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Predicting Risks of Cadmium Toxicity in Salinity-Fluctuating Estuarine Waters Using the Toxicokinetic–Toxicodynamic Model

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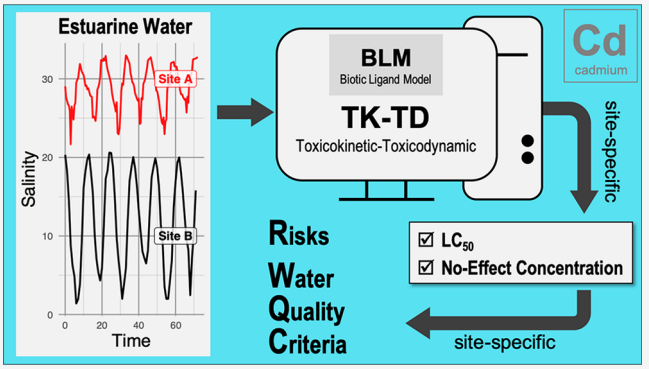
ABSTRACT: In estuaries, salinity fluctuates rapidly and continuously, greatly affecting the bioavailability and thus toxicity of contaminants, especially metals, causing difficulties in deriving site-specific water quality criteria. We developed a method for predicting the toxicity of the metal cadmium (Cd) in estuarine waters of any salinity fluctuation scenario. Cd bioaccumulation and toxicity were measured in an estuarine clam *Potamocorbula laevis* under stable salinities (salinity = 5, 15, 25) and fluctuating salinities (5–25), using the toxicokinetic–toxicodynamic (TK–TD) framework. Cd bioaccumulation decreases with increasing salinity; whereas intrinsic Cd sensitivity of organisms reaches the minimum at an intermediate salinity around 20. At each specific Cd level, interpolating TK–TD parameters measured at the stable salinities well predicts the Cd bioaccumulation and toxicity under fluctuating salinities. To extend the model for various Cd levels, the biotic ligand model (BLM) was integrated into the TK–TD framework. The BLM-based TK–TD model was successfully applied to scenarios of simulated and monitored salinity fluctuations in estuarine waters, for which the median lethal concentrations and no-effect concentrations (2.0–3.1 $\mu g\ L^{-1}$) of Cd were derived. Overall, we integrated the BLM and TK–TD models and provided a useful tool for predicting metal risks and deriving criteria values for salinity-fluctuating estuarine waters.



1. INTRODUCTION

Salinity is the principal factor that shapes community composition of organisms in estuaries.[1,2] It varies temporally and spatially, causing osmotic stresses and affecting bioavailability of contaminants, especially metals.[3−5] Metal bioavailability is affected by salinity through multiple mechanisms, including anion (e.g., Cl^-) complexation[6,7] and cation (e.g., Na^+, Ca^{2+}) competition.[8,9] In addition, salinity alters the intrinsic sensitivity of organisms to metals.[9,10] Together, these effects lead to diverging trends of metal toxicity in response to changing salinity.[3,9,11] Despite these complexities, in estuarine waters, there is an ongoing necessity to derive site-specific water quality criteria for metals using models.[12−14]

The biotic ligand model (BLM) is the most promising model to consider salinity effects owing to its ability to model water chemistry effects.[15−17] In BLM, free metal ion is considered the most bioavailable species; complexation of metal ion by chemical ligands and competition from major cations reduce metal bioavailability. However, even at the same site in an estuary, salinity fluctuates hourly instead of remaining stable, as implicitly assumed in the BLM,[14] creating difficulties in developing and using BLM to derive criteria values. In addition, the majority of previous studies investigated salinity effects on metal toxicity under several stable salinities[3,4,9] or specific salinity-fluctuating scenarios.[18−20] Knowledge provided by such studies is still hard to be directly applied to estuarine waters where salinity fluctuates continuously with infinite diversity in fluctuation amplitudes and patterns.

Integrating BLM with the toxicokinetic-toxicodynamic (TK–TD) model provides an opportunity to better predict metal risks in the salinity-fluctuating estuarine waters. TK–TD model is a flexible framework for modeling the time-course of metal bioaccumulation and toxicity.[21−23] TK relates metal bioaccumulation to metal exposure, describing processes of metal uptake and elimination; TD relates toxicity to metal bioaccumulation, simulating processes of hazard accumulation. Due to its kinetic property, the TK–TD model is especially suitable for modeling contaminant exposure under fluctuating conditions. For example, it has been successfully applied in studying exposures to fluctuating contaminant concentrations.[24,25] Moreover, we effectively used the TK–TD model to delineate dual effects (i.e., water chemistry and physiological

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effects) of salinity on toxicity of Cu[11] and Cd[9] previously. Taken together, integrating BLM into the TK−TD framework, i.e., using BLM in the TK part to simulate water chemistry effects of salinity, will theoretically improve our ability to predict metal risks in salinity-fluctuating estuaries, although it has yet to be tested.

Metal toxicity under fluctuating salinity conditions in comparison to stable salinity conditions has been sporadically investigated; diverse results were obtained. In some cases, salinity fluctuation did not show extra effects that cannot be predicted from the effects observed at constant salinities. For example, salinity fluctuation did not increase Cd toxicity to the mysid *Mysidopsis bahia* when exposed at the no-effect concentration (5 μg L^{-1}) of Cd.[20] In addition, Cd toxicity to the larvae of the estuarine fish *Menidia menidia* under fluctuating salinities (10−30) was similar to that under a constant salinity of 20.[26] However, in several other studies, extra effects of salinity fluctuation were suggested. Davenport[18] observed that the mussel *Mytilus edulis* closed its shell valves when the salinity dropped below 5, making the salinity and metal concentration, to which the mussel was exposed, different from the conditions of the ambient environment. Leung et al.[19] found that the dog whelk *Nucella lapillus* accumulated higher concentrations of Cd but showed lower mortality when exposed under fluctuating salinities rather than under fixed salinities (11, 22, and 33). In summary, it is not clear whether metal bioaccumulation and toxicity under fluctuating salinities can be predicted by measurements done under several stable salinities.

In this study, we measured cadmium (Cd) bioaccumulation and toxicity in the estuarine clam *Potamocorbula laevis* at stable and fluctuating salinities under the TK−TD framework. We first tested whether Cd bioaccumulation and toxicity under fluctuating salinities (5−25) can be predicted by interpolating TK−TD parameters measured at constant salinities (5, 15, and 25). We then integrated BLM into the TK−TD framework to establish a unified model for Cd exposures under any (realistic) fluctuating salinity scenarios. Median lethal concentrations (LC_{50}) and no-effect concentrations (NEC) of Cd under fluctuating salinities were calculated using the model. The method in principle is applicable for other estuarine organisms and pollutants and provides a tool for assessing ecological risks in salinity-fluctuating estuarine waters.

2. MATERIALS AND METHODS

2.1. Organisms and Materials. The clam *Potamocorbula laevis* was collected from the Jiulong River Estuary (24.469917° N,117.930944° E), Fujian Province, China [see Supporting Information (SI) Figure S1 for the map]. Individuals with shell lengths between 1 to 2 cm were used for experiments. The clams were acclimated to laboratory conditions for at least 2 weeks before use. During acclimation, clams were fed the green alga *Chlorella* sp. daily; and seawater was renewed daily. The seawater was collected from Tong'an Bay, Xiamen, China (24.566944° N,117.192472° E), and had an original salinity of around 30 and a pH of 8.0. To be used in experiments, the seawater was filtered by a glass fiber filter (Whatman GF/C) and then a 0.22-μm polypropylene capsule filter (GVS Calyx), and was diluted to desired salinities with ultrapure deionized water (18.2 MΩ·cm).

A stable isotope ^{113}Cd (ISOFLEX, San Francisco, California, U.S.A., dissolved in 5% HNO_3) was used as a tracer for determining Cd toxicokinetics. Freshly prepared exposure solution of desired salinities and ^{113}Cd concentration was adjusted to a pH of 8.0 when necessary with 2 mol L^{-1} NaOH, and equilibrated overnight before use. All plastic (polypropylene or polymethylpentene) containers, before being used, were soaked in 2% HNO_3 for 1 d and washed with reverse osmosis water and then ultrapure water. All experiments were conducted at 22 ± 1 °C with a light/dark cycle of 14:10 h.

2.2. Salinity-Fluctuating Exposure System. A flow-through exposure system was used to simulate salinity fluctuation (see Figure S2). Salinity of the exposure solution varied between 5 and 25, two cycles per day, simulating a semidiurnal tidal cycle. Within each 12-h cycle, salinity was gradually elevated during the first 6 h by peristaltically pumping salinity 30 solution into the exposure container, and then lowered by pumping salinity 0 solution during the second 6 h. To keep the ^{113}Cd concentration constant in the exposure container, ^{113}Cd was added in both (i.e., salinity 30 and 0) influent solutions at the same concentration. The salinity of the exposure solutions was monitored using a conductivity meter (Cond 6+, OAKTON).

Variation of salinity in the exposure beaker can be described by the following equation based on salt mass balance:

$$\frac{d\mathrm{Sal}(t)}{dt} = \frac{Q(t) \times [\mathrm{Sal}_{in}(t) - \mathrm{Sal}(t)]}{V} \quad (1)$$

where $\mathrm{Sal}(t)$ is the salinity at time t; $\mathrm{Sal}_{in}(t)$ is salinity of the influent solution; $Q(t)$ is the flow rate of the influent and effluent solution, which was 2.68 mL min^{-1} in this study; and V is the volume of solution in the exposure beaker, which was maintained constant at 600 mL.

In experiments of constant salinity exposure, a similar flow-through system was used, of which the salinity of the influent solution was held constant at desired levels (i.e., 5, 15, and 25).

2.3. Cd Toxicokinetics. Two sets of experiments were conducted for measuring Cd toxicokinetics. In the first set, Cd toxicokinetics was measured at 2 μg L^{-1} of ^{113}Cd and compared between exposure conditions of fluctuating salinity (5−25) and constant salinities (i.e., 5, 15, and 25). In the second set, Cd toxicokinetics was compared for different ^{113}Cd concentrations (i.e., 2, 20, and 200 μg L^{-1}) under fluctuating salinity.

