Review of Consultant reports for proposed North Totten Inlet Mussel Farm

Prepared by Jack Rensel, Ph.D. Rensel Associates Aquatic Sciences. 03 December 2008

AN ASSESSMENT OF POTENTIAL WATER COLUMN IMPACTS OF MUSSEL RAFT CULTURE IN TOTTEN INLET
by NewFields Northwest

I reviewed and checked each of my prior written recommendations regarding this report and most of them were incorporated or somehow accommodated, a few were not and I either let them go as not significant or restated them herein. The report has becoming a useful, understandable compendium of water column topics dealing with the proposed project and will serve very well for the purposes of writing an EIS. The assumptions and methods are fairly well presented and explicit and both the potential issues of low dissolved oxygen in North Totten Inlet (NTI) and the summertime effect of nitrogen excretion are addressed fairly. For these same issues I provide some additional, but limited suggestions on how to summarize the findings. Grammatical, syntax or less important suggested corrections are provided as "sticky notes" in the pdf file of the report.

I do not foresee much additional work on this besides considering the suggested changes of mine or my fellow ITRs, and then the final report preparation. As Roger Newell points out, we had to re-read the entire document which was certainly very time consuming compared to having been offered a track changes version, as one has to think through all the major and some minor points again. As a result of this, I focused mainly on the dissolved oxygen and nitrogen sections and did not re-inspect every aspect of the food web sections of the report other than any specific passages that I might have commented upon previously. If I am asked to review this report again, please provide a track changes version for the sake of efficiency.

Nutrient Sensitivity (section 4.2)

Rensel Associates and PTI Environmental Consultants (1991) ranked the percentage of time when DIN was depleted (somewhat arbitrarily set at any concentration measured at less than 7 µM) in surface waters from April through October with Windy Point data from the 1980s and found it to be 62% of the data. We did not set any percentage of time as a threshold at 55%, as stated at the beginning of this section. Perhaps Ecology did in the other cited documents, but if so the lumping of my report and Ecology's is confusing. Here I provide a synopsis of the above cited work, and note that it was a broad team effort of consultants, agency biologists and university experts who participated and reviewed the work extensively for USEPA. In that regard, it represented a community effort. Please consider using the following narrative or something similar in place of what you had:

In a report prepared for and with the USEPA Puget Sound Estuary Program, Rensel Associates and PTI Environmental Consultants (1991) compiled the percentage of time when DIN was depleted (defined as any concentration measured at less than 7 uM DIN) in surface waters from monthly observations in April through October, 1981 through 1985 with Windy Point sampling station data and found it to comprise 62% of the data and result in a mean DIN concentration of 2.46 µM. Of the 40 subareas of Puget Sound evaluated, that placed Totten Inlet as the 8th most nutrient sensitive in this tentative ranking. The authors also calculated the percentage of time when the concentration of subsurface (10 and 30 m) dissolved oxygen was less than 5 mg/L in the same time frames. For Totten Inlet, the result was zero percent (for 10 m, as there were no 30 m depths sampled). Other tentative measures of nutrient sensitivity were assessed, such as the period of time when nitrogen to phosphorus ratios were low and nitrogen or both nutrients were not detectable. The authors *pointed out that this was a tentative ranking system and subject to error as there is large variability in both the variability of individual phytoplankton species nutrient kinetics and even among studies of the same species*".

There is no "and" after "Rensel" and before "Associates" as found in this same section. I would appreciate you getting that correct as previously pointed out in the prior review.

Nitrogen flux

1) In section 4.3.3, Nitrogen, the revised report provides the critical summer season estimates that we requested and does an admirable job of presenting the facts without undue interpretation or speculation. But I think the results could be made more clear with a few simple procedures for the purpose of EIS preparation, as follows:

As the text reads now, it sounds like a net gain of total nitrogen will occur for the Totten Inlet system with 6,037 kg added by excretion, but only 3,400 kg removed with harvest. A non-diligent reader could easily assume that there was a net gain of 2x from the operation, which will not be the case. Mass balance considerations indicate that shellfish production must result in **less** total nitrogen in the system as a result of mussel growth and harvest. Otherwise, matter is being created from nothing and the balance of sinks and sources do not equate.

I suggest that the gross amount of total N consumed by the mussels in the algal growing season should be highlighted in the text and bullet summaries provided something like this:

- removal of total N by the mussels from seston and phytoplankton consumption (~14,937 kg/y) representing 100%, and then partitioned into:
- removal from Totten Inlet by harvest (3,400 kg/yr, closer to 23% than 22% apparently),
- fecal/psuedofeces (5,500 kg/y[1]) with some unknown loss to burial, coupled nitrification-denitrification plus some remineralization back to the water column, none of these components know at this time)
- and reintroduction as excretion (6,037 kg/y).

(The values I give above simply represent my quick attempt to extract the correct values from the NewFields Report, and they may not be entirely correct).

Then the same type of layout for summer time only could be presented. Somewhere you need to state the total removal by mussel feeding. I couldn't find it anywhere but calculated it as ~ 14,937 kg/y or whatever your analysis shows.

I find the Rodhouse model flow chart not sufficient to illustrate any of this because percent of volume of C and N flow is more meaningful than rate per m^2. I defer to Roger Newell's comments of using the Rodhouse model as you did, and agree with his suggestions on how to explain and understand your process. Also the Rodhouse figure's decimal points are out of place, so they don't even appear to be decimal points which should be noted in the legend or altered to be more legible. I suggest either a simple bullet section like that above for annual and algal growing season or a flow chart showing the simple totals in a breakout to each component.

