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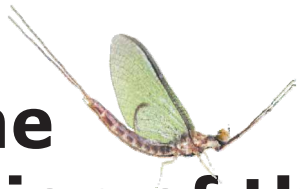

Assessment of the Biological Condition of the New Fork River, in the Vicinity of the Pinedale Anticline Project Area: 2008

An Assessment of Changes among Benthic Macroinvertebrate Assemblages

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Executive Summary

This report follows up several years of evaluation of the ecological condition of the New Fork River within the Pinedale Anticline Project Area (PAPA) using aquatic invertebrate assemblages. In previous years, we have identified some sediment-related impairment occurring between NF40 and NF30. The most likely causes of these relatively localized shifts in dominant species are pipeline river crossings and platform construction in the active hyporheic zone.

The 2008 field survey described by this report collected 8 1-sq. ft. samples from each of nine sites on the New Fork River (and a reference on the East Fork River, NF17). The invertebrates of these samples were identified in the laboratory and used to calculate metrics that are used to evaluate the ecosystem function. These results were also used in 2007; thus, this is the first year in which sufficient replication was used to allow year-to-year comparisons for statistical evaluations of change.

The first analyses in this report describe the spatial changes among sites in 2008. We found that there were many localized differences in the invertebrates species assemblages and that many of these changes were actually statistically significant. These were considered on a case-by-case basis through the report. Most of the differences we found did not support the hypothesis of PAPA-related impairment of the New Fork River in 2008. The most notable exception to this was the dominance of aquatic worms below the pipeline crossing at NF30. This has been an ongoing localized problem that is most likely related to repeated disturbance of the river bed for pipeline crossings and by riparian drilling platforms. The continuity of this shift to a worm-dominated ecosystem may have been exacerbated by a long period of reduced river flows, which have yet to clear the disturbed sediment from the system.

There were also some noteworthy subtle differences among the sites. The farthest downstream site showed dominance in some sediment tolerant organisms, but also had some sensitive species. This change in species abundance was reflected as a shift in the dominance of functional feeding groups—being dominated by collectors in the same proportion as the sediment impaired site NF30.

The comparison of 2008 with 2007 indicated that nearly all sites exhibited improved conditions (net results from multiple ecological metrics) in 2008. The exception was the farthest downstream site NF19, which had a net decline in condition. This was predicted in earlier reports as part of the recovery signature (Marshall 2007, 2008) as sediments were exported from NF30.

Like the rest of this decade, 2008 did not provide a typical sustained spring discharge for the New Fork River. It did provide the greatest spring discharge in recent years, but apparently it was insufficient to purge sediments from NF30 completely. The discharge this year (next report) is the first normal discharge in a decade and we anticipate the best assessment of recovery in the next report.

However, the recovery requires that the additions of sediments between NF30 and NF40 are reduced or halted. If this does not occur, then this could be the early signs of reach-wide, long-term sediment impairment of the New Fork and Green Rivers rather than just part of the recovery signature. Still, it might be worth the PAWG's time to discuss ways of minimizing the impacts of pipeline construction and review erosion control strategies.

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Preamble:

There has been a trend in science writing to favor the active voice over the passive voice—particularly among ecological journals. To some, this may seem informal, but it is generally more concise—allowing more information to be discussed in less space, while promoting greater comprehension. Usually when we prepare single-author papers, the singular first person pronoun, "I" is used extensively. However, this report is the product of much work by the Sublette County Conservation District and their stakeholders so I usually used the plural pronoun, "we."

Acknowledgements:

This report is the product of much work by many persons. I would especially like to thank Kathy Raper, Darrell Walker and Sno Ann Engler for their work on the report, in the field and logistical support. Also the SCCD board of supervisors for their commitment, continued involvement, and funding of the project.

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An Assessment of Changes among Benthic Macroinvertebrate Assemblages

1.0 Background

The purpose of this study was 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 several years and incorporates the Sublette County Conservation District's baseline biological monitoring of the New Fork River. Thus, although the focus is on 2007-2008, this report draws upon nine years of biological data (2000-2008) to describe changes in the ecological condition of the New Fork River.

This study is more complex than a typical bioassessment 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.

Last year 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.

For 2008's field survey, we continued using the improved methods and the improved statistical design. We replaced a reference site that was naturally un-comparable to others (NF01) with a new site (NF80). In the 2007 report, we began laying the foundation for using the improved sampling methods to compare with the composited sampling methods. This will allow calculations to make the data from 2007 and 2008 comparable with data collected earlier and with regional reference criteria used to classify benthic invertebrate data with standards around the state. The culmination of this work will be summarized and published in a separate report—keeping this already long report on the task of describing the current condition of the New Fork River, and how it had changed between 2007 and 2008.

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. States 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 so-called "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 used to assess the New Fork are not new; Ruth Patrick of the Academy of Natural sciences began performing such surveys as early as the 1940's. 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.

Invertebrates are the most commonly used animal assemblage[1] 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 we are specifically monitoring aquatic insects, mussels, snails, worms, crayfish, crustaceans, mites, leeches and similar organisms. But 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 breaking down of complex organic material—and in transferring energy to higher trophic[2] 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.

[1] Assemblages are collections of species living together.
[2] "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 of 2007's PAPA Monitoring Results

The most significant finding of the 2007 report was 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. Mayflies and stoneflies were not as common at the site as they should have been[3].

A tour of riverside PAPA development was 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 taskgroup in September 2008.

Although the site tour indicated that operators were not doing all they could to prevent sedimentation in the New Fork River, it remains unclear how much influence multiple drought years influenced retention of sandy sediments. We do know that the New Fork River has experienced dramatically reduced flows from low precipitation from 2001-2005.
Although the severity of drought reduced in recent years, the river has not recently experienced "normal" levels of sustained high flow consummate with snow melt (Fig. 1.1).

This is important for several reasons. First, a high sustained runoff flow is critical to purge sediments entrained to the system during PAPA development; high flows are part of the natural recovery process for rocky mountain streams, and without them, the effects of development in the PAPA and other surrounding areas may appear exacerbated.

Notice that in 2008, we had greater runoff than the previous year and exceeded the 54-year median flow. However, these flows were not sustained (they lasted only about half of the normal sustained snow-melt runoff period) and may only allow partial export of sediments from disturbed sites (i.e., NF30).

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

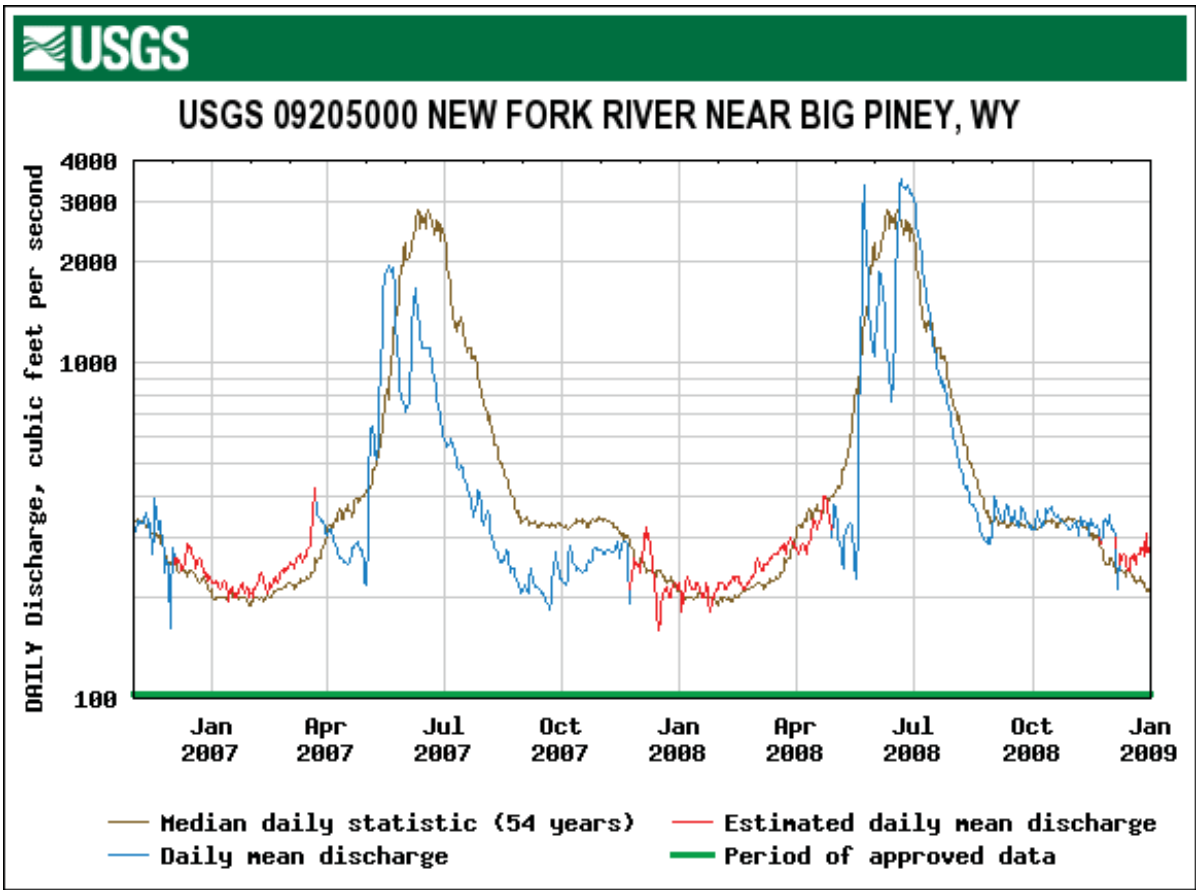

FIGURE 1.1. 2007 New Fork River Discharge. The New Fork River normally exhibits a sustained high flow of nearly 2600-2900cfs for about 30 days. The river sustained only 1900cfs for 1 week in 2007.

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). The 2008 field sampling followed the recommendations from the 2007 report by replacing site NF01 with a new upstream reference (NF80) because NF01 was too different (naturally) to serve as an adequate reference[4].

NF01 New Fork River

NF01 is located ~ 1½ miles downstream of the New Fork Lake Dam, near the outlet of the lake. The dam was breached in 1928 and was subsequently rebuilt. The New Fork Irrigation District maintains a gauging station on the dam. A Boy Scout Camp is located on the lower end of the lake approximately 1 mile from the dam. The upper portion of the watershed above the lake is a wilderness area in the Wind River Mountains. Baseline biological assessment indicated that this site had a unique biological and chemical composition from downstream sites (Marshall 2005a, 2005b). At this location, much of the river's flow is provided as through-fall from New Fork Lake Dam; the effects of dams on river food webs are well documented and numerous (nutrient sink, elevated seston, elevated filter feeders etc.). Additionally the confluence of Willow Creek and Duck Creek increase the conductivity of the New Fork River (Marshall 2005b) and alter the composition of the river's invertebrate communities.

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 make up 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.

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

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 a USGS gauging station and HWY 353. 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 as 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. Thus, this site is the ultimate recovery zone site and we do not anticipate development in 2009 to reach this site.

NF30 New Fork River

NF30 is located downstream of most of the Anticline development and below several pipelines' hyporheic crossings. The site is located on BLM land and has been sampled since the year 2001. A 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.

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 Pinedale Anticline. 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.

NF80 New Fork River

NF80 is located downstream of the confluence of Duck Creek ~ 1 mile and upstream of NF4 ~ 1 mile. NF80 was established to monitor any effects from exploration and development from the upper portion of the Pinedale Anticline Project Area. Upstream of NF80 is the town of Pinedale, a golf course and subdivisions. Both chemical and biological data are collected at this site.

Sublette County Conservation District
Pinedale Anticline Surface Water Sampling Sites

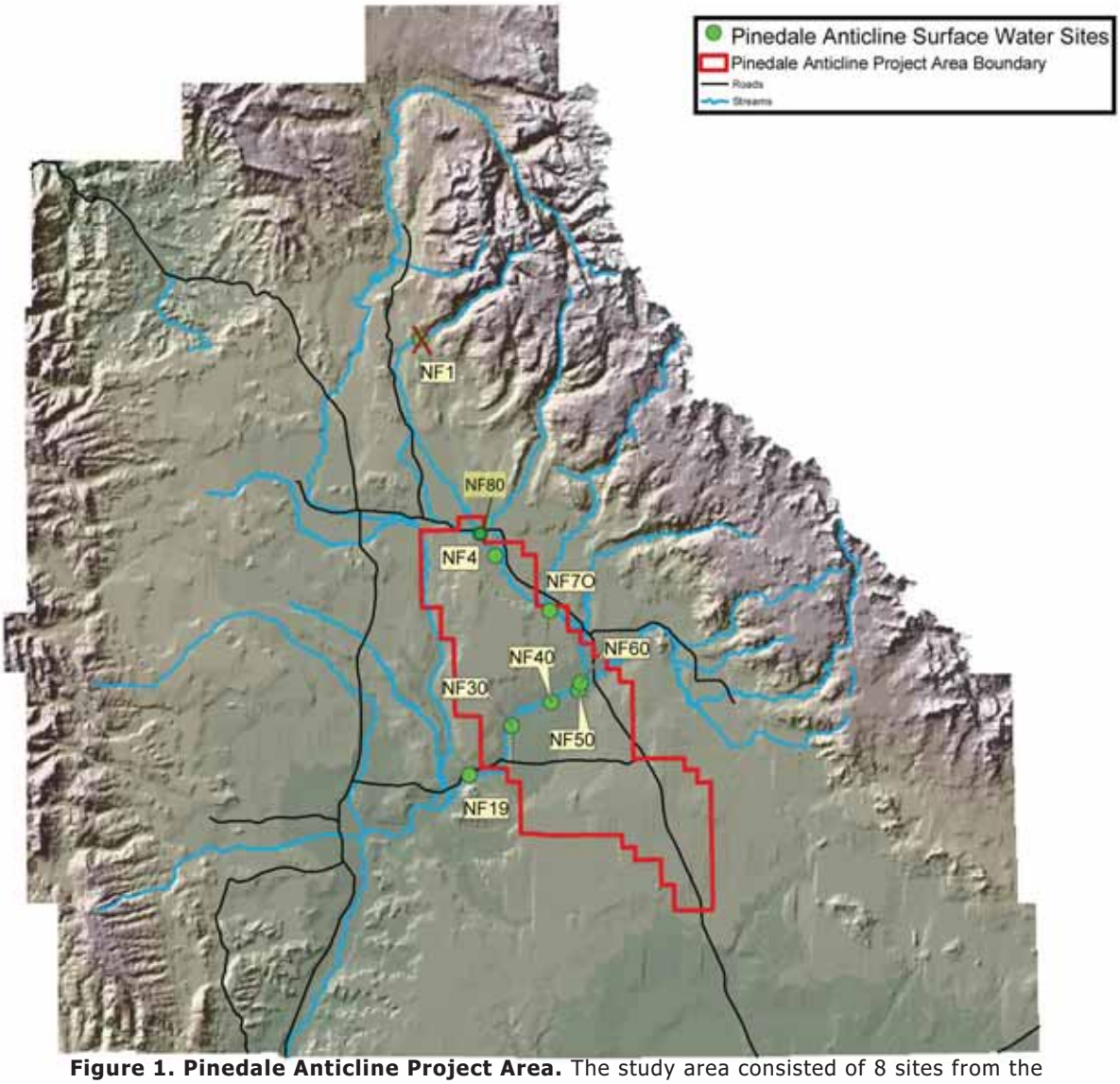

Figure 1. Pinedale Anticline Project Area. The study area consisted of 8 sites from the New Fork River and one site on the East Fork River. The goal is to assess changes in the condition of the New Fork River as it passes though the Pinedale Anticline Project Area (outlined in red) of central Sublette County. Site NF01 is crossed out because it was replaced with site NF80 in 2008.

Disclaimer

The Sublette County Conservation District shall not be held liable for improper or incorrect use of the data described and/or contained herein. These data are not legal documents and are not intended to be used as such.

The information contained in the data is dynamic and changes over time. It is the responsibility of the data user to use the data appropriately and consistently within the limits of the data.

The Sublette County Conservation District provides no warranty, expressed or implied, as to the accuracy, reliability, or completeness of the data. Although these data have been processed successfully on a computer system at the Sublette County Conservation District office, no warranty expressed or implied is made regarding the utility of the data on another system or for general or scientific purposes, nor shall the act of distribution constitute any such warranty.

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2.2. 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 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 needed 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. Graphs throughout the results section of this report have been bisected to clearly show the two 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 are most likely to be in the form of eroded soil and sedimentation in streams. Additionally, if leachate or other industrial chemicals are spilt on soil, their eventual arrival in river systems is likely to correspond to runoff events. Note that there are no direct disposal effluents during the 2008 survey—although Anticline Disposal may have a diffused, effluent active during the 2009 field survey.

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 run off to the lower study area. In 2007, increasing concerns of increasing development in the northern PAPA necessitated a study area that could differentiate these influences from natural variation and anthropogenic influences farther upstream. Thus in 2007, the PAPA assessment added an upper study area.

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, and 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.

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 and Willow Creeks 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 and seem to 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 and Willow Creeks, 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.

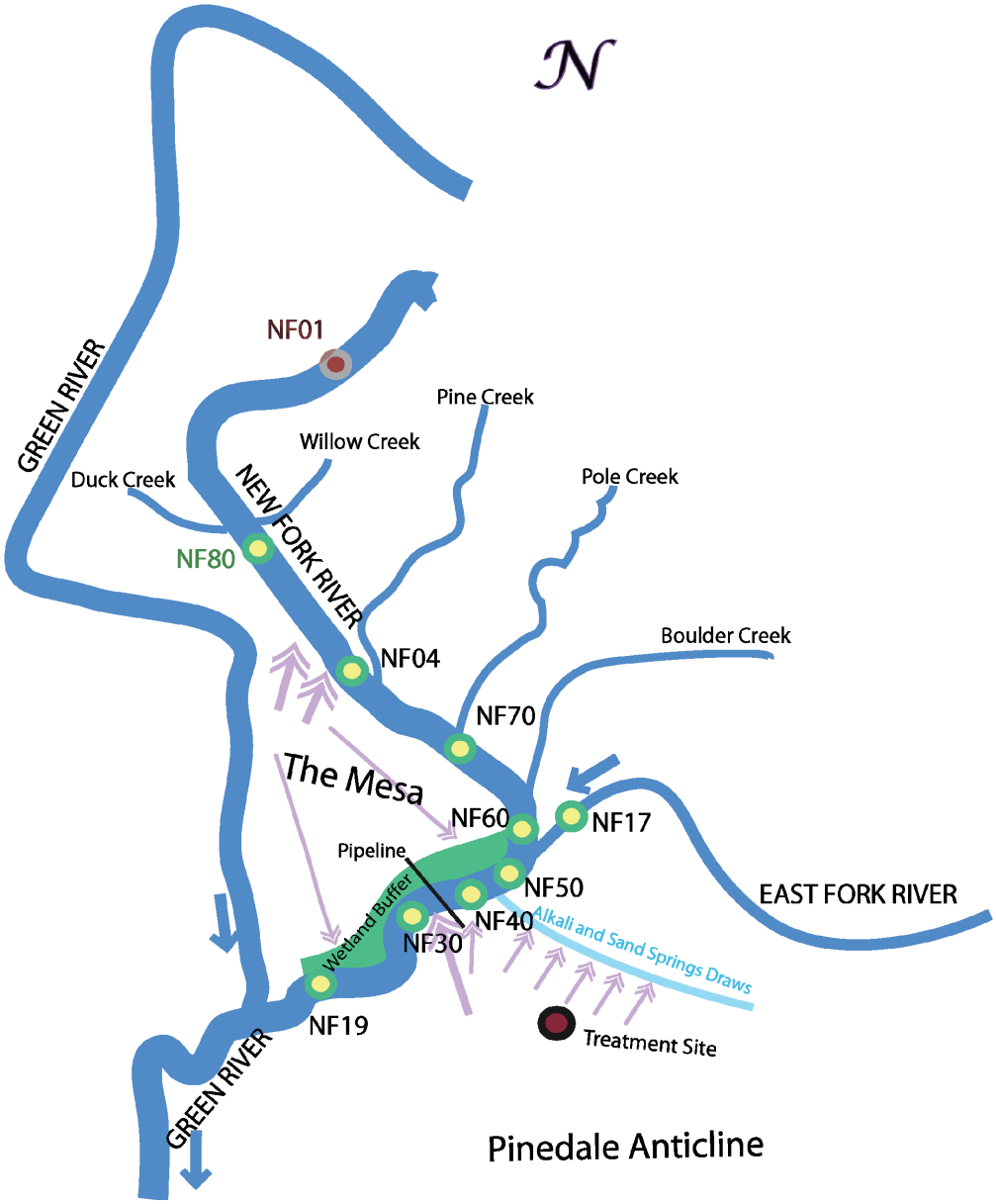

Figure 2.2. Study Site Schematic. This diagram shows the interrelationship among the location of study sites and potential sources of runoff in the PAPA. Double headed arrows indicate potential vectors of influence on the New Fork River from development on the PAPA. Although most of the runoff on the 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. The taxonomic composition of NF01 is significantly different from other sites because it is smaller and influenced by New Fork Dam. Site NF01 was not sampled this year (see text) and has been replaced with a more appropriate reference site: NF80.

