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Dear Ms. Morris,

The following comments are provided in response to input from the Independent Technical Review Committee regarding the *Proposed Scope of Work and Protocols for Aquatic Environment Technical Studies* in support of the Environmental Impact Statement required by Thurston Counties Declaration of Significance for Taylor Resources application for the culture of mussels at the *North Totten Inlet* site. Your November 1, 2001 document (Thurston County ITR Recommendations, 11/01/01) was copied into this letter. My responses to each comment are highlighted in *blue italics*.

A. Potential Impacts to the Benthic Community.

The overall conclusion reached by the Independent Technical Reviewers is that the scope of work and proposed protocols will result in useful information and that the consultant (AES) has demonstrated a high level of knowledge and capability in this regard. However, recommendations are made for refocusing the scope and protocols in several key components, to result in a more appropriate and defensible work product. By eliminating some components and changing others, the net effect on the cost to implement these studies should be similar, or less.

The proposed sampling plan apparently draws considerably from ongoing research work in British Columbia for salmon net-pen growers being conducted by the Taylor Resources' consultant. This work is interesting and important in terms of research to develop new, quicker chemical or physical measures of bottom impacts, but for the mussel culture project, the Independent Technical Reviewers recommend that this task remain focused on monitoring, rather than developing new, previously untested and possibly unreliable metrics, as discussed below.

Response: *The work in British Columbia has been guided and approved by the Technical Advisory Group (TAG) to the Ministry of Water, Land, Air and Parks. This group includes senior scientists from the Canadian Department of Fisheries and Oceans (DFO), Ministry of Water, Land, Air and Parks (MWLAP) and the Ministry of Agriculture, Fisheries and Forests (MAFF). The results of the benthic effects studies, commissioned by the TAG, were published in September 2001 (Brooks, 2001a). The physicochemical endpoints evaluated in support of the development of salmon farm performance standards in British Columbia have been used for nearly a decade by numerous researchers. They are by no means, "previously untested and possibly*

unreliable metrics." Brooks (2001a, 2001b) provides a review of the historic literature regarding the use of sulfides, redox, and TVS (or TOC) by dozens of researchers throughout the world who have used these endpoints in an effort to understand the biological response to organic inputs. For instance, see (Kristensen et al. 2000; Hargrave et al. 1995; Wildish et al. 1999; Brooks 2000c; Johnsen et al. 1993; Cranston 1994; Heijs et al. 1999; Mackin and Swider 1989). Sulfides and redox potential are used in several salmon and mussel aquaculture monitoring programs and as surrogate endpoints for biological monitoring in the DRAFT New Brunswick regulation and in the Scottish Environmental Protection Agency regulation (SEPA, 2000).

Analysis of the proposed scope of services and protocols for assessing potential impacts to the benthic community was initiated by reviewing what was proposed in the documents provided. Brooks (2001a)

The Aquatic Environmental Sciences (AES) *Scope of Services* for benthic impact analysis (August 29, 2001) has an introduction and three bulleted topics representing 1) that a literature survey had been done, 2) how sampling transects will be arrayed and some discussion of parameters that were selected to be measured in a prior survey at the proposed North Totten Inlet site, 3) what parameters are to be collected at an existing site referred to as Deepwater Point (apparently Gallagher Cove).

Response. *Deepwater Point is an existing mussel culture facility in Totten Inlet that is remotely located from Gallagher Cove. It is not Gallagher Cove. The Deepwater Point farm was chosen for this benthic effects study because water depths, sediment types and currents are similar to those at the proposed North Totten Inlet site.*

A separate *Protocols* document (September 1, 2001) gives some details regarding how the sampling will be done. It does not, however, give sufficient quality assurance detail in all cases, similar to what would be required in a Quality Assurance Project Plan for state or federal contracts. A couple of key areas are noted below where this type of information should be presented and appropriate data collected. This type of information is especially critical for the *in situ* measurements referred to as physicochemical measurements, discussed below.

Response. *The quality assurance requirements in the September 1, 2001 protocols for this study are those defined in Puget Sound Estuary Protocols (PSEP 1996)*

The proposal states that a preliminary inventory of benthic invertebrates has been completed at the proposed North Totten Inlet site. The *Scope of Services* document notes that the inventory will be expanded to include additional stations. A major consideration of any benthic sampling design to establish baseline conditions is the selection of station locations, spatial frequency and types of measurements to be collected. Here the Independent Technical Reviewers provide some criticism and suggestions for the proposed work, noting that there is room for custom-design of sampling plans, as long as the plans are straightforward and consistent with study goals and objectives.

A.1 Baseline sampling: Station frequency and replicates

Most probably an important goal of the initial sampling is to establish existing conditions for a baseline measurement at the site of proposed mussel rafts, and a reference area in some area similar to, but remote from the proposed project. If the North Totten Inlet site raft is installed prior to confirming baseline conditions, there will be no means to go back and provide the precondition samples if it is not done correctly. The use of reference locations to determine impacts is, at best, a coarse approximation as there is no guarantee that invertebrate larval recruitment and survival will be similar at a remote location.

Response. *Reference stations are not required for physicochemical comparisons because biologically significant concentrations of sulfide and stressful redox conditions are now known (Brooks 2001a). Therefore, these surrogates can be tied to absolute values (or ranges of values) to assess the need for biological monitoring. The monitoring of infauna will always require an appropriate* ***local*** *reference station because there are too many variables that change from one season to another (see Brooks 2000b) and from one year to another. These factors include, but are not limited to, season as it relates to recruitment, temperature, primary production, etc. and interannual factors related to macroscopic weather patterns. It is inappropriate to assume that future infaunal comparisons to baseline data collected in this study could provide a basis for assessing effects because infaunal communities vary by season and year.*

AES proposes to collect single benthic samples along transects leading away from the rafts. The case is argued in the appendix of the AES *Literature Review* (June 22, 2000) that collection of replicates at any given distance is psuedo-replication, and the replicates should not be collected because the question being asked is not if some regulatory criterion is being exceeded, but rather what are the spatial changes and are there subtle changes that more frequently located but unreplicated samples will detect? This argument may have some merit if the homogeneity of the bottom in terms of sediment grain size, carbon content and invertebrate population structure over small (i.e., 1 to 3-meter) distances has been established. This apparently has not been done. It is not uncommon to do a "pre-study" in each new environment, where a very large number of "replicate" samples are collected at one location. The results are plotted in terms of percent of species recovered for each subsequent grab. Historically, this is has been used as a means to establish how much replication is needed to reach any particular level of confidence that is desired. Without this type of information, sampling after the initial sampling may encounter patchy areas that were not adequately documented, resulting in false positive or negative effects.

Response. *The purpose of the faunal survey at the North Totten site is to characterize the macrofaunal community – not to provide a basis for future inferential regulatory monitoring. Brooks (2000a) reported clines in water depth (40.2 to 85.5 feet) and percent fines in sediments (silt and clay = 37.9 to 56.9%) in a preliminary survey at the North Totten Inlet site. These physical endpoints changed gradually from inshore to offshore. Benthic video suggested a continuous change in these endpoints across the proposed tenure. The question being asked here is, "what benthic resources might be*

compromised by additional organic loading associated with the proposed mussel farm." That question is best answered through a regression approach. The results of this study, as proposed, will describe the benthic community as a function of the evaluated physicochemical endpoints.

In light of the problems associated with pseudoreplication, same station replicates are seldom used in faunal surveys. The U.S. EPA is conducting faunal surveys to characterize habitats in Tillamook Bay, Yaquina Bay, Willapa Bay and Grays Harbor (Dr. Steven Ferraro, US EPA, personal communication). They have stratified the estuaries into three or four "habitat types" such as eelgrass meadows, burrowing shrimp dominated mudflats, and oyster beds. EPA has collected 15 samples (each sample consisting of paired 8 cm diameter cores collected side-by-side). The contents of the two cores at each station are pooled – they are ***not replicates.*** *Two small cores were taken to provide the optimum quadrat size (Steve Ferarro, personal communication). The fifteen samples were collected at randomly determined differential GPS stations within each strata. Each strata may cover thousands of acres. Infauna were screened on 1.0 mm screens and evaluated to Family. The basis for this experimental design is developed in Ferraro et al. (1989, 1991) and Feraro and Cole (1992, 1995). It should be noted that within the context of "Habitat Type", the benthos underlying the proposed North Totten site represents a single habitat. Therefore, the samples collected there, using a systematic random sampling design along specified transects, can all be considered true replicates (not pseudoreplicates) of sediment conditions within a single habitat type.*

The regression approach, relying on single samples collected in a systematic random sampling design, may not be familiar to some environmental surveyors. However, it is frequently used in environmental studies with great success (see Prairie 1996; Ferraro and Cole 1997, Brooks 2001a)

I believe the ITR is referring to rarefraction curves (see Ludwig and Reynolds, 1988 for a general discussion). There are numerous potential errors associated with this method (see Resenberg and Resh, 1993) and this methodology is infrequently used in environmental assessments. In fact, most environmental assessments exclude "rare species" because they provide no statistically significant information useful in understanding pollution effects (see Nash 2001). Under any circumstances these curves have no value in determining the number of samples necessary to achieve some level of confidence or power of a test. That exercise requires an evaluation of the internal variation associated with true replicate samples (see Zar 1984 or Neter et al. 1985).

