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A Comparison of Analytical Digestion Techniques for the Detection of Elemental Tracers Associated with Marine Finfish Aquaculture

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ABSTRACT

Two different digestion techniques for trace-element analysis were carried out on marine sediment samples collected in the Broughton Archipelago, British Columbia, Canada, to compare their utility in terms of identifying excess element concentrations using a geochemical normalization technique. The first technique consisted of a partial digestion of sediment samples using the Strong Acid Leachable Metals (SALM) method, while the second technique carried out a total digestion of sample material using hydrofluoric (HF) and nitric (HNO_3) acids. Relationships observed between HF- and SALM-derived trace-element concentrations for Cd, Ca, Cr, Cu, Fe, Pb, Li, Mg, Mo, Ni, P, Na, S, V, and Zn were stronger than those for trace-elements, Al, As, Ba, Be, Co, Mn, K, Sr, Sn, Ti, and U. Regression analysis of the combined data set of the former group showed a strong correlation and an offset from unity between the congruent trace-element concentrations. The weak relationships observed for the latter group were attributed to resolution associated with detection-limit issues (As, Be, Sn, and U) and/or sample heterogeneity (Al, Ba, Co, Mn, K, and Ti). Geochemical normalization against lithium (Li) allowed for the quantification of "excess" levels of trace-element tracers and redox indicators. Both digestion methods clearly identified samples containing excess levels of both direct (Cu, Zn, P) and indirect (Mo) aquaculture tracers through the Li-normalization technique. Stronger element:Li correlations were observed for SALM methods in comparison to HF. Although the SALM method involves a partial digestion of trace-elements, it is useful in identifying both direct and indirect tracers of aquaculture activities once combined with Li-normalization, which can be used to complement benthic organic enrichment indicators used in environmental monitoring programs.

Keywords: analytical technique comparison, indicators, trace-elements

INTRODUCTION

Chemical tracers of aquaculture activities serve as important tools for assessing effect pathways within ecosystem-based management strategies associated with aquaculture-environmental interactions. For example, sediment tracers of finfish wastes, including trace-elements, organic content, redox potential and porewater ΣH_2S concentrations have been used individually or in concert to identify potential impacts of aquaculture operations in the immediate vicinity of farms in various parts of the world (Chou *et al*. 2002; Schaanning and Kupka Hansen 2005; Smith *et al*. 2005; Wildish *et al*. 2005; Mendiguchia *et al*. 2006; Dean *et al*. 2007; Sutherland *et al*. 2007a, 2007b; Hargrave *et al*. 2008; Basaran *et al*. 2009; Papageorgiou *et al*. 2009). Certain trace-elements serve as direct indicators of waste material (zinc (Zn), phosphorus (P), copper (Cu); Lewis and Metaxas 1991; Sutherland *et al*. 2001; Petersen *et al*. 2005; Yeats *et al*. 2005), while indirect tracers (cadmium (Cd), molybdenum (Mo), uranium (U); Smith *et al*. 2005) are those elements which preferentially accumulate in anoxic, organic-rich sediments associated with aquaculture operations.

Both direct and indirect tracers are naturally-occurring sediment components, and therefore their usefulness as tracers of far-field effects of fish farms will depend on the accuracy and precision of techniques for separating the tracer signal from the natural background signal. Lithium (Li) normalization (Loring 1991; Soto-Jiménez and Paez-Ozuna 2000; Aloupi and Agelidis 2001; Sutherland *et al*. 2007a) effectively reduces the background noise due to variations in the type of source rock and sediment grain size for a number of elements including Cu, Zn, Mo and U whose concentrations in detrital marine sediments are mostly determined by their concentrations in clay minerals. Other normalization techniques will be required for elements such as Ca, the abundance of which is not as simply related to clay mineral composition and grain size. Yeats *et al*. (2005) have shown how this technique can be used to compensate for variations in the background concentrations of Cu and Zn in aquaculture-influenced settings. A second approach to increasing the tracer signal to the background noise ratio is to minimize the dissolution of the background concentrations. While it is not possible to dissolve only the farm waste component of the sediments, some techniques will be better than others at maximizing dissolution of the tracer signal while minimizing dissolution of the background. One such technique that is regularly being used in the detection of metals in marine environments in British Columbia is a Strong Acid Leachable Metals (SALM) method that uses hot concentrated nitric and hydrochloric acid for partial digestion of the samples (SALM; BCMELP 2001). This method liberates environmentally available forms (exchangeable, and those bound to Fe oxides, Mn oxides, sulfides, carbonates) and minimizes dissolution of more refractory minerals (e.g., silicates). Another traditional method involves the dissolution of the entire sediment sample using hydrofluoric acid (HF) in combination with other mineral acids to provide a total elemental concentration.

