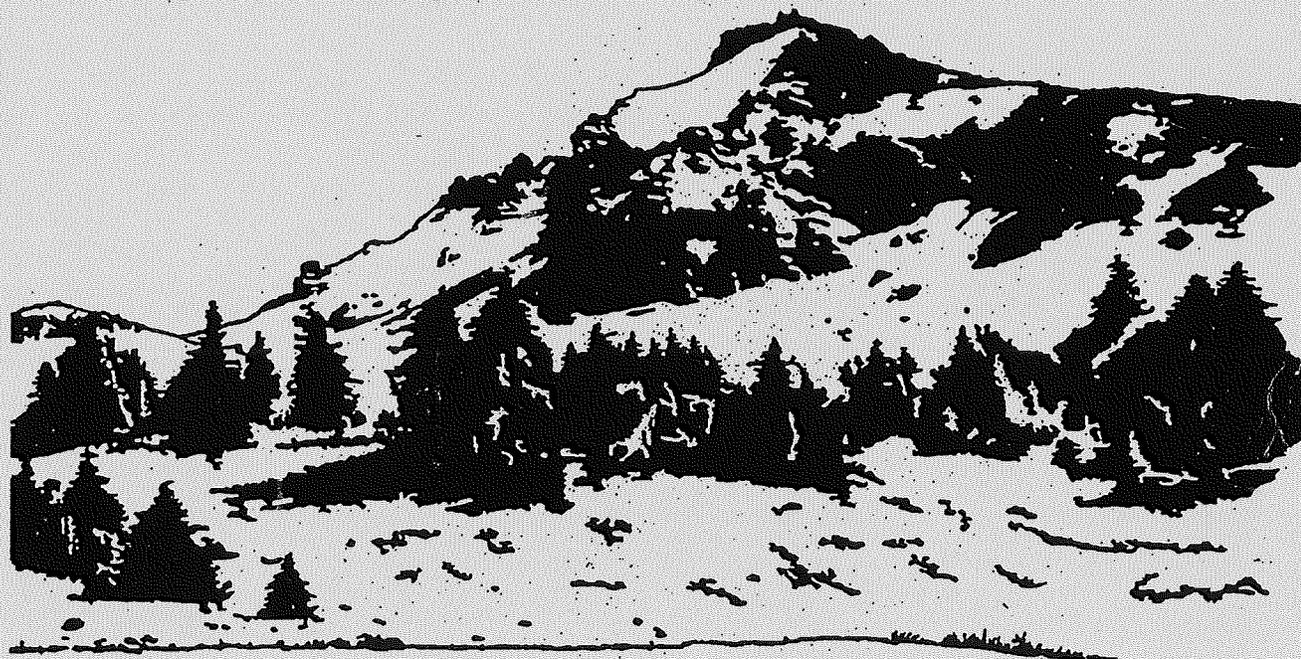


Ecological Effects of Stocked Trout in Naturally Fishless High-Elevation Lakes, North Cascades National Park Service Complex, WA, USA: Phase II

William J. Liss, Gary L. Larson, Torrey J. Tyler, Lisa Ganio,
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Technical Report
NPS/CCSOOSU/NRTR-98/01



United States Department of Interior - National Park Service
Columbia Cascades System Support Office

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April 1999
NPS/CCSOOSU/NRTR-98/01

CA-9000-8-0006
Subagreement 11

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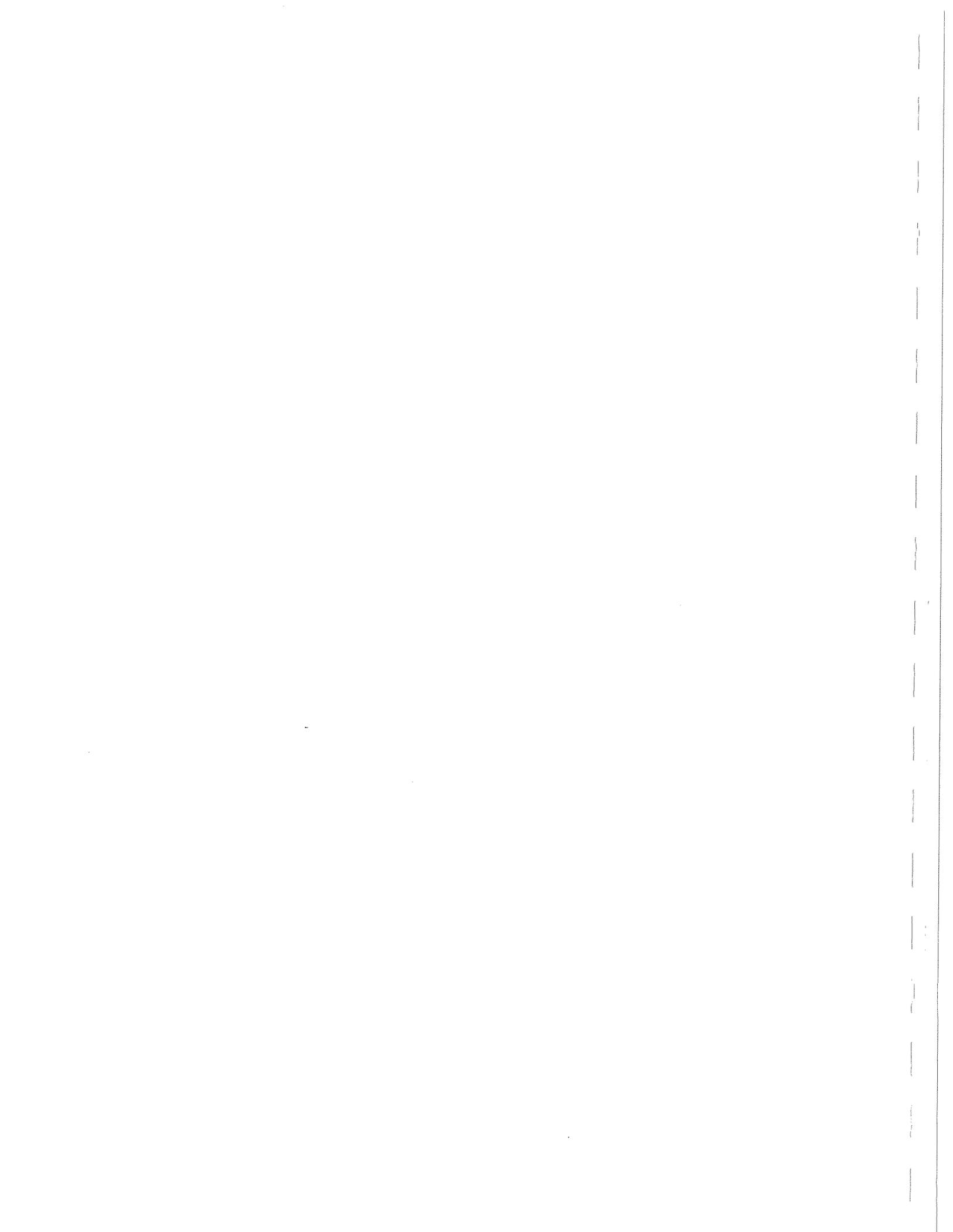
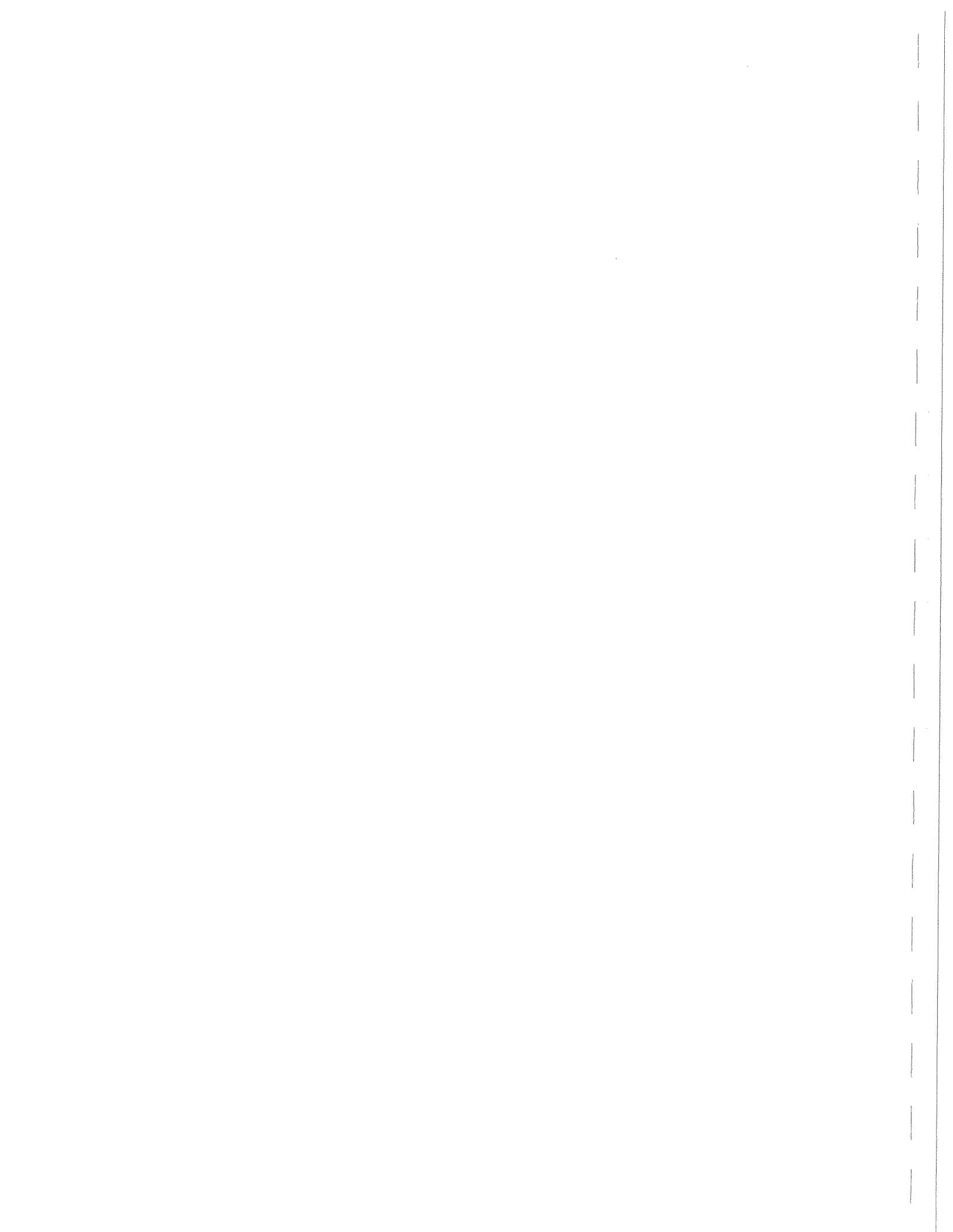


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INTRODUCTION

This research project was initiated in response to a directive from the Director of the National Park Service (NPS) on June 12, 1986, which was related to the stocking of trout into lakes of North Cascades National Park Service Complex (NOCA). The directive was an official response to a January 1986 request from the NPS Pacific Northwest Regional Office (PNRO) for a clear statement regarding fish-stocking policies of the NPS, i.e., stocking of non-native animals, including fish, is not allowed in units of the NPS (NPS 1991). The main elements of the directive included establishment of current fish and aquatic habitat baseline conditions, evaluation of the impacts of fish stocking on fish and wildlife, and determination of changes over time referenced against baseline conditions or against undisturbed conditions. Although mountain lakes in the area now designated as NOCA were originally devoid of fish, the Washington Department of Wildlife had stocked 75 of the 150 lakes thought to be able to support fish populations prior to the establishment of the park in 1968. Stocking was allowed to continue in the two recreational areas of the park between 1968 and 1974. In 1975, PNRO proposed to exempt certain restrictions on some lakes and suggested a gradual phasing-out of stocking in the park. The park and the State of Washington co-signed a variance to the NPS policy of no fish stocking in 1979 so that selected lakes could continue to be stocked with non-native trout at regular intervals in selected lakes. NOCA drafted a new memorandum of understanding (MOU) in 1985 in which the policy variance was dropped. This proposal resulted in considerable debate by the public and Congress. A supplemental agreement to the 1985 MOU was prepared in 1988 and specified the stocking levels of selected lakes through the year 2000. As part of this agreement and after completion of the present research program, the park will prepare an environmental assessment and high-elevation lake management plan by 2000.

The first phase of this research project (1989 - 1991) investigated ecological impacts of stocked trout on naturally fishless lakes in NOCA. Very little was known about the aquatic resources in the park prior to initiation of this research. Results indicated that larval salamanders (*Ambystoma macrodactylum*), the top native vertebrate predators in many fishless lakes in NOCA, were at significantly lower densities in lakes with fish as compared to their densities in lakes without fish. However, larval densities in fishless lakes were variable, which suggested

that abiotic factors were influencing larval abundance. Furthermore, effects of introduced trout on the limnetic food web appeared to be influenced by trout density and size structure and lake nutrient levels. In general, reproducing trout populations maintained higher densities and a more complex age and size structure than did non-reproducing trout populations in lakes that had to periodically stocked with fry. In lakes east of the hydrological divide, densities of the large-bodied and ubiquitous copepod *Diaptomus kenai* were significantly lower in lakes with reproducing fish than in lakes with non-reproducing trout. In small and shallow lakes with elevated nutrient levels, the small-bodied copepod *D. tyrrelli* maintained higher abundances in lakes with high fish densities where large-bodied copepods were absent. Only three of the 15 nearshore macroinvertebrates taxa were found in significantly fewer lakes with vertebrate predators (salamanders and trout) than in lakes with no vertebrate predators. The distribution of one of these taxa, *Desmona*, may be restricted solely by trout predation. The distribution and abundance of rotifer taxa appeared largely unrelated to vertebrate predators.

A panel of aquatic ecologists has peer reviewed the program on an annual basis since 1989. Based on the panel's recommendations, five small and shallow lakes with non-reproducing trout populations (two in the subalpine and three in the forest zone) were selected for manipulation (see below). The lakes had low densities of large-bodied trout. These lakes were sampled during the 1990 field season. Toward the end of the season, the fish were removed by extensive gill-netting and angling. All but one of the lakes was restocked with trout fry in densities comparable to those used by the Washington State Department of Wildlife.

A proposal for this phase of the project (Phase II) was prepared and submitted in 1991. The main objectives were to:

1. Increase our understanding of the interactions between salamanders and stocked trout by:
 - A. Investigating salamander distribution and abundance relative to the presence and absence of trout in major lake ecosystem types in the park;
 - B. Investigating ecological conditions (elevation, aspect, lake morphology, bottom composition, water quality, phytoplankton, zooplankton, and benthos) that influence the occurrence and abundance of salamanders;
 - C. Estimating the proportion of total juvenile salamander mortality attributable to predation by trout.
2. Evaluate changes in community structure and organization in manipulated and control lakes by:
 - A. Determining for each lake, the structure of pelagic (crustacean zooplankton, rotifers,

and phytoplankton) and benthic (benthic macroinvertebrates) systems;

B. Determining for each lake, the abundance, size (length) structure, and diet of trout;

C. Determining for each lake, the species composition, abundance of aquatic life stages, and habitat utilization of salamanders.

Unfortunately, funding for Phase II of the project was delayed until FY1993. For this reason, only a limited amount of funding was available in FY1992. Carry-over funds from Phase I and supplemental funding from NOCA and the PNRO were used to continue data analyses and to sample each manipulated lake twice during the field season. Funding was received in FY1993 and we completed a full field season. In FY1994, we had planned a full field season that included resampling of the manipulated lakes and an emphasis on sampling lakes with non-reproducing trout populations. However, extensive forest fires in the park constrained the field season and few lakes with non-reproducing trout populations were sampled. During the last year of the Phase II project (FY1995), only limited funding was available and we emphasized resampling of the manipulated lakes and preliminary laboratory experiments of trout-salamander interaction; the latter being a recommendation of the peer review panel. In FY1996, a limited amount of carry-over funds and supplemental funding from NOCA were available and we decided to complete the laboratory trout-salamander interaction experiments and resample the manipulated lakes.

Because we were able to only complete one full field season during the period from 1992 to 1995, we re-evaluated the objectives of the Phase II project and the content of the final report. We decided to update our data files and provide more comprehensive analyses of water quality, phytoplankton assemblages, and crustacean zooplankton assemblages than those in the Phase I report. The major thrust of the report, however, remained focused on the first objective of the Phase II project, i.e., increasing understanding of the interactions between salamanders and trout. We included analyses of the salamander field work conducted between 1990 and 1994, and the laboratory experiments on salamander-trout interaction. Data from the manipulated lakes, based on original objective 2, were integrated into the zooplankton and salamander analyses, rather than preparing a separate chapter on this subject. Impacts of non-reproducing trout populations on native fauna remained mostly unresolved owing to our inability to sample lakes of this type in FY1994. However, a proposal (Phase III) was submitted to the USGS - Division of Biological Resources in FY1997 to address the ecological impacts of non-reproducing trout populations on native fauna in NOCA lakes. If funded, this additional work will compliment our existing data to address the overall impacts of introduced trout on native fauna. This third phase of the project

would be completed in a timely manner so that the NOCA staff can prepare the required environmental assessment and high-elevation lake management plan by the year 2000.

ACKNOWLEDGMENTS

We thank the following for their many contributions to this project: Jim Larson, Shirley Clark, Kathy Jope, John Reynolds, Bill Paleck, Jon Jarvis, Bruce Freet, Reed Glesne, and Michael Collopy. We also thank LaVon Mauer for her assistance in preparing the final draft of the report.

Chapter 2.

**Physical and Chemical Characteristics of Lakes
in the Glacially Influenced Landscape of the
North Cascades Mountain Range, Washington State, USA¹**

¹Larson, G. L., G. Lomnicky, R Hoffman, W. J. Liss, and E. A. Deimling. Accepted. Integrating physical and chemical characteristics of lakes into the glacially influenced landscape of the North Cascade Mountain Range, Washington State, USA. Environmental Management.

Abstract: Physical and chemical characteristics of 58 montane lakes in alpine, subalpine, and forest vegetation zones in the North Cascades Mountain Range were assessed between 1989 and 1993. The objectives of the study were to document the time of ice-out relative to lake elevation, determine how a sharp climate gradient west and east of the hydrologic divide affected this relationship for subalpine lakes, and assess how lake water quality was associated with lake elevation, lake depth, and basin geology. As expected, lake ice-out times occurred earlier with decreasing elevation. East-slope subalpine lakes iced-out earlier than did west-slope subalpine lakes because the east slope of the study area was drier and warmer than was the west slope. On average, the lakes were relatively cold, neutral in pH, and low in dissolved substances and concentrations of nitrogen and phosphorus. Although some shallow lakes (depth <10 m) exhibited the highest alkalinities, conductivities, and concentrations of phosphorus and nitrogen, most shallow lakes exhibited low values for these variables that were comparable to values observed in deep lakes. Geology did not play a major role in segregating the lakes based on water quality. Overall, lake temperature, pH, alkalinity, conductivity, and concentrations of total phosphorus and total Kjeldahl-N increased with decreasing elevation. These changes in water quality with decreasing elevation in this temperate mountainous region appeared to correspond with warmer air temperatures, and increased vegetation biomass, soil depth and maturity, dissolved substances, and nutrient availability.

INTRODUCTION

Interest in the limnology of montane lakes began decades ago, e.g., Strom (1931), Ruttner (1937), and Hutchinson (1937). Such pioneering efforts documented the short ice-free period of the lakes and their unproductive condition. In subsequent years a series of limnology studies contributed additional information to the fundamental understanding of the physical and chemical characteristics (water quality) of these lake systems (e.g., Pennak 1955; Pechlaner 1966, 1967, 1971; Loffler 1969; Stout 1969; James and Hubbick 1969; Reed 1970; Zutshi et al. 1980; Larson 1973, Larson et al. 1994; Mosello 1984; Mosello et al. 1991; Aizaki et al. 1987; Psenner 1989; Vass et al. 1989; Boggero et al. 1993; Gosso et al. 1993; and Kovacik and Stuchlik 1994). Collectively, these studies showed that the water quality of montane lakes during the ice-free period was influenced by basin geology, watershed vegetation, local climate and lake depth.

In recent years, there has been a growing interest in characterizing the water quality of montane lakes at a coarser scale than the watershed unit. For groups of lakes distributed across montane landscapes, changes in lake water temperature, pH, alkalinity, and conductivity have been shown to be related to lake elevation (Anderson 1970; Zutshi et al. 1980; Vass et al. 1989; Stout 1993; Lukavsky 1994; Larson et al. 1995). Perhaps the best example of the relationship between elevation and water quality was provided by Nauwerck (1994), who showed that water temperature, pH, conductivity, and concentration of total phosphorus increased and concentration of nitrate-N decreased with decreasing elevation in montane lakes of northern Swedish Lapland. In the present study, we assessed the water quality of montane lakes distributed over an elevational range of 1651 m in a relatively limited geographical area that included alpine, subalpine, and forest vegetation zones, a relatively uniform geology, and sharp west-east climate gradient formed by the hydrologic divide (Lomnický 1996). Our working hypotheses for data analyses were that: (1) increased lake temperature, pH, alkalinity, conductivity, and concentrations of nutrients would decrease with elevation; and (2) water quality would be influenced by lake depth and geology. The objectives of the study were to: (1) document the time of ice-out relative to lake elevation west of the hydrologic divide and describe how the west-east climate gradient affected this relationship for subalpine lakes; (2) assess how lake water quality was associated with lake elevation, and (3) assess how water quality differed between deep and shallow lakes, and between geologic types.

STUDY AREA

North Cascades National Park Service Complex (NOCA) is located in the north-central portion of Washington State (Whatcom and Skagit Counties) in the North Cascades Mountain Range. The northern border of the 204,000 ha park borders Canada (Figure 2.1). Most of the mountain range is underlain by hard crystalline bedrock, primarily gneiss and granite, although some sedimentary and volcanic outcroppings exist (Misch 1977). Regional uplifting, which initially formed the mountain range in the Cenozoic 50 million years ago, created a base template that is highly jointed, faulted, and fractured (Misch 1977). Glacial activity during the Pleistocene and the neoglacial period formed the basins of the study lakes.

Differential precipitation across a hydrologic divide separates the region into two major climatic units (Porter 1976; Jackson and Kimerling 1993). A maritime climate prevails on the west due to the influence of warm, moist air masses from the Pacific Ocean and Puget Sound.

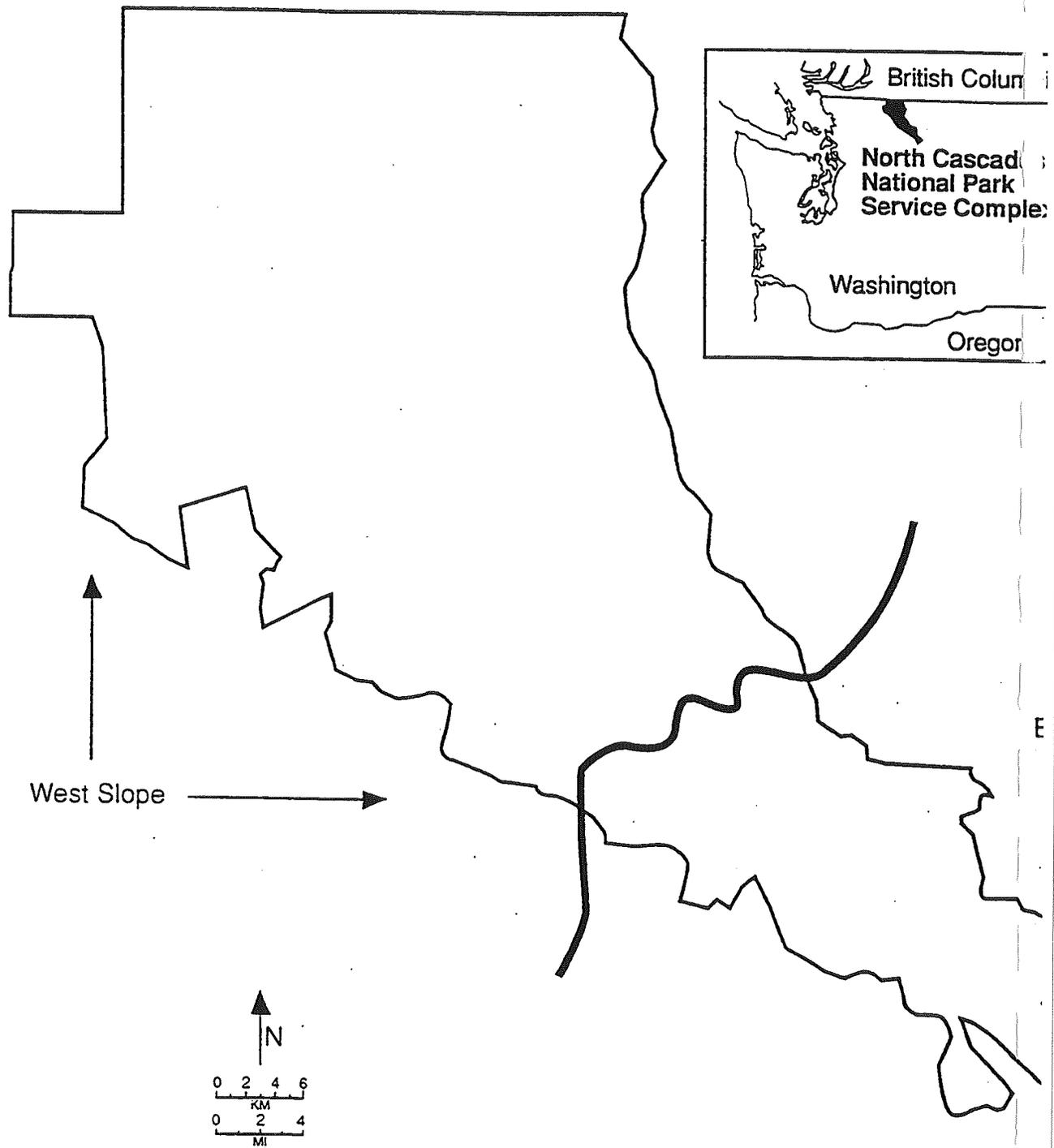


Figure 2.1. Location of the North Cascades National Park Service Complex in Washington. Black line represents the hydrologic crest separating west-slope and east-slope regions of the park.

This climate is characterized by wet, relatively warm winters, and cool summers. A semi-arid continental climate exists on the east and is characterized by colder winters and relatively hotter, dryer summers compared to the west. Throughout the park, ice and snow cap the lakes for many months each winter. Rains during the brief snow-free season are common, particularly on the west. Drainages west of the hydrologic divide empty into Puget Sound, whereas drainages to the east flow into the Columbia River basin.

Lomnický (1996) classified NOCA lakes according to the type of vegetation surrounding the lakes (Table 2.1). Based on a vegetation cover type map developed by Agee and Pickford (1985), Lomnický (1996) identified three main vegetation zones in NOCA: alpine, subalpine, and forest. The forest zone was further subdivided into high-forest and low-forest zones, distinguished by the presence of snow pack until spring (high forest) versus intermittent snow-free periods in winter (low forest). Each vegetation zone occurred at higher elevations on the east slope than on the west slope (Table 2.1).

METHODS

Fifty-eight lakes were sampled from 1989 through 1993 while they were free of snow and ice (Table 2.2). Some lakes were sampled one to three times, whereas others were sampled up to 12 times during the 5-year study. The lakes were located in remote mountainous terrain, and were accessed by hiking or by helicopter. Sampling was conducted from an inflatable boat over the deepest spot in each lake. Water temperatures were determined at a depth of 1 m using an Omega HH70 hand-held thermometer. Water samples were collected 1 m below the surface of each lake using a 1.5-L Van Dorn-style bottle. Filtered (0.7- μ m prewashed Watman GF/C filters) samples were placed in 1-L acid-washed, high-density polyethylene bottles for nutrient analyses. Unfiltered samples collected in 250 ml acid-washed polypropylene bottles were used for analyses of pH, alkalinity, and conductivity. To the degree possible, water samples were taken just before leaving the field to minimize sample deterioration. If samples were kept overnight in the field, the bottles were placed out of the light during the day or in dark bags and kept in the coldest spot that could be found (e.g., inlet stream, snow bank). When departing the field, the samples were wrapped with insulation to maintain coolness, transported out of the field, frozen, and then shipped to the Cooperative Chemical Analytical Laboratory, Oregon State University. Chemical analyses included pH, alkalinity, conductivity, total Kjeldahl-N, nitrate-N, ammonia-N, total phosphorus, and orthophosphate-P (Table 2.3).

Table 2.1. Elevations of all NOCA lakes (Lominicky 1996) and dates of ice-out for study lakes in 1989 by vegetation zone east and west of the hydrologic divide.

Vegetation zone	Statistics	Elevation (m)	Ice-out (Day of the year)
East-slope Lakes			
Alpine	average	1961	NA ¹
	N	3	NA
	SD	145	NA
Subalpine	average	1847	188
	N	24	11
	SD	177	20
Forest High	average	1648	176
	N	7	5
	SD	1	20
Forest Low	average	662	95
	N	1	1
	SD	--	--
West-slope Lakes			
Alpine	average	1601	216
	N	36	8
	SD	250	16
Subalpine	average	1566	205
	N	67	18
	SD	187	16
Forest High	average	1360	182
	N	16	8
	SD	125	16
Forest Low	average	810	138
	N	8	7
	SD	230	20

¹Not available

Table 2.2. Elevations, surface areas, maximum depths, and vegetation zones and the number of times each lake was sampled (1989-93) for NOCA study lakes east and west of the hydrologic divide.

