A Comparison of $\delta^{15}N$ in Aquatic Invertebrates Upstream and Downstream of a Wastewater Treatment Plant in Michigan's Upper Peninsula

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ABSTRACT

Nutrient loading from waste water effluent has become a contributing factor to environmental degradation and eutrophication. Chironomids from the order Diptera and Lepidostomatids from the order Trichoptera were collected upstream and downstream from a point source of wastewater effluent discharge in Michigan's Upper Peninsula. If the effluent there was a significant amount of nitrogen coming from the effluent, we expected to see higher $\delta^{15}N$ values for invertebrates downstream vs. upstream. The invertebrates were dried and tested for the stable isotope ^{15}N. $\delta^{15}N$ values for both invertebrate families were compared and found not significantly different, so $\delta^{15}N$ was pooled from both families and combined within each sampling site. The $\delta^{15}N$ enrichment for each sample site was compared, and the two upstream sites were found to be significantly lower in $\delta^{15}N$ than the two downstream sites. There was a significant increase in nitrogen downstream of the wastewater treatment plant.

INTRODUCTION

Human wastewater effluent contains a suite of organic and inorganic nutrients that are discharged into wetlands and/or water bodies. Modern wastewater treatment plants efficiently remove organic carbon from wastewater, but their efficiency in removing inorganic nutrients is limited (Gucker et al. 2006). Water quality issues in densely populated areas have shifted from problems of organic pollution to that of inorganic nutrient pollution (Smith et al. 1987).

Most sewage treatment plants discharge wastewater containing high concentrations of nitrogen (N), an element that is difficult to remove without expensive tertiary treatment (Jordan et al. 1997). Spray irrigation of wastewater effluent into forests is a technique that is thought to be most effective in preventing nitrogen from entering ground and surface water (Jordan et al. 1997). However, the dumping of effluent into lotic systems is still a common practice.

It has long been thought that lotic systems are self purifying by way of microbial decomposition of organic carbon (Hynes, 1974). Time and further study have brought to light the effects of organic and inorganic pollution on aquatic organisms and communities (Gucker et al. 2006). According to Gucker et al. (2006) organic pollution from wastewater causes benthic invertebrate density and biomass to increase. In their study of a small rural wastewater treatment plant, Gucker et al. found increased NH_4 downstream of the plant and an increase in density and biomass of benthic invertebrates.

In recent years, stable isotope analysis has been used to solve biogeochemical problems in ecosystem analysis because stable isotope data can contribute both source-sink (tracer) and process information (Peterson and Fry 1987). It can also be used to quantify food webs (Steffy and Kilham 2004). The stable isotope $\delta^{15}N$ can be used as an indicator of nitrogen enrichment from wastewater effluent although little attention has been paid to the use of $\delta^{15}N$ in riverine food webs (Obendorfer et al. 1984, Wooten and Power 1993, Power and Deitrich 2002).

A measure of $\delta^{15}N$ in benthic invertebrates can indicate varying nitrogen levels in lotic systems and can be measured in benthic consumers because consumers typically become enriched in $\delta^{15}N$ relative to their food (average 3.4% per trophic level) (Anderson and Cabana 2007). However, the $\delta^{15}N$ value of aquatic consumers can be highly variable and can be affected by factors such as functional feeding groups (e.g., collectors, scrapers, shredders), taxonomic group, and life stage (Branstrator et al. 2000, Zah et al. 2001, Vanderklift and Ponsard et al. 2003). Anderson and Cabana (2007) found that organisms within the same primary-consumer feeding groups that fed on different resources were highly correlated in terms of $\delta^{15}N$. Shredders feed on terrestrial detritus and assimilate their N from bacteria that colonize the detritus. Likewise, collectors collect the fine particulate organic matter produced by shredders and also assimilate their N from colonizing bacteria.

Prior to 1996, The Clark Township Wastewater Treatment Plant sprayed its effluent into a cedar swamp surrounding the plant at the headwaters of Pearson Creek (Map 1). Subsequent to 1996, the effluent has been discharged directly into Pearson Creek. Secondary effluent runs down the creek and into Les Cheneaux Bay. The plant is located in a rural area and the effluent is released into the creek via a culvert underneath

Blind Line Rd. According to Mike Grant of the University of Michigan Biological Station, the treatment plant completes primary and secondary treatment. However, some phosphorus, chloride, and nitrogen are not removed from the water. Wastewater is released twice a year, once in the spring just before Memorial Day and once in the fall after Labor Day.

