Science of the Total Environment 957 (2024) 177477

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Exploring the depuration of metals in the green shore crab (*Carcinus maenas*): Studies with radiolabelled calcium, zinc, and nickel

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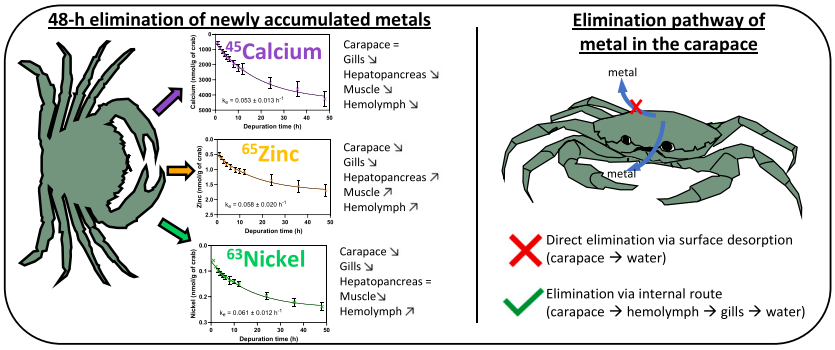
HIGHLIGHTS

- We followed the depuration of newly accumulated metals in clean sea water for ≥48 h.
- Ca was lost from all soft tissues, but not from the carapace.
- Zn and Ni were lost mainly from carapace and gills (increase in other soft tissues).
- Carapace metal loss occurs via internal route (carapace to hemolymph to gills).
- Carapace metal loss occurs via passive physicochemical (not life-mediated) processes.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Julian Blasco

Keywords:
Carapace
Cuticle
Temperature
Excretion
Hepatopancreas
Gills
Newly accumulated metals

ABSTRACT

The depuration of newly accumulated metal from the crab *Carcinus maenas* (inter-moult stage) was studied, with a particular focus on the carapace, in light of recent findings that it is a major site for direct uptake and incorporation of Ca, Zn, and Ni from the external sea water. Crabs were exposed for 24 h to calcium ([Ca] = 389 mg L^{-1} or 9.7 mmol L^{-1}), zinc ([Zn] = 82 μg L^{-1} or 1.25 μmol L^{-1}), and nickel ([Ni] = 8.2 μg L^{-1} or 0.14 μmol L^{-1}) with the addition of radio-labeled metal (^{45}Ca, ^{65}Zn, ^{63}Ni) in sea water (12 °C, 32 ppt), then transferred to clean sea water for ≥48 h. After 24 h of metal exposure, the carapace accounted for ≥85 % of the total body burden of all three newly accumulated metals. For Ca, depurations from the carapace and whole crab were negligible, though levels in soft tissues (gills, hemolymph, and muscle) fell quickly. In contrast, newly accumulated Zn levels in carapace and gills declined by ~60 % over 48 h, while muscle, hepatopancreas, and hemolymph burdens increased, reflecting shifts from gills and/or carapace to internal tissues. Newly accumulated Ni concentrations in the carapace and gills declined by ~50 % over 48 h, reaching >75 % loss by 10 days. As for Zn, Ni levels increased in the hemolymph and initially in the hepatopancreas, indicative of internal redistribution. Acute temperature increase (12 °C to 22 °C) had negligible effects on depuration rates (Q_{10} values ~1.0). Depuration from the carapace was unchanged in recently euthanized crabs, or when the carapace was shielded

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https://doi.org/10.1016/j.scitotenv.2024.177477
Received 6 August 2024; Received in revised form 6 November 2024; Accepted 8 November 2024
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with a membrane. We conclude that physicochemical processes are the rate-limiting steps for metal elimination from the carapace, and that metals newly incorporated in the carapace can leave only through the internal surface of the carapace; the external surface is not a depuration site.

1. Introduction

The green shore crab (*Carcinus maenas*), a model species in ecotoxicology (Griffin et al., 2024; Leignel et al., 2014; Rodrigues and Pardal, 2014), has been used extensively in studies on the uptake and internal distribution of trace metals from the external sea water [reviewed by Bryan (1971, 1968, 1966); Rainbow (2007, 1985); Henry et al. (2012)]. There is general agreement that for Cd (Jennings and Rainbow, 1979; Wright, 1977a; Wright and Brewer, 1979), Zn (Chan and Rainbow, 1993a, 1993b; Nogueira et al., 2024), and Ni (Blewett et al., 2015; Blewett and Wood, 2015; Nogueira et al., 2024), most of the metal is accumulated in the carapace, which is also the major deposition site of Ca and Mg. This is not surprising, as these trace metals can serve as analogues and antagonists of Ca (Hogstrand, 2012; McGeer et al., 2012) and/or Mg (Blewett and Leonard, 2017; Pyle and Couture, 2011). Ca and Mg are the major cationic mineral elements comprising the carapace (Compère et al., 1993; Greenaway, 1985; Travis, 1963), which is by far the largest tissue compartment on the basis of weight (Blewett et al., 2015; Chan and Rainbow, 1993a; Jennings and Rainbow, 1979).

It is traditionally believed that these trace elements follow the same pathway as for Ca, with uptake through the gills, distribution via the hemolymph, and transport through the hypodermis into the carapace (Greenaway, 1985; Neufeld and Cameron, 1993; Wheatly et al., 2002). Metal uptake rates may be highest immediately after moulting, but the long intermoult periods represent the stage in which *C. maenas* spends most of its adult life (Passano, 1960). Using radio-labeled metals, our group has shown recently that, in 24-h exposures, 100 % of newly acquired Zn and Ni, and even 64 % of newly acquired Ca in the carapace of the intermoult green crab are deposited directly from the external sea water (Nogueira et al., 2024). For Zn, only passive physicochemical processes appear to be involved, but there was evidence that 89 % of new Ca uptake and 71 % of new Ni uptake by the carapace was dependent on life processes. Furthermore, these metals were strongly associated with the carapace, as they were not displaced by a rinse with ethylenediaminetetraacetic acid (EDTA) and by an excess of non-labeled metal, suggesting some level of incorporation. Regardless of where and how the metal has been incorporated in the carapace, this raised the question addressed in the present study: Can metals (Ca, Zn, and Ni) in the carapace be returned to the external sea water?

There have been only a few previous studies on metal depuration from *Carcinus maenas*, and none directly addressing this possibility. However, several investigators have suggested that direct desorption of metals adsorbed to the external surface of the carapace, rather than incorporated into it, may occur (Chan and Rainbow, 1993b; Rainbow et al., 1990; Vilquin et al., 1975; Wright, 1977b; Wright and Brewer, 1979). This is an important distinction, as Rainbow (1988) argued that externally adsorbed metal is not metabolically available. Regardless, most investigators have concluded that the major excretion route for Cd and Zn is via the gills, with small fractions via the urine and feces (Bryan, 1966; Chan and Rainbow, 1993b; Rainbow et al., 1990; Wright, 1977b; Wright and Brewer, 1979). There appear to be no previous studies on Ni excretion in crabs. Note that Cd is non-essential (McGeer et al., 2012), unlike Ca and Zn that are widely recognized as essential elements (Hogstrand, 2012), while Ni is likely but unproven to be essential in aquatic animals (Blewett and Wood, 2015; Pyle and Couture, 2011). Regardless of their essentiality status, both Ni and Zn can become toxic to aquatic life at elevated concentrations (Hogstrand, 2012; Pyle and Couture, 2011). In the present study, we sought environmental relevance by studying the depuration of Ca, Ni and Zn in green shore crabs which had been earlier exposed to Zn or Ni at concentrations corresponding to the current US EPA (1995) recommended chronic water quality criteria for the protection of marine life.

Our particular focus was on how these metals leave the carapace. Calcium was examined as it is the major metal component of the crab carapace (Compère et al., 1993, 1992; Greenaway, 1985; Travis, 1963). Nickel and Zn were selected as these two trace elements are currently categorized as priority pollutants by several jurisdictions (e.g., USEPA (Ni and Zn), Environment and Climate Change Canada (Ni and Zn) and the EU (Ni)), and they have often been found at concentrations exceeding toxicity thresholds for marine life in coastal environments (Blewett and Leonard, 2017; Hogstrand, 2012; Pyle and Couture, 2011; USEPA, 1995). Crabs used for this study were adult individuals in intermoult stage.

