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### Comments

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## EFFECT OF AGE AND TISSUE WEIGHT ON THE CADMIUM CONCENTRATION IN PACIFIC OYSTERS (*CRASSOSTREA GIGAS*)

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**ABSTRACT** This study investigated the influence of age and tissue weight on cadmium (Cd) levels in Pacific oysters (*Crassostrea gigas*). Oysters from 4 different age groups (1, 2, 3, and 4 y) were collected at an oyster farm in Willapa Bay, Washington. To examine the effects of age, 60 oysters from each group were sorted into 3 composites of 20, and Cd analysis was carried out on all composite samples. To study the effects of tissue weights, 25 oysters from each of the 4 age groups were collected and analyzed individually for Cd. All oyster Cd concentrations were below the 3.7 ppm ( $\mu\text{g/g}$ ) wet wt Food and Drug Administration (FDA) level of concern and the 2 ppm wet wt Hong Kong limit. There was a moderate correlation between Cd concentration and age ( $R^2 = 0.60$ ). The 1-y-old oysters had significantly lower Cd concentrations than the 2–4-y-old oysters. Tissue weight also influenced Cd concentration ( $R^2 = 0.31$ ). The effect of tissue weight was found to vary with age, with a stronger correlation to Cd levels in the 1- and 2-y-olds and a weaker correlation among the 3- and 4-y-olds. The results indicate that oysters accumulate the majority of their Cd during the first two years of their lives, after which point they become saturated and show only incremental additions of Cd in later years.

**KEY WORDS:** cadmium, Pacific oysters, *Crassostrea gigas*, age, weight

### INTRODUCTION

Cadmium (Cd) is a trace metal that is widely distributed in soil, air, water, and living things (Pinot et al. 2000). It is not an essential metal for plants, animals, or humans and has a long biological half-life (up to 30 y) (Pinot et al. 2000, Satarug et al. 2006). Long-term exposure to Cd can result in health problems such as kidney dysfunction, liver disease, lung cancer and skeletal decalcification (Nordberg 2003, Satarug et al. 2006). Nonoccupational Cd exposure among humans occurs primarily through smoking tobacco and food consumption (Kikuchi et al. 2002, Satarug & Moore 2004).

Bivalves, including Pacific oysters (*Crassostrea gigas*), have a heightened ability to accumulate trace metals and are often used as indicator species for biomonitoring (Olivier et al. 2002, Robinson et al. 2005, Shi & Wang 2004, Sokolova et al. 2005). These marine organisms have developed a system of metal detoxification involving lysosome organelles and the cysteine-rich protein metallothionein (MT) (Sokolova et al. 2005). Whereas lysosomes and MTs reduce the toxic effects of metal exposure, they also allow for survival of organisms carrying elevated body burdens of toxicants, including Cd. Interestingly, the Pacific oyster has been reported to contain a novel gene coding for a form of MT with a higher metal ion binding capacity than that described for other MTs, possibly allowing for an increased ability for this species to accumulate metals (Tanguy & Moraga 2001). Although MT and metals in marine molluscs have been reported to vary with changes in size and/or age, these relationships have been inconsistent, with reports of correlations being direct, indirect, or insignificant (Amiard et al. 1986, Christy 2005, Frew et al. 1989, Leung & Furness 1999, Mackay et al. 1975, Mouneyrac et al. 1998, Nielson 1975, Thomson 1982, Yap et al. 2003).

Because of the potentially harmful effects of Cd, numerous regulatory and health agencies worldwide have established

guidelines for Cd exposure. Although there is some general consensus regarding safe intake levels, there is great variation among maximum permitted Cd levels in shellfish. Based on a maximum daily intake of 55  $\mu\text{g}$  Cd/person, the US Food and Drug Administration (FDA) determined the level of concern for Cd in shellfish (i.e., molluscan bivalves and crustaceans) to be 3.7 ppm ( $\mu\text{g/g}$ ) (FDA 1993). Codex Alimentarius Commission recently adopted a 2-ppm wet wt limit for Cd in marine bivalve molluscs, excluding oysters and scallops (CODEX 2006). Maximum permitted levels in Hong Kong, Australia, and New Zealand agree with the Codex limit; however, in these cases there are no exceptions for Pacific oysters (FAO/NACA 1995, Kruzynski 2004).

