Comments on Section 3 of the PPL Phase IV Report Following the Fly-Ash Spill at PPL Martins Creek LLC

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General Comments

1. Conclusions of Section 3 of the Phase IV report are basically sound, though some of the spatial differences are downplayed. We agree that the results, taken as a whole, provide no compelling evidence of a long-term impact of the fly-ash spill on biological assemblages in the Delaware River.

2. There seem to be trends in water quality (conductance) and some aspects of macroinvertebrate and algal assemblage structure along the study gradient. The authors are correct that these do not neatly implicate the spill (some changes occur downstream of the first downstream station, while others, like macroinvertebrate density, are seen mainly at that station). These trends could be due to other aspects of the facility operation, though the authors do not discuss this point.

3. Several of the statistical analyses are conservative. For example, multiple samples within stations are pseudo-replicates, so use of these as the basis for calculation of errors increases the chance of finding significant differences. Also, as the authors note, they often did not adjust for multiple comparisons to achieve the correct experiment-wise error in individual upstream-downstream comparisons. In these cases, the resulting bias would be toward finding more differences between stations rather than fewer, and for this reason the analyses are conservative.

Benthic Macroinvertebrate Community Assessment

1. On the whole, the benthic macroinvertebrate study was well done. The only result indicating a possible impact downriver from the fly-ash spill was observed in the chironomid community. It is not unusual for metrics to point in different directions, and as the authors note, the results taken as a whole do not indicate impairment of the benthic macroinvertebrate community by the fly-ash spill.

2. Portions of Appendices D-1 to D-7 require taxonomic updates and spelling corrections, as follows:

- Order Haplotaxida instead of Tubificida Enchytraeidae
- Order Haplotaxida instead of Tubificida Naididae
- Order Haplotaxida instead of suborder Lumbricina Lumbricidae
- Order Arhynchobdellida instead of Arynchobdellida Erpobdellidae
- *Prostoma graecense* instead of *Prostoma graescense*
- Family Planorbidae instead of Planorbiidae
- *Micromenetus dilatatus* instead of *Menetus dilitatus* (see AFS Mollusks, 2nd Ed)
- Order Neotaenioglossa instead of Mesogastropoda Hydrobiidae

- *Amnicola limosus* instead of *Amnicola limosa*
- Order Unionida instead of Unionidaa
- Order Acariformes instead of Hydrachnidia

Fish Community Assessment

1. The fish assemblage assessment was well done, and we concur that there is no compelling evidence of a fly-ash impact on the fishery.

Mussel Community Assessment

1. Replace “Eastern elliptio” with “eastern elliptio” (see AFS Common and Scientific Names of Mollusks, 2nd ed.).

2. Station Locations, Field Methods, last paragraph: Did the four- and five-person mussel survey teams consist of three divers and one person recording, measuring, and identifying from a boat, and four mask-and-snorkel collectors with one person recording, measuring, and identifying in the shallower waters (or some combination thereof)? If so, this should be explained.

3. Station Locations, Qualitative Survey, paragraph 2: It appears that the largest recorded mussel individuals came from the subset used for tissue analysis. If so, this should be stated. The word “immediately” should be removed from the last sentence; determining the largest and smallest individuals with so many people in the water, especially in the deeper pools, was probably accomplished after removing and measuring a subset that does not get immediately returned to the substrate.

4. Results, Comparison to Other Delaware River Stations, paragraph 3. In the sentence “Furthermore, Dr. Lellis’ data also suggest that any of the uncommon species...”, change “any” to “most” and add “rare to” before “uncommon species” to read “Furthermore, Dr. Lellis’ data also suggest that most of the rare to uncommon species...” Our recollection of the Lellis (2002) study is that the single specimen of dwarf wedgemussel was taken downstream from the mouth of a tributary stream that supports a population of dwarf wedgemussels, and the tributary stream may have been the source of the single mussel. This would make the presence of the dwarf wedgemussel in the Martins Creek study area very unlikely.

5. Summary, bullet 1: The two pools in the reference and downstream areas support a similar species *composition* (although based on only two species) but not *numbers* (825 for Reference and 325 for Downstream; q.v., mean number of search minutes per mussel, bullet 4 below). The less abundant populations downriver indicate a possible impact.

6. Summary, bullet 2: It was noted that the relative proportion of one common and one uncommon mussel species in one of the downstream pools (97.1 and 2.5 %) and one of the reference pools (97.4 and 2.6 %) is similar to the percentages in Lellis (2002) (97.7 % and 2.0 %). The relevance of comparing two of eight stations with the complete Lellis (2002) study is unclear. Having said that, the percentages of the two most abundant species in the Lellis study is similar to most of the comparisons in the Martins Creek survey, as follows: reference stations

(97.7 % and 2.3 % for the eastern elliptio and alewife floater, respectively), downstream stations (98.2 % but only 1.6 % for the alewife floater), and for the complete survey (97.8 % and 2.0 %, the more accurate comparison and almost the same as the complete Lellis study noted above). These numbers simply reflect that the river is dominated by a single species.

7. Summary, bullet 3: The eastern elliptio was the only species in the contiguous (riffle/run) habitats. This is probably a reflection of the preferred habitats of the thin shelled alewife floater and thicker shelled eastern elliptio, the dominant nature of the eastern elliptio, and the spotty distributions of mussels. Similar numbers were counted at three of the four, not all four of the sites.

8. Summary, bullet 4: The mean number of search minutes it takes to collect a mussel in the pool areas is approximately three times greater in the downstream sites (2.6, mean number of search minutes per mussel) than in the reference pools (0.9), roughly two times greater in the contiguous areas of the downstream stations (16.3) versus the reference region (9.2), and nearly three times greater in the downstream (combined pool and contiguous regions, 4.7) versus upstream reference areas (1.6). A less abundant mussel population downriver indicates a possible impact.

9. Summary, bullet 5: Interpretations of the quantitative data are limited by the fact that only 15 samples were collected (10 in the reference area and only 5 in the downstream area). Although based on a small number of samples, a larger percentage of juvenile mussels (23.7%) was found in the reference area than downstream (9.1%). A lower percentage of juveniles in the downriver region indicates a possible impact downstream.

10. Summary, bullet 6: Finding one rare species (triangle floater) at one of the downstream sites, although important, is not relevant to the issue of whether there is an impact on the mussel community downstream.

11. Summary, bullet 7: The Martins Creek and Lellis (2002) studies are not similar in species composition (eight versus three species—four if you include one species from PF&BC records). Similarities occur in the dominance of the eastern elliptio (97.7 % in the Lellis study and 97.8 % in the Martins Creek investigation), with 2.0 % and 2.1 % of the population consisting of the alewife floater in the Lellis and Martins Creek studies, respectively. The remaining six taxa in the Lellis study, based on 123,255 specimens, constitutes an important but minuscule 0.3%. We recommend adding the words "most of" to the last sentence, as follows: "This suggests that most of these species are likely to be present in similar relative numbers..."

