A Preliminary Investigation of Nutrients and Isotopic Nitrogen in Oregon Chub Habitat Adjacent to Oakridge Sewage Treatment Plant

Final Report

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Introduction

Oregon chub (*Oregonichthys crameri*) is a minnow endemic to the Willamette River drainage, with a current distribution limited to 25 natural and nine introduced populations (Scheerer et al. 2003). The species was listed as endangered in 1993 over its entire range under the Endangered Species Act of 1973, as ammended. Oregon chub prefer slow-moving backwater habitats, such as sloughs, flooded marshes, and beaver ponds, that are common along the Willamette River and its tributaries. While some chub populations are stable or have increased in recent years, others are declining or have been extirpated (Scheerer et al. 2003). In the Oakridge Slough in the Middle Fork Willamette River drainage, the chub population density is low and the population has shown a recent decline in abundance from about 500 fish in 1999 to less than 50 fish in 2002 (Scheerer et al. 2003). Oakridge Slough also has an approximately 1-m thick layer that consists of primarily suspended organic material in various states of decay overlying a hard substrate. Sloughs in the area with more stable chub populations typically have less than 10 cm of organic material accumulated over a solid substrate. Reports from U.S. Forest Service indicate that the slough is undergoing natural eutrophication rates, and reduced water velocity and disconnection from the active flood plan because construction of the upstream dam has reduced flushing events and led to a net increase in organic material. However, the consistency and appearance of the material in Oakridge Slough was observed to be very different from nearby sloughs (P. Scheerer, ODFW, pers. comm.).

Oakridge Slough is located just downstream of Oakridge Sewage Treatment Plant, where sewage received from the town of Oakridge is treated and resulting biosolids are applied to upland sites on U.S. Forest Service land (Figure 1). The Oregon chub recovery plan (U.S. Fish and Wildlife Service 1998) specifically recommends "Monitor(ing) the effects of the adjacent sewage treatment plant practices on water quality in Oregon chub habitat" and "if nutrient rich runoff entering the slough is degrading water quality for the chub, take actions to reduce the impacts." Due to the proximity of the lagoon and biosolids application sites to surface water entering Oakridge Slough, concerns have been raised regarding how current or past practices at the treatment plant could have influenced water quality or organic matter buildup in the slough, and thereby potentially impacting the nearby Oregon chub population or its habitat.

This preliminary investigation was initiated to gather water quality information in Oakridge Slough and the surrounding area to evaluate if water quality is sufficient to support Oregon chub, and to determine if nutrients are enriched to concentrations that would harm Oregon chub, augment chub habitat, or indicate anthropogenic discharges have occurred from sources such as the sewage treatment plant. Specifically, the objectives were to compare nutrient concentrations in the slough to concentrations upstream and to those at or near the treatment plant. In addition, vegetation, biosolids, and detrital samples were collected from specific areas to evaluate levels of stable isotopes of nitrogen that could indicate an anthropogenic source of nitrogen entering the slough. Nutrient enrichment in the slough could augment buildup of organic matter, and the isotopic nitrogen analysis can help evaluate if the nitrogen within the organic matter originated from anthropogenic sources or from a more natural eutrophication process. An attempt was also made to physically characterize the primary constituents of the thick layer of material within the slough.

Methods

Water samples for nutrient and dissolved organic carbon (DOC) analyses were collected at Site 1 (the middle of Oakridge Slough channel near a staff plate), Site 2 (within the culvert that drains groundwater under the biosolids lagoon), Site 3 (a marshy area upstream of the culvert), and Site 5 (upstream of slough about 25 m downstream of the culvert at Site 2) (Figure 1). Site 3 served as a reference site because it was upstream of any potential nutrient inputs resulting from biosolids application and lagoon operations. Samples of inorganic and organic blank water were filtered at the U.S. Geological Survey (USGS), Water Resources Division (WRD) laboratory at the District Office in Portland, Oregon, to serve as field blanks (Site 4) for quality control purposes. Nutrients evaluated included dissolved nitrate nitrogen, dissolved Kjeldahl nitrogen (DKN), soluble reactive phosphorus (SRP), and total dissolved phosphorus. In addition, two samples of the organic material layer were collected from Oakridge Slough (from the top of the layer and from about 0.5-m deep) for visual examination under a dissecting microscope at WRD.

Water for DOC was collected in new 1-quart glass mason jars rinsed with site water; water samples for nitrate, DKN, and phosphorus analyses were collected in 3-L Nalgene jars cleaned with Liquinox®, tap water, 5% hydrochloric acid (HCL), distilled/deionized (DDI) water, and rinsed with site water. Samples were collected on December 20th, 2001 and were placed on ice in a cooler and transferred to the WRD lab. All water samples were filtered through a 0.45-micron capsule filter at the WRD lab within eight hours of collection. Samples were stored at 4 °C until delivery to North Creek Analytical, Inc., Beaverton, Oregon, on January 2, 2002. Nitrate nitrogen, organic carbon, and DKN were analyzed on January 3, 5, and 9, 2002, respectively, by the following U.S. Environmental Protection Agency (EPA) methods: Kjeldahl nitrogen by semi-automated block digestion and colorimetry (EPA 351.2); nitrate nitrogen by ion chromatography (EPA 300.0); and dissolved organic carbon by catalytic combustion or wet chemical oxidation with measurement by a flame ionization detector (EPA 415.1). Accuracy and precision of results were measured using laboratory blanks, duplicates, and matrix spikes for each analyte. For the nutrient concentrations, both the method detection limits and minimum reporting levels were included with results. The method detection limit is the minimum concentration that can be measured and reported with a 99 percent confidence that the analyte is greater than zero. The minimum reporting level is the smallest measured concentration of an analyte that may be reliably reported given the analytical method.

