

A survey of the impacts of fish introduction and removal on zooplankton of alpine lakes in Rocky Mountain National Park, USA

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“These are your fountains and your gardens of life. Kindly assist in keeping them”

-Enos Mills

Abstract.

Studies of fish effects on zooplankton in alpine lakes show mixed or inconclusive results. When effects are detected, they are not uniform across different systems (e.g. fish increase zooplankton taxa richness in some studies but decrease richness or lead to extirpation in others). Teasing out these relationships is important because (non-native) fish removal is becoming a more predominant management directive. We surveyed 33 alpine and sub-alpine lakes in Rocky Mountain National Park in late July 2005 to capture peak copepod and cladoceran production, comparing zooplankton communities of lakes with and without fish across an elevation and productivity gradient. Both redundancy analysis and a priori t-tests (Bonferroni corrected) were used to examine the factors that explained zooplankton community composition both in terms of mean individuals and biomass of each taxa. Fish had significant impacts on both zooplankton biomass and community composition in these lakes. Non-rotifer taxa that were found in lakes with and without fish were significantly smaller in lakes with fish. Zooplankton communities in lakes with fish were almost entirely composed of rotifers, while fishless lakes supported greater numbers and kinds of larger zooplankton but fewer rotifer taxa. Fish presence, however, did not account for differences in total taxa richness or total abundance of individuals. These results show that significant changes in biomass and community composition can be masked if management decisions are based only on comparisons of diversity or raw numbers of individuals. Changes in community composition are particularly important because different zooplankton communities have very different ecological functions.

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1. Introduction.

This study was instigated at the request of Rocky Mountain National Park (RMNP) resource managers in the context of greenback cutthroat trout (GBCT) management program. Our goal was to assess the impacts of past fish on zooplankton communities by surveying as many Park lakes as possible in a short time. We also examined recovery of zooplankton communities in lakes from which fish have been extirpated since stocking cessation. This work takes place in the context of immediate Park management questions as well as an overall set of questions of importance to aquatic ecologists.

1.1. The Greenback Cutthroat Trout Management Plan of Rocky Mountain National Park.

The alpine lakes of Rocky Mountain National Park were fishless until fish stocking began in the late 19th century (a history of stocking in the Park has been compiled by Kennedy et al 2001). All fish stocking of Park lakes was terminated in 1968, but many once fishless lakes still maintain populations of both GBCT (native to the streams of the Park) and non-native fishes. Knapp et al (2001) present a comprehensive review of the general issues surrounding management decisions about fish introductions and removal from alpine systems. Currently, the Park is in the process of determining the impacts of these introductions.

Resource managers of Rocky Mountain National Park have set out a series of plans to protect and restore both GBCT fish populations and their natural habitat. The most recent work in this line is the Greenback Cutthroat Trout Management Plan, which includes a provision for setting GBCT populations in their original ecological context. Pre-stockings, the lakes in RMNP were fishless, while GBCT thrived in Park streams. The GBCT Management Plan seeks to balance the

requirements of understanding this natural context as well as other interests in preserving Park resources.

In accordance with this directive, we surveyed the pelagic zooplankton of alpine lakes in Rocky Mountain National Park east of the Continental Divide. Our primary goal was to establish a baseline understanding of what zooplankton communities are currently like in the Park and to assess the effects of fish stocking and removal (in lakes where fish have died out since stocking cessation). This survey begins to address two key goals of the GBCT Management Plan. First, a zooplankton survey is part of the call for comprehensive accounts of current aquatic habitat in the Park. Secondly, assessments of the effects of fish introduction are necessary to understand how to protect and restore the ecological context of historic GBCT populations in Park streams to an unperturbed and sustainable state.

1.2 Research on zooplankton in alpine lakes.

The introduction of fish into alpine lakes can have dramatic effects on alpine lake ecosystems, particularly because fish introduce a new top predator into an often complex trophic web. Consequently, fish introductions directly or indirectly impact nutrient cycling in lakes (Schindler 2001, Kitchel et al 1993) and primary production (Carpenter 1993, Slusarczyk 1997). These effects often include changes in the size, abundances, and kinds of zooplankton present (Donald 2001). Because zooplankton occupy a sensitive level between top-down (predation) effects and bottom-up processes (algal production, other environmental determinants), understanding changes in zooplankton communities offer important ecological insights into whole lake processes.

Fish generally suppress or extirpate larger zooplankton, and consequently smaller zooplankton (particularly rotifers) increase in numbers, diversity, or both (Toro et al, 2006, review these

responses in European alpine lakes, studies in the United States include Northcote et al 1978, Drake et al 2000, Donald et al 2001). In some cases, fish introduction changes the species composition but not the diversity, while in some systems fish presence actually increases zooplankton diversity (Donald 2001). In almost all previous studies, fish introductions decreased the biomass of key species or the entire population (Northcote et al 1978, Kitchel et al 1980, Carpenter et al 1993). These effects are not universal, however- fish presence was not a significant factor determining zooplankton diversity or community composition in one recent study (Winder et al 2001).

In general, Zooplankton of alpine lakes have shown low resilience to changes due to fish introductions, which is an important consideration for understanding the long term impacts of fish introductions and recovery after removal. Some set recovery estimates at 19 years following fish removal (Donald 2001), other lakes take longer or have not recovered (Toro et al 2006 and McNaught 1999 found no recovery after 1 year and 32 years respectively). Parker et al 2001 found that zooplankton abundance and biomass increased within weeks, but one species (*Hesperodiaptomus arcticus*) did not return.

2. Methods.

This study was intended to be a rapid and intense assessment of the effects of fish on the lakes of Rocky Mountain National Park. Because zooplankton populations can change rapidly over time, our goal was to balance the need to survey as many lakes as possible while avoiding confounding factors relating to change over time due to life history phenology. Furthermore, because ice-free time and other factors related to elevation and lake morphology impact life history and phenology of zooplankton populations, we sought to sample a range of lakes at different elevations and in different drainages. At each lake, we also tried to address the well known difficulties in sampling zooplankton

communities, particularly the problem of patchiness (Avois et al 2000, Avois-Jacquet forthcoming, Kratz et al 1987, Bottrell et al 1976). The methods we employed reflect these constraints in the context of our main research objective: detecting differences in zooplankton communities at peak cladoceran/copepod production between lakes with and without fish populations.

2.1. Lake selection.

All lakes within the Park east of the continental divide were considered for this study. The lakes actually selected were chosen using specific criteria:

- 1) Lakes of particular importance to past and present fish management.
- 2) Lakes of particular importance to visitors.
- 3) Lakes that occur in the same watershed but have different fish histories.
- 4) Sampling the greatest possible variety of lakes along elevation, size, drainage morphology, and productivity gradients.
- 5) Allowing the sampling of the most possible lakes in the given time.

The first three criteria were assessed using information from Park scientists and managers (in particular Terry Terrell, Mary Kay Watry and Cheri Yost). Both historical and contemporary fish management interests were also employed, and the information provided by Chris Kennedy of the U.S. Fish and Wildlife Service was invaluable (Kennedy et al 2001). We parsed the third criterion in two ways. The first distinguished lakes currently supporting fish populations from lakes with no fish, while the second sorted lakes into lakes with fish, lakes without fish, and lakes that were stocked but fish had been extirpated since

stocking cessation in 1968. This last category was meant to help us address the question of whether and to what extent zooplankton communities respond to fish removal.

The fourth criterion was meant to capture the greatest variety of lakes along gradients known to impact zooplankton communities. The range of lakes along these gradients is shown in Figure 1. The environmental gradients of interest were elevation, temperature, productivity as measured by chlorophyll-a, conductivity, and of course the fish status. Longer term studies that ranged over a full year or years would be necessary to fully account for differences in lake morphology, ice-free time, and other factors that influence zooplankton life history. Nonetheless, our range of lakes captured a great variety of different lakes in different drainages in order to account for these factors.

The fifth criterion reflects field conditions, and regrettably eliminated some important lakes. For example, Chasm Lake was not sampled because field effort was more efficient elsewhere, and other lakes (Cub Pond) are not included in the analysis due to laboratory time constraints. These omissions will be addressed in future work (particularly samples that were taken but not analyzed) if time and resources are available. The lakes that were selected, sampled, and for which samples were processed for this report are listed in Table 1.

2.2. Zooplankton sampling.

Key zooplankton populations of Colorado mountain lakes peak in late July to early August (Pennak 1944), particularly the dominant cladocerans and copepods. In order to compare fish effects, we elected to sample during this period. In order to avoid confounding the comparison with changes over time, we sampled as intensively as possible, getting to 34 lakes in 10 days from 15 to 25 July, 2005. It is possible that different slope faces, and consequently different ice-melt times, could affect

zooplankton phonologies in a way that would impact this survey. In an effort to minimize this effect we sampled as many lakes as possible from a wide range of elevations, situated in as many different drainages as possible. It was uniformly sunny and warm over this period with the exception of 24 July, which was stormy and overcast.

