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Ocean acidification does not affect the trophic transfer of Ag, Co, and Zn in the cuttlefish *Sepia officinalis*

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ABSTRACT

Cephalopods are known to efficiently accumulate metals and may therefore play an important role in the trophic transfer of contaminants within marine food webs. However, the influence of environmental changes such as ocean acidification on trace element assimilation and retention in these organisms remains poorly understood. In the present study, the trophic transfer of three trace elements (Ag, Co, and Zn) was investigated in juvenile cuttlefish *Sepia officinalis* under two seawater pH conditions representative of present-day (pH 7.92) and near-future ocean acidification scenarios (pH 7.63). Using radiotracer techniques and a pulse-chase feeding experiment with radiolabelled shrimp, we quantified assimilation efficiencies, depuration kinetics, and tissue distribution of these elements following a single contaminated meal. Juvenile cuttlefish showed high assimilation efficiencies for all three trace elements: 94–100% for Ag and Co, and 77–78% for Zn. Depuration kinetics revealed element-specific retention patterns, with biological half-lives of several weeks to months for Ag and Zn, whereas Co was eliminated more rapidly. Tissue distribution showed a strong organotropism towards the digestive gland, which acted as the main storage compartment for Ag and Co, while Zn showed a wider distribution across tissues. No significant differences in assimilation efficiencies, depuration kinetics, or tissue distribution were observed between pH treatments. These results suggest that moderate ocean acidification scenarios projected for the coming century are unlikely to significantly affect trophic transfer and internal handling of trace elements in juvenile cuttlefish.

1. Introduction

Marine ecosystems are currently undergoing significant physico-chemical changes due to increasing atmospheric CO_2 concentrations. The dissolution of CO_2 in seawater leads to a decrease in ocean pH, a process known as ocean acidification. Since the Industrial Revolution, ocean pH has decreased by approximately 0.1 units, making the oceans nearly 30% more acidic, with projections estimating a further drop of 0.3–0.4 units by the end of the century if emissions continue unabated (Abbasi and Abbasi, 2011; Feely et al., 2019; IPCC, 2019). Such changes in seawater carbonate chemistry may alter metal speciation and bioavailability (Belivermiş et al., 2020; Shi et al., 2016; Stockdale et al., 2016), but they may also affect the physiology of marine organisms, including digestive processes (Pimentel et al., 2015) and ion regulation mechanisms that are closely involved in the uptake and retention of trace elements (Hu et al., 2017; Melzner et al., 2020).

Recent studies suggest that ocean acidification may influence the bioconcentration of waterborne trace elements in marine invertebrates by modifying both environmental chemistry and physiological processes related to acid–base and ionic balance regulation (e.g., Belivermiş et al., 2020, 2016; Romero-Freire et al., 2024). However, for many marine organisms, dietary exposure may represent the dominant pathway for the uptake of trace elements, as shown for Cd, Co, Hg, and Zn (e.g. Mathews and Fisher, 2008; Wang and Wang, 2018). In this context, the trophic transfer of metals largely depends on the assimilation efficiency (AE), defined as the fraction of an ingested element that is incorporated

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into biological tissues (Pouil et al., 2018a; Wang and Fisher, 1999).

As shown in fish, AE is influenced by numerous biotic factors (e.g., food type, metal concentration in food, ontogenetic stage) as well as abiotic factors such as temperature, salinity and pH (e.g., Pouil et al., 2020, 2018b, 2016). Variations in environmental parameters can modify metal speciation in the digestive tract and affect physiological processes involved in ion regulation (Alves et al., 2020; Melzner et al., 2020) and metal assimilation. For example, increasing salinity has been shown to decrease the AE of Ag and Co in euryhaline turbot and diamond sturgeon (Jeffree et al., 2026; Pouil et al., 2020), likely due to changes in metal speciation in the gut lumen and associated osmoregulatory adjustments. Interestingly, Zn AE remained stable across salinity gradients, reflecting strong physiological homeostasis for this essential element (Pouil et al., 2020).

Among marine invertebrates, cephalopods are known to efficiently accumulate trace elements and may reach relatively high concentrations in their tissues (see Penicaud et al. (2017) for review). These organisms occupy a key ecological position in marine trophic webs, acting both as active predators of invertebrates and fish and as prey for numerous predators such large fish, seabirds and marine mammals (Chouvelon et al., 2011; Coll et al., 2013). Despite the difficulty in sampling them quantitatively, evidence suggests that cephalopods comprise a large proportion of the biomass of marine communities (Amaratunga, 1983), and consequently cephalopods may represent an important vector for the trophic transfer of contaminants and toxins within marine ecosystems (e.g., Bustamante et al., 1998; Justino et al., 2023; Lischka et al., 2018). Despite this ecological relevance, the processes governing the bioaccumulation of trace elements in cephalopods remain relatively poorly documented compared to those of fish or other marine organisms (Penicaud et al., 2017).