SRP and total dissolved phosphorus samples were analyzed at the WRD office on December 20 and 26, 2001, respectively. Total dissolved phosphorus was analyzed by persulfate digestion, autoclaving, and colorimetric determination as described in Standard Methods (American Public Health Association et al. 1992). SRP was determined by direct colorimetry following filtration (American Public Health Association et al. 1992). The colorimetric determination involves measuring the light attenuation after reaction of the phosphate ion with molybdate, which turns blue. Concentrations are calculated according to Beer's Law, and using a standard curve prepared separately.

The minimum sample holding times for nitrate (48 hours), DKN, dissolved organic carbon, and total dissolved phosphorus (about 1-2 days unpreserved) between filtration and analysis were not met during this study, and the concentrations of analytes could be affected by storage time. However, all samples were prepared and analyzed in the same manner, and any effects resulting from storage would be comparable among all samples.

Water quality measurements (temperature, conductivity, pH, and dissolved oxygen) were collected at nine locations including the four sites where water samples were obtained (Figure 1). Spot measurements were collected using a Hydrolab® H20® multiparameter water quality instrument (multiprobe). The multiprobe was calibrated on site prior to obtaining measurements using calibration fluids with temperatures similar to those of the site water.

Twelve samples were analyzed for isotopic nitrogen as an indicator of ^{15}N enrichment from anthropogenic sources such as animal wastes. Samples were collected on April 29, 2002, from Oakridge Slough, upstream areas, locations within the biosolids application areas 1 and 2, and the sewage lagoon (Figure 1). A sample of filamentous algae was also collected from Upper Buckhead Slough, a backwater area along the Middle Fork Willamette River that was considered a reference area because it was not directly influenced by agricultural runoff or wastewater discharge (Figure 2). Samples consisted of vegetation (grass, pondweed, or filamentous algae), biosolid material from the sewage lagoon, and the suspended, detrital material from Oakridge Slough. Samples were collected in chemically-cleaned jars, placed in a cooler with ice, and stored at -20 °C at the Oregon Fish and Wildlife Office in Portland until processing.

Isotopic nitrogen samples were processed on May 3, 6, 14, 16, and 30, 2002, at the WRD laboratory. Each sample was placed on a 63-micron sieve and rinsed with DDI water to remove foreign particles from the desired matrix. The biosolids sample (SP08) was not washed because the material was smaller than the sieve size; however, the biosolids material was homogeneous with no foreign material visible. Samples were lyophilized (freeze-dried) for at least 24 hours and ground with a mortar and pestle. Instruments used in grinding were washed and rinsed with Liquinox®, DDI water, and 5% HCL between samples. Samples were then stored at -20 °C until shipment to the laboratory conducting the analysis.

Samples were analyzed at the Stable Isotope Facility, Department of Agronomy and Range Science, University of California at Davis. Stable isotope ratios for nitrogen were measured by continuous flow isotope ratio mass spectrometry (IRMS; 20-20 mass spectrometer, PDZEuropa, Northwich, United Kingdom) after sample combustion to CO_2 and N_2 at 1000 °C in an on-line elemental analyzer (PDZEuropa ANCA-GSL). The gases were separated on a Carbosieve G column (Supelco, Bellefonte, Pennsylvania) before introduction to the IRMS. Sample isotope ratios were compared to those of standard gases injected directly into the IRMS before and after the sample peaks, and the delta ^{15}N of air values calculated. Isotopic composition was expressed in terms of delta (δ) values, which represent permil (‰) differences from the isotopic composition of nitrogen in air (Peterson and Fry 1987) by the equation:

$$\delta^{15}N = \{[^{15}N/^{14}N_{sample} / ^{15}N/^{14}N_{air}] - 1\} \times 10^3$$

Differences between duplicate samples were not greater than 0.3 ‰ and the standard deviation of quality control check samples was <0.3 ‰.

Results and Discussion

Nutrients and organic carbon

Nitrate nitrogen

Nitrate nitrogen is a measure of the concentration of nitrogen contributed by the nitrate anion, which is the relatively stable end product of the nitrification process (biological oxidation of ammonium to nitrite by autotrophic ammonium oxidizing bacteria, e.g., *Nitrosomonas*, and subsequent conversion to nitrate by *Nitrobacter* bacteria; U.S. Environmental Protection Agency 1986). Anaerobic conditions may stimulate the denitrification process (i.e., the conversion of nitrate nitrogen to nitrogen gas, or N_2), and generally waters with low dissolved oxygen values have low nitrate levels (Irwin et al. 1990). Nitrate is highly water soluble compared to other forms of nitrogen and is the form most readily transported to groundwater and other water bodies (U.S. Environmental Protection Agency 1986). Nitrate is less likely to be bound to sediment or suspended particles compared to other nutrients such as phosphate phosphorus, so nitrate transport in rivers is generally not dependent on suspended sediment and is not as easily trapped by sedimentation processes as is phosphorus (Smith et al. 1987). Nitrate concentrations are much less toxic to fish than are nitrite and ammonia. However, at concentrations below levels associated with toxicity to fish, nitrate can contribute to eutrophication and algal blooms when other essential nutrient factors are present (U.S. Environmental Protection Agency 1986).

In Oakridge Slough, nitrate exceeded detection limits at all sites, but fell between the detection and reporting limits for the reference site (Site 3) and barely exceeded the reporting limit at the culvert site (Site 2; Table 1, Figure 3). Nitrate was not detected in inorganic blank water. Nitrate was most elevated (approaching 2 mg/L) in Oakridge Slough at Site 1, a concentration which is nearly 60 times higher than the reference area (Figure 3). Nitrate concentrations at all sites were well below the acute toxicity values for various species of fish (see U.S. Environmental Protection Agency 1986 and Russo (1985) for summary of toxicity values).

