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Monitoring Plant Tissue Nitrogen Isotopes to Assess Nearshore Inputs of Nitrogen to Lake Crescent, Olympic National Park, Washington

Scientific Investigations Report 2016–5054

U.S. Department of the Interior
U.S. Geological Survey

Cover:

Front: Algal mats and spawning redd markers with red alder in background, near the Lyre River outlet, Lake Crescent, Washington. Photograph by Steven Fradkin, National Park Service, April 3, 2003.

Back: Macrophyte reeds above and below lake water surface, east basin of Lake Crescent, Washington. Photograph by James Foreman, U.S. Geological Survey, June 2010.\

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Conversion Factors

International System of Units to Inch/Pound

Multiply	By	To obtain
	Length	
millimeter (m)	0.03937	inch (in.)
centimeter (cm)	0.3937	inch (in.)
meter (m)	3.281	foot (ft)
kilometer (km)	0.6214	mile (mi)
	Area	
hectare (ha)	2.471	acre
square meter (m^2)	10.76	square food (ft^2)
	Volume	
liter (L)	0.2642	gallon (gal)
	Flow rate	
cubic meter per second (m^3/s)	810.7	acre-foot per day (acre-ft/d)
	Mass	
kilogram (kg)	2.205	pound avoirdupois (lb)
kilogram per year (kg/yr)	2.205	pound per year (lb/yr)
	Application rate	
kilograms per hectare per year [(kg/ha)/yr]	0.8921	pounds per acre per year [(lb/acre)/yr]

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as

$$°F = (1.8 \times °C) + 32.$$

Datums

Vertical coordinate information is referenced to the North American Vertical Datum of 1988 (NAVD 88).

Horizontal coordinate information is referenced to the North American Datum of 1983 (NAD 83).

Elevation, as used in this report, refers to distance above the vertical datum.

Supplemental Information

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25 °C).

Concentrations of chemical constituents in water are given in either milligrams per liter (mg/L) or micrograms per liter (µg/L).

Monitoring Plant Tissue Nitrogen Isotopes to Assess Nearshore Inputs of Nitrogen to Lake Crescent, Olympic National Park, Washington

By Stephen E. Cox,[1] Patrick W. Moran,[1] Raegan L. Huffman,[1] and Steven C. Fradkin[2]

Abstract

Mats of filamentous-periphytic algae present in some nearshore areas of Lake Crescent, Olympic National Park, Washington, may indicate early stages of eutrophication from nutrient enrichment of an otherwise highly oligotrophic lake. Natural abundance ratios of stable isotopes of nitrogen ($\delta^{15}N$) measured in plant tissue growing in nearshore areas of the lake indicate that the major source of nitrogen used by these primary producing plants is derived mainly from atmospherically fixed nitrogen in an undeveloped forested ecosystem. Exceptions to this pattern occurred in the Barnes Point area where elevated $\delta^{15}N$ ratios indicate that effluent from septic systems also contribute nitrogen to filamentous-periphytic algae growing in the littoral zone of that area. Near the Lyre River outlet of Lake Crescent, the $\delta^{15}N$ of filamentous-periphytic algae growing in close proximity to the spawning areas of a unique species of trout show little evidence of elevated $\delta^{15}N$ indicating that nitrogen from on-site septic systems is not a substantial source of nitrogen for these plants. The $\delta^{15}N$ data corroborate estimates that nitrogen input to Lake Crescent from septic sources is comparatively small relative to input from motor vehicle exhaust and vegetative sources in undeveloped forests, including litterfall, pollen, and symbiotic nitrogen fixation. The seasonal timing of blooms of filamentous-periphytic algal near the lake shoreline is also consistent with nitrogen exported from stands of red alder trees (*Alnus rubra*). Isotope biomonitoring of filamentous-periphytic algae may be an effective approach to monitoring the littoral zone for nutrient input to Lake Crescent from septic sources.

[1]U.S. Geological Survey.

[2]National Park Service, Olympic National Park.

Introduction

Lake Crescent is a deep oligotrophic lake in northwestern Washington, is renowned for its pristine water quality, and is a primary attraction in Olympic National Park (fig. 1). The surrounding watershed is composed of undeveloped mountainous forestlands except around the perimeter of the lake where development occurred prior to this area being designated as a national park in 1938. About 47 percent of the 40 km of lake shoreline has some form of development, including a regional highway, residential cabins, park resort facilities, campgrounds, and picnic areas, all of which use on-site septic systems to treat domestic sewage. Between 1990 and 2010, Olympic National Park employees observed an apparent increase in the size and number of algal mats in littoral areas of the lake. Filamentous-periphytic algae including *Mougeotia* sp., *Spirogyra* sp., and cyanobacterium *Oscillatoria* sp. have been observed in near-shore areas at several locations around the lake during the winter and spring, (Meyer and Fradkin, 2002). The development of filamentous-periphytic algal mats on cobble and gravel substrate near the Lyre River outlet is of particular concern because this location is the only spawning area of a unique form of rainbow trout (*Oncorhynchus mykiss*), referred to locally as Beardslee trout (Meyer and Fradkin, 2002). Nearshore algal mats also have been reported in other areas of the lake, particularly near Barnes Point, Fairholm Campground, and at some unspecified locations along the north shore (fig. 2). The increased presence and abundance of filamentous algae in freshwater environments is indicative of nutrient enrichment (Jacoby and others, 1991; Tank and Dodds, 2003; Hadwen and Bunn, 2005). An improved understanding of the link between nitrogen inputs around the shoreline of Lake Crescent and the seasonal occurrence of algal mats in some areas is needed by managers of Olympic National Park to protect park resources effectively.

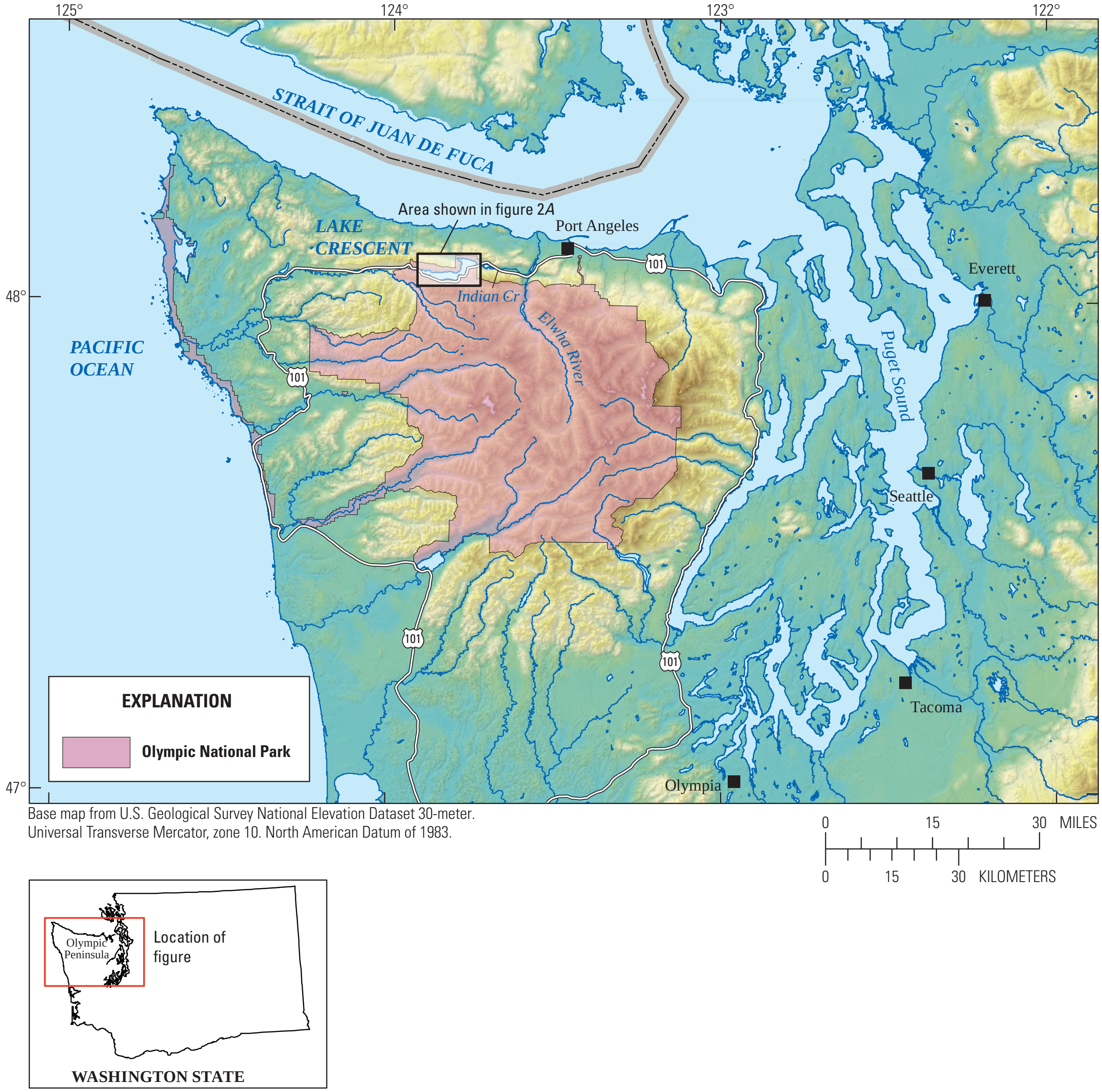

Figure 1. Location of Lake Crescent, Olympic National Park, Washington.

