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# Marine macroalgal community structure, metal content and reproductive function near an acid mine drainage outflow

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**"Capsule":** *Evidence is presented that acid mine drainage affects the structure of marine algal communities.*

**Abstract**

Marine macroalgal communities were examined near the outflow of acid mine drainage (AMD) from the Britannia Mine, British Columbia, Canada. No marine algae were present within 100 m of the mouth of Britannia Creek, which carries the AMD into the marine environment. At greater distances (300–700 m) from this Creek, mean summer cover of filamentous green algae, mostly *Enteromorpha intestinalis*, was $>60\%$, which was significantly higher than at nearby reference stations. At still greater distances (600–1000 m) from Britannia Creek, *Fucus gardneri* dominated algal communities that were similar to those at reference stations. No consistent differences were detected in mean plant length, mean per cent cover or mean oocyte production between *F. gardneri* near Britannia Creek and those at reference stations. Cu body burden in *F. gardneri* near Britannia Creek was five to 17 times higher than in reference plants. 

*Keywords:* *Fucus*; *Enteromorpha*; Acid mine drainage; Copper; Algae

## 1. Introduction

Cu ore was extracted from the mine at Britannia Beach, BC, Canada, from 1902 until the mine ceased operations in 1974. Rain, snowmelt and groundwater now percolate through the mine tunnels, producing an acidic solution of dissolved metals known as acid mine drainage (AMD). A portion of the AMD from the mine flows into Britannia Creek, which in turn flows into Howe Sound 50 km north of Vancouver, BC (Fig. 1). *Fucus gardneri* Silva (Phaeophyceae, Fucales) is an intertidal seaweed that thrives 2 km from the mouth of Britannia Creek but is absent from the shores near the Creek. *F. gardneri* beds provide habitat and food for a variety of benthic invertebrates, some of which are important food sources for chum salmon [*Oncorhynchus keta* (Walbaum)] fry and chinook salmon [*Oncorhynchus tshawytscha* (Walbaum)] fry and smolts. As part of a larger study of the impact of AMD from Britannia Mine on salmon and their habitat, this work examines the distribution and characteristics of the marine algal communities in the vicinity of Britannia Creek, with the intent of quantifying the differences in algal communities: (1) in areas near the Creek, where *F. gardneri* is absent; and (2) in areas more distant from the Creek, where *F. gardneri* is present but appears to be smaller and less predominant.

Chretien (1997) examined the flux of metals from Britannia Creek into Howe Sound. Fig. 2A shows the temporal change in dissolved Cu concentrations in Howe Sound at the mouth of Britannia Creek. The peak in late spring and early summer corresponds with the melting of snow at high elevations, which flushes accumulated AMD from the mine workings (Chretien, 1997). These high levels of Cu also coincide with the freshet of the nearby Squamish River, which lowers the salinity of upper Howe Sound to 3–10‰. The spatial dispersal of dissolved Cu from Britannia Creek in late May 1998 was measured by Grout and Levings (2000; Fig. 2B). Dissolved Cu concentrations near the mouth

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of Britannia Creek are elevated to 40–100 times those at stations 5 km from the Creek. There is also a consistent elevation of Cu at stations up to 1000 m north of Britannia Creek; these levels are two to 20 times those at reference stations.

Many authors have examined the effects of Cu on algae and algal communities in localities other than British Columbia. Strömgren (1979, 1980) tested the effect of Cu on five algal species, and found that growth rates of three *Fucus* spp., as well as two other fucoids, were decreased when exposed to 12–50 μg Cu $l^{-1}$. Andersson and Kautsky (1996) tested the sensitivity of reproductive stages of Baltic Sea *Fucus vesiculosus* (L.) to Cu at various salinities. In 20 μg Cu $l^{-1}$ at salinities both lower (6‰) and higher (20‰) than optimum, germination was reduced by 70–80%. In contrast, oocytes exposed to the same concentration of Cu at optimal salinity (14‰) showed no toxic effects.

Other authors have focused on algae other than fucoids. Castilla (1996) found communities composed almost entirely of *Enteromorpha compressa* (L.) Greville (Chlorophyta) near the Salado River, Chile, where runoff from mine tailings flows into the Pacific Ocean. Reference sites farther from this river were comparatively species-rich. Reed and Moffat (1983) and Correa et al. (1996) have found *E. compressa* to be resistant to high Cu concentrations compared to many brown macrophytes, with the growth rate of plants being unaffected by $\leqslant$600 μg Cu $l^{-1}$.

A series of hypotheses concerning *F. gardneri* plant length, cover, gamete production, Cu body burden and filamentous green algal cover were formulated based on qualitative observations and previous work. *F. gardneri* plant length, cover and gamete production, as well as filamentous green algal cover, were all hypothesized to be lower at stations near Britannia Creek than at relatively unpolluted reference stations. Body burden of Cu in *F. gardneri* was expected to be higher in plants at the former stations relative to the reference stations.

