Water Quality & Biological Response To Rotenone Treatment in Diamond Lake, Oregon

Prepared for the
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Roseburg, OR

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ABSTRACT

Diamond Lake experienced poor water quality and an impaired trout fishery as a consequence of the illegal introduction of tui chub (*Gila bicolor*). Plans were developed to remove the tui chub by partially drawing down the lake and treating the lake with rotenone. The drawdown was initiated in November 2005 and completed in July 2006. Prior to treatment, over 46,700 kg of tui chub were removed from the lake using gillnets and trapnets. The lake was treated with rotenone September 14-16, 2006 using 48,590 kg of powdered rotenone and 32,648 kg of liquid rotenone. The total amount of active rotenone applied was 5,830 kg. Diamond Lake experienced frequent and intense cyanobacteria blooms and associated water quality problems up to 2006. In summer 2006, the phytoplankton community composition was comprised largely of diatoms (first *Fragilaria crotonensis*, then *Synedra*), but the cyanobacterium, *Anabaena,* became dominant just prior to treatment of the lake with rotenone. *Anabaena* continued to bloom through the fall and under the ice in 2006-2007. Following ice-off on April 25, 2007, the diatom, *Synedra,* became dominant, made possible by the high concentrations of ammonia that accumulated during the winter. The bloom of diatoms subsided as high densities of the cladoceran, *Daphnia pulicaria,* grazed down the diatoms. Transparency, which before treatment had averaged 1.6 m in 2006 increased to a maximum of 12.5 m on June 26, 2007. Macrophytes which had grown to a depth of 7 to 8 m depth prior to treatment were present at the deepest part of the lake in 2008. Following the treatment, concentrations of phosphorus remained unchanged in the surface waters, whereas concentrations of total nitrogen declined from 472 µg/L in 2006 to 392 µg/L in 2007 and 298 µg/L in 2008. Mean field pH values declined from 9.5 in 2006 to 8.4 in 2007 and 7.8 in 2008. Chlorophyll *a* concentrations declined from 15.3 µg/L in 2006 to 2.7 µg/L in 2007 and 7.8 µg/L in 2008. The zooplankton community composition changed from a system largely dominated by small cladocerans and rotifers before treatment to a more balanced community after treatment with relatively equal densities of cladocerans, copepods, and rotifers. Large cladocerans such as *Daphnia pulicaria*, which were absent in 2006, achieved a median summer density of 26,648 individuals per cubic meter in 2007 and 7,617 individuals in 2008 and a maximum density of over 77,000 individuals per cubic meter in July 2007. Benthic macroinvertebrates increased from a density of 0.7 g/m^2 in August 2006 prior to the treatment to 22.5 g/m^2 in October 2007 and 18.9 g/m^2 in October 2008. The community composition of benthic macroinvertebrates changed from one dominated largely by chironomids before treatment to a more diverse community comprised of diptera, amphipoda, ephemeroptera, trichoptera, odonata, gastropoda, and bivalvia. The amphipod, *Hyalella*, increased from 1 individual sampled in 2006 to 683 in 2007 and 1,183 in 2008. Condition factors of stocked rainbow trout (*Oncorhynchus mykiss*) were 1.5 in 2007 and 1.3 in 2008. Growth rates of rainbow trout were 19.4 cm in 2007 and 21.0 cm in 2008. In summary, all water quality and fisheries goals of the Diamond Lake Restoration Project were met or exceeded.

INTRODUCTION

Diamond Lake is a 1,226 ha (3,029 ac) lake in the central Oregon Cascades, likely formed from volcanic debris from the Mazama eruption circa 7,686 ybp (Zdanowicz et al. 1999) forming a dam on Lake Creek (Sherrod 1991). It is located about 17.7 km (11 mi) north of Crater Lake National Park. Prior to about 1910 the lake was fishless. It was stocked at that time with rainbow trout and developed into a highly successful fishery and egg-gathering station (Dimick 1954). In the 1940s, tui chub were introduced into the lake, presumably as a bait fish. The tui chub rapidly multiplied, eliminating most of the benthic invertebrates and causing authorities to treat the lake with rotenone in 1954 (Dimick 1957; Bauer 1964). The lake recovered rapidly (Bauer 1976) and once again became a successful trout fishery until 1992 when tui chub were once again found in the lake. The tui chub once again multiplied rapidly, stripping the lake of benthic invertebrates and larger zooplankton taxa (Eilers et al. 2007). In 2001, the lake experienced a major bloom of cyanobacteria (Jones et al. 2007), which continued in subsequent years with varying degrees of severity.

In response to the deteriorating water quality and impaired trout fishery, the Oregon Department of Fish & Wildlife (ODFW) developed a plan to restore the fishery and address the water quality problem simultaneously by eradicating the tui chub. The method selected to remove the tui chub was to drawdown the lake and treat the lake with rotenone, similar to the treatment used on Diamond Lake in 1954. An environmental impact analysis was conducted by the Umpqua National Forest and completed in 2004 (Umpqua National Forest 2004).

The FEIS identified two major goals of the project: (1) improve overall water quality (referred to as Element 1), and (2) improve the recreational fishery (referred to as Element 2). The post-project goals under the water quality element consisted of the following:

- **pH**: less than 10 percent of summer water samples would have pH values that exceed 8.5 (or the pH value determined attainable in the Total Maximum Daily Load [TMDL]calculations).
- **Algae**: The average value of primary production (as represented by chlorophyll *a*) for all samples collected during a three month summer sampling period would be less than or equal to 0.01 mg/L (= 10 *µg/L*) or the level determined attainable in the TMDL calculations.
- **Neurotoxin Production**: *Anabaena flos-aquae* levels would remain below 15,000 cells/mL indicating that the waters of Diamond Lake are safe for water contact recreation.

The post-project goals under the recreational fishery element were:

- **Tui chub**: Tui chub are absent from Diamond Lake or if reintroduced are present in numbers believed small enough to control using limited mechanical methods (nets, seines, disruptions of breeding, etc.) or stocking with predacious fish.
- **Ecological Indices of Lake Health (Eilers 2003a)**: Monitoring data (zooplankton and benthic invertebrate population numbers and community compositions, and appropriate water quality data) indicate recovery of the Diamond Lake food chain is adequate to support a recreational fishery without compromising progress toward achieving water quality standards.
- **Trout**: Annual harvest rates for legal-sized trout increase. Trout growth rates and condition factors return to levels approaching those observed prior to the introduction of tui chub.

The proposed activities under the plan included canal reconstruction, a fall/winter draw down, mechanical fish removal and utilization, a September rotenone (fish toxicant) treatment to eradicate tui chub, fish carcass removal and utilization, water management during the refill period, monitoring, fish restocking, educational activities, and contingency measures for controlling tui chub if they are reintroduced to Diamond Lake in the future.

The canal reconstruction allowed for an 8 ft draw down and construction of new headworks to allow for a controlled drawdown, which was initiated in November, 2005. Full drawdown was achieved in summer 2006, while ODFW and a contractor captured tui chub in nets and removed them from the lake. The outlet to Lake Creek was sealed in early September and ODFW applied 32,648 kg of liquid rotenone and 48,590 kg of powdered forms of rotenone in the lake and tributaries September 14-16, 2006. The total amount of active rotenone applied was 5,830 kg (Truemper 2006). The lake was monitored and water was not released downstream until there were no measureable concentrations of rotenone or its byproducts. The refill of the lake to its pre-treatment level was completed in July 2007. The lake was restocked with rainbow trout in spring of 2007, thus completing one of the last of the major plan activities. One of the continuing responsibilities of the plan was to monitor water quality and biological populations to ensure that the level of trout stocking (Element 2) does not impinge on meeting water quality goals under Element 1 of the plan.

The purpose of this report is to describe the results of water quality and biological monitoring conducted on Diamond Lake from 2006 through 2008 during the treatment and recovery process. The results of the Diamond Lake Restoration Project are summarized by topical area in report. Further analyses of the results are presented in the discussion section which provides a synthesis of the project findings. Other activities which were ancillary to this water quality report are summarized elsewhere. This includes application statistics associated with the rotenone treatment

(Truemper 2006) and amphibian response to the drawdown and treatment (Hayes and Price 2007; Hayes and Rombough 2008). The Umpqua National Forest also funded Portland State University (PSU) to collect water quality data on Diamond Lake starting in 2006. The results of their data collected in 2006 and 2007 are reported elsewhere (Sytsma et al. 2007; Sytsma and Miller 2008). The data collected by PSU in 2008 are reported here under an agreement between the Forest Service and the Oregon Department of Fish & Wildlife (ODFW).

METHODS

1. Field

Water quality in Diamond Lake was determined by sampling at site DLA over the deep portion of the lake. The coordinates for the site are latitude 43.16972º N and longitude 122.15177º W. In-situ measurements were made primarily using an In-Situ Troll 9000 sonde equipped with sensors for barometric pressure, temperature, conductivity, pH, and dissolved oxygen. The unit was calibrated using manufacturer's recommendations for pH, using buffers of pH 7 and 10, in that order. Dissolved oxygen was calibrated using saturated water at the site created by aerating water prior to calibration. Dissolved oxygen measurements were checked against Winkler titrations using a Hach digital titration kit for samples collected at two depths, generally at 1 m and 12 m or 13 m. In cases where the sonde and Winkler values differed by more than 0.5 mg/L from the sonde measurements, the values recorded with the sonde were adjusted based on the bias reflected by the Winkler titrations. The sonde was operated by recording measurements at each meter interval after the readings stabilized. This could range from 30 seconds under warm conditions to over 10 minutes under cold-water conditions for each interval. Repeat observations were recorded at two depths while retrieving the sonde.

Secchi disk measurements were made by lowering a standard 20 cm diameter disk on the shady, sheltered side of the boat and recording the average of the depth of disappearance and the depth of reappearance of the disk. When additional parties were present, the average of all readings was used. Light extinction was measured by percent difference between light intensity with a LI-COR model LI-250A underwater quantum sensor. Measurements were taken on the south side of the boat by recording the surface incident radiation and at each meter below the surface until the 1 percent incident level of light had been reached or exceeded.

Water samples were collected with a peristaltic pump attached to high grade Tygon® tubing, weighted at the opening. Water was pumped through the lines for several minutes to remove any water or particles. The line was then lowered to the prescribed depth and additional water was pumped through the system into a Wildco® 12 L sample churn splitter until it was at least half full. The churn was rinsed and the process was repeated until sufficient volume had been pumped for the necessary aliquots. The churn was mixed while aliquots were filled from the spigot into the pre-rinsed sample bottles. Nalgene® HDPE bottles were used for collection of all analytical measurements. Samples were placed in a cooler on ice and transported back to the office prior to shipping overnight to the laboratory. Samples for analysis of silica, nitrate+nitrite (reported here as nitrate), ammonia, and ortho-phosphorus were filtered. Silica aliquots were kept refrigerated prior to shipping, whereas the nutrient samples were frozen. Prior to 2007, all samples were filtered with Whatman® GF/C glass microfiber 47 mm filters using a Nalgene® manual pump filtration system. In 2007, Geotech® 0.45 micron capsule filters were used operating in-line with the peristaltic pump. Split samples were used to evaluate the differences between filtration methods. Additional samples were collected for split analyses of nutrients by Aquatic Research, Inc. in Seattle. Several blank samples were submitted to CCAL for determination of possible contamination. CCAL periodically analyzed internal duplicates to provide an indication of the precision of the measurement process. The quality assurance project plan (QAPP) developed for the Diamond Lake Recovery Project in 2006 is on file with ODFW, Roseburg, Oregon (Eilers 2006).

2. Analytical Laboratory

The primary analytical laboratory used for analysis of nutrients was the CCAL laboratory located on the Oregon State University campus in Corvallis, Oregon. Analytical methods used for the analyses are summarized in Table 1 and additional details are located on the CCAL website (http://ccal.oregonstate.edu/).

Table 1. Analytical methods and data quality objectives for water quality samples from Diamond Lake from 2006-2008.

#	Analyte	Method	Precision	Accuracy	Quantitation Limit	Notes
1	Temperature	170.1	0.5 C	0.5 C	0.1 C	
2	Conductivity	120.1	≤ 10 %	≤ 7 %	1 µS/cm	
3	Dissolved Oxygen	4500-O C	0.3 mg/L	0.2 mg/L	0.1 mg/L	
4	pH	150.1	0.3 s.u.	0.2 s.u.	0-14 s.u.	
5	Alkalinity (ANC)		10 %	10 %	1 µeq/L	Gran plot
6	Total Phosphorus	SM4500-P	10 %	10 %	0.01 mg/L	
7	Ortho-Phosphorus	SM4500-P E	10 %	10 %	0.001 mg/L	
8	Total Nitrogen	SM4500-P	10 %	10 %	0.01 mg/L-N	
9	Ammonia	SM4500-NH3	10 %	10 %	0.01 mg/L-N	
10	Nitrate	SM4500-NO3	10 %	10 %	0.001 mg/L-N	
11	Silica	SM4500-SiO2	10 %	10 %	0.1 mg/L-SiO2	
12	Calcium	SM4110	5 %	5 %	0.01 mg/L	IC
13	Magnesium	SM4110	5 %	5 %	0.01 mg/L	IC
14	Sodium	SM4110	5 %	5 %	0.01 mg/L	IC
15	Potassium	SM4110	5 %	5 %	0.01 mg/L	IC
16	Sulfate	SM4110	5 %	5 %	0.05 mg/L	IC
17	Chloride	SM4110	5 %	5 %	0.01 mg/L	IC
18	TOC	SM513B	± 20 %	10-15 %	1 mg/L	
19	DOC	SM513B	± 20 %	10-15 %	1 mg/L	

3. Taxonomic Identifications

The water quality monitoring program included collection of phytoplankton, zooplankton, and benthic macroinvertebrates. Taxonomic analyses of phytoplankton samples from Diamond Lake have been conducted by Aquatic Analysts, Inc., Milwaukie, OR since 1992. Aquatic Analysts, Inc. continued to analyze the phytoplankton samples through this study period. Methods used by Aquatic Analysts, Inc. are provided in Appendix A. Quality assurance split samples of phytoplankton were collected and shipped to PhycoTech, Inc., St. Joseph, MI (http://www.phycotechch.com/) and GreenWater Laboratory, Palatka, FL (http://www.greenwaterlab.com/) for comparison.

Zooplankton samples for Diamond Lake have been analyzed by ZP Taxonomic Services, Inc., Lakewood, WA since 1992. Their services were also retained through this study period. Procedures for the analysis of zooplankton samples are provided in Appendix B. Analyses of benthic macroinvertebrates has not been a routine practice since 1980 when collection of benthic invertebrates was discontinued in favor of use of fish metrics for helping to guide fish stocking decisions. Annual sampling of benthic macroinvertebrates was re-instituted in 2004. Samples reported for 2006 were analyzed by Third Rock Consultants, Lexington, KY

(http:www.thirdrockconsultants.com/) and samples from 2007 and 2008 were analyzed by Dr. Michael Cole with ABR, Inc., Forest Grove, OR (http://www.abrinc.com).

RESULTS

1. Quality Assurance Review

A quality assurance review was conducted for the analytical chemistry data and the phytoplankton taxonomic data. The review of the analytical chemistry data showed that the data met or exceeded data quality objectives for nearly all analytes (Appendix D). One exception was for dissolved organic carbon (DOC) where some of the reported values equaled or exceeded the values for total organic carbon (TOC). The source of this apparent error was never fully resolved, although splits with various filters failed to isolate the problem to the field or the laboratory. Consequently, DOC values are not reported here. The results from samples split with another laboratory (Aquatic Research, Inc., Seattle, WA) showed a difference of 4 *µg/L* in the results for total phosphorus (TP). Although this difference was statistically significant ($P < 0.05$), it was not known if this represented a bias between the two laboratories or a deviation of +/- 2 *µg/L* about a mean of zero. Regardless, the magnitude of this deviation was judged to be insufficient with regard to ecological significance.

The review of the phytoplankton data produced results that are more problematic (Appendix C). The analysis of splits among three sets of taxonomists (Aquatic Analysts, Inc., the primary phytoplankton laboratory; and the two laboratories used for quality assurance splits, PhycoTech, Inc. and GreenWater Labs) yielded highly variable results, especially at the species level. There was good agreement among taxonomists for some major diatom taxa such as *Fragilaria crotonensis, Aulocoseira distans* (reported using the older nomenclature as *Melosira distans* by Aquatic Analysts), and *Asterionella formosa,* but poor agreement among taxonomists for species within the cyanobacteria, cryptomonads, chrysophytes, and chlorophytes. One of the key species identified in the project goals, *Anabaena flos-aquae*, was commonly identified as belonging to other taxa by the laboratories conducting the analysis of split samples. The taxonomic difficulties in using morphological characteristics alone to identify cyanobacteria, and this genus in particular, is described by St. Amand et al. (2007) who reported this taxon as *A. lemmermanni*. Although we report *Anabaena flos-aquae* using the data reported by Aquatic Analysts, it is quite possible that phycologists may re-classify this organism in future monitoring of Diamond Lake.

Other major areas of disagreement among the taxonomists for Diamond Lake include the genera *Synedra* and *Cryptomonas.* Because of the lack of concordance among the algal taxonomists, most

of the organisms are reported only to the genera level or even assembled into more general groups in this report.

An examination of variables was conducted to assess possible outliers among some of the variables. The most glaring inconsistency that was identified was the chlorophyll *a* value reported from the last sample collected in 2008. The results show a chlorophyll *a* concentration that was highly irregular with respect to measured Secchi disk transparency (Figure 1) and light extinction (Figure 2). A check with the laboratory showed no basis for the inconsistency. This chlorophyll *a* observation was removed from the data set reported here. The basis for this inconsistency and its implication for the lake and the current sampling design is described in the discussion section of the report.

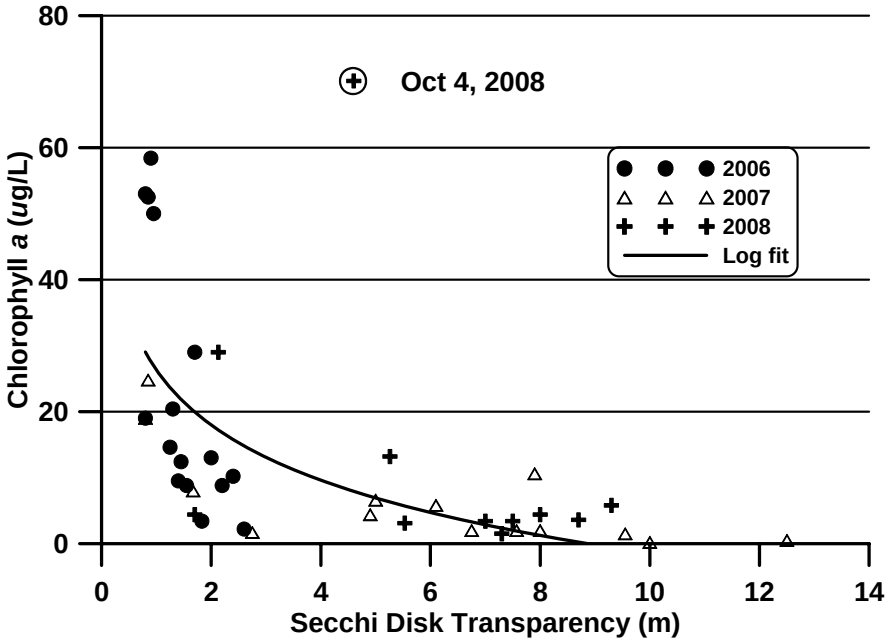

Figure 1.Secchi disk transparency versus chlorophyll *a* for samples from Diamond Lake collected in 2006-2008.

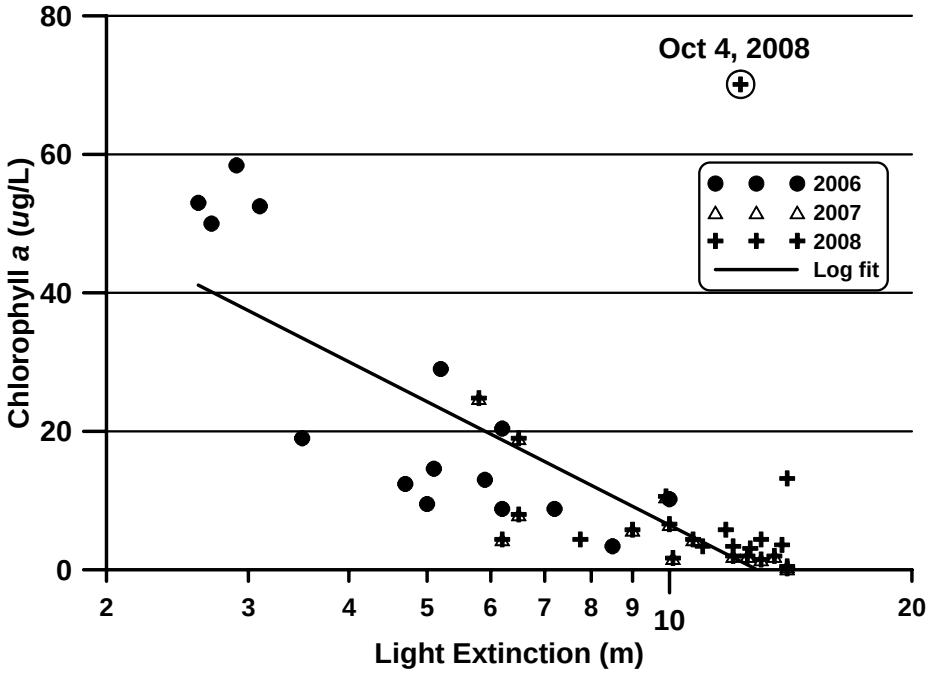

Figure 2. Depth at which light extinction equaled 1 percent of surface light versus chlorophyll *a* for samples collected from Diamond Lake in 2006-2008.

2. Climate and Hydrology

The climatic conditions during 2006-2008 for Diamond Lake deviated relatively little from long-term averages for the area. Total annual precipitation measured at the adjacent SNOTEL site showed that values during the study years differed little from the long-term average of 127 cm (50 in) for the site (Figure 3). Summer daily average air temperature values for 2006-2008 were slightly above the long-term average, but did not approach the variations among years that occurred from 1990-2000 (Figure 4). Dates of ice-off for 2006 and 2007 were close to the longer-term average, but ice-off in 2008 equaled the latest date of ice-off recorded since 1990 (Figure 5). The discharge from Lake Creek was highly altered in 2006-2007compared to typical years as a consequence of the change in lake stage during the draw down and the re-fill periods (Figure 6). Discharge in 2008 was more similar to pre-treatment conditions, although the magnitude of total discharge was somewhat elevated. The effect of the manipulation of lake stage during the study is evident in Figure 7.

Discharge to Diamond Lake from its primary tributary, Silent Creek, increased slightly during the study period (Figure 8). Presumably, flows from Short Creek and groundwater discharge to Diamond Lake followed patterns similar to that observed in Silent Creek. The draw down and re-fill of Diamond Lake made it possible to study aspects of the hydrology of Diamond Lake in ways not easily reproduced under normal lake levels. The computed groundwater discharge/recharge associated with Diamond Lake showed a net discharge to the lake through late summer and a slight period of net recharge to the lake in early fall before the return of rain (Figure 9). Computation of groundwater fluxes made it possible to better define all components of the water budget for the same period (Figure 10).

The observation wells installed around the perimeter of Diamond Lake prior to the treatment helped to define primary groundwater discharge and recharge areas around the lake (Breeden 2004). The groundwater levels were monitored through 2007 to document the return of normal groundwater flow paths (B. Eilers 2008). The results showed a minor alteration in flow paths and groundwater gradients in the vicinity of the lake which quickly returned to pretreatment levels with re-fill of the lake (Figure 11).

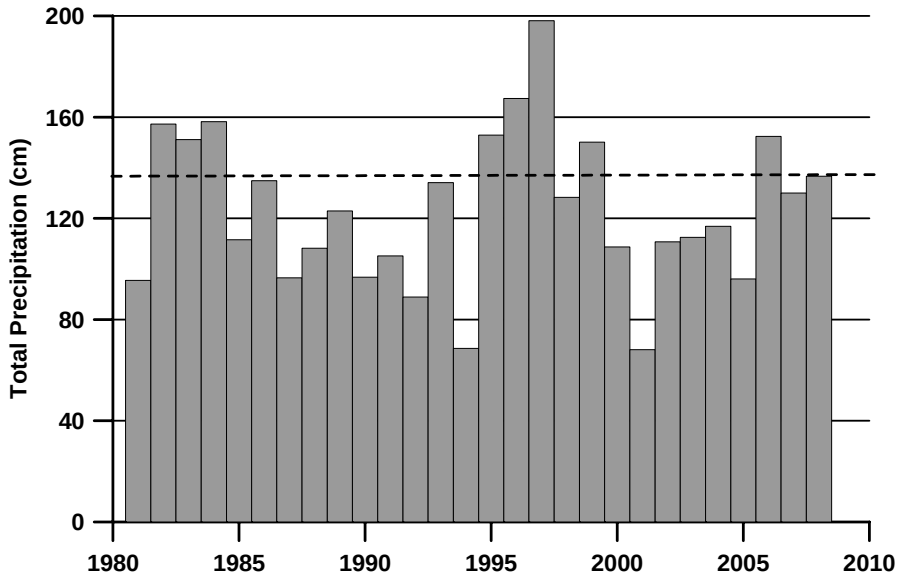

Figure 3. Total annual precipitation measured at the SNOTEL site adjacent to Diamond Lake. The dashed horizontal line represents the mean for the period of record.

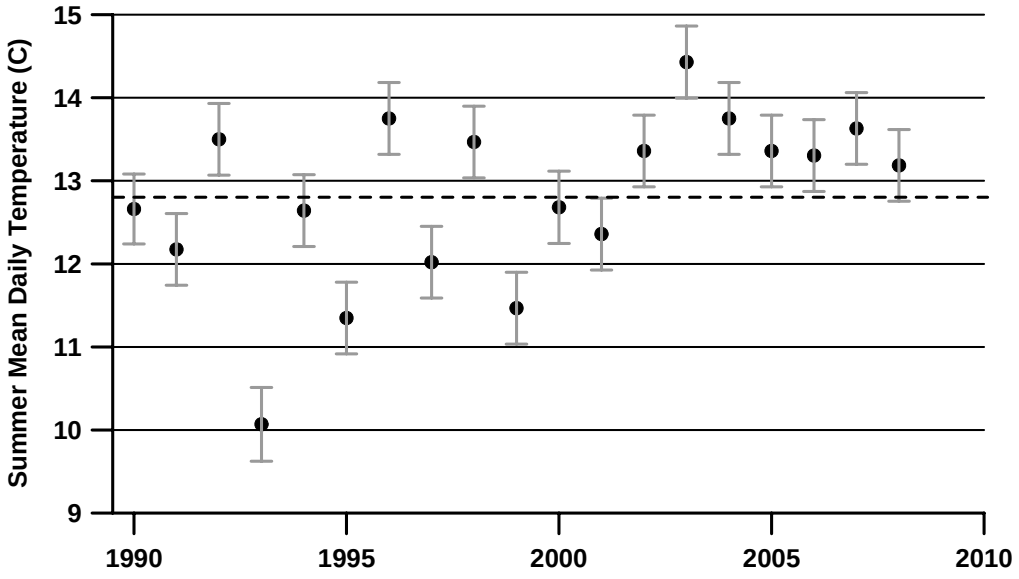

Figure 4. Mean daily temperature measured at the Diamond Lake SNOTEL site for the summer period (June-Sept) from 1990 to 2008. The vertical bars represent the standard errors of the mean. The dashed horizontal line represents the mean for the period of record.

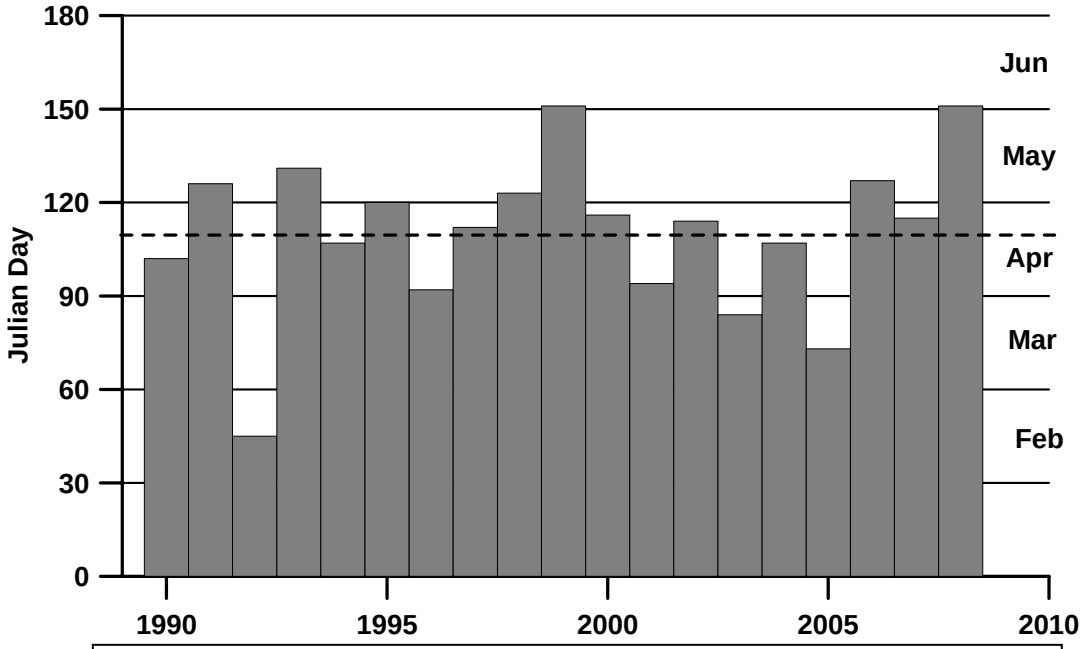

Figure 5. Dates of ice-off for Diamond Lake defined as being completely ice-free. The average date of ice off for this period of record is indicated by the dashed line. Data courtesy of David Loomis.

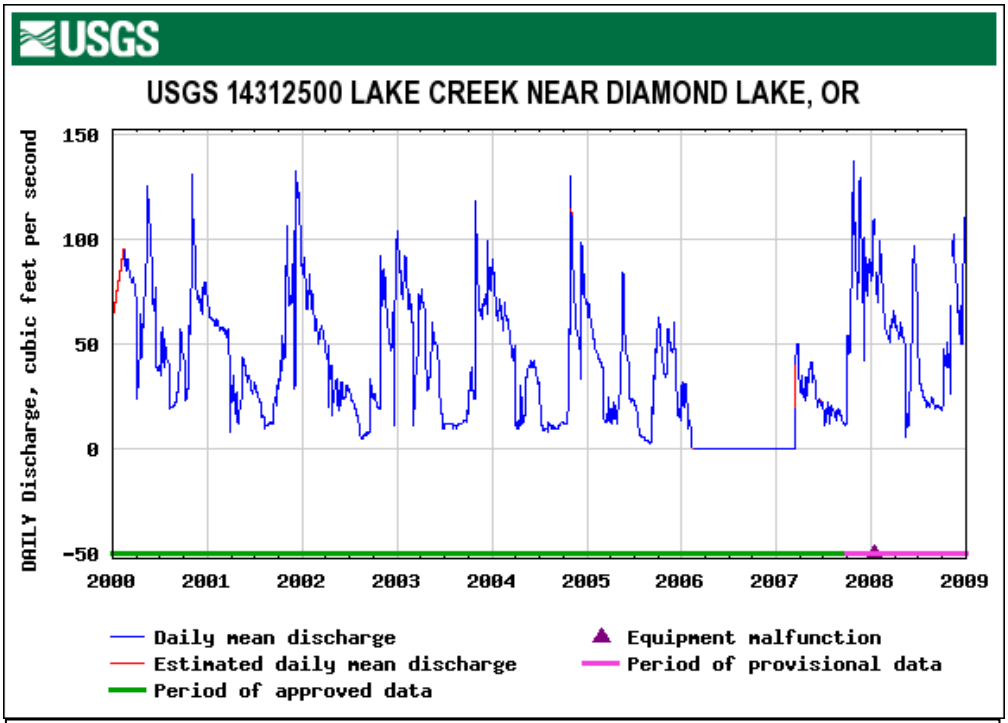

Figure 6. Discharge from Diamond Lake through Lake Creek from 2000-2008. Data provided by USGS.

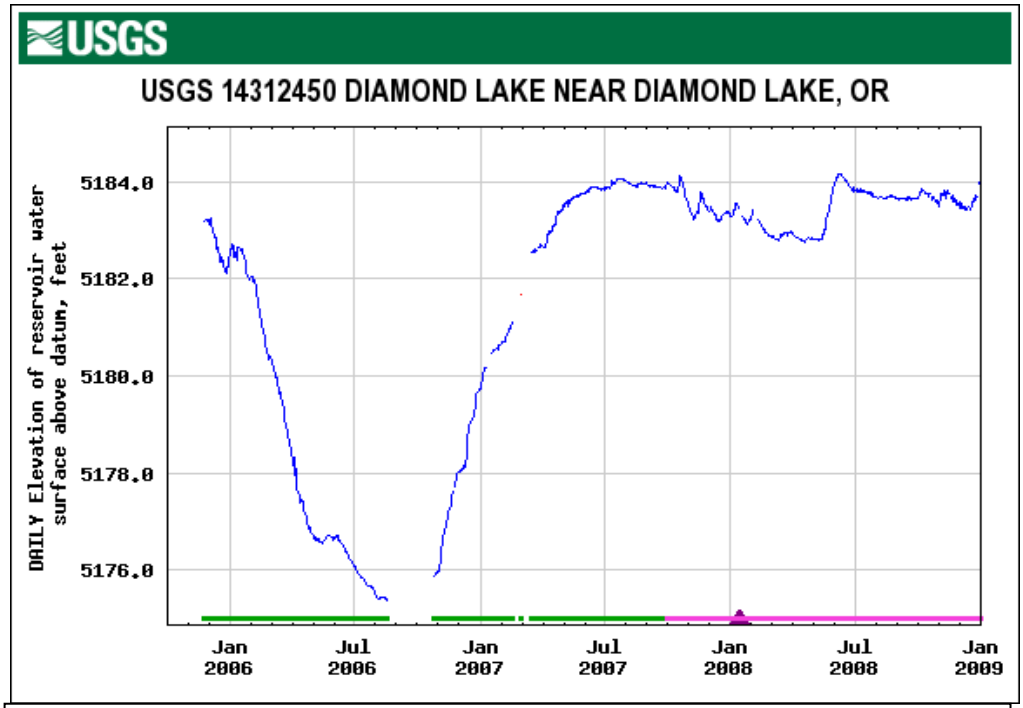

Figure 7. Surface elevation of Diamond Lake from May 2006-December 2008. Data provided by USGS.

