ASSESSMENT OF THE BIOLOGICAL CONDITION OF THE NEW FORK RIVER, IN THE VICINITY OF THE PINEDALE ANTICLINE PROJECT AREA: 2012

AN ASSESSMENT OF CHANGES AMONG BENTHIC MACROINVERTEBRATE ASSEMBLAGES

PREPARED FOR SUBLETTE COUNTY CONSERVATION DISTRICT, PINEDALE, WYOMING

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PREAMBLE:

Although this is a single-author publication, this report is the product of much work by the Sublette County Conservation District (SCCD), their stakeholders and my technicians. The plural pronoun, "we" is used to acknowledge their contributions and insights to the project. The findings of this report are based on empirical data and may or may not represent the views of SCCD or the districts employees.

ACKNOWLEDGEMENTS:

This report is the product of much work by many persons. I would especially like to thank Kathy Raper, Darrell Walker, Eric Peterson and Sno Ann Engler for their work on relating to this project. I am especially grateful for the dedicated field work of Kathy Raper, and the fact that Darrell Walker cares about the project enough to be passionate about methods and findings. Furthermore, I thank the SCCD Board of Supervisors for their commitment, continued involvement and funding of the project.

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APPROVAL:

This report has been reviewed by the Sublette County Conservation District. The work constitutes the final deliverable for the project: **PAPA 2012 REPORT** .

Brett D. Marshall, Director, River Continuum Concepts Date

District Representative Signature Date

March 2014. Revised.

EXECUTIVE SUMMARY

The Executive Summary captures key points from the report, and presents essential elements such as background, methods, results and discussion to the casual reader. It is similar to an "abstract" in scientific publications, but without the same economy of words. It should stand alone as a summary, but may seem redundant, because all elements of this summary are also presented elsewhere in this report. Readers who are especially curious about how and why we determined something are encouraged to read the whole report.

STATEMENT OF PURPOSE

This purpose of this ecological survey is to document the New Fork River's benthic community structure in such a way that the cumulative effect of physical and chemical stressors could be documented and efficiently integrated. Measurements are made at a scale appropriate for the response variables[1] and should not be confused with the methods used to evaluate whole watersheds at geological spatial-temporal scales[2]. Previously, this distinction was not clear and had caused some misunderstanding among reviewers with geomorphology experience who had assumed that our purpose was to supplant geomorphology studies by using macroinvertebrates as a surrogate measure for habitat assessment. Therefore we need to reiterate the purpose very clearly here: *the purpose of this ecological survey is to document the composition of macroinvertebrate assemblages of the New Fork River in such a way that potential ecological perturbations can be assessed with some insights, relating to cause and effect.*

PROJECT HISTORY SUMMARY

This report follows up 13 years of data collection by Sublette County Conservation District using benthic macroinvertebrate communities as indicators of the ecological condition of the New Fork River within the Pinedale Anticline Project Area (PAPA). In previous years, we identified some spatially-localized alterations of community structure which may have been related to natural gas development in the PAPA. Ultimately, all the ecological descriptors indicating significant changes all reflected one underling community-level response: a dramatic and sustained elevation of the relative abundance (and by extension, relative importance) of the oligochaete worms of the genus *Nais*[3]. The effect was primarily localized to one of the nine study sites, below a natural gas pipeline, and existed from 2004-2008. In 2010(Marshall 2011), we found that the abundance of worms was strongly correlated with the abundance of aquatic plants and more weakly associated with sedimentation.

Aquatic worms are usually associated with organic deposits in fine sediments; changes in their abundance can be related to changes in the quantity or quality of fine sediment deposits. The results of past surveys (Marshall 2008, 2009) indicated that there were active sources of erosion between NF30 and NF40 and that these were significantly correlated with changes in the abundance of macroinvertebrate fauna. Additionally, there were two activities related to PAPA development that may have caused these changes in benthic community structure. First, the pipeline capacity was increased annually by adding additional pipes across the New Fork River. Although the additional pipelines were bored beneath the stream bed, riparian activities associated with pipeline placement may have resulted in localized disturbance of the hyporheic zone. Second, there was an increase in the development of drilling platforms in riparian areas where there is clearly hyporheic flow during this period. These platforms may cause ecological perturbation of river ecosystems by affecting hyporheic exchange and sedimentation.

Another historically relevant finding was the presence of *Didymosphenia geminata* at some sites in 2010. The field observations by SCCD were corroborated by the occurrence of this algae species in benthic samples. The impacts of excess *D. geminata* growth often cause a shift in benthic macroinvertebrate community composition similar to the effect of

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[1] Our response variables are metrics that describe aspects of macroinvertebrate community structure.

[2] These would be hydrology or geomorphology surveys where direct measurements of the physical environment are in and of themselves response variables.

[3] *Nais* is a common genus of aquatic worms in the family Naididae (which currently includes the family recently called Tubificidae).

sedimentation. Therefore for this report (and the field season for the next report[4]) we increased the analytical effort to account for the growth of aquatic plants and especially those that might cause elevations in the abundance of aquatic oligochaete worms.

2012 RESULTS SUMMARY

The results of most analyses for 2012 indicated that there were very few changes in benthic community structure – spatially or temporally. However, there were some spatial changes and some temporal shifts in the distribution of benthic macroinvertebrates that need to be considered here.

- Spatial analyses indicated that one site, NF30, had several metrics indicating significant declines (relative to nearby locations) in biological condition of aquatic resources. All of these measures were responses to the same fundamental element of community composition: high relative abundance of aquatic worms (*Nais* sp.). These differences persisted, even when the abundance of worms was corrected for influential habitat variables (such as moss coverage). [q.v., Section 3.2*]
- Analysis of temporal trends indicated two sites appeared to have declined from the previous year in several metrics. [q.v., Section 3.4*]
- NF30 had several metrics indicating significant decline in condition from the previous year, but these responses were to the same fundamental element of community composition: higher worm densities than the previous year. Worms comprised and averaged 48.2% (22-66%) of the benthos at NF30 in 2012, as compared to 4.7% non-insects in 2011. [q.v., Section 3.4*]
- NF50 had several metrics indicating significant decline in biological condition from previous years. The responses were all related to one single fundamental element of community composition: an increase in the relative abundance of aquatic worms (*Nais*). This phenomenon was not as pronounced as observed at NF30 and the abundances of non-insects were not beyond our expectations for New Fork River. However, the increase to 17.8% non-insect abundance was a statistically significant change because the metric was especially low in 2011 (4.8%) with a low variance. [q.v., Section 3.4*]
- Although two sites had significant declines relating to elevated non-insects, one site, NF40, exhibited improved conditions resulting from a decrease in worm abundance. In 2011 a high average non-insect abundance (33.7%) was observed but dropped to 20.3%. [q.v., Sections: 3.2, 3.3, 3.4*]
- We repeated the density analyses introduced to the study in 2011 to determine if the high relative abundances of non-insects were due to decreased densities of insects, or just increases in worm densities. [q.v., Section 3.5*]
    - We found that mayfly, stonefly, and caddisfly densities at these sites were not significantly reduced compared to most other sites. Stonefly density estimates were low at NF30, but this was likely due to the laboratory methods relying on fixed count standardization. [q.v., Section 3.5*]
    - This suggests that the high worm abundances in 2012 (as with 2011) did not occur because all the other "good" insects disappeared. Rather, the environmental conditions favored high abundance of Naididae worms. This means that the micro habitat at NF30 has changed to favor worms, but not necessarily to the detriment of other important insects. [q.v., Section 3.5*]

*A general review of the methods section will be necessary to understand the results and discussion sections of this report.

[4] We analyzed percent cover of algae and other plant types as recorded in SCCD's field notes in 2011. For 2012, we had SCCD collect quantitative periphyton samples for dry weight determination of biofilm data.

CONCLUSIONS AND RECOMMENDATIONS

The benthic macroinvertebrate assemblages of the New Fork River and the East Fork River generally indicated improving conditions at most sites in 2012, relative to 2011. The notable exceptions were the sites NF30 and NF50. All the metrics that indicated significant differences at these sites were caused by abundant small worms.

The New Fork River site downstream of the East Fork River confluence had a moderate, but not exceptional increase in the relative abundance of worms in 2012 that was not shared with sites upstream. At the time of this report preparation, it is unclear what conditions may have caused this shift in benthic community composition; possibilities are discussed below.

NF30 is the New Fork River site downstream of the natural gas pipeline crossings and downstream of the area near the natural gas wells constructed in the riparian corridor—over hyporheic stream channels. Thus, the increase in worms at NF30 could be due to anthropogenic development in the PAPA. However, it may also be related to the dispersion of conditions which caused exceptional worm abundance at NF40, a short distance up river in 2011.

NF40 improved from the previous year, but still exhibited relatively high worm abundance in 2012. The site may be influenced by anthropogenic developments in the riparian corridor, or from anthropogenic activities in the Alkali Draw or the Sands Springs Draw drainages. Alternatively, natural intermittent flow events from these draws could also influence the changes in the abundance of worms.

Regardless of the cause of the high abundance of worms at NF50, NF30, and even at NF40, it is noteworthy that the metrics responded to high worm abundance alone; these metrics did not respond to any reductions in the density of important insect groups in the New Fork or East Fork Rivers.

We conclude that any influences of development in the PAPA on the surface water quality of New Fork and East Fork rivers in 2012 were minor.

RECOMMENDATIONS

We usually conclude this report with several recommendations for the SCCD to improve or adapt the monitoring program so that it remains useful to assess changing environmental conditions. This is an essential element of adaptive management and the adaptive management approach to monitoring: response followed by feedback followed by adaptation of the monitoring program's design. Along these lines, we have recommended the measure and analysis of periphyton biomass for several years. The biomass of epilithic algae is important for trophic structure of benthic macroinvertebrate communities—especially for mid-sized rivers receiving intensive sun light, like the rivers of central and western Sublette County. There are several reasons for this recommendation. First, as stated above, it is a very important base of the food web affecting relative abundance, survival, growth and production of many macroinvertebrates directly or indirectly. Second, the spread of the nuisance algae *Didymosphenia geminata,* which we believe will not be affected by PAPA development, could mimic response signatures of PAPA-related impairments of ecosystem function eventually. Third, discharges from Anticline Disposal, downstream of Sand Springs Draw between NF40 and NF30, could have either a positive or negative effect on the growth of periphyton or other important biofilms in the New Fork River. These could have indirect effects on benthic community structure and need to be documented and should be accounted for in our response models for macroinvertebrate ecology. Additionally, possible spills and other intermittent PAPA activities could affect biofilm growth and indirectly have dramatic effects on the ecology of the system.
We recommended several methods by which these variables could be collected for use in this report. We requested the cheapest option to get the simplest baseline possible, with the potential for more rigorous analyses when necessary. That is, we requested the biomass of epilithic scrapings collected by SCCD. This was approved in a meeting in 2011 for the 2012 field season, but not funded. Samples were collected that would have improved this report, but not analyzed.

This year, we re-iterate the need for some form of accounting for periphyton and other biofilms. This can be done by local labs in Pinedale, or other professional laboratories serving the natural gas and petroleum industries. SCCD may have capacity to conduct some of these analyses in house. We can work with the district on formatting the data for maximal use.

Consider another downstream reference/recovery site. NF19 has served well as the downstream reference and recovery zone for several years (since 2000). However, the SCCD should consider possibly adding another downstream recovery site. In 2012, we observed the downstream migration of worms from NF40 to NF30, and the lack of high worm abundance at

NF19. We have not yet observed any PAPA related impairments at NF19, but if the spread of worm dominated communities continues, the site's value as the downstream reference/recovery site will be compromised. Proactive adaptive monitoring should be prepared for this contingency. Therefore, the SCCD may want to develop baseline conditions at a new site downstream that may eventually serve as the downstream reference if required. There are several ways in which this could be implemented. We will meet with the SCCD and discuss these options so that the board can make an informed decision.

Continue the use of density measurements. This report for 2012 is the second report where we used density measures to elucidate the severity of changes related to the high worm abundances at some sites in the New Fork River. Both times we used these analyses they showed that there are certain limitations to the State's bioassessment criteria and that the severity of community structure changes were much more minor than the relative abundance measures suggested. We recommend the continued use of these measures.

Continue the monitoring program. We have found several sites over several years that had mild community structure changes that could be attributed to PAPA development. The fact that the program can diagnose relatively mild changes is testimony of its robustness. The fact that we can detect them and correctly surmise that these effects are not as dramatic as they first appear is testimony for the completeness of the monitoring design. However, these mild changes need to continue to be monitored to ensure that the cumulative effects do not become catastrophic to the natural resources of Sublette County.

Account for potential effects of Anticline Disposal. Anticline Disposal initiated a permitted discharge to the New Fork River in 2009. The current sites NF30 and NF40 should be adequate to account for any effects of this discharge on the New Fork River. However, the modes of potential effects remain unclear. There are several ways we can address the relevance of this influence. If the district can acquire some data from Anticline on the chemistry and volume of discharges, we may be able to anticipate the kinds of likely responses of the New Fork River ecosystem. Additionally, the SCCD can add some simple field measures to the sampling program that might account for the influence of this discharge. Candidate measures include conductivity and pH. Seston measurements might also be warranted. We should discuss these influences before the next report.

Meet with your consultant. We believe it is important for the integrity of the program that we are actively aware of changes on the ground in Sublette County. Additionally, it is important to ensure there is a venue for the natural gas operators and other stakeholders to share feedback regarding the monitoring program. Traditionally, this has been the role of the Pinedale Anticline Work Group (PAWG) and the related task groups and committees. However, since the disbanding of the PAWG, there has been no direct feedback or interchange with stakeholders. We welcome the opportunity for these exchanges.

RESPONSE TO SCCD QUESTIONS AND COMMENTS ON DRAFT REPORT

PROJECT HISTORY

Earlier reports (Marshall 2008, 2009) did not represent surveys specifically designed to describe erosion. Yet, in the Executive Summary and in the Introduction of this report, you assert that these surveys indicated active sources of erosion. How do you support this statement?

In these reports we found several lines of evidence that supported the occurrence and effects of active erosion near sites NF30 and NF40. These observations were later corroborated by field observations. Although our discussion of these findings eventually included our post-hoc field visit (Marshall 2009), they were actually based originally upon three lines of evidence among data collected by SCCD in 2007 and elucidated in the analysis of the report for that year (Marshall 2008). The first line of evidence was that the quantity of sands in individual benthic grabs, as described by SCCD's field data, was greater at these two sites (NF30, NF40) than at all other sites, this resulted in a statistically significantly lower median particle sizes for samples collected from these sites. The second line of evidence was that when we quantified the interaction of river velocity with sand concentrations, all sites, except NF30 and NF40, exhibited the typical base-flow pattern of sand volume being inversely proportional to flow. That is, in an equilibrium situation sand deposition is typically greater in slower water because faster waters sweep it away. We observed this for all sites except NF30 and NF40. At these two sites we found a positive correlation of sand with velocity—indicating that faster waters had greater concentrations of sands, not less. This relationship was not only contrary to expectations but was statistically significant. This only happens when there is an active source of sediment entrainment (erosion) nearby.

The third line of analytical evidence is actually an indicator of the magnitude of the effects of sedimentation. We observed a statistically significant increase in sediment-tolerant worms at sites NF30 and NF40. This difference in worm abundance among the sites was not statistically significant once statistical models corrected from the influence of fine particles. This means that the increase in fine particles was not only statistically significant and related to sediment, but also sufficiently ecologically significant to statistically influence benthic community structure. That is, this variable clearly demonstrated to have biological consequences. Anecdotally we differentiate these sediment dwelling worms from the worms observed in later surveys because the more recent worms were found among the leaves of aquatic moss plants and were pale green in color—whereas these sediment dwelling worms were a dark tan in color; presumably reflecting some aspect of the differential niches occupied by the animals.

In the project history section of the report, you mention "spatially localized alterations of benthic community structure." Are these specifically related to the pipeline crossing?

Not necessarily. When we initially found these localized deviations in benthic community structure (and function as noted by metrics), we focused on the pipeline because it was the only known riparian disturbance in the region. Also at the time, the Paradise Irrigation Ditch along with a complex wetland system was believed to buffer the New Fork River from all development on the Mesa side of the river. Subsequent field visits (Marshall 2009) had revealed several large platforms being constructed within the buffer zone, and directly on top of active hyporheic river channels in riparian corridors. Construction activities for these platforms involved disturbance and fill of hyporheic stream channels and disturbance of riparian habitats. This and the frequent (annual) disturbance of land adjacent to the pipeline crossing probably had greater influence than the pipeline itself.

In discussion of these changes at the Pinedale Anticline Work Group (PAWG), the influence of the East Fork River was explored. It was suggested that the elevated abundances of fine particles at NF30 and NF40 were caused by the East Fork River, which is known to have finer particles. However, we developed a robust study design that was able to account for the effects of the East Fork River. We found more worms at NF30 and NF40 than we found in the East Fork River itself. Thus, in order for the East Fork River to have caused the observed differences, it would have had to have provided a greater concentration of fines to the New Fork River than are exhibited within the East Fork River itself. This is highly improbable because it is mathematically impossible for the median particle size of two populations combined to be less than the median of the population with the lower median.

It is important to note that sediments are not all homogenous in their potential habitability to sediment dwelling invertebrates. Sediments that have a different ratio of organic: inorganic materials will be favored by different invertebrates. Similarly sediments of different textures or grain-sizes will support different invertebrate taxa. We found that the invertebrate assemblages at NF30 and NF40 deviated significantly from those of the East Fork River, as well as

those from elsewhere in the New Fork River. Thus, the physical measures and the biological measures suggest that the changes are due to environmental changes near the sites, rather than natural influences extending from East Fork River.

Near the end of the Project History Summary, you mention the role of Didymo and that it could have effects on benthic structure and function that are analogous and similar to sedimentation impacts. You mentioned that you increased the analysis of field data to try to account for this. To what baseline did you compare this to?

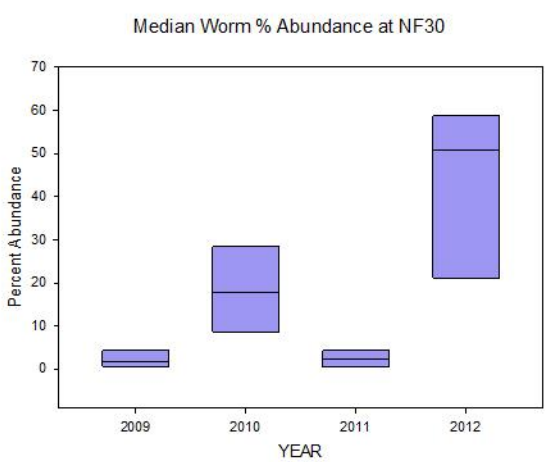
We were only able to compare field measures collected from the sites to each other (Fig. 3.1.10). We've discussed the need for a baseline and the need for quantitative data for several years now. We know Didymo is present in the system because we have identified it in the lab from benthic grab samples and observed it inside the stomachs of some midges. Field observations of Didymo mats can be useful, but I have seen algae experts misidentify other mat-forming diatoms as Didymo in the field; lab identifications are much more accurate. The distinction primarily matters for diagnostic purposes. That is, although the effects of mucilaginous slime mats formed by all stalked diatom species (including Didymo) should have similar effects on macroinvertebrate community structure. Different species of diatoms form mats under different conditions; most occurrences of other species are caused by nutrient enrichment; Didymo is not.

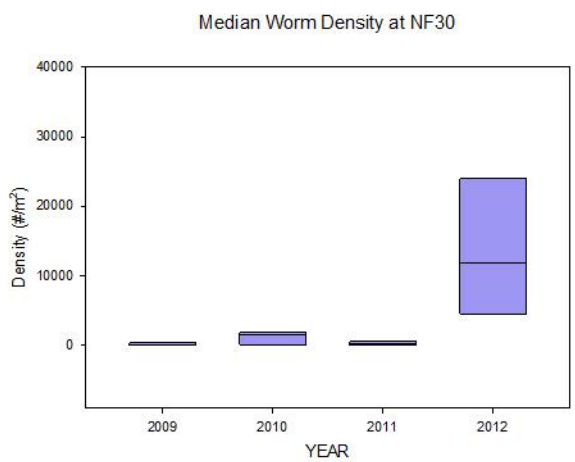
The need for a quantitative baseline for this alga has been discussed in previous reports, proposals, and personal communications with SCCD (Marshall 2012, 2013a, 2013b) but it has not yet been implemented into the PAPA report. We will be glad to design a monitoring program to quantify the abundance of this species in the New Fork River and other streams in Sublette County.

2012 RESULTS SUMMARY

In the 2012 results summary of the Executive Summary you mention that the changes in several metrics are related to increases in worm abundance at NF30. What are the actual numbers of worms from the last three years at NF30?

The primary driver of the metric Non-Insect Abundance was the aquatic worms. If this metric was high, it was always because of worms. If it was low it was always because of worms. Other non-insects (that is non-worm, non-insects) comprised only <5% of the community. Therefore, the graphical representation of worm abundance over time at all sites, including NF30 and NF40, is best represented by Figure 3.4.3. The average percent abundance 2009, 2010, 2011, and 2012 was 2.9%, 19.8%, 2.5%, and 42.9% respectively. The average density of worms for 2009, 2010, 2011, and 2012 was 505/m^2, 1213/m^2, 307/m^2, and 14283/m^2 respectively. The box plots below show the median value and the interquartile ranges for observations at NF30 for each year. The Relative abundance graph shows the same pattern as presented in the temporal trends section of the report (Fig. 3.4.3). The density graph (below) shows that the actual density of worms was much greater than it was in previous years. Metrics are calculated based on relative abundances (%) and richness.





EXEC. SUMMARY RECOMMENDATIONS

You recommend accounting for potential influences of Anticline Disposal by using chemical data to screen for changes related to the discharge. You mention that conductivity of the water might be a good simple measure to screen for influences. We already collect conductivity measures for NF30, NF40, NF19, NF04, NF90 and NF80. Why haven't you conducted an analysis of these measures?

The first and simplest answer is that these data have not been provided for analysis in the PAPA report. This was not an oversight. The SCCD board of supervisors decided early in development of this project to focus on biological / ecological analyses to keep it manageable. An earlier report on baseline conditions of the New Fork River (Marshall 2006) was a voluminous tome including all chemical measures and all biological measures ever collected by SCCD. The complete analysis of biological and chemical trends would double the work and the size of the report. Although the addition of one more covariate would not add dramatically to the work effort, it would add some work and cost to the project.

Before we expand the scope of the project to include these data, it is important to realize the measures collected by the district may or may not actually be able to show relevant changes. The current measures are single grab samples collected quarterly or in conjunction with other forms of sampling. If SCCD collects one conductivity measure upstream on Tuesday, and another on Thursday, and there is a small difference, what does it mean? Does it mean site 1 is different from site 2? Or does it mean that Tuesday was different from Thursday? We don't know. Similarly, we have no idea how representative these measures are of actual conditions. If the Anticline Disposal discharges from 12:00-14:00 and Kathy samples at 08:00 we would not detect a difference. Conversely, if she samples at 13:00 we might be lead to think that the differences among sites are more persistent or more intensive than they really are.

To make these analyses meaningful we would need to install continuous monitor stations upstream and downstream. These samplers can easily provide hourly (or about any time interval) sampling of chemical data. Conductivity is a great measure because it is an inexpensive probe and requires almost no maintenance. Dissolved oxygen probes need to have their solution and membranes changed relatively often when continuously deployed (at least the models I am familiar with). Nutrient probes are expensive and also require maintenance.

The analyses of these data and their integration to the report would increase the scope of the report and result in about an 8% increase in the report's cost. Alternatively it could be provided to the district as a small mini report annually to ensure that the effects of the discharge remain minimal and can be accounted for in drought years. People might actually be surprised at how minor the changes are… and that the discharge might even be beneficial for aquatic life.

In the final conclusion you say, "We conclude that any influences of development in the PAPA on surface water quality of the New Fork and East Fork Rivers were minor." Can't you add, "if negligible at all?"

I am not sure I understand the question. If you mean to imply that there is absolutely positively no effect whatsoever, then the simplest answer is, "no, you cannot add this." It is a matter of the logical structure of hypothesis testing in general and the statistical power of the study combined with the facts that we know that any human activity in a river basin will have some effect on the quantity, quality, or retention of waters in the watershed. The appropriate question asked by environmental monitoring is, "Are the impacts of this activity sufficiently mediated that their effects are not detrimental to the structure or function of the ecosystem?" Then we design a study with sufficient statistical power to detect differences that are ecologically relevant. Moreover, in the previous bullets immediately before the statement upon which this question is based, we discuss several changes that we do believe to be related to development in the PAPA. But even if we did not detect any response at all, the absolute dismissal of any and all influences would be cavalier, unsubstantiated, and the hallmark of bad science. These closing words were carefully chosen to reflect the fact that effects we observed were minor, as indicated by study.

INTRODUCTION: 1.2: REVIEW OF PREVIOUS PAPA REPORT RESULTS.

You state that spring runoff was reduced and then that the elevated runoff eventually reduced the relative abundance of midges and worms in the New Fork River. This needs to be explained. What years specifically?

Each year we have included a graphical summary of the hydrological regime for the current year and previous years in the PAPA report. The USGS manages a flow station on the New Fork River, but the data have not been available for all years; recently the access to data before 2007 was made more difficult. The effects of drought were discussed in the early reports

and the role of elevated flow was discussed in detail for the PAPA 2009 report. It has been discussed each year since. In this year's report, the hydrologic regime was presented in Section 2.2 of this report. Flows were graphically presented for the present report and previous reports in figures 2.2.2-2.2.3.

Specifically, years 2000-2008 were all below average flows. 2009 and 2011 were the first years of sustained high discharge. Importance of seasonal flow surges for the habitat of macroinvertebrates has been published in several excellent papers including insightful perspectives by Poff (1992) and more recently by Death (2010). Also the relationship is discussed by Minshall (1984) and Newbury (1984). Generally the assortment and loosening of substrata is critically important to maintain the pore-spaces and interstices required to support diverse macroinvertebrate assemblages; without these intensive seasonal flow events, the microhabitats fill with fines. Thus, we postulated that the drought exacerbated the effects of any sediments incidentally added to the river during the drought.

The figure numbers in section 2.2 are incorrect.

No. These original figure numbers were correct; you had incorrectly counted a figure from Section 2.1.

ANALYTICAL METHODS, SECTION 2.7.

Embeddedness? Why name variables if we don't see them in the model? Isn't it enough to generally discuss?

This is the methods section of the report. The nature of science writing requires that the methods section is very detailed. Specifically, it should provide enough detail that a person with experience in the field can understand how and why things were done the way they were done and to allow independent replication of our analysis. The measures of embeddedness were collected for this report by SCCD in such a way that they could not be used as covariates because of missing values. If we decided to use this variable for the model we would have had to exclude ~20% of the samples because of missing data. However, just because the variable was not used in the model does not mean it was not analyzed. We did analyze the statistical significance of differences in embeddedness at each (Section 3.1, Fig. 3.1.5). The data were collected as covariables with benthic samples, and are statistically analyzed as variables. Therefore, they have variable names and need to be discussed in sufficient detail to explain how we obtained the values, why there were missing values, and why the variable was not used in the model. The methods section of the report is where this discussion is supposed to occur.

RESULTS

The appendix information about the Principle Components Analysis (PCA) and factor loading was missing in the draft.

The appendices were omitted from the draft because collectively they increase the size of the report dramatically. They have been added to this final draft.

In the section addressing the spatial differences in biological metrics the EPT richness metric, it appears that the only significant difference is an increase in the species richness of the EPT orders at NF60 relative to NF90. Why was this?

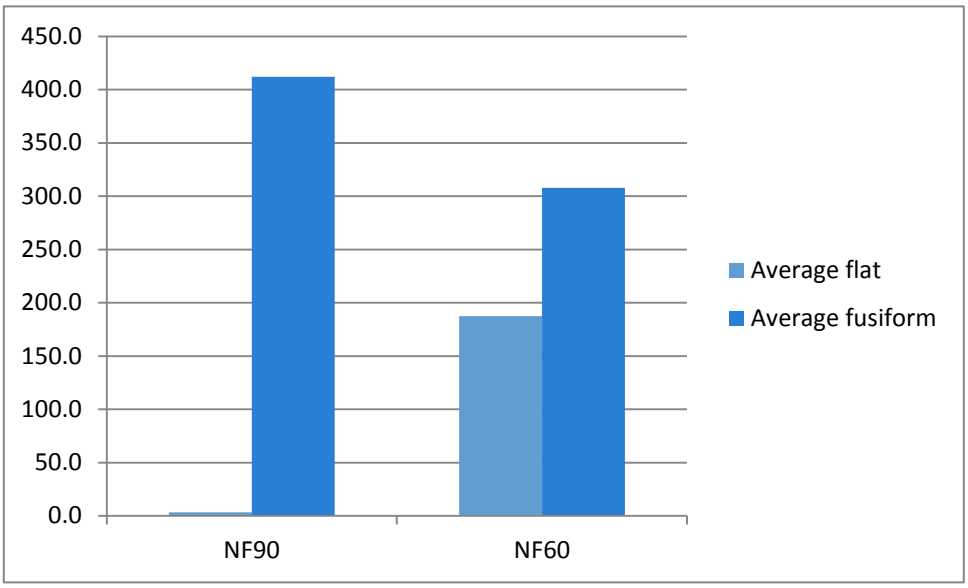
This is a more complicated question than it seems and I did not discuss it in detail because the actual implications of this observation mean very little for our study design. In fact, I actually noted the differences among all sites as only being represented between these two sites to emphasize how little difference there was among the sites and that the metric did not indicate a PAPA-related impairment. The specific result is referenced in section 3.2.3 and illustrated in Figure 3.2.3. The site with the greatest average EPT taxa richness was NF60. It was significantly different only from the site with the lowest NF90. Elevated EPT richness was only related specifically to the differences between these two sites (NF60, NF90). NF60 was not different from any other site and NF90 was not different from any other site.

The short and simplest answer is that there were about an average of 2-3 more mayfly species per sample at NF60 than at NF90. I say, "about" because the comparison of samples uses averages and estimates of variance, and there is no such thing as a fraction of a species. Often it was the Heptageniidae (*Rhithrogena* sp.) or another broad, flat taxon, *Asioplax* sp. (Leptohyphidae (=Tricorythidae)) that was present in samples from NF60 but not in samples from NF90. However, before one concludes that this means there are fewer species at NF90, we need to carefully examine the limitations of this design. Specifically, this study uses elements of bioassessment designs (e.g., metrics, fixed-count subsamples) because the goals were to assess water-quality changes, not to assess population dynamics of a species or of groups of species. Using this design, when one species becomes more abundant it means another species will have reduced relative abundance and may

exhibit abundances below the detection limit of the sampling method. The field, laboratory, and analytical methods used to assess community changes related to water quality are standardized to richness per number of animals. That is, the number of organisms examined is used as the ultimate unit standardization criterion, not the area of stream bottom (as discussed several places in this report). For our study, we have 8 samples from which 200-organsims were identified. To address your question and determine if a specific taxon was lacking, we added all the samples together for each site and contrasted the taxa list for both sites. This effort uses a more complete taxa list than the one used to test hypotheses and is standardized as richness per >1600 organisms. The only mayfly taxon present at NF60 but not at NF90 was *Asioplax*. Thus, *Rhithrogena* occurred in reduced relative abundances but was not absent, and *Asioplax* was very rare or absent (less than 1 individual per 1600 organisms (<0.06%)). These two genera have one thing in common; they have a similar body form. Both taxa are dorsoventrally compressed with bodies that are wide and flat to facilitate life on the flat surface of rocks and flowing water. The river bottom at NF90 is extensively covered by aquatic vascular plants; this likely favors a different body form such as the fusiform bodies of baetid mayflies. Wiersema et al. (2001) described the specific habitat of *Asioplax* (*A. numinuh*) in Texas as being on the top of large flat cobble with thick coatings of periphyton and silt. This habitat was lacking at NF90, therefore its absence from benthic grab samples should not be surprising.

Though there were usually more mayfly species at NF60 than at NF90, we found a baetid mayfly from NF90 that did not occur among the 1600 invertebrates identified from NF60. *Procleon* sp. has the general fusiform body-form common among the Baetidae and has distinctive pigmentation that makes it easy to identify. The specimen occurs among PAPA samples only occasionally and in very low abundances. *Procleon* has a very low tolerance to organic pollution; 1.5 on a scale of 10 (zero being extremely sensitive, ten indicating extreme tolerance). It is a common misconception that the Baetidae are more tolerant than other mayflies (e.g., Hargett and ZumBerge 2006) but this is not true (Hilsenhoff 1987, 1988, Lenat and Penrose 1993). Baetidae have more generations per year than most mayflies, asynchronous larval development and are very active swimmers. They are, therefore, excellent recolonizers of disturbed habitats and young baetids are often the first colonizers of frequently disturbed streams. For this reason, the Baetidae are sometimes excluded from EPT richness or percent EPT measures calibrated at the ecoregional scale (as with Hargett and ZumBerge 2006). The presence of *Procleon* at NF90 provides additional evidence that mayfly richness at NF90 is not suppressed by pollution. Indeed we found no significant difference among these two sites for the HBI which expresses the average pollution tolerance of the benthic community (Tables 3.2.1, 3.2.2, Fig. 3.2.8).

The relative exclusion of flat mayflies from NF90 can be inferred from the figure below which shows the average number of mayflies with clinger (flat) and swimmer (fusiform) body shapes per sample (1sq. ft.). This supports the hypothesis that the difference in mayfly richness at both sites is related to habitat exclusion of the dorsoventrally flattened body form because of natural conditions which favor the fusiform body shape (e.g., extensive macrophyte bed).



There is an incomplete sentence introducing the community dominance metric (5 taxa). Please fix and clarify.

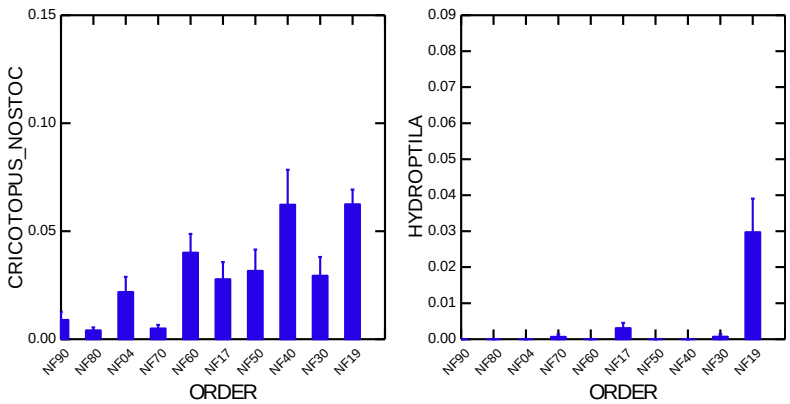
Done. It now reads as follows:
Stribling et al. (2000) found most (75%) reference sites in the Wyoming Basin Ecoregion had <87% Dominance (5 taxa). This was recommended as the 100% biotic integrity threshold for their version of the Wyoming Stream Invertebrate Index (WSII). All of the sites in this survey averaged less than 80% for the five most abundant taxa; they all scored very well for this metric—as well as the "best" streams in the state. High community dominance values usually herald the increasing influence of stressors because the metric increases as tolerant species become more dominant. Low values occur when ecological conditions become hospitable for more species; therefore, the relative abundance of any one taxon (or five taxa) is reduced.

Appendix 3, "Supplementary Graphs," is missing.

The appendices were omitted from the draft because collectively they increase the size of the report dramatically. They have been added to this final draft.

In the Functional Feeding Group graphs, the figure caption indicates that the "Other" functional feeding group shows an increasing role along a downstream gradient in the New Fork River. What species comprised the classification of "Other."

There were two groups of aquatic insects in the "Other" category. One is the midge *Cricotopus (Nostococladius),* which has a symbiotic relationship with the blue-green alga *Nostoc.* The other taxon was the Hydroptilidae caddisfly, *Hydroptila* sp., which sucks the cytoplasm from filamentous green algae, such as *Cladophora*. The only site with significant *Hydroptila* abundance was the downstream site, NF19 (figure below, proportional abundance (0.03=3%), error bars = 1SEM).



In the discussion of the density results, you say, "In 2011 (Marshall 2012), we found that worms controlled how much of the sample was used to attain 200 insects and that this resulted in samples representing different sample areas in the field." What do you mean?

This was an introduction to the problem detailed over an entire two pages in the Methods Section (Section 2.8) which described the reason we conducted an analysis of density in addition to metrics. Though not acceptable in journals (where space is at a premium), technical reports (like this document) often remind readers about the rationale for analyses they are about to read. We rephrased this text to clarify (section 3.5.6). Now it says:

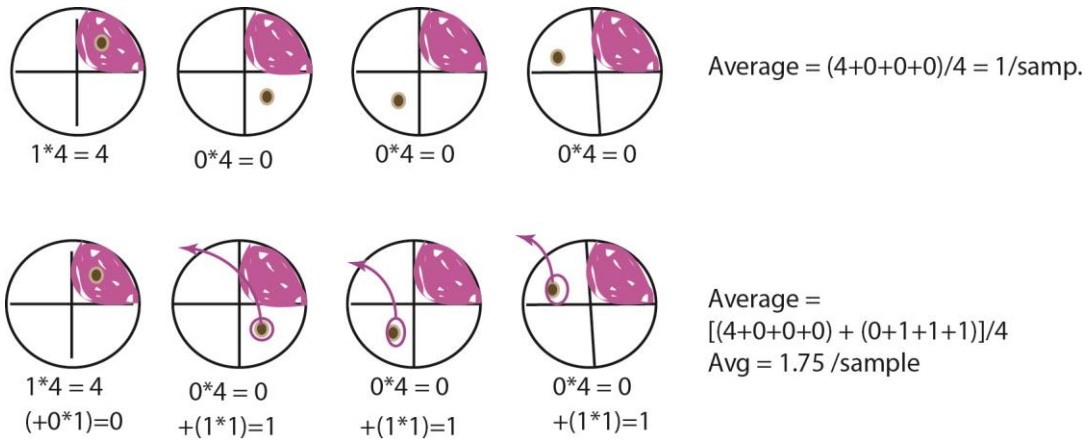
In 2011, (Marshall 2012) we found that worms influenced the amount of the sample that was processed to attain the target standard unit of effort. As a result, the data from each sample represented different proportions of samples, and each sample was 1 square foot. Therefore, the effective area analyzed in the lab varied several orders of magnitude from about 1ft^2 to about 1 square inch. The relationship in effective area with worms was highly significant in 2011 (Fig. 2.8.1, r^2=0.75 $p<0.001$) so we repeated this analysis for 2012.

You mentioned that there is a way which circumvents some of these problems with density by processing the samples differently in the lab. How would this work and what would it cost.