A section of the *Protocols* document labeled "2.0 Study Design" states that:

> "*The purpose of this study is not to evaluate the statistical significance of differences in endpoints (here meaning parameters) between a reference station and treatment stations. The purpose is to evaluate trends in these endpoints as a function of distance from the farm to determine thresholds for biological effects by evaluating the covariance of biological and physicochemical endpoints*."

Note: "Treatment stations" are the stations near the rafts, as opposed to the reference or control stations that would be remote. The "treatment" is the installation and operation of the mussel rafts.

The Independent Technical Reviewers do not entirely agree with this stated goal for this particular project. Instead, the purpose should be to establish baseline conditions where it counts, in the benthic infauna. The physicochemical data collection is of secondary importance, as described below. Determination of trends over a transect is indeed better done with replicates than without, but it is not possible to judge completely, as the details of the proposed analysis are not included.

Response. This section of the ITR's report appears to refer to the Deepwater Point study that is designed to assess the affects of an ongoing mussel culture operation on the benthic community. The collection of pseudoreplicates has no value and would contribute very little to our understanding of the environmental effects created by mussel culture. The ITR may not recognize the value of understanding the relationship between physicochemical surrogate endpoints and biological response, but this author and several regulatory jurisdictions do (DFO Canada and Scotland). The U.S. EPA (David Young, personal communication) is now dedicating considerable effort to understanding the role of sulfides and redox potential in estuarine ecosystems. The analysis of sediments for the surrogate endpoints is inexpensive (about 10% of the cost of a single infaunal sample) and adds to our knowledge of these relationships. Taylor Resources has agreed to retain this element of the study because they can use the surrogate relationships with macrofauna in the future to inexpensively monitor environmental conditions adjacent to their farms.

Section 2.0 goes on to state that equal variances will be assumed for the treatment and reference stations allowing t-tests. A set of two single sentences in the next two bullet items (*Protocols* document, page 2) suggests that these data will be subjected to regression analysis to allow more elaborate techniques and to understand temporal relationships. It is the position of the ITRs that regression analysis cannot be conducted properly without some assessment of homogeneity of variance at and near single-sample stations.

Response. *I refer the ITR to Neter et al. (1985). Regression analysis requires that residuals be normally distributed and that the error variance be constant over all observations (independent variables). This condition is properly termed homoscedasticity. When the error terms (residuals) are unequal as a function of values of the independent variable(s), the condition is referred to as heteroscedastic. Regression analysis is relatively robust with respect to heteroscedasticity. However, when heteroscedasticity is severe, the variable(s) can be transformed to reduce this condition (log, ln, square-root, arcsine(sqrt), etc.). There is no mathematical basis for the ITR's statement that ". . .regression analysis cannot be conducted properly without some assessment of homogeneity of variance at and near single-sample stations."*

The ITRs do not find that the above AES proposed approach is reasonably foolproof to determine baseline conditions. Rather than assuming equal variances, and dismissing replicates at individual stations as psuedo replication (i.e., false replication, not needed and not useful) as is done in the salmon net pen appendix of the AES *Literature Review*, the ITRs believe replicates of at least 3 or 4 samples should be collected at each station,

and if necessary the number of stations reduced to as few as five, with several close to the edge of the rafts. At a minimum, this should be done at the stations beneath, at the perimeter, and 30 m from the rafts for at least the baseline sampling. Costs can also be cut by significantly reducing both the number of stations monitored but more so by reducing the number of parameters measured, as discussed in Section A.2, below. As an aside, there is no discussion of why a 0.1 m^2 Van Veen grab sampler was selected; in the recent past, petite ponar grab samplers have been widely used for this type of work (as is discussed and argued for in the AES *Literature Review*) and the smaller sampler would reduce the cost of infauna analysis.

Response. *The ITR cannot dismiss the inherent problems associated with pseudoreplication. More replicates collected at fewer stations would add little to our understanding of baseline conditions at the new site (North Totten Inlet) and would jeopardize the integrity of the study at the existing site (Deepwater Point). The author agrees with the ITR that more samples collected with a smaller Petite Ponar grab (0.0225 m^2 footprint) would provide a more robust design at similar cost. However, the Department of Ecologies Sediment Management Unit has repeatedly argued that the 0.1 m^2 van Veen is more appropriate because most of the data in Ecologies database was collected with that size grab. I discussed this issue again with Brett Betts in the Sediment Management Unit while writing this response. He was provided with specific information regarding the Totten Inlet study. He discussed the substitution of a Petite Ponar grab with others in his unit and there was a consensus that if Ecology were to make use of the resulting data, it should be collected with the 0.1 m^2 van Veen. Therefore, while the author agrees with the ITR, the study will use the larger van Veen. It is worth noting that if a smaller grab were substituted and more samples collected, those samples should either be collected in an identical design on another transect or the inter sample distance should be decreased with more single samples collected on the same transect. The collection of replicate samples at a single station would not increase the robustness of this study. I refer again to the EPA's carefully developed habitat characterization studies in numerous Pacific Northwest estuaries – study designs that do not include same station replicates.*

The spacing of stations along a given transect has been proposed, but no rationale is given for the near and far stations and the intervals provided. If the mean velocity at the site can be estimated, a simple settling model can be utilized to estimate the area where wastes will most likely accumulate.

Response. *Particulate organic matter is released from intensive aquaculture in a continuum of sizes. The particle's size and shape plus the density of the ambient medium and of the particle determine its settling velocity (see Stokes equations). The point is that organic matter released from all forms of intensive aquaculture will settle with a continuum of velocities with an upper boundary limit likely associated with mussel shell in this case and no practical lower bound. Assuming a single or restricted range of settling velocities likely contributed to the failure of the Department of Natural Resources monitoring program to determine the true spatial extent of the effects associated with salmon farms. Brooks (2001a) has clearly demonstrated that those effects extend to*

distances of at least 200 meters (654 feet) from operating salmon farms. The problems with Washington State's program became evident to this author during monitoring eight of Washington State's 12 operating netpens over the last ten years. Observed effects at Washington State salmon farms extend well beyond the 200' (61 meters) distance established as a Reference Station by DNR.

The author has no desire to repeat the mistakes that Washington State made in its salmon farm-monitoring program. The maximum distance of 125 meters identified in the protocols was based on the author's best professional judgment and experience in monitoring 45 different salmon farms in British Columbia and Washington State. The depth of the Deepwater Point farm and current speeds were considered as factors in that judgment.

The author will add a comment to the protocols indicating that the initial canister and sediment physicochemical data will be used to adjust the spatial extent of the sampling transect. If the initial work indicates that observable benthic impacts are restricted to distances less than 125 meters, then the inter sample spacing will be reduced such that only one or two samples are collected beyond the area of impact.

Recommendation A-1: Initial and possibly later sampling should include replicates to establish the background variability before assuming that a regression model, without replicates to form data points, will provide an accurate assessment of benthic conditions and baseline conditions. The spacing of stations should be based on estimated or actual (existing site) affects distribution data.

Response. *The regression approach will very nicely describe the background variability without replicates. That is especially true in this instance, because shallow physical clines have already been observed at the North Totten site. The maximum distance at which samples are collected at the Deepwater site will be modified based on initial canister and sediment physicochemical results. The collection of same station replicates is unjustified by any review of the current toxicological literature. The ITR is referred to Brooks (2001a) for a demonstration of the power of this study design. The publication is available from the author, from Environment Canada's or DFO Libraries and it is posted on the BC Salmon Farmers website. This comment is not intended to infer that the ITR should review Brooks (2001a) as a part of its deliberations.*

A.2 Selection of physicochemical parameters

The proposed work includes a number of parameters to be collected, but the purpose of each should be stated, not assumed. The Independent Technical Reviewers believe that potentially too many parameters are proposed for measurement, and in some cases, not the correct ones. The protocols appear to be adapted from experimental salmon pen work in British Columbia (and even are stated as such in Section 3.2.10.1 on page 7 of the Protocols document, apparently an oversight). The ITRs agree that the proposed protocols will provide some necessary information to evaluate the impact of the proposed mussel farm, but the scope could be altered to improve the quality and comparability of the data. It is important to recognize that these measurements are secondary in importance to the actual effects on benthic infauna. The physicochemical measurements

are just surrogate indicators of how the benthic infauna is being affected. They may or may not closely correlate with the biological health of the system. For more than a decade it has been known that changes occur to the infauna before measures such as TOC are significantly altered (see Weston 1990, Mar. Ecol. Prog. Ser.).

