Elemental Indicators of Benthic Organic Enrichment Associated with Marine Finfish Aquaculture

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ABSTRACT

Sediment samples were collected from the west and east coasts of Canada and analyzed for trace-element content to identify inorganic tracers and indicators of aquaculture waste material and benthic organic enrichment events. Samples were collected at varying distances from fish-farm sites in the Broughton Archipelago (BA), British Columbia and in Letang Inlet, southwest New Brunswick (SWNB). The results from a principal component analysis revealed that copper (Cu), molybdenum (Mo), phosphorus (P), and zinc (Zn) were consistently associated with established aquaculture indicators of benthic organic enrichment (porewater sulfide, redox potential, and/or organic matter) across different operational (active vs fallow) and oceanographic/bathymetric settings (archipelago vs estuarine). Although cadmium (Cd) and uranium (U) were linked to this aquaculture tracer/indicator grouping in SWNB, they did not show similar trends in BA, likely due to higher background concentrations (Cd) and analytical detection issues (U). In general, these findings suggest that certain trace-elements (Cu, P, Zn) serve as direct tracers of aquaculture waste material (feed, faeces and anti-fouling agents), while other trace-elements (Cd, Mo, U) serve as indirect "redox" indicators of benthic organic enrichment and associated sediment anoxia. Geochemical normalization allowed for the quantification of "excess" levels of tracers and redox indicators and, in turn, permitted correlation of these estimates with other aquaculture indicators (organic matter), spatial gradients, and meiofaunal diversity. In the BA, excess concentrations of Cu, Mo, P and Zn showed strong inverse relationships with distance from net pen systems as well as with meiofaunal abundance (Kinorhyncha). Principal component analysis and Li-normalization facilitates identification of both direct and indirect tracers of aquaculture activities that can be used to complement benthic organic enrichment indicators in environmental monitoring programs.

INTRODUCTION

Chemical tracers of finfish aquaculture wastes are important tools for ecosystem-based management of environmental effects of aquaculture activities. Benthic impacts associated with finfish production include changes in sediment quality and subsequent alterations to microbial, meiofaunal and macrofaunal diversity (Karakassis and Hatziyanni 2000; Wildish and Pohle 2005; Sutherland *et al.* 2007a; Kawahara *et al*. 2009; Terlizzi *et al.* 2010). Certain trace-elements can be used as direct tracers of aquaculture activities and can identify waste particles in the pelagic, benthic, and intertidal environments (Yeats *et al*. 2005). Zinc (Zn), phosphorus (P), and copper (Cu) are examples of trace-elements that serve as direct tracers of waste material in the form of feed and faecal pellets (Zn, P, Cu) and antifouling agents (Cu) (Lewis and Metaxas 1991; Petersen *et al.* 2005). Zn is used to prevent cataract formation in juvenile fish while P and calcium (Ca) are included in feed to ensure proper bone formation (Richardson et al. 1986). Both P and Ca exhibit high concentrations in fish feed pellets, with P becoming enriched relative to carbon in fish faecal waste since the former is not effectively utilized by salmon owing to their inability to digest phytic acid (Lall 1991). Elevated levels of Cu and Zn have been detected in the water column, seafloor sediments, and intertidal environments near aquaculture operations (Uotila 1991; Kempf *et al.* 2002; Belias *et al.* 2003; Chou *et al.* 2004; Schendel *et al.* 2004; Yeats *et al.* 2005; Mendiguchia *et al.* 2006; Dean *et al.* 2007; Black *et al.* 2008). Collectively, P, Cu and Zn can be used to identify the source of waste material contributing to benthic organic enrichment and validate dispersion models designed to predict waste transport and depositional footprints.

Trace-elements that show preferential accumulation under reducing redox conditions may also serve as indicators (indirect tracers) of benthic organic loading events (Macdonald *et al.* 2008; Laing *et al.* 2009). Trace-elements, such as molybdenum (Mo) and uranium (U), for example, are removed from solution in reducing sedimentary porewaters through the precipitation of authigenic sulfides. As a result, these elements have been shown to be linked to organic-rich and/or anoxic sediments sometimes associated with netpen footprints (Smith *et al.* 2005). In general, trace-elements serving as direct tracers and sediment redox indicators can be used to 1) complement existing sulfide/redox classification schemes designed to assess benthic impacts (Wildish *et al.* 2001; Hargrave *et al.* 2008) and 2) assess the potential linkages between redox conditions and aquaculture activities.

Inorganic tracers and indicators of aquaculture activities (Cd, Cu, Mo, P, U and Zn) are naturally occurring sediment components, and therefore their utility 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. Lithium (Li) normalization (Loring 1991; Aloupi and Angelidis 2001) is an effective means to reduce the background noise due to variations in the type of source rock and sediment grain size for a number of elements including Cu, Zn and Mo whose concentrations in detrital marine sediments are mostly determined by their concentrations in aluminosilicates (e.g., clay minerals). Yeats *et al.* (2005) have shown how this technique can be
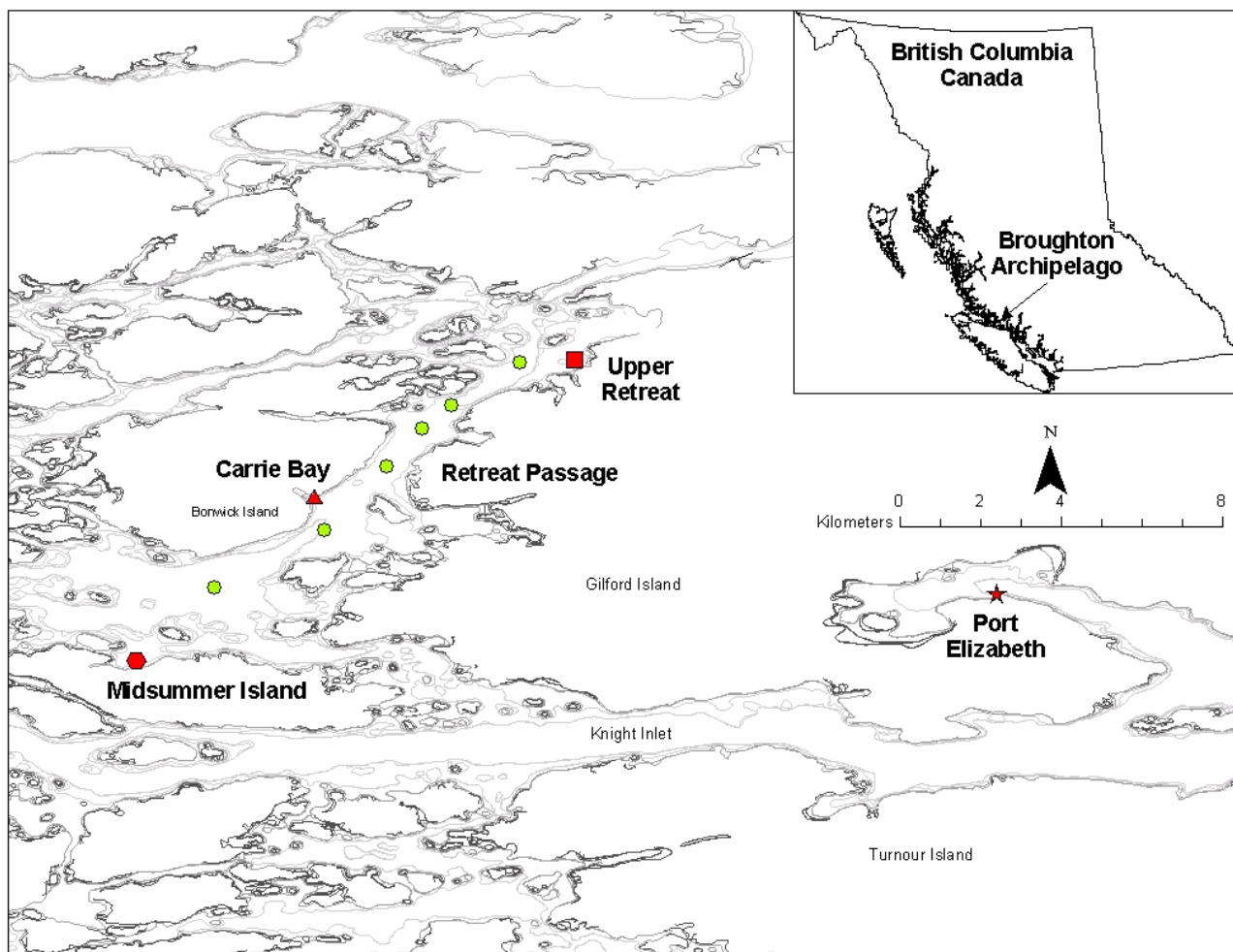
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Fig. 1 Locations of fish farm and reference sites in the Broughton Archipelago, British Columbia, on the west coast of Canada. Green circles = reference stations in Retreat Passage; red square, red triangle, and red hexagon = fish farm sites.