[1] There are two estimates of fecal and pseudofecal production a paragraph apart on page 43 that are close, but off by about 10%

2) A second point about summarizing this nitrogen section: I believe that removal of total nitrogen from the system by the mussel rafts has compounding and significant effects in the long term that should be set off in opposition to the short term effects of more phytoplankton production in NTI from the mussel excretion of ammonia (and urea, which is not mentioned except in the introduction, but should be).

This involves the future volume of phytoplankton production that will not occur due to less total nitrogen in the nitrogen-limited system being available through nitrogen cycling. What is important here is the compounding effect of removing the nitrogen, which has a cumulative, recurring effect throughout the algal growing season. Carbon and all the other components except nitrogen are not limiting and can be fixed, lost and re-fixed throughout the growing season just as nitrogen can be, but with less nitrogen there is less total production in nitrogen-limited system. And there is no doubt from your report and review of the literature that surface waters are nitrogen-limited during portions of each summer and fall.

I do not think you can easily put a value on the reduced phytoplankton production of subsequent N cycling. A qualitative measure of its importance can be gained by considering that the turnover rate of phytoplankton production (ratio of production to standing stock) is rapid, on the order of a few days or more on average, so the removal by harvest prevents the turnover of N many more times per each growing season, not just once as presently implied (or not). The text should say this and point it out in the summary, too, in my opinion. What obfuscates this type of analysis is the apparent high percentage of N that it is seston compared to phytoplankton year around. And I don't understand the turnover rate of the seston to organic living forms and back, but it may be quite slow compared to the turn over time of phytoplankton because of its abundance.

3) A third point is that if there is going to be a discharge of nitrogen from aquaculture into a seasonally nutrient-sensitive water body and the need to minimize the growth effects on phytoplankton, the site should have strong currents and turbulence to dilute the nitrogen to concentrations known to be rate-limiting to uptake and/or growth based on the physical data from the site and facility effects modeling and field observations that will be done at the proposed NTI site. In contrast, discharging in a poorly flushed, more highly stratified and nearshore area of a water body could have a more dramatic and immediate effect on algal populations, which is why we see problems with *Ulva* spp. "sea lettuce" in some nearshore environments of Puget Sound.

The fact that there will be no measurable increase in concentration of dissolved N downstream of the rafts by 70 m or less is one manifestation of the site's suitability, and because the resulting concentrations of N are so low, the remaining phytoplankton in the downstream area will have to struggle to take up nitrogen (based on known half-saturation constants of uptake detailed by EPA's experts at the time (Bowie et al. 1985[2]). Remember that half saturation constants indicate the rate at which ½ the growth or uptake of N is limited and generally falls into the range of <0.1 to ~7 uM for most phytoplankton. That means that the threshold where rate limitation begins to occur is much higher for any given individual species.

[2] Bowie, G.L., Mills, W.B., Porcella, D.B., Campbell, C.L., Pagenkopf, J.R., Rupp, G.L., Johnson, K.M., Chan, P.W.H., Gherini, S.A., and Chamberlin, C.E. 1985. Rates, constants, and kinetics formulations in surface water quality modeling. U.S. Environmental Protection Agency, Environmental Research Laboratory, Office of Research and Development, Athens, Ga. EPA/600/3-85/040.

Comparison of Deepwater Point rafts and proposed NTI rafts, need for biomass estimates

Total biomass of mussels at the Deepwater Point site during various Pacific Shellfish Institute measurements in 2002 and 2003 is not stated in the report to the best of my knowledge. I requested this be included in my prior review as the EIS writer, the county and the public will want to know this in order to qualitatively compare the observed effects at the existing site with the expected effects of the proposed project (despite the difference in conditions between them). I found a table showing density per m^2 of mussels, but the report should not make the reader do the math. It should highlight the total biomass in several locations, in the beginning when the PSI studies are first introduced and later when measured nitrogen, chlorophyll or dissolved oxygen effects are discussed and contrasted between measured and modeled effects. If no biomass estimates are available from the Deepwater Point studies that should be stated.

Amino Acids

See comment of Roger Newell that includes a credible reference stating that mussels do not excrete urea or amino acids, as previously thought. The text should be altered accordingly.

N:P ratios

Again, please use the adjective “molar” or “atomic” when referring to the average N:P ratio of phytoplankton of 16:1. There is always confusion about this unless the reader knows if you are referring to units of moles or total weight. The former is more appropriate.

Flushing rate (Residence Time) discussion section 2.1.2

Please check these flushing rate values and units. Flushing rates are always indexed to some percentage of the water that is replaced, as it takes a very long time to replace all the water in an exponential decay function. Dr. Brooks gives these values in his reports and has it correct in that regard.

Figure 28

Other important points that can be gleaned from the figures include:

1) The surface and subsurface (10m) concentrations of nitrogen are positively correlated to some significant extent, and

2) Subsurface N (of all species) is not appreciably higher or different than surface N, which is a bit surprising if Totten Inlet was highly stratified during measurement. Instead, it is evidence that there is episodic vertical mixing of the water column as a result of wind events. We see this commonly in Puget Sound in the summer with harmful algal blooms too, just a few hours or a day of a low pressure weather front creates enough vertical mixing to disperse blooms and prevent immediate recurrence.