In this context, the extent to which ocean acidification affects the trophic transfer of trace elements remains poorly understood, particularly in cephalopods and during early life stages that are critical for growth and survival. Moreover, the effects of ocean acidification on metal bioaccumulation are highly variable across taxonomic groups, species and the elements considered. For example, no significant effect of elevated $p\mathrm{CO}_2$ on the AE of essential elements was observed in the clownfish *Amphiprion ocellaris* (Jacob et al., 2017), whereas hypercarbia dampens dietary Hg accumulation in meagre *Argyrosomus regius* (Sampaio et al., 2018). Minet et al. (2022) reported no significant effect of elevated $p\mathrm{CO}_2$ on mercury (Hg) toxicokinetics in juvenile cuttlefish, either through dissolved or dietary exposure. However, these results concern a limited number of elements and species and may not necessarily apply to other trace metals with different chemical properties and physiological roles.

The present study aimed to investigate the trophic transfer and toxicokinetics of three trace elements - two essential elements (Co and Zn) and one non-essential element (Ag) - in juvenile cuttlefish under contrasting seawater pH conditions. The common cuttlefish *Sepia officinalis* is the most abundant and commercially important cephalopod species in the Northeast Atlantic. The annual landings in 2015–2020 ranged from 9000 to 13,000 tonnes (Laptikhovsky et al., 2023). Juveniles develop in coastal nursery areas where they feed predominantly on small crustaceans before progressively shifting to a diet composed largely of fish (Pinczon du Sel et al., 2000). As a coastal species, cuttlefish may be impacted by variations in water quality, including metals or radionuclides that can be released by contaminated river plume. Along the Atlantic coasts, littoral nurseries received historically high Ag and Zn releases from the Charente and Gironde estuaries (Lanceleur et al., 2011). In addition, ^{110m}Ag, ^{60}Co, and ^{65}Zn are radionuclides regularly measured, whereas at low levels, downstream of nuclear power plants (Eyrolle et al., 2008). These elements can thus bioaccumulate in biota and transfer along the trophic webs until reaching the voracious young cuttlefish. Combined with others environmental factors, such as seawater pH that vary between nurseries and seasons (Petton et al., 2024), some authors suggest that local environmental changes influence the location of abundance peaks in this species, although causality remains difficult to demonstrate (Pierce et al., 2008).

Using radiotracer techniques and a pulse-chase feeding approach, we quantified assimilation efficiencies, depuration kinetics, and tissue distribution of these elements following a single contaminated meal. This approach allowed us to assess whether near-future ocean acidification scenarios may influence the assimilation, retention and internal distribution of trace elements in juvenile cuttlefish.

2. Materials and methods

2.1. Origin and acclimation of cuttlefish

Juveniles of cuttlefish *Sepia officinalis* (4.5 ± 0.8 g) were collected by dip net in the intertidal eelgrass beds from Arcachon Bay, France, in July 2016, and shipped to the International Atomic Energy Agency premises in the Principality of Monaco. Juvenile cuttlefish were placed in a 700-L plastic tank and acclimated for minimum of 2 weeks to laboratory conditions (open circuit, water renewal: 300 L h^{-1}; 0.45 µm filtered seawater; salinity: 38 p.s.u.; light/dark: 12 h/12h; temperature: 17 °C; ambient pH: 7.95). During this period, the cuttlefish were fed twice a day with living prawns *Palaemon* sp. After this period, cuttlefish were randomly transferred in two 20-L plastic tanks (n = 10, open circuit, water renewal: 20 L h^{-1}; 0.45-µm filtered seawater; salinity: 38 p.s.u.; light/dark: 12 h/12h; temperature: 17 °C, Fig. 1) and acclimated to the target pH values for two weeks prior to a unique radiotracer exposure (i.e. one single-feeding using radiolabelled prawns following by 29 days of depuration as described in section 2.2.2.). The pH and temperature conditions were 7.92 ± 0.04 ($p\mathrm{CO}_2$ of approx. 609 µatm) and 7.63 ± 0.03 ($p\mathrm{CO}_2$ of approx. 1281 µatm), at 16.9 ± 0.1 and 16.8 ± 0.1, respectively. These values were chosen based on the current projections provided by the literature for the next centuries (pH: −0.14 to −0.40; Clark et al., 2024).