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 this site. It soon became clear that this was not sufficient because we needed to account for changes from the New Fork River as well as potential impacts from runoff through Sand Springs and Alkali Draws, which enter the river downstream from the East Fork River and upstream from NF40. Thus several sites were added to account for this gradient.

NF17, 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 recently augmented with replicate samples 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. The difference between NF50 and NF40 may account for runoff flushed through the draws. Direct runoff (as opposed to indirect runoff) from the Anticline would be represented from changes in the condition of NF40 to NF30 (Fig. 2.2).

Most of the land comprising the Mesa drains to the southeast. Thus, it appeared likely that potential runoff and erosion could 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 run off from the southeast edge of the Mesa (Fig 2.2). 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.

2.2. Field methods

In 2007, we altered the 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 (Marshall 2008) report we reported both the historic method and the altered method simultaneously. This report considers primarily the augmented sampling method. This allows us to compare differences among sites in 2008 and to compare those sites with 2007 to evaluate change. This field method was called the "single Surber" (SS) method in earlier reports (Marshall 2008) and we retain that nomenclature this year.

There were also "composite Surber" (CS) samples collected in 2008. The handling of these samples is described below, but their purpose is to calibrate calculations making the SS method comparable with the pre-2007 PAPA surveys, the SCCD baseline biology survey (Marshall 2005a) and WY DEQ bioassessment framework, which uses regional models of variation. These methods are beyond the scope of this report and will constitute a separate report, which future studies may reference.

Single sample methods

In 2007 and 2008, we collected eight single Surber samplers from each site, each of which was processed individually in the laboratory to Wyoming Department of Environmental Quality's Standard procedures (e.g., Stribling et al. 2000). The procedures deviated from DEQ's Standard methods, which were in earlier PAPA assessments, in that DEQ usually "composites" all eight samples into a single sample representing the site. In order for the SCCD field crews to collect replicates , they had to actually disturb 40 ft2 and remove all insects and debris from the bottom of the river. By keeping the samples separate, we can correlate them with environmental variables and increase the statistical power of assessment.

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. So by sampling consistent velocities we prevent this from producing confounding results.

In addition to ensuring flow consistency among velocities, sampling a range of flows at sites allows us to account for the effects of velocity on biological measures statistically. For this to succeed, each site needs 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. 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.

This technique also allowed us to relate other habitat variables directly to biological measurements. Many of these could be related to natural gas development—or due to natural variation. For example, for each sample, the field crew measured the relative substrata size distribution, and embeddedness.

Composite sample methods

SCCD also collected some composite samples similar to those collected before. This was especially important because the focus of this report is to detect PAPA related change and to complete the study we need data that we know are comparable to the baseline data collected from the SCCD baseline study and previous years of PAPA monitoring. These data sources were also used in this report. Each composite sample is made of eight randomly-selected (scattered all over the river bottom) single, square-foot benthic samples that were poured together in the field, preserved and sent to the laboratory for analysis. The problem with this method is that the sample can never be re-separated to correspond to other variables (such as flow, embeddedness, depth, and particle size distribution). Another disadvantage is that this is an incredibly ineffective method to replicate; to attain only five replicates requires the collection of 40 samples. Composite samples were only collected from sites with a history of being sampled with this technique. As with earlier assessments, SCCD collected five replicates using this method so that we could compare from site to site and year to year.

The advantages of this method are minor, given the cost of replicating this method is that the first data sets collected for this project used this method, as did the SCCD baseline surveys and the WY DEQ regional WSII models. This is the last year these samples are collected for the PAPA survey, and they are not discussed in the results of this report—as stated elsewhere, they will be used to calculate correction factors—maintaining the value of historic data.

2.3 Laboratory methods

Biological metrics data from 2000-2004 were entered and validated by SCCD personnel and sent to Brett Marshall for analysis by a professional stream ecologist with 22 years of invertebrate ecology experience. Certified professional laboratories completed all the laboratory analyses and trained SCCD staff collected all field measurements. Thus, this report meets the requirements for credible data defined by the State of Wyoming. Most biological data from 2004-2007 were generated from raw taxonomic data by EcoAnalysts, Inc. according to the taxonomic standards set forth by WY DEQ.

Single samples (as discussed above), which are not used by WY DEQ at this time, were subsampled to allow the identification of 200 organisms to the genus-species level , including midges and worms. If specimen condition or maturity prevented this level of taxonomy they were identified to the lowest practical taxonomic level. If the single samples contained fewer than 200 individuals, the entire sample was identified.

Composite samples are commonly used by WY DEQ. These were subsampled to ensure that 500 individual organisms were identified to the specified levels defined by WY DEQ. This includes species level identification for all taxa, including midges and worms when specimen maturity and condition permits. If the composite samples contained fewer than 500 organisms, the entire sample was sorted.

A complete quality assurance report was submitted to SCCD with the data and indicated that, similar to previous years, all invertebrate laboratory procedures met or surpassed the sorting and taxonomy standards required for these types of data.

In 2008, the contract laboratory, EcoAnalysts, Inc. (Moscow ID) destroyed three samples from NF40. In 2006, the same contract Laboratory destroyed three samples. This impacts the statistical analyses, because the samples were very carefully collected from a range of water flow velocities. By destroying several samples from one site, that site did not have the same range of flow represented. This caused some interaction effects that had to be statistically analyzed and corrected for. Statistical methods are described in the Analytical Methods section (below) and when appropriate they are discussed in the Results section.

2.4 Analytical Methods

Habitat variables

The area contained within each SS 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), and embeddedness.

In similar assessments (Marshall 1997, 1998, 1999, 2007a) conducted for the Academy of Natural Sciences, I have found that compiling substrate size distribution data into a Particle Size Index (PSI) has certain advantages. It correlates well with biological metrics and avoids problems with autocorrelation caused by using all the measures (which are proportional to each other). The index I have used in the past weighs the percentage of each substrate size class, relative to the suitability for invertebrate colonization. For example, many invertebrate species do not like sand—it moves in the river flow and could bury them or grind them up. Larger particles provide more stable, colonizable macroinvertebrate habitat. Optimal balance of providing surface area and stability is attained by the cobble-sized particles. Boulders are stable, but have less surface area per unit volume to accommodate diverse communities.

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

Biological variables

Biological metrics are values calculated from the taxonomic data set (which is a list of the species collected and their abundance) because they summarize the changes in species composition in terms of changes in ecological function. 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 predicts that the functions performed by these species should be conserved—unless the ecosystem's function in 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 by summarizing functional changes. The metrics compared in this report are discussed briefly below. The WSII mentioned throughout the text is the Wyoming Stream Invertebrate Index developed by Wyoming DEQ. It is a method of combining metrics to summarize the ecological condition of streams and rivers in the state and will be discussed in the next section (Section 2.5).

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 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. Thus, three of the metrics used in the WSII are the richness of Ephemeroptera, Plecoptera, and Trichoptera represented separately. We used combined EPT richness metrics to compare NF30 directly to NF40, but the richness of the individual EPT orders was used to calculate the WSII and compare samples to the regional reference condition.

The metric "percent abundance of Trichoptera" is based on the same philosophy as the other EPT measures. Many states, including Wyoming, exclude the family Hydropsychidae from this calculation because members of this family are generally more tolerant than most caddisflies. Thus, this metric excludes these taxa for this study and is calculated by dividing the total number of non-Hydropsychidae Trichoptera by the total number of invertebrates identified and multiplying by 100.

The abundance of chironomid midges is often used as an indicator of environmental perturbation because there are 4000 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 responds to organic enrichment and sedimentation, as well as acid mine waters. 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 Biological Community Index, Community Tolerance Quotient (BCI CTQ) is an index that is calculated by combining the tolerance of invertebrate species to ions with the expectations for the region. High values indicate a community of high tolerance, whereas low values indicate a community dominated by sensitive organisms. High values indicate a community dominated by organisms preferring higher conductivity waters.

In the WSII-2 (Hargett and ZumBerge 2006), BCI is replaced by the Hilsenhoff Biotic Index. This index uses the weighed abundance of organism's tolerance to organic pollution to score the sample from zero to ten. Low scores mean that most organisms are very sensitive to pollution. High scores indicate that most organisms in the sample are very tolerant to pollution. For example, the rat-tailed maggot, Syrphidae, has a tolerance of 10, and can be collected from sewage treatment lagoons. Thus, high values, usually indicate a polluted condition, where as low values usually occur in clean, cool, low-sediment waters. It is important to remember that this metric has been specifically developed to describe the effects of organic pollution. It also responds to some forms of sedimentation, but is pretty ineffective to describe changes in pH, metals, or other ionic changes in water quality.

Semivoltine taxa are taxa that require more than 1 year to reach reproductive maturity. These taxa have generation times of 2 or more years. We found that some of the earlier data (Bollman 2004) confused long-lived taxa with semivoltine taxa and therefore, I recalculated the metric for this analysis. The semivoltine taxa in these samples included some stoneflies, and dragonflies in the family Gomphidae. Earlier calculations had included some long-lived beetles. Although these taxa are long-lived, they may reach maturity in 1 year and should have been classified as univoltine. The rationale for this metric is that these taxa take a long time to reproduce and re-colonize habitats. Thus, streams that are subject to frequent severe, but intermittent, disturbances will support very few semivoltine taxa. The WSII-2 (Hargett and ZumBerge 2006) specifically excludes beetles from the calculation of Semivoltine richness—eliminating potential confusion by neophytes between the terms, "semivoltine" and "long-lived" (some beetles are actually semivoltine).

The Wyoming Stream Invertebrate Index

The Wyoming Stream Invertebrate Index has recently been revised from the criteria used in earlier reports and baseline development (Stribling et al. 2000, Jessup et al. 2002, ZumBerge 2004 Pers. Comm.), to a new form (Hargett and ZumBerge 2006). However at the time of this report we only have limited data available and cannot re-describe the entire baseline conditions of the New Fork River. Therefore, we use both the WSII-1 (Stribling et al. 2000, Jessup et al. 2002, ZumBerge 2004 Pers. Comm.) and the WSII-2 (Hargett and ZumBurge 2006) in this report. The WSII-1 is used to compare with baseline conditions described earlier (Marshall 2005a), whereas the WSII-2 is used to describe the current results in the context of current Wyoming Monitoring Standards. At this time we are unsure of which of the two WSII's versions most accurately reflects the condition of sites in Sublette County, but the WSII-2 is based on much more data. This monitoring project's goal is specifically to detect changes related to PAPA development; this is a decidedly different goal than comparing samples to regional reference conditions (the goal of the WSIIs). We used the WSIIs because they have been found to respond to human stressors as have the metrics of which they are

composed. Thus readers should understand that for this report, the focus is on describing change in the WSIIs and their metrics, rather than the actual condition score derived by the WSIIs. However, when the results of the WSIIs aid the interpretation of change, they are referred to in the text. The sites used in this report are all located in the Wyoming Basin Ecoregion (Fig. 1.3) and used reference criteria appropriate for that region.

The WSII-1 scores 10 metrics (Table 2.1) between zero and 100, then averages the scores to arrive at a numerical value (0-100) describing the ecological condition of the river. WSII values near 100 describe streams that are very similar to the regional reference streams upon which the WSII is based. Values near zero indicate streams that deviate significantly from reference conditions. The numerical scores are also used to derive narrative condition classifications (Table 2.2) to infer the "health" of the stream communities—again, compared to the regional reference. For example, scores close to zero mean that the stream appears severely impaired relative to the reference criteria collected in the year 2000-2001—and the site would be classified as "Very Poor" condition.

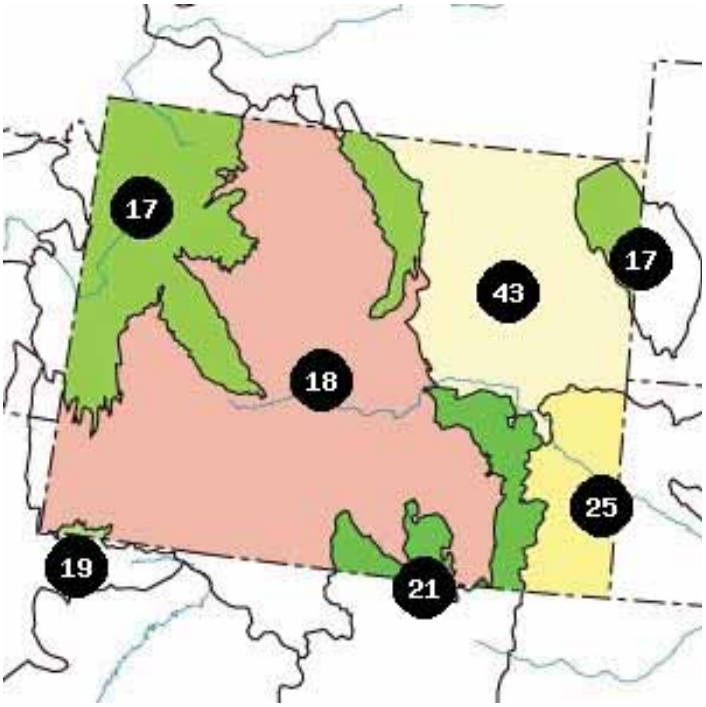

Figure 2.3. Wyoming Ecoregions. All the sites sampled in the New Fork Basin were in the Wyoming Basin ecoregion—denoted by the number 18. All streams in region 18 use the same criteria for the Wyoming Stream Invertebrate Index (WSII-1 and WSII-2).

Table 2.1. WSII-1 Scoring Criteria for the Wyoming Basin Ecoregion. Metric scores are inserted in place of the word "metric" in the scoring formula column. Values in the 5th or 95th percentile are based on the earlier survey of reference streams through out the Wyoming Basin Ecoregion. The average score for all 10 metrics is then used to attain the mean quality relative to the Wyoming Basin streams comprising the reference.

Wyoming Basin Ecoregion Rating Formulae (WSII-1[5])

Metric	Scoring formula	5th or 95th percentile
Total taxa	100*metric / 95th%ile	45
Ephemeroptera taxa	100*metric / 95th%ile	9
Plecoptera taxa	100*metric / 95th%ile	5
Trichoptera taxa	100*metric / 95th%ile	10
% Plecoptera	100*metric / 95th%ile	13
% Trichoptera (no Hydropsychidae)	100*metric / 95th%ile	31.3
% Non-insects	100*(55 - metric)/(55 - 5th%ile)	0.5
% Scrapers	100*metric / 95th%ile	31.8
BCI CTQa	100*(110 - metric)/(110 - 5th%ile)	62.6
Semi-voltine taxa	100*metric / 95th%ile	7

Table 2.2. WSII-1 Scoring Narrative Condition Criteria for the Wyoming Basin. Using the above criteria (Table 2.1), each metric is scored between 0-100. The average of all metrics is used to represent the "condition" of the river or stream surveyed. The average score will range from 0-100, just as the score from each metric and is used to classify the condition according to the criteria in this table. For example, using the WSII-1 a mean Metric score of 85 would be called "Very Good," whereas a mean metric score of 43 would be called "Fair."

Condition	Minimum	Maximum
Very Good	80.5	100
Good	60.9	< 80.5
Fair	40.6	< 60.9
Poor	20.3	< 40.6
Very Poor	0	< 20.3

[5] Based on Stribling et al. 2000, Jessup et al. 2002, ZumBerge pers. comm.. 2004

The revised WSII (2006)

The WSII-2 (Hargett and ZumBerge 2006) is very similar to the WSII-1 (Stribling et al. 2000, Jessup et al. 2002, ZumBerge 2004 Pers. Comm.) except that some of the metrics have been changed, scoring criteria adjusted, and nature of the narrative condition criteria was altered. The specific metrics are discussed in Section 2.4 (Biological Variables; above). The scoring criteria (Table 2.3) are similar—they rate score metrics between zero and 100 based upon comparison with regional reference criteria. The new condition classes (Table 2.4) are "Full-support", "Intermediate," and "Partial/Non-support" of aquatic life use. "Full-support" of aquatic life use indicates that the site (or sample) exceeds the 25th percentile of reference streams. Scores below the 25th percentile of aquatic life uses were split into three equal classifications. The "Intermediate" category is the upper 1/3 of reference sites below the 25th percentile. This roughly translates to the range of the 50th-25th percentile of reference streams. Ancillary data need to be provided to interpret the condition of these sites—although they still exceed the condition of about 50% of the reference sites. Sites that score below the 50th percentile of the reference streams are said to have suffered "substantial anthropogenic perturbations" near the sample location (Hargett and ZumBerge 2006, p.25).

Table 2.3. WSII-2 Scoring Criteria for the Wyoming Basin Ecoregion. Metric scores are inserted in place of the word "metric" in the scoring formula column. Values in the 5th or 95th percentile are based on the earlier survey of reference streams through out the Wyoming Basin Ecoregion. The average score for all 10 metrics is then used to attain the mean quality relative to the Wyoming Basin streams comprising the reference.

Wyoming Basin Ecoregion Rating Formulae (WSII-2[6])		
Metric	*Scoring formula*	*5th or 95th percentile*
Ephemeroptera taxa	100*metric / 95th%ile	9
Trichoptera taxa	100*metric / 95th%ile	9
Plecoptera taxa	100*metric / 95th%ile	6
% non-insects	100*(64 - metric)/(64 - 5th%ile)	0.4
% Plecoptera	100*metric / 95th%ile	22.3
%Non-Hydropsychidae of Trichoptera	100*metric / 95th%ile	31.3
%Collector-gatherer	100*(96 - metric)/(96 - 5th%ile)	100
% Scrapers	100*metric / 95th%ile	38.6
HBI	100*(8.3 - metric)/(8.3 - 5th%ile)	1.9
Semi-voltine taxa (No Coleoptera)	100*metric / 95th%ile	5

Table 2.4. WSII-2 Scoring Narrative Condition Criteria for the Wyoming Basin. Once the metrics are scored according to the criteria in Table 2.3, the average score is used to determine the narrative condition classification from the table below. For example, using the WSII-2, a mean metric score of 56 would be called "Full-Support," whereas a mean metric score of 50 would be called "Intermediate." A mean metric score of 34.5 or less would be called "Partial-Support" or "Non-support." The meaning of these terms is discussed in the text.

Condition	Minimum	Maximum
Full-Support	>51.9	100
Intermediate	51.9	34.6
Partial / Non-Support	<34.6	0

[6] Hargett and ZumBerge 2006.

Statistical Analyses

The goal of this monitoring project has a different goal than comparing 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, our study design was developed to allow us to use the WSII to test sites NF30 and NF40 for changes related to the Anticline. Additionally, we use metrics that have historically been useful in the New Fork River (Marshall 2005a). We used SYSTAT v.12 statistical analysis software for most analyses.

We used Analysis of Variance (ANOVA; Zar 1999) to test for differences among sites in 2008, (treatment = SITE). The ANOVA used the within site averages and variance 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%.

Tukey's HSD test was used to followup ANOVA results to determine which specific sites were significantly different from each other. This test used the same critical p-values used for the ANOVA, allowing for significant difference if the probability of type-1 statisical error was less than 5%.

This report and the 2007 report used methods that allowed more efficient use of statistical analyses than in previous reports. 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 uses 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[7]. 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." This procedure was especially useful to describe which metrics appeared to correlate with velocity or sedimentation.

[7] 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.