The objective of this paper is to determine the effect of digestion technique (SALM vs. HF) on sediment trace-element concentrations. The more complete HF digestion technique has been employed on the east coast of Canada, while the SALM method is commonly used in British Columbia (west coast of Canada). A comparison of direct concentrations of trace-elements as well as Li-normalization results of inorganic tracers of aquaculture were carried out and

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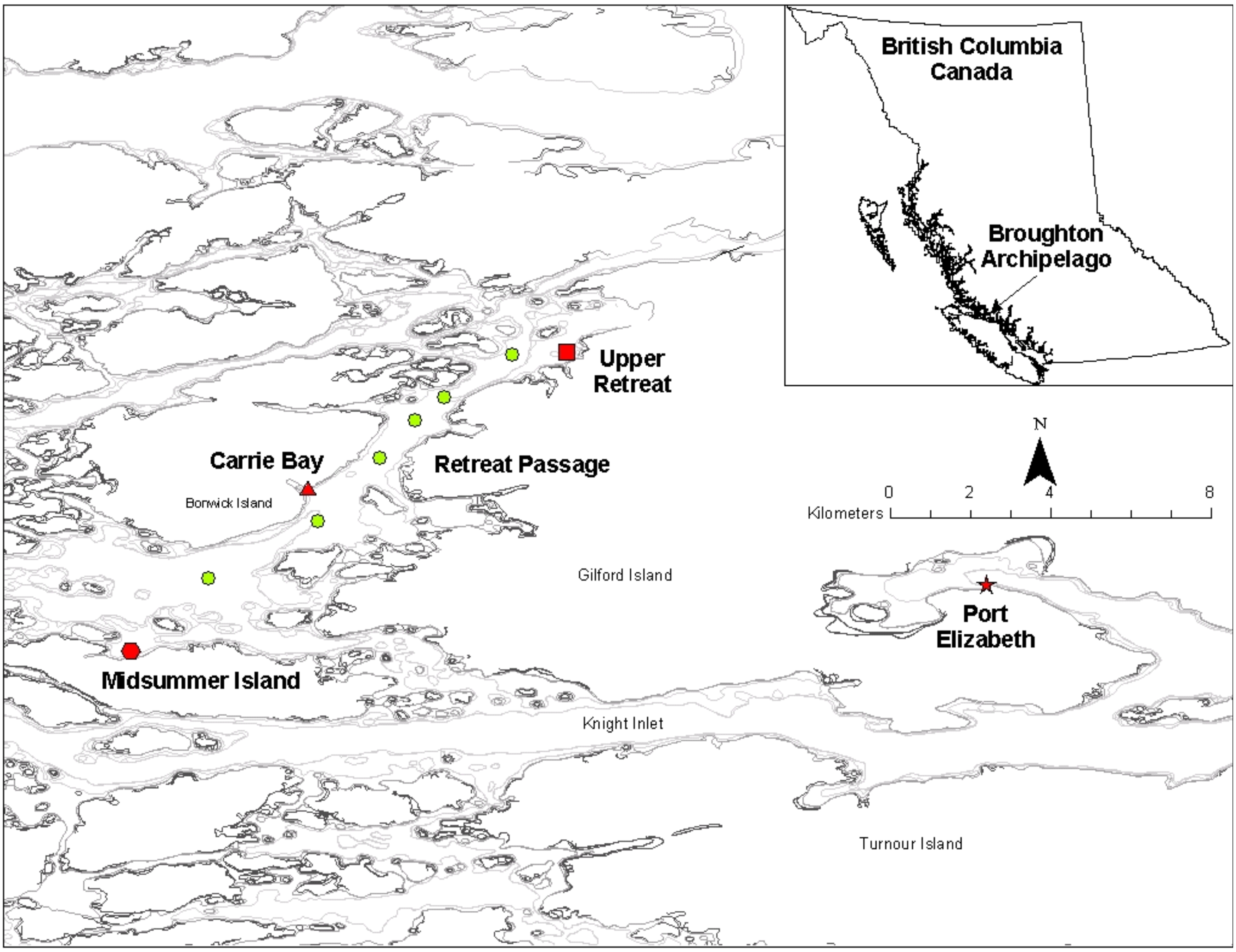

Fig. 1 The locations of fish farm and reference sites in the Broughton Archipelago, British Columbia, Canada. Green circles = reference stations in Retreat Passage; red square, red triangle, and red hexagon = fish farm sites.

their respective benefits and disadvantages discussed as part of the first objective.