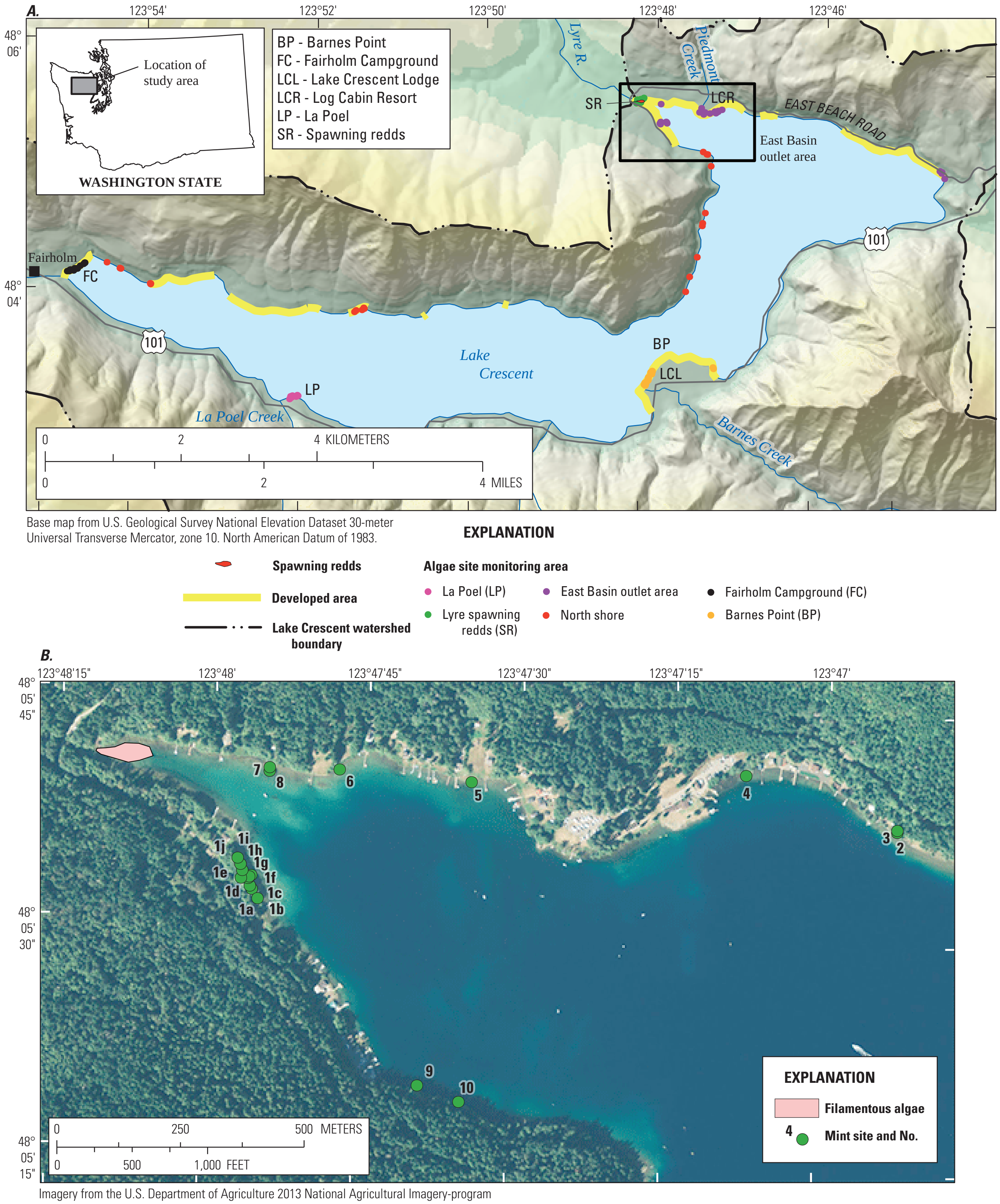

Figure 2. Shoreline locations of (*A*) monitoring sites and (*B*) field mint areas, Lake Crescent, Olympia National Park, Washington. Sampling areas for field mint are in the East Basin/Lyre River outlet area.

Aging and potentially failing septic systems associated with shoreline development have been suggested as a potential source of nutrients entering Lake Crescent through groundwater transport and subsequent discharge to the lake (Meyer and Fradkin, 2002). Residential development of lake shorelines can generate substantial change to the nearshore littoral zone and to the entire lake ecosystems, often through increased inputs of nitrogen and other nutrients. Examples of nutrient-driven eutrophication have been widely reported for many lakes and reservoirs (Goldman, 1988; Edmondson, 1991; Elser and others, 2007). Eutrophication of Lake Tahoe, California and Nevada, a deep-water oligotrophic lake similar to Lake Crescent, was partly due to septic-system effluent entering Lake Tahoe as discharging groundwater flow (Loeb and Goldman, 1979). Even small percentages of shoreline development that use septic systems for sewage treatment have been associated with measurable increases in concentrations of chlorophyll-*a* and other metrics of lake eutrophication (Moore and others, 2003). Other effects of shoreline development that may affect lake water quality include:

- Alteration of vegetation growing near the shore, including removal of conifer trees and installation of grass lawns (Christensen and others, 1996; Francis and Schindler, 2006; Marburg and others, 2006);
- Reduction of large woody debris (Christensen and others, 1996; Francis and Schindler, 2006);
- Decreased macrophyte density in the littoral zone (Radomski and Goeman, 2001); and
- Increased armoring of littoral-zone sediments (Wehrly and others, 2012).

The effect of shoreline development around Lake Crescent has been assessed by comparison of littoral-zone periphyton communities and shoreline vegetation at four developed and undeveloped sites by Rosenberger and others (2008). They measured significantly larger biomass of algae and accumulation of detritus in littoral areas of developed sites, as well as a greater percentage of periphytic algae and detritivores in nearshore communities. The density of trees along the shoreline was not significantly different between developed and undeveloped sites; however, the size of trees was larger at developed sites than at undeveloped sites, and the presence of red alder (*Alnus rubra*) increased compared to conifers (Rosenberger and others, 2008). The increased size of trees and lawns along developed lakeshore areas has been noted by Francis and Schindler (2006) and referred to as “trophy trees.” Increased urbanization was correlated with increased litterfall and the density of deciduous tree species in lowland streams of the Puget Sound (Brett and others, 2005; Roberts and Bilby, 2009). Although the increases in biomass and detritus measured by Rosenberger and others (2008) were consistent with nutrient enrichment, their accompanying measurements of stable isotopes of nitrogen in periphyton samples did not show a significant difference between developed and undeveloped sites as might be expected if nitrogen from septic sources was a significant source of the nutrients at developed sites.

A study of water and nutrient budgets of Lake Crescent identified multiple sources of nutrients entering the lake (Moran and others, 2013). More than 60 percent of nitrogen input to Lake Crescent was from streamflow and atmospheric deposition. The remaining 40 percent of nutrient input was distributed among non-point sources, including vehicular traffic, litterfall, pollen dispersal, nitrogen-fixing plants, and on-site septic systems (Moran and others, 2013). Nitrate concentrations in periodic samples of groundwater measured by Moran and others (2013) were small (typically about 0.5 mg/L of nitrogen) and showed little variability between developed and undeveloped areas, although sporadic and occasional elevated concentrations of as much as 2.9 mg/L of nitrogen were measured. The estimated total nitrogen input from groundwater to Lake Crescent, which included contributions from on-site septic systems, was 1,470 kg/yr or 3.4 percent of the total nitrogen input. The estimates of nitrogen input from septic systems used in the nutrient budget were based on the mass of nitrogen generated in wastewater by the number of person-days that park visitors, staff, and residents were present in the park. These inputs resulted in estimated nitrogen from septic systems of 623 kilograms of nitrogen per year (kg-N/yr) or 1.5 percent of the total nitrogen input to Lake Crescent (Moran and others, 2013). Comparatively, the estimated nitrogen input to the lake derived from nitrogen exported by alder trees within 300 m of the lake shore was 6,900 kg-N/yr, more than 10 times larger than inputs from septic systems. Additional nitrogen inputs to the lake from vegetation include litterfall and pollen deposition.

