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Species Diversity of Black Flies (Diptera: Simuliidae) in Relation to Stream Size Submitted

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Species Diversity of Black Flies (Diptera: Simuliidae)

in Relation to Stream Size

Submitted in Partial Fulfillment of the Requirements for Graduation With Honors to the Department of Natural Sciences at Carroll College, Helena, Montana

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26 March 2002
This thesis for honors recognition has been approved for the Department of Natural Sciences by:

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Dr. Mary Keeffe  Date
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Abstract

Black flies have been studied extensively because they: have an essential world-wide distribution, require a blood meal from humans and other animals to reproduce, transmit blood-bound diseases, have diverse sibling species differentiated by chromosomal rearrangements associated with sex and act as an important food source in fresh water ecosystems. Reproduction is universally tied to fresh water streams in which eggs, larvae, pupae and adults develop. Despite the large number of studies of black fly ecology, genetics and parasitology, essentially nothing is known about factors that determine species diversity of black flies at specific locations. I tested the hypothesis that large streams would have greater species diversity of black flies than medium-sized or small streams by collecting black fly larvae from six streams in west-central Montana (Missouri River and Yellowstone River (large streams); Little Prickly Pear Creek and the Gallatin River (medium-sized streams) and Ten Mile Creek and Camp Creek (small streams) at two-week intervals throughout the summer of 2001. Although I have only two replicates for each of the three size categories, the highest species diversity was observed in medium-sized and small streams and not in large streams as I had predicted. Possibly, small and medium sized streams provide more diverse microhabitats throughout the year than do large streams. Although all species of black fly in this study had been previously described for the province of Alberta, Canada, a new chromosomal cytotype designated, Simulium arcticum IIL-15, was described from both the Yellowstone and Gallatin rivers.
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Introduction

Black flies (Diptera: Simuliidae) are an important source of food for animals in fresh water streams (Dimick and Mote, 1934; Elliot, 1967 and 1973). They are important to medicine due to their role as vectors of disease. A vector of *Onchocerciasis*, also known as African River Blindness, they affect 18 million people in over 36 countries (WHO, 2001). Black flies are also pests whose females require a blood meal to reproduce (Fallis *et al.*, 1974). They are of economic importance due to their effect on livestock as evidenced in northern Alberta, Canada. In a 53,245 hectare survey area nearly $500,000 worth of cattle were lost in 1971 due to harassment, loss of blood, particularly in the young, and secondary infections as a result of bites by black flies (Laird, 1981). Black flies possess giant, polytene chromosomes in larval salivary glands (Rothfels and Freeman, 1966) that exhibit tremendous micromorphological detail from which chromosomal variants (additions, deletions, paracentric inversions, interchanges and centric shifts) have been comparatively deduced (Rothfels, 1979). Moreover, paracentric inversions tend to include sex-determining segments, which differentiate sibling species of black flies in sympatry (Rothfels and Featherston, 1981; Shields and Procurier, 1982).

Simuliids are distributed world-wide with the exception of polar regions and islands lacking freshwater streams (Fallis *et al.*, 1974). Eggs are laid in freshwater streams in summer or fall and either emerge that year or overwinter as eggs or larvae.
Though numerous studies of black flies exist, little is known concerning determinants of local species diversity (Currie, pers. comm.). Many factors may play a role in determining species diversity. I examined whether stream size affected species diversity in black flies and monitored diversity in small, medium and large streams. One might assume that large rivers would exhibit the greatest species diversity at any location since they may be expected to be more diverse in microhabitats. However, Currie (pers. comm.) suggested that he tended to find greater species diversity in small streams but had not tested this hypothesis directly. I compared the population of black fly larvae in small, medium and large streams from May through early August during the summer of 2001. I hypothesized that large streams would have greater species diversities than small streams based on the assumption that the former may be more diverse ecologically.
Materials and Methods

Site Selection:

The purpose of this study was to determine if stream size was correlated with species diversity in black flies during summer. Consequently, I arbitrarily selected sites at large, medium and small sized streams. I choose streams which appeared to be large, medium and small in width. No actual physical characteristics (e. g. width of stream, or flow volume) were measured to categorize stream size. I sampled streams in the three categories in the same way throughout the summer. To better represent these three categories streams of similar size were selected in different regions of west-central Montana. I chose the Missouri River (Lewis and Clark County) and Yellowstone River (Park County) as large-sized streams; the Gallatin River (Gallatin County) and Little Prickly Pear Creek (Lewis and Clark County) as the medium-sized streams and Camp Creek (Gallatin County) and Ten Mile Creek (Lewis and Clark County) as the small-sized streams (Fig. 1).

