STATUS AND ECOLOGY OF A GLACIAL RELICT MOLLUSK, THE ROCKY MOUNTAIN CAPSHELL LIMPET (*ACROLOXUS COLORADENSIS*), IN RELATION TO THE LIMNOLOGY OF LOST LAKE, GLACIER NATIONAL PARK, MONTANA (USA)

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By

Bonnie K. Ellis[1], Leo Marnell[2], Michelle Anderson[1], Jack A. Stanford[1]
Christian Albrecht[3] and Thomas Wilke[4]

[1]Flathead Lake Biological Station
The University of Montana
311 Bio Station Lane
Polson, MT 59860-9659 USA
Phone: (406) 982-3301
Fax: (406) 982-3201
Email: bonnie.ellis@umontana.edu

[2]National Park Service
Glacier National Park
Park Headquarters Science Center
West Glacier, MT 59936 USA

[3]Department of Ecology and Evolution
J.W. Goethe University Frankfurt
Siesmayerstr. 70, D-60054
Frankfurt, Germany

[4]Department of Animal Ecology and Systematics
Justus Liebig University Giessen
Heinrich-Buff-Ring 26-32 (IFZ)
D-35392 Giessen, Germany

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INTRODUCTION

The Rocky Mountain capshell limpet, *Acroloxus coloradensis* (Henderson), was discovered in Lost Lake, a small subalpine pond on the east side of the Continental Divide, in Glacier National Park (GNP), Glacier County, Montana, during the mid-1960s (Russell and Brunson 1967). The reported distribution of *A. coloradensis* is highly disjunct and limited to 17 localities, all in North America. In addition to the Lost Lake population, a colleague of ours (Brian Reid) in 2001 collected *A. coloradensis* in Trout Lake on the west side of the Continental Divide in GNP; and, 11 records exist from Rocky Mountain lakes in Colorado (Walker 1925; Pioneer Environmental Service, Inc. 1993; Riebesell et al. 2001), British Columbia, Canada (Clarke 1981), Alberta, Canada (Mozley 1926, 1930), and Quebec, Canada (Clarke 1970). The type locality is Peterson Lake, near Nederland, Colorado (Walker 1925; Bryce 1970). However, *A. coloradensis* also was reported from 2 widely separated ponds in Ontario (Clarke 1970), 1 pond in Colorado (Riebesell et al. 2001) and Paul and Clifford (1991) collected it from the Beaver River in northeastern Alberta, Canada.

Fossilized *A. coloradensis* was reported in the Berends and Dixon Pleistocene assemblages distributed across southwestern Kansas, portions of Oklahoma and in north central Nebraska (Taylor 1954, 1960). These fossils are in glacial outwash and lacustrine sediments suggesting presence in cold-water lakes and ponds and the modern distribution records are coherent with continental glaciation patterns. Thus, Paul and Clifford (1991) called this capshell a glacial relict species that likely was widely distributed in North America during the Pleistocene.

Owing to its disjunct distribution and relict status, the Rocky Mountain capshell was considered rare by Hubendick (1969) and possibly an endangered species (Wu 1989). In 1992, the US Fish and Wildlife Service was petitioned to list *A. coloradensis* for protection under the Endangered Species Act. However, listing was withheld pending further evaluation. The species was described simply as a species of special concern (Garza 1993; USFWS 1994). In any case based on review and synthesis of collection records, Frest and Johannes (1995) later described *A. coloradensis* as an extremely rare species that warranted protection by management agencies.

The status of *A. coloradensis* in Lost Lake has not been examined since the species was discovered some 40 years ago, and collection records in Lost Lake and elsewhere included few ecology notes or habitat descriptions. Historically fishless, Lost Lake, like many other potential capshell habitats in the Rocky Mountains, has a long legacy of trout (Salmonidae) stocking and heavy use by recreationists for fishing, wading, and swimming. The lake is small and shallow and the capshells are rather fragile, epilithic organisms, perhaps vulnerable to waders and swimmers. Moreover in a preliminary study, Marnell (unpubl.) found capshells in trout guts. Thus, during 2001–2, we quantified the distribution and abundance of capshells in Lost Lake and in relation to a detailed limnological analysis of the lake. We also examined phylogenetic relationships within the Acroloxidae to clarify the status of *A. coloradensis* in a local–global context. We compared the Lost Lake genotype to Eurasian species of the family and examined genetic diversity among Rocky Mountain populations of *A. coloradensis* using nuclear DNA sequencing. Finally, we considered the data in a conservation context. We concluded that Rocky Mountain capshell limpets remain fairly abundant in Lost Lake. No genetic variation was demonstrated among Rocky Mountain populations selected for analysis. They do not appear to be very sensitive to fish predation. Nonetheless, protection of this important population should include returning Lost Lake to its historical fishless condition by intensive gill netting of nonnative trout and carefully managing or eliminating wading and swimming by recreationists.

METHODS AND BACKGROUND

Limnology of Lost Lake

Lake surface area, catchment area, and bedrock geology composition in the catchment were determined using Arc/INFO and a digitized map of bedrock geology in Glacier National Park (Whipple 1992) obtained from the National Park Service GIS Lab and the University of North Carolina's Department of Geography. Lake bathymetry was determined from a spatially explicit set of depth soundings obtained in 1994 by L. Marnell (unpubl.). Hourly water temperature from August 2001 to July 2002 was determined by placement of 2 temperature loggers (Onset StowAway®) at a depth of 0.6–0.7 m in the eastern and southern regions of the lake.

Water column sampling of chemistry and biota was conducted approximately monthly during the ice free season of 2001 (July–October) and 2002 (June–September). An inflatable raft was anchored to the shoreline to access the midlake site without disturbing the sediments. Vertical water column profiles of temperature, pH, specific conductance, dissolved oxygen, oxygen saturation, and turbidity were obtained with an electronic meter (Hydrolab Surveyor). The meter was calibrated with ASTM standards on site prior to sampling and calibration records for all meters and analytical instruments were archived at the laboratory. Secchi disk depth was determined near midday on the shady side of the raft. Acid-cleaned polyethylene or Teflon bottles were rinsed twice before filling with lake water obtained with a VanDorn water bottle from depths of 1 m and 5 m for nutrient, biological, and carbon analyses. Water samples collected for chlorophyll *a* analysis were filtered on site under a dark tarp and immediately frozen on dry ice. In August and October 2001, additional grab samples were collected with sterile gloves at about 0.2 m depth near the south shoreline for trace metals analyses. All samples were transported to the Flathead Lake Biological Station Freshwater Research Laboratory within 5 h.

Dissolved and total organic carbon (DOC, TOC) were determined by persulfate digestion and infrared CO_2 detection (Menzel and Vaccaro 1964). Chlorophyll *a* was determined by acetone extraction (APHA 1998). Total nitrogen (TN) was determined by persulfate digestion (D'Elia et al. 1977). Soluble total metals were determined by ultrasonic nebulization inductively coupled plasma spectrometry at the Environmental Biogeochemistry Laboratory of The University of Montana (EPA Method 200.15): aluminum (Al), arsenic (As), barium (Ba), beryllium (Be), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), nickel (Ni), phosphorus (P), lead (Pb), sulfur (S), silicon (Si), tin (Sn), strontium (Sr), titanium (Ti), vanadium (V), and zinc (Zn). Other analyses followed the methods of APHA (1998) with minor modification (Stanford et al. 1986): sulfate (SO_4), total phosphorus (TP), soluble reactive phosphorus (SRP), nitrite plus nitrate ($NO_{2/3}$-N), ammonium (NH_4-N), and carbonate alkalinity.

Net and gross primary productivity were determined in Lost Lake in August and September 2002 by the analysis of changes in dissolved oxygen using the light/dark bottle technique (after Wetzel and Likens 2000). Replicate bottles were incubated at 5 depths throughout the photic zone that extended to the lake bottom over a midday 2-hour period. Oxygen concentrations were determined by Winkler titration. Mass of oxygen was converted to mass of carbon by multiplying by 0.375 (i.e., the ratio of moles of carbon to moles of oxygen).

Values of $mgC/m^3/h$ were plotted against depth and the area under the curve was integrated to determine photosynthetic productivity through the water column below 1 square meter of water surface ($mgC/m^2/h$). Assuming productivity was proportional directly to light, daily productivity was estimated by computing a diurnal expansion factor by comparison of the area of the insolation during the incubation period with that of the whole day (Wetzel and Likens 2000). A Licor 188 photometer was used to measure insolation as photosynthetically active radiation (PAR in $\mu E/m^2/s$) throughout the day and an underwater sensor was used to measure PAR with depth.

Triplicate vertical zooplankton hauls (5 m to surface) were made with a 64 µm, 30 cm diameter Wisconsin net in August, September, and October 2001 and in June, August, and October 2002 and samples were preserved in 95% ethanol (final preservative concentration of 70%). In the laboratory, zooplankton samples were concentrated or diluted so that at least 50 of the more common species could be counted in each subsample. Subsamples were obtained with a Henson-Stempel pipette and transferred to a Sedgewick-Rafter cell. Cladocera were counted on a Leitz compound microscope at 40X, while rotifers and copepods were counted at 100X. Specimens were identified to the lowest taxonomic level possible (Dussart and Defaye 1995; Edmondson 1959; Fitzpatrick 1983; Smith 2001; Thorp and Covich 2001).

Ten rocks were selected from a depth of 0.75 m from three different regions of the lake in July, August, and September 2002 for determination of periphyton biomass in the littoral zone. The biofilm from a 4 cm^2 area of rock surface was scraped onto a filter and frozen with dry ice on-site. Samples were later analyzed for chlorophyll *a* by acetone extraction (APHA 1998). A general survey of littoral macrophytes was made.

Deep water benthos samples were collected by combining 2 or more Ekman dredge samples, which were then rinsed in the field using a 64 µm mesh sieve and preserved in formalin. In the laboratory, formalin was rinsed out of the samples and all of the large organisms were removed from the entire sample. Then, organisms in a 1:2 to 1:8 subsample (based on density) were picked using 6X magnification on a Leitz stereoscope, with quality control scans at 12X to confirm removal of all organisms. Specimens were identified to the lowest taxonomic level (Burch 1982; Merritt and Cummins 1996; Provonsha 1990; Smith 2001; Wiggins 1996) and enumerated.

Littoral benthos were quantified in September and October of 2001 and August of 2002 by one person disturbing the substratum by hand and a second person sweeping the surrounding water with a 64 µm kick net for a given period of time (1–2 minutes). We carefully reset rocks with limpets attached. Samples were preserved in 95% ethanol (final preservation of 70%). Samples were picked and specimens identified and enumerated as described above for the deep water benthos. Catch per unit effort was calculated and reported as individuals or organisms per minute.

For all biotic samples, a reference collection was prepared and archived in the Flathead Lake Biological Station collections.

Capshell Distribution and Abundance

We quantified location and density of *A. coloradensis* and made ecological notes via SCUBA surveys. With few exceptions in an initial survey, capshells were observed attached mainly to the lower margins and undersides of movable rocks on the lake bottom but exclusively in the near-shore (littoral) zone. Density was determined by placement of a 0.25 m^2 metal quadrat on the lake bottom and counting delimited capshells by hand. Substratum type and depth were noted as well. All rocks within each quadrat were examined and placed outside the template until all layers containing limpets had been removed. The rocks with limpets attached were carefully replaced back into the quadrat after counting. Nine areas around the lake were surveyed. Steep areas and regions with boulders and submerged timber and macrophytes were surveyed by SCUBA and few limpets were found.

Status of Fishes and Predation on Capshells

According to National Park System stocking records, Lost Lake was first planted with 33,600 rainbow trout, *Oncorhynchus mykiss* (nonnative in Glacier National Park) fingerlings, in 1931. Between 1933 and 1943 more than 62,000 cutthroat trout, *Oncorhynchus clarki* (native) fry and fingerlings, were introduced. The most recent documented plantings consisted of 4,000 fingerling (i.e., 10 to 17 cm) rainbow trout stocked between 1964 and 1968. However, we determined from fishing and gill netting that only eastern brook trout *Salvelinus fontinalis* (nonnative) were present at the time our study began. No record exists of this species having been stocked in Lost Lake, but likely they were introduced around 1960 because an angler harvest survey in 1965 showed 22% rainbow trout, 77% brook trout, and 1% other. Park biologist, Robert Wasem, set a gill net in Lost Lake in July 1969 and captured 7 brook trout; average length 178 mm, mean weight 55 gm, average condition factor 0.973. No other species was taken. Perhaps the rainbow and cutthroat trout could not reproduce in the lake, whereas brook trout could. Or perhaps, rainbows and cutthroats were gradually eliminated by competition and predation from the very aggressive brook trout, as has been documented elsewhere (Chilcote 2005). In any case, brook trout netted from the lake in 1994 had several limpets in their stomachs (Marnell, unpubl. data), hence our concern for the potential impact from fish predation.

During the summer 2002 and fall 2004, brook trout were collected from Lost Lake using overnight gill net sets. Three 15 m nylon nets with 0.95 X 1.27 cm mesh and one 23 m nylon net with 0.95 X 1.9 cm mesh were used. Fish collected during the 2002 sampling were frozen for later processing. Fish collected in 2004 were returned to the laboratory fresh, measured, and weighed. The stomachs were preserved in formalin. The contents of these stomachs were examined under a dissecting microscope at 10X–30X magnification. In addition to recording the presence or absence of *A. coloradensis*, other taxa from each stomach were recorded and entered into a data base. The frozen fish from the 2002 sampling were thawed and the contents were examined to determine the presence or absence of *A. coloradensis*. Because of the poor condition of the previously frozen stomachs, other taxa besides limpets were not recorded.

Capshell Phylogeny

Acroloxidae are monophyletic freshwater limpet gastropods characterized by sinistral anatomy (Hubendick 1972), reduced lung, a semidiaulic genital system, and lack of anterior gizzard (Nordsieck 1993). Acroloxidae have an enigmatic distribution pattern. They are restricted to the holarctic region with only 2 recognized species and only 1 considerable radiation (up to 25 species) in ancient Lake Baikal, Russian Federation, and surroundings and few more species in Lake Ohrid and caves in Bosnia (Hubendick 1960). Family status was proposed as early as 1950 by Bondesen (1950) according to egg mass morphology. Subsequently, the distinctness of Acroloxidae from limpets belonging to Ancylidae has been repeatedly stated on the basis of different data like sperm morphology (Burch 1962; Healy 1996), gross anatomy (Hubendick 1962, 1972, 1978) or chromosome morphology (Burch 1962). The degree of convergence in morphology between Acroloxidae and other limpets was considered to be most remarkable by Hubendick (1962). Very little has been known until now regarding within-family relationships. In the Nearctic, there exists a single representative of *Acroloxus*, *Acroloxus coloradensis* (Henderson 1930), which was originally described as *Ancylus hendersoni* Walker (1925).