If vertical stratification was intense, we would expect the subsurface depths to have much higher DIN than the surface (unless the nutricline was much deeper than 10 m), such as I have observed and measured in other Puget Sound Inlets such as Budd and Henderson Inlet, but apparently this is not the case as often in Totten Inlet as elegantly shown by new Dept. of Ecology research discussed below.

Section 4.3.3.6 Phytoplankton response

The first sentence states that nitrogen discharged from the mussel rafts will be rapidly taken up by phytoplankton in the spring and summer after the spring blooms. This will be true at times, but not necessarily at all times. When nutrient depletion of surface waters is most prevalent during calm, warm and sunny periods, it will take significantly longer for excreted nitrogen in that layer to be sequestered because the standing stock of phytoplankton will be reduced (or in the case of late summer – dinoflagellates; at night, many will be at depth trying to sequester deep layer N). For example, see the plot that Kenn Brooks prepared showing 1989 to 1999 chlorophyll a concentrations at surface and subsurface depths at Windy Point Ecology Dept. sampling station. This plot shows summer chlorophyll a concentrations as low as 1 ug/L and I would expect concurrently concentrations would have been even lower at the NTI proposed mussel raft site if measured. A more extreme example of the same thing is nitrogen discharge into tropical seas such as the Caribbean, where modeling and field studies have shown the nutrient plume to persist longer and farther than would be the case in temperate seas with greater phytoplankton abundance and density. When we model phytoplankton uptake from aquaculture excretions, ambient concentrations of phytoplankton and water temperature are the two key sensitivity factors to be considered.

Dissolved Oxygen (Section 3)

Dissolved oxygen (DO) is a major area of concern in all aquaculture siting both for the good of the environment and the good of the cultured species. The report does a good job of presenting and discussing the pertinent information, but I believe there is some fine tuning still needed. Foremost of interest is the dissolved oxygen concentrations and flux at the proposed project site and vicinity in the water column and near the bottom. The former (surface and subsurface) indicate good DO conditions but there is one data set reported by Evans Hamilton Inc. that indicates relatively low DO immediately above the seabottom.

I had a correspondence with Evans Hamilton Inc. (EHI) regional manager oceanographer (Carol Coomes) about the near bottom dissolved oxygen data collected at the proposed project site. She agreed there could have been problems[3]. From inspection of the data, I thought it was probably not accurate as the DO content should have increased to higher levels during stronger tidal flow periods. A tidal effect was seen, but it seemed to be muted. From these discussions with Ms. Coomes, it is clear there were at least two major problems with the data that probably negatively biased the data discussed on page 17 (2nd to last paragraph) and in Appendix A (the 2006 report of EHI) to lower than expected results.

The CTD used was a Hydrolab Co. Datasonde 3, a older model that I used a decade or more ago and still own, but no longer use as it is two generations out of date. While these units are adequate for some purposes, when installed in low current situations they always require the use of a screw-on stirring unit to ventilate the

[3] Email of November 14, 2008 from Carol A. Coomes to Jack Rensel: *Jack, I think it is very probable the flow was restricted for DO measurements. The DataSonde 3 did not have stirrer on it and because it was mounted inside the TRBM and close to the bottom the low DO readings occurred. The cast DO readings were not as low as the in situ measurements and the profiling CTD did have a stirrer attached to it. We have since changed out both the TRBM covers and DO probes for any studies requiring DO measurements. The new covers have slots cut out of all sides to allow water flow. The DO meter is mounted up off the bottom grate at an open slot or often outside the TRBM cover. We also have a box frame with open sides for areas not prone to fishing. Hope this helps. Carol.*

dissolved oxygen probe. Without the stirrer, the probe actually draws the dissolved oxygen level down below ambient conditions as it consumes oxygen. I do not know why the stirrer was not used, but most probably because this older unit stirrer requires a large amount of battery power and it would have exhausted the CTD battery rather quickly unless an external, submersible battery pack was used and even then the deployment period would be limited.

A second problem involved mounting the CTD inside a poorly ventilated "trawl-resistant bottom mount" cage as shown in the EHI 2006 report as Figure 44 and very near the sediment-water interface. Note that the mount has only a few small holes and thus circulation would have been very poor between the inside and outside of the mount. Since those deployments, EHI has altered the unit to allow better flow through and tidal exchange. All moderate- to fine-grained sediments in Puget Sound have dissolved oxygen demand from the respiration of bacteria and other micro- and macro-organisms. It is likely that the bottom mount acted as like a leaky, benthic respirometer and thus was not measuring only the water column near the bottom but the demand of oxygen from within the sediments which resulted in the muted increases of DO concentration readings during the stronger tidal flow periods.

Comparing the water column data for the same day in the EHI (2006) report to the bottom mounted CTD data, all the results are >7 mg/L. If the bottom mounted CTD was performing accurately, we would expect some of the data to at least approach 7 mg/L, but it did not. This is further evidence that the near bottom data was biased and probably not reliable.

Whatever the case, water column monitoring of DO concentrations is not routinely performed by Ecology or anyone else in Puget Sound at 2 cm above the bottom, so I believe that these data should be qualified in the NewFields report with a statement pointing out how they are not comparable to other data and may in fact be biased as discussed above.