Response. *Goyette and Brooks (1999) documented changes in benthic macrofauna correlated with small increases in sediment percent fines and total volatile solids or TOC (± 0.5 percent). Brooks (2001a) has shown that there is no "No Effects Threshold" associated with increasing free sediment sulfides in the range (2 to 20,000 μM). A similar finding is being observed by the U.S. EPA in its estuarine studies (David Young, U.S. EPA Corvalis, personal communication). The point is that TOC or TVS are rather insensitive indicators of effects in benthic macrofauna. In part that is because TOC or TVS comes in a variety of forms with catabolic rates that vary significantly. The TVS itself has little affect on benthic environments. It is the biological oxygen demand (BOD) associated with TVS that creates changes in sediments that influences benthic macrofauna – especially after the sediment's assimilative capacity has been exceeded. Labile TVS in the form of animal waste has high associated BOD. It is metabolized quickly by microbes leading to reduced redox potential and potentially to a shift to anaerobic metabolism leading to increased sulfide concentrations, which are toxic to macrofauna. Refractory forms of TVS have reduced BOD and do not create as dramatic a change. Brooks (2001) found higher TVS at several reference stations associated with drift macroalgae and terrigenous inputs than was observed at the associated farm stations. The result was much high sulfide and much lower redox at the farm stations when compared with the reference station – even though the reference station had higher TVS. The effects of the higher sulfides and reduced redox were very apparent in the macrofauna. Weston (1990) reached some useful conclusions. However, the simplistic approach used in those assessments also led to a number of false assumptions that have been clarified in the last 10 years.*

The author agrees with the ITR that impacts on macrobenthos are the most definitive endpoint for evaluating effects associated with organic loading. However, the processing of macrofaunal samples is time consuming and expensive. By verifying the relationship between the TVS, free sulfides and redox, Taylor Resources can evaluate the extent and degree of effect associated with its culture operations at about one tenth the cost of macrofaunal analyses. Brooks (2001) has clearly and unequivocally defined the macrofaunal response to these surrogates at salmon farms in British Columbia. Interestingly, on a case-by-case basis, TVS was the least predictive surrogate found in this study.

In the opinion of the ITRs, sediment grain size, total organic carbon (TOC) and benthic infauna analysis should be analyzed at least initially to determine the baseline conditions prior to the installation of the proposed mussel farm. The first and last measurements are proposed by AES, but not TOC. It is recommended that the contractor collect total organic carbon (TOC) samples from the grabs, even if it means the exclusion of others such as TVS or ORP, as explained below.

Carbon is the primary waste product demanding oxygen in the sediments, and the direct measurement of carbon is relatively simple. Samples must be collected carefully, but only a small amount of sample is needed. The Washington Department of Ecology (Ecology) has selected TOC as a key monitoring parameter for salmon net pens, as indexed to sediment grain size because background levels of TOC increase with decreasing mean particle size. TVS (total volatile solids) is easier to analyze, but is a poor substitute as a measure of organic input and would not provide comparable data to many prior studies. It has been pointed out that TOC cannot be measured in coarse (i.e., gravel or cobble) bottoms. This is not considered a major drawback as without exception, these constitute erosional, not depositional bottoms. Grab sampling in coarse, erosional bottoms is not recommended as most samplers are incapable of operating properly there (e.g., the jaws of the sampler may not close completely, allowing materials to slip out).

Response. *The author disagrees that TVS is a poor substitute for TOC. In fact, for the following reasons, the author considers TVS to be a much better endpoint than TOC:*

1. *On several occasions the author has received TOC results from accredited laboratories that simply did not make sense. After considerable coaxing from the author, the laboratories searched, and in each case found errors in their analyses or reports. One of these laboratories, after consulting with its parent organization, decided that the uncertainties in the current EPA TOC protocols were severe enough that they would no longer conduct this analysis.*

2. *Sediments must be ground to a powder to be analyzed for TOC. This necessarily excludes material larger than ca. 2.0 mm (gravel). The result is that many types of sediment cannot be analyzed for TOC – leading to inconsistencies in some studies because some of the samples must be analyzed by TVS and others by TOC. Under any circumstances, the exclusion of large material biases the carbon estimates upward (as a percentage of the total sample dry weight).*

3. *Shell hash (sediments containing mostly ground shell) must be digested with acid to remove the calcium carbonate before TOC analysis. If this is not done carefully, then the remaining $CaCO_3$ confounds the analysis. The combustion of shell during TVS analysis can also lead to errors. However, the errors are relative small. Drying clamshell and combusting it at 550 ^oC reveals a TVS of 1.74%. A similar exercise with oyster shell leads to a slightly higher value of 2.11% (Brooks, unpublished). In comparing organic loading in shell hash sediments at treatment stations with similar reference stations, this error represents a constant addition and does not influence the determination of TVS increases at treatment stations.*

4. *TVS can be analyzed inexpensively by producers for in-house monitoring. Believe it or not, salmon producers are conducting a great deal of self-monitoring in Canada. Washington State salmon producers have not found this necessary for compliance with their NPDES permits.*

Having made these arguments, the author is aware of Washington State's commitment to TOC. This issue has been discussed with Taylor Resources and they will fund redox, sulfide, sediment grain size, TVS and TOC analyses for this study. As previously stated, the redox, sulfide and TVS analyses are inexpensive and add a wealth of information that will enable Taylor Resources to inexpensively monitor its operations in the future.

Total sulfides were included as another parameter to be measured for impact determination. This measurement may be a very useful measurement to quantify the effects of floating aquaculture, but it is subject to substantial error if not conducted correctly. It would be particularly useful in environments subject to significant inputs of slowly-degrading carbon not associated with aquaculture, such as wood debris from creeks, rivers or manmade processes that could unfairly bias estimates of the floating aquaculture impact with it's more biologically-available carbon sources. There may be problems with this measurement involving the volatility of the substance and lack of comparability among monitoring techniques. The AES protocols are quite detailed with regard to this parameter, but there is some confusions between the protocol sections. Section 3.2.10.3 states that the analyses will be done in the laboratory, but section 3.2.9.3 discusses analysis in the field. The tense of the entire section is past, not future. Some other monitoring programs typically analyze this parameter on shipboard, in the field, or shortly thereafter (e.g., on shore at the end of the day). Precision may vary greatly depending on time differences and handling procedures, and this should be addressed. Sample spikes and means to verify calibration standard accuracy should be discussed.

Response. *The author agrees that free sulfides must be measured carefully. Wildish (1999) and Brooks (2001b) discuss this issue in detail. Changes in sediment sulfides as a function of holding time are well recognized as important. However, it is not oxidation of sulfides that is a problem. Rather it is continued reduction of sulfate to sulfides, leading to increasing sulfide concentrations, at least in the first 72 hours of holding time, that is the problem (see Wildish 1999). At a recent Protocol Subcommittee meeting in British Columbia, we decided to impose a holding time of one hour for samples prior to sulfide analyses.*

The laboratory referred to is on our research vessel. I will change this wording. Both AES and Environment Canada (Pacific Environmental Science Center) have found that prolonged holding times, even with zinc acetate fixation, leads to spurious sulfide measurements. In fact, we have found that adding two normal zinc acetate to sediment samples in the amounts described in PSEP (1996) results in unrealistically low sulfide concentrations following shipment on ice.

There is at least one farm in Washington State where refractory TVS has repeatedly confounded the results of biological monitoring. At this farm we have observed a positive correlation between several biological endpoints and TOC. The reason is clearly evident in benthic video, which revealed increasing accumulations of eelgrass, macroalgae and terrigenous organic material with increasing distance from the farm.

Having said that, many analyses are difficult and must be done with care. Experience has shown that free sulfide measurements are one of those. However, the author has been training technicians for four years in British Columbia and the techniques can be mastered by many. However, that mastery takes time and experience.

As previously stated, Taylor Resources has agreed to include sulfide in this study in addition to TOC and sediment grain size recommended by the ITR.

ORP (redox potential) is sometimes measured in monitoring programs of this type, but is also subject to problems with precision and methodology. Numerous researchers have concluded that ORP is not a good measure for management and regulation, as reproducibility (i.e., precision) is poor. Moving the probe a single millimeter up or down may result in highly different results. The contractor may elect to measure ORP, but the ITRs do not believe that it should be relied upon for impact determination in this study.