Concerning the method used to regulate the seawater pH, we followed the recommendations of the Guide to best practices for ocean acidification research and data reporting (Riebesell et al., 2010). The pH was monitored every 15 min in each aquarium to within ±0.05 pH units using a pH probe connected a multi-probe aquaristic computer (IKS ComputerSysteme, www.iks-aqua.com) that bubbled pure CO_2 into the aquaria. Temperature in each aquarium was also monitored, using a dedicated probe connected to the same computer (Fig. 1). The pH probes were calibrated weekly using Tris-HCl and NBS buffer solutions. Total alkalinity was measured by titration using Methrom 809 Titrando calibrated with NBS buffers, Tris-HCl (Batch 150) and reference materials (Batch 137) (Dickson, 2016). The $p\mathrm{CO}_2$ was determined from pH, temperature, salinity (38 p.s.u) and total alkalinity (2530 µmol kg^{-1}) measurements using the R package seacarb (Gattuso et al., 2024).

2.2. Experimental procedures

2.2.1. Shrimp radiolabelling

Since crustaceans dominated the natural diet of juvenile cuttlefish (Darmaillacq et al., 2004; Koueta et al., 2002), we used prawns as radiolabelled prey. Preparation of the 80-radiolabelled prawns (approx. 1 to 2 cm in total length) was carried out by exposing them for 7 days to dissolved radiotracers in an aerated 20-L aquarium (closed circuit; shrimp density: 4 shrimps L^{-1}, 0.45 µm filtered seawater; salinity: 38 p.s.u.; light/dark: 12 h/12h; temperature: 20 °C; pH: 8.00). Radiotracers of high specific activity were purchased from Polatom, Poland (^{110m}Ag as AgNO_3 in 0.1 M HNO_3, $T_{1/2}$ = 252 days; ^{57}Co as CoCl_2 in 0.1 M HCl, $T_{1/2}$ = 272 days and ^{65}Zn as ZnCl_2 in 0.1M HCl, $T_{1/2}$ = 244 days). Seawater was spiked with small volumes (>0.2 mL) of radiotracers (nominal activity of 4 kBq L^{-1} for ^{110m}Ag and 5 kBq L^{-1} for ^{57}Co and ^{65}Zn). In terms of stable metal, these concentrations corresponded to 7, 0.05 and 46 ng.L^{-1}. No change in pH was detectable in the aquarium (closed circuit) after the tracer additions. During the 7-day exposure,

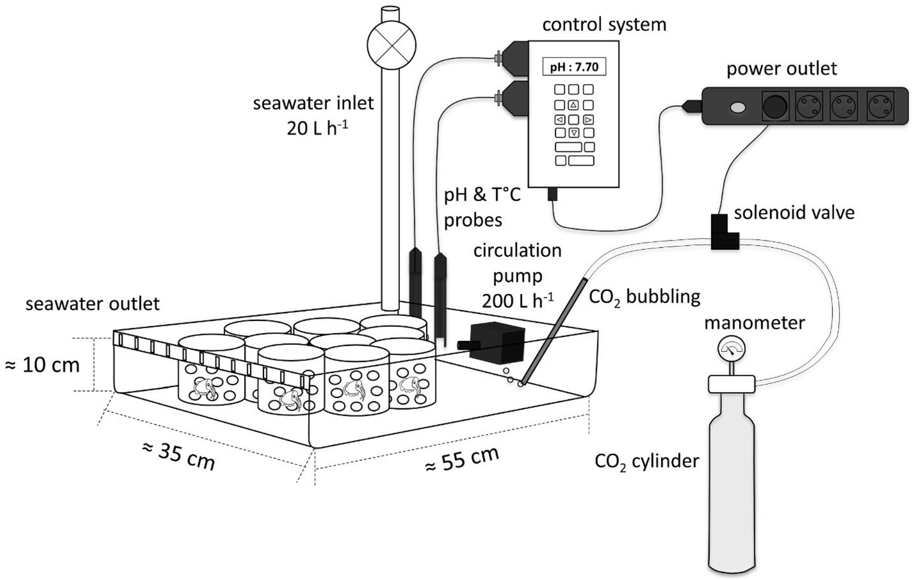

Fig. 1. Schematic view of the experimental setup used during the depuration. Here, is shown the plastic tank where pH is regulated at 7.63.

seawater was renewed and spiked four times to eliminate ammonia generated by shrimp excretion and keep the radiotracer activity constant. The activity of the radiotracers in seawater was checked before and after each seawater renewal, to determine time-integrated activities (Pouil et al., 2016). Each organism was kept isolated during the duration of the experiment in a buoyant cylindrical polystyrene container (10 cm of diameter; drilled to allow for free water circulation) in order to avoid cannibalism and facilitate individuals' identification. The shrimps were fed with non-contaminated minced mussels one time between each water renewal.