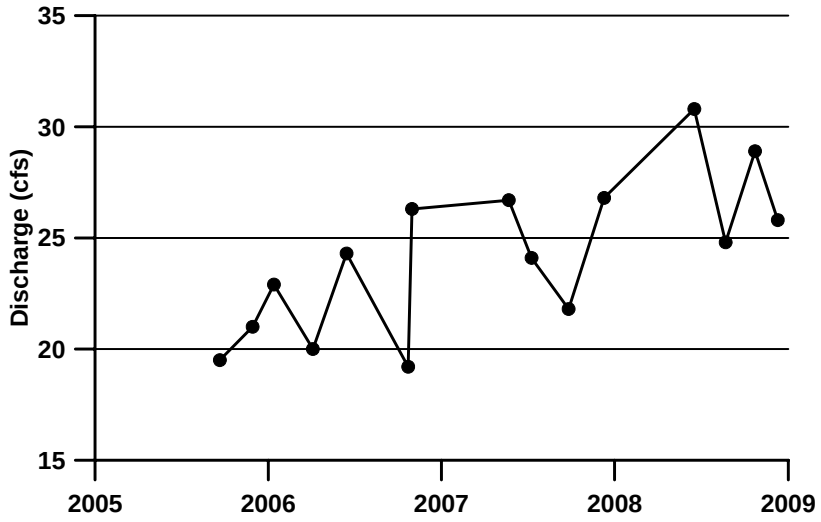

Figure 8. Discharge measurements of Silent Creek collected at FS Road 4795 by USGS from October 2005 through 2008.

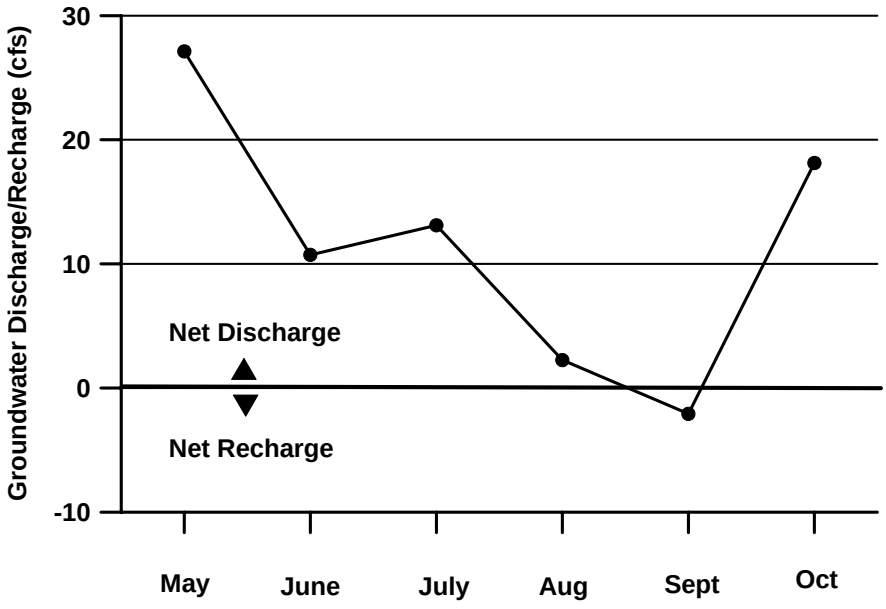

Figure 9. Groundwater discharge to Diamond Lake from May through October 2007 (after B. Eilers 2008).

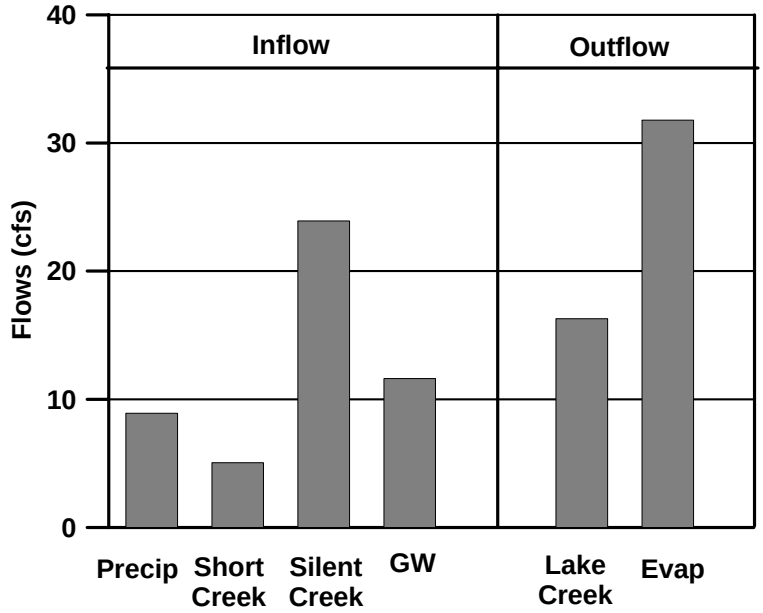

Figure 10. Water budget components of Diamond Lake for the period May-October 2007 (after B. Eilers 2008).

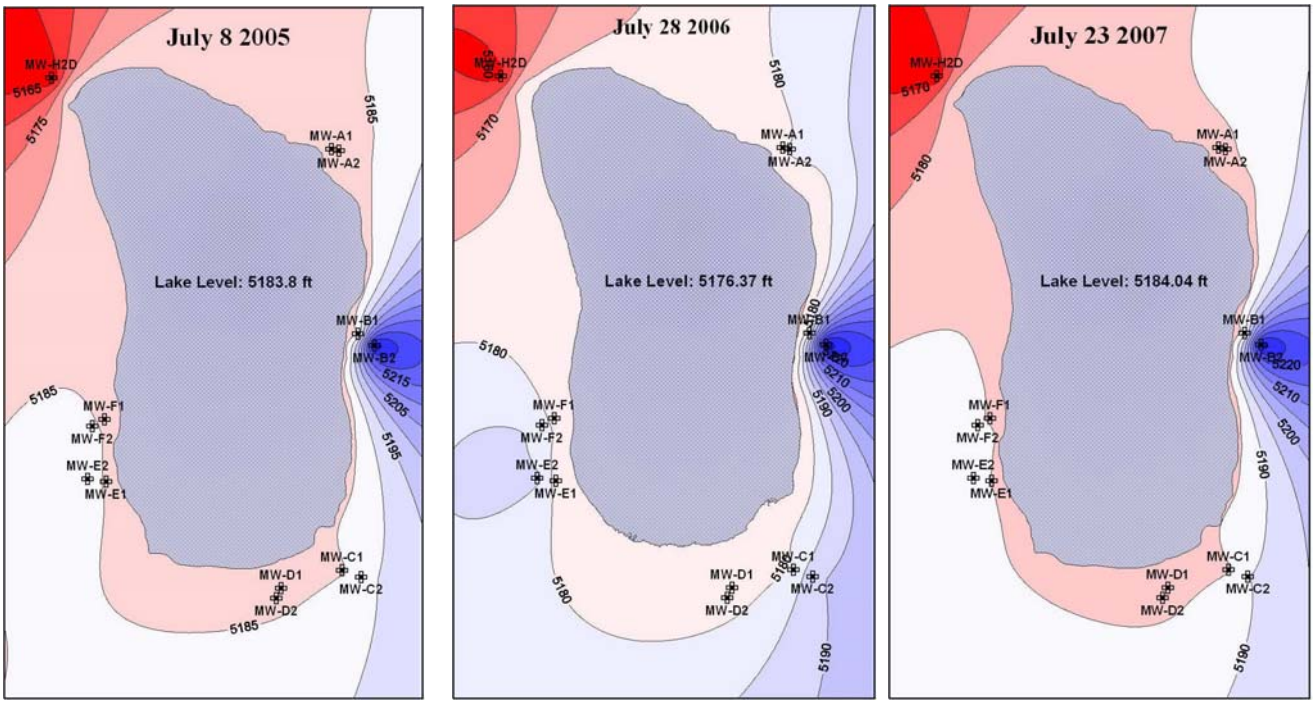

Figure 11. Ground water contours in the vicinity of Diamond Lake in July, 2005-2007 (after B. Eilers 2008).

3. Physical Lake Properties

Temperature regime is a critical component of any lake system. Diamond Lake stratifies in the summer and winter, which makes it a dimictic system. However, the degree of stratification in the summer is weak compared to deeper lakes in Oregon. This is due, in part, to its elevation where the nights are generally cool and the summer season is short. Another contributing factor is its relatively shallow depth (Z_{max} = 14.6 m) for a lake of its surface area. This was further exacerbated in summer 2006 when the lake reached its peak drawdown of about 2.6 m, producing a temporary maximum depth of less than 12 m. Despite this reduction of depth, the lake stratified in mid-June and mixed in early September (Figure 12). Although summer mean daily temperature was similar for 2006-2008 (Figure 4) the lake did not achieve a strong stratification until July in 2007 and relatively warm water (18 C) penetrated deep into the lake to create a small hypolimnion (Figure 13). Additionally, the lake remained stratified for only about six weeks before mixing in mid-August. The stratification returned to a more typical pattern in 2008, although again, the lake mixed in August (Figure 14). When the plots for all three years are combined, it's easy to visualize the periods of summer and winter stratification (Figure 15). Surface temperatures follow a sinusoidal pattern from year to year (Figure 16).

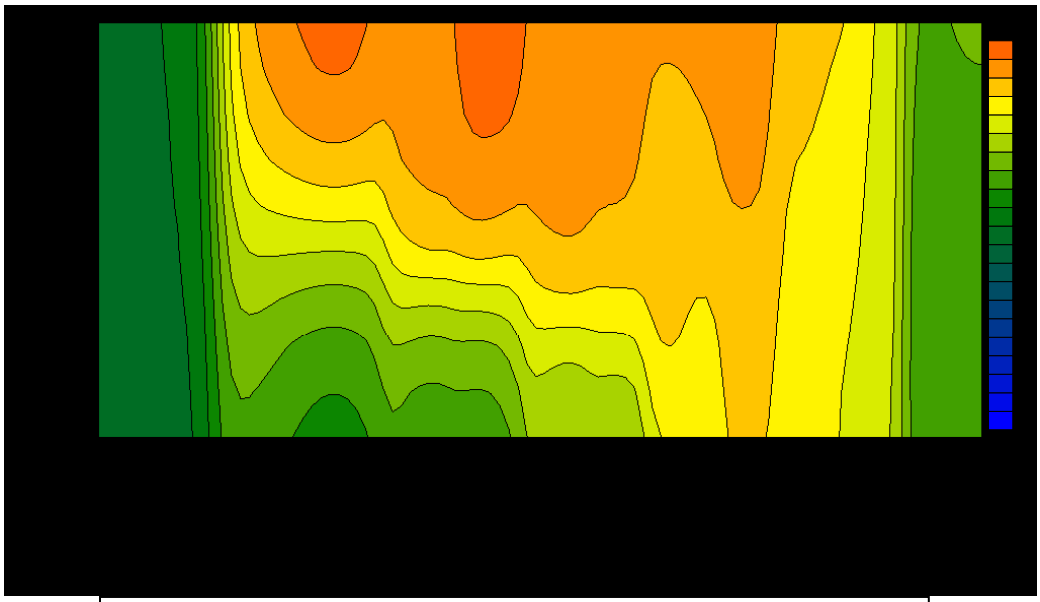

Figure 12. Diamond Lake water temperature from May 2006 to October 2006.

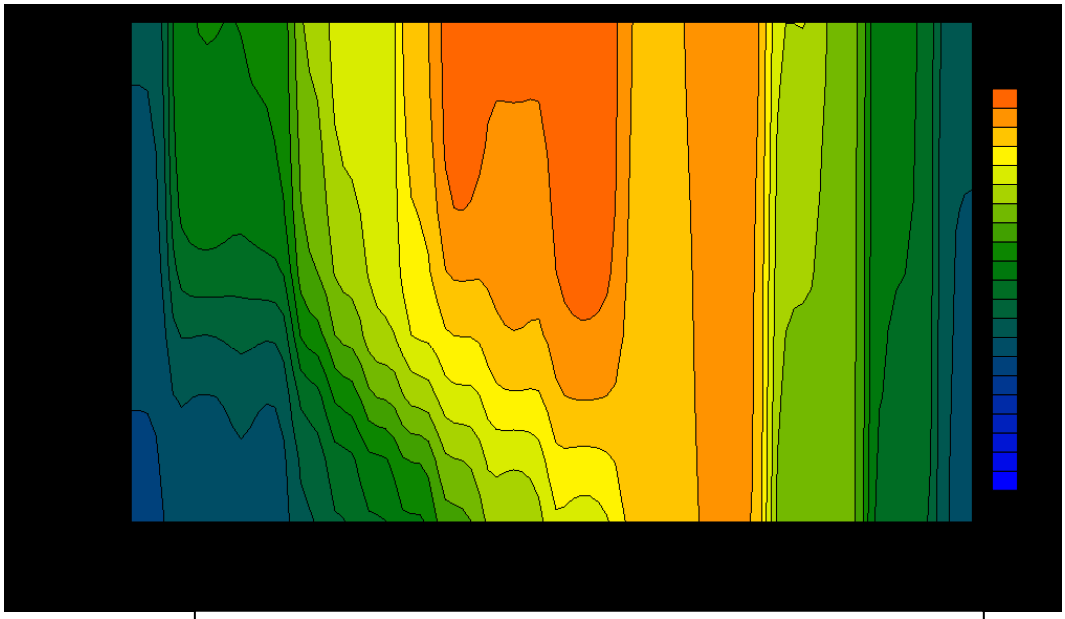

Figure 13. Diamond Lake water temperature from May 2007 to October 2007.

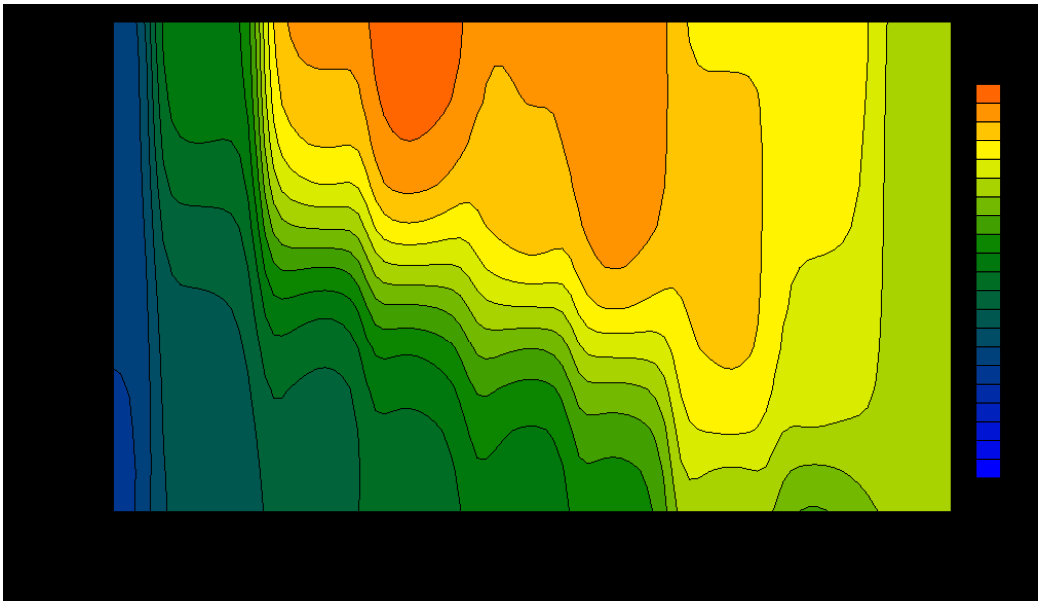

Figure 14. Diamond Lake water temperature from June 2008 to October 2008.

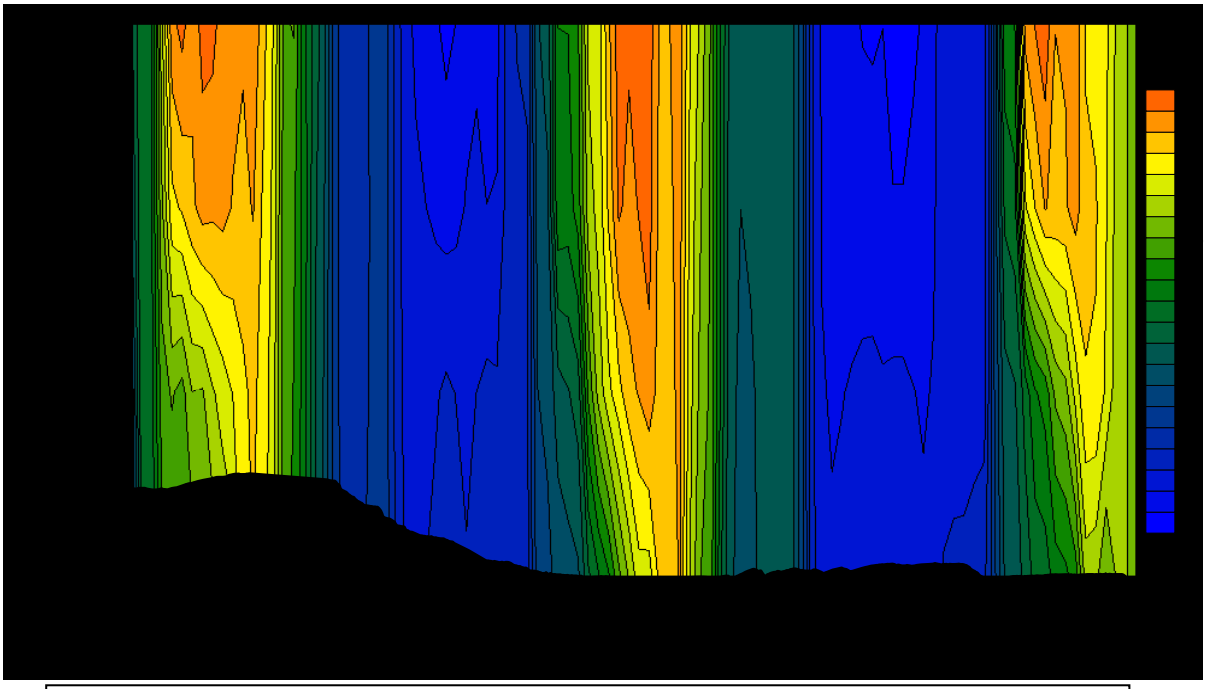

Figure 15. Diamond Lake water temperature from May 2006 to October 2008.

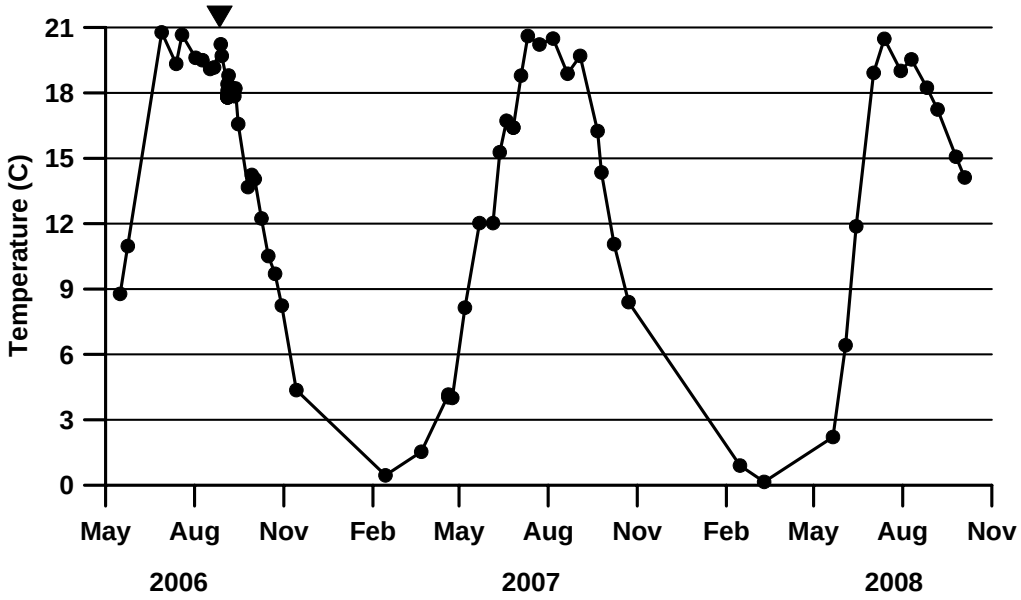

Figure 16. Water temperature at 1 meter depth from May 2006 to October 2008. The inverted triangle (▼) at the top of the figure indicates the application of the rotenone treatment. This convention is repeated in many of the subsequent figures.

The depth of light penetration declined throughout the summer and fall of 2006, reaching a minimum in November following the rotenone treatment (Figure 17). Light extinction increased rapidly in the spring of 2007, reaching a maximum in early July. At this point, sufficient light was present to allow plant growth at all depths in Diamond Lake. Light extinction declined through summer and fall, but the pattern of lower light penetration in the spring and maximum light penetration was achieved in summer 2008. Secchi disk transparency followed a similar pattern observed for light extinction (Figure 18), although Secchi disk transparency is subject to interferences (clouds, waves, different algal types) that do not influence the measurements of light extinction.

A more data-rich set of Secchi disk transparency measurements was made available by weekly observations recorded by staff with Diamond Lake Resort during much of the open-water period (Figure 19 and Figure 20). These measurements were started following the first major *Anabaena* bloom that was documented in July 2001. The results illustrate a typical pattern of a spring bloom of *Synedra* followed by alternating population pulses of *Anabaena* spp. and *Fragilaria crotonensis* that caused the transparency to fluctuate 3 to 5 meters within the open-water from 2002-2005. That pattern was altered in 2006 when the maximum draw down was achieved, tui chub were being harvested prior to treatment, and the rotenone treatment was applied in September 2006. This led to reduced densities of *Anabaena* in the summer of 2006, but a major increase in *Anabaena* following the treatment. The phytoplankton dynamics are discussed in greater detail later in the report.

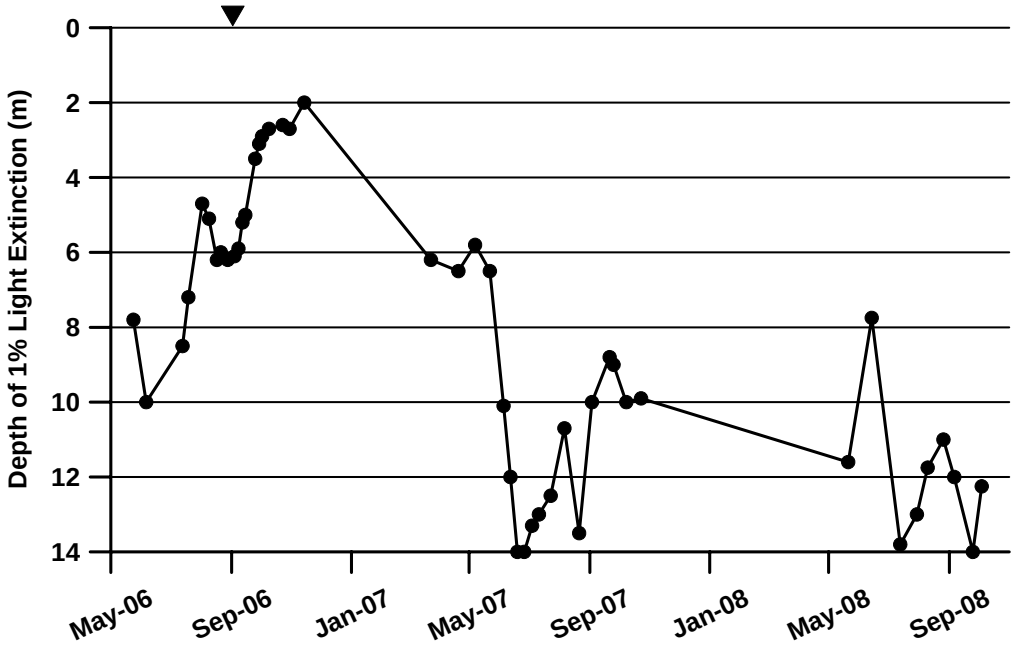

Figure 17. Depth of 1 percent light extinction from May 2006 to October 2008.

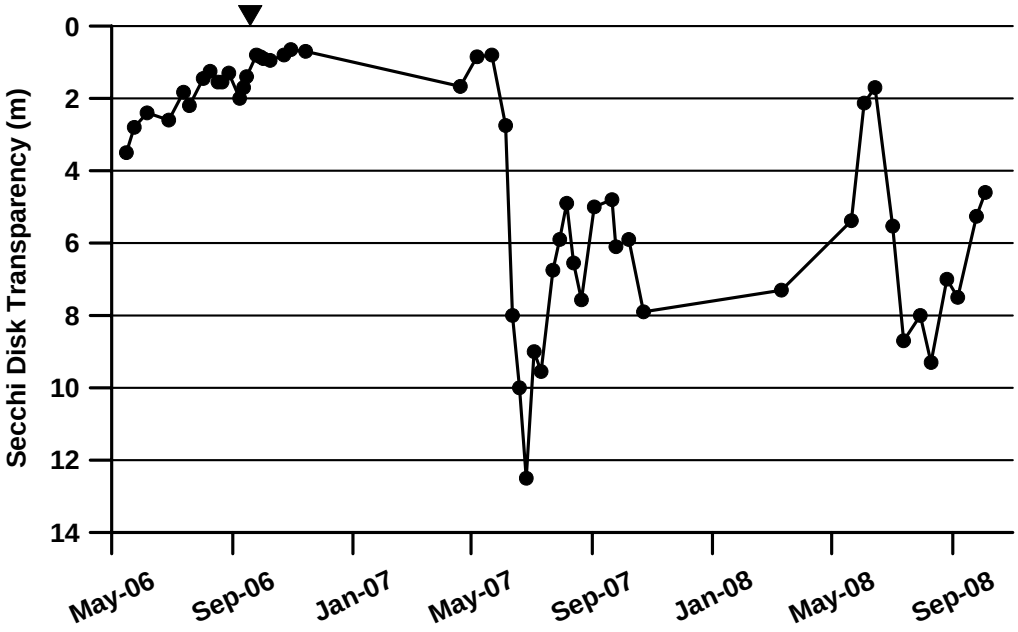

Figure 18. Secchi disk transparency in Diamond Lake from May 2006 to October 2008.

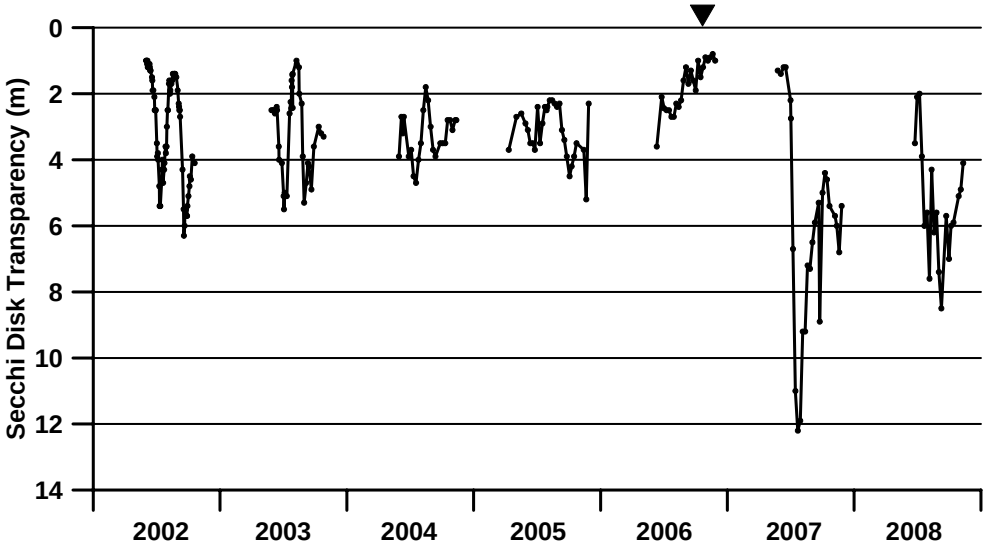

Figure 19. Secchi disk transparency in Diamond Lake from 2002 to 2008. Data courtesy Diamond Lake Resort.

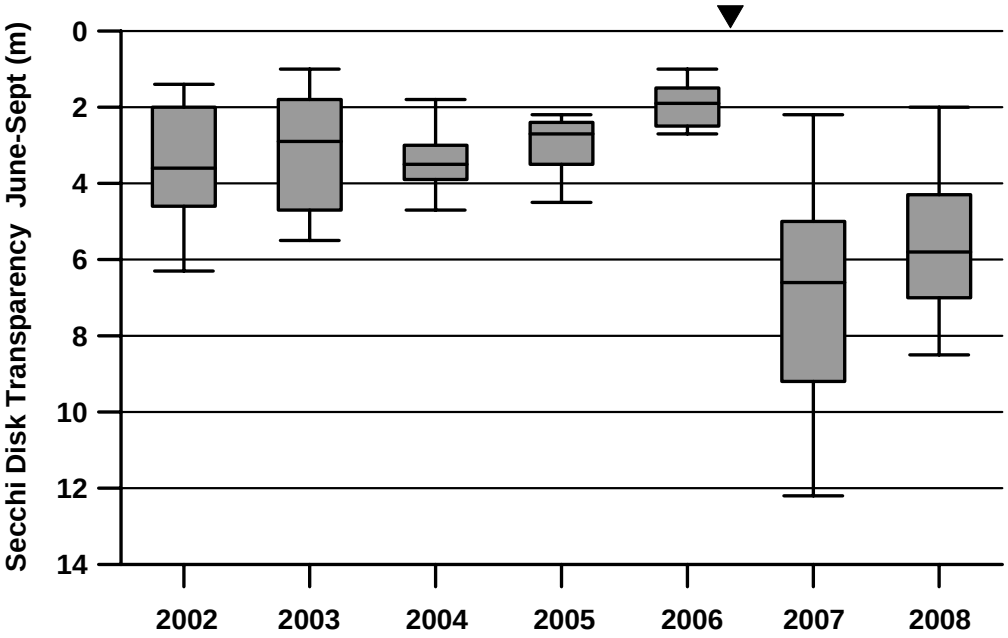

Figure 20. Secchi disk transparency from 2002 through 2008 summarized as boxplots. Data courtesy of Diamond Lake Resort.

Summary statistics for temperature, Secchi disk transparency, and light extinction are provided in

The results show no significant change in surface temperature among years, although the temperature in 2006 is significantly greater than that observed in 2008. Secchi disk transparency and light extinction both show highly significant increase during the study period.

Table 2. Analysis of variance (one-way ANOVA) for selected physical measurement of Diamond Lake from June through September, 2006-2008. Tests for temperature are based on measurements from 1 meter.

Variable	Unit	Year	N	Mean	se	V[a]	F[b]	P	[c]△
Temperature	ºC	2006	17	18.48	0.715	≠	1.64	0.2087	=
		2007	13	17.40	0.818				
		2008	9	16.31	0.983				
Secchi Disk Transparency	m	2006	14	1.63	0.557	≠	26.5	0.0000	↑
		2007	14	7.10	0.557				
		2008	9	6.12	0.695				
Light Extinction	1% Depth (m)	2006	14	5.91	0.506	=	36.1	0.0000	↑
		2007	11	11.34	0.571				
		2008	8	11.86	0.669				

4. Water Chemistry

a. Field Measurements

pH in Diamond Lake is a critical metric with regard to the TMDL (Eilers et al. 2005) and it is recognized as a major project goal under Element 1 (Water Quality). pH in Diamond Lake through spring and fall of 2006 was well above the target limit of 8.5 (Figure 21 and Figure 22). Despite the declining water temperatures, pH continued to increase throughout the water column following the rotenone treatment in September 2006, reaching a maximum value of 10.6 at 0.3 m on October 31, 2006. pH values declined considerably in 2007, with only six observations in the summer exceeding the limit of pH 8.5 and none in 2008. Note that values in spring and fall are not included in the summer period determination of post-treatment goals for pH. None of the measured surface pH values exceeded the target goal of 8.5 in 2008.

Profiles of dissolved oxygen (DO) concentrations (Figure 23) and percent saturation (Figure 24) show a period of elevated values immediately following the rotenone treatment in 2006 and again in June of 2008. The elevated surface values (Figure 25 and Figure 26) reached a maximum of nearly 12 mg/L (140 percent) following the rotenone treatment as primary production spiked under favorable conditions of elevated nutrients and an unseasonably warm, calm fall. The spike in DO during June 2008 followed a late ice-off and an unusual bloom of *Stephanodiscus.* These conditions are described in greater detail in the phytoplankton section of the report.

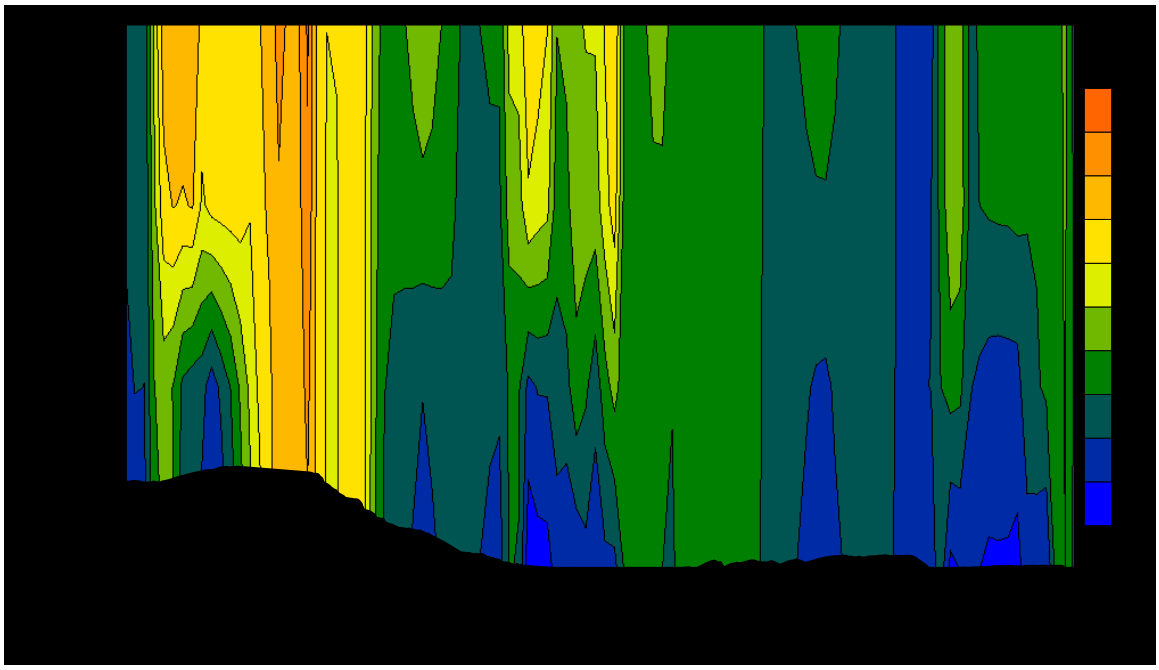

Figure 21. pH profiles in Diamond Lake from May 2006 to October 2008.

