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Abstract: Diet is an important pathway for metal uptake in marine organisms, and assimilation efficiency (AE) is one of the most relevant parameter to quantify trophic transfer of metals along aquatic food webs. The most commonly used method to estimate this parameter is pulse-chase feeding using radiolabeled food. This approach is, however, based on several assumptions that are not always tested in experimental context. The present work aimed at validating the approach by assessing single-feeding and multiple-feeding approaches, using a model species (the turbot *Scophthalmus maximus*). Using the kinetic data obtained from the single-feeding experiment, we tested whether the reconstruction of a multi-feeding was consistent with data provided by an actual multi-feeding performed under the same experimental conditions. Our results validated the single-feeding approach.

Keywords: Fish, Metals, Radioecology, Trophic transfer, Assimilation efficiency

INTRODUCTION

Fish are exposed to various sources of metals, including water and food. It has become increasingly clear that diet represents the main contribution to global accumulation of metals (such as Mn, Cd, and Zn) in marine fish (e.g. [1–3]). Understanding the trophic transfer of metals in fish is therefore key to properly qualify and quantify their accumulation capacities [4]. Bioaccumulation though trophic transfer in fish has been studied in natural environments (e.g. [5–7]), but the relationship between metal concentration in prey and bioaccumulation in consumers/predators is difficult to establish under these conditions. Indeed, metal concentrations in whole-body prey, identified by stomach content analysis, are often compared with those in predator specific tissues without questioning bioaccumulation and biotransformation processes, feeding relationships, and trophic status [7–10]. Experimental approach in controlled conditions is an excellent option to unambiguously assess the transfer of a metal from an organism to another [9], in particular by using radiotracer techniques because of their high sensitivity [11,12].

One of the most relevant parameters to quantify trophic transfer is the assimilation efficiency (AE), i.e. the proportion of metal in the prey that is assimilated by the consumer (e.g. [13–15]). AE can be compared quantitatively among different elements, biological models, food items and environmental conditions. To estimate this parameter, one valuable method used since the 1980s is the pulse-chase feeding (e.g. [16–18]). Briefly, this technique consists of feeding organisms with radiolabeled food (live prey or compounded feed) for a short period of time (typically shorter than their gut transit time) and then to follow the depuration kinetics of the radioisotopes [18,19]. The limited period of feeding ensures that the ingested fraction can be accurately quantified by counting the whole organism and allowing to limit the confounding influence of elimination thus avoids error in the assessment of AE [18-19]. This technique has the advantage to not requiring complete recovery of egested feces, and of allowing easier

estimation of AE than other methods. For example, the mass balance requires quantification of total ingestion, excretion, and egestion (i.e., loss of material in feces after absorption or post-ingestive metabolism) [12,19]. In principle, any radioisotope can be used. However, gamma-emitting radioisotopes are generally preferred as they allow radiocounting the predator alive, which allows limiting the number of individuals to sacrifice and generating data with reduced biological variability [12].

Experimental determination of AE using pulse-chase feeding approach assumes that each food ration is processed in the same way by the organism which is actually the prerequisite of this method as only one ration is followed, and then extrapolated to the entire digestion process. Some evidences indicate that this assumption may not always be satisfied. Indeed, some studies have suggested that concentration of Cd in food can impact its assimilation in sea urchins [18]. Furthermore, methods for determining the AE are variable in the literature [19, 20]. As explained by Wang and Fisher [19], two approaches are commonly used: the short-term and the long-term approaches. In the "short-term approach", the depuration phase is limited to a short period of time (i.e. gut purge phase), usually a few hours; [1,21,22]. Conversely, some authors recommend a "longer-term approach" (i.e. allowing to describe the loss of the fraction that is actually absorbed by the organism and slowly eliminated), for several days, weeks or months [15,23,24]. Using one method or the other may lead to variable AE measurements [19] with, in particular, a higher estimation of AE in the "short-term" experiments.

In this context, the present study aimed at validating the pulse-chase feeding approach (i.e. single-feeding) in the turbot *Scophthalmus maximus* fed with radiolabeled compounded pellets using gamma-emitters (^{109}Cd, ^{57}Co, ^{54}Mn and ^{65}Zn) through a single-feeding vs. a four-feeding experiment.

MATERIALS AND METHODS

Origin and acclimation of organisms

In January 2014, one hundred juvenile turbot *S. maximus* were purchased from a fish farm (France Turbot) and shipped to the IAEA-EL premises in the Principality of Monaco. Fish were acclimated to laboratory conditions for 21 days (open circuit, 500-L aquarium; water renewal: 100 L h^{-1}; 0.45-µm filtered seawater; salinity: 38 p.s.u.; temperature: 15 ± 0.5°C; pH: 8.1 ± 0.1; light/dark: 12h/12h). During the acclimation period, the fish were fed a daily ration of 2% of their estimated biomass with 1.1-mm pellets (Le Gouessant).

Experimental procedure

Radiolabeling of pellets

In order to compare metal AE estimates in *S. maximus* fed by single- or multi-feedings, 1.1-mm manufactured pellets (Le Gouessant) were radiolabeled. Radiotracers of high specific activity were purchased from Isotope Product Lab (^{109}Cd as $CdCl_2$ in 0.5 M HCl, [$T_{1/2}$] = 463.9 days; ^{57}Co as $CoCl_2$ in 0.1 M HCl, [$T_{1/2}$] = 271.8 days; ^{54}Mn as $MnCl_2$ in 0.5 M HCl, [$T_{1/2}$] = 312.2 days; ^{65}Zn as $ZnCl_2$ in 0.1M HCl, [$T_{1/2}$] = 243.9 days). Seventeen grams of dry pellets were dipped for 1 h in 22 mL of seawater previously spiked with 2 kBq mL^{-1} of ^{57}Co, ^{54}Mn and ^{65}Zn, and 4 kBq mL^{-1} of ^{109}Cd. Pellets were then dried for 48 h at 50°C and kept in a dry environment in order to prevent mold growth. The pellets used were radioanalysed (1g dry wet per measurement, i.e. ≈ 650 pellets) prior to each feeding. Activities were 4908 ± 155 Bq ^{109}Cd g^{-1} dry wt, 2305 ± 63 Bq ^{57}Co g^{-1} dry wt, 2215 ± 75 Bq ^{54}Mn g^{-1} dry wt and 2344 ± 79 Bq ^{65}Zn g^{-1} dry wt. In terms of stable metal concentration, these activities were negligible compared to those found in the pellets: they corresponded to 0.15 ng.g^{-1} for Cd, 0.3 ng g^{-1} for Co, 55 pg g^{-1} for Mn and 6 ng g^{-1} for Zn, which represent concentrations at least 3 orders of magnitude lower than those measured in non-radiolabelled pellets (see the details of the methodology in