2.2.2. Exposure of cuttlefish via radiolabelled shrimp

Juvenile cuttlefish (n = 10 per condition) were randomly selected for each experimental treatment. Wet weights were 4.6 ± 1.0 g and 4.4 ± 0.6 g respectively for the cuttlefish exposed to pH 7.70 and pH 8.05). The experiment consisted of a single feeding of cuttlefish in the different experimental conditions with radiolabelled shrimp. During and after the 5-min radiolabelled feeding, an additional cuttlefish was placed in a net in each tank to assess any possible radiotracer recycling from seawater due to leaching from the radiolabelled food or, later on, from cuttlefish depuration. Two hours after the single-feeding, all the cuttlefish (including control individuals of each condition) were whole-body γ-counted alive (radioanalysis details below). Non-fed cuttlefish displayed radioactivity levels below detection limits attesting a negligible recycling of radiotracers from seawater during trophic exposure. After counting, cuttlefish were replaced in separate drilled circular plastic boxes in the same open-circuit aquarium to facilitate individual recognition and were regularly radio-analysed to follow the radiotracer depuration kinetics over 29 days. During the depuration period, cuttlefish were fed one time per day using non-labelled shrimp to cover their nutritional needs.

After the depuration period, 5 individuals per condition were dissected in 7 compartments: (1) the digestive gland, (2) the gills, (3) the head (including arms), (4) the muscles, (5) the cuttlebone, (6) the skin and (7) the remaining tissues, and were separated, weighed (wet wt) and radioanalysed to determine the body distribution and concentration of trace elements. Briefly, tissues were coarsely shredded with surgeon and immersed in 10 mL of nitric acid 0.2 M to promote the release and homogeneous distribution of radiotracers in a 10 mL liquid geometry.

2.3. Radioanalysis

The radioactivity of the tracers was measured using a high-resolution γ-spectrometer system composed of 5 Germanium - N or P type - detectors (EGNC 33-195-R, Canberra® and Eurysis®) connected to a multichannel analyser and a computer equipped with spectra analysis software (Interwinner 6, Intertechnique®). The γ emissions of ^{110m}Ag, ^{57}Co, and ^{65}Zn were detected at 658, 122, and 1115 keV, respectively, without overlap of the respective counting windows. The radioactivity in living cuttlefish and samples was determined by comparison with standards of known activity and of appropriate geometry (calibration and counting as described by Cresswell et al. (2017)). Briefly, phantoms were prepared as follows: the radioactivities measured in living cuttlefish were standardized against activities measured in a whole-body, formal-in-preserved cuttlefish filled ventrally with paper soaked in diluted acid and spiked with known radiotracer activities. Phantoms for seawater and tissues consisted of 10 mL of diluted acid. Both phantoms were spiked with 976 Bq, 1050 Bq, and 996 Bq of ^{110m}Ag, ^{57}Co, and ^{65}Zn, respectively.

Measurements were corrected for background and physical radioactive decay. Living cuttlefish were placed in counting boxes (diameter: 160 mm, height: 80 mm) filled with clean seawater (at the appropriated conditions of pH) during the counting period. Small drilled plastic tubes are placed in the counting boxes that sufficiently limits cuttlefish movement during radioanalyses (Cresswell et al., 2017). The counting period (<30 min) was adjusted to obtain a propagated counting error less than 5% in order to maintain cuttlefish health and ensure normal behaviour. Maximal variations of pH during the counting have not exceeded ±0.1, which is consistent with an oxygen consumption below 1.5 mg L^{-1} over this period, based on the maximum MO$_2$ values (650 mg kg^{-1} h^{-1}) reported for juvenile cuttlefish (Minet et al., 2025).

2.4. Data treatment and statistical analysis

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)]. The depuration kinetics of Zn were best fitted using a two-component exponential model (decision based on ANOVA tables for two fitted model objects and examination of residuals) following the equation:

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

where At and A0 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 components, respectively. The “s” component represents the depuration of the radiotracer fraction that is weakly associated with the organisms and rapidly eliminated (i.e. proportion associated with the faeces). The “l” component describes the depuration of the radiotracer fraction that is actually absorbed by the organism and eliminated slowly. The long-lived component allows estimating the assimilation efficiency (AE) of the radiotracer ingested with food ($AE = A_{0l}$).

For Ag and Co, the first phase of the depuration was very rapid and not fitted by a two-component exponential model thus the “s” component was not included in the model and the equation becomes:

$$A_t = A_{0l} \cdot e^{-k_{el}t}$$

For the two components, a biological half-life can be calculated ($T_{b1/2}$) from the corresponding depuration rate constant according to the relation $T_{b1/2} = \ln2/k_e$. All statistics and graphics were performed using R freeware 4.2.1 (R Development Core Team, 2022). Model constants and their statistics were estimated by iterative adjustments of the models using non-linear models (nls function) from the package “stats”.