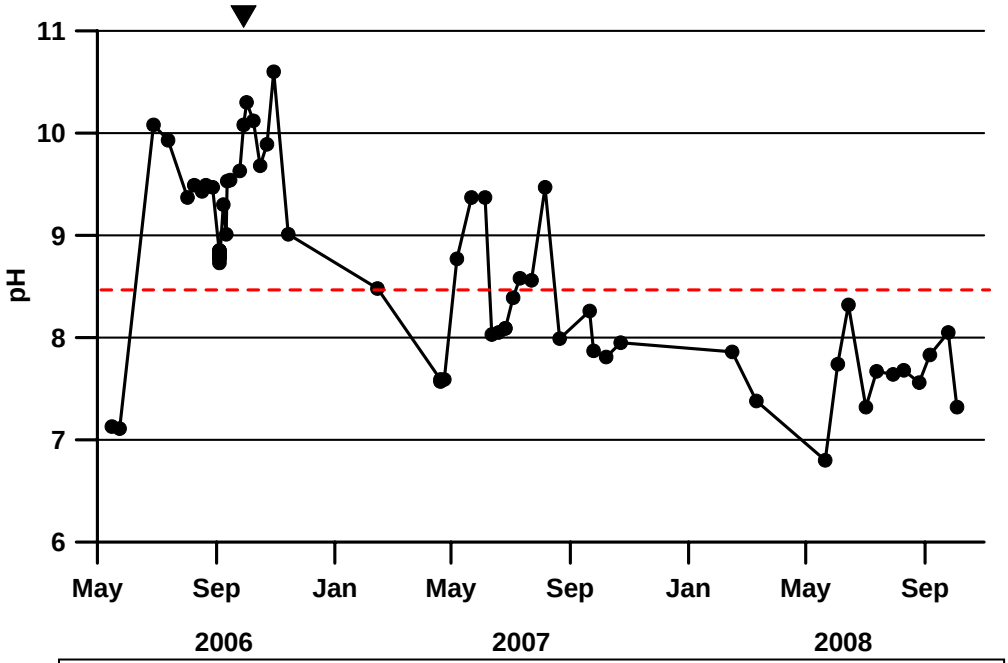

Figure 22. Field pH at a depth of 1 meter in Diamond Lake measured from May 2006 to October 2008.

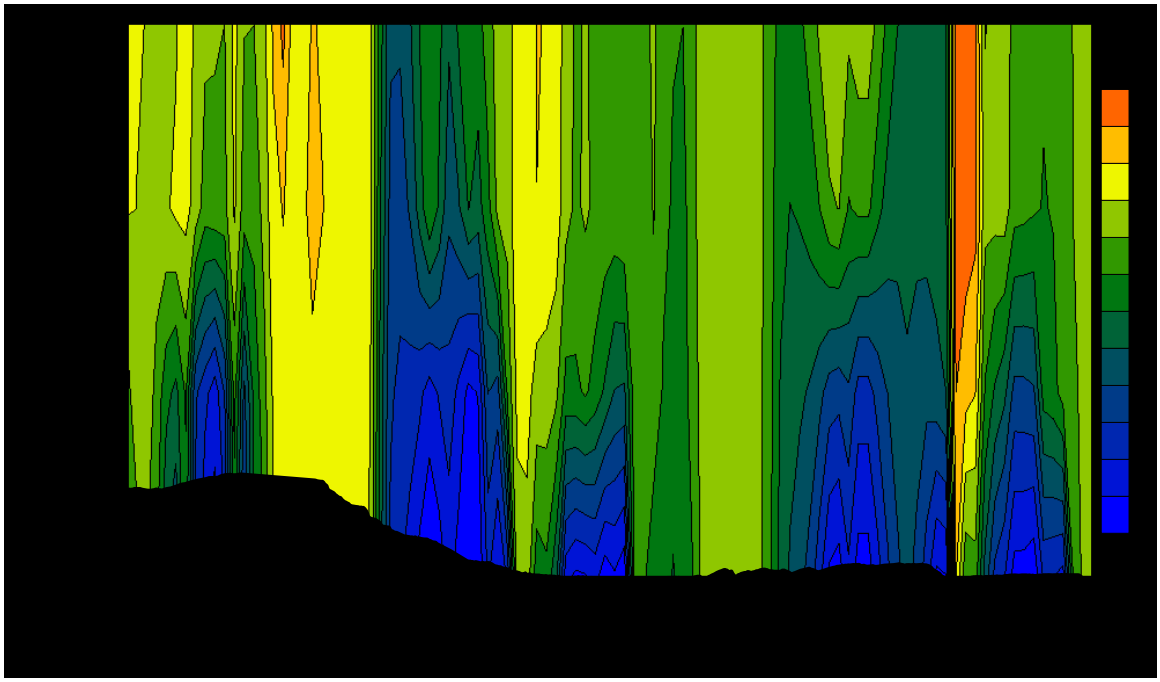

Figure 23. Dissolved oxygen concentrations (mg/L) in Diamond Lake from May 2006 to October 2008.

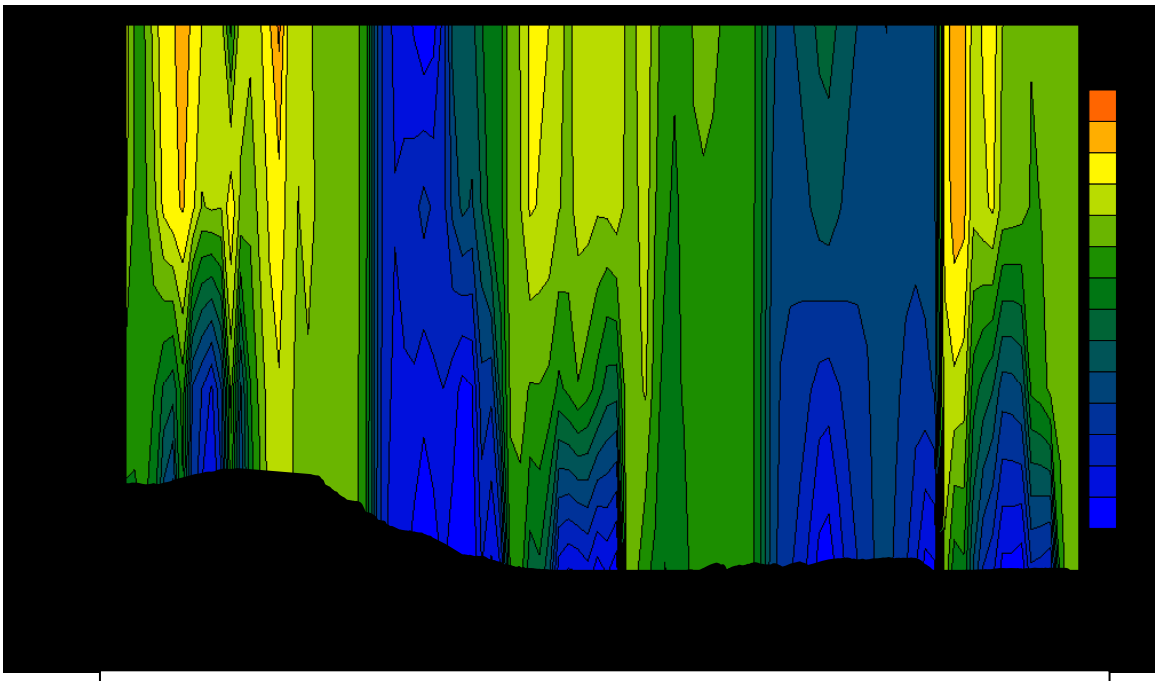

Figure 24. Dissolved oxygen profiles in Diamond Lake from May 2006 to October 2008 (expressed as percent saturation).

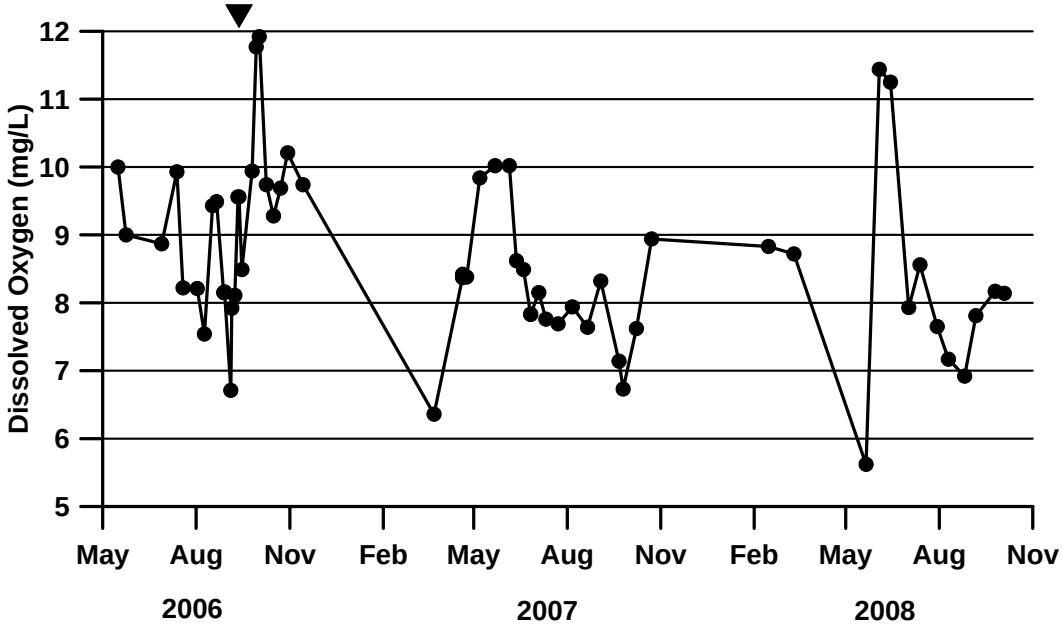

Figure 25. Dissolved oxygen concentrations (mg/L) at a depth of 1 meter in Diamond Lake from May 2006 to October 2008.

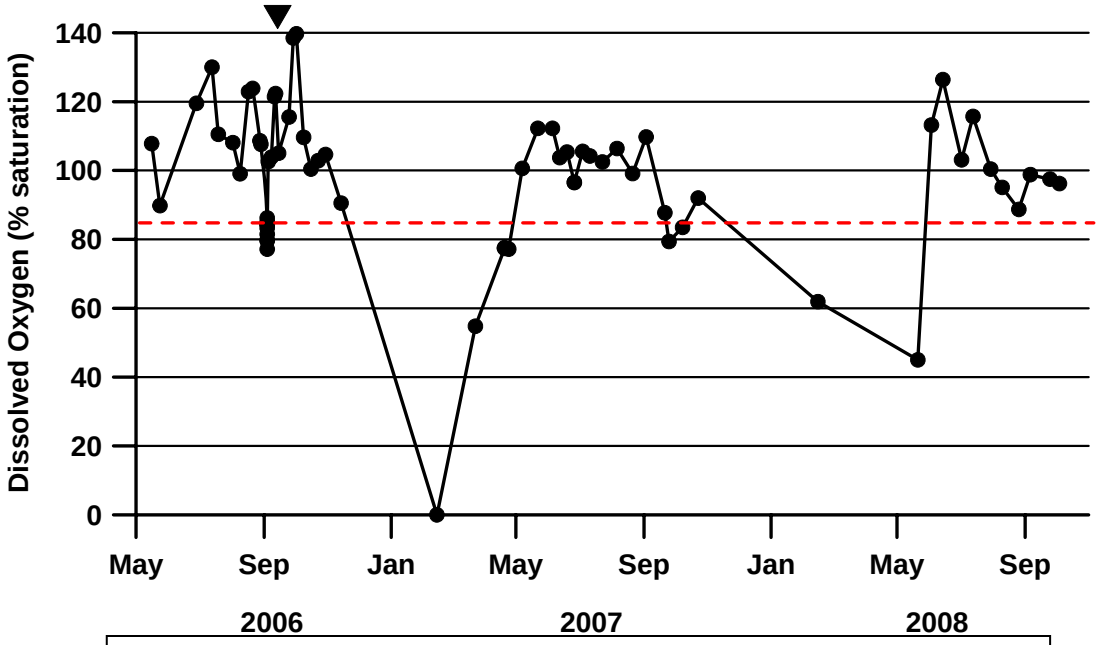

Figure 26. Dissolved oxygen (expressed as percent saturation) at a depth of 1 meter in Diamond Lake from May 2006 to October 2008.

The ANOVA results show that dissolved oxygen expressed as percent saturation showed a significant decline from 2006, whereas DO expressed in concentration units did not show a significant decline. This is attributed to the warmer water temperatures observed in 2006 (Figure 16), resulting in higher percent DO saturation for a given concentration of DO. Field pH measurements show a highly significant decline in mean values from 2006 to 2008.

Table 3. Analysis of variance for dissolved oxygen and pH values measured at a depth of 1 meter in Diamond Lake from 2006-2008.

Variable	Unit	Year	N	Mean	se	V[a]	F[b]	P	△
Dissolved Oxygen	% Sat	2006	17	113.3	2.77	=	4.78	0.0144*	⇩
		2007	13	100.7	3.17				
		2008	9	104.3	3.81				
Dissolved Oxygen	mg/L	2006	17	8.83	0.291	=	1.71	0.1958	=
		2007	13	8.01	0.333				
		2008	9	8.54	0.400				
Field pH	su	2006	14	9.51	0.110	=	54.6	0.0000**	⇩
		2007	12	8.40	0.118				
		2008	9	7.76	0.137				

b. Laboratory Measurements

Laboratory pH values also show a decline from 2006 to 2007 (Figure 27). However, the laboratory values would have had sufficient opportunity to de-gas carbon dioxide, resulting in lower pH values compared to the in-situ measurements (Figure 22). Concentrations of alkalinity in Diamond Lake show a dilution in spring following ice-out and an increase through the summer months (Figure 28). This is consistent with the influx of groundwater observed later in the spring (Figure 9), continuing inputs of alkalinity from the tributaries, and evapoconcentration of solutes in the lake through summer and early fall.

Silica shows no consistent pattern among the three years (Figure 29). Although most silica enters Diamond Lake from its tributaries, just as with alkalinity, silica has a major loss term attributed to use of silica by diatoms and chrysophytes and burial in the sediments.

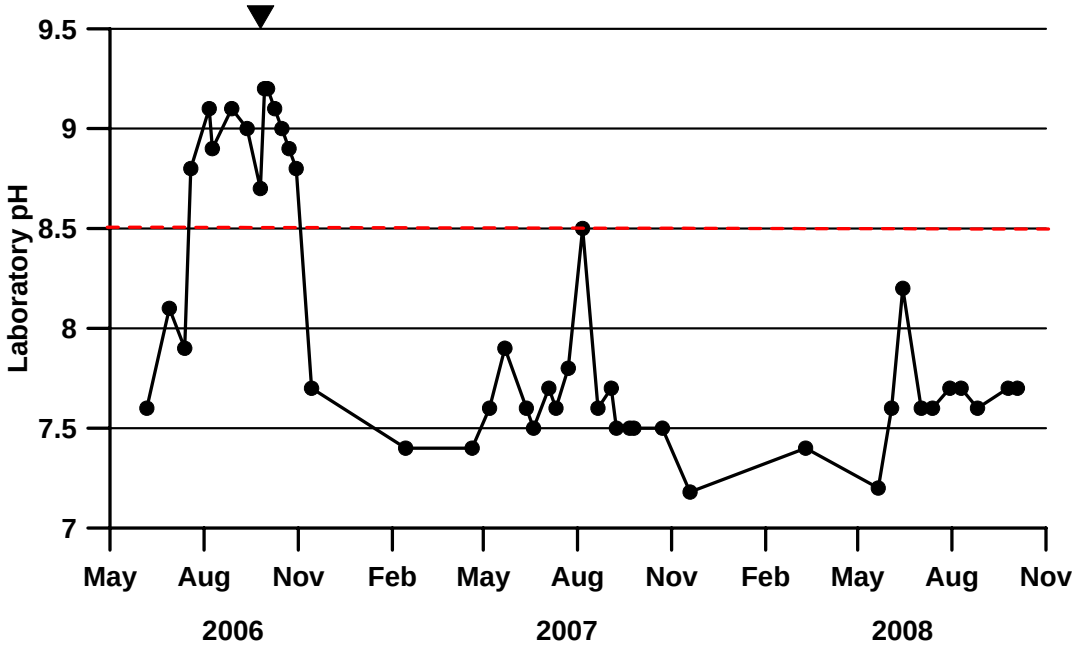

Figure 27. Laboratory pH for samples collected at a depth of 1 meter in Diamond Lake from May 2006 to October 2008.

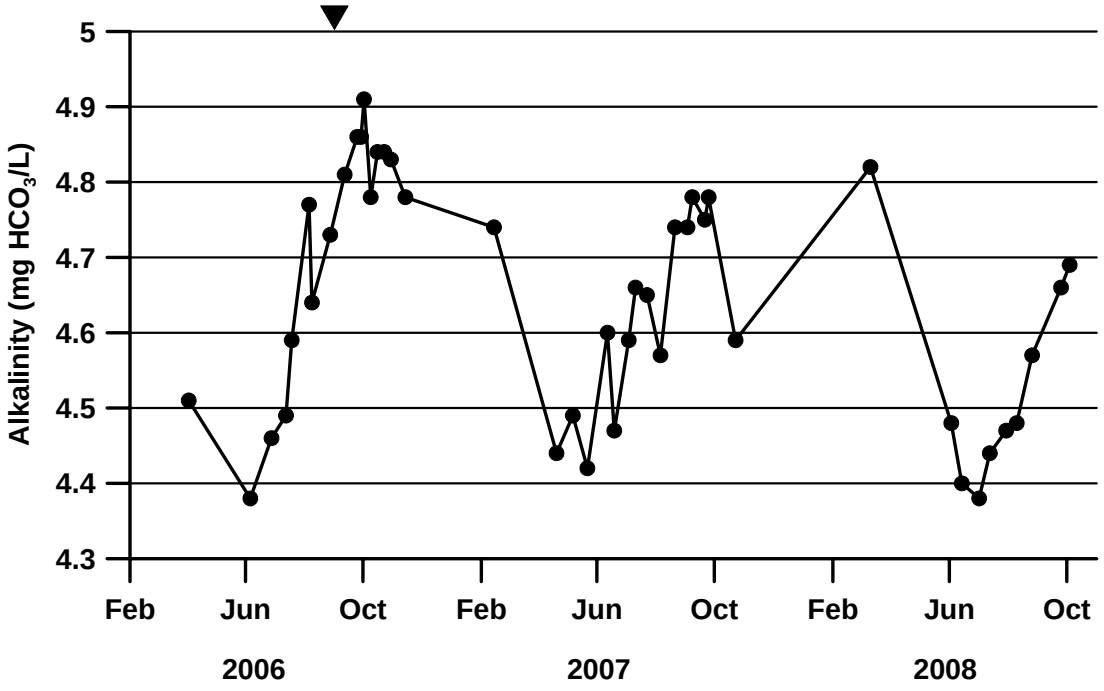

Figure 28. Alkalinity at a depth of 1 meter in Diamond Lake from May 2006 to October 2008.

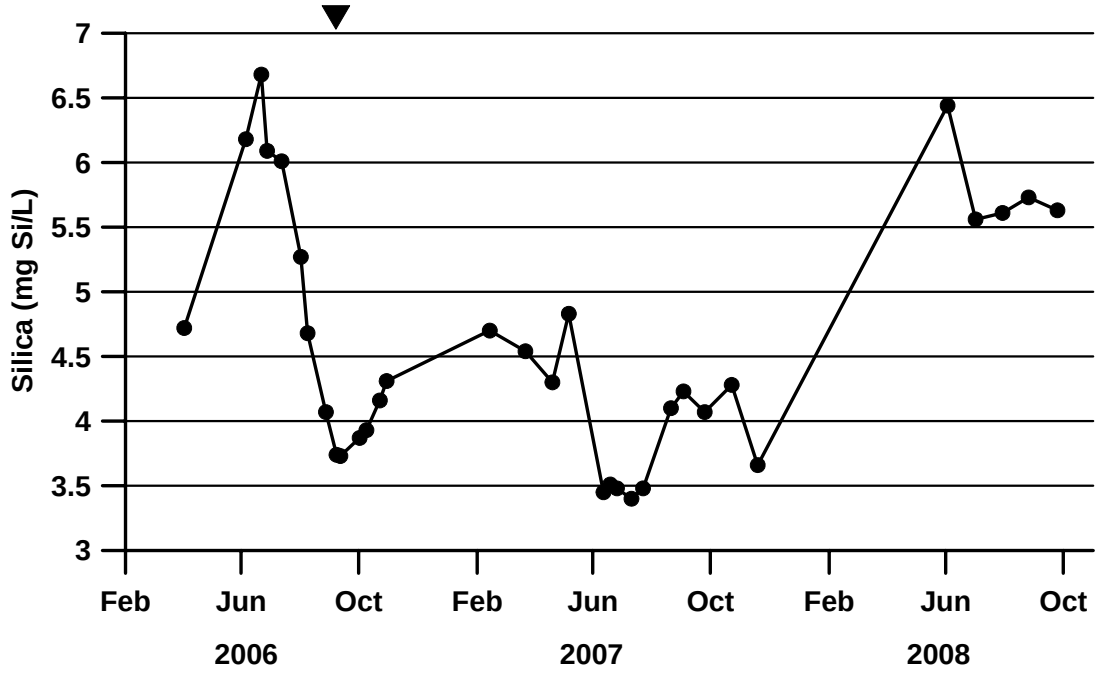

Figure 29. Silica at a depth of 1 meter in Diamond Lake from May 2006 to October 2008.

Total phosphorus (TP) concentrations varied considerably among the three years, with few repeating patterns (Figure 30). Summer TP values were generally from 20 to 30 μg/L, but usually showed large variations during fall mixis or spring mixis after ice-out. Total phosphorus increased only slightly after fall mixis in 2006, but concentrations increased about 20 µg/L in the two months following the rotenone treatment. Surface TP concentrations declined steadily through winter 2006-2007, increased during ice-out and mixis and then declined to values of circa 20 µg/L in summer 2007. Concentrations of TP increased nearly 20 µg/L as the lake de-stratified in August 2007. Maximum surface TP values were recorded in June 2008 when values approached 60 µg/L. These elevated values declined rapidly with the onset of stratification to values about 23 µg/L during the summer of 2008, rising once again during fall mixis. Concentrations of TP at mid-depth were generally similar to those measured at the surface with one notable exception in late July 2007 where mid-depth concentrations rose to values intermediate between those at the surface and bottom values (Figure 31). Concentrations of TP in the bottom waters increased to values nearly an order of magnitude greater than those measured at the surface under winter and summer stratification. The greatest concentrations of TP were recorded during summer stratification of 2007 and winter stratification of 2008.

Concentrations of ortho-phosphorus (PO_4) showed no change during the summers of 2006-2008, however the surface concentrations of PO_4 reached a maximum measured value of nearly 30 µg/L during ice-out of 2008 (Figure 32). Despite this spike, PO_4 declined rapidly in June 2008 and remained near detection limits throughout the summer. Concentrations of PO_4 in the bottom waters reached nearly two orders of magnitude greater concentrations than those observed at the surface (Figure 33). Again, the concentrations of PO_4 in the bottom waters increased in 2007 and 2008 compared to 2006.

Concentrations of total nitrogen (TN) in the surface waters declined from 2006 to 2008 (Figure 34). Concentrations of TN reached a peak in November 2006 of over 900 µg/L, two months following the rotenone treatment and declined steadily to June 2007. TN values increased slightly in the summers of 2007 and 2008, but the overall trend was downwards. Concentrations of TN at mid-depth in the water column were very similar to those measured at the surface, however, those measured in the bottom waters showed large increases during periods of stratification (Figure 35). The peaks in TN have remained fairly steady throughout the three study years at TN values over 1 mg/L.

Concentrations of ammonia remained low in the surface waters during summer periods, but showed large increases under the ice and following the early mixis in August 2007 (Figure 36). This large increase in ammonia under the ice was not observed in winter/spring 2006, prior to the rotenone

treatment. The peaks of winter 2007 and 2008 produced similarly high concentrations of ammonia at about 260-270 µg/L. Concentrations of ammonia in the mid-depths were again very similar to those measured at the surface (Figure 37). However, ammonia concentration in the bottom waters were nearly four times greater than those measured at the surface, thus accounting for much of the total nitrogen observed during these periods (Figure 35).

Concentrations of nitrate were largely below detection limits until September 2007 when they began to increase (Figure 38). Nitrate concentrations peaked in March 2008 under the ice and declined rapidly with ice-out and mixis in June 2008. Concentrations of nitrate at mid-depth approached those at the surface, whereas nitrate concentrations in the bottom waters were usually quite low (Figure 39).

The ratio of TN to TP in the surface waters of Diamond Lake averaged near 20 in 2006, 15 in 2007 and 10 in 2008 (Figure 40). Only briefly in summer 2006 and 2008 did values of TN:TP decline below the Redfield ratio of 7.2 (Redfield 1934). Most of the decrease in the TN:TP ratio is attributed to a decline in the concentration of nitrogen (Figure 34) rather than any significant changes in phosphorus concentrations.

Total organic carbon (TOC) declined from 2006-2008, with some slight deviations in the pattern (Figure 41). The decline in TOC is approximately the same magnitude as observed for TN.

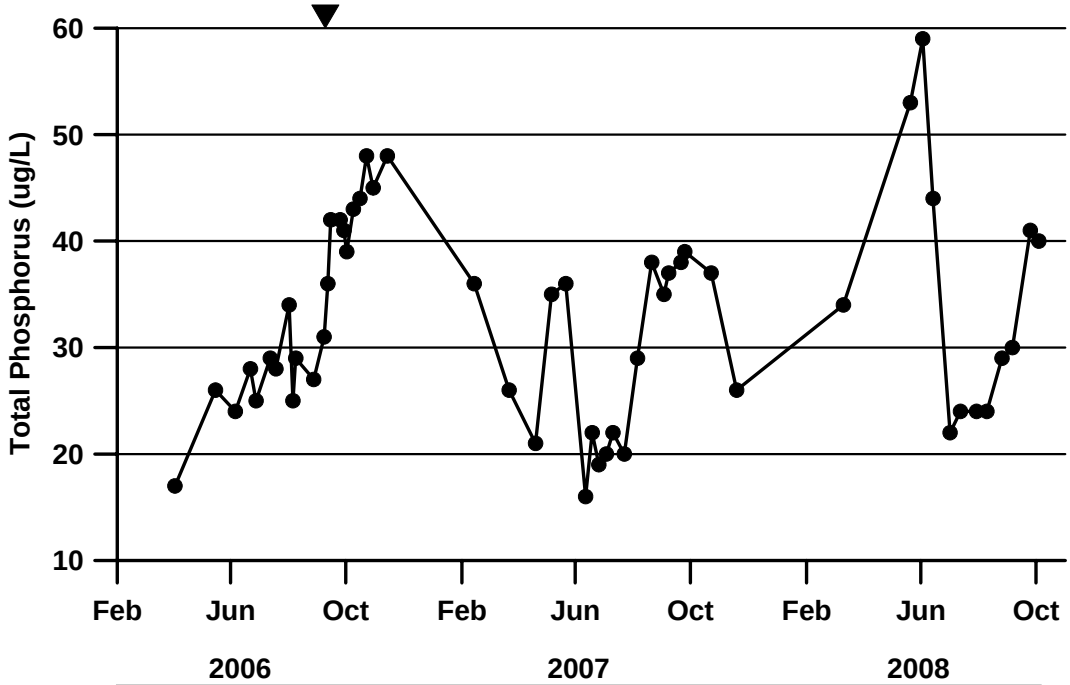

Figure 30. Total phosphorus at a depth of 1 meter in Diamond Lake from May 2006 to October 2008.

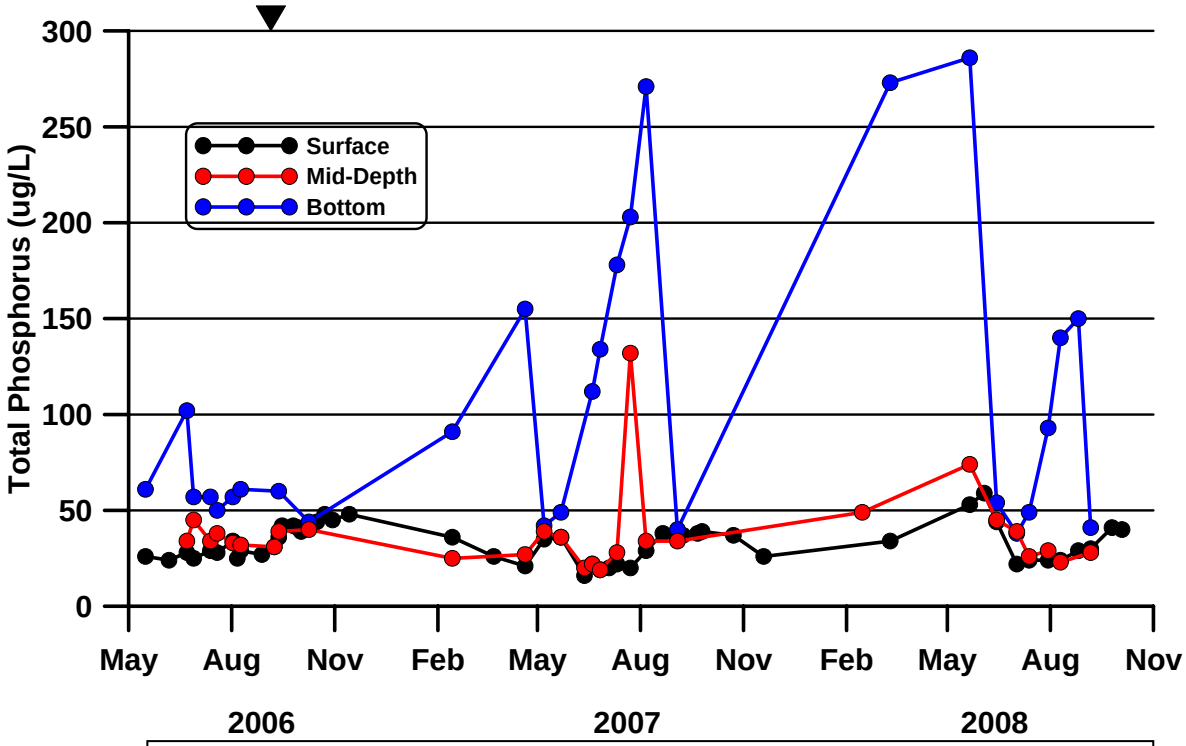

Figure 31. Total phosphorus at surface (1 m), mid-depth, and above the bottom in Diamond Lake from 2006-2008.

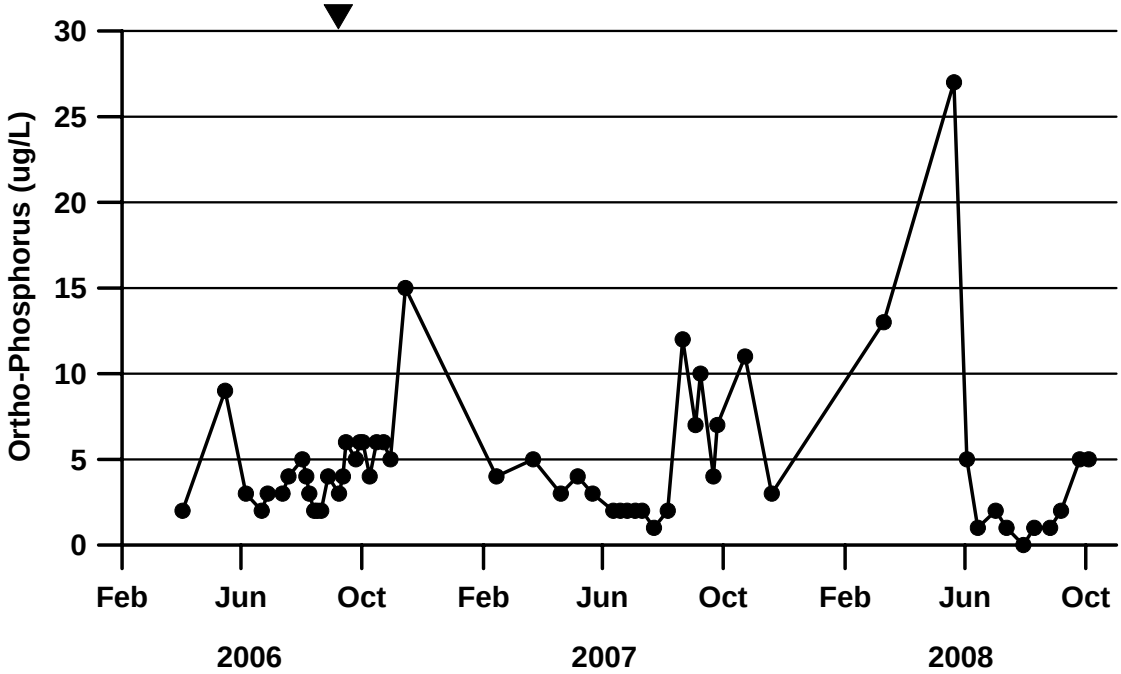

Figure 32. Ortho-phosphorus concentrations at a depth of 1 meter in Diamond Lake from May 2006 to October 2008.

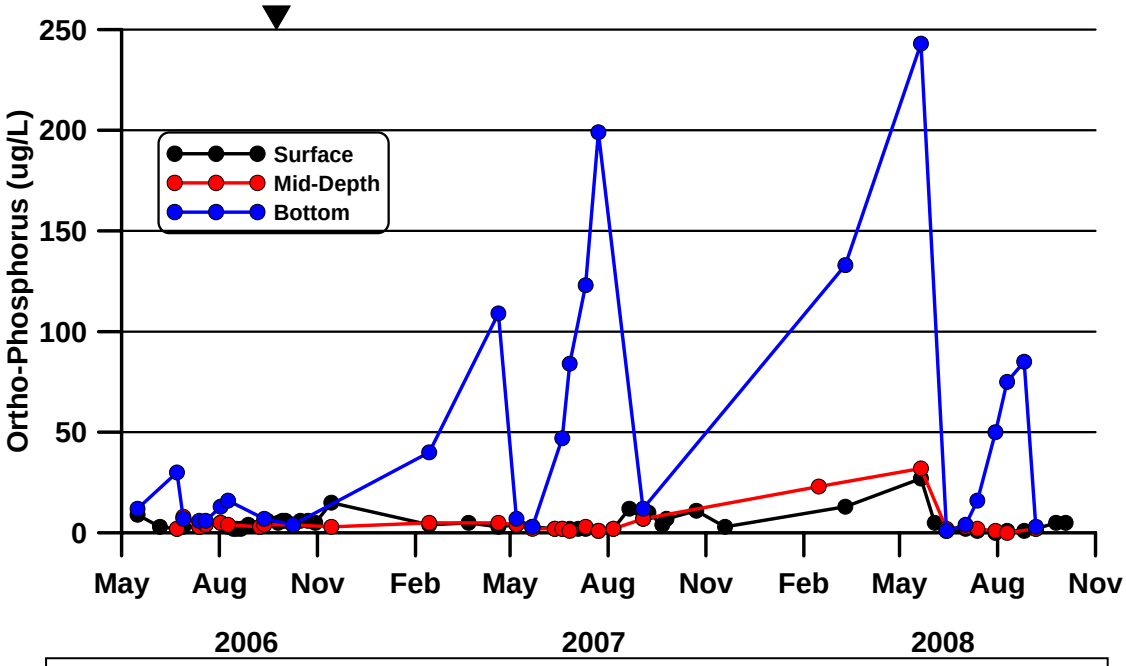

Figure 33. Ortho-phosphorus at the surface, mid-depth, and bottom levels in Diamond Lake, 2006-2008.