12. The mussel study consisted primarily of both diver and shallow-water hand collections that resulted in the collection of 1,293 specimens, with evident juvenile recruitment in the eastern elliptio. The percentages of eastern elliptio and alewife floater, as well as rare species (triangle floater and yellow lampmussel, PF&BC record), in the Martins Creek area, along with the possibility of at least several more rare species occurring in the study area, indicate that the character of the mussel fauna in the upriver regions studied by Lellis (2001, 2002) extends downstream to at least the Martins Creek survey region. Unlike the benthic macroinvertebrate study, the results of the mussel community assessment are inconclusive, with results that may indicate either an impact from the fly-ash spill or merely natural variability in the mussel fauna.

Fish and Mussel Tissue Sampling and Analyses

1. The metals are probably below thresholds for ecological damage, but there does seem to be a difference between upstream and downstream stations, particularly the first downstream station. Tables 3-28 and 3-29 show a fair number of differences where downstream is greater than upstream, particularly for mussels and for metals that were commoner in the fly ash. The summary mentions this possibility of differences at the first downstream station, but it is not given much attention.

2. The detection limit for antimony is stated as 0.05, but values of 0.03 are given for mussels. It seems that antimony, thallium, and vanadium were uniformly below detection, so they are uninformative. They should be removed from Table 3-27 (the values can be kept in Table 3-28) or placed below the others. Leaving them in gives the impression that there is no evidence of difference between up and down, when really there is insufficient information to test for a difference. Visually, this means that the table has more white, giving the impression of fewer effects. Another option would be to use an intermediate gray for metals which are below detection in both upstream and downstream samples for any of the species. This would help with arsenic, which was not detected in the fish, but was in the mussels (and was higher downstream).

3. The authors single out barium and mercury as being higher in the fly ash than in sediments. However, arsenic seems even more concentrated in the fly ash (ratio of 45.1, while barium is 15.0 and mercury is 15.8). Arsenic was significantly higher downstream in the one comparison (mussels) that could be done.

4. There does not appear to be any mention of chronic effects of mercury due to bioaccumulation. It does not seem likely that the spill contributed to mercury bioaccumulation, but it is another mode of effect.

5. The authors complain about using mussels for the metal analyses (Fish and Mussel Tissue Sampling and Analyses, paragraph 3). This statement seems to downplay the importance of the mussel data. Whatever their usefulness for direct calculation of damages, they are probably one of the best groups for establishing that metals did or did not enter food webs as a result of the spill. It should be recognized that there may be different elements of the study design for the two goals of comparing upstream with downstream and estimating food web effects.

6. The inclusion of two species of darters (in different proportions in different samples and stations) adds another possible source of variance in the station comparisons. For comparing food chain effects, it may be reasonable to sample a range of taxa based on availability, while more uniform samples would be best for upstream-downstream comparisons. For some stations (e.g., 199.3-R), there were as many shield darter in the samples as in other samples in which only shield darters were used. If the shield darters were smaller for the stations where both species were used, this further complicates comparisons.

7. Were the suckers that were analyzed too large to serve as prey for osprey? If not, osprey are another potential consumer.

8. Chart 18 is labeled “sucker length vs mercury concentration”. However, it is really sucker length and mercury concentration at each station. Graphs of mercury concentration versus length

(with different symbols for different stations) would be informative. It seems likely that this would explain much of the variability among samples. The mercury-length relationship could be factored out using ANCOVA. The design is not well-balanced, so length and station effects would always be conflated. However, if length is used as a covariate and there is no subsequent significant station difference, that would be a strong argument against a true station difference.

9. Although a review is clearly beyond the scope of work of the study, some discussion of differences in modes of uptake between the biota analyzed (fish versus mussels) and different metals is warranted.

10. The correlation structure among metal concentrations within samples would be informative; e.g., using principal components analysis. Separate analyses upstream and downstream of the spill site might be suggestive. For example, in upstream sites, one would not expect mercury to be correlated with the other metals. Downstream, it would be correlated differently (with stronger correlations with several of the metals) if the spill was a significant source, but otherwise would show the same structure as upstream.

11. Results and Discussion, paragraph 6: The authors note that statistically significant results occur when downstream concentrations are negligibly different. They then say that this might be an artifact. Small but significant differences are not necessarily artifacts; they may simply show that a good job was done of removing various sources of variation, so that a small difference could be detected. They then mention the artifact of a few "hits" among non-detects creating significant differences. This is a valid issue, and the authors might want to flag some of these cases in the tables.

12. Results and Discussion, paragraph 8: "chaotic patterns" are mentioned. "Chaotic" is not a good word to use here, since it carries a variety of meanings, some of which are technical, very specific, and not appropriate to this study.

13. Replace "Eastern elliptio" with "eastern elliptio" (see AFS Common and Scientific Names of Mollusks, 2nd ed.).

14. Fish and Mussel Tissue Sampling and Analyses, paragraph 2: "To address this issue, an assessment of metals concentrations in fish and shellfish was conducted to determine if metals were elevated in fish or mussels...": Use "mussels" rather than "shellfish", as "shellfish" is a general term that can refer to molluscs and/or crustaceans. Also, delete the repetitive "in fish or mussels".

15. Fish and Mussel Tissue Sampling and Analyses, Field Methods, paragraph 2: The eastern elliptio is widespread (in Atlantic drainages from Canada to Georgia, etc.) but not cosmopolitan.

16. Fish and Mussel Tissue Sampling and Analyses, Estimation of Risk Based Concentrations (RBCs), paragraph 3: "*Lontra canadensis"* instead of "*Lontra Canadensis"*. Scientific names are used for the bald eagle and river otter but not for other species (e.g., muskrat, raccoon). We suggest a consistent use of scientific names, or else omit them for species with readily recognizable common names.

Periphyton Community Assessment

1. The stated objective of the periphyton community assessment is to "determine whether there was any impairment to the periphyton community resulting from the release." It is impossible to meet this objective as stated, both because of difficulties in proving that detected impairment (if any) was caused by the release and because there may have been impairment that was not detected due to limitations of the study design and how the data were analyzed and interpreted. The best that can be done is to look for evidence of impairment, primarily by comparing conditions upstream and downstream from the fly-ash spill. It would be useful to include in the introduction a verbal conceptual model or set of hypotheses regarding some of the effects that might be expected as a result of the fly-ash spill (i.e., how would we recognize impairment?). Those effects that could be detected by the study design, and how data are to be interpreted to do so, should be specified. It would also be useful to characterize the magnitude of effects the data analysis is designed to detect. Given the relatively long period from the time of the spill to the time samples were taken (roughly one year), it is reasonable to assume that substantial recovery has taken place and that most effects to be assessed are long-term, persistent changes and more moderate, subtle effects.

2. The word "diatoms" (with an "s") often appears where it should not. For example, "diatom analysis" is correct, not "diatoms analysis".

