# A PILOT ASSESSMENT OF THE ASSOCIATION BETWEEN STONEFLY ASSEMBLAGES AND THE INCIDENCE AND SEVERITY OF WHIRLING DISEASE IN TRIBUTARIES OF THE BLACKFOOT RIVER, MONTANA

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## Introduction

This brief study was intended to investigate whether stonefly assemblages could be useful bellwethers of the presence or severity of whirling disease in streams tributary to the Blackfoot River. At least one previous study (Bollman 1998) demonstrated associations between stonefly richness and certain observational measures related to reach-scale habitat integrity; these measures included streambank stability, condition of the riparian zone, and stream channel morphological elements. Loss of streambank stability, riparian zone integrity, and natural channel morphology may contribute to instream conditions favoring the presence of *Tubifex tubifex*, the oligochaete intermediate host for the whirling disease organism (*Myxobolus cerebralis*). Thus, we hypothesize that metrics describing stonefly assemblages may be useful in predicting the presence and severity of whirling disease.

## Methods

Benthic invertebrates were sampled on July 26-27, 2006 from single riffles in each of 13 tributary streams of the Blackfoot River. A D-frame net with 1000 micron mesh was used. Substrates were disturbed by kicking along transects; sampling effort was timed and distance approximated by stepping off. Table 1 lists sampling sites, the time expended for each sample, and the approximate distance over which substrates were disturbed. Samples were preserved in 95% ethanol at streamside, and delivered to Rhithron Associates in Missoula for sorting and identification of organisms.

In the laboratory, samples were sorted under dissecting stereoscopes, using 10x – 30x magnification. A random selection of 500 organisms was taken from each sample; stoneflies collected in these subsamples were separated from the remaining organisms and preserved. All stoneflies remaining in each sample were then collected, and these were preserved separately.

Stoneflies from both the random subsample and the total sample collection were identified using published keys; specimens were identified to the lowest taxonomic level possible considering the maturity of the animals and the availability of appropriate keys. Generally, at least genus level was achieved; in many cases, species could be determined. Early-instar capniids were left at family level. Two samples yielded a total of 6 extremely immature specimens. These were identified to family level, but were not considered in the subsequent analysis since it was not possible to determine whether they represented unique taxa or were early instars of taxa already included in the taxa lists.

No further analysis of the 500 organism random subsamples was performed, other than the inclusion of stoneflies from those subsamples in the present exploration. All sample fractions were preserved and retained at Rhithron for possible further analysis.

Physical and chemical data as well as data related to incidence (percent of reaches with infection >3 in 2005) and severity (mean MacConnell-Baldwin scale value in 2005) of whirling disease in each stream were collected and compiled by Montana Fish Wildlife and Parks personnel. Correlation matrices (Spearman rank R) were constructed using these data and stonefly data, and these matrices were examined for suggestive associations. In all, 18 metric expressions summarizing the stonefly data were analyzed for correlation with the 2 whirling disease measures. Data from Bear Creek was deleted from the data set, since the status of whirling disease in that stream in 2005 was not known at the time of this study.

## Results

Nineteen stonefly taxa in 7 families were present in the 13 samples. A total of 1348 stoneflies were identified.

Figures 1 and 2 illustrate correlation between stonefly taxa richness and 2 measures of whirling disease incidence and severity. Correlations were not significant, but Chamberlain Creek clearly presents as an outlier in these analyses. When Chamberlain Creek was removed from the dataset, correlation between stonefly taxa richness and measures of whirling disease severity and incidence were significant. ($R= -0.711143$, $p < 0.05$ and $R=-0.736352$, $p < 0.05$).

Three functional feeding groups were represented in the stonefly collection from the 13 samples: shredders were represented by 8 taxa, predators by 10 taxa, and collectors by 1 taxon. There was a significant association of predatory taxa richness with infection severity ($R= -0.616833$, $p < 0.05$), but not with infection incidence. This relationship is illustrated in Figure 3. Other significant correlative relationships were demonstrated between whirling disease severity and sensitive stonefly taxa richness and abundance, and between sensitive taxa richness and richness within the family Chloroperlidae and whirling disease incidence. However, neither sensitive taxa nor Chloroperlid taxa were well-distributed among these sites.

No other significant associations could be demonstrated between either measure of whirling disease and measures of richness, relative abundance, or absolute abundance of various stonefly families, functional groups, or tolerance characteristics.