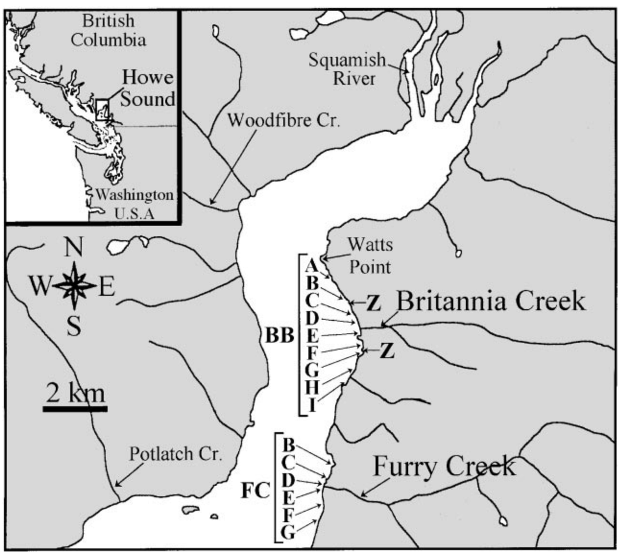


Fig. 1. Map of upper Howe Sound, BC, showing study stations and geographical features referred to in the text. BB, Britannia Beach; FC, Furry Creek. The entire shoreline between the points labelled Z is devoid of *Fucus gardneri*.

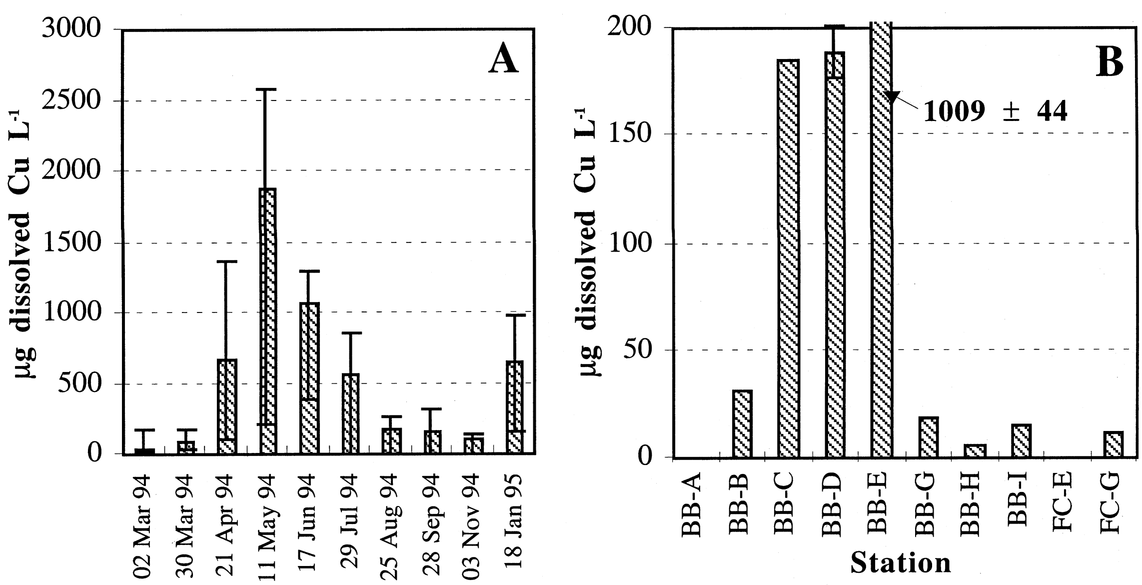


Fig. 2. Concentrations of dissolved Cu in Howe Sound near Britannia Creek. (A) Temporal change in Cu levels from March 1994 to January 1995. Values are means of eight samples taken along a transect near the mouth of Britannia Creek. Error bars are the range of concentrations in each set of samples. Data from Chretien (1997). (B) Spatial dispersion of metals from Britannia Creek in Howe Sound on 28 May 1998. See Fig. 1 for location of sampling stations. Three samples were taken at stations BB-D and BB-E; error terms are ±1 S.E. From Grout and Levings (2000).

## 2. Materials and methods

### 2.1. Transects — algal cover and F. gardneri length

The transect stations are shown in Fig. 1. Stations at Britannia Beach were chosen to assess the presumed gradient of AMD concentrations at increasing distance from Britannia Creek, and a similar gradation was then sought at Furry Creek to control for freshwater inflow. Substratum type can also have important effects on the distributions of *F. gardneri* and other algae; an attempt was thus made to select matching stations in each area with similar substratum types.

Sampling was conducted over 2 or 3 days on the lowest tides of each month. Transect surveys were conducted in June, July, August, October and December 1998. The area of study at each transect station consisted of a 20-m horizontal reach of the intertidal zone. The vertical limits of the study area corresponded to those of the zone where *F. gardneri* is normally found in Howe Sound: typically 1.5–3.0 m above lowest lower low water (LLLW). A horizontal transect line was laid along the top or bottom of the *F. gardneri* zone. Three vertical transects were then randomly placed along the horizontal line and a 25×25-cm quadrat was randomly placed at three points along each vertical line, for a total of nine quadrats per study area. The quadrat was prestrung with nylon string in a grid pattern with squares 1.5×1.5 cm, and 20 of the intersection points were randomly marked with pieces of coloured wire.