We conducted experiments to follow the depuration of radio-labeled calcium (^{45}Ca), zinc (^{65}Zn) and nickel (^{63}Ni) from *Carcinus maenas* over 48 h upon return to clean sea water, with a particular focus on the role of the carapace. The metals had been newly accumulated during 24 h of preceding waterborne exposure at environmentally realistic concentrations, as described in our recent accumulation study (Nogueira et al., 2024). By serial sampling of tissues, we were also able to track the internal movement of the newly accumulated metals during the depuration period. Specific experiments also probed the mechanisms of depuration from the carapace, by examining the effect of temperature and recent euthanasia, with the expectations that life-dependent processes should exhibit a relatively high temperature coefficient Q_{10} (close to 2.0) in contrast to simply physicochemical processes (close to 1.0) (Hoar, 1983), and of course would be blocked in dead crabs. We also examined the effect of shielding a portion of the carapace from contact with the external sea water, with the expectation that this would reduce or eliminate the loss of metals that occurred directly through the surface of the exoskeleton.

2. Materials and methods

2.1. Animal collection

Animal collection and holding conditions were previously described in Nogueira et al. (2024). Briefly, intermoult male green shore crabs (*Carcinus maenas*) (mean weight ± SEM = 24.4 ± 0.5 g) were collected by baited traps from Pipestem Inlet in Barkley Sound (BC, Canada), under licences (XR2772015 and XR2032016) from Fisheries and Oceans Canada. Animals were brought to Bamfield Marine Sciences Centre (Bamfield, BC, Canada) and maintained in outdoor tanks with flow-through sea water at 12 °C under a natural 10 h light:14 h dark photoperiod, for at least one week prior to experiments. This natural sea water (used in all experiments in this study) was pumped from 20 m deep in the Bamfield inlet and filtered through various mesh sizes (finest filtration at 0.32 cm) prior to research use. Seawater composition was as reported by Blewett et al. (2015): 32 ppt salinity, pH 8.1; 475 mM Na^+, 11 mM K^+, 9.7 mM Ca^{2+}, 47 mM Mg^{2+}, 515 mM Cl^-, 2.9 mg L^{-1} dissolved organic carbon (DOC). Crabs were fed every two days with salmon heads, and food was withheld 48 h prior to any experimentation.

2.2. Test solutions

Metal exposure waters were prepared 24 h prior to experiments by spiking sea water with stock solutions of radio-active metal and, for Zn and Ni only, non-radioactive metal. For Ca tests, sea water was spiked with ^{45}Ca (Amersham Biosciences, Inc., USA) at a final activity concentration of 4.5 μCi L^{-1}, while the natural seawater level of Ca

remained unchanged (i.e. 389 mg L^{-1} or 9.7 mmol L^{-1}). For Zn tests, sea water was spiked with non-radioactive Zn ($ZnSO_4.7H_2O$, ACS grade, Fisher Scientific, Toronto, Canada) and radio-active ^{65}Zn (Amersham Biosciences, Little Chalfont, U. K.) at final total and activity concentrations of 82 μg L^{-1} (1.25 μmol L^{-1}) and 4.5 μCi L^{-1}, respectively. For Ni tests, sea water was spiked with non-radioactive Ni ($NiCl_2.6H_2O$, ACS grade, Sigma-Aldrich, St. Louis, M, USA) and radio-active ^{63}Ni (Eckert and Ziegler, Valencia, CA, USA) at final total and activity concentrations of 8.2 μg L^{-1} Ni (0.14 μmol L^{-1}) and 6 μCi L^{-1}. The total Zn and Ni concentrations corresponded to the current US EPA (1995) recommended chronic water quality criteria for the protection of marine life, and were chosen to represent moderately polluted coastal sea water (Hogstrand, 2012; Pyle and Couture, 2011).

2.3. Experiments

2.3.1. Metal depuration kinetics in whole crabs

A first set of experiments was conducted to assess metal elimination rates from pre-loaded crabs, by following the appearance of radioactivity in water over a 48-h depuration period. The radioisotope loading phase was performed as by Nogueira et al. (2024), by submerging crabs for 24 h in aerated test solutions at a density of 1 crab/1.2 L, in plastic containers. Water temperature was maintained at 12 °C by setting test containers in a water bath with running sea water at 12 °C. No water renewal was conducted throughout the 24-h exposure and ammonia levels remained <25 μM. Water samples were collected at the start and end of the 24-h exposure, and filtered (<0.45 μm, nylon membrane filter) for analyses of dissolved metal concentration (20-mL samples), radioactivity (2-mL samples), and ammonia concentrations (1-mL samples).

Following the loading phase, crabs ($n = 7$ per metal treatment) were quickly rinsed in sea water, then placed in individual plastic containers (1 crab per container) with precisely 400 mL of aerated un-spiked sea water at 12 °C. Water in depuration containers was completely replaced after 12, 24, 36 and 48 h to minimize radioisotope re-absorption in crabs. Unfiltered water samples (5-mL) were collected for radioactivity analysis at 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36 and 48 h of the depuration phase. Crabs were blotted dried and weighed (±0.1 g) at the end of the experiment.

2.3.2. Metals movement in tissues

A second set of experiments consisted of a 24-h metal loading phase (exposure to metal-spiked sea water, as in the previous set of experiments), followed by a depuration phase (exposure to clean sea water) during which radioactivity was measured in crab tissues (carapace, gills, muscles, hepatopancreas, hemolymph) at various depuration times (mostly at 0, 3, 12 and 48 h). For each metal, depuration was conducted at 12 °C (control treatment) and 22 °C (warm treatment) to evaluate the temperature effects on internal movement and elimination of radioactivity in crab tissues. Furthermore, radioactivity was measured in the carapace of crabs at 12 °C, after shielding the dorsal surface of the carapace from the water using a rubber membrane (dental dam) (shielded treatment), and after euthanizing the crabs (euthanized treatment) prior to depuration. For the control treatments (live unshielded crabs at 12 °C), crabs (n = 7 at each time in each treatment) were sampled at 0 h, 3 h (Ni and Ca only), 12 h, 48 h, and 10 days (Ni only). The shorter 3-h sampling time for Ni and Ca was added after preliminary experiments showed fast tissue re-distribution and elimination rates for these two radio-labeled metals. For the other treatments (22 °C, shielded and euthanized), crabs (n = 7 at each time in each treatment) were sampled at 0 h, 3 h (Ni and Ca only), 12 h (Zn only), and 48 h.

The loading phase was conducted as in the previous set of experiments. For the carapace shielding treatment, shielding was quickly conducted between the loading and depuration phase. For this, crabs were towel-dried, and held on a restraint board while a rubber membrane was applied using cyanoacrylate tissue cement (Vetbond™, 3 M Corporation, St. Paul, MN, USA), so as to cover the whole dorsal surface of the carapace (about 20 % of the entire surface area of the crab, see Supplementary Fig. SI.1 in Nogueira et al., 2024). For the experiment with dead crabs, cryo-euthanasia was conducted between the loading and depuration phase, by putting the crabs in a freezer (at −20 °C) for a few hours.

