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Axinopsida serricata shell encrustation: A potential indicator of organic enrich ment conditions in sediments in the southern Strait of Georgia, British Columbia, Canada

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29 ABSTRACT

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31 We evaluate the potential of the geographic and within-sediment distribution patterns of rust-32 coloured shell encrustations on an abundant subtidal bivalve, Axinopsida serricata (Carpenter, 33 1864) as geochemical indicators of organic enrichment from marine municipal outfalls. The 34 progressive development of shell encrustation over the life of the animals is suggested by heavier 35 encrustations on large shells compared to smaller shells regardless of the geochemical conditions 36 of the habitat. Heavy encrustations decline in an exponential manner at sediment acid volatile 37 sulphide levels >7 umol/g. Analyses show that the reddish shell encrustations are from an 38 amorphous iron oxide or hydroxy-oxide likely micro-biologically mediated; the oxides appear to 39 be embedded within the inner matrix of an organic layer, with a chemically distinct outer layer. 40 A schematic model is proposed which shows how enrichment of labile organic carbon around outfalls affects the availability of dissolved iron in sediments and leads to less extensive 41 42 encrustations on bivalve shells. Predominantly sandy sediments with low organic loading have 43 much less potential for iron oxide deposition than silty sediments due to greater oxygen 44 penetration into the surface sediment.

45

Shell encrustation appears to be relatively persistent and indicative of long-term conditions, regardless of spatial and temporal fluctuations in sediment geochemistry. With more research on development over the life-cycle of the animal, the pattern of *A. serricata* shell encrustation has the potential to rapidly provide a map of cumulative labile organic loading and oxygen penetration of sediments around municipal outfalls on the west coast of North America where this species is common. However, caution must be used in interpreting results, since

background sediment characteristics (substrate type, bottom currents and sediment transport) can
affect encrustation patterns.

54

Key words: bivalves, shell encrustation, iron oxidation, acid volatile sulphides, sediment, outfall

57 **1.0 Introduction**

58 One of the difficulties in determining the effects of organic enrichment on the biology and 59 chemistry of sediments is relating periodic sediment chemistry measurements to the long-term 60 ambient conditions experienced by the organisms inhabiting the benthos. For environments such as seabeds surrounding municipal outfalls, it would be particularly helpful to develop a simple 61 62 method for evaluating the intensity of organic loading within sediments, integrated over time. 63 The bivalve Axinopsida serricata (F. Thyasiridae) proved to be of interest when, during the 64 processing of taxonomic samples from a monitoring survey of the Iona outfall receiving 65 environment in the Strait of Georgia on the west coast of Canada, it was noted that the shells 66 showed considerable rust-coloured encrustations that appeared to be related to distance from the 67 outfall.

68

A. serricata is a small infaunal bivalve (<5mm), which can be found from southern California to Alaska and is an abundance dominant in a variety of subtidal substrate types and depths (Bright, 1991; Burd, 1992, 2002, 2003a,b; Llansó, 1998; McPherson et al., 2004a,b,c), including the receiving environment of the Iona and Lions Gate outfalls (south-eastern margin Strait of Georgia – McPherson et al. 2007a,b) and the Macaulay Point outfall near Victoria, BC (Burd, 2003b) (see Fig. 1). It appears to be relatively tolerant of conditions near these three major

outfalls, but the population declines within the zone of maximum particulate deposition north of
the Iona outfall (McPherson et al., 2007a) and near the Macaulay Point outfall (Burd, 2003b).
Distributional patterns for particulates from the Lions Gate outfall in Burrard Inlet have not been
clearly defined (McPherson et al., 2007b), however.

79

Acid-volatile sulphides (AVS) have been used to infer general sediment geochemical conditions and particle loadings in the 3 outfall monitoring programs (McPherson et al., 2007a,b; CRD, 2005), and is highest in areas that receive the greatest organic loading. Because AVS values tend to be spatially heterogeneous and ephemeral in sediments (Morse and Rickard, 2004), confident extrapolations from these geochemical parameters to the long-term sediment conditions experienced by the benthos cannot presently be made.

86

Organic deposition from a source such as an outfall depletes oxygen in sediments and, depending 87 88 on the strength of depletion and diffusion of oxygen, other electron acceptors (e.g. nitrate, 89 sulphate and oxides of Mn and Fe) can become important in the oxidation process (Bender et al., 90 1989; Brendel and Luther, 1995). During redox processes, iron and manganese shift between 91 dissolved phase components subject to diffusion and solid-phase precipitated minerals which 92 may be manifest as deposition on shells. Based on the rusty colour of the shell encrustations, 93 and the burrowing nature of this bivalve, it is probable that the encrustations derive from oxides 94 of either iron or manganese, as found in bivalves from other habitats (Swinehheart and Smith, 95 1979; Gillan and De Ridder, 1997; Huettel et al., 1998).

97 The purpose of this paper is to contribute to the understanding of geochemical conditions under 98 which shell encrustation occurs, or does not occur. Shell encrustation patterns may provide 99 insights into the sediment redox conditions and organic loading experienced by the sediments, 100 and provide a more biologically-relevant monitoring tool than spot-measurements of sediment 101 geochemistry. The geographic and within-sediment distribution patterns of shell encrustations on A. serricata in relation to organic enrichment gradients revealed by sediment AVS, were 102 103 examined near 3 municipal outfalls in the Strait of Georgia, BC, and a background area 104 elsewhere in the Strait. The composition of the encrustations, and the mechanisms for producing 105 these encrustations were also explored. Finally, we propose a schematic model to show how shell 106 encrustation on A. serricata might occur under different sediment conditions.

