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Spatial and temporal variations in cadmium concentrations and burdens in the Pacific oyster (*Crassostrea gigas*) sampled from the Pacific north-west

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A R T I C L E   I N F O

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A B S T R A C T

Oysters from the north-west coast of Canada contain high levels of cadmium, a toxic metal, in amounts that exceed food safety guidelines for international markets. A first required step to determine the sources of cadmium is to identify possible spatial and temporal trends in the accumulation of cadmium by the oyster. To meet this objective, rather than sample wild and cultured oysters of unknown age and origin, an oyster "grow-out" experiment was initiated. Cultured oyster seed was suspended in the water column up to a depth of 7 m and the oyster seed allowed to mature a period of 3 years until market size. Oysters were sampled bimonthly and at time of sampling, temperature, chlorophyll-*a*, turbidity and salinity were measured. Oyster total shell length, dry tissue weights, cadmium concentrations ($\mu g\ g^{-1}$) and burdens (μg of cadmium oyster^{-1}) were determined. Oyster cadmium concentrations and burdens were then interpreted with respect to the spatial and temporal sampling design as well as to the measured physio-chemical and biotic variables. When expressed as a concentration, there was a marked seasonality with concentrations being greater in winter as compared in summer; however no spatial trend was evident. When expressed as a burden which corrects for differences in tissue mass, there was no seasonality, however cadmium oyster burdens increased from south to north. Comparison of cadmium accumulation rates oyster^{-1} among sites indicated three locations, Webster Island, on the west side of Vancouver Island, and two within Desolation Sound, Teakerne Arm and Redonda Bay, where point sources of cadmium which are not present at all other sampling locations may be contributing to overall oyster cadmium burdens. Of the four physio-chemical factors measured only temperature and turbidity weakly correlated with tissue cadmium concentrations ($r^2 = -0.13$; $p < 0.05$). By expressing oyster cadmium both as concentration and burden, regional and temporal patterns were demonstrated, which may have been missed if just concentration was determined.

1. Introduction

To meet the ever increasing pressure primarily from the developed nations, for a secure protein source, many countries have turned to finfish and shellfish aquaculture to meet this demand. Canada is no exception with aquaculture being aggressively expanded on both east and west coasts. On the west coast of BC, both provincial and federal governments initiated an aggressive expansion of the shellfish industry, notably for the Manila clam (*Venerupis philippinarum*) and oyster (*Crassostrea gigas*). Hence, it was a major setback to the shellfish industry when several shipments of BC oysters were rejected from the Hong Kong market for containing cadmium, a toxic metal, in excess of regulatory guidelines of 2 $\mu g\ g^{-1}$ cadmium wet weight (Kruzynski et al., 2002; Kruzynski, 2004).

In response to this rejection of product, a number of studies were initiated to address the source of cadmium to BC oysters such that in doing so, remedial and preventative measures could be enacted ensuring that the product could be safely grown without fear of high cadmium levels (Kruzynski et al., 2002; Kruzynski, 2004; Rasmussen et al., 2007; Lekhi et al., 2008; Widmeyer and Bendell-Young, 2008). An important first step in understanding sources of cadmium to oysters on the west coast of BC, is to determine spatial and temporal trends that may provide some insight as to possible routes of exposure, and secondly, to determine if physio-chemical factors such as temperature, turbidity and salinity and food availability influenced uptake and hence amounts of cadmium accumulated within the oysters. Here, at 24 sites located along the west coast of BC, that represented primarily oceanic versus coastal sites an oyster "grow-out" experiment was initiated in 2001. Cultured oyster seed was suspended in the water column and the oyster seed allowed to mature for a period of 3 years until market size. Oysters were sampled bimonthly and at time of sampling, temperature, chlorophyll-*a*, turbidity and salinity were

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measured, as was total shell length. Oyster dry tissue weights and cadmium concentration and burden were determined and interpreted with respect to the spatial and temporal sampling design as well as to the measured physio-chemical and biotic variables. Through such an analysis our objectives therefore were two-fold (1) to determine if regional or temporal patterns in cadmium accumulation by the oyster occurred, and (2) if accumulation was influenced by the measured physio-chemical factors.