Response. *GESAMP (1996) has recommended redox potential for monitoring aquaculture waste.* ***However,*** *the author agrees with the ITR and has recommended that redox potential data continue to be collected in British Columbia, but that it not be included in their regulatory program. The Ministry of Water, Land, Air and Parks has agreed with this recommendation. The ITR is correct in noting that redox potential changes very quickly as a function of depth in sediments (see Mackin and Swider 1989 or Brooks 2001a). That is why these measurements are made in homogenized surficial (top 2.0 cm) sediment samples. The true value of redox is to confirm the range within which sulfide measurement should fall (Brooks unpublished has provided the B.C. government with sulfide – redox regression analyses that include 95% confidence limits. This graph will be used by technicians in the field for confirming the relation between sulfides and redox as part of British Columbia's quality assurance program.*

The quality assurance procedures outlined in the protocols are those required by PSEP (1996). The author refers the ITR to Table C-6. The requirements for a certified reference material, ***matrix spike*** *and surrogates are all listed as* ***not applicable for sulfides****. There is good reason for this. PESC tried spiking sediments with sodium sulfide and found that they could only recover 10 to 20% of the spike. The reason is simple, the* $S^{=}$ *ion associated with sodium sulfide is highly reactive with a host of divalent cations found in sediments (Fe, Mn, etc.). This species is more aggressive than the common sulfide species found naturally in sediments (*HS^- *and* H_2S*) and it complexes quickly. The silver sulfide probes used in these analyses only measure free sulfides (*H_2S*,* HS^-*, etc.). The probes do not measure sulfide metal complexes, which are quickly formed with addition of a spike. Under any circumstances, the quality assurance requirements in these protocols are essentially identical to those published in PSEP (1996).*

The Independent Technical Reviewers can think of no good reason why TOC should be overlooked for the present study as a secondary or surrogate indicator of benthic impact. If there is evidence that the proposed site has excessive wood debris inputs, then it would be less useful, but the ITRs doubt that this is the case, given their familiarity with the inlet and the watershed. It may be true that TVS shows a broader footprint on the bottom than TOC (as indicated in verbal communications with other sources about the work of AES in Canada), but there is no published, peer-reviewed source of such information to

rely on at this point. It would not be surprising if TVS was impacted from a fish farm over wider areas than TOC, as proximate analysis of fish feed and feces show they contain non-organic components, too. But carbon is the source of the "problem" in terms of biological oxygen demand, not inorganic, refractory materials. TVS may eventually be selected as a good measurement for salmon net pen monitoring in British Columbia, but in Puget Sound there is a wealth of TOC background information that does not exist in western Canada. AES may have information correlating TVS to TOC sampling results, but no such documentation was provided to the Independent Technical Reviewers. Moreover, this may have to be done on a site-specific basis.

The ITRs consider it important to keep to the essentials using established, accepted, conservative measures. Standard Methods (APHA 1985, the "bible" of analytical procedures for investigators and laboratories) concludes:

> "*Determination of fixed and volatile solids do not distinguish precisely between inorganic and organic matter because loss on ignition is not confined to organic matter. It includes losses due to decomposition or volatization of some mineral salts. Better characterization of organic matter can be made by such tests as total organic carbon*." (Emphasis added. Note that carbon compounds, with their carbon atom chains, are defined as organic matter.)

Given that the key regulatory agency (Ecology) has selected grain size, TOC and benthic infauna as appropriate measures for monitoring and managing salmon net pens in Puget Sound, the Independent Technical Reviewers believe it would be a mistake to neglect TOC and add several other measurements. TOC analysis is not expensive, particularly for large numbers of samples. Collection of the top two centimeters of sediment has to be done carefully, and the protocols should disclose how this is done, rather than referring to a proprietary method that may not be repeatable by other workers.

Recommendation A-2: TOC measurement should be added to sediment grain size and benthic infauna as key parameters to be analyzed, at least in the initial samplings. This will increase comparability of results to other aquaculture studies and existing regulations in Puget Sound. The other physicochemical parameters are optional, with total sulfides appearing to have significant merit, but if cost is a consideration, they could be deleted. If the other measurements are to be included, the protocols should include more detail regarding methods and quality assurance means. Less emphasis should be placed on the importance of any of these measures, in deference to the more important biological infauna analyses.

Response. *In consideration of the Department of Ecologies history of using TOC, Taylor Resources and the author agree to add this parameter. Sediment samples will be evaluated for TOC, TVS, sulfides, redox potential and sediment grain size.*

A.3 Transect station orientation

It appears that proposed transect locations were a partial subset of what AES is or has done for research on salmon pens in British Columbia, without the more distant stations > 125 m. This is probably fine given the probable relative impacts, but some details are missing from the sampling design, such as: How were the transects for collecting samples determined with regard to dominant currents? There may have been some drogue or current meter direction studies at the proposed or existing sites. The EDAW work may have included this, but this document was not provided to the Independent Technical Reviewers. Determination of dominant direction of flow is not difficult to do, but it is important to establish the transects with regard to the dominant current directions, not bathymetry or guesswork. The direction of flow may change at the beginning and ends of flood and ebb tide, but that should not interfere with the determination of flow during the periods of mid tide when velocity is greatest.

Recommendation A-3: Transect orientation should be based on site-specific tidal current data or estimates from drogue surveys.

Response. *The prevailing current directions at, and through, the Deepwater Point mussel farm have been determined using windowshade drogues. This was conducted as part of the Pacific Shellfish Institute program. That information is being provided to the author and will be used along with a benthic video survey to establish the downcurrent transect for sampling at Deepwater Point.*

A.4 Transect station positioning

As the North Totten Inlet site is not located and positioned, accuracy of the differential global positioning system (DGPS) is very important so that the transect will be located exactly as planned in relation to any future rafts. No quality assurance information was provided with the protocols. The DGPS should be monitored for built-in accuracy measures (if any, such as EFE), and more importantly checked against known, plotted benchmarks or pinpointed structures such as pilings before and after sampling. During monitoring, the GPS should have an alarm or system to notify the user that the differential system is not working. Although accuracy is stated to be 3 m, it is not clear what this represents (95% confidence level?). It may be difficult to place a buoy at the site, given the active opposition to the project, but this should be considered as an additional measure to assure proper positioning.

Response. *The survey vessel is equipped with a Magellan™5000 DLX GPS unit coupled to a CSI GBX-PRO differential beacon receiver. The three dimensional 95% RMS accuracy of the combined units is < ± 3.0 meters. Differential lock is indicated both on the CSI instrument and with a "D" on the Magellan™ 5000. Aquatic Environmental Sciences has a surveyed point in front of our boathouse. The "Pre-cruise Checklist" requires pulling the boat over the survey marker and checking the dGPS system prior to each cruise. We have never found this unit to be off by more than 0.001 minutes longitude or latitude. When available, we have checked the unit against known positions*

using Coast Guard buoys. If there is a surveyed buoy available in Totten Inlet, we will include a check at this station.

The sample locations for the benthic effects study at Deepwater Point will be determined using a premarked ½" polypropylene line attached to the farm and sampling station on the research vessel. The location of the sample is noted using dGPS for future reference.

The purpose of the baseline study at the proposed Deepwater Point site is to characterize the benthic community in the single strata identified in the vicinity of the farm. The purpose is not to identify precise stations for future comparison to determine minor changes in the benthic community. Such an effort would inevitably be thwarted by the constantly changing nature of benthic communities. General comparisons of the composition of future benthic communities can be made with the results of the entire survey, but comparisons to determine statistically significant changes in community structure are best made against an appropriate reference station. Because of the uncertainty of the final mooring of the mussel rafts (within perhaps ± 10 to 20 meters) and their constant movement on currents (another ± 20 to 30 meters), the placement of a buoy would add nothing to this study. The questing being asked at the proposed North Totten site is, "what is the general character of benthic macrofauna and what benthic resources would be put at risk during operation of the proposed mussel farm."

Another point involves the location of the existing raft, and its anchoring arrangement. Will the sampling there be based on where the edge of the rafts are at any given time, or DGPS measurement, or both? As a minor note, polypropylene line should not be used in station positioning as stated in Section 3.2.2 of the protocols, as it stretches too much to be accurate. Dacron line or composites are much more suitable for measuring distances.

Response. *Sample stations will always be referenced to the rafts – not to geographical locations. That is necessary because the position of the rafts changes with time and the farm's footprint is no a thin black line but a fuzzy line drawn with chalk.*

The ITR appears to be confusing polypropylene line with nylon. Polypropylene is recognized for its low stretch and is therefore not recommended for anchor lines or mooring lines. Figure (1) describes the relative stretch of a variety of synthetic lines (http://www.alberta-ck318.freeserve.co.up/rope.htm). Note that the difference in stretch between three-strand polypropylene and three-strand standard polyester (Dacron) is negligible.

Aquatic Environmental Sciences uses ½" polypropylene line with a breaking strength of ~4,700 pounds. We can typically just reef on the line with our 10 hp kicker in reverse. Therefore the tension on the line is estimated at about 100 pounds or 2% of the breaking strength of the line. A vertical black line is provided in Figure (1) at this point. Horizontal lines are provided defining the percent stretch for nylon (3.5%); polypropylene (ca. 2.8%); and Dacron (ca. 2.5%). At 125 meters, the stretch in polypropylene under the stated conditions would be 3.5 meters and the stretch in Dacron would be 3.125 meters. In the context of the other variables that influence station positioning, the difference between Dacron and polypropylene (0.375 m at 125 m with 100 lbs of force) is insignificant. In this same real world, the 3 plus meter stretch in

either line is inconsequential. Under any circumstances, the actual station location is recorded using dGPS with an accuracy of ± 3.0 meters (about the same as the stretch).

One great advantage of polypropylene is that it floats. This is a significant safety attribute when working around anchored structures like salmon and/or mussel farms. All references were consistent with the statement in one line selection chart; "Polypropylene exhibits low stretch and floats indefinitely in salt water." Aquatic Environmental Sciences will continue to use polypropylene transect lines and strongly recommends them to the ITR.

