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Assessment of the Biological Condition of the New Fork River, in the Vicinity of the Pinedale Anticline Project Area: 2009

An Assessment of Changes among Benthic Macroinvertebrate Assemblages

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Executive Summary

This report follows up ten years of evaluation of the ecological condition of the New Fork River within the PAPA using aquatic invertebrate assemblages. In previous years, we have identified some spatially-localized alterations of community structure which may be related to natural gas development in the Pinedale Anticline Project Area (PAPA). Ultimately, all the ecological descriptors indicating significant changes all reflected one underling community-level response: a dramatic and sustained elevation of the relative abundance (and by extension, relative importance) of the oligochate worms of the genus *Nais*. The effect was primarily localized to one of the nine study sites, below a natural gas pipeline, and existed from 2004-2008. This report details the findings from the 2009 field survey.

Nais are usually associated with organic deposits in fine sediments and changes in their abundance can be related to changes in the quantity or quality of fine sediment deposits. The results of past surveys indicated that there were active sources of erosion between NF30 and NF40 and that these were significantly correlated with changes in the abundance of macroinvertebrate fauna. Additionally, there were three factors that indicated that development of the PAPA may have been related to these changes. First, the pipeline capacity was increased annually by adding additional pipes across the New Fork River. Although the additional pipelines were bored beneath the apparent stream bed, the process likely resulted in localized disturbance of the hyporheic zone. Second, during the study period there was an increase in the development of drilling platforms in riparian areas; these platforms are also likely to disturb hyporheic sediments and organisms. Finally, a third source of sediment may have occurred from direct surface erosion from around platforms; in 2008, most of the erosion controls implemented early in PAPA development had failed, and some new sites did not include erosion control measures at all.

2009 was the first year in nearly a decade to experience a "normal" sustained high flow from mountainous snow-melt. Spring run-off is an important natural phenomenon for western rivers and typically results in the sorting and redistribution of organic and inorganic substrata. Nine years of below normal flows may have prevented natural processes from mediating the effects of development in the PAPA. Thus, we anticipated that the results from the 2009 field survey would show a reduction in the abundance of *Nais* among sites and a net decrease in the number of significant differences in related ecological indicators.

The 2009 field survey described by this report collected eight 1-sq. ft. samples from each of eight sites on the New Fork River and one 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 methods were also used in 2007 and 2008. Since we now have three years of replicated sampling from nine sites, we report the statistical assessment of trends graphically rather than in tabular form used in the 2008 report.

There was a significant reduction in the abundance of *Nais* at all sites in 2009. Most dramatic reductions occurred at the site that historically had the greatest abundance of non-insects (mostly *Nais*), NF30--below the pipeline crossing. *Nais* abundance fell from about 30-50% of the invertebrates sampled to about 5%, which is considered about normal for flowing North American freshwater ecosystems.

The results of the 2009 survey document the recovery of the New Fork River from multiple years of drought that may have exacerbated some of the effects from development in the PAPA. Although the changes exhibited by biota over recent years appear to have been mediated by a single season of high spring run off, it is important to remember that prolonged and more extensive development, especially in the riparian zone, may cause

significant alterations to the structure and function of aquatic food webs, which may affect the long term beneficial uses of the New Fork River.

The examination of multi-year trends shows that every site except the East Fork River has had a net improvement in conditions in 2009 (positive metrics minus impaired-type changes in metrics). NF17 had 2 positive metrics (% scraper, and HBI[1]) and 2 negative metrics (% collectors and % dominance). All other sites appeared to have improving water quality in 2009. The Hilsenhoff Biotic Index (HBI) improved at every site in 2009.

The single most important finding of this report is that non-insect dominance near the pipeline crossing is less of a concern that it ever has been in 5 years.

STATEMENT OF PURPOSE

After the 2009 Task Group meeting, it became clear to me that, for some people, I had not made sufficiently clear the purpose of this study. Many federal and state agencies engage in habitat assessment and their familiarity with certain terms caused some confusion. Perhaps, the most confusing thing for some readers is that some of the terms are similar to terms used in geomorphology (cobble, substrata size, and sedimentation). It is not the purpose of this biological survey to serve as a geomorphology or hydrology survey, nor a basic habitat assessment. This survey's purpose has always been to document the river's ecology in such a way that the cumulative effect of both physical and chemical stressors could be documented in a timely manner; measurements are made a scale appropriate for the response variables (aspects of macroinvertebrate community structure), whereas for hydrology surveys, direct measurements of the physical environment are in and of themselves response variables measured at the scale appropriate for larger scale assessments (spatial and temporal).

Apparently some readers had assumed that the purpose of our ecological survey was to replace a geomorphology study by using macroinvertebrates as a surrogate measure for habitat assessment. Although I do not know how widespread this misconception has become, I concur with those readers that the approach would make little sense. Therefore I want to reiterate the purpose very clearly here: *the purpose of this ecological survey is to document the composition of macroinvertebrate assemblages of the New Fork River in such a way that potential ecological perturbations can be assessed with some insight of cause and effect.*

It just so happens that in recent years, localized patchy build up of fine sediment, presumably entrained from hyporheic disturbance, has been the cause of shifts in the composition of macroinvertebrate communities of the lower study area. Thus recent reports have had to discuss the implications.

Schedule for 2010 report

We have already received the invertebrate samples collected for 2010. We will be able to complete the lab work on these samples several months earlier than we did this year. The goal is to complete the report by the first week of September 2011.

[1] The Hilsonhoff Biotic Index (Hilsenhoff 1987) is a well known index providing a weighted average of the mean pollution tolerance of invertebrates in aquatic ecosystems. Maximum values (10) occur only in communities composed entirely of very tolerant organisms (think: sewage sludge), whereas minimum values (0) occur in places where all organisms are very sensitive to pollution (see report text).

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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 (SCCD) and their stakeholders so I usually used the plural pronoun, "we" to acknowledge their contributions and insights to the project.

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 this 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 is to characterize the biological condition of the New Fork River, Sublette County, Wyoming, and to assess impacts related to natural gas development on the New Fork River. This monitoring program has been active for a decade and incorporates the Sublette County Conservation District's baseline biological monitoring of the New Fork River. Although the focus is on 2007-2009, this report draws upon ten years of biological data (2000-2009) 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.

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

Purpose

After the 2009 Task Group meeting, it became clear to me that, for some people, I had not made sufficiently clear the purpose of this study. Many federal and state agencies engage in habitat assessment and their familiarity with certain terms caused some confusion. Perhaps, the most confusing thing for some readers is that some of the terms are similar to terms used in geomorphology (cobble, substrata size, and sedimentation). It is not the purpose of this biological survey to serve as a geomorphology or hydrology survey, nor a basic habitat assessment. This survey's purpose has always been to document the river's ecology in such a way that the cumulative effect of both physical and chemical stressors could be documented in a timely manner; measurements are made a scale appropriate for the response variables (aspects of macroinvertebrate community structure), whereas for hydrology surveys, direct measurements of the physical environment are in and of themselves response variables measured at the scale appropriate for larger scale assessments (spatial and temporal).

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habitat assessment. Although I do not know how widespread this misconception has become, I concur with those readers that the approach would make little sense. Therefore I want to reiterate the purpose very clearly here: *the purpose of this ecological survey is to document the composition of macroinvertebrate assemblages of the New Fork River in such a way that potential ecological perturbations can be assessed with some insight of cause and effect.*

It just so happens that in recent years, localized patchy build up of fine sediment, presumably entrained from hyporheic disturbance, has been the cause of shifts in the composition of macroinvertebrate communities of the lower study area. Thus recent reports have had to discuss the implications.

1.1 Biological Monitoring Background

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

Invertebrates are the most commonly used animal assemblage[2] 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. The monitoring program does not use data from bacteria (they are micro-invertebrates) or fish (they are macro-vertebrates). These groups can also be used for biological monitoring, but their spatial temporal scales of response are not appropriate for the local scale of this project and would not allow impacts to be located.

Invertebrates are incredibly diverse and abundant. They are also critically important because they play critical roles in detrital food webs—including the breaking down of complex organic material—and in transferring energy to higher trophic[3] 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.

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

1.2 Review Previous PAPA Surface Water Biological Monitoring Results

The most significant findings of previous reports indicated that the New Fork River was experiencing a significant active erosion source below the pipeline crossing between NF40 and NF30 (Marshall 2008). The macroinvertebrate assemblages at the site downstream from the pipeline were dominated by sediment tolerant organisms—mostly midges and worms (*Nais*). Mayflies and stoneflies were not as common at the site as they should have been[4].

A tour of riverside PAPA development completed after the report (Marshall 2008) indicated that many of the erosion controls implemented at drilling sites had failed over time and new drilling platforms often lacked erosion controls altogether. Additionally, several drilling platforms had been built in the floodplain, over hyporheic stream channels where they are likely to induce sedimentation of the New Fork River through sub-surface flows. These findings were not in the report, but were presented to the PAPA task group in September 2008 and again in October 2009. Some Water Task Group members (WTG) were incredulous that we had this discussion, but they may have forgotten that there is about a 12-month delay between data collection and final analysis with these reports.

Although the site tour indicated that operators were not doing all they could to prevent sedimentation in the New Fork River, it had been unclear how much 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 until the year considered in this report (Fig. 1.1).

This is important for several reasons. A high sustained runoff flow is critical to purge sediments entering 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.

Note 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).

In this report we document changes in benthic macroinvertebrate assemblages during the first year a normal sustained high discharge event occurred.

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

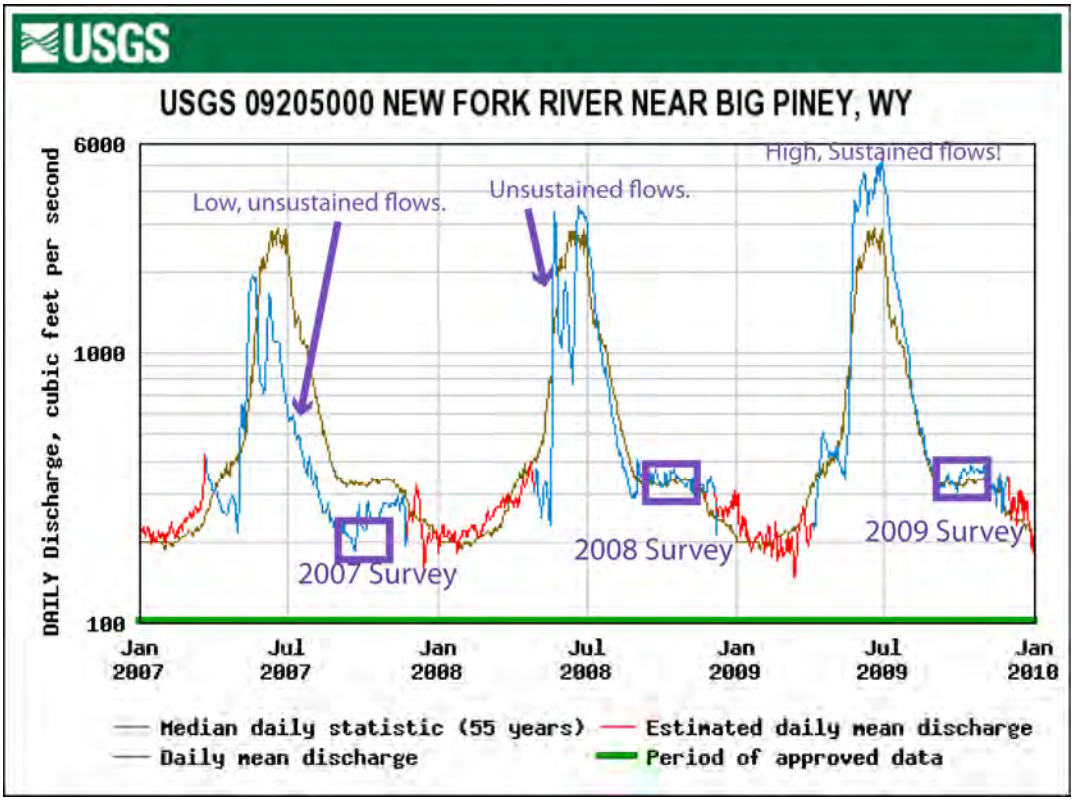

FIGURE 1.1. New Fork River Discharge 2007-2009. The New Fork River normally exhibits a sustained high flow of nearly 2600-2900cfs for about 30 days. The river sustained only 1900cfs for 1 week in 2007. 2009 was the first year in the decade to exhibit both high and sustained spring run off.

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 2009 field sampling followed the regimen of 2008 by replacing site NF01 with a new upstream reference (NF80) because NF01 was too different (naturally) to serve as an adequate reference[5].

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.

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 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. However, its continued status as a down stream reference could be compromised if gas development increases in the area.

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

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

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 (no chemical data available).

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

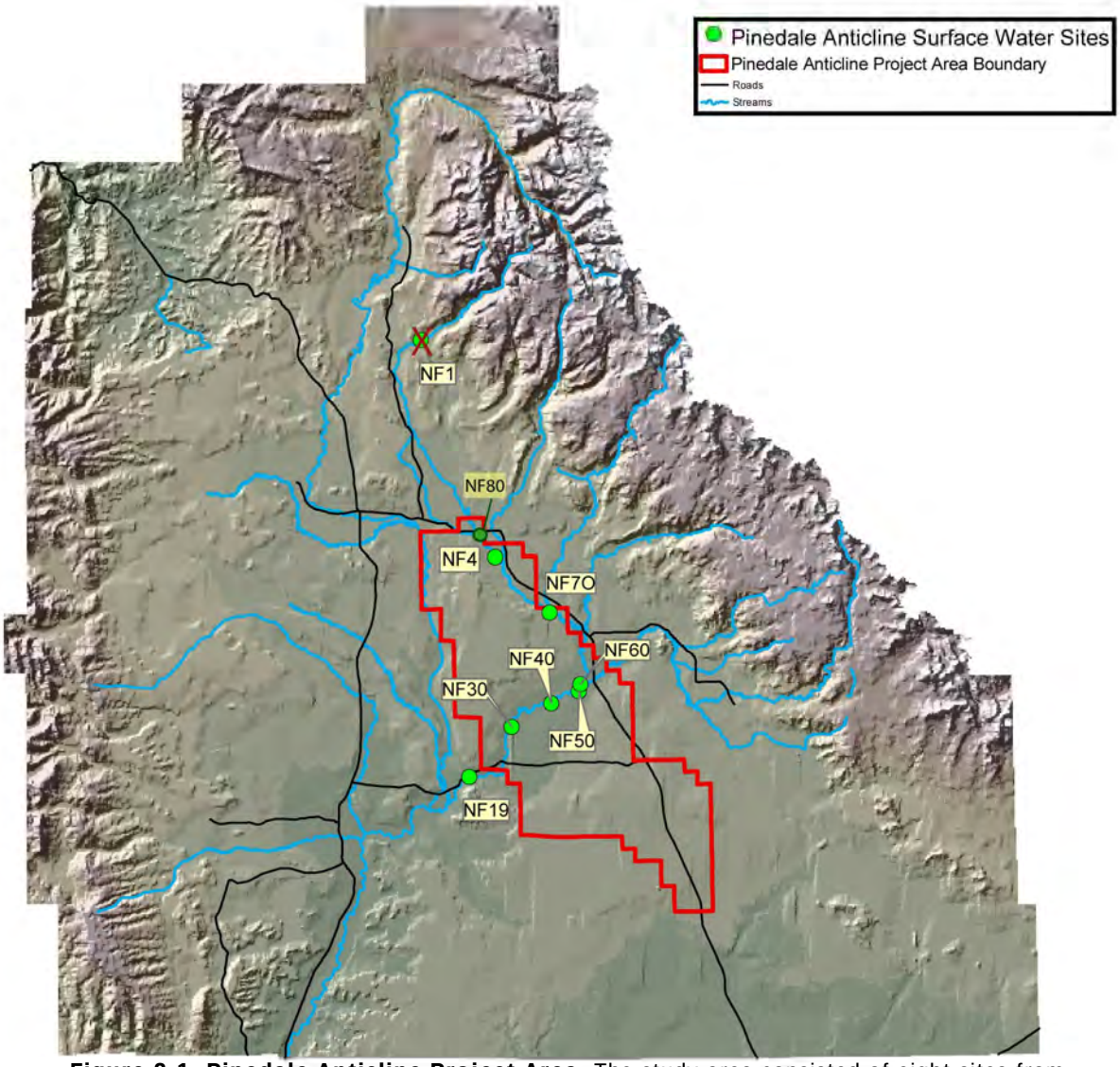

Figure 2.1. Pinedale Anticline Project Area. The study area consisted of eight sites from the New Fork River and one site on the East Fork River. The goal is to assess changes in the condition of the New Fork River as it passes though the Pinedale Anticline Project Area (outlined in red) of central Sublette 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.

