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COMPARING SINGLE-FEEDING AND MULTI-FEEDING APPROACHES FOR EXPERIMENTALLY ASSESSING TROPHIC TRANSFER OF METALS IN FISH

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Abstract: Diet is an important pathway for metal uptake in marine organisms, and assimilation efficiency is one of the most relevant parameters 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 an experimental context. The present study aimed to validate 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, the reconstruction of a multi-feeding experiment was tested for consistency with data provided by an actual multi-feeding performed under the same experimental conditions. The results validated the single-feeding approach. *Environ Toxicol Chem* 2017;36:1227–1234.
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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 the global accumulation of metals (such as Mn, Cd, and Zn) in marine fish [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 [5–7], but the relationship between metal concentrations 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 specific predator tissues without questioning bioaccumulation and biotransformation processes, feeding relationships, and trophic status [7–10]. An experimental approach under 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 for quantifying trophic transfer is the assimilation efficiency—the proportion of metal in the prey that is assimilated by the consumer [13–15]. Assimilation efficiency can be compared quantitatively among different elements, biological models, food items, and environmental conditions. To estimate this parameter, 1 valuable method used since the 1980s is the pulse-chase feeding [16–18]. Briefly, this technique consists of feeding organisms with radiolabeled food (live prey or compounded feed) for a short period (typically shorter than their gut transit time) and then following 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 limiting the confounding influence of elimination, thus avoiding errors in the assessment [18,19]. This technique has the advantage of not requiring complete recovery of egested feces, and of allowing easier estimation of assimilation efficiency than other methods. For example, the mass-balance method requires quantification of total ingestion, excretion, and egestion (i.e., loss of material in feces after absorption or postingestive metabolism) [12,19]. In principle, any radioisotope can be used. Gamma-emitting radioisotopes are generally preferred, however, because they allow the predator to be radiocounted alive, which limits the number of individuals to be euthanized and generates data with reduced biological variability [12].

Experimental determination of assimilation efficiency using the 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 1 ration is followed and then extrapolated to the entire digestion process. Some evidence indicates that this assumption may not always be satisfied. Indeed, some studies have suggested that Cd concentration in food can impact its assimilation in sea urchins [18]. Furthermore, methods for determining the assimilation efficiency are variable in the literature [19,20]. As explained by Wang and Fisher [19], 2 approaches are commonly used: short-term and long-term approaches. In the short-term approach, the depuration phase is limited to a short period (i.e., gut purge phase), usually a few hours [1,21,22]. Conversely, some authors recommend a longer term approach (i.e., allowing description of 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 1 method or the other may lead to variable assimilation efficiency measurements [19] with, in particular, a higher estimation of assimilation efficiency in the short-term experiments.

In this context, the present study aimed to validate the pulse-chase feeding approach (i.e., single-feeding) in the turbot

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Scophthalmus maximus fed with radiolabeled compounded pellets using gamma-emitters (^{109}Cd, ^{57}Co, ^{54}Mn, and ^{65}Zn) through a single-feeding versus a 4-feeding experiment.

MATERIALS AND METHODS

Origin and acclimation of organisms

In January 2014, juvenile turbot *S. maximus* were purchased from a fish farm (France Turbot) and shipped to the International Atomic Energy Agency-Environment Laboratories 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, 8.1 ± 0.1; 12:12-h light:dark cycle). 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. To compare metal assimilation efficiency 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, half-life [$t_{1/2}$] = 463.9 d; ^{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 0.1 M HCl, $t_{1/2}$ = 243.9 d). For 1 h, 17 g of dry pellets were dipped 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 to prevent mold growth. The pellets used were radioanalyzed (1 g dry wt/measurement; i.e., ≈650 pellets) prior to each feeding. Activities were 4908 ± 155 Bq ^{109}Cd g^{-1} dry weight, 2305 ± 63 Bq ^{57}Co g^{-1} dry weight, 2215 ± 75 Bq ^{54}Mn g^{-1} dry weight, and 2344 ± 79 Bq ^{65}Zn g^{-1} dry weight. In terms of stable metal concentration, these activities were negligible compared with 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 nonradiolabeled pellets (see the details of the methodology in the Supplemental Data; 0.6 ± 0.02 μg Cd g^{-1} dry wt, 0.3 ± 0.01 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/treatment) for 1 min to 10 min in 50 mL of seawater and measuring any radioactivity in the seawater. This ratio of pellets to seawater was intentionally high (∼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 (i.e., food) of the fish, 2 additional turbot were used in each treatment as controls to take into account the possibility of radiotracer recycling through seawater (see the *Single-feeding experiment* section).