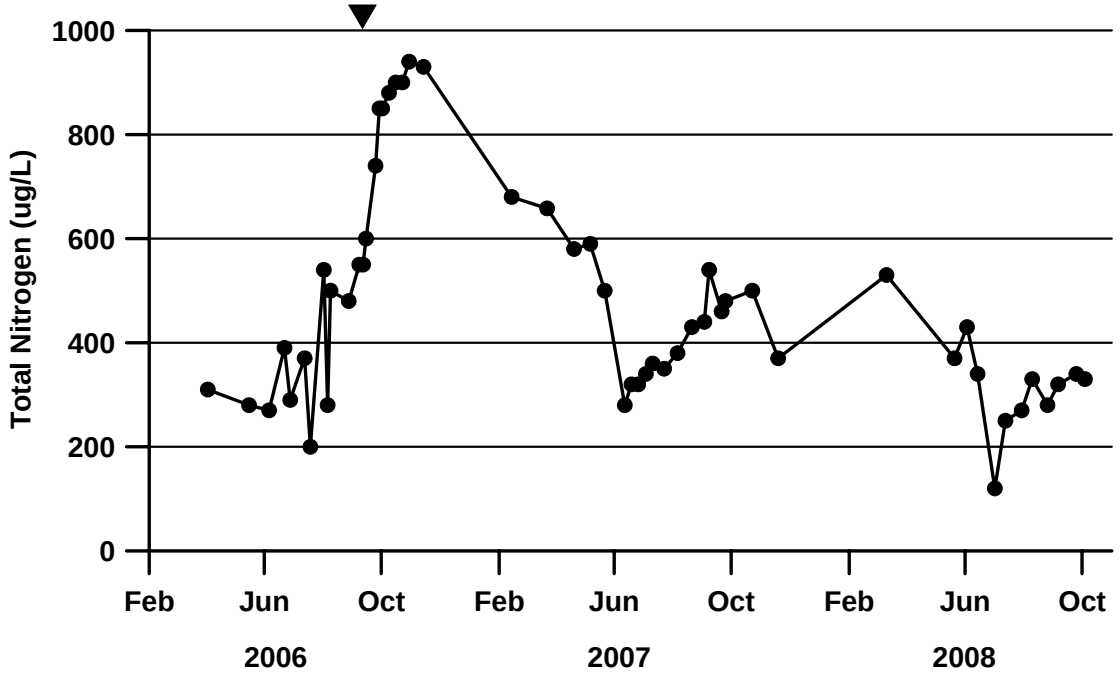

Figure 34. Total nitrogen at a depth of 1 meter in Diamond Lake from May 2006 to October 2008.

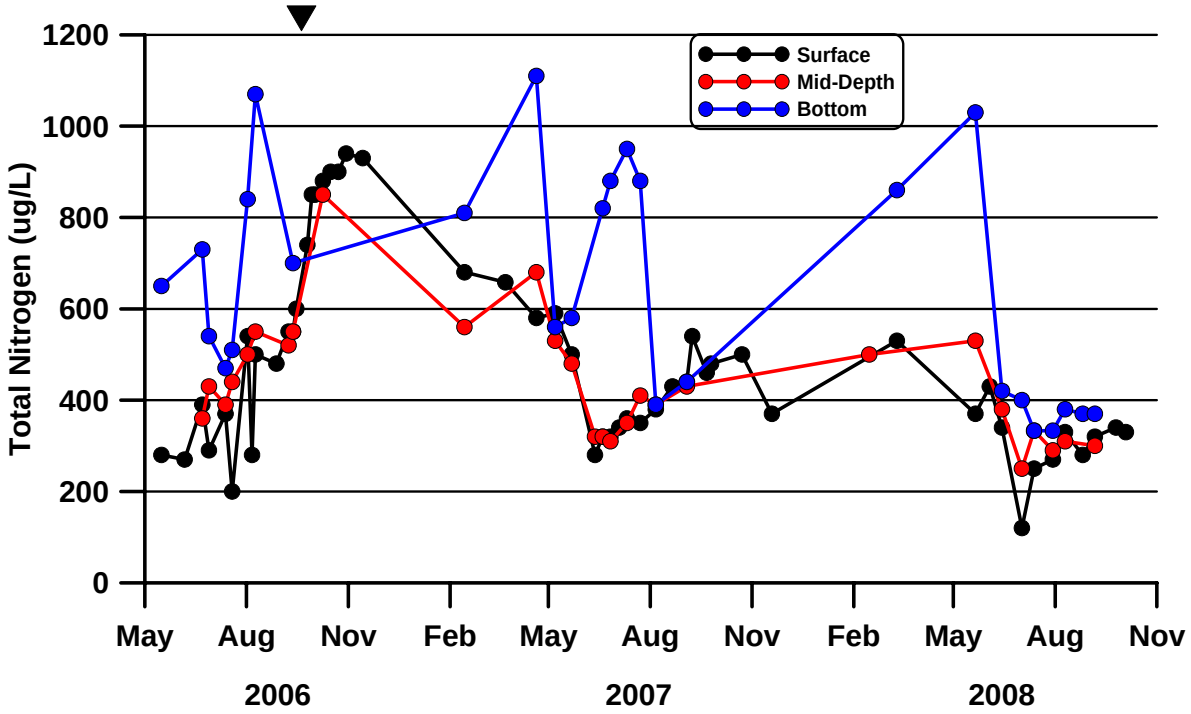

Figure 35. Total nitrogen at surface, mid-depth, and bottom water of Diamond Lake, 2006-2008.

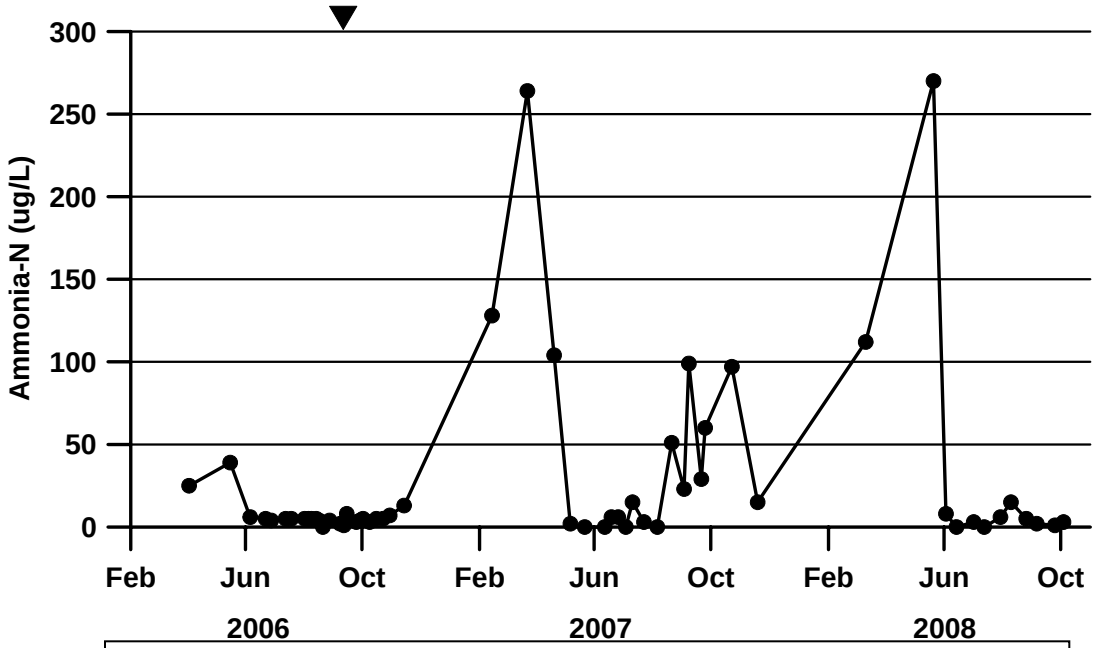

Figure 36. Ammonia concentrations at a depth of 1 meter in Diamond Lake from May 2006 to October 2008.

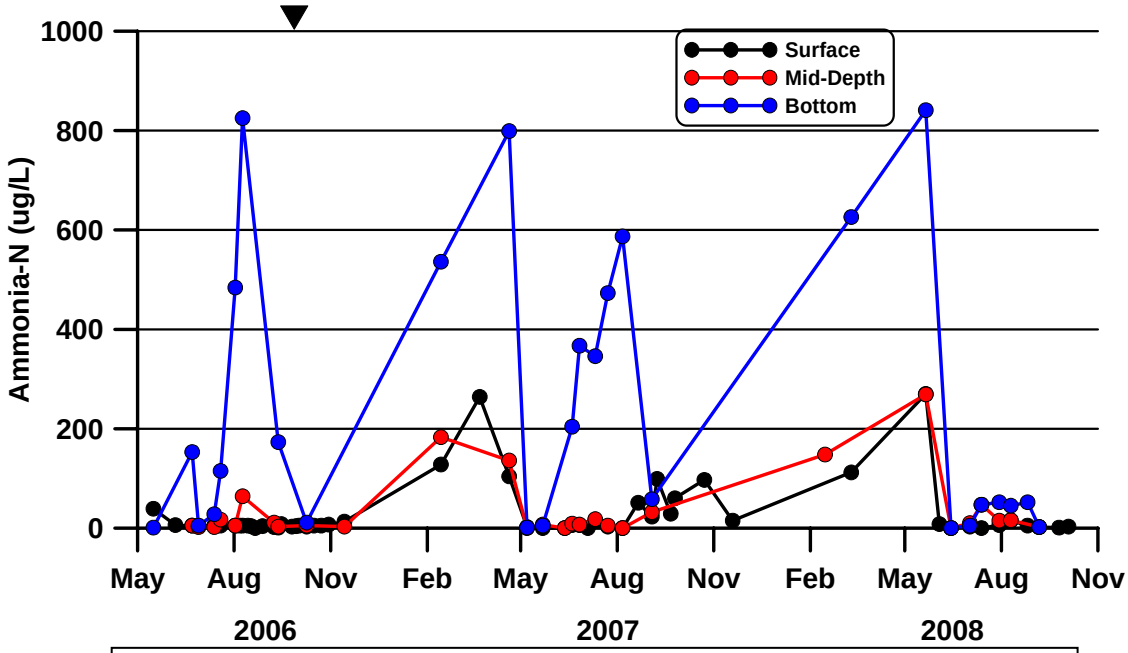

Figure 37. Ammonia concentrations at the surface, mid-depth, and bottom waters of Diamond Lake, 2006-2008.

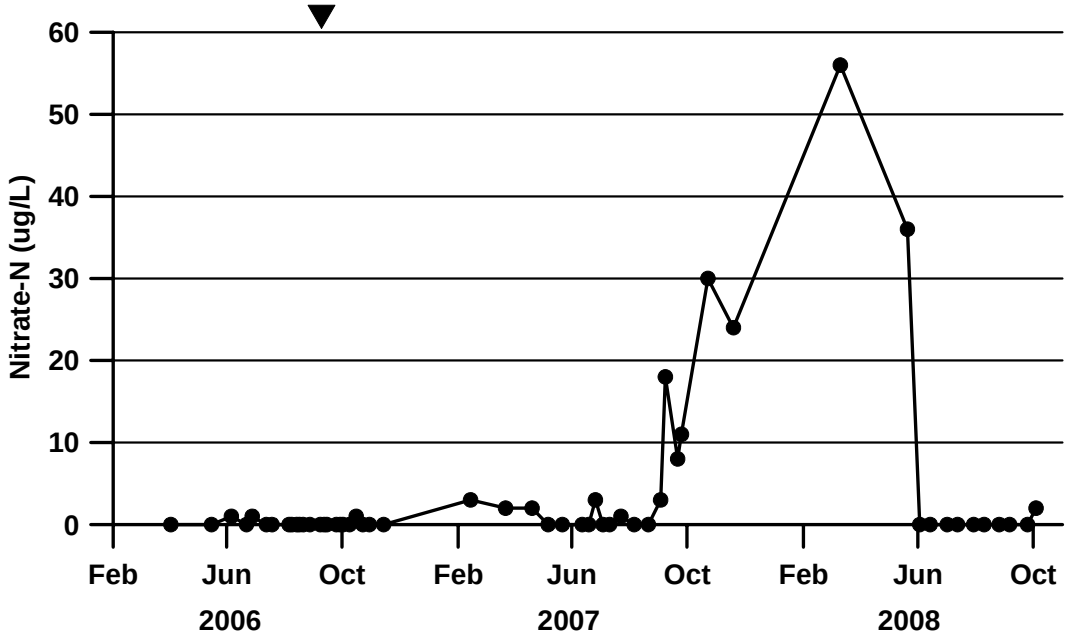

Figure 38. Nitrate concentrations at a depth of 1 meter in Diamond Lake from May 2006 to October 2008.

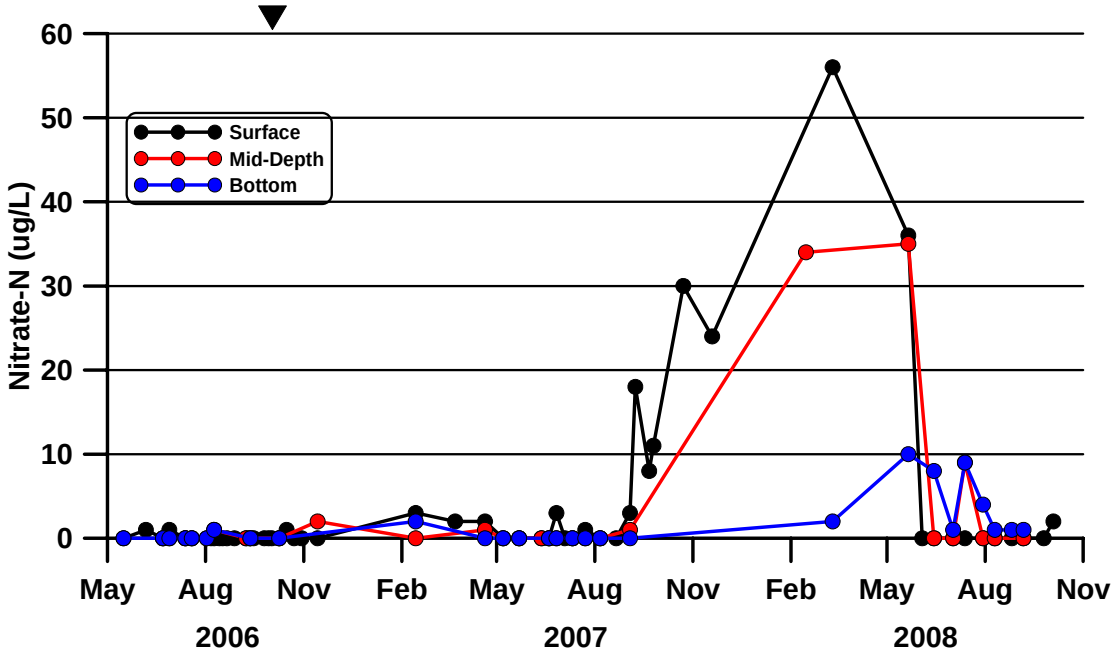

Figure 39. Nitrate concentrations in the surface, mid-depth and bottom waters of Diamond Lake, 2006-2008.

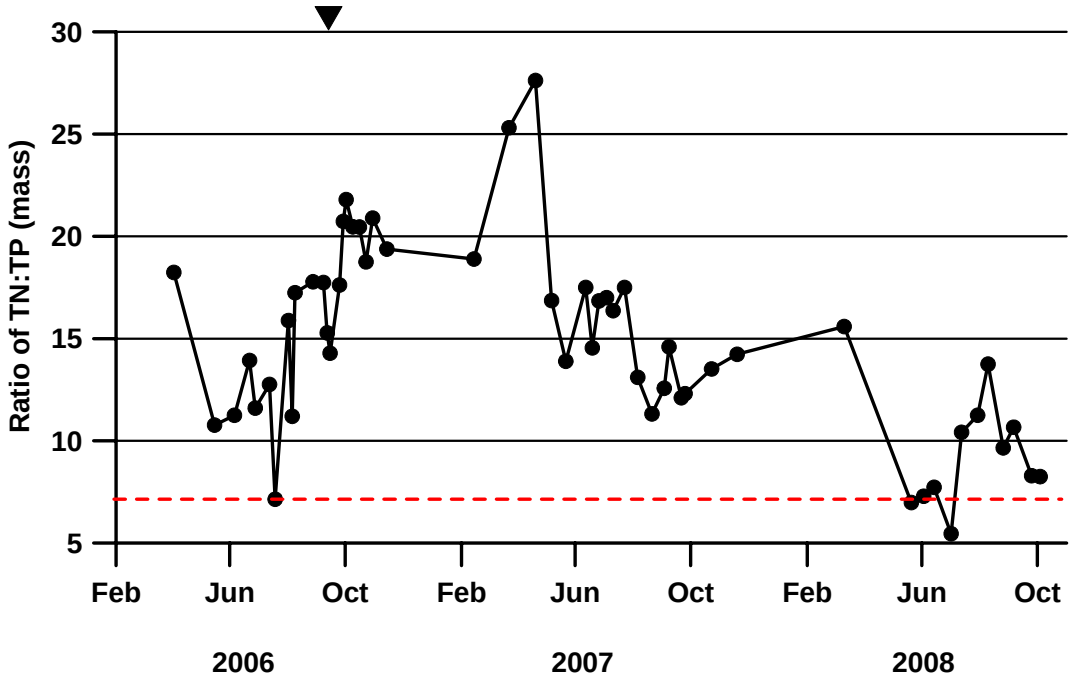

Figure 40. Mass ratio of total nitrogen to total phosphorus at a depth of 1 meter in Diamond Lake from May 2006 to October 2008. The red line shows the Redfield ratio whereby values above the line are generally perceived to be phosphorus-limited and values below the line are perceived to be typically nitrogen-limited.

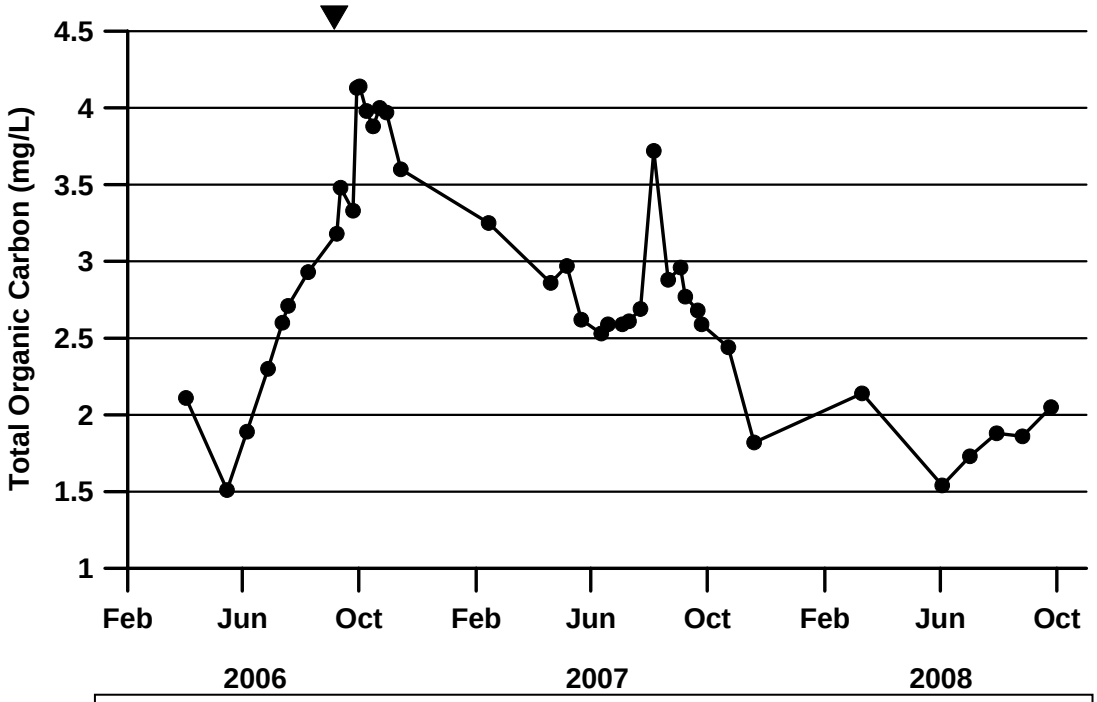

Figure 41. Total organic carbon (TOC) at a depth of 1 meter in Diamond Lake from May 2006 to October 2008.

The analysis of variance of surface water chemistry among years for the June-September season shows that concentrations of total and ortho phosphorus were unchanged (Table 4). In contrast, total nitrogen, total organic carbon, laboratory pH, and alkalinity all showed statistically significant declines from 2006 to 2008. Summer concentrations of ammonia and nitrate showed statistically significant increases from 2006 to 2007, but then declined again in 2008 to values not different from 2006. Nitrate concentrations increased dramatically from fall 2007 to spring, which is not reflected in these statistical comparisons (Figure 38). Silica showed a significant decline from 2006 to 2007, but again the values in 2008 returned to those observed in 2006.

Table 4. Analysis of variance for measurements of water chemistry from samples collected at a depth of 1 meter in Diamond Lake from June 1-September 30 for the period 2006-2008.

Variable	Unit	Year	N	Mean	se	V[a]	F[b]	P	[c]△
Total Phosphorus	µg/L	2006	14	31.5	2.44	=	0.90	0.4173	=
		2007	12	27.9	2.63				
		2008	9	33.0	3.04.				
Ortho Phosphorus	µg /L	2006	17	3.6	0.58	≠	2.62	0.0868	=
		2007	12	4.4	0.70				
		2008	9	2.0	0.80				
Total Nitrogen	µgN/L	2006	14	472	35.8	≠	4.67	0.0166*	⇩
		2007	12	392	38.7				
		2008	9	298	44.7				
Ammonia	µgN/L	2006	17	4.2	4.26	≠	5.35	0.0094**	⇘
		2007	12	24.3	5.07				
		2008	9	4.4	5.86				
Nitrate	µgN/L	2006	16	0.12	0.823	≠	4.82	0.0144*	⇘
		2007	12	3.67	0.950				
		2008	9	0	0				
Silica	mg Si/L	2006	9	5.16	0.26	≠	12.5	0.0003**	⇘
		2007	8	3.72	0.28				
		2008	5	5.79	0.35				
Total Organic Carbon	mg C/L	2006	9	2.78	0.157	=	10.2	0.0007**	⇩
		2007	11	2.70	0.142				
		2008	5	1.81	0.211				
pH (lab)	su	2006	10	8.64	0.124	=	19.0	0.0000**	⇩
		2007	11	7.68	0.118				
		2008	8	7.71	0.139				
Alkalinity	mg/L HCO_3	2006	10	4.66	0.041	=	5.52	0.0101*	⇩
		2007	11	4.67	0.039				
		2008	8	4.48	0.046				

[a] Assumption of variances (equal and not equal)

[b] F statistic

[c] Changes from 2006-2008, whereby direction is indicated with the arrow

Table 5. Concentrations of selected analytes from the top (1 m), middle (usually 7 m), and bottom (12-13m) water of Diamond Lake from May 2006 to October 2008.

Variable	Unit	Year	Top			Middle			Bottom		
			N	Mean	se	N	Mean	se	N	Mean	se
Total Phosphorus	µg/L	2006	23	34.5	1.95	9	36.2	7.54	9	61.0	24.0
		2007	19	29.0	2.14	11	37.8	6.82	10	127.5	22.8
		2008	12	35.3	2.70	7	37.7	8.54	9	124.9	24.0
Ortho Phosphorus	µg /L	2006	25	4.6	0.88	10	4.0	1.89	9	11.2	23.3
		2007	19	4.5	1.01	10	3.2	1.89	10	89.6	22.2
		2008	12	5.2	1.27	7	6.0	2.25	9	57.8	23.4
Total Nitrogen	µgN/L	2006	23	570	38.4	9	510	40.6	8	689	82.5
		2007	19	451	42.2	11	434	36.8	10	742	73.8
		2008	12	326	53.1	7	341	46.1	9	500	77.8
Ammonia	µgN/L	2006	25	8.8	10.8	10	11.7	19.9	9	199	96.0
		2007	19	47.3	12.4	11	36.1	19.0	10	338	91.1
		2008	12	35.4	15.6	7	51.3	23.8	9	186	96.1
Nitrate	µgN/L	2006	24	0.12	2.04	10	0.30	2.03	9	0.11	0.73
		2007	18	5.8	2.36	11	0.18	1.94	10	0.20	0.70
		2008	12	7.8	2.89	7	6.3	2.43	9	4.1	0.73
Silica	mg Si/L	2006	15	4.77	0.20	5	4.93	0.70	4	5.96	0.52
		2007	14	4.00	0.20	2	5.58	--	1	3.94	--
		2008	5	5.79	0.34	2	5.32	--	3	5.87	--
Total Organic Carbon	mg C/L	2006	19	3.18	0.14	8	2.93	0.18	8	2.39	0.24
		2007	16	2.73	0.15	10	2.90	0.16	9	2.28	0.24
		2008	6	1.87	0.25	2	1.73	--	3	1.59	--

5. Phytoplankton

Virtually all aspects of phytoplankton community composition and related metrics showed considerable change from 2006 to 2008. One of the general metrics of system response identified in the post-project goals was that chlorophyll *a* concentrations during the summer be less than or equal to 10 µg/L. Chlorophyll *a* levels during the summer of 2006 exceeded 20 µg/L, yet in the summers of 2007 and 2008, values were well below the 10 µg/L threshold (Figure 42). Chlorophyll concentrations increased dramatically after the rotenone treatment, reaching a peak for this period of 73 µg/L on October 17, 2006. Elevated chlorophyll levels occurred in the spring of 2007 and 2008, when diatom populations increased rapidly following mixis.

Phytoplankton biovolume shows a similar response as chlorophyll, although the blooms of *Fragilaria crotonensis* in summer 2006 did not yield the high chlorophyll values generated by the Anabaena blooms in fall 2006 follow the treatment (Figure 43). A similar response was observed in spring of 2008 when the diatom bloom of *Stephanodiscus* did not generate extremely high chlorophyll levels compared to the biovolume of the bloom. The phytoplankton biovolume was dominated by two groups in Diamond Lake, the diatoms and the cyanobacteria (Figure 44). The cyanobacteria, largely *Anabaena*, were present to any significant degree only in 2006 and then primarily after the treatment. This pattern differed substantially from previous years where the cyanobacteria blooms were mid-summer events (Eilers et al. 2005). The third post-project water quality goal identified in the FEIS related to neurotoxin production expressed as the goal to keep the density of *Anabaena flos-aquae* less than 15,000 cells/mL. The results of the project have so far succeeded in this regard (Figure 45).

Additional information regarding the changes in abundance of the dominant diatom taxa are shown in Figure 46. The *Fragilaria crotonensis* which dominated in early summer of 2006 gave way to species of *Synedra* in late summer and fall. The next major pulse of diatoms was the *Synedra* bloom which occurred in spring of 2007. This bloom was relatively long-lived compared to the brief, but intense bloom of *Stephanodiscus* which occurred in spring 2008. *Aulocoseira distans* occurred throughout the study period, but in relatively modest numbers.

Minor taxa in this context refer to abundance based on algal biovolume. If algal dominance for this assessment were based on cell density, then many of these so-called minor taxa are among the most dominant numerically. However, because they are small algae, their computed biovolumes do not compare with the cyanobacteria and diatoms. Nevertheless, they are important from two aspects: (1) they are ecologically important because they serve as an important food source for some zooplankton and (2) changes in relative abundance of these organisms provide additional information regarding shifts in the lake condition. One of the most abundant taxa, *Cryptomonas*, appears to have declined from 2007 to 2008, although the basis for this determination is relatively weak. *Chromulina*, a chrysophyte, appears to have increased considerably from 2006 to 2007 and 2008. Although dinoflagellates and *Chlamydomonas* (a chlorophyte) have been present in Diamond Lake throughout the study period, they again appear to have increased in 2008. The primary dinoflagellate taxa present were *Dinobryon sertularia* and *Glenodinium*.

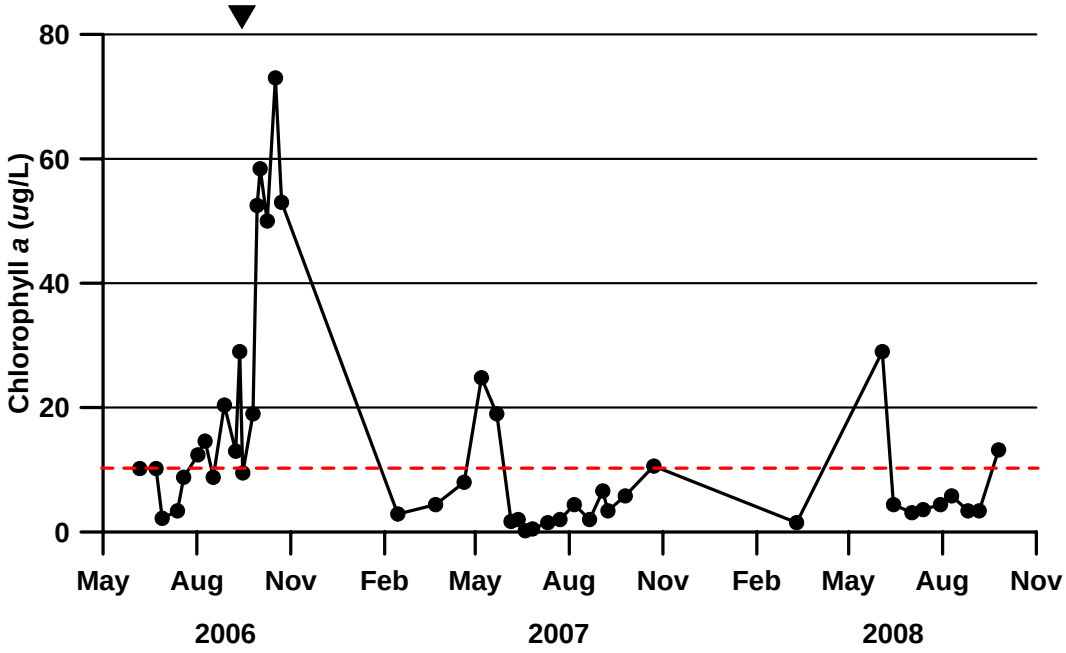

Figure 42. Chlorophyll a at a depth of 1 meter in Diamond Lake from May 2006 to October 2008. The sample results for October 4, 2008 are not plotted.

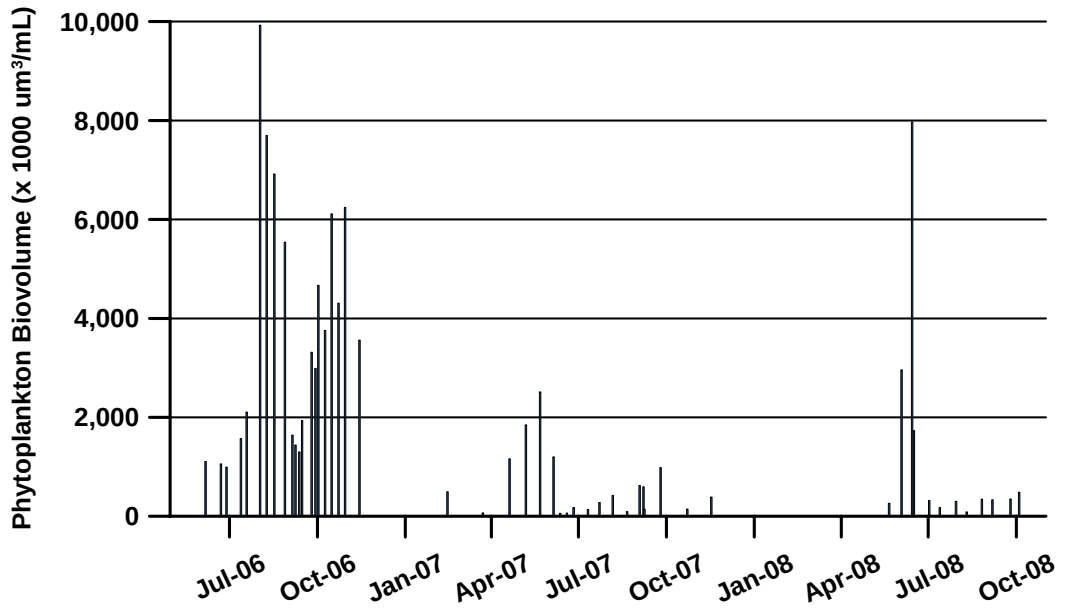

Figure 43. Phytoplankton biovolume at a depth of 1 meter in Diamond Lake from May 2006 to October 2008.

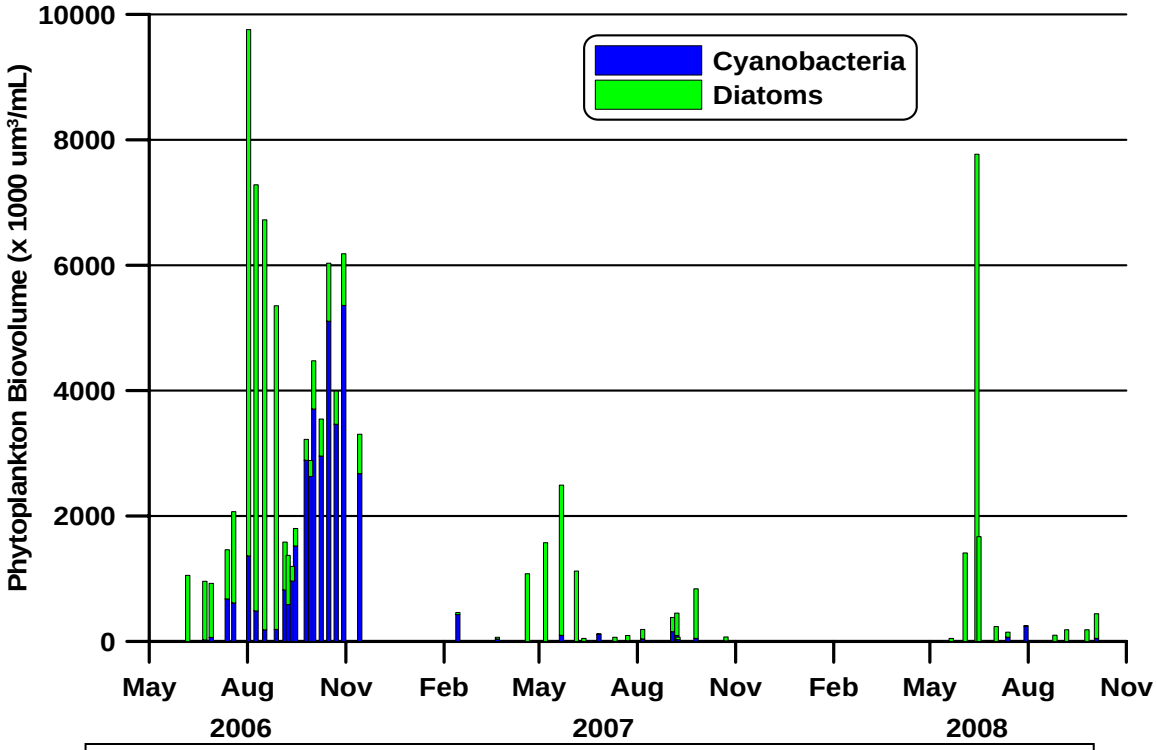

Figure 44. Dominant phytoplankton groups, cyanobacteria and diatoms, in Diamond Lake from 2006-2008.

