UNIVERSITY of WASHINGTON
CIVIL & ENVIRONMENTAL ENGINEERING

February 28, 2011

Brian Nickel
NPDES Permits Unit
U.S. EPA Region 10

Dear Mr. Nickel:

My reply to your memo dated Feb. 25, 2011, has two parts: 1) a response to your methodological suggestions, and 2) a response to your comments on our study design compared to the paper by Elser et al. (1990).

1. We appreciate your suggestions for determining algal toxicity in the effluents we tested. The EPA method you mentioned is probably a simpler experiment than the one we planned. If we obtain funding to do these follow-up experiments, it would be good to first determine how many would be sufficient? Would it be enough to run these experiments only once for each effluent type tested in our initial experiments?

We also agree that simply adding concentrated reagents to the Spokane River samples is a better method (than diluting with 50% synthetic media as we had suggested) for testing whether the apparent low %BAP for these samples is actually due to N or some other type of nutrient limitation.

We doubt that N or micronutrient limitation is the reason why our Spokane WWTP final effluent samples indicated very low %BAP. As you note, the nitrate content of these effluents is likely to be several mg L^{-1} (actually probably > 10 mg L^{-1}) and we will point out the micronutrient content of these effluents is also likely to be several orders of magnitude higher than natural levels. We could be wrong, but our prediction is that these outcomes were not due to toxicity or micronutrient limitation. Ultimately the only way to settle this is to conduct follow-up experiments, which we would be happy to carry out.

You note that the algae in bottle tests might be carbon (*i.e.,* CO_2) limited. This statement could be true for closed bottle experiments such as those described by Elser et al. (1990), but it is very unlikely for the open and continuously shaken bottles we used for our experiments.

At the end of your comments you state: *"The fact that N and P chemistry constantly changes in the environment is the reason EPA recommends nutrient water quality criteria and monitoring be based on total P and total N (EPA 2000a, 2000b)."* If this is actually the official position of EPA I believe it is simply wrong! Equating different types of phosphorus solely on the basis of TP could result in huge mistakes as regards receiving waterbody eutrophication risk. For example, it is well known that the %BAP of typical secondary effluents is in the 80-90% range, whereas the particulate P that dominates the peak flows of most natural and disturbed streams has %BAP in the 20-40% range (Reynolds and Davies 2001, Li and Brett 2011). Equating the TP in for example the secondary effluents for the Spokane WWTP with the sediment laden flows of Hangman Creek would be a very problematic decision.

2. I did a six-year PostDoc in Charles Goldman's lab at UC Davis, and I have coauthored 18 papers with Jim and Goldman and know their paper well. First it should be noted that

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their paper was not about BAP, it was about N versus P limitation. As far as we are aware the EPA (or Standard Methods) has not developed a protocol for determining BAP using natural phytoplankton assemblages. We also note that it is ironic that an EPA scientist seems to be suggesting we should have forgone an experimental protocol developed by EPA (see Miller et al. 1978) in favor of one that has not been vetted by EPA.

You are correct that Elser et al. (1990) criticized the use of algal monocultures for determining which macronutrient controls phytoplankton growth, but you did not also note that the short-term bioassay experiments described by Elser et al. have also been criticized by many well known limnologists as providing meaningless or worse misleading results (e.g., see Schindler et al. 2008). For example, Schindler et al. (2008) contend P is the ultimate constraint on phytoplankton biomass even if short-term bottle experiments suggest N is limiting. Schindler and many other prominent limnologists (see also Carpenter 2008) have argued the only true test of nutrient utilization is whole lake experiments. This shows that every experimental protocol has it critics, as well as limitations (and advantages). [It also shows limnologists like to debate each other]. For example, the classic criticism of whole lake experiments is that they are practically impossible to replicate, so one cannot know whether the outcome is site specific. This is important because it is quite likely the geologic composition of lake watersheds will also influence whether they are N or P limited.

In the Long Lake context we see value in conducting in situ bioassay experiments, and would gladly carry out these experiments. But to be clear, these field experiments would provide different information than the lab experiments we have already carried out. For example, it would be very difficult determine the %BAP in these field experiments in anything but a very course way. However, in a qualitative sense these experiments could be very interesting. For example, if we added 10% effluent to in situ bottle experiments and did not find a significant increase in algal biomass, this would provide strong confirmation that the low %BAP results we obtained in our laboratory experiments was valid.

However, in situations like the present, no single experiment (or series of experiments) can *prove* anything. Every experimental approach that one could imagine has limitations (and critics). Ultimately, the scientists, managers and stakeholders concerned with the ecological health of Long Lake will have to use a **Weight of Evidence** approach to determine whether sufficient information is available to predict how decisions taken in the present will affect the limnological state of this system in the future. For example, as we noted in our Dec. 16, 2010, project presentation, once the dischargers to the Spokane River get to the Limit of Technology (LOT), there will be a dramatic reduction in the amounts and the bioavailability of P discharged from these sources. I presented simple mass balance calculations that showed that if you dilute a cumulative effluent discharge with a flow of 100 cfs and 500 $\mu g\ L^{-1}$ TP into the Spokane River it would more than double the TP concentration in this river during the critical low flow months of July to October. But once the dischargers get down to an average effluent concentration of 50 μg TP L^{-1}, the aggregate discharges will only increase the TP concentration in the Spokane River by about 2 μg TP L^{-1} and these effluents will have much lower %BAP than is currently the case. The post-LOT scenario (*i.e.,* effluents with much lower TP and %BAP) is precisely the type of whole lake experiment advocated by

Schindler, Carpenter and many other limnologists. Based on First Principles and our laboratory experiments, we predict that post-LOT conditions should result in a marked reduction in the spatial and temporal extent of hypoxic conditions in Long Lake. The best test by any measure is going to be the response of Long Lake itself to future conditions. That whole lake "experiment" will be the gold standard of gold standards, and the before and after Dissolved Oxygen profiles from Long Lake will provide the ultimate answer to this discussion.

If funding becomes available, we would gladly conduct in situ bioassay experiments with effluent additions to Long Lake using a design similar to that of Elser et al. (1990). We would also gladly run new experiments to determine if toxicity or limitation by nutrients other than P might have influenced any of our outcomes. If this were to happen it would be important to have broad-based buyoff on the details of the experimental protocol. It would also be a very good idea if the managers and stakeholders for the Spokane River discussed ahead of time how the outcomes of these experiments might influence their decisions. If new data are not going to change anybody's position, then they will not be useful.

Sincerely,

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