The comparison of assimilation of trace elements among the different experimental conditions was performed using Wilcoxon Mann-Whitney non-parametric test on k_{el} and AE calculated for each individual cuttlefish (the best fitting model obtained for the entire set of cuttlefish was applied to individuals; Zar, 1996). The distribution of trace elements across the 7-body compartments of cuttlefish under the different pH conditions was compared using the same statistical analysis. The level of

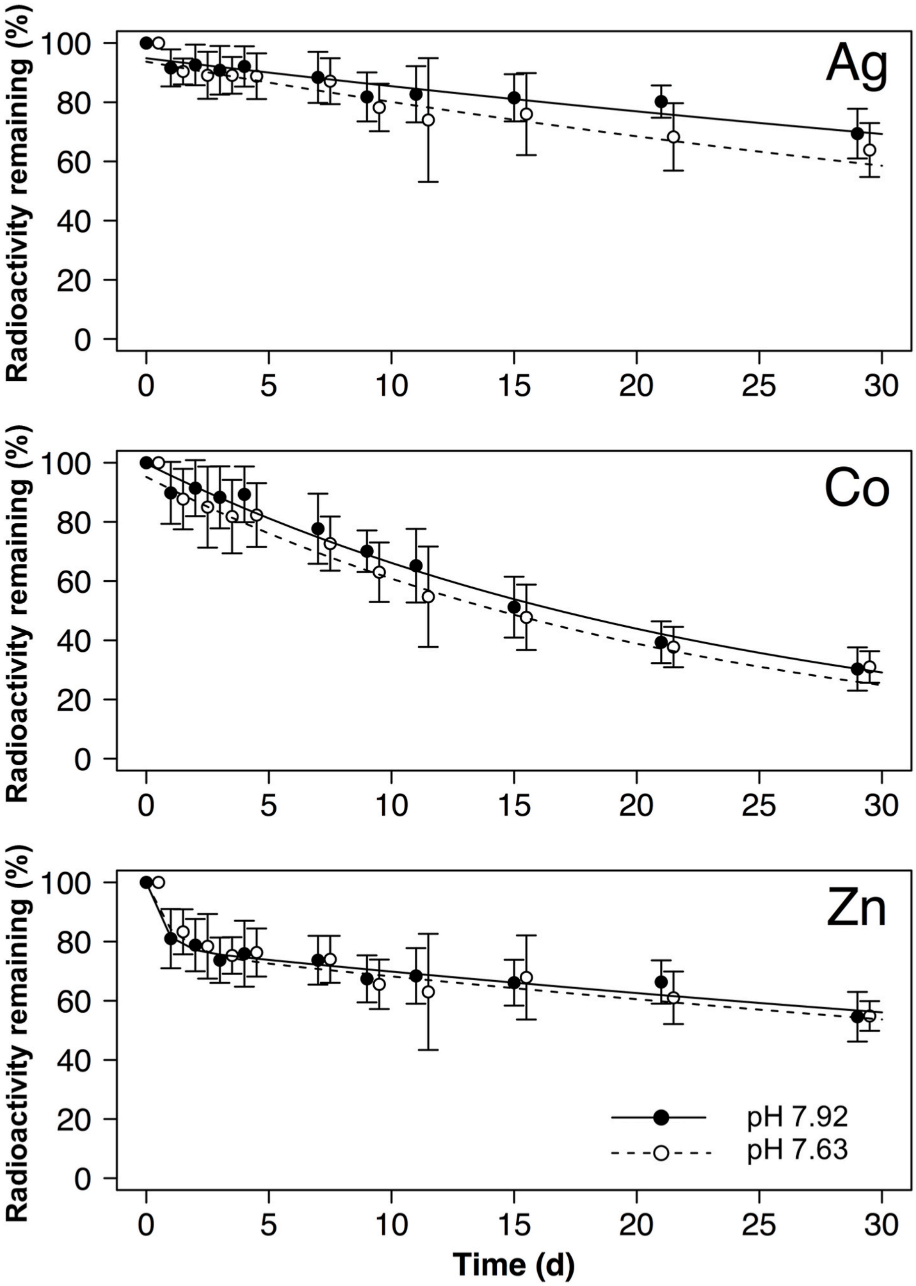

Fig. 2. Influence of pH on the depuration of Ag, Co, and Zn during a 29-d depuration experiment in juvenile cuttlefish *Sepia officinalis* single-fed with radiolabelled prawns. Values, expressed as remaining activities, are means ± SD (n = 10 per condition). Parameters and statistics of depuration kinetics are given in Table 1.

significance for statistical analyses was always set at $\alpha = 0.05$.

