Impact of Municipal Sewage Effluent on Nitrogen and Phosphorus Dynamics and Growth of Resident Filamentous Algae in a Second Order Stream in West-Central Oklahoma

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Abstract: Elevated levels of stream nutrients due to municipal effluent have been identified as major stressors in streams around the world. We investigated the effects of municipal wastewater treatment plant effluent on nitrogen and phosphorus levels in Little Deep Creek, a 2nd order stream in Custer County, OK, USA. Nutrients were monitored in the effluent, an upstream site, and three downstream sites on three sample dates. Concentrations of nitrate-N, ammonia-N and total-P increased significantly downstream of the effluent release indicating a potential change to more eutrophic conditions. N:P stoichiometry indicated a shift from phosphorus limitation at the upstream site to nitrogen limitation at the downstream sites. Estimated nutrient retention length for total-P suggests a reduction in the stream's capacity to process phosphorus. Nitrate-N uptake length indicates little nitrogen retention by the stream, but suggests that ammonia-N is converted to nitrate-N. Growth of *Spirogyra* and *Cladophora* in effluent diluted with stream water showed that low effluent concentrations stimulated growth of both algae. At higher effluent concentrations estimated to exist downstream of the release, growth rate of *Cladophora* was inhibited relative to the rate of *Spirogyra* suggesting that effluent release into the stream could alter competitive abilities of these filamentous algae.

Introduction

Nitrogen and phosphorus have been identified as the most common stressors for streams in the United States (USEPA, 2006). In the US Southern Plains ecoregion 48% of streams reportedly have high total phosphorus levels and 36% have high nitrogen levels compared to reference streams (USEPA 2006). Sources of elevated nitrogen and phosphorus in streams may include both nonpoint and point sources (Waiser et al. 2010), but waste water treatment plant (WWTP) effluent has been found to account for 50-90% of nutrient inputs in watersheds around the world (Haggard et al. 2005). In the United States, 14,748 publicly owned WWTPs treat waste water generated by 238 million people (USEPA 2016). Treated effluents from these plants are discharged into streams and alter stream nutrient conditions. Nutrient levels may remain elevated for distances of 10 to 85 km downstream of WWTP discharges (reviewed by Carey and Migliaccio 2009).

Elevated levels of these nutrients lead to eutrophication of stream and river ecosystems (USEPA 2006) and contribute to dead zones that develop in the Gulf of Mexico and other coastal areas dominated by river inputs (Justić et al. 1995). Eutrophication has been shown to

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increase growth of algae, reduce water clarity, result in harmful diel fluctuations in dissolved oxygen and pH, and increased probability of fish kills (reviewed by Smith et al. 1999). The trophic structure of stream ecosystems has been shown to be altered by nutrient enrichment resulting in concentration and stoichiometric changes in nitrogen and phosphorus (Miltner and Rankin 1998; Evans-White et al. 2009; Dodds and Smith 2016). Excessive growth of the green alga, *Cladophora*, has often been associated with elevated nutrients and eutrophication (Dodds and Gudder 1992; Ensminger et al. 2000). However, the impacts of WWTP effluent and stream eutrophication on *Cladophora* and other filamentous algae have not been closely investigated (Dodds and Gudder 1992; Stevenson et al. 2006).

The purpose of this study was to investigate the effects of effluent from a WWTP of a small city in western Oklahoma on the nitrogen and phosphorus levels on the downstream water. The ability of this second order stream to ameliorate changes in nutrient levels was evaluated. In addition, the potential effects of effluent on growth of two filamentous algae (*Cladophora* and *Spirogyra*) resident in the receiving stream were investigated.

Materials and Methods

Sample Sites. Water sample collection sites (Fig. 1) were established on Little Deep Creek, a second order stream in Custer County OK, relative to the effluent release point of the Weatherford, OK waste water treatment plant. The stream is approximately 14 km in length and is located in the Cross Timbers Transition and the Rolling Red Hills Ecoregions of Oklahoma (Woods et al. 2005). Little Deep Creek carries water the year around and originates in springs in the Weatherford Gypsum Hills formation located west of the city (Fay et al. 1978). Water from Little Deep Creek empties into Deer Creek which in turn empties into the Canadian River. Water carried by Little Deep Creek ultimately ends up being released into the Gulf of Mexico as these streams are a part of the Mississippi River Basin.

