Technical Memorandum 86-68220-11-04

Zebra and Quagga Mussels: Pre- and Post- Impacts of Zebra/Quagga Mussels on the Physical, Chemical, and Biological Attributes in Pueblo Reservoir

prepared by

Davine Lieberman and G. Chris Holdren
Environmental Applications and Research Group (86-68220)
Technical Service Center
P.O. Box 25007
Denver, CO 80225

U.S. Department of the Interior
Bureau of Reclamation

This page intentionally left blank.

Table of Contents

Section **Page**

List of Figures in Appendix A

List of Figures (Continued)

This page intentionally left blank.

1.0 Introduction

Pueblo Reservoir is located on the Arkansas River in Pueblo County about six miles west of Pueblo, Colorado. The reservoir has a concrete dam with over 60 miles of shore line; 5,664 acres of surface water, and a total storage capacity of 357,678 acre-feet.

Zebra mussel veligers were first collected and identified from Pueblo Reservoir in Colorado in November 2007. These findings were tested and confirmed by DNA testing in 2008. Veligers were most numerous during 2008 and included confirmation of both zebra and quagga mussels. During 2009, veliger abundance diminished drastically for unknown reasons. In 2010, there was only one veliger confirmation from the reservoir, and no adult mussels have been found to date. A rigorous collection program, which began in 2008, should have been sufficient to find veligers in each sample tow if they were present in the reservoir.

This study was intended to focus on the consequences that zebra/quaggas could have on limnological aspects of the reservoir. As the study progressed, it was obvious that the main focus of the study could not be undertaken since adults have not been found to date, and the number of veligers decreased as the study progressed. Therefore, the main objective of this study, is to look at the physical, chemical, and biological characteristics of the reservoir, and to suggest what may have occurred in terms of the disappearance of veligers in Pueblo Reservoir. Unfortunately, the data collected was not sufficient to provide a good explanation for the declining veliger numbers in the reservoir.

All figures for the report are included in Appendix A. Monitoring data (Appendix B) for the project is included as an Excel spreadsheet (Pueblo Reservoir.xlsx).

This page intentionally left blank.

2.0 Methods and Materials

Sampling collections at the 6 sampling stations in Pueblo Reservoir were conducted monthly in June 2008 (one sampling event), May through September 2009 (5 sampling events), and March through October 2010 (8 sampling events). There were six sampling stations located at Pueblo Dam (PR-1), North Marina (PR-5), mid-reservoir (PR-2), upreservoir (PR-3), farthest station in uppermost part of reservoir (PR-4), and buoy by South Marina (PR-6).

At all sampling stations, water temperature (°C), pH, dissolved oxygen (mg/L), and specific conductance (µS/cm) depth profiles were taken at one meter intervals from surface to bottom depths, with a YSI multi-parameter probe.

Secchi disk transparency readings were taken with a 20 cm diameter black and white secchi disk with and without the aid of a 26 inch Aqua View II viewscope. The disk was lowered into the water with a metered line until it disappeared from sight, then it was brought up until visible, and then lowered once again until it disappeared. At this point, the depth of the secchi disk was recorded in meters.

A surface and composite sample (0 to 5 m) were collected for chlorophyll *a (*chl *a*) analysis. A flexible swimming pool hose weighted and connected to a measured line at one end was lowered into the water column to a depth of 5 m, the hose end on the boat deck was plugged with a rubber stopper, then the weighted end was pulled up and put into a 20 g bucket, the stopper was removed, and water was drained into the bucket and collected in 1 L Nalgene bottles. All samples were typically filtered immediately on the boat. From each water sample, 250 mL of water was filtered through a 47 mm Whatman GF/C filter, the filter was folded, wrapped in foil, placed in a coin envelope, and immediately stored on dry ice. In the lab, filters were put in the freezer until analyzed for chl *a* and pheophytin using a spectrophotometer according to Standard Methods (Method 10200 H (2), 1998).

Water samples for nutrient analyses and ions were collected with a Kemmerer from one meter below the surface and one meter above the bottom depth. The following nutrients were analyzed: soluble reactive phosphorus (SRP) (0.001 mg/L), method detection limit (mdl), method EPA 353.2), total phosphorus (0.003 mg/L mdl, method EPA 365.1), nitrite-nitrate (0.003 mg/L mdl, method EPA 365.1), ammonia nitrogen (0.003 mg/L mdl, method EPA 350.1) and total Kjeldahl nitrogen (0.003 mg/L mdl, method EPA 351.2). Calcium ion was analyzed using 3111B (0.2 mg/L mdl, Standard Methods, 1998).

Zooplankton samples were collected with a 64 µm mesh Wisconsin-style plankton net from surface to 15 m at most stations, or to one meter above the bottom, if depth was shallower than 15 m. Samples were preserved with Lugol's iodine solution in dark 250 mL Nalgene amber

bottles. Aliquots from samples were placed in an Utermohl chamber where zooplankton were counted at 100x magnification until at least 200 individuals were tallied by BSA Environmental Services. Identification followed Edmundson (1959), Ruttner-Kolisko (1974) and Pennak (1989). Biomass estimates were based on established length-width relationships (Dumont et al. 1975, McCauley 1984, Lawrence et al. 1987). The lengths or the lengths and widths of each species were measured equal to 10 for common species and lesser for more rare taxa. For cladocerans, length was measured from tip of head to end of body (shell spines excluded). For copepods, length was determined from tip of head to insertion of caudal ramus. For rotifers, length was measured from tip of head to end of body (spines, toes, other appendages excluded). In accordance with McCauley (1984), biomass was computed for appropriate number of individuals for each sample location and arithmetic mean biomass was multiplied by species abundance to equal a species biomass per sample.

Composite phytoplankton samples representing 0 to 5 m depth interval were collected using a swimming pool hose. The weighted end of the hose was lowered into the water column to 5 meters and a rubber stopper was inserted into the other end on deck. The weighted end was pulled out of the water and placed into a 20 L bucket; then water was released by uncorking the hose. Composite samples were collected in 250 mL Nalgene amber bottles. Samples were preserved with Lugol's iodine solution to tea color (about 4 mL). Samples were sent to the contractor, BSA Environmental Services for processing. Slides were prepared using standard membrane filtration technique (McNabb, 1960). This technique preserves cell structure and provides good resolution, allowing the samples to be examined and photographed at high magnifications. Samples were thoroughly mixed as a part of the filtering process to ensure that the organisms were evenly distributed.

A Leica DMLB compound microscope (100X, 200X, 400X, 630X, 1000X) was used for enumerating filtered phytoplankton samples. The abundance of common taxa was estimated by random field counts. At least 400 units (colonies, filaments, unicells) were enumerated to the lowest possible taxonomic level from each sample. In accordance with Lund et al. (1958), counting 400 natural units provided accuracy within 90% confidence limits. In addition, an entire strip of the filter was counted at high magnification (usually 630X) along with half of the filter at a lower magnification (usually 400X) to further ensure complete species reporting. Cell biovolumes of all identified phytoplankton taxa were quantified on a per milliliter basis. Biovolumes were estimated using formulae for solid geometric shapes that most closely matched the cell shape (Hillebrand et al., 1999). Biovolume calculations were based on measurements of 10 organisms per taxon for each sample where possible. For taxa with substantial size variation (such as diatoms), size classes were designated arbitrarily to determine average cell size (biovolume). For each taxon, 25 cells were measured from each size class (assuming that sufficient numbers are available). Mean biovolumes within each size class were used to calculate the total biovolume contributed by the taxon to its representative sample (Burkholder and Wetzel, 1989).

3.0 Results

3.1 Physical Data

3.1.1 Station PR-1

In front of Pueblo Dam, water temperatures were taken from surface to bottom during the sampling period in front of Pueblo Dam at Station PR-1 (Figures 2 and 3). Data were not collected during late fall and winter months. During the study period, surface water temperatures ranged from 6.56 to 24.34 °C. Coolest temperatures were typically present in March and warmest temperatures in July. Bottom water temperatures ranged from 4.75 °C to 20.68 °C. Water temperatures during the study period were isothermal at the end of March 2010, the earliest date the reservoir was sampled, and again at the end of September 2009 and 2010. By the beginning of May, surface water temperatures warmed and the reservoir had either undergone spring turnover, like in 2009, or was in the process of undergoing turnover, like in 2010. Waters were typically stratified at this station from May through the end of August, and isothermal by the end of September.

Dissolved oxygen levels tended to degrade in mid-summer, particularly in July and August, as water temperatures warmed in mid-summer. Lowest levels recorded for dissolved oxygen levels were below 2 mg/L and 25 percent saturation from 15 meters to the bottom depth of 30 meters on Aug. 25, 2010. Quagga mussels typically tolerate lower DO levels than zebra mussels, however, Claudi (2009) states that dissolved oxygen in deeper waters of lakes and reservoirs may become a limiting factor during portions of the year. In this case, station PR-1 exhibited low percent saturation of dissolved oxygen in 2009 and 2010 during the study period which may be below levels that Dreissenid mussels can tolerate, and one possible reason that veliger densities have decreased each year since they were discovered in the reservoir in 2007.

The pH levels present during the study period were typical (Figures 2 and 3). Levels in June 2008 in the epilimnion were high (approaching pH 9.0), however they did correspond to DO levels that were greater than 9.0 mg/L, indicating growth in algal material in the upper levels of the water column.

Specific conductance levels ranged from 210 to 380 µS/cm in the 2008/2009 study period (Figure 2), and from 290 to 460 µS/cm in 2010 (Figure 3). Specific conductance levels are influenced greatly by timing and volume of spring run-off from year to year. Specific conductance levels were typically greatest after spring turnover and as the reservoir thermally stratified in early May. This was the period before spring inflow waters entered the reservoir. Specific conductance levels were then diluted in June by spring run-off that entered below surface waters in the metalimnion of the reservoir. Specific conductance levels of inflowing waters were lower than those present in the

reservoir, and therefore tended to dilute existing levels. As run-off lessened, specific conductance levels increased throughout summer and fall months.

3.1.1 Station PR-2

Directly across the reservoir from the North Marina, surface water temperatures ranged from 6.56 °C to 24.34 °C (Figures 4 and 5). Bottom temperatures ranged from 5.00 to 21.65 °C during the study period. Station PR-2 had turned over by end of May and was thermally stratified until September when it turned over again. Dissolved oxygen levels at Station PR-2 were below 2 mg/L and 25 percent saturation in July and August, similar levels present at Station PR-1.

The pH levels present during the study period at Station PR-2 (Figures 4 and 5) were typical and similar to levels present at Station PR-1. Levels in June 2008 in the epilimnion were high (approaching pH 9.0), however they did correspond to DO levels that were greater than 10.0 mg/L, indicating growth in algal material in the upper levels of the water column.

Specific conductance levels ranged from 180 to 380 µS/cm in 2008/2009 study period and from 250 to 460 µS/cm in 2010 (Figures 4 and 5). Levels at PR-2 were lower than at Station PR-1 (Pueblo Dam) and were diluted more from springtime inflow waters due to location of the station (see explanation for Station PR-1). Inflows were detected as lower specific conductance levels below surface waters until August when this was reversed (Figure 4) in 2008/2009 and in 2010.

3.1.1 Station PR-3

At this mid-reservoir station, surface water temperatures ranged from 6.34 to 24.78 °C and bottom temperatures ranged from 5.14 to 22.05 °C (Figures 6 and 7). This station turned over in spring and fall, and thermally stratified from May through September. Low dissolved oxygen levels were below 2 mg/L and at 25 percent saturation DO in July and August and similar to those reported at Stations PR-1 and PR-2.

The pH levels present during the study period at Station PR-3 were typical and similar to levels present at Station PR-1 and PR-2 (Figures 6 and 7). Levels in June 2008 in the epilimnion were high (approaching pH 9.0), however they did correspond to DO levels that were about 9.5 mg/L, indicating possible growth in algal material in the upper levels of the water column.

Specific conductance levels ranged from 160 to 450 µS/cm in 2008/2009 study period and from 230 to 470 µS/cm in 2010 (Figures 6 and 7). In 2008/2009, levels tended to fluctuate a great deal from month to month due to the proximity of this station to inflows entering the reservoir (Figure 6). During 2010 study period, inflows at this station were more easily detected as interflows at 10 meters in June, and then at bottom depths as the summer season progressed. Interflows tended to have greater specific conductance levels than other depths at this station (Figure 7).

3.1.1 Station PR-4

At Station PR-4, the upper-most station, surface water temperatures ranged from 6.67 to 24.88 °C, and bottom temperatures ranged from 6.62 to 23.86 °C (Figures 8 and 9). This station was thermally stratified from May to September. In contrast, dissolved oxygen levels at all other sampling stations, were above 5 mg/L and 60 percent saturation during the entire sampling period.

The pH levels present during the study period were typical however they were also greater than at other station (Figures 8 and 9). This station was shallower and at the upper-most area of the reservoir.

Specific conductance levels ranged from 160 to 470 µS/cm in 2008/2009 study period and from 200 to 550 µS/cm in 2010 (Figures 8 and 9). During the study period, levels tended to fluctuate a great deal from month to month as a result of inflows plunging under the surface to mid-to-bottom depths.

3.1.1 Station PR-5

At the North Marina, surface water temperatures ranged from 5.71 to 24.83 °C and bottom temperatures ranged from 4.90 °C to 22.27 °C (Figures 10 and 11). This station was thermally stratified from May to August. DO levels were below 2 mg/L and 25 percent saturation at the end of August 2010 (Figure 11).

The pH levels present during the study period were typical and similar to levels present at Stations PR-1 and PR-2 (Figures 10 and 11).

Specific conductance levels ranged from 200 to 400 µS/cm in 2008/2009 study period and from 250 to 450 µS/cm in 2010 (Figures 10 and 11).

3.1.1 Station PR-6

Near the mouth of the South Marina, surface water temperatures ranged from 7.31 to 23.54 °C and bottom temperatures ranged from 4.96 to 21.41 °C (Figures 12 and 13).

This station was thermally stratified from May to August. DO levels were below 2 mg/L and 25 percent saturation at the end of August 2010 (Figure 12).

The pH levels present during the study period were typical (Figures 12 and 13) and ranged from 7.8 to 9.0 during 2008/2009, and from 7.7 to 8.7 during 2010 field season.

Specific conductance levels range from 210 to 380 µS/cm in 2008/2009 study period and from 280 to 460 µS/cm in 2010 (Figures 12 and 13).

3.2 Calcium

Calcium levels ranged between 31 to 53 mg/L at the surface waters at all stations and from 22 to 53 mg/L at the bottom depths at all stations (Figure 14). Lowest levels were present in June during high run-off or in August when the reservoir was thermally stratified. Highest calcium levels were recorded in May during isothermal conditions before spring run-off entered the reservoir.

3.3 Nutrients

Phosphorus and nitrogen concentrations in Pueblo Reservoir were generally low and in the oligotrophic to mesotrophic range (Figures 15-19). Total phosphorus concentrations averaged about 0.020 mg/L during the study period, and total nitrogen (TKN plus nitrate+nitrite-N) averaged about 0.38 mg/L (Appendix A).

3.4 Secchi Disk Transparency and Chlorophyll

Box and whisker plots of secchi disk transparency (Figure 20) and chlorophyll *a* (Figure 21) illustrate greatest and lowest levels with the whiskers and ranges of middle values in the boxes. This type of graph is useful when comparing one variable for several different stations and gives a visual representation of differences of that variable between stations. The median value is represented by the horizontal line in the box, the box portion shows 50 percent of the data, 25th percentile or lower quartile is the median of the lower half of the data, 75th percentile or upper quartile is the median of the upper half of the data, and whiskers show the minimum and maximum observation values. A lack of overlap in the interquartile range between boxes represents differences in data.

Secchi disk transparency levels ranged from 0.95 m at Station PR-2 on 23 September 2009 to 9.73 m, also at Station PR-2, on 4 May 2009 (Figure 20 and Appendix A). The greatest water clarity was typically present preceding spring run-off, and immediately after the reservoir stratified. The lowest transparency levels were observed in October after the reservoir turned over in the fall.

Surface chlorophyll *a* concentrations ranged from less than 0.1 µg/L at Station PR-6 on 4 May 2009 to 27.66 µg/L at Station PR-4 on 30 June 2009 (Figure 21 and Appendix A). The lowest levels at the surface were present in May and the highest levels were after spring run-off in June. Composite chlorophyll *a* levels (0 to 5 m) ranged from less than 0.1 µg/L at Station PR-6 on 4 May 2009 to 19.97 µg/L at Station PR-4 on 26 July 2010 (Figure 21 and Appendix A). Composite levels generally tended to be greater than surface levels.

3.5 Phytoplankton

Phytoplankton populations were monitored as part of this study, but no definite conclusions could be drawn from the available data. Diatoms (Bacillariophyta) tended to be the dominant group in terms of both numbers and biomass at most stations (Figures 22-27), although several groups were present during the latter half of 2010.

3.6 Zooplankton

Zooplankton populations were also monitored. Different groups were dominant at different stations during the study period (Figures 28-33). Zooplankton numbers and biomass tended to be higher at Station PR-4 than at any of the other stations.

3.7 Veligers and Algal Toxins

Veligers, the larval stage of dreissenid mussels, were intended to be a major focus of the study, but populations were not sufficient to draw any definite conclusions. Veliger samples showed a decrease in numbers from 2008 to 2009, possibly a result of cooler air temperatures and a milder summer in Colorado in 2009 compared to 2008. Numbers decreased further in 2010. There was insufficient data to determine whether or not dreissenid mussels would become established in Pueblo Reservoir at the time the study was completed.

Algal toxins (microcystin) were also monitored because some studies had indicated algal toxin concentrations increased following introductions of dreissenid mussels. Samples for algal toxin analyses were collected on 3 June 2009 and 24 March 2010 at all stations. Microcystin concentrations were below the detection limit of 0.060 µg/L for all samples analyzed.

This page intentionally left blank.

4.0 References

Burkholder, J.M. and R.G. Wetzel. 1989. Epiphytic microalgae on natural substrata in a hardwater lake: seasonal dynamics of community structure, biomass and ATP content. Arch. Hydrobiological Journal., Suppl. 83:1-56.

Dumont, H.J, I. Van de Velde, S. Dumont. 1975. The dry weight estimate of biomass in a election of Cladocera, Copepda, and Rotifera from the plankton, periphyton, and benthos of continental waters. Oecologia. 19:75-97.

Edmundson, W.T. 1959. Freshwater Biology. 2nd Ed. New York City, NY: Wiley-Interscience.

Hillenbrand, H., C.D. Dürselen, D. Kirschtel, U. Pollingher, and T. Zohary. 1999. Biovolume calculation for pelagic and benthic microalgae. Journal of Phycology 35: 403-424.

Lawrence, S.G., D.F. Malley, W.J. Findlay, M.A. MacIver, I.L. Delbaere. 1987. Method for estimating dry weight of freshwater planktonic crustaceans from measures of length and shape. Can. J. Fish. Aquat. Sci. 44:264-274.

Lund, J.W.G., C. Kipling and E.D. LeCren. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimates by counting. Hydrobiolgia 11:143-170.

McCauley. E. 1984. The estimation of the abundance and biomass of zooplankton in samples. In: Downing, J.A., F.H. Rigler editors. A manual for the assessment of secondary productivity in fresh waters. Oxford (UK): Blackwell Sci. p. 228-265.

McNabb, C.D. 1960. Enumeration of Freshwater Phytoplankton Concentrated on the Membrane Filter. Limnology and Oceanography. 5(1): 57-61.

Pennak, R.W. 1989. Fresh-water invertebrates of the United States protozoa to mollusca. 3rd ed. New York (NY): John Wiley and Sons, Inc.

Ruttner-Kolisko, A. 1974. Plankton rotifers, biology and taxonomy. Die Binnengewasser. 26:1-146.

This page intentionally left blank.

Technical Memorandum 86-68220-11-04

Zebra and Quagga Mussels: Pre- and Post- Impacts of Zebra/Quagga Mussels on the Physical, Chemical, and Biological Attributes on Reclamation Reservoirs

prepared by

Davine Lieberman and G. Chris Holdren
Environmental Applications and Research Group (86-68220)
Technical Service Center
P.O. Box 25007
Denver, CO 80225

U.S. Department of the Interior
Bureau of Reclamation

This page intentionally left blank.

Table of Contents

Section **Page**

List of Figures in Appendix A

List of Figures (Continued)

This page intentionally left blank.

1.0 Introduction

Pueblo Reservoir is located on the Arkansas River in Pueblo County about six miles west of Pueblo, Colorado. The reservoir has a concrete dam with over 60 miles of shore line; 5,664 acres of surface water, and a total storage capacity of 357,678 acre-feet.

