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Simon Pouil[a,b], François Oberhänsli[a], Peter W. Swarzenski[a], Paco Bustamante[b] and Marc Metian[a]

[a] International Atomic Energy Agency - Environment Laboratories, 4a Quai Antoine Ier, MC-98000, Principality of Monaco, Monaco

[b] Littoral Environnement et Sociétés (LIENSs), UMR 7266 - CNRS-Université de La Rochelle, 2 rue Olympe de Gouges, F-17000 La Rochelle, France

* Corresponding author: Simon Pouil

ORCID: 0000-0003-1531-0362

Radioecology Laboratory

IAEA Environment Laboratories

4a Quai Antoine 1er

MC-98000, Monaco, Principality of Monaco

Telephone: +33493311604

E-mail: simon.pouil1@gmail.com

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* were 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 and AEs 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., ^{134,137}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 ($t_{\frac{1}{2}}$ = 30.17 ± 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 behaviour of radionuclides in aquatic environments (Barescut et al., 2008). 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 fish (the marbled rockfish *Sebastiscus marmoratus* and the grunt *Jarbua terapon*). 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 hand-held 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 minutes 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 ℃ 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 ± 3g; salinity 25: 40 ± 4g and salinity 38: 43 ± 3g, 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 muscles ventral (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 $T_{b1/2} = \ln2/k_e$. Statistical

differences between these parameters were then tested using Kruskal-Wallis and Siegel and Castellan non-parametric 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 euryhaline fish, depuration kinetics of ^{137}Cs were followed in single-fed juvenile turbots *S. maximus* acclimated to three salinities conditions (10, 25, 38) for 21 days. During the whole experimental period, only a limited growth of the individuals was recorded and no mortality occurred.

Whole-body depuration kinetics of ^{137}Cs in juvenile turbots were best fitted by a two-phase model (Fig. 1; R^2: 0.81 - 0.97). A small proportion (28 - 43 %, Fig. 1 and Table 1) of the ingested ^{137}Cs was associated with the short-term component. This component was characterized by a rapid loss ($T_{b1/2s} < 2$ d, Table 1). Estimated ^{137}Cs AEs in turbot ranged from 56 % to 72 % (Fig. 1 and Table 1). Assimilated ^{137}Cs was slowly eliminated, with long-term biological half-life ($T_{b1/2l}$) values ranging from 36 to 83 d (Fig. 1 and Table 1). Statistical analyses carried out on individual estimated AEs and k_{el} revealed that salinity affected the trophic transfer of ^{137}Cs with a significantly lower AE and significantly k_{el} at the highest salinity (p<0.05; Fig. 1). Linear regressions were established for AEs and $T_{b1/2l}$ and different salinities (Fig. 2).

Post-feeding body distribution of ^{137}Cs in juvenile turbots at the end of the 21-d depuration period is not significantly different between the three salinity conditions (p>0.05, Fig. 3). Distribution among the body compartments systematically ranked according to the following decreasing order (Fig. 3): Remaining tissues (i.e. remaining skin, skeleton, fins, heart and

muscle residues; 39-40%) > dorsal and ventral muscles (36-38%) >> head (20-21%) >> digestive tract (1-2 %) > liver, kidney and gall bladder (<0.5%).

4. Discussion

Over geologic time scales, fish have evolved to thrive in either fresh- or salt-water environments, or both, and can be categorized simply by their tolerance to salinity (Gamperl et al., 2017). Fish that can tolerate only a very narrow range of salinity are considered stenohaline (e.g., Scombridae). Conversely, fish that can tolerate a wide salinity range at some phase in their life-cycle are considered euryhaline (e.g., Mugilidae, turbot *S. maximus*). The blood composition of vertebrates – including all fish – is thought to be traceable back to the elemental composition of the primordial ocean, when seawater salinity was considerably lower than it is today (Epstein, 1999). Thus, if one would dilute the salinity of ocean water today by about 25%, it would have almost the same salt content as fish blood and would contain similar proportions of Na, K, Ca and Cl. To be able to survive in seawater or a range in salinities and still maintain their blood salt levels, fish have developed specific mechanisms to compensate water lost through osmosis and to remove salts absorbed from the increasingly saline oceans.