Several taxa were collected only in streams with no incidence (i.e. 0% of reaches with >3 on the MacConnell-Baldwin scale) of whirling disease. These were the nemourids *Visoka cataractae* (collected from 2 sites) and *Zapada oregonensis* (one site), the perlid *Calineuria californica* (2 sites), the taeniopterygid *Taeniopteryx* sp. (one site), and perlodids *Isoperla* sp. (one site), *Kogotus* sp. (2 sites), and *Megarcys* sp. (4 sites). It should be noted that *Kogotus* sp. was collected from Arrasta Creek, which had a low mean severity rating (0.02). The low severity rating despite 0% of reaches severely infected suggests that whirling disease probably is present though not widespread in Arrasta Creek. Two taxa were collected only from streams with whirling disease infection: *Pteronarcella badia* (2 sites) and *Claassenia sabulosa* (2 sites).

**Discussion**

Although significant trends could be demonstrated, none of the correlative relationships explored in this study gave results definitive enough to support the hypothesis that characteristics of stonefly assemblages can predict the incidence or severity of whirling disease as measured here. In each analysis, there is considerable overlap of results between infected and uninfected streams.

It seemed justifiable to delete Chamberlain Creek from the correlation analyses, since there are some unique conditions in that watershed immediately upstream of the sampling location. As noted, this site presents as an outlier; despite high incidence of infection in this stream, the sample collected here yielded the highest stonefly taxa richness of any sample in this study. Historic reconstruction that reclaimed altered channel may have resulted in ideal habitat conditions for stoneflies. Reconstructed reaches were located immediately upstream of the sampling location. In addition, two artificial upstream ponds that drain into Chamberlain Creek in the immediate upstream area. These ponds are thought to harbor *t. tubifex* "hotspot" conditions such as warmer water effluent and organic sediments used to line the ponds.

Since there were some taxa that may have been confined to uninfected streams, further investigation of potential indicator taxa in the family Perlodidae may be promising. Further, the taxa that were identified in this study as potential indicators will be relatively easy to identify streamside, if care is taken to sample at appropriate times of year.

## Tables and Figures

**Table 1.** Summary of sampling events: July 2005.

| Sampling date | Waterbody | Time expended (min: secs) | Distance | Description of effort |
|---|---|---|---|---|
| 7/27/2006 | Gold Creek | 6:20 | 36 feet | Single transect (riffle) |
| 7/27/2006 | Bear Creek | 6:20 | 36 feet | Single diagonal transect (riffle) |
| 7/27/2006 | W. Twin Creek | 6:15 | 36 feet | Triple diagonal transect (riffle) |
| 7/27/2006 | E. Twin Creek | 6:20 | 36 feet | Triple diagonal transect (riffle) |
| 7/26/2006 | Cottonwood Creek | 5:00 | 36 feet | Single diagonal transect riffle |
| 7/26/2006 | Monture Creek | 5:30 | 36 feet | Single transect (riffle) |
| 7/26/2006 | Chamberlain Creek | 8:10 | 36 feet | Triple transect (riffle) |
| 7/26/2006 | Arrastra Creek | 6:40 | 36 feet | Triple diagonal transect (riffle) |
| 7/27/2006 | Belmont Creek | 6:04 | 28 feet | Single transect (riffle) |
| 7/26/2006 | Blanchard Creek | 7:15 | 27 feet | Double transect (riffle) |
| 7/26/2006 | Landers Fork | 6:20 | 36 feet | Single transect (riffle) |
| 7/27/2006 | Johnson Creek | 6:00 | 36 feet | Double diagonal transect (riffle) |
| 7/26/2006 | Elk Creek | 6:30 | 36 feet | Triple diagonal transect (riffle) |

