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

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1.0 ABSTRACT. Oysters from the Pacific north-west coast contain high cadmium concentrations (greater than $13.5 \mu\text{g g}^{-1}$ 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, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, 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 two-three 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures were region dependent. Oysters from the most marine influenced sites contained gut and tissue cadmium residues of 30.4 ± 3.08 (S.E.) $\mu\text{g g}^{-1}$ and $6.0 \pm 0.6 \mu\text{g g}^{-1}$, 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, $43.0 \pm 2.4 \mu\text{g g}^{-1}$ and $10.2 \pm 0.68 \mu\text{g g}^{-1}$ gut and tissue respectively with isotopic signatures representative of terrestrial organic matter. This indicates that diet is an important

- 24 source of cadmium to oysters from the Pacific north-west, however its importance is region
25 dependent and cannot be simply ascribed to one source of organic matter alone.
- 26 **Key Words:** Oysters, cadmium, diet, organic matter, stable isotopes

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28 **2.0 INTRODUCTION**

29 The ever increasing global demand for protein coupled with the decline in wild fisheries
30 has resulted in countries such as Canada turning to aquaculture, both shellfish and finfish to meet
31 this demand. Since 1998, on the west coast of Canada, the aquaculture industry has undergone
32 aggressive expansion, in part to meet this global need, but also to gain from the economies of
33 such industry. For the B.C shellfish industry aggressive expansion was planned for the farming
34 of Manila clams (*Venerupis philippinarum*) and oysters (*Crassostrea gigas*) (Kruzynski 2004).
35 Hence, the rejection of three shipments of B.C. oysters from the Hong Kong market, due to high
36 cadmium levels, a toxic metal, in excess of $2 \mu\text{g g}^{-1}$ wet weight was seen as a major setback to
37 what was being forecasted as an important economic driver for the industry (Kruzynski et al.
38 2002, 2004).

39 The outcome of this rejection that is, the potential loss of international markets, triggered
40 a number of research initiatives to address the source of this cadmium to B.C. oysters (Kruzynski
41 et al. 2002, 2004, Rasmussen et al. 2007, Lekhi et al. 2008, Widmeyer and Bendell-Young
42 2008). Further motivation came from Health Canada who issued oyster consumption guidelines
43 of 460 g and 60 g per month for adults and children respectively (Canadian Food Inspection
44 Agency 2003). More recent assessments of Cheng and Gobas (2007) and Widmeyer and
45 Bendell-Young (2008), have indicated that these guidelines need to be much more stringent,
46 especially for target populations such as First Nations.

47 The two main routes of cadmium exposure to the oysters and to bivalves in general are
48 diet and direct uptake from the water column via the gill and mouth, as dissolved cadmium. Both
49 have the potential to contribute with the relative importance of each source dependent on a

50 number of abiotic (e.g., water chemistry) and biotic (e.g., selective filter-feeding by the bivalve)
51 factors. The importance of diet however, has only been clearly demonstrated under controlled
52 laboratory conditions, (e.g., Ettajani et al. 2001, Reinfelder et al. 1997). Demonstrating diet as a
53 route of exposure under field conditions is much more difficult. The most common approach for
54 determining dietary exposure under field conditions is through filtering suspended particulate
55 matter (SPM) from the water column, as a surrogate for what the oyster is filtering from the
56 water column (e.g., Widmeyer and Bendell-Young 2008 and Lekhi et al. 2008). However, oysters
57 are selective filter-feeders (Navarro and Thompson 1995, Cognie et al. 2001, Bayne 2002), and
58 what is removed as SPM is not necessarily what the oyster has filtered from the water column as
59 diet. Widmeyer and Bendell-Young 2008 concluded that suspended and deposited sediments
60 were not good predictors of oyster cadmium concentrations. Similarly, Lekhi et al. 2008,
61 concluded that cadmium concentrations of SPM filtered from the water column (three size
62 fractions of 0.4-3, 3.0-20 and $>20 \mu\text{m}$), were poor predictors of tissue cadmium concentrations in
63 cultured oysters. Indeed, there is yet a way to selectively remove from the seawater, exactly
64 what the oyster has filtered from the water column.

65 It still remains then to assess the role of diet, that is, what the oyster has actually filtered
66 from the water column and ingested, as a source of cadmium to the oyster. Given their highly
67 selective feeding behavior, rather than measure suspended particulate matter (SPM)
68 concentrations within the associated water column as an indicator of oyster diet, we chose to
69 measure cadmium concentrations and the corresponding stable isotope signature of the actual gut
70 contents that is, exactly what the oyster had filtered from the water column. Based on estimates
71 from the literature on gut retention times, it is likely what we are measuring is that what the

72 oyster has filtered within the last 8-10 hours (Chaparro et al. 2001) and truly represents “dietary”
73 exposure.

74 Stable carbon and nitrogen isotope ratios have become valuable tools for ecologists trying
75 to define trophic levels and understand feeding relationships in aquatic communities (Fry and
76 Sherr 1984, Kwak and Zedler 1997, Peterson et al. 1985, Riera et al. 1999). The differential
77 fractionation of heavy (^{13}C and ^{15}N) and light (^{12}C and ^{14}N) carbon and nitrogen isotopes during
78 food assimilation results in consumers exhibiting $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures that are enriched
79 over those of their food source (DeNiro and Epstein 1978, 1981). Further, as the $\delta^{13}\text{C}$ signatures
80 of organic matter will reflect the carbon source from which they are derived, tracing different
81 organic sources through feeding consumers is possible (Stephenson and Lyon 1982). The
82 nitrogen stable isotope signature ($\delta^{15}\text{N}$) exhibits a relatively predictable enrichment of $\approx 3.5\text{‰}$
83 through successive trophic levels (Minagawa and Wada 1984) whereas the enrichment factor for
84 $\delta^{13}\text{C}$ is less consistent (0 to 1.5‰) (Fry and Sherr 1984). Photoautotroph $\delta^{13}\text{C}$ signatures will
85 vary depending on the type of carbon fixation (i.e. C_3 vs C_4 vs CAM) and the inorganic carbon
86 sources available (i.e. CO_2 and HCO_3^-). Riverine phytoplankton ($\sim -35\text{‰}$), estuarine
87 phytoplankton ($\sim -24\text{‰}$) and (terrestrial vegetation ($\sim -28\text{‰}$)) have comparatively light $\delta^{13}\text{C}$
88 signatures (Riera and Richard 1997). Oceanic phytoplankton generally have $\delta^{13}\text{C}$ signatures
89 between -20‰ to -22‰ , while benthic diatoms have relatively heavy signatures of -14‰ to -16
90 ‰ (Riera and Richard 1997). Given these distinct signatures, here we apply stable isotope
91 analysis to determine the origins of organic matter ingested by the oyster to aid in elucidating
92 sources of cadmium to these bivalves.