Salinity is one of the most important characteristics that define coastal and marine ecosystems and has been shown to exert a strong influence on fish physiology (Marshall and Grosell, 2006) and well-being. Indeed, fish accommodate large variations in salinity and can still maintain their blood osmolality (Marshall and Grosell, 2006). To be able to do this, their main osmoregulatory adaptations include (1) regulating their water intake (digestive tract) (2) controlling the production of urine (excretory system), and (3) an active excretion or retention of ions (mainly gills; e.g. Lorin-Nebel et al., 2006). With such physiological mechanisms in place, past studies have shown that salinity can also influence the bioaccumulation and toxicity of select contaminants such as trace elements and radionuclides. For example, the uptake

kinetics of the following dissolved radionuclides, ^{109}Cd, ^{137}Cs, ^{75}Se and ^{65}Zn are sensitive to salinity fluctuations (Ni et al., 2005; Prihatiningsih et al., 2016a; Zhao et al., 2001). Nevertheless, key information on the kinetics of bioaccumulation is still needed regarding the influence of salinity on the the trophic transfer of diverse contaminants, including radiocaesium. In juvenile turbot, the observed ^{137}Cs assimilation efficiencies (AEs) of ^{134}Cs ranged from 56 to 72% and are similar to values measured in a previous study using the same species fed only natural prey (63 ± 2%; Mathews et al., 2008). This similarity in AE values indicates that, even if compounded pellets may lack the refractory components (e.g., exoskeleton or insoluble granules) found in natural prey organisms, the behavior of ^{137}Cs in turbot fed with artificial diet was probably representative of the behavior of elements in natural systems. The AEs of radiocaesium observed in turbot compared also well with previous measurements in other marine and euryhaline fishes. Thus, the averaged AEs of Cs for two species of temperate fish (European seabass *Dicentrarchus labrax* and gilthead seabream *Sparus aurata*) fed on brine shrimp and fish were 74-84% (Mathews and Fisher, 2008; Mathews et al., 2008). Other studies on tropical fish also showed a high Cs AE, including mangrove snapper *L. argenticumaculatus* (78-95%; Zhao et al., 2001), marbled rockfish *S. marmoratus* (70-79%), rabbitfish *S. fuscescens* (51-55%) and grunt *T. jarbua* (73-75%; Pan and Wang, 2016). Nevertheless, Prihatiningsih et al. (2016b) have shown lower Cs AEs in a herbivorous fish, the milkfish *Chanos chanos* (35-37% in average). The generally high AEs observed for radiocaesium in fish (Pouil et al., 2018) raised the importance of trophic transfer in the global bioaccumulation of this radionuclide for this taxon (Zhao et al., 2001).

To the best of our knowledge, there exist no study to date that have investigated the influence of salinity on the ^{137}Cs dietary assimilation in marine fish. Our results confirm that ^{137}Cs AEs in turbot was affected systematically by salinity. Indeed, a significant decrease (-22% compared to the AE measured at the lower salinity) of ^{137}Cs AEs was observed at the highest salinity (38).

In our experimental conditions, linear regressions were established for AEs and $T_{b1/2l}$ and different salinities (Fig. 2). Nevertheless, this relationship should be confirmed in future investigations where the salinity gradient would be wider although the range used in this study was selected both to be realistic with the environment conditions encountered by the species in the wild and avoid mortality. The decrease in ^{137}Cs when the surrounding salinity increases can be related to the osmoregulation processes in marine fish that control the diffusive ion invasion and excreted the excess ions to maintain their osmolarity (Lorin-Nebel et al., 2006). Based on these physiological mechanisms, the lower observed ^{137}Cs AE in turbots maintained in the highest salinity condition can be explained by (1) a limited entrance of dietary ^{137}Cs from the intestinal tract, and/or (2) a higher excretion rate of ^{137}Cs in particular from the gills. The intestine plays an important role in osmoregulation in fish (Grosell, 2006, 2007; Whittamore, 2012) and the digestive processes can be affected by salinity (Fang and Chiou, 1989; Ferraris et al., 1986; Lee-Shing and Shu-Fen, 1989; Tsuzuki et al., 2007). Nevertheless, in this study, our results indicated that, for juvenile turbots, a potential effect of salinity on the digestion process of ^{137}Cs-radiolabelled pellets is not the only explanation on the salinity-dependence of the radiocesium assimilation. Indeed, the comparison of efflux rate constants (k_{el}) reveals that the excretion of ingested ^{137}Cs; occurring mainly during the second phase of depuration (Wang and Fisher, 1999; Pouil et al., 2018), is higher excretion rate of ^{137}Cs in the fish exposed to this experimental condition. Since artificial food as used to fed juvenile turbot, biotransformation cannot occur, thus, radiocaesium in the compounded pellets is most likely found in monovalent ionic form (^{137}Cs$^+$). When fish are exposed to high salinity conditions, the majority of the monovalent ions gained from the gastrointestinal tract are eliminated across the gill surface (Grosell, 2007). The gills are also responsible for eliminating unnecessary Cs$^+$, which is biochemical analog of K$^+$ (Relman, 1956), from the body fluid, presumably through the K$^+$ excretion pathway in the gills (Furukawa et al., 2012). The influence of salinity on the excretion