One way we have found to circumvent these problems is to split the sample into coarse and fine fractions using a 2mm and 0.0002mm (200µm) sieves. A set proportion of each of these fractions is processed in the lab and the fractions are combined or analyzed separately. This involves processing each sample twice for all phases. For example, we'd have to sort a fine portion of a sample, then sort the coarse portion… then we'd mount midges for the fine portion… then mount midges for the coarse portion… etc. For the first year we would expect the cost of samples to double. Also we would need about 100-120 hours to write up the results in a useful and defensible way to ensure the continued long-term continuity of the PAPA data set. We would want to be able to make the data comparable to the earlier years of the monitoring program, while also being able to perform some calculations that improve the data.

Another way, which will not be as quantitative, will be to do a "large-rare" search. This procedure has a technician scan the left-over portion of the sample for 20-30 minutes to recover any species from the sample that they had not found in the fixed-count. The specimens would be placed in a separate vial, and processed separately. This would not allow us to improve our density estimates, but it would help address the issue of missing taxa among samples. It is a subjective and limited approach to visually fractioning. There will be a smaller representation of "coarse fraction" that would be processed in addition to the sample. Thus, even though we would process two fractions through all phases of sorting and taxonomy, the coarse fraction would have received dramatically reduced laboratory effort. This would increase the cost per sample by about $50. However, SCCD needs to understand that the large rare search method always introduces bias of higher abundances; especially for taxa that have lower abundances. For low-abundant taxa, this method will add about 75% error to the density estimates (see figure below).

Another way to do this is to alter the subsampling target to exclude worms from the fixed count. They would still be counted and identified, but they would not have as much control over the level of effort and not as much influence on density estimates. For samples with high worm abundance (~50%), this basically will double sorting time and taxonomy time… doubling the cost of the sample. For samples with more moderate worm abundance (~25%) this will have about a 50% increase in sorting time and taxonomy time. For samples with low worm abundance this should have very minor effects on lab effort. I examined the samples collected in 2012 and estimated the average change in sample cost for this method. We found that changing to this way of processing in 2012 would have increased the average lab effort by 29.21%. Assuming the same numbers of samples are collected in future years, we can make this change for a 29% increase in sample cost; making this the most cost effective method to deal with the problem. There will need to be about 100-120 hours of reporting and analysis to document and ensure compatibility with previous years.



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Assessment of the Biological Condition of the New Fork River, in the Vicinity of the Pinedale Anticline Project Area: 2012

AN ASSESSMENT OF CHANGES AMONG BENTHIC MACROINVERTEBRATE ASSEMBLAGES

1.0 BACKGROUND

The purpose of this study is to characterize the biological condition of the New Fork River, Sublette County, Wyoming, and to assess impacts related to natural gas development on the New Fork River. This monitoring program has been active for thirteen years (2000-2012) and incorporates the Sublette County Conservation District's baseline biological monitoring of the New Fork River. Although the focus is on 2007-2012[5], this report draws upon thirteen years of biological data (2000-2012) to describe changes in the ecological condition of the New Fork River.

This study is more complex than a typical bioassessment[6] because it needs to be; complexity is required to discern natural gas related impacts from natural variation in a very dynamic river system. There are several other forms of human influence (e.g., construction, sewer discharge) in the drainage as well as natural influences (e.g., stream size, substrata composition, mineral springs etc.) from which potential impacts will need to be differentiated. Thus, this study is more complex than many biological monitoring programs in the state, but it is that way because it needs to be to fulfill its purpose.

In 2007 we introduced several new methods to the study, including new sites to help tease out the influence of the sediment laden East Fork River and an altogether new study reach to assess potential impacts of increasing development on the northern portion of the Mesa. Additionally, we introduced an improved sampling method to the study which allowed improved statistical analyses. This report's analyses concentrate on the five years of quantifiable, replicated sampling, while drawing upon 13 years of descriptive trends and changes among sites.

PURPOSE

This survey's purpose is to document the river's benthic community structure in such a way that the cumulative effect of both physical and chemical stressors could be documented in an efficient integrated manner. Measurements are made at a scale appropriate for the response variables (aspects of macroinvertebrate community structure), and should not be confused with the methods used to evaluate whole watersheds at a geological spatial-temporal scale (as for hydrology or geomorphology surveys, where direct measurements of the physical environment are in and of themselves response variables). This distinction was not apparent to some reviewers in past years causing some misunderstanding among reviewers with geomorphology experience; apparently some readers had assumed that the purpose of our ecological survey was to replace a geomorphology study by using macroinvertebrates as a surrogate measure for habitat assessment. Therefore, I want to reiterate the purpose very clearly here: *the purpose of this ecological survey is to document the composition of macroinvertebrate assemblages of the New Fork River in such a way that potential ecological perturbations can be assessed with some insight of cause and effect.*

[5] These years used a more rigorous statistical design.

[6] Bioassessment is a study design used by State Regulatory Agencies using a "regional reference", but with no capacity to describe change.

1.1 BIOLOGICAL MONITORING BACKGROUND

Assessment of the biological condition of surface waters has become a key element in the comprehensive monitoring of water quality in the United States and beyond. State and federal agencies have been refining the techniques for regional assessment for about two decades (e.g., Plafkin et al. 1989, Barbour et al. 1999). However, these "rapid bioassessment protocols" use regional models that are not appropriate to evaluate the site-specific concerns required to assess change in the New Fork River. Site-specific designs, like those we use to assess the New Fork River are not new; Ruth Patrick of the Academy of Natural Sciences began performing such surveys as early as the 1940's. The Academy still conducts detailed faunal surveys to assess the effects of complex perturbations on the ecological processes of streams and rivers across the USA. As computing power has advanced, so too have the methods used to assess change in ecological communities; more complex calculations and simulations are now feasible (Underwood 1997).

Invertebrates are the most commonly used animal assemblage[7] used to describe ecological changes in rivers. "Benthic" is an adjective implying association with the bottom of streams or lakes. The "macro" part of the name means that, for much of the animals' life cycle, they are large enough to be seen without a microscope (though microscopes are required to identify them). Invertebrates are animals without backbones. Thus, studies of benthic macroinvertebrates are specifically concerned with aquatic insects, mussels, snails, worms, crayfish, crustaceans, mites, leeches and similar organisms. The monitoring program does not use data from bacteria (they are micro-invertebrates) or fish (they are macro-vertebrates). These groups can also be used for biological monitoring, but their spatial temporal scales of response are not appropriate for the local scale of this project and would not allow impacts to be located.

Invertebrates are incredibly diverse and abundant. They are also critically important because they play critical roles in detrital food webs—including the breaking down of complex organic material—and in transferring energy to higher trophic[8] levels by serving as food sources. Together, these aspects make macroinvertebrate assemblages excellent indicators of the overall health—or condition—of any ecosystem:

- They are numerous enough to be effectively sampled.
- They are diverse enough to exhibit response signatures.
- They are important and relevant to all "higher" animals.
- They respond rapidly enough to provide early warnings of problems.
- Their response to disturbance is recognized as important by many agencies.

For these reasons, benthic macroinvertebrates are often used to assess the effects of human activities to streams and rivers. Thus, they may be used to describe the impacts of development and to describe the effectiveness of restoration (or mitigation). This is the rationale behind this study.

[7] Assemblages are collections of species living together (a sub-set of community, which is more inclusive).

[8] "Trophic structure" refers to the level of organisms in the food chain (or food webs) and specifically refers to their roles in processing organic matter and moving its energy to other groups of animals. For example, algae, algae eating invertebrates, predatory invertebrates and fish, might represent different trophic levels in a food web.

1.2 REVIEW PREVIOUS PAPA SURFACE WATER BIOLOGICAL MONITORING RESULTS

The most significant findings of previous reports indicated that the New Fork River was experiencing a significant active erosion source below the pipeline crossing between NF40 and NF30 (Marshall 2008). The macroinvertebrate assemblages at the site downstream from the pipeline were dominated by sediment tolerant organisms—mostly midges and worms (*Nais*). mayflies and stoneflies were not as common at the site as they should have been[9].

A tour of riverside PAPA development completed after the report (Marshall 2008) indicated that many of the erosion controls implemented at drilling sites had failed over time and new drilling platforms often lacked erosion controls altogether. Additionally, several drilling platforms had been built in the floodplain, over hyporheic stream channels where they are likely to induce sedimentation of the New Fork River through sub-surface flows. These findings were not in the report, but were presented to the PAPA task group in September 2008 and again in October 2009.

Although the site tour indicated that operators were not doing all they could to prevent sedimentation in the New Fork River, it had been unclear how much multiple years of drought influenced retention of sandy sediments. We do know that the New Fork River had experienced dramatically reduced flows from low precipitation from 2001-2008 with first sustained spring runoff event occurring in 2009 (Figs. 2.2.1, 2.2.2).

Spring run-off is an important natural phenomenon for western rivers and typically results in the sorting and redistribution of organic and inorganic substrata. Nine years of below normal flows may have prevented natural processes from mediating the effects of development in the PAPA. The elevated flow reduced the relative abundance of midges and worms, while increasing the richness of EPT orders and EPT abundance. The net effects were dramatically improved conditions at all sites on the New Fork River, and no-net change at the East Fork River site.

However, our analyses found that the abundances of these worms were greater where aquatic plants flourished (Marshall 2011, 2012).

This year's report examined some other aspects of the composition of benthic communities relating to worm abundance.

Flows were more typical in 2009, 2010, 2011 and 2012. Although run off flows of 2010 were of typical magnitude, they were not sustained for as long (fig. 2.2.2). In 2010, worms increased at NF40. In 2011, worms increased at NF40 again. However, our analysis found them more strongly associated with aquatic mosses than with fine sediments. Since we have no reason to believe moss growth is due to PAPA related impacts, these changes in benthic community structure are probably not indicative of PAPA-related impairments in water quality. Furthermore, analysis of the Density of the various orders (especially EPT orders) indicated that although relative abundance was low at NF40, Density at this location did not appear significantly reduced. This suggests that any actual changes in the condition of the resource at this location were very minor.

[9] The expectations were drawn from upstream sites and from the SCCD baseline monitoring dataset.

2.0 METHODS

2.1 STUDY SITES

Some of the sites used for this survey were part of the SCCD's baseline biological monitoring network for the New Fork River (NF01, NF04, NF17, NF19) and others were added later specifically to assess the influence of PAPA development on the New Fork River (NF30, NF40, NF50, NF60, NF70, NF80, NF90). In 2009, the site NF01 was replaced with a new upstream reference (NF80) because NF01 was too different (naturally) to serve as an adequate reference[10]. For this study, an additional upstream reference (NF90) was added because new development has encroached on NF80.

NF04 NEW FORK RIVER

NF04 is located south of Pinedale ~2 miles and is 50 feet downstream from the South Tyler Bridge. South Tyler is an access road for the PAPA. NF04, when established, was located upstream of the PAPA. A Wyoming Game and Fish Department fishing access and boat-launch are located at the sampling site. NF04 is also located downstream of the confluences of NF02 Willow Creek and NF03 Duck Creek; the confluence of these streams is believed to coincide with dramatic changes in the chemical and biological makeup of the New Fork River (Marshall 2005a). Additionally, increased development on the north end of the Mesa may contribute potential runoff to the New Fork River upstream of this site.

NF17 EAST FORK RIVER

NF17 is located on the East Fork River, ~0.125 miles upstream of the confluence with the New Fork River. The Wyoming Game and Fish Department Boulder Fish Rearing Station is located upstream of NF17. NF17 is located downstream of HWY 191 approximately 5 miles. The East Fork River at NF17 is a sand dominated system with active sediment transportation occurring continually. In combination with several other sites, this site serves as a reference to account for changes downstream because it is a natural source of fine sediments that change the nature of the New Fork River's substrate composition and biology.

NF19 NEW FORK RIVER

NF19 is located on the New Fork River, upstream of the confluence with the Green River ~2 miles. The site is ~1½ miles downstream of HWY 351. Badlands lie adjacent to the New Fork River upstream of NF19. NF19 is downstream from the PAPA. NF19 is the last sampling site in the New Fork River watershed. It serves to describe the condition of the New Fork River before it mixes with the Green River, and to help characterize the nature of upstream changes. Currently, this site is the ultimate recovery zone site and development did not reach this site during the period of this study. However, its continued status as a downstream reference could be compromised if gas development increases in the area.

NF30 NEW FORK RIVER

NF30 is located downstream of most of the PAPA development and below several pipelines' hyporheic crossings. The site is located on BLM land and has been sampled since the year 2001. A reclaimed gravel pit is located west of the sampling site. NF30 is located downstream of the confluence of the East Fork River (NF17) ~3 miles. Five replicated samples were collected at this site from 2004-2008. These samples represent the "study" community that was compared to NF40 and NF50 to describe the effects of development in the PAPA.

[10] NF01 exhibits about 50% of the discharge of NF80 and sites farther downstream. It is influenced by an impounded lake and is dominated by invertebrate species that do not normally occur downstream.

NF40 NEW FORK RIVER

NF40 is located within the PAPA and above the pipelines' crossings. The site is below the confluence of the East Fork River (NF17), Sand Springs and Alkali Draws and upstream from NF30 by about 1.5 miles. Five replicated samples were collected at this site during the years 2004 to 2008, but it was not sampled prior to 2004. These replicated samples originally represented the "control" community for comparisons with NF30 to describe the effects of the PAPA. The site is not an ideal control site because there is potential influence from Sand Springs Draw and Alkali Draw during runoff. This is likely to become more of a problem with planned development in the upper reaches of Sand Springs Draw. Thus, this site is now considered a measurement of the combined influence of Sand Springs and Alkali Draws, when compared to NF50.

NF50 NEW FORK RIVER

NF50 is located downstream of the confluence of the East Fork River (NF17) ~½ mile and upstream of Sand Springs and Alkali Draws. This site was established in 2007 to account for the effects of the East Fork River on the biota of the New Fork River. This is important because NF40 may be influenced by elevated sediment expulsion from Sand Springs and Alkali Draws. If this were to occur, there would be no way to differentiate the effect from the influence of the sand-laden East Fork River. A Bureau of Land Management public fishing access and boat launch area is located at this sampling site. Only biological data is collected at NF50 based upon the decision of the Pinedale Anticline Water Task Group. No chemical data is collected at NF50.

NF60 NEW FORK RIVER

NF60 is located upstream of the confluence of the East Fork River (NF17) with the New Fork River ~3/4 of a mile. NF60 was established in 2007 to describe the condition of the New Fork River before it is influenced by the East Fork River. This is important for documenting the influence of the East Fork River on the New Fork River at NF50. Only biological data is collected at NF60 based upon the decision of the Pinedale Anticline Water Task Group. No chemical data is collected at NF60.

NF70 NEW FORK RIVER

NF70 is located downstream of the confluence of Pole Creek ~¼ mile and downstream of NF04 ~ 4 miles. NF70 was established to monitor any effects from exploration and development from the northern portion of the Pinedale Anticline Project Area. This site measures the cumulative changes related to the gas development and the influence of Pinedale's sewage treatment plant (Pine Creek) which may change over time if facility management should change. Only biological data is collected at NF70 based upon the decision of the Pinedale Anticline Water Task Group (no chemical data available).

NF80 NEW FORK RIVER

NF80 is located downstream of the confluence of Duck Creek ~1 mile and upstream of NF04 ~1 mile. NF80 was established to monitor any effects from exploration and development from the upper portion of the PAPA. Upstream of NF80 is the town of Pinedale, a golf course and subdivisions. Both chemical and biological data are collected at this site.

NF90 NEW FORK RIVER

Site NF90 was added as the upstream control site for the surface water biological monitoring program when new exploration and development occurred upstream of NF80 in 2009. NF90 is located at a BLM public fishing access and is known locally as "The Bull Pasture." It is a low gradient reach with benthic substrata dominated by aquatic vascular plants. NF90 is located north west of Pinedale approximately 5 miles and is upstream of the confluences of both Willow and Duck Creeks. Both biological and chemical data are collected at NF90. NF90 was a reference site used for development of WY DEQ's Wyoming Stream Invertebrate Index.

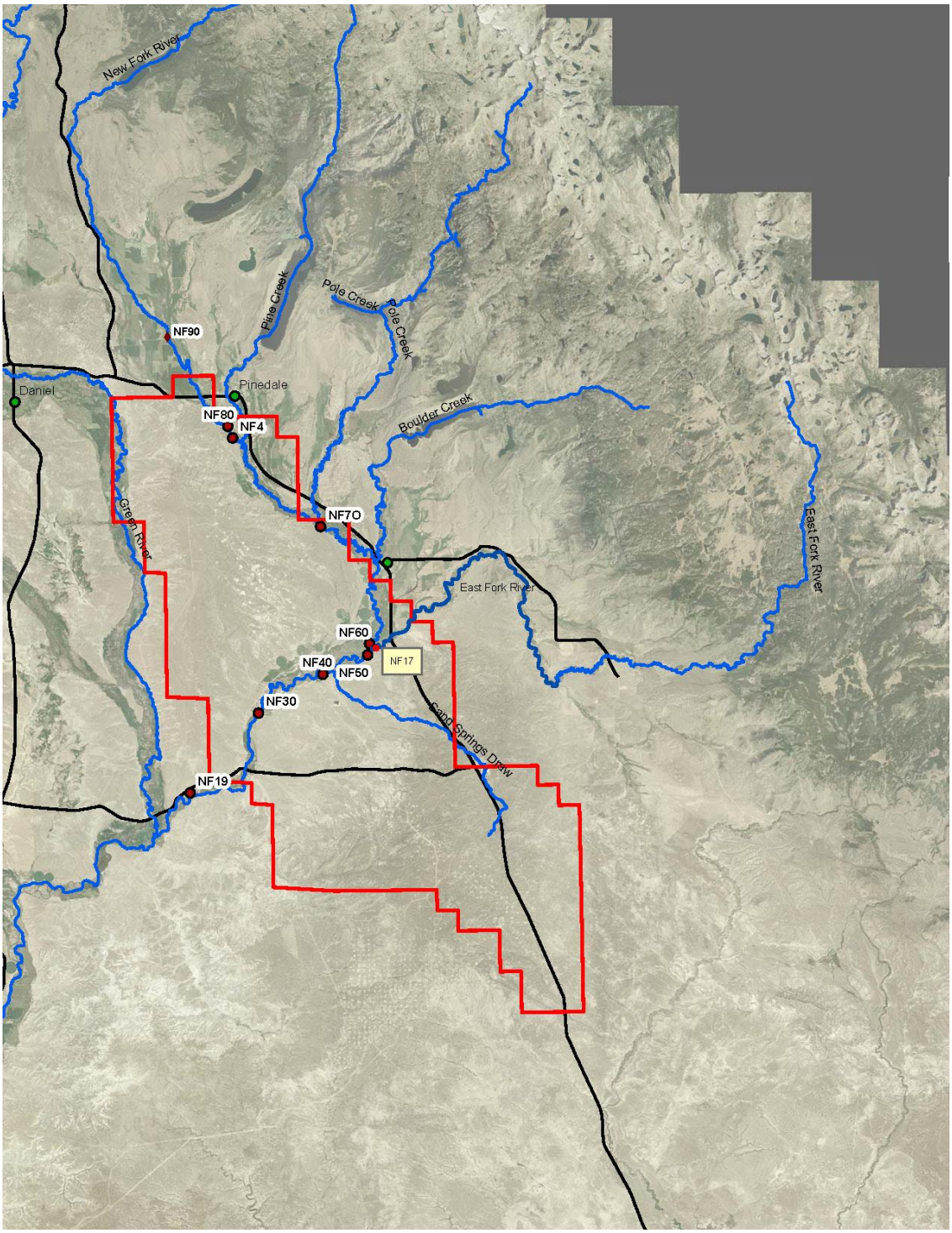

Figure 2.1.1. Pinedale Anticline Project Area. The study area consisted of nine sites from the New Fork River and one site on the East Fork River. The goal is to assess changes in the condition of the New Fork River as it passes though the Pinedale Anticline Project Area (outlined in red) of central Sublette County. In 2010, NF90 was added as the upstream control site for the surface water monitoring program when new exploration and development occurred upstream of NF80 in 2009. This figure was prepared by Sublette Country Conservation District.

2.2 NEW FORK RIVER FLOW CONDITIONS

It is impossible to consider ecological changes in the New Fork River without doing so in the context of the river's annual discharge regime. River flows determine the distribution of flow-loving organisms, the distribution and storage of organic and inorganic substrata, and the ecological success of species exploiting different ecological niches. The flow regimes of rivers of the Sublette County are driven by slow melt which causes a large seasonal discharge pulse in the late spring, followed by lower baseline flows through summer, autumn and winter. This pulse determines the assortment[11] of inorganic substrata for a large part of the year. For the first 8 years of the survey, the New Fork River had below average spring discharge peaks and fine materials accumulated in the interstices of the river bottom. In 2009, we observed the first above average sustained discharge event, which resulted in a clearing of the fines which had accumulated among the interstices (Fig. 2.2.1). Peak runoff flows in 2010 were about average in magnitude but a little shorter in duration than normal (Fig. 2.2.1). The Peak flows in 2011 were the greatest since our invertebrate reports began with a crest about 800% of the 58-year average (Fig. 2.2.1).

Peak runoff flows in 2012 were reduced compared to 2011 and below average (58 years) in duration (Fig. 2.2.2). At the very peak of runoff, the New Fork River crested at about 1,300 cubic feet per second (cfs), with the 58-year average peak flow being near 1,200 (cfs), but this crest was about 20% the average duration of average peak flow (Fig. 2.2.1, Fig.2.2.2). By the time samples were collected in August and September, the base flow of the New Fork River was about equal to the average (58 years) base flow and about equal to the flows during which samples were collected for these annual surveys. However, the base flows of 2011 were about 100 cfs greater than the flows from which samples were traditionally collected. Thus, the actual placement of benthic sampling gear in the thalweg in 2012 was similar to all previous years except 2011. In 2011, a more marginal placement was required because of higher water levels when samples were collected.

[11] The complex mosaic of different sized inorganic particles in the river results from the particles being "assorted" by flow. Assortment in this case refers to the results of this process.

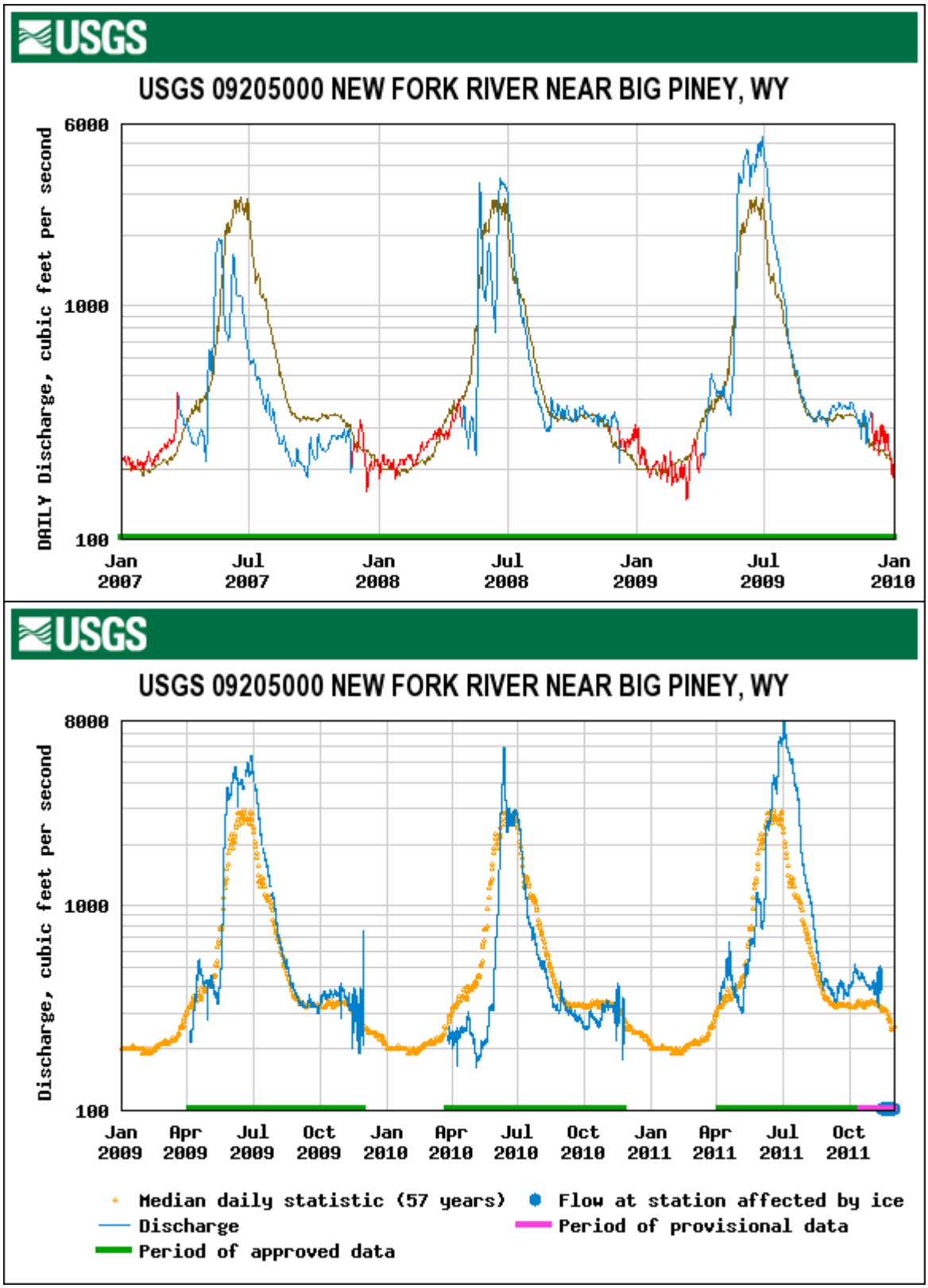

Figure 2.2.1. Historic New Fork River Discharge. The discharge is shown for 2007 through 2011 in cubic feet per second from data acquired from the US Geological Society.

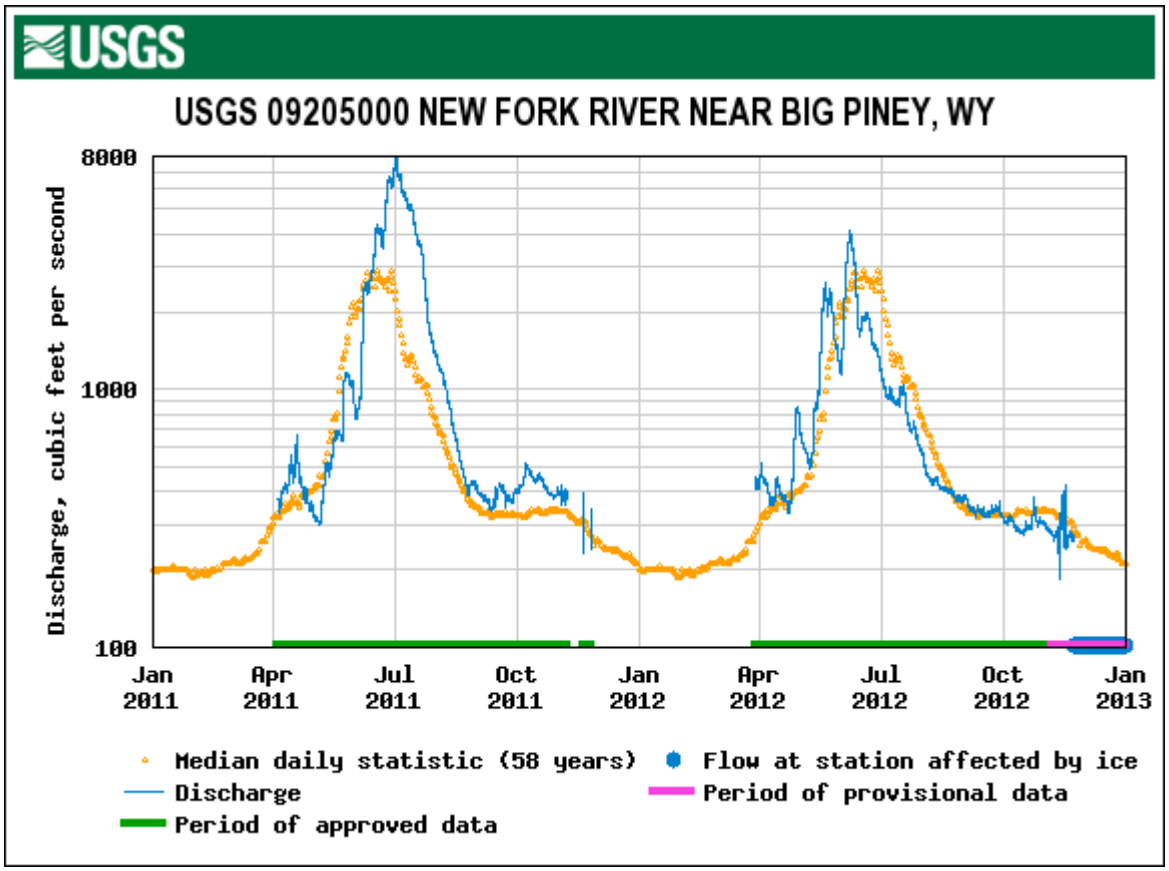

Figure 2.2.2. New Fork River Discharge, 2012. The discharge is shown for 2011 and 2012 in cubic feet per second from data acquired from the US Geological Society. The data displayed in gold is the 58-year average flow.

2.3 HOW THE SITES FIT TOGETHER

The study sites represent a cumulative gradient of effects. Development of this study was a process of evolution from a simple comparison of two sites (NF30 and NF40 (Marshall 2005)) to a more complex study design using statistical procedures to tease out the effects of sources of variation not related to PAPA development. To understand how the sites fit together, we need to consider how the New Fork River might come to be influenced by development on the PAPA. We identified several modes whereby the integrity of the New Fork River could be affected by runoff from development on the PAPA. Currently there are two regions where potential effects of PAPA development are likely to accumulate as measureable impacts. We have separated these two areas into the upper and lower study areas to facilitate discussion and analysis. Additionally, the middle study area was selected to account for ecosystem changes related to the confluence and integration of New Fork River and the East Fork River. Graphs throughout the results section of this report have been trisected to clearly show the three study areas as well as the relative location of study sites along the downstream gradient. We have prioritized these locations based on the likely movement of surface waters during rain and snow melt events. This makes sense because these events are the most likely source of disturbance for surface waters—which, if they were to occur, would most likely to be in the form of eroded soil and sedimentation in streams. Additionally, if leachate or other industrial chemicals are spilled on soil, their eventual arrival in river systems would likely correspond to runoff events. Note that there were no direct disposal effluents prior to 2009, but Anticline Disposal activated a permitted diffused effluent during 2009.

Much of the development on the Mesa occurs in an area where the flow of run-off events is directed southeast, toward the lower study area. Similarly the development in the southeastern PAPA is most likely to experience runoff to the lower study area. In 2007, concerns over intensifying development in the northern PAPA necessitated a study area that could differentiate these influences from natural variation and anthropogenic influences farther upstream. As development in the PAPA increased, so too did the potential impacts further upstream and thus, the upper study area was initiated.

THE UPPER STUDY AREA

Although most of the runoff from the Mesa flows southeast, there is an area on the northern edge of the Mesa which drains northerly. In 2007, three sites (NF01, NF04, NF70) were sampled to account for changes in this study area. In 2008, NF01 was replaced with a more appropriate reference site (NF80) occurring downstream of Willow Creek and Duck Creek. In 2010, we added NF90 to ensure development near NF80 did not bias our upstream reference. We expect NF80 and NF90 to reflect very similar ecological benthic communities unless development begins to degrade the ecological function of NF80.

Although the upper study area is smaller than the lower study area, the gradients are as complex as those occurring in the lower project area. Duck Creek and Willow Creek are known to influence the chemical and biological composition of the New Fork River (Marshall 2005a). These tributaries increase the conductivity of the New Fork River's water and seem to also increase the amount of suspended and organic material.

NF70 integrates the effects of several smaller drainage systems off the Mesa, but is also influenced by Pine Creek and Pole Creek. These tributaries may dilute the waters contributed from Duck Creek and Willow Creek, but the influences of Pine Creek may change from year to year as it serves as a conduit for the Pinedale waste water treatment plant. This makes it difficult to assess PAPA-related influences from other anthropogenic stressors. Thus we added replicates to the NF70 site in 2007 so that statistical procedures could be used to correct for different sources of variation. Over the long-term, the temporal changes occurring at this site relative to NF04 and NF60 (the upstream site of the lower study unit) will be important to diagnose changes within the upper study area and the lower study area as well.

The expected response signature for an extensive degradation of the New Fork River would include a decline in condition at NF80 or NF04 (relative to NF90) persisting farther downstream to NF70. Note that some ecological measures increase in response to ecological degradation, whereas others decrease. We use the word decline as a qualitative term of ecological condition, not as a quantitative measure of actual change.

THE MIDDLE STUDY AREA

Sites of this area are designed to account for the integration of the East Fork River and the effects of the confluence of the East Fork River on the biota of the New Fork River. NF17, the only site on the East Fork River, had traditionally been represented by a single bioassessment sample, which did not allow us to account for variation in the New Fork River that may be related to inputs from this naturally sandy system. This site was eventually augmented with replicate samples in 2009 to allow us to include it as a spatial-temporal variable in the statistical models. Conditions at NF50 should result from a combination of the conditions at NF60 and NF17. Additionally, since NF50 is only a short distance from NF40, it should also serve as a reference for the lower study area above the influence of Sand Springs Draw and Alkali Draw.

THE LOWER STUDY AREA

Originally, the primary concern was runoff directly from development on the southeast section of the PAPA. This was the rationale for the early addition of NF30. NF40 was added later to serve as a benchmark by which to gauge changes at the NF30 site. It soon became clear that this was not sufficient because we needed to account for changes from the New Fork River, the East Fork River and potential impacts from runoff through Sand Springs and Alkali Draws. These two seasonal, ephermeral drainages drain the area occupied by the Anticline Disposal site and contribute to the New Fork River between NF40 and NF50. Thus several sites were added to account for this gradient.

The difference between NF50 and NF40 may account for runoff flushed through the Sand Springs Draw and Alkali Draw. Direct runoff (as opposed to indirect runoff) from the PAPA would be represented from changes in the condition of NF40 to NF30 (Fig. 2.3.1).

Most of the land comprising the Mesa drains to the southeast. Thus, it is likely that potential runoff and erosion would enter the river from the south-eastern edge of the Mesa. However, field investigations in 2006 indicated an extensive wetland system which would buffer the river from the effects of runoff from the southeast edge of the Mesa (Fig 2.3.1). Thus, the most likely source of impacts to the lower study area used to be runoff from the southeast portion of the PAPA — directly (i.e., pipelines or site-runoff to the northwest) or indirectly (via the draws).

In 2007, development on the northwest side of the river encroached into the riparian zone—circumventing the natural wetland buffer. Now, the potential for impairment includes direct runoff and hyporheic disturbance from development on the northwest side of the lower study area.

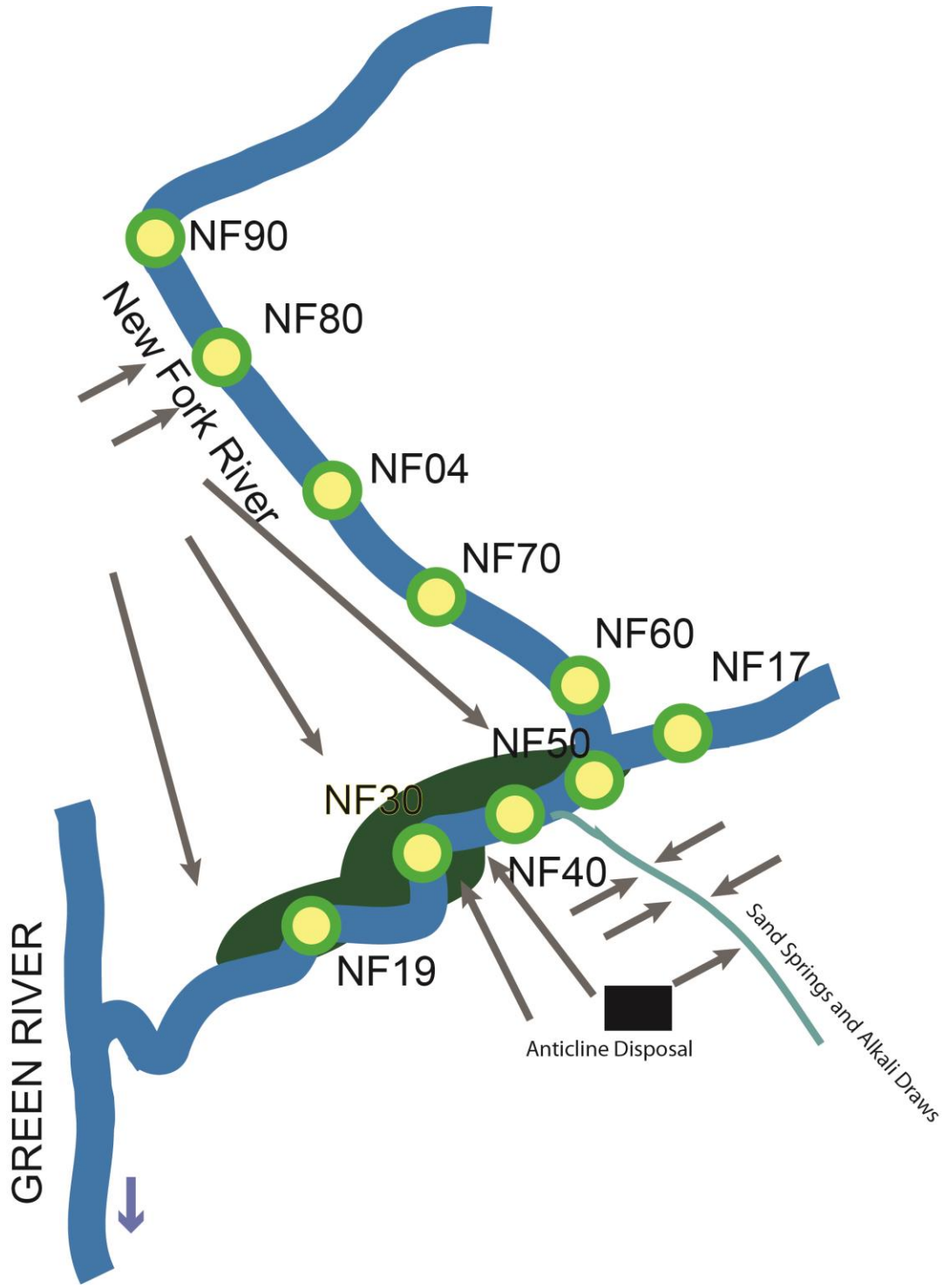

Figure 2.3.1. Study Site Schematic. This diagram shows the interrelationship among the location of study sites and potential sources of runoff in the PAPA. Arrows indicate potential vectors of influence on the New Fork River from development on the PAPA. Although most of the runoff on the Mesa drains to the southeast, this runoff encounters several wetland systems and is unlikely to actually reach the river. The sample sites are marked as circles on the river.

