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Trophic transfer of trace elements in a euryhaline fish, the turbot *Scophthalmus maximus*: Contrasting effects of salinity on two essential elements

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ABSTRACT

Trace elements can be accumulated from coastal environment by aquatic organisms from their food and be transferred throughout the food webs. Studying the effects of salinity on the trophic transfer of trace elements in a euryhaline fish, able to deal with large variations in salinity, is therefore key to understand their dynamics in aquatic environments. In this context, we investigated the potential influence of salinity on the trophic transfer of two essential elements (Mn and Zn) in the euryhaline fish, the turbot *Scophthalmus maximus* using radiotracer techniques. After acclimation to three salinities (10, 25 and 38), turbots were fed with radiolabelled pellets (^{54}Mn and ^{65}Zn). Kinetic parameters of depuration were determined after a 21-d period and trophic transfer factors were calculated. Trophic transfer of Mn at the highest salinity was significantly lower than for the other conditions whereas salinity did not significantly influenced Zn trophic transfer. Differences in the processes involved in the regulation (homeostasis) of the two tested trace elements may explain the contrasting influence of seawater salinity for Mn and Zn.

1. Introduction

Environmental conditions can affect trace element bioaccumulation in aquatic organisms such as fish (Luoma and Rainbow, 2005; Phillips and Rainbow, 1993). Among environmental factors, water salinity is known to influence trace element accumulation by causing (1) changes in trace element speciation and therefore on their bioavailability, and (2) modifications of fish physiology, especially regarding the osmoregulation processes (Ni et al., 2005). Salinity affects biokinetic parameters, such as the uptake rate (k_u) or the efflux rate (k_e) of some trace elements such as Cd, Cs, Se, and Zn in fish (Ni et al., 2005; Zhao et al., 2001). Nevertheless, most of the studies that looked at the potential effects of salinity on metal accumulation focused on waterborne trace elements rather than dietary ones (e.g., Zhang and Wang, 2007; Zhao et al., 2001; Webb and Wood, 2000). Thus, only limited information is available about the influence of salinity on trace element trophic transfer while diet is recognized as the major pathway for most of the trace elements bioaccumulated by fish (e.g., Pouil et al., 2018a; Mathews and Fisher, 2009; Xu and Wang, 2002).

The turbot, *Scophthalmus maximus* (Scophthalmidae), is a demersal fish widely distributed in Western European coastal waters. This species inhabits in a wide range of water salinities with its breeding usually occurring in low-salinity waters (Kuhlmann and Quantz, 1980). The euryhaline nature of this species has been confirmed by Waller (1992) who reported that osmoregulatory disturbances only occurred at a salinity below 6. This euryhaline species, through its ability to move from brackish water to seawater environments and its trophic ecology, is a potential significant vector of trophic transfer of trace elements from coastal to marine ecosystems. Furthermore, turbot became a few years ago a model species for trace element studies on fish. The turbot was recently considered as a biological model for numerous ecotoxicological studies related to the bioaccumulation of dietary trace elements (e.g., Pouil et al., 2015, 2016, 2017a, 2018b). Studying this species is therefore relevant to assess the effect of salinity on the trophic transfer of trace elements in fish.

The present study investigated the possible effects of a wide range of salinities on the assimilation efficiency (AE) of two essential trace elements (Mn and Zn) in a euryhaline fish, the turbot *S. maximus*. Radiotracer techniques were used to determine depuration parameters and body distribution of the selected dietary trace elements in *S.*

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maximus exposed to three salinities (10, 25 and 38). Kinetic parameters data were then used to model the influence of salinity on the potential of biomagnification (Trophic Transfer Factor, TTF) of Mn and Zn in the field.

2. Materials and methods

2.1. Origin and acclimation of fish

Juvenile turbots *S. maximus* were purchased from a fish farm (France Turbot, www.france-turbot.com) and shipped to the International Atomic Energy Agency premises in the Principality of Monaco. The fish were kept in a 700-L aquarium (open circuit, water renewal: 350 L h^{-1}; 0.45 μm filtered seawater; salinity: 38; light/dark: 12 h/12 h). Then, three weeks before the experiment, 24 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 to the targeted values for 10 days before starting experiment. During the acclimation period, the fish were fed a daily ration of 1.5% of their biomass with 1.1-mm commercial pellets (proteins: 55% and lipids: 12%; Le Gouessant, www.legouessant.com).

