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Intertidal mussels do not stop metal bioaccumulation even when out of water: Cadmium toxicokinetics in *Xenostrobus atratus* under influences of simulated tidal exposure☆

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ABSTRACT

Intertidal bivalves are periodically exposed in air. It is tempting to speculate that the organisms would temporarily escape from contaminants when they are out of water and thus have lower risks. In this study, we tested this speculation by investigating cadmium (Cd) toxicokinetics in an intertidal mussel, *Xenostrobus atratus*, under the effects of tidal exposure using simulated tidal regimes. The uptake rate constant (k_u) of Cd ranged from 0.045 L g^{-1} d^{-1} to 0.109 L g^{-1} d^{-1}, whereas the elimination rate constant (k_e) of Cd ranged from 0.029 d^{-1} to 0.091 d^{-1}. Cd bioaccumulation was slightly higher in the continuously immersed mussels than the alternately immersed mussels, but much lower than what would be expected if assuming bioaccumulation being proportional to immersion duration. Cd uptake was observed even when mussels were exposed in air, due to uptake of Cd dissolved in mantle cavity fluid and internalization of Cd adsorbed on mussel tissues. Overall, tidal height showed limited effects on Cd bioaccumulation, consistent with the trend of Cd concentrations found in *X. atratus* collected from different tidal heights. The mantle cavity uptake mechanism is expected to be applicable to other contaminants and bivalves, and should have important implications in risk assessments for intertidal environment.

1. Introduction

Bivalve mollusks living in intertidal zones are subject to alternating immersion and emersion periodically (Andrade et al., 2019; Raffaelli and Hawkins, 2012). When immersed in water, bivalves actively filter water, feed, excrete wastes, and in the meantime accumulate contaminants. When emerged into air, these processes are expected to suspend. If contaminant accumulation stops after emersion, bivalves living at different tidal heights should have very different contaminant exposure and thus different levels of risk. Surprisingly, tide effects (e.g., exposure duration) have not been considered as a modifying factor in biomonitoring programs (Apeti et al., 2012; General Administration of Quality Supervision and Standardization Administration of P. R. China, 2007) or bioaccumulation modeling (Luoma and Rainbow, 2005; Tan et al., 2018; Wang et al., 1996). Intertidal bivalves, especially mussels and oysters, are the most frequently used biomonitors to indicate the status and trend of contamination in coastal waters (Beyer et al., 2017; Melwani et al., 2014; Rainbow and Phillips, 1993). The lack of understanding of tide effects may lead to unrepresentative intertidal sampling and undermine the interpretation of biomonitoring data.

Effects of tidal exposure on metal bioaccumulation have been sporadically studied for many years. Phillips (1976) reported that mussels (*Mytilus edulis*) collected from upper-shore levels had higher concentrations of Cd, Zn, and Pb in winter, but not in summer. Lobel & Wright (1982) also observed higher Zn concentration in *M. edulis* at the upper-shore sites. Later, Mubiana et al. (2006) examined tide effects on ten metals in *M. edulis* and found metal-specific relationships (including positive, negative, and independent) between metal concentration and tidal height. Besides, tide effects were also observed in other intertidal organisms, including

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snails (Gay and Maher, 2003) and barnacles (Al-Thaqafi and White, 1991); however, the effects showed various trends and extent. Taken together, although organisms inhabiting upper-shore levels appear to have shorter exposure duration, they were seldom found to have lower contaminant (e.g., metal) concentrations. Measuring toxicokinetics in organisms at different tidal height may provide insights into this apparent discrepancy.

Toxicokinetics, quantitatively describing the rate of uptake and elimination of metals into and from organisms, is also important for interpreting biomonitoring data (Grech et al., 2017; Rainbow, 1995; Wang and Tan, 2019). It relates metal concentration measured in biomonitors to the level of contamination in the environment, enables comparison of biomonitoring data obtained based on different species over large geographical distances, and provides information on the period over which the contamination levels being reflected by the biomonitors.

Xenostrobus atratus is a eurythermal mussel, widely distributed in coastal waters of east Asia, spanning temperate and tropical regions. It inhabits middle and upper zones of intertidal waters, being tolerant to air exposure, and usually is one of the dominant species (Wang, 1997). These attributes suggest it can serve as a suitable national and international biomonitor like other widely used mussels. However, *X. atratus* currently is underrepresented in biomonitoring studies and information on metal toxicokinetics is lacking for this species. Recently, *Xenostrobus securis*, a closely related species, was found to well reflect metal contamination in water, suspended particles, and surface sediment, and thus is considered as a good biomonitor of coastal waters (Markich and Jeffree, 2019). Additionally, toxicokinetics of Cd and Se were previously studied in *X. securis* (Alquezar et al., 2007).

In this study, we measured toxicokinetics of Cd in *X. atratus*, with special focus on the effects of tide using laboratory simulated tidal conditions. We also field sampled *X. atratus* inhabiting different tidal heights to compare with the toxicokinetic measurements. Our major objectives include: (1) quantifying the effects of tidal height on Cd bioaccumulation for understanding the field data; (2) investigating whether tidal height is an important factor that needs to be considered during intertidal biomonitoring; (3) obtaining Cd toxicokinetics of the *X. atratus* to facilitate its use as a biomonitoring species. Cd is a priority pollutant and generally has higher concentrations in mollusks than in other groups of aquatic organisms (e.g., crustaceans and fishes) (Eisler, 2009). Cd concentrations are low ($\leq$0.1 μg L^{-1}) in unpolluted marine waters, but can be higher than 2 μg L^{-1} in polluted surface waters, including estuarine and coastal waters (U.S. Environmental Protection Agency, 2016). The mechanisms revealed for Cd in this study is expected to be extrapolatable to other metals.

2. Materials and methods

2.1. Organisms and materials

X. atratus were collected during low tide from pillars of Yanwu Bridge, Xiamen, China (24.4380° N, 118.1058° E). The mussels were acclimated to experimental conditions (22 ± 1 °C, salinity 30) for two weeks before being used in experiments. During acclimation, mussels were fed the green algae *Chlorella* sp., and seawater was renewed daily. Individuals of shell length ranging from 0.8 cm to 1.3 cm (soft tissue dry weight 1.4 ± 0.5 mg, mean ± standard deviation) were used for experiments. Seawater (salinity around 30 and pH around 8.0) was collected from Tong'an Bay (24.5669° N, 118.1925° E), Xiamen, China. Seawater used in toxicokinetics experiments was filtered through a 0.22-μm mixed cellulose esters membrane (Xianya, Shanghai, China) contained in a stainless steel 90-mm filter holder (Millipore). Polypropylene containers used in experiments were acid cleaned, i.e., soaked in 5% HNO_3 overnight and then rinsed with deionized water. The stable isotope ^{113}Cd (ISOFLEX, San Francisco, California, U.S.A.) dissolved in 5% HNO_3 was used for preparing Cd exposure solutions. Exposure solutions were adjusted to pH 8.0 if necessary by adding microliters of 4.0 mol L^{-1} NaOH and were equilibrated overnight before use. All experiments were conducted under the temperature of 22 ± 1 °C with a photoperiod of 14 h light: 10 h dark.

2.2. Effects of tide on Cd concentration in field collected mussels

Another batch of *X. atratus* was collected from eleven height intervals of one pillar to investigate the effects of tidal height on Cd bioaccumulation in the field (Fig. S1). Mussels (soft tissue dry weight 3.5 ± 2.4 mg) were sampled from the highest water mark on the pillar (denoted 0 cm) to the ground level at 20 cm intervals, except for the first interval (90 cm) and the last interval (50 cm), where mussel distribution was sparse. From each height interval, 20 mussels were collected, except for the two lowest intervals, where fewer individuals were available. The mussels were immediately dissected when transported to the laboratory; the soft tissues were rinsed with deionized water (18.2 MΩ cm), placed individually in clean ziplock bags, and stored at −20 °C.