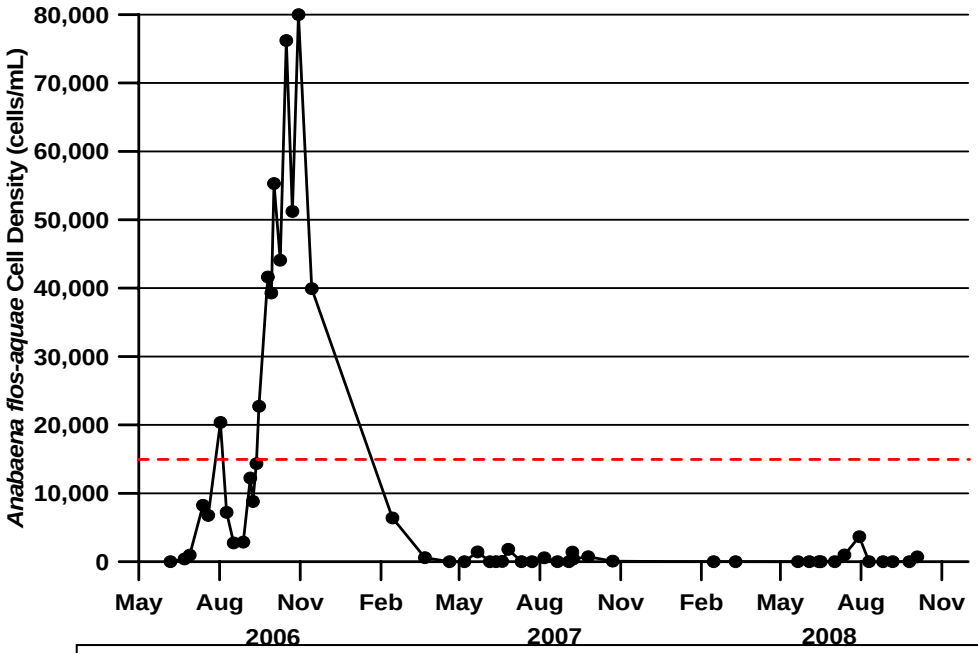

Figure 45. Cell density of *Anabaena flos-aquae* reported for the surface waters of Diamond Lake from 2006-2008. The project goal identified in the FEIS of 15,000 cells/mL is shown as the dashed line.

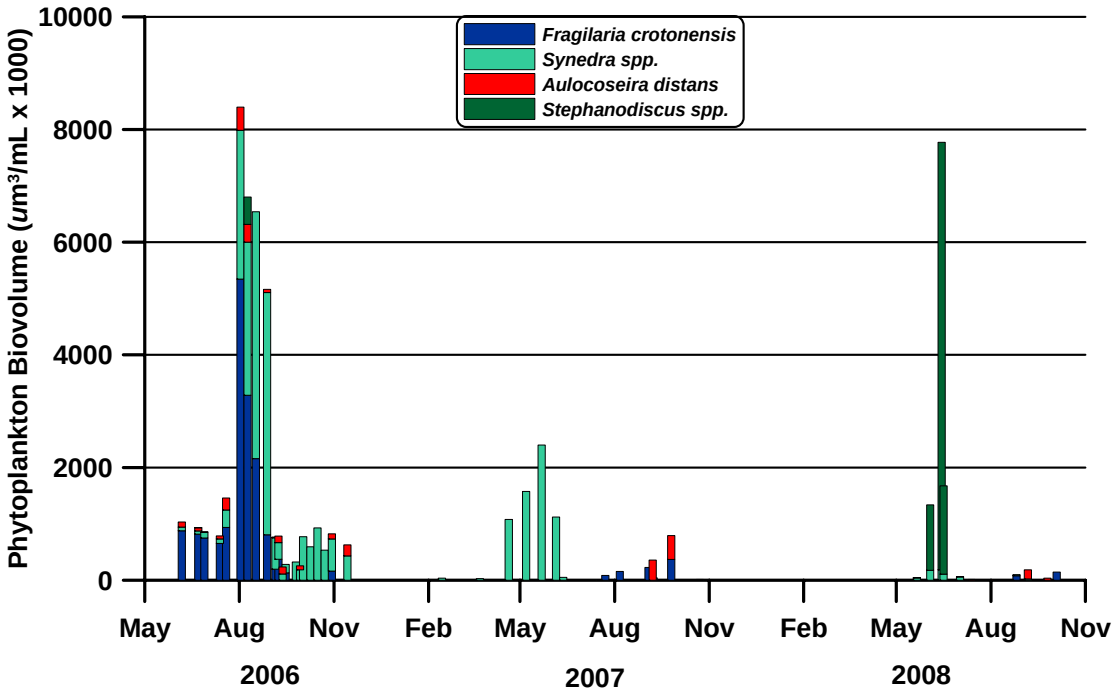

Figure 46. Biovolume of dominant diatom taxa in Diamond Lake from 2006-2008.

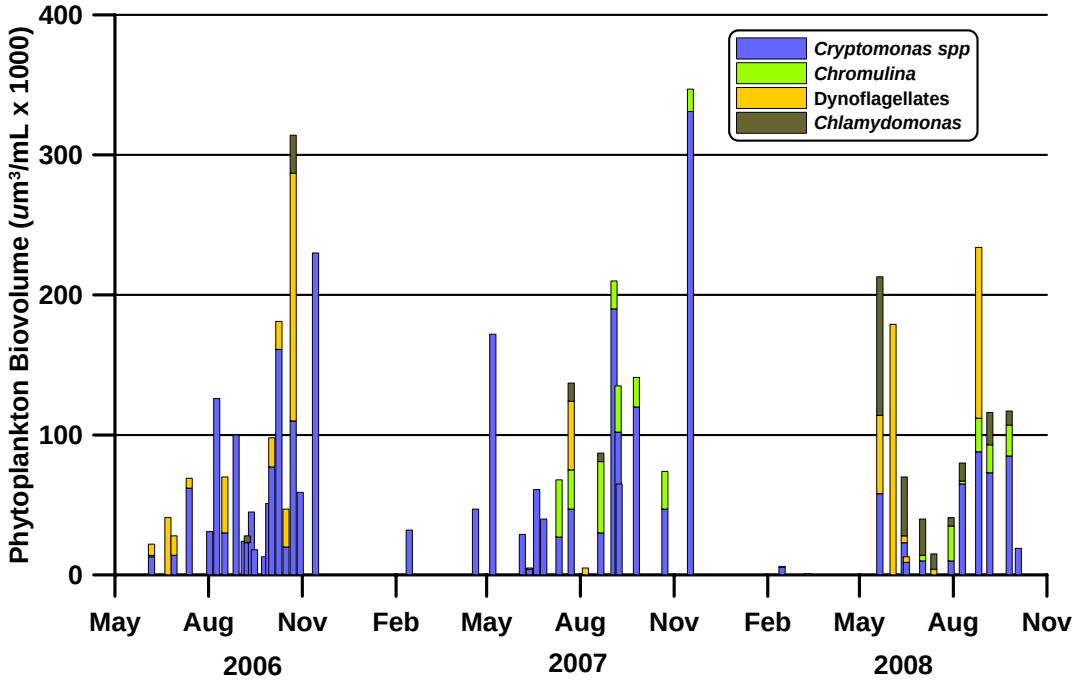

Figure 47. Minor phytoplankton taxa present in Diamond Lake from 2006-2008.

Anabaena spp. was by far the dominant genus of cyanobacteria in Diamond Lake. However, in June 2007 low densities of *Aphanizomenon flos-aquae* were present in samples from 1, 7, and 13 meters. *Aphanizomenon* was also sampled in August 2007 and again in September 2008. However, the sampling methods and taxonomic analyses failed to sample another N-fixing cyanobacteria, *Gloeotrichia echinulata*, that was present in 2007 and 2008. *Gloeotrichia* is a large colonial taxon that can be seen without the aid of magnification. I observed this taxon in the lake and in samples from the lake during late summer of 2007 and 2008. To confirm this, I retrieved the preserved October 4, 2008 phytoplankton sample from Aquatic Analysts and poured it into a glass cylinder for inspection. I retrieved a colony of *Gloeotrichia* and placed it under a microscope for confirmation (Figure 48). The implications of *Gloeotrichia* present in Diamond Lake are explored in the Discussion.

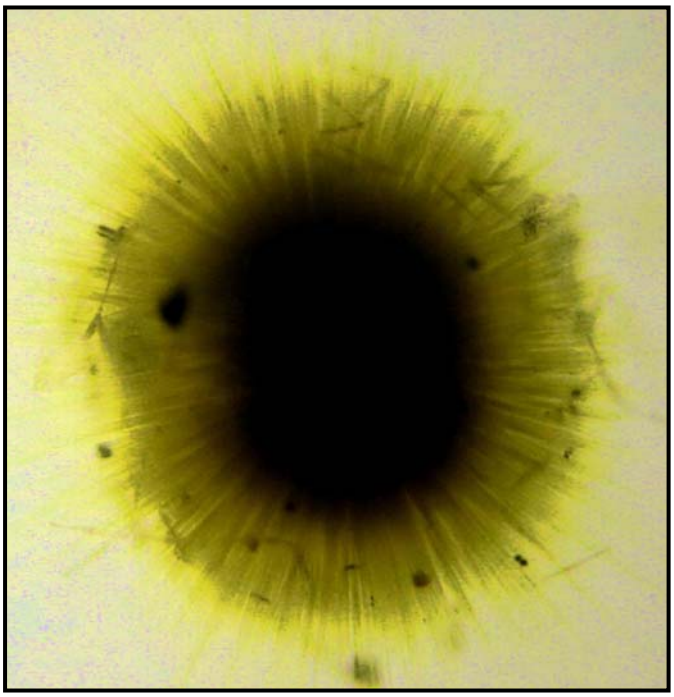

Figure 48. Gloeotrichia colony from a phytoplankton sample collected on October 4, 2008 in Diamond Lake at 1 meter depth.

Table 6. Analysis of variance for selected measures of phytoplankton from samples collected at a depth of 1 meter in Diamond Lake from 2006-2008.

Variable	Unit	Year	N	Mean	se	V[a]	F[b]	P	[c]△
Chlorophyll *a*	µg/L	2006	14	15.3	2.51	≠	5.64	0.0082**	
		2007	11	2.7	2.83				
		2008	9	7.8	3.13				
Phytoplankton Total Biovolume	µm^3 /L	2006	14	3,684,000	589,000	≠	7.35	0.0024**	
		2007	11	382,000	664,000				
		2008	10	1,457,000	697,000				
Anabaena Biovolume	µm^3 /L	2006	14	855,000	156,000	≠	8.33	0.0012**	
		2007	11	29,000	177,000				
		2008	10	31,000	185,000				
Anabaena Cell Density	Cells /L	2006	15	12,570	2200	≠	9.05	0.0007**	
		2007	12	413	2460				
		2008	10	464	2694				

6. Macrophytes

Macrophytes were not systematically sampled lake-wide in 2006 and 2008. However, a hydroacoustic survey conducted in July 2007 showed the extent of macrophytes just as the lake reached full-pool for the first time since the rotenone treatment (Figure 49). The general distribution of macrophytes in mid-summer of 2007 did not differ greatly with the distribution of macrophytes observed in 2002 (Figure 50), with the exception of the obvious loss of some shallow macrophytes associated the drawdown. However, divers sent down in fall of 2007 reported seeing tall macrophytes present in the deepest area of the lake (B. Eilers, personal communication). And when the monitoring buoy and anchor was retrieved in fall 2008, again at the deepest area of the lake, the anchor was covered with abundant macrophytes (Figure 51). The dominant macrophyte attached to the anchor appeared to be *Ceratophylum* (coontail), with a smaller number of *Elodea* (American waterweed) plants.

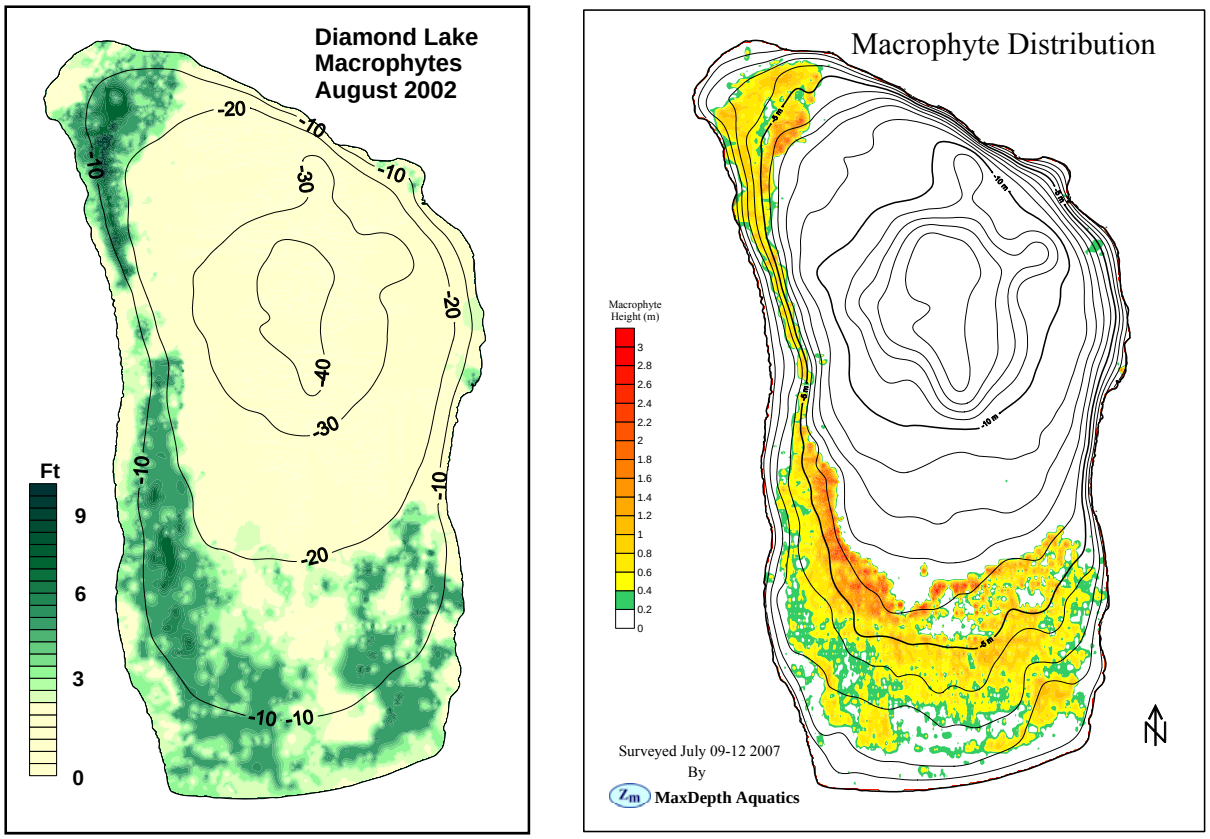

Figure 49. Distribution and canopy height of macrophytes in Diamond Lake based on a hydroacoustic survey, August 2002 (left). After Eilers and Gubala (2003). Distribution and canopy height of macrophytes in Diamond Lake based on a hydroacoustic survey, July 9-12, 2007 (right). After B. Eilers (2007).

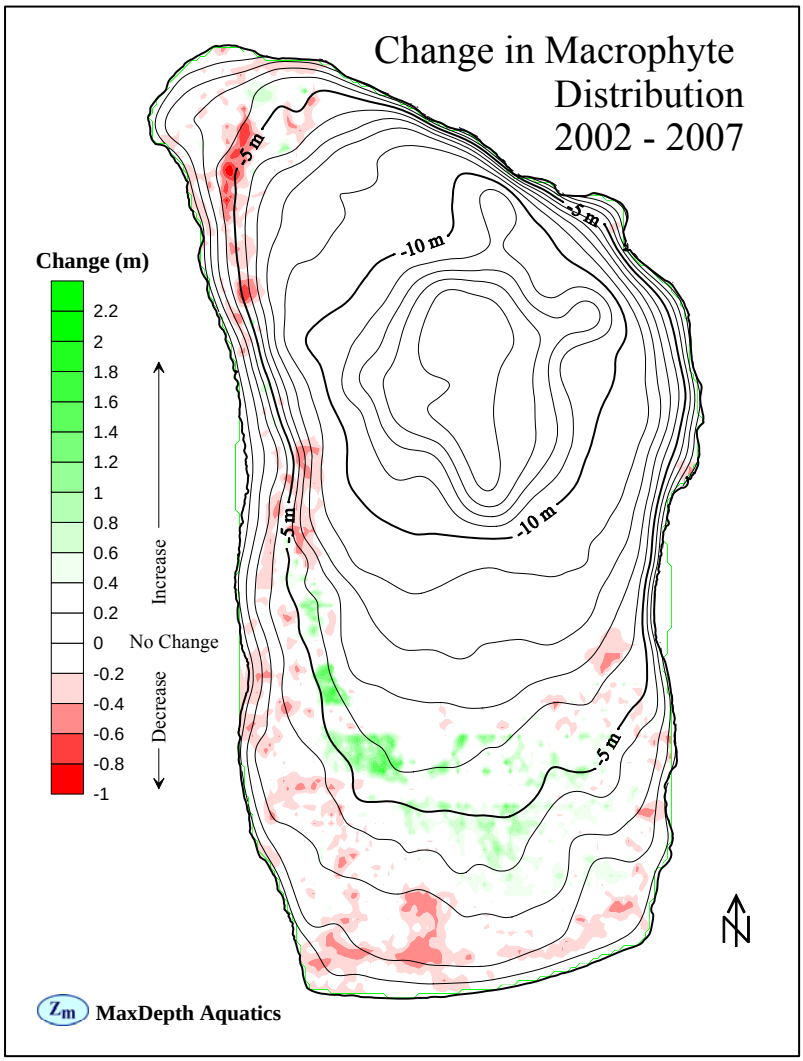

Figure 50. The change in macrophyte distribution and canopy height in Diamond Lake between August 2002

Figure 51. Macrophytes attached to the buoy anchor recovered from Diamond Lake in October 2008 at a depth of 14 meters.

7. Zooplankton

The density of total cladocerans in Diamond Lake declined from 2006-2008 (Figure 52). However, the total cladocerans as a percentage of the number of zooplankton individuals in the samples increased from 2006-2008 (Figure 53). Most of the cladocerans present in 2006 were smaller taxa (*Daphnia galeata mendotae, Diaphanosoma brachyurum, Bosmina longirostris,* and *Chydorus sphaericus*) providing relatively little for resources for trout. In contrast, the dominant cladocerans in 2007 and 2008 were large taxa, *Daphnia pulicaria* and *Daphnia rosea* (Figure 54). The large *Daphnia* were already present in relatively low densities in 1992 at about 1000 individuals per cubic meter when sampling for zooplankton was initiated (Figure 55). The density of these taxa declined another order of magnitude by 2001 and were not sampled in 2002, 2003, and 2006 and only in densities less than 100 individuals per cubic meter in 2004 and 2005. The density of large *Daphnia* reached a peak abundance on June 19, 2007 of over 77,000 individuals per cubic meter. The median density of large *Daphnia* declined from 18,237 individuals per cubic meter in 2007 (June-Sept) to 5,817 individuals in 2008.

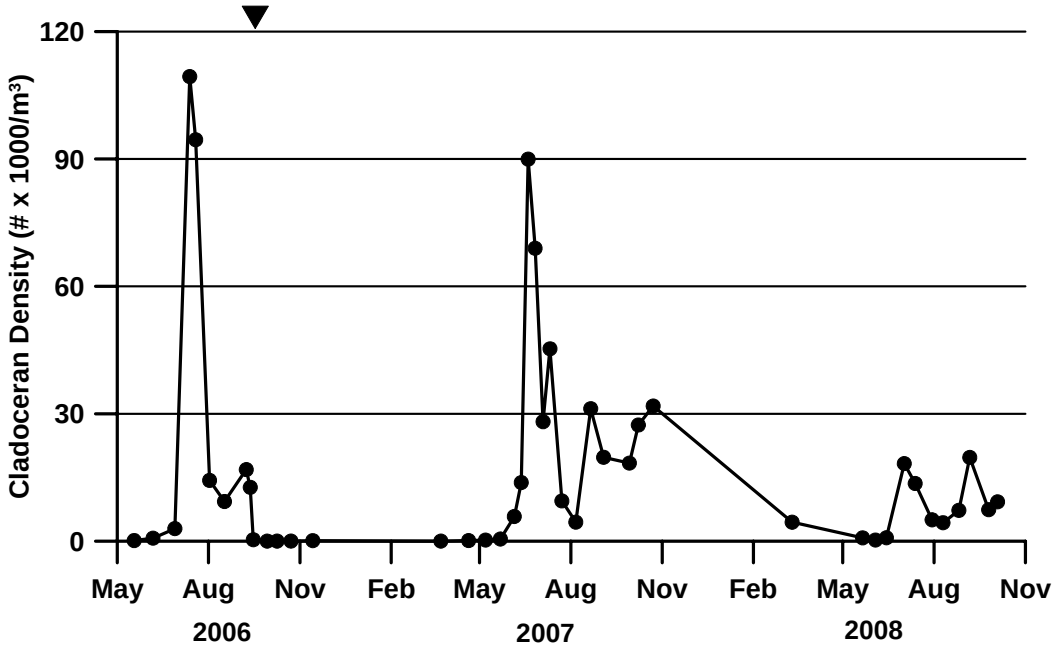

Figure 52. Total cladoceran density in Diamond Lake from May 2006 to October 2008.

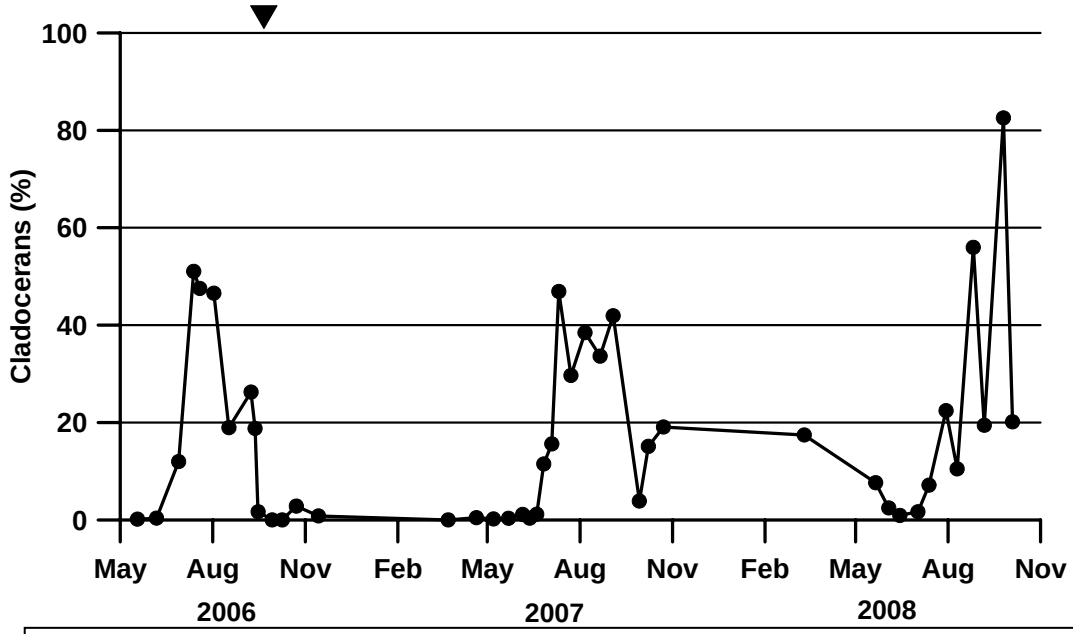

Figure 53. Total cladocerans in Diamond Lake as a percentage of the total number of individuals in the sample from May 2006 to October 2008.

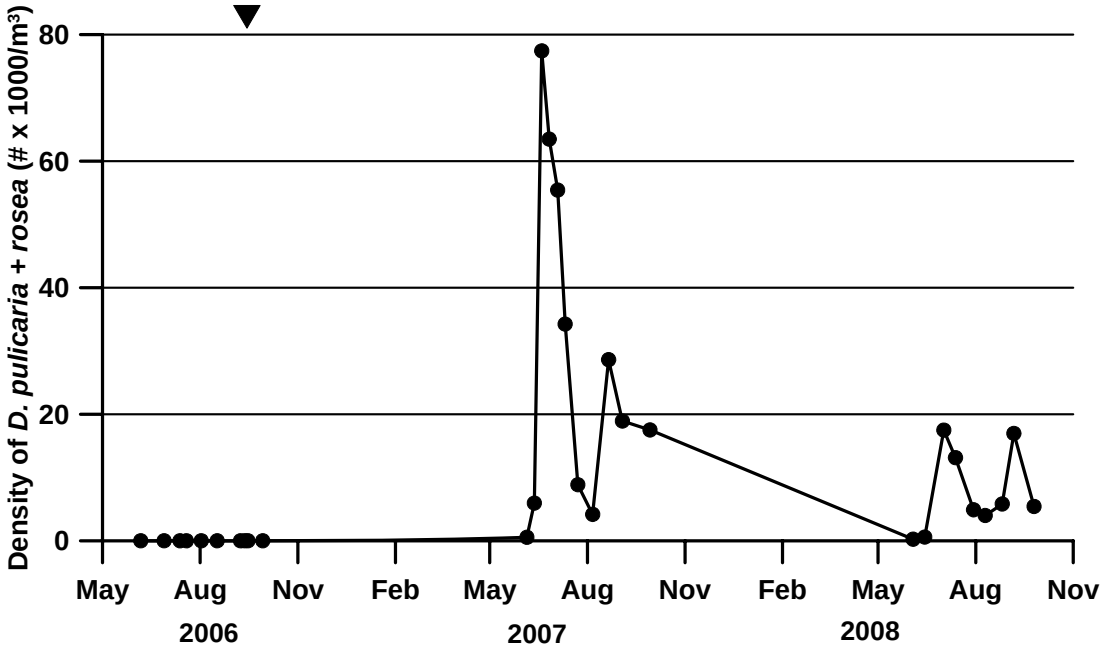

Figure 54. Combined density of *Daphnia pulicaria* and *Daphnia rosea* in Diamond Lake from May 2006 to October 2008 expressed on a linear scale.

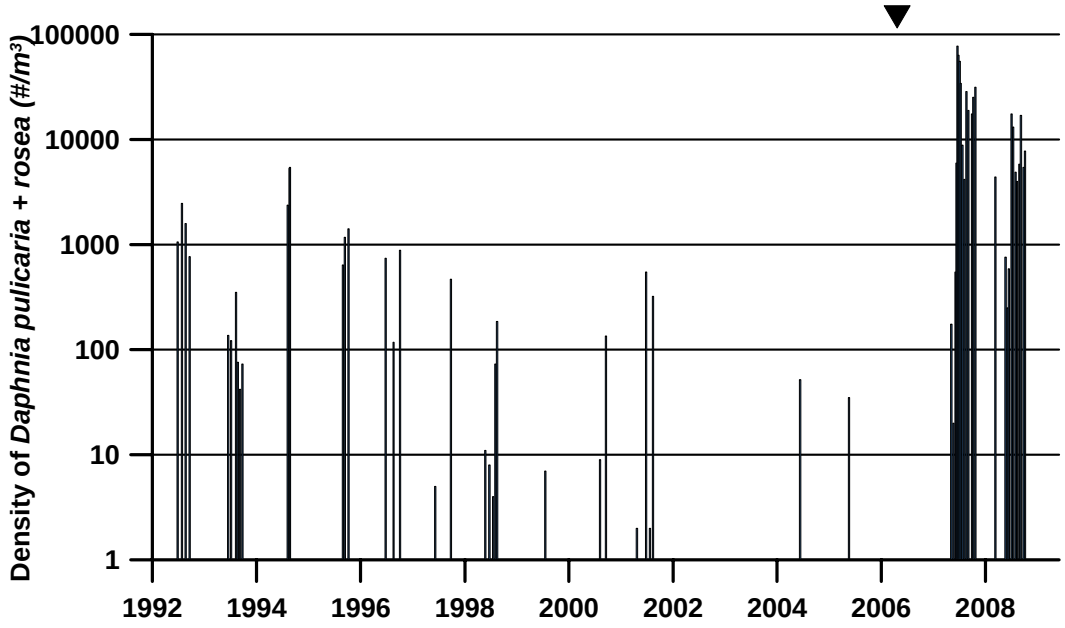

Figure 55. Combined density of *Daphnia pulicaria* and *Daphnia rosea* in Diamond Lake from 1992 to 2008 expressed on a log (base 10) scale.

The density of copepods was generally lower than that observed for the cladocerans, with the exception of a major spike in the abundance of copepods in late June 2007 when the density of copepods exceeded 90,000 individuals per cubic meter (Figure 56). The dominant taxon during this brief period representing 97 percent of the copepods was *Epischura nevadensis*, a large calanoid copepod. This taxon has been observed to be a preferred prey of rainbow trout fry in certain situations (Irvine and Northcote 1982). Copepods only achieved 40 percent of the zooplankton population in August 2007, when copepod density was moderately high and rotifer density was low (Figure 57).

The density of rotifers peaked in late June 2007, when the number of individuals per cubic meter exceeded 600,000 (Figure 58). Over 99 percent of the rotifers during this period were *Keratella cochlearis*. However, rotifers remained the dominant (numerically) zooplankton throughout much of the study period (Figure 59).

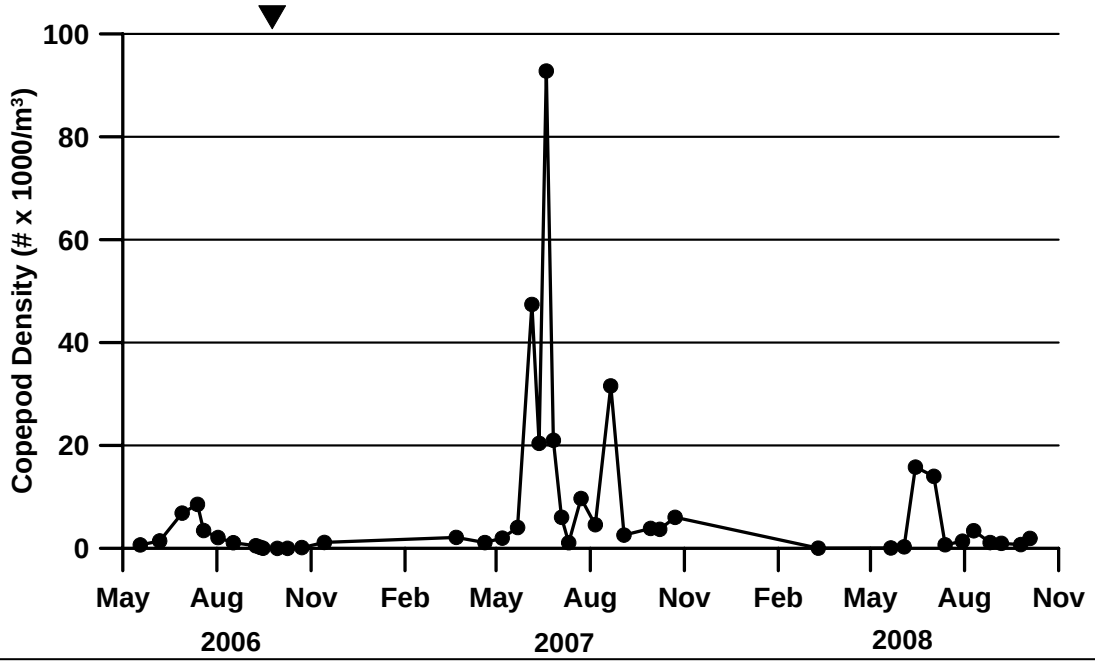

Figure 56. Total copepod density in Diamond Lake from May 2006 to October 2008.

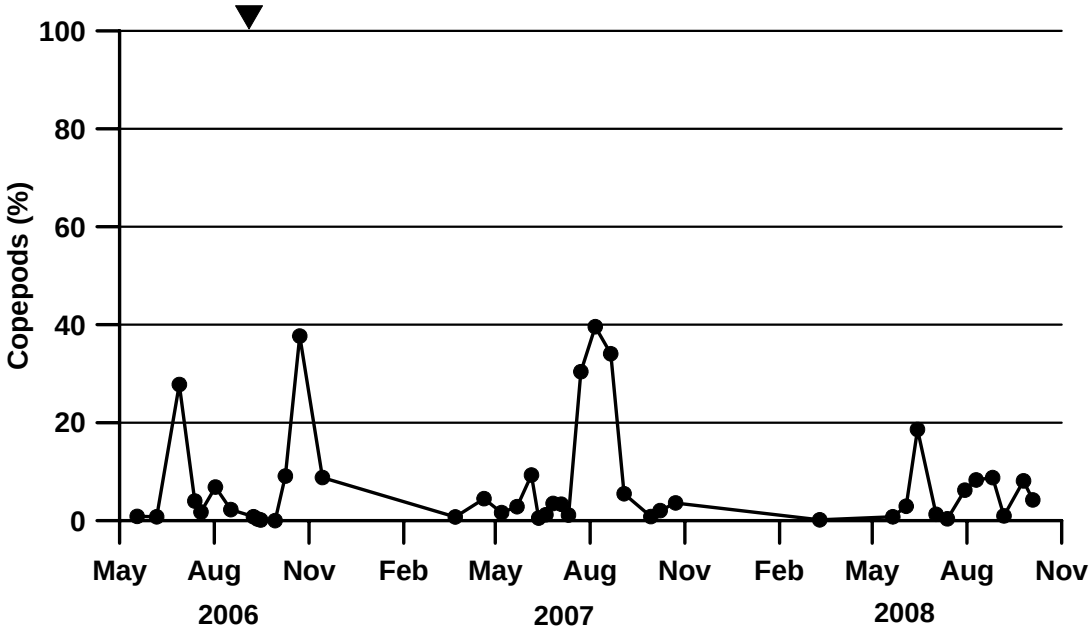

Figure 57. Total copepods in Diamond Lake expressed as a percentage of the total number of individuals in the sample from May 2006 to October 2008.