Freshly collected specimens of *A. coloradensis* from Lost Lake (113.5753°W, 48.6817°N) and Trout Lake (118.2269°W, 52.8783°N), both in Glacier National Park, and an unnamed lake in Jasper National Park (Lake No. 1 in Mozley [1930]; 118.2228° W, 52.8737°N) were immediately preserved in 95% ethanol for the genetics analysis. A combination of DNA sequences from three genes, the conservative nuclear gene for the small subunit rDNA (SSU rDNA; also known as 18S rDNA) and the mitochondrial cytochrome oxidase *c* subunit I (COI) and large subunit rDNA (16S rDNA) were used to infer the position of *A. coloradensis* within the Acroloxidae, while COI was used to estimate genetic diversity and divergences within and among populations of *A. coloradensis*. Four specimens from Lost Lake, 3 from Trout Lake and 7 from the unnamed lake in the Rocky Mountains of Jasper National Park, Alberta, Canada, were analyzed.

The data set for the phylogenetic analysis comprised the widespread European species *A. lacustris* (populations: 1 - Macedonia, 2 - Albania, 3 – Germany), the ancient Lake Ohrid endemic *A. macedonicus*, and type species of the three Lake Baikal endemic Acroloxid genera: *Pseudancylastrum dorogostaiski*, *Gerstfeldtiancylus renardi*, and *Baicalancylus boettgerianus*.

Three representatives of Lymnaeidae (*Lymnaea stagnalis*), Planorbidae (*Planorbarius corneus*), and Physidae (*Physa fontinalis*) from Albrecht et al. (2004) where also included in the phylogenetic analysis. *Siphonaria concinna*, an intertidal limpet, was chosen as outgroup since these marine pulmonates were repeatedly proposed to be basal to limnic basommatophorans (Hubendick 1978; Nordsieck 1992).

Isolation of DNA, PCR, bidirectional sequencing procedures, and primer details followed Albrecht et al. (2004). The protein-coding COI sequences (up to 965 bp long) were aligned using BioEdit 5.01 (Hall 1999). The initial alignments of the 16S and 18S sequences using default settings in ClustalW (Higgins et al. 1996) were refined manually, resulting in fragment lengths of 463 and 1,026 bp, respectively.

Two different data sets were analyzed. The first data set combined 16S, COI, and 18S sequences for a reduced set of taxa, whereas the second data set included only sequences for the two mitochondrial genes (i.e., 16S and COI), but for a larger set of taxa. For phylogenetic reconstructions, Bayesian inference (BI) and the maximum parsimony (MP) approach were chosen. Prior to the phylogenetic analyses, the computer program Modeltest 3.06 (Posada & Crandall 1998) was used in order to find the optimal model of DNA substitution. Bayesian inference was conducted using MrBayes 3.0b4 (Huelsenbeck & Ronquist 2001). First, we compared several independent runs using the default random tree option and the starting parameters suggested by Modeltest to monitor the convergence of the log-likelihoods of the trees. Based on these preliminary analyses, we did a final run using the Metropolis-coupled Markov chain Monte Carlo variant with four chains (one cold, three heated) and 1,000,000 sampled generations with the current tree saved at intervals of ten generations. A 50% majority rule tree was constructed from all sampled trees with the first 1,000 trees (= 10,000 generations) ignored as burn in. MP analyses were performed in PAUP 4.0b10 (Swofford 2002) with heuristic search, 1,000 random-addition-sequence replications, and TBR branch swapping. Equal weight and unordered character states of all characters were assumed.

The phylogeography of the COI haplotypes was inferred using statistical parsimony (SP) (Templeton et al. 1992). The SP network was constructed with the computer program TCS v.

1.06 (Clement et al. 2000). Nucleotide diversities and divergences (corrected according to the K2P-parameter-model) were calculated using MEGA 2 (Kumar et al. 2000) with standard errors estimated by 1,000 bootstrap replications with pairwise deletion of gaps and missing data.

RESULTS

Limnology of Lost Lake

Geology, bathymetry, and hydrology

Lost Lake is a roughly oval shaped basin contained in a shallow glacial cirque located on the north side of the "Going-to-the-Sun Road", the main road bisecting Glacier National Park. It has a maximum depth of 8.2 m and a surface area of 0.8 hectares (Fig. 1). The lake is situated near the north shore of the much larger St. Mary Lake at west longitude -113.5753, north latitude 48.6817 (UTM 310446 east, 5395273 north) at an elevation of 1418 m MSL; thus, it is in the transition zone between the coniferous valley bottom and the subalpine. The lake lies at the base of Goat Mountain below a forested (subalpine fir) headwall. The lower slopes of Goat Mountain are composed of sedimentary rock strata, with Precambrian age Appekunny and Grinnel argillites comprising 40% and 59% of the catchment area, respectively. The catchment is 60 hectares of exposed bedrock (81%) and colluvium (13%) with soil development in the immediate area of the lake allowing forestation. A talus field near the base of the mountain extends to the north shore near mid-lake.

Seeps were evident at the base of the talus slope on the north shore from June through August, but were mostly dry by September. Discharge in the outlet seep was estimated to be <0.5 L/minute in August 2001. Any water discharged from the lake would flow into St. Mary Lake, a part of the Hudsonian drainage. No inlet was apparent on the western shore, even though one is shown on the USGS topographic map for Rising Sun quadrangle (1968), thus, we concluded that water flux was mainly via groundwater pathways to and from the lake. Indeed, temperature measurements made after surface flows ceased in the primary inlet seep in September, 2001, indicated groundwater discharge into the lake because water temperatures in the region of the inlet were 11^{o}C while lake water column was 17^{o}C a few meters distant. The outlet consisted of only a trickle in June of both years, but it is apparent from the terrain that greater discharge had occurred in the past. Snow water equivalents from a Snotel site north of Lost Lake (Many Glacier, elevation 1492 m MSL) indicate that 2001 was about average while 2002 was well above the 1971–2000 historical average for that site. The maximum spring snow water equivalent for Many Glacier in 2001 and 2002 was 35.6 cm and 47.8 cm, respectively, compared to the historical mean of 36.6 cm. Surficial water flux was intermittent and on dry years it is likely that no surface discharge occurs at all. Nonetheless, lake volume remained fairly stable and we classified it as a type of seepage lake (see Pennak 1969).

The littoral area of the lake where capshells were most abundant (Fig. 1) consisted of abundant flat rocks on the eastern and southeastern shores. The northern and western shores, where limpets were much less abundant, are typically deeper with rocks and rubble dispersed among sunken logs and occasional patches of rooted aquatic plants. Numerous flat boulders were strewn among the sand and silt, having fallen into the lake from the nearby talus slope.

Shoreline terrestrial vegetation

The shoreline was almost entirely ringed by brushy vegetation, including mountain maple (*Acer glabrum*), alder (*Alnus* spp.), birch (*Betula* spp.), and redosier dogwood (*Cornus stolonifera*). Surrounding slopes had a patchy forest of lodgepole pine (*Pinus contorta*) mixed with Douglas fir (*Pseudotsuga menziesii*) and subalpine (species) fir at the higher elevations. Allochthonous organic matter including tree boles were present in the lake owing to the steep profile of the north and west shorelines. Trees presumably were carried into the lake by snow and rock avalanches.

Temperature patterns

Lost Lake is dimictic and ice covered from about early December to May (Fig. 2). Maximum water temperature was 21.8°C on August 18, 2001 at approximately 0.65 m depth. Thermal stratification of the water column occurred both years of the study; a thermocline was evident by early June of 2002 between 2 and 5 m (Figs. 3 and 4). The thermocline persisted into July 2002 at 4 to 6 m, but only weak temperature stratification (0.5°C change in 1 m) was present by August and it was very near the lake bottom. Though many shallow lakes and ponds mix completely due to wind action, Lost Lake has considerable protection from high trees and steep mountain slopes to the north. Once temperatures cool in the fall, mixing is more prevalent. In September of 2001, the lake was homeothermal and well mixed.

Light penetration

Light extended to the bottom of the lake during all months of the study, with the bottom receiving about 5% of surface light in summer and 1% in fall (data not shown). Correspondingly, Secchi disk depths were highest in summer (e.g., 5.2 m) and lowest in fall (e.g., 2.75 m). Light penetration would be highly attenuated under ice and snow in winter but was not measured.

Water chemistry and pelagic primary productivity

A decrease in pH and a rapid drop in dissolved oxygen (DO) were observed in the hypolimnion during all months (Figs. 3 and 4). Even when stratification was weak in August, DO was down to 0.63 mg/L at the bottom and pH showed a significant decline with depth as well. By September, the lake began to mix as temperatures cooled, but still a decline in DO and pH was evident. The rapid decline in oxygen with depth was not surprising given the abundant organic matter generated within the water column. Allochthonous inputs from deciduous vegetation and senescence and decomposition of macrophytes in winter also add to the organic matter of the lake. Chlorophyll *a* concentrations were high in the water column (see Tables 1 and 2; maximum of 12 µg/L measured at 5 m in August 2001) owing to algal blooms from mid-summer through early fall. Phytoplankton biomass dynamics were not quantified but 2 species of *Spirogyra* were observed at about 0.5–1 m depth at the north end of the lake by early July and persisted until late summer. The dinoflagellate, *Ceratium* sp., was the more dominant large phytoplankton, as they were very abundant in zooplankton samples from August of both years.

The decrease in pH in the hypolimnion was likely due to higher CO_2 generated via respiration of the sedimenting organic matter. Higher pH in surface waters can also be a result of removal of CO_2 by algal photosynthesis. This was most evident in August, 2001, when a significant shift in pH was observed along with a spike in DO at 4 m, indicating a lens of dense algae at the base of the thermocline. It was also interesting to see the supersaturation of oxygen in the upper water column in June, 2002, indicating vigorous photosynthetic production following ice breakup and increasing light conditions.

From June to August, higher turbidity was observed with depth (Figs. 3 and 4) and may represent sedimenting organic matter slowly concentrating in the hypolimnion. Algal biomass (i.e., chlorophyll *a*) was high and dredge samples revealed a very thick ooze of fine particulate organic matter and detritus over lake sediments. Another biogenic source of turbidity may also be feeding by brook trout on organisms found in the sediments. Brook trout gut content analyses (discussed in detail below) showed that Diptera, found in deep water benthos samples, were common food items. Although we did not observe fine sediments suspended in the water column, it is likely that following large snowmelt events, a considerable amount of silt-size particles are deposited in the lake, washed out of the upslope talus and colluvium. Large clumps of fine sediment particles were observed in dredge samples. However, it seems most probable that the increased turbidity was biogenic in origin. During the period of this study, the inlet was not a substantial source of inorganic particles. In September, winds must have been sufficient to suspend sedimenting organic matter, detritus and possibly bottom sediments, as turbidity was high throughout the water column and all other parameters were similar top to bottom.

The ratio of catchment area to lake surface area is quite high at 75. Typically, a higher ratio is associated with relatively rapid lake flushing and higher nutrient and major ion inputs allowing elevated rates of phytoplankton production (Kalff 2002). However, we lack resolution on the hydraulic residence time due to the lack of ground water seepage rates into and out of the lake. But, nutrient concentrations in the inlet seep were considerably higher than those in the lake, particularly $NO_{2/3}$-N (Table 1), which is typical of ground waters. Total phosphorus concentrations were also high in the inlet, being almost three times that of the surface waters. During the spring, most of the snowmelt seeps into the rubble, through bedrock fractures, traveling underground, picking up additional ions and some portion of this groundwater, and then intercepts Lost Lake. Certainly, very little surface flow reached Lost Lake during this study. Determination of direct atmospheric nutrient inputs to the lake surface were not within the scope of this study, but the National Atmospheric Deposition Program site at West Glacier, Montana (i.e., ~36 km southwest of Lost Lake; 980 m elevation) indicated annual deposition of nitrate plus ammonium nitrogen on the order of 3.18 and 4.59 kg/ha in 2001 and 2002, respectively.

Nutrient and carbon concentrations in Lost Lake were high in comparison to 8 other high elevation lakes in GNP that were sampled from 1984–1990 (Ellis et al. 2002; see Table 2). The same geologic formations of the Belt Series that occur in the Lost Lake catchment occur in the 8 other lake catchments studied, though in different proportions. The Grinnell Formation (59% of Lost Lake catchment) is composed of red argillites interbedded with quartzite, while the Appekunny Formation (40% of catchment) is composed of argillite, quartzite, and some siliceous dolomite (Whipple 1992). Greater dissolution of the Appekunny Formation might be expected, resulting in higher specific conductance and nutrient concentrations. The groundwater inputs to Lost Lake and the lack of continuous surface flow in and out of the lake likely account for the higher concentrations observed in Lost Lake compared to the other Glacier National Park lakes.

Nitrate plus nitrite nitrogen entering the lake via groundwater and atmospheric deposition was rapidly assimilated by the algal community, with median concentrations in the water column being below the detection limit both years of the study (Tables 1 and 2). Nitrogen to phosphorus ratios were high at 26–28 for Lost Lake, indicating that phosphorus is probably limiting. However, this ratio is at the lower end of the range observed for 8 other high elevation lakes in Glacker National Park (i.e., 24–43; Ellis et al. 2002) and $NO_{2/3}$ was always below detection limit. The lake may be limited by nitrogen in late summer, as has been observed for other lakes in the Flathead Basin (Stanford and Ellis 2002). DOC levels were high and averaged about 3 times that of the other high elevation lakes. The more phytoplankton production that occurs in response to nutrient loading, the more DOC should accumulate. Phytoplankton biomass, measured as chlorophyll *a*, averaged 4.96 and 6.94 µg/L at 1 m and 5 m, respectively. Thus, it was not too surprising to find that net primary production was in the range observed for eutrophic lakes (i.e., 1,257 mgC m^{-2} day^{-1}; Table 3). The trophic status of Lost Lake may best be characterized as mesotrophic, though the comparison parameters spanned ranges observed for oligomesotrophic to eutrophic categories (after Wetzel 2001).