MATERIALS AND METHODS

Sample collection and processing

Individual sediment samples were collected at 47 stations within the Broughton Archipelago (BA), British Columbia Canada (**Fig. 1**). The samples were collected as part of a larger project examining potential benthic impacts associated with marine finfish operations (Sutherland *et al*. 2007a, 2007b, 2007c; Sutherland and Yeats 2011). The stations were located at 4 different fish farm sites (red symbols in **Fig. 1**) and along a reference transect located in a nearby channel (represented by green circles in **Fig. 1**). Sampling stations were located at various distances between 0, 30, 60, 90, 300, 1000, and 5000 m from netpen systems. The samples collected at 300 and 500 metres from netpen systems were also considered to be reference or background samples along with those collected from Retreat Passage. Sutherland *et al.* (2007a) showed that trace-element estimates collected at 300, 500, and > 1000 m from the fish farm systems all fell within the confidence limits of a geonormalization regression providing an element-lithium reference ratio. A Van Veen grab was used to collect sediment samples from the seafloor for trace-element analyses. A Van Veen grab is made up of 2 steel buckets hinged together to serve as "jaws" that grab a sample of the seafloor. The grab is lowered to the seabed with the buckets ("jaws") propped open, digs into the seafloor upon impact, and captures a sediment sample upon the ascent of the grab. The relatively large surface area and the strong closing mechanism allow the jaws to excavate relatively undisturbed sediments to a maximum depth of ~25 cm.

Trace metal analysis

Each sediment sample was split into 2 subsamples in order to carry out separate analytical digestion techniques pertaining to total (HF) and partial (SALM) digestion methods. The first set of subsamples (n=47) obtained from sediment sampling were analyzed for trace-elements according to the US EPA method ICP-AES 200.15 using ultrasonic nebulization (US EPA 1994). The digestion process followed that of the SALM method where a sample is first dried at <60°C, then digested in a mixture of concentrated nitric (HNO_3) and hydrochloric (HCl) acids at 90°C for 2 hrs. The extracts are then analyzed for trace element content via inductively coupled plasma atomic emission spectroscopy (ICP-AES). The second set of subsamples obtained from the 47 sediment samples were digested using a microwave assisted HNO_3 and HF digestion procedure that was based on EPA method 3052. The digests were then analysed using a combination of ICP-AES for the majority of elements and inductively coupled plasma mass spectrometry (ICP-MS) for low concentration elements including Sb, La, Tl, Sn and U. Five samples were analyzed in duplicate to ensure quality control. The data were $\log_{10}$ transformed to separate the data points and allow for a closer examination of the systematic shift from unity due to the different digestion methods.

Statistical analyses

A correlation analysis was conducted for each trace-element to compare the concentrations resulting from each digestion method. Trace-elements that showed relatively strong coefficients of determination (r^2>0.4) between digestion methods were combined to provide an overall comparison against unity. Trace-element concentrations were normalized using lithium in cases where trace-

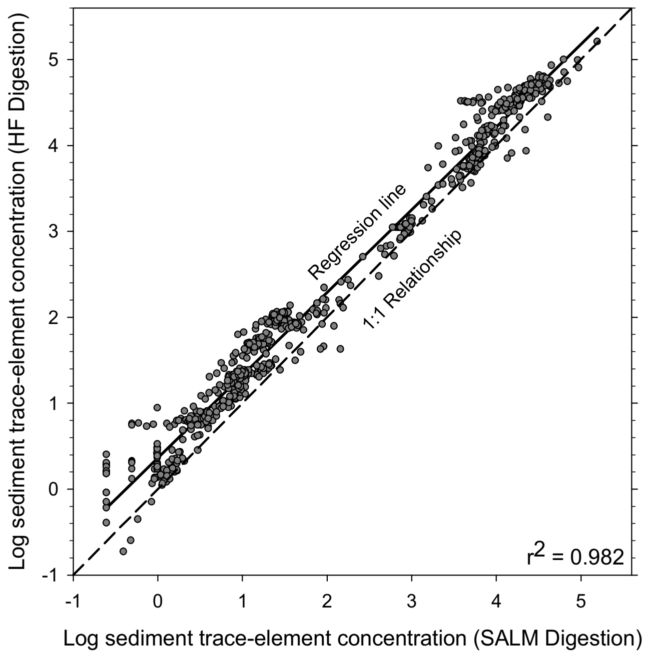

Fig. 2 The relationship between solid-phase trace-element concentrations analysed using HF and SALM digestion methods. Trace-elements presented in this plot include Cd, Ca, Cr, Cu, Fe, Pb, Li, Mg, Mo, Ni, P, Na, S, V, and Zn. HF = hydrofluoric acid, SALM = Strong Acid Leachable Metals.