2.4 FIELD METHODS

Since 2007, we used an improved field sampling plan to enable use of more substantial statistical analyses to differentiate anthropogenic changes in the New Fork River from natural variation—and variation not related to development on the PAPA. In the 2007 report (Marshall 2008), we reported both the historic method and the altered method simultaneously. This report considers only the sampling method. This allows us to compare differences among sites spatially and to compare those sites with earlier years to evaluate change. The replicated sampling method was called the "single Surber" (SS) method in earlier reports (Marshall 2008) and we retained that nomenclature each year since for consistency.

SINGLE SAMPLE METHODS[12]

In the early years of the monitoring program (2005-2006), SCCD collected 5 replicate samples each composed of the aggregate of eight standard Surber samples (1 square foot (0.093m^2), 500µm mesh net) using Wyoming Department of Environmental Quality's (WY DEQ) standard field collection techniques. For the SCCD field crew to collect five replicates, they had to actually sample (i.e., vigorously scrub) 40 ft^2 to gain only the discriminatory power of 5 replicates. We found these methods of sample collection were inadequate for the survey for several reasons, including unacceptable with site variance, taxonomic incompleteness, and efficiency (Marshall 2008, 2013).

In 2007-2012, we collected eight single Surber samples from each site, deviating from the WY DEQ sampling methods only in the act of not compositing in the field. Samples may be electronically composited at another date if desired for comparison to regional reference criteria. By keeping the samples separate, we reduced within-site variance, attained more taxonomically representative samples, and reduced cost of both field and laboratory work. We also were able to use more advanced statistical algorithms to correlate macroinvertebrate measures with environmental variables to aid inferences of cause-and-effect. Again, samples may be electronically composited at a later time if necessary, but once they are composited in the field, this valuable variation is lost forever.

Single samples were collected using a stratified random sampling regime where near-substrate flow measures were used to ensure that the samples from each site fell within a uniform range of flows. This procedure is important for several reasons. First, it ensures that flows are uniform among sites. We know that near-substrata flows can account for a very large amount of variation in aquatic invertebrate assemblages (e.g., Hart and Fonseca 1998, Hart and Finelli 1999) and we know that gas development is not likely to alter flow regimes. Therefore, we can prevent water velocity from confounding our results by sampling a consistent range of velocities. Additionally, sampling a range of flows at sites allows us to account for the effects of velocity on biological measures statistically. This requires each site to have a sufficient range of velocities to encompass a meaningful amount of biological variation, and we need the range to be similar among all sites[13]. This technique is called Analysis of Covariance (ANCOVA; Zar 1999) and it can be accomplished using the General Linear Models (GLM) algorithm (Wilkinson 2006) common among statistical software packages.

Unlike the composited samples commonly used by WY DEQ, our sampling method allowed us to relate other habitat variables directly to biological measurements. Some of these could be related to natural gas development—or due to natural variation. For example, the field crew characterized both substrata composition within samples and the amount of vegetation within samples. Substrata composition could be affected by erosion but we do not anticipate any direct effect of PAPA development on aquatic plants. This hypothesis could be tested by physical habitat monitoring by SCCD.

Within each sample, SCCD staff described the size distribution of inorganic substrata and quantified the relative cover different forms of aquatic vegetation. These data were provided as percent of inorganic substrata covered by the vegetation type, and were very useful covariates explaining significant variation in some invertebrate response metrics.

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[12] In 2012, all samples were composed of single Surber samples, representing 1ft^2, with several replicates from each site. Prior to 2007, some sites in the PAPA were monitored using composite samples where each individual sample was composed of 8 Surber samples. The 2007 report found that there were significant differences in richness and diversity measures attained by the different methods, but that the single samples could be made comparable using rarefaction analyses.

[13] The target range included relatively slower riffle areas (~0.6fps) and faster riffle areas (~1.6fps).

This method is limited by not accounting for thickness of vegetation cover. For example, if 50% of the rock surfaces are covered with 1mm thick *Didymosphenia geminata* mats the DIDYMO variable would be 50%. If 50% of the rock surfaces were covered with 6 inches of *D. geminata* slime, the DIDYMO variable would also be at 50%, even though the 6-inch mat would be much more influential on invertebrate assemblage composition. For this reason, in the last report (Marshall 2011) we encouraged the SCCD to begin gathering more quantitative data for this variable. Covariates are discussed in greater detail in section 2.7 of this report. We re-assert this need in the recommendations section of this report.

2.5 LABORATORY METHODS

Biological metrics data from 2000-2004 were entered and validated by SCCD personnel and sent to River Continuum Concepts for analysis by a professional stream ecologist. Certified professional laboratories completed all the laboratory analyses and trained SCCD staff collected all field measurements. Most biological data from 2004-2007 were generated from raw taxonomic data by EcoAnalysts, Inc. Samples collected in, since 2008 (inclusive) were processed in-house at River Continuum Concepts by a masters-level Aquatic Entomologist with >27 years of experience. Thus, this report meets the requirements for credible data defined by the State of Wyoming.

Single samples (as discussed above), were subsampled to allow the identification of 200 organisms to the lowest practical taxonomic level; usually genus-species level. If the single samples contained fewer than 200 individuals, the entire sample was identified.

Previous laboratories used different levels of taxonomy and caused some artificial inflation of taxa richness. This occurred both within and among contract laboratories; it is a natural part of dealing with large multi-species invertebrate data sets. Sometimes a taxonomist can place a solid species, species-group, or sub-species identity on one or two specimens, but can only identify some to genus level. When the data are compiled, this results in the same organism having three different names. We found this caused a problem in 2008's report (Marshall 2009). We used the same procedure that WY DEQ (Hargett and ZumBerge 2006, Hawkins et al. 2008) and US EPA (Barbour et al 1999) have used to deal with this problem: we defined Operational Taxonomic Units (OTU). Thus, all specimens were analyzed at a standard level of taxonomic effort--which truncates the dataset slightly to an appropriate level that is reproducible for all samples. The details of this process are documented in the Appendix to this report.

2.6 QUALITY ASSURANCE STATEMENT

The generally accepted norm for sorting efficiency (the proportion of the invertebrates actually removed for analysis) is that > 90% of the organisms in a sorted portion must be found and retained. The initial evaluation of the sorting efficiency indicated that all samples were processed at >91% efficiency, with an average efficiency of >97%.

2.7 ANALYTICAL METHODS

PHYSICAL VARIABLES / COVARIATES

The area contained within each benthic sample was described to provide sample-specific habitat data. These data were collected and recorded by SCCD during field collection and added to the analytical data set. These measures included depth, flow (6/10 depth and near substrata), % size composition of inorganic substrata (Wentworth 1922), embeddedness and plant cover (%). Plant cover was divided into several categories (discussed below).

WATER FLOW (VELOCITY)

Flow was measured by SCCD in the field using two methods: the 6/10 (60%) depth method and the near-substrata method. The 6/10 depth method is commonly used for discharge calculations and fisheries assessments because it often describes the average velocity of the water column. However, to provide covariates for this study, we are especially concerned with flows near benthic substrata because these flows are more correlated with the invertebrate community (e.g., Hart and Finelli 1999). Therefore, only the measurements taken near the river bottom were used to define the covariate FLOW, which was used in the GLM algorithm to account for variation among macroinvertebrate metrics. As with all covariates, these measures are intended to explain variation biological variation within and among sites, not to describe the average conditions at sites (as would be the goal of a habitat assessment).

INORGANIC SUBSTRATA

SCCD quantified the relative proportion of the various classes of inorganic particles as described by Wentworth (1922). The categories were visually estimated as Sand, Fine Gravel, Coarse Gravel, Pebble, Cobble, and Boulder. They were estimated as percent of sample volume at about 5-10% intervals. These variables were used collectively to describe the composition of benthic substrata from which invertebrates were collected. We consolidated these data into a histogram displaying the relative contribution of each substrate category. However, these variables are not suitable to be all combined into the covariance model because of autocorrelation problems; if a sample is 100% sand, it is necessary for it to have 0% cobble. An index integrating all these variables is the best way to generate a continuous variable without losing information gathered in the field.

I have found that a suitable substrate particle variable can be generated by weighting the substrate categories biologically. Specifically, integrating these data into a Substrate Particle Index (PSI) circumvents the problems of autocorrelation arising from using all the substrate variables in the modeling procedure. This index was not developed for this project; it has been used for surveys conducted for the Academy of Natural Sciences with great success nationwide (Marshall 1997, 1998, 1999, 2007a). The PSI correlates well with biological metrics and integrates the substrata size-distribution data into a single, biologically relevant variable, thereby avoiding problems with autocorrelation caused by using all the measures.

The PSI weighs the percentage of each substrate size class according to the relative suitability for invertebrate colonization. For example, many invertebrate species cannot inhabit sand habitats in flowing waters because shifting substrata bury insects or damage them through abrasion. Larger particles are more suitable for colonization because they are more-stable and provide ample space for colonization without abrasion among interstices. Optimal balance of surface area and stability is attained by a mix of cobble-sized (or cobble-pebble-sized) particles. Boulders are, of course, more stable but have less surface area per unit volume to accommodate diverse communities and therefore they are weighed down in the PSI. The formula for the PSI used as a Covariate in our analysis of biological metrics is presented below.

PSI= 0*sand + 1*Fine Gravel + 2*Coarse Gravel + 3*Pebble +4*Cobble + 1*Boulder

Embeddedness is another common element of inorganic substrate characterization measured by SCCD from each benthic sample. Embeddedness was measured as the depth of cobble buried by fines, measured in millimeters. The SCCD measured embeddedness on three cobbles and reported the average. There are other methods to estimate or express the degree to which coarse particles are embedded among fines. Other methods usually express embeddedness as a percent measure. The method used by SCCD eliminates the subjectivity of these methods, but may result in missing values when there are no particles to measure. For this reason embeddedness has not been a useful covariate in this survey.

Embeddedness data (variable EMBED) were used to compare the quality of benthic substrata from each benthic grab through one-way Analysis of Variance (ANOVA). This variable was not suitable for use in the Covariance GLM algorithm for data collected in 2012 because of missing values from samples dominated by macrophytes, which prevented measurement (there were no cobbles upon which embeddedness measures could be taken). Therefore, the dataset violated the several statistical assumptions (e.g., slope homogeneity etc.).

AQUATIC VEGETATION

SCCD personnel visually estimated the relative portion of each Surber sampler (1ft^2) that was covered with vascular plants, mosses, and algae. The specific components of the aquatic plant community were described using an informal "morphotype" nomenclature which assigned algae to certain types (designated A1~A20) based on field observations. Notes for "taxonomic" consistency among sites and years are maintained by SCCD and enhanced by having the same field observer each year of the study. Aquatic vascular plants were measured as separate cover estimates describing for macrophytes (Types M1-M7). Results from the 2010 and 2011 report indicated these variables are very influential on biological metric scores and benthic community structure. This year, we examined several aggregate and separate measures to optimize their use as covariates to explain variation in macroinvertebrate metrics. The variable VEG (short for vegetation) was originally used in 2010 and 2011 reports as a sum of all types or aquatic vegetation in sample (A10-A20 and M1-M18). The Variable ALGAE was used to identify the aggregate sum of all types of algae. The variable MOSS was used to represent the branched moss called A4 by SCCD (probably the aquatic moss *Fontinalis*). The Variable MACROPHYTES was used to represent all the vascular hydrophytes (M1-M7) in aggregate. The variable, "PLANTS" was used to combine the variables MOSS and MACROPHYTES because they both function similarly in their ability to provide colonization substrata and refugia. SCCD's A5 taxon consists of "stalked" or "slime-like" diatoms that may physically exclude macroinvertebrate from otherwise suitable substrata. This group is collectively referred to by the variable DIDYMO[14]. The variable has some shortcomings. For example, it is difficult for a subjective variable to quantify certain differences. Values are expressed as % cover based on field observations, but thick coats of slime could have the same percent cover values as thin or patchy algae mats. There were too many zero-value observations for *D. geminata* coverage (DIDYMO) to allow use as a covariate.

Aquatic vegetation variables were used to compare the sites, but only a subset of variables were used as covariates to describe differences among benthic macroinvertebrate assemblages of the New Fork River. Specifically, we used ALGAE, MACROPHYTES, and MOSS. The variables VEG and PLANTS were highly correlated with each other and with other variables. Since they were computationally redundant with the other aquatic vegetation variables, they were excluded from the GLM models used to describe spatial shifts in macroinvertebrate community structure. They were however analyzed in ANOVA to describe differences among sites to help facilitate interpretation of findings.

[14] The variable name DIDYMO is short for *Didymosphena geminata* because this species has often been observed among the detritus in benthic grab samples from the New Fork River, and the algae's large mucalagenous mats are cause for conservation concerns. However, there are other stalked diatom species with similar growth patterns; the values documented in this report are based on field observations without definitive taxonomic identification; there may be species other than *D. geminata* represented in these data.

BIOLOGICAL VARIABLES

Biological metrics summarize the changes in species composition in terms of changes in ecological function and are values calculated from the taxonomic data set (which is a list of the species collected and their abundance). Metrics were used as the response variables for most analyses. This was necessary because the abundances of species change naturally though time and in space due to changes in the environment, inter-species competition and other factors. Ecological theory (e.g. The River Continuum Concept, Vannote et al. 1980) predicts that the functions performed by these species should be conserved—unless the ecosystem's function is impaired. That is, the abundance of each species may change naturally as a response to climatic variation or natural biological cycles, but usually a reduction in the abundance of one species is accompanied by an increase in the abundance of similar species. Thus, measures like the relative abundance of collector-gatherers should be more consistent than the abundance of individual species comprising the collector-gatherer guild. This is how metrics reduce the variability in species abundances while summarizing functional changes. The metrics compared in this report are discussed briefly below (Table 2.7.1).

Taxa Richness is a very common metric that is used to describe the function of terrestrial and aquatic ecosystems. The measure is calculated by counting the number of different species (or similar kinds) in the sample. For aquatic ecology, the underlying philosophy is that more species can live in clean water than in polluted water. Therefore, higher values of taxa richness indicate a "healthier" condition and lower richness values may indicate an impaired condition.

The basis of the Shannon-Wiener Diversity Index has its origins in information theory[15] and is summarized as a value H' which reflects the richness and the abundance of all species in the community. Though the value of diversity indices vary between zero and infinity (in theory), the maximum value in SCCD samples using bioassessment composites and non-composite samples is about 3 (diversity is unitless).To help understand the cause of changes in diversity we also examined one of the major components of diversity: evenness (J'). Evenness is a measure of the equality of abundances of all species in a sample or community. It varies between zero and 1, with values of 1 indicating all species have exactly the same abundance within a sample. Basically, high diversity and evenness reflect healthy communities, whereas low diversity and evenness indicate a numerical dominance by a few species, or very few total species. Shannon Diversity for this report (and all preceding years) was calculated using natural logarithms, but diversity estimates can be converted for comparison with base-2 or base-10 logarithms by use of a scalar correction factor (e.g., Krebs 1998).

The orders Ephemeroptera, Plecoptera and Trichoptera (mayflies, stoneflies and caddisflies, respectively - EPT) are generally considered to be more sensitive to disturbance than other organisms. Although not universally true, many of these organisms need cool, flowing water with high oxygen and low ion concentrations year-round. Thus, one of the most popular metrics in the United States today is the EPT index, which is the taxa richness of these three sensitive orders (e.g., Lenat and Penrose 1993). Because these orders do not always respond uniformly, many states—including Wyoming—have started using the richness of each of the EPT orders as separate metrics. We use the complete EPT richness metric for analyses rather than the separate measures, which are more convoluted for synthesis[16].

The abundance of chironomid midges is often used as an indicator of environmental perturbation because there are >5,000 species known from the northern hemisphere. Some of the common species are very tolerant to certain stressors and reach very high abundances when densities of predatory insects or competitors are reduced in polluted waters. This metric

[15] Information theory is a branch of applied mathematics, electrical engineering, and computer science involving the quantification of information. Information theory was developed by Claude E. Shannon to find fundamental limits on signal processing operations such as compressing data and on reliably storing and communicating data. Since its inception it has broadened to find applications in many other areas, including statistical inference, natural language processing, cryptography, neurobiology, the evolution and function of molecular codes, model selection in ecology, thermal physics, quantum computing, plagiarism detection and other forms of data analysis.

[16] For example if the average Ephemperoptera richness increased by 2, and the stonefly and caddisfly richness each increase by one, in this report we would have spent three pages discussing each metric then ultimately having to discuss them as offsetting changes in EPT richness. Changes in specific taxa where analyzed and are presented as graphs in Appendix 2 with pairwise comparison results presented in the figures.

responds to organic enrichment and sedimentation. Specific taxa comprising the chironomid assemblage can be particularly useful for describing the causes of changes in multi-metric indices (like the WSII) and other metrics.

North American streams are normally dominated in abundance, richness, biomass, and production by aquatic insects. The notable exceptions are high-mineral springs and highly disturbed streams. Thus, high numbers of non-insect invertebrates often indicate that streams are stressed, or that there are unusual circumstances governing the community structure. Some non-insects, such as the ubiquitous amphipod *Hyallela* sp., are very tolerant of stress from high temperatures and elevated salinity. Others, like aquatic earthworms are tolerant to organic or inorganic sedimentation. Thus, specific taxa can be useful to help diagnose the causes or nature of anthropogenic perturbations.

The abundance of dominant taxa usually increases when a few specialist species increase in abundance in polluted or stressed ecosystems, and specialists (or sensitive species) decline. The original expression of this metric was expressed as the relative abundance (%) of the single most abundant species. Latter expressions of the dominance metric included the several most abundant taxa. Thus, the metric is often expressed as the relative abundance of the 1, 2, 3, 4 or 5-most abundant species.

Wyoming's bioassessment criteria, like those used by other states, use the percent abundance of the five most-abundant taxa. Although our values will deviate somewhat from Wyoming DEQ's bio-criteria[17] (e.g., Hargett and ZanBerge 2006), we also used the abundance of the five most abundant taxa to calculate dominance.

There are five common functional feeding groups (FFG) used to classify macroinvertebrate taxa by their roles in processing organic material. The generalists eat fine particles of organic material that require little chewing to fit in their mouth. Their FFG designation is "collectors" and they are further subdivided by their methods of acquiring organic particles into collector-gatherers and collector-filterers. Gatherers are deposit seekers whereas filterers remove fine particles from the water column. Predators are the FFG that preys upon other animals. Scrapers have adaptations to scrape fine layers of algae from rock and other hard surfaces. Shredders play very important roles in breaking up deposits of course detritus and woody debris--resulting in food availability for all other groups. In streams with extreme sedimentation problems, extreme pollution, or even urbanization, the community tends to shift towards FFG composition dominated by collectors and predators.

[17] These samples use a 200 organism count lab effort and might use slightly different OTU's for some calculations.

Table 2.7.1. Biological Metrics and their Expected Response to Disturbances.

Biological Metric	Description	Response to Ecological Perturbation
Taxa Richness	The number of "species"	Decreases as species disappear
Diversity (H')	The Shannon-Wiener Index of Species Diversity	Decreases as species disappear, OR decreases as facultative species increase in abundance
Evenness (J')	Pielou's Index of Evenness	Decreases as a few tolerant species increase their abundances and sensitive species decline
EPT Taxa Richness	The number of "species" from the orders Ephemeroptera, Plecoptera, and Trichoptera	Decreases as species disappear
EPT Relative Abundance	The portion of the community (%) comprised of the orders Ephemeroptera, Plecoptera, and Trichoptera	Decreases as more-tolerant species become more abundant
Chironomidae Relative Abundance	The portion of the community (%) comprised of chironomid midges	Increases as less-tolerant groups become less abundant and a few tolerant midge species increase in abundance
Non-Insect Relative Abundance	The portion of the community (%) comprised of non-insect macroinvertebrates such as worms and snails	Increases as insects become replaced with more tolerant non-insect species
Community Dominance (5 taxa)	The portion of the community (%) comprised of the five most abundant taxa	Increases as a few tolerant taxa begin to dominate the community composition
Hilsenhoff Biotic Index	Tolerance weighted abundance index ranging from zero to 10	Values near 10 indicate dominance by organisms adapted for life in organically rich environments; values near zero indicate dominance by organisms very sensitive to organic material.
Collector-Gatherers	The portion of the community (%) comprised of collector-gatherers	Increases as sand or sediment accumulate in interstices or as specialists become less abundant
Collector-Filterers	The portion of the community (%) comprised of collector-filterers	May increase when organic particles are suspended in the water column, or as specialist become less abundant. OR: Decrease if flow rates decrease.
Scrapers	The portion of the community (%) comprised of invertebrates which scrape algae from substrata	May increase under moderate nutrient additions, or decrease if disturbances cause habitat exclusion (sediment, filamentous algae or other habitat loss)
Shredders	The portion of the community (%) comprised of invertebrates which shred organic particles into finer particles	Usually decreases if other groups become more abundant. OR may increase if disturbances add large organic materials to food webs. OR may respond to changes in the river-bottom's ability to store/retain coarse detritus.
Predators	The portion of the community (%) comprised of invertebrates which eat other animals (vertebrates or invertebrates)	Increase or decrease
Total Collectors	The combined relative abundance (%) of collector-gatherers and collector-filterers	Increases as specialists decline in abundance

STATISTICAL ANALYSES

This monitoring project does not have as a goal the comparison with other streams throughout Wyoming; we want to know if gas development in the Pinedale Anticline Project Area is changing the biology of the New Fork River. This is a much more complicated question than can be answered by the WSII's narrative condition criteria. We know from past experience that there are some natural deviations from the regional references of the WSII. However, we use metrics that have historically been useful in the New Fork River (Marshall 2005a) and in regional bioassessment models. We used SYSTAT v.13 statistical analysis software for most analyses.

We used Analysis of Variance (ANOVA; Zar 1999) to test for differences among sites in 2012, (treatment = SITE). The ANOVA used the within-site averages and variances to determine the likelihood that the levels of each treatment are sufficiently similar to be considered statistically representative of the same population of data. In application, a P-value (probability) that is small means that there is a low probability that the observations are sufficiently similar to belong to the same "group." The convention among research scientists is to use a critical P-value of P=0.05 (5%) as the decision threshold. Thus, if P<0.05, there is >95% likelihood that the compared groups are not homologous. Another way to say this is, that the probability of "type-1 statistical error" is less than 5%; we have a <5% chance to incorrectly conclude that homologous groups are not actually homologous.

Although a very low type-1 statistical error is paramount for sound science, it has been criticized for environmental monitoring because it may cause real and important environmental changes to be obscured by natural variation. To avoid this conundrum, we also examined all metrics with a more-liberal P-value (P<0.10) and called these changes "marginally statistically significant" or "marginally significant." When these terms arise they mean that the result was not significant at the 95%-level, but was at the 90%.

When appropriate, Tukey's HSD test was used to follow-up ANOVA results to determine which specific sites were significantly different from each other. If data characterization indicated that the ANOVA assumption of variance homogeneity was violated, the more robust Tamhane's T2 test was used to determine which sites were significantly different from each other. If the assumptions of normal distribution were violated, the non-parametric ANOVA-equivalent test, the Kruskal-Wallis test was used and followed up with the Dwass-Steel-Crichlow-Fligner test of pairwise comparisons. These tests used the same critical p-values used for the ANOVA, allowing for significant differences if the probability of type-1 statistical error was less than 5%. We summarized the pairwise test results using letters to define groups of sites that were not significantly different from each other. In tables and figures, each site is given a letter (or set of letters) and sites sharing a common letter in the results were not significantly different from each other. For example, a site marked as ABC and a site marked as CDE would not be considered different from each other because they share the letter C. However, a site identified by AB would be significantly different from a site marked C because they share no common letters based on the results of pairwise tests. The graphical summary of each metric is presented in figures showing unadjusted means and confidence intervals. However, the pairwise comparison's, presented in the figures, show the significant differences among sites as tested after correction for the role of significant covariates. This allows readers to see actual means with confidence intervals while also observing the final differences among the sites after all adjustments. The changes in differences among sites caused by correcting for physical conditions are discussed at the end of text discussions of each metric.

This report follows the tradition set by the 2007 (Marshall 2008) and 2008 (Marshall 2009) reports and uses methods that allowed more efficient use of statistical analyses than in previous reports (pre-2007). Among these were methods that allowed us to include habitat measures in analyses of biological data. We used the General Linear Models (GLM) algorithm (available in most statistical software) and used metrics as response variables and all the habitat variables as predictors. The modeling procedure then removes predictor variables (i.e., flow, particle size, embeddedness etc.), which, when tested, do not explain a significant amount of variation in a specific response variable (i.e., metric). These are removed one at a time until only the variables that significantly explain variation in the model being developed and tested are retained.

The process is complete when only variables that are significant—given the other variables in the model--are included[18]. For this reason the procedure is called a "backwards step-wise multiple regression modeling algorithm," but throughout this report we call it simply the "multiple regression," "GLM" or "Analysis of Covariance." This procedure was especially useful to describe which metrics appeared to correlate with velocity or sedimentation.

Select metric scores were plotted against time (years) to ensure temporal trends are considered as well as spatial differences. The metrics from 2012 were compared to the previous years' metric values for each site using the Kruskal-Wallis non-parametric ANOVA because the assumptions of parametric statistics were rarely met by the data, and there were insufficient analyses for regression models or time-series analysis to be meaningful. The metrics chosen for these analyses are robust to modest changes in laboratory effort or operational taxonomic units (OTU's) including damaged specimens and immature specimens. Thus, temporal trends were examined using metrics that did not use richness measures. We used mostly % abundance measures to examine temporal trends (Table 2.7.2). When the Kruskal-Wallis test noted differences, they were explored using the Dwass-Steel-Crichlow-Fligner test for all pairwise comparisons.

Table 2.7.2. Metrics used to Examine Temporal Changes at Each Study Site.

Biological Metric	Description	Response to Ecological Perturbation
EPT Relative Abundance	The portion of the community (%) comprised of the orders Ephemeroptera, Plecoptera, and Trichoptera	Decreases as more-tolerant species become more abundant
Chironomidae Relative Abundance	The portion of the community (%) comprised of chironomid midges	Increases as less-tolerant groups become less abundant and a few tolerant midge species increase in abundance
Non-Insect Relative Abundance	The portion of the community (%) comprised of non-insect macroinvertebrates such as worms and snails	Increases as insects become replaced with more tolerant non-insect species
Community Dominance (5 taxa)	The portion of the community (%) comprised of the five most abundant taxa	Increases as a few tolerant taxa begin to dominate the community composition
Hilsenhoff Biotic Index	Tolerance weighted abundance index ranging from zero to 10	Values near 10 indicate dominance by organisms adapted for life in organically rich environments; values near zero indicate dominance by organisms very sensitive to organic material
Scrapers	The portion of the community (%) comprised of invertebrates which scrape algae from substrata	May increase under moderate nutrient additions, or decrease if disturbances cause habitat exclusion (sediment, filamentous algae or other habitat loss)
Total Collectors	The combined relative abundance (%) of collector-gatherers and collector-filterers	Increases as specialists decline in abundance

[18] For example, a metric such as "%Filterers" might be strongly correlated with velocity and particle size. However, once one of the variables is in the model, the contribution of the other may be non-significant, and that variable is excluded from the model. ALSO NOTE: "given the variables in the model" has a statistical meaning; it is not an incomplete sentence about giving something.

2.8 ANALYSIS OF POTENTIAL EFFECTS ON DENSITIES OF MAJOR AQUATIC INSECT GROUPS

New for 2011, Continued in 2012

2.8.1 THE NEED FOR ANALYSIS OF INSECT DENSITY

For the 2012 analysis, we continued our analysis of aquatic insect density. Elevated abundances of aquatic worms have persisted for several years and raised concern that ecological changes[19] may have negative impacts on the fisheries resources of Sublette County. Therefore, we added some new analyses in 2011's report (Marshall 2012) to address the concerns raised at the 2011's Pinedale Anticline Work Group (PAWG) meeting (regarding 2010's report) and to evaluate the significance of some of our findings. Worms have been found to increase in relation to sedimentation (Marshall 2008, 2009) and in relation to the growth of aquatic plants (Marshall 2010, 2011, 2012). Sedimentation could be related to construction of roads, pipelines, or drilling pads in riparian or hyporheic corridors. However, we do not expect development on the PAPA to directly affect the growth of plants or primary production.

Our study was not designed to replace a detailed model of food web structure[20], because the goals of the study did not require this much rigor or cost. Although our analytical abilities are somewhat limited by initial design, there are some relatively simple analyses we can use to address the concerns about potential impacts of PAPA development on fisheries resources. However, it is important to acknowledge that we do not propose these analyses as a substitute for more rigorous science, but rather as a method to screen for significant detrimental shifts in the abundances of aquatic insects which may have serious consequences for the aquatic natural resources of Sublette County. Thus, we examined the abundance of several groups of aquatic insects using somewhat different methods than those typically used for biological assessment of water quality. These additional analyses provide the PAWG with new resource management information from old (and new) data and improve the study without increasing cost.

The methods used for biological assessment (bioassessment) of surface water quality are somewhat different from those used to describe other aspects of benthic ecology. Specifically, the laboratory procedures are standardized to a fixed-count of organisms and the analyses rely on computational methods calculating on percent composition measures or richness measures, whereas other methods[21] are often used to describe community structure and function. The methods used to assess water quality are particularly different from those used to assess the potential availability biomass for foraging fishes. Bioassessment methods have advantages over volume, mass, nested proportions, or whole-sample processing because they reduce costs, make budgets predictable[22], and help with some technical issues related to comparing species richness estimates. The advantages and disadvantages of different sampling methods (field and laboratory procedures) have been reviewed from the perspective of their application for rapid bioassessment protocols (e.g., Barbour and Gerritsen 1996, Courtemanch 1996, Vinson and Hawkins 1996).

Biological assessment methods focus on richness measures and relative abundance estimates from a standardized unit of effort as defined by the number of species identified. To avoid redundancy (e.g., Barbour et al. 1999) bioassessments don't report both density and relative abundance of a group of insects; the only abundance measure usually used is relative abundance. A decline in the relative abundance of one group of aquatic insects (for example, mayflies) may be caused by an actual decline in density (assumed), or, alternatively by an increase in density of another group of invertebrates (e.g.,

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[19] Regardless of cause (natural or anthropogenic).

[20] Food web structures are summarized as ecological maps of the flow of energy (as organic carbon) through different trophic levels of communities. A true assessment of ecological function requires measures of production rates for different trophic levels (usually mass*area^{-1}*time^{-1}) and includes estimates of the energy flow between various trophic units. However, since there are no clear reference criteria for normal food web structure and function of the New Fork River ecosystems, such a study would be very expensive, without providing clear assessment of effects of development within the PAPA.

[21] Density, biomass or production estimates.

[22] Benthic samples may contain anywhere from ~100-1,500,000 invertebrates. When a laboratory procedure is standardized based on the number of invertebrates identified, the laboratory costs are fixed to the cost of identifying the same number of insects per sample (100, 200, 300, 500, or 1000).

worms). An increase in the density of abundant taxa can cause a decline in the relative abundance of other taxa, even when the density of rarer taxa remains unchanged. Therefore, when a group of insects shows a reduction in relative abundance, it is not necessarily due to a decline in their contribution to the community or in their role in ecosystem function, but it can in fact be due to increases of other species. This type of effect is especially important to understand because our recent findings have found very high abundances of small worms. For instance, in a sample where there are 100 invertebrates (total) and 20 of them are worms, then worms comprise 20% and non-worms comprise 80% of the sample. This also indicates a density of 20 worms/ ft^2 and 80 non-worms/ ft^2 (215/m^2 and 861/m^2). If environmental conditions change and cause dramatic increases to the density of worms, to about 2000[23] per sample, then the percent abundance of non-worms decreases dramatically (80/2080, or 3.8%) even though their total abundance in the ecosystem remains unchanged (80/ sq. ft.). For comparisons of water quality and biological assessment, we are interested in the changes of relative abundance (i.e., the change from 80% to 3.8%) of the community. However, to speculate on the potential impacts on foraging fishes and actual questions of ecosystem function (vs. community composition), we must be more interested in actual changes in density. Ideally we would examine changes in the biomass and production of certain invertebrate groups during periods of greatest fish biomass accumulation (i.e., growth, production, recruitment etc.). But this is beyond the scope of our assessment and these data are not available; we must make inferences based on abundances and densities.

The specific problem has been that during this survey, worms occasionally become so abundant in some samples that they determine how much of the sample is actually processed in the laboratory. This is because when using the standard methods endorsed by state and federal agencies (e.g., Plafkin et al. 1989, Barbour et al. 1999, Stribling et al. 2000, Hargget and ZumBurge 2006), a fixed number of organisms are randomly selected for identification. We examined this relationship in 2011 (Fig. 2.8.1) and found that, in some samples worm abundance was so high that less than 1% of the sample was processed to attain the target number of animals for analysis (Fig. 2.8.1).

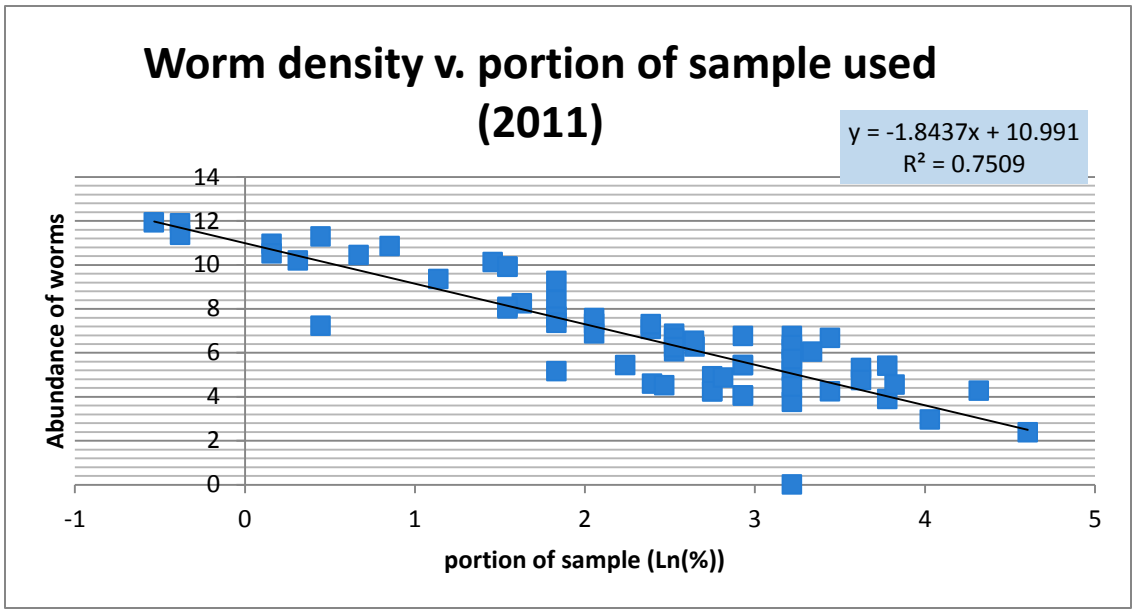

Figure 2.8.1. The abundance of worms had a significant effect on how much detritus was examined in the laboratory. The relationship was logarithmic so the axis represents data transformed using natural logarithms. Samples with many worms used less than 1% of the sample to attain the target effort. An analysis of this problem for 2012 is included in the results section of this report.

[23] Samples from New Fork River, sometimes exceed this density worms (*Nais* sp.).

2.8.2 DENSITY CALCULATIONS AND ANALYSES

The simplest way to circumvent the effects of the high abundance of worms (or any other single taxon) is to convert relative abundance data to densities. That is, convert the percent abundance measures used for metric calculations and bioassessment into abundances per unit area. We corrected for laboratory subsampling and for sample area to estimate the total density (#/m^2)of the orders Ephemeroptera, Plecoptera, and Trichoptera because they are known as important forage for fishes and are sufficiently coarse taxonomically, that under-sampling errors (artificial zeros or below detection limit errors) do not hinder our calculations. Additionally, some taxa with very short life cycles and high biomass turnover can be important in fish production so we included analyses of midge and black fly density.

Densities were calculated for each of the taxa and standardized per 1-meter of river bottom. These values were transformed using natural logarithms, but often failed the Levene's test of variance homogeneity, so the Kruskal-Wallis non-parametric ANOVA was used to test for significant differences among sites. The pairwise comparisons were conducted using the Dwass-Steel-Crichlow-Fligner Test for all pairwise comparisons (DSCFTFAPC).

For the purposes of these analyses, we need to test the hypothesis that there were no dramatic reductions in the density of certain benthic insects even though there were dramatic decreases in their relative abundance (%). Therefore, we were specifically concerned with statistically significant declines in the density of these animals—especially where elevated worm (or black fly) abundance suggested that other species could have become "rare" (low % abundance).

As with other statistical tests, differences were considered statistically significant if tests resulted in a low type-1 statistical error rate with a probability value of $P<0.05$, and we considered error rates of $0.10<P<0.05$ as marginally significant.

The results of the analyses of aquatic insect densities presented here need to be interpreted conservatively—within the intended purpose of the analysis and within the limitations of the monitoring program's elements of design. Specifically, we are screening for extreme reductions in the density of important groups of insects. Comparisons among zones of densities may lead to spurious interpretations. Consider the methods used for biological assessments, recommended by most state regulatory agencies (including Wyoming DEQ; e.g., Hargett and ZumBerge 2006). In the laboratory, samples are processed for a set number of specimens to be identified; with large composite samples[24], the state bioassessment criterion is 500 organisms per 8 square feet of river bottom, or 62.5 insects per square foot. In our replicated design, we did not composite samples, and our criterion was a more-rigorous 200 organisms per square foot of river bottom. The fixed count method of subsampling standardization is ideal to attain richness estimates per unit organism-count (500, 300, 200, 100 organisms (etc.); Barbour et al. 1996), but it is not ideal for determination of total richness, or abundance per unit area (i.e., density) because not all samples are treated uniformly (e.g., Courtemanch 1996). The potential error of multiplying a small number by a large correction factor is at the heart of the potential problem. Consider our specific case in this study: a high density of worms caused us to use only 1% (sometimes less) of the sample to attain 200 insects. In this case, 1 mayfly (subsample) represents 100 mayflies for purposes of density estimation. Conversely, when 100% is used, one mayfly represents only one mayfly for density estimation. This is not a problem in-and-of itself; it is why we use correction factors in aquatic ecology. However, the assumption is that the error is constant among samples; when fixed count methods are used this assumption is invalid. This can cause density estimates from one sample to be attained by counting insects from the equivalent of a 2"x 2" square of river bottom, whereas other sample's estimates would be calculated using an equivalent of a larger sample area (up to 12"x 12" in this study, up to 8 ft^2 in other SCCD monitoring projects). Thus potential errors from clumping and heterogeneous distribution of species are not constant among all samples (e.g., Southwood 1991 and Krebs 1999). The assumption of constant error-rates is a key principle of ecological sampling, and it is built into most sampling plans by using standardized sample methods, usually sample area. Although fixed-count subsampling methods are acceptable for relative abundance measures (used for water quality assessment) they are not ideal for other calculations, such as density and biomass measures (important for fisheries assessments). Our purpose in using density estimates was to assess the effect of dramatic changes in the relative abundance of some insects (especially the EPT taxa) on the actual density of these groups.