In the Willamette Basin, nitrate concentrations are quite variable and are frequently influenced by anthropogenic sources such as agricultural and urban runoff, sewage treatment outfalls, and other discharges. Rinella and Janet (1998) reported nutrient concentrations in water at 51 stations as part of the National Water-Quality Assessment (NAWQA) program within the Willamette and Sandy River Basins, which encompass 12,000 square miles. Nutrient concentrations were associated with land use categories such as agriculture, urban, and forest. Nitrate nitrogen concentrations from 289 water samples at 51 locations ranged from 0.054 to 22 mg/L (median 1.1 mg/L). The upper 10% of the nitrate concentrations exceeded 5.9 mg/L and were associated with sites receiving primarily agricultural runoff. Nitrate concentrations in Oakridge Slough in our study was most similar to values from the NAWQA study in streams influenced by agricultural runoff. For example, in the Pudding River at Aurora, a NAWQA site influenced by runoff from agriculture (58% coverage) and discharge from a sewage treatment plant, the median nitrate in 31 samples was 1.5 mg/L (Rinella and Janet 1998). In contrast, nitrate concentrations in our reference samples were well below the median value (0.15 mg/L, 23 samples) reported in the NAWQA study for a forested reference site in the Oregon Coast Range (Gales Creek near Glenwood), and were below the median value (0.35 mg/L, 22 samples) for a forested site in the Cascade Range (Little Abiqua Creek near Scotts Mills).

Compton et al. (In press) reported that stream nitrate concentrations can be influenced by nitrogen-fixing red alder (*Alnus rubra*); nitrate concentrations were correlated to broadleaf cover dominated by red alder in the Oregon Coast Range. Nitrogen leaches as nitrate into groundwater and surface water once the available amount exceeds ecosystem needs. Flow-weighted averages of monthly nitrate samples in 26 streams sampled by Compton et al. (In press) in 2000 ranged from 0.074 to 2.043 mg/L (mean=0.882 ± 0.544 mg/L), and 8 of 26 (31%) stations sampled exceeded 1 mg/L nitrate. Red alder is present along the stream feeding Oakridge Slough and could contribute to the nitrate concentrations observed in the slough. However, it is very unlikely that the relatively small amount of red alder at the Oakridge site is responsible for the concentrations in the slough, as Compton et al. (In press) found that over 50% alder in the Coast Range was needed to result in a nitrate concentration of 1.6 mg/L. Nitrate concentrations in Oakridge Slough were near the maximum concentrations for coastal streams reported by Compton et al. (In press), and on-site reference values, along with reference concentrations observed in other Cascade Range streams (Vanderbilt et al. 2003), indicate that nitrogen from alder may be only a minor input relative to the concentrations observed in the slough.

Although Oregon Cascade Range streams can receive natural inputs of nitrate nitrogen from sources such as red alder, data from reference streams in the Cascade Range indicate concentrations are typically very low. In addition, primary production of Cascade Range streams is often nitrogen limited (Anderson 2002, Gregory et al. 1987). Long-term monitoring data from H.J. Andrews Experimental Forest in streams within three unlogged watersheds range from 0.001 to 0.004 mg/L; these values are low probably due to high terrestrial biological demand for nitrate and low availability for flushing (Vanderbilt et al. 2003). Recommended nutrient criteria for the Cascade Range region is also much lower than observed in Oakridge Slough. The EPA's recommended criteria for reference conditions for nitrate (NO_2 + NO_3) based on 75 streams from the Cascade Aggregate Ecoregion is 0.005 mg/L (U.S. Environmental Protection Agency 2000), and Ice and Brinkley (2003) recommended a nutrient criteria for nitrate nitrogen of 0.12 mg/L for the western forest region. The nitrate concentrations from the upstream reference area at Oakridge were very low, and the nitrate concentrations in Oakridge Slough appear to be enriched to levels indicative of anthropogenic sources. Because urban and agricultural lands are not present near our study sites, it is likely that runoff from the biosolids application area or from past practices at the sewage treatment plant are sources of nitrate nitrogen or contribute to nitrification in Oakridge Slough.

Dissolved Kjeldahl nitrogen

DKN represents all soluble forms of organic nitrogen plus ammonium. The nitrogen in DKN is considered largely available to biota, and elevated concentrations can indicate nitrogen over-enrichment and eutrophication.

DKN was highest in Oakridge Slough (0.80 mg/L) and lowest at the reference site (0.48 mg/L), although the difference in concentrations between the slough and reference area was much less dramatic for DKN than for the nitrate (Table 1, Figure 3). DKN was also detected in inorganic blank water at the reporting limit.

DKN from Oakridge Slough and upstream sites were similar to the reference or lower concentration values from other studies where anthropogenic sources of nitrogen were researched. In the tributaries of Lake Ray Roberts, a system near Dallas/Ft. Worth, Texas, that was influenced by nitrogen sources resulting from dam building activity, agriculture, and a sewage treatment plant outfall, most Kjeldahl nitrogen concentrations within the disturbed area ranged from 1 to 2 mg/L, with the lowest value (0.45 mg/L) from an undisturbed upstream site and the highest values from the sewage treatment plant during low flow (Institute of Applied Sciences 1988). The NAWQA program in the Willamette Basin (Rinella and Janet 1998) reported median Kjeldahl nitrogen in 259 samples as 0.50 mg/L (range=0.20 to 4.1 mg/L). The highest values in the latter study were in streams influenced by agricultural runoff. In contrast, the recommended criteria (from the 25th percentiles on data from all seasons) for reference conditions for total Kjeldahl nitrogen based on 65 streams from the Cascade Aggregate Ecoregion is 0.05 mg/L (U.S. Environmental Protection Agency 2000).