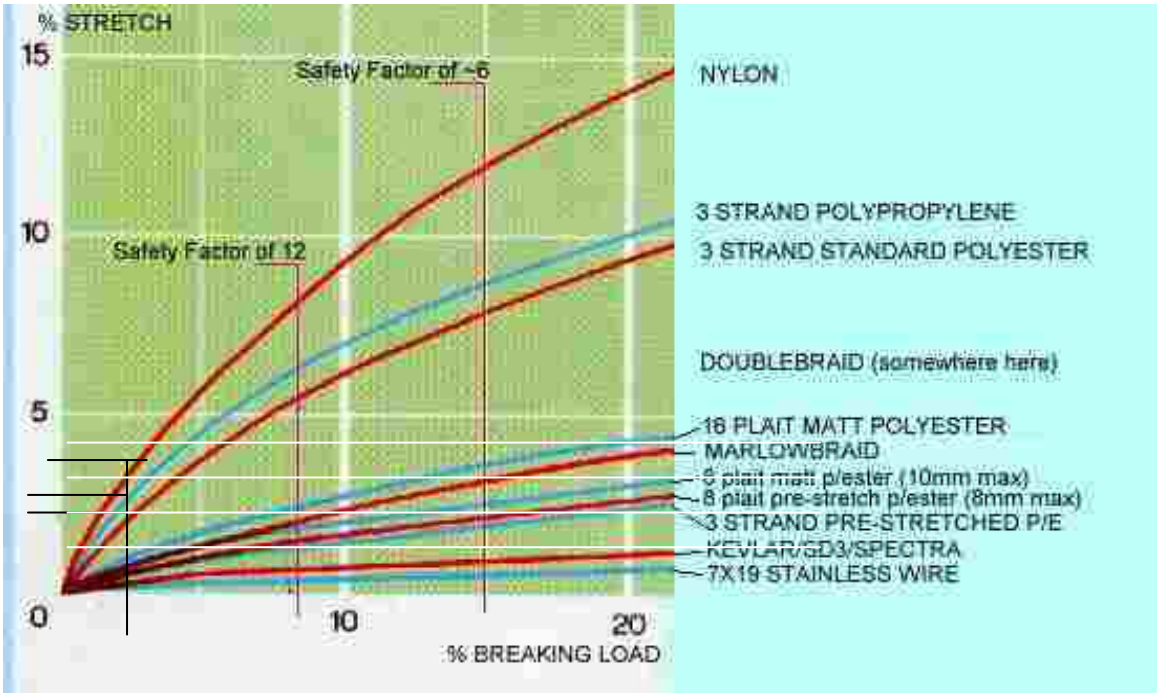

Figure 1. Relative percent stretch of several synthetic lines and wires as a function of percent breaking load. The vertical black line represents a stretching force equal to 2% of the breaking load of polypropylene line and the horizontal black lines represent the percent stretch of nylon; polypropylene and polyester (Dacron).

Recommendation A-4: Quality assurance measures are needed for transect station locating and DGPS use.

A.5 Temporal nature of sampling

It is not explained how sampling will be conducted temporally; i.e., will samples be collected at the same time of year or is multi-season sampling proposed? Will the baseline and the expanded survey be conducted at the same time of year assuming it is too late to expand the work from the baseline samples already collected? Does AES expect the abundance and biomass of the infauna to change seasonally, and if so, will annual sampling account for this? The protocols state that sampling will be done in November 2001, but this is later than normally done for Puget Sound benthic studies. Section 3.3 of the *Protocols* document states that the inventory will be expanded, but it is

not clear if the same stations will be re-sampled, or only if additional stations will be sampled.

Recommendation A-5: Temporal sampling frequency should be discussed and dealt with in the Scope of Services *or* Protocols *document.*

Response. *The baseline survey will be accomplished only once at the proposed North Totten Inlet site. Originally, this work was scheduled for November 2001. Obviously, this cannot be accomplished. The work was scheduled in late fall in recognition of the commonly held view that macroinvertebrate communities are least variable in winter. The Puget Sound Estuary Protocols, in the Benthic Infauna Study Design Considerations section (page 10) states that, "When characterizing adult populations of benthic macroinvertebrates it generally is preferable to sample when population estimates are least variable. Data collected by Lie (1968) suggest that late winter may be the most appropriate time to sample adult populations of benthic macroinvertebrates in Puget Sound."*

It is indeed now too late to combine the three samples previously collected with 12 new samples to obtain a sample size of 15. Therefore, the baseline survey will be expanded to include 16 samples, 8 each on two orthogonal transects intersecting in the center of the proposed tenure and extending to the tenures boundaries in four directions. That is one more sample than the 15 samples considered necessary by the U.S. EPA for characterizing estuarine habitats in the Pacific Northwest. Consistent with our understanding of the variability of benthic macroinvertebrate communities and as recommended in PSEP (1996), this work will be accomplished in the winter of 2001-2002. To reiterate, as stated in the Scope of Work, this work will be accomplished once.

Macroinvertebrate collections at the existing Deepwater mussel farm will also be collected once. Assuming that the same strong relationships exist between physicochemical surrogates (particularly sulfides) and the benthic community near this mussel farm, the remainder of the study will rely on the surrogates to predict the health of the benthic community on a bimonthly basis. All of this is described in Table (1) of the protocols, which I assume was provided to the ITR. A change in the number of samples might occur if the initial data indicates that farm effects are restricted to less than 125 meters or that they extend to greater distances. One of the beauties of sulfide analyses is that they are completed immediately in the field. With experience, a properly trained scientist can determine, in real time, whether or not additional samples need to be collected.

The author does expect that the macrofaunal community will change seasonally (Brooks (2000a). However, collecting benthic samples seasonally is considered beyond the scope of this project. PSEP (1996) covers this issue nicely by stating that, "Given the seasonal variation characteristic of benthic assemblages in general, it is recommended that direct comparisons between samples collected during different seasons be made with appropriate caution, or avoided completely (see my previous comments in this regard). Therefore, studies investigating interannual variation in the characteristics of benthic assemblages should be conducted during the same season (preferably the same month) each year. Emphasis added.

The bottom line here is that Taylor Resources proposes to collect benthic samples during the winter at both the existing and proposed mussel farms – as recommended by PSEP (1996). This will require that future sampling be accomplished during the same season if comparisons are too be made. However, as previously noted, all macrofaunal samples are better compared with an appropriate reference station in addition to comparisons with the baseline data. As long as the samples are collected at the reference and treatment stations at the same time of year, seasonal effects should not be a factor.

A.6 Canister studies

The AES protocol for measuring the rate of biodeposition from mussel cultures involves collection of wastes from the mussel raft using pipe canisters. The ITRs believe the wastes should not be filtered with 50 micron mesh size screen during processing, due to the loss of fine particles. It is relatively easy to let the materials settle in the canister for several hours, and siphon off or provide an exit for the supernatant leaving the solids and less water to be analyzed. Removal of the <50 µm size fraction could bias the results. Findlay et al. (1995) used 500 µm (micron) mesh fractionation to remove really large debris, but had evidence that this did not bias their results. Again, this protocol may be fine, but the Independent Technical Review team was not provided with any method documentation or literature citations.

Regarding the analysis of the canister contents, the same comments above regarding total organic apply here, too. TVS does not necessarily yield a reasonable estimate of carbon loading, and the ITRs believe the impacts and budgeting of effects should deal in that currency.

Response. *As previously noted, TVS is a frequently used endpoint for evaluating organic loading in environmental risk assessments. The citations are too numerous to list. Canister contents resulting from 30-day deployments can be several hundred grams. Measurement of TOC would require subsampling, which complicates the determination of total sample weight. The measurement of TVS is more direct, because the determination can be made on the entire canister contents and not extrapolated from subsamples (see Brooks 2001a).*

Recommendation A-6: Screening of the canister-collected sediments should be avoided in favor of allowing the solids to settle, then decanting or removing the supernatant. If this protocol has been conducted elsewhere with acceptable results, this should be stated or the published literature cited in the AES Protocols *document.*

Response. *In all of the canister samples collected by this author, the contents have settled to the bottom of the canister where they have been preserved, in-situ, with a salt – formaldehyde solution. It is the overlying water that is siphoned off through a Nitex screen to retrieve any suspended particulates. The settled material is then washed from the canister into an appropriate size HDPE bottle and the residue (if any) on the Nitex screen is backflushed into the sample. The total volume has always been less than 2.0 liters. We then allow the contents to settle in the laboratory until the supernatant is clear*

(one or more days). The supernatant is then siphoned through a Nitex screen and the canister contents removed to tarred aluminum boats for drying and combustion.

A.7 Acceptable level of impact

The ITRs understand the need to collect baseline and future affect information. But inevitably, these processes lead to the key question: What is an acceptable level of impact from the project, in this case, to the benthic community? Fortunately, there do exist benthic impact regulations for Puget Sound sediments that include special provisions for aquaculture. Although the regulations are pointed at salmon net pens, the concept and indeed even the criteria may be suitable for mussel raft management and regulation. There are drawbacks to the system, principally that the levels of allowable TOC are too low for some areas (i.e., naturally occurring levels in comparable areas are higher than the allowable levels). But that becomes less important or unimportant if baseline information is available at a floating aquaculture site.