Pacific oysters have been the top aquaculture species harvested worldwide for a number of years, with production figures growing from 1,190–4,377 thousand metric tons in the period from 1990–2003 (Johnson 2005). The United States provides a significant portion of the worldwide oyster supply: a total of 38,506 thousand pounds (meat weight) were harvested in 2004, and US exports of fresh or prepared oysters totaled 7,505 thousand pounds (US \$17.1 million) (Harvey 2006, Johnson 2005). Oyster exports continue to grow, totaling 7,797 thousand pounds (US \$17.9 million) in the year 2005 (Harvey 2006). The observed increases in oyster exports over the last several years have been primarily attributed to increased demand from Asia, which is expected to continue to grow as Asian economies strengthen over the next few years. Washington is one of the top export states in America, and because of its location along the Pacific Rim, a significant amount of its products (including oysters) are destined for Asian countries (CEI 2002; Lin & Schmidt 2000). In Hong Kong, where oysters from the US are highly regarded, there is a strong demand for seafood, with molluscan imports for 2003 totaling 56,061 metric tons (Wolf & Yuen 2004). However, over the past several years some shipments of Pacific oysters from Washington and British Columbia, Canada, have been rejected by Hong Kong because they contained levels of Cd that exceeded 2 ppm (Kruzynski

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2004). Considering that Cd levels in some West Coast Pacific oysters can naturally fall between 1 and 4 ppm, these rejected shipments pose an economic threat to the Pacific Northwest oyster industry, in addition to raising human health concerns (Christy 2005). Although the Hong Kong market prefers relatively large oysters, with typical shucked meat weights around 50 g (wet wt), the effects of oyster tissue weight and age on Cd levels in West Coast Pacific oysters are unknown.

In a collaborative effort to evaluate and minimize economic and human health risks resulting from Cd levels in West Coast Pacific oysters, the Oregon State University Seafood Laboratory, in cooperation with Pacific Shellfish Institute, CA Sea Grant (UC Davis), Integral Consulting, Hong Kong University of Science and Technology, and University of Alaska, carried out a US Department of Agriculture (USDA)-funded project with the following goals: (1) evaluate the spatial distribution of Cd in West Coast Pacific oysters; (2) identify factors that may influence Cd concentrations; (3) evaluate the impact of Cd on the shellfish industry and human health; and (4) provide outreach and extension services.

In response to the first goal, a comprehensive sampling effort was carried out in which composite oyster samples from various growing sites in Washington, Arkansas, and Oregon were analyzed for Cd (Christy 2005). The most extensive sampling was carried out in Washington, in which 92 composites had an average Cd concentration of 1.24 ppm, with 17% of the composites in excess of 2 ppm. In the work presented here, research on the effects of biological factors on Cd levels in oysters was carried out at one of the Washington growing sites: Willapa Bay. Willapa Bay is a shallow, relatively pristine, coastal-plain estuary with intensive oyster cultivation, contributing nearly 10% of total US commercial oyster harvests (Banas et al. 2007, Ruesink et al. 2006). The objectives of the current study were to examine the effects of age and tissue weight on Cd concentration in the US West Coast Pacific oysters cultured in Willapa Bay. Sediment Cd concentrations in the oyster beds were also investigated as an additional potential indicator of oyster Cd levels.

## MATERIALS AND METHODS

### Age Study

#### Sample Collection

Pacific oysters (*C. gigas*) from 4 age groups (1, 2, 3, and 4 y) and corresponding sediments were collected from an oyster farm August 2005 in Willapa Bay, Washington. Although all oysters were bottom-cultured at the same farm, each age group was maintained in a distinct but nearby growing area. The 1- and 2-y-old oysters available for sampling were from triploid-growing areas, whereas the 3- and 4-y-old oysters were from diploid sites. Because of sampling limitations, it was not possible to select sites where all four age classes were of the same ploidy. Size ranges were predetermined for oysters from each age group (Table 1) according to typical oyster shell lengths (longest point from anterior to posterior) observed at the growing beds. These ranges were based on an earlier study, which allowed for collection of oysters within a 5.1-cm shell length range (Christy 2005). Oysters were not collected unless they were within these size ranges. Because of high variability in shell length among the 4-y-old oysters, the size range for this age group was slightly greater than

TABLE 1.

Results of oyster age study showing yr class, shell lengths, oyster Cd concentrations (wet wt), average tissue weights (including nectar), and sediment Cd concentrations (dry wt).