Sample Collection:

On May 15, 2001 I began collecting and returned to each of the six sites at two-week intervals until Aug. 6, 2001 when I terminated my collections. A collection bout was defined as a 30-minute period during which I surveyed the stream for any and all evidence of black flies (larvae, pupa and pupal cases). All submerged microhabitats including rocks, branches of trees, trailing vegetation, etc. were sampled. Individual specimens were removed from their attachment sites with fine forceps and placed immediately into a fresh solution of Carnoy fixative (a 3:1 mixture of cold absolute
ethanol and glacial acetic acid) in a 45 mL screw cap scintillation vial. The exact same location at each stream was sampled during subsequent collections throughout the summer and each site was sampled in the same way.

Determination of Species Diversity:

Individual larvae and pupae were observed under an Olympus Zoom dissecting microscope and keyed to species. Species identifications were made on the basis of a variety of physical characteristics including: shapes of dorsal head patterns, shapes of ventral postgenial clefts and the numbers and shapes of larval respiratory filaments of the gill histoblast. The monograph, *An Annotated List of and Keys to the Immature Black Flies of Alberta (Diptera: Simuliidae)* (Currie, 1986) was used to aid in species identification. Species identity, numbers of individual larvae and numbers of individual pupae for each species were determined for each sample at each site.
Results

Large-sized Rivers:

Species identities and frequencies of individuals for each species identified at each of the seven collection dates are shown for the Missouri and Yellowstone rivers (Figure 2.).

Species diversity was low for each of these large rivers throughout the summer with only *S. vittatum* and *S. arcticum* being present. A major emergence of *S. vittatum* or *S. arcticum* was evident in each river, Missouri and Yellowstone respectively, with peak numbers of larvae occurring on June 24 in the Missouri River and on June 10 in the Yellowstone River. However, my data suggest the possibility of at least two separate emergences of these species at these rivers since the 82 larvae collected in the Missouri and the 235 larvae collected in the Yellowstone may have been at the terminal and beginning distributions of additional generations, respectively. Apparently, *S. arcticum* occurs at extremely low numbers in the Missouri while *S. vittatum* is scarce in the Yellowstone. (Figure 2).

Medium Sized Rivers:

Five species of black fly were observed in Little Prickly Pear Creek and four species were observed in the Gallatin River (Figure 3). Moreover, four species (*S. arcticum, S. vittatum, S. tuberosum and S. decorum*) were common to both streams. *S. canonicolum* was only found in Little Prickly Pear Creek and only at low frequencies (Figure 3). It is possible that at least two generations of *S. arcticum* occur at Little Prickley Pear Creek
since larger numbers of larvae were collected on May 15 than on May 29 (Figure 3). The emergence of *S. vittatum* in each of these streams may peak in middle to late August since numbers of *S. vittatum* were highest on August 6.

Small Sized Streams:

Five species of black fly were observed at 10 Mile Creek and four species were observed at Camp Creek (Figure 4). Species diversity of black flies at 10 Mile Creek was identical to that at Little Prickly Pear Creek with the same five species occurring at both sites (Figs. 3 and 4). Moreover, species diversity at Camp Creek was identical to that of the Gallatin River (Figs. 3 and 4). *S. arcticum* predominates at all four sites (Figs. 3 and 4). Two generations of *S. arcticum* at 10 Mile Creek and two generations of *S. vittatum* at Camp Creek may occur since numbers of larvae collected on August 6 appear to be increasing.

New Cytotype:

A new cytotype of *S. arcticum*, IIL-15, was found in the Yellowstone and Gallatin rivers (Shields, pers. comm.).
Discussion

My original hypothesis was that larger streams might have higher species diversities than smaller streams. The two large streams I sampled, the Missouri and Yellowstone rivers, each had two species with a different species being dominant throughout the collection period. In stark contrast, the two small streams, Ten Mile Creek and Camp Creek, had five and four species, respectively. The medium streams, Little Prickly Pear Creek and the Gallatin River, also had five and four species, respectively. These observations suggest that I must reject my hypothesis that larger streams would have greater species diversity than smaller streams. A more rigorous test of this hypothesis would involve the sampling of larger numbers of large and small streams as well as measuring physical features of the streams, which might suggest other factors that might influence species diversity.