3. Field and Laboratory Methods, paragraph 4: Though the soft-algae analysis should be considered semi-quantitative, the diatom count analyses were quantitative. The diatom results were not presented on a number per area sampled basis, but since counts of each species were made, the results are quantitative.

4. Field and Laboratory Methods, last sentence: "Relative biovolume of each soft algae species was estimated without criteria." Clarify the meaning of "estimated without criteria".

5. Data Analysis, paragraph 1: It is not clear what is meant by a "replicate". Does it mean an algal count? This should be clarified. Also, a reference should be given for the CD. How can a copy be obtained? Raw data should be available for the reader to examine.

6. Data Analysis, Paragraph 2: What does "small sample" mean?

7. Data Analysis, paragraph 3: "It is generally accepted that greater numbers of taxa (or increased species richness) indicate higher biotic integrity." This is not true for periphyton, where the most commonly accepted view is that species diversity increases following moderate disturbance (Intermediate Disturbance Hypothesis). Thus, one might expect species diversity to increase downstream following the fly-ash spill. It is not necessarily true that greater diversity values "indicate higher biotic integrity", and such values therefore should not be interpreted to mean no effect of fly ash.

8. Results, Chlorophyll *a* and Ash-Free Dry Mass: In addition to results for chlorophyll *a* and ash-free dry mass, it would be useful to see results for ash weight, as this might provide a measure of the amount of residual fly ash on the rocks. We also recommend that the chlorophyll *a* and ash free dry mass values presented in Table 39 be re-checked. Most of the values differ markedly from values presented in DRBC (2007) for this region of the Delaware River. This fact

is especially noticeable when one computes the Autotrophic Index (AI) for each site, which is simply ash free dry mass divided by chlorophyll *a* (expressed in the same measurement units). For the site closest to the study region, the value of AI in the DRBC report is 319 (dimensionless). In contrast, values of AI computed from chlorophyll *a* and ash free dry mass values presented in Table 39 (after converting to common units) range from 3204 to 11,548.

9. Results, Diatoms: Does the first sentence and paragraph mean "Only 8 species occurred with an abundance of 5% or more"? If so, this should be clarified. While it is worthwhile to compare relative proportions of dominant species in the upstream samples with those in the downstream samples, the fact that there is relative similarity is not strong evidence of no impact of fly ash. More weight should be given to the less common species, and to the communities as a whole. The quality of the underlying data would support a more sophisticated and reliable comparison than is presented by the authors. In particular, the composition of the entire assemblages from upstream and downstream samples should be compared statistically.

10. Results, Diatoms, paragraph 7: *Achnanthes minutissima* (= *Achnanthidium minutissimum*) is the most common diatom in stream and river diatom assemblages throughout the country. Thus, the fact that it is present in large proportions both upstream and downstream cannot be used to support a conclusion of no effect of the fly-ash spill.

11. Results, Soft Algae, Table 42 and paragraph 2: The aquatic macrophyte *Podostemum ceratophyllum* was found at several locations at upstream reference sites, and made up 31% of the biovolume. However, it was not found in any of the samples from the downstream sites. Might this be an effect of the fly ash (or other aspects of the facility operation)? This possibility should at least be considered, especially since this taxon can be important habitat for benthic invertebrates. Just a quick web search found the following:

> "Comments: *Podostemum* could easily be the poster child of Piedmont rivers (B. Adams, pers. comm.); it is unlikely that many aquatic plant species in the Piedmont have been hit harder. **It is very sensitive to sedimentation** and, accordingly, has declined greatly throughout its range and has been lost from nearly all areas it once occupied in some drainages such as the Upper Neuse Basin of North Carolina (Adams pers. comm.)." (http://www.dnr.state.md.us/wildlife/Md_Veg_Com/123_.asp, emphasis added)

12. Summary: The logic of the interpretations of periphyton results is flawed. Results are selectively and, in some cases, incorrectly interpreted. We see the following problems in the summary bullets.

Bullet 2: Similarity in abundance of the most abundant species is not a reliable measure of disturbance effect. It is flat-out incorrect to say that higher taxa numbers and diversity indicate higher biotic integrity. More likely, this is an indication of moderate impact or of differences in habitat diversity.

Bullet 5: The Siltation Index (SI) values are shown to be higher downstream, as logically would be expected as a consequence of the fly-ash spill. As far as we are aware, there is no evidence that the main areas of continued exposure to fly ash were still located immediately downstream of the spill site at the time of sampling, roughly one year after the spill. If other sections of the Phase IV report provide such evidence, then it should be specifically referred to here. Otherwise, it is questionable whether the results should be interpreted as indicating there is no effect on periphyton because the SI values immediately downstream are not as high as those further downstream. It could well be that the main areas of fly-ash accumulation have migrated downstream as particles are repeatedly entrained into the water column, transported some distance, and redeposited (like other types of particulate matter in rivers, such as fine sediment and seston).

References

Delaware River Basin Commission (DRBC). 2007. *Pilot Study: Implementation of a Periphyton Monitoring Network for the Non-Tidal Delaware River.* Delaware River Biomonitoring Program, Delaware River Basin Commission, West Trenton, NJ.

Normandeau Associates Responses to
Academy of Natural Sciences of Philadelphia Comments
on Section 5 of the PPL Phase IV Report on Martins Creek Ash Release

23 May 2007

General Comments

1. Conclusions of Section 5 of the Phase IV report are basically sound, though some of the spatial differences are downplayed. We agree that the results, taken as a whole, provide no compelling evidence of a long-term impact of the fly-ash spill on biological assemblages in the Delaware River.

Response: It is agreed that the results, taken as a whole, provide no compelling evidence of a long-term impact of the fly ash release spill on biological assemblages in the Delaware River.

2. There seem to be trends in water quality (conductance) and some aspects of macroinvertebrate and algal assemblage structure along the study gradient. The authors are correct that these do not neatly implicate the spill (some changes occur downstream of the first downstream station, while others, like macroinvertebrate density, are seen mainly at that station). These trends could be due to other aspects of the facility operation, though the authors do not discuss this point.

Response: The gradient of environmental conditions along the study reach is an important consideration when evaluating the biological data from the study. It is acknowledged that the biological data likely reflect these changes in environmental conditions, and do not implicate the fly ash release. A detailed assessment of the possible effects of other factors was outside the scope of this evaluation. However, in response to the comment, discussions regarding the possible gradient of environmental conditions have been added to the report.

3. Several of the statistical analyses are conservative. For example, multiple samples within stations are pseudo-replicates, so use of these as the basis for calculation of errors increases the chance of finding significant differences. Also, as the authors note, they often did not adjust for multiple comparisons to achieve the correct experiment-wise error in individual upstream-downstream comparisons. In these cases, the resulting bias would be toward finding more differences between stations rather than fewer, and for this reason the analyses are conservative.