The existing regulations involve a sediment impact zone established under and around a facility to a distance of 30 m from the perimeter. The entire regulations are not repeated here, but sediment grain size and TOC are used as screening tools to detect possible adverse change at that 30 m distance. Alternatively, if baseline or reference levels have been established, too much change in these triggers sampling to see if the benthic infauna is perturbed more than allowed by the Puget Sound benthic impact standards (that apply to all industries, not just aquaculture). This system, although not perfect, is reasonably accurate and has stood up to court challenge and appeals. It is unlikely that the mussel rafts of the proposed size and capacity will eventually be excluded using these existing rules.

Recommendation A-7: Consideration should be given to applying existing floating aquaculture impact criteria for salmon net pens to the impact analysis of the proposed mussel raft.

Response. *The Department of Ecology has authority to manage NPDES permits in Washington State. Director Fitzsimmons, in a letter dated August 18, 1997, to Mr. Arnt Thorkildsen, noted that, "Ecology does not require shellfish farmers to obtain waste discharge permits to grow shellfish on intertidal beds or from rafts. The reason a permit is not required is the shellfish farmers do not need to add fish food (nutrients) to the water to promote shellfish growth. Although a waste discharge permit is not required, shellfish farms must still comply with state water quality standards."*

Ecology reaffirmed its position in an amicus curiae briefing submitted to the Ninth Circuit Court of Appeals (No. 00-35667) in which Ecology stated that, "As the agency responsible for implementing the NPDES program in Washington State, Ecology has considered whether Taylor's mussel rafts require an NPDES permit. Ecology has concluded that the mussel rafts do not require an NPDES permit because Taylor does not add food to the water in order to promote shellfish growth. . . . . "

First, based on Ecologies (and EPA's) repeated denial of the need for NPDES Permits at intensive shellfish culture sites (specifically mussel rafts such as those proposed in this project), it is considered inappropriate for the ITR to suggest that, "the concept and indeed even the criteria (for regulating salmon netpens under NPDES permits) may be suitable for mussel raft management and regulation." There is simply no basis in the history of these issues for such a hypothesis.

Second, Based on thousands of benthic sediment samples collected in British Columbia, it is inappropriate to conclude that baseline information can be substituted for information collected at an appropriate local reference station. Baseline data provides a window on the past and is good for general comparisons. However, for regulatory purposes, there is simply too much variability in both sediment physicochemistry as a function of season, and from year to year, to be able to define meaningful statistically significant differences between existing conditions and baseline data.

The author was asked by both the Canadian federal Department of Fisheries and Oceans and the provincial Ministry of Water, Land, Air and Parks to make recommendations regarding physicochemical benchmarks and biological criteria for inclusion in their salmon farm regulation. The author has refused, contending that allowable biological effects are political decisions that need to be made by Cabinet (or other responsible policy makers). The author has agreed to define appropriate physicochemical benchmarks triggering biological monitoring once the biological criteria have been defined. The same arguments hold here and the author is very reluctant to recommend biological effects criteria – especially when those criteria are already laid out in the Washington State Administrative Code (WAC 173-204-320-(3)(c)).

B. <u>Potential Impacts to the Surrounding Water Column</u>.

The AES *Scope of Services* document defers to the PSI proposal as the description of work to be performed to address Issue B of the Thurston County Declaration of Significance (DS) for the Taylor Resources project.

A reliable estimate of the residence time of water in Totten Inlet is needed to assess the carrying capacity of the inlet for mussel aquaculture and the impact of the Taylor Resources proposed project on water quality. The PSI study relies on the Washington Department of Ecology (Ecology) South Sound circulation model (SPASM). Unfortunately, plans for use of the South Sound model are vague, and the degree of resource commitment from Ecology to the PSI study is unspecified.

Three-dimensional numerical models of circulation require careful verification through comparison with data. Until such verification is done, a model cannot be relied on as a *primary* source of information regarding residence time, although it may still give useful *secondary* information on a possible range of values for the residence time. The primary source should still be an estimate based on the best available data and a clearly stated set of assumptions.

Response. *The National Sea Grant College Program award to the Pacific Shellfish Institute is for development of information relative to, "Ecological Characteristics and Carrying Capacity of Suspended Shellfish Culture Systems." The project identifies Curtis Ebbesmeyer, a physical oceanographer with Evans-Hamilton as the lead scientist for this part of the effort. Dr. Ebbesmeyer will participate in this program during its second year (assuming funding is provided for year two).*

Under any circumstances, Taylor Resources is required to address the flushing of Totten Inlet only as it affects the determination of the inlet's carrying capacity. The carrying capacity model developed in the PSI effort will include a sensitivity analysis. If Totten Inlet is found to be close to its carrying capacity, and if the carrying capacity is very sensitive to flushing times within the range predicted for Totten Inlet, then a closer scrutiny of this issue will be warranted. On the other hand, if Totten Inlet is found to be well below its bivalve carrying capacity and if the model is found to be relatively insensitive to Totten Inlet's flushing rate, then it is the author's opinion that it is unlikely that the Hearing Examiner would require Taylor Resources to expend resources verifying or refining the flushing model as an exercise in basic science. It is inappropriate to make judgments regarding this issue until the carrying capacity model can provide estimates and until appropriate data is developed in PSI's program.

Residence time in Totten Inlet has been estimated in a study by Ebbesmeyer (1998) based on historical hydrographic measurements. More recently, Ecology has collected an extensive set of water quality measurements in the South Sound. This data set could be used for revising residence time estimates in Totten Inlet. Comparison with a previous study will establish a possible range of values for the residence time. Output from SPASM data (as available) would provide additional information about a possible range of values.

Response. *As a member of PSI's team, the author understands that PSI will use all available data for estimating flushing times in Totten Inlet and for evaluating bivalve carrying capacity. This comment will be passed on to the studies director, Dr. Dan Cheney.*

Combining these sources of information, a typical value for water residence time as well as a possible range and degree of and uncertainty can be established. This would require no additional data collection and no model runs other than those already done and those which will be performed as a part of the originally-envisioned PSI study.

Response. *This comment will be passed on to Dr. Cheney.*

Different parts of Totten Inlet may have a quite different water residence time scale. Such differences manifest themselves as gradients in property distributions in the inlet (such as oxygen and nutrient concentrations). The Ecology data set has spatial coverage that would help the assessment of differential residence time from the mouth to the head of the inlet.

Response. *As previously noted, the degree of accuracy in flushing time required to reasonably estimate the proposed mussel farm's impact on other resources and Totten Inlet's bivalve carrying capacity has yet to be determined. An appropriate level of effort will be made to insure that the range of predicted flushing times is sufficient to provide the needed level of confidence in the determination of effects.*

Recommendation B-1: Estimates of residence time in Totten Inlet should be synthesized from previous studies, estimates based on new Ecology data, and existing runs of the SPASM model, with special attention paid to the range of residence time within the Inlet inferred from property gradients. The model should not be used as a primary source of residence time estimates until its validity has been thoroughly demonstrated, which would be beyond the scope of the PSI study or project-specific studies for the Taylor Resources North Totten Inlet mussel culture proposal. Either the PSI study or additional work to be done by a consultant under contract to Taylor Resources should develop an upper and lower range estimate of flushing efficiency for the inlet as a whole and for the proposed mussel raft location, and acknowledge the uncertainty about this parameter. The upper range should represent a worst-case estimate for conservative consideration of the potential impacts of proposed mussel rafts.

Response. *See previous comments.*

C. <u>Potential Impacts to Phytoplankton and Other Aquatic Life in Totten Inlet</u>.

The AES *Scope of Services* document defers to the PSI proposal as the description of work to be performed to address Issue C of the Thurston County Declaration of Significance (DS) for the Taylor Resources project.

C.1 Additional scope of work document needed to "bridge the gap" between PSI study data and the North Totten Inlet mussel culture impact analysis

The four objectives of the PSI proposal are appropriate for an Aquaculture Initiative funded by Sea Grant (which this study is), but differ somewhat from what is needed to assess the project-specific environmental impacts of proposed North Totten Inlet mussel culture rafts on water quality and phytoplankton. The four objectives are more broadly stated to obtain information for shellfish aquaculture, in general. The PSI study will investigate physical, chemical, and biological conditions that result in the best mussel growth. While some of the PSI data will be useful to address the Taylor Resources' mussel raft proposal, methods for "bridging the gap" to address project-specific water quality and phytoplankton impacts have not been presented.

Response. *The purpose of the PSI study is not to, ". . .investigate physical, chemical, and biological conditions that result in the best mussel growth." The purpose, as stated in the first sentence of the Rationale given for the study, is to "….address critical environmental issues affecting the siting, sizing and operation of shellfish farms which utilize suspended culture techniques."*

The stated purpose of the PSI study is no different from Taylor's need to assess the environmental response to the proposed mussel culture operation at North Totten Inlet. Specific protocols supporting PSI's project are being written at this time. They will involve the measurement of a suite of parameters upcurrent, at varying locations within a row of rafts, and downcurrent from an existing mussel farm to determine changes in seston (including phytoplankton and other forms of particulate organic matter consumed by mussels) and in the water's physicochemistry (transparency. turbidity, TVS, chlorophyll α, phaeopigments, dissolved oxygen, DIN, phosphate and silicate). The study will also include similar evaluations at a local reference station. The PSI study will measure organic flux to the benthos and the reflux of nutrients from sediments back into the water column. This information will be combined with measurements of water flux through and around the farm to develop a mass balance assessment of organic carbon, nutrients and oxygen using a simple box model approach that will partition the environment into water and sediment compartments. Specific protocols for PSI's effort will be provided to the ITR when available.

