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Sources of dietary cadmium to the Pacific oyster *Crassostrea gigas*

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ABSTRACT

Oysters from the Pacific north-west coast contain high cadmium concentrations (greater than 13.5 μg g^{-1} dry weight), which exceed consumer guidelines for international markets. Oysters are selective filter-feeders and attempts which have focused on suspended particulate matter (SPM) as a means to assess the importance of diet as a route of cadmium exposure have met with limited success. Here we use actual gut contents as an alternate to SPM to assess if this is a better predictor of cadmium exposure to the oyster via the diet. We also applied stable isotope analysis, $\delta^{13}C$ and $\delta^{15}N$, to determine the origin of organic material (from terrestrial to oceanic) ingested by the oyster. Oyster gut and tissue cadmium concentrations and corresponding isotopic signatures were determined every 2–3 months for 22 months from 10 locations on the west coast of B.C. Gut and tissue cadmium concentrations were correlated ($r^2 = 0.40$; $p < 0.05$), suggesting that diet could account for at least 40% of observed cadmium oysters residues. Oyster gut and tissue cadmium concentrations and $\delta^{13}C$ and $\delta^{15}N$ signatures were region dependent. Oysters from the most marine influenced sites contained gut and tissue cadmium residues of 30.4 ± 3.08 (S.E.) μg g^{-1} and 6.0 ± 0.6 μg g^{-1}, respectively, and a stable isotopic signature typical of marine phytoplankton. In contrast, oysters sampled from regions influenced by coastal processes contained significantly greater concentrations of cadmium, 43.0 ± 2.4 μg g^{-1} and 10.2 ± 0.68 μg g^{-1} gut and tissue, respectively, with isotopic signatures representative of terrestrial organic matter. This indicates that diet is an important source of cadmium to oysters from the Pacific north-west, however its importance is region dependent and cannot be simply ascribed to one source of organic matter alone.

1. Introduction

The ever increasing global demand for protein coupled with the decline in wild fisheries has resulted in countries such as Canada turning to aquaculture, both shellfish and finfish to meet this demand. Since 1998, on the west coast of Canada, the aquaculture industry has undergone aggressive expansion, in part to meet this global need, but also to gain from the economies of such industry. For the B.C. shellfish industry aggressive expansion was planned for the farming of Manila clams (*Venerupis philippinarum*) and oysters (*Crassostrea gigas*) (Kruzynski, 2004). Hence, the rejection of three shipments of B.C. oysters from the Hong Kong market, due to high cadmium levels, a toxic metal, in excess of 2 μg g^{-1} wet weight was seen as a major setback to what was being forecasted as an important economic driver for the industry (Kruzynski et al., 2002, 2004).

The outcome of this rejection that is, the potential loss of international markets, triggered a number of research initiatives to address the source of this cadmium to B.C. oysters (Kruzynski et al., 2002; Kruzynski, 2004; Rasmussen et al., 2007; Lekhi et al., 2008; Widmeyer and Bendell-Young, 2008). Further motivation came from Health Canada who issued oyster consumption guidelines of 460 g and 60 g per month for adults and children, respectively (Canadian Food Inspection Agency, 2003). More recent assessments of Cheng and Gobas (2007) and Widmeyer and Bendell-Young (2008), have indicated that these guidelines need to be much more stringent, especially for target populations such as First Nations.

The two main routes of cadmium exposure to the oysters and to bivalves in general are diet and direct uptake from the water column via the gill and mouth, as dissolved cadmium. Both have the potential to contribute with the relative importance of each source dependent on a number of abiotic (e.g., water chemistry) and biotic (e.g., selective filter-feeding by the bivalve) factors. The importance of diet however, has only been clearly demonstrated under controlled laboratory conditions (e.g., Ettajani et al., 2001; Reinfelder et al., 1997). Demonstrating diet as a route of exposure under field conditions is much more difficult. The most common approach for determining dietary exposure under field conditions is through filtering suspended particulate matter (SPM) from the water column, as a surrogate for what the oyster is filtering from the water column (e.g., Widmeyer and Bendell-Young, 2008; Lekhi et al., 2008). However, oysters are selective filter-feeders (Navarro and Thompson, 1995; Cognie et al., 2001; Bayne, 2002), and what is removed as SPM is not necessarily what the oyster has filtered from the water column as diet. Widmeyer and Bendell-Young (2008) concluded

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that suspended and deposited sediments were not good predictors of oyster cadmium concentrations. Similarly, Lekhi et al. (2008), concluded that cadmium concentrations of SPM filtered from the water column (three size fractions of 0.4–3, 3.0–20 and >20 μm), were poor predictors of tissue cadmium concentrations in cultured oysters. Indeed, there is yet a way to selectively remove from the seawater, exactly what the oyster has filtered from the water column.

It still remains then to assess the role of diet, that is, what the oyster has actually filtered from the water column and ingested, as a source of cadmium to the oyster. Given their highly selective feeding behavior, rather than measure suspended particulate matter (SPM) concentrations within the associated water column as an indicator of oyster diet, we chose to measure cadmium concentrations and the corresponding stable isotope signature of the actual gut contents that is, exactly what the oyster had filtered from the water column. Based on estimates from the literature on gut retention times, it is likely what we are measuring is that what the oyster has filtered within the last 8–10 h (Chaparro et al., 2001) and truly represents "dietary" exposure.

