DR. STEPHEN BROWN, Moderator

In the next presentation, Dr. Scott Cooper will discuss "Evaluation of Acid Deposition Effects on Sierra Nevada Aquatic Invertebrates and Fish".

5. Evaluation of Acid Deposition Effects on Sierra Nevada Aquatic Invertebrates and Fish. Dr. Scott Cooper, U.C. Santa Barbara.

DR. SCOTT COOPER, UC SANTA BARBARA

The basins in the high Sierra Nevada have a limited capacity to neutralize acids. Because these basins receive high amounts of precipitation, there have been concerns that the biota of lakes and streams there may be showing responses to acid deposition. We now know from research funded by the Air Resources Board that acid deposition does occur in the high Sierra Nevada. During times of snowmelt, pH in sensitive lakes is reduced to about 5.5 to 5.8 for periods as long as a week. Furthermore, during summer rainstorms, we often get titrations of sensitive lakes in which ANC goes to nearly zero. As John Stoddard just pointed out, there are also situations where very small water basins may have pH depressions to 5.4 for short periods of time. The question is, do these acid deposition events have any effects on the biota of lakes and streams in the Sierra Nevada, and secondly, if acid loading increases in the state of California, will that have an impact on these sensitive systems?

My research group and I have concentrated on invertebrates and fish in sensitive high Sierra Nevada systems. We have concentrated on fish because they are commercially important; there is an important sports fishery in the high Sierra Nevada. We have concentrated on aquatic invertebrates because they are the major food base for these fish. We have also studied invertebrates and fish because they are important indicators of environmental stress. We can use the species composition of invertebrate and fish assemblages to infer something about environmental stress and its effects on aquatic systems. In addition, when chemically monitoring a system, if you are not sampling frequently enough, acid deposition events may be missed. However, the biota integrates the effects of pulse chemistry. They live through those acid depressions, and immediate changes in chemistry may be reflected in long-term changes in the biota. We might not see these kinds of effects in discontinuous hydrochemical monitoring.

When we were confronted with the question of what are the current and potential effects of acid deposition on high Sierra Nevada lakes and streams, we chose to use a three-pronged approach (Fig. 1). First of all, we had a long-term monitoring program. John Melack has talked a bit about some of the hydrochemical monitoring program. In this particular case, the monitoring program involved choosing a sensitive system and then measuring various physical, chemical, and biological factors through time. We noted if there was any relationship between acid deposition events and changes in these parameters.

this system live anywhere from 6-10 years. It is only because of this longevity that they are able to maintain themselves in this system in this face of tremendous year-to-year variation in recruitment.

Some of our surveys included one-shot sampling of many systems throughout large areas (Fig. 10). We wanted to make sure that the systems we monitored were representative of other lakes and streams in the high Sierra Nevada. We can only determine that by evaluating the similarity of our study systems to other lakes and streams. This also gave us a regional assessment of resources, e.g. trout populations, throughout large areas of the Sierra Nevada. We can look for relationships between water chemistry of a large number of lakes and the biota, including species composition or the abundance of different species. Finally, we can use these kinds of data to come up with regional projections of how changes in acid loading might affect natural resources throughout large areas of the Sierra Nevada.

There are three surveys we conducted as part of our Emerald Lake studies. We sampled seven lakes in the Emerald Lake area over four years to find out if the species composition and abundance of different species changed from year to year (Fig. 11). In general, species composition was pretty stable. We found that the lakes have the same species composition from year to year even though absolute abundances varied depending on parameters like time of snowmelt.

In the second survey, we used the EPA E map procedure to select 30 lakes throughout the high Sierra Nevada (Fig. 12). We sampled one lake from each of those marked hexagons. Knowing how many lakes there are within each area and knowing what percentage of those areas we sampled, we then extrapolated from the sampling regime to calculate the number of lakes that contain each of the major fish species in the high Sierra Nevada. We sampled fish and invertebrates using the same techniques we used in monitoring. Most of the lakes had a pH greater than 6 (Fig. 13). There were two fairly acidic lakes. One lake had a pH of about 4.7, and the other had a pH of 5.2. Most of these lakes were located in the Bench Lake area of the eastern Sierra Nevada and eastern Kings Canyon National Park. The EPA has conducted a fairly comprehensive survey of lakes. They measured water chemistry in a lot of lakes throughout the western US, and they extrapolated to about 1400 lakes in the Sierra Nevada. We got very similar results to the EPA study (Fig. 14). In both cases over 80% of the lakes had ANC's of less than 200 micro equivalents per liter. These are very dilute lakes and very poorly buffered. We did not collect any trout or invertebrates from the two most acidic lakes, otherwise there seemed to be very little relationship between water chemistry and the biota (Fig. 15). Using the EPA's E map procedure, we found that golden and rainbow trout were the two species most frequently collected in lakes throughout the high Sierra Nevada (Table 2). The California Department of Fish and Game has carried out a very aggressive stocking program where they have introduced golden trout to many parts of the high Sierra Nevada.

David Bradford and Tom Jenkins, who carried out most of the sampling, discovered that there

downstream from Emerald Lake (Fig. 5). We also monitored fish populations in a number of other lakes in the Tokapah basin near Emerald Lake. By using a variety of techniques, such as electroshocking in the streams and netting and angling in the lakes we captured fish and marked them. We pumped their stomachs to find out what they were eating, and we sacrificed some of them to age them. We developed a fairly comprehensive data set on the life histories of the fish in the system. We know something about their population sizes from year to year (Table 1), their growth rates (Fig. 6), and their diets (Fig. 7) through an annual cycle.

We used mark-recapture techniques to determine population sizes, and in the stream, we also used depletion techniques. Brook trout is the only species of fish present in Emerald Lake and nearby lakes. Most of these lakes originally did not contain fish, but they were stocked with brook trout. They were also stocked with rainbow trout, but in many of these lakes, brook trout took over, and there are no rainbow trout in the system anymore. Brook trout reproduce naturally in these systems, and they are not stocked anymore. There is a lot of year-to-year variation in the numbers of young-of-the-year in the system but very little variation in the adult populations. Because most of the trout in this system enter the outlet stream to spawn, and because the literature shows that it is the early life history stages of trout, the eggs and the embryos, that are most sensitive to acid stress, we have paid particular attention to spawning and early life history stages in evaluating acid deposition effects. We monitored the entry of fish from both the lake and downstream ponds into the outlets for spawning and we have comprehensive data on spawning migrations (Fig. 8). We have also counted the number of nests made by spawning trout and counted the eggs in representative nests so that we have estimates of the number of eggs produced each year by these fish (Fig. 9). In addition, we have estimates of egg survivorship. We put eggs inside small mesh baskets and surround them with gravel, and then buried them into the substrate at the same depth that trout bury these eggs. Most of the brook trout in this system spawn in September or October. The eggs develop throughout the winter and the fry hatch from eggs usually between January and April. They stay in the gravel during the yolk sac stage. Once the yolk sac is absorbed, they emerge from gravel and become free living usually between April and June. In the winter time we had trouble finding the stream, let alone finding the baskets, and sometimes we had to dig through 8 meters of snowpack simply to get to the stream and dig up egg baskets to measure egg survivorship. There was a lot of year-to-year variation in young-of-the-year densities in this system even though adult populations stayed fairly constant. These high altitude populations of young seem to be prone to catastrophes. In one winter, an avalanche hit Emerald Lake. It acted as a piston forcing tremendous amounts of water through the outlet. The whole outlet was scoured, and all the eggs were washed away. In another year, a drought year, the water was very low, and the interstitial zones froze, and all the eggs in the outlet died by freezing. In another year, the outlet stream dried up, then the eggs died of desiccation. We think that the adults in

because of their artificiality. They divorce an organism from its natural environment and from the other species with which they normally interact and make it very difficult to create the same climate conditions and water chemistries found in the real world. In our experiments, we deployed plastic bags in Emerald Lake. We acidified some of these bags, and left others as unacidified controls (Table 3). We then monitored the responses of phytoplankton and zooplankton to these perturbations. These experiments were short-term and ran for one week to a month. Acid rain contains high concentrations of different kinds of ions. It contains hydrogen ions, nitrate and sulfate, so we wanted to partition the effects of hydrogen ions from the effects of nitrate and sulfate ions. In this system and at the concentrations we used, chloride, potassium, and sodium ions were biologically inert. They did not have an effect on the biota. By adding hydrochloric acid to some of these bags and reducing pH to 5.2, we were studied the effects of hydrogen ions on the biota (Table 3). By adding potassium nitrate and sodium sulfate to some of these bags, we also studied the effects of nitrate and sulfate ions. We compared responses in these bags to bags where sulfuric and nitric acid were added. As a consequence, we could partition the effect of different ions on the biota, and found that there were sensitive species. Daphnia and Diaptomus were two of the very sensitive species in the system. Daphnia was reduced to almost nonexistence below a pH of 5.2. Other taxa showed more graded responses, but many of them show sensitive responses to acidic conditions (Fig. 20). In general, taxa responded to proton but not nitrate and sulfate additions.

We also got some interesting results for Keratella taurocephala which increased at pH 5.2 (Fig. 20). From the literature and other experiments, we know that this response probably represents release from competition with Daphnia. Daphnia is the dominant competitor in these systems. When Daphnia was wiped out by acidic conditions, the Keratella populations went through the ceiling because they were released from competition with Daphnia. You would never see these kinds of in a laboratory toxicity test because the whole community is not present. We repeated these experiments in a second year because we wanted to define precisely the dose-response relationships. We deployed 18 bags in Emerald Lake, and we created a gradient of 6 different pH levels ranging from about 4.7 on up to natural pH which was around 6.3. Daphnia was nearly absent when pH was reduced to 5.2 or less, and above that level, there was a linear increase in the abundance of Daphnia with increasing pH (Fig. 21). Keratella increased as pH declined probably as a result of release from competition with Daphnia (Fig. 21). Using these kinds of results, we were able to construct what is likely to happen to the zooplankton assemblage should acidity increase in these lakes. In our gut analysis of trout, we found that Daphnia was a major component of trout diets in Emerald Lake (Fig. 7). By affecting Daphnia, increased acidity can indirectly affect trout by changing their food base.

The other set of experiments we conducted were in stream side channels. We know from the literature that the eggs and embryos of trout are especially sensitive to acidic conditions. In one set of

were a series of naturally acidic lakes in the Bench Lake area. We set out to sample lakes in the Bench Lake area for fish, invertebrates, zooplankton, and water chemistry to determine if any relationships exist between the biota and water chemistry of naturally acidic lakes versus lakes that were not naturally acidic (Fig. 16). As Aaron Brown pointed out, most of the acidic lakes are acidic because of the weathering of pyrites which produce sulfuric acid. There are basins with acidic lakes and alkaline lakes. There are lakes in very close proximity that vary tremendously in water chemistry.

As an initial part of the study, 104 lakes (all the lakes greater than a hectare) in this area were sampled for fish and amphibians, and their ANC's and pH's were measured. Most of the lakes fell between pH of about 6 and 8, so most of them are near neutral (Fig. 17). However, there were about 10 lakes that had pH's of less than 6. In this talk, acidic lakes are those with pH's of less than 6, and non-acidic lakes are those with pH's greater than 6. We also sampled 33 of these lakes for zooplankton and invertebrates, and did a detailed analysis of water chemistry, measuring ANC, pH, electrical conductance, major ions, and aluminum in these 33 lakes (Fig. 18).

Finally, the third component of our program was field experiments (Fig. 19). Acid tolerances are often fairly species specific, and, when we started these studies, people had not studied many of the taxa that were present in our high altitude lakes. We needed to find out to what degree these taxa tolerated acidity. In addition, we also found in the literature that, within fish species, different strains or populations with different genotypes had different tolerances to acidity. At that time, there were almost no data on the acidity tolerances of fish in the western US. Responses to acid inputs also depend upon the concentrations of other ions like aluminum and calcium. There is a dose-response relationship fairly specific to each situation. We carried out these experiments because we knew almost nothing about acid tolerances under the chemical conditions present in the high Sierra Nevada. These experiments generated dose-response data on how different species responded to different levels of acid input, and we used them to calibrate monitoring and survey programs. If we saw relationships between the abundance of different species and pH through time or space, and we saw a congruent set of relationships from our field experiments, then we could be fairly certain that those relationships were real; they were due to the effects of acidity on these taxa. Finally, the big advantage of these experiments was that they functioned as perturbance tests. Conditions not found in nature could be created. Another thrust of this research was to allow predictions of what would happen if acid loading increased. In these experiments, we actually made bags or experimental units much more acidic than anything we could find in nature, thereby allowing us to predict what would happen if acidity increased in these systems.

We conducted two types of experiments. We did tests in large bags in Emerald Lake, which had a water chemistry representative of high Sierra Nevada systems. I am a very strong believer in doing field experiments rather than laboratory experiments. Laboratory experiments have often been criticized

of these invertebrates are aquatic insects. Things like Baetis increase their drift at pH 5.2 and 4.6. Chironomid larvae actually decreased their drift rates at pH 5.2. They apparently burrow into the substrata to get away from acid conditions, but at pH 4.6, they are killed and drift out of the system. Other mayflies and a stone fly do not show a drift response at pH 5.2, but they do at 4.6. Finally, there were a variety of taxa that showed no response to acid inputs. These drift or toxic responses resulted in wholesale changes in population numbers of some taxa on the bottom (Fig. 28).

Based on all these data, we have two general qualitative conclusions about the current and potential effects of acid deposition on high Sierra Nevada aquatic systems. On one hand, we found that sensitive taxa are present through both time and space throughout the Sierra Nevada. We do not think that episodic acidification is affecting the biota at this time. If there are effects, they are certainly very transient and probably localized. However, if acid loading increases, our experiments showed that we will see substantial changes in species composition and in the structure of food chains in these systems.

Q (AUDIENCE) Your dose-response curve very clearly shows that there is a threshold for the effect in natural acidification; is that correct? You said you seem to get a 0 response and then in an abrupt change. Is that interpreted correctly?

A (DR. COOPER) There is a dummy regression variable that indicates the pH at which densities go to 0. Above that point, there is a significant linear relationship. There is a quantitative effect even from 5.5 up to 6.3, but below pH 5.2 Daphnia is eliminated.

Q (AUDIENCE) You eliminate the species?

A (DR. COOPER) Between 6.3 and 5.5, there is a depression, but no elimination of the species. Below approximately 5.2 there is complete elimination.

Q (AUDIENCE) I guess there were some very alkaline lakes. There was something there about pH 9.3 or 9.4. Can you tell me what the chemistry of this is? Can it be mobilized to keep the other lakes from getting acid?

A (DR. COOPER) Aaron, can I refer that question to you?

A (DR. BROWN) I think those lakes are slightly alkaline. They are in closed basins, so there is no drainage.

Q (AUDIENCE) Alkaline salt then in a volcanic area at a high altitude.

Q (AUDIENCE) You say that the adults of these species died. Do you know what the mechanism is when the pH is depressed?

A (DR. COOPER) You mean for microcrustaceans?

Q (AUDIENCE) Yes.

A (DR. COOPER) The literature suggests that it affects ion regulation, so you are primarily affecting things like the sodium pump and membrane phenomena. That probably accounts for these toxic

experiments, we concentrated on survivorship of the egg stage of golden trout in the face of acid stress. We performed it in the field so that we could replicate natural Sierra Nevada chemistry in terms of calcium, aluminum, and other parameters. We conducted these experiments in small channels in Mine Creek which drains Spuller Lake. We had a gravity flow system that consisted of a series of channels. These were filled with natural spawning gravels, and we placed golden trout eggs into these channels. Some of the channels did not receive acid and the remaining ones received 1 of 5 different levels of acid input. We covered them to keep raccoons out and to shade them because eggs at this stage are usually in the dark, and are very sensitive to light. This experiment was hard to conduct, because it was done during the spawning period. Our acid dosing apparatus kept freezing at night, and we had to dig down through about 4 meters of snow just to set it up. It showed that, at least at these low temperatures, there was very little effect of acid inputs on the survivorship of golden trout eggs (Fig. 22).

We have also done these stream side channel experiments to study the effects of acid inputs on stream invertebrates, because these invertebrates are important food sources for fish. We fixed nets to the outlets of each of these channels to monitor those animals that entered the water column and drifted downstream (drift). We measured drift, and we measured the densities of benthic invertebrates at the beginning and the end of the experiment. We set up 12 channels (Fig. 23). Some of the channels were unperturbed (controls), acid was added to some so that the pH was reduced to 5.2, and we added acid so that pH was reduced to 4.4 in another set. Because the early stages of acidification are often pulses, and occur as episodes associated with snowmelt or with summer rains, we tried to replicate one of these 8-hour acid pulses. We sampled drift throughout all of these experiments for a day before and two days after each event, and we sampled the benthos, those animals living on the bottom, a day before and two days after the experiment (Figs. 24 and 25). We found that there were a variety of sensitive taxa, particularly the mayfly Baetis, which showed sensitive responses to acid input. There was very little difference among treatments until the acidification period, and then there was greatly enhanced Baetis drift in those channels to which acid was added (Fig. 26). After the acidification event, there was more drift from the control channels than from acidified channels, and that was because acidification wiped out the population in acidified channels. There were almost no individuals left to drift in acidified channels, and that accounts for these results.

This was purely a toxicological study. The reason animals drifted out of acidified channels was because they were killed by the acid (Fig. 27). We placed the drift in buckets and counted the numbers of live versus dead animals. Most of the intense drift was due to the fact that acid killed these animals, and they left the system by drifting out of it. The fact that these animals were killed has a lot of implications for the whole population. They were not simply leaving because of a behavioral response to increased acidity. This slide summarizes the drift responses of different invertebrates (Table 4) . Most

Figure 1

CURRENT AND PREDICTED IMPACTS OF ACID DEPOSITION ON AQUATIC SYSTEMS IN THE HIGH SIERRA

I. MONITORING. EMERALD LAKE & NEARBY SYSTEMS.

II. SURVEYS. HIGH SIERRA LAKES AND STREAMS.

III. FIELD EXPERIMENTS.
LAKE BAGS.
STREAMSIDE CHANNELS.

Fig. 1 Study design

effects.

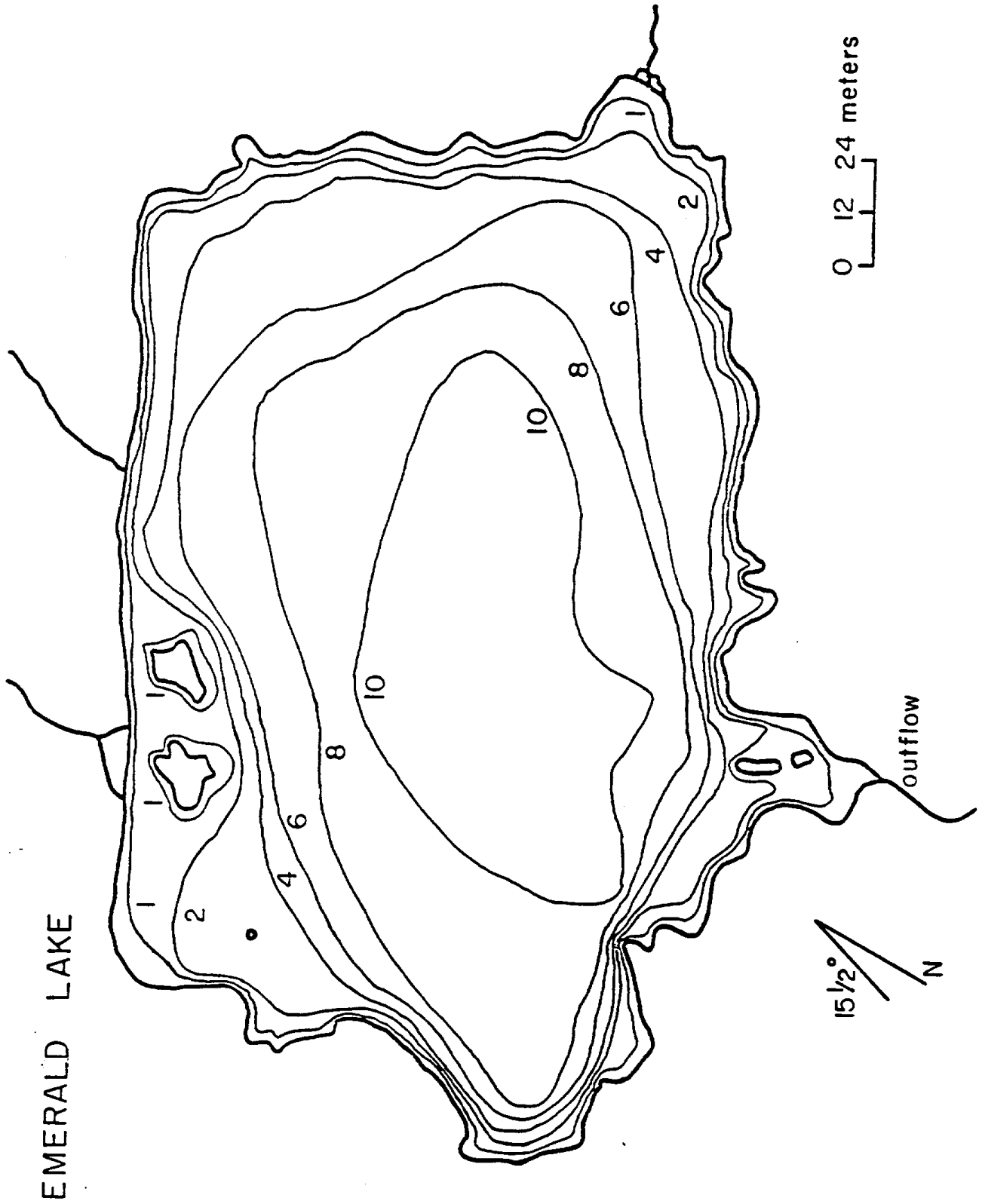


Fig. 3 Map of Emerald Lake

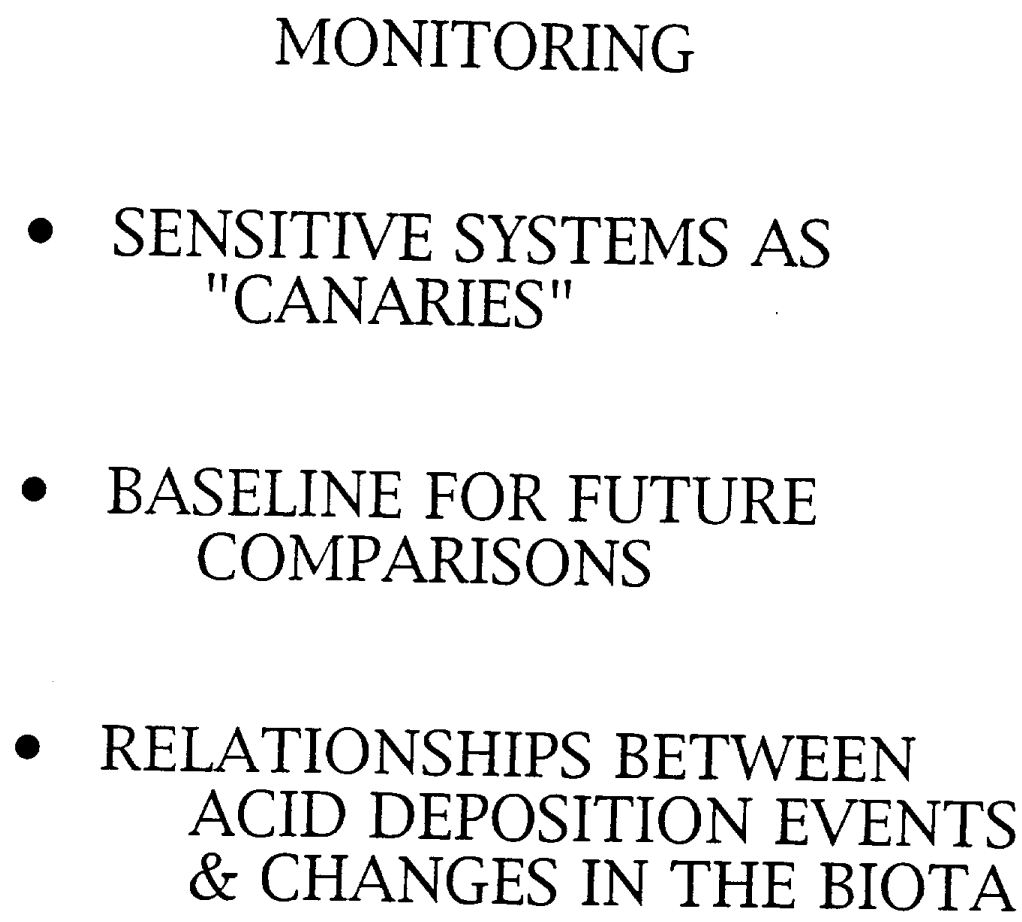


Fig. 2 Rationale for monitoring program

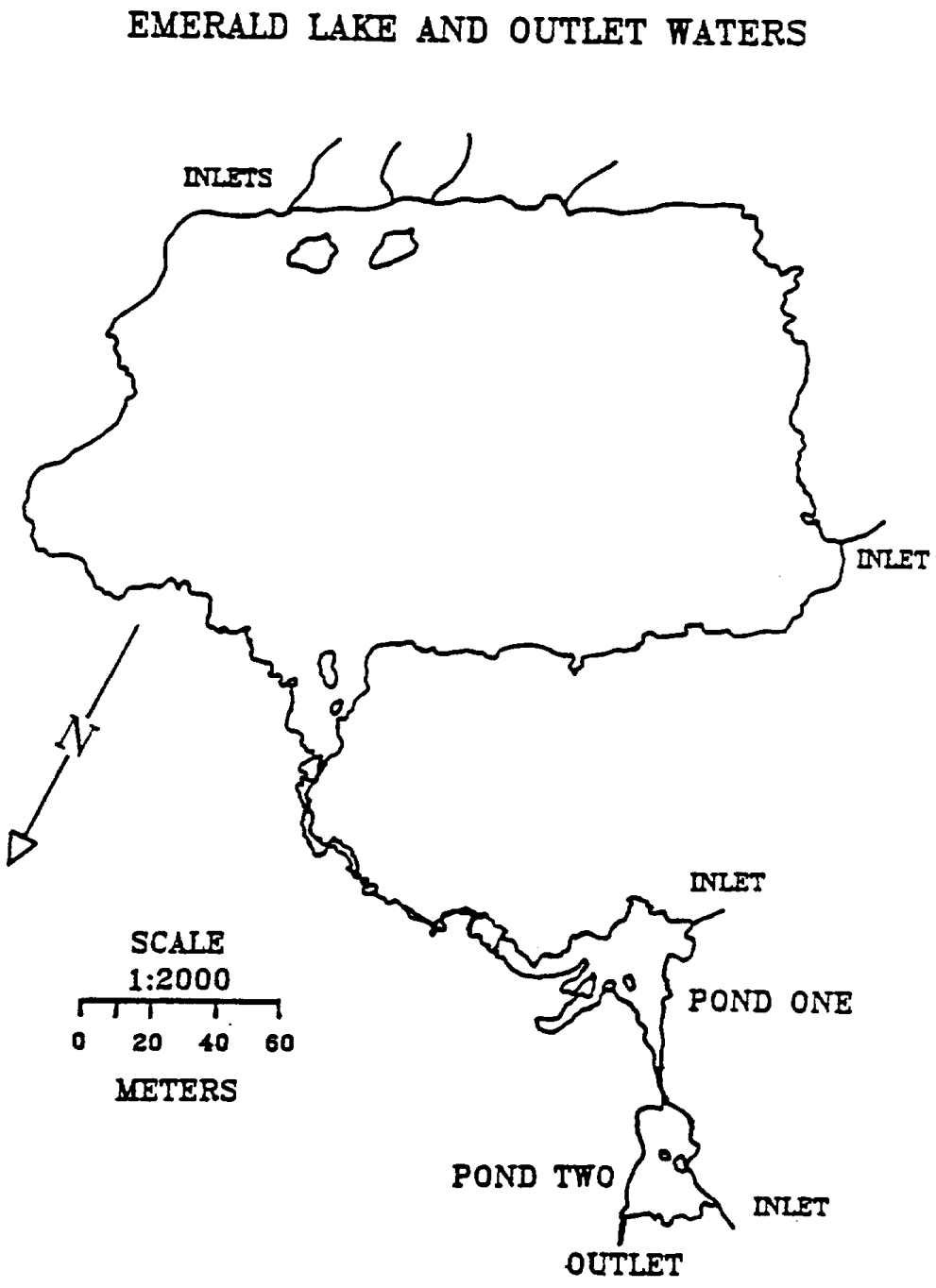


Fig. 5 Map of Emerald Lake and its outlet waters.

Figure 4

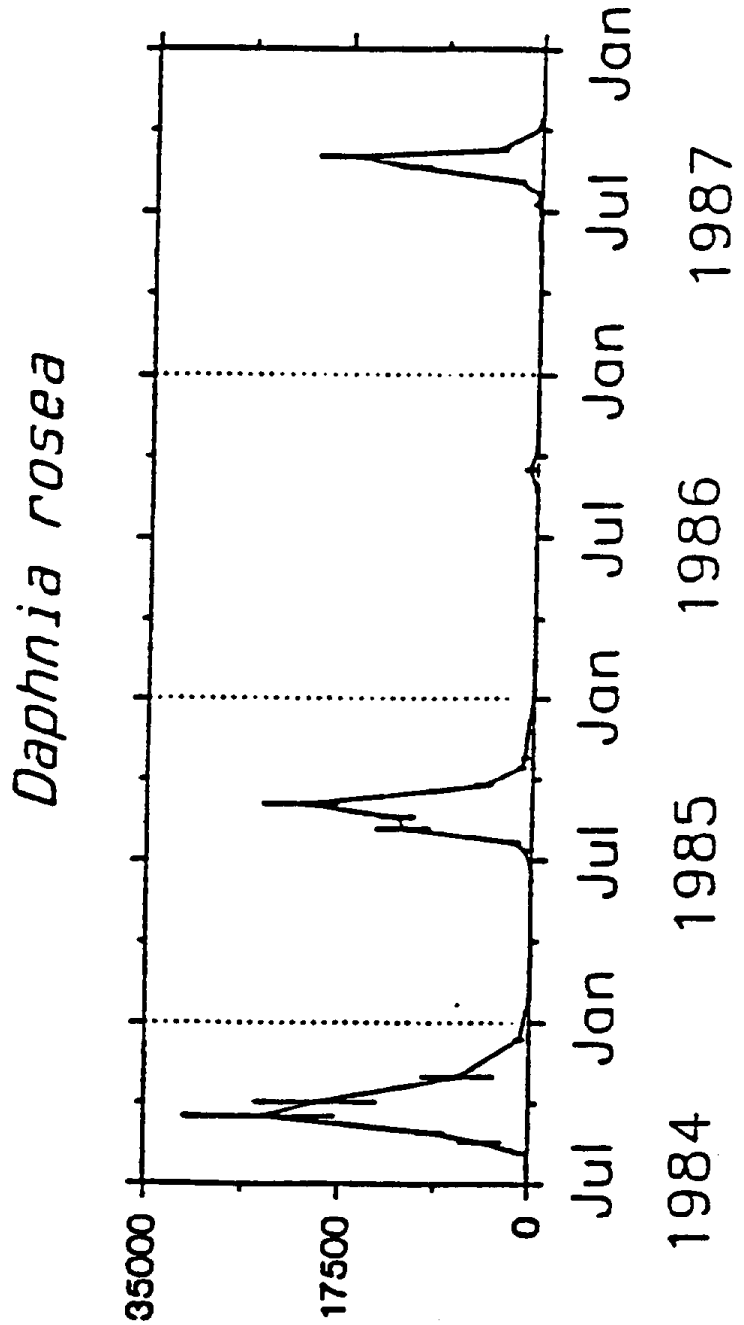


Fig. 4 Population dynamics of *Daphnia rosea* in Emerald Lake, 1984-1987.

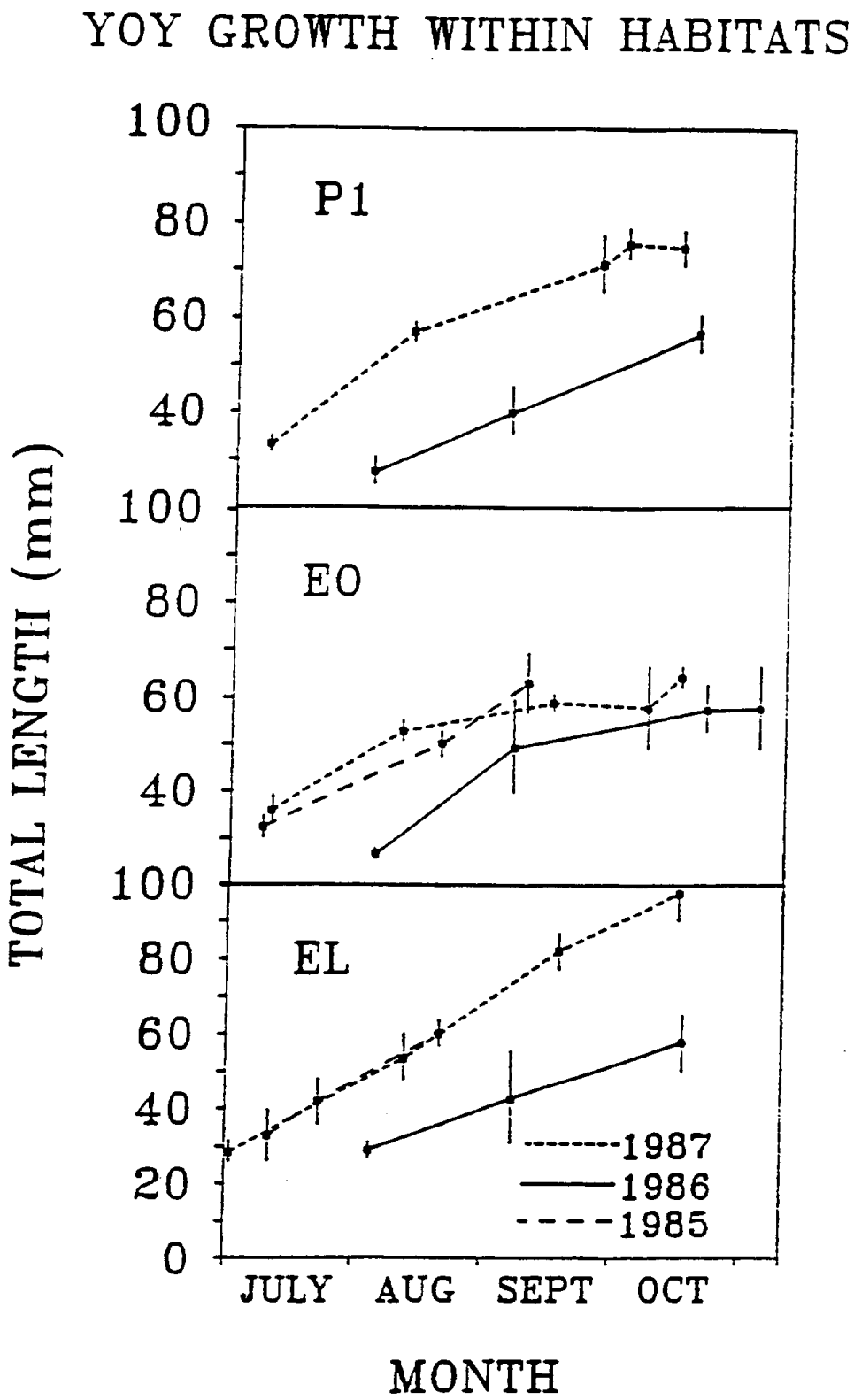


Fig. 6 Young-of-the-year brook trout growth in Emerald Lake (EL), the Emerald outlet (EO), and the first downstream pond in the Emerald outlet (P1), 1985-87.

POPULATION ESTIMATES FOR BROOK TROUT IN THE EMERALD LAKE SYSTEM

HABITAT	YEAR	YOY	ADULT
LAKE	1985	428	1132 (907-1357)
	1986	67	1169 (720-1618)
	1987	323	1036 (736-1336)
OUTLET	1985	198	97 (49-145)
	1986	26	222 (185-259)
	1987	101	190 (131-248)

Population estimates of young-of-the-year (YOY) and adult brook trout in Emerald Lake and its outlet waters.

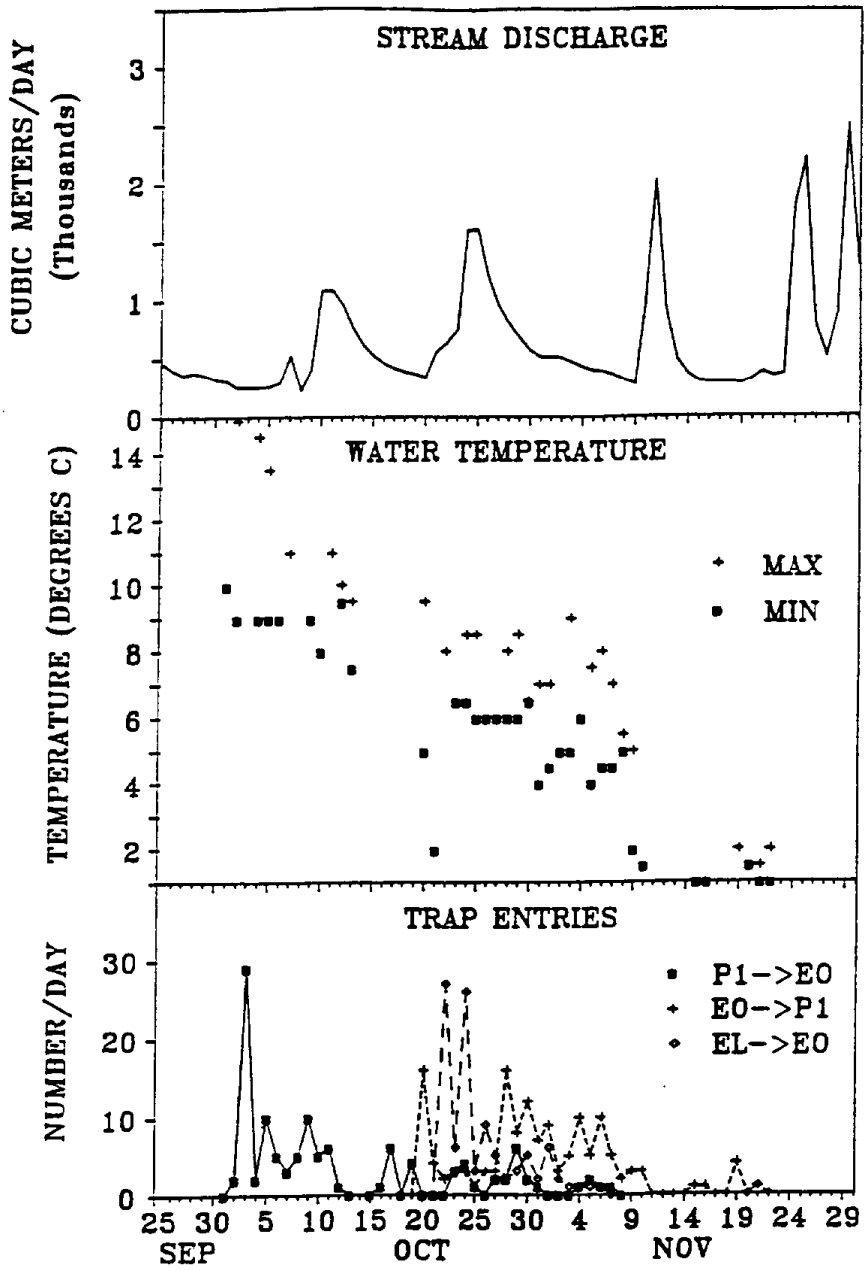


Fig. 8 Discharge and temperature in the Emerald outlet, and movement of trout from Pond 1 (P1) to the Emerald outlet (EO), from EO to P1, and from Emerald Lake (EL) to the Emerald outlet (EO).

Figure 7

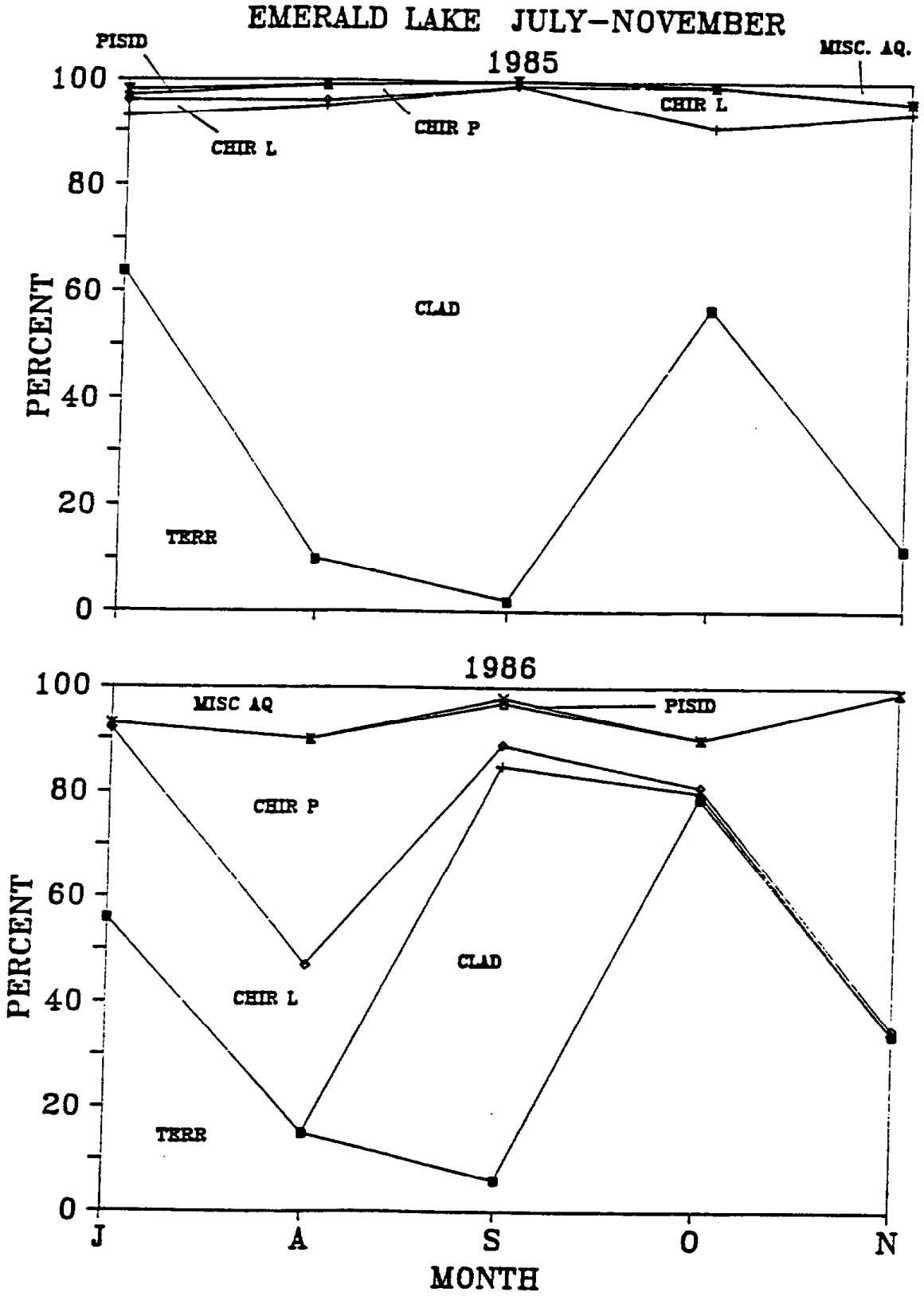


Fig. 7 Summer/Fall brook trout diets (% by number) in Emerald Lake, 1985 and 1986. Code: TERR=terrestrials, CLAD = cladocerans (*Daphnia*), CHIR L = chironomid larvae, CHIR P = Chironomid pupae, PISID = *Pisidium*, MISC AQ = miscellaneous aquatic invertebrates.

SURVEYS

- BASELINE DATA

  REPRESENTATIVENESS OF "CANARY"

  REGIONAL ASSESSMENTS OF RESOURCES

- RELATIONSHIPS BETWEEN WATER CHEMISTRY & THE BIOTA

- REGIONAL PROJECTIONS OF IMPACTS OF ACID DEPOSITION

Fig. 10 Rationale for the surveys.

Figure 9

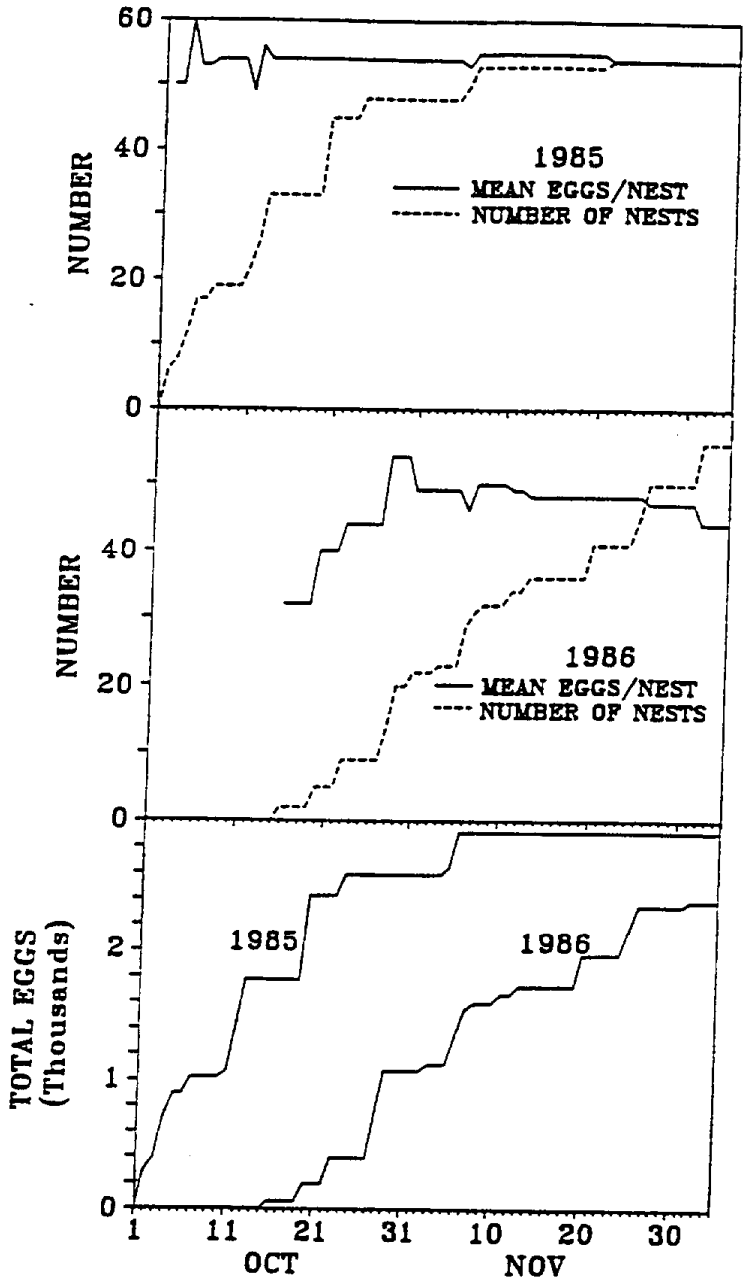
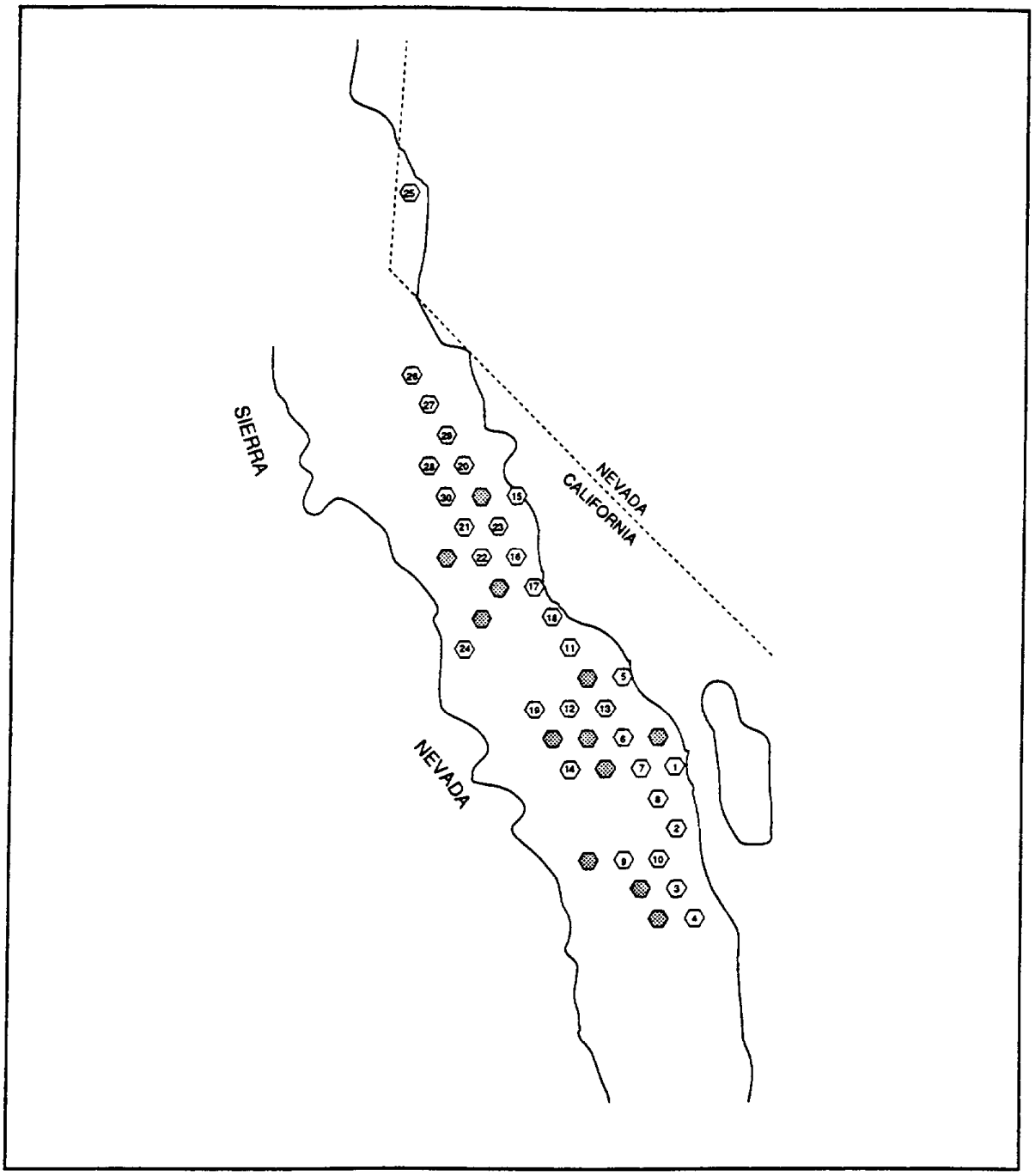


Fig. 9 Number of eggs per nest, number of nests, and total number of eggs in the Emerald outlet, 1985-86.

Figure 12



The portion of the Sierra Nevada containing lakes above 8000' (2439 m) elevation, showing all of the hexagons within the proper altitude range. The number within each sampled hexagon corresponds to the number of the lake sampled within that hexagon.

Fig. 12 Lakes sampled in the High Sierra as part of the second survey using EPA's E map procedures.

Figure 11

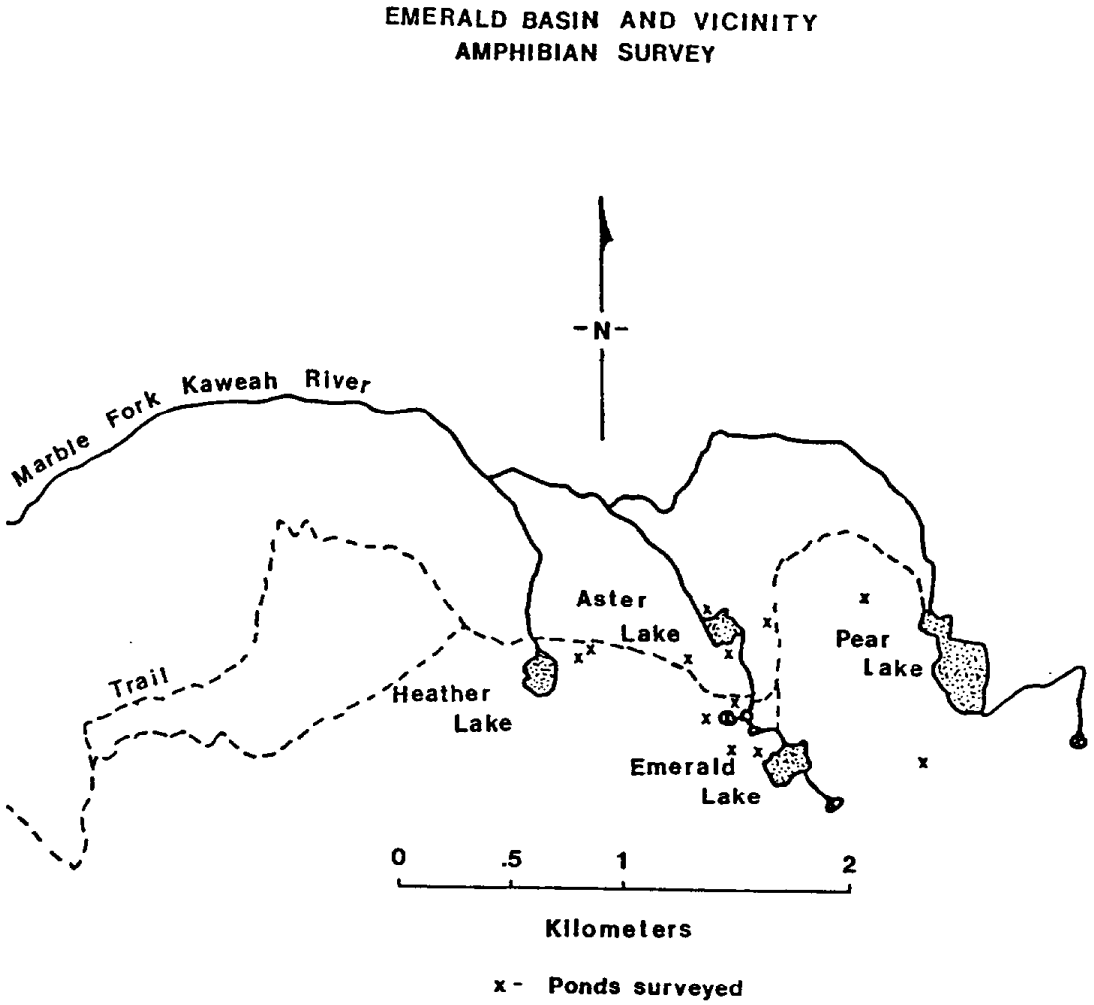


Fig. 11 Map of lakes sampled as part of the Tokopah survey.

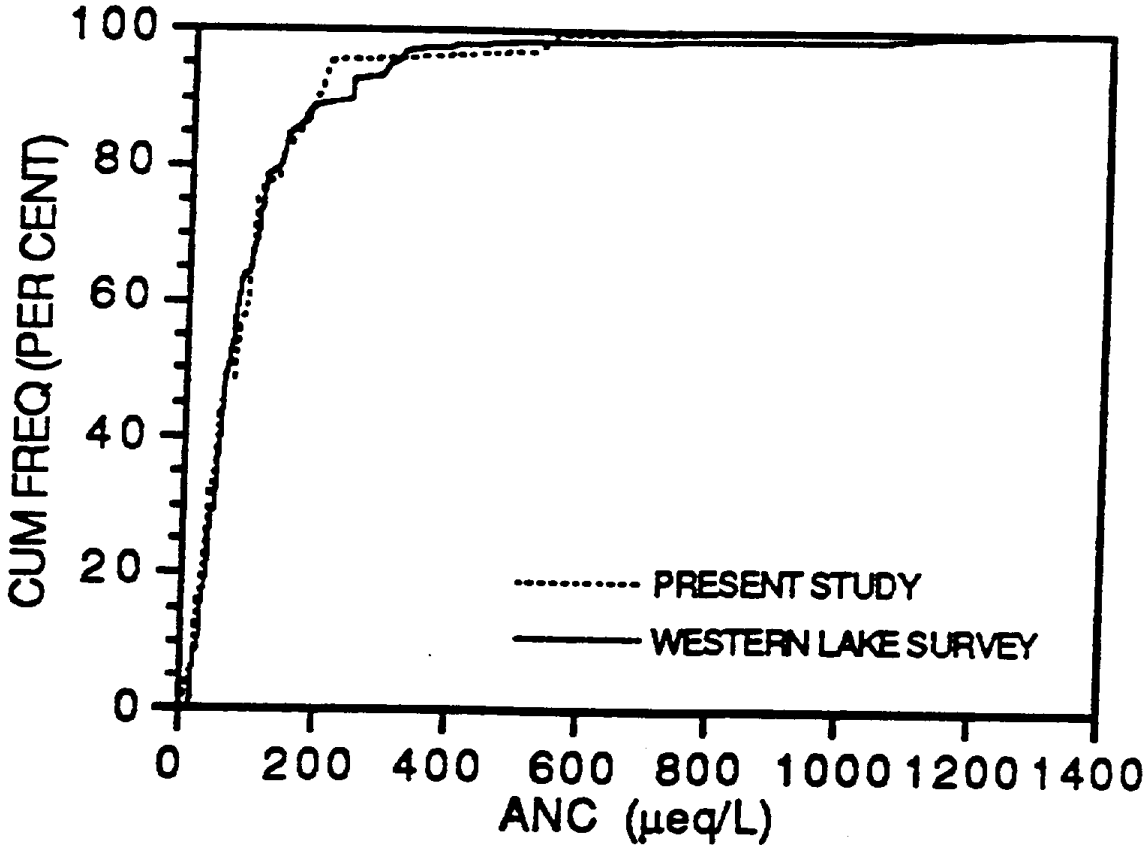


Fig. 14 Cumulative frequency distributions for ANC of the target population of lakes in the second survey and in the EPA's Western Lake Survey.

Figure 13

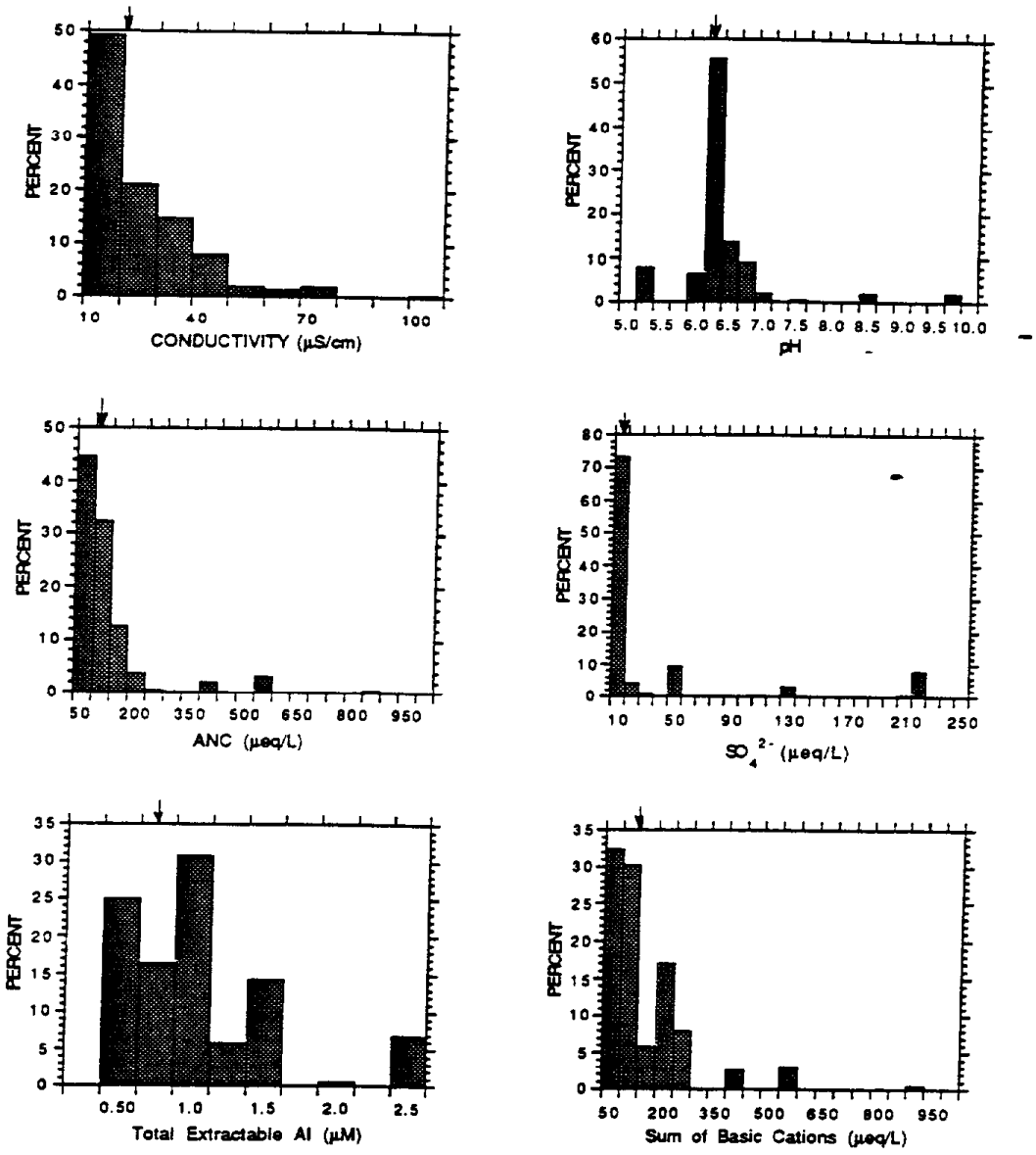


Fig. 13 Frequency distributions of lakes with different chemical characteristics.

Table 2

SPECIES	CATEGORY	ESTIMATED POPULATION SIZE	PERCENT OF POPULATIONS
Golden	Natural Reproduction	469	34
Golden	All Populations	498	36
Rainbow	Natural Reproduction	349	25
Rainbow	All Populations	461	33
Brook	Natural Reproduction	185	13
Brook	All Populations	221	16
Brown	Natural Reproduction	112	8
Cutthroat	Natural Reproduction	7	0.5

Estimated numbers and percentages of High Sierra lakes containing populations of different trout species.

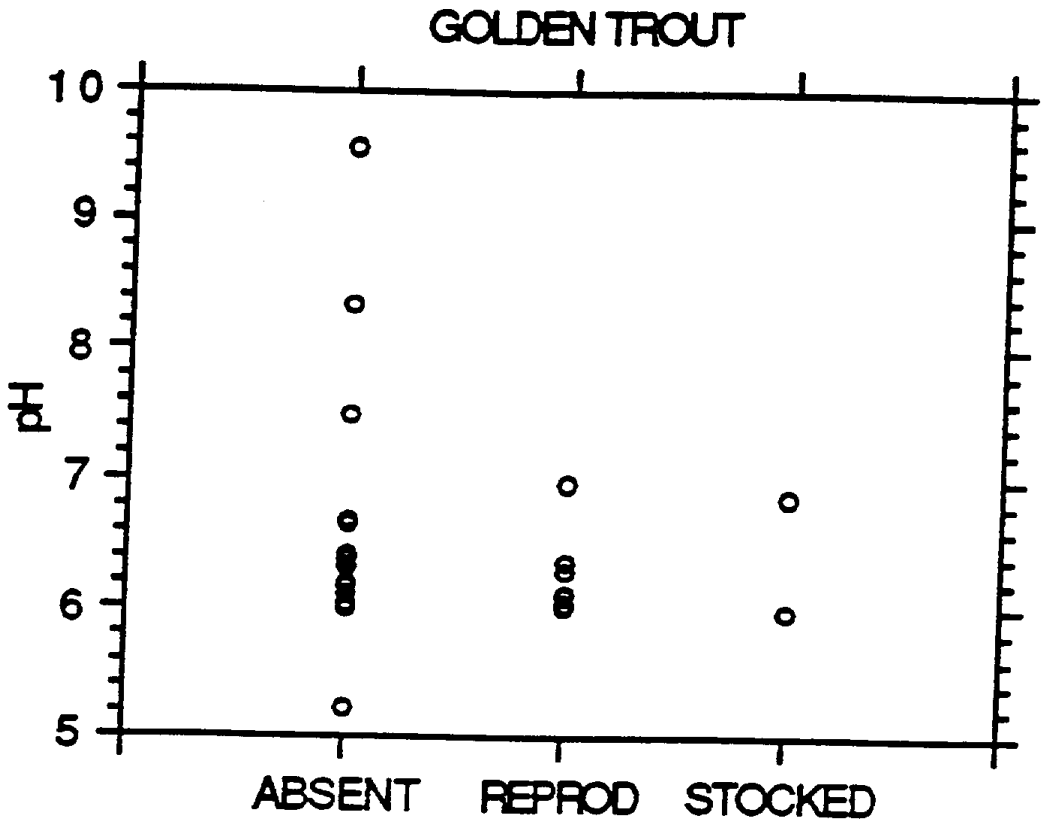


Fig. 15 pH's of lakes with (REPROD = naturally reproducing, STOCKED = maintained through stocking) and without golden trout populations.

Figure 17

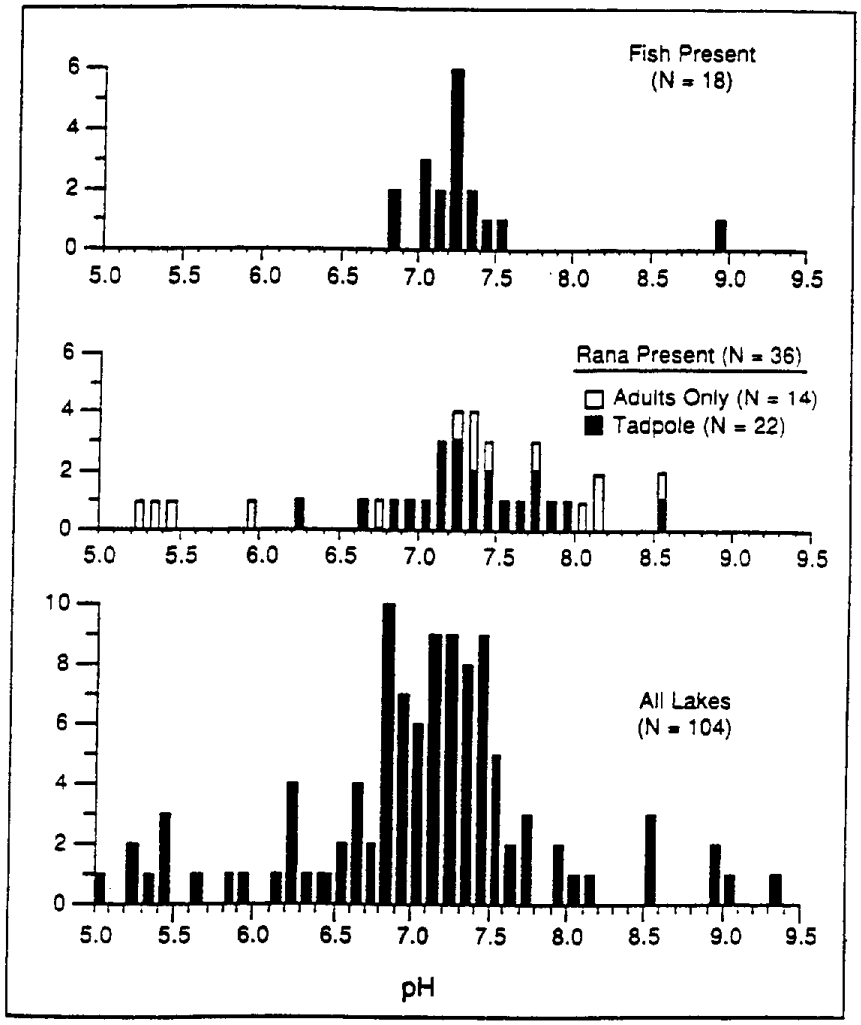


Fig. 17 pH's of all lakes included in the Bench Lake survey (bottom), as well as the pH's of that subset of lakes containing fish (top) or amphibians (middle).