Lake	Elevation (m)	Depth (m)	Surface Area (Ha)	Vegetation Zone ¹	Times Sampled
EAST-SLOPE LAKES					
COON	662	8.2	5.8	4	2
	1270	59.0	49.0	2	2
TRAPPER	1504	4.1	11.9	3	7
WADDELL	1629	2.5	4.3	3	4
BATTALION	1642	12.0	17.7	2	4
DOUFTFUL	1664	16.1	47.3	2	2
GREEN VIEW	1679	3.6	4.0	3	3
DAGGER	1679	5.0	6.1	3	7
MCALESTER	1717	6.3	10.4	3	8
RAINBOW	1789	1.2	5.0	2	8
M132	1800	0.3	2.0	2	6
M131	1863	1.3	8.8	2	7
MR11	1873	0.3	1.5	2	6
MR2	1873	0.2	1.5	2	4
MR3	1931	1.0	2.1	2	4
LOWER TRIPLET	1974	1.4	7.6	2	2
MM11	1988	1.0	4.3	2	4
UPPER TRIPLET	2033	0.2	1.2	2	3
JUANITA					
WEST-SLOPE LAKES					
THUNDER	412	3.0	6.4	4	4
PYRAMID	802	0.3	8.8	4	9
HOZOMEEN	861	38.4	19.0	4	2
WILLOW	870	8.2	3.7	4	2
RIDLEY	958	4.3	9.8	4	1
LOWER PANTHER	1031	0.2	5.8	4	12
UPPER PANTHER	1031	0.1	3.0	4	10
NO NAME	1171	4.4	9.1	3	1
BOUCK	1174	5.2	19.0	3	1
PRICE	1188	16.0	26.2	2	1
LS1	1241	0.4	3.4	3	10
LS2	1243	2.0	4.7	3	10
MONOGRAM	1270	12.7	37.2	2	1
LOWER THORNTON	1357	23.5	33.0	3	
LS3	1365	1.5	4.9	2	2
MORaine	1378	32.5	33.0	1	1
PM53	1382	1.0	9.1	3	1
NERT	1388	1.0	8.2	3	2
MIDDLE THORNTON	1427	5.0	24.0	2	1
WILD	1488	4.6	8.8	2	1
JEANITA	1496	0.5	2.4	3	1
LOWER REVILLE	1525	1.6	3.0	2	1
EP6	1566	3.0	19.8	2	2
MP8	1566	0.9	3.7	1	1
VULCAN	1583	3.3	6.4	2	1
COPPER	1601	5.2	22.6	2	1
EGG	1604	0.8	4.9	2	1
SKYMO	1609	4.3	5.5	2	2
UPPER SKYMO	1610	3.0	4.3	1	2
KLAWATTI	1624	12.0	33.0	1	1
TALUS TARN	1632	0.6	3.6	2	1
OUZEL	1659	2.0	9.8	1	1
SWEET PEA	1687	3.4	27.4	3	1
TAPTO, MIDDLE	1754	0.3	5.5	2	3
TAPTO, WEST	1754	0.8	4.3	2	3
TAPTO, UPPER	1755	4.0	13.1	2	3
BEAR	1769	11.4	46.3	2	1
EILEY	1982	1.0	4.6	1	1
WILEY	2028	2.4	--	1	1
SILVER	2063	65.1	137.0	1	1

¹Alpine (1), subalpine (2), high-forest (3), and low forest (4)

Table 2.3. Analytical procedures used by the Cooperative Chemistry Analytical Laboratory, Oregon State University.

Variable	Method	Detection limits	(Units)
pH	Portable Beckman meter 21. Orion Sureflow Standardized with pH 4 and pH 7 buffers. Final reading recorded after 5 consecutive readings of the same value (usually 30-45 minutes)	0-14	Standard
Alkalinity	Electrometric titration to pH 4.5	0.2	(mg/l)
Conductivity	Wheatstone Bridge, Yellow Springs model 33, corrected to 25°C	0.4	(μ S/cm)
Nitrate-N	Technicon Autoanalyzer, automated cadmium reduction	0.001	(mg/l)
Kjeldahl-N	Nessler's Reagent finish	0.01	(mg/l)
Ammonia-N	Technicon Autoanalyzer, colorimetric automated phenate	0.005	(mg/l)
Total Phosphorus	Persulfate digestion, ascorbic acid finish	0.001	(mg/l)
Orthophosphate-P	Reactive phosphate, ascorbic acid finish	0.001	(mg/l)

Dates of ice-out, defined as the time when the lake surfaces were virtually ice-free, were estimated for most lakes from aerial surveys conducted in 1989 when the field crews were helicoptered to and from sampling locations. The estimated dates of ice-out likely were within 1 to 2 weeks of the actual dates in some cases. For a few lakes not covered by the aerial surveys, estimated dates of ice-out were obtained from backcountry park rangers.

Average values for the water-quality variables were calculated using the STATS module of SYSTAT (Wilkinson 1987). Average pH was calculated from hydrogen ion activity. Regression analyses were used to evaluate dates of ice-out and changes in water quality with increased lake elevation for lakes west of the hydrologic divide using the MGLH module of SYSTAT. West-slope NOCA lakes were used because samples were available from a greater range of lake elevation (i.e., alpine to low-forest lakes), than were east-slope lakes (primarily subalpine lakes). Statistical comparisons of dates of ice-out and the water qualities of east-slope and west-slope lakes in the subalpine vegetation zone were conducted using the Mann-Whitney test (NPAR module of SYSTAT). Level of statistical significance was $\alpha = 0.05$. Discriminant analysis (NCSS vers. 5.03, Hintze 1992) was used to examine how the water quality variables as well as elevation, lake depth, and lake surface area (independent variables) would predict the placement of lakes into four classification categories (dependent variable) based on the four vegetation zones. This was elucidated during the analysis by: 1) the construction of a classification matrix according to the assignment of lakes to the classification categories; 2) determination of classification accuracy by calculation of the percent reduction in classification error due to the independent variables; and 3) determination of the number of lakes misclassified. The discriminant analysis also calculated three canonical variate scores for each lake, and calculated the percent variation attributable to each variate, as well as the correlations between each variate and each independent variable. An initial analysis was performed using all independent variables (Table 2.4). A second analysis was performed using only independent variables found to have significant influence during the first analysis.

RESULTS

Date of ice-out increased with increasing lake elevation on the east slope ($r^2 = 0.259$, $N = 16$, $P = 0.044$) and on the west slope ($r^2 = 0.621$, $N = 39$, $P < 0.001$) of the park. East-slope subalpine lakes iced-out earlier than did west-slope subalpine lakes ($P = 0.01$). This result

Table 2.4. Identification of independent variables having significant influence during the initial discriminant analysis using all eleven variables.

Variable	F-probability
Elevation	<0.001
Surface Area	0.301
Depth	0.184
pH	<0.001
Alkalinity	<0.001
Conductivity	<0.001
Total Kjeldahl-N	<0.001
Ammonia-N	0.113
Nitrate-N	0.011
Total Phosphorus	0.286
Orthophosphate-P	0.035

occurred even though the average elevation of east-slope subalpine lakes was higher than the average elevation of west-slope subalpine lakes (Table 2.1).

On average, NOCA lakes were relatively cold, neutral in pH, and low in dissolved substances and concentrations of nitrogen and phosphorus (Table 2.5). However, considerable variation was observed for most variables. Water temperature, pH, alkalinity, conductivity, total Kjeldahl-N, and ammonia-N of west-slope lakes decreased in concentration and nitrate-N increased in concentration with increased elevation (Table 2.6). Only total phosphorus and orthophosphate-P did not change in concentration relative to elevation (Table 2.6). However, when three lakes (two alpine and one subalpine) receiving turbid-glacial outwash were deleted from the data set, total phosphorus increased with decreasing elevation (Table 2.6). Changes in vegetation zone were related to elevation as well. East-slope subalpine lakes were significantly higher in pH, alkalinity, total phosphorus, and total Kjeldahl-N than were west-slope subalpine lakes (Table 2.7).

Based on the second discriminant analysis, using six water quality variables and elevation as independent variables with vegetation zone as the dependent variable, 98.2% of the among-group variation was found to be associated with the first two canonical variates; 93.3% was associated with variate 1 and 4.9% with variate 2. Canonical variate 1 was positively correlated with elevation and negatively correlated with alkalinity, conductivity, total Kjeldahl-N, and pH (Table 2.8). Canonical variate 2 was most strongly and positively correlated with orthophosphate-P and nitrate-N (Table 2.8). The effectiveness of this model in placing lakes into the correct vegetation zone-based classification categories was somewhat problematic, however, since 13 of the 58 lakes (22.4%) were misclassified.

Collectively, shallow lakes (<10 m in maximum depth) exhibited a wide range of values for alkalinity and conductivity, and concentrations of total Kjeldahl-N and total phosphorus. A greater percentage of shallow lakes maintained high values of the variables than did deep lakes (Table 2.9).

Geology did not play a major role in segregating most NOCA lakes based on water quality. For example, water quality of lakes in granitic or gneiss basins, the primary geologic substrates, did not differ (see Lomnický 1996). The few low-forest lakes in watersheds with greenstone outcroppings were higher ($P = 0.025$) in alkalinity and conductivity than were low-forest lakes in either gneiss or granite bedrock watersheds (Table 2.10).

Table 2.5. Average water quality of west-slope and east-slope NOCA study lakes.

Variable	Units	n	Average	Range	SD
Temperature	°C	51	11.6	1.3-21.0	4.7
pH	Standard	58	7.03	5.9-8.7	--
Alkalinity	mg/l	58	2.6	0.4-20.5	3.4
Conductivity	mg/l	58	24.2	1.9-156.9	29.4
Total Kjeldahl-N	mg/l	58	0.049	0.01*-0.194	0.045
Nitrate-N	mg/l	58	0.007	0.001*-0.035	0.008
Ammonia-N	mg/l	58	0.005	0.005*-0.020	0.004
Total phosphorus	mg/l	58	0.007	0.001*-0.030	0.006
Orthophosphate-P	mg/l	58	0.001	0.001*-0.006	0.001

*detection limits

Table 2.6. Regression analyses of water quality variables and vegetation zone as independent variables and elevation as the dependent variable for west-slope NOCA lakes.

Variable	n	Sign of the Regression Coefficient	r ²	P
Temperature	37	-	0.475	<0.001
pH	40	-	0.363	<0.001
Alkalinity	40	-	0.400	<0.001
Conductivity	40	-	0.425	<0.001
Total Kjeldahl-N	40	-	0.477	<0.001
Nitrate-N	40	+	0.111	0.036
Ammonia-N	40	-	0.148	0.014
Total Phosphorus	40	-	0.061	0.123
Orthophosphate-P	40	-	0.005	0.655
Total Phosphorus ¹	37	-	0.232	0.003
Vegetation Zone	40	-	0.661	<0.001

¹Two alpine lakes and 1 subalpine lake receiving turbid-glacial meltwater were deleted.

Table 2.7. Comparisons of water qualities of west-slope subalpine lakes (n = 16) and east-slope subalpine (n = 9) lakes, NOCA.

Variable	Average Values		P*
	West slope	East slope	
Temperature (1989; °C)	10.9	13.6	0.074
pH (Standard units)	6.7	7.0	0.041
Alkalinity (mg/l)	1.1	1.8	0.026
Conductivity (μ S/cm)	14.9	16.4	0.353
Total Kjeldahl-N (mg/l)	0.024	0.055	0.016
Nitrate-N (mg/l)	0.008	0.007	0.871
Ammonia-N (mg/l)	0.006	0.005	0.780
Total Phosphorus (mg/l)	0.005	0.009	0.002
Orthophosphate-P (mg/l)	0.001	0.001	0.637

*Mann-Whitney U-test

Table 2.8. Correlation of independent variables and canonical variates one and two determined during the second discriminant analysis.

Variable	Canonical Variate 1	Canonical Variate 2
Elevation	0.623	-0.066
pH	-0.324	-0.112
Alkalinity	-0.507	0.241
Conductivity	-0.465	0.151
Total Kjeldahl-N	-0.387	-0.114
Nitrate-N	0.186	0.505
Orthophosphate-P	-0.035	0.784

Table 2.9. Number of shallow (<10 m) and deep (>10 m) lakes exhibiting elevated conductivity alkalinity, and concentrations of total Kjeldahl-N and total phosphorus. Possible numbers of lakes: shallow (38), deep (19).

Variable	Concentration or Level	Number and Percentage of Lakes			
		<10 m	%	≥10 m	%
Conductivity	≥25 μS/cm	14	37	3	16
Alkalinity	≥3 mg/L	12	32	3	16
Total Kjeldahl-N	≥0.08 mg/L	10	26	1	5
Total Phosphorus	≥0.008 mg/L	14	37	3	16

Table 2.10. Comparisons of conductivity and alkalinity of low-forest west-side lakes in basins with gneiss or granite and those in greenstone (n = 3).

Variable	Average Value (Minimum - Maximum)		P
	Gneiss - Granite	Greenstone	
Conductivity μS/cm	48.2 (11.4-20.5)	119.9 (97.2-157.0)	0.025
Alkalinity mg/L	5.4 (2.6-7.5)	48.2 (21.0-65.7)	0.025

DISCUSSION

The results of this study were consistent with previous findings (e.g., Nauwerck 1994) that the water quality of montane lakes was associated with elevation. Increased water temperature, pH, alkalinity, conductivity, concentrations of total phosphorus (without the three lakes receiving turbid glacial effluent) and total Kjeldahl-N, and decreasing concentration of nitrate-N were associated with decreasing elevation.

We believe that the observed changes in lake water quality with decreased elevation were associated with warmer air temperatures, and increased biomass of terrestrial vegetation, soil depth and maturity, dissolved substances, and nutrient availability as described by Aber and Melillo (1991) for temperate forests. The alpine vegetation zone is a cold and desiccated zone covered by ice and snow much of the year. The local environment of these lakes is not moderated extensively by soils and surrounding vegetation. Lake watershed relief tends to be steep, except for lakes perched on ridgetops (Lomnický 1996). Water retention in the basins is limited, except in frozen form, because soils are thin and predominately mineral or nonexistent (Franklin et al. 1973), and steep relief of the watershed probably facilitates the rapid routing of water down slope. Lake ice-free dates in this vegetation zone occur late in the summer season, if at all.

Air temperatures increase with decreasing elevation as the vegetation progresses from the alpine to the subalpine zone. The subalpine zone is a mixture of meadows and stringers of trees that provide added organic input to lakes in the form of needle litter and woody debris (Franklin et al. 1973; 1988). Increased soil depth and maturity leads to enhanced retention of ground water that increases mineral weathering and nutrient availability to lakes (Gorham 1961). Lakes become ice-free earlier than in the alpine zone. Consequently, subalpine lakes tend to warm earlier than do alpine lakes.

Further decreases in elevation and associated increases in temperature promote the establishment of the forest zone. Warmer temperatures lead to reduced periods of winter snowpack, and increased biomass of vegetation and soil depth and maturity in lake watersheds. Increased soil-water interactions elevate nutrient availability to lake systems (Gorham 1961; Hem 1989). Forest lakes, therefore, are higher in pH, alkalinity, conductivity, total Kjeldahl-N, ammonia-N, and total phosphorus than are subalpine and alpine lakes. These lakes, especially low-forest lakes, also are warmer and become ice-free earlier than lakes in other vegetation zones.

Although increased values of most water-quality variables were associated with decreased elevation and changes in vegetation, some of the results did not fit this pattern. For example, nitrate-N decreased in concentration with decreasing elevation in NOCA lakes. This result was similar to those provided by Nauwerck (1994), which may reflect decreased phytoplankton activity with increased lake elevation (Wetzel 1983). The relatively high concentrations of total phosphorus in the three lakes receiving turbid-glacial outwash may have been related to erosional processes (Brundin 1958). The higher alkalinity and conductivity of low-forest lakes in greenstone basins relative to those in gneiss-granitic basins probably was associated with increased concentrations of calcarious materials (Staatz et al. 1972). The reason that east-slope subalpine lakes were significantly higher in pH and alkalinity and concentrations of total Kjeldahl-N and total phosphorus than were west-slope subalpine lakes remains unclear because the geology and vegetation of the watersheds of these two groups of lakes were comparable. Although some shallow lakes exhibited the highest alkalinities, conductivities, and concentrations of nitrogen and phosphorus, the influence of lake depth remains somewhat unclear because many shallow lakes exhibited low values for these variables, as was the case for nearly all deep lakes.

As expected, ice-out times occurred earlier with decreasing elevation. Warmer air temperatures and decreased snow deposition with decreasing elevation were undoubtedly important in producing this pattern (Lomnický 1996). The finding that east-slope subalpine lakes iced-out earlier than did west-slope subalpine lakes was consistent with this conclusion because the climate of the east slope was drier and warmer than that of the west slope (Lomnický, 1996).

In summary, this study provides additional evidence that the water qualities of temperate zone montane lakes are influenced to varying degrees by lake depth, geology, climate, and elevation. We believe that the major shifts in vegetation zone and associated increases, as described by Aber and Melillo (1991), in soil depth and maturity, dissolved substances, and nutrient availability with decreasing elevation at NOCA are primary influences on the observed changes in water quality. Explanations for variation in water quality among lakes in the same category remains elementary, however. Additional attributes of the landscape appear to exert control on water quality based on the low r^2 values from the regression analyses between the water quality variables and elevation, plus the misclassification of about a quarter of the lakes in our discriminant analysis. Further studies of the interrelationships between the morphology of the lakes and the characteristics of their entire watersheds (vegetation, geology, aspect, soil depth

and maturity, and climate) are needed to develop better models that describe the limnological variations of montane lakes across the landscape.

ACKNOWLEDGMENTS

We thank the National Park Service and the USGS - Forest and Rangeland Ecosystem Science Center for the financial support of this project, and especially Mike Collopy, Jim Larson, Shirley Clark, Kathy Jope, Jon Jarvis, Bruce Freet, and Reed Glesne. Stanford Loeb, Stanley Dodson, William Neill, and John O'Brien provided recommendations for the sampling design, Stanford Loeb, Stanley Dodson, and James Petranka reviewed the manuscript, and Lisa Ganio provided recommendations for statistical analyses. Becca Berkey typed and Ruth Jacobs edited the manuscript.

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**Phytoplankton Assemblages in High-Elevation Lakes
in the Northern Cascades Mountain Range,
Washington State USA¹**

¹Larson, G. L., C. D. McIntire, R. E. Truitt, W. J. Liss, R. Hoffman, E. Deimling, and G. Lomnicky. 1998. Phytoplankton assemblages in high-elevation lakes in the Northern Cascades Mountain Range, Washington State USA. *Archives für Hydrobiologie* 142:71-93.

Abstract: Phytoplankton assemblages in high-elevation lakes of North Cascades National Park Service Complex were studied during the open-water period in 1989. Collectively, 93 taxa were identified in 55 samples from 51 lakes. Based on cell densities, cyanobacteria had the highest relative abundance (36.7%), followed by chlorophytes (29.8%), and chrysophytes (19.6%). *Aphanocapsa delicatissima* had the highest proportional abundance (14.0%). Only 15.1% of the taxa occurred in more than 20 samples. Phytoplankton cell densities increased following a gradient of increasing lake-water temperature, alkalinity, and concentration of total Kjeldahl-N associated with decreasing lake elevation. Chrysophytes and cyanobacteria were the most important taxa (relative abundance) in alpine and subalpine lakes, whereas cyanobacteria had the highest relative abundances in high-forest and low-forest lakes. Chlorophytes had their highest relative abundance in high-forest lakes. Although low in relative abundance, diatoms and dinoflagellates were most abundant in alpine lakes. An ordination by correspondence analysis indicated that most alpine, subalpine, and high-forest lakes had similar floras. Although a few subalpine lakes exhibited deviations from this pattern, the main differences in phytoplankton composition were found in a group of low-forest and high-forest lakes. Canonical correspondence analysis (CCA) provided evidence that the distribution of samples and taxa in ordination space was correlated with a gradient of decreasing lake elevation and increasing water temperature, alkalinity, and concentration of nitrogen. When CCA was used to examine relationships among phytoplankton taxa and vegetation zones, a continuous distribution of taxa was found from the low-forest zone to the subalpine zone, with a large number of taxa occurring primarily in the subalpine and high-forest zones. Three phytoplankton taxa occurred primarily in alpine lakes, whereas five taxa co-occurred in alpine, subalpine, and high-forest zones. In NOCA lakes, elevation and associated changes in water quality and concentrations of nutrients, especially nitrogen, appeared to be the primary physical and chemical factors influencing the taxonomic structures of phytoplankton assemblages.

INTRODUCTION

High-elevation lakes in the Pacific Northwest of the United States typically are capped each year by ice and snow covers from late fall to early summer. After the ice and snow covers disappear (the open-water period), the lakes undergo substantial changes in physical, chemical, and biological characteristics (Larson 1973; Hall 1973; Larson et al. 1994; and Larson et al. 1995). Densities and taxonomic structures of the phytoplankton assemblages are influenced by

environmental conditions such as climate, duration of the open-water period, lake morphology, water quality, and geographical location (Larson et al. 1994; Larson et al. 1995). For example, the taxonomic structure of phytoplankton assemblages in high-elevation lakes along the east side of Olympic National Park (OLYM) is related to lake depth and the concentration of nitrate-N. For chain lakes connected by streams in the park, phytoplankton densities increase with decreasing lake elevation. Phytoplankton densities also increase in isolated lakes and chains of lakes as the duration of the open-water period increases (Larson et al. 1995). At Mount Rainier National Park (MORA), phytoplankton densities and the taxonomic structure of assemblages in high-elevation lakes are associated with the duration of the open-water period and geographical location, the latter corresponding to a west-east climate gradient in the park (Larson et al. 1994).

High-elevation lakes in North Cascades National Park Service Complex (NOCA), another national park in the Pacific Northwest, exhibit a range of physical and chemical characteristics depending on their location in alpine, subalpine, high-forest, or low-forest vegetation zones (Lomnicky et al. 1989; Liss et al. 1995). During the open-water period, alpine lakes have low water temperature and high concentrations of nitrate-N relative to low-forest lakes. Decreasing elevation and associated changes in vegetation zone correspond to increases of water temperature, pH, alkalinity, conductivity, the concentration of Kjeldahl-N, and the concentration of ammonia-N (Liss et al. 1995). Given this array of lake characteristics and our knowledge of phytoplankton assemblages in OLYM and MORA, a reasonable hypothesis is that characteristics of phytoplankton assemblages in NOCA lakes correspond to the physical and chemical characteristics of lakes in different vegetation zones. The objective of this paper is to characterize the taxonomic structure of phytoplankton assemblages in NOCA lakes in relation to the physical and chemical characteristics of the lakes.

STUDY AREA

NOCA, located in the north-central portion of Washington State (Figure 3.1), is a rugged and heavily glaciated area in the northern Cascade Mountain Range. The park is 1274 km² and ranges from 330 m to 2700 m in elevation (Liss et al. 1995). Climatic and geologic conditions, soils, and vegetation are diverse. Annual precipitation, originating largely from warm moist winds from the Pacific Ocean, exceeds 100 cm at low elevations and over 350 cm at high elevations. The park area east of the hydrologic crest of the North Cascades Mountain Range receives less precipitation than does the west side. Extensive glacial systems exist because most

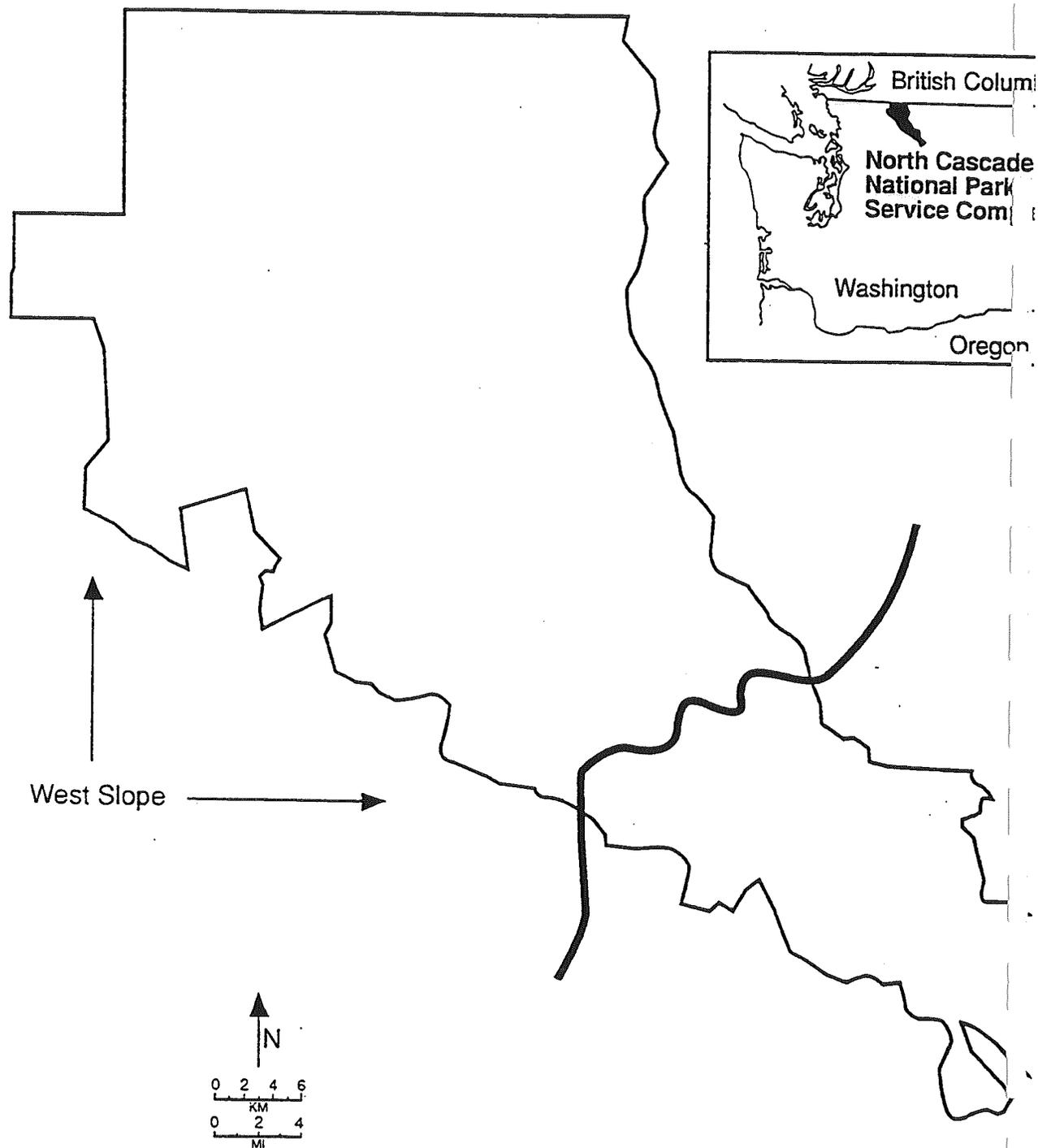


Figure 3.1. Geographical location of North Cascades National Park Service Complex (NOCA) in Washington State. The bold line represents the hydrologic crest separating west-slope and east-slope regions of the park.

of the precipitation is snow. Low-forest, high-forest, subalpine, and alpine zones are the main vegetation zones present (Lomnický et al. 1989; Liss et al. 1995). Lakes in this study are classified based on the vegetation zone surrounding each lake (Table 3.1). The lakes are capped by ice and snow for many months each year. The open-water period typically occurs between mid-June to mid-July and early October, although some alpine lakes do not always become free of ice and snow each year.

METHODS

Sampling

From July through September 1989, 55 phytoplankton samples were collected from 51 NOCA lakes. Four lakes were sampled twice during the field season. The lake sampling schedule was limited by logistical problems associated with working in rugged terrain and much of the sampling effort was constrained by the timing of the open-water periods. For these reasons, low-forest and high-forest lakes were sampled before subalpine and alpine lakes. Some subalpine and alpine lakes were not sampled until September. All phytoplankton samples were collected with a 1.5-L Van-Dorn-style sampler from a depth of 1 m near the deepest location in each lake. The samples were preserved in Lugol's solution, and 500 individual cells were identified to species and counted in each sample using an inverted microscope (McIntire et al. 1996). The minimum size of cells counted was 1 μm .

Unfiltered water samples obtained concurrently with the phytoplankton samples were analyzed for pH, alkalinity, conductivity, and total phosphorus. Water samples also were analyzed for concentrations of orthophosphorus-P, Kjeldahl-N, nitrate-N, and ammonia-N after they were filtered through 0.7 μm prewashed Watman GF/C filters. The water samples were stored in acid washed polypropylene bottles. Water samples were transported out of the field, frozen, and then shipped to the Cooperative Chemical Analytical Laboratory, Oregon State University, Corvallis, Oregon, for analysis. Near-surface water temperatures were recorded with an Omega HH70 series hand-held thermometer. Lake elevation, area, depth, water quality, and nutrient data were provided by Liss et al. (1995).

Data Analysis

For analytical purposes, the data were organized into two files, a matrix of species abundances and a corresponding matrix of environmental data. The species-abundance matrix contained cell density values for 93 species; the environmental data included measurements

Table 3.1. Lake acronyms, elevations, areas, depths, and vegetation zones of NOCA study lakes. Vegetation zones: alpine (1); subalpine (2); high-forest (3); and low-forest (4).