New Data and analyses of near bottom water oxygen, ammonium and other factors

A new interim data report by the Department of Ecology is available that focuses on South Puget Sound (SPS) water quality. The striking graphic to the right shows some of the results and makes it clear that Totten Inlet and in particular Northern Totten Inlet has the best conditions of all of South Puget Sound in terms of near bottom dissolved oxygen. I recommend that the final Newfields report at least briefly reference this report and possibly ask to use this figure.

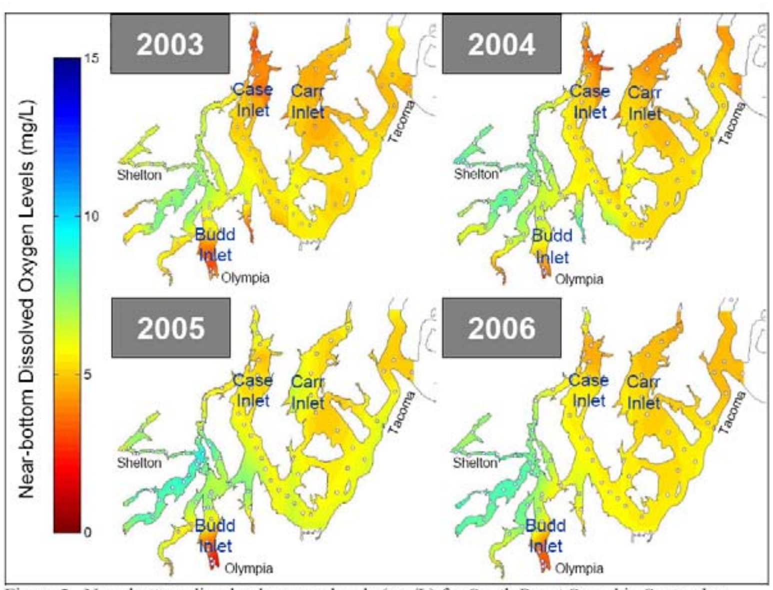
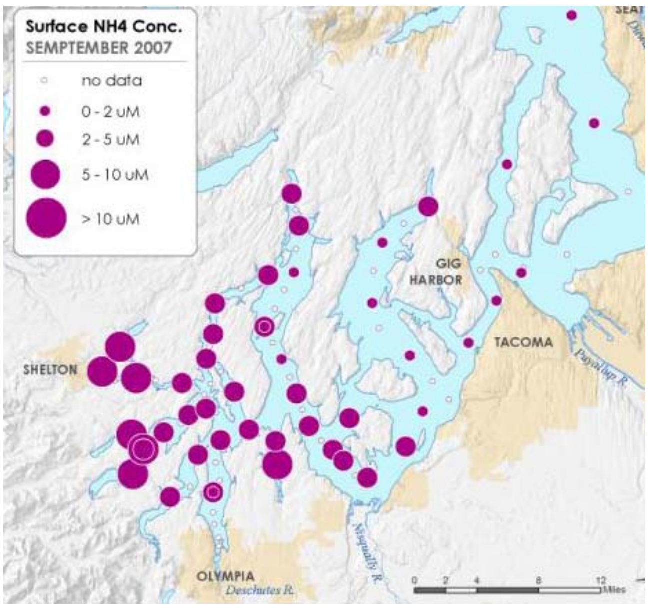
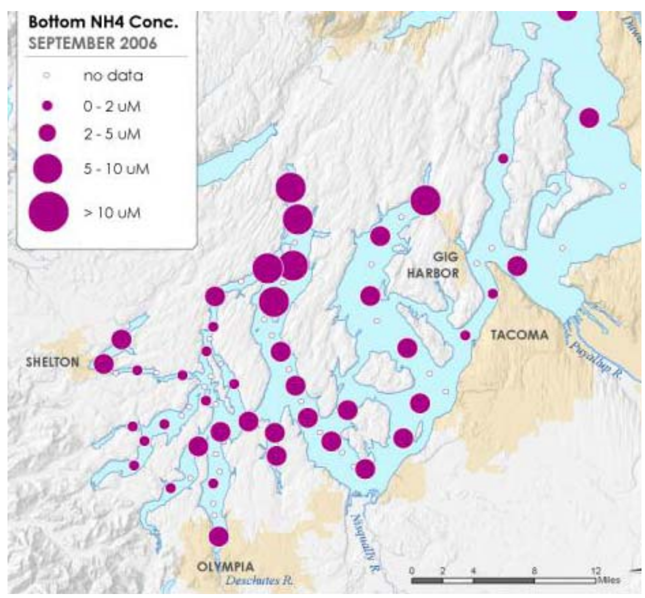


Figure 2. Near-bottom dissolved oxygen levels (mg/L) for South Puget Sound in September. Values have not been corrected with laboratory Winkler results.

See http://www.ecy.wa.gov/puget_sound/docs/SPSDOS_datareport_Jun08.pdf Contact Mindy Roberts, the professional engineer for Ecology and lead author for more details and permission to use the figure. mrob461@ecy.wa.gov There are also other highly pertinent findings regarding nitrogen and near bottom ammonia that show that NTI is very different than the southern portions of Totten Inlet as well as more circulation information not previously available, but the DO results in particular are timely to address the near bottom DO conditions in Totten Inlet discussed above. You will need to obtain the QAPP to see what they mean by near bottom, as it doesn't seem to be defined in the data report but it is a discrete near bottom measurement, not just the deepest of the normal CTD casts.