Figure 16

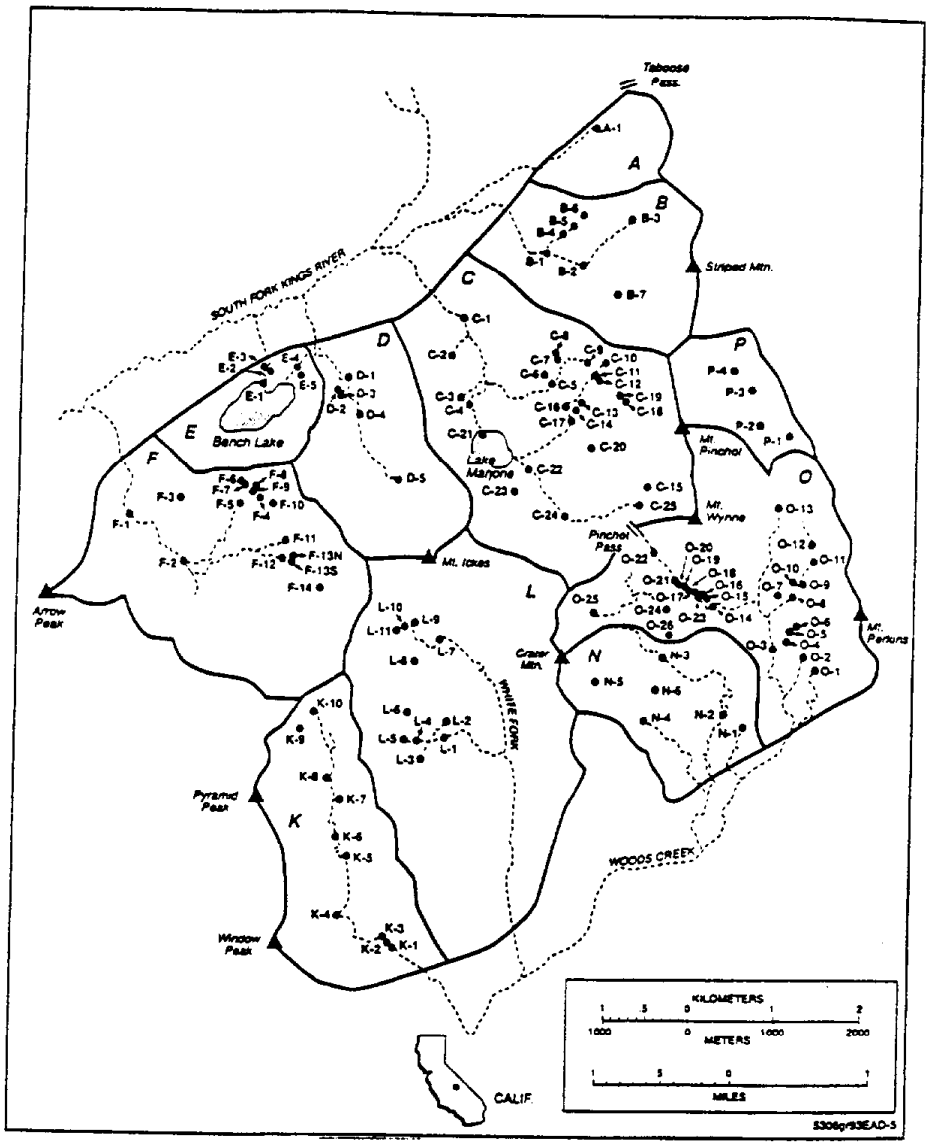


Fig. 16 Map of Bench Lake area, focus of the third survey, which compared the biota and chemistry of acidic vs. non-acidic lakes.

FIELD EXPERIMENTS

- DATA ON DOSE-RESPONSE CURVES
- CALIBRATION OF MONITORING/SURVEY PROGRAMS
- PREDICTION OF IMPACTS OF INCREASED ACID LOADING

Fig. 19 Rationale of field experiments.

Figure 18

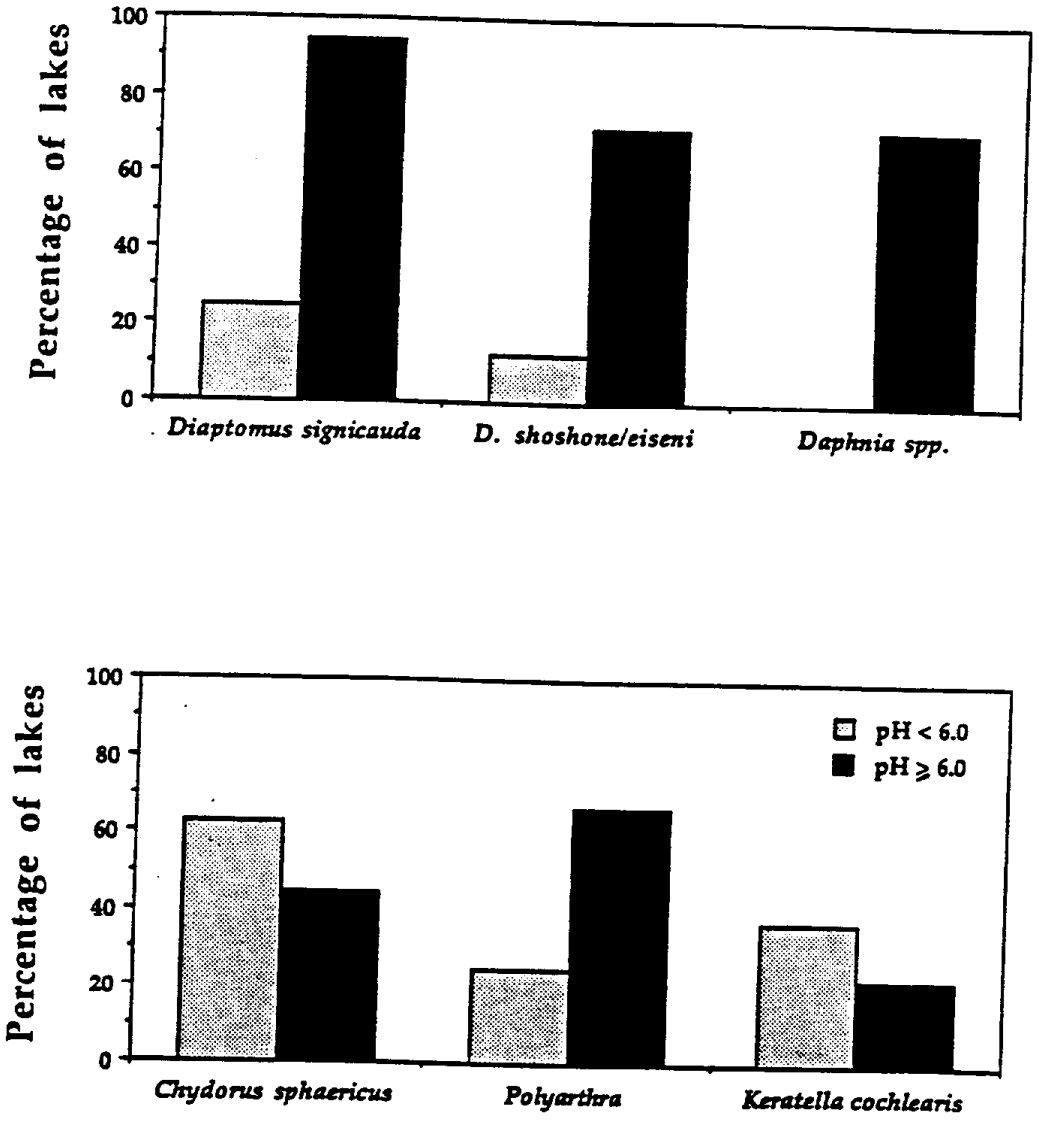
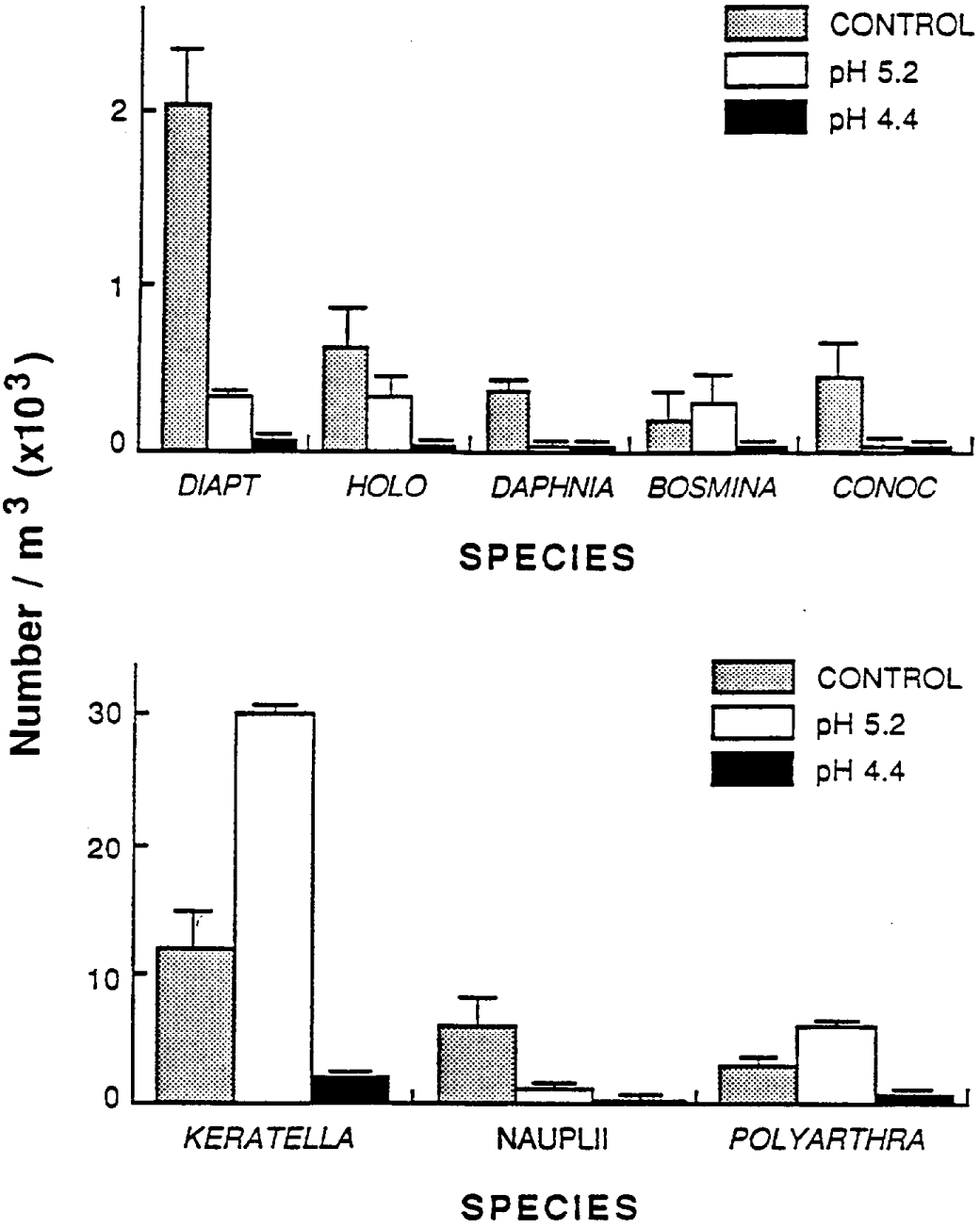


Fig. 18 Percentages of acidic (pH < 6) and non acidic (pH > 6) lakes containing common zooplankton species.

Figure 20



Densities of common zooplankton taxa (X ± 1 SE) in bags assigned to different treatments at the end of Experiment 2.

Fig. 20 Densities of common zooplankton taxa in bags assigned to different pH treatments, Experiment 2.

Table 3

Design of bag experiments. Numbers in parentheses in the body of the table indicate the experimental pHs of sulfuric + nitric acid treatments. The asterisks in Experiment 4 indicate inclusion or exclusion of bottom sediments in control and acid addition bags in a cross-classified design.

Treatment	Expt. 1	Expt. 2	Expt. 3	Expt. 4
Control (pH=6.3)	X	X	X	X*
$H_2SO_4+HNO_3$	X (4.8)	X (5.2)	X (5.2)	X* (5.6)
$H_2SO_4+HNO_3$	X (4.2)	X (4.2)		
HCl (pH=5.7)			X	
HCL (pH=5.2)			X	
$KNO_3+Na_2SO_4$			X	
PO_4			X	
$H_2SO_4+HNO_3+PO_4$ (pH=5.2)			X	

Design of first set of bag experiments. Each experiment lasted from one to five weeks.

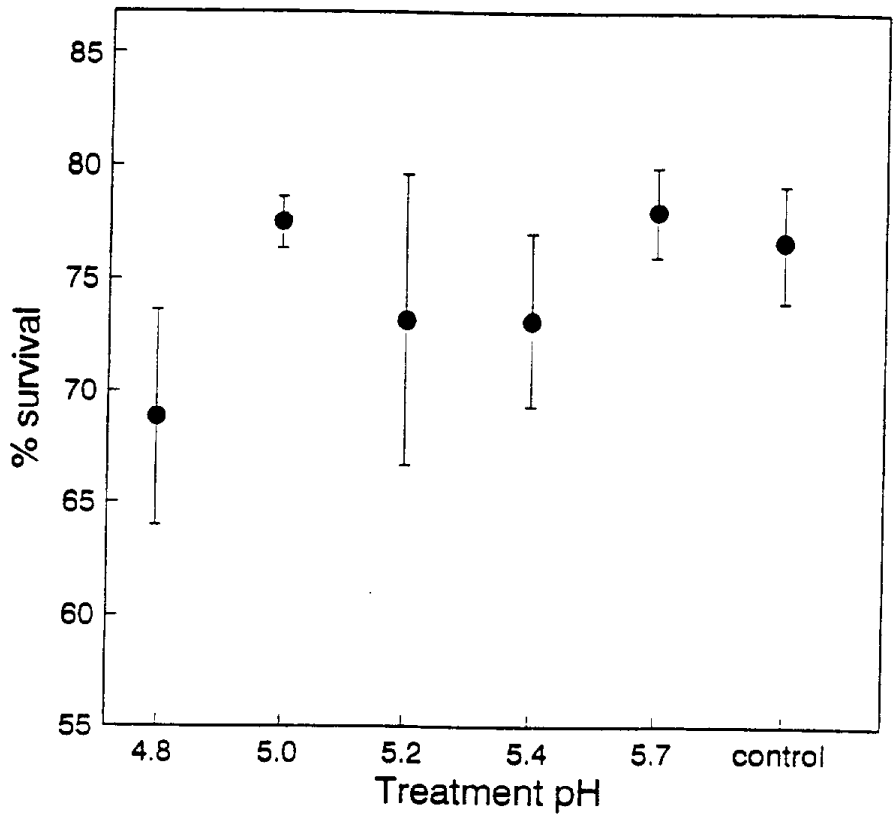
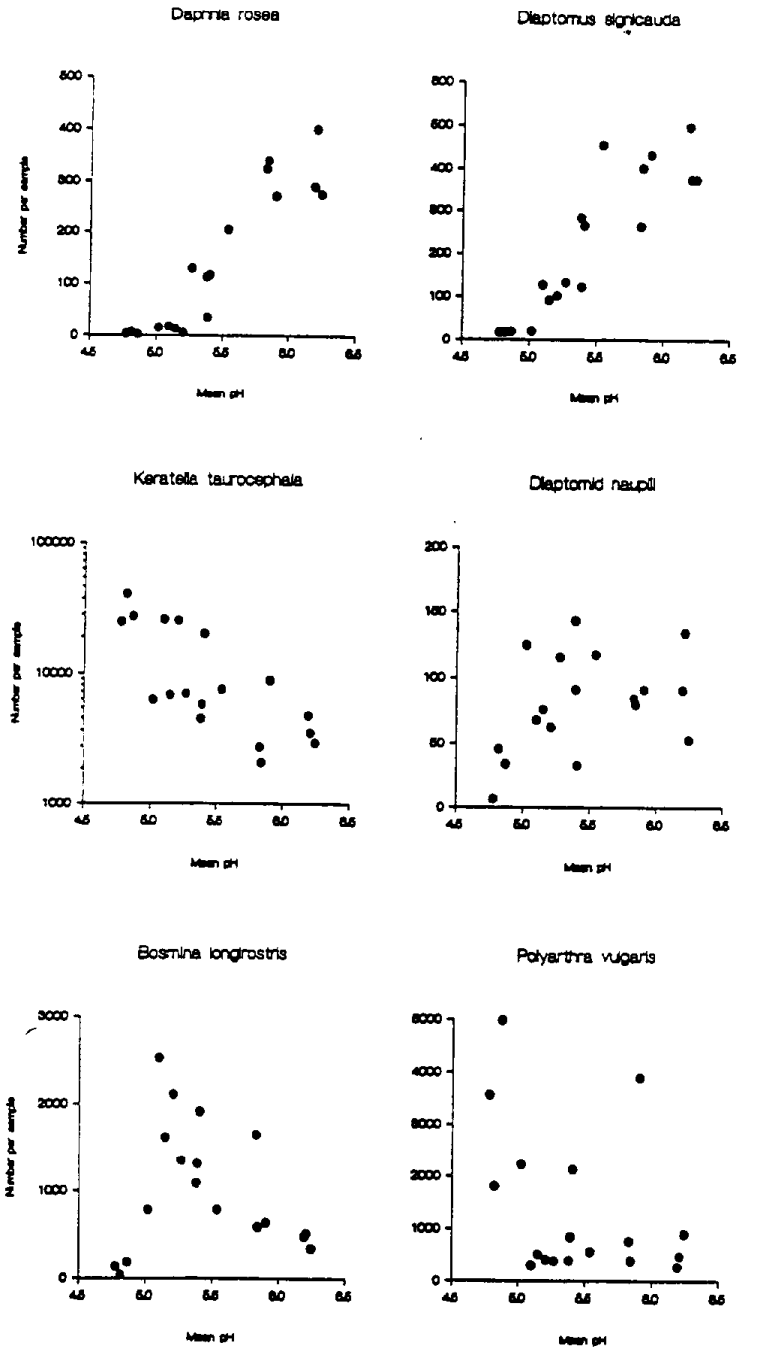


Fig. 22 Survival of golden trout eggs at different pH's.

Figure 21



Mean abundance of each species in each enclosure vs. mean pH of each enclosure after acidification.

Fig. 21 Mean abundance of each species in each bag enclosure versus mean pH of each enclosure after acidification.

Figure 24

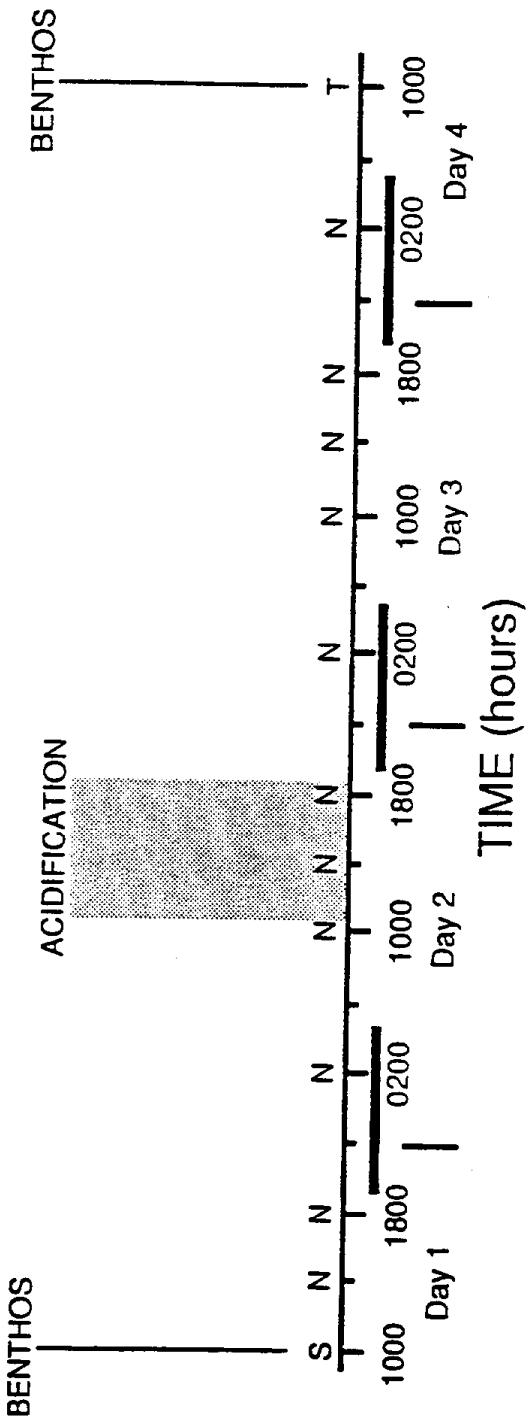
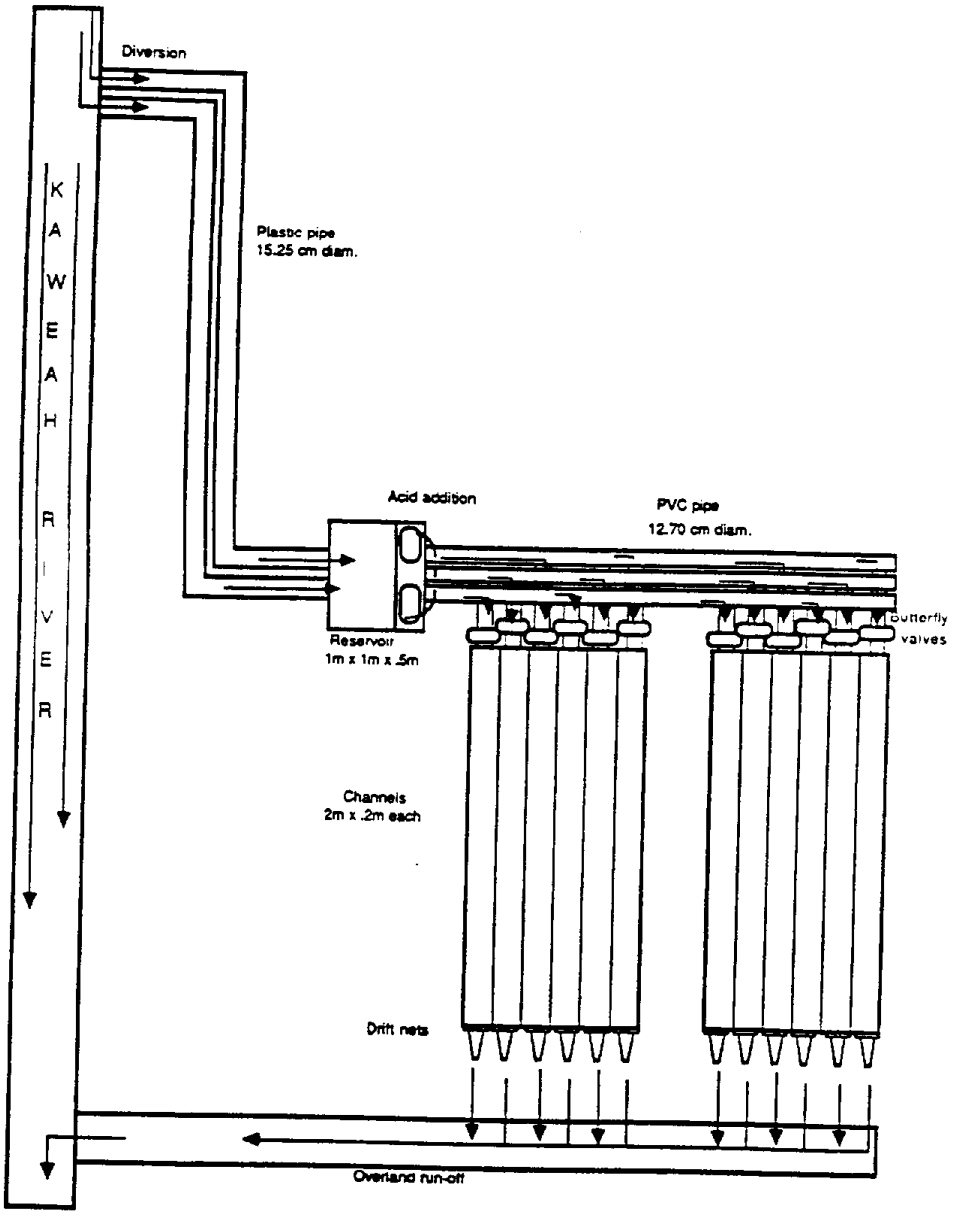


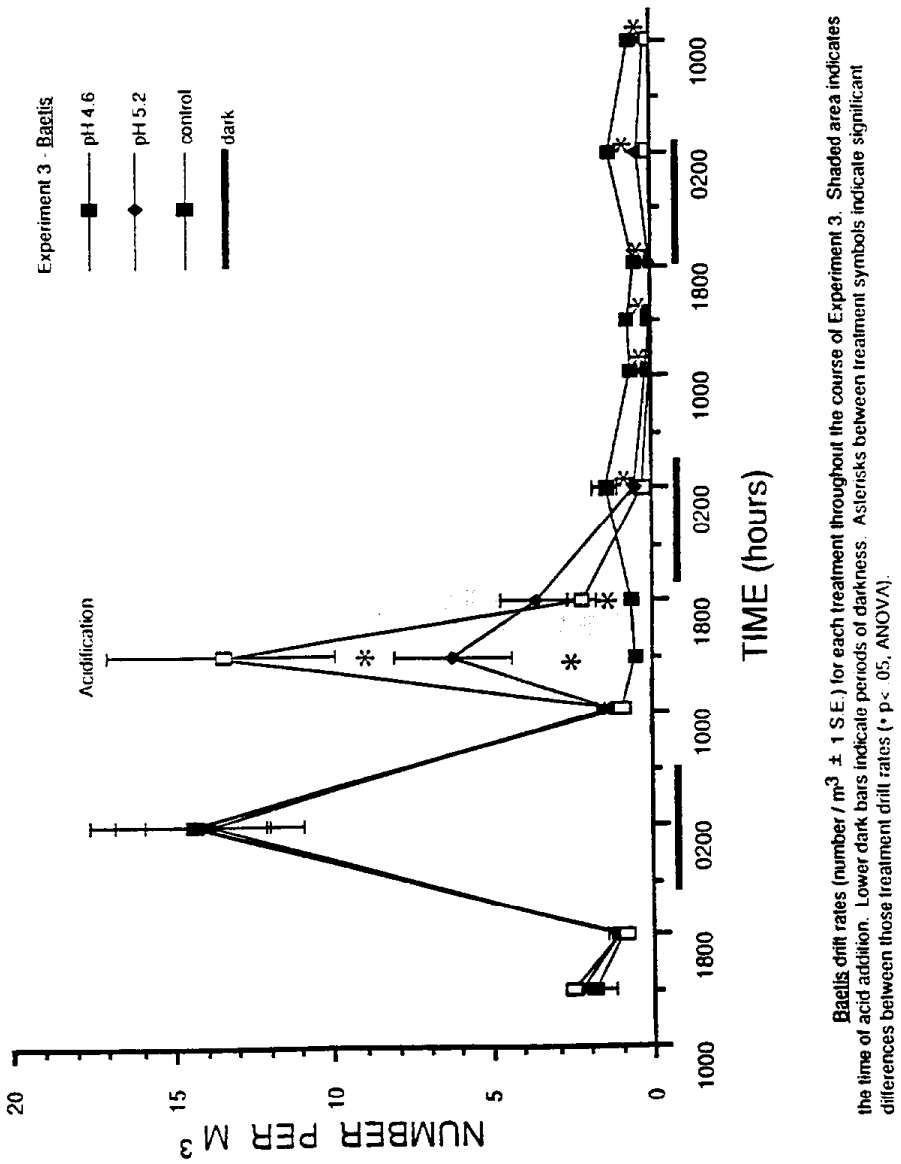
Fig. 24 Sampling design for stream channel experiments. The shaded area indicates the time of acid additions, the dark bars under the time line represent the periods of darkness (night), vertical lines between days indicated 2400 hs (midnight) initial (S) and final (T) benthic sampling times are noted. Drift nets were first set in place at time S, emptied and replaced at each of the "N's" and finally removed at time T.



Design of replicate experimental stream channels set up along the Marble Fork of the Kaweah River, July-October 1986. Arrows indicate direction of water flow

Fig. 23 Design of experimental stream channels used in examining the responses of stream invertebrates to acid inputs.

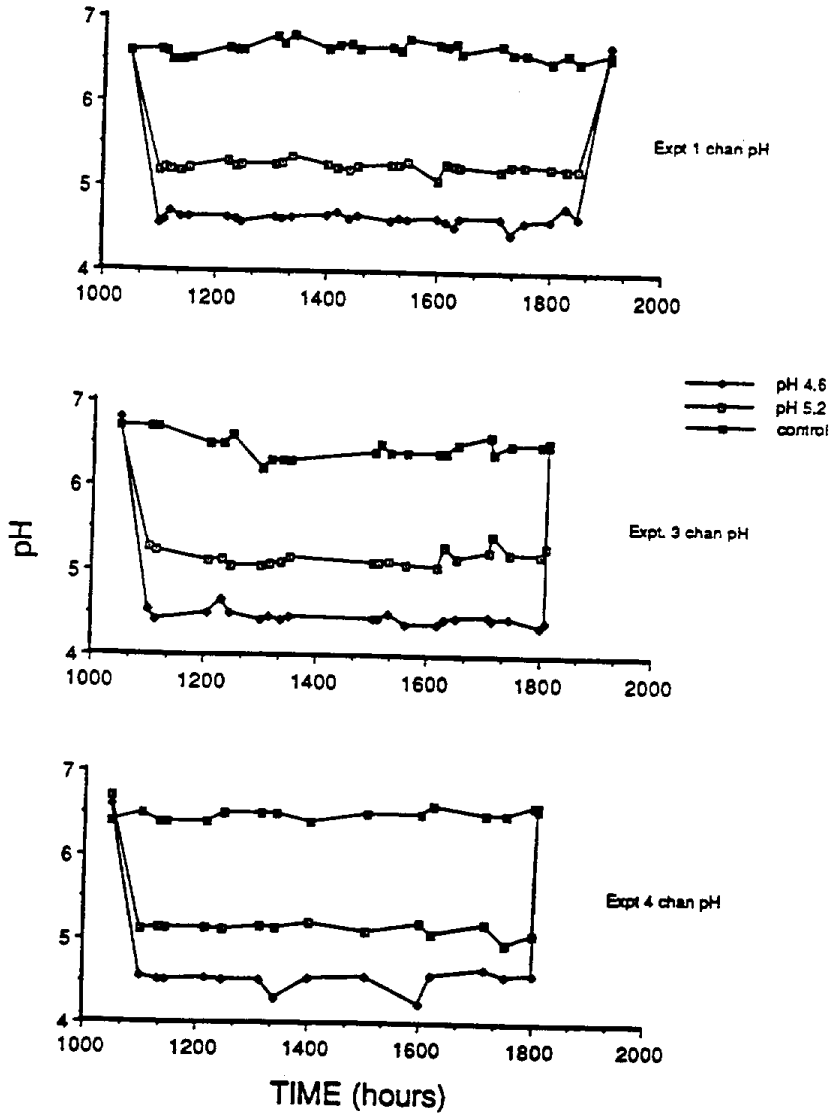
Figure 26



Baetis drift rates (number / m^3 ± 1 S.E.) for each treatment throughout the course of Experiment 3. Shaded area indicates the time of acid addition. Lower dark bars indicate periods of darkness. Asterisks between treatment symbols indicate significant differences between those treatment drift rates (* $p < .05$, ANOVA).

Fig. 26 *Baetis* drift rates for each treatment throughout the course of one of the stream channel experiments.

Figure 25



Treatment pH's from the experimental channels for each of the three reported experiments. Values are from one channel within each treatment level, taken at approximate 15 minute intervals over the 8 hour acidification.

Fig. 25 Treatment pH's for the experimental channels during acid pulses in three experiments.

Table 4

	DRIFT RESPONSE	
	pH 5.2	pH 6.4
Baetis	+	+
Chironomid larvae	-	+
Epeorus (*Paraleptophlebia* *Zapada*)	0	+
Simulium larvae (Chironomid pupae *Amiocentrus*)	0	0

Summary of drift responses of different invertebrate taxa to acid inputs. + = increased drift, - = decreased drift, 0 = no response.

Figure 27

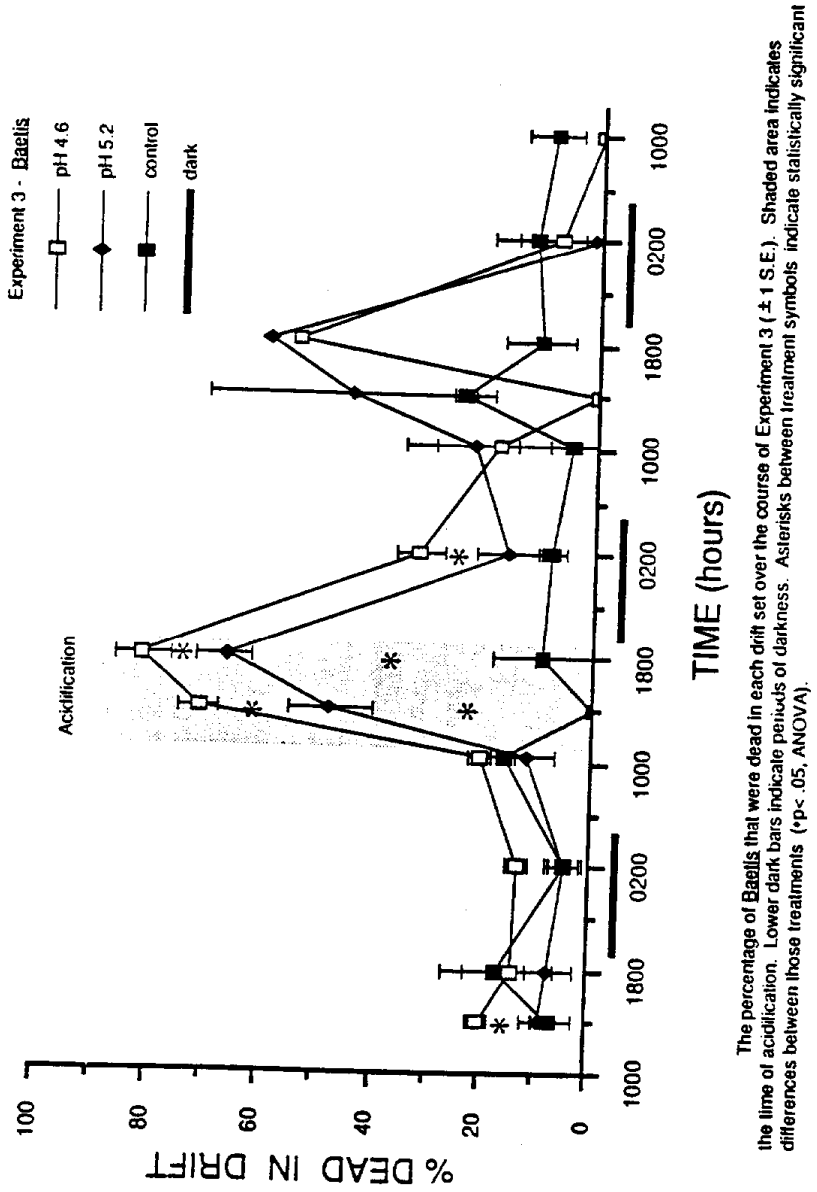


Fig. 27 The percentage of drifting *Baetis* that were dead over the course of one of the stream channel experiments.

DR. STEPHEN BROWN, Moderator

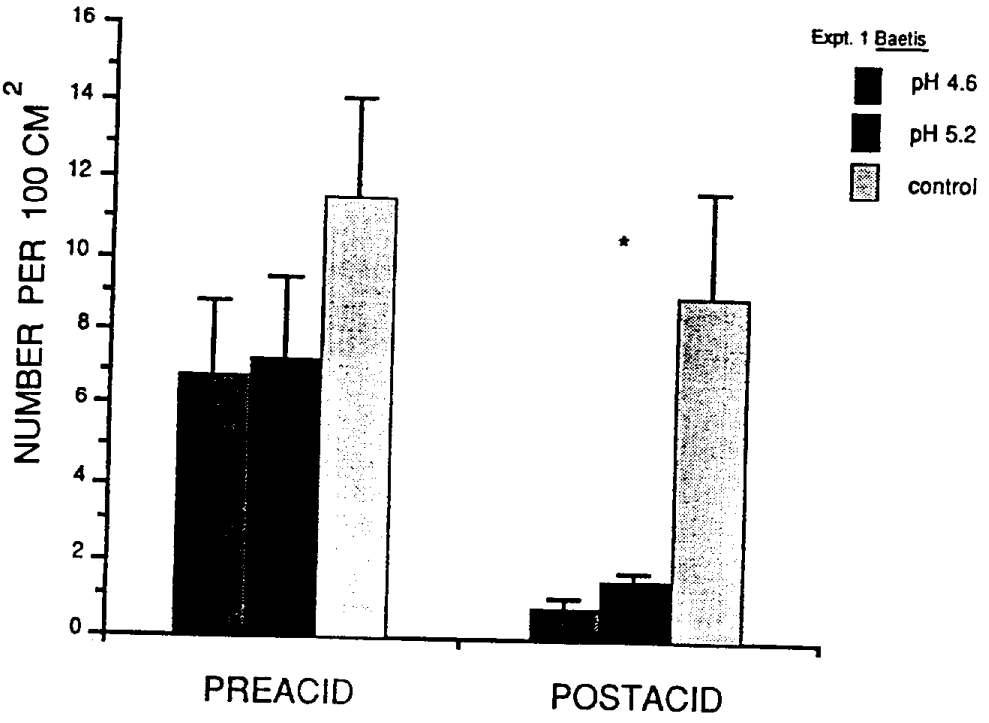
We have one additional talk before lunch, Dr. David Bradford from the US EPA will summarize the effects of acid deposition on amphibians in the Sierra Nevada.

6. Summary of Effects of Acid Deposition on Amphibians in the Sierra Nevada. Dr. David Bradford, U.S. EPA.

DR. DAVID BRADFORD, US EPA

This project was initiated in response to two events in the late 1980's. The first of these was the Air Resources Board interest in acid deposition and, in particular, its potential effects on aquatic systems in the Sierra Nevada. The second was the observation by myself and some other amphibian researchers, that populations of two species of amphibians found in the high Sierra Nevada were declining. There were drainages where these species had disappeared in the previous 10 years and other sites where they had disappeared over the previous 20 to 30 years; and yet there were still other species whose populations seemed to be unchanged. This was a phenomenon that was happening in some other mountain regions of the US, and other parts of the world. At about the same time, it was recognized that it was not just the mountain species that were declining, but there were many lower elevation species that had disappeared or declined in the past couple of decades. However, these montane disappearances were particularly puzzling. In many cases, these species were inhabiting national parks and wilderness areas where there was not any obvious factor operating that could explain the disappearance. With funding from the Air Resources Board to myself and Malcolm Gordon at UCLA, we started our project with three goals in mind, some of which were similar to those Scott Cooper just mentioned. We wanted to determine the sensitivity of the amphibians in the high Sierra Nevada to conditions associated with, or potentially associated with acid deposition. We also wanted to characterize the aquatic chemical conditions for amphibian populations over the Sierra Nevada, so we would have some basis for interpreting what the sensitivity meant. Finally, we wanted to test the hypothesis that acid deposition had been a cause for these population disappearances or declines.

A reason for suspecting acids as a problem was the low observed levels of acid neutralizing capacity in the waters and the existence of a source of acid deposition (Fig. 1). Episodes of low pH had been observed at the time of our studies. The lowest measured value was 5.3 when we initiated our studies, and such low values are known to affect some species of amphibians. The episodes of low pH occurred at a time when amphibians are breeding. These species breed in snowmelt water. They start breeding as soon as the ice is off the water, and amphibian eggs and hatchlings are generally known to be the most acid sensitive stages. A final reason to suspect acidic deposition as a stressor is that the low ionic content of the water affects the ability of amphibians to maintain ion balance in the face of an acid



Benthic densities (number per 100 cm^2 ± 1 S.E.) of Baetis in treatment channels in Experiment 1. Preacid samples were taken 24 hours prior to acid addition, postacid were taken 48 hours after the conclusion of acid addition. Asterisk indicates a significant difference between treatment channels (* p<.05, ANOVA)

Fig. 28 Benthic densities of Baetis in treatment channels in one of the stream experiments before (PREACID) and after (POSTACID) acid additions.

morning, 75 micrograms per liter is a very high level for the Sierra Nevada.

Removing the eggs from these animals requires several tricks, depending on the species. A hormone is injected to induce them to ovulate at the right time, and, if you get them from the field at the right time, they need very little manipulation. We treated them with a reconstituted soft water to mimic Emerald Lake, and this made the chemical composition off the scale for any sort of recommended ionic strength recipe for toxicity testing. We dosed them for 7 days at 15 degrees C, replacing the water every day. Data are shown in figure 4 for the yellow legged frog. pH is on the X axis, and survival through a post treatment period is in the Y axis. The typical response for embryos was a high survivorship until pH was below 5. There was a significant depression at pH 4.5 and the LC50 was in the range of pH 4.4 or 4.3. The tadpoles were hardier and had a lower LC50 than the embryos, and we saw no effects of aluminum. None of the critical pH values for any of these species was close to 5; they were all in the mid 4's or lower (Fig. 5). We did not see the expected toxic effect if acid deposition is the problem. We looked at a couple of sublethal effects. If the pH is decreased, they grow more slowly. The yellow legged frog did show a significant drop in size at pH 5.25, but only for embryos (Fig. 6). Figure 7 shows the sublethal effects that we compiled. We found premature hatching or a little shortening of hatching time in the Yosemite toad at pH 5.0. We do not want to speculate from these data on the ecological effects, because these low pH values are rare events. It is tough to make a case that the sublethal effects are consequential when we do not have any data that indicate that those pH levels are occurring in the Sierra Nevada.

It does not appear from the classical toxicity testing approach that the critical life stages were affected by pH levels that might be occurring in the field (Fig. 5). The declining species are not any different in their sensitivity to the non-decliners. When LC50's or critical pH values are compared, they are not different in the order that you would expect. The values we got for Sierra Nevada amphibians are not really different than those elsewhere, so the fact that they live in very dilute water does not seem to affect their sensitivity. Finally, we did not see any toxic effects of the added aluminum.

In the field, we did a couple of studies. One hypothesis was that the water chemistry differed between sites with amphibians and sites without amphibians (Fig. 8). If acidic conditions were influencing their distribution or a cause of disappearances, we expected to see water chemistry differences. We focussed on pH and ANC because they would reflect on both acidity and on how vulnerable the water would be to acidification. In addition , we measured electrical conductivity (EC) because low EC, reflecting the low ionic strength in the water, could aggravate amphibian sensitivity to acid conditions. Second, we wanted to test the idea that water chemistry was different between sites containing the declining species versus sites containing the non-declining species.

We chose 30 randomly selected areas from a uniform grid and surveyed these over the course

load. Thus, the exceptionally dilute waters in the Sierra Nevada may render amphibians more susceptible to acidic conditions.

Before presenting the studies we did, I would first like to briefly describe some of the natural history of the four amphibian species we studied. The mountain yellow legged frog is often very abundant. They have been observed in some of the lakes in populations of many hundreds, and they are very closely associated with water. They have not been observed wandering around the landscape, and they over-winter in the water under ice. They inhabit alpine lakes at over 11,000 feet elevation, and it is at sites like this where they are often most abundant. Tadpoles take 2-3 summers to reach metamorphosis, so they are exposed to the aquatic situation for three summers and intervening winters, experiencing 0-4°C during winter. This is a different pattern than for many other amphibians which undergo metamorphosis in the same year as the eggs are laid.

The evidence for disappearance of this species came from various sources. Figure 2 illustrates data I gathered in Sequoia and Kings Canyon National Parks in two areas where the number of populations over a 10-year period declined from 26 down to 1. That one is now extinct. Half of these populations happened to be in the area that was the subject of today's reports on water chemistry, namely Emerald Lake and up stream from Emerald Lake in the Marble Fork of the Kaweah River. There are several ongoing studies elsewhere in the Sierra Nevada that are not yet public but they corroborate this pattern of dramatic population declines. The second species is the Yosemite toad, also occurring at high elevation. The Yosemite toad is another species that is disappearing. In the latest evaluation of on-going surveys, it has disappeared from about 50% of historic sites. The third species is the pacific tree frog. This species ranges from low elevation to high elevation. It is not declining and it is still observed at just about every site it used to inhabit. The pacific tree frog spends most of its time on land in moist areas near streams, but they come down into pools for breeding in the spring snowmelt. Typically, these pools will dry up and the tadpoles will have reached metamorphosis by that time. The fourth species is the long toed salamander. This salamander ranges down from the north into the northern part of the Sierra Nevada at high elevation, and it breeds in sites still choked with snow and essentially at 0 degrees C.

We did several studies, including a dose-response study in the laboratory, and field surveys over a broad area. In the dose-response work, we wanted to test the idea that the critical life stages, primarily the eggs or early stage tadpoles, are affected by pH levels that might occur due to acidic deposition (Fig. 3). We assumed that pH 5.0 is a worst case in the Sierra Nevada. This pH is not being observed now. We also wanted to test the idea that these declining species are more sensitive to low pH than the non-declining species, and that amphibians in the Sierra Nevada might be more sensitive to low pH than those found elsewhere. Finally we added aluminum at 75 micrograms per liter to some of our treatments to test the hypothesis that aluminum mobilization was causing the declines. From what we heard this

represented in field waters. Moreover, the animals in the field are exposed to the unusual water conditions year-round, and the pH levels could be lower at other years or times than those when pH was measured. We saw that acid conditions in the field can affect these animals, but we do not really know whether this is because the animals are more sensitive to low pH in winter, whether pH is particularly low during some winters, or whether the animals are affected primarily by some other chemical constituent than hydrogen ions.

There are a couple of ongoing works that are relevant to our studies. One is a study by Cindy Carey at the University of Colorado, Boulder, on the effects of acid on immunosuppression in amphibians. The immunosuppression approach is very appealing, because we are not observing mortalities of sensitive embryo and tadpole stages in the field. Rather, sometime in the middle of summer, or following the winter, all of the adults are found to be gone. This has been observed over and over again for many different amphibian species. The immunosuppression theory is a good one, but it is also a difficult one to study.

Another study is the interaction of acid and other factors. Leslie Long at UC Santa Cruz has shown that a combination of ultraviolet B levels at high elevation and acid conditions increases the sensitivity to both factors. More work is needed that would actually relate that to conditions in the field.

The third topic that is actually more sinister concerns study of some enigmas in the pattern of distribution of disappearances of amphibians in California. In southern California, the mountain yellow-legged frog is gone from Palomar Mountains, and virtually gone from the San Jacinto, San Gabriel, and San Bernardino Mountains. There are just a couple of pockets where these frogs remain, and yet they were well represented prior to the 1960's. In the southern Sierra Nevada, the mountain yellow-legged frog is extinct from the Kaweah and Tule River drainages, which comprise the western facing drainages at the southern end of the Sierra Nevada. Yet just over a divide in the Kings Canyon-Sequoia area, that is, in the upper Kern River basin and South Fork of the Kings River, there are many populations. In addition, in the foothills of the Sierra Nevada, there is a species of lowland foothill yellow-legged frog that formerly occurred from the southern Coast Ranges and southern Sierra north to Oregon. Now the species is absent from the southern Sierra north to around Stanislaus County, except for a couple of pockets between Fresno County and Stanislaus County. These patterns of amphibian declines suggest an association with air flow patterns and air pollution, and air pollution is worse in southern California and in the southern end of the San Joaquin Valley. I raise that point as something to keep in mind, but there are really no established mechanisms for the disappearances of amphibians in this region.

DR. STEPHEN BROWN, Moderator

Questions?

of two summers (Fig. 9). Each area consisted of a circle of 15 square kilometers. We had a protocol for searching for water that contained a species and for searching for water that looked like a good habitat for the species but in which the species was absent. Out of the 30 survey areas (Fig. 10), 5 had no amphibians because there was not enough water, or the water had fish. Fish prey on amphibians, and in these alpine lakes amphibians are not found where fish are present. 25 survey areas did have amphibians. The species present were the pacific tree frog in all 25; two species of toads in 8 (6 with Yosemite toad, 2 with Western toad); the yellow legged frog in 6; and the salamander that comes in from the north in only one.

We had a fair number of sites as a basis for comparison between the two declining species and a common non-declining species. Figure 11 shows data for the widespread tree frog that is not declining, with pH on the X axis and ANC and EC on the Y axis. If acid conditions affect the distribution of populations, we expected the sites where they were present to differ in water chemistry from the sites where they were absent. The analysis of variance within sites and among sites showed no significant difference in the water chemistry associated with presence or absence of this species. This is what you would expect for a non-declining species, however we found this to be true for the declining species as well (Fig. 12). Ignore for the moment the 2 points below pH 5.0 in Figure 12 because this is that naturally acid lakes area. We saw no significant difference and, indeed, no hint of a pattern for the yellow legged frog based on presence or absence. The same is true for the Yosemite toad (Fig. 13). When you combine these data to compare the three species among themselves (Fig. 14), the water chemistry does not differ significantly among the species. Thus, from the stand point of both the laboratory and these field studies, they do not support the hypothesis either that amphibians are declining due to acidification or that they are affected by acid conditions.

We also conducted a second field study in an area of Kings Canyon National Park that is unusual in that it contains naturally acidic lakes, as well as non acidic ones. Results of this study are shown in figure 15. This is a subset of what Scott Cooper showed in his talk because this is 65 out of the 104 lakes. I have not included lakes with fish, because amphibians cannot live in those, or very shallow lakes where the tadpoles and the yellow legged frog cannot over winter under the ice. We are left with 65 lakes that represent potential habitat for the yellow legged frog. We have 8 lakes that fit in the category of pH under 6. When we studied these lakes below pH 6, the only amphibians we saw were adults. We did not note any evidence of reproduction in those lakes. This was particularly dramatic when you see some of these lakes juxtaposed; the water chemistry associated with these acid lakes was preventing these species from reproducing. What is a little puzzling, and different from some of the invertebrate work, is that the pH's measured in these lakes are not toxic to amphibian embryos and tadpoles in the laboratory. However, in the laboratory we did not have all the other chemical constituents

deposition at this point.

Q (DR. MAUTZ) I would like to make a comment. I think that in any laboratory toxicology experiment you are limited to controlling a few variables and evaluating a small sample. Therefore, it would not surprise me at all if you identified a pH that was toxic in a laboratory study, pH 4 for example, that field conditions at somewhat higher pH than that could easily induce the toxic effect on the long-term basis over the lifetime of the animal.

A (DR. BRADFORD) Exactly. That is why we took both a laboratory and field approach to this. The laboratory gives us a basis to make comparisons. The full variation in the field is the reality.

Q (AUDIENCE) These days there is a lot of talk about hormone mimics and particularly their effects on amphibians and other vertebrates. I guess some of the persistent compounds in the atmosphere could have some effects. Have you thought about those possibilities, particularly when you mention locales close to southern California? Conceivably you might have more PCB's or something of that sort in the air emissions and that could have a localized effect.

A (DR. BRADFORD) There is one study going on now investigating estrogenic effects. Gary Fellers is doing surveys in conjunction with these studies. They are using the pacific tree frog, which they can find everywhere, to look at an elevation gradient and a gradient across the state. I believe they are studying an egg-membrane forming enzyme which may be affected. In any case, they are using that as a bioassay, asking the question of whether there is any evidence of effects, but I have not seen any data on that study. It is puzzling that we still have this situation in which adult populations crash, but the reproductive pattern or production of eggs or sperm is not upset. It is as if the adults get sick and die. That argues against the estrogenic reproductive effects idea, but it is certainly one that several people are trying to work on.

Q (AUDIENCE) After hearing both parts of your talk, I was left with a feeling of ambiguity. I have seen some of your published papers, and the indication from the first part of your talk is that acidification is not having an effect on the amphibian population in the high Sierra Nevada. However, when you presented the data from the naturally acidic lakes, all of a sudden I felt like there was an acid effect. What is your conclusion?

A (DR. BRADFORD) The conclusion based on our overall effort with laboratory studies and field studies over the Sierra Nevada is that we see no direct evidence of acid effects. The laboratory work is limited to the specific laboratory conditions we tested, and is comparable to what has been done with amphibians elsewhere. The Sierra Nevada-wide field work brackets broader conditions by comparing conditions where amphibians occur with where they don't occur. If there is something else happening along with acidification, for example, if there is an interaction of UVB and pesticides and acid, it should show up in the analysis we did in the field. We are not seeing any association in the field. From those two perspectives (laboratory and field), we are not supporting the hypothesis that acid is a problem. The situation in the acid lakes area is very different than in the Sierra Nevada in general, and it is a case of chronic acidification. We do not know what the conditions are year-round, and we do not know about amphibian sensitivity to such things as 0 degrees C for six months and occasional low O_2 stress. Also, there are higher concentrations of the many chemical constituents in those waters that are associated with acidic conditions. My interpretation for why amphibians are not present in waters of lets us say pH 5.4, is that they are affected by other aspects of water chemistry, or effects that are different under winter conditions. In any case, I am not arguing that we have data that support a problem of acid

Figure 2

Rana muscosa Sites

Location	Source	Pre-1980		1989-90
Sequoia/Kings Cyn. N.P.:				
Localized Areas	Bradford	26	→	1
Parkwide	Rangers, Others	20	→	11
North of Sequoia:				
Random Survey	Bradford et al.	9	→	2
Scattered Records	Various	14	→	1

Figure 1

WHY SUSPECT ACID DEPOSITION AS A CAUSE OF AMPHIBIAN POPULATION DECLINES IN THE SIERRA NEVADA?

1. AIR POLLUTION SOURCE IN CENTRAL VALLEY.

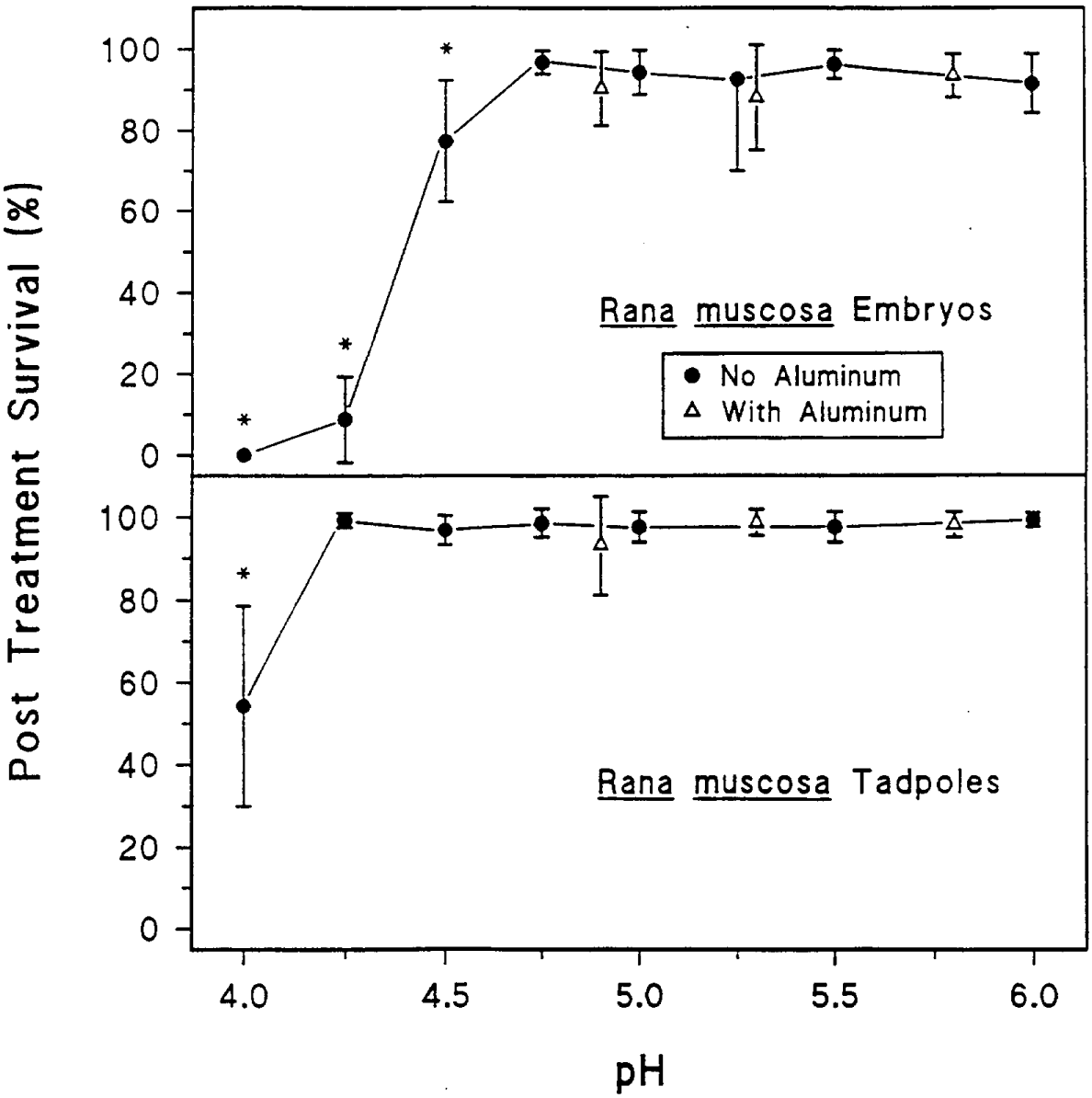
2. EXCEPTIONALLY LOW ACID-NEUTRALIZING CAPACITY OF SURFACE WATERS.

3. EPISODES OF LOW pH OCCUR AT TIME OF AMPHIBIAN BREEDING, i.e., SNOWMELT.

4. MEASURED pH LEVELS (pH = 5.3) KNOWN TO AFFECT SOME AMPHIBIAN SPECIES.

5. LOW IONIC CONTENT OF WATER AFFECTS ABILITY OF AMPHIBIANS TO MAINTAIN ION BALANCE.

Figure 4


Post Treatment Survival (%)
100
80
60
40
20
0
Rana muscosa Embryos
No Aluminum
With Aluminum
Rana muscosa Tadpoles
4.0
4.5
5.0
5.5
6.0
pH

DOSE-RESPONSE STUDIES

HYPOTHESES:

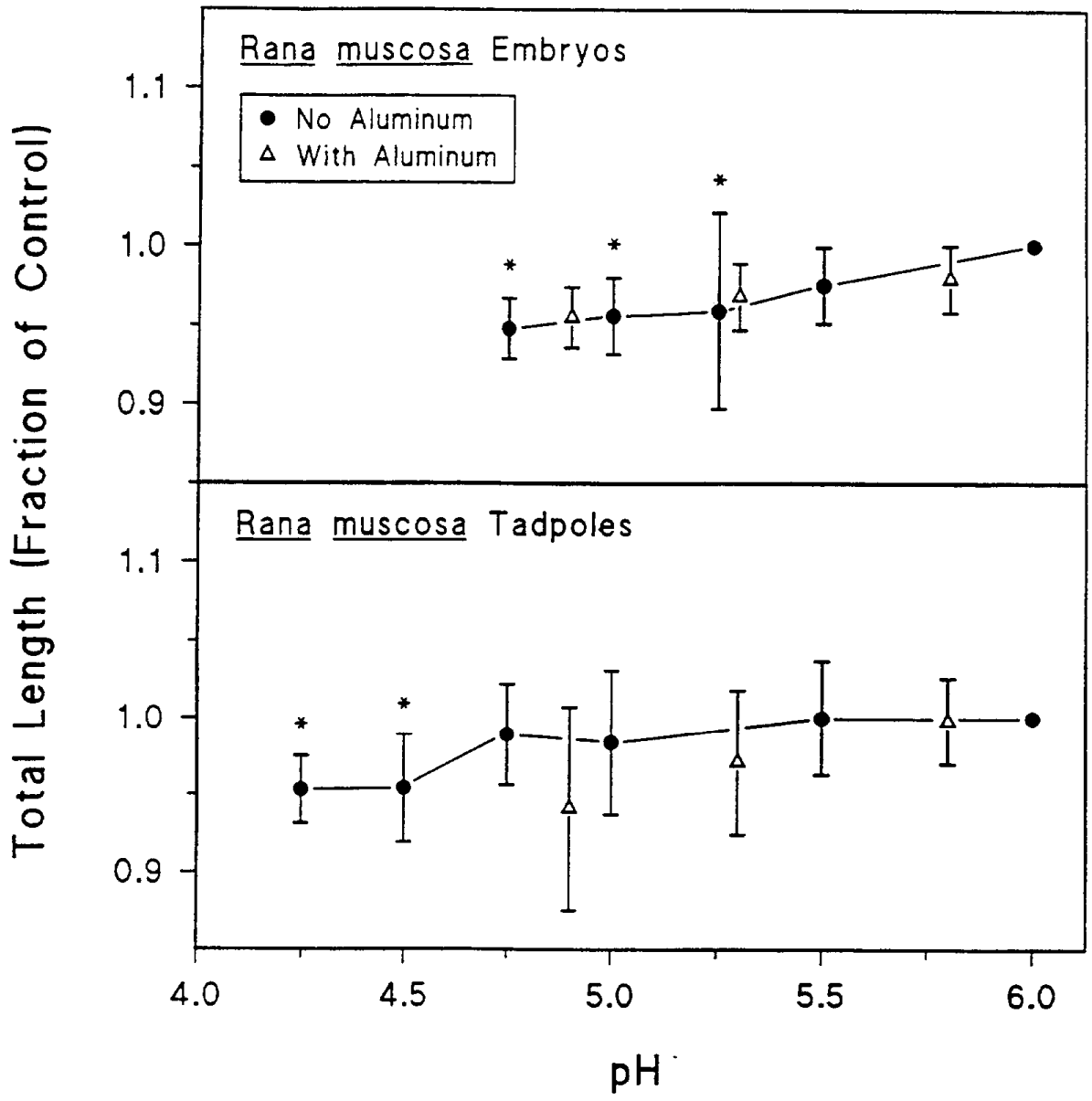
1. CRITICAL LIFE STAGES OF DECLINING SPECIES ARE ADVERSELY AFFECTED BY pH LEVELS THAT MAY OCCUR DURING EPISODES OF ACIDIFICATION IN THE FIELD.