Estimates of *F. gardneri* per cent cover were obtained by recording the number of marked points under which the plant occurred. Per cent cover of filamentous green algae was then estimated by moving the canopy of *F. gardneri* aside and counting the number of points under which these plants occurred. All species of filamentous green algae were grouped into one category for purposes of cover data, and samples were returned to the laboratory and identified using the keys of Gabrielson et al. (1989).

*F. gardneri* plant lengths were then measured. Five plants were selected in each quadrat by picking the individual that was closest to each of five randomly marked points. Plant length was measured as the distance from the holdfast to the end of the longest frond.

### 2.2. Oocyte release

Sampling for oocyte release was conducted concurrently with the transect surveys; collections took place in July, August, October and December 1998. Plants were collected haphazardly from the middle of the *F. gardneri* zone, within 20 m of the transect stations. Plants were sought which appeared to be reproductive, namely those that had swollen receptacles and conceptacles (reproductive tips of plants and gamete-producing cavities, respectively), were dark in colour and were covered in a large amount of exudate (Pollock, 1969). Three samples were collected at each station; plants were pulled from the substratum by hand, placed in open plastic bags and transported on ice to the laboratory. Oocyte release was induced by following the procedures of Pollock (1969) with minor modifications. Oocytes were counted under a dissecting microscope, and counts were normalized to the surface area of the plant tissue that had produced them.

### 2.3. Cu body burden in Fucus gardneri

*F. gardneri* were collected from the middle of their vertical distribution (approximately 2.0 m above LLLW) in July 1998 concurrently with the transect data collection. Three plants were collected at each station. These plants were returned to the laboratory, rinsed and hand-scrubbed three times with seawater and sent to ASL Laboratories (Vancouver, BC) for Cu content analysis. Plants were air-dried in a laminar flow fumehood at ambient temperature, ground and then ashed at 470°C for 24 h in a muffle furnace. Ashed material was digested in a 1:1 mixture of concentrated nitric acid and concentrated hydrochloric acid at 95°C for 2 h. Cu analysis was carried out using flame atomic absorption spectrophotometry.

### 2.4. Statistical analyses

*F. gardneri* per cent cover, plant length, oocyte release, Cu body burden and filamentous green algal cover were all compared across stations using a one-way analysis of variance (ANOVA) with $\alpha = 0.05$. At some stations, certain measurements yielded values of zero in all quadrats; e.g. per cent cover of *F. gardneri* at stations BB-C through BB-F was consistently zero. These measurements cannot be analyzed by ANOVA because they have no variance. These stations were omitted from the ANOVA, but the results can be interpreted based on their obvious contrast with stations with non-zero values for the measurement in question. If significant differences were detected by the ANOVA, a Student–Newman–Keuls (SNK) test was conducted to determine which stations were significantly different from the others.

Some violations of the assumptions of these parametric statistical tests, namely the assumptions of equal variances and of normal distributions, were detected using Levene's test and the Kolmogorov–Smirnov test, respectively. Transforming the data did not eliminate these violations. Most non-parametric procedures, while not requiring normality of the data, still require equal variances in all samples (Underwood, 1997). While ANOVA and SNK tests do make the assumptions listed above, they are very robust to departures from normality and equality of variances (Zar, 1996; Underwood, 1997). Therefore, parametric ANOVA and SNK tests are used despite occasional violations of assumptions.

## 3. Results

### 3.1. *F. gardneri* cover

Per cent cover estimates for *F. gardneri* are presented in Fig. 3, and results of statistical analyses are shown in Table 1. No *F. gardneri* was found at any time at stations BB-C, BB-D, BB-E or BB-F (see Fig. 1 for station locations). Outside of this zone of *F. gardneri* absence, however, no significant differences were detected among estimates of *F. gardneri* cover at any stations, with one exception. This general lack of significant differences occurs despite an apparent trend toward lower cover of *F. gardneri* at stations BB-B, BB-G and BB-H than at FC stations.

### 3.2. Filamentous green algal cover

Per cent cover of filamentous green algae is presented in Fig. 4, and results of statistical analyses are listed in

