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Comparative examination of Lake Spokane groundwater for nutrient discharge by residential development influence

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Comparative Examination of Lake Spokane Groundwater for Nutrient Discharge

by Residential Development Influence

Eastern Washington University Biology Department

Masters of Science: Biology

Advisory: Suzanne Schwab, Camille McNeely, Carmen Nezat

By: Wyatt Plastino

THESIS OF

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APPROVED BY:

MASTER'S THESIS

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Acknowledgments

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Comparative Examination of Lake Spokane Groundwater for Nutrient Discharge by Residential Development Influence

Eastern Washington University Biology Department Advisory: Suzanne Schwab, Camille McNeely, Carmen Nezat By: Wyatt Plastino

Abstract

The goal of this study is to support the implementation of the Total Maximum Daily Load regulation for the Spokane River and Lake Spokane area in Eastern Washington in partnership with United States Geologic Survey and the Washington State Department of Ecology. The influence of residential development along the north shore of the Lake Spokane reservoir in the Suncrest area was examined as a possible nonpoint source of nutrient contamination to shoreline groundwater and as a possible influence on algae growth in Lake Spokane. Surface water and groundwater samples from hand-driven piezometers were collected from three residential development categories. The three categories were distinguished by proximity to residential development and onsite septic systems in relation to the shore line of Lake Spokane: nearshore, terrace and undeveloped/reference. Samples were taken monthly from March through August 2015. Groundwater samples were analyzed for chloride, ammonium, nitrite plus nitrate and orthophosphate through the Washington Department of Ecology's Manchester Laboratory. Surface water samples and a subset of groundwater samples were analyzed for ammonium, nitrite plus nitrate and orthophosphate through Eastern Washington University. Groundwater from areas of residential development in March/April 2015 was significantly higher in nitrite plus nitrate and groundwater from nearshore residential development was significantly higher in orthophosphate in August 2015 (p-value \leq 0.05). This indicates that residential development may be impacting groundwater nutrient concentrations. In conjunction with groundwater sampling, algae growth response to groundwater taken from the three development categories was compared using *Anabaena sp.* as a bioassay indicator for relative nutrient contents. There was significantly higher daily chlorophyll change in groundwater from the nearshore than reference development categories (p-value ≤ 0.05).

Introduction

Eutrophication is the processes by which the excessive addition of nutrients (particularly nitrate and phosphate) to a water body stimulates primary productivity and algal growth, ultimately degrading the overall water quality. Eutrophication is a long standing concern in freshwater bodies due to the associated reduction in biodiversity, loss of aesthetic and recreational value, and potential toxicity to wildlife and humans (Edmonson et al., 1956; Jordan et al., 2007; Withers et al., 2009; McCobb et al. 1999). While eutrophication is a natural stage in the aging process of some water bodies, anthropogenic inputs of nutrients can artificially stimulate and sustain eutrophic conditions (Edmonson et al., 1956). In freshwater bodies, the most common limiting nutrient factor for aquatic growth and eutrophication is phosphorus (Dodson, 2005; Vllenweider 1968). While phosphorus is a natural part of the environment, it can also come from a host of human activities in the watersheds including runoff from impermeable surfaces, manufacturing, agricultural practices and civil and domestic wastewater effluent (Dodson, 2005; Cook et al. 2005). When dissolved nutrient concentrations become excessive in a water body, exponential growth of algae can occur. These algal blooms can deplete dissolved oxygen leading to hypoxic conditions, reduced water clarity, and may produce toxins and odors, all of which negatively impact wildlife, aesthetics and recreation. The reduction of dissolved oxygen in water bodies during eutrophication is caused by respiration during the growth cycle and associated

decomposition upon death. Oxygen depletion can impact invertebrates, fish and amphibians in the water body (Dodson, 2005, Rocha et al. 2015). Furthermore, freshwater and marine Cyanobacteria species such as *Anabaena, Microcystis* and *Aphanizomenon* are capable of producing cyanotoxins including neurotoxins, cytotoxins, endotoxins, and hepatotoxins, while *Karenia brevis* is the marine dinoflagellate responsible for the toxic red tides in the Gulf of Mexico (Paul and Richard, 2014; Texas, 2015). To reduce the occurrence of algal blooms in many managed lakes various agencies may monitor and regulate phosphorus, ammonia and dissolved oxygen. Mandated and empowered by the Clean Water Act local water regulatory agencies implement Total Maximum Daily Load restrictions or water quality attainment plans which establish limits for each nutrient as criteria to maintain or improve water quality. If maximum daily loads are exceeded the sources of these nutrients must be identified and regulated to protect and preserve aquatic life and human health.

To combat the consequences of eutrophication, nutrient water quality criteria were developed as part of the Clean Water Act Section 304(a). The United States Environmental Protection Agency (EPA) has developed protocols and standards to provide guidance for states and tribes adopting water quality criteria (USEPA, 2015). For many impaired water bodies, Total Maximum Daily Load requirements have been established to reduce nutrient inputs from both point and nonpoint sources (GeoEngineers et al., 2011; Moore and Ross, 2010; USEPA, 2015). To enforce total maximum daily load requirements, accurate identification of ecologically relevant sources, both point and nonpoint, contributing to contamination must be identified (Novotny and Olem, 1994; Withers et al., 2009).

