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Bioaccumulation kinetics of cadmium and zinc in the freshwater decapod crustacean *Paratya australiensis* following multiple pulse exposures

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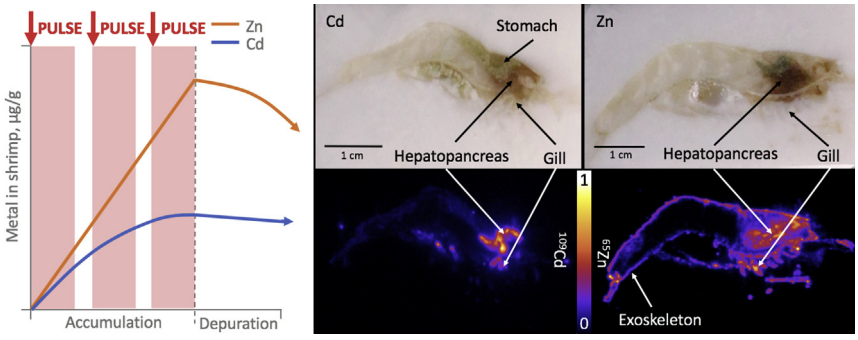
HIGHLIGHTS

- Shrimp accumulated aqueous cadmium and zinc individually and in a binary mixture over three short term (<10 h) pulses
- Over the depuration, zinc efflux was significant and cadmium efflux was minimal
- Both metals observed in gills and hepatopancreas throughout depuration

GRAPHICAL ABSTRACT



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ABSTRACT

Stormwater runoff has been identified as a major source of metal contaminants in urban waterways, where during storm events organisms tend to be exposed to short-term pulses, rather than a constant exposure of contaminants. Current water quality guidelines (WQGs) are generally derived using data from continuous exposure toxicity tests, where there is an assumption that chronic exposures provide a meaningful way of assessing the impacts and effects in organisms as a result of these pulsed storm events. In this current study the radioisotopes ^{109}Cd and ^{65}Zn were used to explore uptake, depuration and organ distribution in the decapod crustacean *Paratya australiensis*, over three short-term (<10 h) exposures. Exposures to radiolabelled cadmium only, zinc only or a mixture of cadmium and zinc were followed by depuration in metal- and isotope-free water for 7 days. Whole-body metal concentrations were determined by live-animal gamma-spectrometry and an anatomical distribution of the radioisotopes was visualised using autoradiography post-mortem. Both metals were significantly accumulated over the pulsed exposure period. In both treatments cadmium and zinc body burden increased at the same rate over the three pulses. Final metal body burden did not markedly differ when shrimp were exposed to metals individually compared to a binary mixture. Over the course of the depuration period, cadmium efflux was minimal, whereas zinc efflux was significant. Autoradiography indicated the presence of both metals in the gills and hepatopancreas throughout the depuration period. These results demonstrate how short-term repeated exposures result in the accumulation of contaminants by shrimp. This study highlights the importance of considering the inclusion of pulsed toxicity tests in frameworks when deriving WQGs.

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1. Introduction

In growing cities, increasing urbanisation tends to be a major contributor to the degradation of urban waterways (Pitt et al., 1995). Urban expansion leads to modification of the natural habitat, altering the quality of the urban aquatic environment (Brown and Peake, 2006). Significantly, stormwater runoff is a major cause of water quality degradation (Burton et al., 2000; Masoner et al., 2019; Zgheib et al., 2012) where variations in surrounding land use, rainfall duration, intensity and frequency have a strong influence on the quality of stormwater output.

Monitoring has revealed that stormwater runoff events tend to occur as multiple pulses each lasting durations of 1–48 h with some pollutant loads being magnitudes of difference above background (Allinson et al., 2017; Walsh et al., 2012). As a consequence, organisms within these environments tend to be exposed to multiple pulses, rather than a constant exposure of contaminants. Water quality criteria (WQC) and guideline values (WQGVs) are typically derived from toxic effects data for continuous exposures, yet, in reality, exposures are more likely to be pulse events for some contaminants. Therefore, the risk posed by pulse contaminant exposures should be considered and routinely assessed (Angel et al., 2018; Burton et al., 2000; Diamond et al., 2006; Hogan et al., 2013; Sinclair et al., 2014).

Stormwater pollutants incorporate a diverse range of compounds including herbicides, pesticides and hydrocarbons (Allinson et al., 2017) and often the priority pollutants identified are metals (Pitt et al., 1995). Metals in stormwater can originate from a range of anthropogenic sources, including vehicle wear, weathering of infrastructure and effluents from surrounding industrial land uses (ANZECC and ARMCANZ, 2000; Brown and Peake, 2006; Makepeace et al., 1995). Specifically, the biologically-essential metal zinc has been identified as a ubiquitous stormwater pollutant (Burton et al., 2000), while the non-essential metal cadmium is another common stormwater pollutant in urban Australian waterways (Allinson et al., 2017).

As the fate and behaviour of metal mixtures in the environment receives increasing attention, understanding the underlying mechanisms behind the toxicity and effect of environmentally relevant metal mixtures has been identified as a priority (Mebane et al., 2020; Van Genderen et al., 2015). Differences in bioaccumulation, final organ distribution and interactions between the non-essential and essential metals cadmium and zinc in decapod crustaceans have been well described (Cresswell et al., 2017, 2015; Nugegoda and Rainbow, 1995; Nuñez-Nogueira et al., 2006; Nuñez-Nogueira and Rainbow, 2005a, 2005b; Rainbow, 2007; Rainbow and Luoma, 2011; White and Rainbow, 1984a, 1984b). However, reviews of metal mixture toxicity have yet to elucidate a distinct pattern in interactions between cadmium and zinc, where toxicity has been reported to be sometimes additive, sometimes less-than or more-than-additive among species (Mebane et al., 2012; Norwood et al., 2003; Vijver et al., 2011). This aspect is becoming increasingly relevant to consider when designing bioassays and developing models of trace metal toxicity (Balistrieri and Mebane, 2014; Mebane et al., 2017; Schmidt et al., 2010). Examining these interactions in the context of pulsed exposures adds another level difficulty in predicting the effect of these metals, where identified knowledge gaps include the lack of short-term pulse exposures with metal mixtures, and the inclusion of multiple pulses during the uptake phase. Recently, attention has been directed towards evaluating and managing acute, pulsed discharge events, such as stormwater runoff, and the associated pollutant load contribution (Allinson et al., 2017; Feldman et al., 2015; Kroon et al., 2016; Patterson et al., 2015). A renewed understanding of the factors that influence the bioaccumulation of metals in aquatic organisms will help assess the risks metals pose under variable conditions, for example, during storm events. Radiotracing, involving the use of gamma-emitting metal radioisotope tracers, has proven to be a valuable tool when used in an ecological context, advancing the study of metal bioaccumulation and retention in aquatic organisms (Cresswell et al., 2017, 2015; Lanctot et al., 2017; Wang et al., 1996) at ecologically relevant trace concentrations (<1 μg/L for cadmium and <50 μg/L for zinc) that may otherwise be undetectable using other analytical approaches.

The primary objective of this study was to investigate the response of an Australian freshwater invertebrate to metal pollutants following three short-term (<10 h) exposures at environmentally relevant stormwater concentrations. Specifically, the accumulation and depuration kinetics, as well as organ distribution of the radioisotope tracers ^{109}Cd and ^{65}Zn were examined in the freshwater shrimp *Paratya australiensis* (Decapoda: Atyidae). This species is abundant and widespread in urban waterways in South-Eastern Australia and thus serves as an ecologically-relevant sentinel species for examining exposures of metals in stormwater runoff (Oulton et al., 2014; Walsh et al., 2001). Live-animal whole-body radiotracing was used to determine the longitudinal accumulation and depuration of each metal. Autoradiography was used to determine organ distribution of newly accumulated isotopes within each shrimp. The combination of pulse exposures and autoradiography is intended to assist in increasing the understanding of essential and non-essential metal bioaccumulation kinetics and organ distribution following multiple short-term pulse exposures.

2. Materials and methods

2.1. Animals

Adult (>10 month) shrimp were collected from an established urban wetland in Caroline Springs, VIC (37°44′45.20″S; 144°43′51.63″E), transported to ANSTO by road, then acclimated for 4 d in the ANSTO Aquatic Ecosystems laboratory in water collected from site. Site water was slowly replaced by water made up using Reverse Osmosis (RO) water at a salinity of 90 ppm using commercially available aquarium salts (Aquasonic, Wauchope, NSW) hereafter referred to as synthetic freshwater (SFW: 0.85 g Cl^-; 0.12 g SO_4^{2-}; 0.47 g Na^+; 56 mg Mg^{2+}; 18 mg Ca^{2+}; 18 mg K^+; 6 mg HCO_3^- in 20 L RO). The amount of trace cadmium and zinc introduced into the synthetic freshwater by the commercially available aquarium salts was 0.005 μg Cd/L and 0.1 μg Zn/L. Shrimp were held in 20 L glass aquaria filled with aerated water that was renewed post feeding. Animals were fed TetraMin® flake (Blacksburg, VA) slurry ad libitum every two days, with all uneaten food siphoned from the tanks 2 h after providing the food. Laboratory conditions were maintained at 21 ± 1 °C on a 12:12 h light:dark regime. Shrimp were visually checked twice daily and holding water physicochemical parameters (temperature, pH, salinity) were tested before each 25% water change every two days, maintained as follows: temperature 21 ± 2 °C; pH 7.2 ± 0.2; salinity 91 ± 4 ppm.