Lake	Lake Acronym	Elevation (m)	Area (ha)	Maximum Depth (m)	Vegetation Zone
Klawatti	KLAW	1624	33.0	12.0	1
MP8	MP8	1566	0.9	3.7	1
Silver	SILV	2063	65.1	137.0	1
Upper Skymo	SKYU	1610	3.0	4.3	1
Wiley	WILE	2028	2.4	4.6	1
Bear	BEAR	1769	11.4	46.3	2
Copper	COPP	1601	5.2	22.6	2
Doubtful	DOUB	1642	12.0	17.7	2
EP6	EP6	1566	3.0	4.9	2
Greenview	GNVW	1664	16.1	47.3	2
Juanita	JUAN	2033	0.2	1.2	2
LS3	LS3	1365	1.5	4.9	2
MM11	MM11	1974	1.4	7.6	2
Monogram	MONO	1270	12.7	37.2	2
MR13	MR13	1789	1.2	5.0	2
MR2	MR2	1873	0.3	1.5	2
MR3	MR3	1873	0.2	1.5	2
MR9	MR9	1813	1.7	4.9	2
Egg	EGG	1640	0.8	4.9	2
Talustarn	TTAR	1632	0.6	3.6	2
Lower Reville	REVL	1525	1.6	3.0	2
Middle Skymo	SKYM	1609	4.3	5.5	2
Middle Tapto	TAPM	1754	0.3	5.5	2
Upper Tapto	TAPU	1755	4.0	13.1	2
West Tapto	TAPW	1754	0.8	4.3	2
Middle Thornton	THRM	1427	5.0	24.0	2
Trapper	TRAP	1270	59.0	49.0	2
Lower Triplet	TRIL	1931	1.0	2.1	2
Upper Triplet	TRJU	1988	1.0	4.3	2
Vulcan	VULC	1583	3.3	6.4	2
Wild	WILD	1488	4.6	8.8	2
Battalion	BATT	1629	2.5	4.3	3
Jeanita	JEAN	1496	0.5	2.4	3
Upper Ketting	KETU	1693	0.6	2.1	3
LS1	LS1	1241	0.4	3.4	3
LS2	LS2	1243	1.0	4.9	3
McAlester	MCAL	1679	5.0	6.1	3
Nert	NERT	1388	1.0	8.2	3
Noname	NONA	1171	4.4	9.1	3
PMS3	PMS3	1382	1.0	9.1	3
Rainbow 1	RAI1	1717	6.3	10.4	3
Rainbow 2	RAI2	1717	6.3	10.4	3
Sweet Pea	SWEE	1687	3.4	27.4	3
Lower Thornton	THRL	1357	23.5	33.0	3
Waddel 1	WAD1	1504	4.1	11.9	3
Waddel 2	WAD2	1504	4.1	11.9	3
Coon	COON	662	8.2	5.8	4
Hozomeen	HOZO	861	38.4	19.0	4
Panther 1	PAN1	1031	0.2	5.8	4
Panther 2	PAN2	1031	0.2	5.8	4
Pyramid 1	PYR1	802	0.3	8.8	4
Pyramid 2	PYR2	802	0.3	8.8	4
Ridley	RIDL	958	4.3	9.8	4
Thunder	THUN	412	3.0	6.4	4
Willow	WILL	870	8.2	3.7	4

of 12 chemical/physical variables, the initial date of the open-water period, and a classification index for vegetation zone. Species richness, species heterogeneity, and total cell densities for all taxa in each sample also were calculated and treated as a separate set of variables for regression analysis. Species heterogeneity was expressed by the Shannon measure of information (Pielou 1975), and species richness was specified as the number of species in a sample of 500 cells. In addition, the niche breadth of each taxon (B_i) was calculated from the expression:

$$B_i = \exp \left[- \sum_{j=1}^k \left(\frac{p_{ij}}{R_i} \right) \log_e \left(\frac{p_{ij}}{R_i} \right) \right],$$

where p_{ij} is the proportional abundance of the i -th taxon in the j -th sample, k is the number samples, and

$$R_i = \sum_{j=1}^k p_{ij}.$$

In this case, values of B_i ranged from 1, when a taxon was present in only one sample, to 55, when it had the same relative abundance in all of the 55 samples.

The chemical/physical variables of the environmental data matrix were analyzed by a principal components analysis (Ludwig and Reynolds 1988). For this analysis, the data were centered, and each variable was standardized by the corresponding standard deviation. An examination of the component loadings indicated that the 12 variables could be reduced to four interpretable components that accounted for 75.6% of the total variance. Relationships between phytoplankton cell density, species richness, and species heterogeneity, and the environmental variables were analyzed by regression analysis. For this analysis, cell density, species richness, and species heterogeneity were designated as the response variables and the explanatory variables were the four principal components derived from the environmental data.

Relationships between the taxonomic composition of the phytoplankton and environmental variables were investigated by ordination methods (Jongman et al. 1987). Preliminary analysis of the species-abundance data indicated that a unimodal response model was appropriate for this purpose and that correspondence analysis without detrending (CA) and canonical correspondence analysis (CCA) provided satisfactory approaches to the analysis of distributional patterns and environmental gradients. For the ordination analyses, cell densities were relativized and expressed as a proportion of the total density in each sample. This

transformation gave the samples equal weight, so lakes with relatively high cell densities did not dominate the analysis. Cell densities also were transformed to their logarithm and relativized, but the results of this analysis were no more interpretable than the analysis without the log transformation. Consequently, the results obtained for the relativized data without the log transformation were used for the presentation below.

To examine similarities in the phytoplankton flora among lakes, the species-abundance matrix was analyzed by CA. In this analysis, the lake (sample) ordinations were displayed, and the axes were scaled to approximate chi-square distances. CCA was used to reveal relative positions of individual taxa and lakes along gradients of selected environmental variables. CCA generated a constrained ordination by selecting linear combinations of environmental variables that maximized the dispersion of species scores along the ordination axes. In this case, the axes were scaled so that the species points were at the centroid of the samples in which they were found, and inter-species distances approximated their chi square distances. A Monte Carlo permutation test for a forward selection of environmental variable was performed to help determine a minimum set of variables that explained the species-abundance data. All ordination analyses and permutation tests were performed by the computer program CANOCO (ter Braak 1987, 1990).

RESULTS

Environmental Variables

A principal components analysis of the 12 chemical/physical variables reduced the interpretable pattern to four independent components of variance (Table 3.2). The first four principal components included the highest component loading (i.e., the correlation between component scores and the variable) for each variable and accounted for 75.6% of the total variance. The first principal component had high-positive loadings (>0.7) for total Kjeldahl N, pH, alkalinity, conductivity, and temperature, and a high-negative loading (<-0.7) for elevation. The second principal component, with high-positive loadings (>0.8) for depth and surface area, was an expression of lake size. The component loadings for the third and fourth principal components clearly indicated that these variables represent concentrations of phosphorus (orthophosphate-P and total phosphorus) and ammonia-N, respectively.

Table 3.2. Principal components analysis of environmental data collected from 51 lakes in North Cascades National Park in the summer of 1989. The analysis included 55 samples of 12 chemical/physical variables. Each variable was standardized by subtracting the mean from each observation and dividing by the corresponding standard deviation. The highest loading for each variable is indicated in bold type.

Variable	Variable Acronym	Component Loadings			
		PC1	PC2	PC3	PC4
Total Kjeldahl-N (mg/l)	TKN	0.82	0.02	0.02	0.07
Total Phosphorus (mg/l)	TP	0.45	0.04	-0.72	0.37
Orthophosphate-P (mg/l)	PO4	0.13	-0.04	-0.90	-0.12
Nitrate-N (mg/l)	NO3	-0.46	0.56	0.14	0.07
Ammonia-N (mg/l)	NH3	0.31	0.11	-0.18	-0.88
pH (standard units)	PH	0.75	0.19	0.07	0.18
Alkalinity (mg/l)	ALKA	0.87	0.26	0.11	0.01
Conductivity (μ S/cm)	COND	0.87	0.26	0.14	0.02
Elevation (m)	ELEV	-0.76	-0.12	-0.19	0.16
Depth (m)	DEPTH	-0.32	0.84	-0.10	-0.05
Area (ha)	AREA	-0.12	0.92	-0.06	0.07
Temperature ($^{\circ}$ C)	TEMP	0.73	-0.16	0.14	0.08
Eigenvalue		4.49	2.09	1.48	1.01
% Variance Explained		37.44	17.39	12.32	8.42

Community Properties

To examine relationships between the environmental variables and three aspects of phytoplankton community structure, total cell density, species richness, and species heterogeneity were regressed against the first four principal components of the chemical/physical variables. For this analysis, the components and response variables were standardized to zero mean and unit variance. Therefore, the explanatory variables were orthogonal, and the standardized regression coefficients were the simple correlation coefficients between each component and the response variable.

The regressions of total cell density, species richness, and species heterogeneity against the four environmental variables were significant ($P < 0.05$), but the multiple correlation coefficients were not particularly high: 0.56, 0.42, and 0.43, respectively (Table 3.3). The strongest relationship revealed by the regression models was between total cell density and the elevation-temperature-alkalinity-total Kjeldahl-N component ($P < 0.01$); there were no significant correlations between cell density and lake size, phosphorus concentration, or ammonia concentration (components 2, 3, and 4). Both species richness and species heterogeneity exhibited a significant positive correlation with the phosphorus component ($P < 0.02$), but were uncorrelated with the other components.

Taxonomic Composition of the Phytoplankton

Ninety-three taxa were observed in the 55 phytoplankton samples during the counting procedure. The relative abundance of each taxon, based on cell density and expressed as a percentage of the total relative abundance (%TRA), was calculated from the expression:

$$\%TRA_i = 100 \left[\frac{\sum_{j=1}^k p_{ij}}{55} \right], \quad \text{where } p_{ij} \text{ was the proportional abundance of the } i\text{-th taxon in the } j\text{-th}$$

sample and k was the total number of samples, 55 in this case. When a taxon was a recognizable entity, but could not be identified to species, or in some cases to genus, it was given an identification number and a drawing was made for future reference. Forty-one taxa had total relative abundance values less than 0.1% and were eliminated from the ordination analysis. Taxa that could be identified to the genus or species level and had relative

Table 3.3. Parameter estimates and ANOVA table for the regression of total cell density, species richness, and species heterogeneity against the first four principal components of the environmental variables listed in Table 3.2. The table also includes a multiple correlation coefficient for each model (R), test statistic (F), and associated probability (P) for each regression.

Response Variable: Total Cell Density			
R = 0.56 F = 5.74 P = 0.001			
Variable	Standardized Coefficient	t-value	P
PC1	0.51	4.34	<0.001
PC2	0.01	0.09	0.931
PC3	-0.05	-0.42	0.678
PC4	0.23	2.00	0.054

Response Variable: Species Richness			
R = 0.42 F = 2.72 P = 0.040			
Variable	Standardized Coefficient	t-value	P
PC1	0.24	1.84	0.072
PC2	0.10	0.80	0.425
PC3	0.32	2.53	0.015
PC4	0.09	0.68	0.502

Response Variable: Species Heterogeneity			
R = 0.43 F = 2.88 P = 0.032			
Variable	Standardized Coefficient	t-value	P
PC1	-0.10	-0.79	0.436
PC2	0.19	1.51	0.139
PC3	0.37	2.90	0.006
PC4	0.06	0.50	0.619

abundance values greater than 0.1% are listed in Table 3.4. These taxa accounted for 80% of the total relative abundance in the species-abundance data matrix.

Of the 52 taxa with relative abundance values greater than 0.1%, there were 3 diatoms (Bacillariophyceae), 19 chrysophytes (Chrysophyceae), 14 chlorophytes (Chlorophyta), 4 cryptomonads (Cryptophyta), 8 cyanobacteria (Cyanophyta), 3 dinoflagellates (Pyrrhophyta), and 1 taxon of unknown taxonomic position. The corresponding relative abundances of these groups of taxa in the entire data set were 1.73% (diatoms), 19.63% (chrysophytes), 29.75% (chlorophytes), 3.25% (cryptomonads), 36.68% (cyanobacteria), and 1.54% (dinoflagellates). Taxa with the highest relative abundance values (Table 3.4) were *Aphanocapsa delicatissima* (14.0%), *Chlorella* (10.8%), and unidentified species of *Diogenes* (8.1%). Other prominent taxa in the samples were *Chromulina parvula* (4.0%), *Chromulina* sp. (5.7%), *Ochromonas sphagnalis* (2.1%), *Ochromonas* sp. (3.6%), *Aphanothece clathrata* (5.9%), *Gloeocapsa gelatinosa* (3.1%), and *Synechocystis* sp. (4.7%). Of the ten taxa identified to the genus or species levels with relative abundance values greater than 2%, seven of these were found in 34 or more lakes (Table 3.4). However, only five of these seven taxa (*Chlorella* sp., *Chromulina* sp., *Ochromonas* sp., *Rhodomonas* sp., and *Aphanocapsa delicatissima*) had niche breadth values greater than 20. *Aphanothece clathrata*, a species with the fourth highest relative abundance value (5.7%), had a relatively low niche breadth (6.3), and was found in only nine lakes, eight of which were in the forest vegetation zone. *Ochromonas sphagnalis*, with a relative abundance of 2.1%, also had a very low niche breadth (2.7) and was found only in three subalpine lakes. Most of the rarer taxa in Table 3.4 (%TRA < 2%) had relatively low niche breadth values, although a few of these occurred in 18 or more lakes and had niche breadth values greater than 12 (*Chlamydomonas globosa*, *Oocystis solitaria*, *Chlorocloster* sp., *Chrysomonadales* sp., *Rhodomonas* sp., and *Peridinium inconspicuum*).

At the level of taxonomic division, cyanobacteria had the highest relative abundance in the entire data set, i.e., the data pooled and considered as a single sample. *Aphanocapsa delicatissima* was the most abundant taxon in the pooled data, followed by unidentified species of *Chlorella* and *Diogenes*. The density of cyanobacteria decreased with increasing lake elevation; corresponding proportional densities of cyanobacteria in the vegetation zones were 49.3% (low-forest), 39.5% (high-forest), 34.1% (subalpine), and 24.8% (alpine; Table 3.5). Relative abundances of chrysophytes ranged from 25.7% (high forest) to 34.3% (subalpine). The relative abundances of chlorophytes ranged from 12.9% (low forest) to 25.0% (high forest).

Table 3.4. List of taxa found in phytoplankton samples collected from 51 lakes in North Cascades National Park in the summer of 1989. The table also includes the relative abundances of each taxon in all samples (see text), the number of lakes in which a taxon was observed (B_o), and niche breadth values for each taxon (B_i). Taxa that could not be identified to the genus level and taxa with a relative abundance of less than 0.1% were eliminated from the table.

Taxon	Acronym	Relative Abundance (%)	Occurrence (B_o)	Niche Breadth (B_i)
DIVISION CHRYSOPHYTA				
<u>Class Bacillariophyceae:</u>				
<i>Cyclotella comita</i> (Ehr.) Kütz.	CYCO	0.60	8	4.71
<i>Cyclotella stelligera</i> Cl. u. Grun.	CYST	0.92	2	1.99
<i>Stephanodiscus</i> sp.	STEP	0.21	10	6.14
<u>Class Chrysophyceae:</u>				
<i>Chlorocloster</i> sp.	CHCL	0.45	29	15.43
<i>Chromulina parvula</i> Conr.	CRPA	4.00	34	14.72
<i>Chromulina</i> sp.	CRSP	5.66	49	37.20
<i>Chrysoikos skujae</i> Naunerck	CSKU	0.27	9	5.93
<i>Chrysomonadales</i> sp.	CMON	0.15	18	15.52
<i>Dinobryon bavaricum</i> Imhof	DIBA	0.88	5	2.27
<i>Dinobryon borgei</i> Lemm.	DIBO	0.11	2	1.74
<i>Dinobryon divergens</i> Imhof	DIDI	0.36	3	2.09
<i>Dinobryon sertularia</i> Ehr.	DISE	0.66	1	1.00
<i>Kephyrion ovale</i> (Lackey) H-P	KEOV	0.37	13	7.03
<i>Ochromonas ovalis</i> Dolf.	OCOV	0.33	8	6.01
<i>Ochromonas pinguis</i> Conr.	OCPI	0.75	1	1.00
<i>Ochromonas sphagnalis</i> Conrad	OCSP	2.09	5	2.65
<i>Ochromonas</i> sp.	OCHR	3.63	38	23.60
<i>Ophiocytium cochleare</i> A. Br.	OPCO	1.36	6	2.71
<i>Ophiocytium parvulum</i> (Perty) A. Br.	OPPA	0.24	15	7.96
DIVISION CHLOROPHYTA				
<i>Chlamydomonas globosa</i> Snow	CHGL	0.20	18	12.46
<i>Chlorella</i> sp.	CHSP	10.83	45	28.53
<i>Crucigenia tetrapedia</i> (Kirch.) West & West	CRTE	0.20	1	1.00
	ELGE	0.33	12	5.44
<i>Elakatothrix gelatinosa</i> Wille.	FRDR	0.31	10	6.57
<i>Franceia droescheri</i> (Lemm) G.M. Smith	HALA	0.28	3	1.90
<i>Haematococcus lacustris</i> (Girod.) Rostafinski	OOPA	0.23	4	1.81
	OOSO	1.26	24	15.40
<i>Oocystis parva</i> West & West	OOSU	0.38	8	5.70
<i>Oocystis solitaria</i> Wittrock	PSEU	1.23	21	7.86
<i>Oocystis submarina</i> Lagerheim	SPSP	0.20	8	4.64
<i>Pseudokephyrion</i> sp.				
<i>Spondylosium</i> sp.				
DIVISION CRYPTOPHYTA				
<i>Chroomonas acuta</i> Utermöhl	CHRA	1.74	22	2.74
<i>Cryptomonas ovata</i> Ehren.	CRYP	0.23	14	7.38
<i>Rhodomonas minuta</i> var. <i>nanoplantica</i> Skuja	RHMI	0.33	2	1.83
	RHSP	0.95	39	27.02
<i>Rhodomonas</i> sp.				

Table 3.4. (continued)

Taxon	Acronym	Relative Abundance (%)	Occurrence (B _o)	Niche Breadth (B _i)
DIVISION CYANOPHYTA				
<i>Anabeana</i> sp.	ANSP	0.55	11	6.19
<i>Aphanocapsa delicatissima</i> West & West	APDE	13.97	41	27.36
<i>Aphanothece clathrata</i> West & West	APCL	5.93	9	6.26
<i>Chroococcus minimus</i> (Keissl.) Lemm.	CHRO	0.19	3	2.36
<i>Dactylococcopsis acicularis</i> Lemm.	DAAC	0.18	3	1.81
<i>Diogenes</i> sp.	DIOG	8.08	34	19.53
<i>Gloeocapsa gelatinosa</i> Kutz.	GLOE	3.07	22	13.36
<i>Synechocystis</i> sp.	SYNE	4.71	38	14.24
DIVISION PYRRHOPHYTA				
<i>Amphidinium luteum</i> Skuja	AMLU	0.18	11	7.53
<i>Gymnodinium</i> sp.	GYMN	0.73	18	7.14
<i>Peridinium inconspicuum</i> Lemm	PEIN	0.63	28	14.51

Table 3.5. Proportional abundances of NOCA phytoplankton by vegetation zone in 51 lakes.

Division ¹	Vegetation Zone ²	Proportional Abundance (%)
BAC	1	7.90
CHL	1	18.95
CHR	1	30.40
CRY	1	2.20
CYN	1	24.75
PYR	1	7.75
UNK	1	8.05
BAC	2	1.59
CHL	2	20.12
CHR	2	34.34
CRY	2	0.90
CYN	2	34.13
PYR	2	1.49
UNK	2	7.42
BAC	3	1.09
CHL	3	25.03
CHR	3	25.71
CRY	3	2.53
CYN	3	39.51
PYR	3	0.61
UNK	3	5.52
BAC	4	3.51
CHL	4	12.87
CHR	4	27.02
CRY	4	3.36
CYN	4	49.29
PYR	4	0.76
UNK	4	3.20

¹BAC (diatoms), CHL (chlorophytes), CHR (chrysophytes), CRY (cryptomonads), CYN (cyanobacteria), PYR (dinoflagellates), and UNK (unknown).

²1 (alpine), 2 (subalpine), 3 (high-forest), 4 (low-forest).

Dinoflagellates and diatoms reached their maximum relative abundances in the alpine zone (7.8% and 7.9%, respectively), but were relatively rare (<4%) in the other vegetation zones. The relative abundances of cryptomonads were low in all vegetation zones, ranging from 0.9% (subalpine) to 3.4% (low forest). Only 14 taxa (15.1%) were found in 20 or more samples. These taxa had niche breadth values of 12 or greater and only 5 taxa had values greater than 20 (range: 23.6 - 37.2). These results suggest that most taxa were limited in distribution and that their densities were uneven among lakes.

Lake Ordinations

CA sample (lake) ordinations were determined by the species-abundance data only, and therefore, the scores revealed the best latent gradients, independent of the measured environmental variables. CA axes 1 and 2 accounted for 19.5% of the variance in the phytoplankton data and had eigenvalues of 0.65 and 0.57, respectively. A plot of axis 2 against axis 1 (Figure 3.2) indicated that most alpine and subalpine lakes, and some of the lakes in the forest zones, had very similar phytoplankton floras. A group of lakes in the forest zones (NERT, THUN, RIDL, WILL, HOZO, and LS2) separated from the other lakes and were located on the right side of axis 1. These lakes all exhibited high relative abundances of *Aphanothece clathrata* >50% of the total cell density in the case of WILL, RIDL, THUN, and NERT. The four alpine lakes were located on the left side of the tight cluster of subalpine lakes. Taxa that had high relative abundances in two or more of these lakes were *Chromulina parvula*, *Gymnodinium* sp., *Aphanocapsa delicatissima*, and *Diogenes* sp. The flora in 4 subalpine lakes was different enough to separate these lakes from the large cluster of alpine, subalpine and some lakes in the forested zones. *Ochromonas sphagnalis* had high relative abundances in three of these outliers (MR9, TRIL, and BEAR), particularly in TRIL where it represented 70.6% of the total cell density. TRIU separated from the cluster primarily because of a high relative abundance (41.4%) of *Ochromonas pinguis*. KLAW was dominated by the cryptomonad *Chroomonas acuta* (76.2%) and the chrysophyte *Chromulina parvula* (13.8%); the relative abundance of *C. acuta* in the other lakes was less than 4.5%. Because of the high dominance of *C. acuta*, Klaw was considered an extreme outlier, and consequently, was eliminated from the final ordination analysis and direct gradient analysis (see below).

NOCA Phytoplankton - 1989 CA Lake Ordinations

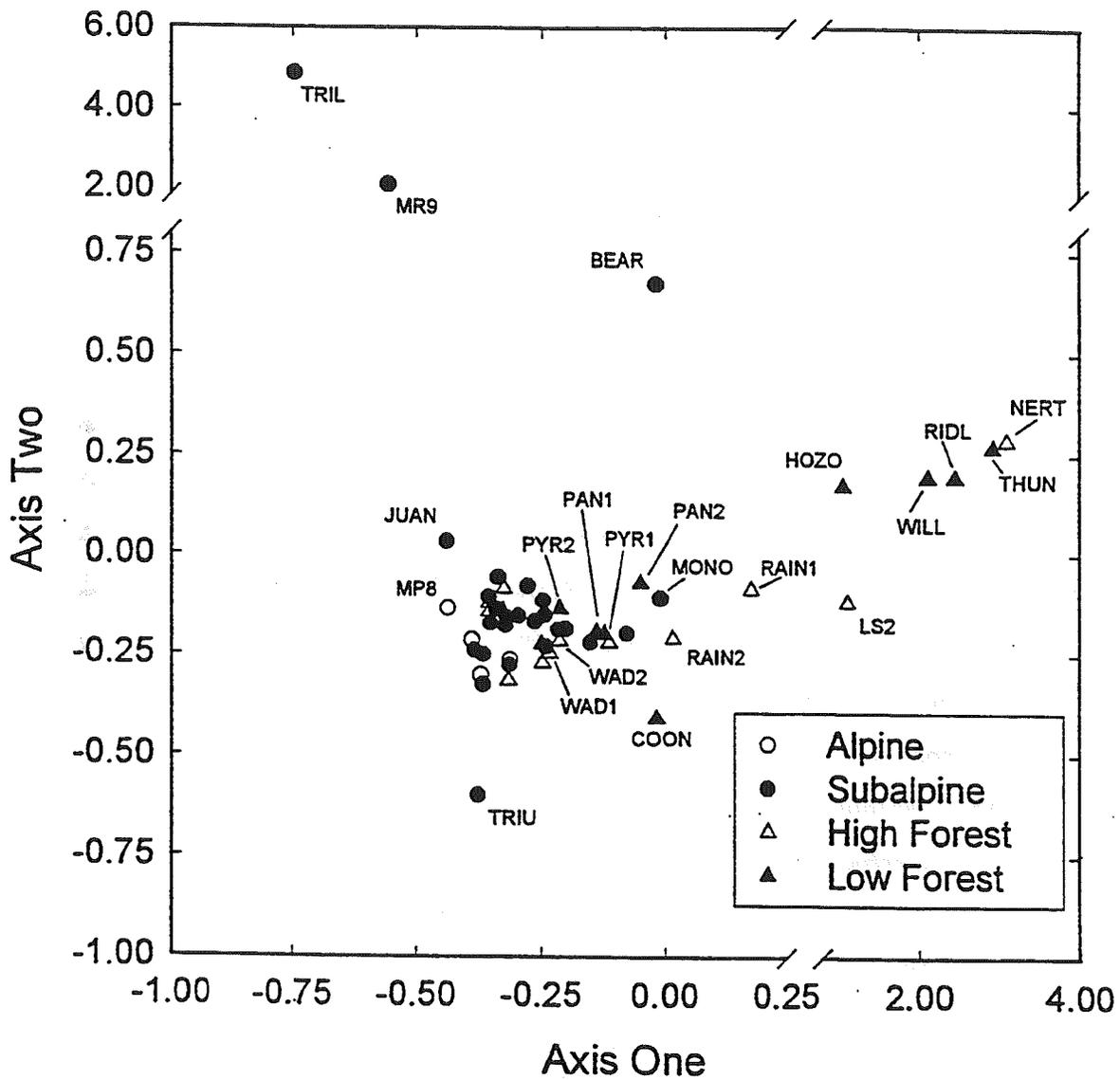


Figure 3.2. Ordination of 54 phytoplankton samples from 50 NOCA lakes by correspondence analysis (CA). Samples were obtained from July through September 1988, and are classified by vegetation zone.

Direct Gradient Analysis

CCA selected linear combinations of a subset of the environmental data that maximized the dispersion of the species scores along ordination axes. Therefore, in contrast to the indirect gradient analysis, the ordination axes were determined by both the species-abundance data and the environmental data matrix. An unrestricted Monte Carlo permutation test was used for the forward selection of environmental variables (ter Braak 1987, 1990). For this test, the number of permutations were 999, and the usual significance level of $P < 0.05$ was relaxed to $P < 0.2$ in order to retain at least one variable from each of the components of variance determined by the principal components analysis (Table 3.6). The degree to which each of the environmental variables individually affected dispersion of taxa along the first ordination axis was revealed by the corresponding eigenvalue (Table 3.6). The permutation tests reduced the number of variables to eight, each of which had an inflation factor of less than 3.0 (Table 3.6). Some variables with eigenvalues of 0.25 or greater (e.g., pH, temperature, and conductivity) were eliminated from the analysis because of colinearity with either alkalinity or total Kjeldahl-N.

CCA axis 1 had an eigenvalue of 0.44 and accounted for 29% of the species-environment relation. The multiple correlation between the environmental variables and this axis was 0.86, which represented some improvement in comparison to the results from the indirect gradient analysis (Table 3.6). CCA axis 1 had relatively high intraset correlations with alkalinity (0.80), total Kjeldahl-N (0.79), and elevation (-0.78). CCA axis 2 had a relatively low eigenvalue (0.25) and was less interpretable. This axis had weak correlations with alkalinity (0.44), time of iceout (-0.49), and nitrate concentration (0.45). CCA axes 1 and 2 together accounted for 45.5% of the species-environment relation.

A graphic display of sample (lake) ordinations in relation to the eight environmental variables indicates that the most prominent gradient is characterized by changes in alkalinity and total Kjeldahl-N with elevation (Figure 3.3). Temperature, conductivity, and pH, variables eliminated from the analysis, have relatively high correlations with alkalinity and/or total Kjeldahl-N (>0.6), and also exhibit changes along an elevation gradient. For interpretative purposes, the axes in Figure 3.3 are scaled so the length of each arrow representing the environmental variables indicates the magnitude of the correlation between these variables and the ordination axes. Therefore, the projections of the sample points on each environmental arc reveals the approximate positions of the lakes relative to the corresponding environmental gradient. For example, RIDL, WILL, THUN, and HOZO are lakes in the low- forest zone and have above average alkalinities and concentrations of total Kjeldahl-N. Lakes with relatively

Table 3.6. List of environmental variables evaluated by an unrestricted Monte Carlo permutation test for the CCA of NOCA phytoplankton data. The first eight variables in the table were retained for the analysis. Relationships between each variable and ordination scores predicted by the regression model are expressed by intraset correlation coefficients.

