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Influence of food on the assimilation of essential elements (Co, Mn, and Zn) by turbot *Scophthalmus maximus*

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ABSTRACT: Food is an important route of metal uptake in marine organisms, and assimilation efficiency (AE) is a key physiological parameter that can be used to systematically compare the bioavailability of different metals from food. This parameter may be influenced by various factors, including diet. The present study aimed to examine the influence of diet on the AEs of 3 essential metals (Co, Mn, and Zn) in the turbot *Scophthalmus maximus.* The pulse-chase feeding method was used with 3 radiolabelled natural prey: fish, shrimp, and ragworm. AE was strongly influenced by the prey and the metal considered. However, the influence of these parameters on AE was variable, and no general trend was observed. The AEs ranged between 5–43% for Co, 23–44% for Mn, and 17–32% for Zn. Results suggest that relationships between metal distribution in the prey (at tissue and subcellular levels) and bioavailability to predator fish is not as obvious as previously assumed based on marine organisms feeding on unicellular or simple pluricellular organisms. Finally, we modelled how *S. maximus* accesses foodborne essential elements, using experimentally derived parameters, the concentration of these elements in prey, and different data on stomach contents from wild turbot. The results emphasised the importance of crustaceans in the nutrition of turbot and showed that crustaceans are generally the most important source of essential metals for turbot, although in some cases, polychaetes can make a large contribution to dietary Co and Mn uptake.

KEY WORDS: Marine fish · Assimilation efficiencies · Natural prey · Depuration · Metals · Nutrition

INTRODUCTION

Fish accumulate metals through different pathways via the water, their food, or direct contact with the sediment (e.g. Warnau & Bustamante 2007, Dutton & Fisher 2011). Over the last decade, food has been increasingly identified as a pathway of major importance for metal intake in fish (Xu & Wang 2002, Mathews & Fisher 2009). However, despite a growing understanding of trophic transfer mechanisms, few studies have focused on the influence of the diet on the assimilation of essential metals in these organisms (Baudin & Fritsch 1989, Garnier-Laplace et al. 2000, Bury et al. 2003).

Essential metals, such as Co, Mn, and Zn, are metabolically required; they are part of the functional groups of various enzymes, play a structural role in respiratory pigments and metalloenzymes, and can act as activating co-factors for various proteins (see e.g. Simkiss 1979, Williams 1981). Fish health can be optimal if essential metals are present in sufficient amounts in their tissues: depletion of these elements

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can provoke pathological impairments and/or physiological alterations, and an excess of essential elements can provoke toxic effects (e.g. Förstner & Wittmann 1983).

One critical parameter for understanding metal trophic transfer in fish is the assimilation efficiency (AE) of the metal from ingested food. If derived under controlled experimental conditions, AE is a first-order physiological parameter that can be compared quantitatively among different metals, organisms, food types, or environmental conditions (Wang & Fisher 1999).

The main objective of the present study was to investigate the influence of diet on essential metal assimilation in a marine predatory fish, the turbot *Scophthalmus maximus*. We compare the AE of 3 essential metals (Co, Mn, and Zn) in *S. maximus*, fed on 3 different natural prey (fish, shrimp, and ragworm) using radiotracer techniques. To better understand assimilation processes for the 3 essential metals, depuration kinetics were determined and AEs estimated after a single feeding with radiolabelled prey (pulse-chase feeding methodology; e.g. Warnau et al. 1996, Metian et al. 2010). Relationships between metal fractioning in prey (tissue and subcellular levels) and metal AEs in their predators have been shown for invertebrates and planktivorous fish (Reinfelder & Fisher 1994, Wallace & Lopez 1996) but not yet for fish fed with complex pluricellular prey. Therefore, tissue and subcellular distribution of essential elements was characterised to assess possible influence on AEs in turbot.

Finally, AE results were combined with stable isotope analyses in the selected prey and with the natural diet of turbot to develop a model that was used to estimate the relative contribution of each prey to the dietary intake of metals.

MATERIALS AND METHODS

Origin and acclimation of organisms

In January 2014, 100 juvenile turbot *Scophthalmus maximus* were purchased from a fish farm (France Turbot, France) and shipped to the International Atomic Energy Agency premises in the Principality of Monaco. Fish were acclimated to laboratory conditions for 21 d (open-circuit, 500 l aquarium; water renewal: 100 l h^{-1}; 0.45 µm filtered seawater; salinity: 38 psu; temperature: 15 ± 0.5°C; pH: 8.0 ± 0.1; 12 h light:12 h dark cycle). During the acclimation period, the fish were fed a daily ration of 2% of their biomass with 1.1 mm pellets (proteins: 55% and lipids: 12%; Le Gouessant, France).

To investigate the influence of diet on essential metal assimilation by *S. maximus*, 3 different natural prey were used: fish (juvenile seabream *Sparus aurata*), shrimp (common prawn *Palaemon serratus*), and ragworm (estuary ragworm *Hediste diversicolor*). Fish were obtained from the hatchery Poissons du Soleil, France; shrimp were purchased from Poissons Vivants, France; and ragworms were purchased from the fishbait supplier Normandie Appâts, France. All prey were acclimated to the same laboratory conditions as the turbot for a minimum of 2 wk prior to experiments. Shrimp and worms were fed a mix of fish feed and crushed mussels, whereas fish were fed 300 µm pellets (Biomar, France). Because body size (weight) is known to affect metal bioaccumulation in marine organisms (Boyden 1974, Warnau et al. 1995, Hédouin et al. 2006), only prey individuals with homogeneous size were used for the experiments (*S. aurata*: 60 d old hatchlings, approx. 1.5 to 2 cm in total length, 0.06 ± 0.01 g wet weight [wet wt]), *P. serratus*: 0.58 ± 0.11 g wet wt, and *H. diversicolor*: 0.82 ± 0.14 g wet wt).