Three replicate beakers were used for each treatment, each containing 24 clams and 600 mL of exposure solution. Two clams were sampled from each beaker every 6 h during the 72-h exposure. The sampled clams were rinsed immediately with 1 mmol L^{-1} ethylenediaminetetraacetate (EDTA, pH 8.0) to stop Cd uptake. Soft tissues were separated from the shells, further rinsed twice with EDTA and twice with ultrapure water, freeze-dried, and weighed. The tissue of each individual (ca. 6 mg dry weight) was placed separately into a 15 mL polypropylene tube and digested with 0.5 mL of 65% HNO_3 at 80 °C for 8 h.

2.4. Toxicity Testing. Toxicity tests of Cd were also conducted in the flow-through system and were compared between the constant salinity (5, 15, and 25) and fluctuating salinity (5−25) conditions. The clams were exposed to 500 μg L^{-1} of Cd for 72 h. Three replicates and one control (with the same salinity but without Cd) were used for each treatment. Each replicate beaker contained 18 clams in 600 mL of exposure solution. Two clams in each beaker were sampled every 3 h for the first 12 h to measure Cd bioaccumulation, following the methods described above. The 8 clams for bioaccumulation measurement were randomly designated at

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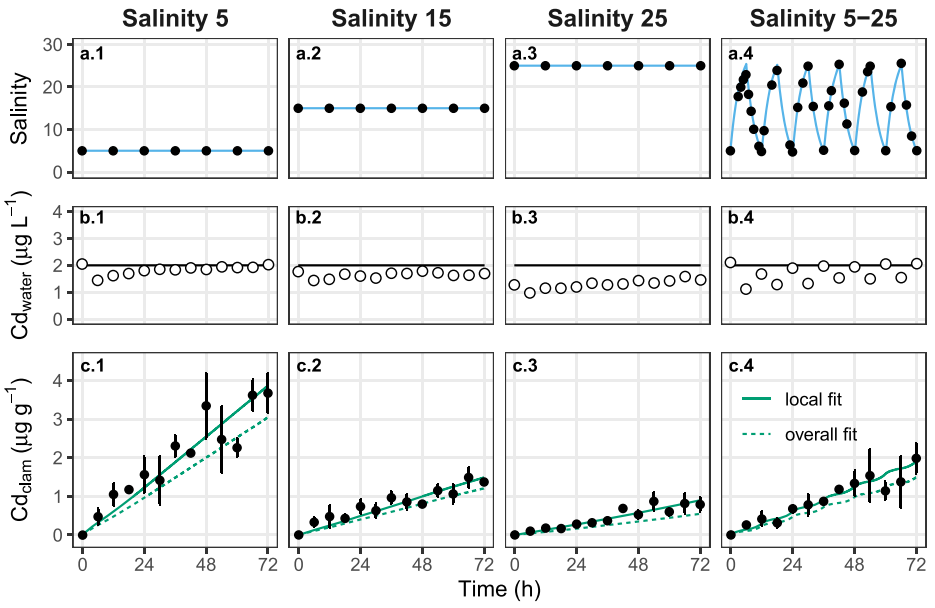

Figure 1. Cd bioaccumulation in the clam *Potamocorbula laevis* under constant and fluctuating salinities. (a) Measured (points) and calculated (lines) salinities; (b) measured (points) and nominal (lines, 2 μg L^{-1}) Cd concentrations in water; (c) measured (points, mean ± standard deviation, n = 3) and model predicted (solid and dashed curves) newly accumulated ^{113}Cd in clams. Local fit: fitting model to the data set shown; Overall fit: fitting model to all data sets shown in Figures 1−3.

the start of the test. The remaining 10 clams were used for toxicity testing. The mortality of the clams was checked every 5 to 8 h, with the dead immediately removed. In the first 12 h of exposure, isotope ^{113}Cd was used to prepare the influent test solution; in the remaining 60 h, common $CdCl_2$ (Sigma-Aldrich) was used instead. Toxicity tests were considered valid only when the mortality in all controls was lower than 10%.[27]

2.5. Chemical Analysis. Concentrations of the ^{111}Cd and ^{113}Cd in clam tissue samples and water were measured using inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7700x). Water samples were collected together with the clam samples and were acidified by adding 100 μL of 7.3 mol L^{-1} HNO_3 per 10 mL of sample. The internal standard ^{115}In (5 μg L^{-1}) was used to correct instrument drift and matrix effects. The standard reference material (SRM 1566b, oyster tissue) was used for quality control and was measured after every 10 to 20 samples to monitor the status of the instrument. Analyses were considered acceptable when the recoveries of ^{111}Cd and ^{113}Cd from the SRM were both within 10% deviation from the certified value of 2.48 μg Cd g^{-1}.

Concentrations of newly accumulated ^{113}Cd in clams were calculated using the following equation:[9,28]

$$\text{new}[^{113}\text{Cd}] = [\text{Cd-113}] \times 12.22\% - [\text{Cd-111}] \times 12.22\% \quad (2)$$

where [Cd-113] (or [Cd-111]) is ICP−MS reported concentration of total Cd (note: not the concentration of the isotope per se) when selecting the isotope ^{113}Cd (or ^{111}Cd) for constructing the calibration curve using the Agilent ICP−MS calibration standard (part number 5183−4688), which is a mixture of Cd natural isotopes of natural relative abundance. The percentage 12.22% is the natural abundance of ^{113}Cd. "[Cd-113] × 12.22%" is the total (i.e., background + newly accumulated) ^{113}Cd in organisms; "[Cd-111] × 12.22%" is the background ^{113}Cd in organisms. A more detailed explanation on the analysis of stable isotope data is available in Tan et al.[9]

Cd concentrations in clams were expressed on a dry weight basis.

2.6. TK−TD Modeling. *2.6.1. Toxicokinetics.* Cd uptake and elimination in clams were described with a one-compartment TK model. Cd concentration in clams [$C_{int}(t)$, μg g^{-1}] is expressed as[9,23]

$$\frac{dC_{int}(t)}{dt} = J_{in}(t) - k_e \times C_{int}(t) \quad (3)$$

where $J_{in}(t)$ (μg g^{-1} d^{-1}) is the uptake rate of Cd; k_e (d^{-1}) is the elimination rate constant. Growth of organisms was ignored for the short-term experiments (see weight of clams in Figure S3). When Cd concentration in exposure solution [$C_w(t)$, μg L^{-1}] is low or varies within a narrow range, J_{in} can be related to $C_w(t)$ by

$$J_{in}(t) = k_u \times C_w(t) \quad (4)$$

where k_u is the Cd uptake rate constant (L g^{-1} d^{-1}) and is dependent on salinity among various other factors.

2.6.2. Toxicodynamics. Mortality of clams in the toxicity tests is considered due to the accumulation of Cd in tissues and is described by a TD model.[9,11,21]

$$\frac{dH(t)}{dt} = \begin{cases} k_k \times (C_{int}(t) - C_{IT}) + h_0, & \text{if } C_{int}(t) > C_{IT} \\ h_0, & \text{otherwise} \end{cases} \quad (5)$$

$$S(t) = e^{-H(t)} \quad (6)$$

where C_{IT}(μg g^{-1}) is the internal threshold concentration, i.e., the highest Cd concentration in tissues that clams can tolerate without elevated mortality; $H(t)$ (dimensionless) is the hazard caused by excessive Cd in tissues (excessive Cd = $C_{int} - C_{IT}$); k_k is the killing rate (g μg^{-1} h^{-1}), i.e., organisms (measured in grams) killed per hour by per μg of bioaccumulated Cd exceeding C_{IT}; h_0 (h^{-1}) is the background hazard rate and was set to 0 in this study as the clams were quite healthy under

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Table 1. Values (Mean ± Standard Deviation) of Model Parameters Estimated under Stable Salinity Conditions[a]

salinity	$k_{u,2}$ (L g^{-1} d^{-1})	$k_{u,500}$ (L g^{-1} d^{-1})	C_{IT} (μg g^{-1})	k_k (mg μg^{-1} h^{-1})
5	0.749 ± 0.038	0.697 ± 0.044	19 ± 2	0.176 ± 0.013
15	0.322 ± 0.019	0.293 ± 0.018	142 ± 9	0.077 ± 0.011
25	0.242 ± 0.022	0.154 ± 0.011	138 ± 9	0.111 ± 0.016

[a] $k_{u,2}$ and $k_{u,500}$: Cd uptake rate constants measured at nominal Cd concentrations of 2 μg L^{-1} and 500 μg L^{-1}, respectively; C_{IT}: internal threshold concentration; k_k: killing rate. See eqs 3−6 for detailed definition of parameters; see Figures 1 and 2 for data from which the parameters were estimated.