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

99 **3.0 METHODS**

100 **3.1 Study Sites.** All sampling sites were located along the southern coast of British Columbia,
101 Canada, within two regions; Barkley Sound an open coastal embayment on the southwest coast
102 of Vancouver Island and Desolation Sound a large Marine Park along the west coast of the
103 mainland at the uppermost north end the Strait of Georgia (Figure 1a and 1b). Four sites were
104 located within Barkley Sound; (1) Effingham Inlet, (2) Seddall Island, located in Useless Inlet,
105 (3) Fatty Basin, a shallow, bowl-shaped, elongated depression, and, (4) Poett Nook, a small,
106 highly protected cove (Figure 1a). The area has a significant freshwater supply via Poett Creek
107 (personal observation). For Fatty Basin, connection to Barkley Sound is via two narrow
108 passages, the “large gut” via Useless Inlet and the “small gut” via Rainy Basin. The constrictive
109 nature of the two connecting passages strongly influences the oceanographic characteristics of
110 the basin.

111 Within the larger region of Desolation Sound six study sites were chosen; (1) Trevenen
112 Bay situated on the east side of Malaspina Peninsula at the south end of Malaspina Inlet, (2)
113 Thor’s Cove within Lancelot Inlet 4 km east of Trevenen Bay separated by Gifford Peninsula,
114 (3) Gorge Harbor a highly protected site, due to its single narrow opening into the Strait of

115 Georgia, on the west side of Cortes Island, (4) Teakerne Arm located at the end of the 7 km
116 inlet, which of the region had the most significant freshwater input via a permanent waterfall,
117 (5) Orchard Bay, located within Waddington Channel on the southwest side of West Redonda
118 Island and, (6) Redonda Bay, situated at the northwest tip of West Redonda Island (Figure 1b).
119 All sites are regions where active shellfish farming is occurring.

120
121 **3.2 Water Chemistry.** Depth profiles of chlorophyll-*a*, salinity, temperature and turbidity were
122 measured at each site coincident with oyster sampling using a multi-parameter probe (YSI 6600
123 multi-parameter sonde). Complete profiles were taken from the air-surface/water interface to
124 approximately 30 meters depth. For regression analysis, 3-5 values from 5-7 meters depth were
125 averaged (S.E. were < 1% for averaged values) to represent water chemistry characteristics for
126 each site.

127 **3.3 Sample analysis, tissue and gut.** *Crassostrea gigas* juveniles of the same age and genetic
128 stock were distributed to all sites in July 2001. The juveniles were grown on clean shells of con-
129 specific attached to two-strand polypropylene strings hung in the water column, a common
130 aquaculture technique termed “string or long-line culture”. For the purposes of this study, 3-10
131 oysters from 5-7 meters depth, were collected from each of the sites at a minimum bimonthly
132 basis beginning in October 2002 until August 2004. Samples were cleaned of epibionts by hand
133 and transferred on ice to the laboratory to be frozen. They were later partially thawed, shucked
134 and dissected into two components: (1) gut contents exclusive of digestive membrane and (2)
135 remaining tissue (herein referred to as “tissue”). For subsequent cadmium analysis, both gut and
136 tissue were dried to a constant weight at 60 °C and homogenized into a fine powder using mortar
137 and pestle.

138 **3.4 Stable Isotope Analysis.** One mg sub-samples of gut and tissue were placed in 9x5 mm tin
139 capsules for CF-IRMS analysis. The University of California Stable Isotope Facility conducted
140 the analysis using a Europa 20/20 stable isotope analyzer. All isotopic data are given in the
141 customary delta notation in units of parts per thousand (‰) relative to the standards Pee Dee
142 Belemnite (PDB) for ^{13}C and atmospheric nitrogen for ^{15}N . Standard deviations for ^{13}C and ^{15}N
143 were ± 0.03 ‰ and ± 0.06 ‰ respectively, for standards analyzed along with the samples.
144 Analytical precision (± 1 S.D.) based on triplicate analyses performed on some samples
145 established good analytical reproducibility (± 0.1 ‰ and ± 0.2 ‰ for ^{13}C and ^{15}N) where

$$146 \quad {}^{13}\text{C}_{\text{sample}} \text{ or } {}^{15}\text{N}_{\text{sample}} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

$$147 \quad \text{and } R = {}^{13}\text{C}/{}^{12}\text{C} \text{ or } {}^{15}\text{N}/{}^{14}\text{N}$$

148 **3.5 Trace Metal analysis.** Sub-samples (gut and tissue) of 150 mg were digested with
149 environmental grade nitric acid in 25 ml acid washed Erlenmeyer flasks. Samples were digested
150 until all organics were converted to CO_2 and then boiled to dryness (0.5 ml). The remaining
151 liquid was diluted with 10 ml de-ionized water and analyzed for cadmium with a Perkin Elmer
152 model 100 Atomic Absorption Spectrophotometer. Procedural blanks and reference material
153 (NIST 1566b Oyster Tissue) were run with the samples as individual samples. The standard
154 deviation of the analyzed reference material was $\pm 0.2 \mu\text{g g}^{-1}$ with accuracy for all samples within
155 10% of the certified values (2.48 ± 0.08 S.E.). All cadmium concentrations are expressed as μg
156 g^{-1} dry weight of sample (gut or tissue).