Trace metals analysis was performed to provide a baseline for Lost Lake and the *Acroloxus* population therein. Sodium concentrations in Lost Lake were outside the range observed for 8 other high elevation lakes in GNP studied over 7 years (i.e., 1.26 mg/L for Lost Lake versus a range of <0.10–0.44 mg/L for other GNP lakes; Ellis unpublished). The source of higher sodium in Lost Lake is unknown. Levels of all other metals appeared to be within the normal range expected from the surrounding geology (Table 4) and aerosol contamination is not indicated from the other variables measured.

Zooplankton

Given the high nutrient concentrations and phytoplankton biomass, the relatively high zooplankton abundance in Lost Lake was expected. Zooplankton abundance ranged from 545 to 827 individuals/L, with a late summer/fall mean of 704 individuals/L. In comparison, the mean late summer/fall zooplankton abundance in 8 other high elevation lakes of GNP ranged from 3 to 446 for the 1984–1990 period of measurement (Ellis et al. 2002). Rotifers comprised more than 74% of the total number of zooplankton on all dates in Lost Lake and reached very high densities in October of both years (>95% of total zooplankton abundance; Fig. 5). The August 2001 samples were poorly preserved and therefore the rotifer abundance was probably an underestimate on that date; the cladocerans and copepods appeared to be unaffected. *Polyarthra* sp. and *Keratella cochlearis* were the most abundant rotifers. Next in abundance were the much less diverse copepods, with copepodites of *Acanthocyclops vernalis* and grouped nauplii dominating (Fig. 6, top panel). Cladocerans were few in number, though the density of

Ceriodaphnia dubia (syn. *C. affinis*) rivaled that of total copepods in August of 2002 (Fig. 6, bottom panel).

Ellis et al. (2002) found the effect of fish being present or absent in the high elevation lakes of Glacier National Park was important in zooplankton community structure. They found that total rotifers and calanoid copepod densities were significantly different between the high elevation fish and fishless lakes ($P < 0.05$, $P < 0.01$, respectively). Lakes containing fish did not have many large zooplankton (i.e., copepods and cladocera) and the crops were usually dominated by rotifers that were too small for fish to forage upon effectively. Clearly, grazing by brook trout in Lost Lake has had a similar effect on the pelagic food. The presence of vertical stratification in Lost Lake may set it apart somewhat from some of the other high elevation lakes examined in the above study. Vertical stratification may allow a refuge for some of the larger cladocera, like *Ceriodaphnia*, which was so abundant in August 2002 (Fig. 6), if they are able to survive the lower levels of oxygen. Reduced oxygen concentrations below the thermocline likely limit brook trout to the upper waters. Submersed macrophytes also provide physical refuges for large zooplankton from predation by fish (Timms and Moss 1984, Schriver et al. 1995). These refuges for zooplankton that feed upon and at times control phytoplankton densities are important to the regulation of littoral phytoplankton communities and their biomass (Irvine et al.1990, Stephan et al. 1998).

Macrophytes

Rooted plants that have much higher rates of organic production than do phytoplankton per unit area often dominate production of organic matter within shallow lakes. Hydrophytic macrophytes were present in Lost Lake but did not appear to dominate the littoral area. Seven species were identified: *Carex vesicaria*, *C. utriculata*, *Myriophyllum spicatum*, *Ranunculus* *aquatilis*, *Potamogeton alpinus*, *P. richardsonii* and *P. friesii*. Not many macrophytes were present in Lost Lake beyond a depth of 1.5 m as loose, flocculent sediment dominated below that depth, which is a poor substratum for macrophyte colonization. Complex competitive interactions of macrophytes, epiphytes (attached microbiota), and phytoplankton occur in shallow lakes. Submersed macrophytes often dominate when total phosphorus concentrations are around 25 µg/L and N:P ratios of the water are high (>>10:1) (Wetzel 2001), which is the case in Lost Lake. The larger submersed plants are very productive and derive most of their nutrients from the sediments. Phosphorus is usually adequate for submersed macrophytes, particularly from the sediments. Anoxic areas within the sediments lead to appreciable bacterial denitrification and losses of nitrogen to the atmosphere. Nitrogen commonly becomes limiting to both submersed macrophytes, epiphytic periphyton communities, and phytoplankton because of heavy biological utilization and from losses by denitrification. High $NO_{2/3}$ concentrations in the inlet and undetectable levels of $NO_{2/3}$ in the water column confirm the rapid uptake of nitrogen by the plant community in Lost Lake. According to Wetzel (2001), further increased loading of P and N can often result in a shift in the submersed plant community to tall species (e.g., *Myriophyllum spicatum*, certain *Potamogeton* spp.), as well as water lilies. Although quantification of macrophyte production was not a part of this study, it did appear that the most dominant macrophyte was the submersed *Myriophyllum spicatum* while *Potamogeton* spp. were also abundant. No water lilies were found.

Littoral periphyton

Profuse growths of benthic algae (*Spirogyra* spp.) occurred in localized areas of the shoreline, while Ekman dredge samples revealed numerous gelatinous masses of blue-green algae on soft bottom sediments (*Aphanothece stagnina*). This blue-green alga often forms almost continuous gelatinous expanses on the bottom of favorable eutrophic habitats (Prescott 1982). Other phytoplankton present in the dredge samples included: *Navicula*, *Rhopalodia*, *Pediastrum*, *Oscillatoria*, *Tetraedron*, *Amphora*, *Gyrosigma*, Centrales spp., *Cosmarium*, *Elakatothrix*, *Tetraedron* spp., *Crucigenia* spp., *Scenedesmus*, *Asterionella*, *Fragilaria*, *Dinobryon*, *Gleocystis vesiculosa*, and *Glenodinium*.

Littoral periphyton biomass on rock surfaces in Lost Lake was lower than might be expected given the high nutrient concentrations in the lake. Values were on par with some of the higher levels recorded for a larger, much less productive lake, Flathead Lake, just south of Glacier National Park (Table 5; Ellis unpublished data). Mean periphyton biomass in Lost Lake in August was 5.83 ± 2.31 $\mu g/cm^2$ chlorophyll *a*. In general, the eastern shore had the highest biomass; there were significant differences between sites in July ($p < 0.05$) and in August between the eastern and southeastern sites ($p < 0.10$; two tailed t-test). There were no significant differences between lake-wide means for the different months.

High grazing of the limited rock substrate in Lost Lake by gastropods and insect larvae likely accounts in part for the lower than expected periphyton biomass. Gastropods often control the composition and amount of periphyton in lakes and ponds. As mentioned previously, the periphyton community may also be limited by nitrogen. Periphyton may also be reduced by heavy human use of the rocky areas of the lake in late summer for wading and swimming.

Littoral macrobenthos

Cladocera, Ostracoda, and Diptera were the most abundant benthic invertebrates (Fig. 7). The Cladocerans were also found in the zooplankton, but *Alona quadrangularis* appeared to be much more abundant in the littoral benthos than in the open water column (see also Fig. 6). The other cladoceran that was abundant in the benthos was *Ceriodaphnia dubia*, but it only occurred in the August 2002 sample. This was the same month when it dominated the open water zooplankton. Ostracods were also very abundant, followed by the Diptera. The Diptera were comprised primarily of Chironomidae. *Acanthocyclops vernalis* and some unknown harpactacoids made up the benthic copepods while *Caenis youngi* was the predominant of 5 Ephemeroptera species. Although less abundant, the large-bodied amphipods comprised the majority of the benthic biomass, with *Gammarus lacustris* and *Hyalella azteca* the dominant species. Many other organisms were present but rare in the littoral benthos (see Fig. 7, lower panel; Appendix Table 1), including bryozoans (*Plumatella* sp.) and sponges (*Spongilla lacustris*). However, the sponges were very abundant on submerged fallen trees. The Rocky Mountain limpet, *Acroloxus coloradensis*, was present but rare in benthos samples. Apparently they adhere so tightly to the rocks that the method of benthos netting did not easily dislodge them. Pieces of oligochaetes were present in all samples, but their condition made identification and enumeration impossible. However, they did appear to be common.

It is worth noting the total absence of both Coleoptera and Notonectidae and the very low numbers of Hemipterans and Odonata in the littoral benthos. These groups are often well represented in small ponds and lakes. Although some of these organisms may have been more abundant on the lake surface in areas that were not sampled for benthos, we did not observe significant surface populations during sampling. It is very possible that brook trout fed on them to such an extent that they remained few in number (see discussion below).

A few organisms were collected during shallow shoreline surveys that were not observed in littoral benthos samples, including an unknown species of leech (Hirudinea). Some species of leeches prey on mollusks, but we never observed a leech attached to a limpet during this study.

Deep-water benthos

The majority of the lake bottom (~>1.5 m depth) consisted of loose fine sediments and detritus. The Diptera dominated densities and biomass of deep water benthos (Fig. 8) while Ephemeroptera were not far behind. The predatory midge, *Chaoborus* sp., was the predominant dipteran. As was observed in the zooplankton, *Caenis youngi* made up the majority of the Ephemeroptera, followed by *Callibaetis* sp., and the major trichopteran was *Mystacides* sp. The same 2 species of Amphipoda that dominated littoral densities were found in the deep benthos as well (i.e., *Gammarus lacustris* and *Hyalella azteca*). The snail *Gyraulus* sp. (Basommatophora) was present but rare in the deep benthos, and the fingernail clam, *Pisidium* sp., was the only species of Veneroida. As in the littoral benthos, pieces of Oligochaetes were present and prevented identification and accurate enumeration, though they appeared to be common. It is interesting that all of these organisms must be adapted to the low oxygen concentrations that were observed in Lost Lake, even if only for a short period should they move in and out of the region.

Capshell Morphology, Distribution, and Abundance

The morphology of *A. coloradensis* in Lost Lake was as described by Burch (1989) and Clarke (1981), with some notable observations. There is a distinct black narrow ring of melanin around the periphery of the mantle just slightly in from the edge (also noted by Clarke 1970). The rest of the mantle is very light in color and clearly visible through the translucent shell of shiny golden-brown. Although thin shells appear to be a common feature for this species, specimens from Lost Lake possessed very thin, delicate shells. The low alkalinity (43 mg/L $CaCO_3$) and calcium concentrations (12–13 mg/L) in Lost Lake may account, in part, for the very thin shells observed. The levels of calcium were far below the range reported by Riebesell et al. (2001) for lakes within Rocky Mountain National Park, Colorado, where *A. coloradensis* was found (i.e., 49–142 mg/L). According to Brown (2001), about 45% of freshwater gastropods are restricted to waters with Ca concentrations >25 mg/L and 95% to levels >3 mg/L.

Dimensions of *A. coloradensis* averaged 3.5 x 2.2 x 0.9 mm (i.e., length x width x height; range = 3.1 x 1.9 x 0.8 to 4.1 x 2.6 x 1.2 mm). *Acroloxus coloradensis* occurred sympatrically in Lost Lake with another limpet, *Ferrissia fragilis* form Basch *isabellae*. *F. fragilis* averaged somewhat larger at 4.7 x 2.9 x 1.4 mm (range = 3.5 x 1.9 x 1.0 to 5.9 x 3.3 x 1.6 mm). The size of *A. coloradensis* from Lost Lake was within the range reported for the Peterson Lake population (Bryce 1970) and the populations from the Quebec lakes (Clarke 1970), but we did not find any specimens that reached 5 mm in length, the upper limit described by Burch (1989). Shell shape of Lost Lake specimens was also similar to those from Peterson Lake and the Quebec lakes with length/height and width/height ratios for Lost Lake *A. coloradensis* of 4.1 and 2.6, respectively. Shell length for the 31 preserved specimens of *A. coloradensis* that were examined in August and September were rather evenly distributed among 4 arbitrary size classes from 3.0 to 4.1 mm, with the largest size class having the fewest representatives. Whether this range in size suggests multiple size classes is not known but a length frequency histogram showed no major breaks as might be represented by different cohorts. Most pulmonates exhibit annual semelparity, with adults that reproduce in the spring and die, thus there is complete replacement of generations. The ability of pulmonates to reproduce in cold water allows adults to breed early in spring and juveniles to grow quickly to adult size before the end of summer. However, some pulmonates (e.g., *Lymnaea stagnalis*) that are typically annual in the warmer waters of Iowa (Brown 1979), often take several seasons to complete their life cycle in the colder waters of Canada (Boag and Pearlstone 1979). Additional seasonal studies are needed in Lost Lake to determine the life history of *Acroloxus coloradensis* there. Surprisingly, the few specimens from Trout Lake that were measured in November suggest there may be at least 2 size classes (i.e., 1.6, 2.4, and 3.2 mm in length), with most individuals in the larger size class (personal communication, Brian Reid). Many studies have implicated periphyton productivity in determining patterns of voltinism (see review in Russell-Hunter 1983), thus it would be interesting to investigate possible differences in life histories of the 2 populations in Lost and Trout Lakes relative to the available resources. Water temperature and hardness were also important factors.

The density of both limpets is presented in Figure 1. Based upon subsamples returned to the laboratory for microscopic analysis, about 70% of the specimens were *A. coloradensis*, so densities of *A. coloradensis* ranged from 0 to about 574/m^2, with a mean for all sites of 164/m^2. This estimate is higher than the 20/m^2 reported by Clarke (1993), but their highly patchy distribution may account for the observed differences. The mean density of *Acroloxus* in Lost Lake was about twice the average maximum densities reported by Riebesell et al. (2001) of 84/m^2 on artificial substrates in Finch Lake, Colorado and 72/m^2 found in Peterson Lake, Colorado by Bryce (1970). However, Wu (1989) found only a single specimen when he returned to the then polluted Peterson Lake in 1989 and Clarke (1993) found only 3 specimens in 1992. Clarke (1970) found only 1–3 specimens per lake or pond in Quebec and Ontario, with densities estimated at 0.17–0.25/m^2 for 2 of the lakes. The maximum density recorded for Lost Lake (i.e., 574/m^2) exceeds all published estimates for *Acroloxus coloradensis*.

Density estimates for Trout Lake and Lake No. 1 were not made, but their distribution appeared to be very patchy. About 50 rocks were examined along 60 m of shoreline in Trout Lake and 25 individuals were found, all on the underside of one rock at the base of an avalanche slope on the south side. In Lake No. 1, an extensive search resulted in 7 specimens, all from under the bark of a submerged spruce log near the south shore. Mozley (1926, 1930) reported *A. coloradensis* from Lake No. 5 (also known as Cutt Lake, personal communication, Ward Hughson), but no specimens were found after a 5-hour search of the shoreline. Mozley did not mention the number of specimens collected in 1925 or the habitat utilized in either Lake No. 1 or No. 5.

