

# **RE-ANALYSIS OF RELATIONSHIPS BETWEEN SEDIMENT CHEMISTRY AND INFAUNAL MACROBENTHIC COMMUNITY RESPONSES, BASED ON BROOKS (2001) DATA**

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## **Introduction**

There is a desire to manage aquaculture operations within coastal British Columbia in a way that waste releases do not result in measurable impacts to the receiving environment beyond the area of operation. Brooks (2001) undertook a detailed study of seabed conditions in the vicinity of nine existing salmon aquaculture operations in coastal British Columbia. Brooks measured aspects of sediment chemistry (sulfide, total volatile solids, redox of the top 2 cm of sediment, etc.) at a series of reference stations ( $\geq 300$  m away) and stations located progressively farther from the net pens (from 0 to 245 m, where 0 m is at the edge of the net pen footprint). The study also included detailed measurement of soft-bottom benthic community structure. Benthos were separated from sediment using a  $(1.0 \text{ mm})^2$  screen at seven of the nine stations

A major objective of the Brooks (2001) study was to define interrelationships between biological responses and sediment chemistry, with a view to establishing appropriate chemical surrogates for the protection of benthic community health around aquaculture operations. In particular, the relationship between magnitude of chemical changes in sediment due to waste loading from individual aquaculture operations and apparent thresholds for biological impairment are anticipated to form the basis for appropriate regulatory/management strategies for encouraging sustainable practices.

The data contained in Brooks currently comprises the major portion of information available for benthic community responses under salmon farms on the west coast of Canada, and will undoubtedly be of interest in establishing appropriate environmental protection goals for sustainable aquaculture. In light of this, an independent re-evaluation of the data produced for the Brooks (2001) study was undertaken in light of concerns by the SAG about the level of interpretation applied in the above-described context. In particular, the data are assessed, and environmentally protective chemical thresholds are considered using a better theoretical understanding of benthic ecophysiology, and using a variety of routinely applied univariate and multivariate procedures.

## **Methods**

### **Results and Discussion**

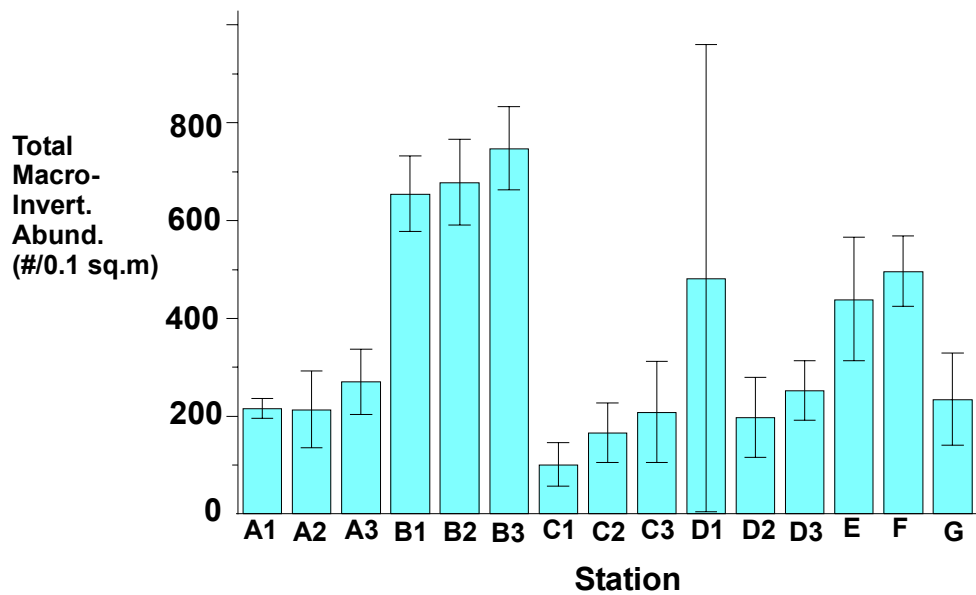
#### *Variability of Benthic Community Structure at Reference Sites*

The Brooks (2001) data available for reference stations include –

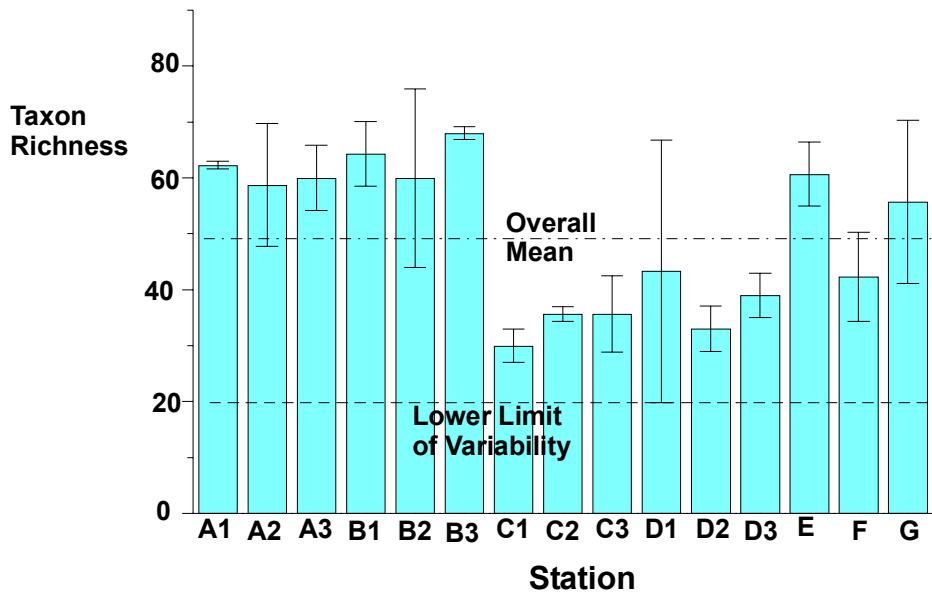
- triplicate grabs at each of three sites for operations A-D;
- triplicate grabs at one site only for operations E, F,G.

Therefore, limited information on both inter- and intra-site variability in benthic community structure is available for four aquaculture operations, whereas only the intra-site variability is evident for the other three.

The results are shown in Figures 1 (total abundance/0.1 m<sup>2</sup>) and 2 (taxon richness in a 0.1 m<sup>2</sup> grab).



**Figure 1: Variation in abundance of macroinvertebrate infauna at reference sites** (error bars represent  $\pm$  95% confidence interval).



**Figure 2: Variation in taxon richness (no. different taxa/grab sample) at reference sites (error bars represent  $\pm$  95% confidence interval).**

A few key issues arise from the background variability of the univariate community summary variables: total abundance and taxon richness.

- abundance tends to be far more variable than richness, as is the case for the vast majority of benthic studies;
- For species richness, the lowest observed species richness in a single 0.1 m<sup>2</sup> grab sample at the reference stations was 21 taxa. The overall average for reference stations was approximately 50 taxa/grab.
- To apply the lowest overall observed taxon richness at all sites as a criterion for distinguishing normal from perturbed conditions, however, would result in severe impairment at some sites (e.g. Site A). In fact, at most sites it is expected that a 10-20% reduction in taxon richness would be statistically detectable based on reference site variability (not 50% as has been previously suggested). Station D1 was the only exception to this.
- This underscores the challenges in applying generic assertions about levels of benthic community impairment in relation to waste discharge in the absence of biological data.

Recent studies by Cross (2001) and WLAP show that the data for the seven sites assessed by Brooks (2001) may exhibit atypically high reference site taxon richness relative to the

larger suite of existing aquaculture sites along the British Columbia coast (Dalby et al, 2001)<sup>1</sup>.

