

INTRODUCTION

Waterways are intimately connected to their surrounding landscapes. As human activities change those landscapes the impacts can be observed in lotic habitats. Due to intensive agricultural activities, fertilizer spills and high-density livestock production, Iowa rivers are among the most impaired in the United States and the Floyd River is no exception (Iowa DNR and EPA 2022). These impacts increase sedimentation, produce low habitat heterogeneity, alter types of allochthonous inputs, and contribute to poor water quality (Herringshaw et al. 2011). Only macroinvertebrate taxa tolerant of these conditions persist in impacted streams, resulting in relatively low taxonomic richness and poor benthic macroinvertebrate index scores (Hilsenhoff 1988). Likewise, following habitat restoration efforts, changes in stream macroinvertebrate community composition offer evidence of success (McDermond-Spies et al. 2014).

We explored the Floyd River macroinvertebrate community by introducing sources of allochthonous input (leaves) into the river at six locations and observing macroinvertebrate colonization. We hypothesized that colonizer richness, abundance, and BMI scores would differ in leaf bags downstream from relatively intact riparian habitats and reaches with high water quality vs. those downstream from degraded reaches. We also hypothesized that leaf species with lower lignin content would support higher abundances of colonizers. Finally, we predicted that there would be significant differences in the composition of macroinvertebrates in the leaf bags after 4 vs. 8 weeks of colonization.

Abstract - Watershed characteristics impact water quality, habitat heterogeneity, and allochthonous inputs. Lotic macroinvertebrates are dependent upon and can be used as indicators of these conditions. Macroinvertebrates tolerant of degraded environments dominate impaired sites, resulting in poor benthic macroinvertebrate index (BMI) scores. We explored the relative importance of habitat characteristics and allochthonous inputs by examining how macroinvertebrate colonization of leaf bags varied with leaf species, water quality, and landscape disturbance. Leaf bags were submerged in the Floyd River at seven locations. After four or eight weeks, we collected the bags for analysis. We hypothesized that colonizer richness, abundance, and BMI scores would be highest in bags downstream from intact riparian habitats and in reaches with higher water quality. We also hypothesized that leaf species with lower lignin content would support higher abundances of colonizers. Midge larvae (*Chironomus*), aquatic annelids, and scuds (*Hyalella*) were the dominant colonizers in all bags, resulting in poor BMI scores at all locations. Richness and abundance did not differ significantly with leaf type or colonization time but did differ significantly with location. Although our results were limited by low water flow and lost bags, we found these initial results interesting and plan to expand the scope of this study in the future.

Table 1. Water quality characteristics.

Characteristic:	Site: Kingbird	E. Div.	470th	490th	110th	130	Sign. Diff. (ANOVA)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
pH	7.33 (0.24)	7.50 (0.41)	7.38 (0.25)	7.50 (0.00)	7.88 (0.75)	7.50 (0.00)	No
DO (mg/L)	8.33 (2.31)	11.00 (1.00)	12.00 (0.00)	10.33 (2.08)	11.67 (0.58)	11.50 (0.71)	No
Nitrite (mg/L)	0.25 (0.35)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.08 (0.11)	0.00 (0.00)	No
Nitrate (mg/L)	1.00 (1.41)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	1.00 (1.41)	0.67 (0.58)	No
Phosphate (mg/L)	0.37 (0.31)	0.20 (0.10)	0.22 (0.10)	0.23 (0.23)	0.43 (0.49)	0.35 (0.07)	No
Ammonia (mg/L)	0.25 (0.00)	0.25 (0.00)	0.25 (0.00)	0.17 (0.14)	0.25 (0.00)	0.25 (0.00)	No
Clarity (cm)	33.00 (23.64)	30.93 (12.85)	35.00 (19.80)	40.00 (28.28)	32.03 (22.85)	45.00 (18.38)	No
Chloride (ppm)	196.33 (9.24)	159.67 (9.71)	149.00 (13.00)	128.67 (24.83)	83.67 (35.95)	43.50 (4.95)	p<0.0001

Chloride concentrations were significantly higher upstream than downstream.

RESULTS and DISCUSSION

There was **no rainfall in our region for the duration of our study resulting in very low discharge** (Fig. 2). As a result, some leaf bags were stranded above the waterline. We also lost several bags to fish "predation" and severed lines. Despite these setbacks, we were able to recover enough bags to compare colonization at 4 of our sites, all 4 species of leaf bags, and compare 4 and 8 week colonization patterns at one site (470th).