Another analytical method we used was analysis of covariance (ANCOVA), which also uses the GLM algorithm. In this case, we used it two ways. First, it tested significant differences in the response slopes of habitat variables responding strangely (velocity vs. % sand). The second use of ANCOVA was to statistically adjust the mean metric values to correct for the influence of water velocity—which is not related to PAPA development, to make PAPA influences more apparent. When this procedure significantly altered the results of the multiple regression or ANOVA, it was discussed in the results. But if there were no significant differences consistent with PAPA related impairment these results are not relevant and not discussed.

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, but we do not anticipate development on or near the Pinedale Anticline to increase 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.

Field crews did a better job of sampling the same range of flows in 2008 than they did in 2007. They managed to do this while sampling the same average flow[8] as the previous year. This is important because deviation in the average value would have been likely to confound analysis among years.

For 2008 there was no significant difference in flow regime sampled at the sites (P=0.343)[9].

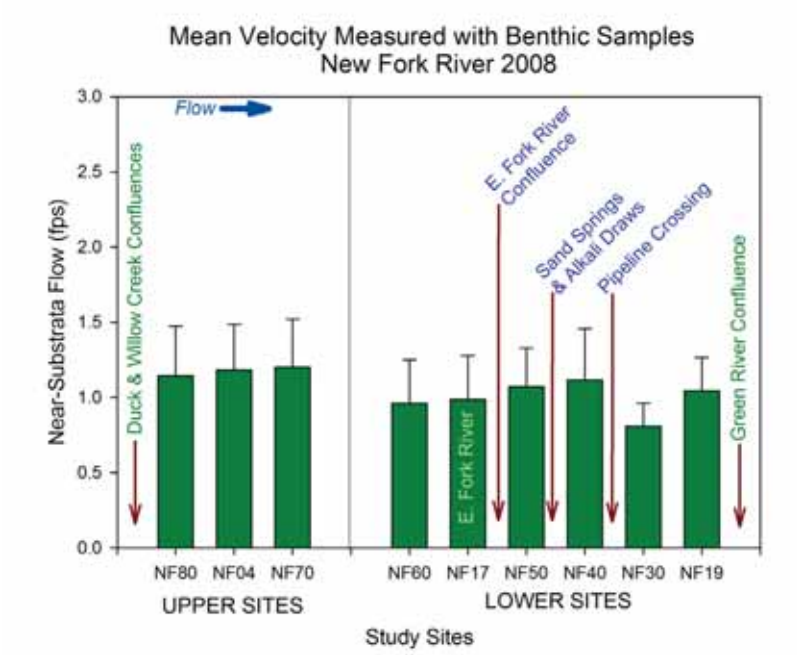

Figure 3.1. Near Substrata flows. The average near-substrata water velocity from which benthic samples were collected is represented by bars. Sites are arranged from upstream to downstream. Error-bars represent 95% confidence interval of the mean.

[8] Recall that "flow," as used here, refers to velocity of water measured as close to the stream bottom as possible to serve as a covariate for benthic analysis.

[9] Recall: there was a significant difference in 2007, but fortunately, there was no interaction effect in the Analysis of Covariance used to compare sites and adjust for flow (Marshall 2008))

The size of particles comprising the stream bottom is important for the success of macroinvertebrates as well as for fish reproduction. The field crew quantified the size composition substrata (Fig 3.2) 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[10] by New Fork River flows. 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 should naturally dominate the East Fork River (NF17).

In 2007 we found that sites NF30 and NF40 exhibited more sand-sediment found in other riffle areas—even those of the sand dominated East Fork River (NF17). In 2008, this pattern continued (Fig. 3.2). Analysis of variance indicated that there was a significant difference in the relative amount of sand in benthic samples ($P<0.001$), and Tukey's HSD test indicated that the difference between NF17-NF30 and NF17-NF40 was not statistically significant ($P=0.708$, $P=0.385$ respectively).

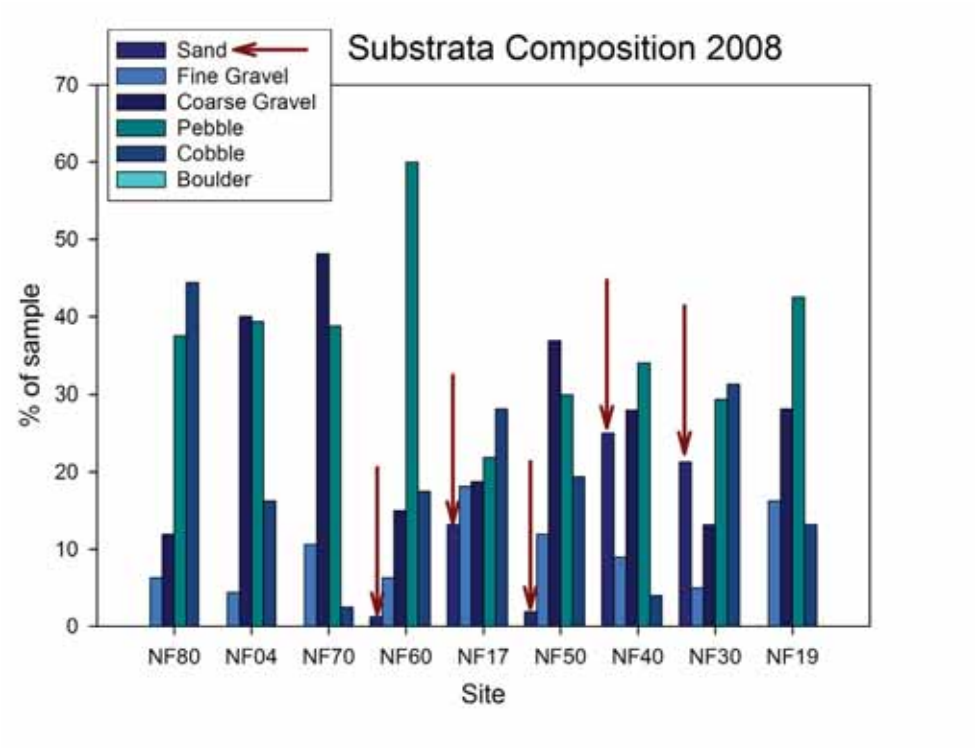

Figure 3.2. Substrata composition. The average percent dominance of different substrate types sampled when sampling was stratified according to flow (Fig 3.1) is represented by bars. Sites are arranged from upstream to downstream. Sites with on-zero dominance of sand are identified with arrows over the "% sand" value.

[10] 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 this study; these values were determined by sampling a individual $1ft^2$ benthic invertebrate samples, across a pre-selected (stratified sampling) range of flows.

In 2007, we found there was an apparent difference in the relationships among sites flow and sand-sediment. The relationships in 2008 were not statistically significant (Fig. 3.3). furthermore the sites had very low r2 values (NF17 r2=0.07; NF30 r2=0.04; NF40 r2=0.02). Together these results indicate the flow-sand model is so weak that a horizontal line (no relationship) fits as well the regression lines modeled by the data.

These results should be interpreted carefully. They mean that the placement of the benthic samplers did not happen to identify the active source of sedimentation apparent in 2007. They do not mean that the excess sand from 2007 has been exported from the system. In fact, the dominance of sand remained higher at NF30 (22%) and NF40 (26%) than NF17 (15%), NF50 (2%) and NF19 (0%). One sample at NF30 was 60% sand and two samples at NF40 had nearly 50% sand dominance—these three samples represent the greatest sand dominance by far—even more so than the sand-dominated East Fork River.

One of the goals of the ecological analyses of this report is to determine if the effects of local erosion on the community composition near the pipeline persist, have dissipated, or intensified from the previous year.

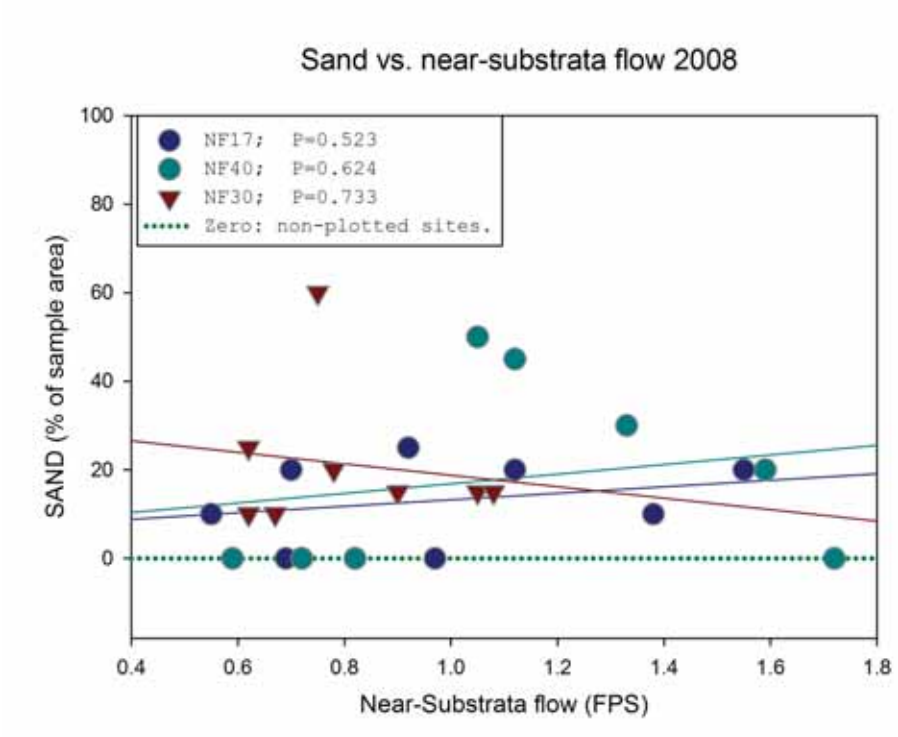

Figure 3.3. Sand-flow regression. Sites with more than one sample containing sand (NF17, NF40, NF30) were used to determine if there was a significant relationship between the amount of sand in the sample and the flow from which the sample was collected. Other sites had seven or more samples collected from zero percent sand-dominated streams.

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.

One problem with the measure is that the nature of the embedding material is not quantifiable, and the measure is subjective. Different results are likely when two (or more) observers record observations for the same habitat—unless they have worked together extensively.

We found no significant differences among the amount of embeddedness reported for all the sites in 2008. Sites below the East Fork River tended to have a greater variation in embeddedness than sites upstream, or than the farthest downstream site (NF19).

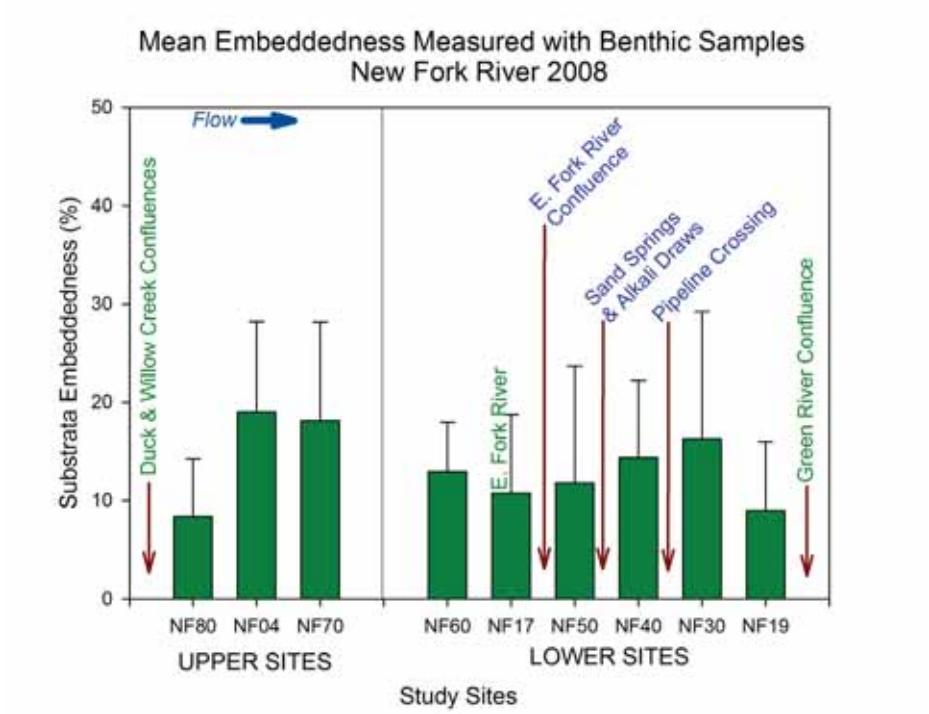

Figure 3.4. Embeddedness 2008. The average embeddedness of sampled (SS) substrate is represented by bars. Error bars represent 95% confidence intervals. Sites are arranged from upstream to downstream.

3.2 Differences among sites 2008 biological metrics

Overall differences among sites

The biological metrics were first screened for significant differences among sites using ANOVA (Table 3.1). Metrics which displayed a significant difference among sites were examined in greater detail to determine if the effects could be due to impairment related to development in the PAPA. All metrics except one (semivoltine[11] Richness) indicated that there was a statistically significant difference among the sites. ($P<0.05$, Table 3.1). Because previous years results indicated sediment-related changes up- and downstream of the pipeline crossing, these differences for 2008 are highlighted. Ephemeroptera Richness, Non-Insect Abundance, and % Scrapers were significantly different in NF30 and NF40.

TABLE 3.1. ANOVA Results. The ANOVA resulted in statistically significant differences among sites for 14 of the 15 metrics tested directly. For metrics that exhibited significant differences, Tukey's HSD was used to identify which sites were significantly different from each other. Sites that were significantly different from each other are noted by different letters in their columns. Sites were not significantly different from each other share at least one letter with similar sites.

		Multiple Comparisons Grouping (Tukeys HSD)								
Metric	P-val	NF80	NF04	NF70	NF60	NF17	NF50	NF40	NF30	NF19
Taxa Richness	<0.001	A	B	BC	BC	AB	B	AB	B	B
E-Taxa	<0.001	AB	BC	A	ABC	AB	AB	**AB**	**C**	AB
P-Taxa	<0.001	A	A	B	B	AB	AB	AB	AB	B
T-Taxa	0.007	A	AB	A	A	AB	AB	AB	B	AB
% E	<0.001	A	AB	AB	B	AB	AB	AB	A	B
% P	<0.001	A	A	C	BC	A	A	AB	AB	B
% T	<0.001	A	A	C	A	A	A	AB	BC	A
%Chironomid	<0.001	A	AB	B	B	A	AB	AB	B	B
%Non-Insect	<0.001	A	A	A	A	A	A	**A**	**B**	A
HBI	<0.001	A	AB	C	AB	AB	AB	AB	A	B
Gatherers	<0.001	A	A	C	AB	BC	B	**C**	**AB**	AB
Filterers	<0.001	A	B	B	B	B	B	B	AB	B
Scrapers	<0.001	A	A	C	ABC	AB	BC	**BC**	**A**	C
Semivoltine	0.622	--	--	--	--	--	--	--	--	--
Dominance(5)	<0.001	A	A	B	AB	AB	AB	AB	A	A
		Upper Study area			Lower Study Area					

[11] Semivoltine Richness is a measure of the number of species which take longer than one year to reach reproductive age. These species are generally considered poor colonizers and streams subject to intermittent disturbances are often lacking semivoltine taxa.

Taxa Richness

The samples (SS) collected and processed in 2008 supported around 30 species (taxa) each—on average. There were significant differences among the sites (Table 3.1, Fig. 3.5). When the differences among sites were divided by study areas they are more easily interpreted. For the upper study area, we found that NF70 supported significantly fewer invertebrate species than NF04, but that the difference between NF70 and NF80 was not statistically significant. Moreover, the site NF70 had fewer species than any other site on average—even though the difference was not statistically significant from most other sites (Fig 3.5, all sites with an "A" over the bar are not significantly different from each other.

For the lower study area, NF30 was significantly different from all other sites in the study unit (Table 3.5). If the mean was lower than the other sites, this might be cause for concern—previously we found (Marshall 2008) that NF30 was slightly-to moderately impaired by sand-sedimentation. However the highest average number of species actually occurred at NF30. High numbers of species usually indicate "healthier" ecosystems. Thus, these results do not necessarily indicate an impact unless the species added to the community are tolerant to disturbances such as sedimentation.

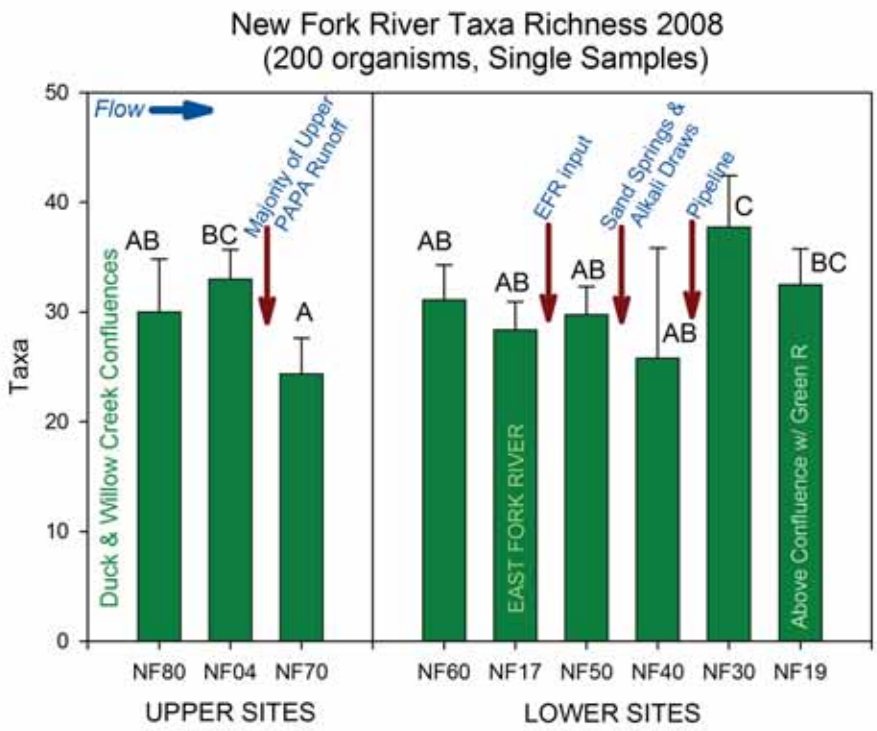

Figure 3.5. Taxa Richness. The number of invertebrate taxa (species) was lowest at NF70 and greatest at NF30. In 2007 there were no significant differences among sites according to taxa richness estimates using SS methods.

Ephemeroptera Richness

The samples (SS) collected and processed in 2008 supported around 3-6 mayfly species (taxa) each—on average. There were significant differences among the sites (Table 3.1, Fig. 3.6). Differences among sites are more easily interpreted when results are divided by study area. For the upper study area, we found that NF70 was supported significantly more mayfly species than NF80, but that the difference between NF70 and NF04 was not statistically significant. Mayfly richness usually becomes elevated in clean, cool, highly oxygenated waters, so this does not indicate a decline in the quality of New Fork River unless the additional taxa are comprised of species that are tolerant to environmental perturbation. NF80 upstream of all PAPA development—is most likely influenced by the quality of tributary waters from Duck Creek and Willow Creek, which have various springs associated with them and elevated conductivity (Marshall 2005). Many mayfly species are sensitive to high conductance water (which often has high metals concentrations).

The lower study area had the greatest mayfly richness at NF60 (just upstream of the East Fork River confluence, a short distance downstream from NF70). The remaining sites were not significantly different from each other. The sand-laden East Fork River had fewer mayfly species than NF60, and all the sites downstream did not exhibit a significant difference among populations. The findings of these analyses are not indicative of PAPA related impairments of New Fork River.