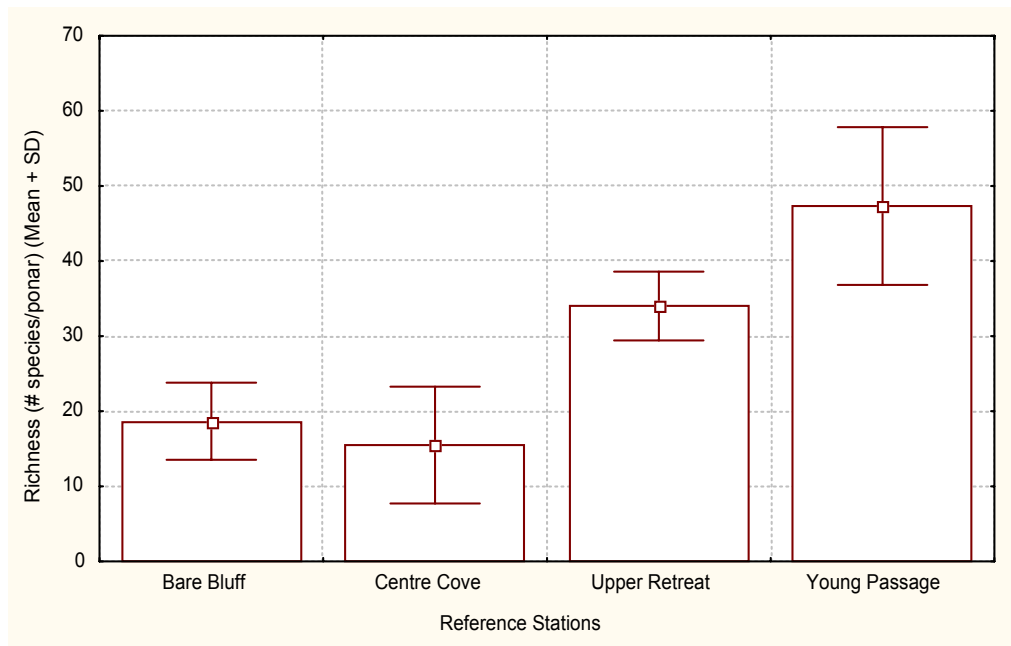
It is important, therefore, that the reader appreciate that generic numerical analyses as applied herein is expected to be valid only to the extent that the seven sites incorporated into the Brooks (2001) study are representative of the larger suite of existing and viable tenure sites. In addition, the statistics used tend to capture the central trends in the available data – something that is appropriate to a generalized case, but which may not reflect conditions at any specific site. There is only limited data available outside of the Brooks (2001) study that is applicable to similar analysis (see Dalby et al, 2001). Information from studies in the Bay of Fundy (Wildish et al, 2001)<sup>2</sup> are of doubtful applicability to the majority of British Columbia sites owing to dissimilarities in tidal regimes and currents.

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<sup>1</sup> Dalby, James, Lloyd Erickson, Eric McGreer, & Bernie Taekema, 2001 (Dec. 13). Preliminary determination of chemical thresholds for the forthcoming British Columbia Aquaculture Regulation. Internal Report to the Scientific Advosiry Group. 20 pp.

<sup>2</sup>Wildish, D., H.M. Akagi, and N. Hamilton. 2001. Sedimentary changes at a Bay of Fundy salmon farm associated with site fallowing. Bull. Aquacul. Assoc. Canada 101-1, (49-56 pp.)

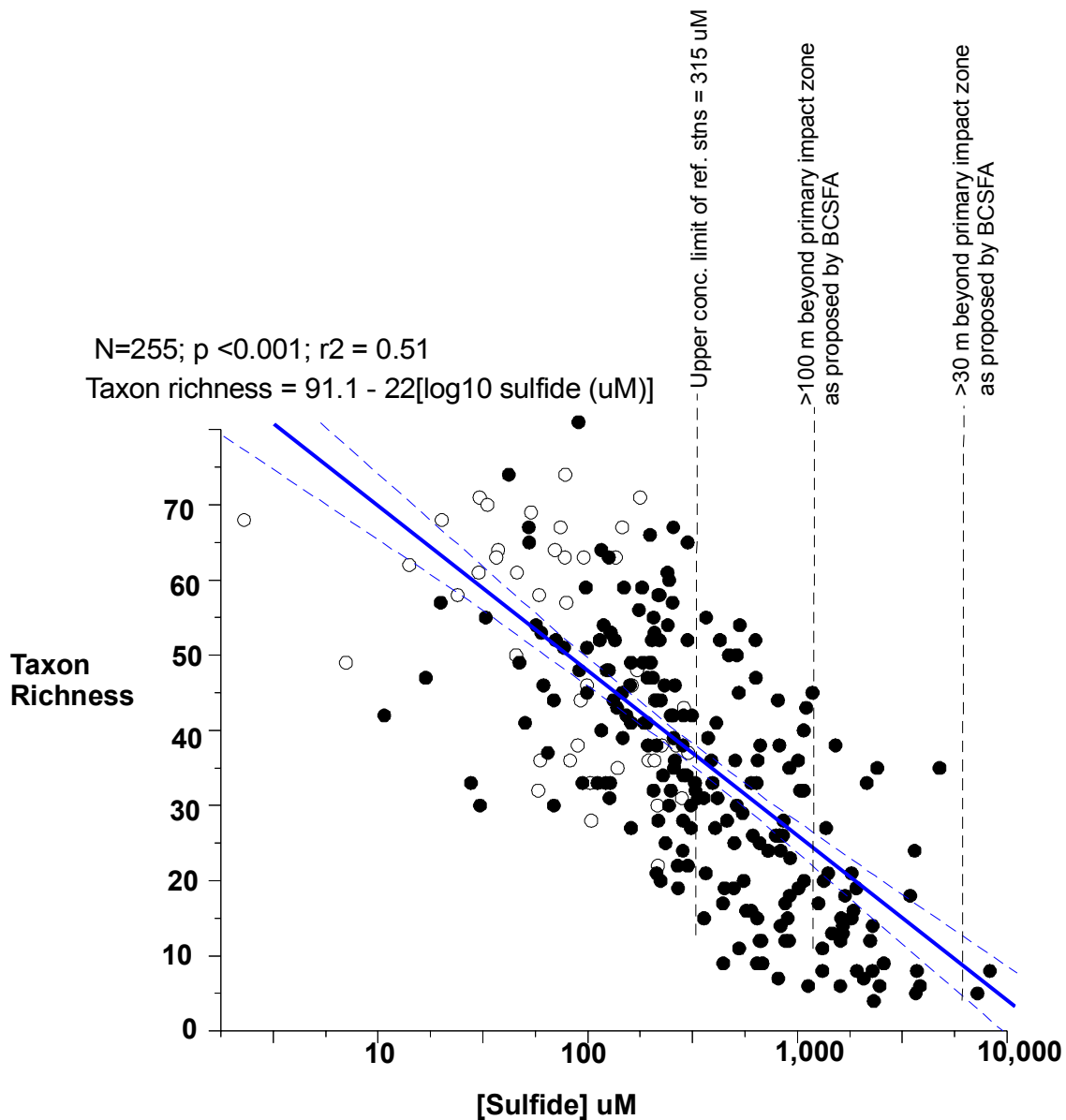
**Fig. 3: Species richness and total number of individuals at 4 reference stations. Data were gathered by MWLAP in Summer 2000 (from Dalby et al, 2001).**



*Univariate measures of community response in relation to sediment chemistry*

Figure 4 graphically illustrates the relationship between sulfide concentrations in the top 2 cm of sediment grab samples (on a  $\log_{10}$  scale) and the associated measured taxon richness, based in the Brooks (2001) data. The overall data set encompasses seven aquaculture sites and 255 individual sediment samples.

The statistical relationship between sediment sulfide concentration and taxon richness accounted for about 50% of the total variability in the overall data set, when the constant is included in the regression model. The variance accounted for is deemed to be quite high given that the sample locations span a wide range of bottom types and depths, current regimes, and different areas of the coast (spanning a range of zoogeographic areas).



**Figure 4: Relationship between benthic community impairment (loss of biodiversity) and sediment sulfide levels (adapted from Brooks, 2001). Open symbols are data for reference sites ( $\geq 300$  m). The log-linear relationship, with upper and lower 95% confidence limits, is shown in blue.**

The predictive relationship and associated uncertainty can in turn be used to predict various levels of biological impairment based on the generic case. First, we need to start with some estimate of the species richness of reference communities at viable aquaculture sites. The average per grab species richness for reference stations in the Brooks (2001)

study was 49.9 or approximately 50 taxa<sup>3</sup>. Using this as a generic estimate of reference area richness, the predicted sulfide concentrations associated with various percent reductions in richness are shown in Table 1:

**Table 1: Predicted reduction in taxon richness across a range of sediment sulfide concentrations**

<b>% reduction / (no. of taxa in a 0.1m<sup>2</sup> grab)</b>	<b>log<sub>10</sub> [s<sup>2-</sup>] (μM)</b>	<b>[s<sup>2-</sup>] (μM)</b>
10% (45)	2.095	125
20% (40)	2.323	210
<b>30% (35)</b>	<b>2.550</b>	<b>355</b>
<b>40% (30)</b>	<b>2.777</b>	<b>599</b>
<b>50% (25)</b>	<b>3.005</b>	<b>1011</b>
<b>60% (20)</b>	<b>3.232</b>	<b>1705</b>
<b>70% (15)</b>	<b>3.459</b>	<b>2878</b>
<b>80% (10)</b>	<b>3.686</b>	<b>4857</b>
<b>90% (5)</b>	<b>3.914</b>	<b>8197</b>

Since this estimate was derived by pooling all of the available data (to derive the generic case), it is reasonable to conclude that approximately half of all sites will exhibit a higher level of biological impairment at a given sulfide concentration while half will exhibit a lower level of impairment relative to predictions.

As mentioned above, a 20% deviation in taxon richness from unperturbed conditions should be detectable at most sites, even using a very limited number of replicates per station. A 30% impairment is predicted to occur at a sediment sulfide concentration of around 360 μM, while a 50% species reduction is predicted to occur at a sulfide concentration of around 1,000 μM. To further place these sulfide concentrations in context, as can be seen from Figure 3 the maximum observed sulfide concentration for the reference station grabs was ≈ 315 μM.