of radiocaesium is also a reason to explain the high ability of freshwater fish to bioaccumulate caesium (Hewett and Jefferies, 1976; Rowan and Rasmussen, 1994; Smith et al., 2002) resulting in its higher biomagnification potential in freshwater ecosystems (Baudin et al., 2000; Garnier-Laplace et al., 2000).

Measurements of the distribution of ^{137}Cs in tissues are important to understand the site-specificity of ^{137}Cs binding and provide additional mechanistic information potentially helping in the interpretation of results from whole-body kinetic measurements. Our results show that the distribution of ^{137}Cs between the tissues of juvenile turbots did not vary significantly with salinity. At the end of the 21-d depuration period, almost all (96-98%) the ^{137}Cs was found in the muscles, the head, and in the remaining tissues (Fig. 3). Thus, edible parts of the fish (i.e. the muscles and the head) represented ~60% of the ingested ^{137}Cs, and thus the component potentially available for human consumers. Mathews et al. (2008) was able to demonstrate by the measurements of weight-normalized distribution of ^{134}Cs the growing role of muscles in the storage of this radionuclide in European seabass during the 21-d depuration period following a ^{134}Cs-radiolabeled feeding. Similar findings were reported in a previous study on mangrove snappers (Zhao et al., 2001) where ^{137}Cs was mainly found in the muscle tissues after a waterborne Thus, muscle tissues may function as a sink for radiocesium in fish.

In summary, this study provides new information on the role of salinity on ^{137}Cs bioaccumulation which can have important risk-assessment implications for humans via seafood safety. The biological half-life of ^{137}Cs, as obtained in the present study in living juvenile turbots, exceeded 80 days and indicates that risks for humans can persist over much longer time scales than the radiocaesium release event. Furthermore, our results highlight the importance of salinity as a critical variable to better understand the dynamics of radiocaesium in aquatic environments and in biota after a contamination event.

5. Conclusion

The influence of ambient salinity values on the bioaccumulation of dissolved ^{137}Cs in fish has been demonstrated in previous experimental works. In this study, for the first time, experimental radiotracer results indicate that the trophic transfer of ^{137}Cs in fish is also influenced by ambient salinity. Thus, this work provides new information useful to understand and model the transfer of radiocaesium throughout foodwebs and aquatic ecosystems.

