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## **Effect of food type on the bioaccumulation and depuration of cadmium in the pacific cupped oyster, *Crassostrea gigas***

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**Key words:** food type; cadmium; ICP-MS; kinetics; *Crassostrea gigas*

### **Abstract**

Cadmium (Cd) is commonly detected in aquatic environment and can accumulate in maricultural organisms. Modification of the food may change the bio-kinetics of trace metals in organisms. The aim of this study was to understand the effects of food type on the uptake and elimination of Cd in different tissues of oyster *Crassostrea gigas*. Oyster groups feeding with different food types were exposed to 10 µg/L Cd for 28 days, and then followed by a depuration of 35 days. One group was added natural seawater rich in algae, the other three groups were fed once daily with *Dicrateria inornata*, *Nitzschia closterium f.minutissima* and mixed alga (*Dicrateria inornata*: *Nitzschia closterium f.minutissima*=1:1), respectively. Oysters were sampled for chemical analysis by inductively coupled plasma mass spectrometry (ICP-MS). The mean concentrations of Cd among different oyster tissue were followed the order as: digestive gland > mantle > adductor muscle. A two-compartment model was used to estimate Cd uptake rates ( $k_1$ ), depuration rates ( $k_2$ ), bioconcentration factor (BCF), and half-lives ( $t_{1/2}$ ) in the three tissues, showing  $k_1$  was considerably higher than  $k_2$ , therefore, the organism have high ability to accumulate Cd. The  $k_1$  in the digestive gland was the highest, followed by mantle and adductor muscle, while the  $k_2$  in the mantle was the lowest. In the present study, the group fed with mixed algae showed the fastest depuration rate and the shortest  $t_{1/2}$  in the important edible part of the oysters (digestive gland), which helps to improve food safety.

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## Introduction

Oyster is one of the most commercially important marine shellfish species for aquaculture in China. The Pacific cupped oyster, *Crassostrea gigas* is a very important maricultural species for abundant nutrition, good taste, rapid growth, and high economic benefits. With the rapid development of economy of coastal area, a great proportion of metal compounds are released into aquatic systems, which might enter aquatic food chains posing long-term risks for aquatic organisms (Mendil et al. 2010). Among the metals, cadmium (Cd) is of concern because it can enter organism through food intake, which inducing cancer in humans (Benavidaes et al. 2005; Nordberg 2010). Human activities, such as, industrial, agricultural and economic development, along the coast, resulted in cadmium concentration of 0.01-42 µg/L in the ocean (Soaress et al. 2008). The spatial-temporal trends of Cd content in oyster soft tissues from 17 main Guangdong coastal bays/ estuaries from 1989 to 2010, results of 268 samples showing the concentrations of Cd varied between not detected and 10.80 µg/g wet weight with average of 1.14 µg/g wet weight. 98.64% of the samples did not exceed the threshold of upper levels (4 µg/g) established by Ministry of Agriculture, China and EPA, USA (Wang et al 2012).

Oysters easily accumulate Cd because they are filter feeders and their mobility is poor. For instance, experimental studies of Cd in diploid and triploid oysters revealed that metal bioaccumulation presented a 20- and 23-fold increase (Marie et al. 2006). It is necessary to study the bioaccumulation and purification of Cd in the aquaculture industry since shellfish can get contaminated for human consumers.

Oysters can eliminate Cd to some extent, but the progress is quite slow and the nutritive value decreases because of metabolism, which is unfeasible in real production unless appropriate purification means are taken to speed up the process. In recent years, studies have been reported for the ecological purification of heavy metals in oysters. Most of the studies mainly focused on the addition of chelating agents, Vitamin C, and chitosan (Cheng 2004; Xie et al. 2013; Zhang et al. 2015). Although there is some feasibility theoretically, limitations existed in practical production, such as high costs or damage to water quality. The practicality should be considered in metal purification. Adjusting the food type of the organisms can accelerate the elimination of heavy metal. For bivalves, single-celled algae such as flattened algae, chlorella and algal spores are more suitable (Wang et al. 2015). The cell wall of these algae is consisted of protein, polysaccharides and lipids, and certain amount of negative charge, which provides functional groups as following: carboxyl, hydroxyl, mercaptan, imidazole, and phosphate radical. These functional groups can combine with metal ions, and increase the vitality of shellfish (Zhou et al. 2006). For example, feeding algae was helpful to the depuration of Cu, Pb, and Cd (Li 2008); the increased salinity and the addition of algae was benefit to the elimination of heavy metal (Cheng 2004).