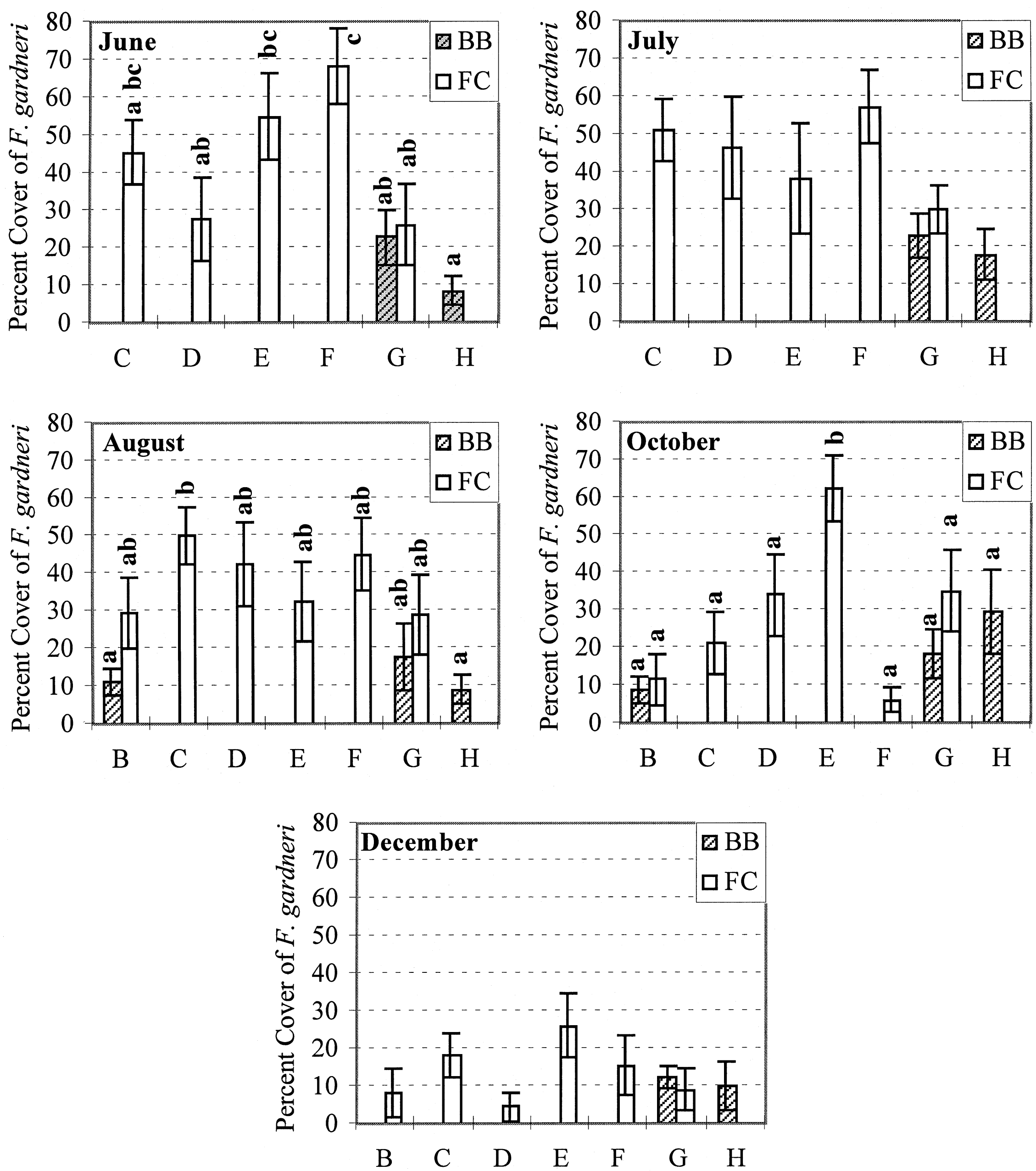


Fig. 3. Per cent cover (mean ± 1 S.E.) of *Fucus gardneri* as measured from June to December 1998. See Fig. 1 for locations of sampling stations. Sampling was not conducted at stations B until August. $n = 9$ for all stations. Letters above bars show results of Student–Newman–Keuls tests: bars that share no letters are significantly different from each other.

Table 1
Statistical analyses of all results[a]

| Parameter | June | July | August | October | December |
|---|---|---|---|---|---|
| *Fucus gardneri* per cent cover | **< 0.001** | 0.051 | **0.008** | **< 0.001** | 0.307 |
| Filamentous green algal cover | **< 0.001** | **< 0.001** | **< 0.001** | 0.199 | **0.002** |
| *Fucus gardneri* plant length | **< 0.001** | **< 0.001** | **0.001** | 0.157 | 0.350 |
| *Fucus gardneri* oocyte release | n/a | n/a | n/a | **0.006** | 0.105 |
| *Fucus gardneri* Cu content | n/a | n/a | **< 0.001** | n/a | n/a |

[a] Values shown are *P*-values of analyses of variance with $\alpha = 0.05$. Significant results are indicated in bold type. Sample sizes are indicated in the figure legends for the appropriate data set.