2. DECLINING SPECIES ARE MORE SENSITIVE TO LOW pH THAN NON-DECLINING SPECIES.

3. AMPHIBIANS IN THE SIERRA NEVADA ARE MORE SENSITIVE TO LOW pH THAN AMPHIBIANS ELSEWHERE.

4. AMPHIBIANS ARE ADVERSELY AFFECTED BY TOTAL INORGANIC ALUMINUM CONCENTRATION OF 75 μg/L.

Figure 6


Rana muscosa Embryos
No Aluminum
With Aluminum
Rana muscosa Tadpoles
Total Length (Fraction of Control)
1.1
1.0
0.9
4.0
4.5
5.0
5.5
6.0
pH

POST-TREATMENT SURVIVAL

Species	Stage	LC_{50}	Critical pH
R. muscosa	Embryo	4.38	4.5
	Tadpole	<4.00	4.0
B. canorus	Embryo	4.70	4.5
	Tadpole	4.32	4.5
P. regilla	Embryo	4.41	4.25
	Tadpole	4.35	4.5
A. macrodactylum	Embryo	4.29	4.25
	Larva	4.34	4.25

Figure 5

FIELD STUDIES

HYPOTHESES:

1. WATER CHEMISTRY (pH, ANC, EC) DIFFERS BETWEEN SITES WITH AMPHIBIANS AND SITES WITHOUT.

2. WATER CHEMISTRY DIFFERS BETWEEN SITES CONTAINING DECLINING SPECIES AND SITES CONTAINING NON-DECLINING SPECIES.

INFORMATION NEEDED FOR RISK ASSESSMENT:

1. RELATIVE ABUNDANCE AND ASSOCIATED WATER CHEMISTRY FOR EACH SPECIES.

Figure 8

SUBLETHAL EFFECTS

	Critical pH	
	Length	Inc. Time
P. regilla		
embryo	4.75	4.75
larva	4.5	-
A. macrodactylum		
embryo	NE	NE
larva	4.75	-
R. muscosa		
embryo	5.25	NE
larva	4.5	-
B. canorus		
embryo	4.75	5.0
larva	4.5	-

Figure 7

Figure 10

	No. Survey Areas (15 km²)	No. Sites Sampled
Amphibians Present	25	143
Amphibians Absent	5	93
TOTAL	30	236
Species Present:		
Hyla regilla	25	126
Bufo spp.	8	20
Rana muscosa	6	21
Ambystoma macrodactylum	1	4

Figure 9

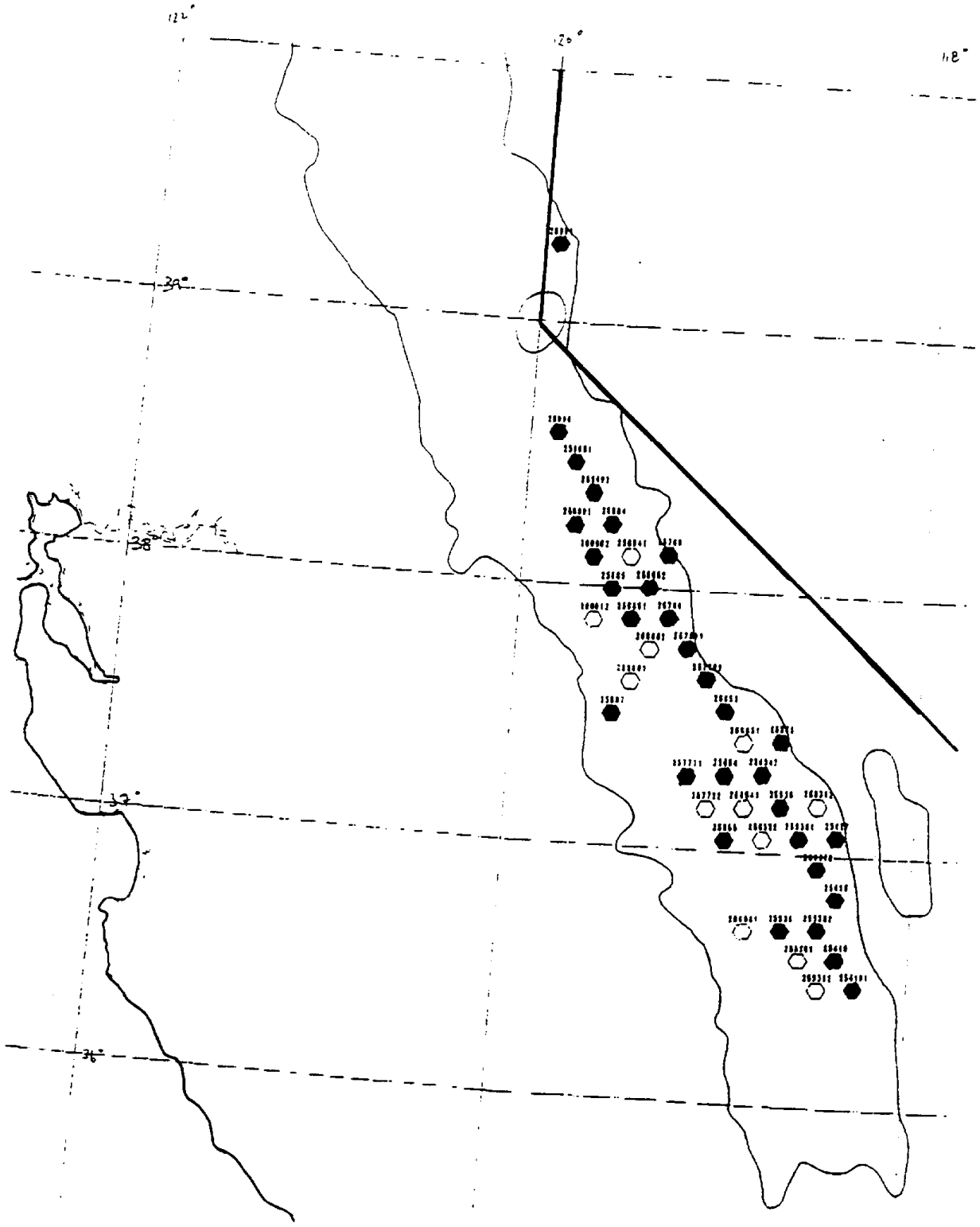


Figure 12

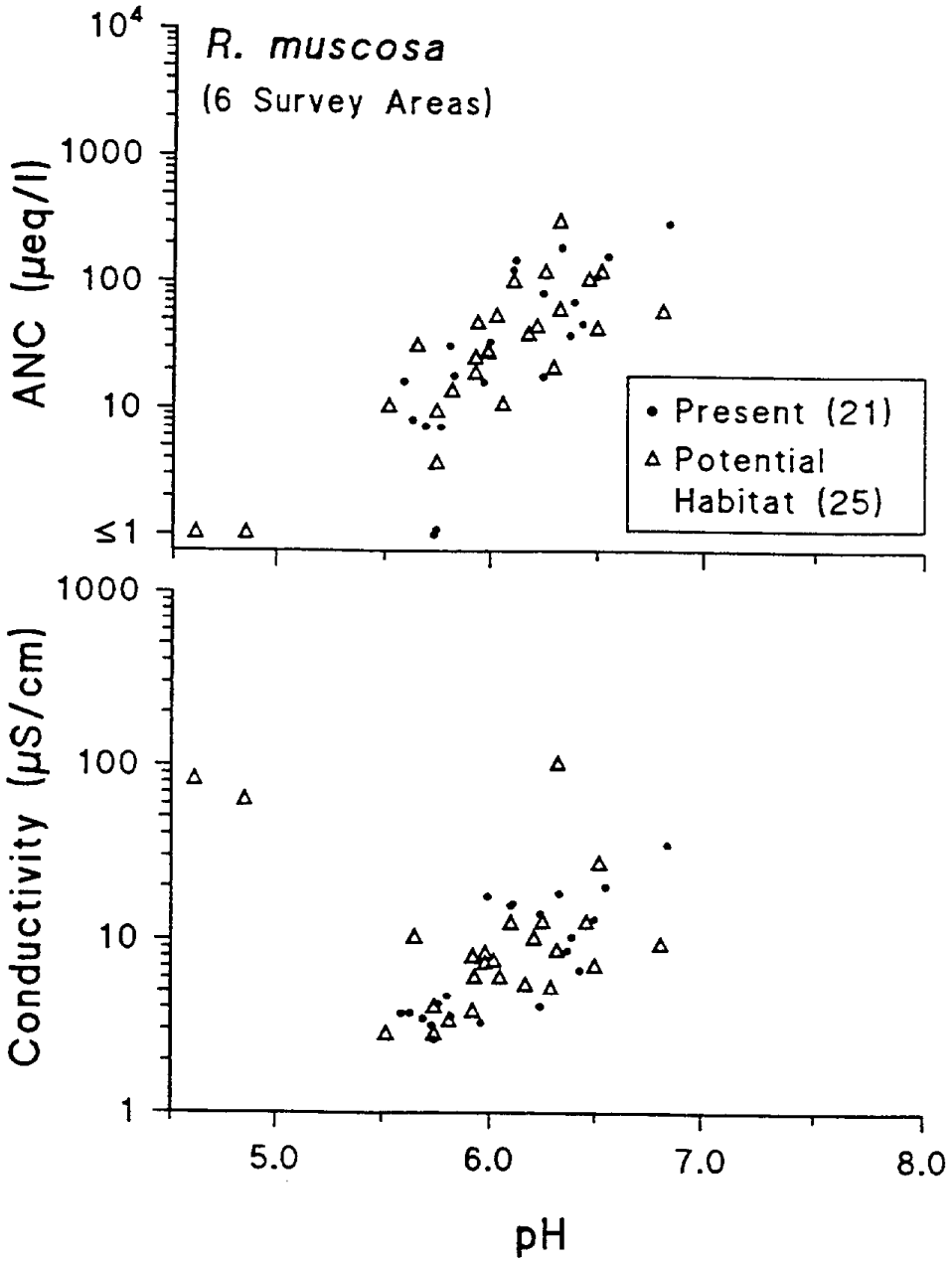

R. muscosa
(6 Survey Areas)
ANC (μeq/l)
10⁴
1000
100
10
≤1
• Present (21)
△ Potential Habitat (25)
Conductivity (μS/cm)
1000
100
10
1
5.0
6.0
7.0
8.0
pH

Figure 11

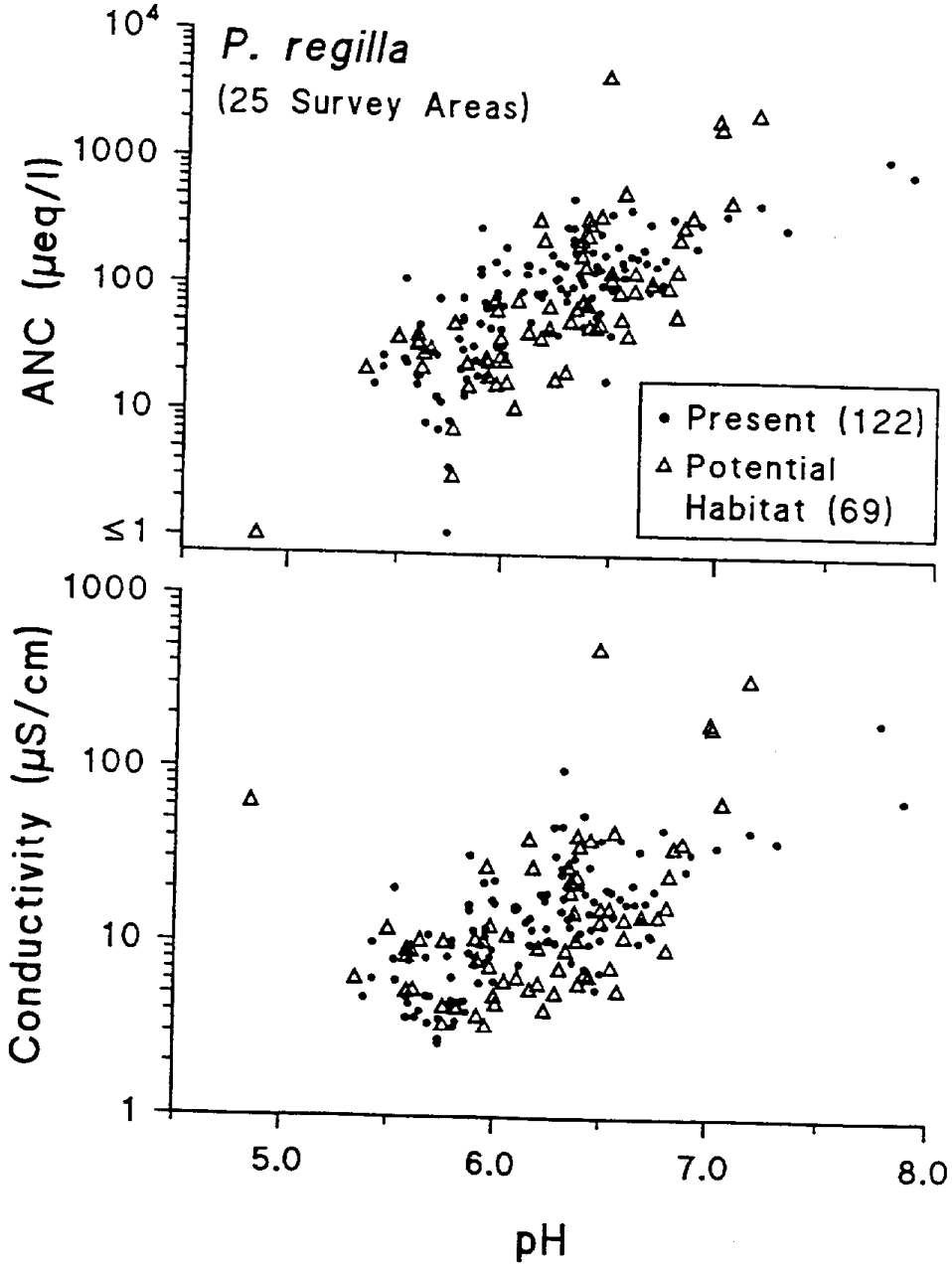

P. regilla
(25 Survey Areas)
ANC (μeq/l)
10⁴
1000
100
10
≤1
• Present (122)
△ Potential Habitat (69)
Conductivity (μS/cm)
1000
100
10
1
5.0
6.0
7.0
8.0
pH

Figure 14

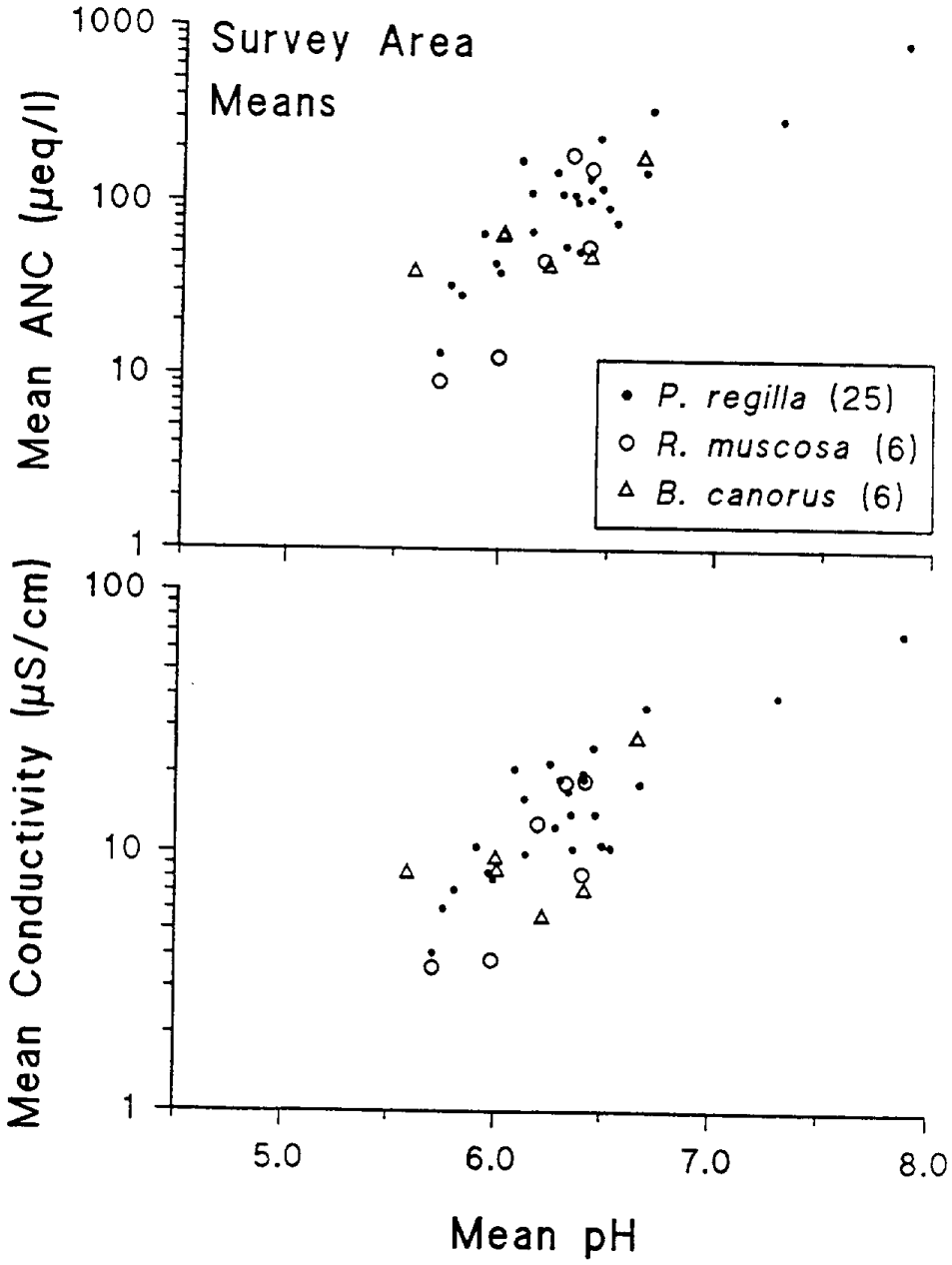
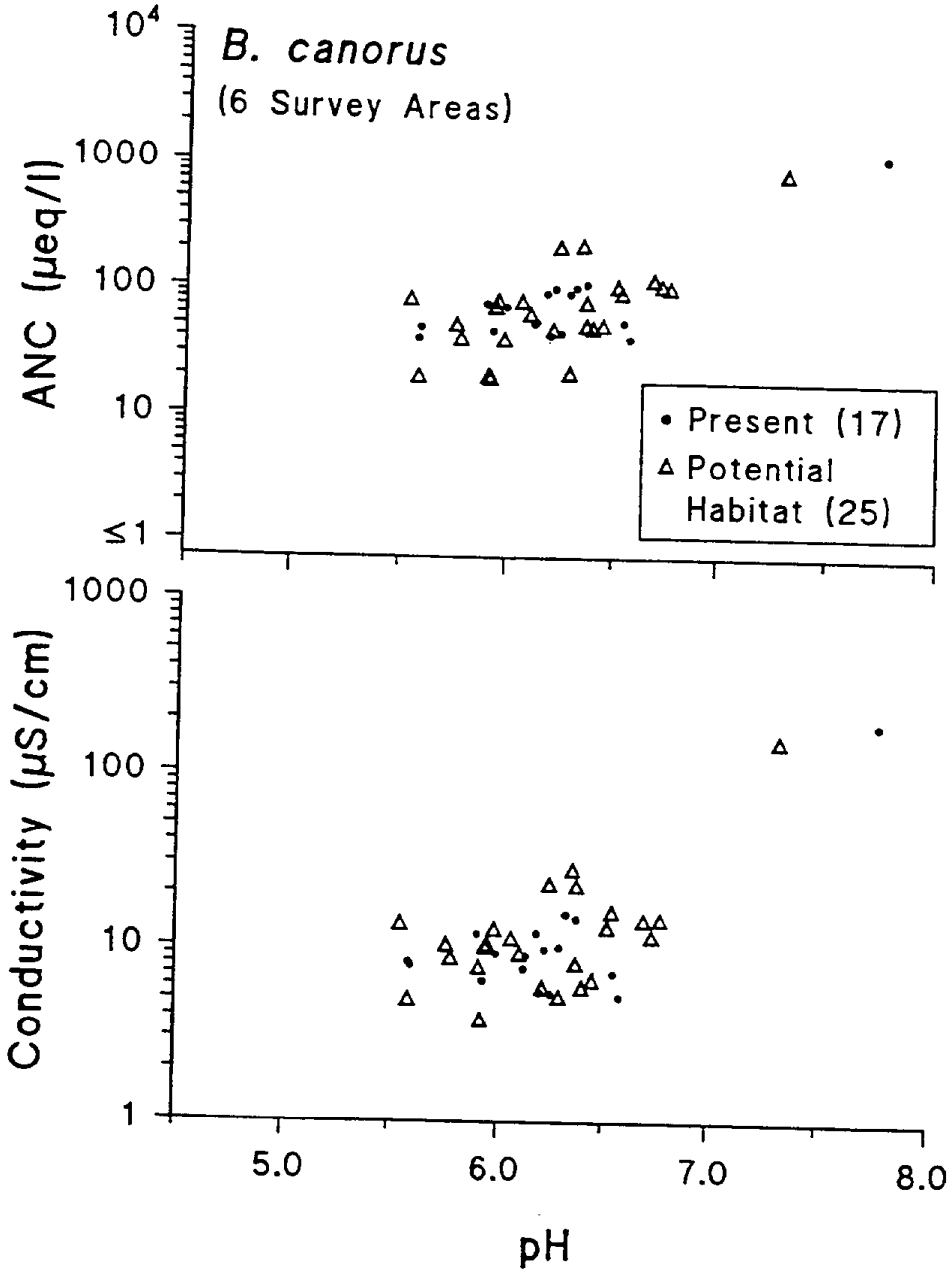

Survey Area
Means
1000
100
10
1
Mean ANC (μeq/l)
P. regilla (25)
R. muscosa (6)
B. canorus (6)
100
10
1
Mean Conductivity (μS/cm)
5.0
6.0
7.0
8.0
Mean pH

Figure 13


B. canorus
(6 Survey Areas)
ANC (μeq/l)
10⁴
1000
100
10
≤1
• Present (17)
△ Potential Habitat (25)
Conductivity (μS/cm)
1000
100
10
1
5.0
6.0
7.0
8.0
pH

DR. STEPHEN BROWN, Moderator

The next speaker is Diana Engle "Assessing the Impact of Acid Deposition in Sierra Aquatic Ecosystems: Tying together the Hydrochemical Biogeochemical and Biological Processes".

7. Assessing the Impact of Acid Deposition in Sierra Nevada Aquatic Ecosystems: Tying Together the Hydrochemical, Biogeochemical and Biological Processes. Dr. Diana Engle, U.C. Santa Barbara.

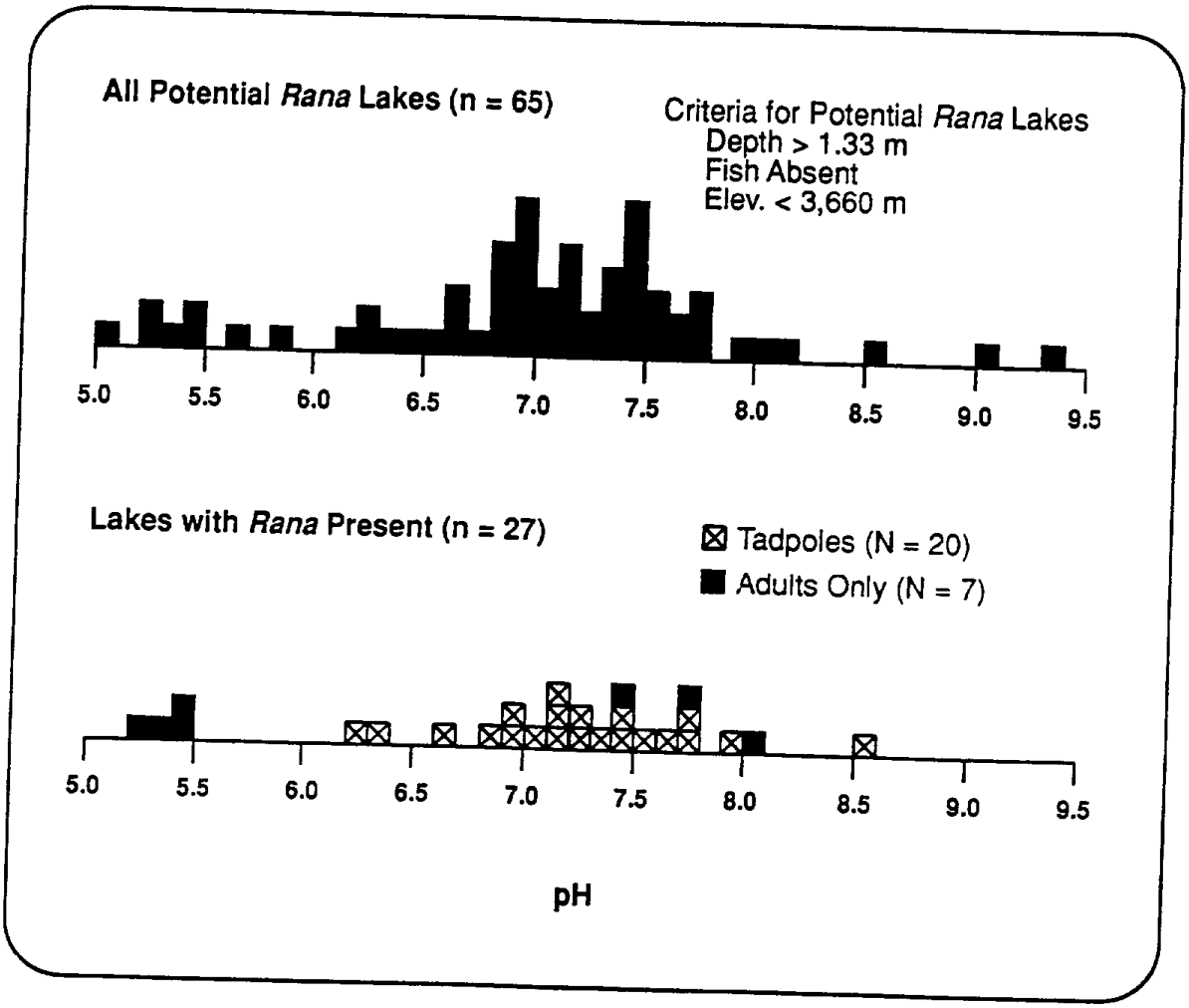
DR. DIANA ENGLE, UC Santa Barbara

For the last year or so, I have been reviewing the reports and publications that stem from the aquatic portion of the research program with the CARB. My job is to synthesize this material into one document. There is an enormous amount of very high quality hydrological data, hydrochemical data, biogeochemical data, soil data, vegetation data, and zooplankton data, but what is missing is the bottom line: whether or not acidic precipitation is ultimately responsible for ecosystem changes. We may know that a multitude of complicated interactions are present, but what is the consequence of these interactions for the biota? The approach I am taking in this synthesis is to determine the timing of different categories of events and determine when biological processes interact with hydrochemical and biogeochemical processes.

I have divided the hydrological year into units, and chose periods to call either snowmelt, summer or autumn/winter. That more or less categorizes what we have seen in a number of different Sierran watersheds. For example, what I refer to as snowmelt, covers the period April, May, and June (Fig. 1). Some leeway might be needed on both sides of that time period if you were examining all processes in a watershed, but it is a ballpark range for the months in which snowmelt occurs. I have listed at least a few of the biological processes which occur with during this time period. Sensitive zooplankton that occur in the water column during those months could be affected by acid in the ion pulse during snowmelt. The larvae of amphibians that use temporary ponds for breeding could be affected as we have already heard. There are different kinds of trout in the Sierra Nevada. There are spring spawning trout and fall spawning trout. The swim up fry of fall spawning trout that emerge from the gravel and start to feed during these months are vulnerable during snowmelt. The swim-up fry of spring-spawning trout do not occur until later in the year, however there are sac fry of these species in the gravel of the streams which have not emerged and could potentially be effected by ionic pulses during snowmelt too.

Figure 2 shows the zooplankton pH ranges. We have already heard about the experiments that were done in Emerald Lake to determine the critical ranges of pH sensitivity for zooplankton in the Sierra Nevada. We found that Polyarthra and Karatella are fairly resistant. I have indicated that by showing the extent of their tolerance down to as low as pH 4. Holopedium and Bosmina are somewhat resistant

Figure 15


All Potential *Rana* Lakes (n = 65)
Criteria for Potential *Rana* Lakes
Depth > 1.33 m
Fish Absent
Elev. < 3,660 m
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
Lakes with *Rana* Present (n = 27)
Tadpoles (N = 20)
Adults Only (N = 7)
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
pH

more sensitive species, occurs almost outside the normal timing of snowmelt discharge. Even if pH does drop in this lake, Daphnia may not be affected, whereas Diaptomus intersects with that period of time and might show the effects of increased acidification more quickly than Daphnia.

In Pear Lake (Fig. 7) the duration of snowmelt discharge and the timing of ice cover also varies from year to year. The pH was very close to the critical pH value for zooplankton in lake outflows in 1991. In this lake, zooplankton (Fig. 8) are actually up in the water column during snowmelt. For the most part, the sensitive species like Diaptomus and Daphnia are not in the water column at that time of year, so this might be a lake in which the effects of increased acidity on zooplankton would be delayed or not observed at all.

A one-time summer visit to get a zooplankton species inventory does not necessarily tell you much when sensitive species is still present. The lake may be experiencing some strong effects of snowmelt discharge, and there may be other sensitive species that were not detected that summer. Ruby Lake (Fig. 9), is the largest and deepest of those seven lakes studied and has the same situation in which the normal timing of snowmelt does not really intercept the timing of population peaks for Daphnia rosea. Among the species that do coincide with snowmelt, Keratella and Polyarthra (Fig. 10) are fairly acid-resistant species.

David Bradford talked in detail about the amphibians, so I will simply summarize what we know (Fig. 11). As Dave said, the embryos and larvae of amphibians are more susceptible to low pH effects than the adults. Between pH 4.5 and 5.0, the growth and recruitment of tadpoles and salamander larvae decline. Below pH 4.5 complete mortality of embryos can occur. I think it is an important point that the occupants of temporary ponds are more vulnerable than those of permanent ponds because they rely on an explosive spring breeding. The permanent pond species adults often establish territories and may breed throughout the summer when they are past the danger point of decreased ANC or acidic pulses. The 5 listed breeding amphibians that do occur at high elevation in the Sierra Nevada are the Yosemite toad, western toad, pacific tree frog, mountain yellow-legged frog, and the long-toad salamander. Of all of these, only two taxa showed recent and rather surprising declines, the mountain yellow legged frog and the Yosemite toad. We have experimental data on these (Fig. 12). The big question is, whether or not pH was a likely factor which contributed to the recent decline of these two amphibians. Experimental evidence reveals that the LC50 of R. muscosa embryos is pH 4.4 and that of tadpoles is a little bit lower than 4.0. In lake survey data, tadpoles are not found in lakes with pH less than 6. High concentrations of other solutes may be involved in this field distribution. For example, high levels of aluminum or sulfate may also occur in the lakes. Out of 12 lakes in which tadpoles were found, trout were absent, so factors other than chemical factors could play a role in distribution. For the toad, B. canorus, LC50's are a little bit higher. Embryos are more resistant than tadpoles. However, in the synoptic survey by

and show increases at lower pH due to competitive release. Conochilus is a little more sensitive, because it is a rotifer species that is common in the lake. The most sensitive zooplankton seem to be the various Diaptomus species and Daphnia which, as Scott showed you, start to decline, right at about pH 5.5 and then pretty much disappear altogether when the pH gets close to 5.

How can we decide which zooplankton are sensitive? We know the results of bag experiments done at a particular time of year which was not necessarily snowmelt time. Using that information, we have to decide whether or not the species that are present during snowmelt might be vulnerable. For Emerald Lake, I was able to acquire the information shown in figure 3. I have listed, for 5 years, the month in which the bulk of snowmelt discharge occurred and the lowest pH was observed in lake outflows. When I could find the information, I included the lowest volume-weighted mean lake pH during those seasons and also at the end of ice cover. The end of ice cover is important because when the lake is ice covered, there could be inverse temperature stratification and stream inflow with an ionic pulse could be carried right across the top of the lake and out to lake outflows without mixing into the water column. This illustrates that co-occurrence of events is not the only factor determining impact on the biota column. Zooplankton may actually avoid an acid pulse if there is ice over the lake at that time.

In figure 4, I have shown adult plankton species chronologies that I developed for these lakes. Scott showed you a graph of Daphnia rosea from Emerald Lake and talked about inter-annual differences in the size of population peaks, as well as differences in timing. For example, in Emerald Lake, Daphnia rosea can be expected to peak at any point between June to late September. Holopedium can be expected to peak, depending on the year, anywhere from April through September. Combining all this information, the species that are present during snowmelt in Emerald Lake are Holopedium (one of the slightly more resistant species), and Keratella tourocephalus, Keratella quadrata (a more vulnerable species), and Polyarthra. In some years population peaks in Daphnia and Diaptomus do intersect with snowmelt, but there are also years in which they peak outside of snowmelt. Analysis of the volume-weighted mean lake pH shows that even when these species are present in the water column during snowmelt, the pH is not low enough be detrimental.

Another one of the seven lakes that have been studied is shown in figure 5. Snowmelt tends to start later in Crystal Lake. At the time of snowmelt discharge, the pH of the water in lake outflows affecting the stream benthos is not the same as the pH of water that comes out of the snowpack. The volume- weighted mean lake pH values are fairly well above the values that might be considered threatening to zooplankton. The typical timing of snowmelt discharge for this lake is shown in figure 6. This lake has different zooplankton dynamics. There are several species which are very erratic in their population dynamics. They can show population peaks in almost any month of the year. There are others which are restricted pretty much to the summer months. In this case, Daphnia rosea, one of the

Another biological process that intercepts or coincides with the summer period, is the effect of through-fall on rainfall chemistry (Fig. 17). The ratio of ammonia to hydrogen ion is greater than 1 in summer precipitation, which is not true in snowfall. In the summertime, ammonium has an important role in the neutralization of strong acid ions such as nitrate, sulfate, and chloride. Without ammonium, summer rain could be as much as 11 times more acidic than it is now. We discovered that foliage retains almost all of the ammonium in summer rain. Foliage doubles nitrate in through-fall via leaching and washing off of dry deposition. The result of this process was that the through-fall has lower ANC and is more acidic than summer rain. The net effect of through-fall on ANC by flora was small on a basin scale, and in the basins we studied, the area covered by vegetation was small. However, I think that through-fall could be important in localized habitats and important for some biota, for example, small ponds. One of the reports mentioned that a number of ponds where tadpoles were not observed were surrounded by live vegetation. If vegetation is increasing acidity, this could have important localized effects on small habitats. One of mechanisms for ANC generation in these watersheds is sulfate retention. Figure 18 lists five main mechanisms available in these watersheds for sulfate retention. The first is sulfate absorption onto iron and aluminum oxide. The second is sulfate precipitation with aluminum to form minerals such as basaluminite, alunite, jurbanite. The third mechanism is microbial incorporation of sulfate into a carbon bonded sulfur and as an ester sulfate. Number four is microbial sulfate reduction followed by volatilization of hydrogen sulfide. This would take place only in water logged soil or lake sediment. The fifth is sulfate uptake by vegetation. This can have a variety of effects. When sulfur is in excess in the environment, sulfate taken up by plants can be stored in foliage as sulfate. Subsequently, it is easily leached from litter, and has a faster turn over-pool in the vegetation. However, when sulfur is in greater demand, it can be incorporated into more resistant compounds in the plant tissue and then be sequestered longer in vegetation. Temperature, moisture and other factors that might affect microbial activity could also have effects on the capacity of these watersheds to retain sulfate. However, in general, sulfur immobilized by the biota is a low percentage of ecosystem sulfur retention; most sulfur retention occurs in the mineral soil.

There were a number of ways the biota mediate hydrogen transformations and nitrate uptake, which is the important mechanism for ANC generation (Fig. 19). Nitrification consumes ANC and produces nitrate, and this particular process has a double effect. It not only puts protons out, but it releases nitrate in the soil solution which could exacerbate nitrate mediated cation leaching. Both protons and a particularly mobile acidic cation are present. Exactly when nitrification takes place during the hydrological year cannot be determined. We do know that it is going to occur after the initiation of snowmelt. Whether or not it will take place under the snowpack is still in question. There is not necessarily a reason to believe it cannot be going on, but Aaron Brown has measured a lack of nitrate

Bradford, Bufo was not found at all.

There are two groups of trout, the fall spawners (Fig. 13) and the spring spawners (Fig. 14). In general, fall spawning trout are slightly more acid resistant than spring spawning trout. The two fall spawning trout species in the Sierra Nevada are brown trout and brook trout. The timing of their fall reproductive activities is a little bit different. Spawning takes place a little earlier in the fall for brown trout and extends over a slightly greater length of time. Hatching for both species occurs under ice through the winter, and emergence from gravel occurs a little earlier for brown trout. Indeed, the swim-up fry for this species come up into the streams at a time at which they could be vulnerable to snowmelt low pH. Brook trout swim-up fry emerge from the gravel a little later, and they may be more at risk from summertime storms (Fig. 15) with low pH. These two trout, even though they are both fall spawners, may be subject to slightly different risks from different kinds of precipitation events. I have listed some of the critical pH values that we have seen from experiments with brook trout (Fig. 13). The adults are pretty hardy; they will not die until pH falls below 4.1, and the same is true for eggs. The most sensitive life stage is the fry. It is the time before emergence and just after emergence that it is most critical. In the Sierra Nevada, there are cases when the pH in stream waters reaches critical values either during spring snowmelt or summertime rain, so there could be an impact on brook trout. Spring spawning trout, include three species; the golden trout, rainbow trout, and cutthroat trout. The adults spawn in June and July, and the eggs are subject to low pH from snowmelt if it occurs that late. Hatching takes place in August, and emergence from gravel of swim-up fry does not occur until later in the summer. In this case, the summertime rainstorms might be a much greater issue of concern, and in these cases, the swim-up fry are at the most sensitive life stage and can be affected by pH levels as high as 5.5, whereas the eggs are much more resistant and will not die until a full pH unit lower is reached. The fertilized eggs of the spring spawning trout are thus susceptible to snowmelt water, although pH would have to be a lot lower than currently recorded. A full pH unit drop is unlikely.

Summer rainstorms can effect a variety of biota including stream invertebrates, larval stages of amphibians using the territories of permanent ponds as breeding sites, and the swim-up fry of stream-spawning trout just mentioned. At least one of the microbial processes can be affected by summer rains. Rates of denitrification are affected by rain or dry spells. Ion fluxes in vegetated zones can be affected by summer rainstorms because of the effects of through-fall.

A review of what Scott told in detail about the stream invertebrates is outlined in figure 16. At the site of acidification in streams due to snowmelt or summer rains, the benthic density of some species of stream invertebrates may decrease. Four main vulnerable species were identified in the stream channel experiments including mayfly nymphs and chironomid larvae. A great percentage of the increased drift observed during acid pulses as short as 8 hours was due to mortality.

what the magnitude of changes in basin ANC could be accomplished just by uptake of strong acid ions by phytoplankton for example. Certainly the sediments are a source of ANC in some of the lakes, but not in every year. I have not tried to figure out, for example, the total uptake of nitrate from the water column.

Q (AUDIENCE) I am speculating about a likely similar influence of acidity on the rate of primary productivity and the rate of survival and successful predation by zooplankton. After all, they are not at the top of the food chain. The zooplankton depend upon the existence of the phytoplankton.

A (DR. ENGLE) I do not know. Scott, did we do phytoplankton counts in the bay experiments?

Q (AUDIENCE#2) There are two issues. Jim Sickman did a lot of measurements of primary production in the lake through time. We did look at species composition of the phytoplankton. There were shifts in species composition in response to acid input, but there was no change, in most cases, in biomass of phytoplankton in those bags. We didn't measure primary production per se, but at least biomass didn't change.

A (DR. ENGLE) In general, it seems that most studies try to use biomass, and most studies try to use phytoplankton biomass as an indicator. Even zooplankton biomass is a difficult issue, because you get subtle shifts in species composition due to differential resistance to acidity. You can even get increases in biomass before you get a subsequent decline. So I think biomass is a really tricky parameter to try to use as an indicator in the acidity problem.

Q (AUDIENCE) Regarding the zooplankton distribution as a function of time. You interpreted those to indicate that the sensitive species weren't affected. Could not you also have interpreted that to say that because they are not occurring when pH is low, near 5.5, that they are actually avoiding that period? Couldn't you use that as evidence of there being an effect? Could you not reverse the mechanism?

A (DR. ENGLE) We have been going out on this limb on an awful lot ever since I began this study. It is true, you can say that the natural population dynamics are such that they are not in a vulnerable period, or you could take a longer view of history on the lake and speculate that they do not appear in the lakes now because of a long term negative effect that has already affected on the distributions. Before I would be willing to go out on that limb, I would have to look at ice cover and a lot of other factors to decide whether or not those species would really take off naturally at those times anyway. Yes, that is an alternative interpretation. It is very risky to take that interpretation, but it is possible.

Q (AUDIENCE) I have a specific point, then I have a question. The specific point is, I think it is important to show the juxtaposition of life history events and when we expect acid deposition

in soil solution under the snowpack. That would lead us to believe that nitrification is not proceeding without time. Plant uptake of ammonium and release of protons is another ANC consuming mechanism. These processes together have been estimated in the Emerald Lake watershed to consume as much as 25% of the acid neutralizing capacity that is produced by geochemical weathering. Denitrification works against nitrification; it increases ANC on a molar basis. However, it cannot occur if soils are frozen or dry. Rates that have been measured in field work were maximal after summer rains. We know denitrification is occurring under the snowpack, but we do not know how important it is. In the Emerald Lake watershed, denitrification is estimated to remove only about 5% of deposition.

We know what and how much leaks out compared to the quantity cycling in the watershed. We know that organic nitrogen dominates nitrogen export in the stream waters. Regardless of the nitrate and the ammonium coming in, being stored in the snowpack, and interacting with talus on its way down to the lake, by the time it leaves the watershed, most of it is in organic form. The amount of nitrogen that is cycling annually in the watershed via uptake by above-ground plant biomass and released subsequently from litter is a greater amount of nitrogen than is falling annually through atmospheric deposition. This implies, that if something else in the watershed changes the net production of vegetation or affects remineralization rates, it could potentially have effects on mass balances regardless of what is going on with snowmelt or the timing of snowmelt or the size of the snowpack.

DR. STEPHEN BROWN, Moderator

Questions?

Q (AUDIENCE) I think what you have shown is very helpful. I would like to consider what I would call time scales or timing for processes described by other speakers from this morning. A couple of them come to mind. What is the time scale for the generation of readily available acid neutralizing capacity in the watershed? What is the size of the clay fraction compared to the size of the hard rock? There is a time scale that is longer than what happens each year. It is a time scale that describes the life of the watershed even though we tend not to think more than about 70 years at a time. The history of the lake is probably much longer, but we do not know how much longer, so these time scales that you talked about are biological and have to be compared with geochemical time scales. I think you have done a very valuable thing by calling them the right time scales.

One thing I am curious about is why the term primary productivity never occurs in this discussion, it seems to me that everything rests on that.

A (DR. ENGLE) Well, I threw in net production, because I was thinking in terms of vegetation - -

Q (AUDIENCE) No, I am not thinking about the vegetation; I am thinking about the lake.

A (DR. ENGLE) The phytoplankton. Well, there has not yet been much thought put into

pH going to mean that the community or species does not reproduce later in the season?

A (DR. ENGLE) I am pretty skeptical about this whole zooplankton issue, because there are also resting spores that come up and seed the water column with recruits after some of these acid pulse events happen for certain species. It is a little uncertain. If you kill an adult fish, it is dead, and it is not going to spawn again, but if you kill 80% of the Daphnia because you get a several hour strong acid pulse, what happens to the other 20% there? Their populations could jump back up in almost no time depending on the algal supply. So yes, it is another complication.

Q (AUDIENCE) I have a response, a comment, and a question. First a response to Jim Morgan's point about putting together a geologic time or reaction time scale. I think there are two reactions that are important here. One is weathering and all the information I have read and calculations I have tried to do based on laboratory studies show that weathering can operate at a speed which is far faster than necessary to neutralize incoming acidity. The same goes for exchange. Exchange capacity is huge relative to the grams of material that are present or the depth of material present, because the surface area of the clay minerals is large and exchange reactions are extremely fast as measured in the laboratories. The part that is the problem, and Roger made a comment about this earlier, and this is what I have been trying to work on, is the hydrochemical problem. It is dependent on where the water goes and with what it interacts. The water does not necessarily see every part of the soil. It may only see a very thin layer or a very thin layer on the surface of the rock. It is turning out to be a very difficult thing to quantify especially at the field level. The comment I have is that your approach is excellent. This time approach to looking at when the species are present is really good. By doing that, a value judgment has been made that the most important value to us is the health of the species. What you have not looked at, and what I am throwing up as a general question, is are there other important things? Is the taste of water important? Is visibility important? ANC would have an effect on that. Is the clarity of water important? I do not know what effects deposition might have on clarity? The fertility of soil at high elevation is important. These ecosystems at high elevation appear to be nitrogen starved. They take up nitrogen and nitrogen disappears when it comes into the watersheds. If we add more nitrogen to those watersheds, will there be an undesirable effect in the long term?

to occur. What surprised me is that there are two sources of acid deposition in these lakes. One is dilution in the snowpack and acid events associated with snowmelt, but there are also summer rainstorms. The one thing that surprised me is when you made a list of things likely to be affected by summer rainstorms, you do not list the zooplankton.

A (DR. ENGLE) I am torn about whether or not the zooplankton data on summer rain is indicative of significant effects.

Q (AUDIENCE) My second general comment is that if you look at acid loading, it is a function of both volume and concentration. In the case of snowmelt, you get a huge volume, but the concentration may not be that great, and if you get dilution effects, you might not expect pH values to fall much lower than 5.5. On the other hand, the summer rainstorms have much lower volumes, but they are much more concentrated. If you were looking for acid deposition effects, would you concentrate on snowmelt, or would you concentrate on summer rainstorms?

A (DR. ENGLE) I am not sure how I would answer that right now, because I have not seen any pH profile of a lake right after a storm, that would give me any indication of what happens to the stream water when it hits the epilimnion of a lake. I do not know what happens to it. I have not seen a comparison of inflow versus outflow during a rainstorm; in other words, how the lake itself serves as a reactor vessel for that water.

Q (AUDIENCE) I think to evaluate that question, you need a lot more meteorologic data, so you know the probability of getting a summer rainstorm and of a given magnitude. That might help answer these questions about where first to look for the impact.

A (DR. ENGLE) When we were working with this material earlier, we felt that it would be too difficult to try to set up a long-term monitoring that was designed to measure the effects of rainstorms, because they are so erratic and unpredictable. One thing we do know for sure that will happen every year is snowmelt.

Q (AUDIENCE) I am trying to understand for these species of the zooplankton and the amphibians you referred to, is it the inlet streams, the lakes, or the outlet streams that have locations where the interest in the pH depression and ANC is greatest?

A (DR. ENGLE) With respect to the benthic invertebrates in those streams either inlet streams or outlet streams would be important. It will depend on the watershed and the size of the streams. Some of the inlets for Emerald Lake are mere trickles, so I do not really know how important they are in terms of benthic productivity for streams.

Q (AUDIENCE) How about watersheds without a lake?

A (DR. ENGLE) Well, then of course, the zooplankton are not an issue.

Q (AUDIENCE) For which species is the 3-5 day short-term scale episodic depression in

Figure 2

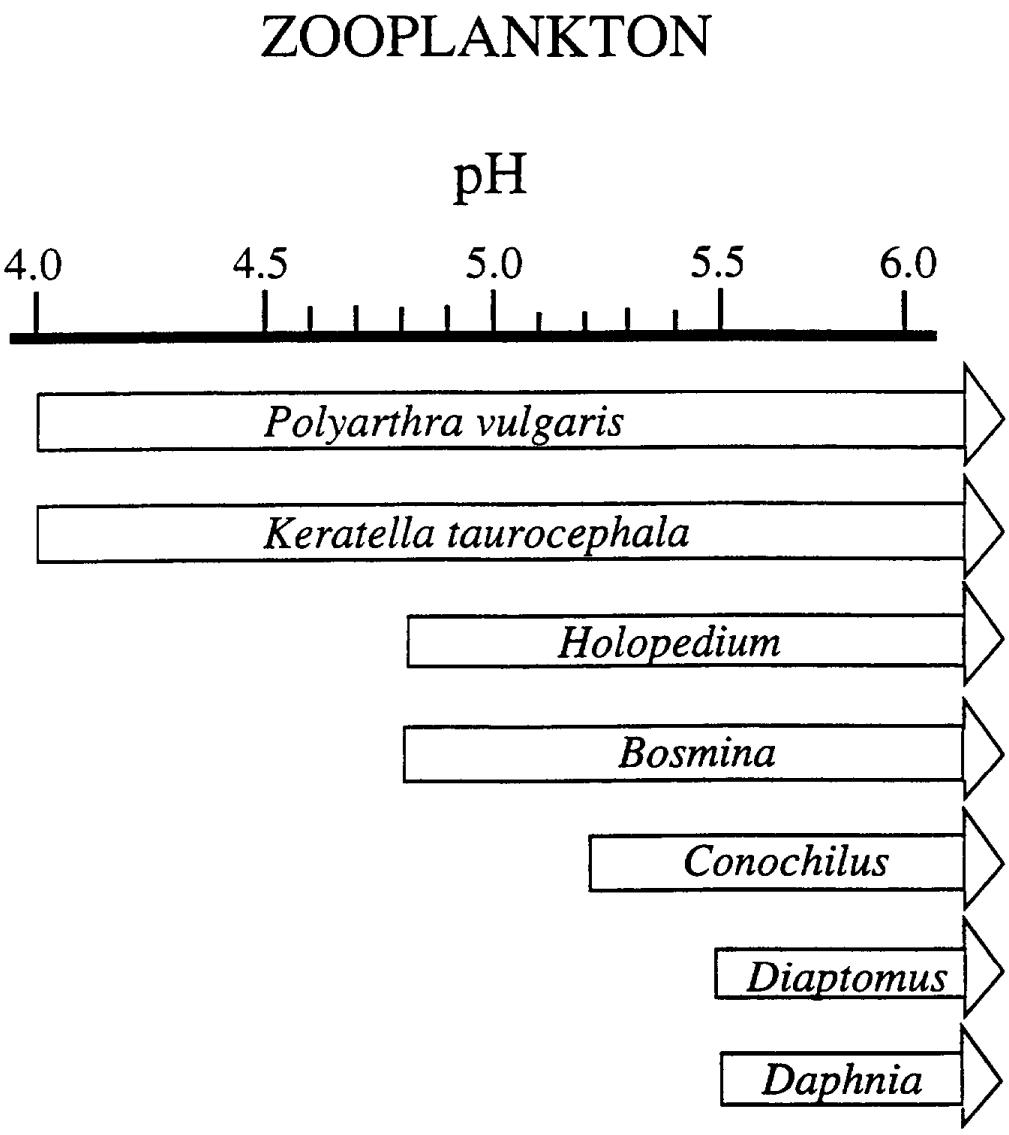
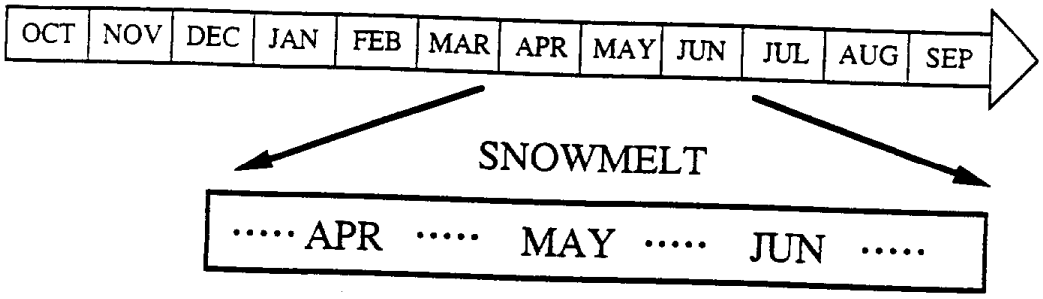

ZOOPLANKTON
pH
4.0
4.5
5.0
5.5
6.0
Polyarthra vulgaris
Keratella taurocephala
Holopedium
Bosmina
Conochilus
Diaptomus
Daphnia

Figure 1



Acidic snowmelt runoff potentially impacts:

* Sensitive zooplankton occurring during snowmelt - the species involved vary between lakes.

* Larvae of amphibians using temporary ponds for breeding.

* Swim-up fry of fall spawning trout.

* Sac-fry of spring spawning trout.

Figure 4

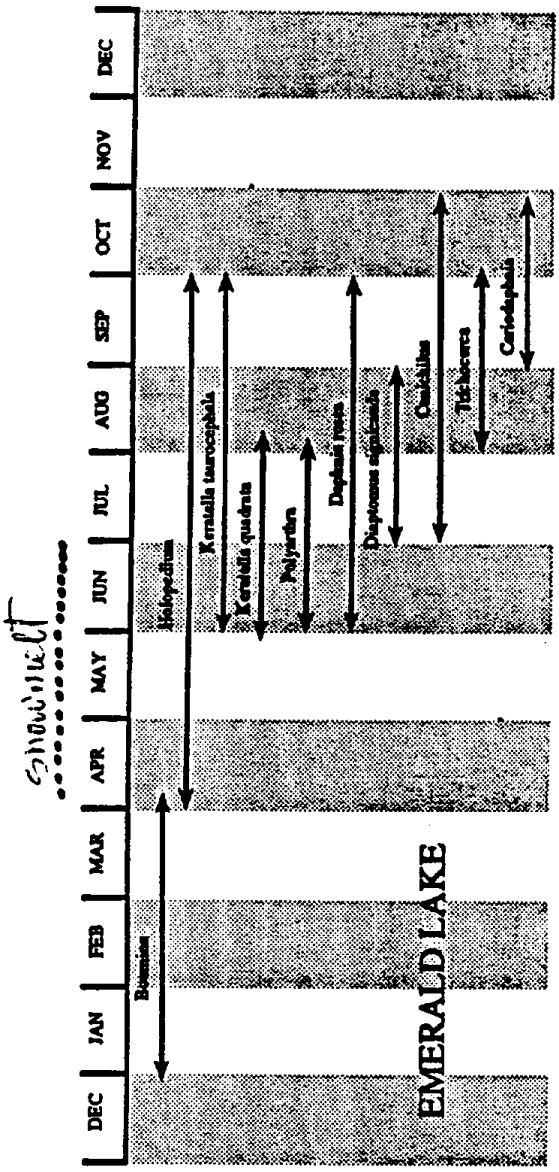

DEC
NOV
OCT
SEP
AUG
JUL
JUN
MAY
APR
MAR
FEB
JAN
DEC
Bosmina
Holopedium
Keratella taurocephala
Keratella quadrata
Polyarthra
Daphnia rosea
Diaptomus sicilis
Ceriodaphnia
EMERALD LAKE

Figure 3

EMERALD LAKE

Year	Months of greatest snowmelt discharge	Lowest pH observed in lake outflow	Lowest volume weighted mean lake pH	End of ice cover
1985	May-June	6.1 (May)	---------	
1986	May-June-July	5.7 (June)	---------	
1987	Apr-May-June	5.7 (May)	---------	
1990	Apr-May-June	5.9 (May)	6.2 (Jun)	end-May
1991	Apr-May-June	5.6 (May)	5.7 (Apr)	end-May

Figure 6

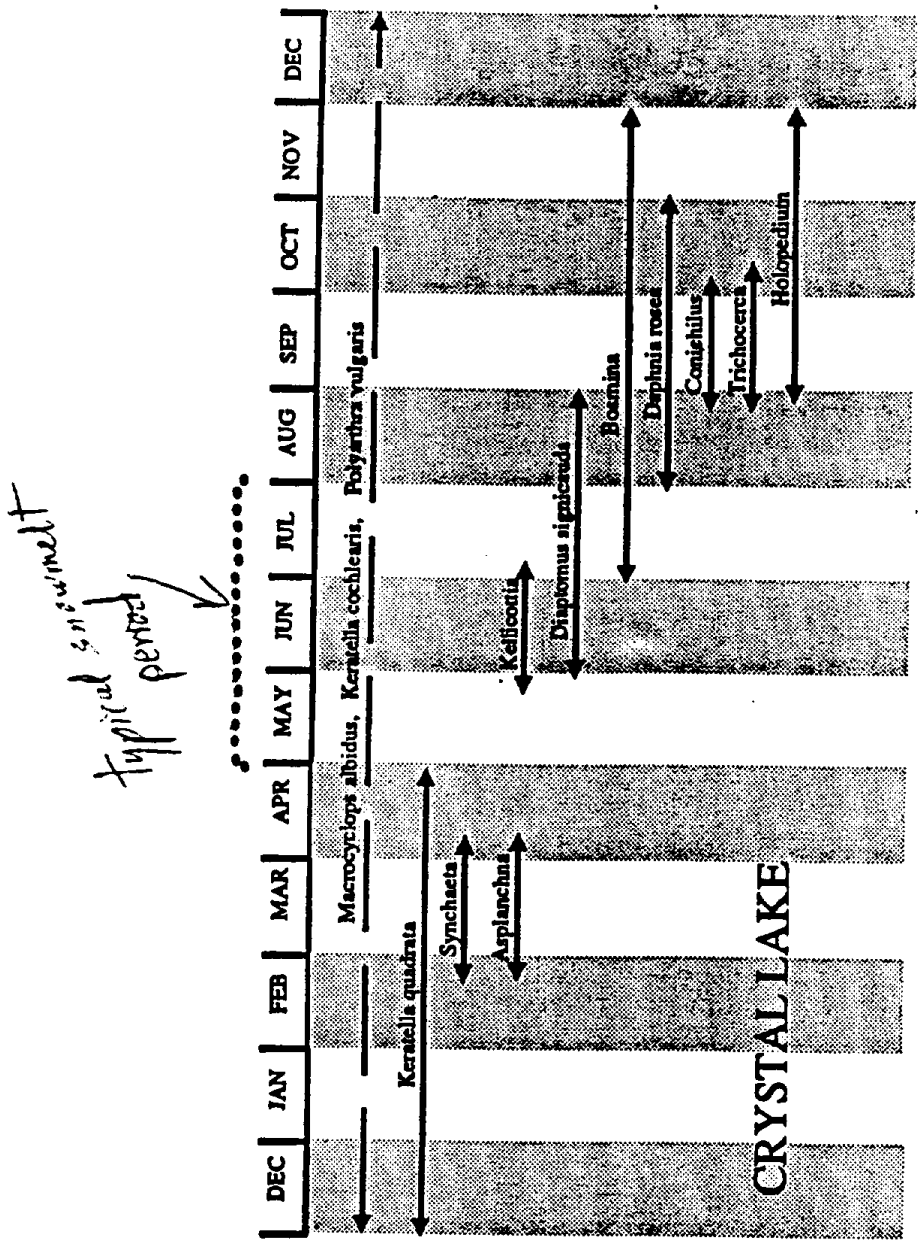

DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
CRYSTAL LAKE
Keratella quadrata
Synchaeta
Anplanchna
Macrocyclops albidus,
Keratella cochlearis,
Polyarthra vulgaris
Kellicottia
Diaptomus sigmicauda
Bosmina
Daphnia retrocurva
Conochilus
Trichocerca
Holopedium
Typical ice-melt period

Figure 5

CRYSTAL LAKE

Year	Months of greatest snowmelt discharge	Lowest pH observed in lake outflow	Lowest volume weighted mean lake pH	End of ice cover
1990	May-June-July	6.0 (repeated)	6.3 (June)	mid-May
1991	May-June-July	5.6 (May)	5.9 (Apr)	end-May

Figure 8

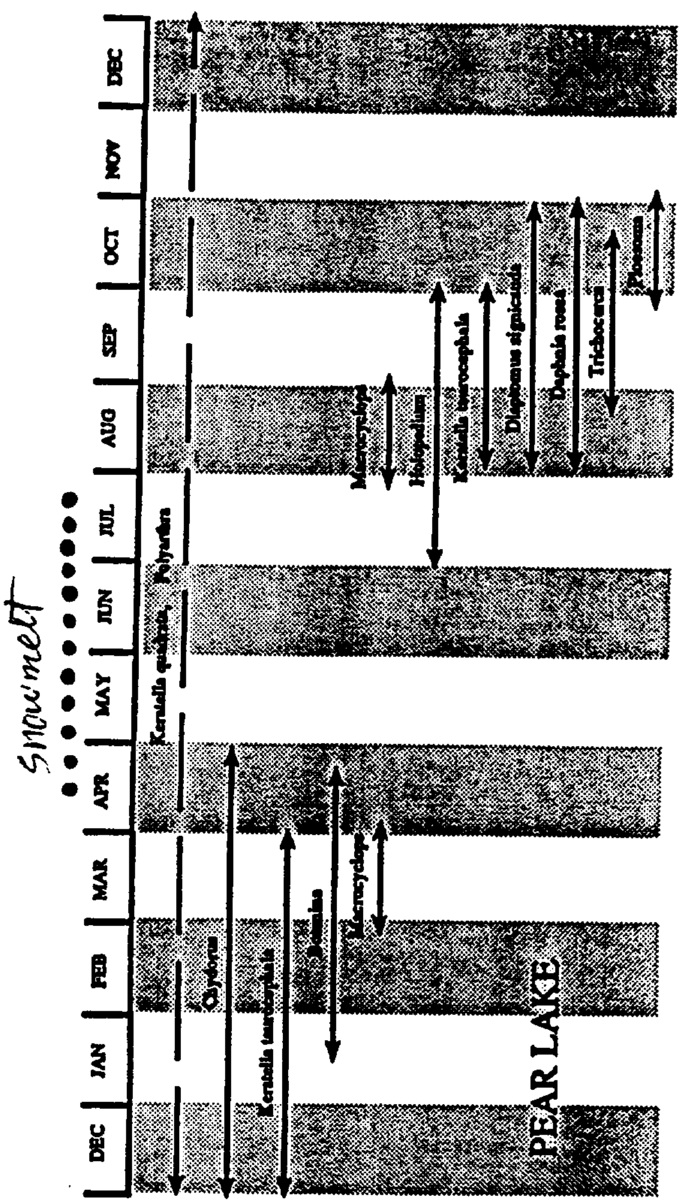

DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
Snowmelt
Keratella quadrata, Polyarthra
Chydorus
Keratella taurocephala
Bosmina
Macrocyclops
Holopedium
Diaptomus signicauda
Daphnia rosea
Trichocerca
PEAR LAKE

Figure 7

PEAR LAKE

Year	Months of greatest snowmelt discharge	Lowest pH observed in lake outflow	Lowest volume weighted mean lake pH	End of ice cover
1990	Apr-May-June-July	5.8 (May)	6.2 (June)	mid-May
1991	May-June-July	5.5 (May)	5.6 (Apr)	end-June

Figure 10

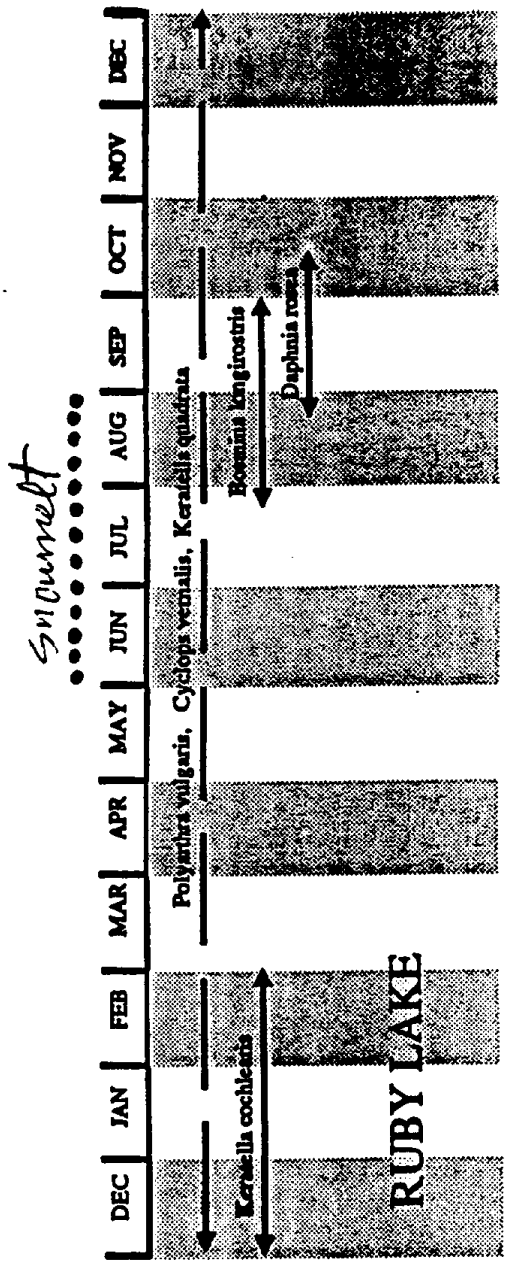

DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
Keratella cochlearis
Polyarthra vulgaris, Cyclops vernalis, Keratella quadrata
Bosmina longirostris
Daphnia rosea
RUBY LAKE

Figure 9

RUBY LAKE

Year	Months of greatest snowmelt discharge	Lowest pH observed in lake outflow	Lowest volume weighted mean lake pH	End of ice cover
1990	June-July-Aug	6.2 (June)	6.1 (June)	end-May
1991	June-July-Aug	5.8 (June)	5.8 (Apr)	end-June

AMPHIBIANS

Is pH likely to be a factor contributing to the recent decline of *Rana muscosa* (mountain yellow-legged frog) and the *Bufo canorus* (Yosemite toad)?

Rana muscosa

Experimental evidence:

* LC 50 of embryos is pH 4.4

* LC 50 of tadpoles is pH 4.0

Survey data: *Rana* tadpoles not found in lakes with pH < 6.0. High concentrations of other solutes may be involved in field distribution (Al, sulfate). Also, in 11/12 lakes where *Rana* tadpoles were found in Bradford's synoptic survey, trout were absent.

Bufo canorus

Experimental evidence:

* LC 50 of embryos is pH 4.7

* LC 50 of tadpoles is pH 4.4

Survey data: *Bufo* not found in Bradford's synoptic survey.

AMPHIBIANS

Generally, the embryos and larvae of amphibians are more susceptible to low pH than adults. Usually, between pH 4.5 - 5.0 the growth and recruitment of tadpoles and salamander larvae decline. Below pH 4.5, complete mortality of embryos can occur.

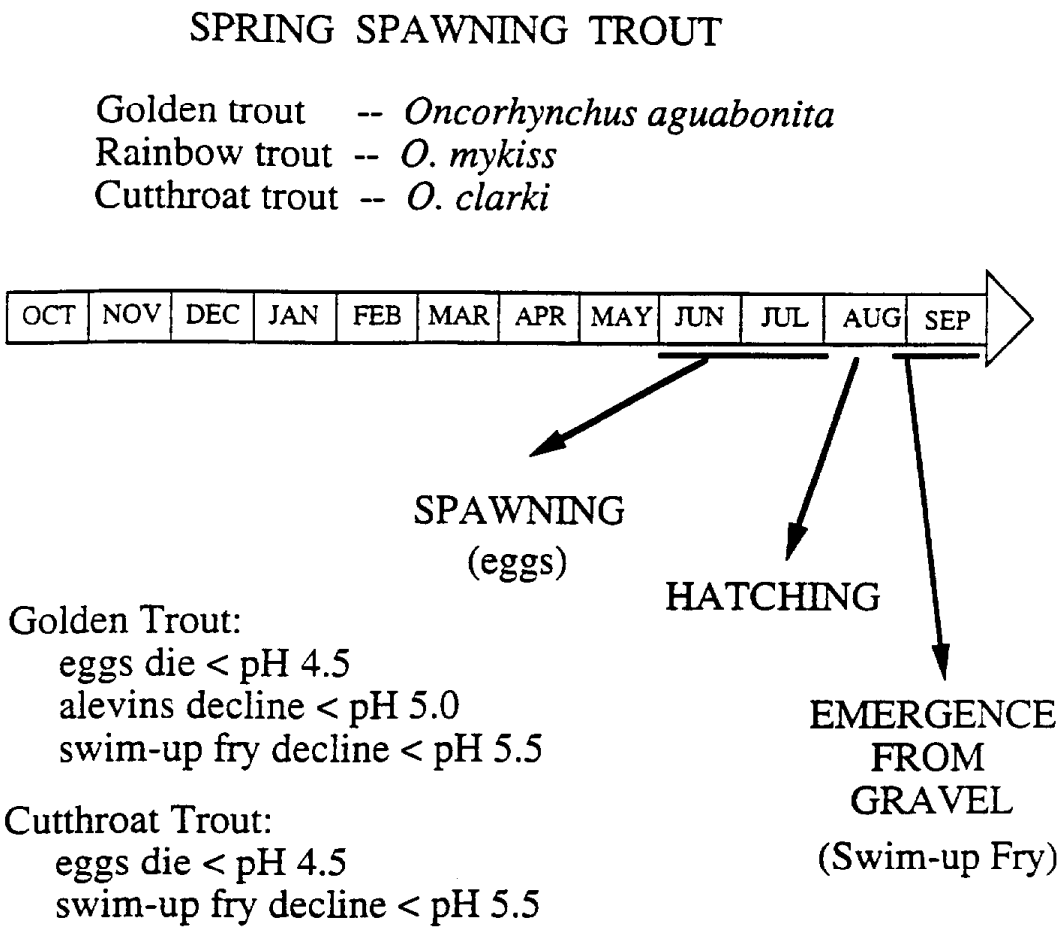
Occupants of temporary ponds are more vulnerable than permanent pond species because they rely on explosive spring breeding tactics and are thus especially vulnerable to snowmelt chemistry.

Adults of permanent pond species establish territories and breed throughout the summer, thus sensitive embryonic stages do not coincide with snowmelt.

There are 5 species of aquatic breeding amphibians that occur at high elevation in the Sierra Nevada:

Bufo canorus (Yosemite toad), *Bufo boreas* (western toad), *Pseudacris regilla* (Pacific tree frog), *Rana muscosa* (mountain yellow-legged frog), *Ambystoma macrodactylum* (long-toed salamander).

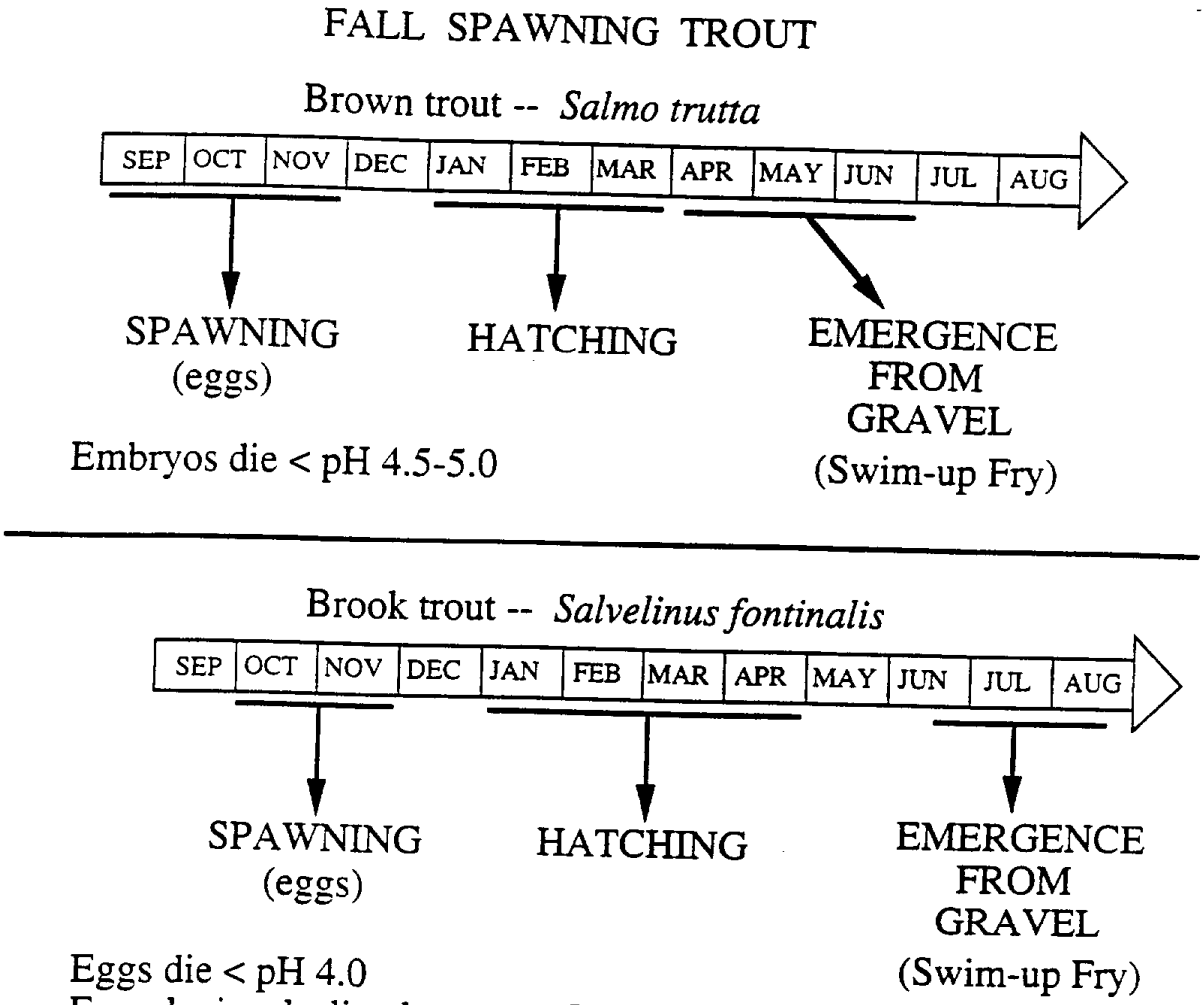
Figure 14



(1) The fertilized eggs of spring spawning trout are susceptible to low pH in snowmelt water. However, surface water pH is currently well above the critical pH during snowmelt in the watersheds studied.

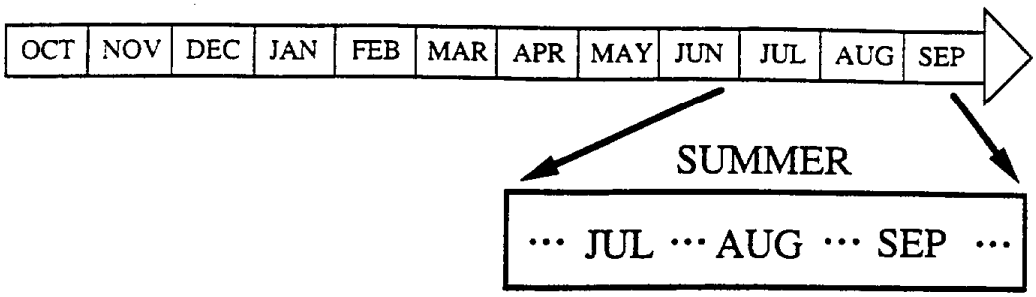
(2) The swim-up fry of these species could be damaged by episodic acidification due to runoff from summer storms.