Point sources of nutrients come from a single location, such as a factory or wastewater treatment plant, and flow continuously or semi-continuously. Point sources are often required to be continually monitored to maintain regulation criteria. Nonpoint sources of nutrients come from widely dispersed locations, such as runoff from impermeable surfaces, like roads and parking lots, or groundwater contamination from cultivated fields and septic systems. Contamination from nonpoint sources tends to fluctuate and, due to their complexity, is often not monitored continuously (Carpenter et al., 1998). The management of nutrient contamination has historically been focused on point sources, although the cumulative impacts from nonpoint sources may be equally important. Modeling, in addition to continuous bank-side analyses examining retention and release of phosphates in soils, has shown nonpoint source contamination can contribute significant amounts of bioavailable phosphorus (Stollenwerk, 1996; Withers et al., 2009; Dudley and May, 2007; Jordan et al. 2007). Therefore, in the interest of reducing impacts to water quality, the regulation and management of freshwater bodies should include nutrient loading from nonpoint sources in addition to point sources.

Nonpoint source nutrient loading through groundwater can be difficult to identify and manage because it is the culmination of many individual components over a large

area that can vary seasonally. Due to the inherent difficulty of quantifying and controlling nonpoint sources their management requires a collective social conscience to recognize and control the multiple and scattered sources by everyone in the watershed, including domestic householders (e.g., minimizing runoff and septic tank overflow and breakthrough) (Withers et al., 2009; Lombardo P. 2006; White 1982). Nonpoint source runoff and septic sewage contaminated groundwater is a nonpoint source that has been shown to contribute sufficient nutrients to contribute to eutrophication in freshwater bodies (McKinley 2010; Stollenwerk, 1996; Pierzynski et al.2000). Therefore, efforts to maintain water quality must include consideration of septic systems, in addition to runoff as nonpoint sources of contamination.

Lake Spokane Background

Lake Spokane (also known as Long Lake) in eastern Washington, is a 24 mile long reservoir of the Spokane River formed by the Long Lake Dam and sourced from Lake Coeur d'Alene in Idaho. It is subject to nutrient inputs from agricultural runoff, industrialization, historical mining, urbanization and septic systems and faces many of the water quality management challenges typical of surface waters impacted by multiple human activities (HDR, 2011; Moore and Ross, 2010). Long Lake Dam, completed in 1915, is a hydroelectric production facility now owned and operated by Avista Corporation. Lake Spokane drains into Franklin D. Roosevelt Lake, an impoundment of the Columbia River (Figure 1).The water level in the Lake Spokane reservoir is managed to maintain minimum flows through most of the year at 1,535 ft above sea level with an annual winter drawdown of 10 feet for aquatic weed management (drawdown was not performed during the course of the study). Mean monthly discharge from years 1892 to 2014 ranges from 17,700 ft³/s in May to 1690 ft³/s in August (Gendaszek et al, 2016). Primary growth in Lake Spokane is limited by phosphorus based on studies examining the response of periphyton to nitrogen and phosphorus, as is common for many freshwater bodies (Patmont et al, 1987, Gendaszek et al, 2016). Sediment composition in the area is mainly coarse textured sediments with underlying basalt bedrock, and the shorelines are composed primarily of a terrace of unconsolidated glacial-fluvial flood sediments composed of sand and gravel with cobbles, boulders and minor silt content (Soltero et al, 1992).

Lake Spokane has a history of eutrophication caused by anthropogenic nutrient inputs over the past 40 years and continues to give rise to seasonal algae blooms, low dissolved oxygen concentrations and occasional outbreaks of toxic Cyanobacteria*.* (Wagstaff and Soltero, 1982; Moore and Ross, 2010). Due to evident water quality violations, starting in the 1990s, segments of the Spokane River and Lake Spokane have been listed on the Washington State Department of Ecology's 303(d) list of impaired water bodies. The 303(d) list is a list of water bodies that are not likely to improve in water quality in two years and require federally mandated remediation efforts (USEPA. 2014). Since the 1990s, through the power of the federal Clean Water Act, the

Washington Department of Ecology has developed a series of strategies to address the water quality issues in the form of total maximum daily load restrictions.

In efforts to meet the goal of the total maximum daily load restrictions, a bi-state budget initiative between Idaho and Washington was formed. The first total maximum daily load for the Spokane River and Lake Spokane was drafted in 2004 but was withdrawn in 2005 due to a developing use attainability analysis petition by the point source dischargers, because of the inability of those dischargers to meet there waste load allocations. The major point source dischargers and their allocations are all documented through National Pollution Discharge Elimination System permits, while the nonpoint sources within the Spokane River including onsite septic systems are not well documented (Cusimano, 2004; Gendaszek et al, 2016). In 2010 the total maximum daily load requirements were revised, and as part of the revision nonpoint sources were added as a component in managing the total nutrient budget of Lake Spokane and the Spokane River to meet a total phosphorus concentration in Lake Spokane of no more than $25 \mu g/L$ (Moore and Ross, 2010). The final and most recent draft of this total maximum daily load includes compliance for waste-load allocations of phosphorus from both point sources and nonpoint sources. In order to meet waste load allocations set by the total maximum daily load requirements, dischargers are to implement technologies and take other actions to reduce point and nonpoint sources of phosphorus to meet the allocated conditions by 2020 (GeoEngineers et al.; 2011; Moore and Ross, 2010).