Differences in the natural abundance ratio of stable isotopes of nitrogen ($\delta^{15}N$) in plant tissue growing in areas of groundwater discharge can, under favorable conditions, provide ecologically relevant information pertaining to the source of plant nitrogen (Kendall and others, 2007), particularly if isotopic differences are large and spatially distinct (Ostrom and others, 2002). Littoral vegetation at the sediment-water interface also can function as a filter sequestering nutrients from discharging groundwater that otherwise would enter the water column (Hagerthey and Kerfoot, 1998; Hagerthey and Kerfoot, 2005; Frandsen and others, 2012). Thus, the measurement of $\delta^{15}N$ of plant material growing at the sediment-water interface in the littoral zone may provide a time-integrated signal of the source of nitrogen entering the lake with groundwater. The $\delta^{15}N$ in plant material from undisturbed ecosystems are typically less than +5 per mil (‰) (Peterson and Fry 1987; Fry, 2008; Craine and others, 2009), whereas the range of $\delta^{15}N$ in plant material that derive nitrogen from animal and human sewage is elevated, often to levels between 10 and 30 ‰ (Finlay and Kendall, 2007). In marine environments, macroalgae with $\delta^{15}N$ signatures greater than the range of undisturbed ecosystems have been linked to animal-derived sources of nitrogen, including livestock and septic systems (Derse and others 2007; Dailer and others, 2010). In freshwater aquatic systems, $\delta^{15}N$ of stream biota were enriched by about 3–4 ‰ in areas served by septic systems compared to sewered areas (Steffy and Kilham, 2004). During seasonal periods of increased tourist activity in a small headwater watershed in Colorado, Kaushal and others (2006) measured increased enriched levels $\delta^{15}N$ in stream algae of about 4 ‰. Stable nitrogen isotope ratios measured in freshwater riparian macrophytes were highly correlated with anthropogenic inputs of nitrogen to river ecosystems (Kohzu and others, 2008) and in estuaries ecosystem (Cole and others, 2004). The $\delta^{15}N$ of wild celery (*Vallisneria americana*) in the littoral areas of two lakes were correlated to increased human population density, suggesting input of nitrogen from septic systems (Benson and others, 2008). Measurements of $\delta^{15}N$ in plant material from multiple locations along the shoreline and littoral zone around Lake Crescent may provide information regarding the level of influence from septic system effluent. If $\delta^{15}N$ of littoral zone plant tissue exceeds the typical range of $\delta^{15}N$ in natural plant material or are significantly elevated above baseline values from undeveloped sites around Lake Crescent, nitrogen from septic systems should be considered a significant potential source of nitrogen.

The U.S. Geological Survey began studies of nutrient inputs to Lake Crescent in Olympic National Park in October 2006 as part of the U.S. Geological Survey-National Park Service Cooperative Water Quality Program. The initial study evaluated the nutrient and water budget for Lake Crescent (Moran and others, 2013). This follow-up study looked more closely at the input of nitrogen from groundwater into Lake Crescent using additional nitrate analysis of groundwater and measurements of nitrogen isotope in plant tissue growing near the lake shoreline. The results of the water-quality analysis of groundwater samples collected as part of the follow-up investigation were reported in the Lake Crescent nutrients budget (Moran and others, 2013).

Purpose and Scope

This report presents the results of an isotopic evaluation of sources of nitrogen used by filamentous-periphytic green algae that form algal mats in some nearshore areas of Lake Crescent. Two questions were addressed:

1. Is the natural abundance ratio of nitrogen in plant tissue growing near the shoreline in developed areas greater than a background range? If so, is it suggestive of a source of nitrogen derived from septic system effluent?

2. Are the nitrogen isotope ratios present in mats of filamentous-periphytic algae growing near the spawning redds of Breadslee trout characteristic of septic sources of nitrogen and are they significantly different from nitrogen isotope ratios of algae present in minimally developed areas of the lake?

For this study, the $\delta^{15}N$ of filamentous periphytic algae growing in the littoral zone and field mint (*Mentha arvensis*) growing near the shoreline of developed and undeveloped areas were measured to establish baseline values and were compared to literature values of $\delta^{15}N$ from various potential sources of nitrogen.

Description of Study Area

Lake Crescent is an elongated lake formed by a landslide that impounded drainage of a deep, narrow valley. The lake occupies an area of 2,030 ha, with a shoreline length of about 40 km. Maximum lake depth is 190 m, with about 50 percent of the lake area overlaying water deeper than 95 m. The surrounding watershed of 11,000 ha is almost entirely within Olympic National Park. Bedrock is composed of faulted and thrusted blocks of marine volcanic and sedimentary rocks that rise steeply from the lake to form jagged, rocky ridges that bound Lake Crescent to the south and north. The land surface below about 500 m elevation has been subjected to Pleistocene glaciation, leaving slopes covered with talus on which dense coniferous forests have developed. The shoreline generally is steep, and often sheer. The lake drains into the Strait of Juan de Fuca through the Lyre River, with a mean annual discharge of 6.06 m^3/s (Moran and others, 2013). About 60 percent of the inflow to the lake is from Barnes, La Poel, and Piedmont Creeks, with numerous smaller streams draining upland areas (fig. 2).

The availability of plant nutrients in Lake Crescent is limited and the lake is classified as oligotrophic. Lake water is dilute, having low concentrations of dissolved constituents. Measurements of specific conductance typically range from 90 to 130 µS/cm at 25 °C. Nutrient concentrations in lake water are low; median concentrations measured by Moran and others (2013) were 0.04 mg/L for nitrogen and 0.008 mg/L for phosphorus. The nitrogen content of groundwater, as measured by Moran and others (2013), also was low, typically less than 0.5 mg/L. In areas of limited shoreline development, groundwater nitrogen concentrations typically were about 0.2 mg/L, whereas in areas with more shoreline development, nitrate concentrations typically were about 0.5 mg/L of nitrogen with sporadically higher concentrations as much as 2.9 mg/L.

The Lake Crescent watershed remains largely undeveloped mountainous forestland. Most development is concentrated in the strip of land surrounding the shoreline of Lake Crescent. Development around the shoreline of Lake Crescent, including numerous resorts and private residences began in the early decades of the 20th century. Development was curtailed in 1938 with the creation of Olympic National Park. As of 2002, about 47 percent of the shoreline has some form of development including a regional highway, and residential cabins, park resort facilities, campgrounds, and picnic areas, all of which use on-site septic systems to treat domestic sewage. Roadway or trail corridors include

U.S. Route 101, a regional highway that borders the southern shore of the lake, and an abandoned railroad grade converted to a pedestrian and bicycle trail that follows the northern shoreline. Residential homes, resorts, or park facilities occupy 23 percent of shoreline area. About 121 private residences, referred to as “inholders-parcels,” remain around the shoreline of Lake Crescent of which about 15 percent are occupied year-round. Maintenance and upkeep of inholder-parcels allows for some level of continued clearing of vegetation and woody debris from shoreline areas. Park facilities include three resort facilities, two campgrounds, several picnic areas, and two boat launch facilities. All these developed areas use on-site septic systems to handle sewage waste that together are estimated to contribute 623 kg-N/yr to Lake Crescent (Moran and others, 2013). In some developed areas, buildings have been removed, allowing the return of native vegetation that is mostly deciduous shrubs and trees including red alder (*Alnus rubra*). The diameter of alder trees in many of these historically developed areas often exceeds 15 cm, suggesting more than 20 years of undisturbed growth (Worthington and others, 1960).

Vegetative land cover around Lake Crescent is dominated by coniferous species, including western hemlock (*Tsuga heterophylla*), Douglas fir (*Pseudotsuga menziesii*), and western red cedar (*Thuja plicata*). Although coniferous forest extends throughout most of the Lake Crescent watershed, near the shoreline, deciduous species including red alder and bigleaf maple (*Acer macrophyllum*) predominate in developed areas. Red alder is a pioneer species that colonizes open areas such as disturbed forestlands and developed areas that are allowed to return to native vegetation (Harrington, 2006). The predominance of red alder in developed areas is likely, in part, an artifact of historical shoreline development. Red alder is an actinorhizal plant species that can fix atmospheric nitrogen, through a symbiotic relation with the bacteria genus *Frankia,* at rates of as much as 100–200 kilograms of nitrogen per hectare per year ([kg-N/ha]/yr) (Binkley and others, 1994). Recent studies have shown red alder to be a substantial source of aqueous nitrogen exported to riparian and watershed ecosystems (Compton and others, 2003; Perakis and others, 2011; Steinberg and others, 2011). The forest understory includes numerous shrub species; field mint is of particular interest, and is abundant and often present at the water’s edge along the Lake Crescent shoreline. Field mint is a perennial shrub, growing as tall as 80 cm with lance-shaped leaves as long as 8 cm. Benthic algae that have been identified in the nearshore littoral zone include green algae of the genera *Mougeotia*, *Spirogyra*, and *Zygnema*, and the filamentous cyanobacterium *Oscillatoria*, (Meyer and Fradkin, 2002; Rosenberger and others, 2008).