107 **2.0 Methods**

108 2.1 Composition and inner/outer structure of shell encrustation

109

Rust-coloured encrustations from the upper valves of a few shells from the 2003 Iona monitoring 110 111 samples (station 15 – see Fig. 1) were scraped and combined to undergo analyses for crystalline 112 metal oxides and trace metals, using X-ray diffraction. The combined scrapings were ground 113 into fine powder in a corundum mortar and smeared onto a zero-diffraction quartz plate with 114 ethanol. Step-scan X-ray powder-diffraction data were collected over a range 3-70°20 with 115 CuKα radiation on a standard Siemens (Bruker) D5000 Bragg-Brentano diffractometer equipped 116 with a diffracted-beam graphite monochromator crystal, 2 mm (1°) divergence and anti-scatter 117 slits, 0.6 mm receiving slit and incident-beam Soller slit. The long sample holder used (43 mm) 118 ensured that the area irradiated by the X-ray beam under these conditions was completely

contained within the sample. The long fine-focus Cu X-ray tube was operated at 40 kV and 40
mA, using a take-off angle of 6°. The X-ray diffractogram was analyzed using the International
Centre for Diffraction Database PDF-4 and Search-Match software by Siemens (Bruker AXS).

123 An intact upper valve of Axinopsida serricata from the 2003 Iona monitoring station 15 (see Fig. 124 1) and from the ER67 location (Satellite Channel) were examined using scanning electron microscopy (SEM) with an energy dispersive spectrometer (EDS) attached. Specimens from 125 126 these two areas were chosen to determine whether or not the shell encrustations were formed in 127 the same way regardless of sediment conditions or location. This analysis determines relative 128 bulk elemental composition of the shell encrustation. Scans were done a) on the external coating 129 of the encrustations for each location; b) on the shell itself from the Iona location (no 130 encrustations) and; c) within the cross section of broken edges of the encrusted shell material for 131 each location.

132

133 2.2 Axinopsida serricata size classes, abundance distribution and shell encrustation related to
134 sediment geochemistry

Axinopsida serricata were extracted from archived grab samples collected during various
monitoring programs over 8 years in the Strait of Georgia (Table 1). Only shells from live
organisms (at the time of sampling) were examined. The general sampling areas, ranging from
32-136 m in water depth, are shown in Fig. 1. Consistent field (sampling) and laboratory
protocols for monitoring benthic infauna were utilized by Biologica Environmental Services Ltd.
(Victoria, BC. Canada), and are described in the original monitoring reports; for example,
benthic samples were collected using a 0.1 m² Van Veen grab and gently washed through a 1

142 mm mesh screen. All specimens were preserved in 70% ethanol. Sediment AVS samples were 143 available from all studies in Table 1 except the 2000 ER67 Ecological Reserve monitoring 144 program conducted in Satellite Channel (Fig. 1). Sediment Acid volatile sulphide (AVS) 145 samples were prepared by decanting the overlying water in the grab sample, removing and 146 discarding the surface layer of potentially oxidized sediment, and then homogenizing the 147 material from the remaining top 2 cm of the grab prior to chemical analysis in accordance with 148 the method described by USEPA (1991). Hydrochloric acid (6.0 N HCl) was added to each 149 sediment sample within a purge and trap system. To measure AVS, the resulting hydrogen 150 sulphide (H₂S) was carried into a basic zinc acetate solution by argon gas and the AVS was 151 determined colourimetrically (using a method in which sulphide reacts with N-N-dimethyl-152 phenylenediamine and the resulting methylene blue is then measured). Concentrations were 153 reported on a µmol/g dry weight basis (see also McPherson et al. 2007a).

154

155 The lengths of A. serricata specimen shells from the preserved samples were measured by 156 passing each specimen over an optical grid with 0.5 mm gradations. The specimens divided 157 naturally into shell length groups as follows: >2.70 mm (size class A: SC-A), and 2.70 mm to 1.0 158 mm. (size class B: SC-B) (see also McPherson et al. 2004a; 2005a; 2006a; 2007a). All 159 specimens from each sample were counted and assigned into four categories based on the level 160 of shell encrustations: no encrustations (0), light encrustations (1), moderate encrustations (2) 161 and heavy encrustations (3) (Fig. 2). An example of a series of shells with moderate-heavy 162 encrustation on the external surfaces is also shown in Fig. 2. In this paper, encrustations were 163 classified as "light" (categories 0 and 1) or "heavy" (categories 2 and 3); data are presented as 164 proportions of shells with light encrustations.

165

166	Data from the 2004 Iona monitoring program were used to show an example of a distribution
167	pattern for abundance and shell encrustation of the two size classes of Axinopsida serricata
168	relative to the organic enrichment gradient around the outfall (as evidenced by sediment AVS -
169	see McPherson et al., 2007a). The proportion of bivalves from all monitoring location (Table 1)
170	samples with light shell encrustation, was plotted against sediment AVS to show trends for the
171	two size classes.
172	COX COX
173	2.3 Axinopsida serricata vertical distribution, shell encrustation and AVS within sediment
174	depth layers
175	In addition to the archived samples from monitoring programs, duplicate 0.1 m ² Smith-McIntyre

grab samples were obtained on October 22/23, 2004 at the Iona monitoring stations 8 and 15
(Fig. 1). Station 8 is located less than 0.5 km north of the Iona outfall, where particulate
deposition from the outfall is known to be at a maximum (Hodgins and Hodgins, 2000), while
Station 15 is located 8 km south of the outfall and outside the influence of the sewage particulate

180 deposition.