Depuration was conducted with crabs in plastic containers under flow-through sea water, except for the 22 °C treatment, which was conducted under static water renewal, at a density of 1 crab/1.2 L in plastic containers filled with aerated sea water warmed at 22 ± 1 °C with heaters. For this latter treatment, water was manually renewed at 6, 12, 24 and 36 h during the depuration phase, to minimize re-absorption of depurated radioisotopes. Crabs were collected at various depuration times (see above paragraph) and quickly rinsed in sea water. Hemolymph was collected with a disposable syringe and 21-gauge needle from the arthrodial membrane of a posterior walking leg, after cryo-anesthesia on ice. Then crabs were blotted dried, weighed (±0.1 g), euthanized at −20 °C and thawed for sampling of dorsal carapace (at a random location), gills, flank muscle and hepatopancreas (HP). The carapace pieces (about 1 cm × 1 cm) were excised from the dorsal surface over the branchial chambers, using a drill (Dremel, Konijnenberg, Netherlands) to collect the whole integument from the epicuticle to the inner membranous layer. In a preliminary test where we stripped this latter layer from the endocuticle (using tweezers) for radiometal analysis, no detectable metal accumulation was observed in it. Thus, it was not analyzed separately from the other layers in subsequent analyses. In shielded crabs, the carapace samples were taken from an area completely covered by the rubber dam. Collected carapace was rinsed in a 10 mmol L^{-1} EDTA solution (disodium dihydrate EDTA salt, ACS grade, Anachemia, Richmond, BC, Canada), then quickly rinsed again in sea water. This rinsing procedure aimed at removing loosely-bound radioisotope from the crab surface. Preliminary tests showed that adding a second rinsing step with non-labeled metal concentration (at a 100-fold higher concentration than the total metal concentration of the loading phase, as used in addition to EDTA by Nogueira et al., 2024) did not desorb further radioisotopes. The gills, muscle, HP, hemolymph and carapace samples were placed in sealed test tubes immediately after collection, then weighed (wet weight, ±0.1 g) within a few hours to prevent water loss from the samples.

2.4. Sample analyses

All analyses were conducted as detailed in Nogueira et al. (2024). Ammonia levels in test waters were checked following Verdouw et al. (1978). Temperature checks were conducted every 6–12 h during experiments, using glass thermometers.

Total dissolved Ca, Zn and Ni concentrations in water samples were measured by inductively coupled plasma mass spectrometry (quadrupole ICP-MS, Agilent 7700×, Santa Clara, CA, USA), in 1 % HNO_3 (*v*/v) (Trace Metal grade, Fisher Scientific) with 10 μg L^{-1} Indium (SCP Science). Calibration was checked with a certified reference water (TM-25.4, Natural Resources Canada, Ottawa, ON, Canada).

The radioactivity of ^{65}Zn (CPM·mL^{-1}) in collected water and weighed crab samples (CPM·g^{-1} ww) was measured by gamma counting (Triathler LSC, Hidex, Mississauga, Canada). The radioactivities of ^{45}Ca and ^{63}Ni in water samples (CPM·mL^{-1}) were measured by liquid scintillation counting (LS6500, Beckman Coulter, Brea, CA, USA) after addition of scintillation cocktail (Optiphase, PerkinElmer). For ^{45}Ca and ^{63}Ni analyses in crab samples (CPM·g^{-1} ww), samples were weighed, digested in 4 N nitric acid (ACS grade, Fisher Scientific, Toronto, Canada) for two days at 65 °C, mixed with scintillation cocktail (Ultima Gold AB™, PerkinElmer, Waltham, MA, USA), then measured by liquid scintillation counting (LS6500, Beckman Coulter). For these latter analyses, quench correction was performed to correct for the counting efficiency differences with that of sea water.

2.5. Data calculations and statistical analyses

Data were analyzed in GraphPad Prism (version 10.0.2). We tested for normality using a Shapiro-Wilk test. With a few exceptions, most of the dataset was found to be normally distributed. When deviations occurred, appropriate non-parametric tests were utilized, as detailed below. Data are presented as mean ± standard error of mean (SEM).

2.5.1. Metal depuration kinetics to the external sea water in whole crabs

From the depuration kinetics experiment, the total amount of newly accumulated metal depurated from a whole crab (nmol g^{-1} crab) was calculated from the cumulative radioisotope concentration in the water samples (in CPM·mL^{-1}) collected during the depuration phase, the volume of the depuration container, the total crab mass, and the inverse of the mean specific activity of each radioisotope in the sea water during the exposure phase (CPM·mL^{-1}/nmol·mL^{-1}):

$$M_{depurated} = \frac{[M_{rad}]_{dep.water} \cdot V_{dep} \cdot [M]_{water}}{[M_{rad}]_{water} \cdot m_{crab}} \quad (1)$$

where $[M_{rad}]_{dep.water}$ is the cumulative radioisotope concentration in the depuration water (in CPM·mL^{-1}), $[M_{rad}]_{water}$ is the radioisotope concentration in the exposure water (in CPM·mL^{-1}), $[M]_{water}$ is the total dissolved metal concentration in the exposure water (in nmol·mL^{-1}), V_{dep} is the depuration water volume (in mL) and m_{crab} is the total mass of crab (in g).

For each metal, the mean total amount of metal depurated (nmol g^{-1} crab) was modelled as a function of time (in h) with a first-order elimination kinetics model (see equation in Supplementary file), allowing the derivation of the elimination rate constant k_e (in h^{-1}).

2.5.2. Temporal trends and treatment effects on metal tissue concentrations

For the second set of experiment, the concentrations of newly accumulated Ca, Zn and Ni in the various crab tissues ($[M]_{tissue}$, in nmol·g^{-1} wet weight) over the exposure phase were calculated based on radioactivity in the digested tissues and the inverse of the mean specific activity (CPM·mL^{-1}/nmol·mL^{-1}) of each radioisotope in the sea water during the exposure:

$$[M]_{tissue} = \frac{[M_{rad}]_{tissue} \cdot [M]_{water}}{[M_{rad}]_{water}} \quad (2)$$

where $[M_{rad}]_{tissue}$ is the radioisotope concentration in the tissue (in CPM·g^{-1} wet weight), $[M_{rad}]_{water}$ is the radioisotope concentration in the test water (in CPM·mL^{-1}) and $[M]_{water}$ is the total dissolved metal concentration in the sea water (in nmol·mL^{-1}).

The concentrations of newly accumulated metals in each tissue (calculated with Eq. (2)) were also used to estimate concentrations of newly accumulated metals in whole crabs ($[M]_{whole\ crab}$, in nmol·g^{-1} wet weight, with Eq. (3)), and in their total soft tissues ($[M]_{soft\ tissues}$, in nmol·g^{-1} wet weight, with Eq. (4)) at each depuration time:

$$[M]_{whole\ crab} = 0.56 \cdot [M]_{carapace} + 0.012 \cdot [M]_{gills} + 0.070 \cdot [M]_{muscles} + 0.031 \cdot [M]_{HP} + 0.32 \cdot [M]_{Hem} \quad (3)$$

with 0.56, 0.012, 0.070, 0.031 and 0.32 representing the respective relative fractional contributions of carapace, gills, muscles, HP (hepatopancreas) and Hem (hemolymph) to the total crab mass, as reported by Nogueira et al. (2024).

$$[M]_{soft\ tissues} = 0.028 \cdot \left[[M]_{gills} + 0.16 \cdot [M]_{muscles} + 0.071 \cdot [M]_{HP} + 0.74 \cdot [M]_{Hem} \right] \quad (4)$$

with 0.028, 0.16, 0.071, and 0.74 representing the respective relative fractional contributions of gills, muscles, HP and Hem to total soft tissues mass (Nogueira et al., 2024).

For each metal, we first evaluated whether there was a significant accumulation in each tissue after the 24-h exposure phase. This was achieved by using either a one sample *t*-test (parametric data) or a one sample Wilcoxon test (non-parametric data) that tested the difference between each mean value and zero. We then evaluated if metal tissue levels changed over time during the depuration phase at both 12°C and 22°C, with a one-way ANOVA (parametric) or a Kruskal-Wallis test (non-parametric). Then, for each metal, the mean concentration of newly accumulated metal in each tissue was plotted as a function of depuration time. Upon visual inspection of temporal trends, data were then fitted with either a linear model, a first-order elimination kinetics model, or a saturation binding model (equations in Supplementary file). In certain cases, metal tissue concentrations did not follow a consistent temporal trend, so these data were not modelled. Effects of temperature on metal movement in tissues were evaluated with extra-sum-of-squares F-tests (when both 12 °C and 22 °C data could be modelled). Further, this regression analysis was conducted on metal concentrations in carapace (measured), whole soft tissues (estimated with Eq. (4)) and whole crabs (estimated with Eq. (3)) after expressing these data as proportions of metal remaining in these compartments at each depuration time, relative to the start of the depuration period. Finally, the same regression analyses were used to evaluate effects of shielding and euthanizing on Zn carapace movement. To evaluate these effects on Ca and Ni carapace movement, we used one-way ANOVAs with Dunnett's multiple comparisons tests (parametric data) or Kruskal-Wallis tests with Dunn's multiple comparisons tests (non-parametric data) at each depuration time (3 h or 12 h, and 48 h), as the Ca and Ni carapace data could not be modelled with time.