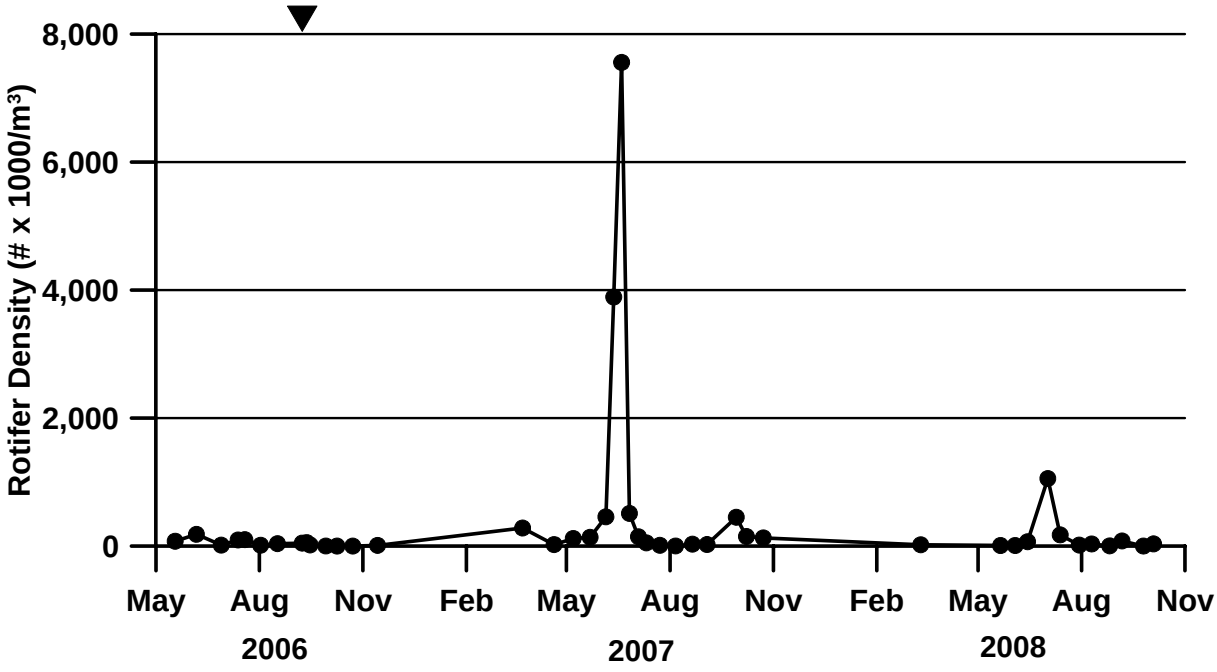

Figure 58. Density of rotifers in Diamond Lake from May 2006 to October 2008.

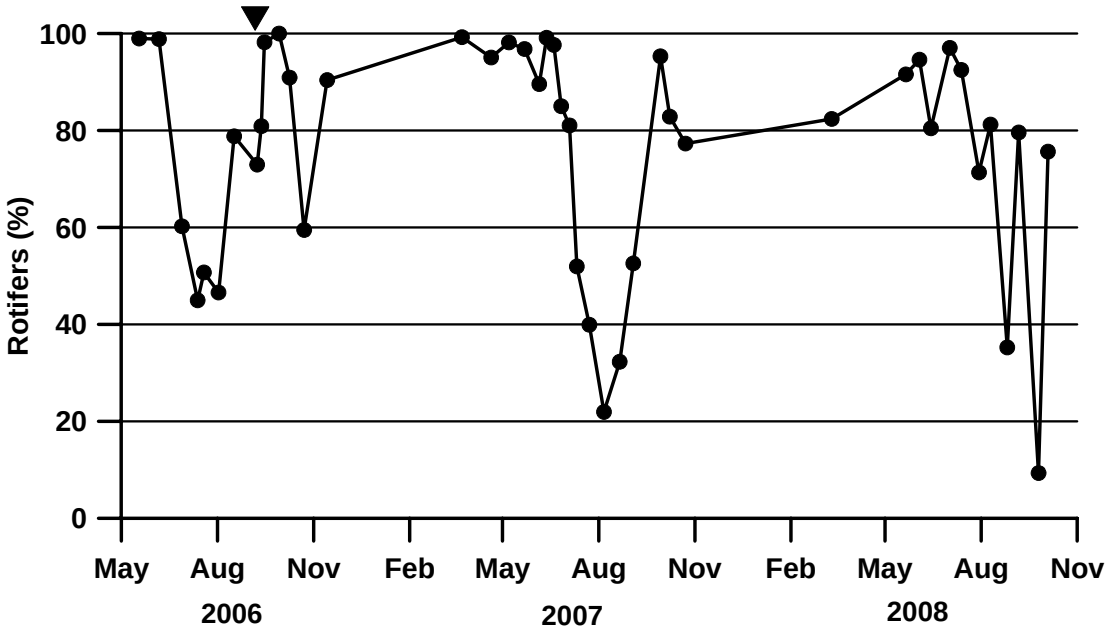

Figure 59. Total rotifers in Diamond Lake expressed as a percentage of the total number of individuals in the sample from May 2006 to October 2008.

Many of the zooplankton taxa are too small to serve as suitable prey species for trout. The predominant prey species for trout in Diamond Lake include the cladocerans, *Daphnia pulicaria, Daphnia rosea, Leptodora kindtii,* and the copepod, *Epischura nevadensis.* Except during late June 2007, when the density of *E. nevadensis* was extremely high, the availability of *D. pulicaria* has largely determined the density of zooplankton available for trout consumption. The percent of zooplankton edible by trout during the study period has varied considerably (Figure 60). During 2006, some increase in edible zooplankton was observed, concomitant with netting efforts to remove tui chub prior to the rotenone treatment. The response of large zooplankton following the removal of the tui chub and before the introduction of rainbow trout fingerlings (June 12, 2007) was impressive. Despite the introduction of 100,000 trout fingerlings in 2007, the percent of edible zooplankton remained high. *Daphnia pulicaria* continued to remain active under the ice in 2007, a behavior that is considered unusual, but not without precedence (Snow 1972). However, the abundance of large *Daphnia* declined rapidly with ice-out (late May) in 2008 and didn't fully recover until September 2008. Additional details on the response of the zooplankton from 2006-2007 are presented in Eilers and Vogel (2008).

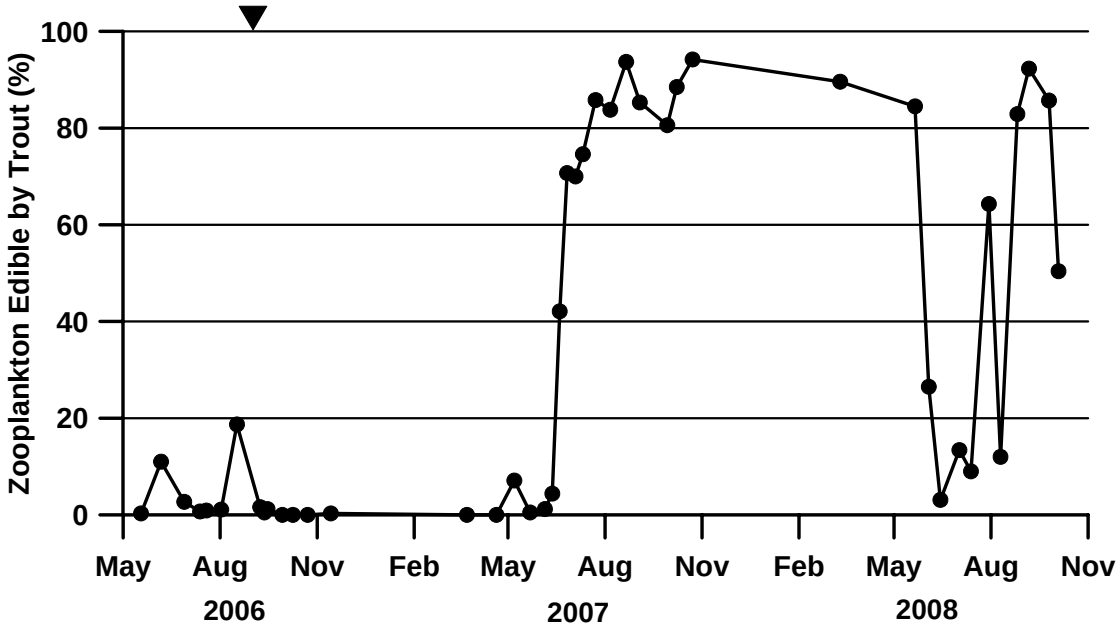

Figure 60. Percentage of zooplankton in Diamond Lake edible by trout from May 2006 to October 2008.

8. Benthic Macroinvertebrates

The abundance of benthic invertebrates has long been considered an important factor contributing to the historically high growth rates of trout in Diamond Lake (Bauer 1976). Sampling of benthic invertebrates had been used by ODFW until 1980 as a guide to appropriate stocking strategies of rainbow trout. That practice was discontinued in favor of trout condition factor as a suitable metric. Consequently, it is unknown when the biomass of benthic invertebrates first began to change. The earliest sampling of benthic invertebrates occurred in 2002 (Eilers 2003b) and it wasn't until 2004 that benthic sampling once again was adopted by ODFW as a key metric for lake sampling (Truemper 2007; Eilers 2008a). The results of the benthic sampling from 2002 to 2008 show a dramatic increase in benthic biomass in 2007 and 2008 (Figure 61). The increase in biomass is nearly linear until August 2008 when biomass declined by 40 percent from a high of 36.9 g/m^2 in July 2008. The biomass continued to decline in October, reaching nearly a 50 percent decline from the biomass measured in July 2008.

The decline in benthic biomass from July to October 2008 appears dramatic, yet when placed in the context of long-term measurements, the change from October 2007 to October 2008 only represents a decline of 16.4 percent (Figure 62). The benthic biomass from 2002-2006 is comparable to that measured from 1949to 1954, prior to the first rotenone treatment and the response in 2007 and 2008 is comparable to the most abundant biomass years from 1955-1979. Not only has the abundance of benthic biomass increased from in 2007 and 2008, but the composition of the benthic community has changed considerably as well. The greatest increase in abundance in benthic organisms from 2006 to 2007 was the massive increase in the density of chironomid larvae (Figure 63). Other notable increases included large gains in non-dipteran insect larvae, amphipods, and increases in leeches and gastropods. The number of benthic taxa increased in 2007 and 2008, presumably as natural recolonization allowed for new (in recent terms) species to find suitable habitats to exploit (Figure 64). Species richness increased with the Chironomidae, the best represented family of benthic taxa, as well as non-chironomid taxa.

The changes in benthic community composition from 2006 to 2007 were as noteworthy with regard to the total benthic biomass as well as the increase in non-chironomid taxa. In 2008, the density of chironomids declined by 83 percent, but the non-dipteran insects more than doubled as did the density of amphipods. A factor analysis of the changes in benthic community composition illustrates that the community was similar from 2004-2006, but changed considerably in 2007 (Figure 65). The community changed again in 2008, by a degree similar to the change from 2006 to 2007.

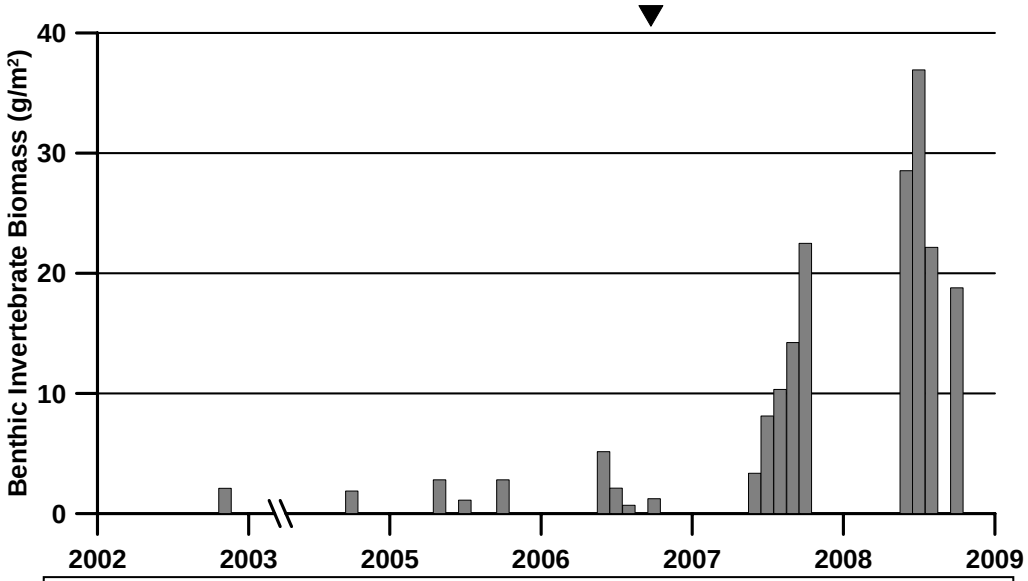

Figure 61. Biomass of benthic macroinvertebrates in Diamond Lake from 2002 to 2008. Data for 2004-2008 provided by ODFW. Data from 2002 was reported by Eilers (2003).

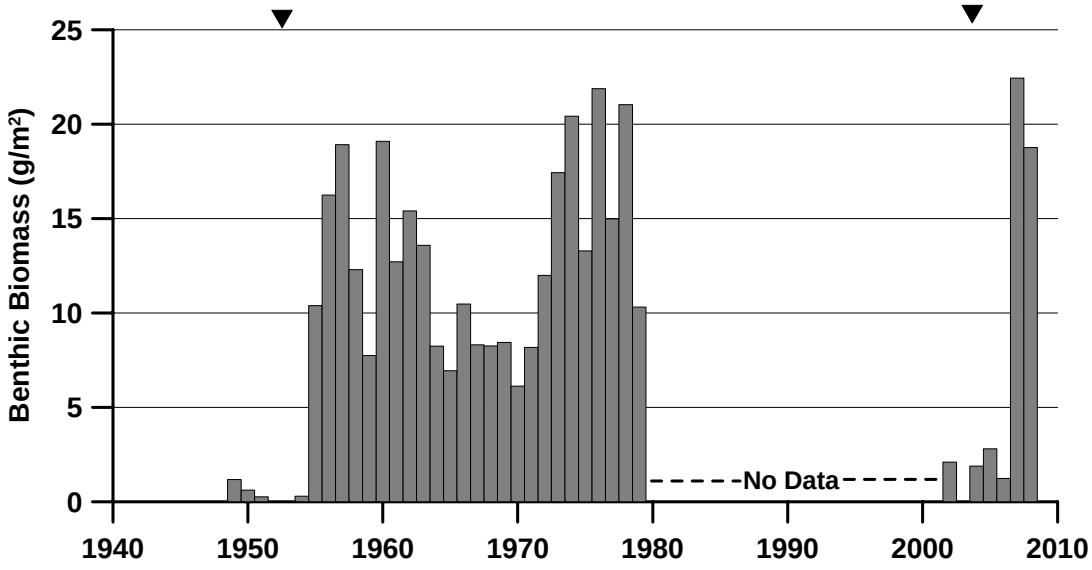

Figure 62. Biomass of benthic macroinvertebrates in Diamond Lake from 1949 to 2008. Data provided by ODFW.

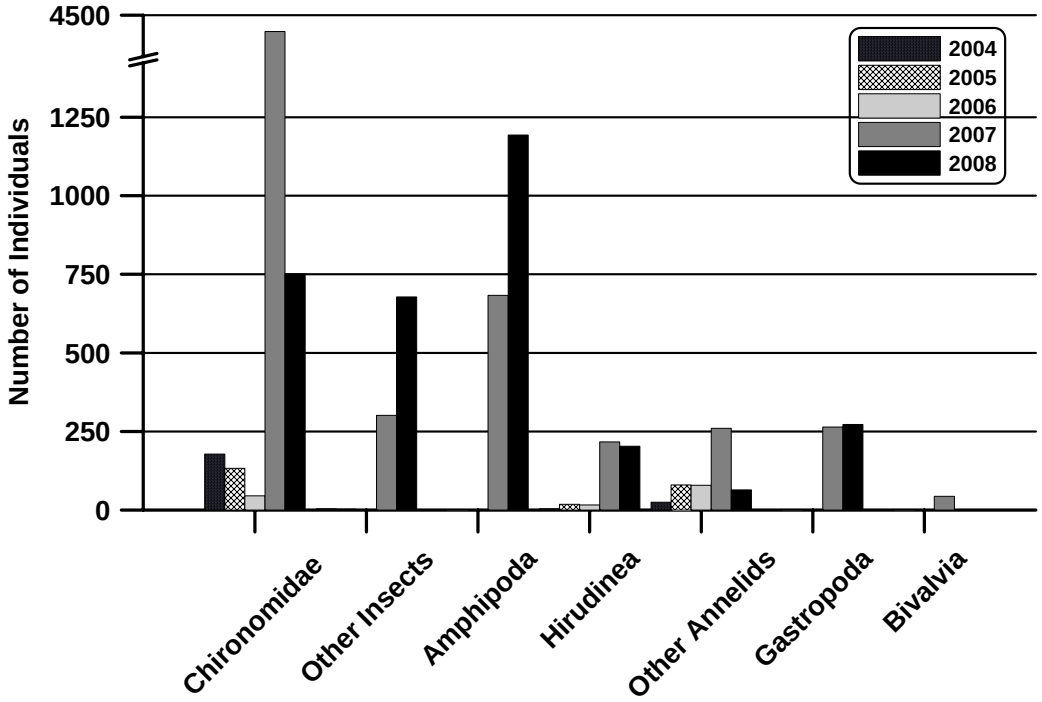

Figure 63. Relative abundance of benthic macroinvertebrate groups in Diamond Lake from 2004 to 2008.

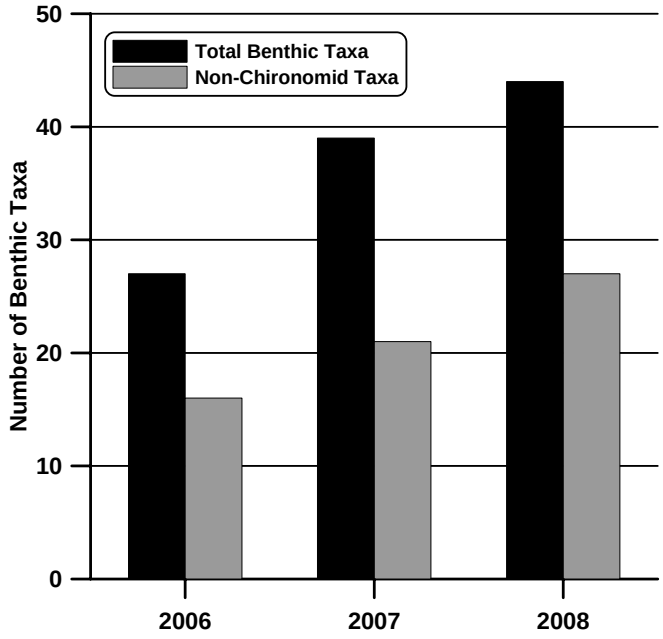

Figure 64. Total number of benthic taxa and non-chironomid (midge) taxa for 2006-2008.

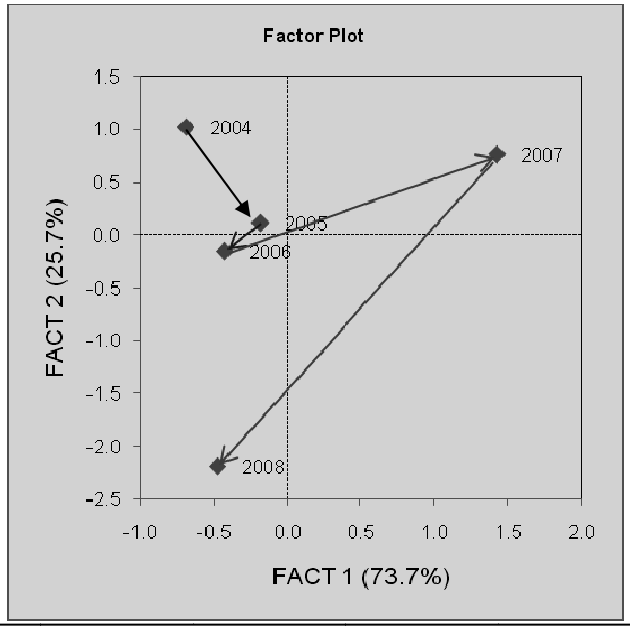

Figure 65. Factor scores based on abundance of dominant groups of benthic macroinvertebrates from 2004-2008.

Two indicators of secondary production used to asses changes in Diamond Lake are density of large *Daphnia* (June-Sept) and total benthic biomass. Those metrics computed for Diamond Lake for the study period show highly significant increases from 2006 to 2007 and 2008 (Table 7).

Table 7. Analysis of variance for two metric of secondary production collected in Diamond Lake from 2006-2008.

Variable	Unit	Year	N	Mean	se	V[a]	F[b]	P	[c]△
Daphnia density	#/m^3	2006	10	0	0	≠	8.71	0.0012**	
		2007	11	28648	4923				
		2008	9	7617	5443				
Benthic Biomass	g/m^2	2006	4	2.3	3.20	=	14.7	0.0011**	
		2007	5	11.7	2.86				
		2008	4	26.6	3.20				

9. Fisheries

a. Tui Chub

By 2006, the fishery in Diamond Lake was totally dominated by tui chub. Because of the role of fish biomass in promoting eutrophication, additional data on the length/weight relationship of the tui chub (Figure 66) and the nutrient content of these fish (Figure 67) were collected. The length/weight relationship for the tui chub follows a well-behaved log-normal relationship with a median weight for this sample of about 25 g/fish. The nutrient content of the tui chub shows a major change during the growth of the fish in which the young have a high nitrogen content and the mature fish have an N:P ratio approaching unity. Most of the change in the N:P ratio is associated with a decline in nitrogen content as the fish ages.

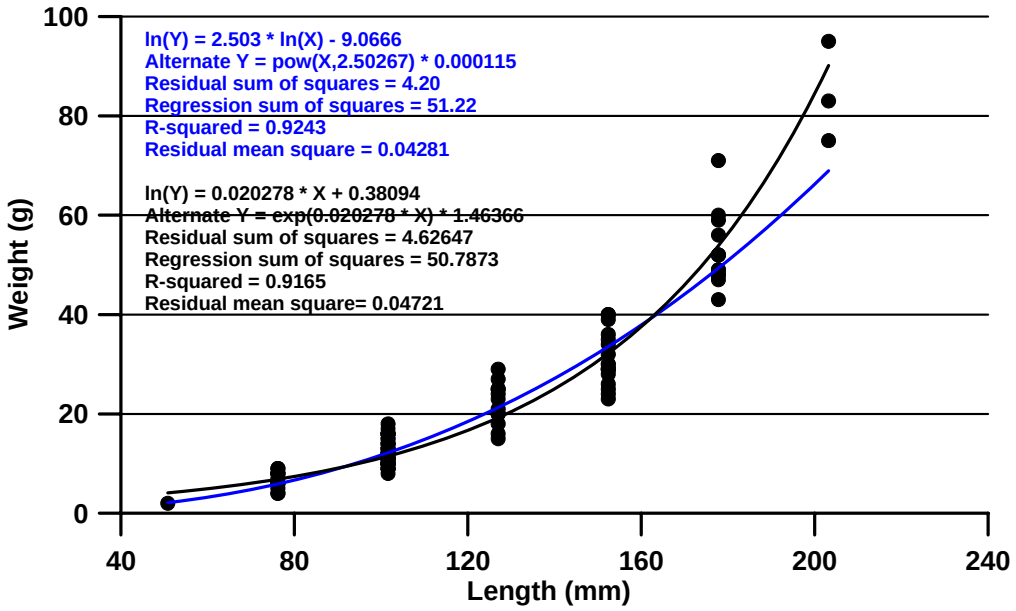

Figure 66. Length-weight relationship for tui chub in Diamond Lake. Data provided by ODFW.

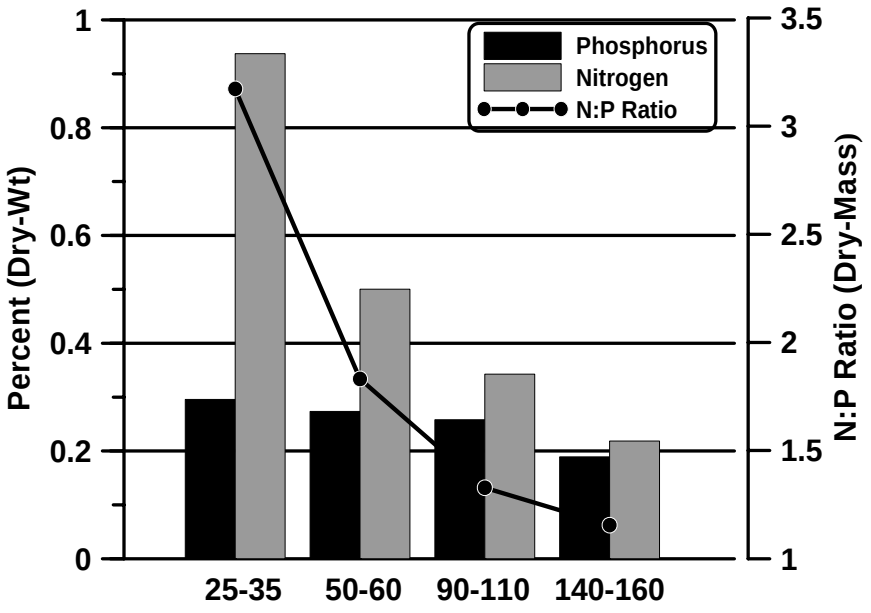

Figure 67. Nutrient content and N:P ratios of tui chub by size class (mm) from fish collected in Diamond Lake in

The decline of the rainbow trout (*Oncorhynchus mykiss*) population, appearing to start in the late 1980s, can be traced to the ascendency of the tui chub (*Gila bicolor*) population. The most consistent data to estimate the relative abundance of tui chub in Diamond Lake is the trap netting conducted by ODFW staff from 1995-2006 (Figure 68). Using data from June and July, it appears that the abundance of tui chub peaked in 2001 and declined somewhat after this. No data for these months are available for 2004 and 2005, however the data collected in 2006 indicates a dramatic decline in abundance of tui chub prior to treatment with rotenone. Some of this decline between June and July could be attributed to the netting of tui chub by ODFW and its contractor. ODFW and its contractor removed about 30,800 kg (68,000 lbs) with trap nets and gill netting during the summer of 2006 (Brick and Truemper 2007). Post-treatment removal of dead tui chub yielded about 15,900 kg (35,000 lbs) of fish (Brick and Truemper 2007). However, it is likely that most of the tui chub were not collected during post-treatment gathering based on likely effectiveness of these methods.

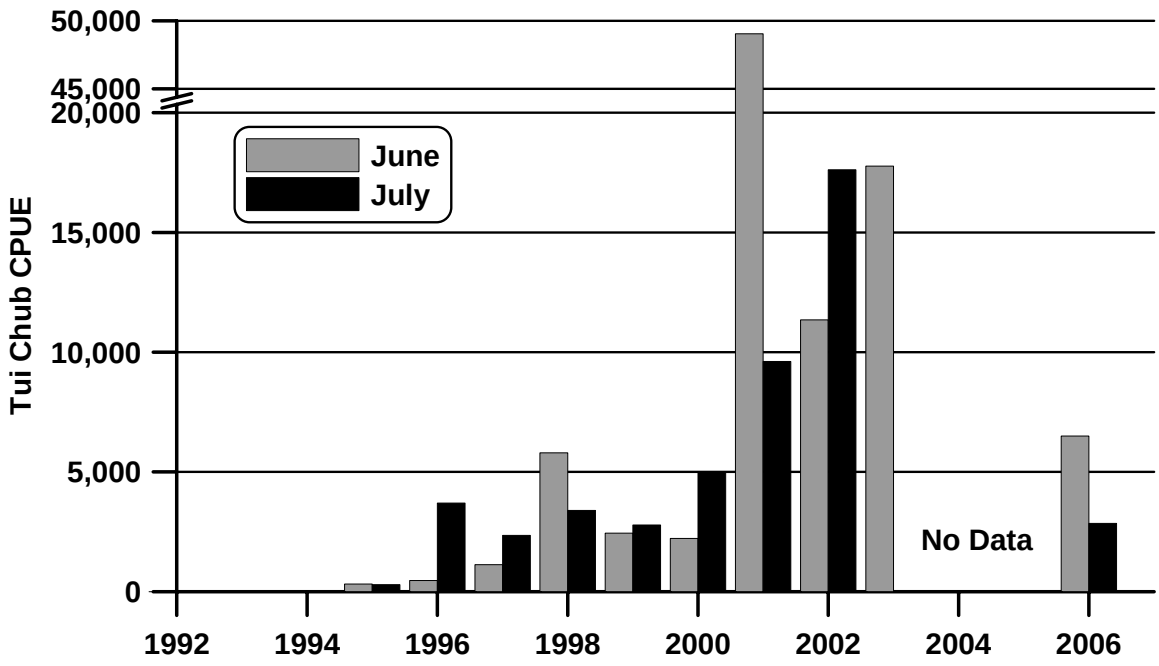

Figure 68. Catch-per-unit-effort (CPUE) of tui chub in Diamond Lake reported by ODFW for the months of June and July from 1995 to 2006.

The biomass of tui chub present in Diamond Lake after treatment can be estimated based on measured increases in the concentration of total phosphorus in the water column as follows:

1. The measured TP in Diamond Lake on September 12, 2006 (the day before treatment) was 36 µg/L. By November 14, 2006, [TP] = 48 µg/L, for an increase of 12 µg/L (which is = 1.2 x 10^{-2} g/m^3).

2. The lake stage on Nov. 14 was 5177.1 ft (USGS DL stage data). Max pool stage is 5183.8 ft, thus the drawdown on Nov. 14 was 6.7 ft (2.042 m).

3. Lake volume at 6.7 ft. drawdown = 50,000 ac-ft, which is equal to 61,500,000 m^3 (6.15 x 10^7 m^3).

4. Increase in TP mass = lake volume X increase in lake concentration
= (6.15 x $10^7 m^3$) x (1.2 x 10^{-2} g/m^3)
= 7.38 x 10^5 g OR 738 kg

5. TP in Fish = 5470 mg/kg (wet weight; ave. analytical results for all fish size classes)
= 5.47g/kg (0.55% wet weight)

6. Mass of fish = 738 kg (increase in TP from #4)/0.0055 (proportion of TP in chub)
= 134,181 kg of fish (295,869 lbs)

7. Corrections for Inflow, Precipitation Inputs of TP (there was no outflow), and phosphorus bound in organic carbon (cyanobacteria + bacteria)

a. Volume of DL at treatment = 46,000 ac-ft = 56,580 m^3
Volume of DL on Nov 14 = 61,500,000 m^3
Increase in Volume = 4,920,000 m^3 (4.92 x 10^6 m^3)

b. Precip from 9/12 – 11/14 = 9.1 in (from DL SNOTEL site)
= 23.1 cm
Vol. of Precip on DL = 1226 ha (x 0.95) [correction for drawdown area]
= 1.165 x $10^7 m^2$ x 0.23m
= 2.68 x 10^6 m^3

c. Vol of Stream & GW inflow = increase in lake vol from precip
(assuming evap ~ zero)
= 4.92 – 2.68 = 2.24 x $10^6 m^3$

d. Mass of TP from Precip = (2.68 x 10^6 m^3) x (10 ug/ TP L)
= 2.68 kg TP

e. Mass of TP from Surface & GW = (2.24 x 10^6 m^3) x (80 ug/ TP L)
= 1.79 x 10 kg
= 17.9 kg TP

f. Mass of TP associated with increase in total organic carbon (TOC)
= 4.14 -3.48 = 0.66 mg/L C increase
= 6.6 µg/L P (assuming 1% P content)
= lake volume (6.15 x 10^7 m^3) x 6.6 x 10^{-3} g/m^3
= 406 kg of P associated with organic matter

8. Corrected Mass of Fish = (738 – 2.68 – 17.9 + 406)/(0.0055)
= 204,258 kg (450,389 lbs)

Note that this assumes that all the fish decomposed to elemental state by November 14, 2006 and that all of the phosphorus from the decayed fish mixed thoroughly in the lake. We have no data from Nov 15, 2006 to Feb 13, 2007 to determine if TP values increased after November 1, although data collected in October 2006 would suggest that TP values had stabilized. Perhaps most problematic is that this approach assumes that TP concentrations in the lake were well-mixed. The lake was isothermal on Nov. 14 and other constituents were the same from top-bottom (DO, pH, conductivity), but some of the phosphorus could have remained on the bottom and not mixed. Also, this estimate does not account for the release of phosphorus from decaying macrophytes during this period. Nevertheless, it offers one approach for arriving at an estimate of the mass of tui chub present in Diamond Lake at the time of the rotenone treatment. If this estimate (204,000 kg of tui chub) is accurate, then the pre-treatment netting and post-treatment collection of dead tui chub removed about 19 percent (46,712 kg/250,970 kg) of the tui chub present in the lake in summer 2006. Using an average weight of 25 g/fish for the tui chub yields about 8,170,000 tui chub killed in the rotenone treatment and about10,039,000 tui chub present in the summer of 2006.

Jackson and Loomis (2004), using CPUE data and population modeling, estimated that there were about 23 million tui chub present in Diamond Lake in 2002. If we assume that both estimates of tui chub abundance (10 million in 2006 and 23 million in 2002) are reasonable approximations, then it follows that there was a 57 percent decline in tui chub abundance between 2002 to 2006. One hypothesis is that this apparent decline represents natural fluctuations in tui chub abundance, perhaps associated with a reduction in the availability of food. However, examination of the tui chub collected in summer 2006 during the netting operations showed that the fish appeared robust and were not stunted. An alternate hypothesis is that much of the decline in the abundance of tui chub during that period was associated with tui chub leaving Diamond Lake through the canal during the draw-down operation from November 2005 through August 2006.

b. Rainbow Trout

Trout condition factor has been used as the sole criterion for fish (and benthic invertebrate) stocking strategies in Diamond Lake since 1980. The condition factor (K) is a dimensionless value based on the relationship between length (L) and weight (W) of a fish, where:

$$K = \frac{100 \bullet W(g)}{L^3 (cm)}$$

High values (e.g., 1.4) indicate fish with a high weight relative to its length. Values below 1.0 are indicative of fish that are very thin for their length. Values between 1978 and 1985 appeared to have stabilized at about 1.4, corresponding to a stocking regime of 400,000 Oak Springs stock rainbow trout fingerlings (Figure 69). After 1985, values began to decline, reaching a minimum of about 0.9 in 1997 when these measurements were discontinued. Following the rotenone treatment, the condition factors returned to values comparable to that observed from 1978-1985.

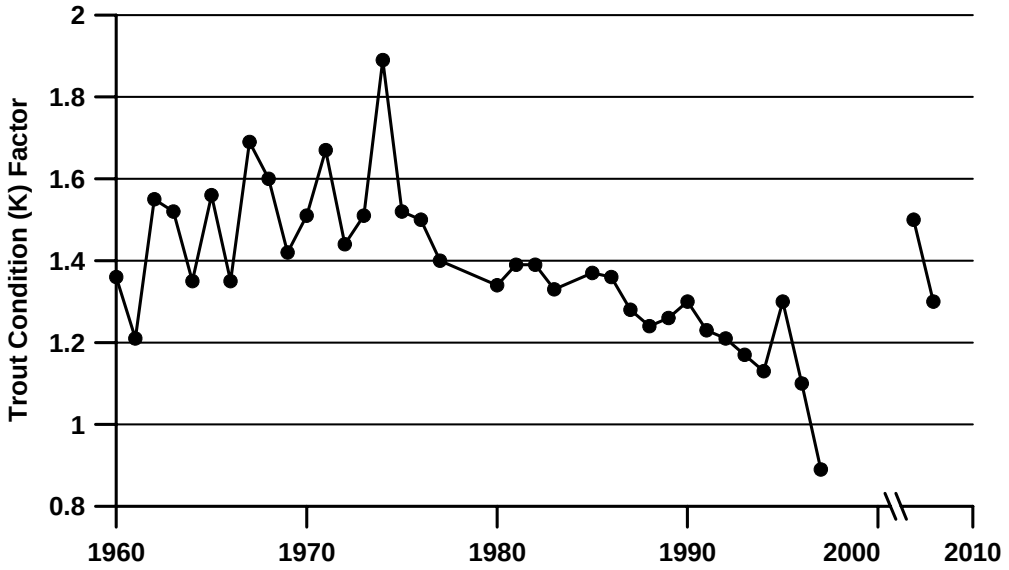

Figure 69. Trout condition factor in Diamond Lake from 1960 to 1997 and 2007-2008. Data provided by ODFW.