Weatherford, OK is a small city with a population of just under 11,000 according to the 2010 U.S. census. Waste water carried by the city's sanitary sewers undergoes primary and secondary treatment in a modern WWTP

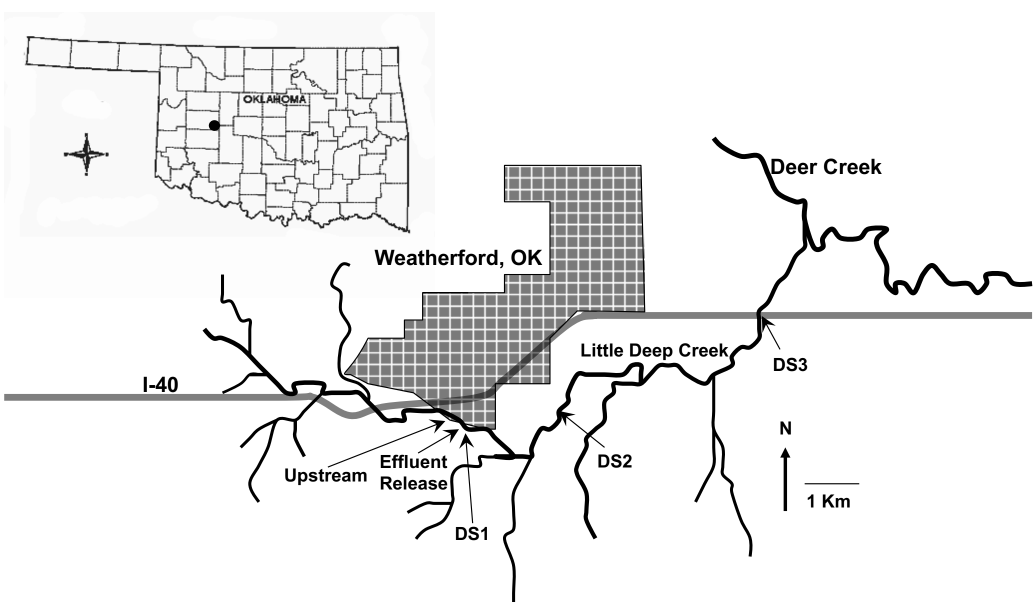

Figure 1. Map showing the location of Little Deep Creek and the sampling sites along the stream channel.

before being discharged into the receiving stream. The WWTP is rated to handle 7,571 m^3 of sewage per day but typically handles significantly less sewage. For example, during this study the average sewage volume processed in April 2012 was 4,000 m^3 per day (maximum = 5061 m^3 per day; minimum = 3021 m^3 per day). Information about the Weatherford WWTP was provided by Mr. Jack Olsen, who was the plants superintendent at the time of the study. Treated effluent is released into Little Deep Creek at latitude 35°31'4.53"N and longitude 98°41'55.23"W. Stream water prior to the addition of the effluent was collected approximately 70 meters upstream of the effluent release (Upstream site). Three downstream sites (Fig. 1) were established to monitor effects of the WWTP effluent addition. The first downstream site (DS-1) was located approximately 70 meters downstream of the effluent release. Sites DS-2 and DS-3 were located 3km and 8.25 km respectively downstream of the effluent release. Stream discharge at the upstream site was estimated on April 02 2012 by direct measurement of stream cross sectional area and average stream flow using a Marsh-McBirney Portable Water Flow Meter (Model 201D).

Stream Conditions and Effluent Nutrient Analysis. Duplicate readings of water temperature, dissolved oxygen, pH, and conductivity were made with a YSI Multi Probe System (Model 556) at the time of water sample collection and other times during the study period. We collected triplicate water samples at each of the Little Deep Creek stream sites and WWTP effluent on three dates (January 23, March 12, & April 02, 2012). Samples were collected in 125 mL sterile sample bags directly from the stream and from the effluent just before it entered the stream. Samples were returned to the laboratory where they were immediately filtered through Whatman 42 filter paper and refrigerated. The samples were analyzed within 4 days of collection for total-P, ammonia-N and nitrate-N using Hach test procedures and a Hach DR 2700 spectrophotometer (Hach Spectrophotometric Procedure Manual, DOC086.98.00801, 2009.

Nutrient uptake lengths for the three nutrients were calculated using the combined averages of the three sample dates at each location. The calculation used was that proposed by Marti et al. (1997), but modified by substituting electrical conductivity for chloride concentrations to determine dilution effects downstream of the effluent release. The formula used was:

$$\mathbf{N_x = N_i \cdot (Cond_x / Cond_i) \cdot e^{-bx}}$$

where N is the nutrient concentration and Cond is conductivity level at station DS-1 (i) and x is the distance where downstream levels are measure (DS-3). The nutrient uptake length is the inverse of the slope (- 1/b) and indicates the rate of removal of the effluent nutrients by stream processes. The substitution of conductivity is valid due to the fact that the upstream water is of high conductivity compared to the WWTP effluent and to surface runoff sources downstream. Furthermore, Vandenberg et al. (2005) found that monitoring of conductivity mirrored changes in chloride levels and could be used to monitor the mixing of effluent with stream water.