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 psu; temperature, 15 ± 0.5 °C; pH, 8.1 ± 0.1; l2:12-h light: dark cycle). 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; if few pellets were uneaten, they were rapidly removed to avoid radioactive leaching from the radiolabeled pellets. Two additional, nonexposed (nonfed) turbot were placed within a net in the same aquarium to check any radiotracer recycling from seawater as a result of 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 individuals (including control individuals) were then γ-counted alive for the first time 2 h 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 nonlabeled pellets once per day (2% of their biomass).

Because 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. No difference in the assimilation efficiency was observed between the 2 fish sizes for any of the elements tested (Supplemental Data, Figure S1).

Multi-feeding experiment. Multi-feeding exposure (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 the *Single-feeding experiment* section. Turbot were individually identified 1 wk before the exposure, 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-d period (1 labeled-pellet feeding every 4 d; Figure 1B). Between each labeled-pellet feeding, fish were fed daily with nonlabeled pellets. The duration (4 d) between 2 labeled feedings was chosen to match up 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 for the ingested radiolabeled 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 d. No mortality was recorded during any of 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 multichannel analyzer and a computer equipped with spectra analysis software (Interwinner 6; Intertechnique). Radioactivity was determined by comparison with standards of known activity and appropriate geometry (calibration and counting). Measurements were corrected for background and physical radioactive decay. Organisms were counted in plastic