2.2. ^{109}Cd and ^{65}Zn pulse exposure: uptake and depuration

^{109}Cd (as $CdCl_2$ $t_{1/2}$ = 462 days, specific activity of stock = 6.97E^{13} Bq/g) was obtained from Eckert and Ziegler Isotope Products Inc., Valencia, CA. ^{65}Zn (as $ZnCl_2$ $t_{1/2}$ = 244 days, specific activity of stock = 9.93E^{08} Bq/g) was produced at Australia's Nuclear Science and Technology Organisation (ANSTO) facility in Sydney, Australia. Both isotope stocks were dissolved in 0.1 M HCl before being further diluted in synthetic freshwater (as above).

Shrimp were introduced individually into square 1.124 L polypropylene containers (Décor, Tellfresh; hereafter referred to as exposure chambers) containing isotope-free (non-radioactive) water for an acclimation period of 24 h. Constant, gentle aeration was provided for all exposure chambers via a compressed airline fed through a hole drilled into the lid. All tests were conducted on a 12:12 h light:dark regime in a temperature controlled room. Acclimation, exposure and depuration water physicochemical parameters were maintained as follows: temperature 21 ± 1 °C; pH 7.1 ± 0.2; salinity 90 ± 2 ppm.

Nominal exposure concentrations were 3.5 μg Cd/L (233 kBq ^{109}Cd/L) and 1240 μg Zn/L (466 kBq ^{65}Zn/L). These concentrations are based on published field data from Melbourne stormwater monitoring locations collected during storm events (Francey et al., 2010). In regard to sublethal concentrations, the concentrations investigated for cadmium falls below recorded 96 h LC50 value determined for the related species *Paratya tasmaniensis* of 60 μg/L (Thorpe and Lake, 1974). For zinc, the test concentration falls above the recorded 96 h LC50 value for *P. tasmaniensis* of 1210 μg Zn/L. However, pilot tests conducted with *P. australiensis* from the same field population did not result in any mortalities when animals were exposed to concentrations of 2000 μg Zn/L. The addition of radiotracers to synthetic freshwater did not significantly impact solution pH (pH 7.1 ± 0.2). Following acclimation, individual shrimp were transferred into separate replicate exposure chambers and were exposed to either a single metal (^{109}Cd or ^{65}Zn) (n = 7) or to ^{109}Cd and ^{65}Zn as a binary mixture (n = 5) over the course of three short-term pulse exposures. During breaks between pulse exposures, shrimp were transferred to individual clean exposure containers filled with isotope-free water. Each exposure chamber contained 400 mL of exposure solution and a polypropylene basket that allowed animals to be removed easily from the solution between pulse exposures and prior to radioanalysis. The exposure (uptake) period, designed to incorporate a range of exposure scenarios that have been recorded during a storm event in the Melbourne region (Walsh et al., 2012), is detailed as follows; 6 h pulse, 6 h break, 9 h pulse, 3 h break, 6 h pulse. Following the final pulse exposure, shrimp were transferred to clean exposure containers filled with isotope-free water for 7 days depuration. Shrimp were not fed over the course of the uptake phase. During the 7 day depuration phase, shrimp were fed every second day, with 100% water changes taking place 2 h post feeding. Subsamples of exposure and depuration solutions were radioanalysed to check for isotope activity and to allow for total metal concentration determination.

Animals were radioanalysed (see the Supporting Information for technique) before and after each pulse, and every 24 h during the 7 day depuration period. Prior to radioanalysis, *P. australiensis* underwent a rinsing procedure where individuals were removed from the exposure chambers and transferred to a rinsing basket and rinsing chamber, then gently dunked 10 times in isotope-free water. This was repeated 4 times in 4 different chambers (i.e. each animal was rinsed 40 times) with the rinsing solution being replaced every third animal. This rinse procedure has been validated (see Supporting Information). At nominated time points throughout the depuration phase, an individual from both single-metal (^{109}Cd and ^{65}Zn) treatments was removed for autoradiography (see Section 2.4 Autoradiography). These nominated autoradiography time points were at depuration time 0-, 24- and 168 h (7 days).

2.3. Autoradiography

Individual shrimp from each single metal treatment group (^{109}Cd and ^{65}Zn) at each nominated sampling point (0, 24 and 168 h depuration) were killed by immersion in ice for 10 min, before being embedded in an inert resin (Cryomatrix, Thermo Scientific), snap frozen in liquid nitrogen, and then stored at −80 °C. Embedded and frozen *P. australiensis* were sectioned using a cryomicrotome (Cryostat Leica CM3050 S, Leica Biosystems). Triplicate sagittal sections (20 μm) were collected, thaw-mounted on gelatine coated slides and dehydrated on a slide warmer at 35 °C for 5 min. Sections were collected every 200 μm or when organs of interest were identified. Organs of interest included the gill, hepatopancreas, abdomen muscle, exoskeleton and antennal gland. Slides were then exposed to a phosphor plate (BAS-SR 2040) for 2 d (^{109}Cd) or 21 d (^{65}Zn) and plates subsequently visualised using a GE Typhoon FLA 7000. Image manipulation to standardise phosphor plate response to presence of isotope activity and false colouring of autoradiographs was conducted with Fiji ImageJ (NIH, https://imagej.nih.gov/ij/).

2.4. Statistical analysis

Prior to any analyses, all data were first analysed for normality (Kolmogorov-Smirnov) and homogeneity of variance (Levene's test) to ensure all chosen tests were appropriate. Differences in weight and total length of shrimp between treatments (^{109}Cd, ^{65}Zn and mixture) were analysed using an Independent Samples *t*-test. Uptake and depuration rates (μg/g/h) for both metals were calculated as the difference between successive body burdens normalised to hour. Analysis of Variance (ANOVA) tests were used to test the differences in radiolabelled metal body burden between treatments (exposure to single or binary mixture) after exposure, and to test differences in radiolabelled metal body burden within treatments throughout the depuration phase (including the time between successive pulse exposures). Differences in rates of uptake between successive pulses, and between exposure treatments were also analysed with ANOVA tests. Tukey post-hoc comparisons were used to determine the specific time points that these differences, if any, occurred. The significance level was set at $\alpha \leq 0.05$ for all tests. Statistical analyses were performed using the statistical software R (R Core Team, 2019) and figures were produced using the package ggplot2 (Wickham, 2009).

3. Results and discussion

3.1. P. australiensis size and survival

Adult shrimp had an average weight of 0.22 ± 0.02 g (ww) and total length of 30.3 ± 1.3 mm (mean ± SD; n = 3–5), with no statistically significant differences between treatments ($p > 0.05$). There was a single unexpected mortality in the ^{65}Zn treatment group at the end of the first pulse exposure. There were two unexpected mortalities in the metal mixture treatment group, one at the end of the first pulse exposure and one during the second pulse. In all cases, each individual moulted approximately 2 h before death. It is speculated that these mortalities were due to the metal pulse overwhelming an individual that may have already had a compromised ability to regulate metals due to recent moulting.

3.2. Temporal assessment of uptake and depuration phase solutions

Random subsamples (10 mL) of exposure and depuration solution (n = 3) in all treatments were radioanalysed at the start of each 'pulse' during the uptake phase, during the breaks between pulses, and prior to feeding every second day throughout the depuration phase to check exposure activity. Mean activity (±SD) of ^{109}Cd and ^{65}Zn measured throughout the pulsed uptake were 228 ± 15 and 458 ± 24 Bq/mL in individual treatments and 231 ± 8 and 462 ± 25 Bq/mL in the mixture treatment, respectively. Radiolabelled metals were detected in the exposure solution at a similar activity among replicates (<10% RSD), and were consistent with nominal concentrations. Analysis of the depuration solution radioisotope activities were consistently below detection limits for all treatments based on 10 min counts, indicating no significant leeching of radioisotopes from the animal into the surrounding solution.

3.3. Uptake of Cd and Zn in live P. australiensis

Low whole-body counts measured during the experimental period that were associated with a high counting error (>5% for ^{109}Cd and >10% for ^{65}Zn; see the Supporting Information) were excluded from all analyses and figures. For Zn only metal treatments, a single outlier individual was identified and removed from all further analyses. This individual was a gravid female carrying a large clutch of eggs and was not considered to give an accurate characterisation of 'normal' organism functioning due to a substantially enhanced metal uptake profile. This individual accumulated 4.5 times more ^{65}Zn compared to the rest of

the treatment cohort (based on average body burden) at the end of the third pulse. In the mixture treatment, two individuals were excluded from all further analysis. These individuals moulted during the pulsed uptake period which subsequently resulted in modified uptake profiles.