This 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.

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This disclaimer applies both to individual use of the data and consolidated use with other data.

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0 2 4 8 12 16 Miles

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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 need to consider how the New Fork River might come to be influenced by development on the PAPA. We identified several modes whereby the integrity of the New Fork River could be affected by runoff from development on the PAPA. Currently there are two regions where potential effects of PAPA development are likely to accumulate as measureable impacts. We have separated these two areas into the upper and lower study areas to facilitate discussion and analysis. 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 spilled on soil, their eventual arrival in river systems is likely to correspond to runoff events. Note that there were no direct disposal effluents during the 2008 survey, but Anticline Disposal activated a permitted diffused, effluent during 2009.

Much of the development on the Mesa occurs in an area where the flow of run-off events is directed southeast, toward the lower study area. Similarly the development in the southeastern PAPA is most likely to experience runoff to the lower study area. In 2007, 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 Creek and Willow Creek are known to influence the chemical and biological composition of the New Fork River (Marshall 2005a). These tributaries increase the conductivity of the New Fork River 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 Creek and Willow Creek, but the influences of Pine Creek may change from year to year as it serves as a conduit for the Pinedale waste water treatment plant. This makes it difficult to assess PAPA-related influences from other anthropogenic stressors. Thus we added replicates to the NF70 site in 2007 so that statistical procedures could be used to correct for different sources of variation. Over the long-term, the temporal changes occurring at this site relative to NF04 and NF60 (the upstream site of the lower study unit) will be important to diagnose changes within the upper study area and the lower study area as well.

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 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 Sand Springs Draw and Alkali Draw. 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 appears 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.

2.3 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 primarily considers 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.

Single Sample Methods

In 2007-2009, we collected eight single Surber samples from each site, each of which was processed individually in the laboratory to Wyoming Department of Environmental Quality (WY DEQ) standard procedures (e.g., Stribling et al. 2000). Collection procedures deviated from WY DEQ’s Standard methods, which were in pre-2007 PAPA assessments, in that WY DEQ usually “composites” all eight samples into a single sample representing the site in the field. For the SCCD field crew 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. Samples may be electronically composited at a latter time if necessary, but once they are composited in the field, this valuable variation is lost forever.

Single samples were collected using a stratified random sampling regime where near-substrate flow measures were used to ensure that the samples from each site fell within a uniform range of flows. This procedure is important for several reasons. First, it ensures that flows are uniform among sites. We know that near substrata flows can account for a very large amount of variation in aquatic invertebrate assemblages (e.g., Hart and Fonseca 1998, Hart and Finelli 1999) and we know that gas development is not likely to alter flow regimes. 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[6]. 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, the field crew measured the relative substrata size distribution, and embeddedness within the area defined by the Surber sampler for each sample.

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

2.4 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. 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 2004-2008 were generated from raw taxonomic data by EcoAnalysts, Inc. Since this contract laboratory lost three samples in 2006 and 2008, we decided to process 2009's samples in-house at River Continuum Concepts.

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

Previous laboratories used different levels of taxonomy and caused some artificial inflation of taxa richness. This occurred both within and among contract laboratories; it is part of dealing with large multi-species invertebrate data sets. Sometimes a taxonomist can place a solid species, species-group, or sub-species identity on one or two specimens, but can only identify some to genus level. When the data are compiled, this results in the same organism having three different names. We found this caused a problem in 2008's report (Marshall 2009). We used the same procedure that WY DEQ (Hargett and Zumberge 2006, Hawkins et al. 2008) and US EPA (Barbour et al 1999) have used to deal with this problem: we defined Operational Taxonomic Units (OTU[20]). Thus, all specimens were analyzed at a standard level of taxonomic effort--which truncates the dataset slightly to an appropriate level that is reproducible for all samples. The details of this process are documented in the Appendix to this report.

2.5 Quality Assurance Statement.

The sorting efficiencies for the 2009 field season averaged 97.5% (± 2.5%) this is the highest average sorting efficiency in the ten years of data collection. Standard convention is that the sorting efficiency should exceed 90%.

2.6 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.

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 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.

There are five common macroinvertebrate functional feeding groups (FFG) used to classify taxa by their roles in processing organic material. The generalists eat fine particles of organic material that require little chewing to fit in their mouth. Their FFG designation is "collectors" and they are further subdivided into collector-gatherers and collector-filterers. Gatherers are deposit seekers whereas filterers remove fine particles from the water column. Predators are the FFG that preys upon other animals. Scrapers have adaptations to scrape fine layers of algae from rock and other hard surfaces. Shredders play very important roles in breaking up deposits of course detritus and woody debris--resulting in food availability for all other groups[7]. In streams with extreme sedimentation problems, extreme pollution, or even urbanization, the community tends to shift towards FFG composition dominated by collectors and predators.

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 2009, (treatment = SITE). The ANOVA used the within site averages and variance to determine the

[7] Shredders release nutrients bound in coarse particles to aid in algae production for scrapers, as well as making fine particles for collectors. By providing sustenance for these groups they also ensure prey is available for predators. If you are interested in FFGs and their role in community ecology, read the River Continuum Concept (Vannote et al. 1980), available at www.rivercontinuum.org.

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 statistical error was less than 5%.

This report follows the tradition set by the 2007 (Marshall 2008) and 2008 (Marshall 2009) reports uses 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[8]. 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.

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

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. This constitutes a stratified sampling scheme that is superior both to completely randomized designs and to subjective stratified sampling methods.

As in 2008, the SCCD field crew did an excellent job of sampling a consistent range of flows in 2009 and sampled the same average flow[9] as sampled in previous years. This is important because deviation in the average value would have been likely to confound analysis among years.

For 2009 there was no significant difference in flow regime sampled at the sites (P=0.995)[10].

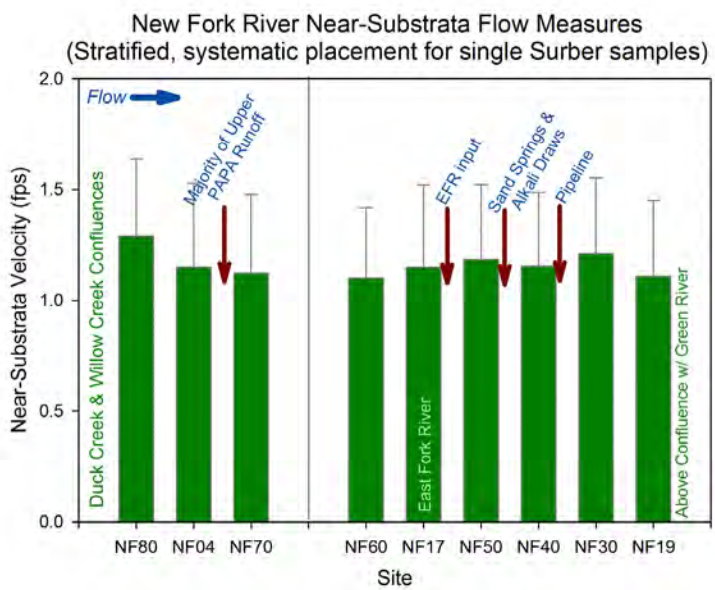

Figure 3.1. Near Substrata Flows. The average near-substrata water velocity from which benthic samples were collected is represented by bars. Sites are arranged from upstream to downstream. Error-bars represent 95% confidence interval of the mean.

[9] 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.

[10] 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[11] 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[12] by New Fork River flows[13]. 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 and 2008, we found that sites NF30 and NF40 exhibited more sand-sediment than found in other riffle areas—even those of the sand dominated East Fork River (NF17). In 2009, this we saw a slight increase in amount of sand in benthic samples from the East Fork River, and a slight decrease in the amount of sand in benthic samples downstream (NF30, NF40). This is could be related to high spring run off in 2009 (Fig. 3.2). ANOVA results indicated there was a significant difference in the amount of SAND[14] in samples from different sites in 2009 ($P<0.0001$). Tukey's LSD test indicated that the only significant differences were NF17, which exhibited significantly more sand than all other sites.

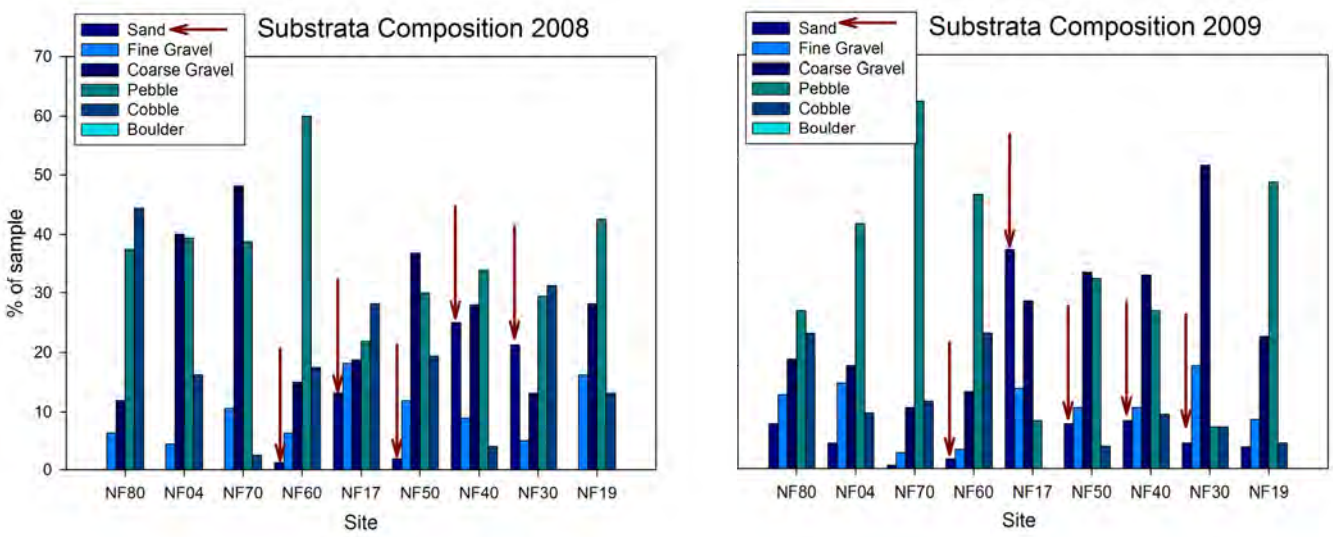

Figure 3.2. Substrata composition. The average percent dominance of different substrate types sampled when sampling was stratified according to flow (Fig 3.1) is represented by bars. Sites are arranged from upstream to downstream. Both 2008 and 2009 are provided for reference.

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

[12] 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.

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

[14] SAND is a variable used in analysis and was generated from field observations of the relative proportion of sand to other substrata in the top 10 cm of sample area.

The Particle Size Index (PSI) is a weighted average of the percent contribution of different substrate sizes (see Analytical Methods). It integrates all substrate types into an independent[15] measure which is known to correlate with macroinvertebrate community structure and abundance. Values of about 300-350 are ideal and are dominated by a complex mix of cobbles and other substrata. Values near zero are dominated by sand and silt. We found that in previous years, differences in community structure were strongly correlated with the PSI.

In 2009, ANOVA indicated there was a significant difference among sites and that the sand-dominated East Fork River had significantly smaller particle sizes in benthic samples, compared to all New Fork River samples collected from similar velocities. Other sites stood out as having larger substrata on average; NF80, NF70 and NF60 were represented by samples with significantly higher PSI values than NF30 and NF40. This is an improvement from previous years where NF30 was lower and not significantly different from NF17.

This provides partial evidence for run-off mediated recovery of the New Fork River system from the accumulation of fines related to PAPA development combined with multiple years of below average flows.

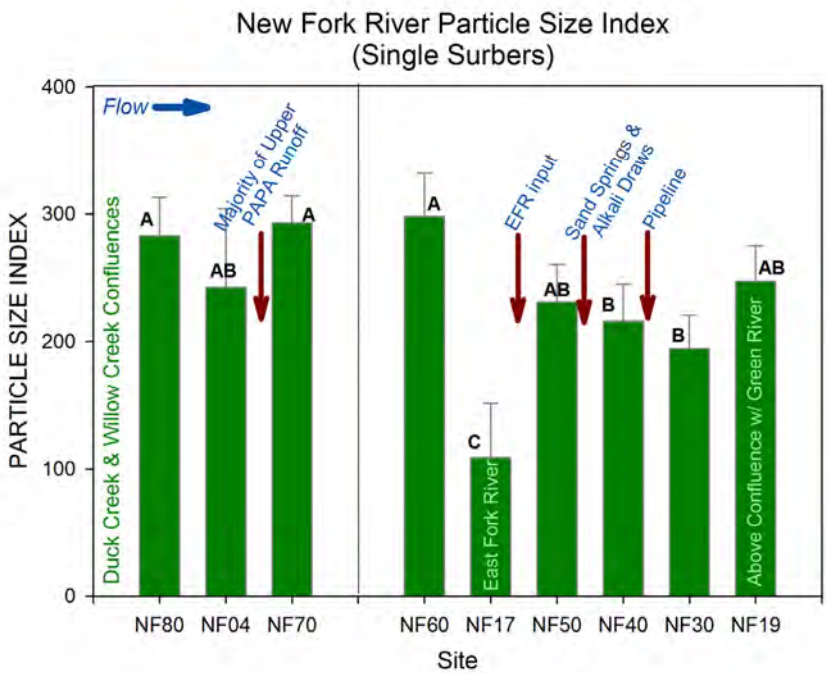

Figure 3.3. Particle Size Index. The particle size index indicated that benthic grabs (Surber samples) from NF17 had significantly lower mean particle size than samples collected from similar water velocity at all other sites (see text). Error bars are 95% confidence intervals. Letters are the final results of statistical tests (see Methods); sites that share a letter are not significantly different from each other (P<0.005).

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

Embeddedness 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.

Embeddedness measures were too incomplete to use as analytical covariates with 2009's macroinvertebrate data (Fig. 3.4); they are presented here only for general reference. Values were not available for several reasons (macrophyte beds, no large substrata to be embedded etc.). Since measures were reported in millimeters of fines around cobbles and pebbles, and since the standard "scrub" depth for Surber samples is 10 cm (100mm), samples consisting entirely of fine material could be reported as 100mm embeddedness. This will be discussed with the SCCD before changes are implemented.

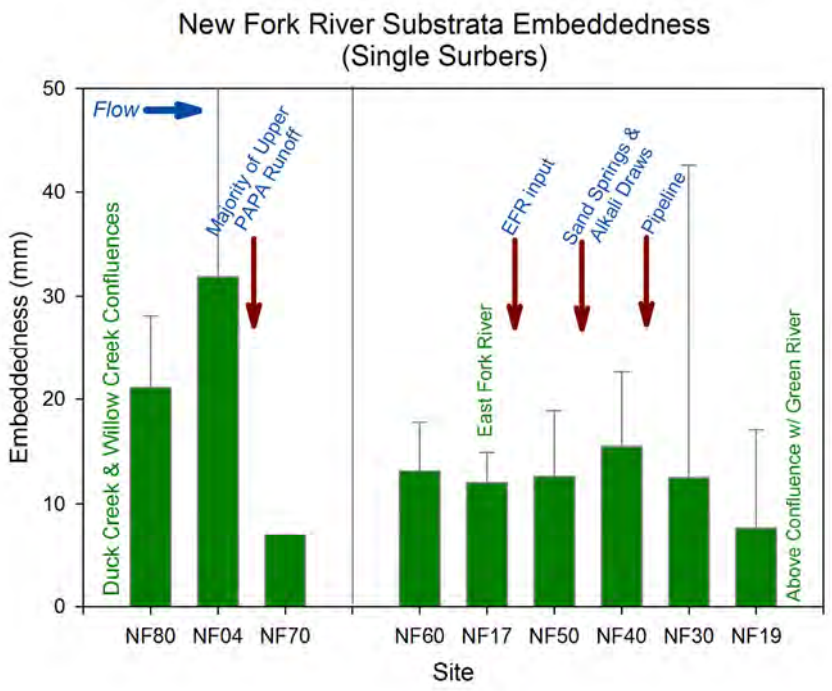

Figure 3.4. Embeddedness 2009. The average embeddedness of sampled (SS) substrate is represented by bars. Error bars represent 95% confidence intervals. Sites are arranged from upstream to downstream.