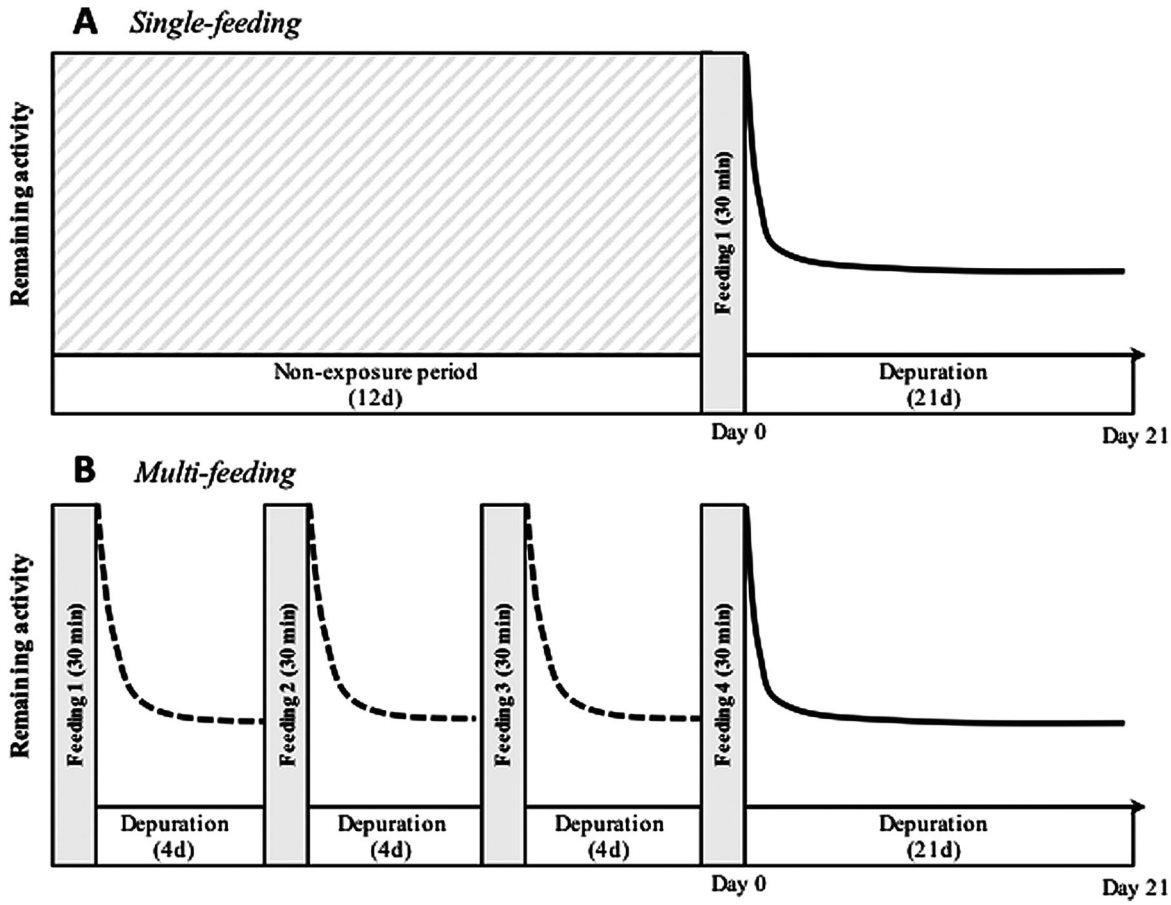

Figure 1. Protocols of (**A**) single-feeding and (**B**) multi-feeding experiments. For each radiolabeled pellet feeding, turbot were fed ad libitum for 30 min and the uneaten food was then removed. For comparison between single-feeding and multi-feeding experiments, we considered day 0 and day 21,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

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 min and 60 min to maintain fish health and ensure normal behavior.

Data treatment and statistical analyses

Validation of the single-feeding approach. To validate the single-feeding approach, we tested whether the reconstruction of a multi-feeding experiment (using the kinetic data obtained from the single-feeding experiment) was consistent with data provided by an actual multi-feeding experiment 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 $\times$ 100 [18]). These kinetics were best fitted using a 2-component exponential model:

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

where A_t and A_0 are the remaining activities (%) at time t (d) and time 0, respectively; k_e is the depuration rate constant (d^{-1}); and the 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 of the radiotracer ingested with food ($AE = A_{0l}$). Thus, assimilation efficiency could be defined as the fraction of the radiotracer pool that is incorporated (tightly bound or not) into the tissues of the organism. 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 significantly different from 0; the l component of the model could therefore be simplified and replaced by a constant [28], and the equation becomes:

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

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 Statistica software 7.0.

Reconstructed versus actual multi-feeding. A theoretical multi-feeding was built using kinetics parameters obtained from the single-feeding experiment 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 similar to a succession of independent single

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 21 d in clean seawater

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

***Probability of the model adjustment: $p < 0.001$.
k_{es} = depuration rate constant (d^{-1}); $t_{b½s}$ = biological half-life (d); AE = assimilation efficiency (%); ASE = asymptotic standard error; R^2 = determination coefficient.

feedings) were calculated for each individual. At this end, the ingested quantities during each feeding were estimated by subtracting the activity measured 2 h before feeding to that measured 2 h after. From these values and the kinetic parameters obtained in the single-feeding experiment (see the *Validation of the single-feeding approach* section), it was possible to calculate the remaining activities after 4 d of depuration for each of 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 with the activities measured at the same times in the actual multi-feeding experiment using the nonparametric Mann–Whitney U test [29]. The level of significance for statistical analyses was always set at $\alpha = 0.05$. All statistical analyses were performed using R software, Ver 3.0.1 [30].