181

Sediment was extracted from the grabs at 1 cm depth intervals, to within 1 cm of the grab sides. This was done to a maximum sediment depth of 10 cm, to provide equal volume samples. Below 10 cm, the wall curvature of the grab begins to reduce the sample volume. From each 1 cm depth layer, a 30 mL sub-sample was removed by plastic spatula and then frozen for chemical analysis of AVS (see methods above), silver and elemental sulfur (for analytical methods see McPherson et al., 2004a). In this study, Ag was measured as an indicator of exposure to municipal outfall

188 particulates (e.g., see Gordon, 1997; Gobeil 1999), whereas elemental sulphur indicates redox

189 condition of sediments. Live speciments of *A. serricata* were preserved in 70% ethanol and later

190 sized and classified according to the degree of shell encrustation.

191

192 **3.0 Results**

Shell encrustation occurred primarily in the vicinity of the inhalant aperture and secondarily in the region of the exhalant aperture, on the outside of the upper shell. Figure 2 shows a close-up photograph of encrustations on the external surface of the upper valve. The density and extent of encrustation was highly variable, and thick layers of encrustation sometimes flaked off during mechanical handling. Despite this, some reddish accretions were typically evident which appeared to be more persistent, even after the loose material was sloughed off.

199 3.1 Composition of Shell encrustation

The X-ray diffraction showed that the metallic crystalline phases of the composite sample of shell scrapings contained rock-forming minerals commonly found in sediment (magnesium, potassium, aluminum), along with a large proportion of aragonite, likely indicative of bivalve shell. No crystalline Fe-oxides or hydroxides were detected. The only phases found that may contain significant Fe are chlorite and dolomite (if solid solution to ankerite is present).

205

The results of the SEM/EDS analysis of shell encrustations for the Iona and ER67 shells are summarized as relative proportions of elements in Table 2. Included are data from the most reliable scans showing a) un-encrusted shell composition; b) outer surface of encrustations, and; c) inner surface (broken edges) of encrustations. All analyses revealed that oxygen was the

dominant element in the encrustations, and the only notable element evident in the scan of bare shell material. The interior part of the encrustation of the Iona shells had much higher iron and calcium levels, and higher phosphorous levels, than the exterior of the encrustation, whereas the reverse was true for aluminum and magnesium. There was no notable difference in relative composition between Iona and ER67 samples.

215

3.2 Distribution of *Axinopsida serricata* size classes and light shell encrustation relative to
 sediment AVS

Figure 3 shows mean abundance for three replicates (number per 0.1 m^2) of the two size classes 218 219 of A. serricata from the 2004 Iona outfall monitoring program samples taken along the 80m depth contour north and south of the outfall (see Fig. 1). Also plotted in Fig. 3 are the 220 221 corresponding mean AVS levels in sample sediments. Figure 3 shows that for both size classes, 222 abundance declined in the region of elevated sediment AVS. The mean abundance of A. 223 serricata in the smaller size class demonstrated a dramatic increase at distances >4 km north and 224 >1 km south of the Iona outfall, with near-zero abundance within the maximum deposition zone for the outfall particulates (McPherson et al., 2005a). Abundance of the larger individuals (>2.7 225 226 mm) showed a more moderate decrease in the maximum deposition zone around the outfall. 227 However, the abundance of both size classes of A. serricata tended to vary in the maximum 228 deposition zone from year to year (McPherson et al., 2007a), and did not always show such a 229 clear pattern in relation to sediment AVS. In addition, other factors clearly affect the abundance 230 of this species, which varies considerably over time and space outside the maximum deposition 231 zone (McPherson et al. 2007a), regardless of sediment AVS levels.

232

There was a clear but variable predominance of shells with light encrustations (i.e. categories 0 or 1) within the maximum deposition zone for the outfall (Fig. 4). By extrapolation, specimens with heavy shell encrustation predominated outside the Iona outfall's deposition zone. However, Fig. 4 also shows that outside the maximum deposition zone for the outfall, the proportion of the small size class of bivalves (1.00 to 2.7 mm) with light shell encrustation (categories 0 or 1) was sometimes higher and more variable relative to the enrichment gradient, than for larger bivalves (>2.7 mm).

240

241 The proportion of the two size classes of Axinopsida serricata with light shell encrustation was 242 plotted against sediment AVS levels (Fig. 5), for all monitoring samples (see Table 1). Figure 5 243 demonstrates that shell encrustations were not linearly related to sediment AVS; rather there was 244 a variable response at sediment AVS levels <7 µmol/g for the larger size class animals, followed by a more consistent increase in proportion at AVS levels higher than this (power regression line 245 fit for all large size class data: $r^2=0.54$, p<0.001). At sediment AVS levels >7 μ mol/g, an 246 247 increasing proportion of the larger size class Axinopsida serricata had light encrustations. A 248 similar pattern was found for the smaller size class, but with a higher variability (power 249 regression line fit for all small size class data: r2=0.36, p<0.01).