Age (yr)	Oyster Shell Length (cm)	Ave. [Cd] ± St dev. (ppm)	[Cd] Range (ppm)	Ave. Oyster Tissue wt (g)	Sediment [Cd] (ppm)
1	6.4–11.4	0.75 <sup>a</sup> ± 0.03	0.72–0.77	16.7 <sup>a</sup> ± 1.2	0.39
2	8.9–14.0	1.04 <sup>b</sup> ± 0.13	0.90–1.16	19.9 <sup>a</sup> ± 0.9	0.26
3	10.2–15.2	1.06 <sup>b</sup> ± 0.04	1.03–1.10	41.7 <sup>b</sup> ± 2.3	0.94
4	14.0–20.3	1.11 <sup>b</sup> ± 0.11	1.04–1.24	79.5 <sup>c</sup> ± 1.7	1.10

Note: Values in the same column labeled with a different superscript letter are significantly different ( $P < 0.05$ ) according to one-way analysis of variance (ANOVA), Tukey test.

for the other age groups. Prior to sample collection, all equipment was acid-washed using a dilute (~5%, wt/wt) solution of nitric acid (HNO<sub>3</sub>) in deionized water to remove any bound metals. This was followed by a thorough rinse with deionized water to remove any remaining acid.

Sample collection was carried out at low tide according to a pre-established sampling protocol (Christy 2005). Upon arrival at each oyster bed, six cones were placed about 10 m apart along a straight line parallel to the waterline. At each cone, the top 15 cm of sediment was collected using a section of PVC pipe with a 10.2-cm diameter. Each of the six core samples was combined in a stainless steel bowl and thoroughly mixed using a stainless steel spoon. A representative sample was then collected in a 4 oz glass jar for Cd analysis. Ten oysters were collected within a 5-m radius of each cone, resulting in a total of 60 oysters collected for each age group. After collection, each oyster was scrubbed with a bristle brush to remove any sand, debris, or biofouling on the shells, then rinsed and sorted. Scrubbing and rinsing were carried out using local seawater collected onsite near each growing bed. After washing, oysters were systematically sorted by groups of 5 into 3 composites of 20. Each composite was placed in a sealable, labeled plastic bag surrounded by an additional plastic bag for reinforcement. Samples were maintained in ice-filled coolers and were delivered the same day to AM Test Laboratories (Redmond, Washington) for Cd analysis.

#### Sample Preparation and Analysis

All oysters were shucked and mixed into their respective composite samples. Composite samples were blended and weighed. Average oyster weights were calculated by dividing the total composite weight by the number of oysters in the composite. Sediment samples and blended oyster composites were digested by open-vessel microwave according to EPA's Office of Solid Waste Manual (SW-846) Method 3050B and analyzed for Cd according to SW-846 Method 6010B, using inductively coupled plasma-atomic emission spectroscopy (ICP-AES). Oyster results were reported on a wet weight basis in order for comparison with the domestic and international limits for Cd in oysters, and sediment samples were reported on a dry weight basis to eliminate the dilution factor presented by seawater. The ICP-AES detection limit was 0.0005 ppm for Cd in oyster tissue (wet wt) and 0.05 ppm for Cd in sediment (dry wt).