2.3. Cd toxicokinetics

Cd toxicokinetics in *X. atratus* were investigated at five ^{113}Cd concentrations, i.e., 0.5 μg L^{-1}, 5 μg L^{-1}, 50 μg L^{-1}, 200 μg L^{-1}, and 1000 μg L^{-1} (see measured concentrations in Table S1). Toxicokinetics at higher Cd concentration (e.g., >5 μg L^{-1}) were not aimed to be environmentally relevant but are useful for explaining and modeling acute toxicity and ecological risk of Cd (Tan et al., 2019). The mussels were exposed to Cd for 0.5 d and then depurated in clean seawater for 13.5 d. During Cd exposure, mussels were not fed; during the depuration period, mussels were fed with *Chlorella* once per day, and seawater was renewed every 1–2 d. There were three replicate beakers for each treatment, and each beaker contained 20 individuals in 600 mL of test solution. Two mussels were sampled from each replicate after 3 h, 6 h, and 12 h of exposure and during the depuration period at intervals of 12 h–84 h. Another six unexposed mussels were also sampled to determine the initial Cd concentration. Mussels sampled during the exposure period were immediately immersed in 1 mmol L^{-1} EDTA to stop the Cd uptake. Soft tissue of mussels was rinsed twice with 1 mmol L^{-1} EDTA and twice with deionized water, placed in clean ziplock bags, and stored at −20 °C (the same for all mussel samples below). Water samples were collected from each treatment after 0 h, 3 h, 6 h and 12 h of exposure, acidified by adding 10 μL of 7.3-mol L^{-1} HNO_3 (prepared by mixing equal volume of concentrated HNO_3 and deionized water) per 10 mL of water sample, and stored at 4 °C before Cd analyses (the same for all water samples below).

2.4. Effects of tide on Cd toxicokinetics

To investigate the effects of tidal exposure on Cd toxicokinetics in *X. atratus*, the mussels were alternately immersed in water and exposed to air during every 12-h tidal period, simulating the semidiurnal tide. The mussels were immersed in water for various durations during every 24 h, including 6 h, 9 h, 12 h, 15 h, and 24 h (continuously immersed), simulating mussels living at different tidal heights (see Table S4 and Fig. S3 for reasons of selecting the durations and more details of experimental design). The mussels were exposed to 5 μg L^{-1} of ^{113}Cd for 2 d and depurated in clean seawater for 7 d. This Cd concentration was selected to ensure reliable measurement based on results from the toxicokinetic

experiments described above. Three replicated beakers were set for each treatment; each beaker contained 30 individuals in 800 mL of exposure solution. In each replicate, the mussels were placed in a perforated polypropylene box hanged in the beaker; the perforated box was elevated out of water during air exposure. During the exposure period, two mussels were sampled from each beaker when they were transferred from water to air or from air to water, except for the continuously immersed mussels, which were sampled at intervals of 4 h–8 h. During the depuration period, mussels were sampled every day. Water samples were also collected for monitoring Cd concentration in exposure solutions (see Table S2 for measured concentrations).

To understand the mechanism why *X. atratus* continued Cd uptake when out of water, Cd uptake were further compared between mussels with or without a transitional clean water exposure between "Cd exposure" and "air exposure". Specifically, two groups of mussels were both exposed to 5 μg L^{-1} of ^{113}Cd for 6 h (see Table S3 for measured concentrations). Afterwards, one group of mussels were immediately exposed in air for 6 h; the other group of mussels were transitionally transferred to clean seawater for 1 h and then exposed in air for 5 h. Three replicated beakers, each containing 600 mL of test solution, were used for each treatment. Ten individuals were sampled from each beaker at the end of Cd exposure, transitional exposure, and air exposure. Ten unexposed mussels were also sampled and measured individually for the initial Cd concentration. Water samples were collected every 2 h for monitoring ^{113}Cd in test solutions.

2.5. Chemical analysis

The whole soft tissues of mussels were freeze dried, weighed, and then digested with 65% HNO_3 at 80 °C for 8 h. All mussels were analyzed for Cd concentrations individually. Samples of the standard reference material (SRM 2976, mussel tissue) were also digested following the same procedures. Concentrations of ^{111}Cd and ^{113}Cd in mussel samples and water samples were determined using the inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7700x). The digested mussel samples and water samples were properly diluted with deionized water or 2% HNO_3 to have a final HNO_3 concentration around 2% and salinity lower than 1 for ICP-MS analysis. The internal standard ^{115}In was used to correct instrument drift and sample matrix effects. A quality control sample was analyzed after every 20 samples to check stability of instrument performance. Analyses were considered acceptable when recovery of ^{111}Cd and ^{113}Cd from SRM were both within the range of 90%–110%.

2.6. Data analysis and toxicokinetic modeling

The newly accumulated ^{113}Cd concentration in organisms was calculated by subtracting the background ^{113}Cd concentration from the total ^{113}Cd concentration (Tan et al., 2019):

$$\text{new}\left[^{113}\text{Cd}\right] = \left[\text{Cd}-113\right]\$12.22\% - \left[\text{Cd}-111\right]\$12.22\% \quad (1)$$

where [Cd −113] and [Cd −111] are ICP-MS reported concentrations (μg L^{-1}) of *total Cd* based on the measurement of isotopes ^{113}Cd and ^{111}Cd, respectively. The value 12.22% is the natural abundance of ^{113}Cd. Therefore, "[Cd − 113] $ 12.22%" represents the total ^{113}Cd in organisms and "[Cd − 111] $ 12.22%" represents the background ^{113}Cd in organisms (more detailed information on stable isotope data analysis is available in Tan et al. (2019)). Tissue Cd concentrations were all expressed on dry weight basis.

A one-compartment toxicokinetic model was used to describe the uptake and elimination of ^{113}Cd in the mussel (Wang and Tan, 2019):

$$\frac{dC_{\text{int}}(t)}{dt} = k_u\$C_w(t) - (k_e + g)\$C_{\text{int}}(t) \quad (2)$$

where $C_{\text{int}}(t)$ (μg g^{-1}) is the concentration of ^{113}Cd accumulated in *X. atratus* at time t; $C_w(t)$ (μg L^{-1}) is the concentration of ^{113}Cd in the test solution; k_u (L g^{-1} d^{-1}) and k_e (d^{-1}) are uptake and elimination rate constants, respectively; g (d^{-1}) is the growth rate constant of the organisms.

The two toxicokinetic parameters (i.e., k_u and k_e) were fitted simultaneously by fitting equation (2) to the uptake and depuration data. Best-fit values and uncertainties were estimated in the software OpenModel (v2.4.2, developed by Neil Crout at Nottingham University) using Marquardt algorithm (Tan et al., 2018). One-way and two-way Analysis of Variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) *post hoc* tests were used for comparing means of Cd concentrations or Cd uptake between different groups of mussels. Significant differences were accepted at $p < 0.05$. Statistical analyses were performed with R (v3.6.1). All figures were generated using the "ggplot2" package of R.

3. Results and discussion

3.1. Effects of tide on Cd concentration in field collected mussels

Cd concentrations in soft tissue of the mussel *X. atratus* ranged from 0.83 μg g^{-1} to 11 μg g^{-1} (mean ± standard deviation: 3.1 ± 1.6 μg g^{-1}; $n = 197$). There was no clear trend in the effects of tidal height on the Cd concentrations (Fig. 1). Significant difference was only found between two height intervals, i.e., the 110–130 cm interval and the 210–230 cm interval ($p < 0.05$). However, the

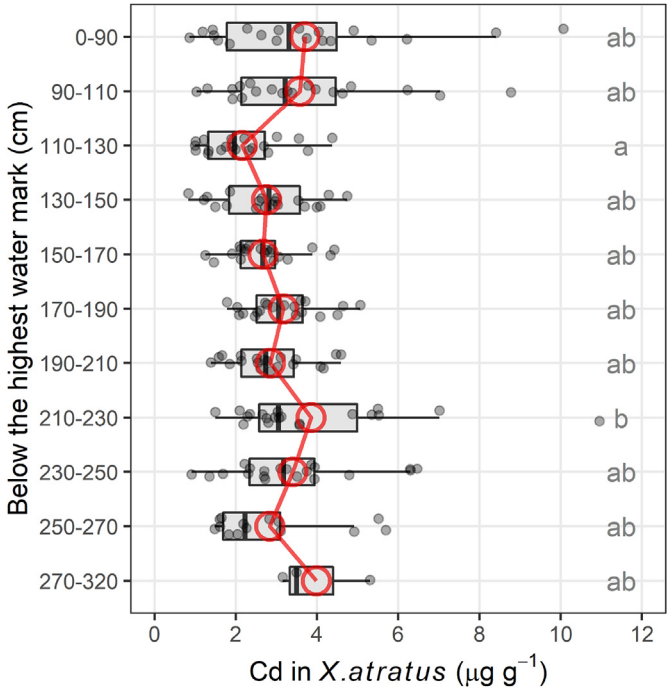

Fig. 1. Cd concentration in the soft tissue of mussels (*X. atratus*). The mussels were collected from 11 height intervals of a pillar of Yanwu Bridge. Each point represents one individual mussel. Height intervals share no common letters are significantly different in mussel Cd concentration ($p < 0.05$, one-way ANOVA followed by Tukey's HSD test).