3.2 Differences among Sites 2009 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 Taxa Richness and Non-insect abundance, indicated that there was a statistically significant difference among the sites. (P<0.05, Table 3.1). Two columns are highlighted (NF40 and NF30) because they represent samples taken upstream and downstream from the pipeline area, and because previous years' results indicated that sediment-related changes occurred at these sites. Only the abundance of collectors and collector-gatherers were significantly different in NF30 and NF40 in 2009.

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. *Denote "marginally significant differences (See Analytical Methods and the Results text discussing the specific metric).

		MULTIPLE COMPARISONS GROUPING (TUKEYS HSD)								
Metric	**P-val**	**NF80**	**NF04**	**NF70**	**NF60**	**NF17**	**NF50**	**NF40**	**NF30**	**NF19**
Taxa Richness	0.312	--	--	--	--	--	--	--	--	--
EPT- Richness	0.032	A	A	A*	A	A	A*	A	A*	A
% EPT	< .001	A	A	B	B	A	A	B	B	B
% Chironomid	<0.001	A	AB	B	B	A	B	B	B	B
% Non-Insect	0.537	--	--	--	--	--	--	--	--	--
Dominance(5)	<0.001	A	B	A	A	A	A	A	A	A
Gatherers	<0.001	AB	AB	B	BC	AC	BC	AC	B	AC
Filterers	<0.001	A	AB	B	B	B	B	B	B	B
Collectors	<0.001	A	AB	B	B	A	B	A	B	A
Scrapers	0.002	B	AB	A	A	B	AB	AB	AB	AB
Shredders	<0.001	B	A	AB	AB	A	AB	B	AB	B
Predators	<0.001	C	AB	AB	BC	AB	A	ABC	BC	ABC
HBI	<0.001	A	AB	B	B	A	B	B	B	B
		Upper Study area			**Lower Study Area**					

Taxa Richness

The samples (SS) collected and processed in 2009 supported around 20 species (taxa) each—on average. This is somewhat fewer than in 2008, because the data were truncated to more appropriate operational taxonomic units to prevent some of the false positives[16] that had to be discussed in earlier reports. Although there sites appeared to vary somewhat in the number of taxa supported, ANOVA indicated that these differences were not statistically significant (Table 3.1, Fig. 3.5). This metric did not show a significant difference among sites in previous surveys either.

Differences in this metric were relatively minor, with means varying among sites by about ±4 taxa from site to site.

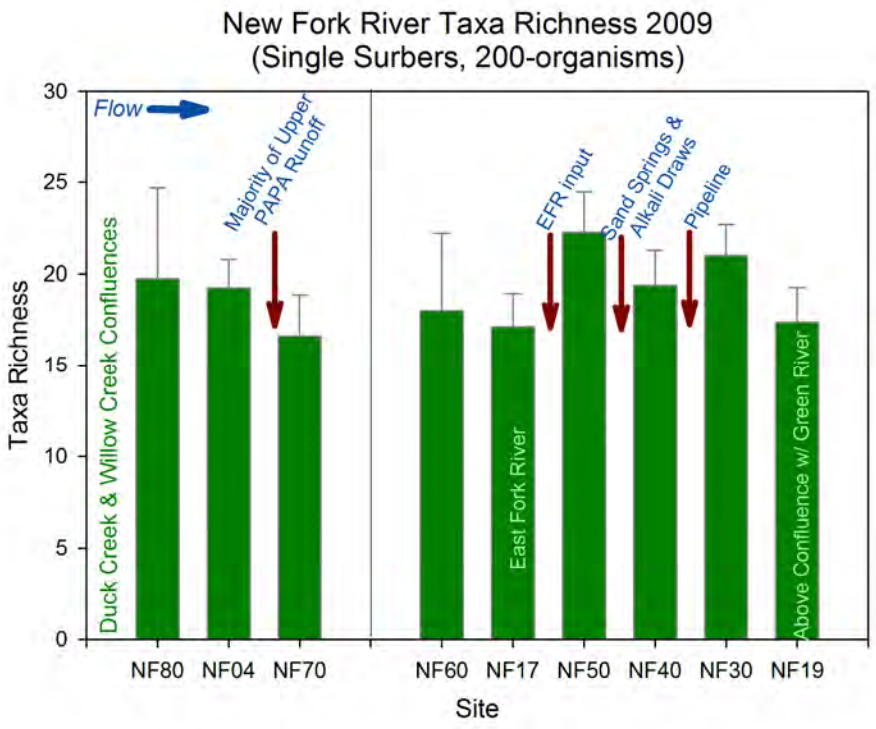

Figure 3.5. Taxa Richness. The number of invertebrate taxa (species) was lowest at NF70 and greatest at NF50. This is the same pattern observed in previous years. Error bars are 95% confidence intervals of the arithmetic mean.

[16] Recall that analysis of 2008's results included some taxa richness inflation by the same organism having several names. We avoided this problem by truncating taxonomic resolution to levels that could consistently be maintained (called Operational Taxonomic Units) to reduce this "laboratory noise."

EPT Richness

The richness of the Ephemeroptera, Plecoptera, and Trichoptera ranged from about 10 to 15 taxa on average. The ANOVA indicated that there some significant differences among the sites, but when the follow-up pairwise comparisons were performed (Tukey's LSD Test), the differences were not significant once they were corrected for the numbers of sites compared. Two sites were considered marginally significantly different from each other (P=0.081); specifically, NF30 supported a marginally significantly[17] greater number of EPT taxa than NF70.

This finding is not consistent with any form of ecological impairment of the New Fork River. The lack of a statistically significant difference in EPT richness underscores the similarity of EPT richness at all sites--most importantly, the richness above the PAPA and below the PAPA are very similar. Additionally, sites that were previously somewhat depressed in this metric (NF30, NF40) exhibited the greatest richness of EPT taxa observed in 2009.

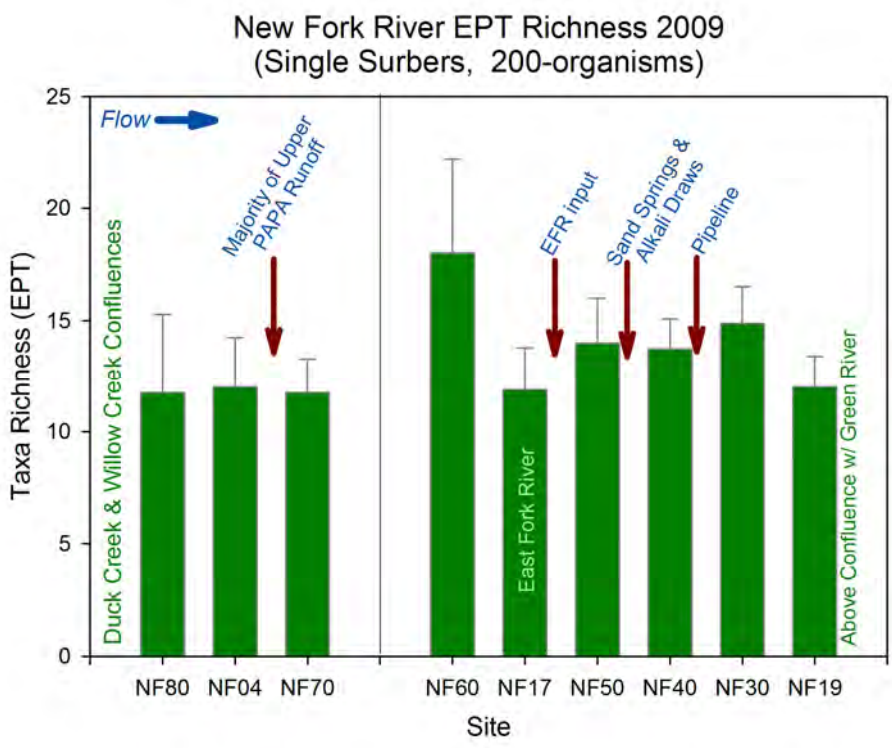

Figure 3.6. EPT Richness. The number of EPT taxa (species) did not vary significantly among sites. Although ANOVA indicated that there was a significant difference among them (Table 3.1) , when multiple comparisons were made among sites, the differences were no longer significant after correction for the number of treatments compared. The difference between NF70 and NF30 and between NF70 and NF50 remained "marginally" significant (0.05<P<0.10). Error bars are 95% confidence intervals.

[17] Yes, it sounds strange, but the significant differences were "marginal" (0.05<P<0.10), so the sites were "marginally significantly different" (Table 3.1).

EPT Abundance

The combined abundance of EPT taxa comprised about 90% of the organisms from some samples and as low as about 30-40% at other sites. ANOVA indicated there were statistically significant differences among sites (P<0.0001). In the upper study area, NF70 had significantly more EPT insects than the two farther upstream sites (NF80, NF04). This happened because NF80 and NF04 had a high abundance of riffle beetles (several genera, but mostly *Optioservus* sp.). Note that the actual density (no./m^2) may not have changed significantly; one side effect of the fixed-count subsampling regimen popular among environmental monitoring programs is that very abundant taxa appear to suppress less abundant taxa. Furthermore, the elevated EPT relative-abundance is consistent with improving water quality, not degrading conditions.

For the lower study area, NF17 and NF50 had significantly fewer EPT organisms than the other lower study area sites. This was due primarily to the high abundance of midges in the East Fork River (NF17) and to a lesser-extent, below the confluence of the New Fork and East Fork Rivers (NF50). In 2009, the confluence of the East Fork River had a greater affect on %EPT than the pipeline crossing, Sand Springs and Alkali Draws, or other development along the New Fork River.

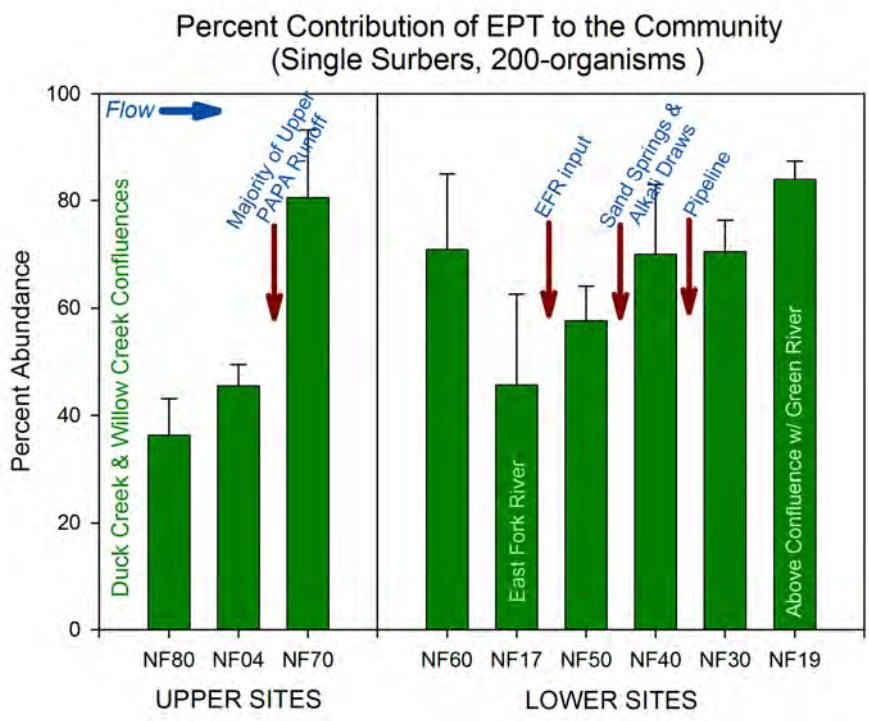

Figure 3.7. EPT Relative Abundance. The abundance of mayflies taxa (species) was greatest at NF19 and lowest at NF80/NF50. NF70 had significantly more EPT fauna than the other upper sites (P<0.005). NF17 had significantly fewer EPT organisms than all other lower study area sites, except NF50 (P<0.005). Error bars are 95% confidence intervals.

Chironomidae Abundance

In 2009, the midges comprised a moderate to small portion of the community on average; the metric ranged from about 40%-10% of the invertebrate assemblage; very similar to the abundance of midges in 2008. There were significant differences among the sites (Table 3.1, Fig. 3.8). At the upper study area, there was a decline in midge abundance along a downstream gradient. In 2009, these differences were only "marginally statistically significant" (P<0.10). 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.

In 2009, the abundance of midges at the lower study area showed a similar pattern of abundance as observed in 2008. Specifically NF17 had many more midges per sample than the other lower study area sites. 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. This metric does indicate a minor improvement over 2008 because during that survey the low midge abundance at NF30 was offset by very high abundance of *Nais* sp (oligochaete worms). In 2009, Nais abundance was much lower on average at NF30 (and NF40).

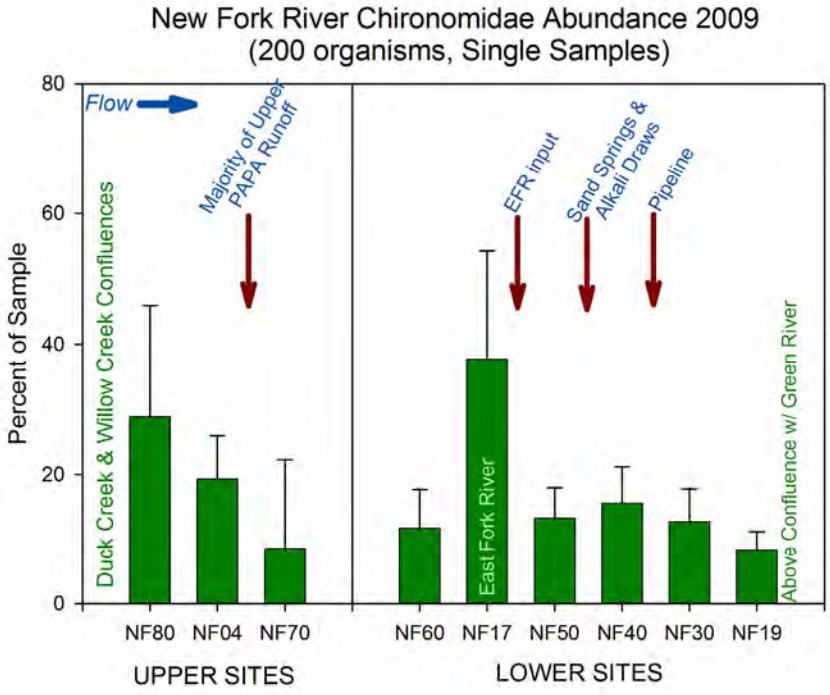

Figure 3.8. Chironomidae Abundance. The abundance of chironomid midges was greatest at NF17 and NF80—both of these sites were significantly different from NF70, NF60, NF30 and NF19. Bars are averages of eight samples, and error bars represent 95% confidence intervals.

Non-Insect Abundance

Each year, non-insect aquatic invertebrates have been dominated by small oligochaete worms (*Nais* sp.). The abundance of these organisms downstream of the pipeline crossing had been abnormally high since 2004. Although this characteristic was localized to the area around the pipeline, it was disconcerting[18] because it could have been related to development (sedimentation) within the PAPA and because it did not seem to be moderating over time. The increase of the abundance of sediment dwelling worms was likely due to an change in the quantity and/or quality or sediment deposits downstream from the pipeline. If these continued to accumulate, non-insect dominance could become much more prevalent in the New Fork River.