The PSI study would benefit from a better definition of the proposed study design for measuring water quality variables. The design for profiling (depth profile, one time) versus data logging (one spot, many times) of variables and the timing of these is unclear (e.g., page 9). Also, further details are needed on calibration and QC for sensors, especially for DO. "The devil is in the details" for these measurements, and automated sensors have known problems.

Recommendation C-1: An additional document should be prepared to specifically address how the results from the PSI study, or other measurements, will address the impacts of the proposed North Totten Inlet mussel rafts on nutrients, chlorophyll, oxygen, and other water and habitat quality variables within Totten Inlet. The new, transitional document between the PSI study and the AES work should include details on sampling design and QA.

Response. *Detailed protocols describing the PSI study will be provided to the ITR when they are developed.*

C.2 Method for assessing mussel raft impacts using PSI study data

The PSI study does not discuss how the impacts of mussel rafts will be assessed. Will two areas in the inlet be compared, one with and one without rafts? How will raft impacts be differentiated from natural variation? How will it be assessed whether the concentrations of nutrients and chlorophyll are different because of rafts?

Recommendation C-2: The transitional document requested in Recommendation C-1, above, must outline how the project-specific impacts of proposed mussel rafts will be assessed using data generated from the PSI study.

Response. *Specifics regarding the PSI proposal will be provided when they become available. If that study design, and the supporting protocols, are found to be inadequate*

to the needs of this EIS, then Taylor Resources will undertake additional studies to fill the gaps. The need for additional studies cannot be determined until PSI publishes its specific protocols.

C.3 Use of historical water quality data

A significant amount of historical water quality data is available for Totten Inlet, collected by the Washington Department of Ecology (Ecology). Recently, Ecology has obtained several intensive seasonal data "snapshots" of Totten Inlet, which also measured nutrient sensitivity of phytoplankton growth. There has been no discussion of the use of these historical water quality data for assessing the environmental impacts of the proposed mussel rafts on water quality and phytoplankton. Use of historical data will be important for establishing baseline conditions and natural variability.

Recommendation C-3: Ecology's historical data should be used to assess the environmental impacts of proposed mussel rafts on water quality and phytoplankton in North Totten Inlet. The transitional document requested in Recommendation C-1, above, should include discussion of how these data will be incorporated and analyzed along with the new measurements.

Response. *The PSI effort will use all data that meets the studies quality assurance objectives. The author has previously communicated quality assurance concerns with some of Ecology's data for Totten Inlet. Those data will be examined, and if they meet muster, they will be used. Otherwise they will not be used.*

C.4 Analysis of phytoplankton species

The PSI study includes analysis of phytoplankton species. If this is to be utilized for the project-specific mussel raft impact assessment, a specific plan is needed, and/or historical comparisons need to be incorporated.

Recommendation C-4: The elements of the PSI study that will and will not be utilized to assess environmental impacts of the proposed mussel raft need to be defined. Specific questions and approaches should be outlined in the transitional document requested in Recommendation C-1.

Response. *As previously noted, the purpose of the PSI study is to provide information essential to producers and regulators regarding the effects on water quality associated with intensive floating bivalve mariculture. Assuming that the PSI study meets the stated goals, the information should provide a basis for evaluating the water column effects associated with the proposed North Totten mussel farm.*

C.5 Use of the SPASM model

Use of Ecology's SPASM model is ill-defined in the PSI study plan. Is funding to be directed towards Ecology for their personnel to use for this application, or does PSI wish to use model themselves? Intentions for the use of the SPSAM model need to be defined.

Recommendation C-5: This element of the PSI study – use of Ecology's SPASM model – needs definition and agreement by all involved parties.

Response. *This is an issue for the PSI study team should take up with the Department of Ecology. The PSI study is independent of Taylor Resources and it is not Taylor Resources responsibility to address the interaction between such an independent study and state agencies. However, as a matter of courtesy, these comments will be passed on to the Pacific Shellfish Institute.*

D. Potential Impacts Associated with Escapement and Propagation of Mussels.

These comments are confined to the issue of mussel escapement and potential impacts of *M. galloprovincialis* on existing shellfish populations, including mussels.

The Thurston County Hearing Examiner's *Findings and Conclusions* indicated concern about escapement of farmed mussels and the potential impacts of escaped mussels on existing mussel populations and other shellfish. The AES *Literature Review* provides a reasonable review of the issue of mussel speciation and the habitat requirements and preferences of the two (sub)species of mussels. The general approach presented in the AES *Scope of Services* document and the AES Protocols document, which consists of documenting the species of existing mussels, followed by monitoring during operation of the mussel raft project, should it be approved, makes sense. It is clear that the first step in assessing potential impacts related to escapement is to determine the relative population levels of each type of mussel in Totten Inlet. Following are comments on the specific approaches proposed to achieve this objective.

D.1 Survey of existing mussel populations in Totten Inlet and establishment of permanent genetic study sites

This issue is addressed on pages 3 and 4 of the AES *Scope of Services* document, and pages 9 to 11 of the AES *Protocols* document. Assessment of escapement will be accomplished by monitoring the relative proportions of the two mussel (sub)species. Both *Mytilus (edulis) trossolus* and *Mytilus (edulis) galloprovincialis* may currently be present in Totten Inlet, and the relative proportion of each mussel type needs to be determined. The proposed number of samples is reasonable for an initial estimate, but the number of mussels that may need to be sampled to detect a particular degree of change in the proportion of populations is not addressed. This degree of sensitivity needed in sampling is an arbitrarily set value, but a sensitivity of 10% (i.e., an ability to detect a change in the proportion of the two mussel species of the magnitude of 10%) would be a reasonable goal. The number of required samples for such a 10% level of sensitivity, sampled from a large population is about 30 mussels per sample. This is consistent with the AES protocol (4.3 Task D3).

The *Scope of Services* document (Task B1) provides for a visual survey of existing mussels, randomly selected mussels from three sites adjacent to existing mussel culture operations, and two additional areas within Totten Inlet, all apparently to be conducted prior to the installation of the proposed mussel raft project. From the mussels collected in these three ways, representative specimens will be speciated by electrophoresis to verify any potential M. *galloprovincialis*. In addition, the *Scope of Services* document (Task B2) calls for an inventory of the existing mussel stocks in Totten Inlet. It is not clear how Task B2 differs from Task B1. With respect to field sampling to address this issue, the *Protocols* document (4.1. Task D1) only refers to the visual survey, from which the genetic identification of 90 mussels will be determined electrophoretically, and three permanent study sites will be established (4.2. Task D2). There is no mention in the protocol of the evaluation of mussels from the additional five sites, as indicated in the *Scope of Services* document, Task B1. The sampling plan needs to be unambiguously defined. The protocol, taken alone, is relatively unambiguous. Using the protocol as the definitive AES proposal, the following comments should be considered.

Response. *Previous field studies by the author have identified M. e. galloprovincialis in Totten Inlet – generally on piers and floats, but occasionally in intertidal areas. Task D1 (in the Protocols) is not a random evaluation to determine the prevalence of mussels with Mytilus edulis galloprovincialis phenotypes in Totten Inlet. It involves a search of the inlet's shoreline, with emphasis on intertidal areas adjacent to the proposed North Totten Inlet site, for mussels that morphologically resemble M. e. galloprovincialis. DNA analysis will be used to confirm (or deny) the presence of galloprovincialis genotypes in the collected mussels. The purpose of this task is to qualitatively determine where galloprovincialis like mussels appear most common in Totten Inlet.*

The purpose of Task D2 is to identify three permanent genetic study sites in Totten Inlet at those locations where the highest numbers of M. e. galloprovincialis were observed in Task D1. It is anticipated that the U.S. Army Corps of Engineers permit for the North Totten mussel farm will require annual monitoring to determine the prevalence of M. e. galloprovincialis. A similar monitoring program was required at the Holmes Harbor mussel farm. Monitoring was completed in Holmes Harbor for two years using identical protocols to those invoked here. The farm was closed following two years of production due to high mussel mortality.

Task D3 defines a baseline genetic survey of randomly selected mussels at each of the three permanent sites to establish the proportion of M. e. galloprovincialis alleles (at PGM-2) in the population before the North Totten Inlet farm goes into operation. The author should have noted that these three permanent sampling stations will be a located in areas where larvae (or juveniles) from the proposed North Totten site would be most likely to recruit.

Sorry for the confusion. The bottom line is that a qualitative non-random search for mussels that are morphologically similar to M. e. galloprovincialis will initially be conducted to identify areas of highest existing numbers (Task D1). Based, in part on that survey, three permanent sampling stations will be identified (Task D2). The baseline

surveys at the three permanent sampling stations defined in Task D3 are designed to provide a random sample inventory of the existing mussel populations at each site.