significant difference was probably confounding effects of tissue weights instead of the effects of tidal height *per se*. Specifically, a positive relationship existed between tissue Cd concentration and the individual tissue dry weight (Fig. S2a); therefore, to eliminate the possible confounding effects of tissue weight, Cd concentrations were normalized to a geometric mean individual weight (i.e., 2.81 mg, Fig. S2b). The normalized Cd concentrations were not significantly different between any two height intervals ($p = 0.129$), which indicated no significant effects of tidal height on Cd concentration in the mussels.

Sessile organisms inhabiting different tidal heights appeared to be exposed to contaminants for different durations and were subject to the behavioral and physiological effects of different intensity (Andrade et al., 2018; Lathlean et al., 2017), which may all directly or indirectly affect metal bioaccumulation. The lack of observed effects of tidal height on Cd concentration in *X. atratus* was thus not expected. Similarly, in the snail *Bembicium nanum* no trend was observed in Cd concentration at different tidal heights, although Cu and Zn were found to be affected, showing a non-monotonic pattern with the variation of tidal height (Gay and Maher, 2003). In contrast, in the mussel *M. edulis*, Cd, Zn, and As concentrations were higher at higher tidal heights, while opposite patterns were observed for Cu, Fe, and Mn, and no clear patterns for Cr, Ni and Pb (Mubiana et al., 2006). The metal-specific patterns suggest that exposure time (the same for different metals) was not the only driving factor of metal bioaccumulation in response to tidal cycle, the physiochemical characteristics and biochemical roles of the metals were also important.

3.2. Cd toxicokinetics

The mussel *X. atratus* were exposed to 0.5–1000 μg L^{-1} of ^{113}Cd for 0.5 d, and subsequently depurated in clean seawater for another 13.5 d. During the exposure period, ^{113}Cd concentration in the mussels increased linearly with time; during the depuration period, ^{113}Cd concentration decreased steadily (Fig. 2). The bioaccumulation of ^{113}Cd at 0.5 μg L^{-1} was too low to be reliably measured, which were mainly attributed to the small size of the mussels (e.g., geometric mean tissue weight of 2.81 mg), the results were thus not presented. The uptake and elimination of ^{113}Cd in *X. atratus* was well described by a one-compartment toxicokinetic model (Fig. 2). Cd uptake rate constant (k_u) was estimated to be 0.045 L g^{-1} d^{-1} to 0.099 L g^{-1} d^{-1}, decreasing with the increasing exposure concentration of Cd (Fig. 2); a constant elimination rate constant (k_e, 0.091 d^{-1}) could be used to describe Cd elimination at different Cd levels, suggesting that the Cd elimination processes were less dependent on exposure concentration of Cd.

Compared to Cd toxicokinetics measured in other bivalves under similar conditions (i.e., temperature, salinity, and Cd concentration), Cd uptake in *X. atratus* was relatively slow (Table S5). Specifically, Cd k_us of *X. atratus* (0.045–0.099 L g^{-1} d^{-1}) were lower than k_us of the larger sized mussels e.g., *Perna viridis* and *Mytilus edulis* (Chong and Wang, 2001; Wang et al., 1996), and k_us of oysters and scallops (Ke and Wang, 2001; Pan and Wang, 2008), whereas similar to k_us measured in clams (Lee et al., 1998; Shi and Wang, 2004; Tan et al., 2019). On the other hand, Cd k_e (0.091 d^{-1}) observed in *X. atratus* was higher than those commonly observed in other marine bivalves (e.g., 0.01–0.04 d^{-1}, Table S5), including oysters (Ke and Wang, 2001; Pan and Wang, 2012), scallops (Pan and Wang, 2008), mussels (Chong and Wang, 2001; Wang et al., 1996), and clams (Chong and Wang, 2001; Lee et al., 1998; Shi and Wang, 2004; Tan et al., 2019). Altogether, it suggests that *X. atratus*, with slow Cd uptake rates and fast Cd loss rates, is a relatively weak Cd accumulator among marine bivalves. In addition, it can be estimated that it takes 33 d [i.e., ln(0.05)/ k_e] for

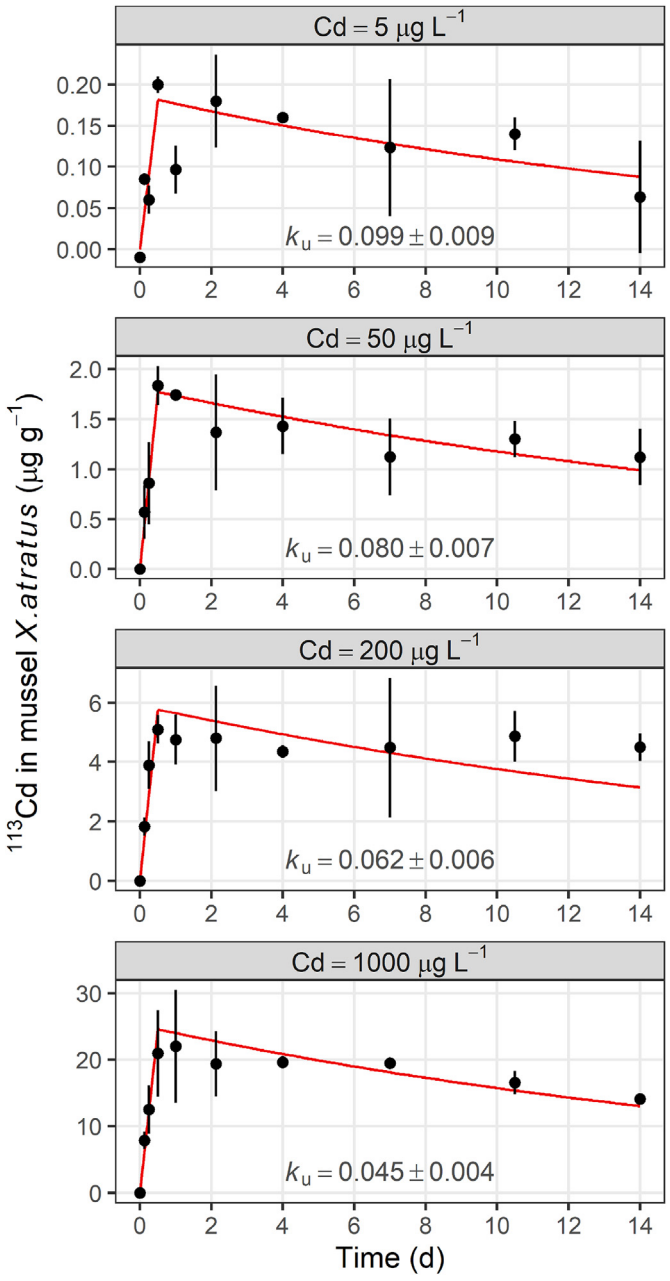

Fig. 2. Uptake and elimination of ^{113}Cd in *X. atratus*. The mussels were exposed to different concentrations of ^{113}Cd (nominal: 5–1000 μg L^{-1}; measured: Table S1) for 0.5 d and then depurated in clean seawater for 13.5 d. Points are measured values (mean ± standard deviation, $n = 3$); curves are model fittings. The same elimination rate constant (k_e, 0.091 ± 0.008 d^{-1}) was assumed for different Cd levels; values of the uptake rate constant (k_u, L g^{-1} d^{-1}) are shown in the figure.