Generally, water chemistry did not differ significantly with location (Table 1). However, **chloride was significantly higher at upstream sites than those downstream**. Agricultural runoff has elevated levels of organic solvents, chloride being a main component (David et al. 2016). Although the chloride levels upstream were excessively high, colonization was highest at the East Division (upstream) site (Figures 3-6). Colonizer richness was fairly low overall (1-8 taxa) and varied significantly with location (Figure 3). For sites and leaf bag types, the **colonizer taxa were indicators of "very poor" water quality** (Hilsenhoff 1988). These taxa may be insensitive to elevated chloride.

Our leaf bags were **dominated by *Chironomus* larvae**. These larvae are tolerant of poor water quality and feed on the fine particulate organic matter that is readily available in the Floyd River. Other frequently observed taxa included the shredder, *Hyalella azteca*, and small filter-feeding crustaceans (ostracods and copepods). Very few other taxa were collected. **Abundances of *Chironomus*, *Hyalella*, ostracods and copepods also varied significantly with location, with highest numbers found at the East Division location** (Figures 4-6).

Higher habitat heterogeneity favors higher richness and abundances of stream macroinvertebrates (Molokwu et al. 2014). **However, given the impaired state of the Floyd River, we were surprised to see significant differences in richness and abundances relative to location in our study**. We believe the higher richness may be directly related to the close proximity of riparian vegetation and well-developed grass buffers upstream from the East Division and 110 St. locations compared to the others (Figure 8). In addition, abundances of the most frequently encountered taxa were significantly higher at the East Division site. Unfortunately, we lacked full sets of leaf bags to compare additional sites and (Kingbird and 130th Streets).

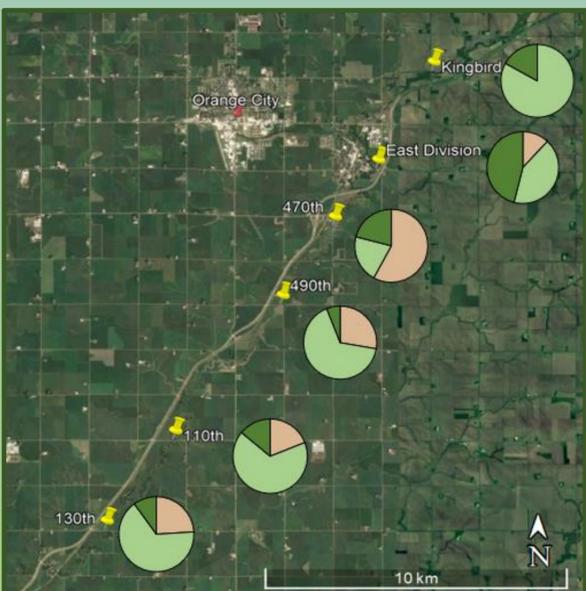


Figure 1. The study locations along the Floyd River in Sioux and Plymouth Counties. Relative bank coverage is included for each location. Legend: Bare, Grass Buffer, Riparian.

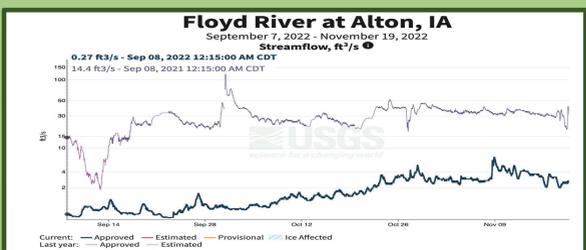


Figure 2. Floyd River discharge during our study (dark line) was much lower than last year (lighter line). This caused problems for our study.

Study Site:

We conducted this study at 6 locations in a reach of Floyd River extending from Kingbird Ave. to 130th St. (Figure 1). The Floyd River is a 4th order stream throughout our study sites (EPA) and classified as a 5th order stream overall. At our study sites, the Floyd watershed drains 161 km² (EPA). The watershed is dominated by agricultural activities. In our study area, the bank conditions were varied, including overgrazed/harvested banks, grass buffer strips, and riparian vegetation. We determined relative bank cover 1 km upstream from each study site using Google Earth Pro (Figure 1).

Materials and Methods:

In early September 2023, we collected leaves from four common riparian tree species found along the banks of the Floyd River in Northwest Iowa, *Fraxinus nigra* (ash), *Acer saccharinum* (maple), *Populus deltoides* (cottonwood), and *Salix nigra* (willow) and air dried them for two weeks. Our 1cm mesh leaf bags each contained 5g of dried leaf blades plus 2-3 small rocks (for weight). On September 20-22, four pairs of bags (1 pair of each species) were introduced to the Floyd River at each of the six study sites by suspending them from bridges with paracord. The initial water level was low, so bags were placed as close to the bed as possible.