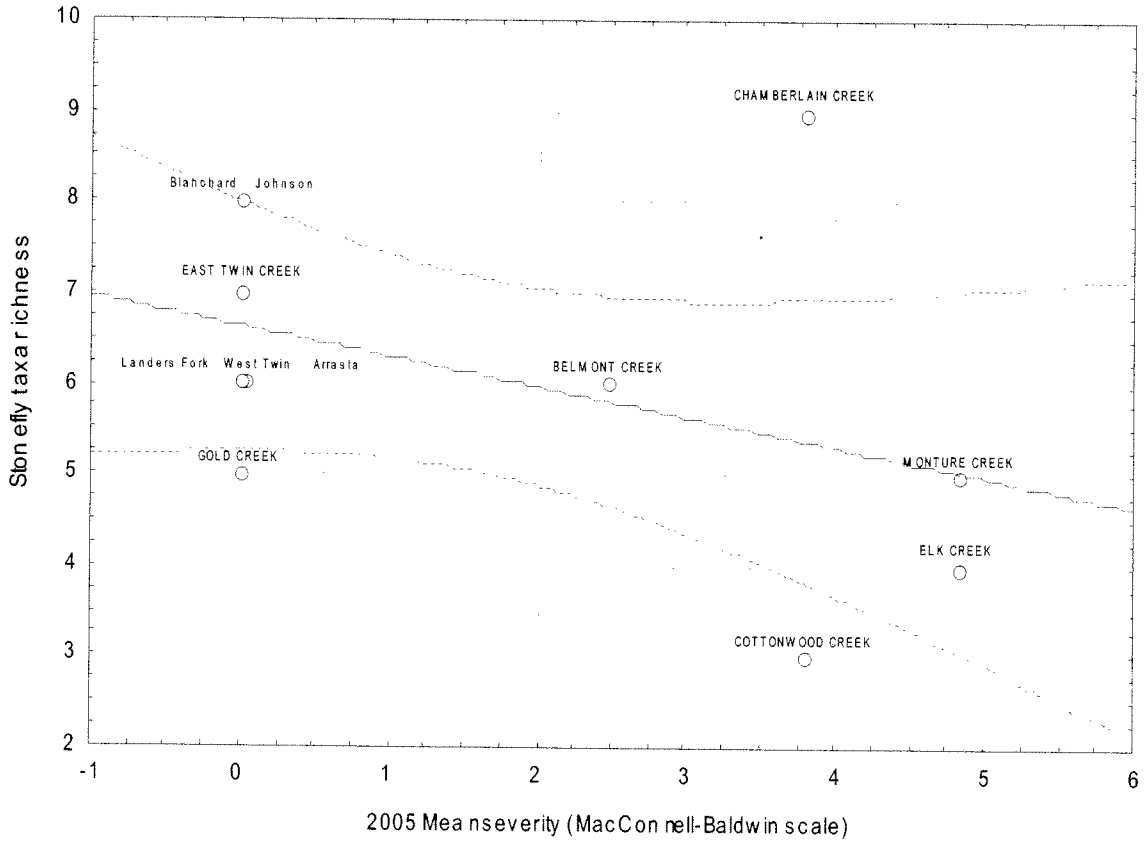


**Figure 1.** Association between stonefly taxa richness and severity of whirling disease (R=-0.46, $p$ >0.05).

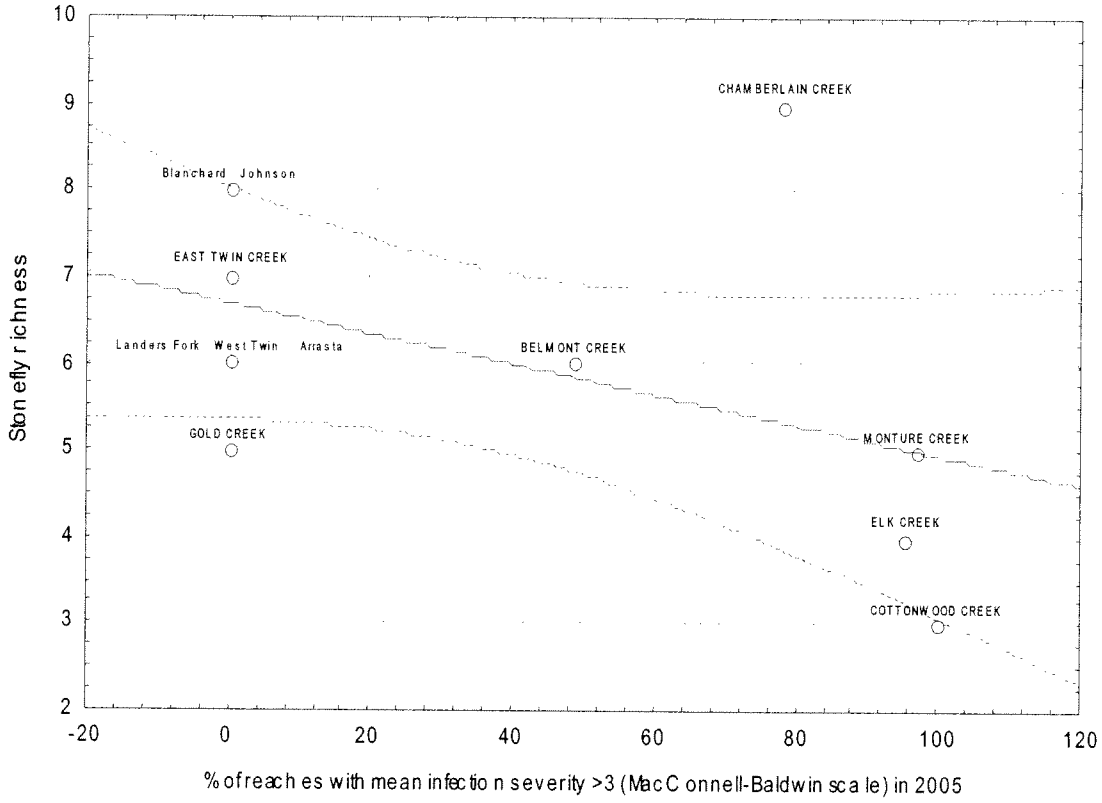


**Figure 2.** Association between stonefly taxa richness and incidence