In 2009, the non-insects remained dominated by *Nais*, but the relative abundance was much lower on average. For example, the non-insect relative abundance at site NF30 dropped from an average of 30% to an average of about 4.4%. Although a few samples (one from NF40 and one from NF17) had somewhat elevated *Nais* abundance, the averages were much lower in 2009 than in recent years and there were no statistically significant differences among sites.

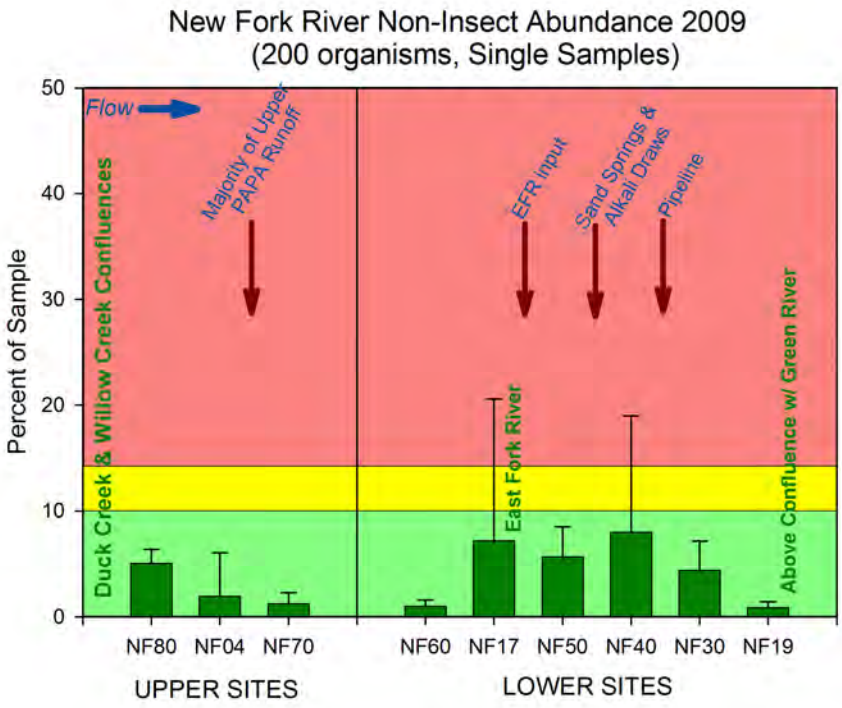

Figure 3.9. Non-Insect Abundance. There were no statistically significant differences among the sites in 2009. Error bars are 95% Confidence Intervals.

[18] Non-insect dominance is often associated with ecological dysfunction in North American freshwater lotic ecosystems.

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.[19] High dominance values indicate that a few taxa have disproportionably high abundance, and can be a sign of ecological perturbation. Because of the operational taxonomic units used in this study, our values may be a little higher than those reported earlier[20].

The upper study area consisted of two sites with high, but not exceptionally high, dominance (5) and one site with much less dominance. The two high sites were only significantly different from the middle upper-study-area site (NF04). That is, NF80 and NF70 were not different from each other, nor were they different from sites of the lower-study area. The unusual observation in 2009 was the low dominance (5) observed at NF04--but low dominance indicates higher diversity--which is usually associated with "healthier" ecosystems.

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.

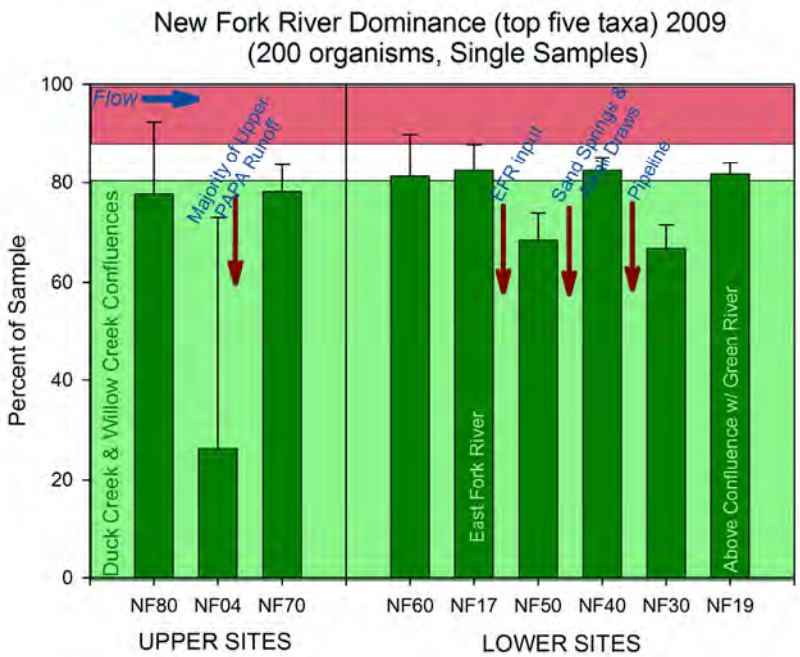

Figure 3.10. Dominance(5). The combined relative abundance of the five-most-abundant taxa (Dominance(5)) was used a measure of community dominance. The only significant difference among sites was NF04, which was significantly lower dominance (good) than all other sites. Error bars are 95% confidence intervals.

[19] Stribling et al. (2000) report most reference sites were less than 85%, and that 50% of reference sites had about 60% or greater dominance (5). Hargett and ZumBerge (2006) did not discuss this metric for the Wyoming Basin Region in the new WSII.

[20] Conservative estimates of richness, usually result in slightly fewer taxonomic units, each comprising a slightly larger amount of the representative community. Thus the sum of our five OTU's might result in a slightly higher Dominance(5) than earlier reported and than used to develop the regional standard of 80.

Abundance of Functional Feeding Groups

Modern aquatic ecology is often concerned with the movement of carbon (energy) through food webs. Thus it is useful to group organisms by their roles in processing organic material; their so-called "functional feeding group." Collector-gatherers are organisms that search out deposits of fine particulate organic material for sustenance. Collector-filterers remove fine particulate organic material from the water column for the same purpose. In streams and rivers, the amount of suspended organic material is linked to the velocity of water. Since both collector-gatherers and collector-filterers consume fine particles of organic matter, the differences in their relative abundances may be related to localized flow conditions. For this reason, it is often useful to consider the combined abundance of filterers and gatherers as the "collector" group[21].

The percent contribution of collector-gatherers, collector-filterers, and combined collectors (filterers + gatherers) displayed very similar values as observed in 2008. The 2009 spatial patterns were nearly overlays of the results from 2008. None of these patterns were indicative of impacts related to PAPA development. Thus, we focus on the combined collectors.

As observed in 2008, the greatest contribution of combined collectors occurred at NF80, downstream of Willow Creek and Duck Creek. The East Fork River, dominated by midges also had high collector abundance, but was not significantly different from the sites downstream from the confluence of East Fork and New Fork Rivers.

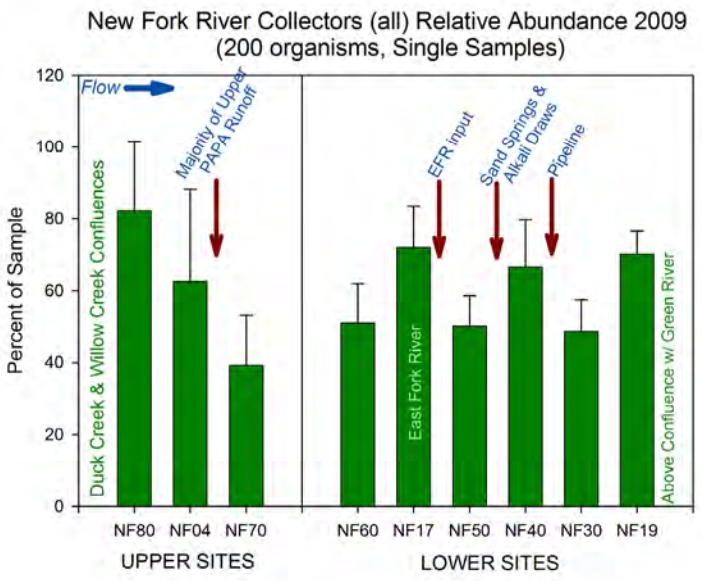

Figure 3.11. Collectors. The combined relative abundance collector-gatherers and collector-filterers. There did not appear to be any significant difference related to PAPA development. The upper study area had significantly fewer collectors at NF70 than at NF80. In the lower study area, NF60, NF40 and NF30 were significantly less dominated by collectors than NF17, NF40 and NF19. Error bars are 95% confidence intervals.

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

Hilsenhoff Biotic Index (HBI):

The HBI rates streams by the abundance-weighted average organic-pollution-tolerance of the entire species assemblage. Thus, a score of 10 occurs only when the entire invertebrate assemblage is extremely tolerant of organic enrichment[22], whereas a score of zero indicates that the entire community is composed of only very sensitive organisms. Generally streams of the Wyoming Basin should have values less than about 4.0[23]. The values obtained for the New Fork River were generally below HBI value of 5, except the site right below the confluences of Duck and Willow Creeks. This pattern was also exhibited in previous years. The site monitored with the best HBI score was NF70, which had HBI values consistent with the best reference sites in the Wyoming Basin Region (again similar to previous years).

The HBI metric reflects other differences in water quality that are apparently unrelated to development in the PAPA. For example, the high values at the two farthest upstream sites (NF80 and NF04) are likely responding to the high-conductivity tributaries a short distance upstream from NF80. In the lower study area, NF17 was influenced by sand-dwelling midges--the others sites in the lower study area were not significantly different from each other.

The HBI did not indicate a significant impairment related to PAPA development.

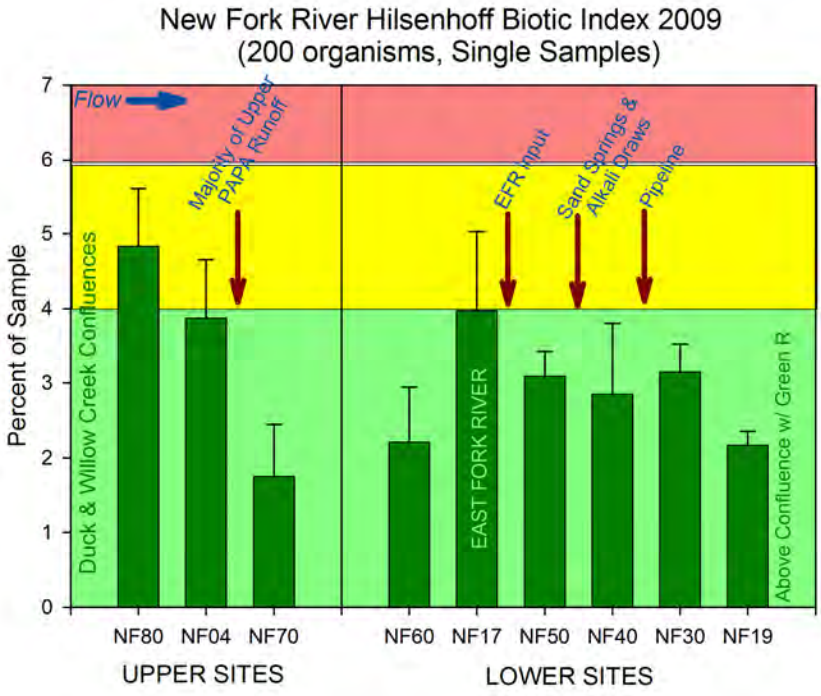

Figure 3.12. Hilsenhoff Biotic Index (HBI). The HBI was near values attained from previous surveys of the New Fork River. High values indicate communities dominated by pollution-tolerant organisms. Bars are averages of eight samples, and 95% confidence intervals.

[22] In reality this would only occur in a pile of sewage sludge or similar enriched, anaerobic environment.
[23] Hargett and ZumBerge (2006) report 95% of reference streams had HBI values greater than 2.7, our earlier studies (Marshall 2006, 2007, 2008, 2009) found that New Fork River sites often have HBI values ~4.0.

Changes Over Time (2007-2009)

Many sites now have amassed three years of data using improved sampling methods. It becomes more useful to assess trends over time. The following line graphs show how the sites have changed over time for select metrics. The metrics based on relative abundance were selected because these are not likely to be significantly affected by the implementation of conservative operational taxonomic units in 2009. For this reason, Taxa Richness, and EPT richness were not examined in this way until the historic database can be edited and truncated to the optimal operational taxonomic units.

Thus the metrics EPT abundance, Percent Non-Insect, Percent Chironomidae, Percent Collectors, Percent Scrapers, Percent Dominance (5), and HBI were used to assess changing community structure over time.

The figures show, for each site the mean metric value, with standard errors. For the final summary, the change from 2008 to 2009 for each metric at each site was assigned a score of -1, 0, or +1, depending if the change was consistent with declining, no-change, or improving water quality, respectively. Then the net sum of the scores for each metric were reported to describe the net change as positive or negative. Note that the 2008-2009 change might be insignificant, even if the overall trend over three years is significant.

Change in the abundance of EPT taxa

The relative abundance of EPT taxa is expected to decline as water quality declines and as more tolerant organisms become more dominant. Thus, when this metric declines, it implies a decline in water quality[24], whereas an increase in the relative abundance of EPT taxa can occur when (or where) water quality improves.

Although NF80 showed no significant trend over the study period[25], all the other sites showed a general trend of improved EPT abundance over the three consecutive years of replicated monitoring. Considering only the changes from 2008-2009, four of the sites (NF60, NF40, NF30, and NF19) showed significant increases in the abundance of EPT taxa and no sites exhibited significant declines in EPT abundance from 2008-2009 (Fig 3.13).

[24] In this context, water quality includes physical conditions of the stream as well as chemical conditions.
[25] It also had fewer observations as the NF80 site was added as an upstream reference in 2008.

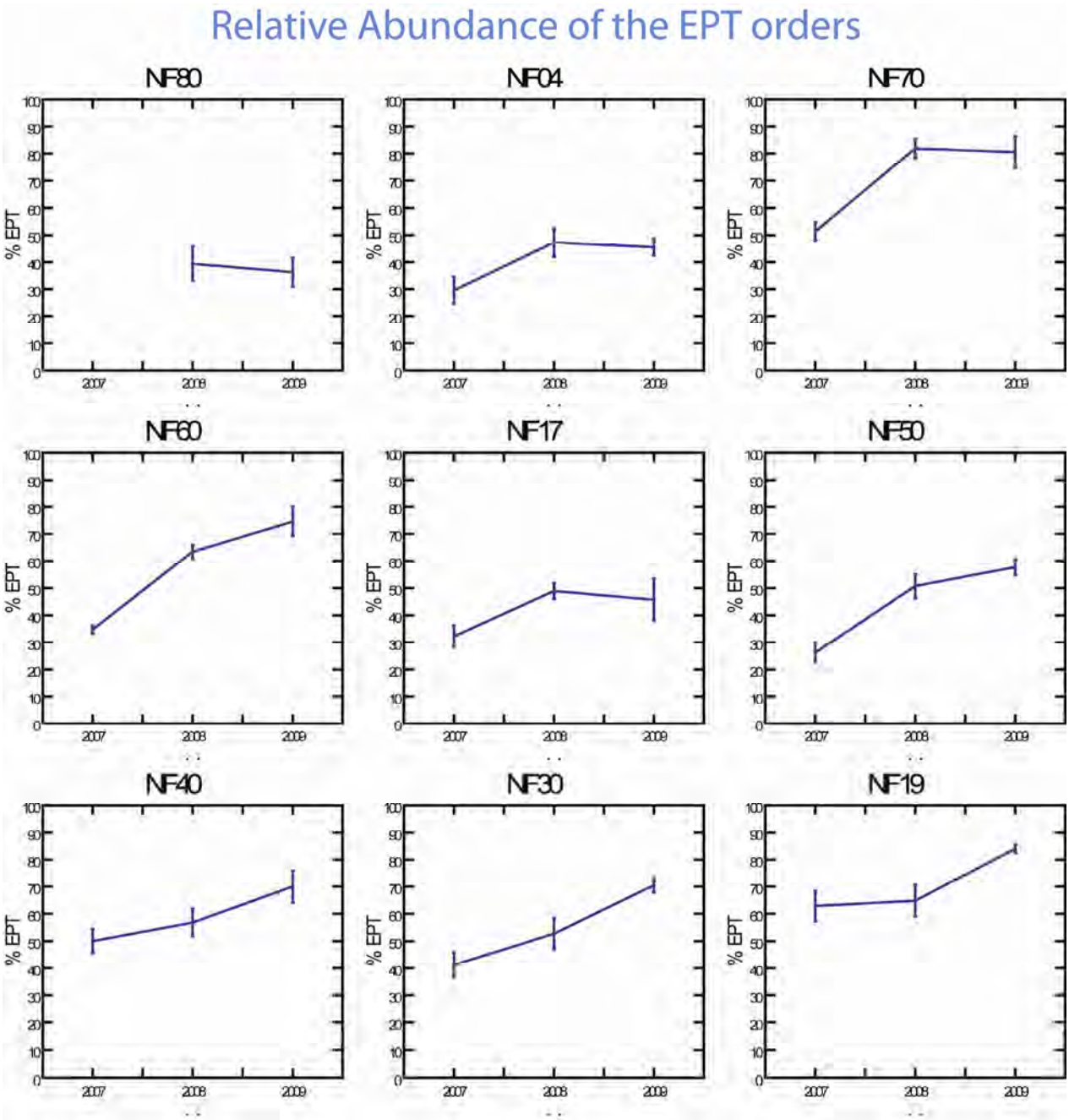

Figure 3.13. Change in EPT Abundance (percent). The abundance of EPT taxa generally showed a significant increase over the three years of the replicated monitoring program. NF60, NF40, NF30, and NF19 showed significant improvements from 2008 to 2009. No sites exhibited significant declines in this metric--over 2007-2009 or over 2008-2009. The figures show the confidence intervals, and means of three years 2007, 2008, and 2009.

