JACOBS PUBLISHERS

OPEN ACCESS

Jacobs Journal of Aquaculture and Research

Research Article

Effects of Salinity and Algal Diets on the Elimination of Cadmium in *Crassostrea rivularis*

Ni Wu, Zhenjv Li, Jieru Cao, Tao Niu, Huasheng Cheng, Tianjiu Jiang*

Research Center for Harmful Algae and Marine Biology, Key Laboratory of Eutrophication and Red Tide Prevention of Guangdong Higher Education Institutes, Jinan University

**Corresponding author: Dr. Tianjiu Jiang, No.601, Huangpu Road (west), Guangzhou, China 510632, Tel: +0086-20-85223112; Email: tjiangtj@163.com*

Received: 06-02-2015

Accepted: 06-30-2015

Published: 07-22-2015

Abstract

The present study aimed to study the effects of salinity (12, 16, 20, 24, and 28) and algal diets (*Platymonas subcordiformis*, *Chlorella* sp. and a mixture of *P. subcordiformis* and *Chlorella* sp.) on the elimination of cadmium (Cd) from oysters (*Crassostrea rivularis*). The Cd accumulation in oysters was also studied. The results showed that the accumulation of Cd in *C. rivularis* was related to the exposure time ($P<0.01$). The elimination rate of Cd from *C. rivularis* increased with increasing salinity. The highest Cd elimination rate of 0.0183 μg/(g·d) was found in the highest salinity group, and 47.6% of the Cd in oysters was removed after 31 days. Compared to oysters fed the single diet, the oysters fed the mixed algal diet exhibited the highest elimination rate of 0.0166 μg/(g·d), and 43.8% of the Cd in oysters was removed after 31 days.

Keywords: Cadmium; Elimination; *Crassostrea rivularis*; Algal Diets; Salinity

Introduction

Oysters are a popular food, and oyster cultures are economically important in the coastal areas of many countries. Metal pollution has been occurring in many developed and developing countries for decades. Oysters are disposed to accumulate metals [1,2], especially cadmium [3-5]. The accumulation of toxic trace elements by shellfish is a worldwide problem that has been discussed for at least the last century [6,7]. The consumption of heavy metal-contaminated oysters may result in chronic and acute toxicity in humans [8]. The consumption of oysters is limited to 5 μg Cd/g dry weight in Europe [9]. In China, the safety standard of Cd in shellfish is less than 4.0 mg Cd/kg wet weight (NY5154-2008, 2008) [10]. To minimize health risks, one of the acceptable methods for purifying contaminated oysters suggested by the National Shellfish Sanitation Program (NSSP) in the USA [11] is transferring the shellfish into a clean environment to remove heavy metals from the shellfish prior to harvest and consumption. However, previous studies on heavy metals eliminated from oysters focused on natural biological cleansing, which transplanted organisms from contaminated areas to conditionally approved waters [12-15]. However, the method has shortcomings, such as longer depuration periods (lasting several months)

and a higher oyster mortality rate [12], which is not wholly appropriate for the industrialized depuration of heavy metals from oysters. Moreover, at present, the depuration of shellfish is mainly targeted toward the pathogenic microorganism [16,17]. No effective faster methods for metal depuration from shellfish have been reported to date. Saed et al. [18] reported that the depuration of metals under laboratory conditions is significantly faster than that achieved in the field. There have been many reports on salinity affecting the communication of metals in shellfish [19-24], but fewer studies have investigated the effects of the elimination of heavy metals, particularly on the effects of algal diets on the elimination of metals.

Reducing or purifying the residual metals in shellfish is an advisable method to make the shellfish achieve the seafood safety standards. *Crassostrea rivularis* was first described by Gould [25] based on a specimen collected from southern China and subsequently used to describe oysters in Japan and China [26]. As a favourite and widely distributed seafood product, *C. rivularis* is one of the most important oyster species cultured in China and has been cultured for centuries [27, 28]. *C. rivularis* is mostly found in low-salinity rivers and estuaries. *C. rivularis* can accumulate metals and has been used as an indicator of marine environmental pollution for decades [21,22,29,30]. In recent years, the pollution from urban, industrial and agricultural sources into the estuarine waters of the Zhujiang (Pearl) River in southern China has been increasing. Water areas containing shellfish habitats have been restricted for production and harvesting. Of the metals found in polluted shellfish, Cd is one of the most toxic metals to humans [31]. In fact, 81.8% of the shellfish from the Zhuhai aquatic product during the fourth quarter of 1996 to the third quarter of 1997 exceeded the seafood safety standards of Cd in shellfish [32]. What is more, average value of Cd concentrations in *C. rivularis* collected from Shantou (2002-2003) and Guangdong (2007) are around 10 mg kg^{-1} day weight [33]. Research on metal purification from shellfish is significant. The aim of the present study is to discuss the effects of salinity and algal diets on Cd elimination from *C. rivularis* in indoor conditions and to provide a theoretical basis and assessment for the industrialized depuration of heavy metals from oysters when the Cd content exceeds the national seafood safety standards.