Supplemental Data; 0.6 ± 0.0 µg Cd g^{-1} dry wt, 0.3 ± 0.0 µg Co g^{-1} dry wt, 66.4 ± 0.2 µg Mn g^{-1} dry wt and 139 ± 4 µg Zn g^{-1} dry wt).

Although the acclimated fish consumed pellets in less than 1 min during regular feeding events, preliminary tests were performed to assess the possible leakage of radioisotopes from the pellets when placed in seawater. These tests consisted of pouring dry radiolabeled pellets (100 mg per treatment) for 1 to 10 min in 50 mL seawater and to measure any radioactivity in the seawater. This ratio pellets/seawater was intentionally high (approx. 10 times higher than in experimental conditions; worst case scenario). The radiotracer leakage from pellets in seawater never exceeded 0.8% and 16% of the initial activity after 1 min and 10 min, respectively. Although these tests confirmed a sole contamination pathway (viz. food) of the fish, two additional turbot were used in each treatment, as controls to take into account the possibility of radiotracer recycling through seawater (see section 2.2.2).

Single-feeding experiment

Single-feeding (protocol detailed in Figure 1A) with radiolabeled pellets was carried out using 5 juvenile turbot (22.5 ± 5.8 g wet wt) randomly picked and transferred into an aerated, open circuit 70-L aquarium (water renewal: 100 L h^{-1}; 0.45-µm filtered seawater; salinity: 38 p.s.u.; temperature: 15 ± 0.5°C; pH: 8.1 ± 0.1; light/dark: 12h/12h). Slits cut into the fins were used to facilitate individual recognition. Turbot were constantly fed with radiolabeled pellets over a 30-min period. During this feeding period, care was taken to ensure an instant ingestion of the pellet provided to the fish and if few pellets were uneaten they were rapidly removed to avoid radioactive leaching from the radiolabeled pellets. Two additional, non-exposed (non-fed) turbot were placed within a net in the same aquarium to check any radiotracer recycling from seawater due to possible radiotracer leaching from the contaminated food or from fish depuration. After the feeding period, all turbot were whole-body γ-counted alive and then

replaced in clean, flowing seawater conditions (parameters as previously described). All the individuals (including control individuals) were then γ-counted alive for the first time 2h after the radiolabeled feeding and then at different time intervals over a 21-d period to follow the depuration kinetics of the radiotracers. The aquarium was cleaned during the counting to avoid contamination from radiotracers contained in feces. Fish were fed non-labeled pellets one time per day (2% of their biomass).

Since body size (i.e. age) is known to affect metal bioconcentration in marine organisms [25], the single-feeding experiment was repeated in the same conditions, using smaller size juvenile turbot (8.05 ± 2.14 g, wet wt). Detailed methods and results are available in the Supplemental Data (out of the main scope of the present study). No difference of AE was observed between the 2 fish sizes for any of the elements tested (see Figure S1).

Multi-feeding experiment

Multi-feeding exposure (see Figure 1B) to radiolabeled pellets was carried out using 5 juvenile turbot (20.2 ± 2.9 g wet wt) kept in the same conditions as described in section 2.2.2. One week before the exposure, turbot were individually identified as described previously. In this experiment, 4 feedings were carried out (each time, turbot were fed for 30 min; the uneaten food was then removed) during a 12-day period (one-labeled pellet feeding every 4 days; Figure 1B). Between each labeled-pellet feeding, fish were fed daily with non-labeled pellets. The duration (4 d) between two labeled feedings was chosen to match with the beginning of the "slowest" part of the depuration phase as determined in the single-feeding experiment, i.e. when the activity in the turbot tended to stabilize. Each fish was whole-body γ-counted alive 2 h before and 2 h after each feeding exposure and then replaced in clean, flowing seawater conditions (parameters previously described). The 2-h period between the feeding and the counting was adjusted to guarantee the minimum digestion process on the ingested 7adiolabelle

pellets and at the same time to avoid their potential regurgitation during handling. Control individuals were placed in the aquarium as previously described and depuration was followed in each individual by daily whole-body γ-counting over 21 days. No mortality was recorded during all the experiments.

Radioanalyses

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 analyzer and a computer equipped with spectra analysis software (Interwinner 6, Intertechnique). Radioactivity 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. Organisms were counted in plastic containers (diameter: 80 mm, height: 50 mm) filled with 150 ml of clean seawater during the counting period. The counting time was adjusted to obtain a propagated counting error less than 5% [26,27]. The counting time varied between 25 and 60 min in order to maintain fish health and ensure normal behaviour.

Data treatment and statistical analyses

Validation of the single-feeding approach

In order to validate the single-feeding approach, we tested whether the reconstruction of a multi-feeding (using the kinetic data obtained from the single-feeding experiment) was consistent with data provided by an actual multi-feeding performed under the same experimental conditions.

