North Totten Inlet Mussel Culture Proposal

Recommendations of the
Independent Technical Review Committee
to Thurston County and Taylor Resources

Regarding the Proposed Scope of Work and Protocols
for Aquatic Environment Technical Studies

November 1, 2001

A team of four Independent Technical Reviewers (ITRs) with specialized expertise in elements of the aquatic environment were selected by mutual agreement between Thurston County and Taylor Resources to assist the County with review and acceptance of the technical work of consultant(s) to Taylor Resources. Aquatic environment technical studies are proposed and will be conducted to address five issues identified in the 9/14/98 Thurston County Declaration of Significance, and in the 6/21/99 Findings and Conclusions of the Thurston County Hearing Examiner. These documents require an Environmental Impact Statement to be prepared for the Taylor Resources' North Totten Inlet Mussel Culture proposal. The Independent Technical Reviewers and their areas of expertise are as follows:

Independent Technical Reviewer	*Area of Expertise*
J.E. Jack Rensel, Ph.D. Rensel Associates Aquatic Science Consultants	Phytoplankton, algal blooms, and effects on benthic organisms and finfish.
Mitsuhiro Kawase, Ph.D. University of Washington School of Oceanography	Physical oceanography: Flushing characteristics (circulation) and water quality (eutrophication).
Jan Newton, Ph.D. University of Washington, School of Oceanography	Biological oceanography: Water quality (nutrients, oxygen) and phytoplankton productivity.
Ralph Elston, Ph.D. AquaTechnics, Inc.	Mussel genetics: potential escapement and competition issues.

The ITRs met on two occasions (September 17, 2001 and October 5, 2001) to review and discuss the following documents:

- Aquatic Environmental Sciences (AES). August 29, 2001. *Scope of Services to Address Aquatic Environment Issues, North Totten Inlet Site Proposed Mussel Culture*.
- AES. September 1, 2001. *Protocols for Implementing the Scope of Services to Address Aquatic Environment Issues, North Totten Inlet Site Proposed Mussel Culture*.
- AES. June 22, 2000. *Literature Review Describing the Environmental Effects associated with the Intensive Culture of Mussels* (Mytilus edulis galloprovincialis).
- Pacific Shellfish Institute (PSI). July 2001. *Ecological Characteristics and Carrying Capacity of Suspended Shellfish Culture Systems*.
- Thurston County Hearing Examiner. June 21, 1999. *Findings of Fact, Conclusions of Law and Decision* in the matter of the Taylor Resources' Shoreline Substantial Development Permit application for construction and expansion of mussel raft installations in Totten Inlet.

The aquatic environment issues of concern to Thurston County, identified in the 9/14/98 Declaration of Significance (DS) document, and reaffirmed in the *Findings and Conclusions* of the Hearing Examiner, are as follows:

A. Potential impacts to bottom-dwelling organisms (the benthic community).
B. Potential impacts to the surrounding water column.
C. Potential impacts to the phytoplankton resource, and the effects this could have on other aquaculture and aquatic life in Totten Inlet.
D. Potential impacts that could be caused by escapement and propagation of mussels.
E. Potential impacts of navigational lighting.

The AES *Scope of* Services document defers to the PSI proposal as the description of work to be performed to address Issues B and C. The PSI study is a Sea Grant Aquaculture Initiative project, not a technical study specifically formulated to address the Taylor Resources' mussel raft proposal. Issue E is not a biological or oceanographic issue, and will be dealt with by others outside the scope of services addressed here.

The consolidated recommendations of the Independent Technical Reviewers with regard to the AES and PSI study plans and protocols for addressing Issues A through D are presented in the remainder of this memorandum.

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.

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.

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).

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.

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.

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.

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.

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.

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.

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.

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.

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.).

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).

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.

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.

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.

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.

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.

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.

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.*

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.

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.*

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.

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.

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.

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.

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.

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.

C. Potential Impacts to Phytoplankton and Other Aquatic Life in Totten Inlet.

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.

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.

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.

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.

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.

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.

D. <u>Potential Impacts Associated with Escapement and Propagation of Mussels</u>.

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.

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.

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).

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.*

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).

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.

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.

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.

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