2. Methods

2.1. Site selection

Twenty-four sites along the west coast of British Columbia were chosen for study (Fig. 1). Active oyster farming was ongoing at all sites. These 24 sites represented a regional distribution from south to north along coastal BC, an aerial length of close to 1000 km. The two most southern sites were (1) Sooke and (2) Sansum Narrows, located at the most southerly tip of Vancouver Island. Moving northward, seven sites were located in Barkley Sound on the most west side of Vancouver Island; (3) Webster Island, (4) Poett Nook, and five sites within (5) Uselet Inlet, (sites 5, 6, 7, 8 and 9 denoted UI-1, UI-2, UI-3, UI-4 and UI-5). Two sites were located in Nootka Sound, north of Barkley Sound and included (10) Kendrick and (11) Tlupana Inlets. Six sites were located in Desolation Sound, located on the west coast of the British Columbia mainland, on the east side of Vancouver Island. These included (12) Trevenen Bay, (13) Thor's Cove, (14) Teakerne Arm, (15) Redonda Bay, (16) Gorge Harbour and (17) Orchard Bay. Continuing northward, one site was located in Quatsino Sound, (18) Hecate Cove, the most northern sampling site on the west side of Vancouver Island. One site was located ca. midway between the most northern and southern sampling sites, (19) Rivers Inlet. Two sites were located on Haida Gwaii, (20) Rennell Sound and (21) Skidgate Inlet. The final three most northerly sites were located just below the Alaska, USA and BC, Canadian border and included (22) Porcher Island, (23) Metlakatla Bay and (24) Lax K'walaams. Each site was visited generally on a bimonthly basis, depending on ease of access and weather conditions.

2.2. Water chemistry

At each sampling occasion, depth profiles at 1 m intervals from the air/water interface, for temperature, chlorophyll-*a*, salinity, and turbidity were determined by Hydroson® (a multi-probe metre). Measurements were taken from the side of the boat, directly beside the oyster long-lines.

2.3. Oysters

All oysters originated from the same seed source (Coast Seafoods, Washington State, USA). There were two grow-out periods. The first was initiated in June 2001 (first grow-out) with samples collected bimonthly until August 2004. As there was concern that amounts of cadmium accumulated by the oysters may be dependent on the particular uptake characteristics of the seed deployed in June of 2001, and to replicate the findings of the first grow-out, a second grow-out period at 13 of the 24 sites was initiated in June

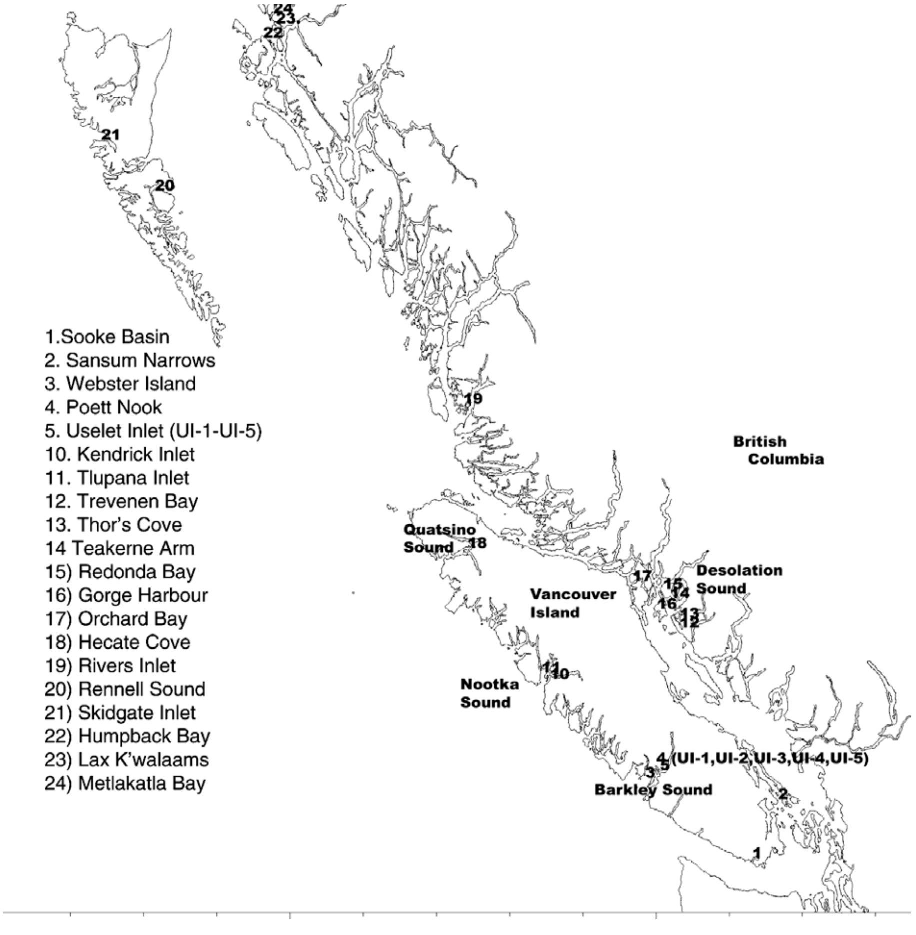

Fig. 1. Map of study area showing the approximate location of the 24 sampling sites.

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of 2003 until August 2004. Benefits from such an experimental approach include knowing genetic history, exact age of the oyster, and being able to compare across the 24 sites, with a known age group with all exposures for all sites beginning at the same time zero. The use of wild and cultured oysters of unknown age and unknown exposure history can confound interpretation of final results. For example, cadmium concentrations have been reported to increase, decrease or stay constant with age, weight and/or length (reviewed in Rasmussen et al. (2007)). Hence, no clear consensus as to the dependence of cadmium concentrations on oyster size has emerged. Use of a grow-out as applied here should help avoid misinterpretation of cadmium concentrations and burdens.