Salinity was measured twice a day in each aquarium using a hand-held conductivity/salinity meter, which was calibrated using conductivity standards encompassing the range of the three selected experimental conditions. Furthermore, in each aquarium, pH and temperature were monitored every 15 min using a continuous measurement system (IKS ComputerSysteme, www.iks-aqua.com). Values of seawater parameters are summarized in Table 1.

2.2. Experimental procedures

2.2.1. Radiolabelling of pellets

Fifteen grams of 1.1-mm pellets were radiolabelled for 1 h in 20 mL of seawater spiked with 1.5 kBq mL^{-1} of ^{54}Mn and ^{65}Zn. Pellets were then dried for 48 h at 50 °C and kept in a dry environment in order to prevent mould growth. In terms of stable metal concentrations in the pellet, these additions of radiotracers corresponded to 10 ng g^{-1} for Mn, 1.4 μg g^{-1} for Zn, i.e. concentrations that are lower than the common concentrations of these metals in the potential prey of the fish (Pouil et al., 2016). Preliminary tests were performed to determine the possible leakage into the water of radioisotopes from the pellets during the feeding. When food was provided, acclimated fish consumed the pellets in < 2 min. Therefore, preliminary tests consisted in pouring radiolabelled dry pellets (100 mg per treatment) for 1, 5 and 10 min in 50 mL seawater and to measure any radioactivity in the seawater (Pouil et al., 2015). The leakage of pellet-radioactivity was under the detection limits even after 10 min immersed in the seawater, respectively. Although these tests confirmed the single-pathway contamination (viz. food) of the fish, one turbot was used in each treatment, as a control to take into account the possibility of ^{54}Mn and ^{65}Zn recycling through water (see Section 2.3).

2.2.2. Exposure of turbots via radiolabelled pellets

A total of 8 acclimatized turbots were randomly selected for each experimental salinity (10: 44.0 ± 2.9 g; 25: 40.0 ± 3.8 g and 38: 42.6 ± 3.4 g). Slits cut into the fins were used to facilitate individual recognition. Each experiment consisted of a single feeding of fish with radiolabelled pellets, a food commonly used in the literature (e.g. Jacob et al., 2017; Zhou et al., 2017; Pouil et al., 2015) to assess trophic transfer of trace elements in fish. 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 or, later on, from fish depuration. Two hours after the 15-min feeding, individual fish were whole-body γ-counted alive and then replaced in the same aquarium to follow subsequent trace element depuration. All the fish (including control individual of each condition) were regularly radioanalysed to follow the radiotracer depuration kinetics over 21 days.

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 kidneys, (5) the liver, (6) the 2 axial muscles (without dorsal skin) and (7) the remaining tissues (including ventral skin, skeleton, fins, heart and muscle residues) and were separated, weighed and radioanalysed to determine the radiotracer body distribution.

2.3. Radiotracers and counting

Radiotracers of high specific activity were purchased from Polatom, Poland (^{54}Mn as $MnCl_2$ in 0.5 M HCl, $t_{1/2}$ = 312 days and ^{65}Zn as $ZnCl_2$ in 0.1 M HCl, $t_{1/2}$ = 244 days). The radioactivity of the tracers was measured using a high-resolution 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 of appropriate geometry (calibration and counting). Measurements were corrected for background and physical radioactive decay. Living organisms were placed in counting tubes (diameter: 160 mm, height: 80 mm) filled with 500 mL of clean seawater (at the appropriated conditions of salinity) during the counting period. The counting time was adjusted to obtain a propagated counting error < 5% (e.g., Rodriguez y Baena et al., 2006) for a maximum of 20 min. As already described by Pouil et al. (2017b), tests were performed prior to the experiment, where fish were placed in similar counting conditions in order to observe their behaviour, i.e. in a counting box for 20 min in the dark. Dissolved O_2 concentration was monitored throughout these tests and was always > 3 mg L^{-1}. No alteration in organism health or behaviour was observed during the tests and then, the experiment.