Stable carbon and nitrogen isotope ratios have become valuable tools for ecologists trying to define trophic levels and understand feeding relationships in aquatic communities (Fry and Sherr, 1984; Kwak and Zedler, 1997; Peterson et al., 1985; Riera et al., 1999). The differential fractionation of heavy (^{13}C and ^{15}N) and light (^{12}C and ^{14}N) carbon and nitrogen isotopes during food assimilation results in consumers exhibiting $\delta^{13}C$ and $\delta^{15}N$ signatures that are enriched over those of their food source (DeNiro and Epstein, 1978, 1981). Further, as the $\delta^{13}C$ signatures of organic matter will reflect the carbon source from which they are derived, tracing different organic sources through feeding consumers is possible (Stephenson and Lyon, 1982). The nitrogen stable isotope signature ($\delta^{15}N$) exhibits a relatively predictable enrichment of ≈3.5‰ through successive trophic levels (Minagawa and Wada, 1984) whereas the enrichment factor for $\delta^{13}C$ is less consistent (0–1.5‰) (Fry and Sherr, 1984). Photoautotroph $\delta^{13}C$ signatures will vary depending on the type of carbon fixation (i.e., C_3 vs. C_4 vs. CAM) and the inorganic carbon sources available (i.e., CO_2 and HCO_3^-). Riverine phytoplankton (∼−35‰), estuarine phytoplankton (∼−24‰) and terrestrial vegetation (∼−28‰) have comparatively light $\delta^{13}C$ signatures (Riera and Richard, 1997). Oceanic phytoplankton generally have $\delta^{13}C$ signatures between −20‰ and −22‰, while benthic diatoms have relatively heavy signatures of −14‰ to −16‰ (Riera and Richard, 1997). Given these distinct signatures, here we apply stable isotope analysis to determine the origins of organic matter ingested by the oyster to aid in elucidating sources of cadmium to these bivalves.

Our objectives are therefore twofold. First we determine cadmium concentrations in the gut (i.e., actual diet) and tissues of oysters, from 10 sites from coastal B.C., sampled every 2–3 months over a 2-year period to determine both the spatial and temporal variability in diet and tissue cadmium concentrations and to assess if gut cadmium does correlate to tissue cadmium concentrations. Second, we apply stable isotope analysis of the gut and tissue to determine the source of diet and thereby the source of cadmium via the diet to the oyster.

2. Methods

2.1. Study sites

All sampling sites were located along the southern coast of British Columbia, Canada, within two regions; Barkley Sound an open coastal embayment on the southwest coast of Vancouver Island and Desolation Sound a large Marine Park along the west coast of the mainland at the uppermost north end the Strait of Georgia (Fig. 1a and b). Four sites were located within Barkley Sound; (1)

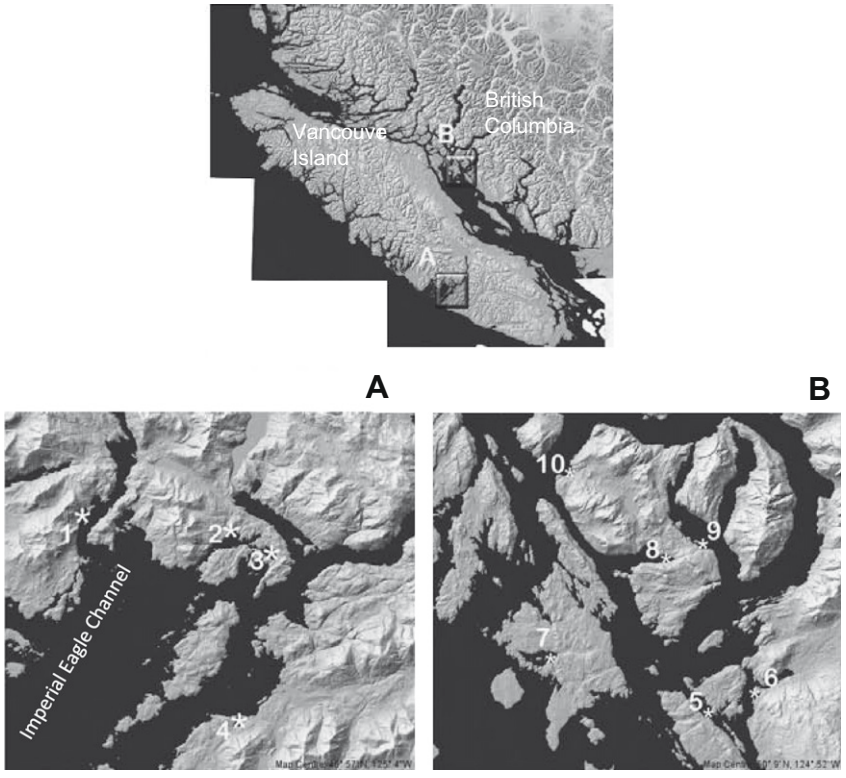

Fig. 1. The location of and sampling locations within the two regions on the west coast of B.C., Canada; (A) Barkley Sound (scale 1:144,546; map:ground), (B) Desolation Sound (scale 1:235,689; map:ground). 1. Effingham Inlet, 2. Seddall Island, 3. Fatty Basin, 4. Poett Nook, 5. Trevenen Bay, 6. Thor's Cove, 7. Gorge Harbour, 8. Teakerne Arm, 9. Orchard Bay, 10. Redonda Bay.

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Effingham Inlet, (2) Seddall Island, located in Useless Inlet, (3) Fatty Basin, a shallow, bowl-shaped, elongated depression, and (4) Poett Nook, a small, highly protected cove (Fig. 1a). The area has a significant freshwater supply via Poett Creek (personal observation). For Fatty Basin, connection to Barkley Sound is via two narrow passages, the "large gut" via Useless Inlet and the "small gut" via Rainy Basin. The constrictive nature of the two connecting passages strongly influences the oceanographic characteristics of the basin.