The nearshore transition from upland areas to littoral zone along the shoreline generally is steep, with few gently sloping beach and littoral areas. Residential development is concentrated in areas of low relief, particularly near the east and west ends of the lake and on the alluvial fan at Barnes Point. In developed areas the shoreline typically has been cleared of shrub vegetation, often include bulkheads near the water line, and beach areas have been cleared of large woody debris. Residential parcels typically have less than 30 m of shoreline. Forest vegetation in developed areas typically has been removed or modified. In non-developed areas, earthen materials in the shoreline transitional zone are composed primarily of bedrock or thin deposits of angular colluvial or glacial sediment containing little organic matter other than fallen trees. Groundwater supply is limited (Walters, 1970), except in the alluvial fans near Barnes Point and Fairholm Campground (fig. 2); some residential parcels use lake water for domestic use. At residential parcels, space for construction of on-site septic systems often is limited to areas near the shoreline; consequently, groundwater-flow paths are short between septic effluent drainfields and the lake. Groundwater flow is controlled largely by precipitation, most of which occurs during the late autumn and winter months.

The nearshore and littoral zone of Lake Crescent is well suited to for the application of $\delta^{15}N$ to distinguish between nitrogen from atmospherically fixed nitrogen sources and nitrogen from septic system wastewater. Use of $\delta^{15}N$ data to track nitrogen sources through ecosystems can be affected by multiple biological and physical processes, which can significantly alter the $\delta^{15}N$ of groundwater confounding interpretation of data (Nadelhoffer and Fry, 1988; Aravena and other 1998; Hogberg, 1997; Bedard-Haughn and others, 2003). Denitrification in particular can lead to highly elevated $\delta^{15}N$; however, this process is microbially mediated occurring under highly anaerobic conditions, which are incompatible with the presence of dissolved oxygen. Groundwater-flow paths discharging to the nearshore littoral zone are typically short and well drained with dissolved oxygen concentrations often greater than 1 mg/L, particularly near the Barnes Point area (Moran and others 2013). The nearshore littoral zone consists primarily of coarse grain sediment, lacking fine grain and organic rich sediments that can lead to redox conditions favorable to denitrification occurring at the sediment-water interface (Hedin and others, 1998). Shoreline sediment near the Barnes Point sampling area typically was composed of fine gravel and larger material. Nitrate concentrations in lake water are very low, typically less than 0.5 mg/L and often undetectable and in groundwater nitrate concentrations were typically less than 0.5 mg/L-N, (Moran and other 2013). Fractionation of nitrogen isotopes during assimilation by plants is a concentration dependent process that is likely to have negligible effect on $\delta^{15}N$ of plant tissue growing with nitrate source concentrations in the range measured in Lake Crescent and near shore groundwater, (Evans and others, 1996; Evans, 2001).

Sample Collection and Analysis

Prior to the collection of plant tissue samples, measurements of the hydraulic gradient across the lakebed-water interface were made to confirm the upward direction of groundwater flow. Hydraulic gradient measurements were made using a seepage meter or manometer board (see Rosenberry and LaBaugh, 2008, for methodology) in littoral areas near the Lyre River outlet, Barnes Point, and Fairholm Campground. Samples of field mint and filamentous periphytic green algae (hereafter referred to as “filamentous periphytic algae”) composed primarily of green algae *Spyrogia* sp. and *Mougeotia* sp., with minor occurrences of *Zygnema* sp. and *Oscillatoria* sp., were collected for analysis of $\delta^{15}N$ during two sampling periods. Field mint, a perennial shrub that commonly is present along the shoreline at many locations around Lake Crescent, also was sampled as a possible alternative to monitoring filamentous-periphytic algae.

Baseline levels, range, and variability of $\delta^{15}N$ values were measured in a preliminary survey in autumn and winter of 2008–09, followed by a 7-month monitoring period between November 2009 and June 2010 that coincided with the seasonal period when nuisance algae mats had been reported to be more prevalent at Lake Crescent. Preliminary survey samples were collected mostly from the more heavily developed areas along East Beach Road and near Lyre River outlet, (referred to as East Basin outlet area) although several samples were obtained near Barnes Point and La Poel (figs. 2*A* and 2*B*). The Barnes Point location was sampled because wastewater from the Lake Crescent Lodge complex that includes an environmental science program for children and teens (NatureBridge) and numerous residential parcels is processed in a 94,000 L capacity sewage-treatment facility, the effluent of which is released to a common drainfield upgradient of Lake Crescent Lodge. The La Poel day-use area was selected as a site to characterize undeveloped coniferous forest areas. This area previously was a campground served by a septic system; the campground was retired in the early 1950s, but still could be leaching septic nitrogen to groundwater (Boyle and Beeson, 1991). As of 2010, this area was restricted to limited day use although repeated observations of toilet tissue near the shoreline suggest some level of ongoing anthropogenic nutrient input. Although the La Poel area cannot be considered pristine, it is likely representative of coniferous bounded lakeshore with only limited developmental effects.

Monitoring samples of filamentous-periphytic algae were collected on seven occasions between November 2009 and June 2010 from six designated monitoring areas around Lake Crescent (fig. 2):

- The Lyre River outlet area including the spawning redds (Lyre redds),
- The shoreline near Log Cabin Resort and along East Beach Road (East Basin Outlet area),
- Barnes Point area including Lake Crescent Lodge and boat ramp area (Barnes Point),
- The Fairholm Campground and day-use area (Fairholm)
- The north shore residential areas (North shore), and
- The La Poel primitive area near the mouth of La Poel Creek (La Poel).

The occurrence of filamentous periphyton mats often was limited to small areas, except in the Lyre River outlet area. Samples were collected from within 10 m of the shoreline and typically at elevations within a few meters depth.

Samples of field mint were collected from plants growing as close to the shoreline as possible, often occurring below the high water mark. The length of shoreline at a mint sampling site did not exceed 20 m. Field mint plants collected from currently occupied residential parcels were typically found near the margins of the parcels and may not have been located directly downgradient from septic drainfield. Samples were collected as intact, unblemished leaves from the apical stem area of individual plants. To assess variation within a single plant, multiple leaf samples were collected for individual analysis from three to four branches from around the main stem near the apex of the plant. Samples were placed in individual labeled plastic bags, placed on ice in a cooler, and returned to the laboratory for preparation for analysis. In the laboratory, leaf material was dried at 60 °C, ground with mortar and pestle, and placed in 4×6-mm tin capsules for stable isotope analysis.

During monitoring events, the shoreline and littoral areas in the designated monitoring areas (fig. 2) were observed from a boat, and 3–5 samples of filamentous-periphytic algae were collected, if available. Algae samples were collected from the sediment on the lakebed surface using either a stainless steel fork or tongs. The latitude and longitude of the sampling locations were recorded using a hand-held Global Positioning System. The depth of overlying water usually was from 0.7 to 2.5 m. In some instances, rocks as large as 15 cm in diameter were collected from the lakebed surface and samples of filamentous green algae were removed using forceps, scissors, or razor blade. In the laboratory, algal samples were washed under a stream of distilled water to remove visible sediment, detritus, or diatoms; dewatered on a 47-mm glass-fiber filter; and then frozen (at -20 °C) until processed for isotope analysis using the same method as for the mint samples.

Stable isotope ratios of the plant and algal tissues were measured using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer at the University of California-Davis Stable Isotope Facility.

The analyses were done using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Limited, Cheshire, United Kingdom). Stable isotope ratios are expressed in delta (δ) notations as the difference in parts per thousand (‰ or “per mil”) from the standard:

$$(\delta^{15}N_{sample}‰) = ([R_{sample}/R_{standard}]-1) \times 1{,}000, \qquad (1)$$

where

R is the ratio of the $^{15}N/^{14}N$ isotopes and atmospheric nitrogen is used as the standard assigned a value of $\delta^{15}N=0$ ‰.

Relative abundance values of $\delta^{15}N$ that are more positive compared to the standard are enriched having a greater proportion of the heavier isotope; conversely, negative or smaller $\delta^{15}N$ values indicate that the samples are depleted of the heavier isotope. Twenty percent of the samples analyzed for this study were submitted in duplicate along with blind reference samples of National Institute of Standards and Technology (NIST) reference material (8573 L-glutamic Acid USGS40 and NIST reference material 1547 peach leaves). The long-term reported laboratory standard deviation for $\delta^{15}N$ measurements is 0.3 ‰.

Variability of $\delta^{15}N$ data was assessed at several levels as part of the preliminary sampling. Instrumental measurement variability was assessed from replicate measurements of the NIST Standard Reference Material 1547, which consists of finely ground peach leaves. The peach leave reference material, although not a standard for $\delta^{15}N$ measurements was assumed to provide homogeneous material of similar organic matter compared to the samples of mint leaves and filamentous-periphytic algae and as such should provide consistent reference material for analysis throughout the study. Variation of $\delta^{15}N$ within the leaves of individual field mint plants (interfoliar variations) as well as between individual field mint plants of the same species from the same location (intersite variation) also was assessed. Interfoliar variation was assessed by separate analysis of four leaves from stems radiating in different directions from the upper half of nine individual mint plants.