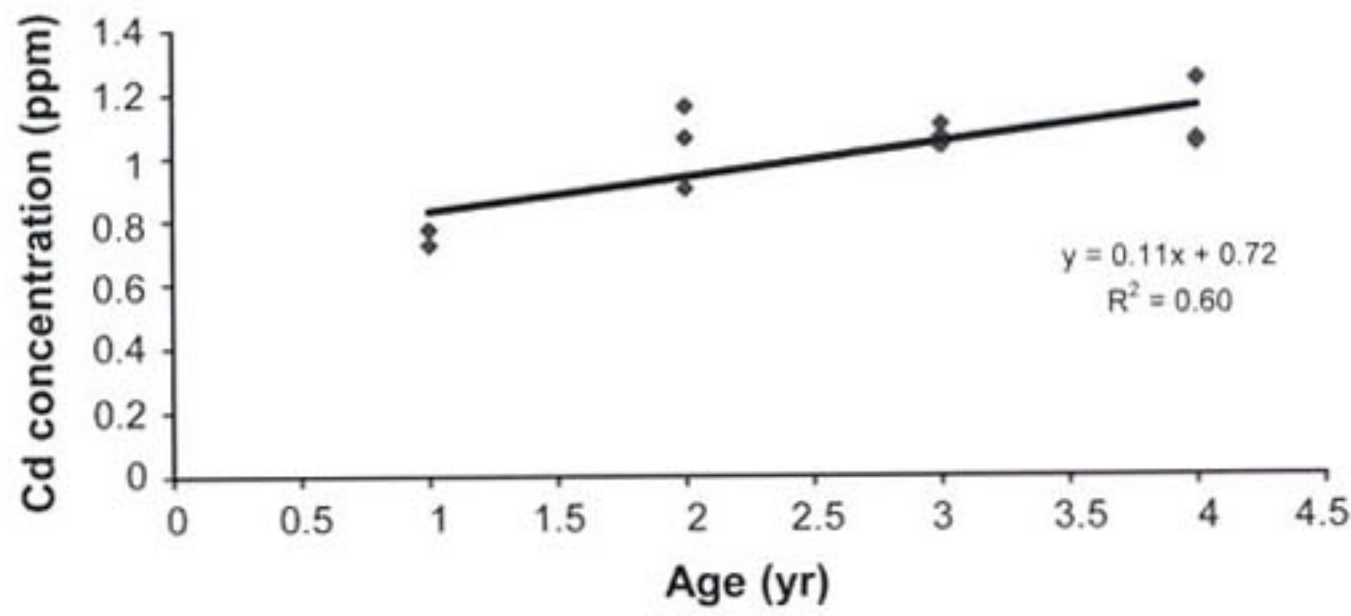


Figure 1. Correlation between Cd concentration and age in Pacific oysters.

**Statistical Methods**

Significant differences in Cd concentrations and shucked oyster weights among age groups were determined by one-way Analysis of Variance (ANOVA), Tukey test, with a significance level set at  $P < 0.05$ . All statistical analyses were carried out with SPSS 13.0 for Windows.

To determine the significance of correlations between oyster Cd levels and age or sediment Cd, graphs were created in which the Cd concentration or overall load (y-axis) was plotted against oyster age class (x-axis). Best fit linear regression lines were developed and the  $R^2$  values were calculated with Microsoft Office Excel 2003.

**Weight Study**

**Sample Collection**

Oysters from 4 age groups (1-, 2-, 3- and 4-y-olds) were collected in August 2005 from an oyster farm in Willapa Bay. These oysters were collected from the same sites outlined in the age study using the same predetermined shell lengths (Table 1). A modification of the sampling protocol described earlier was carried out, in which 25 oysters were collected at each site, resulting in a total of 100 oysters. For the weight study the oysters were treated as individual samples and were not grouped into composite samples as in the age study. After collection, the oysters were scrubbed and rinsed with local seawater and placed in labeled sealable plastic bags. The bagged samples were packed in ice-filled coolers and driven to the Oregon State University Seafood Laboratory (OSU-SFL) in Astoria, Oregon, for sample preparation.

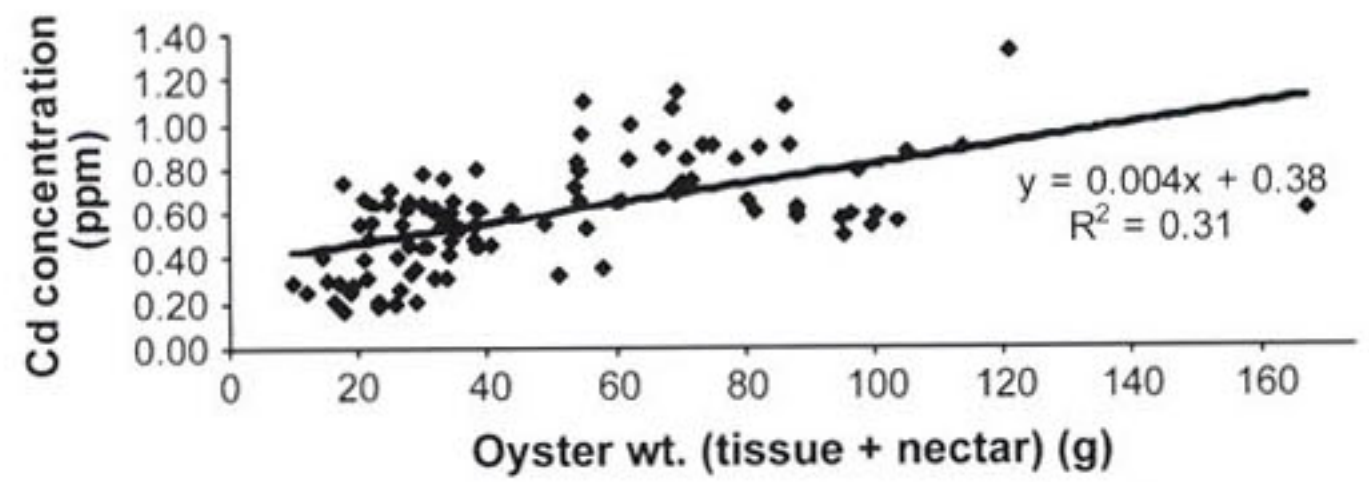


Figure 2. Relationship between Cd concentration and individual oyster tissue weights for all age groups combined.