Research Method

Oyster

C. rivularis (aged 2 years, shell length of 15.6±1.56 cm and weight of 221.6±25.8 g) were collected from Daya Bay culture areas, Guangdong, China (Figure 1), where the yield, quality, and culture areas of *C. rivularis* have been adversely affected by the growing metal pollution. The oysters were selected, cleaned carefully and then maintained in sand-filtered seawater (PY16293, Jiuding Hi-tech Filtering Equipment, Ltd., Beijing) without food and under natural lighting at room temperature (water temperature 17-24°C, salinity 34, pH 7.8-7.9) and were supplied dissolved oxygen by the continuous pumping of air a week prior to the experiment.

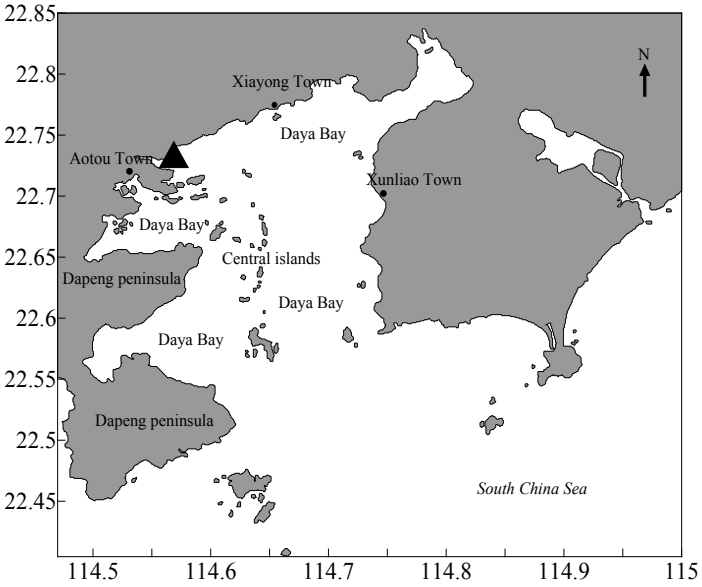

Figure1. Sampling site of *C. rivularis.*

(Note: the triangle is the sampling site in the Daya Bay *C. rivularis* culture areas)

Experimental seawater

Clean, pollution-free seawater attained the quality standards for Grade I seawater [34] after it was filtered through sand and precipitated. The seawater was diluted with distilled water and continuously pumped with air for 48 h before use.

Accumulation of Cd in *C. rivularis*

To obtain a consistently higher Cd concentration in oysters for the elimination experiment, *C. rivularis* was cultured in Cd-polluted seawater. Seawater and seawater with dissolved $CdSO_4 \cdot 8H_{20}$ (AR, purity of 99%, Sinopharm Chemical Reagent, Shanghai, China) with a Cd concentration of 25 μg/L were prepared following the seawater quality standards for marine aquaculture [35] and the protocols described by Lu et al.[21]. Four hundred *C. rivularis* were placed in a cement pool (length of 180 cm, width of 100 cm and height of 80 cm) containing 25 μg/L Cd seawater (salinity 20, pH 7.8-7.9) at a room temperature of 22.2-28.1°C and allowed to accumulate Cd for at least 11 days. Five sampling times were defined: 0, 2, 4, 7, and 11 days. At each sampling point, eight oysters were removed randomly for metal analysis from both the experimental and control groups. The seawater was replaced, and any dead *C. rivularis* were removed daily. The Cd-polluted seawater was replaced with clean seawater from the control groups. No food was provided to any of the groups.

Elimination of Cd from *C. rivularis*

After the accumulation experiment, the remaining live *C. rivularis* were divided into nine groups. Five groups (G_{12}, G_{16}, G_{20}, G_{24}, and G_{28}) and four groups (G_P, G_C, G_{PC} and G_{ct}) were used to understand the effect of salinity and algal diets on the elimination of Cd from *C. rivularis*. The oysters were cultured in a polyethylene barrel containing 120 L of clean seawater under natural illumination at a temperature of 22.5-28°C with a pH of 7.8-7.9 to eliminate metal pollution over a period of 31 days. Each barrel contained 45 oysters.

Effect of salinity on the elimination of Cd from *C. rivularis*

Five different salinities (12, 16, 20, 24, and 28) were chosen to assess the impact of salinity on the elimination of Cd from oysters. During the experiment, the oysters were fed *Platymonas subcordiformis* (cell length of 11-14 μm, width of 7-9 μm and height of 3-5 μm), and the cell density was maintained at 2×10^4 cells/mL. Seven sampling times were defined: 0, 2, 5, 10, 15, 23, and 31 days. At each sampling point, six oysters were randomly collected from each group, and the Cd contents in the soft parts were determined.