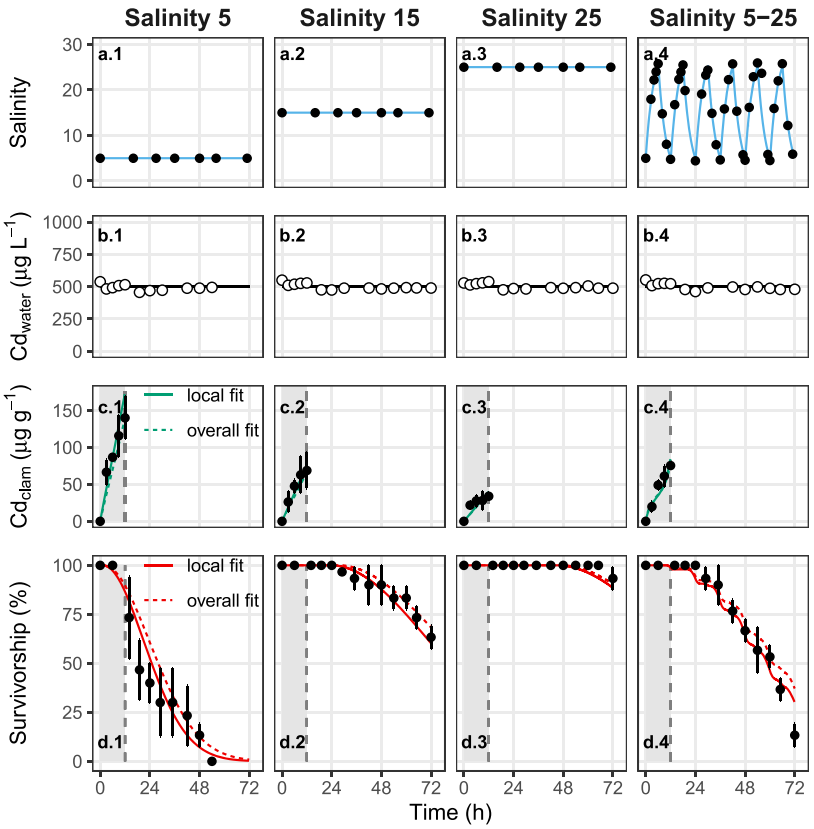

Figure 2. Cd bioaccumulation and toxicity in the clam *Potamocorbula laevis* under constant and fluctuating salinities. (a) Measured (points) and calculated (lines) salinities; (b) measured (points) and nominal (lines, 500 μg L^{-1}) Cd concentrations in water; (c) measured (points, mean ± standard deviation, n = 3) and model predicted (solid and dashed curves) newly accumulated ^{113}Cd in clams; and (d) observed (points, mean ± standard deviation, n = 3) and model predicted (solid and dashed curves) survivorship of clams. Local fit: fitting model to the data set shown in Figure 2; overall fit: fitting model to all data sets shown in Figures 1−3.

laboratory conditions and no appreciable mortality occurred in the controls; $S(t)$ is the survivorship of clams.

2.7. Data Analysis. The effects of salinity on Cd toxicokinetics were quantitatively described with two equations of different complexity. At each specific Cd level, Cd k_u was related to salinity using an empirical equation assuming competitive inhibition of salinity against the uptake of Cd:[11,15]

$$k_u(\mathrm{Sal}) = \frac{1}{a + b \times \mathrm{Sal}} \tag{7}$$

where a and b are empirical constants. Further, the equation was extended to different Cd levels by constructing a BLM-based TK−TD model:[15,23]

$$J_{in} = \frac{J_{max} \times K_{CdBL} \times Cd_{tot} \times f}{1 + K_{CdBL} \times Cd_{tot} \times f + K_{SalBL} \times Sal} \tag{8}$$

where f is the fraction of free Cd^{2+} ion activity of the total dissolved Cd (Cd_{tot}, μg L^{-1}); K_{CdBL} (L μg^{-1}) is the stability constant for the binding of Cd^{2+} to the biotic ligands; similarly, K_{SalBL} (dimensionless) is the hypothetical stability constant for the binding of major cations (e.g., Na^+ and Ca^{2+}) to the biotic ligands; and J_{max} (μg g^{-1} h^{-1}) is the maximum uptake rate of Cd. We call eq 7 the "local fit" (i.e., applicable to a specific Cd level) and eq 8 the "overall fit" (i.e., applicable to all Cd levels).

The TK−TD model was implemented in the free mathematical modeling software OpenModel (version 2.4.2) developed by Neil Crout at University of Nottingham. Values and standard deviations of model parameters were estimated by least-squares optimization using the Marquardt algorithm. Detailed procedures of modeling and parameter estimation is provided in the SI Note S1.

Cd speciation at different salinities was calculated using the speciation modeling program Visual MINTEQ (version 3.0). Measured (rather than nominal) concentrations of Cd in exposure solution were used in data analysis. Calculation of Cd

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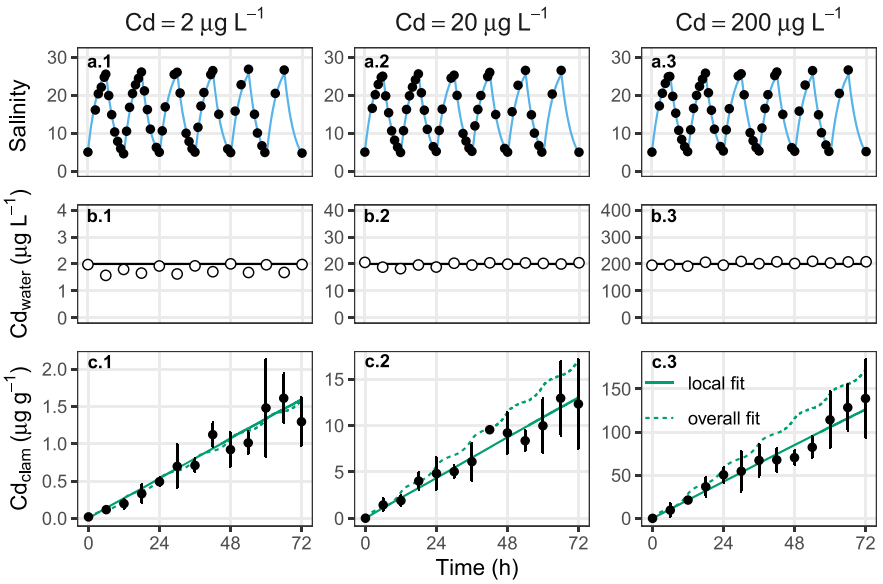

Figure 3. Cd bioaccumulation in clam *Potamocorbula laevis* under fluctuating salinities. (a) Measured (points) and calculated (lines) salinities; (b) measured (points) and nominal (lines, 2, 20, and 200 μg L^{-1}) Cd concentrations in water; and (c) measured (points, mean ± standard deviation, n = 3) and model predicted (solid and dashed curves) newly accumulated ^{113}Cd in clams. Local fit: fitting the model to the data set shown in Figure 3; and overall fit: fitting the model to all data sets shown in Figures 1−3.

LC_{50} and NEC using the calibrated TK−TD model were conducted in R (version 3.4.3).

3. RESULTS AND DISCUSSION

3.1. Cd Bioaccumulation under Constant and Fluctuating Salinities. We first investigated whether Cd bioaccumulation under fluctuating salinities can be predicted by interpolating Cd bioaccumulation measured under constant salinities.

Under constant salinities, Cd bioaccumulation in clams is well described by the one-compartment TK model (Figure 1c.1−c.3, solid curves). The uptake rate constant (k_u) of Cd decreased from 0.749 to 0.322 and 0.242 L g^{-1}d^{-1} when the salinity increased from 5 to 15 and 25 (Table 1). In the model fitting, a fixed value of Cd elimination rate constant (k_e, 0.0382 d^{-1}) was assumed based on our previous finding that Cd k_e was insensitive to seawater salinity.[9] The slow elimination of Cd (~3.8% per day) explains the seemingly *linear* increase of Cd in clams during the 3-d exposure.

Under fluctuating salinities, Cd k_u inferably varied with the fluctuating salinity. We interpolated k_u (at ~2 μg L^{-1} of Cd) for different salinities using eq 7:

$$k_{u,2}(\mathrm{Sal}) = \frac{1}{0.538 + 0.160 \times \mathrm{Sal}} \quad (9)$$

With this empirical equation, Cd bioaccumulation under fluctuating salinity conditions was calculated by integrating eq 3 over time. The calculated values (Figure 1c.4, solid curve) agreed well with the measured values, indicating the feasibility of interpolation in predicting Cd bioaccumulation under fluctuating salinities.

The observed decrease in Cd k_u with increasing salinity is consistent with the complexation and competition effects explained by BLM. Specifically, free Cd^{2+} ion is the most bioavailable species.[7,15,17] The fraction of Cd^{2+} decreases with increasing salinity due to complexation of Cd^{2+} by major anions, mainly Cl^- (Figure S4).[9] The competition of major cations (e.g., Na^+, Ca^{2+}, Mg^{2+}) with Cd^{2+}, occurring at the biouptake sites, also increases with salinity. These inhibitory effects of "complexation" and "competition" on Cd uptake were empirically described using eq 7. Similar inhibitory effects of salinity against Cd bioaccumulation were consistently observed for various other organism species, such as mussels,[29,30] clams,[30] snails,[29] copepods,[31] shrimps,[32] crabs,[33] and fish.[8,34]

Similarly, at the high Cd concentration of 500 μg L^{-1}, Cd k_u decreased from 0.697 to 0.293 and 0.154 L g^{-1} d^{-1} when the salinity increased from 5 to 15 and 25, respectively (Figure 2c, Table 1). Again, we interpolated k_u for various salinities at this Cd level using eq 7:

$$k_{u,500}(\mathrm{Sal}) = \frac{1}{0.345 + 0.218 \times \mathrm{Sal}} \quad (10)$$

The interpolated k_u predicted well the Cd bioaccumulation under fluctuating salinity at this high Cd level (Figure 2c.4), confirming again the feasibility of the interpolation method.

3.2. Cd Toxicity under Constant and Fluctuating Salinities. We further investigated whether Cd toxicity under fluctuating salinities can be predicted by toxicity measured under stable salinity conditions.

Under constant salinities, the clams survived better at higher salinities (Figure 2d), consistent with the effects on Cd bioaccumulation. The survivorship of clams was fitted with the TD model (eqs 5 and 6); and two TD parameters, C_{IT} and k_k, were estimated (Table 1). C_{IT} was 19, 142, and 138 μg g^{-1}; and k_k was 0.176, 0.077, and 0.111 mg μg^{-1} h^{-1} at salinity 5, 15, and 25, respectively.