of whirling disease. (R=-0.51, $p > 0.05$).

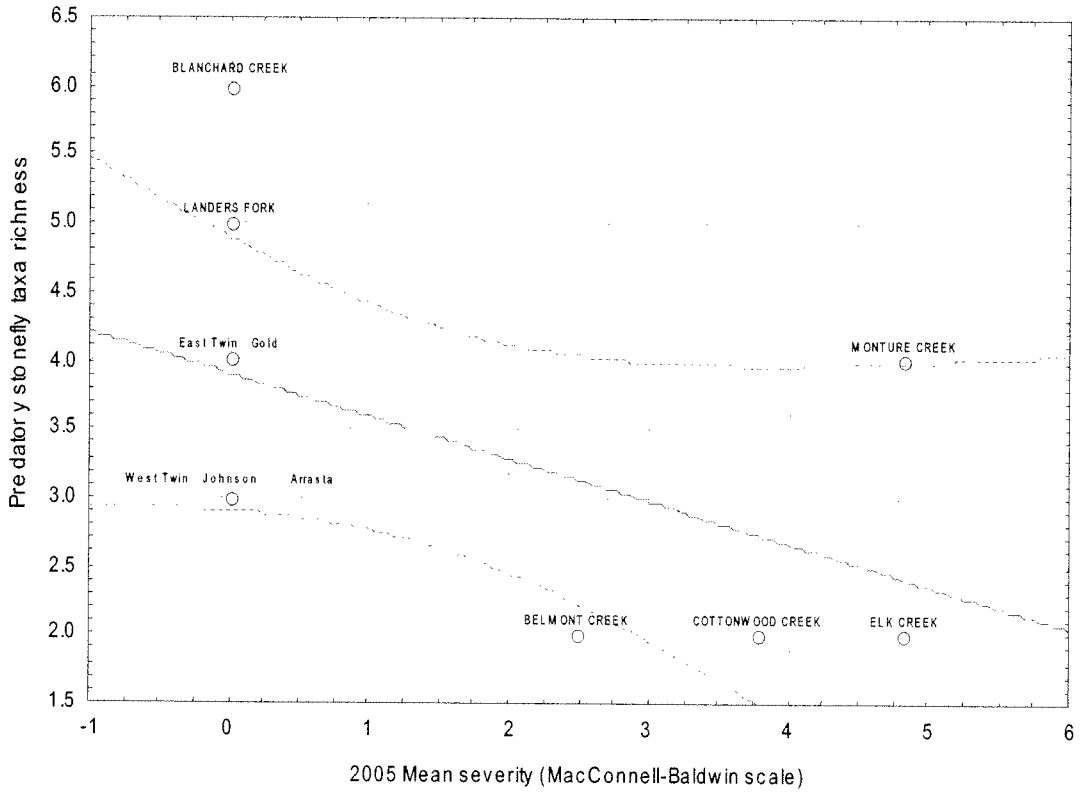


**Figure 3.** Association between predatory stonefly taxa richness and severity of whirling disease. (R=-0.51, $p > 0.05$). Chamberlain Creek was not included in this analysis.