Temperature coefficients (Q_{10}) on metal depuration rates over a 48 h depuration period were calculated from these tissue concentrations at 12 °C and 22 °C with the following equation:

$$Q_{10} = \left(\frac{[M]_{T2,0h} - [M]_{T2,48h}}{[M]_{T1,0h} - [M]_{T1,48h}} \right)^{\left(\frac{10}{T_2 - T_1}\right)} \quad (5)$$

where $[M]_{T1,0h}$ and $[M]_{T1,48h}$ are metal concentrations after respectively 0 and 48 h of depuration in a crab compartment (the carapace, or the whole crab (as calculated by Eq. (3)) or the whole soft tissues (as calculated by Eq. (4))) at temperature T_1 (12 °C) and $[M]_{T2,0h}$ and $[M]_{T2,48h}$ are metal concentrations after respectively 0 and 48 h of depuration in the same crab compartment at temperature T_2 (22 °C).

3. Results

3.1. Exposure conditions

Measured dissolved Ni concentrations in exposure solutions were 7.60 ± 0.30 μg L^{-1} (n = 18), i.e. 93 % of the nominal concentration of 8.2 μg L^{-1}. Measured dissolved Zn concentrations in test solutions were 72.1 ± 4.1 μg L^{-1} (n = 13), i.e. 88 % of the nominal concentration of 82 μg L^{-1}. Clean sea water had 0.29 ± 0.07 μg L^{-1} of Ni and 5.84 ± 0.23 μg L^{-1} of Zn. Over the 24 h of metal exposure, water radioactivity decreased on average by 2.66 ± 0.35 % for Ca, 20.3± 0.9 % for Zn and 10.0 ± 1.1 % for Ni. The metal speciation in the exposure sea water was dominated by free cationic metal (M^{2+}) (55–71 %), as previously calculated in Nogueira et al. (2024). Temperature measurements were within ± 1 °C of targeted values (12 °C and 22 °C) throughout the experiments. Water ammonia concentrations remained ≤25 μM during the experiments. No crabs died during the experiments.

3.2. Metal depuration kinetics from whole crabs

Fig. 1 shows the metal loss over 48 h in clean sea water from the preloaded crabs (measured from the appearance of radiolabelled metal in clean sea water). About 4300 nmol of the Ca (Fig. 1A), 1.7 nmol of the Zn (Fig. 1B) and 0.24 nmol of the Ni (Fig. 1C) that were newly accumulated

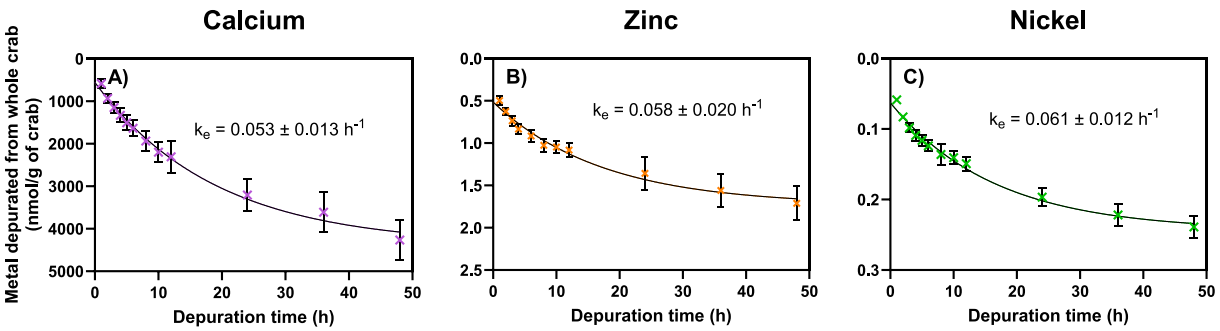

Fig. 1. Cumulative total amount of newly accumulated A) Ca, B) Zn or C) Ni depurated from whole crabs, after a 24 h exposure to respectively ^{45}Ca (and 389 mg L^{-1} total Ca), ^{65}Zn (and 82 μg L^{-1} total Zn), or ^{63}Ni (and 8.2 μg L^{-1} total Ni) at 12 °C, then various depuration times in sea water at 12 °C. At time 0 h, crabs were rinsed with sea water only. Depuration was measured by excretion of newly accumulated metal to the external sea water. Values are mean ± SEM (n = 7). Plain lines are regression lines with first-order elimination kinetics model and k_e is the fitted elimination rate constant.

during the 24 h loading phase were lost per g of whole crabs over 48 h. In this experiment, crabs were rinsed with sea water but not with EDTA prior to the whole-crab depuration kinetics experiment, to prevent physiological disturbance and potential EDTA toxicity to the crabs. Thus, the data of Fig. 1 may include some loss of loosely bound superficial component, which would likely occur early in the depuration period (<1 h), explaining why the first-order elimination models do not meet the Y intercept at 0 (Fig. 1). First-order elimination rate constants (k_e) derived from this time course experiment were very similar for the three metals: 0.053 ± 0.013 h^{-1} for Ca (Fig. 1A), 0.058 ± 0.020 h^{-1} for Zn (Fig. 1B) and 0.061 ± 0.012 h^{-1} for Ni (Fig. 1C).

3.3. Metal tissue concentrations during depuration, at two temperatures

3.3.1. Tissue-specific concentrations

Fig. 2 shows the concentration of newly accumulated Ca, Zn and Ni in various tissues of crabs (n = 6–13), after 24-h exposure to radiolabeled metals at 12 °C (y-intercept), then after various depuration times at 12 and 22 °C. All metal accumulation data in this study are presented on a wet weight basis. If of interest to the reader, dry weight estimates may be obtained using wet:dry ratios measured in previous studies with *Carcinus maenas:* 1.5 for carapace, 9.5 for gills, 4.1 for muscle, 13 for hemolymph (Bjerregaard and Depledge, 2002) and 4.1 for hepatopancreas (Bjerregaard, 1990).

After the 24 h exposure and before depuration (i.e. at 0 h of depuration time, i.e. y-intercepts of Fig. 2), all three metals were significantly accumulated in all the analyzed tissues ($p < 0.01$, one-sample t-tests or Wilcoxon tests). Newly accumulated Ca concentrations in the different tissues were respectively 2–4 and 3–6 orders of magnitude larger than newly accumulated Zn and Ni concentrations (Fig. 2). For example, newly accumulated Ca, Zn and Ni levels in the carapace were respectively 52,800 ± 7400, 11.8 ± 1.93 and 0.740 ± 0.082 nmol g^{-1} ww. At least in part, this reflected comparable differences in exposure concentrations of Ca (9700 μmol L^{-1}) vs. Zn (1.25 μmol L^{-1}) vs. Ni (0.14 μmol L^{-1}). For newly accumulated Ca, the highest concentrations were in the carapace (Fig. 2A), followed by the hemolymph (Fig. 2M), and then the rest of the tissues (Fig. 2D, G, J). For Zn and Ni, the highest concentrations were in carapace (Fig. 2B, C) and gills (Fig. 2E, F), with much lower levels in the other tissues (Fig. 2H, I, K, L, N, O). Because the carapace comprises the major portion (56 %) of the body weight of the crab, it represented the largest percentage of the whole-body burden for all three metals (Ca = 92 ± 1 %, Zn = 85 ± 3 %, Ni = 98 ± 0.3 %).