Change in the Abundance of Non-Insects

Most North American freshwater rivers are dominated by aquatic insects--both in terms of abundance and diversity. Communities that are dominated by non-insect macroinvertebrates occur in unusual circumstances, and may indicate ecological perturbation or naturally saline environments. Thus increases in the %Non-insect metric are considered undesirable, under most circumstances and may indicate declines in water quality. Since we have noted a problem with this metric over recent years, we were especially hopeful that the 2009 survey would result in lower densities of non-insects in the New Fork River.

The abundance of non-insects declined significantly from year to year at the sites where there were high abundances of non-insects in the past. Sites that have historically had low abundance of non-insects also had fewer non-insect invertebrates in 2009 (Fig 3.14).

Considering only changes from 2008 to 2009, six of the nine sites (NF80, NF04, NF60, NF50, NF30, NF19) showed a significant reduction of non-insects in 2009 (Fig 3.14). NF17, NF70, and NF40 showed no significant change from 2008 to 2009. None of the sites showed an increase in the average abundance of non-insect invertebrates, however, one sample from NF17 and one from NF40 did have fairly high abundances (~30-40%) of non-insects (almost entirely *Nais*).

Change in the Abundance of Chironomid Midges

Non-biting midges (Chironomidae) are a very diverse group of macroinvertebrates, with over 4,000 species in North America. Although some species are very sensitive to disturbance and/or pollution, generally midges become dominant in streams that are disturbed or polluted. Thus significant increases in midge abundance can indicate a decline in water quality.

The abundance of chironomids generally exhibited a decline in abundance over the three year period of replicated monitoring and none of the sites displayed an increase in midge abundance (Fig. 3.15). The only exception was NF30 which has consistently had low midge abundance over all three years.

Considering only changes from 2008 to 2009, only two sites had significant declines in midge abundance that were consistent with improving water quality (NF50, NF19). This is because the abundance of midges was lower in 2008 than in 2007.

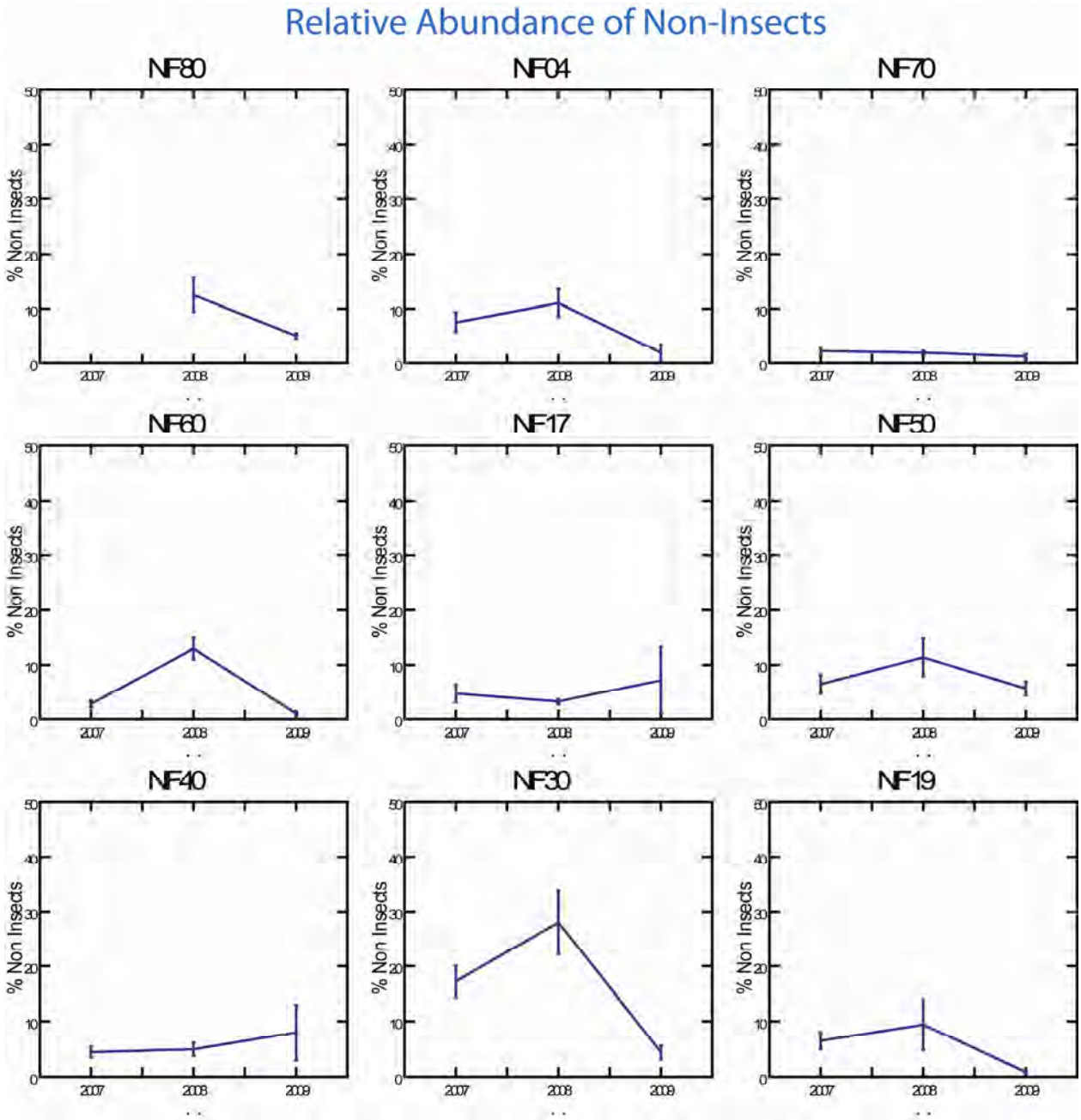

Figure 3.14. Change in Non-Insect Abundance (percent). The abundance of non-insect macroinvertebrates generally showed no trend (if initial values were low) or a decline if values were high in previous years. The figures show the confidence intervals, and means of three years 2007, 2008, and 2009.

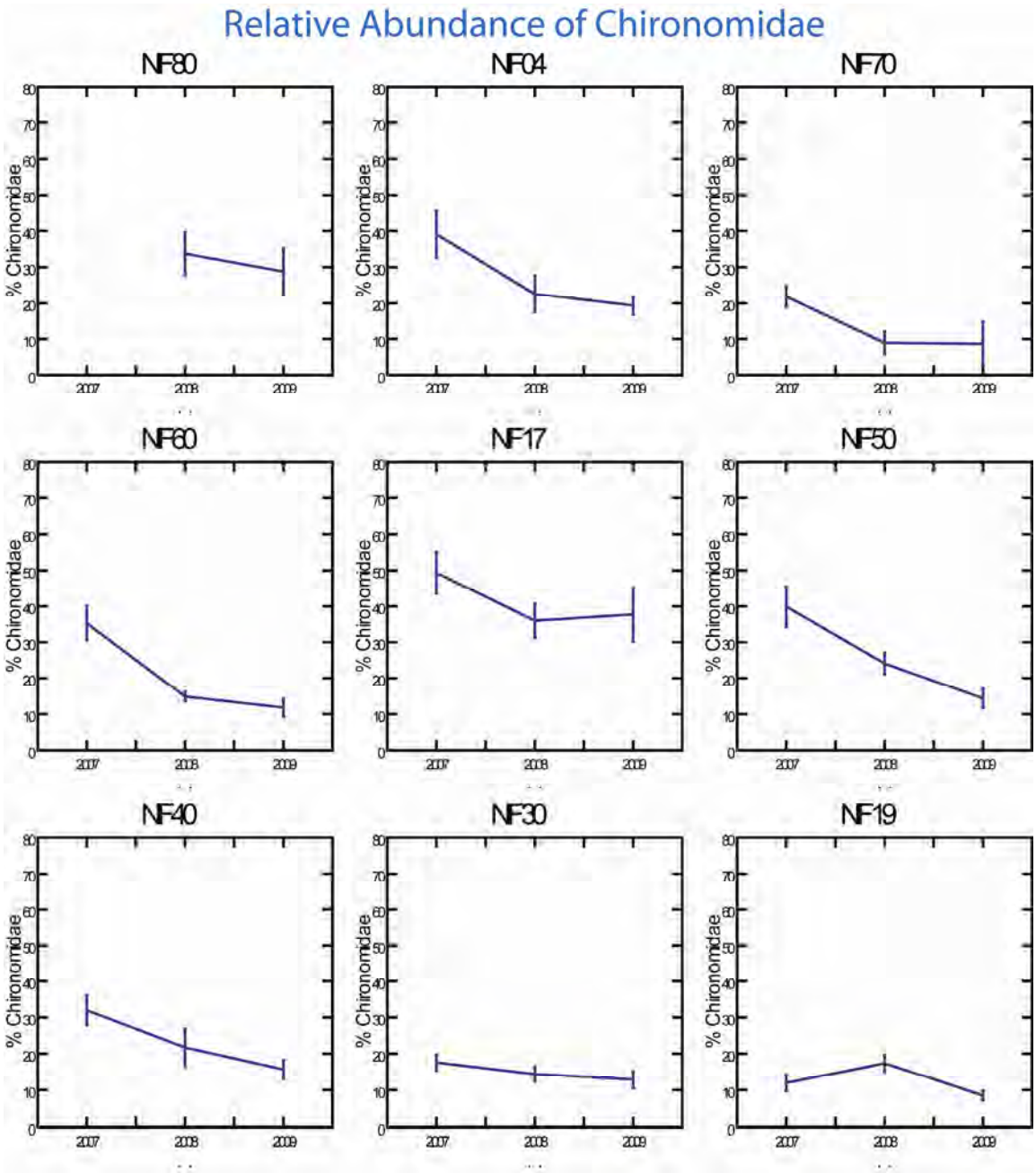

Figure 3.15. Change in Chironomidae Abundance (percent). The abundance chironomid midges generally showed no trend (if initial values were low) or a decline if values were high in previous years. The figures show the standard errors (SEM), and means of three years 2007, 2008, and 2009.

Changes in Collector Abundance

The percent contribution of collectors should respond similar to midges. That is, increases in water quality should result in a decrease in collector abundance, where as declines in water quality can cause an increase in the abundance of collectors and more specialist functional groups (such as shredders and scrapers) decline.

Many of the sites showed no significant trend over the three years of replicated study. NF17 showed a steady increase in collector abundance (Fig 3.16). Some sites declined over the three years of replicated data collection, whereas some sites increased, and others changed insignificant amounts.

Changes in Dominance (5)

Declines in water quality often allow a small group of invertebrates to dominate the ecology of the system; resulting in an increase in dominance. Over the three years of replicated data, most sites showed either insignificant change or a slight increase (Fig 3.17). NF04 was the only site to have a significant decline in dominance over the study period. This metric was marginally increased by use of optimized taxonomic units, which provided a conservative estimate of richness-resulting in fewer taxa of slightly more abundance.

Changes in Scraper Abundance

Scrapers are specialists that scrape algae from large substrata as sustenance. The Wyoming DEQ's Wyoming Stream Invertebrate Index sets the regional goal for the relative abundance of scrapers at about 33-34% (Stribling et al. 2000, Hargett and Zumburge 1996) with the mathematical assumption that more is better[26]. Over the three year study period, most sites showed an increase in the abundance of scrapers (Fig. 3.18).

Changes in the Hilsonhoff Biotic Index

Declines in HBI values indicate that the community has become increasingly represented by species that are know to be sensitive to organic pollution. In many cases, organic pollution was also accompanied by sedimentation when species tolerance values were generated. In 2009, every site showed a significant decrease in HBI; an indication of significant improvement in the taxonomic composition of macroinvertebrates of the New Fork (and East Fork) River (Fig 3.19).

[26] However, in cases of nutrient enrichment the scrapers become dominant as a sign of ecological perturbation. There are also issues of multicolinearity when using both scraper abundance and collector abundance (100% scrapers = 0% collectors) as a measure of assessment. Scrapers are presented for descriptive and diagnostic purposes.

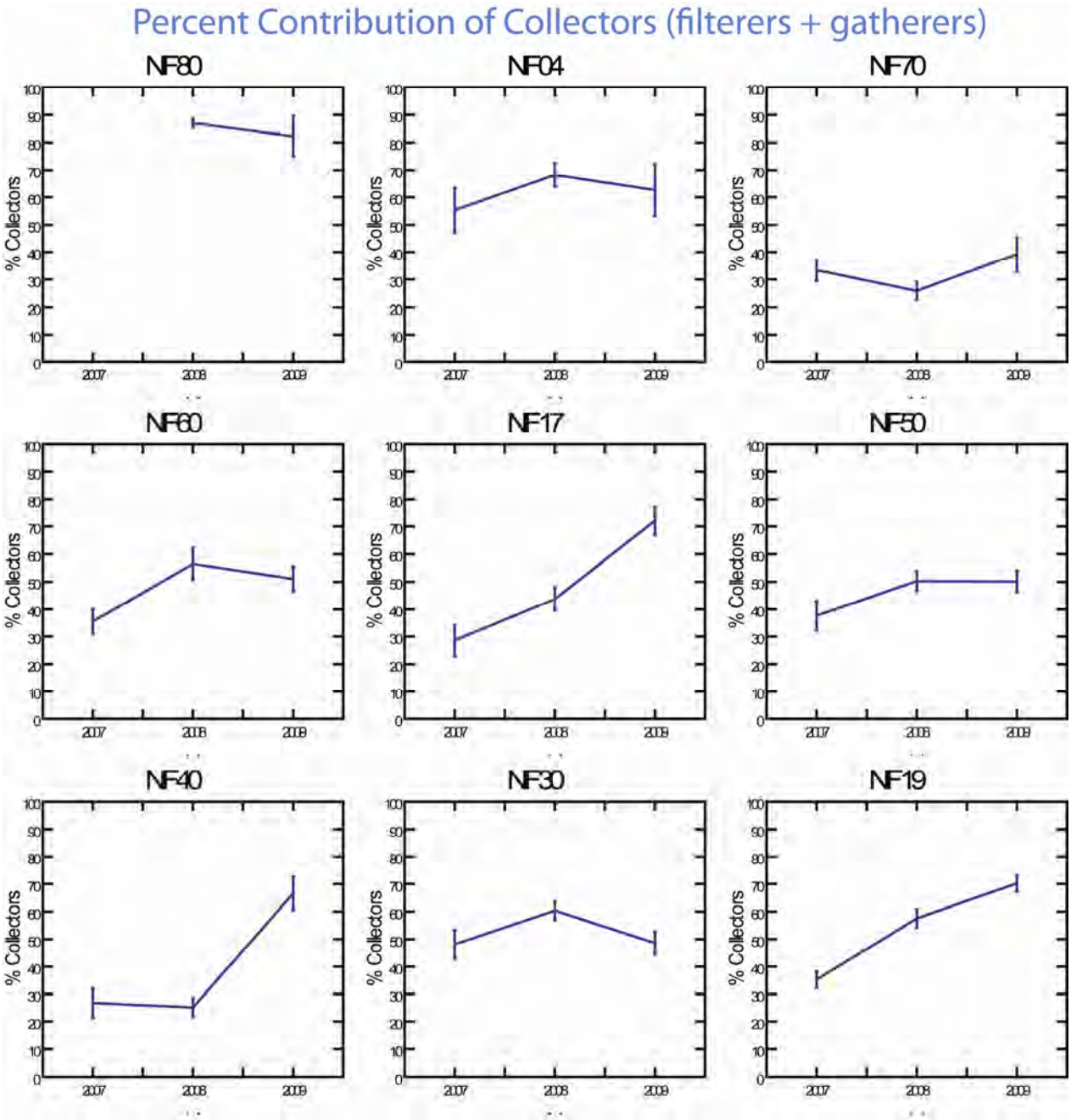

Figure 3.16. Change in Collector Abundance (percent). The abundance of collectors generally showed no trend. Some sites declined, others increased over time. The figures show the confidence intervals, and means of three years 2007, 2008, and 2009.