used to compensate for variations in the background concentrations of Cu and Zn in aquaculture-influenced sediments.

In this paper, we examine the relationship between sediment trace-elements and established indicators of benthic organic enrichment (porewater sulfide ($\Sigma H_2S = H_2S + HS^- + S^=$), redox potential ($E_{NHE}$), and organic matter (OM); Hargrave *et al.* 2008; Papageorgiou *et al.* 2009) at aquaculture operations in the Broughton Archipelago (BA), British Columbia, and in Letang Inlet, southwest New Brunswick (SWNB). This project was not designed to be a case-study describing the driving forces shaping the distribution of trace-elements exposed to various operational and environmental conditions. Rather, this meta-analysis was carried out to propose aquaculture tracers/indicators that are "blind" to site-specific conditions and will serve as robust variables across vastly different oceanographic, bathymetric, and operational settings.

MATERIALS AND METHODS

Sample collection and processing

The Broughton Archipelago is a cluster of islands located on the south-central coastline of British Columbia, Canada. These islands make up the north eastern portion of Queen Charlotte Strait and form an archipelago at the entrance of several mainland fjords. The islands, channels, and fjords create a complex circulation network driven by winds, strong tidal and estuarine flows, and river and glacial runoff (Foreman *et al.* 2006). Sediment samples were collected at four fish-farm locations within the Broughton Archipelago (BA): Carrie Bay (CB); Upper Retreat (UR); Port Elizabeth (PE); Midsummer Island (MI); and Retreat Passage (RP) (**Fig. 1**).

The CB location contains a fallowing, former fish farm site (operation of the CB fish farm ceased in 1998), while UR, PE, and MI contain active fish farms. RP contains 6 far-field stations situated along the middle axis of a channel located central to the fish farm sites. Benthic grab and core samples were obtained at Carrie Bay in September, 2000, and at the remaining sites in January, 2002. Two sampling transects were positioned parallel with the 4 active net-pens longest axis which is also the axis of the dominant current direction. Sampling stations were located at 0, 30, and 300 m from the net-pen perimeter. While the 300 m stations were considered reference stations within each bay ecosystem harbouring an individual fish farm, additional far-field reference stations were located outside the bay along the connecting channel axis (**Fig. 1**: green-circle symbols). Samples were collected from the fallow farm site (CB) in a star pattern radiating out from a central location of this former net-pen system. The 0, 30, 60, and 90 m stations were classified as near-field farm stations, while those greater than a 300-m distance were considered far-field reference stations (Sutherland *et al.* 2007c).

A single Van Veen grab and a gravity corer were deployed at each station depending on substrate suitability and the ability of each sampling method to penetrate the seabed (Lie and Pamatmat 1965; Eleftheriou and Moore 2005). 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. Several surface sub-samples (0-2 cm) were collected from each Van Veen grab sample for organic content, water content, grain-size and sediment chemistry analyses. For sediment grain size, each sediment sample was treated with
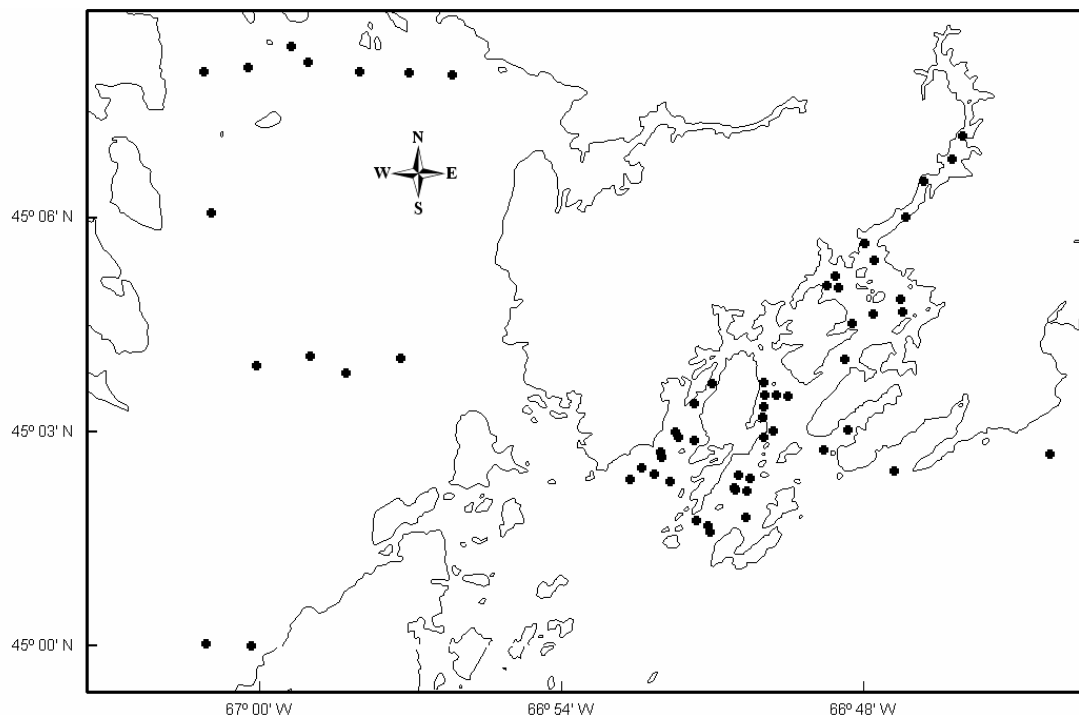
Fig. 2 Locations (represented by black circles) of fish farm and reference sites in Southwest New Brunswick, on the east coast of Canada.