Zebra mussel veligers were first collected and identified from Pueblo Reservoir in Colorado in November 2007. These findings were tested and confirmed by DNA testing in 2008. Veligers were most numerous during 2008 and included confirmation of both zebra and quagga mussels. During 2009, veliger abundance diminished drastically for unknown reasons. In 2010, there was only one veliger confirmation from the reservoir, and no adult mussels have been found to date. A rigorous collection program, which began in 2008, should have been sufficient to find veligers in each sample tow if they were present in the reservoir.

This study was intended to focus on the consequences that zebra/quaggas could have on limnological aspects of the reservoir. As the study progressed, it was obvious that the main focus of the study could not be undertaken since adults have not been found to date, and the number of veligers decreased as the study progressed. Therefore, the main objective of this study, is to look at the physical, chemical, and biological characteristics of the reservoir, and to suggest what may have occurred in terms of the disappearance of veligers in Pueblo Reservoir. Unfortunately, the data collected was not sufficient to provide a good explanation for the declining veliger numbers in the reservoir.

All figures for the report are included in Appendix A. Monitoring data (Appendix B) for the project is included as an Excel spreadsheet (Pueblo Reservoir.xlsx).

This page intentionally left blank.

2.0 Methods and Materials

Sampling collections at the 6 sampling stations in Pueblo Reservoir were conducted monthly in June 2008 (one sampling event), May through September 2009 (5 sampling events), and March through October 2010 (8 sampling events). There were six sampling stations located at Pueblo Dam (PR-1), North Marina (PR-5), mid-reservoir (PR-2), upreservoir (PR-3), farthest station in uppermost part of reservoir (PR-4), and buoy by South Marina (PR-6).

At all sampling stations, water temperature (°C), pH, dissolved oxygen (mg/L), and specific conductance (µS/cm) depth profiles were taken at one meter intervals from surface to bottom depths, with a YSI multi-parameter probe.

Secchi disk transparency readings were taken with a 20 cm diameter black and white secchi disk with and without the aid of a 26 inch Aqua View II viewscope. The disk was lowered into the water with a metered line until it disappeared from sight, then it was brought up until visible, and then lowered once again until it disappeared. At this point, the depth of the secchi disk was recorded in meters.

A surface and composite sample (0 to 5 m) were collected for chlorophyll *a (*chl *a*) analysis. A flexible swimming pool hose weighted and connected to a measured line at one end was lowered into the water column to a depth of 5 m, the hose end on the boat deck was plugged with a rubber stopper, then the weighted end was pulled up and put into a 20 g bucket, the stopper was removed, and water was drained into the bucket and collected in 1 L Nalgene bottles. All samples were typically filtered immediately on the boat. From each water sample, 250 mL of water was filtered through a 47 mm Whatman GF/C filter, the filter was folded, wrapped in foil, placed in a coin envelope, and immediately stored on dry ice. In the lab, filters were put in the freezer until analyzed for chl *a* and pheophytin using a spectrophotometer according to Standard Methods (Method 10200 H (2), 1998).

Water samples for nutrient analyses and ions were collected with a Kemmerer from one meter below the surface and one meter above the bottom depth. The following nutrients were analyzed: soluble reactive phosphorus (SRP) (0.001 mg/L), method detection limit (mdl), method EPA 353.2), total phosphorus (0.003 mg/L mdl, method EPA 365.1), nitrite-nitrate (0.003 mg/L mdl, method EPA 365.1), ammonia nitrogen (0.003 mg/L mdl, method EPA 350.1) and total Kjeldahl nitrogen (0.003 mg/L mdl, method EPA 351.2). Calcium ion was analyzed using 3111B (0.2 mg/L mdl, Standard Methods, 1998).

Zooplankton samples were collected with a 64 µm mesh Wisconsin-style plankton net from surface to 15 m at most stations, or to one meter above the bottom, if depth was shallower than 15 m. Samples were preserved with Lugol's iodine solution in dark 250 mL Nalgene amber

bottles. Aliquots from samples were placed in an Utermohl chamber where zooplankton were counted at 100x magnification until at least 200 individuals were tallied by BSA Environmental Services. Identification followed Edmundson (1959), Ruttner-Kolisko (1974) and Pennak (1989). Biomass estimates were based on established length-width relationships (Dumont et al. 1975, McCauley 1984, Lawrence et al. 1987). The lengths or the lengths and widths of each species were measured equal to 10 for common species and lesser for more rare taxa. For cladocerans, length was measured from tip of head to end of body (shell spines excluded). For copepods, length was determined from tip of head to insertion of caudal ramus. For rotifers, length was measured from tip of head to end of body (spines, toes, other appendages excluded). In accordance with McCauley (1984), biomass was computed for appropriate number of individuals for each sample location and arithmetic mean biomass was multiplied by species abundance to equal a species biomass per sample.

Composite phytoplankton samples representing 0 to 5 m depth interval were collected using a swimming pool hose. The weighted end of the hose was lowered into the water column to 5 meters and a rubber stopper was inserted into the other end on deck. The weighted end was pulled out of the water and placed into a 20 L bucket; then water was released by uncorking the hose. Composite samples were collected in 250 mL Nalgene amber bottles. Samples were preserved with Lugol's iodine solution to tea color (about 4 mL). Samples were sent to the contractor, BSA Environmental Services for processing. Slides were prepared using standard membrane filtration technique (McNabb, 1960). This technique preserves cell structure and provides good resolution, allowing the samples to be examined and photographed at high magnifications. Samples were thoroughly mixed as a part of the filtering process to ensure that the organisms were evenly distributed.

A Leica DMLB compound microscope (100X, 200X, 400X, 630X, 1000X) was used for enumerating filtered phytoplankton samples. The abundance of common taxa was estimated by random field counts. At least 400 units (colonies, filaments, unicells) were enumerated to the lowest possible taxonomic level from each sample. In accordance with Lund et al. (1958), counting 400 natural units provided accuracy within 90% confidence limits. In addition, an entire strip of the filter was counted at high magnification (usually 630X) along with half of the filter at a lower magnification (usually 400X) to further ensure complete species reporting. Cell biovolumes of all identified phytoplankton taxa were quantified on a per milliliter basis. Biovolumes were estimated using formulae for solid geometric shapes that most closely matched the cell shape (Hillebrand et al., 1999). Biovolume calculations were based on measurements of 10 organisms per taxon for each sample where possible. For taxa with substantial size variation (such as diatoms), size classes were designated arbitrarily to determine average cell size (biovolume). For each taxon, 25 cells were measured from each size class (assuming that sufficient numbers are available). Mean biovolumes within each size class were used to calculate the total biovolume contributed by the taxon to its representative sample (Burkholder and Wetzel, 1989).

3.0 Results

3.1 Physical Data

3.1.1 Station PR-1

In front of Pueblo Dam, water temperatures were taken from surface to bottom during the sampling period in front of Pueblo Dam at Station PR-1 (Figures 2 and 3). Data were not collected during late fall and winter months. During the study period, surface water temperatures ranged from 6.56 to 24.34 °C. Coolest temperatures were typically present in March and warmest temperatures in July. Bottom water temperatures ranged from 4.75 °C to 20.68 °C. Water temperatures during the study period were isothermal at the end of March 2010, the earliest date the reservoir was sampled, and again at the end of September 2009 and 2010. By the beginning of May, surface water temperatures warmed and the reservoir had either undergone spring turnover, like in 2009, or was in the process of undergoing turnover, like in 2010. Waters were typically stratified at this station from May through the end of August, and isothermal by the end of September.

Dissolved oxygen levels tended to degrade in mid-summer, particularly in July and August, as water temperatures warmed in mid-summer. Lowest levels recorded for dissolved oxygen levels were below 2 mg/L and 25 percent saturation from 15 meters to the bottom depth of 30 meters on Aug. 25, 2010. Quagga mussels typically tolerate lower DO levels than zebra mussels, however, Claudi (2009) states that dissolved oxygen in deeper waters of lakes and reservoirs may become a limiting factor during portions of the year. In this case, station PR-1 exhibited low percent saturation of dissolved oxygen in 2009 and 2010 during the study period which may be below levels that Dreissenid mussels can tolerate, and one possible reason that veliger densities have decreased each year since they were discovered in the reservoir in 2007.

The pH levels present during the study period were typical (Figures 2 and 3). Levels in June 2008 in the epilimnion were high (approaching pH 9.0), however they did correspond to DO levels that were greater than 9.0 mg/L, indicating growth in algal material in the upper levels of the water column.

Specific conductance levels ranged from 210 to 380 µS/cm in the 2008/2009 study period (Figure 2), and from 290 to 460 µS/cm in 2010 (Figure 3). Specific conductance levels are influenced greatly by timing and volume of spring run-off from year to year. Specific conductance levels were typically greatest after spring turnover and as the reservoir thermally stratified in early May. This was the period before spring inflow waters entered the reservoir. Specific conductance levels were then diluted in June by spring run-off that entered below surface waters in the metalimnion of the reservoir. Specific conductance levels of inflowing waters were lower than those present in the

reservoir, and therefore tended to dilute existing levels. As run-off lessened, specific conductance levels increased throughout summer and fall months.

3.1.1 Station PR-2

Directly across the reservoir from the North Marina, surface water temperatures ranged from 6.56 °C to 24.34 °C (Figures 4 and 5). Bottom temperatures ranged from 5.00 to 21.65 °C during the study period. Station PR-2 had turned over by end of May and was thermally stratified until September when it turned over again. Dissolved oxygen levels at Station PR-2 were below 2 mg/L and 25 percent saturation in July and August, similar levels present at Station PR-1.

The pH levels present during the study period at Station PR-2 (Figures 4 and 5) were typical and similar to levels present at Station PR-1. Levels in June 2008 in the epilimnion were high (approaching pH 9.0), however they did correspond to DO levels that were greater than 10.0 mg/L, indicating growth in algal material in the upper levels of the water column.

Specific conductance levels ranged from 180 to 380 µS/cm in 2008/2009 study period and from 250 to 460 µS/cm in 2010 (Figures 4 and 5). Levels at PR-2 were lower than at Station PR-1 (Pueblo Dam) and were diluted more from springtime inflow waters due to location of the station (see explanation for Station PR-1). Inflows were detected as lower specific conductance levels below surface waters until August when this was reversed (Figure 4) in 2008/2009 and in 2010.

3.1.1 Station PR-3

At this mid-reservoir station, surface water temperatures ranged from 6.34 to 24.78 °C and bottom temperatures ranged from 5.14 to 22.05 °C (Figures 6 and 7). This station turned over in spring and fall, and thermally stratified from May through September. Low dissolved oxygen levels were below 2 mg/L and at 25 percent saturation DO in July and August and similar to those reported at Stations PR-1 and PR-2.

The pH levels present during the study period at Station PR-3 were typical and similar to levels present at Station PR-1 and PR-2 (Figures 6 and 7). Levels in June 2008 in the epilimnion were high (approaching pH 9.0), however they did correspond to DO levels that were about 9.5 mg/L, indicating possible growth in algal material in the upper levels of the water column.

Specific conductance levels ranged from 160 to 450 µS/cm in 2008/2009 study period and from 230 to 470 µS/cm in 2010 (Figures 6 and 7). In 2008/2009, levels tended to fluctuate a great deal from month to month due to the proximity of this station to inflows entering the reservoir (Figure 6). During 2010 study period, inflows at this station were more easily detected as interflows at 10 meters in June, and then at bottom depths as the summer season progressed. Interflows tended to have greater specific conductance levels than other depths at this station (Figure 7).

3.1.1 Station PR-4

At Station PR-4, the upper-most station, surface water temperatures ranged from 6.67 to 24.88 °C, and bottom temperatures ranged from 6.62 to 23.86 °C (Figures 8 and 9). This station was thermally stratified from May to September. In contrast, dissolved oxygen levels at all other sampling stations, were above 5 mg/L and 60 percent saturation during the entire sampling period.

The pH levels present during the study period were typical however they were also greater than at other station (Figures 8 and 9). This station was shallower and at the upper-most area of the reservoir.

Specific conductance levels ranged from 160 to 470 µS/cm in 2008/2009 study period and from 200 to 550 µS/cm in 2010 (Figures 8 and 9). During the study period, levels tended to fluctuate a great deal from month to month as a result of inflows plunging under the surface to mid-to-bottom depths.

3.1.1 Station PR-5

At the North Marina, surface water temperatures ranged from 5.71 to 24.83 °C and bottom temperatures ranged from 4.90 °C to 22.27 °C (Figures 10 and 11). This station was thermally stratified from May to August. DO levels were below 2 mg/L and 25 percent saturation at the end of August 2010 (Figure 11).

The pH levels present during the study period were typical and similar to levels present at Stations PR-1 and PR-2 (Figures 10 and 11).

Specific conductance levels ranged from 200 to 400 µS/cm in 2008/2009 study period and from 250 to 450 µS/cm in 2010 (Figures 10 and 11).

3.1.1 Station PR-6

Near the mouth of the South Marina, surface water temperatures ranged from 7.31 to 23.54 °C and bottom temperatures ranged from 4.96 to 21.41 °C (Figures 12 and 13).

This station was thermally stratified from May to August. DO levels were below 2 mg/L and 25 percent saturation at the end of August 2010 (Figure 12).

The pH levels present during the study period were typical (Figures 12 and 13) and ranged from 7.8 to 9.0 during 2008/2009, and from 7.7 to 8.7 during 2010 field season.

Specific conductance levels range from 210 to 380 µS/cm in 2008/2009 study period and from 280 to 460 µS/cm in 2010 (Figures 12 and 13).

3.2 Calcium

Calcium levels ranged between 31 to 53 mg/L at the surface waters at all stations and from 22 to 53 mg/L at the bottom depths at all stations (Figure 14). Lowest levels were present in June during high run-off or in August when the reservoir was thermally stratified. Highest calcium levels were recorded in May during isothermal conditions before spring run-off entered the reservoir.

3.3 Nutrients

Phosphorus and nitrogen concentrations in Pueblo Reservoir were generally low and in the oligotrophic to mesotrophic range (Figures 15-19). Total phosphorus concentrations averaged about 0.020 mg/L during the study period, and total nitrogen (TKN plus nitrate+nitrite-N) averaged about 0.38 mg/L (Appendix A).

3.4 Secchi Disk Transparency and Chlorophyll

Box and whisker plots of secchi disk transparency (Figure 20) and chlorophyll *a* (Figure 21) illustrate greatest and lowest levels with the whiskers and ranges of middle values in the boxes. This type of graph is useful when comparing one variable for several different stations and gives a visual representation of differences of that variable between stations. The median value is represented by the horizontal line in the box, the box portion shows 50 percent of the data, 25th percentile or lower quartile is the median of the lower half of the data, 75th percentile or upper quartile is the median of the upper half of the data, and whiskers show the minimum and maximum observation values. A lack of overlap in the interquartile range between boxes represents differences in data.

Secchi disk transparency levels ranged from 0.95 m at Station PR-2 on 23 September 2009 to 9.73 m, also at Station PR-2, on 4 May 2009 (Figure 20 and Appendix A). The greatest water clarity was typically present preceding spring run-off, and immediately after the reservoir stratified. The lowest transparency levels were observed in October after the reservoir turned over in the fall.

Surface chlorophyll *a* concentrations ranged from less than 0.1 µg/L at Station PR-6 on 4 May 2009 to 27.66 µg/L at Station PR-4 on 30 June 2009 (Figure 21 and Appendix A). The lowest levels at the surface were present in May and the highest levels were after spring run-off in June. Composite chlorophyll *a* levels (0 to 5 m) ranged from less than 0.1 µg/L at Station PR-6 on 4 May 2009 to 19.97 µg/L at Station PR-4 on 26 July 2010 (Figure 21 and Appendix A). Composite levels generally tended to be greater than surface levels.

3.5 Phytoplankton

Phytoplankton populations were monitored as part of this study, but no definite conclusions could be drawn from the available data. Diatoms (Bacillariophyta) tended to be the dominant group in terms of both numbers and biomass at most stations (Figures 22-27), although several groups were present during the latter half of 2010.

3.6 Zooplankton

Zooplankton populations were also monitored. Different groups were dominant at different stations during the study period (Figures 28-33). Zooplankton numbers and biomass tended to be higher at Station PR-4 than at any of the other stations.

3.7 Veligers and Algal Toxins

Veligers, the larval stage of dreissenid mussels, were intended to be a major focus of the study, but populations were not sufficient to draw any definite conclusions. Veliger samples showed a decrease in numbers from 2008 to 2009, possibly a result of cooler air temperatures and a milder summer in Colorado in 2009 compared to 2008. Numbers decreased further in 2010. There was insufficient data to determine whether or not dreissenid mussels would become established in Pueblo Reservoir at the time the study was completed.

Algal toxins (microcystin) were also monitored because some studies had indicated algal toxin concentrations increased following introductions of dreissenid mussels. Samples for algal toxin analyses were collected on 3 June 2009 and 24 March 2010 at all stations. Microcystin concentrations were below the detection limit of 0.060 µg/L for all samples analyzed.

This page intentionally left blank.

4.0 References

Burkholder, J.M. and R.G. Wetzel. 1989. Epiphytic microalgae on natural substrata in a hardwater lake: seasonal dynamics of community structure, biomass and ATP content. Arch. Hydrobiological Journal., Suppl. 83:1-56.

Dumont, H.J, I. Van de Velde, S. Dumont. 1975. The dry weight estimate of biomass in a election of Cladocera, Copepda, and Rotifera from the plankton, periphyton, and benthos of continental waters. Oecologia. 19:75-97.

Edmundson, W.T. 1959. Freshwater Biology. 2nd Ed. New York City, NY: Wiley-Interscience.

Hillenbrand, H., C.D. Dürselen, D. Kirschtel, U. Pollingher, and T. Zohary. 1999. Biovolume calculation for pelagic and benthic microalgae. Journal of Phycology 35: 403-424.

Lawrence, S.G., D.F. Malley, W.J. Findlay, M.A. MacIver, I.L. Delbaere. 1987. Method for estimating dry weight of freshwater planktonic crustaceans from measures of length and shape. Can. J. Fish. Aquat. Sci. 44:264-274.

Lund, J.W.G., C. Kipling and E.D. LeCren. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimates by counting. Hydrobiolgia 11:143-170.

McCauley. E. 1984. The estimation of the abundance and biomass of zooplankton in samples. In: Downing, J.A., F.H. Rigler editors. A manual for the assessment of secondary productivity in fresh waters. Oxford (UK): Blackwell Sci. p. 228-265.

McNabb, C.D. 1960. Enumeration of Freshwater Phytoplankton Concentrated on the Membrane Filter. Limnology and Oceanography. 5(1): 57-61.

Pennak, R.W. 1989. Fresh-water invertebrates of the United States protozoa to mollusca. 3rd ed. New York (NY): John Wiley and Sons, Inc.

Ruttner-Kolisko, A. 1974. Plankton rotifers, biology and taxonomy. Die Binnengewasser. 26:1-146.

This page intentionally left blank.

Appendix A: Figures

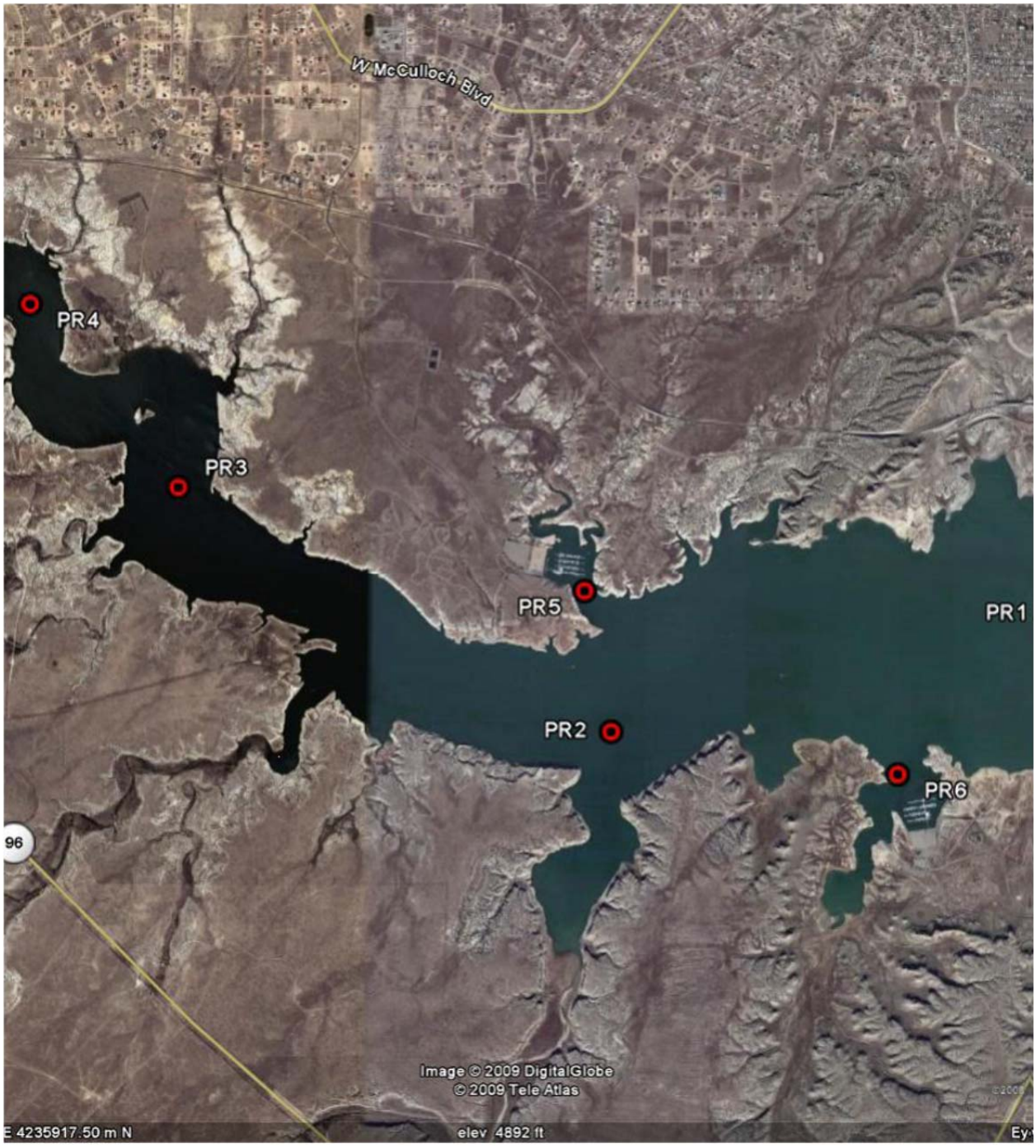

Figure 1 – Pueblo Reservoir sampling locations.