Within the larger region of Desolation Sound six study sites were chosen; (1) Trevenen Bay situated on the east side of Malaspina Peninsula at the south end of Malaspina Inlet, (2) Thor's Cove within Lancelot Inlet 4 km east of Trevenen Bay separated by Gifford Peninsula, (3) Gorge Harbor a highly protected site, due to its single narrow opening into the Strait of Georgia, on the west side of Cortes Island, (4) Teakerne Arm located at the end of the 7 km inlet, which of the region had the most significant freshwater input via a permanent waterfall, (5) Orchard Bay, located within Waddington Channel on the southwest side of West Redonda Island and (6) Redonda Bay, situated at the northwest tip of West Redonda Island (Fig. 1b). All sites are regions where active shellfish farming is occurring.

2.2. Water chemistry

Depth profiles of chlorophyll *a*, salinity, temperature and turbidity were measured at each site coincident with oyster sampling using a multi-parameter probe (YSI 6600 multi-parameter sonde). Complete profiles were taken from the air-surface/water interface to ~30 m depth. For regression analysis, 3–5 values from 5 to 7 m depth were averaged (S.E. were <1% for averaged values) to represent water chemistry characteristics for each site.

2.3. Sample analysis, tissue and gut

C. gigas juveniles of the same age and genetic stock were distributed to all sites in July 2001. The juveniles were grown on clean shells of con-specifics attached to two-strand polypropylene strings hung in the water column, a common aquaculture technique termed "string or long-line culture". For the purposes of this study, 3–10 oysters from 5 to 7 m depth, were collected from each of the sites at a minimum bimonthly basis beginning in October 2002 until August 2004. Samples were cleaned of epibonts by hand and transferred on ice to the laboratory to be frozen. They were later partially thawed, shucked and dissected into two components: (1) gut contents exclusive of digestive membrane and (2) remaining tissue (herein referred to as "tissue"). For subsequent cadmium analysis, both gut and tissue were dried to a constant weight at 60 °C and homogenized into a fine powder using mortar and pestle.

2.4. Stable isotope analysis

Sub-samples (1 mg) of gut and tissue were placed in 9 × 5 mm tin capsules for CF-IRMS analysis. The University of California Stable Isotope Facility conducted the analysis using a Europa 20/20 stable isotope analyzer. All isotopic data are given in the customary delta notation in units of parts per thousand (‰) relative to the standards Pee Dee Belemnite (PDB) for ^{13}C and atmospheric nitrogen for ^{15}N. Standard deviations for ^{13}C and ^{15}N were ±0.03‰ and ±0.06‰, respectively, for standards analyzed along with the samples. Analytical precision (±1 S.D.) based on triplicate analyses performed on some samples established good analytical reproducibility (±0.1‰ and ±0.2‰ for ^{13}C and ^{15}N) where

$$^{13}C_{sample} \quad \text{or} \quad ^{15}N_{sample} = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad \text{and}$$
$$R = {}^{13}C/{}^{12}C \quad \text{or} \quad {}^{15}N/{}^{14}N$$

2.5. Trace metal analysis

Sub-samples (gut and tissue) of 150 mg were digested with environmental grade nitric acid in 25 ml acid washed Erlenmeyer flasks. Samples were digested until all organics were converted to CO_2 and then boiled to dryness (0.5 ml). The remaining liquid was diluted with 10 ml de-ionized water and analyzed for cadmium with a Perkin–Elmer model 100 Atomic Absorption Spectrophotometer. Procedural blanks and reference material (NIST 1566b Oyster Tissue) were run with the samples as individual samples. The standard deviation of the analyzed reference material was ±0.2 $\mu g\, g^{-1}$ with accuracy for all samples within 10% of the certified values (2.48 ± 0.08 S.E.). All cadmium concentrations are expressed as $\mu g\, g^{-1}$ dry weight of sample (gut or tissue).

2.6. Statistical analysis

All statistical analyses were done using JMP v4.03. Initial tests for data normality (normality probability plots) indicated some deviations from normality with data demonstrating some degree of negative skewness, however, not enough to warrant data transformation for the use of non-parametric tests which tend to be less robust.

Relationships between water chemistry parameters (chlorophyll *a*, temperature, turbidity and salinity) and oyster tissue cadmium concentrations were assessed with simple linear regressions. Linear regressions were also used to analyze the relationship between gut and tissue cadmium concentrations. A simple Student's *t*-test was used to test for significant differences in the overall grand mean cadmium gut and tissue concentrations between the two regions. A multiple comparison ANOVA (Tukey–Kramer) was used to determine significant differences in cadmium concentrations among sampling periods within each site. A Tukey–Kramer was chosen because of the unequal number of samples available to test at each site over the course of the sampling. Dual isotope plots with 95% confidence intervals were used to assess differences in $\delta^{13}C$ and $\delta^{15}N$ signatures between sites during sampling periods. Linear regressions were used to analyze the relationship between $\delta^{13}C$ in tissue and gut as well as relationships between gut and tissue cadmium concentrations and $\delta^{13}C$ signatures. The level for significance for all tests was $p < 0.05$.

3. Results

3.1. Correlations between water chemistry parameters and oyster tissue cadmium concentrations

Of the four factors measured (temperature, chlorophyll *a*, salinity, and turbidity), oyster cadmium tissue concentrations were only weakly dependent on temperature ($r^2 = -0.22$; $p < 0.05$) (Fig. 2).