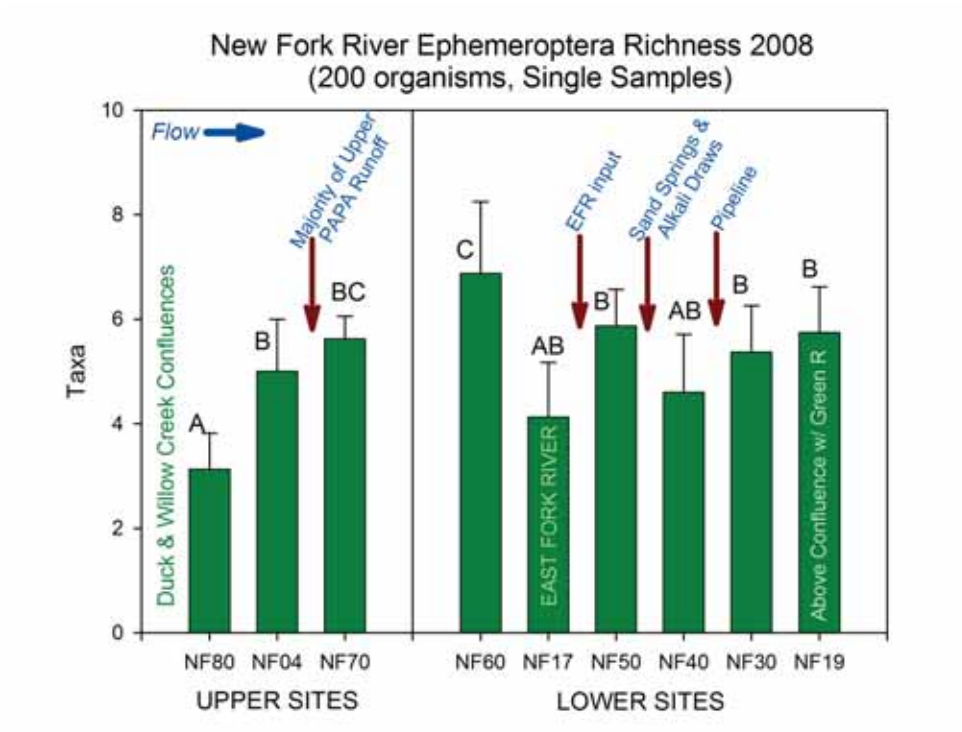

Figure 3.6. Ephemeroptera Richness. The number of mayfly taxa (species) was greatest at NF70 and lowest at NF80. In 2007 there were no significant differences among sites according to taxa richness estimates using SS methods.

Plecoptera Richness

The sites sampled (by SS sampling) in 2008 supported between 0.5-3 stonefly species (taxa) each—on average. There were significant differences among the sites (Table 3.1, Fig. 3.7). Differences among sites are more easily interpreted when results are divided by study area. For the upper study area, we found that NF70 was supported significantly more stonefly species than NF80, but that the difference between NF70 and NF04 was not statistically significant. Stonefly richness usually becomes elevated in clean, cool, highly oxygenated waters, so this does not indicate a decline in the quality of New Fork River unless the additional taxa are comprised of species that are tolerant to environmental perturbation. NF80 upstream of all PAPA development—is most likely influenced by the quality of tributary waters from Duck Creek and Willow Creek, which have associated springs which elevate conductivity (Marshall 2005). Many stonefly species are sensitive to high conductance water (which often has high metals concentrations) however a few species are tolerant to dissolved metals.

The lower study area had the greatest stonefly richness at NF60 (just upstream of the East Fork River confluence, a short distance downstream from NF70). However, none of the sites were significantly different from each other. These results are not indicative of PAPA related impairments of New Fork River.

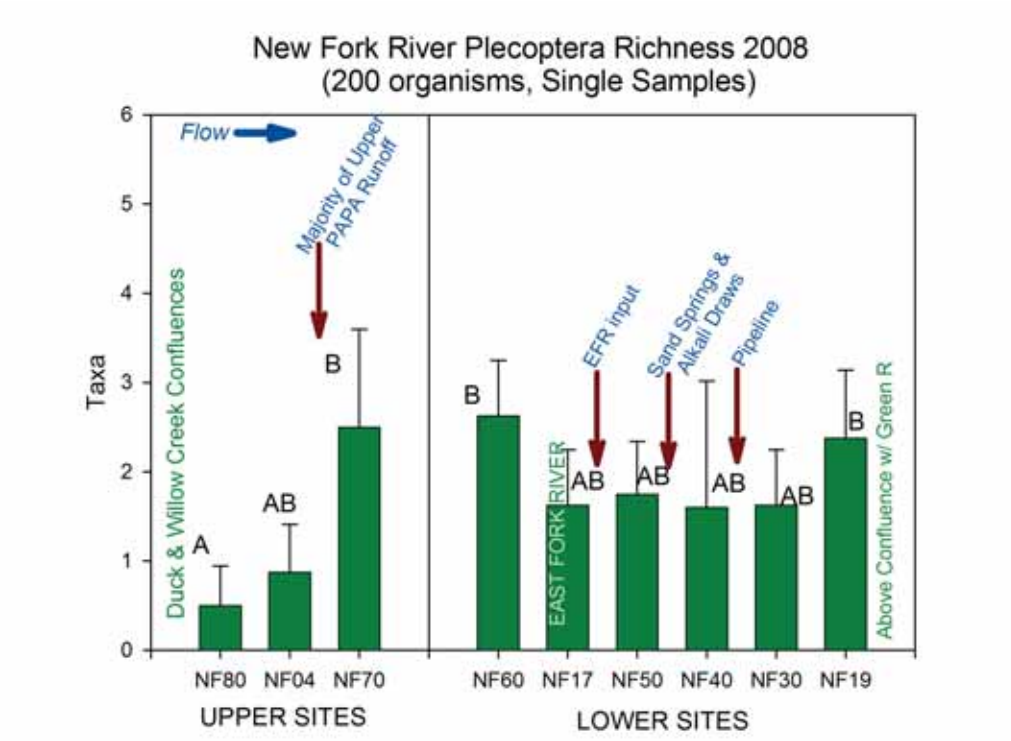

Figure 3.7. Plecoptera Richness. The number of stonefly taxa (species) was greatest at NF70 and lowest at NF80. In 2007 there were no significant differences among sites according to Plecoptera richness estimates using SS methods.

Trichoptera Richness

The SS samples collected in 2008 supported between 4-6 caddisfly species (taxa) on average. There were significant differences among the sites (Table 3.1, Fig. 3.8). For the upper study area, There were no statistically significant differences among sites NF80, NF04, or NF70.

The lower study area had the greatest caddisfly richness at NF30 (just downstream of the pipeline crossing. This site was significantly different from NF60, but not different from the other sites. Similarly the other lower study area sites were not significantly different from each other. The exception was NF60 which was significantly different from the NF30. This was due primarily to differences in taxonomic effort by the technicians at the contract laboratory (EcoAnalysts, Inc.). Specifically, the family Leptoceridae[12] was identified to several levels by technicians: Leptoceridae, *Oecetis* sp. or *Oecetis avara*—these are all equivalent taxonomic units. Thus, the apparent trend of increasing Trichoptera Richness from four taxa (upstream) to six taxa (downstream) is due to inconsistencies in laboratory procedure, not an actual increase in the number of species downstream (Fig. 3.8, Right). In this case, statistical significance is probably not indicative of ecological significance[13].

The findings of these analyses are not indicative of PAPA related impairments of New Fork River.

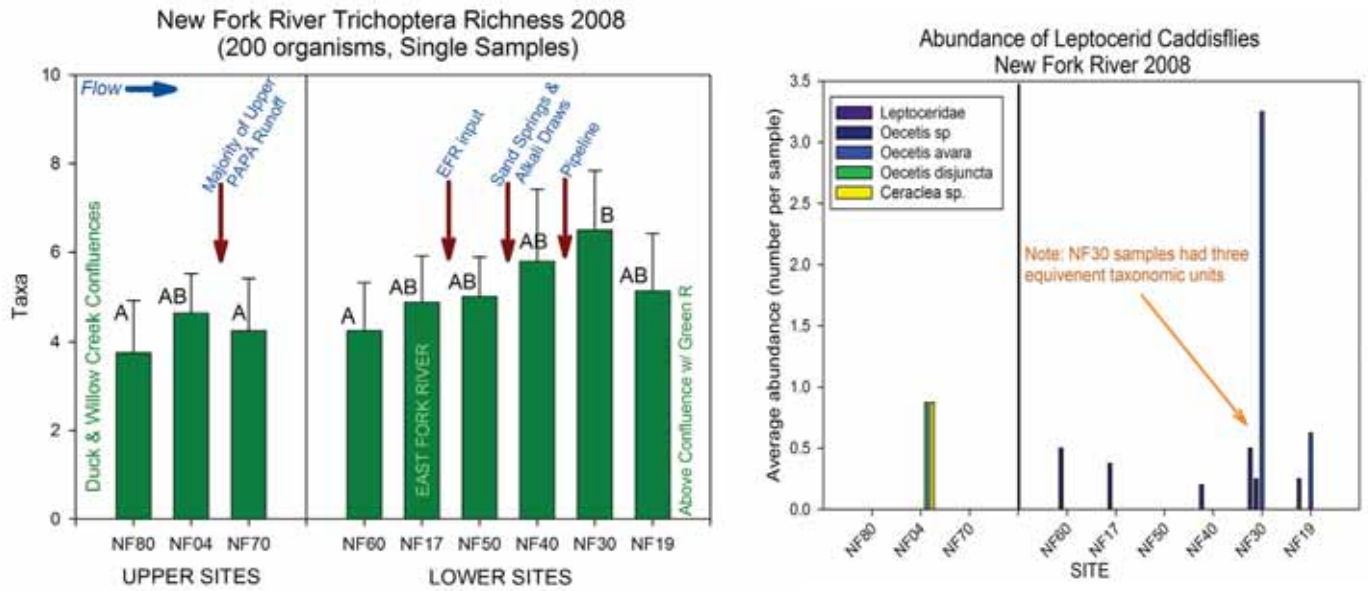

Figure 3.8. Left: Trichoptera Richness. The number of caddisfly taxa (species) was greatest at NF70 and lowest at NF80. In 2007 there were no significant differences among sites according to Trichoptera richness estimates using SS methods**. Right: Leptocerid Caddisflies**. Letptocerid caddisflies were identified to several levels by technicians. At NF30 this resulted in the same taxon having three names (family, genus and species) which inflated the richness estimates and made the NF30 samples appear to support a significantly greater number of Trichoptera taxa than NF60. The difference between the sites was two taxa, with only *Oecetis* sp. being identified at NF60, and all three taxonomic levels being identified at NF30 (3 taxa - 1 taxa = 2 taxa; the difference was due to laboratory effort—and might reflect differential condition of the specimens.

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[12] Leptoceridae are the "long-horned caddisflies." This is a reference to the very long antennae of the adults.

[13] Richness measures count the number of different kinds of animals identified in the samples. When different people work on different samples, or when the condition or maturity of specimens differs among samples, the same animal (species) can have several names, making them appear to be different types of animals. This happened in the upper study area in 2008 where the same bug was called three names in NF30, and only 1 name at NF60, NF17, and NF40. Another taxonomic group also had the same problem (Brachycentridae, *Brachycentrus*, *B. occidentalis, B. americanis*). These inconsistencies accounted for the differences in Trichoptera Richness values reported by the analytical laboratory and just happened to also overly the downstream gradient.

Ephemeroptera Abundance

Mayflies comprised between 22-50% of the communities sampled on average. There were significant differences among the sites (Table 3.1, Fig. 3.9). For the upper study area, we found that NF70 was supported significantly more mayflies than the new upstream reference site, NF80. And the difference between NF70 and NF04 was not statistically significant.

Mayfly richness usually becomes elevated in clean, cool, highly oxygenated waters, so this does not indicate a decline in the quality of New Fork River unless the additional taxa are comprised of species that are tolerant to environmental perturbation. NF80 upstream of all PAPA development—is most likely influenced by the quality of tributary waters from Duck Creek and Willow Creek, which have been suspected of altering the conductivity of the New Fork River because of hard-water springs located along the tributaries (Marshall 2005). Many mayfly species are sensitive to high conductance water and hard waters. The low abundance of mayflies at NF80 may reflect the natural conditions of the New Fork River as it receives waters from these tributaries and the sites downstream represent the dilution by tributaries like Pine Creek, Pole Creek and the East Fork River.

Other than NF30, there were no statistically significant differences observed in the lower study area. NF30 exhibited a significant reduction in mayfly abundance when compared with NF19 (downstream) and NF60 (above the East Fork confluence). The site had the lowest abundance of mayflies of all the sites, but this was likely due to the high abundance of non-insects occurring at NF30 (see below).

These results could be related to impairments related to natural gas development within the PAPA (see below).

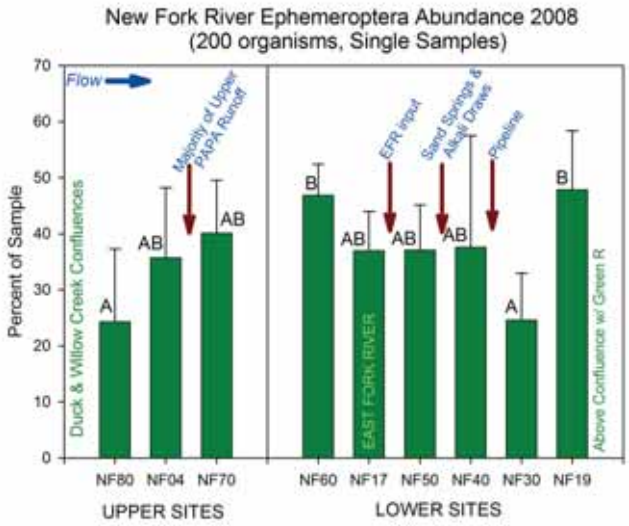

Figure 3.9. Ephemeroptera Abundance. The abundance of mayflies taxa (species) was greatest at NF19 and lowest at NF80/NF30. Bars are means and error bars are 95% confidence intervals.

Plecoptera Abundance

Stoneflies comprised between 1-6% of the communities sampled on average. There were significant differences among the sites (Table 3.1, Fig. 3.10). For the upper study area, we found that NF70 supported significantly more stoneflies than the new upstream reference site, NF80. Although the difference was small (about 4% of the community) it was statistically significant because there was a relatively small amount of within site variation around the means—this is often the case with low-abundance groups.

At the lower study area, we observed a decrease in stonefly abundance in East Fork River and below its confluence with the New Fork River. Stonefly abundance gradually increased below the confluence of the East Fork River, but the only downstream site to have more significantly (statistically significant increase in abundance) more stoneflies than the East Fork River was the farthest downstream site, NF19.

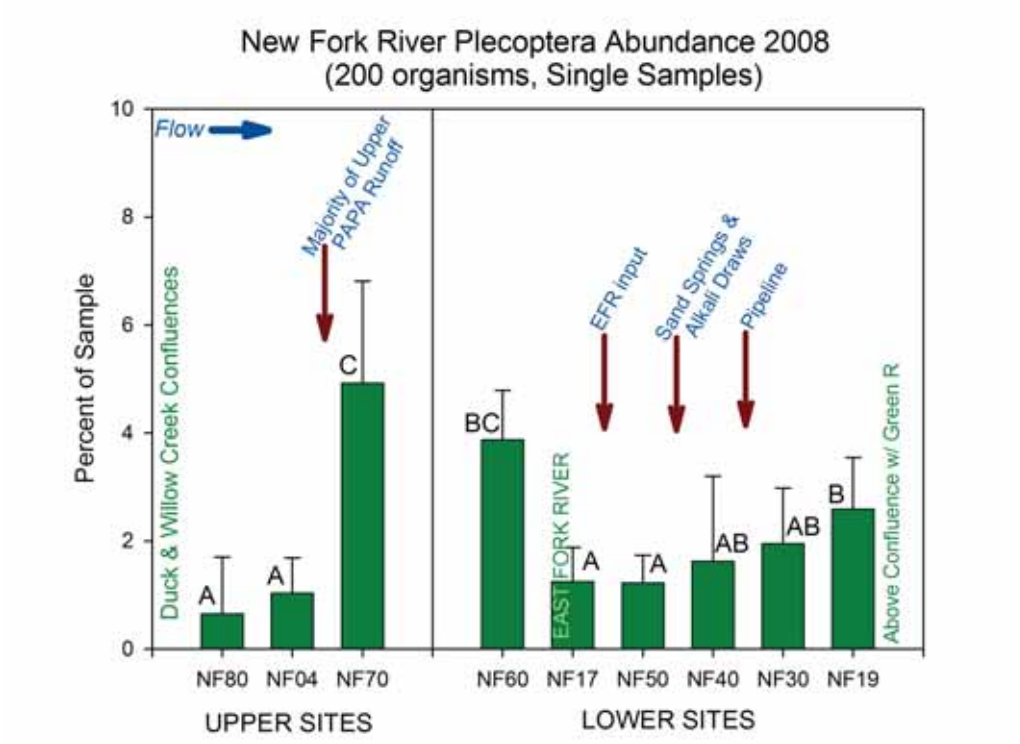

Figure 3.10. Plecoptera Abundance. The abundance of stonefly taxa (species) was greatest at NF70 and lowest at NF80/NF17/NF50. Bars are means and error bars are 95% confidence intervals.

Trichoptera Abundance

Caddisflies comprised about 10-40% of the communities studied on average and there were significant differences among the sites (Table 3.1, Fig. 3.11 Left). There was a dramatic increase in the abundance of caddisflies at the downstream site (NF70). When a trend like this is observed, it is important to determine if the dominant species are especially tolerant to certain disturbances. For example, the hydropsychid caddisflies (*Hydropsyche*, *Cheumatopsyche*) are filter feeders and sometimes benefit from disturbances that increase the amount of suspended organic material in the water column. We found that nearly all the caddisflies increasing this metric at NF70 were *Lepidostoma* (shredders), and are not indicative of disturbance (Fig. 3.11, Right).

The lower study area also showed an increase in caddisfly abundance downstream (Fig. 3.11 left), but this was due to the addition of the small, algae-associated caddisflies of the genus *Hydroptila* (Fig 3.11, right). A similar pattern in *hydroptila* sp abundance was also observed in 2007, and it is probably related to plant material growing among stream substrata.

These changes are not likely to be directly related to development within the PAPA.

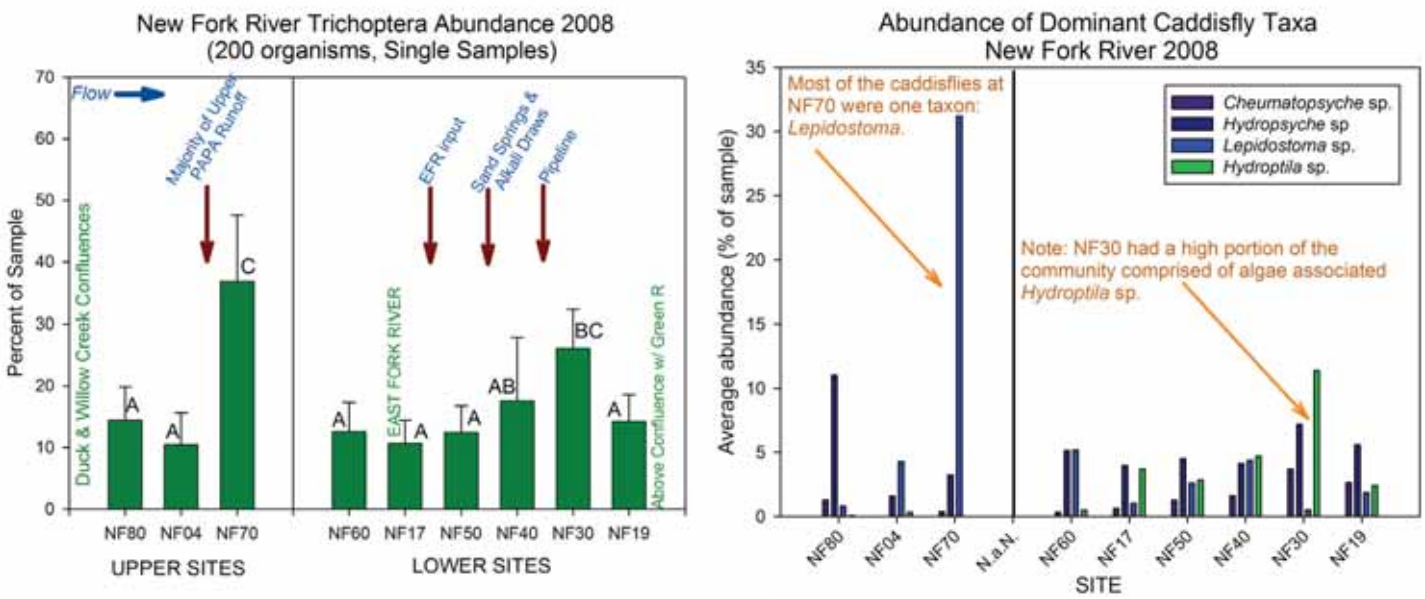

Figure 3.11. Trichoptera Abundance. *Left*. The abundance of caddisfly taxa (species) was greatest at NF70 and lowest at NF04. *Right*; **dominant caddisfly species**. The high abundance of caddisflies at NF70 was almost entirely the shredder *Lepidostoma* sp. The increase in abundance of caddisflies at NF30 was primarily due to increase in the abundance of *Hydroptila* sp., which comprised almost half of all the caddisflies collected from the site (average).