Both C_{IT} and k_k indicate the sensitivity of organisms to internal accumulated Cd: higher C_{IT} or lower k_k reflects lower sensitivity. Both parameters indicate the lowest sensitivity of organisms at the intermediate salinity and the highest sensitivity at the lower end of the salinity range (Figure S5, Table 1), consistent with our previous observations obtained at more (i.e., seven) salinity levels (5−30).[9] These results

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Table 2. Variables and Parameters of the Toxicokinetic-Toxicodynamic Model for Predicting Cd Bioaccumulation and Toxicity under Fluctuating Salinities[a]

model parameters or variables	unit	equations or values
J_{in}, Cd uptake rate	$\mu g\ g^{-1}\ h^{-1}$	$J_{in} = \frac{J_{max} \times K_{CdBL} \times f \times Cd_{tot}}{1 + K_{CdBL} \times f \times Cd_{tot} + K_{SalBL} \times Sal}$
J_{max}, maximum uptake rate of Cd	$\mu g\ g^{-1}\ h^{-1}$	203 ± 30
f, fraction of free Cd^{2+} ion	dimensionless	$f = \frac{1}{0.009993 \times Sal^2 + 0.3867 \times Sal + 2.5048}$
k_e, elimination rate constant	h^{-1}	0.00159 ± 0.00008
K_{CdBL}, stability constant for the binding of Cd^{2+} to uptake sites	$(\mu g\ L^{-1})^{-1}$	0.00057 ± 0.00004
K_{SalBL}, hypothetical stability constant for the binding of major cations to uptake sites	dimensionless	0.00048 ± 0.00006
C_{IT}, internal threshold concentration of Cd	$\mu g\ g^{-1}$	$C_{IT} = -12.3 \times \lvert Sal - 19.8 \rvert + 201$
k_k, killing rate of Cd	mg $\mu g^{-1}\ h^{-1}$	$k_k = 0.0098 \times \lvert Sal - 18.3 \rvert + 0.0448$

[a]See dashed lines in Figures 1−3 for predicted bioaccumulation and toxicity; see Figures 4 and 5 for predicted median lethal concentrations (LC_{50}) and no-effect concentrations (NEC).

confirmed that salinity not only affected Cd bioaccumulation but also affected the organisms' sensitivity to the bioaccumulated Cd; therefore, the observed salinity effects on organism survivorship under Cd exposure were a superposition of the dual effects.

The lowest sensitivity of organisms reached at an intermediate salinity suggests an optimal salinity of the clam *P. laevis* around 15. Deviation from the optimal salinity increased the organisms' sensitivity to Cd hazard. We thus interpolated C_{IT} and k_k for different salinities by assuming a linear relationship between sensitivity and salinity deviation:[9,11]

$$C_{IT} = -12.3 \times |Sal - 19.8| + 201 \quad (11)$$

$$k_k = 0.0098 \times |Sal - 18.3| + 0.0448 \quad (12)$$

The survivorship of clams under fluctuating salinity was in turn predicted (Figure 2d.4, solid curve). The prediction agrees well with the observed survivorship (Figure 2d), again, confirming the feasibility of the interpolation method.

We previously observed similar optimal salinities for *P. laevis* when they were exposed to Cu (salinity 10−15)[11] or Cd (salinity 11−20).[9] Although *P. laevis* is a euryhaline species that can survive a wide range of salinities (2−30),[11] deviation from their optimal salinity still causes sublethal stress. Under the suboptimal salinity conditions, the clams need higher activity of ionoregulation, which involves membrane transporters such as Na^+/K^+ ATPase and Ca^{2+} ATPase.[10,35] Ionoregulation processes are susceptible to the disruption of Cd^{2+};[35] therefore, the clams showed higher sensitivity to Cd toxicity under suboptimal salinities.

3.3. Modeling Cd Bioaccumulation and Toxicity under Fluctuating Salinities. Upon confirming the feasibility of interpolating both TK and TD parameters for fluctuating salinities, we further aimed to extend the model to be applicable for different Cd concentrations. Values of k_e, C_{IT}, and k_k in principle are independent of Cd concentration; however, k_u varies substantially over a wide range of Cd concentrations. As a result, the relationship between k_u and salinity varies at different Cd concentrations, requiring a unique salinity-interpolation equation (e.g., eqs 9 and 10) for each Cd concentration. It is, in practice, impossible to derive such interpolation equations for each Cd level as that which we performed for Cd of 2 and 500 $\mu g\ L^{-1}$. To solve this problem, we extended the fluctuating salinity experiment to different Cd concentrations (i.e., 2, 20, and 200 $\mu g\ L^{-1}$, Figure 3), and then built a unified TK−TD model (i.e., the BLM-based TK−TD model, eq 8) to simulate effects of salinity and Cd concentration simultaneously.

When Cd concentration increased from 2 to 20 $\mu g\ L^{-1}$ and 200 $\mu g\ L^{-1}$, the average Cd k_u under fluctuating salinities decreased, as expected, from 0.293 to 0.232 L $g^{-1}\ d^{-1}$ and 0.220 L $g^{-1}\ d^{-1}$, respectively (Figure 3). This decrease is attributable to the partial saturation of the biotic ligands at higher Cd^{2+} levels, as predicted by BLM.[15]

We calibrated the BLM-based TK−TD model to all Cd uptake data collected in this study (i.e., overall fit, Figures 1−3) simultaneously. Variables and parameters, either as values or as functions of salinity, from the overall fit are summarized in Table 2. Predictions of the model (Figures 1−3, dashed curves) agree well with the observed values, although they are less well compared to the local fits (i.e., separate interpolations) (Figures 1−3, solid lines) as expected.

The manifold effects of salinity on Cd bioaccumulation are represented in the BLM model (eq 8) by different parameters. The anion complexation and ionic strength effects are represented by the parameter f; the cation competition effects are represented by K_{SalBL}. Ideally, cation competition effects should be decomposed into the effects of each major cations (e.g., Na^+, Ca^{2+}, Mg^{2+}, K^+). However, considering the fact that major cation concentrations covary and are in nearly constant ratio to salinity (i.e., conservative elements),[1] we treated the major cations as a whole and as a hypothetical competitor for practicality.

One possible concern on the BLM-based TK−TD model is that salinity effects may be repeatedly counted. But that did not happen. Salinity has dual effects on Cd toxicity, i.e., water chemistry effects and physiological effects; they are separately modeled, and each are modeled only once. The water chemistry effects are considered by the BLM; the physiological effects are not included in the BLM but in the TD model, where salinity effects on organism sensitivity are modeled by relating C_{IT} and k_k to salinity based on the "optimal salinity" mechanism.

3.4. Model Applications in Estuarine Waters. *3.4.1. Deriving Cd LC_{50} under Fluctuating Salinities.* Using the BLM-based TK−TD model (Table 2), we can predict time-course of survivorship of organisms exposed to Cd under fluctuating salinities. Reversely, we can calculate the Cd concentration that leads to 50% of mortality of organisms (i.e., LC_{50}) after any

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(reasonable) duration of exposure (see R code for calculation in the SI). Calculated LC_{50}s for three fluctuating salinity scenarios were presented in Figure 4.

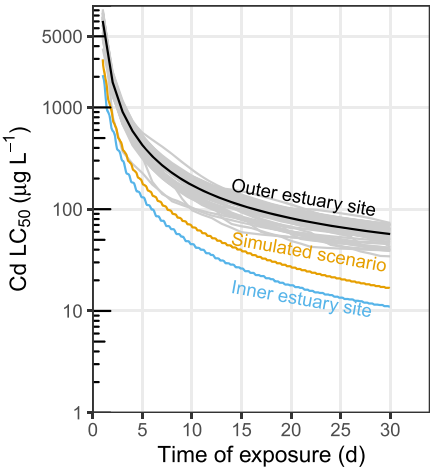

Figure 4. Predicted median lethal concentration (LC_{50}) of Cd to the clam *Potamocorbula laevis* under different scenarios of fluctuating salinity and for different exposure durations. Simulated scenario: fluctuating salinity simulated in this study (see Figure 1a.4); Inner (outer) estuary site: salinity fluctuation monitored at two sites in Jiulong River Estuary (see Figure S1 for the site map and Figure S6 for the salinity information). For the inner estuary site, the 3-d salinity pattern was repeated for the calculation. For the outer estuary site, LC_{50} values were calculated for multiple 30-d intervals selected from the one-year salinity monitoring data (gray lines, n = 48; black line: average). See R code for the method of calculation in the SI Note S2.

The three scenarios include salinity fluctuation simulated in this study (Figure 1a.4, salinity 5−25) and monitored from two sites in the Jiulong River Estuary (see Figure S1 for the site map and Figure S6 for the salinity information). LC_{50} differs remarkably among different scenarios, having lower values, indicating higher risks, at the inner estuary site (salinity mean: 12; salinity range: 0−20) than the outer estuary site (salinity mean: 22; salinity range: 5−33). Even at the same site (e.g., outer estuary site), LC_{50} varies substantially due to the annual fluctuation of salinity.

LC_{50} derived from traditional standard toxicity tests is tied to a specified exposure duration (e.g., 48 or 96 h). LC_{50} decreases with exposure duration; therefore, comparing or compiling LC_{50} values from different studies using different test durations is difficult and even inappropriate. LC_{50} derived from the TK−TD model is free of this problem and is available for any duration of exposure.

