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Comparative study of trophic transfer of the essential metals Co and Zn in two tropical fish: a radiotracer approach

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Abstract

The trophic transfer of two essential metals (Co and Zn) was investigated in two tropical euryhaline fish species (*Monodactylus argenteus* and *Scatophagus argus*), at both the adult and juvenile stages, using the pulse-chase feeding method and radiotracers (^{57}Co and ^{65}Zn). The food selected was brine shrimp (*Artemia salina*) previously exposed to dissolved radiotracers. Depuration kinetics of both elements were followed for 45 d. During this period, ^{57}Co and ^{65}Zn distribution was also determined at different time intervals among 15 different body compartments. Results showed that, in juveniles, the ingested Co was poorly assimilated by both species (AE < 6%), whereas *S. argus* assimilated ^{65}Zn more efficiently than *M. argenteus* (AE = 24% vs. 15%). In terms of body distribution of these essential elements, the trends were similar between adults and juveniles of both species: Co was concentrated and mainly distributed in the liver in *S. argus*, highlighting the role of this organ in the storage of Co. Conversely, in both species, ingested Zn was more diffusely distributed throughout the body. The differences observed between species could be related to a difference in the digestive enzymatic system in each studied species, which in turn is related to their slightly different feeding habits in the natural environment.

Key words: Cobalt, Zinc, Assimilation, Kinetics, Tissue distribution, Interspecies differences

1. Introduction

Essential metals 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 enzymes (see e.g. Simkiss 1979; Williams 1981). For example, Co is a key component of vitamin B12 (cyanocobalamin), which is a coenzyme in a number of cellular processes including the oxidation of fatty acids and the synthesis of DNA (e.g. Blust 2011). Zn has structural and catalytic roles in many proteins and almost 10% of all genes in sequenced fish genomes carry the annotation of Zn binding (e.g. Hogstrand 2011). Healthy conditions of fish are optimal if these elements are present in sufficient amounts in their tissues: depletion in these elements can provoke pathological damages and/or physiological alterations and their excess can also lead to toxic effects (e.g. Rainbow 2002; Förstner & Wittmann 2012).

In fish, food appears increasingly as an important pathway for acquiring essential metals (e.g. Xu & Wang 2002; Mathews & Fisher 2009). For example, the metal intake from the diet in the teleost *Scophthalmus maximus* was estimated to be responsible for more than 70% of the body burden of Co, Mn and Zn (Mathews & Fisher 2009). Despite a growing dataset showing the major role that food plays in essential and non-essential metal accumulation in fish (e.g. Spry et al. 1988; Zhang & Wang 2005), there is still limited understanding of the factors that influence metal assimilation at the interspecific level. Among these factors, feeding strategies have been reported to be crucial in the differential metal assimilation in coexisting species (e.g. Ni et al. 2000).

A key parameter for understanding and modeling metal trophic transfer in fish is the assimilation efficiency (AE). This variable can be easily determined under laboratory conditions, using radiotracer techniques (e.g. Mathews et al. 2008; Pouil et al. 2015). A complementary approach is the determination of the dynamics of inter-organ metal transfer, based on the measurements of metal in the different organs and tissues at different time

intervals during the depuration phase. Although of interest, this approach is not very commonly reported in the literature. It has however been applied for toxicokinetic studies in fish (e.g. Hogstrand et al. 2003) or with other aquatic active predators like cephalopods (Bustamante et al. 2002; 2004).

In this context, the present work investigated the trophic transfer of two essential elements (Co and Zn) in adults and juveniles of two tropical euryhaline fish species, the silver moony *Monodactylus argenteus* and the spotted scat *Scatophagus argus*, using gamma-emitting radiotracers (^{57}Co and ^{65}Zn). This biokinetic study has focused on obtaining transfer rate data for tropical fish species given the fact that the majority of available information has to date come from studies carried out with temperate species. Recent reviews on the topic of trace element and radionuclide transfer in marine organisms have stressed the need for more data on tropical species in order to make valid comparisons on a global scale (see e.g. Fowler & Fisher 2005).

Two levels of biological organization were considered in this study, i.e. the whole organism and the different organs and tissues, in order to evaluate the biokinetic parameters of the metal depuration and to identify metal transfer dynamics among the body compartments during the depuration phase.

2. Materials and Methods

2.1. Acclimation of organisms

One hundred juveniles and 20 adults of each species (silver moony *M. argenteus* and spotted scat *S. argus*) were shipped to the International Atomic Energy Agency-Environment Laboratories' premises in the Principality of Monaco. Fish were acclimated for 3 months to laboratory conditions, adjusted to replicate as closely as possible their natural tropical

environment (2000-L tank for adults and 700-L aquarium for juveniles; open circuit: 200 L h⁻¹ in each tank; 0.45-µm filtered seawater; salinity: 35 p.s.u.; temperature: 25 ± 0.3°C; pH: 8.1 ± 0.1; light/dark: 12h/12h). During the acclimation period for both species, the juvenile fish were fed 3 to 4 times per day with adult brine shrimp (*Artemiasalina*) at a daily ration of 130-290 mg wet weight (wwt) per individual. Adults were fed 4 times per day with compounded pellets (JBL Maripels®) at a daily ration of 1.6-2 g dry weight (dwt) per individual. Virtually no mortality was observed during the acclimation period. Since body size (age) is known to affect metal bioaccumulation in marine organisms (Boyden 1974; Warnau et al. 1996a; Hédouin et al. 2006), only individuals with homogeneous sizes were used in the experiments.

2.2. Experimental Procedure

2.2.1. Radiolabelling of brine shrimp

In order to investigate the trophic transfer of the two essential elements (Co and Zn) by *M. argenteus* and *S. argus*, adult brine shrimp (*Artemiasalina*) were used as prey. Preparation of the radiolabelled brine shrimp was carried out by exposing them for 18 h in aerated 20-L aquaria (approx. 300 g wwt of adult brine shrimp per aquarium). At the end of the exposure period, brine shrimp were briefly rinsed and stored at -20°C. Exposure was repeated using 6 batches of brine shrimp in order to obtain enough radiolabelled food for trophic transfer experiments (with both juvenile and adult fish). Radiotracers of high specific activity were purchased from CERCA, France (⁵⁷Co, [T_{1/2}] = 271.8 days) and Amersham, UK (⁶⁵Zn, [T_{1/2}] = 243.9 days). Stock solutions of radiotracers were prepared in 0.1 M HCl.