sodium hypochlorite (NaOCl) to remove organic matter prior to the grain size analysis. The pipette method was used to determine the silt and clay contents, while wet sieving was used to determine the sand fractions (McKeague 1978). Water content (percent) was calculated using the differential weight values between wet and dry measurements standardized by wet weight. The sediment samples were dried at 55°C for 48 h and desiccated for 2 h prior to dry weight determinations. Organic content was calculated using the differential weight values expressed as percent between dry and ashed measurements (550°C for 2h) standardized by dry weight (Sutherland *et al*. 2007a).

A lightweight gravity corer, similar to that described by Pedersen *et al*. (1985), was used to collect vertical profiles of porewater ΣH_2S and redox potential (E_{NHE}) of the sediment column. The gravity core consists of a transparent plexiglass tube inserted into a stainless steel housing at the top end of the tube. The corer is lowered to the seafloor and allowed to vertically penetrate the sediments to depths ranging from 20 to 70 cm, depending on sediment properties. Upon retrieval of the corer, increased tension in the line triggers an o-ring mechanism that seals the top end of the corer and retains the sample within the core tube upon accent. The core-barrel lining was predrilled with holes at 5-cm intervals in a helical pattern to allow for measurements of E_{NHE} and dissolved sulphide (Wildish *et al*. 2001; Sutherland *et al* 2007a).

The Quoddy region of the Bay of Fundy is located on the east coast of Canada along the southwest coast of New Brunswick (SWNB). Tidal flows are highly sheared along the narrow passages between the islands located in Passamaquoddy Bay (**Fig. 2**). Semi-diurnal tides exist in this region with a range of 2.6 m, while a large tidal range (16 m) is observed towards the head of the Bay of Fundy (Greenberg *et al*. 2006). Sediment samples for metal analysis were collected as part of two separate investigations. The larger and more complete investigation, from the perspective of types of analytical measurements, included the collection of sediment samples in September 1999 at 60 stations from Passamaquoddy Bay and Letang Inlet with a Hunter grab (**Fig. 2**). Stations were chosen based on a distance gradient from the net-pens to incorporate both near-field and far-field environments in each bay system. The top 2 cm of each grab sample were sub-sampled for water content, grain size, organic carbon and trace-element measurements. Sediment cores collected with a gravity corer fitted with a plexiglass core liner that had been predrilled with holes at 5 cm intervals were collected at the same sites and used for measurements of E_{NHE} and dissolved sulphide. With the exception of

trace-element analysis, the analytical methods employed on the east coast were similar to those followed in the Broughton Archipelago (Bugden *et al*. 2001; Sutherland *et al*. 2007a). Metal measurements were also made on sediments collected at 12 unspecified salmon farm locations in the Letang Inlet as described by Chou *et al*. (2002). Meiofauna samples were not collected as part of the SWNB study.

Trace metal analysis

Trace-element analysis for the main BA dataset (n = 145) was carried out according to the US EPA method ICP-AES 200.15 for metal and trace element analysis using ultrasonic nebulization (US EPA 1994) (EXOVA, Surrey, Canada). The digestion process followed that of Strong Acid Leachable Metals (SALM) 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) (EXOVA) (Sutherland *et al*. 2007b). New Brunswick samples (n=70) were digested using a microwave assisted nitric and hydrofluoric acid digestion procedure that was based on EPA method 3052. The digests were analysed using a combination of ICP-AES for the majority of elements and inductively coupled plasma mass spectrometry (ICP-MS) (New Brunswick, Canada) for low concentration elements including Sb, La, Tl, Sn and U. Mercury was digested with HCl/HNO_3 and analysed by cold vapour atomic absorption spectrophotometry. All the metal digestions and analyses were conducted by accredited commercial laboratories.

Since the trace-element analytical techniques differed slightly between the British Columbia and New Brunswick locations, the two coastal data sets were not combined and individual Principal Component Analyses were carried out on each data set in isolation. A comparison of trace-element concentrations and the identification of aquaculture tracers/indicators arising from the two different analytical digestion techniques is presented by Sutherland and Yeats (2011). The New Brunswick digestion method results in total digestion of the samples and higher trace-element concentrations, while the British Columbia method results in the partial digestion of the samples and lower trace-element concentrations. In addition, the Broughton Archipelago data set was divided between active and fallow operational phases (**Figs. 3-5**). This design was carried out to test the robustness of trace-element indicators as-

sociated with benthic organic enrichment within contrasting coastal locations, farm operational phases, and analytical digestion methods. Li-normalization (Loring 1991) was used to identify samples with trace-element concentrations significantly above background, thereby permitting quantification of the aquaculture signature. Yeats *et al.* (2005) has previously shown that the signal to noise ratio and thus the ability to detect elevated concentrations of Cu and Zn is improved if geochemical normalization with Li is used to compensate for natural variation in the background metal concentrations (**Fig. 6**).

Meiofauna analysis

Meiofauna subsamples were collected from intact Van Veen Grab samples using a modified 60-cc syringe-core described by Somerfield *et al.* (2005). The tip of the syringe was cut off and the modified syringe was plunged into the central grab area to collect a sediment core. The uppermost sediment sections (2 × 1-cm depth intervals) were placed in labeled 100-ml jars containing 4% formalin. Meiofauna samples were passed through a 0.5 mm and 0.063 mm set of sieves and extracted from the 0.063 mm fraction using a decantation technique and stained with Rose Bengal (Warwick and Buchanan 1970). The decanted sample was then scanned under 10X and 40X magnification using a Leica Wild M3Z microscope and meiofauna were enumerated. Meiofaunal abundance was standardized according to volume of sediment depth interval (2 cm) with a syringe-barrel diameter of 2.6 cm. The data were then log transformed following the addition of one prior to analysis in order to normalize the data (Green and Montagna 1996).