The isotope data were plotted on normal probability plots to distinguish between baseline and elevated values of $\delta^{15}N$, as noted by prominent discontinuities in the distribution of plotted data. Previous studies have used plots of empirical data distributions to analyze data variation so that threshold values and differences between baseline and elevated values of trace-elements concentrations in contaminated sediments can be distinguished (Sinclair, 1974; Rickert and others, 1977; Rose and others, 1979; Sinclair, 1991) as well as to identify geochemical anomalies (Tennant and White, 1959; Reimann and others, 2005). Cumulative probability plots have been used to distinguish between background and elevated concentrations of nitrate and other water-quality constituents (Panno and others, 2006). Normal probability plots were constructed using the inverse standard normal distribution and Blom’s plotting position (Helsel and Hirsch, 2002). A nonparametric analysis of variance (ANOVA) Kruskal-Wallis test (SYSTATT 13® software) was used to assess if median $\delta^{15}N$ values were different among sampling areas using a post-hoc Conover-Inman pairwise comparison test to identify differences among sampling areas. Likewise, a two-sample t-test was used to compare mean $\delta^{15}N$ values of filamentous-periphytic algae from the background site to filamentous-periphytic algae from the areas of concern near the mouth of the Lyre River.

Nitrogen Isotopes in Plant Tissue

The preliminary survey consisted of measurements of $\delta^{15}N$ in 48 samples of field mint plants and 30 samples of filamentous-periphytic algae (table 1). Sampling sites were concentrated in the Lyre River outlet area and the more heavily developed areas of the East Basin outlet area where septic influence on nearshore aquatic vegetation was expected to be more prevalent (fig. 2*B*). More intensive sampling was done at mint site 1 (fig. 2*B*) to provide data on variation of $\delta^{15}N$ within leaves from different branches of the same plant, (interfoliar variation). Shoreline vegetation at site 1 appeared generally uniform along the 100 m stretch of contiguous shoreline; alder trees and grasses were the predominant vegetation species and field mint plants were present throughout the reach. This area historically had been developed with residential cabins, which subsequently were removed. The area was allowed to return to native vegetation. To provide data on intrasite variability, four composites mint samples were collected from nine shoreline areas of about 30 m in length that were near residential cabins. The distribution of mint plants was less uniform occurring more commonly near the margin of residential parcels. Samples for filamentous-periphytic algae were collected from seven matts in littoral area within 150 m of littoral areas near the Lyre River outlet. The mat areas typically were oblong in shape with dimensions of about 1–2 m. A second set of algae samples (N=12) was collected 3 months after the first set from a single algal mat that measured about 1.5 by 1 m. Additional samples of filamentous-periphytic algae were collected from the Barnes Point area and the La Poel day use area.

Results from the preliminary survey of $\delta^{15}N$ in field mint ranged from -3.74 to +3.31 ‰, with only one value more positive than 3 ‰. This value used as the upper end of the range of plant material from undisturbed natural ecosystem (Peterson and Fry, 1987; Fry, 2008). The standard deviation of $\delta^{15}N$ in leaves sampled from different branches of the same plant showed interfoliar variation in the mint plants samples from at site1 averaged about 0.58 ‰, roughly 10 times larger than the standard deviation variation measured in peach leaf reference material presumed to be homogeneous (table 2).

Table 1. Statistics for stable isotopes of nitrogen ($\delta^{15}N$) in preliminary plant tissue samples of filamentous-periphytic algae and field mint collected at Lake Crescent, Olympic National Park, Washington, October–December 2008.

[All values are in per mil (‰)]

	Number of samples	Minimum	Maximum	Mean	Standard deviation	Median
Algae	30	-1.55	9.44	1.17	2.67	-0.40
Field mint	48	-3.73	3.31	-0.56	1.71	-0.54

Table 2. Variability of abundance ratio of stable isotopes of nitrogen ($\delta^{15}N$) in plant tissue samples of field mint, filamentous-periphytic algae, and peach leaves collected at Lake Crescent, Olympic National Park, Washington, October–December 2008.

[All values are in per mil (‰). NIST, National Institute of Standards; –, no data]

Sampling site	Number of samples	Minimum	Maximum	Average	Standard deviation
Interfoliar variation (field mint)					
Site 1a	4	-0.38	-0.17	-0.24	0.09
Site 1b	4	-1.72	-0.54	-1.11	0.49
Site 1c	4	-1.19	0.20	-0.62	0.61
Site 1d	4	0.83	2.42	1.64	0.87
Site 1e	4	0.29	1.93	0.96	0.72
Site 1f	4	-0.14	0.87	0.53	0.48
Site 1g	4	-0.08	1.65	0.96	0.83
Site 1h	4	0.15	1.35	0.99	0.57
Site 1i	4	-0.70	0.56	-0.02	0.61
Intersite variation (field mint)					
Site 1 average	9	-1.11	1.64	0.34	0.58
Site 2	5	-3.74	-0.83	-2.50	1.17
Site 3	7	-2.70	-1.51	-1.97	0.44
Site 4	5	0.22	1.49	0.83	0.45
Site 5	1	-1.88	-1.88	-1.88	–
Site 6	3	-3.18	-1.72	-2.37	0.74
Site 7	3	-3.54	1.89	-0.75	2.72
Site 8	6	-3.19	2.36	0.15	2.05
Site 9	4	-0.95	3.31	0.43	1.97
Site 10	5	-1.42	-0.24	-0.71	0.61
Filamentous algae					
Lyre Intrasite	7	-1.55	-0.10	-0.74	0.46
Lyre Intramat	12	0.23	1.03	0.53	0.25
La Poel	4	-0.86	0.18	-0.26	0.45
Barnes Point	7	1.37	9.44	5.00	3.24
Reference material					
Peach leaves (NIST 1547)	4	2.00	2.12	2.07	0.06

Interfoliar variations in field mint plants from site 1 was similar to that measured by Dijkstra and others (2003) in forbs and grasses grown in undisturbed forest understory and meadow of aspen-spruce ecosystems. The range of $\delta^{15}N$ measurements at site 1 was larger than measured by Dijkstra and others (2003). Relative abundance and variation of $\delta^{15}N$ in field mint at sites 2–10, many of which were located near existing residential cabins, were similar to site 1. Field mint plants near the margin of residential parcels may not have been directly in the flow path of groundwater discharged from septic system drainfields. The range and variability of $\delta^{15}N$ at some mint sites were larger than might be expected for a single source of nitrogen. With the close proximity of nearby septic system drainfield it is likely that some plants contain some nitrogen derived from septic systems, which may in part explain the large variability in $\delta^{15}N$ measured at sites 7, 8, and 9. Variation in rooting depth may account for some of the variation in $\delta^{15}N$ values because $\delta^{15}N$ varies with depth in forest soils and becomes more enriched at depths to 30 cm (Nadelhoffer and Fry, 1994).

The distribution of normal quantiles of mint data approaches a straight line lacking major inflections or major change in slope (fig. 3). This pattern is indicative of normally distributed data as might be generated from either a single source of nitrogen or a well-mixed composite of multiple sources (Helsel and Hirsch, 2002). The overall range of $\delta^{15}N$ in mint at Lake Crescent generally is consistent with $\delta^{15}N$ data from undeveloped forest land of the Cascade and Coast Ranges reported by Binkley and others (1985). The lack of substantially elevated $\delta^{15}N$ in field mint collected from areas where on-site septic systems are present indicates that monitoring $\delta^{15}N$ in field mint is not an effective approach to identifying input from septic systems to Lake Crescent.

Results from the preliminary survey of $\delta^{15}N$ in filamentous-periphytic algae ranged from -1.55 to 9.44 ‰ (table 1). Although most $\delta^{15}N$ values were within the range of plant material typical of undisturbed natural ecosystem 17 percent of the algae data exceeded that range suggesting a source of nitrogen with elevated $\delta^{15}N$ (Peterson and Fry 1987; Fry 2008). Variation as measured by standard deviation was low except for the samples from the Barnes Point area. Variability within an individual mat (intramat) was about one-half of that measured from seven separate mats located within the 150 m sampling area.