Nutritional characteristics and stable metals in prey

Preliminary characterisation of metal concentration and basic nutritional composition of the prey was carried out prior to radiolabelling. Protein content (using N content), percentage of dry matter (DM), and essential metal concentrations (Co, Mn, and Zn) were measured. To determine the amount of N, samples of food items (n = 3) were freeze-dried (FreeZone 18 l Console Freeze Dry System, Labconco) before being manually crushed. Aliquots of 1 to 5 mg were analysed using a vario EL CHN analyser (Elementar). For each food item, the protein content (expressed as % of DM) was estimated using conversion coefficients from N-values (i.e. 5.58 for fish and 5.60 for the other prey; Tacon et al. 2009). DM content was determined by drying the samples in a ventilated oven at 105°C for 24 h.

For essential element analyses, samples (n = 3 for each prey) of 250 to 1000 mg were digested using 5 ml of 65% HNO_3 and 2 ml of H_2O_2. Acidic digestion was performed overnight at ambient temperature and then heated in a microwave for 40 min, with a temperature increase to 190°C for 20 min, followed by 20 min at 190°C (1600 W). After the mineralisation process, each sample was diluted to 50 ml with Milli-

Q quality water, and an extra 1:5 dilution was prepared. Co and Mn were analysed by ICP-MS (iCAP Q ICP-MS, Thermo Scientific) and Zn by flame atomic absorption spectrometry (SpectrAA 220, Varian). A certified reference material (fish muscle, IAEA 407) was treated and analysed in the same way as the samples. Results were in good agreement with the certified values (Table 1). For each set of analyses, blanks were included in the analytical batch. The detection limits were (µg g^{-1} dry wt) 0.006 (Co and Mn) and 0.5 (Zn). All metal concentrations are given on a dry weight basis (µg g^{-1} dry wt). For the shrimp, antennae, antennules, rostrum, and telson were removed before analysis in accordance with experimental methodology (see 'Exposure of turbot via radiolabelled prey').

Experimental procedures

Radiolabelling of the prey

Preparation of the radiolabelled prey was carried out by exposing them for 7 to 21 d in aerated 20 l aquaria. Radiotracers of high specific activity were purchased from Isotope Product Lab, USA (^{57}Co as $CoCl_2$ in 0.1 M HCl, [$T_{1/2}$] = 271.8 d; ^{54}Mn as $MnCl_2$ in 0.5 M HCl, [$T_{1/2}$] = 312.2 d; ^{65}Zn as $ZnCl_2$ in 0.1M HCl, [$T_{1/2}$] = 243.9 d). Seawater was spiked with the radiotracers (nominal activity of 0.5 kBq l^{-1} per isotope for fish and shrimp exposures and 1 kBq l^{-1} per isotope in the case of ragworm). In terms of stable metal concentrations, these additions corresponded to 0.2–0.4 pmol l^{-1} for Co, 3.7–7.4 pmol l^{-1} for Mn, and 220–440 pmol l^{-1} for Zn, i.e. concentrations that are lower than the background concentrations of these metals in the open ocean (Bruland 1983). Small volumes (10 µl) of the diluted radiotracer solution were added to the aquaria, and no changes in pH were detectable in the aquaria (closed-circuit) after tracer addition. Seawater was regularly renewed and spiked daily to keep the activity as constant as possible. Activity of the metal tracers in seawater was checked daily, before and after each seawater renewal, to determine time-integrated activities (Warnau et al. 1996, Rodriguez y Baena et al. 2006). Prey were fed after each seawater renewal. For shrimp exposure, each organism was kept individually during the whole duration of the radiotracer exposure in a cylindrical plastic container (drilled to allow for free water circulation) to avoid cannibalism (e.g. during moulting) and to facilitate individual recognition. For the ragworm exposure, the walls of the aquarium were obscured, and plastic tubes were added as artificial burrows.

Table 1. Comparison of metal concentration (mean ± SD, n = 3) in reference material (fish muscle, IAEA 407) measured by ICP-MS (Co and Mn) and by flame atomic absorption spectrometry (Zn) with certified values. All the values are expressed in µg g^{-1} dry weight

Element	Measured	Certified
Co	0.08 ± 0.01	0.10 ± 0.02
Mn	2.50 ± 0.07	3.52 ± 0.32
Zn	65.4 ± 0.7	67.1 ± 3.8

Exposure of turbot via radiolabelled prey

Three sets of experiments were conducted for each prey. For each set, 8 to 15 juvenile turbot (11.17 ± 4.76 g) were transferred to an aerated, open-circuit, 70 l aquarium. The number of turbot depended on the amount of contaminated prey available. Slits cut into the fins were used to facilitate individual recognition. One week before the exposure to radiolabelled diet, fish were fed daily with non-labelled prey to acclimate them to this diet. Each experiment consisted of a single feeding of fish with radiolabelled diet (e.g. Metian et al. 2010). Turbot were fed 30 min ad libitum with freshly killed prey; uneaten prey were removed after the 30 min feeding. To facilitate ingestion, shrimp were cut into pieces, and the antennae, antennules, rostrum, and telson removed. After the 30 min feeding, individual fish were whole-body γ-counted alive and then placed in a new aquarium with open-circuit seawater conditions (parameters as previously described for their acclimation) to follow subsequent metal depuration. During depuration, fish were fed daily with non-labelled pellets (2% of their biomass; Biomar 2014) to keep consistent digestive physiology among all individuals. During and 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. After radiolabelled feeding, all the fish (including control individuals) were regularly radioanalysed to follow the radiotracer depuration kinetics over 21 d. After each counting, fish were moved to a new 70 l aquarium with clean water.