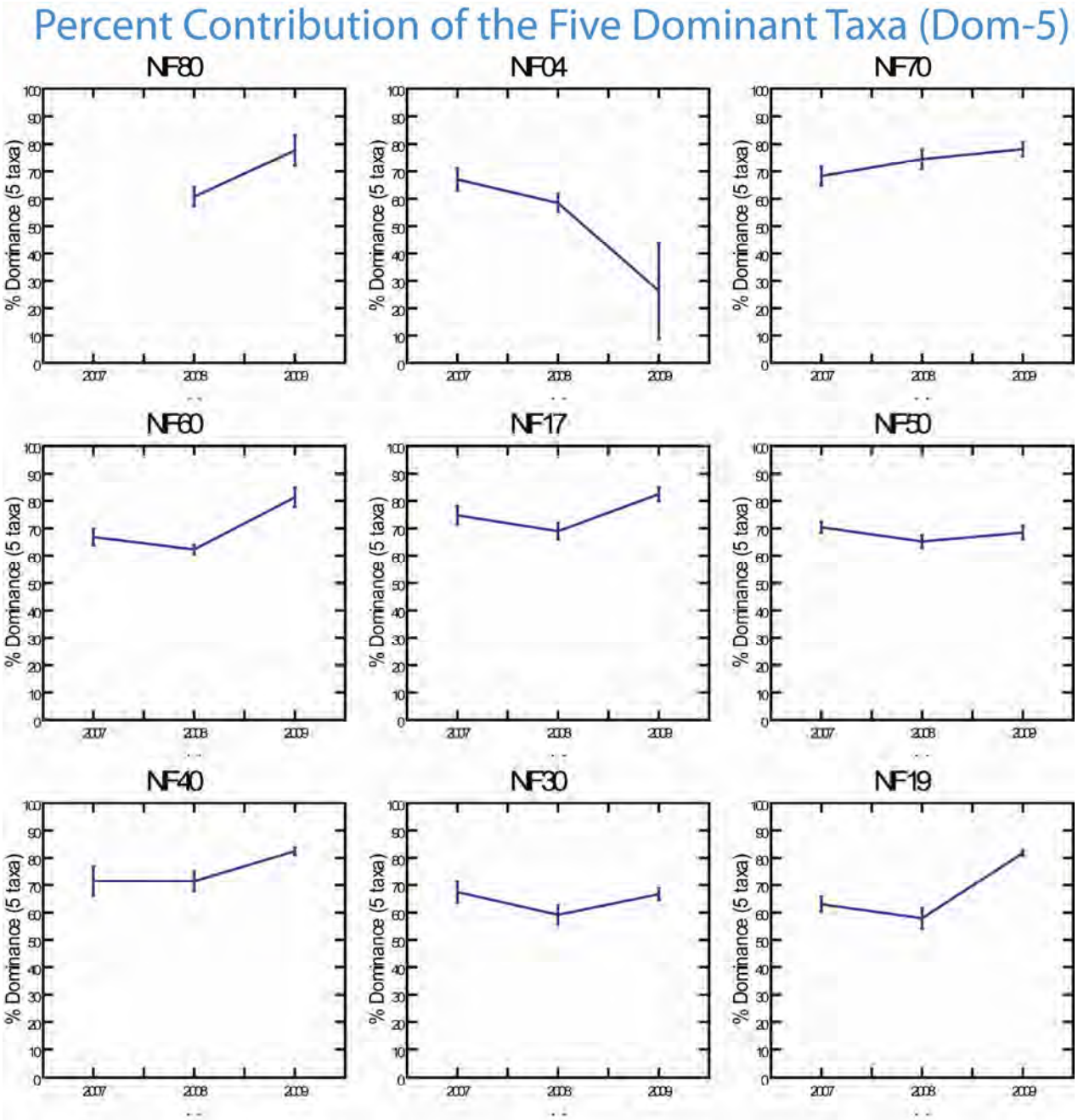

Figure 3.17. Change in Dominance of the Five most Abundant Taxa (percent). The community dominance generally showed no trend; some increased over time, some remained essentially the same. The figures show the confidence intervals, and means of three years 2007, 2008, and 2009.

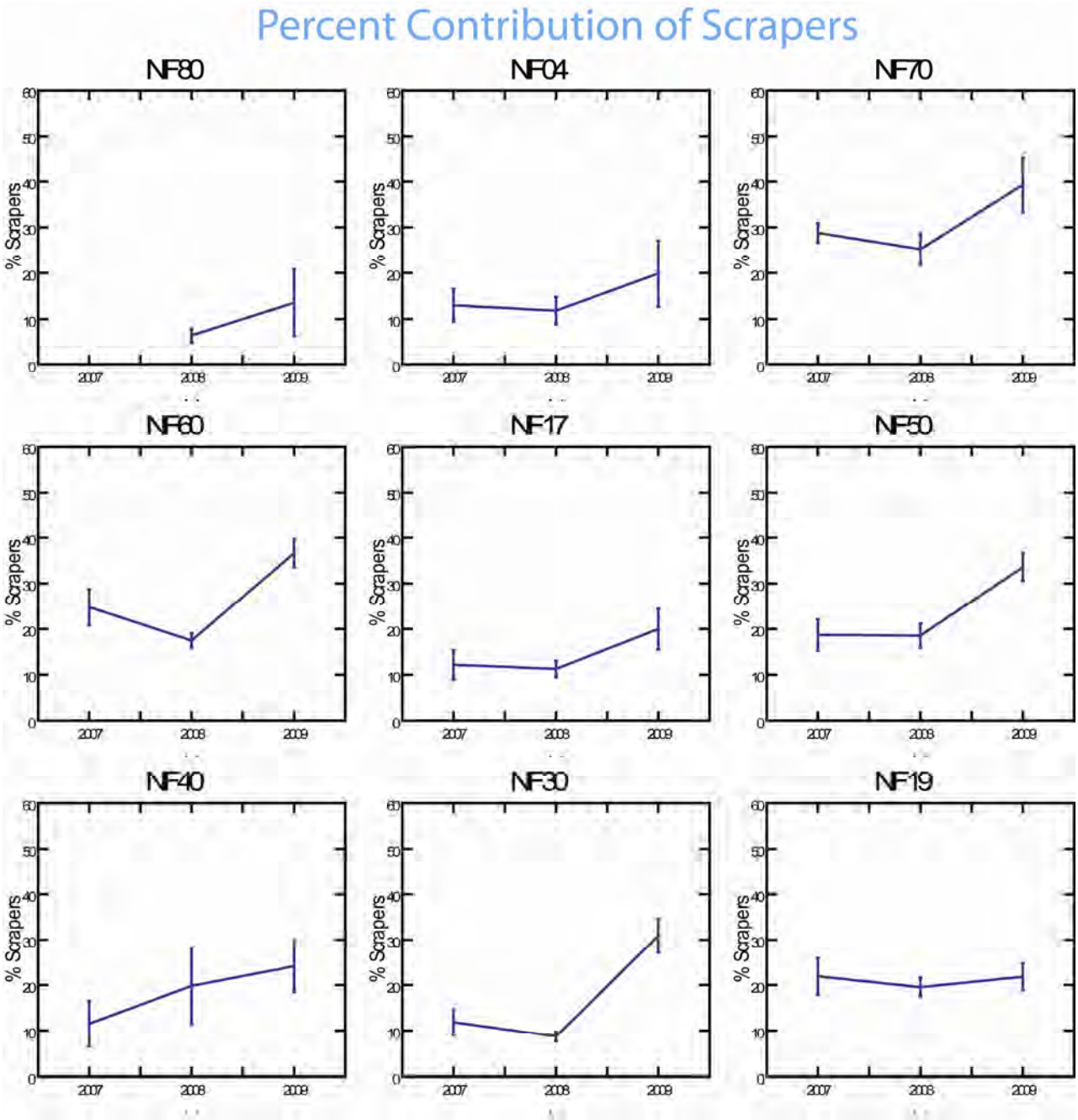

Figure 3.18. Change in Scraper Abundance (percent). The relative abundance of scrapers generally increased in 2009. The WSII threshold of about >34% scrapers is ideal. Most sites increase towards this threshold. The figures show the confidence intervals, and means of three years 2007, 2008, and 2009.

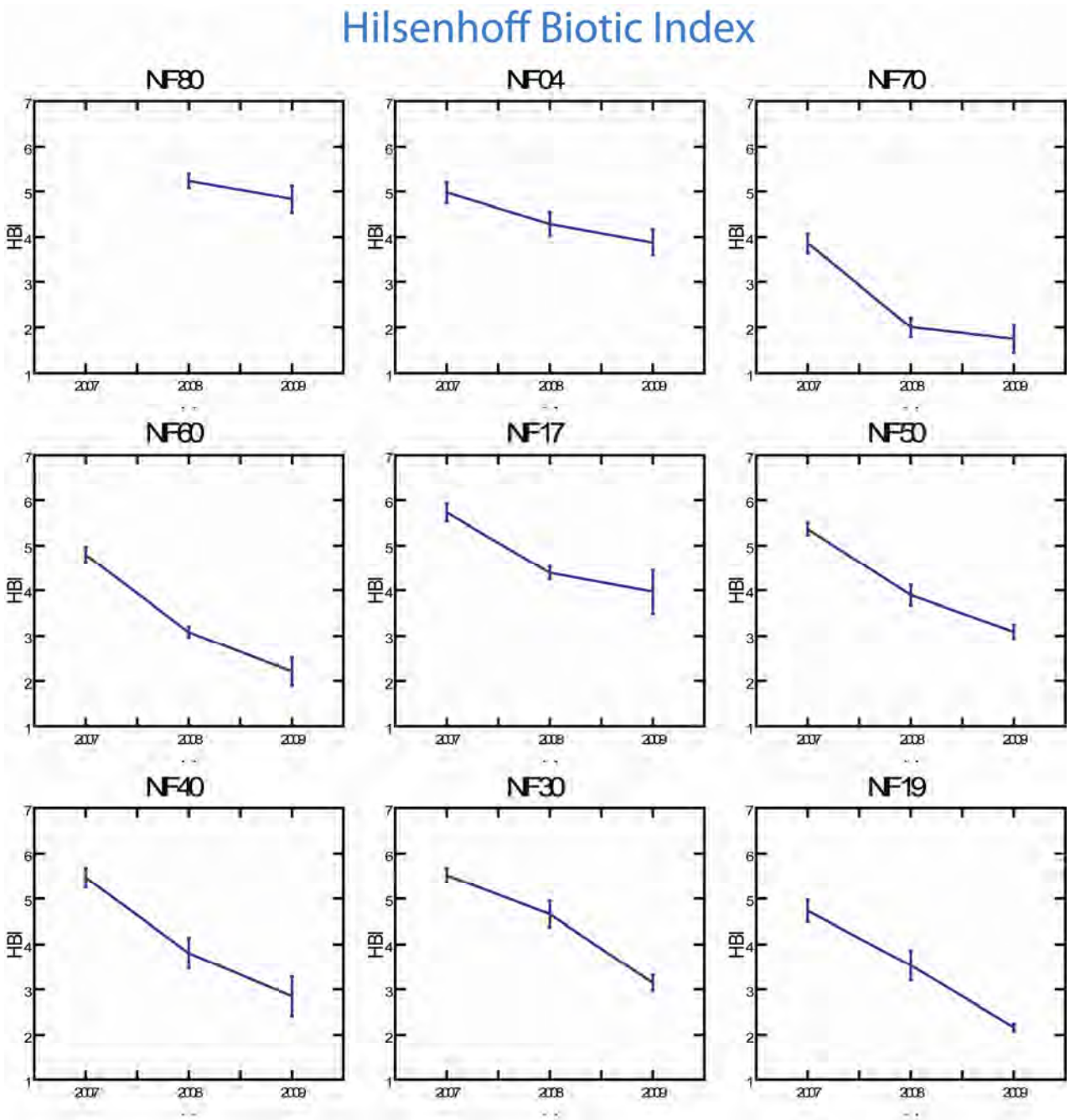

Figure 3.19. Change in Hilsenhoff Biotic Index. The HBI showed a universal decline in 2009 across all sites. A decline in HBI is consistent with improving environmental conditions because it indicates pollution sensitive organisms are more successful than they were previously. The figures show the confidence intervals, and means of three years 2007, 2008, and 2009.

Overview of Changes from 2008-2009

The general trend among all the sites in the New Fork River exhibited biological metrics that suggested improving conditions from 2008 to 2009 (Table 3.2; Figs. 3.14-3.19, Fig. 3.20). The East Fork River netted a neutral change in conditions by having offsetting metrics suggesting improvements and declines. The macroinvertebrate communities of none of the sites indicated significant declines in water quality over time.

The site that exhibited the greatest improvement, was site NF30, which included five metrics suggesting improvements, and no metrics suggesting declines in condition. This is a particularly important finding because this site was the one with which previous findings of degraded substrata and high non-insect abundance were concerned. Our hopes were that the elevated and sustained spring runoff would help the New Fork River purge the sediments that had accumulated below the pipeline, and allow development of a "more natural" benthic fauna at NF30. These results support this expectation.

Most metrics improved more often than they declined. The two exceptions were the abundance of collectors and the measurement of percent dominance. Both of these metrics declined more than they improved (across all sites), however, they were slightly influenced by the change to sustainable operational taxonomic units which resulted in more organisms being classified as collectors and greater abundance in fewer taxonomic units (see laboratory methods).

The most important finding is that non-insect abundance is less of a concern in 2009 than it has been any time in the last five years.

TABLE 3.2. Summary of multi-year trends. This table cross-tabulates trends from the previously discussed metrics (Figs. 3.14 - 3.19) in terms of the hypothesized response to changes in water quality. Measures that suggest improving water or declining water quality were noted with the symbols ☺ or ☹ respectively. Metrics indicating no change over time were noted with the symbol 😐. For example, the relative abundance of EPT orders (%EPT) is expected to *increase* with improving water quality so sites that showed a significant increase in EPT are noted with the symbol ☺. The Hilsenhoff Biotic Index *decreases* with improving water quality, which is indicated with the ☺ symbol; had any of the HBI values increased over time, they would have been noted by the ☹.