Nonpoint source areas of possible significance along the Spokane River and Lake Spokane have been selected for examination to insure they meet the requirements set for the total maximum daily load requirement by 2020. Among these identified sites is Suncrest, a small, dense residential development along the northeastern shore of Lake Spokane containing over 1,300 residences, all relying on onsite septic systems for wastewater disposal (Figure 1). Septic system drain-fields retain phosphorus from wastewater and effluent by sorption and precipitation of phosphorus to sediment particles in the soil of a disposal field (Figure 2A, 2B); (Lombardo, 2006). The attenuation of this phosphorus in the sediment of the disposal field is dependent on the soil's chemical and physical properties, rate of wastewater loading, design and management and the sites' hydrology and proximity to surface water and groundwater. Dependent on these factors, disposal fields can also exceed their sorption capacity, reducing their ability to retain and attenuate additional phosphorus. As a consequence, breakthrough in these systems can occur and additional phosphorus in septic system effluent is able to migrate greater distances, potentially discharging and influencing surface water nutrient contents (Harmon et al. 1996). Many of the onsite septic systems in the Suncrest area are over 40 years old and are reaching the end of their operational timelines (McKinley and Siegrist, 2010; Lowe and Siegrist, 2008). A conservative analysis of septic system failure/breakthrough in the Suncrest septic system area was performed by the engineering company HDR in 2011, resulting in an estimate of approximately 3.0lbs/day of phosphorus loading. In 2011, it was estimated that 150 of the 1,380 Suncrest onsite septic

systems had reached breakthrough, with 440 more systems projected to reach breakthrough in the next twenty years (HDR, 2007, 2011). Groundwater inputs from onsite septic systems in the Suncrest area are therefore a potentially substantial source of phosphorus to groundwater entering Lake Spokane, particularly when combined with potential runoff from impermeable surfaces and fertilizers.

Previous attempts to identify phosphorus and septic influences on Lake Spokane in the Suncrest area include an analysis of lake water for optical brighteners in laundry detergents, but the results were inconclusive (SCCD, 2015). Therefore, current data on the nutrient concentrations in groundwater in the Suncrest development area are needed in order to facilitate management and possible reduction of those sources. My study had two goals. First, through groundwater monitoring, this study was designed as a preliminary investigation to identify possible variation in nutrient concentration in groundwater between sites near residential development containing onsite septic systems and reference sites not containing onsite septic systems. Second, I tested whether potential variation in nutrients between development categories is sufficient to stimulate algal growth, indicating that runoff and potentially septic systems are contributing to diminished water quality. This study also includes an examination of seasonal variability of groundwater nutrient contents. The focus of this study is to support the implementation of the total maximum daily load regulation with implications for approaches to facilitate management and reduction of those nutrient sources in Lake Spokane.

Methods

Sample site

Preliminary investigations to identify sites for more extensive sampling led by the United States Geological Survey (USGS) attempted to identify locations where ground water was potentially influenced by septic system effluent discharge using nitrogenisotope analysis of aquatic vegetation (Gendaszek et al, 2016). In August and September 2014, plant samples of Richardson's pondweed (*Potamogeton richardsoni*) were collected from 84 locations along the Suncrest shoreline. Plants were sent to the University of California-Davis Stable Isotope Facility to be analyzed for $\delta^{15}N$ ratios (Gendaszek et al, 2016) to identify possible locations of septic influence, caused by natural bioaccumulation of ¹⁵N compared to ¹⁴N in human septic effluent. The $\delta^{15}N$ in human septic effluent has been found to be greater than natural environmental background $\delta^{15}N$ ratios; therefore elevated values of $\delta^{15}N$ in plant tissues can be indicative of nitrogen from anthropogenic sources (Cabana et al. 1996; Cole et al. 2004). δ^{15} N analysis indicated the δ^{15} N value from Richardson's pondweed located in nearshore residential development and terrace residential development locations were not significantly different (Wilcoxon rank sum test; p-value 0.29). The $\delta^{15}N$ value in Richardson's pondweed located from undeveloped shoreline in eastern Lake Spokane were significantly less than that from nearshore residential development locations (Wilcoxon rank sum test; p-value 0.004), and terrace residential development locations

(Wilcoxon rank sum test; p-value <0.001). There were also significantly higher $\delta^{15}N$ values in Richardson's pondweed located in an undeveloped shoreline in central Lake Spokane downstream of all other development locations, but this downstream undeveloped shoreline in central Lake Spokane was not used for groundwater sampling during this study.

Study Design

Based on their preliminary investigation of $\delta^{15}N$ ratio and temperature profiles, in consultation with USGS, I selected three areas for more extensive sampling along the north eastern Lake Spokane shoreline that differed in their degree of residential development: nearshore, terrace, and reference (Figure 3). Site development category was based on density of septic systems and proximity to the waterbody: (1) near shore with high residential development and onsite septic systems close to shoreline, (2) terrace with residential development and onsite septic located on a terrace away from the shoreline (3) a reference site lacking residential development or onsite septic systems (Figure 3). The reference category was located upstream of all other development category locations, the upstream most sites of the reference category being near the junction of the Spokane River and Little Spokane River. Terrace category was taken from two locations divided by the nearshore category, one located furthest downstream and the other upstream of the nearshore category. The nearshore category was located between the terrace category and downstream of the reference category (Figure 4). High variation in bank side sediment

composition within and across development categories was noted during field sampling; lentic areas of high silt deposition were present across all development categories. High sediment deposition was most apparent in a wetland section of the reference development category.

Sampling began in March 2015 led by the USGS (Gendaszek et al, 2016) with thirty sampling sites, ten sites per development category, (Figure 5). For the initial sampling event, nineteen of the thirty groundwater samples were taken during March 24- 26, 2015, and the remaining eleven were taken April 29-30, 2015 (Gendaszek et al, 2016). Following the initial sampling, a subset of nine sites from the developmental categories was selected for additional groundwater sampling, three sites per development category. The nine sites selected for seasonal monitoring of groundwater nutrient content were sampled monthly April to August near the 20th of each month. The groundwater samples from the initial sampling event were sent to the USGS National Water Quality Laboratory to be analyzed for chloride, ammonium, nitrite plus nitrate, orthophosphate and total phosphorus (Gendaszek et al, 2016). Groundwater samples from the seasonal monitoring were sent to the Washington Department of Ecology's Manchester Laboratory to be analyzed for chloride, ammonium, nitrite plus nitrate and orthophosphate. Funding for analysis of these groundwater samples for the initial sampling event and seasonal monitoring were provided through the Washington Department of Ecology from March to June. The remaining funding for groundwater seasonal monitoring samples July to

August and sampling equipment for the study were provided through USGS. July to August groundwater samples from seasonal monitoring were sent to the Washington Department of Ecology's Manchester Laboratory and were analyzed for ammonium, nitrite plus nitrate and orthophosphate.