The present study aimed to evaluate the accumulation and purification of Cd in tissues of *C. gigas* under different food types. In this way, the uptake and depuration kinetics of cadmium were experimentally evaluated by employing a two-compartment model.

## Materials and Methods

### *Ethics statement*

All animal experiments were conducted in accordance with the guidelines and approval of the respective Animal Research and Ethics Committees of China. The field studies did not involve endangered or protected species.

### *Oyster and rearing conditions*

Pacific oysters (average 32.67±3.16 mm in shell height, 90.83±8.49 mm in shell length, 38.19±4.26mm in shell width, 91.33 ±11.34 g in body weight) were obtained from Rizhao, Shandong province, China (March 2017). Prior to the experiment, the oysters were acclimated to laboratory conditions for 2 weeks in fully aerated water tanks (70 oysters per 150-liter tank). One group was added natural seawater rich in natural algae, the other three groups were fed once daily with *Dicrateria inornata*, *Nitzschia closterium f.minutissima* and mixed alga (*Dicrateria inornata*:*Nitzschia closterium f.minutissima*=1:1), respectively. The concentration of microalgae given to the oyster after each feeding was close to 2.5\*10<sup>6</sup> cells/mL in each tank.

### *Cadmium exposure and sample collection*

Cadmium CdCl<sub>2</sub> (cadmium chloride anhydrous, CAS: 7790-78-5) were used for conducting the heavy metal exposure experiment. After 2 weeks of acclimation, the contaminant cadmium (Cd) was introduced at 10 µg/L. The concentration of Cd was chosen based on the limit of cadmium in sea water according to GB 3097-1997 and within the dose range previously cited in the literature for laboratory metal exposure experiments (Jo et al 2008; Gueguen et al 2017).

The Cd exposure experiments were carried out in triplicate using 200 L PVC tanks containing 150 L of exposure media (sea water) and 70 individuals per test unit. The animals were randomly allocated into four feeding groups with three replicates. The food type were the same with those in acclimation period. Control conditions corresponded to 3 tanks with no contamination, the oysters were subjected to the same conditions as the exposed groups. Each day, control and experimental tanks were emptied. Each group of oyster were rinsed with non-spiked seawater and transferred into 3 corresponding clean "food tanks", filled with non-spiked seawater and fed for 2 h using the three kinds of food. During this time, control and exposure tanks were rinsed and filled with non-spiked seawater. After 2 h of equilibration, physical and chemical parameters were measured and seawaters were spiked with cadmium to nominal Cd exposure concentration. The accumulation phase lasted for 28 days. A depuration phase of 35 days followed. The temperature of the water was 23-25°C, the salinity of sea water was 30‰. The pH was approximately 7.9, and the oxygen concentration was >7 mg/L because of the aerator pump.

Tissues, including mantle, digestive gland and adductor muscle were carefully collected from three healthy oysters as parallel samples. Once removed from the tanks, the sampled individuals were rinsed in milli-Q water to remove any chemical residue from their body surface. Water samples of 50 mL were collected in polypropylene centrifuge tubes. Samples were taken 0.083, 7, 14, 21 and 28 days after the start of contamination (D0.083, D7, D14, D21 and D28). Contaminations were stopped at D29, and then the animals were transferred to clean medium for the depuration phase. Sampling was done at D35, D42, D49, D56 and D63 during the depuration phase. For each harvested animal, 1 g of each tissue was stored at -20°C. Dead animals were removed daily from the tanks. In order to assess the health status, the condition Index (CI) of the oysters was determined, and was calculated for each individual according to the equation (Strady et al., 2011):