The distribution of normal quantiles of $\delta^{15}N$ in filamentous-periphytic algae data (fig. 3) shows substantial departure from a straight line, closely resembling the intersection of two lines near 1 ‰. Most of the algae data plot in what appears to be a baseline segment that includes roughly 85 percent of values ranging from about -2 to 1 ‰. A separated segment that includes about 15 percent of the algae data ranges from 1 to 9.8 ‰. This skewed distribution of data suggests a bimodal distribution of $\delta^{15}N$ data from the combination of two distinct subpopulations of data (Helsel and Hirsch, 2002; Reinman and others, 2005) and is interpreted as representing two distinct sources of nitrogen being used by filamentous algae. The baseline segment for filamentous-periphytic algae of $\delta^{15}N$ varies from -2 to 1 ‰ and represents an end member of the filamentous-periphytic algae data (fig. 3), which appears to be composed almost entirely of nitrogen from undisturbed forest ecosystem. The elevated segment contains enriched $\delta^{15}N$ values ranging from 1 to 9.8 ‰, approaching the range of $\delta^{15}N$ characteristic of nitrogen from septic system effluent (Kendall and others, 2007). Differences in the average $\delta^{15}N$ of the two segments exceed the range of variation measured among individual mint plants and within the 10 mint sampling sites. Samples with elevated $\delta^{15}N$ values (fig. 3) were interpreted to contain a mixture of nitrogen derived from both an elevated nitrogen source and nitrogen from the forest ecosystem. The lack of an elevated plateau level in the filamentous-periphytic algae data suggests that there are insufficient data points to well-characterize this endmember. The range of $\delta^{15}N$ in filamentous-periphytic algae from littoral areas of the lake may provide a useful tool for monitoring input from septic systems to Lake Crescent.

Monthly Monitoring

Monitoring samples were collected at approximately monthly intervals (November 2009–June 2010) from six monitoring areas around Lake Crescent (fig. 2*A*). The presence of algal mats varied seasonally, and was most abundant in the Lyre redd area during January–March where, as in the Barnes Point and Fairholm Campground areas, algal mats were more abundant during May and June. Typically, 3–4 samples were collected from each area on each sampling visit, except at La Poel where occurrences of filamentous-periphytic algae often was sparse. Coherent algal mats larger than 0.1 m^2 were observed only occasionally in other areas of the lake. For the 6 monitoring areas, 144 algal samples were analyzed for $\delta^{15}N$ (table 3).

The $\delta^{15}N$ of the 144 monitoring samples analyzed ranged from -4.50 to 10.0 ‰ (table 3). The empirical data distribution of the filamentous-periphytic algae monitoring $\delta^{15}N$ data (fig. 4) showed a bimodal pattern similar to that of the preliminary algae data, although there is more curvature in the monitoring data and the position of the inflection in the data occurs at 3 ‰, as compared to 1 ‰ in the preliminary data. The increased curvature may be the result of temporal variation in $\delta^{15}N$, as the collection of monitoring data occurred over a 6-month period, thus incorporating seasonal and spatial variability. Seasonal and spatial variability of as much as 2 ‰ has been reported in the $\delta^{15}N$ of primary producing plant tissue over a broad area of southern Florida (Fourqurean and others, 2005). Similarly, Kaushal and others (2006) reported seasonal variability of about 1 ‰ in baseline $\delta^{15}N$ data for headwater streams in Colorado.

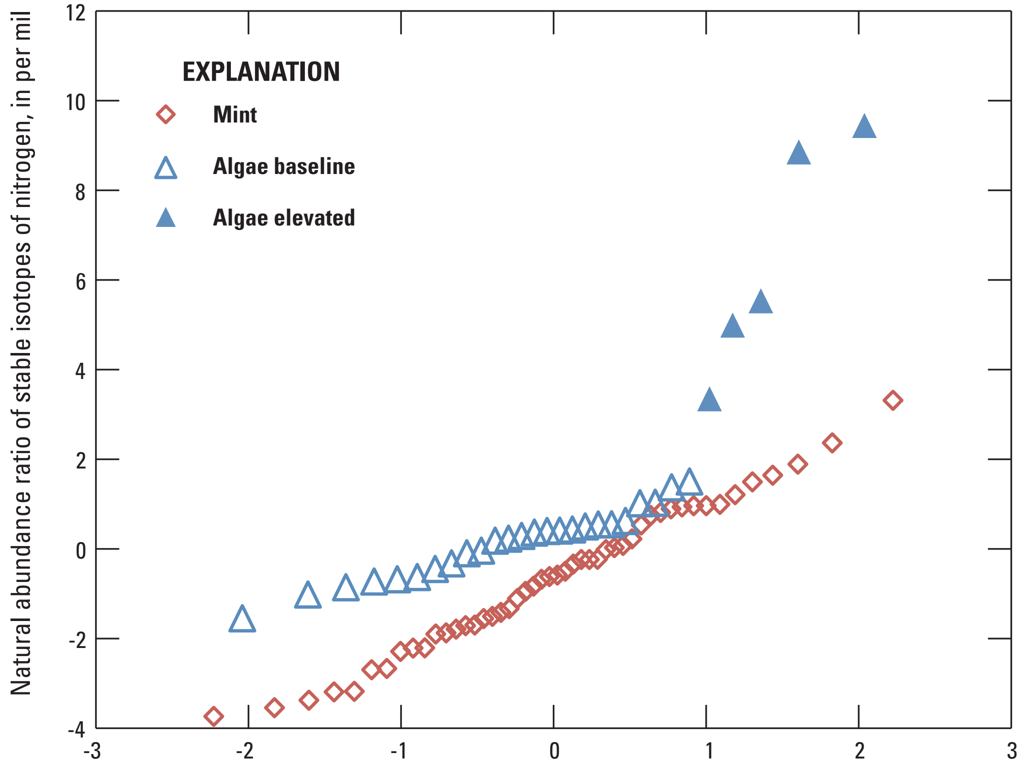

Figure 3. Distribution of normal quantiles of natural abundance ratio of stable isotopes of nitrogen ($\delta^{15}N$) in preliminary plant tissue samples collected at Lake Crescent, Olympic National Park, Washington, October–December 2008.

Table 3. Statistics for abundance ratio of stable isotopes of nitrogen ($\delta^{15}N$) in filamentous-periphytic algal tissue collected from monitoring sites at Lake Crescent, Olympic National Park, Washington, winter–spring 2009.

[All values are in per mil (‰)]

Algae site monitoring area	Number of samples	Minimum	Maximum	Mean	Standard deviation	Median
La Poel	7	-0.96	2.1	0.03	1.01	-0.32
Lyre	36	-1.60	2.9	0.18	1.01	0.20
North shore	32	-1.70	7.1	0.63	1.84	0.09
Fairholm Campground	20	-0.39	7.9	1.90	1.89	1.60
Barnes Point	25	-1.70	10	4.50	3.62	3.90
East Basin outlet area	24	-4.50	7.0	1.40	2.42	0.99

Most monitoring and preliminary filamentous-periphytic algae data (85 and 66 percent, respectively) plot in the baseline segment of the empirical distribution plots (figs. 3 and 4). This segment includes samples from the La Poel site, where anthropogenic inputs are minimal based on present use as minimally developed day use area. Data in the baseline segments of the probability distributions are consistent with the range of $\delta^{15}N$ that has been measured in natural plant material unaffected by nitrogen from wastewater sources (-8 to +3 ‰); Peterson and Fry, 1987; Kendal, 1998; Kendall and others, 2007), as well as of $\delta^{15}N$ measurements of foliage from undeveloped conifer-alder forest of the Pacific Northwest (Binkley and others, 1994), the Oregon Coast Range (Scott, 2004; Scott and others, 2008) and the Sol Duc area west of Lake Crescent in Olympic National Park (Steven Perakis, U.S. Geological Survey, written commun., 2014). Together, these data from the baseline parts of figures 3 and 4 seem representative of the natural level of $\delta^{15}N$ in littoral filamentous-periphytic algae grown in Lake Crescent without significant input of nitrogen from anthropogenic sources.