Repeated measures ANOVAs revealed that shrimp body burden of each metal was significantly affected by time when metals were exposed individually (^{109}Cd: $df_{1,3}$; $F_{37.9}$; $p = 0.008$. ^{65}Zn: $df_{1,4}$; $F_{20.3}$; $p = 0.0108$) indicating that aqueous ^{109}Cd and ^{65}Zn were readily accumulated over the course of the three pulsed exposures (Fig. 1).

Shrimp exposed to ^{109}Cd only appeared to accumulate most of the metal within the first two pulses. On average, shrimp accumulated ^{109}Cd at a rate of 0.043 ± 0.02 μg/g/h and 0.027 ± 0.01 μg/g/h during the first and second pulse respectively, compared to uptake over the third pulse which was at a lower rate of 0.007 ± 0.02 μg/g/h. However, these differences in uptake rate between successive pulses were not found to be significant ($df_{1,2}$; $F_{2.53}$; $p = 0.25$). Whole-body bioaccumulation of cadmium in decapod crustaceans has previously demonstrated a linear uptake rate during continuous exposures over 7–25 days in the

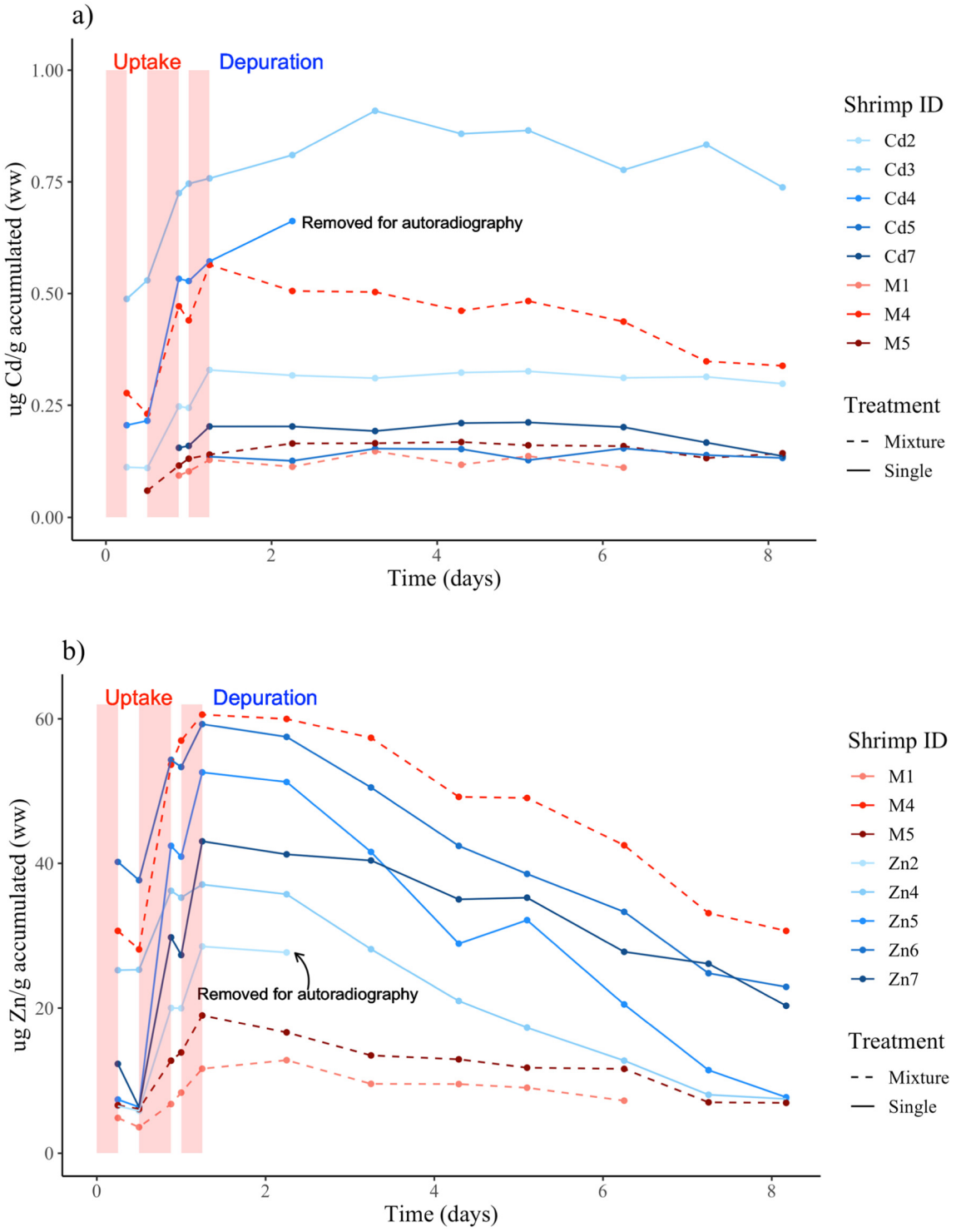

Fig. 1. Amount (μg/g) of accumulated aqueous Cd and Zn in *P. australiensis* exposed to a) ^{109}Cd and b) ^{65}Zn individually or in a mixture over the course of three pulse exposures (indicated by shaded grey rectangles) followed by a 7 day depuration. Data in graphs is the accumulation of each metal at the individual animal level.

genera *Litopenaeus* and *Macrobrachium* (Cresswell et al., 2014a; Cresswell et al., 2017; Cresswell et al., 2015; Metian et al., 2010). Generally, cadmium is regarded as a non-essential metal that is known to not be regulated in aquatic invertebrates (Luoma and Rainbow, 2005), and would present a linear uptake profile without reaching equilibrium until some toxic action was imparted.

In this instance, over the time period analysed, the rate of cadmium uptake did not significantly decrease over the three pulses. The animals used in this study originated from an urban constructed wetland, and therefore have most likely had some form of previous exposure to cadmium. Previous sampling efforts of the site's condition (unpublished data, 2016) have reported sediment metal concentrations to be 0.5 mg Cd/kg where although site water metal concentrations were not measured as part of this research, it can be expected that there would be an evident level of aqueous cadmium at this site. Previous studies have demonstrated the physiological ability in other invertebrate species to excrete or limit new bioaccumulation of cadmium after having undergone metal pre-exposure (Blackmore and Wang, 2002; Chiodi Boudet et al., 2013; Rainbow et al., 2003). The statistical results for cadmium pulsed uptake described in this study indicates linear uptake, where no evidence of an altered uptake was observed for this species over the pulsed exposure period.

Generally, throughout the pulsed exposure period, accumulation of ^{65}Zn increased linearly over time and showed no evidence of approaching a steady state (Fig. 1b). During the first pulse zinc was accumulated at an average rate of 2.8 ± 1.1 μg/g/h. During the second and third pulse the average rate of ^{65}Zn uptake was 1.2 ± 0.9 μg/g/h and 1.4 ± 0.9 μg/g/h, respectively. Although the rate of zinc uptake was greatest during the first pulse, subsequent pulses resulting in similar rates, and the differences in uptake rates between pulses were not found to be statistically significant ($df_{1,4}$; $F_{1.21}$; $p = 0.33$). Although bioaccumulation results indicate an increase in whole-body zinc over the course of the pulse uptake period, this increase is not expected to result in a proportional increase in total body zinc due to an expected efflux of recently accumulated and pre-existing (non-radiolabelled) zinc in the animals. This process has been observed before in the marine decapod *Palaemon elegans* where the concentration of radiolabelled zinc increased, but total zinc concentrations did not significantly change over the uptake period (White and Rainbow, 1984b). It may be presumed that a similar process was occurring in *P. australiensis*, considering the animals were collected from an urban field environment where previously collected *P. australiensis* had body burden values in the range of 13 ± 2 μg Zn/g (n = 5, each sample a composite of 3 individuals). However, this conclusion remains purely speculative without explicit measurements of zinc flux through the animals.

Between pulse exposures, when shrimp were transferred to isotope free water for 6 h (first break) and 3 h (second break) no significant depuration ($p > 0.05$) of either metal was detected from the whole-body of the animals.