3. Results and discussion

3.1. Assimilation efficiencies and depuration kinetics

Our results showed that juvenile cuttlefish efficiently assimilated the trace elements Ag, Co, and Zn from a single contaminated meal, with most of the ingested metal retained in the body's long-term pool (Fig. 2). The AEs were extremely high for all three elements, ranging between 94 and 100% for Ag and Co, and 77–78% for Zn (Table 1). These values indicate that nearly all of the ingested Ag and Co, and the majority of Zn, were absorbed through the digestive process. These high AEs in cephalopods are consistent with previous radiotracer-based studies. For example, Bustamante et al. (2002) shown that juvenile cuttlefish can assimilate 63% of ingested Cd and Zn from radiolabelled brine shrimp while Bustamante et al. (2004) found AE of 67% for Ag and 99% for Co in juvenile cuttlefish fed on the same prey. Prey type can influence the assimilation of trace elements, as demonstrated in other taxa (e.g., Pouil et al., 2016, 2015).

In cephalopods, Minet et al. (2022) showed a strong effect of prey type (fish vs. shrimp) on the whole-body depuration kinetics of Hg in juvenile cuttlefish. Individuals fed with shrimp efficiently eliminated most of the assimilated ^{203}Hg, whereas those fed with fish showed near-infinite retention of the absorbed radiotracer. This difference was related to Hg speciation in the prey: analyses indicated that 82 ± 4% of the ^{203}Hg was present as Me^{203}Hg in fish, compared with only 30 ± 22% in shrimp, which therefore contained a larger proportion of inorganic Hg. Consequently, fish represented mainly a source of methylmercury for cuttlefish under these experimental conditions, while shrimp provided a higher fraction of inorganic Hg that was more readily eliminated. Therefore, variations in prey species and, in some cases, speciation among studies may partly explain the differences observed in assimilation efficiencies, although the overall trends remain broadly consistent (Pouil et al., 2018a).

Following assimilation, the depuration kinetics revealed element-specific retention strategies. Radiotracer elimination profiles were best described by a two-phase model for Zn and a single-phase exponential for Ag and Co (Fig. 2; Table 1). The long-term retention phase accounted for the majority (77–100%) of the load of each metal. Accordingly, biological half-lives ($T_{b1/2}$) in the long-term compartment were on the order of several months for Ag and Zn, compared with only a few weeks for Co. Cuttlefish retained Ag with an estimated $T_{b1/2}$ of 44–66 days and Zn for 58–63 days during the long-term phase, whereas Co was more rapidly lost ($T_{b1/2}$ 15–17 days). This shorter retention time in juveniles contrasts with observations in adult cuttlefish, where dietary Co can be retained for extremely long periods ($T_{b1/2}$ up to 990 days) due to strong sequestration in the digestive gland. Such ontogenetic differences suggest that metal retention capacities may evolve during development, possibly in relation to the progressive functional maturation of the digestive gland and its metal-binding mechanisms. Therefore, the relatively rapid loss of Co observed here in juveniles may reflect limited storage capacity rather than active regulation through enhanced excretion. By contrast, Ag and Zn showed relatively strong retention in the present experiment, suggesting incorporation into storage or detoxification sites within the body. However, Ag retention in cuttlefish can vary markedly depending on diet. For instance, when juvenile cuttlefish were fed brine shrimp, Ag was only weakly retained with a biological half-life of about 13 days, indicating relatively rapid elimination despite efficient initial assimilation. These differences likely reflect variations in metal bioavailability and storage forms in prey. Similar retention patterns have been reported for other trace elements: Hg in juvenile cuttlefish shows $T_{b1/2} \geq 1$ month (Minet et al., 2022), while Cd and Zn may reach ~2 months (Bustamante et al., 2002).The present results are consistent with the major role of the digestive gland in trace-element storage in cuttlefish, where several metals (e.g. Ag, Cd and Zn) are known to accumulate and be associated with intracellular ligands such as metallothionein-like proteins, contributing to their prolonged retention.

The seawater pH/pCO$_2$ can impact intestinal cationic regulation, digestive processes and AE (Melzner et al., 2020). Here, cuttlefish reared under control pH 7.92 and acidified pH 7.63 conditions showed no significant differences in trophic transfer of Ag, Co, or Zn. The proportion of each metal assimilated from the diet was not statistically different between pH treatments (ANOVA, $p > 0.05$), indicating that a ~0.35 unit decrease in seawater pH did not impair or enhance the digestive uptake of these trace elements. The depuration rate constants (k_{el}) for Ag, Co, and Zn likewise did not differ between pH 7.92 and 7.63 ($p > 0.05$). Such resilience to near-future pCO$_2$ levels is consistent with no effect of elevated pCO$_2$ (~1600 µatm, pH 7.54) on the toxicokinetics of both inorganic and organic Hg depuration, either from water or diet (Gentès et al., 2023; Minet et al., 2022). The present findings reinforce that, at least for juvenile cephalopods, moderate ocean acidification scenarios projected for the coming century have minimal impact on the trophic transfer and retention of trace metals. We infer that cephalopods like fish, may have the ability to self-regulate pH shifts in their digestive tract explaining that ocean acidification will not affect the trophic transfer of metals in these organisms (Jacob et al., 2017).