250

The *A. serricata* samples from ER67 (Satellite Channel) did not have concurrent sediment AVS measurements, but sediments in this location are generally considered to be well oxygenated and flushed. The area is also subject to low natural sedimentation and has low silt/clay content (i.e. 10-25% silt/clay, Ellis 1968) compared with the other sampling areas in this study (45-98% silt/clay, McPherson et al., 2007a,b). For simplicity, the ER67 samples were plotted with a

256 presumed AVS = $1.0 \mu mol/g$, although experience with similar habitats suggests that it was 257 probably even lower for these samples (Macdonald et al., in press). The proportion of shells 258 from ER67 samples with light encrustation tended to be higher than those found at low AVS 259 levels for the other 4 sampling locations listed in Table 1.

260 3.3 Shell encrustation and AVS in sediment depth layers

More than 90% of *A. serricata* of both size classes were found within the top 4 cm of sediments (Fig. 6a,b) at station 8 and station 15 (reference area). *A. serricata* in the smaller size class were rare at station 8 (near the outfall), with only 8 individuals found in both grabs.

264

Fig. 6c,d shows the proportion of *A. serricata* with light shell encrustation through all sediment depth layers in the grab samples. Most of the large size class *A. serricata* at station 8 had light shell encrustation regardless of sediment depth, whereas almost none of the large size class of bivalves at station 15 had light shell encrustation. The smaller size class *A. serricata* are shown only for station 15, due to the near-zero abundance of small *A. serricata* in both station 8 samples. At station 15, the proportion of smaller size class bivalves with light shell encrustation ranged from 0 to 0.3.

272

AVS levels in sediments at station 15 were consistently lower than at station 8 (Figs. 6,e,f). The proportion of large size class *Axinopsida serricata* with light shell encrustation was higher in all sediment depth samples regardless of AVS levels at station 8 than at station 15. The small size class bivalves found at station 15 showed more variable shell encrustations relative to AVS levels than for the large size class.

Silver and elemental sulfur were much higher in all depth layers in sediments at station 8 than atstation 15 (Fig. 7).

281 **4.0 Discussion**

282 Encrustations such as those found on A. serricata shells have been documented for sediment-283 dwelling bivalves from various locations globally (Swinehart and Smith, 1979; Dando et al., 1994). Gillan and De Ridder (1997) describe a rust colored biofilm and iron oxide deposit on 284 285 shells of the bivalve Montacuta ferruginosa, which is commensal in burrows of sand urchins 286 (spatangoids). Gillan and De Ridder (1997) found that biofilms are laid down over bivalve shells by a complex microbial community with filamentous bacteria similar to Thiothrix and 287 Beggiatoa, both of which can oxidize sulphide. Similarly, active pumping of water through the 288 289 burrow (since this species lack siphons) by A. serricata to support their metabolism creates two 290 zones (inflow and outflow) of reliable O₂ supply in direct contact with the shell, which would become favoured habitat for microbes that produce biofilms and aid in preserving oxidized iron 291 292 precipitates. Gillan et al. (2000) and Gillan and De Ridder (2004) proposed possible ontogenetic 293 scenarios relating to the development of rust-coloured bivalve shell encrustations.

294

In the current study, the lack of calcium or other elements in the clean shell surface scan, and the differences in outer and inner scans for both locations could be due to the presence of a biological film or periostracum. In bivalves, shell colour is mostly concentrated in the periostracum, a horny layer covering the shell. The encrustations found herein do occur on the outside of the periostracum, but their persistence upon mechanical abrasion of the accretions suggests that the material may also become "cemented" to the shells, possibly within biological films, or the periostracum itself. Swinehart and Smith (1979) found considerable concentrations

of Fe and Mn within the periostracum of freshwater molluscs, with reduced amounts found in the
periostracum of the marine bivalve *Mytilus californianus*. The authors describe the minerals as
being concentrated in amorphous regions in a proteinaceous matrix (see also Gillan and De
Ridder, 1997). How the shell encrustation patterns vary on live versus dead shells has not been
documented.

The depth distribution of the bivalves primarily in the top 4 cm of sediments, appears to be 308 309 related to their size and ability to maintain a viable burrow for bringing overlying oxygenated 310 water to the inhalant aperture. Thyasirids lack siphons, and build an inhalant tube in sediments 311 using their elongated foot (Allen 1958, Slack-Smith 1994). This limits burial depth (see Dufour 312 and Felbeck, 2003), particularly in the non-chemosynthetic species (Dufour, 2005) and is an 313 unusual feature of endobenthic bivalves, making this taxon uniquely situated to reflect 314 geochemical conditions in near-surface sediments. This species is considered by Word (1990) to 315 be an interface detrital (suspension) feeder (see also Zhukova et al., 1992, Dufour 2005). Dufour 316 (2005) did not find any bacterial symbionts in the gills of A. serricata, which suggests that it is 317 unlikely to have any nutritional augmentation from chemoautotrophic symbiosis, as some 318 thyasirid bivalves do.