Variable	Eigenvalue	Inflation Factor	Intraset Correlation Coefficients	
			Axis 1	Axis 2
ALKA	0.35	2.16	0.80	0.44
TKN	0.34	2.54	0.79	0.26
ELEV	0.31	2.14	-0.78	-0.08
ICEO	0.26	2.43	-0.48	-0.49
NH3	0.22	1.24	0.54	-0.18
NO3	0.19	1.34	-0.38	0.45
PO4	0.16	1.23	0.16	0.12
DEPTH	0.16	1.28	-0.10	-0.18
COND	0.32	<i>These variables were eliminated from the CCA analysis</i>		
TEMP	0.25			
PH	0.25			
AREA	0.16			
TP	0.12			
Multiple R			0.86	0.78

CCA Ordination of Lakes and Environmental Variables

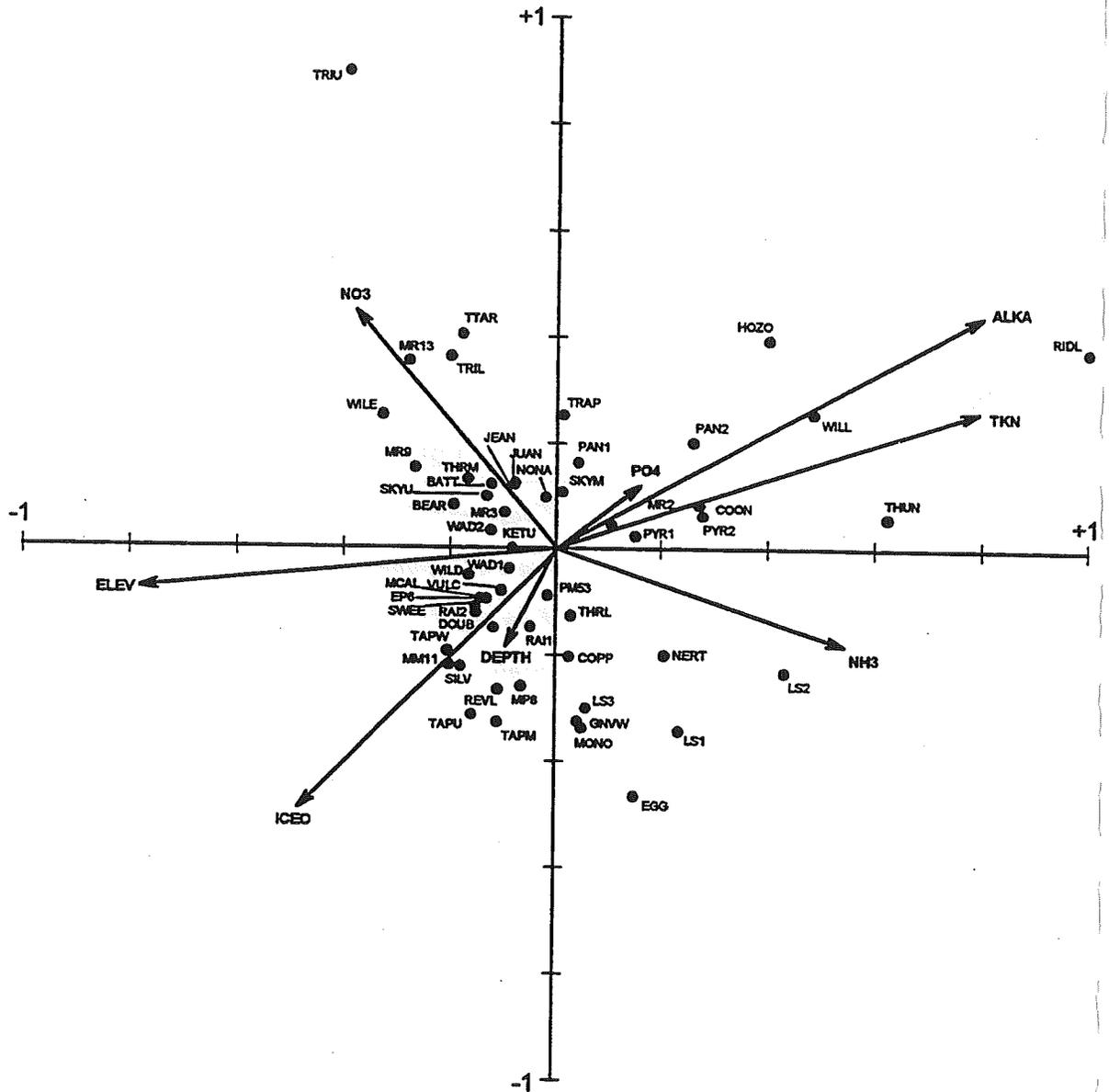


Figure 3.3. CCA ordination of phytoplankton samples from 50 NOCA lakes and associated environmental variables. The length of each arrow indicates the relative importance of the corresponding environmental variable. Positions of the sample along each gradient are determined by the perpendicular projection of the sample points on to each environmental arrow.

high concentrations of nitrate-N include TRIU, MR13, TTAR, TRIL, and WILE. These lakes are found in either the subalpine or alpine zones. Environmental arrows can be extended through the origin in an opposite direction to explore the projection of samples with below average values of each variable. If this is done, the ordination predicts that many of the subalpine lakes and alpine lakes have below average alkalinities and concentrations of total Kjeldahl-N and orthophosphate-P. Furthermore, multiple samples from RAIN (1 and 2), PYRA (1 and 2), PAN (1 and 2), and WADD (1 and 2) indicate that seasonal changes of the taxonomic composition of phytoplankton assemblages were minimal in this study (Figures 3.2 and 3.3).

CCA ordinations of phytoplankton taxa that correspond to the lake configuration (Figure 3.3) are presented in Figure 3.4. Many of these taxa are located near the origin, indicating that they tend to occur in lakes with near average values for the environmental variables. A projection of the taxa points on the alkalinity and total Kjeldahl-N arrows place *Aphanothece clathrata* and *Oocystis submarina* at the positive end of the corresponding gradient, followed by *Cryptomonas ovata*, *Chlorocloster* sp., *Ophiocytium cochleare*, *Cyclotella comta*, *Crucigenia tetrapedia*, and *Oocystis parva*. Five taxa (*Ochromonas sphagnalis*, *Dinobryon borgei*, *Haematococcus lacustris*, *Ochromonas pinguis*, and *Dactylococcopsis acicularis*) tend to occur in lakes with above average concentrations of nitrate-N, whereas *Dinobryon sertularia* has high relative abundance in lakes with a high concentration of ammonia-N.

CCA also was used to examine relationships between phytoplankton taxa and the vegetation zones. For this analysis, the environmental data consisted of a matrix of zeros and ones that represented four nominal variables, one for each of the four zones (alpine, subalpine, high forest, and low forest). These zones were determined by the nature of the surrounding vegetation of each lake and were closely related to lake elevation (Lomnický 1996). A two-dimensional plot of taxa scores and the centroids for each vegetation zone provided an indication of the affinities of the taxa for lakes in the different zones (Figure 3.5).

The general configuration of taxa in Figure 3.5 depicts a continuous distribution of taxa ordered from the subalpine zone, through the high-forest zone to the low-forest zone, with a large number of taxa occurring primarily in both the subalpine and high-forest zones. Taxa most closely associated with the alpine zone were *Haematococcus lacustris*, *Dactylococcopsis acicularis*, and *Chroomonas acuta*; whereas *Oocystis parva*, *Oocystis submarina*, *Cryptomonas ovata*, *Cyclotella comta*, *Aphanothece clathrata*, and *Ophiocytium cochleare* had a greater tendency to occur in the low-forest zone. Five taxa (*Cyclotella stelligera*, *Gymnodinium* sp., *Peridinium inconspicuum*, *Rhodomonas minuta*, and *Chromulina parvula*) that occurred in the

CCA Ordination of Taxa and Environmental Variables

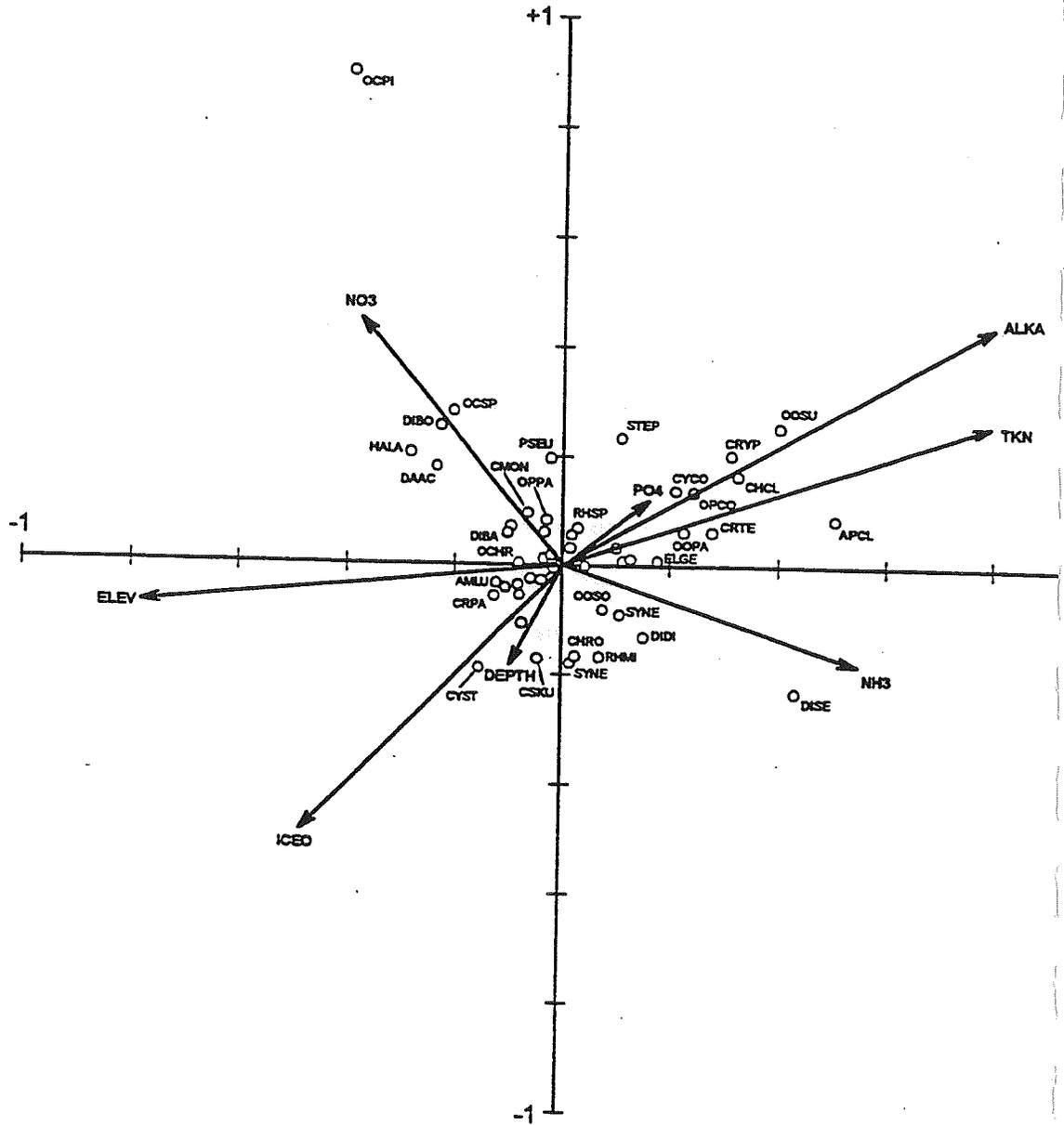


Figure 3.4. CCA ordination of phytoplankton taxa from 50 NOCA lakes and associated environmental variables. Relative positions of the taxa along each environmental gradient are determined by the perpendicular projection of the taxa points on to each environmental arrow.

CCA Ordination of Taxa and Vegetation Zones

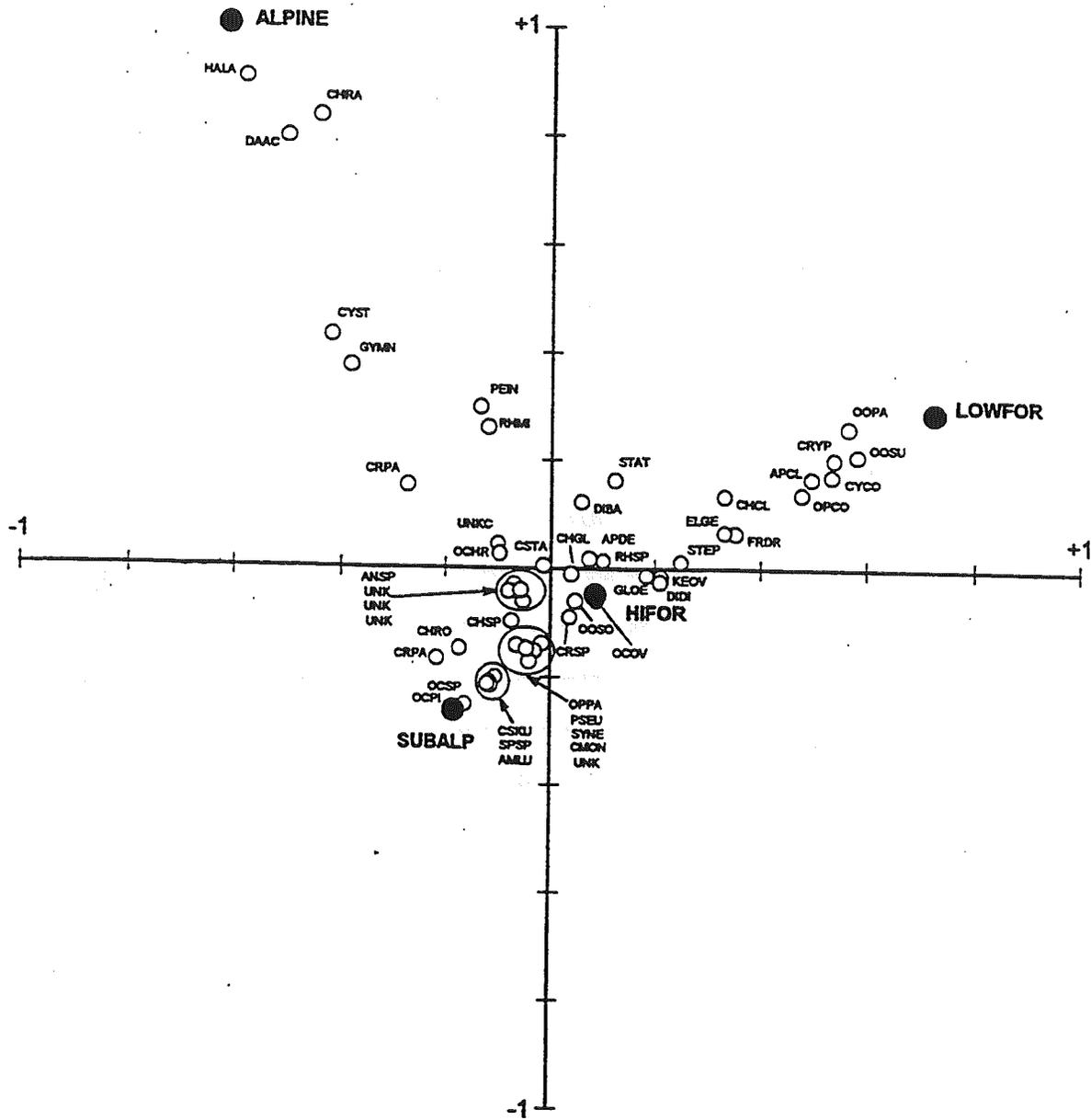


Figure 3.5. CCA ordination of phytoplankton taxa from 50 NOCA lakes in relation to four vegetation zones. In this case, the four zones were treated as nominal variables, and their relationship with the phytoplankton flora is expressed by a biplot of taxa scores and the centroids for the vegetation zones.

subalpine and high-forest zones were able to extend their distribution into some of the alpine lakes.

DISCUSSION

NOCA lakes exhibited a water-quality gradient that gradually changes from low to high concentrations of dissolved solids and total Kjeldahl-N with decreasing lake elevation and increasing water temperature. Phytoplankton cell densities increased along this elevation-temperature gradient. These results suggest that phytoplankton productivity tends to increase with decreasing lake elevation and the associated changes in water quality. Furthermore, both species richness and species heterogeneity were positively correlated with the concentration of total phosphorus. The absence of an elevation-temperature gradient for phosphorus gradient in NOCA lakes was due to the relatively high concentrations of phosphorus in lakes in the low-forest zone and in alpine lakes and 1 subalpine lake receiving turbid-glacial effluent. When the turbid lakes were removed from the database, total phosphorus increased in concentration with decreasing lake elevation (data not shown). Therefore, it appears that the importance of phosphorus to species richness and species heterogeneity was dependent on elevation and temperature in non-glacially-turbid lakes, but for all lakes, both variables were independent of elevation and temperature from a statistical standpoint. These results were similar to those of Nauwerck (1994) who showed that phytoplankton species richness increased with increasing concentration of phosphorus in northern Swedish Lapland lakes.

The increase in relative abundance of cyanobacteria with decreasing lake elevation at NOCA probably corresponds to the associated increases of pH, alkalinity, conductivity, and concentrations of nutrients (Gerloff and Skoog 1957, Fogg et al. 1973, Shapiro 1973, Healey 1982). A similar distributional pattern (based on biomass) was observed by Nauwerck (1994) for cyanobacteria in northern Swedish Lapland lakes. In high-elevation lakes in Mount Rainier National Park (MORA) and Olympic National Park (OLYM), which are located approximately 150 km from NOCA in western Washington, cyanobacteria were low in density (Larson et al. 1994; 1995). However, the elevation range of the lakes sampled at MORA and OLYM was narrow in comparison to NOCA study lakes in that alpine and low-forest lakes were not sampled. In contrast to cyanobacteria, Sandgren (1991) demonstrated that chrysophytes decrease in abundance with increased lake pH, alkalinity, conductivity, and nutrient concentrations. The results of the present study and those of Nauwerck (1994) were consistent with this

generalization. At MORA and OLYM, the phytoplankton assemblages of most lakes were dominated by chrysophytes (Larson et al. 1994, 1995). Although chlorophytes can be a common component of the phytoplankton assemblages in oligotrophic montane lakes (Kosswig 1967, Priddle and Happey-Wood 1983, and Nauwerck 1994), at NOCA, chlorophytes were common in alpine, subalpine and high-forest lakes, but were much less common in low-forest lakes. Cryptophytes, diatoms, and dinoflagelates were in low relative abundances in NOCA lakes, a result consistent with those of Rosen (1981), Nauwerck (1994) and Larson et al. (1994, 1995).

Lake (sample) CA ordinations based on algal taxonomic composition indicated that most alpine and subalpine lakes and some lakes in the forest zones had similar phytoplankton floras. However, four subalpine lakes and a group of low-forest lakes and high-forest lakes, located on the far-right side of axis 1 (Figure 3.2), were separated from the other lakes. Although the second axis was not correlated with the environmental variables, increasing scores on the first axis of the CA were correlated with decreasing elevation and increasing temperature, alkalinity, and concentrations of total Kjeldahl-N and ammonia-N. This gradient was practically the same as the environmental gradient associated with phytoplankton cell densities, and provided additional evidence of an increase in lake productivity with decreasing elevation.

The CCA analysis provided evidence that the spatial distributions of some phytoplankton taxa were correlated with either an elevational-alkalinity-total Kjeldahl-N gradient or dissolved nitrogen (nitrate-N or ammonia-N) gradients (Figure 3.4). A chrysophyte, a chlorophyte, a cryptophyte and a cyanobacteria at the positive end of the elevation-alkalinity-total Kjeldahl-N gradient had their highest relative abundances in low-forest lakes (RIDL, WILL, HOZO, THUN, COON, PAN2; Figure 3.3). Five taxa, 3 chrysophytes, a chlorophyte, and a cyanobacteria, were at the positive end of the nitrate-N gradient. These taxa had their highest relative abundances in alpine and subalpine lakes (WILE, MR13, TTAR, TRIL, TRIU; Figure 3.3). The highest relative abundance of one chrysophyte was associated with above average concentrations of ammonia-N in high-forest lakes (NERT, LS1, LS2; Figure 3.3). The other taxa were located near the origin of the two axes, indicating that their associations with the environmental variables were similar. Thus, within the broad context of the alkalinity-total Kjeldahl-N-elevation gradient, these results suggest that nitrogen species were important variables for identifying taxonomic differences of the phytoplankton assemblages among NOCA lakes.

CCA also was used to examine relationships between phytoplankton taxa and the vegetation zones (Figure 3.5). A continuous distribution of taxa was shown from the subalpine zone through the low-forest zone, with a large number of taxa occurring primarily in the

subalpine and high-forest zones. Three species (*Haematococcus lacustris*, *Dactylococcopsis acicularis*, and *Chroomonas acuta*) occurred primarily in alpine lakes, whereas five taxa (*Cyclotella comita*, *Gymnodinium* sp., *Peridinium inconspicuum*, *Rhodomonas minuta* var. *nanoplantica*, and *Chromulina parvula*) co-occurred in the alpine, subalpine, and high-forest zones. At the division level, cyanobacteria, chlorophytes, diatoms, and cryptophytes were present in lakes at all vegetation zones. However, *Cyclotella stelligera* had its highest relative abundances in alpine and subalpine lakes, and therefore its location on the ordination was between the two vegetation zones. A similar argument could be made for the chrysophyte, *Chromulina parvula*. Overall, chrysophyte taxa had their highest relative abundances in the subalpine lakes and lakes in the forest zones. Dinoflagellate taxa had their highest relative abundances in alpine and subalpine lakes.

Phytoplankton assemblages in NOCA lakes were similar in the average number of taxa per sample and ranges of cell densities to phytoplankton in lakes in OLYM (Larson et al. 1995) and MORA (Larson et al. 1994), but were lower in the total number of taxa (Table 3.7). Duration of the open-water period, geographic location within a study area, lake size, elevation, flushing rate, transparency, water temperature, hardness, and nutrients were some of the variables determined to be important factors influencing the taxonomic structure of phytoplankton assemblages in these and other studies, e.g., Pechlaner (1971), Earle et al. (1986), Earle et al. (1987), Stoddard (1987), Paloheimo and Fulthorpe (1987), Morris and Lewis (1988) and Knoechel and Cambell (1988). In NOCA lakes, elevation and associated changes in water quality and concentrations of nutrients appeared to be the primary factors influencing the taxonomic structure of the phytoplankton assemblages. The role of zooplankton grazing on the densities and taxonomic structures of phytoplankton assemblages in NOCA lakes remains unresolved.

ACKNOWLEDGMENTS

This study was funded by the National Park Service and the USGS - Forest and Rangeland Ecosystem Science Center. Park staff assisted with logistical aspects in the field. Stanford Loeb, Stanley Dodson, John O'Brien, and William Neill provided constructive comments and suggestions of the project. Stanford Loeb, Stanley Dodson and James Petranka reviewed the manuscript. Ruth Jacobs edited and Rebecca Berkey typed the manuscript.

Table 3.7. Total number of phytoplankton taxa, average number of taxa per sample, and the ranges of cell densities in lakes in Olympic National Park, Mount Rainier National Park, and NOCA (1989).

Location	Total number of taxa	Average number of taxa/sample	Range of density per ml	Author
Olympic N.P. (7 lakes)	172	---	311-5216	Larson et al. 1995
Mt. Rainier N.P. (27 lakes)	203	20	1047-5505	Larson et al. 1994
NOCA	93	19	55-38926 ¹ 55-6744 ²	Present work

¹55 samples.

²52 samples.

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Chapter 4.

**Interaction of Diaptomid Copepods and
Introduced Trout In High-Elevation Lakes
In the Pacific Northwest, USA**

A version of this chapter was published as: Liss, W.J., G.L. Larson, E.A. Deimling, L.M. Ganio, R.L. Hoffman, and G.A. Lomnicky. 1998. Factors influencing the distribution and abundance of diaptomid copepods in high-elevation lakes in the Pacific Northwest, USA. *Hydrobiologia* 379:63-75.

Abstract - We investigated the impact of abiotic factors and trout density on distribution and abundance of diaptomid copepods in high-elevation lakes in North Cascades National Park Service Complex (NOCA), Washington, USA. The most common large diaptomid, *Diaptomus kenai* (mean length = 1.88 mm), was able to persist over a wide range of abiotic factors, but the smaller herbivorous diaptomid, *D. tyrrelli* (mean length = 1.18 mm), was restricted to shallow lakes (maximum depth ≤ 10 m) with relatively high concentrations of total Kjeldahl nitrogen and total phosphorous. There was a significant negative relationship between the density of *D. tyrrelli* and the density of large diaptomids, which could imply interaction between large and small diaptomids. The abundance of large diaptomids was significantly lower in shallow lakes with high densities of reproducing trout (>250 fish ha⁻¹) than in fishless lakes, in deep lakes with reproducing trout, or in lakes where trout do not reproduce and are periodically stocked with fry at low densities (average 179 fry ha⁻¹). In lakes where nutrient concentrations were suitable for *D. tyrrelli*, the small diaptomid was often abundant when trout density was high and large diaptomids were either absent or in low abundance. Our research suggests that trout density, nutrient concentration, and lake depth influence food web interactions in high lakes in NOCA.

INTRODUCTION

Calanoid copepods are important members of limnetic communities in high-elevation lakes, sometimes dominating the crustacean zooplankton fauna (Reed and Olive 1958; Patalas 1964; Anderson 1971, 1974, 1980; Stoddard 1987; Larson et al. 1994). Abiotic factors such as lake elevation (Reed and Olive 1958; Patalas 1964; Anderson 1971, 1974), water temperature (Allan and Goulden 1980; Byron et al. 1984; Walters et al. 1987), lake area and depth (Anderson 1974; Stoddard 1987), and water chemistry (Sprules 1975; Byron et al. 1984; Stoddard 1987) influence calanoid distribution and abundance. These factors directly affect survival and reproduction or indirectly affect species abundance by altering food quality and quantity or the intensity of biotic interactions (DeMott 1989; Gliwicz and Pijanowska 1989).

Vertebrate predators, especially fish, can prey selectively on larger, more visible prey and cause a shift in community composition from large-bodied to small-bodied forms (Zaret 1980; Gliwicz and Pijanowska 1989). Impacts of fish predators on zooplankton can vary along a gradient of increasing predation intensity, with the greatest effects occurring at the highest

predation intensities (Brocksen et al. 1970; Stenson 1972; Gliwicz and Prejs 1977; Langeland 1978; Dodson 1979; Zaret 1980; McQueen et al. 1986; Kerfoot 1987; Post and McQueen 1987).

Calanoid copepods are frequently among the larger-bodied taxa of zooplankton in high-elevation lakes (Anderson 1971, 1974; Stoddard 1987; Starkweather 1990), are often highly pigmented (Hairston 1978; Starkweather 1990), and so may be susceptible to fish predation. Anderson (1972) reported disappearance of the large diaptomid *D. arcticus* from a lake in the Canadian Rockies following repeated introductions of trout. Several studies have documented the presence of large diaptomids in fishless oligotrophic lakes and their absence in lakes with fish in the same geographic area (Anderson 1974; Northcote and Clarotto 1975; Dodson 1979; Stoddard 1987; Starkweather 1990; Donald et al. 1994). Trout stocked in fishless lakes in British Columbia led to reduction in body size of *D. kenai* but had little effect on its density (Northcote et al. 1978). Anderson (1980) reported that cutthroat trout (*Oncorhynchus clarki*), a common species in high-elevation lakes in the west, often co-occurs with large diaptomids.

Large and small calanoids also may be vulnerable to predation from invertebrate predators, especially *Chaoborus* (Dodson 1970; Sprules 1972; Fedorenko 1975a,b; Northcote et al. 1978; Giguere 1979; Anderson 1980) and *Gammarus* (Anderson 1980). Large diaptomid copepods, in turn, can prey on other zooplankton including smaller copepods (Anderson 1967, 1970; Dodson 1970, 1974; Sprules 1972; Maly 1976; Giguere 1979; Paul et al. 1995) and rotifers (Williamson and Butler 1986; Williamson 1987; Paul et al. 1995). Zaret's (1980) predation submodel II predicts that high densities of vertebrate predators would eliminate large predatory copepods thus allowing smaller herbivorous copepods to increase in abundance. Such "top-down" effects in food webs may be most evident in oligotrophic systems such as high lakes (Gliwicz and Prejs 1977; Dodson 1979; McQueen et al. 1986; Neill 1987; Carney 1990), particularly when vertebrate predation intensity is high and persistent (Dodson 1979; Gliwicz and Pijanowska 1989).

In this chapter we investigate factors influencing distribution, abundance, and patterns of co-occurrence of large and small diaptomid copepods in high-elevation lakes in North Cascades National Park Service Complex (NOCA), Washington, USA. Specifically, we investigated 1) the effect of abiotic factors on distribution and abundance of large (*Diaptomus kenai* and *D. arcticus*) and small (*D. tyrrelli*) diaptomid copepods, and 2) whether the abundance of large and small

diaptomids copepods differed between lakes with high trout densities, lakes with low trout densities, and lakes with no trout.

METHODS

Study Area

NOCA is located along the crest of the Cascade Range of northern Washington, USA. There is no road access to NOCA lakes; they can be reached only by hiking or helicopter. Many lakes in NOCA were stocked with trout (primarily cutthroat, *Oncorhynchus clarki*, and rainbow, *O. mykiss*) during this century for recreational angling (Jarvis 1987).

Abiotic and Biotic Samples

Twenty-seven lakes were sampled two or more times in at least one year from 1989 through 1993 (Table 4.1). All zooplankton analyses were conducted using only these 27 lakes except determination of the effect of fish on large diaptomids in deep lakes (maximum depth >10 m), where four deep lakes that were sampled only once per year were also included to increase sample size. Lakes were sampled between ice-out (mid-June to early July) and the onset of inclement weather in the fall (approximately mid-September).

For each lake on each sample date, three replicate vertical tows were taken with a 20-cm diameter number 25 (64 μm mesh) zooplankton net. In 1989 only one vertical tow was taken in each lake. Vertical tows were taken near the deepest part of the lake. The net was lowered to within 1 m of the lake bottom and towed upward at a rate of about 0.5 m sec^{-1} . In the field, samples were preserved in 5% neutral sugar formalin (Haney and Hall 1973). In the laboratory, samples were split using a Folsom plankton splitter. A split portion was poured into a settling chamber and left to settle for 24 h. Organisms were identified to species. Organisms of each species were counted using an inverted microscope at 100X magnification. Body length (metasome and urosome) of adult diaptomids of each taxon was measured.

All analyses were conducted using densities of adult diaptomids. In Kettling and Juan de Fuca lakes, adult stages of copepods were not present when the lakes were sampled. For these lakes, density of stage V copepodids was used in data analysis because we were able to infer the species of diaptomid from the body length of the copepodids.

Table 4.1. Occurrence of adult diaptomid copepods in North Cascades National Park Service Complex, 1989-1993.