3.2. Tissue distribution and organotropism of metals

The tissue distribution of trace elements in *S. officinalis* revealed a marked organotropism, with the digestive gland acting as the primary storage compartment (Fig. 3). After the depuration period, the majority of Ag and Co was found in the digestive gland, which accounted for more than 90% of the whole-body burden, whereas only minor fractions were detected in muscular tissues, gills, or other compartments. Notably, the Co detected in the remaining tissues may be associated with the

Table 1

Estimated depuration kinetic parameters of Ag, Co, and Zn in juvenile cuttlefish *Sepia officinalis* acclimated to two pH conditions (7.63 and 7.92; n = 10 per condition) and single-fed with radiolabelled prawns and then maintained for 29 d in natural seawater at the given pH. Depuration parameters: A_0: activity (%) lost according to the short- and long-term exponential component, respectively; k_e: depuration rate constant (d^{-1}); $T_{b1/2}$: biological half-life (d); ASE: asymptotic standard error; R^2: determination coefficient; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Element	Short-term			Long-term			R^2
	A_{0s} ± SE	k_{es} ± SE	$T_{b1/2s}$ ± SE[a]	A_{0l} (=AE) ± SE	k_{el} ± SE	$T_{b1/2l}$ ± SE[a]	
pH 7.63							
Ag	-	-	-	93.70 ± 1.66***	0.016 ± 0.002***	44 ± 5	0.81
Co	-	-	-	95.20 ± 1.87***	0.045 ± 0.003***	15 ± 1	0.95
Zn	22.00 ± 3.17***	1.62 ± 0.71*	0.7 ± 0.3	77.96 ± 1.83***	0.011 ± 0.002***	58 ± 13	0.91
pH 7.92							
Ag	-	-	-	94.86 ± 1.10***	0.010 ± 0.001***	66 ± 7	0.90
Co	-	-	-	99.67 ± 1.57***	0.041 ± 0.002***	17 ± 1	0.96
Zn	22.89 ± 4.11***	1.04 ± 0.45*	0.4 ± 0.2	76.92 ± 2.84***	0.012 ± 0.003***	63 ± 10	0.88

[a] Calculated following the relation $T_{b1/2} = \ln 2/k_e$.