Oysters were sampled from all sites for the first grow-out. For the second grow-out, 13 of the 24 sites included, Webster Island (site 3), Poett Nook (site 4), Useless Inlet UI1 to UI-5, (sites 5–9), Treveren Bay (site 12), Thor's Cove (site 13), Teakern Arm (site 14), Redonda Bay (site 15), Gorge Harbour (site 16), and Orchard Bay (site 17). Sites 12–17, located on the coast of mainland BC were considered to have a greater influence from coastal processes, as compared to sites 1 and 3–11 located on the west coast of Vancouver Island, and on Haida Gwaii (20 and 21) which were primarily influenced by oceanic processes (Fig. 1). At each site, a seeded long-line was deployed. Each line was approximately 8 m long with seeded shells (cultch) inserted at 30 cm intervals beginning ca. 1 m from the surface. Three to five oysters were sampled approximately bimonthly, from shallow (1–3 m depth) to deep (4–7 m depth) positions along the long-line. Oyster shell length (at the maximum length), was recorded in the field at time of sampling. All oysters were transported on ice to the laboratory, where they were frozen whole until ready for analysis. Cultivated oysters are not depurate prior to market, hence we did not depurate oysters prior to freezing and subsequent cadmium analysis. For the first grow-out, June 2001, 2269 oysters were sampled, for the second-grow-out, June 2004, 501 oysters were sampled for a total of 2770 oysters.

2.4. Laboratory analysis

2.4.1. Dry weights

Oysters were thawed at room temperature and blotted with a paper towel. Dissections were done with sterile scalpel blades and acid-washed plastic knives. The shell was forced open with a scalpel and one of the adductor muscles severed to allow for the shell to be completely opened. The second adductor muscle was cut, the complete oyster removed from the shell, and blotted on kimwipe® to remove excess moisture. To obtain dry weights, individual oyster tissue ($n = 2770$) were placed into drying ovens at 60 °C for 2–3 days depending on size. Weight of dried samples was then recorded.

2.4.2. Cadmium analysis

Individual dried samples were homogenized by hand into a fine powder using a mortar bowl and pestle. To prevent cross-sample contamination, both mortar bowl and pestle were lined with weighing paper, which was changed after each specimen. Open-vessel digestions were performed in acid-washed Pyrex flasks. Sub-samples of approximately 0.15 g were digested from each specimen in 10 mL 70% Anachemia Environmental Grade nitric acid (in the instance that the oyster's dry weight was between 0.02 and 0.15 g, the entire oyster was digested). Solutions were brought to a constant temperature of 115 °C, boiled down to approximately 0.3 mL, at which point they were diluted to 10 mL with ddH_2O.

Analysis was carried out via flame atomic absorption spectrophotometry (Perkin Elmer Analyst 100). Internal quality control was based on the analysis of digested Standard Reference Material (SRM) oyster tissue 1566a and 1566b. In each digestion series, either 1566a or 1566b was included, along with a reagent blank. Determine concentrations were in agreement with the SRM cadmium values and no cadmium was detected in any of the reagent blanks. Cadmium limits of detection were 0.02 mg L^{-1}.

2.5. Statistical analysis

All analyses was conducted using Statistical Analysis System made available though Simon Fraser University (c) 2002–2003 by SAS Institute Inc., Cary, NC, USA. Note: SAS (r) 9.1 (TS1M3), Licensed to Simon Fraser University, Site 0009875003. Data normality was assessed through normality plots and when required, data was log transformed to meet the assumptions of the parametric tests. Significance for all tests was set at $p < 0.05$. Oyster cadmium was expressed as concentration (in μg g^{-1}) as well as burden (μg cadmium $oyster^{-1}$). To calculate oyster cadmium burdens, the individual cadmium concentration determined for each oyster was multiplied by the corresponding dry weight of the oyster that is, oyster cadmium concentration in ug $g^{-1} *$ dry weight of oyster in g resulting in μg of cadmium per individual oyster. To assess spatial trends, data collected for each site on a bimonthly basis was pooled and resulting averages plotted for each site; to assess temporal trends, data collected at each site over the 3 years sampling period was pooled and resulting averages plotted for each month sampled.

3. Results and discussion

3.1. First grow-out versus second grow-out oysters

To determine that patterns in cadmium uptake by the oyster were not related to a particular "seed" used at time of grow-out deployment, and to replicate observed trends of the first grow-out, oyster cadmium concentrations averaged over the time of sampling for the common thirteen sites for the first and second grow-outs were regressed. As regional patterns were the same ($r^2 = 0.78$; $p < 0.05$, Fig. 2), and given the limited amount of time that the second grow-out oysters were deployed for, further analysis focused on first grow-out oysters only.