Lake	Elev (m)	<i>D. kenai</i>	<i>D. arcticus</i>	<i>D. tyrrelli</i>
Large Copepods Only				
MR 12	1981	X		
Dee Dee	1922	X		
MR 16	1908	X		
MR 11	1863	X		
MR 13-1	1800	X	X	
MR 13-2	1789	X		
U. Tapto	1755	X		
M. Tapto	1754	X		
W. Tapto	1754	X		
Rainbow	1717	X		
M. Waddell	1642	X	X	
Doubtful	1642	X		
Waddell	1504	X		
Trapper	1270	X	X	
LS 2	1243	X		
Small Copepods Only				
Juanita	2033			X
U. Triplet	1988			X
MR 2	1873			X
Dagger	1679			X
Kettling	1639			X
Large and Small Copepods				
L. Triplet	1931	X		X
MR 9	1813	X	X	X
McAlester	1680	X		X
LS 1	1241	X		X
U. Panther	1031	X		X
L. Panther	1031	X		X
Pyramid	802	X		X

Mean density of adult diaptomids at each sampling occasion was calculated from the replicate vertical tows. Analysis was conducted using the maximum of the mean densities of each species in each year. If a lake was sampled over several years, maximum annual densities were averaged over all sample years.

Lake depth was determined with a handheld sonar gun (Manta FR-100) along transects parallel and perpendicular to the long axis of a lake. Surface area was estimated by digitizing 7.5 min USGS topographic maps. Elevation was determined from these maps.

Each time a lake was sampled, water temperature ($^{\circ}\text{C}$) was measured at 1 m intervals from the lake surface to 30 m over the deepest point in the lake using an Omega 871A digital thermo-couple. Since very few of the lakes in this study were thermally-stratified, water temperature at a depth of 1 m was used in data analysis. Water samples were taken from a depth of 1 m over the deepest point in a lake using a 1.5 l Van Dorn bottle. Frozen filtered and unfiltered water samples were transported to the Cooperative Chemistry Analytical Laboratory at Oregon State University for chemical analysis (total Kjeldahl nitrogen, total phosphorus, ortho-phosphorus-P, nitrate-N, ammonia-N, pH, alkalinity, conductivity). For each lake, values for each of these variables were averaged first for each year and then over all years that the lake was sampled.

To assess fish effects on large copepods, lakes were divided into three categories: lakes with high trout densities, lakes with low trout densities, and lakes with no trout. Lakes were placed in these categories based on mark-recapture estimates and information from stocking records. Absence of fish from lakes was verified by gill-netting, angling, and snorkeling.

In nine lakes, fish density was estimated by mark-recapture (Liss et al. 1995; Gresswell et al. 1997). Fish were captured by angling with lures and artificial flies, fin-clipped, and released. Injured fish (e.g., bleeding from gills or tongue) were not marked. Post-release mortality was probably low because only one of 24 marked fish died after being held overnight in enclosures. Recapture with variable mesh gill nets usually began the day following completion of marking. Abundance was estimated by a single-census Petersen estimator and 95% confidence limits were calculated (Ricker 1975). Both angling and gill-netting tended to under-sample small fish. Consequently, abundance estimates pertain to fish ≥ 177 mm total length. Trout this size are about 2-3 years old (Liss et al. 1995).

Some NOCA lakes in which trout do not reproduce are periodically stocked with fry at low densities. Lack of reproduction was determined from NOCA records (Jarvis 1987) and verified by field observations including lack of suitable spawning habitat, failure to observe fry or smaller fish, and little variation in age and size structure of captured fish. In NOCA, the average density of trout fry stocked in higher-elevation lakes (≥ 1100 m) from 1976 to 1993 was 179 fish ha⁻¹ (range 59.8-375 fish ha⁻¹; N=37) and the average interval between stocking was >5 years (unpublished records, North Cascades National Park Service Complex, 2105 Highway 20, Sedro Wooley, WA, 98284).

Lower elevation lakes (<1100 m) in NOCA are more accessible to anglers and tend to be more heavily stocked than higher elevation lakes. Fry have been stocked at high densities in two small, low elevation (1031 m) lakes, Upper Panther Lake (surface area = 0.1 ha, mean stocking density = 833 fry/ha, range = 500-1000 fry/ha, mean stocking interval = 3.5 years, N = 3) and Lower Panther Lake (surface area = 0.2 ha, mean stocking density = 933 fry/ha, range = 750-1500 fry/ha, mean stocking interval = 3.7 years, N = 4). Upper Panther Lake is located a few meters away from Lower Panther Lake. In late summer 1990 fish were removed from both lakes by intensive gill-netting and angling. Trout density in these lakes was low at the time of fish removal. The lakes are small and clear and absence of trout following removal procedures was verified by observation from shore and snorkeling. In fall 1990, Lower Panther Lake was stocked with fry (*O. clarki*) at a relatively high density (750 fry/ha) whereas Upper Panther Lake was not stocked with fish. Zooplankton and abiotic factors were sampled in Upper and Lower Panther Lakes prior to fish removal in 1990 and in each subsequent summer from 1991 through 1993. In 1992, trout densities in Lower Panther were estimated by mark-recapture.

Larval salamanders (*Ambystoma macrodactylum* and *A. gracile*) were present in many fishless lakes (Liss et al. 1995; Tyler et al. 1998). Salamander predation can influence the structure of zooplankton communities (Dodson 1970, 1974; Sprules 1972; Giguere 1979; Morin 1987). Larval salamander densities were assessed by snorkeling the perimeter of lakes and carefully searching through bottom materials (Liss et al. 1995; Tyler et al. 1998).

Data Analysis

Correlations between densities of diaptomid species and abiotic factors were investigated by determining Pearson Correlation Coefficients and testing the coefficients for significance

($\alpha = 0.05$). To assess the influence of abiotic factors on presence-absence of large and small diaptomid species, lakes were grouped into three categories: lakes with only large diaptomids, lakes with only small diaptomids, and lakes with both large and small diaptomids (Table 4.1). To determine if levels of abiotic factors differed among the three categories, pairwise comparisons of each abiotic factor between categories were conducted using the Kruskal-Wallis test. A Bonferroni adjustment (Miller 1981) was used to maintain the Type I error rate at 0.05 for the three pair-wise comparisons of each abiotic factor. Each comparison was judged to be significantly different if $P \leq 0.017$ ($0.05 \div 3$). We also compared levels of abiotic factors between lakes with the small diaptomid (a group composed of lakes with only the small diaptomid and lakes with both large and small diaptomids) and lakes where the small diaptomid was absent (lakes with only large diaptomids) using the Kruskal-Wallis test ($\alpha = 0.05$).

To determine the relative effects of abiotic factors and large diaptomids on small diaptomid density we fitted a general linear regression model with the logarithm of small copepod density as the dependent variable and selected abiotic factors, large diaptomid density, and lake category (i.e., lakes with only large diaptomids, lakes with only small diaptomids, and lakes with both large and small diaptomids) as independent variables. We compared the slopes of the relationships between the dependent and independent variables for each lake category.

To determine if large copepod density differed statistically between the three trout density categories (i.e., high trout density, low trout density, no fish), pairwise comparisons of large copepod densities between the categories were conducted using the Kruskal-Wallis test. Again, to maintain the Type I error rate at 0.05 we used a Bonferroni adjustment and individual comparisons were judged to be significantly different if $P \leq 0.017$.

Interannual trends in the densities of large copepods in both Upper and Lower Panther Lakes were assessed using regression analysis. To determine if large diaptomid density differed between Upper Panther Lake and Lower Panther Lake, we compared diaptomid densities between the two lakes using the Kruskal-Wallis test ($\alpha = 0.05$).

RESULTS

Diaptomid Copepod Distribution

Five species of diaptomid copepods were found in high-elevation lakes in NOCA. Three of these species, *Diaptomus kenai*, *D. arcticus*, and *D. tyrrelli*, were relatively common and were pigmented red. The other species, *D. lintoni* and *D. leptopus*, were found only in a few low elevation lakes. Analyses were restricted to the three most common species. *Chaoborus* rarely occurs in NOCA high lakes.

Diaptomus kenai (mean length = 1.88 mm) and *D. arcticus* (mean length = 2.04 mm) are the largest zooplankters in NOCA. *Diaptomus kenai* is widely distributed among NOCA lakes while *Diaptomus arcticus* is much less common (Table 4.1).

Lakes with only large diaptomids (*D. kenai* and *D. arcticus*) were principally higher elevation (≥ 1243 m) lakes (Table 4.1). *Diaptomus arcticus* and *D. kenai* co-occurred in some of these lakes. The small herbivorous (Olenick 1983) copepod, *D. tyrrelli* (mean length = 1.18 mm), occurred allopatrically, and sympatrically with large diaptomids, across the entire range of lake elevations (Table 4.1).

Abiotic Factors

The density of *D. kenai* was not significantly correlated with any abiotic factor ($P > 0.05$). In contrast, the density of *D. tyrrelli* had a significant positive correlation with total Kjeldahl nitrogen (TKN; $r = 0.54$, $P = 0.0036$) and total phosphorus (TP; $r = 0.53$, $P = 0.0044$). Correlations of abiotic factors with *D. arcticus* abundance were not determined because the number of lakes with this species was small.

The small copepod was found only in shallow lakes (≤ 10 m) that maintained high epilimnion concentrations of TKN (≥ 0.05 mg l⁻¹; Figure 4.1) and TP (≥ 0.007 mg l⁻¹; Figure 4.2). In contrast, both deep and shallow lakes with low TKN and TP supported only *D. kenai*. *Diaptomus kenai* also occurred allopatrically and sympatrically with *D. tyrrelli* in shallow lakes with high TKN and TP.

Results of comparisons of abiotic factors among lakes with only large diaptomids, lakes with only the small diaptomid, and lakes with both large and small diaptomids were consistent with the correlation analysis. Lakes with only large diaptomids were significantly lower in TKN,

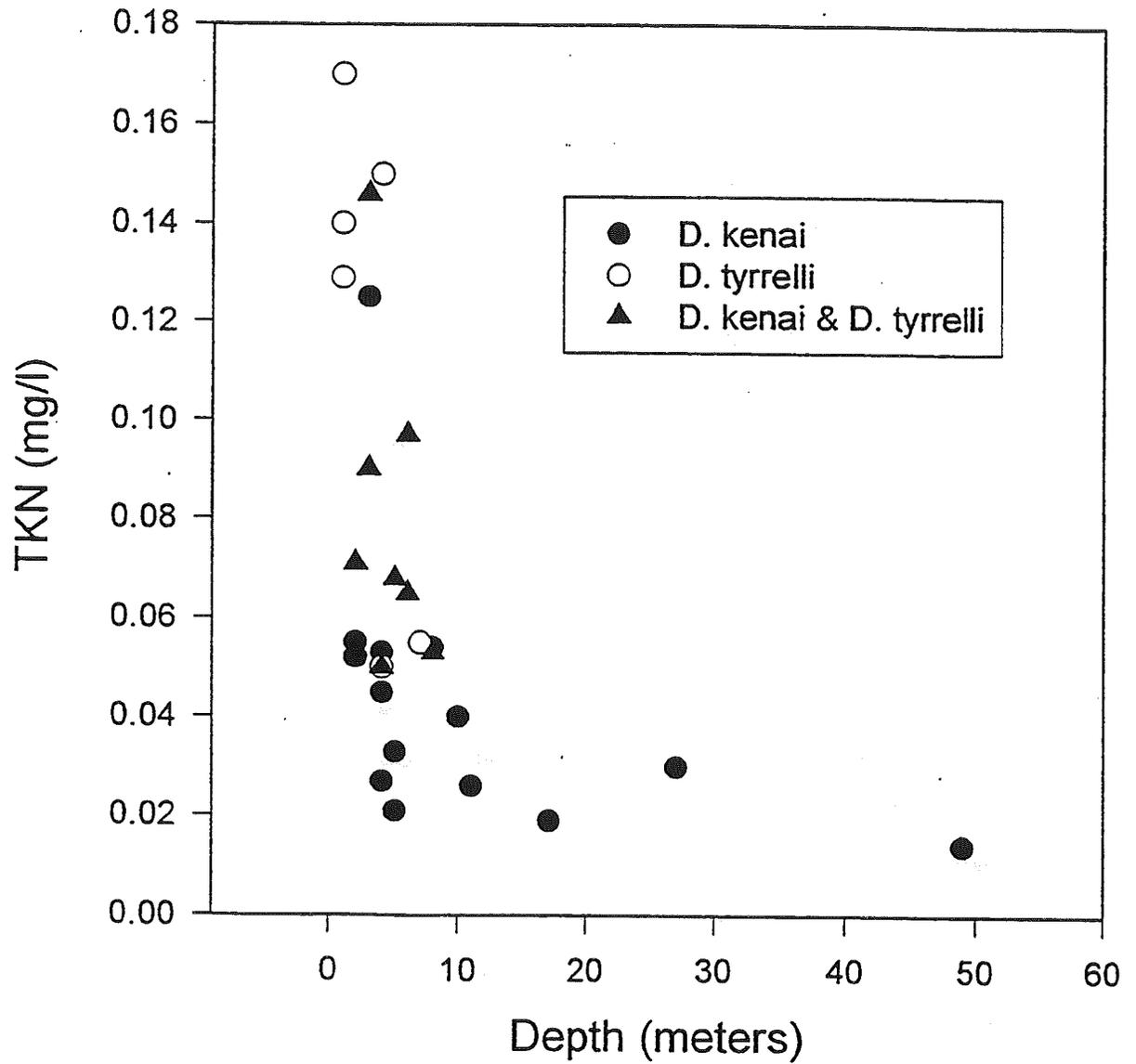


Figure 4.1. Relationship between total Kjeldahl nitrogen (TKN) concentration at one m depth and maximum lake depth for lakes with only *Diaptomus kenai*, lakes with only *D. tyrrelli*, and lakes with both *D. kenai* and *D. tyrrelli*.

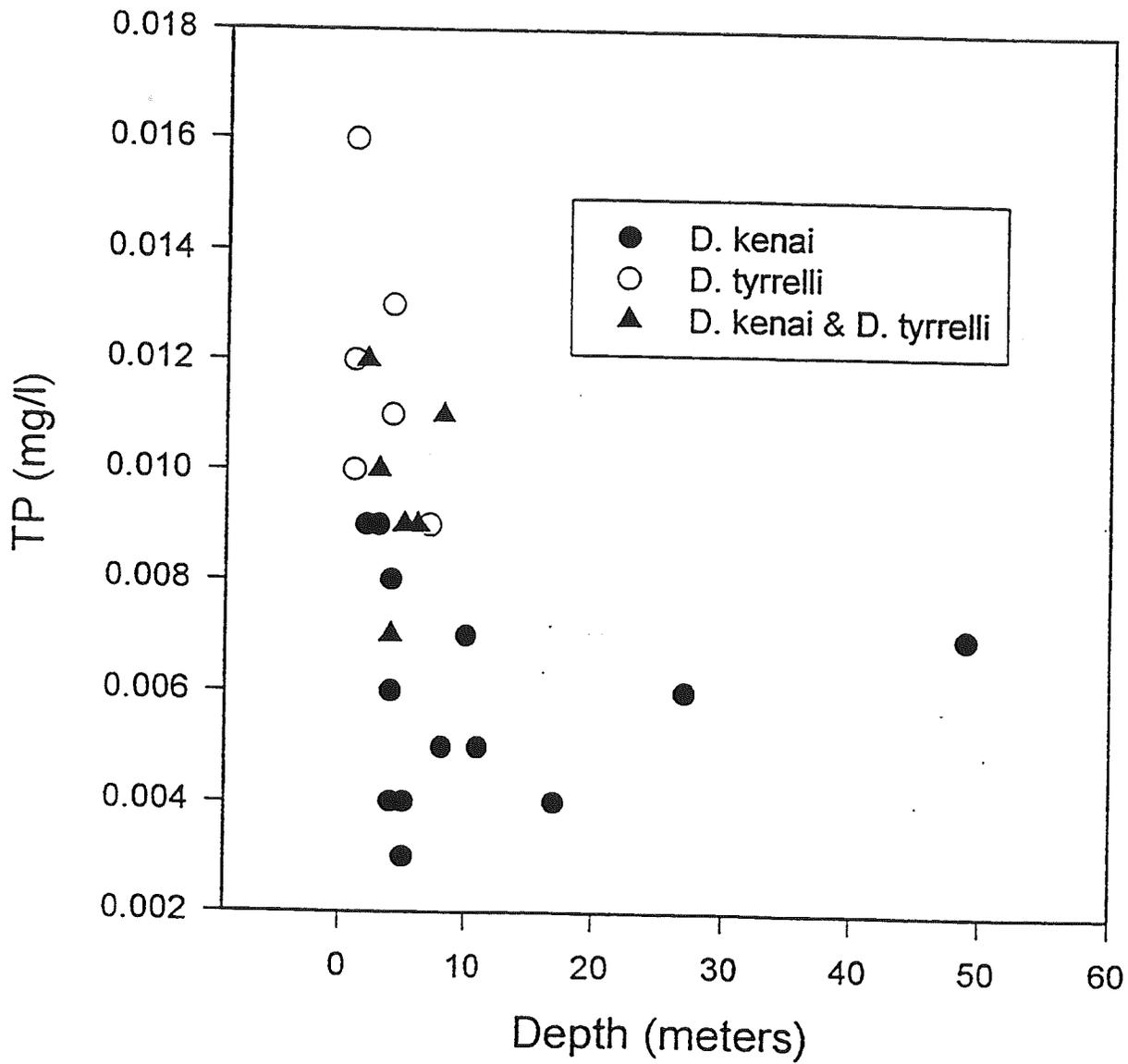


Figure 4.2. Relationship between total phosphorus (TP) concentration at one m depth and maximum lake depth for lakes with only *Diaptomus kenai*, lakes with only *D. tyrrelli*, and lakes with both *D. kenai* and *D. tyrrelli*.

TP, and conductivity than were lakes with only the small diaptomid (Table 4.2; Kruskal-Wallis, $P = 0.0034$, 0.0009 , and 0.017 , respectively). Lakes with only large diaptomids also were significantly lower in TKN and TP than were lakes with both large and small diaptomids (Table 4.2; Kruskal-Wallis, $P = 0.0033$ and 0.0009 , respectively). However, there were no significant differences in any abiotic factor between lakes with only the small diaptomid and lakes with both large and small diaptomids (Table 4.2; Kruskal-Wallis, $P > 0.017$). Lakes with the small diaptomid (a group composed of lakes with only the small diaptomid and lakes with both large and small diaptomids) had significantly higher TKN, TP, pH, alkalinity, and conductivity than lakes where the small diaptomid was absent (Kruskal-Wallis, $P \leq 0.012$).

Copepod Relationships

Relationships between densities of large and small diaptomids was assessed using only lakes with nutrient levels within the range of occurrence of *D. tyrrelli* ($\text{TKN} \geq 0.05 \text{ mg l}^{-1}$, $\text{TP} \geq 0.007 \text{ mg l}^{-1}$, $N = 17$, Figures 4.1 and 4.2). For this set of lakes, we determined if TKN, TP, and large diaptomid density were related to *D. tyrrelli* density in each lake category (i.e., large diaptomids only, small diaptomids only, large and small diaptomids) using a general linear regression model.

For lakes with $\text{TKN} \geq 0.05 \text{ mg l}^{-1}$ and $\text{TP} \geq 0.007 \text{ mg l}^{-1}$, the slopes of the relationships between *D. tyrrelli* and TKN and TP were not significantly different from zero ($P > 0.05$) for each lake category nor were there any significant differences in mean values of TKN and TP between lake categories. Thus, although TKN and TP may have influenced whether *D. tyrrelli* was present in lakes, TKN and TP had no statistically discernible effect on *D. tyrrelli* density in lakes where their concentrations were $\geq 0.05 \text{ mg l}^{-1}$ and $\geq 0.007 \text{ mg l}^{-1}$, respectively. For lakes with both large and small diaptomids, there was a significant negative relationship between the logarithm of *D. tyrrelli* density and large diaptomid density ($P = 0.0003$, B in Figure 4.3). *Diaptomus tyrrelli* densities were highest in lakes where large copepods were absent (S in Figure 4.3).

Fish Predation

Lakes with high fish densities all supported reproducing populations of trout (Table 4.3). In these lakes, trout density was $>450 \text{ fish ha}^{-1}$ in all lakes except Kettling Lake. Fish density

Table 4.2. Mean and range (in parentheses) of abiotic factors in lakes with only large diaptomids, lakes with only small diaptomids and lakes with both large and small diaptomids in North Cascades National Park Service Complex, WA, USA.

	TKN (mg/l)	TP (mg/l)	PO ₄ (mg/l)	NO ₃ (mg/l)	NH ₃ (mg/l)	pH (mg/l)	ALKA (mg/l)	COND (µmbos/cm)	TEMP (°C)	ELEV (m)	DEPTH (m)	AREA (ha)
Large Copepods	0.042 (0.014-0.125)	0.006 (0.003-0.009)	0.001 (0-0.002)	0.008 (0-0.035)	0.005 (0.003-0.008)	6.982 (6.200-7.633)	1.885 (0.466-5.819)	17.210 (4.220-52.674)	13.105 (10.250-15.500)	1702 (1243-1981)	11.273 (2.000-49.000)	6.473 (0.300-59.000)
Small Copepods	0.116 (0.050-0.170)	0.012 (0.009-0.016)	0.002 (0.001-0.002)	0.004 (0.001-0.016)	0.006 (0.005-0.009)	7.294 (6.350-7.850)	2.588 (0.670-4.825)	39.546 (17.338-16.050)	13.463 (11.450-16.050)	1847 (1639-2033)	3.250 (1.200-7.000)	1.550 (0.200-4.000)
Large and Small Copepods	0.080 (0.050-0.146)	0.009 (0.007-0.012)	0.001 (0.001-0.002)	0.002 (0.001-0.005)	0.007 (0.004-0.012)	7.391 (6.646-7.967)	4.115 (0.991-8.067)	36.455 (7.573-72.130)	14.696 (12.700-18.400)	1242 (412-1931)	5.063 (2.100-8.800)	1.463 (0.100-5.000)

Table 4.3. Maximum summer densities of large copepods and *Diaptomus tyrrelli* in lakes with high fish densities, lakes with low fish densities, and lakes with no fish, North Cascades National Park Service, Complex, WA, USA. Cutthroat (*Onchorynchus clarkii*, CT) and rainbow (*Onchorynchus mykiss*, RB) trout were present in the lakes with fish.

Lake	Fish Density (no/ha)	Trout Species	Large Copepod Density (no/l)	<i>D. tyrrelli</i> Density (no/l)
High Fish Density ($< 10\text{m}$ maximum depth)				
Dagger	640 (392-1104) ^a	CT	0.000	6.11
U. Triplet	459 (311-707) ^a	CT	0.000	0.52
L. Triplet	477 (256-976) ^a	CT	0.090	2.22
McAlester	526 (326-1054) ^a	CT	0.030	4.72
	483 (274-933) ^a			
Rainbow	500 (236-1154) ^a	RB	0.002	0.00
	649 (307-960) ^a			
Kettling	254 (201-320) ^a	RB	0.000	10.46
LS2	617 (417-806) ^a	CT	0.120	0.00
	640 (332-1347) ^a			
Low Fish Density				
MR 9	100 (88-111) ^b	RB/CT	0.490	2.91
MR 11 (1991, 1992)	123 ^b	RB	1.190	0.00
MR 13-2	190 (58-363) ^b	RB	1.310	0.00
DeeDee	125 ^b	CT	0.820	0.00
MR 16	92 (63-140) ^a	CT	4.110	0.00
LS1 (1990)	28 ^c	CT	3.400	0.004
No Fish				
MR 12 ^d	0.0		0.330	0.00
MR 11 (1990)	0.0		1.260	0.00
MR 13-1 ^d	0.0		1.040	0.00
U. Tapto	0.0		1.230	0.00
M. Tapto	0.0		0.760	0.00
W. Tapto	0.0		1.510	0.00
M. Waddell	0.0		0.080	0.00
Waddell	0.0		0.840	0.00
Juanita ^d	0.0		0.000	15.74
MR 2 ^d	0.0		0.000	27.61
Pyramid ^d	0.0		2.330	0.73

^aFish density estimated by mark-recapture and 95% confidence limits.

^bAverage density and range of fry stocked, determined from stocking records

^cFish density estimated by Leslie Method (Ricker 1975)

^dFishless lakes with salamander densities ≥ 5.0 larvae/100 m shoreline

was relatively low in only one lake with reproducing trout (MR 16). This lake was placed in the low fish density category. Except for MR 16, trout did not reproduce in lakes with low fish densities.

Large diaptomid densities were significantly lower in shallow (maximum depth <10 m) lakes with high trout densities than in lakes with low trout densities (Table 4.3; Kruskal-Wallis, $P = 0.0026$). However, large diaptomid densities in lakes with high trout densities and in lakes with low trout densities did not differ significantly from large copepod densities in fishless lakes (Kruskal-Wallis, $P = 0.045$ and 0.07 , respectively). A possible explanation for the lack of a significant difference in large copepod densities between lakes with high trout densities and fishless lakes is predation by salamander larvae on large copepods. With the exception of LS 1, salamanders were absent or low in abundance in lakes with fish in Table 4.3 (Liss et al. 1995; Tyler et al. 1998).

When fishless lakes with salamander densities ≥ 5.0 larvae 100m^{-1} of shoreline were excluded from the analysis, there was a significant difference in large diaptomid densities between lakes with high fish densities and fishless lakes (Kruskal-Wallis, $P = 0.0063$). However, even when salamander lakes were excluded, there was still no significant difference in large copepod densities between lakes with low fish densities and fishless lakes (Kruskal-Wallis, $P = 0.42$).

Fish density could be determined effectively by mark-recapture only in lakes with relatively small surface areas (≤ 7 ha) and shallow maximum depths (≤ 10 m). To determine if lake depth influenced interaction of reproducing fish and large copepods we compared large copepod densities in deep lakes (maximum depth >10 m, $N = 6$) with reproducing trout to those in shallow lakes. Large diaptomid densities were significantly higher in deep lakes (mean large copepod density = 0.35 l^{-1} , range = $0.03\text{-}1.04 \text{ l}^{-1}$) than in shallow lakes with reproducing fish (Kruskal-Wallis, $P = 0.0004$), suggesting that lake depth influenced the interaction between large diaptomids and reproducing fish.

Diaptomus tyrrelli was present, often at high abundance, in five of the seven lakes with high trout densities (Table 4.3). The small copepod was absent from most lakes with low trout densities and from most fishless lakes.

Further evidence suggesting that high densities of reproducing trout can lead to reduction or elimination of large diaptomids is provided by comparison of lakes connected by tributary

streams. Rainbow Lake (Table 4.3) is the lowest in elevation of a series of four lakes. Small streams from the three higher elevation lakes, MR 13-1 (no fish), MR 13-2 (non-reproducing fish), and MM 11 (non-reproducing fish), flow into Rainbow Lake. MR 13-1 and MR 13-2 are only a few hundred meters from Rainbow Lake. Large copepods were relatively abundant in MR 13-1 and MR 13-2 (Table 4.3) and they were present in MM 11 (mean density = 0.44 l⁻¹, sampled once in 1989 and 1993). Chemical and physical conditions in Rainbow Lake were suitable for large diaptomids (e.g., TKN = 0.040 mg l⁻¹, TP = 0.008 mg l⁻¹, maximum depth = 10 m) and yet the lake, which supports a dense population of reproducing trout, had virtually no large copepods (Table 4.3) despite ample opportunity for colonization from all of the three higher elevation lakes. Rainbow Lake was sampled three times in 1991, twice in 1989 and 1992, and once in 1993. Adult *D. kenai* were found at low density on only one of these sample dates.

Although Upper and Lower Panther lakes have a history of high fry stocking rates, *D. kenai* was relatively abundant in both lakes (Table 4.4). There was no statistically significant difference in large diaptomid density between Lower Panther Lake, which had been stocked with a high density of fry in fall 1990, and Upper Panther Lake, which was fish-free since fall 1990 (Table 4.4, Kruskal-Wallis, $P > 0.99$).

In Lower Panther Lake, large diaptomid density was higher in 1990 prior to fry stocking than in any subsequent year (Table 4.4). However, there was no significant linear time trend in large diaptomid density in the lake ($P > 0.05$). Mark-recapture estimates indicated that fish density in Lower Panther Lake had been reduced by nearly 60% by 1992 (Table 4.4), probably due largely to angler harvest. Although quantitative estimates of fish density in 1993 are lacking, observations while snorkeling indicate that trout density was low.

In Upper Panther Lake, which had been fish-free since fall 1990, there was a significant negative interannual trend in large diaptomid density ($r = -0.42$, $P = 0.02$) caused by a very high density in 1990. This was one of the highest densities of *D. kenai* observed during the study. A decline in *D. kenai* density is contrary to what would have been expected following trout removal if fish were preying heavily on the large copepod.

Table 4.4. Densities of trout and *Diaptomus kenai* in Upper and Lower Panther Lakes and number of samples per summer (N).

