# The role of salinity in the trophic transfer of $^{137}$Cs in euryhaline fish

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## ARTICLE INFO

## ABSTRACT

In order to better understand the influence of changing salinity conditions on the trophic transfer of $^{137}$Cs in marine fish that live in dynamic coastal environments, its depuration kinetics was investigated in controlled aquaria. The juvenile turbot *Scophthalmus maximus was* acclimated to three distinct salinity conditions (10, 25 and 38) and then single-fed with compounded pellets that were radiolabelled with $^{137}$Cs. At the end of a 21-d depuration period, assimilation efficiencies (i.e. AEs = proportion of $^{137}$Cs ingested that is actually assimilated by turbots) were determined from observational data acquired over the three weeks. Our results showed that AEs of $^{137}$Cs in the turbots acclimated to the highest salinity condition were significantly lower than for the other conditions ($p < 0.05$). Osmoregulation likely explains the decreasing AE observed at the highest salinity condition. Indeed, observations indicate that fish depurate ingested $^{137}$Cs at a higher rate when they increase ion excretion, needed to counterbalance the elevated salinity. Such data confirm that ambient salinity plays an important role in trophic transfer of $^{137}$Cs in some fish species. Implications for such findings extend to seafood safety and climate change impact studies, where the salinity of coastal waters may shift in future years in response to changing weather patterns.

## 1. Introduction

Radioisotopes of caesium (i.e., $^{134}$Cs, $^{137}$Cs) can be discharged into the marine environment from assorted human activities. Recently, the Fukushima Daiichi nuclear power plant accident in Japan led to an unprecedented release of radiocaesium into the environment including the ocean directly adjacent to the accident site (Buesseler et al., 2011, 2012; Estournel et al., 2012). As a result of this catastrophe, there has been a surge in studies on integrated marine ecophysiology and radioecology. Indeed, due to the persistence of $^{137}$Cs ($t_{1/2} = 30.17 \pm 0.03$ yr) in aquatic environments (Pröhl et al., 2006), radiocaesium can be readily bioaccumulated by aquatic organisms at the bottom of the aquatic food chain (e.g. phytoplankton and invertebrates; Heldal et al., 2001; Topcuoğlu, 2001) and can then be transferred to higher trophic levels such as marine fish (Pentreath, 1977). Furthermore, the potential for biomagnification has been highlighted with radiocaesium (e.g. Zhao et al., 2001), which can lead concentrations up 1000 Bq kg$^{-1}$ (338 Bq kg$^{-1}$ of $^{134}$Cs and 699 Bq kg$^{-1}$ of $^{137}$Cs) in fish (Chen, 2013; Iwata et al., 2013; Wada et al., 2016). Such findings confirm (1) the importance of marine organisms as vectors for bioaccumulation and biomagnification of many contaminants, including radionuclides like caesium (Tateda et al., 2013), and (2) a potential health risk to humans from consuming contaminated

seafood (Chen, 2013). A more robust understanding of these processes for radiocaesium in aquatic organisms within higher trophic levels such as fish is thus warranted as there still are fundamental research questions that need to be systematically addressed using both field- and laboratory-based observations. For example, to what effect do key environmental variables such as salinity, pH and temperature impact on the trophic transfer of radiocaesium in commercially-important fish species.

Salinity is a master variable for coastal and marine ecosystems and can play an important role in the chemical speciation of many elements and can also affect in the physiology of fish (Ni et al., 2005). Indeed, in seawater fish can adapt to a wide range in salinity conditions (Lorin-Nebel et al., 2006). Marine fish can compensate high salt content by actively excreting ions in order to maintain their osmolality (*viz.* osmoregulation processes; Lorin-Nebel et al., 2006). Thus, salinity strongly impacts the physiology of fish and hence their ecology and distribution (Wootton, 1991). Furthermore, the salinity of seawater, especially in coastal areas, is also affected by global changes caused in part by anthropogenic activities (IPCC, 2014). It is therefore important to consider this important environmental variable to better understand and predict the dynamic behavior of radionuclides in aquatic environments (Barescut et al., 2009). Nevertheless, our knowledge about the influence of salinity on bioaccumulation of radionuclides in aquatic