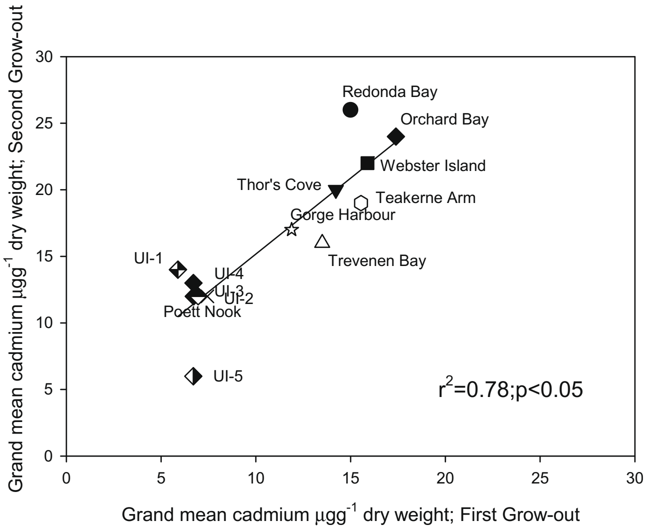

Fig. 2. Relationship between grand mean oyster cadmium concentrations averaged overall sampling periods for 13 of the 24 sites for first grow-out and second grow-out oysters. Regional patterns of cadmium uptake are the same between the two sets of oysters ($r^2 = 0.78$; $p < 0.05$). Unique symbols represent each site.

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3.2. Spatial and temporal trends; length, dry weight, cadmium concentration and cadmium burden

Spatial and temporal trends in oyster length, dry weight, cadmium concentration and burden were assessed by plotting the four variables against each sampling site with all months pooled (Fig. 3a–d) and for each month sampled with all sites pooled (Fig. 4a–d).

Average oyster length and weight over the 3 years sampling period was ca. 90 mm and 3 g with the exception of the most northern sites which were significantly smaller (Fig. 3a and b). Oysters sampled from the more shallow depths were also heavier by approximately two-fold as compared to those sampled at depths greater than 4 m. Oysters sampled from Barkley Sound tended to be heavier as compared to the other sampling sites. Greatest oyster cadmium concentrations ($\mu g\ g^{-1}$) were found in oysters sampled from Webster Island (site 3), on the west coast of Vancouver Island (Fig. 3c). Notable regional groupings of common oyster cadmium concentrations were those sampled from Barkley Sound (Poett Nook and Useless Inlet (UI-1 to UI-5) sites 4 and 5–9, respectively), which were lower than those sampled from the Desolation Sound region (Teakerne Arm, Redonda Bay and Redonda Bay, sites 14, 15 and 17, respectively) which generally were higher than all other sites. These regional differences however are in likely in part due to differences in the dry weight of the oyster from these sites. When expressed as the burden of cadmium given the different average dry masses only the smaller oysters from the most northern sampling sites had much less cadmium burdens as compared to other regions sampled. Without these sites, then a south-north trend became apparent with those oyster sampled from the southern regions containing less cadmium (in grams) per oyster as compared to their northern counterparts (Fig. 3d). Christy (2005) who in a similar survey, in north-west coastal USA, just south of the BC, Canada, Washington, USA border, also noted a slight northward and seaward increase in oyster tissue cadmium concentrations. Comparing the findings of Christy (2005) with those of the current study, oyster cadmium concentrations ranged from 2.97 to 16.25 $\mu g\ g^{-1}$ dry weight on 92 composite samples, values similar to those reported here.

Over the 36 months grow-out period, oysters increased linearly in length and exponentially in dry weight by three- and eight-fold, respectively (Fig. 4a and b). At the end of the study oysters were generally 120 mm in length corresponding to market size. Overall oyster cadmium concentrations demonstrated a seasonality being higher during winter months versus warmer summer seasons. Observed seasonal trends in cadmium concentrations have been related to changes in tissue weight due to the reproductive cycle