#### *Other covariates of benthic community richness*

A multiple linear regression was performed with taxon richness as the independent variable and a variety of predictor variables, including distance from the net pen, log<sub>10</sub> [sulfide], AVS, sediment redox (ORP), station depth, percent silt-clay, salinity, and pH.

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<sup>3</sup> Note previous comments that the average reference site taxon richness from the Brooks (2001) study may be high relative to other existing or potential aquaculture sites, but is the best estimate given that the statistical relationships were derived from the same seven sites.

Salinity might be important, for example, as a surrogate of estuarine influences on community structure.

Interpretations derived from multiple regression analysis are always vulnerable to the order in which independent variables are entered (or removed). A step-wise regression was conducted, manually changing the order of variable entry, and through use of both forward and backward procedures.

The strength of semi-partial correlations between taxon richness and the predictor variables was as follows (n = 255):

Variable	Partial Correlation	F	P
Log <sub>10</sub> s <sup>2-</sup>	-0.711	258.8	0.000
Distance (m) from net pen	0.629	165.8	0.000
ORP	0.604	145.1	0.000
TVS	-0.462	68.6	0.000
% siltclay	0.311	29.3	0.000
Salinity (ppt)	-0.322	27.1	0.000
Depth (m)	-0.162	6.80	0.010
pH	0.116	3.43	0.065

The model that provided the best overall fit was –

$$\text{Taxon Richness} = 74.4 + 0.061 [\text{distance (m)}] - 24.1[\text{silt-clay}] - 13.3 \log_{10}[\text{s}^{2-}] \quad (1)$$

(n=255; R<sup>2</sup>(adj) = 0.619; p < 0.0001)

This can be compared with the model that includes log<sub>10</sub>[s<sup>2-</sup>] alone (Figure 3) which accounted for 50.9% of the total variance of the data set, and a model with distance from the net pen as the sole predictor variable, which accounted for 39.6% of the variation.

When the constant was removed from the model, the overall fit and predictive power was substantially improved:

$$\text{Taxon Richness} = -11.9 \log_{10}[\text{s}^{2-}] + 0.067 [\text{distance (m)}] + 2.15[\text{salinity (‰)}] - 15.6[\text{silt-clay}] \quad (2)$$

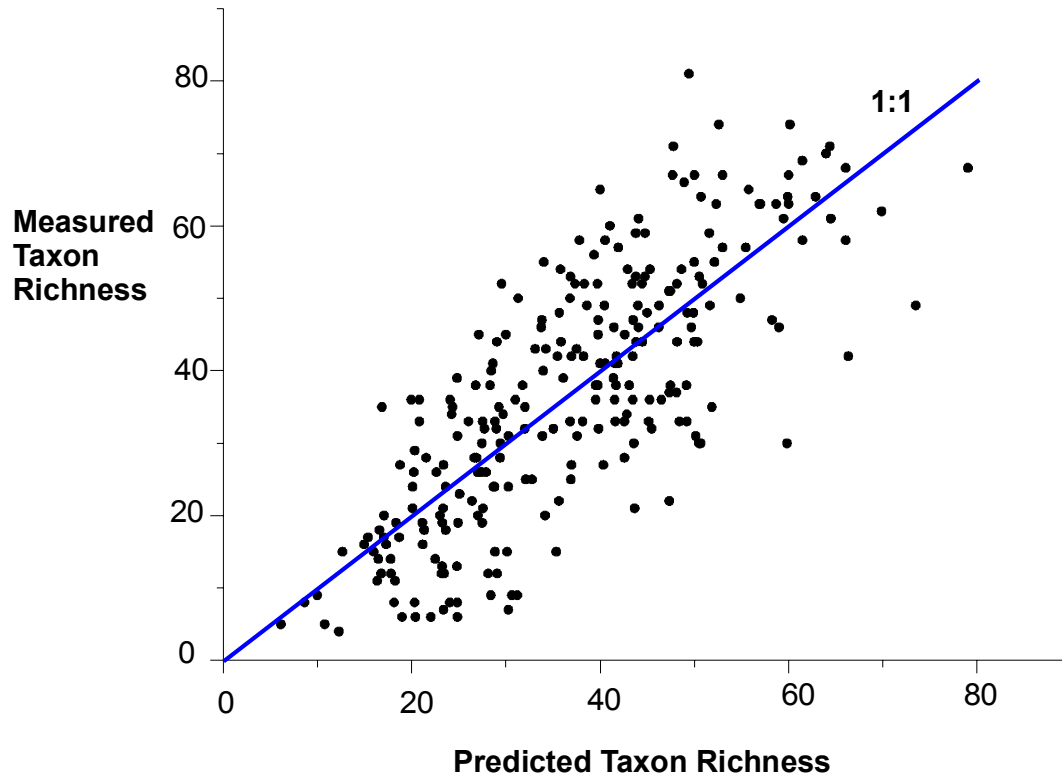
(n=255; R<sup>2</sup>(adj) = 0.929; p < 0.0001)

While both ORP and TVS are correlated with species richness, they are redundant and less powerful predictors than the log<sub>10</sub> sulfide concentration and distance from the net pen. Similarly, although station depth was also significantly correlated with taxon



richness, sediment silt-clay content appears to be a better overall predictor of taxon richness.

Figure 5 shows the actual versus predicted species richness, based on equation (2).



**Figure 5: Predictive value of equation 2.**

There are some interesting implications of this analysis. As might be expected, taxon richness is predicted to decrease at higher sulfide concentrations, and in closer proximity to aquaculture sites. Further, taxon richness is predicted to increase with increasing salinity. Finally, taxon richness is inversely related to the percent silt-clay percentage, which in turn is correlated with station depth. Deeper stations tend to have finer grained sediments, being less prone to sorting and re-suspension from wind-generated wave action. This would, in turn, reduce the rates of pore water diffusion and limit re-oxygenation from above.

Regardless of how the regression was run, there was a relationship between decreasing species richness and proximity to the aquaculture operation that accounted for variation beyond what was associated with sediment sulfide levels. This strongly suggests that other aspects of waste release from aquacultural operations, in addition to presence of sulfides, influences the biodiversity of infaunal macroinvertebrate communities. As suggested previously, it should not be ruled out that byproducts of the heterotrophic microbial decomposition of organic other than sulfides influence the infaunal macrobenthos.

Equation 2 can be used to back-calculate sediment sulfide concentrations within the Brooks (2001) data set that would lead to various levels of taxon richness at an assumed or prescribed distance from the operation, and at sites of specified salinity and sediment grain size. For the purpose of this exercise, a hypothetical aquaculture site was considered to be in a low, medium, or higher salinity environment (28, 30.5 and 32 ‰, respectively), and located over a fine-grained, medium-grained, or coarse grained seabed (silt-clay fraction = 1.0, 0.5, and 0.2, respectively). These levels were operationally defined cut-offs derived directly from an examination of the range of variation across the seven sites in the Brooks (2001). In other words, the assumed ranges of site characteristics were chosen so that they were in the range of the actual site data. The categorizations have little if any theoretical value outside of the context of this study.

**Table 2: Back-calculations of sediment sulfide levels ( $\mu\text{M}$ ) (top 2 cm) associated with various levels of taxon richness, at sites with different salinity or sediment grain size (predicted from Eq'n 2)**

<i>a) Low salinity sites (28 ‰)</i>									
Distance from operation (m)	Fine-grained (1.0 silt-clay)			Medium-grained (0.5 silt-clay)			Coarse-grained (0.2 silt-clay)		
	30	65	100	30	65	100	30	65	100
% of richness relative to assumed reference of 50 taxa									
75	6	9	14	23	36	56	65	100	160
70	9	15	23	37	58	91	110	170	260
65	15	24	38	60	94	150	170	270	430
60	25	39	62	97	150	240	280	440	690
55	40	64	100	160	250	390	450	710	1100
50	65	100	160	260	400	630	730	1200	1800
45	110	170	260	410	650	1000	1200	1900	2900
40	170	270	430	670	1100	1700	1900	3000	4800
35	280	440	690	1100	1700	2700	3100	4900	7700
30	450	710	1100	1800	2800	4400	5100	8000	13000
25	740	1200	1800	2900	4500	7100	8200	13000	20000