[24] Composite samples are formed when several traditional samples are collected and combined into a single sample in the field. The process is irreversible and prohibits many useful statistical analyses. In Wyoming, the most common composite samples are composites of 8 Surber samplers, each of which comprises a sample area of 1 sq. ft.

The first year we performed the analyses, in 2011 (Marshall 2012), we found that insect densities did not actually agree with the results attained from examining relative abundances. Therefore, we decided it was important to continue this line of investigation.

This year we found that other organisms may also become extremely abundant and cause similar effects on the relative abundance measures of other species. Therefore, we also ran the same set of analyses using the dominant taxon's density compared to the effective sample area, as well as describing the effect of worm density.

3.0 RESULTS

3.1 PHYSICAL MEASURES (COVARIATES)

The sampling regime for all other variables was based on selecting samples from a uniform flow range between sites. This was a very important aspect of the sampling plan because it allowed us to stratify the sampling plan without bias (a statistical concern) and allowed us to control an unwanted source of variation on the invertebrate community. That is, invertebrate assemblages are known to respond to water velocity, and we do not anticipate development on or near the PAPA to change the velocity of water in the New Fork River. Thus, if we collected samples from the same approximate range of flows at all sites, we could control for this variation and account for it statistically. This constitutes a stratified sampling scheme that is superior both to completely randomized designs and to subjective stratified sampling methods.

The responses of specific covariates in 2012 are displayed (Table 3.1.1) and discussed individually in the following sections.

TABLE 3.1.1. Difference in Physical Conditions. The physical measures collected with each benthic grab were used as covariates to explain natural variation in biological metrics. Here differences among these sites are tested for each covariate using parametric or non-parametric (as noted) statistical tests.

		Multiple Comparisons Grouping (Tukey's HSD or Non-Parametric equivalent)									
Metric	P-val	NF90	NF80	NF04	NF70	NF60	NF17	NF50	NF40	NF30	NF19
Near-substrata velocity	0.175	--	--	--	--	--	--	--	--	--	--
Substrata covaraiates											
Particle size index	<0.001	AB	AB	B	A	B	A	B	A	AB	B
Embeddedness	0.322	--	--	--	--	--	--	--	--	--	--
Autotroph covariates											
Vegetation [n.p.]	<0.001	AB	AB	B	A	B	AB	B	B	B	B
Algae[n.p.]	<0.001	AB	AB	A	AB	B	AB	B	B	B	B
Macrophytes [n.p.]	<0.001	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Moss[n.p.]	0.005	Aa	a	Aa	Aa	Aa	Aa	A	Aa	Aa	Aa
Plants [n.p.]	<0.001	a	a	A	A	A	A	A	A	A	A
Periphyton biomass	Excluded	--	--	--	--	--	--	--	--	--	--
Didymo	n/a	--	--	--	--	--	--	--	--	--	--
		Upper Study Area				Middle Study Area			Lower Study Area		

n.p.	Indicates the variable failed tests of normal distribution and/or tests of variance homogeneity. Transformations were not able to sufficiently remedy these violations for parametric statistics so the non-parametric alternative, Kruskal-Wallis test (followed by the Dwass-Steel-Crichlow-Fligner Test for all pairwise comparisons).
n/a	Indicates there were insufficient data (too many zero values) for this test to be meaningful.
Excluded	Indicates the samples were collected in the field, but not processed in the lab or included in the analyses at the request of the SCCD and natural gas operators.

BENTHIC FLOW (VELOCITY)

The speed of water near the stream bed can account for more than 90% of the natural variation in benthic community structure (e.g., Hart 1994, Hart and Finelli 1999). It is important to control for this source of variation so that it is not erroneously ascribed as an impact related to natural gas development. Therefore, we used flow as a criterion to stratify our sampling regimen. Since flow criteria were used to determine where benthic samples were collected from, it should not be surprising that we found no statistically significance among the sites (Table 3.1.1, Figure 3.1.1).

There were no statistically significant differences observed in near substrata flows among sites in 2012. Flows sampled in 2012 were more consistent with flows sampled in previous years than the flows sampled in 2011, which were a little greater due to higher flows in the river (Marshall 2012). Multi-year comparisons might notice subtle influences of slightly higher flows in 2011, relative to 2010 and 2012, but this should not affect spatial comparisons among sites in the year 2012 (or any other year for that matter).

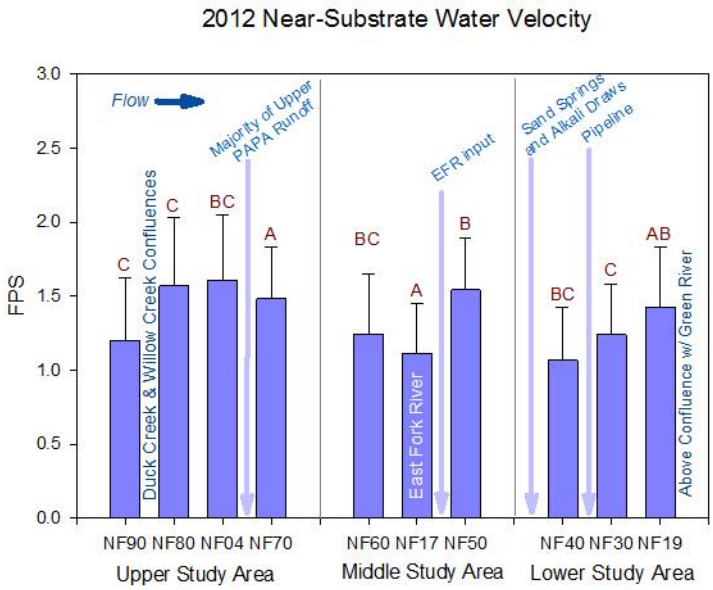

Figure 3.1.1. Near Substrata Water Velocity. The velocity of water measured near the stream bed was used to stratify samples from a uniform range of flows. There were no significant differences among flow regime sampled at each site in 2012. The graph shows average velocities (n=8) and 95% confidence intervals for all benthic macroinvertebrate samples collected in 2012.

SUBSTRATA SIZE COMPOSITION

The size of particles[25] comprising the stream bottom is important for the success of macroinvertebrates as well as for fish reproduction. The SCCD field crew quantified the size composition substrata (Fig 3.1.2) within each Surber sample. Thus, these data do not describe the totality of particle sizes found at each site, but rather where the benthic samples were collected—as assorted[26] by New Fork River flows[27]. Since the effort was standardized by water velocity, the sites should be somewhat similar—unless something other than flow has influenced the distribution of particles in the river. For example, we know from field observations that smaller particles naturally usually dominate the East Fork River (NF17).

Samples in 2012 generally contained less sand than they did in 2011 or 2010. This is considered an improvement, since earlier surveys from 2008 and 2009 (Marshall 2009, 2010) reported active erosional sources near NF30 and NF40, and it appeared that sufficient fine material had accumulated during the drought to affect the community composition of benthic assemblages in those years. The sand constituent of substrata had also decreased in 2011 relative to 2010. Thus the 2012 data reflect a continued short-term trend in the reduced role of sand on benthic invertebrates sampled.

There were also fewer cobbles represented in benthic samples in 2012 (Fig. 3.1.2). Thus the differences among sites were primarily represented among the three middle substrata categories: Pebble, Coarse Gravel and Fine Gravel. We performed a Principle Component Analysis (PCA) and plotted the samples in the coordinates defined by the first two PCA axes to illustrate trends among the sites in the relative composition of these particle-size categories. The plot of factor loadings (Appendix 2.1) indicated that sand had almost no influence on the distribution of observations in PCA space, and that the abundance of cobbles also explained very little of the variation among the sites and samples.

The layout of the samples in the PCA multidimensional space is best represented by the first two principle component axis, which explained >85% of the variation among sites (Fig. 3.1.3). The vectors along each axis show the influence of different size classes on the distribution of points along that axis. For example, cobbles and pebbles tended to pull samples to the right on the primary axis, whereas cobbles and fine gravel tended to pull samples upward on the secondary axis. The variable SAND always had very little influence on the distribution of samples in PCA space and was weighed very close to zero by the PCA. This is because all samples collected were from substrata containing very little sand. If one site was especially different in substrata composition, we would expect the samples from that site to form a separate cluster of points. However, there was generally a great deal of overlap among all sites indicating similarity in substrata composition among them. The downstream site from the upper study area, NF70, had all of its samples occur along the far right of the PCA plot (Fig. 3.1.3) but not in a clearly distinct cluster; many samples from elsewhere in the New Fork River occurred in the same general area in the PCA space. This pattern is due to the prevalence of gravel in the samples collected from NF70 and is corroborated by noting that the site had the greatest average fine gravel and course gravel proportions (Fig 3.1.2). The PCA indicates that this high average was due to a high, but not extreme, amount of gravel in all samples—rather than a few samples with extreme amounts of fine/coarse gravel.

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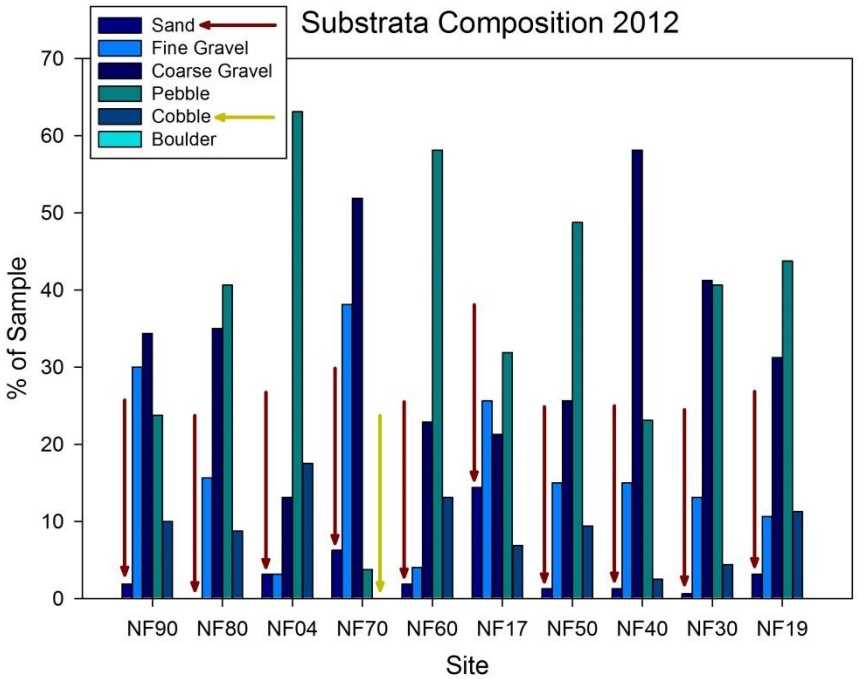
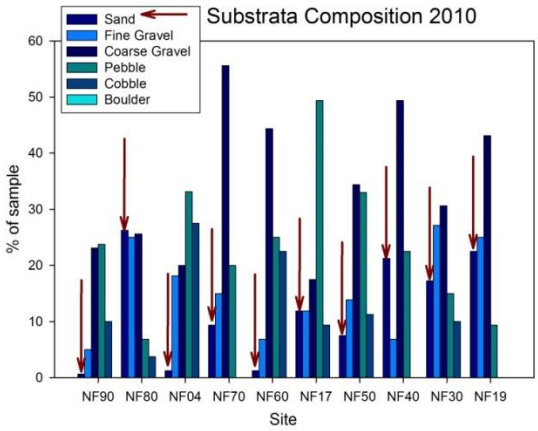
[25] Please note that the scale of these measures is different from those used for geomorphology and habitat assessment. See "purpose statement" in the Executive Summary and Introduction sections of this document for further explanation.

[26] Particles in rivers are assorted by flow. In equilibrium, high flows scour smaller particles away and the dominant remaining particles are larger. In slower flows, the finer particles settle out and become more abundant as substrata. To estimate the site's actual substrate composition, a randomized transect regimen is required (a pebble count). This was not performed for our component of the New Fork River monitoring effort; our data were attained by sampling individual 1ft^2 benthic invertebrate samples, across a pre-selected (stratified sampling) range of flows.

[27] Where flow is the velocity of near-substrata water used to select samples from a consistent range of flows.





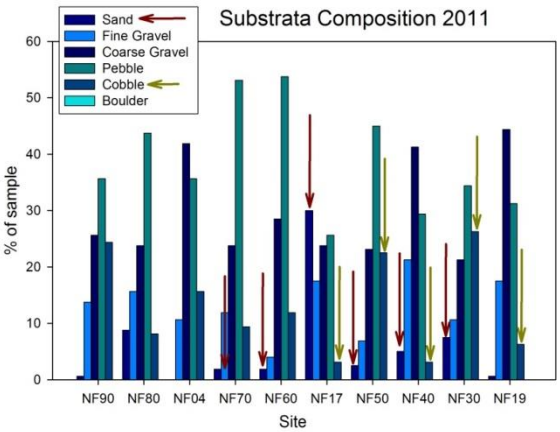


Figure 3.1.2. Substrata Composition. The average percent occurrence of different substrate types sampled when sampling was stratified according to flow (Fig 3.1.1) is represented by bars. Sites are arranged from upstream to downstream. Data from the previous two years (2010, 2011) are provided in addition to 2012 for comparison.

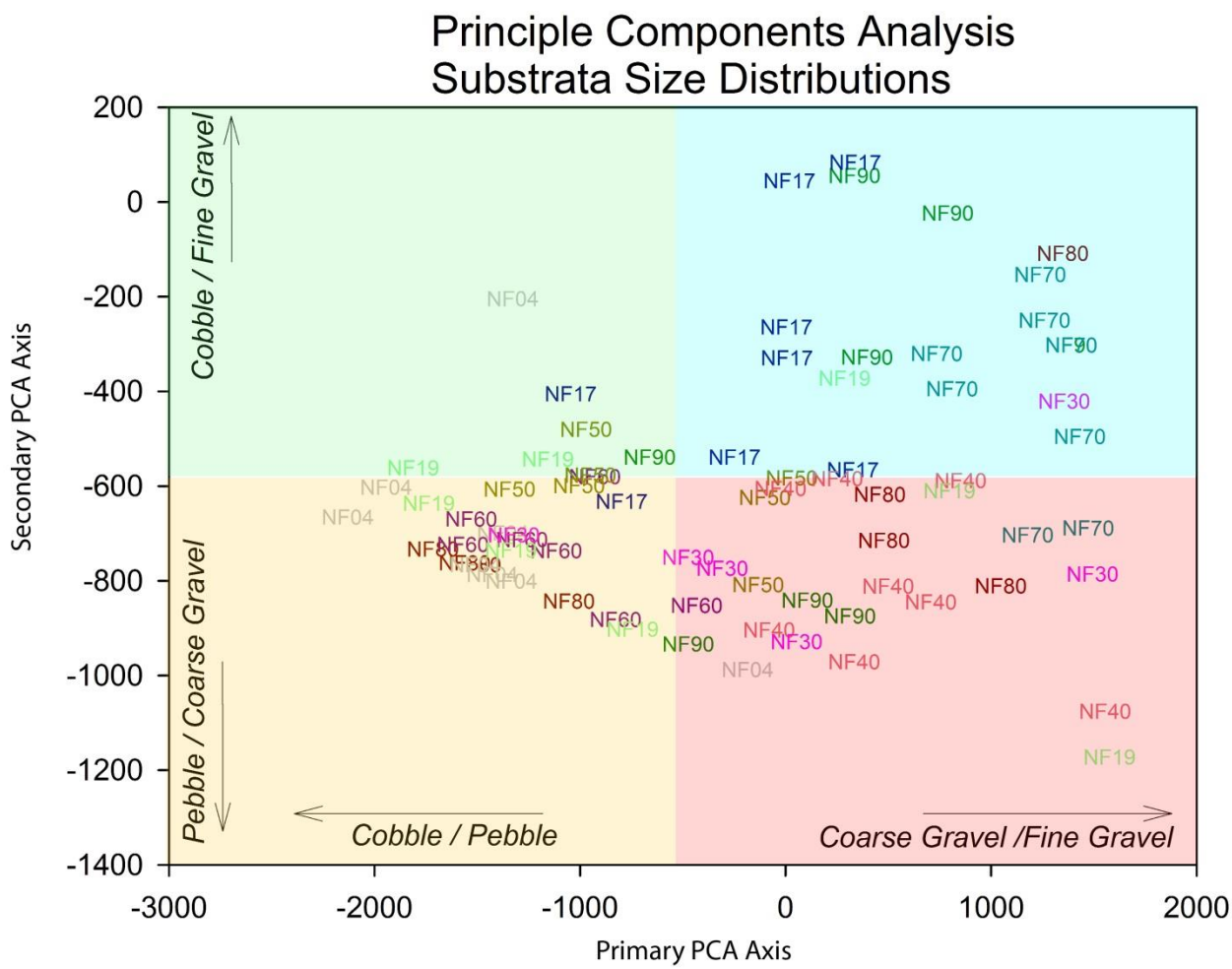


Figure 3.1.3. PCA of Substrata Composition Data. We performed principle component analysis on the substrata composition size-categories. The layout of the samples in this multidimensional space is best represented by the first two principle component axis, which explained >85% of the variation among sites. The vectors along each axis show the influence of different size classes on the distribution of points. For example, cobbles and pebbles tended to pull samples to the right on the primary axis, whereas cobbles and fine gravel tended to pull samples upward on the secondary axis. Notice that the variable SAND always had very little influence on the distribution of samples in PCA space (that is why it is not shown). If one site was especially different in substrata composition, we would expect the samples from that site to form a separate cluster of points.

PARTICLE SIZE INDEX

The Particle Size Index (PSI) is a weighted average of the percent contribution of different substrate sizes (see Analytical Methods). It integrates all substrate types into an independent[28] measure which is known to correlate with macroinvertebrate community structure and abundance. Values of about ~300 are ideal and are dominated by a complex mix of cobbles and other substrata. Values near zero are dominated by sand and silt. We found that in previous years, differences in community structure were strongly correlated with the PSI.

In 2012, the lowest values of the PCI were at NF70, which was dominated by fine gravel and coarse gravel but lacked cobble. Sites with similarly sized substrata were NF17 (the traditionally lowest PSI site) and NF40 (low in recent years). These low sites were only significantly different from NF19, NF50, NF60, and NF04. The reasons for the low PSI values at NF70 are discussed above with the substrate particle size distribution. NF17 had more sand than any other site in 2012, and NF40 had the greatest dominance of coarse gravel.

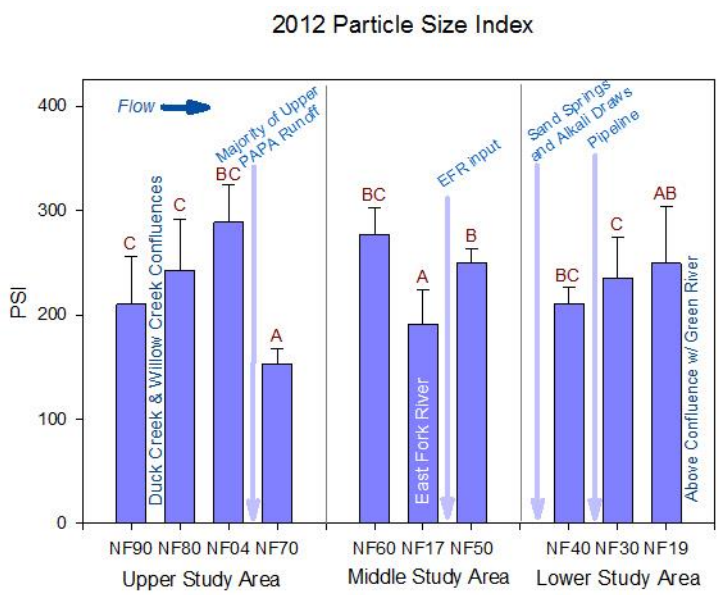


Figure 3.1.4. Particle Size Index. Relative abundance of inorganic particles comprising the sample area was qualitatively evaluated in the field. The relative contributions of different size-classes of particles were used to calculate the PSI. The graph shows average PSI values (n=8) and 95% confidence intervals for all benthic macroinvertebrate samples collected.

[28] Independence is an important consideration for statistics. Computational independence is especially important, since there is no way to hedge the null hypothesis to compensate. For example, multi-colinearity problems would occur if we used all substrate classes for covariates; 100% cobble forces all other substrate size-classes to zero, and a zero percent cobble forces some other measures to non-zero values. Thus, the PSI not only helps explain change in invertebrate abundances, but also circumvents computational problems that would otherwise arise.

EMBEDDEDNESS

Embeddedness is the relative portion of course substrata buried among finer particles. It is a subjective measure expressed as the average percentage of larger particles buried among silt, sand, or very fine gravel. The impact of embeddedness on rivers and streams depends not only on the depth of embedding, but also the fineness of the embedding material and the extent of high embeddedness through the river-system. Finer material denies access to pore-spaces for invertebrates and small fish. If the material is organic in nature, it can deplete oxygen concentrations or facilitate plant growth.

The measurable amount of embeddedness was about the same in 2012 as it was in 2011. That is, it was <20mm of embeddedness. Some of the sites in previous years had average embeddedness values close to 5mm. There were no significant differences in the average embeddedness observed among the sites in 2012 (Table 3.1.1, Fig. 3.1.5).

True embeddedness values of the sites would need to be evaluated by reach-wide habitat analyses. These data are specific only to stratified-random benthic grabs collected from a similar range of flows from the sites.

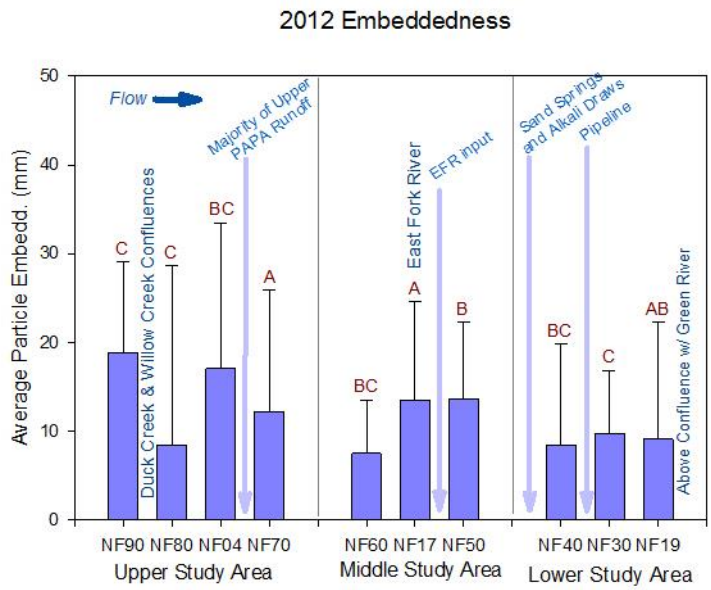


Figure 3.1.5. Embeddedness 2012. The average embeddedness of sampled (SS) substrate is represented by bars. Error bars represent 95% confidence intervals. Sites are arranged from upstream to downstream.

TOTAL AQUATIC VEGETATION COVER

The proportion of total vegetation cover (variable VEG = ALGAE + MACROPHYTES + MOSS) was used as an explanatory variable for the first time in 2010. At that time, we found it to be very useful to explain some variance in macroinvertebrate metrics. However, during the analyses for the PAPA 2011 report (Marshall 2012), we found this covariate never explained a significant portion of the variance in macroinvertebrate metrics when other aspects of physical habitat were included in the model. For the 2012 analysis, we found the variable VEG to be highly auto correlated with other variables (ALGAE, MACROPHYTES, MOSS) and found that using these variables separately provided greater diagnostic power than use of the composite variable VEG. Therefore, this variable was not used as a covariate for final analyses conducted on the 2012 data. It is presented here for consistency with earlier reports and to facilitate comparison. It was used in an ANOVA describing physical differences among sites (Table 3.1.1), but not in the GLM models describing macroinvertebrate response to PAPA development. We included this variable for the ANOVA of physical differences among sites.

The general pattern of vegetation density was very similar to that observed in the previous year. Generally, the upper and lower study areas had more plant cover than the middle study area. In the upper study area, NF70 had significantly less total vegetation than the sites upstream from it but only had significantly less plant material than NF04 because the other sites (NF90 and NF80) had a very wide range of variation (Fig. 3.1.6). There were no significant differences among the vegetation cover of the middle study area, or among the sites of the lower study area. Overall the only significant differences were between NF70 and several other sites (NF04, NF40, NF30, NF19).

One notable difference from the prior year's vegetation cover was that in 2011 and earlier, NF19 had very little plant cover, however in 2012, it had the greatest single average plant cover. The specific components of this plant cover are further discussed with the other plant-related variables, but it is largely due to elevated abundances of the nuisance algae, *Didymosphenia geminata*.

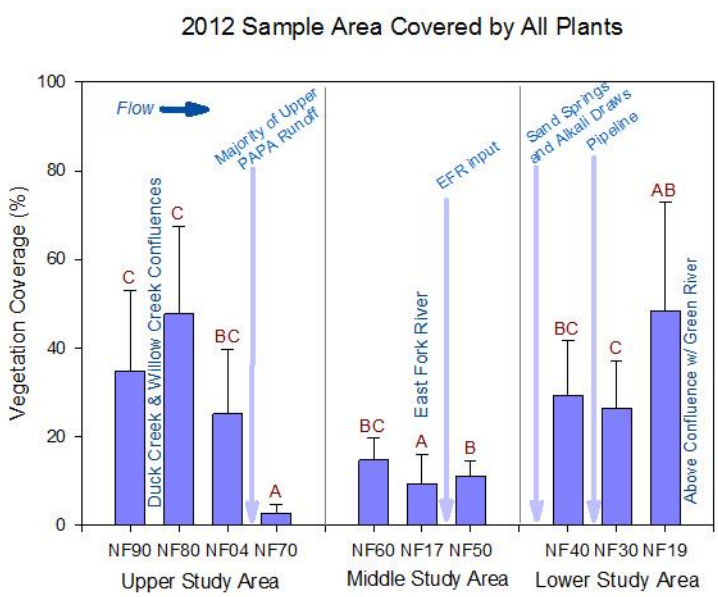


Figure 3.1.6. Plant Cover. The mean relative portion of plant cover from each square foot sampled for macroinvertebrates is presented with 95% confidence intervals. Statistical comparison of differences among sites test required the non-parametric Kruskal-Wallis test and pairwise comparisons with the Dwass-Steel-Crichlow-Fligner test.

MACROPHYTES

Macrophytes are aquatic vascular plants (angiosperms) that grow attached to benthic substrata—typically with roots or holdfasts among finer substrata such as sand, silt or some complex organic-inorganic matrix. Generally, aquatic plants are not fed upon by most stream dwelling macroinvertebrates. Macrophytes generally influence macroinvertebrate assemblages by providing colonizable substrata, flow refugia, or foraging refugia. Thus, foraging among stream dwelling macroinvertebrates is usually facilitated by macrophytes providing accumulations of organic particles or attachment loci for filterers to remove particles from the water column (e.g., Resh and Rosenberg 1984). In some instances, insects may feed on epiphytic algae growing on the leaves and stems of macrophytes. Although most species of aquatic insects dwelling in lotic ecosystems do not feed on macrophyte tissues *per se*, one midge genus common among the macrophytes of the New Fork River, *Polypedilum*, is known to feed on living aquatic vascular plant tissues.

There were too many zero-values to allow statistical testing of differences among sites. As with previous years, macrophytes were most dominant at NF90 and NF80 (Fig. 3.1.7).

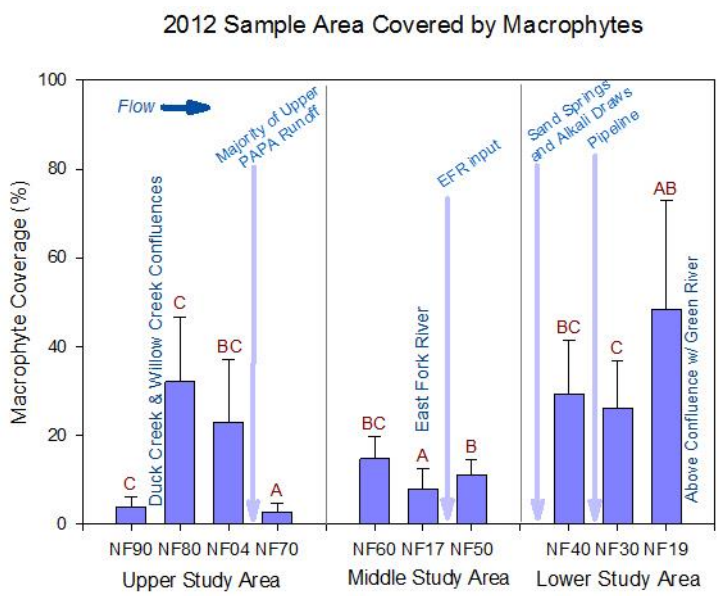


Figure 3.1.7. Macrophyte coverage. The mean relative portion of aquatic moss coverage from each square foot sampled for macroinvertebrates is presented with 95% confidence intervals.

AQUATIC MOSS COVERAGE

The portion of benthic substrata covered with branched aquatic bryophytes was first used as a covariate in 2011. This was measured as the percent coverage per benthic grab sample (1 sq. ft.). This covariate was especially important because mosses are important to certain aquatic invertebrates. For example, our laboratory observations indicate that some naidid worms were found exclusively nestled between the stem and leaf of aquatic mosses. Also, moss is a known habitat for some ephemerellid mayflies which find shelter and food (deposits of fine organic detritus) among the fronds of mosses. Conversely, one of the most abundant mayflies of the New Fork River watershed, *Rhithrogenia* sp., may actually be excluded from habitats with extensive moss growth because of its flat, wide, body form and the need for smooth substrata.

A similar percentage of each benthic sample was covered with moss as observed in recent years. The average percent cover was greatest in the upper and lower study areas, with the middle study area being characterized by very low percent cover by aquatic mosses. MOSS was a very important covariate for many macroinvertebrate metrics (Tables 3.1.1, 3.2.2). There were no statistically significant differences among sites in 2012. There was one marginally significant difference (0.1>P>0.05) between NF50 and NF80 (Fig. 3.1.8); all other sites were not significantly different from each other. There was a wide range of variation, especially at sites with relatively high average moss cover. Therefore, non-parametric statistics were required to test the differences between sites because of deviations from normal distribution and variance heterogeneity.

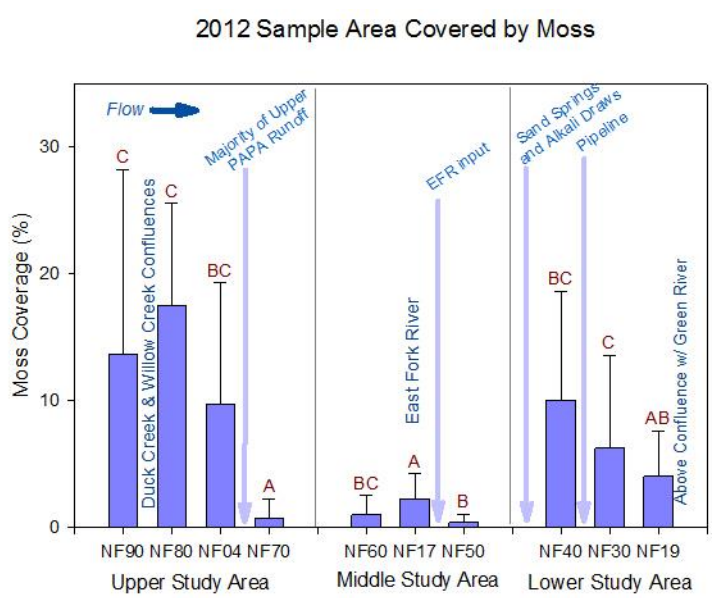


Figure 3.1.8. Moss coverage. The mean relative portion of aquatic moss coverage from each square foot sampled for macroinvertebrates is presented with 95% confidence intervals.

ALGAE

Field observations of algae are not often used as statistical covariates for several reasons. Typically, the density of algae is so low that laboratory analysis is required. Notable exceptions are when algae are sufficiently abundant to be visually observed in the field—such as with filamentous algae or when algae form mats. We used field observations of algae as a covariate that occasionally explained a significant amount of variation in biological metrics. Some limitations of this method are discussed in the methods section of this report. When observed cover-values were high, they usually reflected growth of filamentous algae or of diatom mats such as those of the nuisance algae *Didmosphenia geminata* (Fig. 3.1.9).

Though *D. geminata* is native to North America, since the late 1990's, the species exhibits massive growth spurts in high elevation, low nutrient and cooler waters. These mass growths can form mucus-like mats several centimeters thick that obfuscate the benthic substrata and dramatically alter benthic community structure (e.g., Marshall 2006a). Most of our field observations of *D.geminata* coverage were zero and there were insufficient data to use this variable as a meaningful covariate in our analysis of benthic macroinvertebrates (Fig. 3.1.10). Thus, other than NF19, most of the covariate "ALGAE" was comprised of filamentous algae.

The only significant differences among the sites were between NF04 and two middle zone sites (NF60, NF50). With NF50 and NF60 having significantly less algae coverage than NF04 non-parametric statistics were required to test the differences among sites because the data failed to meet the assumptions of parametric statistics. There were no significant differences among sites within a specific study area (upper, middle, or lower), nor transitioning among study areas (upper-mid (NF70-NF60), mid-lower (NF50-NF40).

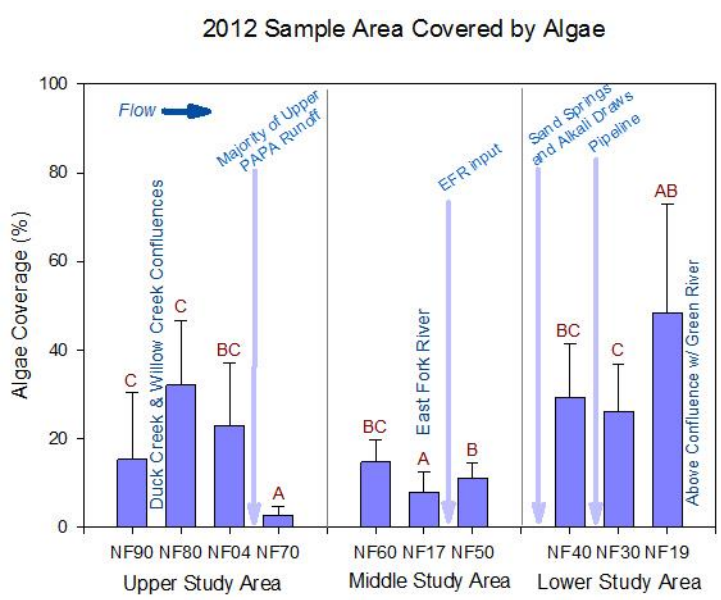


Figure 3.1.9. Algae coverage. The mean relative portion of algae coverage from each square foot sampled for macroinvertebrates is presented with 95% confidence intervals.

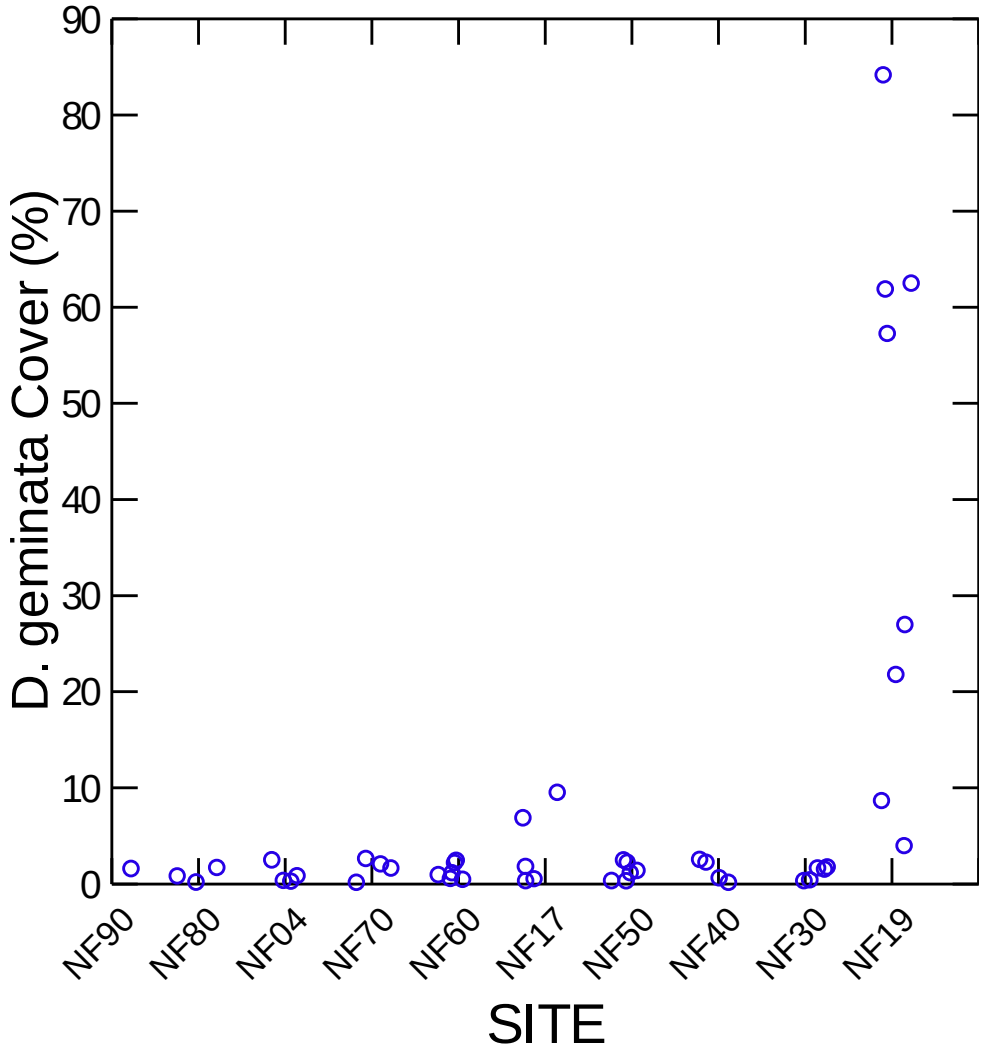


Figure 3.1.10. *Didymospenia geminata* coverage. Field observations of the relative portion of algae coverage of *D. geminata* from each square foot sampled for macroinvertebrates are presented as individual observations.