Part of figures 79 and 89 from the new Ecology data report showing surface and near bottom ammonium concentrations for the worst case timing of the annual cycle. <u>Note how bottom ammonia concentrations are very low in all of Totten Inlet compared to all the other inlets</u>.



Review of ***Supplemental study of dissolved nutrients and particulate organic matter in the waters near the proposed mussel farm in North Totten Inlet, Washington State, USA*** **(undated) by Kenneth M. Brooks**

Most of my concerns about the prior draft were met by the revised report, with a few exceptions. Although there is fundamental disagreement between the author and myself (or with my fellow reviewer, Jan Newton) on these two remaining matters, I stress that there is no argument about analytical methods, but rather study design and interpretation of results. Nor do my remaining concerns negate the usefulness of most other aspects of the subject report.

Importantly, these two disagreements are on issues that appear to have no significant implications to the outcome of the overall findings regarding water column dynamics in Totten Inlet. Also, the two topics discussed below from this supplemental report were not referenced or relied upon in any manner by the primary water column report prepared by NewFields Northwest. Other than these two disputed topics, I believe the author has made a valuable contribution to understanding the dynamics of Totten Inlet and southern Puget Sound. It is common for professional scientists to disagree on specific aspects of their work and I appreciate Dr. Brook's efforts to objectively focus on the data and our critique of it.

Below I present my prior comments from the early 2008 review of the prior report version. The author's response is repeated next, with my follow-up interpretation of the situation and recommendations to the EIS writers.

1. Are phytoplankton nutrient limited in Totten Inlet during the seasonal cycles?

Original Rensel Comment from early 2008:

It is stated on page 14 that "*Phytoplankton production is light limited in the Pacific Northwest from November to the beginning of March*". This is true only for some terminal inlets and bays as the majority of the marine waters of Puget Sound main basin, North Puget Sound and the Strait are light limiting (to algal growth) year round. This is an important point that should not be glossed over. Also there are profound differences between the limiting levels for phytoplankton (in general) versus seaweeds, the latter being much higher.

The report goes on to say: "*It should be noted that at no time were the DIN concentrations < 1.0 μM where the nutrient becomes limiting*". This is without doubt incorrect, the range of ½ saturation constants for phytoplankton uptake or growth limitation (the ambient value at which uptake or growth of algae is restricted to one half the saturated nutrient level) span a very wide range from < 1 μM to > 7 μM with tremendous variation among species[4]. This should be corrected if this is to be an accurate report regarding nutrient dynamics in the subject area. The waters of Totten Inlet are "nutrient sensitive" based on many authoritative studies and reviews, and the incorrect assessment provided here does not alter that fact. Certainly they would be less nutrient-sensitive nearer the north end, given the cooler temperatures, higher DIN levels in the mixed layer, and less vertical stratification compared to the central or southern end. While this is well known for this

[4] Bowie, G.L., Mills, W.B., Porcella, D.B., Campbell, C.L., Pagenkopf, J.R., Rupp, G.L., Johnson, K.M., Chan, P.W.H., Gherini, S.A., and Chamberlin, C.E. 1985. Rates, constants, and kinetics formulations in surface water quality modeling. U.S. Environmental Protection Agency, Environmental Research Laboratory, Office of Research and Development, Athens, Ga. EPA/600/3-85/040.. Still the most often cited and used information source by EPA and others. See also Thomann, R.V. and J.A. Mueller. 1987. Principles of surface water quality modeling and control. Harper Collins, New York, NY.

inlet and all the other inlets of SPS, it is important in the present context to demonstrate the more suitable siting of the mussel rafts that are proposed.

Brooks Response in late 2008:

*In general, we are talking about nutrient limitation of primary production by phytoplankton. We are not talking about specific species. The 1 μM limitation is not in general "incorrect" as asserted by Jack Rensel. It only becomes incorrect when one starts talking about specific species of phytoplankton and that was not within the scope of this paper. Lastly, these investigations were made to **supplement** the NewFields analysis conducted in the absence of the expected PSI reports. There is nothing in Jack Rensel's comments requiring a change to this supplemental report.*

Rensel Follow up in late 2008:

The above brief response, or the subject report, does not explain how the author concluded that Totten Inlet is <u>not</u> nutrient sensitive.

This is one of several conclusions and states, in the section *Factors affecting phytoplankton production in North Totten Inlet: "The nutrient data collected during these studies suggests that phytoplankton production in North Totten Inlet is not nutrient limited at any time of the year".*

This conclusion remains incorrect, for reasons I summarize here:

1) Other, much more definitive analyses such as nitrogen addition experiments from Totten Inlet demonstrate controlled-condition cause and effect for categorizing the area as nutrient sensitive. In short, if you add nitrogen to samples of phytoplankton and get immediate and significant growth effects, the water was nitrogen limited. No debating necessary. We could stop at this point.

2) The author may have confused one measure of nitrogen uptake and growth rate limitation (the "1/2 saturation constant" concentration) as a definitive of nutrient limitation, but it is rather the point at which nutrient uptake of growth is 50% less than it is when unrestricted by sufficient supply rate (which usually accompanies high concentrations). Uptake and growth is reduced less than 50% at much higher concentrations than the ½ saturation constant. For example, a species or community of phytoplankton may have a ½ saturation constant of 1 µM but the threshold where uptake or growth is reduced by limited supply for that same scenario may be 3 µM or greater (assuming that the supply rates to these concentrations are constant, which is often performed in studies by using continuous culture apparatus). The author does not say if he is referring to the ½ saturation constant or the threshold of nitrogen limitation or something else.