Table 2 (continued)

b) Medium salinity sites (30.5 o/oo)									
Distance from operation (m)	Fine-grained (1.0 silt-clay)			Medium-grained (0.5 silt-clay)			Coarse-grained (0.2 silt-clay)		
	30	65	100	30	65	100	30	65	100
% of richness relative to assumed reference of 50 taxa									
75	16	26	41	64	100	160	180	290	460
70	27	42	66	100	160	260	300	470	740
65	43	68	110	170	270	420	490	760	1200
60	70	110	170	270	430	680	790	1200	2000
55	110	180	280	440	700	1100	1300	2000	3200
50	190	290	460	720	1100	1800	2100	3300	5100
45	300	470	740	1200	1800	3000	3400	5300	8300
40	490	770	1200	1900	3000	4700	5500	8600	14000
35	790	1200	2000	3100	4800	7600	8800	14000	22000
30	1300	2019	3200	5000	7800	12000	14000	23000	36000
25	2100	3300	5200	8100	13000	20000	23000	37000	58000
c) Higher salinity sites (32 o/oo)									
Distance from operation (m)	Fine-grained (1.0 silt-clay)			Medium-grained (0.5 silt-clay)			Coarse-grained (0.2 silt-clay)		
	30	65	100	30	65	100	30	65	100
% of richness relative to assumed reference of 50 taxa									
75	31	48	76	120	190	300	340	540	850
70	50	79	120	190	310	480	560	880	1400
65	81	130	200	320	500	780	900	1400	2200
60	130	210	330	510	800	1300	1500	2300	3600
55	210	340	530	830	1300	2100	2400	3800	5900
50	350	540	860	1300	2100	3300	3900	6100	9600
45	560	880	1400	2200	3400	5400	6300	9900	16000
40	910	1400	2300	3500	5600	8800	10000	16000	25000
35	1500	2300	3700	5700	9000	14000	17000	26000	41000
30	2400	3800	5900	9300	15000	23000	27000	42000	66000
25	3900	6100	9600	15000	24000	37000	43000	68000	110000

The predictions suggest that sulfide thresholds for biological effects are actually higher farther away from the aquaculture operation than near the operational footprint, a premise that initially appears highly counter-intuitive. This is driven by the inclusion in the model as a predictor variable of the distance term, however. The term makes a significant contribution to the explanatory power of the regression model only because aspects of waste discharge other than sulfide production undoubtedly influence the benthic community structure. Possible examples include elevated ammonia or methane concentrations, or oxygen depletion, in sediment pore water.

Equivalent concentrations of sulfide are predicted to produce a lower level of biological response farther away from the point of waste discharge because the interactive influence of other stressors would be expected to be less. Had more appropriate chemical and physical surrogates been available, these would have removed the need to use distance as a predictor variable.

From a management perspective, back-calculated levels of sediment sulfide at 100 m or more away from the net pen are the best reflection of the role of sulfide in influencing benthic structure with minimum influence from other waste-related stressors.

Provided that the statistical model explored herein has some value for generalizations beyond the seven sites studies, the multiple regression predictions show how the influence of sulfide on benthic community structure is expected to be highly variable depending on substrate type, local oceanographic conditions and perhaps other conditions not addressed in the Brooks (2001) study. This finding is consistent with the findings by Dalby et al (2001) that sulfide concentrations at specific sites and on specific dates associated with a 50% reduction in species richness relative to reference stations vary from 277 to 2,400  $\mu\text{M s}^{-2}$ , excluding data based on log-linear relationships with very little if any explanatory power (those with an  $r^2 < 40\%$ ).

The variability of benthic community responses to waste discharge across sediment grain size and oceanographic conditions will come as little surprise to the vast majority of marine benthic ecologists. The importance of controlling community variability associated with seabed depth, estuarine conditions or sediment granulometric properties strongly drives the design of field studies.

The site-specific variability, and different experiences of various stakeholders at different site, may be a contributor to disagreements over generic environmentally acceptable thresholds for sediment sulfides. On the other hand, the expected range of variation at sites relative to the generic case shows that adoption of a tiered approach that triggers progressively greater level of site-specific investigative effort will be of benefit to site operators in allowing the inclusion of greater 'realism' into waste management regimes.

### *Multivariate analysis*

Univariate measures of biological community structure are often inadequate for capturing the major structural and functional features, and subtleties of changes in those features over space and/or time. A Principal Components Analysis (PCA) was used to further explore the structure of the benthic community data.

There is a large body of peer-reviewed literature on the appropriateness of various multivariate statistical techniques for use with benthic community data. PCA and similar factor analysis techniques are a subset of the “General Linear Model” type of analyses (which include single and multiple regression as well as ANOVA and MANOVA-type designs). As such, PCA is best suited to data that are continuously distributed, exhibit multivariate normality, and are not unduly influenced by multivariate heteroscedasticity. On the other hand, it is well recognized that PCA outputs and interpretation are generally robust to departures from the model assumption.

Marine benthic community data tend to exhibit a large number of ‘rare’ species that are found at only a few of the sampling stations, while their abundance values are ‘zero’ for the vast majority of samples. Further, there are often a few species that in some samples exhibit extremely high abundances, and marine benthic communities are generally assumed to be log-normally distributed. For these reasons, many researchers advocate for alternative multivariate techniques such as MultiDimensional Scaling (MDS). The approach used herein was to eliminate data for rare species from the data matrix, and run an unrotated PCA on the taxa that collectively accounted for 98% of the total infaunal macroinvertebrate abundance at either stations in close proximity to the net pens (0 to 22.5 m), farther away (30-245 m), or the reference sites (Table 3).

Within close proximity to the edge of the net pens, only three species made up around 90% of the total macroinvertebrate abundance, whereas at the reference stations 67 different taxa accounted for the same cumulative abundance for a similar number of stations (36 stations in close proximity and 44 stations  $\geq 300$  m away). Of particular note is that stations 75 to 225 m beyond the perimeter of the net pen still exhibited a much reduced diversity (32 species accounted for ~90% of the total abundance at 129 stations). At a distance of 30-60 m from the net pens, 5 species accounted for ~90% of the total abundance.

**Table 3: List of dominant infaunal macroinvertebrate taxa under (0-22.5 m), near (30-60 m), farther away (7-225 m) from aquaculture sites, or from local reference sites (>300 m away)**

Stations 0-22.5 m from operation (n=36 stns)				Stations 30-60 m from operation (n=46 stns)			
Taxon	Abund.	% of total abund.	Cum. %		Abund.	% of total abund.	Cum.%
<i>Capitella capitata</i> (1)	59230	48.9%	48.9%	<i>Capitella capitata</i> (1)	19519	33.9%	33.9%
<i>Nebalia pugettensis</i> (1)	41311	34.1%	83.0%	<i>Nebalia pugettensis</i> (1)	13987	24.3%	58.1%
<i>Schistomeringos annulata</i> or <i>pseudorubrovittata</i> (1)	6644	5.5%	88.5%	<i>Schistomeringos annulata</i> or <i>pseudorubrovittata</i> (1)	7203	12.5%	70.6%
<i>Diopatra ornata</i> (1)	4070	3.4%	91.9%	<i>Eusirus</i> sp.(1)	5243	9.1%	79.7%
<i>Eusirus</i> sp.(1)	2579	2.1%	94.0%	<i>Pseudotanais oculatus</i> (2)	4388	7.6%	87.3%
<i>Sigambra tentaculata</i> (1)	2140	1.8%	95.8%	<i>Sigambra tentaculata</i> (1)	3769	6.5%	93.8%
<i>Brada villosa</i> (1)	2138	1.8%	97.6%	<i>Platynereis bicanaliculata</i> (2)	258	0.4%	94.3%
<i>Axinopsida serricata</i> (1)	268	0.2%	97.8%	<i>Lucina tenuisculpta</i> (2)	249	0.4%	94.7%
<i>Alvania</i> sp. (1)	172	0.1%	97.9%	<i>Glycymeris subobsoleta</i> (2)	208	0.4%	95.1%
<i>Scalibregma inflatum</i> (1)	109	0.1%	98.0%	<i>Nephtys cornuta</i> (2)	166	0.3%	95.4%
				<i>Axinopsida serricata</i> (1)	154	0.3%	95.6%
				<i>Alvania</i> sp. (1)	151	0.3%	95.9%
				<i>Jassa falcata</i> (2)	143	0.2%	96.1%
				<i>Metacaprella kennerli</i> (2)	112	0.2%	96.3%
				<i>Pinnixa occidentalis, eburna</i> or <i>schmittii</i> (2)	96	0.2%	96.5%
				<i>Acila castrensis</i> (2)	95	0.2%	96.7%
				<i>Lumbrineris luti</i> or <i>lagunae</i> (2)	91	0.2%	96.8%
				<i>Cancer magister</i> or <i>gracilis</i> (2)	84	0.1%	97.0%
				<i>Ophiodromus pugetensis</i> (2)	82	0.1%	97.1%
				<i>Alia gaussipauta</i> (2)	80	0.1%	97.3%
				<i>Leitoscoloplos pugettensis</i> or <i>Orbinidae</i> (2)	71	0.1%	97.4%
				<i>larval shrimp</i> (2)	61	0.1%	97.5%
				<i>Lucinoma annulata</i> (2)	60	0.1%	97.6%
				<i>Unidentified bivalves and juveniles</i> (2)	55	0.1%	97.7%
				<i>Lepida longicorrata</i> (2)	47	0.1%	97.8%
				<i>Orchomene obtusa</i> or cf. <i>pinguis</i> or <i>dicipiens</i> (2)	43	0.1%	97.8%
				<i>Chaetozone setosa</i> (2)	42	0.1%	97.9%
				<i>Armandia brevis</i> (2)	39	0.1%	98.0%