Effect of algal diets on the elimination of Cd in *C. rivularis*

The oysters were fed three types of algal diets. Group G_P was fed *P. subcordiformis* (2×10^4 cells/mL). Group G_C was fed *Chlorella* sp. (2×10^5 cells/mL, cell length 7-13 μm, width 6-8 μm, height 8-10 μm). Group G_{PC} was fed *P. subcordiformis* (1×10^4 cells/mL) and *Chlorella* sp. (1×10^5 cells/mL). The control group (G_{ct}) received no food. The oysters were fed three times daily to maintain the cell density. At specific intervals (0, 2, 5, 10, 15, 23, and 31 days), six oysters were randomly sampled from each group for Cd analysis. The seawater was diluted to a salinity of 20 for all of the groups.

Metal analysis

The whole soft tissues of oysters were washed with distilled water, removed from their shells, dried with filter paper, weighed and homogenized. We weighed the 0.30-g homogenized samples in quadruplicate for each group, placed the samples and 5 mL of analytical-grade nitric acid into a high-pressure digestion jar, and allowed the contents to predigest overnight at room temperature for 12 hours. The samples were then digested in a microwave digester (MARS-5 system, CEM, USA), the digestates were diluted with up to 50 mL of deionized water, and the Cd concentrations in the samples were measured using an atomic absorption spectrophotometer (Optima 2000 DV). The standard reference material for Cd was provided by the National Standard Material Centre, Beijing, China.

Results

Accumulation of Cd in *C. rivularis*

Exposure to Cd-polluted seawater resulted in the continuous accumulation of Cd in oysters, which followed a net accumulation model (Figure 2). A linear regression analysis demonstrated that the Cd accumulation in *C. rivularis* was positively correlated with the exposure time ($P<0.01$, $y = 0.924x + 3.387$, $R^2 = 0.984$, where y is the Cd concentration in the oyster soft tissues [μg/g] and x is the exposure time [day]). The accumulation rate of Cd in *C. rivularis* was 0.92 μg/(g·d) during 11 days of exposure to the Cd-polluted seawater with a Cd concentration of 25 μg/L.

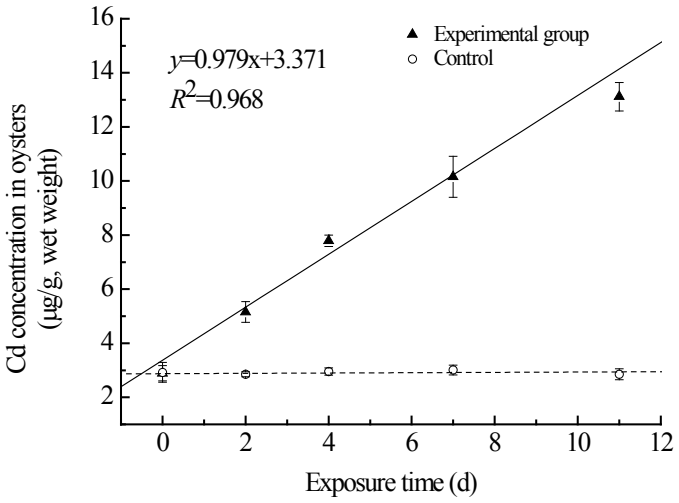

Figure 2. Accumulation of Cd (25 μg/L) in *C. rivularis* at different exposure times.

Effects of salinity on the elimination of Cd from *C. rivularis*

The Cd concentration in oysters decreased over time in all of the salinity groups (Figure 3), but the Cd elimination rates varied between the different salinity groups (Figure 4). The regression analysis indicated that the relationship between Cd in *C. rivularis* and the depuration time could be described as follows:

$C_t^{12} = 12.188e^{-0.0098t}$, $R^2 = 0.801$ ($P< 0.01$);

$C_t^{16} = 11.842e^{-0.0124t}$, $R^2 = 0.803$ ($P< 0.01$);

$C_t^{20} = 11.606e^{-0.0144t}$, $R^2 = 0.824$ ($P< 0.01$);

$C_t^{24} = 11.339e^{-0.0163t}$, $R^2 = 0.826$ ($P< 0.01$);

$C_t^{28} = 11.224e^{-0.0183t}$, $R^2 = 0.839$ ($P< 0.01$);

where C_t^x is the Cd in the soft tissues of *C. rivularis* at salinity *x*, and *t* is the depuration time (day).

The Cd elimination increased with increasing salinity. Group G_{28} with a salinity of 28 exhibited the highest elimination rate (0.0183 μg/(g·d)), and 47.6% of the Cd was removed after 31 days of depuration. In contrast, group G_{12} with a salinity of 12 had the lowest depuration rate (0.0098 μg/(g·d)), and only 27.9% of the Cd was removed after 31 days of depuration (Figure 4).