3.4. Depuration of Cd and Zn in live P. australiensis

Repeated-measures ANOVAs revealed that over the course of the 7 d depuration period, there was no significant efflux of ^{109}Cd from shrimp in the single-metal treatment ($df_{1,4}$; $F_{1.88}$; $p = 0.24$), or of either metal in shrimp from the metal mixture treatment (^{109}Cd: $df_{1,2}$; $F_{3.36}$; $p = 0.21$. ^{65}Zn: $df_{1,2}$; $F_{0.4}$; $p = 0.62$). However, there was a significant efflux of ^{65}Zn from the single-metal treatment with this difference being detected after 144 h (6 d) depuration (df_7; $F_{2.9}$; $p = 0.02$). The efflux rate of ^{109}Cd was found to be −0.01 ± 0.02 μg/g/d when exposed individually, and −0.006 ± 0.02 μg/g/day when exposed in a metal mixture. The ^{65}Zn efflux rate was found to be −4.6 ± 0.6 μg/g/d when exposed individually, and −1.8 ± 1.9 μg/g/day when exposed in a metal mixture. After being transferred to isotope free water for the 7 d depuration period, shrimp lost only 18% of the ^{109}Cd body burden (Fig. 2a) following individual metal exposure. Similarly, following the multi-metal exposure, ^{109}Cd body burden decreased by 13% (Fig. 2b). Slow depuration rates of cadmium have been previously demonstrated for other freshwater decapod crustaceans (Cresswell et al., 2015, 2014b). However, in the marine decapod *Litopenaeus stylirostris*, Metian et al. (2010) observed at 60% reduction in accumulated cadmium over 21 d depuration following a 25 d accumulation. In comparison, shrimp appeared to depurate ^{65}Zn more readily, where over the course of the 7 d depuration period there was a 67% and 38% decrease of the ^{65}Zn body burden after exposure to metal individually and in a mixture respectively (Fig. 2b). It is expected that if there was a longer depuration period (>14 d) in zinc-deficient water this would result in lower whole-body concentrations of ^{65}Zn. This is in contrast to a recent study (Cresswell et al., 2015) where following a 21 d uptake and 14 d depuration in metal-free water, the decapod crustacean *Macrobrachium australiense* retained radiolabelled zinc.

The results demonstrate that in *P. australiensis* there was significant efflux of zinc following exposure, however shrimp did not efflux internalised cadmium to any sufficient degree. Primarily these differences in ^{109}Cd and ^{65}Zn efflux are likely due to the non-essential and essential nature of the two metals, where cadmium is not regulated and is consequently retained more readily than zinc in the body of decapods following exposure (Luoma and Rainbow, 2005).

With regards to short-term exposures, Cresswell et al. (2017) detected a sharp decrease, ~60% within the first 24 h, in whole-body cadmium following a short-term exposure (6 h), followed by a gradual decrease throughout a 7 d depuration period. This is in contrast to the depuration profile following a long-term exposure (7 d) by the same study where there was no decrease in whole-body cadmium during the first 24 h, followed by a decrease of ~50% by day 7. The depuration profile for the long-term exposure in Cresswell et al. (2017) is more in keeping with the depuration profile generated for this current study. This may suggest that uptake of metals over multiple pulses may result in similar depuration kinetics as a long-term (chronic) uptake phase, where shrimp depurate the metal less rapidly following long term exposure compared to a singular short-term (6 h) 'pulse'.

3.5. Effect of mixture

This study reveals that metal accumulation did not differ markedly when shrimp were exposed to individual metals compared to a binary mixture (Fig. 1). When exposed to ^{109}Cd in a mixture, shrimp accumulated the metal at an initial rate of 0.05 ± 0.01 μg/g/h during the first pulse, and a rate of 0.01 ± 0.02 μg/g/h during the second and third pulses, where uptake rates did not differ between pulses ($p > 0.05$). A repeated measures ANOVA did not indicate that cadmium accumulation rate as part of a binary mixture over the uptake period was significant ($df_{1,2}$; $F_{11.3}$; $p = 0.078$). In this case the lack of significance is thought to be in large part due to the limited sample size (n = 3) and high individual variation in cadmium uptake. Further studies with an increased sample size would assist in rectifying this uncertainty.

Similar to the uptake of zinc in the individual metal treatment, shrimp accumulated zinc as part of a binary mixture throughout the pulsed exposure ($df_{1,2}$; $F_{22.4}$; $p = 0.028$). Over the three pulses ^{65}Zn was accumulated at a rate of 1.39 ± 1.2 μg/g/h during the first pulse, which then decreased to 0.81 ± 1.1 μg/g/h and 0.67 ± 1.4 μg/g/h during the second and third pulse, albeit with no statistical differences ($p > 0.05$).

Exposure over three pulses to cadmium and zinc as part of a binary mixture had no significant impact on the total accumulated metal at the end of the uptake phase (^{109}Cd: $df_{5,17}$; $F_{2.05}$; $p = 0.12$. ^{65}Zn: $df_{5,17}$; $F_{3.0}$; $p = 0.28$) compared to shrimp exposed to a single metal only. This suggests that there was negligible competition between the two metals for uptake sites at the gill for the exposure concentrations used (3.5 μg Cd/L and 1240 μg Zn/L). This is consistent with some previous studies in decapod crustaceans, most notably Cresswell et al. (2015) who demonstrated that there were no significant differences between

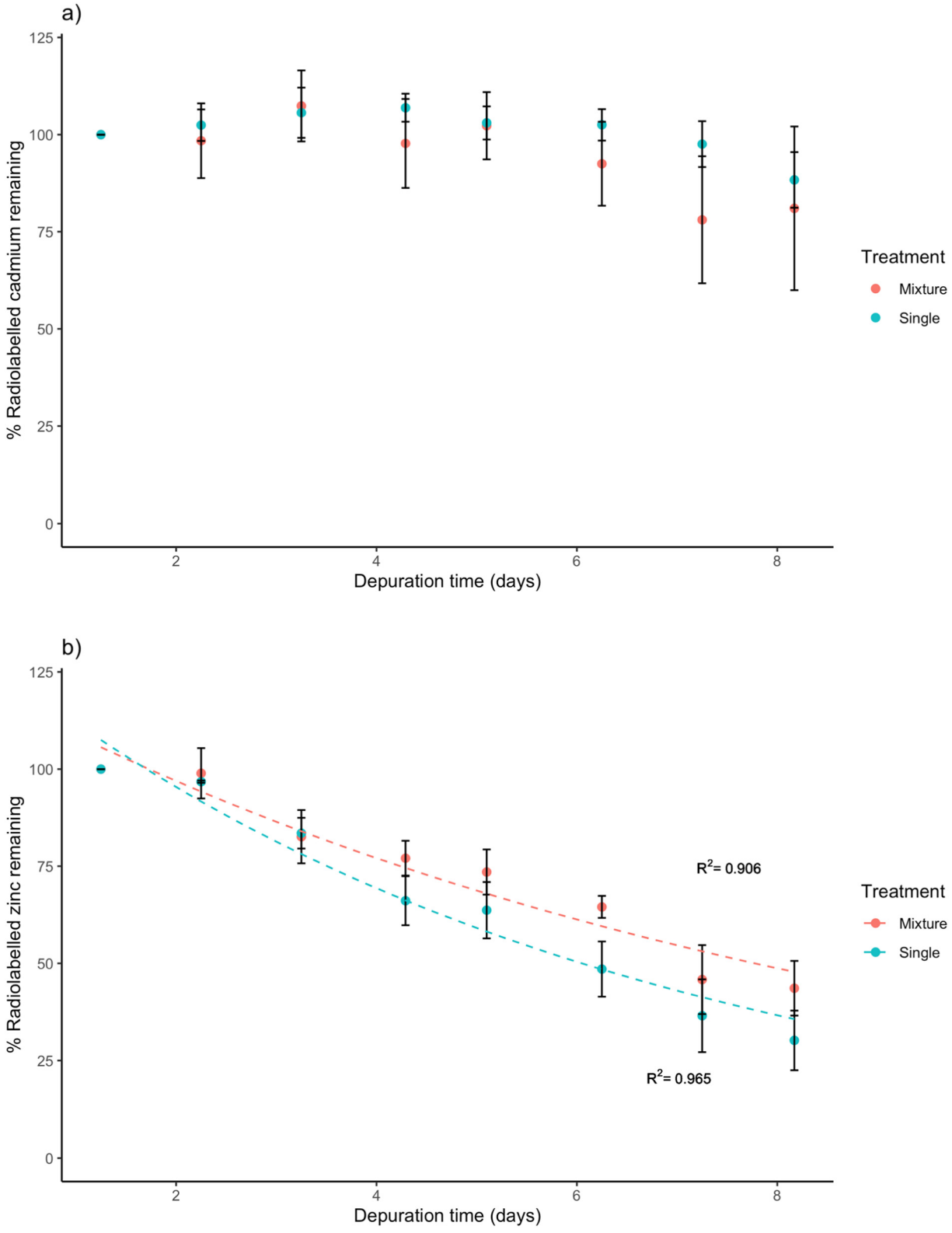

Fig. 2. Percentage of ^{109}Cd and ^{65}Zn remaining in *P. australiensis* over the course of the 7 d (168 h) depuration following exposure to either individual metals or as binary mixtures for a) cadmium and b) zinc. Dashed lines are first-order exponential depuration rates. Data is the mean ± SE (single n = 5; mixture n = 3).