X. atratus to eliminate 95% of newly accumulated Cd from its tissue, which means that Cd concentration measured in *X. atratus* reflects Cd exposure over the preceding ~1 month.

The uptake rate of Cd (J_{in}, μg g^{-1} d^{-1}) over the wide range of Cd concentrations (C_w, ~3–1000 μg L^{-1}) can be well described by a power function (Fig. 3):

$$J_{in} = 0.126 \times C_w^{0.862} \quad (3)$$

A power coefficient (i.e., 0.862) smaller than 1 indicates that Cd uptake rate increases less than proportionally with increasing Cd concentration. Similar equations with power coefficient close to or

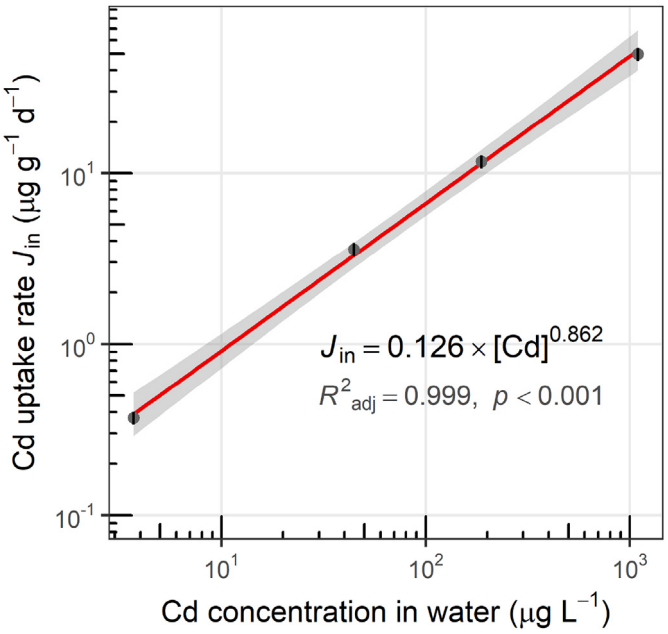

Fig. 3. Relationship between Cd uptake rate (J_{in}) in *X. atratus* and Cd concentration in water. Linear relationship exists between Cd uptake rate and Cd concentration in the double-log scale. Values are mean ± standard error.

slightly smaller than 1 were also reported in a range of other marine bivalves, where much lower exposure Cd concentrations (e.g., 0.1–0.5 μg L^{-1}) were sometimes included to derive the equations (Table S5). The equation derived in *X. atratus* is expected be reliably extrapolated to lower Cd concentrations (e.g., < 1 μg L^{-1}) for modeling and predicting Cd uptake in less contaminated natural waters.

3.3. Effects of tide on Cd toxicokinetics

The mussels were exposed alternately to seawater and air for 48 h with different daily immersion durations (6–24 h), simulating tidal exposures of mussels inhabiting different tidal heights. After 48 h of exposure, Cd in the continuously immersed mussels was 1.11 μg g^{-1}, slightly higher (as expected) than in the intermittently immersed mussels (0.70–0.84 μg g^{-1}, Fig. 4). The estimated average Cd k_u ranged from 0.080 to 0.109 L g^{-1} d^{-1} (Cd uptake rates were also calculated for each interval of immersion and air exposure, see Fig. 5 and discussions below). Values of k_u were very similar among the 6-h, 9-h, and 12-h immersion treatments, slightly but significantly higher in the continuously immersed treatment (one-way ANOVA, $p < 0.05$), and intermediate in the 15-h immersion treatment (Fig. S4). Overall, tidal exposure showed limited effects on Cd bioaccumulation in *X. atratus*, to some extent in agreement with the field data (Fig. 1).

The mussels were then depurated for 7 d under the different tidal regimes (Fig. 4). Again, a shared elimination rate constant (k_e), which was estimated to be 0.029 d^{-1}, was used to describe the Cd elimination processes in different treatments. It should be noted that the shared k_e did not necessarily indicate no effects of tidal exposure on Cd elimination; however, the good agreement between the model and the observed depuration curves suggests that the assumption was a good approximation. More importantly, using a shared k_e would make it much easier for future modeling of Cd bioaccumulation under various tidal conditions. The k_e estimated here is substantially lower than that estimated above in the Cd toxicokinetics experiment (0.091 d^{-1}), which might partly be due

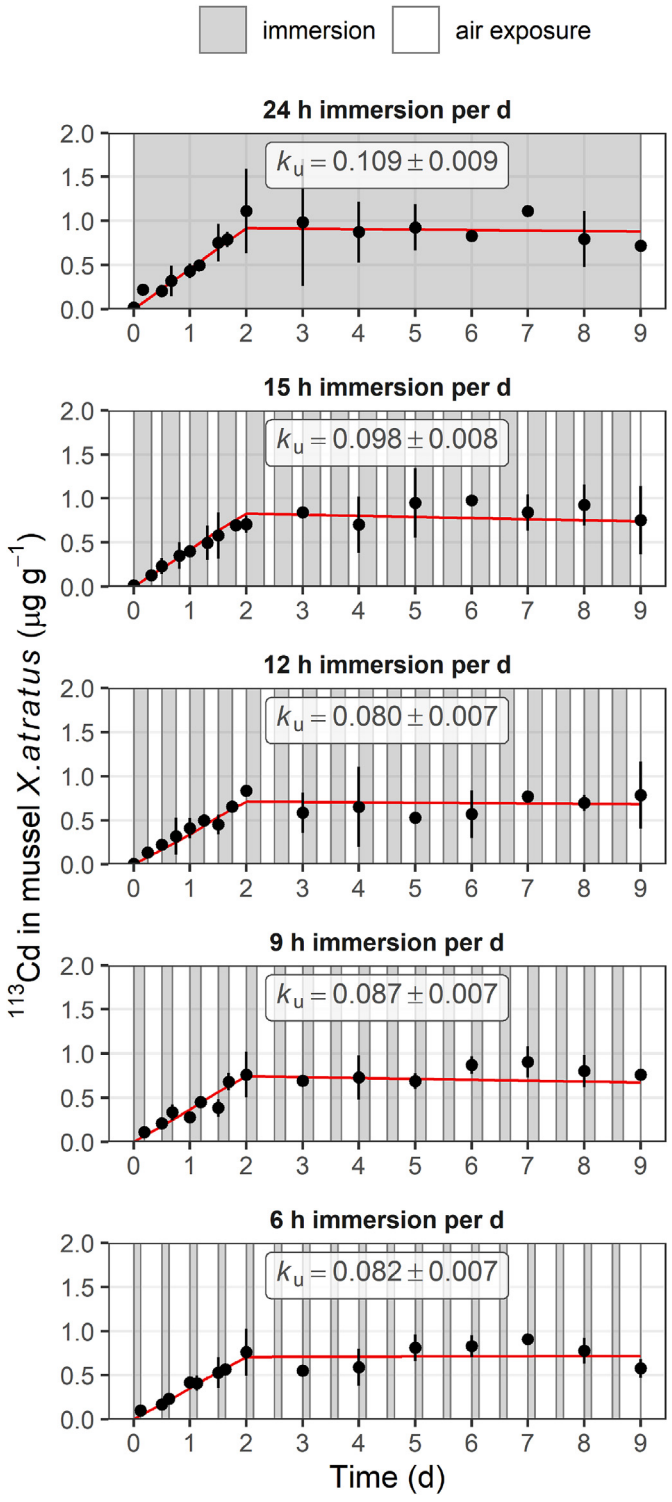

Fig. 4. The accumulation and elimination of ^{113}Cd in *X. atratus* subject to different tidal regimes (i.e., 6–24 h of immersion per d). Mussels were exposed to 5 μg L^{-1} of ^{113}Cd during the first 2 d and subsequently depurated in clean seawater for 7 d. The grey areas represent periods of immersion; white areas represent periods of air exposure. Points are measured values (mean ± standard deviation, $n = 3$); curves are model fittings assuming an average k_u (L g^{-1} d^{-1}) for each treatment and a shared k_e (best-fit value: 0.029 ± 0.012 d^{-1}) for all treatments.