Algal Growth Experiments. We evaluated the potential effect of WWTP effluent on growth rates of two filamentous algae (*Cladophora glomerata*(?) and *Spirogyra grevelliana*[?]) that had previously been isolated from Little Deep Creek. The algae were grown for two weeks in batch cultures containing filter-sterilized (0.2µm microculture capsule filter, PALL Corp.) effluent diluted with filter-sterilized upstream stream water. Dilution treatments tested were 0%, 10%, 25%, 50%, and 100% effluent. Growth chamber (Percival Model E-30B) conditions were 25 C, 150 µmoles photons x m^{-2} x s^{-1}, and 16h:8h photoperiod. Clumps of algal biomass used to inoculate culture flasks were standardized by visually estimating biomass added to porcelain spot plates. Clumps were assigned to treatments using a table of random numbers and initial biomass inoculated was estimated as the average of 8 additional replicate clumps. Growth rate was measured as change in dry mass of algae in 4 replicates cultures per dilution.

Statistical Analysis. Data on stream

conditions, nutrient levels, and algal growth rates were analyzed with one-way and two way ANOVA along with Tukey's multiple comparison to identify differences between sample sites or sample dates using Graphpad Prism version 8.4.3 for MacOS, GraphPad Software, San Diego, California USA, www.graphpad.com. Values presented are means ± 1 standard deviation.

Results

Stream and Effluent Conditions. Temperature and dissolved oxygen were not significantly different between any of the sample sites and WWTP effluent (Fig. 2A & B). The pH at the DS-1 site was significantly lower ($p = 0.0146$) than at the DS-3 site but all other site comparisons yielded no significant differences in pH (Fig 2C). Conductivity measurements exhibited the most differences between the collection sites (Fig. 2D). The WWTP effluent was significantly lower in conductivity compared to the upstream water ($p = 0.001$). This difference is likely due to the different sources of the water. The upstream water originates from springs in the Weatherford Gypsum Hills formation located just to the west of Weatherford and is rich in calcium and sulfate ions. The water in the WWTP effluent originates from wells that draw from the Rush Springs Aquifer which lies below the gypsum layers of the geological formation (Fay et al. 1978). The upstream discharge of Little Deep Creek on April 02, 2012 was measured to be 6,647 $m^3 \cdot d^{-1}$. Using the April average effluent discharge of 4100 $m^3 \cdot d^{-1}$, the WWTP effluent comprised approximately 38% of the downstream flow. Predicted DS-1 site conductivity based on the above dilution factors and conductivity levels in the upstream water and the effluent was 1189 µS which is in good agreement with the measured value of 1139 µS. The dilution of the upstream water by the effluent and also by water from tributaries entering Little Deep Creek downstream of the effluent release (Fig. 1) was apparent in the decreasing conductivity at the DS-1, DS-2 ($p = 0.0003$), and DS-3 sites ($p < 0.0001$) compared to the upstream site.

Collection site nutrient levels. Nitrate-N concentrations at the upstream site were not significantly different on the three sampling dates (Fig. 3A) and averaged 1.42 ± 0.30 $mg \cdot L^{-1}$ (Fig. 3D). The WWTP effluent had significantly ($P < 0.0001$) higher levels of nitrate-N compared to the stream water and averaged 8.44 ± 3.27 $mg \cdot L^{-1}$. Effluent nitrate-N increased significantly ($P < 0.0001$) from the January 23 to April 02 sampling dates (Fig. 3A). The average nitrate-N at the DS-1 site (Fig. 3D) was statistically higher than the upstream concentration ($P < 0.0001$). The DS-1 April 02 sample had significantly higher nitrate-N levels ($P < 0.05$) than either of the January 23 and March 12 samples (Fig. 3A). Nitrate-N concentrations at all downstream sites (Fig. 3D) were significantly higher than the upstream concentration ($P < 0.0001$) and did not vary significantly by date ($P > 0.60$). The DS-3 nitrate-N level was not significantly different from the DS-2 level ($P = 0.92$) but was significantly lower ($P = 0.02$) than the level measured at DS-1 (Fig. 3D). Nutrient uptake length calculated for nitrate-N was unclear in that after accounting for dilution effects, the concentration of nitrate-N increased by about 4% between the DS-1 and DS-3 sites.