**Sample Preparation and Analysis**

On arrival at the OSU-SFL, each oyster was shucked and individually weighed. Weights included the oyster tissue and its surrounding nectar. Each shucked sample (tissue and nectar) was then blended for 1 min in a glass jar using an Osterizer homogenizer. The same procedure was carried out for each oyster to give a total of 100 individual samples. Blended samples were double-bagged in sealed Ziploc Freezer Bags and frozen at  $-18^{\circ}\text{C}$  over the weekend. On the following Monday, the bags were packaged in coolers with ice packs and shipped by FedEx overnight to AM Test Laboratories in Redmond, Washington. At AM Test Laboratories, the 100 oyster samples were individually digested according to SW-846 Method 3050B and analyzed for Cd concentration by ICP-AES, SW-846 Method 6010. Results were reported on a wet weight basis.

**Statistical Methods**

To evaluate the relationship between the individually measured oyster weights and Cd levels, graphs were created with the Cd concentrations on the y-axis and the shucked oyster weights on the x-axis. Best fit linear regression lines were developed and the  $R^2$  values were calculated using Microsoft Office Excel 2003.

**RESULTS AND DISCUSSION**

**Age Study**

As shown in Table 1, all oyster Cd concentrations were below both the 3.7 ppm level of concern set by the FDA and the 2 ppm Hong Kong limit. The 1-y-old oysters had a significantly ( $P < 0.05$ ) lower Cd concentration (0.75 ppm, range