$$CI = \text{Visceral Content (wet weight; g)/Shell (wet weight; g)} * 100$$

#### *Measurement of cadmium levels in tissues and water samples*

Oyster tissues and water cadmium analyses were conducted by ICP-MS using methods similar to those of Gaw et al. (2012) and McRae et al. (2016). Tissues (mantle, digestive gland and adductor muscle), was weighed and placed in a freeze drier for 1 week. 0.2 g freeze-dried tissue was then placed into acid washed polycarbonate vials. Tissues were then digested by adding 5 mL of 10% ultrapure HNO<sub>3</sub> and left for 24 h before refluxing at 85 °C for 1 h. Volumes were adjusted to 20 mL using MilliQ water. If necessary, samples were diluted using 2% ultrapure HNO<sub>3</sub> and placed in acid-washed test tubes to be analysed by ICP-MS (Agilent 7500cx). For quality assurance/quality control purpose, the standard oyster 1566b (National Institute of Standards and Technology) tissue samples were used as the reference and treated with the same procedures. SRM 1566b provided acceptable recovery rates (90%-110%). Data are presented at µg Cd per g dry-weight tissue. Detection limits for tissue analysis were calculated as three standard deviations of the mean blank concentration (0.01 µg/g).

Acidified and filtered water samples taken from the cadmium exposures were directly analysed by ICP-MS (Agilent 7500cx). As for tissue samples, QA/QC was achieved by using procedural blanks (Gaw et al., 2012). Detection limits for water analysis were calculated as three standard deviations of the mean blank concentration (0.01 µg/L).

#### *Bioconcentration factor measurement*

A two-compartment model was applied to the Cd accumulation data (Kahle and Zauke 2002). Model parameters were estimated simultaneously from the following equations, using non-linear iterative least square methods;

For uptake phase ( $0 < t < t^*$ , with  $t^*$  =time of uptake phase (day))

$$C_A = C_0 + C_w (1 - e^{-k_2 t}) \quad (0 < t < t^*)$$

For the clearance phase ( $t > t^*$ )

$$C_A = C_0 + C_w (e^{-k_2(t-t^*)} - e^{-k_2t}) \quad (t > t^*)$$

Where  $C_A$  is mean metal concentration in animals ( $\mu\text{g/g}$ );

$C_0$  is a constant ( $t=0$ );

$C_w$  is the mean measured metal exposure ( $\text{mg/L}$ );

$k_1$  is the rate constant for uptake phase (per day);

$k_2$  is the rate constant for clearance phase (per day).

These constants allow determination of the bioconcentration factor (BCF), because this equation considers that CB reaches a steady state. This way,  $t$  continues to increase ( $t \rightarrow \infty$ ) until  $e^{-k_2t} = 0$ ; in these conditions,  $C_A = k_1/k_2 \cdot C_w$ , in which  $\text{BCF} = C_A / C_w = k_1/k_2$ . Half-lives  $t_{1/2}$  were calculated as  $0.693/k_2$  (Niimi 1987)

### Statistical analysis

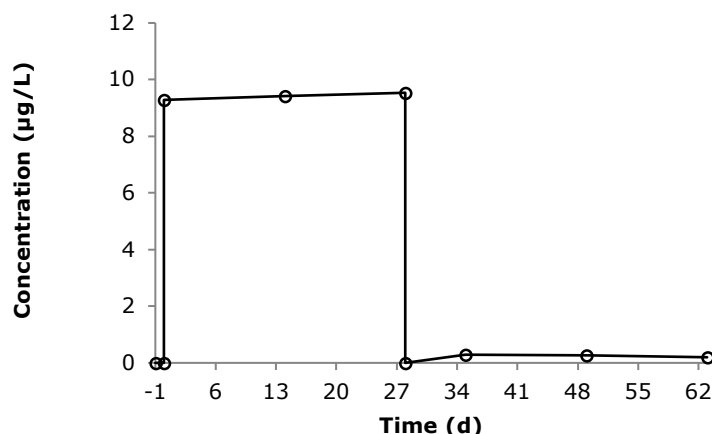
Results are presented as means  $\pm$  standard deviation. The data were subjected to one-way ANOVA. Duncan's test was used to determine significant differences between variables. A significance level of 0.05 was used for all statistical analysis (ie., a probability of  $p \leq 0.05$  was considered to be significant). All analyses were run in the software package SPSS 16.0 and Origin 8 for Windows.