The preferred habitat of both limpets in Lost Lake appeared to be the lower faces and underside of moveable flat rocks (~>5 cm diameter) in relatively shallow water (most <1 m depth, a few 1–1.5 m depth). Some *Ferrissia fragilis* were found on the lower edges of large boulders and on woody debris. Others have reported the occurrence of *A. coloradensis* along shallow lake and pond shorelines (Clarke 1970, Bryce 1970, Riebesell 2001) and from the undersides of rocks (Bryce 1970). Even the one report from a stream noted there was no measurable current velocity and the limpets preferred the only rocky substrate in the vicinity (Paul and Clifford 1991). As mentioned previously, *A. coloradensis* from Trout Lake also was present on the bottom of rocks in shallow water (<30 cm depth), but all specimens from Lake No. 1 were found under the bark of a submerged spruce log.

The presence of *F. fragilis* in close association with *A. coloradensis* is of interest. *F. fragilis* was not reported from Lost Lake in 1967, though other mollusks were. Whether this species was missed in the original investigation or whether it is a newcomer is not known. However, during the present study, it was impossible not to collect both, as they occurred in the same habitat and looked very similar. It is interesting to note that Jokinen (1987) in a study of gastropod distributions found that *F. fragilis* was a species with the greatest dispersal ability (called "super tramps" by Diamond [1975]) and was observed only at sites with low snail diversity, like Lost Lake. Pulmonates are dispersed passively in mud on birds or insects and it seems quite possible that *F. fragilis* could be a recent addition to Lost Lake. It also is not known whether these 2 limpets are competing. It does appear they inhabit the same habitat, the undersides of rocks, although a few *F. fragilis* were also found on logs and big boulders. There is some evidence indicating competition is rare in freshwater snails either due to little niche overlap or differences in resource utilization (Brown 2001). It is known that another limpet (i.e., *Ancylus fluviatilis*) very selectively grazes diatoms (Calow 1973a, 1973b). If *Ferrissia* and *Acroloxus* in Lost Lake are selectively eating different algae and/or detritus, then resource partitioning would allow coexistence. Clarke (1970) reported that in the laboratory, *A. coloradensis* fed upon periphyton from Peterson Lake that was dominated by blue-green algae, but no particular specificity was mentioned. There is also some indirect evidence indicating competition does occur in gastropods inferred from apparent competitive exclusion (Harman 1968).

Acroloxus densities in Lost Lake were highest where flat rocks were piled in layers and where the area was not utilized by people for wading and swimming. Limpets could be found on the underside of rocks throughout a few layers, hence the large densities possible. The optimum habitat of layered rock was found on the eastern and southeastern shallow shores, but reduced densities of limpets were observed where people commonly waded out into the lake to swim (Fig. 1, shaded area). The limpets can be easily crushed between the successive layers of rock from simply walking on the substrate. During the sunny July and August sampling periods, an average of 18 people visited the site each day and about 4 would wade/swim (including dogs). Our presence may have reduced the number of visitors that utilized the area, hence usage might have been even greater. Persons accessing the lake on the northern and western shores, primarily to fish, would have little impact on the limpet population, as the substrate is not optimum for the limpets.

A. coloradensis, like most pulmonates, appears to be well adapted to the wide range of temperatures in Lost Lake (i.e., close to 0° C for almost 5 months and maximum summer temperature near 22° C at 0.65 m depth). There does not appear to be much buffering of water temperature in winter from ground waters, though there was a short period in late January where a rain on snow event may explain the short warming.

Lost Lake is mesotrophic, as were the lakes inhabited by *Acroloxus* described by Clarke (1970). Riebesell (2001) also found the Rocky Mountain lakes with *Acroloxus* to have significantly higher calcium and conductivity than lakes without *Acroloxus*, again suggesting generally more productive habitats. Snail populations in eutrophic habitats have more generations a year, more rapid shell growth, and higher fecundity, but highly eutrophic sites can be detrimental to gastropods (Brown 2001). Habitats that go hypoxic can result in diebacks of gastropod populations. In Lost Lake, it appears that the most suitable limpet substrate at 0–1.5 m is well above the zone of rapid oxygen depletion (i.e., below 4 m; see Figs. 3 and 4), at least in summer. The rather abundant brook trout population suggests that winter oxygen levels are probably not a problem as well.

In the study by Riebesell et al. (2001), all of the 6 Rocky Mountain lakes in Colorado supporting *A. coloradensis* were higher elevation and had higher calcium, lower conductivity, and a lower flushing rate index than Lost Lake. Although Lost Lake is also within the Rocky Mountain Range, it appears that the most suitable conditions for *A. coloradensis* in the northern region may be outside the ranges reported for Colorado.

Status of Fishes and Predation on Capshells

Total number of brook trout gill netted in 2002 and 2004 was 52 and 42, respectively. Mean weight and length of fish in 2002 was 73 g and 201 mm, while in 2004 it was 89 g and 197 mm. The length/weight relationship for brook trout from Lost Lake showed an even distribution of sizes from 40–130 g in 2002 while fish from 2004 showed a bimodal distribution (Fig. 9).

Results of brook trout stomach content analyses are shown in Fig. 10. The break in the 2 size classes of brook trout (i.e., 107–123 mm and 209–259 mm) represent different age cohorts as interpreted from a length frequency histogram (not shown). Feeding habits differed between the smaller and larger fish. The smallest fish fed almost entirely on *Ceriodaphnia dubia* and Formicidae (ants), whereas larger fish had a greater prey base. The smallest size class also fed upon Aphidae (aphids) and Trichoptera. The taxa occurring most commonly in gut samples from the larger cohort were Corixidae (water boatmen), Trichoptera (caddisflies), Formicidae (ants) and Notonectidae (backswimmers). The ants appeared to be dominated by winged males, indicating mass emergence during the sampling period. It was interesting to see that the most abundant prey for adult brook trout were primarily terrestrial or free-swimming aquatic organisms (i.e., Corixidae, Formicidae, Notonectidae) and that the dominant benthos were rare or nonexistent (i.e., Amphipoda, Ostracoda, Cladocera, and Copepoda). The Trichoptera were mostly pupa and would be found on the lake bottom, primarily in the littoral area, as would the Corixidae; while the Formicidae and the Notonectidae would be found most often on the lake surface. The gut content analyses were from brook trout obtained in the summer and fall only. Obviously, surface insects would not be a major component of the winter diet when the lake is ice covered. It is clear from the wide range of diet items (Fig. 8, lower panel) that brook trout could shift to more benthic or pelagic species in winter (e.g., Amphipoda, Diptera, and

Cladocera). Other taxa represented in brook trout stomachs that were not shown in Fig. 10 (i.e., <2% of the diet) included: Ephemeroptera, Gastropoda, Hydracarina, Raphidiidae, and Veneroida.

No specimens of *Acroloxus coloradensis* or *Ferrissia fragilis*, either entire or fragmentary were observed in brook trout gut contents from either of the sampling dates. Although *A. coloradensis* and *F. fragilis* were absent from the samples, other mollusks were observed, including clams and snails. The presence of these groups suggests that brook trout would eat *A. coloradensis* if they were able to find the limpets, which may indeed account for the specimens recovered from brook trout stomachs in 1994. It is also possible that empty shells of dead limpets were consumed during benthic foraging. Certainly, the tendency for the limpets to adhere tightly to the undersides of rocks may ensure their survival in this habitat.

Most experimental evidence supports a strong role for predators in determining gastropod assemblages (Brown 2001). Brook trout might play a role in reducing densities of the other mollusks in Lost Lake (i.e., *Physa gyrina* and *Pisidium casertanum*), as both were found in stomach contents, though in very low numbers. Fish predators can also alter snail foraging behavior (Turner 1997) with snails using refuges more in the presence of fish. It would be interesting to see if either of the limpets in Lost Lake would shift to use of the upper surfaces of rocks if brook trout were removed or whether their selection of the bottom of rocks has more to do with the type of algae and/or detritus present or some other physical parameter (e.g., light).

The presence of nonnative brook trout may impact the limpets in other ways. It is possible that a trophic cascade produced by the brook trout may result in increased pressure on the dominant food of *Acroloxus*, periphyton. Brook trout in Lost Lake feed heavily on trichopterans, corixids, notonectids, and aphids, all large predacious insects. This release in predation pressure could potentially result in an increase in many of their prey (e.g., scrapers, detritivores, and collector gatherers) and these insects could in turn compete with the limpets for periphyton. Interactions between snail predators, snails, periphyton, and macrophytes can be very complex in natural aquatic ecosystems and difficult to predict without a very detailed food web analysis.

Leeches are a known predator of gastropods, but as mentioned previously, none were observed attached to the limpets in Lost Lake. Other important predators include beetles, Hemiptera and Odonata nymphs, and ostracods (Smith 2001), the last of which were very abundant in Lost Lake. Hydra were observed attached to the bodies of *Acroloxus coloradensis* in Lost Lake. Hydra are carnivorous and commonly eat small, open-water plankton and sometimes small fish, but are generally less effective at capturing animals that normally inhabit underwater surfaces (Slobodkin 2001). Some cladocerans and ostracods are immune to their sting, but whether this is the case for *A. coloradensis* is not known.

Capsell Phylogenetic Relationships

Acroloxus within the Acroloxidae

Both MP and BI analyses yielded trees with compatible branching patterns. Acroloxidae were monophyletic and this was highly supported in all analyses. Within Acroloxidae, *Acroloxus coloradensis* is the sister-taxon to the clade comprising the Lake Baikal endemic genera *Gerstfeldtiancylus, Baicalancylus,* and *Pseudancylastrum*, which were monophyletic in both data sets (94% bootstrap support, Fig. 11; 1.00 Bayesian posterior probabilities, Fig. 12). The European clade, comprising the widespread *A. lacustris* and the Lake Ohrid endemic, *A. macedonicus*, is monophyletic and the sister-taxon to the *A. coloradensis*/Lake Baikal species flock clade.

The sister-group relationship of the rare North American *Acroloxus coloradensis* and the Lake Baikal acroloxid species flock is significant. Hitherto, the widespread European *A. lacustris* was assumed to be the sister-species of *Acroloxus coloradensis* (Hubendick 1969). This is also significant as to the proposed relictual status of Baikalian taxa (Sitnikova 1994; Sherbakov 1999). Lake Baikal limpets have been studied for more than one century (e.g., Gerstfeldt 1859; Hubendick 1969). Interestingly, the European ancient Lake Ohrid species, *A. macedonicus*, is not related to Lake Baikal species but rather related to the widespread European *A. lacustris*.

Hubendick (1969) speculated on the phylogenetic status of Lake Baikal acroloxids. He assumed the simpler radulae of the ancient lake endemics to be secondarily simpler compared to *A. lacustris*. The new results hint to an interesting and unusual distribution pattern. Although *A. coloradensis* was shown not to be a cold-water stenothermal species (Paul & Clifford 1991), the general climatic features in Eastern Siberia and the Rocky Mountains are compatible nowadays.

Although cross-Beringian faunal connections have been postulated several times, it is, in fact, not a very common biogeographical pattern (Sanmartin *et al.* 2001). The relictual status and "phylogenetic age" of *A. coloradensis* can not be discerned from the current data available. The fossil record hints to a once more continuous distribution of Acroloxidae in the whole Nearctic region (Tracey *et al*. 1993). The biogeographic connection of the Lake Baikal region and the Rocky Mountains is not easily explained, but could certainly be enlightened when acroloxid species of Far East Russia become available for genetic study.

Rocky Mountain phylogeography and population divergences (COI gene)

All three populations of *Acroloxus coloradensis* were monophyletic; i.e., there was no genetic variation among the 7 specimens from Jasper National Park, among the 3 specimens from Lost Lake or among the 4 specimens from Trout Lake. Moreover, there was no variation between the Lost Lake and Trout Lake populations. However, the Canadian lake population differed from the former two populations by one mutation (corresponding to a genetic distance of 0.017 or 0.17%) (Table 6). A statistical parsimony network showing the geographical distribution of the COI haplotypes found is given in Fig. 13.

One interesting aspect of the present study is the low genetic diversity (variations within populations) and divergences (variation between populations) in *Acroloxus coloradensis*. It should be noted that this is not *per se* a methodological problem as the COI gene chosen, in general, can well resolve population level relationships in gastropods (e.g., Wilke & Pfenninger 2002; Wilke & Duncan 2004).

Although many factors may influence the genetic diversity of a population (e.g., generation time, body size, life history, mode of reproduction, gene flow), the size and phylogenetic age of the population play a significant role in shaping its population structure and diversity. The older a given population is the more mutations can accumulate. In other words, the low genetic diversity and divergences in the *A. coloradensis* populations studied here might reflect their young phylogenetic age. Although it is very difficult to specify a time frame, a Power Test conducted by Wilke (2003) has shown that the COI fragment used here is suitable to resolve internodes in gastropods of about 250,000 years or less. That means the homogeneous *A. coloradensis* populations are most likely younger than that.

Another interesting aspect is the phylogeography of *A. coloradensis* relative to the Continental Divide. The divide runs along the crest of the Rockies, from British Columbia, through the United States, and continues southward into Mexico and Central America. It divides the continent's principal drainage into that flowing eastward and that flowing westward. This significant biogeographical barrier has resulted in the separation of some plant and animal species (e.g., the opossum shrimp *Mysis relicta*).

The present study indicates the distribution and genetic diversity of *A. coloradensis* is not noticeably affected by the Continental Divide. This becomes clear when comparing the Lost Lake population (east of the divide) with the Trout Lake population (west of the divide): both populations show completely identical COI haplotypes. Moreover, the Jasper National Park populations (just east of the divide) differ both from the Trout Lake and Lost Lake populations. It is therefore likely this small genetic difference is not due to processes related to the Continental Divide but simply a matter of geographic distance.

CONCLUSIONS AND RECOMMENDATIONS

We concluded that Rocky Mountain capshell limpets remain fairly abundant in Lost Lake. *Acroloxus coloradensis* in Lost Lake occurred primarily in the eastern and southern regions of the lake (Fig. 1). The preferred habitat of the limpet was the underside of flat rocks (~>5 cm diameter) in relatively shallow water (~<1 m depth). Densities were highest where layers of flat rocks were numerous and the area was not utilized by people for wading and swimming. Densities averaged $164/m^2$ (range = 0 to $574/m^2$). The maximum densities reported for Lost Lake exceed all published estimates for the species. Reduced densities of limpets were observed where people commonly waded out into the lake to swim (Fig. 1, shaded area), as they can be easily crushed between the successive layers of rock from simply walking on the substrate. Persons accessing the lake on the northern and western shores, primarily to fish, would have little impact on the limpet population, as the substrate is not optimum for the limpets.