The purpose of this study is to measure the degree of nitrogen enrichment from Clark Township Treatment Plant wastewater effluent in Pearson Creek via δ^{15}N ratios in benthic invertebrates. Two invertebrate families will be used from similar feeding groups: Lepidostomatidae (Order Trichoptera) from the shredder feeding group, and Chironomid (Order Diptera) from the collector feeding group. We will first ask the question, are these two organisms feeding at the same trophic level? If so, we expect to see statistically similar values for δ^{15}N between feeding groups. The second question we will examine is, is there a significant increase in nitrogen enrichment downstream from the point source of effluent? We will address this question by comparing both feeding groups of invertebrates from two sites upstream from the point source to two sites downstream from the point source. If there is nitrogen enrichment, we expect to see a significantly higher value for δ^{15}N in both downstream samples.

METHODS

Field Methods

Diptera and Trichoptera were collected with a D-frame aquatic net at two sites above and two sites below the point source of waste water effluent (Map 2). Upstream site A was approximately 30 meters upstream from the point source (Map 2). Ten Trichoptera and 5 Diptera were collected there. Upstream site B was on the upstream side of the road from the culvert where the effluent is discharged (point source) (Map 2). Ten Trichoptera and 10 Diptera were collected there. Site C was downstream within 5-10m of the point source (Map 2). Ten Trichoptera and 10 Diptera were collected there. Site D was approximately a mile downstream from the point source, where the creek flows into Cedarville Bay (Map 2). Trichoptera were not found at site D. Twelve Diptera were collected there. The specimens were preserved in 70% ethanol in the field and stored in whirl packs.

Laboratory Methods

The specimens were dried in a 60°C oven. Due to the small size and weight of the Diptera, some of them had to be pooled and run as one sample. For upstream site A, two Diptera were pooled and run in one sample, and three Diptera were pooled and run in another sample for a total of two samples. For upstream site B, three Diptera were pooled in one sample, and four Diptera were pooled in a second sample for a total of two samples. For downstream site C, seven samples of Diptera were tested, and three of those samples pooled two Diptera. For downstream site D, five samples of Diptera were tested. Two out of the five samples pooled three Diptera, and one out of the five samples pooled two Diptera.

For the sake of time, not all Trichoptera collected were tested. Only 6 Trichoptera were run individually at upstream site A. All ten Trichoptera collected were tested at upstream site B. Six out of ten Trichoptera were tested at downstream site C, and no Trichoptera were tested at downstream site D (none were found there.)

Each sample was introduced individually into a Costech Elemental Combustion System and transferred to a Thermo Finnigan Delta Plus XP mass spectrophotometer. The mass spectrophotometer tested for ratios of $\delta^{15}N/\delta^{14}N$ and the ratios were compared to a standard (nitrogen gas in the atmosphere). Instrumental error was ±0.2.

Statistical Analysis

$\delta^{15}N$ ratios for Diptera and Trichoptera at downstream site C were compared with a t-test. A Normal Probability Plot was used to check that the data were normally distributed. The $\delta^{15}N$ ratios for both invertebrates were pooled at each site and we used an ANOVA test to compare means across all sites. A Tukey’s test was used for post hoc comparisons.

RESULTS

Downstream sites had higher $\delta^{15}N$ values than upstream sites. There was no significant difference between $\delta^{15}N$ values for Diptera and Trichoptera at downstream site C (t-test, t = x, of = x, p = 0.057). There was no significant difference in $\delta^{15}N$ values between pooled invertebrates at upstream sites A and B (p = 0.831), between pooled invertebrates at upstream site B and downstream site C (p-value 0.061), and between pooled invertebrates at downstream site C and Diptera at downstream site D (p = 0.932) (Table 1). There was a significant difference in $\delta^{15}N$ ratios between pooled invertebrates at upstream site A and downstream site C (p = 0.028), between pooled invertebrates at upstream site A and Diptera at downstream site D (p = 0.004), and between pooled invertebrates at upstream site B and Diptera at downstream site D (p = 0.005) (Table 1). The average $\delta^{15}N$ ratios between upstream sites A and B, and downstream sites C and D were compared. Upstream site A had an average of 4.0 $\delta^{15}N$, upstream site B had an average of 3.6 $\delta^{15}N$,downstream site C had an average of 5.8 $\delta^{15}N$ and downstream site D had an average of 6.4 $\delta^{15}N$(Figure 1).