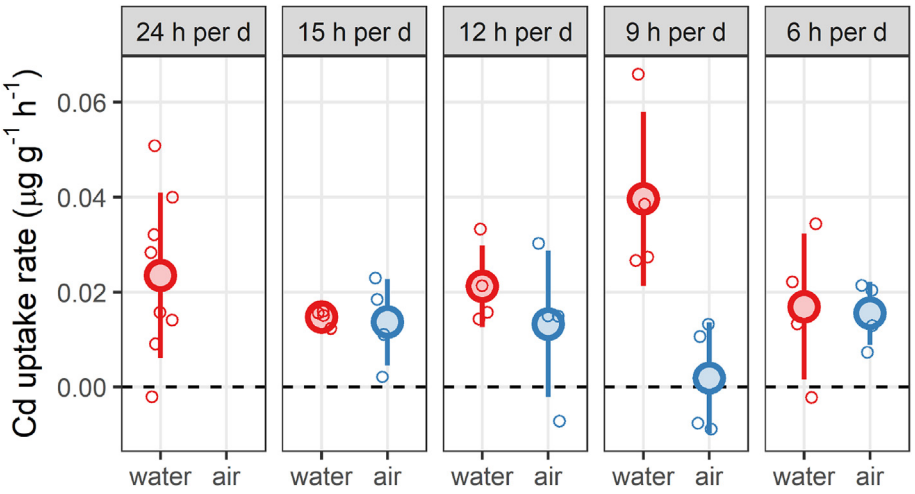

Fig. 5. Cd uptake rates during intervals when *X. atratus* were immersed in water or exposed in air. The mussels were subjected to different tidal regimes (i.e., 6–24 h of immersion per d). See Fig. 4 for more details of the experiment. Larger circles are mean values; error bars represent standard deviation; each smaller circle represents the Cd uptake rate during each exposure interval.

to the substantial discrepancy in the estimated mussel growth rate constants (g) in the two experiments (~0.045 d^{-1} vs. ~0.020 d^{-1}; Fig. S6 and Fig. S7), since k_e and g could not be clearly separated during their estimation (see Equation (2)). Differences in physiological status of the two batches of mussels may also be responsible for the differences in k_e. By comparing to other marine bivalves (Table S5), we speculate that 0.029 d^{-1} should be a better estimation of Cd k_e in *X. atratus* and should be chosen for future modeling work.

If the mussels only accumulated Cd when immersed in Cd-spiked seawater and stopped Cd uptake when out of water, the bioaccumulation pattern would have differed from what was observed in Fig. 4: Cd in the alternately exposed mussels would show zig-zag shaped increases; mussel Cd would be proportional to the total time of immersion (e.g., 4 times higher Cd in mussels from the 24-h group than from the 6-h group). Actually, Cd bioaccumulation in the alternately exposed mussels was steadier and greater than expected, indicating that the mussels did not stop Cd uptake even when out of water.

The Cd uptake rates were calculated for each interval of immersion and air exposure and were presented in Fig. 5. Cd uptake rates were higher in immersed intervals than in air exposure intervals ($p = 0.012$); whereas no significant difference was found between different immersion-duration treatments ($p = 0.87$). In most cases, Cd uptake rates were above zero even when the mussels were out of water, except in the 9-h immersion treatment, where considerably elevated Cd uptake rates were accordingly observed during the immersion intervals.

We speculated that Cd uptake in air-exposure mussels was due to exposure solution enclosed in their mantle cavity when they emerged from water. If the speculation was correct, we could predict that such Cd uptake would disappear if the mussels were depurated for a while in clean seawater before being exposed in air, because the Cd-rich cavity fluid would have been replaced by (almost) "Cd-free" clean seawater. We tested our speculation by comparing Cd uptake in air-exposure mussels with or without a preceding period of clean-water depuration (Fig. 6). Consistent with the prediction, during air exposure, Cd concentration increased in mussels without clean-water depuration (Fig. 6a), whereas slightly decreased in mussels with clean-water depuration (Fig. 6b). Nonetheless, it should be noted that the increase of Cd concentration in Fig. 6a ($p = 0.084$) and the decrease of Cd

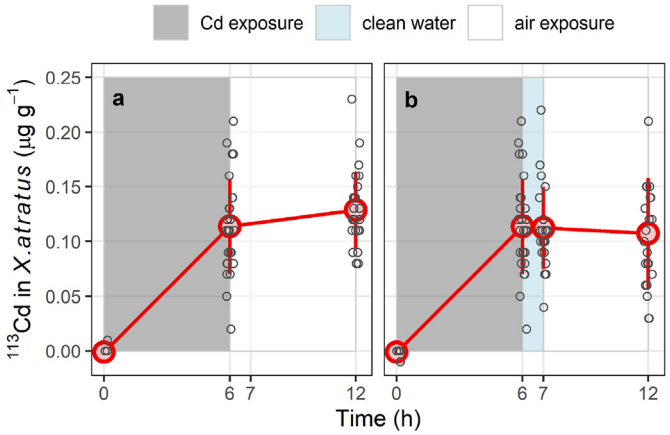

Fig. 6. Concentrations of ^{113}Cd in *X. atratus* tissues after exposures to ^{113}Cd, clean seawater, and air. (a) Mussels were exposed to 5 μg L^{-1} of ^{113}Cd for 6 h and then exposed to air immediately for 6 h; (b) mussels were transitionally depurated in clean seawater for 1 h between the 5 μg L^{-1} of ^{113}Cd exposure and the air exposure. Larger circles are mean values; error bars represent standard deviation; each smaller circle represents one individual mussel. Data of 0 h and 6 h are shared between both panels.

concentration in Fig. 6b ($p = 0.32$) were not statistically significant (between the 6-h and 12-h time point), partially due the large inter-individual variations observed in the experiment.

We estimated whether Cd contained in the cavity fluid could provide enough Cd to achieve the observed Cd bioaccumulation during air exposure. The average Cd k_u were 0.080 L g^{-1} d^{-1} to 0.098 L g^{-1} d^{-1} (Fig. 4), which means that during 1 d, 1 g of mussel tissue (dry weight) accumulated Cd contained in 0.080 L–0.098 L of exposure seawater. Therefore, a middle sized 2.35-mg mussel should have accumulated Cd from 43 μL to 72 μL of solution during the 4.5 h–9 h of air exposure. In the other hand, the average volume of cavity fluid collected from a 2.35-mg mussel was only 21 μL (Fig. S5). The amount of Cd adsorbed on the soft tissue of a 2.35-mg mussel was equivalent to the amount of Cd contained in 31 μL of cavity fluid. Moreover, cavity fluid Cd concentration was 1.4 times higher than the exposure seawater (Fig. S5). Taken together, the amount of Cd enclosed in mantle cavity of a mussel is equivalent to 73 μL [i.e., $(21 + 31) \times 1.4$] of exposure solution, very similar to the volume (i.e., 43–72 μL) required to support the bioaccumulation

during air exposure, which again supports our speculation of mantle cavity uptake. In accordance with our finding, it was found in the clam *Anomalocardia flexuosa* that the amount of radioisotope ^{65}Zn contained in mantle cavity fluid was 38% of the ^{65}Zn accumulated in the soft tissue after 9 d of aqueous exposure (Chen et al., 1993). In other words, Zn contained in mantle cavity of *A. flexuosa* could sustain its Zn bioaccumulation for around 3 d.

The "mantle cavity uptake" mechanism can be used to reconcile results observed in the literature. Coleman (1980) compared Cd accumulation in alternately immersed and drained mussels (*M. edulis*) and continuously immersed mussels. The drained mussels were immersed in exposure solution for half as long as the continuously immersed mussels, but accumulated more than half as much Cd. The disproportional relationship between immersion duration and Cd bioaccumulation could be explained by Cd uptake from mantle cavity fluid, similar to what we observed in *X. atratus* (Figs. 4 and 6a). In contrast, Amachree et al. (2013) exposed *M. edulis* to Cd either continuously or intermittently (i.e., 2-d Cd exposure and 2-d clean seawater) for 14 d; Cd concentrations in tissues of continuously exposed mussels were generally more than 2 fold higher than in the intermittently exposed mussels, which can be explained by the absence of "mantle cavity uptake" during clean seawater exposure, similar to what we observed in Fig. 6b.