3.4.2. Deriving NEC of Cd under Fluctuating Salinities. NEC of Cd is defined as the maximum Cd concentration in water that does not cause mortality of organisms of concern under long-term exposure. Using the TK−TD model, we can calculate NEC as the maximum Cd concentration that ensures Cd accumulated in organisms (C_{int}) constantly below the threshold C_{IT} (i.e., $C_{int} \leq C_{IT}$, Figure 5). Again, R code for the calculation is provided in the SI. In the calculation, we ignored growth dilution in organisms for simplicity and considering the precautionary principle, which would slightly underestimate NEC, making it more conservative when used in ecological risk assessment. Nonetheless, growth dilution can be readily considered in the model by assigning organism growth rates,

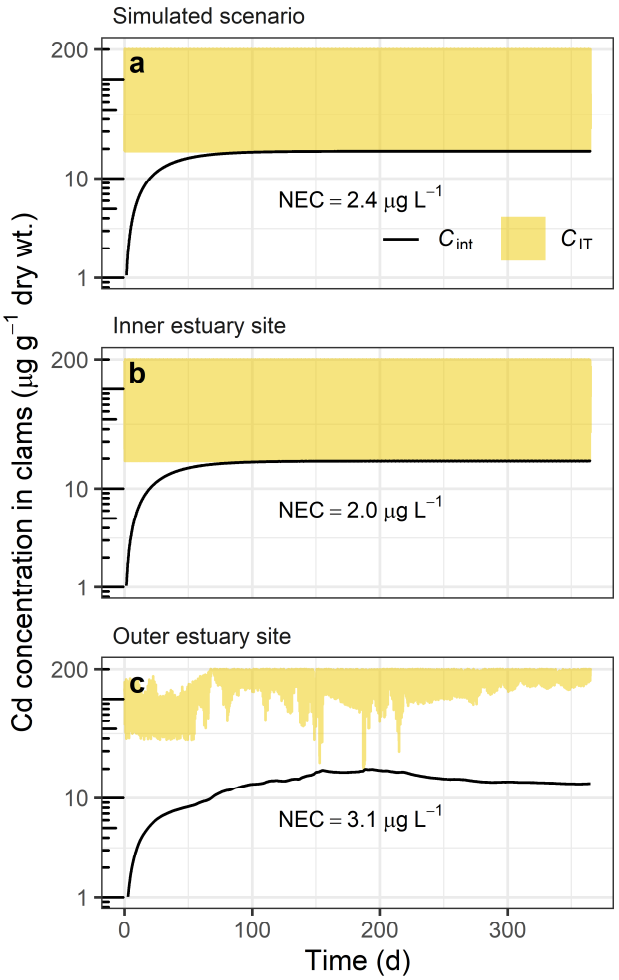

Figure 5. Predicted no-effect concentrations (NEC) of Cd to the clam *Potamocorbula laevis* under three scenarios of fluctuating salinity. Black curves: Cd concentration accumulated in clams (C_{int}); golden areas: instantaneous values of threshold internal concentration (C_{IT}). Others as in Figure 4.

which can be roughly estimated or more elaborately measured from life history observation.[36]

NECs were calculated to be 2.4, 2.0, and 3.1 $\mu g\ L^{-1}$ for the scenarios of fluctuating salinity simulated in this study and monitored at the two sites in Jiulong River Estuary (Figure 5). It should be noted that these NECs apply to survival but not necessarily to more subtle end points such as growth and reproduction. These values of NEC were comparable to Seawater Quality Standard of China (Grade I: 1 $\mu g\ L^{-1}$; grade II: 5 $\mu g\ L^{-1}$), but lower than the criteria continuous concentration (7.9 $\mu g\ L^{-1}$) of Environmental Protection Agency of the United States.[37] In addition to the precautionary nature of NEC, NEC varies within a much narrower range than that of LC_{50}, regardless of the pattern of salinity fluctuation. These attributes of NEC suggest its usefulness in deriving water quality criteria, especially for estuarine waters.

3.5. Implications. The dual effects of salinity on Cd toxicity differ in mechanisms (chemical vs physiological) and trends (monotonic vs nonmonotonic); therefore, they should be and can be separately modeled when predicting Cd toxicity in salinity-fluctuating waters. Water chemistry effects on Cd bioaccumulation were modeled using the BLM; physiological

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effects on organism intrinsic sensitivity were modeled by relating TD parameters to salinity based on osmotic stress. While BLM is more frequently used in freshwaters and for modeling toxicity directly, we used it as a TK model, based on which toxicity was subsequently modeled, making BLM kinetic, more flexible, and better suited to estuarine waters.

The BLM-based TK−TD model provides an efficient scheme to derive effect concentrations (EC_x) and NECs of Cd for salinity-fluctuating waters. This framework can also be readily applicable to predicting risks of other metals. For comparison, we list below several other methods we consider inefficient or incorrect:

(1) Deriving EC_x and NECs by conducting toxicity tests under salinity-fluctuating conditions. Toxicity is dependent on salinity fluctuation amplitudes and patterns, which vary among sites, making extrapolation of the derived EC_x and NECs difficult. Nonetheless, such tests are useful for model calibration or validation.

(2) Deriving EC_x and NECs under several stable salinities, and then select the worst-scenario values for water quality management. Such a precautionary approach may lead to over-protection and inefficient use of resources.

(3) Deriving EC_x and NECs under an average salinity. Under fluctuating salinities, the toxicity threshold C_{IT} varies with salinity almost instantly (Figure 5). In contrast, Cd bioaccumulation C_{int} responds much more steadily due to the cumulative nature of tissue Cd and the long biological half-life of Cd (i.e., 18 d). As a result, C_{int} under fluctuating salinities can be predicted by assuming an "average" salinity (see Figure 3), but C_{IT} cannot. Using average salinity would underestimate risks of Cd in salinity-fluctuating estuarine waters.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c06644.

Map of study area; experimental setup; weight of clams; Cd speciation at different salinities; relationship between C_{IT}(or k_k) and salinity; field monitored salinity; model calibration in OpenModel; and R code for calculating LC_{50} and NEC (PDF)

R code for calculating LC_{50} and NEC (TXT)

Data used for model calibration in OpenModel (XLSX)

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Notes

The authors declare no competing financial interest.

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Supporting information to:

Predicting risks of cadmium toxicity in salinity-fluctuating estuarine waters using the toxicokinetic-toxicodynamic model

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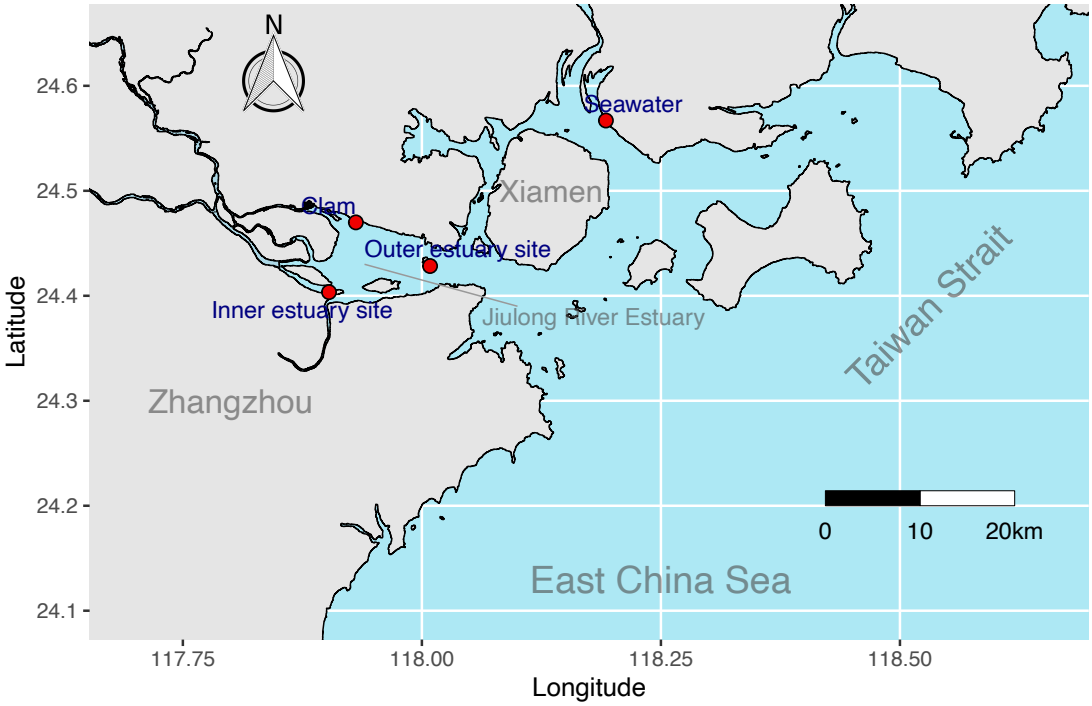


Figure S1 Map of the sites, where the clams (*Potamocorbula laevis*) and the seawater were collected, and two stations, where salinity fluctuation was monitored. See Figure S5 for the monitored salinity.

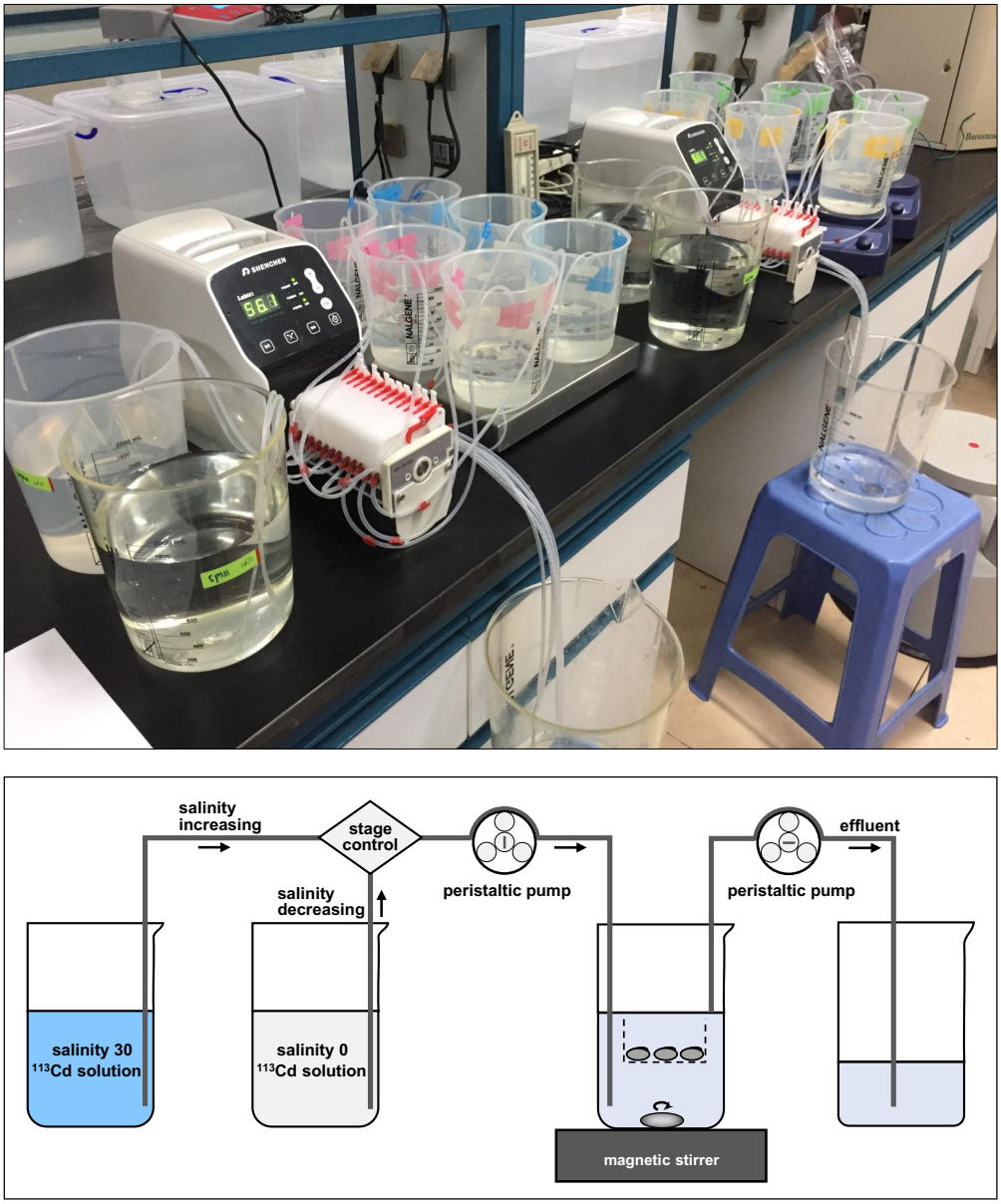


Figure S2 Experimental setup for investigating Cd exposure under fluctuating salinities.