A two-factor variance analysis of the five elimination groups showed that the salinity and depuration time had a notable effect on the elimination of Cd from *C. rivularis* (P<0.01), but there was no interaction between the salinity and depuration time (P>0.05). A repetitive-measure analysis of variance demonstrated that group G_{28} (P<0.01) was significantly different from groups G_{12}, G_{16}, and G_{20}, and a similar finding was found for group G_{24} (P<0.05). In addition, group G_{12} was significantly different from groups G_{16} and G_{20} (P<0.01), and there was also significant differences between groups G_{16} and G_{20} (P<0.05) (Table 1).

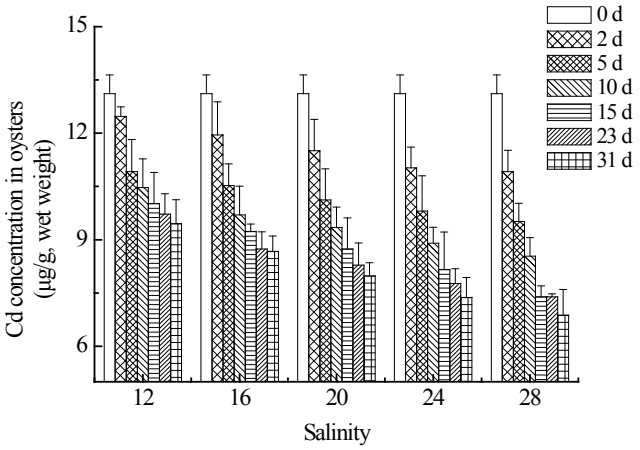

Figure. 3. Effect of salinity on the elimination of Cd from *C.rivularis.*

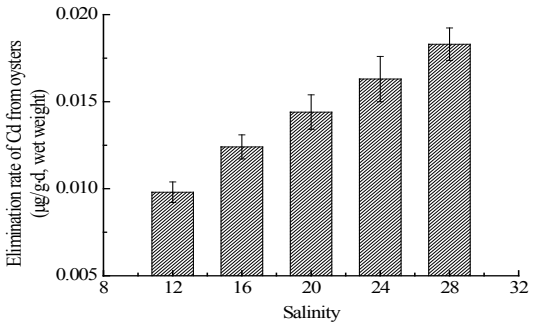

Figure 4. Effect of salinity on the elimination rate of Cd from *C. rivularis.*

Table 1. *P* value of the repetitive-measure analysis of variance between groups with different salinities.

Group	G_{12}	G_{16}	G_{20}	G_{24}	G_{28}
G_{12}	-	0.001	0.000	0.000	0.000
G_{16}	0.001	-	0.019	0.000	0.000
G_{20}	0.000	0.019	-	0.016	0.000
G_{24}	0.000	0.000	0.016	-	0.093
G_{28}	0.000	0.000	0.000	0.093	-

Effects of algal diets on the elimination of Cd from *C. rivularis*

After the oysters were transferred into clean seawater with a salinity of 20 and different algal diets, Cd was eliminated at a low rate (Figure 5). Interestingly, different algal diets resulted in different rates of Cd removal. The relationship between the Cd in *C. rivularis* and the depuration time can be described as follows:

Group G_P: $C_t^P = 11.606e^{-0.0144t}$, $R^2 = 0.824$ (P<0.01);

Group G_C: $C_t^C = 11.639e^{-0.0128t}$, $R^2 = 0.789$ (P<0.01);

Group G_{PC}: $C_t^{PC} = 11.597e^{-0.0166t}$, $R^2 = 0.875$ (P<0.01);

Group G_{ct}: $C_t^{ct} = 11.835e^{-0.0112t}$, $R^2 = 0.814$ (P<0.01).

The decreasing order of the Cd elimination rates were G_{PC}, G_P, G_C, and G_{ct} (Figure 6). The highest elimination rate (group G_{PC}) was 0.0166 μg/(g·d), and 43.8% of Cd was removed after 31 days. In contrast, the lowest elimination rate (group G_{ct}) was 0.0112 μg/(g·d), and only 32.6% of the Cd was removed after 31 days. The elimination rates of groups G_P and G_C were 0.0144 and 0.0128 μg/(g·d), respectively (Figure 5).

A two-factor analysis of variance of the four depuration groups revealed that the algal diets and depuration time had a significant effect on the elimination of Cd from *C. rivularis* (P<0.01), but no interaction was found between the algal diets and the depuration time (P>0.05). Group G_P was significantly different than group G_{ct} (P<0.05) but showed no significant differences with groups G_C and G_{PC} (P>0.05). In contrast, group G_C was significantly different from group G_{PC} (P<0.05) but not from group G_{ct} (P>0.05) (Table 2).