The presence of another limpet, *Ferrissia fragilis*, in Lost Lake is of interest as it was not reported by Russell and Brunson (1967). Whether this species was missed in the original investigation or whether it is a newcomer is not known, but it appears that it shares similar habitat with that of *A. coloradensis*. If competition is occurring between these 2 limpets, it does not appear to have reduced the density of *A. coloradensis* since estimates were made by Clarke (1993).

The Lake is surprisingly productive, owing probably to its nature as a seepage lake. Groundwater seeps into the lake are high in $NO_{2/3}$ and total phosphorus, but both nutrients are rapidly assimilated by lake biota. The trophic status of Lost Lake may best be characterized as mesotrophic, though the net primary production was in the range observed for eutrophic lakes. The lake is dimictic and oxygen is reduced below 4 m as soon as thermal stratification begins, with near 0% saturation on the bottom by August. Fish are limited to nonnative brook trout that feed on an array of zooplankton and benthos. Although the capshells do not appear to be very sensitive to fish predation in summer and fall, it is possible that brook trout are more aggressive in bottom feeding during winter when surface organisms and zooplankton populations are reduced.

Nonetheless, protection of this important population should include returning Lost Lake to its historical fishless condition by intensive gill netting of nonnative trout and carefully managing or eliminating wading and swimming by recreationists.

The sister-group relationship of the rare North American *Acroloxus coloradensis* and the Lake Baikal acroloxid species flock is significant. Hitherto, the widespread European *A. lacustris* was assumed to be the sister-species of *Acroloxus coloradensis*. The biogeographic connection of the Lake Baikal region and the Rocky Mountains is not easily explained but might be elucidated with the genetic study of specimens from the Russian Far East. Both the Lost Lake (east of divide) and Trout Lake (west of divide) populations showed completely identical COI haplotypes indicating that the distribution and genetic diversity of *A. coloradensis* is not noticeably affected by the Continental Divide. Moreover, the Jasper National Park populations (just east of the divide) differs both from the Trout Lake and Lost Lake populations. It is therefore likely this small genetic difference is not due to processes related to the Continental Divide but simply a matter of geographic distance.

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Table 1. Chemistry of Lost Lake at 1 m and 5 m depths in comparison to the inlet seep on June 4, 2002. Alk = alkalinity, SO_4 = sulfate, DOC = dissolved organic, TOC = total organic carbon, NH_4-N = ammonium nitrogen, $NO_{2/3}$-N = nitrate + nitrite nitrogen, TN = total nitrogen, SRP = soluble reactive phosphorus, TP = total phosphorus, Chl *a* = chlorophyll *a*, Phaeo = phaeophytin.

	Inlet seep	Lake 1 m	Lake 5 m
Alk (mg/L $CaCO_3$)	44.9	39.4	42.7
SO_4 (mg/L)	3.38	3.29	3.40
DOC (mg/L)	1.00	1.89	2.02
TOC (mg/L)	1.06	1.97	2.21
NH_4-N (µg/L)	5.9	6.6	5.6
$NO_{2/3}$-N (µg/L)	82.1	3.4	<0.6
TN (µg/L)	307	162	227
SRP (µg/L)	3.0	<0.3	1.6
TP (µg/L)	41.4	14.9	24.9
Chl *a* (µg/L)		2.32	7.09
Phaeo (µg/L)		1.27	2.76

Table 2. Mean (± 1 standard deviation) values for chemical analysis of water samples from 1 m and 5 m depths in Lost Lake, 2001–2002. Sample median and interquartile range (IQR) were computed for variables with values below detection limit (Helsel & Hirsch 2000). Variable abbreviations as in Table 1. Means for 8 other high elevation lakes in Glacier National Park (GNP) sampled from 1984–1990 are also given (Ellis et al. 2002).

	1 m			5 m			GNP Lakes		
	Mean or Median*	SD or IQR*	n	Mean or Median*	SD or IQR*	n	Mean	SD	n
Alk (mg/L CaCO$_3$)	42.1	2.0	5	42.9	1.0	5			
SO$_4$ (mg/L)	3.19	0.09	5	3.20	0.14	5			
DOC (mg/L)	2.37	0.36	8	2.42	0.37	8	0.80	0.31	8
TOC (mg/L)	2.72	0.77	8	2.79	0.71	8	1.05	0.39	8
NH$_4$-N (µg/L)*	5.1	0.1 - 5.1	8	5.3	0.3 - 5.3	8			
NO$_{2/3}$-N (µg/L)*	<0.6	<0.6	8	<0.6	<0.6	8			
TN (µg/L)	238	75	8	261	77	8	76	15	8
SRP (µg/L)	1.9	1.8	8	1.7	1.3	8			
TP (µg/L)	18.6	4.2	8	22.4	6.3	8	5.0	1.2	8
Chl *a* (µg/L)	4.96	3.67	8	6.94	3.03	7			
Phaeo (µg/L)	2.20	1.36	7	2.47	1.19	6			
N:P (µmoles:µmoles)	28		8	26		8	36	7	8

Table 3. Net primary productivity and related characteristics of Lost Lake in comparison to ranges described for lakes of different trophic categories (after Wetzel 2001). Variable abbreviations as in Table 1.

Trophic type	Net primary productivity mgC m^{-2} day^{-1}	Chlorophyll *a* (µg/L)	Light ext coeff (ηm^{-1})	TOC (mg/L)	TP (µg/L)	TN (µg/L)
Oligotrophic	50–300	0.3–3	0.05–1.0	<1–3		
Oligomesotrophic					5–10	250–600
Mesotrophic	250–1000	2–15	0.1–2.0	<1–5		
Mesoeutrophic					10–30	500–1100
Eutrophic	>1000	10–500	0.5–4.0	5–30		
Lost Lake	1257	6.9	0.8	2.8	22	261

Table 4. Trace metals analysis results from grab samples (0.3 m) obtained near south shoreline of Lost Lake in August and October of 2001. Values are in mg/L.

	August	October
Aluminum	<0.01	<0.01
Arsenic	<0.005	<0.005
Barium	0.0305	0.0330
Beryllium	<0.0001	<0.0001
Calcium	12.1	13.2
Cadmium	<0.001	<0.001
Cobalt	<0.003	<0.003
Chromium	<0.005	<0.005
Copper	<0.003	<0.003
Iron	0.0138	0.0036
Potassium	<0.5	<0.5
Lithium	0.0018	0.0024
Magnesium	3.30	3.67
Manganese	0.0007	<0.0005
Molybdenum	<0.003	<0.003
Sodium	1.26	1.21
Nickel	<0.002	<0.002
Phosphorus	<0.01	<0.01
Lead	<0.01	<0.01
Sulfur	1.18	1.17
Silicon	2.31	3.58
Tin	0.003	<0.003
Strontium	0.0354	0.0404
Titanium	<0.005	<0.005
Vanadium	<0.005	<0.005
Zinc	0.0026	0.0048

Table 5. Periphyton biomass (μg/cm^2 chlorophyll *a*) in the southeastern, eastern and northeastern regions of Lost Lake, 2002.

	July	n	August	n	September	n
Southeastern	2.70	3	4.01	3	3.88	3
Eastern	6.29	4	7.33	4	6.24	4
Northeastern	3.03	3	5.64	3	4.07	3
Lake wide mean	4.23	10	5.83	10	4.88	10
Lake wide SD	2.25		2.31		2.75	

Table 6. Nucleotide diversity (diagonal line) and divergences (below diagonal line) of the COI gene within and among populations of *Acroloxus coloradensis* based on K2P distances.

	LL	TL	JP
LL	0		
TL	0	0	
JP	0.0017	0.0017	0

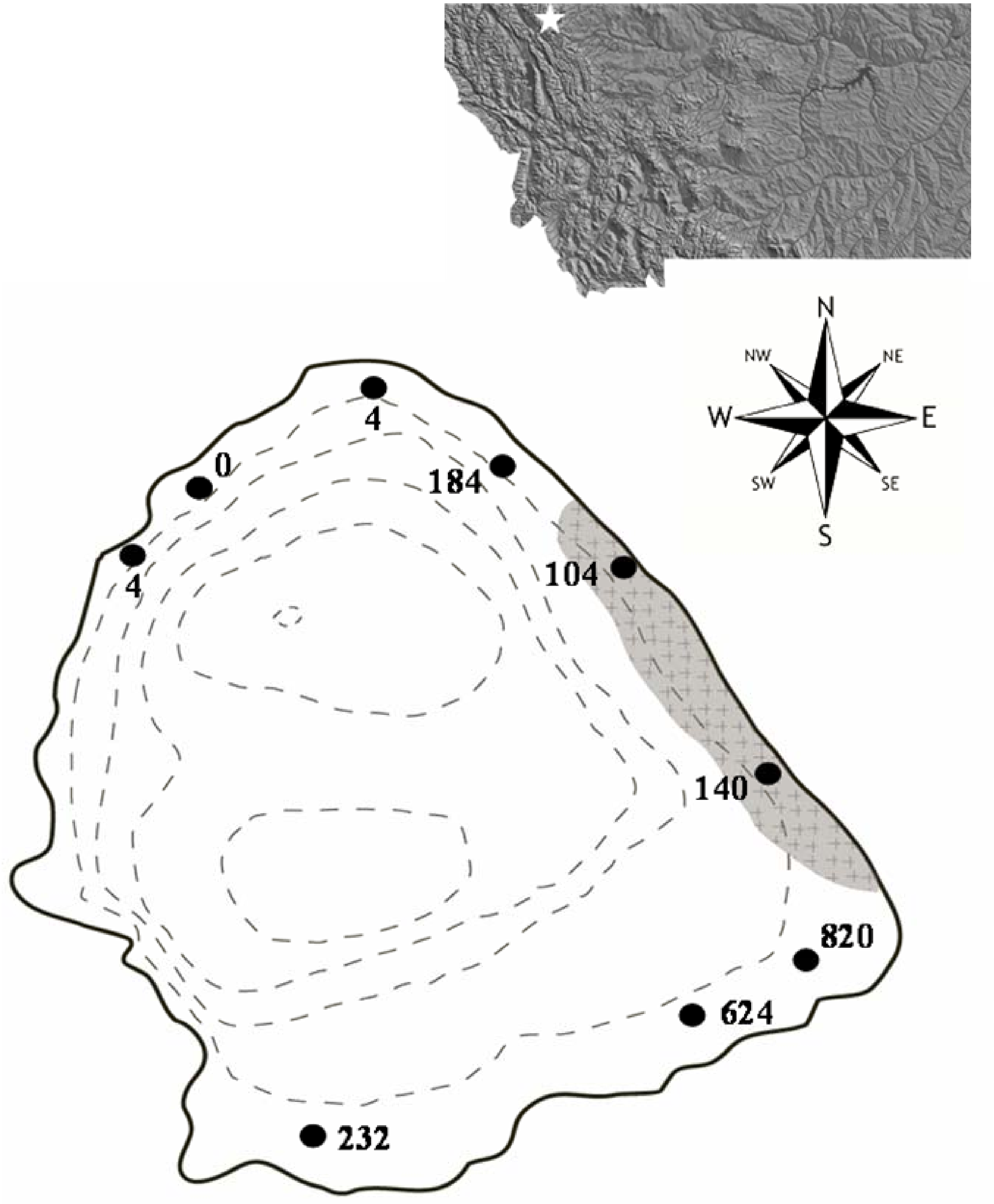


Figure 1. Approximate bathymetry denoted by dotted lines (1.5, 3.0, 4.6, 6.1 and 8.2 m depths). Shaded area corresponds to the area of highest human use. Location of Lost Lake in northwestern Montana is shown in the upper figure (white star). Densities (#/ m^2) of limpets, *Acroloxus coloradensis* and *Ferrissia fragilis* form Basch *isabellae,* in Lost Lake, Montana, 2001 shown next to black circles.

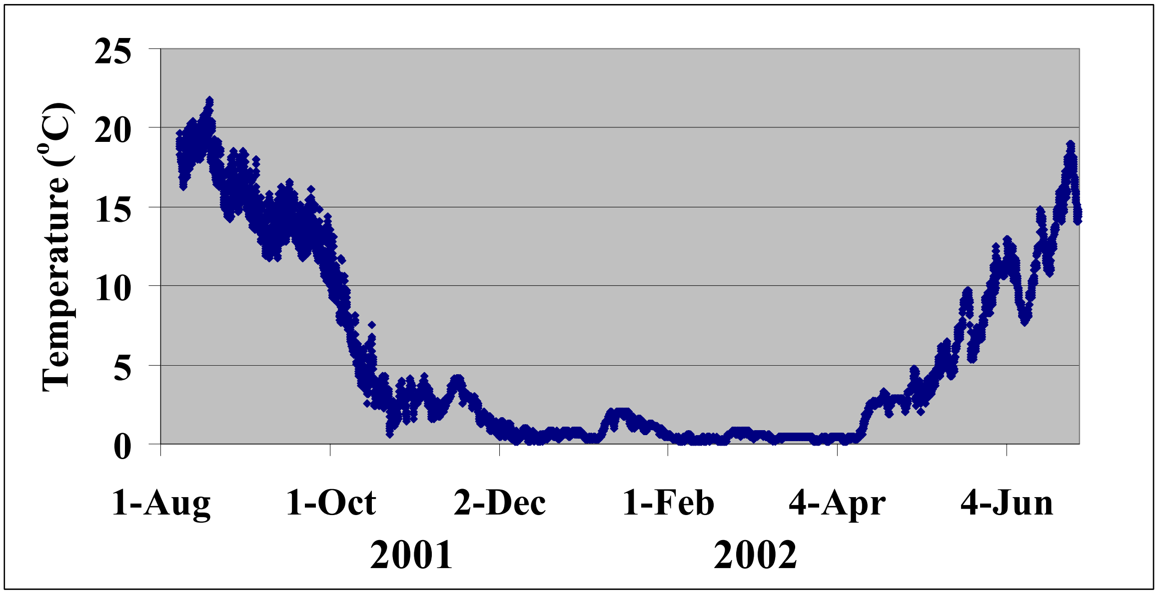


Figure 2. Hourly temperature (° C) at approximately 0.7 m depth in Lost Lake.