Radiotracer compartmentalization in prey

Radiolabelled fish (n = 3) and shrimp (n = 3) were dissected to isolate the hard body parts (skeleton and cuticle) that are assumed less digestible for predators (Reinfelder & Fisher 1994). Samples were radioanalysed to quantify the percentage of activity sequestered in these body parts (i.e. skeleton and cuticle).

Distribution of radioelements between the soluble and insoluble fractions was determined in 4 individuals of each species of prey according to a method adapted from Bustamante & Miramand (2005). This method allows quantification of metals associated with the soluble fraction of the prey (i.e. cytosol; Wallace & Lopez 1996, Bustamante & Miramand 2005). Briefly, 4 contaminated prey stored at –80°C were crushed, and the tissue was homogenised (T25 Ultra-Turrax Basic, IKA) in approximately 10 volumes (0.1 g tissue to 1 ml) of a solution of TRIS-HCl buffer 0.02 M, sucrose 0.25 M with 1 mM PMSF (phenylmethylsulfonylfluoride, as protease inhibitor), and 5 mM DTT (dithiothreitol, as reducing agent), at pH 8.06. The homogenates were centrifuged at 45 000 × *g* for 2 h at 4°C (Sorvall Evolution RC Superspeed Centrifuge, Sorvall instruments) to separate cytosol (i.e. the soluble fraction) from the cellular debris, the organelles, and the metal-rich granules (i.e. the insoluble fraction; Fig. 1). Aliquots of each fraction obtained were radioanalysed to determine the radiotracer's activities. The same procedure was repeated over time for each prey.

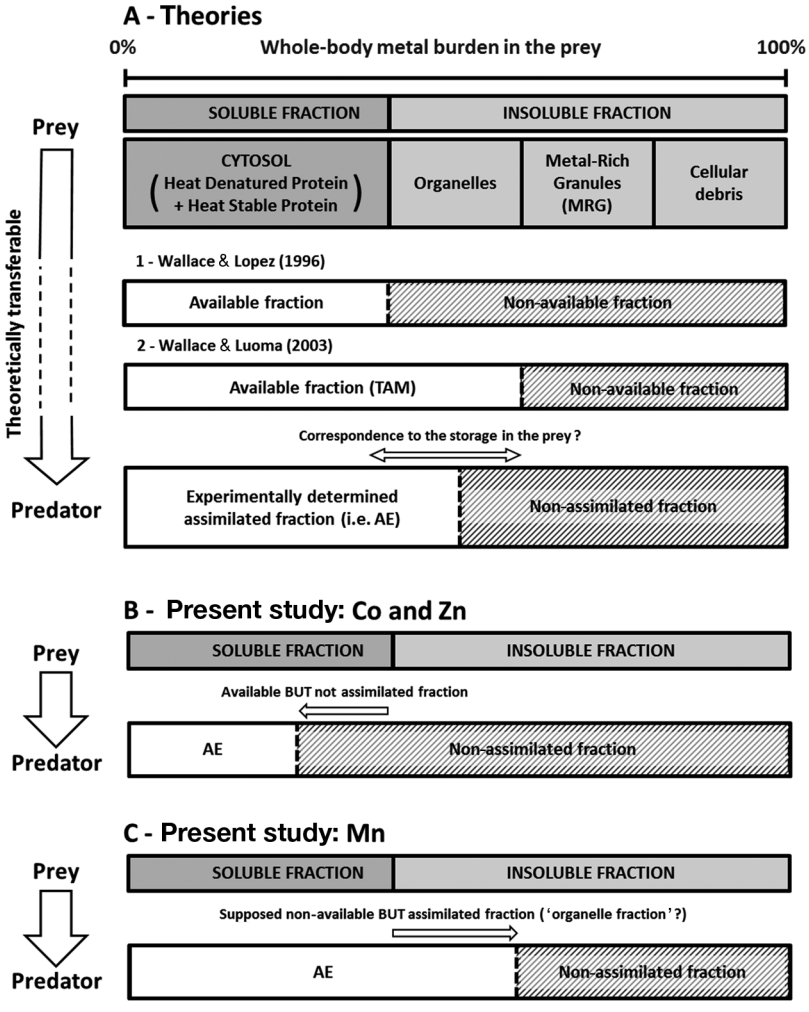

Fig. 1. Definition of the different concepts used in the subcellular fractionation of the metal and the relation between the subcellular fractionation in the prey and assimilation efficiency measured in the predator. (A) Description of theories developed by Wallace & Lopez (1996) and Wallace & Luoma (2003). (B) The present study, where the measured AE in the predator is lower than expected values based on the fraction of the element present in the soluble fraction, i.e. part of the available metal fraction in the prey is not assimilated by the predator. (C) The present study, where the measured AE in the predator is higher than expected values based on the fraction of the element present in the soluble fraction, i.e. part of the non-available metal fraction in the prey is assimilated by the predator

Radioanalysis

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 filled with clean seawater during the counting period. The counting time was adjusted to obtain a propagated counting error <5% (e.g. Rodriguez y Baena et al. 2006). In the case of live turbot, the counting time varied between 25 and 60 min in order to maintain fish health and ensure normal behaviour.

Data treatment and statistical analysis

Depuration kinetics were fitted using a non-linear model. 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; Warnau et al. 1996). The depuration kinetics of the radiotracers were best fitted using a simple exponential model including a constant (Eq. 1). Decision was based on an F-test and examination of residuals:

$$A_t = A_{0s} \cdot e^{-k_e t} + AE \quad (1)$$

where A_t and A_{0s} are the remaining activities (%) at time t (d) and 0, respectively; k_e is the depuration rate constant (d^{-1}), and AE is the assimilation efficiency (%). The first component represents the depuration kinetics of the radiotracer fraction that is weakly associated with the organisms and rapidly eliminated (the subscript s standing for short-lived), whereas the second component refers to the proportion of the radiotracer ingested with food that is actually assimilated by the organism (Warnau et al. 1996). For the short-lived component, a biological half-life can be calculated ($T_{b1/2}$) from the corresponding depuration rate constant according to the relation $T_{b1/2s} = \ln 2/k_e$. Model constants and their statistics were estimated by iterative adjustment of the model and Hessian matrix computation, respectively, using the non-linear curve-fitting routines in the Statistica software 7.0.