Table 3 (continued):

Stations 75-225 m from operation (n= 129 stns)				Stations > 300 m from operation (n= 44 stns)			
	Abund.	% of total abund.	Cum.%		Abund.	% of total abund.	Cum.%
<i>Schistomeringos annulata</i> or <i>pseudorubrovittata</i> (1)	18988	24.5%	24.5%	<i>Axinopsida serricata</i> (1)	2153	14.6%	14.6%
<i>Pseudotanais oculatus</i> (2)	10989	14.2%	38.7%	<i>Glycymeris subobsoleta</i> (2)	1019	6.9%	21.5%
<i>Capitella capitata</i> (1)	10255	13.2%	51.9%	<i>Lumbrineris luti</i> or <i>lagunae</i> (3)	807	5.5%	26.9%
<i>Nebalia pugettensis</i> (1)	4886	6.3%	58.2%	<i>Cooperilla subdiaphana</i> (3)	607	4.1%	31.0%
<i>Eusirus</i> sp.(1)	4145	5.3%	63.6%	<i>Leitoscoloplos pugettensis</i> or Orbinidae (2)	605	4.1%	35.1%
<i>Sigambra tentaculata</i> (1)	3741	4.8%	68.4%	<i>Chaetozone spinosa</i> (3)	568	3.8%	38.9%
<i>Axinopsida serricata</i> (1)	2729	3.5%	71.9%	<i>Chaetozone setosa</i> (2)	452	3.1%	42.0%
<i>Glycymeris subobsoleta</i> (2)	2298	3.0%	74.9%	<i>Acila castrensis</i> (2)	387	2.6%	44.6%
<i>Lumbrineris luti</i> or <i>lagunae</i> (3)	1461	1.9%	76.8%	<i>Sigambra tentaculata</i> (1)	366	2.5%	47.1%
<i>Leitoscoloplos pugettensis</i> or Orbinidae (2)	1114	1.4%	78.2%	<i>Nuculana minuta</i> or <i>cellulitaa</i> (3)	282	1.9%	49.0%
<i>Acila castrensis</i> (2)	1014	1.3%	79.5%	<i>Euclémene zonalis</i> (3)	259	1.8%	50.8%
<i>Rhepoxynius cf. variatus</i> (3)	741	1.0%	80.5%	<i>Schistomeringos annulata</i> or <i>pseudorubrovittata</i> (1)	254	1.7%	52.5%
<i>Pinnixa occidentalis, eburna</i> or <i>schmittii</i> (2)	673	0.9%	81.3%	<i>Alia gaussipauta</i> (3)	248	1.7%	54.2%
<i>Chaetozone setosa</i> (2)	670	0.9%	82.2%	<i>Pseudotanais oculatus</i> (2)	239	1.6%	55.8%
<i>Ophiodromus pugetensis</i> (2)	456	0.6%	82.8%	<i>Pinnixa occidentalis, eburna</i> or <i>schmittii</i> (2)	209	1.4%	57.2%
<i>Euclémene zonalis</i> (3)	450	0.6%	83.4%	<i>Cumella vulgaris</i> or <i>Lucon</i> sp. (4)	209	1.4%	58.6%
<i>Peisidice aspera</i> or similar (3)	439	0.6%	83.9%	<i>Exogone molesta</i> (3)	170	1.1%	59.7%
Unidentified bivalves and juveniles (2)	424	0.5%	84.5%	<i>Tachyrhynchus lacteolus</i> (3)	170	1.1%	60.9%
<i>Alvania</i> sp. (1)	404	0.5%	85.0%	Unidentified bivalves and juveniles (2)	168	1.1%	62.0%
<i>Scalibregma inflatum</i> (1)	359	0.5%	85.5%	<i>Spio cirrifera</i> (3)	161	1.1%	63.1%
<i>Prionospio cirrifera</i> or <i>multibranchiata</i> (3)	348	0.4%	85.9%	<i>Rhepoxynius cf. variatus</i> (3)	153	1.0%	64.2%
<i>Chaetozone spinosa</i> (3)	339	0.4%	86.3%	<i>Prionospio steenstrupi</i> (3)	152	1.0%	65.2%
<i>Exogone molesta</i> (3)	311	0.4%	86.7%	<i>Prionospio cirrifera</i> or <i>multibranchiata</i> (3)	146	1.0%	66.2%
<i>Lepida longicorrata</i> (2)	303	0.4%	87.1%	<i>Peisidice aspera</i> or similar (3)	143	1.0%	67.1%
<i>Lucinoma annulata</i> (2)	293	0.4%	87.5%	<i>Heterophoxus oculatus</i> (3)	142	1.0%	68.1%
<i>Pandora filosa</i> or <i>bilirata</i> (3)	290	0.4%	87.9%	<i>Lucina tenuisculpta</i> (2)	137	0.9%	69.0%
<i>Lucina tenuisculpta</i> (2)	278	0.4%	88.2%	<i>Lucinoma annulata</i> (2)	126	0.9%	69.9%
<i>Terebellides</i> sp. or <i>Laussa venusta</i> (3)	277	0.4%	88.6%	<i>Dentalium</i> sp. (3)	126	0.9%	70.7%
<i>Heterophoxus oculatus</i> (3)	269	0.3%	89.0%	<i>Pandora filosa</i> or <i>bilirata</i> (3)	122	0.8%	71.6%
<i>Platynereis bicanaliculata</i> (2)	263	0.3%	89.3%	<i>Ischyrocerus</i> sp. (3)	120	0.8%	72.4%
<i>Orchomene obtusa</i> or cf. <i>pinguis</i> or <i>dicipiens</i> (2)	263	0.3%	89.6%	<i>Syllis elongata</i> (3)	114	0.8%	73.1%
larval shrimp (2)	243	0.3%	89.9%	<i>Ophiodromus pugetensis</i> (2)	106	0.7%	73.9%
<i>Harmothoe</i> sp.(3)	242	0.3%	90.3%	<i>Nephtys ferruginea</i> (3)	106	0.7%	74.6%