2.4. Kinetic parameters and TTF

Depuration of radiotracers was expressed as the percentage of remaining radioactivity (radioactivity at time *t* divided by the initial radioactivity measured in the organism at the beginning of the depuration period; following methods developed in Warnau et al. (1996)). The depuration kinetics of Mn and Zn were best fitted using a two-component exponential model:

$$A_t = A_{0s} . e^{-k_{es}t} + A_{0l} . e^{-k_{el}t} \quad (1)$$

Table 1
Seawater parameters during the experiment on the assimilation of essential trace elements in juvenile turbots exposed to different conditions of salinity. Values are Means ± SD. Letters denote significant differences.

Condition	Salinity measured	Conductivity (mS cm^{-1})	pH	Temperature (°C)
Low salinity (10)	10.05 ± 0.13[a]	16.99 ± 0.09[a]	8.02 ± 0.04[a]	19.70 ± 0.08[a]
Medium salinity (25)	24.94 ± 0.16[b]	39.27 ± 0.15[b]	7.99 ± 0.07[b]	19.72 ± 0.05[a]
High salinity (38)	37.8 ± 0.05[c]	56.98 ± 0.04[c]	7.98 ± 0.03[c]	19.71 ± 0.12[a]

where A_t and A_0 are the remaining activities (%) at time t (d) and 0, respectively; k_e is the depuration rate constant (d^{-1}). "s" and "l" subscripts are related to the short- and long-lived component, respectively. The "s" component represents the depuration of the radiotracer fraction that is weakly associated with the organisms and rapidly eliminated (i.e. proportion associated with the faeces). The "l" component describes the depuration of the radiotracer fraction that is actually absorbed by the organism and eliminated slowly (Whicker and Schultz, 1982; Reichle, 1967; Hubbell et al., 1965). The long-lived component allows estimating the assimilation efficiency (AE) of the radiotracer ingested with food (AE = A_{0l}; Pouil et al., 2018a; Warnau et al., 1996). For the two components, biological half-life ($T_{b1/2}$) can be calculated from the corresponding depuration rate constant according to the relation $T_{b1/2} = \ln 2/k_e$. Kinetic parameters were determined using the R freeware 3.5.2 (R Development Core Team, 2018) and the 'nlstools' package (Baty and Delignette-Muller, 2015).

To assess the biomagnification potential of Mn and Zn following dietary exposure, trophic transfer factors (TTFs) were also calculated for a specific link in the food chain in which a predator ingests metal in prey as follows:

$$TTF = \frac{AE \times IR}{k_{el}} \quad (2)$$

where AE is the assimilation efficiency of the ingested metal in the fish, IR is the weight-specific ingestion rate of prey (g g^{-1} d^{-1}) and k_{el} is the depuration rate constant (d^{-1}) of the radionuclide out of the predator (see Mathews et al., 2008; Zhao et al., 2001). A TTF > 1 suggests that biomagnification is possible, and TTF < 1 suggests that biomagnification is unlikely (Reinfelder et al., 1998). For these TTF calculations, we considered a range of ingestion rates (IR) by fish likely to be encountered under natural conditions (0.02 to 0.10 g g^{-1} d^{-1}; Zhao et al., 2001).

Statistical comparisons between the three different salinity experiments were conducted using individual depuration kinetics of each element: individual kinetic parameters were obtained using the best fitting model at the global scale to the data of each individual. Then, differences between these parameters were tested using Kruskal-Wallis and Siegel and Castellan non-parametric tests (Zar, 1996). The same statistical tests were used to compare Mn and Zn organotropism of turbots under the different salinity conditions. The level of significance was always set at $\alpha = 0.05$.