Total and soluble reactive phosphorus

Inorganic phosphorus compounds have low solubility and in natural waters phosphorus occurs primarily as phosphate (American Public Health Association et al. 1992, Hem 1985). Phosphorus as phosphate is required for plant growth and is essential for life (U.S. Environmental Protection Agency 1986). Soluble reactive phosphorus, or SRP, usually represents the orthophosphate portion of total phosphate phosphorus in the sample, and is typically thought to be the fraction of phosphorus immediately available to biota. Orthophosphate is the primary form of phosphorus directly available to organisms for uptake and use. Total phosphorus represents the immediately available form plus the phosphorus that may become available through release from sediment, organic material, and ion exchange (Garman et al. 1986). Total dissolved phosphorus represents the SRP plus the dissolved organic phosphorus, and often total dissolved phosphorus approximates SRP. Phosphate can be elevated in waters receiving discharges from treated municipal wastewater due to detergents, animal wastes, and fertilizers. Orthophosphate inputs from sewage and fertilizer releases to surface waters are the most easily used form of phosphorus for algal growth (American Public Health Association et al. 1992). Phosphorus correlates well with the eutrophication in water bodies, as it is often the limiting nutrient (Garman et al. 1986). EPA recommended that total phosphate phosphorus should not exceed 50 µg/L in any stream where it enters a lake or reservoir, nor exceed 25 µg/L within a lake or reservoir (U.S. Environmental Protection Agency 1986). Correll (1998) reported that concentrations of 100 µg/L are unacceptably high for most streams and concentrations of 20 µg/L can be a problem. Wetzel (1983) reported phosphorus in nonpolluted natural waters ranges from 10 to 50 µg/L, but variation is high; freshwater lakes with total phosphorus concentrations above 100 µg/L are considered hypereutrophic, those between 30 and 100 µg/L are often eutrophic (Wetzel 1983). In groundwater, concentrations are typically low (20 µg/L) since phosphorus adheres to soil particles (Garman et al. 1986). However, groundwater in volcanically influenced areas can have naturally elevated phosphorus levels (Dillon and Kirchner 1975).

At all Oakridge sites, total dissolved phosphorus and SRP were present and well above the detection limits (Table 1). Total dissolved phosphorus and SRP were the most elevated (282 µg/L and 237 µg/L, respectively) at Site 2, which apparently drains groundwater moving underneath the cement-lined sewage lagoon. Phosphorus at Site 2 was 7 to 12 times greater than in water from Oakridge Slough or the reference area (Table 1, Figure 4). In contrast, concentrations of both phosphorus forms in Oakridge Slough were only slightly higher than the reference area (Figure 4). Phosphorus concentrations in Oakridge Slough and the upstream sites were within the concentration range found in nonpolluted natural waters, but concentrations near the culvert at Site 2 were elevated well above levels suggesting hypereutrophic conditions (Wetzel 1983). At the time of sampling (late fall), green filamentous algae were observed growing in the waterway just below the drainage from Site 2, but the amount of algal growth did not seem excessive. Elevated phosphorus concentrations at this site indicate that hypereutrophic conditions would be expected in summer unless concentrations declined over time.

A nationwide study by USGS from 1974 to 1981 based on 381 river sampling stations near agricultural or urban areas reported total phosphorus values of 60 µg/L as the 25th percentile, 130 µg/L for the median, and 229 µg/L as the 75th percentile (Smith et al. 1987). In the NAWQA program within the Willamette Basin (Rinella and Janet 1998), the median of total phosphorus at 263 stations was 90 µg/L (range=10 to 7,000 µg/L); the median for SRP in 284 samples was 50 µg/L (range=10 to 5,800 µg/L). Most sites sampled in the NAWQA study had a number of phosphorus sources, so concentrations in Cascade Range streams without sources would be expected to be much lower.

Tributaries of Lake Ray Roberts (north of Dallas/Fort Worth, Texas) influenced by anthropogenic sources of nutrients exhibited most values of total phosphorus within the 200 to 600 µg/L range, whereas 70 µg/L was reported for a undisturbed upstream location and 2,220 µg/L was noted below a sewage outfall during low flow conditions (Institute of Applied Sciences 1988). SRP concentrations varied in the disturbed tributaries from 200 to 500 µg/L, with 0 µg/L and 2,100 µg/L reported for the upstream and sewage outfall locations, respectively.

In the NAWQA sampling at Gales Creek (100% forested), total phosphorus in 23 samples had a median of 20 µg/L, a 25th percentile at the reporting limit (10 µg/L), and a 75th percentile of 30 µg/L. The median for SRP at Gales Creek was 20 µg/L, whereas the minimum value was below the reporting limit and the maximum value was 30 µg/L (Rinella and Janet 1998).

The Pudding River at Aurora, with 58% agricultural cover type and an upstream discharge of a sewage outflow, had median total phosphorus in 31 samples of 110 µg/L, a 25th percentile of 70 µg/L, and a 75th percentile of 190 µg/L. The median for SRP was 70 µg/L, and the 25th and 75th percentiles were 50 and 190 µg/L, respectively (Rinella and Janet 1998).

The EPA's recommended criteria for reference conditions for total phosphorus based on 27 streams sampled the Cascade Aggregate Ecoregion is 9.06 µg/L (U.S. Environmental Protection Agency 2000). This recommended value is below concentrations of total dissolved phosphorus observed at all sites sampled at Oakridge.

The total dissolved phosphorus and SRP values at the culvert location (Site 2) were elevated well above upstream reference samples and reference samples from other investigations. Concentrations at Site 2 are more similar to agricultural sites, disturbed areas, or water influenced by sewage discharge. However, little information is available on the natural levels of phosphorus in groundwater in the area, and the high concentrations could be naturally elevated from volcanic soils. A clear source of the phosphorus, as well as the impacts of the high observed concentrations to the nearby stream and Oakridge Slough, remains unknown. Because phosphorus was not elevated in Oakridge Slough, it is unlikely that phosphorus is directly impacting Oregon chub in the slough. However, the phosphorus concentrations, along with the elevated nitrate nitrogen, could contribute to eutrophication in the slough.

Dissolved organic carbon

DOC was detected in similar concentrations at all sites, and was also present in the organic blank water (Table 1, Figure 3). A calculated typical freshwater concentration of DOC for temperate and arid region rivers is 3 mg/L (Hem 1985), which is near concentrations observed at the study site. DOC concentrations were also similar to those detected in various streams within the Willamette Basin (Rinella and Janet 1998).