Clams were placed in a perforated polypropylene box, which was immersed in 600 mL of exposure solution. The exposure solution was continuously mixed using a magnetic stirrer. Clams were initially exposed at salinity 5. Exposure solution of salinity 30 was peristaltically pumped into the exposure beaker at the flow rate of 2.68 mL min^{-1}. Salinity in the exposure beaker, thereby, gradually increased from 5 to 30 during a period of 6 h. After that, the influent was switched to exposure solution of salinity 0. Salinity in the exposure beaker then gradually decreased from 30 to 5 during the second period of 6 h. Switching influent salinity between 0 and 30 occurred every 6 h, creating salinity fluctuation simulating that of a semidiurnal tide. Salinity in the exposure beaker was intensively checked (see Figure 1-3 for the measured salinity). The volume of exposure solution was fixed at 600 mL, which was achieved by placing the effluent tubing at the 600-mL mark and setting the effluent flow rate slightly higher than 2.68 mL min^{-1}.

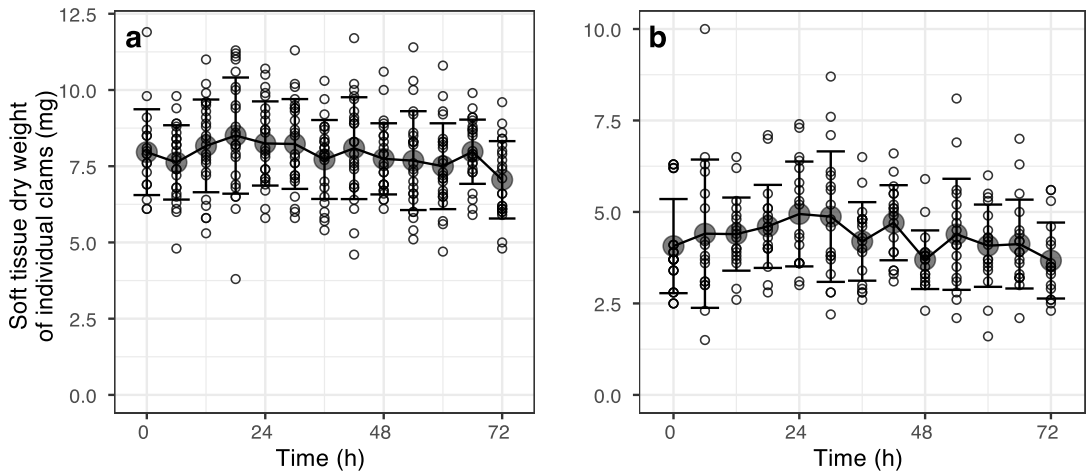


Figure S3 Soft tissue dry weight of individual clams (*Potamocorbula laevis*). (**a**) The clams used in the experiments of Figure 1 (Cd = 2 μg L^{-1}, constant and fluctuating salinities); (**b**) the clams used in the experiments of Figure 3 (Cd = 2, 20, and 200 μg L^{-1}, fluctuating salinities). Open circle: individual measurement; filled circle and error bar: mean ± standard deviation.

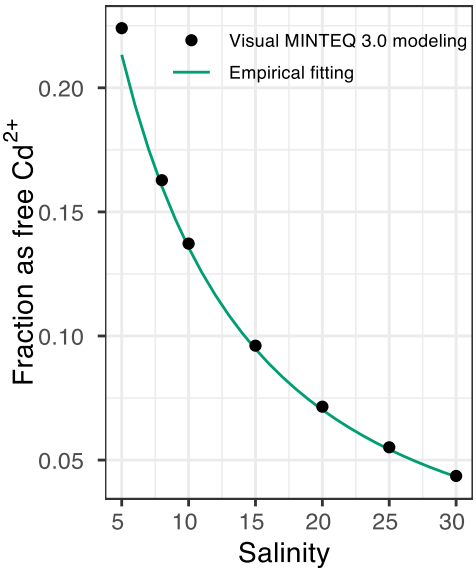


Figure S4 Fraction of dissolved Cd as free Cd^{2+} (*f*) at different salinities. Points are values calculated using Visual MINTEQ 3.0 (https://vminteq.lwr.kth.se) following the methods described by Tan et al. (2019) (reference 9); the curve is an empirical fitting of the calculated values. The empirical equation is $f = 1/(0.009993 \times \mathrm{Sal}^2 + 0.3867 \times \mathrm{Sal} + 2.5048)$. As the Cd concentrations (e.g., 2–500 μg L^{-1}) are around 6 orders of magnitude lower than the dominant complexing ligands Cl^-, *f* is stable across different Cd concentrations.

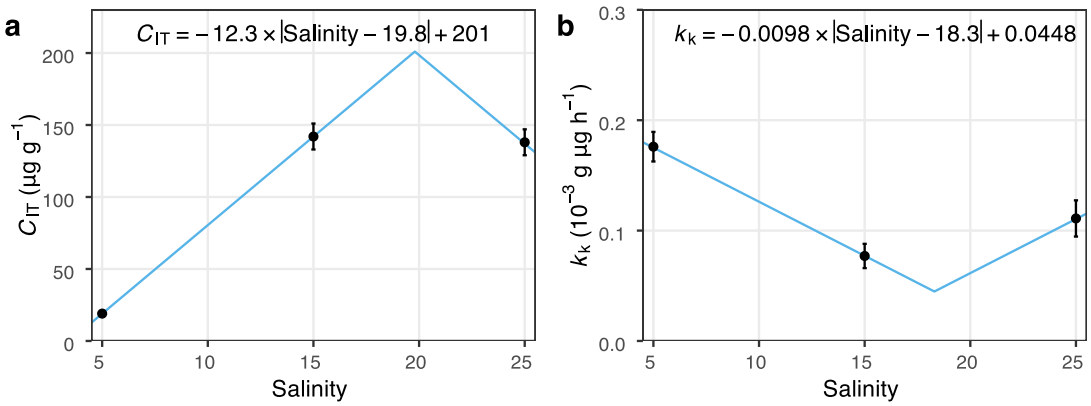


Figure S5 Relationship between (**a**) internal threshold concentration (C_{IT}), (**b**) killing rate (k_k), and salinity. Points are measured values (mean ± standard deviation); curves are fitted values (see equations in the figure).

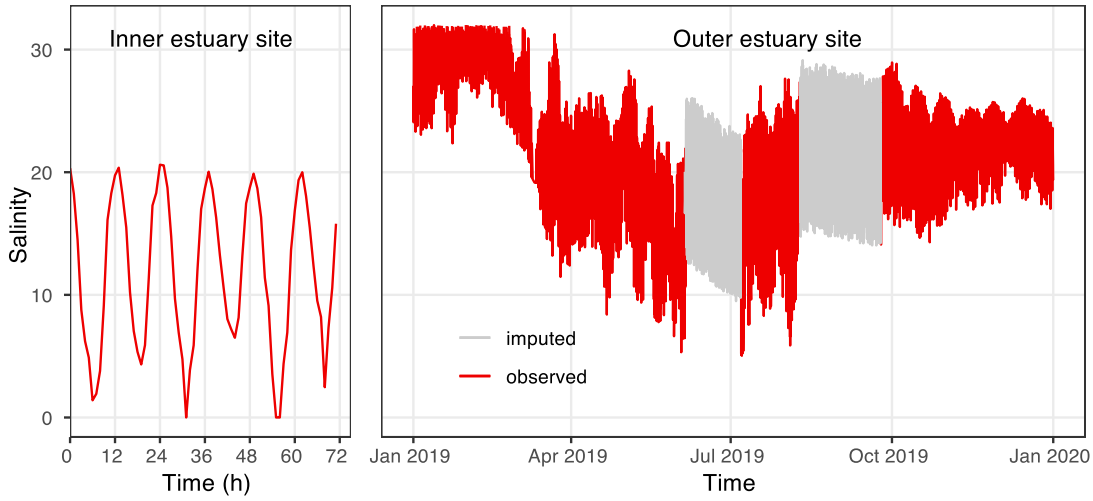


Figure S6 Fluctuation of salinity at two sites in Jiulong River Estuary. At the outer estuary site, missing data were imputed by an average of the random interpolation and linear interpolation. At site the inner estuary site, the salinity pattern was repeated for model calculation where duration was longer than 3 d. Salinity was set to 5 when below 5 for the calculation of LC_{50} and NEC (see Figures 4 and 5) considering the salinity range to which the model was calibrated and the salinity range of estuarine waters where the clam *Potamocorbula laevis* inhabits.