157 **3.6 Statistical Analysis.** All statistical analyses were done using JMP v4.03. Initial tests for data
158 normality (normality probability plots) indicated some deviations from normality with data

159 demonstrating some degree of negative skewness, however, not enough to warrant data
160 transformation for the use of non-parametric tests which tend to be less robust.

161 Relationships between water chemistry parameters (Chlorophyll *a*, temperature, turbidity
162 and salinity) and oyster tissue cadmium concentrations were assessed with simple linear
163 regressions. Linear regressions were also used to analyze the relationship between gut and tissue
164 cadmium concentrations. A simple student's t-test was used to test for significant differences in
165 the overall grand mean cadmium gut and tissue concentrations between the two regions. A
166 multiple comparison ANOVA (Tukey-Kramer) was used to determine significant differences in
167 cadmium concentrations among sampling periods within each site. A Tukey-Kramer was chosen
168 because of the unequal number of samples available to test at each site over the course of the
169 sampling. Dual isotope plots with 95% confidence intervals were used to assess differences in
170 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures between sites during sampling periods. Linear regressions were used to
171 analyze the relationship between $\delta^{13}\text{C}$ in tissue and gut as well as relationships between gut and
172 tissue cadmium concentrations and $\delta^{13}\text{C}$ signatures. The level for significance for all tests was p
173 < 0.05 .

174

175 **4.0 RESULTS**

176 **4.1 Correlations between water chemistry parameters and oyster tissue cadmium**

177 **concentrations.** Of the 4 factors measured (temperature, chlorophyll *a*, salinity, turbidity),
178 oyster cadmium tissue concentrations were only weakly dependent on temperature ($r^2=-0.22$; $p <$
179 0.05) (Figure 2).

180 **4.2 Cadmium concentrations in gut versus tissue.** Although the oyster is highly selective in
181 its feeding, particles can be selected for based on size alone, rather than quality. This is
182 especially true during periods of low particle concentrations (Navarro and Thompson 1995). To
183 ensure that poor quality gut components were not contributing to amounts of cadmium measured
184 in the gut (that is, gut cadmium levels could be related to gut inorganic content rather than
185 associated with organic content), on a subsample of oysters, we determined the relationship
186 between amounts of cadmium measured in the gut with gut inorganic content. Inorganic content
187 was determined by igniting the gut contents at 450°C for 4 hours and weighing the remaining
188 residue. Gut inorganic content ranged from 4-12%, with no relationship between % inorganic
189 content and gut cadmium concentration (Figure 3). Cadmium gut concentrations were shown to
190 be significant positive predictors of cadmium tissue levels accounting for close to 40% of
191 observed tissue cadmium concentrations ($r^2=0.4$; $p < 0.05$; Figure 4).

192 **4.3 Oyster cadmium concentrations; between region and within site comparisons.** Average cadmium
193 concentrations for tissue and gut, for each site and the two regions during the sampling periods are
194 presented in Figures 5a, for gut and 5b for tissue. Standard errors have been omitted for figure clarity
195 however, are presented in Figure 4. Overall oysters sampled from Desolation Sound contained greater
196 amounts of gut and tissue cadmium levels (43.0 ± 2.4 (S.E.) $\mu\text{g g}^{-1}$ and 10.2 ± 0.68 $\mu\text{g g}^{-1}$ respectively)
197 than their Barkley Sound counterparts (of 30.4 ± 3.08 $\mu\text{g g}^{-1}$ and 6.0 ± 0.6 $\mu\text{g g}^{-1}$, respectively) (students t-
198 test; $p < 0.05$). Maximum gut cadmium concentrations of 80.4 $\mu\text{g g}^{-1}$ were determined for oysters
199 sampled from Effingham Inlet in Barkley Sound during January 2004 (Tukey-Kramer; $p < 0.05$) (Figure
200 5a). Within Desolation Sound the significantly highest gut content concentration was 91.9 $\mu\text{g g}^{-1}$ in
201 oysters sampled from Orchard Bay during May 2003 (Tukey-Kramer; $p < 0.05$) (Figure 5a). The greatest
202 tissue cadmium concentrations determined for oysters sampled from Barkley Sound, were again found for
203 those oysters sampled from Effingham Inlet, April 2003 (16.1 $\mu\text{g g}^{-1}$; Tukey-Kramer; $p < 0.05$; Figure

204 5b). For Desolation Sound, maximum oyster tissue cadmium concentrations of $26.1 \mu\text{g g}^{-1}$ (Tukey-
205 Kramer; $p < 0.05$) were again from oysters sampled from Orchard Bay in August of 2004 (Figure 5b).

206 **4.4 Stable isotope analysis.** Dual isotope plots for gut and tissue at each site for all sampling
207 periods are presented by region in Figure 6a for Barkley Sound and 6b for Desolation Sound.

208 The most striking difference between the two regions with respect to the stable isotopic
209 signatures of oyster gut and tissue were the difference in ranges for primarily $\delta^{13}\text{C}$. Oyster gut
210 and tissue from Barkley Sound contained a smaller range of mean carbon isotopic signatures
211 (-21 to -19, Figure 6a) as compared to Desolation Sound where signatures had a much greater
212 range in values (-25 to -19 Figure 6b). Those sites located within Barkley Sound displayed
213 heavier $\delta^{15}\text{N}$ values (7-11, Figure 6a) as compared to Desolation Sound sites which had lighter
214 $\delta^{15}\text{N}$ values (6-9, Figure 6b). Overall both isotopes indicated a distinction in diet between the
215 two regions.