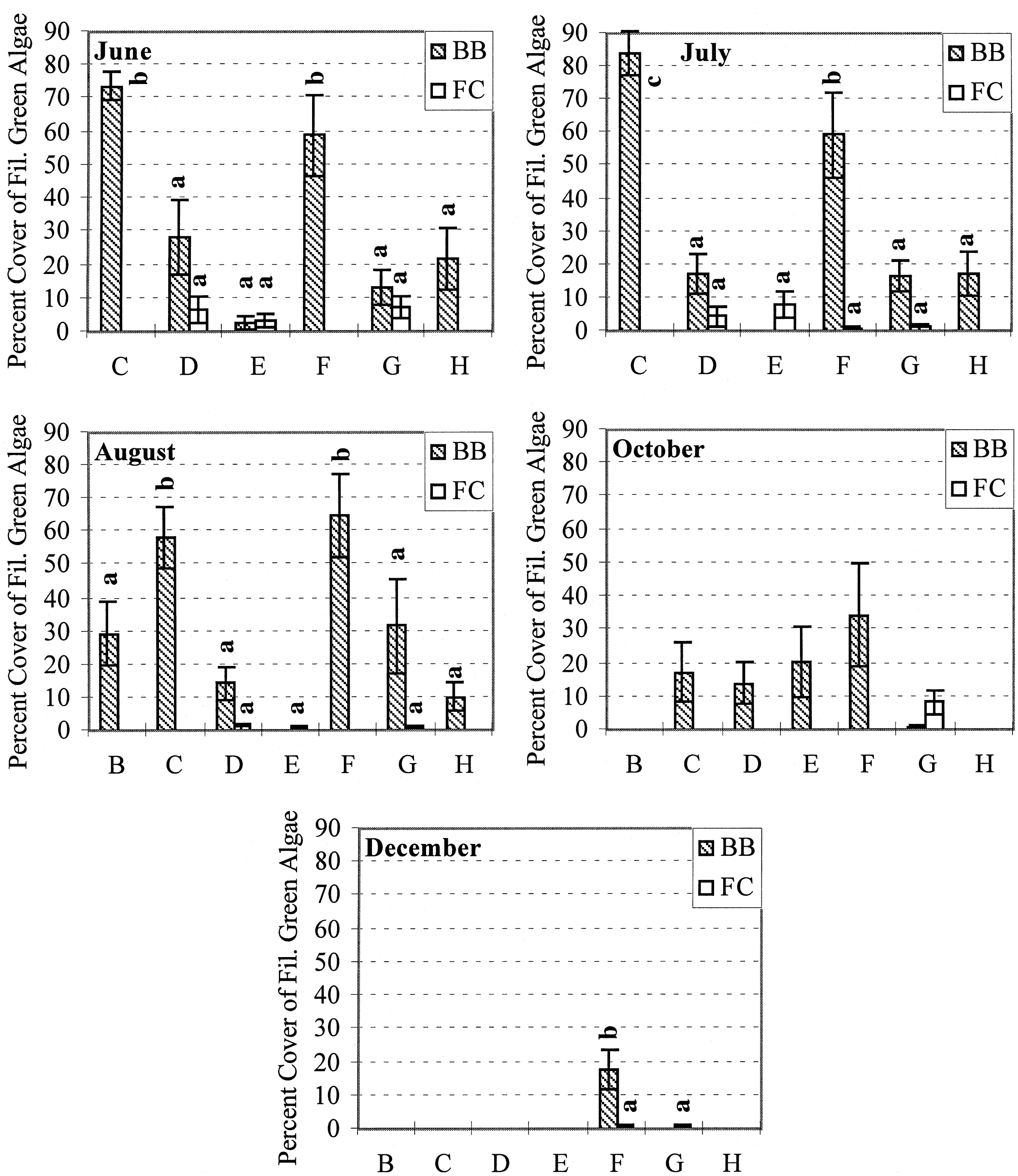


Fig. 4. Per cent cover (mean ± 1 S.E.) of filamentous green algae as measured from June to December 1998. See Fig. 1 for locations of sampling stations. Sampling was not conducted at stations B until August. $n = 9$ for all stations. Letters above bars show results of Student–Newman–Keuls tests: bars that share no letters are significantly different from each other.

Table 1. Filamentous green algae generally comprised two groups: *Enteromorpha intestinalis* (L.) Link and *Ulothrix* spp. Further identification of the latter was not possible due to variability in specimens within the ranges listed in the keys. The assemblages of filamentous green algae at stations near Britannia Creek consisted only of *E. intestinalis*, whereas those in other areas comprised both *E. intestinalis* and *Ulothrix* spp. Filamentous green algal cover tended to be higher at all stations near Britannia Creek than at stations near Furry Creek, and was significantly higher at stations BB-C and BB-F than at all other stations in June, July and August.

### 3.3. *F. gardneri plant length*

Lengths of plants at each station are presented in Fig. 5, and results of statistical analyses are presented in Table 1. *F. gardneri* at Britannia Beach stations tended to be shorter than those at Furry Creek, but, while some differences were detected by the ANOVA, they were not elucidated by the SNK test.

### 3.4. *Oocyte release*

The July and August releases yielded extremely low numbers of oocytes, often fewer than five per individual plant. In contrast, October and December samples yielded very high numbers of oocytes, frequently more than 600 per plant. Therefore, only October and December data are presented and analysed (Fig. 6). Results of statistical tests are presented in Table 1. Significantly more oocytes were released by *F. gardneri* from station BB-B (see Fig. 1 for station location) than at all other stations in October, while there were no differences among other stations. No significant differences were detected among oocyte releases from December samples. There is no general tendency toward higher or lower oocyte releases from plants at Britannia Beach.

### 3.5. *Cu body burden in F. gardneri*

Results of Cu body burden measurements are shown in Fig. 7, and results of statistical comparisons are shown in Table 1. Cu body burdens in *F. gardneri* from all Britannia Beach stations are significantly higher than those in algae from Furry Creek stations, with the highest Cu body burden being found in plants to the north of Britannia Creek.

## 4. Discussion

### 4.1. *F. gardneri cover*

Stations BB-B and BB-G are the nearest locations to the north and south of Britannia Creek, respectively, where *F. gardneri* grows. BB-B is 1000 m north of the Creek mouth, while BB-G is 600 m to the south of the Creek, leaving 1600 m of shoreline completely devoid of this alga. An ANOVA detected significant differences in the cover of *F. gardneri* among stations that supported the alga (BB-B, BB-G, BB-H, and all FC stations) in June, August and October (Table 1), but the SNK test could only elucidate some of these differences (Fig. 3). This lack of consistent differences is probably caused by the high variances that were present at all stations. *F. gardneri* is patchily distributed on the scale of the quadrat used ($25\times25$ cm; pers. obs.), resulting in high variances and, therefore, low power of any statistical tests.