Response: Noted. The Phase IV work plan, which was reviewed and approved by the Natural Resource Damage Assessment Team members and reviewed by the Academy and the DRBC, was designed to maximize the ability to identify possible ash-related impairment.

Benthic Macroinvertebrate Community Assessment

1. On the whole, the benthic macroinvertebrate study was well done. The only result indicating a possible impact downriver from the fly-ash spill was observed in the chironomid community. It is not unusual for metrics to point in different directions, and as the authors note, the results taken as a whole do not indicate impairment of the benthic macroinvertebrate community by the fly-ash spill.

Response: Noted. The benthic macroinvertbrate community assessment was conducted using standard biological methods, and was performed in accordance with the Work Plan. It is agreed that the study results as a whole do not indicate impairment of the benthic macroinvertebrate community by the fly ash release. No changes were made to the text..

2. Portions of Appendices D-1 to D-7 require taxonomic updates and spelling corrections, as follows:

- Order Haplotaxida instead of Tubificida Enchytraeidae
- Order Haplotaxida instead of Tubificida Naididae
- Order Haplotaxida instead of suborder Lumbricina Lumbricidae
- Order Arhynchobdellida instead of Arynchobdellida Erpobdellidae
- *Prostoma graecense* instead of *Prostoma graescense*
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- Order Neotaenioglossa instead of Mesogastropoda Hydrobiidae
- *Amnicola limosus* instead of *Amnicola limosa*
- Order Unionida instead of Unionidaa
- Order Acariformes instead of Hydrachnidia

Response: As requested, revisions were made to Appendix Tables D-1 through D-7, as noted, in most cases. The exceptions are those instances such as “Order Haplotaxida instead of Tubificida Enchytraeidae” where the original order name (Tubificida) was replaced with the suggested substitute (Haplotaxida), but the family name (Enchytraeidae) was left in place so that the hierarchy of order/family/genus names remained consistent with the rest of the data.

Fish Community Assessment

1. The fish assemblage assessment was well done, and we concur that there is no compelling evidence of a fly-ash impact on the fishery.

Response: Noted. The fish community survey was conducted using standard biological methods, and was performed in accordance with the Work Plan. It is agreed that the study results do not indicate fly ash-related impairment of the fish community. No changes were made to the text.

Mussel Community Assessment

1. Replace "Eastern elliptio" with "eastern elliptio" (see AFS Common and Scientific Names of Mollusks, 2nd ed.).

Response: As requested, the changes noted above were made to the text.

2. Station Locations, Field Methods, last paragraph: Did the four-and five-person mussel survey teams consist of three divers and one person recording, measuring, and identifying from a boat, and four mask-and-snorkel collectors with one person recording, measuring, and identifying in the shallower waters (or some combination thereof)? If so, this should be explained.

Response: As requested, additional explanation was added to the text.

3. Station Locations, Qualitative Survey, paragraph 2: It appears that the largest recorded mussel individuals came from the subset used for tissue analysis. If so, this should be stated. The word "immediately" should be removed from the last sentence; determining the largest and smallest individuals with so many people in the water, especially in the deeper pools, was probably accomplished after removing and measuring a subset that does not get immediately returned to the substrate.

Response: As requested, the changes noted above were made to the text.

4. Results, Comparison to Other Delaware River Stations, paragraph 3. In the sentence "Furthermore, Dr. Lellis' data also suggest that any of the uncommon species...", change "any" to "most" and add "rare to" before "uncommon species" to read "Furthermore, Dr. Lellis' data also suggest that most of the rare to uncommon species..." Our recollection of the Lellis (2002) study is that the single specimen of dwarf wedgemussel was taken downstream from the mouth of a tributary stream that supports a population of dwarf wedgemussels, and the tributary stream may have been the source of the single mussel. This would make the presence of the dwarf wedgemussel in the Martins Creek study area very unlikely.

Response: As requested, the changes noted above were made to the text.

5. Summary, bullet 1: The two pools in the reference and downstream areas support a similar species *composition* (although based on only two species) but not *numbers* (825 for Reference and 325 for Downstream; q.v., mean number of search minutes per mussel, bullet 4 below). The less abundant populations downriver indicate a possible impact.

Response: The use of mean values (presented in the comment above) precludes the consideration of natural variability in mussel numbers in upstream areas, and is a poor

method for evaluating potential differences. Alternately, the data indicate that the numbers of mussels observed in the downstream pools (85 and 240 mussels) were within the range of mussels observed in the upstream pools (13 to 812 mussels). This information has been added to the report, and contrary to the last sentence above, does not suggest a possible impact to the mussel community.

6. Summary, bullet 2: It was noted that the relative proportion of one common and one uncommon mussel species in one of the downstream pools (97.1 and 2.5 %) and one of the reference pools (97.4 and 2.6 %) is similar to the percentages in Lellis (2002) (97.7 % and 2.0 %). The relevance of comparing two of eight stations with the complete Lellis (2002) study is unclear. Having said that, the percentages of the two most abundant species in the Lellis study is similar to most of the comparisons in the Martins Creek survey, as follows: reference stations (97.7 % and 2.3 % for the eastern elliptio and alewife floater, respectively), downstream stations (98.2 % but only 1.6 % for the alewife floater), and for the complete survey (97.8 % and 2.0 %, the more accurate comparison and almost the same as the complete Lellis study noted above). These numbers simply reflect that the river is dominated by a single species.

Response: Comparing the proportion of the mussel species in the upstream with the downstream pools, and in the pools and contiguous habitats with the Lellis data establishes the similarity among these data. Certainly this observation indicates that the Delaware River at these locations is dominated by one species upstream and downstream of the ash release. No changes were made to the text.

7. Summary, bullet 3: The eastern elliptio was the only species in the contiguous (riffle/run) habitats. This is probably a reflection of the preferred habitats of the thin shelled alewife floater and thicker shelled eastern elliptio, the dominant nature of the eastern elliptio, and the spotty distributions of mussels. Similar numbers were counted at three of the four, not all four of the sites.

Response: It is very likely that observation of only one species in the contiguous habitats is a reflection of habitat preference. However, we do not agree that similar numbers of eastern elliptio were observed at only three, not all four, of the stations. The differences in numbers among the four stations are not great, nor are the differences among the catch-per-unit-effort data. No changes were made to the text.

8. Summary, bullet 4: The mean number of search minutes it takes to collect a mussel in the pool areas is approximately three times greater in the downstream sites (2.6, mean number of search minutes per mussel) than in the reference pools (0.9), roughly two times greater in the contiguous areas of the downstream stations (16.3) versus the reference region (9.2), and nearly three times greater in the downstream (combined pool and contiguous regions, 4.7) versus upstream reference areas (1.6). A less abundant mussel population downriver indicates a possible impact.