Besides the uptake of aqueous Cd, tidal height theoretically may also affect Cd bioaccumulation by affecting dietary assimilation and organism growth. Specifically, organisms immersed longer should have longer time of filtering food particles from water column and assimilate more dietary Cd; organisms inhabiting higher in the intertidal zone may show slower growth due to increased period of stress and reduced period of feeding, which may elevate tissue Cd concentration. The tidal effects observed in the field collected mussels are the superposition of all these effects.

3.4. Environmental implications

Although effects of tidal height on metal bioaccumulation were previously reported, dependent on the biological species and metals investigated, the effects were usually not substantial and not readily predictable (Al-Thaqafi and White, 1991; Gay and Maher, 2003; Lobel and Wright, 1982; Mubiana et al., 2006). Overall, tidal height is a less important factor than organism size, inter-species differences, and seasonal variations in determining metal bioaccumulation (Lobel et al., 1990; Mubiana et al., 2006; Tang et al., 2017; Weng and Wang, 2014). At present, tidal height is not required to be considered as a bioaccumulation modifying factor when conducting intertidal sampling according to guidelines of many biomonitoring programs (Apeti et al., 2012; General Administration of Quality Supervision and Standardization Administration of P. R. China, 2007). Nevertheless, based on the multiple lines of information mentioned above, we still recommend collecting organisms from different tidal heights, which requires no much additional efforts, to obtain more representative biomonitoring samples.

Besides the "mantle cavity uptake" mechanism, another possible mechanism underlying the decoupling (of immersion duration and Cd bioaccumulation) was the compensation response of the re-immersed mussels. Specifically, when mussels were re-immersed after a period of air exposure, they filtered water more actively than the continuously immersed mussels to relieve stresses caused by air exposure (Coleman, 1980), which may lead to higher Cd uptake rates. Interestingly, this mechanism was only observed in the 9-h immersion treatment. These two mechanisms theoretically should be applicable to other bivalves (and probably gastropods, barnacles) and other contaminants. This to some extent had been evidenced in the mussel *M. edulis*, of which Benzo[*a*]pyrene was found to be 1.2 times higher in air-exposed individuals (6 h exposure + 6 h air-exposed) than in the individuals immersed in clean water (6 h exposure + 6 h in seawater) (Durand et al., 2002).

For bivalves, the duration of contaminant exposure is neither simply the duration of immersion nor the duration of valve opening due to the continued uptake of contaminants in the closed bivalves. This knowledge contributed by the present study has, but not limited to, the following implications: First, during experiments for measuring toxicokinetics in bivalves, the exposure starts when the bivalves open their shells; temporary closing of shells during experiments is unlikely to affect the precision of measurements. Second, when accidental pollution occurs in waters of bivalve aquaculture (e.g., oysters, mussels, and scallops), elevating the shellfish out of water is not effective for stopping further pollution. Instead, the shellfish should be moved into clean or less polluted water as soon as possible. Third, when modeling contaminant bioaccumulation in intertidal bivalves for assessing ecological or health risks, it is acceptable to assume continuous exposure (for simplicity) instead of different exposure times for different tidal heights.

4. Conclusions

During simulated tidal exposures, variations of immersion duration showed very limited effects on Cd bioaccumulation in *X. atratus*. This apparent decoupling of Cd bioaccumulation and immersion duration was attributed to the continued Cd bioaccumulation occurred in mantle cavity after the mussels emerged into air at low tide. The sources of Cd for such bioaccumulation included Cd-rich solution enclosed in the mantle cavity and Cd adsorbed on the surface of mussel tissues. The "mantle cavity uptake" mechanism should be applicable to other contaminants and other intertidal organisms with shells (e.g., bivalves, gastropods, barnacles). The findings of this study would facilitate the use of *X. atratus* as a national and international biomonitor, and lend insights into risk assessments for intertidal zones.

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.

CRediT authorship contribution statement

Zhi Lin: Investigation, Writing - original draft. **Xingting Fan:** Investigation. **Junlin Huang:** Investigation. **Rong Chen:** Conceptualization. **Qiao-Guo Tan:** Conceptualization, Supervision, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.114192.

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Online Supplement to:

Intertidal mussels do not stop metal bioaccumulation even when out of water: cadmium toxicokinetics in *Xenostrobus atratus* under influences of simulated tidal exposure

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1. Measured concentrations of ^{113}Cd in exposure media

Table S1. Measured concentrations of ^{113}Cd in exposure seawater in the Cd toxicokinetics experiment. The nominal concentrations were 5, 50, 200, and 1000 μg L^{-1}. See Figure 2 for results of the experiment.

Time (h)	Nominal concentration (μg L^{-1})			
	5	50	200	1000
0	3.0	46	191	1187
3	2.8	44	188	1128
6	2.8	44	188	1131
12	2.7	43	186	1165

Table S2. The measured concentrations of ^{113}Cd (μg L^{-1}) in exposure seawater in the tidal height effect experiment (see Figure 4 for results of the experiment). The nominal ^{113}Cd concentration was 5 μg L^{-1}; test solutions were renewed after 24 h.

Daily immersion duration (h)	Time of experiment (h)	Cd in water (μg L^{-1})
6	0	4.6
	3	4.1
	15	4.1
	24	4.7
	27	4.6
	39	4.1
9	0	4.2
	4.5	4.1
	16.5	4.4
	24	4.6
	28.5	4.7
	40.5	4.2
12	0	4.2
	6	4.2
	18	4.2
	24	5.3
	30	4.5
	42	4.2
15	0	4.3
	7.5	4.3
	19.5	4.1
	24	4.9
	31.5	4.4
	43.5	4.0
24	0	4.2
	4	4.2
	16	4.1
	24	4.4
	36	4.3
	48	4.1

Table S3. Measured concentrations of ^{113}Cd in exposure seawater in the experiment for comparing Cd accumulation in mussels with or without a clean seawater depuration period between the 6-h Cd exposure and the 5-h or 6-h air exposure. The nominal concentration was 5 μg L^{-1}. See Figure 6 for results of the experiment.

Time (h)	Cd concentration (μg L^{-1})	
	Cd-spiked water → air	Cd-spiked water → **clean water** → air
0	4.1	4.0
2	4.0	3.8
4	4.0	3.9
6	3.9	4.0

2. Effects of tidal height on Cd concentrations in field collected *X. atratus*

Figure S1. The mussel *X. atratus* were collected from different height intervals of one pillar of Yanwu Bridge for investigating effects of tidal height on Cd bioaccumulation.

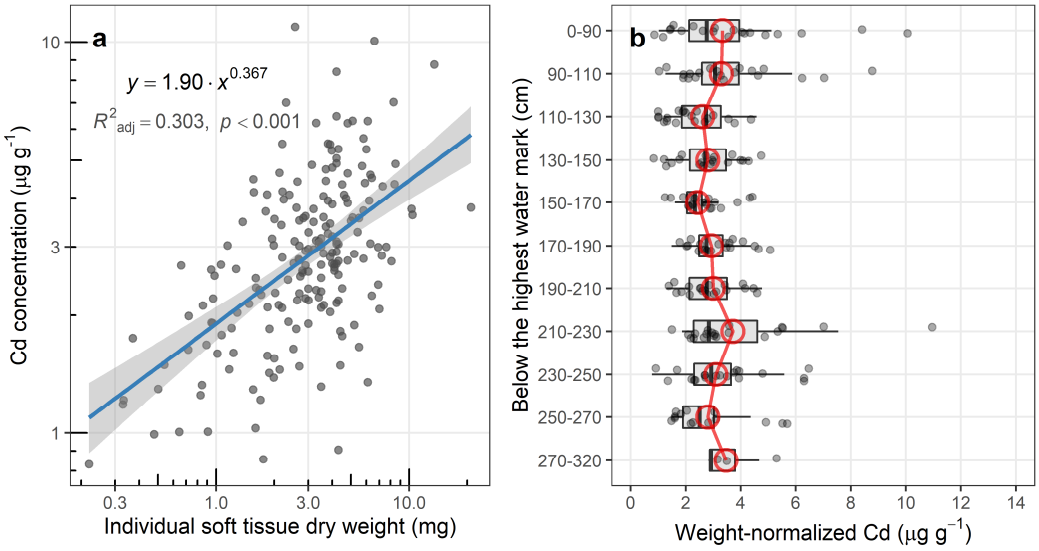

Figure S2. (a) The relationship between tissue Cd concentration in the mussel *X. atratus* and the individual dry weight. (b) Weight-normalized Cd concentration of mussels collected from different height intervals of the bridge pillar. Each point represents one mussel individual; the larger open circles (red) represent average values. A significant linear relationship existed between tissue Cd concentration and the individual weight in the double-log scale. Tissue Cd concentrations were thus normalized to the geometric mean individual weight (i.e., 2.81 mg) using the quantitative relationship showed in panel (a) and were presented in panel (b).