Stations 75-225 m from operation (n= 129 stns)				Stations > 300 m from operation (n= 44 stns)			
	Abund.	% of total abund.	Cum.%		Abund.	% of total abund.	Cum.%
<i>Mysella tumida</i> (3)	236	0.3%	90.6%	<i>Cirratulidae</i> (3)	105	0.7%	75.3%
<i>Tachyrhynchus lacteolus</i> (3)	224	0.3%	90.9%	<i>Scalibregma inflatum</i> (1)	103	0.7%	76.0%
<i>Spio cirrifera</i> (3)	224	0.3%	91.1%	<i>Harmothoe</i> sp.(3)	95	0.6%	76.6%
<i>Goniada brunnea</i> or <i>maculata</i> or <i>annulata</i> (3)	222	0.3%	91.4%	<i>larval shrimp</i> (2)	92	0.6%	77.2%
<i>Dentalium</i> sp. (3)	218	0.3%	91.7%	<i>Lepida longicorrata</i> (2)	82	0.6%	77.8%
<i>Syllis elongata</i> (3)	209	0.3%	92.0%	<i>Macoma secta</i> (3)	79	0.5%	78.3%
<i>Ischyrocerus</i> sp. (3)	198	0.3%	92.2%	<i>Onuphis iridescens</i> or <i>elegans</i> (3)	79	0.5%	78.9%
<i>Alia gaussipauta</i> (3)	193	0.2%	92.5%	<i>Solariella vancouverensis</i> (4)	77	0.5%	79.4%
<i>Nephtys cornuta</i> (2)	186	0.2%	92.7%	<i>Cossura</i> sp.(4)	76	0.5%	79.9%
<i>Armandia brevis</i> (2)	184	0.2%	93.0%	<i>Terebellides</i> sp. or <i>Lanassa venusta</i> (4)	75	0.5%	80.4%
<i>Macoma nasuta</i> (3)	182	0.2%	93.2%	<i>Ampharete</i> sp. (3)	75	0.5%	80.9%
<i>Ophelina breviata</i> (3)	169	0.2%	93.4%	<i>Amage anops</i> (4)	72	0.5%	81.4%
<i>Cooperilla subdiaphana</i> (3)	167	0.2%	93.6%	<i>Eteone tuberculata</i> (4)	68	0.5%	81.9%
<i>Cirratulidae</i> (3)	158	0.2%	93.8%	<i>Eusyllis</i> sp.(4)	67	0.5%	82.3%
<i>Westwoodilla caecula</i> (3)	155	0.2%	94.0%	<i>Euclémene reticulata</i> (3)	66	0.4%	82.8%
<i>Macoma secta</i> (3)	152	0.2%	94.2%	<i>Mysella tumida</i> (3)	65	0.4%	83.2%
<i>Nuculana minuta</i> or <i>cellulitaa</i> (3)	149	0.2%	94.4%	<i>Yoldia scissurata</i> (3)	64	0.4%	83.6%
<i>Prionospio steenstrupi</i> (3)	143	0.2%	94.6%	<i>Platynereis bicanaliculata</i> (2)	63	0.4%	84.1%
<i>Nereis procera</i> (3)	141	0.2%	94.8%	<i>Sternaspis scutata</i> (4)	62	0.4%	84.5%
<i>Ampharete</i> sp. (3)	138	0.2%	95.0%	<i>Pectinaria granulata</i> (3)	61	0.4%	84.9%
<i>Glycera capitata,robusta</i> or <i>convoluta</i> (3)	132	0.2%	95.1%	<i>Laonice cirrata</i> or <i>pugettensis</i> (3)	61	0.4%	85.3%
<i>Polydora</i> (3)	127	0.2%	95.3%	<i>Tiron biocellata</i> (4)	59	0.4%	85.7%
<i>Monoculoides</i> sp. (3)	122	0.2%	95.5%	<i>Terebellides stroemi</i> (3)	53	0.4%	86.1%
<i>Pectinaria granulata</i> (3)	121	0.2%	95.6%	<i>Nicomache lumbricalis</i> (3)	53	0.4%	86.4%
<i>Diplodonta impolita</i> or <i>orbella</i> (3)	108	0.1%	95.8%	<i>Eteone longa</i> (4)	52	0.4%	86.8%
<i>Yoldia scissurata</i>	104	0.1%	95.9%	<i>Alvania</i> sp. (1)	51	0.3%	87.1%
<i>Crab zoea</i> or <i>megalopae</i> (3)	102	0.1%	96.0%	<i>Macoma inquinata</i> (4)	50	0.3%	87.5%
<i>Nereis juveniles</i> or <i>Nereis brandti</i> (3)	102	0.1%	96.1%	<i>Glycera capitata,robusta</i> or <i>convoluta</i> (3)	49	0.3%	87.8%
<i>Glycinde picta</i> (3)	101	0.1%	96.3%	<i>Lumbrineris bicirrata</i> or <i>similibris</i> (3)	48	0.3%	88.1%
<i>Onuphis iridescens</i> or <i>elegans</i> (3)	100	0.1%	96.4%	<i>Sphaerodoropsis biserialis</i> (4)	48	0.3%	88.4%
<i>Euclémene reticulata</i> (3)	99	0.1%	96.5%	<i>Monoculoides</i> sp. (3)	46	0.3%	88.7%
<i>Nephtys ferruginea</i> (3)	95	0.1%	96.7%	<i>Orchomene obtusa</i> or cf. <i>pinguis</i> or <i>dicipiens</i> (2)	44	0.3%	89.0%
<i>Kefersteinia cirrata</i> (3)	93	0.1%	96.8%	<i>Crab zoea</i> or <i>megalopae</i> (3)	44	0.3%	89.3%
<i>Lumbrineris bicirrata</i> or <i>similibris</i> (3)	91	0.1%	96.9%	<i>Eusirus</i> sp.(1)	43	0.3%	89.6%



Stations 75-225 m from operation (n= 129 stns)				Stations > 300 m from operation (n= 44 stns)			
	Abund.	% of total abund.	Cum.%		Abund.	% of total abund.	Cum.%
<i>Phyllodoce</i> sp.(3)	87	0.1%	97.0%	<i>Westwoodilla caecula</i> (3)	42	0.3%	89.9%
<i>Megaluropsus</i> sp.(3)	85	0.1%	97.1%	<i>Goniada brunnea</i> or <i>maculata</i> or <i>annulata</i> (3)	41	0.3%	90.2%
<i>Axiothella rubrinocincta</i> (3)	83	0.1%	97.2%	<i>Macoma nasuta</i> (3)	40	0.3%	90.5%
<i>Maldanidae</i> or <i>Notoproctus pacificus</i> (3)	81	0.1%	97.3%	<i>Glycera</i> sp. or <i>Glycera americana</i> (4)	40	0.3%	90.7%
<i>Eunoe depressa</i> (3)	80	0.1%	97.4%	<i>Diplodonta impolita</i> or <i>orbella</i> (3)	39	0.3%	91.0%
<i>Praxillella affinis</i> or <i>P.</i> (3)	79	0.1%	97.5%	<i>Nitidiscala cf. tincta</i> (4)	39	0.3%	91.3%
<i>Nephtys longosetosa</i> or <i>punctata</i> (3)	78	0.1%	97.6%	<i>Maldanidae</i> or <i>Notoproctus pacificus</i> (3)	38	0.3%	91.5%
<i>Pinnixa tubicola</i> (3)	76	0.1%	97.7%	<i>Ophelina breviata</i> (3)	36	0.2%	91.8%
<i>Laonice cirrata</i> or <i>pugettensis</i> (3)	74	0.1%	97.8%	<i>Unidentified amphipods</i>	36	0.2%	92.0%
<i>Terebellides stroemi</i> (3)	73	0.1%	97.9%	<i>Nephtys cornuta</i> (4)	35	0.2%	92.2%
<i>Nicomache lumbricalis</i> (3)	71	0.1%	98.0%	<i>Polydora</i> sp.(4)	35	0.2%	92.5%
				<i>Syllis juveniles</i> (3)	35	0.2%	92.7%
				<i>Eunoe depressa</i> (3)	34	0.2%	92.9%
				<i>Nebalia pugettensis</i> (1)	33	0.2%	93.2%
				<i>Nereis juveniles</i> or <i>Nereis brandti</i> (3)	32	0.2%	93.4%
				<i>Lumbrineris</i> sp. (3)	31	0.2%	93.6%
				<i>Decamastus gracilis</i> or <i>Heteromastus fillobranchus</i> (4)	29	0.2%	93.8%
				<i>Diopatra ornata</i> (4)	29	0.2%	94.0%
				<i>Byblis millsii</i> (4)	29	0.2%	94.2%
				<i>Parandalia fauveli</i> (4)	28	0.2%	94.4%
				<i>Nephtys longosetosa</i> or <i>punctata</i> (3)	27	0.2%	94.6%
				<i>Cylichna</i> sp. or <i>Crepidula</i> sp.(4)	27	0.2%	94.7%
				<i>Thyasira gouldi</i> or <i>Thracia trapezoides</i> (4)	27	0.2%	94.9%
				<i>Capitella capitata</i> (1)	26	0.2%	95.1%
				<i>Cirratulus cirratulus</i> (4)	26	0.2%	95.3%
				<i>Axiothella rubrinocincta</i> (4)	25	0.2%	95.4%
				<i>Spionidae</i> (4)	25	0.2%	95.6%
				<i>Maera simile</i> (4)	24	0.2%	95.8%
				<i>Crenella decussata</i> (4)	24	0.2%	95.9%
				<i>Lumbrineris zonata</i>	23	0.2%	96.1%
				<i>Ostracoda</i> (4)	23	0.2%	96.2%
				<i>Nereis procera</i> (3)	22	0.1%	96.4%
				<i>Odostomia tenuisculpta</i> (4)	22	0.1%	96.5%
				<i>Syllis spongiphila</i> (4)	22	0.1%	96.7%



Individual taxa are indicated with a bracketed number (1-4) behind the name in Table 3 to indicate whether they were numerical dominants at close proximity stations versus those farther away. Not surprisingly, the indicator taxa of aquaculture waste deposition (especially *Capitella capitata* spp. complex, *Nebalia pugettensis*, and *Schistomeringos annulata* or *pseudorubrovittata*) are endemic species at reference stations as well, but at the reference sites only accounted for 0.18, 0.22 and 1.7%, respectively of the total infaunal macroinvertebrate abundance retained on a (1 mm)<sup>2</sup> sieve.

Overall, even a qualitative analysis of the benthic data - when considering the seven current aquacultural operations holistically - suggests that waste release has resulted in the exclusion of the majority of taxa even in an area 75 to 225 m from the edge of the net pen.

A PCA was performed on the data using Systat 6.0<sup>TM</sup>, after first eliminating rare taxa. Beginning with the 255 stations x 205 individual taxa in the original data set, taxa were eliminated from analysis if they were found in ten or fewer of the 255 stations, or if there were no more than 20 observed in all samples. Following this, all remaining taxa were ranked from highest to lowest abundance in the overall data set, and taxa that accounted for the top 98% of total abundance were retained. For the initial analysis, the PCA was run using an unrotated solution, on the untransformed abundance data. The final data set comprised 116 taxa (variables) x 225 samples (observations).

Five principal components were retained, which consecutively captured 12.2, 6.8, 5.6, 4.5 and 3.8% of the total variance in the data set (total of 32.7%). This is quite low. A further reduction of the data set to eliminate less abundant taxa would likely stimulate a greater loading of the overall variance on fewer principal components.

Five different groups of taxa were identified as loading significantly (factor score > |± 0.5|) on each of the five principal components retained, as shown in Table 4.