Response: There are upstream/downstream differences in the catch-per-unit-effort data when means are computed. However, it is important to note the great variability observed

between the two reference pool stations (0.4 and 28.3 search minutes per mussel) and that the downstream pool data are within this range, in fact, much closer to the low end (2.1 and 4.0 search minutes per mussel). Although in the other contiguous habitats the downstream data (15.0 and 17.0 search minutes per mussel) are not in the range observed upstream (7.5 and 10.9 search minutes per mussel), the differences are not great. Computing means by combining the pool and the contiguous habitats data skews the results because of the influence of Reference Pool Station 195.1-P where a great number of mussels (812) were observed.

The mussel population downstream may appear to be less abundant when means are computed. However, the downstream numbers and catch-per-unit-effort data are within, or close to, the range observed upstream, a measure of the variability in the Delaware River. These data do not suggest impact. No changes were made to the text.

9. Summary, bullet 5: Interpretations of the quantitative data are limited by the fact that only 15 samples were collected (10 in the reference area and only 5 in the downstream area). Although based on a small number of samples, a larger percentage of juvenile mussels (23.7%) was found in the reference area than downstream (9.1%). A lower percentage of juveniles in the downriver region indicates a possible impact downstream.

Response: Although it is noted that a larger number and percentage of juvenile mussels was observed in the upstream (or reference) samples, when conducting statistical testing (Mann-Whitney Test), it was found that the differences are not statistically significant between the upstream and downstream samples. This does not suggest an impact downstream. The text and Table 5-23 have been changed to indicate this absence of a statistically significant difference.

10. Summary, bullet 6: Finding one rare species (triangle floater) at one of the downstream sites, although important, is not relevant to the issue of whether there is an impact on the mussel community downstream.

Response: The observation of one triangle floater is relevant to the effort because it contributes to the similarity of this station's mussel data to that of Lellis (2002). Secondly, the presence of this uncommon species may be of importance to the regulatory agencies. No changes were made to the text.

11. Summary, bullet 7: The Martins Creek and Lellis (2002) studies are not similar in species composition (eight versus three species—four if you include one species from PF&BC records). Similarities occur in the dominance of the eastern elliptio (97.7 % in the Lellis study and 97.8 % in the Martins Creek investigation), with 2.0 % and 2.1 % of the population consisting of the alewife floater in the Lellis and Martins Creek studies, respectively. The remaining six taxa in the Lellis study, based on 123,255 specimens, constitutes an important but minuscule 0.3%. We recommend adding the words "most of" to the last sentence, as follows: "This suggests that most of these species are likely to be present in similar relative numbers..."

Response: Species compositions are more than a checklist of species present, and also include relative proportion of the species. However, definitions of this term may differ among biologists. Also, as requested, the words "most of" and "relative" have been added to the text.

12. The mussel study consisted primarily of both diver and shallow-water hand collections that resulted in the collection of 1,293 specimens, with evident juvenile recruitment in the eastern elliptio. The percentages of eastern elliptio and alewife floater, as well as rare species (triangle floater and yellow lampmussel, PF&BC record), in the Martins Creek area, along with the possibility of at least several more rare species occurring in the study area, indicate that the character of the mussel fauna in the upriver regions studied by Lellis (2001, 2002) extends downstream to at least the Martins Creek survey region. Unlike the benthic macroinvertebrate study, the results of the mussel community assessment are inconclusive, with results that may indicate either an impact from the fly-ash spill or merely natural variability in the mussel fauna.

Response: The data indicate that the mussel communities at downstream stations are within, or very close to, the natural variability observed upstream. This finding is not evidence of impact. No changes were made to the text.

Fish and Mussel Tissue Sampling and Analyses

1. The metals are probably below thresholds for ecological damage, but there does seem to be a difference between upstream and downstream stations, particularly the first downstream station. Tables 5-28 and 5-29 show a fair number of differences where downstream is greater than upstream, particularly for mussels and for metals that were commoner in the fly ash. The summary mentions this possibility of differences at the first downstream station, but it is not given much attention.

Response: Differences in metals levels in tissues were noted but found to be inconsistent across metals and species, leading to the conclusion that the differences did not appear ash-related. In addition, the levels found were evaluated to see if exceedences of any relevant environmental standards occurred. As noted in the comment above, tissue levels were well below problematic levels even under moderately conservative assumptions regarding diet, home range, bioavailability, etc. With these findings, further evaluation of the spatial differences in metals concentrations in tissues was considered unnecessary.

2. The detection limit for antimony is stated as 0.05, but values of 0.03 are given for mussels. It seems that antimony, thallium, and vanadium were uniformly below detection, so they are uninformative. They should be removed from Table 5-27 (the values can be kept in Table 5-28) or placed below the others. Leaving them in gives the impression that there is no evidence of difference between up and down, when really there is insufficient information to test for a difference. Visually, this means that the table has more white, giving the impression of fewer effects. Another option would be to use an intermediate gray for metals which are below detection in both upstream and downstream samples for any of the species. This would help

with arsenic, which was not detected in the fish, but was in the mussels (and was higher downstream).

Response: The detection limit varied from sample to sample, and the table was changed to reflect variability in detection levels. As for denoting the tissue samples that were mostly non-detect, this information was already in the tables (those tissue types were italicized). The comment demonstrates, however, that our approach to denote these samples was not sufficiently clear. Alternate typeface or notation has been provided in the revised tables.

As suggested in the comment, intermediate shading for the non-detects was tried, but it made the tables too difficult to read. Moreover, shading for non-detects is not necessary. As explained in the text accompanying these Tables, the shading in Tables 5-27 through 5-30 is intended to compare tissue concentrations that, nominally, were higher upstream than downstream compared to those that were nominally vice-versa. Thus, the appropriate comparisons were gray squares to black. (Different colors were originally used, but were changed to shades to accommodate potential copying.) Nothing was intended for those cells that were the same upstream and downstream, nor were these cells mentioned or discussed.

3. The authors single out barium and mercury as being higher in the fly ash than in sediments. However, arsenic seems even more concentrated in the fly ash (ratio of 45.1, while barium is 15.0 and mercury is 15.8). Arsenic was significantly higher downstream in the one comparison (mussels) that could be done.

Response: It is assumed that this comment refers to the paragraph beginning "There is also no apparent relationship between fly ash constituents and the metals that are elevated (Table 5-34)." Arsenic in mussels is discussed in the very next paragraph. Nonetheless, a sentence was added to the proceeding paragraph as well.

4. There does not appear to be any mention of chronic effects of mercury due to bioaccumulation. It does not seem likely that the spill contributed to mercury bioaccumulation, but it is another mode of effect.

Response: The TRVs used to estimate safe concentrations for wildlife were based on chronic exposures to methylmercury, the form of mercury that is most readily bioaccumulated in aquatic systems. Thus, the RBCs specifically address chronic effects of mercury that is bioaccumulated. To make this clearer, a sentence was added to the section on RBC that states, "These LOAEL values pertain to chronic exposures and ecologically relevant endpoints."