3.2. Cadmium concentrations in gut versus tissue

Although the oyster is highly selective in its feeding, particles can be selected for based on size alone, rather than quality. This is especially true during periods of low particle concentrations (Navarro and Thompson, 1995). To ensure that poor quality gut components were not contributing to amounts of cadmium measured in the gut (that is, gut cadmium levels could be related to gut inorganic content rather than associated with organic content), on a sub-sample of oysters, we determined the relationship between amounts of cadmium measured in the gut with gut inorganic content. Inorganic content was determined by igniting the gut contents at 450 °C for 4 h and weighing the remaining residue. Gut inorganic

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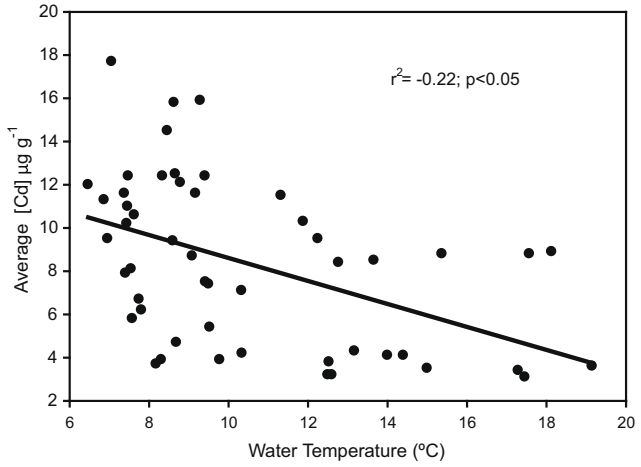

Fig. 2. Average oyster tissue cadmium determined for all sites and sampling periods versus water column temperature taken at 3–5 m depth. $r^2 = -0.22$ and is significant at $p < 0.05$, $N = 68$). Values are means of 3–10 oysters. Standard errors omitted for clarity but are presented in Fig. 4.

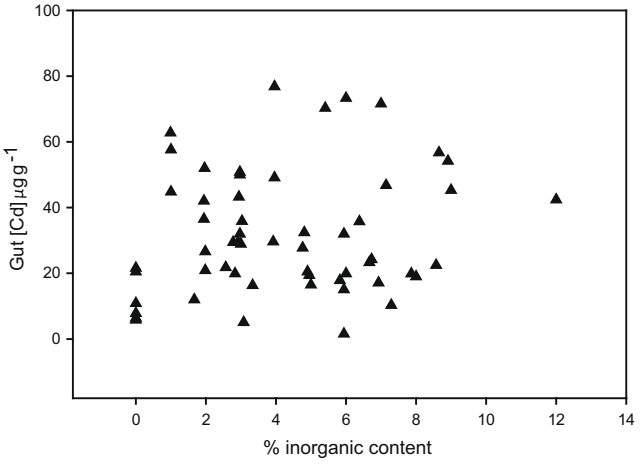

Fig. 3. Cadmium in B.C. oyster gut in $\mu g\ g^{-1}$ dry weight versus gut% inorganic content. $N = 55$ individual oysters.

content ranged from 4% to 12%, with no relationship between % inorganic content and gut cadmium concentration (Fig. 3). Cadmium gut concentrations were shown to be significant positive predictors of cadmium tissue levels accounting for close to 40% of observed tissue cadmium concentrations ($r^2 = 0.4$; $p < 0.05$; Fig. 4).

3.3. Oyster cadmium concentrations; between region and within site comparisons

Average cadmium concentrations for tissue and gut, for each site and the two regions during the sampling periods are presented in Fig. 5a, for gut and b for tissue. Standard errors have been omitted for figure clarity however, are presented in Fig. 4. Overall oysters sampled from Desolation Sound contained greater amounts of gut and tissue cadmium levels (43.0 ± 2.4 (S.E.) $\mu g\ g^{-1}$ and $10.2 \pm 0.68\ \mu g\ g^{-1}$, respectively) than their Barkley Sound counterparts ($30.4 \pm 3.08\ \mu g\ g^{-1}$ and $6.0 \pm 0.6\ \mu g\ g^{-1}$, respectively) (Student's *t*-test; $p < 0.05$.). Maximum gut cadmium concentrations of $80.4\ \mu g\ g^{-1}$ were determined for oysters sampled from Effingham Inlet in Barkley Sound during January 2004 (Tukey–Kramer; $p < 0.05$) (Fig. 5a). Within Desolation Sound the significantly highest gut content concentration was $91.9\ \mu g\ g^{-1}$ in oysters sampled from

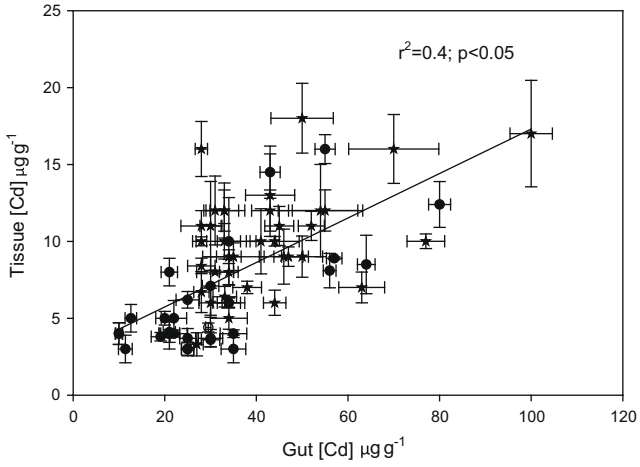

Fig. 4. Relationship between tissue and gut cadmium over all sampling periods for all locations. Values are means of 3–10 oysters ± 1 S.E. $r^2 = 0.4$; $p < 0.05$. ✸ are oysters from Desolation Sound, ● are oysters from Barkley Sound $N = 68$.