Statistical comparisons between the 3 different feeding experiments were conducted using individual depuration kinetics of each element: individual parameters (k_e and AE) were obtained using the best-fitting model at the global scale (Eq. 1) to the data of each individual. Then, differences between these parameters were tested using Kruskal-Wallis and Siegel and Castellan non-parametric tests. The same statistical tests were used to compare the bioavailability of metals in the different prey. The level of significance for statistical analyses was always set at $\alpha = 0.05$. All the statistical analyses were performed using R software 3.0.1 (R Development Core Team 2014).

A model was developed and used to estimate the relative contribution of each prey to the metal intake from food in wild turbot. The model assessing these contributions for each studied essential elements was determined using the following equations:

$$C_{pi} = \sum(AE_{pi} \times Q_{pi} \times IR \times O_{pi} \times BW) \quad (2)$$

$$C_{ri} = (C_{pi} / \sum C_p) \times 100 \quad (3)$$

where C_p (µg d^{-1}; wet wt) is the amount of metal from i prey retained by the turbot (Eq. 2). This value was then expressed as a percentage of total metal intakes from food (Eq. 3). AE_p is the assimilation efficiency (%) in the preddator estimated using Eq. (1) for each prey (p); Q_p (µg g^{-1} wet wt) is the stable metal concentration in prey; IR (% of body weight d^{-1}) is the ingestion rate for fish (range of values used in the literature: 0.1 to 10%; Xu & Wang 2002); O_p (%) is the occurrence of prey in natural diet estimated by stomach contents analysis (Sparrevohn & Støttrup 2008, Florin & Lavados 2010); and BW (g wet wt) is the average body weight of the turbot used in this study. All the values are expressed on a wet weight basis, using the conversion from percentage of dry matter provided in Table 2.

To better capture the variability in the trophic transfer of essential elements to the turbot that can occur in the field, 3 scenarios covering 3 different situations were created. Using the model previously described, these scenarios were implemented on the basis of 3 different diet compositions reported from field surveys (Sparrevohn & Støttrup 2008, Florin &

Table 2. Food composition and nutritional values (mean ± SD). For the shrimps, the antennae, antennules, rostrum, and telson were removed. Protein estimation based on nitrogen content using conversion coefficients (5.58 for fish and 5.6 for the other prey; Tacon et al. 2009)

	Dry matter (DM) (%)	Stable metals (µg g^{-1} dry wt)			Nutritional values (% DM)	
		Co	Mn	Zn	Nitrogen	Protein
Fish	22.2 ± 3.19	0.11 ± 0.01	15.8 ± 1.75	110 ± 1	2.19 ± 0.07	12.2 ± 0.40
Shrimp						
Cephalothorax	29.03 ± 0.43	0.12 ± 0.03	2.78 ± 0.28	71 ± 3	2.72 ± 0.44	15.25 ± 2.44
Abdomen	27.1 ± 0.82	0.04 ± 0.00	1.34 ± 0.12	43 ± 1	3.09 ± 0.30	17.32 ± 1.68
Whole (reconstituted)	27.9 ± 0.63	0.08 ± 0.01	2.02 ± 0.09	56 ± 1	2.93 ± 0.35	16.44 ± 1.94
Ragworm	20.6 ± 1.96	2.21 ± 0.82	43.06 ± 31.33	127 ± 25	0.89 ± 0.59	4.99 ± 3.31

Table 3. Description of the 3 scenarios (Low, Medium, High) used in the model for estimating the relative contribution of prey in the essential metal uptake by turbot and details regarding the values used for the model parameters. AE: assimilation efficiency of the predator; IR: ingestion rate of the predator; *Q*: stable metal concentration in the prey

Parameter	Low	Medium	High
AE	Mean – SD	Mean	Mean + SD
IR	Min	Mean	Max
Q	Mean – SD	Mean	Mean + SD

Lavados 2010). For each of these diets, 3 distinct values were assigned for the ingestion rate of the turbot, the concentration of essential elements in the prey, and the turbot AE for the 3 elements studied (IR, *Q*, and AE; details are provided in Table 3). Briefly, scenario 'low' corresponded to the inclusion into the model of minimal values of these parameters found in the present study (*Q* and AE) or in the literature (IR; Xu & Wang 2002), whereas maximum and average values of the same parameters were respectively used in the 'high' and 'medium' scenarios.

RESULTS

Nutritional characteristics and stable metal concentration in prey

Essential element concentrations and nutritional characteristics estimated for the different food items are given in Table 2. Although these values correspond to rough estimates of these characteristics (n = 3 for each prey), ragworms were the prey with the highest levels for all studied essential elements (Co, Mn, and Zn). For example, Co concentrations reached 2.21 ± 0.82 µg g^{-1} dry wt in ragworms vs. 0.08 ± 0.01 µg g^{-1} dry wt in shrimp and 0.11 ± 0.01 µg g^{-1} dry wt in fish (Table 2). However, ragworms were less nutritious than fish and shrimp, with 5% of protein in dry matter compared to 12 and 16%, respectively (Table 2).

Compartmentalisation of radiotracers in prey

Body distribution

After radiolabelling, ^{57}Co and ^{65}Zn were mainly distributed in the soft parts of fish and shrimp (i.e. whole-body activity minus activities measured in skeleton or cuticle, respectively, of 89 ± 3% and 60 ± 7% for Co and 78 ± 3% and 63 ± 6% for Zn; Fig. 2A). In contrast, storage of ^{54}Mn depended on the considered prey: for fish, this element was mainly concentrated in the soft parts of the body (64 ± 8%), whereas the soft parts of shrimp contained a smaller proportion of Mn (29 ± 8%).