In August a final sampling was done, during which the thirty sample sites from the initial sampling were resampled with the addition of one reference site located in the upstream section of the reference category. These samples were analyzed through Eastern Washington University department for ammonium, nitrite plus nitrate and orthophosphate. Funding for the final resampling analysis was provided through Eastern Washington University. In August a surface water sample was taken at each groundwater sampling location. Rates of groundwater exchange into the Lake Spokane reservoir were not measured over the course of the study; therefore estimates for fluxes of phosphorus and nitrogen into the waterbody were not estimated.

Sampling Procedure

To collect groundwater samples, temporary hand driven point piezometers were installed at each site location to a depth of roughly one meter into the reservoir sediment and within six meters of the shoreline (Figure 6). Piezometers were installed by ramming a two meter by two and a half centimeter diameter steel pipe into the reservoir bed to a depth of one meter with a removable steel drive point. The steel pipe was then inserted with one centimeter diameter polyethylene tubing fitted with a coarse fifteen centimeter

stainless steel wire mesh screen to maintain flow and restrict large particles during groundwater extraction. The steel bar was then removed leaving the polyethylene tubing and fitted stainless steel screen within one meter of the reservoir sediment. Prior to taking a groundwater sample, each piezometer was then developed by pumping and extracting water using a peristaltic pump from the piezometer for 30-60 minutes until clear water was produced. Following piezometer development groundwater samples were then extracted and field filtered using a preconditioned 0.45 µm capsule filter fitted to the peristaltic pump. Filter conditioning was performed by purging unused filters with one liter of deionized water to remove potential manufacturing residue. Sample collection bottles were also rinsed with deionized water followed by filtered native water three times prior to each sample being collected. Surface water samples were collected at each site June to August and field filtered by hand using a conditioned 0.45 µm disk filter. Following collection, water samples were immediately placed in iced coolers.

Over the seasonal monitoring portion of the study, following each sampling event, all samples were shipped in iced coolers within twenty-four hours to the Washington Department of Ecology's Manchester Laboratory to be analyzed. Surface water and groundwater samples analyzed through the Eastern Washington University were kept frozen until analysis January to March 2016. Samples analyzed though Eastern Washington University were analyzed using an ALPPKEM O-I analytical flow analyzer following O-I analytical methodology and management procedures (O-I Analytical,

2009). For each sample, field measurements of specific conductance, dissolved oxygen of groundwater and surface water, temperature and hydraulic gradient were taken. These field measurements were used as a check standard to insure samples were of groundwater in origin and to determine general direction of groundwater flow (Table 1). Installation and operation of the piezometers followed Washington Department of Ecology v.2 standard operating procedures (SinClair and Pitz, unknown). A field blank and replicate was taken during each sampling to insure quality-control of sampling procedures and equipment. Results for all field blanks were reported less than detection limits aside from one during the initial event and one in June for ammonium, results were 0.01 and 0.016 ppm, with <0.01 ppm being the detection limit for ammonium. Preparation of sampling materials and protocol for sampling procedures followed USGS standard practices (U.S. Geological Survey, variously dated; Wagner et al., 2007; Kozr and Kahle, 2013; Wilde, 2004; Gendaszek et al, 2016).

Monthly monitoring of water-levels in monitoring wells near Suncrest show that groundwater flow is generally toward the reservoir, indicating minimal influence of surface water on groundwater samples. However, Soltero et.al (1992) reported significant variation in groundwater flow direction depending on location and reservoir stage. To insure groundwater discharge to surface water and assess groundwater influence direction, the difference in hydraulic head between surface water and groundwater was estimated using a manometer board (Simonds et al., 2004, Gendaszek et al, 2016). The

manometer board is operated by attaching one valve to the installed piezometer and placing the other valve in the surface water above the streambed surface. The water levels in the two opposing valves are then pumped using a peristaltic pump to equal levels. A valve located above the two opposing valves (piezometer and surface water) is then used to maintain pressure on the system while the pressure from the peristaltic pump is removed. The pressure in the system is then momentarily removed by opening and closing the above valve, the system is allowed to equilibrate and a reading is taken of the water levels in the tubing above the two opposing valves; this process was repeated four to five times during each sampling event per site location to develop a reading (Figure 6). To compute the vertical hydraulic gradients the difference between the water levels in the surface water and groundwater is divided by the depth of the midpoint of the screened interval beneath the streambed (Winter et al. 1988; Gendaszek et al, 2016). Groundwater discharge is indicated when the water level inside the piezometer tubing is higher than the water level of the surface water tubing indicating a positive vertical hydraulic gradient value (Gendaszek et al, 2016). A positive vertical hydraulic gradient value indicates flow from groundwater towards surface water, while a negative vertical hydraulic gradient value indicates that surface water is moving towards groundwater (Figure 7). During the initial sampling hydraulic gradient was confirmed at twenty-one of the thirty piezometer locations. Seventeen piezometers reported with positive hydraulic gradient, three with negative and one piezometer was reported with a neutral hydraulic gradient (Gendaszek et al, 2016). Groundwater discharge was confirmed at nine out of nine piezometer

locations each month during seasonal sampling, but seasonal variability and low vertical hydraulic gradient values were noted. During the final sampling hydraulic gradient was confirmed at thirty-one out of thirty-one piezometer locations, twenty-nine piezometers reported with positive hydraulic gradients and two with negative.