The statistical significance of depuration time on metal tissue concentrations at both temperatures are given inside the various graph panels of Fig. 2 (asterisks indicating p values of the one-way ANOVA or Kruskal-Wallis test). In the carapace, the concentration of newly accumulated Ca was overall stable over the depuration time (Fig. 2A). Contrary to Ca, the concentrations of newly accumulated Zn (Fig. 2B) and Ni (Fig. 2C) in the carapace decreased by about half over 48 h of depuration. Nickel first appeared to increase in the carapace within 3 h in clean sea water, then decreased to similar levels at 12 and 48 h of depuration. Yet, about 25 % of the accumulated Ni remained after 10 days of depuration (Fig. 2C). Overall, temperature had no apparent effect on metal depuration in the carapace (Fig. 2A, B, C).

In the gills, the newly accumulated concentrations of all three metals strongly decreased over the depuration period, following a first-order elimination kinetics model (Fig. 2D, E, F). The loss mostly occurred within the first 12 h for Ca and Zn, and was independent of temperature (Fig. 2D, E). On the other hand, significant loss still occurred past 12 h for Ni at 12 °C, and this decrease was significantly accelerated at 22 °C (Fig. 2F).

In the muscle, the concentration of newly accumulated Ca followed a first-order elimination kinetics model, with most of the loss occurring within the first 12 h (Fig. 2G). Zinc concentration increased in the muscles over the depuration period, following a saturation kinetics pattern (Fig. 2H). Nickel significantly decreased in the muscles, but the totality of the loss seemed to occur <3 h (first sampling time point), so the first-order elimination kinetics did not fit the Ni data well (Fig. 2I). By 10 days at 12 °C, about half of the newly accumulated Ni was still present in the muscles (Fig. 2I). Temperature had little apparent effect on the temporal trends of the three metals in the muscles (Fig. 2G, H, I).

In the hepatopancreas, the concentration of newly accumulated Ca was relatively stable over depuration time at 12 °C, but decreased slightly at 22 °C (Fig. 2J). Newly accumulated Zn increased linearly with depuration time in the hepatopancreas at 12 °C, but remained constant at 22 °C (Fig. 2K). There was no significant effect of depuration time on newly accumulated Ni at both temperatures (Fig. 2L). The 12 °C levels were quite variable and suggested an initial increase followed by a decrease to initial levels by 48 h, which persisted up to 10 days (Fig. 2L).

In the hemolymph, newly accumulated Ca concentration followed a first-order elimination kinetics model (as for the gills and muscles), with most of the important loss occurring within the first 12 h (Fig. 2M). While the F-test predicted an effect of temperature on this Ca trend (p = 0.0092), this effect was mostly driven by a data point at 12 h and the extent of the difference between the two temperatures was overall very small (Fig. 2M). As for the HP, the concentration of newly accumulated Zn increased linearly in the hemolymph over the depuration period at 12 °C, but was stable at 22 °C (Fig. 2N). Newly accumulated Ni levels increased linearly in the hemolymph over the 48-h depuration period, with no significant temperature effect (Fig. 2O).

3.3.2. Whole soft tissue, carapace and whole crab concentrations

Re-analysis of the above data, on the basis of whole soft tissues (gills + muscles + HP + Hem), hard tissues (carapace) and whole crabs (gills + muscles + HP + Hem + carapace), revealed distinct overall metal handling patterns by the green shore crab (Fig. 3). In the whole soft

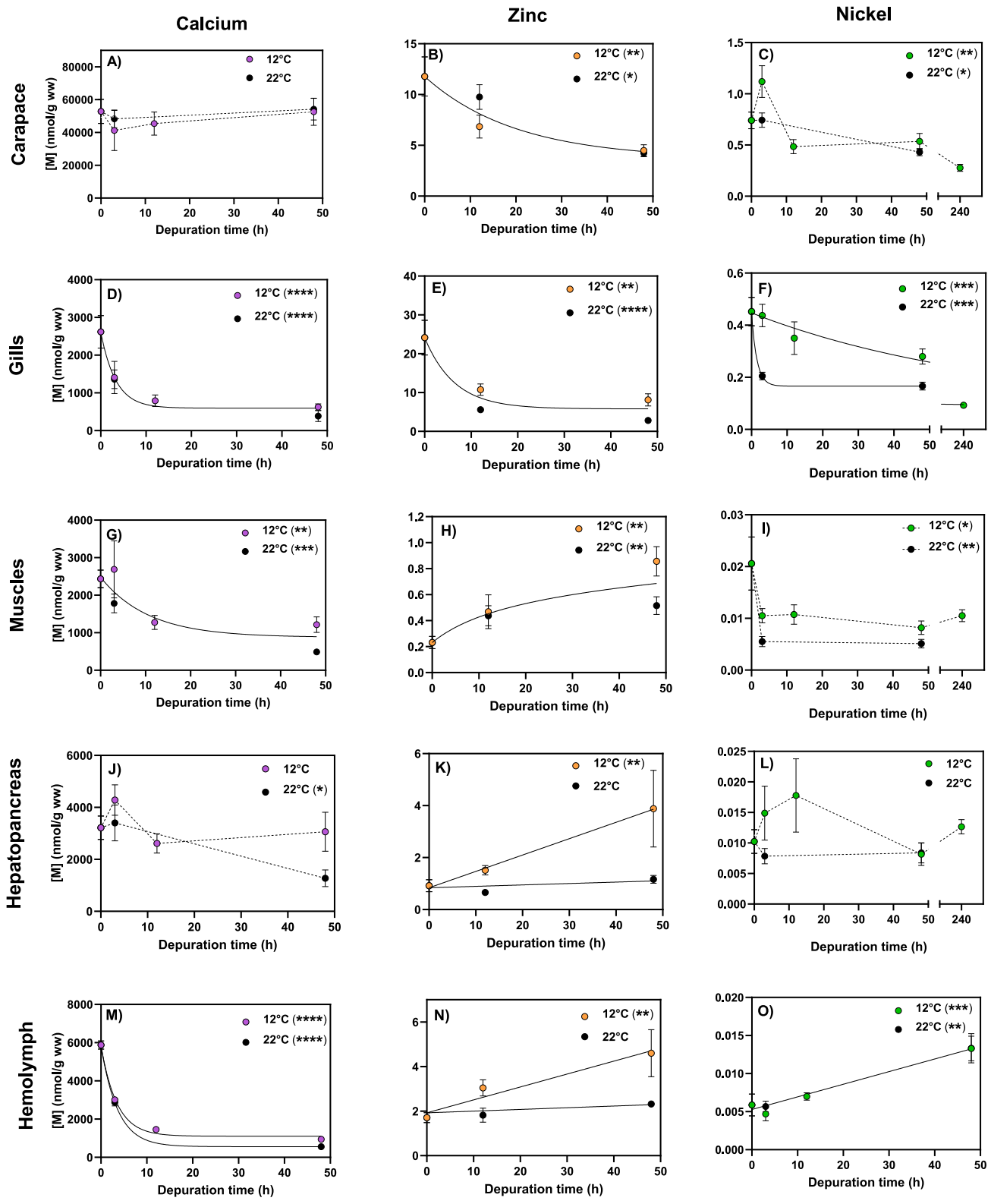

Fig. 2. Concentration of newly accumulated A) Ca, B) Zn and C) Ni in crab tissues, after a 24-h exposure to respectively ^{45}Ca (and 389 mg L^{-1} total Ca), ^{65}Zn (and 82 μg L^{-1} total Zn), or ^{63}Ni (and 8.2 μg L^{-1} total Ni) at 12 °C, then various depuration times in sea water at either 12 or 22 °C. At each sample time, crabs were rinsed with EDTA to remove newly accumulated metal loosely bound to the external surface. Values are mean ± SEM (n = 6–13). Asterisks indicate a significant temporal effect on metal tissue level at a given temperature (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, one-way ANOVA or Kruskal-Wallis test). Dotted lines are connecting lines and plain lines are regression lines with a first-order elimination kinetics model (panels B, D–G, I, M), a saturation binding model (panel H) or a linear model (panels K, N, O). A common model fitting both temperature datasets (panels B–D, G–I, N–O) indicates the absence of temperature effect ($p > 0.05$, F-test).