Zooplankton were sampled using a vertical tow method with a Wisconsin plankton net (mesh size 80 μ m) pulled at 0.5 m/s (Figure 6). Tows were taken from near bottom (but not touching to avoid benthic sampling), or from 10m in deeper lakes. In shallow lakes (<0.5m) horizontal tows were taken. In general, we prefer vertical tows because they sample a column across depths. This method requires taking a small inflatable raft that can be deflated for hiking.

Standard methods of sampling zooplankton are reviewed in (Smith 2001, Thorp et al 2001). Many studies generally employ the method of one vertical tow in the deepest spot of the lake (e.g. Donald 2001), which does not address the patchy distribution of zooplankton. A notorious problem with zooplankton sampling is the high variance due to patchiness and other factors such as diurnal migration. In order to minimize the effects of this variance in the context of comparing different lakes, we optimized the number of samples taken per lake. We took one tow at the deepest spot in the lake plus two additional tows (for a *minimum* of three tows per lake). We also took 1 additional tow for every 5 acres of lake area to account for patchiness. The results of this report are based only on the three initial tows (always including the deepest). There is no evidence that additional tows were necessary for the purposes of the comparisons we are making here. However, in forthcoming analysis we plan to examine how the number of tows impacts inferences about individual lakes (any future work on this will be submitted as an addendum to this report).

Plankton from each tow were rinsed with a small squirt-bottle into 50 mL whirlpacks, preserved with 80% ETOH, and transferred to 20 mL scintillation vials at base camp for safe transport (Figure

7). Each tow was collected and stored separately. ETOH is effective because it is easily transported and is not a hazardous material, but it does impact some aspects of identification (particularly of the aloricate rotifers).

2.3. Zooplankton identification, counting, and measurement.

Zooplankton were identified and counted at the St Croix Watershed Research Station, the environmental research center of the Science Museum of Minnesota. Zooplankton were transferred from the 20 mL scintillation vials to 40 mL centrifuge tubes and diluted to between 21 and 25 mL. This volume was rigorously agitated and then sampled with a 1mL Hensen-Stempel pipette. The 1mL sample was transferred to a 1mL Sedgwick Rafter counting slide. Organisms were identified and counted on an Olympus BX50F4 Microscope at several levels of magnification. For notes on taxonomy, see results below.

Generic identification was established using both Pennak (Smith 2001) and Thorp et al (2001). Other resources include the classic Dodds (1915) and comparative anatomical imaging available online (e.g. An Image-Based Key to the Zooplankton of the Northeast USA version 2.0, Center for Freshwater Biology, Department of Zoology University of New Hampshire, Durham, NH 03824, USA). Online resources were used only as explorations of images of different characteristics used in traditional keys. Zooplankton were identified, counted, and measured in order to calculate biomass.

Three sub-samples were taken from each tow, and three tows were analyzed for each lake. The mean abundance and size measurements of taxa in the three sub-samples was calculated and adjusted to a value per cubic meter based on dilution and vertical tow volume. Length and breadth measurements were converted into biomass using equations listed in Table 2. Final zooplankton data were analyzed in terms of mean abundance per lake of each taxa, mean biomass per lake (mean

abundance x lake specific mean biomass for each taxa), and mean taxa richness per lake. Testing variance was thus between-lake variance, not between-tow or sub-sample, avoiding problems of pseudo-replication.

It should be noted that biomass calculations were made for each tow, and biomass of any given species is then the mean of these tow measurements and is tow-specific. The mean of tow-specific values were combined into lake specific mean biomass values for use in analysis. In most studies, biomass measurements are combined over the entire study area, so that biomass figures for a species are the same across all lakes. This saves time, dramatically reducing the number of necessary measurements. While our methods were time consuming, they were intended to give a much more accurate measurement of differences in biomass relative to fish impacts, which was a primary goal of this survey.

2.4. Conductivity, temperature, and chlorophyll-a.

Temperature (in degrees Celsius) and conductivity (in $\mu\text{S}/\text{cm}^2$) were measured at the point of the deepest tow, at a depth of 1m. Integrated water samples of the vertical water column were taken using a hand pump for chlorophyll *a* analysis. This water was filtered with a hand vacuum pump and filter tower using GF/C filters until the filter was colored. These filters were stored in dark film canisters, placed in a small cooler with ice, and frozen at the earliest opportunity. The filters were ground up and analyzed for chlorophyll-*a* (Arar et al 1997). Samples were also filtered through GF/F filters (two per lake) for future analysis of C:N:P ratio analysis in order to explore nutrient characteristics of the lakes and possible differences caused by fish. If these data become available a supplement to this report will be submitted to the Park, but currently no resources are available for this work.

3. Results.

3.1. Taxa presence by lake.

Taxa present in each lake are listed in Table 3. Overall taxa richness is shown by lake in Table 1. A total of 47 zooplankton taxa were sampled over 34 lakes (Table 3), and species richness of the lakes ranged from 1 to 10.3 (Table 1). In most cases, species identification was attempted. Due to lack of reliable keys and the fluctuating or indeterminate state of zooplankton taxonomy, many taxa are left at the Generic level.

Of particular note are the aloricate rotifers *Collotheca pelagica*, *Conochilus* sp, and *Conochiloides* spp. *C. pelagica* was keyed out consistently in Smith 2001, but there is some possibility that this taxa in samples represents a variant of *Conochiloides*. The latter was definitely present, but the smaller forms were impossible to distinguish from *Conochilus* sp. Consequently, results pertaining to *Conochiloides* should be considered a *Conochilus/Conochiloides* complex. The identification of *C. pelagica* needs confirmation with future sampling using different methods of preservation, as ETOH causes desiccation.

All *Diaptomus* males counted in this report were *Diaptomus shoshone*, although *D. nudus* is also known from the Park and not all individuals were identifiable (particularly because of the predominance of females, for which the keys are less reliable). The calanoid copepod *Epischura* was identified regularly by means of leg structure, but further work is being done in this regard. One problem is that the caudal setae do not resemble *Epischura lacustris* in the experience of the author, and the bodies did not exhibit the characteristic bend at the urosome. Consequently, the designation of *Epischura* is tentative, and could be in error. This problem will be attended to in greater detail in the summer of 2006, and any information will be submitted as an addendum to this report.

Some species of the cladoceran *Daphnia* are difficult to distinguish, and are often considered variants or hybrids. *D. pulex* and *D. pulicaria* were combined for analysis for this reason. *D. schodleri* and *D. catawba* were distinguished, but may be variants of each other or *D. pulex*, a phenomenon that is gaining more interest (Spaak et al 2004). The diversity of *Daphnia* species is interesting and important, but for much of our study the distinctions should be taken in the context of the current state of cladoceran taxonomy and biology. Of particular note, however, is that *D. middendorffiana*, a keystone species in environmental quality assessments (sensitive to bottom-up and top-down disturbances) was found in Green Lake only. The identification of this species was made with a high degree of certainty. All other identifications not noted here are made with confidence. A disk containing reference images accompanies this report.

3.2. A priori T-Tests on fish presence and total zooplankton abundance.

Total zooplankton abundance (individuals per m³) was compared between lakes with fish and without fish using a two sample T-test on log (x+1) transformed data. Bonferroni-adjusted probabilities were chosen as the p-value for these tests because multiple tests were performed. Pooled or non pooled variance was used as appropriate. Results are summarized in Table 4, and the mean abundance comparison between fish and fishless lakes are set out in Figure 8. There was no significant difference in the mean number of zooplankton individuals between lakes with and without fish (Bonferroni-corrected p = 0.634), nor was there a significant difference in taxa richness (Bonferroni-corrected p = 0.399).

3.3. A priori T-Tests on fish presence and zooplankton biomass.

Differences between lakes with and without fish were tested on the biomass of all zooplankton, all copepods, *Diaptomus shoshone*, all cladocerans, *Daphnia* species, and all rotifers. Biomass was measured in $\mu\text{g/L}$. Again, a two-sample T-test was used on log+1 transformed data and p values were Bonferroni corrected because multiple tests were run simultaneously. Results are summarized in Table 4 and means comparing the two fish-states are shown in Figure 8. Overall biomass weighted abundance was significantly lower in lakes with fish (Bonferroni-corrected $p < 0.001$), as was Cladoceran biomass (Bonferroni-corrected $p = 0.014$), *Daphnia* species biomass (Bonferroni-corrected $p = 0.006$), total copepod biomass (Bonferroni corrected $p = 0.001$), *Diaptomus shoshone* biomass ($p < 0.001$). Rotifer biomass was not significantly different between the two types of lakes (Bonferroni-corrected $p = 0.287$).

3.4. Redundancy analyses and fish effects on zooplankton communities: community composition and abundance ($\#/\text{m}^3$).

Overall effects of fish and other environmental variables on community composition were tested using redundancy analysis. Redundancy analysis is a form of constrained ordination that measures the amount of variation in community data explained by different environmental factors. This form of analysis is one of the more common and robust ways of testing the responses of communities composed of multiple species to multiple environmental gradients (Legendre 1998, Legendre et al 1999, Lepš et al 2003). We used mean species abundances of each lake as community variables (dependent variables). Environmental gradients were fish presence/absence, chlorophyll-a concentration (as a proxy for productivity), temperature, conductivity, lake size and elevation. The two axis of the redundancy plot that are tested for relation to the environmental gradients in a

correlation matrix that measures independent correlation of each environmental gradient with the axis. The x-axis correlates with elevation (-0.3449), temperature (0.7035) and conductivity (0.6719). The y-axis correlates strongly with fish presence (-0.6097) and weakly with chlorophyll *a* concentrations (-0.2727).