SITE	% EPT	% Non-Insect	% Midges	% Collectors	% Scrapers	% Dominance (5)	HBI	
NF80	😐	☺	😐	😐	😐	☹	☺	**+2**
NF04	😐	☺	😐	😐	☺	☺	☺	**+4**
NF70	😐	😐	😐	☺	☺	😐	☺	**+3**
NF60	😐	☺	😐	😐	☺	☹	☺	**+2**
NF17	😐	😐	😐	☹	☺	☹	☺	**0**
NF50	☹	☺	☺	😐	☺	😐	☺	**+3**
NF40	☺	😐	😐	☹	☺	☹	☺	**+1**
NF30	☺	☺	😐	☺	☺	😐	☺	**+5**
NF19	☺	☺	😐	☹	😐	☹	☺	**+1**
	+2	**+6**	**+1**	**-1**	**+7**	**-4**	**+9**	

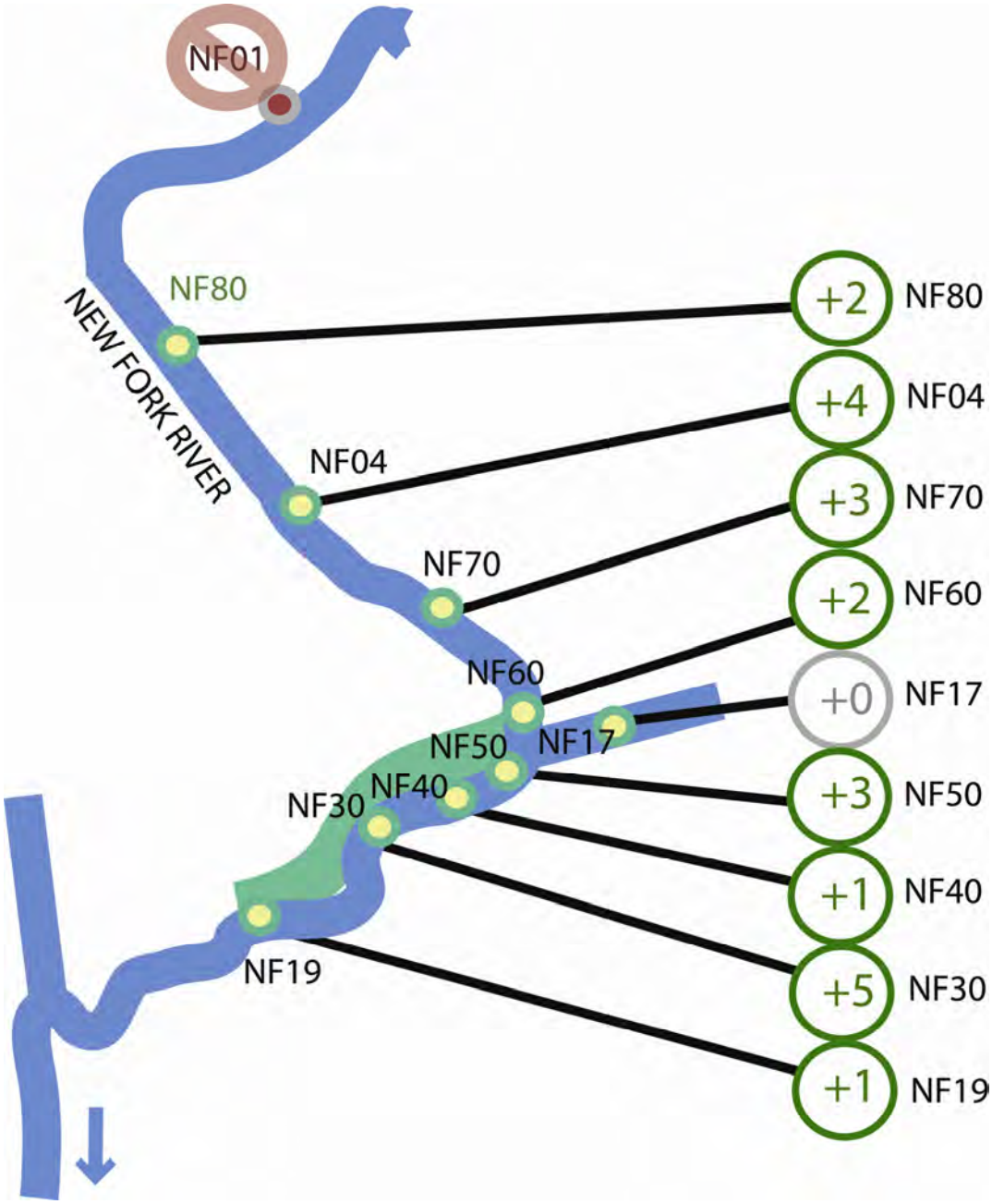

Figure 3.20. Schematic of Sites and Net Changes among Sites. We used the more liberal p-values (P< 0.10) and subtracted the number of measures that indicated declining conditions from those indicating improving conditions. This resulted in a net change score. Positive numbers indicate that more metrics indicated improved conditions in 2009, where as negative numbers indicate that more metrics indicated a decline in conditions in 2009. Note that the communities of none of the sites appeared to respond with a net decline in "quality."

Discussion

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 spatially-localized alterations of community structure which may be related to natural gas development in PAPA. Ultimately, all the ecological descriptors indicating significant changes all reflected one underling community-level response: a dramatic and sustained elevation of the relative abundance (and by extension, relative importance) of the oligochate worms of the genus *Nais*. The effect was primarily localized to one of the nine study sites, below a natural gas pipeline, and existed from 2004-2008. This report details the findings from the 2009 field survey.

Nais are usually associated with organic deposits in fine sediments and changes in their abundance can be related to changes in the quantity or quality of fine sediment deposits. The results of past surveys indicated that there were active sources of erosion between NF30 and NF40 and that these were significantly correlated with changes in the abundance of macroinvertebrates fauna. Additionally, there were three factors that indicated that development of the PAPA may have been related to these changes. First, the pipeline capacity was increased annually by adding additional pipelines across the New Fork River. Although the additional pipes were bored beneath the apparent stream bed, the process likely resulted in localized disturbance of the hyporheic zone. Second, during the study period there was an increase in the development of drilling platforms in riparian areas; these platforms are also likely to disturb hyporheic sediments and organisms. Finally, a third source of sediment may have occurred from direct surface erosion from around platforms; in 2008, most of the erosion controls implemented early in PAPA development had failed, and some new sites did not include erosion control measures at all.

2009 was the first year in nearly a decade to experience a "normal" sustained high flow from mountainous snow-melt. Spring run-off is an important natural phenomenon for western rivers and typically results in the sorting and redistribution of organic and inorganic substrata. Nine years of below normal flows may have prevented natural processes from mediating the effects of development in the PAPA. Thus, we anticipated that the results from the 2009 field survey would show a reduction in the abundance of *Nais* among sites and a net decrease in the number of significant differences in related ecological indicators.

The 2009 field survey described by this report collected eight 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 methods have been used to assess the New Fork River near the PAPA since 2007. This was the first year that trends could be assessed.

There was a significant reduction in the abundance of *Nais* at all sites in 2009. Most dramatic reductions occurred at the site that historically had the greatest abundance of non-insects (mostly *Nais*), NF30--below the pipeline crossing. *Nais* abundance fell from about 30-50% of the invertebrates sampled to about 5%, which is about normal for flowing North American freshwater ecosystems.

The results of the 2009 survey document the recovery of the New Fork River from multiple years of drought that may have exacerbated some of the effects from development in the PAPA. Although the changes exhibited by biota over recent years appear to have been

mediated by a single season of high spring run off, it is important to remember that prolonged and more extensive development, especially in the riparian zone, may cause significant alterations to the structure and function of aquatic food webs, which may affect the long term beneficial uses of the New Fork River.

The examination of multi-year trends at the site shows that every site except the East Fork River has had a net improvement in conditions in 2009 (positive metrics minus impaired-type changes in metrics). NF17 had 2 positive metrics (% scraper, and HBI) and 2 negative metrics (% collectors, % dominance). All other sites appeared to have improving water quality in 2009. The HBI improved at every site in 2009.

The single most important finding of this report is that non-insect dominance near the pipeline crossing is less of a concern that it ever has been in five years.

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Appendix 1. Operational taxonomic units for 2009.
Note that as the database is built these may change.

ORDER	FAMILY	Taxon	O.T.U.
EPHEM	**Baetidae**	**Baetidae**	Baetidae
EPHEM	**Baetidae**	*Acentrella sp.*	Acentrella
EPHEM	**Baetidae**	*Acentrella insignificans*	Acentrella
EPHEM	**Baetidae**	*Acentrella turbida*	Acentrella
EPHEM	**Baetidae**	Acerpenna pygmaea	Acerpenna
EPHEM	**Baetidae**	Acerpenna sp.	Acerpenna
EPHEM	**Ameletidae**	Ameletus sp.	Ameletus
EPHEM	**Leptohyphidae**	Asioplax sp.	Asioplax
EPHEM	**Ephemerellidae**	Attenella margarita	Attenella
EPHEM	**Ephemerellidae**	Attenella sp.	Attenella
EPHEM	**Baetidae**	Baetis bicaudatus	Baetis
EPHEM	**Baetidae**	Baetis flavistriga	Baetis
EPHEM	**Baetidae**	Baetis sp.	Baetis
EPHEM	**Baetidae**	Baetis tricaudatus	Baetis
EPHEM	**Caenidae**	Caenis youngi	Caenis
EPHEM	**Baetidae**	Callibaetis sp.	Callibaetis
EPHEM	**Baetidae**	Centroptilum sp.	Centroptilum
EPHEM	**Heptageniidae**	Cinygmula sp.	Cinygmula
EPHEM	**Baetidae**	Diphetor hageni	Diphetor
EPHEM	**Ephemerellidae**	Drunella coloradensis/flavilinea	Drunella
EPHEM	**Ephemerellidae**	Drunella doddsi	D. doddsi
EPHEM	**Ephemerellidae**	Drunella grandis	Drunella
EPHEM	**Ephemerellidae**	Drunella spinifera	Drunella
EPHEM	**Heptageniidae**	Epeorus albertae	Epeorus
EPHEM	**Heptageniidae**	Epeorus deceptivus	Epeorus
EPHEM	**Heptageniidae**	Epeorus grandis	Epeorus
EPHEM	**Heptageniidae**	Epeorus longimanus	Epeorus
EPHEM	**Heptageniidae**	Epeorus sp.	Epeorus
EPHEM	**Ephemeridae**	Ephemera sp.	Ephemera
EPHEM	**Ephemerellidae**	**Ephemerllidae**	Ephemerellidae
EPHEM	**Ephemerellidae**	Ephemerella inermis/infrequens	Ephemerella
EPHEM	**Ephemerellidae**	Ephemerella sp.	Ephemerella
EPHEM	**Heptageniidae**	Heptagenia sp.	Heptagenia
EPHEM	**Heptageniidae**	Heptageniidae	Heptageniidae
EPHEM	**Baetidae**	Heterocloeon sp.	Heterocloeon
EPHEM	**Leptophlebiidae**	Leptophlebiidae	Leptophebiidae
EPHEM	**Leptophlebiidae**	Leptophlebia sp.	Leptophebia
EPHEM	**Heptageniidae**	Nixe sp.	Nixe
EPHEM	**Leptophlebiidae**	Paraleptophlebia sp.	Paraleptophlebia
EPHEM	**Baetidae**	Plauditus sp.	Pladitus
EPHEM	**Heptageniidae**	Rhithrogena sp.	Rithogena
EPHEM	**Ephemerellidae**	Serratella tibialis	Serratella
EPHEM	**Leptohyphidae**	Tricorythodes sp.	Tricorythodes
ODONATA	**Coenagrionidae**	Coenagrionidae	Coenagrionidae
ODONATA	**Gomphidae**	Ophiogomphus sp.	Gomphidae

ODONATA	**Ophiogomphus**	Gomphidae	Gomphidae
PLECOP	**CAPNIIDAE**	Capniidae	Capniidae
PLECOP	**Chloroperlidae**	Chloroperlidae	Chloroperlidae
PLECOP	**Perlidae**	Claassenia sabulosa	Claassenia
PLECOP	**Perlodidae**	Cultus sp.	Cultus
PLECOP	**Perlidae**	Hesperoperla pacifica	Hesperoperla
PLECOP	**Perlodidae**	Isoperla sp.	Isoperla
PLECOP	**Nemouridae**	Malenka sp.	Malenka
PLECOP	**Perlodidae**	Perlodidae	Perlodidae
PLECOP	**Pteronarcyidae**	Pteronarcella sp.	Pteronarcella
PLECOP	**Pteronarcyidae**	Pteronarcys californica	Pteronarcys
PLECOP	**Pteronarcyidae**	Pteronarcys sp.	pteronarcys
PLECOP	**Perlodidae**	Skwala sp.	Skwala
PLECOP	**Perlodidae**	Sweltsa sp.	Sweltsa
PLECOP	**Taeniopterygidae**	Taeniopterygidae	Taeniopterygidae
PLECOP	**Nemouridae**	Zapada cinctipes	Zapada
PLECOP	**Nemouridae**	Zapada columbiana	Zapada
PLECOP	**Nemouridae**	Zapada oregonensis gr.	Zapada
HEMIP	**Corixidae**	Sigara sp.	Sigara
COLEO	**Dytiscidae**	Agabus sp.	Agabus
COLEO	**Dytiscidae**	Brychius sp.	Brychius
COLEO	**Elmidae**	Cleptelmis addenda	Cleptelmis
COLEO	**Hydrophilidae**	Enochrus sp.	Enochrus sp.
COLEO	**Hydrophilidae**	Helophorus sp.	Helophorus sp.
COLEO	**Hydrophilidae**	Laccobius sp.	Laccobius sp.
COLEO	**Hydraenidae**	Ochthebius sp.	Ochthebius sp.
COLEO	**Haliplidae**	Haliplus sp.	Haliplus
COLEO	**Dryopidae**	Helichus sp.	Helichus
COLEO	**Elmidae**	Heterlimnius sp.	Heterlimnius
COLEO	**Dytiscidae**	Liodessus sp.	Liodessus
COLEO	**Elmidae**	Narpus sp.	Narpus
COLEO	**Elmidae**	Optioservus sp.	Optioservus
COLEO	**Dytiscidae**	Oreodytes sp.	Oreodytes
COLEO	**Elmidae**	Zaitzevia sp.	Zaitzevia
MEGALO	**Sialidae**	Sialis sp.	Sialis
DIPTERA	**Chironomidae**	Ablabesmyia sp.	Chironomidae*
DIPTERA	**Chironomidae**	Boreoheptagyia sp.	Chironomidae*
DIPTERA	**Chironomidae**	Brillia sp.	Chironomidae*
DIPTERA	**Chironomidae**	Cardiocladius sp.	Chironomidae*
DIPTERA	**Chironomidae**	Chaetocladius sp.	Chironomidae*
DIPTERA	**Chironomidae**	Cladotanytarsus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Corynoneura sp.	Chironomidae*
DIPTERA	**Chironomidae**	Cricotopus (Nostoc.) nostocicola	Chironomidae*
DIPTERA	**Chironomidae**	Cricotopus bicinctus gr.	Chironomidae*
DIPTERA	**Chironomidae**	Cricotopus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Cricotopus trifascia gr.	Chironomidae*
DIPTERA	**Chironomidae**	Cryptochironomus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Diamesa sp.	Chironomidae*
DIPTERA	**Chironomidae**	Eukiefferiella brehmi gr.	Chironomidae*
DIPTERA	**Chironomidae**	Eukiefferiella claripennis gr.	Chironomidae*