Subcellular distribution

The majority of ^{57}Co taken up by the prey was located in the soluble fraction, with proportions ranging between 63 and 87%. The highest proportion (87 ± 2%) was measured in the soluble fraction of fish, whereas for shrimp, the soluble fraction contained 63 ± 7% of the Co body burden (Fig. 2B). ^{54}Mn was mainly distributed in the insoluble fraction

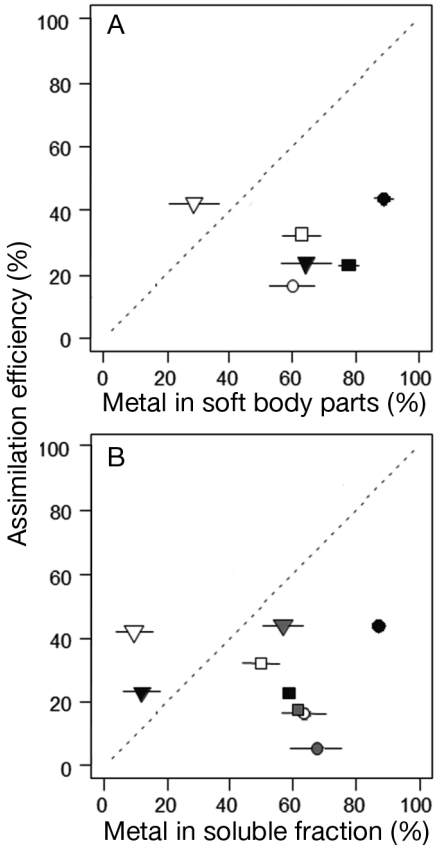

Fig. 2. Relationship between metal fractioning in the prey (quantified by dissection and centrifugation) and assimilation efficiency (AE) in turbot. (A) Comparison between AE in turbot and metals: Mn (triangle), Co (circle), and Zn (square), included in soft body parts of prey fish in black and shrimp in white. (B) Comparison between AE in turbot and metals in soluble fractions of prey fish in black, shrimp in white, and ragworm in grey. Dashed line shows equivalence according to Wallace & Lopez (1996)

of shrimp and fish with 91 ± 6 % and 88 ± 6 %, respectively. In contrast, for ragworms, ^{54}Mn was mainly (57 ± 7 %) present in the soluble fraction (Fig. 2B). The subcellular compartmentalisation of ^{65}Zn in the different prey was variable. In fish and ragworms, most of the ^{65}Zn was located in the soluble fraction (~60 %), whereas it was distributed equally between the soluble and insoluble fractions of shrimp.

Effects of diet on metal assimilation

To evaluate the influence of diet on metal assimilation in turbot *Scophthalmus maximus*, depuration kinetics of the 3 essential metals were followed after a pulse-chase feeding, using radiolabelled food items. The activity level of each element in each prey was measured prior to feeding: the average activities were 50 Bq ^{57}Co g^{-1} wet wt, 19 Bq ^{54}Mn g^{-1} wet wt, and 67 Bq ^{65}Zn g^{-1} wet wt in fish; 22 Bq ^{57}Co g^{-1} wet wt, 13 Bq ^{54}Mn g^{-1} wet wt, and 144 Bq ^{65}Zn g^{-1} wet wt in shrimp without antenna, antennules, rostrum, and telson; and 20 Bq ^{57}Co g^{-1} wet wt, 7 Bq ^{54}Mn g^{-1} wet wt, and 250 Bq ^{65}Zn g^{-1} wet wt in ragworm.

Whole-body depuration kinetics of ^{57}Co, ^{54}Mn, and ^{65}Zn in turbot were always best fitted by a 2-phase model (simple-exponential model and a constant; Fig. 3 and Table 4; R^2: 0.76 to 0.98). The assimilation efficiency (AE) and depuration rate of the 3 radiotracers depended both on the food and metal considered. The major fraction (53 to 95 %) of the 3 elements was rapidly lost ($T_{b1/2s}$ < 1.4 d) regardless of which prey had been ingested.

Estimated ^{57}Co AE varied significantly ($p < 0.05$) according to the prey type (Table 5). ^{57}Co was poorly assimilated by the turbot fed with radiolabelled ragworms (AE = 5.1 ± 1.1 %). Assimilation was elevated when the turbot were fed with juvenile fish (AE = 43.1 ± 12.0 %), and an intermediate situation was observed when they were fed with shrimp (AE = 16.3 ± 4.0 %). Estimated AE of ^{54}Mn also varied with the diet, though to a lesser extent. AE was significantly lower ($p < 0.001$; Table 5) when turbot were fed with fish (AE = 23.0 ± 7.7 %) than when fed with shrimp and ragworms (42.0 ± 6.6 % and 43.7 ± 2.3 %, respectively; Fig. 3, Table 5). Variation of ^{65}Zn AE was less pronounced. The only significant difference occurred for AEs estimated when turbot were fed with shrimp and ragworm ($p < 0.05$; Table 5), ^{65}Zn being more efficiently assimilated from shrimp (AE = 32.2 ± 6.0 %). Regarding depuration rate constants (k_e), values obtained for ^{57}Co and ^{65}Zn when turbot were fed with ragworm were significantly higher ($p < 0.05$) than when fed with the 2 other prey, indicating that their retention was shorter. For ^{54}Mn, no significant difference in k_e was observed ($p > 0.05$; Table 5).