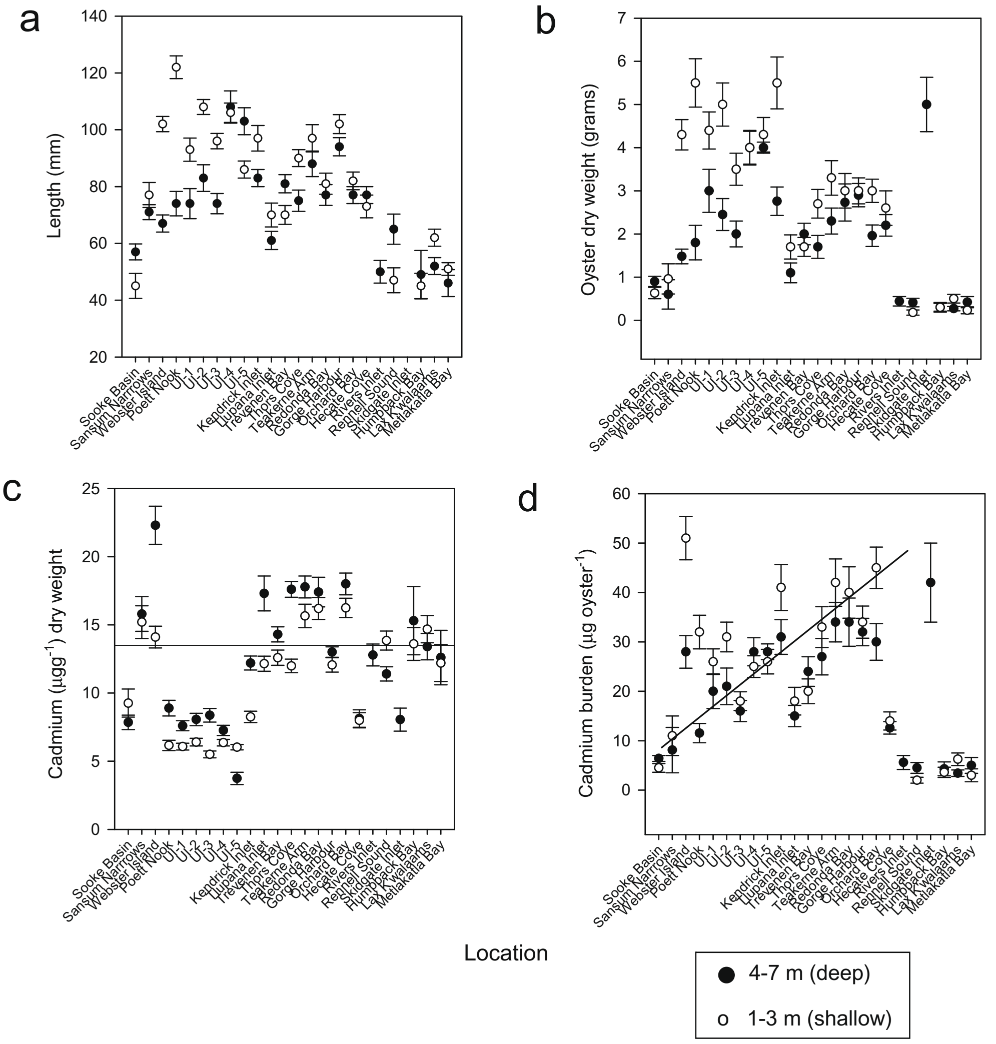

Fig. 3. (a–d). Oyster length, dry weight, cadmium concentration and cadmium burden (mean ± 1 S.E.) for each location. Sites are plotted in a south to north direction. Reference bar on Fig. 3c is the 13.5 $\mu g\ g^{-1}$ dry weight or 2 $\mu g\ g^{-1}$ wet weight reference point for rejection of oysters from the Hong Kong market.

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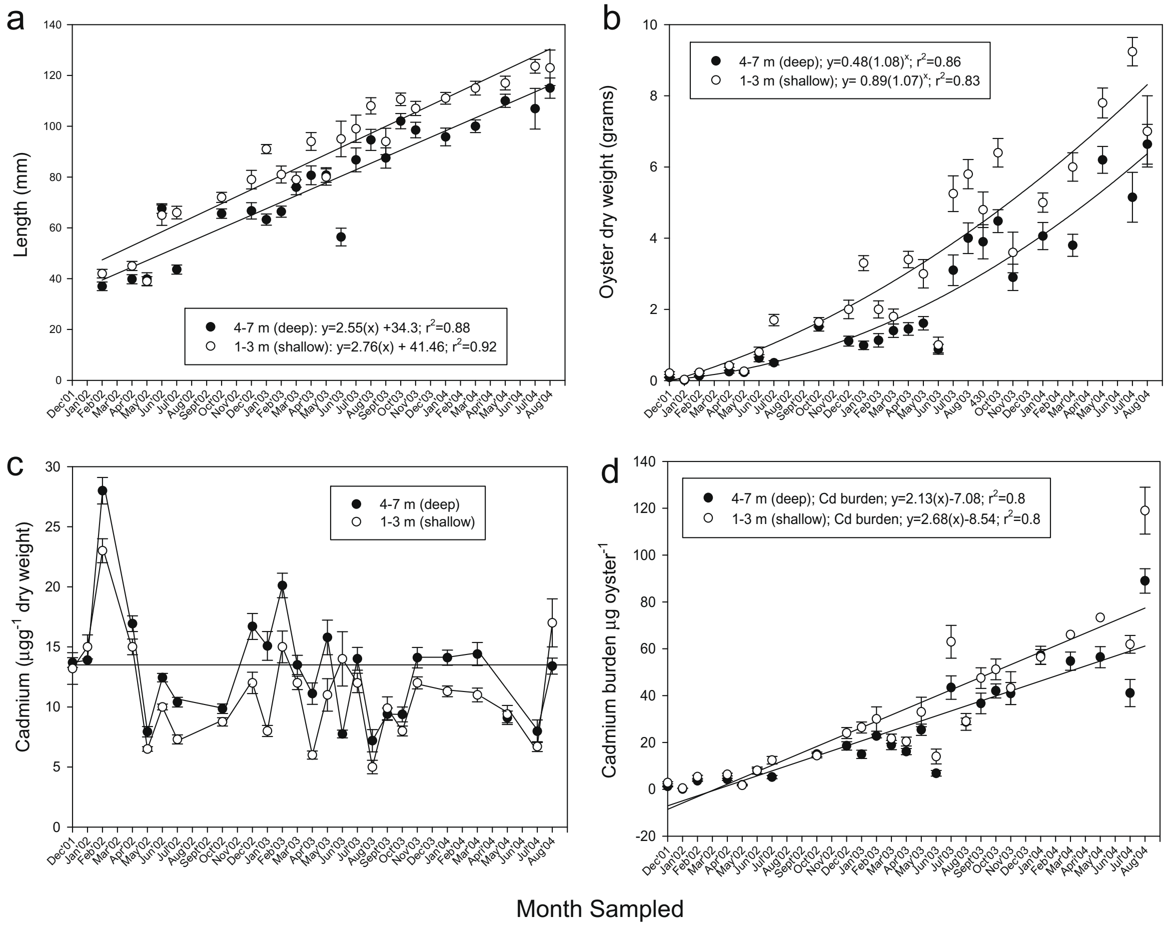