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*100 [18]). These kinetics were best fitted using a two-component exponential model:

$$A_t = A_{0s} \text{ x } e^{-k_{es}t} + A_{0l} \text{ x } e^{-k_{el}t}$$

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 feces). The "l" component describes the depuration of the radiotracer fraction that is actually absorbed by the organism and eliminated slowly [18]. The long-lived component allows estimating the assimilation efficiency (AE) of the radiotracer ingested with food ($AE = A_{0l}$). Thus, AE could be defined as the fraction of the radiotracer pool that is incorporated (tightly bound or not) into the tissues of the organism [18]. In the present study, the depuration of the assimilated fraction of all elements was very slow. The long-term depuration rate constant (k_{el}) was not significant different from 0 and the "l" component of the model could therefore be simplified and replaced by a constant [28] and the equation becomes:

$$A_t = A_{0s} \text{ x } e^{-k_{es}t} + A_{0l}$$

with $A_{0l} = AE$

A short-term biological half-life can be calculated ($T_{b1/2}$) from the depuration rate constant according to the relation $T_{b1/2s} = \ln 2/k_{es}$. Model constants were estimated by iterative adjustment of the model using the Quasi-Newton method in the Statistica software 7.0.

Reconstructed vs actual multi feeding

A theoretical multi-feeding was built using kinetics parameters obtained from the single-feeding and compared with data measured during our multi-feeding experiment. The expected values of our model (i.e. assuming that the experience of multi-feeding is like a succession of independent single-feedings) were calculated for each individual. At this end, the ingested quantities during each feeding were estimated by subtracting the activity measured 2 hours before feeding to that measured 2 hours after. From these values and the kinetic parameters obtained in the single-feeding experiment (see the section Validation of the single-feeding approach), it was possible to calculate the remaining activities after 4 days of depuration for each the feedings. Taking into account the residual activities from the previous feedings, we reconstructed the evolution of the theoretical whole-body activity in the multi-fed turbot (n = 5). Then, reconstructed activities were compared to the activities measured at the same times in the actual multi-feeding experiment using non-parametric Mann-Whitney U test [29]. The level of significance for statistical analyses was always set at $\alpha = 0.05$. All the statistical analyses were performed using the R software 3.0.1 [30].

Complementary data from the multi-feeding experiment

As indicated earlier in section 2.2.3, γ-countings were performed on live turbot before and after each of the 4 feeding events, allowing an individual determination of the gain and loss of the radiotracers between the feedings of the turbot with radiolabeled pellets. Calculation of each new input of pellet-borne tracers transferred to the fish was then possible considering the whole-body activity measured 2h after the ingestion of 10adiolabelled pellet minus the background activity (measurement done before the new feeding; Figure 1B). This can be compared with the whole-body activity after 4 d without new inputs (measurement before the next feeding with

radiolabelled pellets) for which the "background" activity can also be subtracted to allow comparing each feeding as an independent single-feeding.

RESULTS

Depuration kinetics after the single-feeding exposure

Whole-body depuration kinetics of ^{109}Cd, ^{57}Co, ^{54}Mn, and ^{65}Zn in single-fed turbot were always best fitted by a two-phase model (one exponential component model and a constant; Table 1 and Figure 3; R^2: 0.98-0.99). The assimilation efficiency (AE) depended on the investigated metal, with average values ranging from 1% for Co to 23% for Mn (Table 1). As indicated in the Supplemental Data S2, no difference of AE was observed between the 2 fish sizes for all elements tested.

Reconstructed vs. actual multi-feeding

Figure 2 displays the activities of the reconstructed multi-feeding (using model parameters from the single-feeding experiment) as well as those actually measured in the multi-feeding experiment. The comparison of the reconstructed vs. actual data did not reveal any significant difference (p>0.05) in terms of whole-body activities, for all the 4 feedings, whatever the considered metal.

Variability among the successive feedings

The multi-feeding experiment allowed following how the whole-body activity in the multi-fed turbot changed at each feeding (4 feedings in 12 d with radiolabeled pellets) and during the depuration period (Figure 4). The whole-body activity after 4 days and 21 days of depuration were not significantly different (p>0.05; Figure 4, Table 2). At the end of the depuration period

(21 d), total retained activity represented less than 1% of the total ingested activity for Co and up to 23% for Cd.

For the individuals (n=3) that had successively and significantly eaten four times, the multi-feeding exposure led to a linear increase of the whole-body activity in turbot for the four studied elements (Figure 4). In order to get a better understanding of the whole-body metal retention after each feeding, a ratio between ingested and remaining activities was calculated (after subtraction of the "background" activity). Our results indicate that, for each element, this ratio decreased throughout the multi-feeding experiment with values ranking from 3 (Mn) to 56 (Co) for the first feeding against less than 2 (Mn) to 31 (Co) for the 4^{th} feeding (Figure 4, Table 3).

DISCUSSION

The present study aimed at testing the validity of the single-feeding approach commonly used to assess trophic transfer of contaminants in marine organisms [31,32]. The protocol consisted of conducting in parallel two experiments where turbot, kept in controlled laboratory conditions, were exposed to the metals 1 single time or 4 successive times, using radiolabeled pellets. Kinetic data from the single-feeding experiment then allowed building a reconstructed multi-feeding situation and comparing it with the observations made under actual multi-feeding conditions.

The depuration of metal transfer to the turbot through the food was always characterized by a biphasic process. Depuration kinetics from the single-feeding experiment were always best described by a model including exponential component and a constant for all the studied elements. Such biphasic depuration kinetics for metals are commonly observed in aquatic organisms (e.g. [14,18,33]). The constant indicated that the assimilated fraction of metal was

strongly bound after 4 days. Indeed, the whole-body activity was not significantly different between 4 and 21 d after of depuration. This finding corroborates the results from a previous study on the trophic transfer of essential elements in the same fish, using natural prey [15, 28]. Our results also indicate that assimilation efficiency (AE) is metal dependent. AE of Mn was higher than one of the other three elements: AEs of Cd and Zn were very close (respectively 14% and 13%) whereas, in contrast, Co was poorly assimilated (AE ≈ 1%). According to the literature available on this fish, AEs observed in the present study appeared to be relatively low (Table 3; [23]). However, some authors (e.g. [34, 35]) have already highlighted that AEs of metals in fish are highly dependent on the diet composition and on metal speciation and, to date, investigations using commercial food as we did here are still limited to a small number of species and few elements (Table 3; [15,12,34]).