Data analysis

Principal Component Analyses (PCA) were carried out using Systat 10^{TM} software (Systat Software Inc, Illinois, USA) with correlation matrix, varimax rotation and pairwise deletions to account for gaps in the data sets. For PCA analysis, the BA data were split into two subsets based on differences in farming activity, sampling design, and geotechnical properties. The larger data sub-set (n=98) included active farm sites and sampling designs consisting of linear station transects aligned with dominant current directions (UR, PE, MI, RP), while the smaller sub-set (n=47) included a fallow farm site with an associated radial sampling design (CB).

"Excess" concentrations were estimated for aquaculture tracers (Zn, Cu, P, and Mo) by subtracting the background concentration (based on the Li-normalized regression equations) from the total concentration. Background data in the BA were obtained from sampling stations located in two far-field regions: 1) 300-500 m from net-pen positions to examine bay-wide trends and 2) 1000-5000 m from net-pen positions within the connecting channel to examine ecosystem-wide trends (Retreat Passage) (Sutherland *et al.* 2007b). In SWNB, background values were determined using data from surficial sediments collected from throughout the region using all available data from coastal water sites that were remote from obvious pollution sources. Graphically, the excess concentration is shown by the vertical distance from the observed concentrations to the upper confidence limit; mathematically (using Zn as an example) it is described by:

$$Zn_{Excess} = Zn_{observ} - (intercept + slope \times Li_{observ} + t \times SE_{regress})$$

where intercept and slope refer to the background relationship with Li, t is the 't-statistic' factor for the chosen level of significance (in this case P=0.05), and SE$_{regress}$ is the standard error of the background regression. Levels of trace-elements were also compared to sediment toxicity thresholds as defined by CCME (1999).

RESULTS

Principal Component Analysis (PCA)

PCA was used to identify hypothetical factors describing the elemental distributions and their associations with other indicators of environmental conditions. The PCA results (**Table 1**) revealed two distinct factors describing 1) elemental distributions characterized almost exclusively by

Table 1 The results of a Principal Component Analysis of trace-element and environmental data collected in the Broughton Archipelago, British Columbia, and Letang Inlet, New Brunswick. Percent values represent the variation associated with each PCA factor.

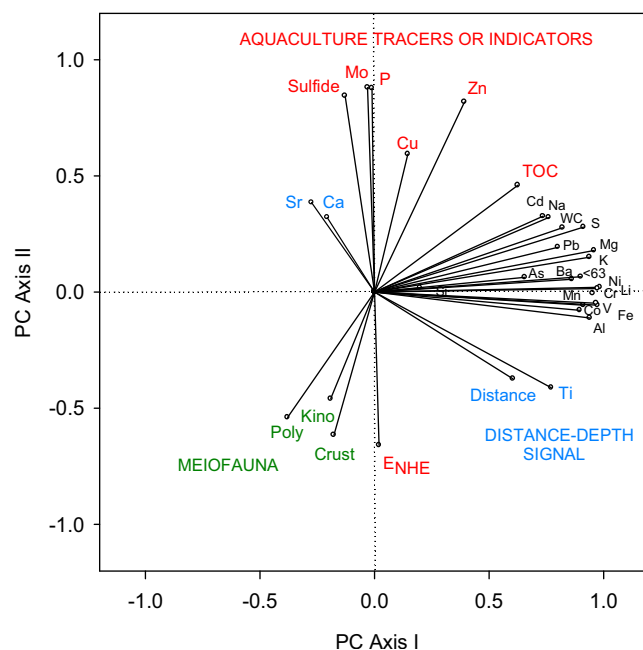| Location	Factor 1 Detrital signal	Factor 2 Tracer – Indicator signal
Broughton Archipelago Active fish farms	49.8%	17.1%
Broughton Archipelago Fallow fish farms	35.0%	20.1%
Letang Inlet Active fish farms	36.2%	23.5%

Fig. 3 Vector plot showing the results of a Principal Component Analysis carried out on sediment data (n=98) collected from active fish farm sites (Upper Retreat, Midsummer Island, Port Elizabeth, and Retreat Passage) located in the Broughton Archipelago, British Columbia. Crust = Crustacean, Distance = distance from netpen, Kino = Kinorhyncha, TOC = total organic carbon, Poly = Polychaete, and WC = water content.

detrital sources and 2) the interaction between aquaculture tracers/indicators and meiofaunal indicators. Only 3 meiofaunal taxa groups (kinorhynchs, crustaceans, and polychaetes) were incorporated into the PCA, since these groups have been shown to be sensitive indicators of benthic organic enrichment associated with finfish operations (Sutherland *et al.* 2007a). Other taxa groups were too low in abundance (tardigrada) or showed high variation in abundance at high sediment sulphide concentrations (nematodes and fora-minifera), rendering these groups not suitable as indicators of organic enrichment.

When considering the tracer/indicator PCA factor, the elements, Cu, P, Mo, and Zn, consistently fell into this category regardless of operational (active vs. non-operational) and oceanographic/bathymetric settings (archipelago vs estuarine). The variance estimates (percentages) in the SWNB data set explained by the detrital signal (36.2%) and tracer-indicator signal (23.5%) were relatively similar to those observed in the Broughton Archipelago. For example, the detrital signals (background) consisted of 35.0% for active fish farm sites and 49.8% for fallow fish farm sites, while the tracer-indicator signals were 20.1, and 17.1%, respectively). These results suggest consistent trends associated with detrital and tracer indicator factors across varying geographic, oceanographic, and operational settings.