Trout stocking in 2007 included 100,000 fingerlings and another 84,400 fish from multiple sources and sizes. This included over 3,600 "trophy" fish weighing about 2.25 kg (5 lb) (Table 8). Stocking rates increased in 2008 to 200,000 fingerlings and nearly 86,000 larger fish (Table 9). From 1970-1992, stocking rates were typically about 400,000 fingerlings, with no stocking of larger fish. This changed in 1992, with the practice of stocking legal-size fish as the condition factor of the introduced fingerlings declined (Figure 69).

The stocking of fingerlings in 2007 and 2008 occurred on June 12 and June 10, respectively. However, these years differed in that ice off occurred on April 25 in 2007 and May 31 in 2008. Thus, the 2007 fingerling stocking occurred 48 days after ice-off, whereas the 2008 fingerling stocking occurred only 10 days after ice-off. Surface temperature on June 12, 2007 was 15.7 ºC, whereas it was 11.9 ºC on June 14, 2008, four days after stocking. The density of *Daphnia pulicaria* on June 12, 2007 was 4,701 individuals per cubic meter, whereas on June 14, 2008, only 588 individuals were present.

The growth rate of fingerling trout stocked in 2007 was 19.4 cm for the period June 12/13-October 31). This was exceeded in 2008 with a growth rate of 21.0 cm for the period June 10 – Oct 29/30. The condition factor average for 2007 was 1.5 (1.28-1.90; n=58), but dropped to 1.3 (1.21 – 1.39; n=14) for 2008. Both condition factor (Figure 69) and growth rate for 2007 and 2008 met the post-project goal under the recreational fishery element.

Table 8. Rainbow trout stocking in Diamond Lake, 2007.

Date	Number	Type	Abbrev	Weight (g)	Size (#/lb)	Length (in)
6/12/07	**100,010**	Fingerling	OS Fing	9.9	46/lb	
5/2/07	330	Trophy	Trop	2270	0.20/lb	
5/3/07	350	Trophy	Trop	2270	0.20/lb	
5/7/07	820	Trophy	Trop	2270	0.20/lb	
5/8/07	800	Trophy	Trop	2270	0.20/lb	
5/9/07	810	Trophy	Trop	2270	0.20/lb	
5/10/07	508	Trophy	Trop	2270	0.20/lb	
Subtotal	**3,618**					
4/26/07	6,090	Cape Cod	CC	156	2.9/lb	~8"
5/1/07	6,480	Cape Cod	CC	168	2.7/lb	~8"
6/7/07	5,034	Cape Cod	CC	189	2.4/lb	~8"
6/8/07	3,815	Cape Cod	CC	189	2.4/lb	~8"
6/9/07	200	Cape Cod	CC	189	2.4/lb	~8"
Subtotal	**21,619**					
5/1/07	4,000	Island Springs	IS	454	1/lb	~12-14"
5/2/07	3,799	Island Springs	IS	454	1/lb	~12-14"
5/3/07	3,700	Island Springs	IS	454	1/lb	~12-14"
5/4/07	6,870	Desert Springs	DS	467	1.03/lb	~12-14"
5/5/07	3,700	Island Springs	IS	454	1/lb	~12-14"
5/6/07	3,700	Island Springs	IS	454	1/lb	~12-14"
5/7/07	3,700	Island Springs	IS	454	1/lb	~12-14"
5/8/07	3,699	Island Springs	IS	454	1/lb	~12-14"
5/9/07	3,700	Island Springs	IS	454	1/lb	~12-14"
5/11/07	7,142	Desert Springs	DS	485	1.07/lb	~12-14"
5/23/07	7,065	Desert Springs	DS	481	1.06/lb	~12-14"
Subtotal	**51,075**					
9/18/07	**6,593**	Eagle Lakes	EL	712	1.57/lb	
6/12/07	**1,547**	Fishwich	FW	197	2.3/lb	~12"
Total	**184,462**					

Table 9. Rainbow trout stocking in Diamond Lake, 2008

Date	Number	Type	Abbrev	Weight (g)	Size (#/lb)	Length (in)
5/30/08	**6,227**	Oak Springs Trophy	OS	709	0.64/lb	~17-18"
6/10/08	**200,100**	Oak Springs Fingerling	OS Fing	10.4	43.5/lb	
8/12/08	**6,005**	Oak Springs	OS	137	3.3/lb	
5/19/08	4,185	Island Springs	IS	454	1/lb	~12-14"
5/23/08	6,808	Desert Springs	DS	463	1.02/lb	~12-14"
5/24/08	4,300	Island Springs	IS	454	1/lb	~12-14"
5/25/08	4,300	Island Springs	IS	454	1/lb	~12-14"
5/26/08	4,300	Island Springs	IS	454	1/lb	~12-14"
5/27/08	4,300	Island Springs	IS	454	1/lb	~12-14"
5/28/08	4,300	Island Springs	IS	454	1/lb	~12-14"
5/29/08	4,300	Island Springs	IS	454	1/lb	~12-14"
6/2/08	6,941	Desert Springs	DS	472	1.04/lb	~12-14"
6/10/08	7,208	Desert Springs	DS	490	1.08/lb	~12-14"
Subtotal	**50,942**					
7/24/08	7,300	Eagle Lakes	EL	151	3/lb	
7/24/08	7,505	Eagle Lakes	EL	151	3/lb	
Subtotal	**14,805**					
5/30/08	1,055	Fishwich	FW	107	4.22/lb	
8/19/08	6,752	Fishwich	FW	97	4.69/lb	
Subtotal	**7,807**					
Total	**285,886**					

Table 10. Trout and other salmonid stocking and harvest in Diamond Lake from 1910-2003 (after Eilers et al. 2007)

Year	Trout Caught	Stocking History			Notes
		Fry (2.5 cm)	Fingerlings (7.5 cm)	Legals (>15 cm)	
1910-38	15,000 to 50,000/yr	1,000,000			Trout up to 4.5 kg common
1939-45		2,000,000			Trout up to 3.6 kg caught; tui chub observed ~1941
1946	12,800	4,000,000			An estimated 68 million tui chub harvested with seine nets or killed by shoreline treatments with rotenone
1947	37,500	3,300,000			
1948	27,900	2,000,000			
1949	9,700	None			
1950	5,800			49,000	
1951	4,000			47,000	
1952	5,300			49,000	
1953	8,500			32,000	
1954	362	None; lake treated with rotenone			~32 million tui chub killed (~360 tonnes)
1955	Closed	530,000			Kamloops strain of rainbow trout stocked; trout caught averaged 33 cm
1956	61,400	250,000			
1957	55,100	300,000			
1958	46,900	1,014,000			
1959	22,600	1,000,000			
1960	33,500	1,063,000			
1961	35,200	1,175,000			
1962-69	265,700[a]		450,000[a]		Strain of trout changed to Oak Springs strain yielding a 10-fold increase in trout caught
1970-78	263,000[a]		380,000[a]		
1979-88	No data, although stocking rates and trout caught remained relatively stable				
1989	167,000				
1992			425,000	5,000	Tui chub found in lake
1993			350,000	14,000	
1994	56,400		425,000	5,000	
1995			412,000	7,500	
1996	70,100		350,000	10,000	
1997	42,000		400,000	7,700	
1998	12,500		395,000	7,500	
1999	5,100		430,000	13,000	
2000	20,000		60,000	53,000	
2001	13,000		50,000	46,000	Lake closures begin with intense *Anabaena* blooms
2002			90,000	65,000	Includes some spring Chinook
2003			90,000	99,000	~30 million tui chub present; includes some spring Chinook

DISCUSSION

The treatment of Diamond Lake with rotenone to remove the tui chub has yielded a large number of major changes in water quality and biological communities. The physical response has been a mean summer (June-Sept) increase in Secchi disk transparency from 1.6 m in 2006 to 7.1 m in 2007 and 6.1 m in 2008. Depth of 1 percent light extinction increased from 5.9 m in 2006 to 11.3 m in 2007 and 11.9 m in 2008. These changes have made it possible for macrophytes to re-colonize throughout the areas of the lake with suitable substrate. The increase in macrophyte extent of coverage has presumably led to a corresponding increase in macrophyte biomass. Greater macrophyte coverage would lead to greater uptake of nutrients by the macrophytes and thus greater competition between phytoplankton and macrophytes for the same resources. Previous sampling of benthic macroinvertebrates has shown that he invertebrates are more abundant in areas with macrophytes (Eilers 2003b). Thus it appears that the expansion of macrophytes into deeper water has provided more cover and more food resources for trout.

In-situ chemistry has shown major changes as well. The percent saturation of dissolved oxygen declined from a summer mean of 113 percent to 101 percent in 2007 and 104 percent in 2008. This reflects the reduction in algal primary production, which has also been offset to some degree by the increase in macrophyte production noted above. Mean summer pH declined from 9.5 in 2006 to 8.4 in 2007 and 7.8 in 2008. By 2008, all surface pH measurements were less than the state water quality standard of pH 8.5. The post-project goal was to have surface pH values be less than 8.5 for 90 percent of observations. This goal has been met.

Changes in water chemistry yielded some surprising results. Summer concentrations of total phosphorus and ortho-phosphorus showed no significant change from pre-treatment to post-treatment. This is consistent with the hypothesis proposed in the TMDL, whereby the high biomass of the tui chub caused accelerated cycling of phosphorus, but not necessarily an increase in phosphorus concentrations (Eilers et al. 2005). Concentrations of total nitrogen showed a significant decline from 2006 (472 µg/L) to 2007 (392 µg/L) and 2008 (298 µg/L). Concentrations of inorganic nitrogen showed no consistent pattern through this period, thus the decrease in total nitrogen can be attributed largely to a decline in organic nitrogen. This is consistent with a decline in algal biomass which sequesters much of the organic nitrogen. Both ammonia and nitrate showed significant increases in concentrations during 2007 compared to both 2006 and 2008. This is largely attributed to an early mixis in August 2007 that mixed ammonia that had accumulated in the hypolimnion in July 2007 into the surface waters. The ammonia was nitrified, contributing to elevated concentrations of nitrate not usually measured in the surface waters of Diamond Lake

during the summer. One of the most surprising changes in the nitrogen dynamics was the increased nitrification that continued under the ice in 2007/2008.

Silica concentrations showed a significant decline in 2007 compared to both 2006 and 2008. It's unclear if this was caused primarily by the equilibration of the groundwater inflows that occurred during the re-fill of Diamond Lake from November 2006 to July 2007 or if other factors such as elevated uptake of silica by diatoms in 2007 were involved. However, densities of diatoms and chrysophytes were low in 2007, suggesting that groundwater inputs played a greater role in affecting the silica concentrations in the lake during 2007. Concentrations of total organic carbon (TOC) exhibited a significant decline from 2007 to 2008, but not from 2006 to 2007. TOC is the sum of all reduced forms of carbon present in the water and includes dissolved organic carbon, algae, bacteria, viruses, and zooplankton. Without additional detail regarding the various compartments of carbon, it's difficult at this time to attribute the decline in TOC to any single source. The ratio of TN:TP is sometimes used to indicate limiting nutrients, whereby a mass ratio of N:P greater than 7.2 indicates P limitation and a values than that indicates N limitation. Nearly all values observed during the study period were considerably greater than 7.2, suggesting P-limitation. This is consistent with concentrations of ortho-phosphorus averaging only 2 to 4 µg/L during the summer.

Another way to visualize the changes in the nutrient dynamics associated with the rotenone treatment is examining concentrations of total nitrogen, total phosphorus, and total organic carbon at major reference points. Since these are total (unfiltered) results, they represent the combination of microorganisms, including phytoplankton, bacteria, and viruses. Figure 70 shows a trophochemical diagram of these nutrients through time. Prior to treatment in August 2006, the values of all three constituents were intermediate to the other reference dates. Shortly after the treatment in October 2006, the concentrations of all three constituents achieved a maximum, but then declined dramatically in July 2007. The change from July 2007 to July 2008 was small compared to the profound chemical changes that occurred following the treatment.

The biological responses to the rotenone treatment were also quite profound. Phytoplankton abundance as represented by chlorophyll *a* declined from a mean of 15.3 µg/L in 2006 to 2.7 µg/L in 2007 and 7.8 µg/L in 2008. The value of 7.8 µg/L chlorophyll in 2008 is somewhat misleading because it includes results from June 2008, which was a late ice-out year. Thus the values from the spring diatom bloom were included in computing this mean concentration. If the chlorophyll value from June 3, 2008 (29 µg/L) is omitted, the mean summer chlorophyll for 2008 drops to 4.9 µg/L. The decline in chlorophyll concentrations to less than 10 µg/L during the post-treatment period satisfies a second post-project goal relating to algae as specified in the TMDL. The June 2008

diatom bloom was unusual with respect to the species composition. Whereas most of the previous spring diatom blooms had been associated with species of *Synedra*, the 2008 spring bloom was almost entirely comprised of *Stephanodiscus*. This later genus may have out-competed the *Synedra* with the greater availability of nitrate in spring 2008.

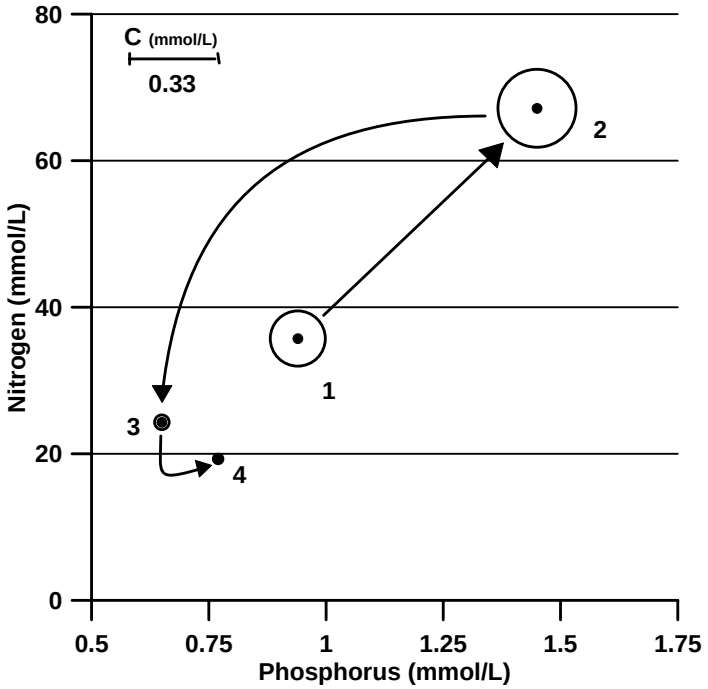

Figure 70. Changes in nutrient concentrations in Diamond Lake shown on four different dates where (1) August 9, 2006, (2) October 30, 2006, (3) July 4, 2007, and (4) July 30, 2008. The diameter of the circles is proportional to the concentration of carbon. After Sterner et al. (1996).

Clearly the most important result of the treatment regarding phytoplankton response is the major reduction of *Anabaena* from the lake. *Anabaena* biovolume declined from an average of 855,000 um^3/mL in 2006 to 29,000 in 2007 and 31,000 in 2008. This represents a 96 percent reduction in *Anabaena* biovolume from pre-treatment to post-treatment conditions. The density of *Anabaena* in Diamond Lake was considerably lower in 2006 compared to 2001-2005. This decline in *Anabaena* in 2006 was most likely caused by the draw-down of 40 percent of the lake volume in 2005/2006 and the likely reduction in the number of tui chub present in 2006 compared to those present in 2001 to 2003. Thus, if the effects of post-treatment response are based on the more stable conditions in 2001-2005, the decline in *Anabaena* biovolume would be even greater. The decline in cell density from 2006 to 2008 was comparable to that observed for Anabaena biovolume. Cell density of *Anabaena* declined 96 percent during post-treatment years from 12,570 cells/mL to 413

in 2007 and 464 in 2008 and no values exceeding 4,000 cells/mL in the summers of 2007 or 2008. This resulted in meeting the target post-project goal of having *Anabaena flos-aquae* cell density remain less than 15,000 cells/mL during the summer.

Although the project was highly successful in reducing the density of *Anabaena*, another N-fixing cyanobacteria taxon was found in Diamond Lake in 2008. *Gloeotrichia echinulata*, a large colonial species was observed on several occasions in Diamond Lake during summer 2008 and examination of a grab sample confirmed its presence (Figure 48). Because of its large size, it can easily fail to be included in samples and subsamples of phytoplankton. *Gloeotrichia* is typically found in eutrophic lakes (Reynolds 1984) and is thus a cause for concern. The analysis of cyanobacterial akinetes in the sediment of Diamond Lake showed that *Gloeotrichia* was present in greater abundance in Diamond Lake than was *Anabaena* from the 1970s through the 1990s (Figure 71). It appeared that *Anabaena* out-competed *Gloeotrichia* when the tui chub became abundant in Diamond Lake circa 2000. Although *Gloeotrichia* is seldom considered a toxigenic genus, there are examples where it has been associated with toxic blooms (Suseela 2008). The current phytoplankton sampling methodology is not adequate to document changes in abundance of a large colonial species such as *Gloeotrichia* and changes should be considered in the sampling protocol to reflect this new information.

The expansion of macrophytes to all depths in Diamond Lake represents a major biological change in post-treatment conditions. This change, made possible by the major improvements in light penetration, affects nutrient recycling, benthic habitat, and fish habitat. The extent, density, canopy height and species composition of the expanded macrophyte community should be monitored because of the ramifications of this development. The earliest measurements available on macrophyte distribution in Diamond Lake indicated that macrophytes (primarily *Nitella*) were present to a depth of 8 m (Lauer et al. 1979). If macrophytes are widespread down to 14 m, it would represent a major departure from conditions that have existed in the lake for over 40 years.

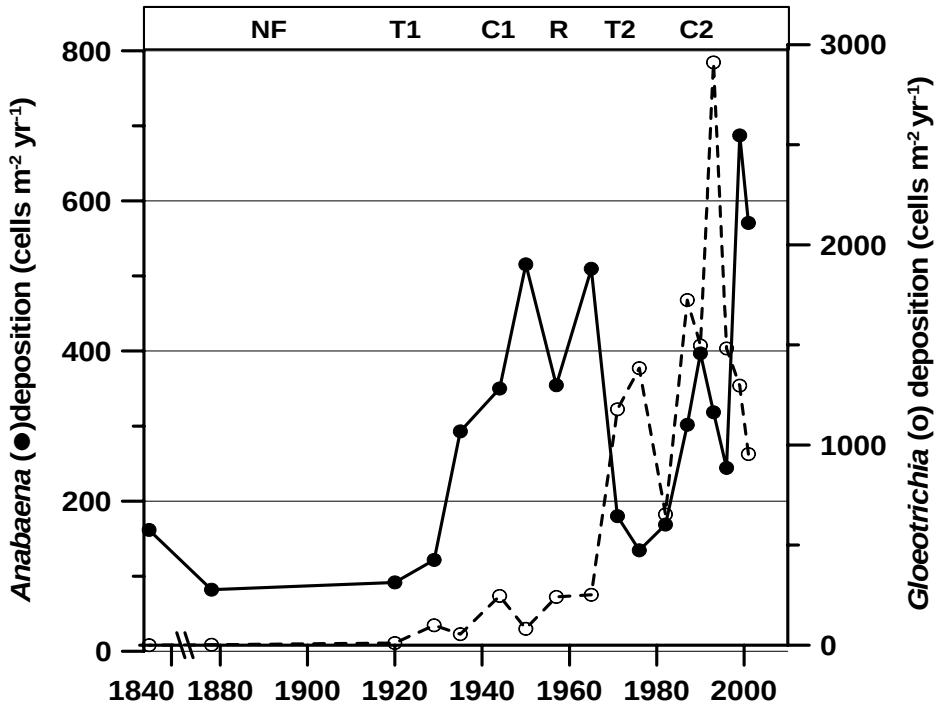

Figure 71. Deposition of *Anabaena* and *Gloeotrichia* in Diamond Lake as a function of year. The abbreviations at the top of the figure refer to: NF = no fish; T1 = 1st introduction of trout; C1 = 1st introduction of tui chub; R = rotenone. After Eilers et al. (2007).

Zooplankton community composition showed a major increase in density of individuals edible by trout and in taxa that have high algal grazing rates. Although the density of total cladocerans decreased from 2006 to 2008, the density of large cladocerans, such as *Daphnia pulicaria* and *D. rosea*, increased from none sampled in 2006 to a mean of 28,648 individuals per cubic meter in 2007. The density of large *Daphnia* remained relatively high in 2008, but declined to 7,617 individuals per cubic meter. A large copepod, *Epischura nevadensis*, was also present in large numbers in 2007 (up to 31,623 organisms per cubic meter on August 21, 2007), but was not sampled after August in 2007 and was present in very low densities in 2008. The results suggest that the stocking of 200,000 trout fingerlings on June 10, 2008 followed ice-out too quickly to allow the population of large daphnids to expand before the onset of heavy predation. This can be resolved in future years by timing the stocking of fingerlings to correspond with 4-6 weeks between ice-out and stocking. This should make it possible for the daphnids to expand, providing a food source for the fingerlings as well as serving as important grazers of the phytoplankton.

The total biomass of benthic macroinvertebrates increased steadily from June 2007 to July 2008 following the treatment. Biomass decreased in August and October 2008 from the peak in July 2008 of 36.9 g/m^2. Although the change of benthic biomass from 36.9 g/m^2 to 18.8 g/m^2 in October 2008 represents a decline of 49 percent, the October value is comparable to the long-term peak biomass values measured from 1956 to 1979 (Figure 62). What is unclear is whether the benthic biomass measured during that 24 year period constituted an optimal level from perspectives other than fisheries. The increase in benthic biomass was accompanied by an increase in the number of benthic taxa, with notable increases in non-dipteran insects and the amphipod, *Hyalella azteca*. Significant gains also were noted among leeches and gastropods. However, the density of chironomids declined by 83 percent between October 2007 and October 2008. The first inclination is to assume that the major decline in chironomid abundance is a direct consequence of predation by trout. However, other highly preferred prey groups showed either substantial increases in abundance (non-dipteran insects and amphipods) or no change (leeches and gastropods). One possible explanation for the decline in chironomids and concomitant increase in preferred prey species is that the chironomids took advantage of the large amount of organic matter remaining in the sediments through 2007. Once that food source was depleted, the density of chironomids returned to a value commensurate with the available food supply. Another sign of a healthy benthic community is the 63 percent increase in the number of benthic taxa and a 68 percent increase in the number of non-chironomid taxa from 2006 to 2008. It is reasonable to expect that additional benthic organisms that were extirpated by the tui chub will continue to re-colonize Diamond Lake over the next several years.

The rotenone project was apparently successful in removing all tui chub from Diamond Lake. Trap netting by ODFW have not yielded any additional tui chub (Truemper and Jackson 2009) and so the presumption is that the eradication program met its primary project goal of eliminating tui chub from the lake. The netting program, however, has found golden shiners (*Notemigonus crysoleucas*) present in Diamond Lake in 2008. Only several hundred of these fish have been collected and it is likely that this species will not flourish in Diamond Lake. Golden shiners were present in Diamond Lake from the 1970s and at no time did the population of these cyprinids become problematic (Truemper and Jackson 2009).

All estimates the number of tui chub in Diamond Lake yielded results with a high degree of uncertainty. Nevertheless, the estimates derived from multiple lines of evidence appear to produce somewhat comparable values. The number of tui chub present in 2002 appears to have been about 20 to 25 million fish (those great than 2 cm). The number of tui chub present in Diamond Lake in 2006 as estimated to be about 8 to 10 million fish. A reasonable explanation for the apparent

decline of tui chub from 2002 to 2006 is that most of these fish left Diamond Lake during the draw-down period from November 2005 to August 2006. The headgate at the canal was closed in early September, thus preventing any additional movement of fish prior to starting the rotenone treatment on September 14, 2006.

The growth rates of fingerling trout stocked in 2007 and 2008 were very high (19.4 and 21.0 cm, respectively). Fingerling trout from 7 to 10 cm stocked in June of each year were being harvested in the fall as 25 to 30 cm fish. The condition factor on these fish was also highly favorable ranging from 1.5 in 2007 to 1.3 in 2008. The number of fish used to generate the condition factor in 2008 (n = 14) was low and thus there is some uncertainty in this value. Nevertheless, both growth rate and condition factor of the stocked trout fingerlings in 2007 and 2008 were highly favorable. Trout survival, the third component of measuring a successful recreational fishery in Diamond Lake, from these two years is not yet available.

The response of a lake to any manipulation yields a variety of responses that can be measured to assess change. A number of lake indices have been created for this purpose. However, many of these indices fail to take advantage of a long history of measurements on Diamond Lake or reflect specific properties of the lake. Prior to the rotenone treatment such a group of lake indices was created to assess changes that would be expected to provide insight into lake processes and help guide the resource management agencies with decisions regarding the density and type of trout stocking. The goal was to create a robust recreational trout fishery without compromising progress towards achieving water quality standards. Implicit in this goal was the recognition that Diamond Lake was not a wilderness lake, but was to be managed for a variety of recreational pursuits.

The first attempt to create such a set of indices for Diamond Lake was produced by Eilers (2003) with funding from ODFW. Once additional data were compiled, this document was revised, specifically to include metrics for the trout fishery (Eilers et al. 2008). The metrics are arranged in four groups loosely representing water quality, primary production, secondary production, and trout success (Table 11). The intent was to generate a range of values of each of the selected metrics that encompassed either what had been experienced in the lake or what was considered possible. For most of these metrics, the index value for a given year is based on the "summer" period which was defined as June through September. We assumed that the index for any metric might be based on a small number of observations, thus we selected the median summer value as the best indication of central tendency. Exceptions to a summer-wide synopsis are metrics which refer to an extreme condition, such as the maximum depth in the lake which anoxia was experienced or the maximum density of *Anabaena* present during the summer.

Table 11. Values used to generate lake condition index values for Diamond Lake (after Eilers et al. 2008). "Epi DO" refers to median epilimnetic DO saturation during the summer. "Hypo DO" refers to the depth in the water column at which anoxia (< 1 mg/L DO) is present. "Chlorophyll" is the epilimnetic concentration of chlorophyll in the summer. "Secchi disk" is the median transparency observed during the summer. "*Anabaena*-median" is the median density of *Anabaena* observed during the summer. "*Anabaena*-maximum" is the maximum *Anabaena* density observed during the summer. "Benthic Biomass" is the average benthic biomass measured at all sites during October. "Amphipods" is the percent of individuals in the October sampling of the benthos that are amphipoda. "Large *Daphnia*" is the median sum of *D. pulicaria* + *D. rosea* present in vertical plankton tows of at least 12 m. "Trout Growth" is the gross increase in fork length of rainbow trout fingerlings stocked within the same calendar year (usually June-October). "K-factor" is the trout condition factor of trout sampled in October. "Trout Survival" is the percent of stocked trout surviving over one year. The "index" value is scaled from 1-10 where 0 is considerable "highly impaired" and 10 is considered "highly favorable".

Index	pH	Epi DO (%)	Hypo DO (m)	Chloro -phyll (µg/L)	Secchi Disk (m)	*Anabaena* (median cells/mL)	*Anabaena* (maximum cells/mL)	Benthic Biomass (lbs/ac)	Amphi -pods (%)	Large *Daphnia* (#/m3)	Trout Growth (in)	K-factor	Trout survival (%)
10	8	83	14	2	8	0	100	250	30	15000	6.5	1.7	80
9	8.1	85	13	4	7	10	1000	200	25	12000	6	1.65	70
8	8.2	87	12	6	6.5	50	2000	175	20	10000	5.5	1.6	60
7	8.3	89	11	8	6	100	3000	150	15	8000	5	1.5	50
6	8.4	91	10	10	5.5	300	4000	125	10	6000	4.5	1.4	40
5	8.5	93	9	12	5	500	5000	100	8	4000	4	1.3	30
4	8.7	95	8	14	4.5	1000	10000	75	6	2000	3.5	1.2	20
3	8.9	97	7.5	16	4	5000	50000	50	4	1000	3	1.1	15
2	9.1	99	7	18	3	50000	100000	25	2	100	2.5	1	10
1	9.3	101	6.5	20	2	100000	500000	10	1	10	2	0.95	5
0	9.5	103	6	22	1	200000	1000000	5	0	0	1.5	0.9	0

The results of the lake condition index applied to 2006-2008 show a dramatic improvement in nearly all metrics used in this index following the treatment in 2006 (Figure 72). pH values continued to improve from 2006-2008, spanning the range of expected values for the surface waters in the summer. In contrast, the epilimnetic dissolved oxygen showed a dramatic increase from 2006 to 2007 and a major decline in the value in 2008. This can be attributed to a diatom bloom (*Stephanodiscus*) in June 2008, following ice-out on May 31. The index was designed to represent summer conditions, yet it appears in cases with late ice-out that it captures the spring diatom bloom that is usually observed in Diamond Lake. The depth of hypolimnetic anoxia decreased substantially after the treatment. Had the comparison been made with data before 2006, when the

lake stage was at its usual level, the pre-treatment value would have been considerably lower than that observed in 2006 when the maximum lake depth was less than 12 m.

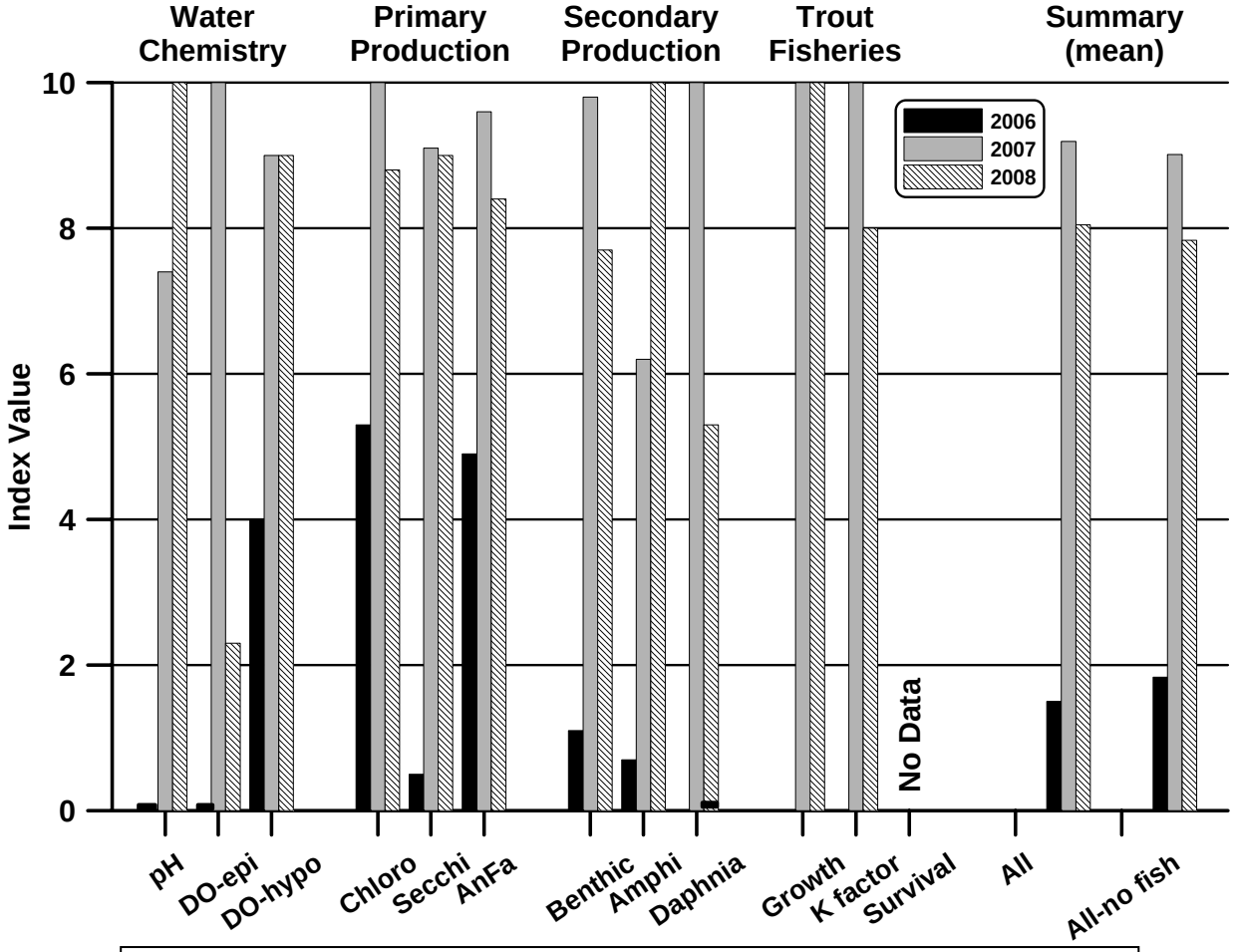

Figure 72. Lake Condition Index for Diamond Lake from 2006-2008 based on the approach presented by Eilers et al. (2008). The average index values are computed using all index values (All) and for all of the index values, except for the fish-related indices (All-no fish).

The lake showed substantial improvement (decline) in chlorophyll concentrations following the treatment. Again, had the pre-treatment data from earlier years been used, the disparity between the pre-treatment chlorophyll index and the post-treatment would have been greater. Secchi disk transparency increased greatly in 2007 and 2008. Use of a median Secchi disk value in 2007 obscured the remarkable peak in transparency which reached 12.5 m on June 26, 2007. The density of *Anabaena* declined by nearly three orders of magnitude from pre-treatment to post-treatment periods. Again, the density of *Anabaena* during the summer of 2006 was considerably lower that

observed during periods from 2001-2005. Nevertheless, the degree of improvement shown in the index value is considerable.
The biomass of benthic invertebrates increased an order of magnitude between 2006 and 2007. There was a noticeable decline in benthic biomass in late 2008 that could either be attributed to a decline in the available organic matter following consumption of the detritus remaining from the 2006 rotenone treatment or it could indicate an increase in fish predation on the benthic organisms. Although the total benthic biomass declined from 2007 to 2008, important prey organisms such as the amphipods increased by a factor of 2, resulting in a large rise in the proportion of amphipoda. Large species of *Daphnia* increased from not sampled in 2006 to impressively high densities in 2007. This was followed by a substantial decline in *Daphnia* in 2008. As noted earlier, it is likely that the decline in *Daphnia* in 2008 was attributed to the stocking of fingerling trout before the cladocerans had an opportunity to exploit their resources. However, with a doubling of the stocking intensity superimposed on this event, it is not possible to sort out the actual cause for the lower densities of *Daphnia* observed in 2008 at this time.