319

The decrease in abundance of *A. serricata* in the maximum deposition zone around Iona (e.g. Fig. 3), and the low abundance of this species in the maximum deposition zone around the Macaulay Point outfall (Burd, 2003b) suggest that sediment geochemical conditions in organically enriched sediments near outfalls can be detrimental. Contaminants associated with outfalls might also play a role in near field bivalve declines, although Ag (Gordon, 1997), nonylphenol ethoxylates (Shang et al., 1999) and PBDEs (Johannessen et al., in press) all show

³⁰⁷

326 enrichments that can be assigned to the Iona outfall, these contaminants are rarely found at levels 327 exceeding provincial and national guidelines at any of the three outfalls (Payne, 2003, 2004, 328 McPherson et al. 2007a,b), and are thus not expected to have adverse effects at this level of the 329 food chain. Some sediment contaminants (e.g., PCBs; Johannessen et al., in press) are unusually 330 low near the Iona outfall, possibly due to dilution from the continuous supply of sediment from the Fraser River. In contrast to the uncertainty related to contaminant effects on biota, Burd et al. 331 332 (2003a,b) describe biotic changes at both the Iona and Macaulay Point outfalls typical of organic 333 enrichment of sediments. This organic enrichment has been inferred from consistently elevated 334 AVS levels (CRD, 2005; McPherson et al. 2007a), from the elevated elemental sulfur and silver 335 in sediments near the Iona outfall, and from the redox profile in a recent sediment core at station 336 8 (Macdonald et al., in press).

337

338 The small size class A. serricata were less tolerant of conditions near the Iona outfall than the 339 large size class (Fig. 3). This may be related to lack of maturity of physiological coping mechanisms or more limited metabolic requirements for smaller specimens, which would 340 341 prevent successful recruitment in the organically enriched sediments. Other possible reasons include reduced predation on large specimens due to changes in juvenile fish and megafauna 342 343 composition in the high outfall deposition area (Metro Vancouver, unpublished Iona monitoring 344 data), or to other unknown reasons related to the life history and variable survival of colonizing 345 individuals. In addition, the population distribution of this species may be complicated in the 346 Iona region by the documented transport of sediment down-slope (see Hodgins and Hodgins, 347 2000). The potential for sediment transport to shift recently settled bivalves in laboratory 348 experiments has been shown by Roegner et al. (1995). Along with potential patchiness in

349 sediment conditions, this could explain why there are some bivalves with heavy encrustations in 350 the maximum deposition zone around the outfall (Fig. 4). Conversely, Gillan and De Ridder 351 (2004) noted that the thick shell encrustation in larger bivalves commensal in echinoid burrows 352 can sometimes get scraped off during burrowing under laboratory conditions, which may help to 353 explain why, in the present study, there was often a small proportion of larger Axinopsida 354 *serricata* with light shell encrustation in areas where most of the bivalves had heavy shell 355 encrustation. To further complicate this picture, the reduced encrustations overall in the smaller 356 size class A. serricata compared with the large size class suggests that encrustations accumulate 357 progressively over the life of these animals. Gillan and De Ridder (2004) also noted that the 358 smallest size class of the commensal bivalve Montacuta ferruginosa had a much higher 359 incidence of uncoated or weakly coated iron shell encrustation than the larger size class, and that 360 the encrustations were thicker in larger specimens, suggesting that they build up over time. The 361 above factors could result in a mosaic of shell encrustation patterns, explaining the variable 362 results in the current study.

363

364 The pattern and intensity of shell encrustations are presumably related to characteristics of the 365 water pumped through the shell over time. Figure 5 suggests that there are geochemical thresholds (AVS >7umol/g) above which shell encrustation frequencies decrease. However, 366 367 between about 3 and 7 umol/g AVS, results were highly variable, which means that periodic 368 measurements of AVS might not provide the most reliable measure of sediment geochemical 369 conditions due to spatial and temporal patchiness in sediments (Morse and Rickard, 2004). For 370 example, in outer Burrard Inlet (Lions Gate sampling area -Fig. 1) samples from 2005, the 371 proportion of larger size class A. serricata with light encrustations was unusually high at one of

372 the stations. Although AVS levels were not very high at this station in 2005, they have

historically been much higher and more variable than at any other station in the survey area. The
proportion of shells with light encrustations in this location remained relatively high throughout
the sampling period (2002-2006) regardless of variability in sediment AVS conditions. This may
partially explain why there is considerable scatter in the relationship between shell encrustation
and sediment AVS (Fig. 5).

378

Results suggest that the pattern of shell encrustation is not ephemeral, but rather a cumulative product of substrate conditions. If there is a progressive increase in shell encrustation over the life of the animals, the degree of encrustation in these animals provides a life-long picture of sediment geochemical conditions, which could be verified and clarified by more in-depth studies of the ontogeny of shell encrustation under varying geochemical conditions over the life of the animals.

385

386 4.1 The biogeochemical mechanism of encrustation

The formation of shell encrustations in *A. serricata* clearly occurs throughout the southern Strait of Georgia and likely in other coastal seas. However, the degree of encrustation and its distribution in the various sampling areas suggests a relationship with sediment composition and organic loading. Although the organic and geochemical composition of the shell encrustations and the mechanisms of microbiological precipitation of iron on shells have been discussed by Gillan et al. (2000), there has been no work done on the geochemical conditions required within the sediments to facilitate the shell encrustation.