Lake	Year	N	Fish Density (no/ha)	Mean Length (mm)	<i>D. kenai</i> Density (no/l)
L. Panther	1990	2	40 ^a 750 ^b	278 fry stocked	2.14
	1991	3	-	-	1.00
	1992	2	320 (195-570) ^c	263	1.36
	1993	3	few	-	1.04
U. Panther	1990	2	60 ^a	242	4.34
	1991	3	0.0		1.33
	1992	2	0.0		0.90
	1993	3	0.0		1.08

^aEstimated by Leslie method (Ricker 1975)

^bStocking density of fry

^cEstimated by mark-recapture: mean and 95% confidence limits

DISCUSSION

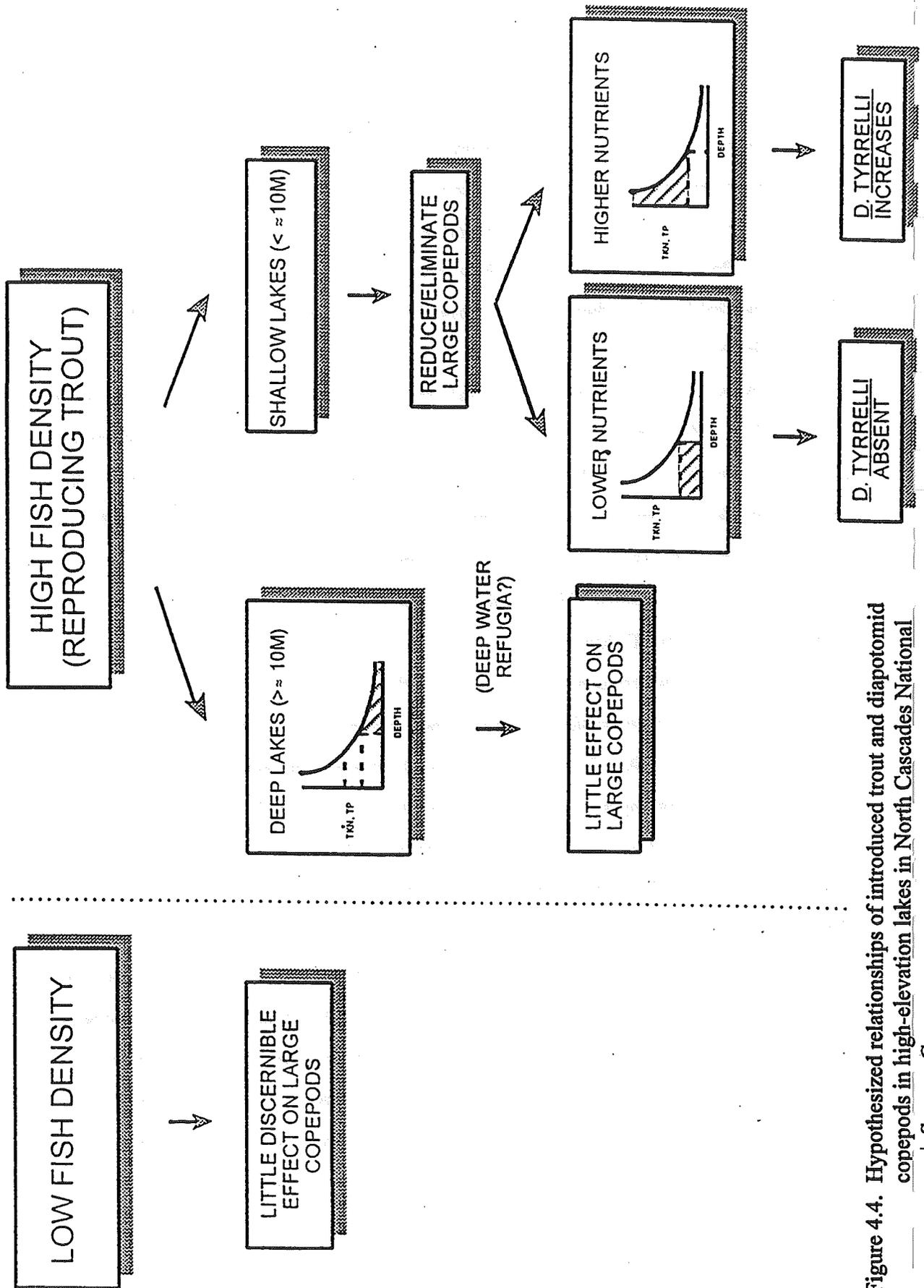
Diaptomus kenai occurred across a relatively wide range of abiotic conditions, which is consistent with its status as the most common crustacean zooplankton in NOCA (Liss et al. 1995). *Diaptomus kenai* is apparently omnivorous (Olenick 1983; Butler et al. 1989), whereas *Diaptomus arcticus* is predaceous on rotifers and other crustacean zooplankton (Anderson 1967, 1970, 1972; Paul et al. 1995). Since *Chaoborus* rarely occurs in NOCA high lakes and cyclopoid copepods are seldom abundant, the two large diaptomid species are probably the top invertebrate predators in the pelagic region of lakes.

In NOCA, *D. tyrrelli* was found only in shallow lakes with relatively high levels of TKN and TP (TKN ≥ 0.05 mg l⁻¹, TP ≥ 0.007 mg l⁻¹). Byron et al. (1984) found that spatial variation of *D. tyrrelli* within Lake Tahoe was correlated with particulate nitrogen, but not with primary productivity, invertebrate predation, or algal biomass. Across its range, *D. tyrrelli* is not restricted to shallow lakes (Anderson 1974; Olenick 1983; Byron et al. 1984), as it appears to be in NOCA, although Olenick (1983) indicated that it was restricted to the upper 2-4 m in a relatively deep British Columbia lake.

Our results are consistent with the view that: 1) introduced trout can reduce or eliminate larger, more visible diaptomid taxa from lakes (Anderson 1974, 1980; Dodson 1979; Stoddard 1987; Starkweather 1990), 2) impacts on large diaptomids varies with fish density, with the greatest effects occurring at high fish densities (Brocksen et al. 1970; Stenson 1972; Langeland 1978; Dodson 1979; McQueen et al. 1986; Post and McQueen 1987) and, 3) impacts on diaptomids due to fish introductions are most likely to be observed in shallow lakes (Gliwicz and Prejs 1977; Donald et al. 1994).

Figure 4.4 is a hypothesis of inferred interrelationships of introduced trout and diaptomid copepods in higher-elevation lakes (≥ 1200 m) in NOCA. Large diaptomid copepods were either absent or low in abundance in shallow lakes with high densities of reproducing trout. Large diaptomid abundance in lakes with low fish densities, most of which maintained non-reproducing trout, was significantly higher than in lakes with high fish densities, but was indistinguishable statistically from fishless lakes (with or without salamanders).

In deep lakes (maximum depth >10 m) with reproducing trout, large copepod densities were significantly higher than in shallow lakes. Perhaps densities of reproducing trout may not



be as high in deep lakes as in shallow lakes, or large diaptomids may find refuge in deeper water during the day and so escape predation from a visually-oriented predator such as trout (Zaret and Suffern 1976; Stich and Lampert 1981; Gliwicz and Pijanowska 1988; Donald et al. 1994).

The small diaptomid, *D. tyrrelli*, occurred in five of seven lakes with high densities of trout where large copepods were absent or low in abundance. In these lakes the small diaptomid frequently reached high abundance. In contrast, *D. tyrrelli* rarely occurred in lakes with non-reproducing fish. In fishless lakes the small diaptomid was abundant only in lakes where large copepods were absent (Juanita and MR 2; Table 4.3).

The tendency for the small diaptomid to occur at high densities in lakes with high fish densities in part may be a consequence of interaction between large and small diaptomids. We observed a significant negative relationship between large diaptomid density and *D. tyrrelli* density for lakes with levels of TKN and TP $\geq 0.05 \text{ mg l}^{-1}$ and $\geq 0.007 \text{ mg l}^{-1}$, respectively. Although the mechanisms underlying this relationship are unknown, the negative correlation could imply interaction between large and small copepods. The replacement of large predatory copepods with smaller herbivorous copepods is consistent with Zaret's (1980) predation submodel II. In a lake in the Canadian Rockies, Anderson (1972) observed large increases in *D. tyrrelli* following elimination of *D. arcticus* by trout. Dodson (1974) and Paul et al. (1995) found significantly higher densities of a small herbivorous cyclopoid copepod in experimental enclosures without *D. arcticus* than in enclosures where *D. arcticus* was present.

Olenick (1983) indicated that *D. kenai* developed more rapidly in a British Columbia lake than did *D. tyrrelli*. We noted a similar pattern in NOCA; *D. tyrrelli* adults often did not appear until late August or September, whereas we commonly found adults of large diaptomids in late July and early August. Thus, in NOCA lakes where the species co-occur, *D. tyrrelli* nauplii could be vulnerable to competition or predation from late-stage copepodids or adults of large diaptomids. Naupliar stages of diaptomids may be particularly vulnerable to both predation and interspecific competition (Anderson 1970; Maly 1976; Olenick 1983; Paul et al. 1995).

Diaptomus tyrrelli occurred only in lakes with higher concentrations of TKN and TP. Leavitt et al. (1994) present evidence that introduced trout can increase phosphorous levels and algal biomass in high-elevation lakes. Thus high fish densities could favor *D. tyrrelli* in NOCA lakes both by reducing densities of large predatory copepods and by increasing nutrient levels in the pelagic zone. However, it is unlikely that high *D. tyrrelli* densities in lakes with fish are a

consequence solely of increased nutrient cycling because the small diaptomid occurred at high densities in some fishless lakes.

The mechanism linking TKN and TP to the distribution of the small diaptomid is somewhat uncertain. TKN and TP are predictors of lake productivity (Lambou et al. 1983; Paloheimo and Fulthorpe 1987). Perhaps increased primary production resulted in greater food availability for the herbivorous small diaptomid.

Although fry were historically stocked at relatively high densities in Upper and Lower Panther Lakes, *D. kenai* was abundant in both lakes. Gliwicz and Pijanowska (1989) assert that reduction or elimination of zooplankton prey by vertebrate predators requires that predation intensity be "persistent." High predation intensity on large copepods may be relatively brief and episodic in lakes that are periodically stocked with high densities of fry if the interval between stocking is relatively long and fish mortality is high (Carpenter et al. 1985). This may be the case in the Panther lakes and it contrasts with lakes with reproducing trout where high fish densities and age and size diversity are more continually maintained. Furthermore, compared to higher elevation lakes, lower elevation lakes in NOCA tend to have greater diversity and abundance of nearshore benthic macroinvertebrates (Hoffman et al. 1996) and zooplankton (Liss et al. 1995), and moderately abundant populations of the salamander *A. gracile*. Thus, in contrast to higher elevation lakes, more alternative foods may be available for trout in lower elevation lakes and predation pressure on large copepods may be reduced.

ACKNOWLEDGMENTS

We are grateful to the former and present members of our Scientific Advisory Panel: Stanford Loeb, Stanley Dodson, Robert Hughes, William Neill, W. John O'Brien, James Petranka, William Platts, and H.B. Shaffer. They were extremely helpful in establishing direction for the research, reviewing annual reports and proposals, and suggesting improvements in research design and interpretation. Stanley Dodson and Nancy Butler provided constructive reviews of the manuscript. Notwithstanding, any shortcomings of the research are the sole responsibility of the authors. This research would not have been possible without the cooperation and logistical support provided by personnel of North Cascades National Park Service

Complex. The work was funded by the National Park Service and the USGS-Forest and Rangeland Ecosystem Science Center.

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**Interaction Between Introduced Trout and
Larval Salamanders (*Ambystoma macrodactylum*)
in High-Elevation Lakes**

A version of this chapter appeared as: Tyler, T., W. J. L. Liss, L. Ganio, G. L. Larson, R. L. Hoffman, G. Lomnicky, and E. A. Deimling. 1998. Interaction between introduced trout and larval salamanders (*Ambystoma macrodactylum*) in high-elevation lakes. *Conservation Biology* 12:94-105.

ABSTRACT -- The larval stage of the long-toed salamander (*Ambystoma macrodactylum*) is the top vertebrate predator in high-elevation fishless lakes in North Cascades National Park Service Complex, Washington, USA. Although most of these lakes were naturally fishless, trout have been stocked in many. The purpose of our research was to determine the effects of physico-chemical factors and introduced trout on abundance and behavior of *A. macrodactylum* larvae. Larval salamander densities were estimated by snorkeling. Snorkelers carefully searched through substrate materials within 2 m of the shoreline and recorded the number of larvae observed and whether larvae were hidden in benthic substrates. Physico-chemical factors were measured in each lake on the same day that snorkel surveys were conducted. In fishless lakes, larval salamander densities were positively related to total Kjeldahl-N concentration and negatively related to lake elevation. Crustacean zooplankton taxa, especially cladocerans, were important food resources for larval *A. macrodactylum*. Crustacean zooplankton and cladoceran densities were positively related to total Kjeldahl-N, suggesting that increased food resources contributed to increased densities of larval *A. macrodactylum*. Differences in larval salamander densities between fish and fishless lakes were related to total Kjeldahl-N concentrations and the reproductive status of trout. Mean larval salamander densities for fishless lakes with total Kjeldahl-N < 0.045 mg/l were not significantly different from mean larval densities in lakes with reproducing trout or in lakes with non-reproducing trout. However, in fishless lakes with total Kjeldahl-N \geq 0.045 mg/l, mean larval densities were significantly higher than in lakes with reproducing trout where fish reached high densities. In fishless lakes with total Kjeldahl-N \geq 0.095 mg/l, mean larval densities were significantly higher than in lakes with non-reproducing trout where trout fry were stocked at low densities. Reduced larval salamander densities in lakes with trout likely resulted from trout predation. There were no significant differences in the percentage of larvae hidden in benthic substrates between fishless lakes and lakes with fish. Our results imply that assessment of fish impacts on amphibians requires an understanding of natural abiotic and biotic factors and processes influencing amphibian distribution and abundance.

INTRODUCTION

There is world-wide concern about declines of some amphibian species (Blaustein and Wake 1990; Wake 1991; Crump et al. 1992; Blaustein et al. 1994a). Amphibian declines may be a consequence of human-related impacts, including chemical pollution, acid precipitation, ozone depletion, habitat destruction and introductions of exotic species (Hayes and Jennings 1986;

Blaustein and Wake 1990; Wake 1991; Wissinger and Whiteman 1992; Blaustein et al. 1994a). There is particular concern over amphibian declines in areas that have been relatively undisturbed by human activity, such as high-elevation locations in western North America (Blaustein and Wake 1990) and U.S. national parks (Bradford 1989; Bradford et al. 1993).

Fish introductions have been implicated in altering amphibian distribution and abundance in high-elevation lakes (Bradford 1989; Bradford et al. 1993; Fellers and Drost 1993; Blaustein et al. 1994a, 1994b). However, fish are not indigenous to many high-elevation lakes in the western U.S. Bahls (1992) reported that 95% of mountain lakes in the western U.S. may have been naturally fishless. Presently, nearly 60% of all high-elevation lakes and about 95% of the larger, deeper lakes now support fish (Bahls 1992).

Although ambystomatid salamander larvae are important native aquatic predators in high-elevation lakes in western North America (Dodson 1970, 1974; Dodson and Dodson 1971; Sprules 1972; Taylor 1983), only a few studies have assessed the impact of fish on their abundance and behavior. Introduced trout can reduce abundance and cause a shift toward nocturnal behavior in the northwestern salamander (*Ambystoma gracile*) (Sprules 1972; Taylor 1983). Studies conducted on ambystomatid species from the eastern U.S. have shown that fish can reduce or eliminate ambystomatid larvae from lakes and streams (Petranka 1983; Semlitsch 1988; Sih et al. 1992), inhibit growth (Semlitsch 1987; Figiel and Semlitsch 1990) and reduce survival (Semlitsch 1987; Sih et al. 1988) of larvae in artificial ponds. Fish may also cause larvae to shift toward nocturnal activity (Sih et al. 1992), restrict activity (Semlitsch 1987; Stangel and Semlitsch 1987; Figiel and Semlitsch 1990), and increase refuge use (Sih et al. 1988, 1992).

Salamander distribution and abundance is influenced by abiotic factors and biotic factors other than fish. Abiotic factors that can influence salamander distributions include elevation (Snyder 1956; Howard and Wallace 1985; Leonard et al. 1993), lake area and depth (Kezer and Farner 1955; Sprules 1974a, 1974b), water temperature (Snyder 1956; Anderson 1968), and conditions in the terrestrial habitat (Sprules 1974a, 1974b). Availability of suitable food resources, such as benthic macroinvertebrates and zooplankton, especially cladocerans and copepods (Anderson 1968; Dodson 1970; Dodson and Dodson 1971; Licht 1975; Freda 1983), also may influence larval salamander abundance and distribution.

The objectives of this research were to: 1) determine relationships between lake chemical and physical characteristics and larval salamander (*A. macrodactylum*) density in each of three lake categories-fishless lakes, lakes with non-reproducing trout, and lakes with reproducing trout,

2) compare the relationships between lake categories, and 3) determine differences in percent larvae hidden in the bottom substrate between lake categories. The research was conducted in North Cascades National Park Service Complex, Washington, USA. Although *A. macrodactylum* is widespread in the Pacific Northwest (Nussbaum et al. 1983; Leonard et al. 1993), very little is known about its distribution within high-elevation areas, variation in larval abundance among lakes, and natural and human factors influencing salamander distribution and abundance.

METHODS

Study Area

North Cascades National Park Service Complex (NOCA) is comprised of North Cascade National Park, Ross Lake National Recreation Area, and Lake Chelan National Recreation Area and is located in the Cascade Range of northern Washington, USA. There are 156 lakes of interest to fisheries managers in NOCA. All of these lakes are low in chemical ion concentrations and are considered oligotrophic. All but one of these lakes were thought to be historically devoid of fish (Jarvis 1987). Many NOCA lakes were stocked during this century with trout, primarily *Oncorhynchus clarkii* and *O. mykiss*, to provide recreational angling opportunity.

Fish

Lakes were grouped into three categories: fishless lakes, lakes with non-reproducing trout, and lakes with reproducing trout. In NOCA fish densities in lakes with reproducing trout are generally much higher than in lakes in which trout do not reproduce (Liss et al. 1995). Average fish density, estimated by mark-recapture in nine lakes with reproducing trout, was 524 fish/ha for fish >177 mm total length (range 250-724 fish/ha except one lake at 98 fish/ha; Liss et al. 1995; Gresswell et al. 1997). Lakes in which fish do not reproduce are periodically stocked with fry at low densities. Average density of trout fry stocked from 1976 to 1993 in 37 high-elevation lakes (≥ 1100 m) was 179 fish/ha (range 60-375 fish/ha) and the average interval between stocking was >5 years (Liss et al. 1995). Lack of reproduction in lakes with fish was determined from NOCA stocking records (Jarvis 1987) and field observations (e.g., failure to observe fry or smaller fish, little variation in age and size structure of captured fish, and lack of

suitable spawning habitat). Presence or absence of trout in study lakes was verified by gill netting, angling, snorkeling, and observations from shore.

Salamander Density

From 1990 through 1994, larval salamander populations were sampled in 20 fishless lakes (NF), 7 lakes with non-reproducing fish (NRF), and 18 lakes with reproducing fish (RF). We sampled lakes from mid-June to mid-September each year, the period of time in which lakes are typically ice-free.

Because of the remoteness of NOCA lakes, relatively short ice-free periods, periodic wildfires, and periods of inclement weather, sampling frequency of lakes varied within and among years. Seventeen lakes (7 NF, 3 NRF, 7 RF) were sampled 2 or more times in at least 1 year, 8 lakes (2 NF, 1 NRF, 5 RF) were sampled once a year over 2 or more years, and 20 lakes (11 NF, 3 NRF, 6 RF) were sampled only once.

Larval salamanders were censused by snorkel surveys. Because of the remoteness of lakes, snorkeling methods provided the best estimates of larval densities given time and equipment constraints. Snorkel methods tend to underrepresent small, cryptic, and benthic individuals in density estimates (Helfman 1983). Therefore, larval salamander density estimates from snorkel surveys are conservative estimates.

From 1990 to 1993, surveys were conducted during daylight. During these surveys, termed "search surveys," a snorkeler carefully searched through substrate materials (i.e., talus, woody debris, fine organic material, and aquatic vegetation) within 2 m of the shoreline and recorded the number of larvae observed. The length of shoreline surveyed was determined following completion of each search survey.

During 1994, four 25 m segments of shoreline were randomly chosen along the perimeter of each lake. The same segments were surveyed on all sampling visits. Each segment was snorkeled along two transects parallel to shore (e.g., Taylor 1983). One transect was approximately 2 m from shore, and the other transect was over deeper water approximately 5 m from shore. During 2 m surveys, the snorkeler counted larvae within an area extending from the shoreline to approximately 2 m offshore. During 5 m surveys, the snorkeler counted larvae approximately 1.5 m to each side of his or her longitudinal axis. The snorkeler did not disturb substrate material during these surveys. Search surveys also were performed along the same segments of shoreline. To determine if the number of larvae observed was different between day and night, each transect was surveyed during mid-afternoon and approximately 30 min after

sunset with the aid of hand-held divelights. When multiple surveys were performed at a lake, salamander density estimates were averaged for each survey technique. Salamander densities were expressed as number of larvae observed per 100 m of shoreline.

Salamander Stomach Contents

Stomachs were collected from 13 salamander larvae from 3 fishless lakes (MR2 [N=9], MR3 [N=2], and MM6 [N=2]) in 1990 and 1991. In the field stomachs and their contents were preserved in 95% ethanol. Organisms found in the stomachs were identified to the lowest possible taxonomic level in the laboratory using a stereomicroscope for benthic macroinvertebrate taxa and an inverted scope at 100x magnification for crustacean zooplankton taxa.

Salamander Behavior

During 1993 and 1994 search surveys, the total lengths (mm) of individual salamander larvae observed during snorkel surveys were visually estimated. Visual size estimates were aided through the use of plastic metric rulers. The number of larvae in each of three size categories (10-30 mm, 31-60 mm, and >60 mm) was determined. Larvae also were categorized according to whether they were hidden or were not hidden in substrate materials (e.g., talus, woody debris), in rock crevices, or among dense vegetation. A larva was classified as "not hidden" if a major portion of its body was visible to the snorkeler prior to the search through substrate materials.

Salamander Species Identification

There are only two species of ambystomatid salamanders (*A. macrodactylum* and *A. gracile*) in NOCA and they rarely co-occur in a lake (Liss et al. 1995). In NOCA *A. gracile* is restricted to low elevation lakes on the west slope of the Cascade Range (Liss et al. 1995).

Salamander larvae were captured using hand-nets during snorkel surveys. Captured larvae were taken to a laboratory and reared to metamorphosis to confirm species identification. When it was not possible to transport larvae from the field, larvae were determined to be *A. macrodactylum* based on larval characteristics (Nussbaum et al. 1983; Leonard et al. 1993); absence of large larvae (>60 mm total length) or neotenes and egg masses characteristic of *A. gracile*; and the presence of pre-metamorphic individuals with adult coloration.

Lake Physical and Chemical Characteristics

Eleven abiotic variables were measured for each lake. A hand-held sonar gun was used to determine maximum depth of each lake. Lake elevations were derived from 7.5' USGS topographical maps, and lake surface areas were determined by digitization of lake shorelines outlined on these maps. Each time a lake was sampled, water temperature and water chemistry samples were collected at 1 m below the lake surface over the lake's deepest point. Water chemistry samples and temperature recordings were gathered over each lake's deepest point to standardize our sampling; thus enabling us to compare measured variables between lakes. Water samples were collected with a 1.5 L van Dorn sampling bottle. Temperature measurements were determined during mid-afternoon using an Omega 871 thermo-couple. Frozen filtered and unfiltered water samples were transported to the Cooperative Chemistry Analytical Laboratory at Oregon State University, Corvallis, for analyses of total phosphorus, total Kjeldahl-N, ammonium-N, and nitrate/nitrite-N concentrations, and alkalinity, pH, and conductivity.

Zooplankton and Nearshore Benthic Macroinvertebrates

Each time a lake was sampled, crustacean zooplankton were collected using a 20-cm-diameter number 25 (64 μm mesh) zooplankton net. From 1990-1993, three replicate vertical tows were collected in each lake on each sampling occasion. Only one vertical tow was performed on each visit to three lakes sampled in 1989. For each vertical tow, the net was lowered to within 1 m of the lake bottom near the deepest point in each lake and retrieved upward at a constant rate. In the field samples were preserved in 5% neutral sugar formalin solution (Haney and Hall 1973). In the laboratory samples were split using a Folsom plankton splitter. Split portions were allowed to settle for 24 hrs and adult zooplankton in these samples were identified to species and counted using an inverted microscope at 100x magnification (Liss et al. 1995). Zooplankton densities were expressed as number/L.

Benthic macroinvertebrates were sampled using a 17-cm-diameter metal sampling tube (Hoffman et al. 1996). Three samples of each major substrate type in the lake nearshore areas were collected. The tube was placed in position over each sampling site and depressed into the substrate. Material was extracted from the tube to an approximate depth of 5 cm and placed into a 250 μm sieve (U.S.A. Standard Tyler No. 60). Material in the sieve was rinsed with water removed from the tube with a large-bore pipette. The material was placed into a plastic container and handpicked for organisms. All organisms were preserved in 70% ethanol. In the laboratory

organisms were identified to the lowest taxonomic level possible using a stereomicroscope. Macroinvertebrate densities were expressed as number/m².

Statistical Analyses

Statgraphics versions 6.0 and 7.0 were used for all statistical analyses. Each statistical test was performed at $\alpha = 0.05$.

To test for differences in average salamander densities between survey techniques (i.e., search, day 2 m, day 5 m, night 2 m, and night 5 m), a Friedman's F-test was performed on salamander densities in fishless lakes with lakes as blocks and survey techniques as treatments.

Only lakes with at least two zooplankton samples in a given year were used for zooplankton analysis. Mean densities for each taxon for each year were calculated. If lakes were sampled over several years, densities were averaged for all samples. Average densities of benthic macroinvertebrate taxa were calculated in the same manner. Pearson correlation matrices were developed to identify significant relationships between selected abiotic variables, zooplankton densities, and benthic macroinvertebrate densities.

Multiple regression was used to determine relationships between abiotic factors and larval density for each fish category (fishless, non-reproducing fish, and reproducing fish). The dependent variable for regression was the natural logarithm of average larval density in each lake, calculated from search surveys. Values for each chemical variable were averaged over all years in which snorkel surveys were performed. Water temperature averages were calculated from temperature measurements recorded on the day of snorkel surveys. A Pearson correlation matrix was developed to examine relationships among abiotic variables used for multiple linear regression analysis. For the correlation matrix, a sequential Bonferroni adjustment was performed on α to eliminate type I error and maintain table-wide significance at $\alpha = 0.05$ (Mille 1981; Rice 1989).

To test for differences in larval densities between fishless lakes and lakes with reproducing and non-reproducing trout, mean larval density and the 95% confidence interval (CI) were determined for each fishless lake from the linear regression model with TKN concentration and lake elevation as independent variables. Mean larval densities and 95% CIs for lakes with reproducing fish and for lakes with non-reproducing fish were also determined. Differences in larval densities between individual fishless lakes and lakes in other fish categories were judged to be significant if 95% CIs did not overlap.

Differences in the proportion of hidden salamanders among larval size classes and among fish categories were investigated using analysis of variance (ANOVA). Proportion of hidden larvae was arcsin-square-root transformed for all ANOVA tests to reduce within-group variability. We tested for differences in proportion of hidden salamanders among larval size classes in fishless lakes. To test for differences in proportion of hidden larvae among fish categories, larvae from all size classes were combined in each fish category and the combined densities were compared between fish categories.

RESULTS

Abiotic Relationships

In fishless lakes in 1994, there were no significant differences in *A. macrodactylum* larval densities between survey types (Table 5.1; Friedman's F-test, $P = 0.34$). Differences in larval densities between survey types in lakes with reproducing fish were not tested statistically since virtually no larvae were observed either during the day or at night. In addition, no larvae were observed in four of eight fishless lakes. Although there were no statistically significant differences between survey types, search surveys appeared to provide the most conservative estimates of larval densities.

Stepwise linear regression for all fishless lakes identified statistically significant relationships between \ln larval density, total Kjeldahl-N (TKN), and elevation ($R^2 = 0.68$, $P = 0.0001$, Figure 5.1):

$$\ln(\text{larval density}) = 4.113 + 58.651(\text{TKN}) - 0.005(\text{elevation}).$$

(2.474) (9.973) (0.002)

The relationship with larval density was positive for TKN and negative for elevation. RD3, a low elevation (802m) fishless lake with high larval density, was withheld from a second regression analysis to determine the influence of this lake's elevation in the model. Stepwise regression when RD3 was omitted identified a statistically significant positive relationship between larval density and both TKN and conductivity ($R^2 = 0.6938$; $P = 0.0001$). Thus, when RD3 was omitted, lake elevation no longer had a detectable influence on larval density.

Total Kjeldahl-N had significant positive correlations with total phosphorus, water temperature, and ammonium-N (Pearson correlation coefficients; $r = 0.74$, $P \leq 0.0001$; $r = 0.51$,

Table 5.1. *Ambystoma macrodactylum* larval density estimates (larvae/100 m of shoreline) for fishless lakes (NF) and lakes with reproducing fish (RF) in 1994.

Fish Category	Lake ^a	DAY			NIGHT ^b	
		Search	2 m	5 m	2 m	5 m
NF	RD3	67	59.7	126	114	76.5
	PM5-3	33	36	13	44.5	44.4
	MC10	59	149	163	61	383
	M6	1	74	29	NP	NP
	MC27	0	0	0	0	0
	MC21-1	0	0	0	NP	NP
	MC4	0	0	0	0	0
	MSH4	0	0	0	0	0
RF	M23	0	0	0	4	0
	LS2	0	0	0	0	0
	M20	0	0	0	0	0
	M7	0	0	0	0	0
	LS7	0	0	0	0	0
	EP9-1	0	0	0	0	0
	PM3	0	0	0	0	0
	MR4	0	0	0	0	0
	M21	0	0	0	NP	NP

^a Letter and number system for lake identification used by NOCA.

^b Night surveys were not performed (NP) in some lakes.