DISCUSSION

The primary goal of this study was to see if the effluent discharged from Clark Township Wastewater Treatment Plant was increasing the nutrient load downstream of the point source. The Diptera family, Chironomidae and Trichoptera family, Lepidostomatidae were chosen for their relative trophic levels.

Lepidostomatidae are shredders who feed on microbes that break down course particulate organic matter and are considered to be primary consumers (Bouchard, 2004). Chironomidae are gathering collectors that feed on microbes that live on fine particulate organic matter (De Hass et al. 2006). Since some degree of omnivory can take place among organisms such as these, Anderson et al. (2007) noted the importance of determining wether or not organisms tested for $\delta^{15}N$ feed on the same trophic level.

If Chironomidae were at a higher trophic level than Lepidostomatidae, we expected them to have significantly higher $\delta^{15}N$ values. If so, they would have to be treated separately in this experiment. Since the $\delta^{15}N$ values between those two families were not statistically different, we could assume that they were feeding on the same trophic level and were able to pool the data from the two families within each site. We did this to get a larger sample size. However, the statistical differences between $\delta^{15}N$ values of invertebrates at each site didn’t coincide with the difference in average $\delta^{15}N$ values of invertebrates between sites.

The first discrepancy was that, statistically, upstream site A was the most different from downstream site D (Table 1). These two sites are the furthest away from each other among all the sites. However, when the average $\delta^{15}N$ values were compared between them, upstream site B was the most different (out of all the other sites) from downstream site D (Figure 1). The second discrepancy was that upstream site A was statistically different from downstream site C (Table 1) and the difference in their average $\delta^{15}N$ values was 1.8. However, upstream site B was not statistically different from downstream site C (Table 1), and yet the difference in average $\delta^{15}N$ values between them was 2.2 (higher than the difference between sites A and C)(Figure 1).

That upstream site B was not statistically different from downstream site C can be explained by the fact that sites B and C were not very far away from each other (approximately 30 ft apart) and were the two sites closest to the point source of effluent discharge. There are millions of gallons of effluent discharged twice and year near these two sites, so it is reasonable to assume that some of the effluent travels upstream for a little while before it flows back downstream. However, the fact that the difference in the average $\delta^{15}N$ values between sites B and C was greater than between sites A and C suggests that site B is not necessarily influenced by effluent discharge. These two discrepancies can not be explained at this time. Further study needs to be done to clarify this. This may suggest that pooling the families has skewed the data somewhat.

Nonetheless, the $\delta^{15}N$ values were higher in invertebrates upstream than they were downstream. They were significantly higher even though effluent is only discharged twice a year. This suggests that nutrient loading from the wastewater treatment plant is having a significant effect not only on the stream, but also on stream biota up the food chain. Since the stream flows into the bay, the effects could presumably reach into tertiary and quarternary trophic levels in the lake. According to Cabana and Rasmussen (1994), $\delta^{15}N$ values in a lake Ontario increased up the food chain from ~9 $\delta^{15}N$ in zooplankton to >16 $\delta^{15}N$ in lake trout in a study done by the Department of Fisheries and Oceans, Canada. It is reasonable to assume that Cedarville Bay could suffer similar consequences.

An increase in nitrogen also affects aquatic plant communities and structures. According to Day et al. (2006), wastewater effluent increased plant productivity in a study area that had received 27 years of wastewater effluent discharge. Similarly, Gucker et al. (2006) found that macrophyte biomass increased 8× between the referenced and wastewater impacted reaches of a stream. In Cedarville, the plant community changes as the stream gets closer to the bay. The site at the bay showed the highest amount of nitrogen of all the sites. Consequently, the bay fauna is dominated by thick stands of *Typha spp*. that span upstream for at least a ¼ mile. This is an indication that nutrient loading has altered the plant community in farthest reaches downstream and has encouraged thick stands of *Typha spp*. to dominate. With the thick stands of *Typha spp*. come decreases in species diversity, and habitat diversity for invertebrates, fish, and birds.

CONCLUSION

The effluent discharge from Clark Township Waste Water Treatment Plant has impacted the local stream biota, which can have repercussions up the food chain. It may also have impacted plant community and structure in the portion of the stream that is closest to the bay and in the bay itself, which further impacts biota by altering habitat structure and diversity. Our results indicate a need for more efficient secondary treatment and the addition of tertiary treatment of the waste water at Clark Township Waste Water Treatment Plant.