Our results displayed in Figure 2 indicate that the reconstruction of a multi-feeding (using the data from a single-feeding, then repeated over time) is consistent with the data provided by an actual multi-feeding performed under the same conditions. These results providing an experimental validation of the single-feeding approach widely reported in the literature since the 1980s [16-18]. It would however be of interest to study the influence of repeated dietary exposures over a longer period of time in order to confirm the trends observed in the present study over 4 feedings. The single-feeding approach has been used to determine AE in various aquatic organisms such as crustaceans, echinoderms, molluscs and fish. In order to get an exhaustive validation of this approach it would also be appropriate to expand the protocol applied in the present study to other biological models exposed to different food items.

In addition to provide the experimental validation of the single-feeding approach, the experimental protocol used in the present study allows better understanding the mechanisms involved in the storage and depuration of trace elements during a multiple trophic exposure. In multi-fed turbot, the whole-body activity increased linearly for all the metals after each of the

4 radiolabeled feedings. Despite the increase in metal concentrations in turbot in response to the multiple-exposure, the percentage of retained activity from each ration was constant during the entire multi-feeding experiment. Some authors have already highlighted that pre-exposure to contaminated food had no effect on the assimilation [35]. Indeed, in the black sea bream *Acanthopagrus schlegeli* and the grunt *Terapon jarbua* AE of Cd and Zn was not influenced significantly following Zn dietary pre-exposure for 1 or 3 wk. Our results indicate that metal storage capacities of turbot are not limited over the 3-week period of exposure. In this context, we can assume that there are neither major changes in metal regulatory mechanisms in this species nor toxic effects of the metal on the assimilation process.

SUPPLEMENTAL DATA

Supplemental Data provide information regarding on the methodology used for stable metal analysis in pellets including the comparison between measured and certificated values in a reference material (Table S1). Thus, we provided kinetics of depuration for two batches of turbots of two different sizes (Figure S1).

ACKNOWLEDGMENT

The IAEA is grateful for the support provided to its Environment Laboratories by the Government of the Principality of Monaco. MW is an Honorary Senior Research Associate of the National Fund for Scientific Research (NFSR, Belgium).

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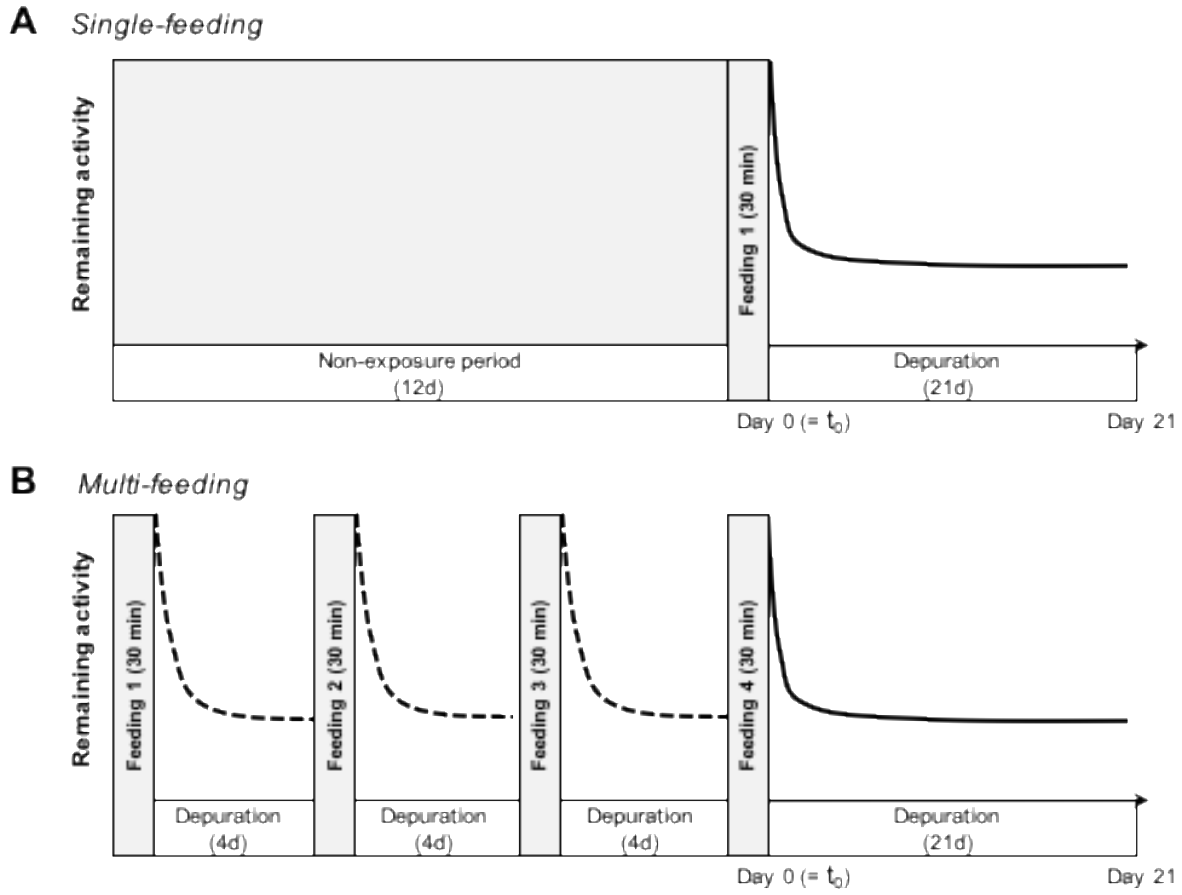

Figure 1. Protocols of (A) single-feeding and (B) multi-feeding experiments. For each radiolabeled pellet feeding, turbot were fed *ad libitum* for 30 min and the uneaten food was then removed. For comparison between single-feeding and multi-feeding experiments, we considered the Day 0 and the Day 21 respectively as the beginning and the end of the depuration period after the last feeding.