3. Results

In order to evaluate how salinity affects the assimilation of essential trace elements in the juvenile turbots *S. maximus*, depuration kinetics of Mn and Zn were followed after a pulse-chase feeding, using radiolabelled pellets. During the whole experimental period (i.e. three weeks of acclimation to the targeted salinity values followed by three weeks of depuration) where the fish were exposed to a gradient of salinities (see the Materials and methods section), only a limited growth of the individuals was recorded and no mortality occurred. Before the single-feeding, the activity level of Mn and Zn was measured in the pellets: 2202 ± 158 Bq ^{54}Mn g^{-1} and 2394 ± 167 Bq ^{65}Zn g^{-1}. During the entire experiment, the exclusive foodborne exposure of the fish to both radiotracers was confirmed (no activity was recorded in the control turbots).

Whole-body depuration kinetics of ^{54}Mn and ^{65}Zn in turbots were best fitted by a two-phase model (R^2: 0.88–0.98; Fig. 1 and Table 2). A large proportion (57–81%, Table 2) of the ingested radiotracers was associated with the short-term component for both the studied elements. This component was characterized by a very rapid loss ($T_{b1/2s} < 1$ d, Table 2). Comparison of short-term depuration rate constants (k_{es}) determined for each individual turbot indicated that there was no significant difference for both studied elements ($p > 0.05$) independently of the salinity conditions. Estimated AEs in turbots ranged from 25% to 43% for Mn whereas Zn was less assimilated (AE < 25%, Fig. 1 and Table 2). Statistical analyses carried out on individual estimated AEs revealed that salinity affected the trophic transfer of Mn with a significantly lower AE at the highest salinity ($p < 0.05$; Fig. 2). In contrast, no significant effect of the salinity was observed for AEs of Zn ($p > 0.05$; Fig. 1). For Mn, long-term efflux rate constants were 2 times higher ($p < 0.05$, Fig. 2) in the turbots maintained at the highest salinity condition (38) with $T_{b1/2l}$ of 46 d while $T_{b1/2l}$ reached 79–86 d in low salinity conditions (10 and 25).

For Mn and, to a lesser extent, for Zn, TTFs calculated for a range of IR were dependent on the salinity conditions (Fig. 3). Depending on the IR, TTFs ranged from 1.0 to 5.7 and 0.3 to 1.7 for Mn at the lowest and the highest salinity conditions, respectively. Zn TTFs ranged from 0.4 to 5.3 with the lowest TTFs observed at the highest salinity (Fig. 3).

Post-feeding distributions of Mn and Zn in turbots exposed to the gradient of salinity, at the end of the 21-d depuration period, is shown in Fig. 4. Similar patterns of Mn and Zn distribution among compartments were observed in turbots exposed to the three salinities ($p > 0.05$). Distribution among the body compartments systematically ranked according to the following decreasing order (Fig. 4): remaining tissues (i.e. remaining skin, skeleton, fins, heart and remaining parts; 44–50%) > head (33–45%) ≫ axial muscles (4–10%) > digestive tract (< 1–9%) ≫ liver (< 2%) > kidneys (< 1.4%) ≫ gall bladder (< 0.2%).

4. Discussion

Salinity is an environmental master factor in coastal marine ecosystems (Smyth and Elliott, 2016). Salinity is known to strongly influence the bioaccumulation of dissolved trace elements in aquatic organisms such as fish through osmoregulatory processes (Wang and Rainbow, 2008; Zhang and Wang, 2007; Ni et al., 2005) and thus affects the dynamics of trace elements in marine environment. Both changes in metal speciation and physiology explained differential trace element uptake rates from water in fish exposed to different salinities (Wang and Rainbow, 2008). Nevertheless, although in their natural environment fish are also exposed to trace elements from the water and eventually from the sediment, previous laboratory studies highlighted that diet is the main pathway of trace element bioaccumulation in fish (Pouil et al., 2018a; Mathews and Fisher, 2009; Xu and Wang, 2002).