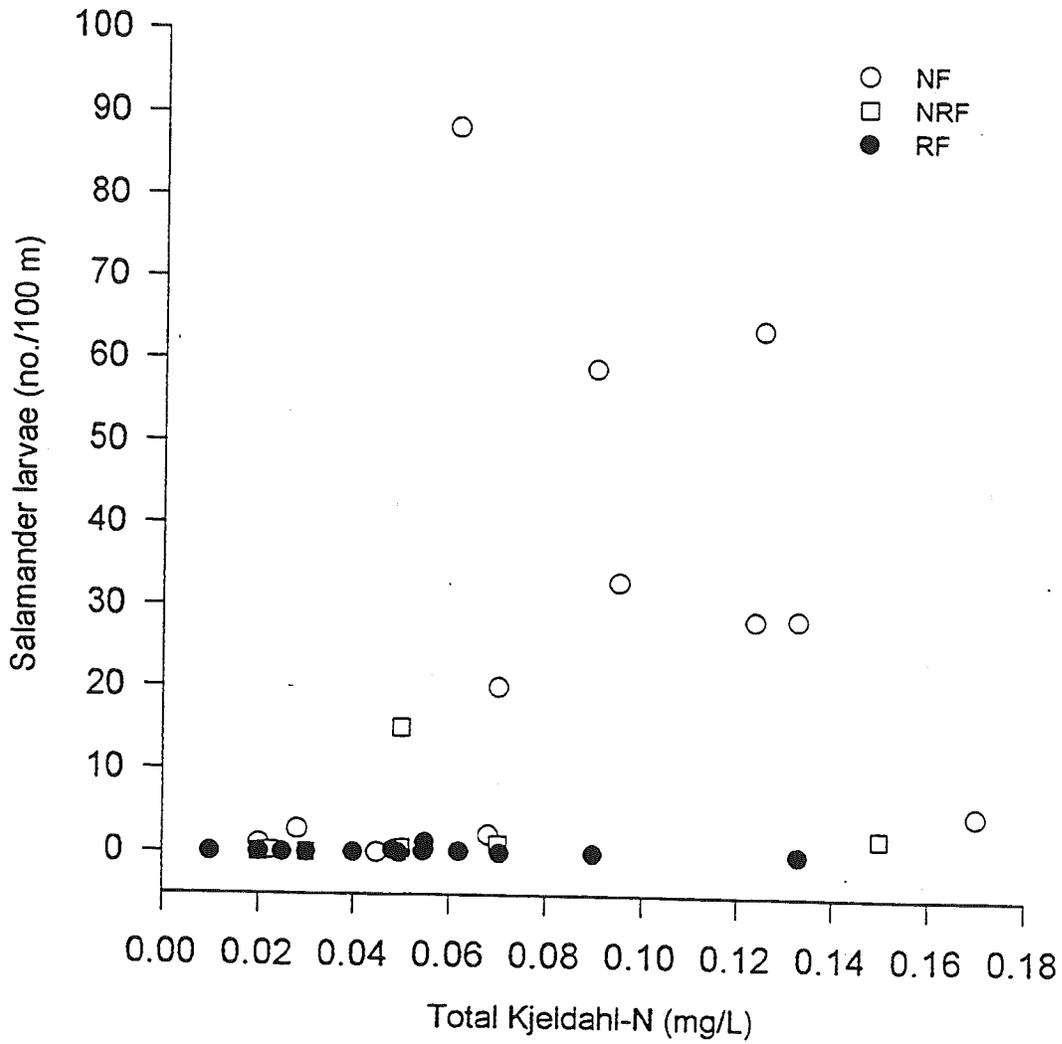


Figure 5.1. Relationships between *Ambystoma macrodactylum* larval densities and total Kjeldahl-N concentrations for fishless lakes (NF; n = 20), lakes with non-reproducing fish (NRF; n = 7), and lakes with reproducing fish (RF; n = 18).

$P = 0.0004$; $r = 0.46$, $P = 0.0017$, respectively). Thus, NOCA lakes with higher TKN concentrations tended to have relatively higher concentrations of both total phosphorus and ammonium-N and higher water temperatures.

Salamander Stomach Contents

Benthic macroinvertebrates were identified in 75% of larval salamander stomachs (Table 5.2). The benthic taxon, Diptera, composed primarily of chironomid larvae, was found in the highest proportion of salamander stomachs. Beetle larvae (Coleoptera) and caddisfly larvae (Trichoptera) also formed significant proportions of larval diets. Crustacean zooplankton also were an important component of salamander diets. Cladocerans were the most common zooplankton taxon found in stomachs.

Crustacean Zooplankton and Benthic Macroinvertebrates

Total crustacean zooplankton density and cladoceran density were positively correlated with TKN (Pearson correlation coefficients; $r = 0.57$, $P = 0.0053$, Figure 5.2a; $r = 0.69$, $P = 0.0003$, Figure 5.2b; respectively). Furthermore, there was a significant positive relationship between percent of cladocera composing the crustacean zooplankton communities and TKN ($r = 0.59$, $P = 0.0040$, Figure 5.2c). At low TKN concentrations (0.0 - ≈ 0.05 mg/L), zooplankton density was very low and the zooplankton communities were composed almost exclusively of copepods. There was no statistically significant relationship between TKN and total benthic macroinvertebrate density (Pearson correlation coefficients, Figure 5.3, $r = -0.3216$, $P > 0.05$) or chironomid density (Figure 5.3, $r = -0.0899$, $P > 0.05$).

Fish Effects

No significant linear relationships between larval salamander density and any abiotic factor were identified by stepwise linear regression for lakes with reproducing fish or for lakes with non-reproducing fish. When larval densities in lakes with reproducing fish were fitted to a regression model with TKN and elevation as independent variables, neither TKN slope nor elevation slope were significantly different from zero (Figure 5.1; $P = 0.68$, $P = 0.39$, respectively). A similar regression analysis of larval densities in lakes with non-reproducing fish also found that TKN slope ($P = 0.35$) and elevation slope ($P = 0.56$) were not significantly different from zero (Figure 5.1).

Table 5.2. Percentage of stomachs from 13 *Ambystoma macrodactylum* larvae that contained benthic macroinvertebrate and crustacean zooplankton taxa.

Taxon	Percent
Benthic Macroinvertebrates	75.0
Diptera	66.7
Chironomidae	41.7
Coleoptera	58.3
Trichoptera	50.0
Ephemeroptera	25.0
Amphipoda	16.7
Plecoptera	8.3
Pelecypoda	8.3
Crustacean Zooplankton	41.7
Cladocerans	41.7
Unidentified	41.7
Imm. Daphnids	25.0
<i>Daphnia rosea</i>	25.0
<i>Ceriodaphnia quadrangula</i>	16.7
<i>Chydorus sphaericus</i>	8.3
Copepods	16.7
Cyclopoids	16.7
Calanoids	8.3
Harpatacoids	8.3

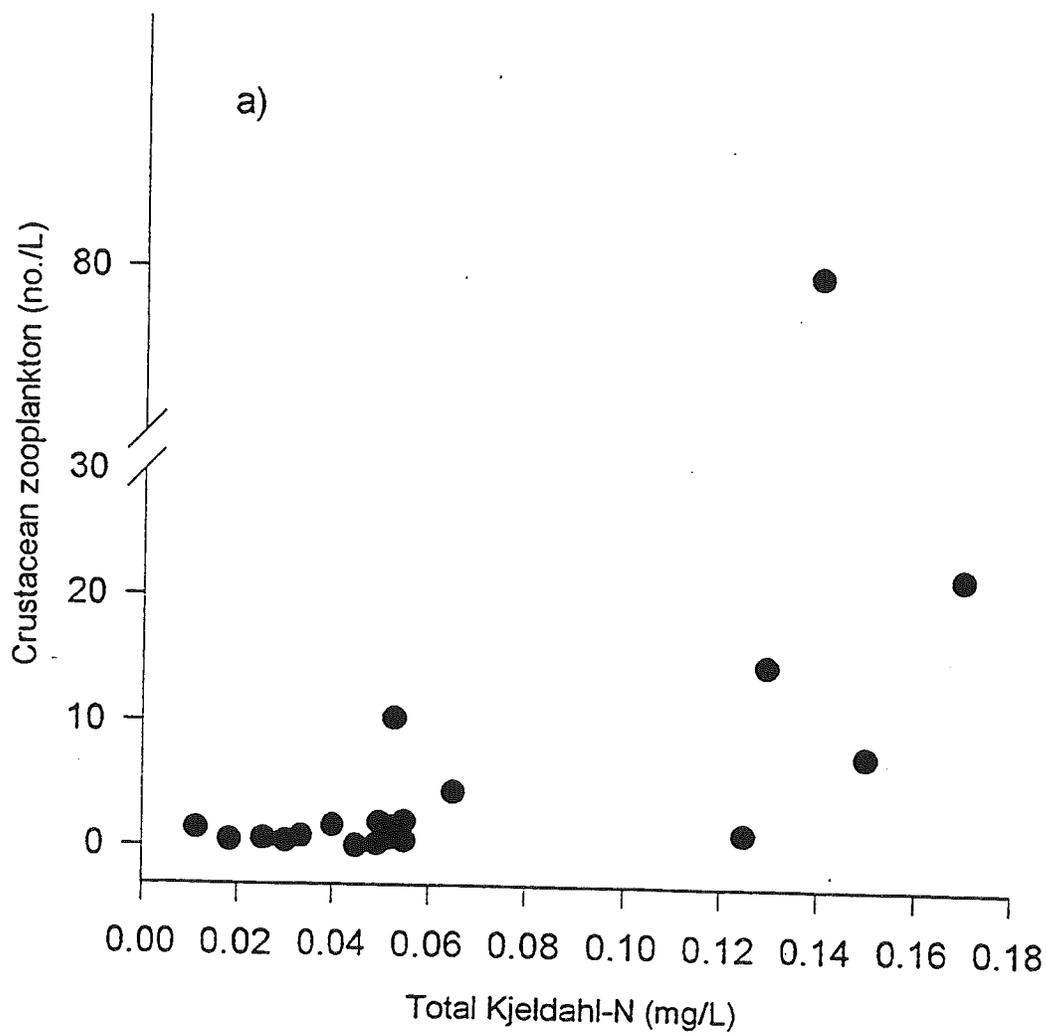
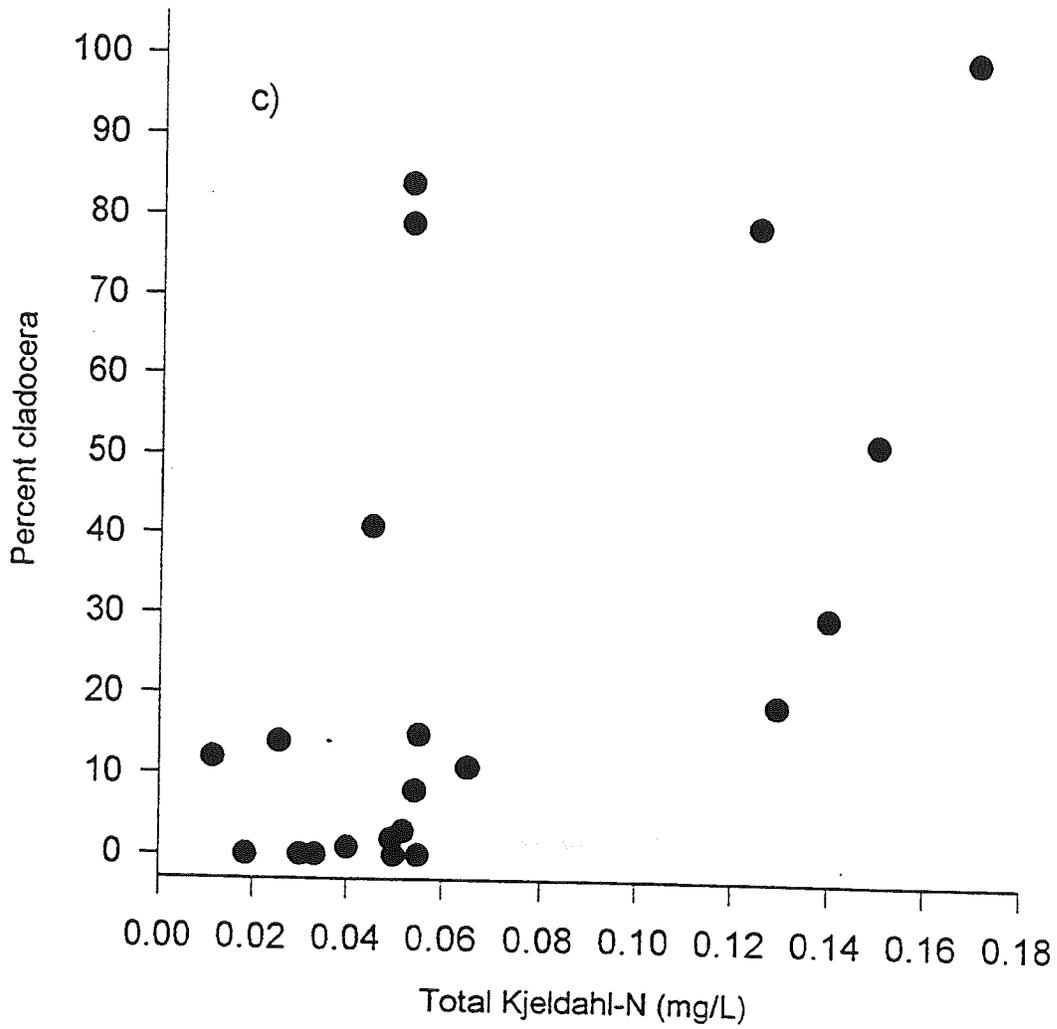
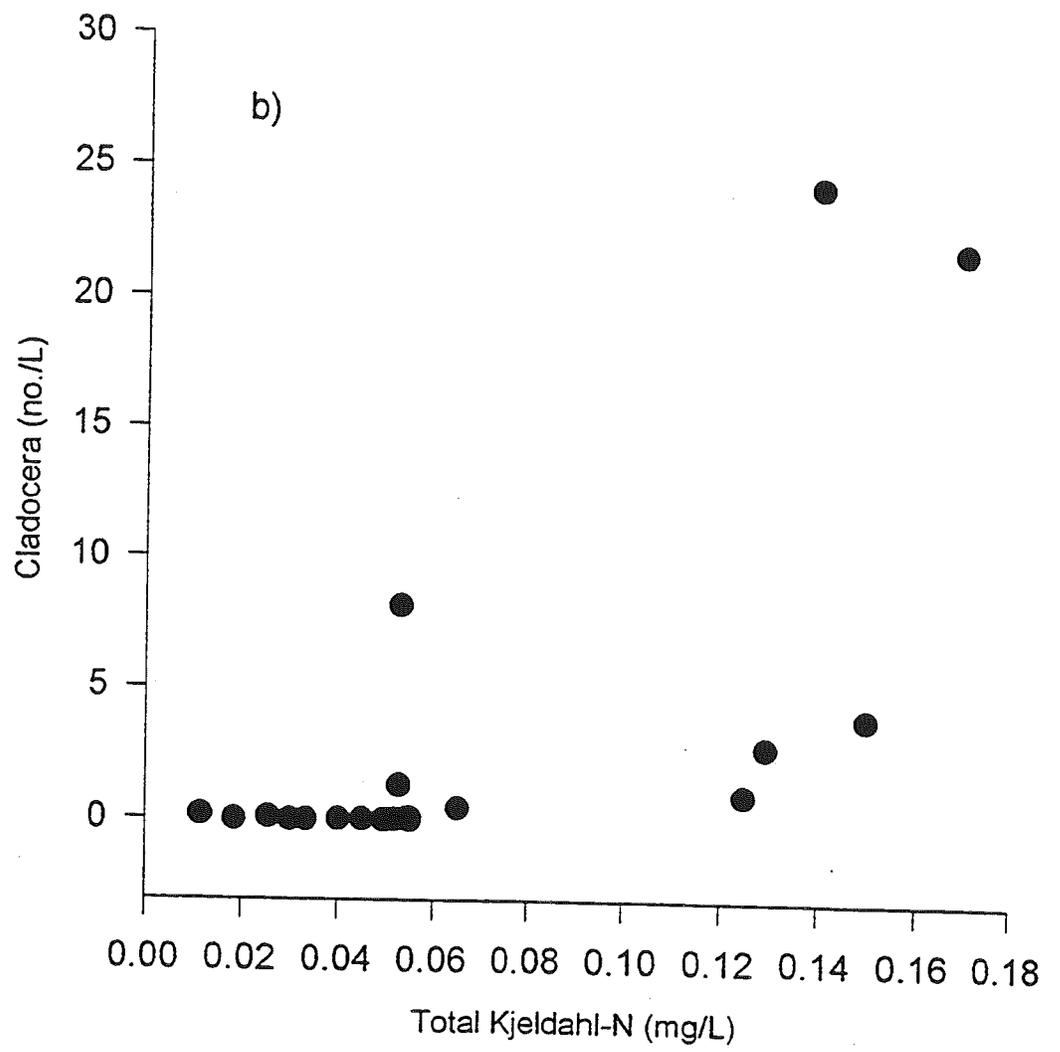


Figure 5.2. Relationships between total Kjeldahl-N concentration and crustacean zooplankton density (a); cladoceran zooplankton density (b); and cladoceran density as a percentage of total zooplankton density (c).





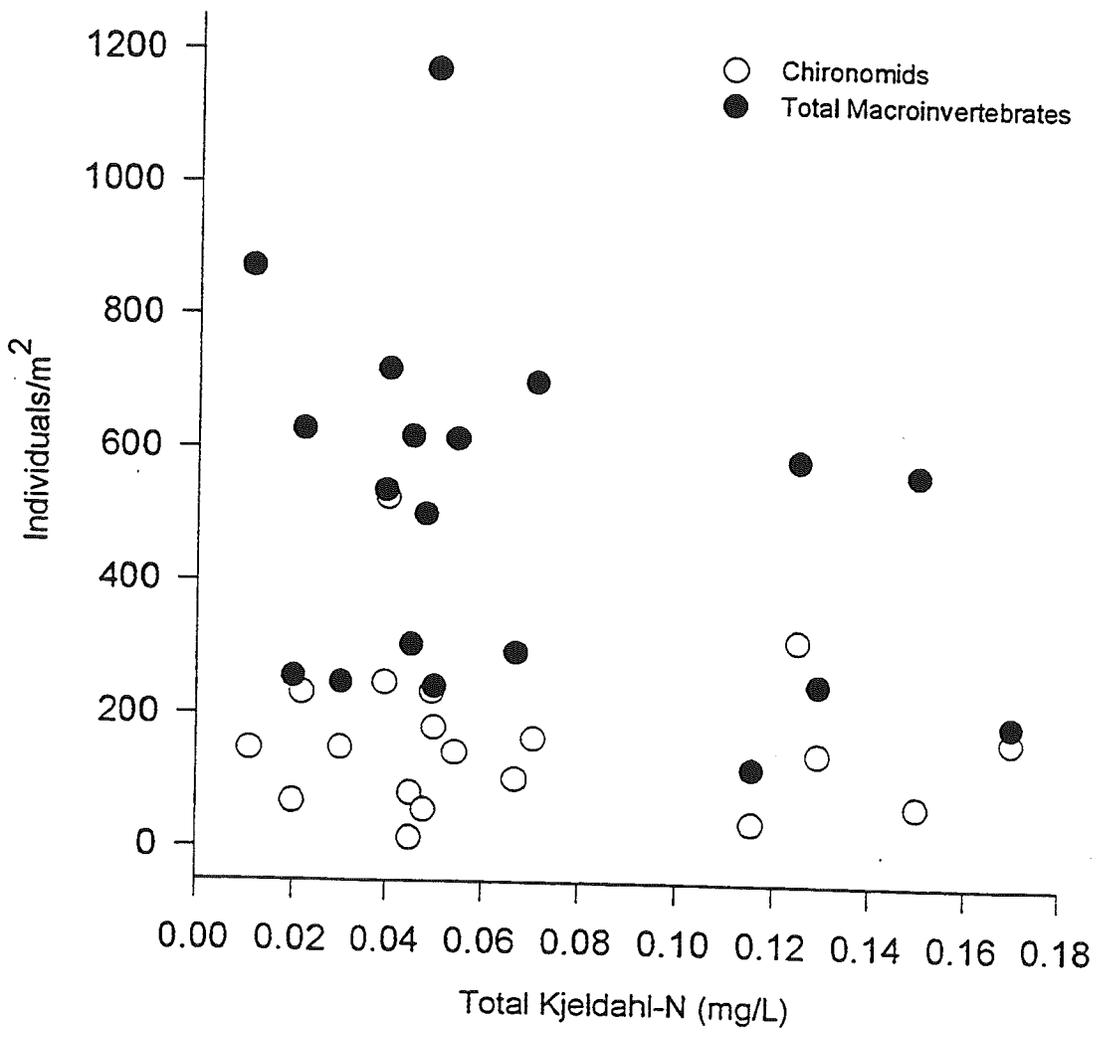


Figure 5.3. Relationship between total Kjeldahl-N concentration and the densities of chironomids and total benthic macroinvertebrates.

Larval densities in lakes with reproducing trout were low, making detection of significant relationships with abiotic variables difficult. Larval densities in lakes with non-reproducing fish also were low and detection of significant relationships between larval density and abiotic variables for these lakes was further limited by a small sample size ($n = 7$).

Because multiple regression revealed no significant relationships between larval density and abiotic factors for either lakes with reproducing fish or for lakes with non-reproducing fish, larval densities from all lakes in each group were averaged and the 95% CI for each group average was determined. To assess fish effects on larval density, the 95% CIs for lakes with reproducing fish and for lakes with non-reproducing fish were compared to 95% CIs for individual fishless lakes determined from the multiple regression model with TKN and elevation as independent variables.

For fishless lakes with $\text{TKN} < 0.045 \text{ mg/L}$, mean larval densities generated by the linear regression equation were not significantly different from mean larval densities in either lakes with reproducing fish or lakes with non-reproducing fish (Table 5.3). However, for all ten fishless lakes with $\text{TKN} \geq 0.045 \text{ mg/L}$, predicted mean larval densities were significantly greater than in lakes with reproducing fish ($P < 0.05$). Of the reproducing fish lakes with $\text{TKN} \geq 0.045 \text{ mg/L}$, none had larval densities that exceeded 1.22 larvae/100m of shoreline. Only four of the ten fishless lakes with $\text{TKN} \geq 0.045 \text{ mg/L}$ had significantly higher mean larval densities than lakes with non-reproducing fish ($P < 0.05$). These fishless lakes all had relatively high concentrations of TKN ($\geq 0.095 \text{ mg/L}$) and relatively high larval densities.

Behavior

In fishless lakes there were no significant differences among the three larval size classes in proportion of larvae hidden in substrate material (Table 5.4; ANOVA, $P = 0.16$). Lakes with non-reproducing fish and lakes with reproducing fish were not tested for differences in proportion of hidden larvae between size classes because few lakes in these categories contained all three larval size classes. There were no significant differences in the proportion of hidden larvae among fish categories (Table 5.4; ANOVA, $P = 0.50$). Although statistical analysis did not identify any significant differences in proportion of hidden larvae among larval size classes or among fish categories, there was a tendency for the percent of hidden larvae to increase as larval size increased in each fish category. Furthermore, there was a tendency for a greater percentage of larger larvae ($>30 \text{ mm}$) to be hidden in lakes with both non-reproducing and reproducing fish than in fishless lakes

Table 5.3. Comparison of 95% confidence intervals (CI) for larval *A. macrodactylum* densities between individual fishless lakes (NF), lakes with non-reproducing fish (NRF), and lakes with reproducing fish (RF).

Fish Category	Lake ^a	TKN (mg/L)	Elevation (m)	Mean larval density ^b	Lower limit 95% CI ^b	Upper limit 95% CI ^b	Difference ^c
NF	MC27	0.01	1488	0.1182	0.0287	0.4874	
	MC21-1	0.02	1528	0.1769	0.0503	0.6225	
	M6	0.02	1632	0.1097	0.0316	0.3208	
	MM6	0.028	1504	0.3158	0.0967	1.0314	
	MA3	0.03	2044	0.0297	0.0051	0.1729	
	MSH4	0.03	1635	0.1945	0.0642	0.5890	
	GM2	0.03	1664	0.1703	0.0557	0.5201	
	MA2	0.04	2127	0.0365	0.0057	0.2344	
	MR6	0.04	1693	0.2679	0.0974	0.7373	
	MM7	0.045	1642	0.4540	0.1751	1.1771	A
	MC4	0.05	1604	0.7249	0.2850	1.8433	A
	RD3	0.0609	802	54.6626	2.8671	1042.1592	A
	MR11	0.068	1863	0.6340	0.2262	1.7771	A
	MR13-1	0.07	1800	0.9522	0.3718	2.4385	A
	MC10	0.09	1556	9.0317	2.9124	30.5844	A
	PM5-3	0.095	1382	28.1399	5.7158	138.5401	B
	MR3	0.1237	1873	15.8833	3.5612	70.8419	B
MR12	0.125	1981	10.4382	2.1128	51.5679	A	
MR2	0.1328	1873	27.0855	5.2653	139.3321	B	
SMI	0.17	2033	115.1137	11.1828	1184.9549	B	
NRF	Average	0.056	1597.4	0.5118	0.0756	3.4666	
RF	Average	0.046	1602.8	0.0821	0.0514	0.1310	

^a Letter and number system for lake identification used by NOCA.

^b Mean larval densities and 95% confidence intervals were determined from a multiple linear regression model using TKN and lake elevation as independent variables.

^c A) indicates a significant difference in larval density between a fishless lake and lakes with reproducing fish, and B) indicates a significant difference in larval density between a fishless lake and both lakes with reproducing fish and lakes with non-reproducing fish.

Table 5.4. Total number of larval *Ambystoma macrodactylum* observed and the number and percent hidden in substrate materials during search surveys of fishless lakes (NF; n = 10), lake with non-reproducing fish (NRF; n = 5), and lakes with reproducing fish (RF; n = 5) in 1993 and 1994.

Fish Category	Larval Size	N ^b	Number Observed	Number Hidden	Percent Hidden
NF	10-30	8	342	131	38.3
	31-60	7	262	68	26
	>60	8	309	152	49.2
NRF	10-30	4	15	6	40
	31-60	3	52	36	69.2
	>60	3	31	28	90.3
RF	10-30	4	14	4	28.6
	31-60	2	4	4	100
	>60	0			

^a Larval size is visually estimated total length (mm).

^b Number of lakes (N) where larvae of each size class were observed.

DISCUSSION

The abundance of larval *A. macrodactylum* in NOCA lakes was related to both lake productivity, as indicated by TKN, and to presence of trout. According to McQueen et al. (1986), the potential productivity at all trophic levels in freshwater lakes is set by nutrient supply. High-elevation NOCA lakes are oligotrophic, or low productivity (Lomnický 1996). Nevertheless, larval density in fishless lakes was positively related to the concentration of TKN. In turn, cell density of phytoplankton, which are fed upon by herbivorous zooplankters, was positively correlated with TKN in NOCA lakes (G. Larson unpublished). Total Kjeldahl-N was also positively correlated with total phosphorus concentration, another indicator of lake trophic state (Wetzel 1983).

Total Kjeldahl-N is a measure of ammonia plus all organically derived nitrogen (Lambou et al. 1983). Organic nitrogen is not readily utilized by algae and bacteria; thus TKN does not play an active role in the energetics of lakes (Goldman and Horne 1983). However, TKN, when correlated with phosphorus concentration has been identified as a good predictor of lake productivity, as measured by chlorophyll density (Lambou et al. 1983) or by total plankton biomass (Paloheimo and Fulthorpe 1987). TKN was also positively correlated with water temperature. Higher water temperature may favor larval salamander growth and survival (Snyder 1956; Anderson 1968).

One link between TKN and larval salamander density appears to be through the pelagic food web. The density of crustacean zooplankton, an important food resource for larval *A. macrodactylum*, increased with increased TKN. Moreover, at high TKN, herbivorous cladocerans, which were identified in more larval stomachs than any other zooplankter, composed a greater proportion of crustacean zooplankton than did copepods. Other researchers also have demonstrated an association between the composition of crustacean zooplankton and nitrogen concentration in high-elevation lakes (Byron et al. 1984; Stoddard 1987; Liss et al. 1995).

Ambystomatid larvae utilize both crustacean zooplankton (Licht 1975; Brophy 1980; Branch and Altig 1981; Freda 1983; Taylor et al. 1988) and benthic macroinvertebrates (Henderson 1973; Licht 1975; Brophy 1980) as food resources. In particular, ambystomatids utilize zooplankton food resources early in larval development (Anderson 1968; Dodson 1970; Dodson and Dodson 1971) and both zooplankton and benthic macroinvertebrates in later stages

of development (Anderson 1968; Dodson 1970; Dodson and Dodson 1971; Freda 1983; Taylor et al. 1988; McWilliams and Bachmann 1989).

Although benthic macroinvertebrates, especially chironomid larvae, were also a large component of larval salamander diets in NOCA lakes, no significant relationship between TKN and either total benthic macroinvertebrate density or chironomid density was detected in NOCA lakes.

The effects of trout on larval salamander density was related to TKN concentration and the reproductive status of trout populations. Larval salamander densities were low (≤ 2.76 larvae/100 m) in lakes with TKN < 0.045 mg/L regardless of whether trout were present or absent. At TKN < 0.045 , no statistically significant differences in mean larval densities were detected between fishless lakes and lakes with fish. In contrast, all fishless NOCA lakes with TKN ≥ 0.045 mg/L had significantly higher measured mean larval densities than did lakes with reproducing trout. Trout usually reach high densities and trout populations have diverse age and size structures in NOCA lakes where fish reproduction occurs (Liss et al. 1995; Gresswell et al. 1997). These results suggest that fish predation is responsible for reducing larval *A. macrodactylum* abundance in lakes with reproducing trout. This conclusion is supported by laboratory and field studies that have demonstrated that fish can reduce the abundance of ambystomatid salamanders or eliminate them from aquatic systems when fish invaded bodies of water where salamanders were present (Sprules 1974a; Thompson et al. 1980; Petranka 1983; Taylor 1984; Semlitsch 1987, 1988; Sih et al. 1988, 1992; Dobler 1994).

Blaustein et al. (1994b) suggested that the fungus *Saprolegnia ferax* caused declines of *Rana cascadae* in the Oregon Cascades and that *S. ferax* could be spread by introduced fish. Although we observed no *A. macrodactylum* eggs or larvae that appeared to be infected with *S. ferax*, the fungus cannot be eliminated as a possible factor contributing to reductions in larval salamanders in NOCA lakes.