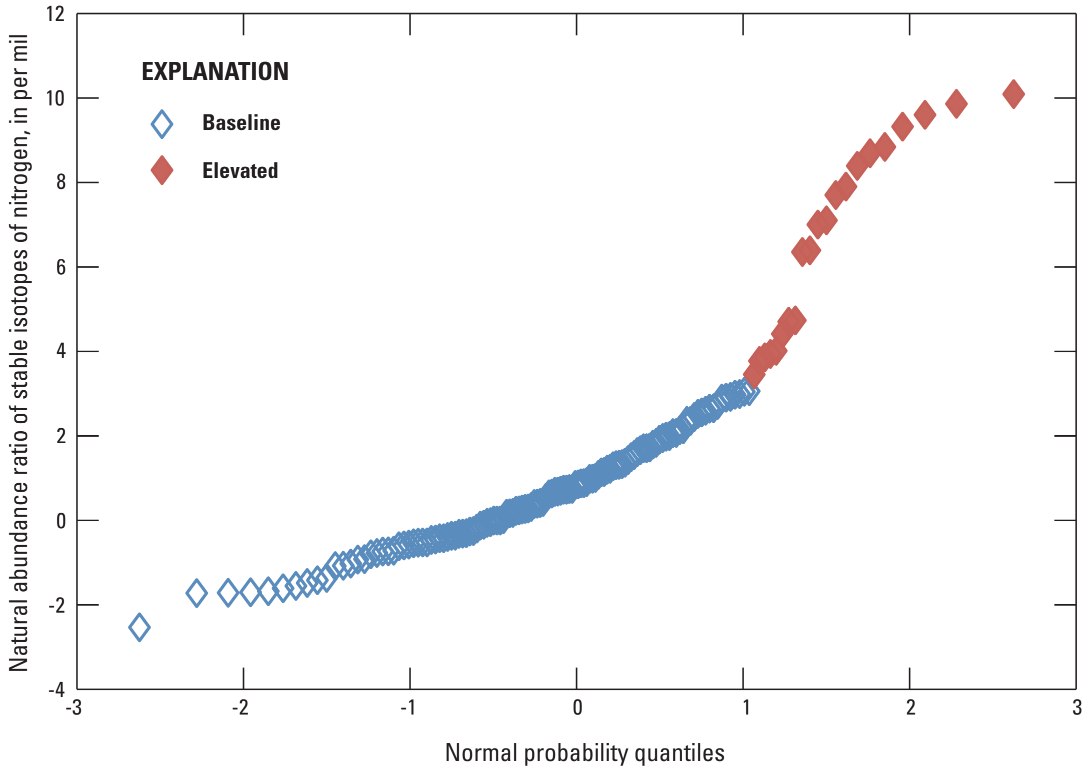

Figure 4. Distribution of normal quantiles of natural abundance ratio of stable isotopes of nitrogen ($\delta^{15}N$) in plant tissue samples of filamentous-periphytic algae from monitoring stations, Lake Crescent, Olympic National Park, Washington, December 2009–June 2010.

Spatial Variation in Stable Isotopes of Nitrogen

Twenty-three algal samples had elevated $\delta^{15}N$ values that ranged from 3 to 10 ‰. About one-half of the samples with elevated $\delta^{15}N$ were collected from the area near Barnes Point, although samples collected from other shoreline areas of the Lake Crescent also were elevated. Data for $\delta^{15}N$ from the six designated sampling areas around Lake Crescent are compared in figure 5. High values of $\delta^{15}N$ in filamentous-periphytic algae were less frequent, present in 10–20 percent of samples from the Fairholm Campground, north shore, and East Basin outlet areas. Elevated values of $\delta^{15}N$ were not measured in samples from La Poel and the Lyre River outlet area. Only seven samples were collected from the La Poel area because of the lack of filamentous-periphytic algae present at that site. Other sites with $\delta^{15}N$ similar to that of the background site included the Lyre River outlet and the north shore area. Comparison of the $\delta^{15}N$ of the six sampling areas using the nonparametric Kruskal-Wallis one-way ANOVA test confirmed that there were significant differences ($p<0.01$) in the $\delta^{15}N$ data between some sampling areas. A post-hoc pairwise comparison (Conover-Inman test) of differences among sampling areas indicated four distinct areas, which are indicated by by letter designations in the box and wisker plots shown in figure 5. The Barnes Point area was significantly different from all other areas ($p=0.01$). By contrast, Fairholm Campground and East Basin outlet areas were similar and distinct from other groups as were the north shore and Lyre River outlet areas. Data from La Poel, Lyre River outlet, and north shore are not statistically different from each other.

The two sites showing the least difference in $\delta^{15}N$ values were the Lyre River outlet area and La Poel. The $\delta^{15}N$ of filamentous-periphytic algae from the Lyre River outlet and La Poel were near the mid-range of -4 to 7 ‰ for a compilation of freshwater algae $\delta^{15}N$ values compiled by France (1995) (n=365) as well as $\delta^{15}N$ values from -1 to +7 ‰ reported by Kendall and others (2007). Because of the interest in the source of nitrogen in the filamentous-periphytic algae mats present in the Beardslee trout spawning area at the outlet of the Lyre River, data from that area were compared using a two-sample t-test with data from the area considered to have the least development. The result of the t-test confirmed the ANOVA analysis indicating that the data from Lyre River outlet and La Poel had a similar distribution ($p=0.68$) and likely were drawn from the same population of nitrogen source material.

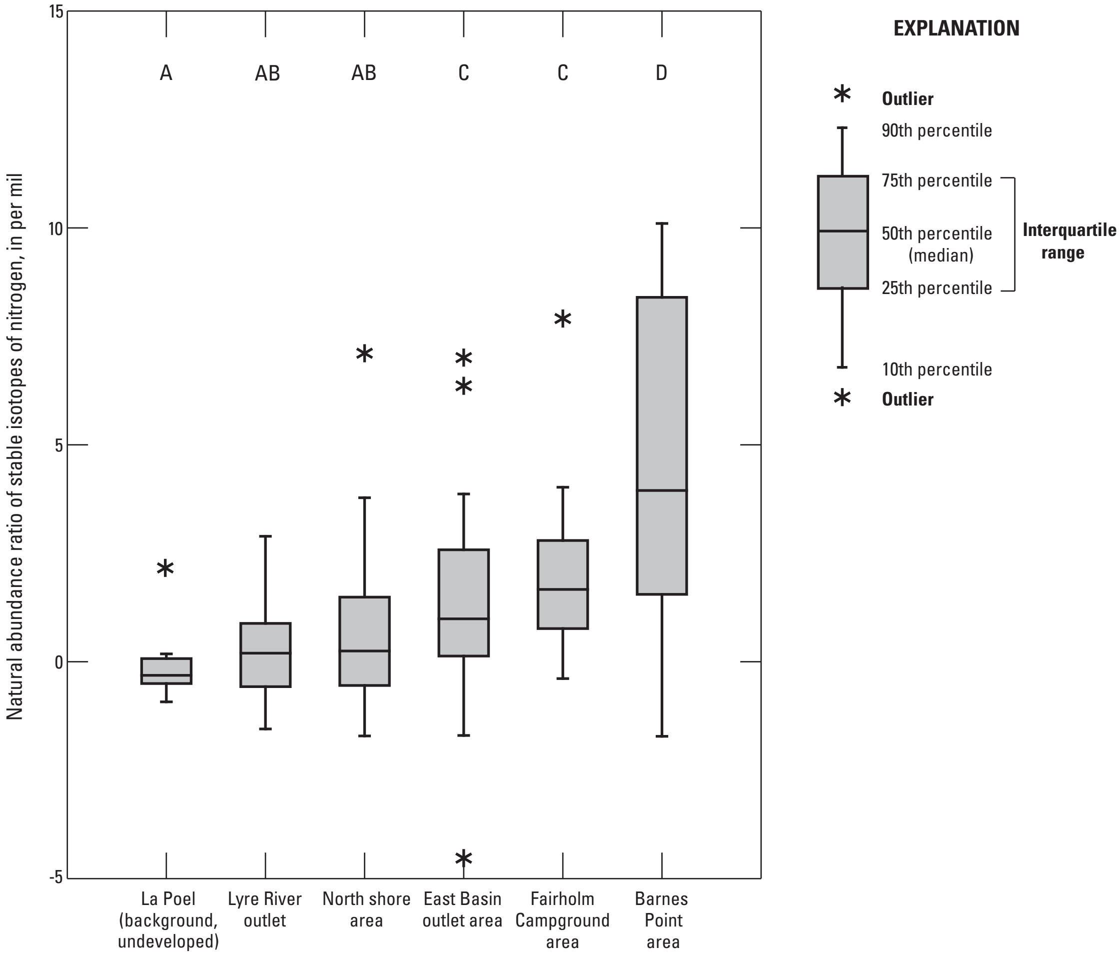

Figure 5. Ranges of natural abundance ratios of stable isotopes of nitrogen ($\delta^{15}N$) of filamentous-periphytic algae from grouped sample locations, Lake Crescent, Olympic National Park, Washington, November 2009–June 2010. Letter designations at top of boxplot indicate a pairwise comparison of differences from Conover-Inman test.

Discrimination of Nitrogen Sources

The distribution of $\delta^{15}N$ data indicates that near shore filamentous-periphytic algae in Lake Crescent use two distinct sources of nitrogen, a widespread baseline source of nitrogen with $\delta^{15}N$ primarily in the range of -2 to 2 ‰, and an elevated source of nitrogen with $\delta^{15}N$, generally greater than 5 ‰. Difference in measured $\delta^{15}N$ were greatest between the least developed site, La Poel day-use area, where $\delta^{15}N$ values were low, and Barnes Point area, where $\delta^{15}N$ was high and where the greatest amount of development has occurred. This relation of elevated $\delta^{15}N$ associated with increased human activity has been reported in many watershed-scale aquatic studies and the source of elevated $\delta^{15}N$ typically is attributed to the input of wastewater either human or agricultural (Cabana and Rasmussen, 1996; Mayer and others, 2002; Cole and others, 2004). Considering the sources of nitrogen included in the nutrient budget for Lake Crescent (Moran and others, 2013), increased human activity and associated septic waste generation was consistent and likely was the source of elevated $\delta^{15}N$ measured in filamentous-periphytic algae. The largest $\delta^{15}N$ values occurred in areas with greatest amount of developed shoreline at Crescent Lake and the greatest intensity of park use and year round activity. Much of the septic waste in the Barnes Point area is treated in a centralized community treatment system with a large septic drainfield located upgradient of the Barnes Point sampling area. Conversely, the lowest $\delta^{15}N$ values were measured in samples collected from La Poel where there is limited activity.