3.2 DIFFERENCES AMONG SITES (2012)

3.2.0 OVERALL DIFFERENCES AMONG SITES

The biological metrics were first screened for significant differences among sites using ANOVA (Table 3.2.1). All metrics indicated that there were statistically significant differences among the sites (P<0.05, Table 3.2.1). Several metrics are highlighted (red) for NF30 because they suggest communities of this location were influenced by some stressors which were apparently less influential at other study sites and these differences were congruent with the hypothesis of PAPA-related impacts to water quality (Table 3.2.1). Thus, diversity, evenness, %EPT, %non-insect, HBI, and % collector-gatherers were all metrics suggesting that the ecological conditions of NF30 were significantly reduced relative to adjacent sites.

After running the GLM stepwise multiple regressions with physical covariates, the means changed very little for most sites (Table 3.2.2). Moreover, the same metrics (diversity, evenness, % EPT, %non-insect, HBI, and %collector-gatherers) suggested impairment at NF30 (Table 3.2.2) relative to nearby sites. The use of covariates helped explain the relative influence of natural variation among the sites and clarified the patterns identified by regular ANOVA.

The following sections address each metric individually. The functional feeding group metrics are discussed simultaneously because they are computationally related.

TABLE 3.2.1. ANOVA Results 2012. The ANOVA resulted in statistically significant differences among sites for all 13 metrics tested directly. Tukey's HSD test was used to identify which sites were significantly different from each other except when the assumption of homogeneity of variance was violated. In this case, Tamhane's T2 test was used. If the Normality assumption was violated the non-parametric (n.p.) Kruskal-Wallis test was used followed by the Dwass-Crichlow-Fligner test to assign pairwise groupings. Sites that were significantly different from each other are noted by different letters in their columns. Sites that were not significantly different from each other share at least one letter with similar sites.

		Multiple Comparisons Grouping (TUKEY'S HSD)									
Metric	P-val	NF90	NF80	NF04	NF70	NF60	NF17	NF50	NF40	NF30	NF19
Total Abundance	<0.001	AB	aB	AB	A	AB	A	AB	B	B	AB
Taxa Richness	0.036	AB	AB	Ab	A	AB	B	AB	AB	AB	AB
Diversity (H') ^{T2}	<0.001	Ab	AB	A	AB	B	AB	AB	AB	**A**	B
Evenness (J') ^{T2}	<0.001	A	Ab	A	AB	B	AB	AB	AB	**A**	B
EPT- Richness	0.021	A	AB	AB	AB	B	AB	AB	AB	AB	AB
% EPT	<0.001	AB	AB	Bc	BC	C	AB	Bc	aB	**A**	C
% Chironomid ^{T2}	<0.001	C	BC	BC	A	Bc	BC	aBC	BC	BC	BC
% Non-Insect ^{T2}	<0.001	AB	AB	AB	A	AB	aB	AB	Bc	**C**	Bc
Dominance(5) ^{T2}	<0.001	BC	BC	C	ABC	A	ABC	ABC	ABC	ABC	A
HBI ^{T2}	<0.001	A	AB	AB	AB	A	A	AB	AB	**B**	A
Gatherers	<0.001	C	C	BC	A	BC	aB	B	BC	**C**	B
Filterers ^{T2}	0.001	AB	AB	AB	AB	AB	AB	B	AB	A	B
Collectors ^{T2}	<0.001	BC	C	bC	A	A	A	A	AB	ABC	A
Scrapers ^{T2}	<0.001	A	Ab	Ab	C	C	C	BC	BC	BC	BC
Shredders $^{n.p.}$	0.280	--	--	--	--	--	--	--	--	--	--
Predators $^{n.p.}$	<0.001	AB	AB	A	A	A	B	AB	B	AB	B
		Upper Study Area				Middle Study Area			Lower Study Area		

TABLE 3.2.2. GLM covariance model 2012. The covariates which explained significant portions of the variance in each metric are indicated by asterisks (*). The P-value evaluates the statistical significance of differences among sites given the covariates included in the model. Tukey's HSD test identified which specific sites were significantly different from each other, given the amount of variance explained by all other variables in the model. Note that the threshold of P<0.15 was only used as a criterion for inclusion in the GLM multiple regression model, not as a threshold for hypothesis testing among sites (q.v., methods section) nor for the evaluation of pairwise groupings. Lower case letters indicate marginally significant differences (0.10>P>0.05) which are identified to avoid type-2 statistical errors. For example, a site identified "aB" is significantly different from a site identified as "C" but only marginally different from a site identified with "A." Sites identified "Aa" are not significantly different from either sites identified as group "a" or group "A."

Metric	P-val	PSI	VELOCITY	MOSS	ALGAE	MACROPHYTES	NF90	NF80	NF04	NF70	NF60	NF17	NF50	NF40	NF30	NF19
Total Density	0.002			***		**	AB	AB	AB	Ac	AB	A	AB	B	BC	AB
Taxa Richness	0.103		**				AB	AB	AB	A	AB	B	AB	AB	AB	AB
Diversity (H')	0.049		***[1]	**			aBc	ABC	ABC	aBC	BC	aBC	BC	ABC	**A**[3]	BC
Evenness (J')	0.001		***[1]	***			A	A	Ab	A	B	AB	AB	B	**A**[2]	B
EPT- Richness	0.021						A	AB	AB	AB	B	AB	AB	AB	AB	AB
% EPT	<0.001			***[1]	*	***	BC	BC	B	C	BC	BC	aBC	B	**A**[2]	BC
% Chironomid	<0.001		**	***		***	B	aB	B	A	aB	B	B	B	B	B
% Non-Insect	<0.001				*	**	A	A	A	A	A	A	A	A	**B**[2]	A
Dominance(5)	0.006		***[1]			*	bC	bC	BC	BC	A	A	AB	AB	BC[3]	A
HBI	<0.001		***[1]	**[1]			AB	B	B	A	ABC	B	ABC	B	**C**[2]	B
Gatherers	<0.001				***	***	C	C	BC	A	BC	A	B	BC	**C**[3]	AB
Filterers	0.085	**[1]	***[1]	**	***		BB	B	AB	AB	AB	AB	AB	AB	A	AB
Collectors	<0.001	***[1]		***[1]	***		BC	C	BC	ABC	ABC	AB	AB	AB	AB	Abc
Scrapers	<0.001	***[1]		***[1]	***[1]		AB	A	AB	AB	AB	AB	B	B	B	aB
Shredders	<0.001	***[1]					A	A	A	B	A	A	A	A	A	A
Predators	<0.001		***				AB	AB	A	A	A	B	AB	B	AB	B
							Upper Study Area				Middle Study Area			Lower Study Area		

[1] This covariate explained a significant portion of the variation in the metric, but also significant interaction with SITE.

[2] These results are consistent with the hypothesized impact of PAPA development on the community structure of NF30.

[3] These results are consistent with a marginally significant impact of PAPA development on the community structure of NF30.

[*] This covariate explained a marginally significant amount of variation (0.15>P>0.10) in the metric.

[**] This covariate explained a significant amount of variation (0.10>P>0.05).

[***] This covariate was very strongly correlated with the metric (P<0.05).

3.2.1 TOTAL ABUNDANCE (DENSITY)

The total abundance of all macroinvertebrates per unit area (density) yielded relatively few statistically significant differences among sites. Although the lowest total abundance (8,170 individuals /m^2) was observed at NF70, this value was not significantly different from the other sites in the upper study area, nor was it significantly different from the two subsequent sites downstream. The site with the greatest average total density was NF40, which supported an average of 39,000 organisms / m^2 (Range: 14,300-111,000). After correction for the influence of two covariates (MOSS, MACROPHYES) NF40 was significantly greater than NF19, NF17 and NF70. There were no significant differences among the sites of the upper or middle study areas. At the lower study area, NF30 and NF40 were not significantly different from each other, but were significantly greater than NF19.

Density is a difficult metric to interpret. Disturbances can lower density if they are severe enough to cause significant mortality to most insect species. Alternatively, disturbances can elevate density if they provide food to a few species or otherwise release them from competition or predation. In this case, both NF30 and NF40 had a relatively high abundance (and high density) of small aquatic worms. Therefore, we propose that elevated density does reflect a negative change in benthic community composition at the lower study area relative to the middle study area, but that density was not significantly different from the macrophyte dominated references at the upper study area. NF19 had more moderate density-reflecting the more typical benthic community structure.

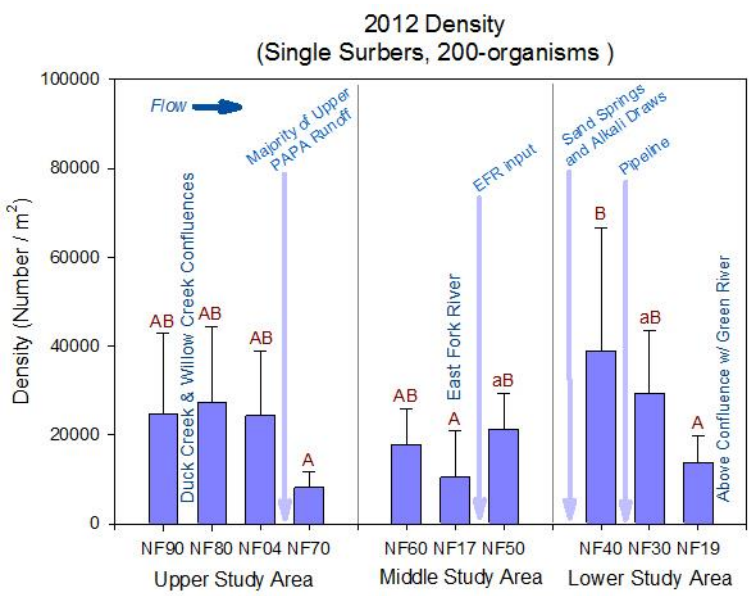


Figure 3.2.1. Total Invertebrate Density. The figure shows the average density per square meter at each site (n=8) and 95% confidence intervals. Sites exhibiting different letters are significantly different from each other. For example sites identified with the letter "A" are significantly different from sites marked "B" but not significantly different from sites marked with "AB."

3.2.2 TAXA RICHNESS

Taxa Richness at the upper study area was relatively homogenious, with all 4 sites having an average richness near 15-20 taxa per sample (Fig. 3.2.2). The middle study area also exhibited no significant differences among the sites. Similarly, there were no significant differences among sites comprising the lower study area.

The only significant differences observed among richness values occurred between the site with the lowest (NF70) average taxa richness and the site with the highest (NF17). This difference cannot be viewed as a PAPA-related impairment because it was caused by a modest increase in the richness of chironomid midges of the East Fork River (NF17)—not a decrease in the richness at a site expected to decline in relation to PAPA related disturbance (Table 3.2.1, Fig. 3.2.2). Therefore, this metric does not indicate any form of PAPA related impairment of the New Fork River.

EFFECTS OF PHYSICAL COVARIATES

The only covariate to significantly influence this metric was the velocity of water near the bottom of the stream. Adjusting for this covariate did not change which sites were significantly different from each other.

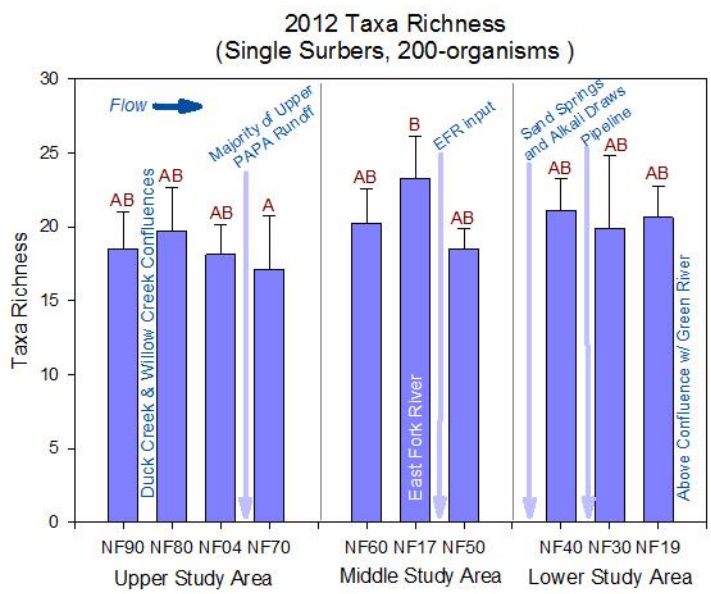


Figure 3.2.2. Taxa Richness. The figure shows the average taxa richness at each site (n=8) and 95% confidence intervals. Sites exhibiting different letters are significantly different from each other. For example sites identified with the letter "A" are significantly different from sites marked "B" but not significantly different from sites marked with "AB."

3.2.3 EPT RICHNESS

We observed no statistically significant differences in the EPT richness among the sites of the upper study area. The observed EPT richness averaged between 7-9 taxa per sample at the upper study area (Table 3.2.2, Fig. 3.2.3).

Although we also observed no significant differences in the EPT richness among the sites of the middle study area, we did observe a slightly greater range of observations (~7-11 taxa) (Table 3.2.2, Fig 3.2.3) compared to the upper study area.

There were no significant differences in the EPT richness among the sites of the lower study area. The range of observed average EPT richness values was ~9-11 taxa per sample (Table 3.2.2, Fig.3.2.3).

The only significant difference among the EPT Richness values observed at the sites was between the upper and middle study areas. Specifically, NF60 had significantly greater EPT richness per sample than NF90. This represents a downstream increase in the richness of the EPT orders—which are generally considered sensitive. Thus, this metric does not indicate impairment of New Fork River related to the PAPA.

EFFECTS OF PHYSICAL COVARIATES

None of the statistical covariates measured explained a significant amount of variation in the EPT richness metric.

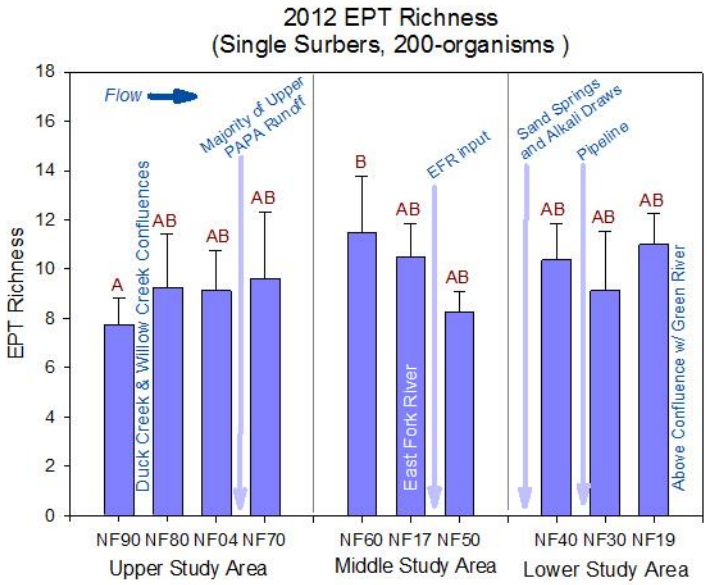


Figure 3.2.3. EPT Richness. The figure shows the average EPT taxa richness at each site (n=8) and 95% confidence intervals. Sites exhibiting different letters are significantly different from each other. For example sites identified with the letter "A" are significantly different from sites marked "B" but not significantly different from sites marked with "AB."

3.2.4 EPT RELATIVE ABUNDANCE

Within the upper study area, the site with the greatest relative abundance of EPT taxa was the site at the downstream boundary of the study area (NF70). It had significantly greater EPT abundance than the site immediately upstream from it (NF04), but was not significantly different from the two preceding sites (which had a slightly lower EPT abundance than NF04, but also had greater variance, which prevented the population from appearing significantly different from NF70). The high values observed at NF70 are actually supportive of the hypothesis of improving conditions downriver.

In the middle study area, we observed that the site upstream from the confluence of the East Fork River (NF60) had greater EPT relative abundance than the sites below the confluence (NF50) or on the East Fork River itself (NF17) but that these differences were not statistically significant.

The lower study area had significantly reduced relative abundance of EPT orders at NF30 relative to the sites immediately upstream and downstream. NF30 had the lowest relative abundance of EPT taxa (17%) study wide. This response signature is consistent with adverse PAPA-related effects at NF30. However, the low metric value was due to a dramatic elevation in the abundance of small moss-dwelling worms (*Nais* sp.) (Section 3.2.5).

Overall, the greatest average EPT relative abundances were observed at NF60 (53%), NF70 (46%), and NF19 (50%). NF70 was significantly different from NF04, NF40, and NF30—all of which had significantly fewer mayflies, stoneflies and caddisflies.

This metric suggests a localized decline in ecological conditions at NF30. However, this is the first year of depressed EPT abundance at the site in recent years. The same phenomenon was observed a short distance upstream at NF40 the previous year (2011). To keep the scale of this effect in perspective, it was caused by an increase in the density and relative abundance of worms (*q.v.*, sections 3.2.5 Non-insect abundance), but it does not reflect a significant decline in the density of these orders (*q.v.*, Section 3.5).

EFFECTS OF PHYSICAL COVARIATES

Several covariates explained a significant amount of variation in EPT abundance among sites. Specifically, the plant material metrics were correlated with EPT relative abundance and explained a large portion of the variance. MOSS and MACROPHYTES were highly significant ($P<0.05$) whereas the variable ALGAE explained less variance ($0.15>P>0.1$) [29]. However, after correction for these covariates, differences among the sites persisted (Fig. 3.2.4, Table 3.2.2). Before correction for covariates, the differences among the sites were similar, but more complicated. Specifically, sites NF40 and NF30 were marginally significantly different ($0.10>P>0.05$), though after covariate correction, they were considered significantly different ($P<0.05$). Additionally, NF50 was marginally significantly different from NF60 and NF19 before accounting for covariates (Table 3.2.1), but after correction NF50 was not different from either of these sites (Table 3.2.2., Fig. 3.2.4).

[29] This is explained in Table 3.2.2.

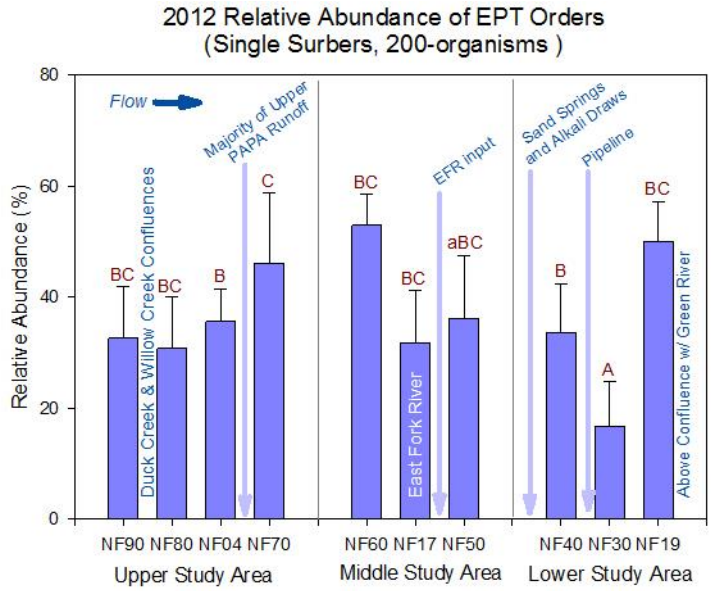


Figure 3.2.4. EPT Relative Abundance. The figure shows the average relative abundance of EPT taxa at each site (n=8) and 95% confidence intervals. Sites exhibiting different letters are significantly different from each other. For example sites identified with the letter "A" are significantly different from sites marked "B" but not significantly different from sites marked with "AB."

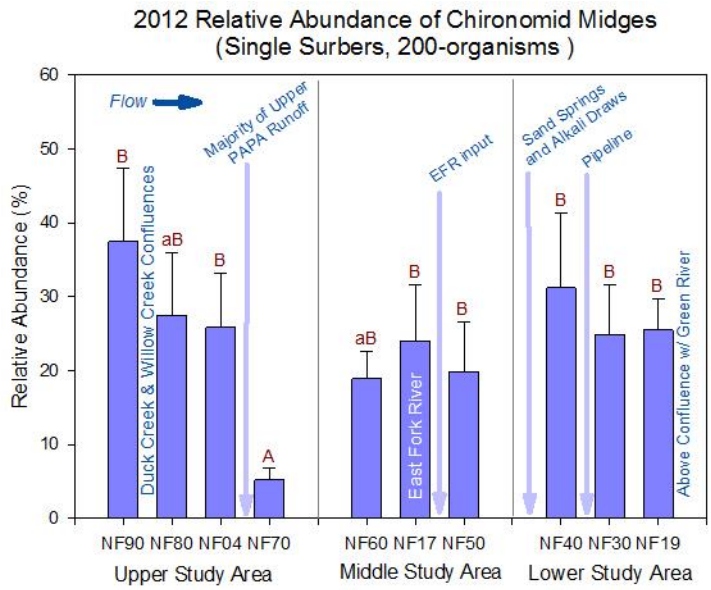


Figure 3.2.5. Chironomid Midge Relative Abundance. The figure shows the average relative abundance of chironomid midges at each site (n=8) and 95% confidence intervals. Sites exhibiting different letters are significantly different from each other. For example sites identified with the letter "A" are significantly different from sites marked "B" but not significantly different from sites marked with "AB."

3.2.5 CHIRONOMID MIDGE RELATIVE ABUNDANCE

The chironomid midges comprise an amazingly diverse group of insects with about 5000 species known to science, representing all benthic functional feeding groups. They naturally occur in a variety of aquatic and semiaquatic habitats and collectively exhibit a wide spectrum of environmental tolerances from very sensitive species to extremely tolerant species. In bioassessment when chironomid midges (collectively) become dominant, it usually represents an increase in the abundance of tolerant organisms. In Sublette County Wyoming, this may indicate an increase in the trophic status of streams (nutrient enrichment) or sedimentation. Thus, we expect higher levels of midge relative abundance at disturbed sites than at less disturbed sites.

Chironomid midges had the greatest relative abundance at sites with elevated plant material (which generally provides habitat and refuge from predation). At the upper study area, NF70 had the lowest relative abundance of chironomid midges (Fig. 3.2.5) and also a very low amount of vegetation cover (Figs. 3.1.6, 3.1.7, 3.1.8, 3.1.9). This site was considered significantly reduced relative to all other sites (within the upper study area and among all study areas) but the differences between NF70 and the sites NF80 and NF60 were only considered marginal ($0.1>P>0.05$) after correction for covariates (Table 3.2.2, Fig 3.2.5). NF70 also had very low chironomid abundance in 2011. These differences reflect the natural differences in the amount of macrophytes among the sites of the upper study area.

There were no significant differences among sites in the middle study area.

There were no significant differences among the sites of the lower study area, nor were there any significant differences between the sites of the middle or lower study area.

The only significant differences observed in midge relative abundance were between a site with very low abundance (NF70) and the sites with greater abundance and lower variance (NF60, NF80). Furthermore, the sites with the average greatest midge relative abundance comprised <40% of the community sampled on average. Thus, even the highest midge abundance we observed (NF90) is not exceptionally high on average, especially given the abundance macrophytes at the site.

This metric provided no evidence of declines in the ecological condition of the New Fork River.

EFFECTS OF PHYSICAL COVARIATES

When the physical covariates were used to account for the physical conditions from which samples were collected (Table 3.2.2), we found that three covariates explained significant portions of the variance in the abundance of chironomid midges (Table 3.2.2). Specifically, the variables MOSS and MACROPHYTES explained a very high amount of variation in midge abundance ($P<0.05$) and the variable VELOCITY explained a more moderate amount of variance ($0.10>P>0.05$). Though the discussion above relates to the data corrected for the influence of these variables, the same sites were considered significantly different from each other before covariate corrections.

3.2.6 RELATIVE ABUNDANCE OF NON-INSECTS

Aquatic insects usually dominate healthy North American rivers. Often non-insects increase in abundance in disturbed or perturbed ecosystems; however they also become abundant naturally in certain unusual situations. For example, non-insects may dominate benthic fauna of Wyoming streams when influenced by sediment entrainment, salinity, nutrient enrichment, thermal disturbances, or significant input from mineral springs.

There were no significant differences among the sites of the upper study area. Although there was a relatively wide range of average non-insect relative abundances among the sites, there was also a high amount of variance within each site.

The middle study area was relatively homogeneous with averages ranging from about 9% (NF17) to about 17% (NF50). Within site variance was generally lower among middle sites than either the upper or lower sites.

Site NF30 had dramatically elevated non-insect abundance compared to the sites upstream and downstream. The differences were statistically significant (Fig. 3.2.6).

Study-wide non-insect relative abundance was greatest at NF30 of the lower study area. This site was significantly greater than all other sites and there were no other significant differences among the other sites.

This metric is also the key to the apparent response of other metrics. For example, the decline in the relative abundance of mayflies, stoneflies and caddisflies occurred because of a dramatic increase in the density of non-insects (small worms). If the percent abundance of one group goes up, the percent abundance of other groups must go down. In this case, the density of non-Insects at NF30 was much greater than other sites, and much greater than observed in the previous year. Thus, this metric describes a potential impact but is also responsible for all the other apparent impacts.

Our examination of the density of macroinvertebrates suggests that the absolute abundance for sensitive aquatic insect orders appears largely unchanged (see section 3.5 below). This indicates that the effect of non-insects on ecosystem function is probably less dramatic than these metrics suggest. That is, this metric and the other responding metrics appear to suggest a dramatic reduction in the roll of sensitive aquatic insects and an increase in the abundance of non-insects, but the density of these groups appears generally unchanged among sites. However, continued monitoring is essential to ensure a mild impairment does not become more serious.

EFFECTS OF PHYSICAL COVARIATES

When the covariates were used to account for the physical conditions from which samples were collected (Table 3.2.2), we found that two covariates explained significant portions of the variance in the abundance of non-insect invertebrates (Table 3.2.2). Specifically, the covariate MACROPHYTES explained a very large amount of variation in the metric ($P<0.05$) and the variable ALGAE explained a marginal amount of variation in the metric ($0.15>P>0.1$). Before correction for these variables, NF30 was still significantly different from all other sites, although the significance of the differences between NF30 and its adjacent sites (NF40, NF19) was marginal ($0.1>P>0.05$). Additionally, the site with the lowest abundance in non-insects (NF70) was significantly different from all the sites in the lower study area and marginally different from the East Fork River (NF17). This is because NF70 had very low amounts of plant cover. When corrected for the influence of macrophytes, NF70 was only significantly different from NF30. This did not result in changing the differences within study areas in a way that would change our hypothesis-based conclusions within study areas. Thus, the change in significant groups does not represent a hidden impact at NF70, but rather the low abundance of both aquatic plants and non-insects—which is considered to reflect a healthier community.

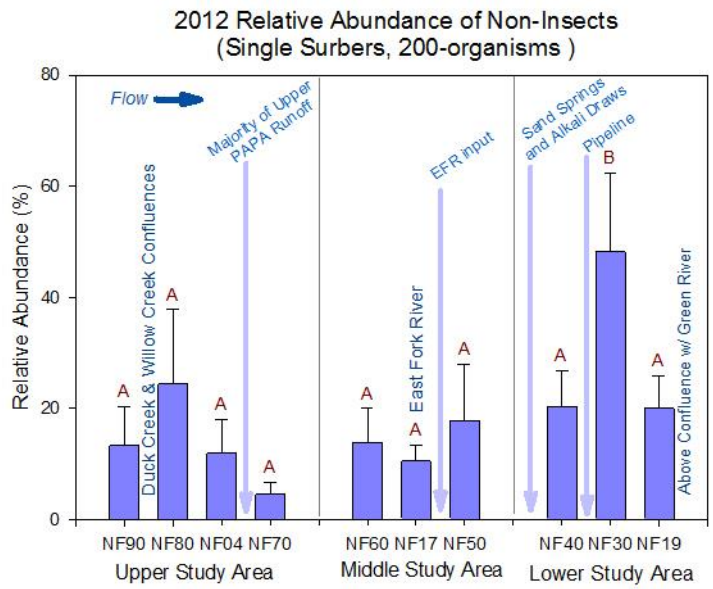


Figure 3.2.6. Non-Insect Relative Abundance. The figure shows the average relative abundance of non-insects at each site (n=8) and 95% confidence intervals. Sites exhibiting different letters are significantly different from each other. For example sites identified with the letter "A" are significantly different from sites marked "B" but not significantly different from sites marked with "AB."

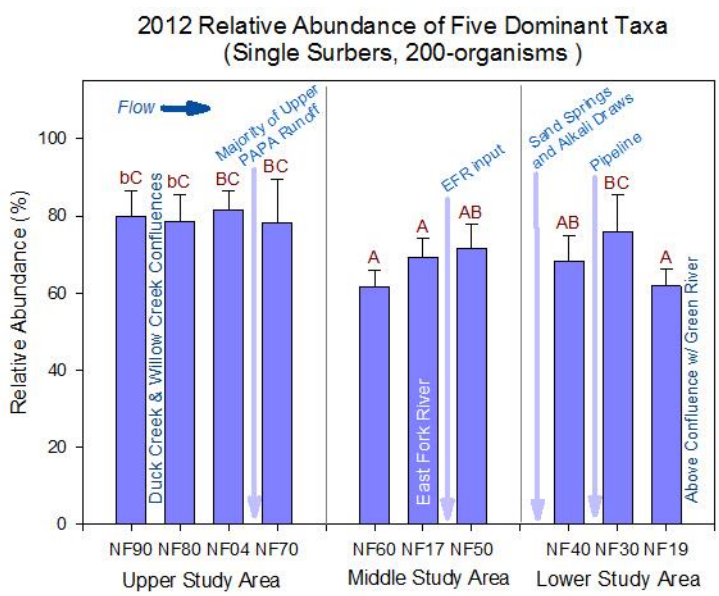


Figure 3.2.7. Community Dominance (5 Taxa). The figure shows the average combined relative abundance of the 5 most abundant taxa in each sample from each site (n=8) and 95% confidence intervals. Sites exhibiting different letters are significantly different from each other. For example sites identified with the letter "A" are significantly different from sites marked "B" but not significantly different from sites marked with "AB."

3.2.7 COMMUNITY DOMINANCE (5 TAXA)

Stribling et al. (2000) found most (75%) reference sites in the Wyoming Basin ecoregion had <87% Dominance (5 taxa). This was recommended as the 100% biotic integrity threshold for their version of the WSII. All of the sites in this survey averaged less than 80% for the five most abundant taxa; they all scored very well for this metric. High community dominance values usually herald the increasing influence of stressors because the metric elevates as tolerant species become more dominant. Low values occur when ecological conditions become hospitable for more species; therefore the relative abundance of any one taxon (or five taxa) is reduced.

The upper study area had the greatest average dominance values, and there was very little difference among the individual sites of the study area. There were no significant differences among the sites of this study area.

The middle study area generally had lower community dominance than the upper study area. There were no significant differences among the sites of the middle study area. The East Fork River (NF17) and the New Fork River (NF60) above the East Fork confluence had significantly lower dominance than the sites of the upper study area and significantly lower dominance than NF30 (Fig. 3.2.7).

In the lower study area, sites NF40 and NF19 had relatively low community dominance compared to the worm-dominated benthos of NF30. NF30 had statistically significantly greater community dominance than NF19, but was not significantly different from upstream NF40.

The values were lower than any previously observed in the New Fork River—and these values are less than the threshold for WY DEQ's reference streams. The values at NF30 were affected by worm dominance, but still were not significantly different from the sites of the upper study area. Therefore, the dominance of the five most-abundant taxa (DOM5) does not provide evidence of any ecological impairment related to natural gas development in the PAPA.

EFFECTS OF PHYSICAL COVARIATES

When the physical covariates were used to account for the physical conditions from which samples were collected (Table 3.2.2), we found that two covariates explained significant portions of the variance in the abundance of non-insect invertebrates (Table 3.2.2). Specifically, the covariate VELOCITY explained a very large amount of variation in the metric ($P<0.05$) and the variable MACROPHYTES explained a marginal amount of variation in the metric ($0.15>P>0.1$). After accounting for the significant environmental variables (Table 3.2.2, Fig. 3.2.7), the differences among the sites changed only slightly compared to the differences in the non-adjusted means (Table 3.2.1). The differences among sites in the upper and middle study areas did not change. Before adjusting for the significant covariates, the community dominance values of the lower study area did not exhibit any significant differences (Table 3.2.1). After adjustment for the covariates, NF30 was considered significantly greater than NF19, though the means were not dramatically different. Inter-study area differences also became more apparent after correction for the environmental variables. The main specific inter-study area differences that were revealed after environmental correction, were that NF90 and NF80 were both marginally significantly different from several sites (NF17, NF50 (marginal), NF40 (marginal), NF19). Correcting for the influence of water velocity and plant coverage made the worm-dominated community at NF30 more apparent.

3.2.8 HILSENHOFF BIOTIC INDEX (HBI)

The Hilsenhoff Biotic Index reflects the average pollution tolerance of a community (see Methods) with high values reflective of organic enrichment, sedimentation or other disturbances.

All the sites had much lower HBI values than the previous year, indicating a reduction in the relative abundance of tolerant organisms in 2012, and thus, the contribution of species generally considered sensitive increased in 2012.

There were no significant differences among the sites of the upper study area. NF80 and NF04 had the highest average values at about 4.5. Values less than 5 indicate more sensitive or facultative taxa than tolerant taxa.

There were no significant differences among the sites of the middle study area. All the sites of this area had similar HBI values, with only a slightly higher value (near 4.5) attained at the lower site (NF50) within the study area (Fig. 3.2.8).

NF30 had significantly greater HBI values than the other lower study area sites in 2012. NF30 was the only site (study wide) to exceed an HBI value >5.0—indicating that tolerant organisms were more abundant than sensitive organisms. After covariate correction the average HBI tolerances from NF30 were significantly different from all sites except those at NF50 and NF60.

This metric suggests a slight ecological disturbance near NF30 that could be related to natural gas development in the PAPA. The cause of this higher HBI value was the high abundance of small worms at NF30.

EFFECTS OF PHYSICAL COVARIATES

When the physical covariates were used to account for the physical conditions from which samples were collected (Table 3.2.2), we found that two covariates explained significant portions of the variance in the HBI (Table 3.2.2). Specifically, the covariate VELOCITY explained a very large amount of variation in the metric ($P<0.05$) and the variable MOSS explained a significant amount of variation in the metric ($0.10>P>0.05$). Before correction for these covariates (Table 3.2.1), NF30 was not considered significantly different with as many sites as it was afterwards (Table 3.2.2).

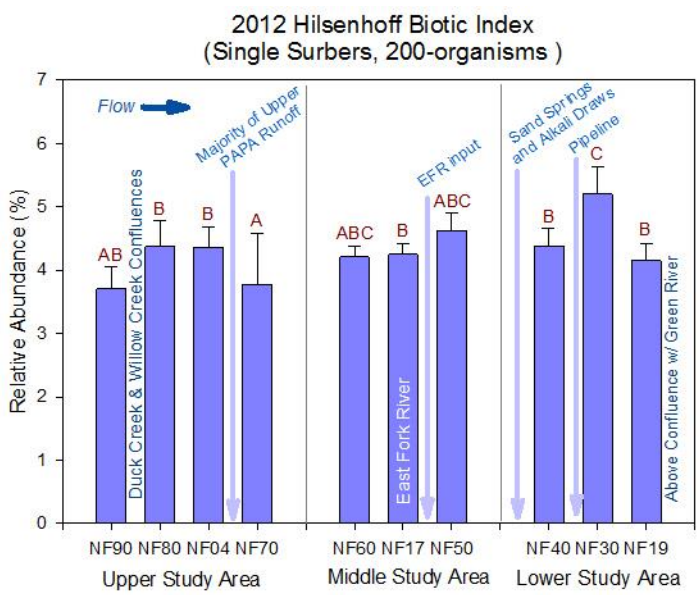


Figure 3.2.8. Hilsenhoff Biotic Index (HBI). The figure shows the average HBI value at each site (n=8) and 95% confidence intervals. Low values indicate community dominance by sensitive organisms, whereas high values indicate dominance by tolerant organisms. Sites exhibiting different letters are significantly different from each other. For example sites identified with the letter "A" are significantly different from sites marked "B" but not significantly different from sites marked with "AB."

3.2.9 RELATIVE ABUNDANCE OF COLLECTORS

Modern aquatic ecology is often concerned with the movement of carbon (energy) through food webs because this is at the core of ecosystem function. Thus, it is useful to group organisms by their roles in processing organic material, or their "functional feeding group." Anthropogenic disturbances that alter the function of food webs are intended to be prevented under the auspices of the Clean Water Act and are sometimes manifested by shifts in the abundance of organisms of different functional feeding groups.

Collector-gatherers are organisms that search out deposits of fine particulate organic material for sustenance. Collector-filterers remove fine particulate organic material from the water column for the same purpose. In streams and rivers, the amount of suspended organic material is linked to the velocity of water. Since both collector-gatherers and collector-filterers consume fine particles of organic matter, the differences in their relative abundances may be related to localized flow conditions. For this reason, it is often useful to consider the combined abundance of filterers and gatherers as the "collector" group[30]. For the purposes of this report, we used the total collectors as a metric, because it has a clear response signature and the changes among the relative contribution (%) of other functional feeding groups are often driven by changes in this single functional feeding group. Other groups are discussed in Section 3.3, and supplementary graphs are presented in the appendix.

As with many previous years, the upper study area, with high densities of aquatic macrophytes and many depositional micro-habitats, exhibited the greatest relative abundance of collectors. Although NF70 had about 20% lower abundances of collectors than the other upper study areas, there were no significant differences among the sites of the upper study area because the variation was moderately high (Fig. 3.2.9).

The middle study area had fewer collectors on average than the upper study area. There were no significant differences among the sites of the middle study area.

There were no significant differences in the contribution of collectors to the communities of the lower study area. Also, the sites of the lower study area were not significantly different from the middle study area.

The only significant inter-study-area differences were between the sites with the greatest collector abundance (NF80) and some sites with lower collector abundance (NF17, NF30).

This metric does not provide any direct evidence of ecological impairment of the New Fork River related to natural gas development in the PAPA. However, it does also make the sites appear more similar than they really are.

EFFECTS OF PHYSICAL COVARIATES

When the physical covariates were added to the GLM algorithm to account for the physical conditions from which samples were collected (Table 3.2.2), we found the covariates PSI, MOSS and ALGAE explained highly significant amounts of variation in the metric ($P<0.05$). Before correcting for the influence of significant covariates NF30 was not significantly different from any other site surveyed. After correction, it was significantly less than NF80. Correction for covariates made interpretation of significant differences more straight forward, but it did not alter the conclusions for this metric.