Ammonia-N concentrations at the upstream site showed significant differences between the three sample dates (Fig. 3B) with the March 12 date being significantly higher than the other dates ($P < 0.05$). Ammonia levels were below the detectable levels on the April 02 date. The WWTP effluent ammonia-N average concentration was significantly higher ($P < 0.0001$) than at the upstream site (Fig. 3E) and showed a high degree of variability between sample dates (Fig. 3B). The January 23 sample concentration was significantly higher ($P < 0.0001$) than concentrations for the March 12 and the April 02 sample dates (Fig. 3B). The DS-1 ammonia-N levels were significantly higher ($P < 0.0001$) than the levels measured at the upstream sample and significantly lower than the effluent levels ($P < 0.0001$) (Fig. 3E). The DS-2 and DS-3 ammonia-N levels were not statistically different than the upstream levels ($P = 0.23$, DS-2; $P = 0.51$, DS-3) but were significantly lower than levels at the DS-1 site ($P < 0.0001$). Nutrient uptake length for ammonia-N was

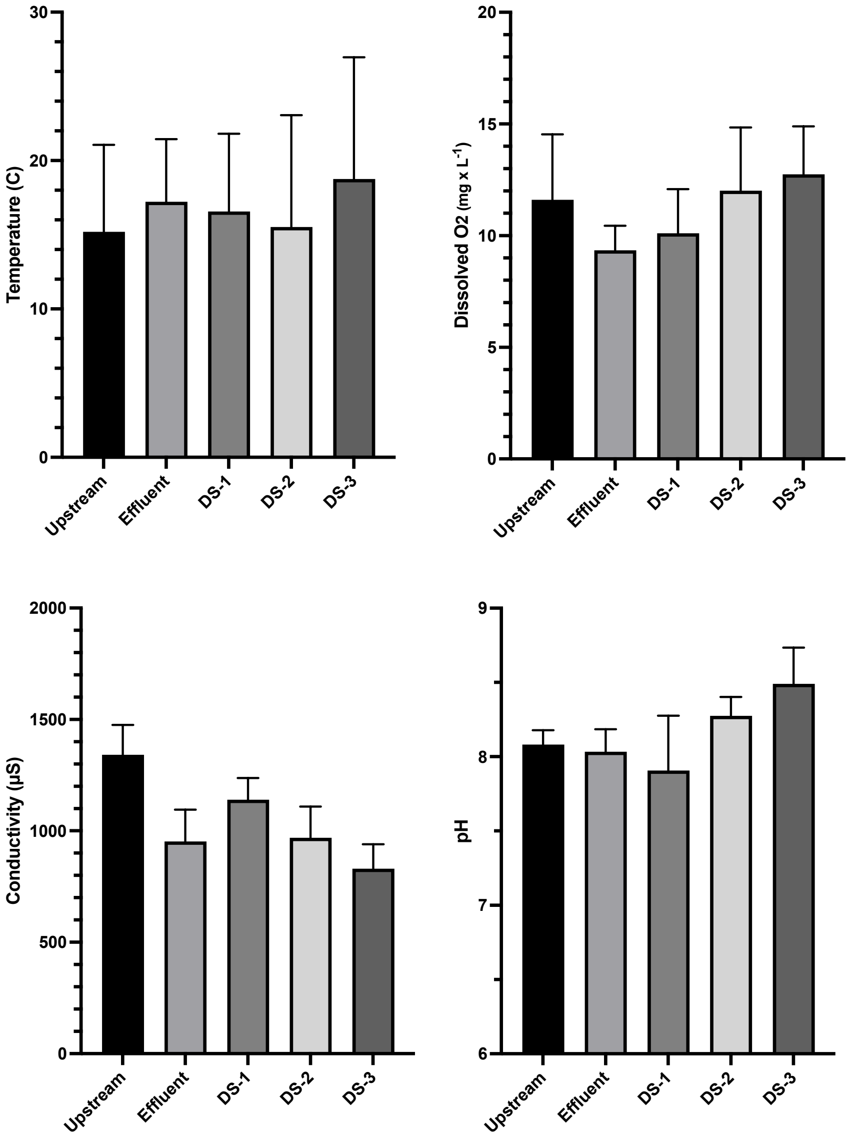

Figure 2. Physical/chemical conditions at the five sample sites. Bars represent means of measurements made on the three sample dates. Error bars represent one standard deviation.

calculated to be 11.2 km. Ammonia-N showed a 39% reduction in concentration between the DS-1 and DS-3 sites after accounting for estimated dilution effects.