Water quality parameters

Water quality parameters of temperature, conductivity, DO, and pH were spot checked at several locations (Figure 1) during water sampling. Temperature was similar at all sites except the Middle Fork Willamette River, which was approximately 3 °C lower than the other sites (Table 2). Temperatures at all sites were within the range (0 to15 °C) considered protective of salmonids (Wydoski and Whitney 1979), and therefore would likely be protective of Oregon chub.

Oakridge Slough exhibited very low DO (2.2 mg/L) deep in the organic material layer and lower DO at the east end of the slough compared to upstream sites (Table 2). Greater biological activity resulting from nitrate and phosphorus enrichment in the slough could cause eutrophication and oxygen depletion as algae use up oxygen during decay. Low DO and anaerobic conditions could also enhance denitrification processes in the slough, although nitrification would likely be the dominant process, and the nitrate concentrations in the slough were sufficient to support nitrification. The low DO observed in the deep sample in the slough also could have been caused by interference of the probe's stirrer by the thick organic material present, allowing depletion of DO near the probe's membrane and an unreliable result. Oregon chub are adapted to low DO conditions, and it is unlikely that the DO observed would negatively impact chub.

Conductivity was highest in Oakridge Slough and much lower in the upstream and river samples. Conductivity is a measure of the ability of water to conduct an electric current (Institute of Applied Sciences 1988), and is related to salinity and total dissolved solids because the ions in solution allow electrical current to be transmitted through water. The greater conductivity of Oakridge Slough would correspond with greater total dissolved solids at the slough compared to other sites.

The pH values at all locations were similar to normal values for streams and rivers (6.0 and 8.5, respectively; Stumm and Morgan 1996). These pH values are considered to be within ranges suitable for Oregon chub and would not be expected to influence results of nutrient sampling.

Isotopic nitrogen

Evaluations of isotopic nitrogen are generally conducted to better identify sources of nitrogen in watersheds or catchments, as human activity has greatly altered the nitrogen cycle in nature and has increased nitrogen loading in rivers (see Kendall 1998 and Mayer et al. 2002 for reviews). Evaluating the isotopic ratio of the two stable nitrogen isotopes, ^{14}N and ^{15}N, can help identify point and non-point sources to aquatic systems, such as nitrogen inputs from agricultural areas (Kendall 1998). The difference between the isotopic ratio in a sample compared to the standard ratio in air (the abundance of ^{15}N in air is constant), as described by the equation in the Methods section above, provides a value of ^{15}N enrichment, expressed as $\delta^{15}N$.

Although the ^{14}N isotope is predominant in nature (about 99.3% of all nitrogen), certain conditions can cause enrichment of the ^{15}N isotope (Kendall 1998). Organisms preferentially use the lighter isotope instead of the heavier isotope, and anything produced by an organism is isotopically lighter than the material not used (the reactant). The nitrate produced during nitrification generally has a lower $\delta^{15}N$ value than the ammonia (reactant) left behind. In contrast, anaerobic conditions and denitrification processes (such as in groundwater) can result in ^{15}N enrichment and larger $\delta^{15}N$ values. As the reactant is used up, the values of the product and leftover reactant change predictably, and different sources of nitrate often have distinct nitrogen isotopic compositions (Kendall 1998). Animals can be enriched in ^{15}N relative to their diet and wastes, as described in the following material quoted from Kendall (1998), which can also be found online at http://wwwrcamnl.wr.usgs.gov/isoig/isopubs/itchch16.html:

> "The increases in $\delta^{15}N$ in animal tissue and solid waste relative to diet are due mainly to the excretion of isotopically light N in urine or its equivalent (Wolterink et al. 1979). Animal waste products may be further enriched in ^{15}N because of volatilization of ^{15}N-depleted ammonia, and subsequent oxidation of much of the residual waste material may result in nitrate with a high $\delta^{15}N$. By this process, animal waste with a typical $\delta^{15}N$ value of about +5 ‰ is converted to nitrate with $\delta^{15}N$ values generally in the range of +10 to +20‰ (Kreitler 1975; 1979), and human and other animal waste become isotopically indistinguishable under most circumstances (an exception is Fogg et al. 1998)."

This process results in much larger $\delta^{15}N$ in areas where animal wastes and wastewater are discharged (Mayer et al. 2002, Jordon et al. 1997, Kendall 1998, Lake et al. 2001). Fertilizer and animal wastes are isotopically distinct, having $\delta^{15}N$ values of 0±2 ‰ and +15±5 ‰, respectively (Kendall 1998). The isotopic compositions of primary producers such as non-nitrogen fixing plants are then reflected by higher level consumers, and the $\delta^{15}N$ values of plants and organisms will reflect the patterns in the interplay of source signatures and fractionations (Kendall 1998). However, $\delta^{15}N$ values from some sources do overlap, and denitrification can form a trend towards increased $\delta^{15}N$, which approach the range indicative of animal wastes (Mayer et al. 2002).

At the Oakridge site, the reference materials revealed very low enrichment of ^{15}N, with $\delta^{15}N$ values in algae of -0.43 to +0.34 ‰ from the upstream reference site and Upper Buckhead Slough, respectively, and -0.63 ‰ in grass clippings upstream of the sewage lagoon (Table 3). These $\delta^{15}N$ values are similar to those expected from atmospheric nitrate deposition or nitrate-containing fertilizers (Mayer et al. 2002). In contrast, the $\delta^{15}N$ values from the biosolids and vegetation samples in Oakridge sewage lagoon (Table 3) were within the range of $\delta^{15}N$ values (+15±5 ‰) indicative of animal waste sources (Kendall 1998). The vegetation sample (pondweed) from the surface of the lagoon exhibited the most elevated $\delta^{15}N$ of +13.8 ‰ (i.e., the vegetation was enriched with ^{15}N from the sewage lagoon biosolids). In addition, grass clippings from two of the biosolids application areas (Figure 1) were moderately enriched with ^{15}N (Table 3). All non-reference samples of grass, algae, and suspended material from Oakridge Slough showed evidence of ^{15}N enrichment (i.e., much higher $\delta^{15}N$ values) compared to corresponding grass and algae reference samples, and were much higher than $\delta^{15}N$ values (0±2 ‰) indicative of atmospheric or agricultural fertilizer sources. However, the $\delta^{15}N$ in all the Oakridge Slough samples were lower than observed in the vegetation and biosolids from the sewage lagoon (Table 3). The $\delta^{15}N$ of the algae sample from the culvert site, where the highest concentrations of total dissolved phosphorus and SRP in water were observed (Figure 4), was just slightly below the $\delta^{15}N$ value in the biosolids sample from the lagoon (Table 3). The intermediate $\delta^{15}N$ values in