3. Effects of simulated tidal exposure on Cd toxicokinetics

Table S4. The estimated average daily immersion time of mussels inhabiting different height intervals of the bridge pillar. The estimation was conducted based on tidal height information of Xiamen in December, 2018, using the software Tides (v 3.7, www.arachnoid.com/tides)

Below the highest water mark (cm)	Average daily immersion time (h)
0-90	0-6.0
90-110	6.0-7.2
110-130	7.2-8.3
130-150	8.3-9.2
150-170	9.2-10.0
170-190	10.0-10.9
190-210	10.9-11.8
210-230	11.8-12.4
230-250	12.4-13.3
250-270	13.3-13.8
270-320	13.8-16.0

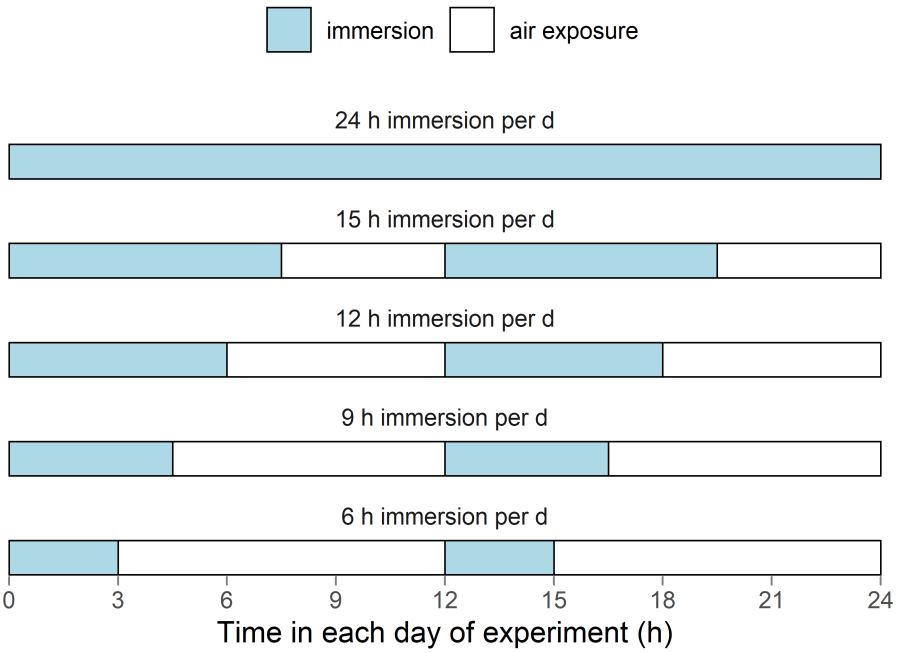

Figure S3. Schematic diagram of the tidal exposure experiment. Mussels were alternately immersed in water and exposed to air every day, simulating the semidiurnal tide. The daily immersion time varied from 6 h to 24 h, simulating tidal exposure of mussels inhabiting different tidal heights.

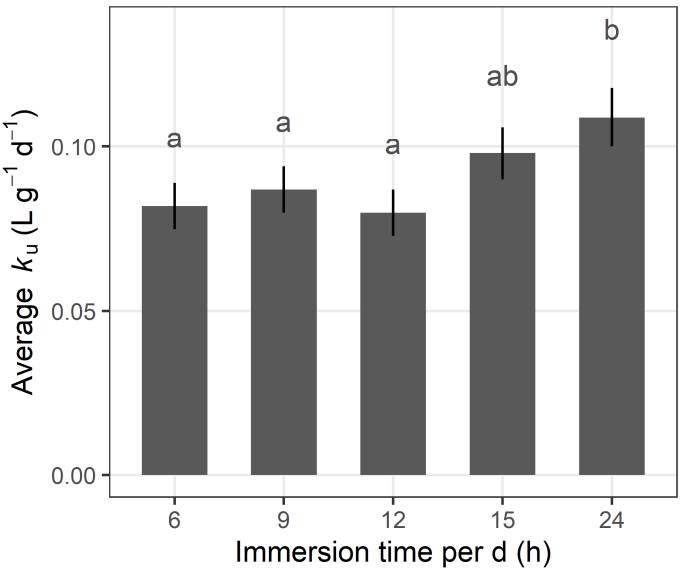

Figure S4. The average Cd uptake rate constant (k_u) of *X. atratus* exposed to 5 μg L^{-1} of ^{113}Cd with different immersion durations per d (i.e., 6-24 h). Results are mean ± standard deviation. See Figure 3 for more details of Cd toxicokinetics under tidal effects.

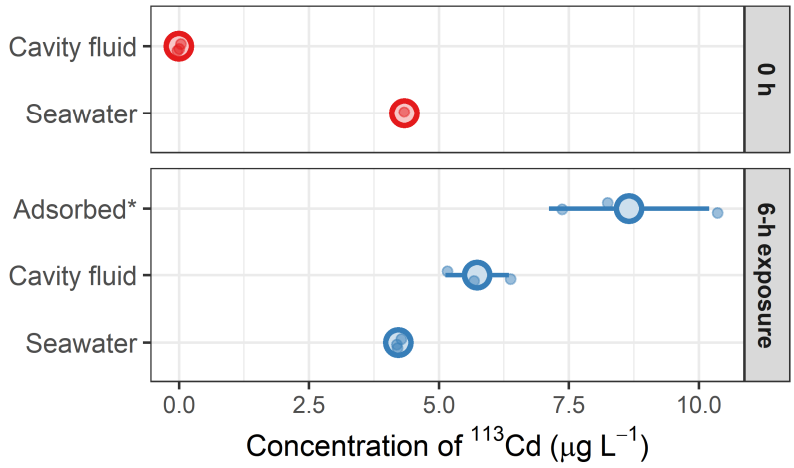

Figure S5. The concentration of ^{113}Cd in seawater, mantle cavity fluid of *X. atratus*, and adsorbed on the soft tissue of *X. atratus* before and after the 6-h exposure to 5 μg L^{-1} (nominal) of ^{113}Cd. Cavity fluid (5.7 ± 0.6 μg L^{-1}) of exposed mussels had 1.4 fold higher concentrations of ^{113}Cd than the exposure seawater (4.2 ± 0.1 μg L^{-1}). The amount of ^{113}Cd adsorbed on the tissues was 1.5 fold higher than that dissolved in the cavity fluid. The average length and height of the 60 mussels used in the experiment were 9.4 ± 0.6 mm, and 5.5 ± 0.4 mm, respectively. The average dry weight of soft tissues of 10 mussels was 23.5 ± 1.9 mg; the average volume of mantle cavity fluid collected from 10 mussels was 207 ± 21 μL.

*Adsorbed: the concentration was calculated by assuming that the adsorbed ^{113}Cd was dissolved in the cavity fluid (i.e., mass of adsorbed ^{113}Cd / volume of cavity fluid).