5. The authors complain about using mussels for the metal analyses (Fish and Mussel Tissue Sampling and Analyses, paragraph 3). This statement seems to downplay the importance of the mussel data. Whatever their usefulness for direct calculation of damages, they are probably one of the best groups for establishing that metals did or did not enter food webs as a result of the spill. It should be recognized that there may be different elements of the study

design for the two goals of comparing upstream with downstream and estimating food web effects.

Response: The statement simply reflects the fact that the mussel tissue sampling was done at the direction of the Trustees but without a clear understanding of how the data was to be used to assess impacts on the Delaware River. This was in contrast to all the other data collected, including mussel community assessment data, where the relevance of the data to assessing impacts to the Delaware River was clear. Nonetheless, the statement was modified to accommodate the commenter's concerns.

6. The inclusion of two species of darters (in different proportions in different samples and stations) adds another possible source of variance in the station comparisons. For comparing food chain effects, it may be reasonable to sample a range of taxa based on availability, while more uniform samples would be best for upstream-downstream comparisons. For some stations (e.g., 199.3-R), there were as many shield darter in the samples as in other samples in which only shield darters were used. If the shield darters were smaller for the stations where both species were used, this further complicates comparisons.

Response: As noted in the first response above, the primary goal of the darter sampling was to assess potential effects on fish-eating wildlife. Darter concentrations were well below levels that could be considered problematic. Thus, further refinement of spatial trends is not warranted.

7. Were the suckers that were analyzed too large to serve as prey for osprey? If not, osprey are another potential consumer.

Response: The suckers were generally too large to serve as prey for osprey. According to the Alaska Department of Fish and Game (2005), ospreys rarely catch fish over 16 inches (400 mm) long. Most (63%) of the suckers collected in the present study were larger than this upper range.

Alaska Department of Fish and Game, 2005. Osprey. Available at: www.adfg.state.ak.us/pubs/notebook/bird/osprey.php. Accessed 07 May 2007.

8. Chart 18 is labeled "sucker length vs. mercury concentration". However, it is really sucker length and mercury concentration at each station. Graphs of mercury concentration versus length (with different symbols for different stations) would be informative. It seems likely that this would explain much of the variability among samples. The mercury-length relationship could be factored out using ANCOVA. The design is not well-balanced, so length and station effects would always be conflated. However, if length is used as a covariate and there is no subsequent significant station difference, that would be a strong argument against a true station difference.

Response: The graph suggested by the comments shows scattered data and provides no meaningful presentation (Attachment 1). The original graph is much clearer, and will benefit further from the suggested change in title.

As suggested, an ANCOVA was conducted on sucker mercury concentrations using fish length as a covariate, after which the three contrasts used in the original ANOVAs were rerun. The results of this ANCOVA along with results of the the original ANOVA are presented in Attachment 2. As shown there, consideration of fish length removed the significant position effects found with ANOVA, and the third contrast, which compared the first downstream sample to the three upstream samples, was also no longer significant when fish size was considered. These same effects were noted when fish weight was used as a covariate. In view of these results, the following text was added.

> "The mercury-fish length relationship can potentially be factored out using Analysis of Covariance (ANCOVA). When the effects of sucker length on mercury concentrations are considered with ANCOVA, there is no longer a significant elevation of mercury in the third contrast ($p = 0.14$, ANCOVA). As suggested by data in Chart 5-18, elevated concentrations of mercury in downstream suckers is apparently due to fish size rather than position effects."

ANCOVA results for other tissue types and mercury are also presented in Attachment 2. ANCOVA also changed the significance level of the bass mercury relationship, which became statistically significant when fish size was considered. Additionally, using length as a covariate, the first contrast became significant. That is, the three upstream stations now had significantly higher mercury concentrations than the three downstream stations. However, this effect was not seen when weight was used as covariate, and the result was not mentioned in the text. ANCOVA had no effect on the contrast results for darters and mussels.

9. Although a review is clearly beyond the scope of work of the study, some discussion of differences in modes of uptake between the biota analyzed (fish versus mussels) and different metals is warranted.

Response: A brief discussion was added as a footnote.

10. The correlation structure among metal concentrations within samples would be informative; e.g., using principal components analysis. Separate analyses upstream and downstream of the spill site might be suggestive. For example, in upstream sites, one would not expect mercury to be correlated with the other metals. Downstream, it would be correlated differently (with stronger correlations with several of the metals) if the spill was a significant source, but otherwise would show the same structure as upstream.

Response: The statistical analyses are already extensive and rigorous. Since tissue concentrations were well below problematic levels, the value of even more analyses is unnecessary. Nonetheless, a principal components analysis was performed with the Sucker data, and the graph of this PCA is provided as Attachment 3 to these responses. It does not appear to show any meaningful upstream-downstream differences in the relationship of mercury bioaccumulation to that of fly-ash related metals.

11. Results and Discussion, paragraph 6: The authors note that statistically significant results occur when downstream concentrations are negligibly different. They then say that this might be an artifact. Small but significant differences are not necessarily artifacts; they may simply show that a good job was done of removing various sources of variation, so that a small difference could be detected. They then mention the artifact of a few "hits" among non-detects creating significant differences. This is a valid issue, and the authors might want to flag some of these cases in the tables.

Response: The issue raised above was already discussed in a footnote, which said

"In some cases, for example thallium in mussels (Table 5-32) and arsenic in suckers (Tables 5-33 and 5-34), this effect occurred because most of the results were below the limit of detection. Since less than detection results were set equal to the detection limit, variance in samples with most values below the detection limit was artificially low. Therefore, it took only a few slightly higher values to cause the negligible difference to be significant."

12. Results and Discussion, paragraph 8: "chaotic patterns" are mentioned. "Chaotic" is not a good word to use here, since it carries a variety of meanings, some of which are technical, very specific, and not appropriate to this study.

Response: "Chaotic patterns" was changed to "disorder."

13. Replace "Eastern elliptio" with "eastern elliptio" (see AFS Common and Scientific Names of Mollusks, 2nd ed.).

Response: As requested, these changes were made to the text.

14. Fish and Mussel Tissue Sampling and Analyses, paragraph 2: "To address this issue, an assessment of metals concentrations in fish and shellfish was conducted to determine if metals were elevated in fish or mussels...": Use "mussels" rather than "shellfish", as "shellfish" is a general term that can refer to molluscs and/or crustaceans. Also, delete the repetitive "in fish or mussels".

Response: As requested, these changes were made to the text.

15. Fish and Mussel Tissue Sampling and Analyses, Field Methods, paragraph 2: The eastern elliptio is widespread (in Atlantic drainages from Canada to Georgia, etc.) but not cosmopolitan.

Response: As requested, these changes were made to the text.

16. Fish and Mussel Tissue Sampling and Analyses, Estimation of Risk Based Concentrations (RBCs), paragraph 3: "*Lontra canadensis*" instead of "*Lontra Canadensis*".

Scientific names are used for the bald eagle and river otter but not for other species (e.g., muskrat, raccoon). We suggest a consistent use of scientific names, or else omit them for species with readily recognizable common names.