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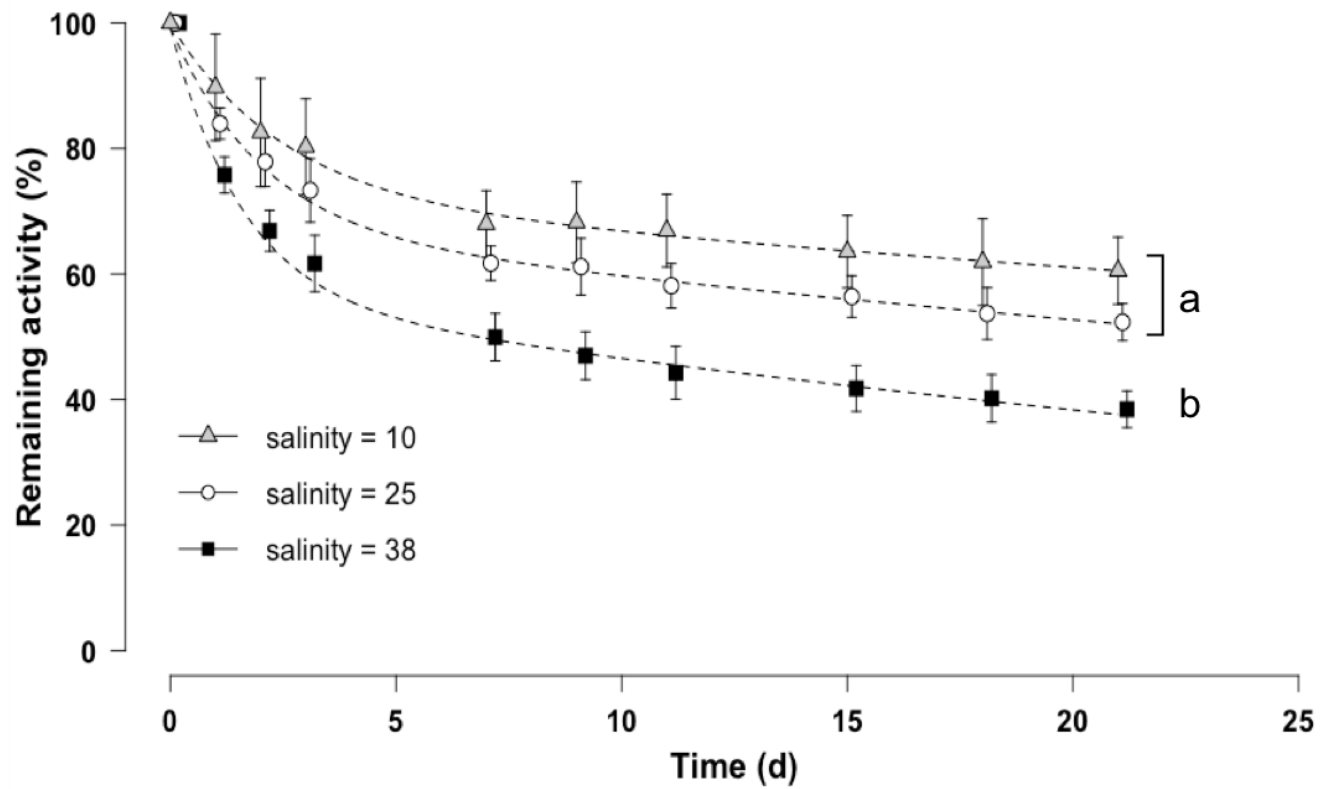
Figure 1. Influence of salinity on whole-body depuration of ^{137}Cs in juvenile turbots (n = 7; percent remaining activities, mean ± SD). Parameters and statistics of depuration kinetics are given in Table 1. Letters indicated significant differences (p < 0.05).
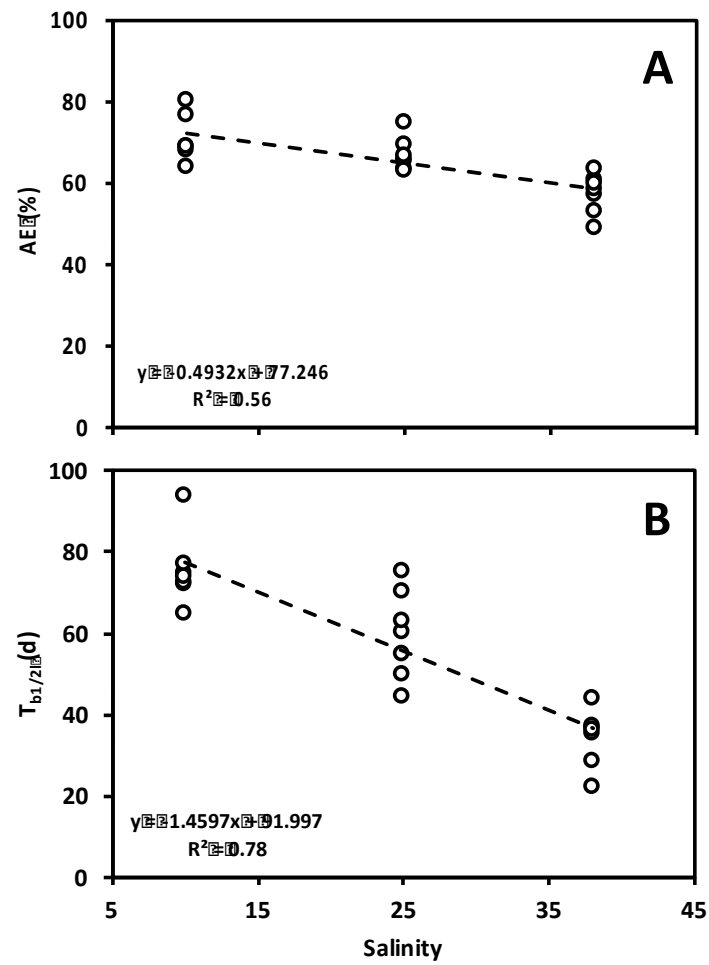
Figure 2. Individual assimilation efficiencies, AEs (A) and long-term biological half-time, $T_{b1/2l}$ (B) of ^{137}Cs of each juvenile turbot acclimated to three salinity conditions (10, 25 and 38). The best fitting model obtained for the entire set of turbots (see Fig. 1 and Table 1) was applied to individuals.
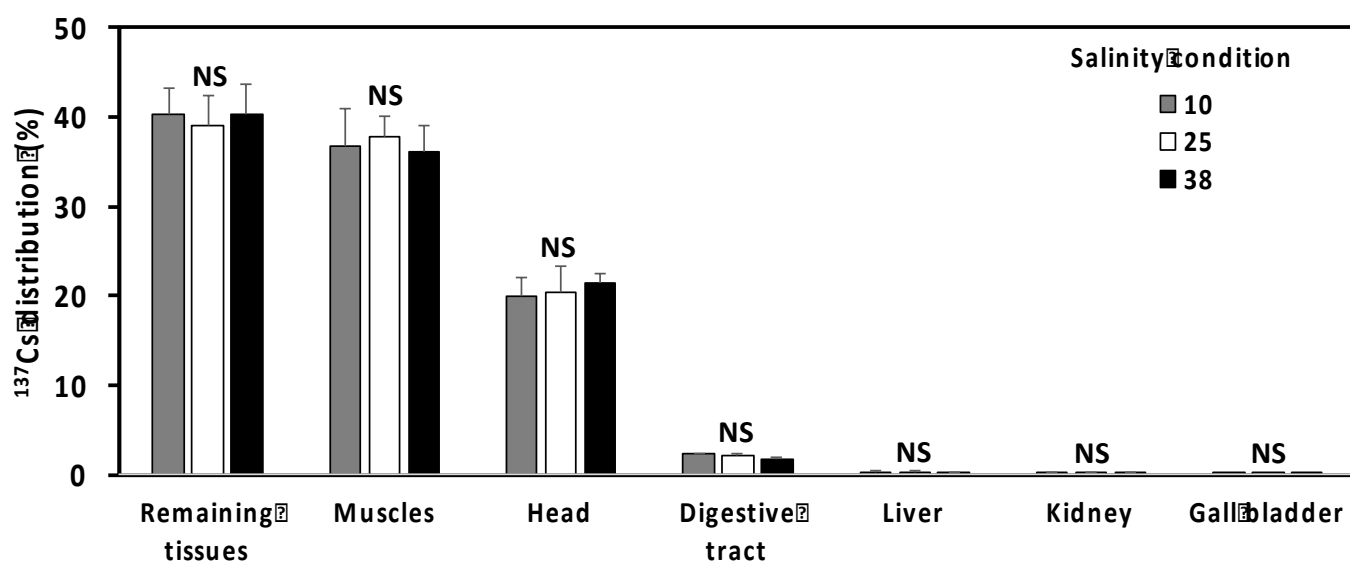
Figure 3. Distribution (%) of ^{137}Cs in the tissues of juvenile turbots acclimated to three salinity conditions (10, 25 and 38), single-fed with ^{137}Cs-radiolabelled pellets and then maintained for 21d in non-contaminated seawater at the given salinity. Values are means ± SD (n=4).