Seawater was spiked with the radiotracers (nominal activity of 3 kBq L⁻¹ for Co and 2 kBq L⁻¹ for Zn). Small volumes (1 mL) of the diluted radiotracer solution were added to the aquaria (close circuit), and no change in pH and salinity was detectable after the tracer addition. At

the end of the exposure period, average activities in the brine shrimp were 239 Bq g⁻¹ ww for ⁵⁷Co and 40 Bq g⁻¹ ww for ⁶⁵Zn.

2.2.2. Exposure of juvenile fish

One batch of 86 juvenile silver moonies and one of 96 juvenile spotted scats (8.4 ± 1.4 g and 4.8 ± 1.4 g ww, respectively) were each transferred into a 70-L aquarium (open circuit: 100 L h⁻¹; aerated, 0.45-μm filtered seawater; salinity: 35 p.s.u.; temperature: 25 ± 0.3 °C; pH: 8.1 ± 0.1 ; light/dark: 12h/12h) prior to the exposure to radiolabelled brine shrimp.

The experiment consisted of a single exposure to radiolabelled brine shrimp via ingestion (thawed prey, single feeding method also called pulse-chase feeding; see e.g. Metian et al. 2010; Pouil et al. 2015). Juvenile fish were fed *ad libitum* for 1.25 h in a closed circuit system (247 g ww and 255 g ww of radiolabelled brine shrimp distributed to silver moony and spotted scat, respectively). Two hours after the beginning of ingestion, all fish were whole-body γ-counted alive and each batch of fish was removed and divided into two groups. Twenty-one individuals were transferred into a 20-L aquarium containing clean, flowing seawater (parameters as previously described). The remainder of the fish were returned into the initial 70-L aquarium. No regurgitation of the ingested radiolabelled brine shrimp was observed.

The 21 individuals from the 20-L aquarium were weighed separately (for individual recognition) and γ-counted alive (see section 2.3) at different time intervals over 45 d to follow the whole-body depuration kinetics of ⁵⁷Co and ⁶⁵Zn. The aquarium was cleaned during each counting period to avoid contamination from radiotracers contained in the faeces. Fish from the 70-L aquarium were regularly sampled (n=3) using the same protocol, anesthetized and dissected (see section 2.2.4) after γ-counting.

2.2.3. Exposure of adult fish

Eighteen adult silver moonies and 13 adult spotted scats ($81 \pm 12\text{g}$ and $193 \pm 35\text{g}$ wwt, respectively) were fed *ad libitum* in a 70-L aquarium during 6 h with thawed radiolabelled brine shrimp (i.e. 11 feedings for 30 min each time) in order to maximise the ingested activity in the fish (total distribution of 795 g of radiolabelled brine shrimp for both species). Due to the size of adult individuals, γ -counting was not performed with living whole organisms; thus, 3 fish were randomly collected at different time intervals and dissected during the 45-d depuration period. For this purpose, fish were anesthetized, sacrificed and dissected into 15 body compartments (see details in section 2.2.4).

2.2.4. Body distribution of metals in fish

Juvenile fish maintained in the 70-L aquarium and all adults were regularly sampled, anesthetized using eugenol, and dissected in order to compare the metal organotropism in the tissues of both species. At each sampling time, 3 individuals (for both juveniles and adults) were dissected into the following body compartments: (1) muscles with the skin, (2) stomach and pyloric caeca, (3) intestine, (4) liver, (5) gall bladder, (6) pancreas and spleen, (7) kidneys, (8) heart, (9) gills, (10) eyes, (11) brain, (12) skeleton (13) head, and in addition for adult fish (14) gonads and (15) red gland. All compartments were separated, weighed (wwt) and radio-analysed (see section 2.3) to determine the radiotracer distribution among the various tissues. During all the experiments, no mortality occurred.

2.3. Radiotracers and Counting

The radioactivity of the tracers was measured using a high-resolution γ -spectrometer system composed of 3 Germanium N type detectors (EGNC 33-195-R, Intertechnique[®]) connected to

a multi-channel analyser and a computer equipped with spectra analysis software (Interwinner 4, Intertechnique®). The radioactivity in live organisms and tissue samples was determined by comparison with known standards of appropriate geometry (calibration and counting). Measurements were corrected for background and physical radioactive decay. Live organisms were placed in polystyrene counting tubes (diameter: 80 mm, height: 50 mm), filled with clean seawater during the counting period. The volume of seawater was kept as low as possible to ensure at the same time the welfare of the fish and a constant geometry during the counting period (i.e. reduced fish movement). The counting time was kept as short as possible and adjusted to obtain a propagated counting error less than 5% (e.g. Rodriguez y Baena et al. 2006; Metian et al. 2009). In the case of radioanalysis of live fish, counting times generally ranged between 15 and 55 min. Tests were performed prior to the experiments, where fish were placed in similar counting conditions in order to observe their behaviour, i.e. in a counting box for 1 hour in the dark. Dissolved O₂ concentration was monitored throughout these tests and was always > 3 mg L⁻¹. No alteration in organism health or behaviour was observed.

2.4. Data treatment and statistical analyses

2.4.1. Whole-body depuration kinetics in juvenile fish

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; Warnau et al. 1996b). The depuration kinetics of the radiotracers were best fitted using a two-component exponential model (Eq. 1), with a decision based on F test and ANOVA tables (Hédouin et al. 2010):

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

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' are the subscripts for the 'short-lived' and 'long-lived' components, respectively. The short-lived component represents the depuration kinetics of the radiotracer fraction that is weakly associated with the organisms and rapidly eliminated (e.g. fraction in faeces), whereas the long-lived component describes the depuration kinetics of the radiotracer fraction that is assimilated by and tightly bound to the organism (Warnau et al. 1996b). The long-lived component allows assessing the assimilation efficiency (AE) of the radiotracer ingested with food ($AE = A_{0l}$). For each exponential component (s and l), a biological half-life can be calculated ($T_{b/2s}$ and $T_{b/2l}$) from the corresponding depuration rate constants (k_{es} and k_{el} , respectively) according to the relation $T_{b/2} = \ln 2 / k_e$. The model was fitted using non-linear routines and model constants were estimated by iterative adjustment of the model, using the Quasi-Newton method in the Statistica[®] software 7.0.