The ordination plot explains a significant amount of the species-environment relationship (for Monte-Carlo permutation tests of the first ordination axis, F ratio = 6.790, p-value = 0.0050 (Figure 2)). The eigenvalue for the x axis was $\lambda = 0.221$, and for the y axis $\lambda = 0.048$. This ordination explains 35.6% of the variation in species composition and abundance. Fish presence (y-axis) explains 13.5% of the variation in this ordination, while other environmental gradients (x-axis) explain 58.4%. The slight correlation between fish absence and chlorophyll-a concentrations is due to the number of fishless lakes that are smaller, warmer, and more productive (e.g., Embryo, Chipmunk). The inverse relationship between the elevation gradient and conductivity and temperature is to be expected in alpine systems.

3.5. Redundancy analyses and fish effects on zooplankton communities: biomass-weighted community composition.

Redundancy analysis was also used to analyze biomass-weighted zooplankton community composition, measured as mean species abundance per lake multiplied by the mean biomass of that species as measured from that lake. Two ordination diagrams were constructed. The first shows individual species responses to environmental variables (Figure 3), while the second shows differences in zooplankton communities by lake (Figure 4). The former diagram shows how variance in individual species biomass is explained by the ordination plot, and the latter shows how variance in biomass weighted community composition for each study lake is explained by the ordination plot.

The ordination explains a significant amount of the zooplankton species-environment relationship (for Monte-Carlo permutation tests of the first ordination axis, F ratio=6.311, p=0.0050). The x axis in this ordination is most correlated with fish presence (-0.7011), temperature (0.6720) and conductivity (0.5648). The y-axis is most correlated with chlorophyll a concentration (-0.3630) and elevation (0.4690). The eigenvalue for the x axis was $\lambda = 0.208$, and for the y axis $\lambda = 0.076$. The ordination explained a total of 38% of the variation in zooplankton species-environment relationships. Of this, 54.6% is explained by the x axis (which most strongly correlates with fish presence), and 41.2 % is explained by the y axis, which correlates most strongly with the environmental gradients related to elevation..

3.6. Detecting effects of fish removal and assessing recovery.

Some lakes in the Park were fishless, stocked, and subsequent to stocking cessation the fish were naturally extirpated. To test if these lakes have reverted to pre-fish stocking zooplankton communities is beyond the powers of this survey (see below). However, we did test to see if the zooplankton of these lakes were more like fishless lakes or lakes with fish. No significant differences were detected between recovering lakes and lakes that never had fish. We examined these differences with a principle component analysis ordination of biomass-weighted zooplankton community data (Figure 5). The x-axis separates fish/fishless lakes ($\lambda = 0.227$) while the y axis represents the environmental variables ($\lambda = 0.120$). Lakes recovering from fish presence align clearly with fishless lakes, and there is a distinct difference between lakes with and without fish. Notable exceptions are the highly productive Bear and Pear lakes that contain fish and very robust zooplankton communities that are like those of fishless lakes.

4. Discussion.

Results from previous studies of fish effects on zooplankton of alpine lakes are variable, ranging from finding no effects to local extirpation of key zooplankton species and significant changes in community composition. It is no surprise that these accounts show such a variety, primarily because trophic effects and bottom-up impacts on zooplankton are extremely complex, and the zooplankton themselves interact in elaborate ways. In this context, it is all the more important that the results of this study are so clear.

There was no difference between fish presence and absence on the zooplankton taxa richness or abundance. However, when biomass weighted abundance and community composition are considered, the results are very clear. Lakes with fish had significantly lower overall zooplankton biomass. Differences in overall zooplankton biomass, but not mean diversity or abundance, are explained by differences in community structure in the two types of lakes. Lakes with fish tend to have a diverse population of small rotifers and very few cladocerans or copepods. On the other hand, lakes without fish support a greater diversity, abundance, and most importantly, greater biomass of cladocerans and copepods.

In other words, *size* matters- the changes in biomass are extremely distinct while changes in richness or abundance are not (given the resolution of the study), and *kind* matters because shifts in zooplankton community caused by fish introductions are quite dramatic. Essentially, fish additions represented a replacement of the top predators of the trophic system, and the consequences of this shift for nutrient cycling and other ecological functions of the lakes remain to be explored.

4.1. The target of the inferences made from these data.

It is important to address the target of the inferences we are making in this study. The entire study design was meant for a rapid yet comprehensive assessment. The variability of zooplankton populations both spatially and temporally is a major concern in studies of zooplankton. In order to get an objective representation of the zooplankton in a *specific* lake requires intensive sampling, and even then such sampling must be done frequently over long periods of time to capture the true variability, composition, phenology and dynamics of the zooplankton community.

Many traditional and contemporary studies of zooplankton, however, rely on a single vertical tow at the deepest point of the lake. This is an economical method, but can underestimate the zooplankton diversity by up to 50% (Arnott et al 1998). In order to address this phenomenon but still attain our study goals, we increased the number of vertical tows and scaled that number to lake size. More importantly, the targets of our analysis are objective comparisons *between* lakes. The zooplankton numbers (diversity, abundance, biomass weighted abundance) are as robust as possible, but in effect they should be understood as indices. The targets of our inferences are explanatory factors that account for differences between lakes using the variance in the zooplankton community measures. It would not be unprecedented to use our results as a measure of what is indeed in many similar studies. Nonetheless, individual lake zooplankton community properties are not the intended target of the inferences made here, which are directed towards differences between lakes.

4.2. Effects of fish on zooplankton species, abundance and biomass.

A summary of zooplankton taxa present in each lake is presented in Table 3, adding a good number of species to current rosters of zooplankton found in Park lakes. It is also noteworthy that RMNP contains a diverse array of zooplankton communities in its lakes, and we can explain much

of this diversity with our results. As expected, the elevation gradient against conductivity, temperature, and productivity is a key determinant of zooplankton community structure, and this is shown in all of our plots. Fish presence, however, is at least equally if not more important (if biomass is considered) for explaining differences in zooplankton communities between lakes.

There was no significant difference in mean abundance (number of individuals) or mean diversity between lakes with and without fish (Table 2, Figure 8). However, mean biomass was significantly lower in lakes that had fish than in fishless lakes. Total number of individuals of zooplankton can be similar but the smaller rotifers are more abundant in the presence of fish than bigger species of crustaceans. Breaking this down a bit, the mean rotifer biomass was not lower in lakes with fish, while the mean biomass of larger crustaceans (Cladocera. and *Diaptomus shoshone*) were significantly lower in lakes containing fish.

These results are striking. Fish presence increases rotifer diversity, while knocking down or eliminating populations of larger crustaceans (Copepoda and Cladocera), which are either eliminated from the lake, present in suppressed numbers, or kept at a small size (early instar) because of predation pressures. Lakes with fish have communities of zooplankton that with more smaller rotifers and lower numbers of larger zooplankton. This shift in zooplankton community structure is best seen on the redundancy analysis plots.

Figure 2 shows how individual species abundances vary with the gradients we tested. The species found in the presence of fish (bottom left) are all rotifers, while the species that cluster toward the fishless end of the plot (top right) are all larger crustaceans. Fish clearly have a significant effect on the populations in these lakes, contra other studies that found only elevation matters (Winder et al 2001). Smaller crustaceans, particularly cyclopoid copepods, are found primarily in the more productive, lower elevation lakes. One outlier is *Daphnia middendorffiana*,

found only in Green Lake during our survey, so it shows a strong preference for high elevation. This species is also sensitive to fish predation and other environmental changes, serving as an ‘indicator species’ (Havas et al 1983).

A similar pattern is shown when species abundances are weighted by lake-specific biomass measurements for each species (Figure 3). Both plots explain a good deal of total variation in zooplankton abundance or biomass (35.6% and 38%, respectively). The differences between the two plots, however, are telling. In the abundance plot, the fish-fishless axis explains only 13.5% of the variation that the plot captures. If lake specific biomasses of each taxa are considered, however, the fish-fishless axis explains 54.6% of the variation treated by the plot. Clearly, fish presence does affect the zooplankton community composition, but the effect on size is a critical factor. We strongly recommend that any future work on zooplankton in RMNP use biomass calculations in order to detect these effects. Because zooplankton are, in general, size-selective omnivores, biomass matters to the role they play in ecosystem processes. Shifting a community from large crustaceans to small rotifers changes the function of the zooplankton in the overall ecosystem context. The exact nature of these changes in Park lakes remain to be studied.

Our analysis of the overall biomass-weighted zooplankton community composition of each lake makes the effects of fish even more obvious (Figure 4). This analysis is the same as that shown in Figure 3, but across another dimension showing lakes rather than individual species. The spread of lakes across the environmental gradients shows that RMNP contains a diverse variety of zooplankton communities along the expected elevation/conductivity, temperature, and productivity gradient. What is most striking is the distinct gap between the zooplankton communities in lakes with and without fish, which are well separated in this plot. In both lakes with and without fish, there

is a range across the elevation-productivity gradient, but the fish status of the lakes show two distinct groups.

