Supporting information to:

Making acute tests more ecologically relevant: cadmium bioaccumulation and toxicity in an estuarine clam under various salinities modeled in a toxicokinetic-toxicodynamic framework

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Material and Methods

2.1 Organisms and materials

In the laboratory, the clams were fed with the green algae *Chlorella* sp. and the seawater was refreshed every day. Seawater was collected from a clean site in Tong'an Bay (24.566944° N, 118.192472° E), Fujian Province, China. Seawater used in experiments was firstly filtered through a glass fiber filter (Whatman GF/C) and then through a 0.22-μm polypropylene cartridge filter (Calyx Capsule).

2.2 Cd bioaccumulation

At the beginning of exposure and each subsequent time point, two clams from each replicate were sacrificed for measuring Cd concentrations. The two clams were separately measured and the results were averaged for data analysis. Water samples were also collected for monitoring Cd concentrations in the exposure seawater. Specifically, 3 mL of water was sampled from each of the three replicated beakers; the three replicate water samples were pooled into one sample, which was then acidified and preserved by adding 90 μL of "1+1" HNO_3 (i.e., one volume of concentrated nitric acid mixed with one volume of deionized water, ~7.3 mol L^{-1}).

When being sampled, the clams were rinsed immediately with 1 mmol L^{-1} of EDTA (pH 8) to stop the Cd biouptake and to remove surface adsorbed Cd. Soft tissue was dissected from each clam, further rinsed twice with 1 mmol L^{-1} EDTA and deionized water, placed separately into clean polyethylene ziplock bags, and frozen at −20 ºC. The clams were subsequently freeze dried, weighted, and digested using concentrated HNO_3 at 80 ºC for about 8 h. The standard reference material (SRM 1566b, oyster tissue) was also digested following the same procedures.

Table S1. List of experiments, including their objectives, experimental conditions, and the related table and figures.

No.	Objective	Duration	Salinity	Nominal Cd (μg L^{-1})	Measured Cd	Results
1	Cd uptake and elimination kinetics at a *low* Cd concentration	12 h (uptake) & 300 h (depuration)	5, 8, 10, 15, 20, 25, 30	5	Table S2	Fig. 1, Fig. 2
2	Cd uptake rate at a *high* Cd concentration	12 h	5, 8, 10, 15, 20, 25, 30	550	Table S3	Fig. 2, Fig. S5
3	Cd uptake rate at a range of Cd concentrations	12 h	5, 15, 25	1, 3, 10, 50, 200, 1000	Table S4	Fig. 5, Fig. S6
4	Cd toxicity at a high Cd concentration	100 h	5, 8, 10, 15, 20, 25, 30	550	Table S5	Fig. 3, Fig. 4

Table S2. Measured concentrations of ^{113}Cd (μg L^{-1}) in the exposure seawater of different salinities used in the accumulation experiment (No. 1 in Table S1).

Time	Salinity						
(h)	5	8	10	15	20	25	30
0	4.9	4.6	4.4	4.3	4.6	4.4	4.2
3	3.0	3.1	3.3	3.3	3.7	3.6	3.6
6	2.3	2.5	2.7	2.9	3.3	3.4	3.5
9	1.9	2.2	2.5	2.8	3.1	3.3	3.3
12	1.7	2.0	2.4	2.5	2.9	3.2	3.2

Table S3. Measured concentrations of ^{113}Cd (μg L^{-1}) in the exposure seawater of different salinities used in the accumulation experiment (No. 2 in Table S1).

Time	Salinity						
(h)	5	8	10	15	20	25	30
0	556	549	544	549	544	542	537
3	529	528	523	530	529	534	525
6	512	519	513	526	555	533	525
9	511	506	513	524	530	530	529
12	508	515	514	528	533	528	525

Table S4. The measured Cd concentrations in the exposure seawater for determination Cd uptake rate at a range of Cd concentrations and different salinities (No. 3 in Table S1).

Salinity 5						
Time (h)	Nominal Cd (μg L^{-1})					
	1	3	10	50	200	1000
0	1.02	2.8	10.3	53	211	1087
6	0.80	2.3	8.5	44	181	977
12	0.75	2.2	8.1	42	175	962

Salinity 15						
Time (h)	Nominal Cd (μg L^{-1})					
	1	3	10	50	200	1000
0	1.00	3.0	10.2	52	208	1090
6	0.87	2.7	9.4	47	189	1029
12	0.82	2.5	9.0	46	188	1028

Salinity 25						
Time (h)	Nominal Cd (μg L^{-1})					
	1	3	10	50	200	1000
0	0.83	2.7	10.2	51	213	1080
6	0.76	2.5	9.7	49	203	1058
12	0.68	2.6	9.6	49	202	1048

Table S5. Measured concentrations of Cd (μg L^{-1}) in the exposure seawater of different salinities used in toxicity tests (No. 4 in Table S1).