Table 1. Estimated depuration kinetic parameters of ^{137}Cs in juvenile turbots acclimated to three salinity conditions (10, 25 and 38; n = 8 per treatment) and single-fed with ^{137}Cs-radiolabelled pellets and then maintained for 21d in unspiked seawater at the given salinity. Depuration parameters: A_0 (=AE): activity (%) lost according to the short- and long-term exponential component, respectively; k_e: depuration rate constant (d^{-1}); $T_{b1/2}$: biological half-life (d); ASE: asymptotic standard error; R^2: determination coefficient. Probability of the model adjustment: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Salinity	Short-term			Long-term			R^2
	$A_{0s} \pm$ ASE	$k_{es} \pm$ ASE	$T_{b1/2s} \pm$ ASE*	A_{0l} (=AE) $\pm$ ASE	$k_{el} \pm$ ASE	$T_{b1/2l} \pm$ ASE*	
10	27.7 ± 5.0***	0.40 ± 0.13**	1.7 ± 0.6	72.1 ± 4.9***	0.008 ± 0.004*	83.1 ± 43.3	0.81
25	32.2 ± 2.5***	0.50 ± 0.08***	1.4 ± 0.2	67.1 ± 2.4***	0.012 ± 0.002***	57.4 ± 11.6	0.95
38	42.9 ± 2.5***	0.63 ± 0.08***	1.1 ± 0.1	56.4 ± 2.4***	0.019 ± 0.003***	36.0 ± 5.7	0.97

* Calculated following the relation $T_{b1/2} = \ln2/k_e$