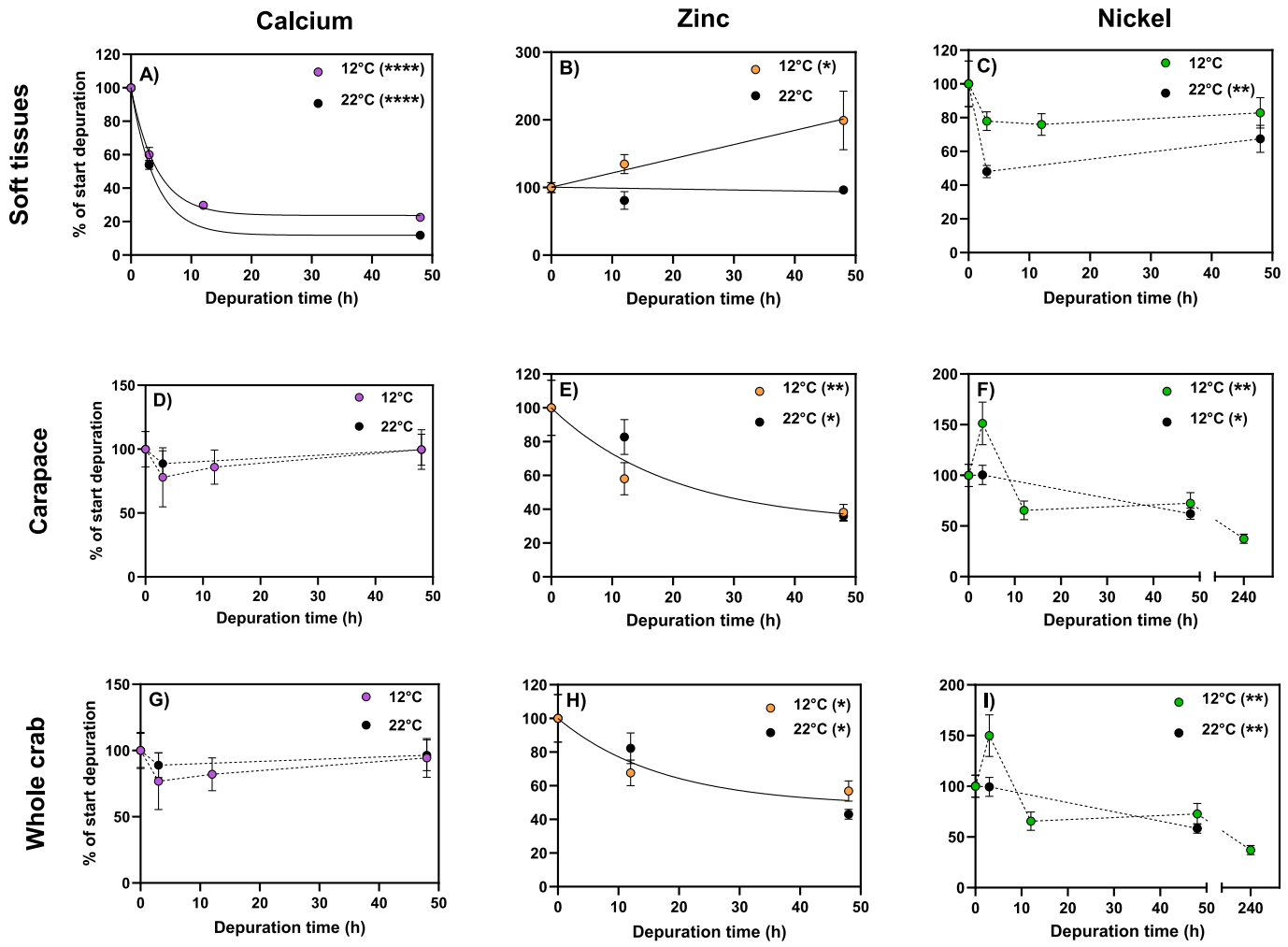

Fig. 3. Proportion of newly accumulated A) Ca, B) Zn and C) Ni remaining in all soft tissues, carapace and whole crabs after a 24-h exposure to respectively ^{45}Ca (and 389 mg L^{-1} total Ca), ^{65}Zn (and 82 μg L^{-1} total Zn), or ^{63}Ni (and 8.2 μg L^{-1} total Ni) at 12 °C, then various depuration times in sea water at either 12 or 22 °C. At each sample time, crabs were rinsed with EDTA to remove newly accumulated metal loosely bound to the external surface. Values are mean ± SEM (n = 6–13). Whole crab and soft tissues data were estimated with Eqs. (3) and (4). Asterisks indicate a significant temporal effect on metal tissue level at a given temperature (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, one-way ANOVA or Kruskal-Wallis test). Dotted lines are connecting lines and plain lines are regression lines with first-order elimination kinetics model (panels A, E, H) or linear models (panel B). A common model fitting both temperature datasets (panels E, H) indicates the absence of temperature effect ($p > 0.05$, F-test).

tissues, newly accumulated Ca (Fig. 3A) was largely and quickly eliminated over 48 h in Ca-45-free sea water. The remaining Ca proportion was 30 % and 20 % of the initial levels at 12 °C and 22 °C, respectively at 48 h (slight temperature effect, $p = 0.0244$, F-test) (Fig. 3A). Compared to Ca, the proportion of newly accumulated Ni (Fig. 3C) in whole soft tissues was decreased to lower extent (only significant at 22 °C), with most of the elimination occurring within the first 3 h of depuration. After 48 h, the remaining Ni proportion was 80 % and 60 % of initial levels, at 12 °C and 22 °C respectively (Fig. 3C). On the other hand, newly accumulated Zn appeared to increase in the whole soft tissues at 12 °C over the depuration phase, but was stable at 22 °C (Fig. 3B).

In the carapace, newly accumulated Ca was retained (Fig. 3D), while newly accumulated Zn (Fig. 3E) and Ni (Fig. 3F) decreased to 40 % and 65 % respectively over 48 h in clean sea water. Ni then further decreased to 40 % by day 10 of depuration (Fig. 3F). There was no evidence of a temperature effect (Fig. 3D–F).

In whole crabs (Fig. 3G–I) proportions of metal depurated were very close to the proportions presented in the carapace in the above paragraph (Fig. 3D–F). More precisely, the remaining metal proportions in whole crabs after 48 h of depuration were 95 % for Ca, 60 % for Zn and 70 % for Ni. This similarity with the carapace patterns was expected, as the carapace accounts for most of the crab total mass (56 %) and contains high metal concentrations relative to the other tissues (cf. Fig. 2). This compartment contained over 85 % of Ca, Ni and Zn total body burden, as previously reported (Nogueira et al., 2024).

From this dataset, we estimated that about 1800, 3.3 and 0.12 nmol of Ca, Zn and Ni were respectively eliminated per g of whole crabs over 48 h of depuration at 12 °C. These losses occurred mainly from the soft tissues for Ca and Ni and from the carapace for Zn. Considering the important uncertainties around these estimates, they agreed relatively well (within a factor of ~2) with the direct measurements from the depuration kinetics experiment using whole crabs and radioactivity appearance in water (cf. Fig. 1).

For each crab compartment, Q_{10} values for depuration rates over 48 h of depuration (Table 1) are close to 1.0 overall.

3.4. *Effects of shielding and euthanization on metal carapace levels during depuration*

There was little evidence that shielding the carapace from the external sea water or euthanization of the crab prior to the depuration period affected metal concentrations in the carapace over the 48-h

Table 1
Temperature coefficients (Q_{10}) calculated between the 12 and 22 °C depuration rates (calculated over the 48 h depuration period) for soft tissues, carapace and whole crabs (data in Fig. 3). No depuration was observed for Zn in the soft tissues at 12 °C, so a Q_{10} could not be calculated for this metal in this crab compartment.