Although all 10 fishless lakes with TKN ≥ 0.045 mg/L had significantly higher measured mean larval densities than did lakes with reproducing trout, only 4 of these 10 fishless lakes had significantly higher larval densities than lakes with non-reproducing trout. Lakes with non-reproducing trout were periodically stocked with low densities of fry (Liss et al. 1995). Thus, fish densities in lakes with non-reproducing trout are likely lower, and fish population age and size structure less complex, than in lakes with reproducing trout. Moreover, significant differences in mean larval densities between fishless lakes and lakes with non-reproducing trout were detected only in lakes with high TKN concentrations (TKN ≥ 0.095 mg/L), where the

highest mean larval densities in fishless lakes were predicted. Comparison of larval densities between fishless lakes and lakes with non-reproducing trout was hampered by small sample size ($n = 7$) of lakes with non-reproducing trout. Further research on the effects of non-reproducing trout on larval salamanders in high-elevation lakes is needed.

Metapopulation processes may be important in regional persistence of amphibian populations (Sjogren 1991; Bradford et al. 1993). Metapopulations are spatially structured systems of local populations connected by dispersal (Hanski and Gilpin 1991). Gill (1978) suggested that red-spotted newt (*Notophthalmus viridescens*) populations in the northeastern U.S. resemble a core-satellite metapopulation. In metapopulations with core-satellite structures, there is considerable variation in population abundance among local populations (Harrison 1991; 1994). Core populations are large populations that occupy high quality habitat and have relatively low probabilities of extinction. Satellite populations are smaller populations that are more susceptible to extinction than are core populations (Harrison 1991; 1994). Core populations can provide stable sources of dispersing individuals that recolonize satellite habitats where local extinction has occurred (Sjogren 1991; Harrison 1994). Although very little is known about metapopulation processes of high-elevation *A. macrodactylum*, it is possible that the relatively large local populations of *A. macrodactylum* that occur toward the upper end of the TKN gradient in NOCA lakes may function as core populations that provide stable sources of dispersing individuals capable of recolonizing habitats where smaller populations have gone extinct (Sjogren 1991; Harrison 1994). Therefore, we speculate that introduced trout could indirectly influence regional population distribution of *A. macrodactylum* by inhibiting recolonization of habitats where extinction has occurred either through reduction or elimination of critical core populations or by impeding dispersal between habitats (Sjogren 1991; Bradford et al. 1993).

The proportion of larvae hidden in benthic substrates tended to increase with both larval total length and fish presence, although the increases were not statistically significant. In both fishless lakes and lakes with fish, a greater proportion of larger salamander larvae were hidden than were smaller larvae. Anderson (1967) reported that metamorphosing larval *A. macrodactylum* became more secretive and congregated in nearshore areas of mountain lakes in California. The tendency for a greater proportion of larger larvae to be hidden may be related to approaching metamorphosis as amphibians may be particularly susceptible to predation at this critical stage of development (Wassersug and Sperry 1977; Arnold and Wassersug 1978).

Although not a statistically significant trend, it appeared that a greater proportion of larvae were hidden in lakes with fish than in fishless lakes. *Ambystoma gracile* appear to be more

reclusive in lakes with fish, staying hidden during daylight hours and possibly restricting foraging to night (Efford and Mathias 1969; Sprules 1974a; Taylor 1983). Shifts in behavior and habitat use to avoid intra- and interspecific predation, while increasing probability of larval survival (Figiel and Semlitsch 1990), may reduce food consumption and growth by decreasing foraging efficiency (Semlitsch 1987; Figiel and Semlitsch 1990). Eventually larvae may need to leave refuge to forage and so become vulnerable to predation (Sih et al. 1988). Thus, refuge use within a lake may not necessarily ensure long-term survival.

Our results imply that assessment of fish impacts on amphibians requires an understanding of natural abiotic and biotic factors and processes influencing amphibian distribution and abundance (Pechmann et al. 1991; Blaustein et al. 1994b). In NOCA, detection of significant differences in larval salamander densities between fishless lakes and lakes with fish was related to TKN concentration which apparently influenced larval food resource availability and, thus, larval density. Significant differences were detectable only in lakes with high TKN where predicted larval densities were high. Furthermore, detection of differences in larval densities between fishless lakes and lakes with trout was related to the reproductive status of trout populations, which likely was indicative of trout density and age and size structures of trout populations.

ACKNOWLEDGEMENTS

We are grateful to the former and present members of our scientific advisory panel: S. Loeb, S. Dodson, R. Hughes, W. Neill, W. J. O'Brien, J. Petranka, W. Platts, and H. B. Shaffer. Beatty and two reviewers provided helpful comments that improved the manuscript. This research would not have been possible without the co-operation and logistical support provided by the personnel of North Cascades National Park Service Complex. This work was funded by the National Park Service and the USGS-Forest and Rangeland Ecosystem Science Center.

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Chapter 6.

**Experimental Analysis of Trout Effects on Survival,
Growth, and Habitat Use of Two Species of Western
Ambystomatid Salamanders¹**

¹Tyler, T. J., W. J. Liss, R. L. Hoffman, and L. M. Ganio. 1998. Experimental analysis of trout effects on survival, growth, and habitat use of two species of Ambystomatid salamanders. *Journal of Herpetology* 32:345-349.

ABSTRACT -- Introduced fish have been implicated as reducing abundance or eliminating ambystomatid salamanders from montane lakes in western North America. We tested the null hypotheses that survivorship, growth, and refuge use of larvae reared for 30 days did not differ between artificial ponds with trout and without trout. Larval survivorship for both *A. macrodactylum* and *A. gracile* was significantly lower in ponds with trout than in fishless ponds. Both species had significantly lower snout-vent lengths in ponds with trout than in fishless ponds at the conclusion of the experiments. Only *A. gracile* had significantly lower body mass in ponds with trout than in ponds without trout. For both species, substrate locations of larvae were significantly influenced by trout at the conclusion of the experiments. Larvae of both species were found in a narrower range of substrates in ponds with fish than in control ponds. Our findings support inferences from field studies that trout have negative impacts on larval *A. macrodactylum* and *A. gracile*.

INTRODUCTION

Larval ambystomatid salamanders are important vertebrates native to montane lakes of western North America (Dodson 1970, 1974; Dodson and Dodson 1971; Sprules 1972), and function as top carnivores in fishless lakes (Taylor 1983). Since the late 1880's, trout (*Oncorhynchus* sp.) and char (*Salvelinus* sp.) have been introduced to many high-elevation lakes in the western USA that were once fishless (Bahls 1992). Several survey studies have provided evidence that trout and char can eliminate ambystomatid salamanders from or reduce their abundance in montane lakes (Burger 1950, Blair 1951, Sprules 1974, Taylor 1983).

A combination of field and laboratory studies demonstrate the effects of fish on native ambystomatids of eastern and midwestern USA. Several field studies show that fish, particularly predatory fish, can reduce or eliminate larval ambystomatids from ponds, lakes, and streams (Thompson et al. 1980, Petranka 1983, Sexton and Phillips 1986, Semlitsch 1988, Sih et al. 1992). Predatory fish can also inhibit growth (Semlitsch 1987, Figiel and Semlitsch 1990) and reduce survival of larval ambystomatids in controlled experiments (Semlitsch 1987, Sih et al. 1988). Furthermore, fish can influence larval ambystomatid behavior. In the presence of fish, larvae may increase nocturnal activity (Sih et al. 1992), restrict activity to benthic substrates (Semlitsch 1987, Stangel and Semlitsch 1987, Figiel and Semlitsch 1990, Jackson and Semlitsch 1993, Sih and Kats 1994), or increase time spent in refuge (Sih et al. 1988, 1992).

Rainbow trout (*O. mykiss*) regularly prey upon perennibranchiate northwestern salamanders (*Ambystoma gracile*) in Marion Lake, British Columbia, Canada (Efford and Mathias 1969). Fish predation by this and other salmonids is likely responsible for lower densities of *A. gracile* in lakes with fish versus no fish (Sprules 1974, Taylor 1983). Gilled *A. gracile* may behaviorally adapt to fish by increasing nocturnal activity (Taylor 1983, 1984).

Effects of fish on larval long-toed salamanders (*A. macrodactylum*) and *A. gracile* have been inferred from comparison of abundance and behavior of larvae in fish versus fishless lakes. For example, in mountain lakes of northern Washington, USA, densities of *A. macrodactylum* are significantly lower in lakes with stocked trout than in fishless lakes (Tyler et al. 1998). We are unaware of any experimental studies that have documented the effects of fish on *A. macrodactylum* and *A. gracile* larvae. The objective of this research was to quantify the impact of trout on survivorship, growth, and refuge use of larval *A. macrodactylum* and *A. gracile* in controlled laboratory experiments. Specifically, we tested the null hypotheses that survival, growth, and refuge use of larvae did not differ between artificial ponds with fish and without fish.

METHODS

Experiments were conducted in a 10x12 m building at Oak Creek Laboratory of Biology. Air temperature in the building was not controlled. Twelve Rubbermaid brand livestock watering tanks were used as artificial ponds. Each oval tank was constructed of fiberglass and plastic composites and had a capacity of 1136 L (166x152x60 cm). Each tank was filled with well-water to a depth of 38 cm (water volume of each tank was \approx 616 L). Substrates that simulated those typical of lakes of the northern Cascade range (i.e., talus or rock slide debris, woody material such as submerged tree limbs and branches, submerged vegetation, and open areas) were established in each artificial pond.

Before the addition of water, each artificial pond was filled to a depth of 4 cm with washed sand. Additional substrate materials were placed over the sand bottom in each pond to simulate talus, woody material, and aquatic vegetation. The amount of each substrate type in each pond was roughly equal. To simulate talus, 12 quarry rocks, approximately 2 L each (determined by water displacement), were arranged in each pond in the shape of a rectangular based pyramid (40x60x20 cm). To simulate woody material, 13 tree limbs (seven with 8-10 cm diameters and six with 2 cm diameters) were bound together with nylon cord in the same general

shape as the talus and anchored to the bottom of each pond with a concrete pipe (44 cm long and 15 cm diameter). Eight plastic aquarium plants (36 cm high) were attached to plastic disks 30 cm in diameter, which were anchored with sand. Total area for each tank bottom was 16,217 cm²; wood and rock each occupied 2400 cm², artificial aquatic vegetation occupied 706.9 cm², and open sand occupied the remaining area (10,710.1 cm²).

Collection of Larvae

Recently hatched *A. macrodactylum* larvae (15-20 mm total length) used in laboratory experiments were collected by dip-net on 16 and 18 April 1996, from a low-elevation, ephemeral pond in Benton County, Oregon. Embryos of *A. gracile* were collected on 28 May 1996, from Pika Lake in the Cascade mountain range, Linn County, Oregon. Additional *A. gracile* embryos were collected on 14 June 1996, from Upper Panther Lake, a lake in the northern Cascade range of Washington, USA.

Prior to the start of each experiment, embryos and larvae were held in 40-L aquaria filled with well-water maintained at 8-11 C. Larvae were exposed to 12 hr of artificial light each day and were fed live *Tubifex* sp. ad libitum twice a week.

Experiment Description

Experiments to determine effects of trout on survival and growth of larval *A. macrodactylum* and *A. gracile* were performed separately for each salamander species. One trout was placed in each of six treatment ponds while six ponds without trout served as controls. Experiments were performed for 30 d for each salamander species. Rainbow trout (*O. mykiss*) obtained from Oregon State University, Department of Food Science and Technology, were used for each experiment. Treatment and control ponds were randomly selected.

Twenty larvae were stocked in each artificial pond. Prior to stocking, the snout-vent length (SVL) of each larva was measured. Larvae were randomly assigned to 12 groups of 20 individuals each. Each group of 20 larvae were then weighed collectively and randomly assigned to a treatment or control pond. Fork length (FL; distance from tip of snout to the fork of the tail) and body mass of each trout was recorded [*A. macrodactylum* experiment, FL mean = 211.7 mm (206-217 mm), mean mass = 112.2 g (107-121 g); *A. gracile* experiment, FL mean = 215.3 mm (212-220 mm), mean mass = 129.2 g (114-146 g)]. One trout was released into each treatment pond 5 hr after larvae had been added.

Artificial light was provided to all tanks from 0700 - 1900 hr each day. All tanks were inoculated with 24 g of *Tubifex* at least one day prior to the start of each experiment. During each 30 d experiment, 12 g of *Tubifex* were dispersed throughout each tank three times per week. Tanks were inspected for abnormalities (i.e., water loss, death of larvae or trout, etc.) each day during the experiments.

After each 30 d experiment, trout were removed from treatment ponds and substrate materials were enclosed with mesh screens and carefully dismantled. Number of larvae in each substrate was recorded as substrates were dismantled. Group mass and individual SVLs of surviving larvae were measured. Observations of substrate use by larvae prior to termination of the experiments were not possible since this would have required dismantling substrates while the experiments were in progress. Average daily ambient water temperature at noon was 10.8 C (9.5-11.6 C) for the *A. macrodactylum* experiment and 18.3 C (16.3-19.3 C) for the *A. gracile* experiment. The difference in daily water temperature between experiments was a result of seasonal change in air temperature in the laboratory buildings.

Statistical Analyses

Differences in larval survival between treatment and control ponds were tested by Analysis of Variance (ANOVA) using arcsin square-root transformed proportion of larvae surviving in each pond. Average SVL and average mass of surviving larvae were compared between treatment and control ponds using unpaired Wilcoxin tests. Statgraphics version 7.0 was used for ANOVA's and Wilcoxin tests. Differences in the substrate locations of larvae between treatment and control ponds were tested by generating full and reduced generalized linear models for both species (Ramsey and Schafer 1997). The log-transformed location of larvae in each pond was the dependent variable and the proportions of larvae in each substrate were independent variables for the models. Full models included the binary response variable of trout presence and absence. The binary response variable was excluded from reduced models. Drop in Deviance tests were then used to determine whether fish presence and absence was a significant variable in describing variation in substrate location of larvae (Ramsey and Schafer 1997). Substrate location of larvae was analyzed using SAS version 6.10. All tests were performed at $\alpha = 0.05$. One control pond in the *A. gracile* experiment was omitted from statistical analyses due to a disease outbreak.

RESULTS

Larval Survival

The proportion of *A. macrodactylum* surviving in ponds with trout was significantly lower than in ponds without trout (Tables 6.1 and 6.2). All *A. macrodactylum* larvae in control ponds survived the 30 d experiment (Table 6.1). Maximum number of surviving *A. macrodactylum* larvae in ponds with trout was 4. *Ambystoma gracile* survivorship was also significantly lower in ponds with trout than in control ponds (Tables 6.1 and 6.2). However, survivorship of *A. gracile* larvae was more variable than that of *A. macrodactylum* in both control ponds and ponds with trout (Table 6.1). Survivorship of *A. gracile* and *A. macrodactylum* larvae were not statistically compared because experiments were not run concurrently and the results could be confounded by experimental conditions.

Larval Growth

Both *A. macrodactylum* and *A. gracile* in ponds without trout had significantly greater SVLs than larvae in ponds with trout (*A. macrodactylum*, $P = 0.0071$; *A. gracile*, $P < 0.0001$, Wilcoxin tests; Table 6.3). Prior to the start of the experiments, SVLs were not significantly different between treatment groups (*A. macrodactylum*, $P = 0.83$; *A. gracile*, $P = 0.27$, Wilcoxin tests). Mean larval body mass also was not significantly different between treatment groups at the beginning of the experiments (*A. macrodactylum*, $P = 0.17$; *A. gracile*, $P = 0.24$, Wilcoxin tests; Table 6.3). At the end of the 30 d experiment, larval *A. gracile* in ponds without trout had significantly greater body mass than did larvae in ponds with trout ($P = 0.0081$, Wilcoxin test). However, larval *A. macrodactylum* did not differ in body mass between treatments ($P = 0.11$, Wilcoxin test; Table 6.3).

Substrate Use

Substrate location of larval *A. macrodactylum* on day 30 was significantly influenced by the presence of trout ($P < 0.0001$, $\chi^2 = 693.3$, d.f. = 4, Drop in Deviance test). Larval *A. macrodactylum* surviving 30 d in ponds with fish were found only in rock substrates (Table 6.4). In contrast, larval *A. macrodactylum* in control ponds were present in all substrates and in the open at the conclusion of the experiment. Substrate location of larval *A. gracile* was also influenced by trout on the final experiment day ($P < 0.0001$, $\chi^2 = 632.08$, d.f. = 4, Drop in Deviance test). Larval *A. gracile* in ponds with trout were found either in rock or woody

Table 6.1. Mean number and proportion (p) of *Ambystoma macrodactylum* (AM) and *A. gracile* (AG) surviving in artificial ponds with trout and without trout.

Species	Trout	N ^a	Mean	Range	p
AM	Yes	6	2.0	0-4	0.10
	No	6	20.0	20	1.00
AG	Yes	6	5.0	1-11	0.25
	No	5	18.6	14-20	0.93

^a Number of ponds used for survivorship analysis; one fishless tank with *A. gracile* was omitted from statistical analysis due to disease outbreak.

Table 6.2. Summary of ANOVA on proportion of larval *Ambystoma macrodactylum* (AM) and *A. gracile* (AG) surviving 30 d experiments in artificial ponds with trout and without trout.

Species	Source of Variation	df	Sum of Squares	Mean Square	Test of Significance
AM	Fish	1	5.167	5.167	$\underline{F} = 216.858$ $\underline{P} < 0.0001$
	Error	10	0.238	0.024	
	Total	11	5.405		
AG	Fish	1	2.322	2.322	$\underline{F} = 34.094$ $\underline{P} = 0.0002$
	Error	9	0.613	0.068	
	Total	10	2.934		

Table 6.3. Mean snout-vent lengths (mm) and weights (g) of larval *A. macrodactylum* and *gracile* in ponds with fish and without fish at the start and end of each 30 d experiment. Snout-vent lengths were measured for each larva. Mean weights were calculated by dividing the total weight of all larvae by the number of larvae in each pond.

Species	Fish	Snout-Vent Length		Weight	
		Start	End	Start	End
AM	Yes	19.79 ± 1.90 (n = 120)	24.83 ± 2.33 (n = 12)	0.51 ± 0.04 (n = 6)	0.89 ± 0.26 (n = 6)
	No	19.85 ± 1.78 (n = 120)	26.73 ± 1.91 (n = 120)	0.48 ± 0.06 (n = 6)	1.17 ± 0.07 (n = 6)
AG	Yes	19.38 ± 2.29 (n = 120)	28.87 ± 2.79 (n = 30)	0.46 ± 0.03 (n = 6)	1.37 ± 0.26 (n = 6)
	No	19.05 ± 2.17 (n = 100)	33.89 ± 3.07 (n = 93)	0.42 ± 0.05 (n = 5)	2.59 ± 0.12 (n = 5)

Table 6.4. Mean (range) of surviving larval *A. macrodactylum* (AM) and *A. gracile* (AG) associated with each substrate at the conclusion of laboratory experiments. Larvae classified as open were exposed on sand substrate or in the water column.

Species	Trout	Substrate				Total
		Wood	Rock	Veg. ^a	Open	
AM	Yes	0	2.0 (0-4)	0	0	2.0 (0-4)
	No	7.8 (6-11)	4.2 (1-8)	4.5 (0-8)	3.5 (0-10)	20.0
AG	Yes	3.0 (0-8)	2.0 (0-5)	0	0	5.0 (1-11)
	No	9.6 (5-16)	1.2 (0-3)	5.4 (4-8)	2.4 (0-5)	18.6 (14-20)

^a Submerged vegetation.

materials (Table 6.4). *Ambystoma gracile* in control ponds were found primarily in wood and vegetation, but also occurred in rock substrates and in open areas. At the termination of the laboratory experiments no larvae of either species in ponds with trout were found in vegetation or in the open.

DISCUSSION

Several studies have used controlled experiments to demonstrate fish predation on eastern ambystomatid larvae (Petranka 1983, Semlitsch 1988, Sih et al. 1992), and numerous researchers have reported that the presence of fish reduces the abundances of larval ambystomatids (Burger 1950, Blair 1951, Sexton and Phillips 1986, Semlitsch 1988).

In the present study, survivorship of *A. macrodactylum* and *A. gracile* larvae was significantly lower in artificial ponds with trout than in fishless ponds. These findings support hypotheses from field studies that larval abundance of both species is reduced in the presence of trout (Sprules 1974; Taylor 1983, 1984; Tyler et al. 1998). Trout predation is likely responsible for reduced larval survival in artificial ponds. However, reduced foraging opportunities due to the threat of predation may have resulted in starvation of larvae, thus, influencing larval survival.

At the termination of the experiments, larval *A. macrodactylum* and *A. gracile* occurred in a narrower range of habitats in artificial ponds with trout than in ponds without trout. The ability to detect predators and select habitats providing refugia from predation has been documented for larvae of several eastern ambystomatid species (Kats et al. 1988, Sih et al. 1992, Sih and Kats 1994), including increased refuge use (Sih et al. 1988) and decreased activity levels in the water column (Stangel and Semlitsch 1987, Figiel and Semlitsch 1990). In the present study, *A. macrodactylum* in ponds with trout were observed only in rock substrates while larvae of *A. gracile* in ponds with trout were found in both rock and wood substrates. Larvae of both species in fishless ponds were observed in all habitats, including open areas. Of the substrates provided, rock and woody material likely provided larvae with the greatest protection from predation.

Larval size of both *A. macrodactylum* and *A. gracile* was influenced by trout; both species exhibited greater SVLs in control ponds than in fish ponds at the conclusion of experiments. However, only *A. gracile* had greater body mass in fishless ponds than in ponds with trout. Semlitsch (1987) and Figiel and Semlitsch (1990) observed reduced growth in SVL of larval *A. maculatum* when raised in tanks with a predatory sunfish.

Reduced foraging opportunities associated with increased refuge use or competition between larvae and trout for a limited food resource could have reduced the growth of larval *A. macrodactylum* and *A. gracile* (Semlitsch 1987, Sih et al. 1988, Figiel and Semlitsch 1990). However, we were unable to determine which of these mechanisms was more important.

Our findings support inferences from field studies that trout can reduce survival of *A. macrodactylum* and *A. gracile* larvae. Our findings also indicate that trout can affect larval habitat selection and growth rates. Slower growth rates may reduce salamander survival either through increased length of the larval period and, thus, increased susceptibility to mortality factors in the aquatic environment (e.g., predation, lake desiccation, or lake freeze). Slower growth rates may also decrease larval size at metamorphosis, thus increasing susceptibility to terrestrial threats (e.g., desiccation, amphibian requirements of osmoregulation and thermal regulation).

ACKNOWLEDGMENTS

We are grateful to the former and present members of our scientific advisory panel: S. Loeb, S. Dodson, R. Hughes, W. Neill, W. J. O'Brien, J. Petranka, W. Platts, and H. B. Shaffer. This research would not have been possible without the co-operation and logistical support provided by the personnel of North Cascades National Park Service Complex. This research was funded by the National Park Service and the USGS-Forest and Rangeland Ecosystem Science Center.

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SUMMARY

Lake Water Chemistry

Time of ice-out increased from low-forest to alpine lakes. Eastside lakes iced-out earlier than did westside lakes. Epilimnetic temperatures were warmest in low-forest lakes and coolest in alpine lakes. Classification did not order lakes relative to chemical characteristics, although westside low-forest lakes differed significantly from other lake classes and were most productive. Little seasonal and annual variation for most chemical characteristics were found. However, chemical differences did mirror environmental and physical differences among lakes. High phosphorus levels separated glacially influenced lakes. Total Kjeldahl-N and total phosphorus decreased with increasing lake depth. Decreasing lake elevation generally was associated with increased water temperatures, pH, alkalinity, conductivity, and nutrients.

Phytoplankton

1. Phytoplankton assemblages from 64 lakes included 153 taxa. Based on cell density counts for all samples, *Aphanocapsa delicatissima* had the highest proportional abundance (67.5%). Sixty percent of the taxa occurred in few than 20 of the samples (177).
2. Large seasonal and annual variations in the taxonomic structure of the phytoplankton assemblages were observed.
3. When classified by forest type, alpine lakes had the lowest number of taxa per sample and subalpine and high-forest the highest. Proportional cell densities of chlorophytes and chrysophytes were highest in alpine and subalpine lakes, whereas cyanobacteria dominated high-forest and low-forest lakes.

4. In general, lake elevation, and changes in water quality associated with changes in lake elevation, and concentrations of nutrients were most closely associated with the taxonomic structure of the NOCA phytoplankton assemblages.

Introduced Trout

In NOCA trout densities in lakes with reproducing trout were generally much higher (range 250-724 fish/ha) than in lakes with non-reproducing trout, where trout fry are periodically stocked at low densities (mean 179 fry/ha with mean interval between stocking > 5 years).

Larval Salamanders

General Conclusion

Field and laboratory studies support the view that introduced trout can reduce abundance or even eliminate long-toed salamander larvae (*Ambystoma macrodactylum*) from fishless high-elevation lakes in NOCA. However, the effects of trout appear to be influenced by the reproductive status of trout and lake chemistry.

Specific Conclusions

1. In fishless lakes, mean larval salamander densities, assessed by snorkeling, were positively related to total Kjeldahl-N (TKN), an indicator of the trophic status of lakes. Higher TKN increased abundance of crustacean zooplankton, an important food resource of larval salamanders, likely contributed to increased larval abundance.

2. Differences in mean larval salamander densities between fishless lakes and lakes with trout was related to TKN concentrations and the reproductive status of trout. Mean larval salamander densities in fishless lakes with TKN <0.045 mg/l were not significantly different from larval densities in lakes with reproducing trout or in lakes with non-reproducing trout. However, in lakes with TKN ≥0.045 mg/l, mean larval densities were significantly higher in fishless lakes than in lakes with reproducing trout. In lakes with

TKN \geq 0.095 mg/L larval densities were significantly higher in fishless lakes than in lakes with non-reproducing trout.

3. In laboratory studies, survival of larval *A. macrodactylum* (and *A. gracile*) over a 30-day period was significantly lower in experimental ponds with trout than in ponds without fish, corroborating results from field studies. Mean body size of surviving larvae also was significantly lower in ponds with trout than in fishless ponds.

4. In lakes with fish there was a tendency for a greater proportion of larvae to be hidden in substrate materials on the lake bottom than in fishless lakes, although the difference between fishless lakes and lakes with fish was not statistically significant. Laboratory studies revealed significant differences in substrate utilization of surviving larvae between fishless experimental ponds and ponds with fish.

Crustacean Zooplankton

General Conclusion

Results of field studies are consistent with the view that introduced trout can eliminate or reduce abundance of large diaptomid copepods possibly resulting in increased abundance of smaller herbivorous diaptomids in high-elevation lakes in NOCA. These effects of trout appear to be influenced by trout density, lake depth, and lake chemistry.

Specific Conclusions

1. The abundance of large diaptomid copepods (*Diaptomus kenai* and *D. arcticus*) was significantly lower in shallow lakes (maximum depth \leq 10 m) with reproducing fish than in lakes with non-reproducing trout or fishless lakes. There was no significant difference in large diaptomid density between lakes with non-reproducing trout and fishless lakes.

2. The abundance of large diaptomids was significantly lower in shallow lakes with high trout densities than in deep (maximum depth $>$ 10m) lakes with reproducing trout.

3. A small herbivorous diaptomid, *D. tyrrelli*, was found only in shallow lakes with relatively high concentrations of TKN (>0.05 mg/l) and total phosphorous (>0.007 mg/l).

4. For lakes with chemical concentrations suitable for the small copepod, *D. tyrrelli* density was inversely related to large diaptomid density which could imply interaction of large and small diaptomids. *D. tyrrelli* often was abundant in shallow lakes with high trout densities where large diaptomids were either absent or low in abundance, whereas in lakes with non-reproducing trout and in fishless lakes where large diaptomids were abundant, the small copepod was usually absent.

Global Conclusions Related to Effects of Introduced Trout On Native Biota

1. Our field studies suggest that introduced trout may have the greatest impact on native biota in shallow lakes (≤ 10 m maximum depth) where reproducing trout reach high densities. Shallow fishless lakes with higher concentrations of TKN are productive habitats for larval salamanders. When reproducing trout reach high densities in these lakes both larval salamanders and large diaptomid copepods are absent or persist at low abundances and the small herbivorous diaptomid *D. tyrrelli* may be abundant.

2. Trout appear to have the least impact on native biota in deep lakes (>10 m maximum depth) with non-reproducing trout. These lakes generally have low concentrations of TKN and are not productive habitats for larval salamanders. Since larval densities are low in fishless lakes with low TKN, it is difficult to detect statistically significant differences in larval densities between fishless lakes and lakes with fish. In deep lakes large diaptomid copepods may find refuge from trout predation in deeper water during the day and so escape predation from a visually-oriented predator such as trout.

3. Lakes with non-reproducing trout will be a crucial component of NOCA's high lakes management plan because the lakes are common within NOCA. Furthermore, many anglers prefer to fish in lakes with non-reproducing trout because trout densities are low and fish often reach a large size. Lakes where trout are incapable of reproducing because they lack adequate spawning areas may offer the most options for future management. In these lakes fish densities can be regulated by controlling both stocking densities and the interval between

stocking. If deleterious effects are observed, fish can be eliminated in a few years through cessation of stocking. Our field studies suggest that non-reproducing trout have little impact on large diaptomid copepods and only affect larval salamanders in lakes that have high TKN (>0.095 mg/l). However, wildfire during 1994, our last scheduled full field season, limited our opportunity to sample lakes with non-reproducing trout. The sample size of lakes with non-reproducing trout was small ($N = 7$), most of the lakes were sampled only once, and the lakes that were sampled occupied only a narrow range along the TKN gradient. Thus our conclusions on effects of non-reproducing trout are tentative and further research is needed.