Effects of salinity on the trophic transfer of trace elements have been poorly investigated in the literature. Ni et al. (2005) found no significant differences in the Assimilation Efficiency (AE) of Cd, Se and Zn in the mudskipper *Periophthalmus modestus* fed on radiolabeled polychaetes and acclimated to a gradient of salinity from 10 to 30. Zhou et al. (2017) found more contrasting results with Cu AEs measured in the white-spotted spinefoot *Siganus canaliculatus* fed on $CuSO_4$-spiked commercial pellets decreased from a salinity of 33 to 10 but increased at lower salinity. In the present study, we found that Zn AEs determined in juvenile turbots at the three different salinities (10, 25 and 38) were similar but Mn AE was significantly lower in the fish exposed to the highest salinity (38) showing that the effects of salinity on the trophic transfer of trace elements in fish are species- and element-dependent.

Zn is one of the most important essential trace elements for fish due to its structural and catalytic roles in > 300 proteins and it serves as a cofactor in many enzymatic systems, playing a vital role in lipid, protein, and carbohydrate metabolism (Bury et al., 2003; Watanabe et al., 1997). Thus, this element is directly involved in growth, reproduction, development and immunity in fish (Watanabe et al., 1997; Tacon, 1987). Although the mechanisms of Zn transfer from the gut lumen to the internal compartment (absorption) are not fully elucidated yet, it seems to be dominated by active processes involving specific transporters (Bury et al., 2003). This element is, among other things, accumulated into cells through specific channels (ZIP family; Hogstrand, 2011; Bury et al., 2003). However, as concentrations can easily be toxic, steady-state cytosolic Zn concentration is controlled by an efflux

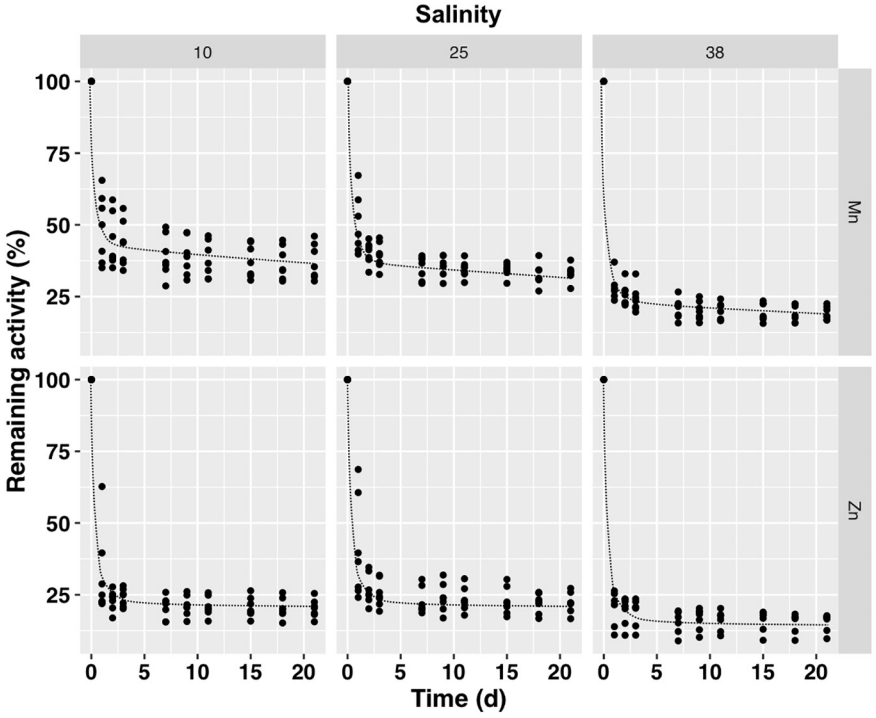

Fig. 1. Influence of salinity on whole-body depuration of ^{54}Mn and ^{65}Zn in juvenile turbot (n = 7; % remaining activities). Parameters and statistics of depuration kinetics are given in Table 2.

transporter of the ZnT family that transports Zn from the cytosol outside the cells (Hogstrand, 2011; Bury et al., 2003). An excess of Zn can be excreted mainly via the bile, intestinal sloughing (Handy, 1996) or the gills (Hardy et al., 1987). Thus, at both organismal and cellular levels Zn status in fish is actively and tightly controlled. In the present experiment, the ^{65}Zn concentrations remained constant in tissues in the fish exposed to the gradient of salinity with ~2.6–3.2 Bq ^{65}Zn g^{-1} fresh weight (FW) demonstrating the existence of internal Zn homeostasis. This mechanism may explain the consistency observed for the proportion of assimilated dietary Zn in turbot (19–24%; this study) and mudskipper (5–7%; Ni et al., 2005) despite variations of salinity.