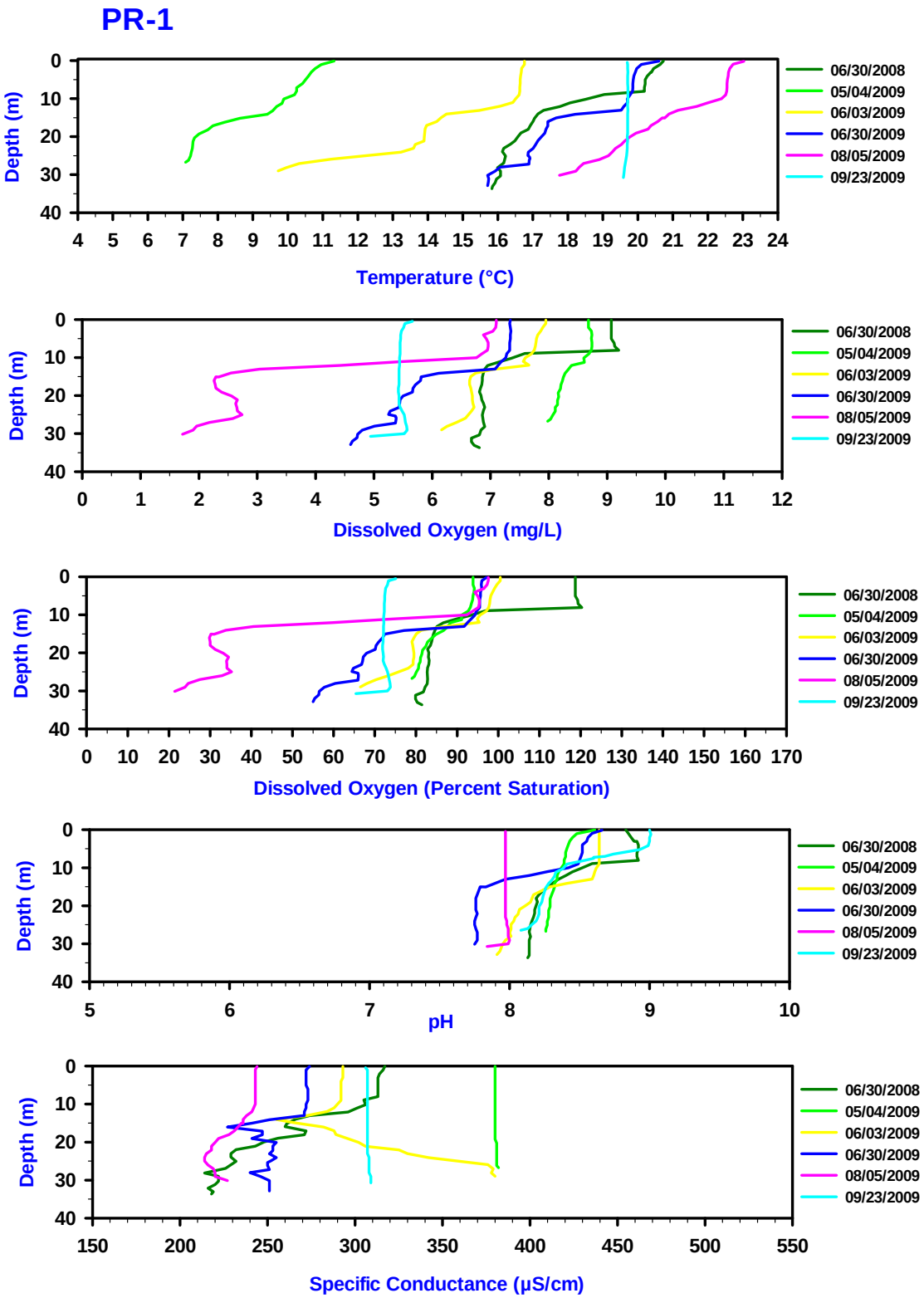

Figure 2 – 2008 and 2009 profiles for Pueblo Reservoir Station PR-1.

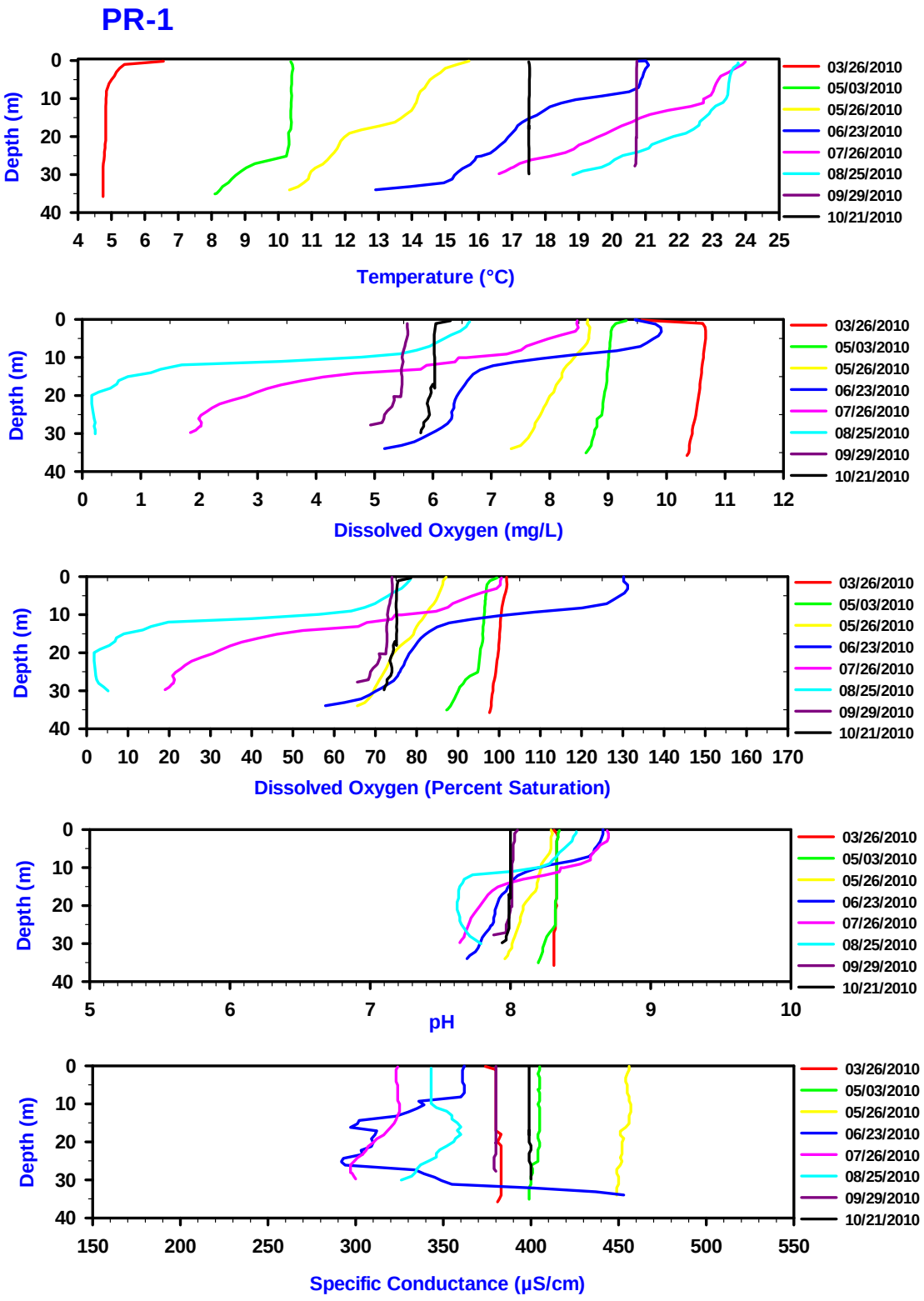

Figure 3 – 2010 profiles for Pueblo Reservoir Station PR-1.

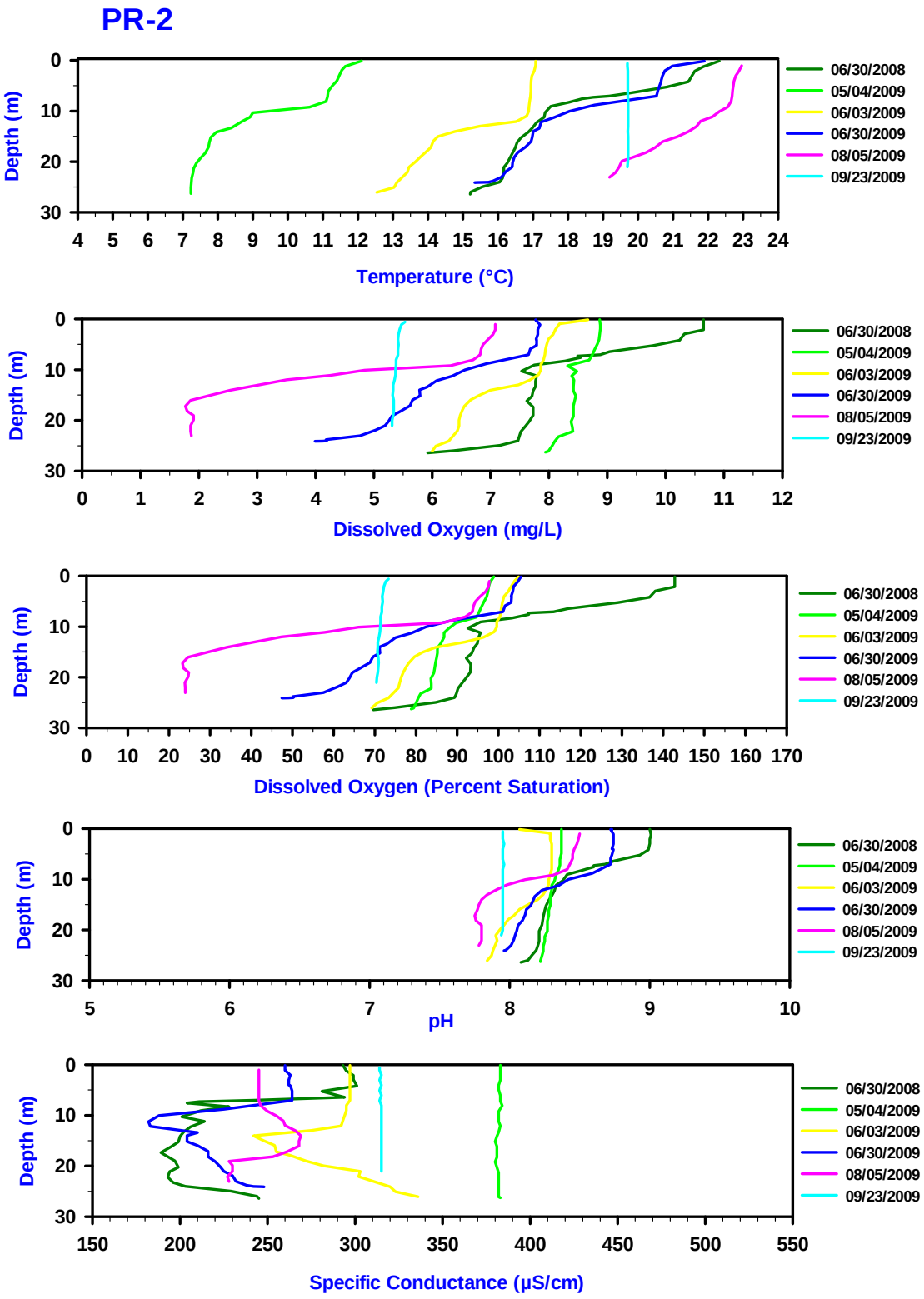

Figure 4 – 2008 and 2009 profiles for Pueblo Reservoir Station PR-2.

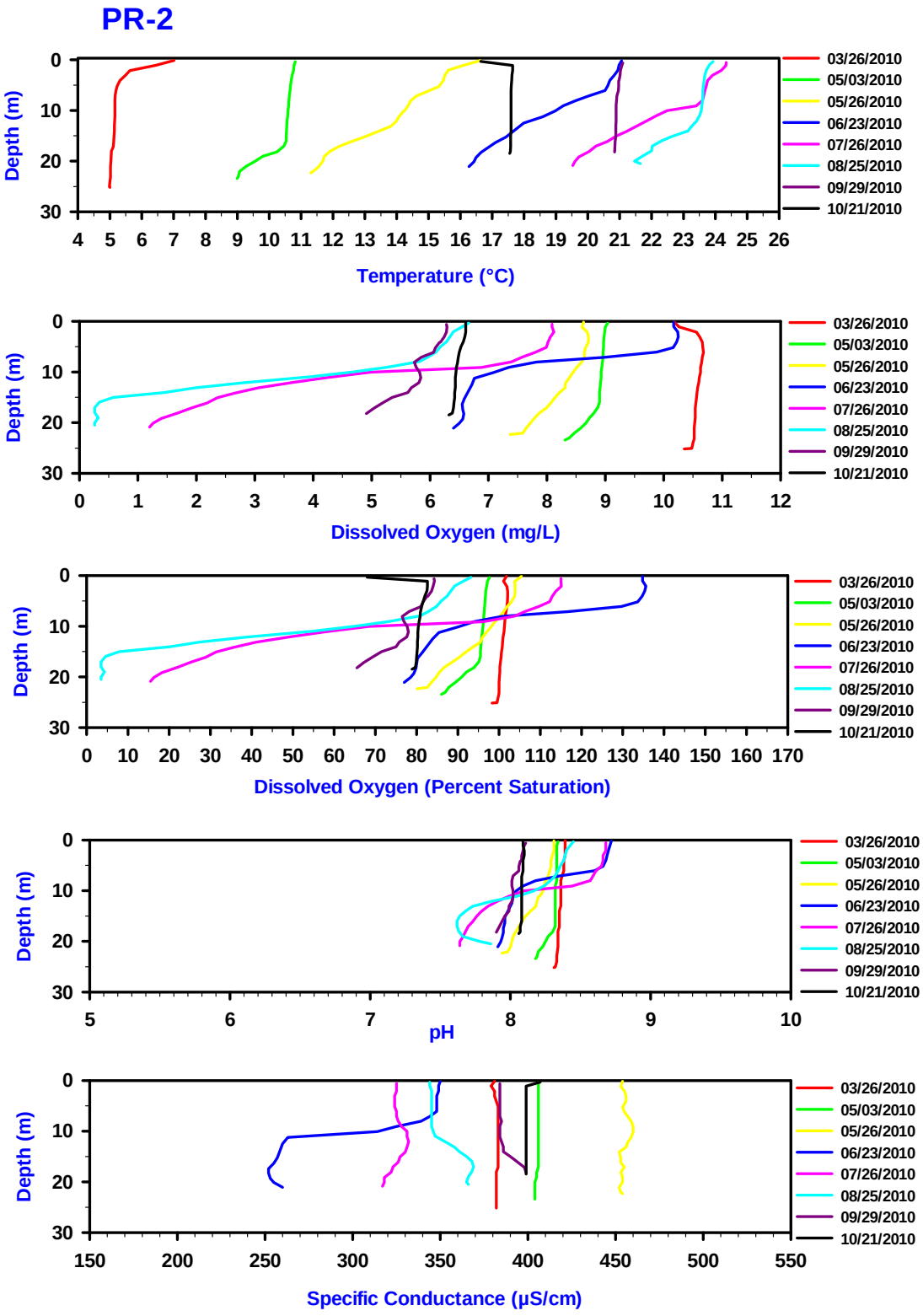

Figure 5 – 2010 profiles for Pueblo Reservoir Station PR-2.

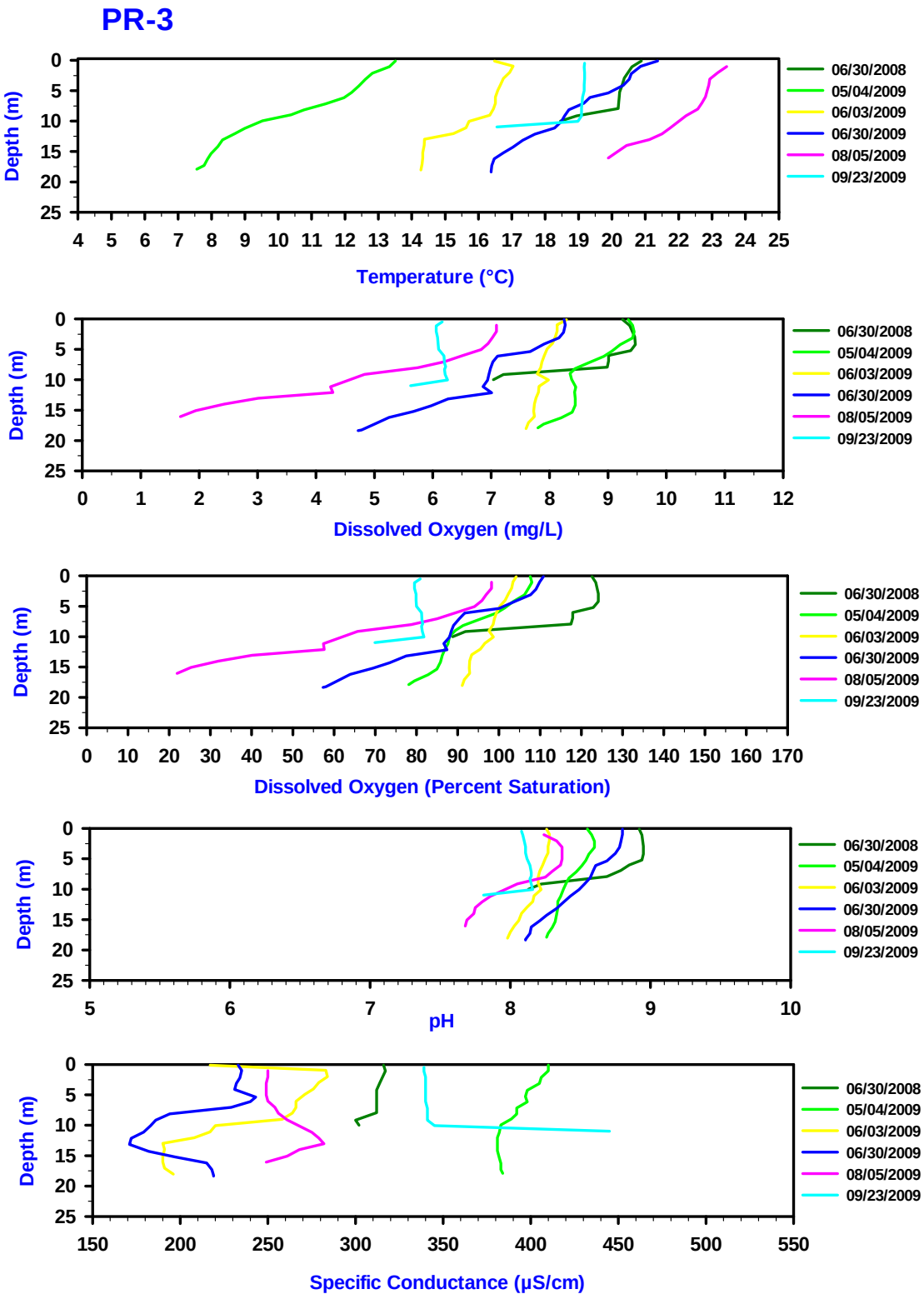

Figure 6 – 2008 and 2009 profiles for Pueblo Reservoir Station PR-3.