organisms is still incomplete. As already shown for invertebrates, such ion exchange reactions influence bioaccumulation kinetics of $^{137}$Cs in fish (e.g. Ke et al., 2000). Previous studies have also demonstrated that lower salinity ambient water facilitates the uptake of dissolved $^{137}$Cs in diverse marine organisms, including some fish species (Pan and Wang, 2016; Zhao et al., 2001). Surprisingly, to date, the direct influence of salinity on the trophic transfer of radiocaesium has not been quantitatively investigated in fish.

The importance of the dietary pathway in the bioaccumulation of radiocaesium by fish has been highlighted in the past (e.g. Zhao et al., 2001). Different modeling approaches have shown that food can play an important role in the radiocaesium bioaccumulation of the mangrove snapper *Lutjanus argentimaculatus* and the turbot *Scophthalmus maximus,* especially at elevated radiocaesium concentrations in prey (Mathews and Fisher, 2009; Zhao et al., 2001). Such findings were confirmed by Pan and Wang (2016) who found that the food pathway was the dominant vector in the $^{137}$Cs accumulation in the omnivorous (the rabbitfish *Siganus fuscescens*) and carnivorous (the marbled rockfish *Sebastiscus marmoratus* and the grunt *Jarbua terapon*) fish. Mechanistic understanding of trophic transfer of radiocaesium is possible through measurements of several physiological parameters described in kinetic model, including the assimilation efficiency (AE) from the ingested radiolabeled food (Wang and Fisher, 1999).

In this context, the present study investigates the possible effects of changing salinity on the assimilation efficiency (AE) of radiocaesium in a euryhaline fish, the turbot *S. maximus*. Controlled radiotracer techniques were used to determine $^{137}$Cs depuration parameters in laboratory aquaria using juvenile turbots previously acclimated at three distinct salinities (10, 25 and 38) after a single feeding with $^{137}$Cs-radiolabelled compounded pellets.

## 2. Materials and methods

### 2.1. Acclimation of fish and experimental conditions

In 2016, juvenile turbots *S. maximus* were purchased from a fish farm (France Turbot, France). Fish were acclimated to laboratory conditions for at least 6 months (constantly aerated, open-circuit 700-L plastic tank; the water exchange rate was set at $350 \, L \, h^{-1}$; salinity = 38; temperature = 20 ± 1 °C; pH = 8.0 ± 0.1; and the light/dark cycle 12 h/12 h). During the acclimation period, the fish were fed a daily ration of 1.5% of their biomass with 1.1-mm pellets (proteins: 55% and lipids: 12%; Le Gouessant, France). All experimental fish were individually identified by slits cut into their pelvic and caudal fins. Then, three weeks before the experiment, the fish were randomly placed in three 20-L aquaria (n = 8) and acclimated to the target salinities (10, 25, 38). During the first days of acclimation, salinities were gradually decreased and then stabilized for 10 days before the experiment.

Salinity was measured twice per day in each aquarium using a handheld conductivity/salinity meter, which was calibrated regularly using conductivity standards encompassing the full range of the three selected experimental waters. For the entire experiment, the measured mean salinity values were 10.1 ± 0.1, 24.9 ± 0.2 and 37.8 ± 0.1, corresponding to conductivity values of 17.0 ± 0.1, 39.3 ± 0.2 and 57.0 ± 0.1 mS cm$^{-1}$, respectively. Furthermore, pH and temperature were monitored in each aquarium every 15 min using a continuous measurement system (IKS ComputerSysteme, www.iks-aqua.com). Variations in pH between the three salinity conditions did not exceed 0.1 during the course of the experiments.