A trend is evident in the data, however: in all months except December, the per cent cover of *F. gardneri* at all the Britannia Beach stations was lower than at all Furry Creek stations, although the differences were not statistically significant (Fig. 3, Table 1). This suggests that there may be some effect of AMD on the cover of *F. gardneri* beyond the 1600-m section from which the plants are absent, but that this difference was not detectable with the methods and sample sizes used in this study. The biological importance, in terms of algal biomass and associated fauna, of the difference observed at these stations is debatable; direct measurement of biomass might be a more appropriate way of determining this biological significance. The absence of *F. gardneri* from the 1600 m surrounding Britannia Creek, however, is not in dispute.

Any relationship between the distribution of AMD and the absence of *F. gardneri* that might be inferred from these observations is exclusively correlative. However, AMD appears to be the most likely cause of the disrupted distribution of this alga near Britannia Creek. All other creek mouths observed in upper Howe Sound, including Furry, Potlatch and Woodfibre Creeks (see Fig. 1 for locations), are dominated by a thick cover of *F. gardneri* (pers. obs.). An alternative explanation for the dearth of *F. gardneri* at Britannia Creek relative to Furry Creek might be the higher turbidity and lower salinity the plants would encounter at the former location due to its proximity to the Squamish River, which dominates the salinity regime in upper Howe Sound during freshet. However, extremely dense populations of *F. gardneri* were found growing to the north of Britannia Creek at Watts Point (pers. obs.). As well, the Woodfibre Creek area encounters higher turbidity and lower salinity than Britannia Beach during freshet (Stockner et al., 1977; Grout et al., 1999). Despite these environmental features, however, the mouth of Woodfibre Creek has *F. gardneri* cover in excess of 80% in some places (pers. obs.). Salinity and turbidity, therefore, do not appear to be viable explanations for the lack of *F. gardneri* at Britannia Beach.