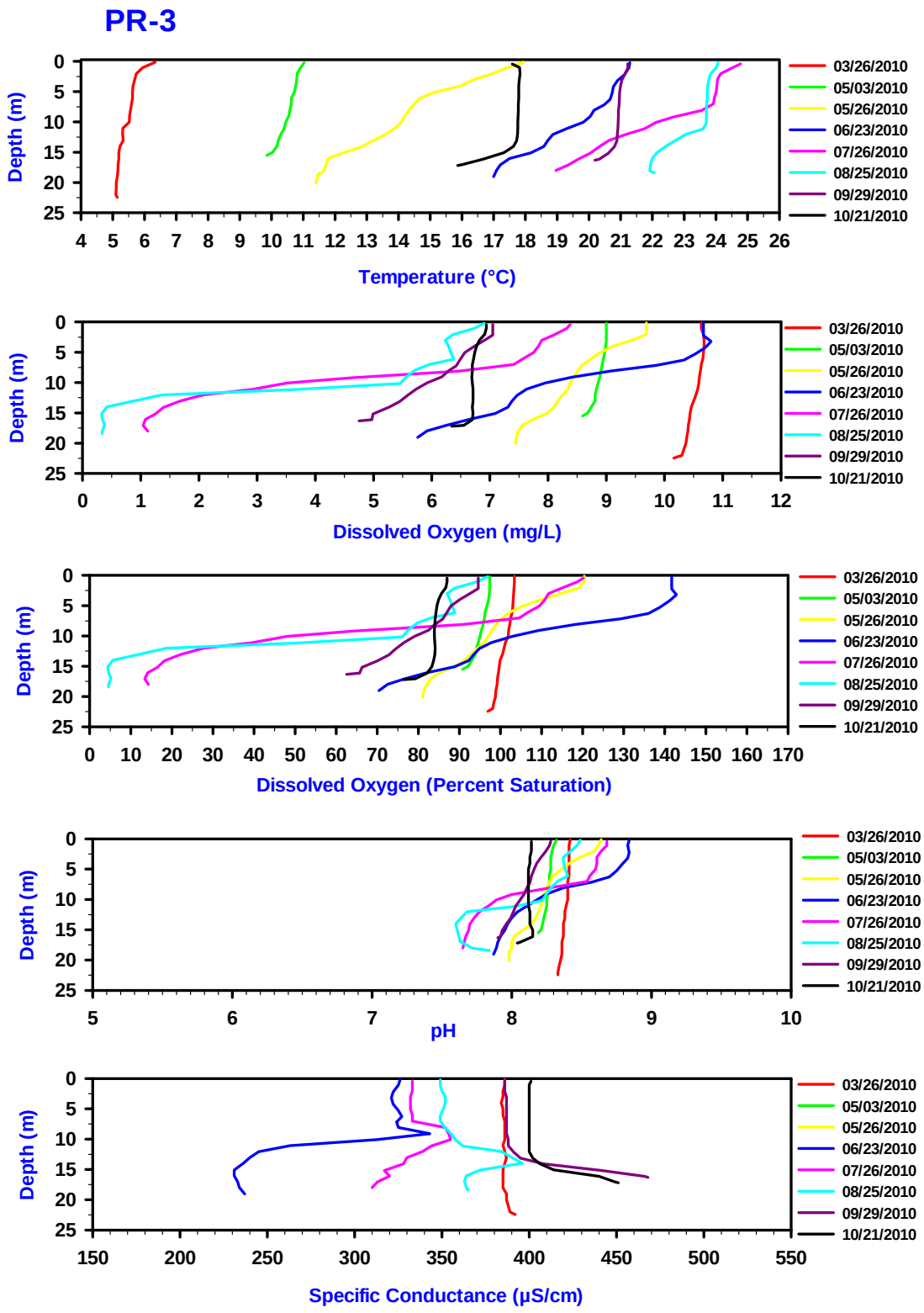

Figure 7 – 2010 profiles for Pueblo Reservoir Station PR-3.

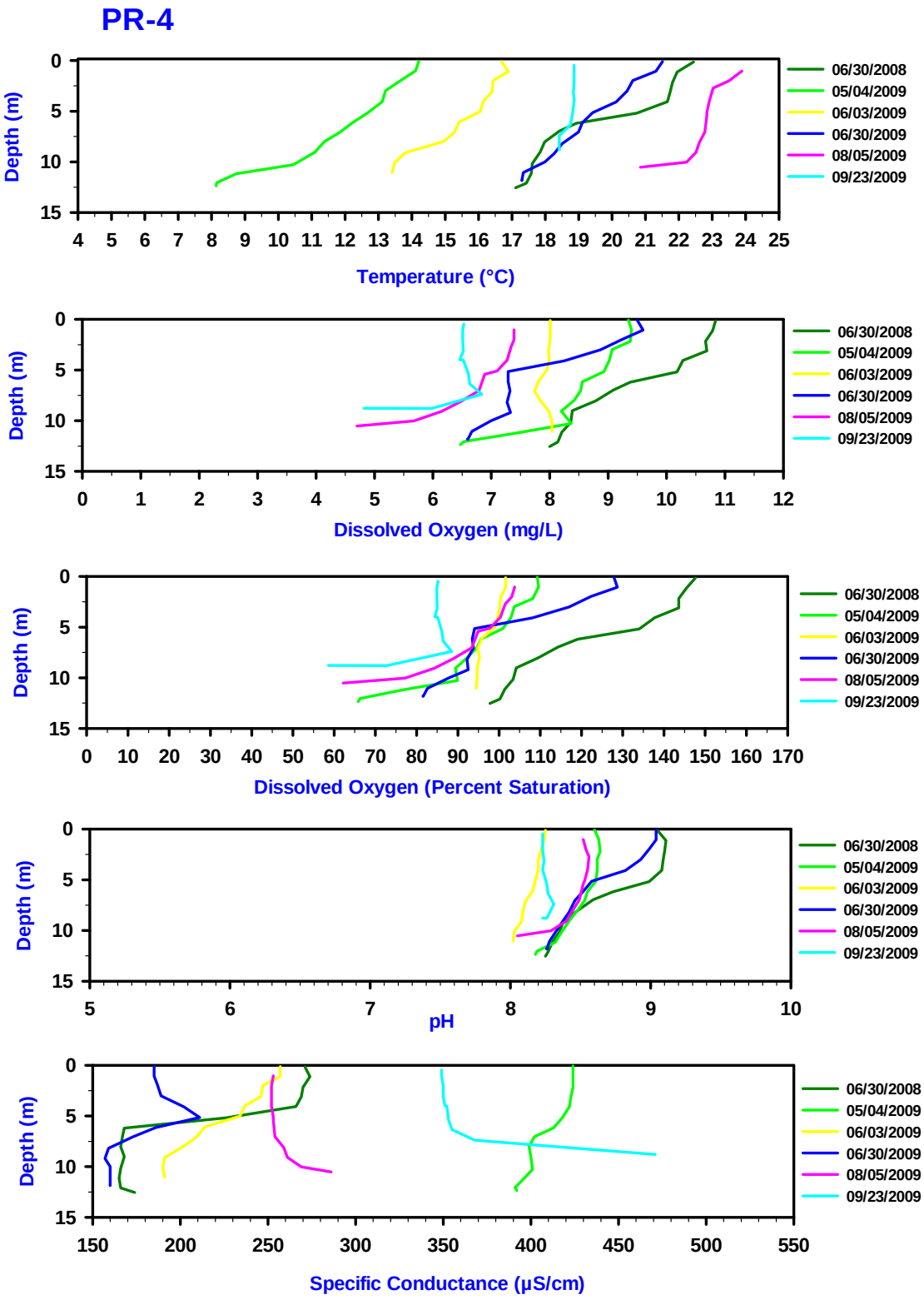

Figure 8 – 2008 and 2009 profiles for Pueblo Reservoir Station PR-4.

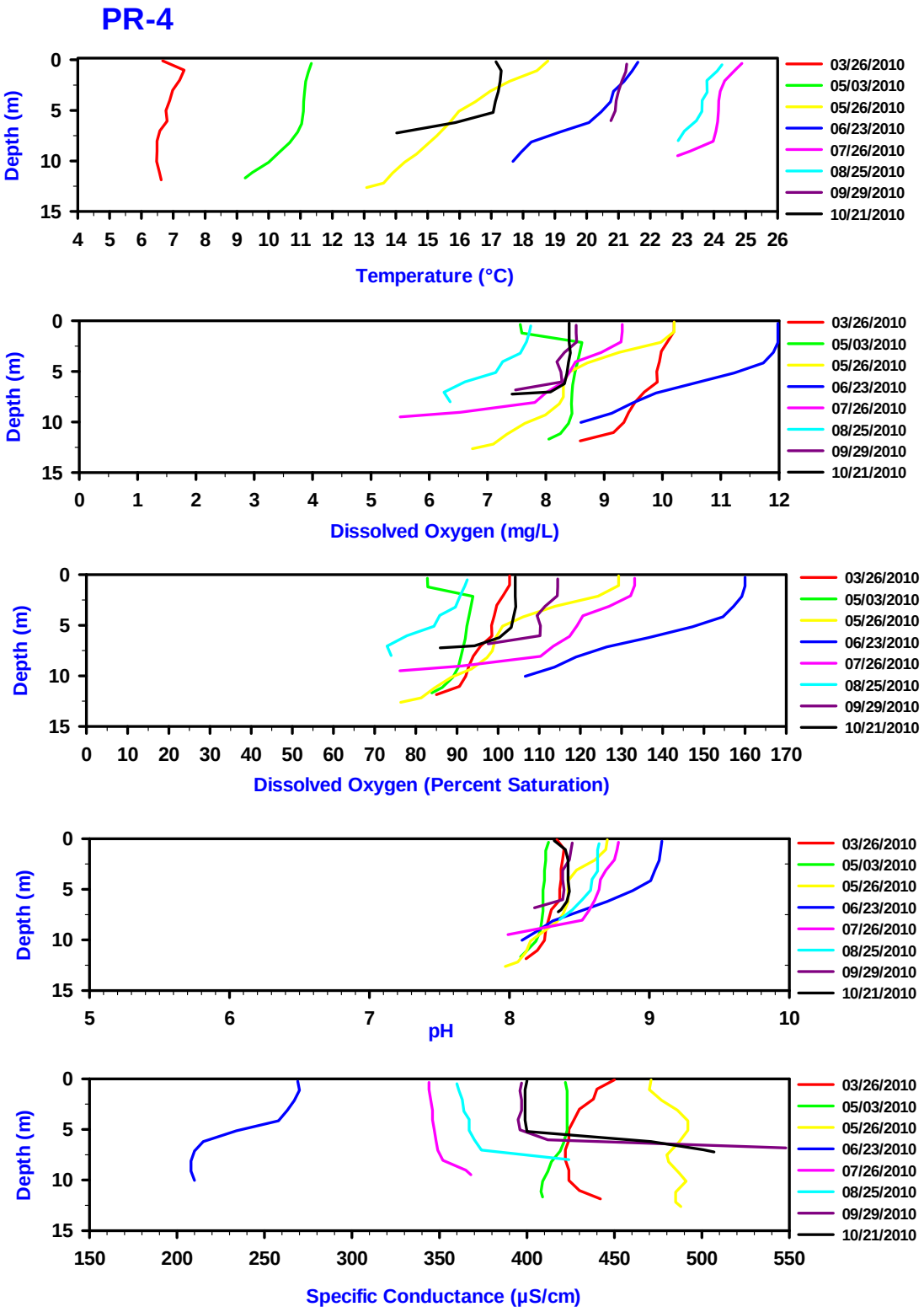

Figure 9 – 2010 profiles for Pueblo Reservoir Station PR-4.

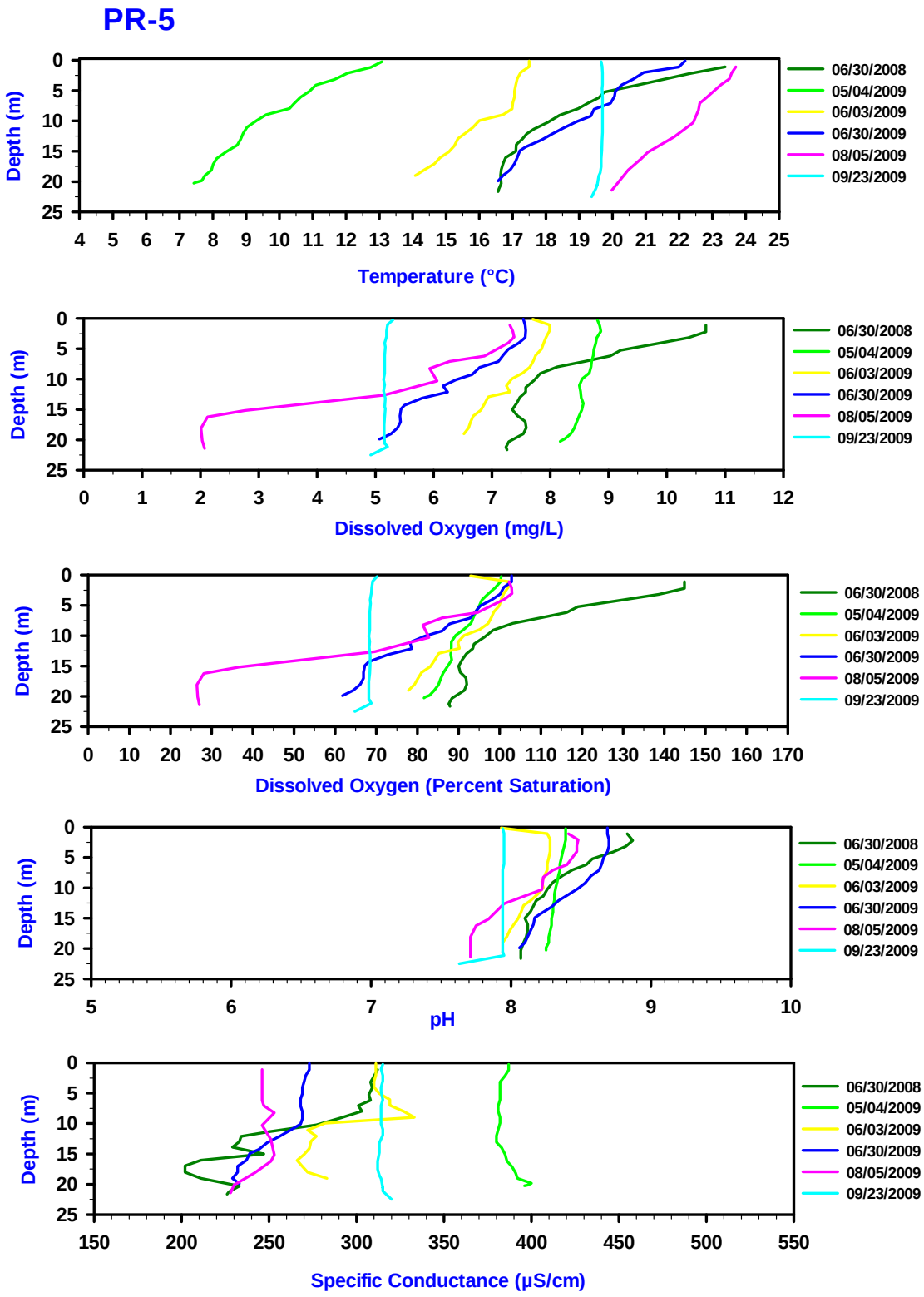

Figure 10 – 2008 and 2009 profiles for Pueblo Reservoir Station PR-5.

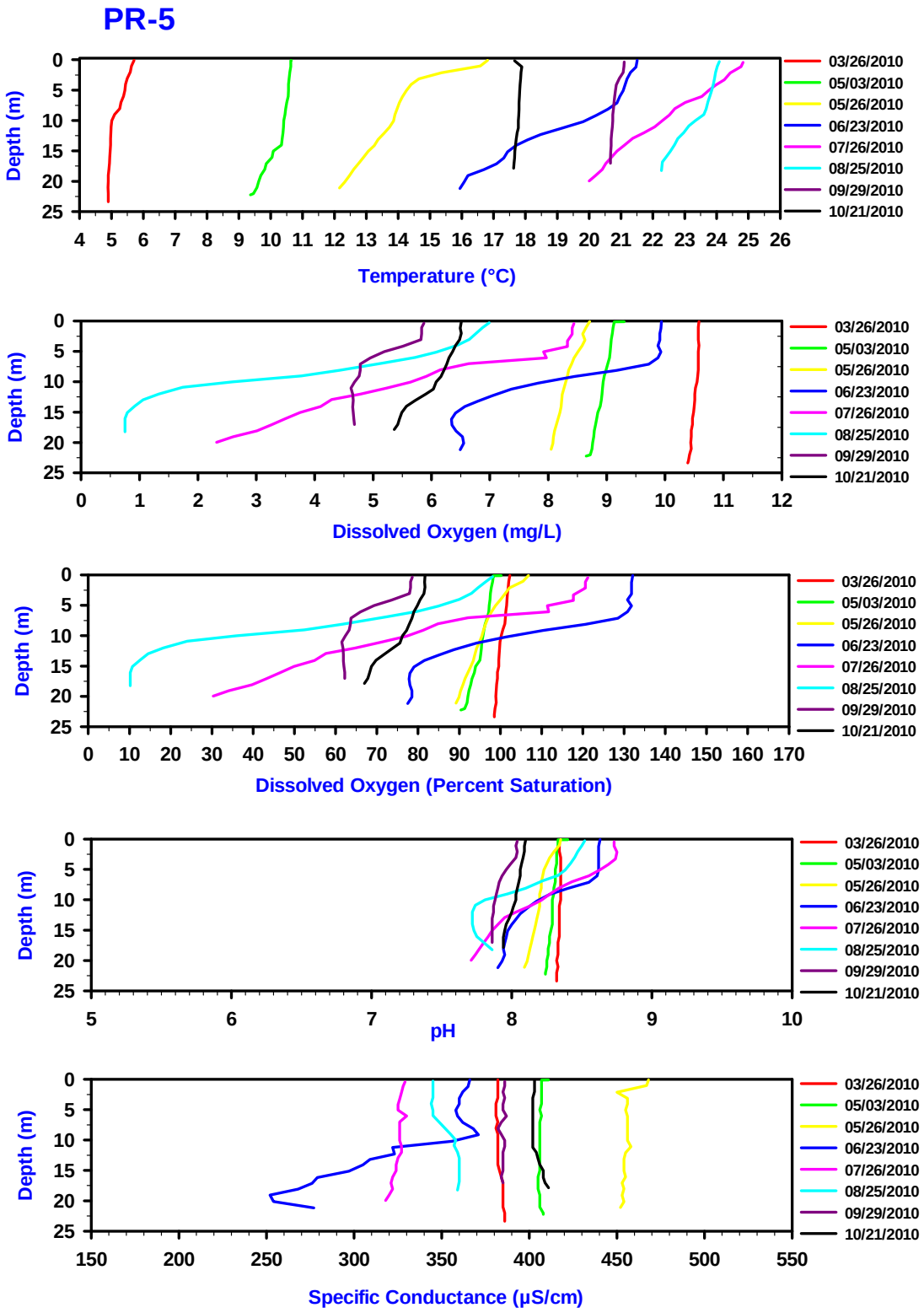

Figure 11 – 2010 profiles for Pueblo Reservoir Station PR-5.

