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Maternal transfer of anthropogenic radionuclides to eggs in a small shark

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ABSTRACT

Maternal transfer of radionuclides to progeny is one of the least known sources of contamination in marine biota and more information is needed to assess its radiological significance. A radiotracer study on spotted dogfish, *Scyliorhinus canicula*, evaluated the hypothesis that four anthropogenic radionuclides (Cobalt-60, Zinc-65, Americium-241 and Cesium-134) could be maternally transferred to eggs and each of their major components during maternal ingestion of radiolabelled food. The linear regressions between cumulative radioactivity that had been maternally ingested and the level in subsequently laid eggs were used to derive maternal-to-egg transfer factors (mTFs). These maternal transfers varied over an order of magnitude and were ranked ^{134}Cs > ^{65}Zn > ^{60}Co > ^{241}Am. This ranking was the same as their relative assimilation efficiencies in radiolabelled food consumed by adults. Among these four radionuclides the potential radiological exposure of embryos is accentuated for ^{65}Zn and ^{134}Cs due to their predominant transfer to egg yolk where they are available for subsequent absorption by the embryo as it develops prior to hatching from the egg capsule. Thus, for cartilaginous fish like shark, the potential radioecological consequences of a pulsed release of these radionuclides into the marine environment may extend beyond the temporal duration of the release.

1. Introduction

The maternal-to-embryo exposure pathway has been a topic of importance within the International Commission on Radiological Protection with regard to human transplacental transfer of radionuclides (ICRP 88, 2001), because of the extreme radiosensitivity of the embryo and foetus (Von Zallinger and Tempel, 1998). Studies among other mammal species have emphasised the species-specific differences in their transplacental radionuclide dynamics (Von Zallinger and Tempel, 1998). More recent studies on levels of radioactive cesium in cattle from the evacuation zone of the Fukushima Daiichi NPP have drawn attention to elevated levels in the organs of foetuses and infants compared to their mothers (Fukuda et al., 2013). Given the continued interest in this transfer pathway of demonstrated significance (Von Zallinger and Tempel, 1998) then exploratory studies in other taxa, in addition to terrestrial mammals, would seem valuable.

There are increasing efforts to better assess the risks to biota in the aquatic environment that result from the releases of radionuclides from nuclear facilities, through a greater knowledge of their environmental transfers to biota and the significance of their consequent radiation exposure. This process is facilitated by the use of databases of transfer factors, which are currently dominated by values for adult life stages (eg. Howard et al., 2013; IAEA, 2004) and require better representation of many phylogenetic groups, eg. cartilaginous fishes. Moreover, fish eggs are included as ICRP RAPs (Reference Animals and Plants) (ICRP, 2008) but there are no data to determine radionuclide transfers to fish eggs (ICRP, 2009).

Transfer factors derived from field-based data characteristically represent the aquatic organism's accumulation of a radionuclide via the environmental transfers from water, food and sediment. These three traditional environmental pathways of radionuclide exposure and transfer to marine fishes have also been frequently investigated under laboratory conditions, but the pathway of maternal transfer to their eggs and later developmental stages has received the least attention. Exposures of the embryonic phase, the life-stage that is typically more vulnerable to toxicological impacts of trace metals

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and radionuclides (Blaylock and Griffith, 1971; Weis and Weis, 1991), has received more attention in teleost fish (Mothersill et al., 2010), compared to their cartilaginous relatives with their characteristically reduced reproductive potential (Camhi et al., 1998). In the bull ray, *Pteromylaeus bovinus*, Hg transfer from mother to embryos has been found under field exposure conditions (Horvat et al., 2014) but to our knowledge no previous experimental studies have been undertaken on maternal transfer and distributions of radionuclides in the eggs of cartilaginous fish. With respect to other transfer pathways there is growing evidence that Chondrichthyans (car tilagenous fish) may be more susceptible than Teleosts (bony fish) to accumulation of trace metal contaminants from food and seawater (De Boeck et al., 2010; Jeffree et al., 2006a, 2010; Leah et al., 1991a,b; Mathews et al., 2008).

We have chosen the spotted dogfish, *Scyliorhinus canicula*, (Order Carcharhiniformes, Family Scyliorhinidae), to test the hypothesis that anthropogenic radionuclides can be maternally transferred to progeny. The four radionuclides used in this study, Zinc-65, Cobalt-60, Americium-241 and Cesium-134, are typically associated with effluents entering the marine environment from coastal nuclear facilities. *S. canicula* belongs to the Family Scyliorhinidae, which includes over 100 species that occur worldwide in temperate and tropical seas (Compagno, 1999). It has a low natural capacity to increase (r), which is typical of cartilaginous fish, producing a relatively small number of large, heavily yolked eggs. The eggs of *S. canicula* have long periods of embryonic development, which are characteristic of dogfish species (Castro et al., 1998), and contrast with eggs of pelagic marine teleosts which typically show reduced duration of development by at least an order of magnitude (Paully and Pullin, 1988). Such a mode of reproduction is typical of oviparous Chondrichthyans which comprise about 40% of the sharks and all the skates (Wourms and Demski, 1993).