Note S1: Calibration of TK-TD Model in the Software OpenModel

Below is a brief introduction of the procedures and information needed to calibrate the TK-TD model in the software OpenModel (version 2.4.2). The model equations were represented by the "main code"; model parameters were optimized by fitting the equations to the observed data that input to the software through "data sheets". Marquardt algorithm and default settings of the software were used for parameter estimation. Detailed information on how to use of the software, including definition of "merit function", conducting "parameter estimation", and so on is available in the manual "OpenModel User Guide" accompanying the software. The complete model file (OMMLx format) can be obtained by contacting the corresponding author Qiao-Guo Tan (email: tanqg@xmu.edu.cn). OpenModel is developed and maintained by Neil Crout at the University of Nottingham, and can be downloaded from http://openmodel.info for free.

1. Main code

The "main code" is the model equations written in OpenModel scripts. Each code chunk describes Cd bioaccumulation at a combination of Cd level and salinity scenario.

```
//---------- Salinity in water -------------------------
salinity = watersalinity.salinity(t)

//fraction of free Cd2+ ion in dissolved Cd is a function of salinity
f = 1/(0.009993*salinity^2+0.3867*salinity+2.5048)

//========== Results shown in Figure 1 ==================
//---------- Cd = 2 µg/L, constant salinity = 5 ---------
Cd2_5psu = water.Cd2_5psu(t)
f5 = 1/(0.009993*5^2+0.3867*5+2.5048)
Cint2_5psu.rate = f5*Cd2_5psu/(a+b*Cd2_5psu*f5+c*5)-ke*Cint2_5psu

//---------- Cd = 2 µg/L, constant salinity = 15 --------
Cd2_15psu = water.Cd2_15psu(t)
f15 = 1/(0.009993*15^2+0.3867*15+2.5048)
```

```
Cint2_15psu.rate = f15*Cd2_15psu/(a+b*Cd2_15psu*f15+c*15)-
ke*Cint2_15psu

//---------- Cd = 2 µg/L, constant salinity = 25 --------
Cd2_25psu = water.Cd2_25psu(t)
f25 = 1/(0.009993*25^2+0.3867*25+2.5048)
Cint2_25psu.rate = f25*Cd2_25psu/(a+b*Cd2_25psu*f25+c*25)-
ke*Cint2_25psu

//---------- Cd = 2 µg/L, fluctuating salinity 5-25------
Cd2_fpsu = water.Cd2_fpsu(t)
Cint2_fpsu.rate = f*Cd2_fpsu/(a+b*Cd2_fpsu*f+c*salinity)-
ke*Cint2_fpsu

//========== Results shown in Figure 3 ==========
//---------- Cd = 2 µg/L, fluctuating salinity 5-25------
Cd2 = water.Cd2(t)
Cint2.rate = Cd2*f/(a+b*Cd2*f+c*salinity)-ke*Cint2
//---------- Cd = 20 µg/L, fluctuating salinity 5-25-----
Cd20 = water.Cd20(t)
Cint20.rate = Cd20*f/(a+b*Cd20*f+c*salinity)-ke*Cint20

//---------- Cd = 200 µg/L, fluctuating salinity 5-25----
Cd200 = water.Cd200(t)
Cint200.rate = Cd200*f/(a+b*Cd200*f+c*salinity)-ke*Cint200

//========== Results shown in Figure 2 ==========
//---------- Cd = 500 µg/L, constant salinity = 5 -------
Cd500_5psu = water_Cd500.5psu(t)
Cint500_5psu.rate = Cd500_5psu*f5/(a+b*Cd500_5psu*f5+c*5)-
ke*Cint500_5psu
//---------- Cd = 500 µg/L, constant salinity = 15 ------
Cd500_15psu = water_Cd500.15psu(t)
Cint500_15psu.rate = Cd500_15psu*f15/(a+b*Cd500_15psu*f15+c*15)-
ke*Cint500_15psu

//---------- Cd = 500 µg/L, constant salinity = 25 ------
Cd500_25psu = water_Cd500.25psu(t)
Cint500_25psu.rate = Cd500_25psu*f25/(a+b*Cd500_25psu*f25+c*25)-
ke*Cint500_25psu
//---------- Cd = 500 µg/L, fluctuating salinity 5-25----------
Cd500_fpsu = water_Cd500.fpsu(t)
Cint500_fpsu.rate = Cd500_fpsu*f/(a+b*Cd500_fpsu*f+c*salinity)-
ke*Cint500_fpsu
```

2. Symbols defined in the model

In the "main code" presented above, three types of symbols are defined, including variables, ordinary differential equations (ODEs), and parameters. Variable values are either provided (observed) or calculated; values of ODE variables are calculated through integration; parameter values are estimated by fitting the model to the observed data.

Symbol	Description	Unit	Experimental Condition
Variables			
Cd2_5psu	Measured dissolved Cd concentration	μg L^{-1}	Cd = 2 μg L^{-1}, salinity = 5
Cd2_15psu			Cd = 2 μg L^{-1}, salinity = 15
Cd2_25psu			Cd = 2 μg L^{-1}, salinity = 25
Cd2_fpsu			Cd = 2 μg L^{-1}, fluctuating salinity = 5−25
Cd500_5psu			Cd = 500 μg L^{-1}, salinity = 5
Cd500_15psu			Cd = 500 μg L^{-1}, salinity = 15
Cd500_25psu			Cd = 500 μg L^{-1}, salinity = 25
Cd500_fpsu			Cd = 500 μg L^{-1}, fluctuating salinity = 5−25
Cd2			Cd = 2 μg L^{-1}, fluctuating salinity = 5−25
Cd20			Cd = 20 μg L^{-1}, fluctuating salinity = 5−25
Cd200			Cd = 200 μg L^{-1}, fluctuating salinity = 5−25
salinity	Salinity	dimensionless	All experiments
f	Fraction of free Cd^{2+} ion	dimensionless	All fluctuating salinity conditions
f5			Salinity = 5
f15			Salinity = 15
f25			Salinity = 25
ODEs			
Cint2_5psu	Cd concentration in organisms	μg g^{-1}	Cd = 2 μg L^{-1}, salinity = 5
Cint2_15psu			Cd = 2 μg L^{-1}, salinity = 15
Cint2_25psu			Cd = 2 μg L^{-1}, salinity = 25
Cint2_fpsu			Cd = 2 μg L^{-1}, fluctuating salinity = 5−25
Cint500_5psu			Cd = 500 μg L^{-1}, salinity = 5
Cint500_15psu			Cd = 500 μg L^{-1}, salinity = 15
Cint500_25psu			Cd = 500 μg L^{-1}, salinity = 25
Cint500_fpsu			Cd = 500 μg L^{-1}, fluctuating salinity = 5−25
Cint2			Cd = 2 μg L^{-1}, fluctuating salinity = 5−25
Cint20			Cd = 20 μg L^{-1}, fluctuating salinity = 5−25
Cint200			Cd = 200 μg L^{-1}, fluctuating salinity = 5−25
Parameters			
a	$1/(J_{max} \cdot K_{CdBL})$	see eq. 8	All experiments
b	$1/J_{max}$		
c	$K_{SalBL}/(K_{CdBL} \cdot J_{max})$		
ke	Cd elimination rate constant	h^{-1}	

3. Data Sheets

Water salinity, measured dissolved Cd concentrations, and Cd concentrations in organisms were input to the model through "data sheets". Model parameters were estimated by fitting the calculated Cd bioaccumulation to the measured Cd bioaccumulation. The data contained in "data sheets" are provided in the Excel file of the Supporting Information.

Name of data sheet	Description	Function
watersalinity	Salinity at different timepoints	Providing values for variable salinity
water	Measured dissolved Cd concentration	Providing values for variables Cd2_5psu, Cd2_15psu, Cd2_25psu, Cd2_fpsu, Cd2, Cd20, Cd200
water_Cd500		Providing values for variables Cd500_5psu, Cd500_15psu, Cd500_25psu, Cd500_fpsu
Cint	Measured Cd concentration in organisms	For fitting the following ODEs: Cint2_5psu, Cint2_15psu, Cint2_25psu, Cint2_fpsu, Cint2, Cint20, Cint200
Cint_Cd500		For fitting the following ODEs: Cint500_5psu, Cint500_15psu, Cint500_25psu, Cint500_fpsu

Note S2: R code for calculating median lethal concentrations (LC_{50}) and no-effect concentrations (NEC) of Cd using the BLM-based TK-TD model

Below we use the fluctuating salinity data simulated in this study (see Figure 1a.4) as an example to show the procedures to calculate LC_{50}s and NECs. It should be noted that in real estuarine waters, salinity fluctuates daily and annually in much more complex patterns. The simple pattern used here is for method demonstration; however, this method can be applied to any salinity fluctuation scenarios.

The R code for calculation is in blue font.

A text version of the R code is available in the other file of the Supporting Information.