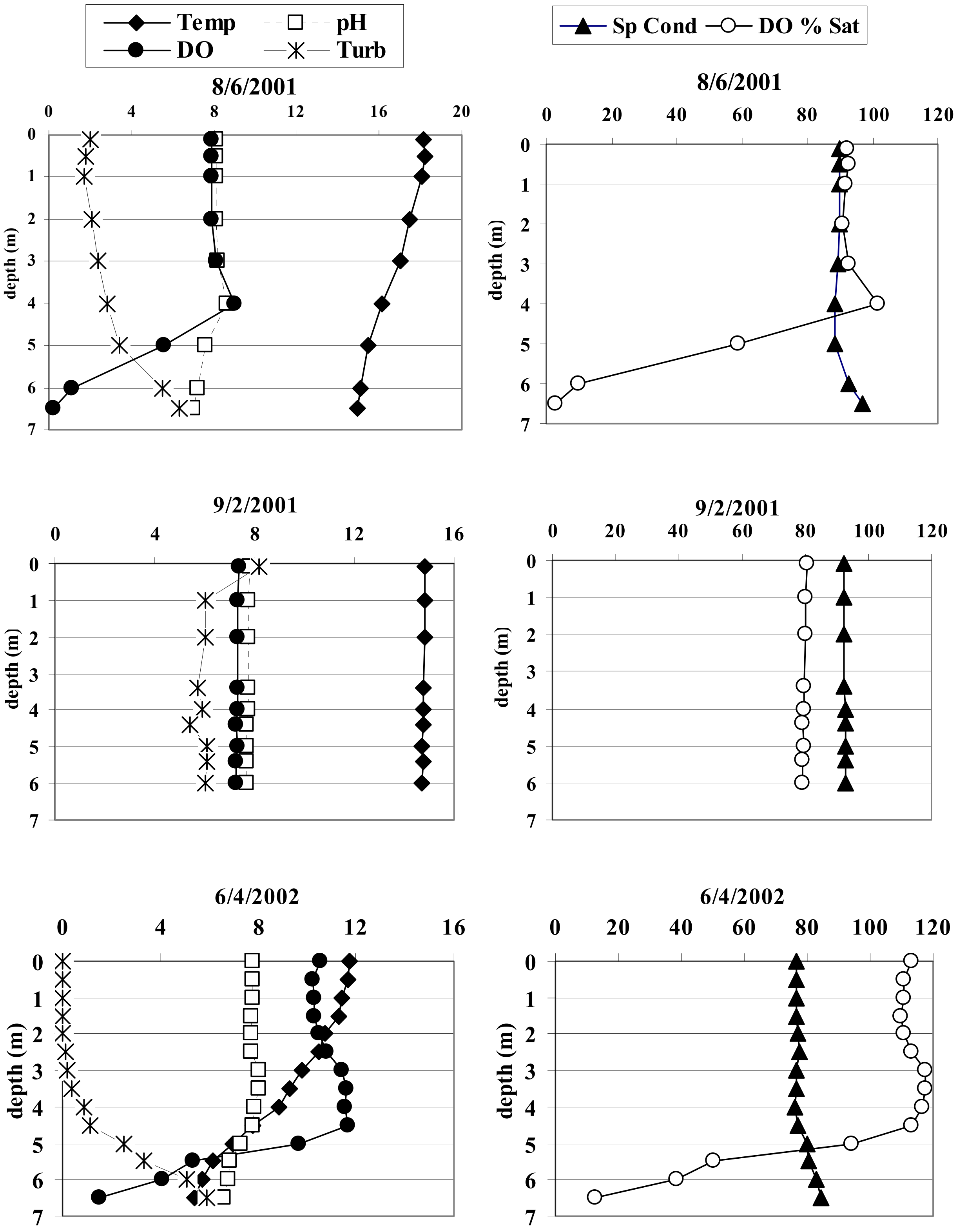


Figure 3. Water column profiles of temperature (°C), pH (units), DO (mg/L dissolved oxygen), Turb (NTU turbidity), Sp Cond (µS/cm specific conductance) and DO %Sat (% saturation of dissolved oxygen) in Lost Lake in August and September 2001 and June 2002.

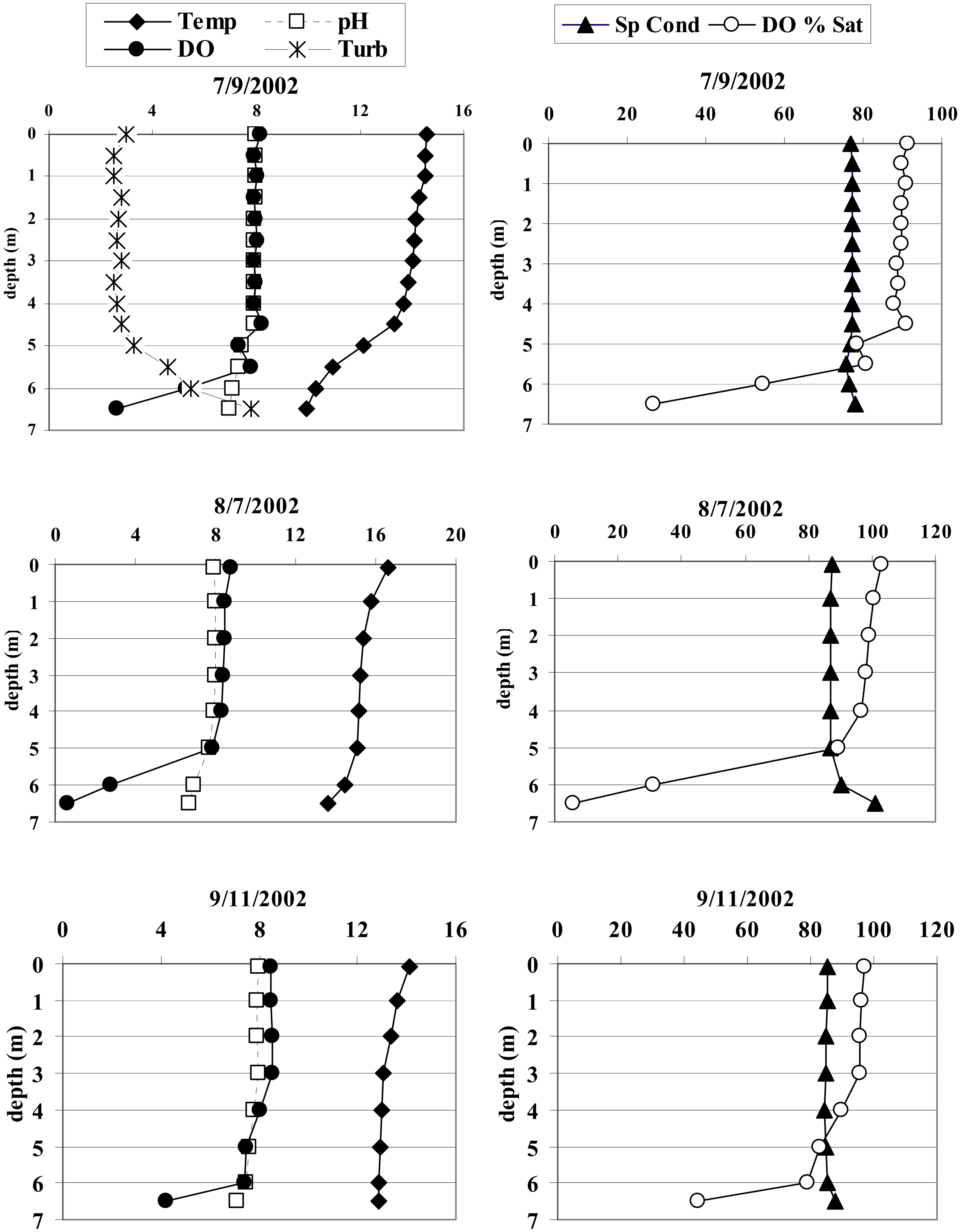


Figure 4. Water column profiles of temperature (^{o}C), pH (units), DO (mg/L, dissolved oxygen), Turb (NTU, turbidity), Sp Cond (µS/cm, specific conductance) and DO %Sat (% saturation, dissolved oxygen) in Lost Lake from July through September, 2002.

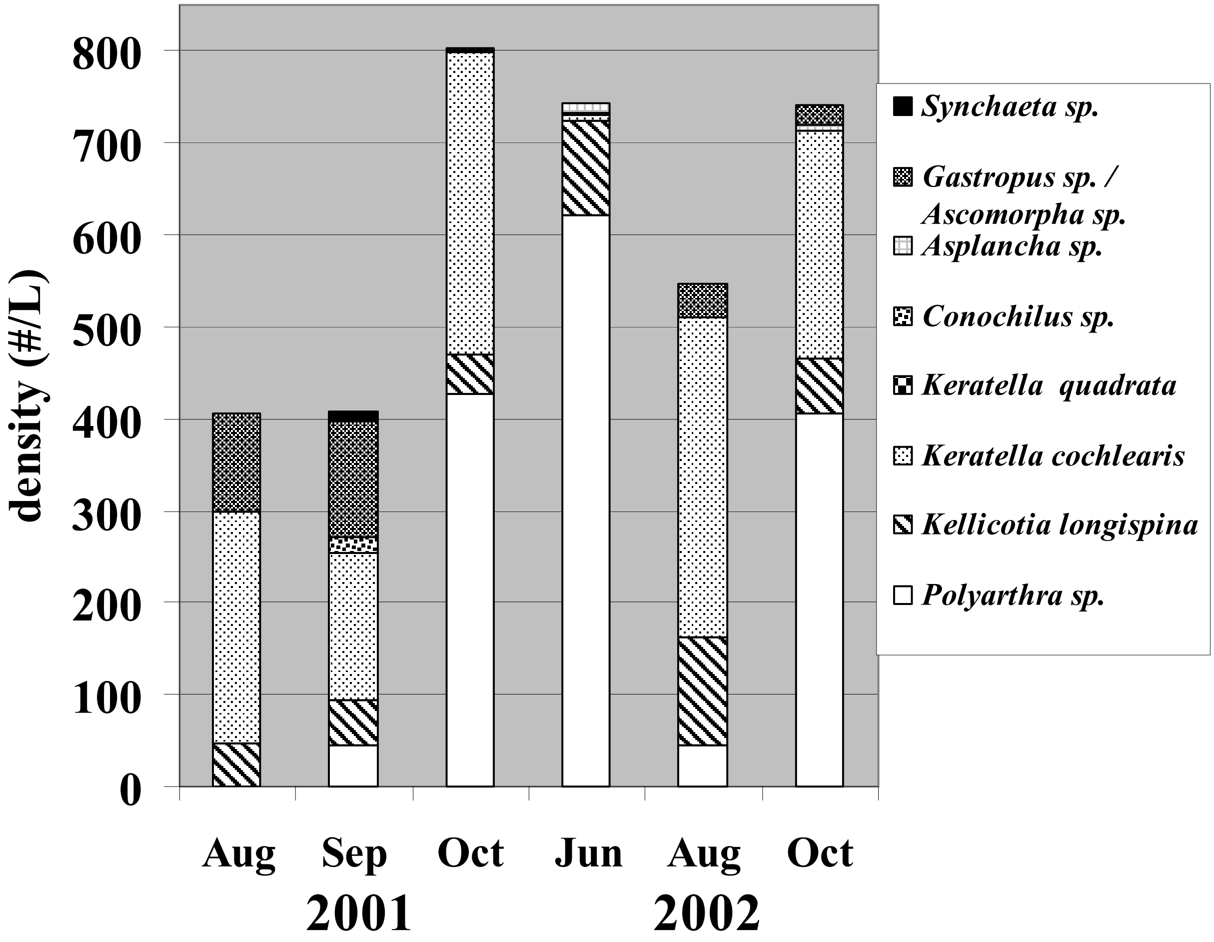


Figure 5. Abundance of rotifers in the zooplankton of Lost Lake (0–5 m net hauls). Note that the August 2001 sample is likely an underestimate (see text).

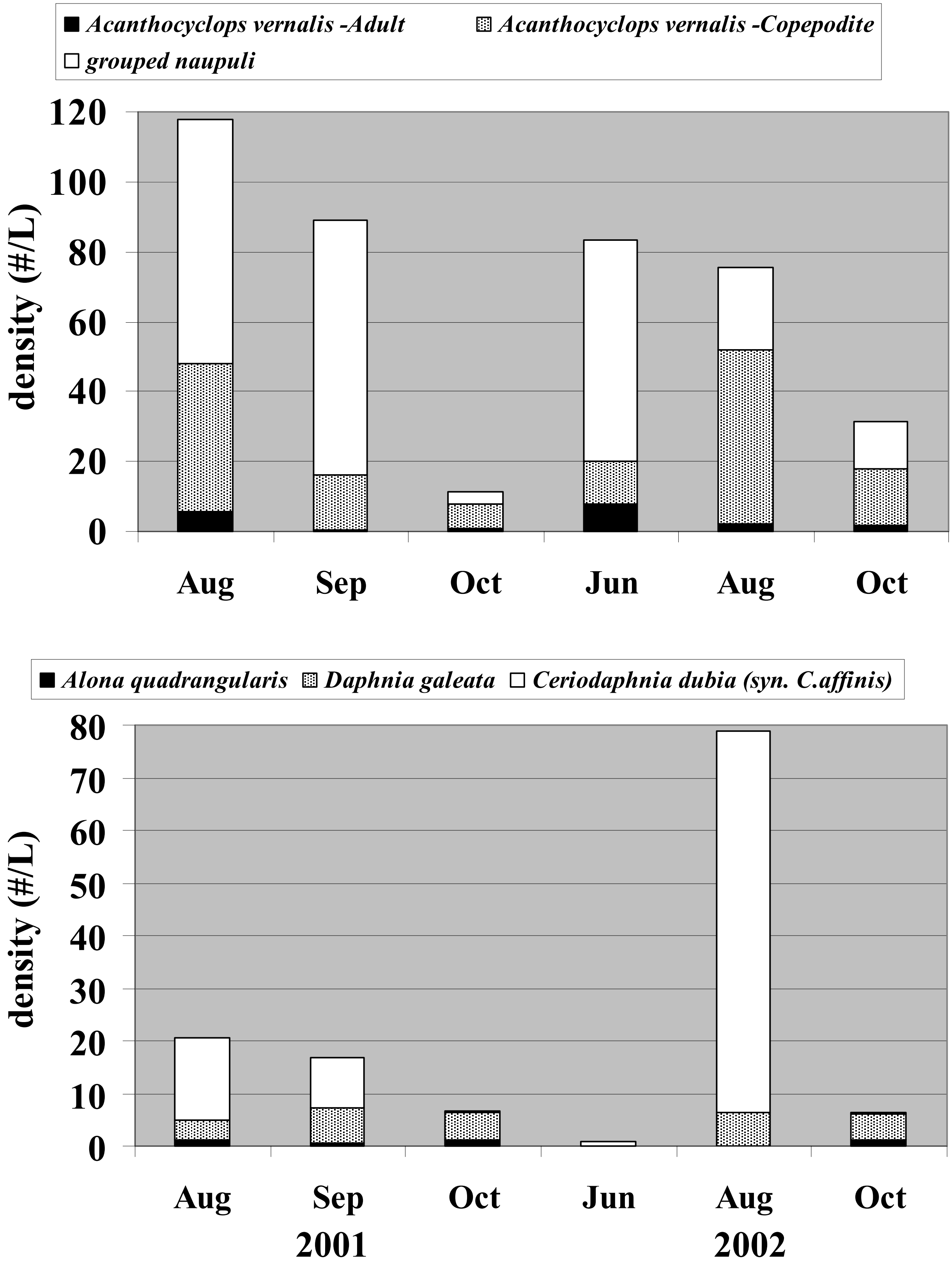


Figure 6. Abundance of copepods (top panel) and cladocerans (bottom panel) in zooplankton of Lost Lake.