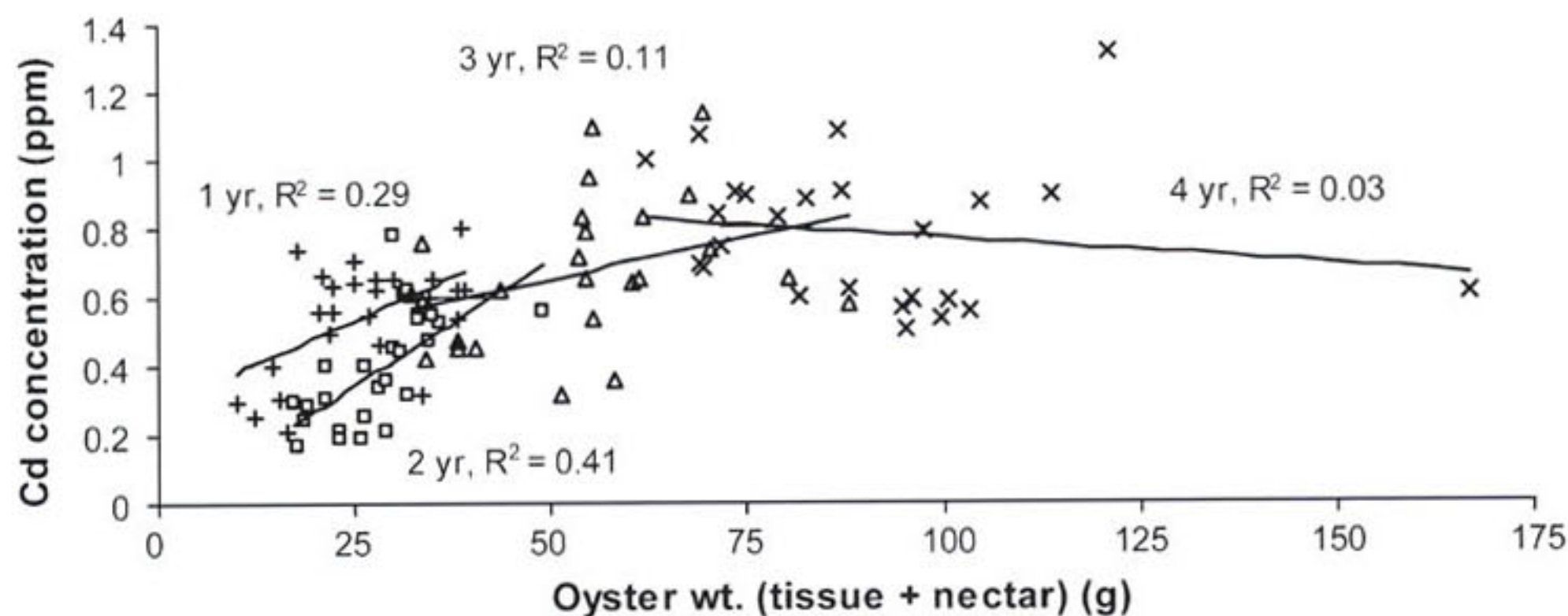


Figure 3. Relationship between the Cd concentration and individual oyster tissue weights, grouped by age class. +, 1 yr old; □, 2 yr old; Δ, 3 yr old; ×, 4 yr old.



0.72–0.77 ppm) compared with the older age classes, and there was relatively high variability in Cd concentrations among the 2- and 4-y-olds (ranges 0.90–1.16 ppm and 1.04–1.24, respectively). Although there was no statistical difference among the Cd concentrations in the 2-, 3-, and 4-y-olds, there were slight increases with each successive increase in yr class, with average Cd concentrations of 1.04, 1.06, and 1.11 ppm, respectively. There was a moderate correlation between age and Cd concentration, with an  $R^2$  value of 0.60 (Fig. 1). These results indicate some influence of age on Cd concentration in West Coast Pacific oysters, especially during the first few years of life.

The composite oyster weights showed no statistical difference between the 1- and 2-y-olds; however, both the 3- and 4-y-olds were significantly heavier ( $P < 0.05$ ). This may be because of differences in sexual maturation and gonad development among the age groups. Sediment Cd concentrations ranged from 0.26–1.10 ppm, with no strong correlation to average oyster Cd levels, in agreement with previous studies (Christy 2005, Hayes et al. 1998).

### Weight Study

As shown in Figure 2, there was a slight correlation of individual oyster weights with Cd concentration, with an  $R^2$  value of 0.31 for all 100 oysters analyzed in the tissue weights study. However, when the oysters were separated by yr-class, it became apparent that the relationship between weight and Cd concentration varies with age (Fig. 3). There was a moderate correlation for the 1- and 2-y-olds ( $R^2 = 0.29$  and  $0.41$ , respectively), whereas the  $R^2$  values for the 3- and 4-y-olds dropped to 0.11 and 0.03, respectively. These results suggest that the oysters accumulate Cd as they grow, with the Cd concentration possibly reaching a saturation point after 3–4 y of growth. Variations in Cd accumulation trends could be caused by a number of growth-related changes in areas such as physiology, gonad development, food preferences, and MT/lysosome detoxification systems.

### Comparison With Other Studies

Previous studies investigating the influence of oyster age on Cd levels have reported mixed results (Table 2). In agreement with the present study, Frew et al. (1989) reported a positive correlation between shell thickness (an indicator of age) and Cd concentration ( $R^2 = 0.42$ ) in the dredge oyster (*Ostrea lutaria*). Pacific oysters grown in Posyet Bay, Japan also exhibited a tendency to accumulate Cd with age (Khristoforova & Chernova 1989). The study monitored Cd levels in spat and 1-y-old oysters over the course of a year. The observed increased Cd concentrations in older oysters were suggested to be a result of a reduced ability of the oysters to excrete Cd combined with increased MT binding and sequestration.

On the other hand, Robinson et al. (2005) recently reported no effect of age on Cd concentrations in 1.3- and 3-y-old oysters (*Saccostrea glomerata*) harvested in Australia, and Pacific oysters harvested in Tasmania were reported to decrease in Cd concentration as they aged (Thomson 1982). The Pacific oysters were transplanted as spat into two growing areas and they were monitored over the course of one growing season. An earlier study on Sydney rock oysters (*Crassostrea commercialis*) cultivated in New South Wales also reported a tendency for Cd

TABLE 2. A comparison of studies examining the relationship between age and Cd concentration in oysters.