Response: As recommended, scientific names were removed for species with common names.

Periphyton Community Assessment

1. The stated objective of the periphyton community assessment is to “determine whether there was any impairment to the periphyton community resulting from the release.” It is impossible to meet this objective as stated, both because of difficulties in proving that detected impairment (if any) was caused by the release and because there may have been impairment that was not detected due to limitations of the study design and how the data were analyzed and interpreted. The best that can be done is to look for evidence of impairment, primarily by comparing conditions upstream and downstream from the fly-ash spill. It would be useful to include in the introduction a verbal conceptual model or set of hypotheses regarding some of the effects that might be expected as a result of the fly-ash spill (i.e., how would we recognize impairment?). Those effects that could be detected by the study design, and how data are to be interpreted to do so, should be specified. It would also be useful to characterize the magnitude of effects the data analysis is designed to detect. Given the relatively long period from the time of the spill to the time samples were taken (roughly one year), it is reasonable to assume that substantial recovery has taken place and that most effects to be assessed are long-term, persistent changes and more moderate, subtle effects.

Response: Noted. It is understood that the study cannot conclude that absolutely no impairment to the periphyton community has occurred. In response to this comment, the text has been reworded to clarify that there was no discernable population-level impact. It is also important to note that the periphyton community assessment was developed in coordination with the Trustees, and the Academy reviewed and commented on the proposed plan and even provided training for the field personnel.

2. The word “diatoms” (with an “s”) often appears where it should not. For example, “diatom analysis” is correct, not “diatoms analysis”.

Response: As requested, these changes were made to the text.

3. Field and Laboratory Methods, paragraph 4: Though the soft-algae analysis should be considered semi-quantitative, the diatom count analyses were quantitative. The diatom results were not presented on a number per area sampled basis, but since counts of each species were made, the results are quantitative.

Response: The term “semi-quantitative” for the diatom count analyses was used because the term “quantitative” should apply only to expressions of numbers per unit area of river substrate sampled. However, it is understood that counts of each species were made and that the samples may, in fact, be considered quantitative by others. The text has been

revised by rewording the sentence and removing the term "semi-quantitative."

4. Field and Laboratory Methods, last sentence: "Relative biovolume of each soft algae species was estimated without criteria." Clarify the meaning of "estimated without criteria".

Response: The term "estimated without criteria" means that percentages in 5% increments were estimated for each taxon (based on the analyst's opinion), rather than using a system similar to the one employed in estimating relative abundance (explained in the report text). This methodology is described in Section 6.4.3 in the Patrick Center's "Procedure for Semi-Quantitative Analysis of Soft Algae and Diatoms", Procedure No. P-13-65 Rev. 0 (11/02), which formed the basis for the soft algae and diatom analyses.

5. Data Analysis, paragraph 1: It is not clear what is meant by a "replicate". Does it mean an algal count? This should be clarified. Also, a reference should be given for the CD. How can a copy be obtained? Raw data should be available for the reader to examine.

Response: The term replicate refers to one of ten individual samples collected at each station. This is explained more clearly in the second paragraph of *Field and Laboratory Methods* on page 46. The data are available on a CD which accompanies this report. The text is changed accordingly.

6. Data Analysis, Paragraph 2: What does "small sample" mean?

Response: "Small sample" in this instance refers to modification of the Shannon Diversity computation in order to account for error when a relatively small number of cells are identified out of a replicate sample that potentially contains many thousands of cells. Information on the small sample modification is available in:

Hutcheson, K. 1970. A test for comparing diversities based on the Shannon formula. *Journal of Theoretical Biology* 29:151-154.

Bowman, K.D., K. Hutcheson, E.P. Odum, and L.R. Shenton. 1971. Comments on the distribution of indices of diversity. *Statistical Ecology* 3:315-366.

This explanation has been added as a footnote to the report.

7. Data Analysis, paragraph 3: "It is generally accepted that greater numbers of taxa (or increased species richness) indicate higher biotic integrity." This is not true for periphyton, where the most commonly accepted view is that species diversity increases following moderate disturbance (Intermediate Disturbance Hypothesis). Thus, one might expect species diversity to increase downstream following the fly-ash spill. It is not necessarily true that greater diversity values "indicate higher biotic integrity", and such values therefore should not be interpreted to mean no effect of fly ash.

Response: The statement that for periphyton, "the most commonly accepted view is that

species diversity increases following moderate disturbance (Intermediate Disturbance Hypothesis)" is incorrect. In fact, in Chapter 6 – Periphyton Protocols in the USEPA's *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish* (Barbour, et. al. 1999) there is no mention of the Intermediate Disturbance Hypothesis in its discussion of Species Richness or Shannon Diversity. In addition, the chapter says "High species richness is assumed to indicate high biotic integrity because many species are adapted to the conditions present in the habitat. Species richness is predicted to decrease with increasing pollution because many species are stressed".

Currently, the PA Department of Environmental Protection has a periphyton sample collection protocol, but not for laboratory sample processing or subsequent data analysis. Therefore, we reviewed the Commonwealth of Kentucky's *Methods for Assessing Biological Integrity of Surface Waters in Kentucky* (July 2002). This document uses the Total Number of Diatom Taxa (an estimate of diatom species richness) and Shannon Diversity as metrics in assessment of periphyton. This methodology (page 37) also states that "high species richness is assumed to be the case in an unimpacted site, and species richness is expected to decrease with increasing pollution".

The use of species richness and Shannon Diversity in assessment of periphyton is widely accepted and justified as applied in the present instance. However, the text was edited slightly to more clearly reflect the text in Barbour, *et. al.* (1999).

8. Results, Chlorophyll *a* and Ash-Free Dry Mass: In addition to results for chlorophyll *a* and ash-free dry mass, it would be useful to see results for ash weight, as this might provide a measure of the amount of residual fly ash on the rocks. We also recommend that the chlorophyll *a* and ash free dry mass values presented in Table 5-39 be re-checked. Most of the values differ markedly from values presented in DRBC (2007) for this region of the Delaware River. This fact is especially noticeable when one computes the Autotrophic Index (AI) for each site, which is simply ash free dry mass divided by chlorophyll *a* (expressed in the same measurement units). For the site closest to the study region, the value of AI in the DRBC report is 319 (dimensionless). In contrast, values of AI computed from chlorophyll *a* and ash free dry mass values presented in Table 5-39 (after converting to common units) range from 3,204 to 11,548.

Response: The chlorophyl a and ash-free dry mass measurements and the related data computations were re-checked by the lab and found to be free of errors. The differences likely reflect that only one measurement was made by the DRBC, in comparison to the 80 measurements made during the Phase IV study.