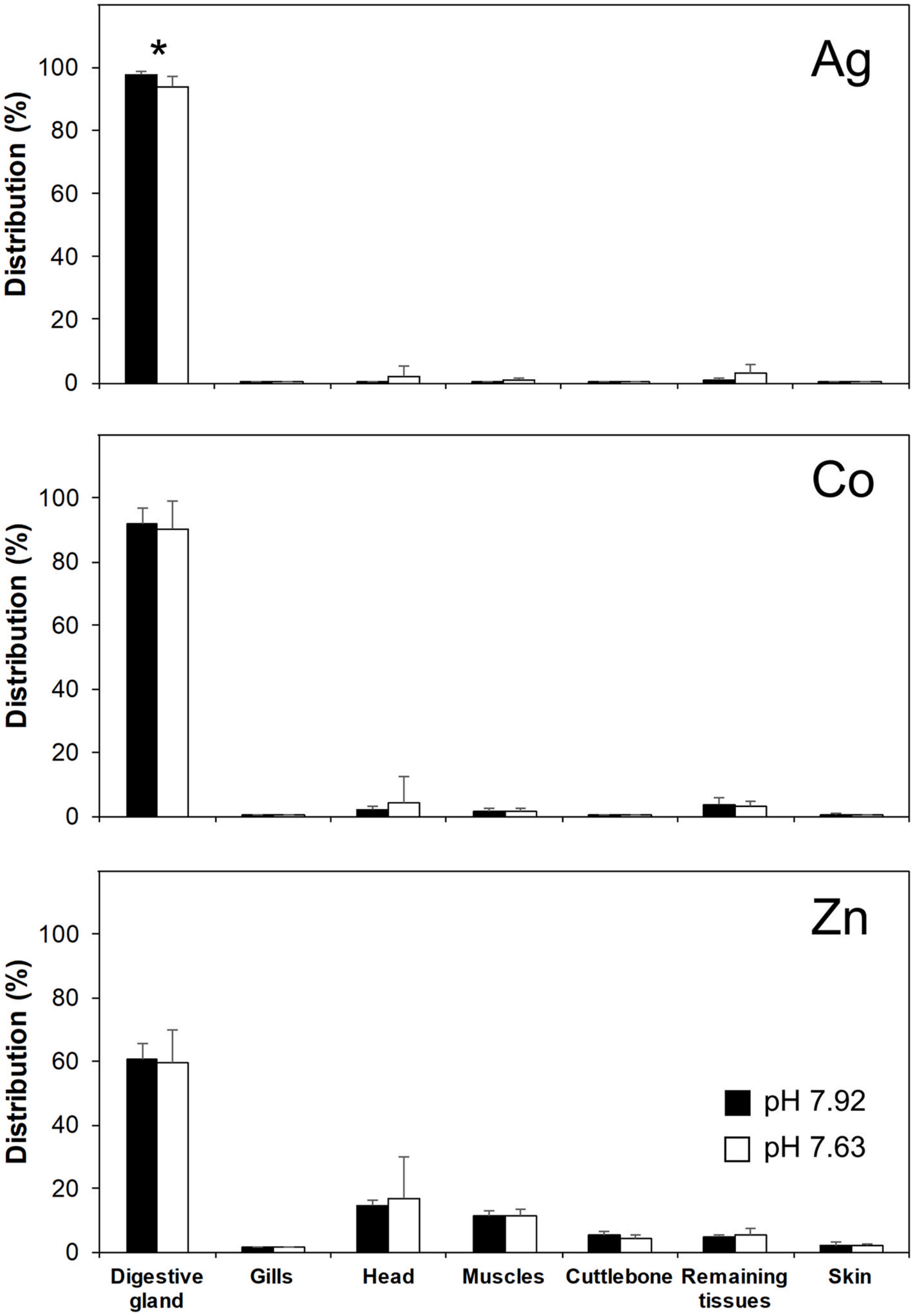

Fig. 3. Distribution of Ag, Co and Zn in seven body-compartments in juvenile cuttlefish *Sepia officinalis* single-fed with radiolabelled prawns and then depurated for 29 days. Values, expressed as percentages, are means ± SD (n = 5 per condition). * : $p < 0.05$.

branchial hearts, which are known to allow a peculiar accumulation of this metal (Miyazaki et al., 2001). Overall, the tissue distribution pattern is consistent with previous studies showing that the digestive gland is the main site of trace element accumulation and detoxification in cephalopods (see Penicaud et al. (2017) for review).

Such preferential accumulation reflects the physiological role of the digestive gland, which functions as both a digestive and storage organ. Metals taken up through the trophic pathway are rapidly transferred to this organ, where they can be bound to intracellular ligands such as metallothionein-like proteins or sequestered into insoluble granules, limiting their circulation toward sensitive tissues. This mechanism has been widely reported in cephalopods and other molluscs and is considered a major detoxification pathway (Amiard et al., 2006; Boucaud-Camou and Boucher-Rodoni, 1983; Bustamante et al., 2002, 2006; Penicaud et al., 2017; Rodrigo and Costa, 2017).

In contrast to Ag and Co, Zn showed a more distributed pattern across tissues in juvenile cuttlefish (Fig. 3) Although the digestive gland remained the main storage site, a substantial fraction of Zn was also detected in muscular tissues and in the head and arms. This broader distribution, also observed in fish, likely reflects the essential role of Zn in numerous physiological processes, including enzymatic activity and cellular metabolism, which requires its incorporation into functional tissues rather than exclusive storage (Pouil et al., 2017). However, in adult cuttlefish, approx. 80% of the total Zn body burden was retained in the digestive gland after food exposure, suggesting a key role of this tissue in the Zn regulation within the organism (Bustamante et al., 2002).

In juvenile cuttlefish, no significant differences in tissue distribution were observed between the control and acidified conditions. The relative proportions of each element among tissues remained similar under both pH treatments, suggesting that near-future ocean acidification scenarios do not significantly alter the internal handling and storage pathways of trace metals in juvenile cuttlefish. Comparable observations were previously reported for Hg toxicokinetics in juvenile cuttlefish, where elevated $p\mathrm{CO}_2$ did not modify organotropism of accumulated Hg (Minet et al., 2022).

Overall, these results confirm that the digestive gland plays a central role in the regulation and detoxification of trace metals in *S. officinalis*, while essential elements such as Zn are more widely distributed among metabolically active tissues at this stage of life. The stability of these distribution patterns under acidified conditions suggests that the physiological mechanisms controlling metal storage remain robust under projected ocean acidification scenarios.

CRediT authorship contribution statement

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

Declaration of competing interest

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

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Data availability

Data will be made available on request.

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