Fig. 4. (a–d). Mean oyster length, dry weight, cadmium concentration and cadmium burden (average ± 1 S.E.) for each month sampled. Reference bar in Fig. 4c is as in Fig. 3c.

and corresponding patterns of energy storage and mobilization (Costil et al., 2005; Dridi et al., 2007) with an increase in tissue weight associated with sexual maturation and generally occurring in spring–summer. Although the Pacific oyster does spawn during July and August, it requires a temperature of greater than 20 °C to do so. Throughout our 3 years sampling period, water temperature only once was greater than 20 °C, hence spawning would be unlikely during this time. As well, analysis of the increase in oyster mass with season for all sites indicates only one real deviation from a general increasing growth trend, June of 2003 where there was a marked decrease in oyster mass. If tissue cadmium concentrations were in part dependent on relative tissue mass, this decrease should have corresponded with an increase in cadmium concentrations, which did not occur.

Recently Lekhi et al. (2008) noted a similar seasonal trend for oysters sampled over a period of 1 year from one location on the west coast. These authors suggested that the higher and lower oyster cadmium concentrations in winter and summer, respectively may be in part due to growth dilution due to seasonal changes in tissue mass. However, as in our study, in the study of Lekhi et al. (2008), corresponding dry weights of the oyster did not support the hypothesis of growth dilution as a probable explanation for the observed seasonality in oyster cadmium concentrations. Oysters sampled in July with the lowest cadmium concentrations had dry weights comparable to oysters sampled in June and August which contained cadmium concentrations similar to those sampled in winter and spring months. If growth dilution was occurring, then given the constant mass of the oysters over June, July and August, oyster cadmium concentrations should have been low for all months.

When cadmium is expressed as a burden of cadmium oyster^{-1} and in contrast to concentrations, no seasonality or differences in depth of the cultivars was noted. Rather, oyster cadmium burdens increased linearly over the three year grow-out period (Fig. 4d). Relative oyster tissue mass then does influence concentrations of cadmium, and is likely the reason for higher concentrations of cadmium in oysters sampled from the deeper depths versus shallower depths, but cannot explain the seasonal trends in concentrations.

3.3. Differences in cadmium accumulation rates among sites

To compare cadmium accumulation among sites, cadmium burdens for each sampling location was regressed against month (Fig. 5). Slopes of the resulting regressions (µg cadmium oyster^{-1}) month^{-1} were then compared to determine at which site cadmium accumulation by the oyster was the greatest (Fig. 6). These included Webster Island, within Barkley Sound and Teakerne Arm and Redonda Bay located within Desolation Sound. These two regions are geographically distinct, with Barkley Sound strongly influenced by oceanic processes, whereas sites within Desolation Sound are more influenced by upland processes. Greater accumulation rates at these sites suggest a point source of cadmium to these oysters, which is not present at the other locations. This was most notable for oysters sampled from Webster Island which had the greatest concentrations and burdens as compared to all other sites.