elements were identified as aquaculture tracers of waste material (Smith *et al.* 2005; Yeats *et al.* 2005; Sutherland *et al.* 2007a). Background or reference samples were used to create a Li-normalized regression equation for each trace-element. Background data in the BA were obtained from sampling stations located in two designated far-field regions: 1) 300-500 m from net pen positions within a fish farm bay and 2) ~ 1000 m from netpen positions in Retreat Passage.

RESULTS AND DISCUSSION

The use of two digestion techniques was explored to identify elevated concentrations of both direct (Cu, P, and Zn) and indirect (Cd, Mo and U) indicators of aquaculture activities on the east and west coasts of Canada. These techniques include a “total” digestion method (HNO_3/HF) designed to quantitatively digest a sediment sample and a “partial” digestion method (SALM) designed to digest a large proportion of a sediment sample based on a strong-acid digestion procedure. Trace-element concentrations are not only influenced by natural factors associated with marine sediments, such as elemental sources, grain size distributions, and redox conditions, but also by the type of analytical digestion methods employed (Luoma 1990). Measurements from both the SALM and HF digestions of the sediment samples provide a direct comparison of trace-element concentrations, since the results are based upon analysis of the same samples. The elemental concentrations resulting from the HF digestion were observed to be generally higher than those measured by SALM digestion due to the more aggressive nature of the HF method when considering the combined data set (**Fig. 2**). Elements that were excluded from the cross-digestion correlation include those elements that 1) fell below or near their individual detection limits across stations undergoing SALM digestion (As, Be, Sn, and U), and 2) did not provide a $r^2 > 0.4$ (Al, Ba, Co, Mn, K, and T). Strong cross-digestion correlations were observed for Cu, P, and Zn, which serve as direct tracers of aquaculture waste, as well as for Cd and Mo, which serve as indirect tracers of owing to their associations with organic enrichment events (**Figs. 3, 4**). However, uranium did not provide the resolution to provide a cross-digestion correlation, likely due to the partial digestion and relatively higher detection limit associated with the ICPAES analysis. Uranium was identified as an indicator of anoxic conditions associated with marine finfish aquaculture through the use of HF digestion techniques on the east coast of Canada (Smith *et al.* 2005). Correlation coefficients and equations for cross-digestion comparisons are presented in **Table 1**.