Time	Salinity						
(h)	5	8	10	15	20	25	30
0	522	532	528	529	530	526	531
24	392	412	426	446	473	477	473
48	367	386	390	416	439	453	463
48*	530	525	526	529	526	524	522
88	513	490	468	480	491	504	496
100	n.d.	n.d.	468	472	488	502	498

* Exposure solution refreshed at this time point; n.d.: not determined.

Table S6. Standard values of Cd listed in "Sea water quality standard (GB 3097—1997)" of China for different environmental function areas.

Sea water quality standard	Applicable to environmental function areas	Standard value of Cd (μg L^{-1})
Grade I	Sea fishery waters, marine nature reserves and the protected areas for rare and endangered marine species.	1
Grade II	Aquaculture area, sea bath, sea sports or entertainment areas where people have direct exposure to sea water; industrial water in direct relation to human food.	5
Grade III & IV	General industrial water areas and costal scenic spots; Waters of port and marine development areas.	10

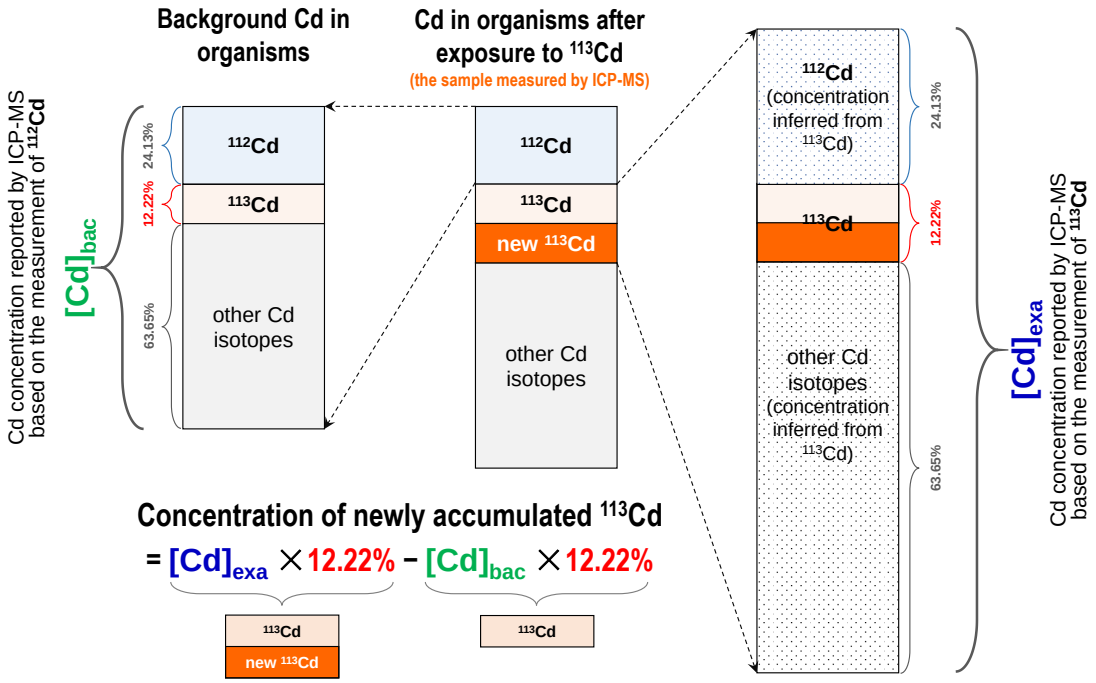

Figure S1. Explanation on how to analyze the ICP-MS data to calculate the concentration of newly bioaccumulated ^{113}Cd.