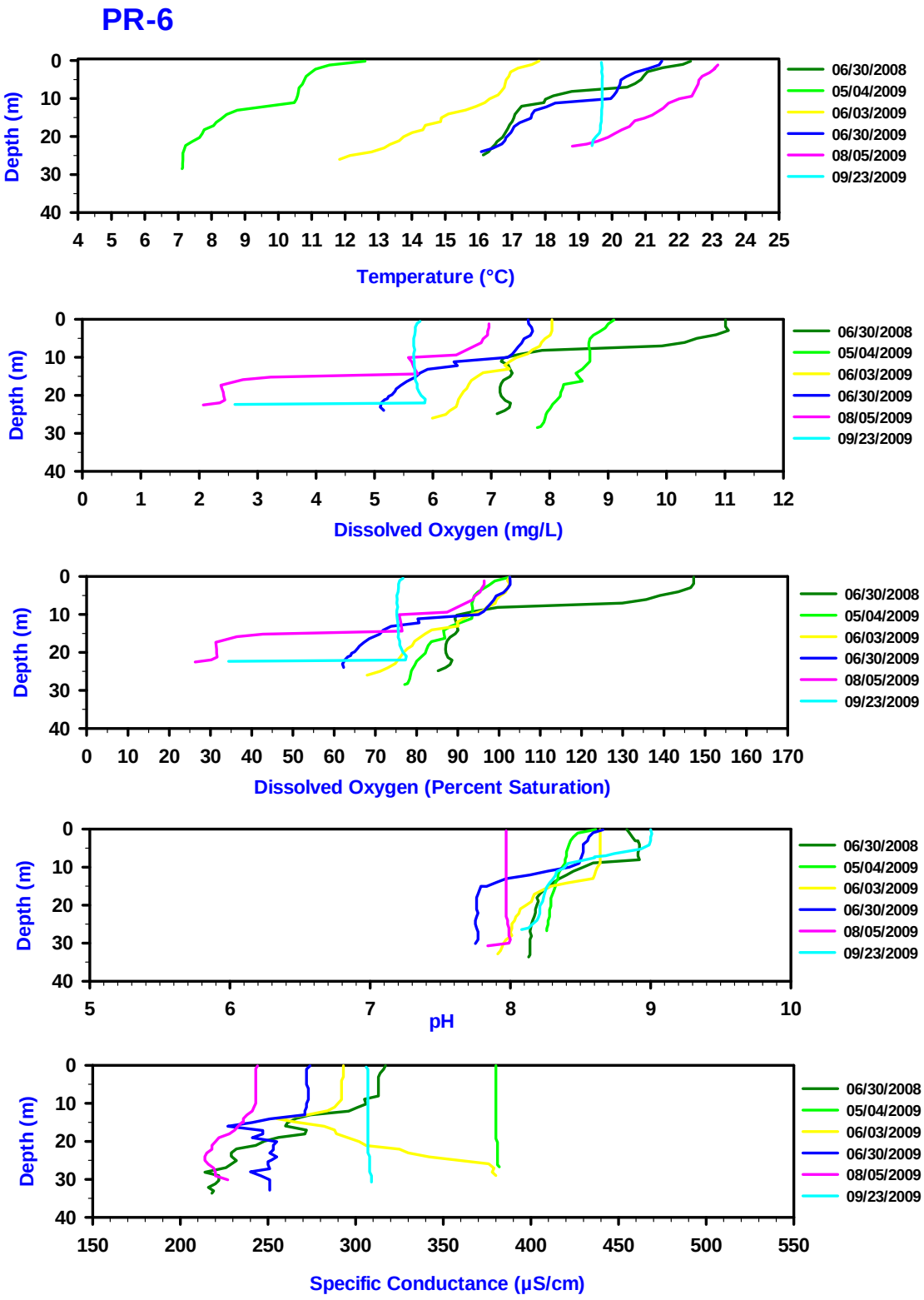

Figure 12 – 2008 and 2009 profiles for Pueblo Reservoir Station PR-6.

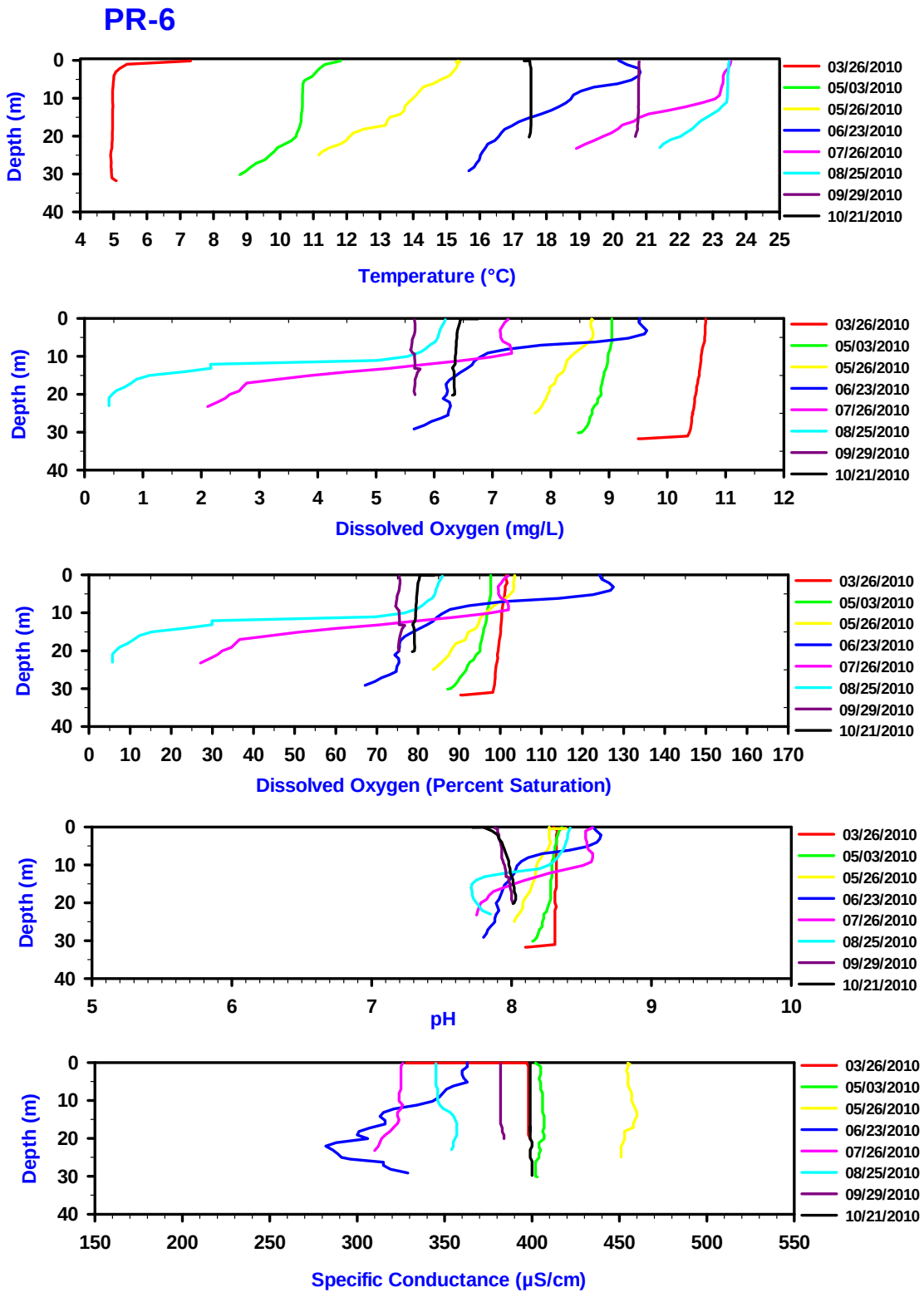

Figure 13 – 2010 profiles for Pueblo Reservoir Station PR-6.

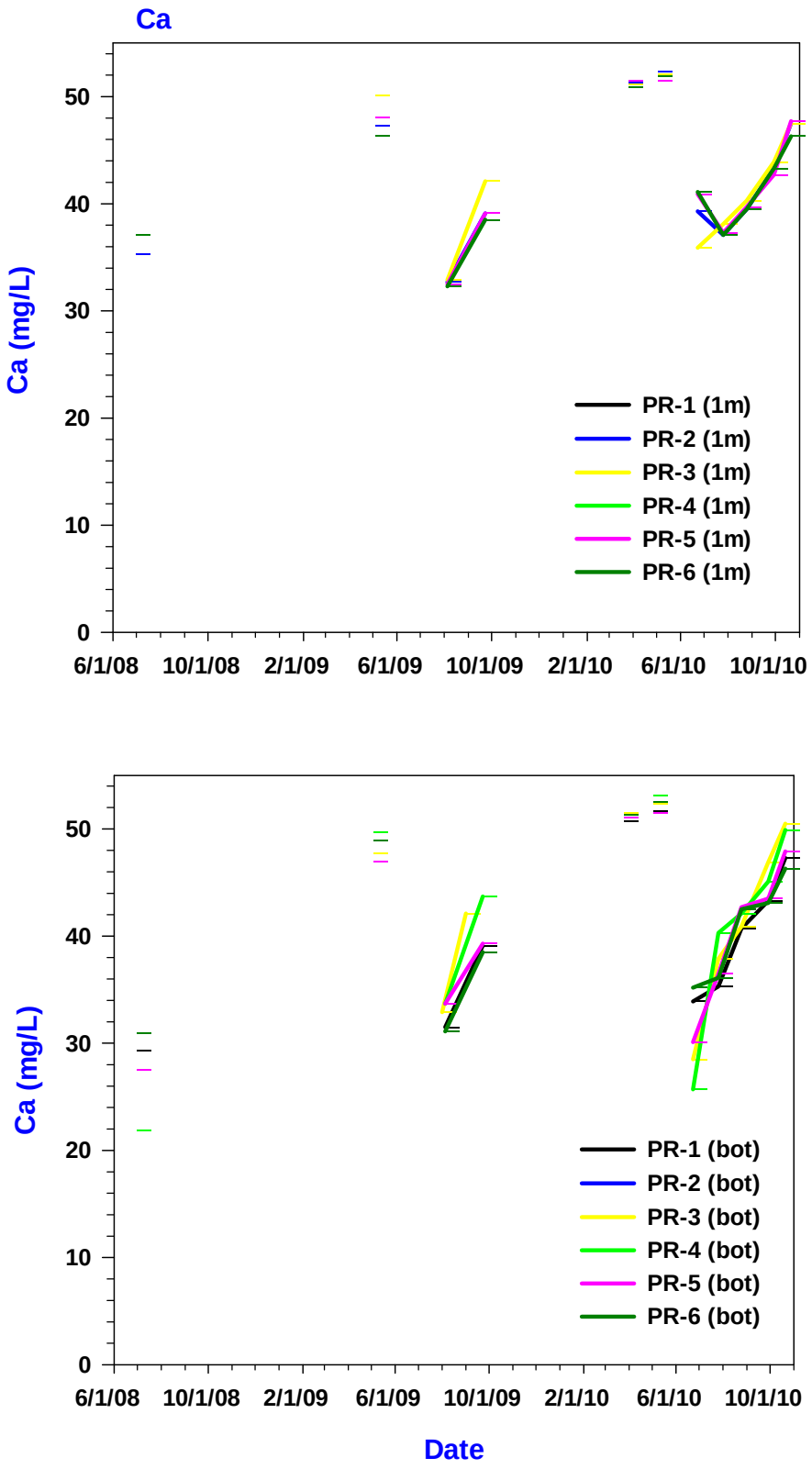

Figure 14 – Pueblo Reservoir calcium concentrations, 2008-2010.

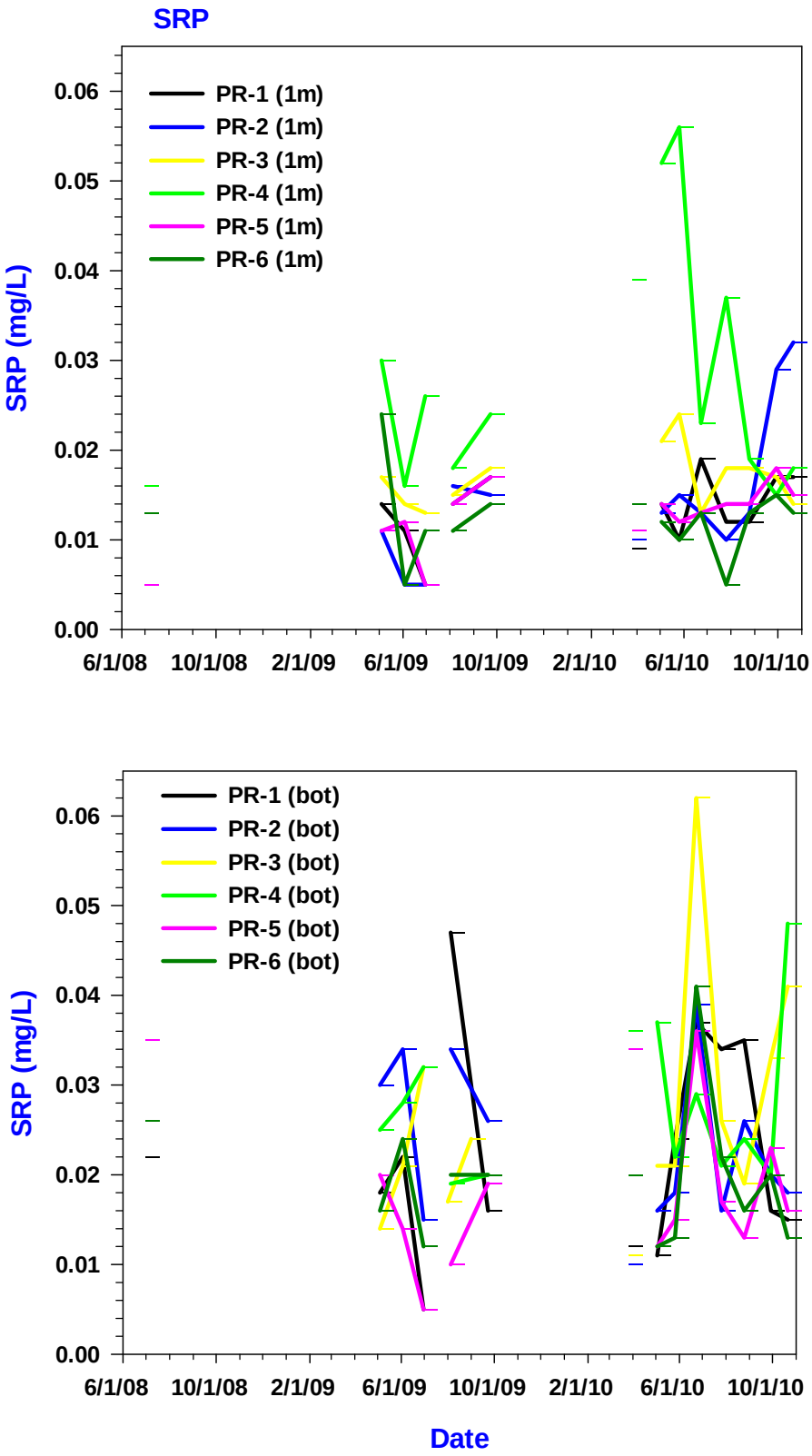

Figure 15 – Soluble reactive phosphorus concentrations in Pueblo Reservoir, 2008-2010.

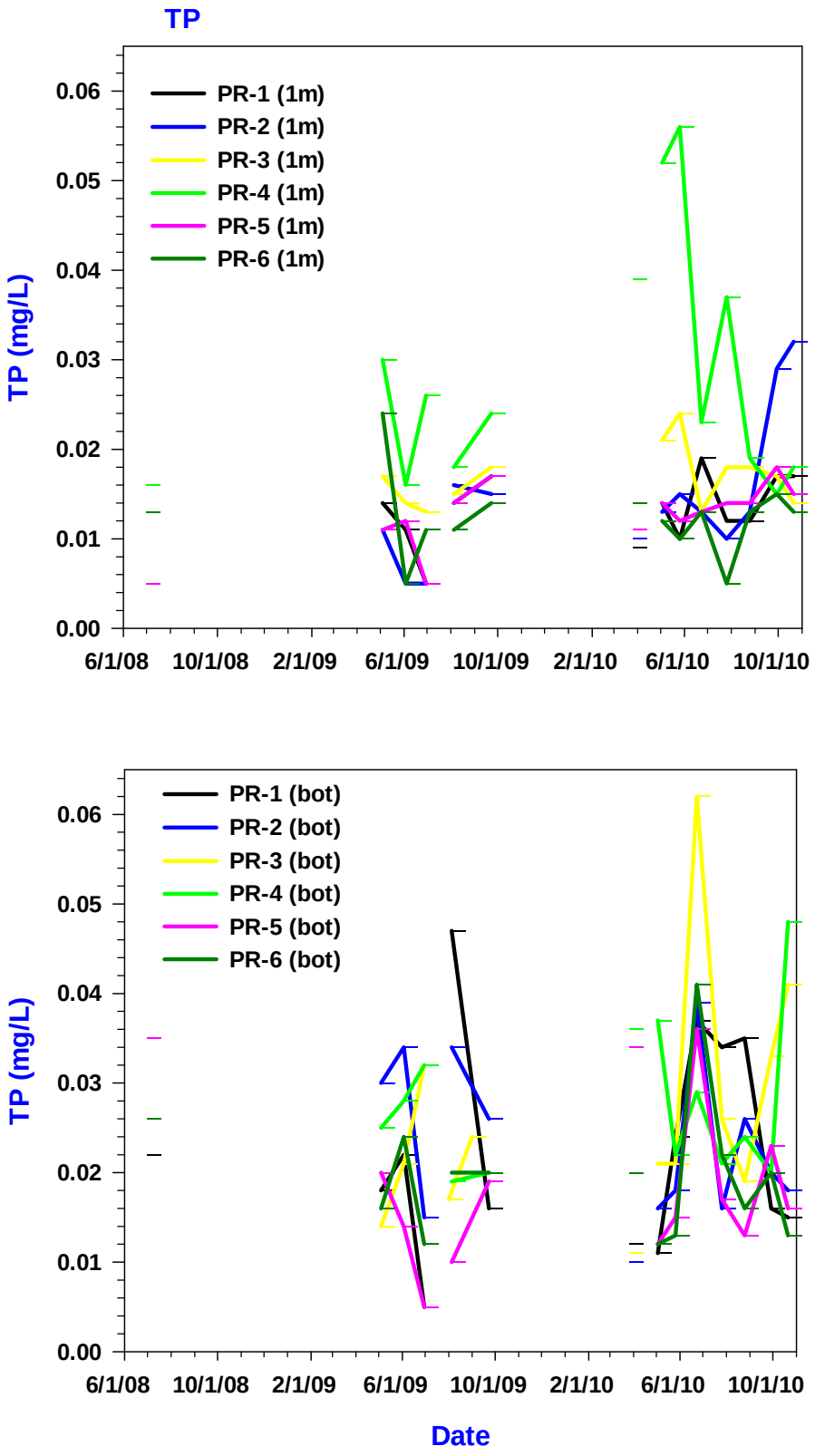

Figure 16 – Total phosphorus concentrations in Pueblo Reservoir, 2008-2010.

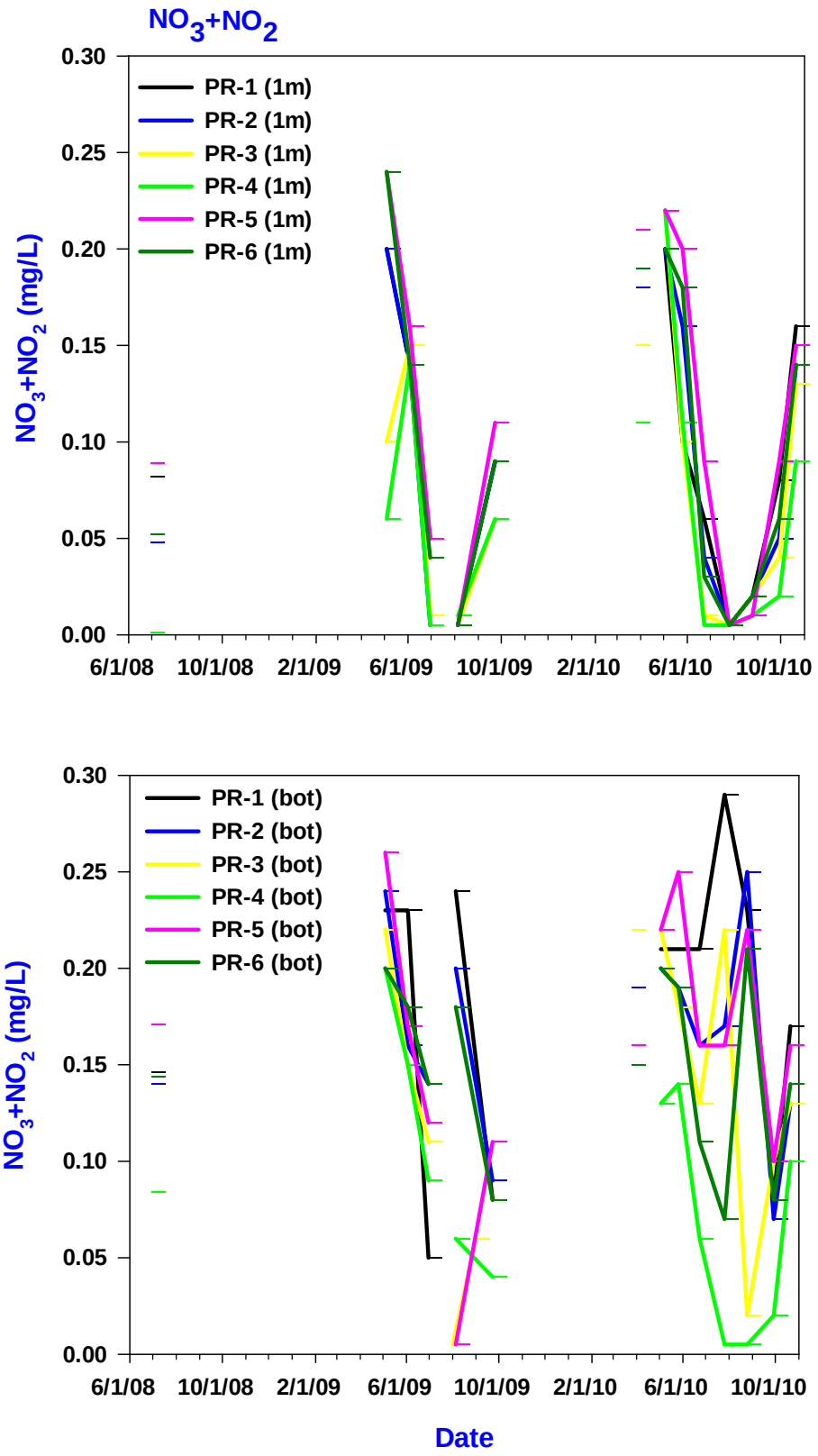

Figure 17 – Nitrate + nitrite-N concentrations in Pueblo Reservoir, 2008-2010.