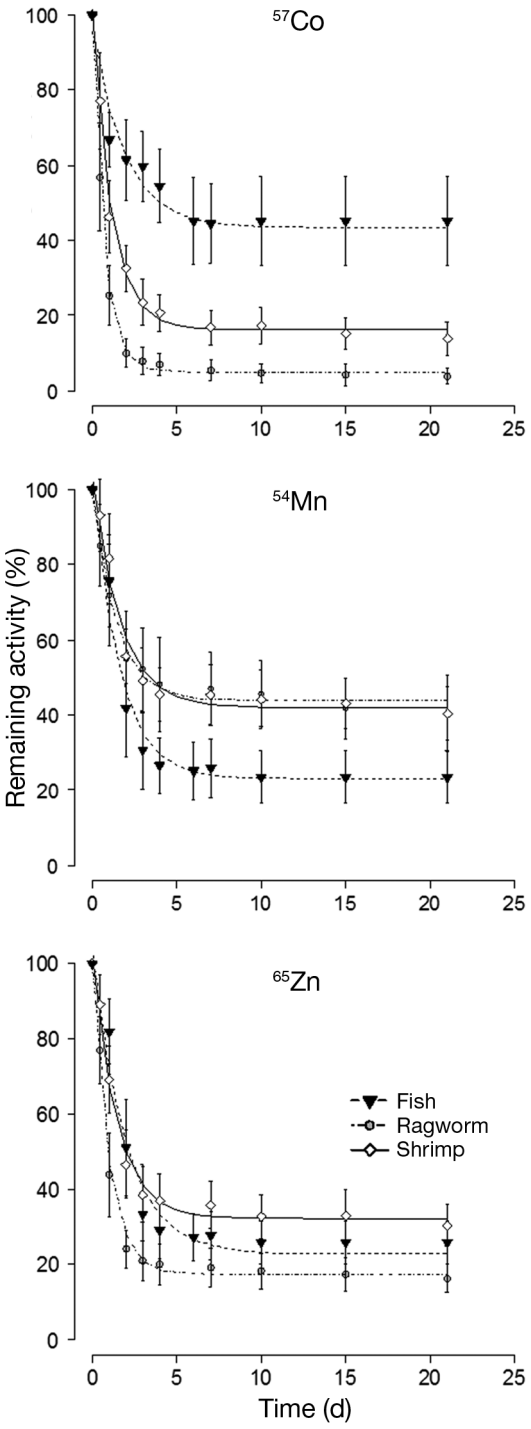

Fig. 3. Influence of type of food (see Table 2) on whole-body depuration of ^{57}Co, ^{54}Mn, and ^{65}Zn in turbot (% remaining activities, means ± SD). Parameters and statistics of depuration kinetics are given in Table 4

Table 4. Estimated depuration kinetic parameters of ^{57}Co, ^{54}Mn, and ^{65}Zn in turbot exposed to the radiotracers by 3 different types of food (fish, shrimp, ragworm; n = 8 to 12 per treatment) and then maintained for 21d in unspiked seawater. k_e: depuration rate constant (d^{-1}); $T_{b½s}$: biological half-life (d), AE: assimilation efficiency (%); ASE: asymptotic standard error; R^2: determination coefficient. Probability of the model adjustment: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

Feed	Short-term k_e ± ASE	Short-term $T_{b½s}$ ± ASE	Long-term AE ± ASE	R^2
^{57}Co				
Fish	0.52 ± 0.10***	1.33 ± 0.24	43.46 ± 1.94***	0.76
Shrimp	0.87 ± 0.05***	0.79 ± 0.05	16.30 ± 0.92***	0.97
Ragworm	1.43 ± 0.07***	0.48 ± 0.02	5.04 ± 0.78***	0.98
^{54}Mn				
Fish	0.63 ± 0.05***	1.10 ± 0.09	23.10 ± 1.47***	0.88
Shrimp	0.61 ± 0.05***	1.14 ± 0.09	41.99 ± 1.20***	0.93
Ragworm	0.71 ± 0.10***	0.98 ± 0.14	43.79 ± 1.62***	0.89
^{65}Zn				
Fish	0.51 ± 0.04***	1.35 ± 0.12	22.80 ± 1.63***	0.94
Shrimp	0.69 ± 0.04***	1.00 ± 0.03	32.20 ± 1.00***	0.96
Ragworm	1.05 ± 0.06***	0.66 ± 0.04	17.39 ± 0.94***	0.97

Outputs of the model on metal intake

The relative contributions of the different prey in the daily trophic intake of stable metals in turbot under 3 natural diets are shown in Fig. 4. When the diet of turbot was composed of fish and crustaceans, the latter taxon provided the highest essential element intake (Fig. 4A,B). However, when polychaetes were included in the diet (even in a small proportion, viz. 28%), they contributed the largest proportion of Co and Mn (38 to 58% and 40 to 78%, respectively, depending on the scenario; Fig. 4C).

DISCUSSION

Our results show that assimilation efficiencies (AEs) are metal-dependent and affected by the food items. Ranges of AE of ^{57}Co, ^{54}Mn, and ^{65}Zn in turbot for the 3 different prey considered were respectively 5–43%, 23–44%, and 17–32%. Although trophic transfer of Co and Mn is poorly documented in fish, the information available shows that the AEs or remaining activities (multi-feeding experiments) reported for carp *Cyprinus carpio* (Baudin & Fritsch 1989), rainbow trout *Oncorhynchus mykiss* (Baudin et al. 2000), silversides *Menidia* sp. (Reinfelder & Fisher 1994) and turbot *Scophthalmus maximus* (Mathews et al. 2008) are always lower than the ones determined in the present study. The values obtained for ^{65}Zn are in accordance with the literature on marine and brackish fish fed with zooplankton (Ni et al. 2000, Xu & Wang 2002, Zhang & Wang 2005) or juvenile fish (Mathews et al. 2008), where reported AEs were between 5 and 31%. To the best of our knowledge, the effect of food type on the AE of Co and Mn has never been studied in fish. However, several studies have demonstrated that the AE of Zn in a predator fish can be affected by the food composition. For example, changes in AE were reported for the glassy *Ambassis urotaenia* (AE between 9 and 15%) and the mudskipper *Periophthalmus cantonensis* (AE between 11 to 31%) when respectively fed with *Artemia* sp. and *Acartia spinicauda* (Ni et al. 2000). According to these authors, differences in AE would be explained by metal storage in specific locations in the prey and would explain the tight correlation observed between AE and elemental distribution in the soft tissues of zooplankton prey.