4.3. Fish removal and zooplankton recovery.

It is difficult to assess the rates or extent of zooplankton recovery from fish introductions without a fish removal experiment. A key problem is the lack of information about what was in the lake before fish introduction. Without this knowledge, it is unclear if any zooplankton have been locally extirpated. We can say, however, that zooplankton communities in lakes after fish removal fall very much more in line with communities in fishless lakes than those in lakes with fish (Figure 5). In order to truly address this question, either pre-fish introduction data would be needed, or an experimental removal combined with frequent monitoring would be required. Furthermore, we have no good information on fish density and its effects on lake zooplankton communities. We can say with some confidence, however, that the zooplankton communities in lakes that are recovering from fish removal are clearly more similar to fishless lakes in our analysis, a positive indication that of patterns of recovery. Individual lake systems and individual species recovery cannot be assessed at this time..

4.4. Lakes of special importance identified by this survey.

The grouping of lakes in Figure 5 also shows some interesting exceptions to the patterns we have found in this survey. Notably, Bear and Pear lakes have very diverse (and not just within the rotifers) and somewhat larger zooplankton populations than other lakes with fish. This effect is surprising and we cannot address the underlying reasons in this survey, other than the somewhat lower elevations of these lakes. Other possible sources of these differences could be catchment size, type and abundance

of macrophytes, retention time and other factors affecting productivity. Bear Lake had the highest overall zooplankton diversity of all the lakes (10.33).

Another notable lake is Bluebird Lake. The zooplankton in Bluebird were the largest found in the survey, remarkably so for such a high elevation lake. Bluebird has an impressive natural barrier to fish introduction, and we would strongly recommend that the Park continue to maintain its fishless status.

Finally, Green Lake was the only lake in which we found *Daphnia middendorffiana*. This species is sensitive to fish predation and is also sensitive to other environmental changes such as acidification or other changes in water chemistry. In some contexts, this species is also surprisingly robust. Nonetheless, we expected to find this species in more lakes. It is very possible that our short sampling burst was not sufficient to capture all species, but on the other hand when it was present (as in Green Lake), *D. middendorffiana* was relatively abundant and dominated that system. This suggests that the high elevation lakes, while the least productive, still support interesting and important zooplankton populations.

4.5. Management implications.

The work we have done in this report successfully identifies key differences in zooplankton communities between lakes with and without fish. It also suggests that lakes from which fish had been extirpated do recover, or at least look much like the zooplankton in fishless lakes. In addition to this information, our conclusions have several management implications that are worth summarizing:

- 1) In future surveys of zooplankton, patchiness must be accounted for. At least 3 vertical tows seem necessary, and we hope to analyze the effects of using between 5 and 10 vertical tows. Obviously this drastically increases sample acquisition and processing times but also increases the value of the data obtained.
- 2) Our survey was meant to compare lakes at peak zooplankton activity, particularly for the crustaceans. This is a good start, but a complete understanding of these communities and the effects of fish introductions or removal will require longer term analysis across seasons and over several years.
- 3) Measuring biomass is extremely important for detecting changes in zooplankton communities due to fish introductions. Changes in biomass and species composition alone were significant even when diversity was not significantly different between lake types. Furthermore, our tests for shifts in community structure were more robust when we looked at biomass weighted abundance data. Simply checking taxa diversity or numbers of individuals does not give the complete picture or can lead to weaker analyses.
- 4) A more complete survey of higher elevation lakes may show *D. middendorffiana* is more common than we found it to be. The value of this species as an indicator remains to be explored, and could be valuable information to RMNP managers and scientists.
- 5) Any future removal in fish populations should most definitely be preceded and followed by an intensive look at the zooplankton community. This would be invaluable information for Park scientists and managers and constitute a major contribution to scientific understanding of alpine systems in general.
- 6) Fish management decisions that may place native fish in fishless lakes for storage during removal of non-natives from streams is strongly discouraged. Lakes like Bluebird Lake would be

dramatically changed by fish introduction. The effects of the changes in zooplankton community on other aspects of water quality (e.g., clarity) are unknown at this point.

- 7) It would greatly improve the precision of scientific answers to management questions if Park lakes were studied long-term. Important information that would be useful include retention times (important for life cycles of species), ice-cover period effects on phenology of biological communities, water chemistry monitoring (nutrients and algal pigments), fish species and densities, and macrophyte species and densities. The changes in these features over time would allow scientific and management questions to be answered with greater confidence and clarity.
- 8) Several questions are still unanswered. First, the difference between native and non-native fishes remains to be explored. Because most trout have similar feeding behavior, we would not expect much raw difference with respect to effects on zooplankton, but different habits and survival rates could have important consequences for their effects on the system as a whole. Secondly, the effects of fish density remain unexplored. As sport fishing is encouraged as a removal technique for non-native fish, changes (presumably reductions) in density offer a unique opportunity to further understand the lakes of RMNP.

4.6. Future products.

This research has been presented to scientists, the public, and the staff of RMNP at the Rocky Mountain National Park Research Conference (Lafrancois 2006a) . A more complete scientific account has been accepted for presentation at the Ecological Society of America 91st Annual Meeting (Lafrancois 2006b). This report has also been submitted to Park scientists and managers, along with a compact disk containing some digital microscope pictures of key zooplankton

identifying attributes. Due to technical difficulties and lack of time, scale stamps were not available for most of these pictures, but in unlabelled shots the field of view is uniformly 650 micrometers wide. A Powerpoint presentation has been included that outlines the various zooplankton groups, and was intended for children ages 6-10, but is suitable for adults.

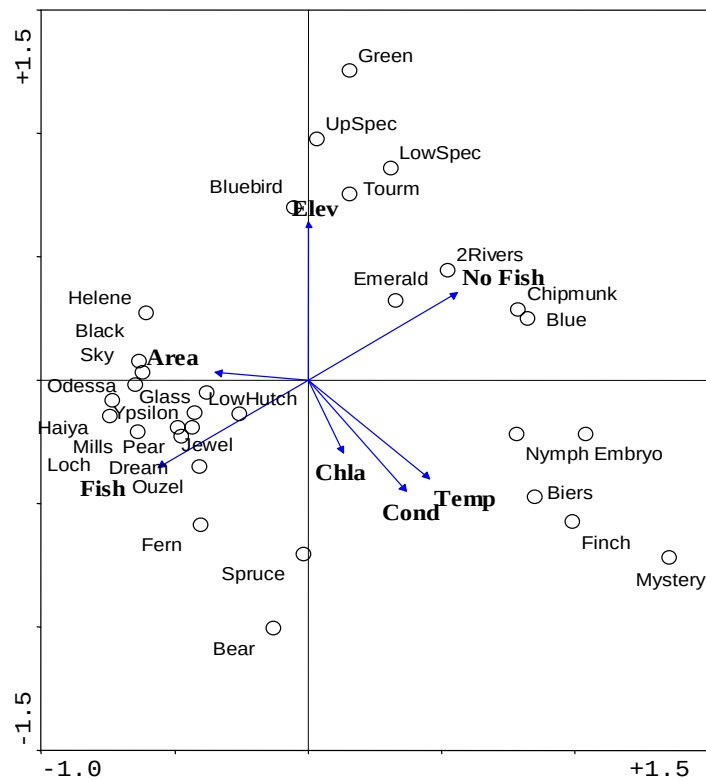
Future products planned this summer include a set of digital pictures of zooplankton taken on a dissecting scope, allowing a full view of the various taxa more suitable for public interest presentations. Two papers are being written based on this work, one for submission to a scientific journal and one intended for Park Science, both expected to be submitted by August 2006.

5. Conclusion.

Our brief but intensive survey has shown that RMNP contains a very diverse range of zooplankton communities in its lakes. As might be expected by the predominance of these effects in the literature, zooplankton communities were affected by elevation, temperature, conductivity, chlorophyll a concentrations, and fish presence or absence. The former environmental features explain more community variation if only abundance is measured, but the latter explains more if biomass is considered. Our work shows that fish presence drives zooplankton biomass down and changes species composition, but does not significantly affect overall diversity or mean numbers of individuals. These effects are largely attributable to changes in zooplankton community structure, as fish presence strongly favors a diverse community of smaller rotifers and fish absence allows the presence of larger crustaceans. With some qualification, we can say that zooplankton communities from the lakes where fish have been extirpated since stocking cessation in 1968 are much more like fishless lakes. It is difficult to assess recovery rates or expectations without further experimentation.

6. Figures.

Figure 1. Principle component analysis plot of lake grouping along environmental gradients. The plot shows lakes sampled from Rocky Mountain National Park east of the Continental Divide across lake size (acres of area), elevation, temperature, chlorophyll-a concentration, conductivity, and fish presence. Notice that elevation forms a gradient against productivity (indicated by chlorophyll a concentration), temperature, and conductivity. This plot is meant to show the range of lake types used in the analysis of zooplankton populations.