2.4.2. Distribution among body compartments (juveniles and adults)

For juveniles and adults of the two fish species, distribution of Co and Zn among the body compartments was determined over time and compared using the non-parametric Mann-Whitney U test (Zar 1996). Furthermore, in order to quantify the transfer of each element over time among the internal body compartments, the ratio of the concentration of Co and Zn in each organ and/or tissue ($Bq\ g^{-1} wwt$) over the whole-body ($Bq\ g^{-1} wwt$) was calculated (sicconcentration index, I_c , as defined by Rouleau et al. 2000). Values of $I_c > 1$ indicate that a tissue is enriched in Co or Zn compared to the average whole-body metal concentration. This index complements the information provided by metal body distribution and is useful for quantifying and comparing the storage capacities of the different body compartments in fish whose weight and ingested activity were variable. Comparisons of I_c values among body

compartments were carried out using the Kruskal-Wallis non-parametric test, followed by a multiple-comparison test of Siegel and Castellan (Zar 1996).

The level of significance for all statistical analyses was always set at $\alpha = 0.05$. All the statistical analyses were performed using either the Statistica[®] software 7.0 or R freeware 3.0.1 (R Development Core Team 2014).

3. Results

3.1. Depuration kinetics and assimilation efficiencies of ⁵⁷Co and ⁶⁵Zn in juveniles

The whole-body depuration kinetics of ⁵⁷Co and ⁶⁵Zn following the ingestion of radiolabeled *A. salinas* by the juveniles of both fish species were best described by a two-component exponential model (Table 1 and Fig. 1). Assimilation efficiency (AE) and retention capacities are metal- and species- dependent. Both species assimilated poorly ⁵⁷Co (AE <6%), whereas spotted scat assimilated ⁶⁵Zn more efficiently than silver moony (AE = $24.2 \pm 1.0\%$ vs. $14.7 \pm 0.9\%$, respectively; $p < 0.05$). Assimilated ⁵⁷Co and ⁶⁵Zn were slowly eliminated, with biological half-life ($T_{b1/2}$) values ranging from 33 to 55 d for ⁵⁷Co and from 67 to 83 d for ⁶⁵Zn (Table 1).

3.2. Body distribution and body dynamic of metals

During the 45-d depuration phase, dissections were carried out regularly in order to determine the metal distribution among selected body compartments (see section 2.2.4). Figures 2, 3 and 4 describe the distribution of Co and Zn, respectively, in the 4 main body compartments (i.e. stomach, intestine, liver, and muscles with skin representing 50-98% and 40-99% of total activity for Co and Zn, respectively) and in the remaining parts (i.e. the sum of the remaining body compartments) during the depuration phase in juveniles and adults of both species. The

detailed distributions in each body compartment are provided in the supplementary material for this article. Considering element distribution, the depuration may be divided into three phases. The first phase (days 0-1), very rapid, corresponds to the passage of the food into the stomach which contains up to 80% of the radioactivity body burden at the end of the feeding period (85 min). The second phase (days 1-4) is characterized by the occurrence of the digestive processes. During phases 1 and 2 (i.e. the first 4d of depuration), almost all the activity was distributed in the stomach and the intestine (up to 95% of the total body burden). Then, after 4 days a third phase can be depicted and corresponds to element translocations among the main compartments when whole-body activities are stable (i.e. when the absorbed fraction has stabilized; Fig. 1). Gradually, the liver and the muscles with skin become then the predominant compartments in terms of trace element burden. Distributions are however relatively stable after day 10 except for Co in *S. argus*. Indeed, the percentage of ^{57}Co body burden in the liver tended to decrease between days 10 and 45 in both juvenile and adult fish, whereas a concomitant increase was observed in the muscles with skin (Fig. 2 and Fig. 4). Distribution patterns over time were similar between the species, juveniles or adults, for ^{65}Zn (Fig. 3 and Fig. 4), but not for ^{57}Co in which case the distribution in the liver was significantly higher in *S. argus* than in *M. argenteus* (Fig. 2 and Fig. 4; $p < 0.05$).

In terms of concentration index, I_c , Tables 2 and 3 provide a ranking of the I_c for Co and Zn, respectively, in each body compartment during the depuration phase in juveniles and adults of both species. The results indicate that after 2 days ^{57}Co was mainly concentrated in the liver for *S. argus* juveniles and adults (Table 2). In the case of *M. argenteus*, there was no significant difference between the calculated I_c values of juveniles and adults for all the compartments, although the liver and the pancreas seem to play an important role in the storage of ^{57}Co during the depuration phase. For ^{65}Zn , regardless of the species and the life stage, the highest

values of I_c were always measured in the stomach and the intestine during the first 24 h then, after this period, in the gall bladder, the pancreas and spleen (Table 3; $p < 0.05$).

4. Discussion

The tropical silver moony, *Monodactylus argenteus*, and the spotted scat, *Scatophagus argus*, were selected to investigate the assimilation, retention and transfer among organs and tissues of Co and Zn ingested from radiolabeled food under controlled laboratory conditions. Experimental approaches commonly used, based on whole-body kinetics (e.g. Zhao et al. 2001; Mathews & Fisher 2009; Pouil et al. 2015), do not allow easily discerning the dynamics of metal transfer among organs and tissues. This fact underscores the originality of the present study since it combines different approaches to provide new information regarding the mechanisms of assimilation and the subsequent dynamics of translocation of these trace metals among the tissues and organs of these euryhaline fish.

At the whole-body level, results show that AEs of Co are quite similar for the two fish species whereas they differ for Zn. Indeed, ranges of AE for ^{57}Co and ^{65}Zn in silver moony and spotted scat were 5-6% and 15-24%, respectively. These AEs are in accordance with those reported in the literature for other species of marine fish, for which AEs ranged from 2 to 44% for Co and 1 to 52% for Zn (Table 4).