It is well documented that storage forms and location of metals in prey determine the bioavailability of these elements for predators (e.g. Wallace & Lopez 1996, Wallace & Luoma 2003, Meyer et al. 2005) and impact the AE. To investigate the possible relationship between storage or location of Co, Mn, and Zn in the prey and the AE of these elements in turbot, the measured AE were compared with metal distribution

Table 5. Comparison of assimilation efficiency (AE, %) and depuration rate constant (k_e, d^{-1}) of ^{57}Co, ^{54}Mn, and ^{65}Zn in turbot exposed to the radiotracers by 3 different types of food (fish, shrimp, ragworm; n = 8 to 12 per treatment) and then maintained for 21 d in unspiked seawater. Underlines indicate that the values (means ± SD) are not significantly different ($p > 0.05$). Statistical comparisons between the 3 different feeding experiments were undertaken using individual depuration kinetics of each element: individual kinetic parameters (k_e and AE) were obtained using the best fitting model at the global scale (Table 4) to the data of each individual

Tracer	Fish	Shrimp	Ragworm
AE			
^{57}Co	43.1 ± 12.0	16.28 ± 4.04	5.14 ± 1.14
^{54}Mn	23.0 ± 7.73	41.99 ± 6.61	43.17 ± 2.34
^{65}Zn	21.7 ± 6.85	32.19 ± 6.02	17.94 ± 1.92
k_e			
^{57}Co	0.59 ± 0.24	0.93 ± 0.25	1.57 ± 0.10
^{54}Mn	0.69 ± 0.21	0.63 ± 0.13	0.78 ± 0.16
^{65}Zn	0.53 ± 0.10	0.71 ± 0.12	1.02 ± 0.12

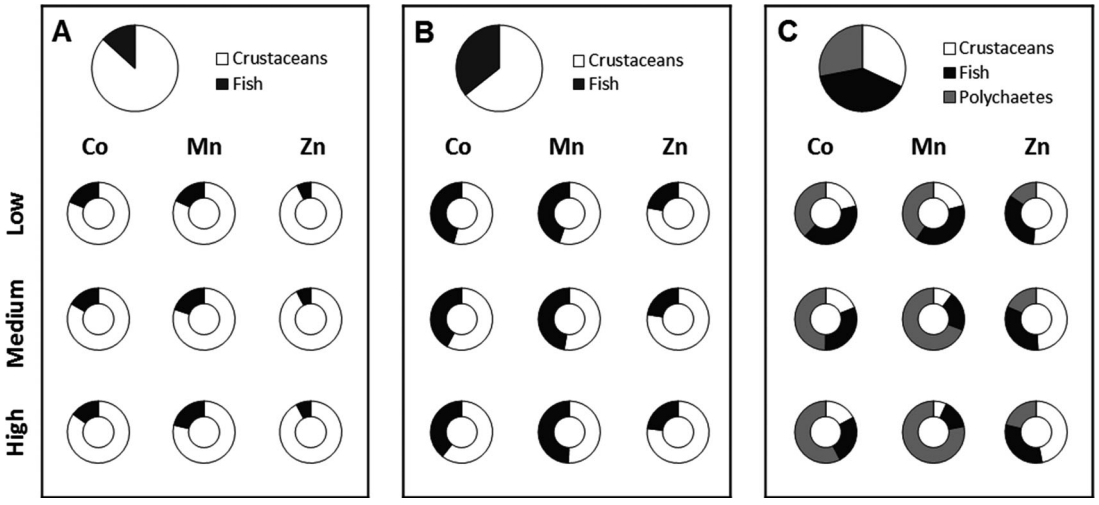

Fig. 4. Relative contributions of the different prey to the daily intake of stable metal from food in turbot under 3 natural diets. Three different scenarios were considered to reflect the variability of parameter values. Scenario 'Low' takes into account lower concentration values of metals in prey with a low ingestion rate and reduced assimilation. Conversely, in the scenario 'High', the maximum values of these parameters are considered. Finally, the average values were used in the 'Medium' scenario

in the prey determined by (1) dissection (tissue distribution) and (2) ultracentrifugation (i.e. subcellular distribution, i.e. soluble vs. insoluble fraction). Several hypotheses have been examined to explain the relationship between the bioavailability of metals, their fractioning in prey, and their assimilation in predators (Rainbow et al. 2011). Our results, obtained using complex pluricellular prey, were compared with the 2 main hypotheses often reported in the recent scientific literature for organisms fed with unicellular or simple pluricellular prey. The first hypothesis assumes that the AE of the predator can be estimated from the percentage of metal in the non-exoskeleton fraction, or soft body parts, of the prey (Reinfelder & Fisher 1994). Such a relationship has been reported for ^{109}Cd, ^{57}Co, ^{75}Se, and ^{65}Zn in the silversides *Menidia menidia* and *M. beryllina* fed with zooplankton. The second hypothesis assumes that the proportion of bioavailable metals for predators is related to the quantity of metal associated with the cytosolic fraction of the prey (Fig. 1; Wallace & Lopez 1996). The metal-available fraction was further considered to be better reflected if the fraction of the metal associated with organelles was added to the cytosolic fraction (i.e. the concept of trophic available metal [TAM]; Wallace & Luoma 2003). In the case of metal bioavailability to predatory fish, Zhang & Wang (2006) found a positive relationship between the TAM fraction in a variety of prey organisms (barnacles, bivalves, fish viscera, and zooplankton) and the AE of Zn and Se in the grunt *Terapon jarbua.* Nevertheless, no strict equivalence between TAM in prey and AE in fish has been found yet, in contrast to invertebrates, and the determination of the metal-available fraction from one trophic level to another is still intensely studied or discussed in the scientific literature (e.g. Rainbow et al. 2011, 2015).