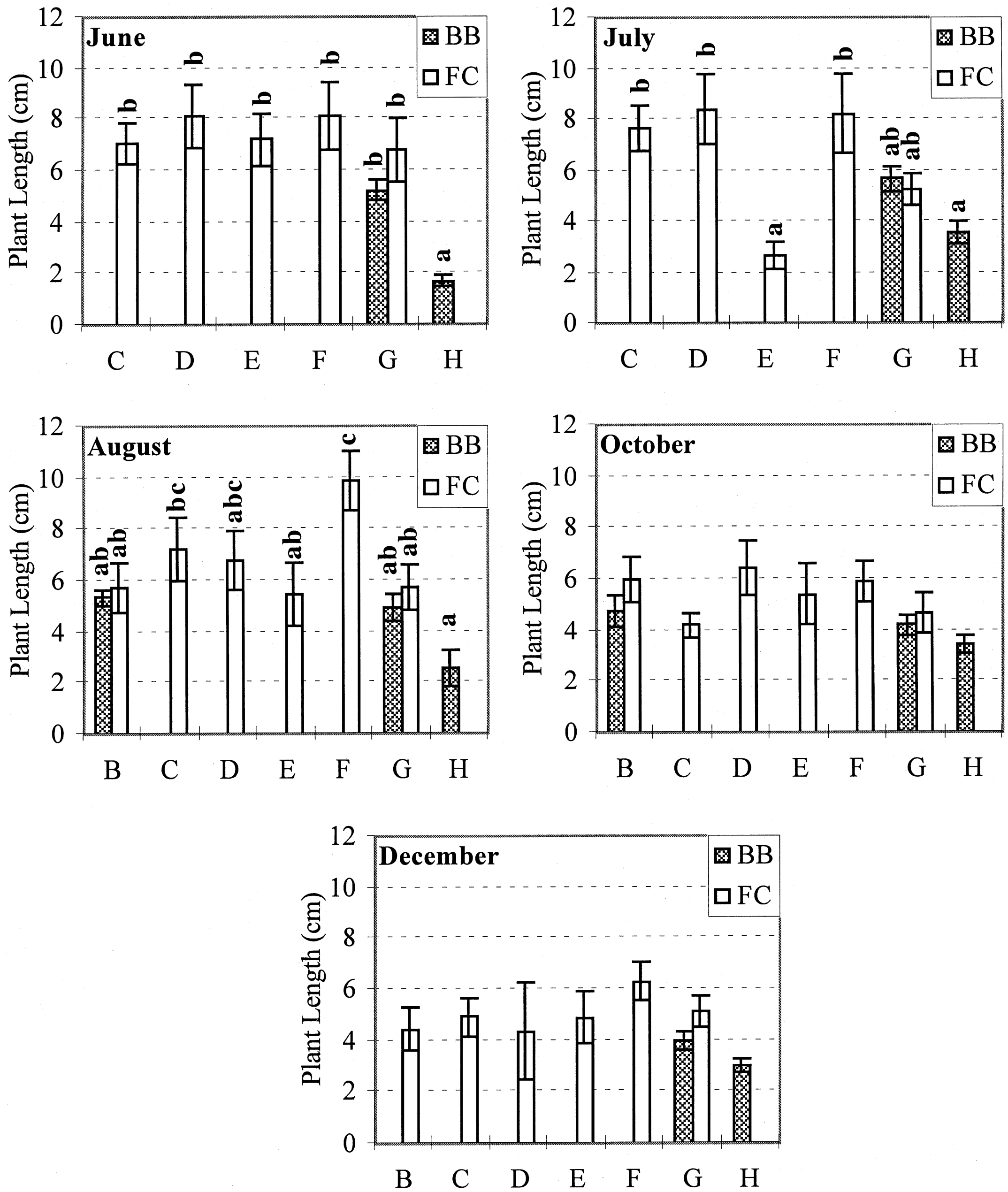


Fig. 5. Plant length (mean ± 1 S.E.) of *Fucus gardneri* as measured from June to December 1998. See Fig. 1 for locations of sampling stations. Sampling was not conducted at stations B until August. *n* varied from 5 to 9 (mean = 7.9). Letters above bars show results of Student–Newman–Keuls tests: bars that share no letters are significantly different from each other.

### *4.2. Filamentous green algal cover*

Many possible mechanisms may be responsible for the observed distribution of filamentous green algae (Fig. 4). It seems that *E. intestinalis* can tolerate higher concentrations of AMD than can *F. gardneri*, resulting in its growth much closer to Britannia Creek. As mentioned above, several authors have found that *Enteromorpha* spp. are much more tolerant of Cu than other algae (Reed and Moffat, 1983; Correa et al., 1996), and that they can dominate Cu-polluted areas (Castilla, 1996). Any comparison of these studies to the current one might be tenuous, however, as the speciation of the metal from Britannia Creek is likely different from that in Reed and Moffat's (1983) laboratory experiments. Furthermore, the high temporal variability of dissolved metal levels in Howe Sound and the different species of algae used in the two studies makes any direct comparisons difficult.

### *4.3. F. gardneri plant length*

The results of the *F. gardneri* plant length measurements are similar to those for *F. gardneri* cover (Section 4.1). There is a trend toward shorter plant lengths at

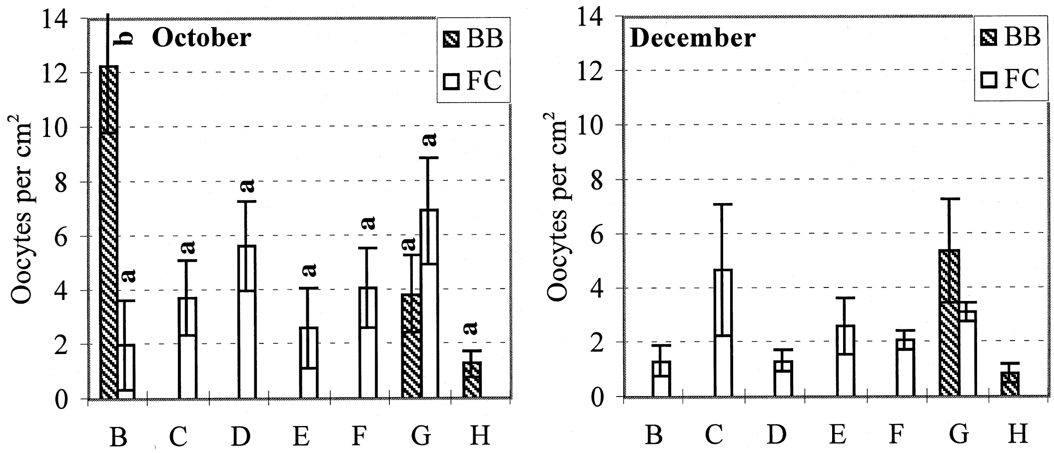


Fig. 6. Oocyte release (mean ± 1 S.E.) from *Fucus gardneri* as measured in October and December 1998. See Fig. 1 for locations of sampling stations. $n=3$ at all stations in October; $n=5$ at all stations in December except FC-G, BB-G ($n=3$) and FC-C ($n=4$). All values are normalized to the surface area of the source plant material. Letters above bars show results of Student–Newman–Keuls tests: bars that share no letters are significantly different from each other.

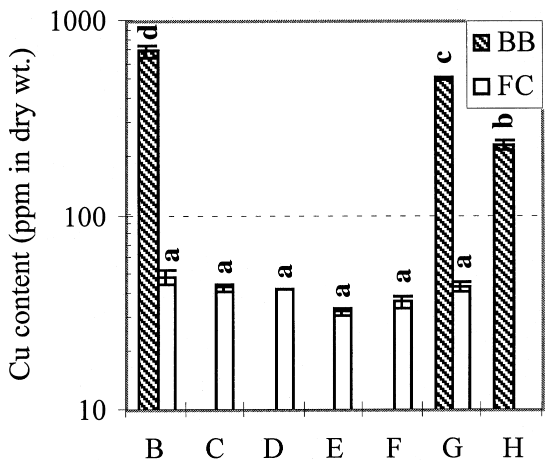


Fig. 7. Cu body burden (mean ± 1 S.E.) in dry weight of *Fucus gardneri* collected August 1998. See Fig. 1 for locations of sampling stations. $n=3$ at all stations. Letters above bars show results of Student–Newman–Keuls tests: bars that share no letters are significantly different from each other.

some Britannia Beach stations relative to Furry Creek stations, but this trend is less pronounced and less consistent than that for *F. gardneri* cover (Fig. 5). These differences might be statistically significant if larger sample sizes were used, but the biological significance of such differences could again be questioned.