Total phosphorus concentrations at the upstream site were not significantly different ($P > 0.90$) on the three sample dates sampled (Fig. 3C) and averaged 0.07 ± 0.017 mg · L^{-1} (Fig. 3F). The WWTP effluent total phosphorus was significantly higher than the upstream water ($P < 0.0001$) (Fig. 3F) and averaged 2.04 ± 0.73 mg · L^{-1} (Fig. 3D). Total phosphorus in the effluent varied significantly between sample dates ($P < 0.0001$), and was lowest in the March 12 sample and highest in the April 02 sample (Fig. 3C). Total phosphorus concentrations measured at the three downstream sites were significantly higher than in the water at the upstream site ($P < 0.0001$), and differed significantly from the effluent ($P < 0.0001$) and each other ($P < 0.003$) (Fig. 3F). Nutrient uptake length for

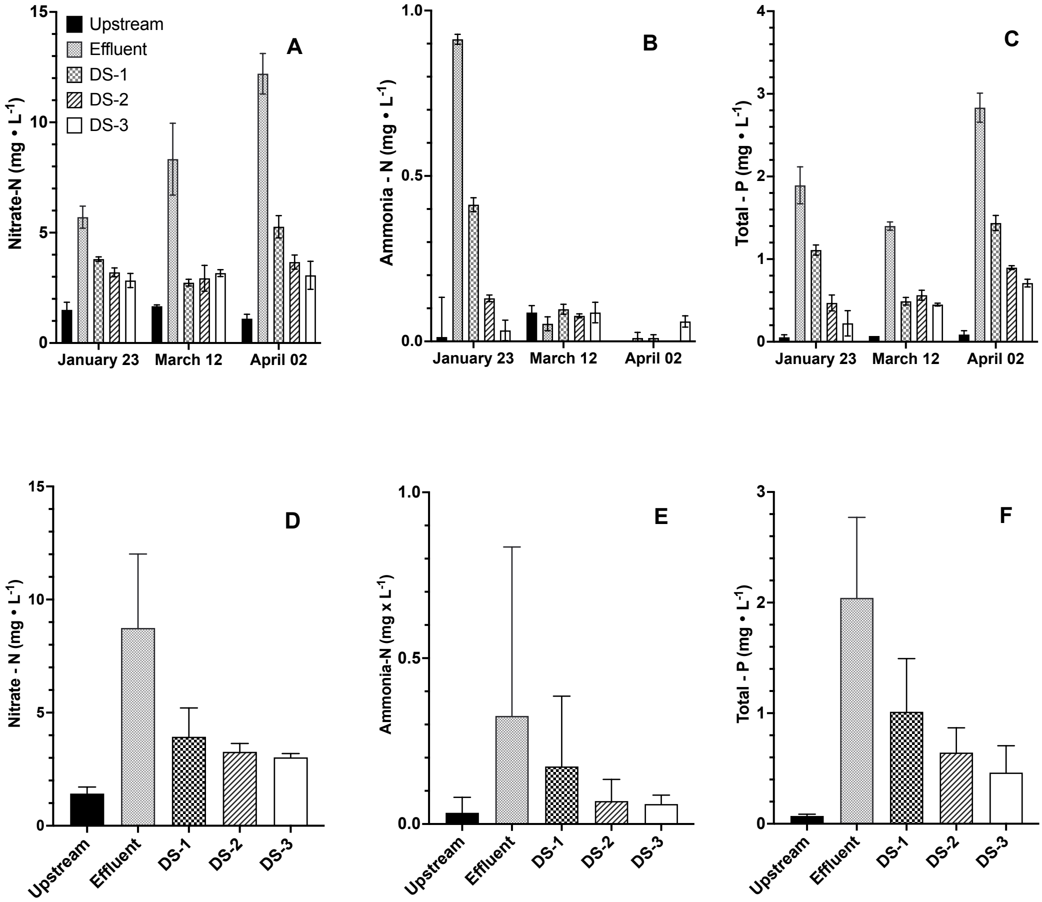

Figure 3. Nutrient concentrations measured at the stream sample sites on the three sample dates (Figs 3A-C). Comparison of nutrient concentrations at the five sample sites over the course of the study by combining measurements from the three sample dates (Figs 3D-F). Bars represent means based on triplicate samples and error bars represent one standard deviation.

total phosphorus was calculated to be 17.6 km. Total phosphorus showed a 27% reduction in concentration between the DS-1 and DS-3 sites after accounting for dilution effects.

The ratio of total nitrogen to total phosphorus (N/P) was calculated by adding the nitrate-N and ammonia-N and dividing by the total phosphorus for each sample and the results are presented in Figure 4. The upstream site water had a N/P ratio of 21.9 ± 8.18. The WWTP effluent had a significantly lower ratio of 4.59 ± 1.27 ($P = 0.003$). Ratios in the downstream samples (DS-1, DS-2, DS-3) were also significantly lower than the upstream ratio ($P < 0.01$) but not significantly different from the effluent or each other ($P > 0.72$).