In the present study, no clear relationship was detected between AEs and metal fractioning in the prey either at a tissue (dissection) or subcellular (ultracentrifugation) level (Fig. 2A,B). Therefore, our results do not support the hypotheses of Reinfelder & Fisher (1994) and of Wallace & Lopez (1996) in the case of turbot fed with complex pluricellular prey. Indeed, for Co and Zn, values of AE for the predator were lower than those expected from both hypotheses, which advocate for equivalence between AE and the metal fraction in the soft parts of the prey (Figs. 1A & 2A) or the metal-soluble fraction in the prey (Fig. 2B). The fraction of Co and Zn contained in the supposed bioavailable compartments of prey (i.e. soft tissues and soluble fraction) was not assimilated by the turbot (Figs. 1B & 2A,B). This overestimation of the bioavailable fraction of trace elements by measuring metals in the cytosolic fraction indicates that the 'TAM fraction' is not applicable to assess the trophically available fractions of Co and Mn in a natural prey of the turbot (Fig. 1A). One potential explanation could result from the ecology of turbot. Indeed, the optimal temperature of juvenile turbot (approx. 15°C as used in the present study) is lower than the temperature used for the examples men-

tioned previously (*Menidia* sp. and *Terapon jarbua*, respectively raised at 18 and 20°C). Acknowledging the positive relationship between temperature and the activity of the digestive enzymes in fish (Xiong et al. 2011), low temperature may lead to a low enzyme activity in turbot, resulting in a less efficient digestion of food and thus lower AEs than expected.

Interestingly, Mn was the only essential element for which the measured AE was greater than what was expected by theory (Figs. 1C & 2B), when the turbot were fed with shrimp or fish. In this specific case, the cytosolic fraction underestimated the fraction of the prey assimilated by the turbot (% soluble < AE). Therefore, the TAM theory (which adds the fraction in the organelles to the fraction present in the cytosol) may be relevant (Fig. 1), although our results cannot prove the equivalence of TAM and AE. Alternatively, our data suggest that other insoluble subcellular compartments of the prey found in the soft tissues (e.g. compare Fig. 2A,B) can be assimilated by the turbot (Fig. 1). For example, previous studies using invertebrate predators (i.e. 2 neogastropods fed with various species of molluscs and crustaceans) have shown that a part of the metal assimilated from the food was also associated with 'metal-rich granules' and 'cellular debris' of the prey (Cheung & Wang 2005, Rainbow et al. 2007). Indeed, metals bound in metal-rich granules appear to be more susceptible to the 'assimilatory powers' of neogastropod molluscs than those of other invertebrates, like decapod crustaceans (Wallace & Lopez 1997, Wallace & Luoma 2003, Rainbow et al. 2006, 2011). In this context, further studies are needed to assess which parts of the insoluble fraction compartments (which include organelles, cellular debris, and metal-rich granules; Fig. 1A) must be taken into account to accurately assess the trophically available fraction of Mn in a predator fish like the turbot.

Our results obtained in controlled conditions help understand the influence of diet on metal AEs. They also provide preliminary information on the contribution of each prey to the total intake of essential metals per ration in the fish, when taking into account the variation of natural diet assessed in the field. The natural diet of the juvenile turbot is mainly composed of crustaceans (in particular decapods), fish (mostly adults and larvae of small pelagic species; Fig. 4), and eventually polychaetes, although their relative proportion is variable and habitat- and season-dependent (Sparrevohn & Støttrup 2008, Florin & Lavados 2010). When combining our results from radiotracer experiments and the level of stable elements measured in typical prey, we estimate that, although polychaetes (ragworms) represent only 28% of the stomach contents of turbot, polychaetes contribute 38 to 58% and 40 to 78% to the total intake of Co and Mn, respectively, in the 'Low' and 'High' scenarios (Table 2, Fig 4C). Ragworms tend to concentrate metals in the marine environment (Table 4). Our results confirmed other field investigations (see reviews of Eisler 2009a,b) and showed higher concentrations of Co, Mn, and Zn in polychaetes than fish and crustaceans. In the case of Mn, the high contribution of polychaetes can be explained by the high Mn AE observed in the turbot fed with ragworms. In contrast, these turbot poorly assimilated Co, and the contribution of polychaetes was related to the high concentration of stable Co in this species. Another aspect revealed by our assessment is the limited contribution of fish to the intake of Zn (always <24%) despite the fact that this prey can represent up to 36% of the stomach contents of juvenile turbot (Fig. 4B). Shrimp generally provide a major part of the essential elements from food and are also the prey that have the highest protein level (Table 3), highlighting the nutritional and ecological importance of crustaceans in the diet of the turbot.

CONCLUSIONS

This study provides new information on essential element assimilation in a marine fish. Our results suggest that diet composition plays a significant role in the assimilation of essential elements ingested with food in the turbot *Scophthalmus maximus*. It also highlights that the supposed relationships between AE in predator and metal fractioning in prey are not necessarily confirmed when complex pluricellular food items are considered. Our simple model, based on the relative contribution of the different prey to essential metal uptake, emphasises the importance of crustaceans in the nutrition of turbot, although when seasonally available, polychaetes can make a disproportionately high contribution to dietary Co and Mn uptake by turbot.

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