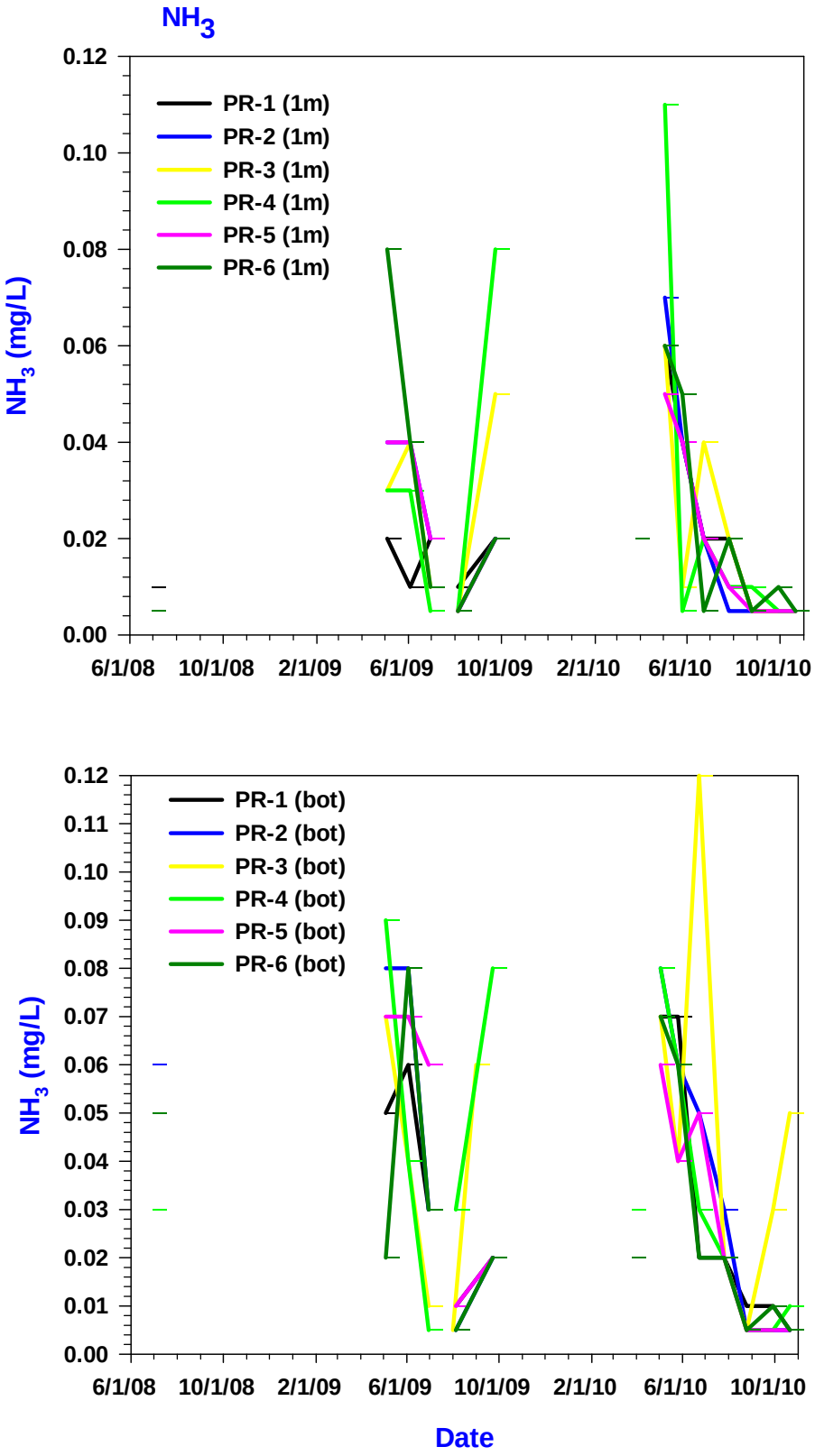

Figure 18 – Ammonia-N concentrations in Pueblo Reservoir, 2008-2010.

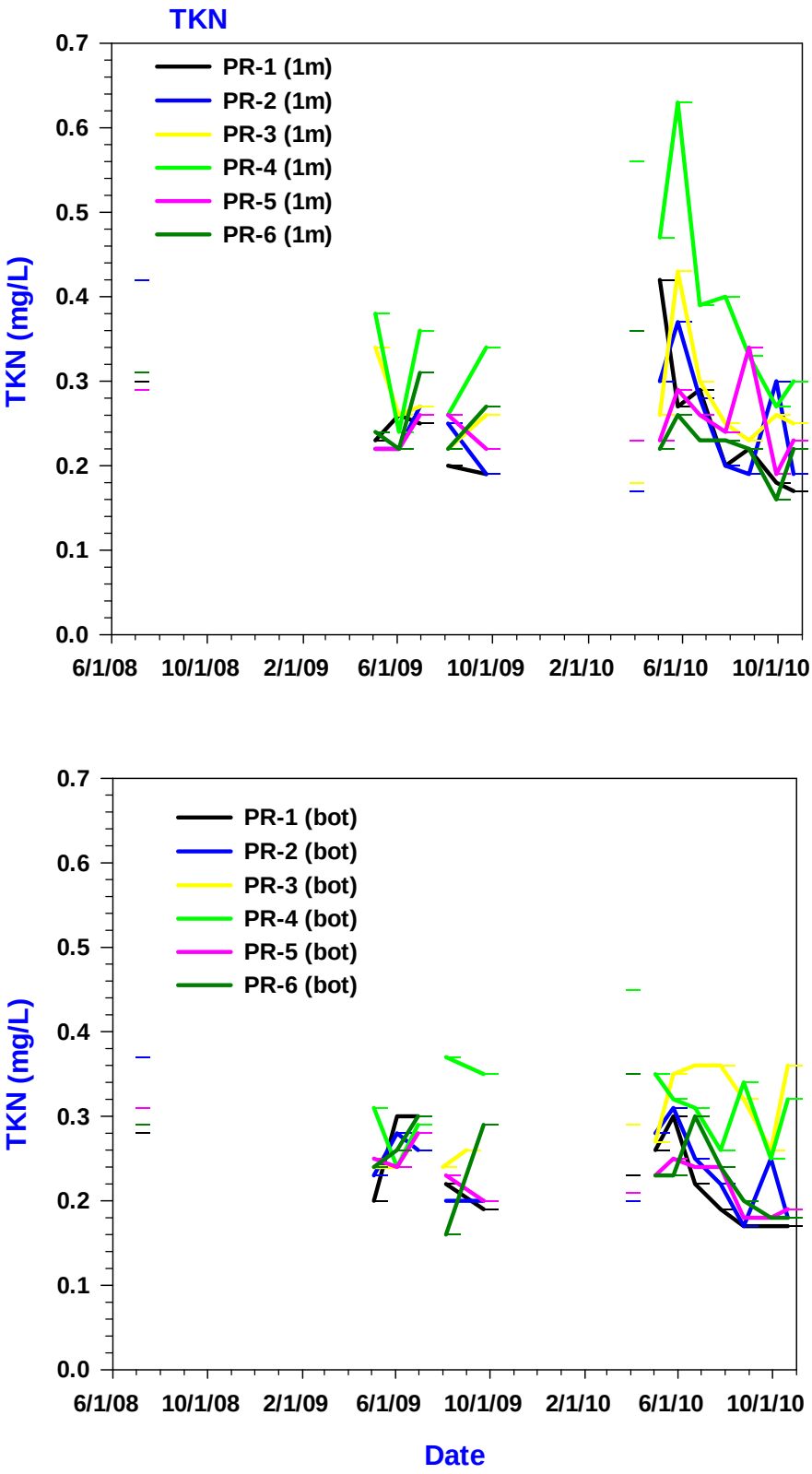

Figure 19 – Total Kjeldahl Nitrogen concentrations in Pueblo Reservoir, 2008-2010.

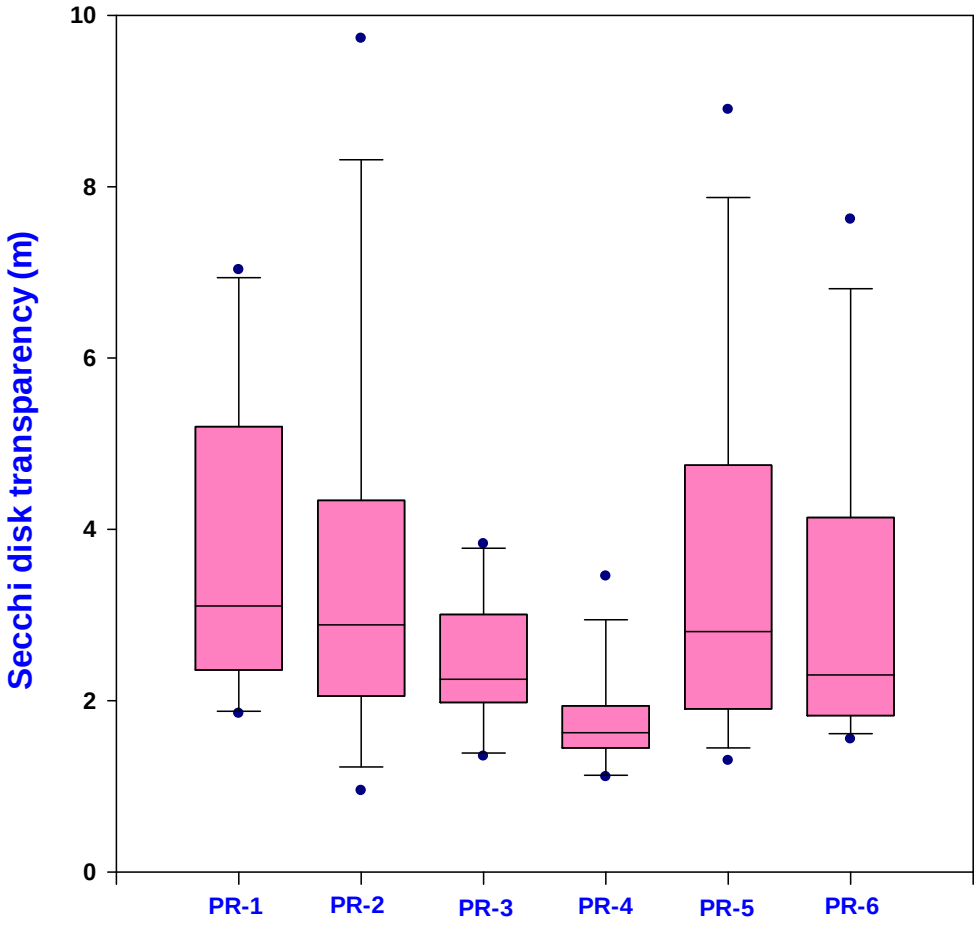

Figure 20 - Secchi disk transparency box plots at Pueblo Reservoir Stations PR-1, PR-2, PR-3, PR-4, PR-5, and PR-6 at 0-5m and 1m depths.

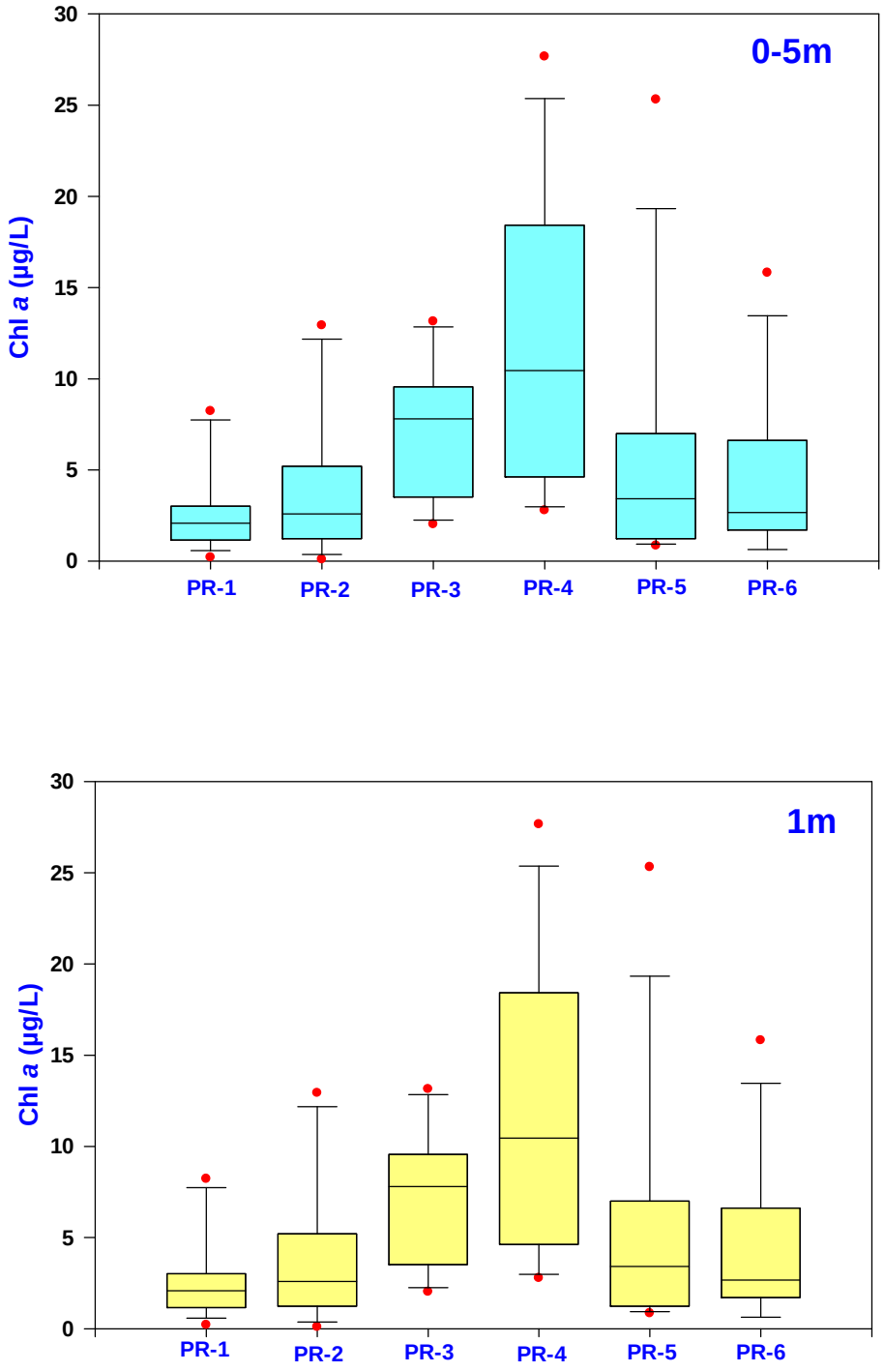

Figure 21 - Chlorophyll *a* box plots at Pueblo Reservoir Stations PR-1, PR-2, PR-3, PR-4, PR-5, and PR-6 for 0-5m and 1m depths, 2008-2010.

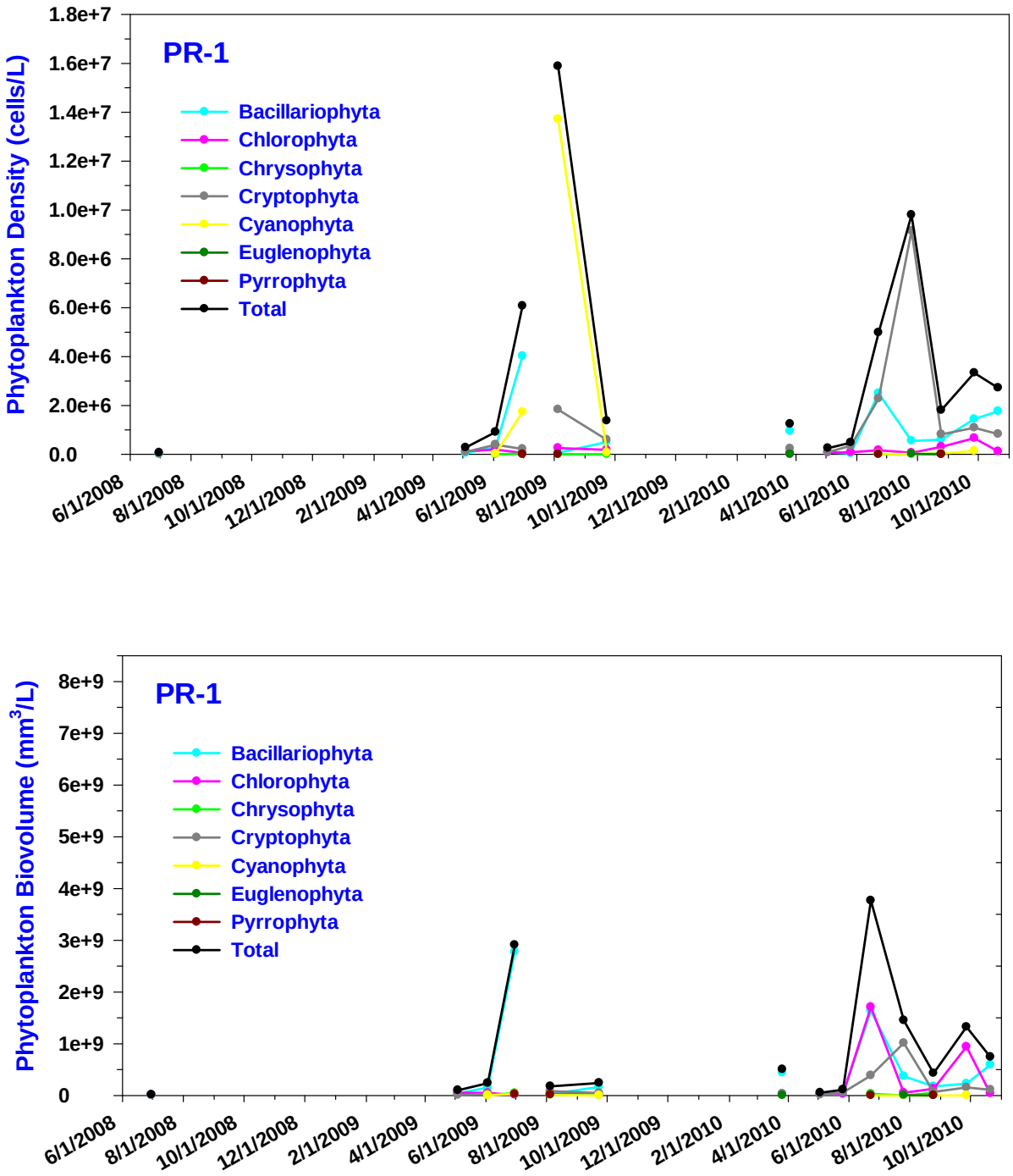

Figure 22 – Phytoplankton levels at Pueblo Reservoir Station PR-1, 2008-2010.