Chironomidae Abundance

In 2008, the midges comprised a moderate to small portion of the community on average; the metric ranged from about 40%-10% of the invertebrate assemblage. There were significant differences among the sites (Table 3.1, Fig. 3.12). At the upper study area, there was a decline in midge abundance along a downstream gradient. The upstream reference (NF80) had significantly greater midge abundance than the downstream site (NF70), but neither of these sites (NF80, NF70) were significantly different from the middle site (NF04). Elevated midge abundance can indicate environmental perturbation, or increases in sediment (inorganic or organic; and suspended or depositional). Thus the decline at NF70 is not indicative of environmental perturbation.

Among the lower study sites, NF17 (East Fork River) had the greatest average dominance by midges—which was significantly greater that NF60, NF30, and NF19. The lowest dominance of midges was observed at NF30. In North American mountain streams, high dominance by midges usually occurs in perturbed benthic communities or under unusual situations such as fine sediment (as is the case with the East Fork River). Thus, this metric does not indicate PAPA-related degradation of the New Fork River at any location. However, it should also be noted that the reason for low midge dominance at NF30 is due to the high abundance of non-Insects at that site[14] (see below).

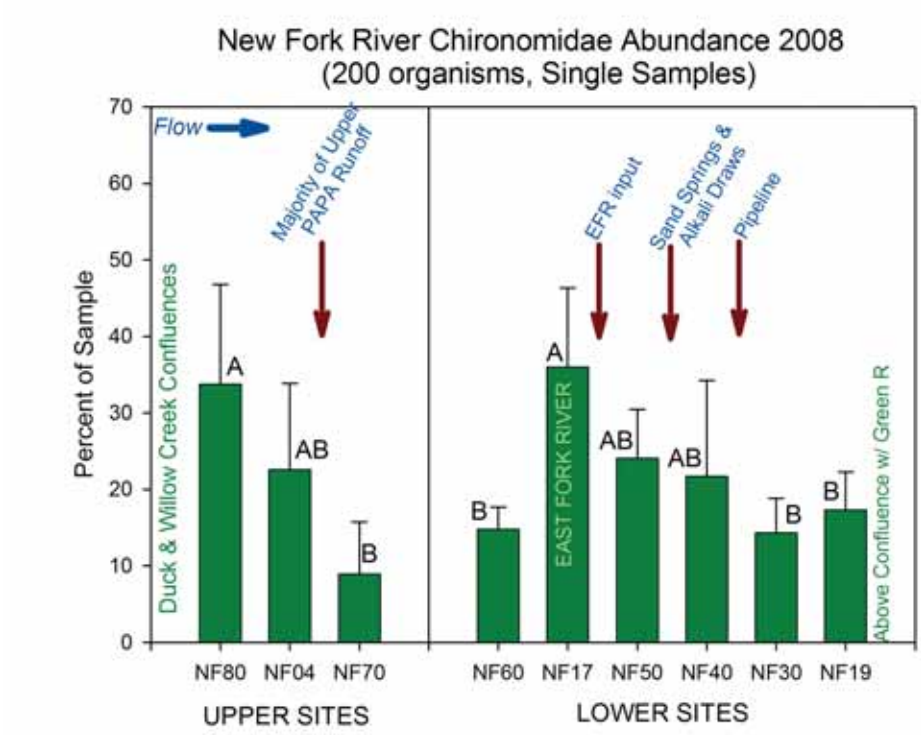

Figure 3.12. Chironomidae Abundance. The abundance of chironomid midges was greatest at NF17 and NF80—both of these sites were significantly different from NF70, NF60, NF30 and NF19. Bars are averages of 8 samples, and error bars represent 95% confidence intervals.

[14] Recall that relative abundance is a percentage measure, and if one group increases dramatically, one or more other measures must decrease (there are only 100 percents!).

Non-Insect Abundance

In 2008, the non-insect invertebrates comprised about 5-30% of benthic assemblages on average. The macroinvertebrate communities of North American streams are typically dominated (>90%) by aquatic insects. The New Fork River has frequently had average values near 10-12%--possibly due to the combination of highly erodible geology and reduced spring runoff discharges (related to drought). The upper study area showed a decrease in the relative abundance of non-insects from NF80 to NF70. This is reflective of a general "improvement" in water quality as the mineral laden waters of Duck and Willow Creeks are diluted by Pine and Pole Creeks.

The lower study area showed a modest increase in non-insect abundance downstream of the irrigation district return waters (NF60). Although the average contribution of non-insects was lower in the East Fork River, these differences were not statistically significant. The only statistically significant difference in non-insect abundance was the dramatic increase at NF30, which was significantly different from every other site.

This response suggests localized impairment of the community composition of the New Fork River near NF30. Likely causes are either hyporheic disturbance from drilling in the active sub-surface river bed, or repeated hyporheic disturbance from pipeline installation (or both).

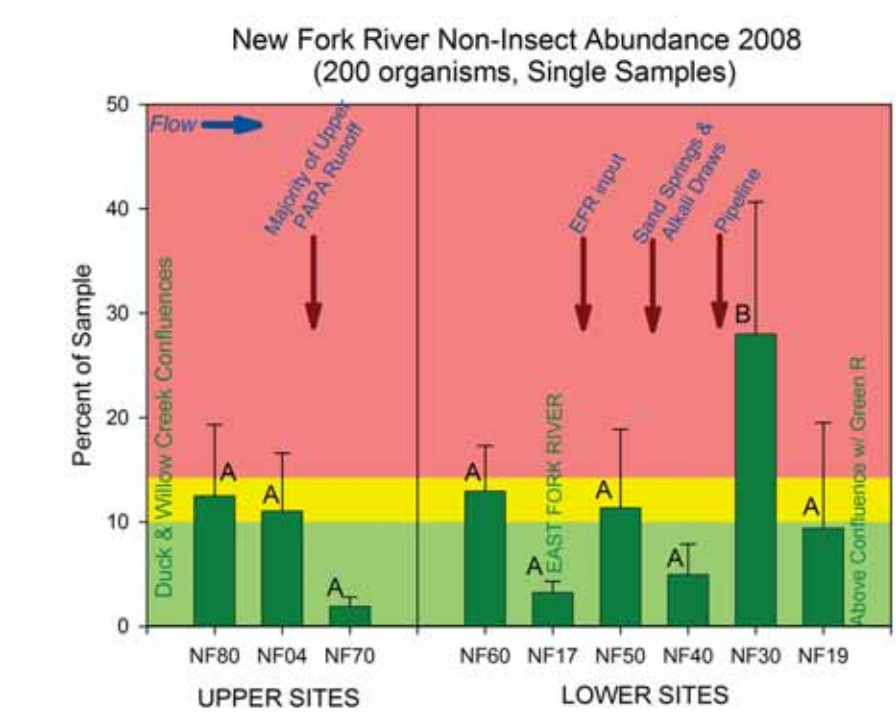

Figure 3.13. Non-Insect Abundance. NF30 had significantly more non-insects than all other sites and was likely due to hyporheic disturbance by pipeline installation or drilling through the active sub-surface stream channel. Green area represents normal fresh water streams in North America. Yellow buffer area allows for the observations of slightly higher than normal values observed in the New Fork Basin. Red are is considered a high percentage of non-insects (the line between red and yellow was subjective). Bars are averages of 8 samples, and error bars represent 95% confidence intervals.

Hilsenhoff Biotic Index

The HBI rates streams by the average organic-pollution-tolerance. A score of 10 occurs only when the entire invertebrate assemblage is extremely tolerant of organic enrichment[15], whereas a score of zero indicates that the entire community is composed of very sensitive organisms. Generally streams of the Wyoming Basin should have values less than about 4.0[16]. The values obtained for the New Fork River were generally below HBI value of 5, except the site right below Duck and Willow Creeks' confluences. The site monitored with best HBI score was NF70, which had HBI values consistent with the best reference sites in the Wyoming Basin Region.

At the lower study area, the HBI was higher in the East Fork River, and remained somewhat elevated in the New Fork River downstream from the East Fork confluence. The difference between NF30 and adjacent sites were not statistically significant. The modest increase in HBI at NF30 was related to non-insects[17] which dominated that site.

Other than a minor (non-statistically significant) increase in HBI at NF30, which was related to the non-insect dominance reported earlier, this metric did not indicate impairment related to PAPA-development.

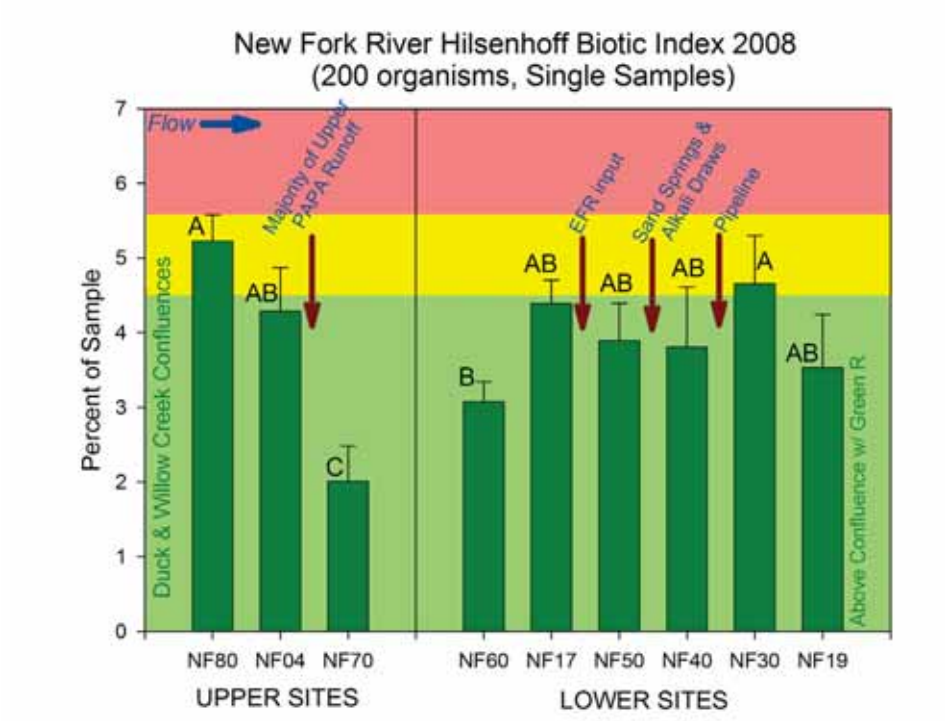

Figure 3.14. Hilsenhoff Biotic Index (HBI). The HBI was near values attained from previous surveys of the New Fork River. High values indicate communities dominated by pollution-tolerant organisms. Bars are averages of 8 samples, and error bars represent 95% confidence intervals.

[15] In reality this would only occur in a pile of sewage sludge or similar enriched, anaerobic environment.

[16] Hargett and ZumBurge (2006) report 95% of reference streams had HBI values greater than 2.7, earlier studies on these sites (Marshall 2006, 2007, 2008) found that New Fork River sites often have HBI values ~4.0.

Collector-Gatherers Contribution

Collector-gatherers are generalists. They usually increase in abundance where organic enrichment co-occurs with sedimentation. The upper study area had the greatest abundance of collector-gatherers (~60%) downstream from Duck and Willow Creeks.

The upper study area showed a substantial decrease in collector-gatherer abundance after the influence of Duck and Willow Creeks were diluted by Pine and Pole Creeks (NF70). NF70 was significantly less dominated by collector-gatherers than the other upstream sites.

Most of the lower study area sites were not significantly different from each other. The exception was NF40, which was significantly different from all other lower sites except the East Fork River (NF17).

The results of this metric did not indicate a significant impairment related to development of the PAPA.

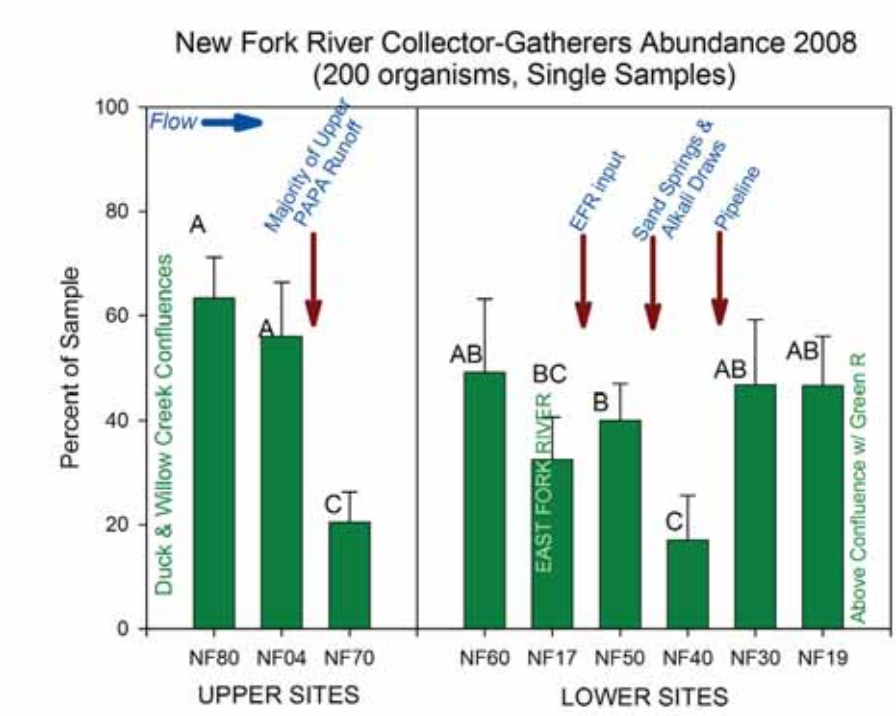

Figure 3.15. Contribution of Collector-Gatherers. Collector-gatherers usually comprised from >50% of New Fork River benthic assemblages. The notable exception was NF80 and NF04, where they comprised more (~60%) on average. Two sites (NF70, NF40) had lower abundance of collector-gatherers (~20%). Bars are averages of 8 samples, and error bars represent 95% confidence intervals.

[17] Non-insects usually have higher HBI tolerance than insects, especially aquatic worms—which comprised most of the non-insects collected.

Collector-Filterers Contribution

Collector-filterers are similar to collector-gatherers, except that they feed on particles suspended in the water's flow. Thus, low abundances of collector-gatherers can occur in faster water were collector-filterers become more abundant. They usually increase in abundance where organic enrichment co-occurs with sedimentation in fast-flowing water.

The upper study area had the greatest abundance of collector-gatherers (about a quarter of the community) downstream from Duck and Willow Creeks. At the downstream end of the upper study area was lowest abundance of collector-gatherers observed in 2008. The only site that was significantly different from the others was NF80, which had significantly more collector-gatherers than all other sites except NF30.

There were no statistically significant differences among the sites of the lower study area. The results highlight the nature of particulate material of the Willow Creek and Duck Creek, but do not indicate any significant impairment of the New Fork River related to development in the PAPA.

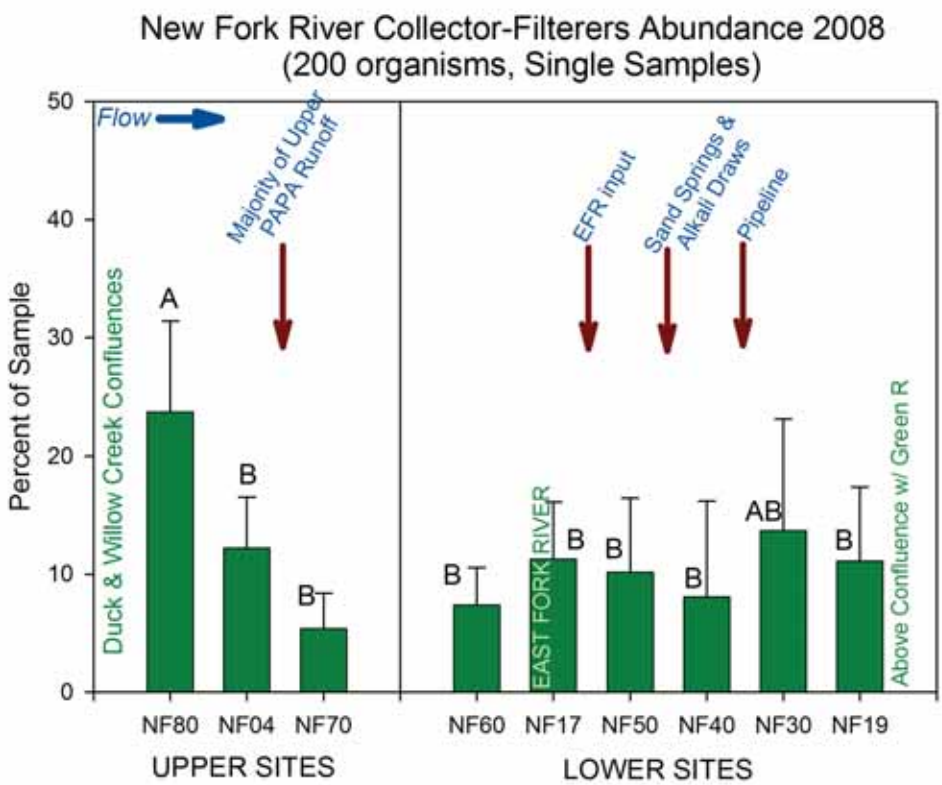

Figure 3.16. Contribution of Collector-Filterers. Collector-filterers usually comprised from 5-15% of New Fork River benthic assemblages. The notable exception was NF80, where they comprised more on average. Bars are averages of 8 samples, and error bars represent 95% confidence intervals.

Collectors (Filterers + Gatherers)

Collector-filterers and collector-gatherers eat the same types of food: fine particles of organic material. The difference is how they attain it. Gatherers usually seek out deposits of organic material from eddies and other slower-flow areas, whereas filterers consume finer organic particles suspended in rapidly flowing water. Thus sometimes the difference between them can be due to flow differences at the sites sampled. We did not expect this to be a problem with this study because flow rates were stratified to be equal at all sites.

When we added the collector-filterers to the collector-gatherer abundance data, the pattern of abundance remained very similar to that observed for collector-gatherers. Also significant differences among sites remained unchanged.

Although the results indicate that collectors comprised about 90% (high) of the NF80 assemblages, they did not reveal any impairment of the New Fork River related to PAPA development.

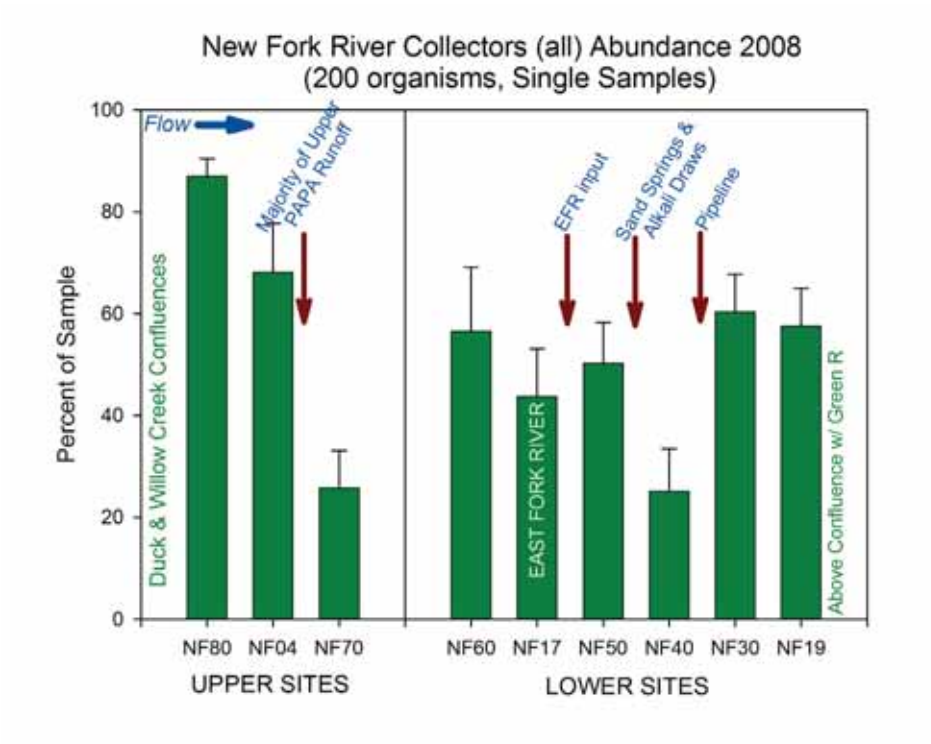

Figure 3.17. Contribution of Collectors (Filterers+Gatherers). Total collectors comprised a very high portion of the assemblages of the upstream site and rather low portions of NF70 and NF40 (low is "good"); the other sites were about ~60%, as expected. Significant differences mirrored those for collector-gatherers.