## Results

After the exposure of cadmium, the average mortality of *C. gigas* in the natural seawater, *Dicrateria inornata*, *Nitzschia closterium f. minutissima* and mixed algae were 3.76%, 2.52%, 2.91% and 3.03%. The CI throughout the experiment was  $27.32 \pm 4.79$ , and there was no significant difference between conditions for each sampling period, which indicated the oysters were of good physiological state during the whole experiment (Geffard et al 2007). The experimental data for water concentration and the concentrations of Cd measured in mantle, digestive gland and adductor muscle of oysters are shown in **Figures 1-4**, and the modelling results are shown in figures 5-7. The background levels of Cd concentration in mantle, digestive gland and adductor muscle of oysters were  $0.75 \pm 0.07$ ,  $1.5 \pm 0.26$ , and  $0.45 \pm 0.06 \mu\text{g/g}$ .

### Cd measurement in water

The exposure concentration of Cd in water was kept stable during the accumulation and depuration phase, which were within 93 to 95% of the nominal values of  $10 \mu\text{g/L}$ . No significant variation was observed over time.



**Figure 1** Cadmium concentration in the water

### Cd bioconcentration in different tissues of oyster

The distribution of Cd was studied in digestive gland, mantle and adductor muscle (**Figures 2-4**). The accumulation of Cd rose dramatically until attaining maximum concentrations. After 28 days' accumulation, no subsequent decrease of Cd in *C. gigas* was observed. Accumulation of cadmium was the highest in the digestive gland of the oyster, followed by mantle and adductor muscle.

In digestive gland, the significant increase in the group fed with *Dicrateria inornata* occurred at day 21, where the concentration was  $5.40 \pm 1.31 \mu\text{g/g}$ . However, the significant increase in all the other three groups occurred at day 14, and the concentration was  $5.17 \pm 0.17 \mu\text{g/g}$  in the group with natural seawater,  $3.93 \pm 0.61 \mu\text{g/g}$  in the group fed with *Nitzschia closterium f. minutissima*, and  $3.80 \pm 0.85 \mu\text{g/g}$  in the group fed with mixed algae ( $P < 0.05$ ) (**Figure 2**).

In mantle, the significant increase occurred at day 14 in the group fed with *Dicrateria inornata*, where the concentration was  $2.63 \pm 0.47 \mu\text{g/g}$ ; while all occurred at day 21 in the group with natural seawater, fed with *Nitzschia closterium f. minutissima*, and fed with mixed algae, the corresponding concentration was  $3.57 \pm 0.64$ ,  $2.13 \pm 0.38$ , and  $3.30 \pm 1.08 \mu\text{g/g}$  ( $P < 0.05$ ) (**Figure 3**).

In the adductor muscle, the significant increase in the group fed with *Dicrateria inornata* occurred at day 28, where the concentration was  $1.80 \pm 0.52 \mu\text{g/g}$ . However, the significant increase in all the other three groups occurred at day 21, the concentration was  $1.19 \pm 0.22 \mu\text{g/g}$  in the group fed without food,  $1.00 \pm 0.54 \mu\text{g/g}$  in the group fed with *Nitzschia closterium f. minutissima*, and  $1.27 \pm 0.69 \mu\text{g/g}$  in the group fed with mixed algae ( $P < 0.05$ ) (**Figure 4**).

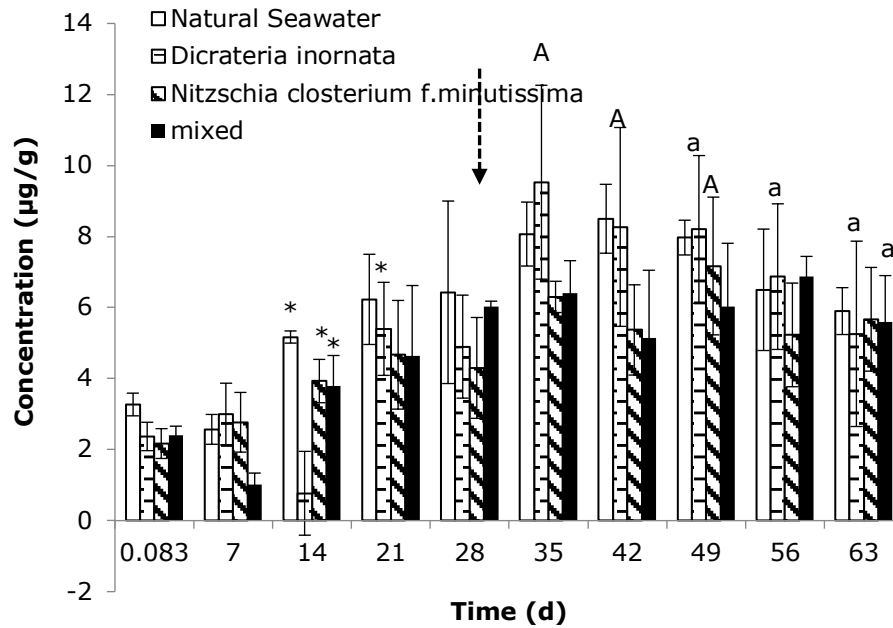
#### *Cd depuration in different tissues of oyster*