The first grouping is made up of polychaetous annelida and bivalve or gastropod molluscs that were notably absent from stations near aquaculture operations. The covariation of this group suggests similar shared habitat preference and tolerances and/or co-facilitation of settling and physiological activities. The strong co-occurrence of various taxa suggests that biological communities tend toward being biologically structured (climax-type communities) as opposed to physically structured (early successional stages).

The second grouping is dominated by amphipod crustaceans and a few polychaete taxa, a possible indication of a common feeding guild. As for the first group, these taxa tended to be absent at stations within 60 m of the edge of the net pen (Table 3).

**Table 4: Loadings of taxa on principal components (a: annelid; m: mollusk; c: crustacean)**

<b>Taxon</b>	<b>PC-1</b>	<b>PC-2</b>	<b>PC-3</b>	<b>PC-4</b>	<b>PC-5</b>
<i>Chaetozone setosa</i> (a)	<b>0.7827</b>	0.2942	-0.1581	-0.0040	-0.0180
<i>Lumbrineris luti</i> (a)	<b>0.7369</b>	-0.1409	0.1096	-0.2835	-0.0071
<i>Axinopsida serricata</i> (m)	<b>0.7161</b>	0.3748	0.1375	0.0588	0.0547
<i>Glycymeris subobsoleta</i> (m)	<b>0.6556</b>	0.4359	-0.2836	0.0315	-0.1642
<i>Tachyrhynchus lacteolus</i>	<b>0.6235</b>	0.3133	-0.3739	0.2895	-0.0380
<i>Chaetozone spinosa</i> (a)	<b>0.6126</b>	0.1834	-0.1357	0.2672	0.1006
<i>Solariella vancouverensis</i> (m)	<b>0.6037</b>	0.3023	-0.2394	0.2992	0.0853
<i>Euclimene zonalis</i> (a)	<b>0.6031</b>	-0.0414	0.1781	0.3369	0.1257
<i>Cooperilla subdiaphana</i> (m)	<b>0.5982</b>	0.2939	-0.2197	0.2553	0.0472
<i>Sphaerodoropsis biserialis</i> (a)	<b>0.5813</b>	0.1364	0.0787	-0.2716	0.0341
<i>Leitoscoloplos pugettensis</i> or Orbinidae (a)	<b>0.5767</b>	0.0556	0.3155	0.3603	0.0945
<i>Nephtys ferruginea</i> (a)	<b>0.5702</b>	-0.2358	-0.1419	0.2979	0.0959
Maldanidae or <i>Notoproctus pacificus</i> (a)	<b>0.5693</b>	0.0521	-0.0614	0.1456	0.0651
<i>Pandora filosa</i> (m)	<b>0.5692</b>	0.3203	-0.3385	0.2290	-0.0816
<i>Axiothella rubrinocincta</i> (a)	<b>0.5677</b>	-0.0151	-0.0147	-0.1162	0.0604
<i>Scalibregma inflatum</i> (a)	<b>0.5674</b>	0.0707	-0.2312	0.0297	-0.0461
<i>Spio cirrifera</i> (a)	<b>0.5647</b>	-0.0780	0.0739	-0.2185	0.0690
<i>Cylichna</i> sp. or <i>Crepidula</i> sp.(m)	<b>0.5247</b>	0.3171	-0.1151	0.1385	-0.0222
<i>Macoma inquinata</i> (m)	<b>0.5214</b>	-0.0057	0.0389	0.2269	0.1724
<i>Nitidiscala</i> cf. <i>tincta</i> (m)	<b>0.5147</b>	0.3104	-0.1998	0.1575	0.0174
<i>Syllis elongata</i> (a)	<b>0.5133</b>	-0.3083	0.2714	-0.5163	0.1315
<i>Orchomene obtusa</i> or cf. <i>pinguis</i> or <i>dicipiens</i> (c)	0.0858	<b>-0.6487</b>	-0.2160	0.0274	0.0421
<i>Westwoodilla caecula</i> (c)	0.0857	<b>-0.6472</b>	-0.0395	0.1096	0.0307
<i>Lepida longicorrata</i> (c)	0.2048	<b>-0.6290</b>	-0.2616	0.0857	0.1045
<i>Lumbrineris luti</i> or <i>lagunae</i> (a) 0.0947	<b>-0.6252</b>	-0.1847	-0.0076	0.0722	
<i>Monoculoides</i> sp. (c)	0.1638	<b>-0.6152</b>	-0.1601	-0.0040	0.0249
<i>Ischyrocerus</i> sp. (c)	0.2400	<b>-0.6103</b>	-0.2787	0.1106	0.0696
<i>Decamastus gracilis</i> or <i>Heteromastus fillobranchus</i> (a)	0.2338	<b>-0.6043</b>	-0.0972	-0.0457	0.1238
<i>Megaluropsus</i> sp. (c)	0.1226	<b>-0.5889</b>	-0.1498	-0.0641	0.0363
<i>Peisidice aspera</i> or similar (a)	0.2757	0.0956	<b>0.6761</b>	-0.1979	0.0539
<i>Heterophoxus oculatus</i> (c)	0.0040	-0.0282	<b>0.6537</b>	<b>0.5158</b>	0.0439
<i>Sternapsis scutata</i> (a)	0.0072	-0.0198	<b>0.5291</b>	0.3324	-0.0845
<i>Harmothoe</i> sp. (a)	0.1053	-0.0425	<b>0.5109</b>	-0.0628	-0.1312
<i>Euclimene reticulata</i> (a)	0.4160	0.0893	0.2923	<b>-0.5812</b>	0.0177
<i>Sigambra tentaculata</i> (a)	-0.3319	0.2371	-0.0313	-0.0698	<b>0.7299</b>
<i>Capitella capitata</i> (a)	-0.3067	0.2126	-0.1175	-0.0826	<b>0.6811</b>
<i>Eusirus</i> sp. (c)	-0.2644	0.1876	-0.0806	-0.0718	<b>0.6400</b>
<i>Metacaprella kennerli</i> (c)	-0.2555	0.1780	-0.0772	-0.0741	<b>0.6240</b>
<i>Jassa falcata</i> (c)	-0.2274	0.1711	-0.0885	-0.0812	<b>0.5827</b>
<i>Schistomeringos annulata</i> or <i>pseudorubrovittata</i>	-0.3267	0.1997	-0.0353	-0.0371	<b>0.5230</b>
<i>Lucina tenuisculpta</i> (m)	-0.0039	0.1993	0.1706	0.0881	<b>0.4941</b>

The taxa that loaded on the fifth principal component (*Capitella capitata*, *Schistomeringos* sp., *Sigambra tentaculata*, *Eusirus* sp.), include a number of those previously identified as numerical dominants at stations very close to aquaculture operations, and which are deemed to be tolerant to sulfides and probably other conditions resulting from excessive levels of organic carbon inputs to the sediment. *Nebalia pugettensis* is notably absent from this group, which may suggest that this species responds to slightly different conditions than the rest, and may indicate a different suite of sediment conditions as a result of organic C loading. In fact, *N. pugettensis* was only found at Farm site C and D, and was absent at the other sites, even where *C. capitata* exhibited high abundances. Conditions that lead to increased *N. pugettensis* abundance merit further consideration.

The tendency for *Lucina tenuisculpta* to co-vary with the rest of this group is not surprising, given that it can facultatively obtain food from symbiotic sulfide-oxidizing bacteria housed in its gill tissue.

**Figure 5: Lack of covariation between *Capitella capitata* and *Nebalia pugettensis***

The advantage of using PCA is that principal component scores are produced for each sample in the data set. The scores can then be examined for their mathematical relationships