Cd has eight naturally occurring stable isotopes (e.g., ^{112}Cd, ^{113}Cd). In the Cd bioaccumulation experiments, clams were exposed only to the isotope ^{113}Cd and no other Cd isotopes. The ^{113}Cd detected in the clams had two origins: one was that newly accumulated from the exposure seawater, the other was that originally existed (or the background ^{113}Cd). ICP-MS (Agilent 7700x) detects signals of specific Cd isotopes while reports concentration of the total Cd instead of that specific Cd isotope. ICP-MS calculates total Cd concentration by dividing the isotope concentration with its abundance. In our experiment, ICP-MS was calibrated with a "normal" Cd solution (Agilent, part number 5183e4688), which has natural abundance of isotopes. Therefore, if a sample has natural abundance of Cd isotopes, measuring any isotope would lead to the same reported total Cd concentration. However, if a sample was enriched with an isotope (Figure S1, middle panel), for example ^{113}Cd, measuring ^{113}Cd would exaggerate the real total Cd concentration (Figure S1, right panel); whereas measuring other isotopes (e.g., ^{112}Cd) would underestimate the real total Cd concentration (Fig. S1, left panel). We use this difference to calculate the concentration of newly accumulated ^{113}Cd.

In Figure S1, total Cd concentrations reported by measuring ^{112}Cd and ^{113}Cd were denoted as $[Cd]_{bac}$ and $[Cd]_{exa}$, respectively. $[Cd]_{bac}$ was the concentration of background Cd; *background* ^{113}Cd thus was "$[Cd]_{bac} \times 12.22\%$". $[Cd]_{exa}$ was the exaggerated Cd concentration; *total* ^{113}Cd thus was "$[Cd]_{exa} \times 12.22\%$". The concentration of newly accumulated ^{113}Cd was the difference between the *total* and *background* ^{113}Cd.

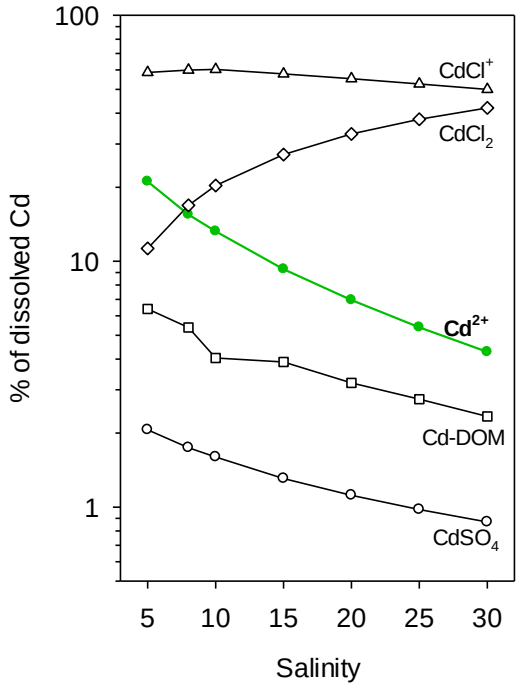

Figure S2. The species distribution of Cd at different salinities. Cd speciation was calculated in the software Visual MINTEQ 3.0 (https://vminteq.lwr.kth.se). Cd concentration was assumed to be 5 μg L^{-1} for the calculation. Temperature and pH were set to 22 ^{o}C and 8.0, respectively. The concentrations of major ions (e.g., Na^{+}, Cl^{-}, K^{+}, Ca^{2+}, Mg^{2+}, SO_4^{2-}) were estimated according to the constituents of surface seawater of salinity 35 (Pilson, 2013, there Table 4.1). The Stockholm Humic Model (SHM) was selected for modeling complexation effects of the dissolved organic matter (DOM). Default settings for DOM were used, except that: (1) concentration (mg L^{-1}) ratio of active DOM to dissolved organic carbon (DOC) was set to 2; (2) 100% of active DOM was assumed to be fulvic acid. Measured concentrations of DOC were used for the calculation. See Figure S3 for the measured DOC concentrations at different salinities.

Pilson, M.E.Q. *An Introduction to the Chemistry of the Sea*. Second ed. 2013: Cambridge University Press.