	Calcium	Zinc	Nickel
Whole soft tissues	1.14	–	1.89
Carapace	1.05	1.03	1.37
Whole crab	0.63	1.32	1.53

depuration period in uncontaminated sea water (Fig. 4). Only a weak effect of shielding and euthanization was observed for Ni (Fig. 4C) after 3 h of depuration ($p = 0.0154$, one-way ANOVA), with a significant decrease in Ni carapace level observed in shielded ($p = 0.0125$, Dunnett's test) and euthanized ($p = 0.0415$, Dunnett's test) crabs compared to the control treatment. Regardless, these effects were opposite those that would occur if shielding or euthanization were to block depuration from the carapace. These decreases were no longer significant at 48 h of depuration.

4. Discussion

4.1. Methodological considerations in interpretation of the data

There are some important caveats in interpretation of the data which relate to the experimental design. The first is that the ^{65}Zn and ^{63}Ni protocols were essentially "pulse" experiments, whereas the ^{45}Ca protocol was a "pulse-chase" experiment. By this we mean that for the former, the crabs were exposed to spiked Zn and Ni for 24 h, in the presence of radio-labeled metals, followed by return to sea water that was nominally free of these metals. Whereas for Ca, after maintaining the crabs in a normal seawater Ca concentration (9.7 mmol L^{-1}) with the presence of radio-labeled Ca for 24 h, animals were returned to normal sea water that contained the same concentration of Ca in the unlabeled form. On a relative basis, this might elevate Ca depuration rates relative to Zn or Ni rates, because the availability of more incoming non-labeled metal from the environment might accelerate the displacement of labeled metal.

The second is that all the rate and internal movement data are expressed in terms of newly accumulated metal, based on the specific activity - i.e. "radioactivity (CPM mL^{-1})/total metal (nmol mL^{-1})" in the original exposure water (Eq. (1)). However, once the newly accumulated metal enters the crab, its specific activity may decline by an unknown amount as the newly accumulated metal equilibrates with "background" unlabeled metal already present in the tissues. The extent of this equilibration is unknown, but it means that movement and depuration rates would likely be underestimated, i.e. a conservative error.

Finally, estimates of metal distribution in the various biological compartments (Eqs. (2) and (3)) assume that metal is uniformly distributed across the whole exoskeleton. This assumption appears correct across the dorsal carapace, which was sampled at random locations in individual crabs and yielded relative standard error ~ 13 % for the mean accumulation of all three metals. However, as areas other than the dorsal shell were not sampled (e.g. ventral carapace), heterogenous metal accumulation across the whole exoskeleton could not be verified, adding to the uncertainties around the distribution estimates.

4.2. Metal accumulation: tissue-specific patterns

Our accumulation data agree with previous studies. Notably, while influxes of metals are at their highest in the mineralizing exoskeleton of postmoult crabs, substantial influxes of metals have also been observed in the carapace of intermoult crabs (e.g. Jennings and Rainbow, 1979; Chan and Rainbow, 1993a, 1993b; Blewett and Wood, 2015; Blewett et al., 2015; Henry and Kormanik, 1985; Neufeld and Cameron, 1993; Nogueira et al., 2024; Roer, 1980). In general, tissue-specific concentrations of newly accumulated metals after 24-h exposure (i.e. time 0 h in the current study; Fig. 2) agreed well with those reported at this same time point in our parallel study on metal bioaccumulation (Nogueira et al., 2024). The two studies were conducted on different batches of crabs collected at the same site over two successive field seasons. As discussed by Nogueira et al. (2024), the very different absolute concentrations of newly accumulated metals (Ca > Zn > Ni) largely reflected comparable differences in metal concentrations in the exposure solutions. Overall, these data confirm that newly accumulated Ca concentrations were greatest in the carapace, then the hemolymph, and smallest in the gills, muscle and hepatopancreas (Fig. 2). In partial contrast, Zn and Ni concentrations were highest in both carapace and gills, but much lower in the other tissues (Fig. 2).

4.3. Metal depuration: whole crab and tissue-specific patterns

The overall rates of depuration of newly accumulated metal from the intact live crabs over 48 h at 12 °C, as measured by the appearance of radioactivity in the external sea water were similar on a relative basis for Ca, Ni, and Zn (Fig. 1), with comparable first order rate constants (0.053 to 0.061 h^{-1}). However, this similarity disguises many differences. First, as noted in Section 4.1, the fact that the Ca experiment was a "pulse-chase" protocol may have accelerated its depuration rate – i.e., if the experiment had been simply a "pulse" protocol (impossible as Ca-free depuration water would have killed the crab!), lower rates may have been recorded.

Second, the tissue-specific patterns of depuration differed greatly among the three metals. Newly accumulated Ca was lost only from soft

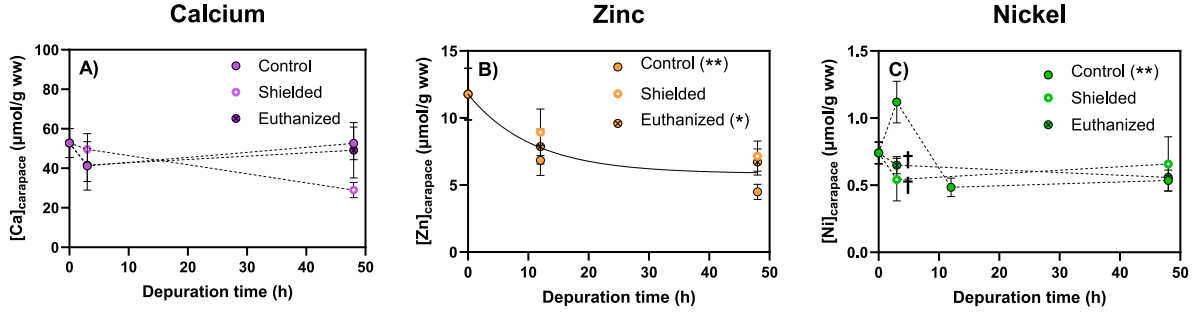

Fig. 4. Concentration of newly accumulated A) Ca, B) Zn and C) Ni in crab carapace, after a 24-h exposure to respectively ^{45}Ca (and 389 mg L^{-1} total Ca), ^{65}Zn (and 82 µg L^{-1} total Zn), or ^{63}Ni (and 8.2 µg L^{-1} total Ni) at 12 °C, then various depuration times in sea water. At each sample time, crabs were rinsed with EDTA to remove newly accumulated metal loosely bound to the external surface. Values are mean ± SEM (n = 7–13). Asterisks indicate a significant temporal effect on metal carapace level for a given treatment (* $p < 0.05$, ** $p < 0.01$, one-way ANOVA or Kruskal-Wallis test). Daggers (3-h Ni data only) indicate a significant difference from control treatment (Dunnett's test). Dotted lines are connecting lines. Plain lines (Zn data) show regressions with first-order elimination kinetics model, with no effect of shielding or euthanization ($p > 0.05$, F-test).

tissues (gills, muscle, hepatopancreas, and hemolymph; Figs. 2D, G, J, M; 3A), with no detectable decrement in the carapace burden (Figs. 2A, 3D). Thus, the loss of Ca from whole crabs observed in Fig. 1A can be mostly attributed to soft tissue depuration, and possibly also to some desorption of loosely-bound metal from the crab exoskeleton (as no EDTA rinse was conducted prior to depuration in this series in Fig. 1). Thus since 64 % of the newly accumulated Ca that was actually incorporated into the carapace had been taken up directly from the water (Nogueira et al., 2024), it simply stayed there, as confirmed by the carapace shielding results (Fig. 4A).