the rates of uptake or total amount of bioaccumulated metal in the freshwater prawn *M. australiense* after 21 d of exposure to 2.1 μg Cd/L and 11.6 μg Zn/L individually and in a mixture. In contrast, Nugegoda and Rainbow (1995) determined that in the euryhaline decapod *Palaemon elegans*, when exposed to 20 μg/L of cadmium and zinc over 96 h, the rate of zinc uptake decreased while the rate of cadmium uptake increased relative to single-metal exposures. These described differences in synergistic and antagonistic interactions between cadmium and zinc may be due to a number of factors including interspecies variation, physiological differences between marine and freshwater species, and the relative proportion of each metal in a mixture (Buchwalter and Luoma, 2005; Hare, 1992; Lanctot et al., 2017;

Mebane et al., 2017; Norwood et al., 2003; Nugegoda and Rainbow, 1989). Metal interactions and bioavailability can also be modified by exposure to variations in water chemistry, where differences in salinity, hardness, mixture ratios and nutrient additions can have marked influences on animal uptake and depuration rates (Poteat et al., 2012; Wang and Rainbow, 2008; Wang and Dei, 2001; Wang and Fisher, 1999). Fluxes in physicochemical parameters such as pH, dissolved organic carbon and salinity would occur in environments regularly affected by pulse stormwater events, where it is well recognised that metals, as well as other pollutants, exist as complex mixtures (Makepeace et al., 1995; Masoner et al., 2019). Measures of toxicity are required to be governed by guidelines that take these fluxes into account, and research into understanding the factors that influence the uptake and retention of metals in complex mixtures is necessary. As such, pulse exposure studies that consider the bioaccumulation kinetics of metals in aquatic organisms help in improving the overall assessment of the impacts of intermittent short-term discharges on the aquatic environment.

3.6. Organ localisation of bioaccumulated metals

The presence of ^{109}Cd and ^{65}Zn was assessed by autoradiography in the gill, hepatopancreas, abdominal muscle and exoskeleton of *P. australiensis*. While ^{109}Cd and ^{65}Zn were observed in the gills and hepatopancreas, it is unknown if these organs are a site of metal loss as images only allowed for the indication of the presence or absence of metals, where the concentration of cadmium and zinc in these organs was not assessed.

3.6.1. Cadmium

On an individual organ scale, ^{109}Cd was present in the gills and hepatopancreas directly following the three pulse exposures (Fig. 3a) and at day 1 depuration (Fig. 3b), with the majority of detectable ^{109}Cd registered in the hepatopancreas at day 7 depuration (Fig. 3c). The presence of ^{109}Cd in the gills and hepatopancreas of the animal immediately following exposure, strongly implies that the major route of bioaccumulation of dissolved cadmium is through the gills. Other routes of cadmium uptake have been observed via the stomach and gastrointestinal tract through the process of imbibing (Cresswell et al., 2015; Fox, 1952), however there was no conclusive evidence of this process taking place in the current study. These findings also suggest that a proportion of cadmium accumulated by the gills remained associated with this organ, as the autoradiographs indicate a detectable presence of the metal in the gill tissue up until 1 d after the last pulse. Given the relatively labile binding of cadmium to the gills, this organ is expected to play a part in the initial desorption of the metal following a short-term pulse exposure. Indeed Cresswell et al. (2017) detailed the fast depuration rate of cadmium from the gill following a short-term (6 h) pulse in which the organ displayed an initial loss of 64% within the first 24 h, where it is assumed that during this initial period loosely bound cadmium in the gills was rapidly desorbed to the surrounding clean water. The initial decrease in cadmium in the gill suggests that these organs are a site of metal absorption and desorption for *P. australiensis*, as opposed to a site of net metal storage as suggested by Nuñez-Nogueira et al. (2006). The absorption and desorption of cadmium across the gill following a short-term 'pulsed' exposure has been demonstrated by Cresswell et al. (2017) for the decapod crustacean *M. australiense*.

Given that cadmium whole-body burden did not significantly decrease over the course of the depuration phase, it can be assumed that only a small amount of the metal bound to the gill surface was lost by desorption to the surrounding water, with the majority being transported across the basolateral membrane into the haemolymph to the hepatopancreas for detoxification via the circulatory system. However, as organ-specific metal burdens where not determined over the depuration period, it is not possible to confirm exactly which process had taken place.

Following transfer from the gills to the hepatopancreas, cadmium is likely bound to metallothionein (MT), which is involved in the cellular regulation and detoxification of the metal (Nuñez-Nogueira et al., 2006). It is here that the duration of exposure potentially affects the depuration rate of the metal from the hepatopancreas, where different metal regulation pathways are enabled if there is a continuing transfer of cadmium from the gills (i.e. chronic exposure) or if there is no further transfer (i.e. short-term exposure). Following a 6 h pulse exposure of 0.56 μg Cd/L to the decapod crustacean *M. australiense*, Cresswell et al. (2017) reported a substantially lower concentration in the hepatopancreas after 24 h of depuration, which was subsequently followed by an increase between 1 and 20 d depuration. This is in direct comparison to a long-term 7 d exposure in the same study (Cresswell et al., 2017) where 88 ± 6% of cadmium remained in the hepatopancreas after 21 days depuration. In this current study, autoradiographic image results illustrate the presence of ^{109}Cd in the hepatopancreas of the animal following 24 h depuration. This is expected to be due to the repeated nature of the pulse uptake period, where following the initial pulse and intermittent break, there was a second pulse that may have subsequently induced pathways resulting in the retention of cadmium in the hepatopancreas, rather than the metal being excreted (Nuñez-Nogueira et al., 2006). In this way, a multiple pulsed exposure may result in the same chronic exposure detoxification mechanisms described in Cresswell et al. (2017) as there is further transfer of cadmium from the gills to the hepatopancreas in each subsequent metal pulse. Future investigations into metal concentration fluxes in the hepatopancreas between pulses, and the hours directly following a series of pulses, would be required to confirm these assumptions.

3.6.2. Zinc

The spatial distribution of ^{65}Zn is shown directly following three pulse exposures (day 0 depuration; Fig. 3e), at day 1 (Fig. 3f) and day 7 depuration (Fig. 3g), where ^{65}Zn was present in the gills and hepatopancreas throughout the depuration period. While the rinsing method employed in this study was successful at removing loosely bound zinc tracer from the shrimp exoskeleton, autoradiographic images indicate that ^{65}Zn was associated with the exoskeleton at days 0 and 1 of depuration (Fig. 3e, f). Previous studies have noted that the highest proportions of zinc body content are found in the exoskeleton of decapods directly following exposure (Nugegoda and Rainbow, 1988; Nuñez-Nogueira and Rainbow, 2005a). Given the relatively short pulsed uptake period, it is expected that in this case zinc had undergone passive absorption to, and subsequent desorption from the exoskeleton, without any of the metal being transported into the underlying soft tissues from the exoskeleton (Nuñez-Nogueira and Rainbow, 2005a; White and Rainbow, 1984a). The whole-body depuration kinetics and the visual reduction of zinc associated with the exoskeleton by the end of the experiment suggests that the desorption of zinc from the exoskeleton took place sometime before 7 days depuration. The adsorption of zinc to the exoskeleton of animals has been shown to be significantly altered by the presence of major ions such as manganese through the complexion/association with oxides (MnO_x) that have high sorptive capacities (Poteat et al., 2012). In this case, it may be possible that the added manganese originating from the aquarium salts may have influenced the adsorption of zinc to the shrimp exoskeleton. Similarly, as animals were collected from the field, they may have entered the experiment with some kind of oxide phase already present on the exoskeleton.

Abdominal muscle tissue, making up a large proportion of the wet weight of decapods, can also contain a significant proportion of accumulated zinc (around 28%) (Nuñez-Nogueira and Rainbow, 2005a), where the metal is required in the muscle tissues to fulfil essential metabolic requirements (Rainbow, 2002). In this study, directly following exposure, zinc is present in part of the abdominal muscle tissue following transport via the haemolymph after being taken up by the gills.