Mn is necessary for the normal functioning of brain and for lipid and carbohydrate metabolism. This element has a key role as a cofactor for enzymes and as a structural element of metalloenzymes. As a cofactor or component of several key enzyme systems, Mn is also directly involved in bone formation, regeneration of red blood cells and reproduction (Watanabe et al., 1997; Tacon, 1987). Interestingly, we demonstrated that for Mn both AE and k_{el} were significantly affected by salinity. Indeed, while the lowest Mn AE was measured in turbots acclimated to the highest salinity (38), in the same condition efflux rate constant (k_{el}) was significantly higher indicating a lesser Mn retention for fish from this treatment (i.e. $T_{b1/2l}$ two times lower compared to the other treatments). Such differences can be explained by a less tight homeostasis for Mn compared to Zn as suggested by the differences observed in the whole-body ^{54}Mn concentrations in fish depending on the salinity with values of ~5 Bq ^{54}Mn g^{-1} FW for the turbot acclimation to the low salinity conditions while ^{54}Mn concentrations decreased to ~3 Bq ^{54}Mn g^{-1} FW in turbots maintained at a salinity of 38. Nevertheless, the mechanisms of transport and absorption of Mn from food in fish are poorly reported and further investigations are needed to support this assumption.

Our results for Mn and Zn revealed the contrasting effects of the

Table 2
Estimated depuration kinetic parameters of ^{54}Mn, and ^{65}Zn in turbots acclimated to three salinity conditions (10, 25 and 38; n = 7 per treatment) and exposed to the radiotracers during a single-feeding with radiolabelled pellets. After the radiolabelled feeding, turbots were maintained for 21d in unspiked seawater at the given salinity. Depuration parameters: A_{0s} and A_{0l} (=AE): activity (%) lost according to the short- and the long-lived exponential component, respectively; k_e: depuration rate constant (d^{-1}); $T_{b1/2}$: biological half-life (d) [$T_{b1/2}$ = ln2/k_e]; ASE: asymptotic standard error; R^2: determination coefficient.

Salinity	Short-term			Long-term			R^2
	A_{0s} ± ASE	k_{es} ± ASE	$T_{b1/2s}$ ± ASE	A_{0l} ± ASE	k_{el} ± ASE	$T_{b1/2l}$ ± ASE	
Mn							
10	56.93 ± 3.44*	2.14 ± 0.53*	0.32 ± 0.08	43.05 ± 2.24*	0.008 ± 0.004*	78.93 ± 37.75	0.88
25	61.63 ± 2.20*	1.62 ± 0.18*	0.43 ± 0.05	38.35 ± 1.51*	0.008 ± 0.003*	85.92 ± 32.86	0.95
38	75.44 ± 1.61*	2.91 ± 0.40*	0.24 ± 0.03	24.56 ± 1.03*	0.015 ± 0.004*	46.24 ± 11.17	0.98
Zn							
10	77.29 ± 2.64*	2.12 ± 0.28*	0.33 ± 0.04	22.71 ± 1.68*	0.006 ± 0.006^{NS}	+ ∞	0.95
25	75.63 ± 3.27*	1.55 ± 0.20*	0.47 ± 0.06	24.39 ± 2.20*	0.005 ± 0.007^{NS}	+ ∞	0.93
38	81.02 ± 1.82*	2.90 ± 1.12*	0.18 ± 0.05	18.98 ± 1.12*	0.008 ± 0.007^{NS}	+ ∞	0.98

Probability of the model adjustment (not applicable to Tb1/2s and Tb1/2l):
^{NS} $p > 0.05$.
* $p < 0.001$.