Complementary data from the multi-feeding experiment. As indicated in the *Multi-feeding experiment* section, 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 2 h after the ingestion of radiolabeled 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 radiolabeled pellets) for which the so-called background activity can also be subtracted to allow comparison of 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 2-phase model (1 exponential component model and a constant; Table 1 and Figure 2; $R^2 = 0.98$–0.99). The assimilation efficiency depended on the metal investigated, with average values

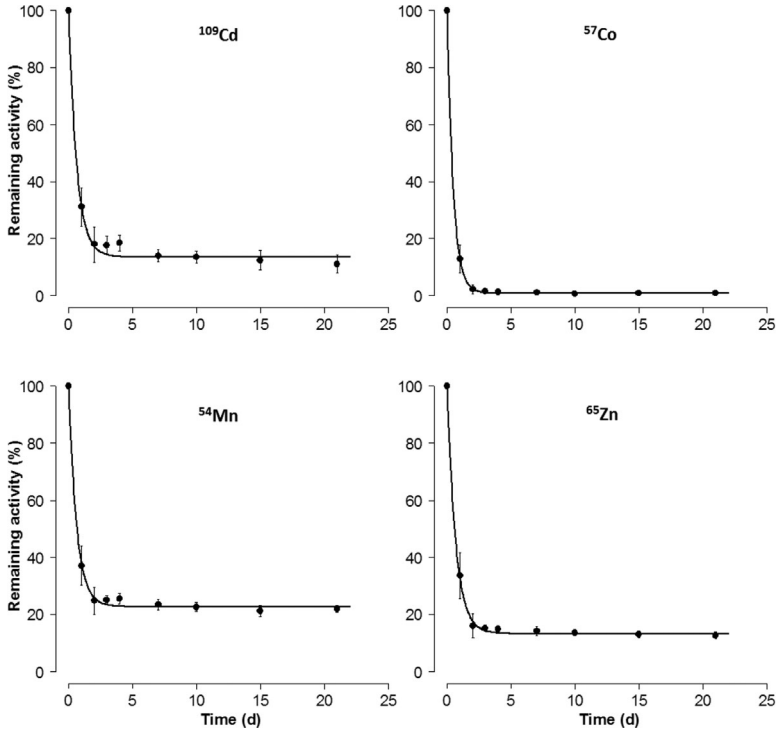

Figure 2. Kinetics of the whole-body depuration of ^{109}Cd (**A**), ^{57}Co (**B**), ^{54}Mn (**C**), and ^{65}Zn (**D**) in single-fed turbot (% remaining activities; means ± standard deviation, $n = 5$). Parameters and statistics of depuration kinetics are given in Table 1.

ranging from 1% for Co to 23% for Mn (Table 1). As indicated in the Supplemental Data, no difference in assimilation efficiency was observed between the 2 fish sizes for any element tested.

Reconstructed versus actual multi-feeding

Figure 3 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. Comparison of the reconstructed versus actual data did not reveal any significant difference ($p > 0.05$) in terms of whole-body activities for any of the 4 feedings, no matter which metal was considered.

Variability among the successive feedings

The multi-feeding experiment allowed us to determine how whole-body activity in the multi-fed turbot changed at each feeding (4 feedings over 12 d with radiolabeled pellets) and during the depuration period (Figure 4). The whole-body activities after 4 d and 21 d of depuration were not significantly different ($p > 0.05$; Figure 4 and 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 less than 23% for Cd.

For the individuals ($n = 3$) that had successively and significantly eaten 4 times, the multi-feeding exposure led to a linear increase in whole-body activity for the 4 elements studied (Figure 4). To gain a better understanding of 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, and less than 2 (Mn) to 31 (Co) for the fourth feeding (Figure 4 and Table 3).