The growth rate of the trout fingerlings in both 2007 and 2008 was extremely high. Almost as impressive was the condition factors measured in 2007. The condition factor declined in 2008, although the cause of the decline is unclear. Three possibilities are offered: (1) the decline in chironomids and total benthic biomass caused a reduction in available food for the trout, (2) the decline in large cladocerans in 2008 also limited growth, and (3) the lack of high densities of *Daphnia* when the fingerlings were stocked contributed to low availability of food during a critical period for the fingerlings. Data on trout survival are not yet available for analysis.

The data show that the lake is still in transition. There have been major fluctuations in abundance of *Daphnia* with a 73 percent decline in mean abundance from 2007 to 2008. The apparent decline of benthic biomass is also cause for approaching the continued recovery of the lake with some degree of caution. Although *Anabaena* densities remained low, they doubled from 2007 to 2008, and concentrations of chlorophyll tripled during the same period. The stocking of fingerling trout too soon following ice-out in 2008 has also illustrated that the timing of actions needs to be considered in the post-treatment era, in addition to the magnitude of the management action. The presence of *Gloeotrichia* also needs to be addressed through a modification of the phytoplankton sampling protocols. Although the return of *Gloeotrichia* is not considered as serious as would a return of higher densities of *Anabaena*, the presence of *Gloeotrichia* suggests that productivity levels are rising in Diamond Lake. Additionally, indices to assess changes in Diamond Lake using measurements intended to characterize summer conditions should be adjusted to include the dates of June15-September 15, instead of June 1-September 30 as used in previous efforts of this kind.

In many respects, the rate and magnitude of recovery of Diamond Lake following the rotenone treatment exceeded the expectations of most biologists and water quality specialists involved in the project. All six water quality and fisheries post-project goals were met or exceeded. Water clarity reached levels not previously recorded and mean surface pH values declined from 9.5 in 2006 to 7.8 in 2008. Chlorophyll values were considerably lower than the state water quality goals and all targets in the TMDL established by DEQ were met or exceeded. *Anabaena* densities were no longer a concern and benthic biomass returned in under two years to values not measured since the 1970s. *Daphnia pulicaria*, absent prior to the treatment, returned to the lake in very high densities. The growth rate and condition factors of the stocking fingerling trout reached levels not seen since the 1980s. Thus, in nearly all respects, the rotenone treatment of Diamond Lake was a major success.

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APPENDICES

A. Methods used by Aquatic Analysts, Inc. for the identification and enumeration of phytoplankton in Diamond Lake 2006-2008.

May 4, 2004

Sample Handling
Sample Collection and Preservation

Phytoplankton is collected by filling bottles with natural water samples. Samples are collected at either discrete depths, or integrated through the photic zone of lakes. A volume of 250 ml is sufficient for most samples.

These samples are preserved with 1% Lugol's solution immediately after collection. Refrigeration is not necessary, and holding times are a year or more.
Sample Tracking

All samples received in the laboratory are immediately logged into a Sample Receipt Log. All samples are stored in a dedicated area until they are processed. After samples are processed and analyzed and data reports have been submitted to clients, samples are placed in storage for at least one year.
Sample Preparation

Permanent microscope slides are prepared from each sample by filtering an appropriate aliquot of the sample through a 0.45 micrometer membrane filter (APHA Standard Methods, 1992, 10200.D.2; McNabb, 1960). A section is cut out and placed on a glass slide with immersion oil added to make the filter transparent, followed by placing a cover slip on top, with nail polish applied to the periphery for permanency. A benefit to this method is that samples can be archived indefinitely; we have over 18,000 slides archived.

Microscopic Analyses
Algae Identifications

Aquatic Analysts has an extensive library of algae literature, including journal reprints, standard reference books, and internet reference sites. We also maintain files, notes, and photographs of algae we've encountered during the past 29 years of identifying algae. Most algae are identified by cross-referencing several taxonomic sources.
Enumeration

Algal units (defined as discrete particles - either cells, colonies, or filaments) are counted along a measured transect of the microscope slide with a Zeiss standard microscope (1000X, phase

contrast). Only those algae that were believed to be alive at the time of collection (intact chloroplast) are counted. A minimum of 100 algal units are counted. (Standard Methods, 1992, 10200.F.2.c.).

Biovolume Estimates

Average biovolume estimates of each species are obtained from calculations of microscopic measurements of each alga. The number of cells per colony, or the length of a filament, is recorded during sample analysis to arrive at biovolume per unit-alga. Average biovolumes for algae are stored in a computer, and measurements are verified for each sample analyzed.

Data Analyses and Reports

Sample Reports

Results of sample and data analyses are provided to the client in electronic format (email and/or CD disk), and in hard copies. Deliverables include individual sample reports, similarity indices, data summaries, combined species lists, and a brief narrative discussion of the results.

Individual sample reports include sample identification, a trophic state index, total sample density, total sample biovolume, and a list of algae species with their absolute and relative densities and biovolumes. All data are reported in Excel format.

Data summaries include sample identification, total density, total biovolume, the trophic state index, and the top 5 most common algae species (codes) and their relative densities. The summary format (Excel) allows for easy calculations and graphs of algae sample data.

Combined species lists of all species within related groups of samples allow greater sensitivity in comparing different lakes, sites, dates, or depth. Algae species are compiled according to their relative densities.

Trophic State Index

A Trophic State Index based upon phytoplankton biovolume has been developed from a data set of several hundred lakes located throughout the Pacific Northwest (Sweet, 1986, Report to EPA). The index was derived in a similar fashion as Carlson (1977) derived indices for Secchi depth, chlorophyll concentration, and total phosphorus concentration. The biovolume index ranges from 1 for ultra-oligotrophic lakes to 100 for hyper-eutrophic lakes. Values agree well with Carlson's indices.

The index is defined as:

$$TSI(\text{biovolume}) = (\text{Log-base } 2 \quad (B+1)) * 5$$

Where B is the phytoplankton biovolume in cubic micrometers per milliliter divided by 1000.

Similarity Index

A similarity index is useful in comparing phytoplankton communities between two samples. The index compares the relative abundances of each species present in two samples and yields a value ranging from 0 for totally dissimilar samples, to 100 for identical samples. The formula for the index (modified from Whittaker, 1967) is:

Similarity Index = 100 - (Sum of DIFFERENCE / 2)

Where DIFFERENCE is the absolute value of the difference of the percent density of a given species in two samples.

Quality Assurance

Microscope Calibration

Aquatic Analysts use a Zeiss Standard phase-contrast microscope primarily with a 1000X magnification for identification and enumeration of algal samples. The diameter of the field of view at 1000X magnification is 0.182 mm. The effective area of a filter is 201 millimeters square.

Algae are enumerated along a measured transect, measured accurately to 0.1 mm with a stage micrometer. The algal densities are calculated from the area observed (transect length times diameter of field of view), the effective filter area, and the volume of sample filtered.

The microscope was calibrated using a standard concentration of latex spheres provided by EPA (Cincinnati, OH). The concentration of these spheres was 12,075 per milliliter. Duplicate preparations of the standard spheres were analyzed; the average result was 11,700 spheres per milliliter (96.9 percent). The computer program used to calculate algae densities compensates for this 3.1% error.

Replicates

Replicate algae samples are analyzed at the client's request. We encourage blind replicates for approximately 10% of all samples collected. Replicates are assessed for algae abundance (relative mean difference of densities) and species composition (similarity indices, species lists).

Independent Analyses

Aquatic Analysts has participated in the analyses of split algae samples on several occasions, with general agreement between samples in terms of algae density and algae species compositions.

Internal Data Verification

A custom computer program handles all calculations and data analyses. Final sample reports are compared with laboratory bench sheets before releasing data.

Data summaries, tables of similarity indices, abundance graphs, and combined species lists are searched for inconsistencies, outliers, and interrupted patterns that may indicate possible errors.

Archives

Aquatic Analysts maintains an herbarium of all microscope slides analyzed (over 18,000 to date). These may be reviewed if questions arise after data are reported. In addition, all computer data (sample tracking data, raw count data, final reported data, data analyses, narrative reports) are archived on CD's in permanent storage.

B. Methods for used by ZP Taxonomic Services for Identification and Enumeration of Zooplankton Organisms Collected for the Diamond Lake Project 2006-2008

Standard Zooplankton Counting and Assessment Methodology Used by ZP's Taxonomic Services, Allan Hayes Vogel, sole prop.

The standard zooplankton sample enumeration uses the following methodology. Samples are first split with a Folsum plankton splitter until an approximate subsample size of 400 total individual arthropods and 100 individuals of the most abundant species are reached. If the initial split does not achieve both of these criteria, then increasingly larger splits are enumerated until both criteria were met, or until the entire sample is counted. All rotifers and protozoans in the split are completely enumerated as well unless their numbers significantly exceed 400 individuals; in which case, a separate rotifer subsplit is made, then counted for rotifers and protozoans. The statistical methodology for this approach is based upon Edmondson and Winberg (1971, p. 178), and assumes that the sampling methods (both in the field and during the splitting) follow a Poisson distribution. This assumption is violated for larger species such as *Chaoborus* and *Leptodora*; thus, all individuals of those taxa found in a sample are enumerated. The selected values of 400 and 100 individuals provide a maximum statistical standard error of the mean of 5 and 10 percent, respectively (The formula used is: $s = 1/\sqrt{N}$). While only the confidence limits for total numbers and most abundant species are set by this procedure, the standard error of the mean for each species can be determined from the original tallies, using the previous formula for the Poisson distribution. Results are reported in numbers per cubic meter, along with the standard error for each value (also in units of numbers per cubic meter).

The standard zooplankton enumeration is done with a Wild M-3 microscope at 32X magnification. Samples are counted in an open counting chamber with six parallel channels following the procedures described in Edmondson and Winberg (1971, p. 131). Species identifications are made at higher levels of magnification under a compound microscope as needed. General taxonomic identifications follow Edmondson (1959), Pennak (1989), and Thorp and Covich (1991). Specific group references used include Berner (1994), Brooks (1957), Brandlova, et al. (1972), Deevey and Deevey (1971), DeMont and Hebert (1994), Dumont and Pensaert (1983), Hebert (2001), Korovchinsky (1992), Patterson (1996), Pontin (1978), Ruttner-Kolisko (1974), Stemberger (1979) and Taylor, et al. (2002). Identifications are to species for all adult and subadult crustaceans, excepting harpacticoid copepods and ostracods, and for most rotifers. Immature copepods through copepodite stage IV are identified as far as their developmental stage allows. Confirmation of the identifications is made using appropriate past local investigations.

For length-frequency studies, crustacean lengths are taken following the protocols described in Edmondson and Winberg (1971. Specifically, cladocerans are measured from the top of the head (helmet included) to the posterior edge of the carapace excluding any tail spine or mucro, and copepods are measured from the end of the cephalothorax to the end of the caudal rami, exclusive of the setae.

The basic quality control method used for enumerating zooplankton samples is the standard error value. Standard error is an estimator of within-sample variability; it is not a between-sample estimator of the population variance such as the statistical parameter, standard deviation, provides. Statistical analyses of past replicated counts have indicated that the standard error values adequately estimate between 90 and 98% of all within-sample variability. Since within-sample variability is <u>not</u> between-sample variability, it is recommended that several replicate samples be collected as part of any standard field research effort to assess between-sample variability. This recommendation is made because between-sample replicates taken at the same time and place have significantly higher variability due to plankton patchiness and species "swarms".

Quality assurance and control for within-sample variability is maintained by routine re-analysis of 2-3% of all samples examined. The samples re-analyzed are selected at random, using a random number generator and the unique sequence number of each sample analyzed. (Unless specifically requested, the results of these re-analyses are not provided.) For major projects (> 500 samples), 5-10% of the samples are re-enumerated and identified a second time “out house”.

An estimate as to the intensity of planktivory based upon the density and relative abundance of the edible species present index is normally made for each sample as well as Dodson's (1992) index calculated for each lake, provided all necessary background information is available. Evaluation of the availability of the different zooplankton species as food items for particular species of fish is derived from an ongoing literature review starting with Brooks (1969) and continuing with Kerfoot (1980), Zaret (1980), and Carpenter and Kitchell (1993). This evaluation is kept up-to-date by regular reviews of recently published zooplankton predation studies in Limnology and Oceanography and the Proceedings of the International Association of Theoretical and Applied Limnology (Verh. int. Ver. Limnol.) as well as the results of articles such as Eilers, et al. (2007). The earlier literature has been summarized in Canale, et al. (1975, 1976).

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Analytical Data

The results of the duplicate analyses run by CCAL showed only one analyte, total phosphorus, which exhibited a statistically significant difference between duplicate samples (Table 2). In this case, the mean difference of 4 µg/L is small with respect to the measurement process (Table 1) and also small relative to ecological changes in the lake.

The blank samples submitted to CCAL, although few in number, showed consistent results (Table 3). Total organic carbon (TOC) and alkalinity showed consistent measurable concentrations in the blanks. However, once again, these concentrations are one to two orders of magnitude lower than the concentrations encountered in Diamond Lake. One issue is that concentrations of TOC in the tributaries are close to the values reported in the blank samples. The alkalinity value measured in the blank (which theoretically should have been zero) also presents a problem for the measurement of alkalinity in the snow samples.

The results of the split samples from CCAL and Aquatic Research, Inc. show good agreement and no apparent bias between the laboratories (Table 4). Total nitrogen analyses show moderately high variability, but these were for samples with concentrations up to 1 mg/L TN. The results for total phosphorus had the highest level of significance (P= 0.093), suggesting that the laboratories had relatively good precision, but may have a slight bias.

The comparison of the two types of filters show no significant difference in the results for the three filtered analytes tested (Table 5). Although silica was not included in this round of comparisons, we have no reason to conclude that a change in the filtration process has altered the measurement of dissolved constituents measured for this project.

Table 2. Statistical analyses of duplicate samples run by the CCAL laboratory. The P value is based on a paired T test with $H_0 = 0$.

Analyte	Units	N	Mean Difference	Standard Error	P Value
TN	*µg/L*	27	17.6	15.0	0.251
TP	*µg/L*	26	3.92	1.80	0.039*
NO_3	*µg/L*	18	0.11	0.14	0.430
NH_3	*µg/L*	21	1.24	1.51	0.421
PO_4	*µg/L*	17	0.12	0.12	0.332
Si	mg/L	8	0.023	0.022	0.349
TOC	mg/L	13	0.048	0.118	0.692
pH	su	14	0.007	0.007	0.336
Alkalinity	mg/L HCO_3	13	0.017	0.015	0.289
Sp. Cond.	*u*S/cm	14	0.16	0.18	0.379

Table 3 Analytical results from the CCAL laboratory of blind blank samples of reagent grade deionized water.

Analyte	Units	N	Mean	Minimum	Maximum
TN	*µg/L*	4	5	0	10
TP	*µg/L*	5	1.2	0	4
NO_3	*µg/L*	4	0	0	0
NH_3	*µg/L*	4	0	0	0
PO_4	*µg/L*	4	0	0	0
Si	mg/L	1	0	0	0
TOC	mg/L	5	0.23	0.12	0.4
pH	su	5	5.7	5.6	5.8
Alkalinity	mg/L HCO_3	5	0.3	0.34	0.27
Sp. Cond.	*u*S/cm	5	1.1	1	1.3

Table 4. Statistical analyses of split samples between CCAL and Aquatic Research, Inc. The P value is based on a paired T test with $H_0 = 0$.

Analyte	Units	N	Mean	Standard Error	P Value
TN	*µg/L*	10	62.5	51.7	0.257
TP	*µg/L*	10	12.1	6.5	0.093

NO_3	*µg/L*	11	0.36	0.45	0.440
NH_3	*µg/L*	11	4.73	3.89	0.253
PO_4	*µg/L*	10	1.7	2.2	0.459
Si	mg/L	6	0.18	0.15	0.279
TOC	mg/L	6	0.29	0.25	0.298

Table 5. Statistical results for paired T test with $H_0 = 0$ for differences between samples filtered with Whatman glass fiber filters and Geotech 0.45 micron capsule filters.

Analyte	**Units**	**N**	**Mean**	**Standard Error**	**P Value**
NO_3	*µg/L*	14	0.07	0.22	0.752
NH_3	*µg/L*	14	5.0	6.2	0.434
PO_4	*µg/L*	13	2.08	2.42	0.407

In 2006, results for dissolved and total dissolved organic carbon (DOC and TOC) were similar. It was expected that there would be a measurable difference between the dissolved and total phases because of the abundant algal population present in 2006. A comparison of DOC and TOC for the 2006 samples showed no significant difference between the analytes, regardless of the filters used for processing the samples (Figure 1). We were unable to explain this apparent discrepancy and therefore discontinued the measurement of DOC in 2007 and 2008.

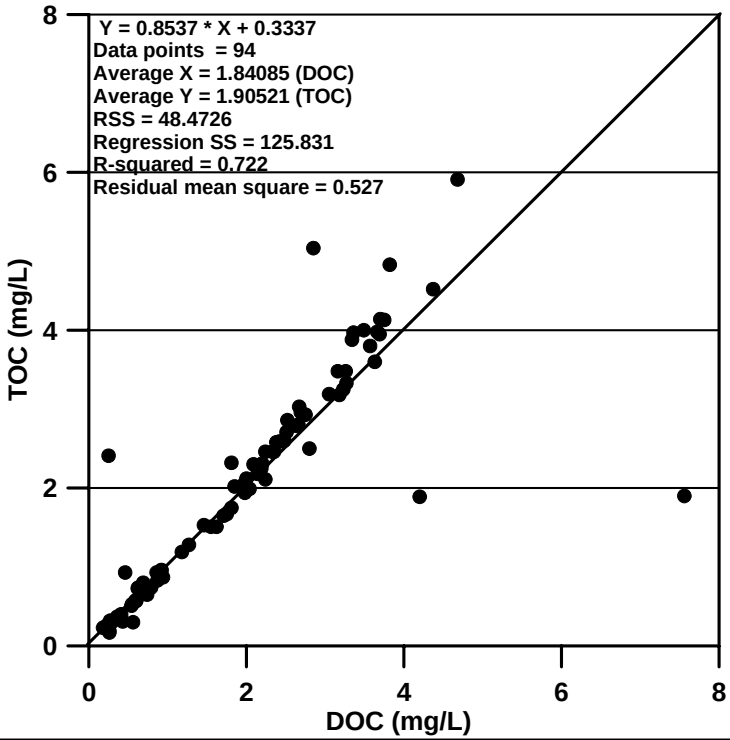

Figure 1. Comparison of TOC and DOC for samples from the Diamond Lake Restoration Project collected in 2006. A paired T test of the differences in the means was not significant (T= 0.84; P = 0.402).

Phytoplankton Taxonomy

The split phytoplankton samples were evaluated for comparability with respect to number of taxa represented, cell density, total biovolume, and species composition. PhycoTech sample results showed a statistically significant ($P \leq 0.05$; Wilcoxon one-tailed paired sample test) greater number of taxa present, cell density, and total biovolume compared to Aquatic Analysts (Figures 1-3). Statistical comparisons between the samples analyzed by GreenWater and the other two laboratories were not conducted because of the small sample size. Large differences in cell density between the two laboratories can be explained, in part, because Aquatic Analysts does not count picoplankton and PhycoTech (and GreenWater) does count these very small taxa. However, when density of dominant genera was examined, it was also observed that PhycoTech reports greater cell density (as does GreenWater).

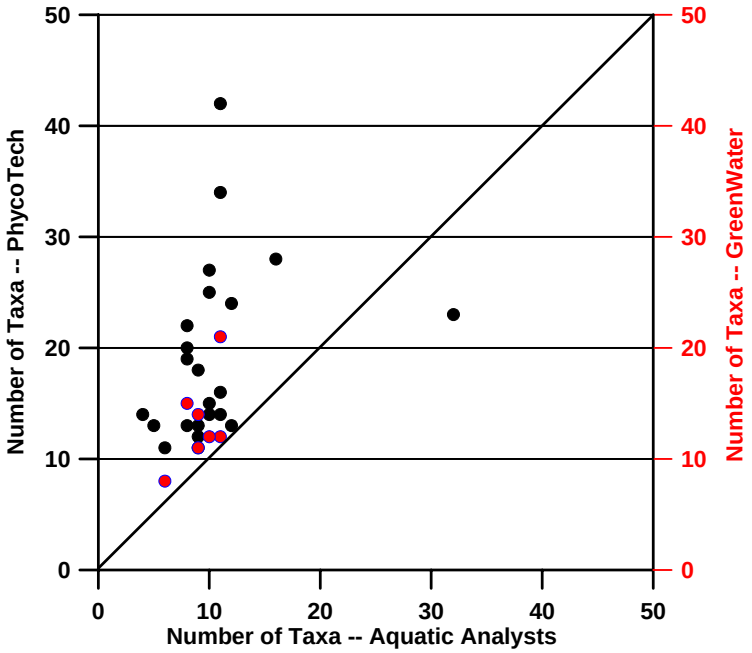

Figure 1. Number of phytoplankton taxa recorded by PhycoTech and Aquatic Analysts (black) and GreenWater and Aquatic Analysts (red) based on split samples collected in Diamond Lake in 2006 and 2007.

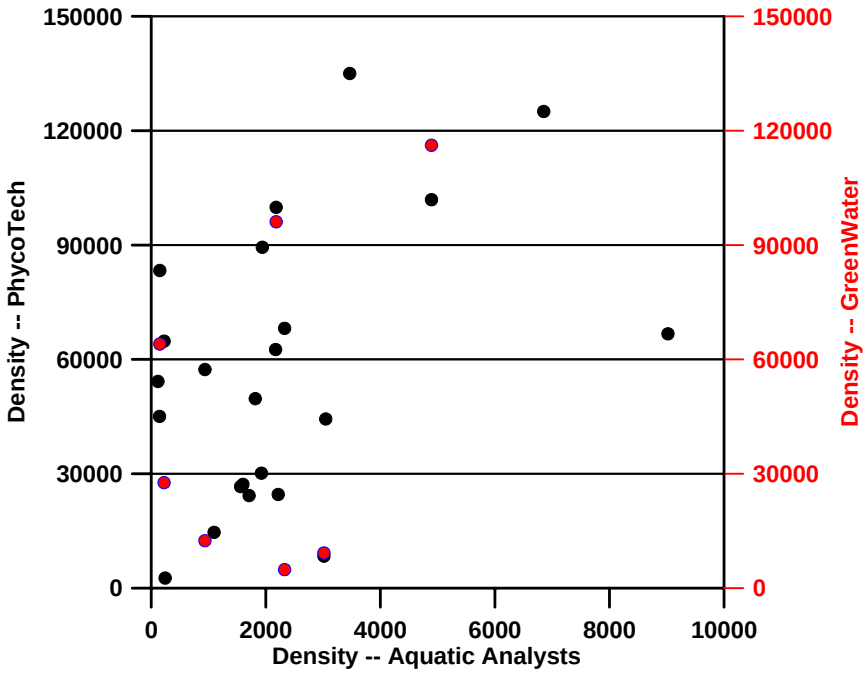

Figure 2. Algal cell density (#/mL) from phytoplankton samples recorded by PhycoTech and Aquatic Analysts (black) and GreenWater and Aquatic Analysts (red) based on split samples collected in Diamond Lake in 2006 and 2007. Note that the axes are scaled differently.

A comparison of dominant taxa, based on biovolume, between the samples analyzed by the two laboratories showed poor agreement between dominant taxa. Both laboratories identified the same species as dominant in only 4 of the 23 (17%) split samples. The most common discrepancies were noted for *Anabaena and Synedra*. Aquatic Analysts identified most of the cyanobacteria as being dominated by *A. flos-aquae*, whereas PhycoTech typically identified these as *A. circinalis*. Aquatic Analysts identified most of the *Synedra* as *S. delicatissima* and *S. radians*, whereas PhycoTech most commonly identified the dominant *Synedra* as *S. ulna*. When the comparison is based on genera, the laboratories identified the dominant genera as the same in 17 of the 23 samples (74%).

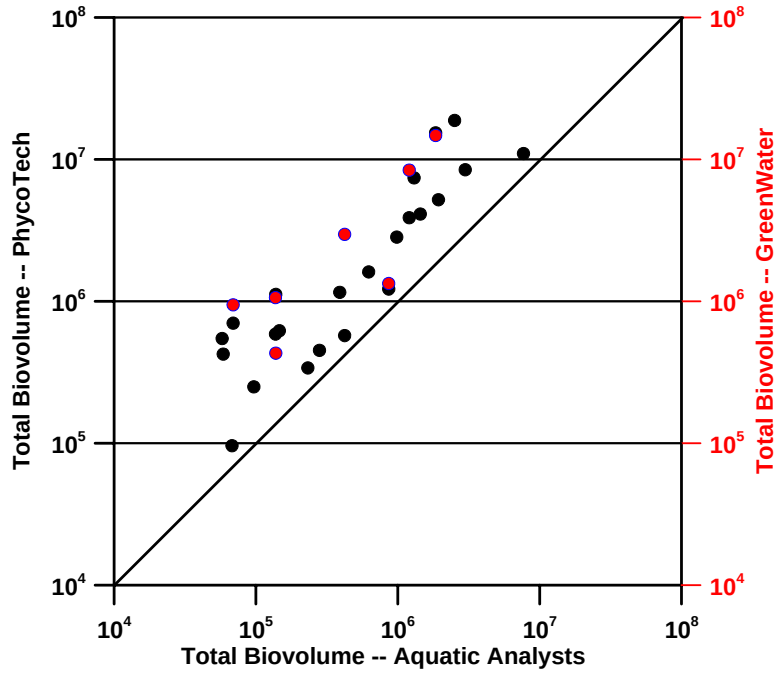

Figure 3. Total biovolume (μm^3/mL) of phytoplankton reported by PhycoTech and Aquatic Analysts (black) and GreenWater and Aquatic Analysts (red) based on split samples collected in Diamond Lake in 2006 and 2007.

The sample taxa for these 23 split samples were grouped into ecologically important groups for Diamond Lake and compared based on percent biovolume. The results show generally similar trends in phytoplankton groups (Figure 4). The split samples show a diatom-dominated lake in July, 2006, transitioning to a cyanobacteria-dominated system in September 2006. The lake moves to a strongly *Synedra*-dominated system in spring 2007 and rapidly changes to a much more diverse

assemblage in June, 2007. On an individual sample basis, there are distinct differences that are noteworthy. In February and March 2007, under the ice, Aquatic Analysts continued to identify the dominant cyanobacteria taxon as *Anabaena flos-aquae*, whereas PhycoTech identified the cyanobacteria as *Oscillatoria limnetica*. This taxon is more likely to be *Oscillatoria* based on its common occurrence in ice-covered lakes. PhycoTech also identified a greater number of taxa in less common groups such as Euglenophyta and Chlorophyta.

The comparison of the seven sets of triplicate samples shows general agreement among the three laboratories when general groups of phytoplankton are considered (Figure 5). However, GreenWater tended to show greater biovolumes of cyanobacteria and chlorophytes and lower biovolumes of chrysophytes compared to the other taxonomists. When comparing genera and species among the three laboratories, there are major differences, even among dominant taxa. All three sets of taxonomists agreed on identification of *Synedra* as the dominant taxon in several samples, but there was no agreement among the laboratories with regard to species. In the February, 2007 sample taken under the ice, all three taxonomists identified cyanobacteria as the second most abundant group, but Aquatic Analysts identified it as *Anabaena flos-aqua,* PhycoTech as *Oscillatoria limnetica*, and GreenWater as *Geitlerinema/Jaaginema*. The likelihood of the taxon being either *Geitlerinema* or *Jaaginema* is low because these genera are typically attached, benthic forms (Wehr and Sheath 2003). Other occurrences of *Anabaena* were not identified as the same species in any of these samples. Aquatic Analysts typically identified most of the *Anabaena* as *A. flos-aquae*, PhycoTech as *A. circinalis*, and GreenWater as *A. mendotae*.

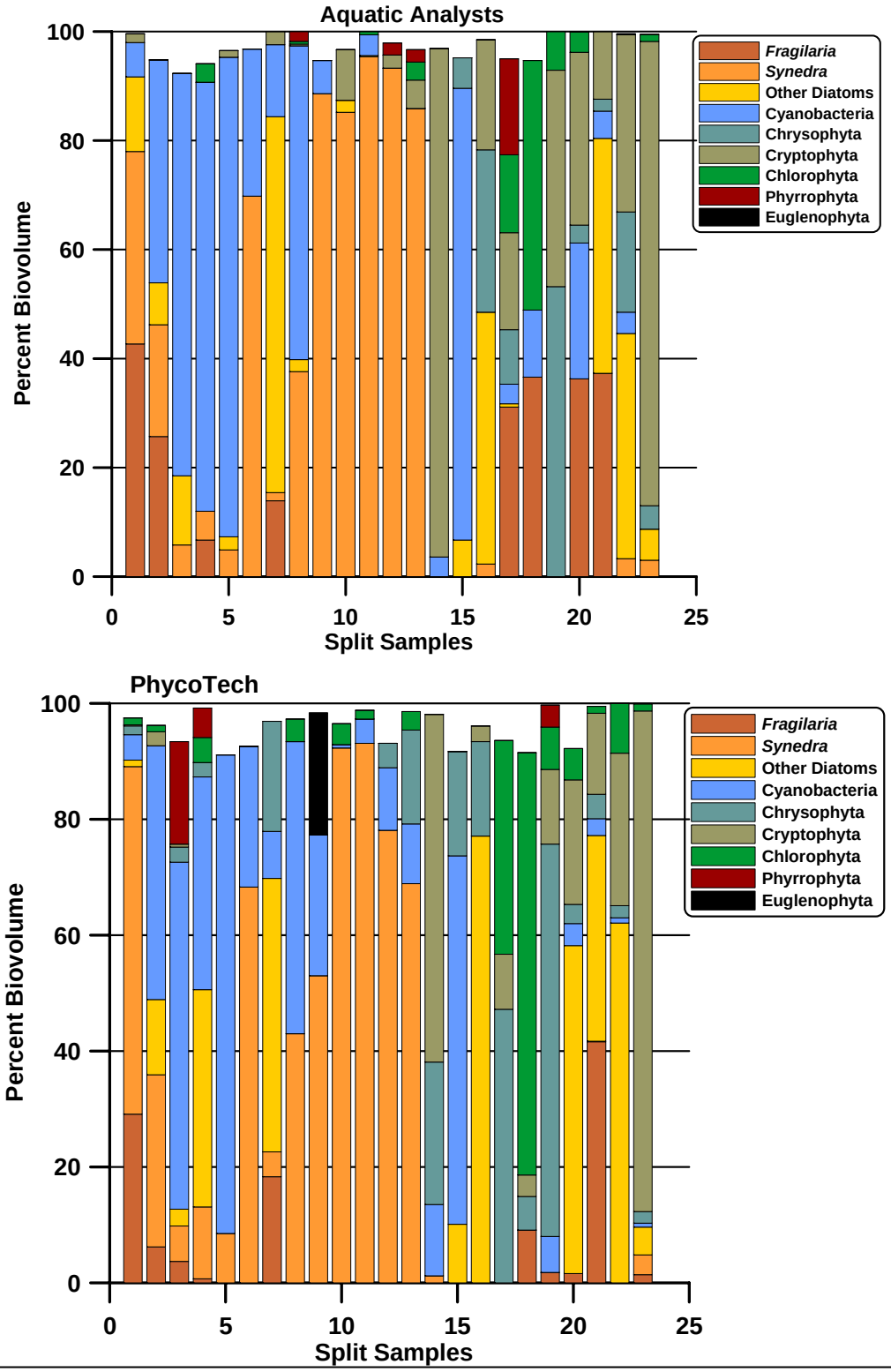

Figure 4. Comparison between ecologically important groups among dominant taxa (based on biovolume) for split samples between Aquatic Analysts (top) and PhycoTech (bottom) in Diamond Lake for 2006 and 2007. The samples are arranged by date, starting in July, 2006.

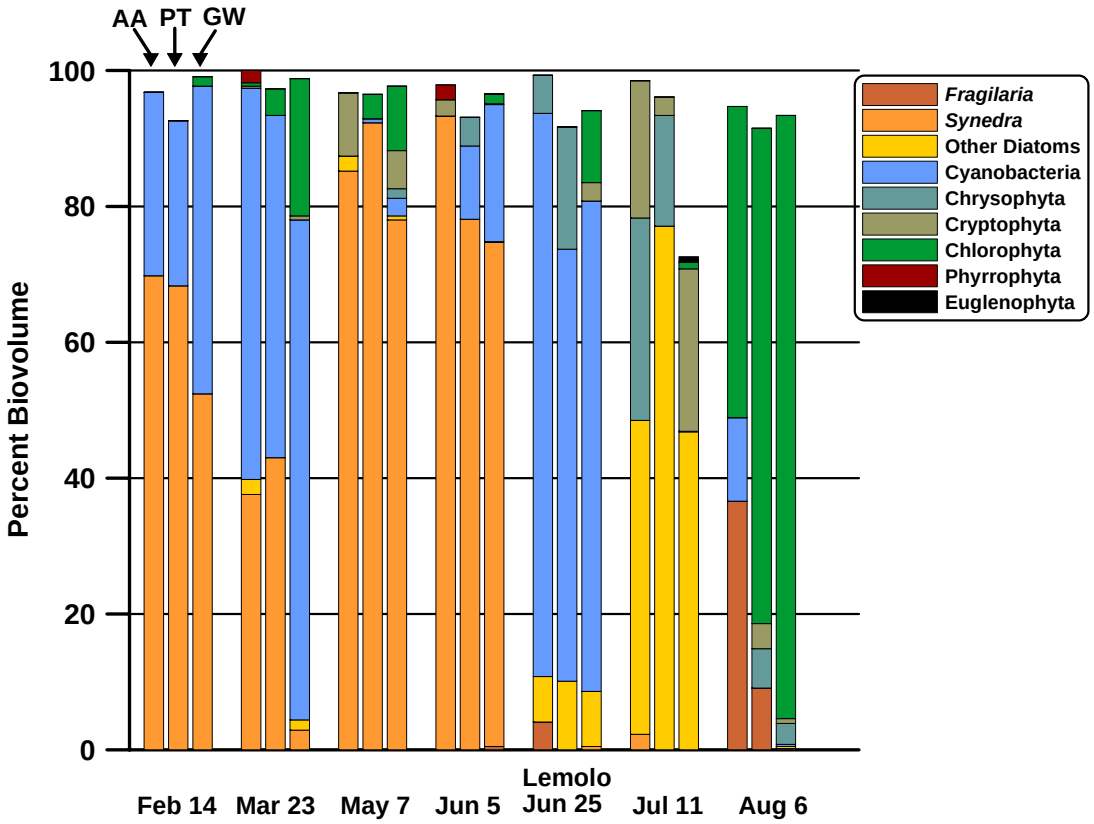

Figure 5. Comparison of seven split samples among the three laboratories showing the dominant taxa and groups of phytoplankton. The first set in each set of triplicates is Aquatic Analysts, the second is PhycoTech, and the third is GreenWater. All samples are 1 meter samples from site DLA, except for the one sample from Lemolo Lake.