Oakridge Slough and other samples compared to the reference samples indicates that a source enriched with ^{15}N is being used by algae in the slough and in the stream near the lagoon. The enrichment could be the result of the denitrification process, although this would be unlikely because algae in the Upper Buckhead reference slough were not using a source enriched with ^{15}N, and denitrification is not sufficient to result in the enriched nitrogen the algae are using in Oakridge Slough. Enrichment could also be the result of uptake of nitrogen that originated from the biosolids associated with the lagoon or the waste management process, or from nitrogen forms entering the nearby stream and slough habitats following biosolid application to the upland areas (Figure 1). The mixing of groundwater dominated by terrestrial inputs with the sewage could result in the diminishing $\delta^{15}N$ values observed in samples collected downgradient from the sewage lagoon.

Material from Oakridge Slough

Visual inspection of the surface sample from Oakridge Slough revealed the material to be primarily comprised of filamentous algae of the genus *Melosira*, as well as *Oscillatoria* or *Phormidium*. Many protozoa, rotifers, and diatoms were also identified. The sample from the deeper organic material layer consisted primarily of broken and hollow parts of filamentous algae, probably *Melosira*, and diatoms. Fewer single-celled organisms and rotifers were present in this sample. Inorganic material (crystalline structure) was also observed, primarily in the deeper sample.

Conclusions

The nutrient analysis indicates that nitrate nitrogen is enriched in Oakridge Slough, and total dissolved phosphorus (primarily as phosphate) in surface water from the culvert underneath the lagoon is sufficient to sustain algal growth. Nitrate and phosphorus concentrations are well below values considered directly harmful to Oregon chub. However, the nitrate concentration in Oakridge Slough was nearly 60 times higher than from the upstream reference area. The nitrate and phosphorus concentrations could augment eutrophic conditions, which could result in increased biological oxygen demand in late summer, or limit the habitat available to Oregon chub due to increased plant and algal growth. Further sampling is needed during different times of the year to determine whether concentrations of nutrients are sustained at these locations or change over time.

Samples from the sewage lagoon are enriched with isotopic nitrogen to levels considered indicative of animal waste sources. Samples collected outside the lagoon, including samples from Oakridge Slough, were also isotopically enriched, although less so than samples from the lagoon. This indicates that the algae in the Oakridge Slough and in the stream adjacent to the lagoon at Site 2 derive their nitrogen from a source enriched with ^{15}N. Materials from the sewage lagoon are the most likely identifiable source of the locally enriched isotopic nitrogen, as it is unlikely that conditions for denitrification (resulting in higher $\delta^{15}N$ values) are present at all sites where enrichment was observed. Some additional sampling during different times of the year would help further elucidate the sources of enriched nitrogen, and whether or not there are continuing inputs of nitrogen into Oakridge Slough that would negatively influence chub habitat.

The suspended material that comprises the layer in Oakridge Slough appears to be predominantly decaying filamentous algae and diatoms, with some inorganic material. The material is thick and limits the available habitat for Oregon chub to the upper 5 cm of the water column. The conditions in the layer appear to be characterized by low dissolved oxygen, although more accurate readings are needed for confirmation.

Recommendations

The results of this study indicate that nitrate concentrations are enriched in Oakridge Slough, and phosphorus is elevated in water from the culvert underneath the lagoon, compared to reference concentrations. These nutrients are not elevated to levels that would directly harm Oregon chub, but concentrations could augment algal growth and continue to limit chub habitat in the slough. Nitrate concentrations are similar to levels indicative of anthropogenic sources, and enrichment of the isotope ^{15}N within the slough is indicative of animal wastes. Nitrate could be entering the nearby waterway from application of biosolids to upland areas, or nitrification could be enhanced in the slough due to sewage waste-related organic matter entering the waterway following overflow of biosolids from the lagoon during flood events, spills, a leaking lagoon liner, or from past operations when the lagoon did not have a cement lining. Further characterization is needed to determine if nitrate from biosolids applications are currently entering the nearby waterway and slough, especially if restoration efforts of the slough habitat are conducted in the future. The following recommendations are provided to better protect Oregon chub in Oakridge Slough:

1) Monitor phosphorus on a seasonal basis at Site 2, as well as in nearby groundwater, to determine if the elevated concentrations observed could cause eutrophication in summer months and if similarly high concentrations are naturally found in groundwater in the area.

2) Establish a plan to monitor nitrate levels in shallow groundwater near the biosolids application site, the slough, and other nearby areas to determine if nitrate concentrations are augmented as a result of biosolids application. Characterization of isotopic nitrogen in the groundwater samples should be conducted to better determine the source of nitrate in the samples.

3) Monitor nitrate concentrations within the slough and upstream to determine if concentrations change seasonally. Algal growth could be measured during nitrate sampling events to better evaluate if eutrophication is associated with nitrate.

4) Monitor dissolved oxygen concentrations in the Oakridge Slough over time to obtain more accurate readings and to better evaluate if concentrations are protective of Oregon chub.

5) Additional characterization of the material in the Oakridge Slough should be conducted to further determine its origin, and fecal-indicator bacteria tests would help determine if the material was associated with sewage.