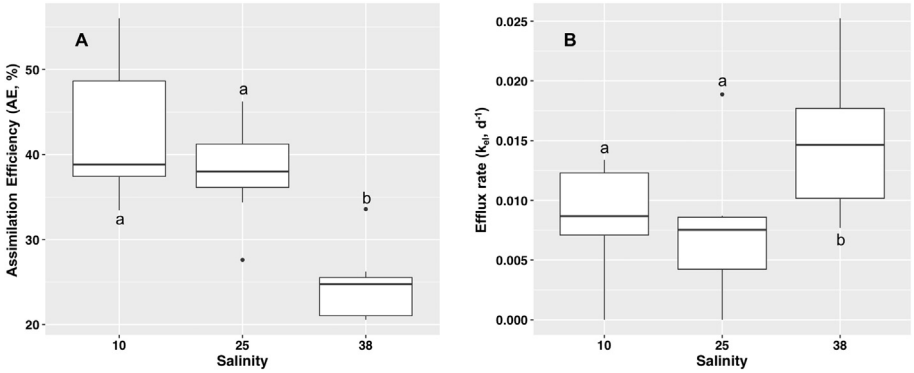

Fig. 2. Comparison of (A) assimilation efficiencies (AEs) and (B) efflux rate constant (k_{el}) of Mn calculated for each individual turbot acclimated to three salinity conditions. The best fitting model obtained for the entire set of turbots (see Fig. 1 and Table 2) was applied to individuals. Letters denote significant differences between the salinity conditions.

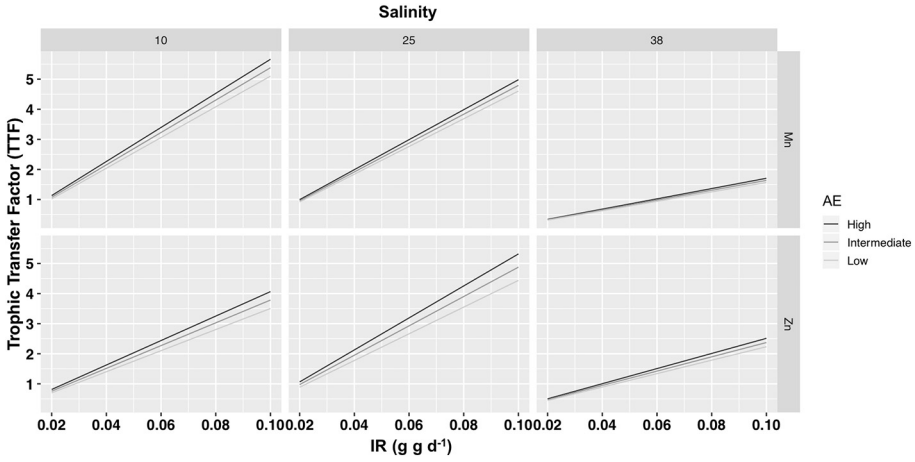

Fig. 3. Trophic transfer factors for juvenile turbots acclimated to three salinity conditions (10, 25 and 38) and fed with radiolabeled pellets at different assimilation efficiencies (AE) and ingestion rates. "High" AE (mean + SD), "intermediate" AE (mean), and "low" AE (mean - SD) values are reported in Table 2.