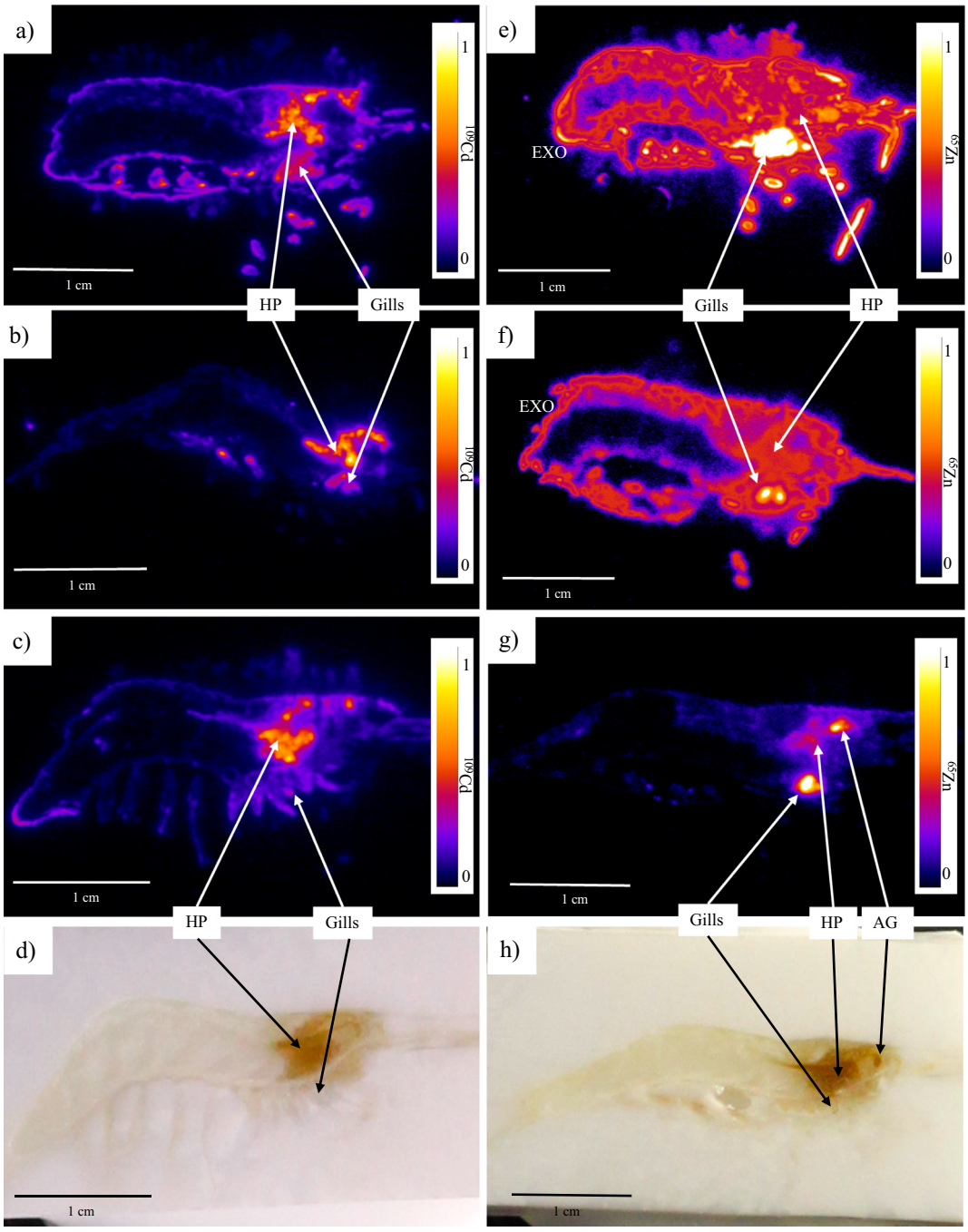

Fig. 3. False colour autoradiographs of radioisotopes in sagittal whole tissue sections of *P. australiensis* in the gills and hepatopancreas immediately following three pulse exposures for a) ^{109}Cd and e) ^{65}Zn; 24 h depuration for b) ^{109}Cd and f) ^{65}Zn and 7 d into the depuration phase c) ^{109}Cd and g) ^{65}Zn with corresponding 20 μm tissue section for d) ^{109}Cd and h) ^{65}Zn exposed shrimp at day 7 depuration. AG = antennal gland; EXO = exoskeleton; HP = hepatopancreas. Scale bars on autoradiographs represent 1 cm. False colouring indicates the absence (0) or presence (1) of ^{109}Cd or ^{65}Zn.

The presence of ^{65}Zn was maintained in the gills throughout the 7 d depuration period, where the gills are probably used as sites of absorption and desorption (Nuñez-Nogueira et al., 2006; Nuñez-Nogueira and Rainbow, 2005a). In a study conducted by Bryan (1968) in which the concentration of zinc in various organs was measured in 18 species of decapods, the gills appeared to be the main site for metal absorption and desorption, where zinc loss across the gills is important for the regulation of zinc haemolymph concentration. Gills have been established as sites of zinc accumulation, where the metal is most likely bound to MT or metallothionein-like proteins (MTLP) and deposited in gill epithelial cells, nephrocytes and haemocytes (Nuñez-Nogueira et al., 2006). However, given the short 'pulsed' uptake, it is possible that the majority of zinc present in the gills directly following exposure and at day-1 depuration has mostly absorbed onto the surface of the gills, rather than incorporated into the gill cells attached to proteins and/or transferred to the haemolymph and relocated to the hepatopancreas.

The decapod hepatopancreas is known to play a key role in the detoxification of trace metals, where zinc within the hepatopancreas has been found to induce MT or MTLP production (Amiard et al., 2006; Pedersen et al., 2014) and is subsequently stored in phosphorus-rich granules (Nuñez-Nogueira and Rainbow, 2005a). However, while it is likely that the hepatopancreas was in some way involved in the detoxification of the metal, since ^{65}Zn was present in this organ throughout the depuration period, the main site of zinc loss was most likely the

gills. However, because the metal burden in discrete organs was not measured in this study it is not possible to conclude exactly what processes have taken place.

Cresswell et al. (2015) reported the spatial distribution of ^{65}Zn in the decapod crustacean *M. australiense* and concluded that the antennal gland contained the greatest density of zinc by 3 orders of magnitude following a 3-week exposure to 11.6 μg Zn/L and a 2-week depuration. The antennal gland is an excretory organ in decapod crustaceans that filters the haemolymph, acting as a temporary passage site involved in zinc excretion (Nuñez-Nogueira and Rainbow, 2005a). In this current study at day 7 depuration there was indication that the antennal gland was involved in the excretion of zinc from the animal, where the presence of ^{65}Zn was detected where the antennal gland was expected to be on the tissue section and corresponding autoradiographic image (Fig. 3g, h). This presence of activity in the antennal gland suggests that the animals were processing the accumulated zinc and had likely begun excretion. The excretion via the antennal gland is likely to be relatively fast as there was a significant reduction in whole-body ^{65}Zn during the depuration period. However, as metal burden in the antennal gland was not measured as part of this study it is not possible to conclude exactly how much reduction in whole-body ^{65}Zn was due to desorption from the exoskeleton or excretion from the antennal gland and/or gills.

4. Implications and future research

This study investigated the accumulation and efflux of ^{109}Cd and ^{65}Zn in the decapod crustacean *P. australiensis* over three short-term pulse exposures. As expected, shrimp body burden for both metals was significantly affected by time, as they accumulated each metal throughout the pulsed exposure period. For both treatments, cadmium and zinc displayed linear uptake over the three pulses. For this species at the concentrations investigated, metal accumulation did not markedly differ when shrimp were exposed to individual metals compared to a binary mixture. During the depuration period, cadmium efflux was minimal, whereas zinc efflux was significant. Autoradiography indicated the presence of both metal radioisotopes in the hepatopancreas, one of the main detoxification tissues in decapod crustaceans. Metals were also found in the gills of the animal, suggesting that these organs are an important site for cadmium and zinc influx and efflux. These results demonstrate how short-term repeated exposures result in the accumulation of contaminants by aquatic organisms.

This study highlights the important consideration for incorporating pulsed toxicity tests in frameworks when deriving WQG values. Research into the accumulation and depuration kinetics of contaminants during pulses, especially when contaminants are presented in a mixture, helps improve our overall understanding of fluctuating contaminant exposure to aquatic organisms. Previously, pulse exposure studies have been used to set site specific guidelines for ecosystems that are impacted by intermittent pulsed discharge (Sinclair et al., 2014), where a rigorous programme of pulse exposure tests was undertaken to quantify the effect of short-term pollutant guideline exceedances (Hogan et al., 2013). Pulse exposures can also be codified into setting guidelines by considering time averaged concentrations (TACs), used to describe metal toxicity as a factor of time. Previous studies that utilise TACs reveal that pulsed exposures may result in a greater or lesser toxic effect compared to equivalent chronic exposures, depending on the species and contaminant investigated (Angel et al., 2018, 2010; Diamond et al., 2006; Holdway et al., 1994; Williams and Holdway, 2000).

In the case of storm events there is generally a poor site-specific understanding of the duration and frequency of pulsed events, and the biological effects that may result from these exposures. Few studies have directly investigated the changes in metal lability over the course of pulsed storm events and the resulting impact these fluxes can have on metal bioavailability and uptake. There is a need for more detailed information in regard to changes in metal speciation during storm events, where the use of passive samplers to measure metal lability over time could provide a means to quantify these changes.

Future tests would benefit from investigating uptake and depuration kinetics following pulse exposures to metal mixtures in higher order sensitive aquatic species such as amphibians and fish. Considerations such as these will help improve the application of pulsed WQG values for a range of target ecosystems.

CRediT authorship contribution statement

Sarah McDonald: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **Tom Cresswell:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing - review & editing. **Kathryn Hassell:** Conceptualization, Methodology, Supervision, Writing - review & editing.