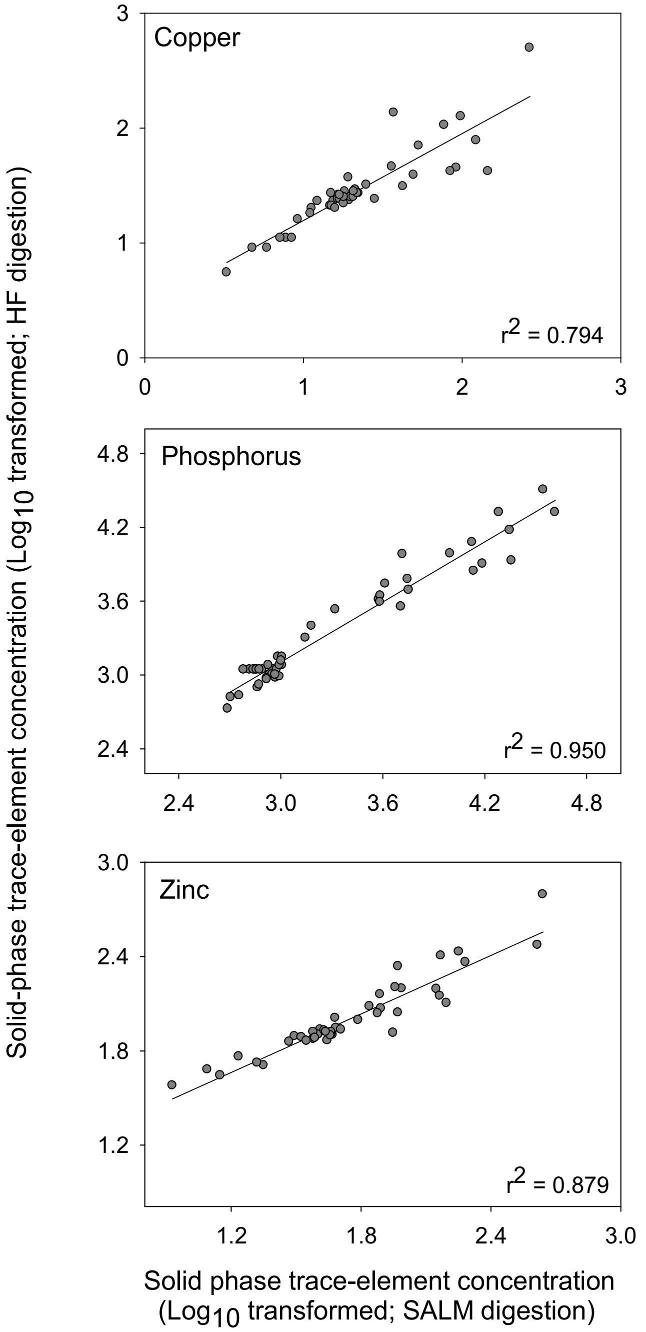

Fig. 3 The relationship between solid-phase trace-element concentrations analysed using HF and SALM digestion methods for direct tracers of aquaculture waste material (copper, phosphorus, and zinc). HF = hydrofluoric acid, SALM = strong acid leachable metals.

Table 1 Correlation equations for the relationship between trace element concentrations analyzed by HF digestion and SALM digestion. V = variable, r^2 = coefficient of determination, N = number of samples, #includes 60 duplicates, *includes 5 duplicate samples.

V	Equation	r^2	N
All	Log(y) = 0.9623*log(x) + 0.3638	0.982	780#
Cd	Log(y) = 1.2727*log(x) + 0.0345	0.746	47*
Cu	Log(y) = 0.7568*log(x) + 0.4371	0.794	47*
Mo	Log(y) = 0.6115*log(x) + 0.4470	0.730	47*
P	Log(y) = 0.8162*log(x) + 0.6548	0.950	47*
Zn	Log(y) = 0.6209*log(x) + 0.9180	0.879	47*

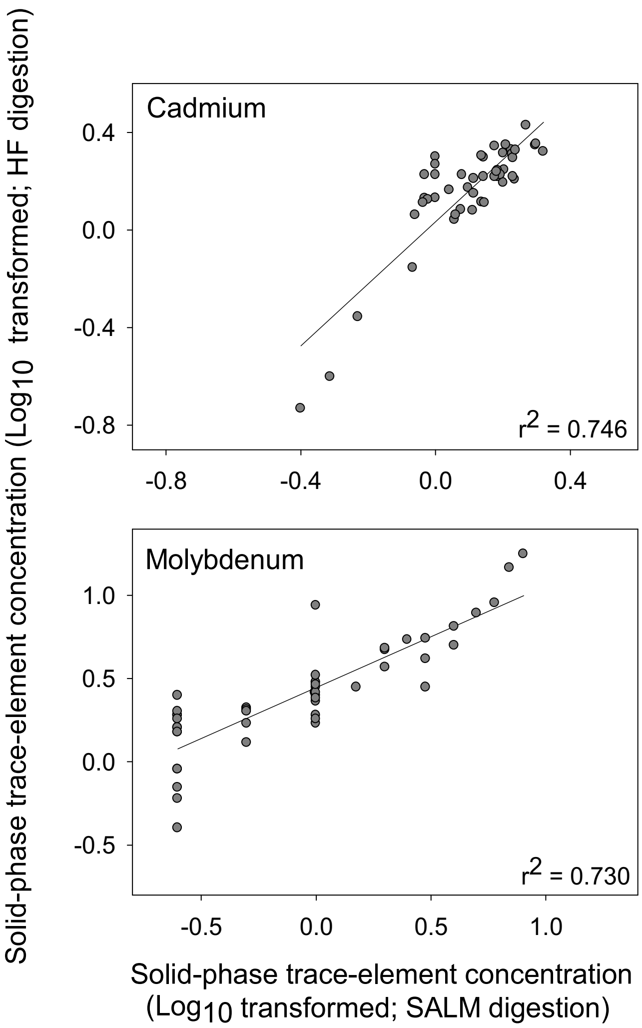

Fig. 4 The relationship between solid-phase trace-element concentrations analysed using both HF and SALM digestion methods. Cadmium and molybdenum serve as indicators of benthic organic enrichment events. HF = hydrofluoric acid, SALM = strong acid leachable metals.