Scraper Abundance

Scrapers are algae-eating invertebrates. Wyoming Basin streams often have about 30-40% scrapers.[18] We found the greatest proportion of scrapers at NF70 which was significantly different from NF80 and NF04. The five common Functional Feeding Groups[19]are Collector-gatherer, Collector-filterer, Shredder-detritivore, Scraper, and Predator. Because the relative abundance of each is inter-related, the data for all Fuctional Feeding Groups are presented below.

At the lower study area, we found that NF30 was significantly different from adjacent sites because the community was dominated by worms (collector-gatherers).

The results did not reveal any significant alteration of the relative abundance of scrapers related to PAPA development.

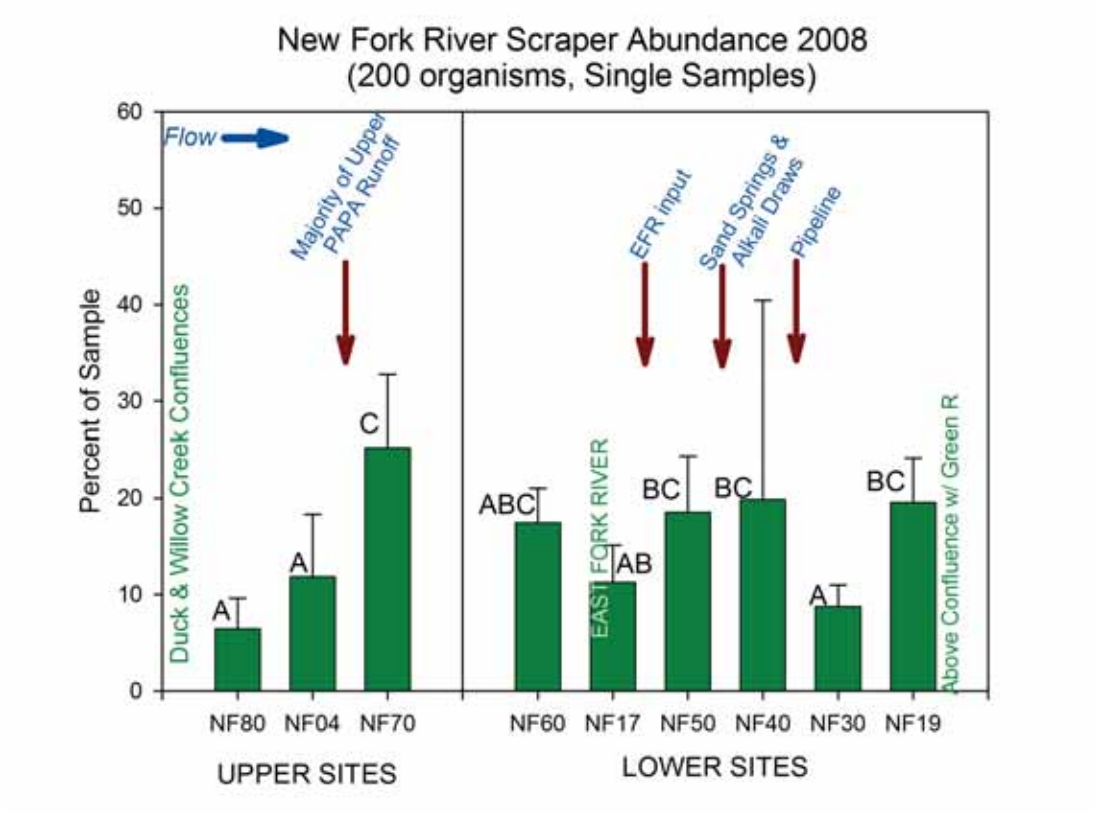

Figure 3.18. Contribution of Scrapers. Scrapers comprised low portions of the community at NF80 and NF30. Bars are averages of 8 samples, and error bars represent 95% confidence intervals.

[18] Hargette and ZumBerge 2006 report that 95% of Wyoming Basin Rivers have less than 38% scrapers. SCCD baseline data suggests that 15-25% is normal for the New Fork River (Marshall 2005).

[19] There are many thousands of species of aquatic invertebrates. Ecologists often group them according to their roles in processing organic material (or "Functional-Feeding Group") to assess functional ecosystem changes.

Other Functional Feeding groups

The River Continuum Concept (Vannote et al. 1980) describes how river community function is expected to change as a function of natural longitudinal gradients. For example, small mountain streams are naturally very different in appearance from large coastal rivers. These apparent differences also reflect the form of sustenance available for invertebrates, resulting in a gradient of changes in species… and in the relative proportion of each of the five major functional feeding groups.

Ecological perturbations can often cause a shift in the relative amount of dominance by different functional feeding groups. We observed several significant differences among the sites in terms of the abundance of some functional feeding groups. Since the relative abundance of one group is related to the others, we decided to review the percent abundance of all five functional groups simultaneously using pie charts (Fig. 3.19). We believe this makes some of the interactions among these groups more clearly than discussing the various combinations of all of these groups separately.

The first thing to note is the new upstream reference site (NF80) and NF04 are entirely dominated by collectors. The other sites all have much lower collector dominance (Fig 3.19). This happens when there is a source of rich fine particulate organic matter entrained to the waterway. The source may be natural or human-caused, but it is not considered to be related to development on the PAPA.

Another important pattern affecting the abundance of other functional groups is the very high proportion of "shredders" from NF70, NF17 and NF40. This result could be misconstrued by some readers to indicate that the river supported large numbers of stoneflies, or other shredder-detritivores. This result is actually a little misleading in that it was strongly influenced by a chironomid midge that is associated with blue-green algae (*Cricotopus* (*Nostococladius*) sp.). This species has a symbiotic relationship with nodules of nitrate fixing bacteria—it is associated with living algae, rather than the allochthonous detritus usually associated with shredders. At NF70, the dominant shredder is Lepidostoma sp.—a traditional "shredder-detritivore," whereas at NF17 and NF40, the dominant shredder is actually a herbivore (Fig. 3.19). This is discussed further under the Synthesis of 2008 Results section of this report (below).

The dramatic increase in collectors at NF30 is reflective of the aquatic worms dominating the assemblages at that site (Figs. 3.18, 3.19). When viewed from a Functional Feeding Group perspective, a similar pattern appeared at the farthest downstream site (NF19). This outcome was expected in earlier reports (Marshall 2007, 2008) as part of the natural abatement of accumulated sediments at NF30. Thus this finding could be part of the early stages of the New Fork River recovery process. The total collector abundance (gatherers and filterers) is similar to the observation at NF30 (Fig 3.18) but the taxa composing the collectors are more sensitive (esp. the mayfly *Rhithrogena*) and those that are sediment tolerant, are more ubiquitous and benign than the worms dominating NF30 (e.g., *Tricorythodes* sp.).

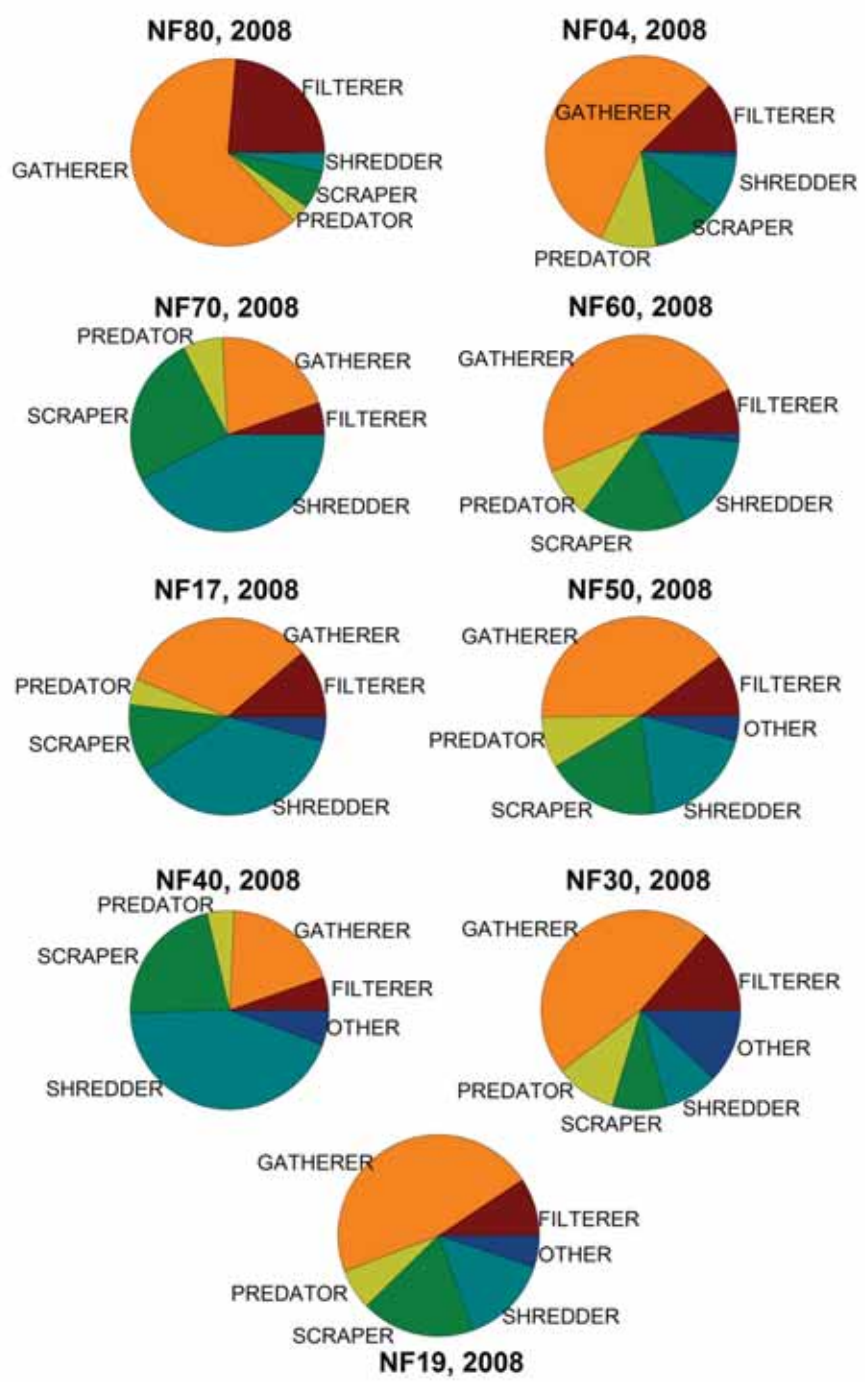

Figure 3.19. Contribution of All Functional Feeding Groups. The relative abundance of the five major Functional Feeding Groups at all of the study sites. When aquatic ecologists discuss "shredders" they usually refer to "shredder-detritivores," which consume course particulate organic material (greater than 1mm). The contract laboratory classified *Cricotopus nostocicola* as "shredder" when they generated data, and this accounted for most the shredders at the locations with high (>20%) shredder abundance (Fig. 3.19). Although not incorrect, readers should note that this finding does not reflect abundance of "typical" shredders which form links to riparian food webs.

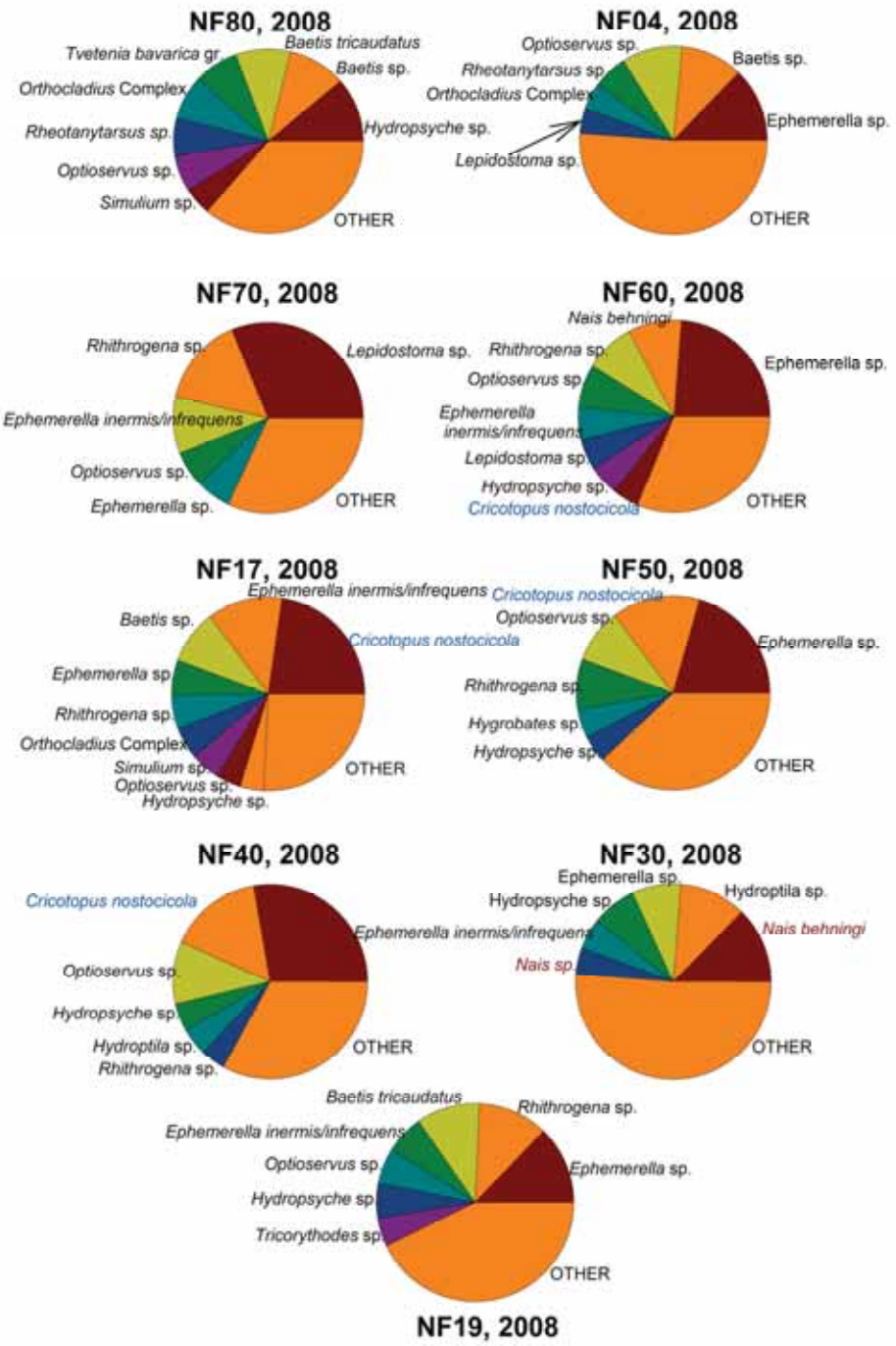

Figure 3.20. Dominant Taxa. The dominance of all species comprising more than 4.0% of each sample are presented. The data are averages of eight samples. The loss of *Cricotopus nostocicola* and its replacement with worms (*Nais*) at NF30, is again evidence of scour by sandy sedimentation, and could be due to PAPA development between NF40 and NF30. The presence of *Rithrogena* sp. at most sites is a good sign because this mayfly is very sensitive to many forms of organic and inorganic chemical pollution; this underscores the local nature of any perturbation around NF30.

Dominance (5 taxa)

Dominance is the measure of how much the dominant species (or group of species) contribute to the entire sample. We used the top 5 taxa, which has been most commonly used in Wyoming and surrounding states. This value should be less than about 80% of the community.[20] High dominance values indicate that a few taxa have disproportionally high abundance, and can be a sign of ecological perturbation.

The upper study area showed NF70 had significantly greater dominance than the other two upper study area sites. This is consistent with the hypothesized response of the upper study area to stressors but it was not significantly different from the values reported at in the previous year (Table 3.2). Since these are new sites, the upper study area is best served by describing year-to-year changes (see discussion).

The lower study area had no significant differences in community dominance (5). Thus the results of this test did not indicate any degradation of New Fork River related to PAPA development. However, while the overall dominance of the five most-abundant taxa does not constitute an impairment of NF30 (or any other site), the species composition of the dominant taxa (fig 3.19) does. Specifically the loss of Nostoc, and Rhythrogena and the addition of worms and hydroptilids as dominant taxa is unusual and undesirable.

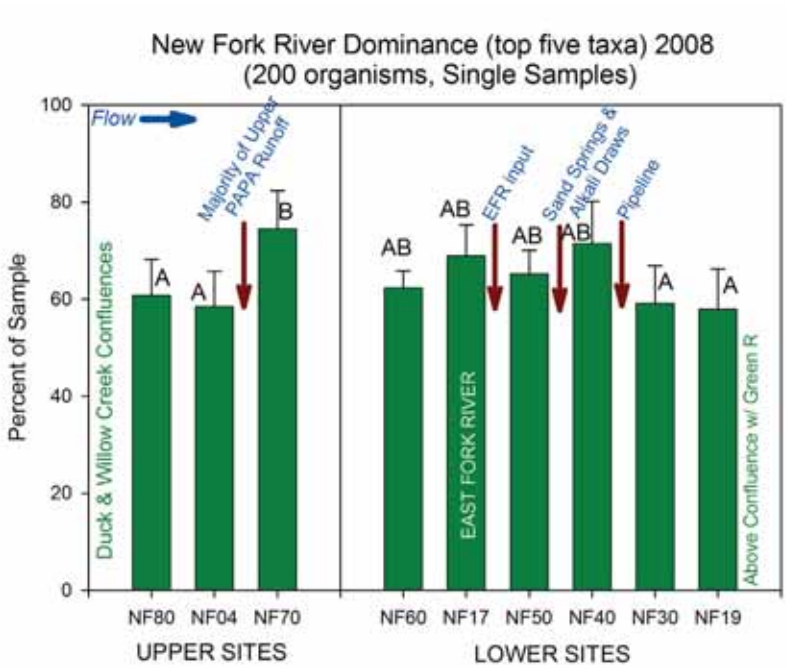

Figure 3.21. Community Dominance (top 5 taxa). The relative abundance of the dominant taxa at each site includes all taxa comprising >4.0% of the assemblage.

[20] Stibling et al. report 95%of reference sites were less than 85%, and that 50% of reference sites had about 60% or greater dominance (5). Hargett and ZumBurge (2006) did not discuss this metric for the Wyoming Basin Region in the new WSII.

Synthesis of 2008 results

Most of the metrics that were significantly different among sites did not actually describe impairment of the New Fork River. We found that there were many localized differences in the invertebrates species assemblages and that many of these changes were statistically significant. This often occurs when there are many overlapping gradients. These were considered on a case-by-case basis through the report (above). Most of the differences we found did not support the hypothesis of PAPA-related impairment of the New Fork River in 2008.

The most notable exception to this was the dominance of aquatic worms below the pipeline crossing at NF30. This has been an ongoing localized problem that is most likely related to repeated disturbance of the river bed for pipeline crossings and by riparian drilling platforms. The multi-year continuity of this shift to a worm-dominated ecosystem may have been exacerbated by a long period of reduced river flows, which have yet to clear the disturbed sediment from the system.

The Blue-green algae *Nostoc* sp. grows as a small dark rubbery nodule on top of stones. It requires a symbiotic midge (*Cricotopus nostococola*) that was collected at all sites. A review of the dominant taxa suggested that it was very abundant at NF50, NF60, NF17, and NF40 (Fig. 3.20). We decided to examine this group more closely to determine if it was excluded by scouring sediments introduced from PAPA related disturbances near NF30 (Fig 3.22). The results are not conclusive. *Nostoc* sp. (and *C. nostococola*) increased dramatically below the return water from the irrigation district and in the East Fork River, maximizing at below the East Fork-New Fork confluence—then dropping off sharply below Alkali and Sand Springs Draws. Since NF30 and NF40 are similar to NF19, and NF70 (and a lesser extent to NF60), it appears to be unrelated to PAPA development[21]. However, if development on the southeastern side of the PAPA has somehow caused an increase in the export of sand through Alkali and Sand Springs Draws, this response could be related to development of the PAPA[22].