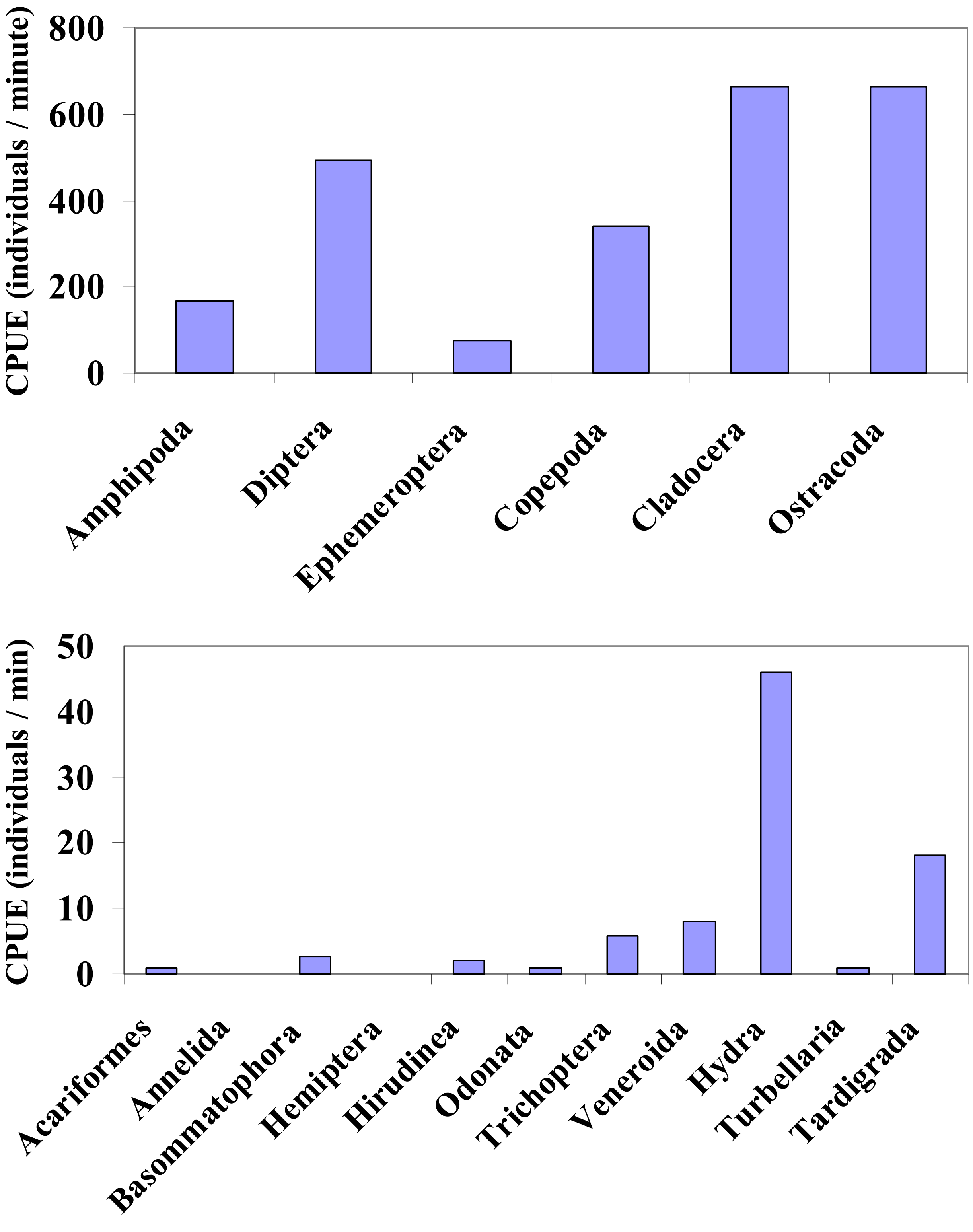


Figure 7. Mean catch per unit effort (CPUE; individuals per minute) of benthos in the shallow (<1 m depth) littoral zone of Lost Lake. Data are means from collections in August, September, and October 2001–2002. Note change in scale between panels.

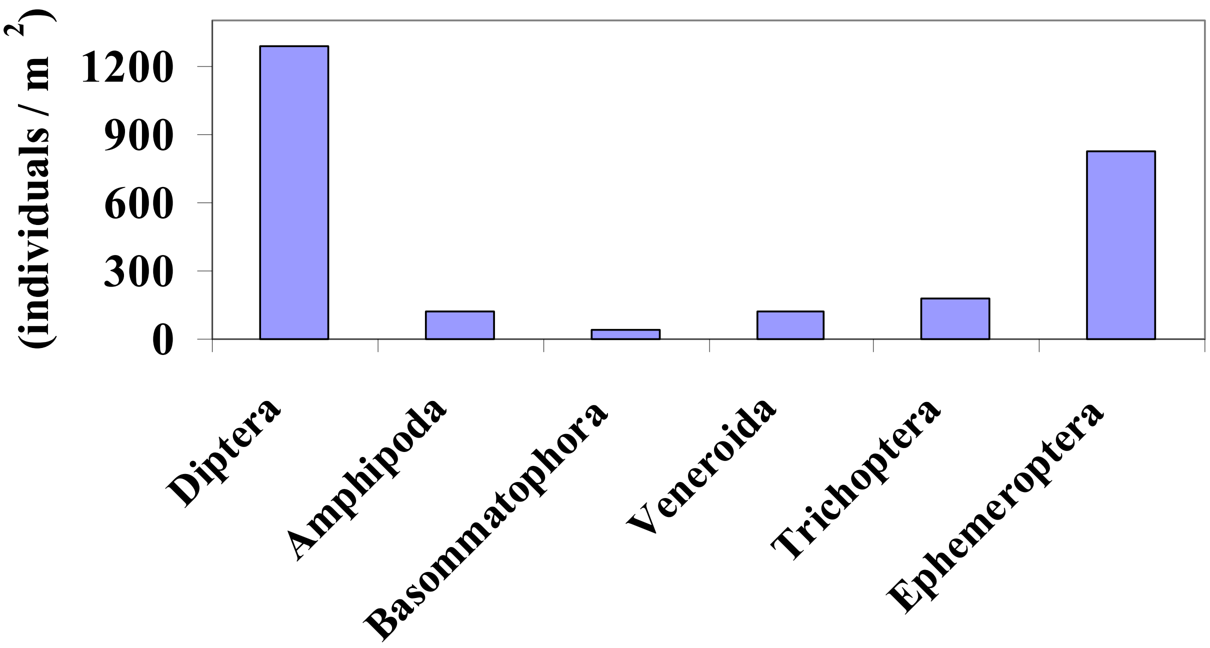


Figure 8. Mean density of deep water benthos (>5 m) in Lost Lake. Data are means from collections in August and September, 2001–2002.

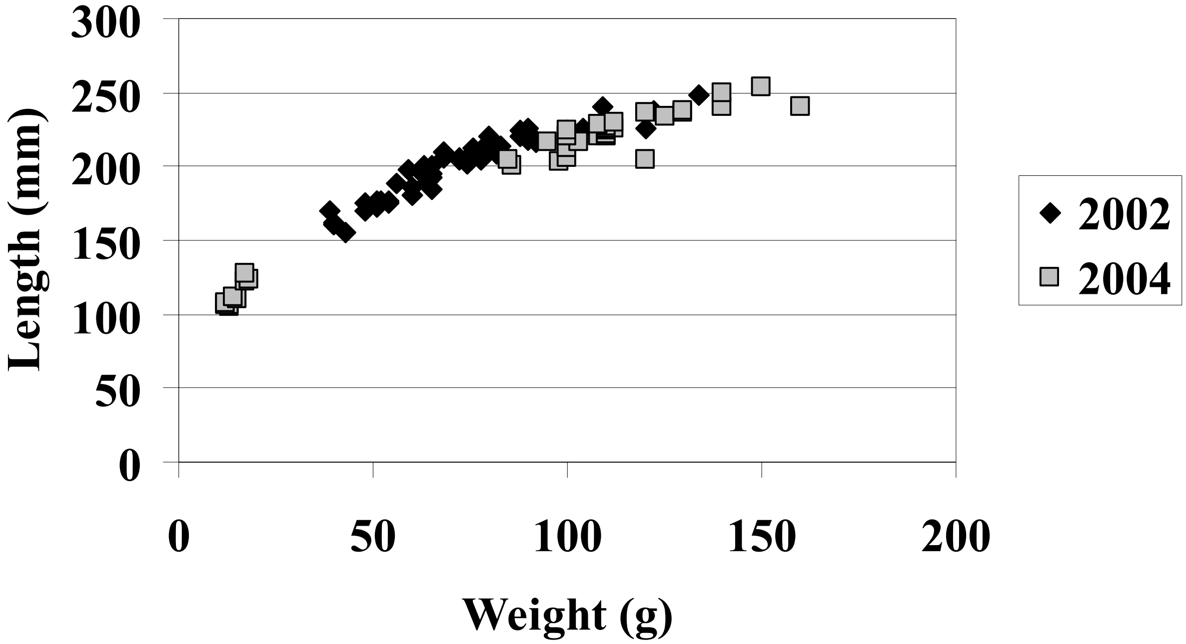
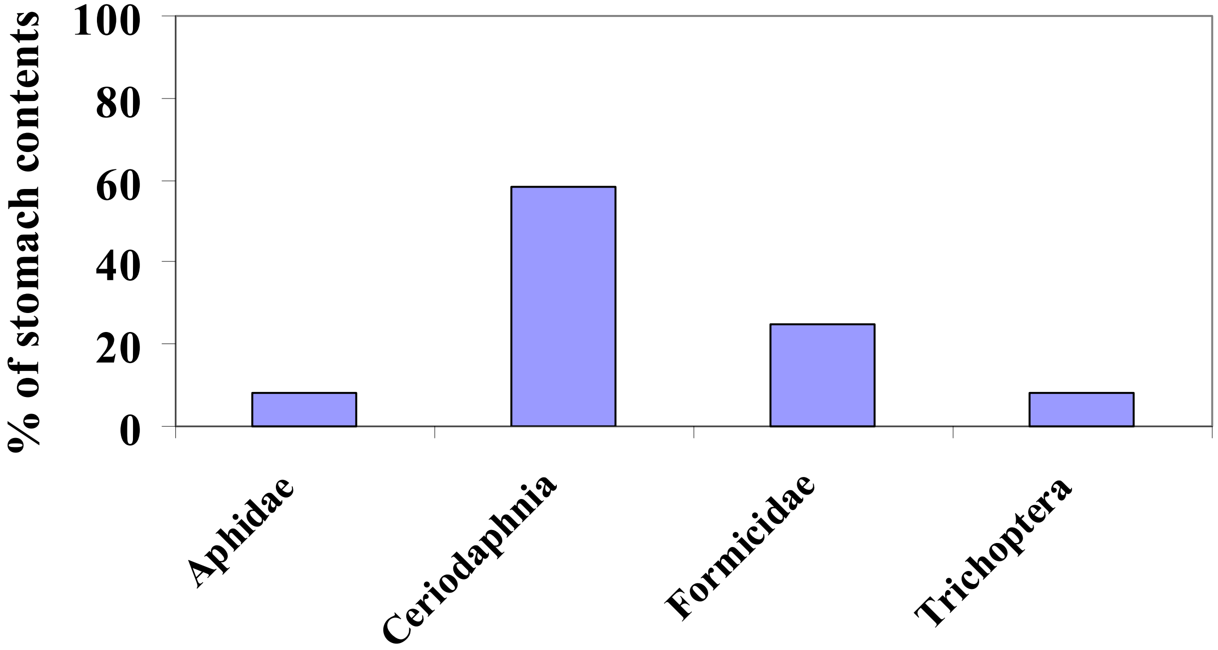


Figure 9. Length/weight distribution of brook trout in Lost Lake in 2002 and 2004.