9. Results, Diatoms: Does the first sentence and paragraph mean "Only 8 species occurred with an abundance of 5% or more"? If so, this should be clarified. While it is worthwhile to compare relative proportions of dominant species in the upstream samples with those in the downstream samples, the fact that there is relative similarity is not strong evidence of no impact of fly ash. More weight should be given to the less common species, and to the communities as a whole. The quality of the underlying data would support a more sophisticated and reliable comparison than is presented by the authors. In particular, the composition of the entire

assemblages from upstream and downstream samples should be compared statistically.

Response: The first sentence means that each of eight species occurred with an abundance of 5% or more. The sentence has been reworded to state the fact more clearly.

As the report section indicates, there is great similarity in the identity of the taxa representing 5% or more of the diatoms identified in the reference and downstream samples. Such similarity does not suggest impact of the fly ash release.

It is important to include all of the taxa, not just those identified in abundance, in the periphyton community assessment. This is accomplished in computation of and statistical analysis of the metrics in subsequent paragraphs of this report section.

10. Results, Diatoms, paragraph 7: *Achnanthes minutissima* (= *Achnanthidium minutissimum*) is the most common diatom in stream and river diatom assemblages throughout the country. Thus, the fact that it is present in large proportions both upstream and downstream cannot be used to support a conclusion of no effect of the fly-ash spill.

Response: The findings that *Achnanthes minutissima* is present in large proportions in the reference and downstream samples and that there is no statistically significant difference between the two sets of samples do not suggest impact of the fly ash release. The conclusion has been reworded to state that there is no evidence of an effect from the fly-ash release rather than that there is no effect.

11. Results, Soft Algae, Table 5-42 and paragraph 2: The aquatic macrophyte *Podostemum ceratophyllum* was found at several locations at upstream reference sites, and made up 31% of the biovolume. However, it was not found in any of the samples from the downstream sites. Might this be an effect of the fly ash (or other aspects of the facility operation)? This possibility should at least be considered, especially since this taxon can be important habitat for benthic invertebrates. Just a quick web search found the following:

"Comments: *Podostemum* could easily be the poster child of Piedmont rivers (B. Adams, pers. comm.); it is unlikely that many aquatic plant species in the Piedmont have been hit harder. **It is very sensitive to sedimentation** and, accordingly, has declined greatly throughout its range and has been lost from nearly all areas it once occupied in some drainages such as the Upper Neuse Basin of North Carolina (Adamspers.comm.)." .

Response: There is no clear explanation for the absence of leaf fragments of the submerged plant *Podostemum ceratophyllum* from the downstream soft algae samples as indicated in Table 5-42. However, as noted from Table 5-42, leaf fragments were absent from 18 of the Reference replicate samples (= 60% of the total replicates) and found to be very abundant or dominant in only 9 replicates (= 30% of the total replicates). This indicates patchy distribution on the river bottom and patches of the plant may have been missed in the downstream periphyton sampling. The field notes are inconclusive with respect to the presence of this species at the Reference or the Downstream stations because it clings to the substrate, looks like moss, and would not have been easily distinguished by the field crew.

The appearance of *P. ceratophyllum* leaf fragments in the Reference replicate samples is shown below:

Upstream (or Reference) Stations

Station 205.0-R		Station 199.3-R		Station 194.5-R	
Sample ID (Replicate)	Relative Abundance	Sample ID (Replicate)	Relative Abundance	Sample ID (Replicate)	Relative Abundance
1NJ01	Rarely seen	2NJ01	None	3NJ01	Common
1NJ02	None	2NJ02	None	3NJ02	None
1NJ03	None	2NJ03	None	3NJ03	Dominant
1NJ04	Common	2NJ04	None	3NJ04	None
1NJ05	Very abundant	2NJ05	None	3NJ05	None
1PA01	None	2PA01	None	3PA01	Dominant
1PA02	None	2PA02	None	3PA02	Dominant
1PA03	Dominant	2PA03	None	3PA03	Very abundant
1PA04	None	2PA04	None	3PA04	Dominant
1PA05	Dominant	2PA05	None	3PA05	Dominant

It is clear that the plant's appearance in the soft algae samples was patchy at the Reference stations. Leaf fragments were absent from all replicates collected at one Reference Station (199.3-R), present in only five replicates at Station 205.0-R, and identified in greatest abundance at Station 194.5-R.

The Maryland Department of Natural Resources website was reviewed. The observation that *P. ceratophyllum* is "very sensitive to sedimentation" came from a personal communication, not a literature reference. This suggests that the supporting information is anecdotal.

In any case, the distribution of this plant species on the river bottom undoubtedly is patchy and defined by a wide range of habitat parameters and river conditions. The absence of *P. ceratophyllum* in the downstream periphyton samples appears to be unrelated to the fly ash release.

12. Summary: The logic of the interpretations of periphyton results is flawed. Results are selectively and, in some cases, incorrectly interpreted. We see the following problems in the summary bullets.

Response: The comments regarding flawed logic and incorrect interpretations are incorrect. The periphyton study measured biomass (chlorophyl a and ash-free dry mass), identified the most abundant taxa, measured species richness and diversity, and computed several other metrics that are listed by the USEPA for interpretation of periphyton data.

In all cases, none of the results of these analyses, including statistical testing where warranted, suggested impact of the fly ash release.

Bullet 2: Similarity in abundance of the most abundant species is not a reliable measure of disturbance effect. It is flat-out incorrect to say that higher taxa numbers and diversity indicate higher biotic integrity. More likely, this is an indication of moderate impact or of differences in habitat diversity.

Response: For bullet 2, see response to comment #7 of this section.

Bullet 5: The Siltation Index (SI) values are shown to be higher downstream, as logically would be expected as a consequence of the fly-ash spill. As far as we are aware, there is no evidence that the main areas of continued exposure to fly ash were still located immediately downstream of the spill site at the time of sampling, roughly one year after the spill. If other sections of the Phase IV report provide such evidence, then it should be specifically referred to here. Otherwise, it is questionable whether the results should be interpreted as indicating there is no effect on periphyton because the SI values immediately downstream are not as high as those further downstream. It could well be that the main areas of fly-ash accumulation have migrated downstream as particles are repeatedly entrained into the water column, transported some distance, and redeposited (like other types of particulate matter in rivers, such as fine sediment and seston).

Response: Other studies will be referenced within the Phase IV report. For example, the results of these studies indicate strong geochemical evidence that the first high-flow event exhausted the vast majority of deposited ash. Noting that the fly ash release occurred on August 23-26, 2005 and the first high flow event in the Delaware River started on October 8, 2005, this evidence indicates that the vast majority of deposited ash likely was washed away within seven weeks of the fly ash release and approximately 10 months before the periphyton samples were collected. Therefore, it is unlikely that fly ash was present in the river between the two pairs of downstream stations, contributing to the higher Siltation Index values computed at the two stations located most downstream.

References

Delaware River Basin Commission (DRBC). 2007. *Pilot Study: Implementation of a Periphyton Monitoring Network for the Non-Tidal Delaware River.* Delaware River Biomonitoring Program, Delaware River Basin Commission, West Trenton, NJ.