216 **4.5 Using $\delta^{13}\text{C}$ isotopic signatures to assess sources of diet and hence cadmium to B.C.**

217 **oysters.** All sampling dates were pooled and average cadmium levels and $\delta^{13}\text{C}$ signatures for
218 both oyster components were calculated for each site and relationships between the carbon
219 isotope signature and cadmium levels for gut and tissue determined. The relationship between
220 the isotopic signatures for gut and tissue was also determined. $\delta^{13}\text{C}$ in tissue and gut was highly
221 correlated (Figure 7; $r^2=0.93$; $p < 0.05$) indicating the role of $\delta^{13}\text{C}$ signatures of organic matter
222 tracing different organic sources through consumers. A significant negative relationship was
223 found between both gut and tissue cadmium and $\delta^{13}\text{C}$, (Figures 8a and 8b; $r^2=0.67$ and 0.4
224 respectively; $p < 0.05$). Oysters sampled from sites located in Desolation Sound with higher
225 cadmium concentrations had depleted $\delta^{13}\text{C}$ signatures indicative of more terrestrial sources as

226 compared to samples from Barkley Sound, a region more influenced by marine sources of
227 organic matter as indicated by the $\delta^{13}\text{C}$ (Riera and Richard 1997).

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230 **5.0 DISCUSSION**231 **5.1 The importance of diet as a source of cadmium to B.C. oysters.**

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

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

249 selection) and will vary dependent on which factor is more important at a particular time or in a
250 particular region.

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

262

263 **5.2 Use of stable isotopes to determine the source of diet to the filter-feeding oyster.**

264 Kaehler et al. (2000) through stable isotope analysis was able to demonstrate that
265 although phytoplankton (oceanic derived particulate organic matter POM) was an important
266 dietary component for both zooplankton and benthos, kelp-derived organic matter accounted for
267 > 30% of the nearshore animal's diet. Kang et al. (1999) also using stable isotopes were able to
268 identify food sources of the suspension feeding cockle (*Cerastoderma edule*). These authors
269 reported two major food sources that were age and season dependent. Bouillon et al. (2000) also
270 successfully used stable isotope analysis to determine sources of suspended organic matter to
271 zooplankton in an estuarine mangrove. These authors were able to determine that despite large

272 amounts of terrestrial and mangrove detritus present in the water column, the locally produced
273 phytoplankton were more important carbon sources for the zooplankton. Riera et al. (1999) used
274 stable isotope analysis to provide a quantification of the relative importance of organic matter of
275 different origins to suspension feeding bivalves. Surveys of bivalves along estuarine gradients
276 have pointed to a trend of increasing incorporation of terrestrially derived detritus into the diet
277 near upper reaches of the estuary (Incze et al. 1982, Stephenson and Lyon 1982, Riera and
278 Richard 1996, 1997). Recently Hill et al. (2006) determined biogeographic and near-shore off-
279 shore trends in isotopic ratios of intertidal mussels and their food sources, both macroalgae and
280 SPM around the coast of southern Africa. These authors noted, for mussels, as we found for
281 oysters in this study, distinct geographic variation in tissue carbon signatures. However, tissue
282 trends were not readily related to carbon values of SPM, again demonstrating that SPM filtered
283 from the water column likely does not represent what the filter-feeding bivalve is actually
284 selecting and hence ingesting as diet. Interestingly, Hill et al. (2006) noted that SPM exhibited
285 trends of $\delta^{13}\text{C}$ depletion from near shore to offshore, suggesting a shift from a signature
286 influenced by macroalgae to one more representative of oceanic phytoplankton.

287 **5.2.1 Barkley Sound:** Within the region two significant site patterns were found. Effingham
288 Inlet across all sampling time periods appeared to exhibit a diet comprised entirely of oceanic
289 marine phytoplankton ($-20 \delta^{13}\text{C}$, $7.60 \delta^{15}\text{N}$) (Riera and Richard 1997). Given the sampling
290 sites location, the mouth of the inlet, and the considerable influence Imperial Eagle Channel has
291 on the food sources available at this site, this would be expected. Contrasting this was the
292 heavier $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures found within oysters sampled from for Fatty Basin ($-19.7 \delta^{13}\text{C}$,
293 $8.39 \delta^{15}\text{N}$) possibly due the influence of benthic microalgae in their diet. Because of differences
294 in the carbon and nitrogen pools available to them, benthic microalgae are readily

295 distinguishable (in terms of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures) than terrestrial vascular plants and
296 phytoplankton (Kang et al. 1999). Unlike the other three sites within Barkley Sound, Fatty
297 Basin is a shallow elongated depression readily mixed through wind and tidally action (Baillie
298 and Welsh 1980; De Jonge and van Beusekom 1992). This mixing would result in the
299 suspension of benthic microalgae making it available to filter feeders. Mixing would also allow
300 for recently deposited particulate organic matter that has been degraded by consumers and
301 isotopically enriched, to be resuspended and made available again as a food source to the
302 oysters. Both processes would result in heavier $\delta^{15}\text{N}$ values as observed here.

303 **5.2.2 Desolation Sound:** In general across all the sampling periods the sites Teakerne Arm,
304 Orchard Bay and Redonda Bay contained carbon signatures ($\delta^{13}\text{C}$ of -22.9, -23.0 and -22.4
305 respectively) that indicated that the diet of oysters from these locations contained more terrestrial
306 organic matter as a food source as compared to oysters sampled from Barkley Sound ($\delta^{13}\text{C}$ range
307 of -19.48 to -20.1) (Riera and Richard 1997). Orchard Bay oysters appear to have had the most
308 significant portion of their diet derived from upland origins i.e., the most depleted of the carbon
309 signatures. Oysters sampled from Gorge Harbor however, showed no terrestrial dietary influence
310 with signatures indicative of marine sources of organic matter ($\delta^{13}\text{C}$ of -20.16). It should be
311 noted that Gorge Harbor likely receives the most significant input from the Strait of Georgia, in
312 terms of oceanic derived organic matter, and it is likely that their diet is consistently based on
313 these inputs.