Bioassay

In conjunction with groundwater sampling, an in vitro comparative groundwater bioassay of algal growth potential was conducted June, July and August of 2015. The purpose of this study was to compare the relative algal growth potential of the groundwater between the development level categories by comparing their ability to support algal growth. Existing algal communities were first removed from the water samples using a 0.10 μ m filter. In June and July filtered groundwater from non wetland reference and filtered groundwater from nearshore residential developed sites were mixed into a continuum between 100% nearshore residential development and 100% non wetland reference in 25% increments (Figure 8). In August the same procedure was used to compare groundwater from nearshore residential development and terrace residential development sites.

The bioassays were performed in 50 ml flasks and each dilution was replicated five times. Each 50 ml flask was inoculated with 2 ml of a stock Alga-Gro©Freshwater *Anabaena* sp. ordered from Carolina Biological Suppy. The inoculated samples were randomly placed on a shaker table set at 80 rpms in a greenhouse. At intervals of two to three days, 2 ml samples from each flask were analyzed for chlorophyll concentrations as an indicator of algal growth using a Turner Trilogy fluorometer.

Analysis

All statistical testing was performed using R v. 3.2.5 statistical software. Nutrient concentrations of groundwater and surface water in March/April and August were tested with the null hypothesis that there is no significant difference between groundwater or surface water nutrient concentrations from different development categories using an alpha of 0.05. For March/April sampling, results for ground water nutrient concentrations (chloride, ammonium, nitrite plus nitrate, orthophosphate and total phosphorus) between development categories (nearshore, terrace and reference) were compared using a one way ANOVA and Tukey HSD test, with groundwater nutrient concentration as response and development category as a predictor (n=10 per development category). All data were log-transformed plus one prior to statistical testing. For August, surface water nutrient concentrations by development category were incorporated as an additional predictor/factor. The August results were tested using a two way ANOVA and Tukey HSD test with groundwater nutrient concentration (ammonium, nitrite plus nitrate and orthophosphate) as response and development category(nearshore, terrace, reference) and water source (groundwater and surface water) as predictors; ($n=10$ per development category).

The results for March/April and August were further examined by dividing the reference development category into two categories - those located at the downstream section of the reference category and those in the upstream section. The reference sites located in the downstream section of the reference category were located in a wetland, highly lenthic zone characteristic of fine sediment composition and high rates of deposition. The resulting two categories were labeled non wetland reference (n=4) and wetland reference (n=6) for March/April and (n=5, n=6) for August. March/April and August results were again subjected to the same statistical methods with the addition of the new development categories wetland reference and non wetland reference.

Results for seasonal variation in nutrient concentrations were tested with the null hypothesis that there is no significant variation in groundwater nutrient concentrations among different development categories over time using an alpha of 0.05. Seasonal groundwater nutrient concentration results were subjected to a repeated measures ANOVA with groundwater nutrient concentration as response and sample month as a random effect (n=3 for each development category).

The bioassay results were tested with the null hypothesis that there is no significant variation in chlorophyll production between groundwater treatment categories (0% nearshore, 25% nearshore, 50% nearshore, 75% nearshore and 100% nearshore). Daily chlorophyll change rates for each sample were calculated for each bioassay at peak chlorophyll production prior to senescence. Comparative daily chlorophyll change rates

for groundwater treatment categories were compared using an ANOVA and Tukey HSD test with groundwater treatment category as the factor and chlorophyll daily production rates as the response, alpha of 0.05 (n=5 per treatment category).

Results

In March there was no significant difference in concentrations of chloride, ammonium, phosphorus, or orthophosphate (p>0.05) in ground water samples from the three different development categories. Nitrite plus nitrate groundwater concentrations from nearshore development categories were significantly higher than groundwater from reference category locations. Nitrite plus nitrate groundwater concentrations from the terrace sites were intermediate between the reference and nearshore sites and not significantly different than either of those categories (Figure 9).

In August, nitrite plus nitrate groundwater concentrations from terrace development categories were higher than groundwater from nearshore and reference categories and surface water from all three categories (Figure 10). Orthophosphate concentrations in ground water from the near shore and reference categories were significantly higher than surface water from all three categories. Orthophosphate concentrations were also higher in ground water from the reference than ground water from terrace category (p-value < 0.05) (Figure 11).

When examining the data, high variance within the reference category was noted particularly elevated chloride, orthophosphate and total phosphorus levels in a proportion of the samples. Further examination of these reference site samples with elevated groundwater nutrient concentrations indicated that many were located within proximity of a wetland (Figure 11, 12, 15, 16). The data were then re-analyzed treating the wetland and non-wetland sites as two distinct categories.

When dividing the reference category between non wetland reference and wetland reference categories (n=4 and n=6 respectively) for March/April, groundwater nutrient concentrations for chloride and orthophosphate were elevated in the wetland reference category. Chloride was significantly higher in the wetland reference category compared to all other development categories including the non wetland reference (p-values < 0.05) (Figure 15). Orthophosphate and total phosphorus were significantly higher in ground water from the wetland reference development category compared to non wetland reference (p-values < 0.05), but were not significantly different between any other development categories (p-value \geq 0.05), (Figure 12, 16). Nitrite plus nitrate concentrations were significantly higher in groundwater from nearshore development category compared to wetland reference (p-values < 0.05), but was not significantly different between any other development categories (p-value > 0.05), (Figure 9). Again, when splitting the reference category in March/April there was no significant difference

in groundwater for ammonium between development categories (p-value > 0.05) (Figure 13).