In the group with natural seawater, after the beginning of the depuration, mantle showed a significant increase between days 35 and 49 compared to day 28 ( $P < 0.05$ ), and followed by a significant decrease at day 63 ( $P < 0.05$ ). Digestive gland did not show significant changes during the depuration phase ( $P > 0.05$ ). Adductor muscle showed a significant increase at day 42 ( $P < 0.05$ ), and followed by a decrease ( $P > 0.05$ ).

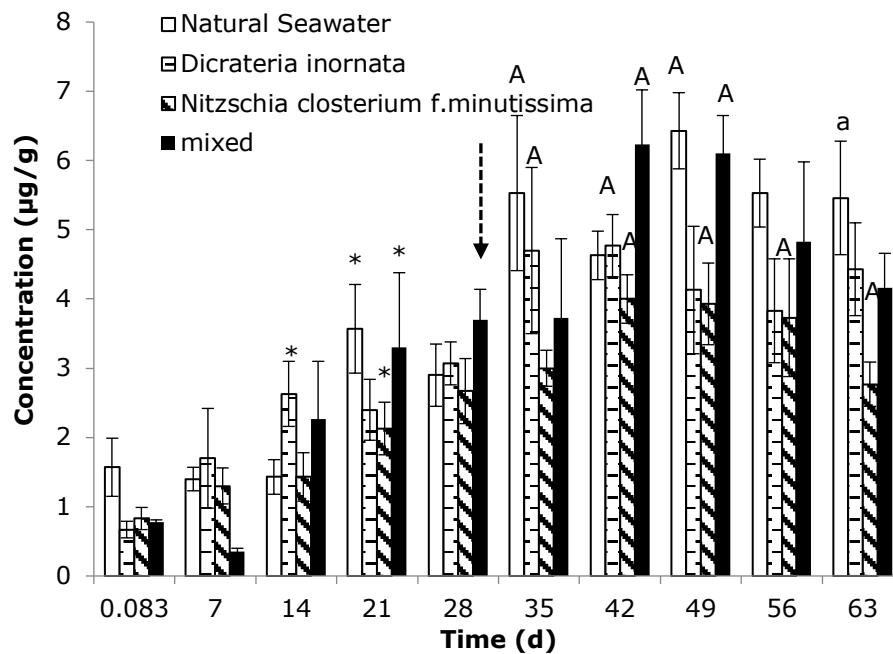
When oysters fed with *Dicrateria inornata*, mantle showed a significant increase between days 35 and 42 compared to day 28 ( $P < 0.05$ ), and followed by a decrease ( $P > 0.05$ ). Digestive gland showed a significant increase at day 35 compared to day 28 ( $P < 0.05$ ), and followed by a decrease ( $P > 0.05$ ). Adductor muscle showed an increase at day 42 ( $P > 0.05$ ), and followed by a significant decrease ( $P < 0.05$ ).

When oysters fed with *Nitzschia closterium f. minutissima*, mantle showed a significant increase between days 42 and 63 compared to day 28 ( $P < 0.05$ ). Digestive gland showed a significant increase at day 49 compared to day 28 ( $P < 0.05$ ), and followed by a decrease ( $P > 0.05$ ). Adductor muscle showed a significant increase at day 42 compared to day 28 ( $P < 0.05$ ), and followed by a sharp decrease at day 63, but it was not significant ( $P > 0.05$ ).