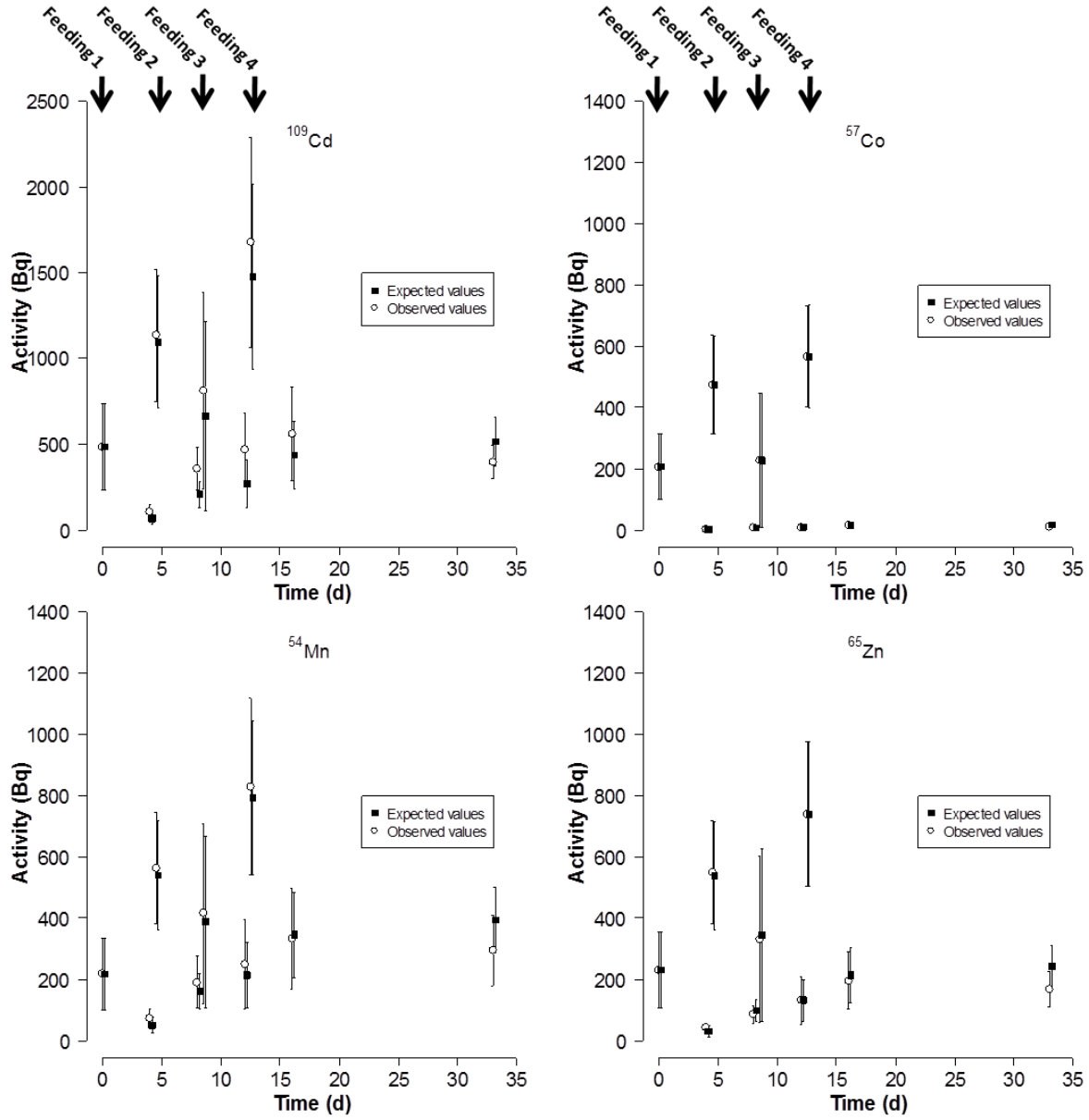

Figure 2. Comparison of activities measured at different times in multi-feeding experiment and those expected using the kinetic parameters obtained from single-feeding experiment experiment (i.e. assuming that the experience of multi-feeding is similar to a succession of independent single-feedings; see "Material and Methods" section for more details). Values (Bq) are means ± SD; n=5.