Figure 13



The most sensitive live stages of the brook trout are larval stages, thus recruitment failure is probably responsible for the disappearance of this species from acid stressed systems. In the Sierra, emerging brook trout larvae may be affected by low pH runoff from summer rain storms. Sac fry may be impacted by snowmelt runoff.

Figure 16



STREAM INVERTEBRATES

Episodic acidification of streams due to snowmelt or summer rains may decrease the benthic density of some species of stream invertebrates.

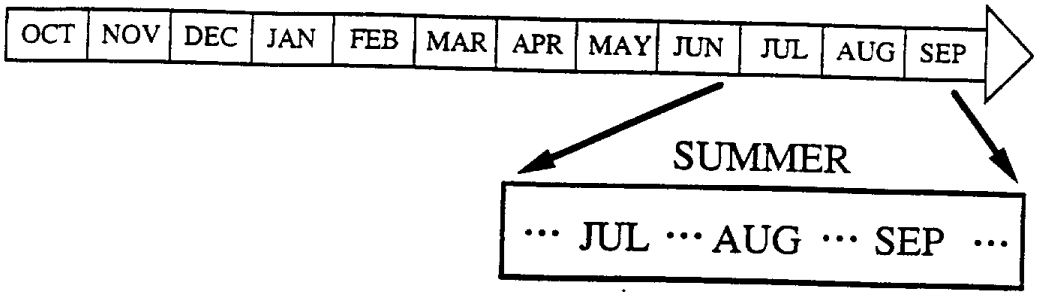
Vulnerable species identified in experimental work in the ELW:

* *Baetis* mayfly nymphs
* *Paraleptophlebia* mayfly nymphs
* *Epeorus* mayfly nymphs
* chironomid larvae

Response of vulnerable species:

When pH was lowered to 5.0, and below, for as little as 8 hours, the drift rates of vulnerable species increased, and much of the increase was due to mortality (i.e. drift was killed by low pH).

Figure 15



Summer Rainstorms can potentially affect:

* Stream benthic invertebrates

* Larval stages of amphibians using territories of permanent ponds as breeding sites

* Swim-up fry of spring-spawning trout

* Denitrification rates (via soil moisture)

* Ion fluxes in vegetated zones via throughfall

Mechanisms for Sulfate Retention

(1) Sulfate adsorption onto Fe- and Al-oxides.

(2) Sulfate precipitation with Al to form such minerals as basaluminite ($Al_4OH_{10}SO_4$), alunite ($KAl_3OH(SO_4)_2$), and jurbanite ($AlOHSO_4$).

(3) Microbial incorporation of sulfate into C-bonded S and ester-SO_4.

(4) Microbial sulfate reduction, followed by volatilization of H_2S (in waterlogged soil, or lake sediments).

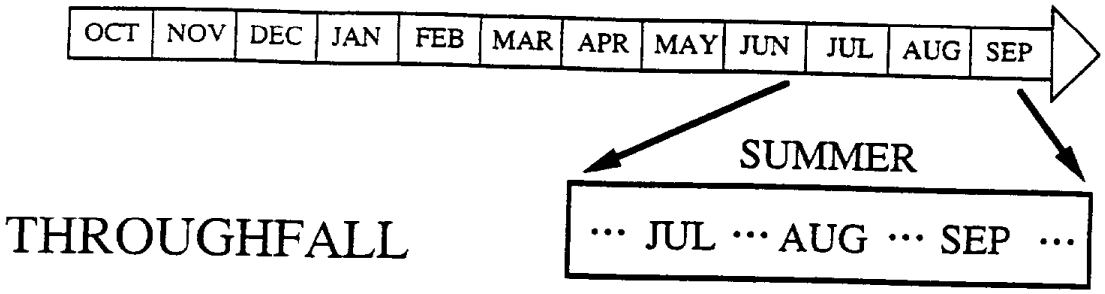
(5) Sulfate uptake by vegetation:

   a. when S is in excess, sulfate can be stored in foliage and is subsequently easily leached from litter

   b. S can be incorporated into more resistant compounds

NOTE: S immobilized by biota is generally a low % of ecosystem S retention - most S retention occurs in the mineral soil.

Figure 17



(1) NH_4^+/H^+ is > 1 in summer precipitation - thus NH_4^+ has an important role in the neutralization of strong acid ions (NO_3^-, SO_4^{2-}, Cl^-) in summer rain. Without NH_4^+, summer rain would be 11X more acidic.

(2) Foliage retains almost all NH_4^+ in summer rain.

(3) Foliage doubles NO_3^- in throughfall (via leaching and wash-off of dry deposition).

RESULT:

Throughfall has lower ANC and is more acidic than summer rain. The net effect of throughfall on ANC flux is small on a basin scale (because the area covered by vegetation is small). However, throughfall could affect localized habitats important to some biota, e.g. ponds surrounded by vegetation.

Figure 20

LOST LAKE

Year	Months of greatest snowmelt discharge	Lowest pH observed in lake outflow	Lowest volume weighted mean lake pH	End of ice cover
1990	April-May-June	5.7 (Apr)	5.8 (June)	end-May
1991	May-June	5.5 (June)	5.5 (Apr)	mid-June

Biologically Mediated N Transformations

(1) Nitrification consumes ANC *and* produces nitrate,

(NH_4^+ -----> NO_3^- and 2 H^+)

thus can exacerbate nitrate-mediated cation leaching.

(when?--probably after initiation of snowmelt)

(2) Plant uptake of NH_4^+ releases H^+

Note: These processes are estimated to consume ca. 25% of the ANC produced in the ELW by geochemical weathering.

(3) Denitrification - increases ANC on a molar basis. Can occur if soils are not frozen or dry. Maximal rates occur after summer rains. Could be important under snowpack. So far, in the ELW, denitrification is estimated to remove only 5% of deposition.

PERSPECTIVE:

** Organic-N dominates N export in stream waters.

** The amount of N cycling annually in the watershed via uptake by above-ground plant biomass and release from litter is *greater* than annual atmospheric N deposition. Implication - changes in net production of vegetation or remineralization rates could affect mass balance of N.

Figure 22

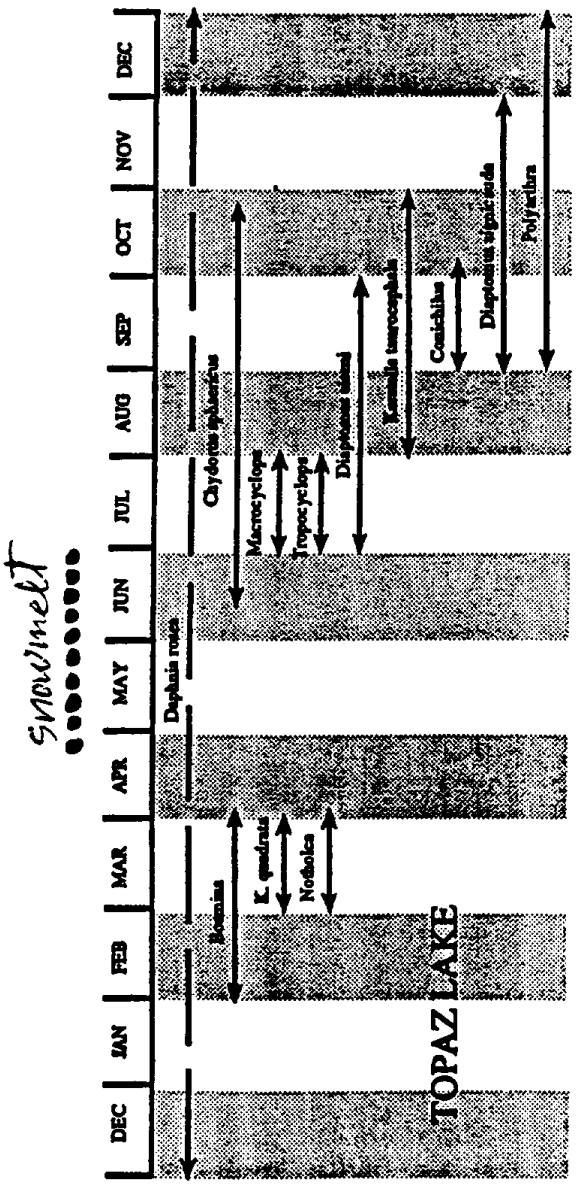

DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
TOPAZ LAKE
Snowmelt
Bosmina
K. quadrata
Notholca
Daphnia rosea
Chydorus sphaericus
Macrocyclops
Tropocyclops
Diaptomus sicilis
Keratella taurocephala
Conochilus
Diaptomus signicauda
Polyarthra

Figure 21

TOPAZ LAKE

Year	Months of greatest snowmelt discharge	Lowest pH observed in lake outflow	Lowest volume weighted mean lake pH	End of ice cover
1990	Apr-May-June	6.1 (June)	5.9 (June)	mid-May
1991	May-June	5.6 (July)	5.6 (Apr)	end-June

D. SESSION 4. HUMAN HEALTH **MR. DANE WESTERDAHL, Moderator**

MR. DANE WESTERDAHL

When we are young, it was thought that acidity meant sulfuric acid and acid sulfates. Also, when we were very young, we initiated a program on acidity that originally had $100,000 total funding towards health. However, very soon thereafter, Mike Hoffman made some findings that acid fogs in Corona Del Mar were substantially acidic. It really raised the interest of the SAC at the initial meeting, and it was thought that the acid problem may have broader effects than those on lakes and watersheds. At the initial meeting of the SAC, I remember quite well, we all thought we needed to take a look at the effects of acid fogs on human health. However, at the same initial meeting, the SAC said, "We need you to answer some questions before you can begin. What are acid fogs? How do you measure acid fogs? How can you generate them for uses in laboratory chambers? Before you may begin, we want to do a pivotal study that answers these questions." That pivotal study came from a request for proposals, and the ERT did the study. They reviewed everything we knew about the physics and chemistry and generation and measurement methods of fogs. It turned out to be very complicated to make a controlled fog in a chamber. We will be hearing about the work that followed that from John Balms, but essentially the first phase of the ARB study was to learn how to make a fog and measure it. The second phase was to determine how to install a fog generation system in a working chamber. Both of those efforts delayed the initiation of the clinical work, but they were both very important. You will hear more about that work, the actual experimental work later. Another study that began very early in the Kapiloff acid deposition program, was a study at UC Irvine with Steve Colome as the principle investigator. It was our first major health study while the chamber work was going on, and it was an ambitious effort. We were trying to measure things that are difficult to measure. We were trying to corral 100+ asthmatics, follow them on a daily basis, and measure their lung function and medication use multiple times per day. We began clinical exposure studies as soon as the UCSF chambers were finished, and we began animal exposure studies soon thereafter. What did we learn from the initial Kapiloff program? As I said, our concern at the beginning was that acidity in California was not the same as the rest of the country viewed acidity, which elsewhere is primarily sulfuric acid and sulfates. We were concerned about nitric acid acidity, and we were concerned about it in fog. What did we learn from our extensive work with acid fogs? John Balmes will talk in detail about what we have learned, but what I have learned is that acid fogs are very complex. Their impact on human physiology is also very complex. One of the important things about an acid fog may not be just its acidity, but also the water content and the effect of water droplets on human physiology. Fog itself is a potent effector of airway response.

After the Kapiloff program, we had a little bit of breathing space to decide what we were going to do in the AAPP program. After reviewing what we had learned and what else had been learned in

Figure 23

SPULLER LAKE

Year	Months of greatest snowmelt discharge	Lowest pH observed in lake outflow	Lowest volume weighted mean lake pH	End of ice cover
1990	June-July	6.1 (June)	6.1 (June)	end-May
1991	June-July	5.7 (June)	5.8 (July)	end-June

to SO_2, and it was thought that maybe they would be specifically sensitive to acid sulfate aerosol. Asthma is an airway disease characterized both by a tendency to have narrowing of the airways and inflammation of the airways. Asthmatics, by definition, have a physiologic characteristic called airway hyper-responsiveness in which their airways are twitchy and ready to narrow upon inhalation of noxious stimuli. Everybody's airways will narrow with high exposure to noxious stimuli. Asthmatics are characterized by an enhanced tendency to airway narrowing.

The first studies with regard to asthmatics and sulfuric acid aerosol did suggest that asthmatics would respond to acid sulfate. In 1983, two studies, one by Jane Koenig's group at the University of Washington in Seattle, using allergic adolescent asthmatics, and then another study from Mark Utell's laboratory at the University of Rochester, using asthmatic adults, did suggest that there was some similarity with sulfur dioxide in the response of asthmatics to sulfuric acid aerosols. The first study that came out of the UCSF acid exposure work was by Jonathan Fine (Fig. 1). He studied airway narrowing in asthmatics with two different types of acids. The study suggested that the hydrogen ion concentration of the aerosol that was titratable was important; that is, that the acidity of the aerosol was more important than the chemical species. Subsequently, even before the chamber was built, we did other studies under my leadership with sulfuric acid aerosol and asthmatics, and we were unable to confirm the findings of Koenig et al. in our population of asthmatics. I should provide a caveat here, in that characterization of severity of asthma is a tricky business, and there may have been differences across laboratories with regard to the severity of asthma and the type of asthma. In general, the asthmatics studied in our laboratory were very mild asthmatics who did not need a lot of medication and did not need to go to the hospital or to emergency rooms very often. They were probably less severe cases than Koenig et al. had studied. The other special characteristic about Jane Koenig's asthmatics, was that they were adolescents and specifically recruited to be allergic asthmatics. In any event, a series of studies in our laboratory and also some work from Jack Hackney's group, really suggested that not all asthmatics responded to sulfuric acid in the same way. Asthmatics have a pretty impressive response to SO_2, and you can almost use sulfur dioxide challenges as a way to pick out asthmatics in a population. That is not the case with sulfuric acid. There seems to be, as I have indicated, variability in response, and some asthmatics will respond and others will not (Fig. 1). A 1990 study from Jack Hackney's laboratory was actually a direct replication of the original 1983 Koenig study; they recruited allergic asthmatics that were adolescents; and they could not reproduce Jane's work.

I just wanted to give you a sense of the magnitude of the effects seen in the initial 1983 studies that suggested that asthmatics were sensitive to sulfuric acid aerosol. Data from Jane Koenig's study showed there was no decrement in lung function during sulfuric acid exposure at rest, but there was about an 8% decrement in expiratory flow rate after 10 minutes of exercise. It was statistically significant, but

the country from extensive experimental work we formulated a research plan. There were suggestions that acids, measured and employed in biostatistical evaluation of epidemiological studies performed all over the world, were having an explanatory role in adverse effects seen in these studies. We were also seeing that materials other than fogs were being reported as possible effectors. We decided our best focus was with fog, and with acid vapors and acid aerosols in combination with common air pollutants, such as ozone, that we see in California. We did work both with and without ozone in most of the studies you will hear about. We sponsored both brief and prolonged exposure studies, and you will hear more about these from the investigators. One last study that is very important, we took to the board yesterday for approval. This is a children's health study. This study is a major initiative of the Air Resources Board and will run approximately a total of 10 years. It is now in its third year. It was viewed at the beginning as an opportunity to address the effects of acidity as part of a complex atmosphere, and a portion of the study was funded out of the funds for acidity research. It was a challenge to us at the time we initiated it as to how best characterize the atmosphere to which the children in this study were exposed. We did a considerable amount of work developing samplers, evaluating available samplers, and developing new samplers that we could afford to operate as part of a ongoing study to look at the health effects on children. I have taken long enough to introduce the historical background. We will go on to John Balmes, who has done most of the clinical work done at UCSF.

1. Clinical Studies. Dr. John Balmes, UC San Francisco.

DR. JOHN BALMES, UC San Francisco

I am charged with talking about clinical studies. I actually prefer the term, controlled human exposure studies, since the clinical relevance may or may not be there depending on the specific protocol and specific findings. The original acid aerosol work done at UCSF was really done under the direction of Dean Shepard who has now moved on to more basic research. I was recruited to take over control of the exposure study. The first couple of studies done at UCSF were really primarily Dean Shepard's, and they involved sulfuric acid aerosol with mouth piece exposures before the chamber was built. I am going to concentrate on the nitric acid vapor work that we have done in recent years.

Sulfuric acid aerosols were initially studied in an exposure study following the model of sulfur dioxide work which had preceded it by a few years. Given that sulfur dioxide emissions are one of the primary causes of acid sulfate pollution, it made a certain amount of sense. The SO_2 work was done both at UCSF by Homer Boushey and Dean Shepard and southern California by Jack Hackney's group at Rancho Los Amigos Hospital. We learned that asthmatic individuals seem to be particularly sensitive

Westerdahl's suggestion, we recruited subjects sensitive to ozone, since only a segment of the population and not everyone will have acute lung function changes when they breathe ozone. We selected subjects to be specifically sensitive to ozone, and the basic hypothesis was to see if nitric acid fog pre-exposure would enhance the effect of ozone. The initial 2 hours of exposure was with nitric acid fog, a non-acidic fog, or just plain air, and there was no change in lung function across the 2 hour pre-exposure period. Then, there was a break followed by ozone exposure, and we found decrements in lung function after ozone exposure, because we selected subjects sensitive to ozone. However, we were not able to enhance the ozone effect on lung function by fog exposure. As a matter of fact, the pre-exposure associated with the greatest subsequent decrement induced by ozone was the air exposure. Both the acid fog and non-acidic fog actually had somewhat protective effects. The first set of conclusions that I would share with you about our work is that nitric acid did have an effect on bronchoconstriction at sufficiently high concentrations, and it appeared to be relatively similar in potency to sulfuric acid aerosol. At 500 micrograms per cubic meter fog (mass median aerodynamic diameter about 6 microns) followed by 0.2 parts per million ozone, we saw an ozone effect, but we did not enhance that ozone effect by pre-exposure to the nitric acid fog.

Q (AUDIENCE) One point of clarification. Is that 500 micrograms of HNO_3 or HNO_3 + water?

A (DR. BALMES) That is 500 micrograms of HNO_3.

Q (AUDIENCE) Then, are the sulfuric acid and the nitric acid aerosol numbers about the same?

A (DR. BALMES) Yes.

Since we had not found much effect with either sulfuric acid aerosol or nitric acid aerosol, and given that nitric acid is often in the vapor form in the Los Angeles basin, the ARB prevailed upon us to study nitric acid vapor. We looked at the effect of nitric acid vapor alone and then in combination with ozone (Fig. 3). This measure of lung function is forced expiratory volume in one second, which is how much air you can blow out with a maximal effort in one second. It is probably the most reliable single lung function test. There was no difference between the air and nitric acid vapor and no change over the four hour exposure. It looked like we induced a mild increase in airway resistance with nitric acid vapor exposure versus air. The level of exposure to nitric acid vapor was 500 micrograms per cubic meter in this study which, as you know, is well above what we ever get in the south coast air basin. In the combined exposure versus ozone alone, i.e. 500 micrograms per cubic meter nitric acid vapor plus 0.2 parts per million ozone or just ozone alone, we got the expected decrement in $FEV_{1.0}$ across the ozone exposure. There was a trend towards a greater effect with the combined exposure, but it was not statistically significant in regard to $FEV_{1.0}$. For airway resistance, we got an increase with ozone

its clinical significance is minimal. This kind of decrement is not likely to make an asthmatic symptomatic. In the Utell at al. study, with an exposure to 1000 micrograms per cubic meter of sulfuric acid aerosol, there was about a 20% decrease in airway conductance which again was statistically significant compared to the control aerosol, but it is again of a magnitude that is not likely to make most asthmatics symptomatic. The other thing I would like to point out here is that there was not really a good dose-response relationship. Most bronchoconstrictor stimuli, including sulfur dioxide, have a pretty nice dose-response. In this case, however, with more than halving the exposure concentration, there was very little difference in the magnitude of change in airway conductance.

In the first acid aerosol study that I was involved with at UCSF, we were able to induce bronchoconstriction in a population of fairly healthy asthmatics. We were able to make dose-response curves for the population of asthmatics, and if we gave enough acid aerosol, we could get bronchoconstriction. We showed that you could induce bronchoconstriction by inhalation of a hyposmolar aerosol. If asthmatics are given water to breathe, you can induce bronchoconstriction, and if we added acid to this aerosol, we could change the dose-response curve to make asthmatics respond a little bit sooner than they otherwise would have. Basically, there was not much difference in the potency of nitric acid or sulfuric acid or a 1-to-1 combination of the two. With all three of the acid aerosols that we used, we were able to shift the dose-response curve compared to the bronchoconstrictor potency of the control saline stimulus. Since most of the work I am going to present suggests that there is not much effect of acid aerosols, I do not want to give the impression that breathing sulfuric acid or nitric acid aerosol will not cause bronchoconstriction. If you give enough, you can get bronchoconstriction, but the question is really whether ambient levels are likely to cause bronchoconstriction.

Q (AUDIENCE) What levels were you using here?

A (DR. BALMES) The levels were rather high. Actually, the highest level of our nebulizer would have been over 10 milligrams per cubic meter of either sulfuric acid or nitric acid.

Since we were not getting much bronchoconstriction in asthmatics, even when we were giving the aerosols in high concentration by mouth piece which would bypass the normal scrubbing mechanism of the nasal airways, we did not expect that the chamber fogs at anywhere near ambient concentrations would induce bronchospasm. The highest level we generated in our chamber was 1000 micrograms per cubic meter of sulfuric acid, and we were not able to see any kind of bronchoconstrictor effect on our young, relatively healthy asthmatics. We then moved to a combination atmosphere study where we gave nitric acid fog at the initial exposure followed by an ozone exposure (Fig. 2). This was theoretically supposed to model something that might occur in the eastern side of the Los Angeles basin where you might have an acid fog in the morning followed by some ozone exposure later in the day. At Dane

bronchoscopy measurements were made. We used 150 micrograms per cubic meter nitric acid vapor content, which is lower than we had used previously, although it is still higher than you would ever see in the south coast air basin or elsewhere. We also used a 0.2 parts per million ozone exposure. The reason we keep using this level is that it does occur in the real world even though it is on the high end, and we were pretty familiar with the lung function and inflammatory effects of this exposure. We exposed people for four hours each for four days in a row (Fig. 4). Lung function measurements were made before and after each four-hour exposure, and we did bronchoscopy for bronchoalveolar lavage and actually did bronchial biopsy (in which you take a piece of the airway wall) on the fifth day, 18 hours after last exposure. After a month, we repeated this whole protocol with either the sham exposure or the actual pollutant exposure. We were blinded to which one it was, and exposures were performed in random order.

Q (AUDIENCE) How many subjects were there?

A (DR. BALMES) Today we have 9 subjects, we are shooting for 15, so this is an interim report. All the rest of the data I have spoken about are published; these are the first unpublished data.

This study had multiple arms, but the arm I am going to share with you is the arm we did first. Figure 5 shows ozone versus a combination of ozone and nitric acid. After the first day, the mean decrement in $FEV_{1.0}$ for 9 subjects was about 10%, pretty much what we had seen before. After day 2, that had increased to about 20%, so we have progression of lung function change. Then on day 3, it is only 5%, and on day 4 there is practically no effect at all. This is nothing new or unexpected. Other people have looked at multiple-day exposures to ozone and observed physiologic tolerance develop.

Q (AUDIENCE) Are the present changes in reference to the day before or to the start of the experiment?

A (DR. BALMES) That is a good question. They are compared to the start of each day. Then, the question would be, is there a gradual decrement in the baseline each day? There is an initial decrement over the first two days, and then by day 3 and 4 it was back to baseline. These comparisons are across each exposure.

There is, even after day 1, a fairly good recovery of the baseline. With our combined exposure, it looks like it may be a little bit worse, but that trend was all driven by one subject. Everybody with the combined exposure except for this one subject, had almost the identical pattern that they had with ozone alone. However, this one subject did show a lack of attenuation of the $FEV_{1.0}$ response across the four days. The 2nd day was the worst, but on days 3 and 4 this subject did not return to the pre-exposure baseline. Thus, in 8 of 9 subjects, there were no enhancements of lung function changes induced by ozone alone resulting from the combined exposure.

The preliminary bronchoalveolar lavage findings are included in figure 6. Total white blood cells

exposure, but nitric acid vapor seemed to protect against the ozone effect to some extent.

Q (AUDIENCE) Are you talking about asthmatic patients now?

A (DR. BALMES) No, these are normal subjects. I should have clarified that. Our nitric acid vapor studies are in normal individuals, but they were almost hyper-normal because they had to be able to exercise for four hours with a 10-minute break each hour, and the level of exercise was jogging or cycling at a fairly mild level. I had to do this exposure myself, and one had to be fairly fit to last four hours.

The next day following the four hour exposures, we did a bronchoscopy. This a fairly standard diagnostic procedure that pulmonologists do routinely. It involves passing a fiberoptic scope through the vocal cords, via either the mouth or the nose, and down into the airways. You can wedge this small scope into the orifices of lobes and segments of lobes in the lung, and then you can wash saline in and out of a segment of the lung and look for the cellular and biochemical constituents in that fluid as a way of studying the degree of respiratory injury or inflammation. We studied air versus nitric acid vapor and then ozone versus the combined nitric acid and ozone exposure. The end-points were first, the count of cells in the lavage fluid that were alveolar macrophages. These are amoeba-like cells, and as their name suggests, they are big eaters of particles inhaled into the airways. The second was the percent neutrophils, and these cells are normally not represented in a very high percentage in the airway lining fluid. These neutrophils are summoned from the bloodstream into the lung with certain types of inflammation, but there was no effect of nitric acid vapor. Ozone reproducibly causes influx of neutrophils into the airways. The combination was not any worse than ozone alone. Total protein content or concentration in the lavage fluid is a marker of how leaky the airway epithelium is. With an injury such as you get with ozone, there is increased leakiness and bloodstream constituents, such as proteins, leak into the airway lumen. There was no effect of nitric acid vapor on total protein concentration. There was an ozone effect; ozone induced a doubling of the total protein concentration. Albumin, one the most important blood specific proteins, was also increased after ozone, but not after nitric acid vapor, and there was no enhancement of the ozone effect by the combination exposure.

In our laboratory, exposure to nitric acid vapor at a pretty high concentration, 500 micrograms per cubic meter for four hours during exercise did not cause any acute lung function effects or enhance the acute lung effects of ozone. We found no evidence of an inflammatory response to nitric acid vapor, nor was there enhancement of ozone-induced inflammation. Even though there was dwindling excitement about further studies of nitric acid vapor at this point, we did propose a multi-day exposure study, because of some of the results of animal toxicologic work. It is pretty hard to put people in chambers for as long as you can put rats or mice in chambers, but we did figure out a protocol which we thought would work (Fig. 3B). This involved a four-hour-per-day, 4-day exposure, and then on the fifth day,

bronchoconstriction.

Q (AUDIENCE) In these human exposure experiments, I don't think you can expect to see an effect in a single acute exposure or in a four dose exposure. In addition, a pollutant like ozone has completely different effects when you use an acute versus a chronic dose.

A (DR. BALMES) Well, I would be the first to agree with you that you cannot study chronic effects on humans with controlled human exposure studies. You have to do epidemiologic studies. You have to study populations in the natural environment with epidemiologic studies if you want to investigate chronic effects in humans. Then, epidemiologic studies have their own set of problems, because you cannot control all the variables. You are going to hear about chronic exposures in animal studies, and I do not disagree with you. In some ways, it would have been nice to have the animal work first before planning human studies, but there is not always the luxury of having those kinds of data in advance. You are going to hear about the NYU and UCI animal data next, but Dane just reminded me that Dean Shepard who initiated these studies did do some animal work. He did sulfuric acid exposures in guinea pigs, and actually, those were not chronic exposures; they were acute exposures, and it took a fair amount of acute sulfuric acid exposure to get airway narrowing in the guinea pigs which is similar to what we found in humans.

found in lavage fluid showed no difference between the exposures. The percent neutrophils were basically the same but slightly higher in the ozone alone group. The lactate dehydrogenase concentration in the lavage fluid, which is an index of cell injury, was a little higher with the combination exposure. The total protein concentration (the measure of leakiness of the airway epithelium) showed no difference. I interpret these data in 9 subjects as basically showing no difference between the combined exposure and exposure to ozone alone.

One arm of this study is to compare single-day ozone exposure versus four-day exposure, to see if there is cumulative injury with the four-day ozone exposure. So far, it does not look like there is that much cumulative injury. The bronchoscopy findings after four days of ozone exposure are not much different than after one day.

The final data I will show are not yet published, but they will be presented at the American Thoracic Society meetings in May 1995. In this study, we gave a pre-exposure to asthmatic individuals (allergic asthmatics) of 250 micrograms per cubic meter nitric acid vapor plus 0.2 parts per million ozone for one hour. This was then followed by an exposure to the specific allergen to which they are allergic. We actually recruited subjects to be allergic to the house dust mite, which is a common allergen. These individuals received the pre-exposure followed by exposure to allergen versus a sham pre-exposure followed by allergen. The combined nitric acid and exposure did not enhance either the early airway narrowing or the late inflammatory response to the allergen. The bottom line is that we have found very little health effects, either in terms of lung function or airway inflammation, from any of the acid atmospheres that we have studied including sulfuric acid aerosol both in fog and smaller size haze aerosol, nitric acid aerosol, nitric acid vapor, or the combination of ozone and nitric acid vapor. I am echoing the morning session with regard to the health effects of atmospheric acidity, but I must emphasize we are only studying acute effects. Acute effects are all we can study in these controlled human exposure studies.

Q (AUDIENCE) If I had to pick a component of acid fog that I would be suspicious of, it is the hydroxymethane sulfonic acid. I wondered if you had any comments on that?

A (DR. BALMES) I could have planted that question. In the interest of time, and I hope I do not go much over my 25 minutes, I have left out a couple of studies. We studied hydroxymethane sulfonic acid after Mike Hoffman showed the presence of a fairly high concentration of that species. That is an interesting compound with regard to potential effects on the airways. It dissociates to both formaldehyde and SO_2 which we know have negative effects on the airways. We exposed a group of young, relatively healthy asthmatics. I think 10 were exposed to a pretty high concentration of HMSA, but I cannot remember the exact concentration offhand. I believe it was at the top end of the range of concentrations that Mike Hoffman had described. We found no effect in terms of enhancing or causing

Figure 2

NITRIC ACID AEROSOLS

- HNO_3 appears to enhance hypoosmolar aerosol-induced bronchoconstriction as much as H_2SO_4 (Balmes et al., 1988)
- Sequential exposure to HNO_3 fog (500 $\mu g/m^3$) for 2h followed by 0.2 ppm O_3 did not enhance the acute lung function effects of O_3 alone (Aris et al., 1991b)

Figure 1

HEALTH EFFECTS OF ATMOSPHERIC ACIDITY

CLINICAL STUDIES

John R. Balmes, MD
Human Exposure Laboratory
Lung Biology Center
University of California, San Francisco

SULFURIC ACID AEROSOLS

- Initial evidence suggested that asthmatic subjects were more sensitive to the acute bronchoconstrictor effects of H_2SO_4 (Koenig et al., 1983; Utell et al., 1983)
- Titratable acidity appears more important than ionic species re: acute bronchoconstrictor effect (Fine et al., 1987)
- Subsequent evidence suggested that there is considerable variability of sensitivity to H_2SO_4 aerosol among asthmatic subjects (Aris et al., 1991a; Avol et al., 1990)

Figure 4

MULTI-DAY STUDY PROTOCOL

- Daily exposure to HNO_3 vapor (150 $\mu g/m^3$) plus O_3 (0.2 ppm) or O_3 alone for 4h for 4 days
- Lung function measurements before and after each daily exposure
- Bronchoscopy for BAL and bronchial biopsy 18h after last exposure
- Repeat 4-day exposure to other atmosphere following identical protocol

Figure 3

NITRIC ACID VAPOR

- Exposure to HNO_3 vapor (500 $\mu g/m^3$) for 4h did not induce acute lung function effects or enhance the acute lung function effects of O_3 in normal subjects (Aris et al., 1993)
- This HNO_3 vapor exposure did not induce evidence of inflammation in bronchoalveolar lavage (BAL) fluid or enhance the acute inflammatory effects of O_3 (Aris et al., 1993)
- Exposure to HNO_3 vapor (250 $\mu g/m^3$) plus O_3 (0.2 ppm) for 1h did not enhance either the early or late-phase response to inhaled allergen in sensitized asthmatic subjects (Chen et al., 1995)
- Multi-day exposure to HNO_3 vapor (150 $\mu g/m^3$) plus O_3 (0.2 ppm) for 4h did not induce greater lung function changes than multi-day exposure to O_3 alone
- Multi-day exposure to HNO_3 vapor (150 $\mu g/m^3$) plus O_3 (0.2 ppm) for 4h did not induce greater BAL evidence of inflammation than multi-day exposure to O_3 alone

Figure 6

MEAN BAL FINDINGS

	O_3 ± SE	O_3/HNO_3 ± SE
Total leukocytes (x 10^4/mL)	19.2±5.0	19.9±4.1
Neutrophils (%)	8.5±2.1	6.1±2.4
LDH (U/L)	-9.7±1.3	-11.3±2.3
Total protein (μg/mL)	0.16±0.02	0.16±0.01

Figure 5

MEAN DAILY CHANGE IN FEV1

	O_3	O_3/HNO_3
Day 1	-11.6%	-10.8%
Day 2	-19.6	-21.3*
Day 3	-4.8	-6.4*
Day 4	-1.1	-2.3*

* One subject had markedly less attenuation of FEV1 response with the combined exposure (-11.9, -33.7, -27.5, -21.7%)

are in combination. We performed exposures under very clean conditions, using nose only exposures so we do not get any neutralization of the nitric acid by ammonia production that is outside the body of the animals. If there was any neutralization, it was going to be a physiological process occurring in the airways as the animals inhale.

We found a number of significant responses in the one month dose-response study that ranged in concentration up to 450 micrograms per cubic meter of nitric acid. We found very few solid significant responses in the nine month exposure but a number of interesting trends that fit some of the patterns of the disease states we were studying. We did not find anything in the disease end-points related to asthma. With bronchitis, we were interested in the epithelial lining of the airways, and particularly events that are related to mucous production. Figure 4 shows a measure of glycoprotein in bronchoalveolar lavage fluid. In the one month dose-response study, we got a very strong dose-response relationship, with a significant effect even at the low 50 micrograms per cubic meter concentration. However, when we went to the more extended exposure in the nine month study, we did not see a significant effect in any of the exposure groups. We did not see any substantial changes in the populations of the secretory cells in the epithelial lining, so it looks like this response is mediated simply by the increased glycoprotein production rates of the secretory cells and not due to any changes in cell proliferation.

We looked at a broader category of pulmonary disease states that included conditions that might be related to increased incidence of respiratory infections. One of these measures is the permeability of the epithelium lining the respiratory tract; it is a measure of how readily foreign particles and compounds penetrate through this epithelial permeability barrier and get into the underlying tissues and into the blood stream. In the nine month exposure, we found a significant response in the nose (Fig. 5). There was a main effect of nitric acid appearing in both the nitric acid alone and in the combination groups. This was permeability in the mucosa of the nasal passages, and this permeability barrier was compromised by exposure to nitric acid.

The other class of end points we studied, also related to infection, involved alveolar macrophages. These are the cells that John Balmes described. They are an extremely important pulmonary defense mechanism. They identify and intercept foreign particles and microorganisms, essentially keeping the lungs clean of these sort of infectious agents and foreign particles. The variety of macrophage functions we studied are summarized in figure 6. I want to look first at Beta glucuronidase. This compound is released into lung lining fluid when macrophages are damaged, and in the nine month exposure study, there was a significant main effect of ozone in elevating beta glucuronidase. In the nine month study exposure we analyzed some of the end points at 3 months into the exposure. There was also a trend for increased beta glucuronidase at the 3 month end point.

2. Animal Studies. Dr. William Mautz, U.C. Irvine, and Dr. Richard Schlesinger, New York University.

MR. DANE WESTERDAHL, Moderator

Our first speaker on animal exposure studies will be William Mautz from UC Irvine, and the next speaker will be Richard Schlesinger from NYU.

DR. WILLIAM MAUTZ, UC Irvine

I am going to describe the results of a project that is part of an ongoing effort at our Air Pollution Health Effects Laboratory to study the effects of oxidant and acid air pollutants on the respiratory system. Much of our previous work was with sulfate acids. This study, however, was of nitric acid vapor and ozone, and we studied the effects of these compounds alone and in combination. It was a chronic exposure study of rats, and we worked with concentrations that were near the levels of common ambient exposures. Usually in animal toxicology studies, you want to use somewhat higher concentrations, so that you can get some definitive biological responses and then elucidate the mechanisms of toxic action. By going to low concentrations, you risk not really seeing much. In fact, if you did see very strong responses in healthy young animals at low concentrations, you would expect in the human population at large, that some of the more sensitive people might be dropping dead in the streets. Nevertheless, to look for the small effects, we employed a multi-disciplinary approach examining a broad range of biological responses. In parallel with this study was another one employing the same concentrations and exposure protocols at New York University. Rich Schlesinger will talk about that in a few minutes. It employed a different animal model and similar biological end points that overlap, but were not entirely the same as our own. We looked at biological end points that were related to four different kinds of pulmonary disease states (Fig. 1). There is not time here to go over all the details of these, so I will highlight the significant effects or trends in the study.

There were two major exposure experiments (Fig. 2); one of them was a one month dose-response study looking at three different graded concentrations of nitric acid vapor, and the second one was a nine month exposure study looking at 50 micrograms per cubic meter of nitric acid vapor alone, 0.15 parts per million ozone alone, and the combination of the two compounds. Basically, we used the results of that initial one month study to set the concentration at 50 micrograms per cubic meter for the extended 9 month study. We also used an episodic exposure protocol to model the way that air pollution occurs in episodes. We exposed the animals for four hours a day, three days a week for these two periods in the study. For the nine month exposure in particular, we have an experimental design that allows us to look at the interaction between these two compounds. We can do a two way analysis of variance (Fig. 3). We have all the cells covered, so we can see if there is a main effect of nitric acid, a main effect of ozone, and we can look for an interaction effect between the two compounds when they

alveolar duct junction and evaluated tissue density. The "x" axis is increasing ever more distant circles from the junction as illustrated in figure 7. There was a significant effect here from ozone and then a trend for elevated density of tissues in ozone and nitric acid. These are very small changes, but nonetheless indicating that there was perhaps a change in lung elasticity which could increase tissue density in fixed lung preparations.

Q (AUDIENCE) Did they analyze all exposure groups?

A (DR. MAUTZ) Not exactly. They were selective about which tissues they analyzed. They first looked at the some groups at high magnification and then selected groups for further analysis. In the first analysis they compared ozone and nitric acid and then in another analysis set with a different stain they studied the combination exposure and at ozone alone. Because all groups were not included in each analysis, we cannot apply a balanced statistical design to look at the main effects and the interaction effects.

I have implied that a lot of these changes we did observe are related to changes in the lung elasticity. We measured lung compliance directly in the nine month study and figure 11 shows the results at three months into the nine month study, and at nine months. There was a significant main effect of ozone in reducing lung compliance; however, when we looked at the end of nine month study (Fig. 11), we do not see a significant alteration in lung compliance. There was another very sensitive measure we made that is related to both changes in elasticity and in changes in remodeling of cellular architecture. This was a measurement of the trapped gas volume in freshly excised lungs. When the chest is opened, and the lungs collapse, the airways collapse and trap a volume of gas in the peripheral parts of the lung. If there have been changes in the cellular structure of the fine airways or in the elasticity in the lung tissues, this gas volume tends to be larger. There was a significant effect of nitric acid increasing excised lung gas volume for both the three month sacrifice time and the nine month sacrifice (Fig. 12).

Finally, I want to mention two other kinds of measurements that were not directly related to the disease states we studied, but they are interesting because of the striking results. Figure 13, illustrates lung heat-shock proteins and these responded to all of the exposures at nine months. Heat-shock proteins are induced when tissues are placed under stress, either by heat stress for which they were originally named, or a toxic stress due to a chemical exposure. We do not really know what this means in terms of any sort of risk to human populations or disease states. Heat-shock proteins, may serve to protect other proteins from being denatured under heat stress. They may involve some signaling function of the immune system. Nevertheless, it was quite surprising that, at these low concentrations of pollutants, we got a strong heat-shock protein stress response. Finally, in the laboratory of Drs. Sindhu and Kikkawa, we looked at the cytochrome P-450 system in the lung (Fig. 14). The cytochrome P-450 system is a

Another macrophage function we studied was Fc receptor binding capacity. This represents the capacity of macrophages to recognize foreign protein and bind it. In the one month dose-response exposure, there was a depressed capacity for performing that function at the high end, although, in the nine month study, we did not see any significant effects. Finally, there is the whole function of phagocytosis itself, recognizing and engulfing a foreign particle. We tested the ability of macrophages to phagocytose latex microspheres. In the one month study, we saw some depressed phagocytosis among the classes of macrophages that ingested a large number of these particles. In the nine month study, we did not see significant effects, however in all these exposure groups, there are trends for reduced levels of phagocytosis.

Q (AUDIENCE) Nine months in a rat is equivalent to how much in humans?

A (DR. MAUTZ) Oh, about a third of a lifetime.

We also found a trend for reduction in the mucociliary clearance mechanism for removing particles from the lung in the nine month study. Again, all these features that I have talked about are related to the ability of the respiratory system to resist infection.

The fourth disease state we looked at was pulmonary fibrosis and emphysema. Here, we are focusing on the fine structure of the lung. Figure 6 illustrates a section of the lung right at the terminal bronchiole-alveolar duct junction, the region where the tubular airways end, branching into the alveolar duct, and finally, the microscopic air sacs were gas exchange takes place. In 5 μm sections, we looked at an average dimension of alveoli as average cord length of lines randomly laid on the section (Fig. 8). We also looked at average thickness of the alveolar septal walls (Fig. 9), and we sent tissues up to UC Davis where one of our collaborators, Kent Pinkerton, looked at higher magnification at this bronchiole-alveolar duct junction for changes in the density of alveolar tissue. In the one month dose-response study, we saw a decline in alveolar cord length. The average size of the alveoli were smaller, but in the nine month exposure, we did not observe a significant effect on that measure. We saw the reciprocal pattern in the thickness of alveolar septa. Figure 9 shows that there was a significant increase in septal thickness in the one month dose-response exposure but no significant change in the nine month study. These kind of changes are probably due to changes in the elasticity of the lung tissues, which then influence how the lungs will inflate at constant pressure when we prepare them for histological analysis.

Q (AUDIENCE) There is no telling the source strength in the lower chart. In the upper chart (Fig. 9), concentration seems to be a variable. What concentration is being used in the lower? You picked one concentration?

A (DR. MAUTZ) One concentration throughout. For the 9 month exposure, there was 50 micrograms per cubic meter HNO_3, 0.15 parts per million O_3, and then the combination of the two.

Figure 10 shows the results from UC Davis. They looked along the length of the bronchiole-

Figure 1

BIOLOGICAL EFFECTS ANALYZED AND RELATION TO PULMONARY DISEASE

1. ASTHMA.

BREATHING PATTERN

BRONCHIAL MAST CELL DENSITY

BRONCHOALVEOLAR EPITHELIAL PERMEABILITY

2. BRONCHITIS.

TRACHEAL SEROUS CELL DENSITY

TRACHEAL EPITHELIAL GLYCOPROTEIN DENSITY

BRONCHOALVEOLAR LAVAGE FLUID GLYCOPROTEIN

EPITHELIAL CELL KILLING AND TURNOVER, TRACHEA AND BRONCHI

3. RESPIRATORY TRACT INFECTION.

MUCOCILIARY CLEARANCE

EPITHELIAL PERMEABILITY (NASAL AND BRONCHOALVEOLAR)

BRONCHOALVEOLAR LAVAGE CELL DIFFERENTIAL

BRONCHOALVEOLAR LAVAGE BIOCHEMISTRY

MACROPHAGE VIABILITY

MACROPHAGE FC RECEPTOR BINDING ANALYSIS

MACROPHAGE PHAGOCYTIC CAPACITY

4. PULMONARY FIBROSIS/EMPHYSEMA.

PULMONARY IRRITANT REFLEX BREATHING PATTERN

QUASI-STATIC COMPLIANCE

EXCISED LUNG GAS VOLUME

LUNG MORPHOMETRIC ANALYSIS

LUNG COLLAGEN DEPOSITION AND BIOCHEMISTRY

LAVAGE FLUID ELASTASE INHIBITORY CAPACITY

EPITHELIAL CELL KILLING AND TURNOVER, BRONCHIOLES AND ALVEOLI

large set of enzymes that are involved in metabolizing foreign chemical compounds in the body. The macrophage defense system handles things at the particle or cellular level, while the cytochrome P-450 system handles chemical compounds at the molecular level. We found that ozone exposure induced the 2B1 system of enzymes increasing the reaction performing the N-demethylation of benzphetamine. That has been observed in the liver before, but this is the first time it has been observed induced in lung tissue. This may represent a marker for exposure to ozone or a similar oxidant. In the bottom panel, we have an elevation of benzo[A]pyrene metabolism induced by exposure to all O_3 and HNO_3 groups. Functionally, the cytochrome P-450 system is designed to modify chemical compounds so that they can be excreted more easily. However, depending on the chemical compounds metabolized, that system sometimes generates compounds that are even more toxic or even carcinogenic. The metabolism of benzopyrene is an example. Now with the current state of knowledge, there is no way to say what kind of added cancer risk this could represent. Nevertheless, these low level exposures are turning on this cytochrome P-450 system in the lung.

In conclusion, for the model pulmonary disease processes we studied using healthy animals at rest, we generally found rather small responses, mostly nonsignificant trends and no synergistic interactions with ozone and nitric acid vapor. However, a number of these trends do fit patterns for 2 of the 4 disease states we studied, and we might then hypothesize that exposure to ozone and nitric acid carries with it the increased risk of respiratory tract infections and small alterations in lung elasticity or fine structure. Now these small effects we observed here in healthy animals imply that, for the exposure of the human population at large, and particularly for sensitive members of the human population, we might see much stronger effects over a long term exposure.

Q (AUDIENCE) If you imagine changes in elasticity under certain conditions, I wonder if you can separate the effects of the exposure on tissue versus the possible effect on lung fluids?

A (DR. MAUTZ) We made our compliance measurements in saline to remove any of the effects of lung fluid surface tension, so these are tissue effects. In fact, we performed the compliance measurements following the pulmonary lavages, so that we could add more measurements to the same rats. We found some changes in fine structure of the lungs, and that could be related to changes in compliance as well. One of the other major things we went after was to determine if there were changes in lung collagen, both by doing a biochemical analysis of collagen in the lung and a histochemical analysis of the tissue density of collagen by staining and microscopy. Neither of those two end-points showed any significant change, so that did not support the idea that, if there was a change in compliance or elasticity, it was due to collagen.

Figure 3

TWO WAY ANALYSIS OF VARAINCE FOR EFFECT OF HNO_3, O_3, AND $HNO_3 + O_3$.

OZONE	NITRIC ACID: NO	NITRIC ACID: YES
NO	PURIFIED AIR	HNO_3
YES	O_3	$HNO_3 + O_3$

Figure 2

HNO_3 AND O_3 CONCENTRATIONS GENERATED FOR EXPOSURE GROUPS IN THE 1 MONTH DOSE-RESPONSE AND 9 MONTH EXPOSURES. Exposures were episodic: 4 hours/day, 3 days/week. Data are mean ± SD, n of daily averages.

	Target Concentration	Measured Concentration
1 Month Dose-Response Exposure Groups		
1. Purified Air		
2. 50 ($\mu g/m^3$) HNO_3		51.7 ± 13.8, 12
3. 150 ($\mu g/m^3$) HNO_3		170.6 ± 55.9, 12
4. 450 ($\mu g/m^3$) HNO_3		460.5 ± 88.5, 12
9 Month Exposure Groups		
1. Purified Air		
2. 50 ($\mu g/m^3$) HNO_3		51.1 ± 7.4, 120
3. 0.15 ppm O_3		0.151 ± 0.003, 120
4. 50 ($\mu g/m^3$) HNO_3 + 0.15 ppm O_3		49.9 ± 7.0, 120 0.152 ± 0.003, 120

Figure 5

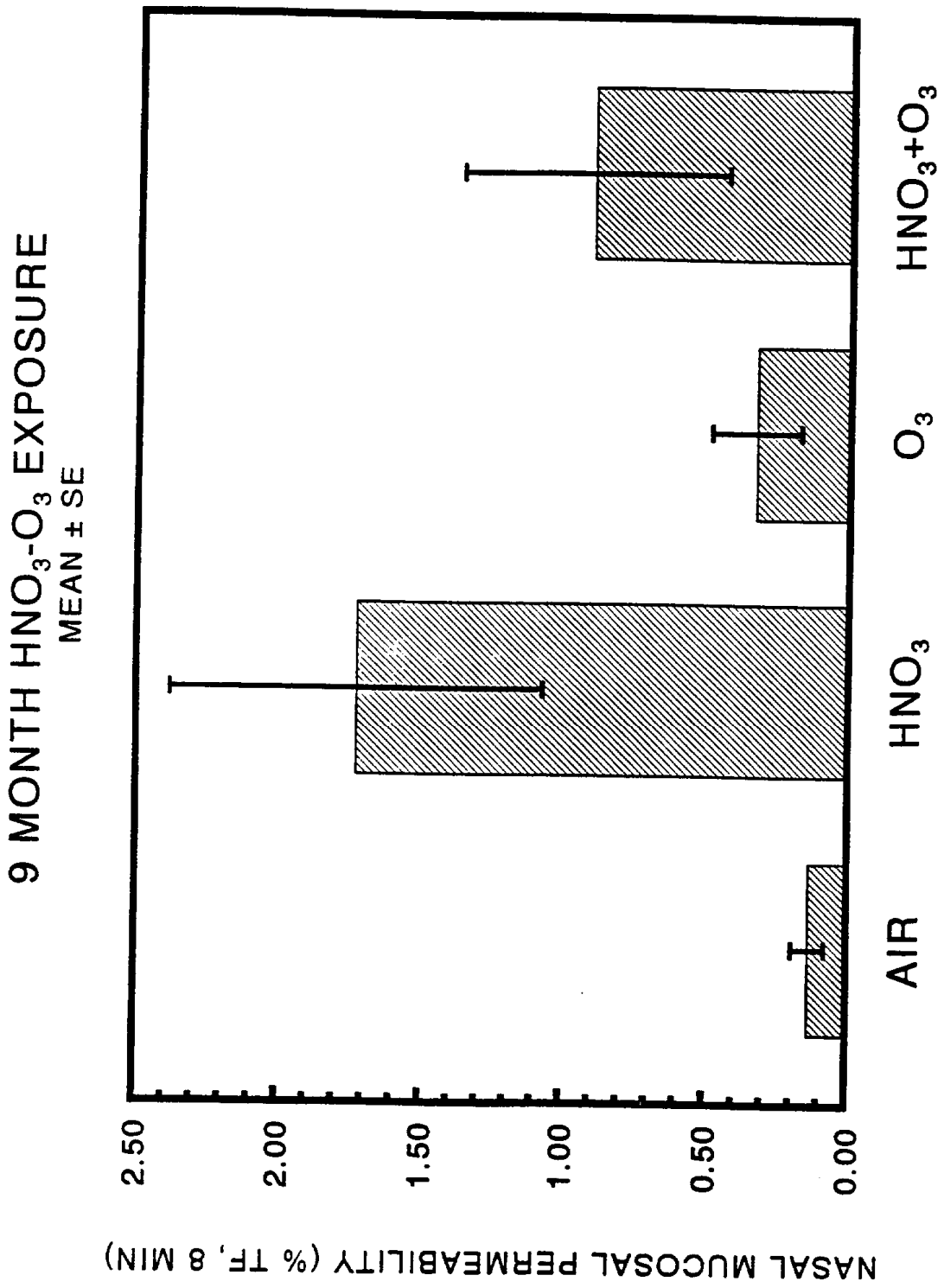
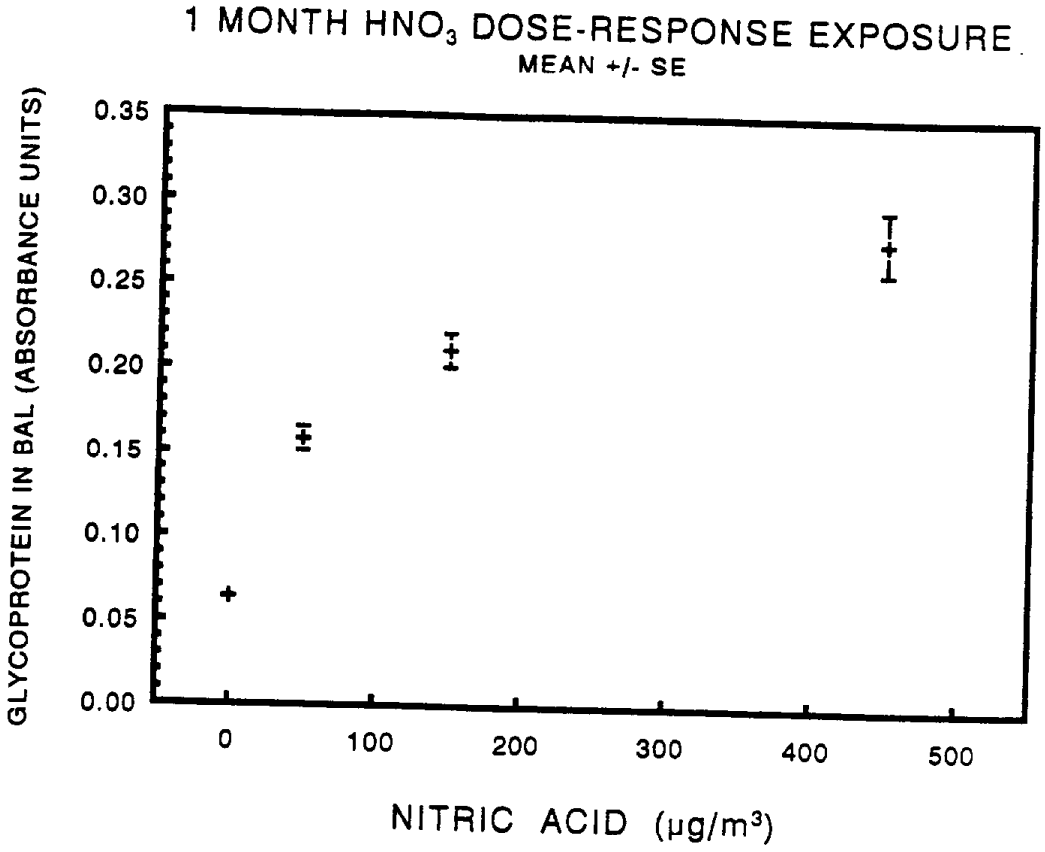

9 MONTH HNO_3-O_3 EXPOSURE
MEAN ± SE
NASAL MUCOSAL PERMEABILITY (% TF, 8 MIN)
2.50
2.00
1.50
1.00
0.50
0.00
AIR
HNO_3
O_3
HNO_3+O_3

Figure 4


1 MONTH HNO_3 DOSE-RESPONSE EXPOSURE
MEAN +/- SE
GLYCOPROTEIN IN BAL (ABSORBANCE UNITS)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
100
200
300
400
500
NITRIC ACID (µg/m³)

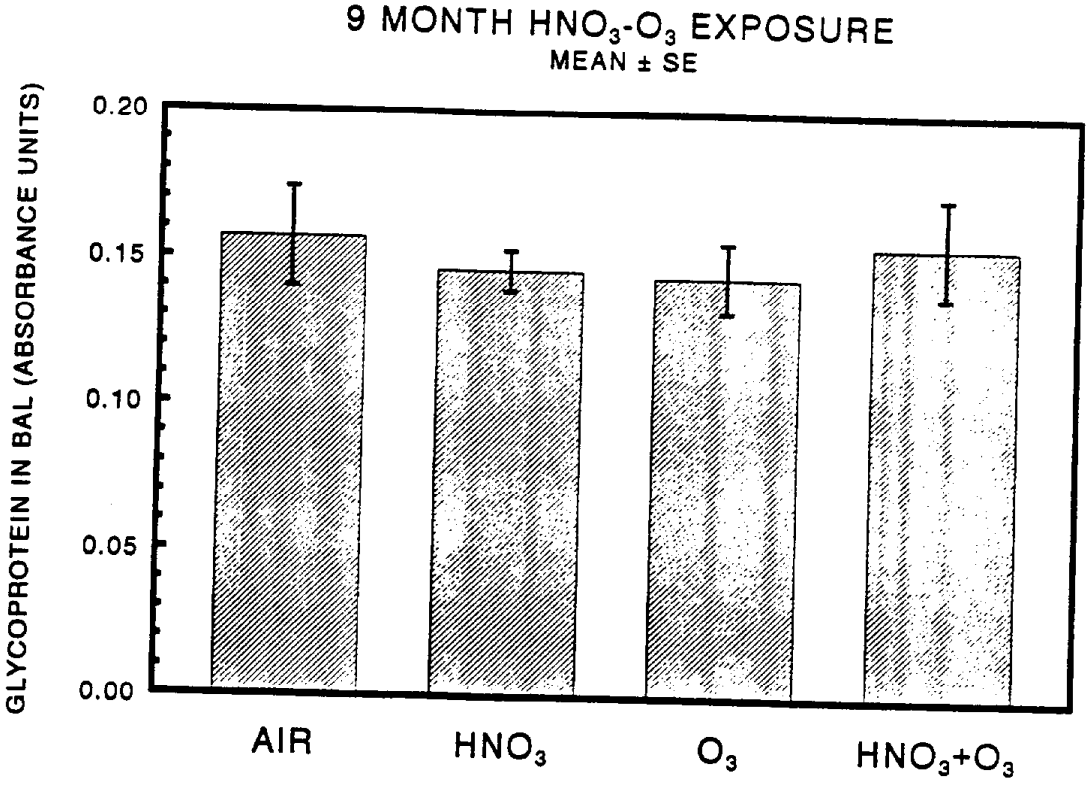

9 MONTH HNO_3-O_3 EXPOSURE
MEAN ± SE
GLYCOPROTEIN IN BAL (ABSORBANCE UNITS)
0.20
0.15
0.10
0.05
0.00
AIR
HNO_3
O_3
HNO_3+O_3

Figure 7

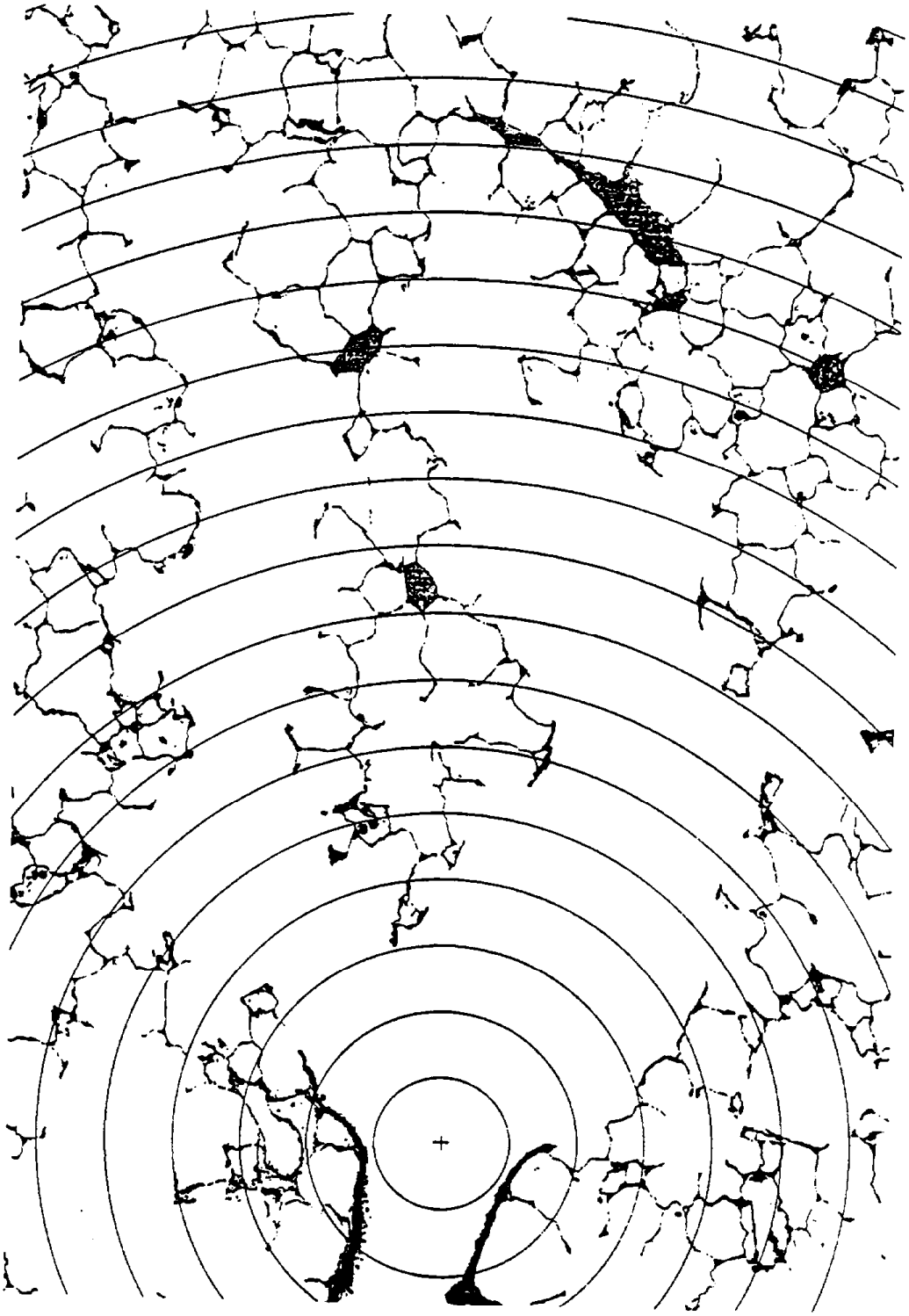


Figure 6

PULMONARY MACROPHAGE FUNCTION AND LAVAGE FLUID BIOCHEMISTRY OF RATS EXPOSED IN 1 MONTH HNO_3 DOSE-RESPONSE AND 9 MONTH EXPOSURE TO HNO_3 AND O_3 ALONE AND IN COMBINATION. The 9 month exposure includes groups of animals analyzed at 3 and 9 months. Data are mean ± SE.

1 Month Dose-Response Exposure to HNO_3

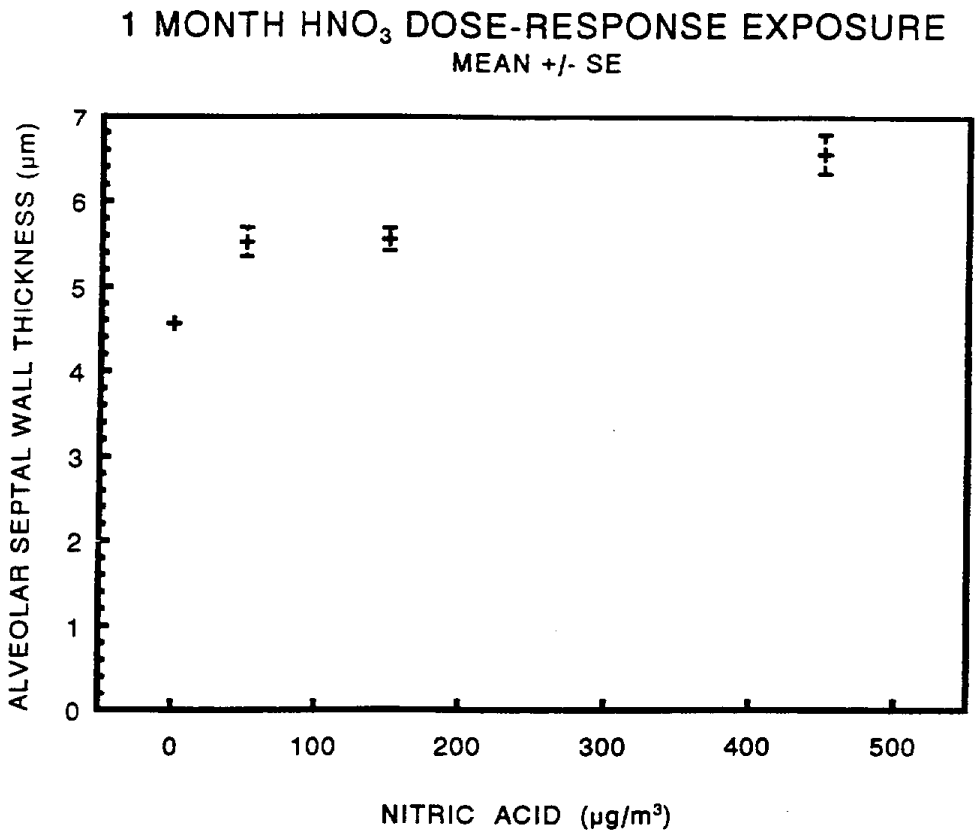
	Purified Air	HNO_3 Concentration 50 $\mu g/m^3$	150 $\mu g/m^3$	450 $\mu g/m^3$
Fc Receptor Binding Capacity (% Rosettes)	43.0 ± 2.8	42.8 ± 1.9	42.6 ± 2.6	36.2 ± 1.6[A]
Phagocytosis (% of cells)				
> 2 particles	85.9 ± 3.4	82.1 ± 6.1	87.4 ± 4.4	86.8 ± 5.3
> 7 particles	16.8 ± 5.1	6.3 ± 1.5[A]	17.0 ± 3.4	9.0 ± 1.8[A]
ß-Glucuronidase (activity units)	3.73 ± 0.41	3.88 ± 0.33	3.03 ± 0.43	4.12 ± 0.54

9 Month Exposure

	Purified Air	50 $\mu g/m^3$ HNO_3	0.15 ppm O_3	$HNO_3 + O_3$
Fc Receptor Binding Capacity (% Rosettes)				
3 Month	47.4 ± 1.9	46.1 ± 2.3	52.0 ± 2.3	49.7 ± 2.1
9 Month	56.8 ± 2.4	56.5 ± 2.9	56.7 ± 2.9	56.6 ± 3.2
Phagocytosis (% of cells)				
3 Month				
> 2 particles	44.3 ± 4.8	43.7 ± 2.9	42.5 ± 3.5	33.8 ± 1.6
> 4 particles	17.9 ± 3.7	17.7 ± 1.8	19.9 ± 3.6	12.0 ± 1.2
9 Month				
> 2 particles	74.2 ± 6.8	72.4 ± 4.6	66.2 ± 6.5,8	63.7 ± 6.6
> 4 particles	54.9 ± 9.4	50.0 ± 5.8	40.9 ± 9.2,8	38.2 ± 6.2
ß-Glucuronidase (activity units)				
3 Month	2.10 ± 0.29	1.78 ± 0.24	2.28 ± 0.31	2.38 ± 0.35
9 Month	1.50 ± 0.18	2.02 ± 0.28	2.12 ± 0.26	2.70 ± 0.35[A]

[A]Significantly different from purified air control ($p < 0.05$).