Kruzynski et al. (2002) recently suggested possible sources of cadmium to oysters cultured on the coast of BC. For oysters sampled from sites located in Desolation Sound, given their close proximity to terrestrial influences, possible sources could include

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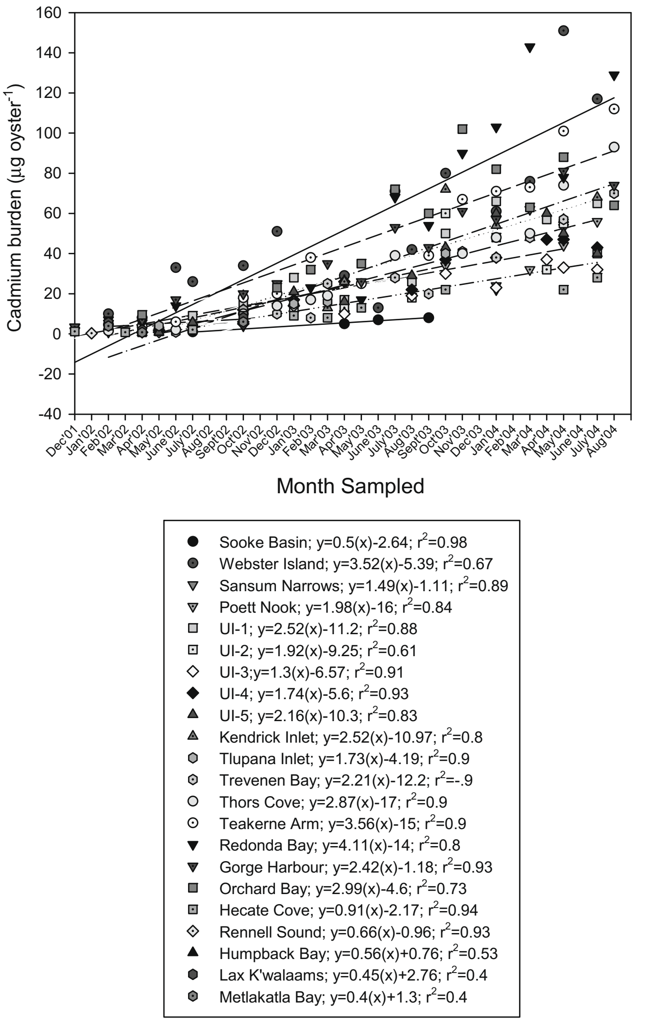

Fig. 5. Cadmium accumulation among sites by month for 22 of 24 locations. (Rivers and Skidgate Inlets were omitted due to insufficient data). All regressions are significant at $p < 0.05$.

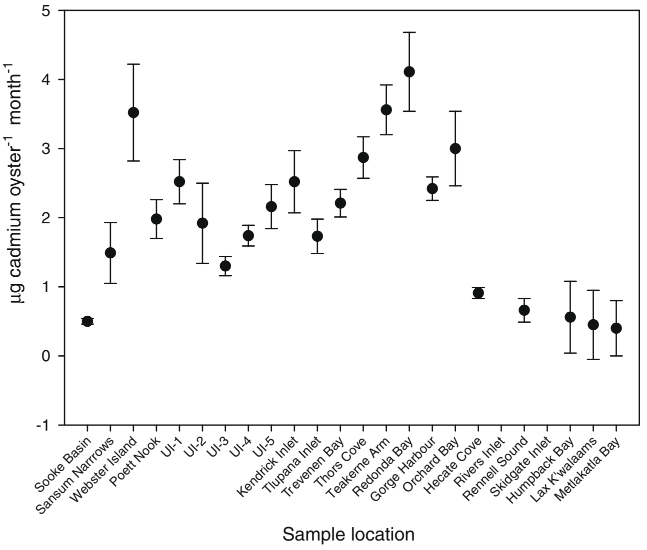

Fig. 6. Oyster cadmium accumulation by site (µg cadmium oyster^{-1}) month^{-1} ± 1 S.E. for 22 of 24 sites.

cadmium contaminated phosphate fertilizers and local septic tanks. Oysters sampled from Webster Island however, are more influenced by oceanic processes rather than direct anthropogenic influences. Possible sources at this one site could also be related to forestry practices within this region e.g. forest canopy removal with resulting erosion of soils naturally high in cadmium. Cadmium contaminated fertilizer applied during reforestation could also contribute to observed oyster cadmium concentrations at this site.