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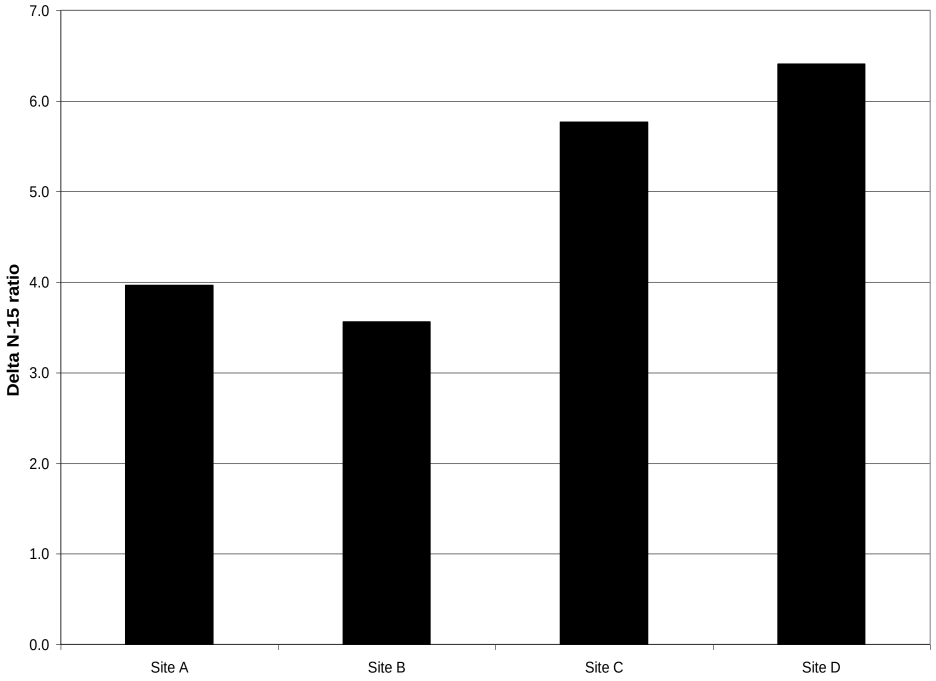
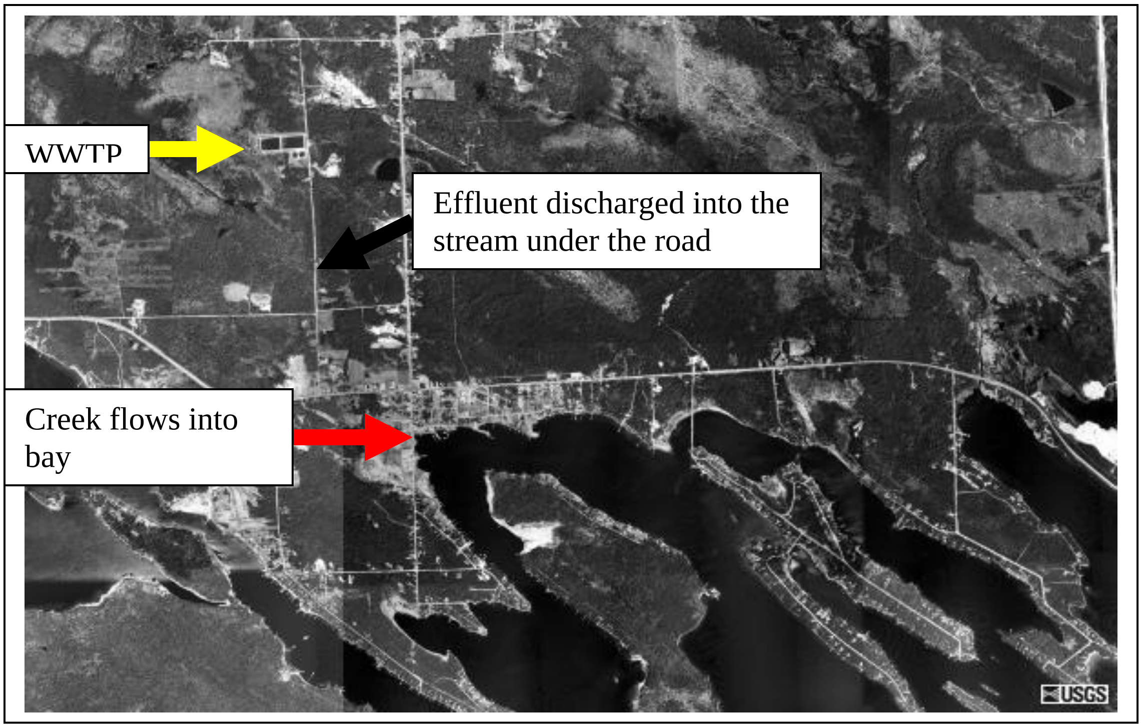
Table 1 A Tukey's test showed no significant difference in $\delta^{15}N$ ratios between site A and B and between site C and D. There was a significant difference in $\delta^{15}N$ ratios between site A and C, between A and D, between B and C, and between B and D.