Figure 9


1 MONTH HNO_3 DOSE-RESPONSE EXPOSURE
MEAN +/- SE
ALVEOLAR SEPTAL WALL THICKNESS (µm)
0
1
2
3
4
5
6
7
0
100
200
300
400
500
NITRIC ACID (µg/m³)

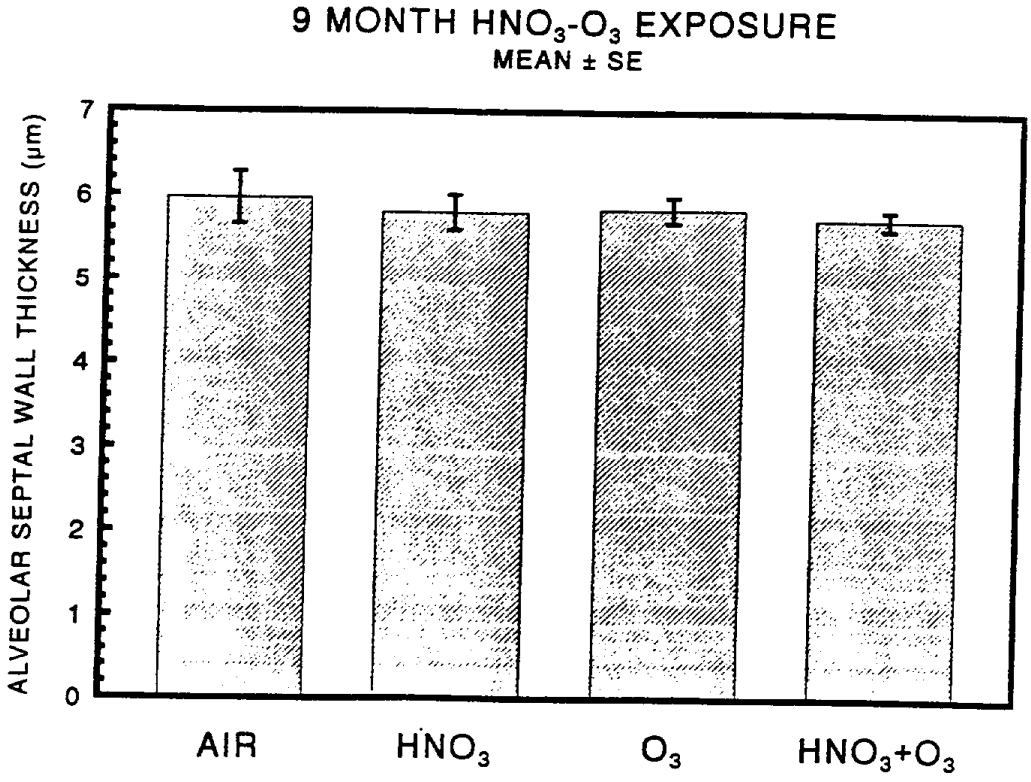
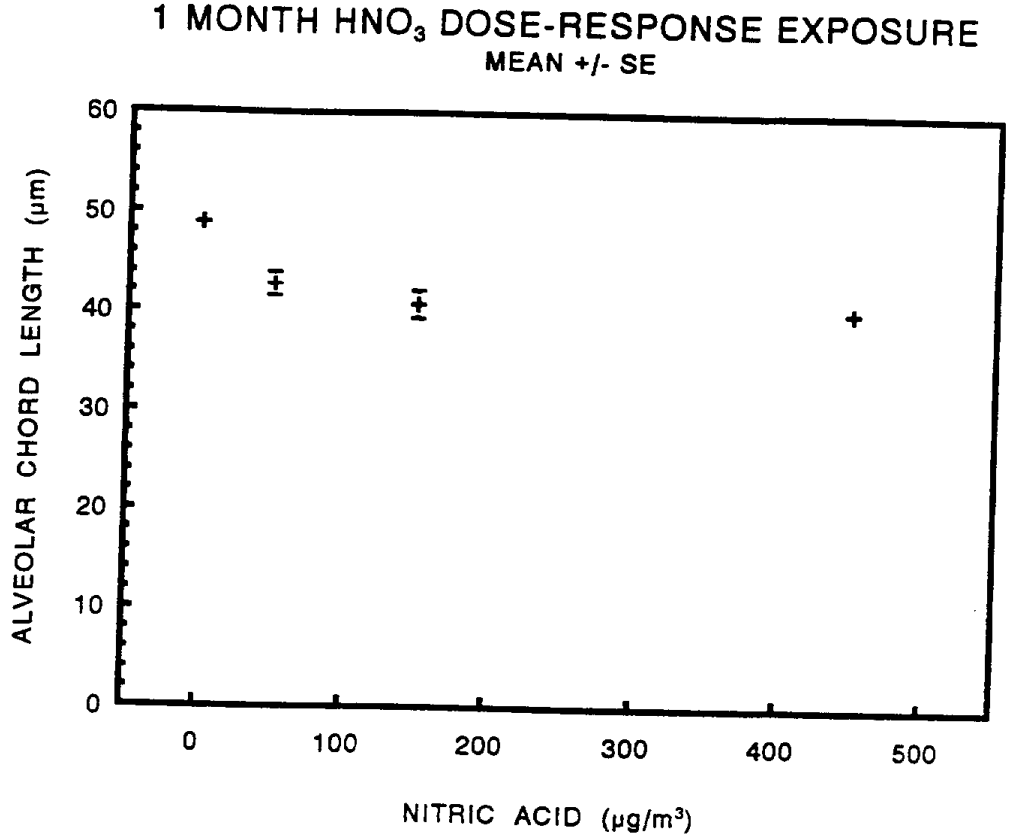

9 MONTH HNO_3-O_3 EXPOSURE
MEAN ± SE
ALVEOLAR SEPTAL WALL THICKNESS (µm)
0
1
2
3
4
5
6
7
AIR
HNO_3
O_3
HNO_3+O_3

Figure 8


1 MONTH HNO_3 DOSE-RESPONSE EXPOSURE
MEAN +/- SE
ALVEOLAR CHORD LENGTH (µm)
60
50
40
30
20
10
0
0
100
200
300
400
500
NITRIC ACID (µg/m³)

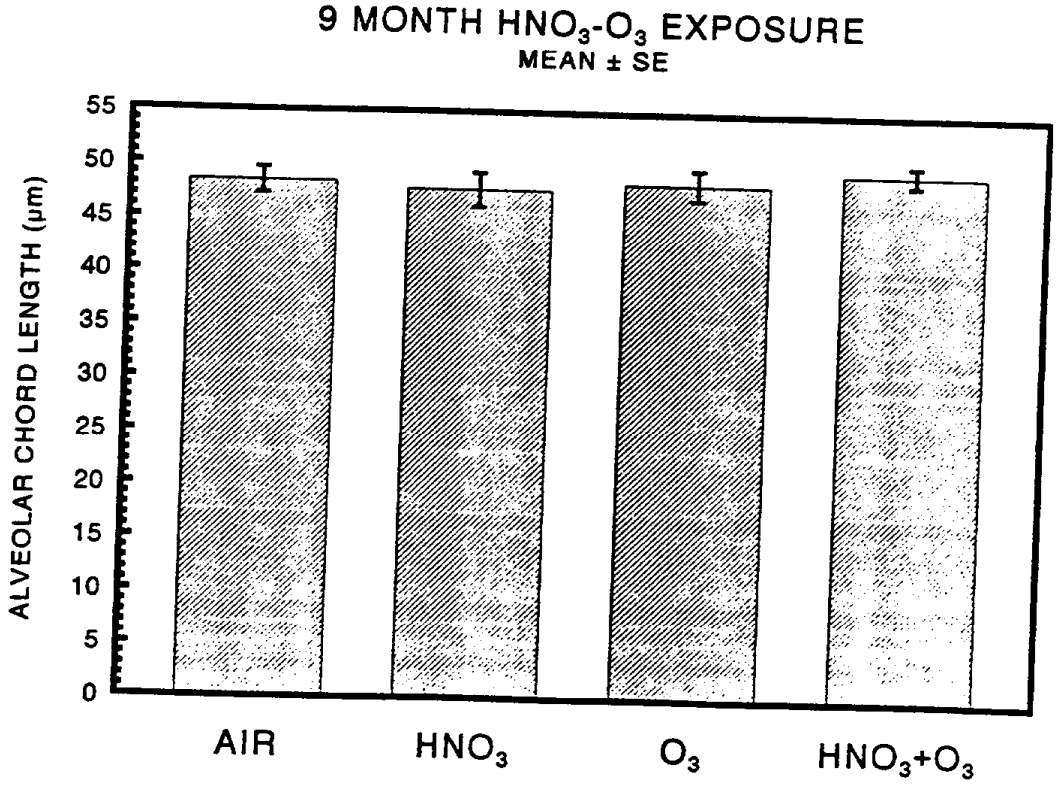
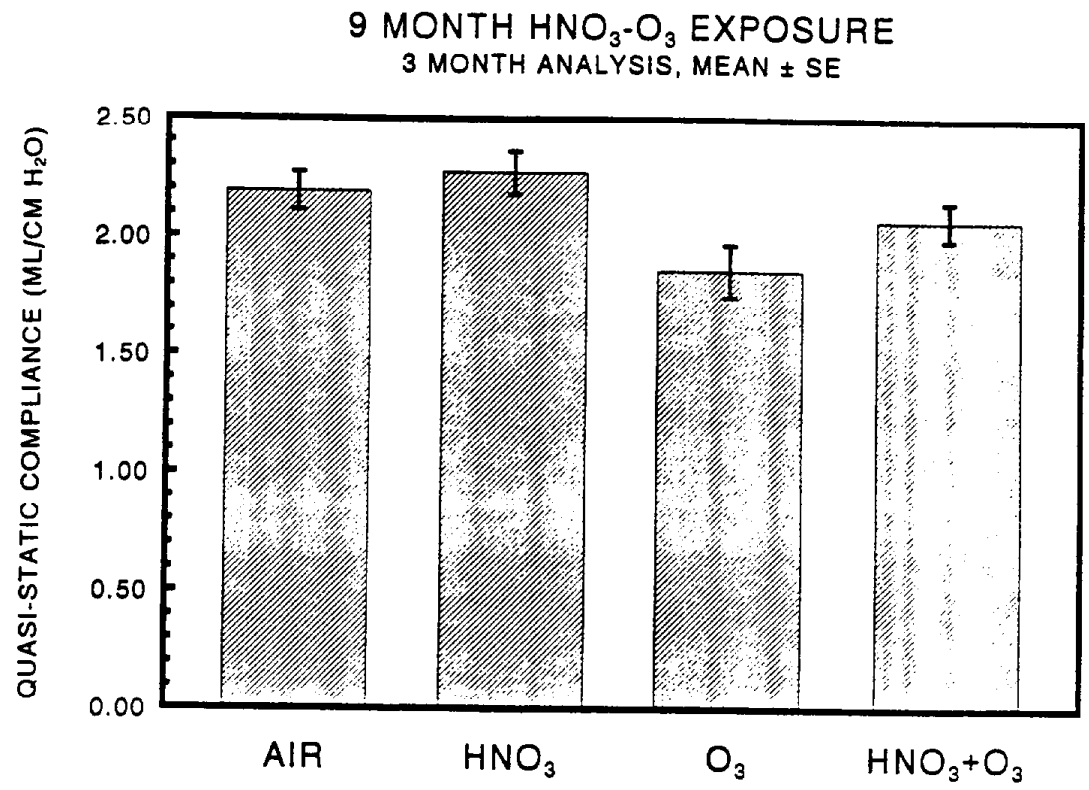

9 MONTH HNO_3-O_3 EXPOSURE
MEAN ± SE
ALVEOLAR CHORD LENGTH (µm)
55
50
45
40
35
30
25
20
15
10
5
0
AIR
HNO_3
O_3
HNO_3+O_3

Figure 11


9 MONTH HNO3-O3 EXPOSURE
3 MONTH ANALYSIS, MEAN ± SE
QUASI-STATIC COMPLIANCE (ML/CM H2O)
2.50
2.00
1.50
1.00
0.50
0.00
AIR
HNO3
O3
HNO3+O3

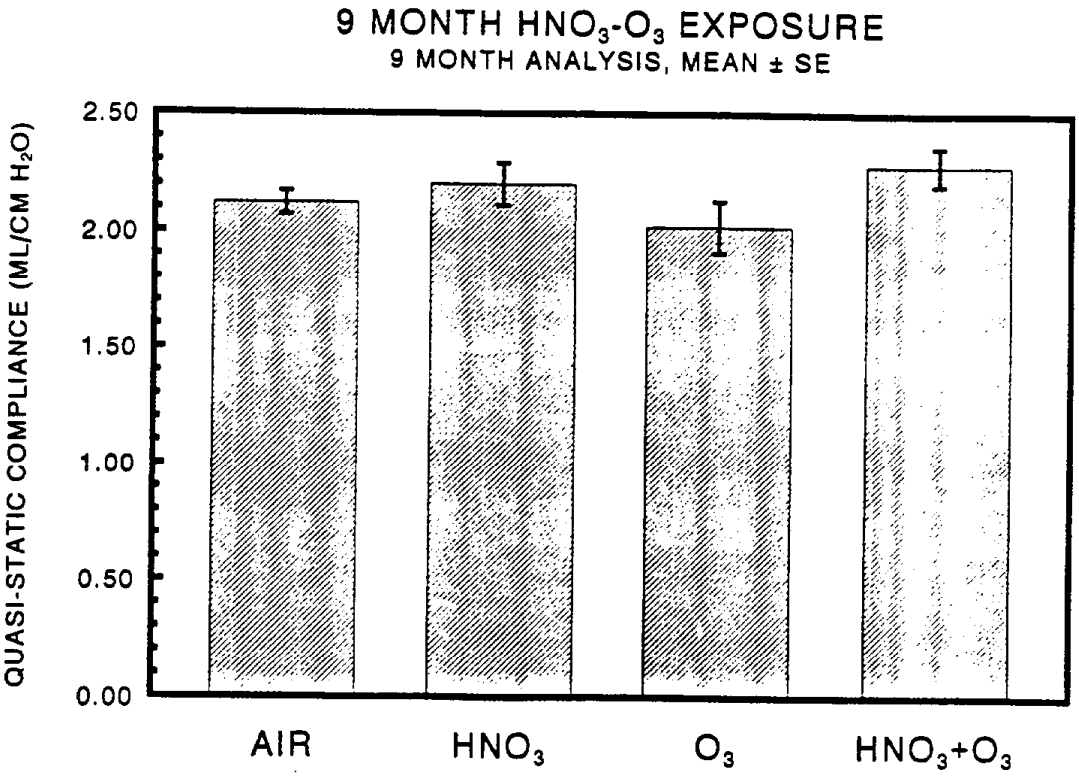

9 MONTH HNO3-O3 EXPOSURE
9 MONTH ANALYSIS, MEAN ± SE
QUASI-STATIC COMPLIANCE (ML/CM H2O)
2.50
2.00
1.50
1.00
0.50
0.00
AIR
HNO3
O3
HNO3+O3

Figure 10

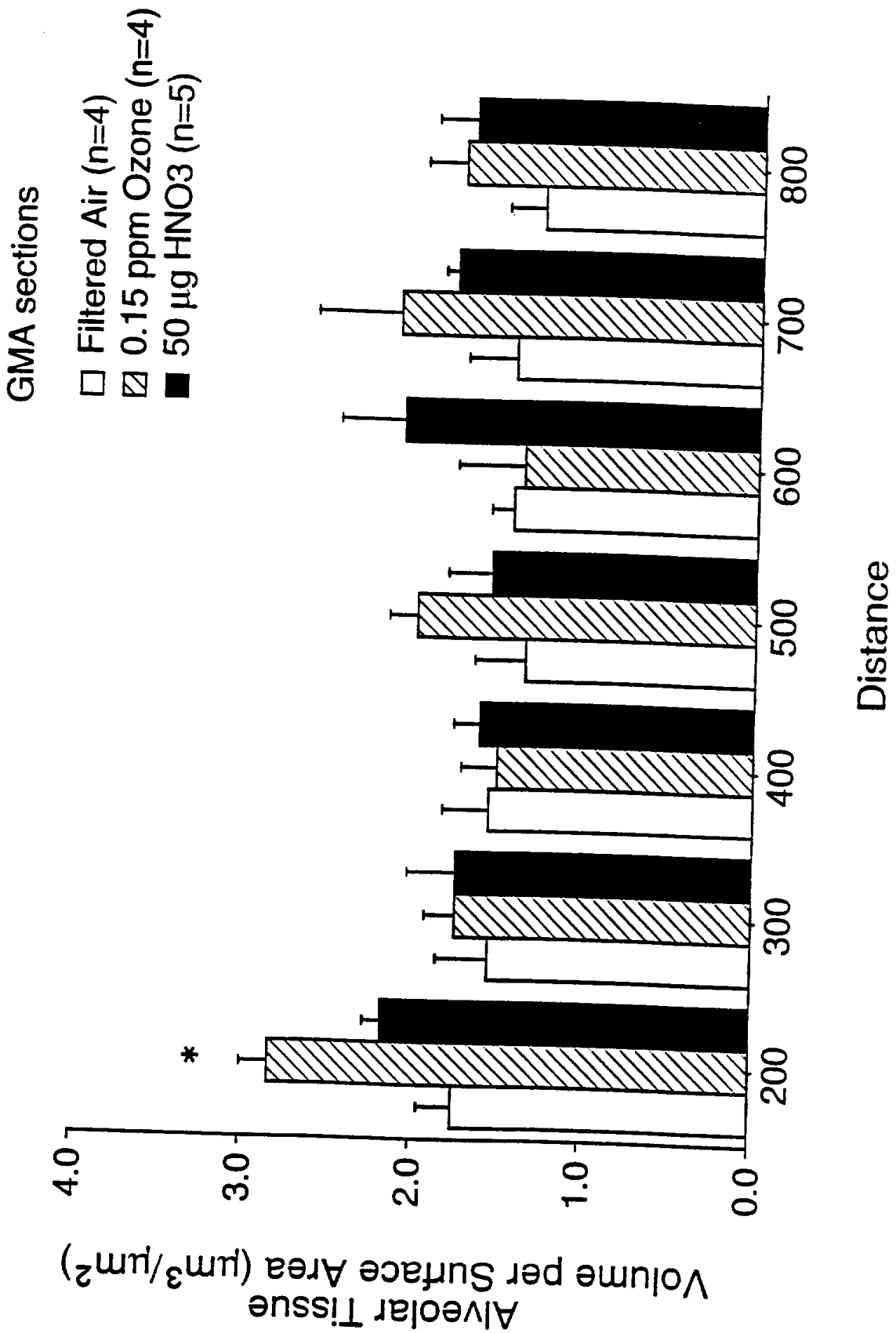

GMA sections
Filtered Air (n=4)
0.15 ppm Ozone (n=4)
50 µg HNO3 (n=5)
*
Alveolar Tissue Volume per Surface Area (µm3/µm2)
4.0
3.0
2.0
1.0
0.0
200
300
400
500
600
700
800
Distance

Figure 13

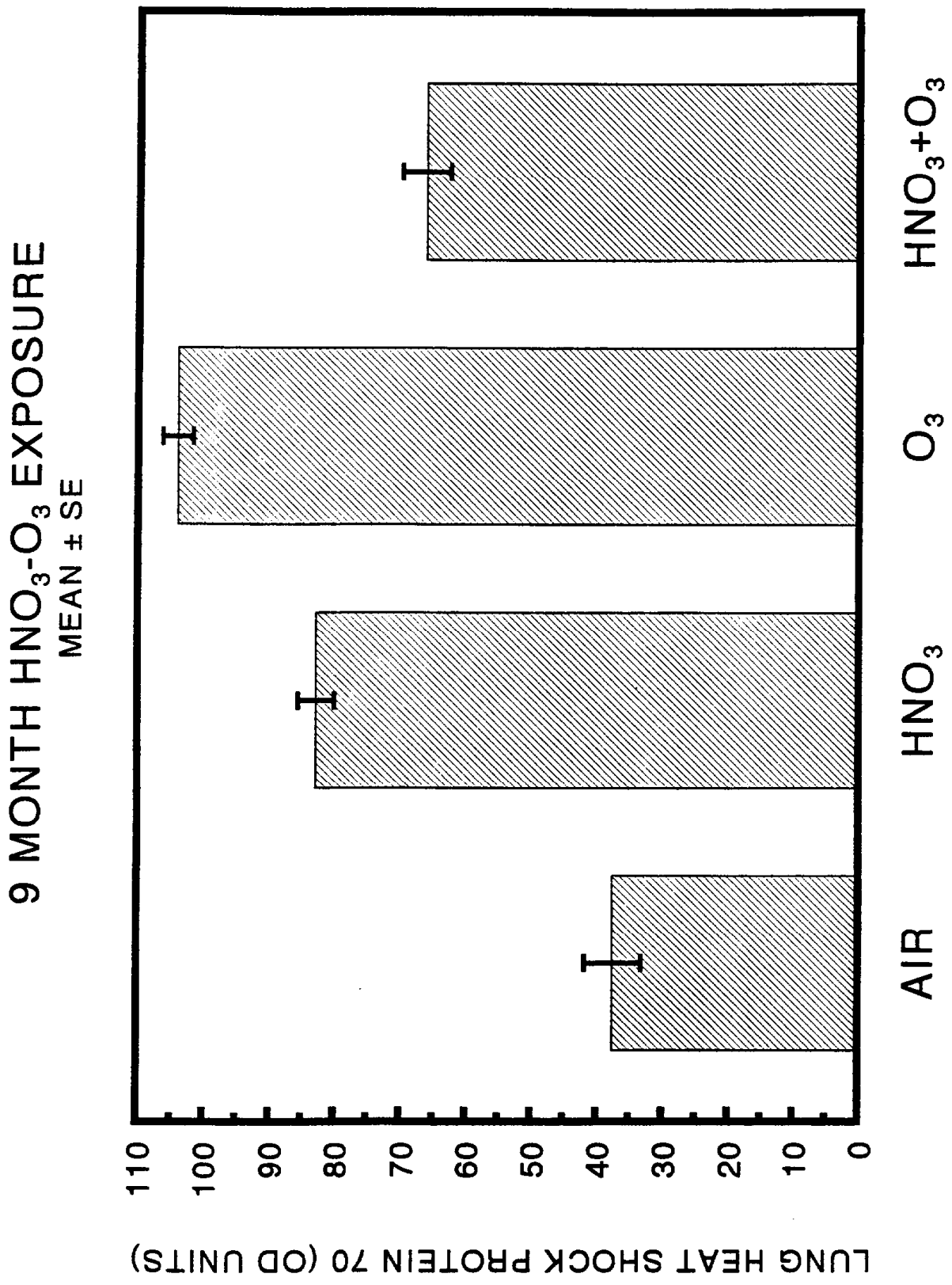
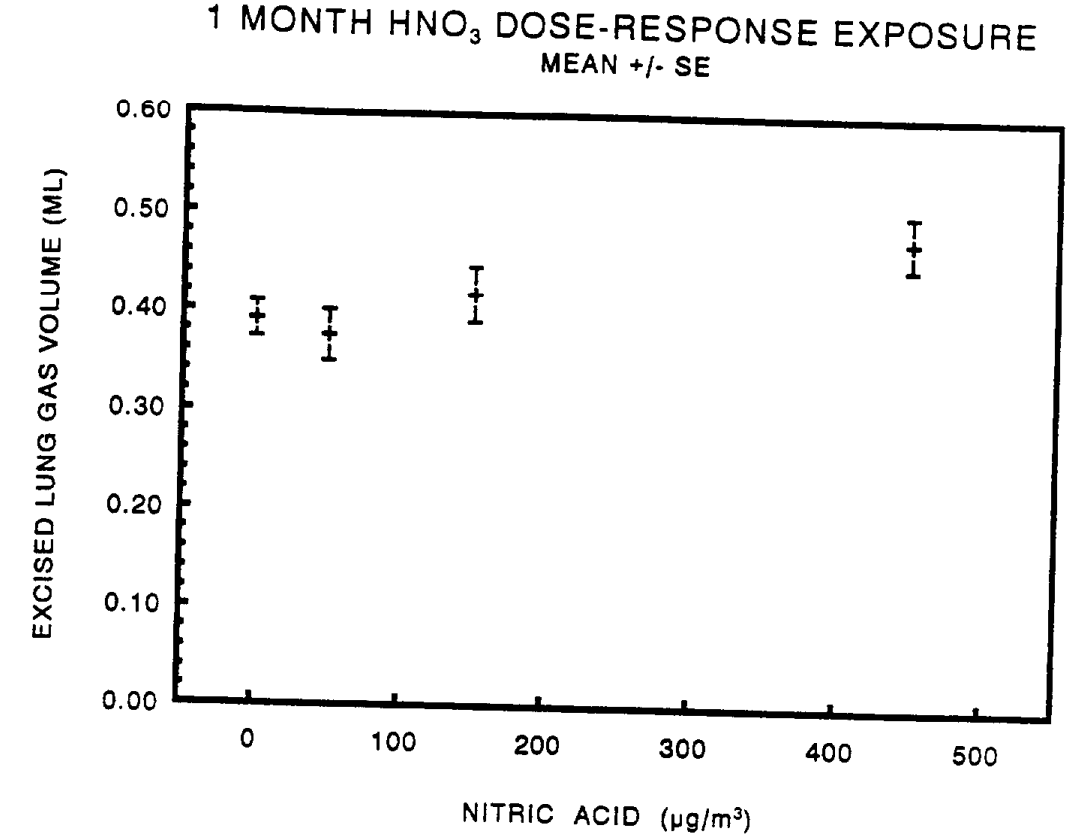
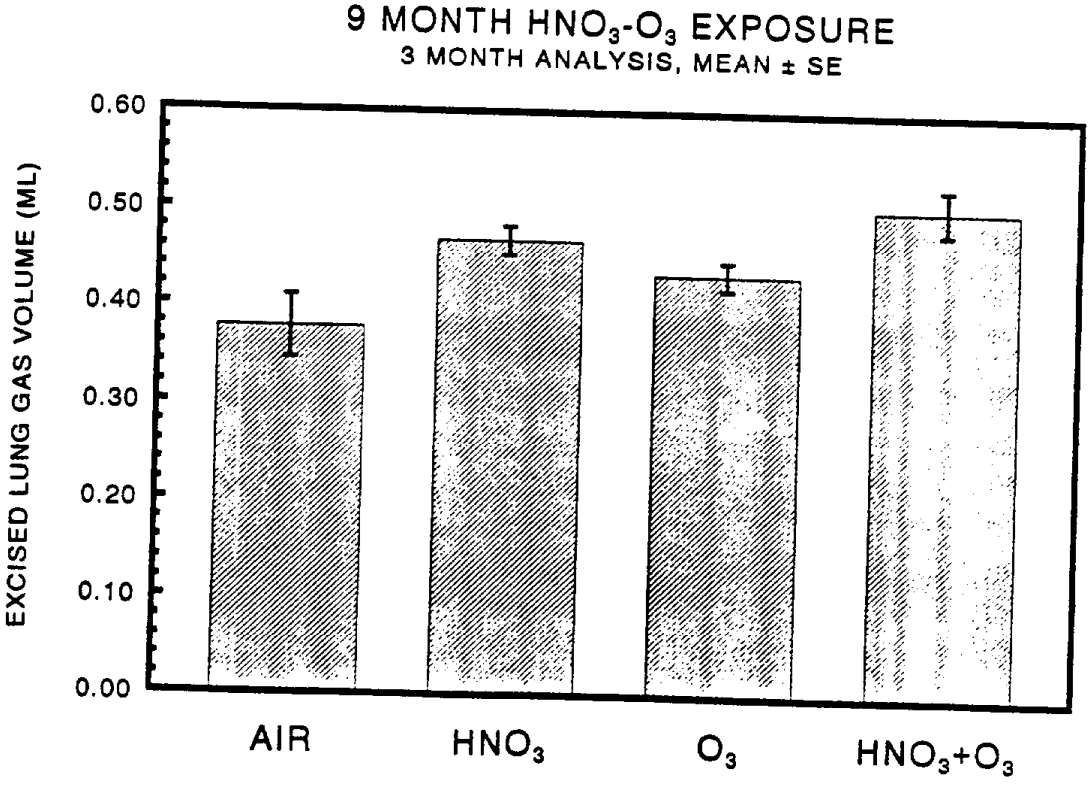

9 MONTH HNO_3-O_3 EXPOSURE
MEAN ± SE
LUNG HEAT SHOCK PROTEIN 70 (OD UNITS)
0
10
20
30
40
50
60
70
80
90
100
110
AIR
HNO_3
O_3
HNO_3+O_3

Figure 12


1 MONTH HNO_3 DOSE-RESPONSE EXPOSURE
MEAN +/- SE
EXCISED LUNG GAS VOLUME (ML)
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
100
200
300
400
500
NITRIC ACID (µg/m³)


9 MONTH HNO_3-O_3 EXPOSURE
3 MONTH ANALYSIS, MEAN ± SE
EXCISED LUNG GAS VOLUME (ML)
0.60
0.50
0.40
0.30
0.20
0.10
0.00
AIR
HNO_3
O_3
HNO_3+O_3

DR. RICHARD SCHLESINGER, NYU Medical Center

As Bill said, our HNO_3 and O_3 animal exposure protocols were exactly the same. We used rabbits instead of rats and the idea was to do a cross species extrapolation which could then be used to compare to acute studies in humans that were done by Dr. John Balmes. In our scaling of time, Bill was talking about months, and I am using weeks but the periods were the same. There was a one month, a three month, and a nine month exposure, and these end points did not change. Lactate dehydrogenase is an index of cellular cytotoxicity. We did not get any changes in protein in lavage suggesting that there was no change in permeability in the airway. Bill found permeability changes in the nose, however.

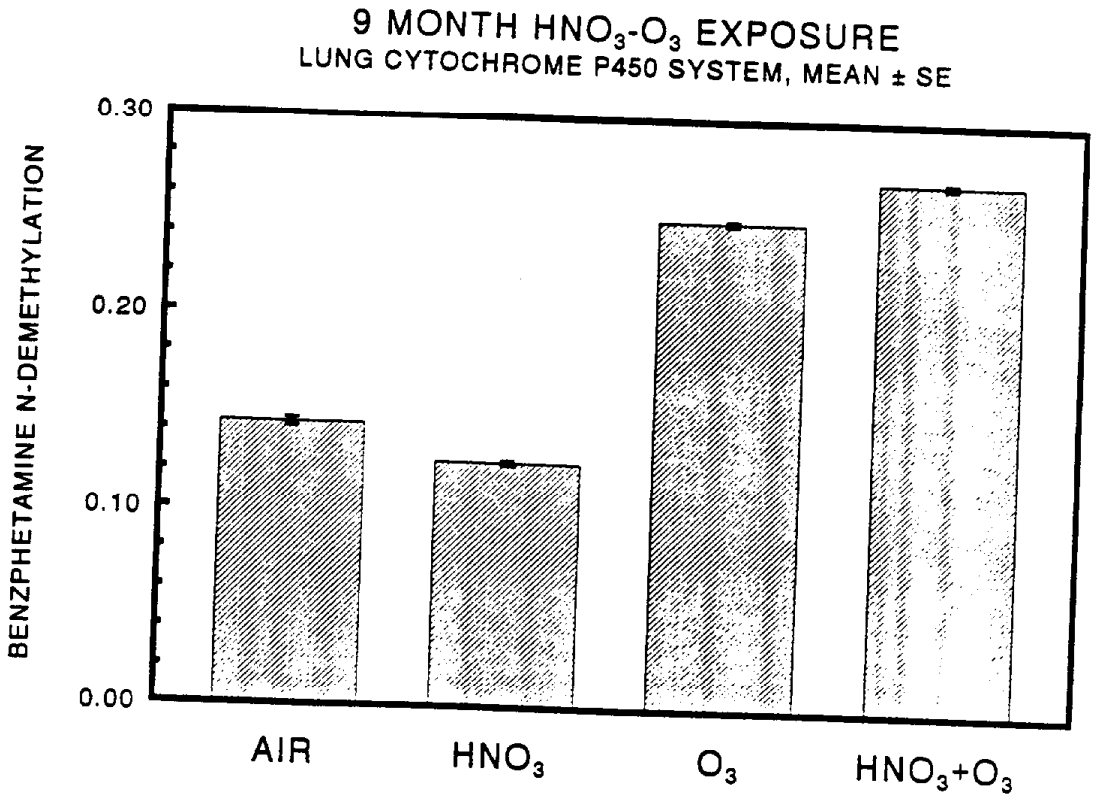
(DR. MAUTZ) We also looked at permeability in tracheobronchial and pulmonary airways, and we also found no significant changes there.

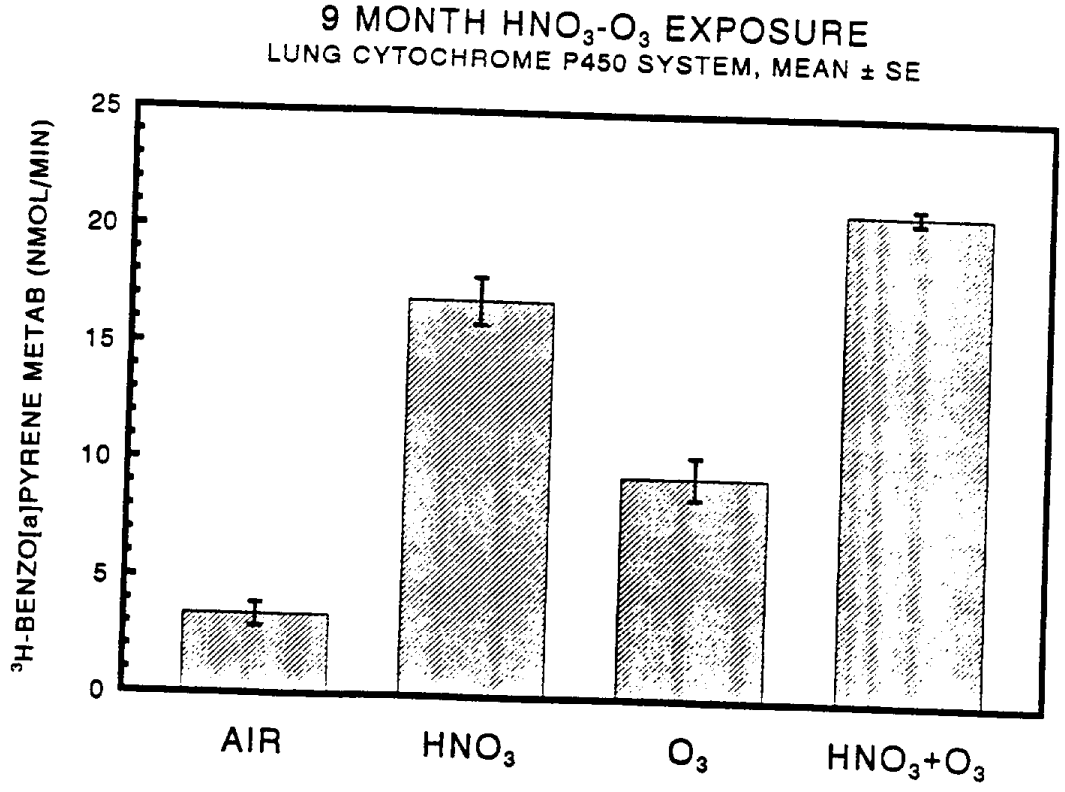
DR. RICHARD SCHLESINGER

We did not get any change in lavage differential cell counts, meaning there was no influx of inflammatory cells in the airways below the head. There were no changes in total cell numbers, so there was evidence from lavage assays that there was no inflammation caused by the acid or the ozone or the acid ozone mixture at 50 micrograms per cubic meter HNO_3. There was no change in the ability of these macrophages to ingest inert particles, suggesting that their defense function in this regard was not changed. There was no change in tracheal responses. John Balmes talked about airway reactivity. We did an assay of airway reactivity whereby, instead of using the whole animal, we took sections of tracheal bronchi and challenged them with the bronchoconstricting agent. This was the in vitro equivalent of in vivo hyper-reactivity studies, and the tracheas did not behave any differently after exposure to acid or ozone. We did not find the changes that were observed in the rat. There was no change in septal wall thickness, and no change in alveolar dimension. There was no evidence of inflammation from a pathological standpoint under light microscopy. Because we did not see anything with the light microscope, we did not do electron microscopy.

In the four week exposure, we did note some changes, most of which resolved after long term exposure. We did some assays of cytokines, chemical mediators in the lavage fluid, as a screening assay. As Bill said, this is really a very descriptive screening study, because nobody knew what nitric acid would do. We picked a few of the important cytokines. TNF is tumor necrosis factor, and this mediator is involved in anti-tumor activity. It is a pro-inflammatory agent and a very potent chemical in the lung. We found that in the four week exposure, there was a reduction in the cytokine activity at the two high concentrations. Superoxide is a reactive oxygen species that is produced by macrophages. It is involved in the macrophage's ability to kill microbes such as bacteria. A reduction in the activity of superoxide or a reduction in the production of superoxide by macrophages is presumed to reflect a reduction in the ability of macrophages to handle infectious agents. We saw a reduction in superoxide activity of

Figure 14


9 MONTH HNO3-O3 EXPOSURE
LUNG CYTOCHROME P450 SYSTEM, MEAN ± SE
BENZPHETAMINE N-DEMETHYLATION
0.30
0.20
0.10
0.00
AIR
HNO3
O3
HNO3+O3


9 MONTH HNO3-O3 EXPOSURE
LUNG CYTOCHROME P450 SYSTEM, MEAN ± SE
3H-BENZO[a]PYRENE METAB (NMOL/MIN)
25
20
15
10
5
0
AIR
HNO3
O3
HNO3+O3

After four weeks of exposure, we found that the airways were hyporeactive at 150 and 450 micrograms per cubic meter of acid but not at 50 micrograms per cubic meter. After 40 weeks of exposure, we found that the 50 micrograms per cubic meter acid was effective in producing this hyporeactivity or reduced responsiveness to a bronchoconstrictor. The ozone had no effect, and the mixture had no effect, so when you add the ozone, this acid effect disappeared. There was a trend, even at 50 micrograms per cubic meter acid, toward hyporeactivity, something we saw definitively only at the high concentrations. If you are looking for an explanation, we do not quite have one yet. Clearly, there are some endogenous respiratory chemicals that have been implicated in hyporeactivity; nitric oxide is one. If one wanted to go out on a very long limb, which we did in the paper, you can speculate that perhaps the nitric acid is reacting with some amino acids and producing some fairly stable compounds which are related to nitric oxide and have been shown to cause hyporeactivity. Aside from that, I cannot explain why nitric acid causes a response which is exactly opposite to that of most of the other acids that we have studied.

One of the dogmas of nitric acid research was that it is a very soluble and reactive material; therefore, it is going to be scrubbed out completely in the upper respiratory tract, and nothing is going to reach the lung. Now, it is clear from the exposures done here at Irvine and from our exposures, that we did get effects on macrophages, and we did get effects in the bronchi and trachea, and there was alveolar remodeling. Clearly, enough of the nitric acid was getting through the head to impact upon the lung. We were interested in figuring out how this could be with such a highly reactive and soluble vapor. We put together a mock upper respiratory tract system of the rabbit, and the idea was to see what happens to the nitric acid vapor when humidity was raised and ammonia was added. In the intact respiratory system, the lungs are at about 99% relative humidity, and there is a substantial amount of endogenous ammonia in the head from bacterial degradation of food in the mouth but also in the nose. Nitric acid is reacting with ammonia. We built conditioning tubes, and we generated nitric acid into these tubes. We had a humidifier which could add water vapor, and we had an ammonia permeation tube which could add metered amounts of ammonia. The hypothesis was that in human airways and in the presence of ammonia, the nitric acid vapor would become an aerosol, and this aerosol would then penetrate into the lung. We monitored the reaction state of this mix with an ammonia monitor, a hygrometer for humidity, a nitric acid monitor, and a condensation nucleus counter for measuring whether any particles are produced. We had other equipment to measure particle size. Essentially, we were putting in vapors and trying to determine what came out the other end. We started with 800 micrograms per cubic meter of nitric acid, about twice the highest level we used in the short study, at five different relative humidities. We used high concentrations to make sure we could resolve something, and this showed that something particulate in nature was coming out. We saw an increase in particle

macrophages recovered from the animals at all three levels after the one month exposure.

One of the purposes of the study was to look at interactions. I want to point out that we did some interaction studies, but they were not predictive. This is very similar to what has been found in other studies and also in our laboratory with acidic sulfates. The type of interaction you get depends upon the biological end-point and upon the concentration of the two chemicals that you are studying. Peroxide production is an example. Hydrogen peroxide is another reactive oxygen species produced by macrophages and is involved in antibacterial defense. In the four week exposure at 50 micrograms per cubic meter, nitric acid caused a production of this material. The ozone did nothing, and the mixture showed a slight reduction which was not statistically significant. The results of the interaction test came out as antagonism. The acid changed it, and the mixture did not change it, so the effect was mitigated. For superoxide production, as we saw before, nitric acid at 50 micrograms per cubic meter reduced superoxide and ozone did nothing, so this is an antagonism. We found no interaction with TNF activity. Basically, we saw antagonism or no interaction. There were basically no interactions except at a couple of end-points, so in the short-term exposures, there were very few interactions between the two materials.

There was one end point that was consistently altered throughout the study. This was the airway reactivity that I mentioned before, but now we are studying the bronchus. The tracheal reactivity was not altered, but the bronchial activity was. In our previous studies with sulfuric acid, there was usually a consistent finding that the inhalation of acidic sulfates caused the airways to be hyper-reactive. If you challenge them with a bronchoconstricting agent, pollutant exposed airways will respond at a lower level of the challenge agent than airways exposed to air. In other words, the airways become twitchy, and this is a hallmark of asthma as John Balmes mentioned. With nitric acid, the responsiveness to the bronchoconstrictor, acetylcholine, was hyporeactive. The airways had a smaller response to the bronchoconstrictor following acid exposure than before exposure, and this occurred at the two highest acid concentrations after four weeks exposure. This is an unexpected finding considering most acid exposures cause hyper-reactivity. When we exposed the animals from the four week exposure to the mixture, we could not measure a bronchoconstrictive effect. The acetylcholine had no effect on the airway, so something strange was going on. We went into our 12 and 40 week exposures, and, as I showed before, most of the end-points had no effect. Jerry Last at UC Davis looked at another disease entity, fibrosis. He measured the breakdown product of collagen which is a protein in the lungs that may be involved in fibrosis if its turnover is more rapid than normal. This breakdown product is hydroxyproline, and the study showed that there was no change in collagen metabolism in the lung after 40 weeks to 50 micrograms per cubic meter of the acid, the ozone, or the mixture. No change in collagen metabolism suggests that the exposure had no effect on potential fibrosis in the airways of the lung.

with a 12-month exposure. There were increased secretory cells in the small airways and increased mucous production. This was also true with sulfuric acid. As I mentioned, there was hyper-reactivity in both the in vivo measurements as well as in vitro measurements, and there was hyper-reactivity in vitro with sulfuric acid levels as low as 75 micrograms per cubic meter. It is clear to us that the effects of sulfuric acid were due solely to the hydrogen ion and not to the sulfate. The fact that the nitric acid caused hyporeactivity may suggest that nitrate may be involved or that nitric acid is reacting in a way that sulfuric acid is not. That is a more complicated effect, compared to effects due solely to changing pH in the airways of a lung. Nitric acid may, in fact, react within the lung to cause some of the changes that we saw.

Q (AUDIENCE) Regarding that antagonism you see with ozone, do you have some thoughts on how something like that can happen?

A (DR. SCHLESINGER) We have been seeing antagonism with ozone, with nitric acid, and with sulfuric acid, and the big problem is trying to determine the mechanism of interaction. We cannot figure out what the mechanism of action of the two acids is. With sulfuric acid, you can explain synergism easier than antagonism, so at this stage, we do not quite understand what is going on. However, as I said, the antagonism was in a couple of end-points, and most of the time there was no interaction at all.

Q (AUDIENCE) Is it possible that nitric acid vapor somehow can get converted to nitric oxide?

A (DR. SCHLESINGER) Well, there was one possibility. There was a suggestion that the nitric acid could react with amino acids, and produce a compound that had been shown to have long lasting bronchodilator properties. That was the basis of our supposition about why the nitric acid caused hyporeactivity in the airways.

Q (AUDIENCE) One final question. Ozone is known to oxidize many proteins, because it is a very strong oxidizer. Do you believe that nitric acid can also do something like that?

A (DR. SCHLESINGER) That is another possibility. Unlike sulfuric acid, nitric acid is more reactive in that regard.

size with increasing relative humidity which is not surprising. The particle size of what was coming out was somewhere between 0.1 and 0.2 micrometers, so there were particles being formed, and the particle size was growing as the humidity was going up. Next, we added ammonia at 400 parts per billion which is a valid number for concentration of ammonia in the mouth. The resident time in that conditioning tube was 0.2 seconds, which is the time the acid would mix with the humidity or the ammonia. We picked that because that was the residence time in the head of the rabbit at the low rates they breathe. The particles we measured had nitrate. There was an increase in the production of particles from the vapor phase nitric acid, and this increased further as ammonia was added at both low and high humidity. At 77% relative humidity with 400 ppb ammonia and 800 micrograms per cubic meter nitric acid, we had about 75% nitrate in the particles.

There were three possibilities here. In the humid atmosphere of the lung, pure nitric acid vapor could become pure nitric acid droplets and be deposited on the airways. Nitric acid could react with the ammonia forming ammonium nitrate, which is not acidic, but perhaps the ammonium nitrate would act as a vehicle for absorbed nitric acid and therefore deliver the acid down to the deep lung. We did look at an inert aerosol, with an inert particle, to determine if it could act as a vehicle for nitric acid. We found that if you add another aerosol (NaCl) to nitric acid, the percentage of particles with nitrate increases. Again, this is not surprising. The acid is probably reacting with chloride and sodium nitrate is formed. The conclusion from that part of the study was that our original supposition that the nitric acid vapor reached the deep lung as a particle is probably true. Most likely, it reaches that level as a combination of very small nitric acid particles, perhaps 0.1 micrometers or so, and ammonium nitrate. Clearly, the nitric acid does reach the lung, because we did see biological effects. We could not do the studies at 99% humidity, because we would develop a fog in the conditioning chamber and everything would wash out. As the ammonia levels go up, we see an increase in particle production. That may explain some of the dosimetry. Whereas it was previously thought that most of the acid would be scrubbed out in the nose, it is clear that it is not.

The other issue is the potency of the nitric acid. There were no earth shattering responses. Some of the effects on macrophage defense function that we saw early on disappeared by the end of the 40-week exposure. There was no inflammation. There were no histopathological changes. We did see consistent hyporeactivity. I thought I would compare it to the sulfate concentrations for which we have data. Remember, for the same equivalent, for the same mass concentration of nitric acid as sulfuric acid, you are going to get about 20% more hydrogen ions from the sulfuric acid. Sulfuric acid seems to be more potent than nitric acid. In some of the studies at UC Davis with ozone, 10 micrograms per cubic meter acid with ozone has produced changes in collagen metabolism. These are short-term studies of three days, and ours were longer. There was an increase in the type of response and a changing response

matrix of exposure patterns for the four key pollutants. Ideally, there would have been at least one representative for every possible high-low combination for the four pollutants. The assessment was based on 86 communities' monitoring site data, and it turned out that not all possible exposure combinations even existed in the study region. The final assessment was narrowed down to 12 communities. Atascadero, Santa Maria, and Lompoc communities were relatively non-polluted. The scheme for population enrollment was developed during phase two. 1800 4th graders and 900 each of 7th and 10th graders with an equal number from each community were enrolled. The plan for phase three is to enroll another 1800 4th graders. The children will be followed through high school graduation or whenever the study ends.

The health effects assessments include a questionnaire which is designed to capture information on each child's personal data: where they were born, any problem at the time of birth, basic information on health problems such as asthma, residence history, household characteristics, socioeconomic data, and general demographic data. Questionnaires are administered each fall. The pulmonary function testing is done in the school room each spring. The last area of data collection for health is school absence monitoring. We are looking for acute respiratory illness by following up on school absences; the medical staff calls parents when the child has been absent.

One of the hallmarks of this particular project is the emphasis on exposure assessment. All too often we rely on a community air monitor, which raises a fundamental issue when we see a health effect in epidemiology. There has always been the fundamental question: "does that represent an individual's exposure?" This particular project has an ambient monitoring component, a microenvironmental component which includes indoor and outdoor measurements at schools and homes, time activity assessment, and selected personal monitoring. Essentially, all of this information is used to develop and validate an exposure model. This will provide us a closer estimate of exposure and hopefully a closer estimate of the actual dose that is affecting the response. It is not going to give us a dose, but it will get us closer to that point.

In phase two, about 3600 children have been enrolled and followed in both 1993 and 1994. Each child has completed lung function tests, a health and general information questionnaire, activity questionnaire, and illness and absence monitoring has been implemented. A key issue of the study design was to select communities that had approximately equivalent demographics, and the communities were selected where the populations would have maximum stability. This was done by looking at 1990 census data. If you are trying to follow individuals over a long period of time, you want them to stay in relatively the same place. We were projecting a 10% loss to follow-up, (i.e., children moving out of the area). As it turned out, we got 14%, but about 4% of those we tracked into the other communities we were studying.

MR. DANE WESTERDAHL, Moderator

Our final speaker is Helene Margolis. She will give a brief status report on two epidemiologic studies that I briefly mentioned earlier. She has been analyzing data with Mike Lebowitz and others from the initial phase of study, and we now have an opportunity to look at the effects on real people including children. That Children's Health Study went before the Air Resources Board yesterday for consideration and approval for the next phase. That next phase is 42 months of study and it was approved by the board. She will tell you more about that process too.

3. Status of Epidemiological Studies. Mr. Dane Westerdahl, CARB and MS Helene Margolis, CARB.

MS HELENE MARGOLIS, Air Resources Board

The children's health study, as Dane indicated, had been initiated through the long-term exposure health effects research program. Today you have heard discussions about the acute effects and the short-term effects but the fundamental issue in this study is the long-term effects. When we began planning this study much of the research indicated that there probably were no acute effects of nitric acid. One does not have the ability to conduct a long term controlled human exposure study. In a large population however, there may be effects that you can detect.

The Children's Health Study is the core project for this long-term exposure health research program. Dr. John Peters of USC is the principal investigator. The goal of the project, and ultimately the program, is to identify and quantify the long-term exposure health effects, the specific pollutants alone and in combination, and to examine the influence of host characteristics (i.g. individual susceptibility). The project was divided into three phases. The first year was a methods assessment and protocol refinement period. The second phase was a cross-sectional study that is just reaching concluding points, and we expect to have a final report within the next three months. The final stage is a longitudinal study. This study is planned for five years of field work with two years of analyses. Part of the major effort of the first phase was to address very specific questions. Available resources limited the number of pollutants in the study design. Focusing on too many pollutants limits the ability to answer questions. The four pollutants included as a result of phase one efforts were ozone, PM10, NO_2, and nitric and hydrochloric acids. In addition, PM 2.5, both mass and chemistry, as well as formic and acetic acids were considered important both as potential health effectors and for regulatory consideration. The study, is being conducted on children for a number of reasons. First of all, they are known to be vulnerable to the health effects of air pollution. They may be more susceptible, and their exposure is potentially greater, because they are outdoors more and tend to be more active while they are outdoors. As a result of the first phase, it was determined that 12 communities should be included, from Alpine in San Diego County to Santa Barbara and San Luis Obispo Counties. The communities were selected based on a

MS HELENE MARGOLIS, ARB "Discussion on Human Health"

This study was initiated by Dr. Ron Fairster at UCI and Dr. Colome in 1985 under partial sponsorship of the acid deposition program. There is a fair amount of evidence that asthma can be exacerbated by ambient pollutants. There is not very much information as to which pollutants, at what concentrations, or under what conditions are prompting this responsiveness, so this project was started as a panel study. It took place in Orange County and utilized the Anaheim monitoring station. The intent was to recruit medically managed asthmatics. The inclusion criteria were rigorous. The subjects had to be current nonsmokers who quit at least two years prior to entry into this study and have smoked less than 10 packs per year since quitting. They were adults age 16-60. There had to be present a clear cut air flow obstruction based on two very critical pulmonary function tests and no other significant health problems except related problems such as atopy. The subjects had to reside within a 15 mile radius of the South Coast Air Quality Monitoring District Station in Anaheim. The subjects were recruited through local clinics and through the American Lung Association Asthma Program. The monitoring station is in the center of the study region. Nine of the residences were outside of the 15 mile radius, but the individuals were so willing to participate the investigators felt they should keep them in.

95 subjects were enrolled in the study, and this summary information is for the 61 who remained active through enough of the study that we could use them in analyses. The mean age was 43, and these are the FEV1% and FEV1/FEV% for the study subjects. These parameters were established in the clinical setting. They monitored the subjects over time after collecting baseline data. Peak expiratory flow tests were performed on a daily basis. Each subject was given a peak expiratory flow monitor, they were trained to use it, and they took this measurement each morning, evening, and before they went to sleep. This tracked their status. Additional information was collected through a questionnaire pertaining to their smoking history. The following is the preliminary results of our analyses. The pollutants (ozone, NO_2, SO_2, and CO) were monitored continuously. PM10 data were collected every six days for the last year of the study. It was not available prior to that. In addition, we looked at total sulfate, nonvolatile sulfate, and sulfuric acid and a combination measurement of ammonium bisulfate and sulfate.

For the analyses, the air quality data were divided into seasons so that we could look at the effects of each pollutant in a given season. In addition, pollen and fungi species were collected for a portion of the study period.

(AUDIENCE) One other thing that might be noted is that there were nitric acid measurements performed as part the study.

When we looked at the nitric acid, we found that the measurements were reliable for only about one month, and this was at the end of the study when many of our participants had faded out. There may be other analyses, such as time series analyses, that we can utilize to take advantage of those data, but

For purposes of this study, it was necessary to develop a two week integrated sampler for acids and for PM 2.5. This monitor was developed by Dr. Suzanne Herring. Based on what we knew at that point with respect to acute effects of the acids, we felt as though this two week integrated sampler would give us the type of information we needed. If we have an acid effect we will have the opportunity later to collect ambient acidity data for a shorter time resolution. We utilized existing monitoring sites as much as possible in the 12 community ambient air quality network, but we needed to establish five new ones to monitor ozone, NO_2, and PM10 continuously. In addition, we collected indoor and outdoor ozone using a timed exposure sampler that was developed specifically for this project. We collected indoor/outdoor ozone data at 48 schools and multiple classrooms per school. In addition, individual level ozone exposure was measured using a personal monitor developed by Drs. Geyh and Koutrakis at Harvard.

One of the main thrusts of the project initially was to define ozone exposure and determine the feasibility of getting personal ozone data. We also planned to get an idea of what the children's lifetime exposures were. This was done using an historical data base. The individual level for each child's exposure to ozone, NO_2, and PM were estimated. Work to develop and validate an exposure model has been initiated, we have just begun the analysis, and they will be reported in the next few months. We have seen some interim results. The feasibility of this study has been well demonstrated. Participation and compliance is always a big issue, especially in long term epidemiologic studies, however these children were more than happy to participate. The questionnaire has 101 questions, and out of the 3600 that we received, only 104 did not have enough data. The demographics were suitable, and the ambient air quality matrix meets the needs of the study design. Historical air pollution exposure estimates showed that there was more variability between individuals than we would have expected. This may allow us to tease apart some of the confounding data found at the community level.

Q (AUDIENCE) Is that the program your talking about?

A (MS. MARGOLIS) Yes, exactly. As long as they lived in California or areas in the United States that had ambient air quality data bases, we were able to access it on a month-by-month basis, collect the data, and average it for that child. Another very important observation was that the indoor concentration of ozone at the schools were low relative to outdoor concentrations. One of the strategies for protecting public health, at least for ozone, is when there is an alert in Los Angeles, the recommendation is bring the children inside. it is potentially very protective. Essentially, that is the crux of where we are with this study.

night coughs were associated with total pollen and total fungus spore count levels.

We are in the process of using a random effects model with these data, this will allow us to utilize the time series data. Dr. Lippmann may have knowledge of other data sets that are comparable, but the availability of these day-in and day-out measurements is quite valuable.

Q (AUDIENCE) How do you deal with the lag between exposure and measurements of response?

A (MS MARGOLIS) That was dealt with in terms of the day-to-day variation. The amplitude percent mean of the morning accounted for the afternoon lag. In other words, that is your lag period.

Q (AUDIENCE) I assume that the morning measurement is related to yesterday's exposure but not to the last 2 or 3 days exposure.

A (MARGOLIS) That is right, and because we were using season averages, again we have not gotten to that point where we are actually using the daily data to account for that. This was just giving us an estimate of the degree of variability.

Q (AUDIENCE) Likewise, the measurements presumably relate to today's exposure.

A (MS MARGOLIS) Right. And again--those are inherent assumptions that we are not making at this point.

Q (AUDIENCE) To get at those kinds of problems, have you attempted to do temporal auto correlation analysis?

A (MS MARGOLIS) It is in process. That is what we are doing now. As I indicated, these are very preliminary analyses. Basically, everything was Z scored to unitize it, and now we are going back to the individual, back to the more time resolved data, and actually looking at this. It was more of a screening process to ask the questions. Are there things here that are showing up? Are the data sufficiently sensitive to pick this up?

Q (AUDIENCE) In terms of sensitivity, you mentioned that five out of the six data sets are preliminary findings and have low power because of limited reportage. Did you do a power analysis? What sorts of power were associated with the tests you conducted?

A (MS MARGOLIS) Regarding the power analyses, I do not have the numbers with me, but essentially they were not sufficient, and that is why it was dropped down. They were done, and, as I said, we looked at whether or not they were sufficiently statistically powerful to proceed. These were the only ones that came up with group averaging with the Z scoring that would provide the power that was acceptable.

Q (AUDIENCE) The area that you selected for recruiting the subjects is 15 miles radius from Anaheim. I imagine you are selecting a region which is highly variable from the standpoint of air

at this point, we are not very optimistic.

Preliminary re-analysis was in collaboration with Mike Lebowitz and Steve Colome. We found that we had good reliable data. It is a very clean data set. For these particular analyses, we divided the data into six seasons, the winters of 1985, 1986, 1987, and the summers of 1986, 1987, and 1988. The pollutant variables that were examined were; ozone one hour maximum and six hour maximum, NO_2 and SO_2 one hour maxima and 24-hour means, total sulfate for a 24 hour mean, and nonvolatile sulfate for a 24 hour mean. From the health perspective, two indices of response were created: 1) calculated amplitude/mean percent which provided a measure of diurnal variation i.e., how these individuals progressed in the course of a day, and 2) a coefficient of variation (morning-to-morning) which provided a measure of day-to-day variation in the peak expiratory flow. In addition, a Z score for each person's peak expiratory flow variables (morning, dinner, and evening) within each season was generated. This unitized all the PEF variables for all individuals and minimized the need to examine co-variants and auto-correlation elements further. There were preliminary analyses of symptom data. The patients kept daily diaries that included information on specific symptoms, general health, and geographic location. The preliminary analyses of symptom data considered the effects of specific pollutants on shortness of breath, night cough, night wheeze, and wheezing during the daytime within a given season. The analyses used were ANOVA and ANCOVA.

For brevity I will just summarize the significant findings. Peak expiratory flow (PEF) responsiveness was found to be season specific. There were no statistically significant associations between peak expiratory flow variables and air pollutants, pollen or the fungi in the winters 1985 and 1987 or in the summers of 1986 and 1988. By the end of the study we lost statistical power because some the people dropped out, and there are missing air quality data. However, in additional analyses that we have planned, we will use time series which may allow us to build up the power by including these people and the periods of time. In the winter of 1986, an increase in the amplitude/mean percent was associated with SO_2. In the summer of 1987, the dinner and evening time periods were associated with total sulfate and SO_2. The morning PEF was associated (i.e. either greater variability, or that there was variability from the previous day, or it was depressed) with NO_2 in the winter. October to February was essentially a winter period. Day and night cough and wheeze were consistently but not significantly associated with measurements of ozone with both the daily maximum and the six hour maximum. There was an increase in reporting of day and night wheeze that was associated with increased daily and total sulfate levels. Night cough was associated with daily total sulfate levels and chest colds. Wheeze during the day was associated with levels of daily SO_2 maximum. In winters of 1985, 1986, and 1987, shortness of breath was consistently associated with the daily mean and the maximum levels of NO_2. A chest cold was less consistently associated with NO_2 daily mean and maximum. Finally, chest cold, wheeze, and

Q (AUDIENCE #2) We have a monitoring site at each community.

Q (AUDIENCE) That is still grossly insufficient.

A (MS MARGOLIS) You are absolutely correct if you really want to know the precise individual exposure. An epidemiology study is not a controlled exposure study. The best we can do is not misclassify an individual's exposure, and, beyond that, any refinement that we can get in terms of what the exposure level is and the conditions of the exposure is a benefit. It is data that you use in conjunction with the other data, the animal data and the human exposure data and controlled exposure data, to say this is this consistent with what we are seeing.

Q (AUDIENCE) There is an a complete and augmented monitoring station for each small community that is involved in the study at the 12 communities, augmented in terms of adding, PM's, adding two week integrated samplers and adding any other air quality data that is available from the community in terms of ambient ozone, NO_2 and the like. These data are applied to their daily exposure assessment and to their lifelong exposure assessment, so we actually did not just set up five sites, we actually made 12 complete sites for 12 communities that already had monitoring.

Q (AUDIENCE) I understand, but I am a little confused, because it seems to me that the reality of that what you are doing is indices which I can accept, but you are certainly not getting absolute measures of exposures for any member of your communities.

A (MS MARGOLIS) No, but we are refining it. Most epidemiological studies have relied on the community exposure, that is just the monitoring. It is virtually impossible if you are following 3600 children in 12 communities to be running after them with little personal monitors and measure it exactly. What you can do, is collect your ambient exposure so you have a general idea of the exposure. We are working towards individual monitoring. There is a whole science now dedicated to it. You compare the community monitor to the school monitor. Then you take those data and you go to the personal data; and you have that more time resolved, because you can track what the child is doing. Once you get to that point where you have these three types of data, you can say this child is very active versus that child who is not very active and begin to fold that into the analyses. There are a lot of assumptions along the way, but you are refining that much further along. You are absolutely correct, however, that there is that concern.

Q (DR. LIPPMANN) I may add that the ARB is only supporting this study to about a million a year, and that is grossly insufficient to do what needs to be done, but it is the only thing that is feasible. I am speaking a bit facetiously here; in this day and age that is actually quite good support. There is a lot more modeling and model validation going on. It has already turned up that indoor school levels, even with the windows open, are remarkably lower than had been estimated. So you can determine from school presence or absence whether to assign any cumulative exposure to ozone for that

pollution. On one side you have cleaner coastal areas like Newport Beach. On the other extreme, you may have Asuza within the 15 mile radius. Does that add variability to your samples, or have you given thought to that?

A (MS MARGOLIS) We have certainly given thought to it, because it is an inherent problem. We were surprised that we found the observation about the SO_2, because we had such low concentrations. We are proceeding with analyses to look at back trajectories, because it could be a surrogate for another pollutant coming in from the refineries at Long Beach. You cannot just assume that because this is showing up, that it is, in fact, an association, especially when you know that there are not consistent data to support that, animal work or the controlled exposure work for example.

Q (DR. LIPPMANN) It seems to me that the one study you most want to compare the results with is the Ostrow Denver study in which they measured a few other things including a limited number of hydrogen ion measurements which they extrapolated to all days using sulfate and other compounds. It may well be there is the same conclusion that they drew from their data, which was hydrogen ion in the aerosol was the most closely associated parameter. You might look at the extent to which, in this case, the SO_2 was acting as a source and surrogate for the acid content. Then you may want to look at the coastal communities differently from the more inland ones, because in the winter, I presume that you have the acid fogs in the morning along the coast much more than inland, and you might expect that the highest responses would be among those coastal communities as opposed to those more inland. Knowing what you now know from the southern California study about the nature of the chemistry of the aerosol in various communities, you might want to try segregating the populations into the different specific air sheds and see if you get different responses where you are more likely to have the particulate hydrogen ion free in the coastal communities versus those more inland.

A (MS MARGOLIS) When we get to the individual analyses, we may be able to see that there is a natural break in the distribution of the individuals, but with the population being at only 61 unless we get extraordinarily lucky it will be difficult, especially because all of the analyses could not be done on all 61 for the different time periods.

Q (AUDIENCE) But if the effect is large, it may come out.

A (MS MARGOLIS) Yes. Thank you.

Q (AUDIENCE) Let us go back to your children's study. In relation to these last couple of questions, I may have missed something, but it is not clear to me how you are going to deal with this fundamental question about exposure. You described five new monitoring sites, but clearly that is way insufficient. I mean you said that was a big issue, and it is always a issue. What are you doing in the children's studies to deal with the issue of what the exposure really is?

A (MS MARGOLIS) You are absolutely correct...

A (MS MARGOLIS) Absolutely. The questionnaire covers a large number of variables including a number of bedrooms in a home, the type of heating, the presence of a microwave. There are multiple purposes for having questions like, that because if they have a microwave, they may not be using the gas stove as much. There are a whole series of questions to address other possible exposures to pollutants of concern that may be occurring from other sources indoors. There is also an activity questionnaire. We are capturing a lot of information, not just about health history and current health status, but also on potential confounders.

Q (AUDIENCE) Is it making that totally confounded because on one hand, socioeconomic status will affect things like nutrition, the amount of health care and so on. On the other hand, it may determine where they live which in turn determines their exposure to air pollution.

A (MS MARGOLIS) Yes. The communities were very carefully selected in part based on air quality. They were stratified on air quality based on ozone, PM and then acids, and subsequently also NO_2. Then, of the best candidates from those stratifications, we looked at 1990 census data and tried to find comparable communities of socioeconomic grouping and time of residence. In fact, we are finding more diversity within communities, but they are comparable. So you are absolutely correct. All of those points are of concern.

DR. DANE WESTERDAHL, Moderator

Thank you Helene. According to our agenda, we have a 30 minute period for discussion of general health issues followed by an up to 45 minute more or less general discussion and closing remarks.

DR. JOHN HOLMES

I do think that we are behind schedule, and we have already had a good deal of discussion on this very ambitious study. Unless there is any major untouched area or anything else that ought to be brought up, I am prepared to express my thanks to our colleagues from UC Irvine, especially to Steve Brown and Brent Takemoto and to my ever faithful Noni Weir who put all this together, and to all the speakers and all the people who contributed so actively to the discussions that have gone on in the last two days.

It is not often that we can bring together this many people who looked at a single problem from so many different angles. I think that as we return to Sacramento and digest all of this information and try to put it together, we will find that we may have learned a lot more than we thought we had just looking at the whole thing project by project. I think there is a synergy that will emerge from all of the scientists in all the different fields who have made contributions to our program. As I have said, we will take what we have learned here and begin the process of preparing the assessment for the governor and the legislature along with recommendations that will be prepared by ourselves working with the Scientific

child for that day. Weekends have been examined. Some children stay in the neighborhood and some do not. They are reporting that. If they go to another place where there is a monitor, they are getting some index of that and plugging it into the adjustment factor for that child. With all the personal monitoring being done, the school monitoring being done, and the characterization of the residence in terms of its infiltration, this is a quite reasonable best shot at getting closer to the individual exposure. It will be the community monitor that establishes what is out there that a child can be exposed to, but it is not going to be the sole index of the analysis.

There is a compromise on the acids. They couldn't afford to do continuous acid monitoring, and the question was should they go to every sixth day or every twelfth day? I think I suggested that no, it is better to get a cumulative measure at the very minimum as well as some temporal resolution by season. The compromise was to do 26 samples per year and collect each over 2 weeks for each sample. That can be modeled with individual samples and selected seasons and times.

This is the first study where the temporal variation in PM_{10} will be consistent with acid, ozone, and NO_2. A lot of times in air pollution epidemiology studies you tend to reduce the power to see a particle effect, because, if you were lucky, you had every day, 24 hour measurements, and if you weren't, you had measurements every sixth day. It was not a fair competition among which pollutant was of greater interest.

Within the overall budget and feasibility, this will be as definitive study as could be reasonably done. I think the team has shown by the percentage of available days to be measured and by having taken the time to develop and get these new monitors tested and established in the field, that this is the best of its kind and the best hope we have. Those of us who are looking at this from the national scene and know the need for these kinds of data are quite pleased that this is fulfilling what needs to be done.

Q (DR. WHITTENBERGER) If one looks at all of the epidemiological studies that have been done in California at air pollution sites since the 1950's, one can see a modest number of studies have been done. They have all been grossly inadequate in terms of quantification of personal exposure. This is the first epidemiological study on a large scale that has been developed in California as Dr. Lippmann has described. I would like to add that Dane Westerdahl, who put in 12 years of effort, and Ms. Margolis with five years of effort and Dr. Holmes for supporting them deserve a great deal of credit for getting this project under way. I think it is a very great accomplishment.

A (MS MARGOLIS) Thank you.

Q (AUDIENCE) I was just thinking a little bit about the variables that might confound relationships between air quality and human health. In your children's study when you administer the questionnaire, do you come up with some index of their socioeconomic status.

APPENDIX 1

Dry Deposition of Atmospheric Nitrogen Compounds to Mixed Coniferous Forest at Barton Flats of the San Bernardino Mountains

Andrzej Bytnerowicz

Advisory Committee. The committee and the ARB staff have an obligation to deliberate on the need for air pollution standards and other matters related to state government policy on air quality. So we have a lot of responsibility, but we have a lot of input from all of you, and I appreciate it very much. So with that, unless there are further announcements. Bill, thanks very much.

(DR. MAUTZ) Thank you, all the participants in the conference.

(DR. HOLMES) Thank you all very much. Have a good weekend.

intensive studies in the middle of 1993 summer.

Results

Concentrations of air pollutants at different levels of the tower at plot 2 and the monitoring station (located at plot 1) are presented in Figures 1 through 4.

Deposition fluxes of nitrate and ammonium to the branches of the ponderosa pine seedlings at different heights on the tower are presented in Figures 5 and 6, respectively.

Results of a comparison of nitrate and ammonium deposition to branches of mature pines and pine seedlings at different heights on the tower are presented in Figures 7 through 12.

Differences in nitrate and ammonium deposition to branches of pine seedlings between the individual plots are presented in Figures 13 through 16.

Deposition values of nitrate to branches of mature trees and seedlings of all the three species in plots 1, 2, and 3 during the 1st intensive study period are presented in Figures 17 through 19, and for ammonium in Figures 20 through 22.