During the study, we measured water pH, dissolved oxygen, phosphorus, nitrate/nitrite, ammonia, chloride, and temperature, and turbidity at each site. On October 13, we collected one leaf bag of each leaf species from each site and preserved the contents in 70% ETOH. We removed the remaining bags on November 8.

Processing involved cleaning debris off the bags and leaves over a 250 µ mesh and examining the debris for macroinvertebrates under dissecting microscopes. Resulting data were analyzed with paired Ttests, ANOVAs, and Tukey's Honestly Significant Difference post-hoc tests.

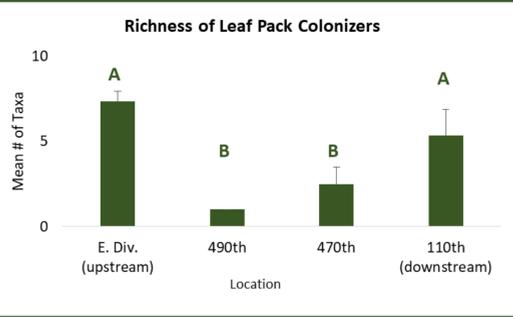


Figure 3. Mean richness of leaf pack colonizers differed significantly with location (ANOVA F 3, 9 = 26.9 p<0.001; Tukey's HSD p<0.05). Different letters indicate significantly different mean and standard deviations are indicated by error bars.

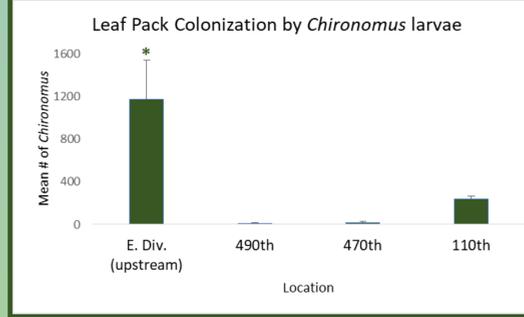


Figure 4. Mean *Chironomus* larval colonization of leaf packs was significantly higher at the East Division location (ANOVA F 3, 9 = 30.8, p<0.001; Tukey's HSD p<0.01). The asterisk indicates significantly different mean and standard deviations are indicated by error bars.

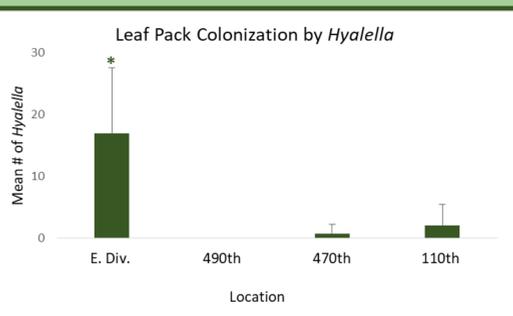


Figure 5. Mean *Hyalella* colonization of leaf packs was significantly higher at the East Division location (ANOVA F 3, 9 = 7.2, p<0.01; Tukey's HSD p<0.05). The asterisk indicates significantly different mean and standard deviations are indicated by error bars.

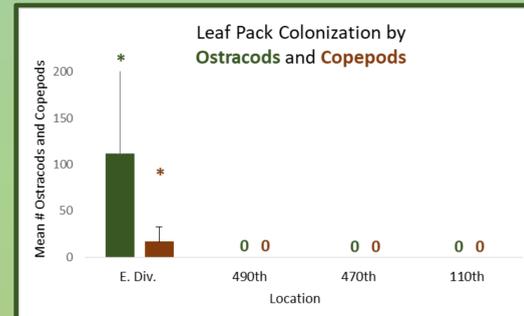


Figure 6. Mean ostracod and copepod colonization of leaf packs was significantly higher at the East Division location than at other locations (ANOVAs p<0.05). The asterisk indicates significantly different means and standard deviations are indicated by error bars.

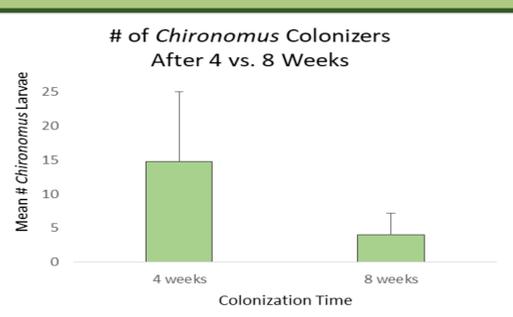


Figure 8. The mean number of *Chironomus* larvae in leaf packs did not differ significantly after 4 vs. 8 weeks of colonization (Paired TTest p=0.09).