DISCUSSION

The present study aimed to test the validity of the single-feeding approach commonly used to assess trophic transfer of

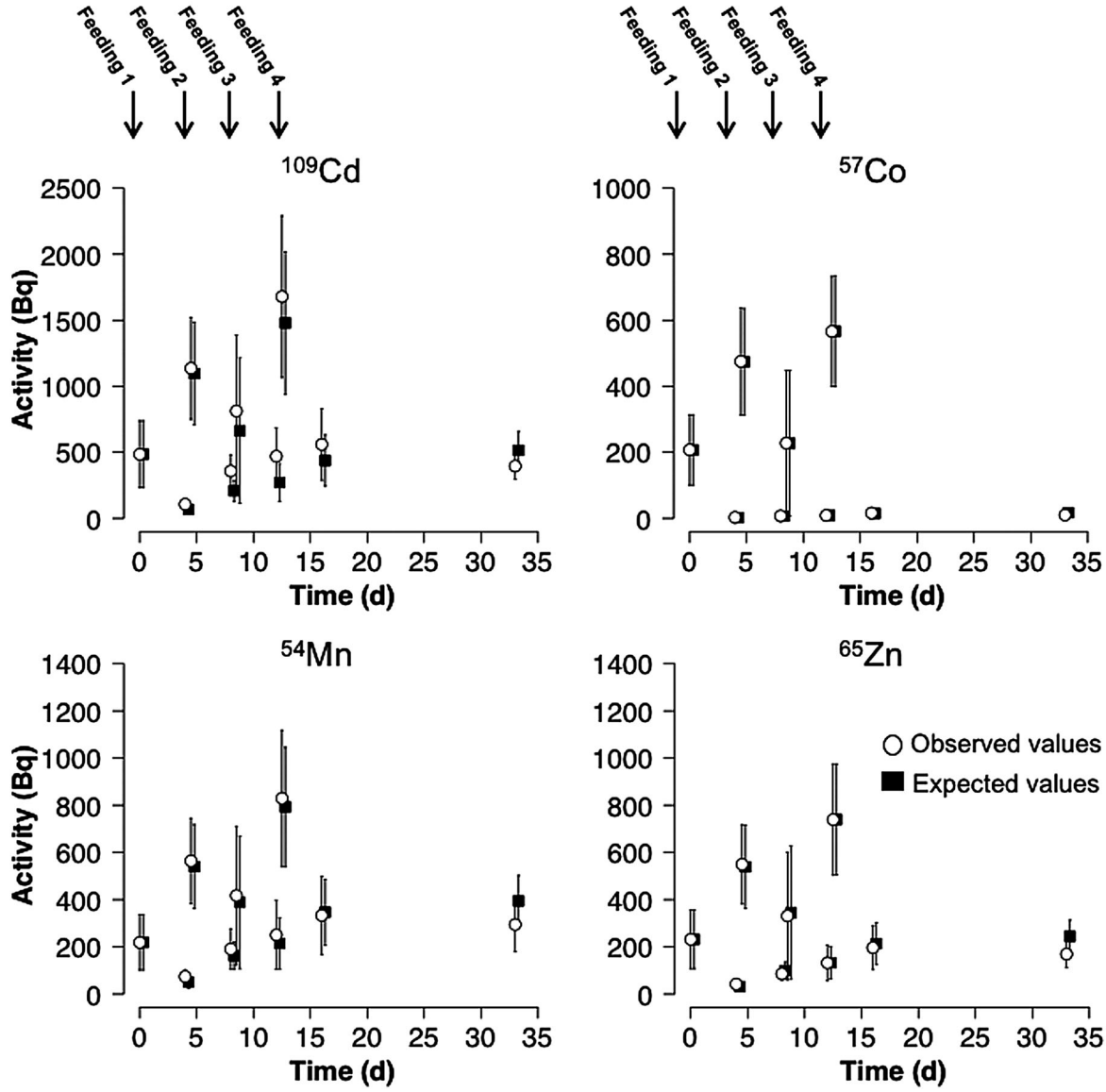

Figure 3. Comparison of activities measured at different times in the multi-feeding experiment and those expected using the kinetic parameters obtained from the single-feeding experiment (i.e., assuming that the experience of multi-feeding is similar to a succession of independent single-feedings; see *Materials and Methods* section for more details). Values (Bq) are means ± standard deviation, $n = 5$.