Orchard Bay during May 2003 (Tukey–Kramer; $p < 0.05$) (Fig. 5a). The greatest tissue cadmium concentrations determined for oysters sampled from Barkley Sound, were again found for those oysters sampled from Effingham Inlet, April 2003 ($16.1\ \mu g\ g^{-1}$; Tukey–Kramer; $p < 0.05$; Fig. 5b). For Desolation Sound, maximum oyster tissue cadmium concentrations of $26.1\ \mu g\ g^{-1}$ (Tukey–Kramer; $p < 0.05$) were again from oysters sampled from Orchard Bay in August of 2004 (Fig. 5b).

3.4. Stable isotope analysis

Dual isotope plots for gut and tissue at each site for all sampling periods are presented by region in Fig. 6a for Barkley Sound and 6b for Desolation Sound. The most striking difference between the two regions with respect to the stable isotopic signatures of oyster gut and tissue were the difference in ranges for primarily $\delta^{13}C$. Oyster gut and tissue from Barkley Sound contained a smaller range of mean carbon isotopic signatures (−21 to −19, Fig. 6a) as compared to Desolation Sound where signatures had a much greater range in values (−25 to −19 Fig. 6b). Those sites located within Barkley Sound displayed heavier $\delta^{15}N$ values (7–11, Fig. 6a) as compared to Desolation Sound sites which had lighter $\delta^{15}N$ values (6–9, Fig. 6b). Overall both isotopes indicated a distinction in diet between the two regions.

3.5. Using $\delta^{13}C$ isotopic signatures to assess sources of diet and hence cadmium to B.C. oysters

All sampling dates were pooled and average cadmium levels and $\delta^{13}C$ signatures for both oyster components were calculated for each site and relationships between the carbon isotope signature and cadmium levels for gut and tissue determined. The relationship between the isotopic signatures for gut and tissue was also determined. $\delta^{13}C$ in tissue and gut was highly correlated (Fig. 7; $r^2 = 0.93$; $p < 0.05$) indicating the role of $\delta^{13}C$ signatures of organic matter tracing different organic sources through consumers. A significant negative relationship was found between both gut and tissue cadmium and $\delta^{13}C$ (Fig. 8a and b; $r^2 = 0.67$ and 0.4, respectively; $p < 0.05$). Oysters sampled from sites located in Desolation Sound with higher cadmium concentrations had depleted $\delta^{13}C$ signatures indicative of more terrestrial sources as compared to samples from Barkley Sound, a region more influenced by marine sources of organic matter as indicated by the $\delta^{13}C$ (Riera and Richard, 1997).

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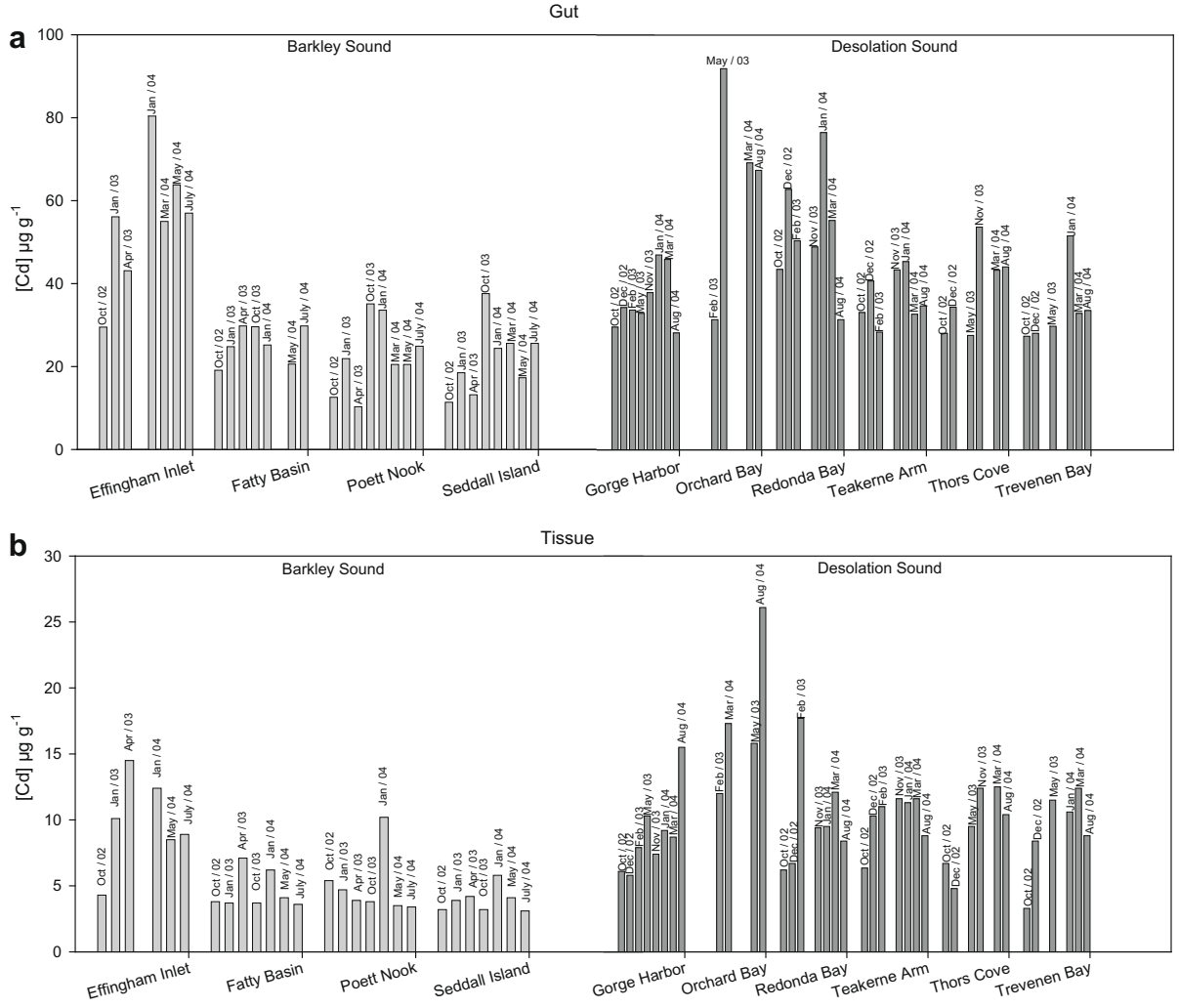