314 **6.0 Conclusions** Based on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis and determined tissue and gut cadmium
315 concentrations, two main conclusions can be drawn; 1) gut contents correlated with cadmium
316 residues found in oysters and 2) the type of organic matter (terrestrial or oceanic) being filtered
317 and ingested by the oyster is region and site dependent, hence diet can contribute greater or

318 lesser amounts of cadmium to the filter-feeding oysters depending on region and the site within
319 the region. In Effingham Inlet, where both diet and tissue concentrations of cadmium in oysters
320 were the greatest, oyster diet was comprised almost exclusively of marine phytoplankton. This
321 was in contrast with oysters sampled from within the same region but at different sites which had
322 much lower cadmium tissue and gut concentrations with diets appearing to be more mixed with
323 other sources of marine organic matter (e.g., marine diatoms). Oysters sampled from regions that
324 were less directly influenced by oceanic processes had gut carbon signatures characteristic of
325 terrestrial organic matter, with corresponding high cadmium concentrations in both the gut and
326 tissue.

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

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338

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445



446 **List of Figures**

447

448 Figure 1. The location of and sampling locations within the two regions on the west coast of
449 B.C., Canada; A) Barkley Sound (scale 1:144,546; map:ground), B) Desolation Sound (scale
450 1:235,689; map:ground). 1. Effingham Inlet, 2. Seddall Island, 3. Fatty Basin, 4. Poett Nook,
451 5. Trevenen Bay, 6. Thor's Cove, 7. Gorge Harbour, 8. Teakerne Arm, 9. Orchard Bay, 10.
452 Redonda Bay.

453 Figure 2. Average oyster tissue cadmium determined for all sites and sampling periods versus
454 water column temperature taken at 3-5 m depth. $r^2=-0.22$ and is significant at $p < 0.05$, $N=68$.
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468 Values are means of 3-10 oysters.

469 Figure 7. Relationship between tissue and gut carbon signatures. 📍 are oysters from Desolation

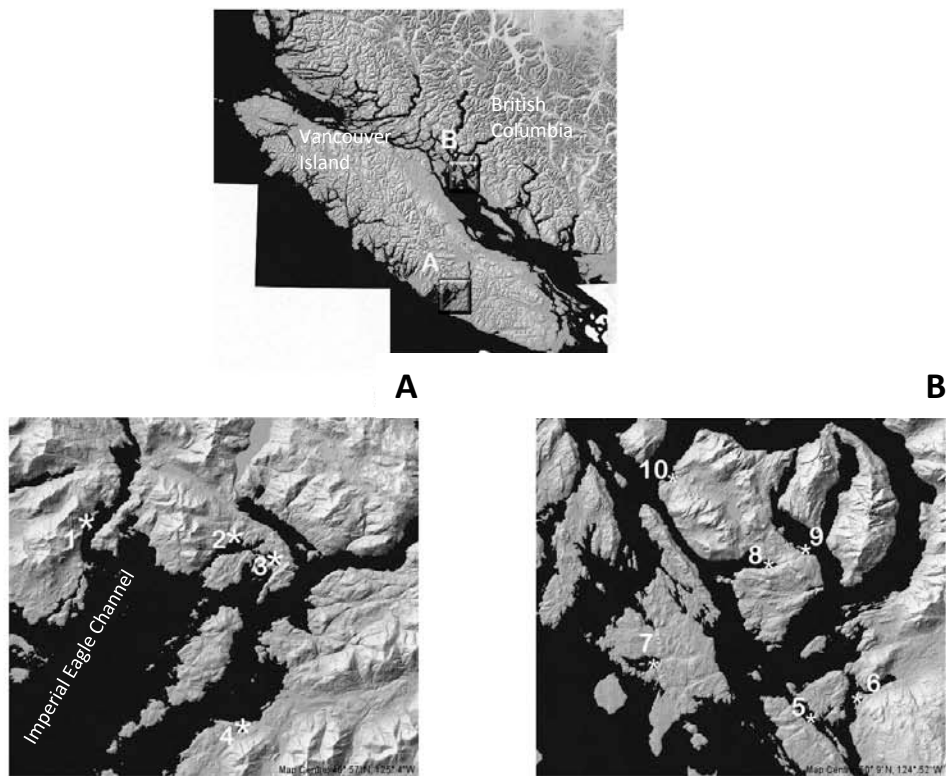
470 Sound, 📍 are oysters from Barkley Sound, $r^2=0.93$; $p < 0.05$. Values are averages over all

471 sampling times, \pm S.E. Samples sizes are provided in Figure 8.

472 Figure 8. Relationships between carbon signatures and cadmium gut (a) and tissue (b)

473 concentrations. Values are averages over all sampling periods for each site \pm S.E. Samples sizes

474 provided in figure legend.