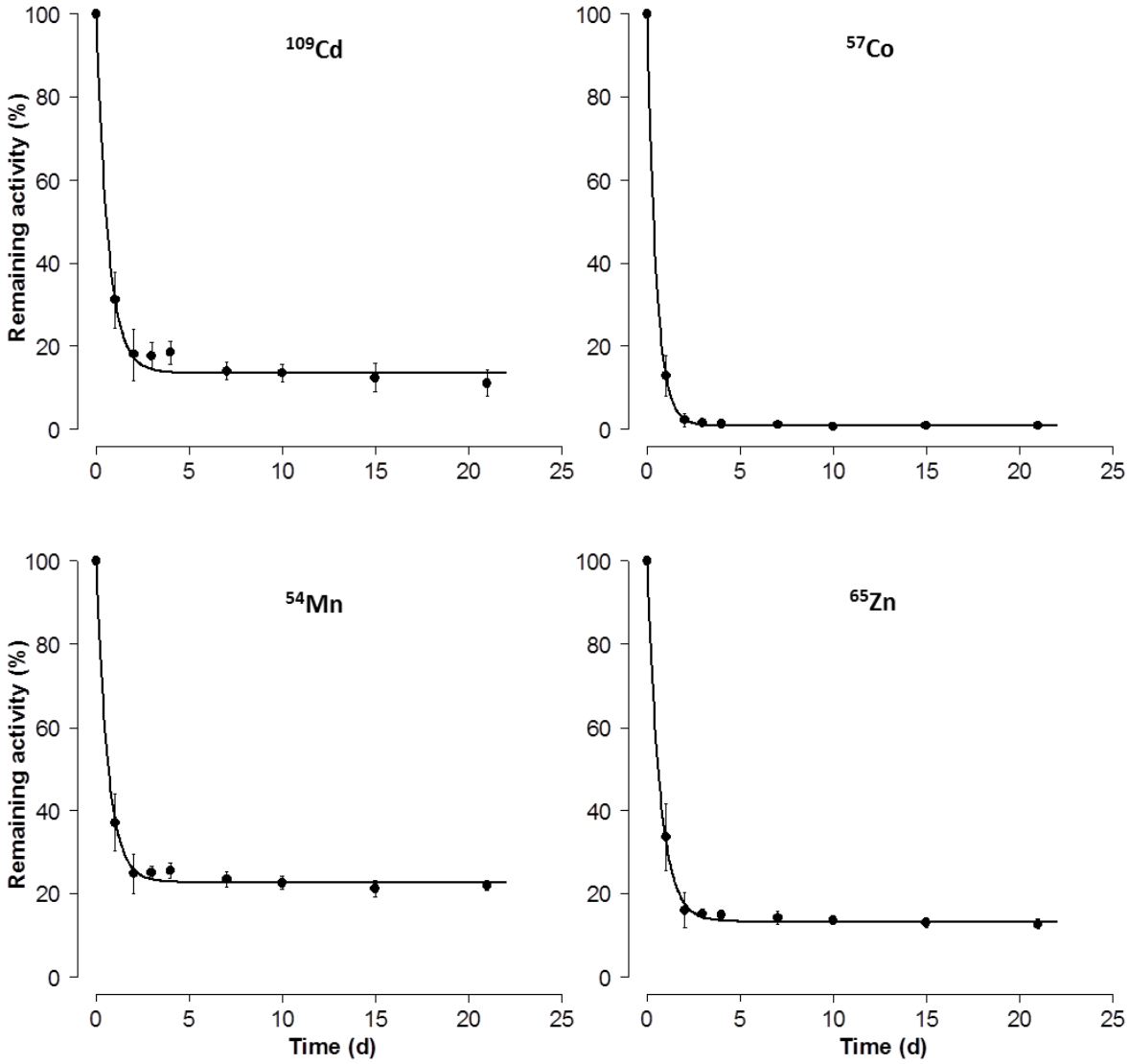

Figure 3. Kinetics of the whole-body depuration of ^{109}Cd, ^{57}Co, ^{54}Mn and ^{65}Zn in single-fed turbot (% remaining activities, means ± SD, n=5). Parameters and statistics of depuration kinetics are given in Table 1.

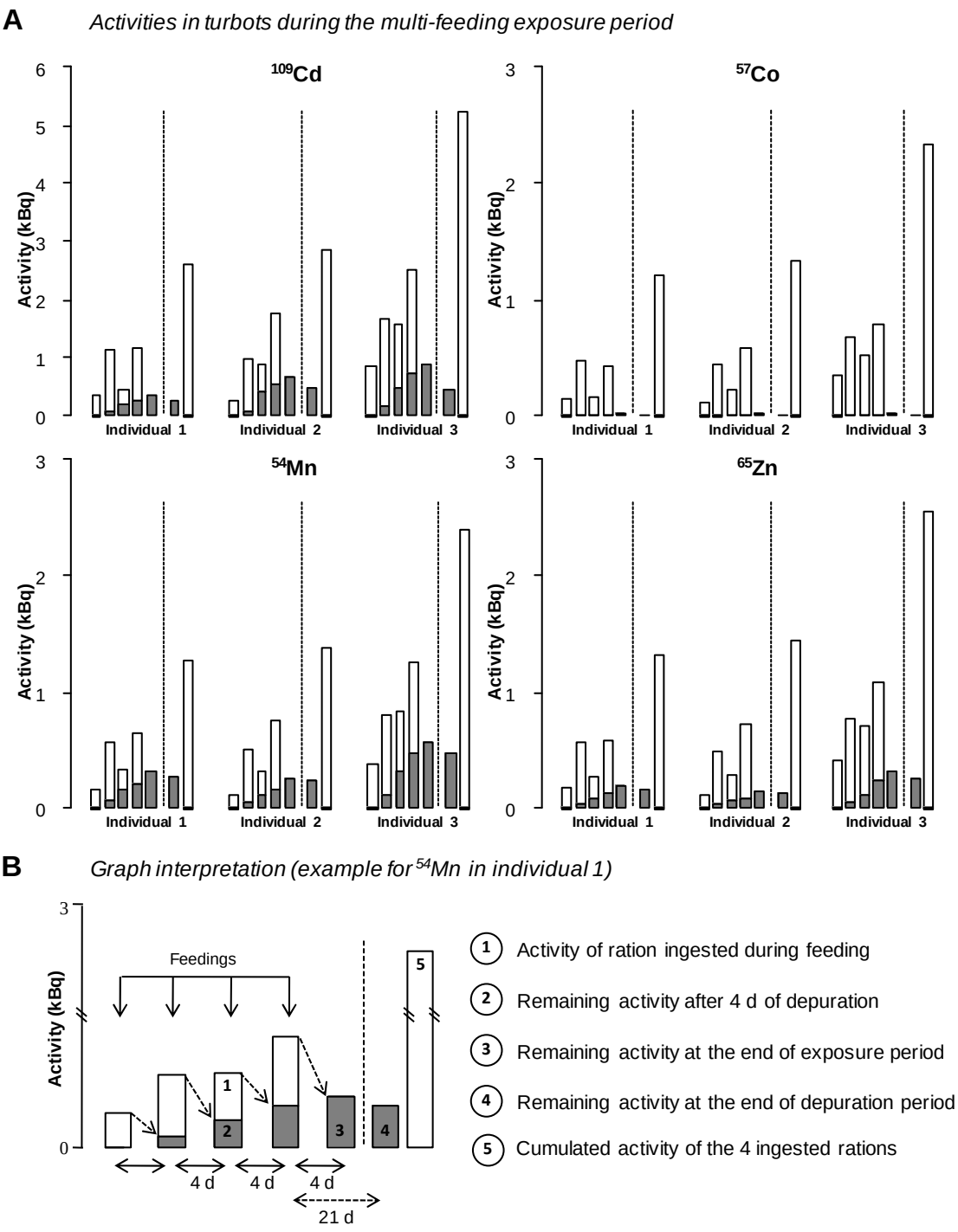

Figure 4. Uptake of ^{109}Cd, ^{57}Co, ^{54}Mn and ^{65}Zn by the turbot during the multi-feeding exposure (12d). Only the three individuals having eaten during the 4 radiolabeled pellet feedings are represented. Values (kBq) are means ± SD; n=3.