Fig. 5. (a) and (b) Average cadmium concentrations in gut and tissue for each site at for the two regions. Date of sampling has been provided for seasonal comparisons. Values are means of 3–10 oysters. Standard errors omitted for clarity but are presented in Fig. 4.

4. Discussion

4.1. The importance of diet as a source of cadmium to B.C. oysters

Cadmium concentrations determined for oyster gut contents were correlated with cadmium tissue levels ($r^2 = 0.4$; $p < 0.05$) suggesting that diet was an important contributor to oyster tissue cadmium concentrations. The importance of dietary uptake as a source of cadmium to filter-feeding bivalves has been demonstrated under laboratory conditions for a number of bivalves, including mussels (e.g., Arifin and Bendell-Young, 2000), oysters and clams (e.g., Ettajani et al., 1992; Reinfelder et al., 1997), supporting our findings here.

Roesjadi and Klerks (1989) have reported that the digestive membrane may be tissue components enriched with cadmium due to the presence of cadmium rich metallothioneins. For our study, removal of gut contents was done ensuring that none of the digestive membrane was included. Thus, measured cadmium gut concentrations could be attributed to just what the oyster had ingested with this gut cadmium accounting for 40% of the variation in oyster cadmium tissue residues. It is likely the remaining variation in oyster tissue cadmium residues (exclusive of gut contents) can be attributed to uptake of cadmium by the oyster in its dissolved form, either through the mouth or gill (e.g., Roesjadi and Robinson, 1994; Lekhi et al., 2008). What is most difficult to determine under field conditions however, is the relative importance of diet versus dissolved cadmium to determined cadmium residues. This is dependent on a complex interplay of both abiotic (e.g., salinity and temperature) and biotic factors (e.g., filter-feeding rate and diet selection) and will vary dependent on which factor is more important at a particular time or in a particular region.

For example, we found that oyster tissue cadmium residues were inversely related to temperature, possibly indicative of a slight seasonality effect on determined tissue cadmium. Lekhi et al. (2008) also noted a temperature affect on tissue cadmium levels, where for one location on the west coast of B.C., cadmium levels in oysters tissue sampled monthly over a period of a year were greatest during periods of lower temperature, i.e., winter, as compared to summer. It remains unclear why oysters would tend to have greater cadmium tissue residues during the colder winter months as compared to the warmer seasons. One possibility suggested by Lekhi et al. (2008) is during winter months, when filter-feeding is less, there is a net weight loss in tissue mass which would lead to an apparent increase in cadmium residues. Recently Bendell and Feng (2009) however, indicated that seasonal increases in oyster tissue cadmium could not be readily ascribed to changes in oyster mass alone.

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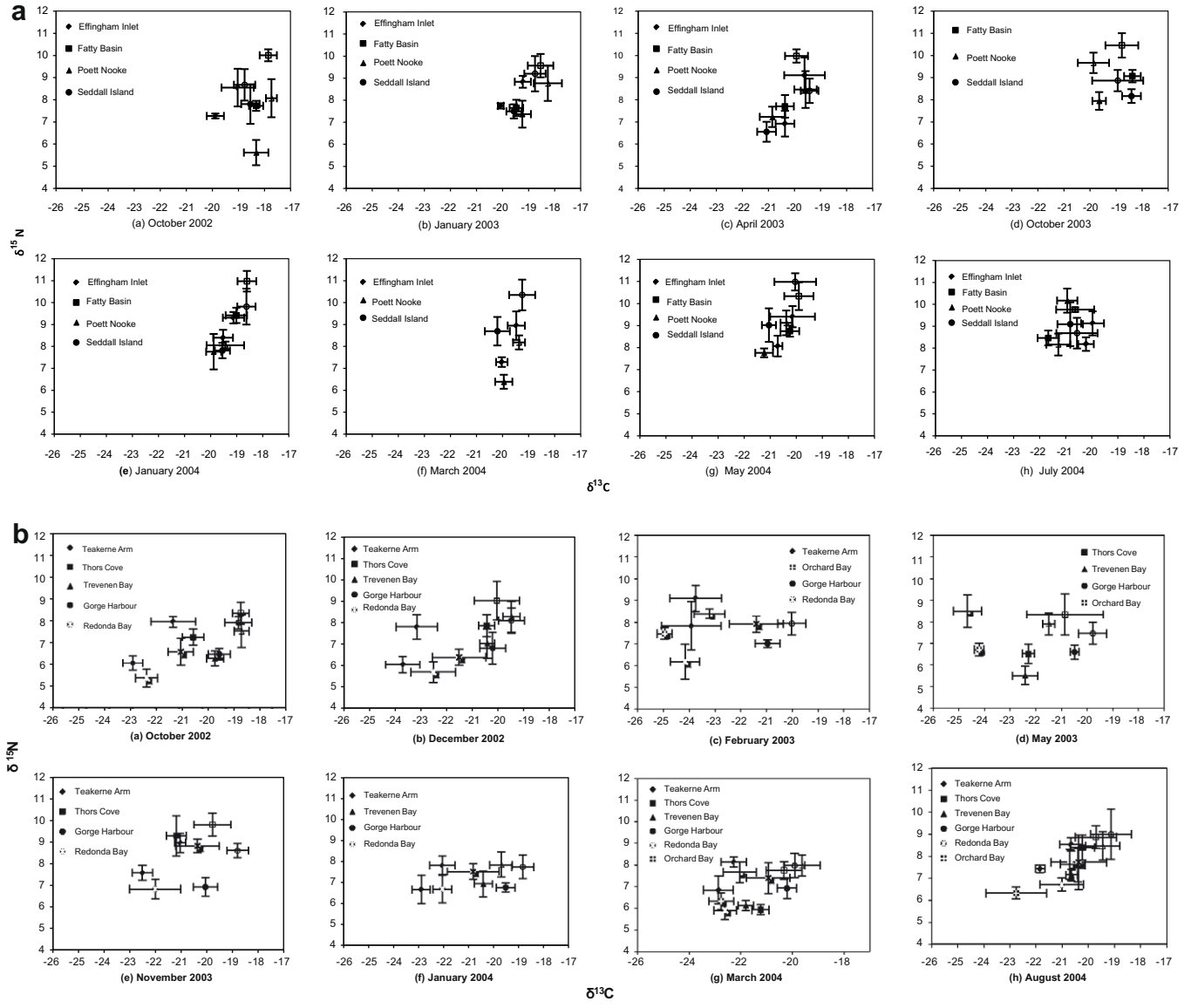