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Figure 1

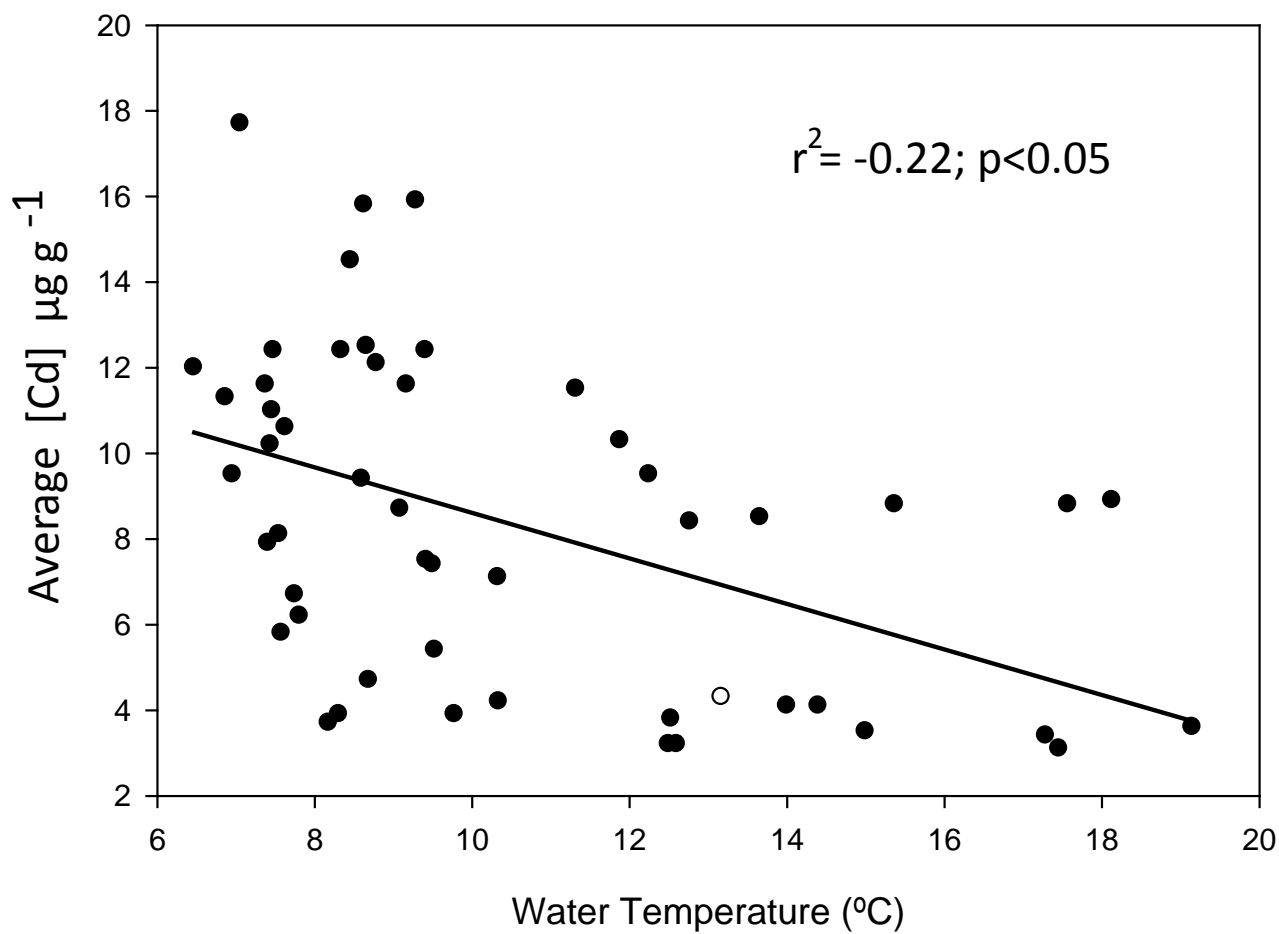


Figure 2

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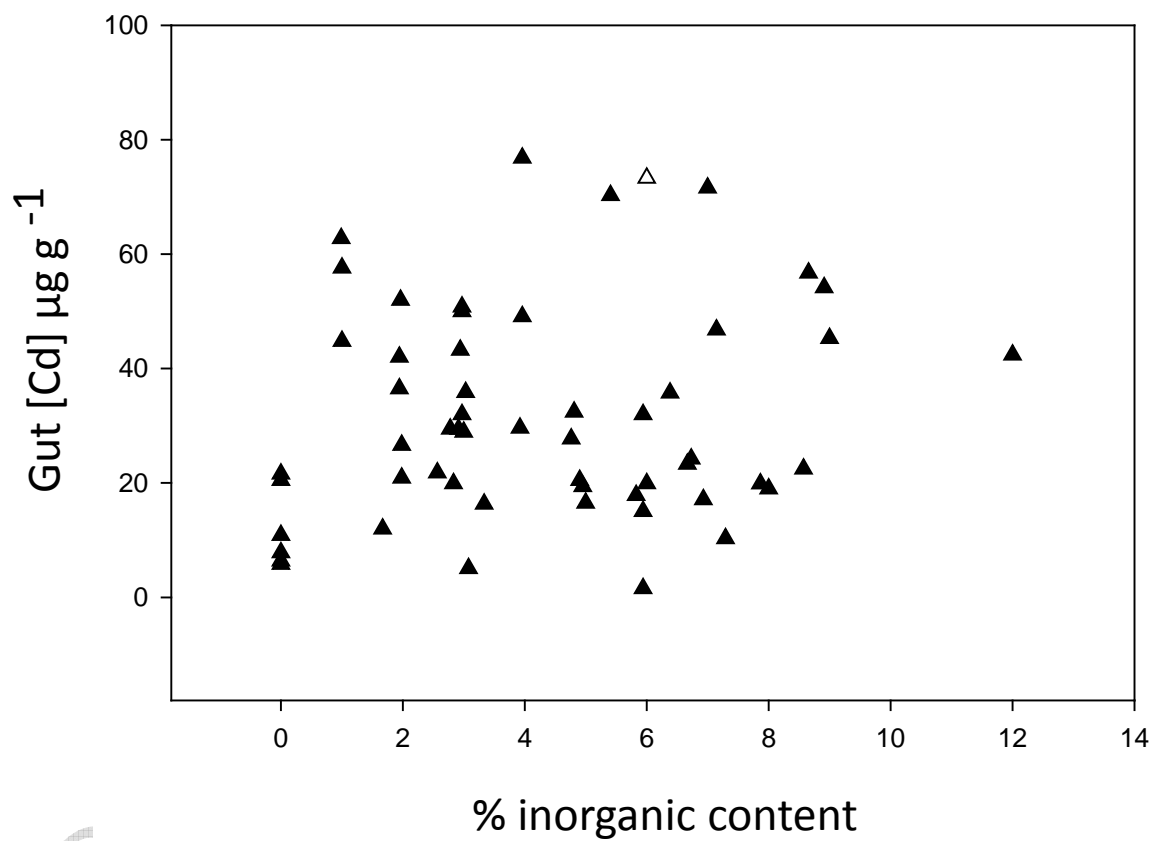