Many factors could explain the variations of AEs. Wang (2001) proposed in his review a ranking of these factors classified in 3 different categories: (1) environmental quality (such as food quality and quantity), (2) metal geochemistry and (3) feeding physiology and biology (such as ingestion rate and gut passage time). Although only bivalves were considered in that review, these factors are generally relevant for fish as well. In the present study, interspecific difference in Zn AE was observed. Indeed, results indicated a higher AE for *S. argus*. Exposure conditions (i.e. the use of radiolabeled brine shrimp as unique food for trophic transfer in both species maintained under the same experimental conditions) did not allow

addressing categories (1) and (2) mentioned by Wang (2001). Although both species are omnivorous, as indicated by their identical Fishbase trophic levels (3.0 for the both species; Froese and Pauly 2015a, b), differences exist in their trophic ecology. Indeed, analyses of the stomach contents from field surveys indicated that detritus is the main diet of *M. argenteus* (Blaber 1980; Rainboth 1996), whereas *S. argus* feeds mainly on zoobenthos such as worms and nekton such as finfish (Mills & Vevers 1989; Monkolprasit 1994; Jeyasselan 1998). These observations suggest that spotted scat has a stronger predatory behavior than silver moony. Dietary habits are related to specific mixes of digestive enzymes or at least to variability of some enzymes' activity (e.g. Furne et al. 2005). For example, among 6 fish species, Hidalgo et al. (1999) have demonstrated that amylase displayed higher enzymatic activity in omnivorous species than in carnivorous predators. Thus, in the present study, the higher Zn AE measured in *S. argus* could be due to a more efficient enzymatic system for the digestion of brine shrimp than in silver moony. In short, spotted scat might be better "equipped" to deal with digesting complex multicellular prey.

This suggested interspecific variation in the fish digestive enzyme array cannot explain the trends observed for Co. For this element, AEs were very low and very similar between the two fish species. Differences, however, occurred in terms of body distribution and I_c . The results highlight the major role played by the liver in Co storage in spotted scat. This organ is involved in the mechanisms of detoxification and storage of Co, as previously shown in the European plaice *Pleuronectes platessa* fed radiolabeled annelid worms *Arenicola marina*, where Amiard-Triquet & Amiard (1974) reported a low Co AE with almost all the activity found in liver and kidney. Thus, trophic ecology could lead to the occurrence of specific mechanisms of storage and detoxification. Further investigation is however needed to obtain a better understanding of the mechanisms of detoxification and storage of Co in the two species examined in this study.

Regarding the potential effect of the life-stage, juveniles and adults of both species had comparable patterns of distribution and concentrations (i.e. “concentration index”) for Co and Zn, i.e. the major fraction of both elements was found in the same organs and the metal concentrations in the organs were similar between the two life-stages. These comparable patterns can result from a similar metabolism in juveniles and adults (viz. similar digestive activity and AE) although there were differences in size (adults of silver moony and spotted scat were respectively 10 and 40 times larger than juveniles). In addition, it is important to note that adults of both species were not sexually mature at the moment of the experiment. This would potentially affect the results due to the non-negligible allocation of some essential elements to reproductive organs, especially in mature females with oocyte production (e.g. Protasowicki 1986; Rajkowska & Protasowicki 2013).

Conclusion

The biokinetic approach at two levels of biological organization, (1) the whole organism and (2) the different organs and tissues, has provided an improved understanding of the mechanisms responsible for the difference in Co and Zn assimilation efficiency observed between *M. argenteus* and *S. argus*. Dissections carried out at different times during the depuration provided new information about the dynamics of metal translocation in the different organs and tissues. Thus, changes observed in the Co distribution between the liver and the muscles in *S. argus* are a good example of the contribution of this radiotracer-based methodology. These results also demonstrated interspecific variations in the assimilation and tissue distribution of Co and Zn in tropical marine fish.

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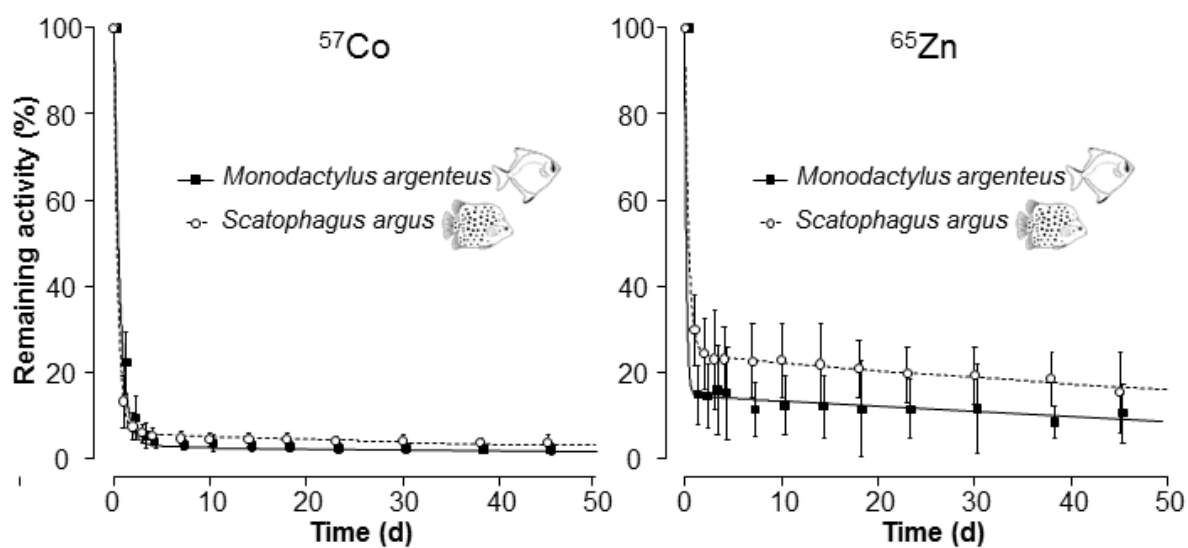


Figure 1. Whole-body depuration of ^{57}Co and ^{65}Zn after a single-feeding with radiolabelled brine shrimp in juvenile silver moony (*Monodactylus argenteus*, n=21) and spotted scat (*Scatophagus argus*, n=21) expressed as percentage of remaining activities (means \pm SD). Parameters of depuration kinetics and their statistics are given in Table 1.