3) Importantly, the measurement of nitrogen concentrations used in this study does little to inform us about rate functions of nitrogen use by phytoplankton. Simply measuring dissolved inorganic nitrogen concentrations tells one very little about flux of nitrogen, which can be fast or slow. Dissolved inorganic nitrogen concentration is one indicator of nutrient status of a water body used in the past but because nitrogen rapidly cycles between organic and inorganic forms, both particulate and dissolved, measurement of DIN alone tells us about probability of nutrient limitation (which is greater as the concentration decreases) but there remains uncertainty unless rate functions are determined. By measuring both nutrient and phytoplankton concentrations, we have a snapshot of what is occurring but not the details that are necessary for the kind of conclusion discussed here. See Appendix A for more detail.

2. Is Totten Inlet as a net consumer or producer of dissolved inorganic nitrogen?

Original Rensel Comment in early 2008:

Totten Inlet dissolved inorganic nitrogen (DIN) concentrations were slightly lower at the mouth of the inlet on ebb tide than during the flood tide during sampling from August to November 2005. Although apparently some of the sample dates only had a single tidal sampling, the author asserts that this suggests that Totten Inlet was a "net consumer" of dissolved inorganic nitrogen. This may be true for dissolved nitrogen, but it is important to consider nitrogen in total and not just the "tip of the iceberg" of one of the dissolved components (DIN) used by phytoplankton and disregards another dissolved form (urea) which was not measured directly or indirectly approximated through total nitrogen minus DIN measurements and calculations. Similar statements were made about phosphorus which are also not valid for the similar reasons (i.e., Total P was not measured although orthophosphate is the primary species of chemical to measure for determining available P to algae at any given moment of sample collection).

Total nitrogen (all dissolved and particulate forms) was not measured (from which dissolved can be subtracted to estimate particulate N + urea or other non DIN forms), so it is not conclusive to assume that the inlet was a net consumer of nitrogen overall. *Nitrogen may flux rapidly from dissolved to particulate form in the food web during the nitrogen cycle.* I also suggest emphasizing (as it already discusses in other sections) losses of particulate or dissolved N could be due to a combination of phytoplankton production, grazing, sedimentation and loss to the atmosphere of nitrogen gas.

Furthermore, I believe it is important for the report to note that such a flood versus ebb tide analysis conducted on the same day suffers from inevitably re-sampling of the same water later when it returned during the flood tide. The ITRs believed it was important to determine reflux rates and provided an easy method to establish the rate in our 2002 memo, but no estimates of reflux (i.e., return rates) were conducted, so the matter remains uncertain. My guess is that reflux would have been relatively low given the strength of tidal flow and mixing in Pickering and Squaxin Passage, but I have no means to be certain.

No information was given on tidal amplitude for the sampling periods in this report. I may have missed it in some other document. This may not be essential in this case, but it could be if the tides selected were the lesser of the exchanges in this mixed semidiurnal tidal area.

Brooks Response in late 2008:

Dissolved nitrogen and phosphorus versus total phosphorus and nitrogen. Plants use inorganic nutrients not particulate organic matter for growth. I have previously read the PESEP report Nutrients and Phytoplankton in Puget Sound prepared by Rensel Associates and PTI Environmental Services and I am aware of the arguments made in the section entitled Review of Existing Nutrient Monitoring Protocols.

The reasons for not including particulate and dissolved nitrogen in calculating nutrient availability to phytoplankton are many. We are interested in the nutrients available to primary producers in the inlet. Those are dissolved nutrients – not particulate nutrients. Particulate forms of N and P include living phytoplankton and zooplankton. The phytoplankton may be consumed by shellfish or finfish and sequestered (shellfish) or moved out of the inlet by migrating fish. Particulate N and P also include suspended detritus and the bacteria that attacks it (seen as marine snow). Detritus comes in many forms, some of which are labile (animal waste) and some of which are more refractory (eelgrass, some macroalgae, terrigenous particulates, etc.) An undetermined proportion of the particulate N and P that Rensel refers to eventually settles to the bottom where it is consumed *by detritivores, bacteria or buried in sediments. Most of the organic loading accumulating in Totten Inlet sediments was at one time a part of the seston. Other portions of the particulate N and P pool may eventually be catabolized and returned to the water column in a dissolved form (DIN or PO_4 that is available for phytoplankton and/or macroalgae uptake) or in may be released to the atmosphere as N_2. The cycle times may be days, weeks, months or years. The point is that cell division times in Totten Inlet are short when primary productivity is not light limited and it is the nutrient availability during those one or two days that is important – not the potential nutrients available in living phytoplankton, zooplankton or detritus.*

Nearly all research involving phytoplankton dynamics that I am aware of assesses DIN and phosphate along with other nutrients. In closing, the report clearly states that Totten Inlet appears to be a net consumer of dissolved nutrients.

Rensel follow up in late 2008:

The question being framed is not entirely relevant. A more relevant question would be: **Is Totten Inlet as a net consumer or producer of total nitrogen (all forms of nitrogen, not just dissolved inorganic nitrogen)?** As explained below.