### 4.4. Oocyte release

Only one oocyte release experiment (in October) showed any significant differences, with plants at BB-B producing more oocytes than those at any other station. The most likely explanation for this result is that the *F. gardneri* populations were undersampled, and the October result was obtained by chance. No conclusions can be drawn, however, without more extensive study at all stations.

It is important to note that the sampling method employed precludes any conclusions about the reproductive viability of populations at any station. The haphazard selection of plants which appeared reproductive, as in the current investigation, only allows inferences about the relative numbers of eggs released by plants once they have become reproductive. Potential effects of AMD on the ability of plants to reach this stage are not addressed here.

Another important consideration is the viability of the oocytes produced at stations BB-B, BB-G and BB-H. It is possible that these oocytes would not germinate in any area as a result of AMD toxicity during their pre-release development. In addition, spermatozoa may be detrimentally affected by AMD, leading to a reduced reproductive capacity for the plants that is not detected by counting oocytes. There are no conclusive differences among the stations that support the original hypothesis that plants near Britannia Beach would produce fewer oocytes. However, this does not rule out other possible reproductive effects.

### 4.5. Cu body burden in F. gardneri

There have been many previous studies of metal content in *Fucus* spp., although most have examined habitats in Europe and fucoid algae other than *F. gardneri*. Riget et al. (1995) found 1.3–3.3 ppm Cu in dry weight of *Fucus vesiculosus* in unpolluted water in Greenland. Other studies in Europe have found up to 20 ppm Cu in dry weight of *Fucus* spp. in what are referred to as unpolluted waters (Foster, 1976; Bryan, 1983; Ho, 1984). Bryan (1983) also examined the metal content in *Fucus* spp. in the mine water-polluted Fal Estuary and found 293 ppm Cu in dry weight.

The levels of Cu in *F. gardneri* in this study agree well with those measured by Dunn et al. (1992) in Howe

Sound, who found up to 960 ppm Cu in dry weight of *F. gardneri*. Overall, the Cu concentrations found in Howe Sound-*F. gardneri* appear to be substantially higher than those found in similar seaweeds in Europe. This is likely due in part to higher levels of Cu in Howe Sound than in water in other places. However, *F. gardneri* may not be directly comparable to the species examined in Europe, as it may accumulate more metal from similar concentrations of water. As well, differences in salinity, pH and other parameters affecting Cu speciation and uptake may differ in the areas considered.

### 4.6. Statistical methods

The statistical methods appear to give a reasonable representation of the results for both the cover of filamentous green algae and *F. gardneri* body burden of Cu: the differences detected using the statistical tests are readily apparent in the graphs (Figs. 4 and 7). In contrast, the analyses of *F. gardneri* cover and, to a lesser extent, *F. gardneri* plant length appear to contain errors. In five of 10 analyses of these two community parameters, the ANOVA detected significant differences but the SNK test failed to elucidate them (Table 1). Where this is the case, one of two errors is occurring: (1) a Type I error is occurring in the ANOVA; or (2) a Type II error is occurring in the SNK test. In the case of *F. gardneri* cover, a Type II error appears to be more likely, as examination of Fig. 3 reveals a smaller per cent cover of *F. gardneri* at BB stations than at FC stations. In the case of *F. gardneri* plant length, however, the trend toward shorter plants at BB stations is less pronounced and less consistent; therefore, either type of error is possible.

Perhaps the most important problem in the above analyses is that the power of the methods used was insufficient to detect subtle differences that may exist in some community parameters (e.g. in *F. gardneri* cover and plant length). This does not appear to be a problem in the statistical methods themselves, but rather in the survey methods employed. Due to the patchiness of the intertidal at the scale examined, larger and more quadrats would likely have reduced the standard errors of the measurements taken. This would have increased the power of statistical tests to detect differences among algal communities at each station. Practical limitations precluded such expansion of the sampling programme.

## 5. Conclusions

A series of hypotheses regarding algal communities near the AMD-polluted Britannia Creek were presented in the introduction. *F. gardneri* plant length, cover and gamete production and filamentous green algal cover were all expected to be lower at stations near Britannia Creek than at relatively unpolluted reference stations. Body burden of Cu in *F. gardneri* was expected to be higher in plants at the polluted stations relative to the reference stations. No marine algae are found at the mouth of Britannia Creek, while filamentous green algae dominate at greater distances (300–700 m) from this Creek mouth. Thus, AMD appears to strongly influence algal community structure within the 1600-m zone from which *F. gardneri* is absent. However, there is no evidence of significant AMD effects on per cent cover, plant length or oocyte production of algae in areas where *F. gardneri* exists. Cu body burden of the *F. gardneri* growing nearest the Creek is five to 17 times that of plants growing 5 km from Britannia Creek. These results provide strong evidence for the role of AMD in structuring algal communities near Britannia Creek.

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