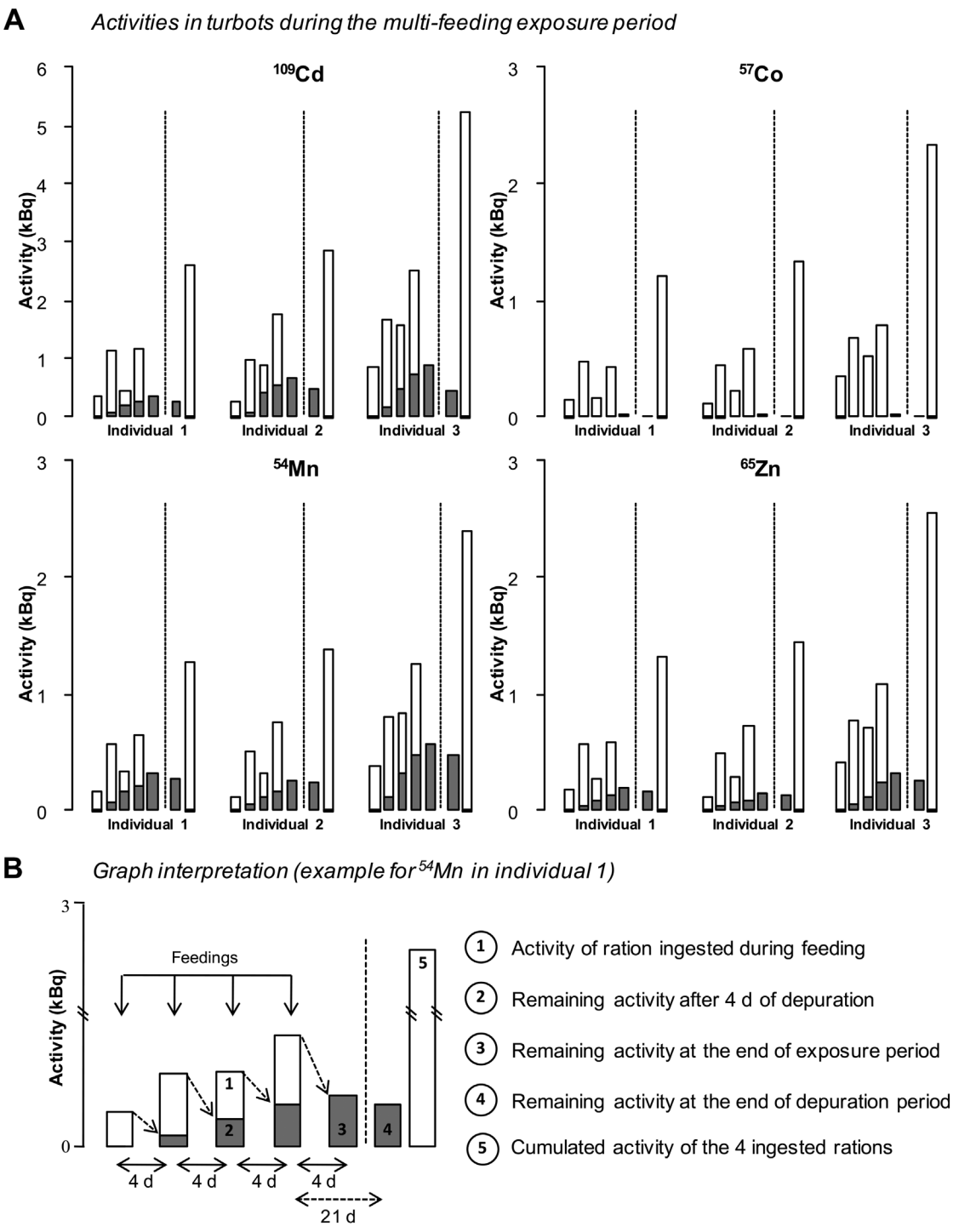

Figure 4. (**A**) Uptake of ^{109}Cd, ^{57}Co, ^{54}Mn, and ^{65}Zn by the turbot during the multi-feeding exposure (12 d). Only the 3 individuals that ate during the 4 radiolabeled pellet feedings are represented. Values (kBq) are means ± standard deviation, $n = 3$. (**B**) Graph interpretation.

contaminants in marine organisms [31,32]. The protocol consisted of conducting 2 experiments in parallel whereby turbot kept under controlled laboratory conditions were exposed to metals 1 single time or 4 successive times, using radiolabeled pellets. Kinetic data from the single-feeding experiment then allowed us to build a reconstructed multi-feeding situation and compare it with the observations made under actual multi-feeding conditions.

Depuration of metal transferred 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 an exponential component and a constant for all elements studied. Such biphasic depuration kinetics for metals are commonly observed in aquatic organisms [14,18,33]. The constant indicated that the assimilated fraction of metal was strongly bound after 4 d. Indeed, whole-body activity was not significantly different between 4 d and 21 d of depuration. This finding corroborates the results from previous studies on the trophic transfer of essential elements in the same fish, using natural prey [15,28]. Our results also indicate that assimilation efficiency is metal dependent. The assimilation efficiency of Mn was higher than

Table 2. Different ratios calculated between ingested and retained activity during the multi-feeding and single-feeding exposures[a]

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 d (unitless; mean ± SD)				
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 (unitless; mean ± SD)	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

[a]Only the 3 individuals that ate during the 4 radiolabeled pellet feedings are included in the calculations.

that of the other 3 elements: the assimilation efficiencies of Cd and Zn were very close (14% and 13%, respectively), whereas Co was poorly assimilated (assimilation efficiency ≈ 1%). According to the literature available on this fish, the assimilation efficiencies observed in the present study appeared to be relatively low (Table 3 [23]). However, some authors [34,35] have already highlighted that assimilation efficiencies of metals in fish are highly dependent on composition of the diet and on metal speciation; to date, investigations using commercial food, as we did in the present study, are still limited to a small number of species and to a few elements (Table 3 [15,12,34]).