The response comment of the author only describes some of the pathways that nitrogen takes. The single point made, that cell division times of phytoplankton is fast does not acknowledge that the bulk of the total nitrogen, as measured in the water column by the established methods, also cycles rapidly at about the same rate. Measurement of total nitrogen in the water column is an existing, defined parameter collected by many researchers and agencies. It only included particulate and dissolved nitrogen, not fish and invertebrates and does not represent the entire pool of nitrogen in the water column as Dr. Brooks argues. Just as total phosphorus measurements in lakes and freshwater systems is a primary pillar of trophic and enrichment status, total nitrogen in seawater characterizes the potentially available pool of nitrogen that can be rapidly converted to dissolved inorganic nitrogen and also includes organic nitrogen that is utilized by many phytoplankton (e.g., urea). The conclusion reached (that Totten Inlet is a net consumer of dissolved nitrogen, is therefore not supported by the data. The conclusion is also tenuous because:

1) The very small (4.9%) difference between flood and ebb tide DIN concentration is not convincing that this is a significant <u>biological</u> difference, regardless of its <u>statistical</u> significance, even if I believed the methods used were correct to answer this question (which I do not).

2) There were significant problems with the study plan including where and when the data were collected, the types of data collected and the amount of data collected, as described below. The study design was incomplete to determine what the author wished to do.

3) As in the prior issue, the author confused measurement of concentrations of one form of nitrogen (DIN) with understanding of the entire flux of total nitrogen in and out of the inlet. The question I framed above is the relevant one if this question must be posed and answered, but I think it largely an academic construct as explained below in the appendix to these comments.

I recommend that the EIS writers disregard the supplemental report's conclusions that Totten Inlet is a net consumer of dissolved inorganic nitrogen and the other conclusion that the inlet is not nutrient limited for the production of phytoplankton. The separate analyses presented by NewFields Northwest in their report are adequate to address carrying capacity issues and the effects of the proposed project on nutrients and phytoplankton.

Appendix A. Are phytoplankton nutrient limited in Totten Inlet during the seasonal cycles?

See summary of comments and responses in the main body of the above letter report. Some details provided here:

A concentration of one μM is thought to be representative of nitrogen limitation by the author. When questioned, we are told that this is incorrect if we consider only certain species of phytoplankton and that that it was not within his scope of work to address this issue. But since the author is proposing a entirely new paradigm of nutrient sensitivity determination, the onus is on him to support his conclusions. And he has chosen not to do so.

When I first examined this issue with others for USEPA in the late 1980s, I found the classic and still relevant literature indicated that phytoplankton limitation of about 50% of uptake or growth occurred from below 1 μM to about 1.5 μM for diatoms but was higher for dinoflagellates and I reported that with references in my report. In late summertime and early fall during quiescent, warm periods of most concern, dinoflagellates are very common in SPS inlets and some are probably harmful, or at least noxious, as first discovered by R. Cardwell (to shellfish larvae) and even shrimp and fish (as I discovered later and published in the early 1970s).

Half saturation constants for different dinoflagellate species have extensive ranges, both among species and within species from different studies but an average that can be calculated from data shown in Bowie et al. 1985 indicates an average of 4.3 to more than 8 μM depending on which set of data is used. This is higher than the average summertime values found at any depth in most summers in Totten Inlet and again, this is the 50% growth limited point, not the initial threshold for uptake of nitrogen or growth limitation that would be even higher. Moreover, macroalgae, especially *Ulva* spp. which can be a nuisance species in Puget Sound and is common in Totten Inlet has even higher half saturation constants than all types of phytoplankton. It is also not novel to find that dissolved inorganic nitrogen levels in Totten Inlet never declined during the selected periods of study to less than 1 μM. For the 40 subareas of Puget Sound I examined for USEPA, none of them declined below this level either as I pointed out.

As I also pointed out in the USEPA report, it is meaningless to try to quantify nutrient sensitivity of subareas of Puget Sound based on average nitrogen concentrations. I said: "*there is uncertainty regarding the approximate concentrations of nitrogen (or other nutrients) that may limit the growth of locally dominant algal species when nutrients, not other factors, are growth limiting. At the present time, the arbitrary use of any one selected concentration of as a threshold of growth reduction could therefore be inaccurate and misleading*". Little has changed since this extensively peer-reviewed report was published in 1991 except that there has been several more definitive nutrient-addition experimental studies that included Totten Inlet that clearly demonstrate nutrient limitation, as discussed below.

In light of these findings, I cannot accept the conjecture that nitrogen is never limiting to Totten Inlet (or other SPS inlet) phytoplankton. Michaelis-Menten or Monod kinetics are underpinnings of phytoplankton ecology well tested and accepted for nearly a century and the observations in the subject report are not sufficient by any measure to reconsider them.

It is not solely my prior conclusions that indicate that nitrogen is the limiting nutrient to algal growth in the growing season for Totten Inlet, but that of the scientists of the University of Washington and the Washington Department of Ecology who repeatedly have studied the issue. Nutrient sensitivity of aquatic systems is best quantified by nutrient addition bioassays, such as the Department of Ecology previous performed showing a massive increase of carbon fixation (and therefore cell growth rate) compared to control bottles when nitrogen was added to discrete water samples from Totten Inlet in July (see Washington Dept of Ecology 2003b as cited in the NewFields Northwest report. Such increases are usually accepted as definitive of nitrogen limitation.

The author's conclusions not only conflict with Department of Ecology and other prior studies, but are not consistent with his own statements in the same unpublished report (e.g., page 41: "Based on conditions observed in Totten Inlet during this study, it is assumed that nitrogen is the nutrient limiting phytoplankton production").