In the Broughton Archipelago, Cu, P, Mo, and Zn covaried with previously established benthic organic enrichment indicators (porewater ΣH_2S and redox potential
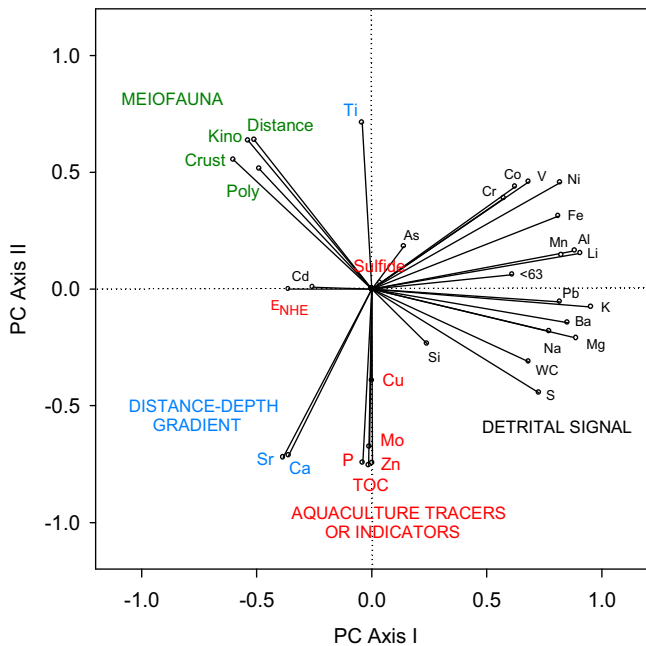
Fig. 4 Vector plot showing the results of a Principal Analysis carried out on sediment data (n=47) collected from a fallowed fish farm site (Carrie Bay) located in the Broughton Archipelago, British Columbia. Crust = Crustacean, Distance = distance from netpen, Kino = Kinorhyncha, TOC = total organic carbon, Poly = Polychaete, and WC = water content.
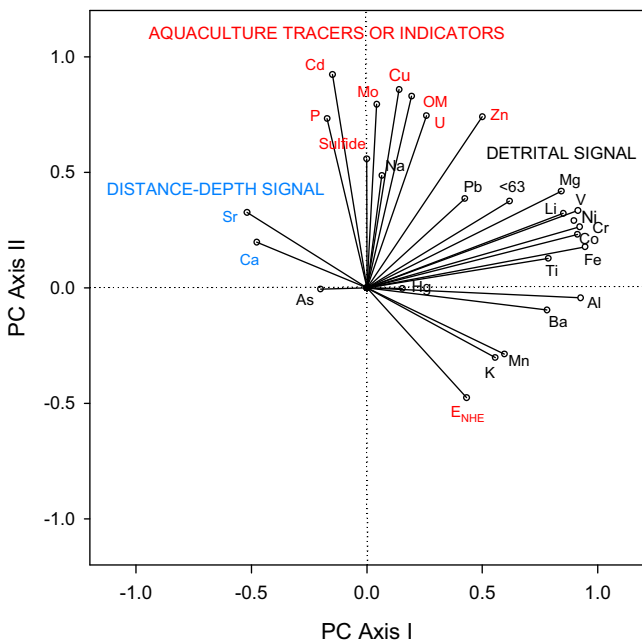

Fig. 5 Vector plot showing the results of a Principal Component Analysis carried out on sediment data (n=66) collected from Letang Inlet, southwest New Brunswick. OM = organic matter.

(E_{NHE})) as well as meiofauna taxa at the active fish farm sites. At the fallow fish farm site, Cu, P, Mo, and Zn were directly correlated with TOC, while the meiofauna taxa were more strongly associated with porewater ΣH_2S, redox potential (E_{NHE}), and abundance of fine sediments (**Figs. 3, 4**), suggesting a shift in the meiofaunal response towards detrital variables as part of the benthic recovery process. The observed inverse relationship between netpen distance and both Ca and Sr likely reflects the prominent shell-dominated substrate and possible waste material (faeces and feed pellets) located in the nearshore zone in this area of the BA. Although fewer sediment variables were measured in the SWNB data set, the PCA shows a very similar picture
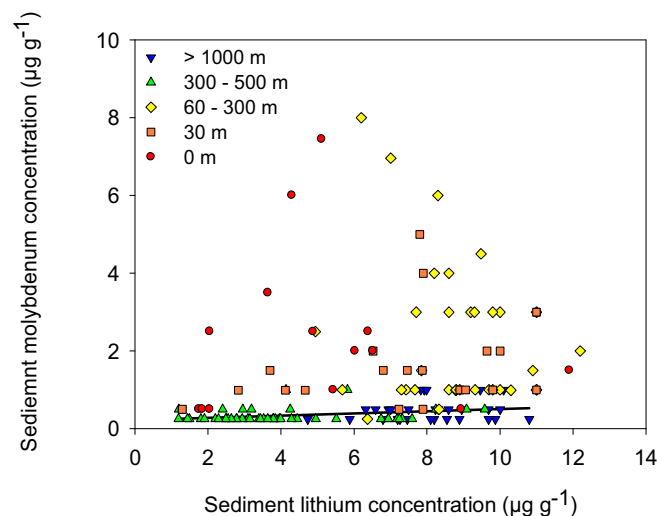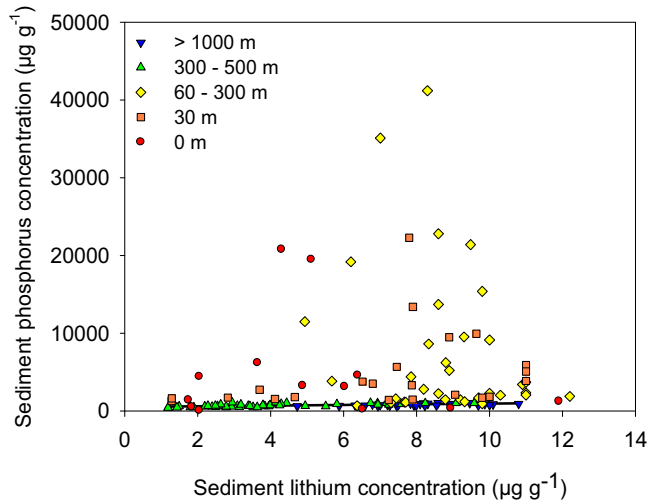

Fig. 6 Sediment phosphorus and molybdenum concentrations normalized to lithium concentration.

with Cd, Cu, Mo, P, U and Zn directly associated with OM while porewater ΣH_2S is inversely linked to E_{NHE} (**Fig. 5**).

Indirect trace-element indicators of benthic organic enrichment

For the total BA dataset, linear relationships between Li and P (r=0.64) and Li and Mo (r=0.33) for the far-field samples define the background regression (**Fig. 6**). The p value used in these analyses was set a 0.05. The large proportion of near-field samples that deviate significantly in excess of the background regression reveals the added contribution associated with organic enrichment. Similarly, in SWNB, Cd (r=0.71), Mo (r=0.79), P (r=0.92) and U (r=0.93) all showed linear relationships with Li for far-field samples, with a proportion of the near field samples showing significant positive deviation from the background.