The results displayed in Figure 3 indicate that the reconstruction of a multi-feeding (using the data from a single feeding and then repeating over time) is consistent with the data provided by an actual multi-feeding performed under the same conditions. These results provide 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 to confirm the trends observed in the present study over 4 feedings. The single-feeding approach has been used to determine the assimilation efficiency in various aquatic organisms such as crustaceans, echinoderms, mollusks, and fish. For a definitive 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 providing experimental validation of the single-feeding approach, the experimental protocol used in the present study allows a better understanding of the mechanisms involved in the storage and depuration of trace elements during a multiple trophic exposure. In multi-fed turbot, whole-body activity increased linearly for all the metals after each of the 4

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

		Metal (AE)				
Species	Food	Cd	Co	Mn	Zn	Ref
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]

radiolabeled feedings. Despite the increase in metal concentrations in turbot in response to 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 assimilation [35]. Indeed, the assimilation efficiency of Cd and Zn in the black sea bream *Acanthopagrus schlegeli* and the grunt *Terapon jarbua* was not influenced significantly following Zn dietary pre-exposure for 1 wk or 3 wk. Our results indicate that metal storage capacities of turbot are not limited to the 3-wk 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—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3646.

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Data Availability—Data, associated metadata, and calculation tools are available from the corresponding author (m.metian@iaea.org).

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