It is curious that this conclusion of nitrogen limitation never occurring is stated, but then not applied to the conclusions of the report or utilized by other Taylor Shellfish consultants. If nutrients were not limiting to algal production at any time in Totten Inlet, there would be no potential problem with nitrogen discharge and resulting algal blooms. The water body would be similar to the Strait of Juan de Fuca where DIN concentrations and flux supply rates are always much above not only ½ saturation constants for algal uptake and growth, but even the threshold of any nitrogen limitation. This is not the case with Totten Inlet by any stretch of the imagination.

The author has not changed his position or opinion despite our critique of this untenable conclusion, so we can agree to disagree. I do not recommend further reply or rebuttal as it will apparently serve no purpose. My position on this matter does not diminish the importance of other aspects of this supplementary work, except for the remaining topic discussed below.

Appendix B. Is Totten Inlet as a net consumer or producer of dissolved inorganic nitrogen?

I provide more details here:

The author has made a fundamental mistake in study design and analysis. I reiterate that it is not logical or correct to use dissolved inorganic nitrogen (DIN) concentrations as a tracer or measure of nitrogen flux as the author has attempted, simply because it is only one highly dynamic component of the overall nitrogen cycling process. This is a fundamental of phytoplankton ecology but often overlooked. See our recent review of eutrophication in coastal waters of the United States that makes this same exact point while noting that flux is more difficult to measure, but essential to understanding nutrient dynamics[5]. Additionally, to make an estimate of the total nitrogen pool that is available for rapid flux to DIN, one can filter the sample to exclude fish and macroinvertebrates and obtain a representation of total nitrogen that is likely to flux rapidly back into the DIN pool.

DIN supply rates can vary tremendously with other factors, but the concentration may concurrently, at times, appear static as concentration gives no measure of flux rate. The particulate nitrogen pool includes seston that rapidly provides DIN through bacterial remineralization with turnover times similar to phytoplankton production. As seston is apparently far more important in terms of the total nitrogen and carbon pool in Totten Inlet than living phytoplankton, it is potentially very important in the flux of dissolved nitrogen to algae. Yes of course some may be refractile or labile but we know that at least some of it is labile and available for ingestions

[5] Anderson, D.M., J.M. Burkholder, W.P. Cochlan, P.M. Glibert, C.J. Gobler, C.A. Heil, R. Kudela, M.L. Parsons, J.E. Jack Rensel, D. W. Townsend, V.L. Trainer and G. A. Vargo. 2008. **Harmful algal blooms and eutrophication: Examples of linkages from selected coastal regions of the United States**. Harmful Algae, 8: 39–53.

and metabolism, otherwise the existing cultured shellfish in rafts and extensively on the shores of the inlet would not grow in winter.

The explanation for this supposed phenomenon is given on page 16 of the report where a non-existent Figure 7a is cited. (There is a figure 7, but it doesn't involve DIN, perhaps the intended figure was number 13). Figure 13 and the written argument involve evaluating the timing of ammonium and nitrite peaks as an indicator of when nitrogen remineralization is occurring. This is inexplicably linked to a measurement of DIN on ebb vs. flood tide ("The mean DIN on ebb tides (0.176 mg N/L) was significantly (p = 0.001) lower than the 0.185 mg N/L observed on flood tides indicating that over the period from August 24, 2005 until December 12, 2005…."). Then the next statement is that this shows that "… Totten Inlet was a net consumer of South Puget Sound dissolved nitrogen".

Other statements are made that because at times ammonium was greater than nitrate concentrations, that ammonium must have been sustaining phytoplankton in Totten Inlet. This is highly speculative because simply measuring the concentration does not characterize the flux rates of a nutrient. This is why tracing techniques have been developed, such as carbon 14 spiking methodologies, have been developed. But there are other legitimate methods.

Indeed, if chlorophyll *a* <u>and</u> total nitrogen was repeatedly and consistently measured well within North Totten Inlet and compared to non-refluxed water from outside the inlet to show significant differences, there would be a correlative case at least to suggest that waters of the inlet were somehow different than the outside waters. But as discussed on page 13 of the subject report, there were no significant differences of phytoplankton concentration as determined by chlorophyll *a* measurement of standing stock. Again, total nitrogen, as routinely sampled and analyzed by the Department of Ecology, was unfortunately not determined. Very small but apparently significant differences in orthophosphate and DIN were detected on flood versus ebb tides and this is of interest, but on its own, and given the limited sampling and confusion about use of data from days when only one tide stage was sampled (see Table 1) is not a compelling case to accept the conclusion.

There are obvious implications if we accept the statement that Totten Inlet is not nutrient limited, but I do not. Nor do the authors of the primary water column report, NewFields Northwest, who did not seek to include or discuss this possible finding.

In the author's view, determining if Totten Inlet is a net consumer or producer of (dissolved inorganic) nitrogen is important to address carrying capacity issues (i.e., will the proposed project unduly reduce phytoplankton production and threaten food resources for the food web). Although intellectually this is an interesting question, if we are discussing total nitrogen, I think answering the question precisely is not important relative to the issues at hand. There is more than adequate evidence that the proposed project will not over tax phytoplankton supplies for higher levels of the food web <u>and</u> the reduction of phytoplankton biomass and total nitrogen in the inlet associated with the project will help reduce the risks of eutrophication.