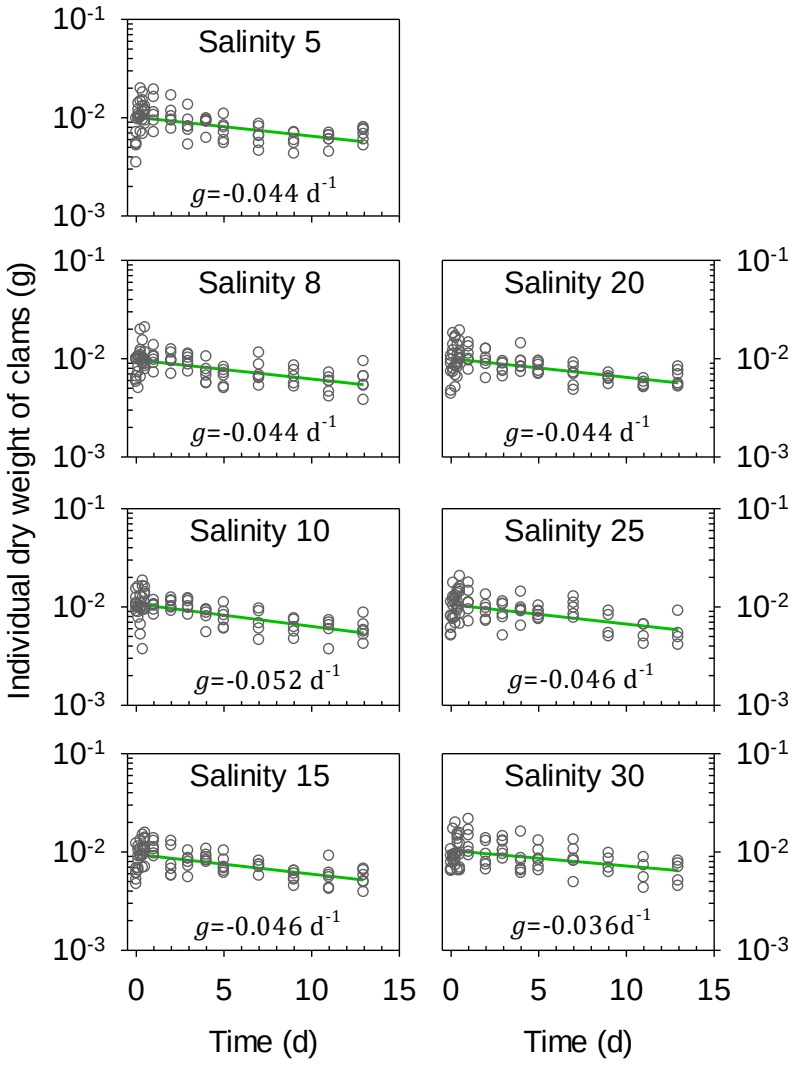

Figure S3. The dry weight of soft tissues of individual clams during the uptake-depuration experiment (experiment No. 1 listed in Table S1). An exponential growth model, $y(t) = y_0 \cdot e^{g \cdot t}$, was fitted to the data. Negative growth (i.e., loss of weight) was observed in all cases; the growth rate constant (g, d^{-1}) ranged from − 0.036 d^{-1} to − 0.052 d^{-1}.

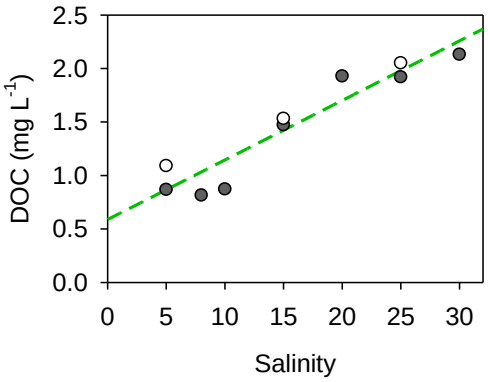

Figure S4. Concentration of dissolved organic carbon (DOC, mg L^{-1}) in the exposure seawater of different salinities used in the present study. Seawater of different salinities used in this study was prepared by diluting salinity-30 seawater with deionized water, leading a decrease of DOC from ~2 to ~1 mg C L^{-1}. The complexation of Cd by dissolved organic matter (DOM) is weak when compared to other metals (e.g., Cu, Hg, Ag). In our calculation, only 2.3% to 6.4% of Cd was complexed by the DOM; the percentage of complexation decreased with increasing salinity (Figure S3). This weak complexation effects should have not substantially confounded the trend of the salinity effects.