Heterogeneity of the environment may also be an important ecological component of species diversity in black flies. MacArthur (1961) found that habitat heterogeneity is positively correlated to species diversity. This means that if there are different niches for each species more than one can coexist. Throughout my collections I found *S. vittatum* attached more predominantly to rocks in the stream bed while I found *S. arcticum* primarily on tree branches and trailing vegetation. The remaining species appeared to be randomly distributed on rocks, branches and trailing vegetation of the collection sites. This may indicate that if an area has many attachment possibilities there may be more
species at that site. A determination of the ecological factors influencing species diversity might include future study of the types and extent of various sub-strata upon which eggs of black flies might attach.

Intermediate disturbance may also play a role in why smaller rivers have more species than large rivers. Sousa (1979) found that inter-tidal zone areas have a high level of species diversity. This was attributed to the disturbance caused by the tide moving in and out. He found that intermediate levels of disturbance had the highest species diversity. This may apply to black fly species diversity because the small and medium sized rivers may have an intermediate level of disturbance (*i.e.* freeze over; deep, fast spring runoff, and running very shallow at the end of summer) when compared to the large rivers (*i.e.* no freeze over and have a more consistent water level).

Though I sampled small, medium and large sized-streams in this study, I sampled about the same area per stream. This may have introduced a sampling bias in favor of small streams since proportionately larger percentages of those smaller streams would have been sampled. In other words, to sample equally, I may have had to sample a larger and more representative proportion of the medium and large-sized streams.

No new morphological species of black flies were discovered in this study. However, in collections from the Yellowstone and Gallatin rivers on June 24, 2001 a new cytotype of *S. arcticum* was discovered (Shields, pers. comm.). In this cytotype, males are heterozygous for a paracentric inversion, IIL-15, in the long arm of chromosome II. This inversion reverses the orientation of section 57 in the long arm of chromosome II (Shields, pers. comm.). The discovery of this new cytotype follows the descriptions of
numerous cytotypes and cytospecies of *S. arcticum* in Alaska (Shields and Procunier, 1982) and in Montana (Shields, pers. comm.). The distribution of IIL-15 *arcticum* could be determined by additional collections in the Yellowstone and Gallatin rivers and their tributaries.

This study began on May 15 and ended in early August. It is possible that different patterns of species diversities might have been observed if the study were conducted throughout the annual cycle since some species of black flies over-winter in the larval stage (Shields, pers. comm.).
Figure 1.

1. Missouri
2. Yellowstone
3. Little Prickly Pear
4. Gallatin
5. 10 Mile Creek
6. Camp Creek
Figure 2. Species diversities, and individual numbers of black flies sampled on seven collection dates on A) the Missouri and B) Yellowstone rivers, Montana.

A)  
<table>
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<th>Date</th>
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<th>29-May</th>
<th>10-Jun</th>
<th>24-Jun</th>
<th>8-Jul</th>
<th>22-Jul</th>
<th>6-Aug</th>
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<tr>
<td>S. vittatum</td>
<td>82</td>
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<td>217</td>
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<td>139</td>
<td>168</td>
<td>167</td>
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<tr>
<td>S. arcticum</td>
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<td>0</td>
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**Missouri**

![Missouri graph](image)

B)  
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<th>29-May</th>
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<th>24-Jun</th>
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<tr>
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<td>235</td>
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**Yellowstone**

![Yellowstone graph](image)
Figure 3. Species diversities and individual numbers of black flies sampled on seven collection dates at A) Little Prickly Pear Creek and B) the Gallatin River, Montana.

**A)**

<table>
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<tr>
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<th>29-May</th>
<th>10-Jun</th>
<th>24-Jun</th>
<th>8-Jul</th>
<th>22-Jul</th>
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<td>148</td>
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<td>52</td>
<td>93</td>
<td>307</td>
<td>32</td>
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<td>3</td>
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<tr>
<td>S. tuberosum</td>
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<td>57</td>
<td>9</td>
<td>11</td>
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<td>0</td>
</tr>
<tr>
<td>S. decorum</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>54</td>
<td>0</td>
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<td>S. canonicolum</td>
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<td>10</td>
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**Little Prickly Pear**

**B)**

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<th>8-Jul</th>
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<td>248</td>
<td>297</td>
<td>54</td>
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<td>54</td>
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<tr>
<td>S. tuberosum</td>
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<td>0</td>
<td>0</td>
<td>15</td>
<td>21</td>
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<tr>
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<td>0</td>
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**Gallatin**
Figure 4. Species diversities and individual numbers of black flies sampled on seven collection dates at A) 10 Mile and B) Camp creeks, Montana.
Literature Cited