[30] I refer to this as "combined collectors" or “total collectors” to avoid confusion with either of the other collector functional feeding groups (either gatherers or filterers).

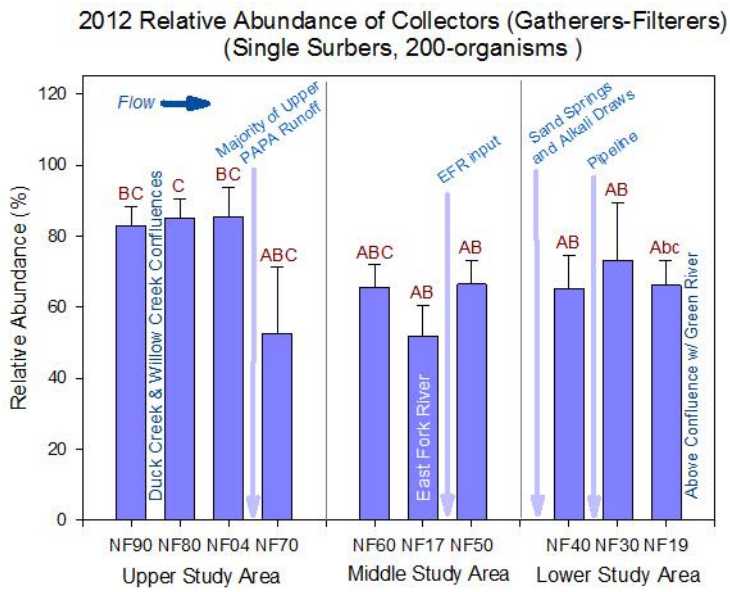


Figure 3.2.9. Contribution of Collectors. The figure shows the average contribution of collectors (%)I value at each site (n=8) and 95% confidence intervals. Sites exhibiting different letters are significantly different from each other. For example, sites identified with the letter "A" are significantly different from sites marked "B" but not significantly different from sites marked with "AB."

3.3 ABUNDANCE OF FUNCTIONAL FEEDING GROUPS (2012)

There are five common functional feeding groups used to generalize the functional roles of macroinvertebrates in processing organic material: collector-gatherers, collector-filterers, scrapers, shredders and predators. A site group generally comprises a very small portion of benthic assemblages. In considering the response of individual metrics (Section 3.2), we considered the combined abundances of collector-gatherers and collector-filterers. Here we consider all the function feeding groups simultaneously. Supplementary graphs in the appendix statistical tables, previously presented, describe the statistical differences of each group among zones (Tables 3.2.1, 3.2.2).

Collector gatherers generally comprised greater than half the invertebrates collected from the thickly vegetated sites of NF90, NF80 and NF40. The only site with low macrophytes abundance to support greater than 50% collector-gatherer abundance was NF30, which was dominated by small worms of the genus *Nais sp*. (Fig. 3.3.1).

The abundances of scrapers were greater when there were fewer aquatic macrophytes. This is to be expected because scrapers generally feed on epilithic diatom films, which may be sparse in macrophyte beds. Scrapers were also very abundant in the East Fork River (NF17). When scrapers were abundant in 2012, they were most often the glossosomatid caddisflies *Glossosoma* or *Protoptila*.

Another important spatial change among the sites in 2012, was the abundance of "other" taxa. Traditionally these organisms comprised about 0-2% of total Functional Groups. In 2012, these "other" organisms comprised an increasing role down river, beginning at NF60 and maximized at NF19 and NF30.

Shredders (primarily *Lepidostoma*) and predators (mostly *Isoperla*) had relatively constant contributions to the communities among sites.

Traditionally, very few members of the "other" functional feeding group occur in the New Fork River. In 2012, they were represented by hydroptilid caddisflies which feed on algal cytoplasm and the chironomid midges which live symbiotically within the blue-green algae *Nostoc* – the *Cricotopus* (*Nostococladius*) midges. By far the more abundant of the two groups were the *Nostoc*-dwelling midges (Figs. 3.3.2, 3.3.3). The Hydroptilids were only abundant at NF19 (3%), whereas *Nostoc*-loving midges showed a gradient of increasing abundance downstream. Because blue green algae (including *Nostoc*) become abundant in nitrogen-limited ecosystems, we consider this a positive trend in the New Fork River and not an indicator of ecological degradation. However, it does underscore the sensitivity of the system to nitrogen additions. Note that some databases, such as the EDAS (TetraTech Inc.) databases commonly used by state regulatory agencies incorrectly assign these midges as shredders.

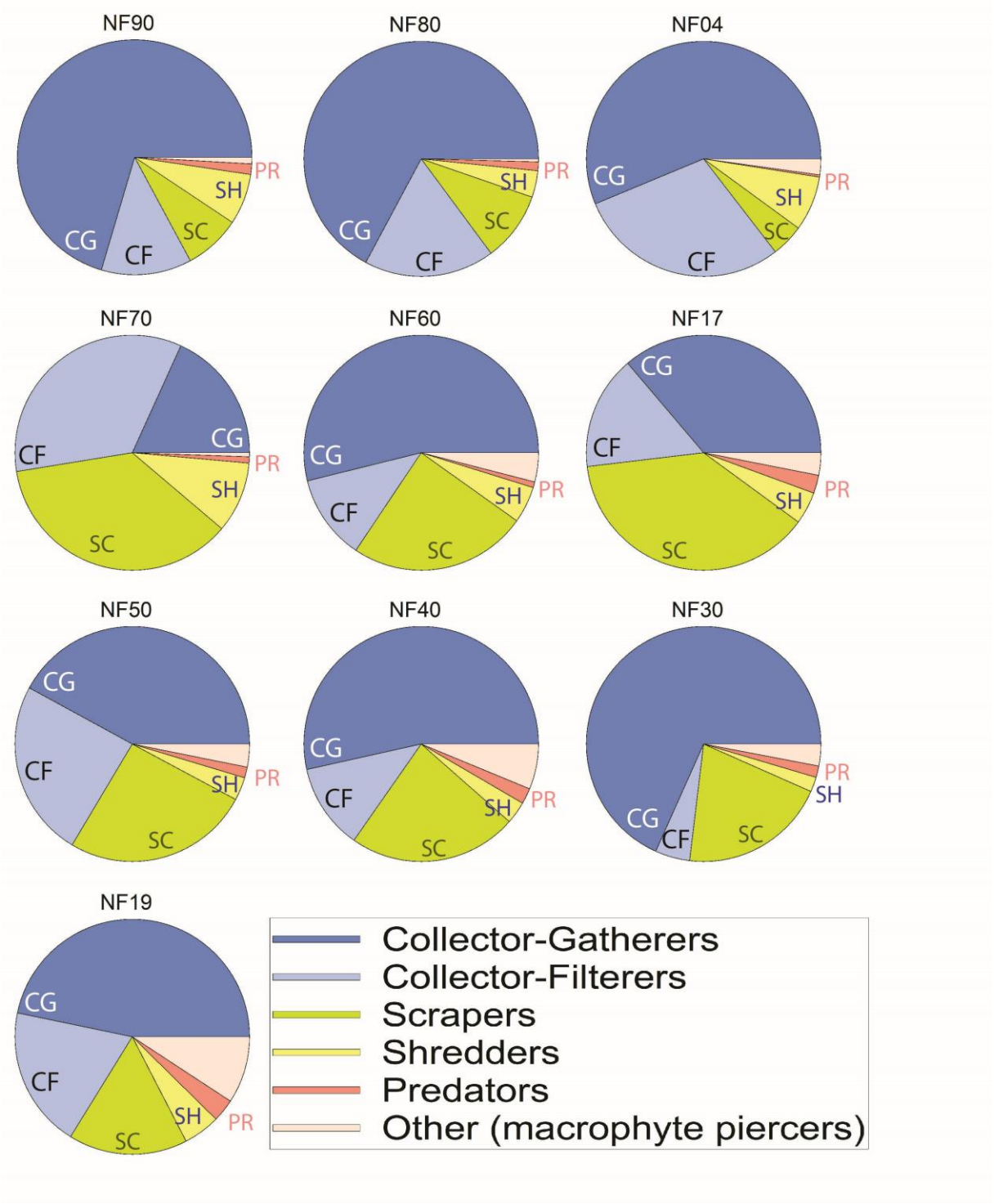


Figure 3.3.1. Contribution of Functional Feeding Groups to Benthic Assemblages. The plot begins at 3:00 on the pie chart and progresses counter-clockwise in the order described in the legend. The portions of each section are also labeled with code identifying the corresponding functional feeding group as follows (CG, collector-gatherers; CF, collector-filterers; SC, scrapers; SH, Shredders; PR, predators). Macrophytes piercers comprised a small portion of some samples, these sites show a larger "other" category, which grew along a downstream gradient.

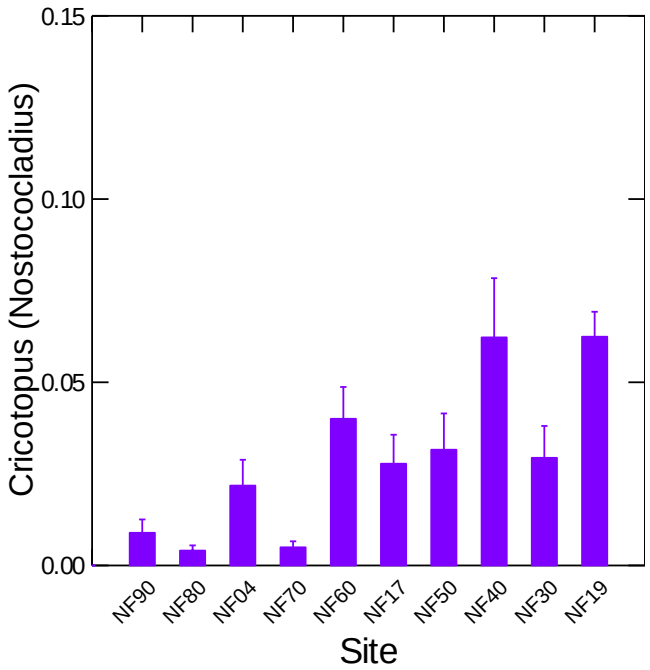


Figure 3.3.2. *Nostococladius* abundance. The abundance of obligate *Nostoc* symbiont midges of the *Cricotopus* (*Nostococladius*) group are shown as average per site. Error bars show one SEM. 95% confidence intervals equals 2xSEM.

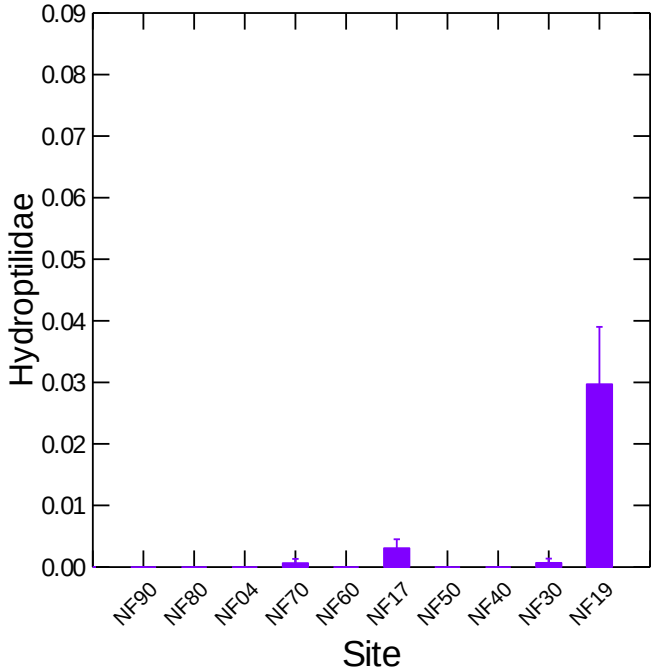


Figure 3.3.3. *Hydroptilidae* abundance. The abundance of macrophytes piercers is shown per site as means ± 1 SEM. 95% confidence intervals ~2xSEM.

3.4 CHANGES OVER TIME (2007-2012)

The previous sections examined spatial variation among study sites sampled only in 2012. If all sites declined in condition from the previous year(s), changes in river condition would be over looked by analysis of spatial variation alone. Therefore, it is important to compare each site with previous years to ensure multi-year trends are not overlooked. As with previous years' analyses, we used metrics that are not as sensitive to modifications in sampling protocol (single Surbers vs. composite Surbers) or laboratory methods (200ct vs. 500ct sample processing). The measures that were least sensitive to changes in methods were: % EPT, % Chironomidae, % non-insect abundance, % collectors, % scrapers, % dominance (5 taxa), and Hilsenhoff Biotic Index (Marshall 2008).

3.4.1 EPT RELATIVE ABUNDANCE

Two sites had significantly different EPT relative abundances from the previous year (Table 3.4.1). NF90 had significantly greater ($P<0.05$) EPT abundance in 2012, compared to 2011. NF30 had significantly reduced EPT abundance in 2012, compared to 2011. NF30 thus showed a significant impairment which could be related to development in the PAPA. The site fell from an average of 59% EPT to an average of 16% EPT. This appears to be very dramatic, but our follow up (Section 3.5) suggests that this shift was due to elevated worm abundance, not due to actual declines in the density of mayflies, stoneflies and caddisflies.

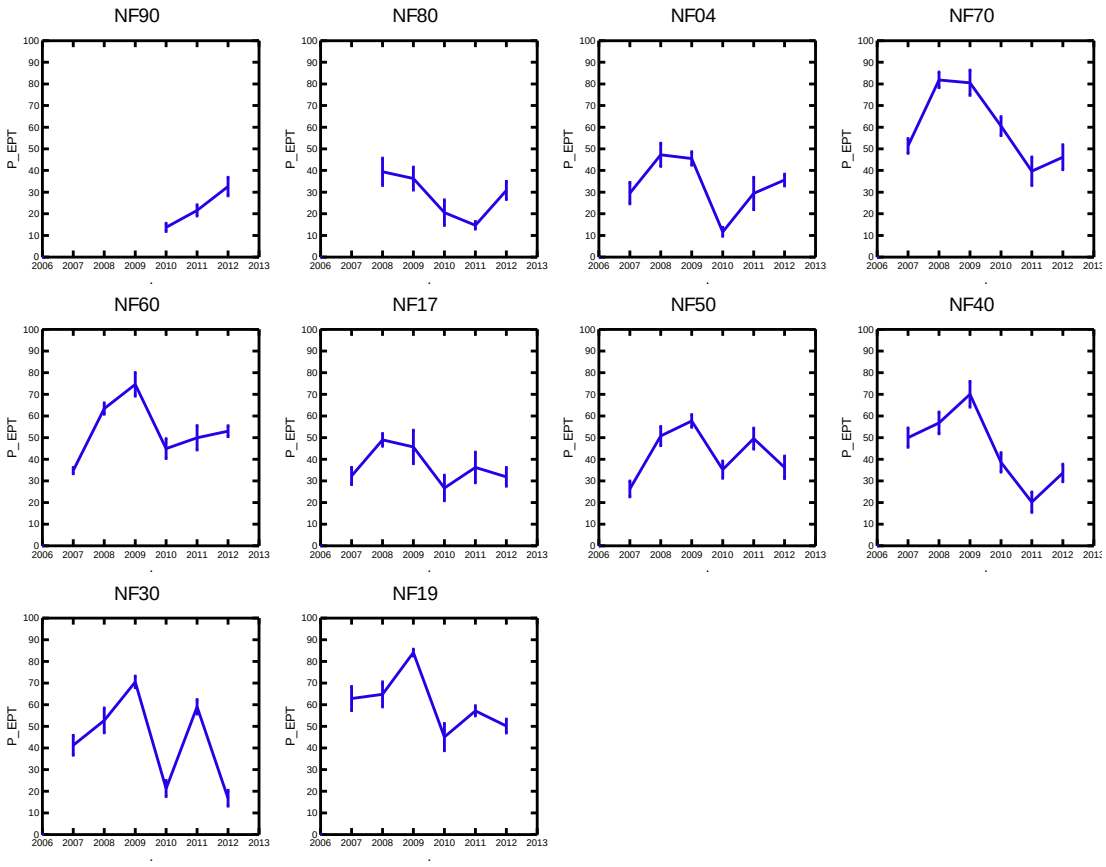


Figure 3.4.1. Changes in EPT Abundance over Time. Lines connect average EPT relative abundance over time and express error as the Standard Error of the Mean (SEM). 95% confidence Intervals are described @ 2x the SEM.

3.4.2 TRENDS IN CHIRONOMID MIDGE RELATIVE ABUNDANCE

Most sites had no significant change in the relative abundance of chironomid midges in 2012. The notable exception was the site NF90 (Table 3.4.1). NF90 had a significant decrease in midge relative abundance with midges comprising only 38% of samples collected in 2012, but 60% in 2011. This constitutes an improvement in benthic community structure. Moreover, none of the sites exhibited dramatically elevated midge abundance in 2012, even sites with prevalent macrophytes growth.

This metric proved no evidence of declining ecological condition of the New Fork River in 2012.

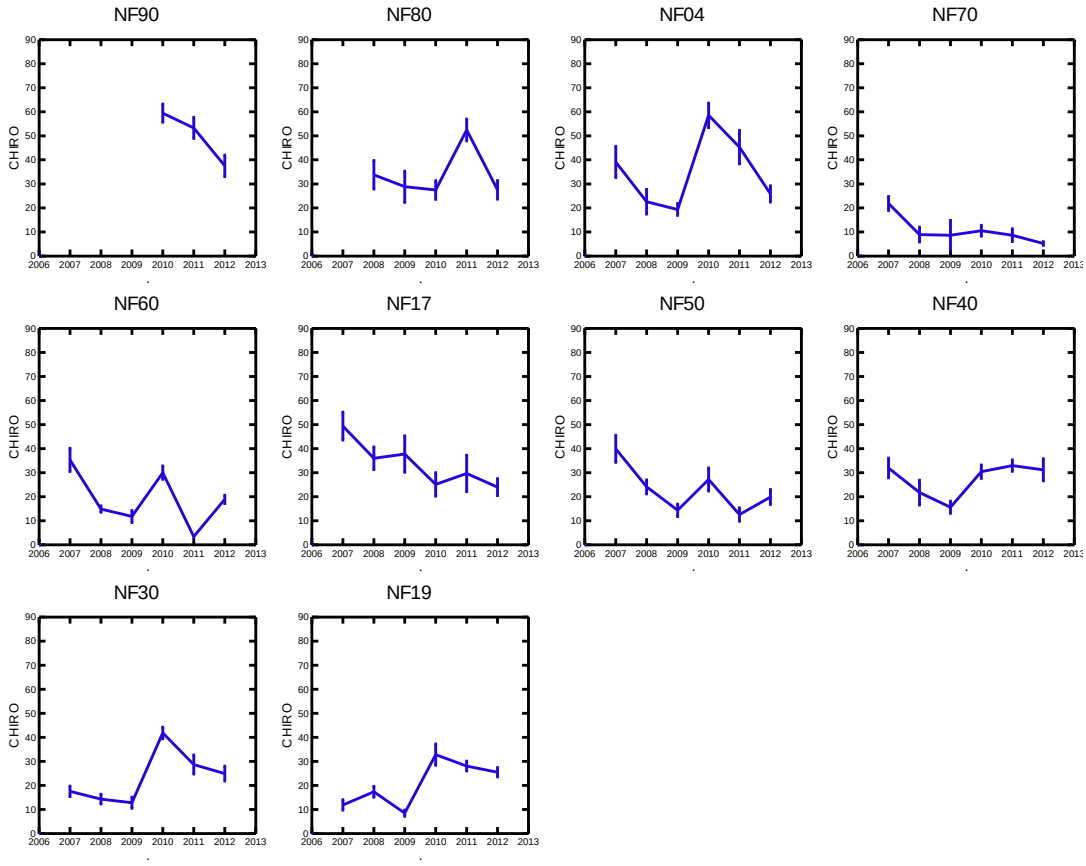


Figure 3.4.2. Changes in Chironomidae Abundance over Time. Lines connect average midge relative abundance over time and express error as the Standard Error of the Mean (SEM). 95% confidence Intervals are described @ 2x the SEM.

3.4.3 TRENDS IN NON-INSECT RELATIVE ABUNDANCE

Several sites had significantly greater non-insect relative abundance in 2012, relative to the previous year. Specifically NF17, NF30, NF50 and NF70 all had significantly greater non-insect relative abundance in 2012, compared to 2011 (Table 3.4.1, Fig. 3.4.3). Three of these sites exhibited comparatively low non-insect abundance, and the statistical significance reflects the low initial values and relatively low variance among observations. Specifically the statistically significant increases observed at NF17, NF50 and NF70 are superficial and do not represent dramatic shifts in the abundance of benthic species, whereas NF30 did exhibit a dramatic increase in the role of non-insects from about 5% in 2011, to an average of about 50% in 2012. These observations were due to the occurrence of moss/sediment dwelling *Nais* worms. Note that a similar phenomenon was observed at NF40 in 2011, but that non-insect abundance appeared to decrease there in 2012. Thus, this observation could reflect migration and mediation of conditions observed at NF40 in 2011, as the conditions producing high worm abundances are exported down river.

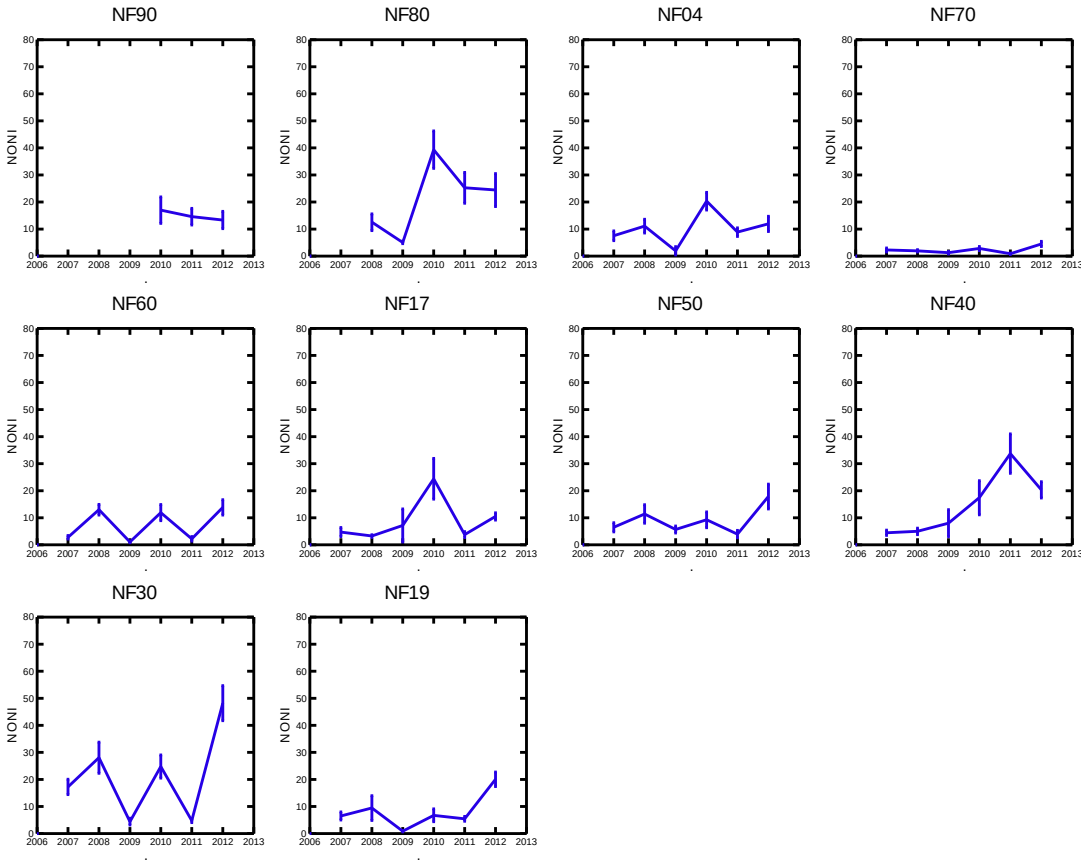


Figure 3.4.3. Changes in Non-Insect Abundance over Time. Lines connect average non-insect abundance over time and express error as the Standard Error of the Mean (SEM). 95% confidence Intervals are described @ 2x the SEM.

3.4.4 TRENDS IN THE CONTRIBUTION OF COLLECTORS TO THE COMMUNITY

The contribution of collectors (gatherers and filterers) was significantly different from 2011 at only one site (NF40). NF40 displayed a significant decline in the abundance of collectors in 2012 (Fig. 3.4.4). This is consistent with an improvement in water quality because collectors usually collectively benefit from disturbance. Values in 2012 dropped to about 65% from the 80% values observed in 2011.

These observations underscore the improved conditions at NF40 in 2012. Thus, this metric did not indicate any impairment of the New Fork River related to operations in the PAPA during 2012.

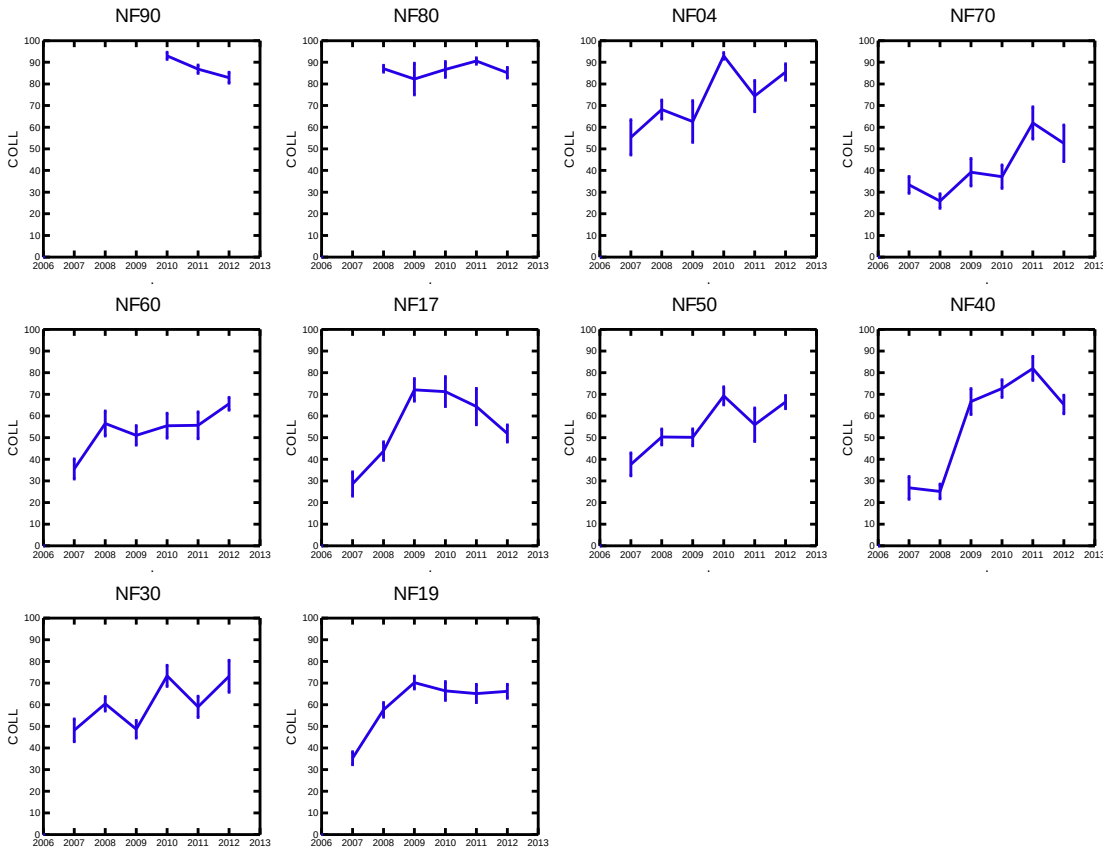


Figure 3.4.4. Changes in Collector Abundance over Time. Lines connect average relative abundance of collectors over time and express error as the Standard Error of the Mean (SEM). 95% confidence Intervals are described @ 2x the SEM.

3.4.5 TRENDS IN COMMUNITY DOMINANCE (5-TAXA) OVER TIME

In 2012, all sites had lower average dominance values compared with 2011. At most sites, these differences were not statistically significant. However, at NF17, NF40 and NF19, these decreases in dominance were statistically significantly ($P<0.05$). All of these differences represent a net improvement in water quality and biotic integrity. It is noteworthy that site NF30, which had dramatic increases in worm dominance in 2012 also had a slight decrease in the average role of the 5 dominant taxa in 2012 (Fig. 3.4.5).

These changes constitute basin-wide improvements in 2012 and do not indicate any impairment of the New Fork River related to development in the PAPA.

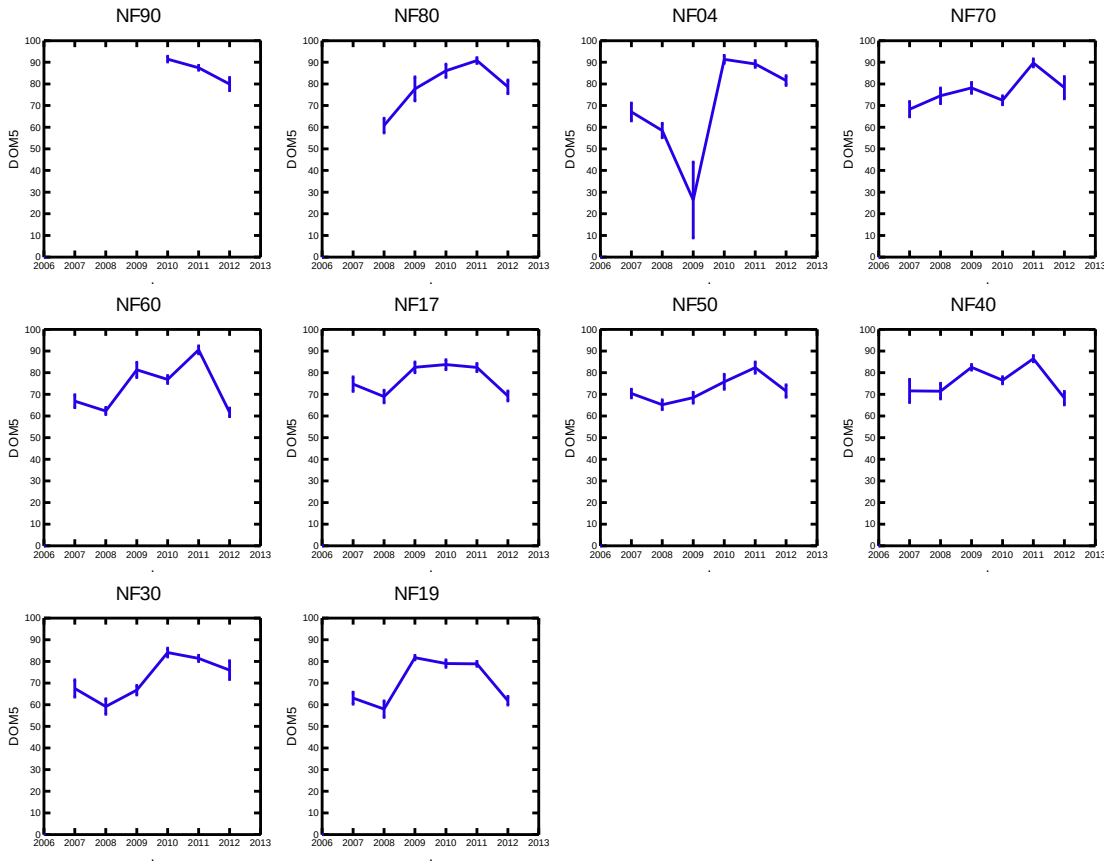


Figure 3.4.5. Changes in Community Dominance over Time. Lines connect average community dominance (5 taxa) over time and express error as the Standard Error of the Mean (SEM). 95% confidence Intervals are described @ 2x the SEM.

3.4.6 TRENDS IN HILSENHOFF BIOTIC INDEX VALUES OVER TIME

Most of the sites had no significant differences in 2012, compared to 2011 (Fig. 3.4.6). Two sites (NF90, NF80) had significant decreases in HBI scores and two sites had significant increases (NF50, NF30). Decreases in HBI represent improvements. Thus, these findings describe the improvements at NF90 and NF80 which were dominated by tolerant collector gatherers in 2011. Both sites had HBI values greater than 6 in 2011 and scores closer to 4 in 2012 (NF80, 4.25; NF90, 3.7).

NF50 had significantly greater HBI values in 2012 (4.5) relative to those in 2011 (3.6). However, these values were less than those observed at the site in 2007, and about the same as those observed in 2010 (4.55). The HBI values observed at NF50 in 2012 increased about the same amount (compared to 2011) as those of NF60, and are similar to the normal range of variation at the site. This was due to a significant increase in the density and relative abundance of non-insects at NF50 which increased to 17.8% in 2012.

The HBI values from NF30 increased dramatically in 2012 because of the tremendous increase of small Naididae worms, which comprised more than 50% of most samples. This represents a much more dramatic change in relative abundance than those observed at NF50. Additionally, NF30 was the only site to score above HBI=5.0 in 2012.

Overall, HBI scores suggested improvements at most locations studied. The exceptions were NF50 and NF30, which had increased relative abundance of tolerant worms (spatially and temporally).

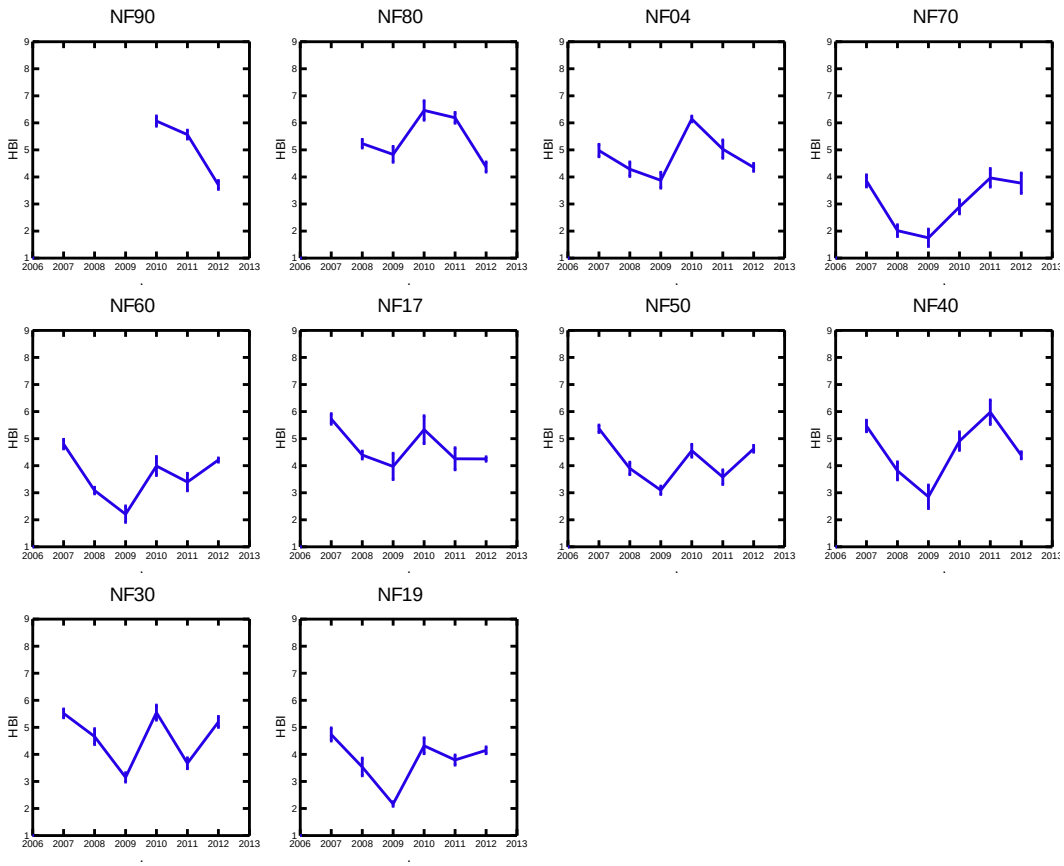


Figure 3.4.6. Changes in HBI over Time. Lines connect average HBI over time and express error as the Standard Error of the Mean (SEM). 95% confidence Intervals are described @ 2x the SEM.

3.4.7 CHANGE IN SCRAPER RELATIVE ABUNDANCE OVER TIME

Most sites showed no significant change in scraper abundances in 2012, compared to 2011 (Table 3.4.1, Fig. 3.4.7). The sites that did have significant changes in 2012 all had significant increases (NF90, NF70, NF17, NF40). Increases are generally considered as desirable because when nutrients or sediment become excessive, scrapers are often excluded from epilithic algae by plant growth or sediments. For this reason, Wyoming DEQ considers the ideal stream to have ≥38.6% scrapers in the Wyoming Basin ecoregion (Hargett and ZumBerge 2006). The sentiment expressed by WY DEQ in adopting this criterion in the naive idea that they do consider it possible for there to be too many scrapers in a Wyoming Basin river. Therefore, many would consider the increase in scraper relative abundance to represent an improvement in water quality and biotic integrity. Though this may not be a universal truth, at the scale of our observations, it is true that greater scraper abundance is desirable, especially considering the basin-wide dominance of collectors in 2011. Thus, this metric described significant improvements at four sites (NF90, NF70, NF17, NF40). The scrapers we observed were primarily the two closely related glossosomatid genera *Glossosoma* and *Protoptila*. Both genera graze closely attached epilithic algae from relatively clean inorganic substrata. *Protoptila* usually occurs in slightly warmer streams with slightly finer substrata (Wiggins 1996).