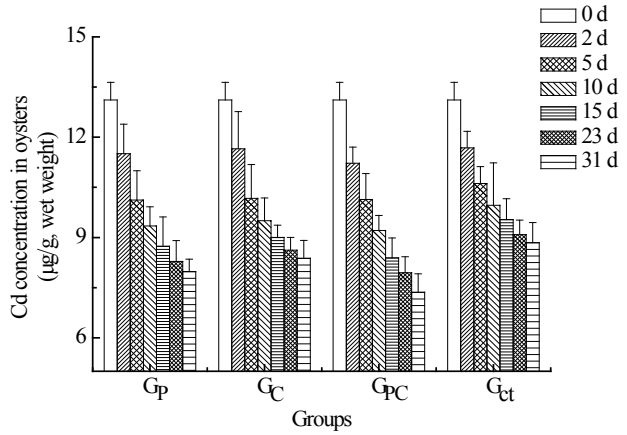

Figure 5. Effect of algal food on the elimination of Cd from *C. rivularis.*

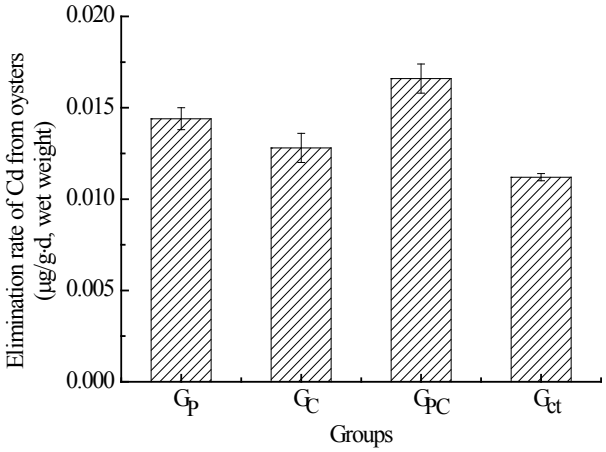

Figure 6. Effect of algal diet on the elimination rate of Cd from *C. rivularis.*

Table 2. *P* value of repetitive-measure analysis of variance between groups with different algal diets.

Group	G_P	G_C	G_{PC}	G_{ct}
G_P	-	0.313	0.210	0.013
G_C	0.313	-	0.035	0.086
G_{PC}	0.210	0.035	-	0.001
G_{ct}	0.013	0.086	0.001	-

Discussion

After the oysters were exposed to seawater containing 25 μg/L Cd for 11 days, the accumulation rate of Cd in *C. rivularis* was 0.92 μg/(g·d). This value is much higher than the subsequent depuration rate, which is 50-fold faster than the highest depuration rate. This finding is also suggested by the works of other researchers. George and Coombs [36] found that the excretion rate of Cd by *Mytilus edulis* was 18-fold slower than the accumulation rate. Arini et al. [37] reported that after a 24 day exposure in Cd-contaminated water, only 73% Cd is eliminated from *Corbicula fluminea* after one year and Cd half-life in the bivalve is estimated around 240 days. The initial Cd concentration used in our study was higher than that used by Denton and Burdon-Jones and Lu et al. [21, 38]. The higher the level of contamination, the more important the Cd binding to cytosolic ligands becomes, and the faster the elimination of cytosolic metals than the insoluble fraction [14].

The elimination kinetics was influenced by environmental factors, such as the salinity, humic acid concentrations, temperature, and feeding rate [39, 40]. Therefore, it is valuable to study the effects of environmental factors on oyster purification and to find a better condition to allow the polluted oysters to eliminate metals faster and more efficiently. Our results suggested that the salinity affected the Cd elimination in *C. rivularis*, and the rate increased as the seawater salinity increased, which is consistent with the results reported by Van Dolah et al. [39]. However, the difference between groups G_{24} and G_{28} was insignificant (P>0.05) (Table 1). It is possible that the higher salinities do not have an obvious effect on the elimination of Cd in *C. rivularis*. The effect of the salinity on the elimination of metal from molluscs appears to differ among species investigations. It appears not to have an effect in the Black-lip oyster *Saccostrea echinata* [38]. There are several methods for the elimination of Cd from aquatic animals, such as defecation, egestion, detoxification, and excretion. The excretion of Cd from different aquatic animals is different [41-43]. However, even from the same species under different conditions, the effects of salinity are different. Lu et al. [21] demonstrated that the elimination of Cd from *C. rivularis* decreased with increasing salinity. Different environmental conditions are more likely to cause the observed discrepancy. Studies have reported that the residual cadmium in oysters is dependent on a complex interplay of both abiotic (such as salinity and temperature) and biotic factors (such as filter-feeding rates and diet selection) and that the level is determined by the dominant factor at a particular time or in a particular region [3,4].