Results of deposition of nitrate and ammonium to branches of mature ponderosa pine, California black oak, and white fir measured at the forest floor during the intensive study period in summer 1993 are presented in Table 1.

Estimated values of conductance of HNO_3 to ponderosa pine branches at different heights on the tower during the 1993 season are presented in Figure 23.

Part II. ESTIMATION OF NITROGEN DRY DEPOSITION TO FOREST.

Assumptions

1. Based on the results presented in Table 1, correction factors for estimating deposition fluxes of oak and fir were calculated:

	Pine	Oak	Fir
NO_3^-	1.00	1.89	0.69
NH_4^+	1.00	1.17	0.47

Dry Deposition of Atmospheric Nitrogen Compounds to Mixed Coniferous Forest at Barton Flats of the San Bernardino Mountains.

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INTRODUCTION

In this draft report only the results for the 1993 season (for which the most comprehensive data set is available) are presented. Field measurements are presented in Part I. Calculations of dry atmospheric deposition are presented in Part II. Preliminary calculations for the 1992 and 1994 seasons confirmed the general trends observed during the 1993 season and will be included in a final report.

Part I. FIELD MEASUREMENTS

Methods

Deposition measurements were performed during three photochemical smog seasons (1992, 1993 and 1994). They included monthly rinsing of branches exposed for about 1-week long periods and determinations of concentrations of HNO_3 vapor, HNO_2 vapor, NH_3 and particulate NO_3^- and NH_4^+ on the monitoring tower located in plot 2, Barton Flats area.

All calculations of deposition fluxes have been derived from the results of branch rinsing of ponderosa pine seedlings located at four different levels of the tower (29 m - at the top of the crown; 24 m and 16 m - in the middle of the crown; and 12 m - at the crown base). The tower was located near a stand of mature ponderosa/Jeffrey pines. In addition, starting at a stage of the complete development of needles on the mature trees (end of July), deposition to branches of mature trees at three different levels of the tower (12, 16, and 24 m) was determined.

In order to calculate deposition to other important tree species for the study area (white fir and California black oak) and to gain information on the horizontal deposition gradient, branches of mature trees and seedlings of pine, fir and oak at the forest floor level were rinsed at study plots 1, 2, and 3 during two periods of

NO_2 - 0.63.

Deposition to Ponderosa Pine

Surface deposition of washable NO_3^- and NH_4^+ to foliage.

General equation: $F_{mature\ tree} = F_{seedling} \times K$

where:

$F_{seedling}$ - average deposition flux of NO_3^- or NH_4^+ to foliage of seedlings on the tower during the entire polluted season

K - correction factor for extrapolating seedling deposition flux to deposition to mature trees

Deposition: F x t x S x 10000/4800

where:

F - deposition flux

t - time of deposition

S - surface area of foliage

10000/4800 - extrapolation from plot area (4800 m^2) to 1 ha (10000 m^2)

Polluted season

$F_{NO3} = 16.71$ ug N m^{-2} h^{-1} x 1.01 = 17.08 ug N $m^{-2}h^{-1}$

$F_{NH4} = 7.84$ ug N m^{-2} h^{-1} x 1.34 = 10.51 ug N m^{-2} h^{-1}

$F_{NO3 + NH4} = 17.08 + 10.51 = 27.58$ ug N m^{-2} h^{-1}

Deposition of nitrate and ammonium = 27.58 ug N m^{-2} h^{-1} x 24h x 182.5 days x 1827 m^2 x 10000/4800 = 460 g ha^{-1}

Clean season

Due to a lack of determinations of deposition to branches during the clean season the following additional assumptions were made:

Deposition of NO_3^- was proportional to ambient concentrations of HNO_3 vapor, and therefore the average deposition flux for the clean season (F_{NO3}) was calculated based on the average ambient concentrations of HNO_3 during the polluted and clean seasons:

2. Based on comparisons of deposition to foliage of the ponderosa pine seedlings and ponderosa pine mature pine trees the following correction factors were derived: NO_3^- - 1.01; NH_4^+ - 1.34. These factors have been used for extrapolating the seedling data to mature trees (in order to estimate total deposition to foliage of trees the seedlings' results were multiplied by the above values).

3. Total surface foliage area values for plot 2 was: ponderosa pine - 1827 m^2, white fir - 4424 m^2, and California black oak - 2832 m^2. These values present a stand leaf area index (LAI) of 1.89 determined with a litterfall analysis (Mark Fenn, unpublished).

5. Surface area for ponderosa pine branches is about 4.5% of the foliar surface (based on experimental measurements on ponderosa pines in the Colorado Rockies, Mike Arbaugh, personal communication). Therefore the area of branches was equal to: 1827 m^2 x 4.5% = 82.2 m^2.

6. Deposition flux to branches for $H^{15}NO_3$ vapor was about 7.9 x higher to branches than to foliage (Marek Krywult, personal communication).

7. No literature information on NH_3 deposition to bark is available and we assume that this deposition is similar to deposition of HNO_3 vapor.

8. The total ground surface area of plot 2 is 4800 m^2.

9. Calculations were done for 2 seasons: (a) polluted season (April 15 - October 15), and (b) clean season, the remainder of the year.

10. Concentrations of N pollutants used for estimates of stomatal uptake (12 h daytime averages): HNO_3 - 3.624 ug m^{-3} (polluted season) and 0.508 ug m^{-3} (clean season); NH_3 - 1.422 ug m^{-3} (polluted season) and 0.329 ug m^{-3} (clean season); NO_2 - 2.764 ug N m^{-3} (polluted season) and 1.341 ug N m^{-3} (clean season).

11. Internal uptake of pollutants were calculated based on stomatal conductance to H_2O measurements. Approximate values for ponderosa pine during the polluted season were 0.065 cm s^{-1} and 0.035 cm s^{-1}, respectively (Patrick Temple, personal communication). Based on a comparison of gas exchange of ponderosa pine and white fir (Takemoto and Bytnerowicz, 1993) we assumed the same values for white fir. For California black oak during the polluted season we assumed stomatal conductance of 0.13 cm s^{-1} (Kramer and Kozlowski, 1979).

12. Correction factors for stomatal conductances of individual gases were derived by dividing a square root of molecular weight of H_2O by a square root of a molecular weight of a gas of interest. The corrections factors were as follows: HNO_3 - 0.54; NH_3 - 1.09;

Internal stomatal uptake

Polluted season

HNO_3 deposition flux = 0.065 cm s^{-1} x 0.535 x 3.624 ug HNO_3 m^{-3} = 2.34 m s^{-1} x 0.535 x 3.624 = 4.54 ug HNO_3 m^{-2} h^{-1} = 1.01 ug N m^{-2} h^{-1}.

HNO_3 deposition = 1.01 ug N m^{-2} h^{-1} x 1827 m^2 x 182.5 days x 12 h x 10000/4800 = 8.4 g ha^{-1}.

NH_3 deposition flux = 0.065 cm s^{-1} x 1.09 x 1.422 ug HNO_3 m^{-3} = 2.34 m h^{-1} x 1.09 x 1.422 = 3.63 ug NH_3 m^{-2} h^{-1} = 2.82 ug N m^{-2} h^{-1}.

NH_3 deposition = 2.82 N m^{-2} h^{-1} x 1827 m^2 x 182. 5 days x 12 h x 10000/4800 = 23.5 g ha^{-1}.

NO_2 deposition flux = 0.065 cm s^{-1} x 0.63 x 2.764 ug NO_2 m^{-3} = 2.34 m h^{-1} x 0.63 x 2.764 = 4.08 ug NO_2 m^{-2} h^{-1} = 1.24 ug N m^{-2} h^{-1}.

NO_2 deposition = 1.24 ug N m^{-2} h^{-1} x 1827 m^2 x 182.5 days x 12 h x 10000/4800 = 10.3 g N ha^{-1}.

Clean season

HNO_3 deposition flux = 0.035 cm s^{-1} x 0.535 x 0.508 ug HNO_3 m^{-3} = 1.26 m s^{-1} x 0.535 x 0.508 = 0.343 ug HNO_3 m^{-2} h^{-1} =0.077 ug N m^{-2} h^{-1}.

HNO_3 deposition = 0.077 ug N m^{-2} h^{-1} x 1827 m^2 x 182.5 days x 12 h x 10000/4800 = 0.7 g N ha^{-1}.

NH_3 deposition flux = 0.035 cm s^{-1} x 1.09 x 0.329 ug NH_3 m^{-3} = 1.26 m h^{-1} x 1.09 x 0.329 = 0.452 ug NH_3 m^{-2} h^{-1} = 0.2352 ug N m^{-2} h^{-1}.

NH_3 deposition = 0.352 ug N m^{-2} h^{-1} x 1827 m^2 x 182.5 days x 12 h = 3.0 g N ha^{-1}.

NO_2 deposition flux = 0.035 cm s^{-1} x 0.63 x 1.341 ug NO_2 m^{-3} = 1.26 m h^{-1} x 0.63 x 1.341 = 1.064 ug NO_2 m^{-2} h^{-1} = 0.324 ug N m^{-2} h^{-1}.

NO_2 deposition = 0.324 ug N m^{-2} h^{-1} x 1827 m^2 x 182.5 days x 12 h x 10000/4800 = 2.6 g N ha^{-1}.

Total stomatal uptake of N pollutants by ponderosa pine = 8.4 + 23.5 + 10.3 + 0.7 + 3.0 + 2.6 = 48.5 g ha^{-1} yr^{-1}.

3.624 ug HNO_3 m^{-3} - 17.08 ug N m^{-2} H^{-1}

0.354 ug HNO_3 m^{-3} - F_{NO3}

F_{NO3} = 0.354 x 17.08/3.624 = 1.668 ug N m^{-2} h^{-1}

Deposition of NH_4^+ was proportional to ambient concentrations of NH_3, and therefore the average deposition flux for the clean season (F_{NH4}) was calculated based on the average ambient concentrations of NH_3 during the polluted and clean season:

1.422 ug NH_3 m^{-3} - 10.51 ug N m^{-2} h^{-1}

0.268 ug NH_3 m^{-3} - F_{NH4}

F_{NH4} = 0.268 x 10.51/1.422 = 1.980 ug N m^{-2} h^{-1}

$F_{NO3 + NH4}$ = 1.668 + 1.980 = 3.648 ug N m^{-2} h^{-1}

Deposition of nitrate and ammonium = 3.648 ug N m^{-2} h^{-1} x 24h x 182.5 days x 1827 m^2 x 10000/4800 = 60.8 g N ha-1

Surface deposition to branches

Polluted season

Nitrate deposition = 17.08 ug N m^{-2} h^{-1} x 7.9 x 82.2 m^2 x 182.5 days x 24 h x 10000/4800 = 101.2 g N ha^{-1}

Ammonium deposition = 10.51 ug N m^{-2} h^{-1} x 7.9 x 82.2 m^2 x 182.5 days x 24 h x 10000/4800 = 62.3 g N ha^{-1}

Clean season

Nitrate deposition = 1.67 ug N m^{-2} h^{-1} x 7.9 x 82.2 m^2 x 182.5 days x 24 h x 10000/4800 = 9.9 g N ha^{-1}

Ammonium deposition = 1.98 ug N m^{-2} h^{-1} x 7.9 x 82.2 m^2 x 182.5 days x 24 h x 10000/4800 = 11.7 g N ha^{-1}

Total surface (washable) deposition to ponderosa pine foliage and branches = 460 + 60.8 + 101.2 + 62.3 + 9.9 + 11.7 = 705.9 g N ha^{-1} yr^{-1}.

Internal stomatal uptake

Polluted season

HNO_3 deposition flux = 0.13 cm s^{-1} x 0.535 x 3.624 ug HNO_3 m^{-3} = 4.68 m h^{-1} x 0.535 x 3.624 = 9.07 ug HNO_3 m^{-2} h^{-1} = 2.02 ug N m^{-2} h^{-1}.

HNO_3 deposition = 2.02 ug N m^{-2} h^{-1} x 2832 m^2 x 182.5 days x 12 h x 10000/4800 = 26.1 g N ha^{-1}.

NH_3 deposition flux = 0.13 cm s^{-1} x 1.09 x 1.422 ug N m^{-3} = 4.68 m h^{-1} x 1.09 x 1.422 = 7.25 ug N m^{-2} h^{-1} = 5.97 g N m^{-2} h^{-1}.

NH_3 deposition = 5.97 ug N m^{-2} h^{-1} x 2832 m_2 x 182.5 days x 12 h x 10000/4800 = 77.2 g N ha^{-1}.

NO_2 deposition flux = 0.13 cm s^{-1} x 0.63 x 2.764 ug NO_2 m^{-3} = 4.68 m h^{-1} x 0.63 x 2.764 = 8.15 ug NO_2 m^{-2} h^{-1} = 2.48 g N m^{-2} h^{-1}.

NO_2 deposition = 2.48 ug N m^{-2} h^{-1} x 2832 m^2 x 182.5 days x 12 h x 10000/4800 = 32.0 g N ha^{-1}.

Clean season

Foliage not present - deposition assumed to be equal to 0.

Total stomatal uptake of N pollutants by California black oak = 26.1 + 77.2 + 32.0 = 135.3 g ha^{-1} yr^{-1}.

Deposition to White Fir

Additional assumptions

1. Surface area of branches was about 4.5% of the foliage (similar to pine). Therefore the area of the branches was: 4424 m^2 x 4.5% = 199.1 m^2.

2. Deposition flux to branches was about 7.9x higher than to the foliage (similar to pine).

3. Due to a lack of determinations of deposition to branches during the clean season it was assumed, similarly as for the pine, that deposition of NO_3^- was proportional to ambient concentrations of HNO_3 vapor and that deposition of NH_4^+ was proportional to ambient concentrations of NH_3 (see the above section for pine for more details).

Deposition to California Black Oak

Additional Assumptions

1. Surface area of branches was about 10.1% of the surface area of the foliage (Marek Krywult, personal communication)

2. Deposition flux to branches is 3.73 x higher to branches than to foliage (Marek Krywult, personal communication)

Surface deposition of washable NO_3^- and NH_4^+ to foliage

Polluted season

F_{NO3} = 17.08 ug N m^{-2} h^{-1} x 1.89 = 32.28 ug N m^{-2} h^{-1}

F_{NH4} = 10.51 ug N m^{-2} h^{-1} x 1.17 = 12.30 ug N m^{-2} h^{-1}

$F_{NO3 + NH4}$ = 32.28 + 12.30 = 44.58 ug N m^{-1} h^{-1}

Deposition of nitrate and ammonium = 44.58 ug N m^{-2} h^{-1} x 24 x 182.5 x 2832 x 10000/4800 = 1152 g N ha^{-1}

Clean season

No foliage present, no deposition occurring.

Surface deposition of washable NO_3^{-1} and NH_4^+ to branches

Polluted season

Nitrate deposition = 17.08 ug N m^{-2} h^{-1} x 1.89 x 3.73 x 10.1% x 2832 m^2 x 182.5 days x 24 h x 10000/4800 = 314.3 g N ha^{-1}

Ammonium deposition = 10.51 ug N m^{-2} h^{-1} x 1.17 x 3.73 x 10.1% x 2832 m^2 x 182.5 days x 24 h x 10000/4800 = 119.7 g N ha^{-1}

Clean season

Nitrate deposition = 1.668 ug N m^{-2} h^{-1} x 1.89 x 10.1% x 3.73 x 2832 m^2 x 182.5 days x 24 h x 10000/4800 = 30.7 g N ha^{-1}

Ammonium deposition = 1.980 ug N m^{-2} h^{-1} x 1.17 x 10.1% x 3.73 x 2832 m^2 x 182.5 days x 24 h x 10000/4800 = 22.6 g N ha^{-1}

Total surface (washable) deposition to California black oak foliage and branches = 1152 + 314.3 + 119.7 + 30.7 + 22.6 = 1639.3 g ha^{-1} yr^{-1}.

Internal stomatal uptake

Polluted season

HNO_3 deposition = 1.01 ug N m^{-2} h^{-1} (the same value as for the pine) x 4424 m^2 x 182.5 days x 12 h x 10000/4800 = 20.4 g N ha^{-1}.

NH_3 deposition = 2.82 ug N m^{-2} h^{-1} (the same value as for the pine) x 4424 m^2 x 182.5 days x 12 h x 10000/4800 = 56.9 g N ha^{-1}.

NO_2 deposition = 1.24 ug N m^{-2} h^{-1} (the same value as for the pine) x 4424 m^2 x 182.5 days x 12 h x 10000/4800 = 25.1 g N ha^{-1}.

Clean season

HNO_3 deposition = 0.072 ug N m^{-2} h^{-1} (the same value as for the pine) x 4424 m^2 x 182.5 days x 12 h x 10000/4800 = 1.5 g N ha^{-1}.

NH_3 deposition = 0.352 ug N m^{-2} h^{-1} (the same value as for the pine) x 4424 m^2 x 182.5 days x 12 h x 10000/4800 = 7.2 g N ha^{-1}.

NO_2 deposition = 0.324 ug N m^{-2} h^{-1} (the same value as for the pine) x 4424 m^2 x 182.5 days x 12 h x 10000/4800 = 6.5 g N ha^{-1}.

Total stomatal uptake of the N pollutants = 20.4 + 56.9 + 25.1 + 1.5 + 7.2 + 6.5 = 117.6 g ha^{-1} h^{-1}.

Ground Deposition

Assumptions: flat surface; deposition equal to average deposition to 3 species in plot 2 ($F_{NO_3^- + NH_4}$ = 29.63 ug N m^{-2} h^{-1} for the polluted season and 2.86 ug N m^{-2} h^{-1} for the clean season).

Polluted season

29.63 ug N m^{-2} h^{-1} x 10000m^2 x 182.5 days x 24 h = 1297.8 g N ha^{-1}.

Clean season

2.86 ug N $m^{-2}h^{-1}$ x 10000m^2 x 182.5 days x 24 h = 125.3 g N ha^{-1}.

Total ground deposition = 1297.8 + 125.3 = 1423.1 g ha^{-1} yr^{-1}.

Total estimated atmospheric dry deposition of N pollutants to forest in Plot 2 = 705.9 (surface deposition to pine) + 48.5 (stomatal uptake by pine) + 1639.3 (surface deposition to oak) + 135.3 (stomatal uptake by oak) + 634.2 (surface deposition to fir) + 117.6 (stomatal uptake by fir) + 1423.1 (ground deposition) = 4703.9 g N ha^{-1} yr^{-1}.

Surface deposition of washable NO_3^- and NH_4^+ to foliage

Polluted season

F_{NO3} = 17.08 ug N m^{-2} h^{-1} x 0.69 = 11.79 ug N m^{-2} h^{-1}

F_{NH4} = 10.51 ug N m^{-2} h^{-1} x 0.47 = 4.94 ug N m^{-2} h^{-1}

$F_{NO3 + NH4}$ = 11.79 + 4.94 = 16.73 ug N m^{-2} h^{-1}

Deposition of nitrate and ammonium = 16.73 ug N x 182.5 days x 24 h x 4424 m^2 = 324.0 g N ha^{-1}.

Clean season

F_{NO3} = 1.668 ug N m^{-2} h^{-1} x 0.69 = 1.15 ug N m^{-2} h^{-1}

F_{NH4} = 1.980 ug N m^{-2} h^{-1} x 0.47 = 0.93 ug N m^{-2} h^{-1}

$F_{NO3 + NH4}$ = 1.15 + 0.93 = 2.08 ug N m^{-2} h^{-1}

Deposition of nitrate and ammonium = 2.08 ug N m^{-2} h^{-1} x 182.5 days x 24 h x 4424 m^2 = 40.3 g N ha^{-1}.

Surface deposition to branches

Polluted season

Nitrate deposition = 17.08 ug m^{-2} h^{-1} x 0.69 x 7.90 x 199.1 m^2 x 182.5 days x 24 h x 10000/4800 = 169.1 g N ha^{-1}

Ammonium deposition = 10.51 ug N m^{-2} h^{-1} x 0.47 x 7.90 x 199.1 x 182.5 days x 24 h x 10000/4800 = 70.9 g N ha^{-1}

Clean season

Nitrate deposition = 1.668 ug N m^{-2} h^{-1} x 0.698 x 7.90 x 199.1 m^{-2} x 182.5 days x 24 h x 10000/4800 = 16.5 g ha^{-1}

Ammonium deposition = 1.980 ug N m^{-2} h^{-1} x 0.47 x 7.90 x 199.1 x 182.5 days x 24 hours x 10000/4800 = 13.4 g N ha^{-1}

Total surface (washable) deposition for white fir foliage and branches = 324.0 + 40.3 + 169.1 + 70.9 + 16.5 +13.4 = 634.2 g N ha^{-1} yr^{-1}.

Table 1. Deposition of nitrate and ammonium ions to branches of mature trees at the forest floor level during the intensive studies ($ug\ m^{-2}\ h^{-1}$).

Plot Number	Pine		Oak		Fir	
	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4
July 19-23, 1993						
1	12.88 (4.68)	4.10 (1.38)	36.31 (16.79)	7.96 (4.22)	13.45 (1.11)	2.92 (0.75)
2	12.65 (3.99)	4.14 (1.13)	35.30 (11.32)	5.74 (1.61)	9.32 (6.45)	1.78 (1.55)
3	18.33 (9.95)	4.96 (2.37)	22.23 (8.94)	4.75 (2.44)	7.80 (4.80)	1.64 (1.54)
July 23-30, 1993						
1	13.21 (3.26)	3.80 (0.92)	27.90 (5.70)	5.53 (1.56)	15.06 (1.29)	2.90 (0.76)
2	16.10 (1.45)	4.84 (0.55)	30.37 (6.80)	5.08 (0.50)	8.18 (1.36)	1.21 (0.34)
3	19.58 (6.98)	4.71 (1.72)	24.89 (8.74)	4.16 (1.42)	10.08 (3.34)	2.14 (0.52)
Average from 2 periods	15.46	4.43	29.22	5.18	10.65	2.10

Summary of the above calculations for three species of trees is presented in Table 2. In Table 3 a summary of calculations for a forest stand level using two values of a leaf area index (LAI=1.89 determined with a litterfall analysis and 3.82 obtained with a ceptometer method (Mark Fenn, unpublished) and wet deposition is presented.

REFERENCES

Kramer, P. J., and T. T. Kozlowski. 1979. Physiology of Woody Plants. Acdemic Press, Inc., Orlando, 811pp.

Takemoto, B., and A. Bytnerowicz. 1993. Effects of acidic fog on seedlings of Pinus ponderosa and Abies concolor: foliar injury, physiological and biochemical responses. Environ. Pollut., 79, 235-241.

Table 3. Calculated total nitrogen deposition at Plot 2 during the 1993 season (g N ha^{-1} yr^{-1}) - summary.

Parameter	Foliage rinsing (LAI=1.89)	Foliage rinsing (LAI=3.82)
NO_3 washable	2039 (2000 throughfall)	4121
NH_4 washable	941 (1000 throughfall)	1902
HNO_3 stomatal	57	115
NH_3 stomatal	168	340
NO_2 stomatal	77	156
Rain, summer	420	420
Rain+snow, winter	110	110
Ground	1423	1423
Total	5235 (5255)	8587

Table 2. Nitrogen dry deposition to trees at Plot 2, Barton Flats, SBNF, during the 1993 season (g N ha^{-1} yr^{-1}) assuming LAI = 1.89.

Parameter	Pine	Oak	Fir	Total
NO_3 washable, foliage	313	834	250	1397
NO_3 washable, branches	111	345	186	642
NH_4 washable, foliage	208	318	114	640
NH_4 washable, branches	74	142	85	301
HNO_3 stomatal	9	26	22	57
NH_3 stomatal	27	77	64	168
NO_2 stomatal	13	32	32	77
Total	755	1774	753	3282

at the forest floor level during the July 19 - 23, 1993 intensive study.

Fig. 16. Deposition of NH_4^+ to foliage of ponderosa pine seedlings at the forest floor level during the July 23 - 30, 1993 intensive study.

Fig. 17. Comparison of NO_3^- deposition to foliage of mature trees vs. seedlings of various species at plot 1 during the July 19 - 23, 1993 intensive study.

Fig. 18. Comparison of NO_3^- deposition to foliage of mature trees vs. seedlings of various species at plot 2 during the July 19 - 23, 1993 intensive study.

Fig. 19. Comparison of NO_3^- deposition to foliage of mature trees vs. seedlings of various species at plot 3 during the July 19 - 23, 1993 intensive study.

Fig. 20. Comparison of NH_4^+ deposition to foliage of mature trees vs. seedlings of various species at plot 1 during the July 19 - 23, 1993 intensive study.

Fig. 21. Comparison of NH_4^+ deposition to foliage of mature trees vs. seedlings of various species at plot 2 during the July 19 - 23, 1993 intensive study.

Fig. 22. Comparison of NH_4^+ deposition to foliage of mature trees vs. seedlings of various species at plot 3 during the July 19 - 23, 1993 intensive study.

Fig. 23. Estimated conductance (K_1) of HNO_3 to ponderosa pine foliage for different height on the tower during the 1993 polluted season.

Figure Captions.

Fig. 1. Concentration of HNO_3 vapor during the 1993 polluted season at different heights on the tower (Plot 2) and the monitoring site (Plot 1).

Fig. 2. Concentrations of NH_3 during the 1993 polluted season at different heights on the tower and the monitoring site.

Fig. 3. Concentrations of NO_3^- in a fine particulate fraction (<2.2 um diameter) during the 1993 polluted season at different heights on the tower and the monitoring site.

Fig. 4. Concentrations of NH_4^+ in a fine particulate fraction (<2.2 um diameter) during the 1993 polluted season at different heights on the tower and the monitoring site.

Fig. 5. NO_3^- deposition flux to foliage of ponderosa pine seedlings on a tower.

Fig. 6. NH_4^+ deposition flux to foliage of ponderosa pine seedlings on a tower.

Fig. 7. Comparison of NO_3^- deposition to foliage of mature trees vs. seedlings at 12 m height on the tower.

Fig. 8. Comparison of NO_3^- deposition to foliage of mature trees vs. seedlings at 16 m height on the tower.

Fig. 9. Comparison of NO_3^- deposition to foliage of mature trees vs. seedlings at 24 m height on the tower.

Fig. 10. Comparison of NH_4^+ deposition to foliage of mature trees vs. seedlings at 12 m height on the tower.

Fig. 11. Comparison of NH_4^+ deposition to foliage of mature trees vs. seedlings at 16 m height on the tower.

Fig. 12. Comparison of NH_4^+ deposition to foliage of mature trees vs. seedlings at 24 m height on the tower.

Fig. 13. Deposition of NO_3^- to foliage of ponderosa pine seedlings at the forest floor level during the July 19 - 23, 1993 intensive study.

Fig. 14. Deposition of NO_3^- to foliage of ponderosa pine seedlings at the forest floor level during the July 23 - 30, 1993 intensive study.

Fig. 15. Deposition of NH_4^+ to foliage of ponderosa pine seedlings

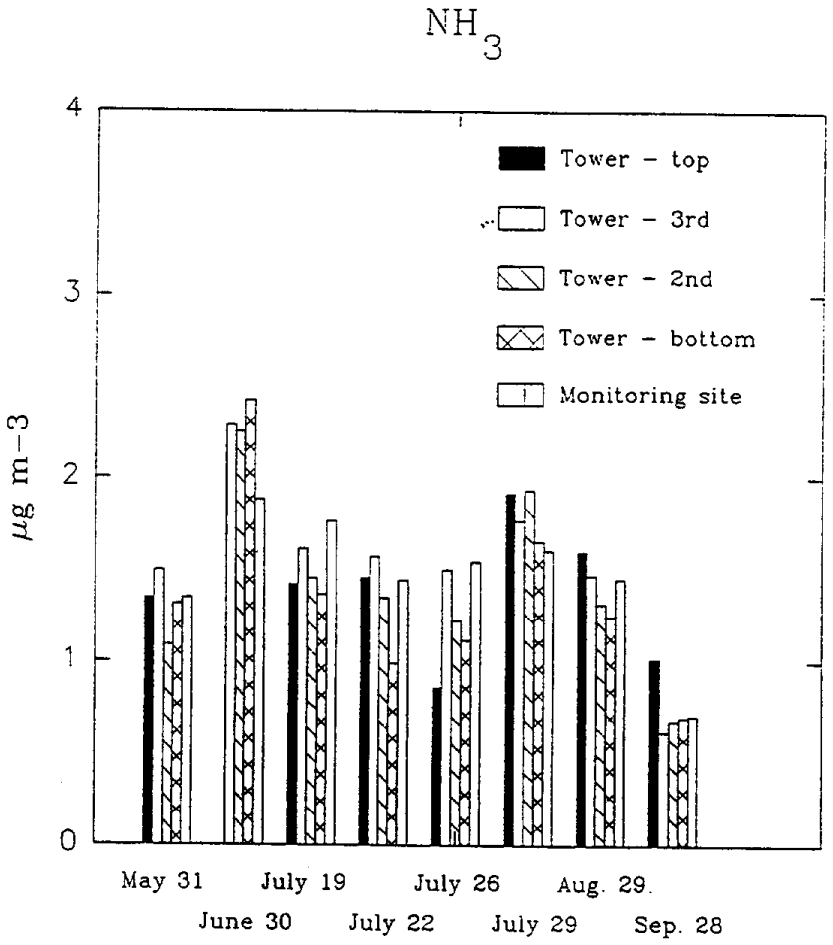

Fig. 2. Concentrations of NH_3 during the 1993 polluted season at different heights on the tower and the monitoring site.

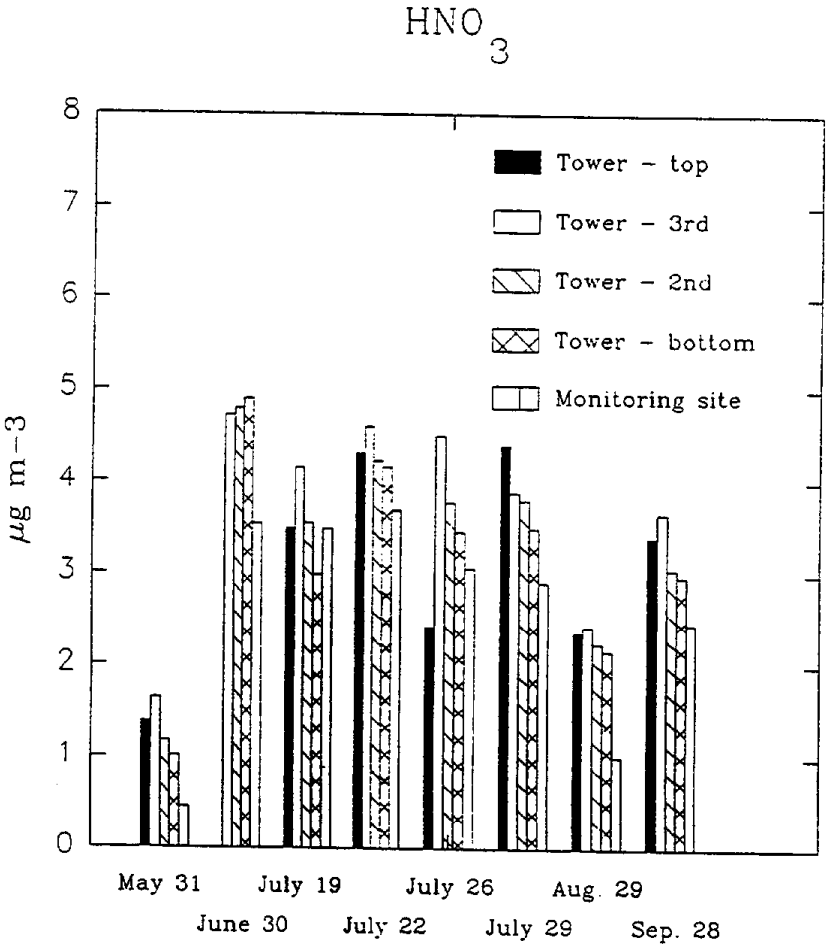

Fig. 1. Concentration of HNO_3 vapor during the 1993 polluted season at different heights on the tower (Plot 2) and the monitoring site (Plot 1).

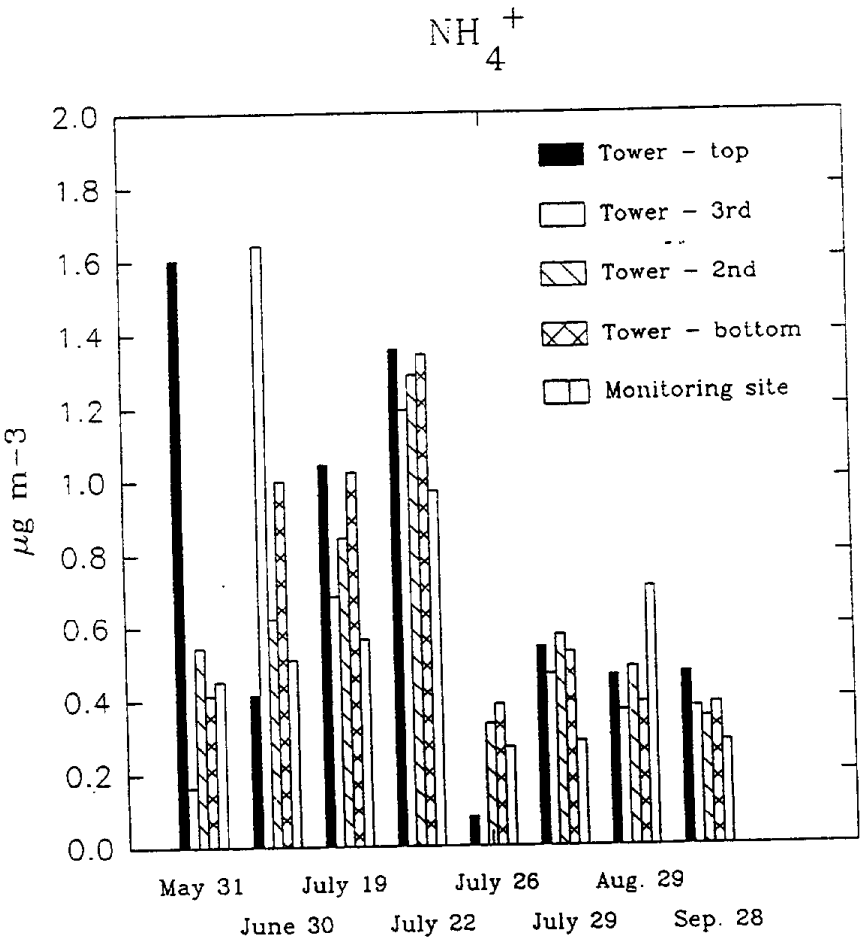

Fig. 4. Concentrations of NH_4^+ in a fine particulate fraction (<2.2 um diameter) during the 1993 polluted season at different heights on the tower and the monitoring site.

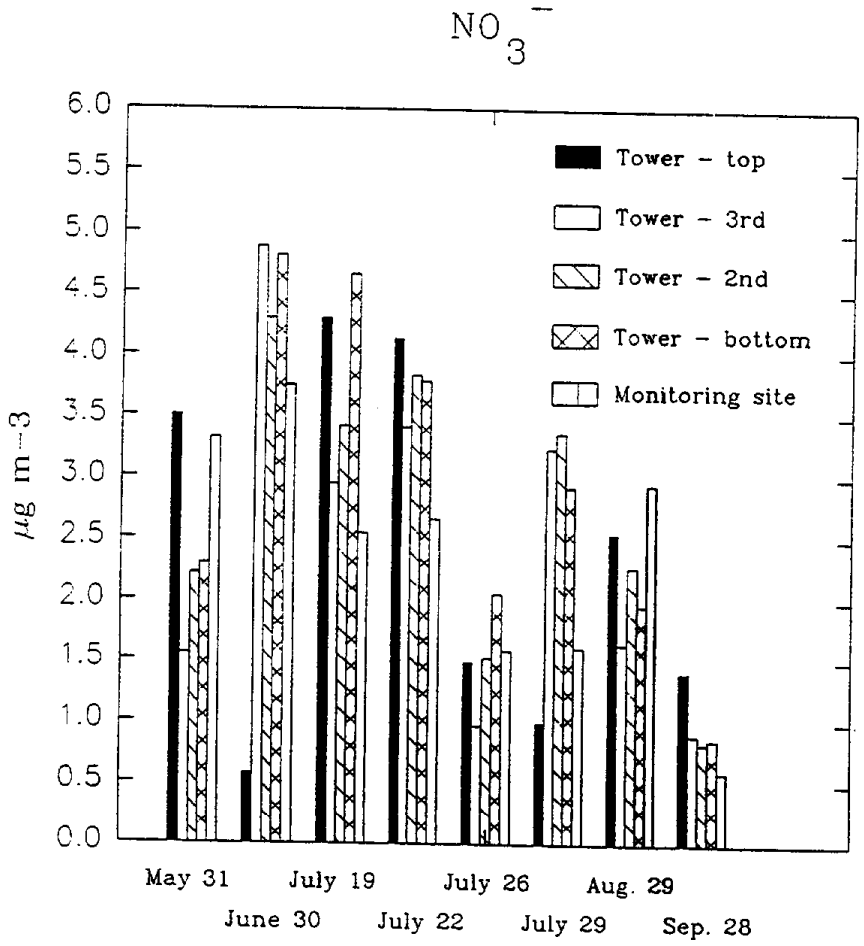

Fig. 3. Concentrations of NO_3^- in a fine particulate fraction (<2.2 um diameter) during the 1993 polluted season at different heights on the tower and the monitoring site.

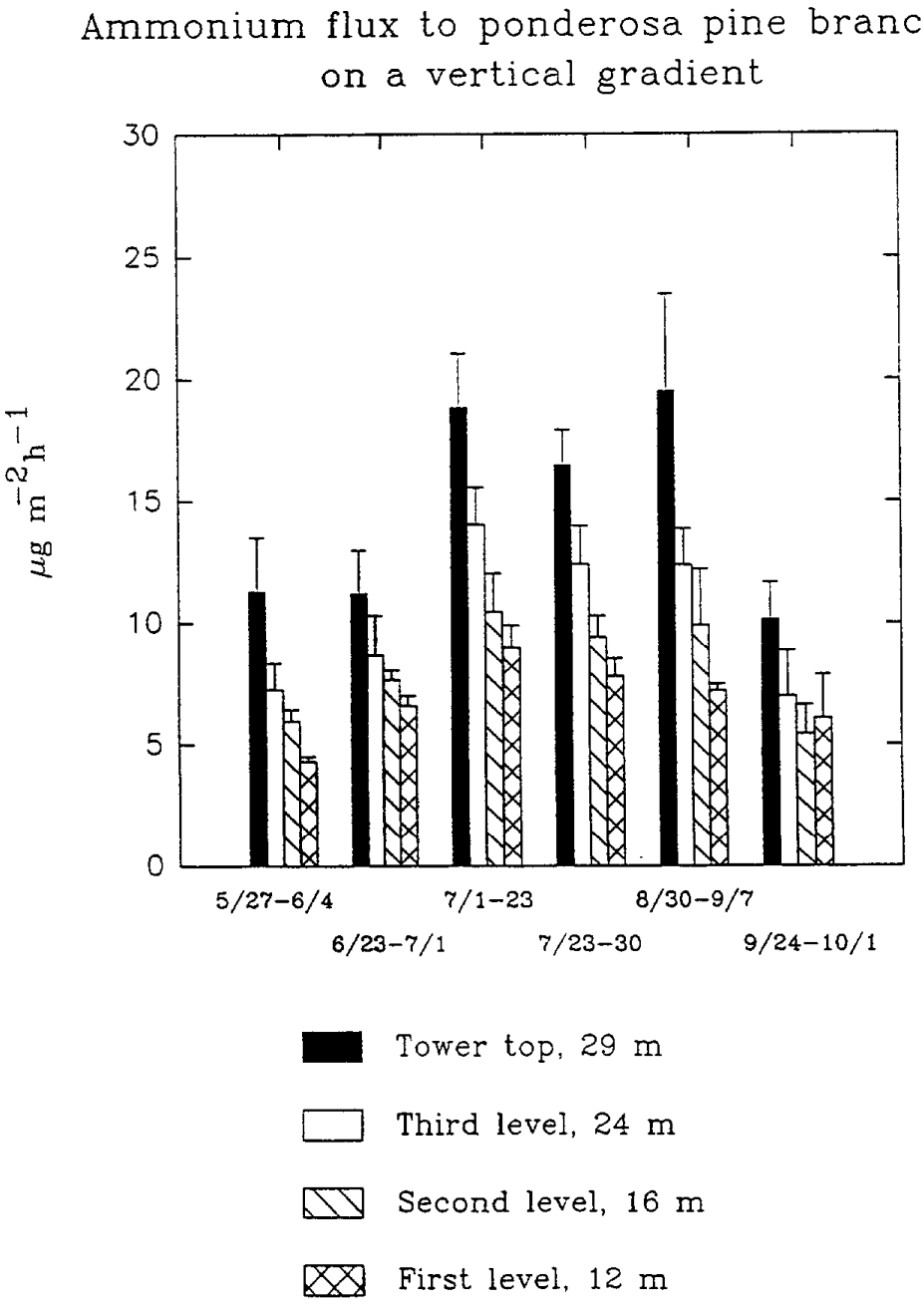

Fig. 6. NH_4^+ deposition flux to foliage of ponderosa pine seedlings on a tower.

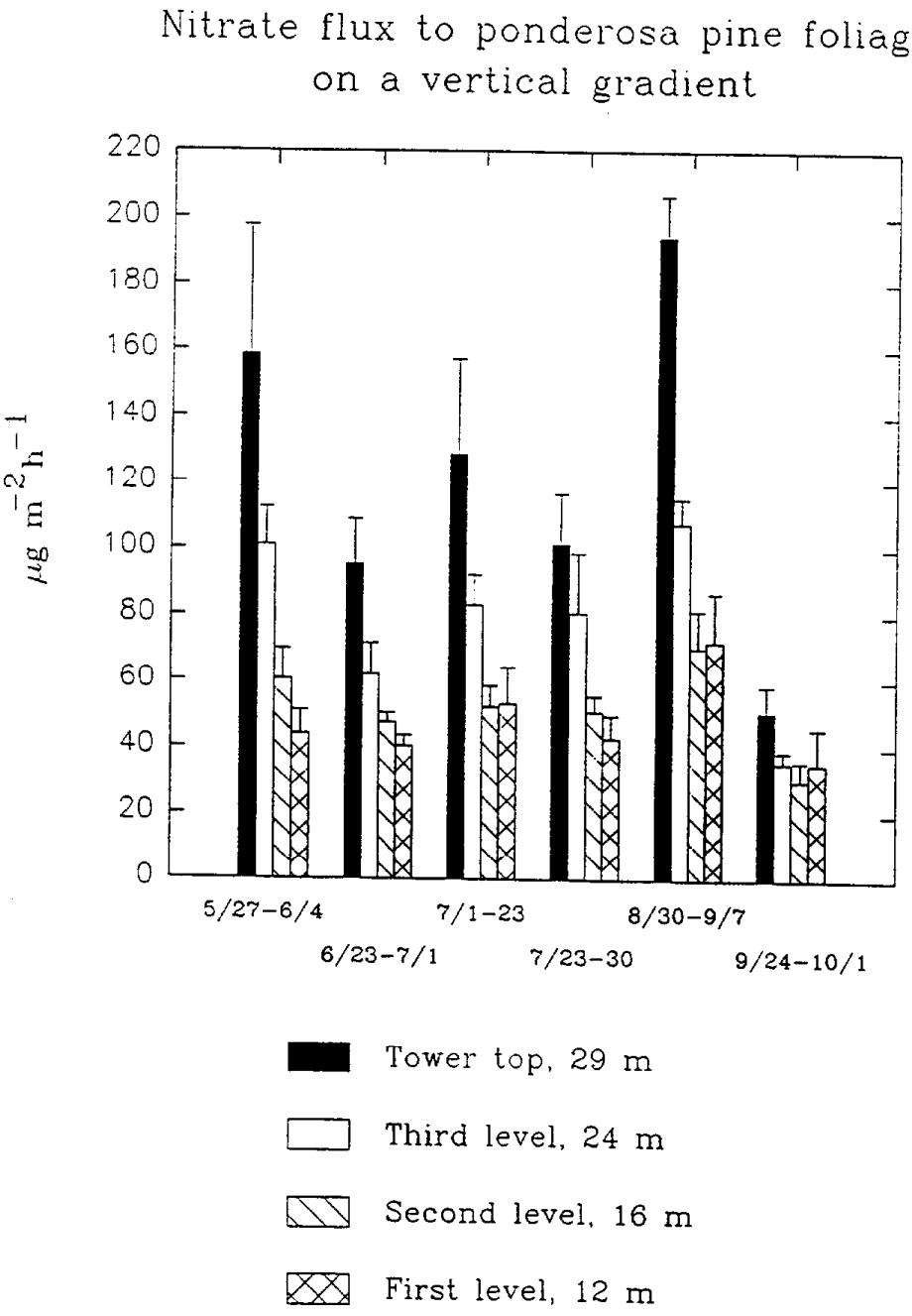

Fig. 5. NO_3^- deposition flux to foliage of ponderosa pine seedlings on a tower.

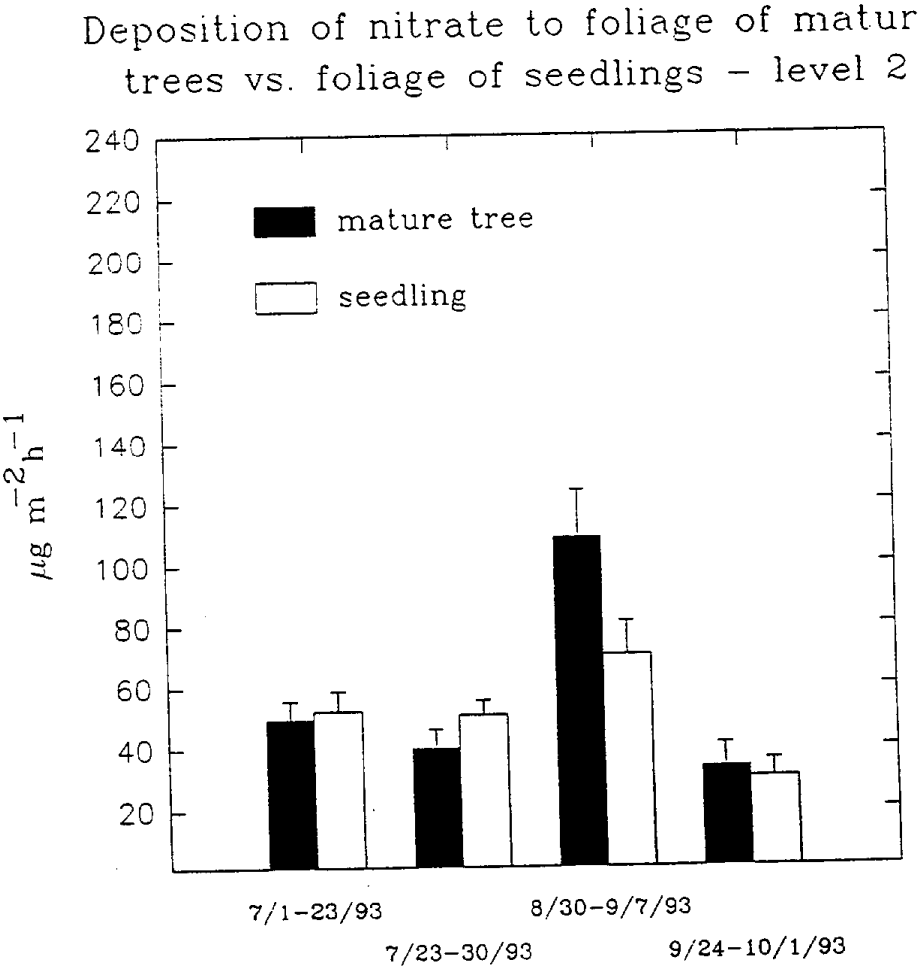

Fig. 8. Comparison of NO_3^- deposition to foliage of mature trees vs. seedlings at 16 m height on the tower.

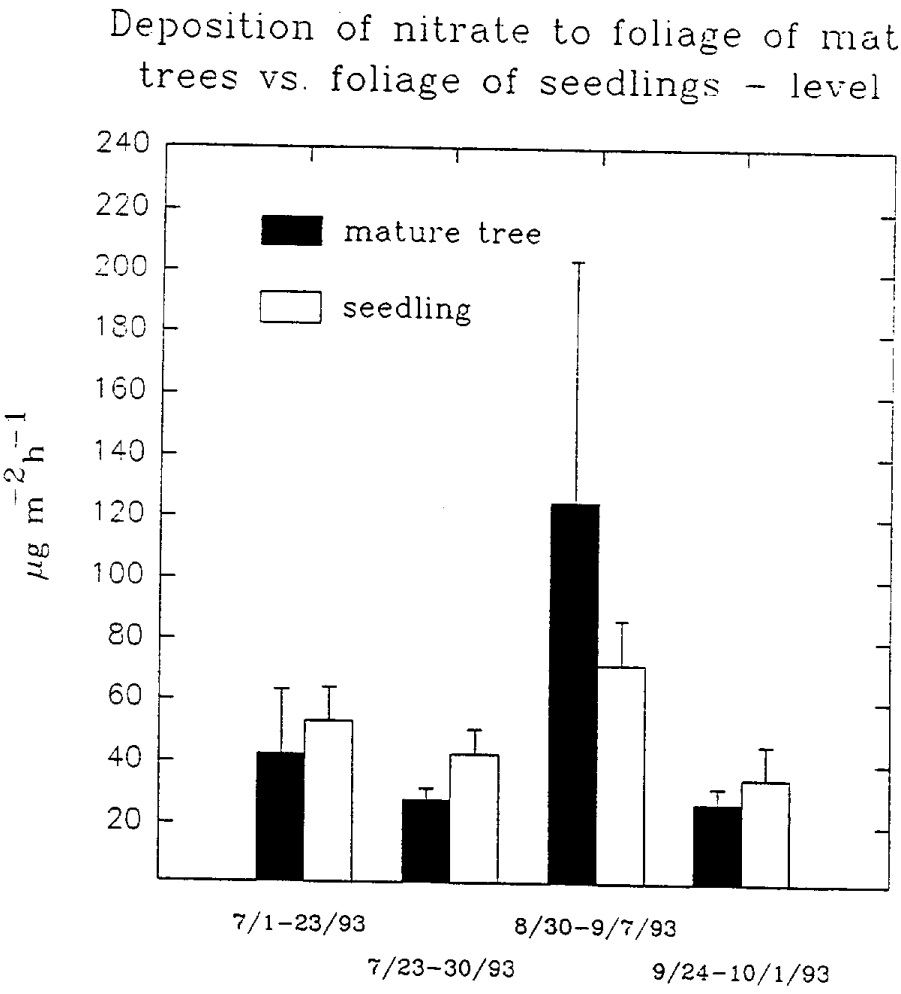

Fig. 7. Comparison of NO_3^- deposition to foliage of mature trees vs. seedlings at 12 m height on the tower.

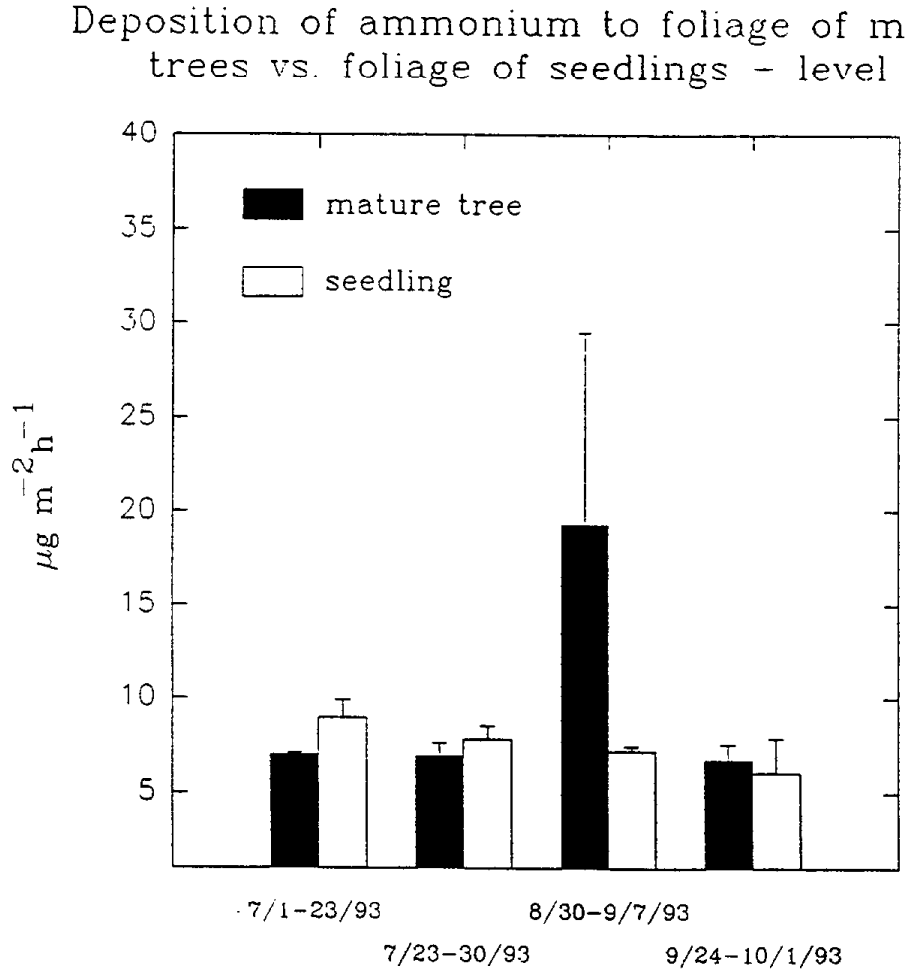

Fig. 10. Comparison of NH_4^+ deposition to foliage of mature trees vs. sedlings at 12 m height on the tower.

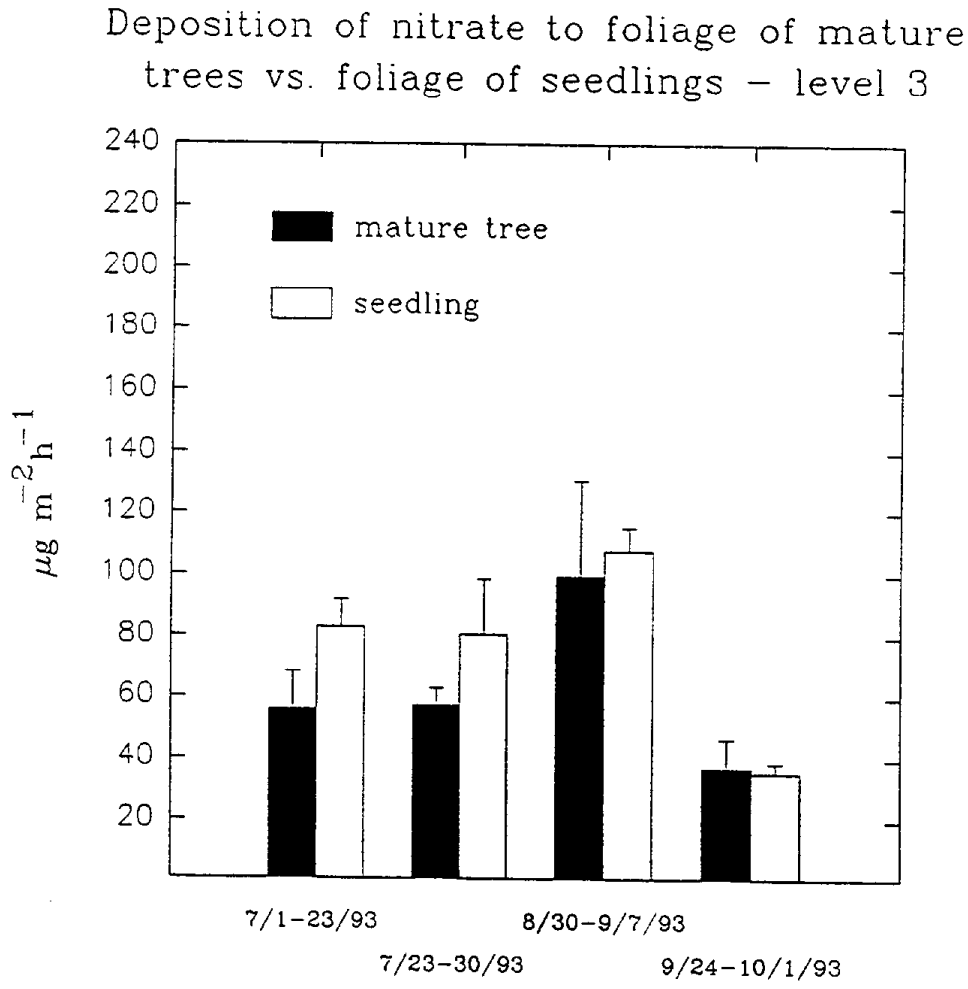

Fig. 9. Comparison of NO_3^- deposition to foliage of mature trees vs. seedlings at 24 m height on the tower.

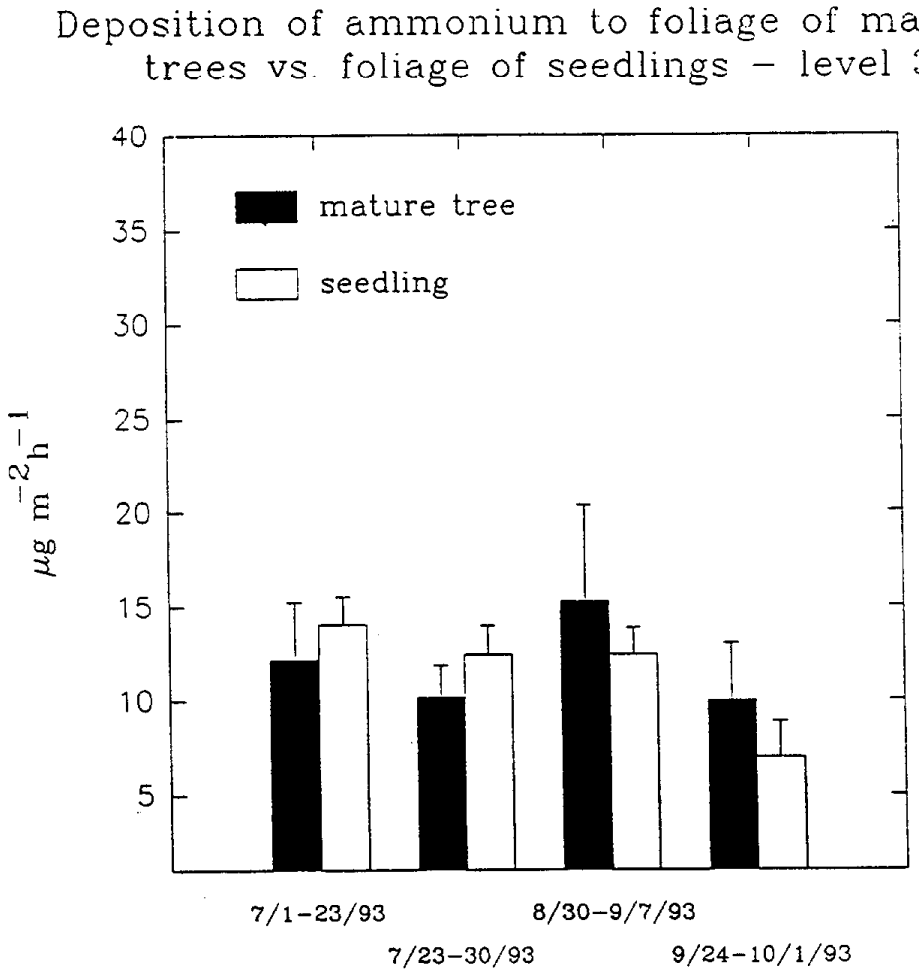

Fig. 12. Comparison of NH_4^+ deposition to foliage of mature trees vs. seedlings at 24 m height on the tower.

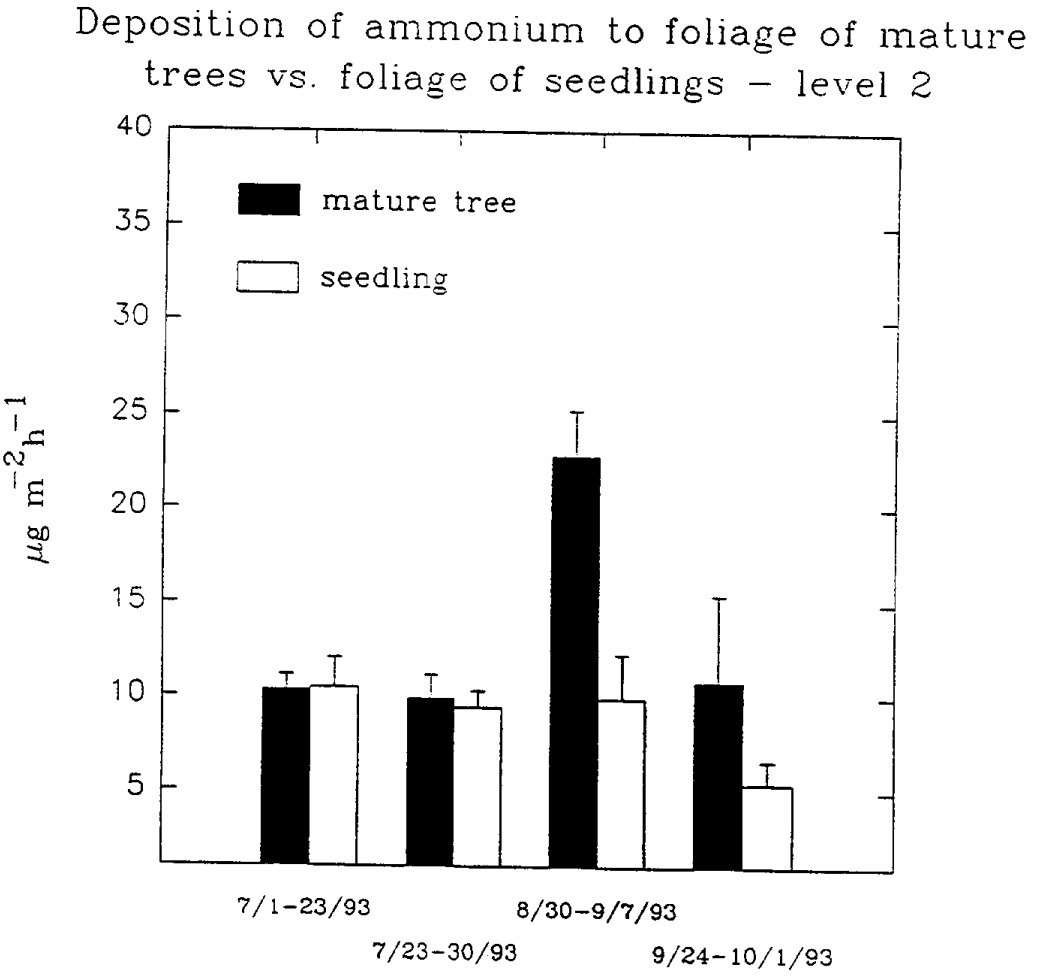

Fig. 11. Comparison of NH_4^+ deposition to foliage of mature trees vs. seedlings at 16 m height on the tower.

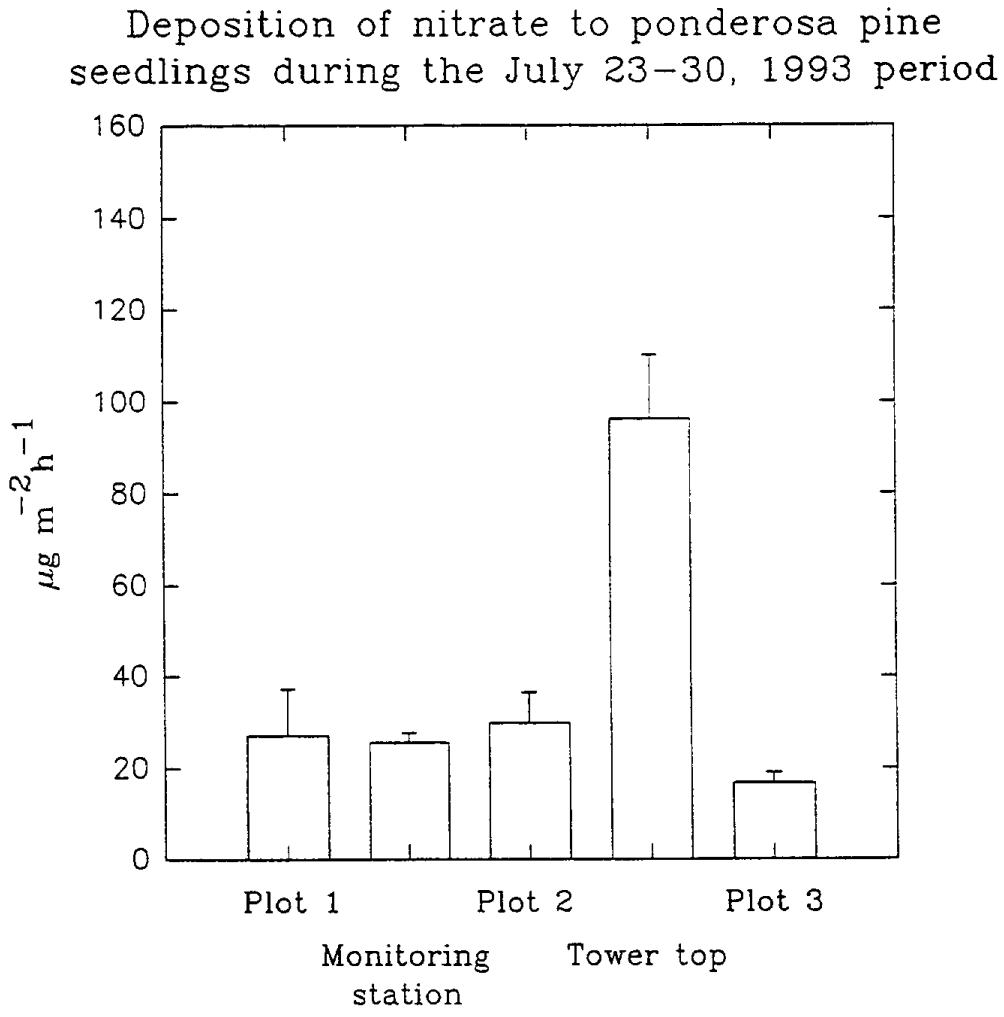

Fig. 14. Deposition of NO_3^- to foliage of ponderosa pine seedlings at the forest floor level during the July 23 - 30, 1993 intensive study.

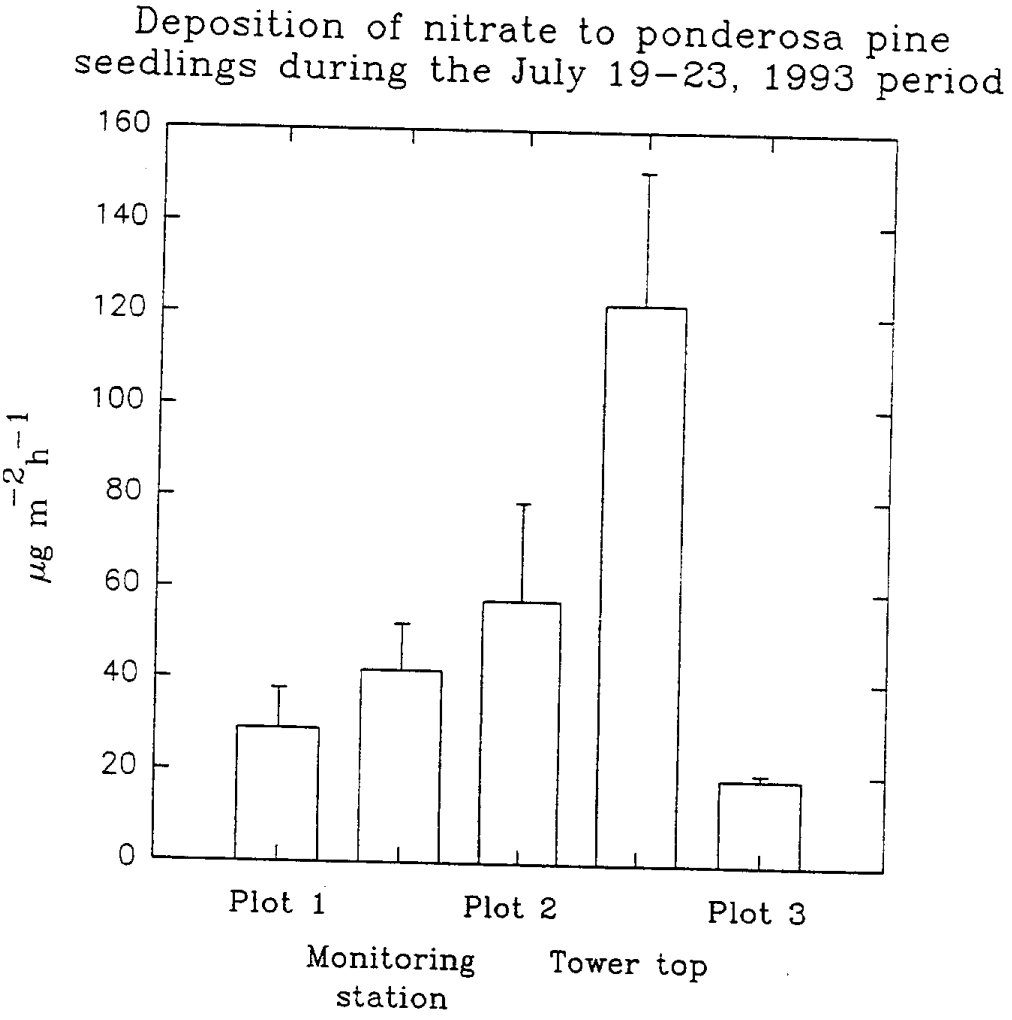

Fig. 13. Deposition of NO_3^- to foliage of ponderosa pine seedlings at the forest floor level during the July 19 - 23, 1993 intensive study.