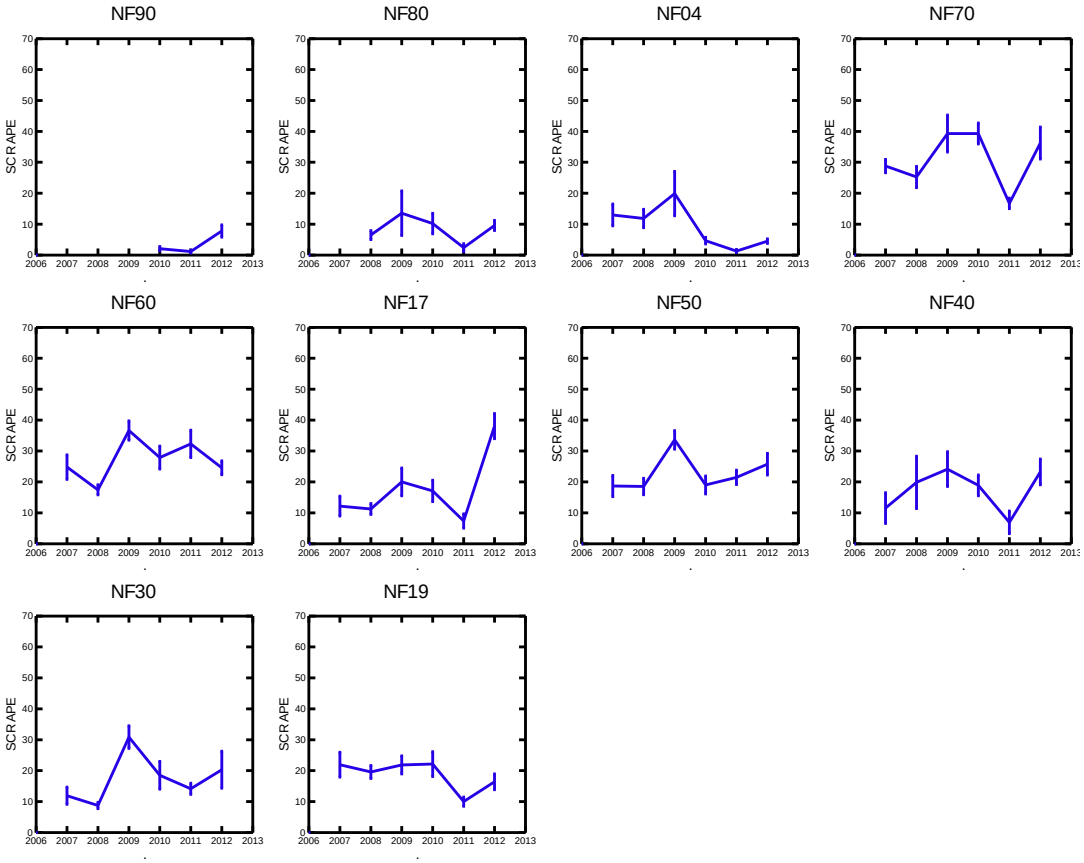


Figure 3.4.7. Changes in Scraper Abundance over Time. Lines connect average relative abundance of scrapers over time and express error as the Standard Error of the Mean (SEM). 95% confidence Intervals are described @ 2x the SEM.

3.4.8 SUMMARY CHANGES FROM 2011 TO 2012

To summarize the net change from year to year, we rated sites that were significantly different from the previous year (P<0.05) as being positive (improvement in water quality) or negative (indicating declining water quality). The positive and negative metrics were added together to produce an indicator of net-change. We looked at net change by site and basin wide by examining which metrics had large net change (delta) across all sites.

Only one metric showed net negative changes in 2012. This was non-insect abundance which indicated 5 sites changed negatively in 2012. All other metrics showed no net change or a positive net change (Table 3.4.1 delta).

Most sites showed zero or positive "site net change" (Table 3.4.1). Only two sites had negative net changes, NF50 (Net -2) and NF30 (Net -3). NF50 and NF30 both indicated negative impacts due to the abundances of small worms in 2012. These changes seemed especially drastic at NF30, at which worms comprised >50% of the invertebrates sampled on average. However, the next section of this report (Section 5.3) shows that these changes did not necessarily indicate the loss of more sensitive species.

Table 3.4.1. Change in metrics from previous year. The significance of changes among years were evaluated using the non-parametric rank sums test for two comparisons, the Mann-Whitney U-Test (two treatment equivalent of the Kruskal-Wallis Non-Parametric ANOVA) because the assumptions required for parametric statistics could not be satisfied with transformations. If analysis resulted in statistically significant differences among zones, they were identified with either a "+" or a "-"reflecting whether the change reflects an improvement or decline (respectively) in water quality. Cells identified with "N.S." indicate differences with the previous year were not statistically significant. Values indicated with an asterisk (*) indicate the difference between years considered "marginally significant" (0.10>P>0.05).

	Upper Study Area				Middle Study Area			Lower Study Area			
	NF90	NF80	NF04	NF70	NF60	NF17	NF50	NF40	NF30	NF19	delta
% EPT	+	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	–	N.S.	0
% Chironomid	+	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	+1
% Non-Insect	N.S.	N.S.	N.S.	–	N.S.	–	–	N.S.	–	–	–5
% Dominance (5)	N.S.	N.S.	N.S.	N.S.	N.S.	+	N.S.	+	N.S.	+	+3
HBI	+	+	N.S.	N.S.	N.S.	N.S.	–	N.S.	–	N.S.	0
% Collectors	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	+	N.S.	N.S.	+1
% Scrapers	+	N.S.	N.S.	+	N.S.	+	N.S.	+	N.S.	N.S.	+4
Site Net Change	+3	+1	0	0	0	+1	–2	+3	–3	0	

Net Change in Metrics over Time (2011-2012)

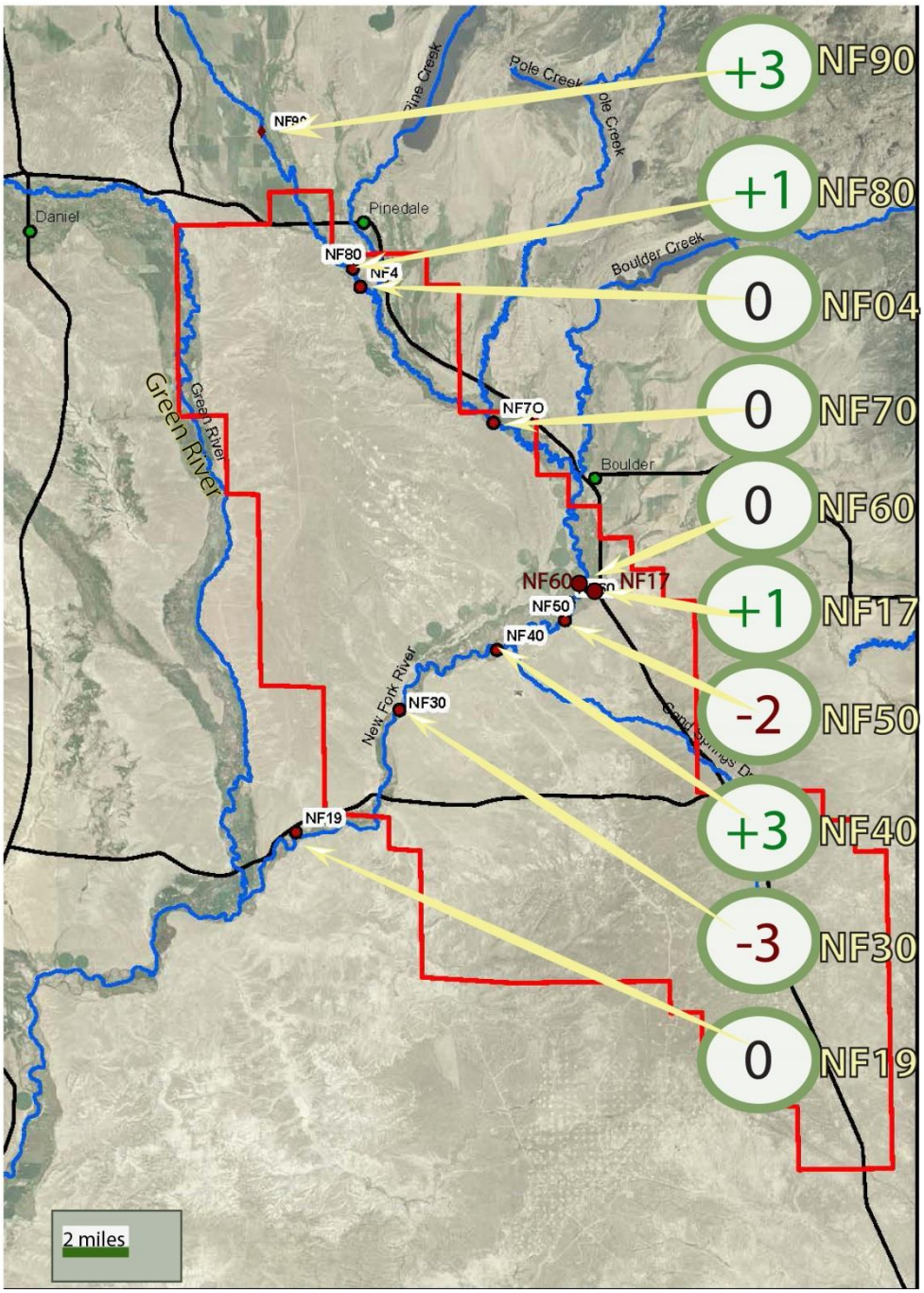


Figure 3.4.8. Spatial Representation of Net Changes since 2011. The number of biological metrics indicating declines in water quality was subtracted from the number of metrics indicating significant improvements in water quality are shown in circles. Net negative numbers indicate that declines were more numerous than significant improvements, whereas positive numbers indicate that improvements were more common than declines.

3.5 SITE DIFFERENCES IN THE DENSITY OF MAJOR AQUATIC INSECT GROUPS

One of the reasons densities are not often used in biological assessments is that they are highly variable and inconsistent among years. For example, one species may not be sufficiently abundant to be detected some years. Additionally, when some species are abundant, their density may be highly variable within a short distance. Furthermore, subsampling can reduce many species below detectible limits and cause large errors due to multiplication (see Methods). To circumvent these effects we pooled the invertebrates into broad categories: mayflies, stoneflies, caddisflies, chironomid midges, and black flies. The use of common names here is to avoid confusion with relative abundance of these groups (such as EPT) which we refer to by order. The density of these groups was highly variable and the data failed the assumptions required for most parametric statistical tests even after transformation, so a non-parametric analogue of the Analysis of Variance test (ANOVA), the Kruskal-Wallis non-parametric ANOVA was used. The Dwass-Steel-Crichlow-Fligner test for all pairwise comparisons was used to determine which sites were specifically different from which other sites.

The results of the analyses of aquatic insect densities presented here need to be interpreted conservatively—within the intended purpose of the analysis and within the limitations of the monitoring program's elements of design. Specifically, we are screening for extreme reductions in the density of important groups of insects. Comparisons among zones of densities may lead to spurious interpretations. The origins of these limitations are discussed in greater detail in the Methods section of this document (Section 2.8.2). In short, the assumption that error is standardized and homogenous among treatments may be invalid for density estimates. Therefore, the comparison among sites for anything other than a cursory evaluation of densities is discouraged.

Table 3.5.1. Differences in Aquatic Insect Density. Densities (abundance per m^2) for the following groups of aquatic insects were compared using non-parametric statistical tests described above. Sites with identical letters for the Dwass-Steel-Crichlow-Fligner test were not significantly different from each other. Lowercase letters indicate marginally significant differences from sites not sharing the same lowercase letter. For example, a site identified with group membership "a" is marginally significantly different (0.10<P<0.05) from sites marked with an "A" but not significantly different from sites marked "Aa."

	KRUSKAL WALLIS	Dwass-Steel-Crichlow-Fligner Test for All Pairwise Comparisons									
Metric	P-val	NF90	NF80	NF04	NF70	NF60	NF17	NF50	NF40	NF30	NF19
Mayfly Density	<0.001	BC	BC	BC	AB	C	A	BC	C	AB	AB
Stonefly Density	<0.001	AB	AB	A	AB	B	AB	B	B	A	AB
Caddisfly Density	<0.174	-	-	-	-	-	-	-	-	-	-
Midge Density	<0.001	bC	bC	BC	A	BC	B	BC	BC	BC	BC
Black Fly Density	<0.001	B	B	B	B	B	A	B	AB	A	A
		Upper Study Area				Middle Study Area			Lower Study Area		

3.5.1 MAYFLY DENSITY

The Kruskal-Wallis non-parametric ANOVA test suggested that there were significant differences among the study sites (P>0.001; Table 3.5.1, Fig. 3.5.1), and the Dwass-Steel-Crichlow-Fligner test indicated NF30 had significantly fewer mayflies than NF40 and NF60 but not with other sites. Mayfly density per sample averaged from a low of 1,500 ind/m^2 (NF70) to a high average density of 6,600 ind/m^2 (NF40). Thus, NF30 was not the lowest site and its significant differences were in comparison with the sites with the greatest density. Thus, the observed increase in non-insect relative abundance at NF50 and NF30 was not due to a dramatic observed reduction in mayfly density (Fig. 3.5.1).

3.5.2 STONEFLY DENSITY

Stonefly densities were typically much lower than mayfly or caddisfly densities. Average densities ranges from 19 ind/m^2 (NF30) to 566 ind/m^2 (NF40). Stoneflies often have low densities and/or patchy distribution. Samples at NF30, were the lowest stonefly densities were recorded, used about 2% of the sample to attain the target number of invertebrates (or about 0.0018m^2) about a 1.6x1.6 inch square of river bottom. Thus, the low density of stoneflies at NF30 could very easily be due to under sampling for large and patchily-distributed stoneflies.

3.5.3 CADDISFLY DENSITY

Caddisfly density was high in 2012, with individual samples containing between 250-9,200 caddisflies/m^2. Sites averaged between 1,800-4,000 caddisflies/m^2. There were no statistically significant differences in caddisfly densities among sites (Table 3.5.1, Fig. 3.5.1). The most abundant caddisflies were Hydropsychidae, Glossosomatidae, and Lepidostomatidae. Thus, the high relative abundance of non-insects at NF30 did not occur because NF30 lost large numbers of caddisflies.

3.5.4 CHIRONOMIDAE DENSITY

The densities of midges were high at all sites with individual observations ranging from 215-35,000 midges/m^2. The greatest average midge density occurred at sites dominated by macrophyte growth (NF90, NF80, NF04) or moss growth (NF40). NF30 supported significantly greater midge density than the site with the lowest density of midges (NF70) but otherwise was not significantly different from the other sites. NF50 was significantly different from the site with the lowest midge density (NF70) but otherwise was not different from the other sites. Thus, NF50 and NF30 did not lose exceptional numbers of midges, causing non-insect abundances to increase.

3.5.5 BLACK FLY DENSITY

Black flies ranged from 0-11,500 larvae/m^2 per sample. Average black fly densities varied greatly among sites ranging from 300-4,400 larvae/m^2 (Table 3.5.1., Fig. 3.5.1). Most sites are not significantly different from each other. Three sites (NF30, NF19, NF17) were significantly lower than all other sites except NF40. These sites were not significantly different from each other.

Blackfly density was relatively low at NF30, but also at NF19 and NF17, but since blackflies have patchy distributions, this may reflect the inconsistencies of samples representing widely variable effective areas (Fig. 3.5.1).

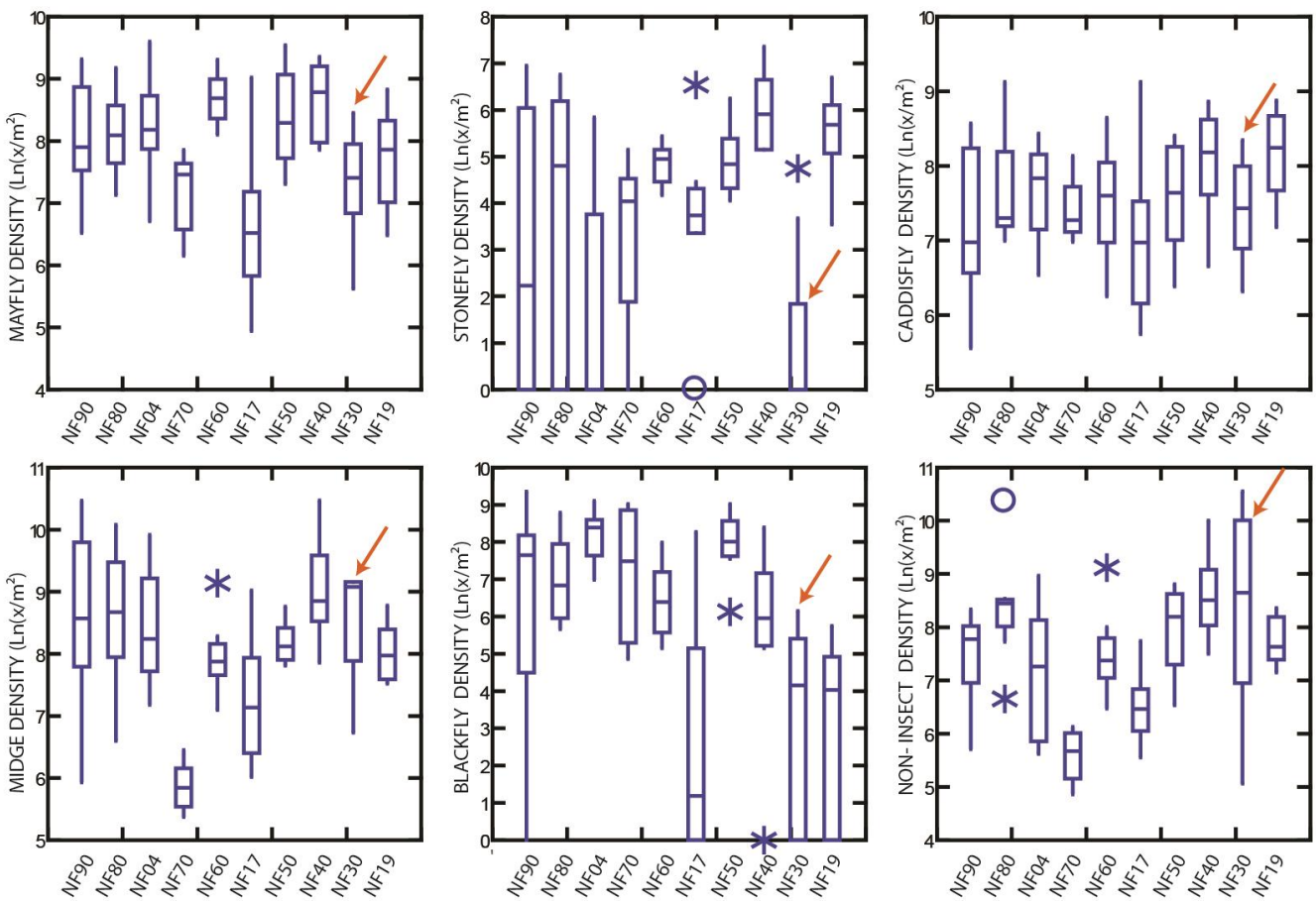


Figure 3.5.1. Overview of the density of aquatic insects. Figures show the box-plot distributions of density (individuals per m^2) for mayflies, stoneflies, caddisflies, midges, and black flies as transformed using natural logarithms. Non-insect density is also provided but notes the scale is greater by an order of magnitude than those of mayflies, stoneflies, caddisflies and black flies. Minor outliers are represented by asterisks (*), major outliers by circles. The site of greatest concern, NF30, is highlighted by an arrow.

3.5.6 DISCUSSION OF DENSITY ANALYSES AND RESULTS

In 2011, (Marshall 2012) we found that worms influenced the amount of the sample that was processed to attain the target standard unit of effort. As a result, the data from each sample represented different proportions of samples, and each sample was 1 square foot. Therefore, the effective area analyzed in the lab varied several orders of magnitude from about $1ft^2$ to about 1 square inch. The relationship in effective area with worms was highly significant in 2011 (Fig. 2.8.1, r^2=0.75 p<0.001) so we repeated this analysis for 2012.

In 2012, we also found a significant effect of worms on the portion of sample used (Fig. 3.5.2) and on the area of river bottom used to estimate densities (Fig. 3.5.3). However, the relationship was weaker than in 2011 (Fig. 3.5.2, r^2=0.295) because some samples had other abundant taxa, controlling subsampling effort; usually black fly larvae. When we changed the response variable from worm density to the density of the most abundant single taxon (Fig. 3.5.3) the relationship was once again highly significant (r^2=P<0.001, r^2=0.78).

Only one group of aquatic insects suggested that the elevated non-insect abundance at NF30 could have been related to a decline in the abundance of sensitive insect species. Specifically the density of stoneflies was significantly lower at NF30 than sites with the greatest density of stoneflies. Additionally, there were more zero-values at NF30 than at the other sites with zero values (NF90, NF80, NF04, NF17; Fig. 3.5.1). However, this finding needs to be carefully considered relative in the context of standard lab effort.

The importance of this cannot be over stated for discussing them among site changes in density of organisms with patchy distributions (e.g., Southwood 1991, Krebs 1999). Since our low estimates of stonefly density at NF30 were from the equivalent of a 1.6 inch x 1.6 inch square and many stoneflies may be 1.6 inches long. The absence of stoneflies in some samples should be expected in some sites, like those from NF30, in which only small portions (~2%) of the samples were used, especially when compared with samples representing 50-100% of samples area.

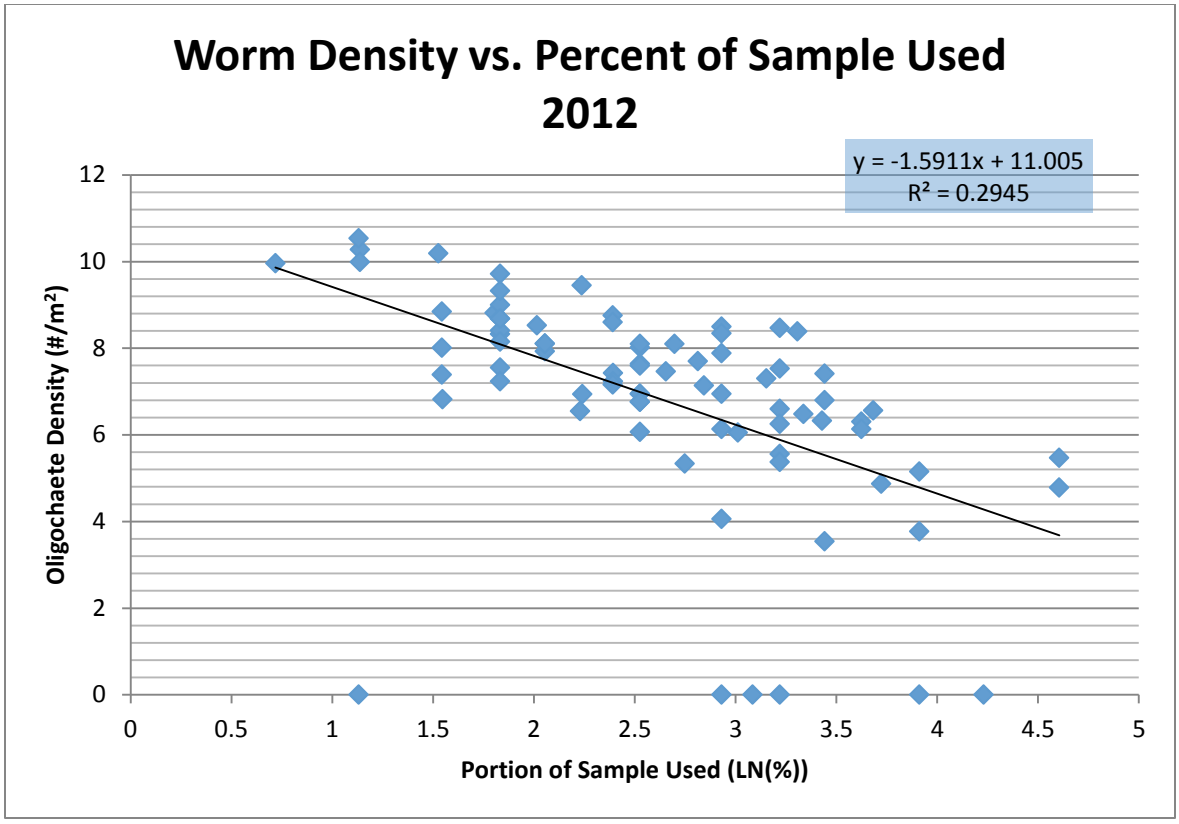


Figure 3.5.2. Effect of Worm Density on Subsampling Effort. The figure shows the relationship between worm density and the portion of the sample used to achieve the subsampling target in the laboratory. This analysis was improved upon in subsequent figures by using the density of the single most abundant taxon (Fig. 3.3.3) because occasionally other taxa were very abundant (note the 6, zero values in this figure).

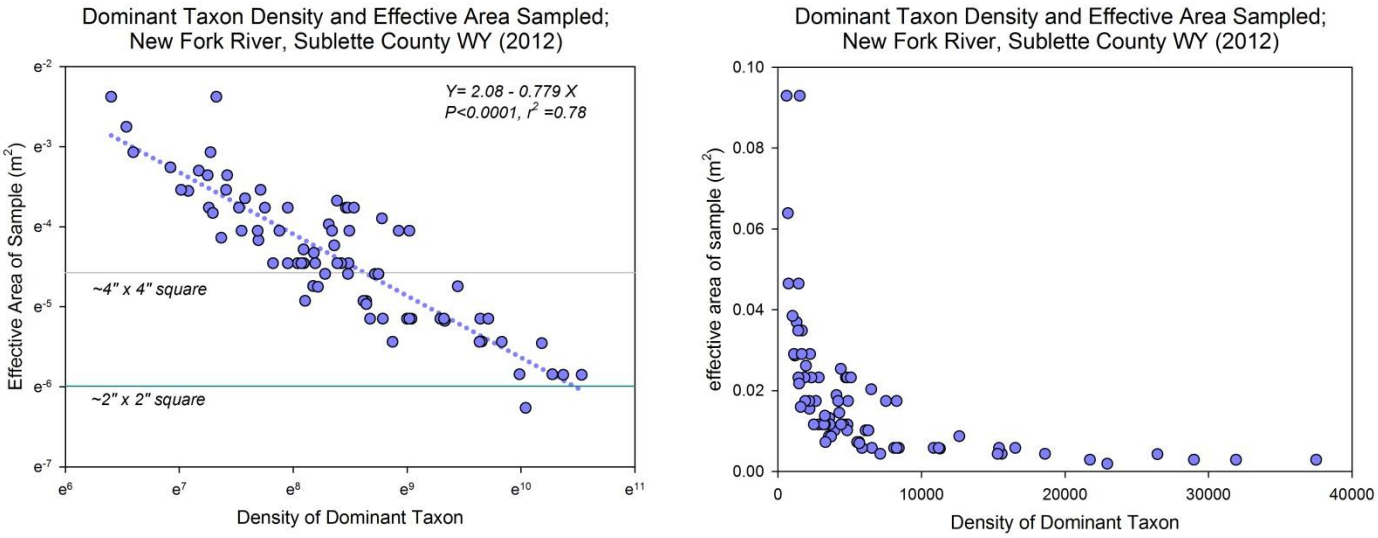


Figure 3.5.3. Relationship between dominant taxon density and portion of sample used. The figure on the right shows the actual dual logarithm relationship. The figure on left uses a natural-log scale to linearize the relationship for analysis.

4.0 RESULTS SUMMARY

The results of most analyses for 2012 indicated that there were very few changes in benthic community structure – spatially or temporally. However, there were some spatial changes and some temporal shifts in the distribution of benthic macroinvertebrates that need to be considered here.

- Spatial analyses indicated that one site, NF30, had several metrics indicating significant declines (relative to nearby locations) in biological condition of aquatic resources. All of these measures were responses to the same fundamental element of community composition: high relative abundance of aquatic worms (*Nais* sp.). These differences persisted, even when the abundance of worms was corrected for influential habitat variables (such as moss coverage).
- Analysis of temporal trends indicated two sites appeared to have declined from the previous year in several metrics.
- NF30 had several metrics indicating significant decline in condition from the previous year, but these responses were to the same fundamental element of community composition: higher worm densities than the previous year. Worms comprised and averaged 48.2% (22-66%) of the benthos at NF30 in 2012, as compared to 4.7% non-insects in 2011.
- NF50 had several metrics indicating significant decline in biological condition from previous years. The responses were all related to one single fundamental element of community composition: an increase in the relative abundance of aquatic worms (*Nais*). This phenomenon was not as pronounced as observed at NF30 and the abundances of non-insects were not beyond our expectations for New Fork River. However, the increase to 17.8% non-insect abundance was a statistically significant change because the metric was especially low in 2011 (4.8%) with a low variance.
- Although two sites had significant declines relating to elevated non-insects, one site, NF40, exhibited improved conditions resulting from a decrease in worm abundance. In 2011 a high average non-insect abundance (33.7%) was observed but dropped to 20.3%.
- We repeated the density analyses introduced to the study in 2011 to determine if the high relative abundances of non-insects were due to decreased densities of insects, or just increases in worm densities.
    - We found that mayfly, stonefly, and caddisfly densities at these sites were not significantly reduced compared to most other sites. Stonefly density estimates were low at NF30, but this was likely due to the laboratory methods relying on fixed count standardization.
    - This suggests that the high worm abundances in 2012 (as with 2011) did not occur because all the other "good" insects disappeared. Rather, the environmental conditions favored high abundance of naidid worms. This means that the micro habitat at NF30 has changed to favor worms, but not necessarily to the detriment of other important insects.

CONCLUSIONS AND RECOMMENDATIONS

The benthic macroinvertebrate assemblages of the New Fork River and the East Fork River generally indicated improving conditions at most sites in 2012, relative to 2011. The notable exceptions were the sites NF30 and NF50. All the metrics that indicated significant differences at these sites were caused by abundant small worms.

The New Fork River site (NF50) downstream of the East Fork River confluence had a moderate, but not exceptional increase in the relative abundance of worms in 2012 that was not shared with sites upstream. At the time of this report preparation, it is unclear what conditions may have caused this shift in benthic community composition; PAPA development is a possibility.

NF30 is the New Fork River site downstream of the natural gas pipeline crossings and near the natural gas wells constructed in the riparian corridor—over hyporheic stream channels. Thus, the increase in worms at NF30 could be due to anthropogenic development in the PAPA. However, it may also be related to the dispersion of conditions which caused exceptional worm abundance at NF40, a short distance up river in 2011.

NF40 improved from the previous year, but still exhibited relatively high worm abundance in 2012. The site may be influenced by anthropogenic developments in the riparian corridor, or from anthropogenic activities in the Alkali Draw or the Sands Springs Draw drainages. Alternatively, natural intermittent flow events from these draws could also influence the changes in the abundance of worms.

Regardless of the cause of the high abundance of worms at NF50, NF30, and even at NF40, it is noteworthy that the metrics responded to high worm abundance alone; these metrics did not respond to any reductions in the density of important insect groups in the New Fork or East Fork Rivers.

We conclude that any influences of development in the PAPA on the surface water quality of New Fork and East Fork rivers in 2012 were minor.

RECOMMENDATIONS

We usually conclude this report with several recommendations for the SCCD to improve or adapt the monitoring program so that it remains useful to assess changing environmental conditions. This is an essential element of adaptive management and the adaptive management approach to monitoring: response followed by feedback followed by adaptation of the monitoring program's design. Along these lines, we have recommended the measure and analysis of periphyton biomass for several years. The biomass of epilithic algae is important for trophic structure of benthic macroinvertebrate communities—especially for mid-sized rivers receiving intensive sun light, like the rivers of central and western Sublette County. There are several reasons for this recommendation. First, as stated above, it is a very important base of the food web affecting relative abundance, survival, growth and production of many macroinvertebrates directly or indirectly. Second, the spread of the nuisance algae *Didymosphenia geminata,* which we believe will not be affected by PAPA development, could mimic response signatures of PAPA-related impairments of ecosystem function eventually. Third, discharges from Anticline Disposal, near Sand Springs Draw, could have either a positive or negative effect on the growth of periphyton or other important biofilms in the New Fork River. These could have indirect effects on benthic community structure and need to be documented and should be accounted for in our response models for macroinvertebrate ecology. Additionally, possible spills and other intermittent PAPA activities could affect biofilm growth and indirectly have dramatic effects on the ecology of the system.

We recommended several methods by which these variables could be collected for use in this report. We requested the cheapest option to get the simplest baseline possible, with the potential for more rigorous analyses when necessary. That is, we requested the biomass of epilithic scrapings collected by SCCD. This was approved in a meeting in 2011 for the 2012 field season, but not funded. Samples were collected that would have improved this report, but not analyzed.

This year, we re-iterate the need for some form of accounting for periphyton and other biofilms. This can be done by professional laboratories serving the natural gas and petroleum industries. SCCD may have capacity to conduct some of these analyses in house. We can work with the district on formatting the data for maximal use.

Consider another downstream reference/recovery site. NF19 has served well as the downstream reference and recovery zone for several years (since 2007). However, the SCCD should consider possibly adding another downstream recovery site.

In 2012, we observed the downstream migration of worms from NF40 to NF30, and the lack of high worm abundance at NF19. We have not yet observed any PAPA related impairments at NF19, but if the spread of worm dominated communities continues, the site's value as the downstream reference/recovery site will be compromised. Proactive adaptive monitoring should be prepared for this contingency. Therefore, the SCCD may want to develop baseline conditions at a new site downstream that may eventually serve as the downstream reference if required. There are several ways in which this could be implemented. We will meet with the SCCD and discuss these options so that the board can make an informed decision.

Continue the use of density measurements. This report for 2012 is the second report where we used density measures to elucidate the severity of changes related to the high worm abundances at some sites in the New Fork River. Both times we used these analyses they showed that there are certain limitations to State's bioassessment criteria and that the severity of community structure changes were much more minor than the relative abundance measures suggested. We recommend the continued use of these measures.

Continue the monitoring program. We have found several sites over several years that had mild community structure changes that could be attributed to PAPA development. The fact that the program can diagnose relatively mild changes is testimony of its robustness. The fact that we can detect them and correctly surmise that these effects are not as dramatic as they first appear is testimony for the completeness of the monitoring design. However, these mild changes need to continue to be monitored to ensure that the cumulative effects do not become catastrophic to the natural resources of Sublette County.

Account for potential effects of Anticline Disposal. Anticline Disposal initiated a permitted discharge to the New Fork River in 2009. The current sites NF30 and NF40 should be adequate to account for any effects of this discharge on the New Fork River. However, the modes of potential effects remain unclear. There are several ways we can address the relevance of this influence. If the district can acquire some data from Anticline on the chemistry and volume of discharges, we may be able to anticipate the kinds of likely responses of the New Fork River ecosystem. Additionally, the SCCD can add some simple field measures to the sampling program that might account for the influence of this discharge. Candidate measures include conductivity and pH. Seston measurements might also be warranted. We should discuss these influences before the next report.

Meet with your consultant. We believe it is important for the integrity of the program that we are actively aware of changes on the ground in Sublette County. Additionally, it is important to ensure there is a venue for the natural gas operators and other stakeholders to share feedback regarding the monitoring program. Traditionally, this has been the role of the Pinedale Anticline Work Group (PAWG) and the related task groups and committees. However, since the disbanding of the PAWG, there has been no direct feedback or interchange with stakeholders. We welcome the opportunity for these exchanges.