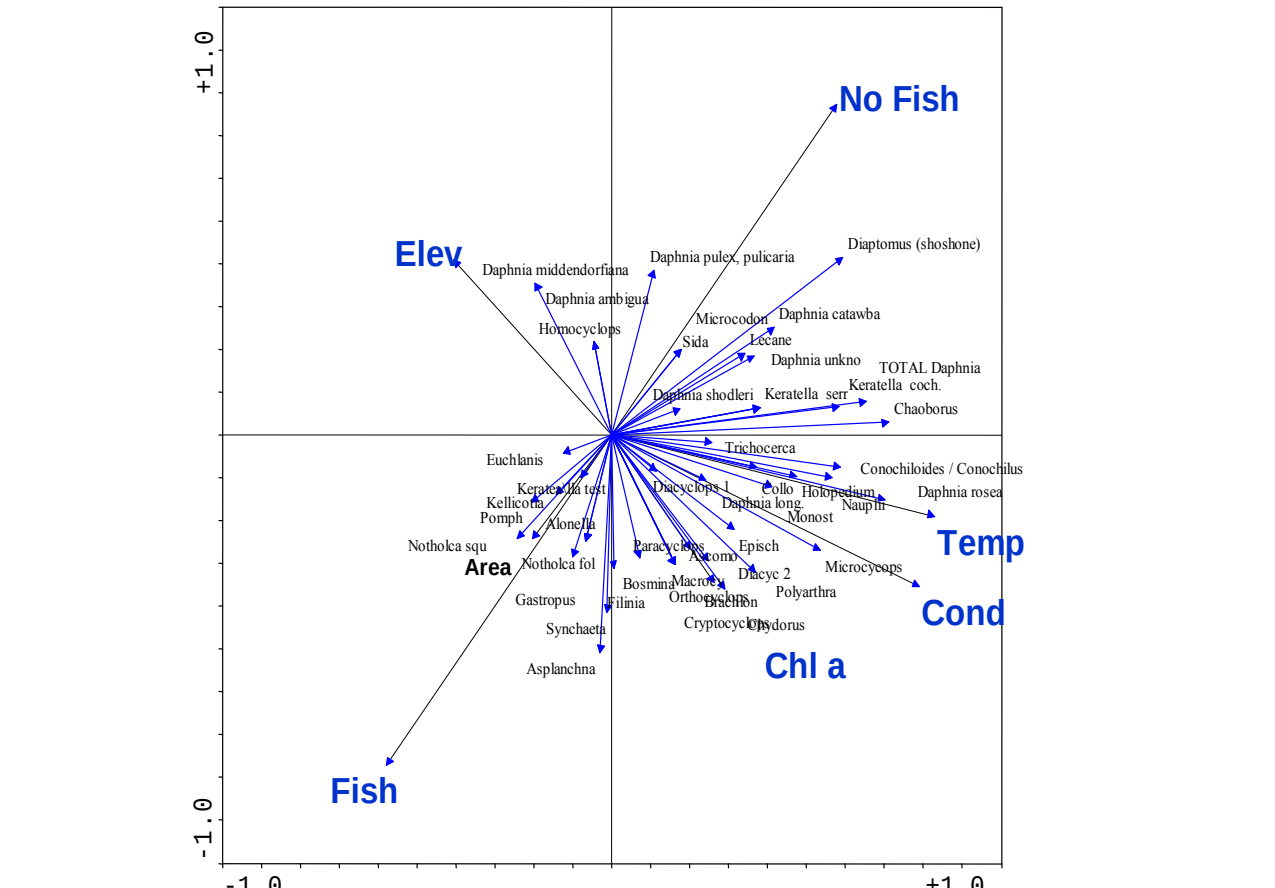


Figure 3. Redundancy plot of zooplankton biomass ($\mu\text{g}/\text{m}^3$) along environmental gradients and fish presence/absence from lakes samples in Rocky Mountain National Park 15-25 July, 2005. $F = 6.311$, $p = 0.005$. Fish presence/absence explains 54.6% of the variation of biomass-weighted zooplankton composition while the environmental gradients explain 41.2%; total of all variance explained is 38.0%.

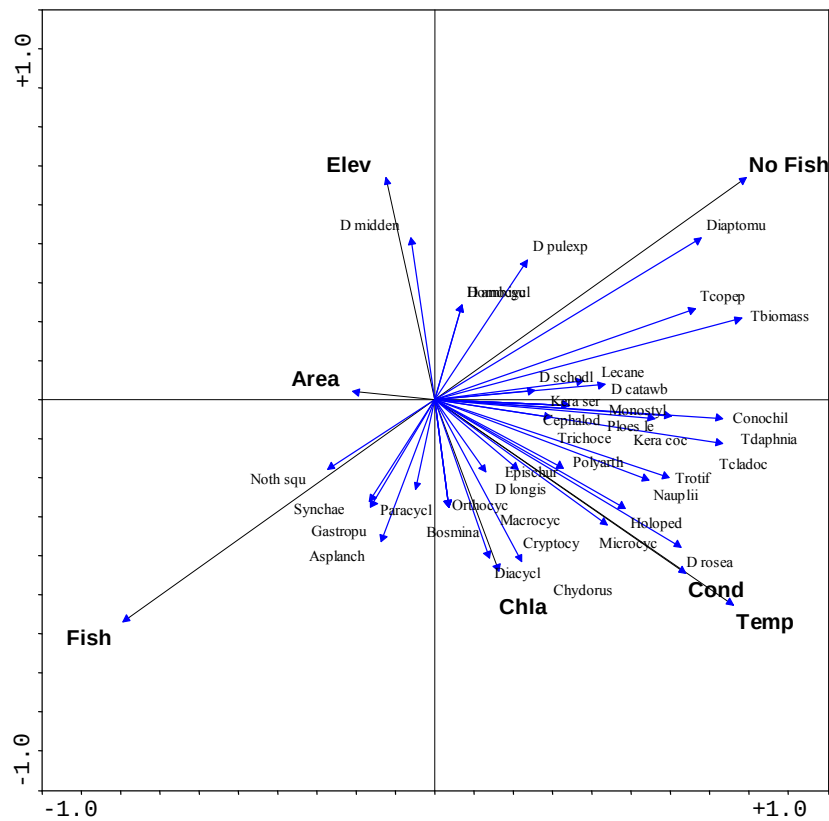


Figure 4. Redundancy plot of zooplankton biomass ($\mu\text{g}/\text{m}^3$) along environmental gradients and fish presence/absence from lakes samples in Rocky Mountain National Park 15-25 July, 2005. Lakes are shown along gradients based on the analysis shown for individual species in Figure X-1. Zooplankton communities of the lakes ranged across the elevation versus temperature - conductivity - productivity gradient, but are distinctly different based on fish presence (as above, fish status explains 54.6% of the variance of zooplankton composition and biomass shown in this plot, while the environmental gradients explain 41.2%; total of all variance explained is 38.0%; $F = 6.311$, $p = 0.005$).

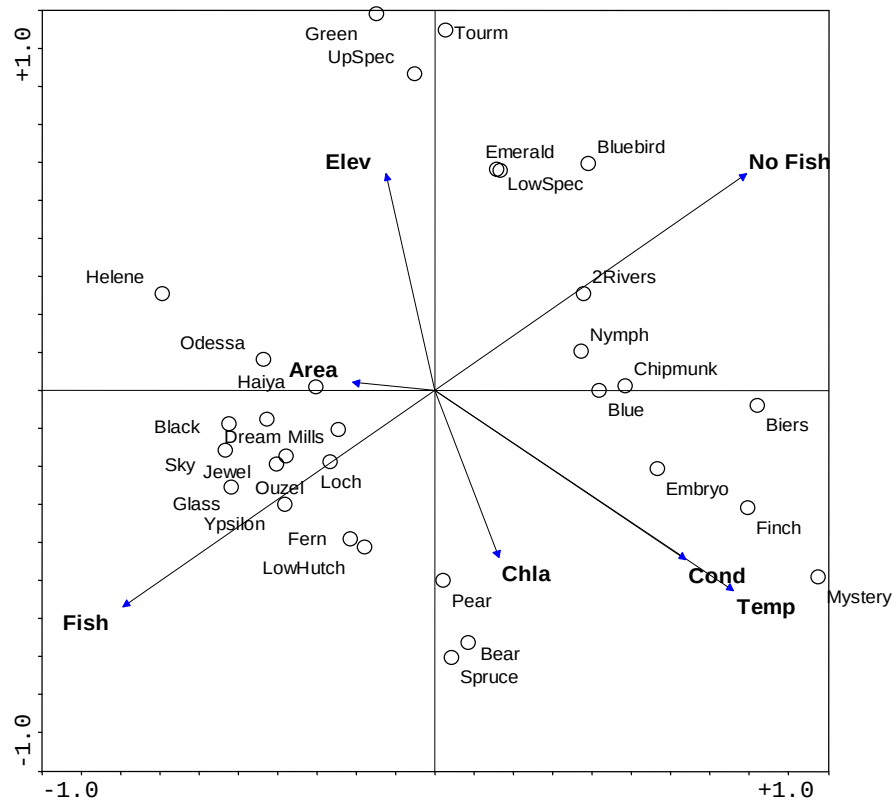


Figure 5. Principle component analysis of biomass weighted zooplankton community composition of lakes in Rocky Mountain National Park surveyed 15-25 July 2005. Lakes with a solid circle contain fish, empty circles show fishless lakes, and empty triangle indicate lakes that were stocked, but fish died out subsequent to the 1968 cessation of stocking. Bluebird, Finch, Emerald, and Bierstadt are lakes that were stocked, but fish died out since stocking cessation. These lakes have a zooplankton biomass weighted community that is similar to the fishless group. (x-axis = fish presence/absence, $\lambda = 0.227$, y-axis = elevation gradient against temperature, conductivity, and chlorophyll a concentration, $\lambda = 0.120$).