Algal Growth Assay. Both of the filamentous algae isolated from Little Deep Creek showed nearly identical growth rates in the upstream water (*Spirogyra* = 0.121 ± 0.009 and *Cladophora* = 0.120 ± 0.008, $\mu \cdot d^{-1}$) (Fig. 5). Growth rate of *Spirogyra* increased 36% to 0.166 ± 0.003 $\mu \cdot d^{-1}$ when grown in a mixture of 10% WWTP effluent in upstream water ($P < 0001$). The elevated growth rate did not change when *Spirogyra* was grown in mixtures that had increased levels of effluent (25%, 50%, and 100%). *Cladophora* also exhibited a significant increase in growth rate in the 10% effluent treatment (0.152 ± 0.002 $\mu \cdot d^{-1}$, P <

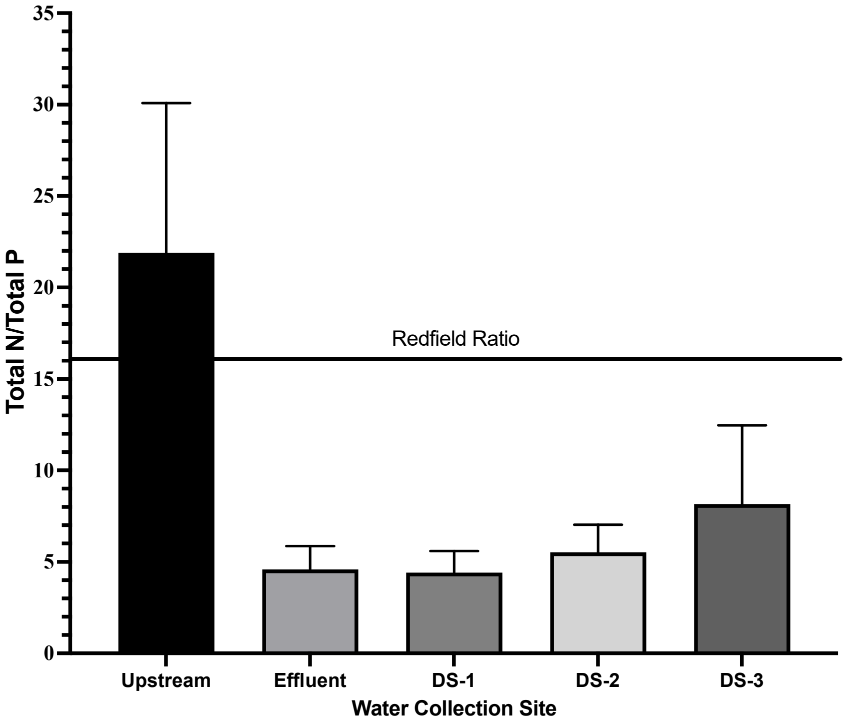

Figure 4. Comparison of N/P ratios at the five samples sites based on combined measurements on the three sample dates. Total nitrogen was obtained by summing nitrate-N and ammonia-N. Bars represent means and error bars represent one standard deviation.

0.0001) that was 27% greater than the growth rate in the upstream water. However, in contrast to the response seen in *Spirogyra*, *Cladophora* dropped back to a rate statistically identical to the growth rate in upstream water in both the 25% and 50% effluent treatment. *Cladophora* growth rate dropped to 60% of the rate measured in upstream water in the 100% effluent treatment ($0.072 \pm 0.008\ \mu \cdot d^{-1}$, $P < 0.0001$).

Discussion

The effluent discharge from the Weatherford WWTP significantly affected the nutrient conditions in Little Deep Creek with respect to both concentration and N:P stoichiometry. Concentrations of nitrate-N, ammonia-N, and total phosphorus were increased on average by 177%, 900%, and 1342% respectively at the DS-1 site compared to the Little Deep Creek upstream water. Similar increases in downstream nutrient levels due to WWTP effluent have been reported in other studies (Andersen et al. 2004; Ekka et al. 2006; Migliaccio et al. 2007; Popova et al. 2006; Waiser et al. 2011). The release of effluent greatly altered the trophic status of Little Deep Creek. Nutrient levels in the upstream water (total N = 1.42 mg $\cdot$ L^{-1}; total P = 0.07 mg $\cdot$ L^{-1}) place Little Deep Creek into the mesotrophic category (total N = 0.7 to 1.5 mg $\cdot$ L^{-1}; total P = 0.025 to 0.075 mg $\cdot$ L^{-1}) proposed by Dodd et al. (1998). In contrast, nutrient levels at the DS-3 site (total N = 3.08 mg $\cdot$ L^{-1}; total P = 0.46 mg $\cdot$ L^{-1}) located 8.25 Km downstream of the effluent release indicate the trophic status was well into the eutrophic range.