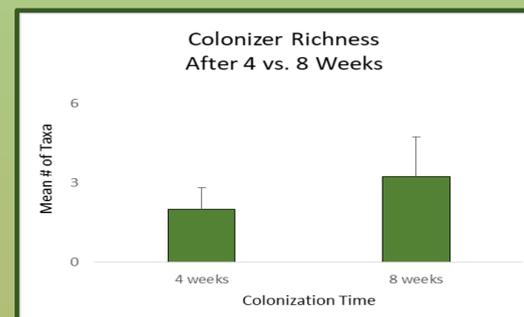


Figure 9. The mean number of taxa in leaf packs did not differ significantly after 4 vs. 8 weeks of colonization (Paired TTest p=0.24).

Table 3. Colonizer richness and *Chironomus* larval abundance did not vary significantly with leaf pack species. Lignin content from Ostrofsky (1997). Note: East Division differed significantly from the other locations; therefore, it was not included in these analyses. Other taxa were not analyzed because they occurred primarily in East Division samples.

Leaf Pack Species (%Lignin)	Richness Mean(+/-SD)	<i>Chironomus</i> Larvae Mean(+/-SD)
<i>Acer saccharinum</i> (21%)	3.00(2.0)	82.67(110.3)
<i>Fraxinus nigra</i> (31%)	3.75(1.5)	99.33(145.5)
<i>Populus deltoides</i> (25%)	3.75(1.5)	11.67(7.1)
<i>Salix nigra</i> (37%)	4.75(3.1)	113.00(114.1)

ANOVA p=0.92 p=0.67

CONCLUSION

Sioux and Plymouth Counties are heavily dependent on agriculture. Often, farmers plow right up to river and creek banks, destroying riparian vegetation and forgoing buffer strips in order to get a few more rows of corn. Agricultural activities degrade water quality and lotic habitat integrity. As a result, streams and rivers in this area heavily degraded and do not readily attract the attention of ecologists. However, it is important to gather baseline data from even heavily degraded habitats if we want assess ongoing negative impacts for comparison with future landscape changes. We believe colonization studies can offer insights into those changes.

References:
David, M.B. et al. (2016). Chloride Sources and Losses in Two Tile-Drained Agricultural Watersheds. J. Environ. Qual. 2016 Jan;45(1):341-8.
EPA (2022). Decision document of the 2022 Iowa Clean Water Act Section 303(d) List.
Herringshaw, C. et al. (2011). Land use impact on stream invertebrate assemblages. Am. Mid. Nat. 165(2):274-293.
Iowa DNR (2022). Integrated Report including the 2022 Impaired Waters List.
Hilsenhoff, W. (1988). Rapid field assessment of organic pollution with a family-level biotic index. J. N. Am. Benth. Soc. 7(1):65-68.
McDermond-Spies et al. (2014). Family-level benthic macroinvertebrate communities indicate successful relocation and restoration of a NE Iowa stream. Eco. Restor. 32(2):161-170.
Molokwu, N. et al. (2014). Effects of substrate on the benthic macroinvertebrate community: and experimental approach. Eco. Eng. 73:109-114.
Ostrofsky, M. (1997). Relationship between chemical characteristics of autumn-shed leaves and aquatic processing rates. J.N. Am. Benth. Soc. 16(4):750-759.



Figure 7. Extent of upstream riparian vegetation and buffer zones relative to the colonization sites.

Because the richness and colonizer abundances at the East Division site was significantly higher than the other locations, those samples were not used to compare differences in colonization of bags containing different leaf species. We did include leaf bags from Kingbird and 130th St. in these analyses. **Overall colonizer richness and number of *Chironomus* larvae did not differ significantly with leaf type** (Table 3).

The lignin content of the leaves we used varied considerably (Ostrofsky 1997) so we expected to see some differences in colonizer richness and abundance. With threats to ash trees from the emerald ash borer, we were particularly interested in colonizer use of ash leaves. However, the majority of colonizing taxa do not feed on coarse particulate organic matter. They may not be sensitive to lignin content because they used the leaf packs as substrate rather than food resources.

Due to the loss of bags, we could only compare 4-week and 8-week leaf bag colonization at the highly degraded 470th St. location. **Richness increased and number of *Chironomus* colonizers decreased from 4 to 8 weeks of colonization time**. However, these changes were not significant (Figures 8 and 9). We look forward to repeating this part of the study in the future.