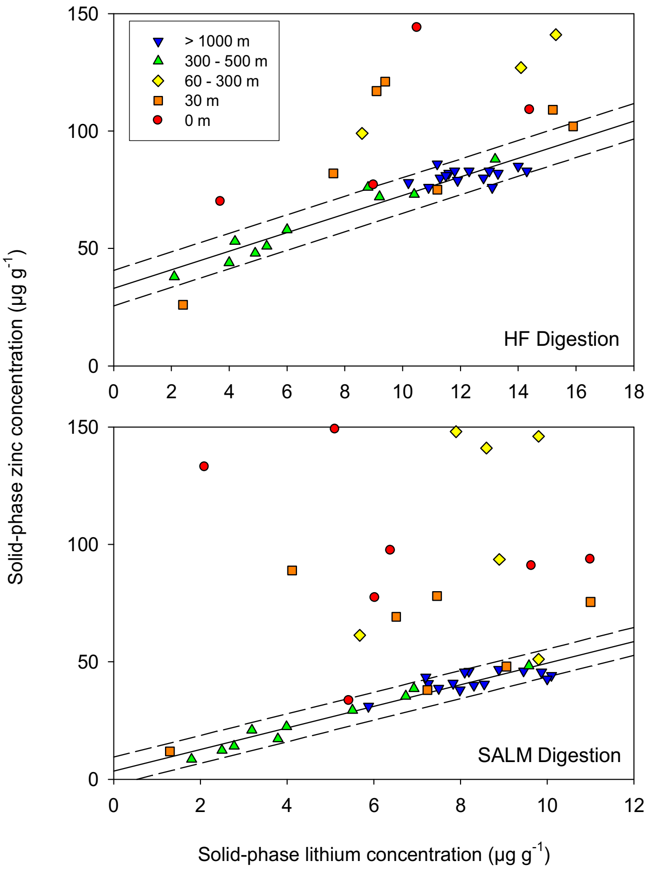

Fig. 5 The relationship between solid-phase zinc and lithium according to netpen-station distances and analytical digestion methods (HF and SALM digestion). HF = hydrofluoric acid, SALM = strong acid leachable metals. Note the Y-axes have been truncated to focus on the observations at low zinc concentrations (see **Fig. 7** for full data set analyzed by SALM digestion).

The geochemical normalization of sediment trace-elements using Li, Al, Rb, Fe or other metals as mineralogical and grain size normalizers has mainly been conducted using 'total' digestion techniques (Allen and Rae 1986; Windom *et al*. 1989; Loring 1991; Morse *et al*. 1993; Daskalakis and O'Connor 1995; Bothner *et al*. 1998). Recent attempts to normalize sediment trace-elements to identify "direct tracers" of aquaculture waste (Zn, Cu) using "partial" digestion techniques have been successful (Yeats *et al*. 2005; Sutherland *et al*. 2007a). For tracers like Cu and Zn, which are potentially toxic, above-background concentrations can be distinguished from those stations considered to be reference or background concentrations. Results presented here and in Sutherland and Yeats (2011) show that Li-normalization is also effective with both digestion techniques for Cd, Mo, and P. This geonormalization approach can be used to validate depositional footprints derived from model predictions (i.e., DEPOMOD), steer sampling designs of environmental monitoring programs, assess potential benthic toxicity issues, and track benthic recovery of fallowing practices.

The differences between the SALM and HF techniques in terms of their abilities to detect excess concentrations of elements associated with aquaculture activities were tested using the Li-normalization technique. **Figs. 5** and **6** show the Li-normalized data for both Zn and Cu, respectively, outlining the "reference" baseline regression as well as the above-baseline concentrations that reflect anthropogenic inputs. The proportion of baseline and above-baseline values appeared to be relatively similar between both digestion treatments. However, slightly higher r^2 values were observed for the baseline regressions derived from the SALM technique relative to those resulting from the HF technique (**Table 2**). This reduction in scatter and associated confidence limits will allow for greater sensitivity in terms of detecting above baseline values.

In addition to stronger correlations of the background element, lithium, the SALM digestion technique is consistent with the metal analytical techniques employed by the Canadian Council of Ministers of the Environment (CCME 1999) sediment quality guidelines. These guidelines call for 'near-total' extraction methods (*aqua-regia*, nitric acid or hydrochloric acid) and not the complete digestion technique afforded by HF digestion. Thus, values falling above the baseline regression derived by a "partial" digestion technique (SALM) can be classified according to the probable effects level (PEL) and threshold effects level (TEL) identified by CCME (1999). The thresholds for each element are used to evaluate the extent to which adverse biological effects would likely take place upon exposure of benthic invertebrates to elements in marine sediments (Sutherland and Yeats 2011). As shown in **Figs. 7** and **8**, concentrations that are greater than both background and the sediment qua-

Table 2 Correlation equations for the geonormalized relationships of zinc vs. lithium and copper vs. lithium for the far-field background samples. r = correlation coefficient, N = number of samples.