In this radiotracer uptake experiment, we determined whether there was maternal transfer of four anthropogenic radionuclides (^{65}Zn, ^{60}Co, ^{241}Am and ^{134}Cs) to eggs of *S. canicula*, during 61 days of the maternal exposure to radio-labelled food, and their distributions among three egg components (case, yolk and jelly).

2. Materials and methods

2.1. Radioanalysis of tissues and water samples

A high-resolution gamma spectrometry system was used for all radiometric analyses of mussel, dogfish, egg and seawater samples, consisting of four coaxial Germanium (N- or P-type) detectors (EGNC 33-195-R, Intertechnique; 40–70% efficiency). The detectors were connected to a multi-channel analyser and a personal computer employing spectral analysis software (Interwinner 6, Intertechnique). The radioactivity levels of samples were determined by comparison with known standards of appropriate geometry, including a phantom dogfish egg, and were corrected for background and physical decay of a given radioisotope. Counting times were adapted to obtain count rates with relative propagated errors of less than 5%, viz. typically 10–30 min for whole egg radioanalyses and 1–12 h for seawater. The radioanalytical techniques used in this study were identical to those used in previous experiments on shark juveniles, eggs and their components (Jeffree et al., 2006a,b; 2007, 2008).

2.2. Acclimation and experimental feeding of female dogfish with radiolabelled food

Eight female dogfish provided by the Roscoff Biology Station, Britanny, France, were chosen within a total length range of 70–80 cms to ensure their sexual maturity (Ivory et al., 2004; Rodriguez-Cabello et al., 1998). These individuals were transported overnight in seawater 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 maintained with no mortality in running, carbon-filtered Mediterranean seawater in a 3000 L aquarium for 30 days at 16° ± 1 °C. Acclimation conditions were virtually identical to those used during the subsequent long-term exposure experiment, and with a similar feeding regime. 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 IAEA.

It is typical of oviparous Chondrichthyans that a large proportion of the females are not laying eggs in any one year (Hamlett and Koob, 1999). Therefore, since not all sexually mature females of *S. canicula* would be expected to contain egg capsules (Rodriguez-Cabello et al., 1998), they were scanned by ultrasound to determine those individuals that contained developing eggs within their ovaries, and a subset of four with developing eggs was chosen for the experimental exposures to radiolabelled food.

Under natural conditions, dogfish are known to feed opportunistically on various macrobenthic fauna, bivalve and gastropod molluscs being the dominant prey items and increasing in dietary frequency with increasing size of dogfish (Lyle, 1983). For this experiment, we chose *Mytilus edulis* as the experimental food both because of the importance of bivalves in the dogfish diet, and the well-established ability of *M. edulis* to accumulate a range of trace elements and radionuclides in its soft tissues (Farrington et al., 1983; Fisher et al., 1996).

Assimilation efficiencies (AE) for the uptake from food (from a single feeding) of the four radionuclides used in the present investigations have previously been determined for *S. canicula* by Mathews et al. (2008). For dogfish fed with radiolabelled juvenile Mediterranean sea bream (*Sparus auratus*), the following percent AE and average loss rate constants in % loss. day^{-1}, in brackets, were reported as follows: Am, 6.0 (0.3); Co, 11.0 (nd); Cs, 73.0 (0.8); and Zn, 20.0 (1.7), i.e. AE's varied by up to more than a factor of ten.

2.2.1. Radio-labelling of mussels

Approximately 1000 *M. edulis* (5–6 cm length) were exposed to seawater labelled with high specific activity ^{241}Am, ^{60}Co, ^{134}Cs and ^{65}Zn for one month in a 300 L aquarium at 16°±1 °C, pH 8.00. These radionuclides had been purchased from Amersham, UK (^{241}Am, ^{60}Co, ^{134}Cs) and Isotope Product Laboratory, USA (^{65}Zn), and were added to the experimental aquarium every second day for 4 weeks in order to maintain constant activities. No change in pH was detectable after the addition of these isotope solutions. To ensure their more realistic and thorough radio-labelling, mussels were also fed every second day with spikes of 10^4 cells/ml of unlabelled *Isochrisis galbana* that the mussels effectively filtered from the radiolabelled water within 30 min.

The activity concentrations of each radioisotope were checked daily both before and after each seawater renewal, so as to determine their mean time-integrated values, which were as follows: ^{60}Co; 0.5 kBq/L, ^{65}Zn; 0.6 kBq/L, ^{134}Cs; 0.6 kBq/L, and ^{241}Am; 0.1 kBq/L. Their mean accumulated activities per individual mussel after one month of exposure were: 150 kBq of ^{60}Co, 180 kBq of ^{65}Zn, 180 kBq of ^{134}Cs, and 30 kBq of ^{241}Am.

At the end of this uptake phase, the soft tissues of all mussels were dissected, homogenised and then divided into four aliquots that were then weighed and frozen. Just prior to beginning the feeding experiment, the respective radioisotopic activity concentrations in mussel tissues were quantified. The average mussel tissue (wet weight)-to-water concentration factors attained were: ^{65}Zn, 333; ^{60}Co, 35; ^{241}Am, 61; and ^{134}Cs, 2. At each feeding time portions of mussel tissue (c. 20 gms) were taken from each of the