The elevated levels of nitrogen and phosphorus downstream of the effluent release are at levels that could potentially have effects on the stream macroinvertebrates and fish communities. For example, several studies have reported critical nutrient threshold values of total N = 1.0 mg $\cdot$ L^{-1}, total P = 0.06 mg $\cdot$ L^{-1} (Evans-White et al. 2009); total N = 0.61 mg $\cdot$ L^{-1}, total P = 0.06 mg $\cdot$ L^{-1} (Miltner and Rankin 1998); total N = 0.39 - 0.98 mg $\cdot$ L^{-1}, total P = 0.10 mg $\cdot$ L^{-1} (Chambers et al. 2012) above which

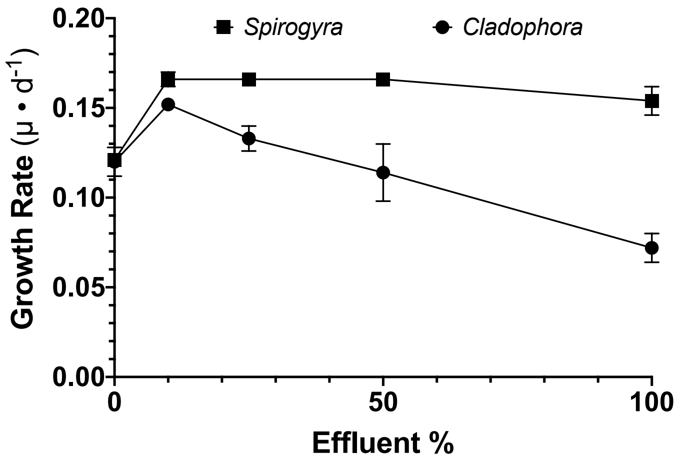

Figure 5. Growth rates of *Cladophora* sp. and *Spirogyra grevelliana* measured in dilutions of WWTP effluent. The 0% dilution is upstream water and other dilutions are made with effluent and upstream water. Symbols represent means and error bars represent one standard deviation. Four replicate cultures were measured for each data point.

macroinvertebrates and fish assemblages may be affected. In this study, total nitrogen and total phosphorus at all the downstream sites greatly exceeded these critical levels, making it likely that the downstream biological community in Little Deep Creek is impacted by the elevated nutrient concentrations. Furthermore, N:P ratios at the downstream sites indicate a shift from phosphorus limitation in the upstream water to a strong nitrogen limitation below the effluent release (Fig. 5) due to high levels of phosphorus relative to nitrogen in the WWTP effluent. Shifts in nitrogen and phosphorus stoichiometry have been linked to shifts in stream assemblages of algae (Stelzer and Lambert 2001; Felisberto et al. 2011) macroinvertebrates (Evans-White et al. 2009, Yun and An 2016) and fish (Yun and An 2016). Based on published research, the nutrient changes in Little Deep Creek caused by the effluent release from the Weatherford, OK WWTP are likely to impact the downstream biological communities.

Biological and physical-chemical processes in streams have been shown to alter and remove nutrients moving downstream (Chaubey et al. 2007) and that low order streams like, Little Deep Creek, play a significant role in this nutrient processing in stream systems (Peterson et al. 2001). One measure of the effectiveness of nutrient processing is the nutrient retention length in the stream. Previous studies have reported that in pristine streams nutrient uptake lengths are short indicating a high efficiency of nutrient retention. Nutrient uptake lengths were measured to be 0.37 km for PO_4-P and 0.549 to 1.839 km for NO_3-N in two headwater streams in Idaho (Davis and Minshall 1999). In two second order streams (one in North Carolina and one in Oregon), phosphorus (SRP) uptake lengths ranged from 0.032 to 1.00 km and NO_3-N ranged from 0.017 to 1.278 km (Munn and Meyer 1990). In a second order stream in Arkansas, the range of measure nutrient uptake lengths were 0.036 to 0.309 km for PO_4-P, 0.018 to 0.197 km for NH_4-N, but NO_3-N increased downstream indicating no retention of NO_3-N (Chaubey et al. 2007).