When dividing the reference category between non wetland reference and wetland reference categories (n=5 and n=6 respectively) for August, again there was no significant difference in groundwater for ammonium between development categories (pvalue > 0.05) (Figure 14). Nitrite plus nitrate concentrations were higher in groundwater from terrace categories than groundwater from nearshore and wetland reference categories and surface water from nearshore, terrace and wetland reference categories (pvalue < 0.05) (Figure 10). Surface water nitrite plus nitrate concentrations in non wetland reference categories were higher than surface water from nearshore, terrace and wetland reference categories (p-value < 0.05) (Figure 10). Orthophosphate groundwater concentrations in wetland reference categories were significantly higher than groundwater from the three other ground water development categories and surface water orthophosphate concentrations from all four development categories (p-value < 0.05). While significantly lower than the wetland reference category groundwater orthophosphate concentrations from nearshore groundwater category were significantly higher than groundwater from the terrace and non wetland reference and surface water from all four development categories (p-value < 0.05), (Figure 12).

For seasonal variation in nutrient concentrations no significant difference between groundwater nutrient concentrations among different development categories over time

was found for ammonium, orthophosphate and nitrate plus nitrate from March to August 2015 ($n=3$ per development category) (p-value > 0.05) (Figure 17, 18, 19).

For both the June and July bioassay, addition of 50% or more nearshore ground water to reference groundwater resulted in significantly higher chlorophyll daily change rates than pure reference groundwater or 25% nearshore goundwater (p-value ≤ 0.05) (Figure 20). However, there was no significant difference with comparison of nearshore to terrace groundwater in August (p-value > 0.05).

Discussion

The primary goal of this study was to continue analysis of groundwater nutrient contents between residential development categories following preliminary investigations lead by Gendaszek et al, 2016. Those preliminary investigations sought to identify groundwater sampling locations with both absence and presence of septic wastewater influence for future groundwater sampling by comparing δ 15N values of Richardson's pondweed down gradient of different land use categories. Due to the natural bioaccumulation of 15N in human waste, septic system waste water contains greater ratios δ 15N that are distinguishable from atmospheric δ^{15} N ratios (Gendaszek et al, 2016; Kreitler and Browining, 1983; McClelland and Valiela, 1998.) In similar studies this source of anthropogenic enriched 15N from waste water has been traced in fresh water systems using macrophyte communities (Finlay and Kendall, 2007; Peterson and Fry, 1987; Cole et al. 2007). The results for $\delta^{15}N$ analysis indicated that Richardson's

pondweed down gradient of all development categories (nearshore, terrace, reference and a downstream undeveloped reference) were enriched in δ^{15} N (containing isotopically heavier ratios of nitrogen) relative to atmospheric δ^{15} N ratios (Gendaszek et al, 2016). Additionally, values for δ^{15} N of Richardson's pondweed from the undeveloped reference category was significantly lower than those from nearshore, terrace and downstream undeveloped reference categories. No significant difference between δ^{15} N values of Richardson's pondweed downgradient of nearshore or terrace categories was detected. However, all development categories were significantly lower than the downstream undeveloped reference category (Gendaszek et al, 2016). These results suggest that septic system influence may be present in groundwater from residentially developed (terrace and nearshore) category areas. However, neither groundwater nor surface water nutrient exchange or hydraulic gradients were measured during the growth period of the sampled Richardson's pondweed. Due to nitrogen's high mobility, the source of the integrated δ^{15} N is not clear and may reflect surface water concentrations, which may explain the discrepancy between the upstream and downstream undeveloped reference categories (Gendaszek et al, 2016; Gunter, 1998; Fetter, 2001).

Having identified potential influence of septic systems within residentially developed areas in comparison to an upstream undeveloped reference site, sampling began in March and April 2015 to determine if groundwater nutrient concentrations differed between these residential development categories: nearshore, terrace and

reference. Samples were collected from thirty piezometer locations (ten per development category) within the same range of the δ^{15} N analysis excluding the downstream reference category. There was no significant variation between nearshore and terrace residential development for chloride, ammonium, nitrite plus nitrate, orthophosphate and total phosphorus. However, nitrite plus nitrate concentrations were higher in groundwater from nearshore residential development in comparison to undeveloped reference category (Figure 9). This suggests that nearshore residential development is influencing groundwater nutrient concentrations with respect to nitrogen. There was no significant variation in groundwater total phosphorus or orthophosphate concentrations between residentially developed land (nearshore and terrace development categories) in comparison to the undeveloped reference category. These results indicate that phosphorus is being retained by the soil in nearshore and terrace residential development categories. The results also show that there is not evidence of septic system phosphorus breakthrough and runoff conditions in the sampled area during March and April of 2015 (Gendaszek et al, 2016; White, 1982).

Further examination of the March and April sampling was performed by separating the reference development category between sites located in a wetland and those upstream not located into a wetland $(n=6$ and $n=4$ respectively). When separating the reference categories, the groundwater from the wetland reference category was elevated with respect to chloride, orthophosphate and total phosphorus (Figure 15, 16,

11). Elevated levels of phosphorus in the wetland reference category groundwater may be the result of deposition and build up of phosphors bound to suspended soil particles as the water speed decreases in the wetland area (White, 1982). Water turbulence was not measured at the site locations; therefore, the source of phosphorus cannot yet be attributed to deposition. Overall, these results indicate that site soil composition and water residence time require further consideration and examination to identify nutrient sources, as deposition and retention of phosphorus in the soil is a possible factor (Gunter, 1998; Fetter, 200; White, 1982). Measurements for hydrologic gradient were taken during the study and indicated general direction of groundwater flow to surface water. However, because Lake Spokane is a managed reservoir and also experiences natural seasonal variations in annual lake stages, the direction of groundwater flow may vary and deposited soils may influence groundwater concentrations. Therefore, in addition to groundwater exchange rates, monitoring and assessment of groundwater flow direction is needed between development categories to estimate nutrient fluxes (Gendzszek et al., 2016; Dudley, 2007; Jordan et al., 2007).