395 The results of this study suggest that increasing enrichment of labile organic carbon related to 396 municipal outfall particulate deposition (as evidenced by sediment silver distributions; Gordon 397 1997 and Fig. 7), reduces the concentration of oxygen in the sediments (as evidenced by 398 elemental sulfur distributions – Fig. 7) and leads to reduced encrustations on the bivalve shells. 399 The process of advecting oxygenated water in through the anterior, inhalent siphon and out 400 through the exhalant siphon affects the redox environment surrounding the bivalve's burrow. In 401 particular, given the location, coloration and composition of the encrustations documented here 402 (Fig. 2), it appears that bivalve ventilation produces encrustations by bringing together dissolved 403 oxygen and dissolved iron (Fe II), a process known to lead to ferric iron precipitation in 404 permeable sediments (Brendel and Luther, 1995; Huettel et al., 1998). This process may be 405 mediated on the bivalve shells in part by microbes (e.g. Gillan and De Ridder, 2004) and the 406 coincident formation of a biofilm, which would help to protect precipitated Fe from re-dissolving 407 during subsequent episodes of hypoxia in surrounding sediments.

408

409 The distribution of shell encrustations as a function of distance from an extraordinary labile 410 carbon source (Fig. 4) together with a notable relationship between AVS and shell encrustation 411 (Fig. 5) suggest that the complexity in the shell encrustation process derives from the manner in 412 which the redox cycle affects iron phases (dissolved/particulate) in sediments.

413

Before proposing a relationship between the oxygen/iron rich encrustations observed on *A*. *serricata* shells, and sediment geochemistry, it is necessary to examine how redox chemistry
might produce dissolved iron (Fe²⁺) and precipitated Fe phases (Fe³⁺ oxy-hydroxide phases).
Metabolism of organic matter involving the consumption of oxygen creates a gradient in the

418 porewater which then supports diffusion of O_2 from bottom water into the sediments. At a depth 419 in sediments where O_2 has been completely consumed, other electron acceptors may be 420 employed in metabolism, but with decreasing energetic return (Bender et al., 1989). Accordingly MnO₂ may be reduced producing dissolved Mn²⁺ which can diffuse upward to re-421 422 precipitate when it reaches dissolved O_2 , and NO_3^- can likewise be reduced during the process of 423 denitrification. With considerably less free-energy return, iron oxy-hydroxides associated with solid phases can be reduced to produce soluble Fe^{2+} (Brendel and Luther, 1995), at a depth below 424 the availability of dissolved O_2 . When other electron acceptors have been exhausted, SO_4^{2-} may 425 be used but this provides a very poor energetic return to microbes (Emerson and Hedges 2003) 426 and also releases S^{2-} (sulphide) as an important byproduct. Therefore, shell encrustation could 427 occur because Fe^{2+} may precipitate when it comes in contact with dissolved O₂ and also when it 428 comes in contact with S₂- pyritization (Berner, 1982). 429

430

The processes outlined above are organized vertically in sediments because O_2 is supplied from the sediment surface and because microbes have a preference for O_2 as an electron acceptor due to the available free-energy return. Dissolved Fe^{2+} , which may be produced below the oxic zone within the sediments during metabolism, can then diffuse upward to precipitate in oxy-

hydroxides, but it may also be sequestered by precipitation as sulphides which occur at depth in sediments provided organic metabolism is sufficiently robust to produce S^{2-} . AVS in sediments is clear evidence of S^{2-} production and precipitation (Morse and Rickard, 2004). Thus the depth at which dissolved Fe²⁺may buildup in sediment porewater is bounded above by oxic conditions where Fe²⁺ is oxidized to Fe³⁺ and precipitated as oxyhydroxide, and below by sulphidic

440 conditions where Fe^{2+} is precipitated as sulphide.

441

Figure 8 is a proposed schematic diagram which shows how Fe^{2+} buildup in sediments may be 442 443 affected by labile organic carbon loading and O_2 penetration. A. servatica live predominantly in 444 the top 4-cm of the sediments, and pump water to supply themselves with O₂. Therefore, it is the supply of dissolved Fe^{2+} in porewater, and not O_2 , that controls whether or not strong 445 446 encrustation occurs. To construct this figure we have used measured AVS profiles from dated 447 cores for the Iona monitoring stations 8 (near the outfall) and station 15 (reference area), (Macdonald et al., in press) and a hypothetical AVS profile at Satellite Channel. Reasonable 448 porewater Fe²⁺ profiles are proposed based on literature (Reimers et al., 1996; Huettel et al., 449 450 1998). Note that annually measured AVS levels have been relatively consistent at the 2 Iona sampling stations over time (McPherson et al., 2007a). 451

452

As we lack sediment geochemical data for Satellite Channel, it is only possible to propose the 453 454 sediment variables that must exist there in support of the comparatively lighter shell encrustation. compared with the other three locations. This difference may be related to availability and 455 concentration of Fe^{2+} through porewater advection (Huettel et al., 1998). It is likely that 456 predominantly sandy sediments in Satellite Channel (75-90% - Ellis, 1968) with low organic 457 loading would have low potential to produce iron oxide shell encrustation because O₂ penetration 458 into the surface sediment would be facilitated, thus precipitating out Fe^{2+} in the region the 459 bivalves tend to inhabit (upper 4 cm of sediments), and making pore-water Fe²⁺ less available for 460 microbial precipitation onto bivalve shells. Therefore, Fe^{2+} can build up in porewater only to 461 462 small concentrations in a restricted zone somewhat below the main habitat of A. serratica. At Iona station 15, sediments are less porous, and have more mud (low O₂ diffusivity) with higher 463