Although Ca and Sr also showed elevated concentrations at some of the near-field stations on both coasts, the normalization of Ca and Sr with Li resulted in curvilinear background regressions. Deviations from the baseline relationship likely result from aquaculture inputs as well as Ca- and Mg-bearing carbonates derived from carbonate minerals or shell debris. Inorganic carbon (C_{inorg}) concentrations, however, can be used to account for the $CaCO_3$ component before calculating the Ca_{Excess} concentration from the background relationship with Li. A similar approach should work for Sr, providing a reasonable estimate of the Sr:C ratio in carbonate minerals can be derived. Based on observations of the BA sample set, the Sr:C ratio would appear to
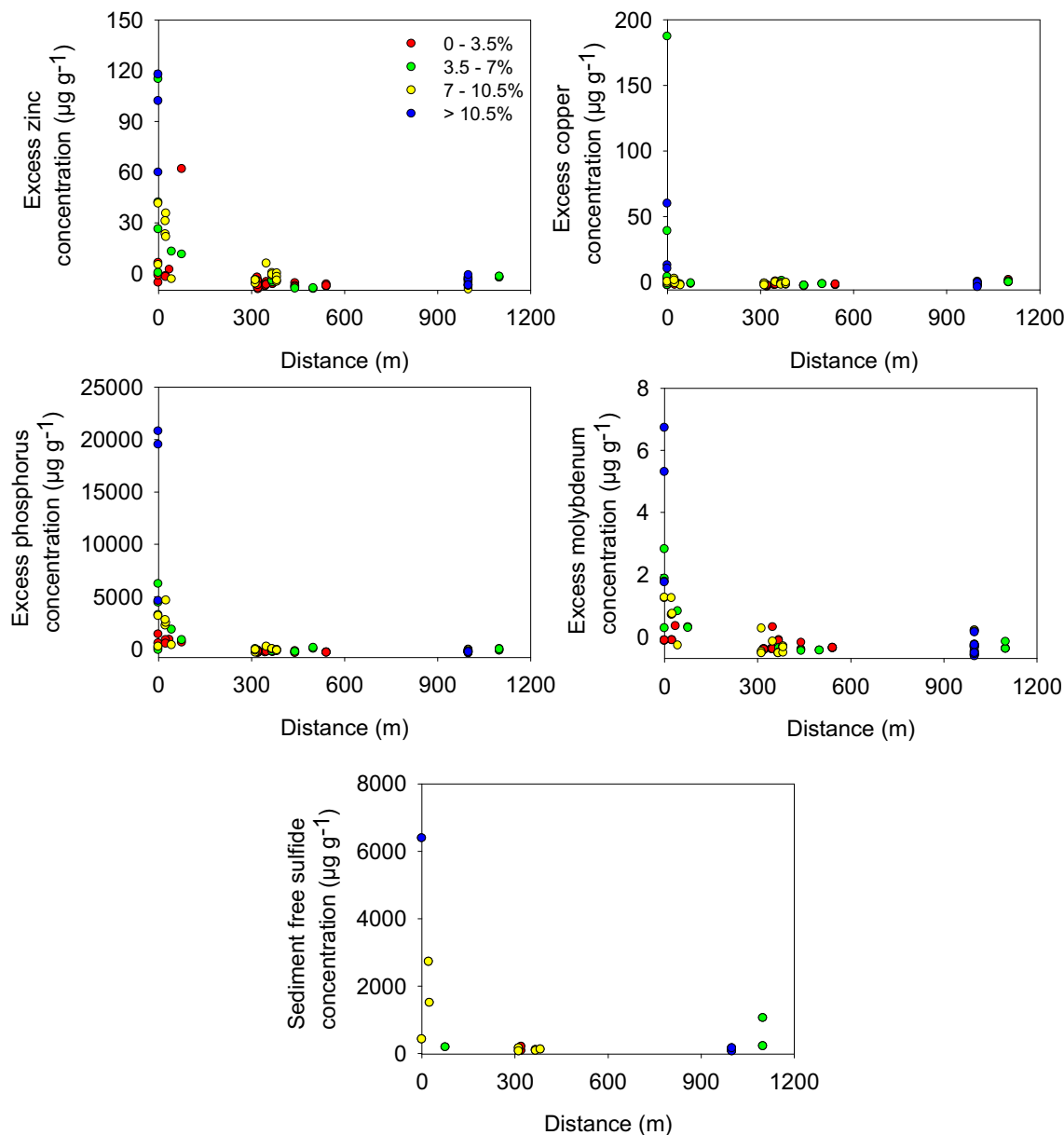
Fig. 7 The relationship between Zn_{Excess}, P_{Excess}, Cu_{Excess}, Mo_{Excess} and dissolved ΣH_2S concentrations vs. distance for Port Elizabeth, Midsummer Island, Upper Retreat and Retreat Passage. Data are categorized by organic content categories (red = 0-3.5%; green = 3.5-7%; yellow = 7-10.5%; blue = >10.5%).

be ~0.005. However, the practical usefulness of Ca or Sr as aquaculture tracers is limited due to 1) the considerable uncertainty associated with estimating Mg-carbonate contributions and 2) the degradation of far-field Li-regressions by inclusion of the C_{inorg} 'correction'. On both coasts, most of the other elements determined in the ICP analyses gave statistically significant ($p<0.05$) simple linear regressions between the concentrations of metal and lithium for both near- and far-field samples.

The plots of excess trace-elements vs. distance in the BA illustrate a rapid decline in elemental concentrations within the first 100 m as well as the overall lack of dependence of excess metal concentrations on organic content (**Fig. 7**). An increase in "excess" metal was observed to be related to a decrease in meiofaunal abundance across a range of increasing porewater ΣH_2S concentrations (**Fig. 8**). The occurrence of trace-elements exceeding sediment quality guidelines is shown in **Fig. 9**.

DISCUSSION

Inorganic aquaculture tracers and indicators

Quantifying trace-element concentrations in sediments is an important component of marine environmental assessment programs designed to identify metal contamination within ecosystems. Both direct and indirect tracers of aquaculture activities have been identified in this study through a Principal Component Analysis (**Figs. 3-5**). For example, the elements, Cu, Mo, P, and Zn, were consistently identified within the hypothetical tracer/indicator factor of the PCA for various aquaculture activities (active vs. fallow) and oceanographic/bathymetric settings (archipelago vs. estuarine) on both the east and west coasts of Canada. In addition, Cu, Mo, P, and Zn were associated with different combinations of previously established aquaculture indicators of benthic organic enrichment (ΣH_2S, E_{NHE}, and/or OM) in the BA and SWNB (Holmer *et al.* 2005; Wildish *et al.* 2005; Sutherland *et al.* 2007a; Papageorgiou *et al.* 2009). The results of this study expand upon observations carried out on Mo, P, and Zn profiles in a few aquaculture sediment cores (SWNB; Smith *et al.* 2005) and support the notion that Cu, P and Zn serve as inorganic tracers of waste deposition and Mo serves as an indicator of sediment anoxia.