Following the initial sampling in March and April, groundwater nutrient concentrations (ammonium, nitrite plus nitrate and orthophosphate) were sampled monthly from March to August 2015 at the three residential development categories (nearshore, terrace and reference). Testing revealed no significant variation between groundwater nutrient concentrations. Lack of significant variation may be due to the small sample size (n=3 per development category). No distinct trends appear over time aside from a spike in ammonium concentrations in July and August in nearshore groundwater. This spike may be related to a large fish die off, thought to be correlated with rapid changes in surface water temperature.

Despite lack of significance in seasonal variation in August 2015, the thirty sample sites from the initial sampling in March and April were resampled with the addition of one non wetland reference site located in the upstream section of the reference category. Both surface water and groundwater samples were collected from each site and analyzed for ammonium, nitrite plus nitrate and phosphorus. Prior to splitting the reference category, there was significantly more nitrite plus nitrate in groundwater from terrace development categories than nearshore and reference categories and surface water from all three development categories. Additionally, nitrite plus nitrate in surface water from the non wetland undeveloped reference was higher than surface water from all other development categories (Figure 10). The source of elevated nitrite plus nitrate in the terrace groundwater and non wetland reference surface water categories is unclear, due to nitrogen's high mobility in groundwater (Gendaszek et al, 2016; Gunter, 1998; Fetter, 2001). Larger sample size and possibly seasonally expanded examination of groundwater nutrient concentrations is needed to confirm these results and their source. In August elevated levels of orthophosphate in reference categories were again associated with sites located within the wetland reference category. Orthophosphate concentrations in the nearshore category groundwater were also significantly higher than groundwater from the

terrace and non wetland reference categories as well as surface water from all three development categories (Figure 11). In combination with nitrogen-isotope analysis of Richardson's pondweed these results indicate that septic system breakthrough conditions may exist in the nearshore development category in terms of phosphorus when compared to terrace and non wetland reference categories (Gendzszek et al., 2016; HDR, 2007/2011; McKinley and Siegrist, 2010). Therefore, seasonal monitoring with large sample size and assessment of groundwater nutrient exchange rates is needed between development categories to estimate nutrient fluxes and pinpoint possible causes of seasonal differences (Dudley, 2007; Gendzszek et al., 2016; Jordan et al., 2007).

In addition to comparative ground water nutrient concentration analysis, groundwater was tested to see if differences in nutrients were sufficient to influence growth of aquatic organisms using *Anabaena sp.* as a bio indicator to compare the relative nutrient potential of the groundwater between the development level categories. For both the June and July 2015 bioassay, the addition of 50% nearshore concentration to reference groundwater resulted in significantly higher chlorophyll daily change rates. This indicates that groundwater from nearshore residential development categories can accelerate algal growth, potentially negatively impacting water quality. There was no significant difference in algae growth potential between groundwater from nearshore and terrace development areas.

Summary and Conclusions

Preliminary investigations lead by USGS using nitrogen-isotope analysis of Richardson's pondweed indicate that the presence of septic system influence is less within the upstream undeveloped (reference) area in comparison to developed categories (nearshore/terrace) (Gendaszek et al., 2016). Sampling in March/April 2015 indicates nitrogen concentrations in groundwater from the reference category were less than the nearshore development category. These results suggest that nearshore development is influencing groundwater nutrient concentrations with respect to nitrogen in comparison to the undeveloped reference category. No significant difference was found in March/April for orthophosphate or total phosphorus between developed and undeveloped categories, indicating that septic system breakthrough conditions (in terms of orthophosphate and total phosphorus) do not exist.

By splitting the reference category between wetland and non wetland reference categories, results indicated that elevated levels of orthophosphate and total phosphorus in the reference category were associated with sites located within a wetland area. This indicates that site soil composition and water residence time require further examination, as deposition and retention of phosphorus in the soil is a possible factor. Hydrologic gradients taken during the study indicated a trend of groundwater flow to surface water. However, groundwater flow directions may vary seasonally, allowing deposited soils to

influence groundwater. Therefore, in addition to groundwater exchange rates, monitoring and assessment of groundwater flow direction is required.

Seasonal monitoring of groundwater indicates that there was not significant variation in groundwater nutrient concentrations between March and August 2015. However, re-sampling in August 2015 indicated that orthophosphate in groundwater from nearshore development categories is elevated in comparison to groundwater from terrace and non wetland reference categories as well as surface water from all development categories. In combination with nitrogen-isotope analysis of Richardson's pondweed, these results indicate that septic system breakthrough conditions may exist in terms of phosphorus.

Examination of groundwater algae growth potential indicates no significant difference between residentially developed categories. However, there is significantly higher daily chlorophyll change rates between groundwater from nearshore and non wetland reference development categories in June and July 2015. This indicates that groundwater from nearshore development areas may have elevated alga growth potential in comparison to non wetland reference categories.

Given the results of this study indicate elevated levels of phosphorus in groundwater from residentially developed nearshore categories in comparison to terrace and non wetland reference categories, seasonal monitoring and assessment of groundwater nutrient exchange rates is needed between development categories to estimate nutrient fluxes.