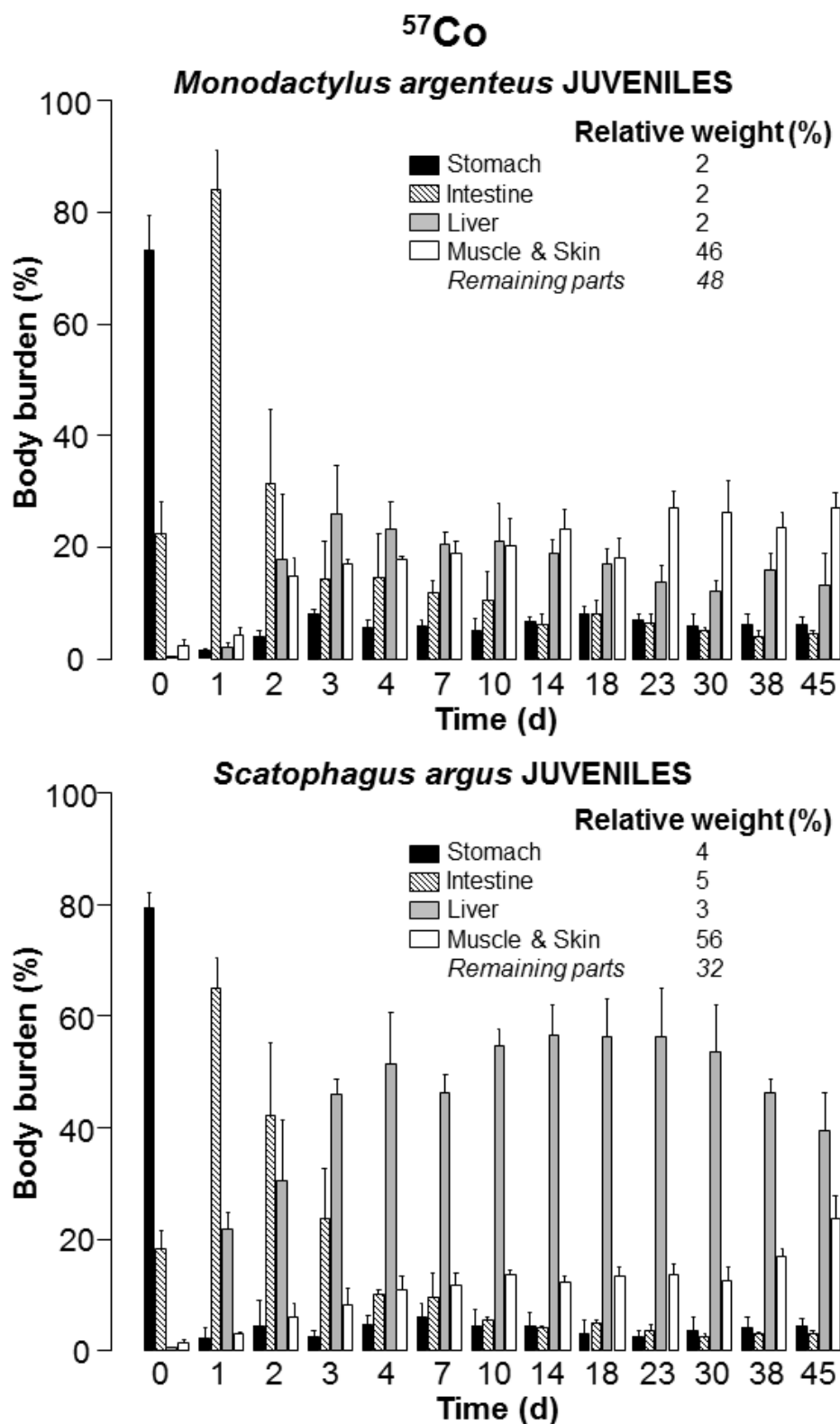


Figure 2. ⁵⁷Co distribution among 4 body compartments (intestine, stomach, liver, and muscles with skin) of juvenile silver moony (*M. argenteus*) and spotted scat (*S. argus*) over the depuration phase after feeding with radiolabelled brine shrimp. At each time three fish were dissected. All the values are expressed as percentage of the whole-body activity (means + SD).