Methods of the experiment: Three replicates, each containing 10 individuals of *X. atratus* in 300 mL of ^{113}Cd-spiked seawater, were used. The nominal concentration of ^{113}Cd was 5 μg L^{-1}. After 6 h of exposure, the mussels were first rinsed twice with 1-mmol L^{-1} EDTA and twice with deionized water, and were dissected on a piece of clean Teflon board. For each replicate, the cavity fluid (of the 10 mussels) that flowed onto the Teflon board was pooled and collected into a centrifuge tube and centrifuged at 7690 *g* for 15 min. The supernatant was collected, acidified, diluted, and measured for ^{113}Cd. The soft tissues of the 10 mussels were pooled and washed in 1 mL of 1-mmol L^{-1} EDTA. The EDTA solution was also centrifuged and measured for ^{113}Cd after proper acidification and dilution. Before the exposure, cavity fluid was also collected from three samples of 10 mussel individuals for comparison.

4. Estimation of growth rate constants of *X. atratus* during experiments

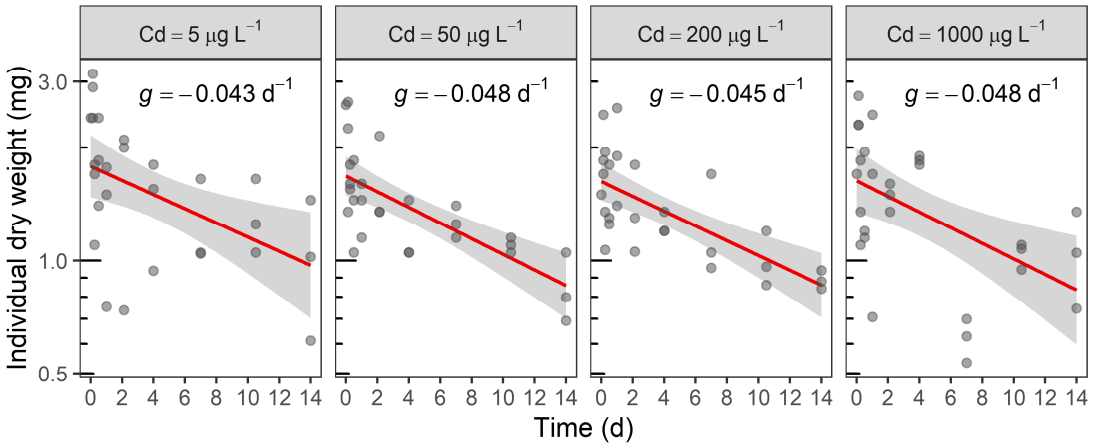

Figure S6. The soft tissue dry weight of *X. atratus* individuals during the experiment for measuring Cd toxicokinetics at different Cd concentrations (i.e., 5-1000 μg L^{-1}). An exponential growth model was used to fit the growth data; negative growth rate constants (*g*) were derived due to decreases in tissue weight of the organisms during experiment. The value of *g* measured from each treatment was assigned to the corresponding treatment for estimating Cd toxicokinetics (See Figure 2 for the Cd toxicokinetics). Each point represents one mussel individual.

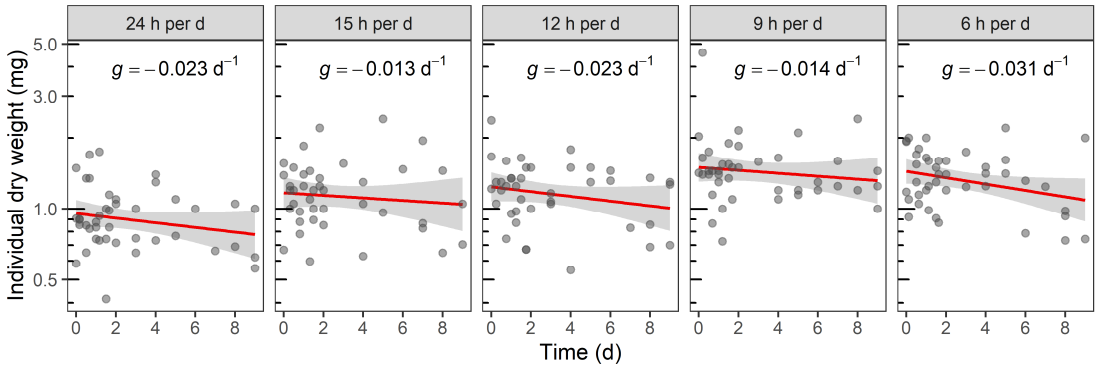

Figure S7. The soft tissue dry weight of *X. atratus* individuals during the experiment for investigating tidal height effects on Cd toxicokinetics. The value of *g* measured from each treatment was assigned to the corresponding treatment for estimating Cd toxicokinetics. See Figure 4 for the Cd toxicokinetics in *X. atratus* when exposed to different tidal regimes. Others as in Figure S6.

5. Toxicokinetics of Cd in marine bivalves (Table S5).

Species		k_u (L g^{-1} d^{-1})	k_e (d^{-1})	J_{in} (μg g^{-1} d^{-1})	Salinity	Temperature (°C)	C_w (μg L^{-1})	Reference
Mussel	*Xenostrobus atratus*	0.045-0.099	0.029-0.091	$0.126 \cdot C_w^{0.862}$	30	22	3-1000	This study
Mussel	*Xenostrobus securis*	~0.037[a]	~0.029[a]	n.a.	31	18	~0.4[b]	Alquezar et al., 2007
Mussel	*Mytilus edulis*	0.346-0.384	0.011-0.014	$0.365 \cdot C_w^{1.049}$	28	15	0.1-10	Wang et al., 1996
Mussel	*Perna viridis*	0.206	0.020-0.029	$0.206 \cdot C_w^{0.934}$	30	18	0.5-50	Chong & Wang, 2001
Mussel	*Septifer virgatus*	0.286	n.a.	$0.286 \cdot C_w^{1.022}$	30	18	0.5-50	Wang & Dei, 1999
Oyster	*Crassostrea gigas*	~0.047	0.0025	n.a.	37	17	0.5	Boisson et al., 2003
Oyster	*Crassostrea hongkongensis*	0.08-0.12	0.012	n.a.	25	20	2	Pan & Wang, 2012
Oyster	*Crassostrea rivularis*	0.719	0.014	$0.719 \cdot C_w^{0.770}$	15	23	0.5-50	Ke & Wang, 2001
Oyster	*Saccostrea cucullata*	0.343	n.a.	$0.343 \cdot C_w^{0.767}$	20	28	0.5-20	Blackmore & Wang, 2004
Oyster	*Saccostrea glomerata*	0.534	0.004	$0.534 \cdot C_w^{0.884}$	30	23	0.5-50	Ke & Wang, 2001
Scallop	*Chlamys nobilis*	0.2-1.2	0.005-0.026	$0.359 \cdot C_w^{1.016}$	~30	~20	0.5-100	Pan & Wang, 2008
Clam	*Macoma balthica*	0.032	0.018	$0.032 \cdot C_w^{1.039}$	20	10	0.11-11	Lee et al., 1998
Clam	*Mactra veneriformis*	0.129-0.145	0.025	$0.140 \cdot C_w^{0.871}$	~30	~20	0.5-50	Shi & Wang, 2004
Clam	*Ruditapes philippinarum*	0.050-0.051	0.029	$0.050 \cdot C_w^{0.992}$	~30	~20	0.5-50	Shi & Wang, 2004
Clam	*Ruditapes philippinarum*	0.064	0.010-0.013	$0.064 \cdot C_w^{0.977}$	30	18	0.5-50	Chong & Wang, 2001
Clam	*Potamocorbula amurensis*	0.125	0.011	$0.125 \cdot C_w^{0.956}$	20	10	0.11-11	Lee et al., 1998
Clam	*Portamacorbula laevis*	0.057-0.149	0.038	n.a.	30	22	5-550	Tan et al., 2019
Clam	*Portamacorbula laevis*	n.a.	n.a.	$0.180 \cdot C_w^{0.880}$	25	22	0.7-1000	Tan et al., 2019

k_u: uptake rate constant of dissolved Cd; k_e: elimination rate constant of Cd from organisms; J_{in}: uptake rate of Cd; C_w: Cd concentration in exposure solution; n.a.: not available; values with "~" were not explicitly provided in the reference but were estimated based on relevant information contained in the reference. [a] estimated by reanalyzing data of the study, assuming wet weight to dry weight ratio of 6; [b] estimated by assuming specific activity of ^{109}Cd to be 2 μCi μg^{-1}

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