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Tables

Table 1 Environmental measurements and nutrient concentrations in ground and surface water samples collected from Lake Spokane, 2015

Figures

Figure 1 Site location Suncrest on the Spokane River in Eastern Washington

Figure 2A Modern septic system design

Figure 2B Phosphorus Cycling in Aquatic Ecosystems

Figure 3 Simplified figure comparing residential development category types in comparison to shoreline sampling location. Relative reduction of density and proximity to shoreline of residence and onsite septic systems from nearshore to reference site categories.

Figure 4 Relative development category locations on north Lake Spokane shoreline in the Suncrest development area: nearshore category in red solid, terrace category in yellow dashed squares, and reference category in blue dashed dots.

Figure 5 Sample site locations of shallow piezometers, 30 locations near north shoreline of Lake Spokane in the Suncrest area, located in northeastern Washington. Nearshore residential development red dots, terrace residential development yellow triangles, undeveloped reference blue diamonds.

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Figure 6 Manometer board design, and piezometer layout. Piezometers are shallow miniature wells pounded into the shore line. Manometer board uses difference in hydraulic head (opposing pressure gradients) between groundwater and surface water to estimate direction of groundwater flow see Figure 8 for visualization of groundwater flow.

Figure 7 Hydraulic gradient: Manometer board uses difference in hydraulic head (opposing pressure gradients) between groundwater and surface water to calculate direction of groundwater flow. Above hydraulic head in groundwater is higher than surface water thus flow of water from groundwater to surface water. Below surface water hydraulic head is higher than groundwater pressure, thus flow of water from surface water to groundwater.

Figure 8 Bioassay dilution continuum in 25% increments. Left to right 100% undeveloped reference (blue) to 100% nearshore residential development (red); dilution continuum was replicated five times for each bioassay.

Results Figures

Figure 10 Nitrite plus nitrate in ppm groundwater and surface water by development category (standard error) August results. n=10 for each development category, n=11 for reference. Reference site divided into wetland reference and non wetland reference subcategories (ground water and surface water) n=6, n=5. Nitrite plus nitrate groundwater concentrations from terrace development categories significantly higher than groundwater from nearshore and reference categories and surface water from nearshore, terrace and reference categories, (two way ANOVA; p-value < 0.05). When splitting reference, Nitrite plus nitrate groundwater concentrations from terrace development categories were higher than groundwater from nearshore and wetland reference categories and surface water from nearshore, terrace and wetland reference categories (two way ANOVA; p-value ≤ 0.05)

Figure 11 Orthophosphate in ppm groundwater by development category (standard error) March results. n=10 for each development category. Reference site divided into wetland reference and non wetland reference subcategories (ground water and surface water) n=6, n=4 respectively. When splitting reference, groundwater from wetland reference is significantly higher than groundwater from non wetland reference category, (ANOVA; p-value ≤ 0.05).

Figure 12 Orthophosphate in ppm groundwater and surface water by development category (standard error) August results. n=10 for each development category, n=11 for reference. Reference site divided into wetland reference and non wetland reference subcategories (ground water and surface water) n=6, n=5. Nearshore category groundwater significantly higher than surface water from all development categories. Reference categories groundwater significantly higher than terrace groundwater and, surface water from all development categories; two way ANOVA; p-value < 0.05. When spiting the reference, groundwater in wetland reference category significantly higher than groundwater from the three other ground water development categories and surface water Orthophosphate concentrations from all four development categories (two way ANOVA; p-value < 0.05).

Figure 13 Ammonium in ppm by development category, (standard error) March results. n=10 for each development category. Reference site divided into wetland reference and non wetland reference subcategories (ground water and surface water) n=6, n=4 respectively. No significant difference between development categories, (ANOVA; p-value \geq 0.05).

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Figure 14 Ammonium in ppm by development category, (standard error) August results. n=10 for each development category, n=11 for reference. Reference site divided into wetland reference and non wetland reference subcategories (ground water and surface water) n=6, n=5. No significant difference between development categories or surface water, (ANOVA; p -value > 0.05).

Figure 15 Chloride in ppm groundwater by development category (standard error) March results. n=10 for each development category. Reference site divided into wetland reference and non wetland reference subcategories (ground water and surface water) n=6, n=4. When splitting reference, groundwater from wetland reference category higher than groundwater from nearshore, terrace and non wetland categories, (ANOVA; p-value \leq 0.05).

Figure 16 Phosphorous in ppm groundwater and surface water by development category (standard error) March results. n=10 for each development category. Reference site divided into wetland reference and non wetland reference subcategories (ground water and surface water) n=6, n=4. Wetland reference significantly higher than non wetland reference development category, (ANOVA; p-value \leq 0.05).

Figure 17 Average in groundwater Ammonia concentrations by site development category, March to August 2015, n=3 per development category. No significant difference between development categories over time (repeated measures ANOVA; p-value \geq 0.05).

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Figure 18 Average in groundwater orthophosphate concentrations by site development category, March to August 2015, n=3 per development category. No significant difference between development categories over time (repeated measures ANOVA; p-value \geq 0.05).

Figure 19 Average in groundwater nitrite plus nitrate concentrations by site development category, March to August 2015, n=3 per development category. No significant difference between development categories over time (repeated measures ANOVA; p-value \geq 0.05).

Figure 20 Relative chlorophyll production of groundwater, continuum of 25% concentrations from near shore to reference development categories (standard error); July results. Significantly higher in daily chlorophyll change in groundwater starting with 50% addition of nearshore groundwater to 100% reference ground water, (ANOVA; p-value ≤ 0.05).