Fig. 6. (a) and (b) Dual isotope plots of average $\delta^{13}C$ and $\delta^{15}N$ signatures for gut (solid) and tissue (open) for oysters sampled from Barkley (a) and Desolation (b) Sounds during each sampling period over the 2-year study. Bi-directional error bars are 95% confidence intervals. Values are means of 3–10 oysters.

4.2. Use of stable isotopes to determine the source of diet to the filter-feeding oyster

Kaehler et al. (2000) through stable isotope analysis was able to demonstrate that although phytoplankton (oceanic derived particulate organic matter POM) was an important dietary component for both zooplankton and benthos, kelp-derived organic matter accounted for >30% of the nearshore animal's diet. Kang et al. (1999) also using stable isotopes were able to identify food sources of the suspension feeding cockle (*Cerastoderma edule*). These authors reported two major food sources that were age and season dependent. Bouillon et al. (2000) also successfully used stable isotope analysis to determine sources of suspended organic matter to zooplankton in an estuarine mangrove. These authors were able to determine that despite large amounts of terrestrial and mangrove detritus present in the water column, the locally produced phytoplankton were more important carbon sources for the zooplankton. Riera et al. (1999) used stable isotope analysis to provide a quantification of the relative importance of organic matter of different origins to suspension feeding bivalves. Surveys of bivalves along estuarine gradients have pointed to a trend of increasing incorporation of terrestrially derived detritus into the diet near upper reaches of the estuary (Incze et al., 1982; Stephenson and Lyon, 1982; Riera and Richard, 1996, 1997). Recently Hill et al. (2006) determined biogeographic and nearshore offshore trends in isotopic ratios of intertidal mussels and their food sources, both macroalgae and SPM around the coast of southern Africa. These authors noted, for mussels, as we found for oysters in this study, distinct geographic variation in tissue carbon signatures. However, tissue trends were not readily related to carbon values of SPM, again demonstrating that SPM filtered from the water column likely does not represent what the filter-feeding bivalve is actually selecting and hence ingesting as diet. Interestingly, Hill et al. (2006) noted that SPM exhibited trends of $\delta^{13}C$ depletion from near shore to offshore, suggesting a shift from a signature influenced by macroalgae to one more representative of oceanic phytoplankton.

4.2.1. Barkley sound

Within the region two significant site patterns were found. Effingham Inlet across all sampling time periods appeared to exhibit

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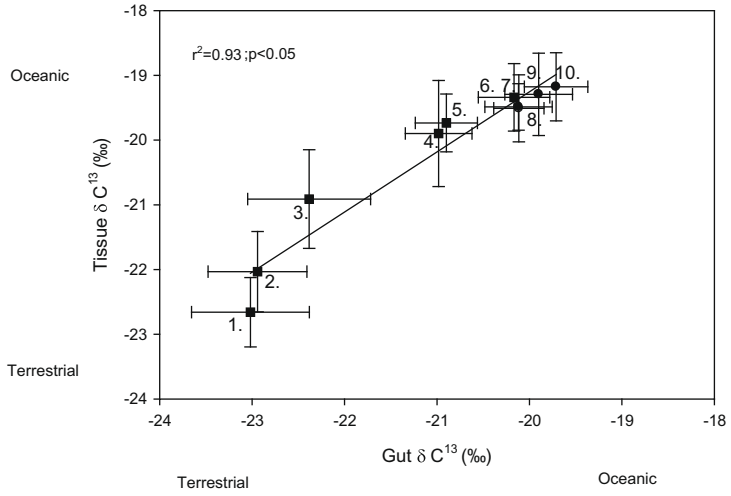

Fig. 7. Relationship between tissue and gut carbon signatures. ■ are oysters from Desolation Sound, ● are oysters from Barkley Sound, $r^2 = 0.93$; $p < 0.05$. Values are averages over all sampling times, ±S.E. Samples sizes are provided in Fig. 8.