3.4. Oyster cadmium concentrations in relation to temperature, salinity, turbidity and chlorophyll-a

As water quality characteristics were taken continuously from surface water to depth, three values each at 1 m depth (i.e. 0.75, 1 and 1.25 m) and seven (i.e. 6.75, 7 and 7.25 m) were averaged for each site at each sampling period. Stepwise multiple regression where average cadmium concentrations and burdens for each site were entered into the regression model indicated that only temperature combined with turbidity negativity correlated to tissue cadmium concentrations ($p < 0.05$; Fig. 7). The combined r^2 was only -0.13, hence only 13% of the variation in cadmium concentrations are ascribed to these two variables. This would be consistent with the seasonal trends previously noted, where higher concentrations of cadmium are found in oysters during the colder seasons i.e. winter as compared to the warmer seasons. Surprisingly, there was no relationship noted for chlorophyll-*a*, a measure of productivity or food availability. Cadmium accumulation by bivalves can occur either via the gill through uptake of dissolved cadmium from the water column, or from their food, phytoplankton, provided through natural oceanic processes. If phytoplankton was the primary source of cadmium to oysters from the west coast, a strong correlation would be seen between phytoplankton abundance and oyster cadmium concentrations. Price and Morel (1990), reported that the marine diatom, *Thalassiosira weissflogii*, was able to physiologically substitute cadmium for zinc and maintain a 90% maximal growth rate. Subsequent work with other phytoplankton species has shown that cadmium can mimic zinc as an algal nutrient within a narrow species-specific range of zinc and cadmium concentrations (Lee and Morel, 1995). Because of their rapid growth during episodic "blooms", phytoplankton can often deplete essential trace metals such as zinc. Growth of the bloom may therefore be maintained by an increase in cadmium assimilation in lieu of zinc. Phytoplankton blooms are linked to coastal upwelling, which provide nutrient (including cadmium and zinc) rich deep waters to the photic zone, and are usually restricted to the spring and late summer months. Due to this higher natural cadmium concentration, cadmium could be accumulated to a greater degree by phytoplankton, particularly during blooms thereby providing a source of cadmium to the filter-feeding oyster. However, this was not observed.

Alternatively, dissolved cadmium may be a more important route of metal exposure to the oyster rather than its food. For example, Lekhi et al. (2008), demonstrated for one location on coastal BC, that dissolved cadmium could account for 50% of the cadmium determined in cultured oysters. These authors also concluded, based on observations at this one location, that uptake of dissolved cadmium via the gill was the primary route of cadmium exposure to BC oysters, a conclusion supported here, by the absence of a relationship between chlorophyll-*a* and cadmium oyster concentrations. However, Christy (2005), who determined cadmium concentrations in cultivated oysters collected from the intertidal region, from 31 sites along the north-west coast of the USA, did not find any relationship between oyster cadmium concentrations and concentrations of dissolved cadmium in water which were always less than 0.0005 mg L^{-1}.

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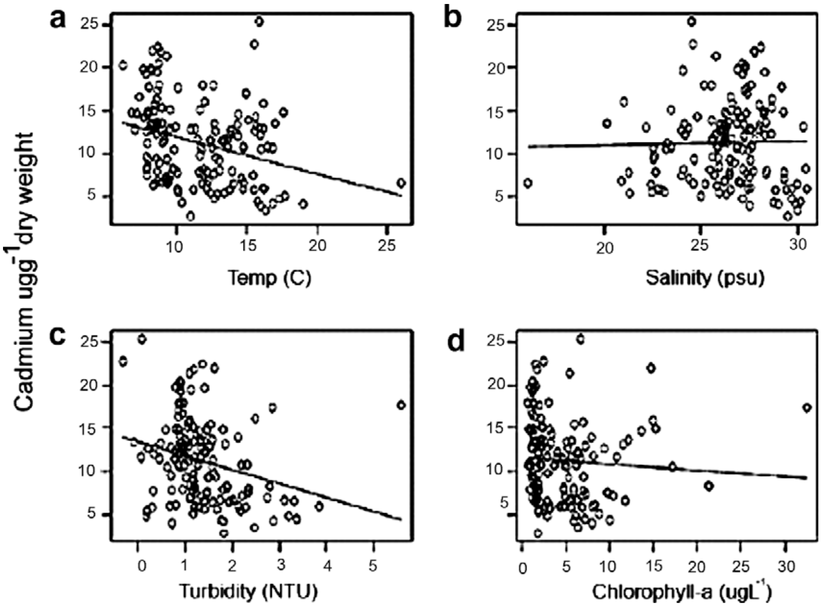

Fig. 7. Multiple regression between oyster cadmium concentrations and temperature, salinity, turbidity and chlorophyll-*a*. Only temperature and turbidity were significantly correlated to oyster cadmium concentration ($r^2 = -0.13$; $p < 0.05$).

4. Conclusions

Cadmium concentrations and burdens in oysters sampled from coastal BC were spatially and temporally dependent. When expressed as a concentration, there was a marked seasonality with concentrations being greater in winter as compared to summer. No clear spatial trend however was evident. When expressed as a burden which corrects for differences in tissue mass, there was no seasonality, however cadmium oyster burdens increased from south to north. Comparison of cadmium accumulation rates per oyster among sites indicated three locations, Webster Island, on the west side of Vancouver Island, and two within Desolation Sound, Teakerne Arm and Redonda Bay, where point sources of cadmium at these sites which are not present at all other sampling locations may be contributing to oyster cadmium burdens. By expressing oyster cadmium both as concentration and burden, regional and temporal patterns were demonstrated, which may have been missed if just concentration was determined.

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