Wright et al. [44] suggested that the tissue metal concentrations can be highly dependent on both environmental conditions and the physiological status of individuals. Food is another factor. Ruelas-Inzunza and Páez-Osuna [45] listed five potential causes for fluctuations in the metal concentrations in seashells, one of which is the food supply. Christie et al. [46] also suggested that the diet is an important contributor to the oyster tissue cadmium concentrations. In our previous studies and in the studies conducted by Van Dolah et al. [39], oysters were fed *P. subcordiformis* and *Isochrysis galbana* during the depuration experiment instead of no food, as in the studies conducted by Denton and Burdon-Jones and Lu et al.[21,38].

Our results suggested that the elimination of Cd from oysters is related to food. The Cd elimination rates in the groups administered food supplies were all faster than that of the control group with no food supply (Figure 5). However, the mechanisms underlying diet-facilitating metal elimination have not yet been determined. Oysters require energy for different tasks, such as: distribution of metal in tissues, turnover of soluble and insoluble metals, synthesis and metabolism of proteins that bind or metabolize heavy metals. Microalgae, such as *P. subcordiformis* and *Chlorella* sp., are easily ingested and assimilated and have high dietary value. It is likely that an algal diet can supply energy and nutrition to allow the oyster to synthesize the enzymes and proteins that function in metal removal.

Interestingly, different algal diets have different effects on Cd elimination from *C. rivularis*. The oysters fed the mixed algal diet were significantly different from the groups with no food supply (Table 2). Oysters fed the mixed algal diet exhibited the highest elimination rates, followed by the *P. subcordiformis* group, and the *Chlorella* sp. group exhibited the lowest rate (Figure 6). This may be caused by differences in the energy and nutrients supplied by the three diets. Studies have revealed that *P. subcordiformis* is a better food than *Chlorella* sp.[47]. *P. subcordiformis* has been used in the commercial breeding of aquatic organisms, including *Mercenaria mercenaria* [47], *Acartia pacifica* [48], and *Apocyclops borneoensis* [49]. It has also been reported that a mixed algal diet is better than a single algal diet. Zhang et al. [47] suggested that the growth and survival of young *M. mercenaria* fed mixed algae were better than that obtained with the single algal diet. Epifanio [50] also found that the breeding of *M. mercenaria* fed a mixture of *Isochrysis galbana* and *Platymonas suecica* was better than that obtained with *P. suecica* alone. Huang and Huang [49] achieved similar results using *P. subcordiformis*, *Chlorella* sp. and mixed algae as food. A mixture of two appropriate algae can supply an adequate mix of nutrients to promote the growth and survival of marine organisms [51]. Thus, the better growth and survival could result in the oysters purifying metal more effectively. The results may serve to optimize the removal of heavy metal from commercially important species. This, in turn, has important consequences for food safety production in Chinese coastal waters.

Conclusion

There was a positive correlation between the Cd accumulation in *C. rivularis* and the exposure time. Cd elimination from *C. rivularis* began when they were moved from Cd-polluted seawater into clean seawater.

The Cd elimination rates from *C. rivularis* increased as the salinity increased.

The algal diets had a notable effect on the elimination rate of Cd from *C. rivularis*. Compared with single algal food, mixed algal food (*P. subcordiformis* and *Chlorella* sp.) was more helpful for *C. rivularis* to eliminate Cd.

These results may serve to optimize the removal of Cd from a commercially important species. It also has important consequences for the safe production of food from Chinese coastal waters.

Acknowledgements

This work was supported by the National Nature Science Foundation of China (No. 41276091), the Technology Research Program of Guangzhou City (2014J4100010, 201508020100), the Technology Research Program of Guangdong Province (2013B010404027), and the Science and Technology for Key Projects of Guangdong Province (No. 2003C20411).

1. References

Ke C, Wang W-X. Bioaccumulation of Cd, Se, and Zn in an estuarine oyster (*Crassostrea rivularis*) and a coastal oyster (Saccostrea glomerata). Aquatic Toxicology. 2001, 56(1): 33-51.

2. Shulkin V, Presley B, Kavun VI. Metal concentrations in mussel Crenomytilus grayanus and oyster Crassostrea gigas in relation to contamination of ambient sediments. Environ Int. 2003, 29(4): 493-502.

3. Bendell LI, Feng C. Spatial and temporal variations in cadmium concentrations and burdens in the Pacific oyster (Crassostrea gigas) sampled from the Pacific north-west. Mar Pollut Bull. 2009, 58(8): 1137-1143.

4. Lekhi P, Cassis D, Pearce C, Ebell N, Maldonado M et al. Role of dissolved and particulate cadmium in the accumulation of cadmium in cultured oysters (Crassostrea gigas). Sci Total Environ. 2008 2009, 393(2-3): 309-325.