To date, only a few studies have also shown that trace-elements can be used to complement observations pertaining to aquaculture impacts based on ΣH_2S and other measures of organic enrichment (Chou *et al.* 2002; Schaan-
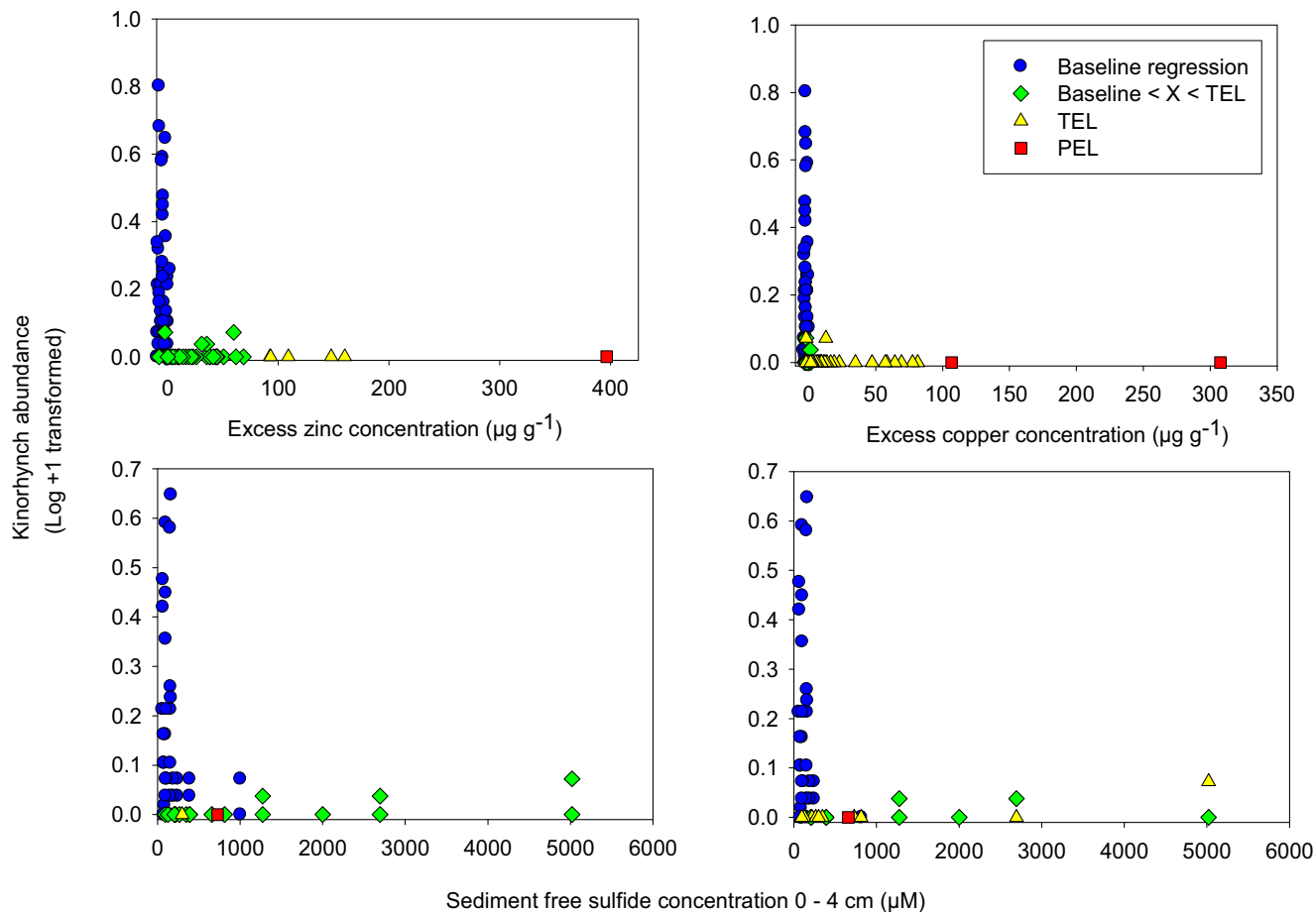
Fig. 8 The relationship between kinorhynchs vs. Zn_{Excess}, Cu_{Excess}, and dissolved ΣH_2S concentrations. The data have been classified according to the Canadian Council of Ministers of the Environment (CCME) sediment quality guidelines. TEL = Threshold Effects Level; PEL = Probable Effects Level.
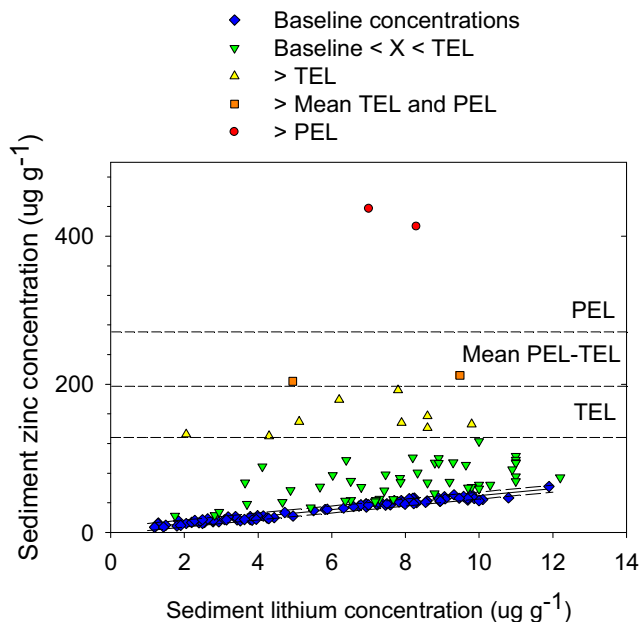

Fig. 9 Li-normalized sediment zinc concentrations plotted according to the Canadian Council of Ministers of the Environment (CCME) sediment quality guidelines consisting of Probable Effects Level (PEL) and Threshold Effects Level (TEL).

ning and Kupka Hansen 2005; Smith *et al.* 2005). For example, Smith *et al.* (2005) have shown that elevated concentrations of Cd, P, Mo and U are correlated with those of Zn and Cu in a limited number of sediment cores from the immediate vicinity of farms in southwestern New Brunswick. Chou *et al.* (2002) used Principal Component Analy-

sis (PCA) that included Cu and Zn data to complement characterization of sediments as normal, hypoxic and anoxic based on ΣH_2S and E_{NHE} measurements. In Norway, ANOVA was used to distinguish categories of sites using various parameters including Cu, P and Zn (Schaaning and Kupka Hansen 2005). Although P is present in relatively high concentrations in both feed pellets and faecal matter (Petersen *et al.* 2005; unpublished data), P is also known to show sensitivity to reducing conditions in coastal marine sediments, complicating the use of this element as solely a tracer of waste material or an indicator of organic enrichment (Sundby *et al.* 1992).

Although Cd was also identified as an inorganic tracer in SWNB (Smith *et al.* 2005), it is not associated with the other identified aquaculture tracers in the BA. The higher background concentrations of Cd observed in the BA relative to those observed in southwest NB may be responsible for masking potential aquaculture-related signals, limiting the utility of this element as an indicator of organic enrichment. Other areas in British Columbia exhibit relatively high concentrations of Cd in sediments not impacted by anthropogenic activities (Pedersen *et al.* 1989; McNee 1997). The separation of Ca and Sr from the remaining trace-element tracers/indicators in the PCA vector plots (**Figs. 3-5**) may be driven by a naturally-occurring near-shore distribution of shell-hash that is prominent in the BA. Other normalization techniques will be required for elements such as Ca and Sr, since their concentrations are not simply related to clay mineral composition and grain size alone.