Figure 3

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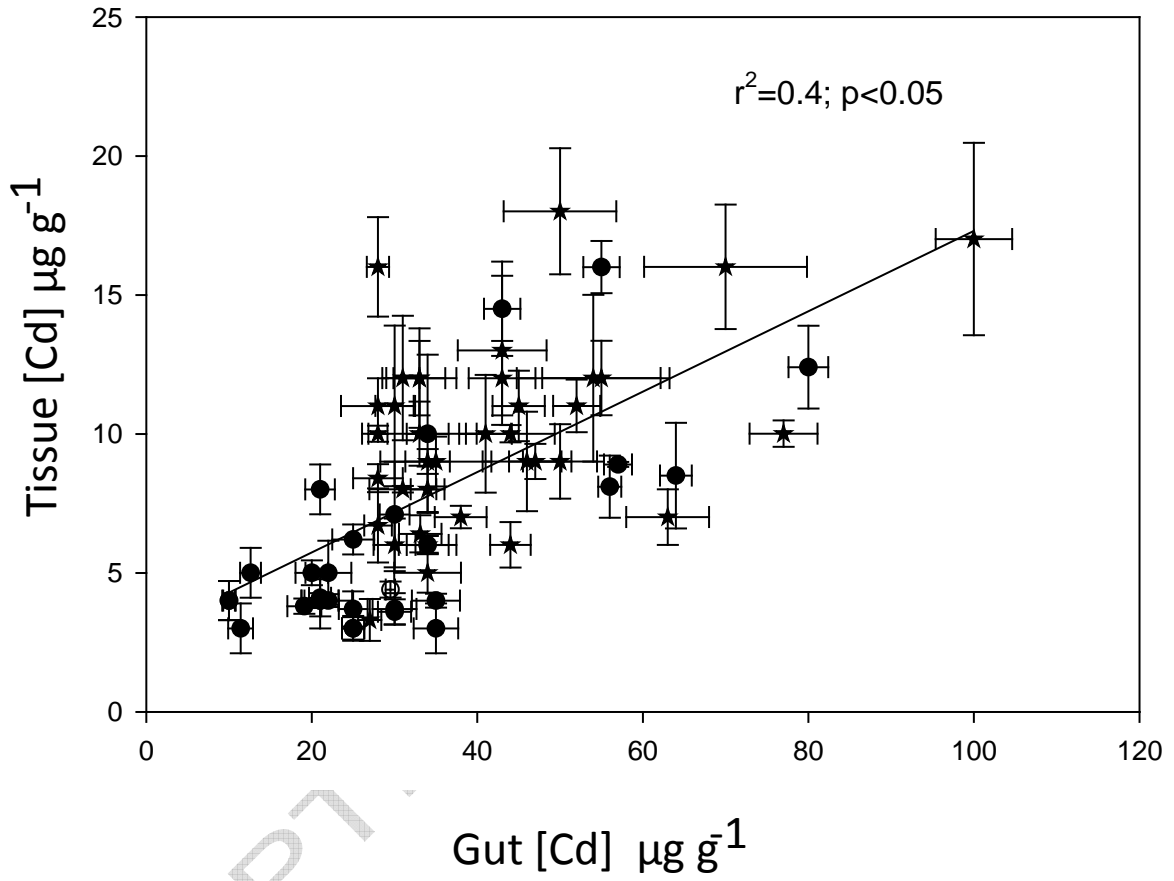
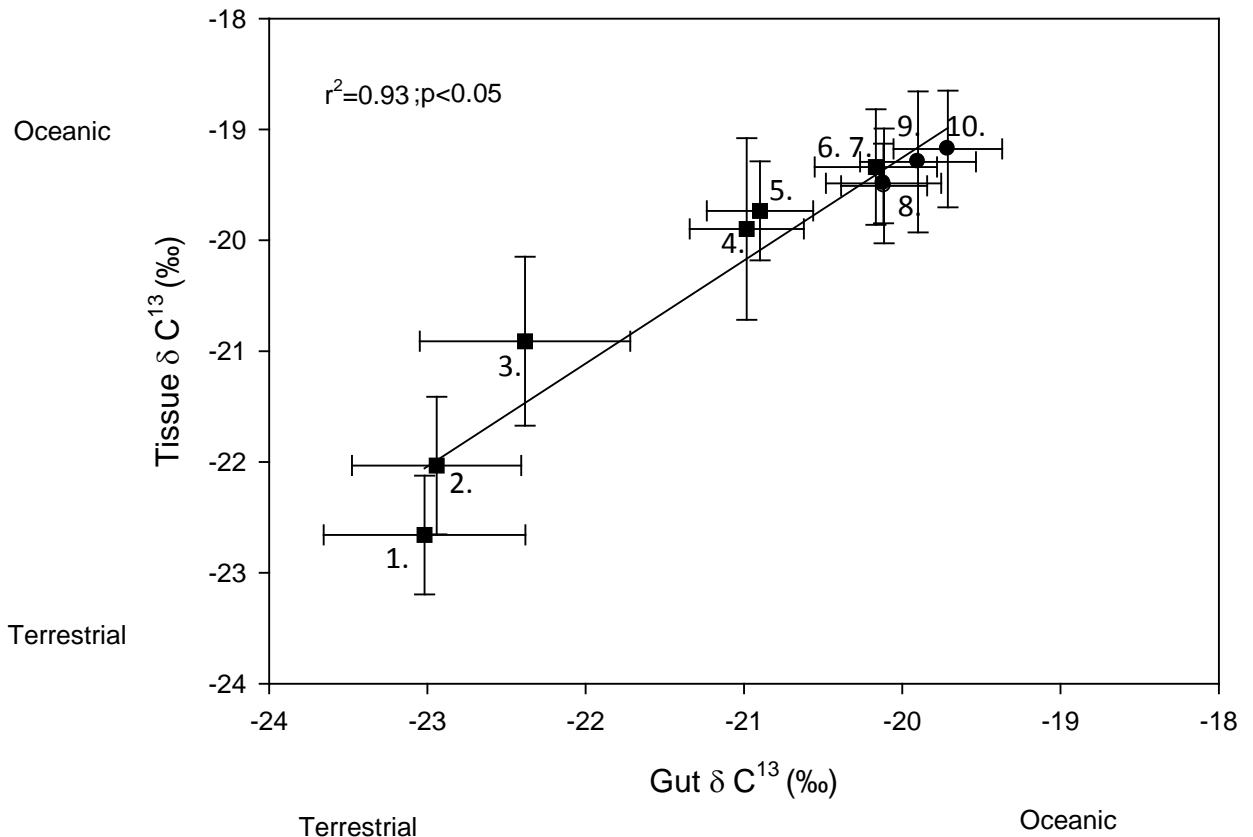