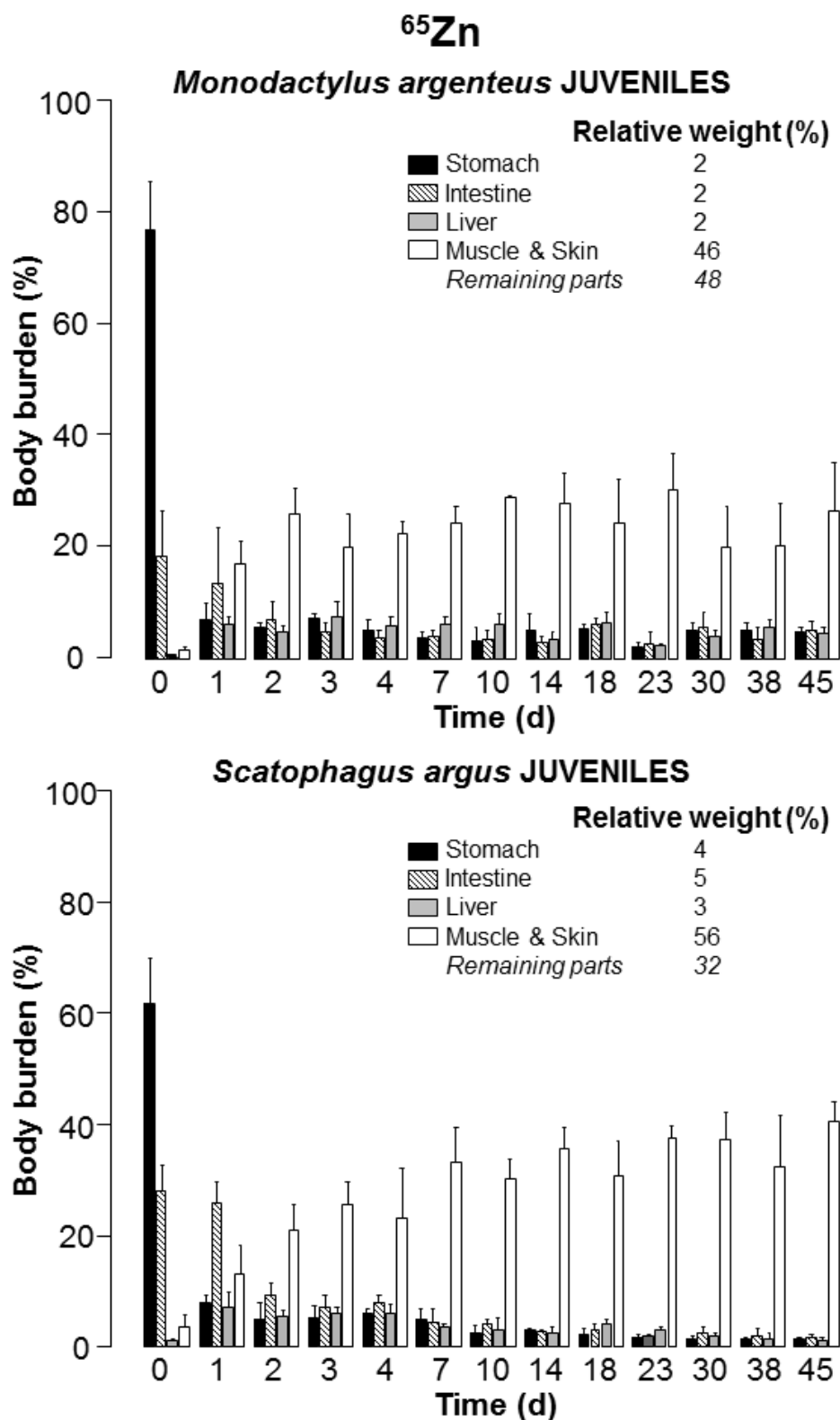


Figure 3. ^{65}Zn distribution among 4 body compartments (intestine, stomach, liver, and muscles with skin) of juvenile silver moony (*M. argenteus*) and spotted scat (*S. argus*) over the depuration phase after feeding with radiolabelled brine shrimp. At each time three fish were dissected. All the values are expressed as percentage of the whole-body activity (means + SD).

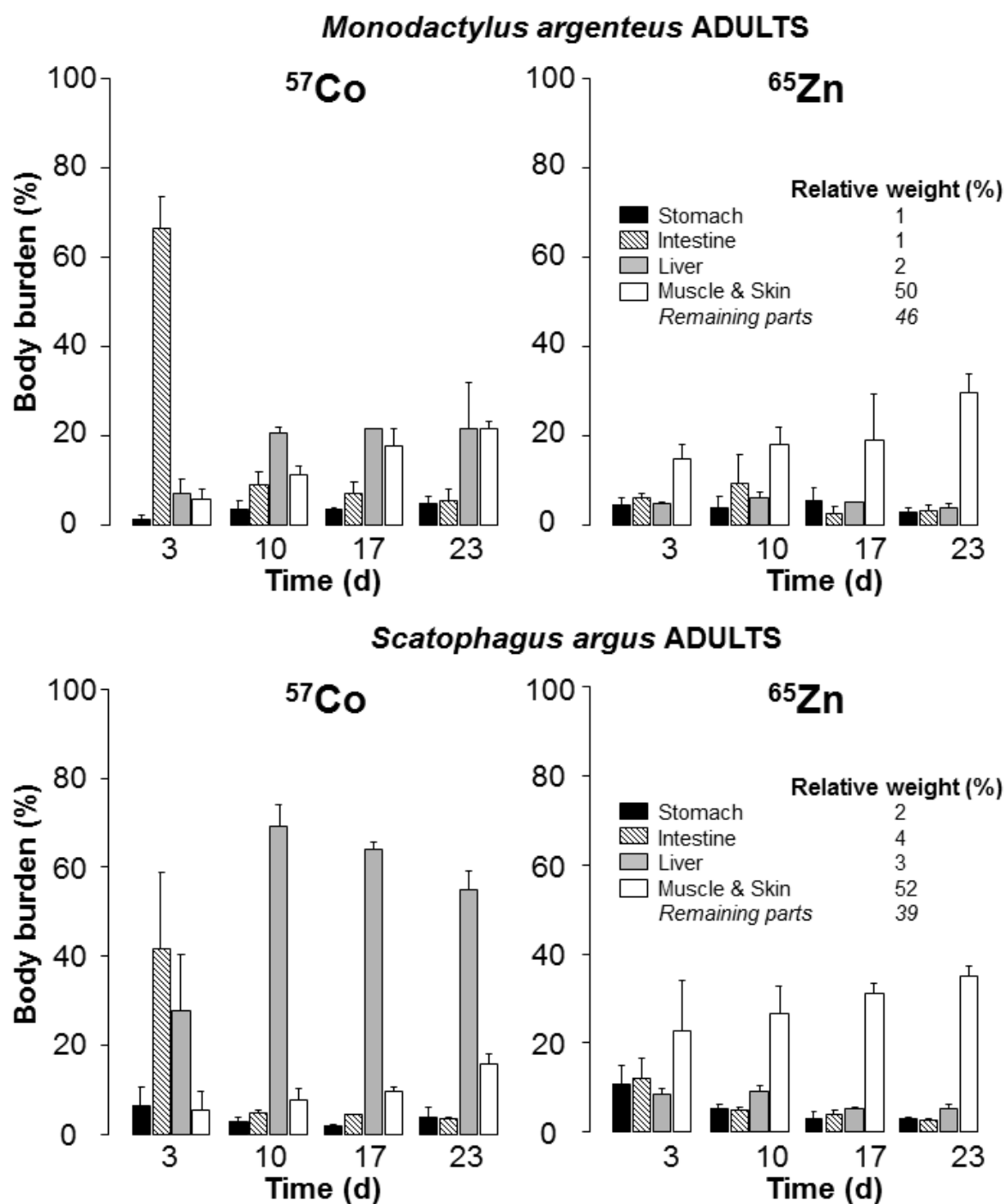






Figure 4. ^{57}Co and ^{65}Zn distribution among 4 body compartments (intestine, stomach, liver and muscles with skin) of adult silver moony (*M. argenteus*) and spotted scat (*S. argus*) over the depuration phase after feeding with radiolabelled brine shrimp. At each time three fish were dissected. All the values are expressed as percentage of the whole-body activity (means + SD).

Table 1. Estimated depuration kinetic parameters of ^{57}Co and ^{65}Zn in silver moony (*M. argenteus*) and spotted scat (*S. argus*) after a single feeding with radiolabelled brine shrimp and then maintained for 45d in a clean flowing seawater. Depuration parameters: A_{0s} and A_{0l} : activity (%) lost according to the short- and the long-lived exponential component, respectively; the A_{0l} value is also the assimilation efficiency (AE; %), $T_{b/2}$: biological half-life (d). ASE: asymptotic standard error; R^2 : determination coefficient of kinetics.