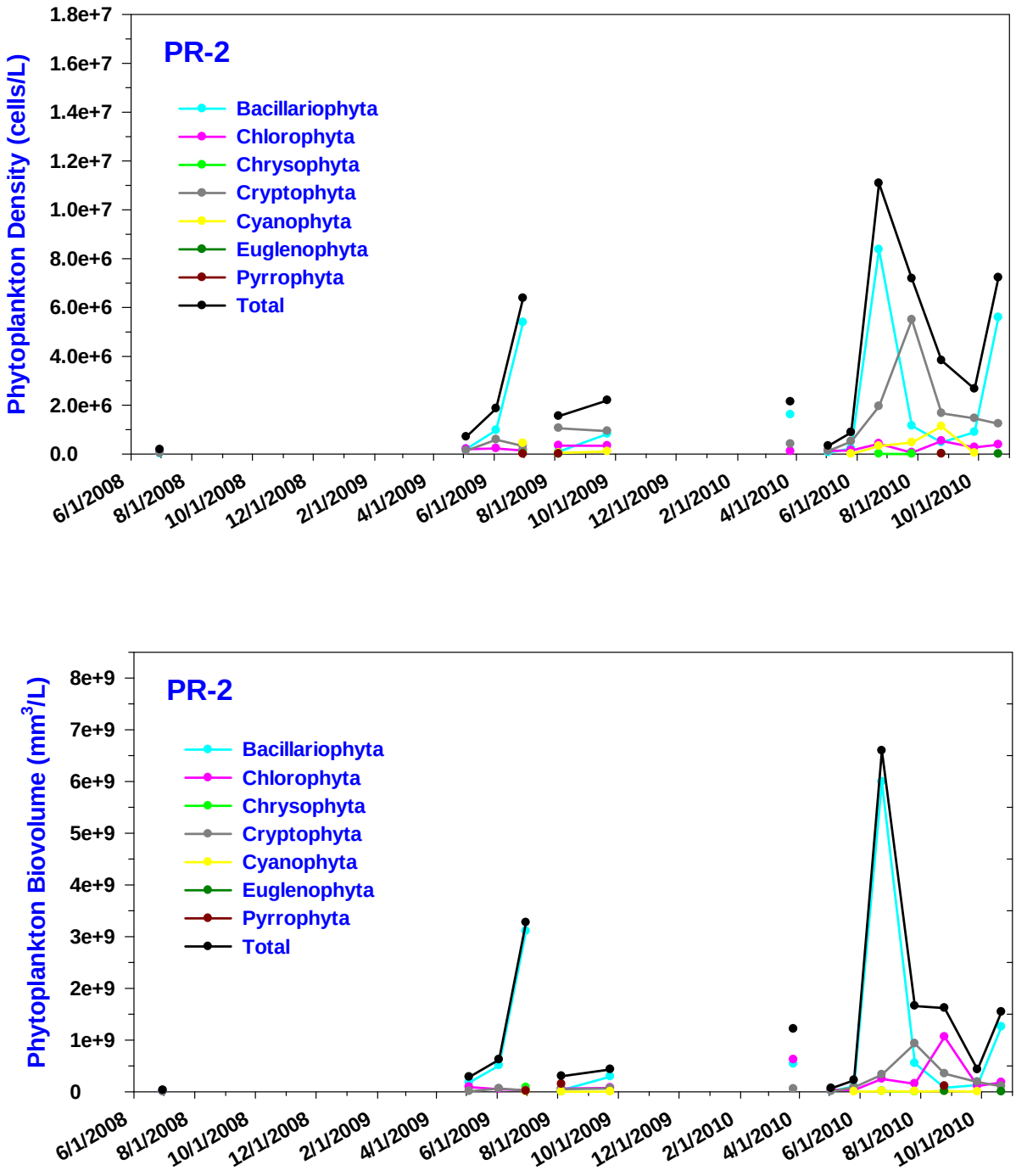

Figure 23 – Phytoplankton levels at Pueblo Reservoir Station PR-2, 2008-2010.

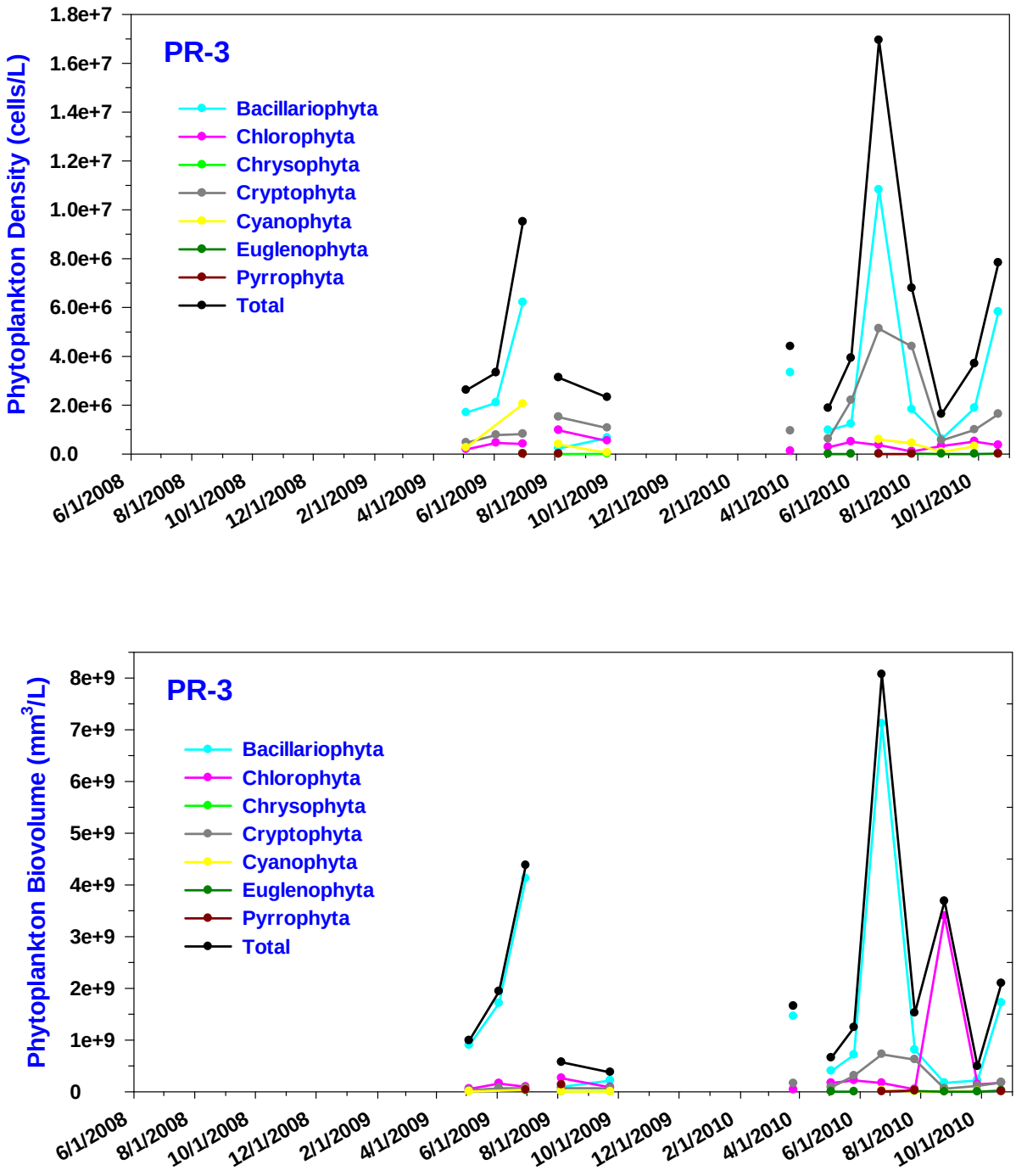

Figure 24 – Phytoplankton levels at Pueblo Reservoir Station PR-3, 2008-2010.

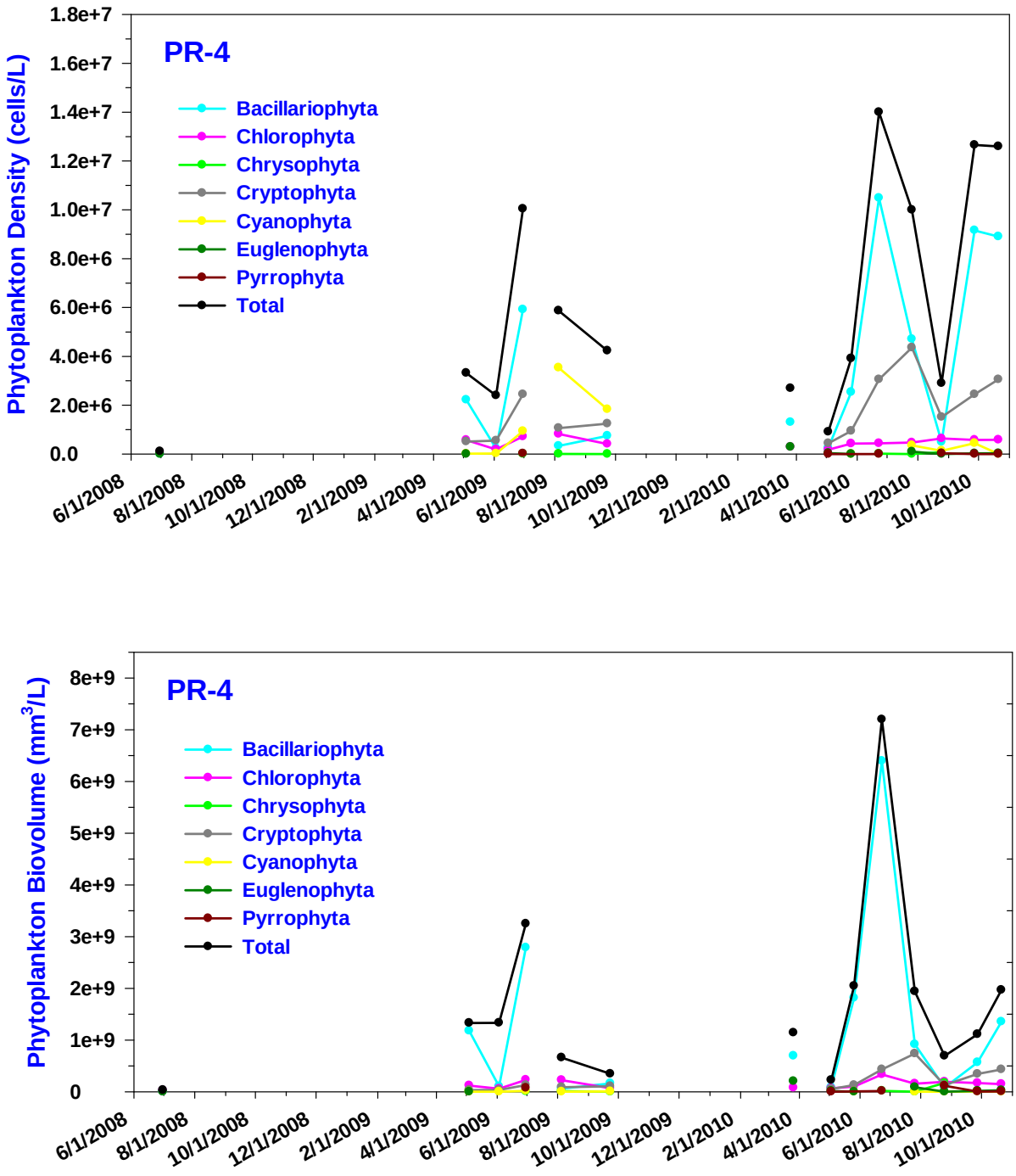

Figure 25 – Phytoplankton levels at Pueblo Reservoir Station PR-4, 2008-2010.

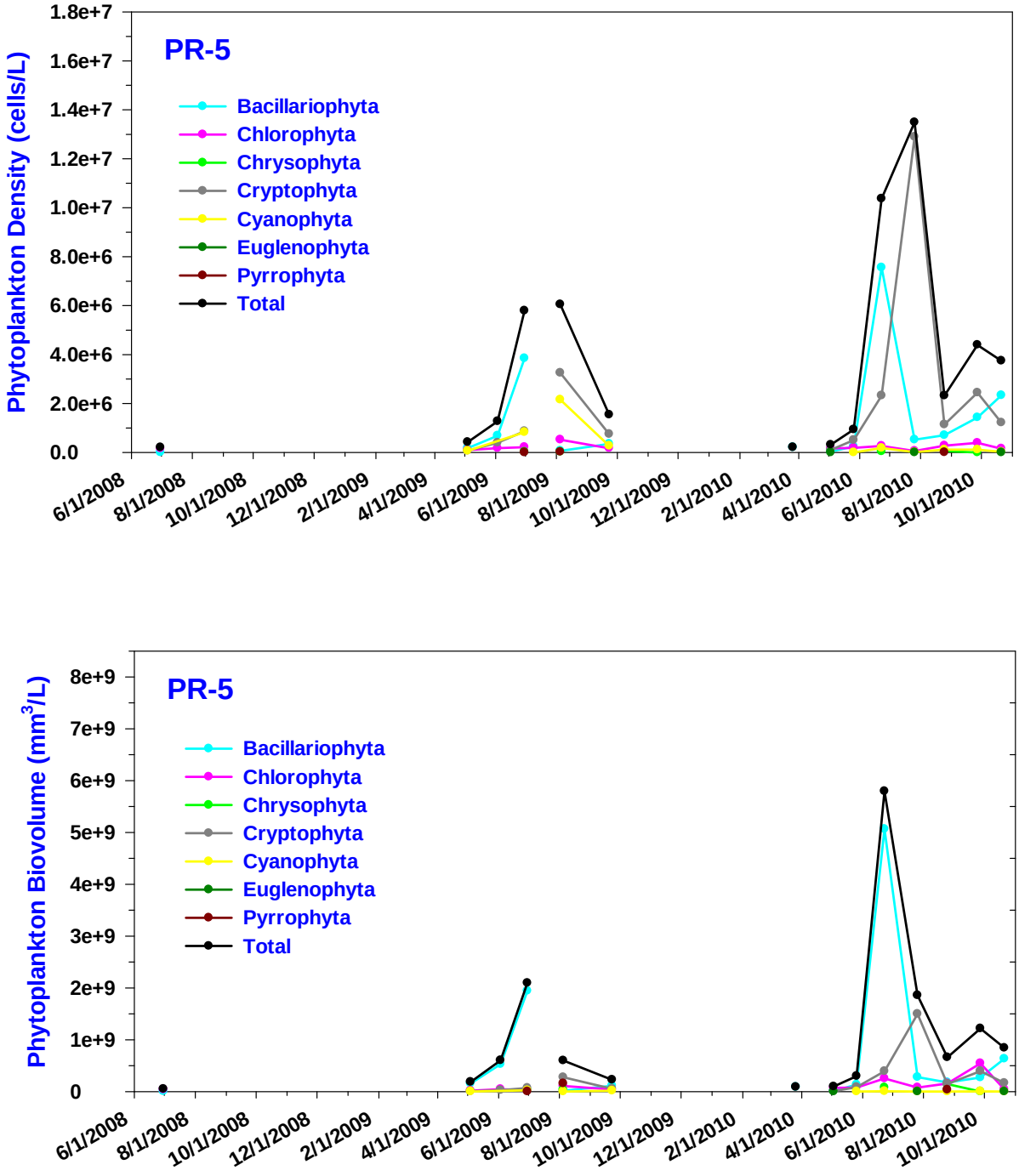

Figure 26 – Phytoplankton levels at Pueblo Reservoir Station PR-5, 2008-2010.

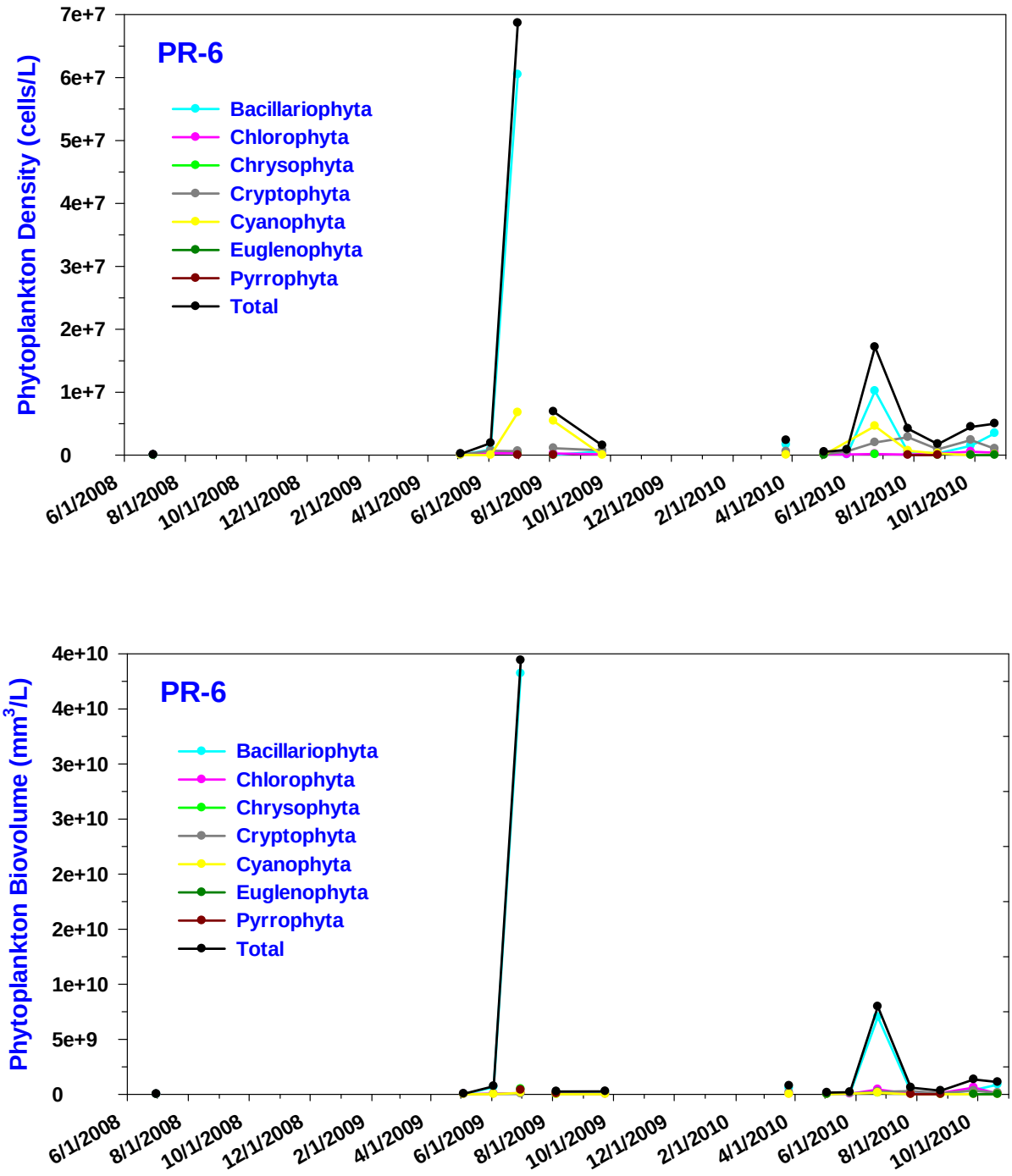

Figure 27 – Phytoplankton levels at Pueblo Reservoir Station PR-6, 2008-2010.

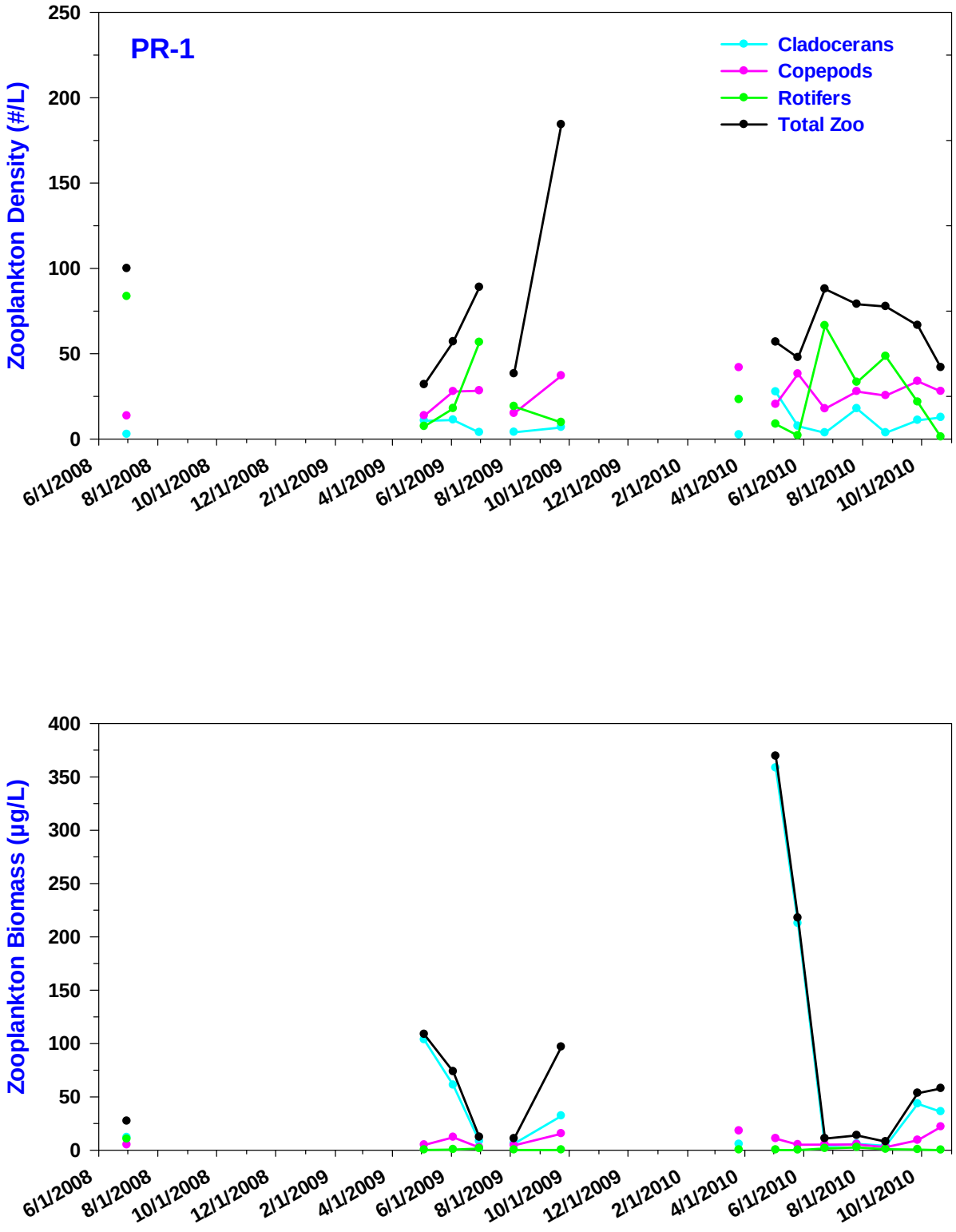

Figure 28 – Zooplankton levels at Pueblo Reservoir Station PR-1, 2008-2010.