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Table 1. Nutrient and carbon concentrations in water samples from sites near Oakridge Sewage Treatment Plant.

Parameter	Site 1 (Oakridge Slough)	Site 2 (culvert under lagoon)	Site 3 (upstream reference)	Blank water	Method detection limit	Reporting limit
Nitrogen & carbon (mg/L)						
Nitrate-nitrogen	1.89	0.106	0.032	ND[a]	0.005	0.1
DKN[b]	0.80	0.60	0.48	0.52[c]	0.26	0.5
DOC[d]	2.0	2.3	2.5	2.0[e]	0.1	1.0
Phosphorus (µg/L)						
SRP[f]	28.1	237	19.3	NA[g]	0.9	NR[h]
Total P	39.5	282	33.9	NA	3.1	NR

[a] Not detected.
[b] Dissolved Kjeldahl nitrogen.
[c] Inorganic blank water.
[d] Dissolved organic carbon.
[e] Organic blank water.
[f] Soluble reactive phosphorus.
[g] Not analyzed.
[h] Not reported.

Table 2. Water quality parameters obtained from spot readings of surface water at various sites near Oakridge Sewage Treatment Plant.

	Site 1 Oakridge Slough					Site 5 Stream	Site 2 Lagoon culvert	Site 3 Stream	Middle Fork Willamette
Parameter	Mid-channel	Mid-deep[a]	Side inflow[b]	Staff plate[c]	East end	Stream edge	Culvert channel	Upstream of culvert	Near edge at outfall
Temp (°C)	9.4	9.8	9.4	9.7	9.9	9.5	9.6	9.7	6.6
Diss. O_2 (mg/L)[d]	8.3	2.2	8.6	5.9	5.3	8.3	7.0	6.1	12.9
Conductivity(µS)	90	80	≃85	≃80	≃72	62	62	60	≃54
pH	6.2	6.0	6.2	6.1	6.0	6.2	6.1	6.0	7.0

[a] Middle of channel, deep in the organic matter.
[b] In small side channel that flows into slough.
[c] Near staff plate in mid-channel.
[d] Dissolved oxygen.

Table 3. Isotopic nitrogen composition, expressed as δ values or permil (‰) differences from the isotopic ratio of nitrogen in air, from various locations near Oakridge Sewage Treatment Plant and on- and off-site reference areas.

Sample Number	Result (‰)	Location	Sample type
RF02	0.34	Upstream reference site	Filamentous algae
B10	-0.43	Upper Buckhead Slough (reference slough)	Filamentous algae
SS06	4.73	Oakridge Slough	Filamentous algae
SS06-DUPLICATE	4.42	Oakridge Slough	Filamentous algae
SS06A	3.84	Oakridge Slough	Detrital material split from filamentous algae in sample SS06
SD07	2.71	Oakridge Slough	Suspended detrital material
SP08	9.54	Sewage lagoon	Biosolids
SP08-DUPLICATE	9.64	Sewage lagoon	Biosolids
SP08A	13.8	Sewage lagoon	Pondweed split from Biosolids in SP08
DC04	1.06	25-m downstream from culvert discharge	Filamentous algae
C05	8.85	Culvert sample	Filamentous algae
RF03	-0.63	Upstream reference site	Grass clippings
R01	5.34	Biosolids application Site 1, 8 m S of riser	Grass clippings
R09	7.19	Biosolids application Site 2; 8 m N of riser	Grass clippings