Tracer	Species	Short-term		Long-term		R^2
		$A_{0s} \pm \text{ASE}$ (%)	$T_{b/2s} \pm \text{ASE}$ (d)	$A_{0l} (=AE) \pm \text{ASE}$ (%)	$T_{b/2l} \pm \text{ASE}$ (d)	
^{57}Co	<i>M. argenteus</i> 	$95.4 \pm 0.7^{***}$	$0.42 \pm 0.01^{***}$	$4.58 \pm 0.44^{***}$	$32.7 \pm 9.0^{***}$	0.99
	<i>S. argus</i> 	$94.2 \pm 0.6^{***}$	$0.28 \pm 0.01^{***}$	$5.77 \pm 0.30^{***}$	$54.5 \pm 12.5^{***}$	0.99
^{65}Zn	<i>M. argenteus</i> 	$85.3 \pm 1.9^{***}$	0.12^{NS}	$14.7 \pm 0.9^{**}$	$66.8 \pm 21.2^{***}$	0.95
	<i>S. argus</i> 	$75.8 \pm 1.9^{***}$	$0.27 \pm 0.04^{***}$	$24.2 \pm 1.0^{***}$	$83.1 \pm 19.3^{***}$	0.94

Significance of estimated parameters:

$^{\text{NS}}$ $p > 0.05$ (not significant)

** $p < 0.01$

*** $p < 0.001$

Table 2. Concentration index (I_c) of ^{57}Co , throughout the 45-d depuration of juvenile silver moony (*M. argenteus*) and spotted scat (*S. argus*) after a single feeding with radiolabelled brine shrimp. I_c is the ratio between the activity concentration ($\text{Bq g}^{-1}\text{wwt}$) in the considered compartment and the activity in the whole fish ($\text{Bq g}^{-1}\text{wwt}$) multiplied by 100. A selection of body compartments having the highest and lowest I_c values ($p < 0.05$) has been made. EYE: eyes, GAL: gall bladder, GIL: gills, GON: gonads, HRT: heart, INT: intestine, KID: kidney, LIV: liver, MUS: muscle, PAN: pancreas + spleen, RED: red gland, SKE: skeleton, STO: stomach. Values are means or means \pm SD; $n=3$. Details of the data are available in supplementary materials.



Species		Time (d)																
		0	1	2	3		4	7	10		14	17	18	23		30	38	45
		Juvenile	Juvenile	Juvenile	Juvenile	Adult	Juvenile	Juvenile	Juvenile	Adult	Juvenile	Adult	Juvenile	Juvenile	Adult	Juvenile	Juvenile	Juvenile
 <i>M. argenteus</i>	Highest I _c values	Max: STO (12±2)	Max: INT (39±3)	Max: INT (19±11)	Max: KID (13±1)	Max: INT (63±4)	Max: HRT (14±6)	Max: HRT (28±19)	Max: GAL (21±13)	Max: LIV (21±13)	Max: PAN (30±28)	-	Max: PAN (47±36)	Max: LIV (18±2)	Max: LIV (16±11)	Max: PAN (60±9)	Max: PAN (48±2)	Max: PAN (51±34)
		Min: INT (8±2)		Min: PAN (7±2)	Min: STO (4±1)		Min: LIV (10±2)	Min: LIV (15±1)	Min: STO (4±1)	Min: HRT (7±2)	Min: LIV (16±5)		Min: LIV (12±3)	Min: HRT (10±2)	Min: STO (6±3)	Min: LIV (8±2)	Min: LIV (8±3)	Min: LIV (7±2)
	Lowest I _c values	Max: PAN (<1)	Max: KID (2)	Max: STO (3)	Max: GIL (2)	Max: GAL (6)	Max: INT (10)	Max: KID (7)	Max: GIL (2)	Max: GON (5)	Max: GIL (2)	Max: GON (6)	Max: STO (4)	Max: GAL (7)	Max: GON (6)	Max: STO (4)	Max: STO (4)	Max: STO (4)
		Min: EYE (<1)	Min: EYE (<1)	Min: EYE (<1)	Min: EYE (<1)	Min: MUS (<1)	Min: SKE (<1)	Min: SKE (<1)	Min: EYE (<1)	Min: MUS (<1)	Min: EYE (<1)	Min: MUS (<1)	Min: SKE (<1)	Min: EYE (<1)	Min: MUS (<1)	Min: SKE (<1)	Min: SKE (<1)	Min: SKE (<1)
 <i>S. argus</i>	Highest I _c values	Max: STO (9±2)	Max: INT (13±1)	Max: LIV (13±6)	Max: LIV (14±2)	-	Max: LIV (18±1)	Max: LIV (26±3)	Max: LIV (18±4)	Max: LIV (30±2)	Max: LIV (33±6)	Max: LIV (21±1)	Max: LIV (21±1)	Max: LIV (25±4)	Max: LIV (29±6)	Max: LIV (17±2)	Max: LIV (17±3)	Max: LIV (15±2)
		Min: INT (3±2)	Min: LIV (8±3)	Min: INT (10±7)														
	Lowest I _c values	Max: LIV (<1)	Max: KID (<1)	Max: KID (4)	Max: INT (7)	Max: LIV (10)	Max: HRT (4)	Max: INT (3)	Max: KID (2)	Max: INT (9)	Max: HRT (3)	Max: BRA (5)	Max: HRT (3)	Max: PAN (4)	Max: RED (4)	Max: HRT (4)	Max: HRT (5)	Max: HRT (6)
		Min: EYE (0)	Min: EYE (<1)	Min: EYE (<1)	Min: EYE (<1)	Min: MUS (<1)	Min: EYE (<1)	Min: EYE (<1)	Min: EYE (<1)	Min: MUS (<1)	Min: EYE (<1)	Min: MUS (<1)	Min: EYE (<1)	Min: EYE (<1)	Min: MUS (<1)	Min: EYE (<1)	Min: EYE (<1)	Min: EYE (<1)

Table 3. Concentration index (I_c) of ^{65}Zn , throughout the 45-d depuration of juvenile silver moony (*M. argenteus*) and spotted scat (*S. argus*) after a single feeding with radiolabelled brine shrimp. I_c is the ratio between the activity ($\text{Bq g}^{-1}\text{wwt}$) in the considered compartment and the total activity in the fish ($\text{Bq g}^{-1}\text{wwt}$). A selection of the compartments having the highest and lowest of I_c values ($p < 0.05$) has been made. BRA: brain, EYE: eyes, GAL: gall bladder, GIL: gills, HRT: heart, HED: head, INT: intestine, KID: kidney, LIV: liver, MUS: muscle, PAN: pancreas + spleen, RED: red gland, SKE: skeleton, STO: stomach. Values are means or means \pm SD; $n=3$. Details of the data are available in supplementary materials.