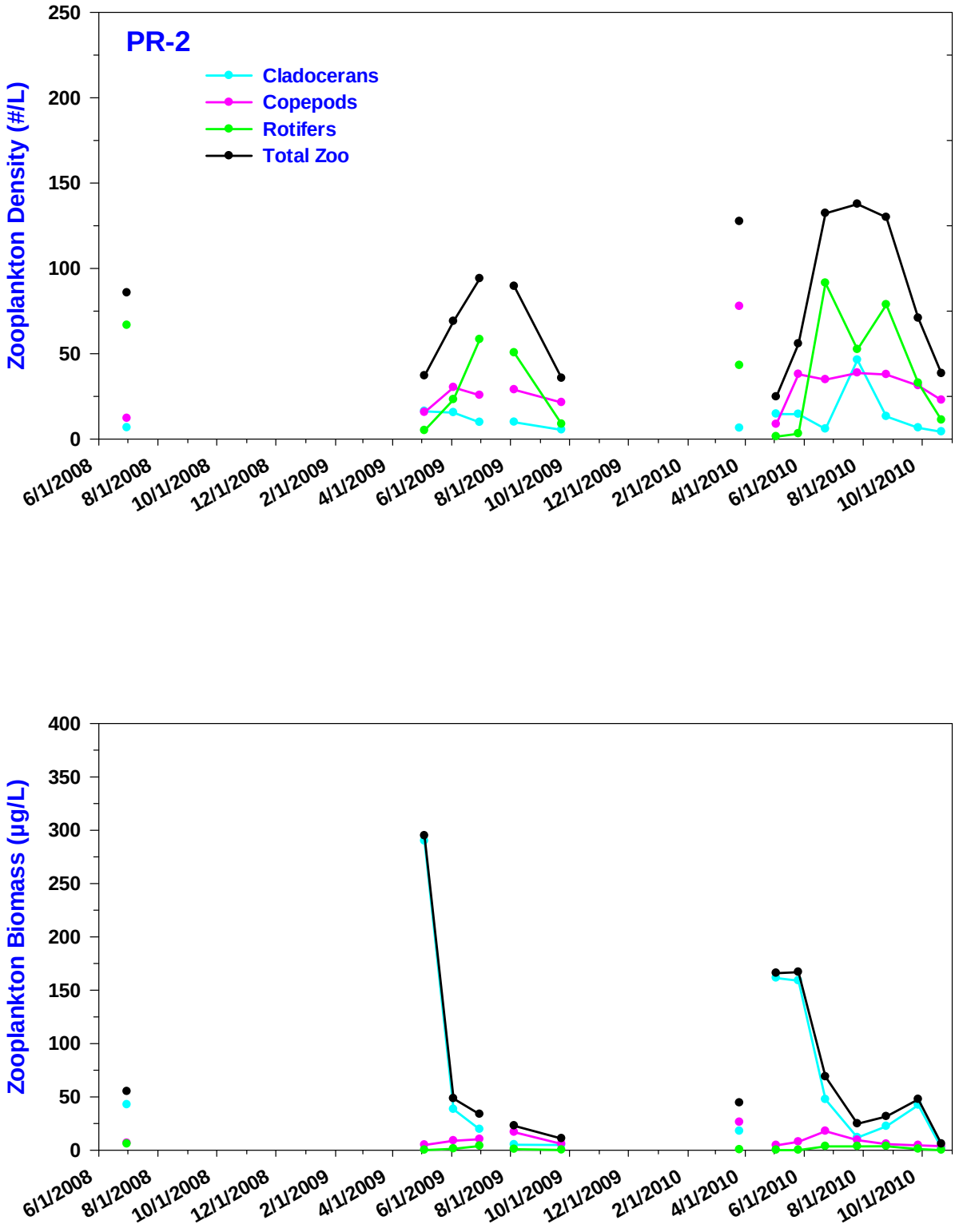

Figure 29 – Zooplankton levels at Pueblo Reservoir Station PR-2, 2008-2010.

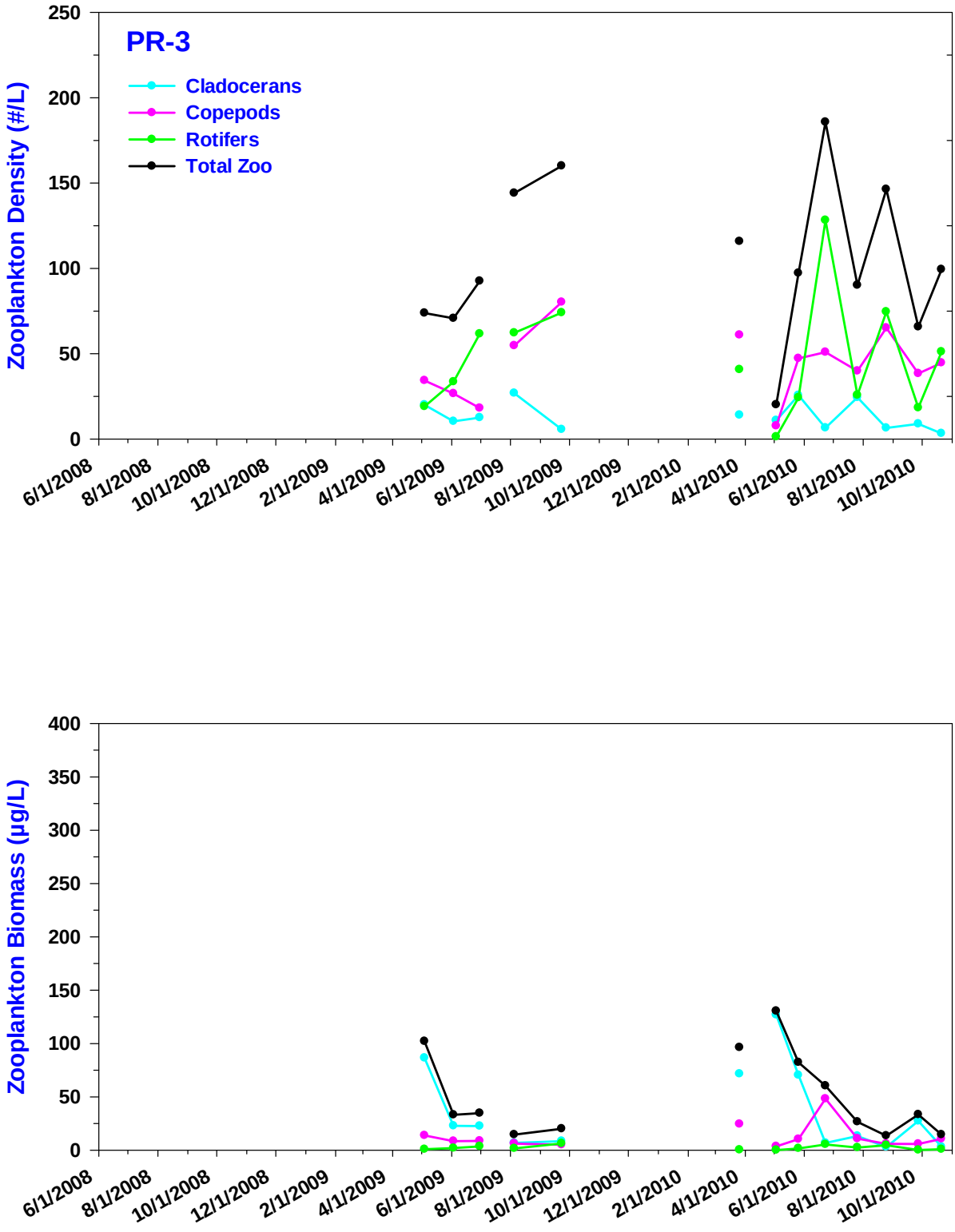

Figure 30 – Zooplankton levels at Pueblo Reservoir Station PR-3, 2008-2010.

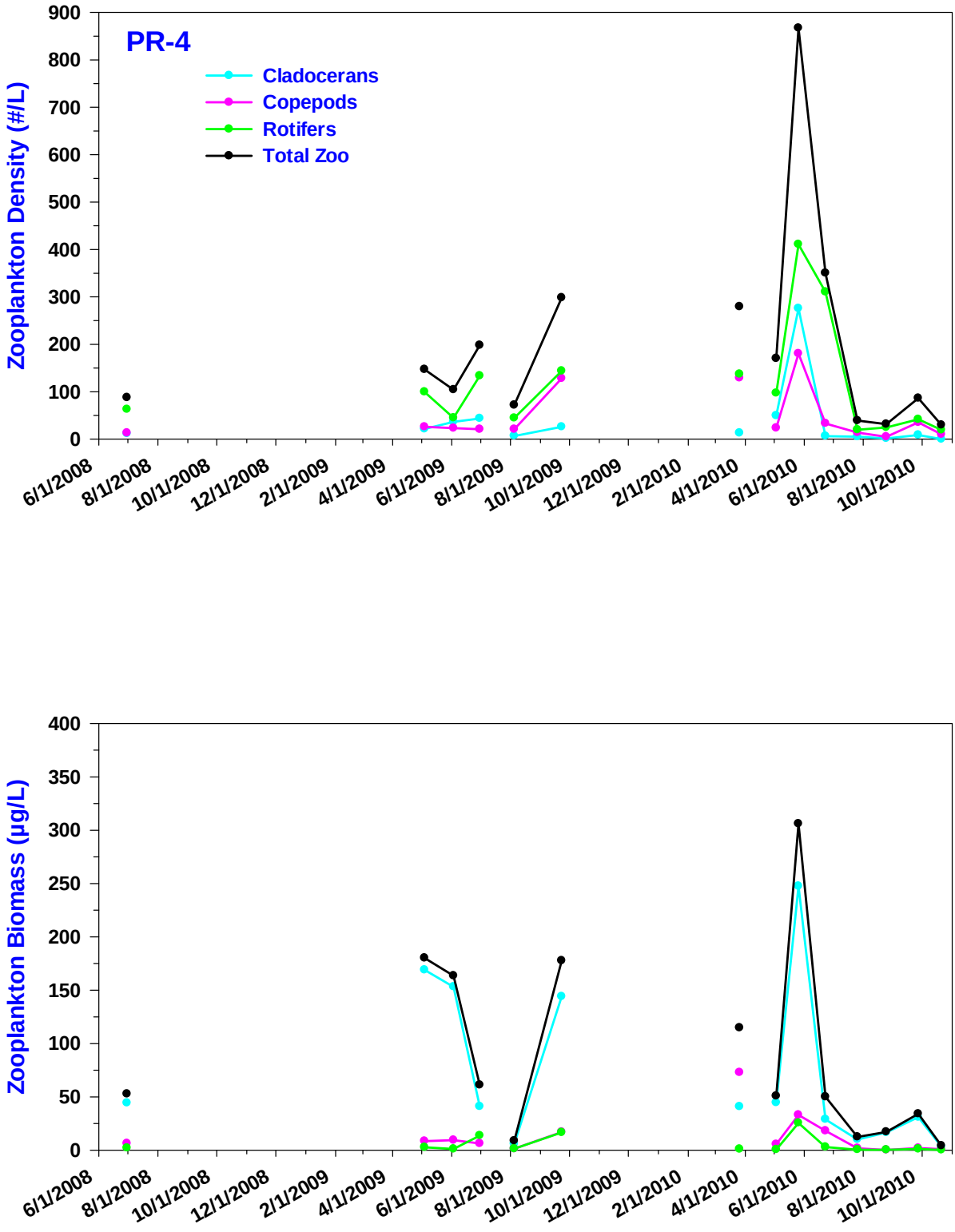

Figure 31 – Zooplankton levels at Pueblo Reservoir Station PR-4, 2008-2010.

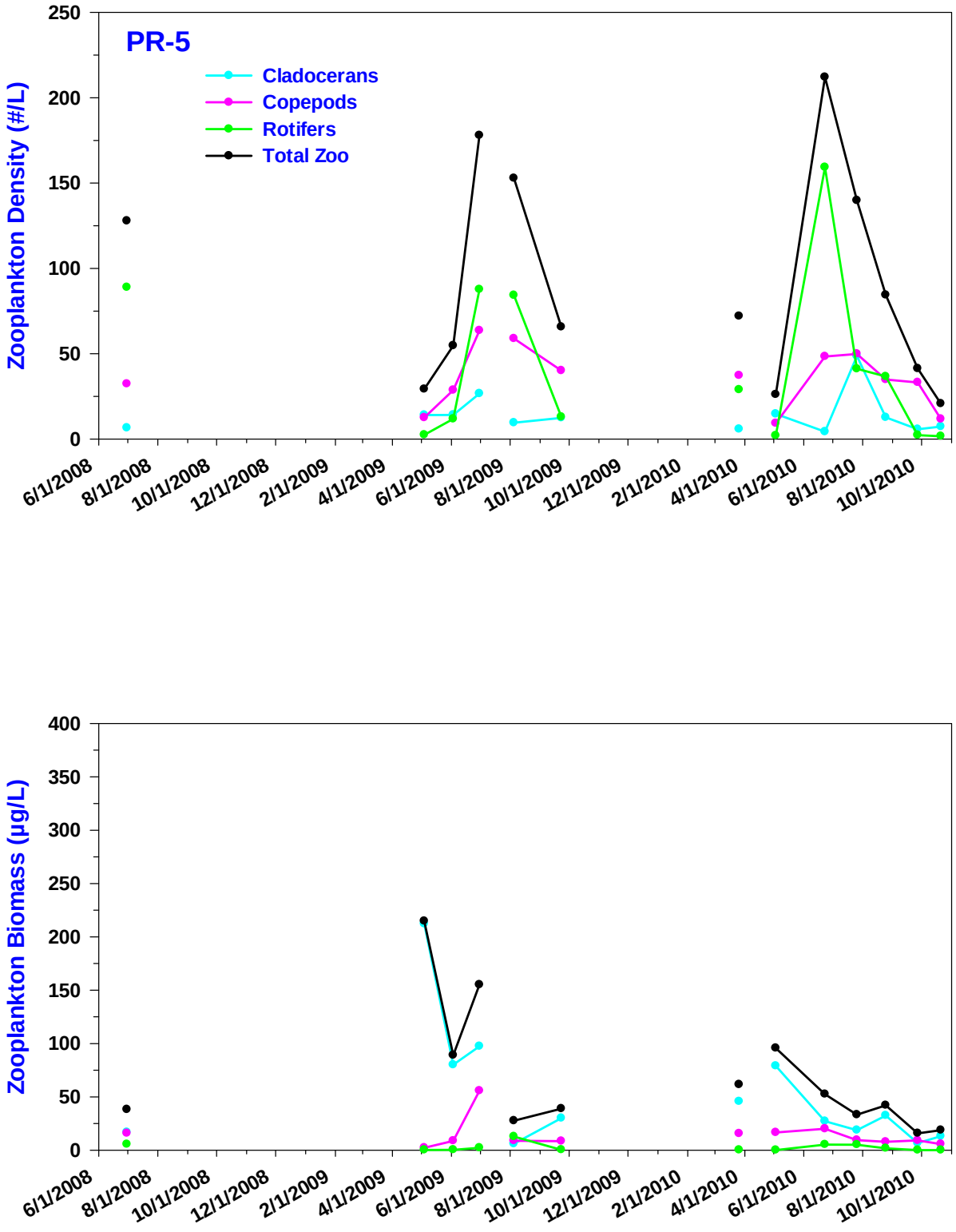

Figure 32 – Zooplankton levels at Pueblo Reservoir Station PR-5, 2008-2010.

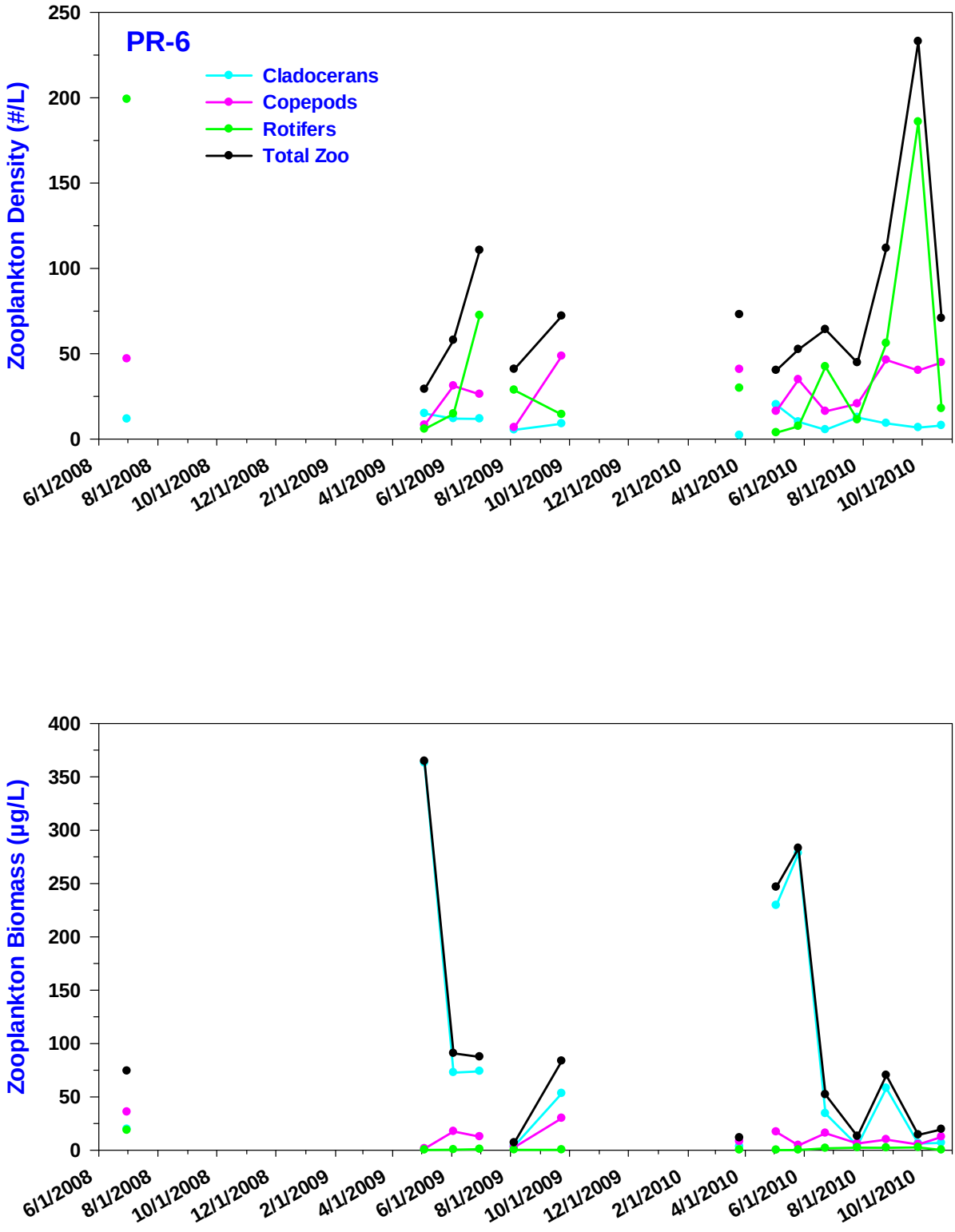

Figure 33 – Zooplankton levels at Pueblo Reservoir Station PR-6, 2008-2010.