Sample	Location	N	Cd Levels	Ages	Correlation of [Cd] With Age	Reference
Pacific oyster ( <i>C. gigas</i> )	Willapa Bay, WA, USA	12 composites of 20 oysters each	0.72–1.24 ppm (wet wt)	1, 2, 3, and 4 y	$R^2 = 0.60$	Present study
Oyster ( <i>S. glomerata</i> )	Numerous sites in Australia	48 oysters	From <0.5 to ~3.0 ppm (dry wt)	1.3 and 3 y	No effect of age on (Cd)	Robinson et al. (2005)
Dredge oyster ( <i>O. lutaria</i> )	Foveaux Strait, New Zealand	60 oysters	n/a (dry wt)	n/a (shell thickness used to indicate age)	$R^2 = 0.42$	Frew et al. (1989)
Pacific oyster	Posyet Bay, Japan	18 oysters	2.7–6.3 ppm (dry wt)	Spat and 1-y oysters analyzed for 1 y	Tendency towards Cd accumulation with age	Khristoforova & Chernova (1989)
Pacific oyster	Pipeclay Lagoon and Dart Island, Tasmania	15 oysters from each site for each sampling interval	n/a (dry wt)	Analyzed throughout the first growing year	Tendency for decreases in (Cd) with growth	Thomson (1982)
Sydney rock oyster ( <i>C. commercialis</i> )	New South Wales estuaries	20 oysters per age group	0.1–0.5 ppm (wet wt)	1.5, 2.5 and 3.5 y	Decrease in (Cd) with age	Mackay et al. (1975)



TABLE 3.

A comparison of studies examining the relationship between tissue weight and Cd concentration in oysters. Tissue weights are listed in terms of wet wt unless otherwise indicated.

Sample	Location	N	[Cd]	Tissue Weight Range (g)	Correlation of [Cd] With Weight	Reference
Pacific oyster ( <i>C. gigas</i> )	Willapa Bay, WA, USA	100 oysters	0.17–1.32 ppm (wet wt)	9.9–167.0	$R^2 = 0.31$ for all samples (correlation varies with age group [see Figure 3])	Present study
Pacific oyster	Washington State, USA	92 composite samples (20 oysters per composite)	0.4–2.5 ppm (wet wt)	16.7–80 (ave. wt calculated from the composite wt)	$R^2 = -0.08$	Christy (2005)
Oyster ( <i>S. glomerata</i> )	Australia	100 oysters for large cohort, 20–30 oysters for smaller cohorts	From ~0.1 to ~5 ppm, dry wt (estimated from available graphs)	From ~0.5 to ~2.8, dry wt (estimated from available graphs)	No sig. relationship in complete data set, but direct correlations within some cohorts (up to $R^2 = 0.69$ ) (only a portion of the data was provided)	Robinson et al. (2005)
Pacific oyster	Gironde estuary, France	40 oysters	Up to 6 ppm (wet wt)	n/a	In the soluble fraction: $R^2 = -0.46$ and $-0.44$ at 2 sites; no correlation at a 3rd site	Mouneyrac et al. (1998)
Dredge oyster ( <i>O. lutaria</i> )	Foveaux Strait, New Zealand	60 oysters	n/a (dry wt)	n/a	$R^2 < 0.01$	Frew et al. (1989)
<i>C. virginica</i>	mid-Chesapeake Bay region, USA	30–34 oysters per sampling, harvested annually for 2 y	From ~0.1 to ~19 ppm (wet wt)	~2 to ~35	Inverse correlation for body weight and ln [Cd] in 15 out of 24 sample groups	Phelps et al. (1985)
Pacific oyster	Pipeclay Lagoon and Dart Island, Tasmania	15 oysters from each site for each sampling interval	n/a (dry wt)	Analyzed throughout the first growing year	Decreased [Cd] with growth	Thomson (1982)
<i>O. edulis</i>	Menai Strait, N. Wales	38 oysters	n/a	0.02–2.5 g dry wt	$r = 0.98$ (log <sub>10</sub> graph)	Boyden (1977)
	Restronguet Creek, Cornwall	24 oysters	n/a	0.34–6.63 g dry wt	$r = 0.94$ (log <sub>10</sub> graph)	Boyden (1977)
Pacific Oyster	Menai Strait, N. Wales	39 oysters	n/a	0.01–4.3 g dry wt	$r = 0.99$ (log <sub>10</sub> graph)	Boyden (1977)
	Outer Harbour, Poole, Dorset	22 oysters	n/a	0.02–4.0 g dry wt	$r = 0.95$ (log <sub>10</sub> graph)	Boyden (1977)
	Restronguet Creek, Cornwall	30 oysters	n/a	0.07–11.6 g dry wt	$r = 0.92$ (log <sub>10</sub> graph)	Boyden (1977)
Sydney rock oyster ( <i>C. commercialis</i> )	New South Wales estuaries	59 oysters	0.1–0.5 ppm (wet wt)	2–14 g (ages 1.5, 2.5 and 3.5 y)	$r = -0.58$	Mackay et al. (1975)
Dredge oyster ( <i>O. lutaria</i> )	Foveaux Strait, New Zealand	15 oysters	~2.5 to ~7.3 ppm (wet wt)	2–30 g	Direct correlation between [Cd] and body weight until about 8–10 g; no correlation in larger oysters (up to ~30 g)	Nielson (1975)