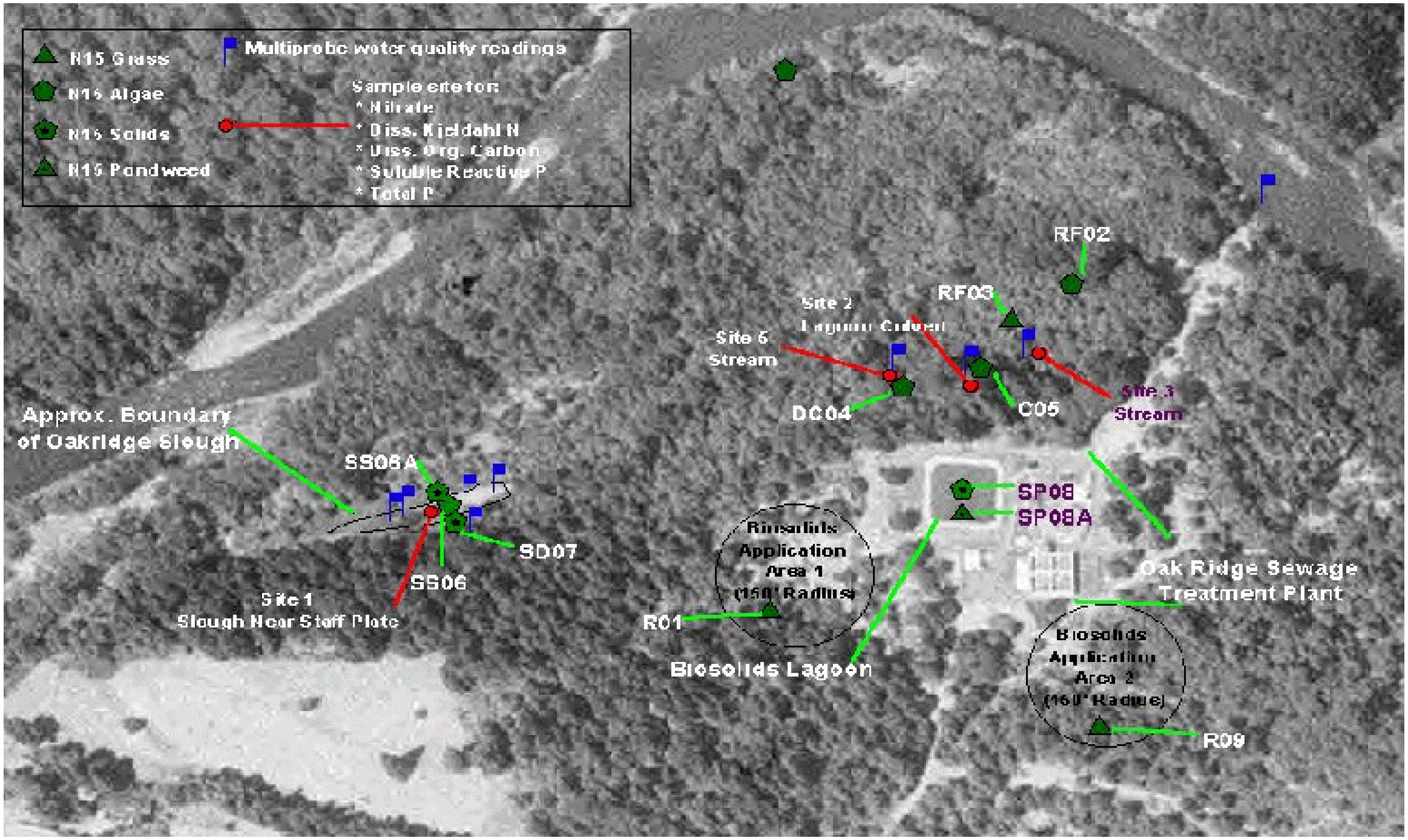

Figure 1. Sampling sites for nutrients, water quality, and isotopic nitrogen near Oak Ridge Sewage Treatment Plant.

Figure 2. Sample location for isotopic nitrogen in surface algae from Upper Buckhead Slough (reference site).

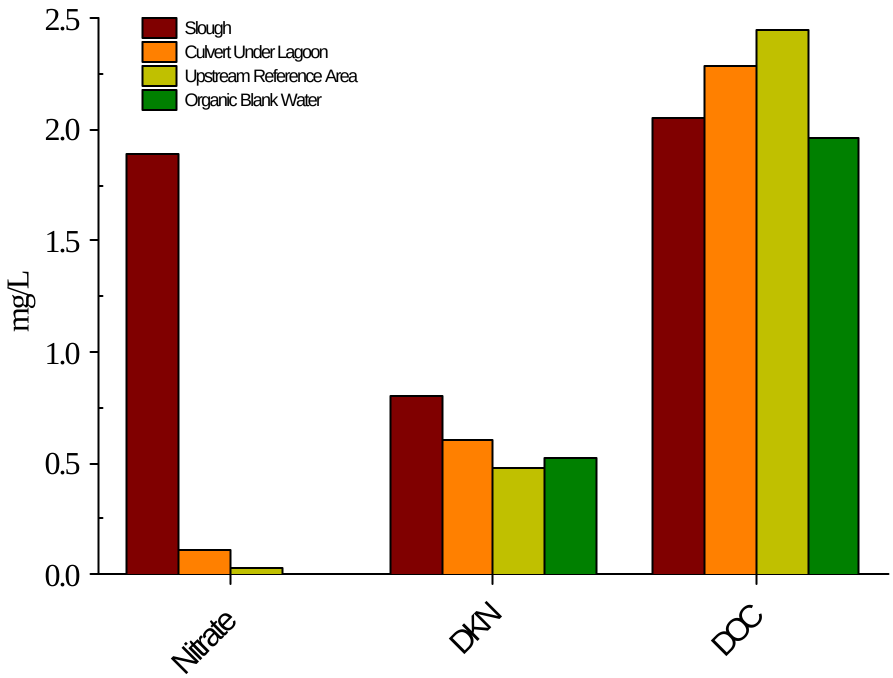

Figure 3. Concentrations of nitrate, dissolved Kjeldahl nitrogen (DKN), and dissolved organic carbon (DOC) from three sites near Oakridge Sewage Treatment Plant and in blank water.

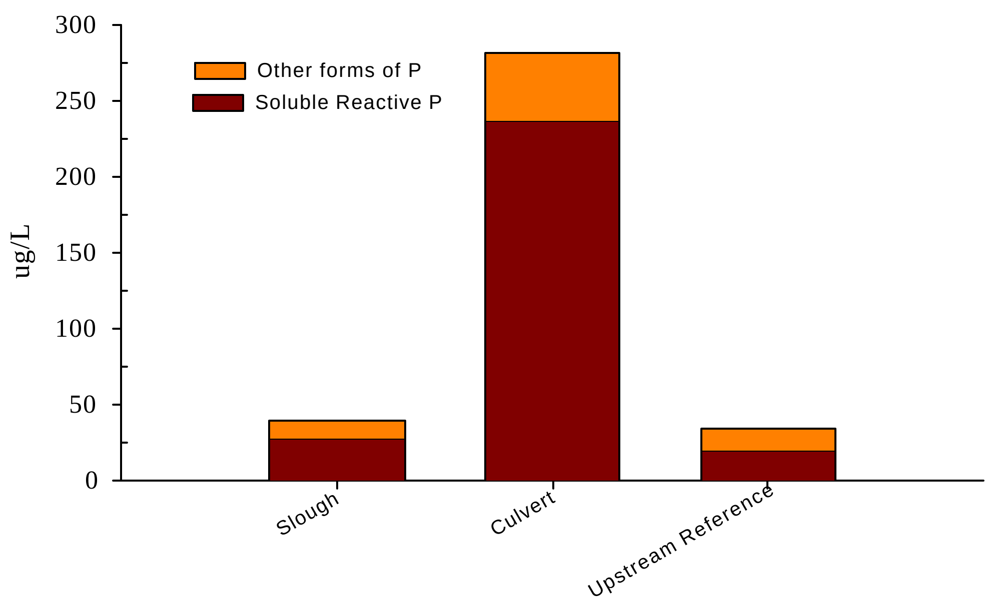

Figure 4. Soluble reactive phosphorous and other forms of phosphorus (together representing total dissolved phosphorus) at three sites near Oakridge Sewage Treatment Plant.