The approach to conduct an initial visual survey has merit and should be completed prior to the selection of any permanent study sites, as is the stated intent in the protocol. The number of mussels proposed for definitive speciation (n=90) is reasonable for this initial survey. The visual survey needs to have detailed documentation in terms of location, so that results can be used to select permanent study sites. The subsequent selection of three permanent study sites is also reasonable but, as noted above, the sampling sensitivity (numbers or individuals sampled per site per sample occasion) needs to be stated.

Response. *The number of mussels to be collected at each permanent sampling station is defined in paragraph 4.3.1. A total of 10 mussels will be collected from within a 0.1 m^2 quadrat, which is placed at three randomly selected positions on the 10 cm grid. A total of 30 mussels will be collected at each permanent sampling site. A total of 90 mussels will be electrophoreses from the three permanent sampling stations.*

Based on information from the ITR coordinator that the Task D1 visual search did not occur in July 2001 as indicated in the *Protocols* document, it is understood that the permanent study sites will not be selected until sometime in Summer 2002. It is further understood that the ITR committee will conduct a site inspection prior to final study site selection, after completion of the visual survey and first procedure to definitively speciate representative mussels (electrophoresis or alternative method – see below).

Response. *The ITR's understanding is correct. The qualitative samples will be collected in the Spring of 2002 and recommendations for three permanent sample sites made. Baseline inventories at the three permanent sample sites will be completed following site approval by at least one member of the ITR.*

The method proposed for sampling within the selected permanent sampling sites (4.3. Task D3) are appropriate.

Recommendation D-1: Revise the Protocols *document to be consistent with the* Scope of Services *document with regard to the five sites in addition to three permanent study sites where visual surveys of existing mussel populations will be conducted. Also state in the* Protocols *document the number of individuals proposed for definitive speciation at each of the three permanent study sites, and explicitly state the desired statistical sensitivity where proportions of the two types of mussels will be monitored.*

Response. *See the author's previous comments. The number of mussels proposed for definitive speciation (30 per long-term study site = 90 total) is stated in paragraph 4.3.1 (Task D3) of the protocols.*

The protocols used in this effort are identical (except for location) with those previously approved by the U.S. Department of Fish and Wildlife, the National Marine Fisheries Service and the U.S. Army Corps of Engineers for a similar mussel culture operation in Holmes Harbor, Washington. The Holmes Harbor farm was approved and

put into operation. Monitoring continued there for two years but was terminated when the farm was taken out of production due to high mussel mortality.

The federal government invoked a benchmark requiring reconsideration of the culture of Mytilus edulis galloprovincialis in Holmes Harbor if the proportion of randomly collected mussels at three permanent survey sites reached ten percent. As noted in Brooks (1991), the probability of observing at least one event in "n" tries given some prevalence of a disease or allele in a population (p) is:

$$P_{>1} = \{1 - (1 - p)^n\}$$

In this case, p was stipulated by the federal government at 0.10 and n = 30 random samples provided a probability of 95.8% of observing at least one *M. e. galloprovincialis* in each sample at the specified prevalence of 0.10.

D.2 Differentiation of *Mytilus (edulis) galloprovincialis* from *Mytilus (edulis) trossolus*

The AES study plan provides for differentiation of the two species or strains of mussel prior to introduction of the mussel rafts ("the project") and while the aquatic environment technical studies are underway. The AES proposal calls for a visual survey of mussels in the pre-project phase, and confirmation of visually-identified mussels using an allozyme method (PGM-2 locus). Later, as a monitoring measure if/when the mussel rafts are in place, the identity and proportion of the two mussel species will be confirmed at three selected permanent study sites using the same method (which will have been sampled prior to the mussel raft project, in order to establish pre-project conditions). *This is correct.*

Although the cited Brooks thesis used the PGM-2 locus, the ITRs found only one published reference to the use of this locus as being diagnostic in the literature and others that did not find this locus to be diagnostic. Brooks may well be correct, but given the lack of general confirmation in the scientific peer-reviewed literature and the fact that newer technology is more definitive and, based on preliminary inquires, can be done for an equivalent cost, the ITRs recommend the use of DNA markers to differentiate mussel species. This will provide a stronger scientific basis for any results that are obtained.

Response. *Differentiation of Mytilus edulis trossulus and Mytilus edulis galloprovincialis using the PGM-2 locus requires very careful preparation of the tissues using specific protocols. The author is unaware of any researchers reporting a failure of this locus to be diagnostic and requests those citations refuting the diagnostic capabilities of PGM-2 from the ITR. Having said that, the author agrees with the ITR that protein electrophoresis has been replaced by DNA analysis since the work with PGM-2 was published in 1991. The author also agrees that current DNA techniques are easier and require less finesse.*

Dr. Patrick Gaffney, an internationally-recognized bivalve geneticist, was consulted by a member of the ITR committee in regard to the differentiation of these mussel species using DNA markers. He provided the following text, subject to minor editing:

Mussels of the genus *Mytilus* and their hybrids can be identified by means of nuclear DNA markers, using PCR-amplified gene fragments. Use of several independent markers is recommended to both increase certainty of identification and enable the detection of backcross hybrids. Use of four markers is optimal, two of which have proven reliable in our laboratory in past projects on *Mytilus* identification, and two additional ones which have been used successfully in British Columbia mussels (Heath et al. 1995). The first two markers are based on the glue gene responsible for the polyphenolic adhesive protein that mussels use to attach their byssal threads to the substrate, and were developed by Rawson et al. The second set are based on ITS and PLIIa gene fragments. Amplification of mussel genomic DNA with primers JH-73 and JH-5 yields a single 240 bp band in M. trossulus, a 255 bp band in *M. edulis*, and a 202 bp band in *M. galloprovincialis*.

Primers JH-54 and JH-5 also yield diagnostic PCR products. Amplification of *M. edulis* DNA produces two fragments, 350 bp and 380 bp in length, which are believed to be alleles. PCR of *M. galloprovincialis* DNA produces two different products, 300 bp and 500 bp in length. These do not appear to be allelic, and some amplifications yield only the 300 bp fragment. A single 240 bp fragment results from amplification of *M. trossulus* DNA with these primers.

ITS primers yield a single ~1250bp product, which after digestion with the restriction enzyme Hinf I provides distinct profiles for *M. trossulus* vs. *M. galloprovincialis* or *M. edulis* mussels.

PLIIa primers yield a ~475bp product, which on digestion with Hha I yields distinct profiles for *M. trossulus* vs. *M. galloprovincialis* or *M. edulis* mussels.

Recommendation D-2: Use the nuclear DNA marker method of species differentiation rather than the allozyme method proposed.

Response. *The author agrees with the ITR that current DNA techniques should be employed for these studies. This issue has been discussed with Taylor Resources and either AES will use an appropriate DNA technique or Taylor will subcontract this work to another competent researcher.*

Procedure

Genomic DNA is extracted from mantle tissue snips from individual mussels using commercial kits (either Qiagen Qiaquick or Gentra's Puregene). These kits reproducibly yield high-quality DNA from bivalve tissues, and are used routinely in our laboratory. Extra tissue snips are typically archived for potential future DNA extraction (in 95% ethanol). Genomic DNA preparations are used as templates for PCR amplification using

the primers described above, with thermal cycling protocols optimized in the laboratory. Appropriate negative controls are run to detect PCR contamination.

PCR products are electrophoresed using high-resolution agarose gels or precast acrylamide gels (BioRad Criterion) to visualize species-specific banding patterns and enable accurate sizing of all PCR products. Each mussel is scored at all four loci, which allows conclusive identification of pure species and hybrids.

Summary. *The following changes will be made to the protocols in consideration of the comments and concerns raised by the ITR:*

- *The number of sediment samples collected at the proposed North Totten site will be increased from 12 to 16. That is one more than is being used by EPA to characterize estuarine habitats in the Pacific Northwest. The three previously collected samples will not be included in this analysis;*
- *Total Organic Carbon will be added to the suite of sediment physicochemical parameters evaluated at both North Totten and Deepwater Point;*
- *The maximum extent of farm effects downcurrent from the Deepwater Point farm will be determined in the first canister and physicochemical analyses and adjustments made in the maximum distance at which future samples, including the infaunal samples, are collected;*
- *Because of concern expressed by Ecologies Sediment Management Unit, sediments will continue to be collected using a 0.1 m^2 van Veen grab;*
- *The text of the protocols will be expanded to clarify text where the ITR has indicated confusion;*
- *As a member of the Pacific Shellfish Institute team responsible for the Sea Grant project, the author will forward specific protocols to the ITR as they are developed. ITR members with an interest in that study are encouraged to communicate with Dr. Dan Cheney, the studies coordinator;*
- *Quality assurance requirements will reflect those required by PSEP (1996). Additional text regarding dGPS protocols and quality assurance, as provided in this response, will be included in the study protocols.*
- *Mussels collected in the genetic study will be identified using an appropriate DNA marker rather than PGM-2.*

I hope these responses address the questions raised by the ITR.

Sincerely,

Dr. Kenneth M. Brooks
Aquatic Environmental Sciences

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