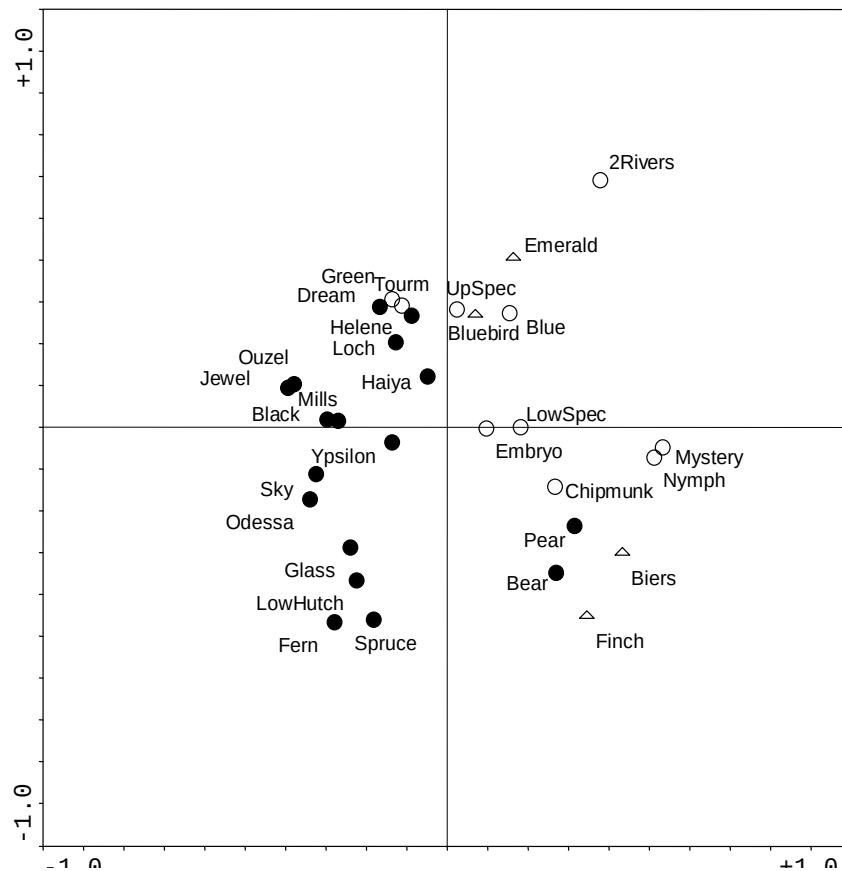


Figure 6. Accessing the lakes of Rocky Mountain National Park was achieved by hiking in with an inflatable raft. Zooplankton were sampled with a Wisconsin Tow net using a vertical tow method from 15-25 July 2005. Pictured are Toben Lafrançois and Cindy Glase, photo by Brenda Moraska Lafrançois.



Figure 7. Zooplankton collected from lakes in Rocky Mountain National Park 15-25 July 2005 were rinsed from the net into whirlpaks for transport to the base camp, where they were transferred into 20 mL scintillation vials. Size and abundance differences between lakes are apparent even at this scale (but with caution since some of the tows were longer than others, so the samples represented here consist of concentrations from different volumes).

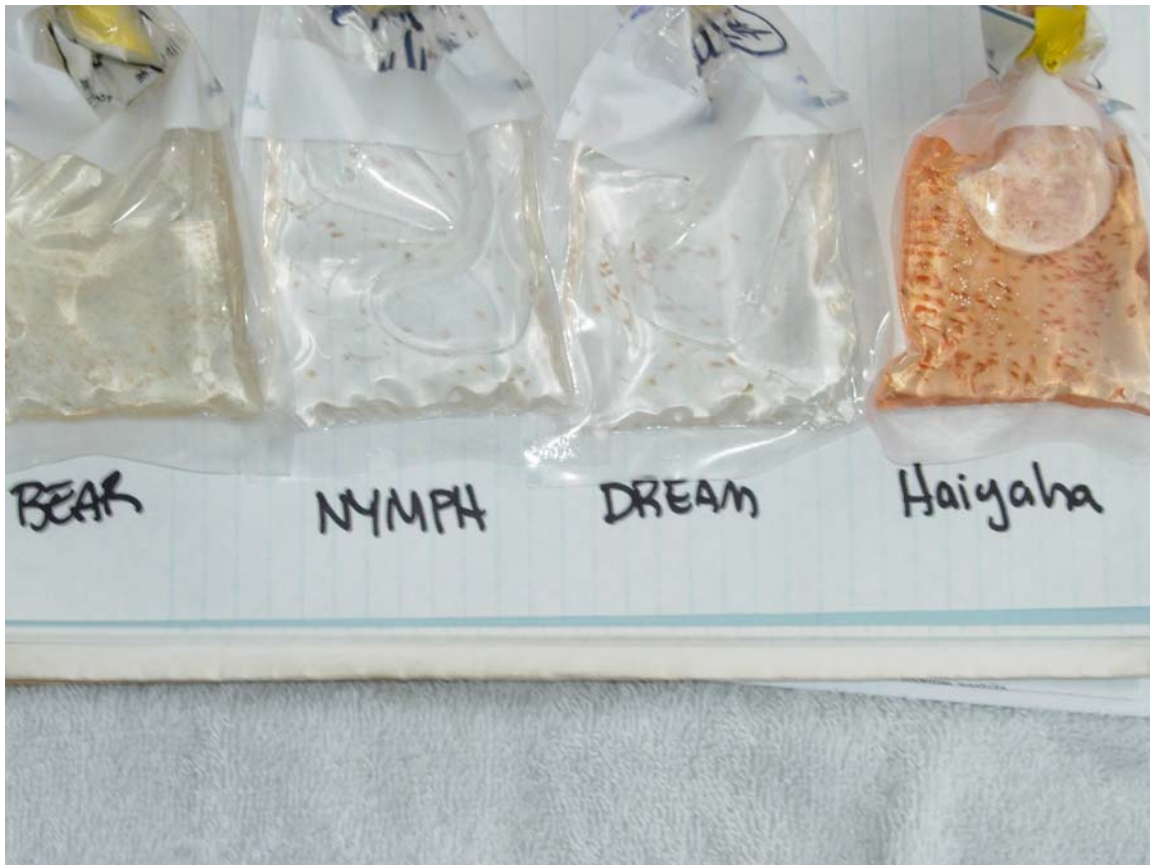
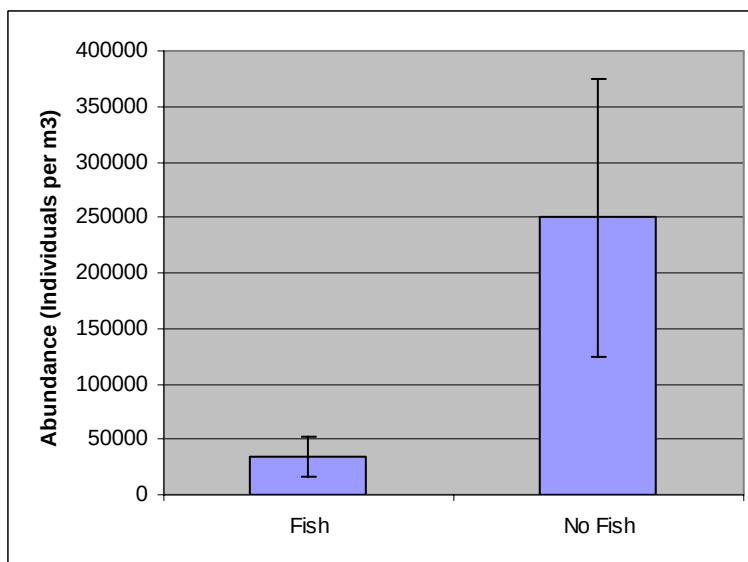
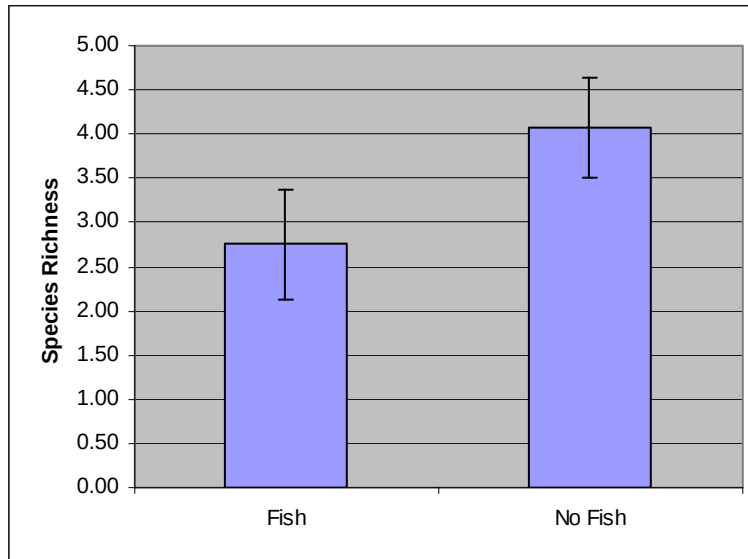
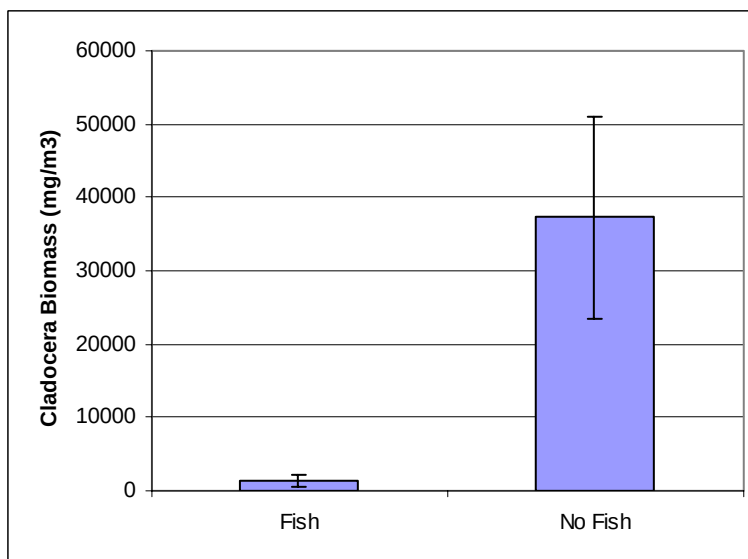
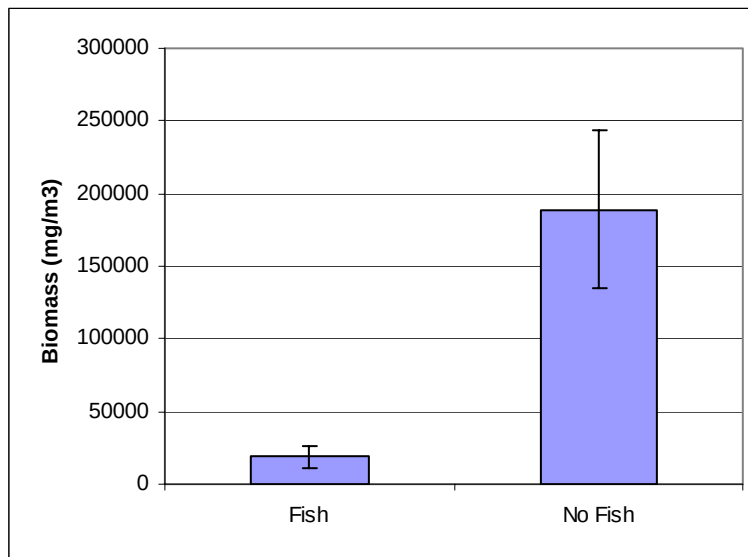