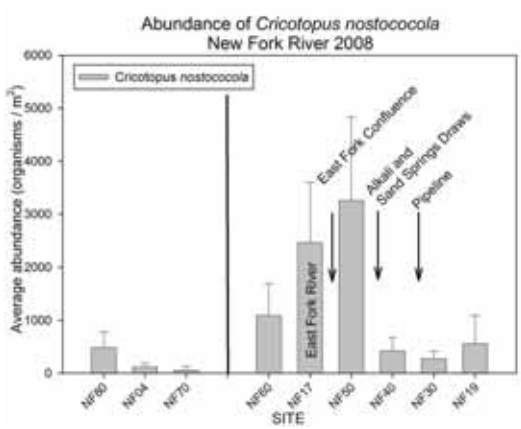

Figure 3.22. Density of *Cricotopus nostocola*. The density of *C. nostococola* is presented as abundance per square meter of stream bottom. Bars are averages of 8 samples, and error bars are 95% Confidence intervals.

[21] Blue green alga, like *Nostoc*, fix nitrogen from elemental sources. This usually means that they occur where nitrate is a limiting factor for plant growth. Thus, there may be a phosphorus source between NF70 and NF60, or there might be nitrate depletion between the sites. There are insufficient data to determine the actual cause and nutrients are not the focus of this study. However, pipeline related scour is unlikely because Nostoc reduction occurred above the pipeline as well.

[22] Recall that NF40 and NF30 did have elevated concentrations of sand (Fig. 3.2).

Two other taxonomic units stood out among the dominant taxa (Fig. 3.19) for specific analysis: *Rhithrogena* (a chemically sensitive mayfly) and Aquatic worms (sediment dwelling). *Rhitrogena* declined below duck and willow creek (it is sensitive to metal ions and high conductivity water[23]) then rebounded after the river water was diluted below Pine and Pole Creeks. When statistical analyses were applied to these data, they failed the test of normality and homogeneity of variance; thus, the Kruskal-Wallace non-parametric ANOVA was used to diagnose differences in the density of *Rhithrogena* using the ranks of individual samples[24]. The contribution of the East Fork River did not change *Rhithrogena* density significantly (NF60 vs. NF50), and other than the sites where *Rhithrogena* was absent, there were no site-specific significant differences in their density (P>0.05).

Aquatic worms comprised about 6,000 organisms per square meter on average at NF30 but individual samples had ~10,000 worms per square meter. Other sites, had averages up to about 3,000[25] worms per square meter. Thus, it appears that the sediment influences on biotic composition of NF30 persisted through 2008 and although some of the changes persisted downstream to NF19 (Fig 3.19), the lower site was not as impaired by sediments because they supported a more typical community (Figs. 3.20, 3.23).

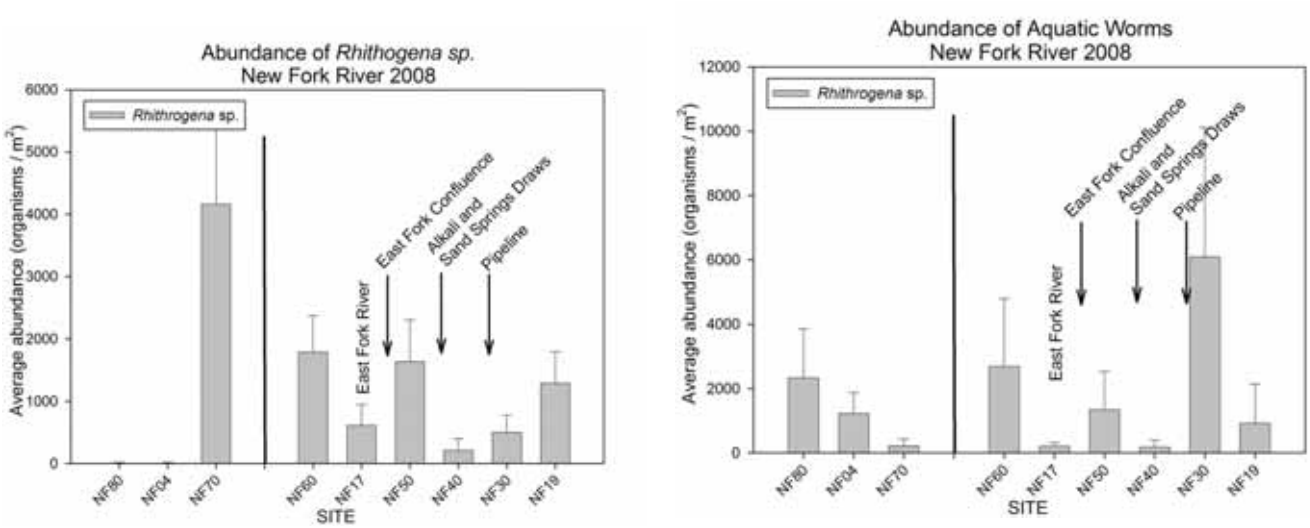

Figure 3.23. Density of some dominant taxa. The density of *Rhithrogena sp.* and aquatic worms (all worm taxa combined) presented as abundance per square meter of stream bottom. Bars are averages of 8 samples, and error bars are 95% Confidence intervals.

[23] There are chemical receptors on the gills that allow *Rhithrogena* to maintain ion balance in low-conductivity water—this causes problems with blood chemistry in higher conductivity waters, such as those entering the New Fork at Willow and Duck Creeks.

[24] The Kruskal Wallace test is tolerant to violations in homogeneity of variance and deviations from the "normal" distribution.

[25] NF80, had 2,200 worms/m^2 and below irrigation return water (NF60) there were 3,000 worms / m^2; most sites had fewer than 1,000 worms per m^2.

The New Upstream Reference NF80

This was the first year we used the NF80 as a reference site. Our findings repeatedly single this site out as "unusual." The influence of inflow from Duck and Willow Creeks significantly and conspicuously altered the composition of invertebrate assemblages of the New Fork River. This was also observed during evaluation of the SCCD baseline dataset and is not suspected to have anything to do with development on the PAPA.

This might bring into question the potential use of this site as a reference for the upper study area. The site is more comparable with other sites than the old reference (NF01), but the influence of tributaries creates an apparent disturbance signature—independent of PAPA development. However, since the upper study area's lower site (NF70) supports assemblages that appear completely recovered from the tributaries' influence we can use the deviation of NF70 from NF80 and NF04—and the changes in this deviation to evaluate the cumulative effects of the PAPA. For example, if NF70's benthic community were to gradually become more similar to NF80, it could be evidence of PAPA related impairment, or it could indicate a decline in the water quality of Pine or Pole Creeks. Regardless of the cause, the relative amount of difference among the sites would be used to evaluate change for the upper study area by comparing changes in similarity of NF70 to NF80. Specifically, when NF70 becomes more similar to NF80, it will indicate an impairment that could be due to development in the upper PAPA or somewhere else near Pinedale.

Biological Metrics: Comparison of 2008 with 2007

A Multivariate Analysis of Variance (MANOVA) was conducted using Particle Index, and near-substrata flows as covariates. Dependent variables for this test were selected to contain the same biological information as the tests used for the within 2008 ANOVA, GLM and ANCOVA tests, while reducing the number of variables to reduce the likelihood of spurious correlations. The dependent variables were TAXA RICHNESS, EPT Richness (combined richness of Ephemeroptera, Plecoptera, and Trichoptera), %CHIRONOMIDAE, %NON_INSECT, WSII-1.

The results indicated that there was a significant SITE effect ($P<0.0001$), a significant YEAR effect ($P<0.0001$) and a significant interaction term ($P<0.001$). These results indicate that the sites were significantly different, the years were significantly different and the response of individual sites over time was significantly different. These kinds of results are notoriously complex to interpret. To simplify interpretation we examined each metric over time, and each site for changes among years (below) and briefly discuss the implications for the effects of PAPA development on the New Fork River. However, readers are cautioned that the exact p-values should be interpreted cautiously because of significant interaction terms. To help with this the site-by-discussion (following pages) of change at each site is evaluated using the regular p-critical value ($P<0.05$), but also a more liberal p-critical to ensure that minor errors do not result in failing to detect ecologically relevant changes—these finding are called "marginally significantly different."

The total number of improving metrics and declining metrics were counted for both $p<0.05$ and $p<0.10$ as an overall index of change between years.

Changes at NF04

Site NF04 was the farthest upstream site that was sampled in both 2007 and 2008. When the scientific convention of a 5% chance of type-1 statistical error is maintained (P<0.05), we found that the abundance of EPT taxa (mayflies, stoneflies, and caddisflies) increased in 2008. This represents an "improvement" in condition that was also reflected in the WSII (which is a weighted average of 10 metrics).

When the type-1 error probability is relaxed slightly (this reduces the likelihood of type-2 statistical error—the probability of failing to detect a real difference), two other indicators suggested (HBI, %Chironomidae) improved ecological conditions at this site.

Thus we have 2- 4 measures indicating that site NF04 has improved conditions from 2007, and no significant indications of ecological decline at NF04 over the year since it was last monitored. This was true even though the particle index indicated there were significant declines in the amount of ideal substrata sampled.

Table 3.2 Change at NF04. The test of significant changes at site NF04 is presented. The Z-test assumed unequal variances for all metrics and sites because Levene's Test indicated that different metrics violated this assumption at different dates. To ensure that all findings use the same comparison criteria, we used the test that did not assume equality of variances for all comparisons. We used the standard scientific criteria to reject the null hypothesis of no change from 2007 to 2008 (i.e, if P<0.05, reject null). Since there were some significant interactions among the responses of sites over time and because we used the slightly more conservative test assuming non-equal variances, we also used the more liberal criteria to reject the null hypothesis (i.e. if P<0.10, reject null). The results from the relaxed rejection criteria (with a greater likelihood of detecting differences) are presented in parentheses. Arrows describe if the 2008 mean increased or decreased since 2007.

NF04	Metric	P-value	Change from 2007 to 2008	Condition Change
Biological Measures	Taxa Richness	0.860	=	n/a
	EPT-Taxa	0.916	=	n/a
	% EPT	0.010	↑	Improved
	%Non-Insect	0.351	=	n/a
	%Chironomidae	0.082	= (⇩)	n/a (impr.)
	%Collectors	0.327	=	n/a
	HBI	0.096	= (⇩)	n/a (impr.)
	Dominance (5)	0.196	=	n/a
Integrative Indices	WSII-1	0.960	=	n/a
	WSII-2	0.077	= (⇧)	n/a (Impr.)
Physical covariates	Flow	0.337	=	n/a
	Embeddedness	0.774	=	n/a
	Particle Index	0.033	↓	Decline

	Significance	
	P < 0.05	P < 0.10
Biological "Improvements" since 2007	1	(4)
Biological "Declines" since 2007	0	(0)

Changes at NF70

Site NF70 was the downstream site of the upper study area. When the scientific convention of a 5% chance of type-1 statistical error is maintained (P<0.05), we found that the abundance of EPT taxa (mayflies, stoneflies, and caddisflies), Midge abundance, and HBI all indicated improved conditions in 2008. This finding was also reflected in the WSII (which is a weighted average of 10 metrics).

When the type-1 error probability is relaxed slightly (this reduces the likelihood of type-2 statistical error—the probability of failing to detect a real difference), the results remained unchanged at this site.

Thus 4 measures indicated that site NF70 has improved ecological conditions from 2007, and no significant indications of ecological decline at the site over the year since it was last monitored. This was true even though the particle index indicated there were significant declines in the amount of ideal substrata sampled.

Table 3.3 Change at NF70. The test of significant changes at site NF70 is presented. The Z-test assumed unequal variances for all metrics and sites because Levene's Test indicated that different metrics violated this assumption at different dates. To ensure that all findings use the same comparison criteria, we used the test that did not assume equality of variances for all comparisons. We used the standard scientific criteria to reject the null hypothesis of no change from 2007 to 2008 (i.e, if P<0.05, reject null). Since there were some significant interactions among the responses of sites over time and because we used the slightly more conservative test assuming non-equal variances, we also used the more liberal criteria to reject the null hypothesis (i.e. if P<0.10, reject null). The results from the relaxed rejection criteria (with a greater likelihood of detecting differences) are presented in parentheses. Arrows describe if the 2008 mean increased or decreased since 2007.

NF70	Metric	P-value	Change from 2007 to 2008	Condition Change
Biological Measures	Taxa Richness	0.108	=	n/a
	EPT-Taxa	0.669	=	n/a
	% EPT	<0.001	↑	Improved
	%Non-Insect	0.678	=	n/a
	%Chironomidae	0.006	↓	Improved
	%Collectors	0.135	=	n/a
	HBI	<0.001	↓	Improved
	Dominance (5)	0.203	=	n/a
Integrative Indices	WSII-1	0.698	=	n/a
	WSII-2	<0.001	↑	Improved
Physical covariates	Flow	0.382	=	n/a
	Embeddedness	0.778	=	n/a
	Particle Index	0.033	↓	Decline

	Significance	
	P < 0.05	P < 0.10
Biological "Improvements" since 2007	4	(4)
Biological "Declines" since 2007	0	(0)

Changes at NF60

Site NF60 was the upstream reference—controlling for the influence of the East Fork River—of the lower study area. When the scientific convention of a 5% chance of type-1 statistical error is maintained (P<0.05), we found that the abundance of EPT taxa (mayflies, stoneflies, and caddisflies), Midge abundance, and HBI all indicated improved conditions in 2008. This finding was also reflected in the WSII (which is a weighted average of 10 metrics). However we also found two metrics (collector abundance, non-insect abundance) that indicated declined ecological conditions over time.

When the type-1 error probability requirement was relaxed slightly (this reduces the likelihood of type-2 statistical error—the probability of failing to detect a real difference), the results remained unchanged at this site.

Thus three measures indicated that site NF70 has improved ecological conditions from 2007, and two measures indicated statistically significant ecological decline at the site over the year since it was last monitored.

Table 3.4 Change at NF60. The test of significant changes at site NF60 is presented. The Z-test assumed unequal variances for all metrics and sites because Levene's Test indicated that different metrics violated this assumption at different dates. To ensure that all findings use the same comparison criteria, we used the test that did not assume equality of variances for all comparisons. We used the standard scientific criteria to reject the null hypothesis of no change from 2007 to 2008 (i.e, if P<0.05, reject null). Since there were some significant interactions among the responses of sites over time and because we used the slightly more conservative test assuming non-equal variances, we also used the more liberal criteria to reject the null hypothesis (i.e. if P<0.10, reject null). The results from the relaxed rejection criteria (with a greater likelihood of detecting differences) are presented in parentheses. Arrows describe if the 2008 mean increased or decreased since 2007.

NF60	Metric	P-value	Change from 2007 to 2008	Condition Change
Biological Measures	Taxa Richness	0.562	=	n/a
	EPT-Taxa	0.144	=	n/a
	% EPT	<0.001	↑	Improved
	%Non-Insect	<0.001	↑	Declined
	%Chironomidae	0.002	↓	Improved
	%Collectors	0.009	↑	Declined
	HBI	<0.001	↓	Improved
	Dominance (5)	0.173	=	n/a
Integrative Indices	WSII-1	0.750	=	n/a
	WSII-2	0.286	=	n/a
Physical covariates	Flow	0.084	=	n/a
	Embeddedness	0.889	=	n/a
	Particle Index	0.168	=	n/a

	Significance	
	P < 0.05	P < 0.10
Biological "Improvements" since 2007	3	(3)
Biological "Declines" since 2007	2	(2)

Changes at NF17

Site NF17 is the East Fork River site. The East Fork is the largest tributary in the study area which exports a large amount of sand to the New Fork River system. Conditions at downstream sites should represent a combination of the conditions at NF17 and NF60. When the scientific convention of a 5% chance of type-1 statistical error is maintained (P<0.05), we found that the abundance of EPT taxa (mayflies, stoneflies, and caddisflies) and HBI indicated improved conditions in 2008. This finding was also reflected in the WSII (which is a weighted average of 10 metrics). However, we also found one metric (collector abundance) that indicated declined ecological conditions since 2007.

When the type-1 error probability requirement was relaxed slightly (this reduces the likelihood of type-2 statistical error—the probability of failing to detect a real difference), another metric, midge abundance (%Chironomidae) was also found to suggest improving ecological conditions since the prior year. This metric also improved in the New Fork River, above the confluence with the East Fork River.

Thus 3-4 measures indicated that site NF70 has improved ecological conditions from 2007, and one measures indicated statistically significant ecological decline at the site over the year since it was last monitored.

Table 3.5 Change at NF17. The test of significant changes at site NF17 is presented. The Z-test assumed unequal variances for all metrics and sites because Levene's Test indicated that different metrics violated this assumption at different dates. To ensure that all findings use the same comparison criteria, we used the test that did not assume equality of variances for all comparisons. We used the standard scientific criteria to reject the null hypothesis of no change from 2007 to 2008 (i.e, if P<0.05, reject null). Since there were some significant interactions among the responses of sites over time and because we used the slightly more conservative test assuming non-equal variances, we also used the more liberal criteria to reject the null hypothesis (i.e. if P<0.10, reject null). The results from the relaxed rejection criteria (with a greater likelihood of detecting differences) are presented in parentheses. Arrows describe if the 2008 mean increased or decreased since 2007.

NF17	Metric	P-value	Change from 2007 to 2008	Condition Change
Biological Measures	Taxa Richness	0.743	=	n/a
	EPT-Taxa	0.668	=	n/a
	% EPT	0.003	↑	Improved
	%Non-Insect	0.387	=	n/a
	%Chironomidae	0.076	= (⇩)	n/a (impr.)
	%Collectors	0.037	↑	Declined
	HBI	<0.001	↓	Improved
	Dominance (5)	0.182	=	n/a
Integrative Indices	WSII-1	0.883	=	n/a
	WSII-2	<0.001	↑	Improved
Physical covariates	Flow	0.768	=	n/a
	Embeddedness	0.599	=	n/a
	Particle Index	0.400	=	n/a

	Significance	
	P < 0.05	P < 0.10
Biological "Improvements" since 2007	3	(4)
Biological "Declines" since 2007	1	(1)

Changes at NF50

Site NF50 is the site downstream of the confluence of the East Fork River.
Conditions should represent a combination of the conditions at NF17 and NF60. When the scientific convention of a 5% chance of type-1 statistical error is maintained (P<0.05), we found that the abundance of EPT taxa (mayflies, stoneflies, and caddisflies), the richness of EPT taxa and the HBI indicated improved conditions in 2008. This finding was also reflected in the WSII (which is a weighted average of 10 metrics). None of the metrics tested indicated declined ecological conditions since 2007.

When the type-1 error probability requirement was relaxed slightly (this reduces the likelihood of type-2 statistical error—the probability of failing to detect a real difference), two other metrics, midge abundance (%Chironomidae) and Community dominance was also found to suggest improving ecological conditions since the prior year. One metric, the abundance of collectors suggested the conditions declined since 2007.

Thus, 4-6 measures indicated that site NF50 has improved ecological conditions from 2007, and 0-1 measures indicated statistically significant ecological decline at the site over the year since it was last monitored.