1. Calculate LC_{50}

1.1 Load packages

```
library(ggplot2) # package for plotting
library(deSolve) # package for solving differential equations
```

1.2 Load salinity data

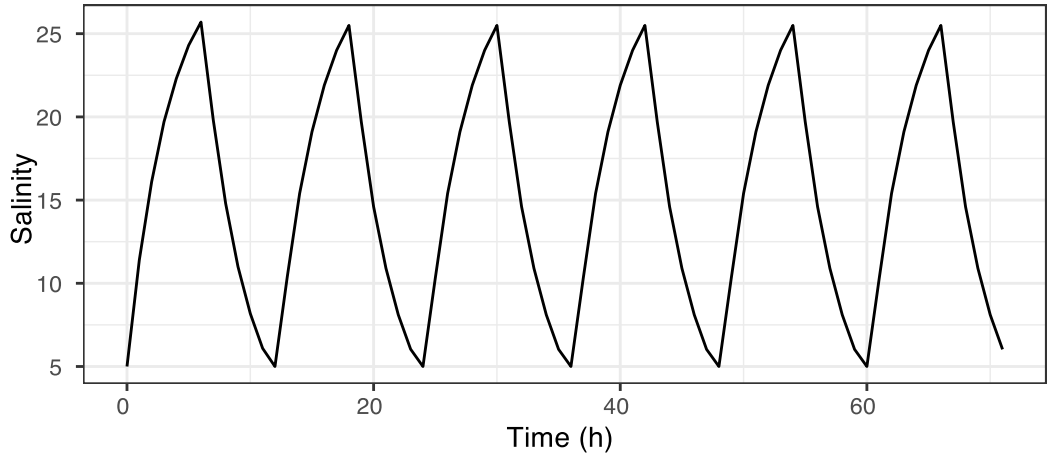
```
salinity_data <- data.frame(Time = c(0:71), salinity =
c(5,11.4,16.1,19.7,22.3,24.3,25.7,19.8,14.8,11.0,8.18,6.09,5,10.4,15
.4,19.1,21.9,24,25.5,19.7,14.6,10.9,8.11,6.04,5,10.3,15.4,19.1,21.9,
24,25.5,19.7,14.6,10.9,8.11,6.03,5,10.3,15.4,19.1,21.9,24,25.5,19.7,
14.6,10.9,8.11,6.03,5,10.3,15.4,19.1,21.9,24,25.5,19.7,14.6,10.9,8.1
1,6.03,5,10.3,15.4,19.1,21.9,24,25.5,19.7,14.6,10.9,8.11,6.03))
```

1.3 View the salinity data

```
head(salinity_data) # view the head of the data frame
```

```
##   Time salinity
## 1    0      5.0
## 2    1     11.4
## 3    2     16.1
## 4    3     19.7
## 5    4     22.3
## 6    5     24.3
```

```
# plot the salinity data
ggplot(salinity_data) +
  theme_bw() +
  geom_line(aes(Time, salinity)) +
  labs(x = "Time (h)",
       y = "Salinity")
```



1.4 Repeat the salinity data

The 3-d salinity data is repeated into the one-year data.

```
salinity_data <- rep(salinity_data$salinity,122)
```

1.5 Linear interpolation of salinity data to get a function of salinity vs. time:

```
salinity <- approxfun(x = seq(from=0,to=8783,by = 1), y =
salinity_data)
```

1.6 Set values for model parameters and stability constants

```
Jmax <- 203           # maximum uptake rate of Cd, μg g-1 h-1
K_CdBL <- 0.0005699      # stability constant for the binding of Cd2+
to uptake sites, (μg L-1)-1:
K_SalBL <- 0.0004763     # hypothetical stability constant for the
binding of major cations to uptake sites, dimensionless
ke <- 0.00159         # elimination rate constant of Cd, h-1
h0 <- 0               # background hazard rate, h-1
```

1.7 Set initial variable values

```
initial <- c(Cint = 0, Hazard = 0)
```

1.8 Define the toxicokinetic-toxicodynamic (TK-TD) model

```
TKTD <- function (t, y, parameters) {
    Cint <- y[1]
    Hazard <- y[2]
    Cd_water <- parameters[1]
    Sal <- salinity(t)
    f <- 1/(0.009993*Sal^2 + 0.3867*Sal + 2.5048)
    Jin <- (Jmax*K_CdBL*f*Cd_water)/(1 + K_CdBL*f*Cd_water +
K_SalBL*Sal)
    dCint<- Jin-ke*Cint
    kk <- 0.0098*abs(Sal - 18.3) + 0.0448
    CIT <- -12.3*abs(Sal - 19.8) + 201
    dHazard<- kk/1000*max(Cint - CIT,0) + h0
    list(c(dCint, dHazard))
  }
```

1.9 Calculate LC_{50}

```
LC50 <- NULL # create empty vector for storing calculated values
```

1.9.1 Set initial values for optimization

```
parms0 <- c(Cd_water = 2000)
```

1.9.2 Calculate LC_{50} in 30 days

Here LC_{50} values are calculated for different exposure durations, i.e., 1 d to 30 d at a 1-d interval. This step may take several minutes.

```
for(i in 1:30){
    times <- seq(from = 0, to = i*24, by = 1)
    ssq = function(parms0){
      Cd_water <- parms0[1]
      out.fit <- ode(y = initial, times = times, func = TKTD, parms =
c(Cd_water)) # integrate TK-TD model to calculate Cd bioaccumulation
and organism survivorship
      n <- length(times)
      Survivorship_final <- exp(-out.fit[,3])[n]
      ssqres <- (Survivorship_final - 0.5)^2  # target of
optimization: survivorship = 50%, i.e., the definition of LC50
      return(ssqres)
    }
    fit.LC50 <- optim(par = parms0,fn = ssq) # optimize to calculate
LC50
    LC50[i] <- as.numeric(fit.LC50$par)
    parms0 <- c(Cd_water = LC50[i])
  }
```

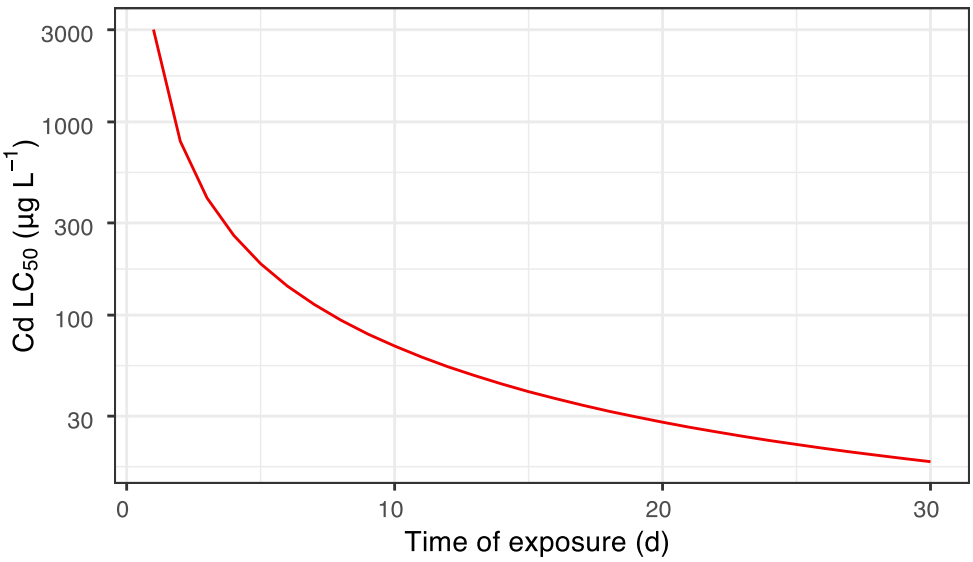
1.10 View the calculated LC_{50}

```
LC50_caculate <- data.frame(time = c(1:30),LC50 = LC50)

head(LC50_caculate) # view the head of the data frame

##   time      LC50
## 1    1 3007.03125
## 2    2  794.77833
## 3    3  403.71479
## 4    4  258.21582
## 5    5  184.49571
## 6    6  141.25453
```

```
# plot the calculated LC50
ggplot(data = LC50_caculate) +
  theme_bw() +
  geom_line(aes(time,LC50),color = "red2") +
  scale_y_log10() +
  labs(x= "Time of exposure (d)",
       y="Cd LC"[50]~"("*mu*g~L^{-1}*")")
```



2 Calculate NEC

2.1 Set initial values

```
parms0 <- c(Cd_water = 0.5)
initial <- c(Cint = 0)
```

2.2 Calculate the internal threshold concentration (C_{IT}) as a function of time

```
times <- seq(from = 0,to = 8783,by = 1)
CIT <- approxfun(x = times,
                 y = -12.3*abs(salinity_data - 19.8) + 201)
```

2.3 Define the toxicokinetic (TK) model to simulate Cd bioaccumulation

```
TK = function (t, y, parameters) {
  Cint <- y
  Cd_water <- parameters[1]
  Sal <- salinity(t)
  f <- 1/(0.009993*Sal^2 + 0.3867*Sal + 2.5048)
  Jin <- (Jmax*K_CdBL*f*Cd_water)/(1 + K_CdBL*f*Cd_water +
K_SalBL*Sal)
  dCint<- Jin-ke*Cint
  list(c(dCint))
}
```

2.4 Define the function to calculate NEC

```
ssq = function(parms0){
  Cd_water <- parms0[1]
  out <- ode(y = initial, times = times, func = TK, parms =
c(Cd_water)) # integrate the TK model to calculate Cd Cint
  Cint <- out[,2]
  CIT <- CIT(times)
  ssqres <- (max(Cint - CIT, na.rm = T) - 0)^2 # target of
optimization: maximum Cint = CIT, i.e., Cint ≤ CIT
  return(ssqres)
}
```

2.5 Calculate NEC

This step may take around 1 minute.

```
NEC <- optim(par = parms0,fn = ssq) # optimize to calculate NEC
Cd_water <- NEC$par
NEC$par

## Cd_water
## 2.390625
```

2.6 Calculate Cd bioaccumulation when exposed to Cd concentration of NEC

```
params <- c(Cd_water = NEC$par)
# integrate to calculate Cd bioaccumulated
d <- ode(y = initial, times = times, func = TK, parms = params)
CIT <- CIT(times)
data_NEC <- data.frame(d,CIT)
```

2.7 Plot the comparison of Cint and CIT when exposed to Cd concentration of NEC

```
ggplot(data_NEC) +
  theme_bw() +
  geom_line(aes(time/24, CIT), alpha = 0.3, color = "red2") +
  geom_line(aes(time/24, Cint)) +
  scale_y_continuous(trans = "log10") +
  labs(x = "Time (d)",
       y = expression("Cd concentration in organisms"~("*mu*g~g^{-1}
  *")")) +
  annotate(geom = "text", x = 160, y = 0.8,
           label = expression(italic(C)[int]<=italic(C)[IT]~""%=>%""
  ~bold(NEC==2.4~mu*g~L^{-1})))+
  annotate(geom = "label", x = 170, y = 70, label = expression("Inter
  nal threshold concentration:"~italic(C)[IT]), color = "red3", alpha
   = 0.7, size = 4)+
  annotate(geom = "text", x = 175, y = 3.2, label = expression("Cd co
  ncentration in organisms:"~italic(C)[int]), size = 4)+
  geom_segment(aes(x = 20, y = 10, xend = 55, yend = 4.5), size = 0.2)
```