Equation	r	N
Zn (HF) = 31.9 + [3.99 x Li (HF)]	r = 0.948	31*
Zn (SALM) = 3.68 + [4.49 x Li (SALM)]	r = 0.959	28
Cu (HF) = 1.08 + [1.96 x Li (HF)]	r = 0.974	31*
Cu (SALM) = -0.12 + [2.16 x Li (SALM)]	r = 0.986	28

*3 HF samples were run in duplicate

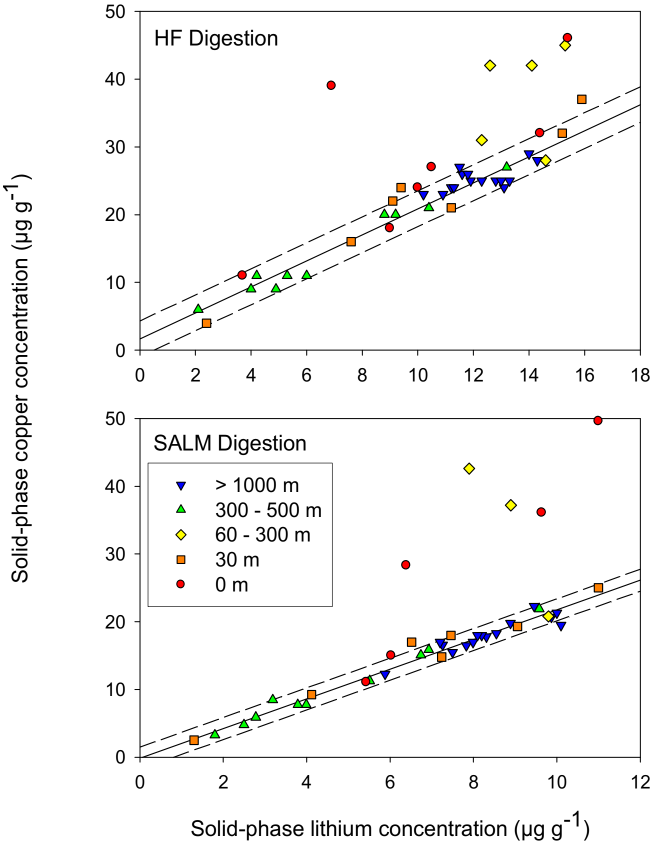

Fig. 6 The relationship between solid-phase copper and lithium according to netpen-station distances and analytical digestion methods (HF and SALM digestion). HF = hydrofluoric acid, SALM = strong acid leachable metals. Note the Y-axes have been truncated to focus on the observations at low copper concentrations (see **Fig. 8** for full data set analyzed by SALM digestion).

lity guidelines were all collected within 300 m of the pen locations. No CCME sediment quality guidelines exist for any of the other tracer/indicator elements described in this report. In the United States, methods documents associated with the NOAA Status and Trends program (Lauenstein and Cantillo 1993) and the EPA National Coastal Assessment (EPA 2000) specify total metal digestion for marine sediment samples. Thus, for our samples, HF results should be used for any comparisons to US effects range low (ERL) or effects range median (ERM) sediment quality guidelines. In Europe, the protocols for the monitoring of marine sediments and biota do not include any effects level criteria, but simply compare sediment contamination levels to background concentrations (OSPAR 2008) using a normalization approach that is consistent with the one described in this paper.

The addition of new aquaculture tracers, such as Cd, Mo, and U, raises new issues related to analytical sensitivity required for the practical application of geochemical normalization methods. Sufficient sensitivity is required to measure background concentrations of both the tracer element and the normalizer element. Since Mo and U are present in relatively low concentrations depending on the digestion technique and local environment, these elements require low detection limits. The required SALM sensitivity for U is uncertain because all analysed samples were less than detection limit, but the similarity of U and Mo concentrations in the HF extracts and more generally in sedimentary rocks (Fairbridge 1972) would suggest that the required sensitivity for U will be similar to that for Mo. Of the commonly available analytical techniques, only graphite furnace atomic absorption spectrophotometry (GFAAS) or inductively coupled plasma mass spectrometry (ICP-MS) afford sufficient sensitivity for analysis of Cd and U. A combined ICP-AES/ICP-MS approach with SALM digestion would appear best suited to meet both the sensitivity requirements for identifying tracers using the normalization technique, and maximize signal to noise ratios for the tracer signals.