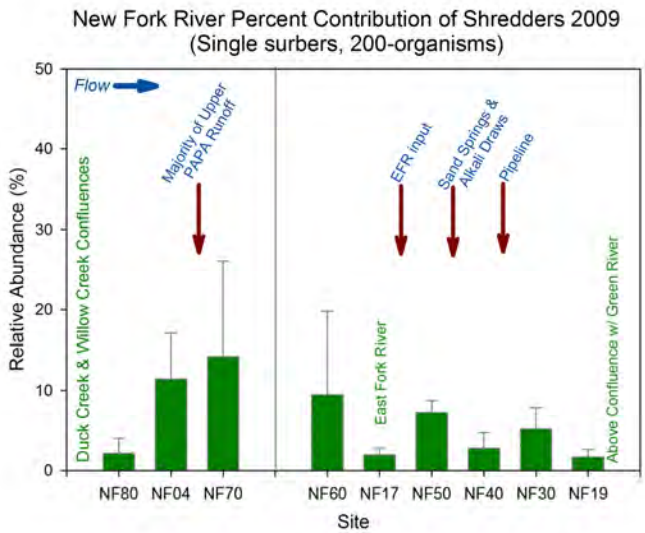
DIPTERA	**Chironomidae**	Eukiefferiella coerulescens gr.	Chironomidae*
DIPTERA	**Chironomidae**	Eukiefferiella devonica gr.	Chironomidae*
DIPTERA	**Chironomidae**	Eukiefferiella gracei gr.	Chironomidae*
DIPTERA	**Chironomidae**	Eukiefferiella sp.	Chironomidae*
DIPTERA	**Chironomidae**	Euryhapsis sp.	Chironomidae*
DIPTERA	**Chironomidae**	Heleniella sp.	Chironomidae*
DIPTERA	**Chironomidae**	Hydrobaenus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Lopescladius sp.	Chironomidae*
DIPTERA	**Chironomidae**	Micropsectra sp.	Chironomidae*
DIPTERA	**Chironomidae**	Micropsectra/Tanytarsus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Microtendipes pedellus gr.	Chironomidae*
DIPTERA	**Chironomidae**	Nanocladius sp.	Chironomidae*
DIPTERA	**Chironomidae**	Odontomesa sp.	Chironomidae*
DIPTERA	**Chironomidae**	Orthocladius (Euortho.) rivulorum	Chironomidae*
DIPTERA	**Chironomidae**	Orthocladius (Euorthocladius) sp.	Chironomidae*
DIPTERA	**Chironomidae**	Orthocladius Complex	Chironomidae*
DIPTERA	**Chironomidae**	Orthocladius sp.	Chironomidae*
DIPTERA	**Chironomidae**	Pagastia sp.	Chironomidae*
DIPTERA	**Chironomidae**	Parakiefferiella sp.	Chironomidae*
DIPTERA	**Chironomidae**	Paralauterborniella nigrohalteralis	Chironomidae*
DIPTERA	**Chironomidae**	Parametriocnemus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Paratanytarsus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Pentaneura sp.	Chironomidae*
DIPTERA	**Chironomidae**	Pentaneurini	Chironomidae*
DIPTERA	**Chironomidae**	Phaenopsectra sp.	Chironomidae*
DIPTERA	**Chironomidae**	Polypedilum sp.	Chironomidae*
DIPTERA	**Chironomidae**	Potthastia gaedii gr.	Chironomidae*
DIPTERA	**Chironomidae**	Potthastia longimana gr.	Chironomidae*
DIPTERA	**Chironomidae**	Procladius sp.	Chironomidae*
DIPTERA	**Chironomidae**	Pseudochironomus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Rheocricotopus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Rheosmittia sp.	Chironomidae*
DIPTERA	**Chironomidae**	Rheotanytarsus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Stempellina sp.	Chironomidae*
DIPTERA	**Chironomidae**	Stempellinella sp.	Chironomidae*
DIPTERA	**Chironomidae**	Stictochironomus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Sublettea sp.	Chironomidae*
DIPTERA	**Chironomidae**	Synorthocladius sp.	Chironomidae*
DIPTERA	**Chironomidae**	Tanytarsus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Thienemanniella sp.	Chironomidae*
DIPTERA	**Chironomidae**	Thienemannimyia gr. sp.	Chironomidae*
DIPTERA	**Chironomidae**	Tribelos jucundum	Chironomidae*
DIPTERA	**Chironomidae**	Tvetenia bavarica gr.	Chironomidae*
DIPTERA	**Chironomidae**	Tvetenia discoloripes gr.	Chironomidae*
DIPTERA	**Tipulidae**	Antocha sp.	Antocha
DIPTERA	**Athericidae**	Atherix sp.	Atherix
DIPTERA	**Ceratopogonidae**	Bezzia/Palpomyia sp.	Bezzia/Palpomyia sp.
DIPTERA	**Stratiomyidae**	Caloparyphus sp.	Caloparyphus sp.

DIPTERA	**Ceratopogonidae**	Ceratopogoninae	Ceratopogoninae
DIPTERA	**Empididae**	Chelifera/Metachela sp.	Chelifera/Metachela sp.
DIPTERA	**Empididae**	Clinocera sp.	Clinocera sp.
DIPTERA	**Tipulidae**	Cryptolabis sp.	Cryptolabis sp.
DIPTERA	**Ceratopogonidae**	Dasyhelea sp.	Dasyhelea sp.
DIPTERA	**Tipulidae**	Dicranota sp.	Dicranota sp.
DIPTERA	**Empididae**	Hemerodromia sp.	Hemerodromia sp.
DIPTERA	**Tipulidae**	Hexatoma sp.	Hexatoma sp.
DIPTERA	**Tipulidae**	Limnophila sp.	Limnophila sp.
DIPTERA	**Muscidae**	Muscidae	Muscidae
DIPTERA	**Empididae**	Neoplasta sp.	Neoplasta sp.
DIPTERA	**Tipulidae**	Pedicia sp.	Pedicia sp.
DIPTERA	**Psycodidae**	Pericoma/Telmatoscopus sp.	Pericoma
DIPTERA	**Ceratopogonidae**	Probezzia sp.	Probezzia
DIPTERA	**Tipulidae**	Rhabdomastix fascigera gr.	Rhabdomastix
DIPTERA	**Sciomyzidae**	Sciomyzidae	Sciomyzidae
DIPTERA	**Simuliidae**	Simulium sp.	Simulium
DIPTERA	**Tipulidae**	Tipula sp.	Tipula
DIPTERA	**Empididae**	Wiedemannia sp.	Wiedemannia
TRICHOP	**Brachycentridae**	Amiocentrus aspilus	Amiocentrus
TRICHOP	**Apatanidae**	Apatania sp.	Apatania
TRICHOP	**Arctopsychidae**	Arctopsyche grandis	Arctopsyche grandis
TRICHOP	**Brachycentridae**	Brachycentridae	Brachycentridae
TRICHOP	**Brachycentridae**	Brachycentrus americanus	Brachycentrus
TRICHOP	**Brachycentridae**	Brachycentrus occidentalis	Brachycentrus
TRICHOP	**Leptoceridae**	Ceraclea sp.	Ceraclea
TRICHOP	**Hydropsychidae**	Cheumatopsyche sp.	Cheumatopsyche
TRICHOP	**Glossomatidae**	Culoptila sp.	Culoptila
TRICHOP	**Limnephilidae**	Dicosmoecus gilvipes	Dicosmoecus gilvipes
TRICHOP	**Philopotamidae**	Dolophilodes sp.	Dolophilodes
TRICHOP	**Glossomatidae**	Glossosoma sp.	Glossosoma
TRICHOP	**Helicopsychidae**	Helicopsyche sp.	Helicopsyche
TRICHOP	**Hydropsychidae**	Hydropsyche sp.	Hydropsyche
TRICHOP	**Hydroptilidae**	Hydroptila sp.	Hydroptila
TRICHOP	**Hydroptilidae**	Hydroptilidae	Hydroptilidae
TRICHOP	**Lepidosomatidae**	Lepidostoma sp.	Lepidostoma
TRICHOP	**Brachycentridae**	Micrasema sp.	Micrasema
TRICHOP	**Leptoceridae**	Nectopsyche sp.	Nectopsyche
TRICHOP	**Limnephilidae**	Neothremma sp.	Neothremma
TRICHOP	**Hydroptilidae**	Neotrichia sp.	Neotrichia
TRICHOP	**Hydroptilidae**	Ochrotrichia sp.	Ochrotrichia
TRICHOP	**Leptoceridae**	Oecetis avara	Oecetis
TRICHOP	**Leptoceridae**	Oecetis disjuncta	Oecetis
TRICHOP	**Leptoceridae**	Oecetis sp.	Oecetis
TRICHOP	**Limnephilidae**	Oligophlebodes sp.	Oligophlebodes
TRICHOP	**Hydroptilidae**	Oxyethira sp.	Oxyethira
TRICHOP	**Hydropsychidae**	Parapsyche elsis	Parapsyche elsis
TRICHOP	**Glossomatidae**	Protoptila sp.	Protoptila
TRICHOP	**Rhyacophilidae**	Rhyacophila brunnea gr.	Rhyacophila
TRICHOP	**Rhyacophilidae**	Rhyacophila coloradensis gr.	Rhyacophila

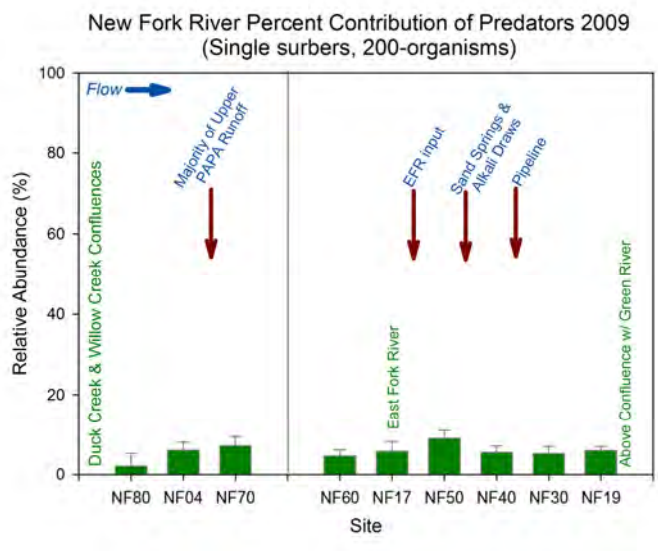
TRICHOP	**Rhyacophilidae**	Rhyacophila hyalinata gr.	Rhyacophila
TRICHOP	**Rhyacophilidae**	Rhyacophila pellisa/valuma	Rhyacophila
TRICHOP	**Rhyacophilidae**	Rhyacophila sp.	Rhyacophila
TRICHOP	**Rhyacophilidae**	Rhyacophila vofixa gr.	Rhyacophila
LEPIDOP	**Pyralidae**	Petrophila sp.	Petrophila
NONI	**Gastropoda**	Ferrissia sp.	Ferrissia
NONI	**Gastropoda**	Fluminicola sp.	Fluminicola
NONI	**Gastropoda**	Fossaria sp.	Fossaria
NONI	**Gastropoda**	Gyraulus sp.	Gyraulus
NONI	**Gastropoda**	Lymnaeidae	Lymnaeide
NONI	**Gastropoda**	Physa sp.	Physa
NONI	**Gastropoda**	Planorbidae	Planorbidae
NONI	**Gastropoda**	Valvata lewisi	Valvata lewisi
NONI	**Bivalva**	Pisidium sp.	Sphaeriidae
NONI	**Bivalva**	Sphaeriidae	Sphaeriidae
NONI	**Bivalva**	Sphaerium sp.	Sphaeriidae
NONI	**Bivalva**	Unionacea	Unionacea
NONI	**Oligo**	Chaetogaster diastrophus	Chaetogaster
NONI	**Oligo**	Enchytraeidae	Enchytraeidae
NONI	**Oligo**	Erpobdellidae	Erpobdellidae
NONI	**Hirudinea**	Glossiphonia complanata	Glossiphonia
NONI	**Oligo**	Haplotaxis sp.	Haplotaxis
NONI	**Hirudinea**	Helobdella stagnalis	Helobdella stagnalis
NONI	**Oligo**	Lumbricina	Lumbricina
NONI	**Oligo**	Lumbriculidae	Lumbriculidae
NONI	**Oligo**	Nais behningi	Nais
NONI	**Oligo**	Nais bretscheri	Nais
NONI	**Oligo**	Nais sp.	Nais
NONI	**Oligo**	Ophidonais serpentina	Ophidonais serpentina
NONI	**Oligo**	Slavina appendiculata	Slavina appendiculata
NONI	**Oligo**	Tubificidae w/ cap setae	Naididae (Tubificidae)
NONI	**Oligo**	Tubificidae w/o cap setae	Naididae (Tubificidae)
NONI	**Acari**	Acari	Acari
NONI	**Acari**	Atractides sp.	Acari
NONI	**Acari**	Aturus sp.	Acari
NONI	**Acari**	Hygrobates sp.	Acari
NONI	**Acari**	Lebertia sp.	Acari
NONI	**Acari**	Oribatei	Acari
NONI	**Acari**	Protzia sp.	Acari
NONI	**Acari**	Sperchon sp.	Acari
NONI	**Acari**	Sperchonopsis sp.	Acari
NONI	**Acari**	Torrenticola sp.	Acari
NONI	**Amphipoda**	Gammarus sp.	Gammarus sp.
NONI	**Amphipoda**	Hyalella sp.	Hyalella sp.
NONI	**Nematoda**	Nematoda	Nematoda
NONI	**Turbellaria**	Polycelis sp.	Polycelis sp.
	Turbellaria	Turbellaria	Turbellaria

*Rabeni and Wang. 2001. Bioassessment of streams using macroinvertebrates: are Chironomidae necessary? *Environmental Monitoring and Assessment* 71: 177–185, 2001

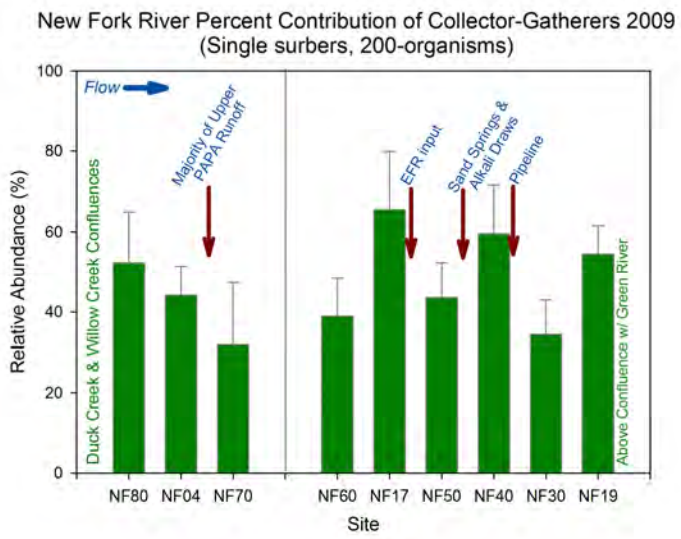
APPENDIX 2: Supplemental Graphs. The relative abundance of different functional feeding groups is wrought with problems of non-independence of observation and multicolinearity. For this reason we decided, *a priori*, to examine collectors and scrapers. Still, for diagnostic purposes, it is useful to consider which groups went up when others went down. For this reason, we added these graphs to enable interested readers to infer the types of changes that accompanied changes in the metrics discussed in the main body of the report.



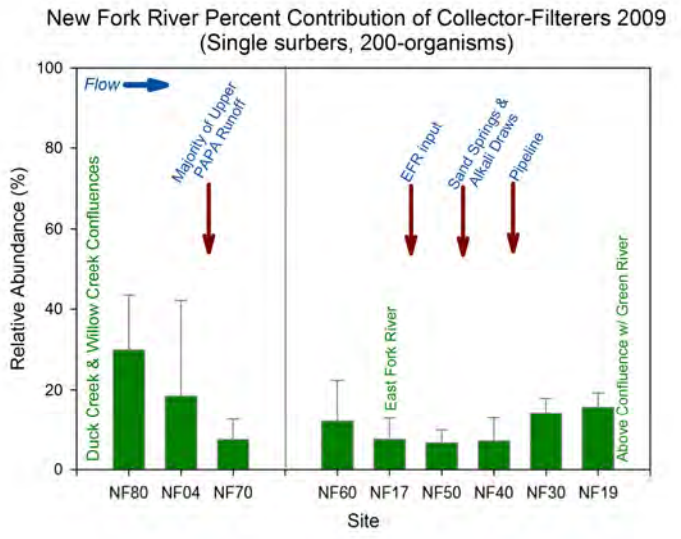
Appendix Figure 2.1. Percent Shredders. The percent contribution of shredders to benthic communities of the New Fork River in 2009. Error bars are 95% confidence intervals.



Appendix Figure 2.2 Percent Predators. The percent contribution of predators to benthic communities of the New Fork River in 2009. Error bars are 95% confidence intervals.



Appendix Figure 2.3. Percent Collector-Gatherers. The percent contribution of collector-gatherers to benthic communities of the New Fork River in 2009. Error bars are 95% confidence intervals.



Appendix Figure 2.4. Percent Collector-Filterers. The percent contribution of collector-filterers to benthic communities of the New Fork River in 2009. Error bars are 95% confidence intervals.