### 2.2. Experimental procedures

#### 2.2.1. Radiolabelling of pellets

Radiolabelling of the compounded pellets was performed as already described in the literature (Pouil et al., 2015) using $^{137}$Cs. Briefly, 15 g

of pellets were dipped for 1 h in 20 mL of filtered seawater spiked with $^{137}$Cs. Then, $^{137}$Cs radiolabelled pellets were dried for 48 h at 50 °C to prevent nutritional loss and mold growth. Before the single-feeding, the activity level of $^{137}$Cs in the pellets was 4947 ± 421 Bq g$^{-1}$. Potential discharge of the radioisotopes into seawater, which may then lead to a double exposure of the fish (food and water) was tested and confirmed not to be an issue as long as the fish consumed the food pellets rapidly (within ~5 min). This was confirmed to be the case after each feeding.

#### 2.2.2. Exposure of turbot via radiolabelled pellets

Juvenile turbots were randomly selected for each experimental salinity (salinity 10: 44 ± 3 g; salinity 25: 40 ± 4 g and salinity 38: 43 ± 3 g, n = 8 for each experiment). Slits cut into the fins were used to facilitate individual recognition. Each experiment consisted of a single feeding of fish with radiolabelled pellets. After the labelled feeding, an additional turbot was placed in each aquarium to assess any possible radiotracer recycling from seawater due to leaching from the radiolabelled food and later, from fish depuration (e.g. Jacob et al., 2017; Pouil et al., 2015). Two hours after the feeding, individual fish were whole-body γ-counted alive and then replaced into the same aquarium to follow subsequent metal depuration. All the fish (including control individuals of each condition) were regularly γ-counted to follow the $^{137}$Cs depuration kinetics over the 21-d experiment.

After the depuration period, 4 individuals per condition were dissected in 7 compartments: (1) the digestive tract, (2) the gall bladder, (3) the head (including gills), (4) the kidney, (5) the liver, (6) the dorsal and ventral muscles (without dorsal skin) and (7) the remaining tissues (including dorsal skin, skeleton, fins, heart and muscle residues) and were separated, weighed (wet wt) and radio-analysed to determine the $^{137}$Cs body distribution and concentration.

### 2.3. Radioanalysis

Radioanalyses were carried out using a γ-spectrometer system composed of 5 Germanium - N or P type - detectors (EGNC 33–195-R, Canberra® and Eurysis®) connected to a multi-channel analyser and a computer equipped with a spectra analysis software (Interwinner 6, Intertechnique®). The radioactivity in living organisms and samples was determined by comparison with standards of known activity and appropriate geometry (Cresswell et al., 2017) and corrected for background and physical radioactive decay (Rodriguez y Baena et al., 2006).

### 2.4. Data treatment and statistical analysis

Depuration kinetics of $^{137}$Cs were best-fitted using a two-component exponential model (see Warnau et al., 1996) adjusted by non-linear regression routines and iterative adjustment (Statistica® 7) and AEs were determined according to methods already described (Warnau et al., 1996; Pouil et al., 2017).

Individual kinetic parameters (AE, $k_{es}$ and $k_{el}$) of turbots maintained in three salinity conditions were obtained using the best-fitting model at the global scale to the data of each individual (e.g. Belivermiş et al., 2015; Pouil et al., 2016). A biological half-life can be calculated ($T_{b1/2}$) from the corresponding depuration rate constant according to the relation $T_{b1/2} = \ln 2/k_e$. Statistical differences between these parameters were then tested using Kruskal-Wallis and Siegel and Castellan nonparametric tests (Zar, 1996). Distribution of $^{137}$Cs in the 7-body compartments of turbots under the different salinity conditions was compared using the same statistical analysis. The level of significance for statistical analyses was always set at α = 0.05. All the statistical analyses were performed using R software 3.0.1 (R Development Core Team, 2014).

## 3. Results

In order to evaluate how salinity affects $^{137}$Cs trophic transfer in a