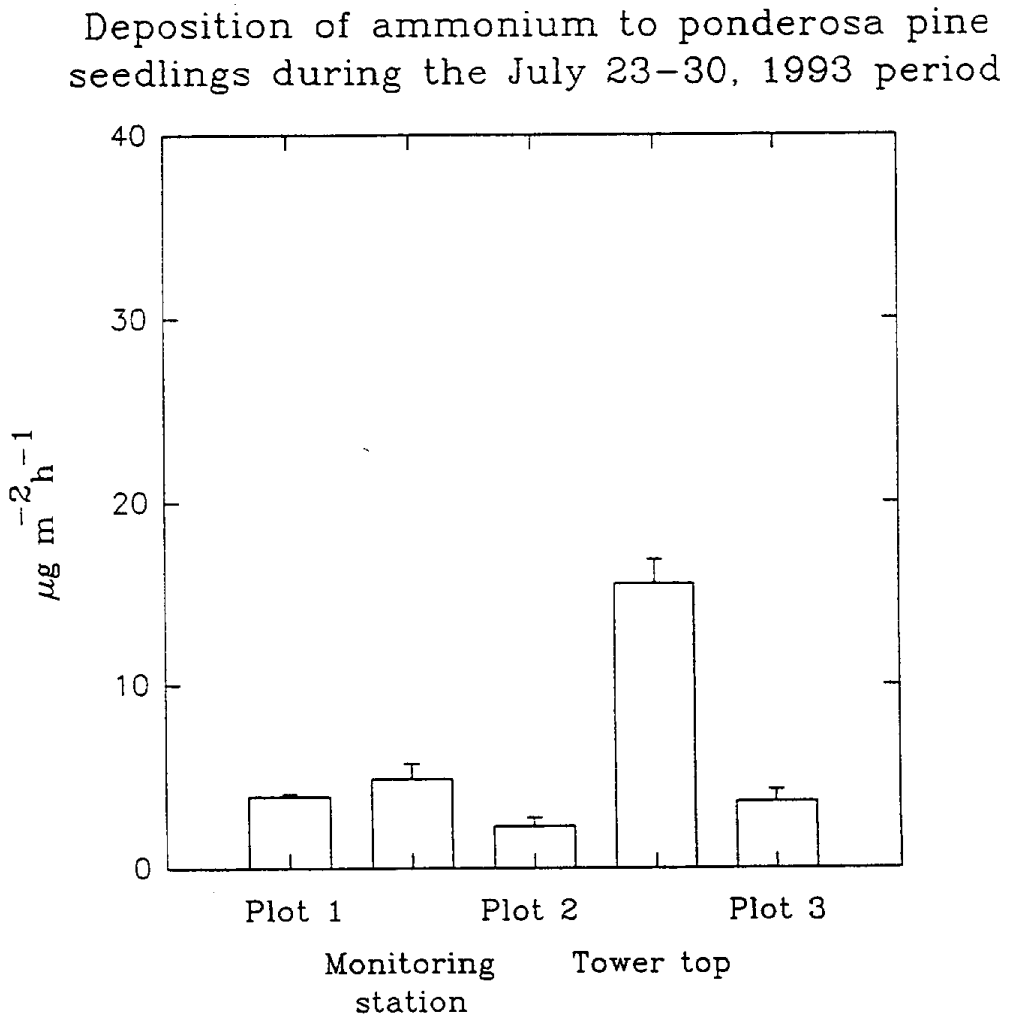

Fig. 16. Deposition of NH_4^+ to foliage of ponderosa pine seedlings at the forest floor level during the July 23 - 30, 1993 intensive study.

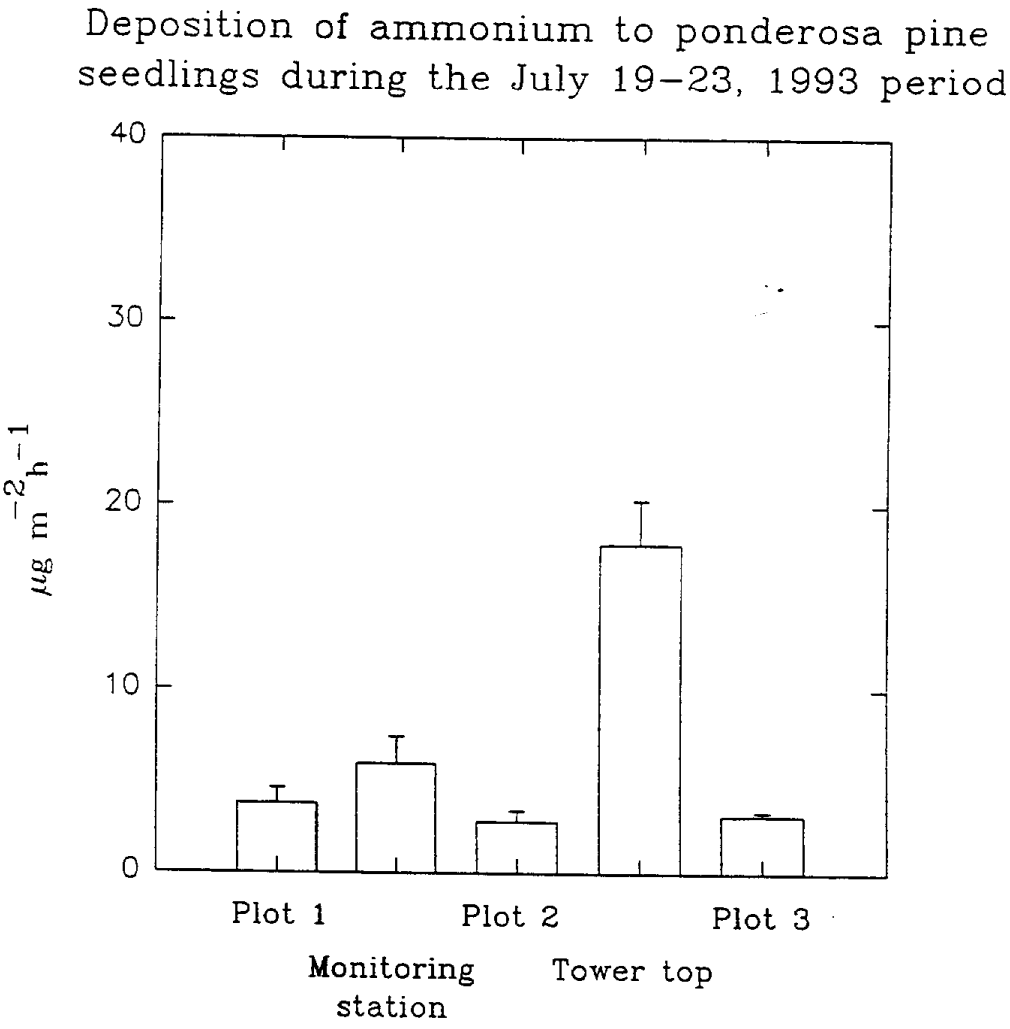

Fig. 15. Deposition of NH_4^+ to foliage of ponderosa pine seedlings at the forest floor level during the July 19 - 23, 1993 intensive study.

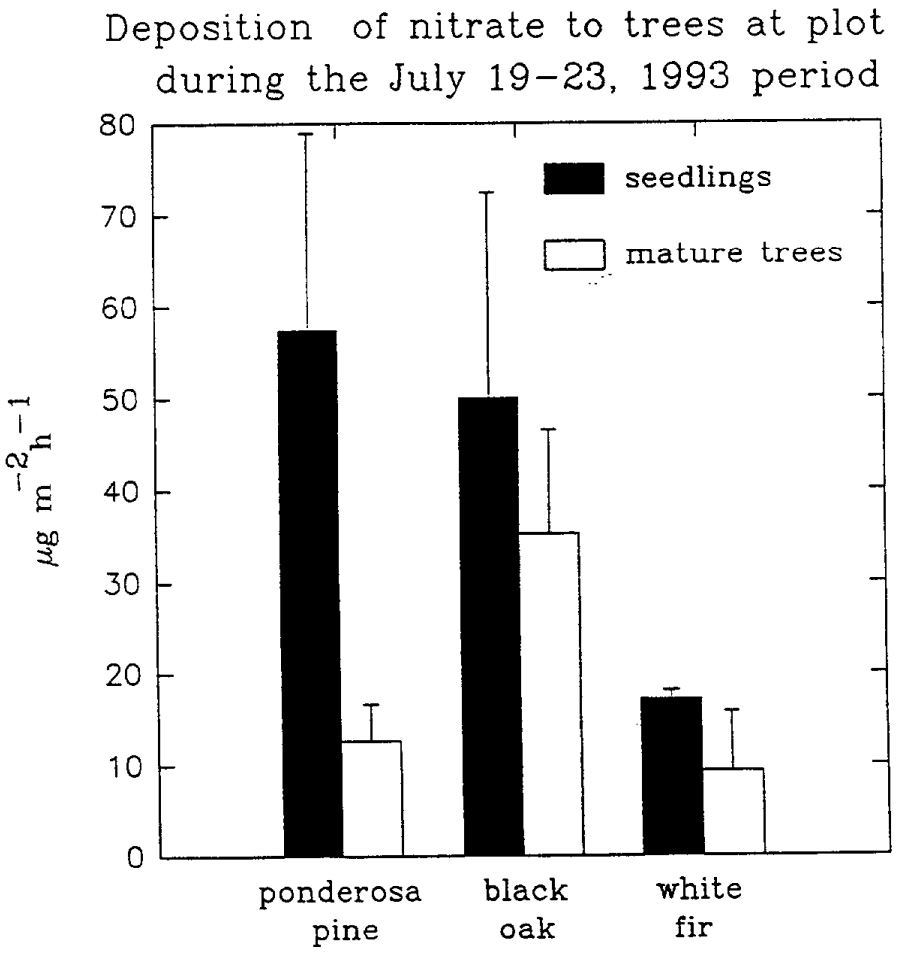

Fig. 18. Comparison of NO_3^- deposition to foliage of mature trees vs. seedlings of various species at plot 2 during the July 19 - 23, 1993 intensive study.

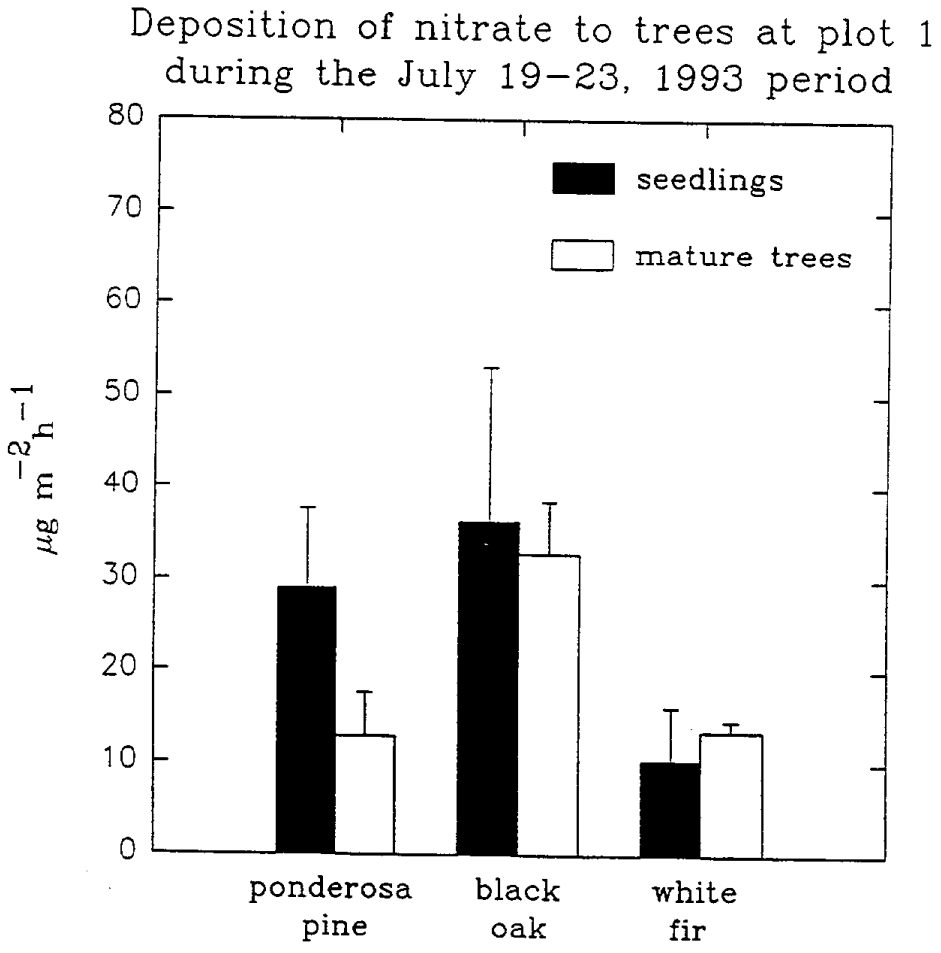

Fig. 17. Comparison of NO_3^- deposition to foliage of mature trees vs. seedlings of various species at plot 1 during the July 19 - 23, 1993 intensive study.

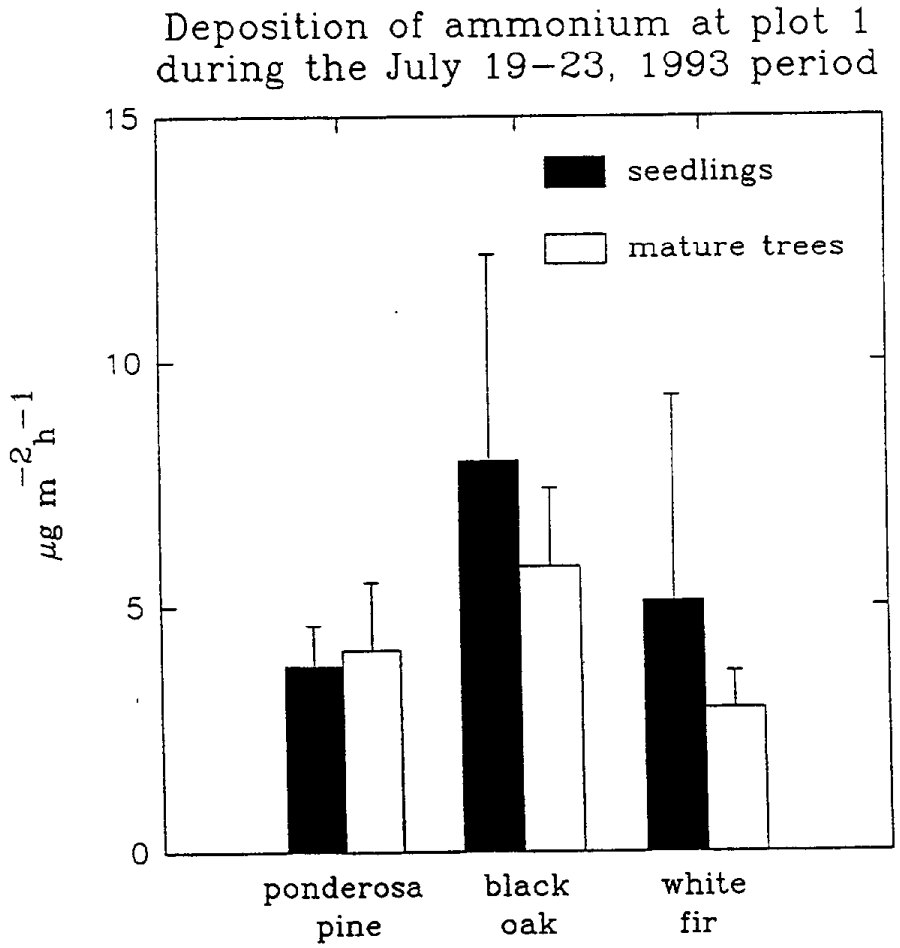

Fig. 20. Comparison of NH_4^+ deposition to foliage of mature trees vs. seedlings of various species at plot 1 during the July 19 - 23, 1993 intensive study.

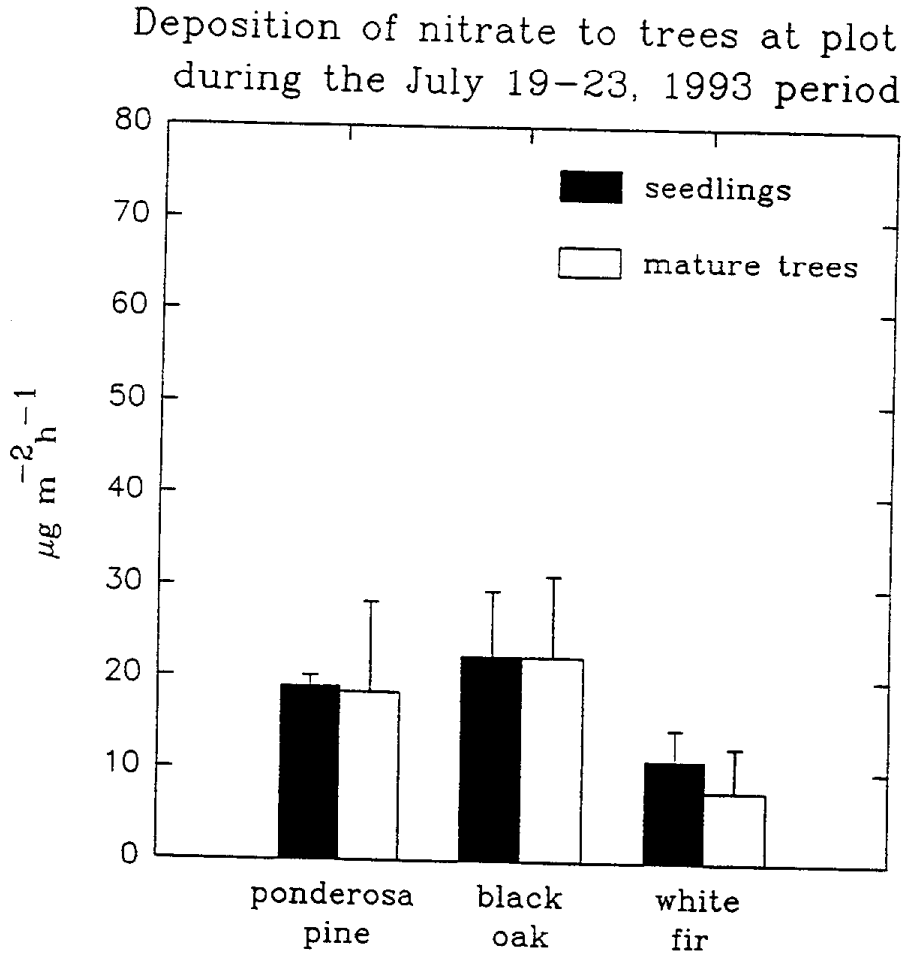

Fig. 19. Comparison of NO_3^- deposition to foliage of mature trees vs. seedlings of various species at plot 3 during the July 19 - 23, 1993 intensive study.

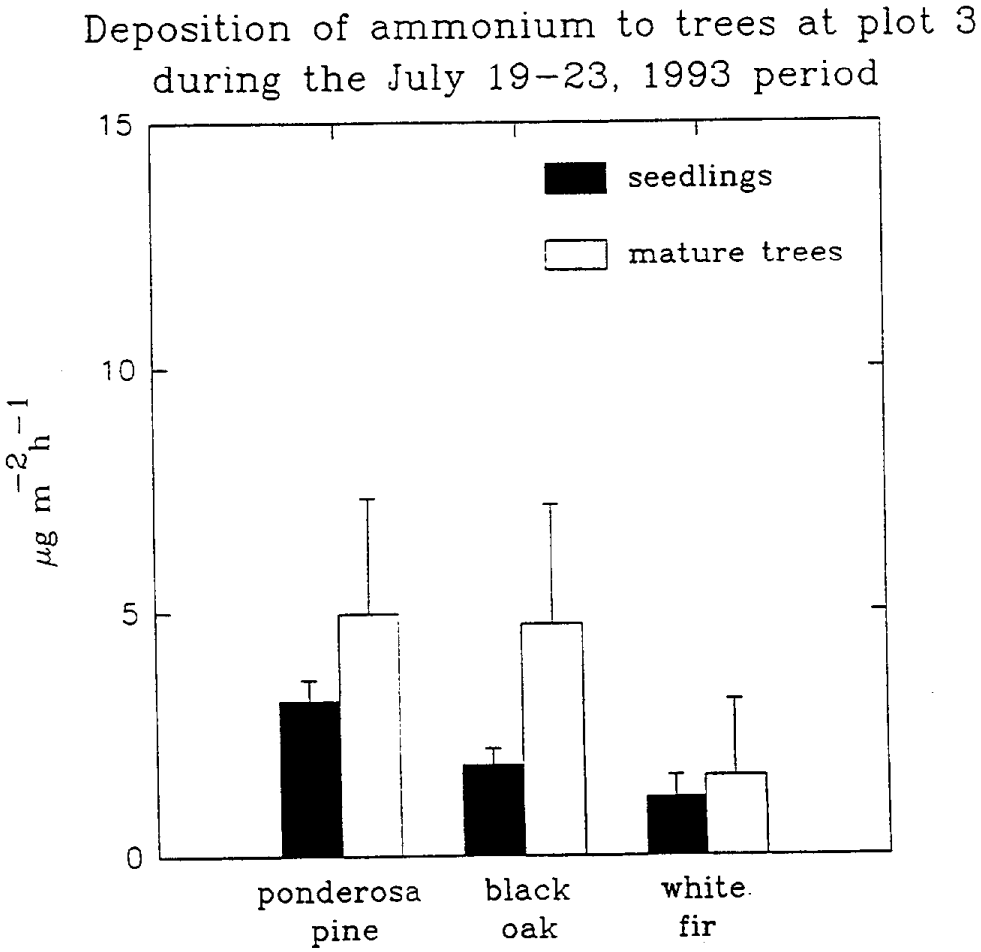

Fig. 22. Comparison of NH_4^+ deposition to foliage of mature trees vs. seedlings of various species at plot 3 during the July 19 - 23, 1993 intensive study.

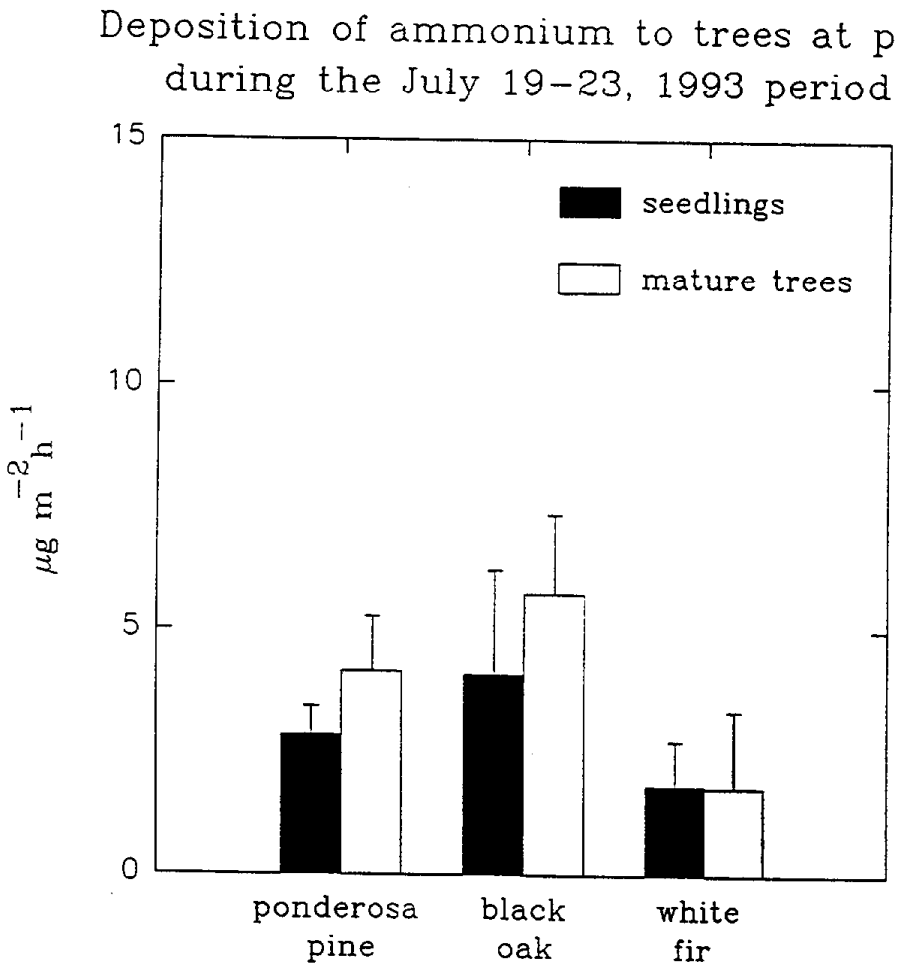

Fig. 21. Comparison of NH_4^+ deposition to foliage of mature trees vs. seedlings of various species at plot 2 during the July 19 - 23, 1993 intensive study.

APPENDIX 2

Patterns of Gas Exchange and Ozone Uptake in Pines at Barton Flats

Patrick V. Temple

3.0 PATTERNS OF GAS EXCHANGE AND OZONE UPTAKE IN PINES AT BARTON FLATS

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3.1 Introduction

The photochemical air pollutant ozone (O_3) is responsible for significant adverse effects on foliar injury and growth of forest trees, particularly ponderosa and Jeffrey pines, in the mountains of southern and central California. Efforts to establish ambient air quality standards for O_3 that will protect trees from injury have been hampered by uncertainties in establishing clear dose-response relationships between atmospheric concentrations of O_3 and foliar injury and growth responses of trees. A major component of these uncertainties is the observation that the adverse effects of O_3 are determined not by atmospheric concentrations but by the biologically-effective dose of O_3 absorbed by the tree. Uptake of ambient O_3 by plant foliage is determined by the gas exchange characteristics of the tree, which are controlled primarily by rates of stomatal conductance. Factors that determine the rate of stomatal conductance will also determine the rate of absorption of the biologically effective dose of O_3 derived from atmospheric concentrations. Thus, in order to link ambient air monitoring of O_3 with predicted or observed effects on foliar injury or tree growth, it is necessary to determine the amount of O_3 absorbed by the tree over the monitoring period.

Stomatal conductance is a highly complex physiological parameter, under the control of a suite of exogenous and endogenous factors. Current attempts to model stomatal conductance have emphasized exogenous or environmental control variables, particularly light, relative humidity, soil water, and temperature. However, endogenous factors, such as species-specific rates of conductance, or difference in rates of conductance between seedlings and mature trees, may play an even greater role in determining O_3 uptake. Relatively few studies of rates of stomatal conductance have been conducted in the field, and even fewer have measured conductance in the canopies of mature trees for extended periods of time.

3.2 Objective

The objective of this research was to provide specific information on rates of stomatal conductance of different age classes of ponderosa and Jeffrey pines in relation to seasonal changes in environmental conditions, particularly soil water, and to endogenous factors, particularly tree age and developmental stage of the foliage. These data were then used to derive monthly and annual budgets for absorbed O_3 dose, which

will be used to link ambient O_3 concentrations with measurements of foliar O_3 injury in the field.

3.3 Methodology

The research was conducted at the Barton Flats tower site, at 2150 m in elevation in the San Bernardino mountains. Systematic measurements of leaf stomatal conductance were taken on a population of Jeffrey pines, consisting of seedlings <0.5 m in height, saplings 2 to 3 m in height, and mature trees accessed from the tower to a height of approximately 30 m. Intensive measurements were taken on a individual seedling underneath the mature tree canopy and on an individual sapling growing in the clearing 10 m from the base of the tower. Comparative measurements with other seedlings and saplings indicated that rates of stomatal conductance in the seedling and sapling were representative of their age classes of pines. Measurements of stomatal conductance at the tops of mature trees could only be taken from the tower, so the representativeness of these measurements could not be determined. However, foliage from the three trees accessible from the tower usually had rates of stomatal conductance that were in agreement to within $\pm 10\%$, suggesting that these measurements may also have been representative of the population of mature pines at that site.

Beginning in May 1992, rates of stomatal conductance were measured on seedlings, saplings, and mature trees using a steady-state porometer (Model LI-1600, Licor Corp., Lincoln, NE) equipped with a cuvette head especially designed for conifer foliage. Measurements were conducted weekly or biweekly from May to October of 1992, 1993, and 1994, and monthly during the winters of 1992-3 and 1993-4, except when the site was inaccessible. Rates of conductance were measured on three needle fascicles of each age class of foliage, between 0900 and 1200, when rates of conductance were at their maximum. The fascicle diameter was measured with a digital electronic microcaliper (Mitutoyo Corp., Japan) and the leaf area of the needles was calculated using the formula:

$$\text{Area } (\text{cm}^2) = 2\,R\,L\,(n + \pi),$$

where R = fascicle radius (cm), L = needle length (cm), and n = number of needles per fascicle, 3 for both ponderosa and Jeffrey pine. Simultaneous measurements of light intensity, relative humidity, and ambient and leaf temperatures were taken with sensors attached to the porometer cuvette.

Concurrent measurements of volumetric soil water contents in the clearing and under the mature tree canopy at the tower site were obtained using an instrument that employed the principle of time-domain reflectometry (TRASE Soil Moisture Meter, SoilMoisture Corp., Santa Barbara, CA). Because of the rocky mature of the soil at the tower site, measurements of soil water were confined to the upper 15 cm of the soil profile.

Data on ambient O_3 concentrations in the area were obtained from a monitoring station located at Barton Flats. Average monthly O_3 for daylight hours was calculated for each of the three years of the study. Rates of stomatal conductance as measured for water vapor were converted to rates of conductance for O_3 and the average daily rate of conductance was calculated for each month. Flux of O_3 to foliage was calculated as average daily O_3 concentration (μmol mol^{-1}) times daily conductance rate (mol O_3 m^{-2} day^{-1}) = O_3 flux, in μmol m^{-2} day^{-1} for each month of the year.

3.4 Results and Discussion

3.4.1 Patterns of Precipitation and Conductance

Stomatal conductance in the seedlings, saplings, and mature ponderosa and Jeffrey pines at the Barton Flats tower site were strongly influenced by annual and seasonal patterns of precipitation both before and during the three years of the study. Annual precipitation in the area in the previous eight years (1984 to 1991) had averaged 30% below normal. This prolonged drought affected the foliar characteristics of the needles, which were significantly shorter than normal. The trees also retained greater numbers of annual whorls of foliage, so that the Jeffrey pines in 1992 retained 7 or more years of foliage, rather than the typical 4 to 5 years of needle retention. The 1990 needle cohort was very small, and in some cases was missing, in response to the extremely dry year of 1989, which had only 30% of the long-term average precipitation.

In contrast, precipitation in 1992 was 50% greater than the long-term average. Summer rains during July-August increased soil water contents that had been depleted earlier in the summer (Fig. 1). Peak stomatal conductance for the seedling and sapling trees occurred in early July but by early August conductance had decreased significantly, and then it gradually declined during the rest of the year (Fig. 1). The peak rates of conductance for the young trees measured in 1992 were the highest observed during the three years of the study, which may have reflected recovery from low conductance during the drought years. Peak stomatal conductance for the foliage of mature trees was less than half that of the younger trees, but by mid-August there were no differences in rates of stomatal conductance among the three age classes of trees (Fig. 1). The relationship between rates of stomatal conductance in the trees and amount of water in the soil was not clear, because peak stomatal conductance had occurred before the onset of the summer rainy period, and conductance rates declined at the time that soil water contents remained high (Fig. 1).

Annual precipitation in 1993 was greater than that of 1992, averaging 57% higher than the long-term mean. A late seasonal storm in early June increased the water content of the soil to levels higher than those at the beginning of the year, and soil water remained relatively constant from July to October (Fig. 2). Patterns of stomatal conductance in the trees were significantly different than those observed in 1992 (Fig. 2). Peak conductance in seedlings increased gradually in April and remained relatively

constant until late August, then declined. Stomatal conductance in the sapling showed a similar pattern, except that the decrease in rates of conductance began earlier in August (Fig. 2). Conductance in foliage of the mature trees fluctuated throughout the summer, but the decline in conductance rates began in July (Fig. 2). Rates of conductance in mature tree foliage averaged 30% lower than those of the seedling and sapling trees for the entire growing season.

In contrast to the previous two years, annual precipitation in 1994 was 42% below the long-term average and little rain fell during the summer months. Soil water reserves became depleted early in the summer (Fig. 3), and this lack of available soil water impacted rates of gas exchange in the trees. Rates of conductance in the seedling, sapling, and mature trees began to decline in mid-June and rates of gas exchange remained low for the remainder of the year for each age class of pines (Fig. 3). Seedlings had the highest rate of conductance during the active period from April to June, but after June the three age classes of pines had similar rates of conductance (Fig. 3). During the period in which the trees were active (early April to mid-June) rates of conductance in the mature trees averaged 35% lower than in seedlings, but 22% higher than in the sapling.

Ozone Flux to Pine Foliage

Ambient ozone concentrations at the field site varied relatively little during the three years of the study (Fig. 4). Highest daylight ozone concentrations averaged 83 ppb in June and 79 ppb in July. Ozone levels were slightly lower than average in July 1992 because of the prevalent cloudy and rainy conditions during that time. Ozone concentrations during the winter months, November to March, averaged 47 ppb. Although ambient ozone concentrations were relatively uniform from 1992 to 1994, ozone flux to pine foliage varied widely over the three year period, because of the highly variable patterns of stomatal conductance described above. The mean daily ozone flux from 1992 to 1994, averaged on a monthly basis, to one-year-old needles of a seedling Jeffrey pine growing in partly shaded conditions under the canopy of the mature pine trees is shown in Fig. 5. Flux rates were highest in 1992, with a peak flux rate of 141 umol O_3 m^{-2} d^{-1} for the month of June. The high flux rate for June was a response not only to the ambient ozone concentrations, which also peaked in June (Fig. 4), but also to the number of hours of daylight which were also greatest in June. In 1993, ozone flux averaged 37% lower than 1992 over the year. Ozone flux was also more uniformly distributed over the growing season. Flux of ozone in 1994 peaked in June, as in 1992, but peak flux rates were 32% lower in 1994 than in 1992. Flux of ozone declined rapidly in July 1994, and continued to decline throughout the summer, reflecting the drought-stressed conditions in 1994. On a yearly basis, average monthly ozone flux in 1994 was similar to 1993, although seasonal patterns of ozone uptake differed significantly between the two years.

Ozone flux rates to the sapling were similar to those measured to the seedling. Flux rates in 1992 averaged twice those of 1993 and 1994, with highest flux rates during the months of May, June, and July (Fig. 6). These three months accounted for 70% of all ozone uptake for the year. In 1994, the drought conditions reduced ozone flux rates in July, August, and September and relatively little ozone uptake occurred during those late summer months. On an annual basis, ozone uptake in the sapling in 1994 was similar to that of 1993, and flux rates in the sapling were similar to those of the seedling over the three years of the study.

The seasonal patterns of ozone uptake in the mature trees differed significantly among the three years of the study (Fig. 7). In 1992, ozone uptake was maximum in June, and this one month accounted for 30% of total annual ozone flux. Peak ozone uptake in the mature trees was 32% lower than that of seedling and sapling trees, and on an annual basis, ozone flux to the mature trees was 30% lower than to seedlings and saplings in 1992. Ozone flux to mature trees was lower in 1993, particularly early in the year, and mean annual ozone uptake averaged 44% lower than in 1992. However, in the dry year of 1994, ozone flux rates were higher early in the year, and on an annual basis ozone flux to mature foliage averaged only 10% lower than fluxes to seedlings and saplings.

Conclusions

1. Ozone flux to pine foliage was highly variable, both from year-to-year and within the year.

2. Ozone flux to foliage was highest in June, and declined rapidly after June particularly in a dry year such as 1994. From 60% to 70% of total annual ozone uptake occurred in the months of May, June, and July.

3. Flux of ozone to foliage of mature pine trees averaged 30% lower than flux to seedlings and saplings during peak periods in early summer. Later in the summer and in dry years, ozone flux to mature trees was equal to or only slightly lower than flux to foliage of other age classes of trees.

4. Total amount of precipitation the previous winter was correlated with total ozone uptake in 1992 (high precipitation) and 1994 (low precipitation), but not in 1993, a year of high precipitation but low ozone uptake.

Table 1. Annual ozone uptake in one-year-old foliage of three age classes of Jeffrey pines at Barton Flats, San Bernardino Mountains, 1992-1994.

	Ozone Uptake (mmol O_3 m^{-2} yr^{-1})		
	Seedling	Sapling	Mature trees
1992	13.5	16.1	10.4
1993	9.2	8.4	5.8
1994	10.8	7.4	8.2

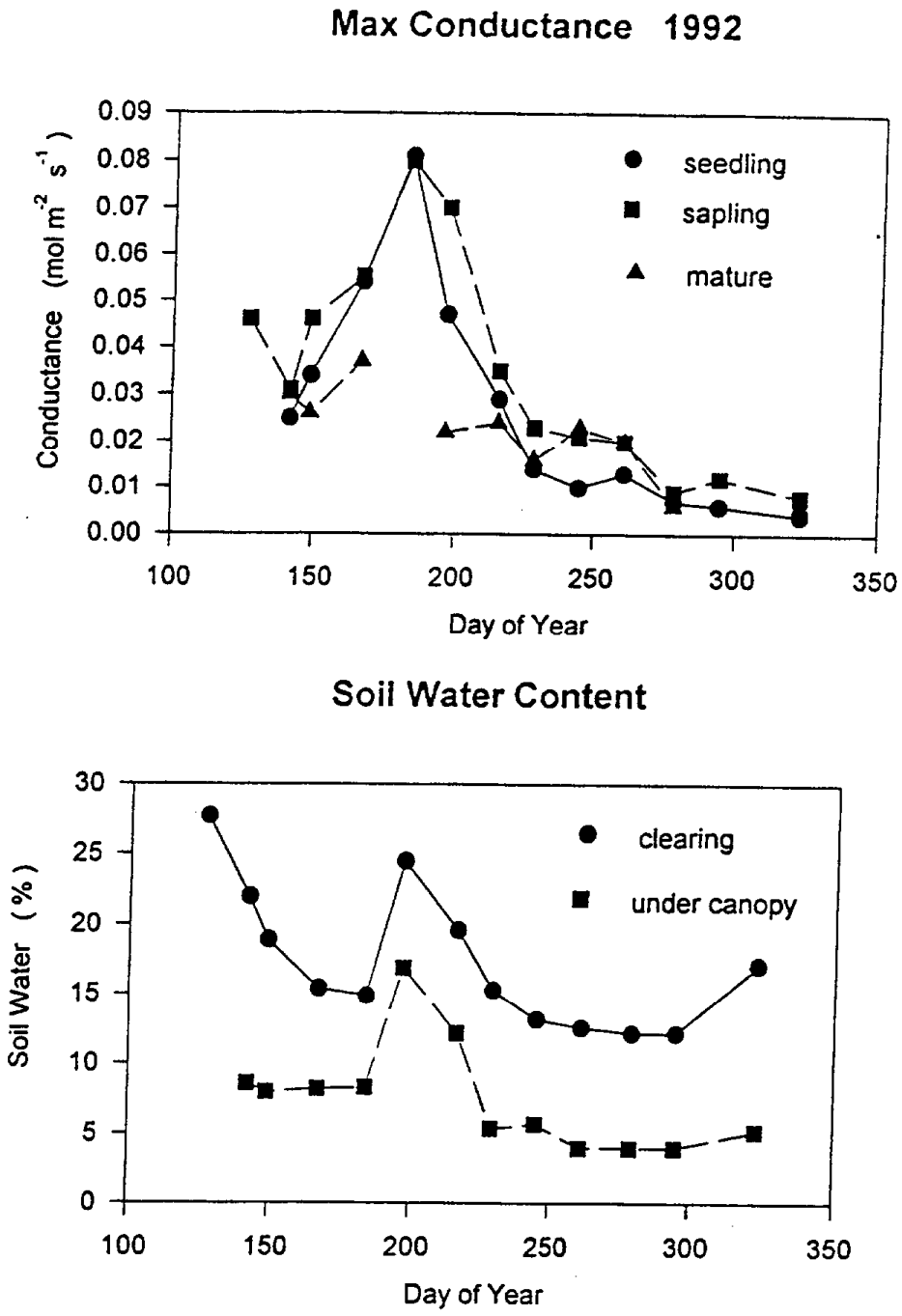


Figure 1. Maximum rates of stomatal conductance in one-year-old needles of three age classes of Jeffrey pines, and soil water content (0 to 15 cm) at the Barton Flats tower site in 1992.

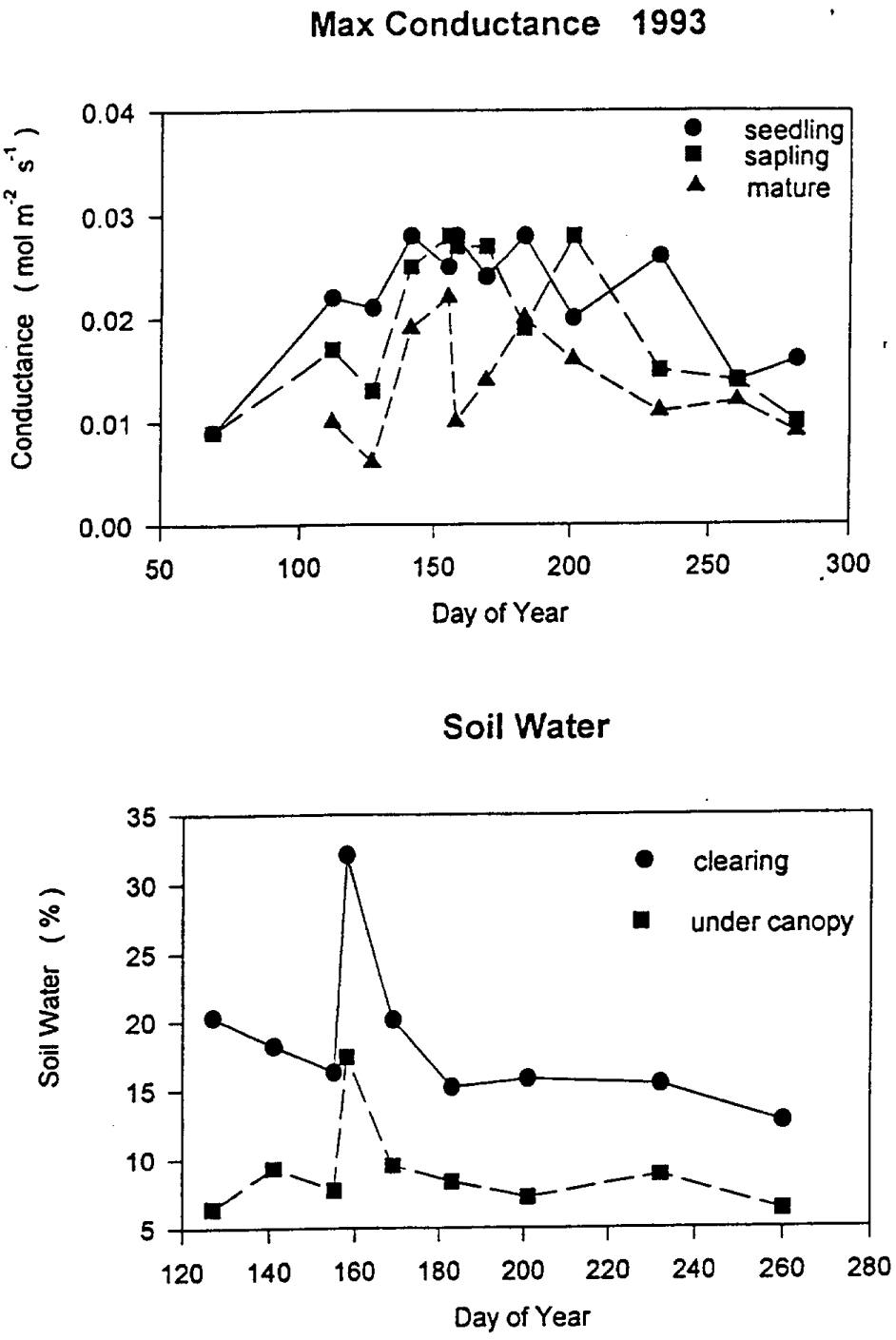


Figure 2. Maximum rates of stomatal conductance in one-year-old needles of three age classes of Jeffrey pines, and soil water content (0 to 15 cm) at the Barton Flats tower site in 1993.

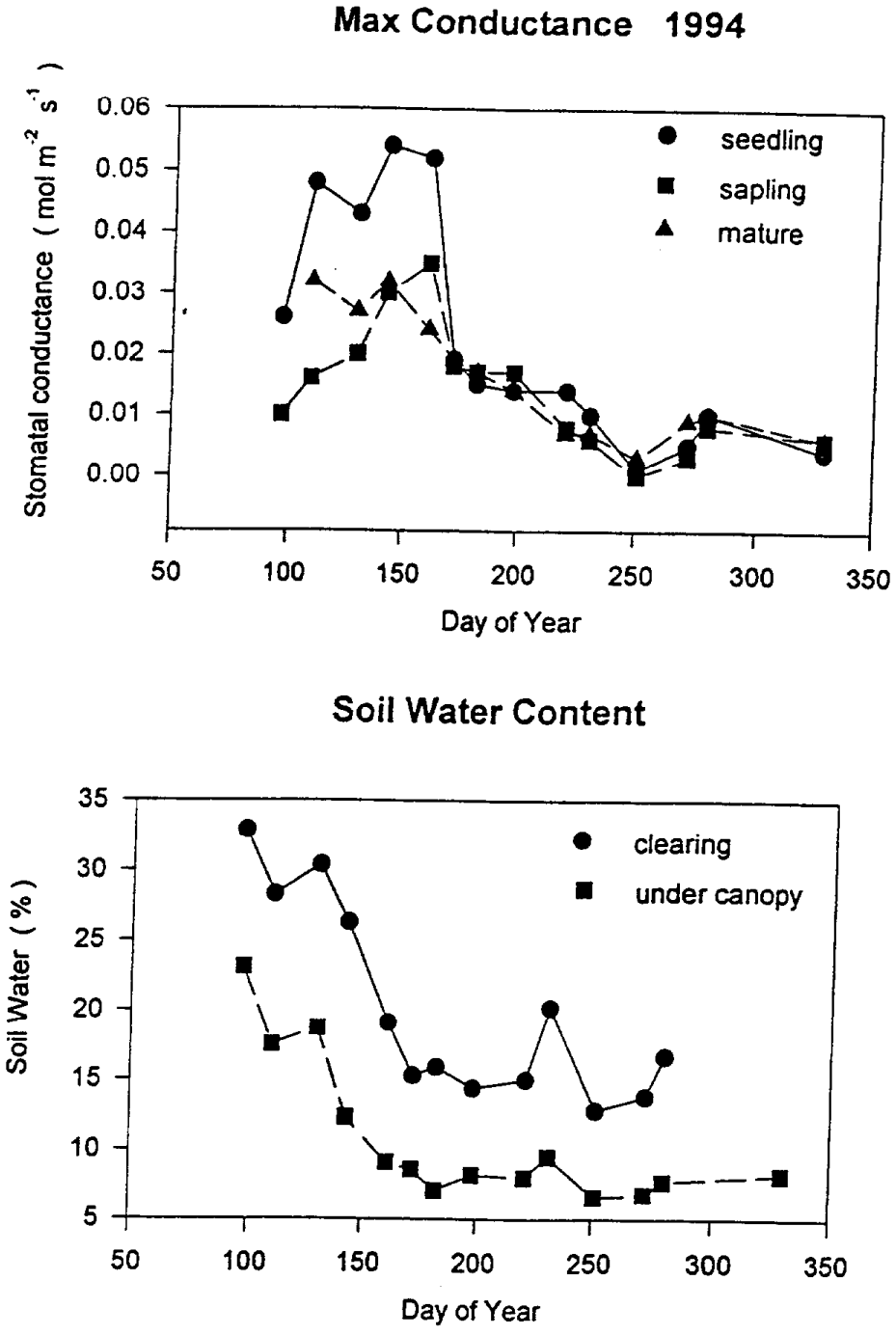


Figure 3. Maximum rates of stomatal conductance in one-year-old needles of three age classes of Jeffrey pines, and soil water content (0 to 15 cm) at the Barton Flats tower site in 1994.

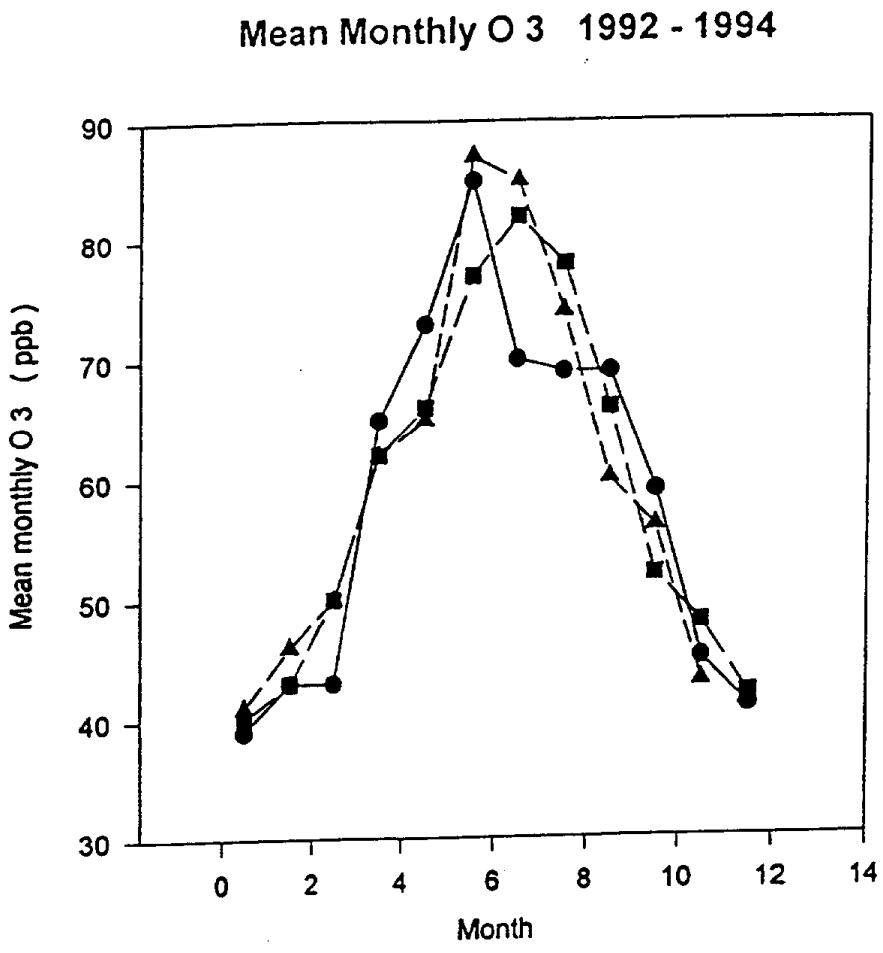


Figure 4. Mean monthly ambient ozone concentrations at Barton Flats, 1992 to 1994.

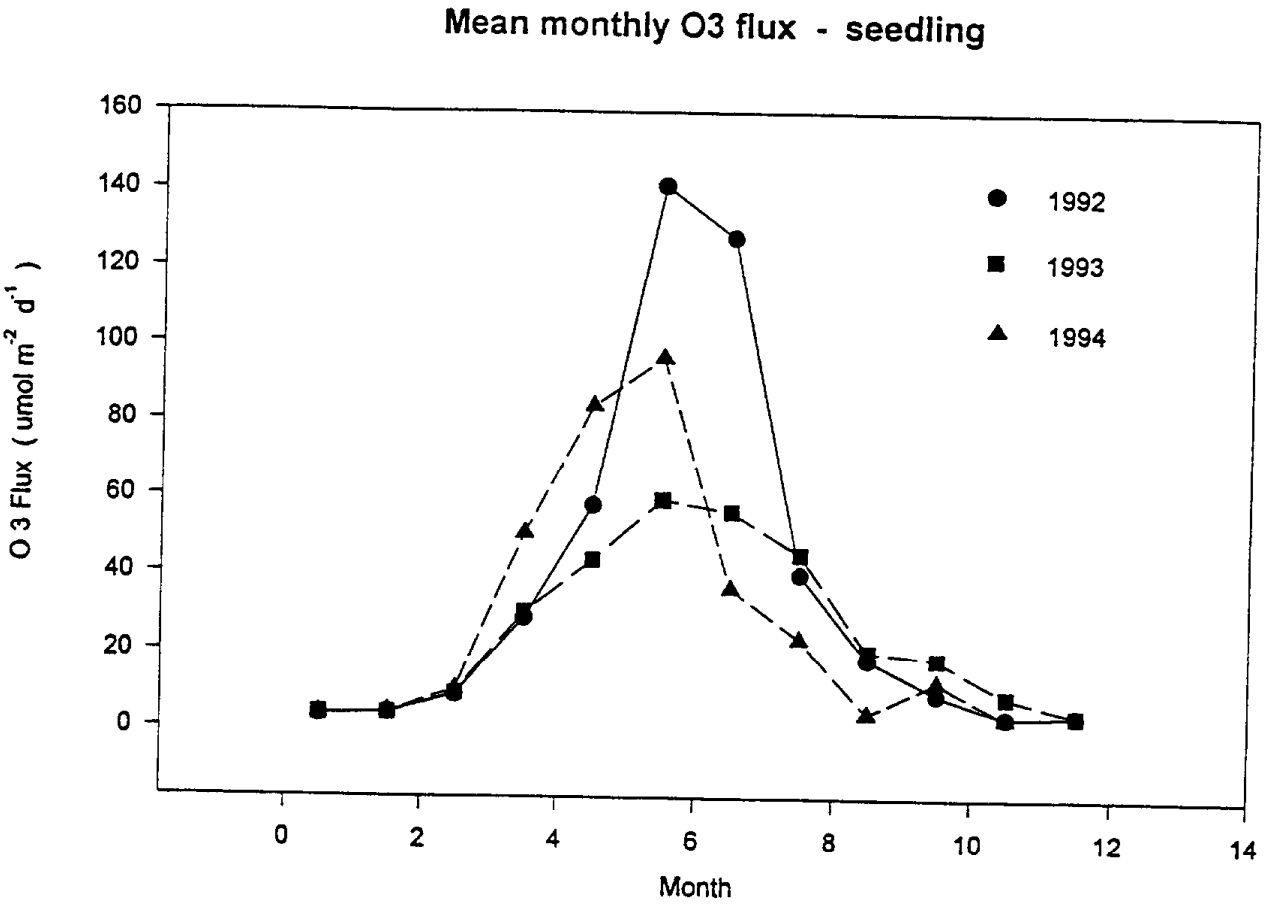


Figure 5. Monthly mean daily ozone flux to one-year-old foliage of seedling Jeffrey pine at Barton Flats, 1992 to 1994.

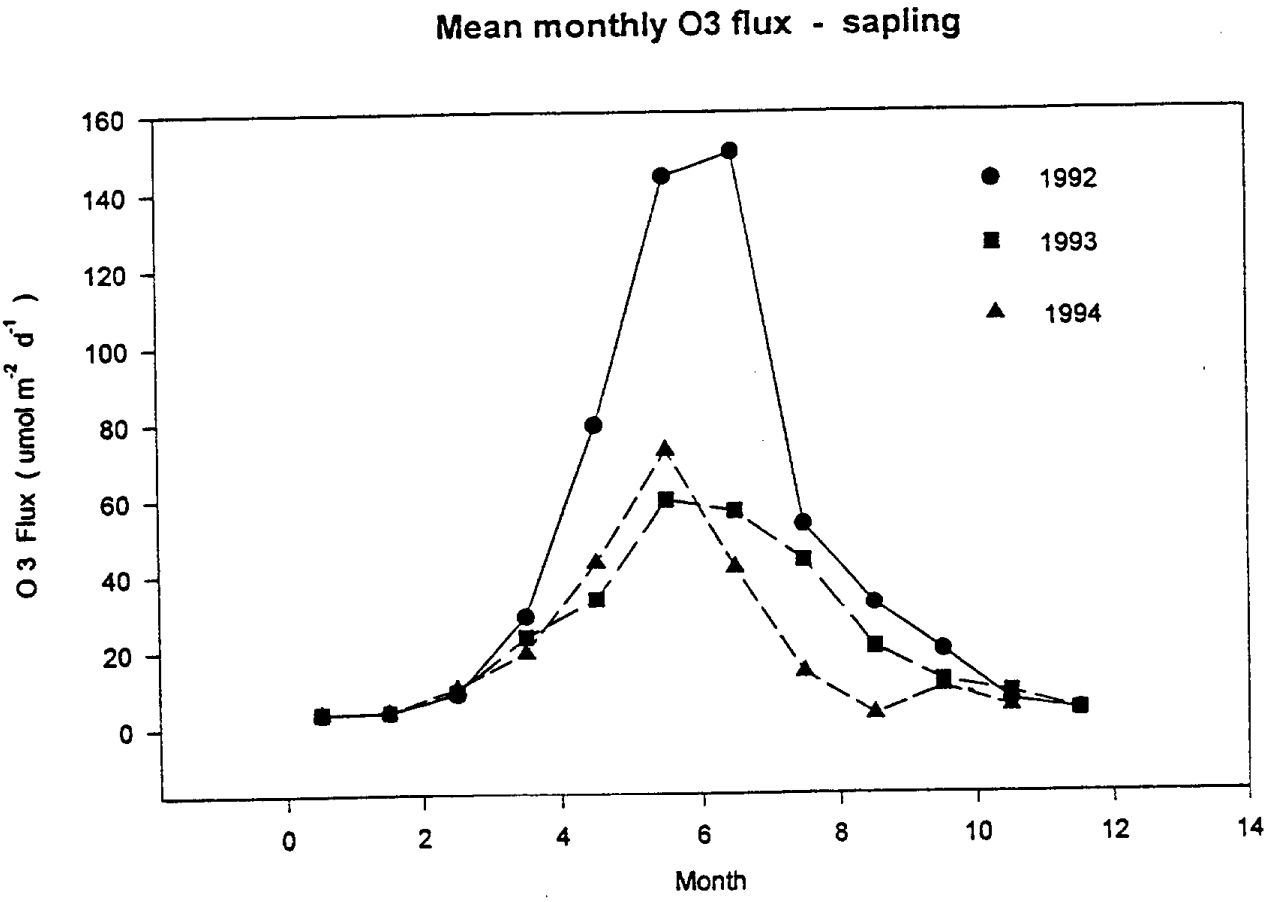


Figure 6. Monthly mean daily ozone flux to one-year-old foliage of sapling Jeffrey pine at Barton Flats, 1992 to 1994.

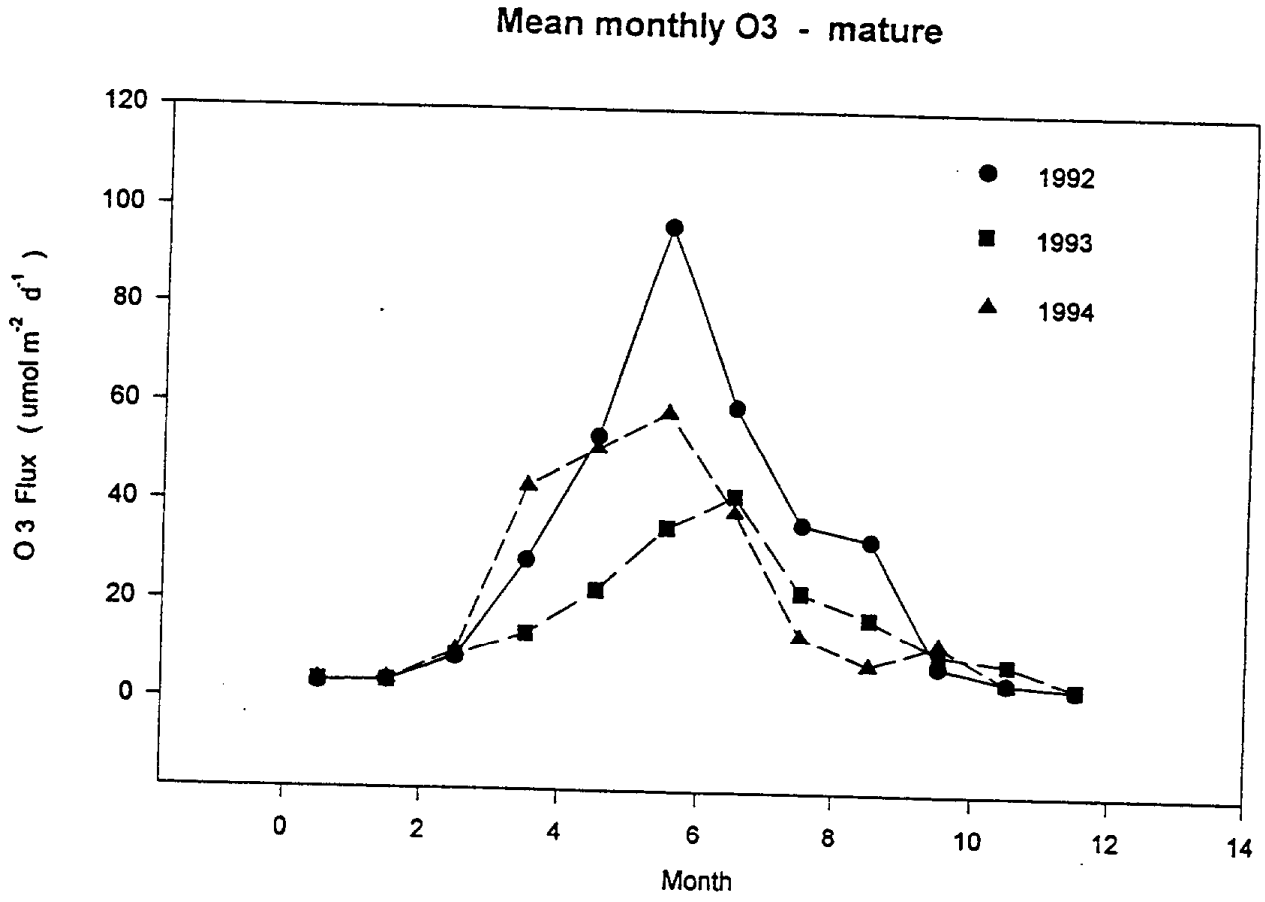


Figure 7. Monthly mean daily ozone flux to one-year-old foliage of mature Jeffrey pine at Barton Flats, 1992 to 1994.

APPENDIX 3

Field Studies in the Eastern Sierra Nevada

Dale W. Johnson and Randy Dahlgren

Field Studies in the Eastern Sierra Nevada

Dale W. Johnson

Nevada Experiment Station (Hatch) Funding
in collaboration with

Randy Dahlgren, U.C. Davis
Shorty Boucher U.C. Berkeley (formerly)
Andrzej Bytnerowicz, USFS Riverside

Soil Solution and stream pH, HCO_3^-, NO_3^-, and SO_4^{2-} concentrations.

	pH	HCO_3^-	NO_3^-	SO_4^{2-}
	----------	$umol_c\ L^{-1}$	----------	
Little Valley, NV*				
Pine	7.1±0.1	417±94	<0.5	273±46
Ceanothus	6.7±0.1	306±47	3±3	107±15
Mountain Alder	7.0±0.2	688±192	<0.5	181±59
Franktown Creek	7.1±0.04	73±15	3±2	3±0.2
Sagehen, CA				
Red fir	7.0±0.1	335±32	73±12	21±3
Sagehen Creek	7.0±0.2	1102±123	1±1	3±0.3

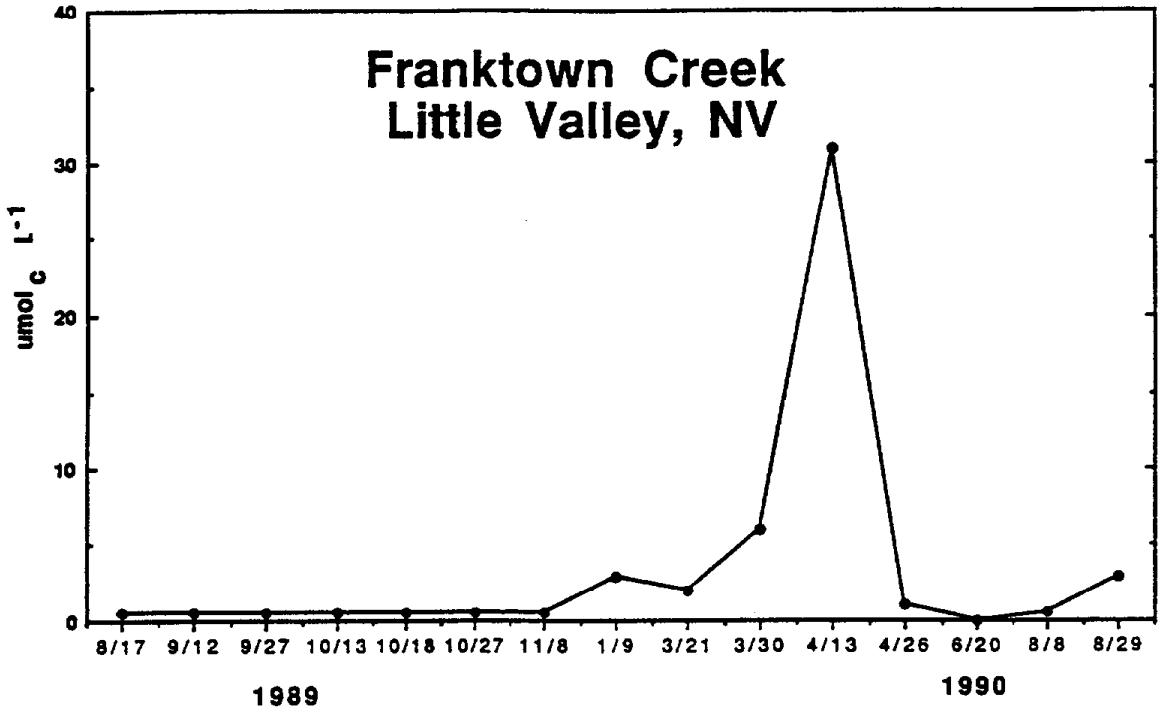
* Data from Johnson (in press)

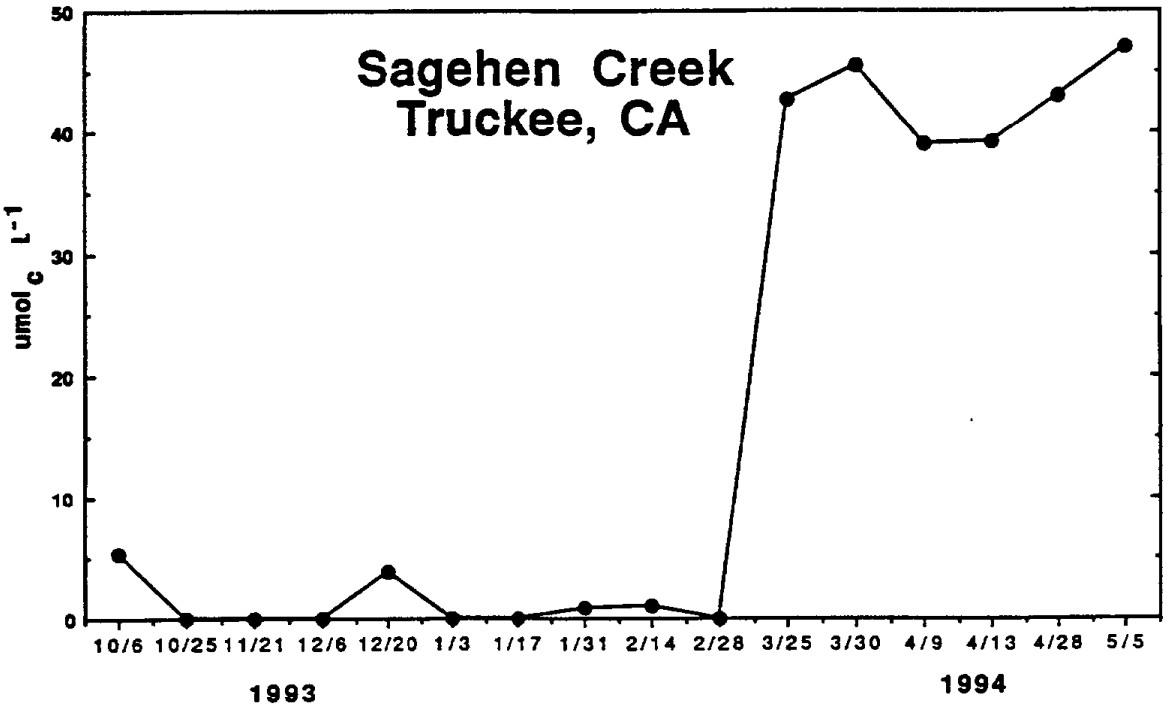
Soil extractable P (NH_4F/HCl) in Little Valley (jeffrey pine) and Sagehen (red fir) soils.