Figure 4

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- 1. Orchard Bay
- 2. Teakerne Arm
- 3. Redonda Bay
- 4. Trevenen Bay
- 5. Thor's Cove
- 6. Gorge Harbor
- 7. Effingham Inlet
- 8. Poett Nook
- 9. Seddall Island
- 10. Fatty Basin

Figure 7

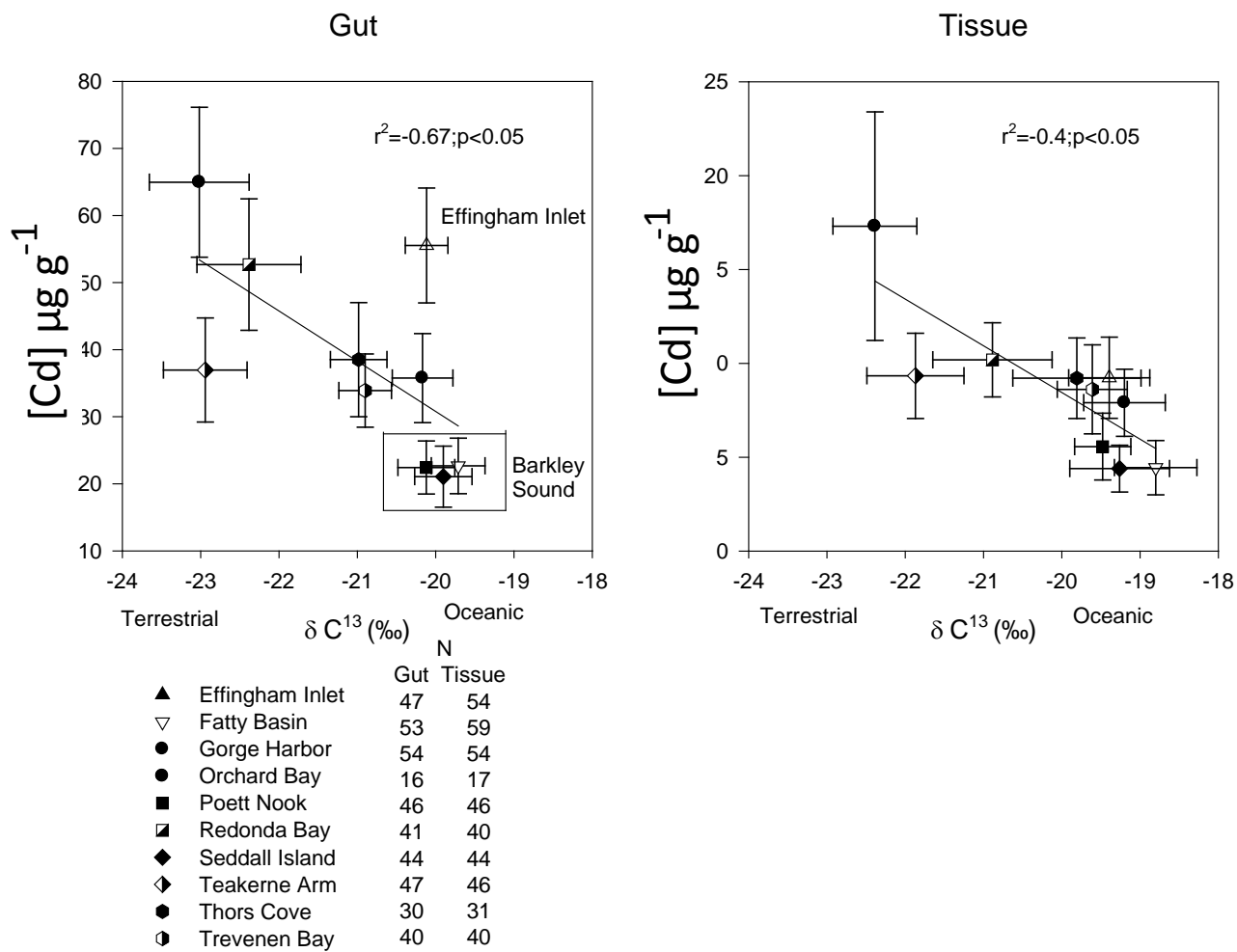


Figure 8