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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Appendix A. Supplementary data

Further information on live-animal radioanalysis protocols, radioanalysis detection and error limits, method for production of phantom using 3-D technologies, validation of the rinse method and calculating animal movement error during radioanalysis. Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.137609.

References

Allinson, M., Zhang, P., Bui, A., Myers, J.H., Pettigrove, V., Rose, G., Salzman, S.A., Walters, R., Allinson, G., 2017. Herbicides and trace metals in urban waters in Melbourne, Australia (2011–12): concentrations and potential impact. Environ. Sci. Pollut. Res. 24, 7274–7284. https://doi.org/10.1007/s11356-017-8395-9.

Amiard, J.C., Amiard-Triquet, C., Barka, S., Pellerin, J., Rainbow, P.S., 2006. Metallothioneins in aquatic invertebrates: their role in metal detoxification and their use as biomarkers. Aquat. Toxicol. 76, 160–202. https://doi.org/10.1016/j.aquatox.2005.08.015.

Angel, B.M., Simpson, S.L., Jolley, D.F., 2010. Toxicity to *Melita plumulosa* from intermittent and continuous exposures to dissolved copper. Environ. Toxicol. Chem. 29, 2823–2830. https://doi.org/10.1002/etc.347.

Angel, B.M., Goodwyn, K., Jolley, D.F., Simpson, S.L., 2018. The use of time-averaged concentrations of metals to predict the toxicity of pulsed complex effluent exposures to a freshwater alga. Environ. Pollut. 238, 607–616. https://doi.org/10.1016/j.envpol.2018.03.095.

ANZECC, ARMCANZ, 2000. National Water Quality Management Strategy, Australian Guidelines for Urban Stormwater Management. ANZECC & ARMCANZ, Canberra, ACT.

Balistrieri, L.S., Mebane, C.A., 2014. Predicting the toxicity of metal mixtures. Sci. Total Environ. 466, 788–799. https://doi.org/10.1016/j.scitotenv.2013.07.034.

Blackmore, G., Wang, W.X., 2002. Uptake and efflux of Cd and Zn by the green muscle *Perna viridis* after metal preexposure. Environ. Sci. Technol. 36, 989–995. https://doi.org/10.1021/es0155534.

Brown, J.N., Peake, B.M., 2006. Sources of heavy metals and polycyclic aromatic hydrocarbons in urban stormwater runoff. Sci. Total Environ. 359, 145–155. https://doi.org/10.1016/j.scitotenv.2005.05.016.

Bryan, G.W., 1968. Concentrations of zinc and copper in the tissues of decapod crustaceans. J. Mar. Biol. Assoc. 48, 303–321. https://doi.org/10.1017/s0025315400034500.

Buchwalter, D.B., Luoma, S., 2005. Differences in dissolved cadmium and zinc uptake among stream insects: mechanistic explanations. Environ. Sci. Technol. 39, 498–504. https://doi.org/10.1021/es0404421.

Burton, G.A., Pitt, R., Clark, S., 2000. The role of traditional and novel toxicity test methods in assessing stormwater and sediment contamination. Crit. Rev. Environ. Sci. Technol. 30, 413–447. https://doi.org/10.1080/10643380091184228.

Chiodi Boudet, L., Polizzi, P., Romero, M.B., Robles, A., Gerpe, M., 2013. Lethal and sublethal effects of cadmium in the white shrimp *Palaemonetes argentinus*: a comparison between populations from contaminated and reference sites. Ecotoxicol. Environ. Saf. 89, 52–58. https://doi.org/10.1016/j.ecoenv.2012.11.008.

Cresswell, T., Simpson, S.L., Smith, R.E.W., Nugegoda, D., Mazumder, D., Twining, J., 2014a. Bioaccumulation and retention kinetics of cadmium in the freshwater decapod *Macrobrachium australiense*. Aquat. Toxicol. 148, 174–183. https://doi.org/10.1016/j.aquatox.2014.01.006.

Cresswell, T., Smith, R.E.W., Nugegoda, D., Simpson, S.L., 2014b. Comparing trace metal bioaccumulation characteristics of three freshwater decapods of the genus *Macrobrachium*. Aquat. Toxicol. 152, 256–263. https://doi.org/10.1016/j.aquatox.2014.04.015.

Cresswell, T., Simpson, S.L., Mazumder, D., Callaghan, P.D., Nguyen, A.P., 2015. Bioaccumulation kinetics and organ distribution of cadmium and zinc in the freshwater decapod crustacean *Macrobrachium australiense*. Environ. Sci. Technol. 49, 1182–1189. https://doi.org/10.1021/es505254w.

Cresswell, T., Mazumder, D., Callaghan, P.D., Nguyen, A., Corry, M., Simpson, S.L., 2017. Metal transfer among organs following short- and long-term exposures using autoradiography: cadmium bioaccumulation by the freshwater prawn *Macrobrachium australiense*. Environ. Sci. Technol. 51, 4054–4060. https://doi.org/10.1021/acs.est.6b06471.

Diamond, J.M., Klaine, S.J., Butcher, J.B., 2006. Implications of pulsed chemical exposures for aquatic life criteria and wastewater permit limits. Environ. Sci. Technol. 40, 5132–5138. https://doi.org/10.1021/es0604358.

Feldman, D.L., Sengupta, A., Stuvick, L., Stein, E., Pettigrove, V., Arora, M., 2015. Governance issues in developing and implementing offsets for water management benefits: can preliminary evaluation guide implementation effectiveness? WIRES Water 2, 121–130. https://doi.org/10.1002/wat2.1061.

Fox, M.H., 1952. Anal and oral intake of water by crustacea. Exp. Biol. 29, 583–599.

Francey, M., Fletcher, T.D., Deletic, A., Duncan, H., 2010. New insights into the quality of urban storm water in south eastern Australia. J. Environ. Eng. 136, 381–390. https://doi.org/10.1061/(ASCE)EE.1943-7870.0000038.

Hare, L., 1992. Aquatic insects and trace metals: bioavailability, bioaccumulation, and toxicity. Crit. Rev. Toxicol. 22, 327–369. https://doi.org/10.3109/10408449209146312.

Hogan, A.C., Trenfield, M.A., Harford, A.J., van Dam, R.A., 2013. Toxicity of magnesium pulses to tropical freshwater species and the development of a duration-based water quality guideline. Environ. Toxicol. Chem. 32, 1969–1980. https://doi.org/10.1002/etc.2251.

Holdway, D., Barry, M.J., Logan, D.C., Robertson, D., Young, V., Ahokas, J.T., 1994. Toxicity of pulse exposed fenvalerate and esfenvalerate to larval Australian crimson-spotted rainbow fish (*Melanotaena fluviatilis*). Aquat. Toxicol. 28, 169–187.

Kroon, F.J., Thorburn, P., Schaffelke, B., Whitten, S., 2016. Towards protecting the Great Barrier Reef from land-based pollution. Glob. Chang. Biol. 22, 1985–2002. https://doi.org/10.1111/gcb.13262.

Lanctot, C.M., Cresswell, T., Melvin, S.D., 2017. Uptake and tissue distributions of cadmium, selenium and zinc in striped marsh frog tadpoles exposed during early post-embryonic development. Ecotoxicol. Environ. Saf. 144, 291–299. https://doi.org/10.1016/j.ecoenv.2017.06.047.

Luoma, S.N., Rainbow, P.S., 2005. Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. Environ. Sci. Technol. 39, 1921–1931. https://doi.org/10.1021/es048947e.

Makepeace, D.K., Smith, D.W., Stanley, S.J., 1995. Urban stormwater quality: summary of contaminant data. Crit. Rev. Environ. Sci. Technol. 25, 93–139. https://doi.org/10.1080/10643389509388476.

Masoner, J.R., Kolpin, D.W., Cozzarelli, I.M., Barber, L.B., Burden, D.S., Foreman, W.T., Forshay, K.J., Furlong, E.T., Groves, J.F., Hladik, M.L., Hopton, M.E., Jaeschke, J.B., Keefe, S.H., Krabbenhoft, D.P., Lowrance, R., Romanok, K.M., Rus, D.L., Selbig, W.R., Williams, B.H., Bradley, P.M., 2019. Urban stormwater: an overlooked pathway of extensive mixed contaminants to surface and groundwaters in the United States. Environ. Sci. Technol. 53, 10070–10081. https://doi.org/10.1016/j.chemosphere.2017.04.058.

Mebane, C.A., Dillon, F.S., Hennessy, D.P., 2012. Acute toxicity of cadmium, lead, zinc, and their mixtures to stream-resident fish and invertebrates. Environ. Toxicol. Chem. 31, 1334–1348. https://doi.org/10.1002/etc.1820.

Mebane, C.A., Schmidt, T.S., Balistrieri, L.S., 2017. Larval aquatic insect responses to cadmium and zinc in experimental streams. Environ. Toxicol. Chem. 36, 749–762. https://doi.org/10.1002/etc.3599.