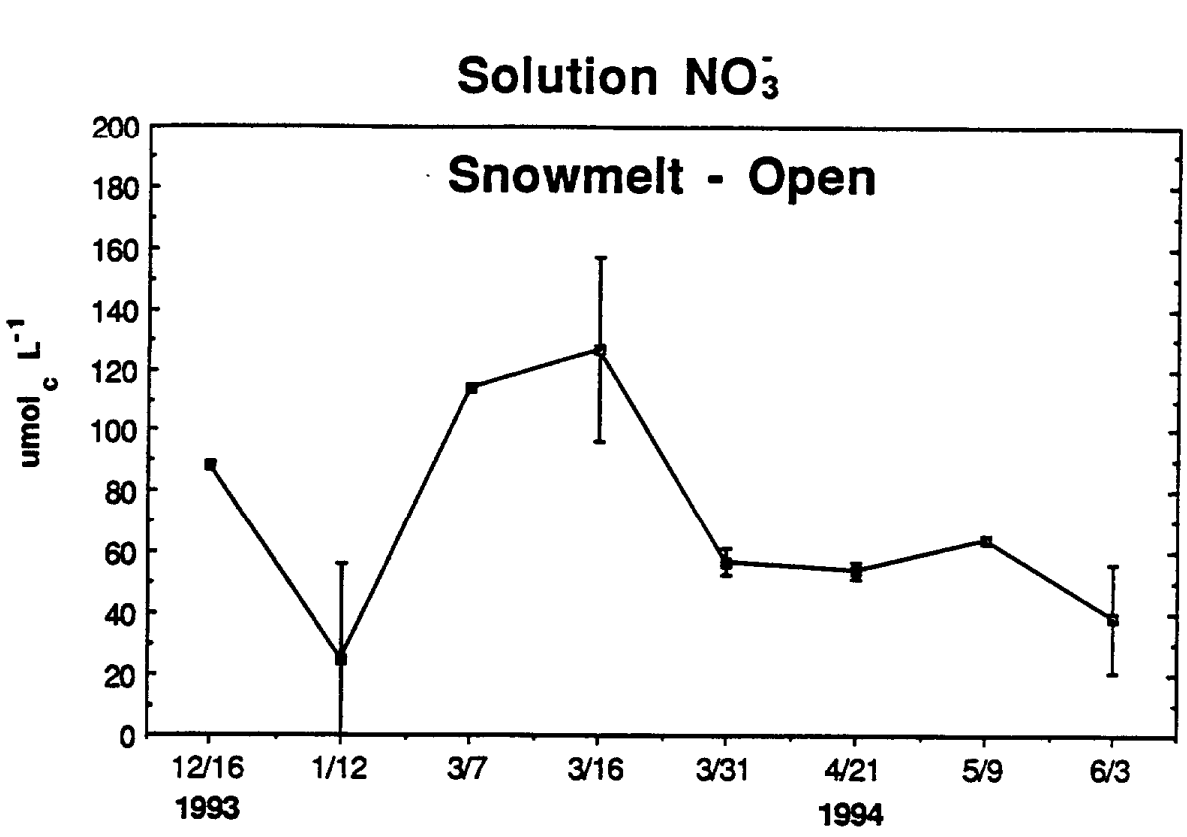
Little Valley*		Sagehen	
Horizon and depth (cm)	Extr. P	Horizon and depth (cm)	Extr. P
A (0-20)	101±21	A (0-10)	<0.5
BA (20-40)	106±36	B (10-18)	<0.5
BC (40-60)	69±12	BC (18-45)	<0.5

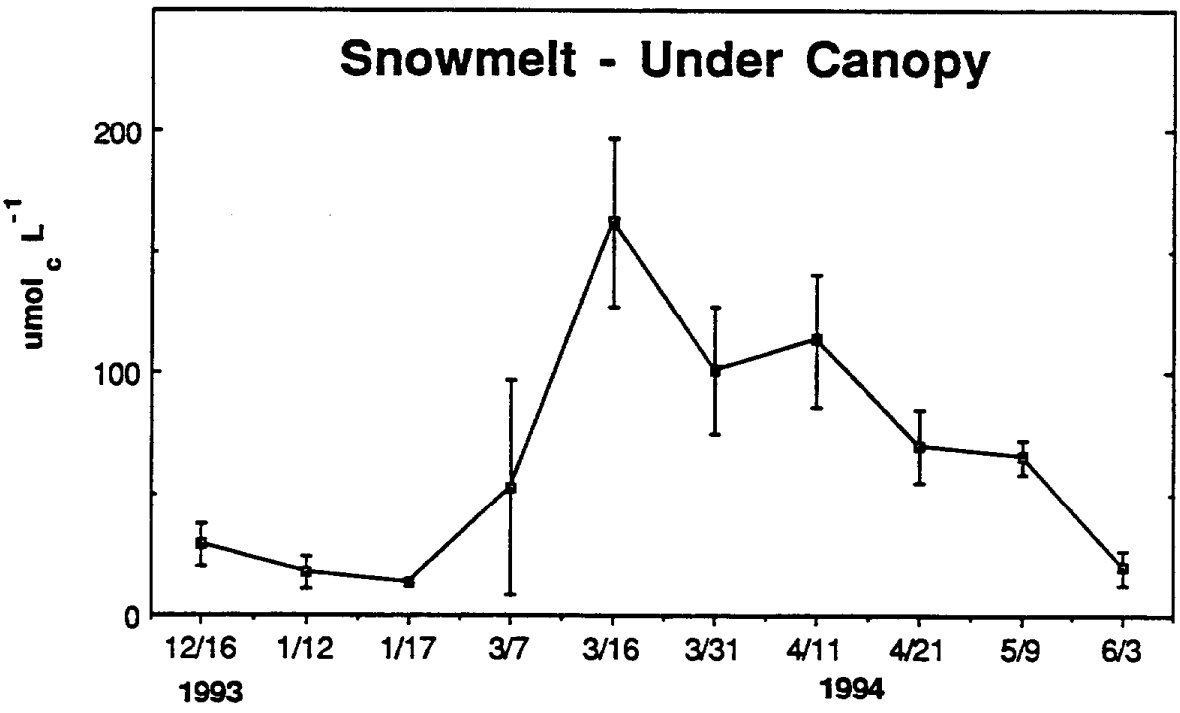
* Data from Johnson (in press)

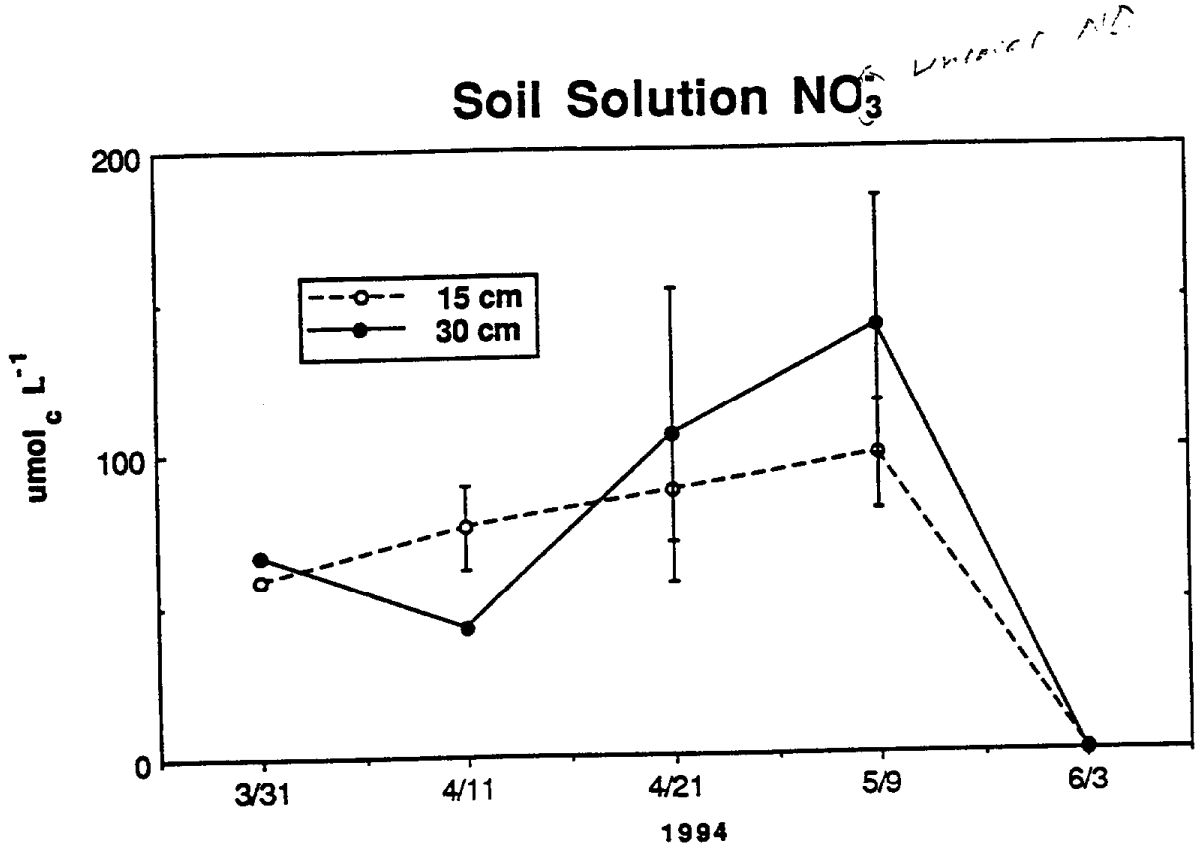
Streamwater NO_3^-





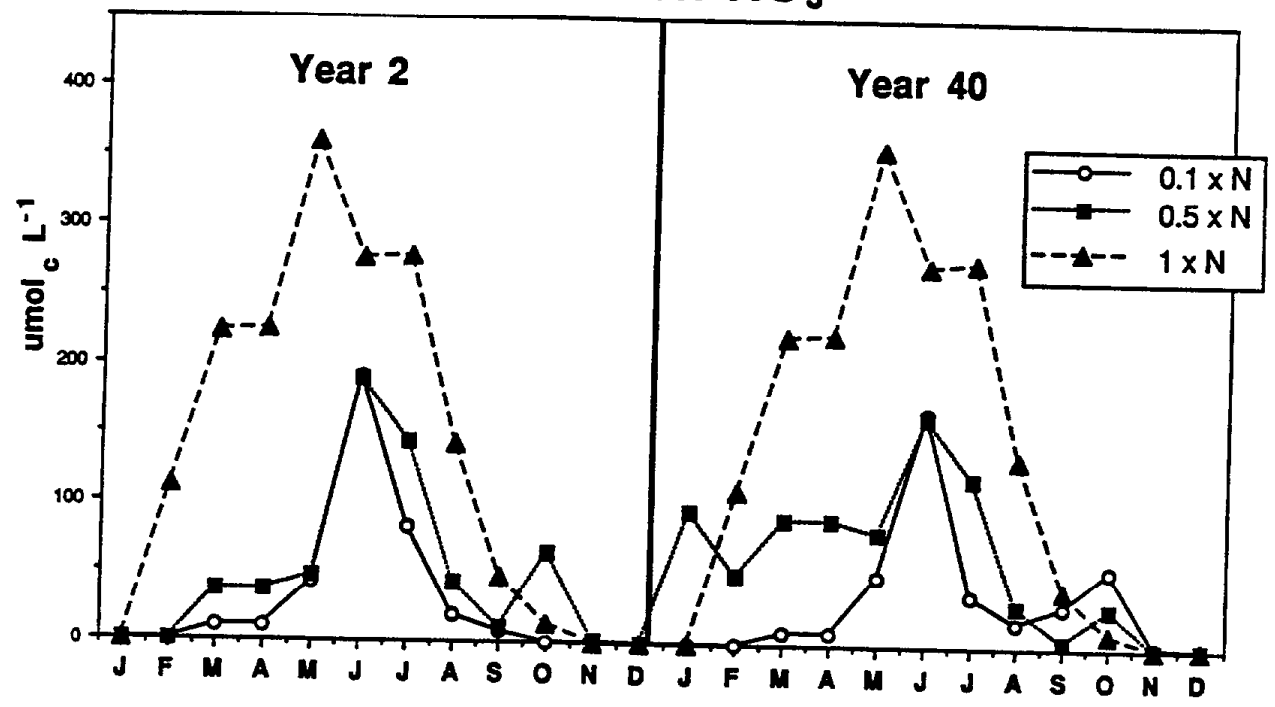

Solution NO_3^-
Snowmelt - Open
umol$_c$ L^{-1}
200
180
160
140
120
100
80
60
40
20
0
12/16
1/12
3/7
3/16
3/31
4/21
5/9
6/3
1993
1994

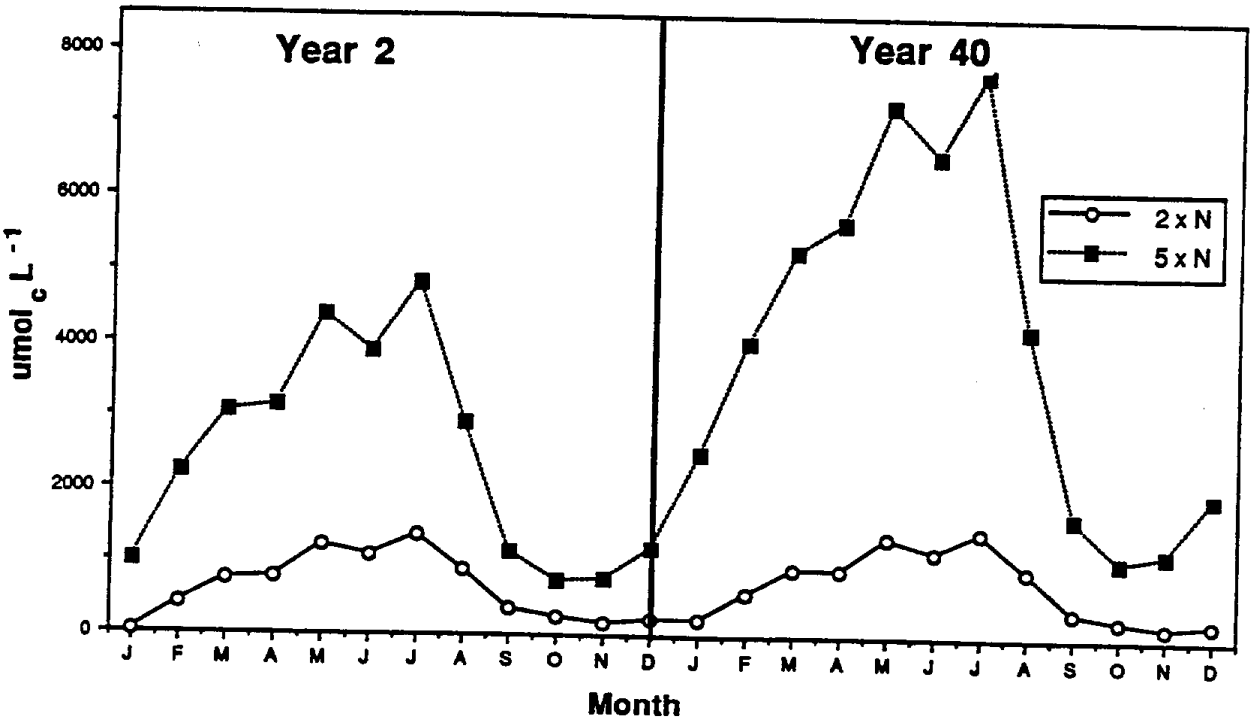

Snowmelt - Under Canopy
umol$_c$ L^{-1}
200
100
0
12/16
1/12
1/17
3/7
3/16
3/31
4/11
4/21
5/9
6/3
1993
1994


Soil Solution NO_3^-
200
100
0
umol$_c$ L^{-1}
15 cm
30 cm
3/31
4/11
4/21
5/9
6/3
1994

Soil Solution Concentrations

B Horizon NO_3^-





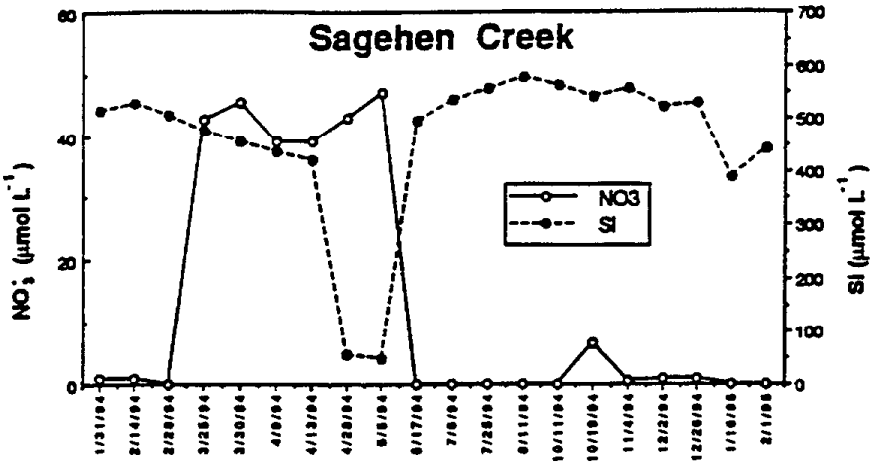


Figure 1. Nitrate and Si concentrations in Sagehen Creek.

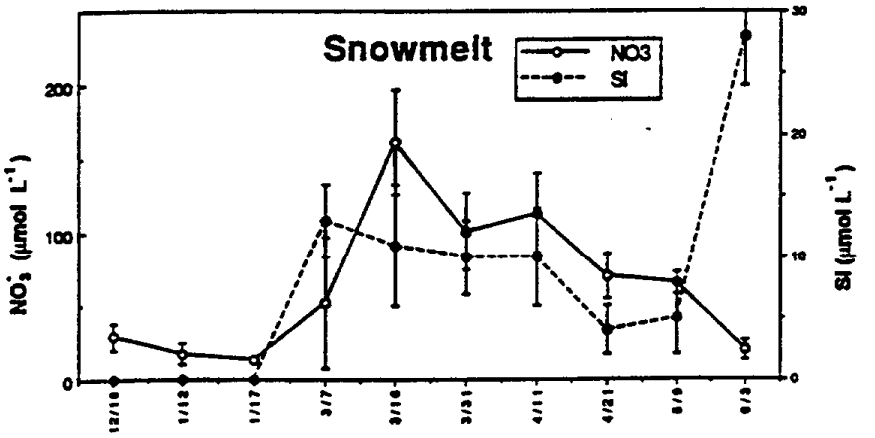


Figure 2. Nitrate and Si concentrations in snowmelt from a red fir plot at Sagehen.

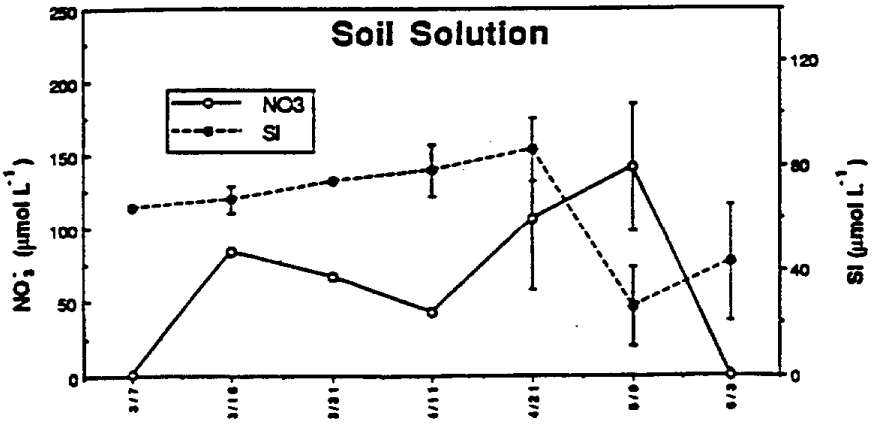


Figure 3. Nitrate and Si concentrations in soil solution (30 cm) at Sagehen.

5

Ayy 3

Summary and Conclusions (cont.)

Field studies from eastern Sierra Nevada:

Streamwater NO_3^- pulses during snowmelt at Little Valley and Sagehen

High NO_3^- concentrations in snowmelt and soil solution at Sagehen, but not at Little Valley

Hypotheses:

1) Temporal disconnect between snowmelt and uptake at Sagehen

2) Low P status at Sagehen

3) Species effect (red fir at Sagehen vs jeffrey pine at Little Valley)

APPENDIX 4

Simulated Effects of N Deposition Rates on Growth and Nutrient Cycling in a Ponderosa Pine Forest

Dale Johnson, Mark Poth, Mark Fenn, Paul Miller, and Andrzej Bytnerowicz

Simulated effects of N Deposition Rates on Growth and Nutrient Cycling in a Ponderosa Pine Forest

Dale Johnson, Mark Poth, Mark Fenn, Paul Miller, and Andrzej Bytnerowicz

Introduction

While N deposition rates in the semi-arid western US are typically low relative to the eastern US or Europe, there are notable exceptions. Fenn and Bytnerowicz (1993) report total N deposition rates ranging from 6 to 30.7 kg ha^{-1} $year^{-1}$ in the San Bernardino Mountains of southern California. Recent estimates of fog deposition suggest that total N inputs my exceed 50 kg ha^{-1} yr^{-1} at one site (Camp Pavika) (Fenn and Lenninger 1995). Elevated soil solution nitrate concentrations have also been found at several sites in the San Bernardino Mountains (Fenn and Poth, unpubl. data). In order to gain some understanding of the potential effects of varying N inputs on ponderosa pine ecosystems, we have calibrated the Nutrient Cycling Model (NuCM) for the Barton Flats intensive study site and run simulations of N inputs ranging from near pristine (1.9 kg ha^{-1} yr^{-1}) to heavily polluted (95.6 kg ha^{-1} yr^{-1}) levels.

Site and Methods

Field Site

The San Bernardino Mountains begin at the Cajon pass, which cuts between them and the San Gabriel Mountains, approximately 80 kilometers east of Los Angeles. The San Bernardino Mountains extend eastward for another 80 kilometers. Barton Flats, the site calibrated for these simulations, lies near the center of the mountain range. The elevation is 1946 m and mean annual precipitation of 540 mm (Arkley 1977, Fenn and Dunn 1989). Vegetation consists primarily of ponderosa pine (*Pinus ponderosa*) with occassional whte fir (*Abies concolor*) and oak (*Quercus kellogii*).

NuCM Model Calibration

NuCM is a stand-level nutrient cycling model developed as part of the Electric Power Research Institute's Integrated Forest Study (Liu et al 1991; Johnson and

Lindberg 1991). The forested ecosystem is represented as a series of vegetation and soil components. The model provides for both an overstory and understory, each of which can be divided into canopy, bole, and roots. Tree growth in the model is a function of user-defined stand developmental stage and the availability of nutrients and moisture. The model routes precipitation through the canopy and soil layers, and simulates evapotranspiration, deep seepage, and lateral flow. Nutrient pools associated with soil solution, the ion exchange complex, minerals, and soil organic matter are all tracked explicitly. The processes which govern interactions among these pools include user-specified rates for decay, nitrification, anion adsorption, cation exchange and mineral weathering.

NuCM was calibrated for the Barton Flats site using data collected from the site for vegetation and soil nutrient concentrations, soil and litter mass, and soil solution concentrations. At the time of this exercise, no data for biomass from the Barton Flats site was available, so the data on a ponderosa pine stand from Klemmedson (1975) was used. Procedures outlined in the User's Manual (Munson et al 1992) were used in the calibration; details are described elsewhere (Liu et al 1991; Johnson et al 1994).

Results

As expected, increases in simulated N deposition resulted in increases in simulated NO_3^- leaching[1] (Table 1). However, simulated NO_3^- leaching rates increased substantially only after N deposition rates of 2 x current rates or higher. Cumulative net ecosystem N retention was actually lower at 0.1 x N (63% of input) than at 0.5 x N or 1 x N (87 and 89% of input, respectively), even though NO_3^- leaching rates increased progressively with N deposition throughout these scenarios (Table 1). Apparently, the NO_3^- leaching rate at 0.1 x N was near a baseline level. Simulated vegetation and forest floor N contents increased progressively with N deposition from 0.1 to 1 x N, accounting for the retention of N in the ecosystem (Table 2). Between 0.1 and 1 x N, vegetation N content increased by 34.59 kmol ha^{-1} (86%), forest floor N increased by 6.54 kmol ha^{-1} (22%), and the sum of vegetation, forest floor and soil exchangeable N increased by 41.13 kmol ha^{-1} (59%) (Table 2). At N deposition levels of 2 x N and 5 x N, net

[1]Over 99% of total N leaching occurred as NO_3^-; thus, NO_3^- leaching can be equated with total N leaching for all practical purposes.

Table 1. Simulated net ecosystem balances of N, P, Ca, K, Mg, and S under varying N deposition scenarios. (kmol ha^{-1} 40 yrs^{-1})

Scenario	0.1 x N	0.5 x N	1 x N	2 x N	5 x N
Nitrogen					
Deposition	5.46	27.31	54.60	109.25	273.10
Leaching	2.02	3.47	5.65	49.11	203.44
Balance	3.44	23.84	48.95	59.62	69.65
% Retention	63%	87%	89%	55%	25%
Calcium					
Deposition	0.47	0.47	0.47	0.47	0.47
Leaching	5.20	3.67	4.07	8.46	30.15
Balance	-4.74	-3.20	-3.60	-7.99	-29.68
% Retention	-	-	-	-	-
Potassium					
Deposition	0.06	0.06	0.06	0.06	0.06
Leaching	7.19	5.54	5.86	6.61	10.61
Balance	-7.13	-5.48	-5.80	-6.54	-10.55
% Retention	-	-	-	-	-
Magnesium					
Deposition	0.09	0.09	0.09	0.09	0.09
Leaching	3.57	2.53	2.84	5.75	16.92
Balance	-3.48	-2.44	-2.75	-5.66	-16.83
% Retention	-	-	-	-	-

ecosystem retention decreased to 55 and 25% of input, respectively (Table 1). There were some increases in vegetation (8.58 kmol ha^{-1}, or 11%) and forest floor (1.52 kmol ha^{-1}, or 4%) N contents from 1 to 2 x N, but no further increases at 5 x N (Table 2). There was a large relative increase in exchangeable N from the 2 to the 5 x N scenarios (8.14 kmol ha^{-1}, or 16,380%) but the absolute magnitude of this increase was too small to cause a large increase in ecosystem N retention (the sum of vegetation, forest floor, and exchangeable N increased by only 7%, all in the form of exchangeable N) (Table 2).

The patterns of simulated leaching and N retention closely corresponded to those in growth. Simulated growth increased substantially from the 0.1 to 0.5 and 1 x N scenarios, indicating N deficiency at less than 1 x N input (Figure 1). Growth rate also increased slightly from the 1 to 2 x N scenarios, but there was no further growth increase from the 2 to the 5 x N scenario.

Increases in N deposition from 0.1 to 1 x N caused unexpected reductions in simulated base cation (K^+, Ca^{2+}, Mg^{2+}) and ANC leaching rates (Figures 2 and 3). This was due to complicated set of chemical interactions which resulted in lower total anion concentrations with increasing N deposition from 0.1 to 1 x N during winter months (October through February) when most leaching occurred. Figures 4 and 5 illustrate these interactions on a seasonal basis for years 2 and 40 of the simulations. Although summertime soil solution NO_3^- concentrations increased with increasing N deposition from 0.1 to 1 x N (Figure 4), this had little effect upon leaching rates because water flux was low during this period. Soil

Table 2. Simulated nutrient distribution after 40 years under varying N deposition scenarios. (kmol ha^{-1} and kmol ha^{-1} yr^{-1})

Scenario	0.1 x N	0.5 x N	1 x N	2 x N	5 x N
N Deposition	**0.14**	**0.68**	**1.37**	**2.73**	**6.83**
Nitrogen					
Vegetation	39.97	53.96	74.56	83.14	83.14
Forest Floor	29.62	33.57	36.16	37.68	37.68
Soil, Exch.	≤0.01	≤0.01	≤0.01	0.05	8.19
Total	69.59	87.53	110.72	120.87	129.01
Calcium					
Vegetation	17.93	21.68	23.96	30.68	31.82
Forest Floor	3.82	4.29	4.08	4.75	4.87
Soil, Exch.	191.30	188.70	186.40	174.90	152.80
Total	213.05	214.67	214.44	210.33	189.49
Potassium					
Vegetation	10.97	13.52	14.93	18.91	19.46
Forest Floor	7.46	8.70	8.13	9.97	10.39
Soil, Exch.	19.41	16.68	15.98	8.54	3.69
Total	37.84	38.90	39.04	37.42	33.54
Magnesium					
Vegetation	5.90	7.39	8.11	10.68	10.68
Forest Floor	2.36	2.67	2.52	2.96	2.96
Soil, Exch.	43.94	43.57	42.95	38.16	28.79
Total	52.20	53.63	53.58	51.80	42.43

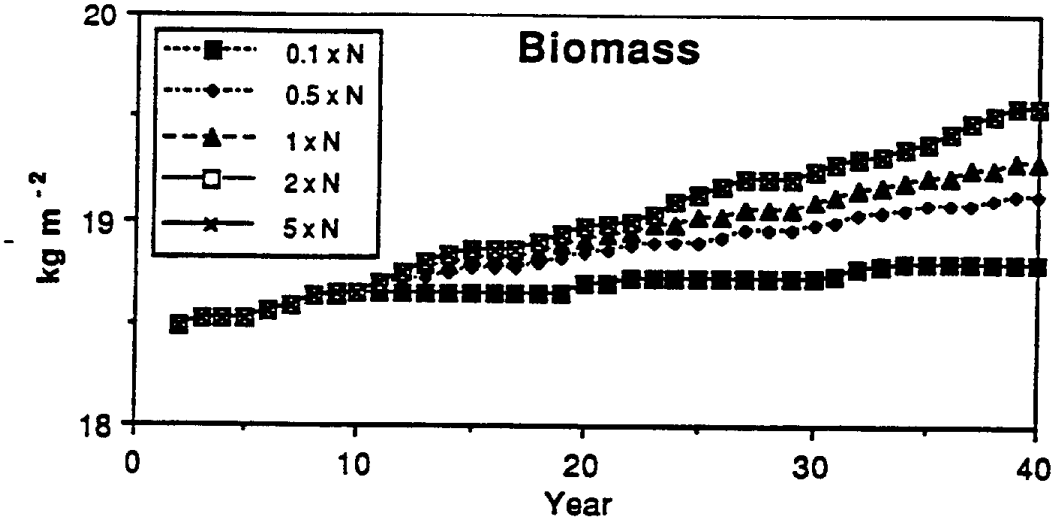


Figure 1. Simulated biomass under various N deposition scenarios at Barton Flats.

solution pH was reduced with increasing N deposition (Figure 5). The pH reductions were due to a combination of reduced base saturation (especially later in the simulation; Figure 6) and the "salt effect", whereby increases in mineral acid anion concentration (NO_3^- in this case) cause H^+ concentration to increase (Reuss and Johnson 1986). These reductions in soil solution pH directly caused reductions soil solution HCO_3^- and indirectly caused reductions in soil solution SO_4^{2-} because of increased pH-dependent SO_4^{2-} adsorption (not shown). The net effect was a reduction in soil solution total anion and cation concentrations with increasing N deposition from 0.1 to 1 x N during the winter months.

Although K^+, Ca^{2+}, and Mg^{2+} leaching rates decreased from 0.1 to 1 x N for the reasons noted above, soil exchangeable K^+, Ca^{2+}, Mg^{2+} pools decreased continuously from 0.1 to 5 x N deposition (Table 2). The decreases from 0.1 to 1 x N were due to uptake and accumulation in vegetation, which offset the reductions in leaching. As N deposition rates increased from 1 to 5 x N, base cation leaching rates increased again, and at 5 x N, there were large decreases in soil exchangeable base cation pools (Tables 1 and 2). The decrease in exchangeable pools was much larger for K^+ (decreased by 77% from 1 to 5 x N), than for Ca^{2+} and Mg^{2+} (decreased by 18 and 23%, respectively, from 1 to 5 x N) (Table 2).

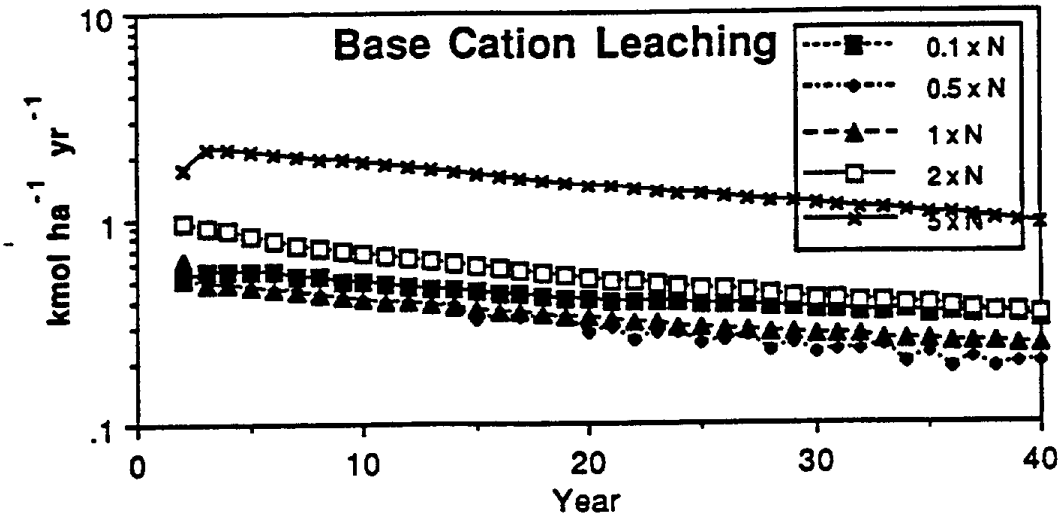


Figure 2. Simulated base cation leaching under various N deposition scenarios at Barton Flats.

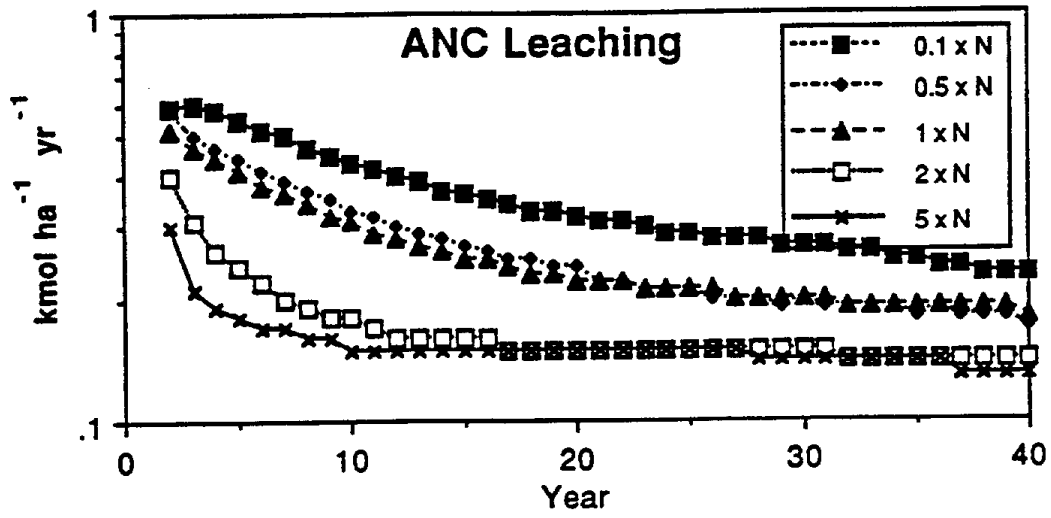


Figure 3. Simulated ANC leaching under various N deposition scenarios at Barton Flats.

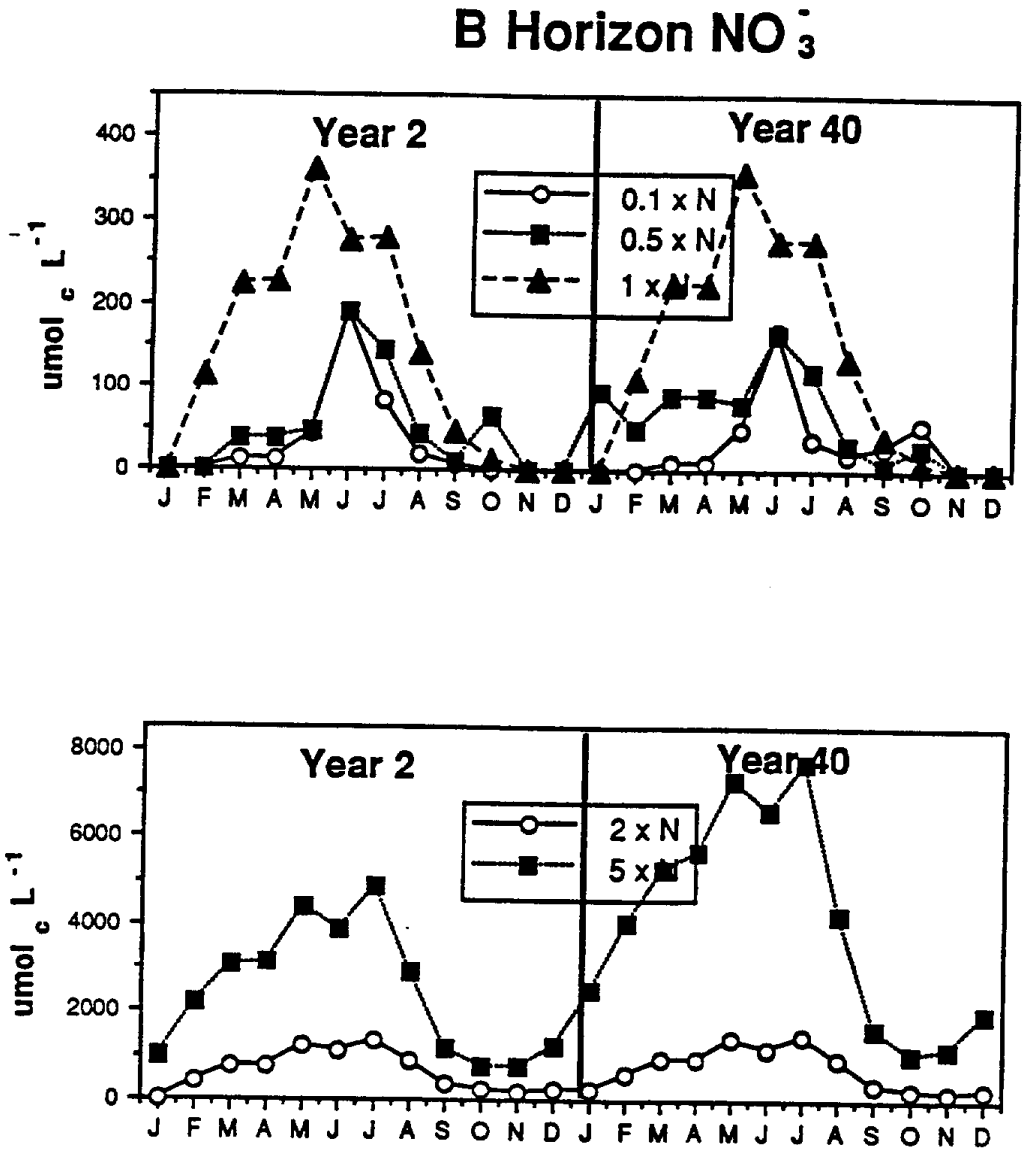


Figure 4. Simulated B horizon nitrate concentrations in the Barton Flats site.

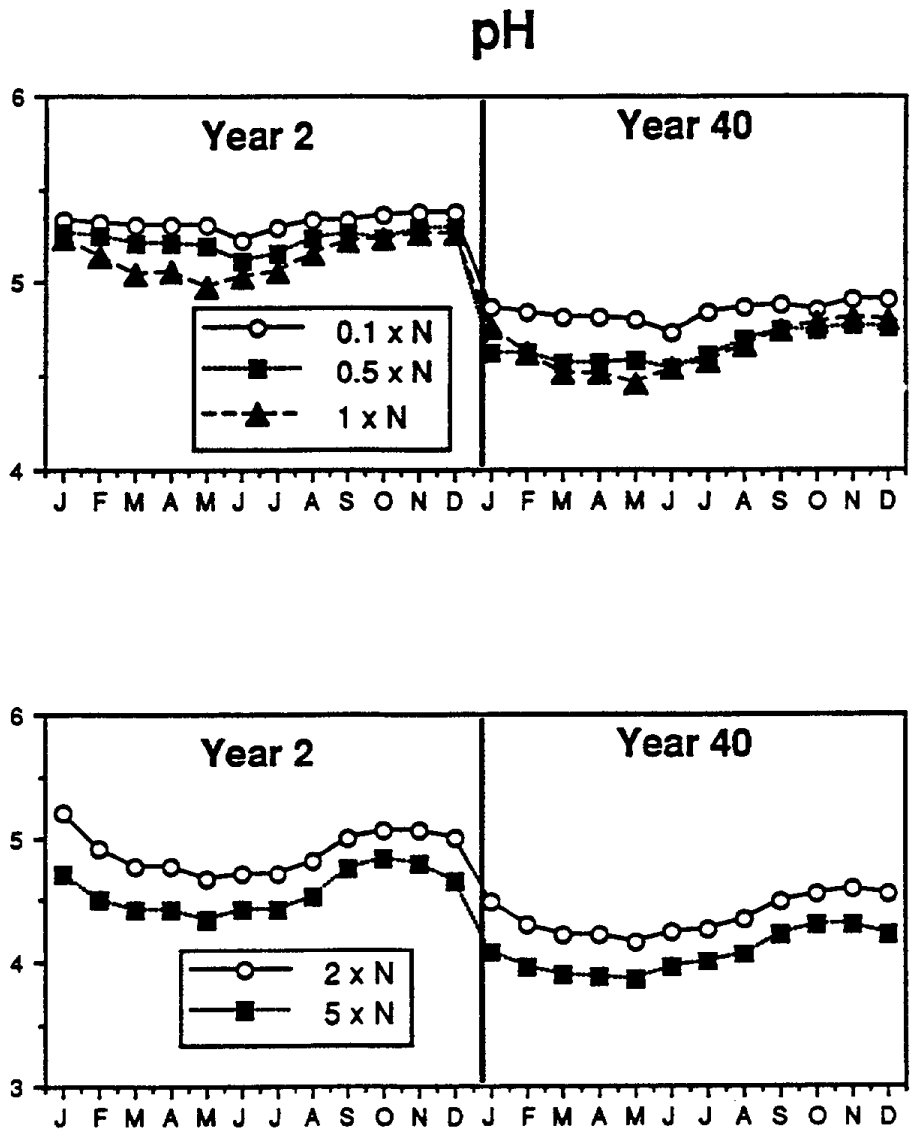


Figure 5. Simulated B horizon pH in the Barton Flats site.

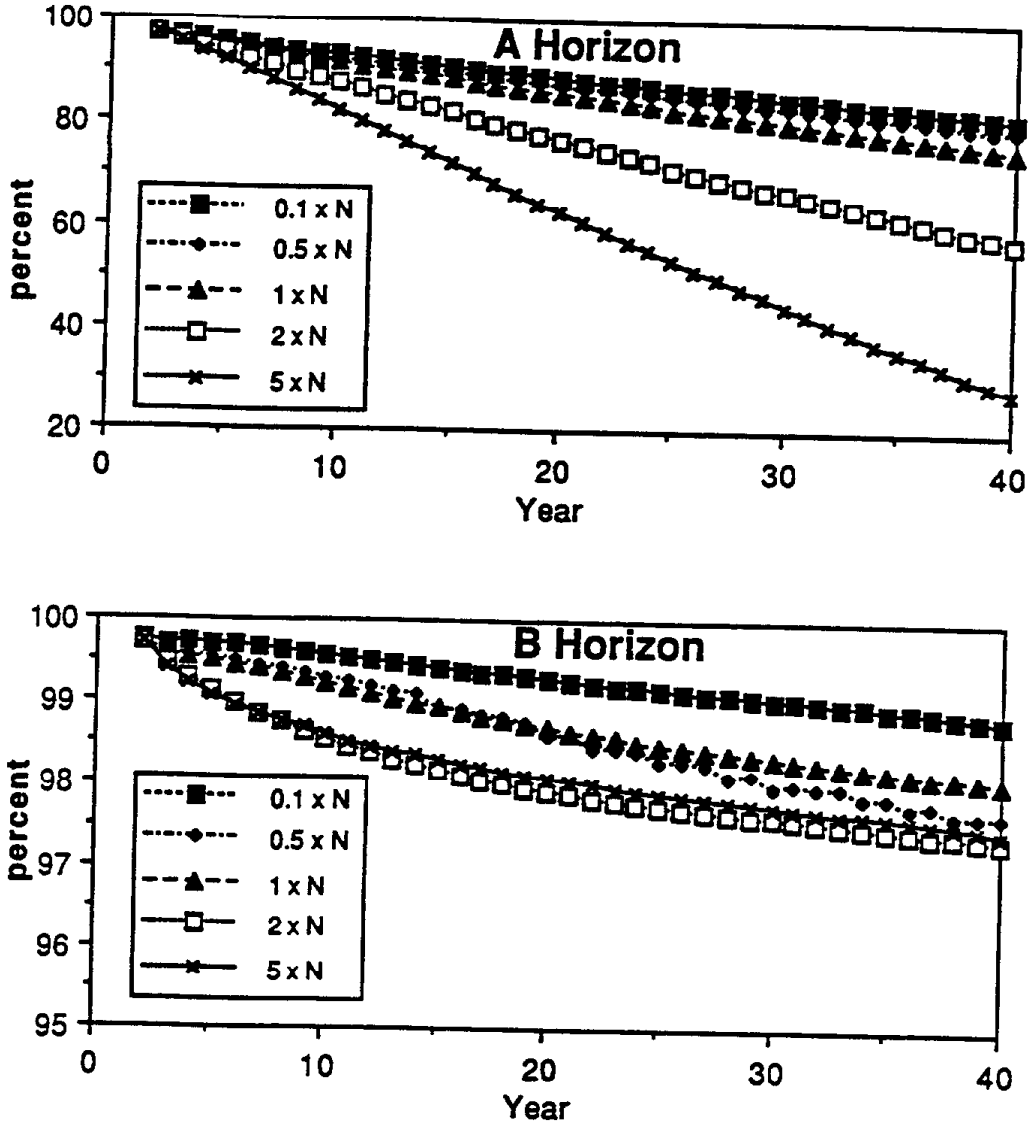


Figure 6. Simulated base saturation under various N deposition scenarios at Barton Flats.

Discussion

The NuCM simulations for Barton Flats produced some results that were expected and some that were unexpected. The reductions in N retention and increases in NO_3^- leaching with increasing N deposition were certainly expected; the only question was at what levels of N deposition the effects would occur. There was a continuous increase in NO_3^- leaching with N deposition, but the leaching rates were relatively low until N deposition increased beyond 1 x N (19.2 kg ha^{-1} yr^{-1}). At some level between 2 and 5 x N deposition (38.4 and 95.6 kg ha^{-1} yr^{-1}), growth responses to N ceased and N retention remained constant. Thus, N "saturation" occurred at some point above 38.4 kg ha^{-1} yr^{-1}, if N "saturation" is defined as the inability to accumulate further N. Below 38.4 kg ha^{-1} yr^{-1}, a pronounced N deficiency occurred, as would also be expected as the norm in less polluted semi-arid forests of this type. The threshold at which N "saturation" occurs in a given simulation will vary with input values for vegetation N uptake (which in turn are determined by the maximum allowable growth and N concentrations in trees) and the degree of soil N retention. In the absence of any further data for these parameters in varying N deposition regimes, we have left them constant.

Although growth response to N occurred up through 38.4 kg ha^{-1} yr^{-1}, soil solution NO_3^- concentrations peaked at relatively high midsummer values, especially in the high N deposition scenarios, but also in the lowest N deposition scenario. These peak NO_3^- concentrations at low N deposition would pose no serious threat for ground- or surface water pollution, however, because of the lack of water movement at that time. Field data recently collected from various sites in the San Bernardino Mountains confirm that summertime soil solution NO_3^- concentrations are very high (thousands of μmol_c L^{-1}) in the more polluted sites (Fenn and Poth, unpubl. data).

The reductions in Ca^{2+}, Mg^{2+}, and K^+ leaching to reduced N deposition rates were unexpected. These reductions were an indirect result of complicated interactions among soil solution pH, base saturation, NO_3^-, SO_4^{2-} and HCO_3^- concentration which could only have been explored through simulation modeling. The general direction of the responses in soil solution pH, SO_4^{2-}, and HCO_3^- concentration are of course consistent with the soil chemical theory built into the model, but the magnitudes of the responses and their interactions with NO_3^- and

base cations were not intuitively predictable, very interesting, and worthy of testing either with field or laboratory column studies.

Summary and Conclusions

In summary, the NuCM simulations for Barton Flats suggest the following:

1. Reductions in N deposition to the Barton Flats site will result in the development of N deficiency, whereas increases will result in N saturation and groundwater pollution of NO_3^-.

2. N saturation will lead to K deficiencies because of large reductions in soil available pools. However, this cannot be given as a prediction because of the unknown rates of K weathering from soil primary minerals, for which there is no data. Increasing K weathering rates in the model would greatly slow the depletion of soil exchangeable K^+ pools.

3. Reducing N deposition rates below 19.8 kg ha-1 yr-1 would cause increases in the rate of base cation leaching from soils due to increases in soil solution HCO_3^- and SO_4^{2-} concentrations. This non-intuitive result could be tested with laboratory column studies.

4. Soil exchangeable K^+, Ca^{2+}, Mg^{2+} pools decreased continuously from 0.1 to 5 x N deposition due to uptake and accumulation in vegetation at the low end of the scale and to increased leaching at the high end of the scale.

5. Summertime soil solution NO_3^- concentrations become very high (> 6,000 $\mu mol_c\ L^{-1}$) with high N deposition rates, and are also significantly elevated even under low N deposition rates.

Given the uncertainties inherent in both the model formulation and the data used to calibrate it, we must advise the reader not to use these simulation results as quantitative predictions of what specific response will occur at a specific N deposition load (for example, we cannot state that soil solution NO_3^- will increase to 100 $\mu mol_c\ L^{-1}$ at an N loading of 1.37 kmol $ha^{-1}\ year^{-1}$). Instead, we offer the results of these simulations as indicators of what is expected with

changing N loadings based upon our best current knowledge of nutrient cycling within this ecosystem. From a scientific perspective, the results of these simulations present some interesting hypotheses that could be tested in either a field, or in some cases, a laboratory setting.

Acknowledgments

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APPENDIX 5

Nutrient Cycling in Forest of the Eastern Sierra Nevada

Dale W. Johnson, Randy Dahlgren, Andrzej Bytnerowicz,
and Virginia Boucher

NUTRIENT CYCLING IN FORESTS OF THE EASTERN SIERRA NEVADA

Dale W. Johnson, Randy A. Dahlgren, Andrzej Bytnerowicz, and Virginia Boucher

INTRODUCTION

In comparison to more humid regions, there is a paucity of information about nutrient cycling in forests of arid and semi-arid regions. There are several reasons to increase our knowledge of nutrient cycles in forests of the eastern Sierra Nevada. First, this knowledge would allow intelligent assessments of forest management practices such as harvesting, burning, and site preparation as well as the effects of exogenous influences such as fire, air pollution, and climate change on these forests. Second, these forests act as filters for nutrients, especially nitrogen, which might otherwise enter the sensitive surface waters of the region. The long-term deterioration of water quality due to increased nutrient inputs to Lake Tahoe has been clearly documented (Goldman, 1981). These nutrient inputs are thought to result primarily from increased land development and associated erosion, with additional contributions from atmospheric deposition and N-fixation by riparian mountain alder (Alnus tenuifolia) (Coats et al 1976; Leonard et al 1980; Byron and Goldman, 1989). With N and H^+ inputs increasing in the Tahoe Basin (Byron et al 1991), it is important to gain some knowledge of the capacity of forest ecosystems in the basin to filter these inputs. At the same time that atmospheric inputs are increasing, the capacity of forests to retain N has likely been reduced due to substantially increased mortality and fires, in part due to drought and insect attacks.

RESEARCH RESULTS TO DATE

Field research to date (funded primarily with Hatch funds through the Nevada Agricultural Experiment Station, with co-funding from NSF's EPSCoR program and from the National Council of the Paper Industry for Air and Stream Improvement, Inc.) has been concentrated in forests in Little Valley, NV and Sagehen Creek Watershed, CA. The Little Valley site has an overstory of jeffrey pine (Pinus jeffreyi) with an occasional white fir (Abies concolor). Understory vegetation was absent. Mean annual temperature near the valley floor is 5° C and mean annual precipitation is 550 mm,

50% of which falls as snow. Soils are classified as the Corbett series, typic frigid Xeropsamments derived from granite. The Sagehen site has an overstory of 80-160-year-old California red fir (Abies magnifica) with occasional white fir (Abies concolor) and an understory of pinemat manzanita (Arctostaphylos nevadensis) with Ribes and Wyethia spp. Elevation at the site is 2100 m. Mean annual precipitation (MAP) and temperature (MAT) for site are not known; values for Sagehen field station at lower elevation are 4.8° C for MAT and 870 mm for MAP, more than half of which is in the form of snow. Soils were classified as the Waca series, loamy-skeletal, mixed, frigid Andic Xerochrepts derived from andesitic lahars and tuff.

In Little Valley, we followed up the studies of Coats et al (1976) in the Tahoe Basin and investigated the effects of N fixation by both mountain alder and snowbush (Ceanothus velutinus) on soils and soil solutions in comparison to jeffrey pine. (No previous studies have been conducted on soil solution chemistry beneath snowbush.) In contrast to the results of Coats et al (1976), we found no evidence of excess NO_3^- beneath either mountain alder or snowbush; soil solution NO_3^- concentrations were near trace levels under all three species (Table 1) (Johnson 1985). There was, however, a sizable NO_3^- peak in streamwaters from nearby Franktown Creek during snowmelt (Figure 1, top). More recent studies in a red fir stand at Sagehen Creek, CA showed a similar peak in streamwater NO_3^- during snowmelt (Figure 1, bottom), as well as elevated NO_3^- concentrations in both snowmelt and soil solutions (Table 1)[1]. Indeed, the soil solution NO_3^- concentrations at Sagehen were nearly as high as in a polluted red spruce site in the Great Smoky Mountains of North Carolina (Johnson et al 1991). As previously noted by Berg (1992), snowmelt is a significant source of the NO_3^-, which can then either enter soils or runoff directly to streamwaters. At Sagehen, there were pulses of both NH_4^+ and NO_3^- during snowmelt which were reflected in both soil solution[2] and streamwater (Figures 1 and 2). Only in June, when snowmelt NH_4^+ and NO_3^- concentrations decreased and biological N uptake presumably commenced, did soil solution NO_3^- drop to levels typical of N deficient ecosystems.

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[1] Only one snowmelt collection was made at Little Valley at the end of an unusually large snowpack accumulation in the winter of 1992 -93, and it had NO_3^- concentrations ranging from <1 to 11 $umol_c\ L^{-1}$. Unfortunately, limited funding precluded analyses of Little Valley solutions for NH_4^+ or DON.

[2] Space limitations preclude showing NH_4^+ in soil solutions in Figure 2; however, soil solution NH_4^+ concentrations closely paralleled NO_3^- concentrations.

We hypothesize that the reasons for the differences in soil solution NO_3^- between the Little Valley and Sagehen sites is due to higher elevation, which in turn results in higher N inputs and later initiation of N uptake by vegetation. Initial samples for air

Table 1. Soil Solution and stream pH, HCO_3^-, NO_3^-, and SO_4^{2-} concentrations. (Standard errors are given.)

	pH	NH_4^+	NO_3^-	SO_4^{2-}
		———	$umol_c\ L^{-1}$	———
Little Valley, NV*				
Pine	7.1±0.1	N.D.	<0.5	273±46
Ceanothus	6.7±0.1	N.D.	3±3	107±15
Mountain Alder	7.0±0.2	N.D.	<0.5	181±59
Franktown Creek	7.1±0.04	N.D.	3±2	3±0.2
Sagehen, CA				
Snowmelt	6.8±0.03	32±5	33±4	55±8
Red fir soil solution	7.0±0.1	68±3	73±12	21±3
Sagehen Creek	7.0±0.2	34±26	11±5	3±0.3

* Data from Johnson (in press)

Table 2. Soil extractable P (NH_4F/HCl) in Little Valley (jeffrey pine) and Sagehen (red fir) soils. (Standard errors are given.)

Little Valley*		Sagehen	
Horizon and depth (cm)	Extr. P ($\mu g\ g^{-1}$)	Horizon and depth (cm)	Extr. P ($\mu g\ g^{-1}$)
A (0-20)	101±21	A (0-10)	<0.5
BA (20-40)	106±36	B (10-18)	<0.5
BC (40-60)	69±12	BC (18-45)	<0.5

* Data from Johnson (in press)

quality show that concentrations of ammonia (NH_3), Nitric acid vapor (HNO_3), and particulate nitrate (NO_3^-) were about 3 times higher at the Little Valley site compared with the Sagehen site. The concentrations at the Little Valley site were similar to the

values at the mixed coniferous forest locations near Kings Canyon, western Sierra Nevada (Bytnerowicz and Riechers, in press). HNO_3 concentration at Sagehen wAS about 2 times higher than the average summer concentration at the clean site of the eastern Sierra Nevada (Miller and Walsh, 1991). We hypothesize that initial snowmelt solutions at Little Valley also had high NH_4^+ and NO_3^- concentrations, but that N was taken up by the forests of Little Valley, which were becoming biologically active at the time of snowmelt, whereas the colder, higher-elevation red fir stands were not yet actively taking up N at this time.

Another major contrast between the Little Valley and Sagehen sites is with respect to soil available P status. Little Valley soils have extractable P (0.5 M HCl/1 M NH_4F; Olson and Sommers, 1982) levels that are an order of magnitude greater than those typical of more humid region forest soils (Johnson and Lindberg, 1991), whereas extractable P in the Sagehen soils below detection limits (Table 2). We hypothesize that the reason for this difference lies in the soil P adsorption capacity, which in turn is a function of soil parent material (decomposed granite in Little Valley, andesitic lahar at Sagehen).

Clearly, snowpack rather than soils are the dominant sources of NO_3^- and NH_4^+ in both soil solution and streamwater. We hypothesize that there is a temporal discoupling between N release from snowpack and biological uptake in high elevation fir forests of the eastern Sierra Nevada. When soil moisture conditions are most favorable for biological activity (winter/spring), the soil and air temperatures are limiting; when soil and air temperatures are most favorable (summer) for biological activity, soil moisture conditions are limiting. Thus, there are only brief periods (e.g., following melting of the snow pack and fall period before snow pack accumulation) when biological activity is not severely inhibited by temperature and moisture conditions. Because of this temporal discoupling, we hypothesize that these high elevation systems are incapable of taking up snowmelt-produced NO_3^- and NH_4^+ because these input pulses occur before active N uptake begins. Lower elevation pine forests are able to take up snowmelt NO_3^- and NH_4^+ because they are more active at the time of NO_3^- and NH_4^+ release.

We hypothesize that the Andic soils at Sagehen have a high P adsorption capacity which has caused a potential P limitation and slow rates of P cycling compared to Little Valley.

A proposal has been submitted to the USDA Competative Grants program to test these hypotheses.

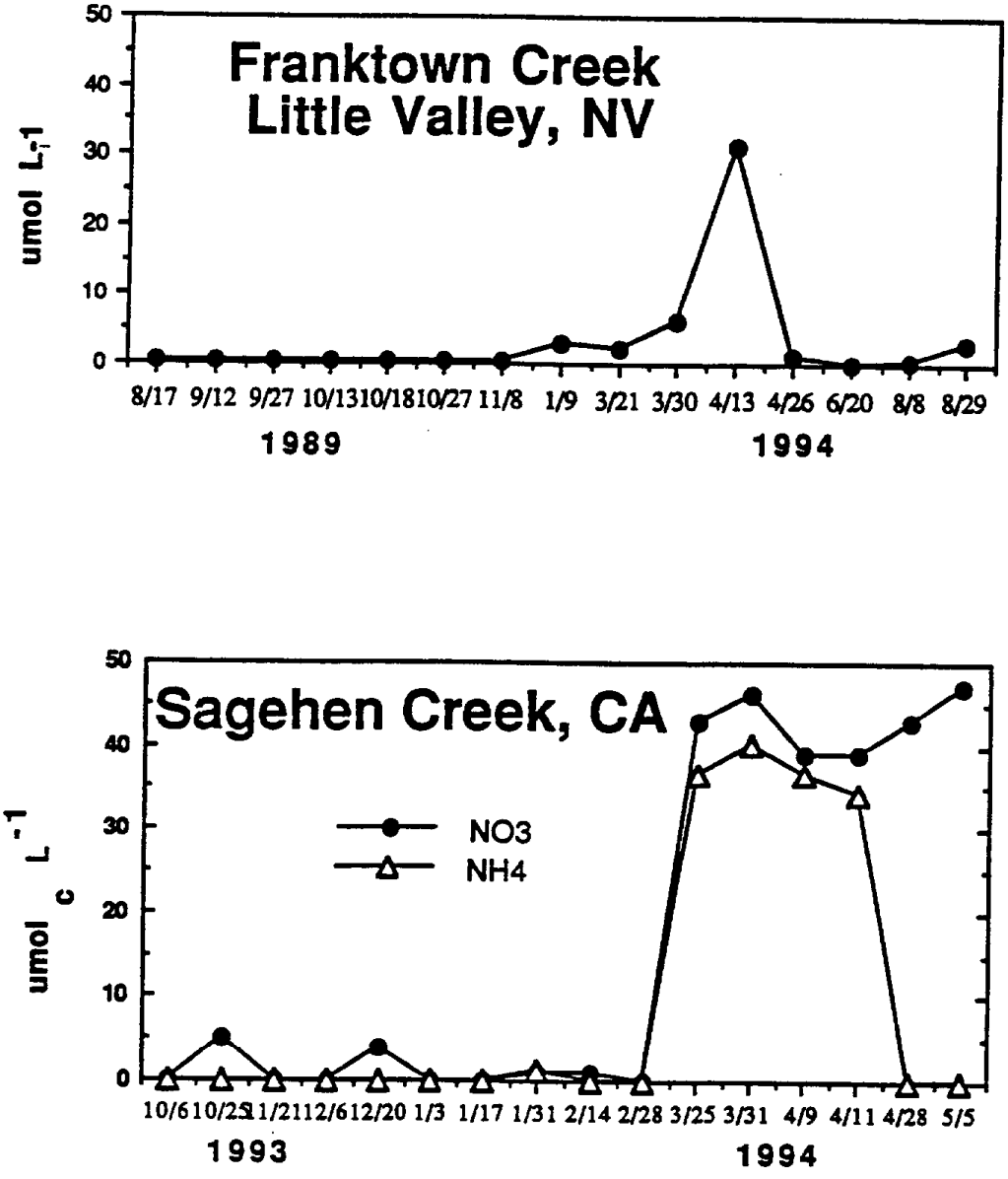

Figure 1. Streamwater NH_4^+ and NO_3^- concentrations in Franktown Creek in Little Valley, NV and Sagehen Creek, CA

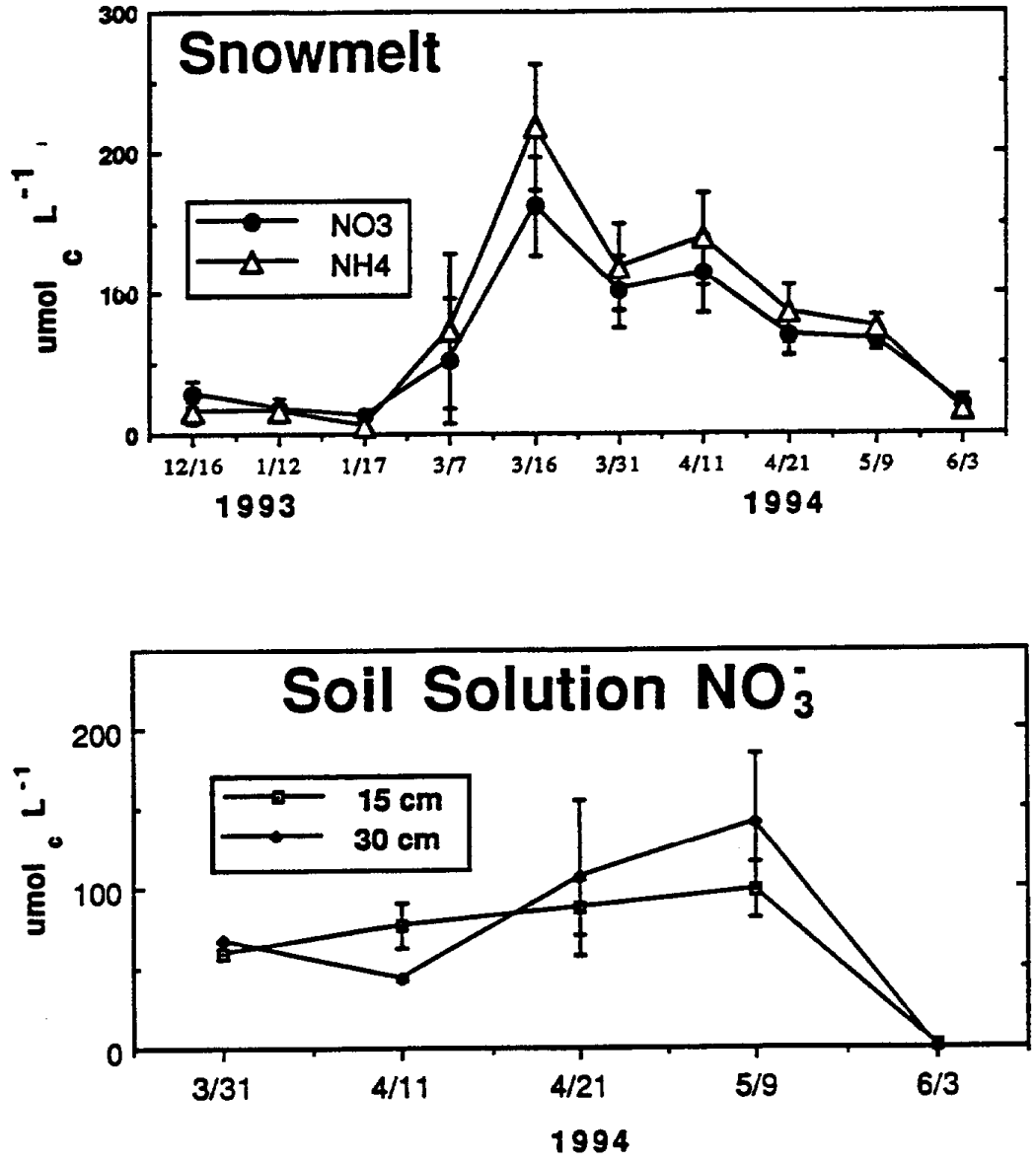

Figure 2. Snowmelt NO_3^- and NH_4^+ (top) and soil solution (bottom) NO_3^- concentrations at Sagehen. Space limitations preclude showing NH_4^+ in soil solutions; however, soil solution NH_4^+ concentrations closely paralleled NO_3^- concentrations.

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