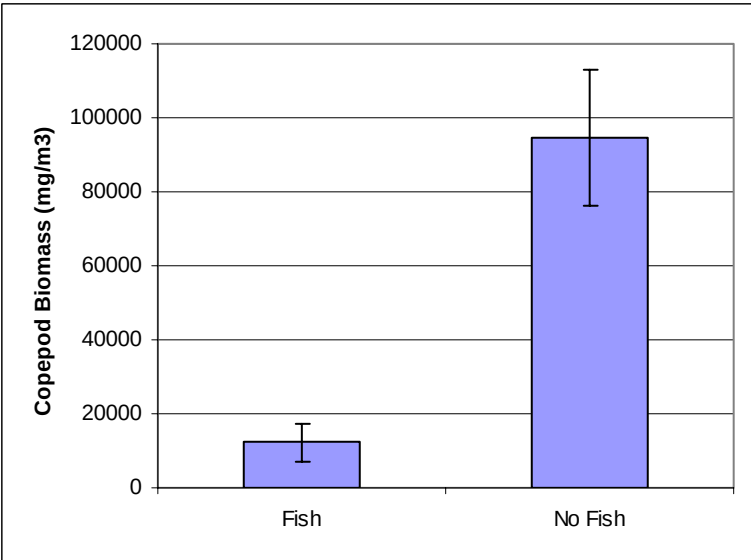
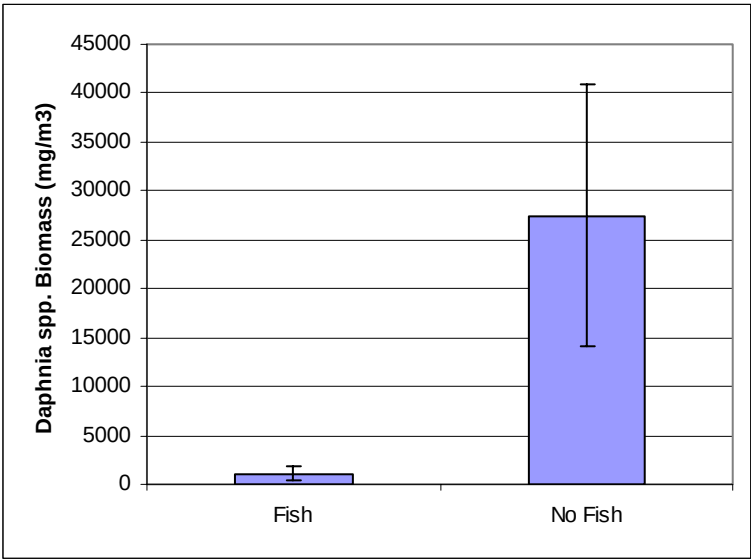
Figure 8. Differences in mean zooplankton community measures and key species biomass means between lakes with and without fish from lakes sampled in Rocky Mountain National Park 15-25 July, 2005. Comparisons with white backgrounds are significantly different (Bonferroni corrected $p \leq 0.05$), those in grey are not (Bonferroni corrected $p > 0.05$). P-values of the relevant statistical tests are summarized in Table 4.



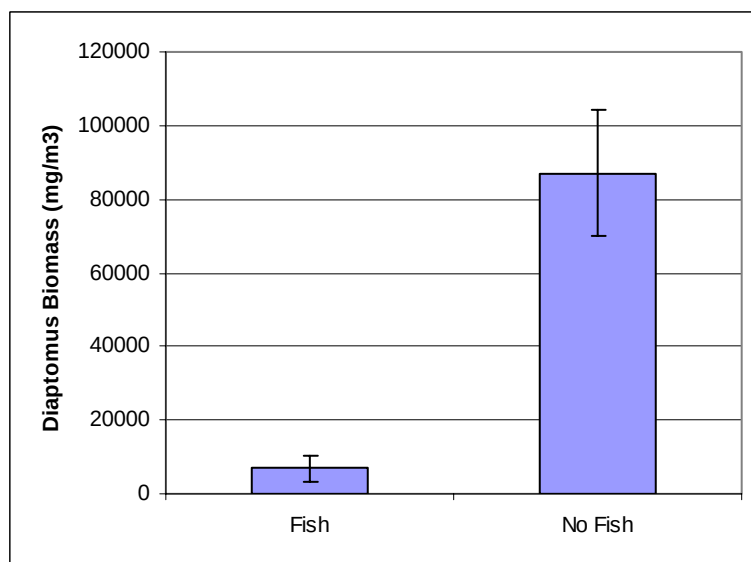
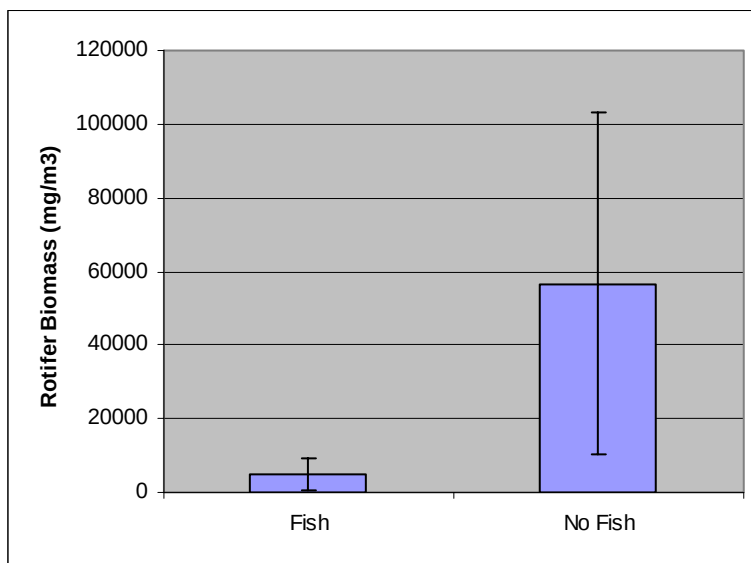
(Figure 8 cont'd)



(Figure 8, cont'd)



(Figure 8, cont'd)



7. Tables.

Table 1. Lake list and environmental data from lakes in Rocky Mountain National Park taken during the survey period 15-25 July 2005. Lakes listed first currently have fish, lakes that had introduced fish populations that have died out are listed next, and finally fishless lakes. Fish presence has a value of 1 if fish are present, 0 if not. Fish recovery status values are similar, with 2 indicating a lake that was stocked but the fish died out after stocking cessation.