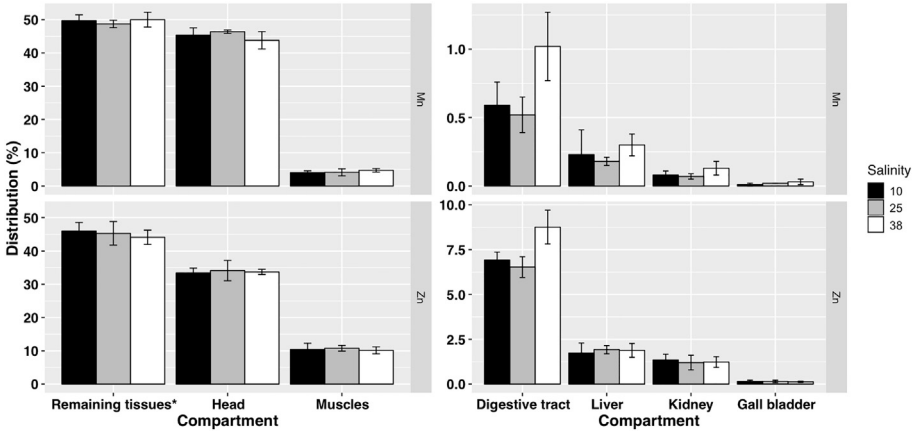

Fig. 4. Distribution (%) of ^{54}Mn, and ^{65}Zn in juvenile turbots acclimated to three salinity conditions (10, 25 and 38), exposed to the radiotracers during a single-feeding with radiolabelled pellets and then maintained for a 21-d depuration period in unspiked seawater at the given salinity. Values are means ± SD (n = 4). *The remaining tissues included remaining skin, skeleton, fins, heart and muscle residues.

salinity on the trophic transfer of trace elements in fish. We assumed that such differences may be reasonably explained by the physiological mechanisms involved in the homeostasis of essential trace elements in fish. In addition to whole-body kinetic determination, we also performed fish dissection at the end of the 21-d depuration period. Indeed, measurements of the distribution of Mn and Zn provide additional mechanistic information potentially helping in the interpretation of results from whole-body kinetic measurements. Our results show that the distribution of Mn and Zn between the tissues of juvenile turbots did not vary significantly with salinity. More than > 98% Mn and > 88% of Zn were found in the muscles, the head, and in the remaining tissues. Similar findings were reported in previous studies (Pouil et al., 2017a) where > 95% of the Mn and > 86% of the Zn were found in the same tissues. The absence of changes in distribution of these elements in the body compartments could be related to the fact that the experimental context is reflecting non-polluted conditions (i.e., no excess of Mn and Zn in the diet) and rather reflects normal physiological processes (Pouil et al., 2017a).

Altogether, the results of this study highlighted the effects of salinity that could potentially lead to a change in the transfer of Mn and Zn within aquatic food webs. Indeed, the estimated TTFs ranging from 1 to 6 at salinity of 10 were 3 times lower at the highest salinity (38) demonstrating that, salinity, in addition to playing an important role in the bioaccumulation of dissolved trace elements, affects also their transfer from diet. Consequently, attention should be paid on this environmental variable for obtaining a better understanding of the

dynamics of trace elements within food webs in coastal marine ecosystems subject to variable inputs of freshwater and trace elements. Nevertheless, care needs to be taken in how to interpret and expand on these results. Indeed, the methodological approach used in this study allowed determining the kinetics of depuration of dietary Mn and Zn in living fish acclimated to a gradient of salinity. However, further investigations are needed to fully understand the influence of salinity on the physiological mechanisms involved in assimilation of the studied trace elements. Furthermore, in our study, turbots were acclimated to stable salinities while salinity changes can occur abruptly in coastal environments (Smyth and Elliott, 2016). These variations are likely to affect the physiology of the organisms that are exposed to them and, consequently, to affect the trophic transfer of the trace elements.

5. Conclusion

In summary, our study showed that salinity differently impacted the AE of Mn and Zn, two essential elements in the juvenile turbots, although this species is euryhaline (i.e. species with a large salinity tolerance). Indeed, Mn AE was higher at lower salinities (10 and 25) than at high salinity (38) while Zn AE was not affected by the salinity conditions. These differences were likely caused by the physiological changes rather than the changes in trace element speciation. After the 21-d depuration period, tissue distributions were similar both for Mn and Zn in turbots acclimated to the three salinities. Given the evidence that food is the major pathway of trace element bioaccumulation in marine fish, salinity would be one important environmental variable driving the trophic transfer of some trace elements in coastal aquatic ecosystems.

Credit authorship contribution statement

Simon Pouil:Conceptualization, Methodology, Formal analysis, Data curation, Investigation, Writing - original draft, Writing - review & editing. **François Oberhänsli**:Conceptualization, Methodology, Resources. **Paco Bustamante**:Conceptualization, Supervision, Writing - original draft. **Marc Metian**:Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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