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APPENDIX 1: TAXA DICTIONARY AND O.T.U.S

ORDER	Taxon	O.T.U.	FFG	HBI
Ephemeroptera				
Baetidae		Baetidae	1	4
	Baetis	Baetis	1	4
	Acentrella	Acentrella	1	4
	Callibaetis	Callibaetis	1	4
	Centroptilum	Centroptilum	1	4
	Paracloeodes	Paracloeodes	1	4
	Procloeon	Procloeon	1	4
	Diphetor hageni	Diphetor hageni	1	4
	Hetercloeon	Hetercloeon	1	4
	Fallceon quilleri	Fallceon quilleri	1	4
	Acerpenna	Acerpenna	1	4
Ameletidae			1	0
	Ameletus	Ameletus	1	0
Ephemerellidae			1	2
	Attenella sp.	Attenella sp.	1	2
	Ephemerella	Ephemerella	1	1
	Drunella	Drunella	1	1
	Drunella grandis	Drunella grandis	1	2
	Drunella doddsi	Drunella doddsi	1	1
	Serratella	Serratella	1	2
Caenidae			1	7
	Caenis	Caenis	1	7
Heptageniidae			3	4
	Heptagenia	Heptagenia	3	4
	Rhithrogena	Rhithrogena	3	0
	Leucrocuta	Leucrocuta	3	1
	Cinygmula	Cinygmula	3	4
	Ecdyonurus	Ecdyonurus	3	4
	Epeorus	Epeorus	3	0
Leptohyphidae			1	5
	Tricorythodes	Tricorythodes	1	5
	Tricoryhyphes	Tricoryhyphes	1	5
	Asioplax	Asioplax	1	5
Leptophlebiidae			1	2
	Choroterpes	Choroterpes	1	4
	Neochoroterpes	Neochoroterpes	1	4
	Paraleptophlebia	Paraleptophlebia	1	1
	Leptophlebia	Leptophlebia	1	2

Ephemeridae			1	6
	Hexagenia	Hexagenia	1	6
Odonata			5	6
	Aeshna umbrosa	Aeshna umbrosa	5	5
Gomphidae			5	1
	Ophiogomphus	Ophiogomphus	5	1
	O. severus	Ophiogomphus	5	1
Coenagrionidae		Coenagrionidae	5	6
	Argia	Argia	5	6
Hemiptera			1	9
Corixidae			1	9
	Sigara	Sigara	1	9
Gerridae			5	10
	Trepobates	Trepobates	5	10
	Limnoporus	Limnoporus	5	10
Veliidae			5	6
	Rhagovelia	Rhagovelia	5	6
Plecoptera				
Pteronarcyidae			4	0
	Pteronarcys	Pteronarcys	4	0
	Pteronarcella	Pteronarcella	4	0
Taeniopterygidae			4	2
	Doddsia occidentalis	Doddsia occidentalis	3	2
	Taenonema	Taenonema	3	2
Capniidae			4	1
Nemouridae			4	2
	Amphinemura	Amphinemura	4	2
	Malenka sp.	Malenka sp.	4	2
	Visoka Cataractae	Visoka cataractae	4	1
	Zapada sp.	Zapada sp.	4	2
	Zapada cinctipes	Zapada sp.	4	2
	Zapada columbiana	Zapada sp.	4	2
	Zapada haysi	Zapada sp.	4	2
Perlidae			5	1
	Hesperoperla pacifica	Hesperoperla pacifica	5	1
	Claassenia subsalusa	Claassenia subsalusa	5	3
	Doroneuria sp.	Doroneuria sp.	5	1
Perlodidae			5	2
	Skwala	Skwala	5	2
	Kogutus	Kogutus	5	2

	Megarcys	Megarcys	5	2
	Isoperla	Isoperla	5	2
Chloroperlidae			5	1
	Sweltsa	Sweltsa	5	1
	Haploperla	Haploperla	5	1
	Suwallia	Suwallia	5	1
Megaloptera			5	6
0	Sialis sp.	Sialis sp.	5	4
Lepidoptera			3	3
Pyralidae	Petrophila	Petrophila	3	3
Trichoptera				
Helicopsychidae			3	3
	Helicopsyche	Helicopsyche	3	3
Brachycentridae			2	2
	Amiocentrus aspilus	Amiocentrus aspilus	3	2
	Brachycentrus	Brachycentrus	2	1
	B. americanus	B. americanus	2	1
	B. occidentalis	B. occidentalis	2	1
	Micrasema	Micrasema	4	2
Rhyacophilidae			5	0
	Rhyacophila (gills)	Rhyacophila (gills)	5	0
	Rhyacophila w/o gills	Rhyacophila w/o gills	5	0
Glossosomatidae			3	0
	Anagapetus	Anagapetus	3	0
	Agapetus	Agapetus	3	0
	Glossosoma	Glossosoma	3	0
	Culoptila	Culoptila	3	0
	Protoptila	Protoptila	3	1
Lepidostomatidae			4	1
	Lepidostoma	Lepidostoma	4	1
Hydroptilidae			6	6
	Ochrotrichia	Ochrotrichia	1	4
	Agraylea	Agraylea	6	8
	Orthotrichia	Orthotrichia	6	6
	Neotrichia	Neotrichia	6	4
	Hydroptila	Hydroptila	6	6
Hydropsychidae			2	4
	Hydropsyche	Hydropsyche	2	4
	Cheumatopsyche	Cheumatopsyche	2	5
	Diplectrona modesta	Diplectrona modesta	2	0
Arctopsychidae			2	2
	Arctopsyche grandis	Arctopsyche grandis	2	2

	Par psyche	Par psyche	5	1
Leptoceridae			5	4
	Oecetis	Oecetis	5	8
	Nectopsyche	Nectopsyche	4	3
	Trianoides	Trianoides	4	6
	Ceraclea	Ceraclea	5	3
Polycentropodidae			2	6
	Polycentropus	Polycentropus	2	6
	Cyrnellus fraturnus	Cyrnellus fraturnus	2	4
	Cernotina	Cernotina	2	5
Philopotamidae			2	3
	Dolophilodes	Dolophilodes	2	1
Apataniidae			3	1
	Apatania	Apatania	3	1
Uenoidae			3	2
	Neothremma	Neothremma	1	0
	Neophylax	Neophylax	3	2
	Oligophlebodes	Oligophlebodes	3	2
Limnephildae			4	4
	Limnephilus	Limnephilus	4	5
	Psychoglypha	Psychoglypha	1	1
	Dicosmeoecus	Dicosmeoecus	4	1
	Hesperophylax	Hesperophylax	4	0
	Ptilostomis	Ptilostomis	4	1
Coleoptera			6	
Haliplidae			1	7
	Peltodytes	Peltodytes	3	5
	Brychius	Brychius	3	7
Dytiscidae			5	5
	Hygrotus	Hygrotus	5	5
	Laccophilus	Laccophilus	5	5
	Liodessus	Liodessus	5	5
	Rhantus binotatus	Rhantus binotatus	5	5
	Oreodytes	Oreodytes	5	5
Hydrophilidae			5	5
	Tropisternus lateralis	Tropisternus lateralis	5	5
	Helophorus	Helophorus	4	7.9
Dryopidae			4	3.2
	Helichus	Helichus	4	3.2
Elide			3	4
	Optioservus	Optioservus	3	4
	Cleptelmis	Cleptelmis	3	4

	Zaitzevia	Zaitzevia	3	4
	Dubiraphia	Dubiraphia	1	4
	Heterlimnius	Heterlimnius	3	4
	Macronychus	Macronychus	3	4
Diptera			5	9
	Chironomidae	Chironomidae	1	6
	Tanypodinae	Tanypodinae	5	7
	Ablabesmyia	Tanypodinae	5	7
	Conchapelopia/Helopelopia	Tanypodinae	5	7
	Procladius	Tanypodinae	5	7
	Labrundina	Tanypodinae	5	7
	Natarsia	Tanypodinae	5	7
	Pentaneura	Tanypodinae	5	7
	Rheopelopia	Tanypodinae	5	7
	Tanypus	Tanypodinae	5	7
	Theinemannimyia grp.	Tanypodinae	5	7
	Diamesinae / Prodiamesinae	Diamesinae	1	2
	Diamesa	Diamesinae	1	2
	Pagastia	Diamesinae	1	2
	Potthastia	Diamesinae	1	2
	Sympothastia	Diamesinae	1	2
	Prodiamesa	Diamesinae	1	2
	Odontomesa	Diamesinae	1	2
	Orthocladiinae	Orthocladiinae	1	7
	Brillia	Orthocladiinae	1	7
	Corynoneura	Orthocladiinae	1	7
	Cricotopus	Orthocladiinae	1	7
	Cricotopus (Nostoc.)	Cricotopus (Nostococladius)	6	7
	Cricotopus/Orthocladius	Orthocladiinae	1	7
	Eukiefferiella	Orthocladiinae	1	7
	Lopescladius	Orthocladiinae	1	7
	Nanocladius	Orthocladiinae	1	7
	Orthocladius	Orthocladiinae	1	7
	Thienemanniella	Orthocladiinae	1	7
	Tvetenia	Orthocladiinae	1	7
	T. vitracies grp.	Orthocladiinae	1	7
	T. bavarica grp.	Orthocladiinae	1	7
	Parametriocnemus	Orthocladiinae	1	7
	Paratrichocladius	Orthocladiinae	1	7
	Rheocricotopus	Orthocladiinae	1	7
	Saetheria	Orthocladiinae	1	7
	Limnophyes	Orthocladiinae	1	7

	Pseudosmittia	Orthocladiinae	1	7
	Synorthocladius	Orthocladiinae	1	7
	Paracladopelma	Orthocladiinae	1	7
	Heterotrissocladius	Orthocladiinae	1	7
	Psilometriocnemus	Orthocladiinae	1	7
	Parakiefferella	Orthocladiinae	1	7
	Plattysmittia	Orthocladiinae	1	7
	Orthocladiinae-A	Orthocladiinae	1	7
Chironominae		Chironominae	1	6
	Chironomini	**Chironomini**	1	6
	Axarus	Chironomini	1	6
	Chironomus	Chironomini	1	6
	Cryptochironomus	Chironomini	1	6
	Demicryptochironomus	Chironomini	1	6
	Dicrotendipes	Chironomini	1	6
	Endochrionomus	Chironomini	1	6
	Gillotia	Chironomini	1	6
	Glyptotendipes	Chironomini	1	6
	Microtendipes	Chironomini	1	6
	Phaenopsectra	Chironomini	1	6
	Polypedilium	Chironomini	1	6
	Paralauterborniella	Chironomini	1	6
	Tanytarsini	**Tanytarsini**	2	7
	Cladotanytarsus	Tanytarsini	2	7
	Micropsectra	Tanytarsini	2	7
	Paratanytarsus	Tanytarsini	2	7
	Rheotanytarsus	Tanytarsini	2	7
	Stempellinella	Tanytarsini	2	7
	Sublettea	Tanytarsini	2	7
	Tanytarsus	Tanytarsini	2	7
Blephariceridae	Blephariceridae	Blephariceridae	3	0
Deuterophlebidae	Deuterophlebia	Deuterophlebia	3	0
Tipulidae			4	4
	Antocha	Antocha	1	4
	Dicranota	Dicranota	5	3
	Tipula	Tipula	4	4
	Hexatoma	Hexatoma	5	2
	Cryptolabis	Cryptolabis	5	4
	Pedicia	Pedicia	5	6
Dixidae			4	1
	Dixella	Dixella	4	1
	Dixa	Dixa	4	1

Dolochopodidae			5	4
Simuliidae			2	6
	Simulium	Simulium	2	6
Phoridae			2	6
	Pericoma	Pericoma	1	4
Ceratopogonidae			5	6
	Palpomyia/Bezzia	Palpomyia/Bezzia	5	6
	Probezzia	Probezzia	5	6
Diptera: Brachycera				
Athericidae			5	2
	Atherix	Atherix	5	2
Empididae			5	6
	Chelifera	Chelifera	5	6
	Clineocera	Clineocera	5	6
	Hemerodromia	Hemerodromia	5	6
	Neoplasta	Neoplasta	5	6
	Oreogeton	Oreogeton	5	6
Muscidae			5	7
	Limnophora	Limnophora	5	7
Tabanidae			5	8
Stratiomyidae			1	10
Non-Insects				
	Naididae	Oligochaeta	1	10
	Naididae (tubificid)	Oligochaeta	1	10
	Naididae (nais)	Oligochaeta		
	Lumbricidae (Eisenilla)	Oligochaeta	1	10
	Pristina	Oligochaeta	1	10
	Lumbriculidae	Oligochaeta	1	7
	Oligochaeta	Oligochaeta	1	10
	Gastropoda	Gastropoda	3	7
	Planorbidae	Planorbidae	3	7
	Hydrobiidae	Hydrobiidae	3	7
	Physa	Physa	3	8
	Ferrissia	Ferrissia	3	6
	ACARI	ACARI	5	8
	Tricladida	Tricladida	5	4
	Nemertea	Nemertea	5	8
	Gammarus	Gammarus	1	4
	Hyalella	Hyalella	1	8
	Sphaeriidae	Sphaeriidae	2	8
	Isopoda	Isopoda	1	6
	Nematoda	Nematoda	5	5

Hirudinea: Macrobdellidae	Hirudinea	5	8
Hirudinea: Erpobdellidae	Hirudinea	5	8
Hirudinea: Glossiphoniidae	Hirudinea	5	8
Helobdella stagnalis	Hirudinea	5	6.7
Hirudinea	Hirudinea	5	8

APPENDIX 2: PCA OF VARIANCE EXPLAINED BY INORGANIC PARTICLES

Matrix to be Factored

	COBB	PEBB	CGRAV	FGRAV	SAND
COBB	1				
PEBB	-0.046	1			
CGRAV	-0.415	-0.622	1		
FGRAV	-0.245	-0.694	0.177	1	
SAND	-0.109	-0.209	-0.196	0.278	1

Latent Roots (Eigenvalues)				
1	2	3	4	5
2.155	1.266	1.032	0.547	0

Empirical Upper Bound for the First Eigenvalue	:	2.571

Chi-Square Test that All Eigenvalues are Equal

N	:	80
Chi-Square	:	2,701.24
df	:	10
p-Value	:	0

Chi-Square Test that the Last 2 Eigenvalues are Equal

Chi-Square	:	2,513.84
df	:	2.764
p-Value	:	0

Latent Vectors (Eigenvectors)

	1	2	3
PEBB	-0.605	-0.102	-0.423
FGRAV	0.541	0.313	0.012
CGRAV	0.478	-0.561	0.078
SAND	0.186	0.687	-0.387
COBB	-0.28	0.322	0.816

Standard Error for Each Eigenvector Element

	1	2	3
PEBB	0.067	0.264	0.109
FGRAV	0.082	0.142	0.222
CGRAV	0.122	0.122	0.324
SAND	0.16	0.227	0.389
COBB	0.139	0.45	0.183

Component Loadings

	1	2	3
PEBB	-0.888	-0.114	-0.43
FGRAV	0.794	0.352	0.013
CGRAV	0.702	-0.632	0.079
SAND	0.272	0.773	-0.393
COBB	-0.411	0.363	0.829

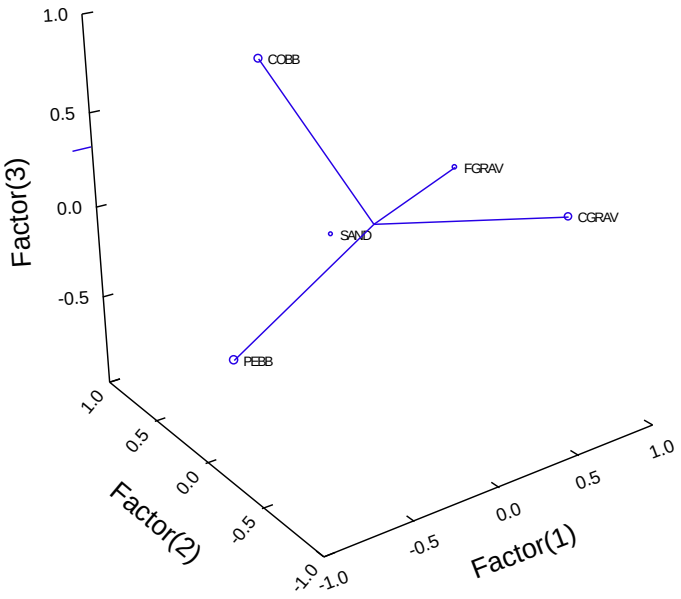
Variance Explained by Components		
1	2	3
2.155	1.266	1.032

Percent of Total Variance Explained			Portion of variance explained by 3 first axes
1	2	3	
43.096	25.324	20.647	89.067 %

Differences: Original Minus Fitted Correlations or Covariances

	PEBB	FGRAV	CGRAV	SAND	COBB
PEBB	0.013				
FGRAV	0.057	0.245			
CGRAV	-0.037	-0.037	0.102		
SAND	-0.048	-0.206	0.133	0.173	
COBB	-0.057	-0.046	0.048	0.013	0.013

Factor Loadings Plot

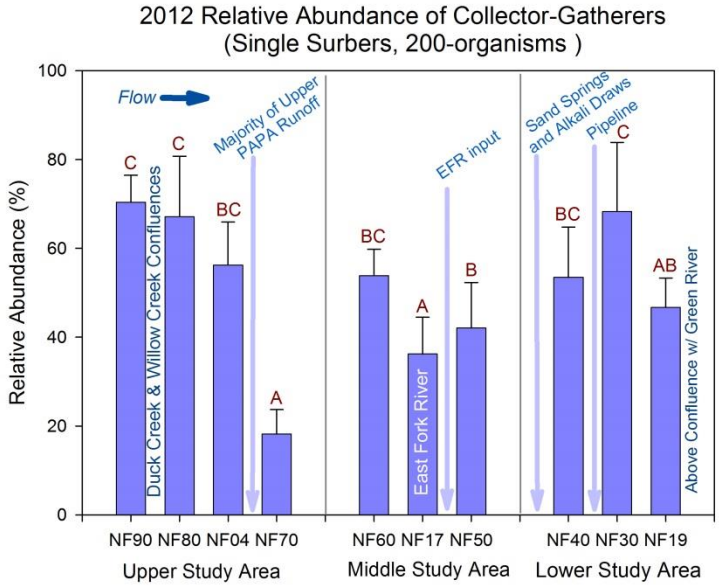


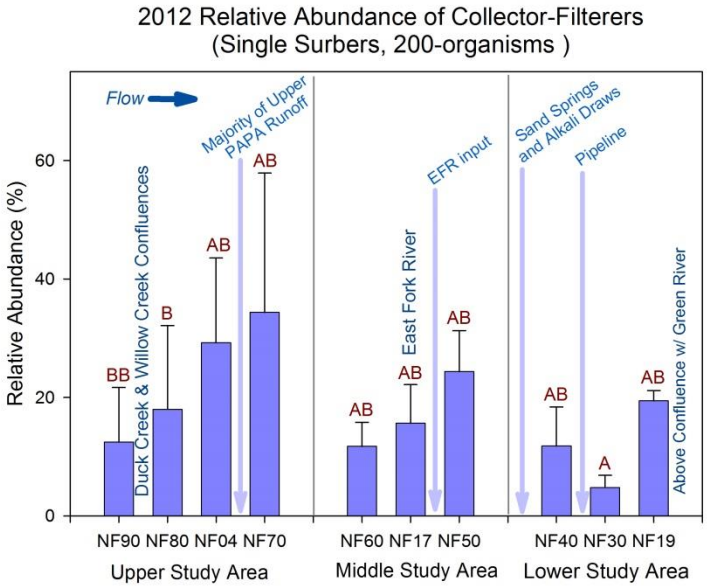
SITE	PC 1	PC 2	PC 3	TSQUARE	Prob.
NF90	2.410	-0.201	0.421	2.898	0.425
NF90	-1.386	1.563	2.530	9.021	0.039
NF90	1.158	-0.085	-0.045	0.630	0.893
NF90	1.247	1.735	1.960	6.819	0.093
NF90	1.565	2.218	-0.905	5.814	0.138
NF90	0.621	-1.355	-0.054	1.632	0.663
NF90	-0.401	-1.355	-0.421	1.696	0.649
NF90	0.391	-1.240	-0.153	1.307	0.736
NF80	1.004	-0.778	0.000	0.946	0.820
NF80	0.953	-1.009	0.015	1.225	0.755
NF80	-2.145	0.220	0.589	2.509	0.489
NF80	2.512	0.261	0.391	3.131	0.390
NF80	-1.780	-0.661	-1.015	2.814	0.438
NF80	-1.474	-0.257	0.526	1.329	0.731
NF80	-1.550	-0.777	-0.916	2.405	0.508
NF80	1.048	-0.359	1.818	3.812	0.302
NF04	-2.318	0.725	1.571	5.301	0.169
NF04	-0.939	0.032	2.166	4.955	0.194
NF04	-2.107	0.480	1.310	3.905	0.291
NF04	-1.958	-0.315	-1.129	3.094	0.395
NF04	-2.239	-0.430	-1.214	3.900	0.292
NF04	-1.550	-0.777	-0.916	2.405	0.508
NF04	-1.864	0.335	0.673	2.140	0.558
NF04	-0.583	2.830	-2.213	11.224	0.016
NF70	2.123	1.212	-1.009	4.237	0.256
NF70	1.642	0.336	-0.156	1.365	0.723
NF70	2.459	1.104	-0.199	3.806	0.302
NF70	2.408	0.873	-0.184	3.325	0.363
NF70	2.410	-0.201	0.421	2.898	0.425
NF70	2.307	-0.663	0.451	3.014	0.407
NF70	2.205	-1.125	0.481	3.478	0.342
NF70	1.618	-0.316	0.154	1.316	0.734
NF60	-0.926	0.003	1.430	2.379	0.513
NF60	-1.437	0.003	1.247	2.464	0.497
NF60	-1.653	0.090	0.412	1.439	0.706
NF60	-1.039	-0.777	-0.733	1.499	0.692
NF60	-1.039	-0.777	-0.733	1.499	0.692
NF60	-2.124	0.312	0.583	2.501	0.491
NF60	-1.499	-0.546	-0.931	2.118	0.562
NF60	-0.505	0.948	-1.464	2.905	0.424
NF17	-0.105	2.365	0.113	4.436	0.237
NF17	0.472	1.494	0.150	1.888	0.608
NF17	1.390	3.797	-0.527	12.550	0.010
NF17	1.282	3.176	-1.594	11.187	0.016
NF17	0.900	1.525	-1.043	3.267	0.370
NF17	-0.504	-0.125	-0.860	0.846	0.843
NF17	1.003	0.295	-0.605	0.889	0.833
NF17	-0.631	0.452	-0.989	1.293	0.739
NF50	-0.656	-0.200	-0.679	0.677	0.882
NF50	0.134	0.626	0.751	0.864	0.839
NF50	-0.707	-0.431	-0.664	0.805	0.853
NF50	-1.218	-0.431	-0.847	1.530	0.685
NF50	-0.733	-0.010	-0.959	1.140	0.775
NF50	-0.120	0.089	0.962	0.909	0.829
NF50	-0.707	-0.431	-0.664	0.805	0.853
NF50	-0.626	0.248	1.875	3.635	0.323
NF40	0.774	-0.662	-0.099	0.634	0.892
NF40	0.110	-1.355	-0.237	1.510	0.690
NF40	1.132	-1.355	0.129	2.061	0.573
NF40	2.000	-2.049	0.541	5.453	0.160
NF40	0.569	-1.586	-0.039	2.139	0.558
NF40	0.902	-1.240	0.030	1.592	0.672
NF40	1.515	-0.778	0.183	1.576	0.675
NF40	0.108	1.047	0.456	1.072	0.790
NF30	2.153	-1.356	0.496	3.841	0.298
NF30	-1.269	-0.662	-0.832	1.764	0.634
NF30	-0.063	-0.849	0.745	1.109	0.782
NF30	-1.269	-0.662	-0.832	1.764	0.634
NF30	2.332	-0.011	0.141	2.543	0.483

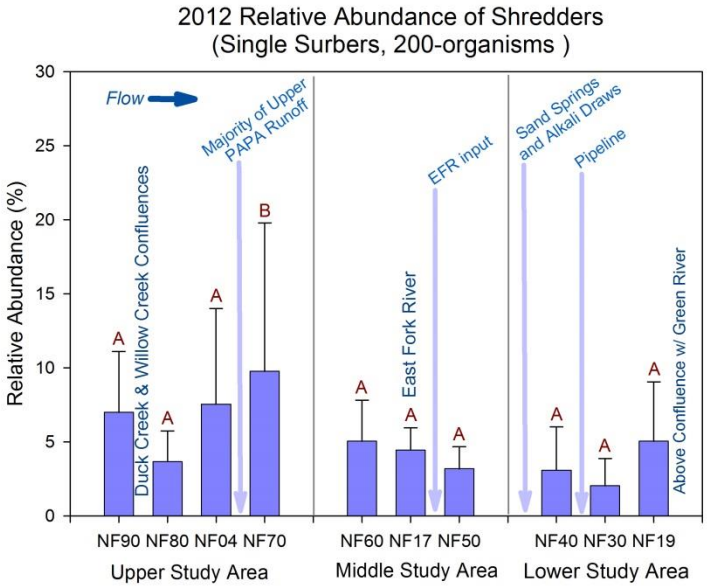
NF30	-0.069	-1.008	-0.351	0.925	0.825
NF30	-0.682	-0.142	0.793	0.842	0.844
NF30	-1.269	-0.662	-0.832	1.764	0.634
NF19	1.948	-2.280	0.555	6.165	0.121
NF19	1.489	-0.357	-0.111	1.141	0.774
NF19	1.105	0.757	-0.634	1.410	0.712
NF19	-1.729	-0.430	-1.030	2.561	0.480
NF19	-2.205	1.505	3.735	17.556	0.001
NF19	-1.704	0.222	-1.340	3.126	0.391
NF19	-0.937	-0.315	-0.763	1.049	0.796
NF19	-1.679	0.511	0.117	1.528	0.686

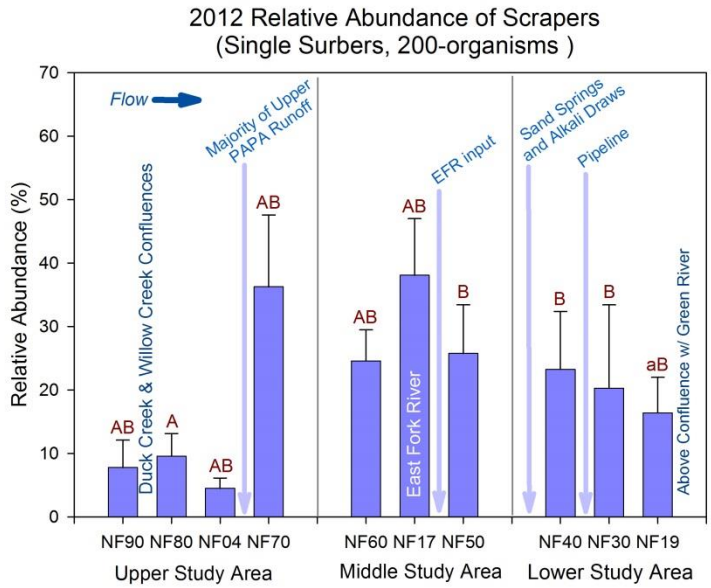
APPENDIX 3: SUPPLEMENTARY GRAPHS

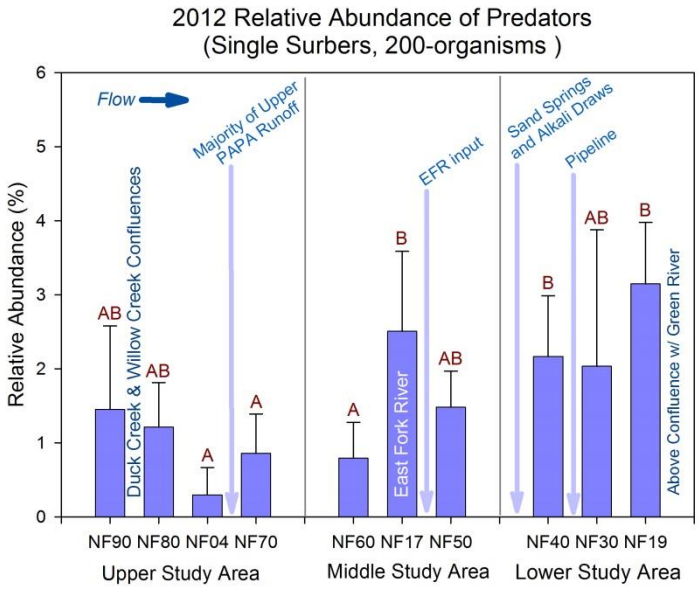
PERCENT ABUNDANCE OF INDIVIDUAL FUNCTIONAL FEEDING GROUPS



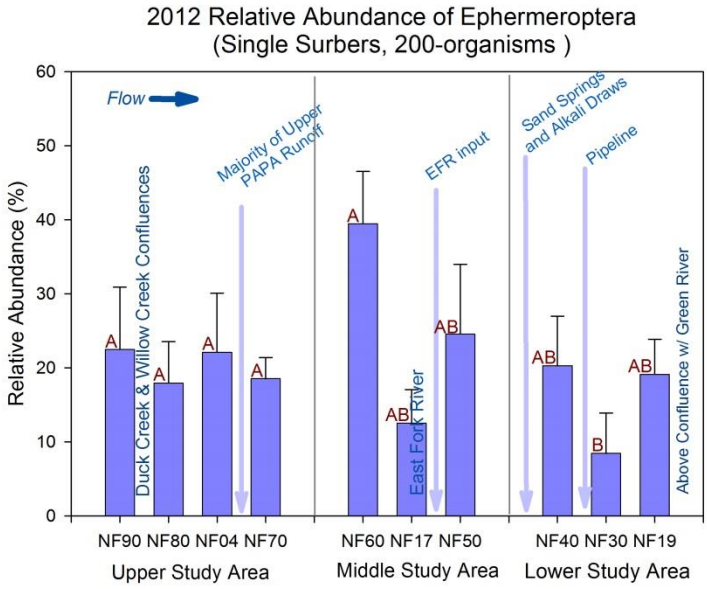


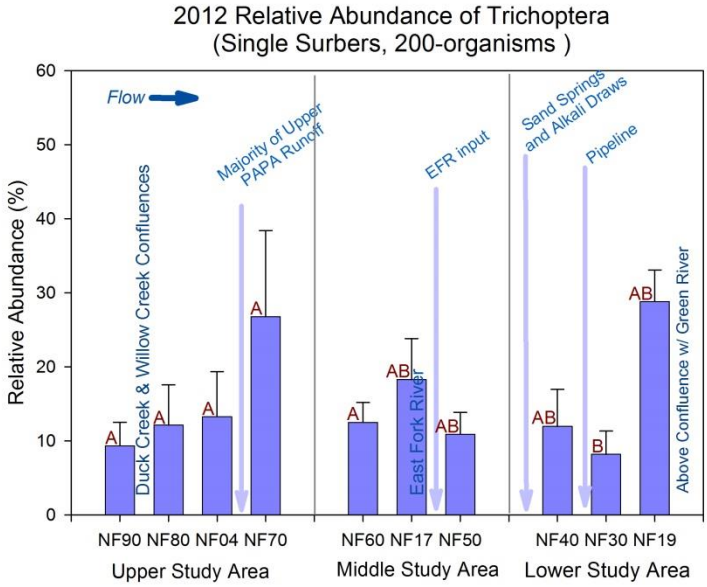

2012 Relative Abundance of Shredders
(Single Surbers, 200-organisms)
Relative Abundance (%)
Flow
Majority of Upper PAPA Runoff
Duck Creek & Willow Creek Confluences
EFR input
East Fork River
Sand Springs and Alkali Draws
Pipeline
Above Confluence w/ Green River
NF90 NF80 NF04 NF70
NF60 NF17 NF50
NF40 NF30 NF19
Upper Study Area
Middle Study Area
Lower Study Area

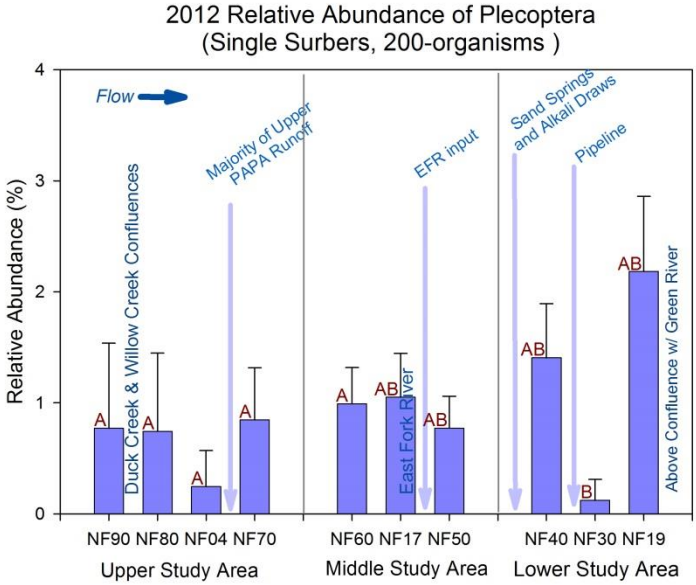

2012 Relative Abundance of Scrapers
(Single Surbers, 200-organisms)
Relative Abundance (%)
Flow
Majority of Upper PAPA Runoff
Duck Creek & Willow Creek Confluences
EFR input
East Fork River
Sand Springs and Alkali Draws
Pipeline
Above Confluence w/ Green River
NF90 NF80 NF04 NF70
NF60 NF17 NF50
NF40 NF30 NF19
Upper Study Area
Middle Study Area
Lower Study Area



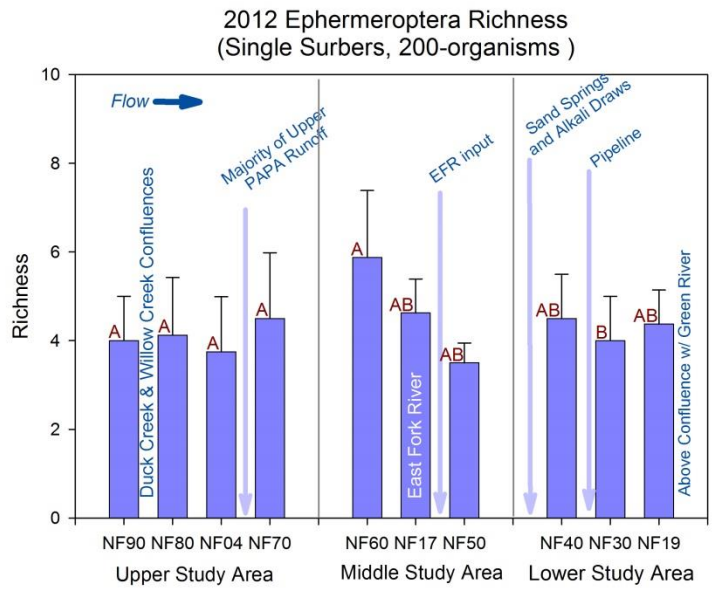
INDIVIDUAL COMPONENTS OF EPT RELATIVE ABUNDANCE: ↓

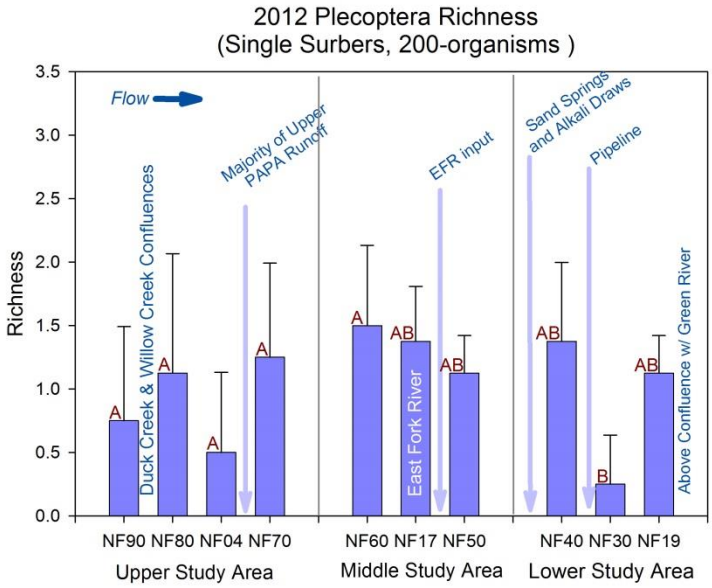


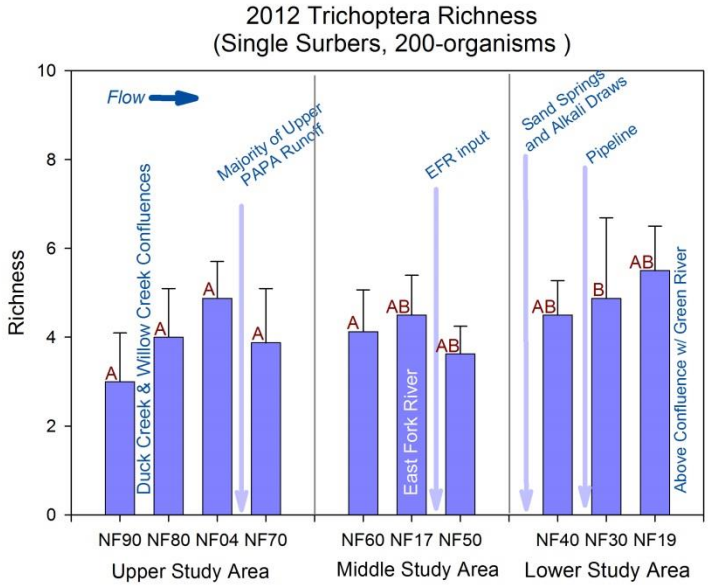

2012 Relative Abundance of Trichoptera
(Single Surbers, 200-organisms)
Relative Abundance (%)
Flow
Duck Creek & Willow Creek Confluences
Majority of Upper PAPA Runoff
East Fork River
EFR input
Sand Springs and Alkali Draws
Pipeline
Above Confluence w/ Green River
NF90 NF80 NF04 NF70
Upper Study Area
NF60 NF17 NF50
Middle Study Area
NF40 NF30 NF19
Lower Study Area

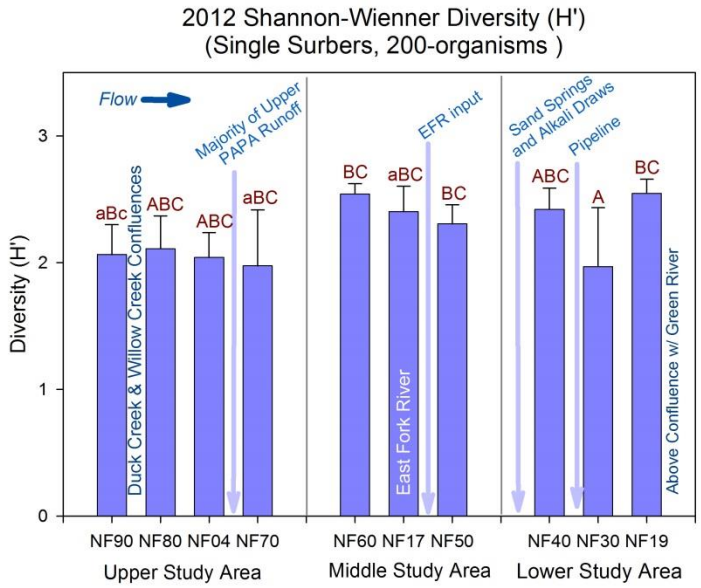

2012 Relative Abundance of Plecoptera
(Single Surbers, 200-organisms)
Relative Abundance (%)
Flow
Duck Creek & Willow Creek Confluences
Majority of Upper PAPA Runoff
East Fork River
EFR input
Sand Springs and Alkali Draws
Pipeline
Above Confluence w/ Green River
NF90 NF80 NF04 NF70
Upper Study Area
NF60 NF17 NF50
Middle Study Area
NF40 NF30 NF19
Lower Study Area

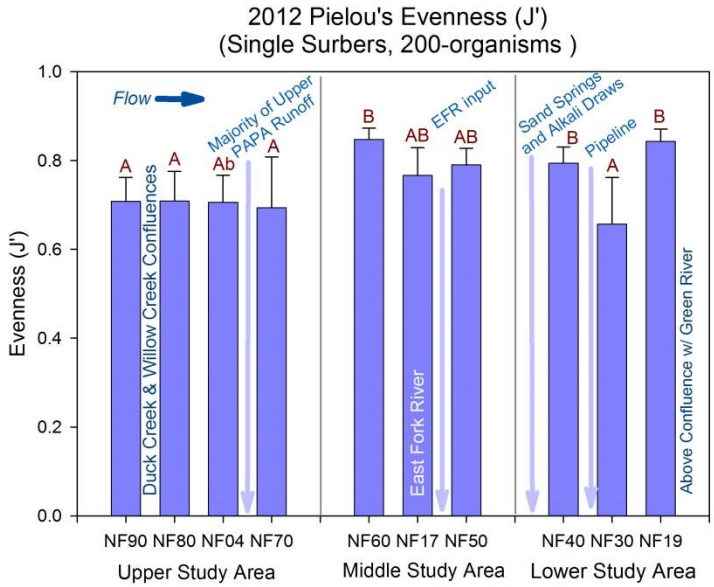
INDIVIDUAL COMPONENTS OF EPT RICHNESS: ↓






2012 Trichoptera Richness
(Single Surbers, 200-organisms)
Richness
Flow
Duck Creek & Willow Creek Confluences
Majority of Upper PAPA Runoff
East Fork River
EFR input
Sand Springs and Alkali Draws
Pipeline
Above Confluence w/ Green River
A
A
A
A
A
AB
AB
AB
B
AB
NF90 NF80 NF04 NF70
Upper Study Area
NF60 NF17 NF50
Middle Study Area
NF40 NF30 NF19
Lower Study Area


2012 Shannon-Wienner Diversity (H')
(Single Surbers, 200-organisms)
Flow
Majority of Upper PAPA Runoff
EFR input
Sand Springs and Alkali Draws
Pipeline
Duck Creek & Willow Creek Confluences
East Fork River
Above Confluence w/ Green River
Diversity (H')
0
1
2
3
aBc
ABC
ABC
aBC
BC
aBC
BC
ABC
A
BC
NF90 NF80 NF04 NF70
NF60 NF17 NF50
NF40 NF30 NF19
Upper Study Area
Middle Study Area
Lower Study Area


2012 Pielou's Evenness (J')
(Single Surbers, 200-organisms)
Flow
Majority of Upper PAPA Runoff
EFR input
Sand Springs and Alkali Draws
Pipeline
Duck Creek & Willow Creek Confluences
East Fork River
Above Confluence w/ Green River
Evenness (J')
0.0
0.2
0.4
0.6
0.8
1.0
A
A
Ab
A
B
AB
AB
B
A
B
NF90 NF80 NF04 NF70
NF60 NF17 NF50
NF40 NF30 NF19
Upper Study Area
Middle Study Area
Lower Study Area