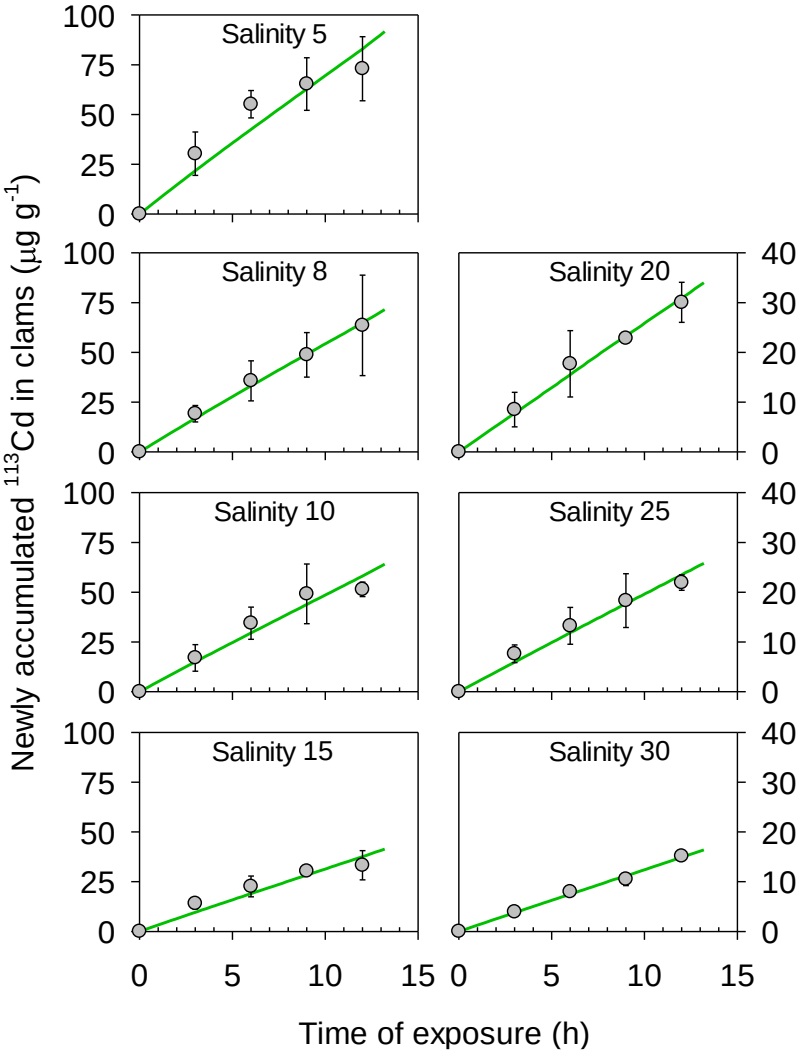

Figure S5. The accumulation of ^{113}Cd in the calm *P. laevis* exposed to a high Cd concentration (nominal 550 μg L^{-1}) at different salinities (experiment No. 2 listed in Table S1). The points are measured values (mean ± standard deviation, n = 3) and the curves are model fits. An efflux rate constant (k_e) of 0.0382 d^{-1} was assumed for the fitting.