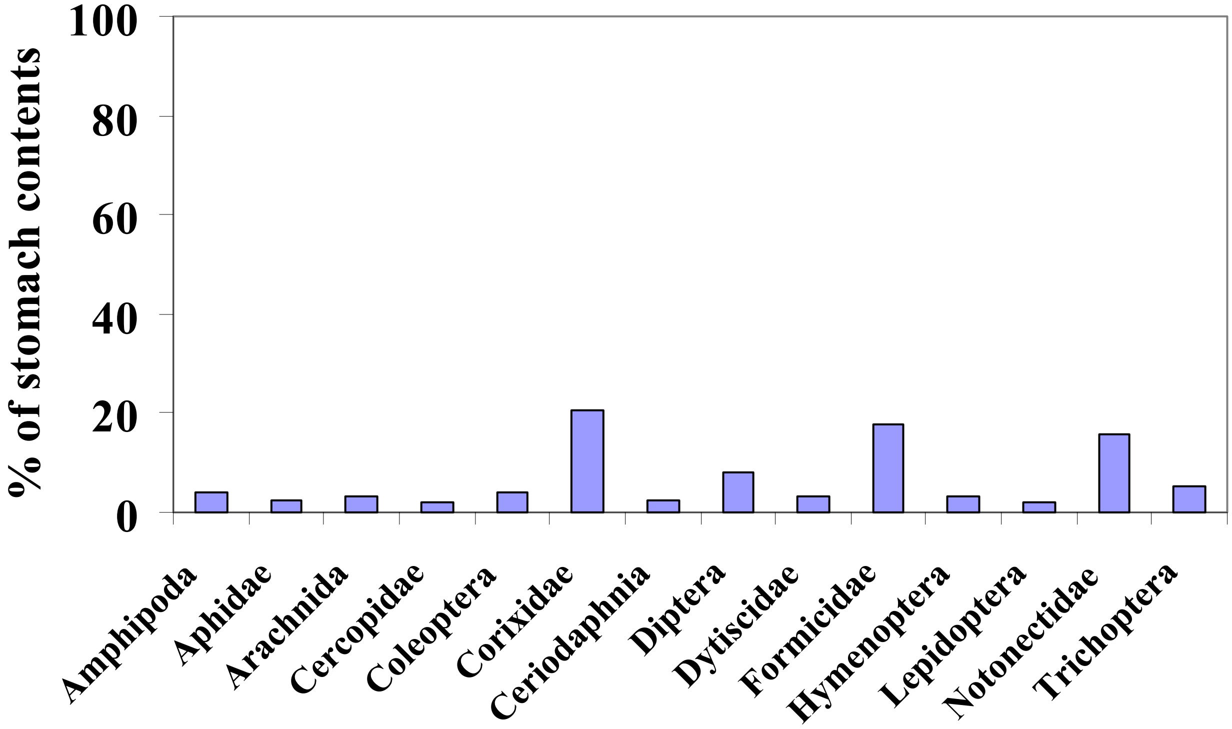


Figure 10. Organisms common in the diet of brook trout. Top panel represents brook trout 107–123 mm in length (~ year 1) while the bottom panel is brook trout 209–259 mm in length (~ year 3+). Only organisms comprising more than 2% of the diet are shown in the bottom panel.

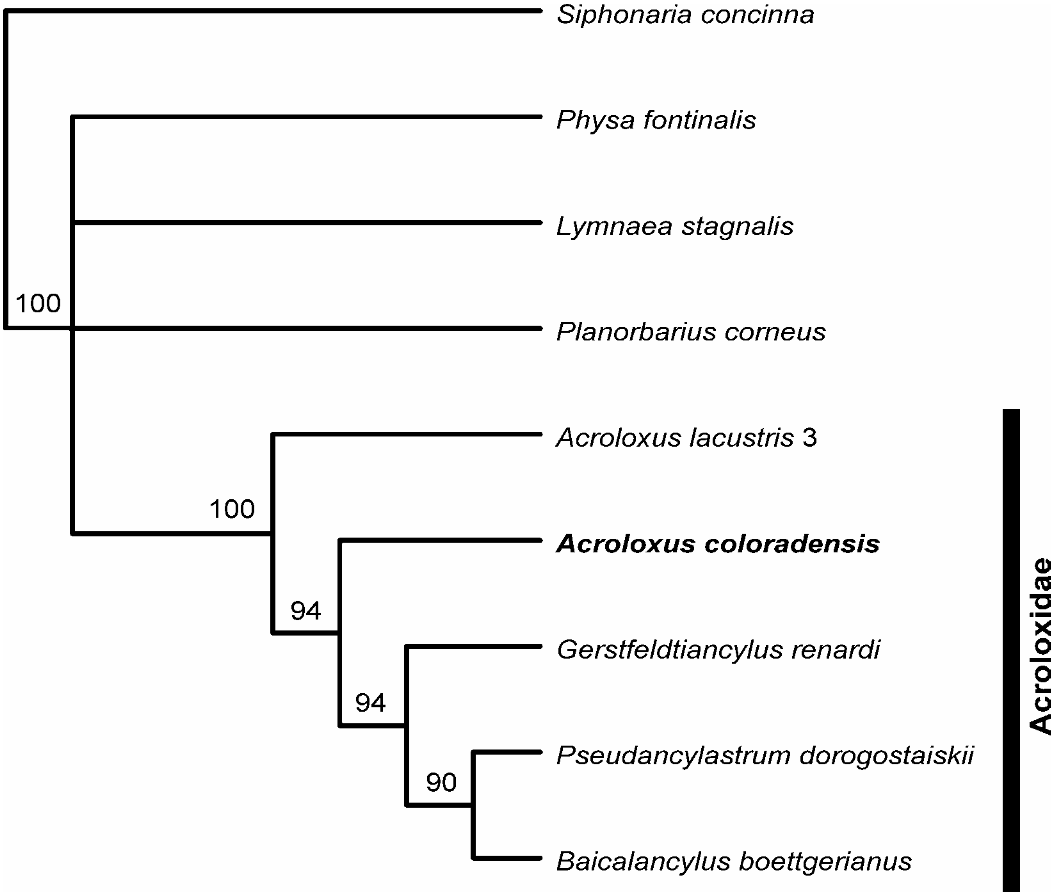


Figure 11. Maximum Parsimony cladogram for Acroloxidae based on 2,490 nucleotide positions of concatenated 18S, COI and 16S DNA sequences. *Acroloxus coloradensis* is from Jasper National Park - JP (Alberta, Canada) and *A. lacustris* 3 from Germany. *Siphonaria concinna* was used as outgroup. Bootstrap support (1,000 replications) is provided at the nodes. *Physa*, *Lymnaea*, and *Planorbariu*s are included as additional hygrophilan taxa here.

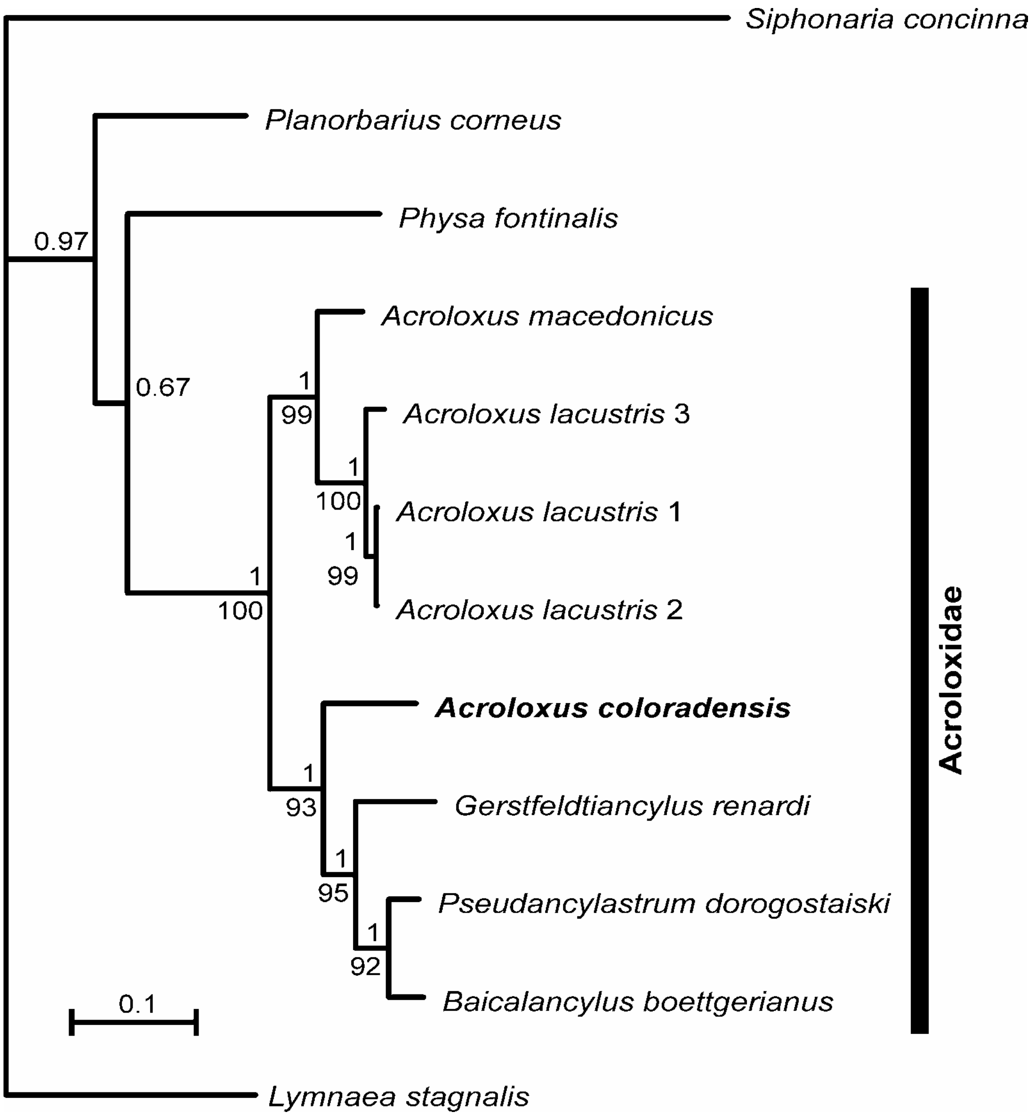


Figure 12. Bayesian inference phylogram for Acroloxidae based on combined COI and 16S sequences (1,428 bp). The tree was rooted with the outgroup *Siphonaria concinna*. The scale bar indicates the expected number of substitutions per site according to the model of sequence evolution applied. Bayesian posterior probabilities and MP bootstrap support (>50%) are provided above and below the branches, respectively.

Figure 13. Statistical parsimony network of COI haplotypes of *Acroloxus coloradensis* from Jasper National Park, Trout Lake and Lost Lake. The only substitution found in the genetic data set of 14 specimens (indicated by a black bar) separates the Jasper National Park population from the two populations from Montana.

Appendix Table 1. Macrobenthos present in Lost Lake, 2001–2002.

Phylum	Class	Order	Family	Genus	Species	Common Name For Group
Annelida	Clitellata	Hirudinea*	Unknown	Unknown	sp.	Leech
Annelida	Clitellata	Oligochaeta*	Unknown	Unknown	sp.	Oligochaetes
Annelida	Polychaeta	Unknown	Unknown	Unknown	sp.	Segmented worm
Arthropoda	Arachnida	Hydracarina	Unknown	Unknown	sp.	Water mite
Arthropoda	Cephalocarida	Cladocera	Chydoridae	Alona	quadrangularis	Water flea
Arthropoda	Cephalocarida	Cladocera	Daphniidae	Daphnia	galeata	Water flea
Arthropoda	Cephalocarida	Cladocera	Daphniidae	Ceriodaphnia	dubia	Water flea
Arthropoda	Cephalocarida	Cladocera	Macrothricidae	Ilyocryptus	sp.	Water flea
Arthropoda	Copepoda	Cyclopoida	Cyclopidae	Acanthocyclops	vernalis	Copepod
Arthropoda	Copepoda	Harpactocoida	Unknown	Unknown	sp.	Copepod
Arthropoda	Copepoda	Unknown	Unknown	Unknown	sp.	Copepod
Arthropoda	Insecta	Diptera	Ceratopogonidae	Bezzia	sp.	Biting midges
Arthropoda	Insecta	Diptera	Chironomidae	Unknown	sp.	Flies
Arthropoda	Insecta	Diptera	Unknown	Unknown	sp.	Flies
Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	youngi	Mayfly
Arthropoda	Insecta	Ephemeroptera	Baetidae	Callibaetis	sp.	Mayfly
Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Paraleptophlebia	debilis	Mayfly
Arthropoda	Insecta	Ephemeroptera	Baetidae	Unknown	sp.	Mayfly
Arthropoda	Insecta	Ephemeroptera	Unknown	Unknown	sp.	Mayfly
Arthropoda	Insecta	Hemiptera	Corixidae	Unknown	sp.	Water boatmen
Arthropoda	Insecta	Odonata	Aeshnidae	Aeshna	sp.	Dragonfly
Arthropoda	Insecta	Odonata	Unknown	Unknown	sp.	Dragonfly
Arthropoda	Insecta	Trichoptera	Limnephilidae	Hesperophylax	sp.	Caddisfly

*Subclass

Appendix Table 1. (Continued) Macrobenthos present in Lost Lake, 2001–2002.

Phylum	Class	Order	Family	Genus	Species	Common Name For Group
Arthropoda	Insecta	Trichoptera	Limnephilidae	Glyphopsyche	irrorata	Caddisfly
Arthropoda	Insecta	Trichoptera	Lepidostomatidae	Lepidostoma	sp.	Caddisfly
Arthropoda	Insecta	Trichoptera	Leptoceridae	Unknown	sp.	Caddisfly
Arthropoda	Insecta	Trichoptera	Leptoceridae	Mystacides	sp.	Caddisfly
Arthropoda	Insecta	Trichoptera	Leptoceridae	Oecetis	sp.	Caddisfly
Arthropoda	Insecta	Trichoptera	Unknown	Unknown	sp.	Caddisfly
Arthropoda	Malacostraca	Amphipoda	Gammaridae	Gammarus	lacustris	Scud
Arthropoda	Malacostraca	Amphipoda	Hyalellidae	Hyalella	azteca	Scud
Arthropoda	Malacostraca	Amphipoda	Unknown	Unknown	sp.	Freshwater shrimp
Arthropoda	Ostracoda	Unknown	Unknown	Unknown	sp.	Seed shrimp
Cnidaria	Hydrozoa	Hydroida	Hydridae	Hydra	sp.	Hydra
Ectoprocta	Phylactolaemata	Plumatellida	Unknown	Unknown	sp.	Bryozoa
Mollusca	Bivalvia	Veneroida	Pisidiidae	Pisidium	casertanum	Fingernail clam
Mollusca	Gastropoda	Basommatophora	Acroloxidae	Acroloxus	coloradensis	Limpet
Mollusca	Gastropoda	Basommatophora	Planorbidae	Gyraulus	sp.	Snail
Mollusca	Gastropoda	Basommatophora	Physidae	Physa	gyrina	Snail
Platyhelminthes	Turbellaria	Unknown	Unknown	Unknown	sp.	Flatworm
Porifera	Demospongiae	Haplosclerida	Spongillidae	Spongilla	lacustris	Sponge
Tardigrada	Unknown	Unknown	Unknown	Unknown	sp.	Water bear

*Subclass