464 carbon flux supported by terrestrial (Fraser River particulates) and marine carbon. Here, the 465 oxygen penetrates shallowly, and AVS accumulates weakly (due to a moderate supply of carbon) below about 3 cm. As a result, Fe^{2+} can build up strongly in A. serratica's prime habitat. 466 467 Finally, in the right-hand panel, Station 8 (near the Iona outfall) exhibits low oxygen 468 permeability (or more rapid biological use of oxygen than can be balanced by diffusion into 469 sediment – see Macdonald et al., in press) and high organic loading, supported partly by outfall 470 particulates (Hodgins and Hodgins, 2000) and partly by marine and terrestrial organic carbon (Macdonald et al., 2005). In this case, the zone of Fe^{2+} build up is again constrained narrowly 471 472 near the sediment surface because of strong AVS production supported by high organic carbon 473 loading. Redox boundaries shown in Fig. 8 would vary seasonally or interannually with carbon 474 loading (Gobeil et al., 2001). Furthermore, sedimentation tends to bury Fe sequestered in solid-475 phase sulphides, whereas when oxyhydroxides near the surface become buried, the iron can be reduced and remobilized into pore water. 476

477 **5.0 Conclusions**

478

The shell encrustations on the *Axinopsida serricata* from both locations were primarily iron oxides or hydroxy-oxides possibly biologically mediated; the oxides appear to be mostly embedded within an "inner" iron and phosphorus-rich matrix with a different external composition, possibly suggesting a biofilm coating the encrustations. The encrustations appear to be persistent regardless of short-term or small scale changes in sediment geochemisty. These facts suggest that the rust-coloured encrustations on *A. serricata* shells are similar to those described by Gillan and De Ridder (1997).

487 How can Axinopsida serricata shell encrustation be used to diagnose sediment geochemical 488 conditions of interest? Here, we show that the frequency and intensity of A. serricata shell 489 encrustation, particularly in the larger animals, has the potential to provide a map of cumulative 490 labile organic loading and potential oxygen penetration of sediments around a municipal outfall, 491 where sediment AVS levels are >7 umol/g. However, given the many variables that can 492 potentially affect the shells of these animals, it is likely that this approach would only be feasible 493 in areas where abundance of the bivalves is naturally high, and sufficient samples are collected to 494 see a pattern in spite of the variability. In addition, interpretation of results must take into 495 account oxygen permeability of sediments (typically substrate type), as well as possible 496 confounding due to sediment transport, which may move animals in and out of the organic 497 enrichment zone.

498

Given a pre-survey of shell encrustation within a relatively homogenous and low current
substrate regime, a more thorough biogeochemical/contaminant sampling program can be
planned in projected areas of organic enrichment impact for sediment geochemistry and benthic
ecology. With a better understanding of the development of the encrustations over the life of the
bivalves, they may ultimately provide a useful tool to assess integrated organic input in locations
where loading is patchy in time or space, for example around fish farms.

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507

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- 515

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680 Figure Captions

681

682	Figure 1. (Right) Locations from which Axinopsida serricata specimens, collected as part of
683	previous monitoring studies, were used for shell encrustation classification. Historical sediment
684	chemistry data were obtained from monitoring reports specific to these locations. Left panel
685	shows details of the two stations (stations 8 and 15), located along the 80 m depth contour north
686	and south of the Iona outfall, that were sampled in this study to obtain A. serricata for
687	classification of shell encrustation and to determine sediment depth at which these bivalves are
688	found, as well as chemical analysis of sediments from specific depths in the grabs.
689	Figure 2. Illustration of the range of shell encrustation for A. serricata corresponding to the
690	following categories: no encrustations (0), light encrustations (1), moderate encrustations (2)
691	and heavy encrustations (3).
692	Figure 3. Abundance of Axinopsida serricata (number per $0.1m^2$) at the 2004 Iona outfall
693	monitoring stations and corresponding sediment AVS levels in relation to distance (km) from the
694	Iona outfall (McPherson et al. 2005a). The Iona outfall is located at 0 km; negative and positive
695	distances in km are south and north of the outfall, respectively.
696	Figure 4. Proportion of Axinopsida serricata shells with either no encrustations (Category 0) or
697	light encrustations (Category 1) in relation to distance (km) north and south of the Iona outfall
698	(data combined for 2001-2006). The Iona outfall is located at 0 km; negative and positive
699	distances are km south and north of the outfall, respectively.
700	Figure 5. Empirical relationship between the proportion of Axinopsida serricata shells with light

encrustations (category 0 or 1) and sediment AVS for all sample areas. AMP in the figure key

702 stands for the Ambient Monitoring Program in south Georgia Strait (Wright et al. 2008). CRD 703 2001 samples are from Macaulay Point area. Included are 7 samples from Satellite Channel 704 location (ER67); Since AVS data are not available, an estimated value of 1.0 umol/l has been 705 given to these samples simply to show how they compare with the samples from the other four 706 locations. A is for the large size class A. serricata; B is for the small size class 707 Figure 6. Large (A) and small (B) size class abundance of A. serricata per each 1 cm layer of 708 sediment in Smith-McIntyre grab. Each section had about the same surface area and volume. 709 The low value at the surface in one station 15 replicate was confounded by the presence of a 710 large Dungeness crab in the sample; Proportion of abundance of "light" encrustation (categories 711 0/1) large (C) and small (D) size class A. serricata in layers at stations 8 and 15. The low value 712 for adults at station 8 at 8 cm depth represents only 2 animals found in that layer; Comparison of 713 light encrustation A. serricata (category 0/1) with sediment AVS in grab layers for large (E) and 714 small (F) size classes for stations 8 and 15. Values for small size class bivalves for station 8 are 715 not shown because there were so few specimens that proportions were meaningless.