Mebane, C.A., Chowdhury, M.J., De Schamphelaere, K.A.C., Lofts, S., Paquin, P.R., Santore, R.C., Wood, C.M., 2020. Metal bioavailability models: current status, lessons learned, considerations for regulatory use, and the path forward. Environ. Toxicol. Chem. 39, 60–84. https://doi.org/10.1002/etc.4560.

Metian, M., Hedouin, L., Eltayeb, M.M., Lacoue-Labarthe, T., Teyssie, J.L., Mugnier, C., Bustamante, P., Warnau, M., 2010. Metal and metalloid bioaccumulation in the Pacific blue shrimp *Litopenaeus stylirostris* (Stimpson) from New Caledonia: laboratory and field studies. Mar. Pollut. Bull. 61, 576–584. https://doi.org/10.1016/j.marpolbul.2010.06.035.

Norwood, W.P., Borgmann, U., Dixon, D.G., Wallace, A., 2003. Effects of metal mixtures on aquatic biota: a review of observations and methods. Hum. Ecol. Risk. Assess. 9, 795–811. https://doi.org/10.1080/713610010.

Nugegoda, D., Rainbow, P.S., 1988. Zinc uptake and regulation by the sublittoral prawn *Pandalus montagui* (Crustacea: Decapoda). Estuar. Coast. Shelf Sci. 26, 619–632.

Nugegoda, D., Rainbow, P.S., 1989. Effects of salinity changes on zinc uptake and regulation by the decapod crustaceans *Palaemon elegans* and *Palaemonetes varians*. Mar. Ecol. Prog. Ser. 51, 57–75.

Nugegoda, D., Rainbow, P.S., 1995. The uptake of dissolved zinc and cadmium by the decapod crustacean *Palaemon elegans*. Mar. Pollut. Bull. 31, 460–463.

Nuñez-Nogueira, G., Rainbow, P.S., 2005a. Kinetics of zinc uptake from solution, accumulation and excretion by the decapod crustacean *Penaeus indicus*. Mar. Biol. 147, 93–103. https://doi.org/10.1007/s00227-004-1542-0.

Nuñez-Nogueira, G., Rainbow, P.S., 2005b. Cadmium uptake and accumulation by the decapod crustacean *Penaeus indicus*. Mar. Environ. Res. 60, 339–354. https://doi.org/10.1016/j.marenvres.2004.12.001.

Nuñez-Nogueira, G., Mouneyrac, C., Amiard, J.C., Rainbow, P.S., 2006. Subcellular distribution of zinc and cadmium in the hepatopancreas and gills of the decapod crustacean *Penaeus indicus*. Mar. Biol. 150, 197–211. https://doi.org/10.1007/s00227-006-0350-0.

Oulton, L.J., Taylor, M.P., Hose, G.C., Brown, C., 2014. Sublethal toxicity of untreated and treated stormwater Zn concentrations on the foraging behaviour of *Paratya australiensis* (Decapoda: Atyidae). Ecotoxicology 23, 1022–1029. https://doi.org/10.1007/s10646-014-1246-2.

Patterson, J.J., Smith, C., Bellamy, J., 2015. Enabling and enacting 'practical action' in catchments: responding to the 'wicked problem' of nonpoint source pollution in coastal subtropical Australia. Environ. Manag. 55, 479–495. https://doi.org/10.1007/s00267-014-0409-5.

Pedersen, K.L., Bach, L.T., Bjerregaard, P., 2014. Amount and metal composition of midgut gland metallothionein in shore crabs (*Carcinus maenas*) after exposure to cadmium in the food. Aquat. Toxicol. 150, 182–188. https://doi.org/10.1016/j.aquatox.2014.03.009.

Pitt, R., Field, R., Lalor, M., Brown, M., 1995. Urban stormwater toxic pollutants - assessment, sources and treatability. Water Environ. Res. 67, 260.

Poteat, M.D., Diaz-Jaramillo, M., Buchwalter, D.B., 2012. Divalent metal (Ca, Cd, Mn, Zn) uptake and interactions in the aquatic insect *Hydropsyche sparna*. J. Exp. Biol. 215, 1575–1583. https://doi.org/10.1242/jeb.063412.

R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria URL. http://www.R-project.org/.

Rainbow, P.S., 2002. Trace metal concentrations in aquatic invertebrates: why and so what? Environ. Pollut. 120, 497–507.

Rainbow, P.S., 2007. Trace metal bioaccumulation: models, metabolic availability and toxicity. Environ. Int. 33, 576–582. https://doi.org/10.1016/j.envint.2006.05.007.

Rainbow, P.S., Luoma, S.N., 2011. Metal toxicity, uptake and bioaccumulation in aquatic invertebrates—modelling zinc in crustaceans. Aquat. Toxicol. 105, 455–465. https://doi.org/10.1016/j.aquatox.2011.08.001.

Rainbow, P.S., Blackmore, G., Wang, W.X., 2003. Effects of previous field-exposure history on the uptake of trace metals from water and food by the barnacle *Balanus amphitrite*. Mar. Ecol. Prog. Ser. 259, 201–213.

Schmidt, T.S., Clements, W.H., Mitchell, K.A., Church, S.E., Wanty, R.B., Fey, D.L., Verplanck, P.L., San Juan, C.A., 2010. Development of a new toxic-unit model for the bioassessment of metals in streams. Environ. Toxicol. Chem. 29, 2432–2442. https://doi.org/10.1002/etc.302.

Sinclair, A., Tayler, K., van Dam, R., Hogan, A., 2014. Site-specific water quality guidelines: 2. Development of a water quality regulation framework for pulse exposures of mine water discharges at a uranium mine in northern Australia. Environ. Sci. Pollut. Res. Int. 21, 131–140. https://doi.org/10.1007/s11356-013-1922-4.

Thorpe, V.J., Lake, P.S., 1974. Toxicity bioassays of cadmium in selected freshwater invertebrates and the interaction of cadmium and zinc on the freshwater shrimp, *Paratya tasmaniensis* Riek. Aust. J. Mar. Freshwat. Res. 25, 97–104.

Van Genderen, E., Adams, W., Dwyer, R., Garman, E., Gorsuch, J., 2015. Modeling and interpreting biological effects of mixtures in the environment: introduction to the metal mixture modeling evaluation project. Environ. Toxicol. Chem. 34, 721–725. https://doi.org/10.1002/etc.2750.

Vijver, M.G., Elliott, E.G., Peijnenburg, W.J.G.M., de Snoo, G.R., 2011. Response predictions for organisms water-exposed to metal mixtures: a meta-analysis. Environ. Toxicol. Chem. 30, 1482–1487. https://doi.org/10.1002/etc.499.

Walsh, C.J., Sharpe, A.K., Breen, P.F., Sonneman, J.A., 2001. Effects of urbanization on streams of the Melbourne region, Victoria, Australia. I. Benthic macroinvertebrate communities. Freshw. Biol. 46, 535–551.

Walsh, C.J., Fletcher, T.D., Burns, M.J., 2012. Urban stormwater runoff: a new class of environmental flow problem. PLoS One (9), e45814 https://doi.org/10.1371/journal.pone.0045814.

Wang, W.X., Dei, R.C.H., 2001. Effects of major nutrient additions to metal uptake in phytoplankton. Environ. Pollut. 111, 233–240.

Wang, W.X., Fisher, N.S., 1999. Effects of calcium and metabolic inhibitors on trace element uptake in two marine bivalves. J. Exp. Mar. Biol. Ecol. 236, 149–164.

Wang, W.X., Rainbow, P.S., 2008. Comparative approaches to understand metal bioaccumulation in aquatic animals. Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol. 148, 315–323. https://doi.org/10.1016/j.cbpc.2008.04.003.

Wang, W.X., Fisher, N.S., Luoma, S., 1996. Kinetic determinations of trace element bioaccumulation in the mussel *Mytilus edulis*. Mar. Ecol. Prog. Ser. 140, 91–113.

White, S.L., Rainbow, P., 1984a. Zinc flux in *Palaemon elegans* (Crustacea: Decapoda): moulting, individual variation and tissue distribution. Mar. Ecol. Prog. Ser. 19, 153–166.

White, S.L., Rainbow, P.S., 1984b. Regulation of zinc concentration by *Palaemon elegans* (Crustacea, Decapoda): zinc flux and effects of temperature, zinc concentration and moulting. Mar. Ecol. Prog. Ser. 16, 135–147.

Wickham, H., 2009. ggplot2: Elegant Graphics for Data Analysis. Springer, New York.

Williams, N.D., Holdway, D.A., 2000. The effects of pulse-exposed cadmium and zinc on embryo hatchability, larval development, and survival of Australian crimson spotted rainbow fish (*Melanotaenia fluviatilis*). Environ. Toxicol. 15, 165–173.

Zgheib, S., Moilleron, R., Chebbo, G., 2012. Priority pollutants in urban stormwater: part 1 - case of separate storm sewers. Water Res. 46, 6683–6692. https://doi.org/10.1016/j.watres.2011.12.012.