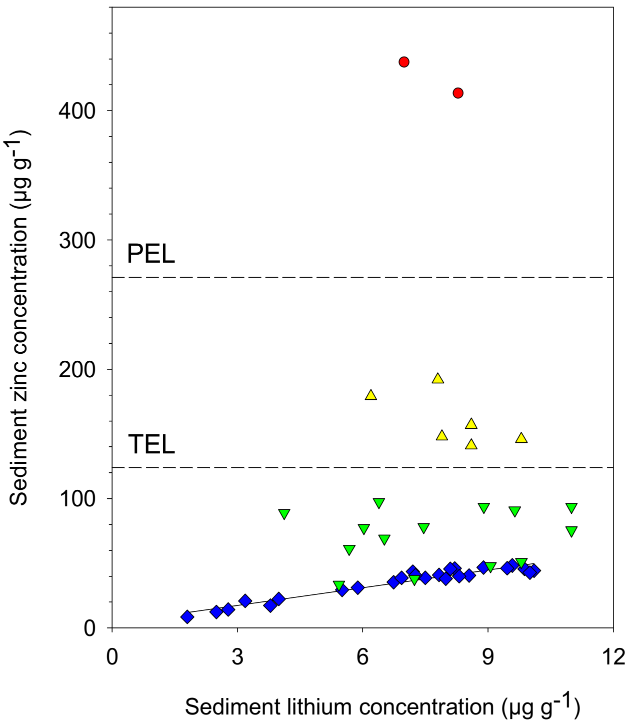

Fig. 7 The relationship between solid-phase zinc and lithium concentrations. Data are colour-coded according to Canadian Council of Ministers of the Environment sediment quality guidelines. PEL = probable effects level; TEL = thresholds effects level. Data were analyzed according to a Strong Acid Leachable Metals digestion technique (SALM).

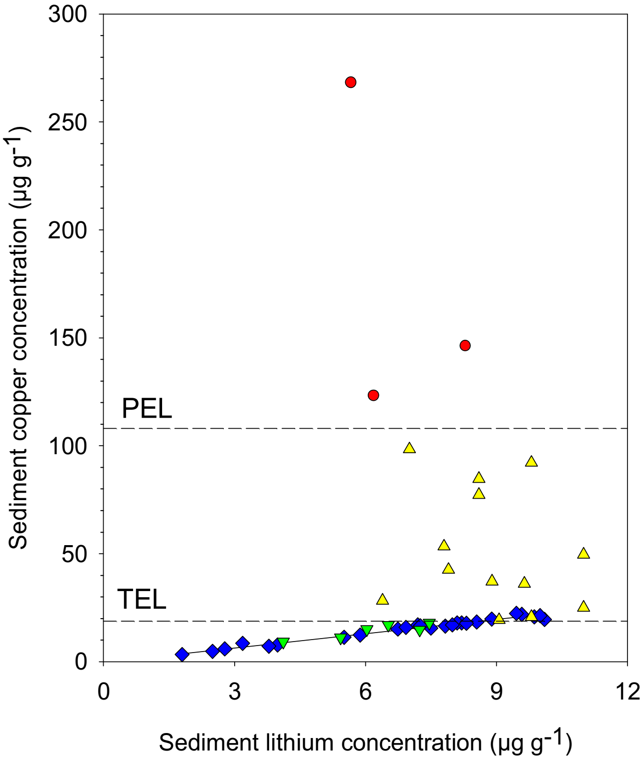

Fig. 8 The relationship between solid-phase copper and lithium concentrations. Data are colour-coded according to Canadian Council of Ministers of the Environment sediment quality guidelines. PEL = probable effects level; TEL = thresholds effects level. Data were analyzed according to a Strong Acid Leachable Metals digestion technique (SALM).

CONCLUSIONS

Inorganic tracers (Cu, P, Zn) and indicators (Cd, Mo) of aquaculture activities can be used to assess potential benthic impacts across various operational modes of finfish aquaculture in different geographic settings. Because background concentrations of Cd, Cu, Mo, P, and Zn can be effectively accounted for using Li-normalization, this method provides an effective means to define aquaculture-derived signatures. The comparison of SALM and HF digestion results shows that both techniques give essentially a similar spatial distribution of the tracer elements. Li effectively accounts for the grain size variability in the SALM data and gives slightly tighter correlations between Cu or Zn and Li for far-field samples. Very sensitive analytical techniques (ICPMS) are required to measure Cd, Mo and U at the concentrations that are encountered in these sediments using SALM.

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