Lake:	Fish presence:	Fish recovery	Elevation (ft)	Area (acres)	Temp (°C)	Conductivity (µs/cm2)	Chl a (µg/l)	Taxa richness:
Bear	1	1	9475	11.2	13.2	22.9	2.371	10.33
Black	1	1	10620	9.2	8.3	6.8	2.261	1.33
Dream	1	1	9900	5.5	9.3	11.1	1.052	2
Fern	1	1	9530	9.2	12.7	10.2	2.134	3
Glass	1	1	10820	4.6	8.8	10.9	2.817	1
Haiyaha	1	1	10230	15.6	7.1	14.2	0.997	2.17
Helene	1	1	10580	2.8	5.3	10.3	0.377	0.33
Jewel	1	1	9950	4.6	12	8	0.882	1.83
Loch	1	1	10180	14.6	9.5	11.5	1.834	1.8
Lower Hutchinson	1	1	10852	7.4	12.5	16.8	1.317	3.8
Mills	1	1	9940	15.6	10.6	8.2	1.019	2.67
Odessa	1	1	10020	8.4	8.6	9.6	0.267	1.17
Ouzel	1	1	10010	6.4	8.7	13.5	2.044	0.5
Pear	1	1	10582	16.5	15	12.4	1.421	5.67
Spruce	1	1	9670	3.7	18.8	14.1	0.978	1.8
Sky	1	1	10900	11.2	6.4	10	3.375	1.17
Ypsilon	1	1	10540	7.4	10.8	9.8	2.31	6.17
Bierstadt	0	2	9416	7.4	18	22.5	0.787	7.83
Bluebird	0	2	10978	22	9.1	11.2	1.559	3.17
Emerald	0	2	10080	6.3	10	9.4	1.556	4
Finch	0	2	9912	7.4	17.7	23.7	3.065	5.17
Blue	0	0	11140	2.8	15.3	16.3	2.64	1.67
Chipmunk	0	0	10660	1	19.9	8.8	1.203	5.33
Embryo	0	0	10360	0.1	18.2	18	2.706	2.83
Green	0	0	11550	3.1	6.3	7.7	0.075	1.5
Lower Spectacle	0	0	11350	7.4	10.5	10.1	1.082	5.33
Mystery	0	0	10200		19.6	33.3	2.213	7.17
Nymph	0	0	9700	0.9	15.1	13	2.496	5.83
Tourmaline	0	0	10590	6.8	5.9	12.4	0.072	1.17
Two Rivers	0	0	10600	5	16.8	6.9	1.052	3.83
Upper Spectacle	0	0	11360	11.2	5.1	8.5	2.01	2.25

Table 2. Equations used to estimate zooplankton biomass surveyed from Rocky Mountain National Park, 15-25 July 2005, and sources for the general equations.

Taxa	Formula	Reference(s)
Daphnia pulex	$W=e^{(1.4663+3.1932\ln L)}$ (mm, μ g)	McCauley 1984
Daphnia shoedleri, other Daphnia spp.	$W=e^{(2.30+3.10\ln L)}$ (mm, μ g)	McCauley 1984
Chydoridae and Cladocera	$W=e^{(1.7512+2.6530\ln L)}$ (mm, μ g)	Bottrell et al 1976
Calanoid copepods	$W=7.9 \times 10^{-7} L^{2.33}$ (μ m, μ g)	Dumont et al 1975
Diacyclops sp	$W=4.9 \times 10^{-8} L^{2.75}$ (μ m, μ g)	Dumont et al 1975
Holopedium gibberum	$W=e^{(5.3976+2.0555\ln L)}$ (mm, μ g)	McCauley 1984
Copepod nauplii	$W=e^{(.6977+.469\ln L)}$ (mm, μ g)	McCauley 1984
Cyclopoid copepods	$W=4.9 \times 10^{-8} L^{2.75}$ (μ m, μ g)	Dumont et al 1975
Rotifers	(ellipsoid volume converted to dry mass)	Doohan 1973, Pace et al 1981.

Table 3. Zooplankton species present in a survey of lakes east of the Continental Divide in Rocky Mountain National Park, 15-25 July 2005.

	Bear	Chipmunk	Ypsilon	LowSpec	UpSpec	Haiya	Dream	Emerald	Nymph	Mills	Jewel	Black	Blue	Green	Biers	LowHutch	Bluebird	Ouzel	Mystery	Glass	Loch	Sky	Pear	Finch	Spruce	Fern	Odessa	Helene	Tourn	2Rivers	Embryo
Alonella sp.																X															
Ascomorpha sp.	X		X			X									X																
Asplanchna sp.	X															X						X	X								
Bosmina longirostrus	X																														
Brachionus sp.	X																														
Chaoborus (albicus) flavicans									X						X				X					X							
Cephalodella sp.															X																
Collotheca pelagica	X		X	X					X										X		X			X							
Conochiloides/Conochilus spp.	X	X	X	X	X		X	X	X	X	X		X		X	X	X		X		X		X	X	X	X		X		X	
Chydorus sp.											X								X				X		X						
Cryptocyclops sp.	X																														
TOTAL Daphnia species	X	X		X				X	X				X	X	X	X	X		X	X			X	X	X	X	X			X	
Daphnia ambigua				X										X	X	X	X			X	X		X	X	X	X	X				X
Daphnia catawba								X	X										X												X
Daphnia longispinus	X								X																						
Daphnia pulex-pulicaria								X									X														X
Daphnia rosea	X	X		X				X							X	X	X		X	X			X	X	X	X	X				X
Daphnia schoedleri				X													X						X								X
Daphnia middendorffiana														X			X						X								
Daphnia (unidentifiable)													X		X																X
Diacyclops sp1																								X		X	X				
Diacyclops sp2																							X	X	X	X					
Diaptomus shoshone*	X	X		X	X	X	X	X	X				X	X	X		X		X		X		X	X				X	X	X	X
Epischura sp.	X			X		X		X	X						X	X				X			X	X	X						
Euchlanis sp.											X										X		X	X	X						
Filinia terminalis	X		X																												
Gastropus sp.			X																				X			X					
Holopedium gibberum	X								X	X		X	X						X				X	X							X
Homocyclops ater				X																											
Keratella cochlearis	X	X	X	X	X		X	X	X	X	X				X	X	X	X	X	X	X	X	X	X					X		X
Keratella testudo	X		X					X																							
Keratella serrulata															X																
Kellicotia longispinus			X																												
Lecane sp.															X																X
Macrocyclus sp.	X																														
Microcodon sp.								X																							
Microcyclus sp.	X		X										X		X	X			X												
Monostyla sp. (see Lecane)	X														X																
nauplii	X	X	X	X	X	X		X	X	X		X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Notholca squamula											X					X						X									
Notholca folicea																X															
Orthocyclops modestus	X																														
Paracyclops sp.	X									X																					
Polyarthra sp.	X																						X	X							
Pompholyx sp.			X							X	X																				
Ploesoma lenticulare															X																
Sida sp.								X																							
Synchaeta sp.	X		X													X															
Trichocerca sp.			X												X																

Table 4. Bonferroni corrected p-values of *a priori* T-tests comparing zooplankton taxa and community measures between lakes with and without fish samples in July 2005 in Rocky Mountain National Park.

Test	Mean in lakes with fish	Mean in lakes without fish	Mean in lakes with fish (log x+1)	Mean in lakes without fish (log x+1)	T	Degrees of freedom	p value	Bonferroni adjusted p value
Taxa richness	2.749	4.077	1.15	1.53	2.046	29	0.05	0.499
Total abundance (individuals per m ³)	34,812	249,588	8.562	10.025	1.902	29	0.067	0.672
Total biomass (µg/ m ³)	18,638	37,251	8.250	11.710	6.195	23.7	<0.001	<0.001
Cladoceran abundance (individuals per m ³)	792	5,627	3.666	6.539	2.441	29	0.021	0.210
Cladoceran biomass (µg/ m ³)	1,398	37,251	2.650	8.244	3.440	29	0.002	0.018
Copepod abundance (individuals per m ³)	6,361	10,318	6.348	8.146	2.666	23.9	0.014	0.135
Copepod biomass (µg/ m ³)	12,327	95,646	7.169	11.202	4.954	18.1	<0.001	0.001
<i>Diaptomus</i> biomass (µg/ m ³)	6791	86,995	3.253	11.133	6.932	17	<0.001	<0.001
Rotifer abundance (individuals per m ³)	27,129	231,976	6.880	8.437	1.144	25.9	0.293	1.0
Rotifer biomass (µg/ m ³)	4,912	56,736	4.500	7.217	2.223	24	0.0361	0.359

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