concentration to decrease with age (Mackay et al. 1975). Oysters at 1.5 y had an average Cd concentration of 0.34 ppm, whereas oysters at 2.5 and 3.5 y were both reported to have average Cd concentrations of 0.21 ppm.

As with studies of age and Cd levels, reports on the effects of tissue weight have thus far been highly varied (Table 3). Early research into the effects of tissue weight on Cd concentration reported positive correlations between the  $\log_{10}$  of the Cd concentration when plotted against the dry tissue weights of a number of populations of Pacific oysters and *Ostrea edulis* (Boyden 1977). Similar to the results of the present tissue weight study, a direct correlation was reported for Cd concentration and body weight in smaller dredge oysters (Foveaux Strait, New Zealand), but not heavier ones (Nielson 1975). The authors suggested that the larger oysters reach an equilibrium point at which the rate of Cd uptake is equivalent to the rate of Cd excretion. Another study reported a positive correlation ( $r = 0.73$ ) between tissue weight and overall Cd load in Sydney rock oysters; however, the correlation with Cd concentration was negative ( $r = -0.58$ ) (Mackay et al. 1975). A number of additional studies have reported a lack of correlation between Cd concentration and tissue weight (Christy 2005, Frew et al. 1989, Mouneyrac et al. 1998, Robinson et al. 2005), whereas others have reported a tendency for Cd levels to decrease as weight increases (Boyden 1974, Mouneyrac et al. 1998, Phelps et al. 1985, Thomson 1982). Although one study reported no correlation, it did find that certain cohorts within its larger set of data showed a positive influence of weight on Cd concentration (Robinson et al. 2005).

The high variability reported in the literature for the influences of age and tissue weight on Cd levels in oysters indicates complex relationships with multiple factors involved. Differences in results may be because of variations in certain intrinsic and extrinsic properties, including species, ploidy, habitat, age and weight ranges, season, source of Cd exposure, and metal detoxification systems. For example, gonad tissue has been reported to have significantly different Cd concentrations than the rest of oyster tissue, with significant seasonal variations (Paez-Osuna et al. 1995, Robinson et al. 2005). Therefore, age and tissue weight may prove to have a different relationship with Cd levels in oysters with varying levels of gonad development. A recent study reported slightly different

trends in Cd accumulation for diploid and triploid oysters (Robinson et al. 2005). In general, triploids had lower and less variable Cd concentrations than diploids, possibly because of the lack of gonad development and spawning among the triploids. Whereas Robinson et al. (2005) did not report whether there were significant differences between the Cd levels in the diploids and triploids, ploidy may prove to complicate the relationship between age, tissue weight, and Cd concentration. In the present study, the 1- and 2-y-old oysters were triploids, whereas the 3- and 4-y-olds were diploids. Although further investigation is required, the stronger correlations found for tissue weight and Cd levels in the younger oysters may have been partially influenced by their ploidy characteristics.

## CONCLUSION

In conclusion, there was a slight effect of age and tissue weight on Cd levels. The 1-y-old oysters had significantly lower ( $P < 0.05$ ) Cd concentrations as compared with 2-, 3- and 4-y-olds, and the  $R^2$  value for age and Cd concentration was 0.60. Sediment Cd levels did not seem to have a direct influence on oyster Cd concentrations, indicating that more environmental factors are involved. Oyster tissue weight was found to influence Cd concentration to some extent ( $R^2 = 0.31$ ) when all 100 samples were plotted together. However, when the data were divided by age groups, the relationship between tissue weight and Cd levels was found to vary with age: there were stronger correlations among the 1- and 2-y-olds and decreasing correlation values among the 3- and 4-y-olds. Taken together, these results indicate that oysters accumulate Cd as they grow during the first two years of their lives, after which point the Cd levels may reach a saturation point during the 3rd and 4th years of life. Differences in ploidy may also have an effect on the accumulation of Cd in oysters; however, further research is necessary to investigate this relationship.

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