Table 1. Estimated depuration kinetic parameters of ^{109}Cd, ^{57}Co, ^{54}Mn and ^{65}Zn in turbot exposed to the radiotracers by single-feeding with labeled pellets (n = 5 per treatment) and then maintained for 21d in clean seawater. k_{es}: depuration rate constant (d^{-1}); Tb½s: biological half-life (d); AE: assimilation efficiency (%); ASE: asymptotic standard error; R^2: determination coefficient.

Tracer	Experiment	Short-term		Long term	R^2
		k_{es}± ASE	$T_{b½s}$ ± ASE	AE ± ASE	
^{109}Cd	SF	1.59 ± 0.10***	0.44 ± 0.03	13.8 ± 0.7***	0.98
^{57}Co	SF	2.12 ± 0.06***	0.33 ± 0.01	1.0 ± 0.3***	0.99
^{54}Mn	SF	1.62 ± 0.09***	0.43 ± 0.02	22.9 ± 0.5***	0.99
^{65}Zn	SF	1.50 ± 0.08***	0.46 ± 0.02	13.4 ± 0.6***	0.99

Probability of the model adjustment:

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

Table 2. Different ratios calculated between ingested and retained activity during the multi-feeding and the single-feeding exposures. Only the three individuals having eaten during the 4 radiolabeled pellet feedings are included in the calculations.

Experiment			^{109}Cd	^{57}Co	^{54}Mn	^{65}Zn
Multi-feeding experiment	Ingested activity (Bq, Mean ± SD)		2975 ± 1301	1338 ± 640	1405 ± 581	1048 ± 448
	Remaining activity after the 4th feeding (Bq, Mean ± SD)	Depuration (4 d)	643 ± 264	19 ± 3	380 ± 166	225 ± 92
		Depuration (21 d)	391 ± 119	12 ± 1	322 ± 125	183 ± 62
	Ratio between ingested and remaining activity after 4 days	Feeding 1	4.64 ± 1.38	55.47 ± 11.81	2.75 ± 0.56	5.29 ± 2.23
		Feeding 2	3.61 ± 1.76	65.89 ± 15.71	3.14 ± 1.00	6.50 ± 1.20
		Feeding 3	1.09 ± 0.36	26.41 ± 13.09	1.08 ± 0.27	2.14 ± 0.75
		Feeding 4	2.10 ± 0.34	30.86 ± 6.28	1.70 ± 0.51	3.06 ± 1.10
	Ratio between cumulated activity in the 4 feedings and remaining activity at the end of the depuration period		9.31 ± 2.92	138.40 ± 42.58	5.27 ± 0.50	9.70 ± 1.35
Single-feeding experiment	Total ingested activity (Bq, Mean ± SD)		1410 ± 440	614 ± 202	637 ± 219	663 ± 224
	Remaining activity (Bq, Mean ± SD)	Depuration (4 d)	265 ± 99	8 ± 3	164 ± 60	101 ± 37
		Depuration (21 d)	160 ± 77	6 ± 3	142 ± 51	86 ± 35
	Ratio between ingested and remaining activity at the end of the depuration period		9.64 ± 2.58	116.93 ± 41.82	4.56 ± 0.27	7.91 ± 0.80

Table 3. Comparison of metal dietary assimilation efficiencies (AEs; Means in %) in marine and brackish water fish species.

Species	Food	Metal (AE)				References
		Cd	Co	Mn	Zn	
Acanthopagrus schlegelii (Blackhead seabream)	Artificial diets	8-14			15-26	[22]
	Mullet muscle	41			42	[22]
	Mussel tissue	20			25	[22]
	Squid viscera	40			14	[22]
	Brine shrimp	5-10			12-34	[36]
Ambassis urotaenia (Banded-tail glassy perchlet)	Brine shrimp nauplii	27-33			15-17	[37]
	Copepods	14-15			5-9	[37]
Dicentrarchus labrax (European seabass)	Seabream juveniles	23	21	33	38	[24]
Lutjanus argentimaculatus (Mangrove red snapper)	Brine shrimp	10			15	[1]
	Clam tissue	9			30	[1]
	Copepods	6			20	[1]
	Manilla clam	7			19	[1]
Menidia sp. (Silversides)	Copepods	3	2		6	[38]
Periophthalmus cantonensis (New Guinea mudskipper)	Brine shrimp larvae	15-26			11-21	[37]
	Copepods	10-22			21-31	[37]
Scophthalmus maximus (Turbot)	Seabream juveniles		27		22	[23]
	Ragworms		5	44	17	[33]
	Seabream juveniles		44	23	23	[33]
	Shrimp		16	42	32	[33]
Sparus aurata (Gilthead seabream)	Brine shrimp nauplii	45	21	25	18	[23]
Terapon jarbua (Jarbua terapon)	Barnacles	3			2	[34]
	Copepods	6			23	[34]
	Clams	9			36	[34]
	Fish viscera	6			52	[34]
	Mussels	4			22	[34]

SUPPLEMENTAL DATA

Stable metals in pellets

For essential element analyses, samples (n = 3) of 250 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 (1600W). After the mineralization process, each sample was diluted to 50 mL with milli-Q quality water and an extra 1:5 dilution was prepared. Cd, 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 analytical batch. The detection limits were (µg g^{-1} dwt): 0.025 (Cd, Co, Mn) and 2 (Zn). All metal concentrations are given on a dry weight basis (µg g^{-1} dwt).