The elevated $\delta^{15}N$ data from the Barnes Point area vary as much as 10.0 ‰ and are consistent with data on septic effluent sources of $\delta^{15}N$. In groundwater, plumes from septic system drainfields $\delta^{15}N$ can be variable, ranging from 0 to 25 ‰ with typical average values in the range of 7 to 10 ‰ (Aravena and others, 1993; Fogg and others, 1998; Hinkle and others, 2008). The $\delta^{15}N$ of macroalgae was elevated about 5.6 ‰ in streams draining an unsewered urban areas compared to adjacent sewered areas (Steffy and Kilham, 2004). A similar level of enrichment of $\delta^{15}N$ in stream algae occurred for some headwater streams during ski season in the Breckenridge Ski area of Colorado (Kaushal and others, 2006). The $\delta^{15}N$ of freshwater algae grown exclusively on septic wastewater can be as large as 17.1 ‰ (Kaushal and others, 2006).

The possibility of elevated $\delta^{15}N$ from other anthropogenic sources of nitrogen identified by Moran and others (2013) as contributing nitrogen input to Lake Crescent includes fertilizers and exhaust emissions from vehicle traffic on roadways. Elevated $\delta^{15}N$ from vehicular nitrogen oxide (NOx) has been measured in grasses adjacent to vehicle roadways, with higher values closer to roadway and lower values farther from the roadway (Laffray and others, 2010). Locations where filamentous-periphytic algae were present and elevated $\delta^{15}N$ was measured typically were greater than 400 m from the roadway through dense forest and not likely to be heavily affected by vehicle NOx. However, filamentous-periphytic algae also were detected along the shoreline adjacent to the U.S. Route 101 near Fairholm. Consumer lawn and garden fertilizer use in the Crescent Lake area is expected to be small, although if present, $\delta^{15}N$ of fertilizer likely would not be distinguishable from background levels, as inorganic fertilizers typically have a mean $\delta^{15}N$ near 0 ‰ (Bateman and Kelly, 2007).

These considerations and comparative analyses of the sampling area data indicate that it is not likely that effluent from septic systems is contributing to the growth of nuisance algal mats observed near the Beardslee trout spawning redds.

Potential Source of Nitrogen in Littoral Zones

Red alder is common in developed riparian areas surrounding Lake Crescent particularly in historically developed shorelines that have been allowed to revegetate (fig. 6). Studies of nearshore areas of Crescent Lake have concluded that alder occurrence is related to the abundance of periphyton at Crescent Lake. Rosenberger and other (2008) determined that developed sites had greater number and size of alder trees, whereas Redberg (2012) concluded that alder cover had a significant influence on the growth and biomass of periphyton associated with developed sites. In the nutrient budget for Lake Crescent, red alder was estimated as the third largest source contributing 16 percent of nitrogen input to the lake. It is possible that red alder is a significant source of nitrogen supporting much of the growth of filamentous-periphytic algae in many littoral areas, except the Barnes Point area. The median $\delta^{15}N$ value of the 121 filamentous-periphytic algae samples that make up the baseline data group is 0.4 ‰, close to the atmospheric value of 0.0 ‰. Plants that fix atmospheric nitrogen generate $\delta^{15}N$ in a narrow range, typically between -2 and 2 ‰ (Shearer and Kohl 1986). Red alder is a pioneering species that naturally revegetates disturbed forested areas, primarily after a forest fire or logging (Harrington, 2006) and also revegetates in areas disturbed by development (Roberts and Bilby, 2009). Development of new shoreline parcels on Lake Crescent was curtailed following the establishment of Olympic National Park in 1938, and many previously developed areas have been allowed to revegetate naturally. Red alder stands reach maximum biomass and soil nitrogen accumulation within about 20 years of establishment and maintain these amounts as long as red alder dominates the site (Luken and Fonda, 1983).

Red alder can act as a source of nitrogen to aquatic systems through two primary mechanisms. Bacteria associated with root systems of red alder fixes large amounts of atmospheric nitrogen (100–200 [kg-N/ha]/yr) that can result in accumulation of soil nitrogen of about 35 (kg-N/ha)/yr (Bormann and others, 1994). Soils beneath stands of red alder in the Pacific Northwest rapidly become highly enriched in nitrogen (Luken and Fonda, 1983; Binkley and others, 1992; Bormann and DeBell, 1981) and may export nitrogen to surrounding watersheds, significantly influencing in-stream nitrogen concentrations (Stottlemeyer, 1992; Wigington and others, 1998; Bechtold and others, 2003; Compton and others, 2003; Volk and others, 2003; Sigleo and others, 2010; Hart and others, 2013).

Red alder detritus and fallen alder trees in the littoral zone are a source of nitrogen. Alder tree detritus, leaves, twigs, and trunks contain more nitrogen than non-nitrogen fixing evergreen species (Volk and others, 2003; Hart and others, 2013). Late-season litterfall from deciduous species such as red alder in autumn can create a large pulse of nitrogen-rich detritus to the littoral zone that might be used as a source of nutrients to periphyton (Harmon and others, 1990; Pieczynska, 1993). The timing of these pulses of increased groundwater discharge of soil nitrogen, coupled with the decay of deciduous litterfall, may trigger the January–February spike in algal mat development in the Lyre River outlet area and Fairholm Campground areas of Lake Crescent. The studies by Volk and others (2003) and Bechtold and others (2003) were done in drainage basins adjacent to Lake Crescent and provide geographically relevant models of processes that may be occurring at the lake. The seasonal variations in nitrogen flux from alder stands were monitored in the Queets River Basin, Washington (Bechtold and others, 2003).

Figure 6. Red alder trees along Lake Crescent shoreline, Olympic National Park, Washington. (*A*) Residential development along East Beach Drive, (*B*) undeveloped area along southern shore of East Basin outlet area, (*C*) Log Cabin Resort recreational vehicle area, (*D*) residential development along Barnes Point, (*E*) Fairholm Campground area (note fallen alder in littoral zone), and (*F*) detritus and wrack line along southern shore of East Basin outlet area. Photographs taken by (*A*) S.C. Fradkin, National Park Service, February 11, 2012; (*B*) S.C. Fradkin, February 28, 2006; (*C*) S.E. Cox, U.S. Geological Survey, September 28, 2007; (*D*) S.E. Cox, September 28, 2007; (*E*) S.E. Cox, September 28, 2007; and (*F*) S.C. Fradkin, January 3, 2007.

They determined that nitrogen readily leached form alder stands during both simulated and actual rainfall events, strongly influencing the flux of nitrogen from alder stands. The peak period of nitrogen export is November–January because of the combined effects of higher nitrogen content and higher streamflows than during the rest of the year. A similar seasonal timing of nitrogen export was reported by Steinberg and others (2011) for the Hood Canal area of Washington, and by Wigington and others (1998) and Hart and others (2013) for the Oregon Coast Range. In streams that flow into Lake Crescent, peak concentrations of nitrogen were measured in October and November, whereas the peaks in total nitrogen mass delivered to the lake were measured from November through January during periods of high flow (Moran and others, 2013). Volk and others (2003) compared nitrogen input from riparian zones of a second-order lowland headwater stream in the Hoh River Basin; litterfall biomass along alder-dominated riparian zones was 348 g/m^2 compared to 104 g/m^2 in conifer-dominated streams. When coupled with the greater nitrogen content of alder litter, the total annual nitrogen input along alder stream reaches was about eight times greater than along similar conifer-dominated stream reaches (Volk and others 2003). High concentrations of nitrogen in alder litter from the Skokomish River Basin rapidly decomposed and released nitrogen (Edmunds and Tuttle, 2010). Similar results were obtained by Hart and others (2013) for streams in the Oregon Coast Range, where the combination of vertical and lateral litter input combined such that total nitrogen inputs from red alder riparian canopies were two times greater than litter input from comparable streams with Douglas fir-dominated riparian canopies.

Summary

The stable isotope of nitrogen ($\delta^{15}N$) signature of filamentous-periphytic algae growing in nearshore zones around much of Lake Crescent is derived largely from nitrogen fixed by plants, except in the Barnes Point area where elevated nitrogen isotope ratios indicate a substantial input of septic systems effluent. Filamentous-periphytic algae growing in trout spawning areas near the Lyre River outlet show little variation, and $\delta^{15}N$ is consistently in the range of natural forest ecosystems, thereby indicating little measurable influence of nearby septic system effluent. The $\delta^{15}N$ signature data confirm that the overall input of septic sources to the nitrogen budget of Lake Crescent is small, and data are consistent with previous study results that alder trees present near the shoreline of Lake Crescent are likely a major contributor of nitrogen to the nearshore littoral areas. This study also showed that the $\delta^{15}N$ of field mint is not a good indicator of the presence of septic system effluent in the Lake Crescent nearshore.

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