Species		Time (d)																
		0	1	2	3		4	7	10		14	17	18	23		30	38	45
		Juvenile	Juvenile	Juvenile	Juvenile	Adult	Juvenile	Juvenile	Juvenile	Adult	Juvenile	Adult	Juvenile	Juvenile	Adult	Juvenile	Juvenile	Juvenile
 <i>M. argenteus</i>	Highest I_c values	Max: STO (13±1)	Max: KID (40±27)	Max: GAL (47±19)	Max: PAN (25±13)	-	Max: GAL (34±23)	Max: GAL (61±41)	Max: GAL (79±72)	-	Max: PAN (50±22)	Max: PAN (198±93)	Max: PAN (88±61)	Max: PAN (53±44)	-	Max: PAN (102±78)	Max: PAN (100±71)	Max: PAN (88±20)
		Min: INT (7±3)	Min: GAL (22±13)	Min: PAN (26±16)	Min: GAL (22±6)		Min: HRT (13±8)	Min: HRT (26±15)	Min: KID (20±3)		Min: GAL (29±7)	Min: HRT (22±3)	Min: KID (25±8)	Min: GAL (31±28)		Min: HRT (14±10)	Min: HRT (31±14)	Min: KID (17±7)
	Lowest I_c values	Max: PAN (3)	Max: HRT (21)	Max: INT (4)	Max: HRT (10)	Max: PAN (50)	Max: BRA (4)	Max: KID (7)	Max: LIV (7)	Max: GAL (24)	Max: STO (4)	Max: BRA (4)	Max: BRA (7)	Max: LIV (3)	Max: RED (66)	Max: BRA (6)	Max: BRA (4)	Max: BRA (6)
		Min: SKE (0)	Min: EYE (<1)	Min: EYE (<1)	Min: EYE (<1)	Min: MUS (<1)	Min: SKE (<1)	Min: SKE (<1)	Min: SKE (<1)	Min: MUS (<1)	Min: SKE (<1)	Min: MUS (<1)	Min: SKE (<1)	Min: SKE (1)	Min: MUS (<1)	Min: SKE (<1)	Min: SKE (<1)	Min: HED (<1)
 <i>S. argus</i>	Highest I_c values	Max: STO (7±0)	Max: INT (6±2)	-	-	-	-	-	-	-	-	-	-	Max: HRT (6±2)		Max: PAN (11±7)	-	-
		Min: INT (5±3)	Min: LIV (2±1)													Min: MUS (3±0)		
	Lowest I_c values	Max: HRT (<1)	Max: HED (1)	Max: KID (9)	Max: PAN (6)	Max: KID (7)	Max: PAN (5)	Max: PAN (5)	Max: HRT (3)	Max: LIV (4)	Max: PAN (4)	Max: HRT (8)	Max: GAL (7)	Max: MUS (4)	Max: HRT (5)	Max: EYE (2)	Max: HRT (5)	Max: PAN (13)
		Min: SKE (<1)	Min: BRA (<1)	Min: BRA (<1)	Min: BRA (<1)	Min: SKE (<1)	Min: BRA (<1)	Min: BRA (1)	Min: GAL (<1)	Min: MUS (<1)	Min: BRA (<1)	Min: MUS (<1)	Min: INT (<1)	Min: INT (<1)	Min: MUS (<1)	Min: INT (<1)	Min: INT (<1)	Min: INT (<1)

Table 4. Comparison of Co and Zn dietary assimilation efficiencies (AEs; means in %) in marine and brackish water fish species.

Species	Trophic level*	Food	Metal (AE)		References
			Co	Zn	
<i>Marine</i>					
<i>Acanthopagrusschlegeli</i>	3.2±0.45	Artificialdiets		15-26	Wang et al. (2012)
		Mullet muscle		42	Wang et al. (2012)
		Mussel tissue		25	Wang et al. (2012)
		Squidviscera		14	Wang et al. (2012)
		Oyster tissue		4	Zhang & Wang (2007)
<i>Scophthalmus maximus</i>	4.4±0.0	Seabream juveniles	27	22	Mathews et al (2008)
		Ragworms	5	17	Pouil et al (2016)
		Seabream juveniles	44	23	Pouil et al (2016)
		Shrimp	16	32	Pouil et al (2016)
		Commercial pellets	1	13	unpublished data
<i>Dicentrarchuslabrax</i>	3.5±0.5	Seabreamjuveniles	21	38	Mathews& Fisher (2008)
<i>Lutjanusargentimaculatus</i>	3.6±0.5	Brineshrimp		15	Xu & Wang (2002)
		Clam tissue		30	Xu & Wang (2002)
		Copepods		20	Xu & Wang (2002)
		Manilla clam		19	Xu & Wang (2002)
<i>Sparusaurata</i>	3.7±0.0	Brine shrimp nauplii	21	18	Mathews et al. (2008)
<i>Brackish</i>					
<i>Ambassisurotaenia</i>	3.4±0.4	Brine shrimp nauplii		15-17	Ni et al. (2000)
		Copepods		5-9	Ni et al. (2000)
<i>Menidiasp</i>	2.6-3.9	Copepods	2	6	Reinfelder& Fisher (1994)
<i>Monodactylus argenteus</i>	3.0± 0.33	Brine shrimp	5	15	this study
<i>Periophthalmusmodestus</i>	3.2±0.4	Brine shrimp larvae		11-21	Ni et al. (2000)
		Copepods		21-31	Ni et al. (2000)
<i>Scatophagusargus</i>	3.0±0.35	Brine shrimp	6	24	this study
<i>Teraponjarbua</i>	3.9±0.5	Barnacles		2	Zhang & Wang (2006)
		Copepods		23	Zhang & Wang (2006)
		Clams		36	Zhang & Wang (2006)
		Fish viscera		52	Zhang & Wang (2006)
		Mussels		22	Zhang & Wang (2006)

* Data obtained from FishBase (Froese & Pauly, 2015c)

(<1)