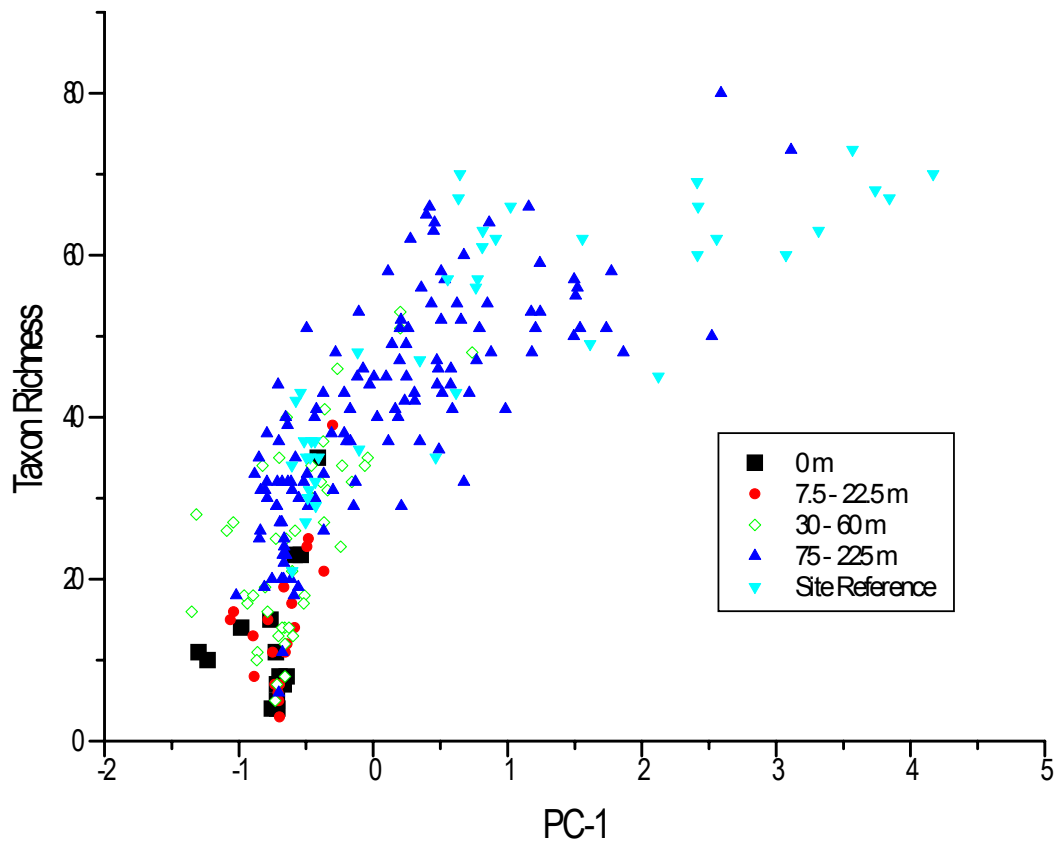
with other variables not included in the multivariate model. Table 5 shows the Pearson correlations (those statistically greater than zero) between aspects of the physical-chemical environment, and benthic community structure as captured in the PCA.

**Table 5: Correlation between multivariate community structure and physical-chemical variables**

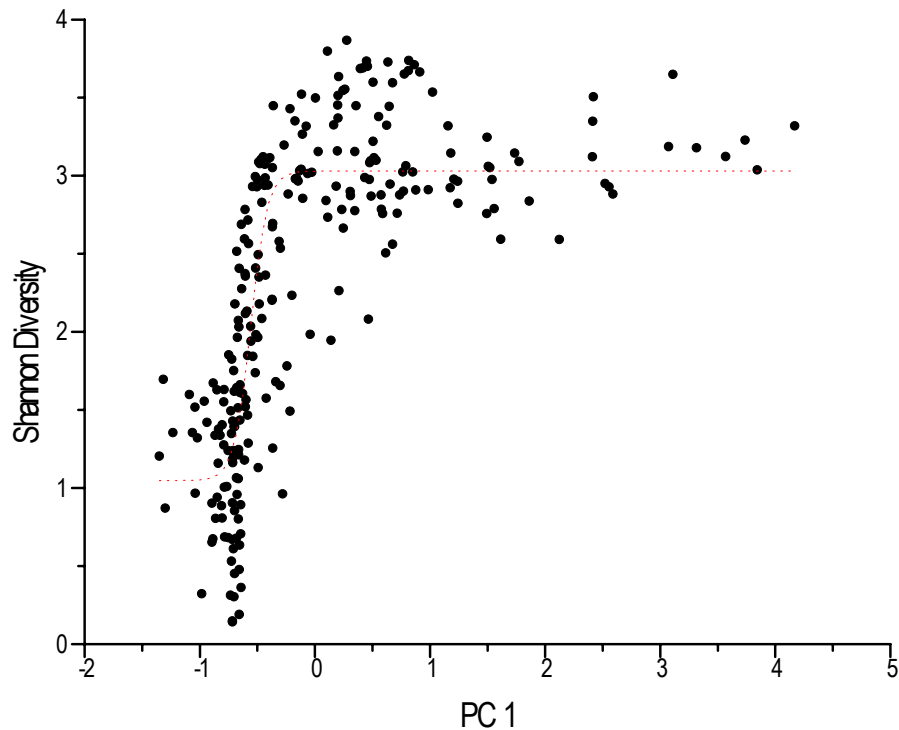
	PC-1	PC-2	PC-3	PC-4	PC-5
<b>Abundance</b>	-0.25	0.23			0.67
<b>Taxon Richness</b>	0.77	-0.35			
<b>Shannon Diversity</b>	0.61	-0.42			
<b>Pielou's Evenness</b>	0.43	-0.37			-0.25
<b>Distance from net pen (m)</b>	0.46		0.27		
<b>Depth (m)</b>	-0.25		0.39		0.24
<b>Sediment sulfide (uM)</b>	-0.35				
<b>pH</b>					-0.28
<b>Redox (ORP)</b>	0.52	-0.24		0.27	
<b>TVS</b>	-0.45	0.38	0.31		
<b>Silt-clay content</b>	-0.35	0.36	0.27		
<b>Dissolved O2</b>	0.33		-0.45		
<b>Salinity</b>	0.44	-0.32	-0.47		

All taxon groupings, as previously described, exhibited variations in abundance that were correlated with surrogate measures of sediment chemistry, depth, proximity to freshwater inputs, and so on. In all cases, however, the strength of the correlation was low (% of variance explained  $\leq 50\%$ ).

The first principal component captures loss of diversity based on a decline in abundance of the taxa that loaded on PC-1. A similar picture emerges when comparing PC-1 scores to taxon richness (Figure 6) or Shannon Diversity (Figure 7). A score of “zero” on the first principal component appears to be a threshold for severe shifts in the overall structure of benthic communities in the vicinity of aquaculture operations.



**Figure 6: Relationship between PC-1 scores and Taxon Richness measured in sediment samples collected in the vicinity of aquaculture operations (based on data from Brooks, 2001)**

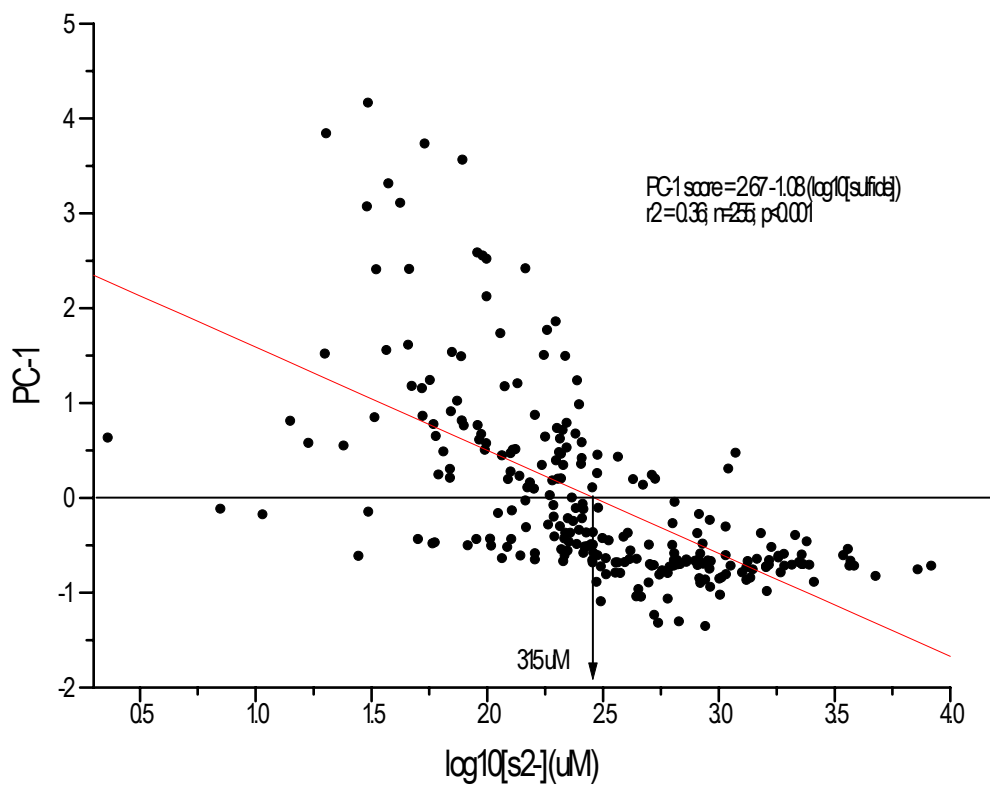


**Figure 7: Relationship between PC-1 scores and Shannon Diversity measured in sediment samples collected in the vicinity of aquaculture operations** (based on data from Brooks, 2001)

Figure 8 shows the relationship between one aspect of the multivariate community structure, as captured on PC-1, and sediment sulfide concentrations. There is a large amount of spread in the bivariate relationship, and a log-linear fit captures only 36% of the variation between PC-1 and sediment sulfide concentrations. This is consistent, however, with the earlier conclusions that sulfide cannot solely explain shifts in benthic community structure in the vicinity of coastal aquaculture sites.

Overall, a sediment sulfide concentration of  $\sim 315$   $\mu\text{M}$  is deemed to be a reasonable threshold beyond which the benthic community is substantially altered.





**Figure 8: Relationship between sediment sulfide and multivariate community response.**