Figure 7. Differences in mean sediment elemental sulfur and silver concentrations with depthlayers for stations 8 and 15.

Figure 8. A schematic diagram showing how the flux of labile organic carbon to the sediment surface influences *A. serricata* (represented by small circles grouped on each set of profiles) shell encrustation. In the left-hand panel, low organic flux into porous sediments (such as those found in Satellite Channel) creates a deep oxic surface zone where Fe²⁺ precipitates as Fe oxyhydroxides maintaining low [Fe²⁺] in *A. serratica*'s prime habitat. In the middle panel, moderate organic loading together with less permeable sediments (such as found near Iona station 15 off

the south arm of the Fraser River), creates a zone favourable to Fe^{2+} in the pore water 724 725 sandwiched between the surface oxic layer where Fe oxy-hydroxides are precipitated and a 726 deeper, mildly sulphidic layer (AVS buildup) where Fe sulphides are precipitated. This narrow, Fe^{2+} enriched zone lies in *A. serratica*'s habitat. In the right hand panel, high organic loading, 727 728 supported partly by outfall particulates (such as that found at Iona station 8 near the outfall), rapidly consumes oxygen and supports strong SO_4^{2-} reduction in near-surface sediments as 729 evidenced by high AVS buildup. Because all living A. serratica bivalves pump O₂ containing 730 731 water through their siphons, we propose that the shell encrustation process is controlled by the diffusion of dissolved Fe²⁺ in porewater for which the conditions in the middle panel are most 732 733 favorable. MAT 734 735

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Figure 6a-f Burd, Macdonald, Wright, van Roodselaar, Fig. 1



Figure 1





Burd, Macdonald, Wright, van Roodselaar, Fig. 3

Distance from outfall (+N,-S km)

Figure 4

Burd, Macdonald, Wright, van Roodselaar Fig. 4





Burd, Macdonald, Wright, van Roodselaar

Figure 6a-f Burd, Macdonald, Wright, van Roodselaar



Burd, Macdonald, Wright, van Roodselaar, Fig. 7



Burd, Macdonald, Wright, van Roodselaar, Fig. 8



Figure 8

Project	Study Area	Year	Depth Range	<i>A. serricata</i> Abundance range	Sediment Geochemical	Reference
Ambient Monitoring	S. Goorgia Strait	2003	(m)	(# per 0.1m ²)	Data	McPhorson of al
Program (DFO, NRC, GVRD)	(outside Burrard Inlet)	2003, 2004, 2006	00 - 100	5-99	les	2004c,Wright et al. 2008
Assessment of the Proposed GSX Pipeline	ER67 Ecological Reserve (Satellite Channel)	2000	73 - 86	49-263	No	Burd and Glaholt, 2000
Iona SO Receiving Environment Monitoring Program	E. Strait of Georgia - 16 km gradient N and S of the SO	2002	80	163-978	Yes	McPherson et al., 2003
Iona SO Receiving Environment Monitoring Program	E. Strait of Georgia - 16 km gradient N and S of the SO	2003	60 - 120	12-1541	Yes	McPherson et al., 2004a
Iona SO Receiving Environment Monitoring Program	E. Strait of Georgia - 16 km gradient N and S of the SO	2004	80	81-965	Yes	McPherson et al., 2005a
Iona SO Receiving Environment Monitoring Program	E. Strait of Georgia - 16 km gradient N and S of the SO	2005	80	194-624	Yes	McPherson et al., 2006a
Iona SO Receiving Environment Monitoring Program	E. Strait of Georgia - 16 km gradient N and S of the SO	2006	80	101-608	Yes	McPherson et al., 2007a
CRD SO Monitoring Program*	Macaulay Pt SO: Juan de Fuca Strait	2001	54 - 67	8-274	Yes	CRD 2003, 2004, 2005, Paine et al. 2004
Lion's Gate SO Monitoring Program	Outer Burrard Inlet	2004	32 - 87	42-445	Yes	McPherson et al., 2005b
Lion's Gate SO Monitoring Program	Outer Burrard Inlet	2005	32 -87	34-355	Yes	McPherson et al., 2006b
Lion's Gate SO Monitoring Program	Outer Burrard Inlet	2006	32 -87	40-501	Yes	McPherson et al., 2007b

Table 1. Summary of specimen and data sources used to examine *Axinopsida serricata* shell deposits on the BC coast. Key: SO = sewer outfall; *includes nearby reference area (Parry Bay).

Elements	shell only (no deposits)	Outer Coating Iona	Inner Coating Iona	Outer Coating ER67	Inner ER67
	Atomic %	Atomic %	Atomic %	Atomic %	Atomic %
0	97.51	83.1	78.1	80.3	74.63
Mg	.52*	1.47	1.39	0.85	1.41
AI	1.09	4.03	1.33	2.49	1.48
Si	.84*	7.59	3.33	6.72	5.95
Р	34*	1.33	4.52	0.73	3.66
S	.37*	0.13*	.37	1.02	0.53
К	.01*	0.67	.33	0.32	0.49
Ca	0.00*	0.9	2.36	5.14	2.73
Fe	0.00*	0.78	8.26	2.42	9.11
Total	100	100	100	100	100

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Table 2. Comparison of the relative elemental composition, determined using SEM/EDS, of Axinopsida serricata shell deposits. Atomic % is the relative percent composition of noted elements. * indicates < 2 sigma or below detectable range.