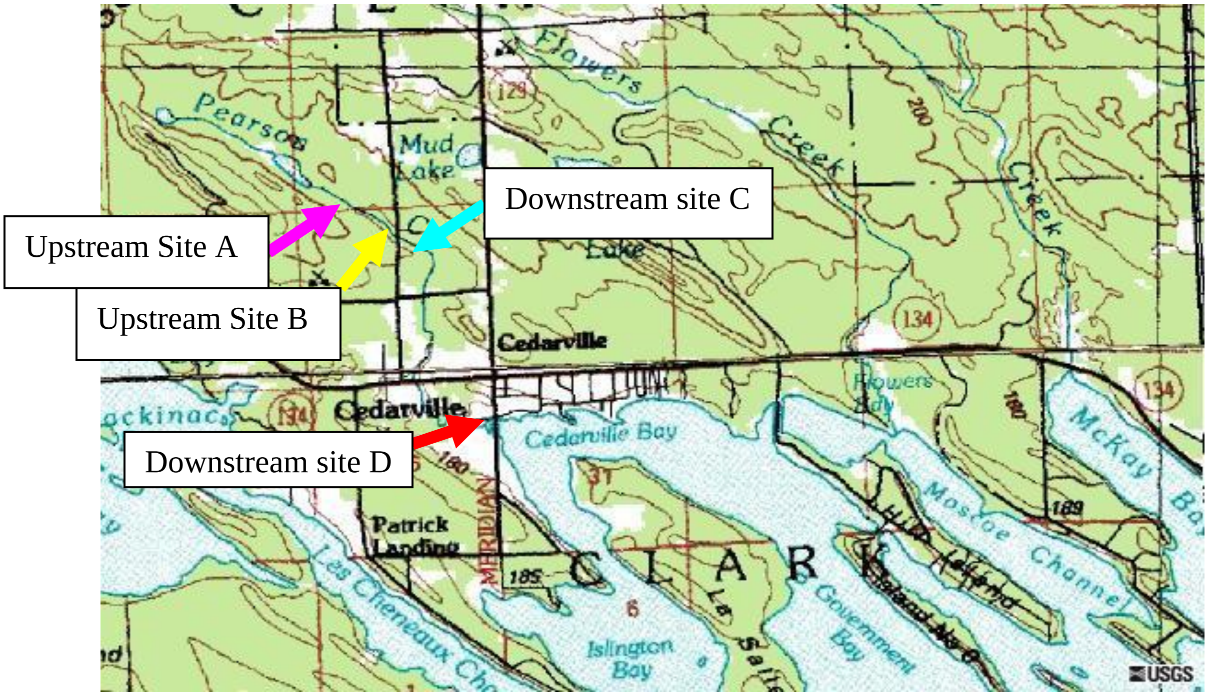
Sites Compared		p-value
A	B	0.831
	C	0.028
	D	0.004
B	A	0.831
	C	0.061
	D	0.005
C	A	0.028
	B	0.061
	D	0.932
D	A	0.004
	B	0.005
	C	0.932

Figure 1 The average $\delta^{15}N$ ratios between sites A, B, C, and D were compared. Downstream sites C and D both showed invertebrates with higher $\delta^{15}N$ ratios than upstream sites A and B.





Map 1 An aerial photo of the sampling site at Cedarville, MI. Indicated here are the waste water treatment plant (WWTP), the point source of effluent discharge, and the area at which the creek flows into Cedarville Bay.



Map 2 A topographic map of the sampling site at Cedarville, MI. Site A is indicated by the pink arrow, site B is indicated by the yellow arrow, site C is indicated by the turquoise arrow, and site D is indicated by the red arrow.