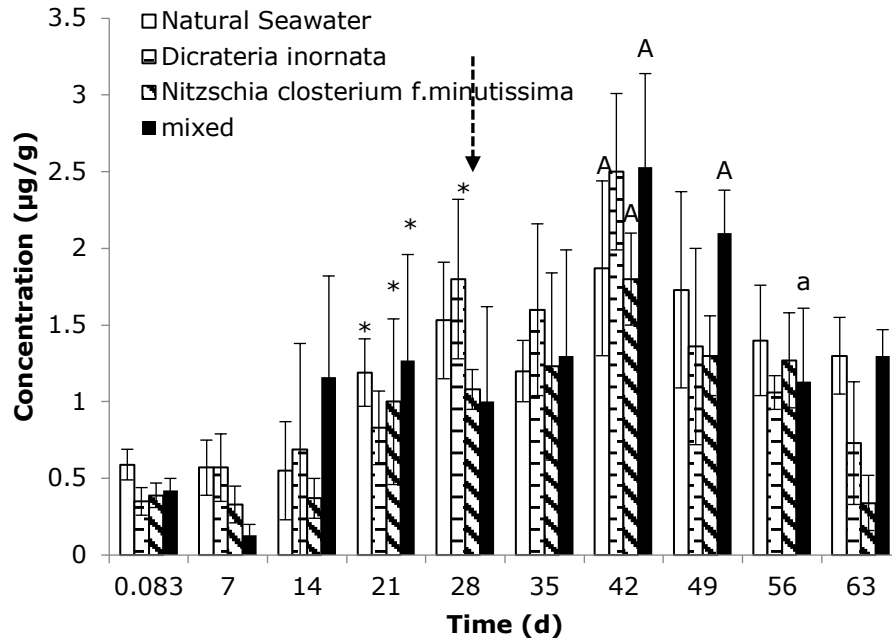
In the group fed with mixed algae, mantle showed a significant increase between days 42 and 49 compared to day 28 ( $P < 0.05$ ), and followed by a decrease ( $P > 0.05$ ). Digestive gland did not show significant changes between days 35 and 56 ( $P > 0.05$ ), then a significant decrease occurred ( $P < 0.05$ ). Adductor muscle showed a significant increase between days 42 and 49 compared to day 28 ( $P < 0.05$ ), and followed by a significant decrease at day 56 ( $P < 0.05$ ).



**Figure 2** Cadmium concentration in *C. gigas* at different food type in digestive gland. Arrows represent the end of the contamination phase and the beginning of the decontamination phase (D28). Values are the means  $\pm$  standard deviation (SD) ( $n = 3$ ). Asterisks represent significant differences ( $P < 0.05$ ) between the same experimental groups in the contamination phase when compared to day 0.083. Uppercase letters represent significant differences ( $P < 0.05$ ) between the same experimental groups in the decontamination phase when compared to day 28. Lowercase letters represent significant differences ( $P < 0.05$ ) between the same experimental groups when compared to the peak value.



**Figure 3** Cadmium concentration in *C. gigas* at different food type in the mantle. Arrows represent the end of the contamination phase and the beginning of the decontamination phase (D28). Values are the means  $\pm$  standard deviation (SD) ( $n = 3$ ). Asterisks represent significant differences ( $P < 0.05$ ) between the same experimental groups in the contamination phase when compared to day 0.083. Uppercase letters represent significant differences ( $P < 0.05$ ) between the same experimental groups in the decontamination phase when compared to day 28. Lowercase letters represent significant differences ( $P < 0.05$ ) between the same experimental groups when compared to the peak value.



**Figure 4** Cadmium concentration in *C. gigas* at different food type in the adductor muscle. Arrows represent the end of the contamination phase and the beginning of the decontamination phase (D28). Values are the means  $\pm$  standard deviation (SD) ( $n = 3$ ). Asterisks represent significant differences ( $P < 0.05$ ) between the same experimental groups in the contamination phase when compared to day 0.083. Uppercase letters represent significant differences ( $P < 0.05$ ) between the same experimental groups in the decontamination phase when compared to day 28. Lowercase letters represent significant differences ( $P < 0.05$ ) between the same experimental groups when compared to the peak value.

The accumulation and depuration kinetics parameters were shown in **Table 1**. In digestive gland, uptake rate constant  $k_1$  was higher in control groups compared to groups fed with algae, but there was no difference between groups fed with *Dicrateria inornata* or *Nitzschia closterium f. minutissima*. Depuration rate constant  $k_2$  was highest in the group fed with mixed algae, but there was no difference between groups fed with groups fed with no algae or *Nitzschia closterium f. minutissima*. The BCF value in the group fed with *Dicrateria inornata* was highest, which was 3472. The time needed for the organisms to eliminate half of the amount of cadmium was 20.63 d in the group fed with mixed algae, which was shorter than the  $t_{1/2}$  in other groups.

