}Am.

Life stage	Exposure pathway	Temperature (C)	Length (cm) *Mass (g wet weight)*	Age or developmental stage	Reference
Egg	Aqueous	17.0	*2.9–6.4*	Pre-hatchling (Stages 32–34; Ballard et al., 1993)	Jeffree et al. (2006b)
Egg	Maternal transfer via diet	16.0	5.5–6.3 *5.3–7.2*	Blastodisc (Stage 4; Ballard et al., 1993)	Jeffree et al. (2015)
Juvenile	Aqueous	16.5	8.5–16	3-4 months	Jeffree et al. (2006a)
Juvenile	Aqueous	16.5	20–30 *16–261*	12-24 months (Ivory et al., 2004)	Jeffree et al. (2010)
Juvenile	Dietary	16.0	*Mean=8*	≤3 months	Mathews et al. (2008); Mathews and Fisher (2009)

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