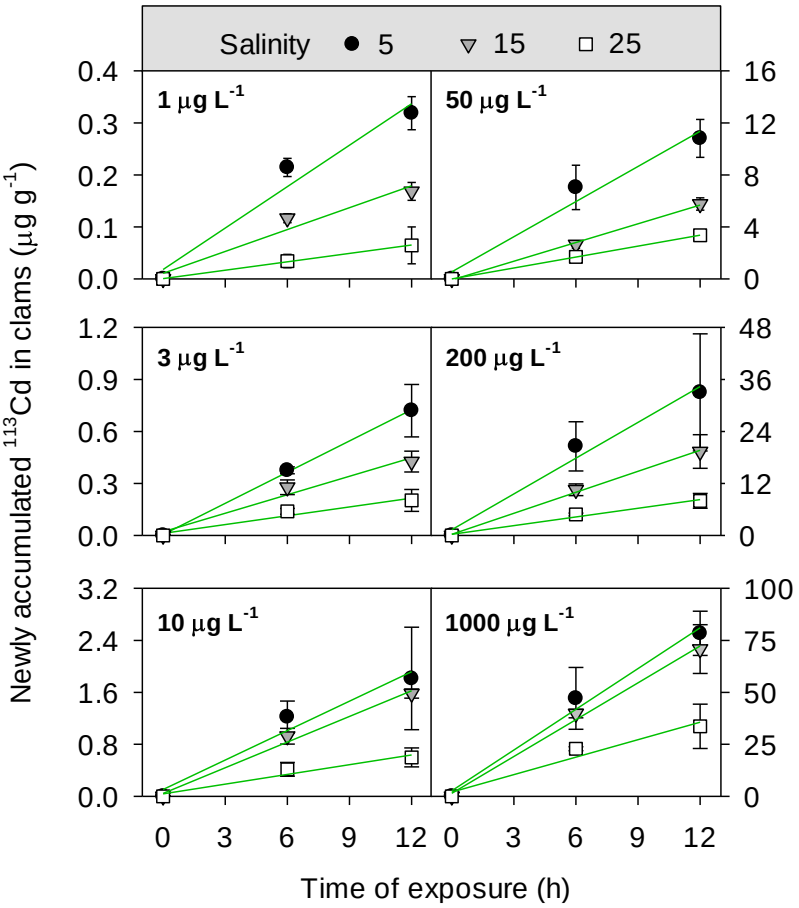

Figure S6. The accumulation of ^{113}Cd in the clam *P. laevis* during the 12-h exposure to different ^{113}Cd concentrations (nominal: 1-1000 μg L^{-1}) at different salinities (i.e., 5, 15 and 25) (experiment No. 3 listed in Table S1). Others as Figure S5.

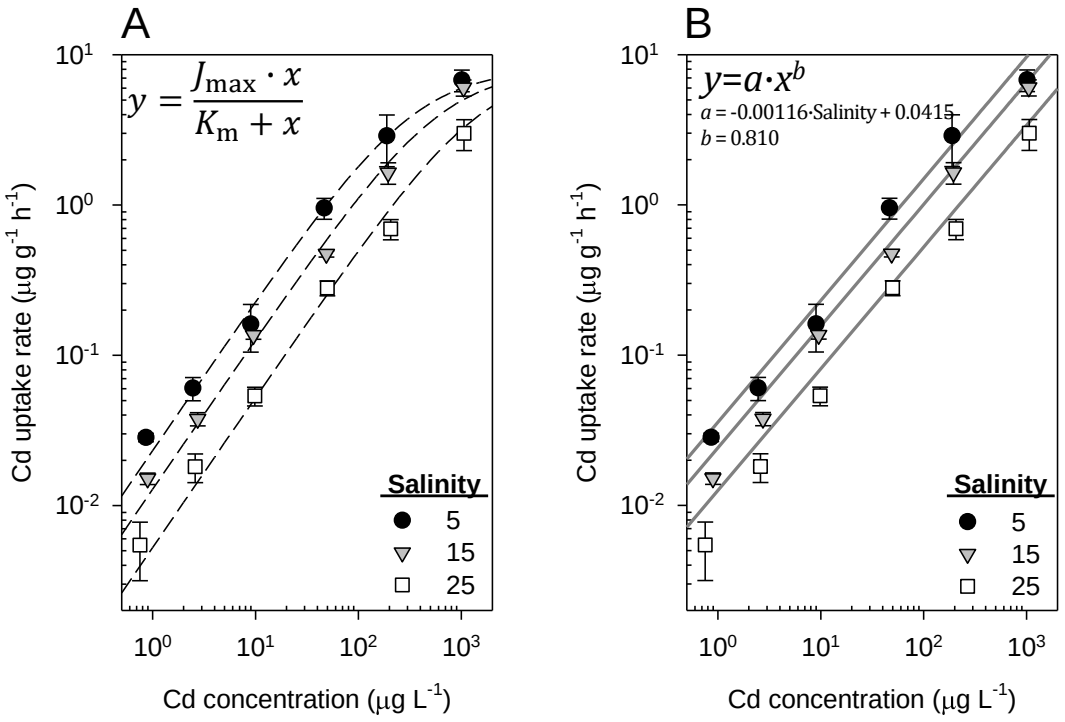

Figure S7. The relationship between Cd uptake rates in the clam *P. laevis* and Cd concentration in seawater. The uptake rates were measured at three different salinities (i.e., 5, 15, and 25). The relationship was described with Michaelis-Menten equations (A) or power functions (B).

A: The same J_{max} was assumed for different salinities in the Michaelis-Menten fitting; J_{max} = 8.07 ± 1.78 μg g^{-1} h^{-1}; K_m was 348 ± 94 μg L^{-1}, 629 ± 168 μg L^{-1}, and 1539 ± 407 μg L^{-1} for salinity 5, 15, and 25, respectively.

B: The coefficients a and b were updated over those of Figure 3 for better prediction of survivorship in the toxicity tests (Figure 4): b = 0.810; $a = -0.00116 \cdot \text{Salinity} + 0.0415$.