The use of the Li-normalized technique to determine "excess" levels of trace-elements provides sensitivity suitable for examining spatial distributions of aquaculture tracers/indicators. In general, a consistent relationship between the tracer elements Cu_{Excess}, Mo_{Excess}, P_{Excess}, Zn_{Excess} and distance from netpen systems is observed across all sites in the BA (**Fig. 7**). Other studies have also shown a decrease

in the total concentration of one or more of these elements with distance from aquaculture operations (Uotila 1991; Belias *et al.* 2003; Brooks *et al.* 2004; Smith *et al.* 2005; Dean *et al.* 2007). The correlations between porewater ΣH_2S and sediment-bound trace-elements Cu_{Excess} (r=0.77), Zn_{Excess} (r=0.74), Mo_{Excess} (r=0.75) and P_{Excess} (r=0.83) are similar for the active farming areas in the BA (n=47), while those collected in SWNB show a stronger association of Mo_{Excess} (r=0.87) and U_{Excess} (r=0.67) with ΣH_2S, than for Zn_{Excess} (r=0.36) or Cu_{Excess} (r=0.30) with ΣH_2S, suggesting that there is a difference between the distributions of the direct tracers of waste deposition (Cu and Zn) and tracers of anoxia (ΣH_2S, Mo and U).

Although fewer data exist for free sediment sulfide concentrations relative to trace-element data, it appears that high sulfide values coincide with excess elemental tracer concentrations proximal to net-pen systems. Sutherland *et al.* (2007b) demonstrated that sediments classified as hypoxic and anoxic according to sulfide concentrations were mostly associated with excess Zn concentrations. These observed associations between direct and indirect tracers would depend on the size, duration, and frequency of production cycles as well as on hydrodynamic and benthic conditions. The occurrence of indirect tracers depends on the development and sustainability of reducing conditions in porewaters.

Redox potential and free sediment sulfide concentrations are sometimes used as indicators for assessing aquaculture-derived benthic organic enrichment (Holmer *et al.* 2005; Wildish and Pohle 2005). However, Wildish *et al.* (2004) have noted that redox measurements may not be entirely reliable and called for other benthic enrichment indicators to complement the use of the ΣH_2S classification system. In addition, Hargrave *et al.* (2005) have questioned the application of a single indicator approach for measuring environmental status or risk, since no single indicator can address complex or cumulative ecosystem interactions. Trace-elements may serve as a complementary tool to ΣH_2S impact classifications since 1) certain elements (Cu, P, Zn) are direct tracers of aquaculture-derived waste material; 2) other elements (Mo, U) reflect benthic organic enrichment and redox conditions; 3) excess elemental concentrations can be easily identified using geochemical normalization techniques; and 4) excess elemental concentrations show strong trends with distance from farm, ΣH_2S gradients, and faunal abundance.

Since trace elements in marine sediments are generally stable following collection, samples can be collected simply from the top 2-cm of a benthic grab sample and transported to a laboratory for analysis. Conversely, porewater ΣH_2S methods requires a more complex *in situ* calibration-analysis set-up due to the volatile nature of ΣH_2S and short lifespans of the calibration and buffer solutions (Wildish *et al.* 1999, 2001). Additional effort is required since vertical profiles from sediment cores provide lower variance in the E_{NHE}-ΣH_2S relationship relative to that observed for surface samples collected from benthic grab samples (Cranford *et al.* 2006). The extra demands associated with the *in situ* sampling conditions of E_{NHE}-ΣH_2S may restrict the number of successful samples collected within an environmental monitoring program due to constraints associated with sampling in remote areas, small boat platforms and coarse substrate conditions. Furthermore, direct tracers of aquaculture activities (Cu, Zn, P) provide additional information in terms of the depositional pattern of waste feed, faeces, and antifoulant agents in the surrounding environment (Petersen *et al.* 2005). It will thus be useful to have an easily and reliably measured parameter such as Zn_{Excess} to follow the waste feed and faeces signal and Mo_{Excess} to augment limited or possibly unreliable ΣH_2S measurements.

Meiofaunal response to aquaculture tracers or indicators

Meiofaunal diversity has been shown to be reduced in the presence of high concentrations of Zn and Cu in estuarine environments (Van Damme *et al.* 1984; Fichet *et al.* 1999) as well as in the presence of finfish farms (Sutherland *et al.* 2007a; Grego *et al.* 2009; Mirto *et al.* 2009). In this study, the relationship between meiofaunal abundance and excess Cu and Zn in the BA shows a sharp increase in meiofaunal abundance at non-excess levels of these elements (**Fig. 8**). In contrast, the plot of kinorhynch abundance vs. ΣH_2S shows more scatter at high ΣH_2S relative to that observed at high Zn or Cu concentrations. Also, a number of samples have both low kinorhynch abundance and low ΣH_2S concentrations. It appears that at high ΣH_2S, sulfide toxicity may inhibit kinorhynch abundance, but at low ΣH_2S, other factors, such as Zn or Cu, organic content, or sediment texture, govern organism abundance. Whether or not high Zn (or Cu) concentrations are contributing to toxicity at low ΣH_2S is difficult to assess, but concentrations of Zn (and Cu) that exceed toxicity thresholds at some net-pen sites may imply that these elements have an effect on meiofaunal abundance (**Fig. 9**). This postulate is supported by observations of samples which exhibit low kinorhynch abundance, low ΣH_2S concentrations and relatively-high concentrations of Zn and Cu.

CONCLUSIONS

Direct tracers (Cu, P, Zn) and indirect indicators (Mo, U) of aquaculture activities can be used to assess benthic impacts across various operational modes of finfish aquaculture in different geographic settings. Li-normalization of trace-element abundance allows for the determination of "excess elemental concentrations" which represents a useful parameter to correlate with environmental and faunal variables. Since Li-normalized distributions of Cu, P, Mo, and Zn provide a sensitive indicator of organic enrichment, they can provide better measures of aquaculture impacts away from the immediate vicinity of net-pen sites than do the more direct measurements of organic content.

Ca and Sr show some potential as tracers of aquaculture wastes but their distributions are complicated by their presence in carbonate shell debris. Cu shows some unique distributional features that probably reflect its use as an antifoulant agent. Since Zn_{Excess}, P_{Excess} and Mo_{Excess} show similar trends regarding spatial distribution and relationships with organic enrichment indicators and faunal responses in the BA, they will be the most useful inorganic tracers of farm wastes. ΣH_2S is a direct measure of benthic organic enrichment but does not represent farm waste directly. Given the sampling logistics associated with ΣH_2S measurements, Mo_{Excess} would provide a useful complement to or surrogate for ΣH_2S in environmental monitoring programs, while Zn_{Excess} or P_{Excess} would provide a more direct measure of farm wastes.

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