5. Ng T Y-T, Stupakoff I, Christy A, Cheney D, Wen Xiong W et al. Cadmium accumulation and loss in the Pacific oyster Crassostrea gigas along the west coast of the USA. Marine Ecology. 2009, Progress Series 401, 147-160.

6. Boyce R, Herdman WA. On a green leucocytosis in oysters associated with the presence of copper in the leucocytes. Proceedings of the Royal Society of London. 1897, 62: 30-38.

7. Orton J. Summary of an account of investigations into the cause or causes of the unusual mortality among oysters in English oyster beds during 1920 and 1921. Journal of the Marine Biological Association of the United Kingdom (New Series). 1923, 13(1): 1-23.

8. FAO/WHO. Evaluation of certain food additives and contaminants Forty-first report of the Joint FAO/WHO Expert committee on food additives. World Health Organization, Geneva, 1993, pp. 28-35.

9. Commission E. Commission Regulation (EC) No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs, European Commission, Brussels. 2001, pp. 1-13.

10. NY5154-2008. Agricultural industry standard of the People's Republic of China, Free-pollutant food, oyster. Agriculture Ministry of China, China. 2008.

11. Supan J. Container-relaying of oysters: an alternative to depuration and relaying. Molluscan Shellfish Depuration. CRC Press, Boston, MA, 1991, pp. 205ą215.

12. Chan K, Cheung R, Leung S, Wong M. Depuration of metals from soft tissues of oysters (Crassostrea gigas) transplanted from a contaminated site to clean sites. Environ Pollut.1999, 105(3): 299-310.

13. do Amaral M C R, de Freitas Rebelo M, Torres J P M, Pfeiffer W C. Bioaccumulation and depuration of Zn and Cd in mangrove oysters (Crassostrea rhizophorae, Guilding, 1828) transplanted to and from a contaminated tropical coastal lagoon. Mar Environ Res. 2005, 59(4): 277-285.

14. Geffard A, Amiard J, Amiard-Triquet C. Kinetics of metal elimination in oysters from a contaminated estuary. Comp Biochem Physiol C Toxicol Pharmacol. 2002, 131(3): 281-293.

15. Wallner-Kersanach M, Theede H, Eversberg U, Lobo S. Accumulation and elimination of trace metals in a transplantation experiment with Crassostrea rhizophorae. Arch Environ Contam Toxicol. 2000, 38(1): 40-45.

16. Pan ZZ, He W, Wu WJ, Ding ZK, Li YM et al. Using ozone-ultraviolet combination system to purify Paphia schnellian for killing microorganisms. Journal of Southern Agiculture. 2011, 42: 94-97.

17. Yang H, Lou Y J. Shellfish purification status and the progress of purification technology. China Fisheries. 2004, 5: 72-73.

18. Saed K, Ismail A, Omar H, Kusnan M. Heavy metal depuration in flat tree oysters Isognomon alatus under field and laboratory conditions. Toxicological & Environmental Chemistry. 2004, 86(3): 171-179.

19. Chong K, Wang W-X. Comparative studies on the biokinetics of Cd, Cr, and Zn in the green mussel Perna viridis and the Manila clam Ruditapes philippinarum. Environ Pollut. 2001, 115(1): 107-121.

20. Lee BG, Wallace WG, Luoma SN. Uptake and loss kinetics of Cd, Cr and Zn in the bivalves Potamocorbula amurensis and Macoma balthica: effects of size and salinity. Marine Ecology-Progress Series.1998, 175: 177-189.

21. Lu CH, Xie W Z, Zhou GJ. Studies on Crassostrea rivularis as a biological indicator of cadmium pollution. Journal of Fishery Sciences of China.1998, 5(2): 79-83.

22. Lu CH, Zhou GJ, Xie WZ. Accumulation and elimination of Crassostrea rivularis to lead. Marine Environmental Science.1999, 18, 33-38.

23. Wang W-X, Fisher NS, Luoma SN. Kinetic determinations of trace element bioaccumulation in the mussel Mytilus edulis. Marine Ecology Progress Series. 1996, 140: 91-113.

24. Wright DA, Zamuda CD. Copper accumulation by two bivalve molluscs: salinity effect is independent of cupric ion activity. Marine environmental research. 1987, 23(1): 1-14.

25. Gould A. Descriptions of shells collected by the North Pacific Exploring Expedition, Proceedings of the Boston Society of Natural History. 1861:14-40.

26. Wang H, Guo X, Zhang G, Zhang F. Classification of jinjiang oysters Crassostrea rivularis (Gould, 1861) from China, based on morphology and phylogenetic analysis. Aquaculture. 2004, 242(1-4): 137-155.

27. Guo X, Ford S E, Zhang F. Molluscan aquaculture in China. Journal of Shellfish Research. 1999, 18(1): 19-31.

28. Que HY, Guo XM, Zhang GF, Zhang FS. Tetraploids induced by inhibiting polar body I with cytochalasin B in Jinjiang oyster, Crassostrea rivularis. Oceanologia et Limnologia Sinica. 2005, 36: 437-444.