Streams that receive elevated nutrients from point sources have significantly longer uptake lengths than nonpolluted streams (Martí et al. 2004). Martí et al. (2004) hypothesized that streams receiving high levels of nutrients from waste water treatments plants would have lower nutrient retention efficiencies due to saturation of the stream communities with the nutrients and their results supported this hypothesis. Streams in their study that showed a decline in nutrient concentrations downstream from nutrient inputs had measured uptake lengths of 0.140 to 29.00 km for dissolved inorganic nitrogen and 0.140 to 14.00 km for phosphate. In addition, 40 - 45% of the streams they studied showed no significant decline in nutrients measured suggesting nutrient saturation of these streams. Haggard et al. (2005) also found a significant impact of a WWTP on the nutrients of Columbia Hollow, a 3rd order stream in the Ozark Plateau in Arkansas. In this study, uptake lengths were estimated between 6.80 to 13.40 km for soluble reactive phosphorus and 0.40 to 1.40 km for ammonia-N. Downstream nitrate-N resulted in negative values in Columbia Hollow which the authors interpreted as indicating no significant uptake and retention of nitrate by the stream. Little Deep Creek in our study had estimated uptake lengths of 17.60 Km for Total P and 11.20 Km for ammonia-N. Nitrate-N did not decline downstream of the effluent release suggesting that the stream community was saturated by nitrate-N like many of the streams in the Martí et al. (2004) study and in the Haggard et al. (2005) study. These results suggest that high nitrogen and phosphorus inputs from the WWTP have impaired the ability of Little Deep Creek to efficiently process and remove these nutrients as hypothesized by Martí et al. (2204).

With respect to nitrogen levels in Little Deep Creek, differences seen between the three sample dates (January 23 to April 02) show a decline in ammonia-N that coincides with an increase in nitrate-N in the samples from the WWTP effluent and the downstream samples (Fig 3A & B), suggesting that nitrification increased over this time period as temperatures increased. Shammas (1986) reported that nitrification was sensitive to temperature changes and measured significant drops in ammonia oxidation rates in nitrifying sludge at temperatures below 15 C. This suggests that ammonia-N levels during the late spring and summer months would be at their lowest levels due to high levels of nitrification. Our results appear similar to those of Haggard et al. (2005) in that nitrogen dynamics in Little Deep Creek appear to be dominated by conversion of ammonia-N to nitrate but little evidence of nitrogen retention by the stream.

Municipal sewage effluent may affect algae in receiving streams by elevating concentrations of growth stimulating nutrients (Carey and Migliaccio 2009) but also by introducing toxic substances that may by inhibitory to growth (Smital et al. 2011). Exposure of *Cladophora* and *Spirogyra* isolated from Little Deep Creek to different dilutions of sewage effluent and upstream water produced both growth stimulation and inhibition that was dependent on the dilution level and the type of alga. Both algae exhibited significant increases in growth rate at the lowest effluent concentration (10%). *Spirogyra* growth rate remained at this elevated level as effluent concentration increased up to 100%. In contrast, the growth rate of *Cladophora* declined with increasing effluent concentration and was inhibited 40% below the rate in the control treatment (upstream water) in 100% effluent. This inhibition of *Cladophora* growth at high WWTP effluent levels suggest some minor toxicity is present in the effluent. These results suggest that the release of WWTP effluent into Little Deep Creek could alter competitive interactions between these two filamentous algae. Since these algae exhibited identical growth rates in the unaltered upstream water, they would normally be in a competitive balance with neither being able to outgrow the other, all else being equal. We estimate that the released WWTP would be diluted to approximately 38% by mixing with the upstream flow (see Results). Growth rate of *Spirogyra* would be 34% greater than that of *Cladophora* at this effluent dilution level, suggesting an enhanced competitive ability for *Spirogyra* downstream of the effluent release.

Our results indicate a significant elevation in concentrations of nitrogen and phosphorus in Little Deep Creek downstream the WWTP effluent release compared to upstream concentrations. These alterations in nitrogen and phosphorus concentrations and in the N/P ratio suggest a likely effect on the composition and functioning of the downstream biological communities, based on previously published studies. The high inputs of the nutrients in the effluent have impaired the ability of the stream to remove the nutrients as indicated by large effective uptake lengths of total-P and ammonia-N. No uptake of nitrate-N was measured over the 8.25 km sampling length in the study which indicates that Little Deep Creek would act as a point source of nitrate-N for Deer Creek, the receiving stream. The algal growth experiment further supports the potential for the WWTP effluent to alter interactions between organisms in Little Deep Creek through differential effects on growth rates and toxicity. Our study shows that the impacts of small municipal WWTPs can have significant effects on the functioning of stream communities in low order streams.

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