In mantle,  $k_1$  was lowest in the group fed with *Nitzschia closterium f. minutissima*.  $k_2$  was lowest in the group fed with no algae, while there was no difference between groups fed with *Dicrateria inornata* or *Nitzschia closterium f. minutissima*. The BCF value in the group fed with no algae was highest, which was 3234. The time needed for the organisms to eliminate half of the amount of cadmium was 85.34 d in the group fed with *Dicrateria inornata*, which was shorter than the  $t_{1/2}$  in other groups.

In adductor muscle,  $k_1$  and  $k_2$  were both lowest in the group fed with no algae. The BCF value was much lower than that in digestive gland and mantle. The  $t_{1/2}$  was lowest in the group fed with *Dicrateria inornata*, which was shorter than that in other groups.



**Table 1** The accumulation and elimination kinetic parameters of Cd<sup>2+</sup> under different food type in oyster

Tissues	Food	k <sub>1</sub>	k <sub>2</sub>	r <sup>2</sup>	BCF	t <sub>1/2</sub>
Digestive gland	natural seawater	26.60	0.023	0.955	1156	30.12
	<i>Dicrateria inornata</i>	17.95	0.005	0.751	3472	134.0
	<i>Nitzschia closterium</i> f. <i>minutissima</i>	17.23	0.023	0.884	758.1	30.49
	mixed	20.12	0.034	0.834	599.1	20.63
Mantle	natural seawater	11.32	0.004	0.888	3234	198.0
	<i>Dicrateria inornata</i>	10.95	0.008	0.930	1348	85.34
	<i>Nitzschia closterium</i> f. <i>minutissima</i>	7.858	0.006	0.932	1305	115.1
	mixed	12.26	0.006	0.879	2122	119.9
Adductor muscle	natural seawater	3.584	0.011	0.830	323.2	62.49
	<i>Dicrateria inornata</i>	6.212	0.038	0.672	162.1	18.09
	<i>Nitzschia closterium</i> f. <i>minutissima</i>	3.591	0.028	0.570	126.4	24.40
	mixed	4.421	0.016	0.638	281.9	44.20

Note: k<sub>1</sub> is the rate constant for uptake phase (per day); k<sub>2</sub> is the rate constant for clearance phase (per day); BCF corresponds to k<sub>1</sub>/k<sub>2</sub>; t<sub>1/2</sub> were calculated as 0.693/k<sub>2</sub>; r<sup>2</sup> is the coefficients

### Discussion

In organisms, the main target organ for accumulation of Cd was digestive gland, because it is the principal interphases among the animal, food, and water. In the present study, the accumulation of Cd in the digestive gland greatly increased, up to 2-fold of that in mantle. This is in agreement with the results of a study conducted previously (Hindarti et al. 2000). The uptake rates in digestive gland was also approximately 2 times of those in mantle, and 5 times of those in adductor muscle.

Another primary tissue of oysters that take in metal contaminants is mantle (Luo et al. 2014). It contacts with the external environments directly and provides a large number of blood vessels, suggesting that it is the major barrier between the environment, hemolymph and internal organs. In present study, the Cd concentration in mantle was average 1.97 µg/g after 14 days' accumulation, which was 19.7 µg/g if we converted the metal concentrations to a dry weight basis using a wet to dry weight ratio of oysters (8-10) measured in the present study. It is also the case in previous study (Liu and Wang 2016). In *Mytilus edulis* after 15 days accumulation, the Cd concentration in mantle was also achieved a relative high concentration of 22 µg/g dry weight, which showed that mantle could be a useful supplemental biological tissue for exposure to toxicants.

Cd concentration in adductor muscle was relative low, and did not exceed the 2 µg/g safety limit at most sampling points. The reason for this is possibly that its physical property is quite solid and then limits the entry of metals.

Experimental data were fitted to a two-compartment model. Coefficients (r<sup>2</sup>) of determination for most groups were higher than 0.8, indicating a good fitting to the model (**Table 1**).