Table 3.6 Change at NF50. The test of significant changes at site NF17 is presented. The Z-test assumed unequal variances for all metrics and sites because Levene's Test indicated that different metrics violated this assumption at different dates. To ensure that all findings use the same comparison criteria, we used the test that did not assume equality of variances for all comparisons. We used the standard scientific criteria to reject the null hypothesis of no change from 2007 to 2008 (i.e, if P<0.05, reject null). Since there were some significant interactions among the responses of sites over time and because we used the slightly more conservative test assuming non-equal variances, we also used the more liberal criteria to reject the null hypothesis (i.e. if P<0.10, reject null). The results from the relaxed rejection criteria (with a greater likelihood of detecting differences) are presented in parentheses. Arrows describe if the 2008 mean increased or decreased since 2007

NF50	Metric	P-value	Change from 2007 to 2008	Condition Change
Biological Measures	Taxa Richness	0.656	=	n/a
	EPT-Taxa	0.006	↑	Improved
	% EPT	<0.001	↑	Improved
	%Non-Insect	0.195	=	n/a
	%Chironomidae	0.022	↓	Improved
	%Collectors	0.052	= (⇧)	n/a (decl.)
	HBI	<0.001	↓	Improved
	Dominance (5)	0.083	= (⇩)	n/a (impr.)
Integrative Indices	WSII-1	0.574	=	n/a
	WSII-2	0.018	↑	Improved
Physical covariates	Flow	0.424	=	n/a
	Embeddedness	0.749	=	n/a
	Particle Index	0.179	=	n/a

	Significance	
	P < 0.05	P < 0.10
Biological "Improvements" since 2007	5	(6)
Biological "Declines" since 2007	0	(1)

Changes at NF40

Site NF40 is the site downstream of the confluence of ephemerally flowing Sand Springs Draw and Alkai Draw. Conditions should be similar to NF50, except for the influence of these two draws—which may convey sediment from natural sources, PAPA development, and other development in the upper watershed. When the scientific convention of a 5% chance of type-1 statistical error is maintained (P<0.05), we found that the abundance of EPT taxa (mayflies, stoneflies, and caddisflies) and the HBI indicated improved conditions in 2008. This finding was also reflected in the WSII (which is a weighted average of 10 metrics). None of the metrics tested indicated declined ecological conditions since 2007.

When the type-1 error probability requirement was relaxed slightly (this reduces the likelihood of type-2 statistical error—the probability of failing to detect a real difference), another metric, midge abundance (%Chironomidae) was also found to suggest improving ecological conditions since the prior year. No metrics indicated declining conditions when critical p-values requirements were relaxed.

Thus, 3-4 measures indicated that site NF50 has improved ecological conditions from 2007, and no metrics indicated statistically significant ecological decline at the site over the year since it was last monitored.

Table 3.7 Change at NF40. The test of significant changes at site NF40 is presented. The Z-test assumed unequal variances for all metrics and sites because Levene's Test indicated that different metrics violated this assumption at different dates. To ensure that all findings use the same comparison criteria, we used the test that did not assume equality of variances for all comparisons. We used the standard scientific criteria to reject the null hypothesis of no change from 2007 to 2008 (i.e, if P<0.05, reject null). Since there were some significant interactions among the responses of sites over time and because we used the slightly more conservative test assuming non-equal variances, we also used the more liberal criteria to reject the null hypothesis (i.e. if P<0.10, reject null). The results from the relaxed rejection criteria (with a greater likelihood of detecting differences) are presented in parentheses. Arrows describe if the 2008 mean increased or decreased since 2007.

NF40	Metric	P-value	Change from 2007 to 2008	Condition Change
Biological Measures	Taxa Richness	0.868	=	n/a
	EPT-Taxa	0.221	=	n/a
	% EPT	0.242	=	n/a
	%Non-Insect	0.544	=	n/a
	%Chironomidae	0.071	= (⇩)	n/a (impr.)
	%Collectors	0.950	=	n/a
	HBI	0.001	↓	Improved
	Dominance (5)	0.695	=	n/a
Integrative Indices	WSII-1	0.849	=	n/a
	WSII-2	0.002	↑	Improved
Physical covariates	Flow	0.119	=	n/a
	Embeddedness	0.281	=	n/a
	Particle Index	0.958	=	n/a

	Significance	
	P < 0.05	P < 0.10
Biological "Improvements" since 2007	2	(3)
Biological "Declines" since 2007	0	(0)

Changes at NF30

Site NF30 is the site downstream of pipeline crossings. Conditions should be similar to NF40, except for the influence of pipeline crossings—which has been implicated with degraded ecological conditions at this site for several years in the past (Marshall 2008). When the scientific convention of a 5% chance of type-1 statistical error is maintained (P<0.05), we found that Total Taxa Richness, abundance of EPT taxa (mayflies, stoneflies, and caddisflies) and the HBI indicated improved conditions in 2008. This finding was also reflected in the WSII (which is a weighted average of 10 metrics). None of the metrics tested indicated declined ecological conditions since 2007.

When the type-1 error probability requirement was relaxed slightly (this reduces the likelihood of type-2 statistical error—the probability of failing to detect a real difference), two metrics, non-insect abundance and total collectors indicated significantly declining ecological conditions since the prior year. One additional metric suggested improving conditions with the more liberal testing constraints: EPT taxa richness.

Thus, 4-5 measures indicated that site NF30 has improved ecological conditions from 2007, and 0-2 metrics indicated statistically significant ecological decline at the site over the year since it was last monitored. One covariate (embeddedness) measured also indicated that there was a significant decline in condition in 2008.

Table 3.7 Change at NF30. The test of significant changes at site NF30 is presented. The Z-test assumed unequal variances for all metrics and sites because Levene's Test for variance homogeneity indicated that different metrics violated this assumption at different dates. To ensure that all findings use the same comparison criteria, we used the test that did not assume equality of variances for all comparisons. We used the standard scientific criteria to reject the null hypothesis of no change from 2007 to 2008 (i.e, if P<0.05, reject null). Since there were some significant interactions among the responses of sites over time and because we used the slightly more conservative test assuming non-equal variances, we also used the more liberal criteria to reject the null hypothesis (i.e. if P<0.10, reject null). The results from the relaxed rejection criteria (with a greater likelihood of detecting differences) are presented in parentheses. Arrows describe if the 2008 mean increased or decreased since 2007.

NF30	Metric	P-value	Change from 2007 to 2008	Condition Change
Biological Measures	Taxa Richness	0.008	↑	Improved
	EPT-Taxa	0.077	= (⇧)	n/a (impr.)
	% EPT	0.116	=	n/a
	%Non-Insect	0.100	= (⇧)	n/a (decl.)
	%Chironomidae	0.266	=	n/a
	%Collectors	0.056	= (⇧)	n/a (decl.)
	HBI	0.018	↓	Improved
	Dominance (5)	0.113	=	n/a
Integrative Indices	WSII-1	0.903	=	n/a
	WSII-2	0.077	= (⇧)	n/a (impr.)
Physical covariates	Flow	0.138	=	n/a
	Embeddedness	0.059	= (⇩)	n/a (decl.)
	Particle Index	0.830	=	n/a

	Significance	
	P < 0.05	P < 0.10
Biological "Improvements" since 2007	2	(4)
Biological "Declines" since 2007	0	(2)

Changes at NF19

Site NF19 is farthest downstream site, a short distance before the New Fork River contributes its flow to the Green River. This site describes the condition of the New Fork River before it influences the Green River. When the scientific convention of a 5% chance of type-1 statistical error is maintained (P<0.05), we found that Total Taxa Richness, abundance of EPT taxa (mayflies, stoneflies, and caddisflies) and the HBI indicated improved conditions in 2008. This finding was also reflected in the WSII (which is a weighted average of 10 metrics). None of the metrics tested indicated declined ecological conditions since 2007.

When the type-1 error probability requirement was relaxed slightly (this reduces the likelihood of type-2 statistical error—the probability of failing to detect a real difference), two metrics, non-insect abundance and total collectors indicated significantly declining ecological conditions since the prior year. One additional metric suggested improving conditions with the more liberal testing constraints: EPT taxa richness.

Thus, 1 measure indicated that site NF19 has improved ecological conditions from 2007, and 2-3 metrics indicated statistically significant ecological decline at the site over the year since it was last monitored. One covariate (embeddedness) measured also indicated that there was a significant decline in condition in 2008.

Table 3.8 Change at NF19. The test of significant changes at site NF19 is presented. The Z-test assumed unequal variances for all metrics and sites because Levene's Test indicated that different metrics violated this assumption at different dates. To ensure that all findings use the same comparison criteria, we used the test that did not assume equality of variances for all comparisons. We used the standard scientific criteria to reject the null hypothesis of no change from 2007 to 2008 (i.e, if P<0.05, reject null). Since there were some significant interactions among the responses of sites over time and because we used the slightly more conservative test assuming non-equal variances, we also used the more liberal criteria to reject the null hypothesis (i.e. if P<0.10, reject null). The results from the relaxed rejection criteria (with a greater likelihood of detecting differences) are presented in parentheses. Arrows describe if the 2008 mean increased or decreased since 2007.

NF19	Metric	P-value	Change from 2007 to 2008	Condition Change
Biological Measures	Taxa Richness	0.135	=	n/a
	EPT-Taxa	0.499	=	n/a
	% EPT	0.803	=	n/a
	%Non-Insect	0.534	=	n/a
	%Chironomidae	0.080	= (⇧)	n/a (decl.)
	%Collectors	<0.001	↑	Declined
	HBI	0.007	↓	Improved
	Dominance (5)	0.264	=	n/a
Integrative Indices	WSII-1	0.019	↓	Declined
	WSII-2	0.240	=	n/a
Physical covariates	Flow	0.601	=	n/a
	Embeddedness	0.374	=	n/a
	Particle Index	0.021	=	n/a

	Significance	
	P < 0.05	P < 0.10
Biological "Improvements" since 2007	1	(1)
Biological "Declines" since 2007	2	(3)

Summary of New Fork River Change (2007-2008)

The results of the 2008 survey indicate that most of the sites sampled in both 2007 and 2008 were generally improved in 2008. This included Site NF30 but did not include NF19, the farthest downstream site (Fig 3.24). Most sites had multiple indications of improvement in 2008. This is likely because of improving climatic conditions, such as an increased snow-melt discharge (Fig. 1.1).

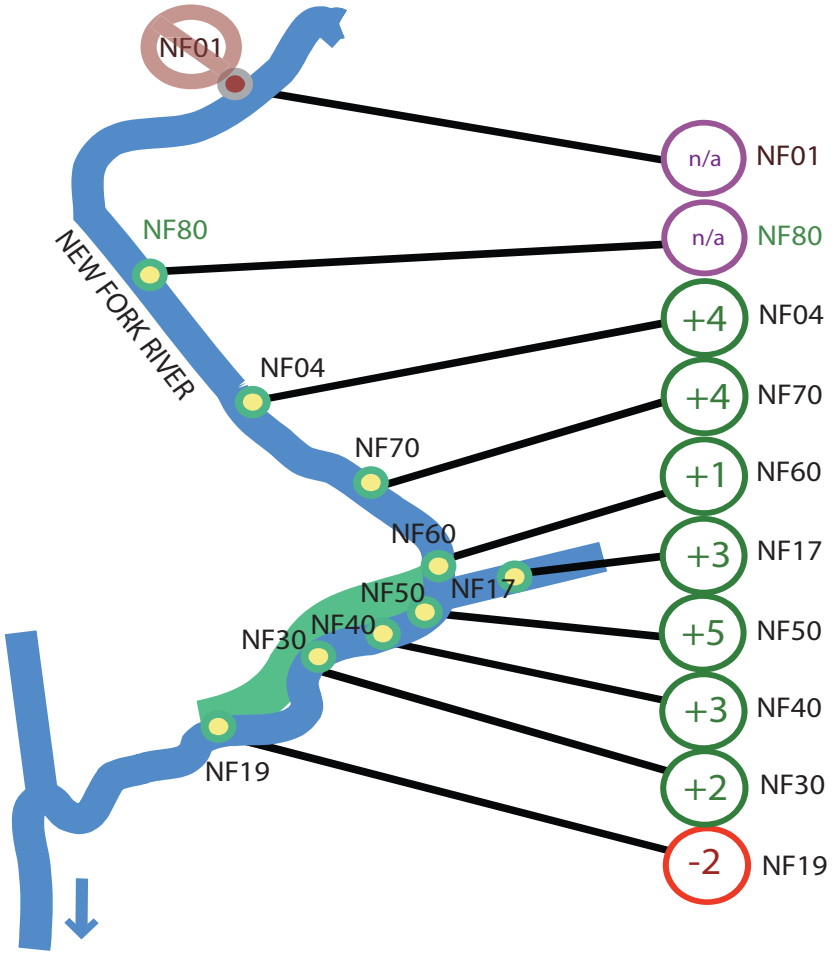

Figure 3.24. Schematic of Sites and Net Changes among Sites. We used the more liberal p-values (P< 0.10) and subtracted the number of measures that indicated declining conditions from those indicating improving conditions. This resulted in a net change score. Positive numbers indicate that more metrics indicated improved conditions in 2008, where as negative numbers indicate that more metrics indicated a decline in conditions in 2008.

Discussion

Most of the metrics examined in 2008 did not indicate any impairment of the New Fork River that could be attributed to development on the PAPA. The notable exception was the same metric that has been indicating impairments in previous years: the relative abundance of non-insects to the assemblages. Specifically, the sediment-tolerant worms reached very high abundances (6,000-10,000/m^2) and were correlated with the accumulations of sand among substrata.

Our analysis of dominant species and functional feeding groups suggested that sediment tolerant collectors are also becoming more dominant farther downstream near the confluence with the Green River. However, more sensitive species also occurred and non-insects were not dominant at NF19. This was the part of the recovery response described in the earlier reports (Marshall 2006, 2007) as the expected results when sediments were exported from NF30 downstream. This is expected for recovery because NF30 cannot recover from excessive sediment additions until these sediments are exported. However, the recovery requires that the additions of sediments between NF30 and NF40 are reduced or halted. If this does not occur, then this could be the early signs of reach-wide, long-term sediment impairment of the New Fork and Green Rivers.

The comparison of 2008 and 2007 surveys indicated that most sites showed an improvement in condition in 2008. This included NF30 but did not include NF19. There are two important implications from this analysis. First, it corroborates the changing condition of NF19 discussed in the previous paragraph. Second, it indicates that there was—other than NF19—a regional improvement to the condition of the New Fork River that persisted even though some of the covariates had a contrary change ("declined condition"). The most likely reason for this is the increase in spring runoff in 2008 relative to 2007 (Fig. 1.1). Although not sustained, the snow-melt flows were greater in 2008 than in the previous five years. Benthic communities are especially dependant on regular seasonal scouring; it maintains high levels of production needed to support a healthy food chain and fishery.

We expect that 2009's report will document improvement of the condition of the New Fork River, because 2009 had the first above average sustained snow-melt discharge since the year 2000 (Fig. 3.25). Still, it might be worth the PAWTG's time to discuss ways of minimizing the impacts of pipeline construction and ways to review erosion control strategies.

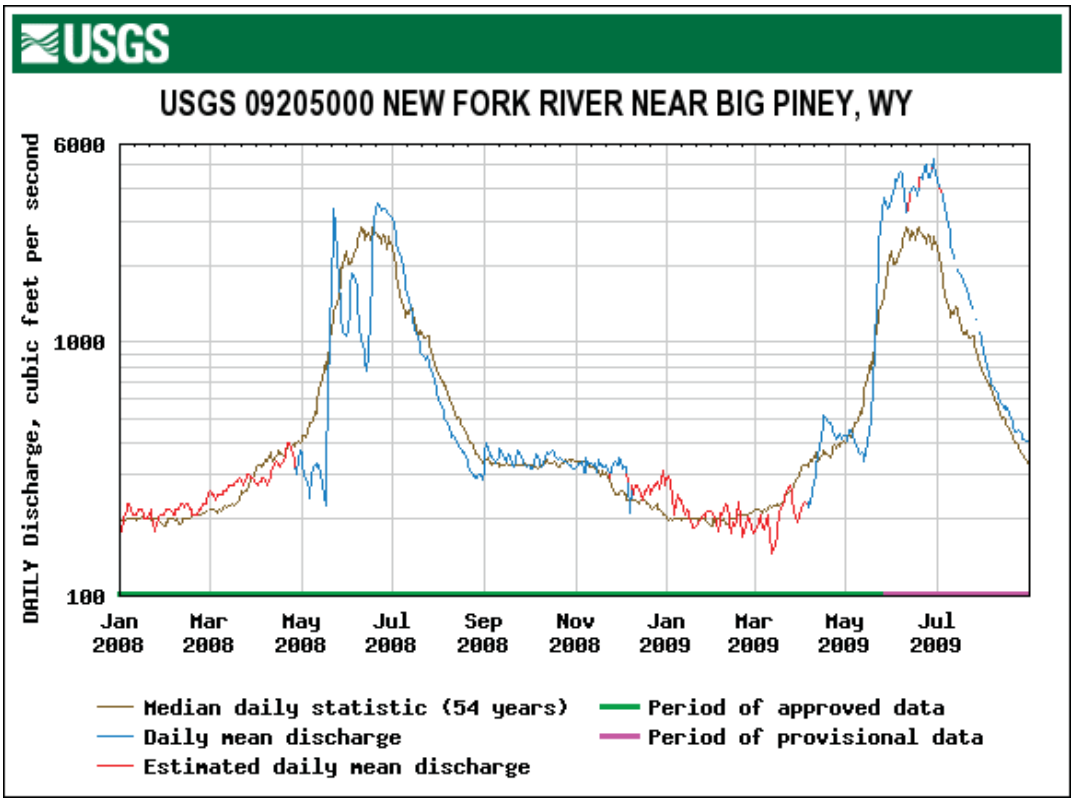

FIGURE 3.25. 2008-2009 New Fork River Discharge. The New Fork River normally exhibits a sustained high flow of nearly 2600-2900cfs for about 30 days. The river sustained 4000 cfs for a normal more than 30 days 2009. This should improve conditions for the biota described in next year's report.

Literature Cited

Hargett, E.G. and J, R. ZumBerge. 2006. Redevelopment of the Wyoming Stream Integrity Index (WSII) for Assessing the biological condition of wadable streams in Wyoming. Wyoming Department of Environmental Quality, Cheyenne WY.

Hart D. D. and C. M. Finelli. 1999. Physical-biological coupling in streams: The pervasive effects of flow on benthic organisms. Annual Review of Ecology and Systematics. 30: 363-395.

Fonseca, D. M. and D.D. Hart. 1996. Density-dependent dispersal of black fly neonates is mediated by flow. OIKOS 75(1):49-58.

Marshall, B.D. 2005a. An Assessment of the Biological Condition of the New Fork River, near the Pinedale Anticline Project Area, Sublette County, Wyoming: 2004 Survey Results. Prepared for Sublette County Conservation District

Marshall, B.D. 2005b. An Assessment of Chemical and Biological Trends in the New Fork River Basin, Sublette County, WY: December 2005 Survey Results. Prepared for Sublette County Conservation District.

Marshall, B.D. 2006. An Assessment of the Biological Condition of the New Fork River, near the Pinedale Anticline Project Area, Sublette County, Wyoming: 2005 Survey Results. Prepared for Sublette County Conservation District and the Pinedale Anticline Water Task Group, Pinedale Wyoming.

Marshall, B.D. 2007. An Assessment of the Biological Condition of the New Fork River, near the Pinedale Anticline Project Area, Sublette County, Wyoming: 2006 Survey Results. Prepared for Sublette County Conservation District and the Pinedale Anticline Water Task Group, Pinedale Wyoming.

Marshall, B.D. 2008. An Assessment of the Biological Condition of the New Fork River, near the Pinedale Anticline Project Area: 2007. Prepared for Sublette County Conservation District and the Pinedale Anticline Water Task Group, Pinedale Wyoming.

McCune, B., and M. J. Mefford. 1995. Multivariate analysis on the PC-ORD system. Version 2.0. MjM Software, Gleneden Beach, Oregon, USA.

Stribling, J.B., B. K. Jessup. J. Gerritsen. 2000. Development of biological and physical habitat criteria for Wyoming streams and their use in the TMDL process. Prepared for U.S. Environmental Protection Agency, Region VIII, Denver Co.

ter Braak, C. J. F. 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. Ecology 67:1167-1179.

ter Braak. 1994. Canonical community ordination. Part I: Basic theory and linear methods. Ecoscience 1:127-140.

WYDEQ.2008b. WYOMING'S METHOD FOR DETERMINING WATER QUALITY CONDITION OF SURFACE WATERS AND TMDL PRIORITIZATION CRITERIA FOR 303(d) LISTED WATERS. Wyoming Department of Environmental Quality, Water Quality Division, Watershed Section. http://deq.state.wy.us/wqd/watershed/Downloads/305b/Final%20Methodology.pdf

Zar, J. H. 1999. Biostatisitcal Analysis (4th edition). Prentice Hall Pub. Upper Saddle River, NJ 07458.

ZumBerge, J. 2004. Personal contact. Wyoming Department of Environmental Quality, Sheridan, WY. An MS Excel file of bioassessment criteria, modifying the WSII for all ecoregions.