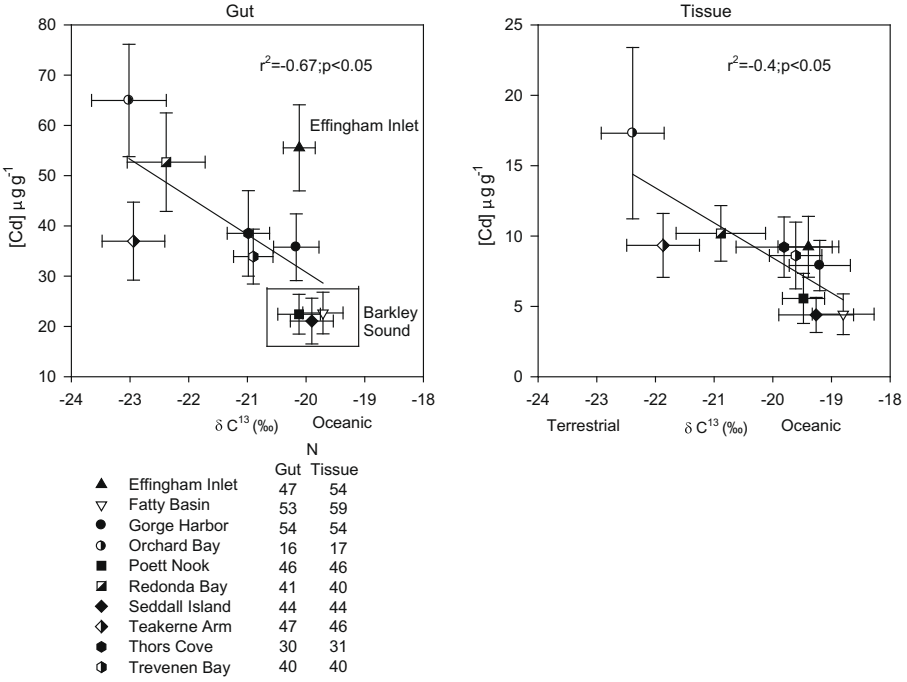

Fig. 8. Relationships between carbon signatures and cadmium gut (a) and tissue (b) concentrations. Values are averages over all sampling periods for each site ±S.E. Samples sizes provided in figure legend.

a diet comprised entirely of oceanic marine phytoplankton (−20 δ^{13}C, 7.60 δ^{15}N) (Riera and Richard, 1997). Given the sampling sites location, the mouth of the inlet, and the considerable influence Imperial Eagle Channel has on the food sources available at this site, this would be expected. Contrasting this was the heavier δ^{13}C and δ^{15}N signatures found within oysters sampled from for Fatty Basin (−19.7 δ^{13}C, 8.39 δ^{15}N) possibly due the influence of benthic microalgae in their diet. Because of differences in the carbon and nitrogen pools available to them, benthic microalgae are readily distinguishable (in terms of δ^{15}N and δ^{13}C signatures) than terrestrial vascular

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plants and phytoplankton (Kang et al., 1999). Unlike the other three sites within Barkley Sound, Fatty Basin is a shallow elongated depression readily mixed through wind and tidal action (Baillie and Welsh, 1980; De Jonge and Van Beusekom, 1992). This mixing would result in the suspension of benthic microalgae making it available to filter-feeders. Mixing would also allow for recently deposited particulate organic matter that has been degraded by consumers and isotopically enriched, to be resuspended and made available again as a food source to the oysters. Both processes would result in heavier $\delta^{15}N$ values as observed here.

4.2.2. Desolation Sound

In general across all the sampling periods the sites Teakerne Arm, Orchard Bay and Redonda Bay contained carbon signatures ($\delta^{13}C$ of −22.9, −23.0 and −22.4, respectively) that indicated that the diet of oysters from these locations contained more terrestrial organic matter as a food source as compared to oysters sampled from Barkley Sound ($\delta^{13}C$ range of −19.48 to −20.1) (Riera and Richard, 1997). Orchard Bay oysters appear to have had the most significant portion of their diet derived from upland origins, i.e., the most depleted of the carbon signatures. Oysters sampled from Gorge Harbor however, showed no terrestrial dietary influence with signatures indicative of marine sources of organic matter ($\delta^{13}C$ of −20.16). It should be noted that Gorge Harbor likely receives the most significant input from the Strait of Georgia, in terms of oceanic derived organic matter, and it is likely that their diet is consistently based on these inputs.

5. Conclusions

Based on the $\delta^{13}C$ and $\delta^{15}N$ analysis and determined tissue and gut cadmium concentrations, two main conclusions can be drawn; (1) gut contents correlated with cadmium residues found in oysters and (2) the type of organic matter (terrestrial or oceanic) being filtered and ingested by the oyster is region and site dependent, hence diet can contribute greater or lesser amounts of cadmium to the filter-feeding oysters depending on region and the site within the region. In Effingham Inlet, where both diet and tissue concentrations of cadmium in oysters were the greatest, oyster diet was comprised almost exclusively of marine phytoplankton. This was in contrast with oysters sampled from within the same region but at different sites which had much lower cadmium tissue and gut concentrations with diets appearing to be more mixed with other sources of marine organic matter (e.g., marine diatoms). Oysters sampled from regions that were less directly influenced by oceanic processes had gut carbon signatures characteristic of terrestrial organic matter, with corresponding high cadmium concentrations in both the gut and tissue.

Our findings suggest that the type of organic matter (terrestrial or oceanic) and amounts of cadmium associated with the organic matter on which the oyster is feeding, is region and site dependent. This then makes it difficult to predict where the oyster will be at greatest exposure via the diet to elevated cadmium levels, unless one goes specifically to the site of interest and measures dietary exposure directly at that site. Importantly, by measuring what the oyster had ingested, rather than SPM, we were able to demonstrate the importance of diet as a source of cadmium to the oyster, a route of exposure that would have been missed if SPM collected from the water column had been used as an indicator of dietary exposure alone.

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