Table S1. Comparison of metal concentration (mean ± SD, n = 3) in reference material (fish muscle, IAEA 407) measured by ICP-MS (Cd, Co and Mn) and by flame atomic absorption spectrometry (Zn) with certified values. All the values are expressed in µg.g^{-1} dwt.

Element	Measured	Certified
Cd	0.133 ± 0.002	0.189 ± 0.019
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

Single-feeding experiments and size-effect

The first experiment was a single exposure to radiolabelled pellets (single feeding method) carried out using juvenile turbots (n=15, 8.05 ± 2.14 g, wet weight) randomly picked and transferred in an aerated, open circuit, 70-L aquarium. The same protocol was repeated in the same conditions using larger juvenile turbot (n=5, 22.5 ± 4.6 g, wet weight).

Whole-body depuration kinetics of ^{109}Cd, ^{57}Co, ^{54}Mn, and ^{65}Zn in turbots exposed by single-feeding were best fitted by a two-phase model (simple-exponential model and a constant; R^2: 0.94-0.99). The retention of the four radiotracers depended on the studied metal. The major fraction (77-99%; Fig. 2) of the four elements is very rapidly lost ($T_{b½s}$ < 2.2 d; Table).

We observed no significant different ($p>0.05$) between the AE of turbots exposed to the radiotracers by single-feedings during the experiment 1 (smaller individuals: 8.05 ± 2.14 g) and experiment 2 (larger individuals: 22.5 ± 4.6 g). However, comparison of k_{es} between the two single-feedings indicates that the values of this parameter were significantly lower ($p<0.01$) in the smaller turbots (Fig. S1).

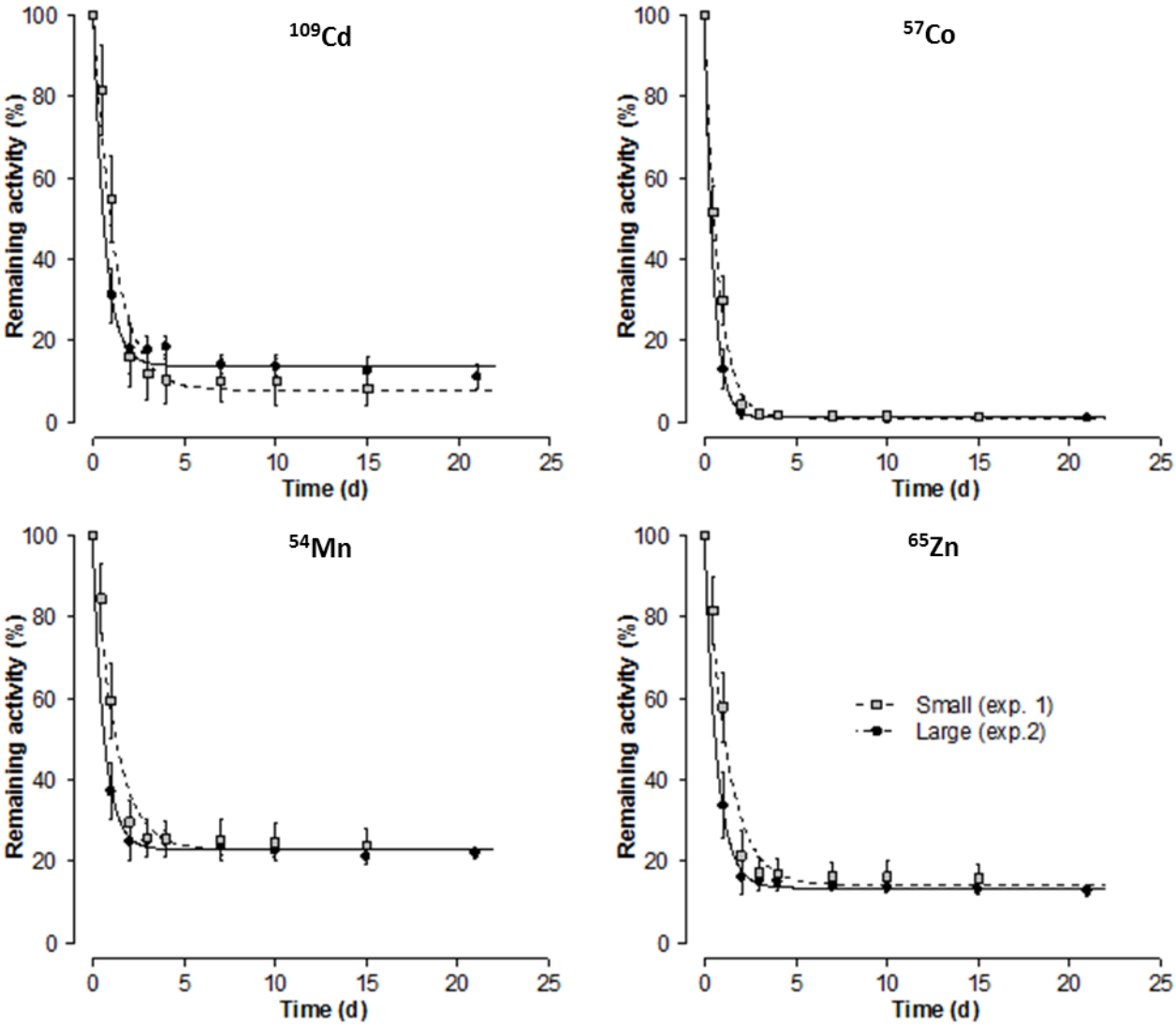

Figure S1. Kinetics of the whole-body depuration of ^{109}Cd, ^{57}Co, ^{54}Mn and ^{65}Zn in juvenile turbot (% remaining activities, means ± SD, n=5-15) of two different sizes after a single-feeding with radiolabeled pellets.