In contrast, newly accumulated Zn was lost largely from the carapace where the concentration declined by about 60 % over 48 h (Figs. 2B, 3E). There was no net loss from the whole soft tissues (Fig. 3B), though gill burden declined markedly (Fig. 2E), whereas muscle, hepatopancreas, and hemolymph burdens all increased (Fig. 2H, K, N). Clearly new metal shifted from gills and/or carapace to internal tissues during the depuration period. Niyogi et al. (2016), using ^{65}Zn in a perfused gill study, demonstrated that a large amount of newly accumulated Zn is held in the gills, and this moves only slowly into the hemolymph. This is very different from the behaviour of ^{45}Ca, which passes readily through perfused crab gills (Lucu, 1994; Pedersen and Bjerregaard, 1995). Martin and Rainbow (1998) reported that ^{65}Zn continued to accumulate in the hemolymph of green crabs for 7 days after a 24-h waterborne exposure. Chan and Rainbow (1993a) performed a similar depuration experiment after a much longer initial exposure period (10 days) to radio-labeled Zn at about the same concentration as in the present study. They reported that during depuration, newly accumulated Zn moved from gills to the hepatopancreas, muscles and other soft tissues, as in the present study. However, in contrast they reported no net loss from the whole animal and minimal loss from the carapace. Perhaps the radio-labeled metal becomes more firmly incorporated after 10 days. In the present study, external shielding had no effect on the loss of newly accumulated Zn from the carapace (Fig. 4B), suggesting that excretion must have occurred via an internal route through the hemolymph and out through the gills (Bryan, 1966; Chan and Rainbow, 1993b).

The depuration pattern of newly accumulated Ni exhibited some similarity to that for newly accumulated Zn, with losses from the carapace (Figs. 2C, 3F) and gills (Fig. 2F), and increases in the hemolymph (Fig. 2L) and hepatopancreas (transiently) (Fig. 2O). Muscle levels initially decreased (Fig. 2I), in contrast to those of Zn which increased (Fig. 2H). This again indicates fluxes of newly accumulated metal between tissues during the depuration period. As with ^{65}Zn (Niyogi et al., 2016), perfused gill studies with ^{63}Ni have shown that high levels of newly accumulated Ni are held in the gills, and are only slowly released into the hemolymph (Blewett et al., 2015). Overall, the data suggest a pattern of regulation, which would support the potential essentiality of Ni (Blewett and Leonard, 2017). The weak to absent effect of external shielding (Fig. 4C) indicates an internal pathway for Ni loss from the carapace via the hemolymph, though there appears to be no information on whether gills, urine, or faecal excretion are the main excretion routes for Ni.

Interestingly, 100 % of both newly accumulated Zn and newly accumulated Ni incorporated into the carapace are taken up through its external surface (Nogueira et al., 2024), yet the present results show that loss of these metals occurs only through its internal surface. This suggests that metals entering via this route can move to the hemolymph and other internal tissues and are presumably available for metabolic processes. This finding contrasts with the traditional view that metals stored in the carapace are not biologically available (Rainbow, 1988; Rainbow et al., 1990), but agrees with the findings of Bergey and Weis (2007) on the fiddler crab (*Uca pugnax*), that in addition to Ca, three trace elements (Zn, Cu, and Pb) are reabsorbed from the carapace prior to moulting. It also supports the report of Nogueira et al. (2024) that external shielding of the dorsal carapace tended to decrease the accumulation of all three metals in internal soft tissues in *Carcinus maenas*. The present findings also show that incorporation into the carapace from the external sea water is essentially a one-way street. Newly accumulated metals can leave only through the internal surface; the external surface of the carapace is not a depuration site. This answers the question raised in the Introduction. However, it is worth noting that moulting of the exuviae may be an important route for depuration of metals that are not fully reabsorbed from the carapace (Bergey and Weis, 2007; Chan and Rainbow, 1993b), especially since the green crab may undergo 18 moults in its lifetime (Crothers, 1967)!

4.4. Mechanisms of depuration in intermoult C. maenas

With only a few exceptions, the temperature-dependence of depuration processes was remarkably low, with similar rates at 12 °C and 22 °C (Figs. 2, 3), yielding Q_{10} values close to 1.0 (Table 1). These indicate that the rate-limiting steps in depuration are set by physicochemical rather than biological processes (Hoar, 1983). This conclusion was reinforced by the finding that euthanization had no effect on the rates of depuration from the carapace, where most of the newly incorporated metal was stored (Fig. 4). For calcium, a simple explanation is that it is stored in the carapace as carbonate precipitates, and that redissolution of Ca from these precipitates is a limited physicochemical process due to the minimal proton influx occurring during the intermoult period (Roer, 1980). For transition metals (Zn and Ni), while they may also form carbonate (and oxide) precipitates in crustacean cuticles, they are most often considered to form chemical bonds with Ca^{2+}-binding sites in the chitinous matrix (nitrogen, hydroxyl and sulfur containing functional groups) (Gilbert and Avenant-Oldewage, 2018; Keteles and Fleeger, 2001). Ni and Zn loss from the carapace might have been the result of ion exchange, with Ca^{2+} displacing Zn^{2+} and Ni^{2+} from these functional groups upon return of the crab to clean sea water (Morales-Barrera and Cristiani-Urbina, 2022; Pradhan et al., 2005). On the other hand, the remaining Ni still associated with the carapace after 10 days in clean water may be in a more stable biomineral form (e.g. nickel carbonate).

This differed clearly from the original accumulation processes, where analyses of temperature effects revealed higher Q_{10} values (Ca > Ni > Zn) indicating a mix of biologically mediated (i.e. life-dependent) and physicochemical functions setting the rates (Nogueira et al., 2024). Indeed, with respect to the carapace alone, “passive” physicochemical mechanisms of incorporation dominated entirely for Zn, with “active” biologically mediated uptake processes being more important for Ca and Ni.

4.5. Perspectives

Recently, de Almeida Rodrigues et al. (2022) have emphasized the importance of crabs for monitoring metal pollution in the marine environment. Given the increasing use of *Carcinus maenas* as a model species in ecotoxicology (Griffin et al., 2024; Leignel et al., 2014; Rodrigues and Pardal, 2014), and its invasive nature, with populations and distributions now expanding throughout the world (Frederich and Lancaster, 2024; Yamada, 2001; Yamada et al., 2005), it is ideally suited for this purpose. de Almeida Rodrigues et al. (2022) focused particularly on the hepatopancreas as the tissue of choice for screening. However, the present investigation and our companion study (Nogueira et al., 2024) have cast new light on the carapace as the major storage site for metals, and the mechanisms by which uptake and depuration occur in this tissue, the latter with no temperature effect. Unlike the hepatopancreas, the carapace can easily be sampled non-lethally, and is regularly renewed by moulting, providing a dynamic picture of pollution. We recommend that the carapace of *Carcinus maenas* be included in future biomonitoring programs.

Currently, trapping efforts are being deployed in North America to control the populations of green shore crab that wreak havoc on coastal ecosystems (McKenzie et al., 2022), and various commercial markets are being evaluated for the trapped crabs (human consumption, bait for

lobster fisheries, fertilizer, bioplastic). At the same time, crabs are very tolerant to metals and are often found in heavily contaminated areas such as harbours. Understanding the fate of metals accumulated in *C. maenas* may offer valuable insights for their potential commercial uses. Notably regarding human consumption, this study and our other recent work (Nogueira et al., 2024) indicate that most of the toxic inorganic metals accumulate in the hard shell, which is generally not used for food. The present study also indicate that it may takes several days of exposure in clean sea water to depurate the metals from the soft tissues of crabs collected in contaminated areas, as trace metals stored in the carapace are slowly released into the soft tissues, prior to exiting the crab.

CRediT authorship contribution statement

Anne Crémazy: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lygia S. Nogueira:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Chris M. Wood:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, 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.

Acknowledgements

Supported by NSERC (Natural Sciences and Engineering Research Council of Canada) Discovery grants to CMW (RGPIN-4732012, RGPIN-201703843, RGPIN-202303714) and to AC (RGPIN-2019-04400), and a CAPES (Coordenaçao de Aperfeiçoamento de Pessoal do Ensino Superior, Brasília, DF, Brazil) postdoctoral grant to LSN. Thanks to the staff of Bamfield Marine Sciences Centre, especially the Research Coordinator, Dr. Eric Clelland, for their assistance, to Dr. Greg Goss (University of Alberta) for crab collection, and to Vivian Lai (UBC) and Dr. Marghaleray Amini (UBC) for ICPMS analyses.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.177477.

Data availability

Data will be made available on request.

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