29. Jia XP, Lin Q, Li CH, Lin YT. Study on the ecological environment of fishing industry and polluted effect of biological resources in southern China. China Ocean Press, Beijing. 2004.

30. Lu CH. Oyster Ostrea rivularis as an indicator of heavy metals pollution. Journal of Oceanography in Taiwan Strait. 1994, 13: 14-20.

31. FAO/WHO. Application of Risk Analysis to Food Standards Issues: Report of the Joint FAO/WHO Expert Consultation. World Health Organization, Geneva, Switzerland.1995.

32. Huang HY, Xu YS, Wang LL, Bai YL, Zhang CH et al. Contamination of mercury, lead, cadmium and arsenic in

the aquatic products from Zhuhai. China Public Health. 1998, 14: 23-24.

33. Pan K, Wang WX. Trace metal contamination in estuarine and coastal environments in China. Sci Total Environ. 2012, 421-422: 3-16.

34. GB3097-1997. State Standard of the People's Republic of China, Sea water quality standard. Environment science Press, Beijing China, 1997. pp. 16-17.

35. NY5052-2001. Agricultural industry standard of the People's Republic of China, Pollution-free food, marine aquaculture water quality, China, 2001.

36. George S, Coombs TL. The effect of chelating agents on the uptake and accumulation of cadmium by Mytilus edulis. Marine biology. 1977, 39: 261-268.

37. Arini A, Daffe G, Gonzalez P, Feurtet-Mazel A, Baudrimont M. Detoxification and recovery capacities of Corbicula fluminea after an industrial metal contamination (Cd and Zn): a one-year depuration experiment. Environmental Pollution. 2014, 192: 74-82.

38. Denton G, Burdon-Jones C. Influence of temperature and salinity on the uptake, distribution and depuration of mercury, cadmium and lead by the black-lip oyster Saccostrea echinata. Marine biology.1981, 64(3): 317-326.

39. Van Dolah FM, Siewicki TC, Collins GW, Logan JS. Effects of environmental parameters on the elimination of cadmium by eastern oysters, Crassostrea virginica. Arch Environ Contam Toxicol. 1987, 16(6): 733-743.

40. Zaroogian GE. Studies on the depuration of cadmium and copper by the American oyster Crassostrea virginica. Bulletin of environmental contamination and toxicology.1979, 23(1): 117-122.

41. de Conto Cinier C, Petit-Ramel M, Faure R, Garin D, Bouvet Y. Kinetics of cadmium accumulation and elimination in carp Cyprinus carpio tissues. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol.1999, 122(3): 345-352.

42. Kim S-G, Jee J-H, Kang J-C. Cadmium accumulation and elimination in tissues of juvenile olive flounder, Paralichthys olivaceus after sub-chronic cadmium exposure. Environ Pollut.2004, 127(1): 117-123.

43. Wang F, Zhao YF, Wu YC, Lv JC, Liu CF et al. Cadmium accumulation and elimination in Chlamys farreri. Journal of Zhanjiang Ocean University.2005, 25: 95-98.

44. Wright D, Mihursky JA. Trace metals in Chesapeake Bay oysters: Intra-sample variability and its implications for biomonitoring. Marine environmental research.1985, 16(3), 181-197.

45. Ruelas-Inzunza J, Páez-Osuna F. Comparative bioavailability of trace metals using three filter-feeder organisms in a subtropical coastal environment (Southeast Gulf of California). Environ Pollut. 2000, 107(3): 437-444.

46. Christie J, Bendell L. Sources of dietary cadmium to the Pacific oyster Crassostrea gigas. Mar Environ Res. 2009, 68(3): 97-105.

47. Zhang T, Yang HS, Liu BZ, Li BQ, Zhang FS. Effects of environmental factors on the survival and growth of Juvenile Hard Clam Mercenaria mercenaria (Linnaeus, 1758). Oceanologia et Limnologia Sinica. 2003, 34:142-149.

48. Gao YH, Lin B. Effects of some factors on feeding rates of Acartia pacifica. Journal of Xiamen University (Nature Science). 1999, 38: 751-757.

49. Huang JQ, Huang HY. Effects of several unicellular algaes upon the reproduction of Apocyclops borneoensis population. Marine Sciences.1999, 4: 47-50.

50. Epifanio C. Growth in bivalve molluscs: nutritional effects of two or more species of algae in diets fed to the American oyster Crassostrea virginica (Gmelin) and the hard clam Mercenaria mercenaria (L.). Aquaculture.1979, 18(1): 1-12.

51. Chen SJ, Liu M. Discussion on the selection techniques of fish bait copepod. Fujiang Fish. 1994, 2: 36-37.