In all the three tissues, the peak concentrations of Cd achieved at D42 or D49, which should occurred at ≤ D28 theoretically. It is inferred that some Cd was reabsorption or transformed after being metabolized by oysters. Although Cd concentration decreased during the depuration phase, the tissues of *C. gigas* were unable to eliminate the accumulated metal completely (**Table 1**). This was also found in the previous study in *C. gigas* (Geffard et al. 2002) (t<sub>1/2</sub>=137 d), and the case in other species, such as *C. virginica* (t<sub>1/2</sub>=70 d) (Roesijadi 1994), *M. edulis* (t<sub>1/2</sub>= 120 d and t<sub>1/2</sub>= 300 d) (Viarengo 1989; Ebianno and Langston 1993).

The uptake rate of Cd at different food type in the *C. gigas* (7.858-12.26 /d in mantle; 17.23-26.60 /d in digestive gland; 3.584-6.212 /d in adductor muscle) was comparable to those observed in other oysters, i.e., 7.8-20.256 /d in *Crassostrea rivularis* (Zhou et al 2012); 19.572-23.364 /d in *Ostrea plicatula Gmelin* (Li 2008). In other marine bivalves, the uptake rates were also observed, i.e., 32 /d in *Ruditapes decussatus* (Serafim and Bebianno 2007); 7.318-13.63 /d in *Tegillarca granosa* (Li 2008).

In the present study, the depuration rates at different food type ranged from 0.004 to 0.008 /d in mantle and 0.005 to 0.034 /d in digestive gland, and 0.011-0.038 /d in adductor muscle.

Similar depuration rates were reported in other oysters, i.e., 0.007 /d in *Crassostrea rivularis* (Zhou et al 2012); 0.0103-0.0110 /d in *Ostrea plicatula Gmelin* (Li 2008). In other marine bivalves, there was also a lower depuration rate, i.e., 0.005 /d in *Ruditapes decussatus* (Serafim and Bebianno 2007); 0.0101-0.0109 /d in *Tegillarca granosa* (Li 2008).

The BCF measured in *C.gigas* at different salinities were more comparable in the range of 1305-3234 in the mantle, 599.1-3472 in the digestive gland, and 126.4-323.2 in the adductor muscle. Similar BCF were found in other oysters and marine bivalves, i.e., 1365.6715-2788.7511 in *Crassostrea rivularis* (Zhou et al 2012); 1783.7-2336.4 in *Ostrea plicatula Gmelin* (Li 2008); 724-1873 in *Tegillarca granosa* (Li 2008).

It followed a different accumulation and elimination pattern in different tissues after different food type. Digestive gland is the main part of accumulating and holding metals. The digestive gland is an important edible part in oysters. In the present study, the group fed with mixed algae showed the fastest depuration rate and the shortest  $t_{1/2}$  in the digestive gland, which can effectively speed up the excretion of cadmium to improve food safety. Previous study has reported that the composition and phytoplankton species present in the food may be important aspects affecting depuration of heavy metals (Li 2008). For instance, feeding *Platymonas* sp is beneficial for the depuration of Pb, feeding algae mixed with *Platymonas* sp and *Chlorella* sp is beneficial for the depuration of Cd, and algae species showed no effect for the depuration of Cu. Cadmium was lost mainly through feces, and an increased food ingestion rate would likely result in an increase in cadmium egestion rate (Pavlaki et al. 2017). Variations of algae species may result in differences of enzyme activities, detoxification mechanisms and nutrients maldistribution in shellfish (Chen et al. 2013; Qiu. 2015). Vitamin C in algae can promote the production of reduced glutathione from oxidized glutathione in shellfish, which is beneficial for binding with metal ions and excreting (He et al. 2014). In green algae, Cd-binding proteins were isolated from the cell lysates of Cd-treated *Chlorella*, which were capable of binding 40-50% of intracellular cadmium. The formation of Cd-binding proteins made Cd nonpoisonous, and cannot be absorbed by organisms until being eliminated. Except for the special cell wall structure (Zhou et al. 2006), which made the algae adsorb Cd, the formation of Cd-binding proteins was also regarded as the biochemical mechanism (Yang et al. 1985).

### Conclusion

The present study elaborated the accumulation and elimination of cadmium kinetics at different food type. Two-compartment model is a useful tool for estimating the behavior of Cd in oyster aquaculture. Highest concentration occurred in digestive gland, followed by mantle and adductor muscle. Tissues have a strong ability to bind Cd, and eliminate Cd more slowly. It showed a different accumulation and elimination pattern in different tissues.

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